NUTS

NMR Data Processing Software

for

Microsoft Windows-95/98/NT/2K/XP and Macintosh

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Table of Contents

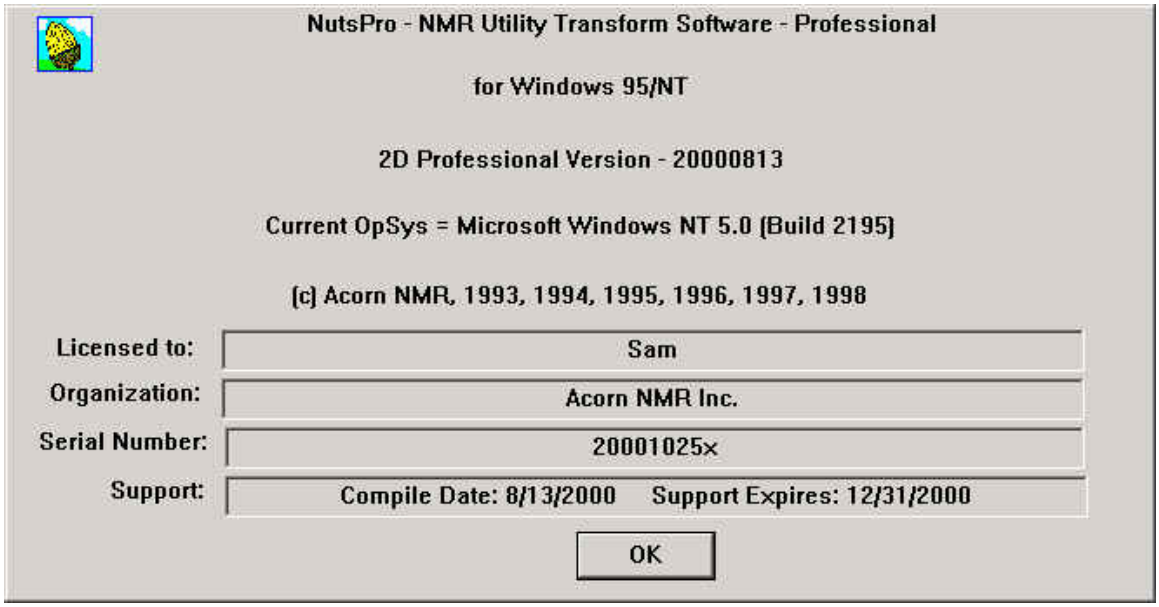
Installation

NUTS Installation for Windows

When you execute the downloaded *win_nuts.exe* file, the window below is displayed. *Note the folder* to which the files will be unzipped. Click on **Unzip**, enter the password. Files will be placed into the folder you specified. When unzipping is finished, close the window.

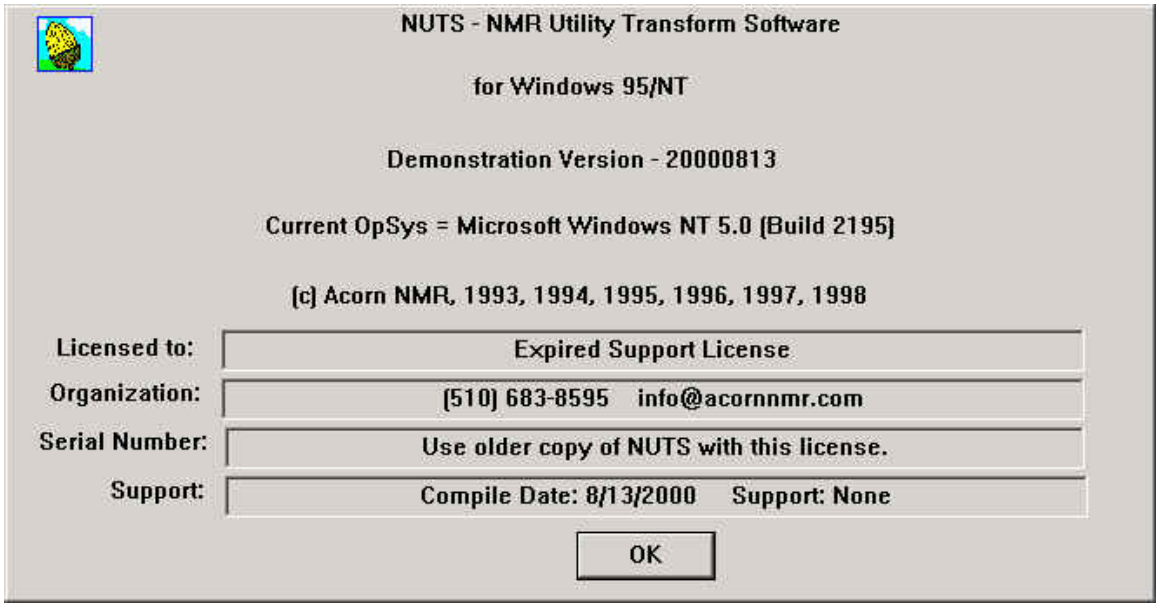
You received 2 attached files with the email message containing installation instructions. Save those 2 files to the folder which now contains the unzipped NUTS files.

If you have followed the instructions you received with your license file, and everything worked as it should, you should see a screen similar to this when you start NUTS, with your name showing:

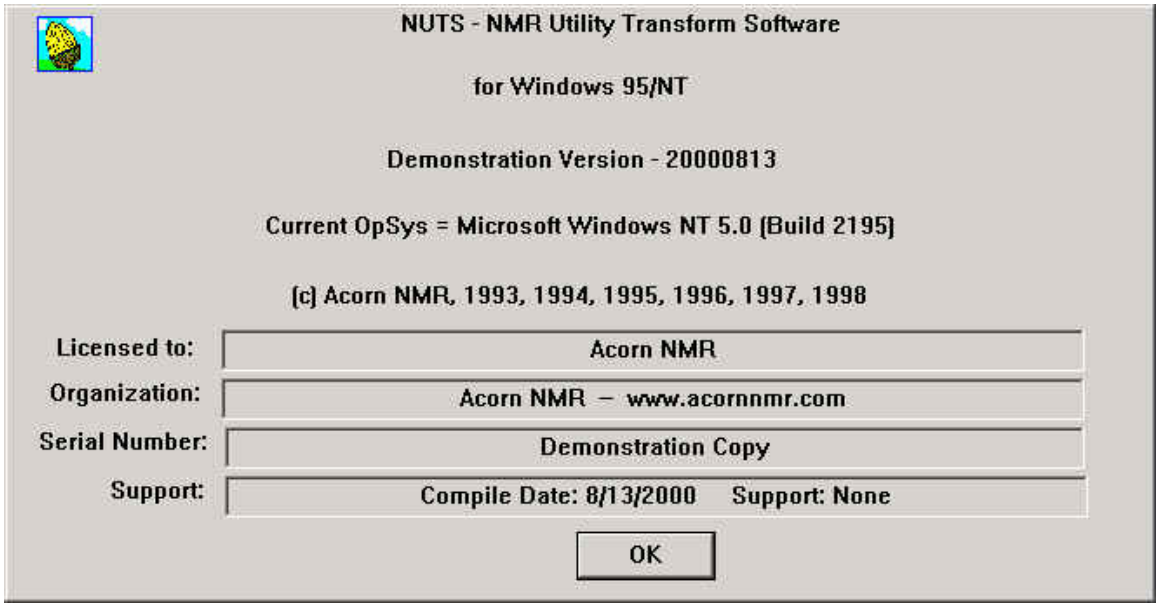


If your name is on this screen, and the correct Version is shown (Lite, 1D, 2D or Professional), then installation was correctly completed, and you can begin using NUTS.

If, instead, you see a screen that says "expired support license", as shown below, then you are attempting to run a copy of NUTS that is not authorized by your license file, because your support period has expired. You either need to renew support, or reinstall your older copy of NUTS whose compile date falls within your support period.



If you see a screen that says Demonstration Copy, as shown below, then the license file has not been correctly installed.



Not all features of NUTS will operate because NUTS is not able to read your license file.

Possible reasons for failure of license installation are:

1. You have not saved the 2 files (which you received via email) into the nuts folder
2. The files were saved, but one or both was saved with an incorrect name
3. The files were corrupted in the course of emailing or transferring between computers after receipt

Most often, corruption of files in the course of transferring between computers occurs when transferring to or from a Macintosh, because the Mac OS insists on modifying files to assign them to an application. This modification consists of appending a "resource fork" onto the beginning of the file, consisting of 128 bytes - the OS literally *changes* the file! To avoid corrupting these files, remember ***always*** to transfer files as binary.

Do not attempt to open either of the files supplied with your installation instructions. They are encrypted binary files, and cannot be viewed with a text editor or word processing program.

In an attempt to help users determine why the license installation failed, the latest version of NUTS (dated 11/1/00 or later) will examine the files in the nuts folder and will display an error message on startup intended to point out the cause of the problem.

Possible messages are:

There is no license file in the nuts folder.

Sometimes, an attempt to save a new license file fails to overwrite an older one, and instead, the new file is named "license1.nmr". This means NUTS will read the old license, not the new one.

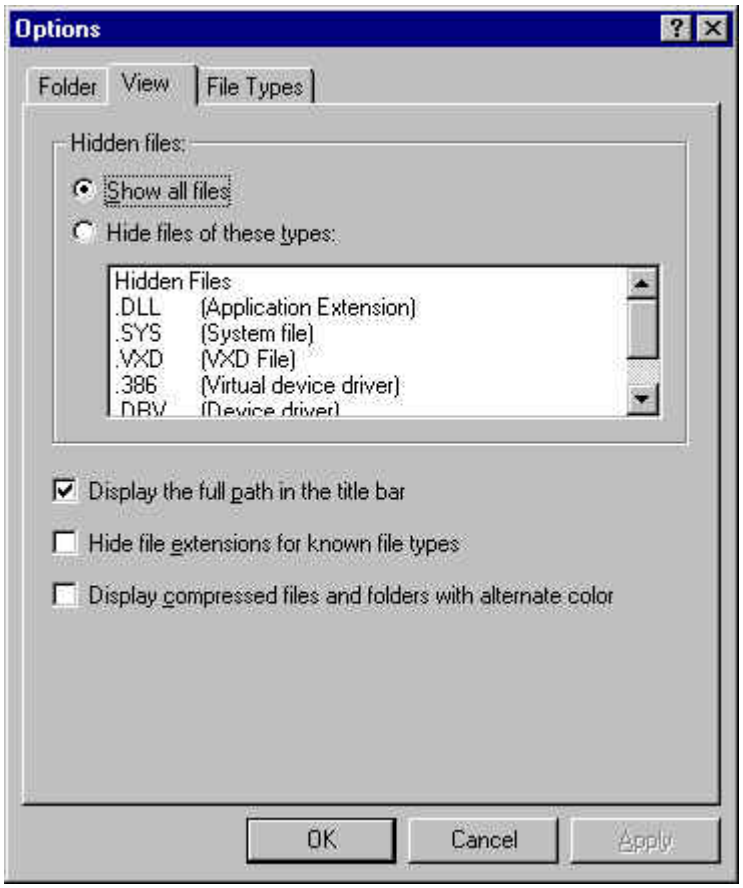
If you observe either of these failures, you need to examine the list of files in the NUTS folder to determine and correct the cause of the problem.

Start NUTS and execute the command **DI**. This opens the Notepad and lists all files in the NUTS folder. Two files were sent with your installation instructions. Both files must be listed, and must have the file names unaltered.

The reason for using this DI command rather than Windows Explorer is that, by default, Windows is often configured such that not all files are listed, and full file names are not always shown. To view the folder contents using a file manager, the first step is to be sure your file manager is displaying a full and correct list of files.

To do this, open *My Computer*. Select **folder options** from the **Tools** menu (Win 2000) or the **View** menu (Win98), or **options** from the **View** menu (NT 4.0). Click on the **View** tab. You should see a screen that looks like one of the following:

Win NT



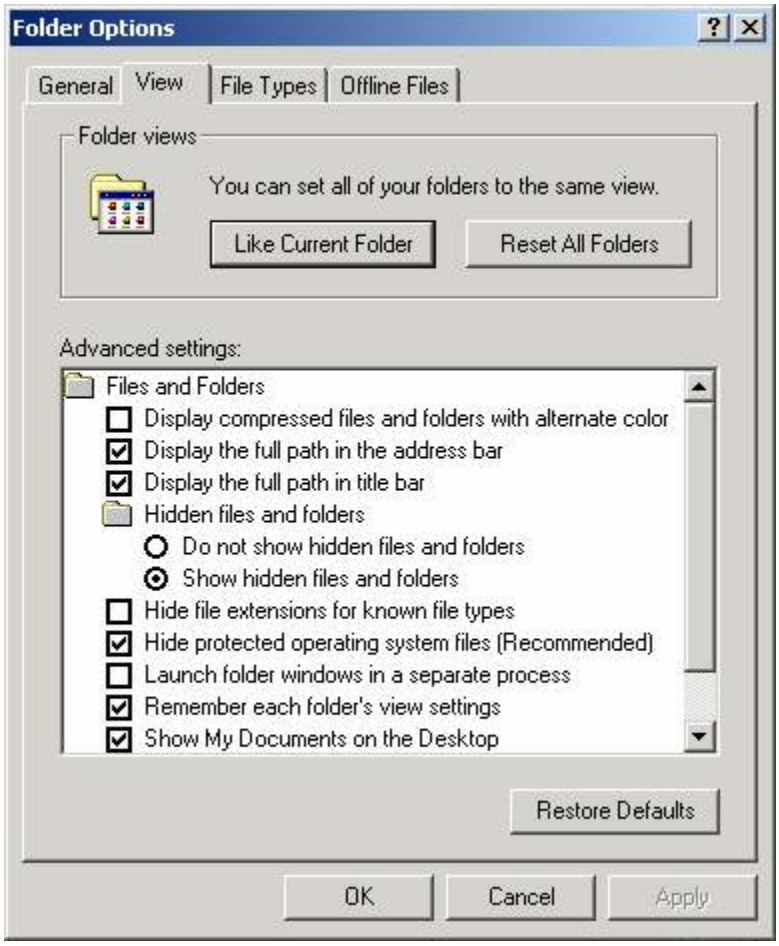
Check the button that says "Show all files".

Be sure the button saying "Hide files of these types" is ***NOT*** checked.

Be sure the box saying "Hide file extensions for known file types" is ***NOT*** checked.

Win 2000 or Win98

Check the button that says "Show hidden files and folders".



Be sure the box saying "Hide file extensions for known file types" is ***NOT*** checked.

Once the file manager options have been correctly set, examine the list of files in the NUTS folder. The 2 files sent to you via email as attached files should be there, with the correct file names. If not, refer back to the instructions emailed with your license file.

Macintosh installation

The NUTS Installer for the PowerMac installs program files, a few sample data files and macros, the updated MacNUTS Help files and the Microsoft system files (which are installed in Extensions).

This is a self-extracting Stuff-It archive that contains an Installer. To extract files, open the file using Stuff-It Expander (which can be downloaded free.) Then double-click on the Installer icon, read the displayed information, and click on Install.

The Installer places a few files into the Extensions folder, and creates a folder called MacNUTS into which the program and supporting files are placed.

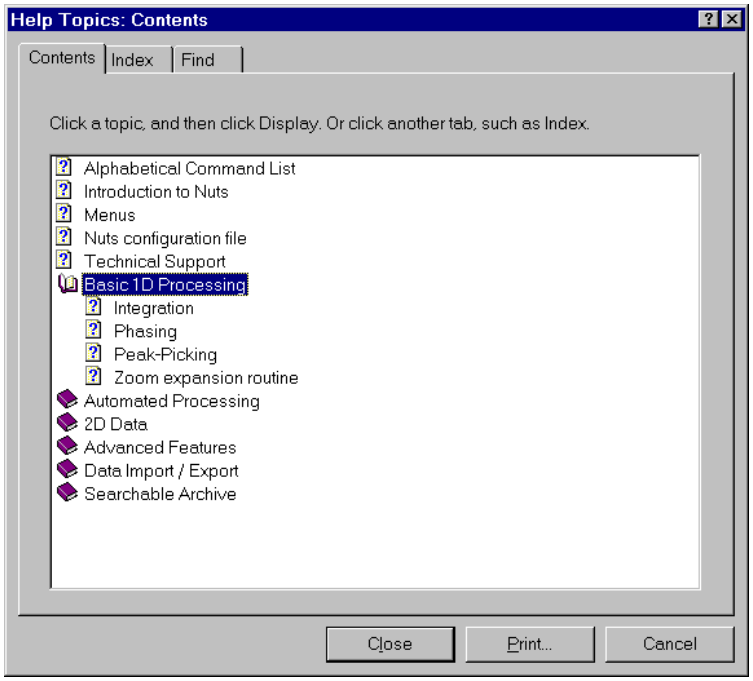
Help files

We have changed the format of Help files from the old "WinHelp" to the new "HTML Help". HTML Help has some very nice navigation features, but its implementation is more complicated. The latest versions of both HTML Help and WinHelp include step-by-step instructions for many NUTS operations, illustrated with screen captures. The conversion to a Help file for the Mac required that the screen captures be reduced to black and white, but otherwise will be the same as the Win version. The Mac version is now included in MacNUTS.sea (4/3/99)

The usefulness of Help files is dependent in part on having good tools for locating the desired piece of information within a large collection of topics. Help files provide one or more of 3 ways to find specific information:

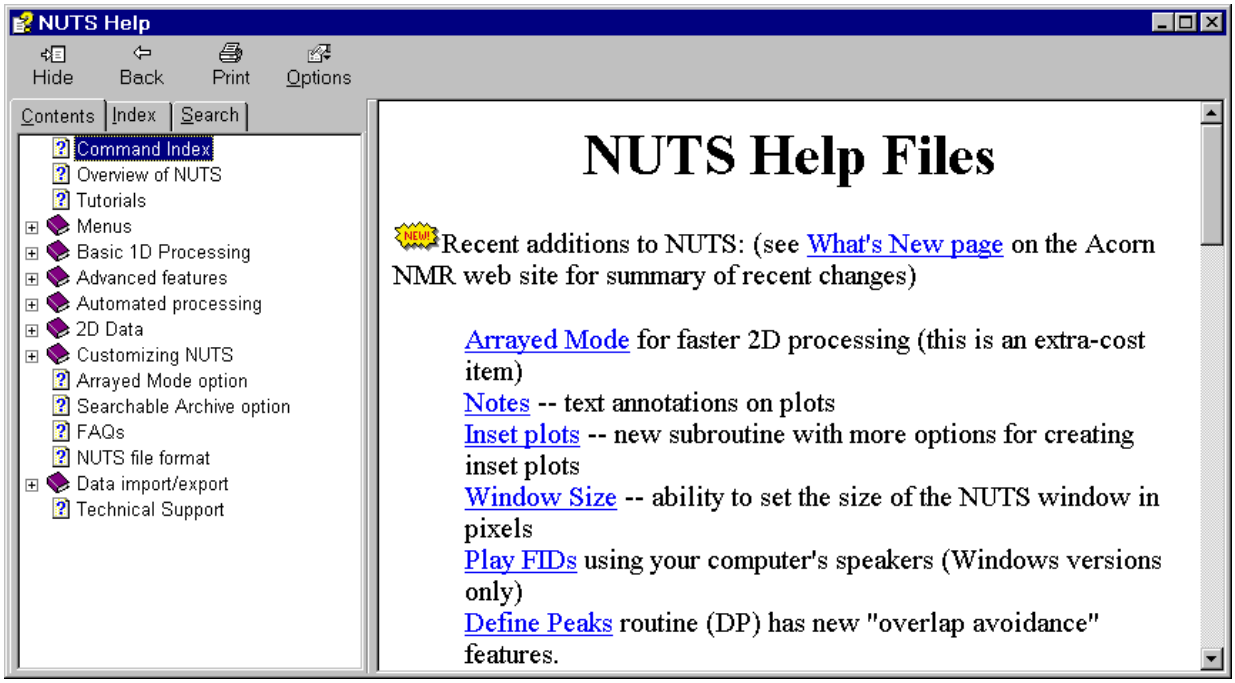
> **Table of Contents** -- a list of topics organized by subject
> **Keyword Index** -- search a list of keywords created by the author of the Help files
> **Full Text Search** -- search for occurrence of a word or phase anywhere in the text of the Help files

Older versions of WinHelp ("WinHelp3.1") and Help on the Mac included only keyword searching. Recent versions of WinHelp ("WinHelp95") provide all 3 of these tools, but the full text search is annoying because you have to wait after selecting "Find" for the program to build the searchable word list.



WinHelp95 window

The new HTML Help is similar to the collection of HTML files on the www.acornnmr.com web site. However, it is not just a collection of HTML pages; it is "compiled", meaning that a collection of HTML pages plus index and table of contents is compressed into a single file. One improvement which results is that the word list for full text searching is created at the time of compilation, eliminating that annoying wait.



HTML Help window

The functionality of a compiled HTML Help file is provided by ActiveX, Microsoft's implementation of Java. That means that the Help file can only be used with Win95/98/NT/2K. Also, it requires some system files to be installed on your computer.

For details about HTML Help, see Microsoft's web site.

Requirements for Win2000 and WinXP:

The HTML Help viewer is already part of the operating system. You don't need to do anything.

Requirements for Win95/98/NT:

Microsoft Internet Explorer (IE) 3.0 or above must be installed on the computer. (Microsoft is supposedly coming out with a setup program to install the "Internet

Explorer runtime engine", for users who do not have Internet Explorer. This is not yet available.) While IE must be installed on the computer, it does not have to be the default browser. IE needs to be installed to add operating system functions used by the HTML help system.

A few other files must be installed into the Windows or Windows/system folder. These are installed by executing a file called **hhupd.exe**, which is included in the nuts zip distribution file. This installs the following files, referred to as "HTML Help Runtime Components":

FILE	*DESCRIPTION*	*LOCATION*
HHCTRL.OCX	HTML Help ActiveX control	Windows/system folder
ITSS.DLL	DLL that handles compiled HTML	Windows/system folder
ITIRCL.DLL	Full-text search DLL	Windows/system folder
HH.EXE	HTML Help viewer	Windows folder

WinNT users - you must have Administrator privilege to install these system files.

Failure to install these files will prevent the compiled Help file from displaying properly and will cause NUTS to close in a most inelegant manner.

The compiled HTML Help file is distributed in the Nuts95.zip installation file. For those users who do not have IE and don't wish to, the alternatives are to access the Nuts Help web page or to install the older style WinHelp file by downloading Nuts.hlp and Nuts.cnt from the. They must be placed in the same folder as the Nuts program. You must then edit the file so that the default Help file is WinHelp by setting the HTMLHELP flag to FALSE.

HS – Help swap

It is also possible to swap the Help file selection within NUTS using the new **HS** (Help Swap) command, which toggles between WinHelp and HTML Help.

Win 3.11 and PowerMac

The compiled HTML Help file can be displayed directly only with the Windows 9x/NT operating system. Other operating systems use the WinHelp version.

The documentation on HTML Help states that it is possible to use Java applets to create a dual window similar to that displayed on Windows 9x/NT. We have found this to be too slow to be functional, and have not implemented it. This has forced us to maintain the

WinHelp version for use on the Mac. This Help file for the Mac is now included in the MacNUTS Installer, or can be downloaded separately.

If you are curious, here's our summary of Java applets for displaying HTMLHelp: Currently this method can display a table of contents and index, but cannot perform a full-text search. To use this Java method, the user must have installed a version 4 browser. On the PowerMac, both Internet Explorer 4 and Netscape 4 work. On Win3.11, only Netscape 4 works. Like most Java stuff today, viewing the help files with a browser using Java is slower than viewing the HTML help files directly with ActiveX on a Windows 9x/NT platform. However, with a reasonably fast PC performance is acceptable. On older and slower PCs the Java performance is a serious drawback. For example, a 486 DX4/100, under Windows 3.11 it took *over 11 minutes* to display the index! It appears to take ~1 second for each entry in the table of contents or index. For the 40 or so items in the table of contents, the wait isn't too bad, but the index contains about 400 keywords.

Alphabetical Command List

Listed below are commands active in the "base level" of NUTS (as opposed to within subroutines). Most are 2-letter commands that are executed immediately, without requiring <ENTER>. As of 5/15/99, there is an optional command mode that allows use of longer commands, including arguments as appropriate, and requiring <ENTER> before the command is executed. Where an equivalent longer command exists, it is listed together with the corresponding 2-letter command. See detailed explanation of this "non-2-letter command" mode.

Longer commands

NUTS has an optional command mode in which commands can be more than just 2 letters long. When in the "non-2-letter" command mode, a carriage return is required after any command is typed before it is executed. This applies only to the base level commands, and not to commands within any of the subroutines.

2F – Turn off 2-letter command mode

2N – Turn on 2-letter command mode

The default command mode can be specified in the nuts.ini file, with the following lines:

CR_FOR_COMMANDS = FALSE or CR_FOR_COMMANDS = TRUE

When this flag is set to TRUE, the longer command mode is active, and all commands will require an <ENTER> before they execute.

The reasons for the change are shortage of "logical" 2-letter commands, and to provide additional flexibility (such as the ability to specify arguments on the command line).

In the new mode of operation, the commands can be up to 32 characters long and some commands can take arguments. The same two letter commands as before still work but they require an <ENTER> before they are executed. Commands selected from a menu automatically detect the mode of operation and supply the <ENTER> when necessary. Links and Macros will need modification to execute properly. The simplest solution for existing macros is to add a **2N** command at the beginning. To use the longer commands in a link or macro, a comma is used to tell Nuts to insert an <ENTER>.

All commands, whether long or short, are listed in the command list.

See also: 3D processing, macros

It is possible to undo many NUTS operations. This works only in the base level of NUTS, not in the subroutines. Before a command is executed, the current data is copied into a temporary 10-layer file cache. This functions like the stack in a reverse polish calculator. Each time a command is executed, the data is added to the stack, and previous entries roll up. The user can then recall each previous version sequentially, causing the stack to roll down, using Ctrl-Z.

The user can choose to enable or disable the Un-Do process. This can be done while NUTS is running, using UN and UF to turn Un-Do on and off, respectively. This can also be done in the nuts.ini file, with the following line:

UNDO = FALSE or UNDO = TRUE

In a link or macro, or when processing 2D data in non-arrayed mode, saving of data to the Un-Do cache is automatically disabled.

UnDo will work for 2D data while in the Arrayed Mode. However, the time required to write copies of a large 2D file probably makes this inadvisable, and it is suggested that Un-Do be disabled before processing 2D data.

UN -- Un-Do On

UF -- Un-Do Off

Ctrl-Z -- recall previously saved data

Note that Ctrl-Z while in the Zoom subroutine is a completely unrelated command (zeroes the data in the expanded region).

Introduction to NUTS

This section is an introduction to the basics of navigating around the program, scaling the display, using the cursor, setting the chemical shift reference, plotting and copying.

In addition to the operation of the program from the menus, a command line user interface is also active. When the menus are pulled down with the mouse and the menu choices are displayed, the keyboard letters corresponding to each command are also shown. This "learn as you go" approach allows the user interested in operation of NUTS from the command line to become familiar with the necessary commands in the course of normal operation and minimizes the frustrations of searching through manuals. Some of the more advanced features are not available from the menus. On-line Help is also available.

See Getting Started section below.

Base Level Operation

The base level of operation is the starting point when the NUTS program is run. Many functions of NUTS, such as Zoom and Integration, operate as subroutines of the base level. Sample files are available for download, and can be opened in NUTS by using the menu and selecting **File/Open**. A dialog box appears and allows the user to select an NMR file. If the file is an FID it will need to be transformed (FT) and phased. If the file was a spectrum (previously processed and stored) it will be displayed and is ready for further perusal. The spectrum can be printed using the **File/Print** menu option, which displays a Print Setup dialog box. The Zoom subroutine is used for horizontal expansions, to display a selected region of the spectrum. The horizontal scroll bar at the bottom of the screen will shift the display left and right if an expanded region is currently displayed.

The NUTS base level of operation is characterized by the following general features.

Vertical scaling The spectrum will be automatically scaled to display the tallest peak full height. Vertical scaling can be done using the scroll bar on the right. Vertical scaling can also be done with the "<", ">", up and down cursor control keys, PAGE UP, or PAGE DOWN keys. At any time, the vertical scale can be reset to zero with S0 or the largest displayed peak can be made full height with Control-Y. See also scaling the display.

Cursor A mouse cursor (ARROW) will be shown on the display and its position is a function of the mouse movements. If the left mouse button is depressed and held, a full window crosshair will replace the mouse cursor and its x and y position displayed in real time in the lower right corner of the window as the mouse is moved. The x position is shown as data point number, Hertz, and PPM. The vertical position is shown as a percent of the tallest peak in the spectrum. This information is displayed as long as the mouse button is depressed.

The cursor information can optionally be displayed in difference mode. While holding down the left mouse button, click the right mouse button. The current x position of the vertical crosshair will be marked and an additional line of readout will be displayed at the bottom right of the window. This additional line of display shows, in real time, the current cursor x position relative to the marked position. As the mouse is moved, while still holding down the left mouse button, this difference display is updated. This is useful for quickly measuring coupling constants, line widths, etc. This line also shows the average of the frequencies of the marker and crosshair cursors, for easy determination of the chemical shift of mutiplets such as doublets.

All functions return to the default base state of operation when the left mouse button is released. Users with a single-button mouse can press the period key on the keyboard in place of the right mouse button.

This is illustrated in the section below about using the cursor.

Setting Chemical Shift Reference

The easiest way to set a peak to a specific chemical shift is to hold down the left mouse button, place the cursor on the peak and type **O** (for offset). A dialog box appears which allows you to set the frequency in either Hz or PPM. Note that this function operates at the base level of NUTS and not in the Zoom subroutine.

If the current file is a 2D file, the same procedure is used to set the shift in both dimensions.

If the chemical shift of the reference peak is to be set to zero, the SZ command provides a quicker way to set the reference. Use Zoom to expand around the peak and SZ sets the value of the largest peak in that region to zero.

The way Nuts keeps track of the chemical shift is via the O1 (offset) parameter. (Note that this does not have the same meaning as in Bruker software.) O1 is the offset, in Hz, from the center of the spectrum to 0 ppm.

SZ -- Set Frequency to Zero

Sets the frequency of the largest peak in the currently displayed region to Zero. This can be used to set the chemical shift reference by first Zooming in on the TMS peak, then typing SZ. This now works for 2D data as well. If the data is displayed as a contour plot or intensity plot, then the largest displayed peak will be set to a shift of zero in both dimensions. Note that in cases of limited digital resolution, the zero value may not appear in the center of the chosen contour, and will need to be adjusted using the cursor and the Offset (**O**) command, as described in the preceeding paragraph.

PR -- Position Reference

Sets the frequency of the largest peak in the currently displayed region to a value previously set in a macro with the Ask Shift command.

Plotting The displayed spectral region is plotted using the **File/Print** menu selection. The NMR data will be plotted as it is currently being displayed. Spectral parameters will be plotted at the bottom of the NMR plot. These parameters are spaced expecting a Landscape 8.5" x 11" plot. If the printer is not currently in the landscape mode, it should be placed into the landscape mode with the printer dialog box when printing. The printed spectrum will have the characteristics of the displayed spectrum. The spectrum height will fill the plot the same as the display window. The axis type will be the same as the displayed axis type. If an integral is being displayed with values on the screen, it will be plotted with values on the paper. There is a Page Setup option under the File menu which allows several plot features to be set.

PL -- Plot

Prints the displayed spectrum to the currently selected printer. The first time PL is executed (unless a Print Setup has already been performed) a Print Setup dialog box is displayed, allowing selection of printer device and print parameters. Once printer parameters are selected, clicking OK causes the plot to be printed. Thereafter, when the PL command is entered, printing is performed directly. The printer parameters can be changed at any time by selecting Print Setup from the File menu. Printing can also be performed from the File menu.

The user can set several options, including whether or not to print parameters on the bottom of plots, whether or not to draw a box around plots, margins, color printing and line thickness by selecting Page Setup under the file menu. NUTS plots are configured for 8.5x11 paper in Landscape orientation and are sized to be as large as possible. By default, plots include the parameter list and box, and all colors on the screen are mapped to black for printing.

Page Setup allows larger margins to be set. Even when margins are set to zero, the plot will always have small margins on all sides. The size of these margins is dependent on the particular printer and printer driver being used. Setting margins to non-zero values adds the entered values to the default small margins. Note that margins are set in mm.

There is also an option in the Page Setup box for making 2D plots square, making it easier to view data from homonuclear experiments. The plot will be the maximum size possible, which is about 7 inches, plus axes, for 8.5x11 paper. This option affects only 2D plots.

Fonts for the different types of text can be changed by choosing Set Fonts from the Edit menu.

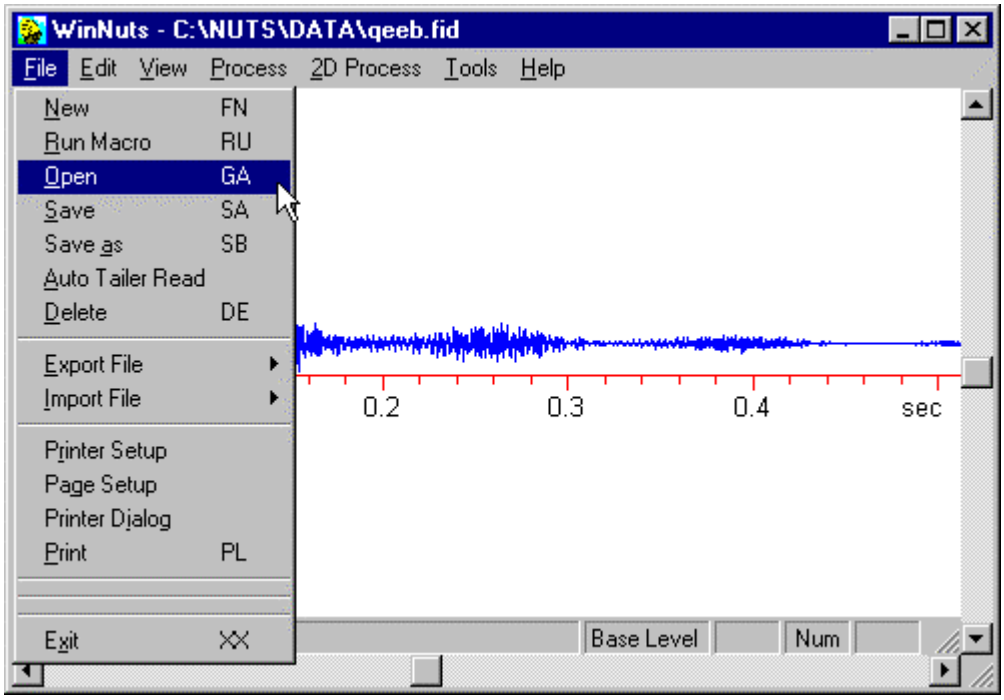
Some of these options can be set in the NUTS.INI file, so that they are invoked every time NUTS is run.

Copy and Paste to the Windows Clipboard The currently displayed spectrum can be placed in the Windows clipboard using the Edit / Copy menu option. From the clipboard, it can be placed into other Windows programs, such as word processing programs for inclusion in reports, using Paste. The picture is a bit map and the result will have the best quality if the NUTS window is set at maximum size, completely filling the screen, before the Edit / Copy operation is performed. NUTS also provides the option of copying the spectrum as a Metafile, rather than a bitmap, which results in a better quality picture. See details about copying below.

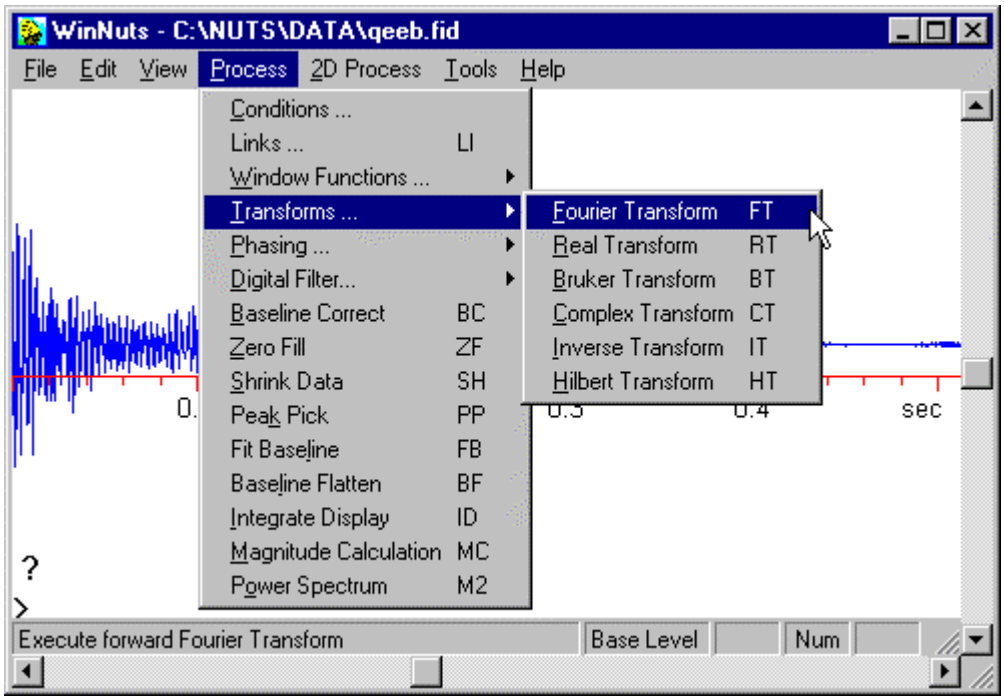
See also: Zoom, Phasing, Integration, Peakpicking

Getting started – open a file and perform basic processing

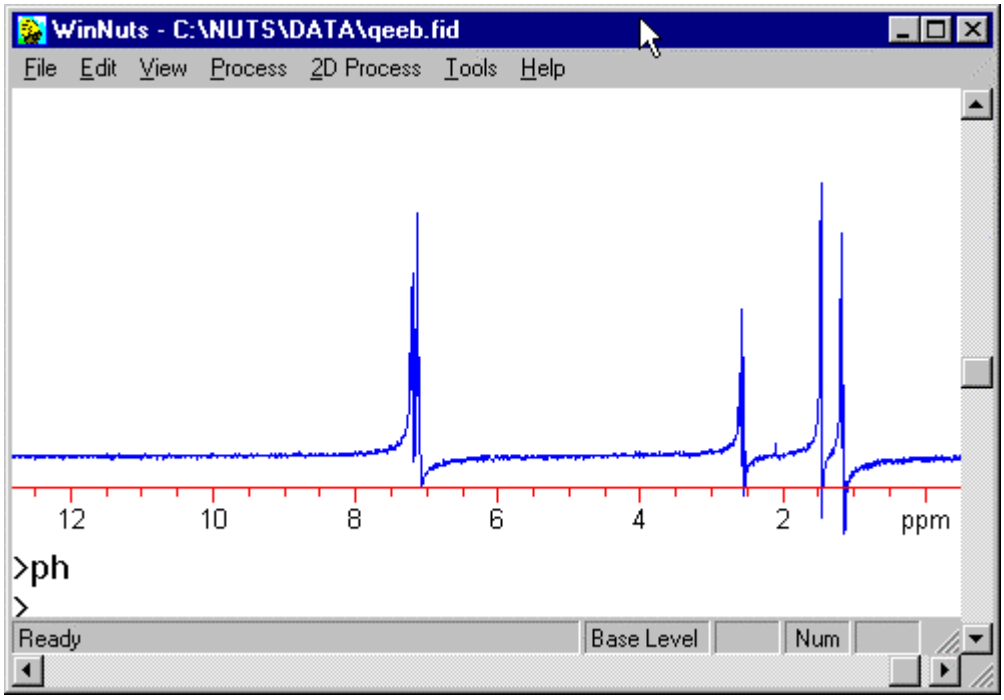
This section illustrates some of the basic operations in NUTS, including FT, automatic phasing, automatic integration and peak picking.



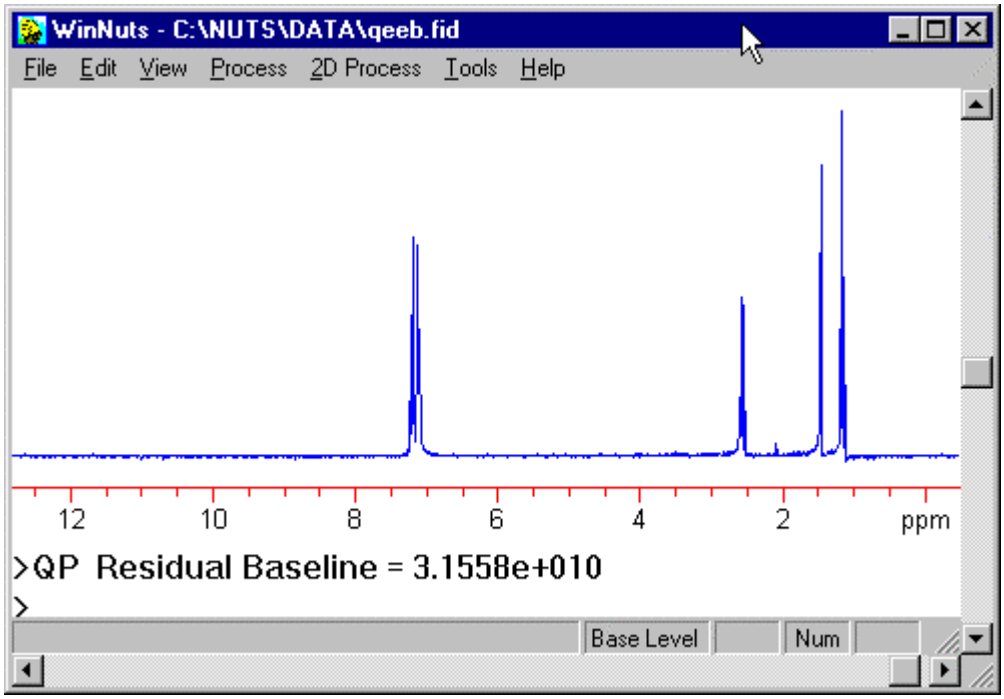
Choose Open from the File menu and select the file corresponding to the FID data.



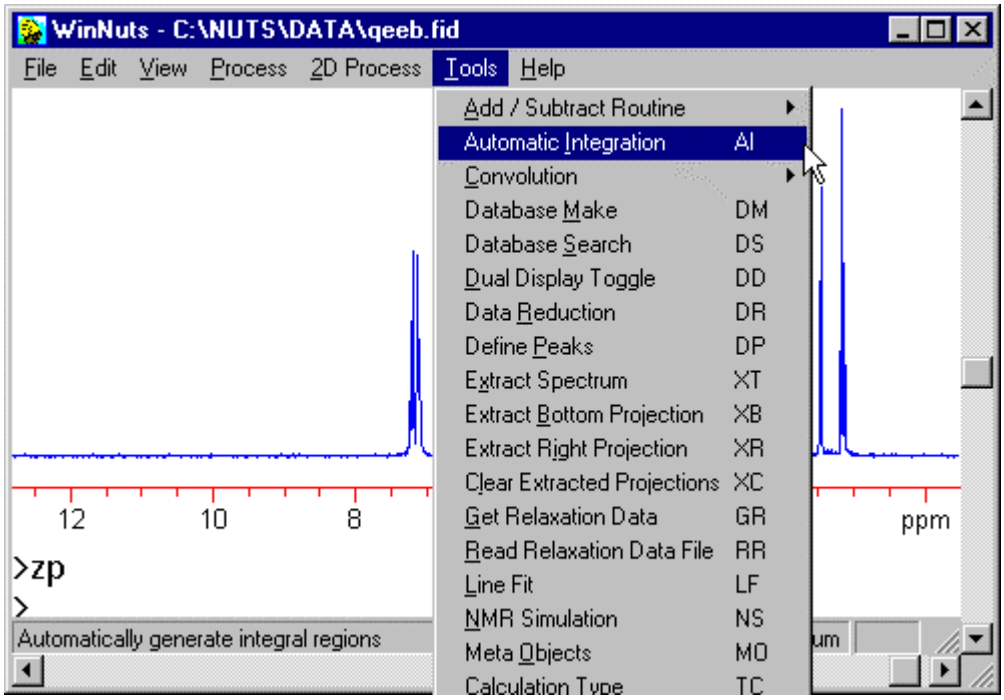
Select Fourier Transform from the Process menu.



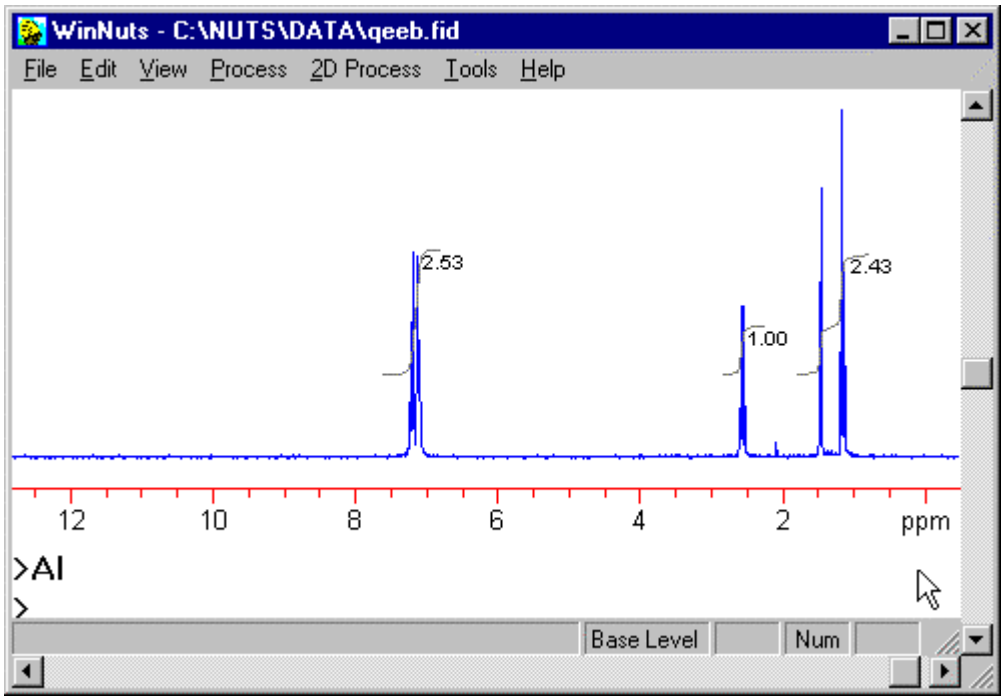
The simplest way to phase is using automatic phasing. Choose Quick Phase from the Process/Phasing menu.



The resulting phase is fairly good, and can be manually touched up using interactive phasing methods. See section on phasing.



Choose Automatic Integration from the Tools menu.

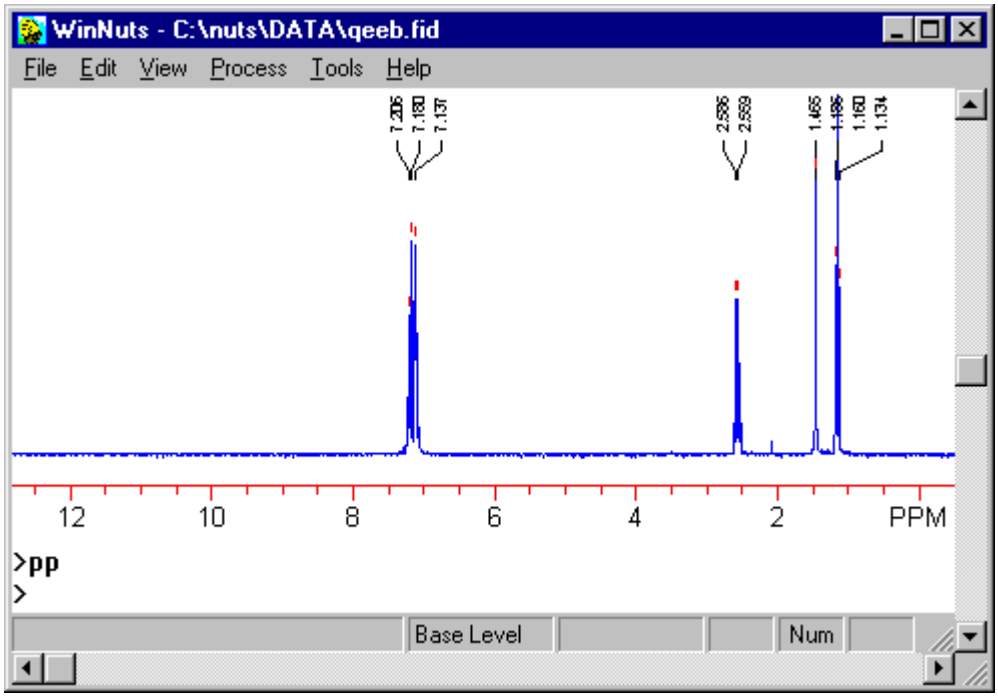


NUTS has automatically set the smallest integral to 1 and created separate integrals for peaks which are sufficiently separated.

Numerical values can be reset and additional integrals defined manually using the integration subroutine.

Display of integrals can be toggled on and off from the View menu selection Show Integrals.

(Note that if the smallest integral is negative, due to poor phasing, integral labels will not be displayed.)



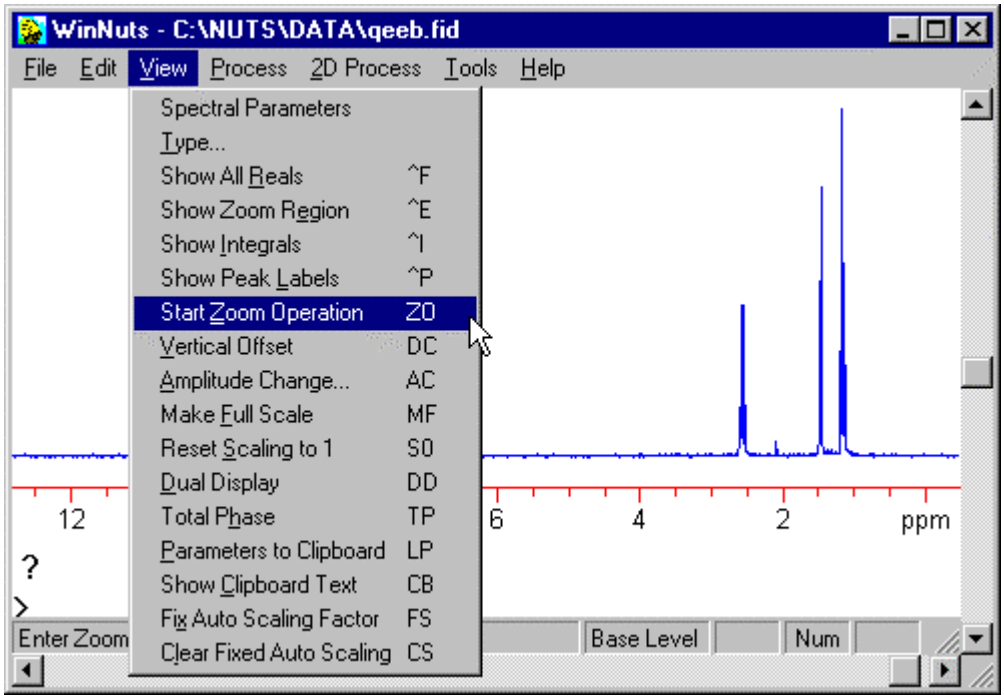
Select Peak Pick from the Process menu. All peaks above the threshold are temporarily indicated with small vertical red lines, and peak labels are displayed at the top of the screen. See Peak Picking for details, including how to set the threshold and options for peak labels.

When PP is executed, a peak list is automatically placed into the Clipboard, from which it can be pasted into any text editor for printing. The list can also be placed on the screen by selecting Show Clipboard Text from the View menu.

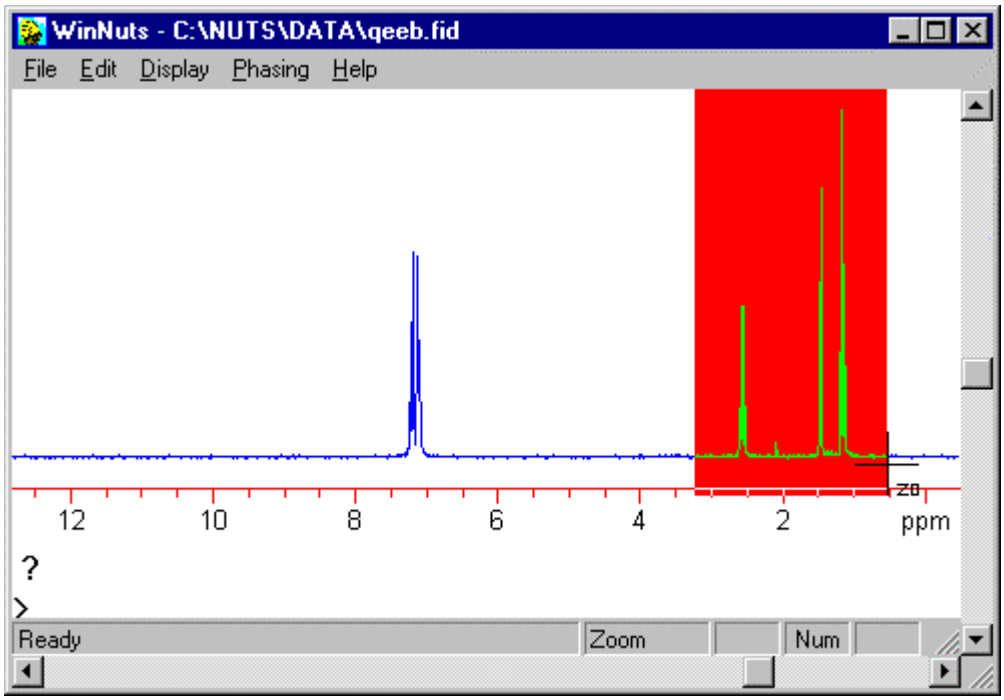
Display of the peak labels can be toggled on and off with Ctrl-P.

Using the cursor and zoom routine

This section illustrates use of the Zoom expansion routine, and using the cursor to set the chemical shift reference and to measure chemical shifts and coupling constants.



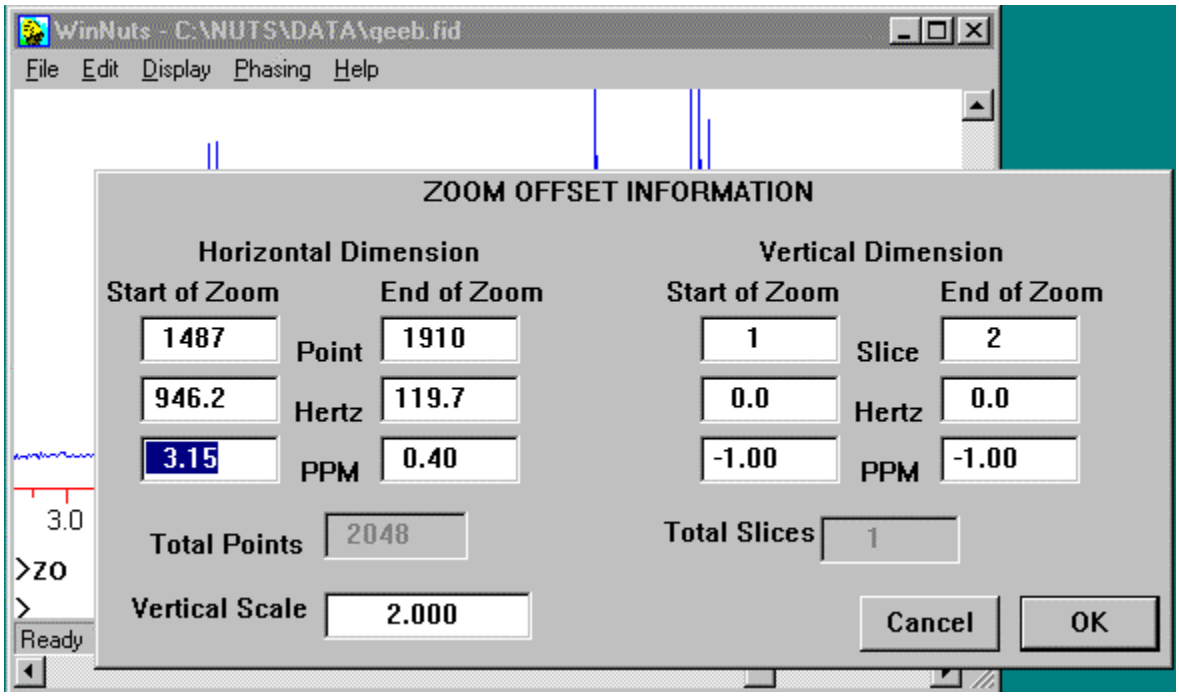
Choose Start Zoom Operation from the View menu. Note that typing **ZO** on the keyboard is equivalent.



To select a region for expansion, press and hold the left mouse button, and drag across the chosen region, which is highlighted in red.

To display this expanded region, choose Zoom Region from the Display menu or type **Ctrl-E**.

To return to displaying the full spectrum, choose All Reals from the Display menu, or type **Ctrl-F**.

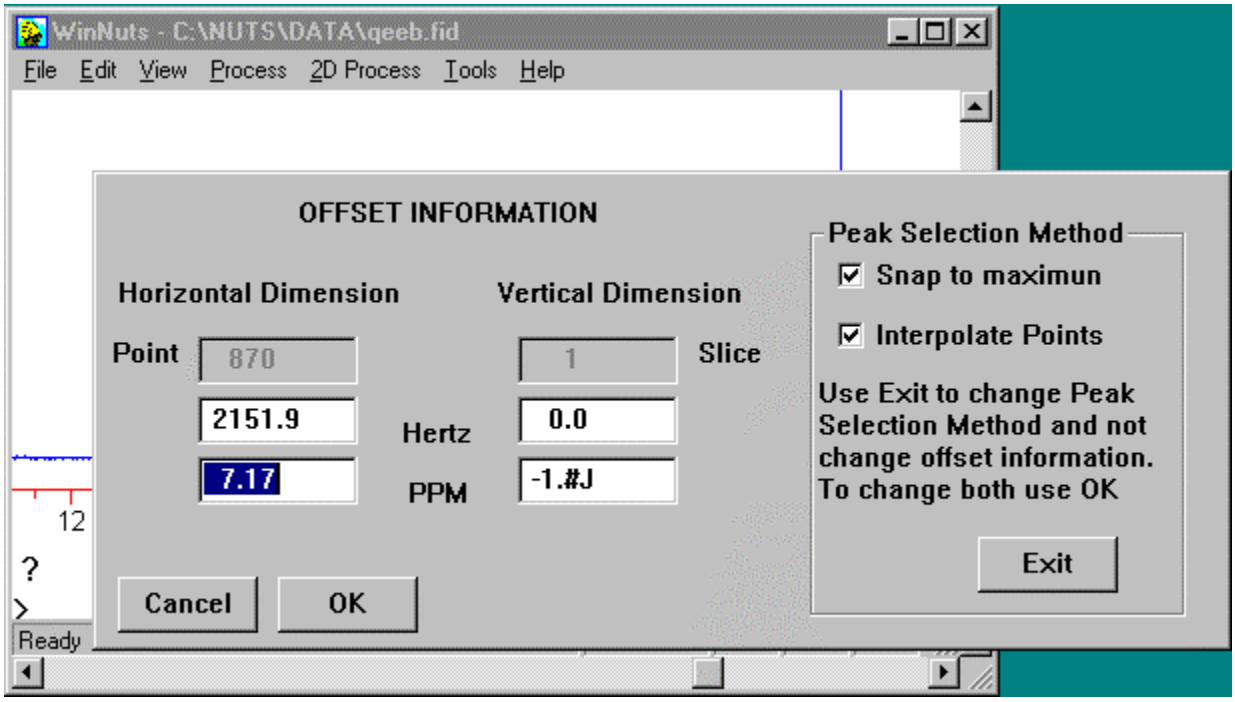


Specific frequency limits can be entered by choosing Set Frequency Limits from the Display menu, or by typing **F**. Limits can be set in points, Hz or ppm.

The right hand half of this dialog box applies to the second dimension of 2D data.

Exit the Zoom subroutine by selecting Exit Zoom from the File menu or by typing <Enter>. The currently expanded region remains on the screen.

For details, see description of the Zoom subroutine.

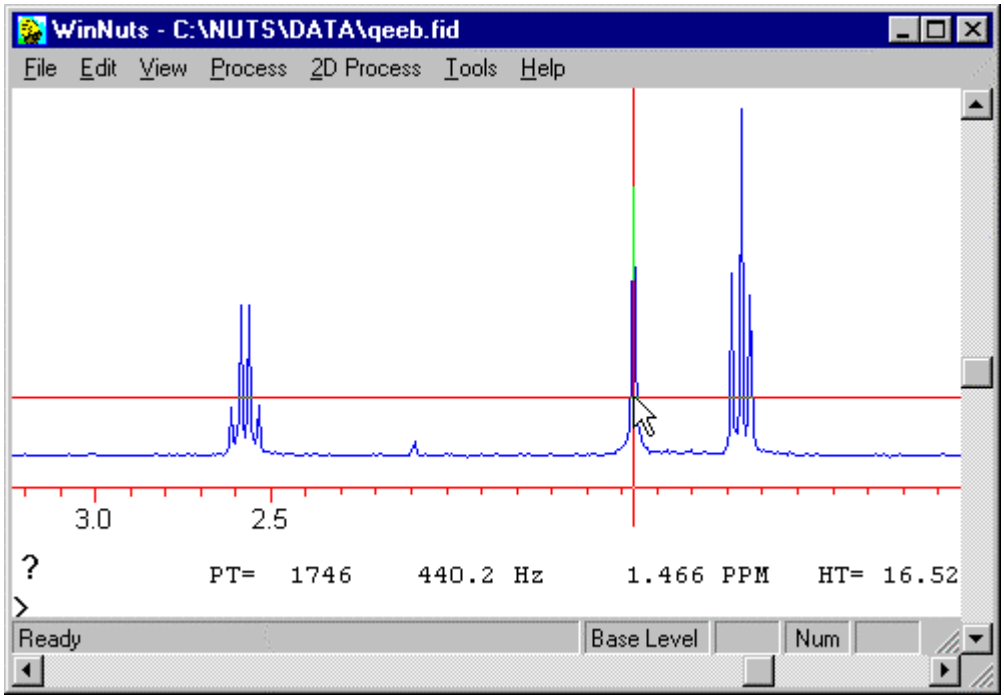


The cursor can be used to set a chemical shift reference. This is done from the base level of NUTS, not from within the Zoom routine.

Press and hold the left mouse button, and place the cursor on the reference peak. While holding down the mouse button, type **O** (Offset) on the keyboard.

A dialog box is displayed allowing the frequency at the cursor position to be entered. As before, the section labeled Vertical Dimension applies to 2D data.

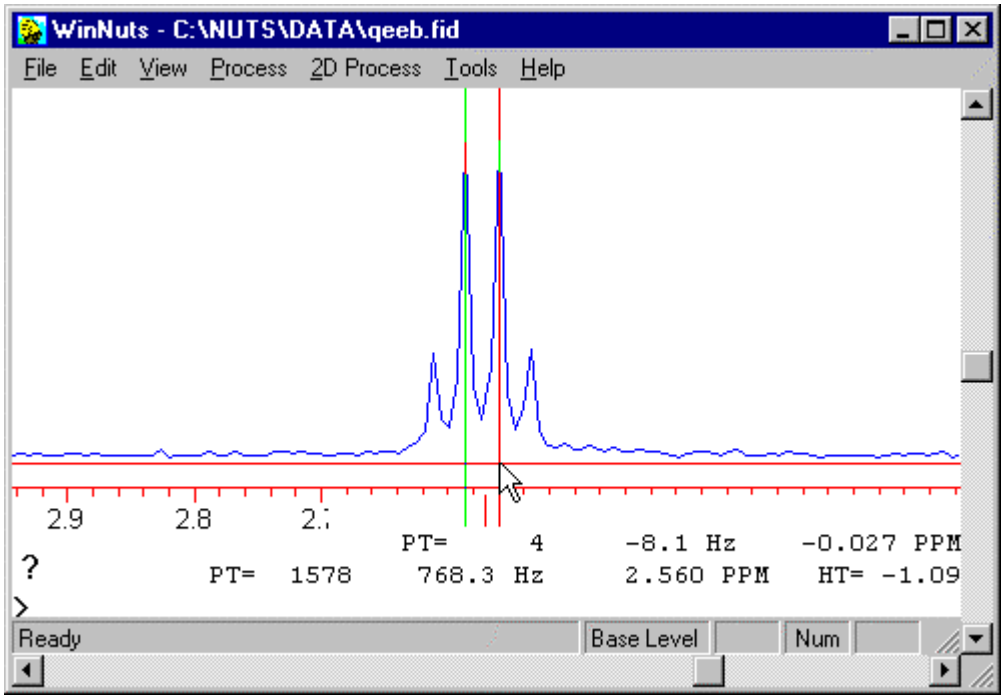
See also: SZ and PR commands.



The cursor can be used to display the frequency of any point in the spectrum.

Press and hold the left mouse button to display a cross-hair cursor. The location of the cursor is displayed at the bottom of the screen as point number, Hz and ppm.

The height of the horizontal cursor is also displayed, expressed as a percentage of the tallest peak in the spectrum.



Frequency differences can be measured using the cursor in difference mode.

Press and hold the left mouse button and position the cursor on the first peak of interest. Click once with the right mouse button, without releasing the left button. This sets a marker cursor (green).

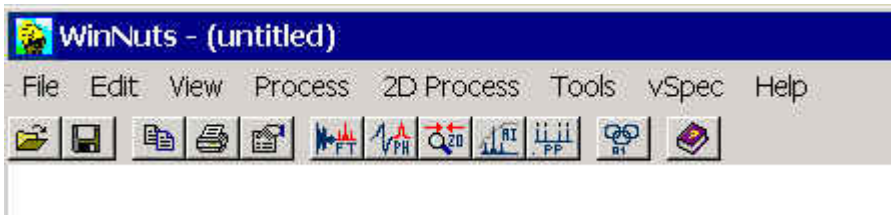
(Users with a single button mouse should press the period key on the keyboard instead of clicking the right mouse button.)

While still holding down the left mouse button, move the cursor. The position of that red cursor is shown as before, but above it is displayed the frequency difference between the marker cursor and the current red cursor position.

Menus

Toolbar

Starting with version 980310, NUTS has a toolbar with icons for common commands.



The corresponding commands are, starting at the left:

File Open (GA)
File Save (SA)
Copy as bitmap (Ctrl-C)
Print (PL)
Page Setup
Fourier Transform (FT)
Phase by Mouse (PH)
Enter Zoom subroutine (ZO)
Automatic Integration (AI)
Peak Pick (PP)
Run Link #1 (A1)
Help on Nuts

Menus

Within the NUTS base level, the menu choices are shown below, along with commands which can be found within each menu. Details can be found by following the links down through layers of menus.

File -- opening and saving files, run macro, printing, page setup and exit Nuts

Edit -- copying spectra, setting fonts, left and right shift, zeroing data, spectrum reverse

View -- Acquisition parameters, Zoom, vertical offset, scaling, dual display

Process -- window functions, Fourier transforms, phasing, Link command strings, baseline correction, integration

2D Process -- intensity and contour plots, step-wise viewing of slices, projections, symmetrize

Tools -- Add/subtract, reference deconvolution, linefitting, spectrum simulation

vSpec (Virtual Spectrometer) -- Simulation of FT-NMR data acquisition.

Help -- Open on-line Help, toggle on/off Helper windows, NUTS data file format, technical support information, About Nuts

File Menu Commands

New (FN) -- Used with spin simulation (NS) routine, to define spectral parameters.

Run Macro (RU) -- Execute a Nuts macro for automated processing.

Open (GA) -- Open a NUTS file

Save (SA) -- Save a file in NUTS format

Save As (SB) -- Save a file using a new name.

Auto Tailer Read -- Sets a flag to automatically read items from the file tailer when file is opened.

Auto Tailer Overwrite --

Delete (DE) -- Delete a file.

Email File (EF) -- Email the current NUTS data file (Win95/98/NT only).

Export -- Export data as ASCII file in one of 5 possible formats, or as JCAMP.

Import -- Import foreign data by automatically detecting the file type (IM).

Print Setup -- Opens dialog box for choosing printer and print options.

Page Setup -- Allows selection of margins, pen width, color and other display and print options.

Printer dialog -- Opens print dialog box.

Print (PL) -- Print the currently displayed region.

Exit (**XX**) -- Exit the Nuts program.

Edit Menu Commands

Copy bitmap to clipboard (**Ctrl-C**) -- See Copying Spectra for description of this and subsequent copy commands.

Copy standard metafile to clipboard (**Alt-Shift-C**)

Copy enhanced metafile to clipboard (**Alt-Shift-E**)

Copy printer Device Context enhanced metafile to clipboard (**Alt-Shift-P**)

Copy standard metafile to file (**Ctrl-Alt-C**)

Copy placeable metafile to file (**Ctrl-Alt-L**)

Copy enhanced metafile to file (**Ctrl-Alt-E**)

Copy printer device context ehnanced metafile to file (**Ctrl-Alt-P**)

Zero Zoom Region (BZ) -- Zero all points in expanded region.

Zero Full Data Region (ZE) -- Zero all data points.

Zero Reals (ZR) -- Zero all real points.

Zero Imaginaries (ZI) -- Zero all imaginary points.

Left Shift (LS) -- Shift data one point to the left.

Right Shift (RS) -- Shift data one point to the right.

Spectrum Reverse (SR) -- Reverse spectrum left to right.

Swap Real & Imag (RI) -- Exchange real and imaginary halves of the data.

Invert Imaginaries (II) -- Invert all imaginary data points.

Set Fonts -- Set fonts for axis (**FA**), clipboard (**FC**), integral labels (**FI**), command line (**FL**), parameters on bottom of plot (**FM**) and peak labels (**FP**).

UnDo (Ctrl-Z) -- UnDo last command.

View Menu Commands

Spectral Parameters -- Acquisition parameters imported with the data, as follows:

Comment (**CO**) -- Up to 80 characters used for description of data.
User (**US**) -- User's name or initials.
Date (**DA**) -- Date data were acquired.
Frequency in 1st Dimension (**F1**) -- Spectrometer frequency in MHz.
Frequency in 2nd Dimension (**F2**) -- Spectrometer frequency for 2nd dimension in MHz.
Offset Frequency in 1st Dimension (**O1**) -- Offset in Hz from center of spectrum to 0 ppm.
Offset Frequency in 2nd Dimension (**O2**) -- Offset in Hz from center of spectrum to 0 ppm, for 2nd dimension.
Sweep Width in 1st Dimension (**W1**) -- Spectral width in Hz.
Sweep Width in 2nd Dimension (**W2**) -- Spectral width in Hz, for 2nd dimension.
Slice (SL) -- Slice number of currently displayed slice.
Experiment (**EX**) -- Name of pulse program used to acquire data.
Number of Acquisitions (**NA**) -- Number of scans acquired.
Pulse Width (**PW**) -- Observation pulse width in usec.
Pulse Delay (**PD**) -- Relaxation delay in sec

Type -- Allows selection of Display Full or Zoomed region, Real and/or Imaginary data points, and axis label (ppm, Hz, points or none)

Show All Reals (Ctrl-F) -- Set display to show full spectrum.

Show Zoom Region (Ctrl-E) -- Set display to previously defined frequency limits.

Show Integrals (Ctrl-I) -- Toggle on/off display of integral trace.

Show Peak Labels (Ctrl-P) -- Toggle on/off display of peak labels defined in DP routine.

Start Zoom operation (ZO) -- Enter Zoom expansion subroutine

Vertical Offset (DC) -- Allows position of spectrum's baseline to be moved up the screen.

Amplitude Change (AC) -- Allows entry of a multiplying factor to scale display.

Make Full Scale (MF) -- Reset display scale to make the largest displayed peak full scale.

Reset Scaling to 1 (S0) -- Remove all scaling factors.

Dual Display (DD) -- Toggle on/off display of a spectrum which has been loaded into the dual display buffer.

Multiple Buffer Display (BU) -- Enter Buffers subroutine for display of multiple spectra.

Total Phase (TP) -- Display the values of zero- and first-order phase which have been applied.

Parameters to Clipboard (LP) -- Copy spectrum parameters to the Windows clipboard for display on the screen or pasting into other applications.

Show Clipboard Text (CB) -- Toggle on/off display of Windows clipboard on the screen (text only).

Fix Auto Scaling Factor (FS) -- Disables automatic adjustment of the display scale, so that different data sets can be compared.

Clear Fixed Auto Scale (CS) -- Removes the effects of the FS command.

Process Menu Commands

Conditions -- Displays a parameter box allowing setting of parameters for window functions, peak picking and phasing.

Links (LI) -- Allows definition of 10 Linked command lists.

Window functions:

Exponential Multiply (**EM**) -- Applies exponential apodization function using previously defined Line Broadening value.
Gaussian Multiply (**GM**) -- Applies gaussian apodization function using previously defined Line Broadening value.
Lorentzian/Gaussian Multiply (**LG**) -- Applies Lorentzian/Gaussian resolution enhancement.
TRAF function (**TF**) -- Applies Traficante resolution enhancement.
Trapezoidal Multiply (**TM**) -- Applies trapezoidal apodization function using previously defined parameters.
Sine Multiply (**MS**) -- Applies sine apodization function with phase defined by S#.

Transforms:

Fourier Transform (**FT**) -- Forward Fourier transform.
Real Transform (**RT**) -- Real Fourier transform.
Bruker Transform (**BT**) -- For Bruker sequential data.
Complex Transform (**CT**) -- Complex Fourier transform.
Inverse Transform (**IT**) -- Inverse Fourier transform.
Hilbert Transform (**HT**) -- Inverse Fourier transform starting from real data.

Phasing:

Auto Phase (**AP**) -- Automatic adjustment of zero- and first-order phase.
Quick Phase (**QP**) -- Automatic adjustment of zero- and first-order phase.
Phase Correct (**PC**) -- Manual phasing using previously set values of PA and PB.
Phasing Expanded (**PE**) -- Phase using 2 previously defined spectral regions.
Phasing by Mouse (**PH**) -- Phase entire spectrum using mouse buttons to adjust 2 phase parameters.
Phase Same (**PS**) -- Apply same phasing as used on previous spectrum.
Zero Phase (**ZP**) -- Remove all phase adjustments.

Digital Filter:

Digital Low Pass (**DL**) -- Apply low pass filter.
Digital High Pass (**DH**) -- Apply high pass filter.

Baseline Correct (BC) -- Remove DC offset and tilt of entire spectrum.

Zero Fill (ZF) -- Double the number of data points by adding zeros.

Shrink Data (SH) -- Reduce the number of data points by deleting points from the right-hand end, as defined by the S@ parameter.

Peak Pick (PP)-- Select peaks above minimum height and place peak list into the clipboard.

Fit Baseline (FB) -- Enter polynomial baseline fitting subroutine.

Baseline Flatten (BF) -- Remove DC offset and tilt of displayed spectral region.

Integrate Display (ID) -- Enter integration subroutine.

Magnitude Calculation (MC) -- Computes magnitude spectrum of the current data set so all peaks appear positive.

Power Spectrum (M2) -- Computes power spectrum of the current data set so all peaks appear positive.

2D Processing Menu Commands

Set 2D scale (SS) -- Calibrates intensity scale for use in calculating contour levels.

View 2D slices (VW) -- Stepwise viewing of slices of a 2D data set.

Transpose data (TD) -- Rotate data by 90 degrees.

Project 2D file (PJ) -- Calculate horizontal projection of currently displayed region.

Intensity Plot (IP) -- Display quick intensity map of 2D data.

Contour Plot (CP) -- Calculate and display contour map of 2D data.

Get Slice (SL) -- Display a selected slice of the 2D data set.

Symmetrize (SY) -- Symmetrize the data to remove artifacts.

Tools Menu Commands

Add/Subtract Routine:

> Enter Add/Subtract Subroutine (**AS**) -- Enter subroutine and display buffer spectrum above current spectrum.
> Load Add/Subtract Buffer (**AL**) -- Copy current spectrum into buffer.
> Edit Add/Subtract Parameters (**AM**) -- Allows setting buffer multiplier, vertical offset and frequency offset.

Convolution:

> Apply Convolution filter (**CA**) -- Apply previously calculated function to displayed FID.
> Calculate Convolution filter (**CF**) -- Calculate reference deconvolution function from displayed peak.
> View Convolution filter (**CV**) -- Display previously calculated function.
> Get Convolution filter -- Read previously saved function from file.
> Save Convolution filter -- Save calculated function to a file.

Searchable Archive:

> Database Make (DM) -- Create archive file from set of NUTS spectra
> Database Search (DS) -- Search NUTS archive file

Relaxation:

> Read Relaxation Data (RR) -- Read list of intensity vs. time from a file.
> Data Reduction (DR) -- Display relaxation data as intensity vs. time plot.

Get Relaxation Data (GR) -- Measure intensity of displayed peak in each slice of the current 2D data set.

Extract:

Extract Spectrum (XT) -- Extract displayed region to a new data set.
Extract Line (XL) -- Extract tallest peak in displayed region to a new data set.
Extract Bottom Projection (XB) -- Save displayed region as horizontal projection for a 2D plot.
Extract Right Projection (XR) -- Save displayed region as vertical projection for a 2D plot.
Clear Extracted Projections (XC) -- Clear previously defined projections.

Automatic Integration (AI) -- Perform automatic integration on displayed region.

Define Peaks (DP) -- Enter Define Peaks subroutine for manual peak picking.

Line Fit (LF) -- Enter line fit subroutine for peak deconvolution.

NMR Simulation (NS) -- Enter spin simulation subroutine.

MetaObjects (MO) -- Enter subroutine for handling graphical objects.

Calculation Type (TC) -- Native (for Macintosh without coprocessor) or Win32 (default).

Stacked Plot (SP) -- Display stacked plot of a 2D or arrayed 1D data set.

Inserts (Inset Plots) (IS) -- Enter subroutine for creating inset plots

Notes (NO) -- Enter subroutine for creating text annotations

Math Routines (MA) -- Display "calculator" window for performing math functions.

Shim (SM) -- Enter subroutine for shimming simulator.

vSpec Menu Commands

Virtual Parameters (VP) -- Opens a dialog box to set acquisition parameters.

Get Sample (GS) -- Specifies the file from which frequencies will be read.

Zero & GO (ZG) -- Reads the file containing frequencies and generates the NMR data.

Note that these commands and the vSpec menu are disabled in the Anasazi Instruments OEM version of NUTS.

Help Menu Commands

Help on NUTS - Displays Help in either WinHelp or HTML Help formats.

Helper Windows - Toggles on/off display of Helper window in the MO subroutine.

Technical Support - How to reach us.

Data File Format - Description of the NUTS file format.

Set NetTime - Synchronize your computer's time to an Internet Time Server (Win95/98/NT only).

About NUTS - Displays license information, support date and compile date.

Viewing acquisition parameters

The following parameters can be examined and/or changed from the View/Spectral Parameters menu or by typing the 2-letter names. In both cases, a dialog box appears displaying the chosen parameter and related parameters. Many of these parameters are printed on plots. The NUTS data translation attempts to translate and insert into the NUTS file header as many parameters from the original file as possible. Some parameters refer to the total data set, such as Date and Number of scans. For others, two sets of parameters are listed for the two dimensions, such as Spectrometer Frequency and Sweep Width.

CO - Comment (eg., sample name or number)

UID - Unique identifier

DA - Date of data acquisition.

EX - Name of pulse experiment used to acquire data.

F1, F2 Spectrometer frequencies in the first and second dimensions.

NA Number of scans acquired.

O1, O2 Spectrum offsets in the first and second dimensions.

PD Pulse delay used in data acquisition.

PW Pulse width used in data acquisition.

SF Spectrometer Frequency.

SW Sweep width in Hz.

US User name or initials

W1, W2 Sweep width in the first and second dimensions.

Offsets (O1 and O2)

Each of these values is the difference, in Hz, between the center of the spectrum and 0 ppm. This is how NUTS keeps track of the chemical shift scale. For 1D data, normally, the chemical shift reference is set using the cursor or, for TMS, the SZ command. However, offset values may be entered directly. For 2D data, the cursor can be used to set the shift reference; see details in the section on 2D data processing.

Number of points

The number of points in the data set, listed for both dimensions.

Dwell Time

This is calculated from the sweep width.

Acquisition Time

This is calculated from the sweep width and the number of data points.

Domain

NUTS must keep track of whether the data is an FID (Time domain) or a spectrum (Frequency domain). Occasionally, NUTS fails to correctly identify when the data represent an FID or a spectrum, and this can be corrected by entering the appropriate word in the Domain box. Save the change by executing a UH (update header) command.

Data Type

There are 3 possible values for this parameter: Complex, Real and TPPI. The latter is used to indicate that the data were acquired using Bruker's sequential acquisition, and for 2D data which was acquired using TPPI in the indirect dimension.

2D Nomenclature

Keep in mind that NUTS will label the dimensions as 1 and 2, with 1 always being the dimension that is *currently displayed* horizontally. So when you are viewing, for example, a HETCOR spectrum from a 300 MHz instrument, with the carbon dimension displayed horizontally, that is dimension 1. So F1 (SF for dimension 1) will be 75 MHz. When you do a TD, the ^{1}H dimension is now horizontal, so dimension 1 is ^{1}H, and F1 is now 300 MHz.

It's easiest to ignore 1 and 2, and just look at the parameters window, keeping in mind that the left column of parameters applies to the dimension that you have displayed horizontally and the right column applies to the vertical dimension.

Note that a common practice is to label the indirect dimension as "1" (even though it's the secondary dimension, and is processed second) because the corresponding t_1 period in the pulse sequence (the incremented time variable) occurs before the acquisition time. To avoid confusion, the NUTS documentation uses the terms *direct* and *indirect* to refer to the different dimensions, which is unambiguous.

If considering only 1D data, the parameters can have the obvious names SF, SW and OF. But with 2 dimensions, we need to add labels 1 and 2. But in Nuts, everything is 2 letters. So these parameters became:

SF ==> F1 and F2

SW ==> W1 and W2

OF ==> O1 and O2 (NOT to be confused with Bruker's O1 and O2, which are defined differently!)

The NUTS offset is defined as the number of Hz between the center of the spectrum and 0 ppm. This is the parameter that keeps track of the chemical shift referencing.

Display

Control-A -- Axis label

Toggles the axis label among the choices: points, Hz, PPM and none. Direct selection is available by choosing Type from the View menu. The font used for the axis label can be set with FA or by choosing Set Fonts from the Edit menu. The default axis type and its font can be set in the NUTS.INI file.

Control-D -- Points/Lines toggle

Toggles the display between drawing the data points and drawing lines to connect the points. The default is to draw lines between the data points. This applies to the real part of the data. Typing Ctrl-D a second time returns the display to the original state.

Note that redrawing the screen while displaying points can become quite slow as the number of points in the display region becomes large.

AxisPen

When NUTS is in the non two-letter command mode, the command "axispen" may be used to change some of the characteristics of the axis line. Examples are:

Change the screen only axis line to a width of 2
axispen screen 2

Change the axis line to a width of 2 only when printing
axispen print 2

Change the axis line to a width of 2 for both screen display and printing
axispen both 2

Change the color of the axis line to red
axispen 255 0 0

Change the color of the axis line to green
axispen 0 255 0

Change the color of the axis line to blue
axispen 0 0 255

UD -- Update Display

Forces NUTS to repaint the screen. This can be used in macros to allow the user to monitor progress of the macro.

QD -- Quick Display

Changes the display mode to a compressed display, which paints the screen faster. This is the default mode of display and is faster than displaying every point with the Slow Display (SD) mode. The difference in display speed between QD and SD becomes more apparent as the size of the data file increases.

SD -- Slow Display

Changes the display mode to displaying every point. This display mode is slower than the compressed display under the Quick Display (QD) mode. The difference in display speed becomes larger as the size of the NMR data set increases. By default, NUTS uses the faster Quick Display.

WS -- Window Size

Allows the user to set the size of the NUTS screen in pixels. This is useful for copying spectra as bitmaps or doing screen captures. It is desirable not to resize a bitmap after it has been created, because this can cause distortions. So the NUTS screen can be set to the desired size for the final image before copying.

Control-B -- Toggle on/off Clipboard Display

CB -- Clipboard on/off

Toggles on and off display of text currently saved in the Windows clipboard. This can be used to place information such as peak lists on plots. This command is also available from the View menu. Ctrl-B is active at all times, including in subroutines. To use this in a link or macro, use "^B".

By default the text is displayed in the upper left corner of the screen. This can be changed by holding down the left mouse button and placing the cross-hair cursor at the position desired for the top left corner of the text region. While still holding down the mouse button, type **C**. Because this involves using the cursor, it must be done from the NUTS base level, not within a subroutine.

When peak picking is performed (with PP), the peak list is automatically placed in the Windows clipboard. If clipboard display is turned on, this list appears on the screen as soon as the display is refreshed. To get a list of integral values, display the integral (with ID) and (after defining sub-regions), type **T** to copy the integral information into the clipboard. Toggling Ctrl-B on then displays the integral information. The integral list can also be placed into the clipboard without entering the integration routine using the IL command.

A list of spectral parameters can also be displayed by typing **ctrl-L** (which places a list of parameters into the clipboard).

To edit the displayed text, first paste the text into the Windows Notepad or other text editing program. Perform the necessary editing, then select and copy to the clipboard the text you want on the plot. Return to NUTS and toggle clipboard display on. This can be used to place a title, sample description or other text on a plot.

The font used for the displayed text can be set with FC or by choosing Set Fonts from the Edit menu. It can also be set in the NUTS.INI file

LP -- Parameters to clipboard

Copies the list of acquisition and processing parameters shown below to the clipboard. Once LP has been executed, Ctrl-L toggles its display on and off. LP also creates a note which can be edited in the Notes subroutine (NO), allowing font and position on the screen to be changed. This is the same result as the P subcommand inside the Notes routine.

```
C:\NUTS\DATA\NT.FID
Sample A13-402
 13FEB90
USER: WWC
PTS1d = 8192
F1  =  361.211121 MHz
SW1 = 2801.10 Hz
O1  =  1201.90 Hz
LB1 =  0.00  Hz  Line broadening
NA =  128        Number of Acquisitions
PW =   7.70 usec Pulse Width
PD =   5.00 sec  Recycle Delay
TP A =  0.00     Total Phase applied (A=zero-order, B=first order)
   B =   0.00
```

A list of parameters can also be printed on the bottom of plots. This option can be selected by choosing Page Setup from the File menu or set in the NUTS.INI file.

Control-L -- Display parameters

Toggles on/off display of the list of acquisition parameters created with **LP** (or Notes subcommand P). If the list hasn't been created, Ctrl-L does nothing. Ctrl-L also interacts with a related command, Ctrl-N, which toggles on/off display of all text boxes defined in the Notes routine. If Ctrl-N is toggled off, then Ctrl-L turns on/off display of the parameter list. If Ctrl-N is toggled on, Ctrl-L does nothing.

Control-N -- Display Notes

Toggles on/off display of all text boxes defined in the Notes subroutine. This is the same as the Notes subcommand **S**, but is active outside of the subroutine.

Scaling data

These commands adjust the display of the data, and do not actually change values of data points.

Scaling the spectrum display

The vertical scale of the displayed data can be adjusted in several ways: by using right hand scroll bar, with the greater than and less than keys (> and <) for changes by factors of 2, with the Page Up and Page Down keys and with the cursor up and down arrow keys for finer adjustment.

There are also several commands that change or affect the display scale:

AC Amplitude change; enter multiplying factor
Ctrl-Y Scales the tallest displayed peak to be full scale.
MF Scales the tallest displayed peak to be full scale.

S0 Scale Zero; sets scroll bar to zero
FS Fix scale
CS Clear fixed scale

It is important to understand that these commands affect only display of the data. They do not change the values of the data points. NUTS does all operations in floating point, rather than integer, arithmetic. This eliminates the need for a scaling or normalization constant which is necessary for data represented as integers, because there is a limited dynamic range for integers. Therefore, the absolute values of the data points in NUTS can be directly compared from spectrum to spectrum without the need for fixing Absolute Intensity.

AC -- Amplitude Change

Opens a dialog box for input of a vertical scaling factor. This command is also available from the View menu.

When used in a macro, the current value for the scaling factor is applied, without asking for user input. Therefore, to use AC in a macro, first use the command SET AC to enter a value, then use the AC command to apply that scaling.

The scaling factor is applied to the spectrum as it is currently displayed. This allows the user to change the vertical scale by a specific factor. The vertical scale can also be adjusted with the right hand scroll bar, with the Page Up and Page Down keys, with the up and down cursor keys and with the < and > keys.

Control-Y -- Set Vertical Scaling

MF -- Make Full command

Adjusts the vertical scaling so that the largest peak in the currently displayed region becomes full scale. This command is also available from the View menu. This command is active in all subroutines. To use Ctrl-Y in a Link or Macro, use " ^Y ". The vertical scale can also be adjusted with the right hand scroll bar, with the Page Up and Page Down keys, with the up and down cursor keys and with the < and > keys. The Amplitude Change (AC) command allows input of a specific scaling factor.

FS -- Fix Scaling

This disables automatic scaling of the data display, allowing a series of spectra to be displayed with the same vertical scale. In its default operation, NUTS scales the data using two different scaling factors. One scaling factor is determined by the position of the right scroll bar. The other scale factor is automatically determined by NUTS and is the absolute difference between the minimum and maximum of the current spectrum. When the FS command is given, the latter number is fixed at the current level until the CS

(Clear Scaling) command is given. This allows other spectra to be loaded and their amplitudes directly compared.

This scaling affects display only. NUTS performs all operations in floating point, not integer math. This obviates the need for applying and keeping track of a scaling or normalization constant in order to compare data. Note that setting minimum height for peak picking using the cursor does not work properly when FS is enabled.

CS -- Clear fixed Scaling

Clears display scaling previously fixed by FS command.

S0 -- Scale Zero

Returns the right-hand scroll bar to zero and removes all vertical scaling applied with either the scroll bar or keyboard (page up/down or <> keys).

DC -- DC offset for display

Allows the user to override the default value for the vertical position of the spectrum on the screen. This is useful for displaying spectra with negative peaks. After entering the DC routine, a scroll bar on the left side of the NUTS window appears and can be used to adjust the vertical position of the spectrum display. Typing <Enter> exits the DC routine, and the new vertical position of the spectrum display remains the default until reset or the program is restarted. To reset the vertical position back to the bottom of the screen, enter the DC routine and type **0** (zero). Then type <Enter> to exit the DC routine.

File handling

This section describes how NUTS keeps track of file names, opening and saving data files and the file "tailer".

A data file (either an FID or a spectrum) can be read into NUTS using the **File/Open** menu command or, equivalently, the **GA** command. The GA command can be used for both 1D and 2D data sets. The current working directory is established each time a file open or file save command is completed. A file can be saved using the same name (writing over the original data) or a new name. These are the Save and Save As operations, respectively, found under the File menu. The equivalent 2-letter commands are **SA** and **SB**, respectively. Either command brings up a dialog box allowing a file name to be entered. The difference between the two commands has to do with the file name which NUTS assigns to the currently displayed data. The currently displayed file is always designated as file A and its file name is displayed at the top of the screen and is printed on plots. Saving the data with a new name using **SA** will cause the name at the top of the screen to change. The same operation using **SB** leaves the name at the top of the screen unchanged. This allows NUTS to keep track of two different file names, and becomes important in operations which involve processing of a series of files, by

allowing the processed data to be saved using a new name rather than being written over the original file.

The Import (**IM**) command (also available from the File menu) operates on data which has not been translated into the NUTS file format. NUTS will attempt to identify the data's source and apply the appropriate translation. The translated file is created with the same name, but with a $ appended to the beginning, so that the original data is never altered. This new file becomes the current file in NUTS. The translated file is always written to disk, to the current data directory. See also the section on importing data.

The default directories for file importing and for the file Open/Save operations (eg, **GA** and **SA**) can be different and can also be set in the NUTS.INI file. (Note that this does not work correctly on the Mac.) If a file is imported from a different directory, this new directory becomes the import directory, and will be the directory displayed for subsequent **IM** commands. Similarly, if a file is read from or saved to a different directory from the default data directory, this operation changes the data directory for subsequent Open and Save operations. The WP command (Which Path) displays the current directories for Read/Write and for Import, so the user can keep track of them.

The S2 command is available for making copies of a 2D file and for saving a 2D file after modifying an individual slice.

The user needs to be aware that the command **GA** (open file A) when applied to a 2D file behaves slightly differently in macros or Links from when it is entered directly. The difference lies in whether NUTS reads just a slice of the 2D data or reads both the slice and the data header. While in a Link (either by itself or within a macro), NUTS reads the data header only once, for the first slice. This makes reading subsequent slices faster. When GA is used to read a 2D file in a Link, NUTS assumes that the user loaded the first slice manually (with GA) immediately before executing the Link. This reads in the file header and all is well. If the same Link is embedded in a macro, NUTS can be forced to read the file header by inserting the following line just before the line containing the Link:

```
Set SL 1
```

The take-home message is that in macros always set SL to one before a Link in which GA will operate on a 2D file. When executing a Link not in a macro, always do a GA on the starting file just before executing the Link.

In Macros:

When using the Set command to specify a file name, the default path name is the current working directory. The current working directory is established each time a **GA** or **GB** command is completed. Therefore, always execute a **GA** after an **Ask FileA** command:

Ask FileA
GA

This establishes the working directory, and subsequent **Set FileX** commands will use that directory.

If the user wants to specify a different path for a file in a Set command, use

Set FullFileA name

Note that if a new path is specified for file A or B, the working directory will be changed when a GA or GB command is next executed.

See also: Linked Command Lists, 2D Processing, Importing data, Exporting data

GA -- Get data set A

Opens the dialog box for reading a file. The last name used for File A, if any, will be the default selected name.

If the file name entered corresponds to a file which is not in the NUTS CDFF format, a dialog box asks if the file should be imported. If the user answers Yes, NUTS will attempt to detect the file type and apply the appropriate translation. This is the same operation performed by the Import (**IM**) command.

GA can be used in a Link for processing a 2D data set or a series of 1D spectra. In this case, the Link will end with an IN command, which causes the processing to loop back to the beginning of the Link and will increment either the slice number (for a 2D file) or the file extension (for 1D files) so that the next slice or spectrum is read in.

See also: Long command names, Importing data, Linked command lists, 2D Processing.

GB -- Get data set B

Opens the dialog box for loading a file. The last name used for File B, if any, will be the default selected name.

SA -- Save dataset A

Saves the current data set under the File A name or a different name. This command is also available from the File menu.

SB -- Save dataset B

Saves the current data set, allowing the entry of a different file name. The name of the currently displayed file, designated as File A, is unchanged. This command is available from the File menu.

EF -- Email File

On Windows versions only, a file can be emailed, provided an email client has been set up. The current NUTS data file is attached to an email message, and the user specifies the email address and can optionally add a message. The file is sent from the disk, so it is necessary to save the current file before executing the EF command. This command is available from the File menu.

See also: Paperless data distribution

DI – Display a directory of the NUTS folder

(Windows version only) This command opens the Windows Notepad and displays a directory listing of the files in the NUTS folder.

File Tailer

The peak list, integral list and some additional data are saved in the data file. (Originally, this was saved in a file "tailer", appended to the end of the data file. With conversion to NUTS file format Type 3, this information is now stored in the file Header, but this command retains its original name.)

A flag found in the File menu determines whether or not information is automatically read from the tailer when a file is opened. This option can also be set in the nuts.ini file.

The NUTS commands which pertain to this are:

TA -- Read all information from file tailer.
TS -- Save tailer information to a separate ASCII file. An example is shown below.
IF -- Enter other information to be saved in tailer.
T+ -- Replace the existing file tailer with the contents of a specified text file.
T- -- Remove file tailer

If integral regions have been defined at the time a file is saved, they are saved in the file tailer. Similarly, if peaks have been selected in the **DP** subroutine, that peak list is saved. The comment line (**CO**) and the path to the file are also saved in the tailer. The additional information which can be saved, using the **IF** command, includes 3 lines of text, a molecular formula and nucleus type.

Any inset plots or text notes which have been defined are also saved with the file.

One note of caution: Because integral and peak lists, inset plots and notes are not cleared when a new data set is opened, it is possible to save irrelevant information in the file tailer of that new file when it is subsequently saved. Also, some peaks or integrals previously defined may fall outside the spectral limits of the second file. In this case, the invalid peaks are reset to the closest valid data point, and invalid integral regions are not displayed.

File Header Type 3 provides for an expandable file header for parameters with a flexible format. For Type 3 files, the "tailer" information is no longer saved in the tailer, but is instead included in the header.

TA – Read Tailer

Reads all information from the file tailer. From within the Integration subroutine, integral regions only can be recalled from the tailer from the Edit menu, or using the keyboard command **R**.

TS – Tailer Save

Creates a text file containing the information which is saved in the file tailer when a Nuts file is saved. A sample of a file Tailer, as saved using **TS**, is:

```
INFORMATION
Name1=data from Sam
Name2=stuff
Name3=CDCl3
Comment=Sample # K-47-B
Formula=C6H10N
Nucleus=H1
Path=D:\NUTS\DATA\sam\$DATA
END_INFORMATION
PEAKLIST
LINE HZ PPM INTENSITY REL INT XOFFSET YOFFSET HorV LABEL
1 2307.23 7.687 120753536 10.149 0.000 0.000 V 4
2 2097.74 6.988 111956936 9.410 0.000 0.000 V 5
END_PEAKLIST
INTEGRALS
REL_VALUE = 1.589616e-009
START_PT START_HZ END_PT END_HZ VALUE LABEL POSITION
6394 8.432 6514 8.281 0.986642 1
6681 8.072 6814 7.905 1.000000 1
6912 7.782 7053 7.605 2.183908 1
7499 7.045 7638 6.870 2.465544 1
7243 7.366 7433 7.127 6.768002 1
END_INTEGRALS
END_TAILER
```

T+ -- Tailer add

A text file can be used to replace the tailer information of the currently displayed data set. The file needs to be in the format of the example above. This feature can be used to edit

information already in a file tailer, by first saving the tailer (**TS**), then editing it with any text editor, saving the changes and then using **T+** to read back in the modified tailer. As soon as **T+** is executed, the modified tailer is saved on disk, using the file name of the currently displayed data set.

T- -- Remove tailer

IF -- File Information

This command brings up a dialog box which allows entry of supplementary information which will be saved in the file "tailer" when the spectrum is saved. This information can be retrieved later with the **TA** command or from the File menu when the file is opened within Nuts. This information is also used when creating a searchable archive file.

User, date, comment and the path to the file are entered automatically from existing information for the current data set.

See also: Creating a searchable archive

SV – Solvent

Enter text for solvent. This is saved in the file tailer.

DE -- Delete file

Brings up a dialog box allowing the user to select a file to be deleted. It does not ask for confirmation before deleting the file.

RL – Read License File

This command reads a special encrypted text file and generates a new LICENSE.NMR file. This is an attempt to aid users who want to get a new LICENSE.NMR file by email, but who cannot handle attached binary files. Before RL is executed, any current LICENSE.NMR in the same directory as the NUTS.INI file must be erased or (better) renamed. RL will read a specially prepared file which MUST be named LICENSE.TXT and MUST be in the same directory as the NUTS.INI file. When NUTS is started and the RL command entered, a new LICENSE.NMR file will be created which will have the information from the encrypted LICENSE.TXT file. This operation needs to be performed only once.

UUEncode – Encode a binary file as text

UUDecode – Decode a uuencoded file

Uuencoding is a common way of converting a binary file as text, facilitating sending the file via email. The command must be executed from the non-2-letter command mode. The command syntax is

uuencode *filein fileout*

uudecode *filein fileout*

Command log for recording processing

Starting Sept, 2001, NUTS has the ability to keep a record of commands used in processing each data file.

cmdlog (or commandlog) – Command Log

This is a non-2-letter command which controls the command logging operation of NUTS. Logged commands are stored in the NUTS header (only if NUTS Header Type 3 is used) when the file is saved. If a file is opened that already has a command log, the log will be read and additional commands will be appended.

An example of the command log section of a NUTS header is shown below. The date and time are the first entry. When the file is opened, the file name with complete path is listed. The data was processed with 2 Hz of linebroadening, FT and quick polynomial baseline correction.

```
##$CMD_LOG
Wednesday August 29, 2001 17:32:16
ga C:\nuts\data\qeeb.fid
lb 2.000000
em 2.000000
ft
qb
##$END_CMD_LOG
```

The command log can be enabled or disabled by an entry in the NUTS.INI file:

NUTS_LOG_COMMANDS = TRUE

If this entry is commented out, set to anything other than TRUE, or is absent, then NUTS, by default, will not log the commands.

The command logging can be turned on with the command

cmdlog on

and command logging can be turned off with the command

cmdlog off

The "cmdlog" command without any argument will bring up a dialog box from which the user selects a file, then notepad.exe will be started and the command log for the selected file will be displayed.

Searching data files

HeaderSearch (or equivalently, hdrsearch) – Search files by keyword

This is a non-2-letter command that allows searching all NUTS files in a designated directory for a specified value for one of the parameters in the file header.

The command syntax is:

hdrsearch *keyword* value

where *keyword* is any of the keywords in the NUTS header (type 3 files only).

A file-open dialog box is displayed, allowing the user to select which directory should be searched. Select any file in that directory. The search results are saved as a text file called **uid_scan.txt** in the default Windows temp directory. When the search is complete, the search results are displayed using Notepad.

For example, to search for all files containing the word benzene in the title field,

hdrsearch ##TITLE benzene

The search is a sub-string search, so all files containing the word benzene in the title field will be listed.

UID - Unique Identifier

A new parameter has been created which can be used to identify individual data sets, or to group related data files together by assigning them all the same UID. The UID is displayed on the Acquisition Parameters screen. The identifier can be any text string. A selected directory can be searched for a specified UID.

The UID is stored when a file is saved (type 3 header only) and is recalled when a file is read, if it exists in the header. The UID can be entered from the Acquisition Parameters screen or from the command line (in non-2-letter command mode), as shown here:

uid string

To search for all files with sub-string matched to "SearchUID" (non-2-letter command):

uid -s SearchUID

and select any file in the desired search directory. The search results are saved as a text file called uid_scan.txt in the default Windows temp directory. When the search is complete, the search results are displayed using Notepad.

passwd - Password

Associated with the UID is the non-2-letter command "**passwd**". The idea is that the user can choose to restrict editing of the UID field by requiring a password to change it. If a password has been set, changing the UID requires the password to be input. The passwd command allows a password to be defined.

Examples of usage:

passwd <new password> <old password, if password currently exists>

if no password has been defined, or if UID does not now exist, the UID can be set regardless of password:
uid <UID>

if UID exists and password is set
uid <NEW_UID> <root passwd>

Eliminating paper

Time and paper can be saved by the NMR facility by sending data back to its customers electronically. The key to gaining customer acceptance of this is making data viewing easy for the customer. A Windows computer can be set up to launch NUTS and load the spectrum when the user clicks on the file. NUTS can now email a file, making this even easier for the NMR facility.

NUTS must be installed on the customer's computer or on a server to which (s)he has access. The file extension must be Associated with the NUTS program on the customer's computer. This is done from the Windows Explorer by clicking on the file to select it, and then choosing Open With from the File menu. This lets you specify the application which will be used to open this type of file. The same thing can be done from most file managers, by selecting Associate under the File menu. Now, when the user clicks on a file with this associated file extension, NUTS is launched and the data loaded. (Of course, this requires that all NMR files have the same file extension.)

NUTS can be customized to automatically display the data in different ways, depending on the customer's preference. Customization can be done using the nuts.ini file and by creating an "auto-exec" macro, which is always run when NUTS is started. For example, let's say the NMR facility processes the data, including defining integrals and picking

peaks. The file is saved and emailed to the customer. The customer's copy of NUTS has been customized to run the following auto-exec macro:

```
NUTSMacro for data display
; read the file "tailer"
ta
; display integrals
an
; display peak labels
^p
; define zoom limits
set zof1ppm 12
set zof2ppm 0
set displayzoom
end
```

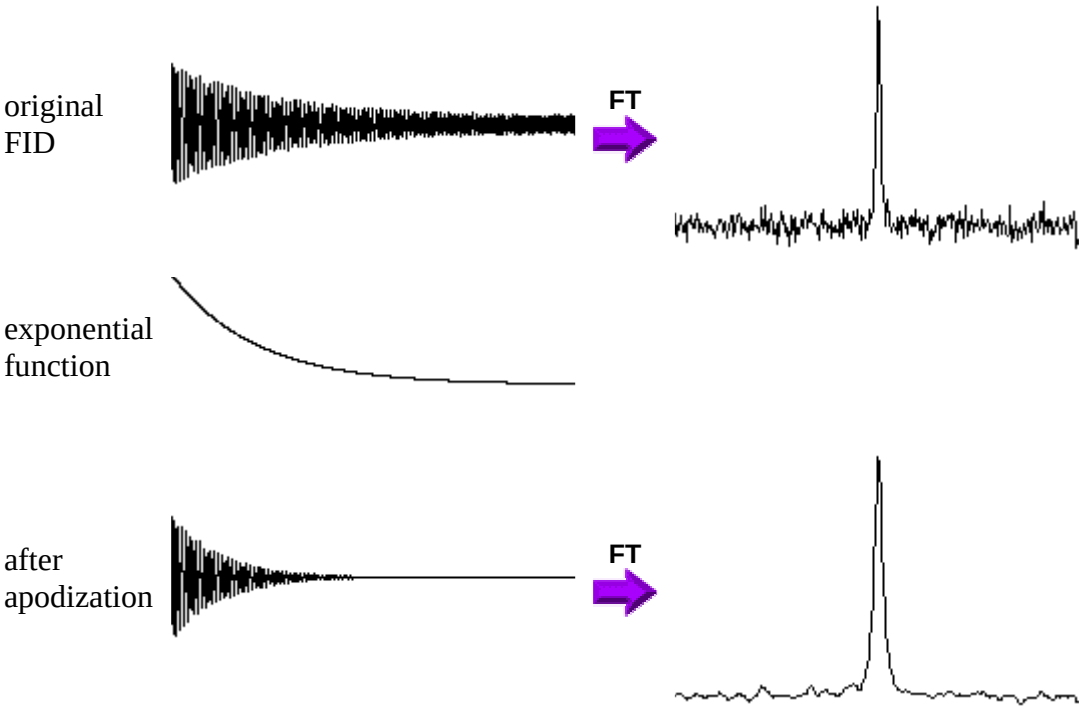
When the customer clicks on the icon for the attached file in his email message, NUTS is launched, the spectrum loaded and displayed 12-0ppm with integrals and peak labels. (S)he is then free to examine, plot, etc.

The NMR facility may prefer to send data directly from the spectrometer, rather than from a PC running NUTS. This is complicated by the fact that most modern spectrometers do not save data as a single file, but rather as a directory containing multiple files and even multiple subdirectories. A simple solution may be to export the data in JCAMP format, and email that single file to the customer. The customer can Associate the JCAMP file extension with NUTS, and the file will be automatically imported and displayed when the customer clicks on the file name or icon, as above.

Basic 1D processing details

Apodization - application of a weighting, or "window", function to an FID

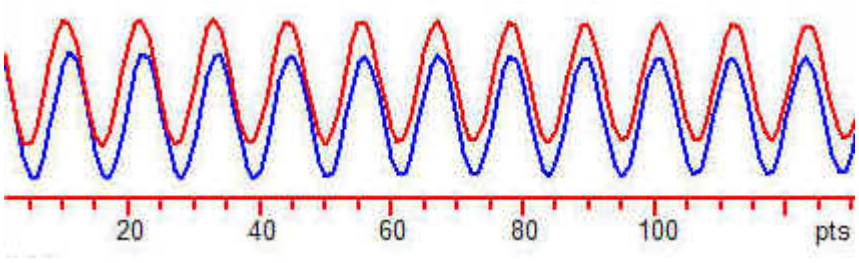
The time-domain signal (FID) of a lorentzian peak decays exponentially with time, due to relaxation. The noise level is constant over the entire FID. As a result, the signal-to-noise ratio (S/N) is greater at the beginning of the FID. The S/N of the resulting spectrum can be improved by "weighting" the beginning of the FID more heavily than the end. This is done by multiplying the FID by a decaying exponential function.



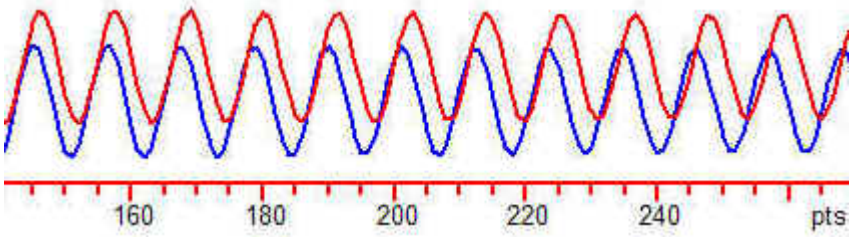
Of course, you don't get something for nothing - the improvement in S/N comes at the expense of resolution. The ability to resolve closely-spaced peaks requires "watching" for long enough to allow their sinusoids to diverge, so the later data points are necessary for resolution. Application of an exponential function broadens peaks and can obscure small splittings.

For example, the 2 sinusoids shown below differ by a few Hz.

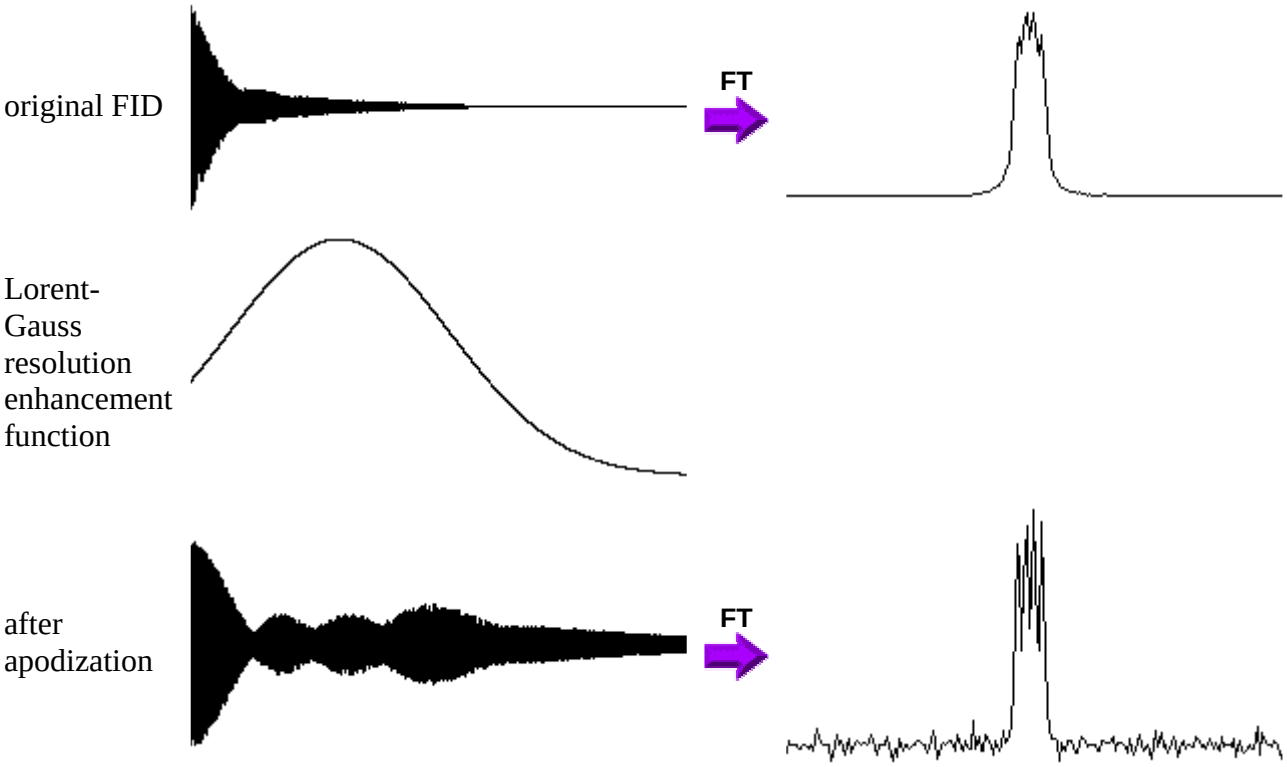
If only the first 100 data points are observed, they appear to be at identical frequencies, so are not resolved.



They only begin to be distinguished at 250 points.



An exponential is the most common function in processing 1D spectra. Other window functions are designed to enhance resolution, with some loss in S/N.



Applying a window function involves a trade-off between signal-to-noise and resolution, requiring an evaluation of the characteristics of the specific data and the information desired from it.

See also: Details of these and other window functions.

Applying window functions in NUTS

This section describes the apodization options provided by NUTS.

See also: Introduction to apodization, Reference deconvolution, Creating custom window functions, interactive application of window functions

Before applying a window function or doing an FT, the FID may need to be corrected for any offset from zero.

BC -- Baseline Correction of the FID

If the data is time domain data (FID), this command averages the last 10% of the points of a complex FID set and then removes that DC offset from each FID. (A different DC

offset between the 2 channels can result in a glitch in the center of the spectrum, see example below.)

Note that this command acts differently if the current data is a spectrum, rather than an FID.

BA -- Baseline Average

This is an alternative to **BC** for removing DC offset between the real and imaginary halves of the data. Instead of calculating the average of just the last 10% of the data (as **BC** does), **BA** calculates the average of *all* data points, then subtracts this average from each point. This command only makes sense for FID data.

A quick way to see the shape of a chosen function is to open the FID to which it will be applied and execute the 21 command. This sets the value of all data points to one. Then apply the chosen function to see its shape.

Note that some apodization functions are dependent on time, so the total shape applied depends on the acquisition time of the FID. By contrast, the sine functions (MS) apply one half of a sine wave over the entire FID, regardless of the acquisition time.

WV -- Window View

This command allows interactive adjustment of parameters defining various window functions. The FID, the apodization function and the spectrum after apodization are shown simultaneously. Parameters can be adjusted and the new window function automatically applied, and the screen is updated. See details in the next section.

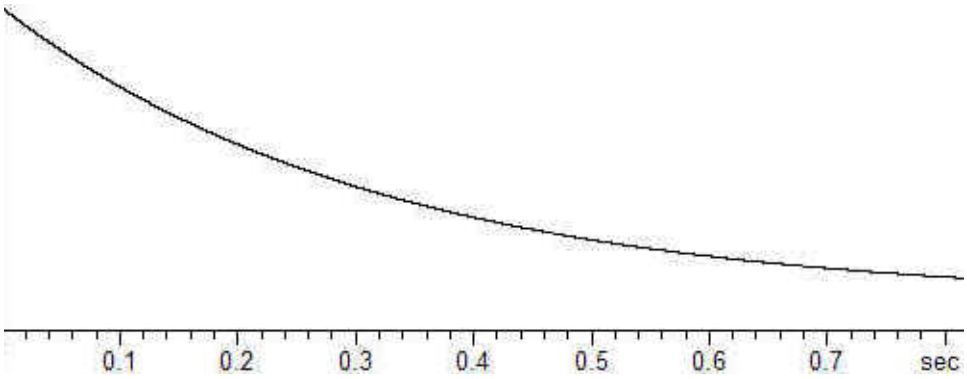
LB -- Linebroadening

This parameter defines the decay of the exponential weighting function applied to the FID with the EM command, to enhance signal-to-noise. The value for the linebroadening (**LB**) must be set first. LB is set via a dialog box brought up by typing LB or selecting Conditions under the Process menu. The exponential multiplication is executed either by typing EM or by selecting it from the Window Functions menu within the Process menu.

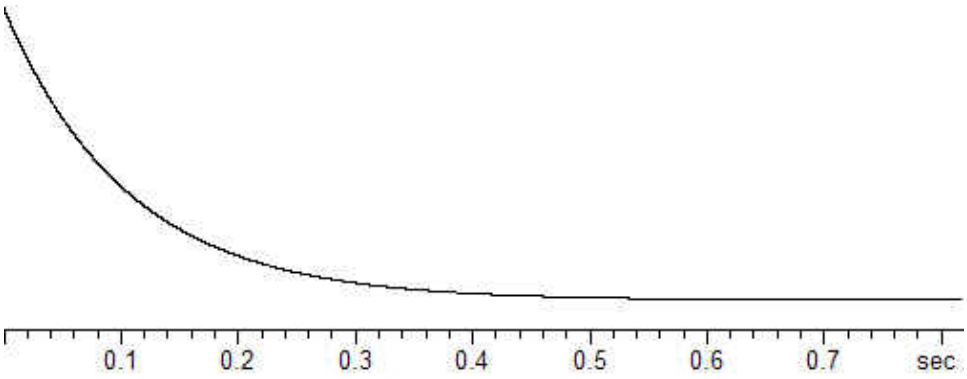
EM -- Exponential Multiplication

Applies an exponential weighting function to the FID to enhance signal-to-noise. The value for the linebroadening (**LB**) must be set first. LB is set via a dialog box brought up by typing LB or selecting Conditions under the Process menu. The exponential multiplication is executed either by typing EM or by selecting it from the Window Functions menu within the Process menu. Note the other available window functions which are listed in this menu.

The larger the value of LB, the faster the window function drops toward zero.



LB = 1



LB = 3

GM -- Gaussian Multiplication

Apodization function for Signal-to-Noise enhancement. This is similar to exponential multiplication (**EM**) but results in a Gaussian rather than Lorentzian lineshape. The amount of line broadening is set with **LB**.

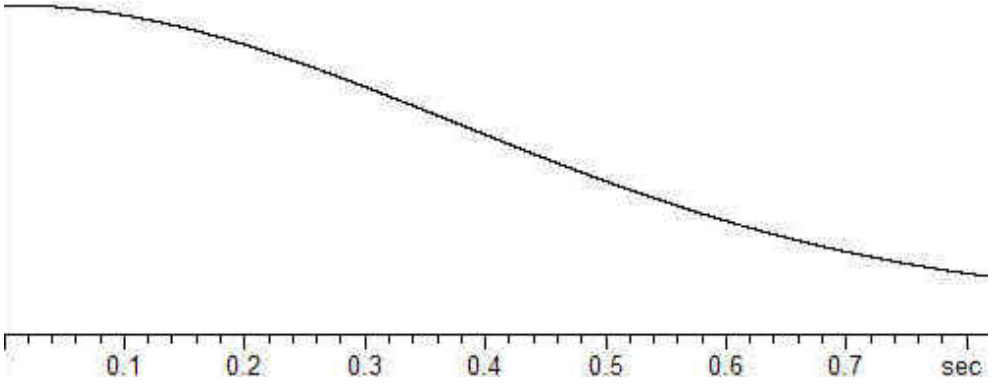
The GM command uses the following equation:

G(t) = exp[-(PI*LB*time)^2 / (4*ln(2))]

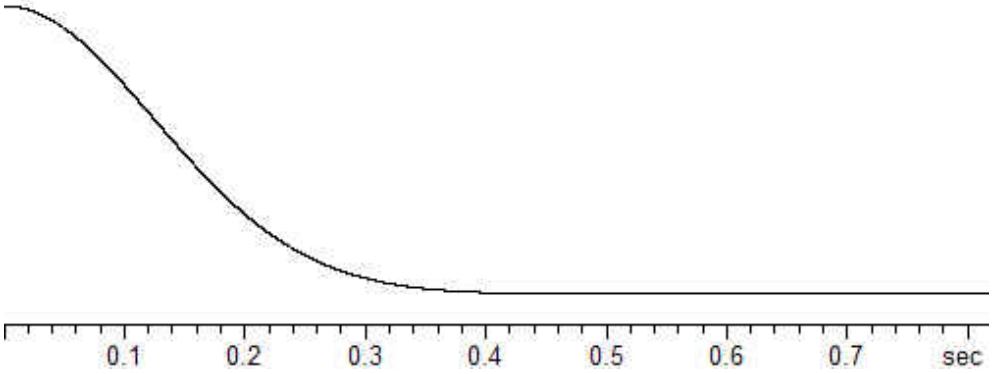
Ref: equation [9], p543, in G.A.Pearson, "Optimization of Gaussian Resolution Enhancement", **J.Mag.Res**. **74**, 541-545 (1987).

Do not confuse this with Lorentzian/Gaussian resolution enhancement.

As with EM, the larger the value of LB, the faster the window function drops toward zero.



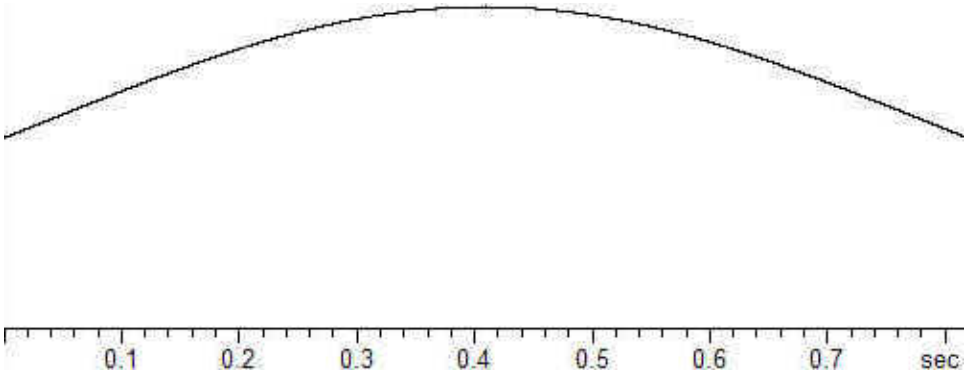
LB = 1



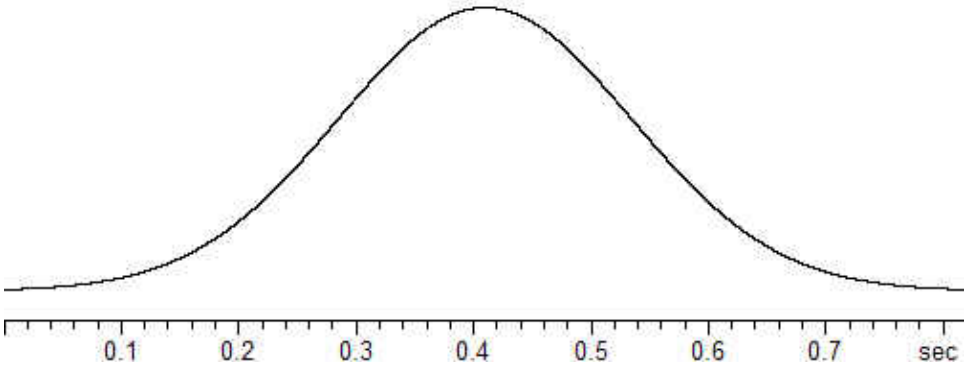
LB = 3

SG -- Shifted Gaussian

Apodization function which is a gaussian shape centered at one-half the acquisition time. The shape is determined by the value of LB. Larger LB results in faster roll-off of the function.



LB = 1



LB = 3

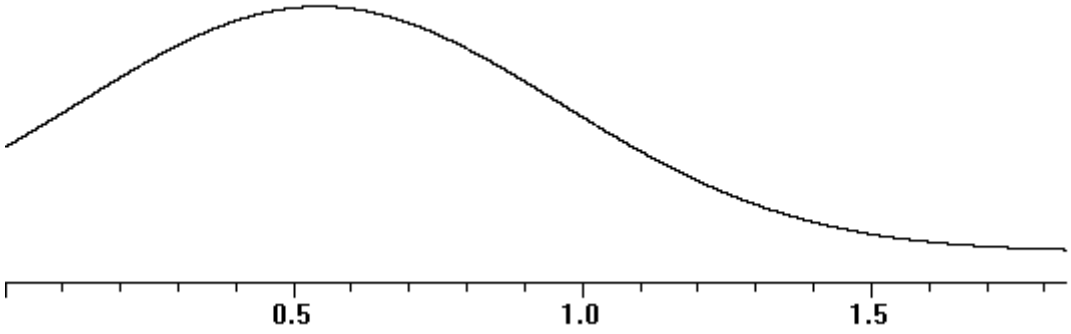
IG -- Incrementally Shifted Gaussian

This is a gaussian function for 2D data, in which the center of the gaussian is shifted based on the slice number.

LG -- Lorentzian/Gaussian resolution enhancement

This command multiplies the FID by a function which combines a Lorentzian using a negative line broadening with a Gaussian. The composite function has the shape shown below. Two parameters must be specified before LG can be executed: **LB** (line broadening, the same parameter used by the EM command) and **GF** (Gaussian factor). LB must be negative for the Lorentzian/Gaussian function. If LB is set to a positive number, NUTS will use the negative of that value. GF is a number between 0 and 1 and defines where the maximum of the function will be, as a fraction of the acquisition time. (This is the same as the Bruker GB parameter). Reasonable starting values are LB = -1 and GF = .3, and then adjust empirically.

This command is also available from the Process menu under Window Functions.



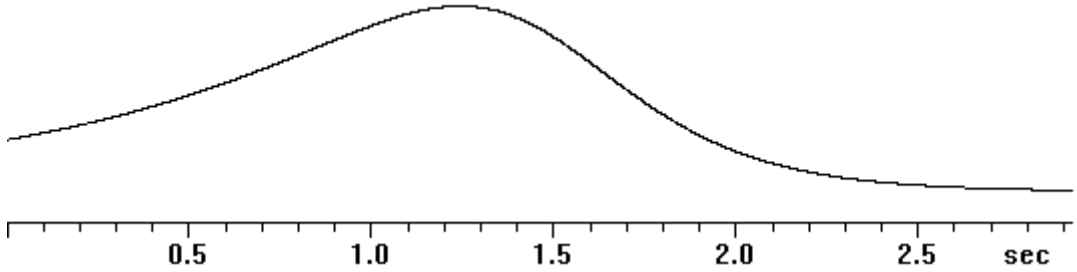
Reference: A.G.Ferrige and J.C.Lindon, **J. Magn. Reson.**, **37**, 337 (1978).

GF -- Gaussian Factor for LG command

This is one of the 2 required parameters for Lorentzian/Gaussian resolution enhancement. It must be a number between 0 and 1. (If its value is set outside these limits and the LG command is executed, NUTS will reset the GF value.) The GF value sets position of the maximum of the Gaussian function, expressed as a fraction of the acquisition time.

TF -- TRAF resolution enhancement

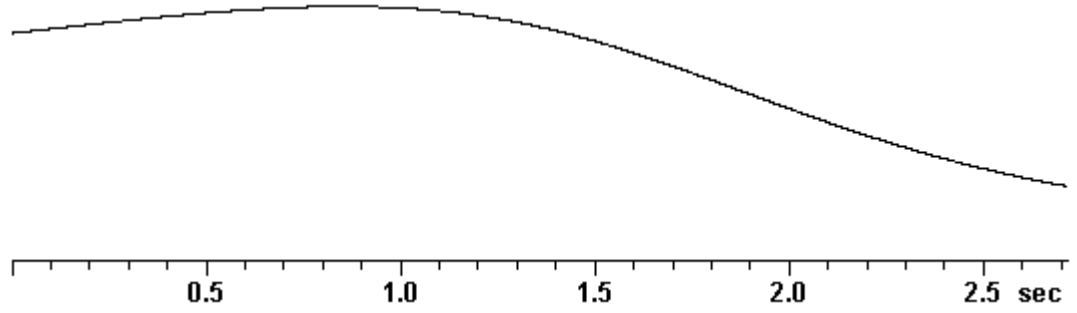
Performed on a FID. The function has the shape shown below. The user must input a value for **LB**, which is an estimate of the natural linewidth. The TRAF function provides resolution enhancement with minimal loss of signal-to-noise.



Reference: D.D.Traficante and D.Ziessow, **J. Magn. Reson.**, **66**, 182-186, (1986).

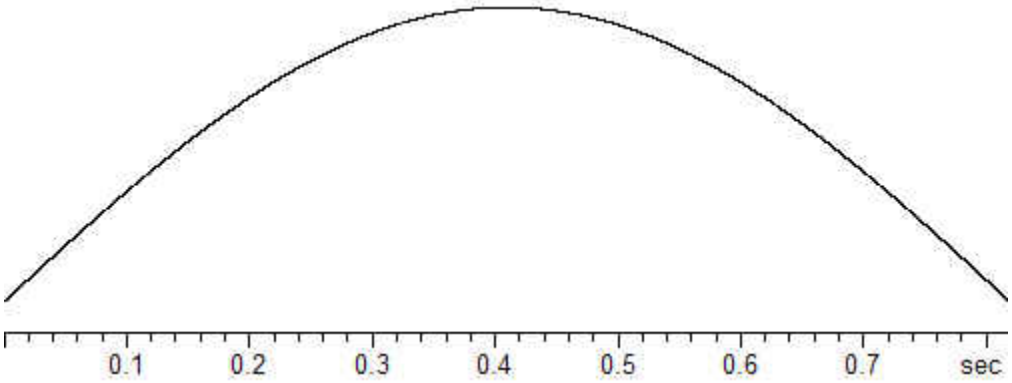
JT -- S-TRAF resolution enhancement

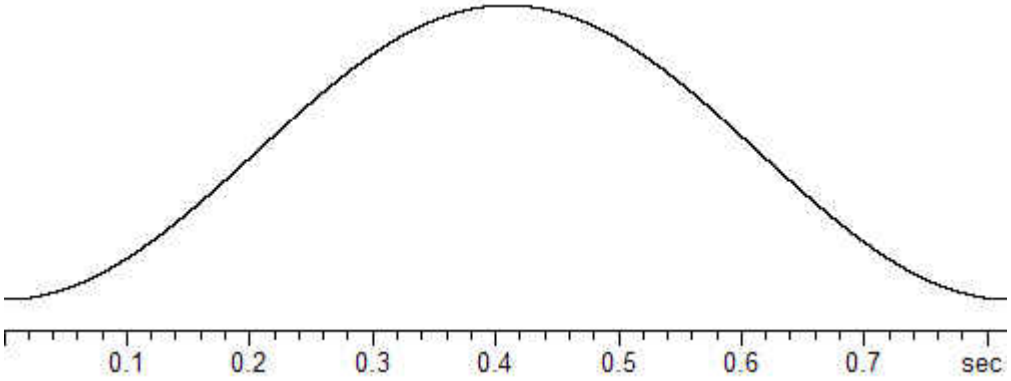
Performed on a FID. The function has the shape below. The user must input a value for LB, an estimate of the natural linewidth. Shown below is LB = .3



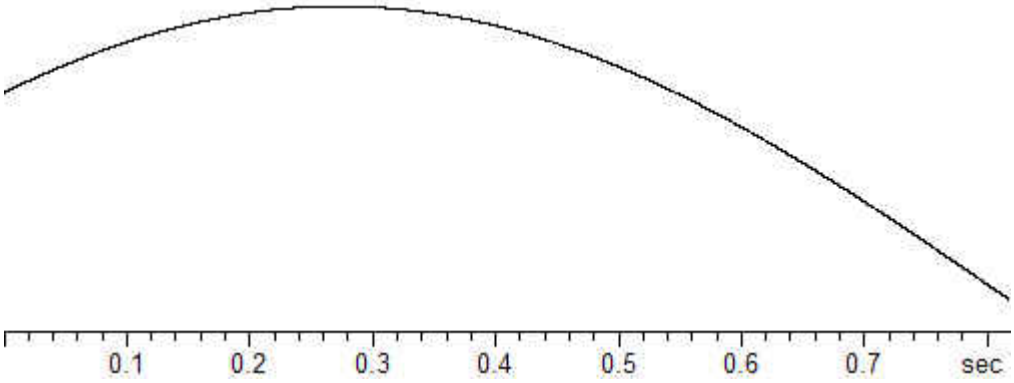
MS -- Multiply Sine

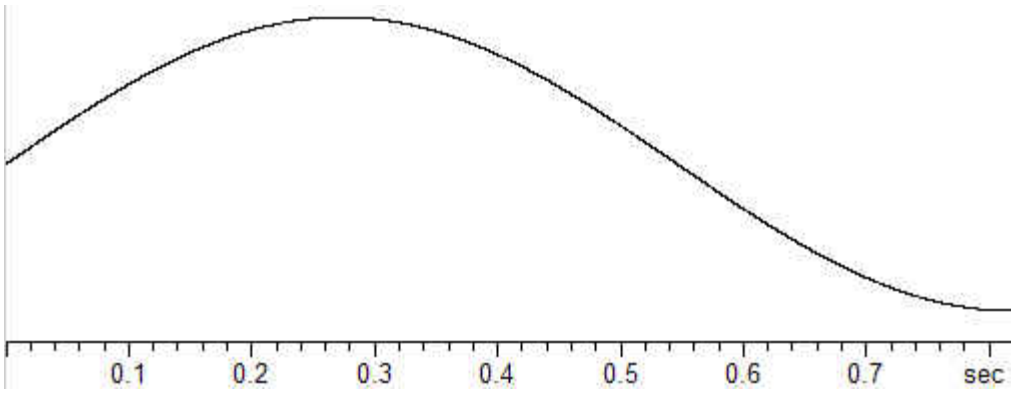
Multiply the reals and imaginaries by a window function which is the first half of a sine wave. Executing the command twice gives a sine squared window function. These 2 figures show the shape of sine and sine squared functions.



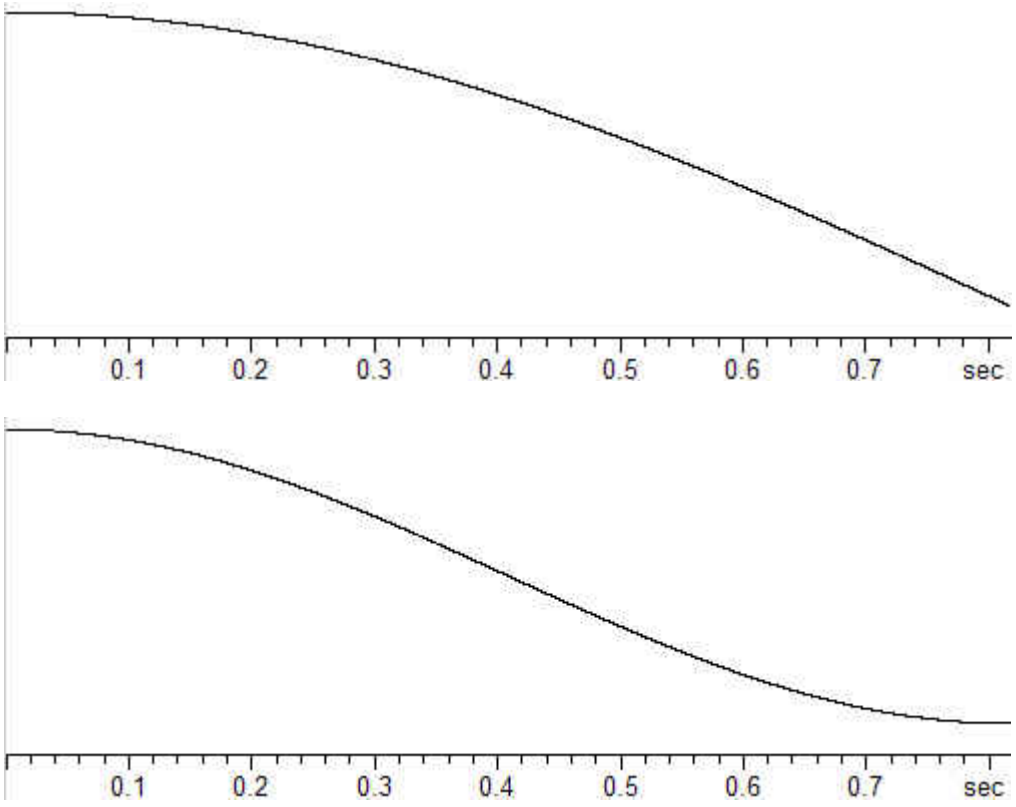


The sine function may have a starting phase different from zero. This phase angle must be set with the **S#** command before MS is executed. The next 2 figures show the shape of sine and sine squared functions with phase (S#) of 45.





The next 2 figures show the shape of cosine and cosine squared functions, which are sine functions with phase (S#) of 90.



This is also available from the Process/Window Functions menu. Note the other window functions which are available from this menu.

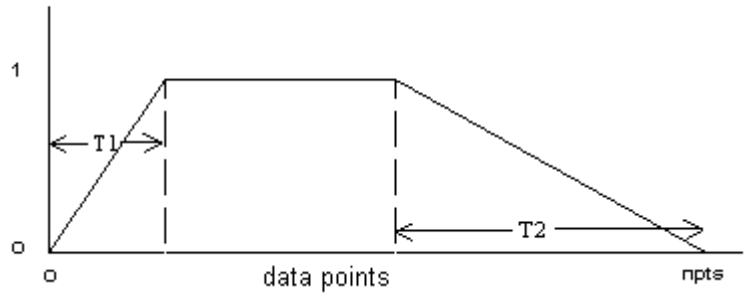
S# -- Phase shift for sine multiplication

Used in conjunction with the Multiply Sine (**MS**) apodization function. The phase shift is entered in degrees. This can also be set within a macro, for example, for 2D processing.

The only valid values for S# are between 0 and 90, inclusive. Entering a value less than 0 or greater than 90 will cause NUTS to set the value to 0 or 90, respectively.

TM -- Trapezoidal Multiplication

Multiplies FID by a trapezoidal shaped function defined by parameters T1 and T2. The first T1 number of points are scaled linearly from zero to one. The last T2 number of points are scaled linearly from one to zero to avoid truncation of the FID. Other points are unchanged. This command is available from the Process/Window Functions menu. Note the other available window functions which are listed in the menu, including sine multiplication (MS), Lorentzian/Gaussian (LG) and Traficante function (TF) for resolution enhancement.



T1 -- Trapezoidal multiplication parameter

Defines the number of data points, starting from zero, to be scaled by the TM command.

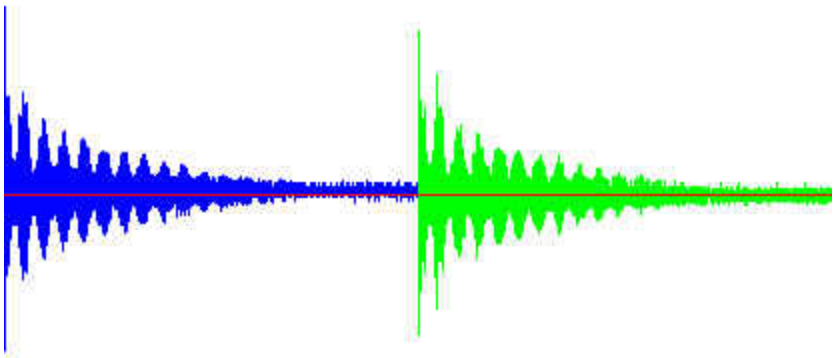
T2 -- Trapezoidal multiplication parameter

Defines the number of points at the end of an FID to be scaled by the TM command.

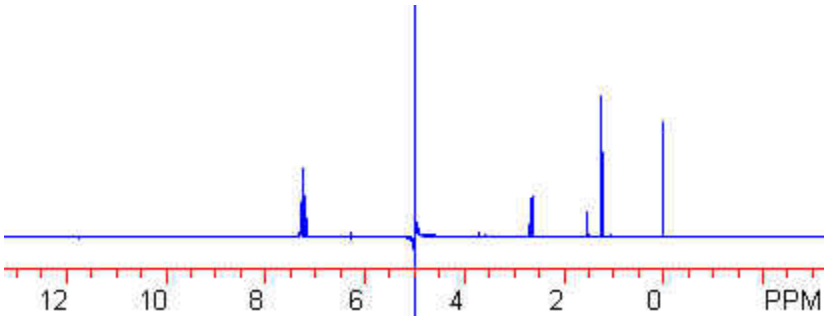
Baseline correcting the FID

Both real and imaginary parts of the FID should decay to zero by the end of the acquisition, but sometimes that "zero" level is not truly zero, and the offset may not be the same for both channels. This mis-match creates a glitch in the center of the resulting spectrum.

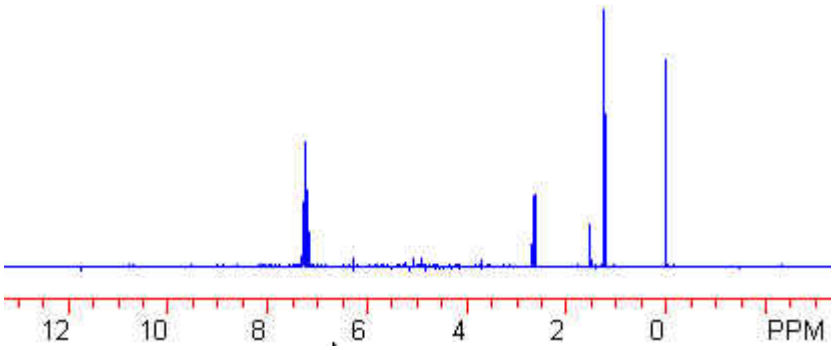
In the figure below, the red line is zero. Note that the imaginary half of the FID (in green) decays to zero, but real half (blue) decays to a value that is slightly above zero.



This "DC offset" appears to be small, but is large enough to create a significant "center glitch" after FT, as shown below.



A BC command, issued before the FT, is used to adjust the DC level of each channel to real zero, and will eliminate the center glitch. Below is the same data, processed with BC before FT.



There is an exception to this - if the FID has NOT decayed to zero (e.g., truncated 2D data), applying a BC may *create* a DC offset, and cause a glitch to appear. A dramatic example is shown for a 3D data set.

Creating customized apodization functions

DB -- Data to Buffer

Copies current data to the convolution filter buffer. This is useful for creating and applying customized apodization functions. (Do not confuse this with the Add/Subtract buffer.)

An apodization function can be created in different ways. One option is to build it within Nuts by first setting the current data points all equal to one with the **21** command. Alternatively, the ASCII import routine could be used to import a function created within a different application.

Once an apodization function is created, it can be copied to the convolution buffer with the **DB** command. The function is then viewed with the Convolution View (**CV**) command and applied with the Convolution Apply (**CA**) command. The latter 2 commands were created originally for use with functions created by the reference deconvolution operation (**CF**), but are also used for the general case of applying a function to an FID.

The data stays in the convolution buffer until replaced by another file or until Nuts is closed. The data may be copied back to the current data file with the Buffer to Data (**BD**) command.

BD -- Buffer to Data

Copies contents of the convolution filter buffer to the current data set, replacing the current data. The buffer must first have been loaded with the Data to Buffer (**DB**) command.

21 -- Convert spectrum to one

Replaces all data points in the current spectrum by one. The current spectrum is lost. Note that the line may appear off scale vertically. If the line is not visible, reduce the vertical scale with the right scroll bar, the Page Down key or the < key.

This command is useful for creating customized apodization functions. Once an apodization function is created, it can be copied to the convolution buffer with the Data to Buffer (**DB**) command. The function is then viewed with the Convolution View (**CV**) command and applied with the Convolution Apply (**CA**) command. The latter 2 commands were created originally for use with functions created by the reference deconvolution operation (CF), but are also used for the general case of applying a function to an FID.

2L -- Convert spectrum to line

Replaces the current spectrum with a line having a DC offset from zero. The actual value of the data points is 4096. The current spectrum is lost. Note that the line may appear off scale vertically. If the line is not visible, reduce the vertical scale with the right scroll bar, the Page Down key or the < key.

This command is useful for viewing apodization functions to optimize parameters. Dual Display can be used to view a trial apodization function simultaneously with a FID by first reading in the FID, placing it in the Add/Subtract buffer with the AL command and typing **DD**. Then execute **2L**, which puts a line on the screen, and apply your chosen apodization function. Each time you want to display a different function, first type **2L** again to re-set the line. To apply an apodization function to a FID, you must first read in the FID.

AD -- Add DC

Adds a constant to all data points. Unlike DC, this command actually changes the data, not just where it is displayed on the screen. The amount added is equal to one-third of the difference between the largest and smallest point in the data set.

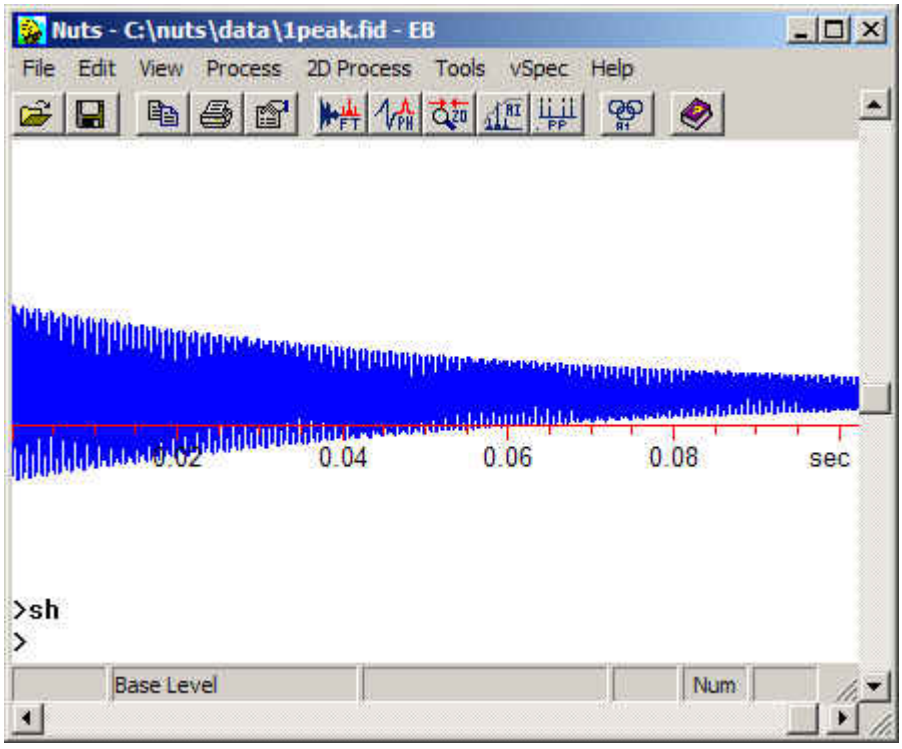
See also window functions, reference deconvolution

Zero-filling, shifting and shrinking data

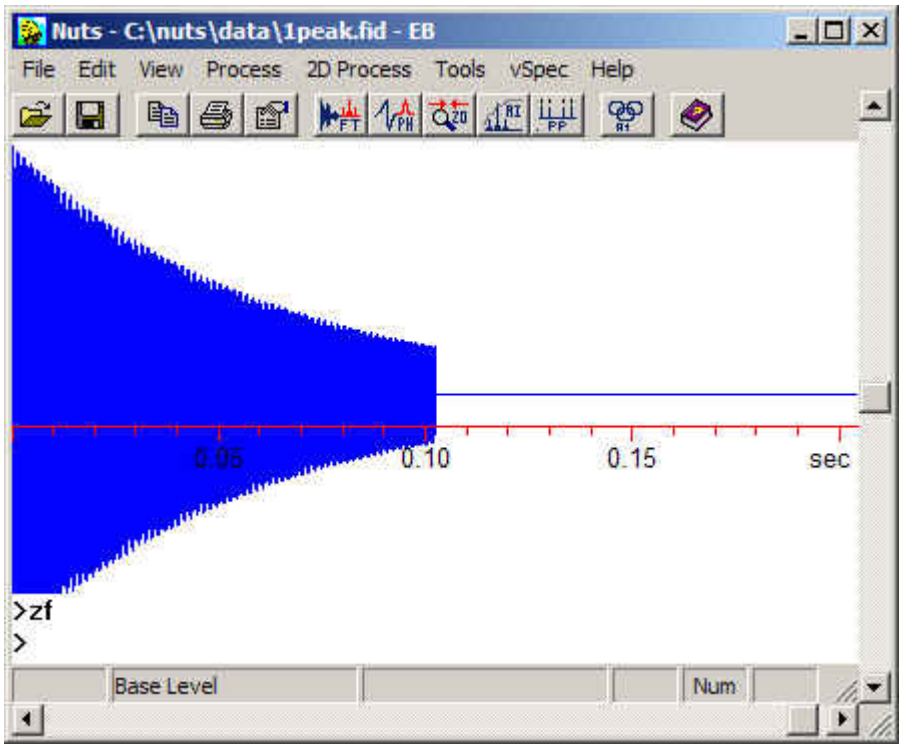
ZF -- Zero Fill

This command doubles the 1D data size by adding zeros to the end of the current data set. If the number of data points collected is not a power of 2, ZF zero-fills to the next higher power of 2, rather than doubling the number of points. This is useful for resolution enhancement and in processing 2D experiments where few data points were collected. (Note that if the data size is not a power of 2, a zero-fill operation is automatically performed when an FT is executed.)

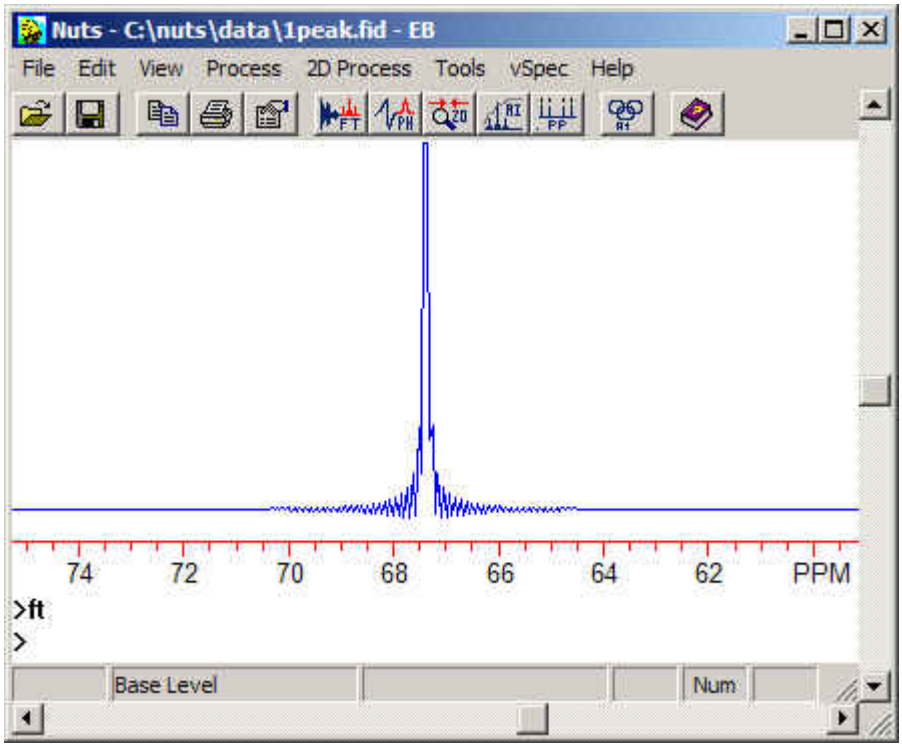
If the FID has not decayed to zero, executing ZF will introduce a discontinuity, resulting in distortion of the spectrum.



When the FID shown above is zero-filled, a discontinuity is created, as seen below.



After FT, the peak has "sinc wiggles" resulting from FT of the discontinuity.



To avoid this, a window function should be applied (usually exponential multiplication) to bring the end of the FID smoothly to zero before zero-filling.

Normally, ZF is used on time-domain data. If applied to frequency-domain data, the values of number of data points, sweep width and offset frequency are updated to be correct for the new data size.

See also: Linear Prediction (LN)

SH -- SHrink data

Reduces the number of data points in a 1D data set. By default, the size is reduced by half the number of points, and the last half of the data is lost. This is (sort of) the reverse of zero-filling. The data can be reduced by a different number of points, as determined by the **S@** parameter.

S@ -- Shrink by

Sets the number of data points by which the current data set will be reduced on execution of the **SH** command. If S@ is set to zero or a negative number, SH will reduce the data size by a factor of 2, its default operation. This default can be altered by setting the S@ parameter to a positive number.

The particular situation for which this was added was 2D data consisting of a number of slices not equal to a power of 2, which had been automatically zero-filled out to the next

power of 2 by the spectrometer, introducing truncation artifacts. SH (with non-zero value of S@) can be used to discard the zeroed points, allowing Linear Prediction (**LN**) of the data to avoid truncation.

/2 -- Reduce spectrum by factor of 2

The number of data points is halved, by discarding 25% of the points at each end of the spectrum. This obviously only makes sense for frequency-domain data. It is common to acquire data with a larger spectral window than needed, to reduce baseline artifacts. This command allows the excess baseline to be discarded and the file size reduced. The reported value for spectral width is adjusted. The operation can be un-done with the following command.

In the non-2-letter command mode, a parameter can be used to specify a different number of points to be discarded from each end, rather than using the default.

The command **brickwall** is equivalent to /2.

Note that the command behaves differently when applied to an FID.

**2 -- Expand data by factor of 2*

The number of data points is doubled by adding zeroes at each end. This obviously only makes sense for frequency-domain data. This is the inverse of the previous command.

In the non-2-letter command mode, a parameter can be used to specify a different number of zeroed data points to be added to each end, rather than using the default.

RD -- Rotate Data

Performs a circular shift of data points a specified number of times. A circular shift means that a point is removed from one end of the spectrum, the data are shifted by one data point, and the point is added to the other end of the data. (Do not confuse RD with left shift (LS) and right shift (RS)).

This is normally used for Bruker data from the Avance series of spectrometers (DnX models) which have been digitally filtered and "decimated" prior to saving the FID. The initial points of the FID are zero. A circular left shift must be performed before the FT. The number of points to shift is calculated from the Decimation Number found in the Bruker file header, but the user can change the number of points.

When RD is executed directly, a dialog box appears allowing the user to adjust parameters. When RD is included in a Link or Macro, the default or last entered value is used, without prompting for user input.

Note that any zero-filling or apodization must be performed before the RD. See details on how to process data with RD.

See also: Special considerations for Bruker data

LS -- Left Shift data

Shifts the real and imaginary data left one point and assigns the last point the value of zero.

RS -- Right Shift data

Shifts the real and imaginary data right one point and assigns the first point the value of zero.

Fourier Transform commands

Conversion from time domain data (FID) to frequency domain data (spectrum) is accomplished via a forward Fourier Transform. There are 3 types of forward transforms, but NUTS should be able to determine which is appropriate for the current data set, and will apply the correct function when Fourier Transform (FT) is selected.

FT -- Fourier Transform

Does a forward Fourier Transform of the current data set. If the data set is real, a Real FT is performed. If the data set is complex, a Complex FT is performed. If the data is a complex interleaved data set (acquired as alternating real and imaginary points, rather than as simultaneous pairs), a Bruker FT is performed.

Using the non-2-letter command mode, the type of transform can be specified by entering FT followed by one of the following arguments:

complex
complex_norotate
complex_nosort
hilbert
inverse
real
sequential

BT -- Bruker Transform

Bruker data from older spectrometers (using the Aspect computer) are usually acquired using a single A-to-D converter, so real and imaginary points are acquired sequentially,

rather than simultaneously. The data is sampled at twice the sampling rate and the data points are placed alternately into the 2 channels to achieve quadrature detection. Therefore, the n^{th} real data point and the n^{th} imaginary data point are not acquired at the same time. This necessitates performing the FT differently. If the data set was converted properly, NUTS will correctly identify the data as "TPPI" (Time-Proportional Phase Incrementation) and typing FT automatically performs the appropriate transform. Typing **BT** forces NUTS to do a "Bruker Transform" regardless of how the data were acquired.

See also: Special considerations for Bruker data

DF -- Digital Filter and FT

This command is an alternative to RD plus FT for digitally filtered data.

First, some background: Digitally filtered FIDs have zeroes at the beginning of the data, and FT creates a very wiggly baseline that requires very large linear phase correction (hundreds or more degrees). To avoid this, the zeroes are removed by a cyclic rotation, performed with the RD command. NUTS determines how many points to rotate from parameters found in the original data file.

However, sometimes the information found in the data header is incorrect or insufficient for NUTS to determine the correct number of points. The DF command examines the beginning of the FID to determine where the "real" start of the data should be, and calculates the linear phase correction needed, then executes an FT and applies that phase correction. This will not work properly for all data, so users are advised to experiment.

See also: Rotate Data (RD)

TJ -- Transform JEOL

JEOL spectrometers also have the option of acquiring data using a digital filter (see explanation for DF command, above). Newer versions of the Delta software manipulate the data to compensate for the distorted ends of the spectrum. The Delta software "knows" that 10% of the spectral window at each end will be distorted. To avoid possible loss of peaks at the edge of the window, the spectral width actually acquired is increased automatically. After the FT, 10% of the data points at each end are automatically discarded, so the resulting spectral width is the value originally input by the user. However, this results in a reduction of the number of data points in the final spectrum from the user's settings. For example, if the user sets a spectral width of 6000 Hz and 16384 data points, the spectral width acquired will be (6000 / 0.8) = 7500 Hz. After FT, 10% of the data at each end is discarded, giving a final spectral width of 6000 Hz, but the final number of data points is reduced to 13107. This is a consequence of needing to perform the FT on data which is 2^n number of points. The digital resolution the user intended was 0.37 Hz/pt but the final spectrum is 0.46 Hz/pt. Note that this may create problems in homonuclear 2D experiments if it is desired to perform symmetrization, as the number of data points is changed in the process.

The TJ command in NUTS performs the same operation as the Delta software does during processing. As described above, the early data points are zero, but we have not identified a parameter in the data header that would let NUTS know how many points need to be cyclically rotated, using the RD command. NUTS examines the early data points in the FID to determine where the "real" start of the data is. An FT is performed, and then a linear phase correction is applied based on how many zeroed points were found at the beginning of the FID. Lastly, 10% of the data points at each end of the spectrum are discarded.

CT -- Complex Fourier Transform

Forces NUTS to execute a complex FT, regardless of the nature of the current data set. Normally, it is better to use **FT** and let NUTS apply the type of Fourier Transform which is appropriate.

RT -- Real Transform

Performs a forward real Fourier transform of the current data set. Any data in the imaginary part of the current data set will be zeroed and ignored. Normally, NUTS correctly identifies when data are real and applies a real FT when the FT command is given. Typing RT will override this and apply a real FT regardless of the nature of the data.

IT -- Inverse Transform

Performs an inverse complex transform of the current frequency domain data set to generate an FID.

HT -- Hilbert Transform

Hilbert inverse Transform allows the creation of a complex FID from a reals only spectrum.

Phasing

NUTS offers several different tools for phasing, including automatic phasing routines that use Simplex optimization, phasing of the entire spectrum using the mouse, phasing using an expanded display of 2 spectral regions and applying specific numerical phase values.

Follow the links to see a step-by-step illustration of the phasing commands, illustrated with screen captures.

Summary of phasing commands:

AP Auto Phase. Automatically adjusts zero- and first-order phase.
QA Quick A Phase. Automatically adjusts zero- order phase only.

QP Quick Phase. Automatically adjusts zero- and first-order phase.
PA Phase A. Input zero-order phase correction to be applied with PC.
PB Phase B. Input first-order phase correction to be applied with PC.
PC Phase Correction. Adjusts the phase of spectrum by the values in PA and PB.
PI Incremented phase. Applies phasing equal to PA and PB multiplied by the slice number.
PE Phasing Expanded. Mouse phasing routine using 2 expanded regions.
PH Phasing using mouse. Left & right mouse buttons control A and B phase parameters.
PS Phase Same. Applies previously determined phase correction to the current spectrum.
TP Total Phase. Displays values of phase correction which have been applied.
ZP Zero Phase. Sets both zero- and first-order phases to zero.

See also: Phasing 2D data, Auto-phase details

Often the best first step in phasing is to make initial phase correction using only zero-order correction, until the phase is close to correct. This is most easily done either with **QA** (automatic phasing, zero-order only) or with **PH**, using only the left mouse button to apply zero-order correction. Adjust phase until the spectrum has approximately the correct phase, then use PH or PE to make final adjustments. This avoids the possibility of inadvertently entering too large a value for the first order phase correction, resulting in an undulating baseline. (If that happens, use **ZP** to reset the phase and start over.)

Spectral baselines can be improved and phasing simplified by adjusting data acquisition parameters so that the first-order phase correction needed is close to zero. This is done by adjusting the delay before the first data point is acquired (DE in Nicolet or Bruker software).

Users who prefer up/down mouse movements to left/right mouse movements can select this in the NUTS.ini file.

AP – Automatic phasing

QP – Quick Automatic phasing

QA – Automatic phasing of zero-order only

There are several ways to phase a spectrum with NUTS. The first way is to use the **QP** (Quick Phase) or **AP** (Auto Phase) command which adjust the zero- and first-order phase corrections. The 2 commands use slightly different algorithms, and one may work better than the other for any specific spectrum. These routines work well if there is a lot of baseline (no peaks) at the ends of the spectrum, if there is no DC offset for the spectrum and if the baseline is reasonably good. There is also an automatic phasing command (**QA**) which adjusts the zero-order phase only, which is best used to give a good starting point for additional phasing. If the phase obtained is not perfect using the automatic

routines, manual phase adjustment becomes necessary. Automatic phasing does not work very well when the spectrum contains very narrow lines (eg., a ^{13}C spectrum). A simple way around this is to process the spectrum with 3-4 Hz of linebroadening, FT and phase. Then recall the spectrum, reduce the linebroadening, FT and phase using **PS**, which applies the previously determined phase parameters.

The auto-phase routines were re-written and improved in March, 2003. See details below.

PH - Mouse phasing

The spectrum phase can also be adjusted using the mouse. The user can start the mouse phasing routine from the menu selection Process / Phasing by Mouse or with the **PH** command. This is usually applied to the entire spectrum at once, but can be used on an expanded region if that is the current display when the PH routine is entered.

On entering the phasing routine a "reduced data point" display of the data will be shown to make screen updates faster. When the left mouse button is depressed, dragging the mouse left and right adjusts the zero-order (or A) phase of the spectrum. When the right mouse button is depressed, dragging the mouse left and right adjusts the first-order (or B) phase of the spectrum.

By default, the phasing pivot point for the B correction is the left end of the spectrum display. This can be changed, but must be done before entering the PH routine. From the Nuts base level, hold down the left mouse button, place the cursor where the pivot point should be, and type P. When the phase is properly adjusted, the user types the <ENTER> key and the new phase adjustments for the spectrum are applied, and the PH routine is exited. Under some conditions there is a slight difference in the phase for the full data point spectrum and the "reduced data point" display. Repeating the PH command will usually correct the problem.

The coarseness of the mouse adjustment of phase can be reduced by 3x by holding down the Control key while phasing. A default setting can also be made in the nuts.ini file.

See details below on how to use PH.

The PH routine was modified to allow a second-order correction to be applied. When second-order phase correction is applied, the pivot point is *always* the far left edge of the spectrum, for simplicity. To use this, type 2 on the keyboard while in the PH routine. Any mouse movement will result in application of second-order correction - ***Use Caution!*** Pressing either mouse button returns to the normal mode of applying zero- and first-order correction.

The values of zero-, first- and second-order correction are shown at the left edge of the screen during phasing.

PE –Phasing expanded regions

The spectrum phase can also be adjusted with the Phase Expanded (**PE**) routine, in which 2 regions of the spectrum are phased in turn. This is usually the best choice of phasing routine for larger data sets, especially to do a "touch-up" after automatic phasing, because it more easily allows careful examination of the phase. (Since the total phase is defined by 2 parameters, determining the correct phase in 2 spectral regions is sufficient to calculate the phase for the entire spectrum. This is similar to Bruker's and Nicolet/GE's cursor phasing process.)

To begin, two regions of the spectrum are selected using the Zoom routine. First, display the entire spectrum using Ctrl-F. Then, enter Zoom, select a region by holding down the left mouse button and wiping across the spectrum. Type "1" to select this as region #1. (Don't expand the display to the selected region, just highlight the chosen region and type 1.) Then select another region and type "2". This defines the 2 regions to be phased.

Now, exit Zoom and enter the Phase Expanded routine with the **PE** command. Region 1 is displayed first by default. Press and hold the left mouse button and adjust the phase of region 1 with left and right mouse movements. The pivot point (where linear correction is zero) is automatically set to the tallest peak in region 1. Adjusting the phase with the left mouse button applies a zero-order correction to the entire spectrum. Then, press and hold the right mouse button and, likewise, adjust the phase of region 2. When both regions are adjusted properly, exit with an <ENTER>. The NUTS program will calculate and apply the proper A and B phase correction to the full spectrum. The selected regions will remain in effect until re-defined, they become invalid, or the NUTS program is exited. Typically, two regions with easy to phase peaks at opposite ends of the spectrum are selected for this operation. In some cases, care needs to be used to keep the B phase correction from getting too large (> 360 degrees) which results in an undulating baseline. You can check this by typing TP (Total Phase). The B phase parameter should be small, and should definitely NOT exceed 360 degrees. Should this happen, the Zero Phase (**ZP**) command can be used to reset the phases to zero so you can start over.

The coarseness of the mouse adjustment of phase can be reduced by 3x by holding down the Control key while phasing. A default setting can also be made in the nuts.ini file.

See details below on how to use PE.

PS – Phase same

For a series of spectra acquired with the same spectrometer conditions, the phase correction is usually the same for each. After the phase correction has been determined for one spectrum, use the **PS** (Phase Same) command to apply the same correction to subsequent spectra. Some minor adjustment may be necessary, which can be done using any of the above methods.

PC – Specifying numerical phase values

The spectrum phase can also be adjusted manually from the keyboard. The amount of phase correction for the A and B phasing parameters (zero- and first-order corrections, respectively; the pivot point is at the far left end of the spectrum) can be entered with the **PA** or **PB** commands or from the Process / Conditions menu selection dialog box. After values for A and B phase correction have been entered, the **PC** command (or the Process / Phase Correction menu selection) will apply those phase corrections each time the command is issued. That is, PC adds to any phasing which has already been done. The total amount of phase correction done to the spectrum will be noted in the Process / Conditions dialog box (view with the **TP**, Total Phase, command) and will be saved with the spectrum when the file is saved to disk.

Some filters on Varian and JEOL spectrometers create spectra which cannot be phased with only a zero and first order phase correction. The NUTS phasing routines were modified to include a second-order phase correction. In the non 2-letter command mode, the PC (and PhaseCorrect) command can take 3 arguments. The first argument is the zero order phase amount, the second argument is the first order phase amount and the third argument is the second order phase amount. This takes practice!

PA - Phase A. Input zero-order phase correction to be applied with PC.

PB - Phase B. Input first-order phase correction to be applied with PC.

PI – Incremented phase

Some solids multidimensional experiments (such as MQ-MAS) require a phase correction which is proportional to t_1, rather than being the same for all slices. The **PI** command applies phasing equal to the values of PA and PB multiplied by the slice number. PI can be used in the non-2-letter command mode to specify the phase correction on the command line. If one argument is given, it is used as the A phase value when doing the phase incrementing. If two arguments are given they are used as the A and B phase values when doing the phase incrementing. If three arguments are given they are used as the A, B and C phase values when doing the phase incrementing.

PI is also used in processing Bruker Sates-TPPI data. These 2D data require inversion of every other slice, which can be done by setting PA to 180 and applying PI.

The PI command was modified to implement diagonal peak suppression in COSY data (ref: ***The NMR Newsletter,*** August, 2000). If the first argument is "-cosy", the command does a special case of phase incrementing which allows the diagonal of a 2D data set to be phased to the desired addition three arguments as above.

TP - Total Phase. Displays values of phase correction which have been applied.

ZP - Zero Phase. Sets both zero- and first-order phases to zero.

How audio filters can affect phasing

Audio filters serve to exclude signals at frequencies greater than the spectral width. The ideal filter would have no effect on signals within the spectral window (the "pass-band"), and would eliminate all frequencies outside it (the "stop-band"). A sharp cut-off minimizes the amount of noise that folds back into the spectrum, maximizing the signal to noise ratio. Real filters fall short of this ideal, and can be the source of phase, amplitude and baseline distortions.

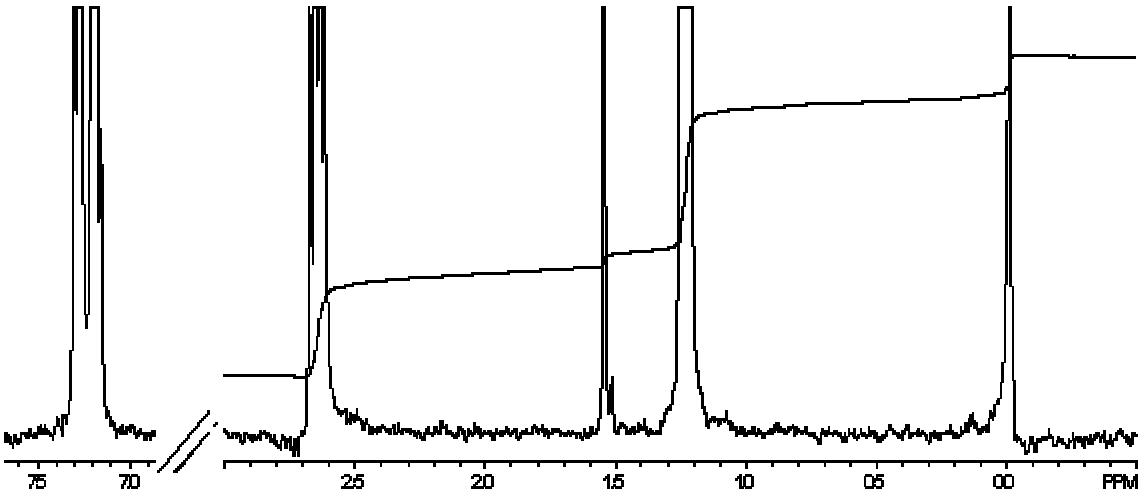
The two most common types of filters used in NMR spectrometers are Bessel and Butterworth. A Butterworth filter yields the flattest amplitude response in the pass-band with a sharp cut-off when entering the stop-band. This gives the best integration, but its slower pulse response time leads to distortions of the first few points in the FID, causing baseline distortions.

A Bessel filter has more amplitude roll-off in the pass-band than a Butterworth filter, meaning that peaks near the edges of the spectral window are attenuated, resulting in inaccurate integration. However, the better pulse response time of the Bessel filter causes less distortion of the early points in the FID and gives flatter baselines.

In addition to the amplitude response in the pass-band and the response time, filters also distort the phase in the pass-band. Bessel and Butterworth filters have typically been used in NMR because these distortions are of low order, and can be corrected with zero- and first-order phase correction. Residual distortions can be observed as slightly out-of-phase peaks near the filter pass-band cut-off (near the ends of the spectrum), usually avoided by widening the spectral width so these distortions do not interfere with peaks of interest. This type of phase distortion can have significant detrimental effects on NOESY spectra - see illustration.

In the course of providing technical support to NUTS customers, we receive sample data sets whose origins run the gamut of spectrometer models and manufacturers. We have observed spectra with much more severe phase distortions than described above. After phasing with the normal zero- and first-order correction, the remaining out-of-phase peaks are not observed merely at the extreme ends of the spectrum, but well into the spectral window.

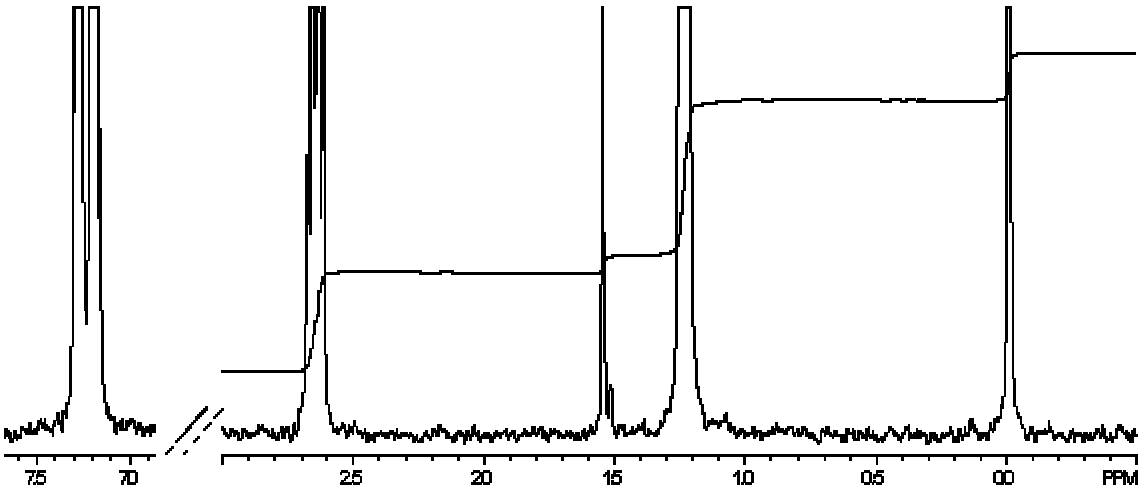
In the spectrum of ethylbenzene below, note that the aromatic and methyl peaks are correctly phased, but TMS and methylene peaks have phase distortions of opposite sign. (The spectrum is shown with increased vertical scale so the phase irregularities are clearly visible). It is not possible to phase all peaks simultaneously using zero- and first-order correction. The distortion in this case is approximately 7 degrees, and is clearly sufficient to interfere with integration. Note that both ends of the displayed integral are flat, meaning that the commonly employed "drift and tilt" integral adjustment cannot compensate.



Plots created by processing on the spectrometers often appear correctly phased, leading us to surmise that the spectrometer software is doing something in addition to "traditional" phase correction. The obvious first attempt at resolving this is addition of a second-order term to the phase correction.

The spectrum above resulted from zero- and first-order phase correction of –121 and 37 degrees, respectively. The pivot point is the downfield edge of the spectrum.

The same spectrum is shown below after phase correction using zero-, first- and second-order values of –155, 185 and –130 degrees, respectively. It appears that addition of one more term is sufficient to yield an acceptable spectrum.



It appears that in the search for ever-increasing signal-to-noise, sharper cut-off filters, which cause undesired phase shifts in the pass-band, are being used. As with many other

aspects of NMR hardware, this is a trade-off, and each spectroscopist needs to be aware of the choices being made.

Magnitude and Power Calculation

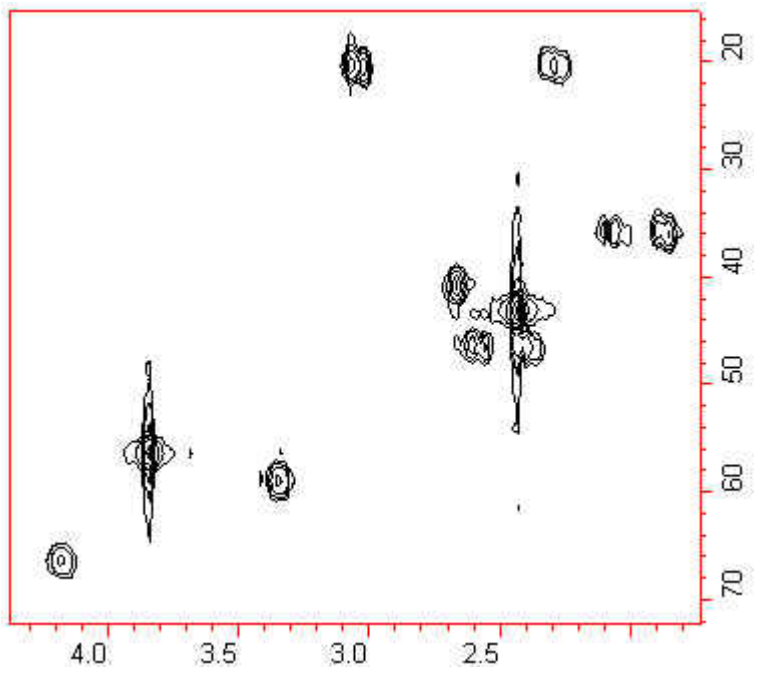
MC -- Magnitude Calculation

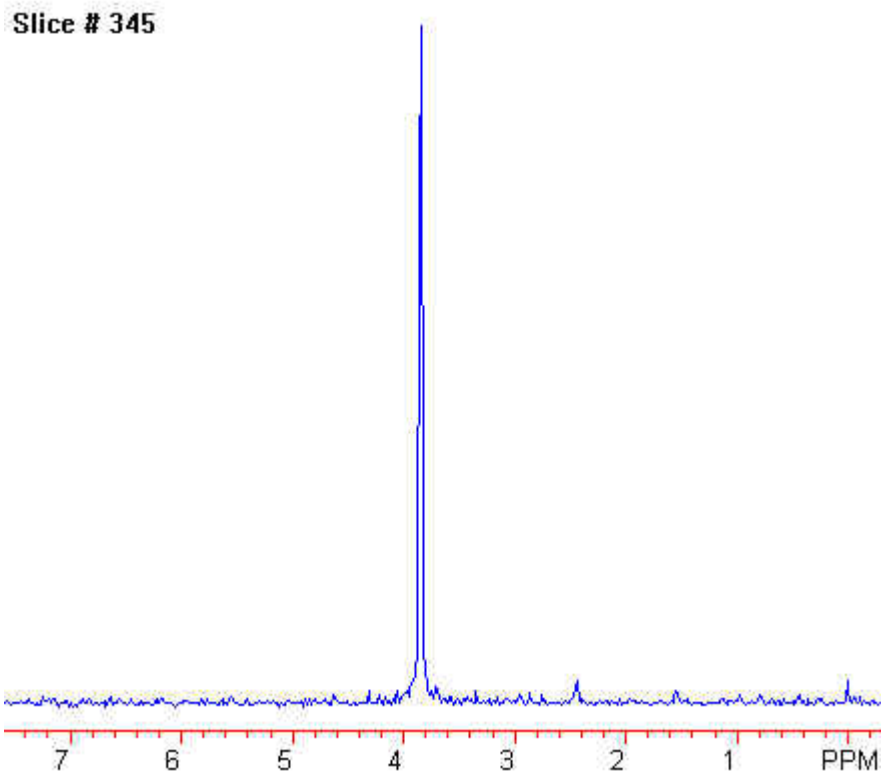
Computes the magnitude spectrum of the current data set so all peaks appear positive. Useful when data is not pure absorption phase. The magnitude spectrum is calculated as

SQRT(Re2 + Im2)

where Re and Im refer to the Real and Imaginary parts of the complex number, respectively.

Below are shown a contour plot of part of the HMQC spectrum of codeine, and the slice through the methoxy peak, processed with MC as the last step. Compare to the same data processed with M2, below.





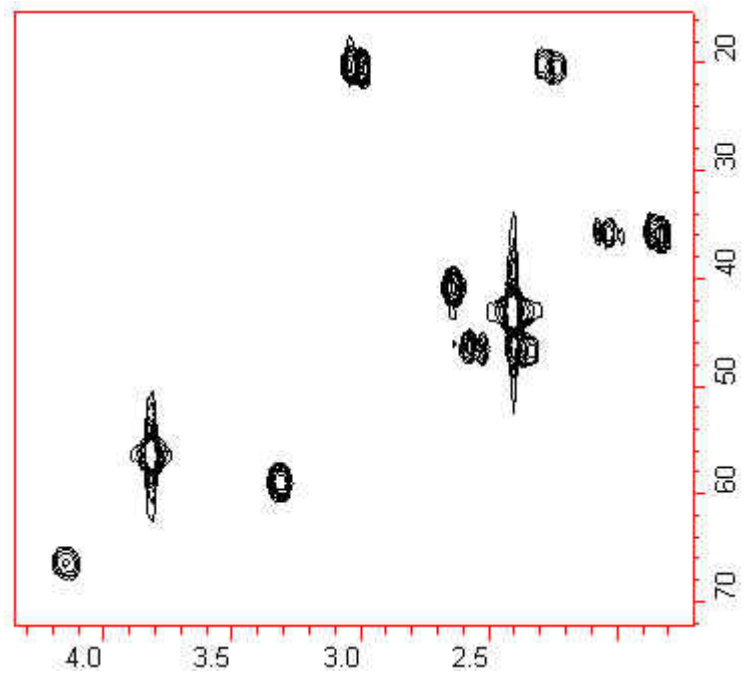
M2 -- Power spectrum

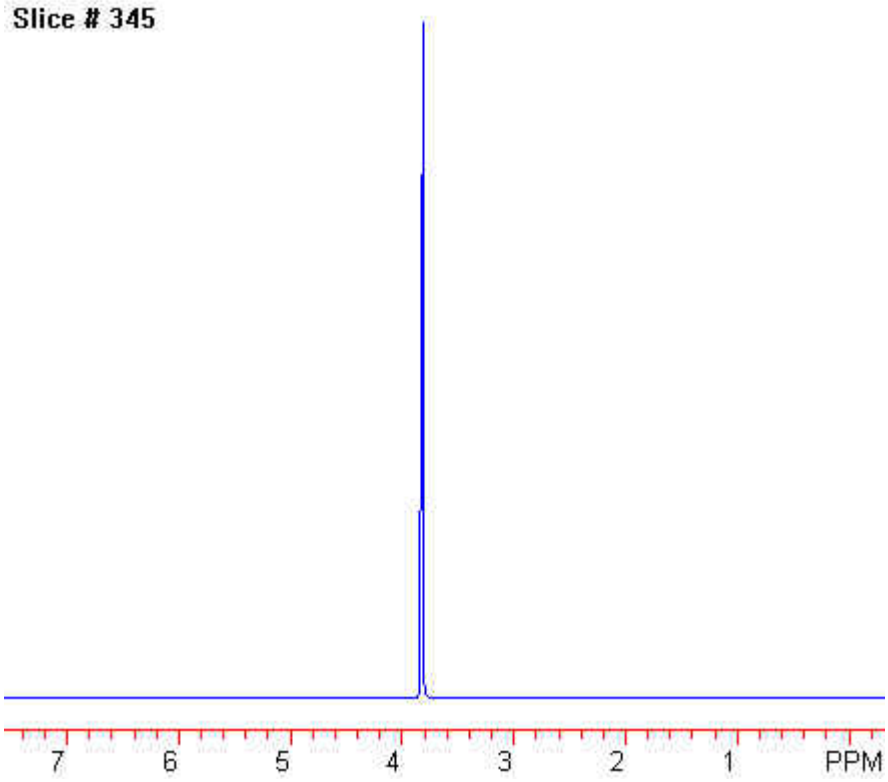
An alternative to a Magnitude Calculation (**MC**). The power spectrum is calculated as

Re2 + Im2

where Re and Im refer to the Real and Imaginary parts of the complex number, respectively.

Below are shown a contour plot of part of the HMQC spectrum of codeine, and the slice through the methoxy peak. This was processed using M2 as the last step. Compare to the same data processed with MC, above.





The M2 command actually does a calculation which converts a time domain signal directly to a power spectrum without the need for an FT. This will work properly only with standard complex data sets, not with real or Bruker TPPI data sets. This was done to speed up 2D processing.

RO -- Square Root of data

This command takes the square root of the data points of both the reals and imaginaries. If the data points are negative the point is replaced with the negative of the square root of the negative of the points value.

Auto-phasing

The autophase routines (QA, QP and AP) were re-written to give faster and better results. In the course of testing algorithms, several parameters for the AP command were created. While in most cases, users have no need of these extended commands, they are available, and are described here for completeness.

Each spectrometer generates characteristic distortions of phase and baseline, so finding an algorithm that works in all cases is difficult. The user should experiment with commands and parameters to see what works best for his/her data.

Part of the process involves a decision as to which data points are baseline and which are peaks. One option is to ignore points at each end of the spectrum, which is useful for data that is digitally filtered, because these spectra commonly have badly distorted points at each end (see example).

In the descriptions below, "A" phase refers to zero-order and "B" phase refers to first-order phase correction terms.

QA adjusts only the zero-order phase parameter. QP

The AP and QP commands can take arguments, when executed in the non-2-letter command. mode. They are described here for AP, but can be applied in the same manner with QP.

QP is a subset of AP and therefore somewhat faster and slightly less accurate.

AP ?
will display possible arguments.

AP log
will do the AP process and log the steps the AP routine takes, written to a file in the current data directory.

AP stepA #
sets the initial step size for the zero order phase.

AP stepB #
sets the initial step size for the first order phase.

AP A
will phase only the zero order phase.

AP look
will exchange the current data for the automatically generated reduced data point data buffer used by the AP routine.

AP enhanced #
will set the AP routine to use advanced baseline detection when # is one. When # is zero the AP process will not use advanced baseline detection. This feature works better with spectra which having peak-free baseline at each end.

AP IgnorePts #
will set the number of points on each end to ignore in the phasing process to #. By default (IgnorePts = -1) the AP process ignores 5% of the data points on each end of the spectrum. When the data set to be phased has peaks close to the ends it is better to set this number to the minimum allowed by the data's baseline.

AP RMS #
will set the number of RMS noise multiples to use in the advanced baseline peak detection mode. Default is 100 times.

AP Pts2Ave #
will set the number of points to average when making the reduced data set for the AP process. The default value is zero which allows the AP process to automatically determine how much to reduce the data based on initial data sizes. QP reduces the data more than AP. If this argument is set to one, then the data is not reduced and the process is slightly better, but a lot slower.

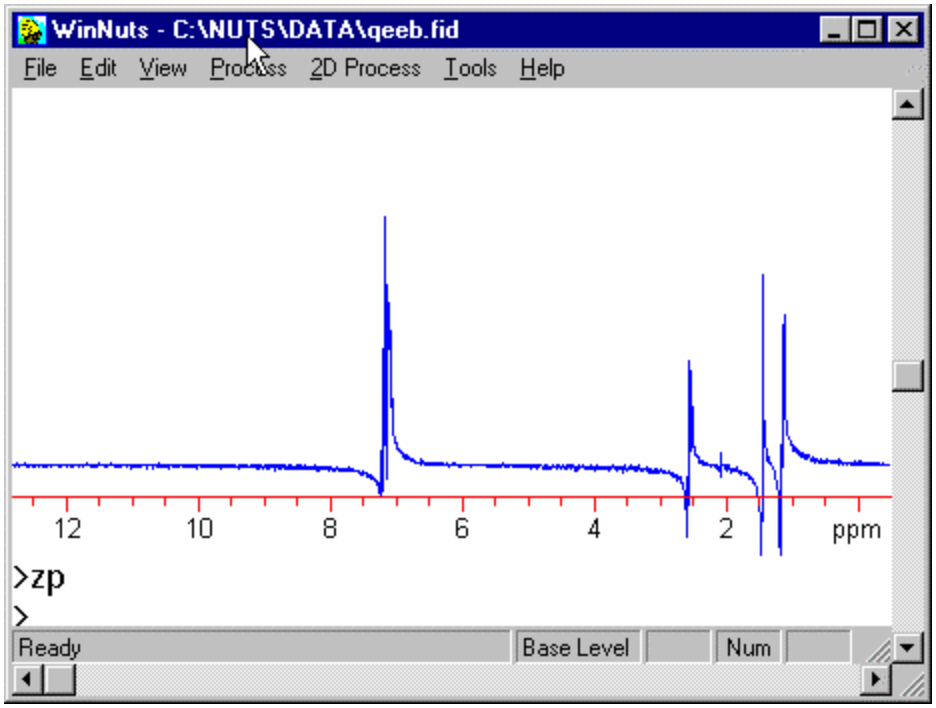
AP quality
does not do an automatic phase process but returns the value used by the AP process for the quality of the current spectrum's phase.

AP Simplex
does the AP process using the Simplex method and creates a log to the current data directory.

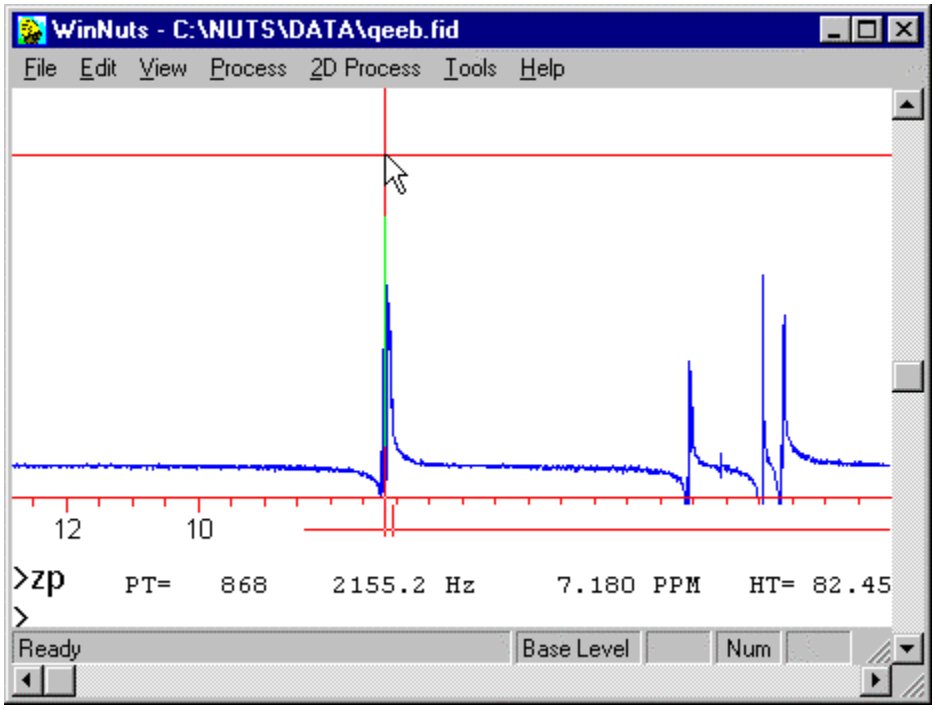
AP map
sets the first order phase to -360 degrees and does a zero-order optimization. It then increases the B phase in 10 degree steps and optimizes the zero order phase at each step until the first order phase reaches positive 360 degrees. It then corrects the current data set to the best phase values it found in this mapping process. This process also creates a log to the current data directory.

Phasing using the mouse (PH)

This subroutine allows the user to adjust the zero-order and first-order phase correction using the 2 mouse buttons. Users with a single button mouse should hold down the shift key, then press the mouse button when instructions call for use of the right mouse button.

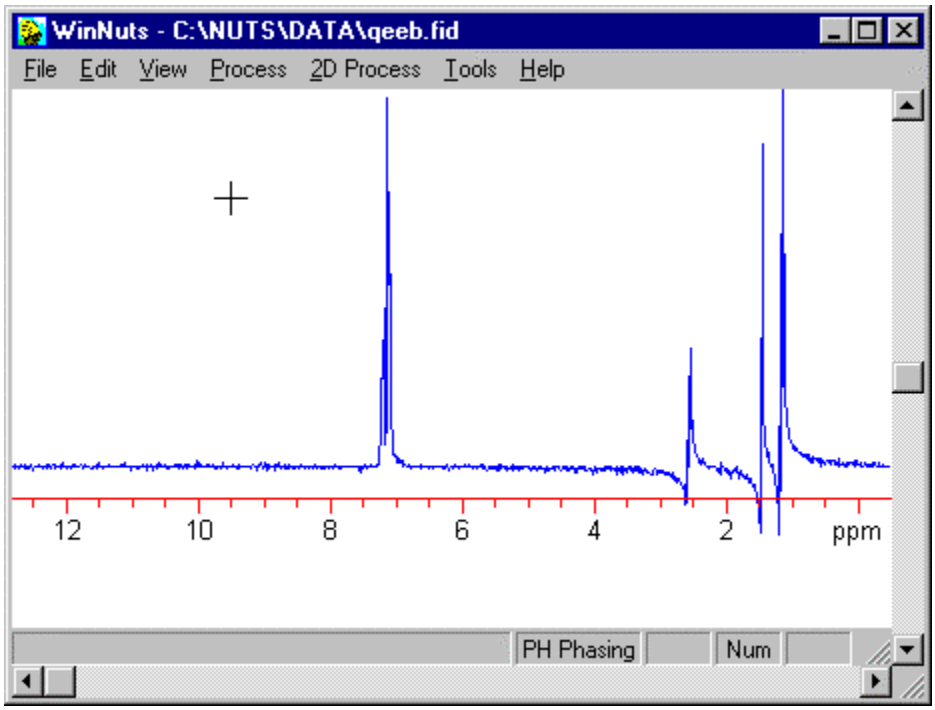


Start with a spectrum that requires phasing. The first step is to set the pivot point, which is the point in the spectrum at which the first-order phase correction is zero. A peak located at the pivot point can be phased with only the zero-order phase.

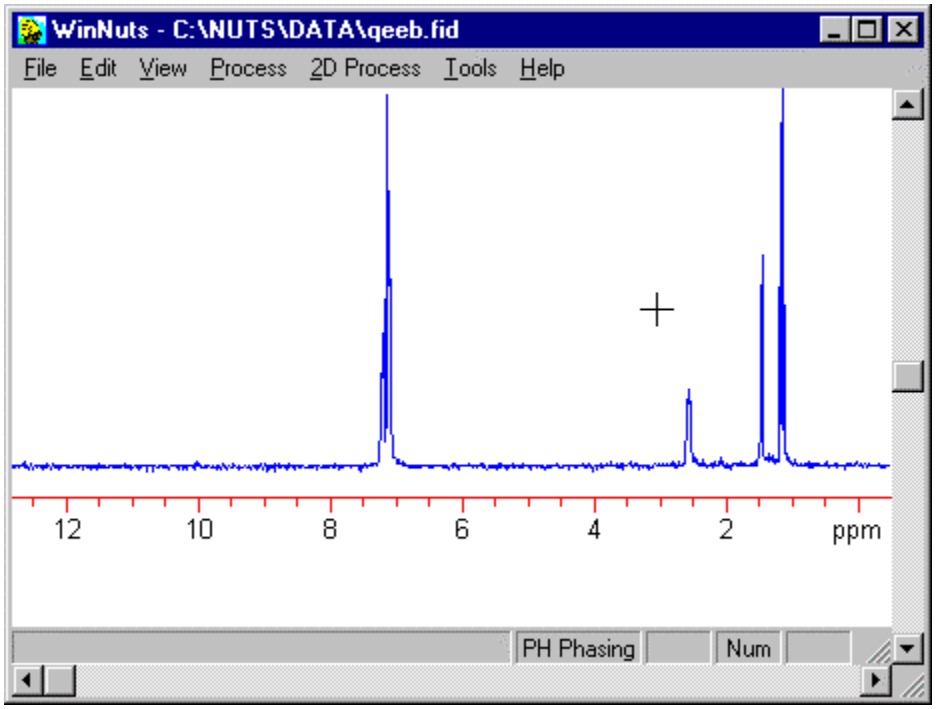


To set the pivot point:

1. Press and hold the left mouse button to display a full-window cross-hair cursor
2. Place the cursor on a chosen peak (on the aromatics in this figure)
3. While still holding the mouse button down, type **P** on the keyboard to set the pivot point.



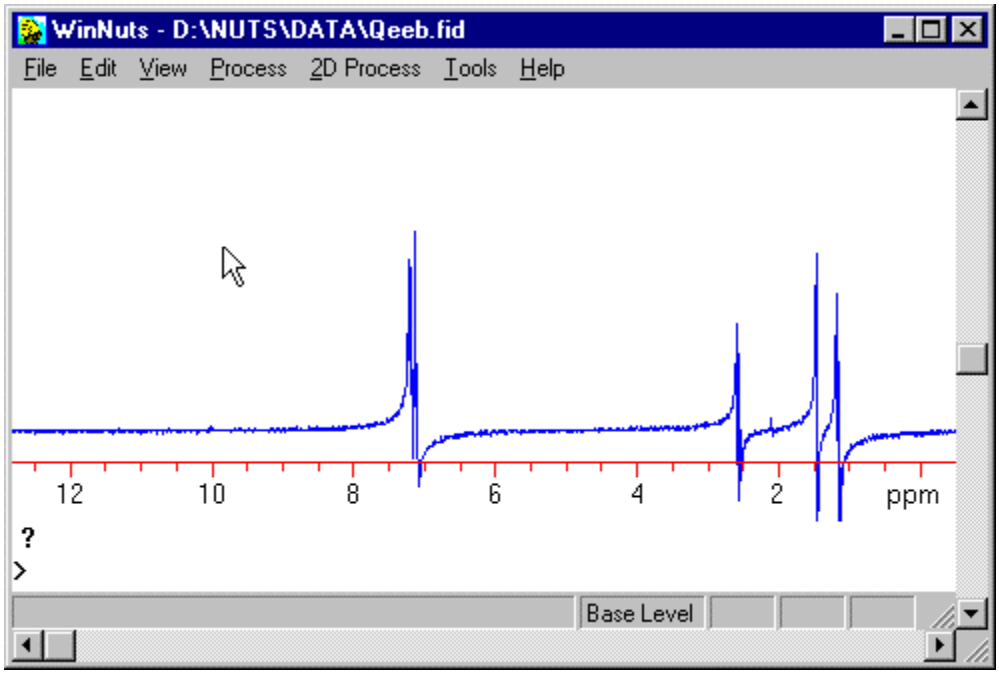
Enter the PH routine by typing PH or from the Process/Phasing menu. Adjust the phase of the peak at the pivot point by holding down the left mouse button and moving the mouse left and right.



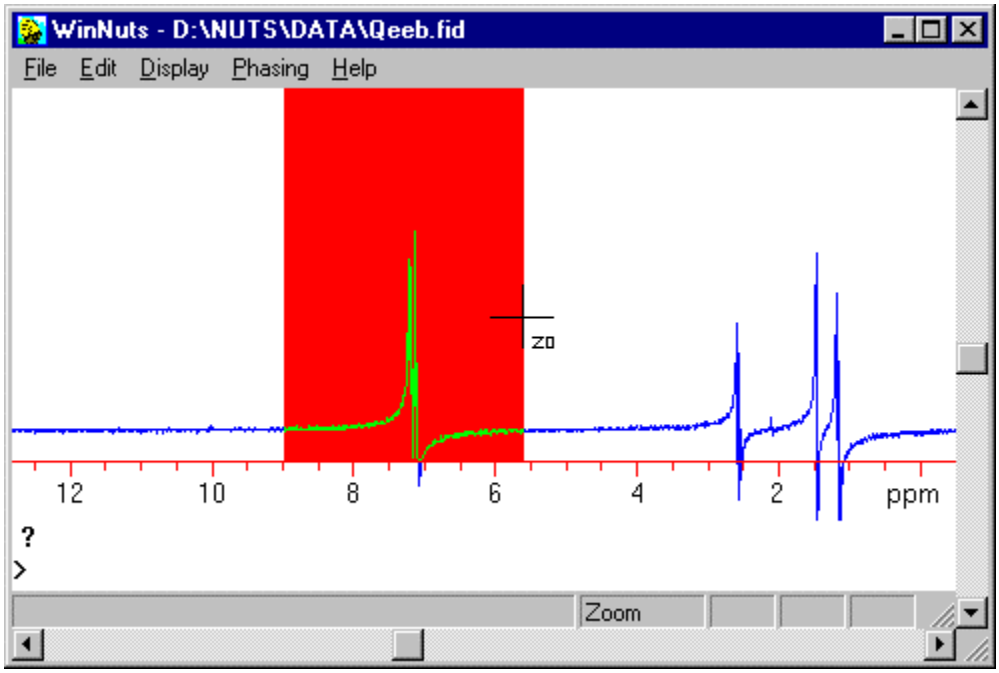
When that peak is correctly phased, phase another part of the spectrum by holding down the right mouse button and moving the mouse left and right. You can go back and forth as necessary to adjust both regions of the spectrum. Exit the PH routine and apply the correction by typing <ENTER>.

Phasing with expanded display (PE)

This routine allows adjustment of phase using an expanded display of 2 spectral regions to adjust the 2 phase parameters. The expanded display can provide a more detailed view for slight phase adjustments.



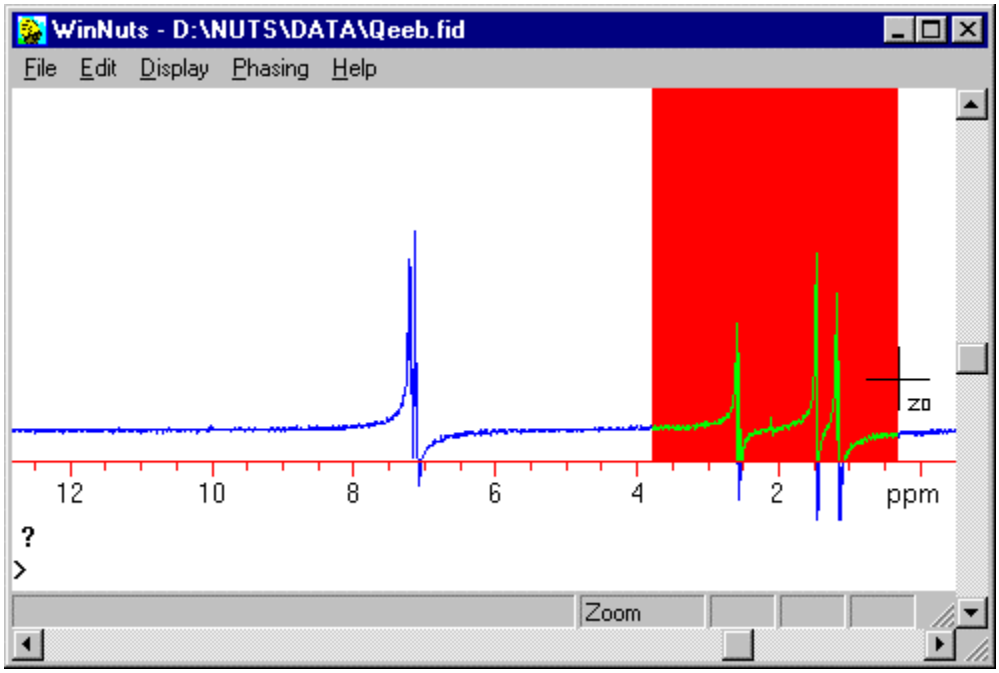
Starting with a spectrum which needs phasing, the first step is to define 2 regions which will be used for phasing.



Enter the Zoom routine and select a region toward the downfield (left) end of the spectrum.

Select by holding down the mouse button and dragging. Do not expand to these display limits.

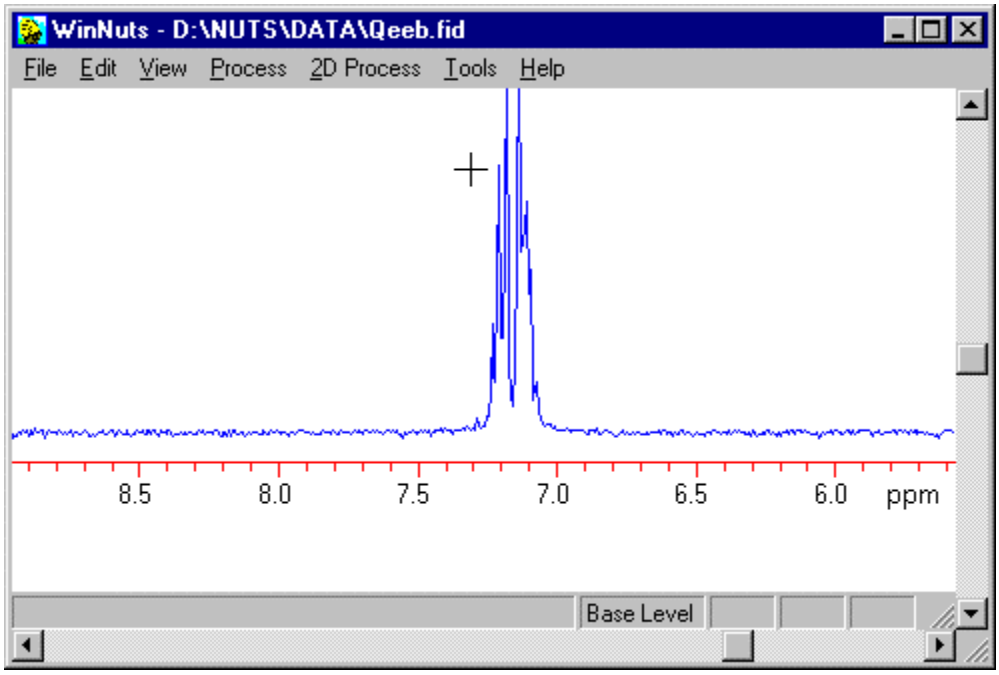
Type 1 to assign this to be region 1.



Choose a second region near the other end of the spectrum, again by dragging while holding down the left mouse button.

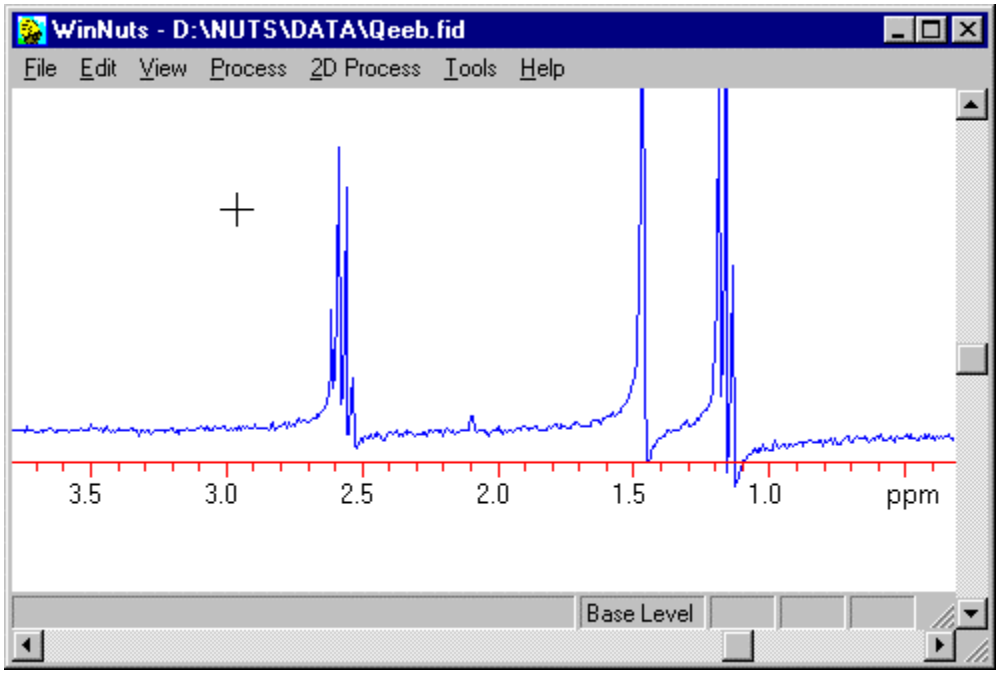
Do not expand to these limits.

Type 2 to assign this to be region 2.



Enter the PE routine by typing PE or from the Process/Phasing menu. The pivot point is automatically set to the tallest peak in this region. (This is the point at which the linear correction is zero.)

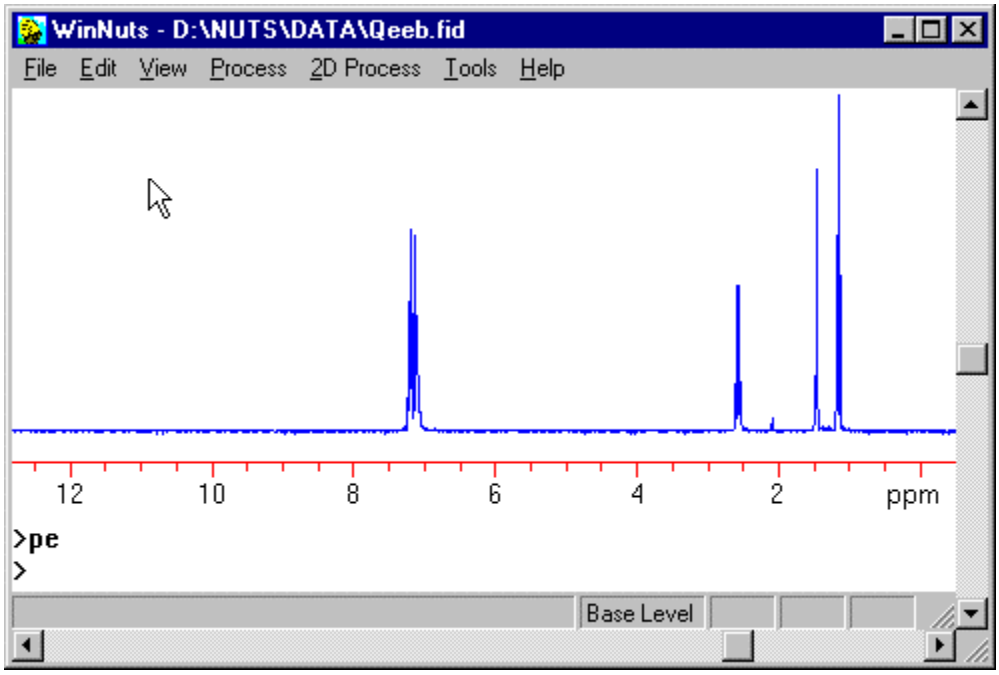
Press and hold the left mouse button. Region 1 is displayed, and its phase adjusted by moving the mouse left and right while holding down the mouse button. This applies a zero-order correction to the entire spectrum.



Once Region 1 is phased, release the left mouse button and press and hold the right mouse button. (Users with a single-button mouse should hold down the shift key then press the mouse button when right mouse button operations are called for.)

When the right mouse button is pressed, the display jumps to Region 2. Phase this region by holding down the right mouse button and moving the mouse left and right.

You can go back and forth by alternating pressing left and right mouse buttons, until phasing looks correct for both regions.



Exit the phasing routine by typing <ENTER>.

The entire spectrum is displayed, with the phase correction.

Baseline correction

Factors affecting baselines

The more information a spectroscopist tries to extract from a spectrum, the more important a flat baseline becomes. Examples of areas where baseline distortion creates unwanted difficulties are:

Small signals in the presence of larger signals
Spectral integration
Difference Spectra
Water Saturation
2D experiments, especially NOESY

In some cases, "baseline distortion" can arise from real NMR signals. Broad signals, which can be wider than the observation sweep width, create "distortion". Examples of this are probe background, wide-line ^{2}H spectra of solids or ^{31}P surface coil spectra. When these special cases of "real signal" are identified and eliminated from the discussion, there are still many cases where instrumentally induced baseline distortion complicates data interpretation. Examples of sources of these instrumental baseline distortions are:

Digitizer overload (Clipping)
Preamp and/or receiver overload
Audio filters
Slow recovery of RF stage from overload
Probe acoustical ringing

These are difficult problems to diagnose and often more than one problem is present at a time. The approach is to isolate each variable as much as possible and repair or replace the offending module or find a work-around solution. An easy one to find and eliminate is clipping of the digitizer. Every spectroscopist knows how to detect and eliminate this one, but let's use it as an example of a particular type of baseline distortion which arises from "overloading". Using a simple sample such as Ethyl Benzene while observing protons, when the digitizer is not overloaded, the spectrum has a normal straight baseline. If the signal amplitude is increased to the point where the first few points of the FID are clipped, the signals will appear to sit in a shallow "hole" in the baseline. The more the signal is clipped, the deeper the hole.

Other areas of the spectrometer's receiver can "clip" or overload besides the digitizer. When there is too much signal for an amplifier or mixer to handle and remain in its linear range, the high amplitude areas of the FID are suppressed relative to the low amplitude areas. This creates an amplifier "clip" which results in a baseline distortion similar to digitizer clipping. Another problem with this type of overload is the creation of "extra" peaks in the spectrum by intermodulation mixing of signals from the probe. For the simple case of two strong signals in the spectrum with this type of overloading, the two signals will mix to yield sum and difference signals as well as the main signals. This can be easy to see with wide sweep widths when the signals are confined to a small region near the center of the spectrum. In other cases, it is more confusing since the signals can "foldback" from outside the spectral window and not appear to be either a sum or a difference. But for this discussion, the main area to notice is the shallow well around the peaks. This type of RF overload occurs most easily when running strong samples such as proteins in water. Very slight RF or digitizer overload has been used to create a very slight "hole" around the residual water signal to reduce the water "tail" in water saturation experiments.

Another type of baseline distortion arises from the distortion of the first few points of an FID. This can come from probe acoustical ringing or, more commonly, filter distortion. If the first few points of the FID are distorted, then during the mathematical processes of Fourier Transform and phase correction, a baseline roll is introduced. When there is one large signal (water) in the spectrum, its small distortion leads to a very curved baseline when examined at the amplitude of most of the interesting spectral peaks.

To minimize this problem, it is best to use a good Butterworth filter set at least 50% wider than the acquired spectrum. A Bessel filter has a better impulse response and produces even less distortion but with it's more gradual rolloff there is a slight reduction in signal to noise.

Either with the proper audio filter or without, a reduction in the baseline curvature can be obtained by adjusting the time between the end of the pulse and the digitizer trigger (DE in Bruker, GE and Nicolet terms) such that the B (first order) phase correction is near zero. For an FID with a distorted first point, this will reduce the mathematical outcome of phasing to a DC offset of the baseline, which is easier to correct. If the digitizer does sequential, rather than simultaneous, data acquisition for each channel during quadrature detection (some Brukers), the transmitter phase also needs to be adjusted to give an A (zero order) phase correction near zero to achieve a similar result.

Linear baseline corrections

NUTS has several commands for correcting baselines that need only DC correction and/or linear tilt. (DC refers to an offset of the spectrum's baseline from true zero that is not dependent on frequency; in other words, the entire spectrum has the same offset.) In most cases, the baseline also has curvature, so linear correction is not sufficient, and a polynomial correction is necessary.

BC -- Baseline Correction of the spectrum

If the data is frequency domain data (spectrum), this command averages the first and last 64 points of the real half of the data and removes the DC bias and linear tilt between the start and end. This may or may not be sufficient to give good integration.

Note that this command acts differently if the current data is time domain data (an FID).

BF -- Baseline Flatten

This removes the DC component of the baseline and any linear tilt within the Zoom frequency limits. This is done by averaging points at each end of the zoom region, the adjusting so that these average values are at zero. The user can set the number of points to be averaged (with 2A) or use default values.

Data outside the Zoom frequency limits are adjusted, with DC correction only, to remain continuous with the Zoom region (see also FR). Note that the display does not need to be expanded to apply BF. The region just needs to be defined with the cursor or with Zoom subcommand **F**. The same operation as BF can be performed within the Zoom subroutine using the **B** subcommand.
See example of BF application below.

FR -- Flatten Region

This command is almost the same as **BF**, with one exception:
In contrast to BF, data outside the Zoom frequency limits is not changed. This can result in discontinuities in the baseline at both ends of the Zoom region. One situation in which this is preferable is when baseline correcting and integrating selected regions of a spectrum. The FR command can be used to correct a chosen region, without affecting

integrals in any other part of the spectrum.
See example of FR application below.

2A -- Number of points to average

Lets the user control how many points are used to by the BF and FR commands. An entry of zero lets the program automatically determine the number of points to average, according to the following:
If the selected region is more than 256 points then 16 points are used for averaging.
If the selected region is less than 256 points and greater than 64 points then 8 points are used for averaging.
If the selected region is less than 64 points and greater than 16 points then 2 points are used for averaging.
If the selected region is less than 16 points then the end points are used directly without averaging.
If the number of points set by the user is greater than the entire selected region then the entire selected region is averaged.

This can be set in a macro using **Set Points_to_average**.

BR -- Baseline correct Bruker digitally filtered data

This command performs an operation similar to BC, but ignores the extreme ends of the spectrum when choosing points on which to base the correction. This is needed with digitally filtered data, due to the distortion of the ends of the spectra. By default, 2% of the data points at each end are ignored. See details below, including description of additional options.

JE -- Baseline correct JEOL digitally filtered data

This performs the same operation as the BR command, but discards 5% of the data points at each end, instead of 2%, as with BR.

See also: TJ, /2

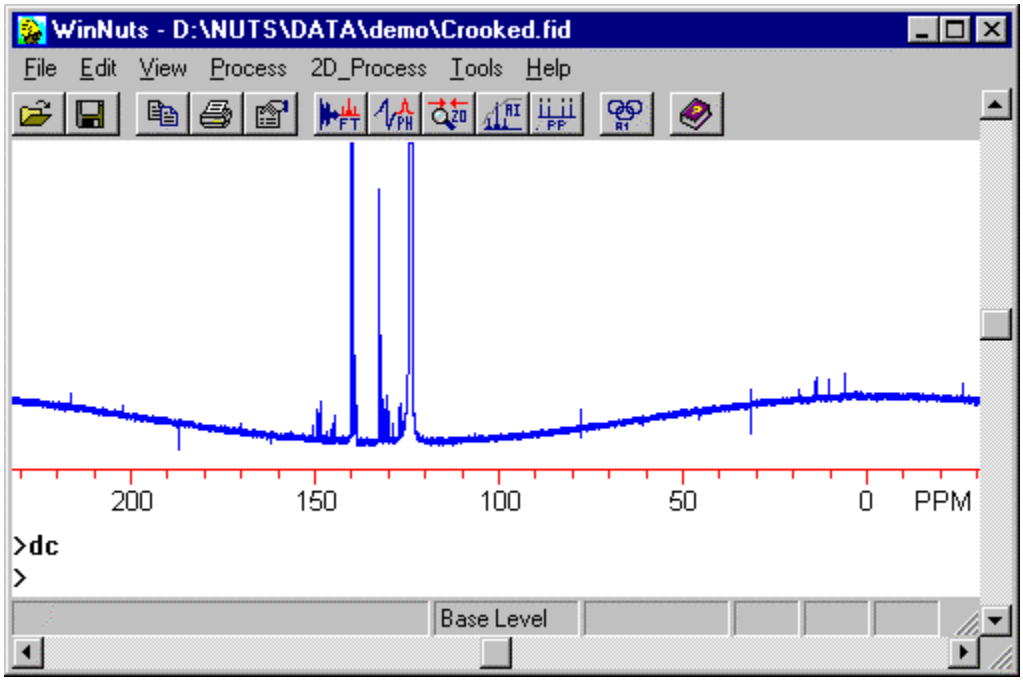
Correcting selected spectral regions

Baseline correction with BF and FR

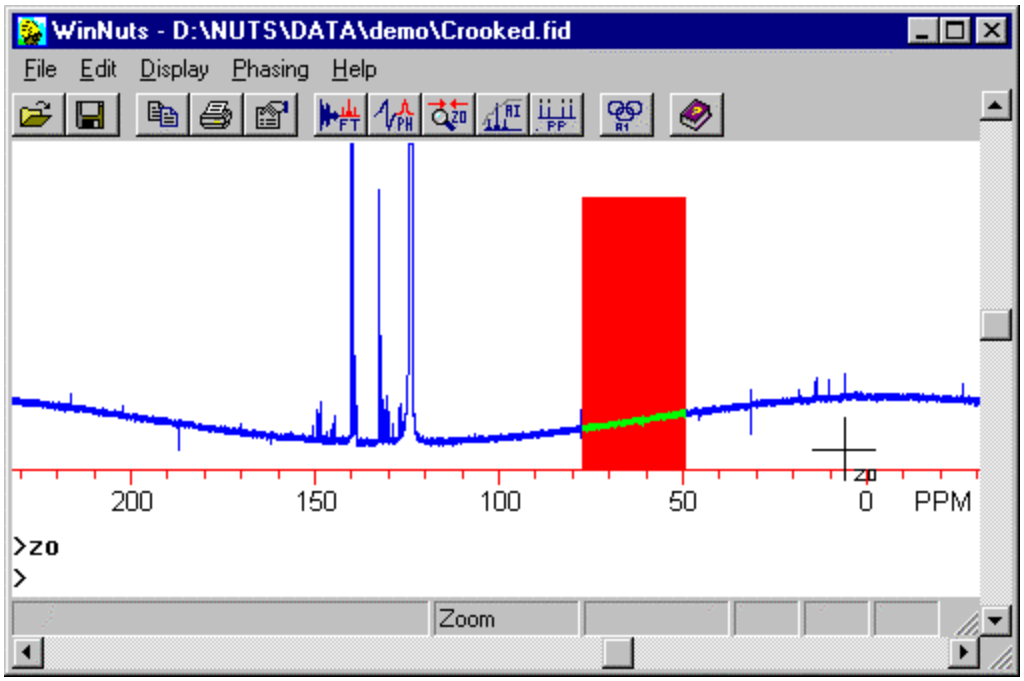
It is important to understand the differences among the choices for baseline correction. The page illustrates the results of 2 similar commands, BF (Baseline Flatten) and FR (Flatten Region). Both remove DC and linear tilt within the current Zoom limits, but they differ in how the remaining parts of the spectrum are affected.

Both commands act by adjusting the Zoom region to bring both ends of the region to zero. FR does not alter parts of the spectrum outside the chosen Zoom region. This can

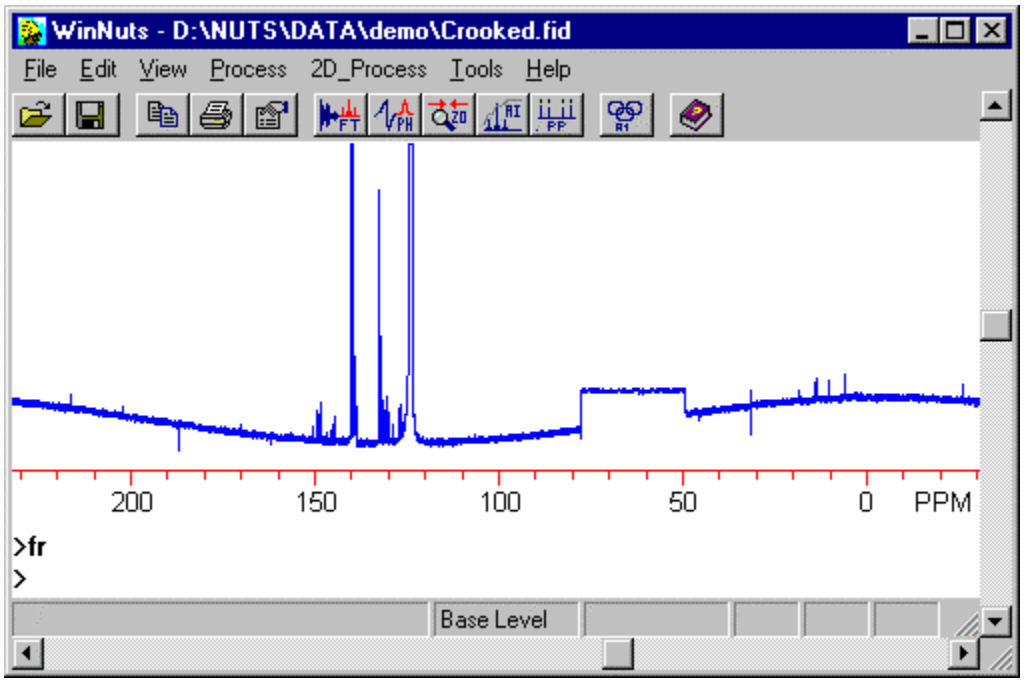
result in severe discontinuities at the ends of the Zoom region. BF avoids these discontinuities by applying a DC offset to the parts of the spectrum on each end of the Zoom region, such that the baseline remains continuous. It is important to understand that applying correction with BF will change the baseline of the entire spectrum, which will affect integrals.



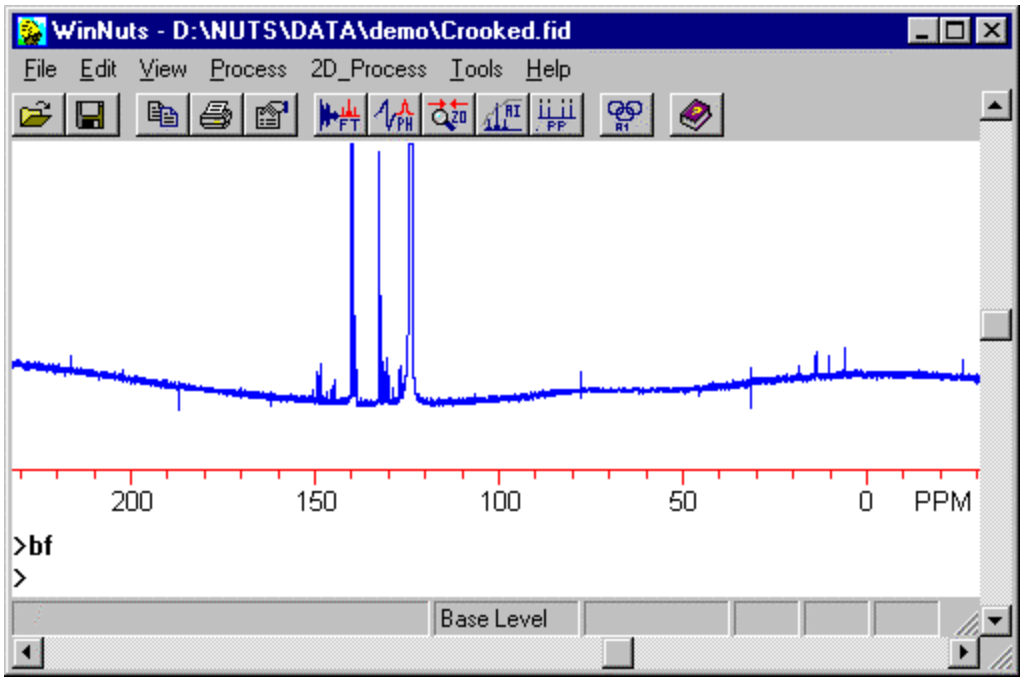
Start with a spectrum with a severely rolling baseline.



A small section of the spectrum is selected with Zoom. (Note that it is not necessary to expand display to these Zoom limits.)



Results of applying FR.



Results of applying BF. (Same spectrum and same Zoom limits.)

See also:

2A – To Average – This allows the user to specify how many points are averaged at each end of the Zoom region to determine the adjustment required to flatten the chosen region.

FB – interactive polynomial baseline correction

QB – quick (non-interactive) polynomial baseline correction

BC – Baseline Correction – removes DC and tilt of the entire spectrum.

Polynomial baseline correction

This section describes various options for correcting a curved baseline.

Sometimes, only a linear correction is required - see options.

In addition to linear and polynomial corrections, backwards linear prediction, which corrects distortions of the first few points in an FID, can be used to flatten the baseline. The baseline can also be corrected from inside the integration routine, using the subcommand **B**. See tips for correcting really bad baselines.

FB -- Fit Baseline

This command enters a subroutine which fits the baseline with a 5th order polynomial. As a starting point, NUTS selects a set of baseline points as follows: The spectrum is divided into 64 regions and those regions which contain just noise are selected. The data points within each selected region are averaged to give one point for input into the polynomial fit. The 64 selected regions are displayed in inverse video, usually red. The decision as to what constitutes noise is based on the RM multiplier value (same parameter as is used for peak picking). If the user finds that the regions being selected as noise are not appropriate, the RM value may be changed. The user can also select or un-select individual regions using the mouse. Place the cursor on a region of the baseline to be selected and click once with the left mouse button. This is a toggle process, so clicking on a selected region will un-select it. The **S** subcommand selects all regions, and the user can then un-select regions containing peaks.

By default, the FB routine divides the spectrum (or expanded region) into 64 regions (or fewer, if very few data points are displayed). It is possible to override this, and specify the number of points in each region. This can be used to define narrower baseline regions, useful when the spectrum is very crowded, with only small segments of baseline between peaks. This is done in the non-2-letter command mode. The FB command will accept either one argument, or 2 arguments. The first argument specifies the number of data points in each region. If there is a second argument, it is a multiplier for the RM parameter, used in the automatic determination of which regions contain peaks, and which are baseline.

If an expanded region is displayed at the time the FB subroutine is entered, the fit will be confined to only that part of the spectrum. This allows a spectrum with very a distorted baseline to be corrected in sections. Be sure to execute a **Ctrl-F** first if the fit should be to the entire spectrum.

For historical reasons, 2 options are available for calculating the best fit polynomial: Simplex and Least Squares. Results from the 2 methods appear to be indistinguishable, and the least squares method is more than 10 times faster. However, sometimes the least squares method fails to converge, and simplex can be used instead.

To initiate the fitting process, type **C** for Simplex calculation or **L** for Least Squares, both of which are available from the Edit menu. (Note that if Enter is typed before initiating a fit, the routine is exited without altering the baseline.) The equation used is a 7th order polynomial whose coefficients are adjusted to fit the spectrum. As the Simplex optimization proceeds, the results of each cycle are displayed. The Error is the square of the difference between the calculated curve and the curve formed by the set of input points. This is displayed so that the user can monitor the process as the Error function converges to a minimum. The iteration can be stopped by typing **Q**. The coefficients of the calculated polynomial are printed on the screen at the end of the fitting operation.

The calculated polynomial can be displayed by selecting Draw Polynomial from the Display menu or by typing **P**. The region selection can be changed and the fit recalculated by repeating the fit (C or L command).

When satisfied with the calculated baseline, typing Enter will apply the correction and exit the FB subroutine. To abort the process and exit without altering the baseline, choose Quit from the file menu or type **X**.

If the correction is made on a part of the spectrum, rather than the entire spectrum, there arises the question of what should be done to the rest of the spectrum. If no adjustment is made to the data outside the displayed region, discontinuities may be introduced at the ends of the corrected region. A logical way to address this is to apply a DC correction to the adjacent regions to avoid discontinuities. However, that would result in changing the baseline, and therefore to the integrals, in parts of the spectrum other than the selected region. The best choice in this case depends on the characteristics of the spectrum and the information being extracted from it, so NUTS allows the user to choose how this situation should be handled.

The option to keep the baseline continuous, and avoid discontinuities, can be set in the nuts.ini file, with the following entry:

CONTINUOUS_BASELINE = TRUE

To prevent any changes to the spectrum outside of the displayed region, use the following entry in the nuts.ini file:

CONTINUOUS_BASELINE = FALSE

Changes to the nuts.ini file do not become effective until NUTS is restarted. To change this option while NUTS is running, the following non-2-letter commands can be used:

FB -c on

enables the DC adjustment of the baseline to eliminate discontinuities.

FB -c off

disables the DC adjustment of baseline, so that regions outside the current display region are unchanged.

These settings apply to both FB and FX.

It is possible to save a "mask" describing which regions of a spectrum should be used in calculating a polynomial baseline correction. This would be used for automated processing of very similar spectra. The user selects the regions in the usual manner, then saves those selections to a text file with the sub-command **M** or by selecting File/Save Mask on the menu while in the FB routine. The mask is then applied with the FX command.

If FB is used in a Link or macro, the automatically selected regions are used, the fit is calculated and the resulting correction applied, all without user interaction. Normally, the type of fit used in this automated mode is polynomial fit using least squares. However, the user has the option of changing this to either Simplex polynomial fit or Fudge mode (equivalent to F subcommand). This is set in the nuts.ini file.

To perform polynomial baseline corrections in arrayed mode, use the FX command. In arrayed mode, the FB command acts only on the displayed slice. This is useful for correcting baselines of selected slices.

FB also has the option of correcting the baseline by forcing each region to be flat by removing DC and tilt separately for each region. This is done with the subcommand **F** (stands for "fudge"). This is useful when the baseline distortions are of too high order to be corrected with a polynomial. See example below.

See details below on how to use FB.

Subcommands:

C Calculate Simplex fit to 5th order polynomial
L Perform Least squares fit to 5th order polynomial
F Fudge baseline by correcting DC and tilt for each region separately
3 Linear spline baseline correction
A Apply default correction and exit FB
M Save selected baseline regions as a "mask" to a text file
R Read a previously saved "mask" file
P Draw polynomial
Q Quit Simplex optimization
S Select all regions
U Un-select all regions
X Exit without applying correction
<ENTER> apply correction and exit

FX -- Fit Baseline

Polynomial baseline correction that works in arrayed mode.

For 1D spectra or when not in arrayed mode, FX is equivalent to QB. It applies a polynomial correction to the displayed slice using default parameters.

When applied to a 2D spectrum while in arrayed mode, FX will execute a polynomial correction independently for each slice.

When in the non-2-letter command mode, the FX command accepts an argument which is the file name of a "mask" file to use when calculating the polynomial fit. The mask must first have been created in the FB subroutine. The mask must be for the same 1D data size

as the current data. If the mask was created while in a zoomed display mode, this command will use the same display region for the polynomial fit and NUTS will end up displaying that zoomed region when the command completes. The mask can be applied to 1D or 2D data.

Why use a mask? More reproducible baseline correction can be obtained, useful in defining a protocol for processing QC data, to insure that the same conditions are used each time. In the case of 2D data, the automatic selection of baseline regions can result in poor choice of baseline regions for some slices, causing serious baseline distortion. The recommended procedure is to examine slices using View (VW) to determine which regions represent "good" baseline throughout the dataset, and then use FB to select those regions and create a mask. The mask is then applied with

2f
fx *maskfile* <ENTER>
2n <ENTER>

where *maskfile* is the file name supplied when the mask was saved to disk.

By default, the "fx mask" command performs a polynomial fit. This can be invoked with any of the following 3 equivalent commands:

FX *mask*
FX –p *mask*
FX –1 *mask*

Optionally, linear baseline "fudge" can be performed instead, identical to the FB routine's **F** sub-command, with either of the following equivalent commands:

FX –f *mask*
FX –2 *mask*

A third option is linear spline correction, identical to the FB routine's **3** sub-command, with either of the following equivalent commands:

FX –s *mask*
FX –3 *mask*

QB -- (or BASELINE) Quick Baseline correction

When executed as a 2-letter command, this performs a least squares polynomial baseline correction, just as would be done within the FB subroutine. There is no user interaction with this command.

In the non-2-letter command mode, QB can take optional parameters and performs a different kind of correction. If no parameters are given, then the routine automatically

selects regions and corrects the baseline by a least squares fifth order polynomial. If other parameters are supplied, QB performs a different type of baseline correction, involving "deducing" a baseline much as the user might do "by eye", and then subtracting that baseline from the spectrum. To do this, the spectrum is divided into segments, and each segment is compared to the rms noise level to determine if it contains peaks.

Use of QB for this type of correction takes up to 3 parameters, as follows:

The first parameter specifies the number of data points for each segment into which the spectrum is divided. (In the default QB and FB corrections, the spectrum is divided into 64 segments). A multiple of rms noise used to distinguish peaks from noise; by default, this is the value of the RM parameter, set in the peak picking routines.

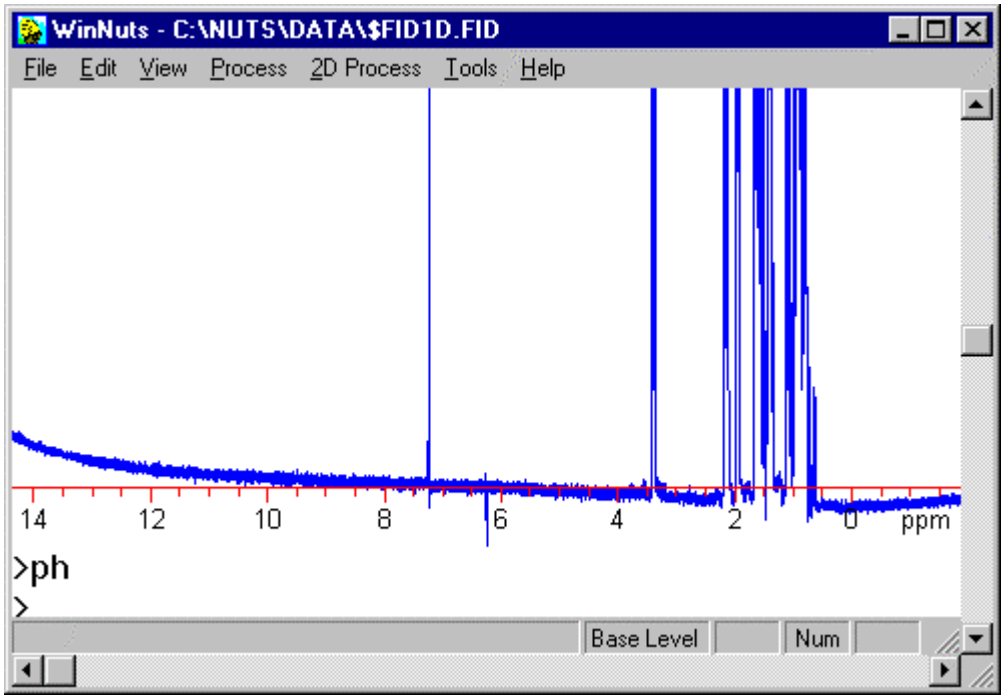
If a second argument is entered, it is used as the multiple of rms noise, instead of using RM, to distinguish peaks from noise.

The only valid value for a third argument is "show", which displays the "deduced" baseline. This action replaces the current data set with the deduced baseline.

This correction is illustrated below.

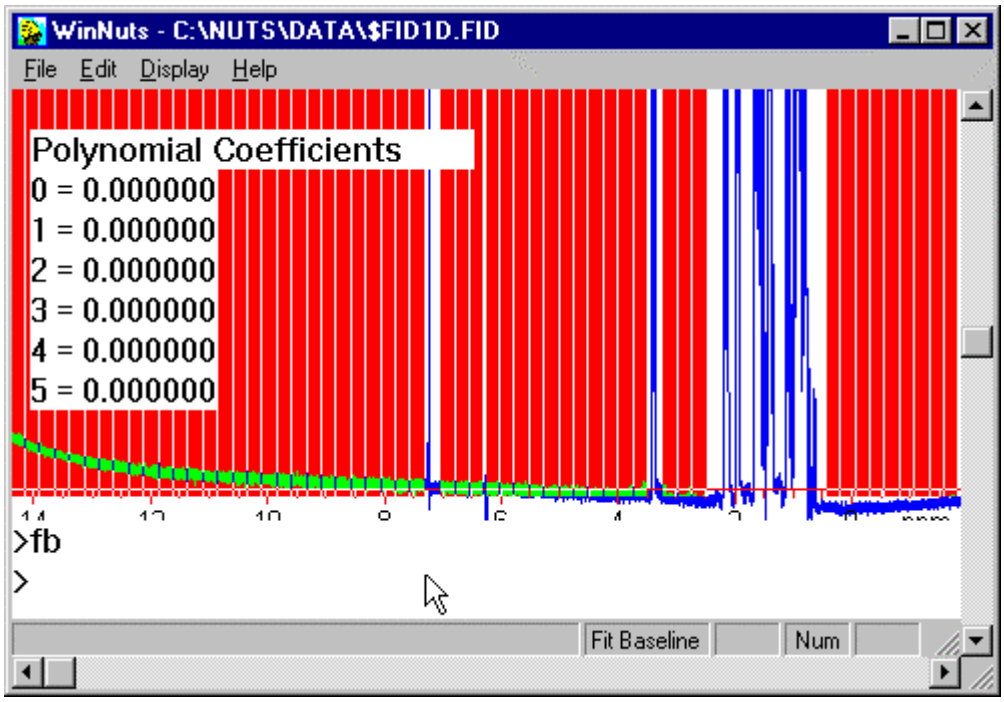
Illustrated example of polynomial baseline correction

This subroutine calculates a 5th order polynomial fit to the baseline.



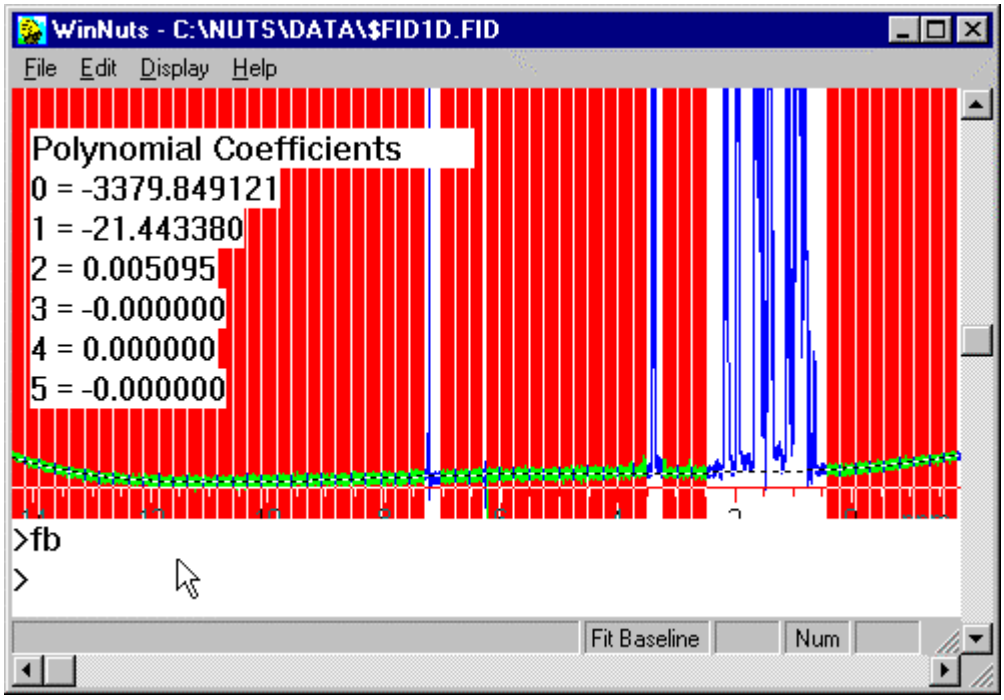
This is the most practical way to correct a baseline which needs more than simple tilt correction.

Type **FB** or select Fit Baseline from the Process menu.



On entering the subroutine, NUTS divides the spectrum into 64 regions. Those regions which consist of only noise are colored red.

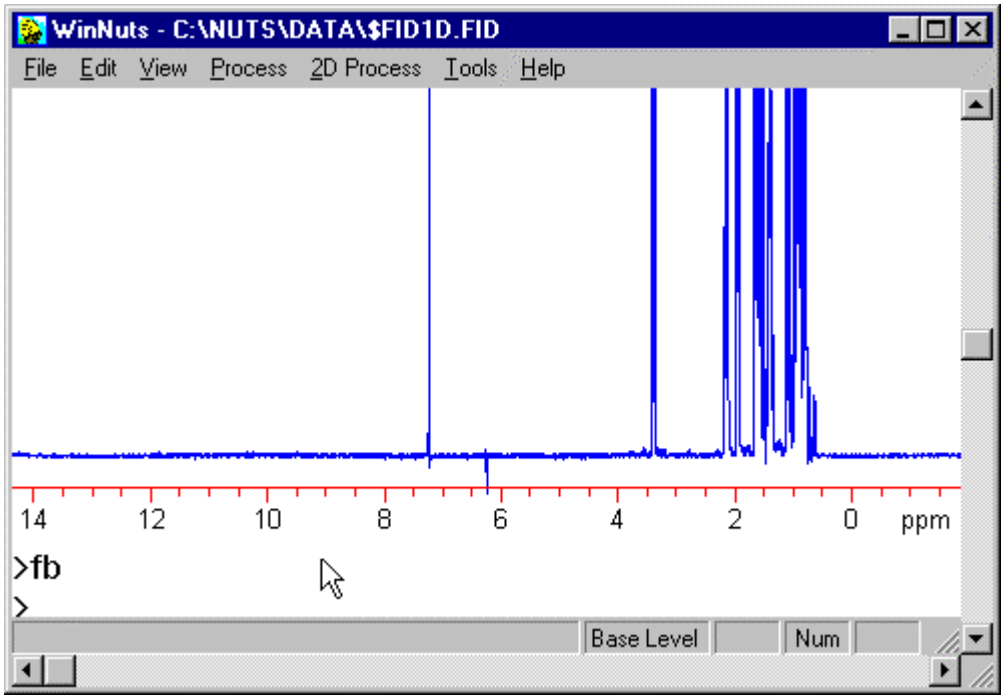
The decision as to what constitutes noise is based on the RM multiplier value (same parameter as is used for peak picking). Regions can be selected or de-selected by clicking with the left mouse button.



Perform a least squares fit by typing **L** or by choosing Calculate Fit from the Edit menu.

The calculated polynomial can be drawn by typing **P** or selecting Draw Polynomial from the Display menu.

If the match to the baseline is not sufficient, try selecting or de-selecting some regions, and repeat the fit.



Exit the routine with <Enter>, which applies the correction.

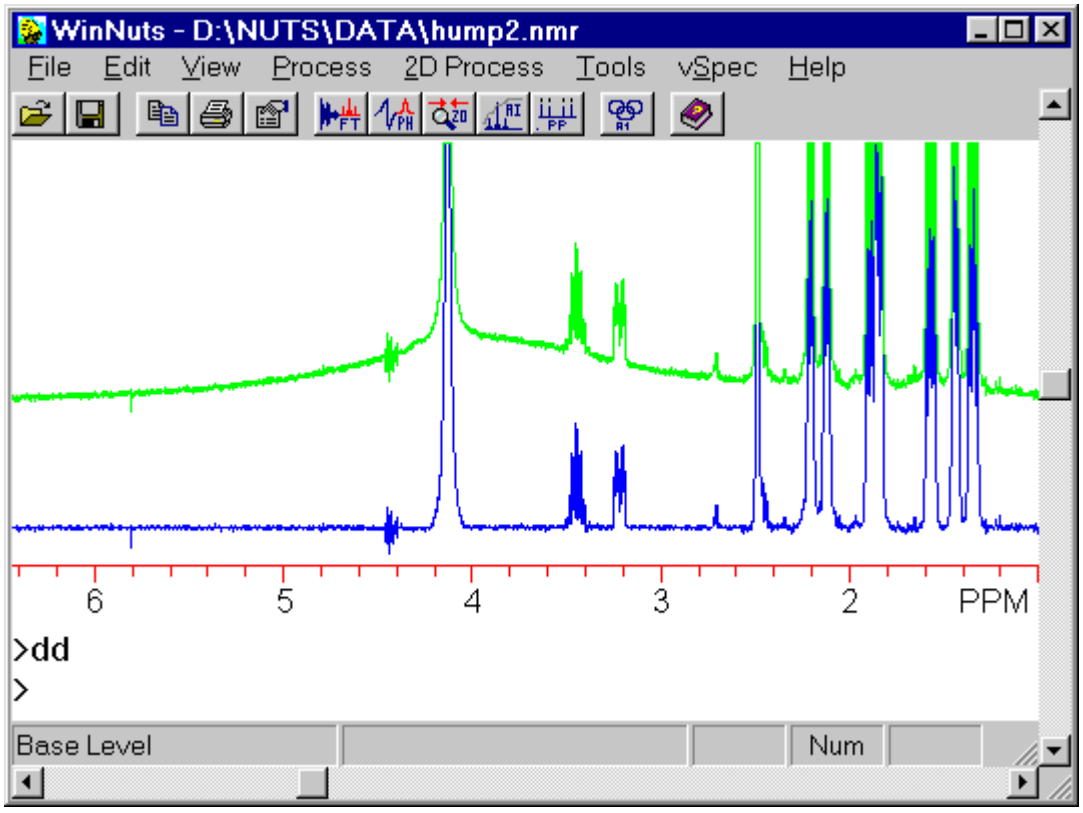
Exiting the routine with **X** quits without applying the correction.

See also: QB -- Quick polynomial baseline correction. This command performs the same least squares fit to a 5^{th} order polynomial, but no user interaction is possible.

Another option which works well in some cases is a "deduced" baseline.

"Deduced" baseline

Some baseline distortions arise from broad peaks due to exchangeable protons or a polymer component in the sample. Even though they are "real", they can be undesirable and interfere with integration. An example is shown here in the top trace.



A person can look at this spectrum and readily deduce where the "baseline" should be.

The same process can be accomplished by software. Some of the steps are similar to the procedure used by the FB routine.

The process involves automatically distinguishing regions of just baseline from regions containing peaks, interpolating where the baseline should go underneath each peak, then subtracting this "deduced" baseline from the spectrum. The process of determining the difference between noise and peaks requires an estimate of the noise level in the particular spectrum. NUTS automatically determines the rms noise of the spectrum. The spectrum is broken into segments, and a determination made as to whether or not each segment contains any peaks. A segment of the spectrum is determined to contain a peak if its (maximum - minimum) value exceeds a defined multiple of the rms noise. By default, Nuts will use as that multiplier the RM value (same as is used in peak-picking), but the user can specify a different value. By default, Nuts divides the spectrum into 64 segments, but the user can specify how many points each segment should contain.

This correction is implemented in NUTS as a modification of the QB command. When issued without parameters, QB applies a non-interactive polynomial correction, equivalent to that performed by FB using default parameters.

To use the "deduced" baseline correction, the user must first place Nuts into the non-2-letter command mode, by typing **2F**. There are 3 optional parameters:
The first parameter specifies the number of points for each segment. The smaller the number, the more segments into which the spectrum is divided. If no other parameter is specified, the current value of the RM parameter will be used to determine which segments contain noise.
A second parameter can be specified, which is the value of the multiplier for the rms noise.

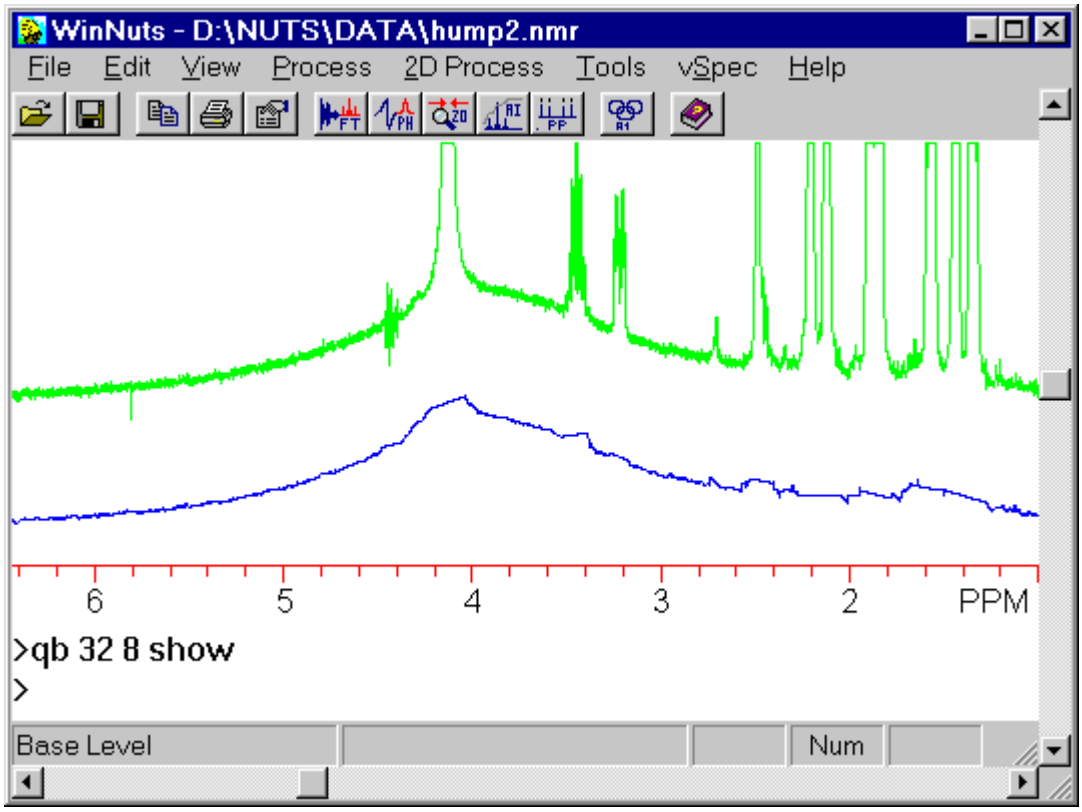
When QB is used with 2 numerical parameters, the baseline is calculated and subtracted, and the current spectrum is replaced by the corrected spectrum. (Users might want to enable Un-Do, to facilitate trial-and-error adjustment of parameters.)

The lower trace in the figure above was obtained using the QB command with the following parameters:

QB 32 8

In this case, each segment contained 32 data points, and segments were determined to contain peaks if their (maximum - minimum) data values exceeded 8 times the rms noise.

To examine the "deduced" baseline itself, the QB command is issued with the optional 3rd argument of "show". The result is shown in the lower trace. The current spectrum has been replaced by this calculated baseline. To apply the correction, the spectrum needs to be opened again and QB applied without the "show" parameter, or use Un-Do to back up one step.



Care in parameter selection is important to avoid distorting the low level lineshapes of peaks that can result in integration errors.

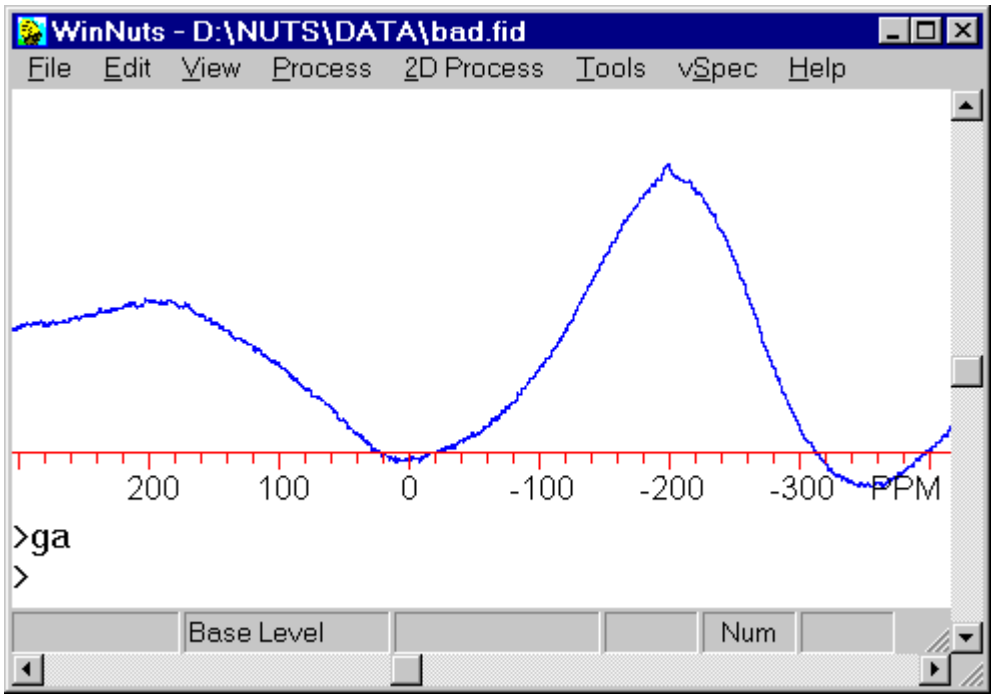
Really bad baselines

Some spectra have baselines that cannot be fit with a polynomial. The options for working with such a spectrum are:

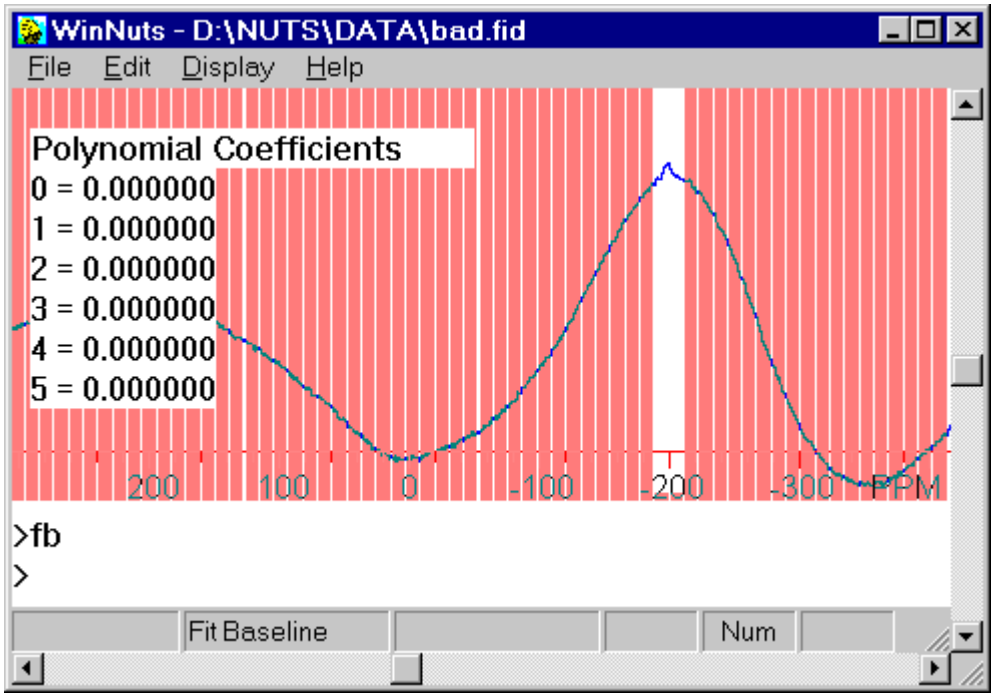
1. Backwards linear prediction to correct the early points in the FID which are causing the distortion. As many as 12 points may need to be corrected. Experiment with parameters to obtain the best results.
2. Divide the spectrum into multiple sections, and apply polynomial correction in the FB routine to each section separately. The baseline curvature within each section may then be of sufficiently low order to be corrected. The best procedure is to select each section of the spectrum using the Zoom subcommand **F**, specifying the sections by points, not by frequency limits. For example, the first section would be point 1 to point n, and the second section would start at point n+1. This should avoid discontinuities at the edges of each section.

3. Humps can sometimes be removed by subtracting a "deduced" baseline. See example in the previous section.
4. Within FB, the **F** subcommand will remove DC and tilt for each selected region separately, forcing each region to be flat. Note that this correction cannot be un-done. This is illustrated below.

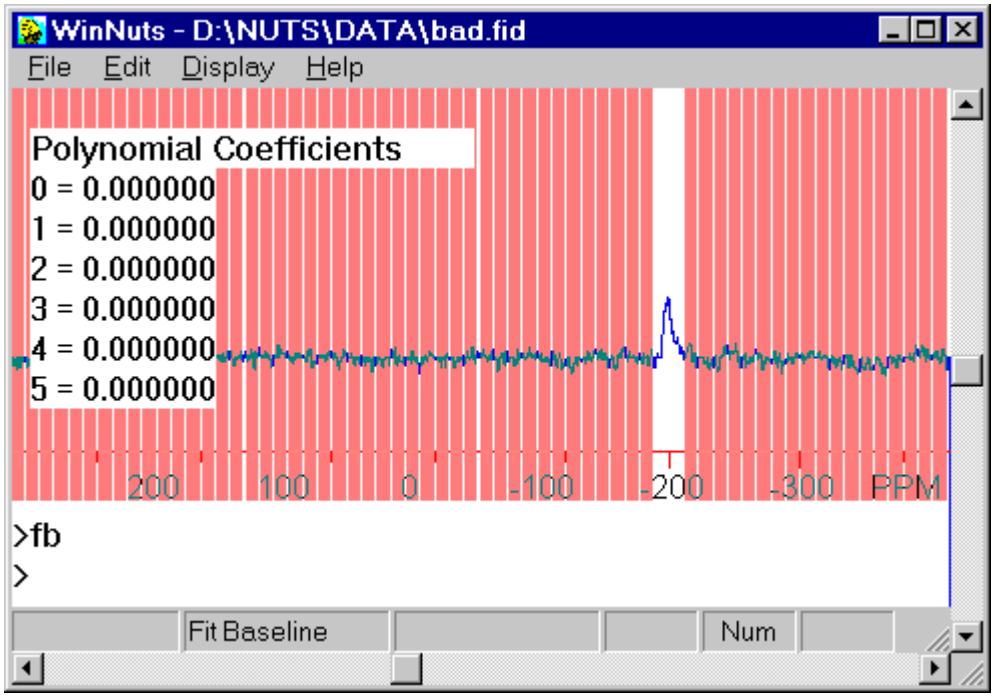
Not all spectra can be corrected with a polynomial, such as the spectrum below. The FB routine includes a "fudge" operation which can be used to improve the situation.



There is a "real" peak at -200ppm.

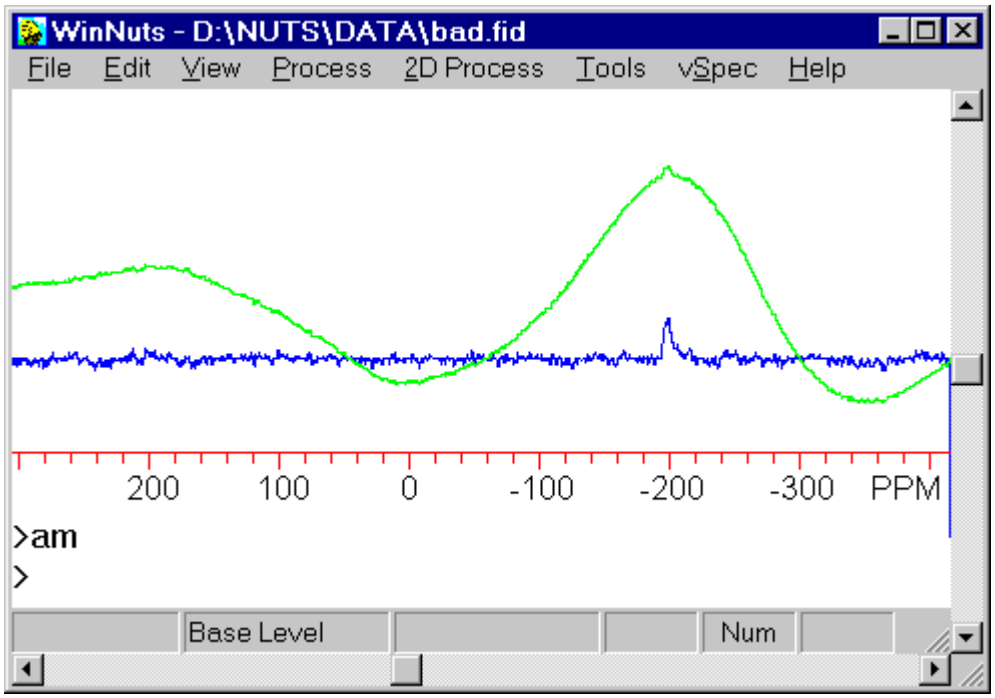


Enter FB, use **S** to select all regions, then un-select the regions around -200ppm.



The **F** ("fudge") subcommand applies a DC-and-tilt correction to each region separately, forcing each to be flat. The un-selected regions are corrected based on adjacent regions, to avoid discontinuities. Once **F** is applied, it cannot be un-done. It can be applied to

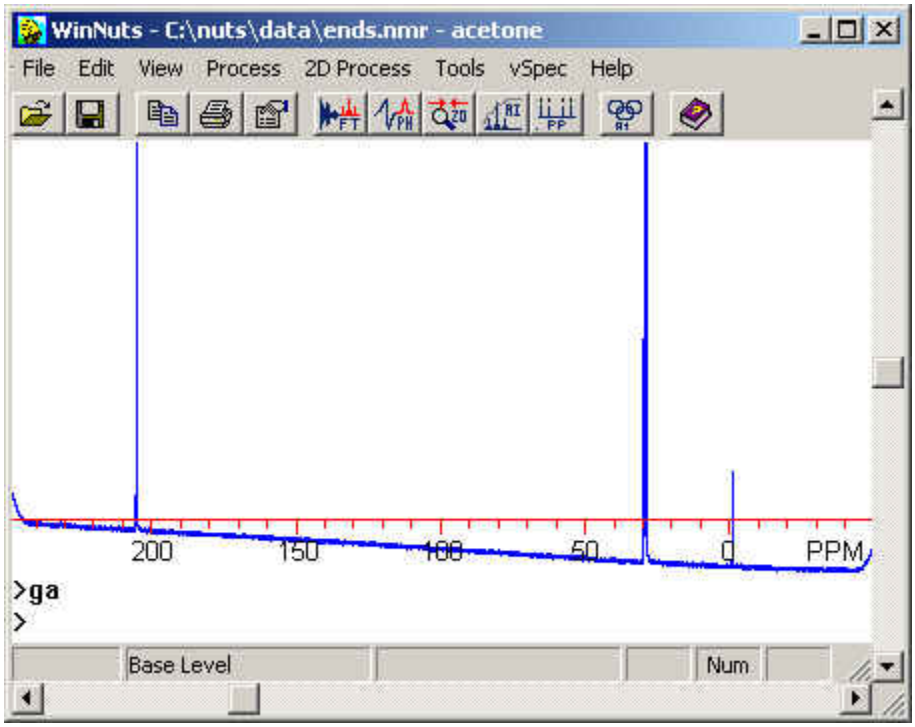
part of a spectrum, but this will create discontinuities at the edges. Note that the results of this type of correction are very sensitive to where the boundaries between selected and un-selected regions fall. Integration of such a corrected spectrum should be viewed with skepticism!



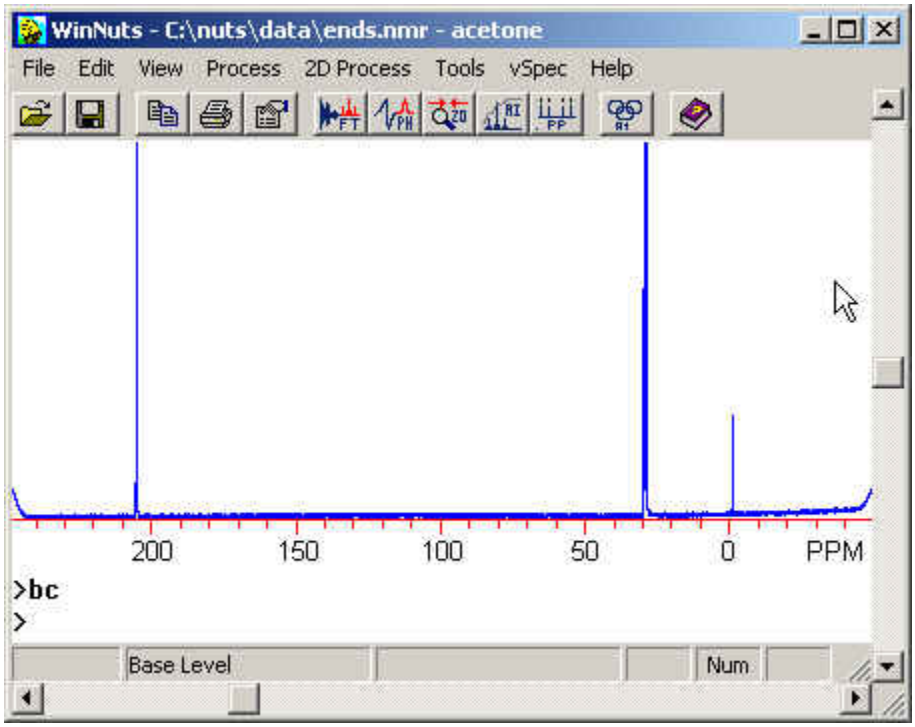
Above is a before-and-after comparison, showing the results of the "fudge" operation.

Correcting baselines of digitally filtered spectra

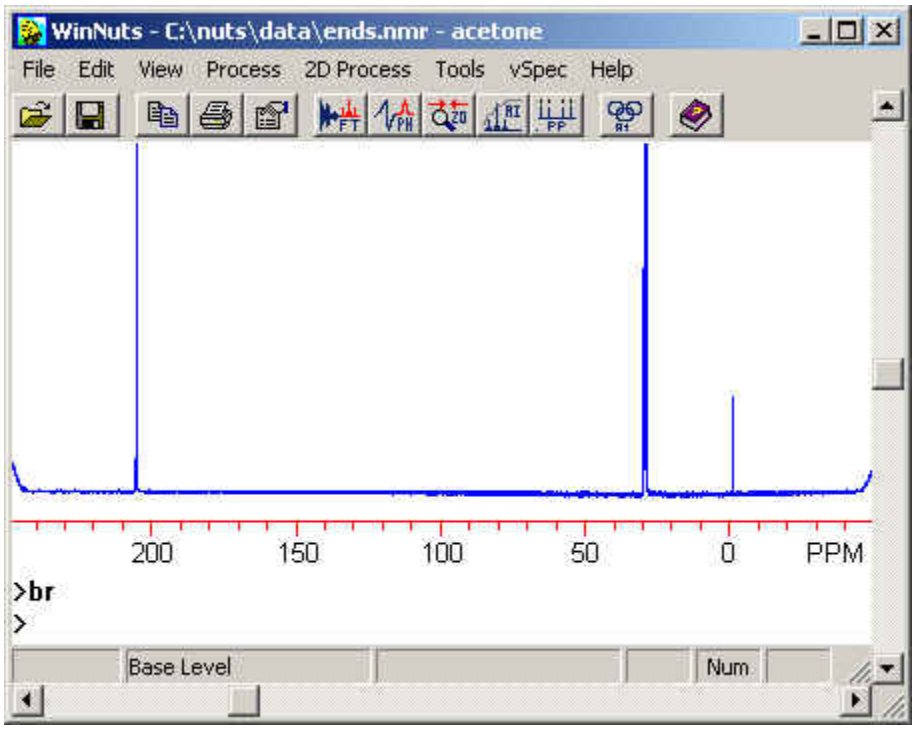
An artifact of digitally filtered data is an abrupt curve at the ends of the baseline.



A tilted baseline, as seen above, is normally easily removed with the BC command, which removes DC offset and linear tilt by assuming that the ends of the spectrum are zero. However, for spectra such as this, the ends are not zero and using BC results in a spectrum whose baseline is not truly at zero, as shown below:



The BR command performs an operation similar to BC, but disregards the ends of the spectrum, so that the correction is based on points that are really zero. The result, seen below, is a much better correction.



By default, the **BR** command (or equivalent non-2-letter command **Bruker**) ignores 2% of the data points at each end. A similar command, **JE** (for Jeol) ignores 5% of the data points at each end.

The command has some additional options that can be invoked in the non-2-letter command mode.

If the number of distorted points doesn't match the 2% or 5% defaults for the BR or JE command, it is possible to specify the number of points on each end to be ignored. This requires 2 arguments: the first is "pts" and the second is the number of points to be ignored. For example, to ignore 350 data points at each end, the command would be

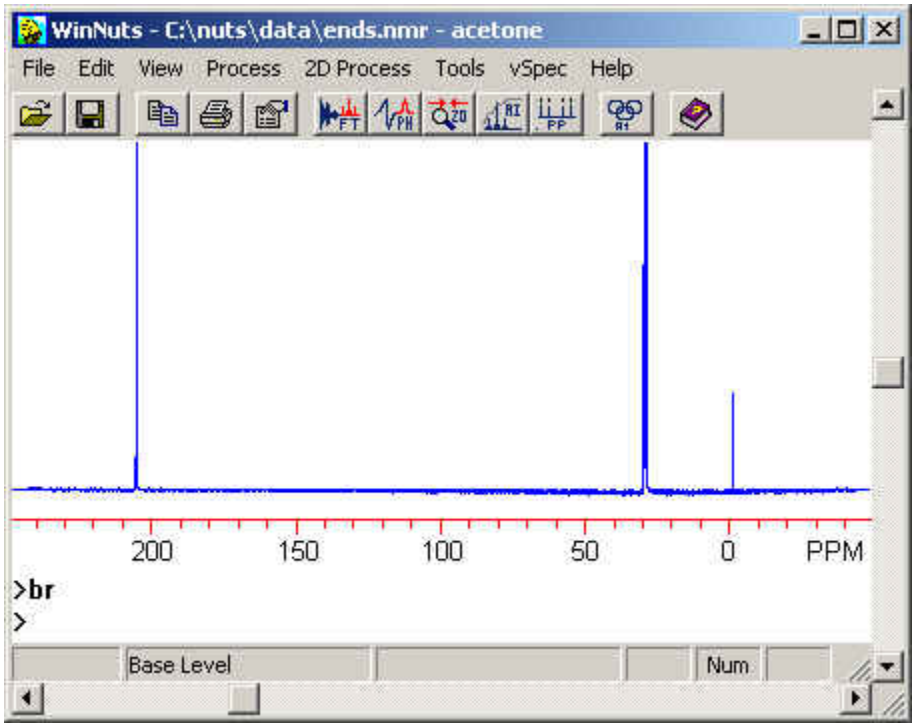
br pts 350

It is also possible to replace the distorted end points with zeroes. This requires 2 arguments: the first is "ZeroPts" and the second is either 1 (to turn ON zeroing of the end points) or 0 (to turn OFF zeroing of the end points).

To choose to zero the ends points,

br zeropts 1

This command does not change the spectrum, it simply selects the zeroing option for subsequent BR commands. The result of the BR command with zeroing turned on is shown below:



Once zeroing of the end points is turned on, it remains on for subsequent BR (or JE) commands until turned off.

It is also possible to discard a specified number of data points at each end of the spectrum, using the /2 command.

Integration

Overview

ID – Enter Integration subroutine

NUTS includes a manual integration subroutine and an automatic integration function. This Overview is a description of the former. It is recommended that users read through this section, as the manual tools are often useful to supplement the automatic method. A summary of subcommands active in the integration subroutine is given below. See details below on how to integrate.

The currently displayed region can be integrated with the menu selection Process / Integrate, with the **ID** command from the NUTS base level or with the Zoom subcommand **I**. After entering the integrate subroutine, the displayed region will have the standard NMR integration line. The first time the integration routine is entered, the integral extends over the entire spectrum and is automatically scaled such that the integral will be full scale at the end of the display. Any changes which are made in scaling or by defining sub-integrals are retained when the integration routine is exited and re-entered. On entering the integration routine, the menu choices change to display commands which are active. All commands can be accessed using the menus, with the exception of those which involve using the mouse. The commands are also available as single-letter keyboard commands.

Adjusting the integral scale -- The scroll bar on the left can be used to adjust the vertical scale of the displayed integral. If the integral display goes off the top of the display, it will wrap around to the bottom of the display. Note that changing the vertical scale of the integral trace does not change the integral values.

While in the ID routine, the **Z** subcommand brings up another scroll bar on the left to adjust the beginning level of the integral. A single <Enter> exits this offset adjustment routine.

Plotting can be done from within the integration routine (P subcommand, or File/Plot menu selection). Whether plotted from within the integration routine, or from the NUTS base level, the plotted spectrum will have the integral plotted on it as it appears on the screen.

The ID routine is exited with the <Enter> key.

Defining separate sub-integrals -- Certain of the integral routine commands are initiated by clicking the left mouse button to display a vertical cursor. For example, the integral trace can be broken into sub-integral regions of interest using this approach. Click the left mouse button once to produce the vertical cursor, click a second time at the beginning of the region to be integrated, and click a third time at the end of the region of interest to complete the sub-integral. Repeat as needed. Once a single sub-integral has been defined, the integral is only displayed for defined sub-integrals. To toggle between this display and the entire integral, type **F**. The sub-integrals are not lost on exiting the integration subroutine.

Setting integral values -- The value of a chosen sub-integral can be set to a user-defined value. Place the cursor on one of the sub-integrals (by clicking once with the left mouse button) and type **V**, which brings up a dialog box in which you can set the value of the selected sub-integral. Once a sub-integral value has been defined, each sub-integral is labeled with its value. The integral values remain unchanged until a sub-integral is explicitly set to a new value, regardless of changes in the vertical scale of either the integral trace or the spectrum. Setting the value of a sub-integral to zero removes all labels.

The integral labels can be displayed vertically, which reduces overlap of the labels. This can be set in the nuts.ini file, or while in the integration subroutine. Integration subcommand **V** displays the labels vertically, subcommand **H** displays the labels horizontally. Note that the **V** subcommand is also used to set the value of a sub-integral, but that command is only invoked when a vertical cursor is displayed and overlaps the chosen sub-integral, so there is no conflict.

By default, the label appears just below the end of each sub-integral trace. The labels can be moved to the top of the screen by typing **M**. This is a toggle command, so typing it again moves the labels back to below the end of the trace.

The integral font can be set with FI or by choosing Set Fonts from the Edit menu. (The user must exit the Integration subroutine to change the font.) Sometimes sub-integrals are so close together that the labels overlap, making them hard to read. To remedy this, the individual labels can be moved up or down slightly. Three positions are defined and are chosen with the keys **1**, **2** and **3**, as follows. Place the cursor on the sub-integrals whose label is to be moved (by clicking the left mouse button once). Typing **1** moves the label up slightly from its initial position. Typing **3** moves the label down slightly from its initial position. Typing **2** returns the label to its initial default position. See illustration below on how to adjust label positions.

Removing sub-integrals -- Single sub-integrals can be deleted by placing the cursor on a sub-integral (by clicking once with the left mouse button) and typing **D**. At any time in the integral subroutine, typing **C** will clear the set of sub-integrals and display the entire integral. Subcommand **L** will delete the last-created sub-integral.

Saving integrals -- The positions of all sub-integrals and their values are saved with the spectrum, so that when the spectrum is recalled at a later time, these integrals can be displayed without having to be redefined manually. This information is saved in a file "tailer", meaning it is appended to the end of the file. See the description of file tailers for more information. An example of the integration information contained in the tailer is:

```
INTEGRALS
REL_VALUE = 3.755704e-006
START_PT  START_PPM END_PT    END_PPM         VALUE   LABEL POSITION
   809     7.560      951      6.636         5.593325    1
  1540     2.801     1623      2.261         2.000000    1
  1722     1.616     1767      1.323         1.923050    1
  1769     1.310     1848      0.796         3.017987    1
END_INTEGRALS
```

The integral values listed in the VALUE column are the result of scaling all integrals by setting the second integral to 2. Absolute comparison of integral values between different spectra is possible using the "REL_VALUE" parameter, which relates the absolute area to the chosen scaled value.

Creating an integral list -- A list of all currently defined sub-integrals and their values can be placed into the clipboard for pasting into a document, for editing, printing or transfer to another application, such as a spreadsheet. The list looks like:

```
NUMBER          FROM           TO        VALUE
   1          7.56 PPM      6.64 PPM      5.59
   2          2.81 PPM      2.27 PPM      2.00
   3          1.62 PPM      1.33 PPM      1.92
   4          1.32 PPM      0.80 PPM      3.02
```

This is accomplished with the Integration sub-command **T**. All sub-integrals are included in the list, whether or not they are currently visible on the screen. The **T** command orders the sub-integral regions from largest to smallest chemical shift, so the list comes out in order, regardless of the order in which the sub-integrals were defined. Once **T** has been typed, the list can be pasted into the Windows Notepad or any word processing program or text editor. From the NUTS base program level, after exiting Integration, the **IL** command is another way to place the list of integrals into the clipboard. Note that if changes are made to the integrals, the information in the clipboard is not automatically updated - it is necessary to do this manually. Any text contents of the clipboard can be displayed on the NUTS screen by typing Ctrl-B (or CB), which is a toggle, so typing it again will turn off display of the text. This can also be done from the View menu.

The integral list can be created using either spaces or tabs to separate the columns. With space-separated columns, when a fixed size font is used, the columns line up. (Best for pasting into Notepad). Tab-separated columns are best for pasting into a spreadsheet program. The choice is determined by a setting in the nuts.ini file, and can be toggled between the 2 options with the **TB** command.

Flattening the integral -- If the integral is not "flat" enough, the region must have its baseline adjusted. NUTS provides several baseline adjustment options. Type <Enter> to leave the Integration routine, correct the baseline with one of the options described in the section on baseline correction. One more baseline correction option is available within the Integration routine, which lets the user correct DC offset and tilt while viewing the effect on the integral trace in real time. From within the integration routine, typing **B** enters this baseline correction routine. Press and hold the left mouse button. Moving the mouse left and right performs a zero-order correction (DC level). The same operation with the right mouse button performs a first-order (tilt) correction. Type <Enter> to keep the correction or **Q** to quit, both of which exit the baseline correction routine to Integration. The correction is performed only on the currently displayed region. This function applies a correction to the spectrum, rather than just adjusting the appearance of the integral trace. Because the ends of the region are altered, this operation may have an effect on adjacent regions. See illustration below on baseline correction within Integration.

The easiest way to integrate an entire spectrum is to integrate expanded regions of the spectrum separately, in order to be able to see spectral details. Expand a region using the Zoom routine, enter the integrate routine and define the sub-integrals. Then either pan to another spectral region using the scroll bar at the bottom of the spectrum or exit the integration subroutine (with <Enter>) and select another expanded region. On re-entering the integration subroutine, the previously defined sub-integrals are still there and defining additional sub-integrals adds to those previously defined. After all sections are integrated, plot limits can be selected, the integrals displayed (using **Ctrl-I**) and the fully integrated spectrum can be plotted.

N.B. If any of the options which involve baseline correction of only the currently displayed region are used, other regions of the spectrum are altered, and their integral values will change. It is preferable to baseline correct the entire spectrum with FB before beginning integration.

The alternative is to integrate each section of the spectrum separately, copying the integral list to the Notepad for each section, and using the Notepad to build a complete integral list of the entire spectrum. While in the integration routine, copy the current region's integral information to the clipboard (**T**) and paste into the Notepad. Then clear the sub-integrals (**C)** before moving on to the next region. This is because whenever T is typed, all sub-integral information is copied to the clipboard, not just those in the currently displayed region. If baseline correction is applied to the current region, it will change the baseline (and therefore the integral values) in other parts of the spectrum. Clearing sub-integrals does not affect the integral scaling.

Subcommands:

> **Left Mouse Button Click** Displays a vertical cursor. Used to define sub-integrals and to select a sub-integral.
> **V** When typed while vertical cursor is displayed, brings up a dialog box allowing

the user to sets the value of the sub-integral at the cursor location. If the value is set to zero, the integral values are not displayed.
C Clears the sub-integrals, displaying one integral line for the entire spectrum.
D Deletes the selected sub-integral in the sub-integral list at the cursor location.
L Deletes the last created sub-integral.
Q Removes the cursor and aborts a sub-integral selection process.
T Transfers the current sub-integral list to the clipboard for pasting into Notepad or other document.
R Read integral information from file "tailer". The integral information must have previously been saved with the file (see TA command).
M Toggles location of the printed sub-integral label among the end of the integral line, the top of the page and the bottom of the page. Applies to both screen display and plot.
1, 2 & 3 Moves location of the printed sub-integral label at the current cursor location to one of 3 vertical positions. Used to remove overlap of the labels of closely spaced integrals. Applies to both screen display and plot.
V Displays sub-integral labels vertically
H Displays sub-integral labels horizontally
Z Brings up another left scroll bar to adjust the beginning level of the integral line. An <ENTER> removes the second left scroll bar. Another **<ENTER>** exits the Integration sub-routine.
F Toggles between showing the sub-integrals and the full spectrum integral.
B Starts an integral/baseline correction process to provide a zero order and first order correction to the integral display. A BF or FB command before entering the integration routine is recommended to get a flat integral. If these don't work, the B sub-command of the ID routine allows use of mouse movements to correct the integral display to make it flat. If the B sub-command is typed while displaying sub-integrals, the system is toggled to full integral display (See F sub-command). Within this baseline correction routine, the baseline is adjusted as follows:
Left Mouse Button Down Mouse movement left and right does zero order integral correction.
Right Mouse Button Down Mouse movement left and right does first order integral correction.
Q Aborts the process and ignores corrections made.
<Enter> Applies the baseline correction and exits the B sub-command.
See illustration below for how to use the B routine.

Unlike other integral correction routines, this operation changes the baseline of the data, rather than just tilting the integral trace. The changes are permanent, not limited to the integral display. Because the integral is so sensitive to subtle baseline imperfections, this can be a very useful way to correct the baseline.

AI -- Automatic Integration

This command displays an integral of the entire spectrum, with the integral broken into integral regions, and labels each region with its relative value. When this command is

executed, any previously defined integral regions and scaling are overridden, whether they were created with AI or manually within the Integral Display (**ID**) subroutine. This command is available from the Tools menu. It is advisable to correct the baseline (with FB) before attempting integration.

NUTS determines where to break the integral in the following manner. The spectrum is divided into 256 equal segments (with the requirement that each segment contain at least 16 points; if not, fewer segments are chosen). NUTS determines the RMS value of all points in each segment and selects the minimum of those values as representing the noise level. Only segments whose RMS value is at least 10 times this noise level will be considered to contain peaks and are included in an integral. Starting from the left end of the spectrum, the first segment found to contain a peak will set the start of an integral region. Each subsequent segment will be included in that integral region if it contains a peak. When a segment is encountered which does not contain a peak, the integral region is ended. This procedure continues for the rest of the spectrum.

The smallest integral so defined is assigned a value of 1 and all other regions scaled accordingly. Following automatic integration, it is possible to enter the Integral Display subroutine and make changes, such as deleting unwanted integral regions, defining new regions or rescaling by setting the value of a chosen region. See the description of the **ID** routine, above, for details.

Display of the integral trace can be toggled on and off by typing **Ctrl-I** or selecting Show Integrals from the View menu. This works whether integral regions have been defined by either the automatic or manual methods. The commands **AN** and **AF** can also be used to turn On and Off the integral display, respectively. The latter 2 commands can be used in Links and macros. Ctrl-I is active at all program levels, including within subroutines. To use Ctrl-I in a Link or macro, use ^I.

IL -- Integral List

Transfers a list of sub-integral values to the clipboard, from which it can be pasted into the Notepad or other document for editing and printing. This is equivalent to the sub-command **T** within the Integration subroutine. All sub-integrals are included in the list, whether or not they are in the currently displayed region.

Control-I -- Toggle on/off integral display

This is the same as the Display Integral option under the View menu. This can also be accomplished with the commands **AN** and **AF**, which turn integral display on and off, respectively. To include this in a Link or macro, use " **^I** ".

Note that the Tab key executes a Ctrl-I, and so performs the same function.

AN -- Turn on integral display

Displays the integral trace. If integral regions were previously defined, either automatically (with AI) or manually in the **ID** subroutine, these regions will be displayed. Otherwise, the integral trace will encompass the entire spectrum. The integral trace can be turned off by typing **AF**. This operation can also be performed by selecting Show Integrals from the View menu or by typing **Ctrl-I**, both of which function as a toggle.

AF -- Turn off integral display

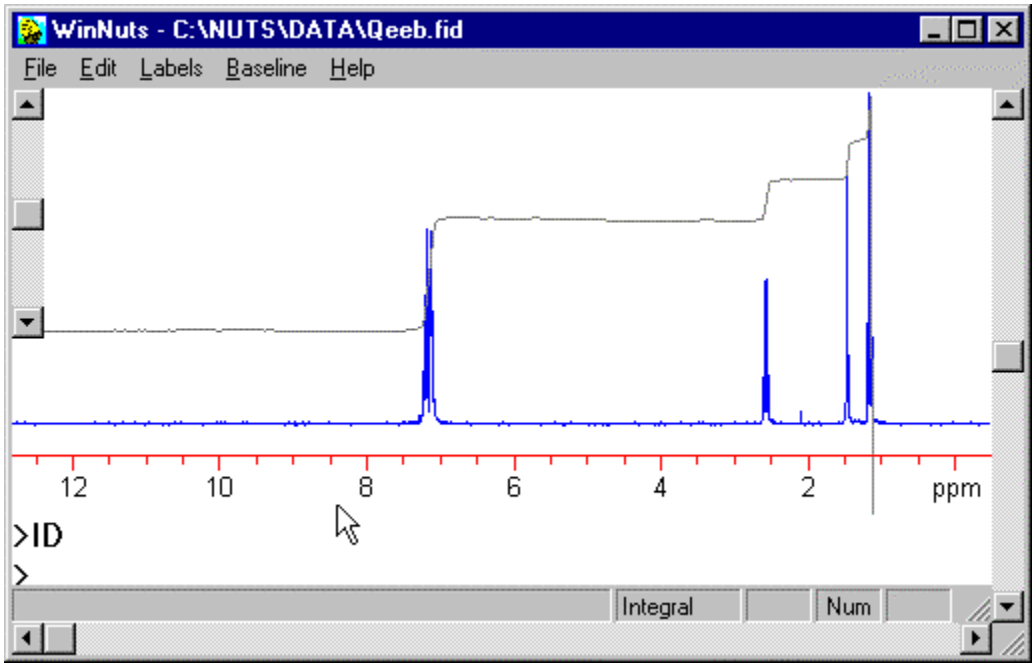
Removes display of the integral trace. The integral trace can be turned on by typing **AN**. This operation can also be performed by selecting Show Integrals from the View menu or by typing **Ctrl-I**, both of which function as a toggle.

Illustrated example

Before attempting integration, it is usually necessary to correct the baseline, so that the integral trace is flat. See baseline correction commands BC, BF and FB.

Other topics within Integration:

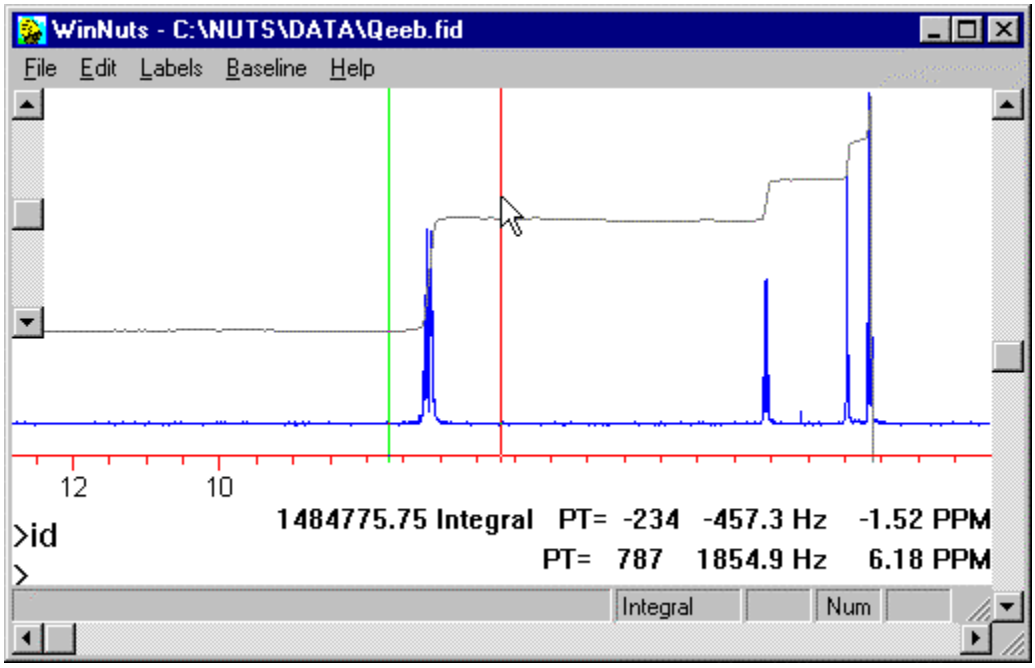
Changing the position of the integral labels
Baseline correction within the integration routine



Enter the Integration subroutine by typing **ID** or by selecting Integrate Display from the Process menu.

A continuous integral trace is displayed covering the entire spectrum. If the integral goes off scale, it will wrap around to the bottom of the screen, as seen here at for the most upfield peak.

The scroll bar on the left adjusts the vertical scale of the integral trace.



Defining subintegrals

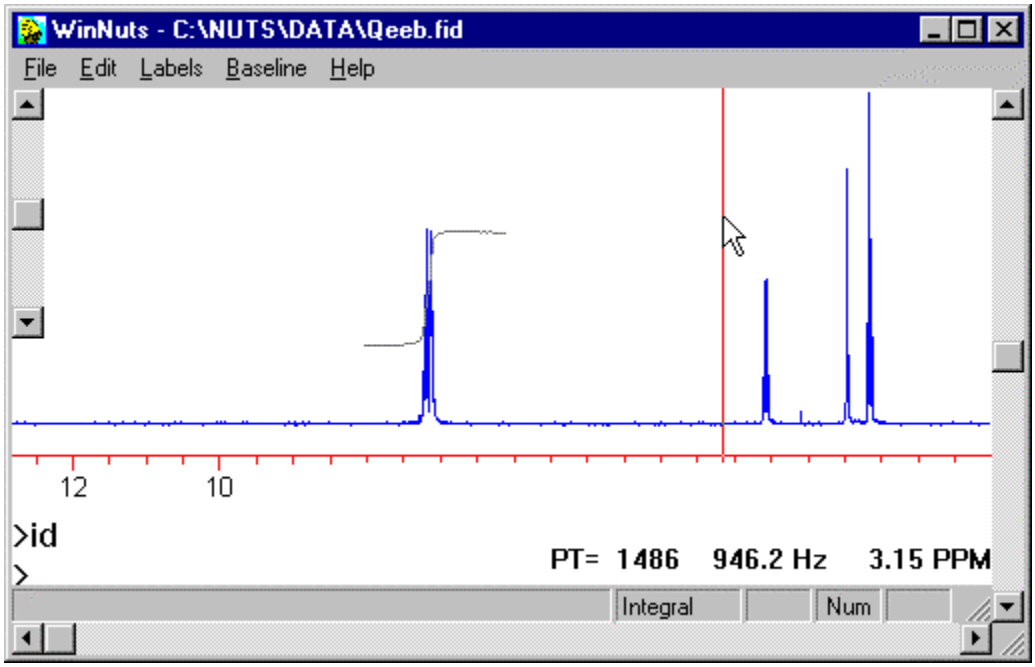
Click the left mouse button once to display a vertical red cursor.

Place the cursor on one side of the chosen peak (either side is OK)

Click the left mouse button again to define one end of the subintegral (green)

Move the cursor to the other side of the peak and click the left mouse button again

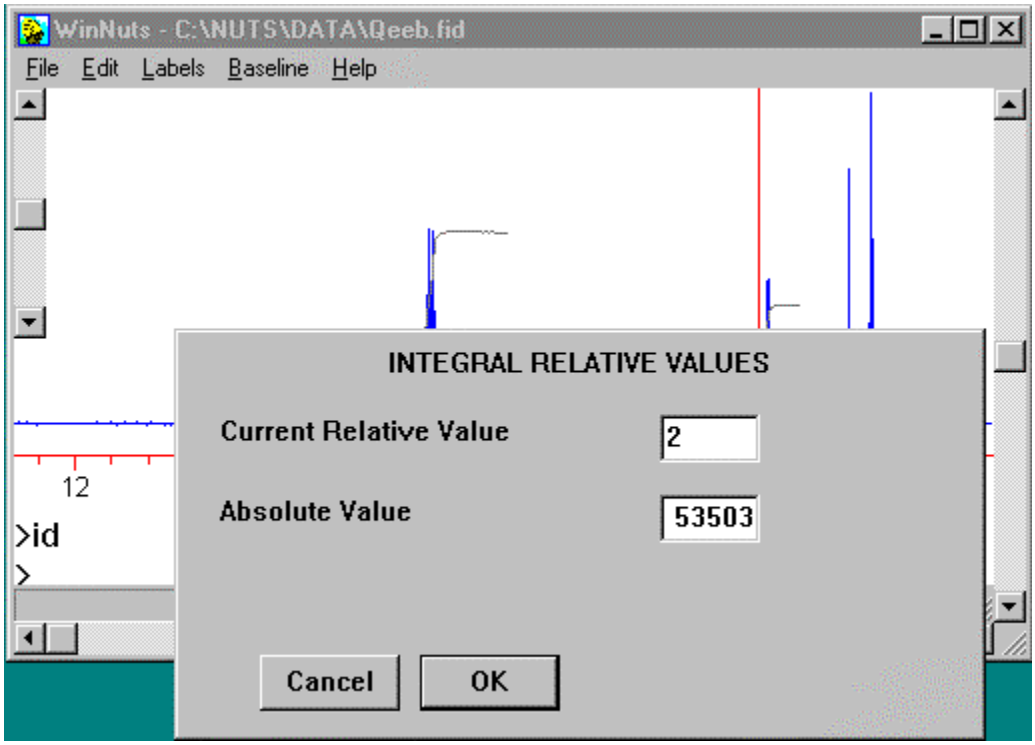
Notice that the location of the cursors is displayed at the bottom of the screen, along with the value of the subintegral being defined.



As soon as the first subintegral has been defined, only subintegrals are displayed, not the full trace. (Display can be toggled between display of subintegrals and full trace by typing F, also available from the Edit menu.)

Additional subintegrals are defined in the same manner.

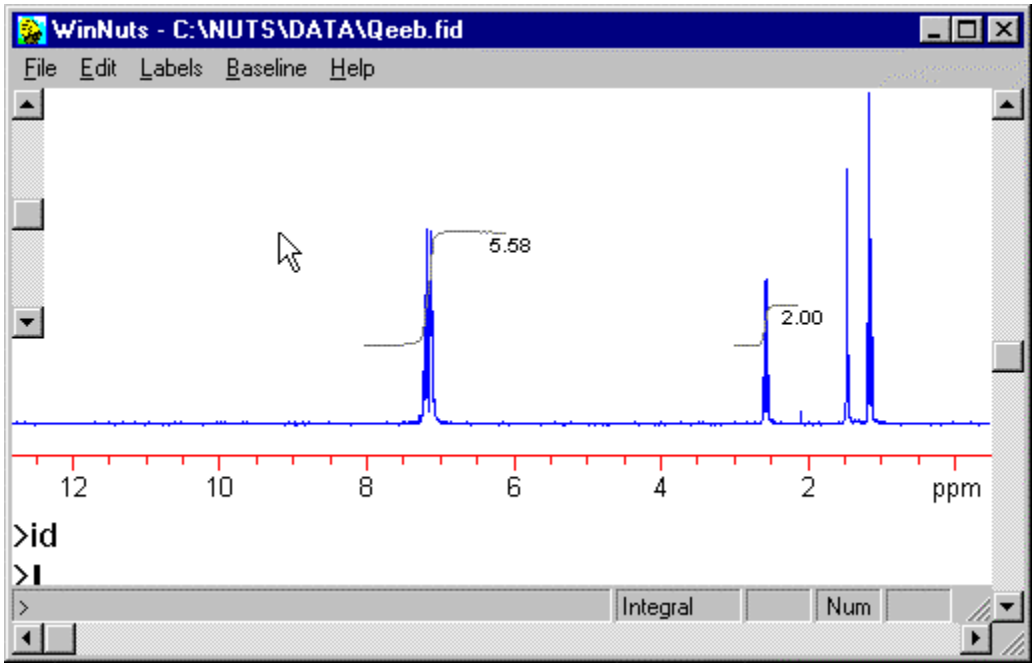
All subintegrals can be deleted using the **C** command. A selected integral can be deleted by placing the vertical red cursor on that integral and typing **D**.



To assign the value of a chosen subintegral

Click the left mouse button once to display the vertical red cursor

Place the cursor on the chosen subintegral and type V (Value) on the keyboard, which displays a box allowing a value for relative area to be entered.



Once a subintegral's value has been assigned, all defined subintegrals are labeled with their relative areas. These labels can be removed by setting the Value of any subintegral to zero.

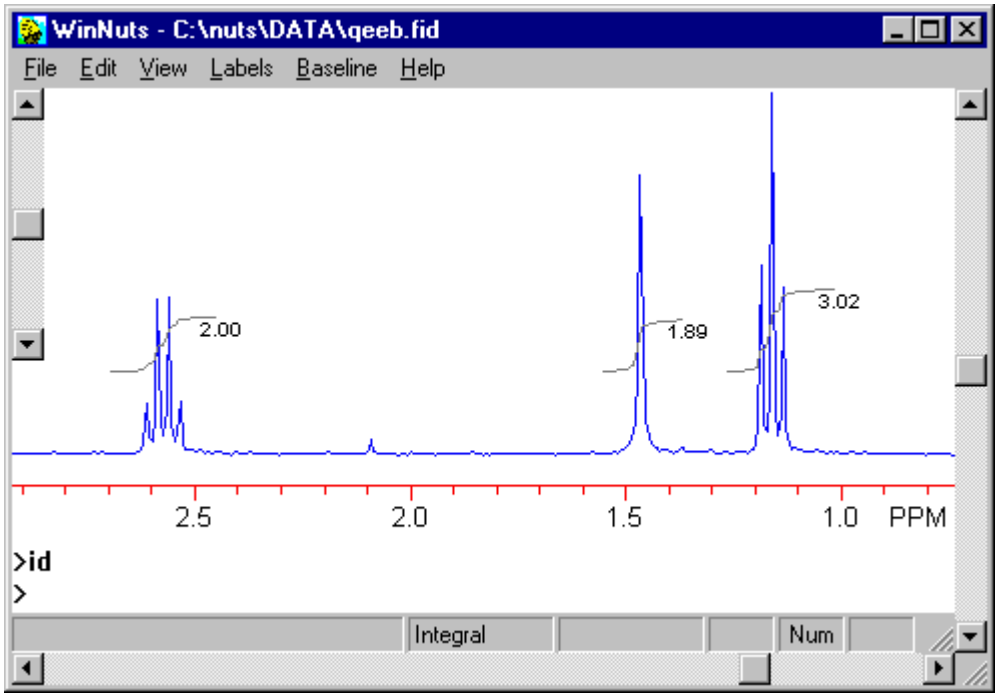
The displayed integral values remain unchanged, even if the vertical scale of the spectrum or of the integral trace is changed, until explicitly reset by the user.

Subintegrals remain defined when the integration subroutine is exited, and even when a new data set is opened.

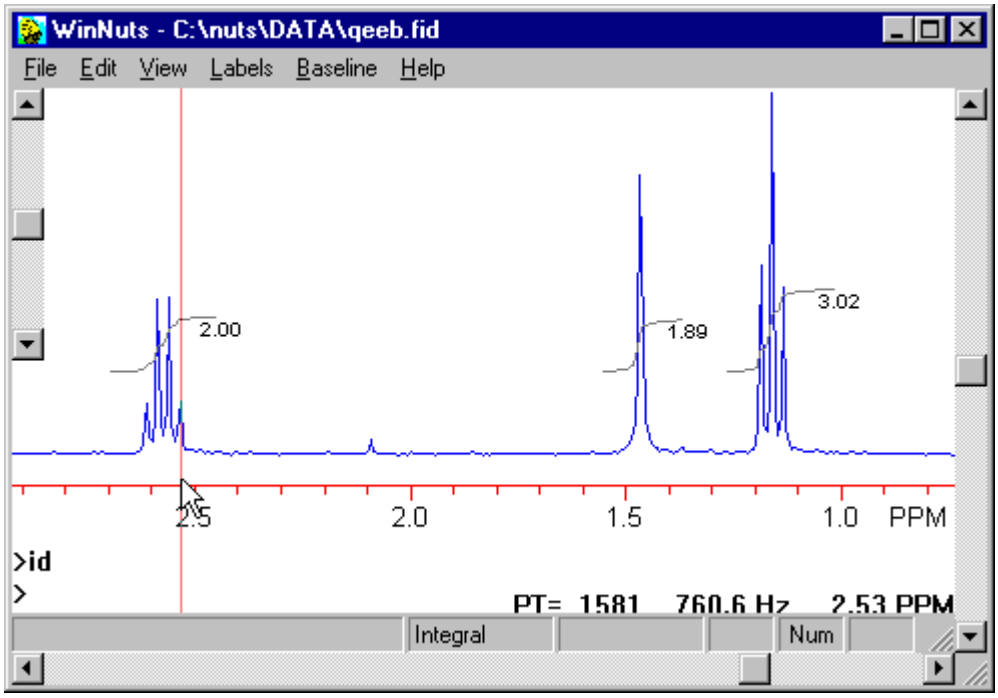
As with all subroutines, typing <ENTER> exits the integration routine.

Display of the integral trace can be toggled on and off without entering the integration subroutine by selecting Show Integrals from the View menu, or by typing **Ctrl-I**.

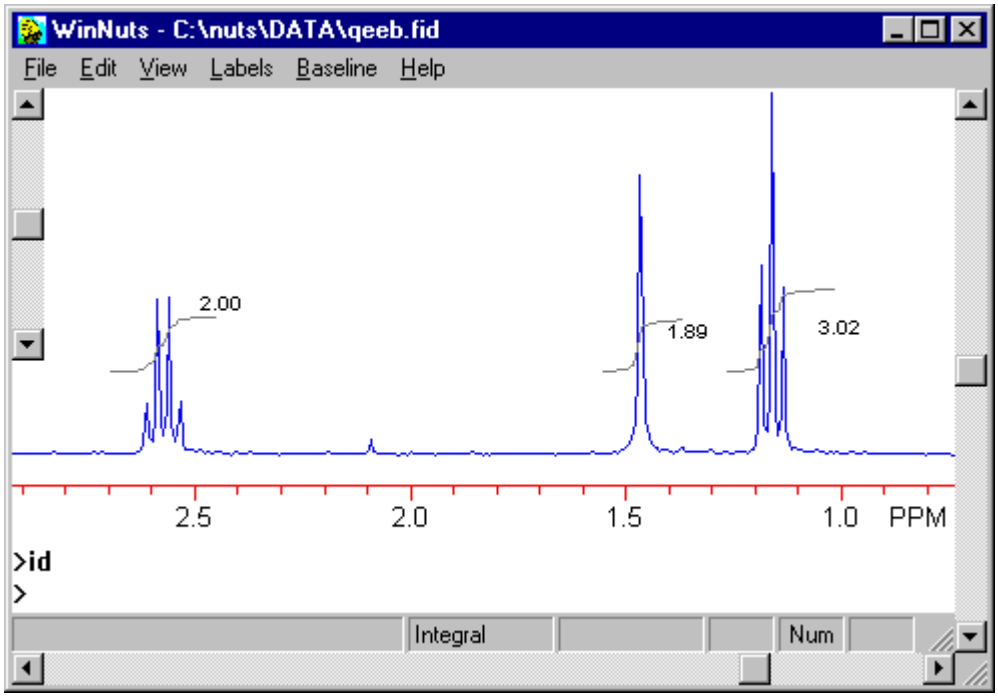
Repositioning integral labels



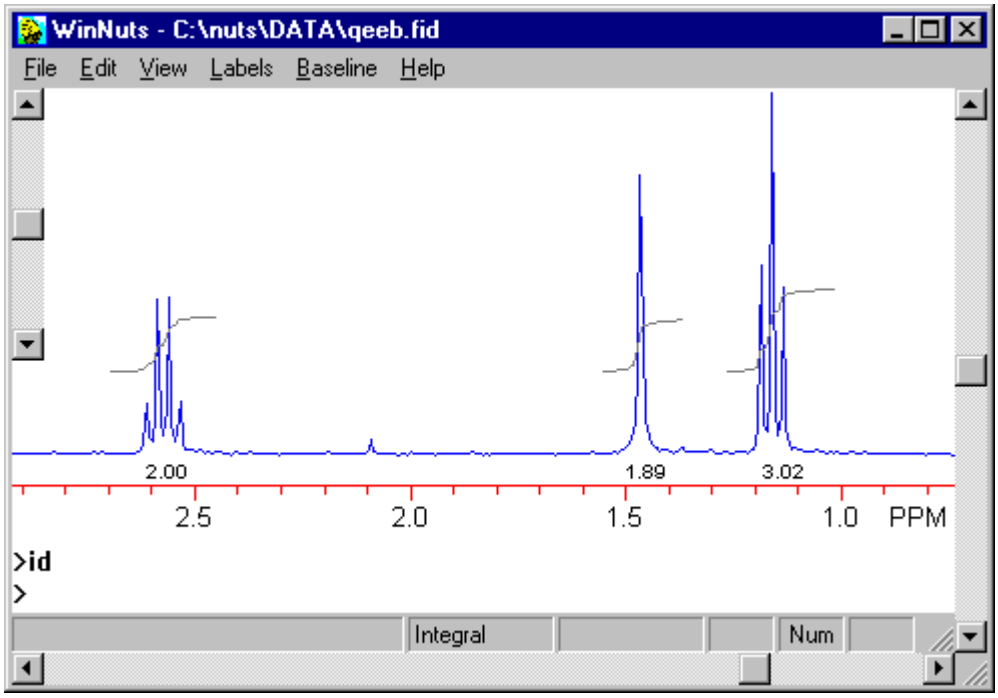
By default, numerical integral labels are placed just below the end of each integral trace. The labels can be repositioned to avoid overlap.



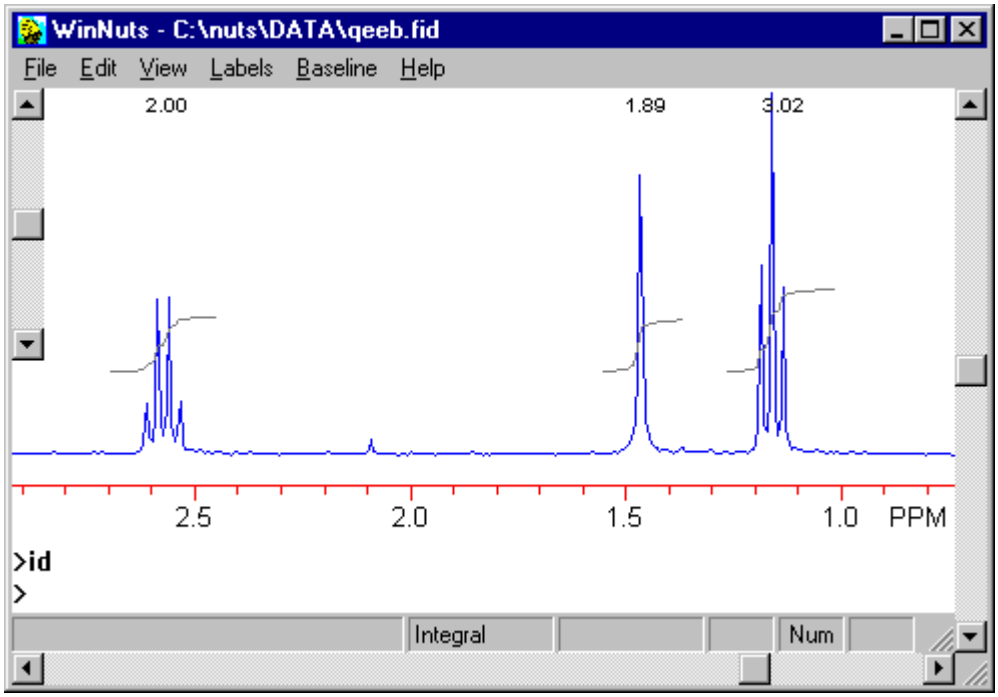
Each label can independently be moved vertically a small amount. To do this, select the chosen integral by clicking the mouse button once to display a vertical red cursor, and placing that cursor on the chosen integral. There are 3 vertical positions available, which are set by typing the chosen number (1, 2 or 3).



The above figure illustrates the 3 positions, 1,2 and 3, from left to right. 1 is just above the end of the integral trace. 2 (default) is just below the integral trace. 3 is slightly lower still.



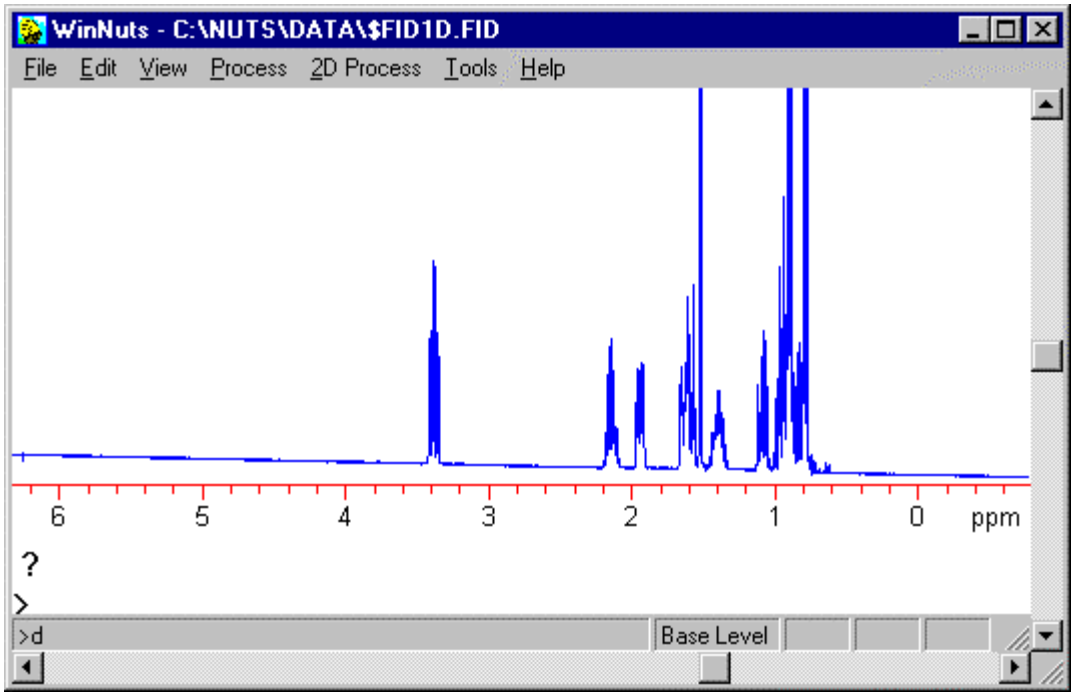
The labels can also be placed below the spectrum or at the top of the screen. The subcommand M toggles among the 2 positions (end of trace, top of screen, below spectrum).



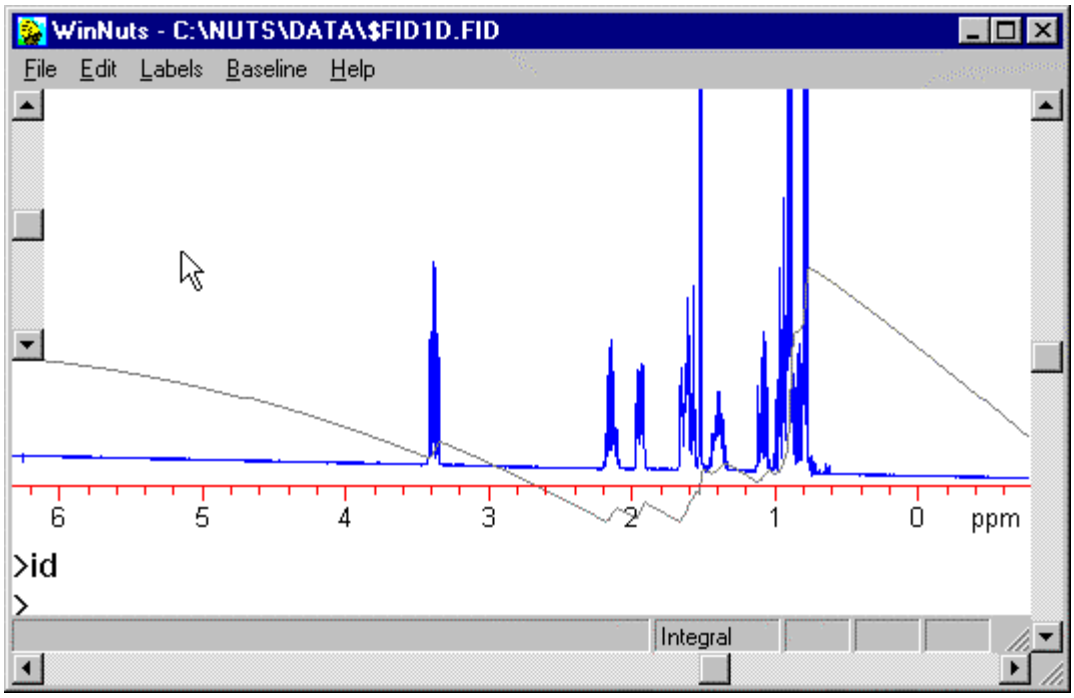
Labels can also be displayed vertically, which can be useful in reducing overlap of labels. Integration subcommand **V** displays labels vertically, and subcommand **H** displays the labels horizontally. The default orientation can also be set in the nuts.ini file.

Using integral display for baseline correction

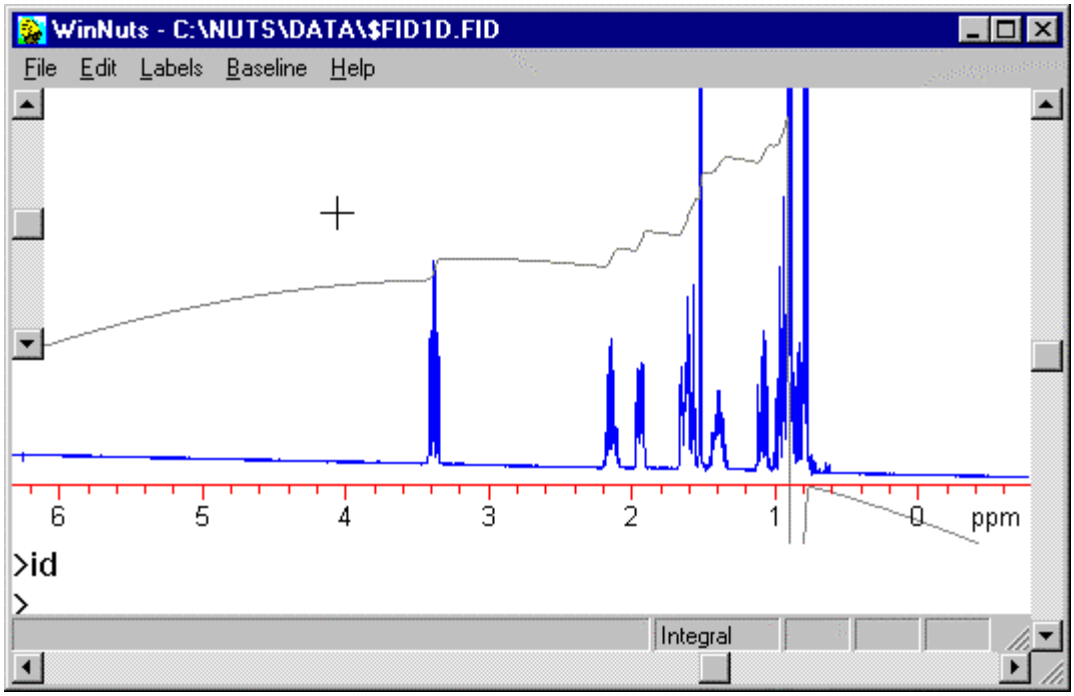
When the integration line is not flat, it means the spectrum's baseline is not flat. This can be corrected using one of the NUTS baseline correction tools, but it can also be corrected from within the integration subroutine. The integral is very sensitive to slight tilt in the baseline, so viewing the integral while making the correction is often very helpful. It is important to understand that this operation does not just adjust the displayed integral trace to be flat. It actually changes the data, permanently.



This spectrum clearly needs baseline correction.



On entering the Integration routine, the integral trace is far from flat.



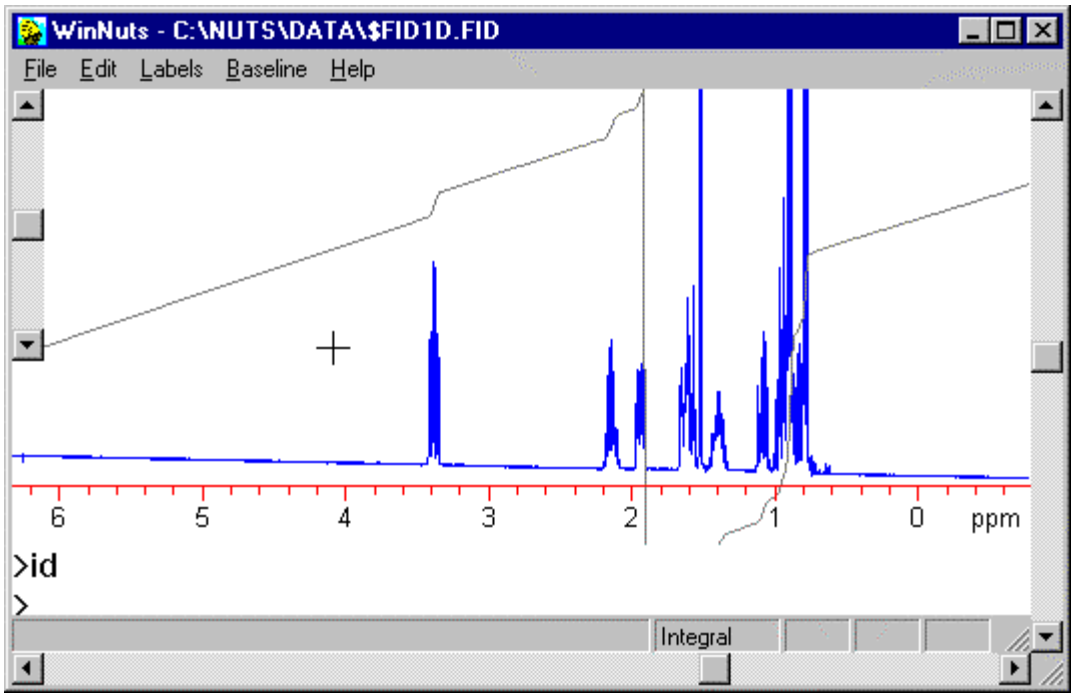
Enter the Integration baseline correction routine by typing B.

The left mouse button controls zero-order correction (DC offset of the baseline, which appears as a tilt of the integral trace).

The right mouse button controls the linear correction (linear tilt of the baseline, which appears as a curved integral trace).

Start by holding down the left mouse button and move the mouse left and right to try to make the integral trace flat in regions of the spectrum that have no peaks.

Clearly, this baseline requires more than just zero order correction.

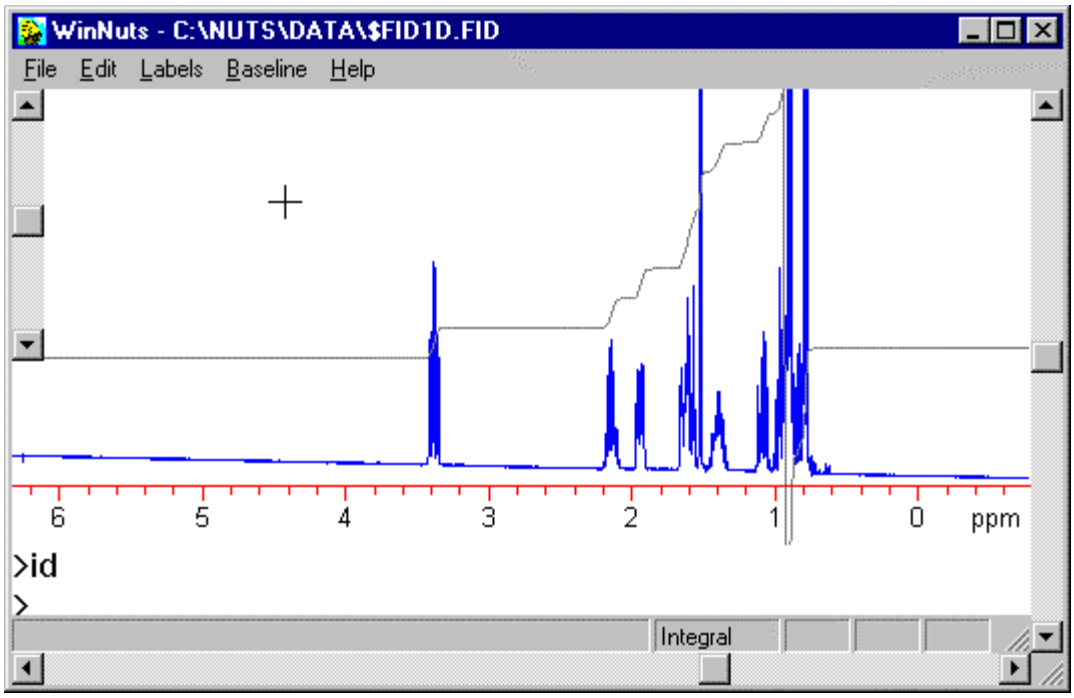


Hold down the right mouse button and move the mouse left and right to try to make the tilt the same over the entire spectrum.

The curvature that was evident above is now gone.

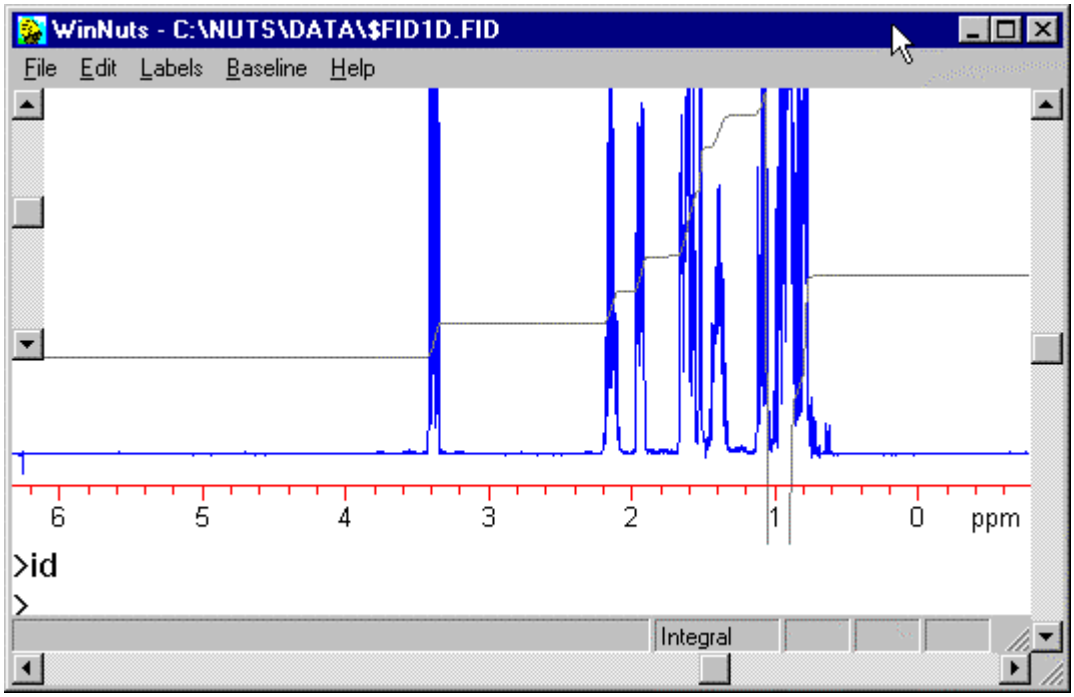
Users with a single-button mouse should hold down the shift key and use the mouse button when the instructions call for use of the right mouse button.

(Caution should be exercised when applying a linear correction, as large curvature can inadvertently be entered.)



The final correction is made with the left mouse button to remove the tilt seen in the previous screen.

When a satisfactory integral trace is obtained, type <Enter> to exit the baseline correction routine and apply the correction to the spectrum.



Notice how much flatter the baseline is than in the first screen.

Zoom expansion routine

ZO – Enter the Zoom subroutine

The Zoom subroutine allows the user to expand a spectrum horizontally to view details more easily. That is, you can "zoom" in and out. The user can enter the Zoom routine of NUTS in several ways: from the menu View / Zoom selection, from the command line with the command "ZO" and by double clicking the left mouse button.

Use of the Zoom subroutine is illustrated in the Introduction section, above.

On entering the Zoom subroutine, the menu choices change to display commands which are currently active. All commands can be accessed from the menus, with the exception of those which involve using the mouse.

Once in the Zoom subroutine, the mouse cursor changes into a small crosshair labeled ZO. While pressing and holding the left mouse button, "drag" the mouse and a region of the screen will be selected in inverse video. To jump to this expanded display, click the right mouse button or type **Ctrl-E**. The display window can be shifted left and right using the horizontal scroll bar at the bottom of the screen. The speed of this process depends on how many points are in the currently displayed region and on the speed of the computer. This may be impractical with slower computers. The horizontal scroll bar can be turned off in the NUTS.INI file.

The right mouse button toggles between full and expanded display while in the Zoom subroutine. While viewing an expanded region, the left mouse button can be used to select a still smaller region, and the right mouse button will then jump to this new expansion (Zoom within Zoom). The user can switch the display (from the Zoom routine or the NUTS base level) using **Ctrl-F** for full display or **Ctrl-E** for the expanded region display. While in Zoom, a specific frequency range of interest can be entered from the keyboard by typing **F**, which brings up a dialog box for entry of frequency limits in points, Hz or ppm.

The Zoom routine is exited with the <ENTER> keyboard key, returning the user to the NUTS base level. The current expanded region remains displayed on exiting Zoom.

Subcommands:

B Baseline Flatten by removing the DC level and linear tilt between the zoom limits. See also correcting the baseline.
F Brings up a dialog box allowing entry of specific points or frequency limits for the expanded display.
I Enter the integral (ID) subroutine. <Enter> will return to Zoom from the ID routine.
D Tilt right end of displayed region Down. Use upper case **D** for larger changes, lower case **d** for smaller changes.

U Tilt right end of displayed region Up. Use upper case **U** for larger changes, lower case **u** for smaller changes.
Baseline tilting in Zoom is illustrated below.
Ctrl-Z Zero the data in (reals and imaginaries) between the start of the Zoom region and the end of the Zoom region. See also zeroing data.
1 Select current Zoom region for region 1 in PE phasing routine.
2 Select current Zoom region for region 2 in PE phasing routine.
0 - 9 Select the current Zoom region and assign it an identifying number 0-9. The region can be later recalled with the corresponding command Z0-Z9.
Enter Exits the Zoom subroutine

EZ -- Enter limits for Zoom regions

Opens a dialog box for setting the frequency limits of the 10 Zoom regions. This accomplishes the same thing as setting regions within Zoom using 0-9.

Control-E -- Expanded display

Toggles the spectrum display to expanded display using the previously selected Zoom region. This is also available by selecting Type from the View menu. **Control-F** returns the display to the full spectrum.

Control-F -- Full display

Displays the entire spectrum (all real points). To jump to an expanded display, using previously defined frequency limits, type Ctrl-E. These commands operate in all subroutines and from Base level of program. This is also available by selecting Type from the View menu.

LZ -- Last Zoomed region

Changes the left and right frequency limits to the previous values. This allows zooming in on a peak and then jumping back to the previous view.

Z0 - Z9 -- Display a previously defined spectral region

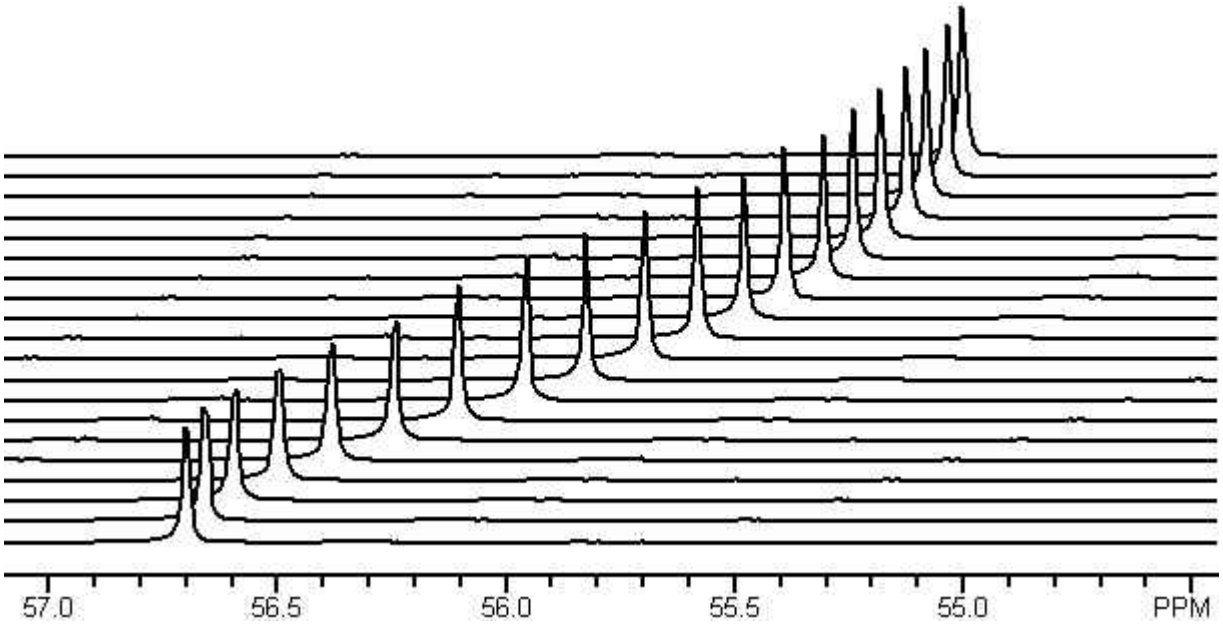
Recall one of ten separate, previously defined zoom regions. Up to 10 such regions can be defined, and those frequency limits saved for later recall. The frequency limits are defined in one of 3 ways:

1. with the EZ (Enter Zoom limits) command
2. within the Zoom subroutine using subcommands 0 - 9
3. inside a macro with the command **Set Zoom_Region**

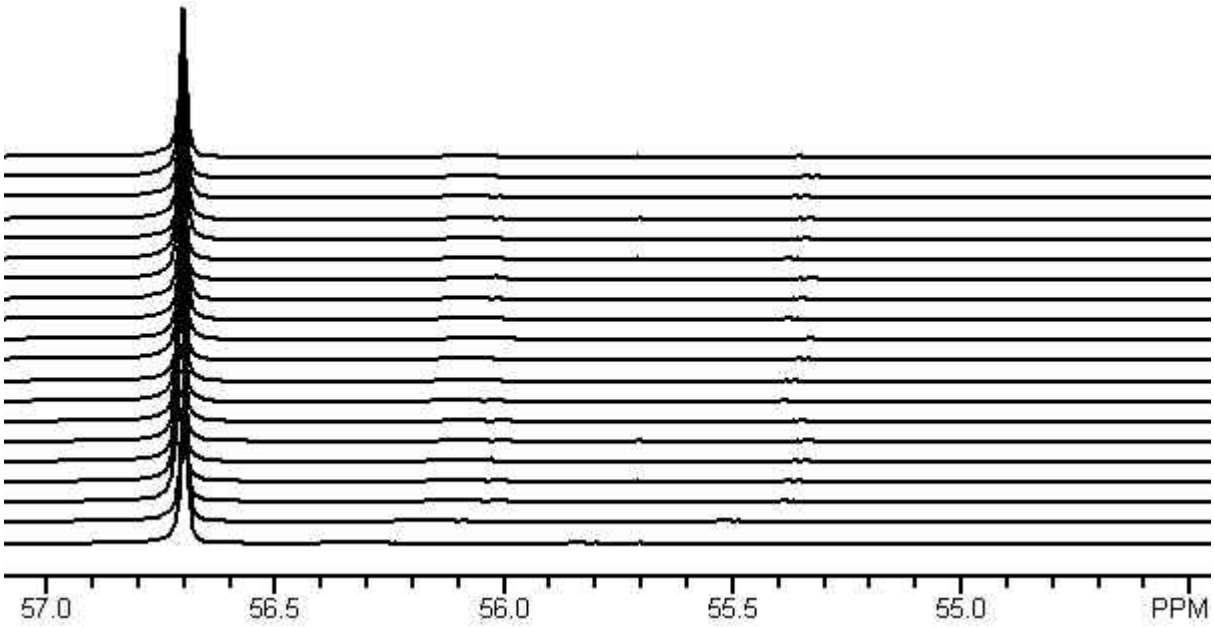
Commands Z0-Z9 recall the corresponding region. This is useful in designing automated processing.

R0 - R9 -- **Register spectra**

This routine " registers" an arrayed set of 1D spectra, so that the chemical shift scale of all spectra is the same. This would be used, for example, when a series of spectra are acquired without a field/frequency lock, resulting in the spectra not lining up properly, as shown here:



First, choose a region of the spectrum whose largest peak will be used to align the spectra. The desired region is selected by setting the region while in ZOom with the # key where # is a digit between 0 and 9 (or, equivalently, by using the EZ command). The corresponding R# command will find the tallest peak in the region, and left or right shift the remaining spectra to make the tallest peak in this region of each subsequent spectrum have the same chemical shift. The ends of the shifted spectra are lost or set to zero. The data set above now looks like:



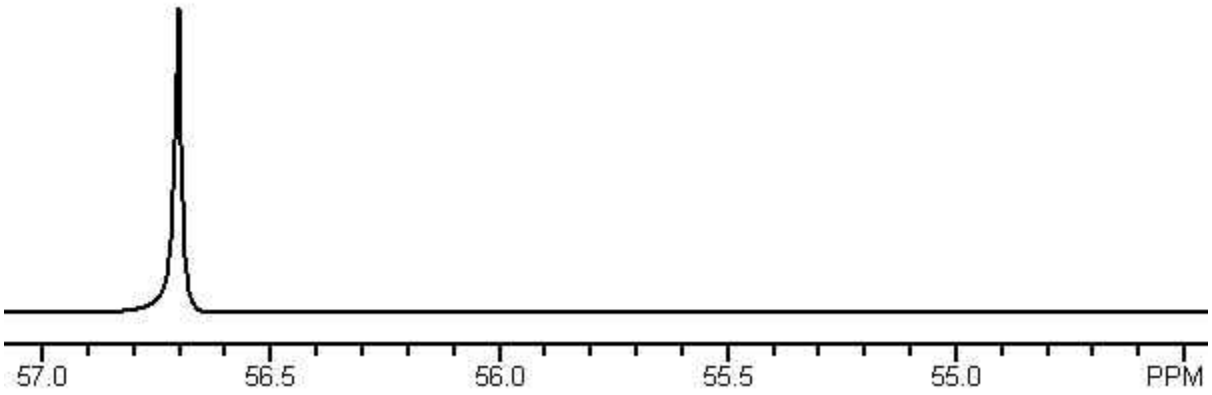
This routine works in the non-arrayed mode or Complex Arrayed Mode.

Y0 - Y9 -- **Sum block averaged spectra**

These commands work in the Arrayed Mode only. "Block averaged" data are collected as a series of 1D spectra and stored as slices of a 2D file. The series of files needs to be summed to yield a single spectrum, but it is necessary to compensate for any field shifts that might have occurred during data acquisition, so that peaks line up correctly before being summed.

In the Zoom routine, select a region containing a peak which can be used for chemical shift registration, and assign it to a numbered Zoom region using one of the Zoom subcommands **0 - 9**. Exit Zoom. Execute the Y# command (# is a number with the same value as the chosen zoom region). This performs the sum with appropriate adjustment so that the tallest peak in the chosen Zoom region lines up for each spectrum. The result will be a 1D spectrum, which has not yet been saved to the disk. When the summing is complete, NUTS also exits the arrayed mode, because the data has been converted to a 1D file, and arrayed mode is no longer appropriate.

The data set shown above results in this summed 1D spectrum:

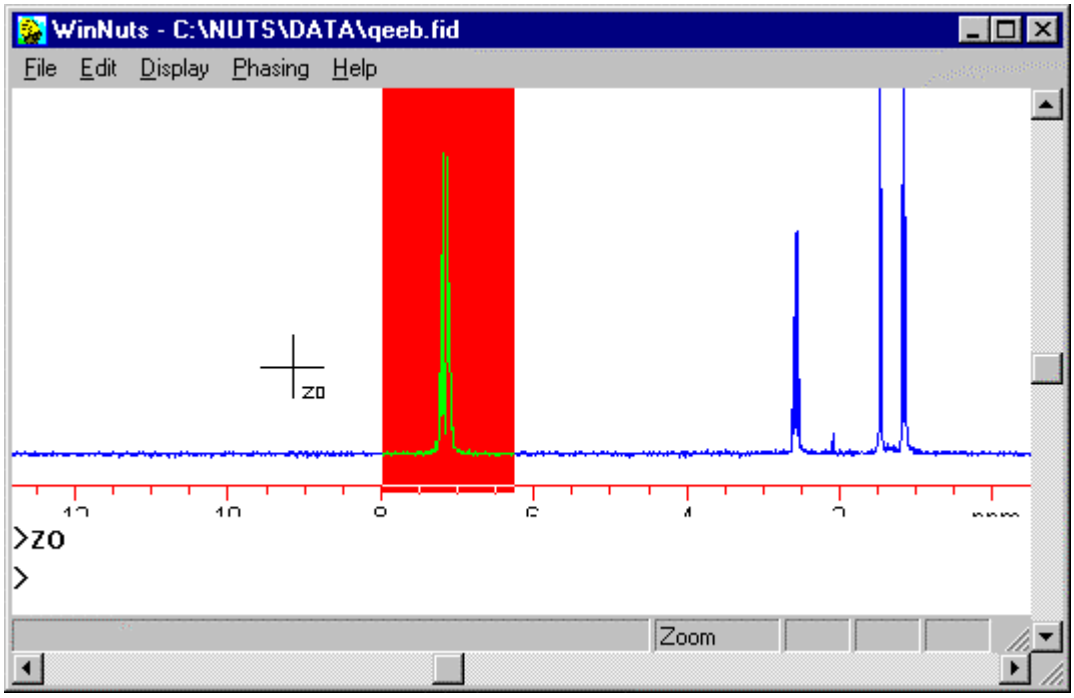


Baseline tilting in Zoom

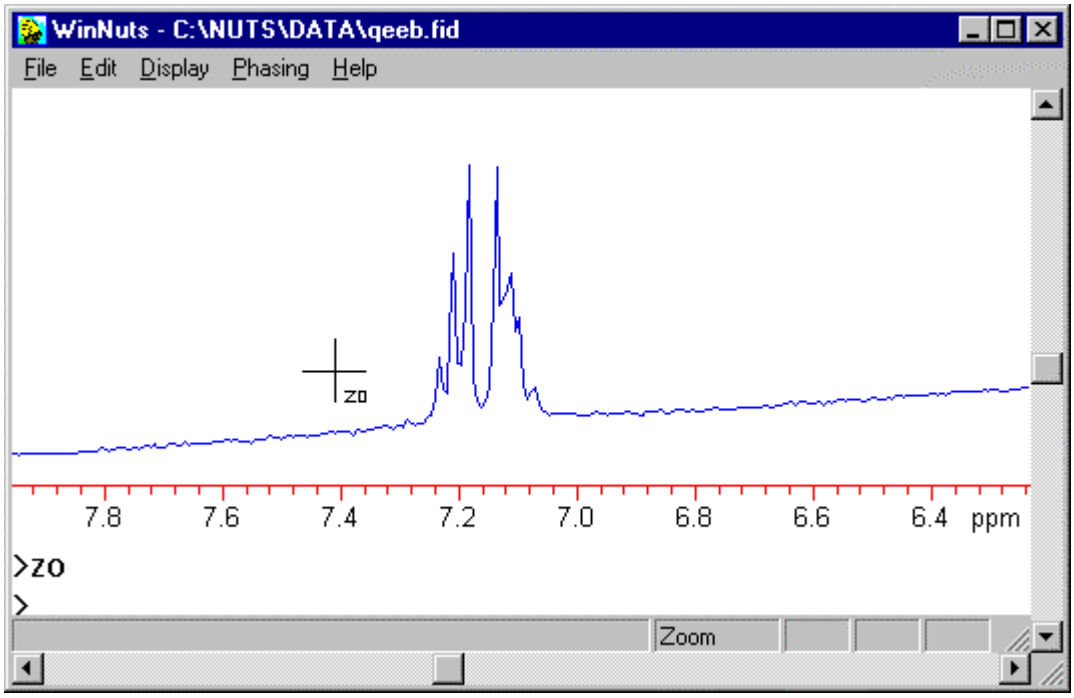
Zoom subcommands D, U, d and u

These subcommands, while useful in special situations, are not commonly used. However, they can be executed accidentally, and it is important for the user to understand what has occurred. One common case is typing DD (for Dual Display) while within the Zoom routine. The result is not the desired one.

These subcommands operate only on the currently displayed region. They act to tilt the right edge of the displayed region up or down in large or small steps, depending on whether upper case or lower case is used.

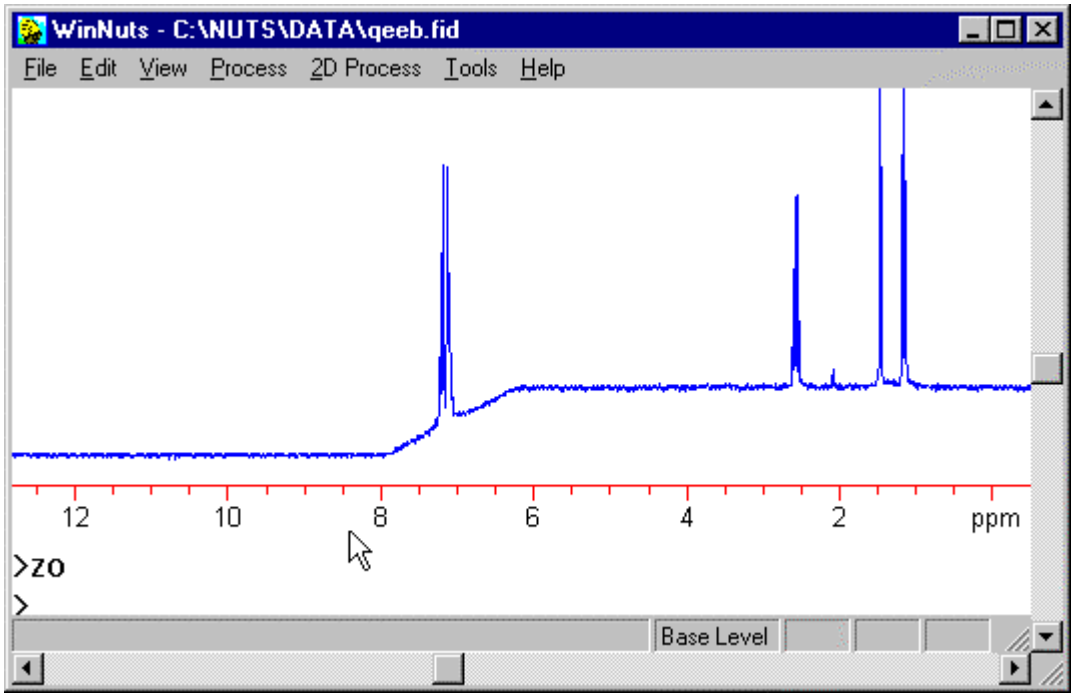


The aromatic region is selected for expansion.



The chosen expansion was displayed by typing Ctrl-E.

The U key was typed several times to make the effect very obvious. The right end of the spectrum has been tilted up.



After exiting Zoom and displaying the entire spectrum, the total result can be seen.

The expanded region was tilted, and the upfield part of the spectrum has been offset so that the baseline is continuous.

Peak picking

PP – Peak pick

Commands and parameters that affect peak picking (details below):

MH - Minimum Height, the threshold for selecting peaks

RM - RMS noise parameter, to distinguish between a peak and noise

ZL – Zero Peak Pick List, clears the list of peaks

Ctrl-P -- Toggle on/off display of peak labels

PN - Peak labels on, to display peak labels

PF - Peak labels off

CB or Ctrl-B -- Toggle on/off display of clipboard text on the screen (see also: Notes subroutine)

The Define Peaks subroutine provides more options for peak picking and labeling.

This operation selects all peaks in the displayed region, indicating which peaks have been selected with vertical lines on the display. Peaks are selected if they meet 2 criteria: peak height must exceed the minimum height (**MH**) value and the peak must decrease by **RM** multiples of RMS noise after reaching a maximum. (This latter is to avoid picking multiple "peaks" for a broad peak in a noisy spectrum.)

When PP is executed, a peak list is placed into the Clipboard. For example,

```
Interpolated Peak Listing
PEAK    POINT   HEIGHT    REL.HT    HZ       PPM
 1      1574    113459    43.49    776.17   2.586
 2      1578    120172    46.07    768.21   2.559
 3      1746    217882    83.52    439.81   1.465
 4      1789    136120    52.18    356.09   1.186
 5      1793    266988   102.35    348.20   1.160
 6      1797    127982    49.06    340.29   1.134
```

Height is peak height in absolute units, Rel. Ht is peak height relative to the tallest peak in the spectrum set to 100. Peak frequency is reported in Hz and ppm.

The peak list can be created using either spaces or tabs to separate the columns. With space-separated columns, when a fixed size font is used, the columns line up. (Best for pasting into Notepad). Tab-separated columns are best for pasting into a spreadsheet program.

The choice is determined by a setting in the nuts.ini file, and can be toggled between the 2 options with the **TB** command.

When the file is saved, the peak list is saved in the file "tailer", so it can be recalled later.

This information can be pasted into and edited or printed from any text editor or word processor, such as the Notepad. The peak list (or any other text in the Clipboard) can be placed on the screen (in the upper left corner, by default) using the Ctrl-B command, which displays the contents of the clipboard on the screen. The font for the clipboard display is set with FC, from the Edit menu or within the NUTS.INI file. The list will be easiest to read if the font is a fixed-width font, such as Courier, so that the columns line up.

The PP command also displays peak labels on peaks. By default, the labels are placed at the top of the screen, but this can be changed in the nuts.ini file. The display of peaks is toggled off (an on again) using Ctrl-P. The peak labels can be edited and other parameters

selected using the DP subroutine. The list can be removed with the Zero List (**ZL**) command.

By default, NUTS uses interpolation to find the peak maximum, which gives a more accurate value for the peak frequency. The interpolation can be turned off from the dialog box which allows setting of the chemical shift reference. From the NUTS base level, press and hold the left mouse button and type **O** to bring up this dialog box.

Peak frequencies or other text labels can be displayed above each peak. To do this, the peaks must be selected within the DP subroutine, rather than with the PP command.

Setting the threshold (minimum height, MH) -- The Minimum Height value is expressed as a percentage of the tallest peak in the spectrum. The simplest way to determine the optimum value for **MH** is by using the cursor from the base level of NUTS (not in Zoom). Press and hold the left mouse button and place the horizontal cursor line at the height you want for the threshold and type **M**. Note that if Fixed Scaling (FS) of the display has been turned on, setting Minimum Height with the cursor will not work properly. Minimum height may also be entered by typing **MH**, which brings up a dialog box and allows a value to be entered. If the peak selection misses peaks which have small splittings, try a smaller value of **RM**.

As of April, 2000, the MH command has been modified to allow use in the non-two letter command mode. The command can be MH or MinHt. If it has no arguments, then the standard dialog box comes up and allows the user to enter an MH value. If it has one argument, that argument is converted to a number between zero and 100.0 and the MH value is set to that number. If it has two arguments, and the second argument is "rms", then the MH value is set to the first argument times the current rms noise of the spectrum.

For example,

mh 5 sets the Minimum Height to 5

mh 5 rms sets Minimum Height to (5 * rms value)

Zeroing data

ZE -- ZEro data set

Replaces all data points with zeroes and the previous data are lost. A dialog box requests confirmation before executing.

BZ -- Baseline Zero

Zeros the region within the Zoom frequency limits without changing the rest of the data. Applies to both the reals and imaginaries. The Zoom subcommand ^Z (**Control-Z**) does the same thing.

ZR -- Zero Reals

Replaces the real half of the data by zeroes. Useful for some types of 2D processing.

ZI -- Zero Imaginaries

Replaces the imaginary half of the data by zeroes. Useful for some 2D processing.

ZN -- Zero Negative points

This command zeros all negative data points of both the reals and imaginaries.

The **Z** subcommand of the View routine will zero all points in the displayed slice. It does not ask for confirmation.

See also: editing 2D data

Miscellaneous tools

FP -- FID Play

Creates a .wav file from the currently displayed FID and plays it through the computer's speakers. You may be surprised at how much you can tell about your data just listening. This works whether the data is an FID or a spectrum, but it only makes sense to use it with FIDs. This is only implemented on the Windows versions greater than version 971106, not on the Mac or OS/2. Obviously, your computer needs to be equipped for sound.

SR -- Spectrum Reverse

Reverses spectrum right-to-left.

RI -- Exchange Real & Imaginary data

This can be performed on both FIDs and spectra. When performed on a FID, the spectrum after FT will look different, depending on whether the data were acquired by simultaneous acquisition of data points into the 2 quadrature channels or by sequential acquisition of data points alternately into the 2 channels (as is done on many Bruker spectrometers). In the former case, the spectrum will be reversed. In the latter case, artifacts will appear in the spectrum which resemble quadrature images. A flag can be set in the Nuts.ini file which causes RI to be executed automatically whenever data is imported.

See also: Special considerations for Bruker data

II -- Invert Imaginaries

Performs a 180 degree phase shift on the imaginary half of complex data. This is useful if the sense of direction with the mouse movements while phasing seems backwards. If performed on an FID, this will reverse the resulting spectrum after FT.

SN -- Signal to Noise

Calculates the ratio of the tallest peak in the currently defined Zoom region to the RMS noise of the baseline. The user must first define the zoom region, to be sure which peak is being used in the calculation. Even if the entire spectrum is displayed (with Ctrl-F), the calculation is performed on the tallest peak within the current Zoom frequency limits. Nuts automatically determines the RMS noise of every spectrum, so the user does not need to define a noise region.

NF -- Calculate system noise figure

Measurements must first be made as described:

First replace the NMR spectrometer's probe with a 50 Ohm metal film resistor. At the gain and frequency settings of interest for the NMR instrument take one scan of data with the resistor at room temperature and save the file with a file name like "HOT.NMR". Cool the resistor in liquid nitrogen and take another scan and save this file with a file name like "COLD.NMR".

In NUTS, enter the NF command and follow the directions. NUTS will load the two data files and calculate the system noise figure. In the non-2-lettered command mode the user can also enter the name of the hot file as argument 1 and the name of the cold file as argument 2.

More information on system noise figure can be found in the Hardware section below.

IV -- InValidate the data

NUTS uses a sophisticated data compression algorithm to minimize the time required to display data on the screen. Occasionally, NUTS fails to redraw the screen following some operation. IV can be used to force NUTS to recalculate and redraw the screen.

See also: UD

XL -- Extract Line

Extracts the real and imaginary sections of a spectrum defined by the zoom region such that the tallest peak in the zoom region is at the center of the NMR spectrum. Points

outside the zoom region will be zeroed. This is useful in creating a reference deconvolution function. See details below on how to use XL.

XT -- Extract Spectral Region

This command uses the currently defined frequency limits to extract a spectral sub-region from a spectrum. Use Zoom to set the frequency limits and type Ctrl-E to display the chosen region. On executing **XT**, this region is extracted. (Note that this may result in a data set whose size is not a power of 2). The values of Sweep Width, Offset and Number of Points are therefore modified. See details below on how to use XT.

This command was created for a specific problem, as described below, but is available for the user to implement for other uses as needed.

A set of kinetics data had been acquired over time on a spectrometer operating without lock. Some field drift occurred during the course of the experiment, so that successive spectra did not line up properly in a stacked plot. In each spectrum, the region 10ppm to 0 ppm was selected after setting the shift reference, and **XT** was executed. Each resulting spectrum was saved under a new file name (using SB) with sequential file extensions. The complete data set was converted to a 2D file***** and when plotted with SP, the peaks were displayed with the correct chemical shift and lined up nicely.

***** Viewing a series of 1D files is often best done with utilities available only for 2D files. (These utilities are available in both 1D and 2D versions of NUTS.) Converting a series of 1D spectra to a 2D file is simple, provided they have sequential file extensions (such as file.004, file.005, file.006, etc). Create and execute Link consisting of the command string

GA SC IN

NUTS will prompt for the file name for the first 1D file and for the file name for the resulting 2D file. To view the file, first read in the 2D file with **GA**.

X0 – X9 -- Extract pre-defined spectral region

These 10 commands each perform an extraction (similar to XT command) of a region previously defined by the corresponding Zoom region (defined using **0 – 9** while inside Zoom, with the EZ command or by the macro command **Set Zoom_Region**). To perform an extraction from inside a macro, use the **Do Extract_PPM** command.

2S -- 2-Point Smooth

Performs a 2-point running average to reduce apparent noise in the currently displayed data set. This is useful, for example, to remove the "wiggles" at the base of narrow peaks following resolution enhancement. This command is available only as a keyboard command. A similar command, **3S**, performs a 3-point smoothing operation.

3S -- 3-Point Smooth

Performs a 3-point running average to reduce apparent noise in the currently displayed data set. This is useful, for example, to remove the "wiggles" at the base of narrow peaks following resolution enhancement. This command is available only as a keyboard command. A similar command, **2S**, performs a 2-point smoothing operation.

SO -- Smooth

This two letter command (non-2-letter command SMOOTH) was added to allow more flexibility in a smooth operation. With no arguments (or in the 2-letter command mode), a three point smooth is done. In the non-2-letter command mode, the first argument is used as the number of points to smooth. An optional second argument is the number of times to repeat the smooth operation.

CH -- Chloroform lineshape display

Used for viewing the lineshape at the base of the peak. This command performs two actions: The frequency scale is adjusted to make the largest peak in the currently displayed region equal to zero (see SZ command) and the vertical scale is increased by a factor of 80.

CL -- Chloroform lineshape

Displays horizontal lines on the screen and plot at 0.55% and 0.11% of the largest peak in the currently displayed region. The command is a toggle, so entering it a second time turns off the displayed lines.

With the lines displayed, the cursor can be used to read off width at each height.

AO – All subroutines off

Exits all subroutines and returns NUTS to the Base Level

XX – Exit NUTS

Closes the NUTS program.

Advanced 1D Features

Automation

Command "links"

LI - Linked Command Lists

This section describes the use of "Links", a simple form of automated processing.

Sequences of commands can be strung together and executed with a single command. This can be used as a shortcut for frequently used command strings or for processing 2D spectra or a series of 1D spectra. The current version of NUTS provides for 10 such command strings, labeled L0 through L9. Typing **LI** or **L#** (where # is a number between 0 and 9) brings up a dialog box and allows editing of the command strings. Spaces are ignored when the string is edited, but can be inserted to make the list more readable. The Linked Lists can be defined in the NUTS.INI file, in which case, their definitions are established each time NUTS is run.

Note that the following description applies only for NUTS in the "2-letter command" mode. Versions of NUTS newer than 5/15/99 have the option of longer commands, which requires that all commands be terminated with <ENTER>. An explanation of modifying Links for this new command mode is given below. See also detailed description of this new command mode in the Commands section at the beginning of this manual.

A command string can be executed by clicking on the corresponding button in the dialog box and then clicking OK. Equivalently, the command string can be executed by tying the command A#, where # corresponds to the chosen L#. A simple example of a useful Link would be

BC FT AP

which performs baseline correction of the FID followed by an FT and then automatically phases the spectrum, with a single command.

For processing 2D data or a series of 1D spectra, the Link is terminated with the IN command, which loops back to the first command in the Link and increments the slice number (for 2D data) or the file extension (for 1D spectra). By default, the link will continue to loop until no more files are found.

It is possible to limit the number of times a Link is executed by setting the Link Limit. This can also be set for Links embedded in macros.

Control characters in Links and Macros are input using the ^ character (shift-6). For example, ^**I** (to represent Ctrl-I) can be used to toggle integrals on and off.

^M is used to execute a carriage return, as is needed to exit a subroutine.

To import data in a Link, use the IM command, rather than GA.

When using GA in a Link where File A is a 2D file, **always** execute a GA manually just before executing the Link. The reason for this is to force NUTS to read the file header so that all parameters will be set correctly.

Packing 1D spectra into a "2D" file

The following link will create a 2D file from a series of 1D files. The 1D files must have sequentially numbered file extensions.

GA SC IN

NUTS will prompt for the first in the series of 1D files to be read, and then will ask for a file name under which to save the 2D data file it will create. It will stop when it cannot find the next file in the series. The new 2D file must be opened to view the data.

L0 - L9 -- Edit Link

Displays the current links (command lists) for examination and editing. A chosen sequence is executed with the command A#. These command sequences can also be set in the NUTS.INI file.

A0 - A9 -- Execute Link

Executes the commands in the corresponding link. The links have default values which are set by the NUTS.INI file. If there is no NUTS.INI file or if the links are not set in the NUTS.INI file then all links are set to

BC EM FT PS

To edit one of the links, type L# (eg., L8). This opens up a screen showing the current definitions of all 10 links and allows them to be edited. Spaces make the list more readable and are ignored.

IN -- INcrement slice number or file extension

Used in Links for incrementing file names and looping back to the beginning of the Link. It is always the last command in a Link which is executed multiple times. IN behaves slightly differently depending on whether the files being read and written are 1D or 2D files. When the file is a 1D file, IN causes the file extension to be incremented. When the file is a 2D file, IN increments the slice number. When all files or slices have been processed, the IN command beeps and exits the link.

By default, the Link will be repeated until NUTS fails to find the next file or slice. However, it is possible to limit the number of times the Link is executed by specifying a Link Limit in the Link dialog box. For Links embedded in macros, this is set with the command

Set LinkLimit

See also: 2D Processing

IA -- Increment slice for file A

Advances the slice counter of a 2D file. This command was created for use in processing interleaved hypercomplex (States type) 2D data, in which pairs of FIDs are processed, then combined. After the first FID of a t1 quadrature pair has been processed, the counter must be incremented before reading in the second FID. After the reconstructed t1 interferogram is stored with **ST**, the linked command list is terminated with the **IN** command, which both increments the file counter and loops back to the start of the link.

In Arrayed Mode, the IA command advances the slice counter and makes that the current slice.

IE -- Increment file Extension

Used in Links to increment the file extension. This operates on FileA and FileB. This should not be confused with the IN command, which both increments the file extension and loops back to the start of the Link. If all files are to be processed in the Link, the IN command is used. The only time one might use IE is to perform multiple increments within a single loop of the Link.

For example, start with a series of 40 1D files with sequential extensions. The user decides he/she only wants to process every other file. The link would then look like

GA BC EM FT SC IE IN

The 2D file created by this Link would have 20 slices, corresponding to every other file from the 1D series.

(Added 8/21/00) When used in the non-two-letter command mode, IE (or IncrementExt) can now take arguments. With no argument, the command acts as described above (increments the file extensions of FileA, FileB and the Import File name if they have numerical file extensions which can be incremented). The new functionality allows the use of arguments. If IE is followed by either "A" or "B" with no second argument, then the extension for file A or B, respectively, will be incremented by one. If there is a second argument it will be used as the amount by which the file extension is incremented. If the second argument is "2" the file extension will be incremented by 2, etc. Negative

numbers are allowed as long as the decremented (negative increment) extension will be greater than zero.

IE [A or B] [amount]
IncrementExt [A or B] [amount]

It is important to understand the subtle distinction between the commands "IA" and "IE A". IA increments the ***slice counter of a 2D file*** to the next slice. "IE A" changes the current name of file A by incrementing its file extension (provided file A's name has a numerical file extension).

The use of IE in this mode is illustrated in a sample macro below.

EI – increment extension for file export

A separate file name can be set as the target for file exports. The EI command is used to increment the numerical file extension, so exporting a series of files can be accomplished in a Link or Macro

Using Links in non-2-letter command mode

When NUTS is operating in the new command mode, which allows use of commands longer than 2 letters, Links must be modified to tell the program to insert <ENTER> after each command. This is done by inserting a comma after each command. Spaces between commands are ignored. For example, the following link will run correctly in the new command mode:

GA , BC , EM , FT , AP ,

Macros

NUTS has the capability of executing a series of commands contained in a text file.

RU – Run macro

The macro is executed from the File menu or using the keyboard command **RU**. Several sample macros are available below and at http://www.acornnmr.com/. Note that these sample macros require that NUTS be operating in the 2-letter command mode. The newer command mode, which allows use of commands longer than 2 letters, requires that each command be terminated with <ENTER>. Commas must be inserted following each command, which NUTS will interpret as an <ENTER>.

The text file can be created in the Windows Notepad or any word processor and saved as an ASCII file. To execute a macro, type **RU** or choose Run Macro from the file menu. For convenience, the macros can be mapped to **Control-Fx** keyboard keys. The

assignments are made in the Nuts.ini file. A macro can also be executed automatically when Nuts is started. The macro to be run is set in the Nuts.ini file, with a line such as

AutoExecMacro = C:\NUTS\MAC\GET_EB.MAC

The first line of a NUTS macro must be

NUTSMacro

A line can be any valid NUTS command or command string, the same as those used in Links. NUTS will ignore blank lines or any line which starts with a space or semicolon. The last line of the macro should be END.

NUTS looks at the first word on each line of a macro and expects to find either a valid NUTS command or **SET**, **ASK**, **DO**, **LOOP**, **CALL** or **QUESTION**, as described below.

Set is used to set the value of some parameter. The format of the Set command is

Set parameter [value]

Some parameters require a value to be specified; others do not. For a list of valid parameters, see below.

Ask is used to request user input. The format of the Ask command is:

Ask parameter where "parameter" can have one of the following values:

FileA, FileB, FileC, FileImport, ExportFile, Shift, SL, LB, GF, T1, T2 and S#

When a value for SL (2D slice number) is entered with the Ask operation, the slice will be read with the next GA command, not immediately.
When a value is entered for Shift, the shift value is not set until a PR command is executed. PR sets the largest peak in the displayed region to the value which the user has previously entered.

Do is used to perform an action. The following are valid uses of Do in a macro:

Do DeleteFile filename This deletes a file with name "filename". Wildcards are allowed on the Windows versions but only a single name is allowed on the Macintosh versions. Note that NUTS does not ask for confirmation, so use with caution!

Do Extract_PPM f1 f2 This is used to perform an extraction of a spectral region defined by f1 and f2 (in ppm). This is similar to the XT command.

Do Sleep This command causes the macro to pause. The amount of time must first be specified with Set Sleeptime x, where x is time msec.

Do increment_lf_get_filename Increments file extension for file used by LF routine to open an existing file.

Do increment_lf_write_filename Increments file extension for file used by LF routine to write a file.

Do increment_export_filename Increments file extension for file used by export commands

Do Math -- performs Math functions. There are numerous commands which perform automated calculations. These are described in the Math section.

Loop is used to execute several lines in a macro a certain number of times. The format is

Loop x y where x is how many times the loop is executed, and y is how many macro lines comprise the loop

Call is used to lauch an external program. NUTS calls a .pif file, which must be "windowed" (no window will be shown). When specifying the path for the .pif file, all backslashes must be doubled. This is available on in the Windows version of NUTS. Example:

call c:\\copyfile.pif

Question – It is possible to have a macro pause during execution to display a question and wait for user response.
Each question needs to start with one line which is "QUESTION". The following lines are for the question - up to 15 lines - then four addition lines

First line starts with YES_BUTTON <*desired action*>
Next line starts with NO_BUTTON <*desired action*>
Next line starts with CANCEL_BUTTON <*desired action*>
4th and LAST line needs to be END_QUESTION

<*desired action*> can be one of three things

1. ABORT
2. CONTINUE
3. New macro name to start

Sample macro:

```
nutsmacro
QUESTION
Click YES to continue the macro execution with the next line after this
question.
Click NO to abort the macro execution.
Click CANCEL to abort the macro execution.
YES_BUTTON CONTINUE
NO_BUTTON ABORT
CANCEL_BUTTON ABORT
END_QUESTION
ft
end
```

Control characters in Links and Macros are input using the ^ character (shift-6). For example, **^I** (to represent Ctrl-I) can be used to toggle integrals on and off.

^M is used to execute a carriage return, as is needed to exit a subroutine.

Sample macros are supplied which can be used as a starting point for creating customized macros. When writing a new macro, it is advisable to test each new part (by commenting out parts not needing testing) to be sure it does what the user expects. The display is not updated during macro execution (to save time), so it is not possible to see the results of each step (although commands are echoed on the command line); hence the recommendation for testing.

Some of the sample macros perform operations which result in files being written at intermediate stages. The macro sets default names for these files so the user does not need to enter them. Any such macro will overwrite those files the next time it is run, so files which the user wishes to save must be renamed before the macro is run again.

Adhering to two rules in writing macros will help avoid problems:

1. **Always** follow "Ask FileA" (or "Ask FileB") with a line consisting of "GA" (or "GB"). See explanation below.

2. When using GA (or GB) in a Link where File A (or File B) is a 2D file, **always** precede the Link with a line consisting of "Set SL 1". The obvious reason for this is to be sure the slice pointer is set to the beginning of the 2D file. See complete explanation below.

Sample macros are included with NUTS and are in a subdirectory of the NUTS directory called mac.

Rule 1:

When using the Set command to specify a file name, the default path name is the current working directory. The current working directory is established each time a GA or GB command is completed. Therefore, executing a GA after an Ask FileA command:

Ask FileA

GA

establishes the working directory, and subsequent Set File commands will use that directory.

If the user wants to specify a different path for a file in a Set command, use

Set FullFileA name

Note that if a new path is specified for file A or B, the working directory will be changed when a GA or GB command is next executed.

Rule 2:

The user needs to be aware that the command GA (open file A) when applied to a 2D file behaves slightly differently in macros or Links from when it is entered directly. The difference lies in whether NUTS reads just a slice of the 2D data or reads both the slice and the data header. While in a Link (either by itself or within a macro), NUTS reads the data header only once, for the first slice. This makes reading subsequent slices faster. When GA is used to read a 2D file in a Link, NUTS assumes that the user loaded the first slice manually (with GA) immediately before executing the Link. This reads in the file header and all is well. If the same Link is embedded in a macro, NUTS can be forced to read the file header by inserting the following line just before the line containing the Link:

Set SL 1

FF - Find File

This command allows batch processing of an entire directory of NMR data files. This only makes sense if used in a macro, such as the following:

```
NUTSMacro batch processing
ff ft sa in
end
```

When the macro is executed, NUTS will ask for a file to open. By selecting a file, the user defines the directory whose data is to be processed in batch mode. NUTS will attempt to identify and open each file in that directory. If a file is encountered that cannot be opened, NUTS will display an error message, then continue. The processed data is saved into a subdirectory of the chosen directory called **_Target**.

Some spectrometer software (eg, Varian) does not save data as a single file, but as a directory which contains several files. The FF command will open each subdirectory of the selected directory and look for identifiable NMR data files, and will import any it finds. This does not work with Bruker XWin data, because the fid file is 2 directory layers down.

The FF command can run into initialization/de-initialization problems if it does not complete its operation of opening every file in the directory. A FF command line argument (reset) can be used to force the FindFile status to be de-initialized.

SET parameters

Parameters which can be set in macros with the SET command.

File names:

FILEA File A; current working directory will be used; See note below

FILEB File B; current working directory will be used; See note below

FILEC File C; current working directory will be used; See note below (File C is used for 2D processing)

ExportFile Name of file to be exported by E1, E2, E3, E4 or E5 command.

FILEIMPORT To set the file name of data to be imported

FULLFILEIMPORT To specify a complete path name for data to be imported

FULLFILEA To specify a complete path for File A; See note below

FULLFILEB To specify a complete path for File B; See note below

FULLFILEC To specify a complete path for File C; See note below

LF_GET_FILENAME To specify filename used by LF routine to read an existing Lines file

LF_WRITE_FILENAME To specify filename used by LF routine to write a Lines file

Display:

DC To set the DC (vertical display) offset of the data

DISPLAYALL Display both real and imaginary points

DISPLAYFULL Display real points

DISPLAYIMAG Display imaginary points

DISPLAYZOOM Expand spectrum to previously set frequency limits

Fonts:

FONT_AXIS +10 To change the font size of the clipboard displayed on screen (in this case, by one point)

FONT_CLIP +10 To change the font size of the clipboard displayed on screen (in this case, by one point)

FONT_PEAK_HORIZONTAL +10 To change the font size of the horizontal peak labels (in this case, by one point)

FONT_PEAK_VERTICAL +10 To change the font size of the vertical peak labels (in this case, by one point)

FONT_INTEGRAL_HORIZONTAL +10 To change the font size of the vertical peak labels (in this case, by one point)

FONT_INTEGRAL_VERTICAL +10 To change the font size of the vertical peak labels (in this case, by one point)

FONT_CmdLine +10 To change the font size of the screen's command line (in this case, by one point)

Axis:

AXISHZ Set axis units to Hz

AXISNONE No axis label

AXISPPM Set axis units to PPM

AXISPT Set axis units to points

Zoom limits:

ZOF1PT Set left hand frequency limit in units of points for Zoom region

ZOF2PT Set right hand frequency limit in units of points for Zoom region

ZOF1PPM Set left hand frequency limit in units of ppm for Zoom region

ZOF2PPM Set right hand frequency limit in units of ppm for Zoom region

ZOF1HZ Set left hand frequency limit in units of Hz for Zoom region

ZOF2HZ Set right hand frequency limit in units of Hz for Zoom region

ZOF1SL Set lower slice limit in second dimension for 2D Zoom region

ZOF2SL Set upper slice limit in second dimension for 2D Zoom region

ZOF12DPPM Set bottom frequency limit in ppm in second dimension for 2D Zoom region

ZOF22DPPM Set top frequency limit in ppm in second dimension for 2D Zoom region

ZOF12DHZ Set bottom frequency limit in Hz in second dimension for 2D Zoom region

ZOF22DHz Set top frequency limit in Hz in second dimension for 2D Zoom region

ZOOM_REGION Allows the user to define up to 10 Zoom regions, similar to Zoom sub-commands 0-9. Syntax is

Set Zoom_Region n f1 f2

where n is the region identifier (0-9) and f1 and f2 are frequency limits in ppm.

Window functions:

GF Gaussian Factor used in Lorentzian/Gaussian resolution enhancement.

LB Linebroadening used with EM, GM, LG and TF commands

S# Set phase shift for sine apodization (MS).

T1 First point for trapezoidal multiplication (TM)

T2 Second point for trapezoidal multiplication (TM)

Linear Prediction:

LNpts Number of points to be predicted by Linear Prediction (LN)

LNmdim Number of points on which Linear Prediction (LN) is based

LNnsig Maximum number of frequencies to be predicted by Linear Prediction (LN)

LNdirection (FORWARD or BACKWARD) used by Linear Prediction (LN)

Integration:

INTEGRAL CLEAR clear all defined integral regions

INTEGRAL 3.0 5.0 to define an integral region (in this case, from 3 to 5)

INTEGRAL 4.2 1.5 5.0 to define and integral (in this case from 4.2 to 1.5) and also assign its value (5, in this case)

Other:

AC Set value for Amplitude Change (AC) command.

AM Set value for Add/Subtract multiplier (AM) command.

ARRAY_ON Enter Arrayed Mode for 2D (original arrayed mode only, does not initiate "pairwise" arrayed mode)

ARRAY_OFF Exit Arrayed Mode for 2D

DP_INFO_STRING Enter text which will be entered in the Label field for all peaks when DP is executed.

LINKLIMIT To specify the number of loops for a Link containing IN. The default is to loop until no more files can be found.

MH Minimum height for peak picking

OF1 Sets the o1 frequency (the frequency of the center of the spectrum) to the specified value, in hertz.

OF2 Sets the o2 frequency (the frequency of the center of the spectrum in the second dimension) to the specified value, in hertz.

PA Zero-order phase correction used with PC

PB First-order phase correction used with PC

POINTS_TO_AVERAGE Sets the number of points to be used with baseline correction commands BF and FR. This is the same as the 2A command.

RDpts n where n is the number of pts to rotate with RD command

RDdirection left (or right) used by RD command

RM RMS noise multiplier used in peak picking

S@ Number of points by which SH will reduce the data set

SHIFT 2.5 To set the chemical shift of the largest peak in the current region (in this case, to 2.5 ppm). If a second value is supplied, separated by a space, this command can be used to set the shift in both dimensions at the same time. The values specified must be in ppm.

SL sets 2D slice number

Sleeptime x Sets the number of milliseconds that the macro will pause when Do Sleep is used.

SPPLOTX Sets x offset for an SP plot.

SPPLOTY Sets y offset for an SP plot.

Note:

When using the Set command to specify a file name, the default path name is the current working directory. The current working directory is established each time a GA or GB command is completed. Therefore, it is a good practice to execute a GA after an Ask FileA command:

Ask FileA

GA

This establishes the working directory, and subsequent Set FileX commands will use that directory.

If the user wants to specify a different path for a file in a Set command, use

Set FullFileA name

Note that if a new path is specified for file A or B, the working directory will be changed when a GA or GB command is next executed.

Sample macros

This file contains NUTS macros for automated processing. Note that these macros require that NUTS is operating in the 2-letter command mode. The new command mode, allowing use of commands longer than 2 letters, requires that all commands be terminated with <ENTER>. This new command mode provides additional flexibility in creating macros, but NUTS must be in the older 2-letter command mode to run the macros shown here. This can be done by executing 2N before running these macros.

The following macros included below. Others can be found on the web site.

Completely automated 1D processing, including integration and inset plots of downfield regions
Example of Looping and Sleep in a macro
Summing slices of a 2D data set

See also:

description of macros
Arrayed Mode 2D processing
processing Varian 2D data
processing TPPI data
processing magnitude hetcor data
DEPT editing for Varian data

This macro was created to process a large set of ^{1}H data to create "survey" spectra. See Help on each command for details.

```
NUTSMACRO
;FT, auto-phase and baseline correct
set lb 0.2
; set zoom region 5 to 10->0 ppm, for plotting
set zoom_region 5 10 0

;set zoom region 6 for aromatics (7 to 10 ppm)
set zoom_region 6 10 7
```

```
;set zoom_region 7 to downfield region (10 to 15 ppm)
set zoom_region 7 15 10

; first, clear previous integrals and inset plots
; then import, FT and phase
; the next line must be changed, to set the number of times the loop
will be executed
loop 100 44
pf idc^m isc^m ^f af mf
ff bc ft ap it em ft qb

; initialize size & position for first IS inset
2f
is xsize .55,
is ysize .5,
is xpos 0,
is ypos 0,
2n,

; display aromatics full scale and create inset
z6 ^e mf isa^m

; initialize size & position for downfield IS inset
2f
is xsize .25,
is ysize .1,
is xpos 1,
is ypos 0,
2n,

; increase vertical scale 3x and create inset
set ac 3
ac
z7 ^e isa^m

; display 10->0, adjust scale, PP and enter integration
z5 ^e
set ac 0.1
ac

; set MH for peak picking
set mh 10
pp

; auto-integrate
set mh 1
ai
an

;save processed file
sa

end
```

This macro steps through planes of a 3D data set, allowing 1 sec between display of planes. The Loop command takes 2 parameters: the number of times the loop is repeated, and the number of lines that comprise the loop. So this macro executes the 2 operations (sleep and]) 48 times.

(The "]" command increments to the next plane. Note that this command is active only in Arrayed 3D Mode.)

```
NUTSMacro   display planes
; first, set time to sleep, in msec
set sleeptime 1000

; define loop,  execute the next 2 lines, 48 times
loop 48 2
do sleep
]
end
```

The command **sum** requires that NUTS be in the "non-2-letter" command mode. This is set/unset with the 2F and 2N commands, respectively. The **sum** command takes 2 arguments, which are the first and last slices to be included in the sum. Note that the sum becomes the current data (1D) set. If in arrayed mode, save the data before performing the sum.

```
NUTSmacro sum slices
; request name of 2D data set, and open it
ask filea
ga

; turn off 2-letter command mode
2f

; sum slices 3 through 10, inclusive
; note that each line in this command mode must be terminated with ","
sum 3 10,

; return to 2-letter command mode
2n,
end
```

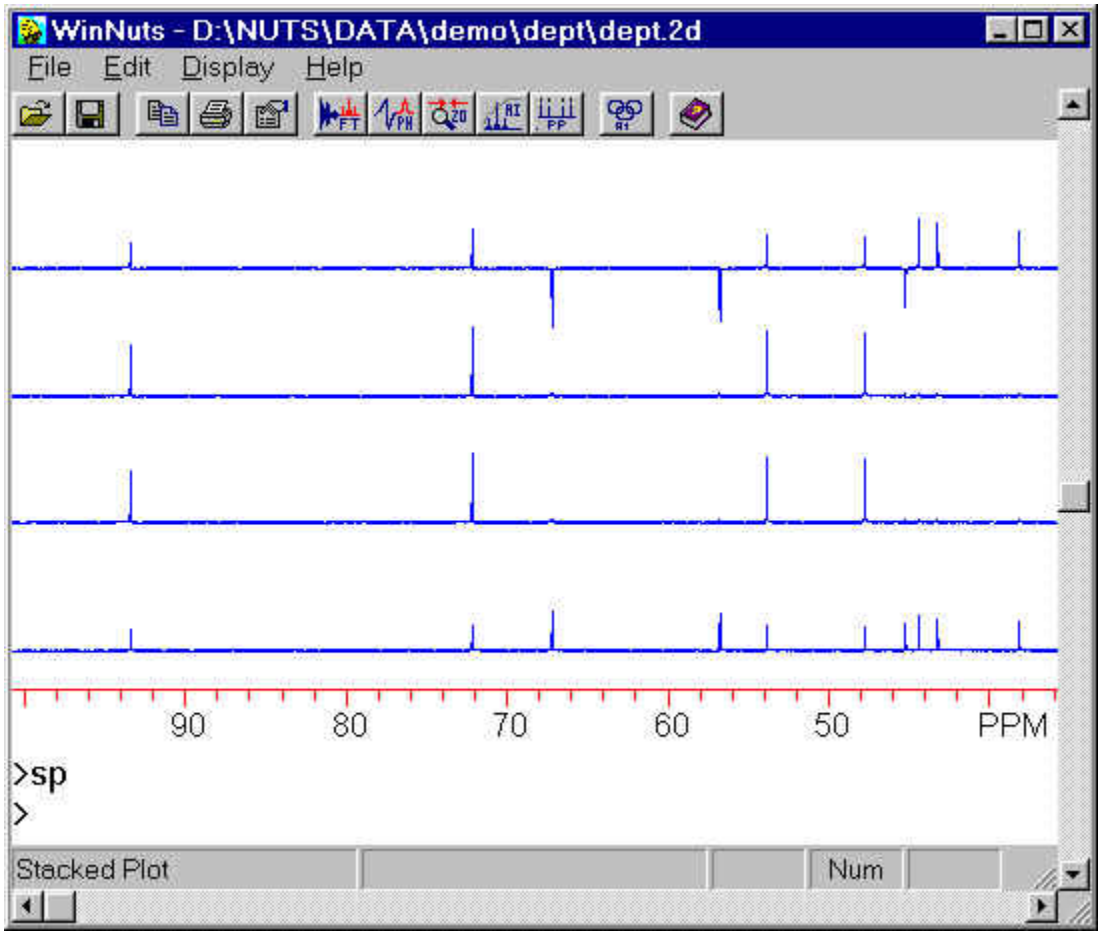
And another example, illustrating use of the IE (increment file extension) command to perform a series of sums. This creates 4 1D files (sum.001 thru .004), each of which is the sum of 3 slices from the original 2D data.

```
NUTSMACRO sum by 3s
ask filea
set sl 1
set fileb sum.001
ga
2f
sum 1 3,
```

```
sb,
ie b,
ga,
sum 4 6,
sb,
ie b,
ga,
sum 7 9,
sb,
ie b,
ga,
sum 10 12,
sb,
2n,
end
```

Processing Varian DEPT data

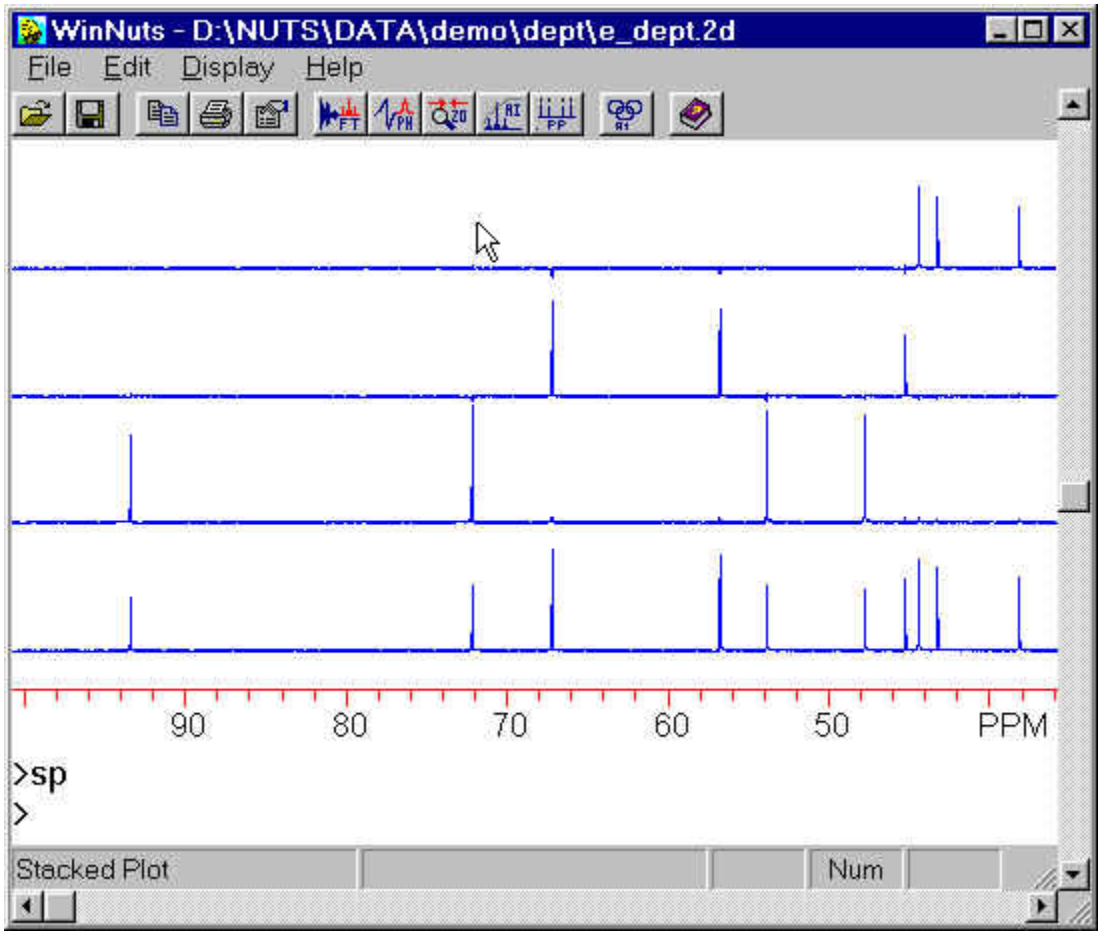
Varian's automated DEPT pulse program generates an arrayed experiment with 4 slices, acquired with phase of the last pulse equal to 45, 90, 90, 135 degrees. The macro below FTs all slices, then combines them to yield an edited stacked plot.



After FT but before "editing", the slices are (top to bottom):

CH_2 inverted, CH and CH_3 up (final pulse = 135)

CH only (final pulse = 90)
CH only (final pulse = 90)
all carbons (final pulse = 45)



After "editing", the slices are

CH_3 only
CH_2 only
CH only
all carbons

Before running this macro, import the data, FT and phase the first spectrum, to determine the phase correction.

```
NUTSMacro edited DEPT
; for Varian std DEPT sequence
; where slices 1 thru 4 are acquired with angle of last
; pulse = 45, 90, 90, 135
; first, import arrayed data, FT 1st slice and phase
; final result is stacked plot with slices
; displayed, from top to bottom:
; CH3
```

```
; CH2
; CH
; all protonated
; macro asks for file name for imported FID array
ask filea
ga
set sl 1
set filec dept.2d
; first, FT all slices
ga bc em ft ps sc in
set filea dept.2d
ga
set sl 1
ga
; 1st slice contains all peaks positive
; multiply it by 3 and save
set am 2
al
as
set fileb dept.1
sb
; slice 4 + 1.3*slice 1 removes CH2
ga
set sl 1
ga
set am 1.3
al
set sl 4
ga
as
; slice 2 minus this leaves only CH3
; but inverted
set pa 180
set am -1
al
set sl 2
ga
as
set fileb dept.4
pc
sb
; slice 3 minus 2*slice 4 eliminates CH peaks,
; leaves CH2 up, CH3 down
ga
set sl 4
ga
set am -2
al
set sl 3
ga
as
; now add this to inverted CH3 only
set am 1
al
set sl 1
set fileb dept.4
gb
```

```
as
set fileb dept.3
sb
; save 2 x slice 2 for CH only
set filea dept.2d
ga
set sl 2
ga
al
as
set fileb dept.2
sb
; finally, combine edited slices into 2D file
set filea dept.1
set filec e_dept.2d
ga
ga sc in
set filea e_dept.2d
ga
ss
mf
set ac .5
ac
set spplotx 0
set spploty 2
sp
end
```

Add/Subtract and Dual Display

This section describes how to display 2 spectra on the screen simultaneously (Dual Display) and how to add and subtract 2 spectra.

See details below on how to use Add/Subtract.

DD -- Dual Display

Toggles dual display on and off. A spectrum must have previously been placed into the Add/Subtract buffer using the **AL** command. Dual Display is also available from the View menu. When DD is activated, the spectrum previously stored in the Add/Subtract buffer is displayed above the current spectrum. The spectra can be vertically scaled together in the same manner as for single spectra, and Zoom can be used for expansion. The spectrum in the DD buffer can be scaled by typing **AM**. This brings up a dialog box that allows a multiplying factor to be entered. Within this same dialog box, the horizontal and vertical offsets of the spectrum in the buffer can also be adjusted. Be careful to exit Zoom before executing this command, as NUTS will interpret DD as 2 baseline adjustment operations.

AS -- Add/Subtract Subroutine

This is a subroutine that allows addition and subtraction of the current spectrum and a second spectrum which has been loaded into the add/subtract buffer. First, a spectrum (or

FID) is loaded into the Add/Subtract buffer with the command **AL**. Then a second spectrum is opened with **GA**. Note that the spectrum in the buffer remains in the buffer and unchanged until another spectrum is loaded into the buffer with AL.

Typing **AS** or choosing Add/Subtract from the Tools menu enters the Add/Subtract subroutine. The menu choices change to those that are active in the subroutine. The spectrum in the buffer is displayed above the current spectrum. Subcommands (listed below) within the subroutine are single letter commands executed immediately, and are available either from the keyboard or from the menus. Typing <Enter> exits the subroutine.

AS can be used in a link or macro, but behaves differently. In this case, the subroutine is not entered. Instead, addition of the buffer spectrum and the current spectrum is automatically executed without further input from the user. The current value of the buffer multiplier (AM) is used. To perform subtraction, set **AM** to -1 before running the link or macro.

The spectra can be added or subtracted by typing **+** or **-**, respectively, or by choosing Add or Subtract from the Edit menu. The resulting spectrum (sum or difference) becomes the current spectrum. The spectrum in the buffer is unchanged. This operation can be un-done by executing the inverse operation.

The difference between the spectra (current spectrum minus buffer spectrum) can be displayed "on the fly" by typing **D** or by choosing Difference from the Display menu. With the difference spectrum displayed, parameters such as Multiplier and left/right offset can be adjusted and the difference spectrum is updated in real time to reflect the changes. Note that Difference mode affects display of the data. Use - (minus sign) to make the subtraction permanent.

The buffer spectrum can be scaled by entering a multiplying factor. The initial value is one. It can be changed by typing **M** or choosing Change Multiplier from the Edit menu.

The buffer spectrum can be shifted left or right by one point using the left and right cursor keys. To shift it by larger steps, hold down the shift key while using the left and right cursor keys, which moves the buffer spectrum in steps of 10 points. The buffer spectrum can also be shifted by any desired amount by typing **O** or choosing Change Offset from the Edit menu, which brings up a dialog box allowing the user to set the offset (in points).

The vertical offset of the buffer spectrum can be adjusted by typing **V** or choosing Change Vertical Position from the Edit menu. The offset is expressed as a percentage of the screen, so that 10 displays the buffer spectrum 10% above the bottom. The vertical offset of the current spectrum can be changed by exiting the AS subroutine, typing **DC** and adjusting the offset using the left scroll bar. When the desired adjustment has been made, type Enter to exit the DC routine and re-enter the AS routine.

Subcommands

B -- Display Both buffer spectrum and current spectrum
D -- Display Difference between the spectra in real time (current spectrum minus buffer spectrum)
M -- Change Multiplier for buffer spectrum
O -- Change horizontal offset of buffer spectrum (in points)
S -- Display current Spectrum only
V -- Change Vertical offset of buffer spectrum
+ (plus sign)-- Add spectra and make resulting sum the current spectrum
- (minus sign) -- Subtract spectra and make resulting difference the current spectrum
Ctrl-C -- Copy screen to Windows clipboard as bitmap. See copying spectra.
Alt-C -- Copy screen to Windows clipboard as Metafile

AL -- Load Add/Subtract buffer

Places the current spectrum into the buffer.

AM -- Add/Subtract Multiplier

Enter the value by which the spectrum stored in the Add/Subtract buffer will be multiplied.

See also: Buffers subroutine

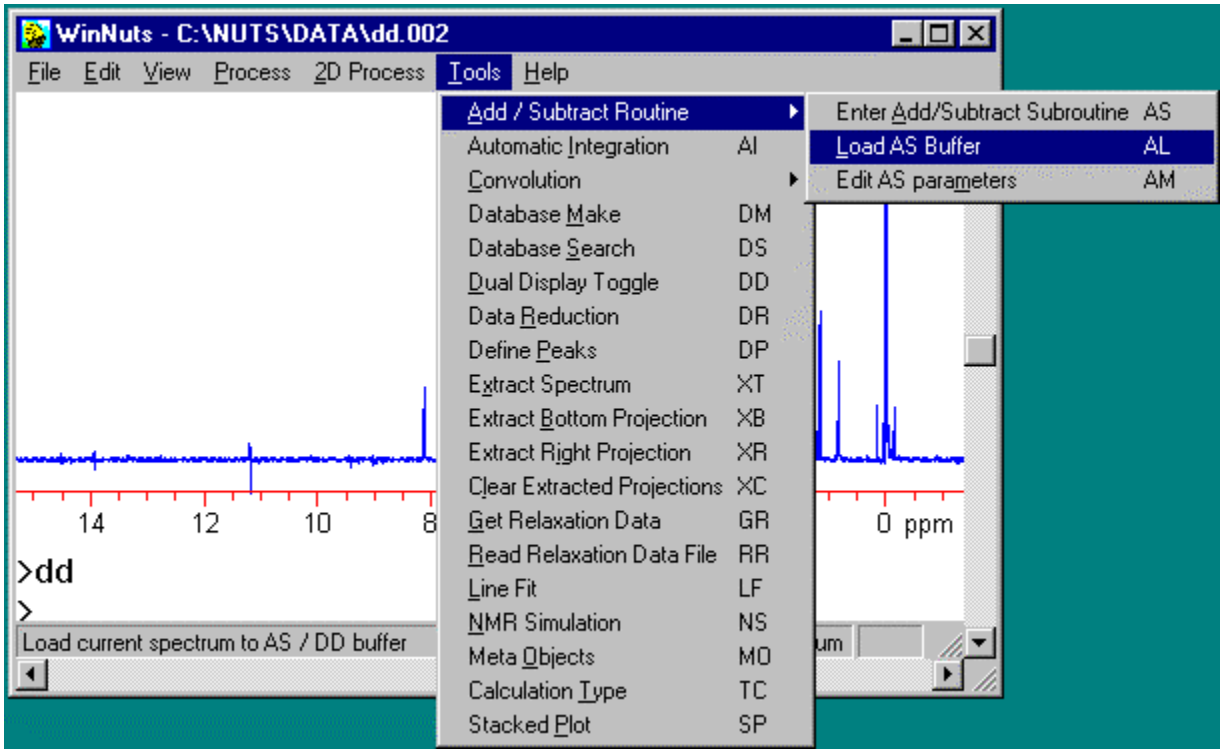
Illustrated example of AS routine

Dual Display allows 2 spectra to be displayed simultaneously. They are scaled together and Zoom expansion applies to both.

The Add/Subtract subroutine allows 2 spectra to be added together or subtracted, and the result becomes the current spectrum.

To display more than 2 spectra, create a 2D data set containing the desired files, and use a stacked plot.

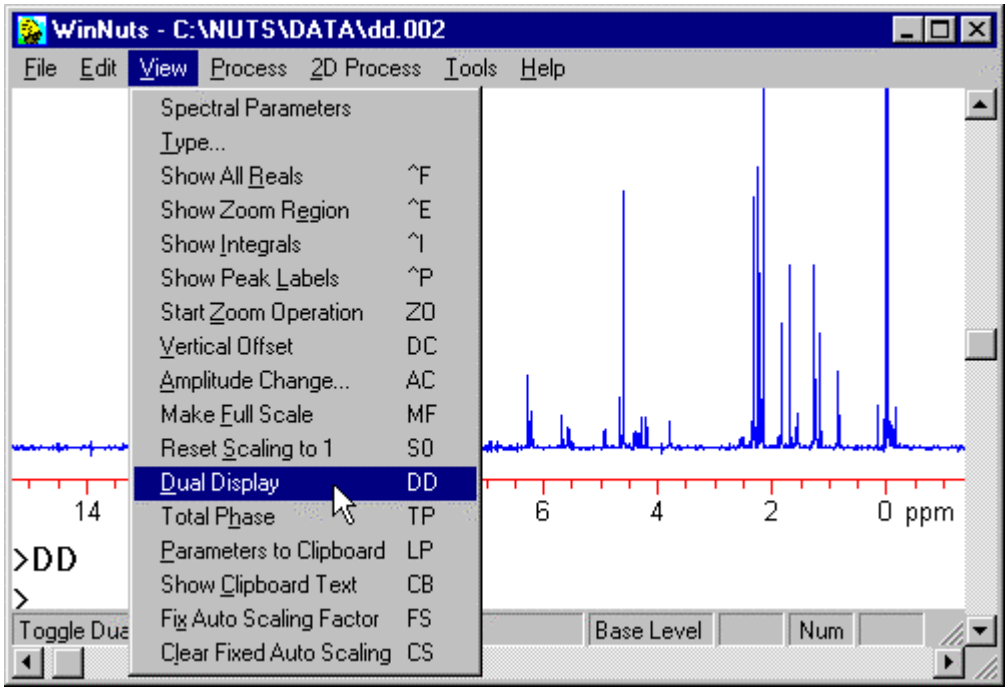
Note that these features are not included in NUTS Lite.



Open the first spectrum to be compared.

Select Load AS buffer from the Tools/Add Subtract Routine menu, or type **AL**.

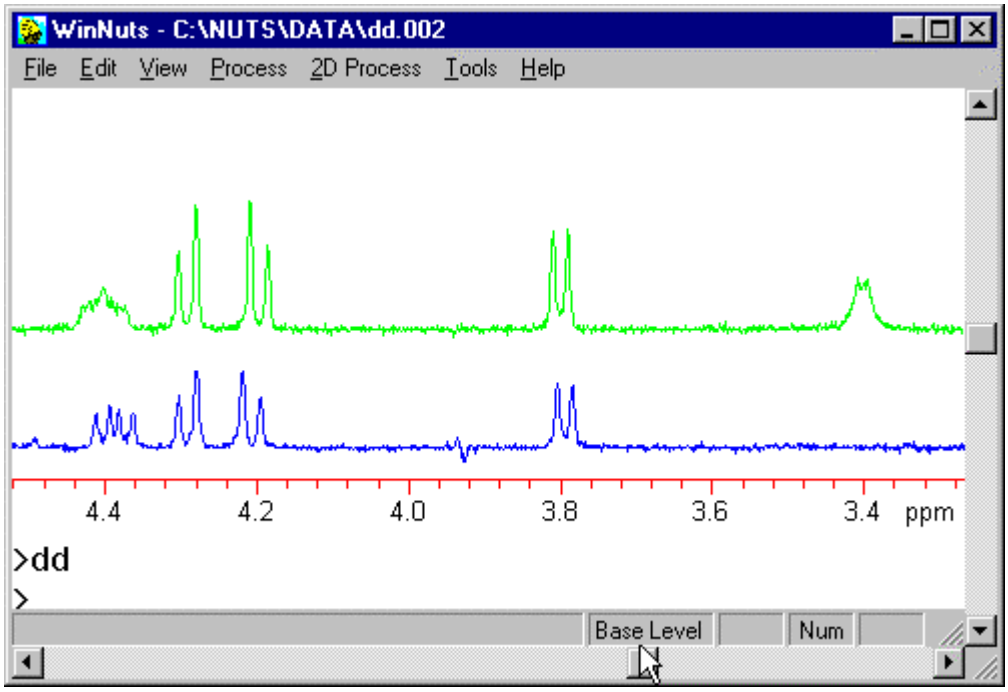
This places a copy of the current spectrum into the Add/Subtract buffer.



Open the second spectrum to be compared.

The contents of the buffer can be displayed above the current spectrum by selecting Dual Display from the View menu, from the Tools menu or by typing **DD**. The buffer is shown in green.

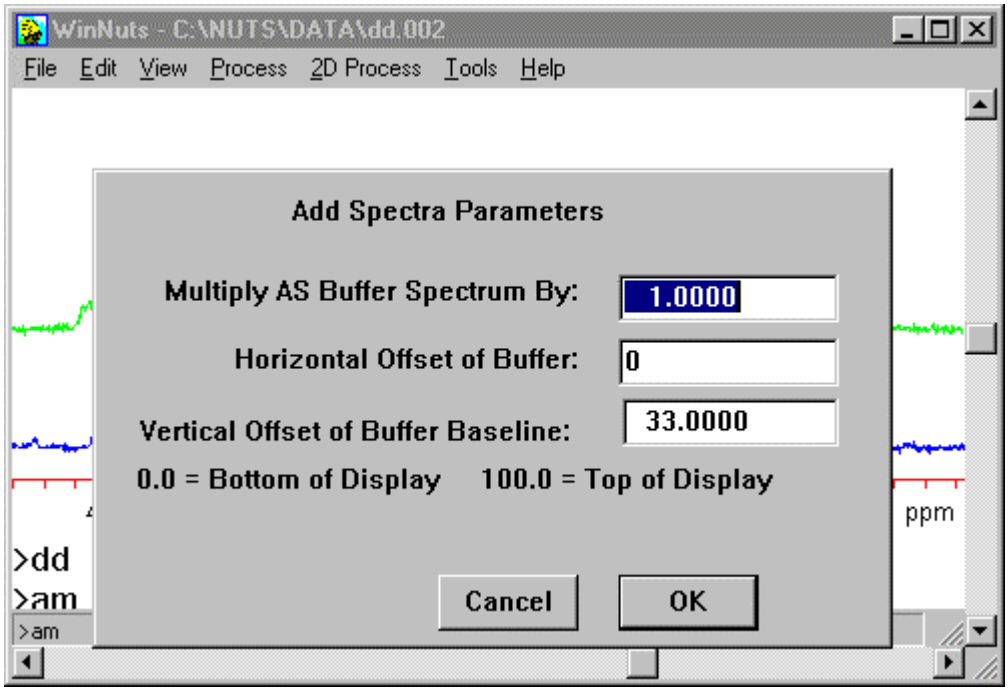
The **DD** command is a toggle, so executing it again will turn off display of the buffer spectrum. The data remains in the buffer, however.



Use Zoom to expand the two spectra together.

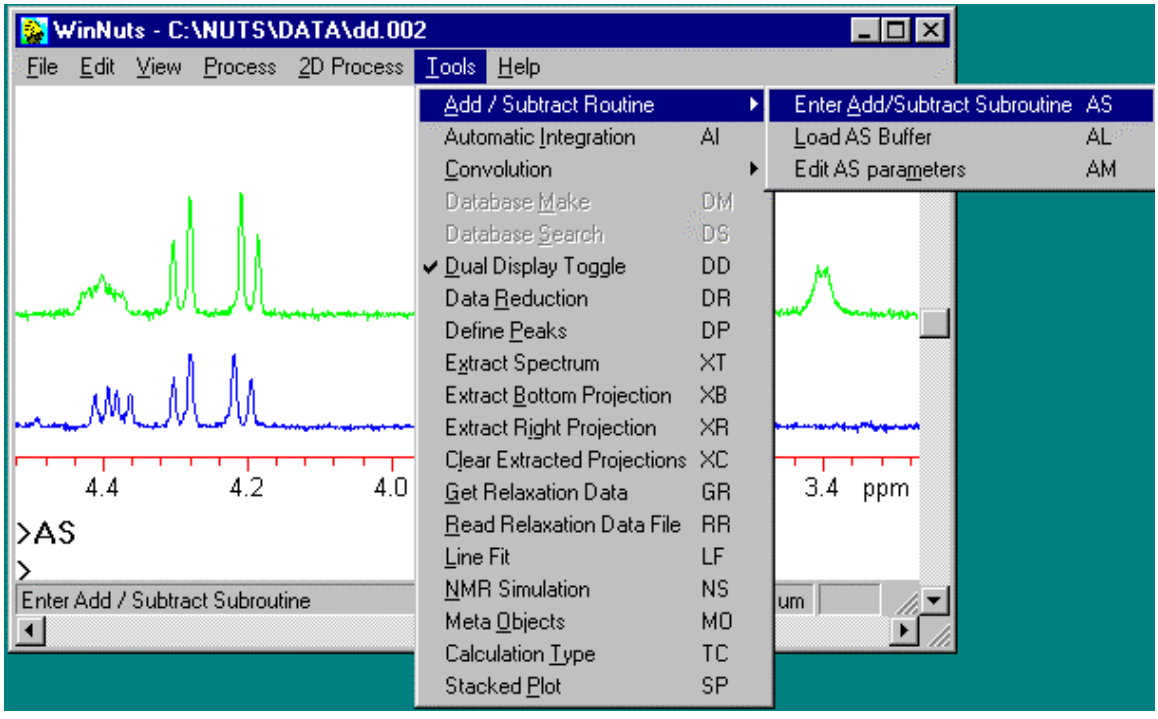
Use the right hand scroll bar, Page Up/Down keys or < > keys to scale the spectra vertically.

Use the scroll bar at the bottom of the screen to pan both spectra.



Use the **AM** command to

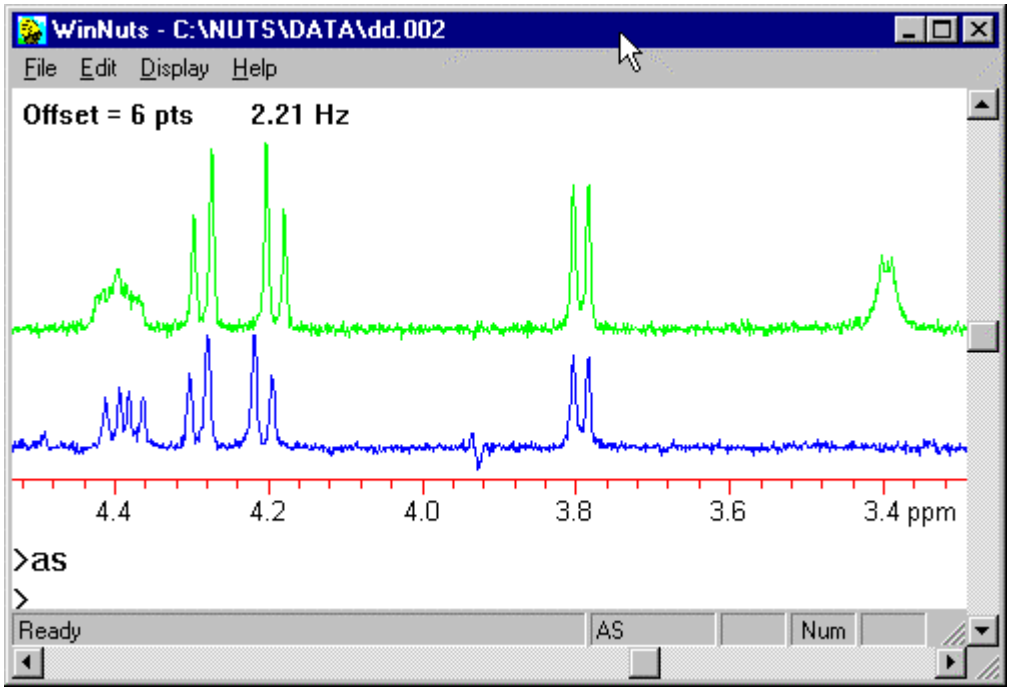
1. Enter a scaling factor for the buffer spectrum
2. Enter a horizontal offset, for use in cases where the spectra do not line up exactly
3. Change the vertical position of the buffer spectrum



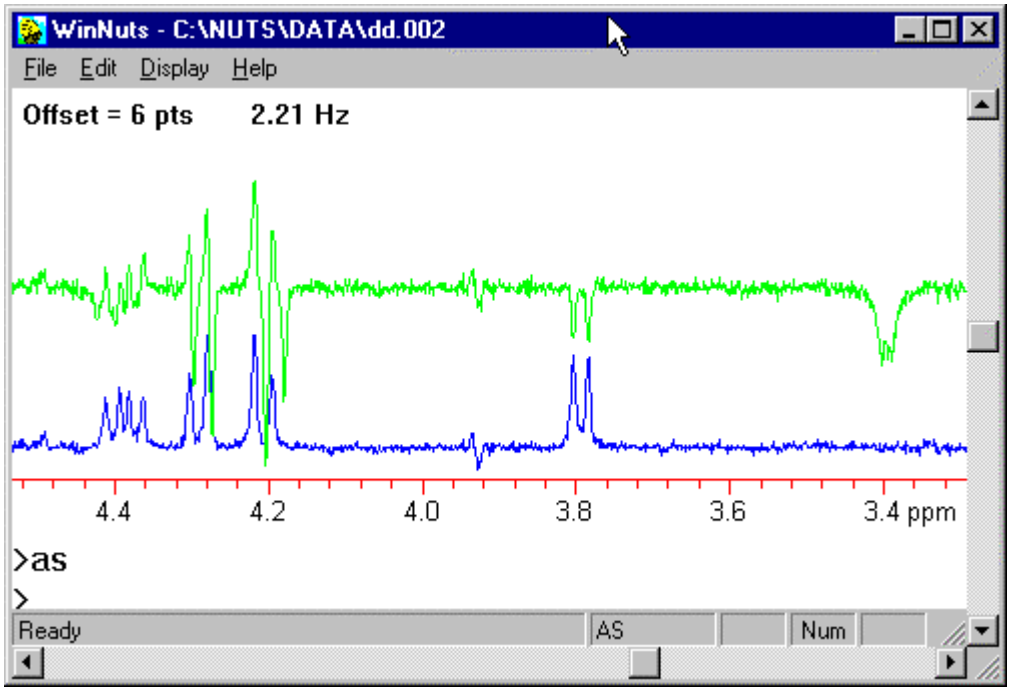
The Add/Subtract subroutine provides more options than the Dual Display mode.

Enter the subroutine from the Tools menu, or by typing **AS**.

Once in the subroutine, you can change the buffer spectrum's multiplier, horizontal offset and vertical position from the Edit menu, (just as was done with the **AM** command above).



It is also possible to shift the buffer spectrum horizontally using the left and right cursor arrow keys. Each key click moves the buffer spectrum one point. Holding down the shift key and using the same cursor keys moves the spectrum 10 points at a time.



From the Display menu, you can choose to display the difference (current spectrum minus buffer spectrum). This is the most convenient way to make adjustments to the vertical scale and horizontal offset to match the 2 spectra for good subtraction.

Choosing Both from the Display menu reverts to display of the buffer spectrum in green, instead of the difference.

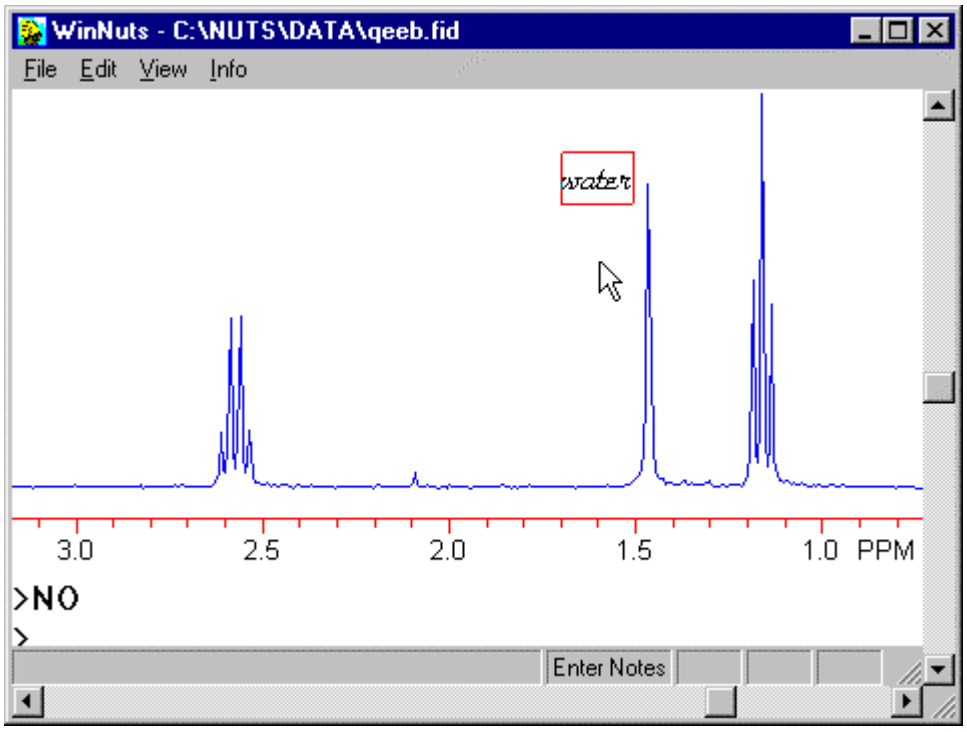
Once all desired adjustments have been made, select Add or Subtract from the Edit menu to replace the current spectrum with the sum or difference (current minus buffer). Either operation can be undone while still in the AS routine by performing the opposite operation.

Once addition or subtraction has been executed and the routine exited (with <Enter>), the current data set has been replaced by the sum or difference.

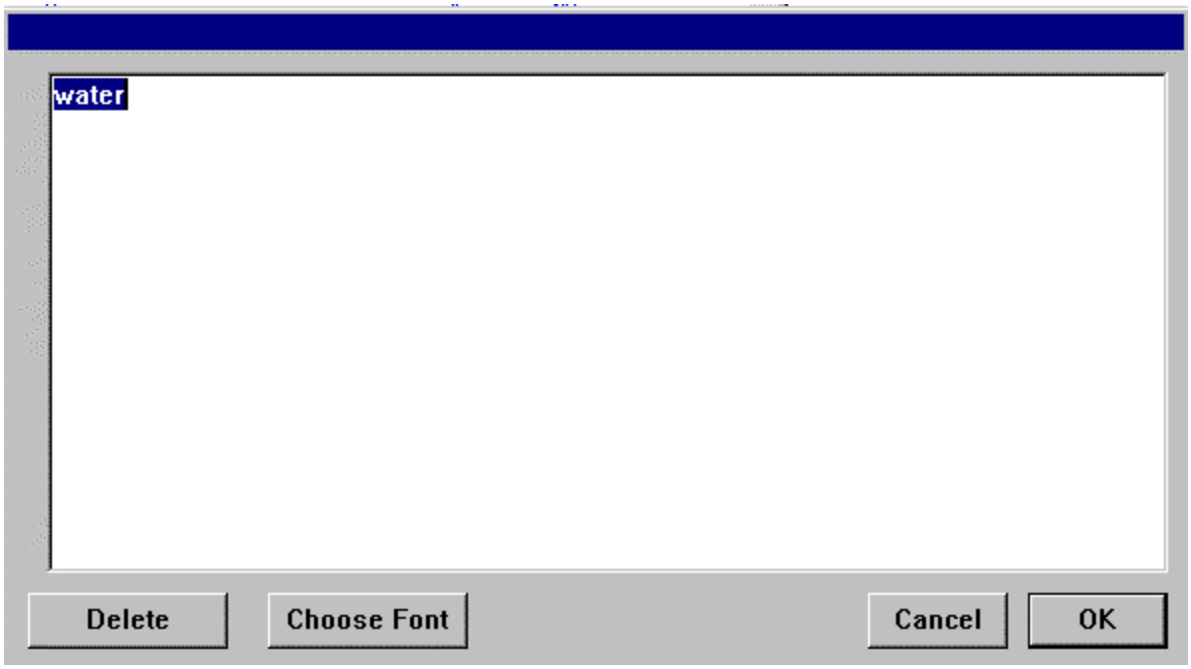
Text annotation

NO – Notes subroutine

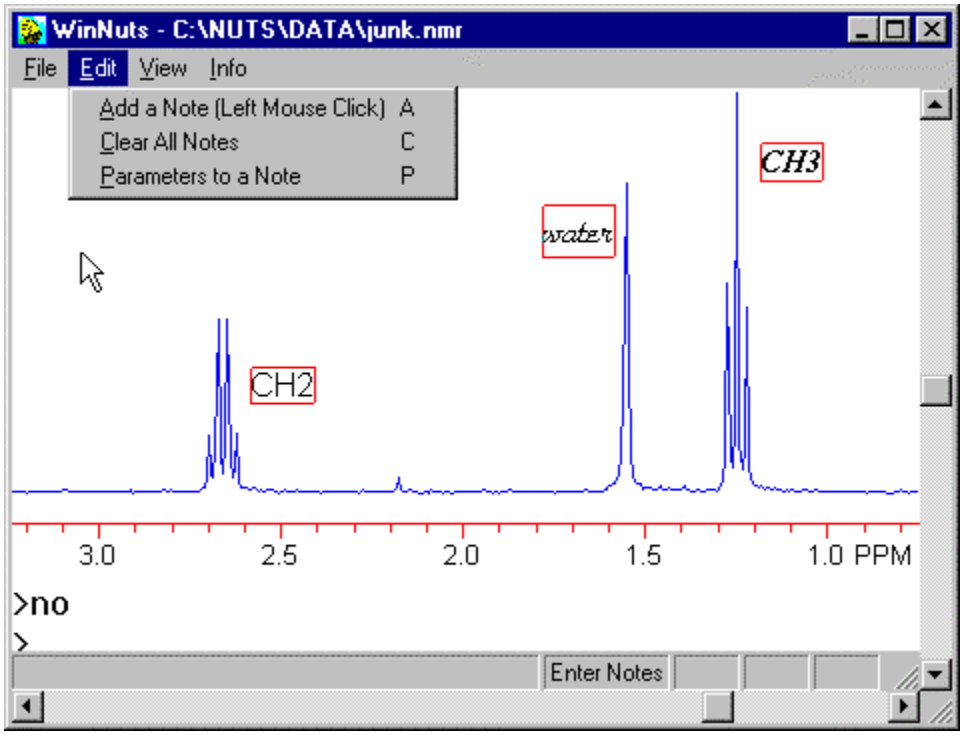
This allows the user to define multiple text boxes, which are "tied" to a point in the spectrum, but can be repostioned with the mouse. **NO** enters the routine (also from the Tools menu).



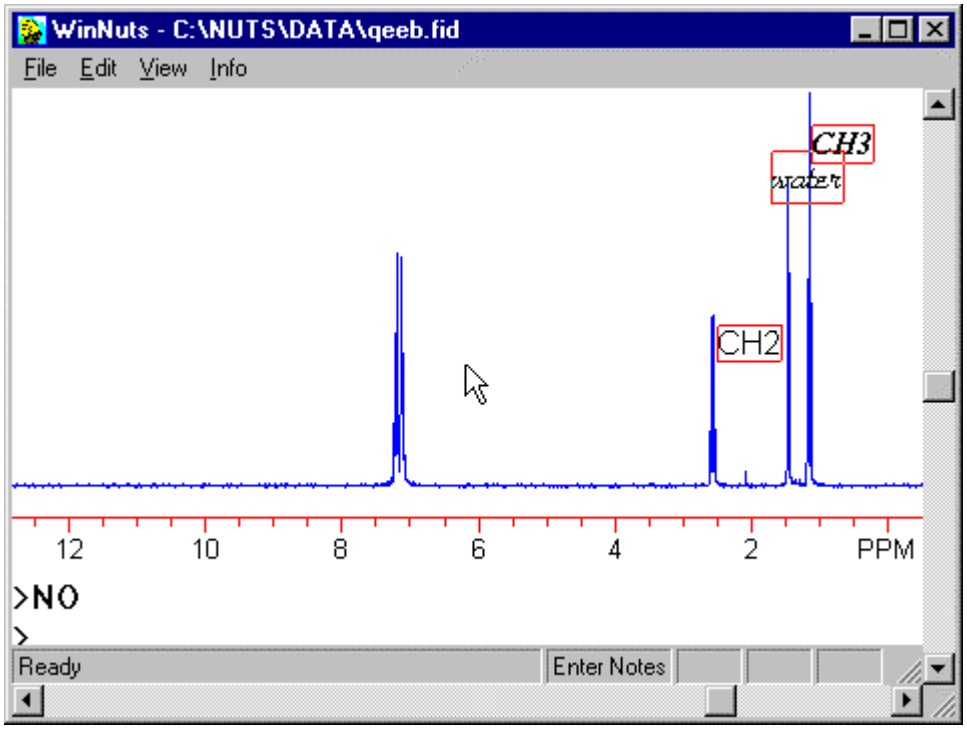
Click with the left mouse button or type **A**. This brings up the dialog box below, allowing the user to enter desired text.



Text can be multiple lines. Click on Choose Font to select font for this note only. The font does not change in this display, but will be correct on the screen.
Click OK to close this box.



Additional notes are added in the same manner. An existing note can be edited by clicking on it with the right mouse button.
A chosen note can be moved by pressing and holding the left mouse button and dragging to a new position. The note box cannot be resized; its size is determined by its contents.
All notes can be cleared by typing **C** or from the Edit menu.
Typing **P** creates a special note box containing the acqusition parameters (equivalent to LP from the NUTS base level)



Notice how the text boxes are repostioned when Ctrl-F is typed. Each note is "attached" to a point in the spectrum, so moves when the displayed region is changed. This means it is possible to select a display region such that some notes are not displayed. This is different from the way other objects are positioned, such as the clipboard text display and objects created in MO. In those cases, the object is tied to a position on the screen, regardless of which part of the spectrum is displayed.

Text which has been copied to the clipboard can be pasted into a Notes box with Ctrl-V (Windows 95/NT) or Command-V (Mac). (This does not work under Windows 3.11).

<**Enter**> exits the subroutine, leaving the notes displayed.
All notes can be toggled on/off from outside the NO routine using **Ctrl-N**.

Subcommands:

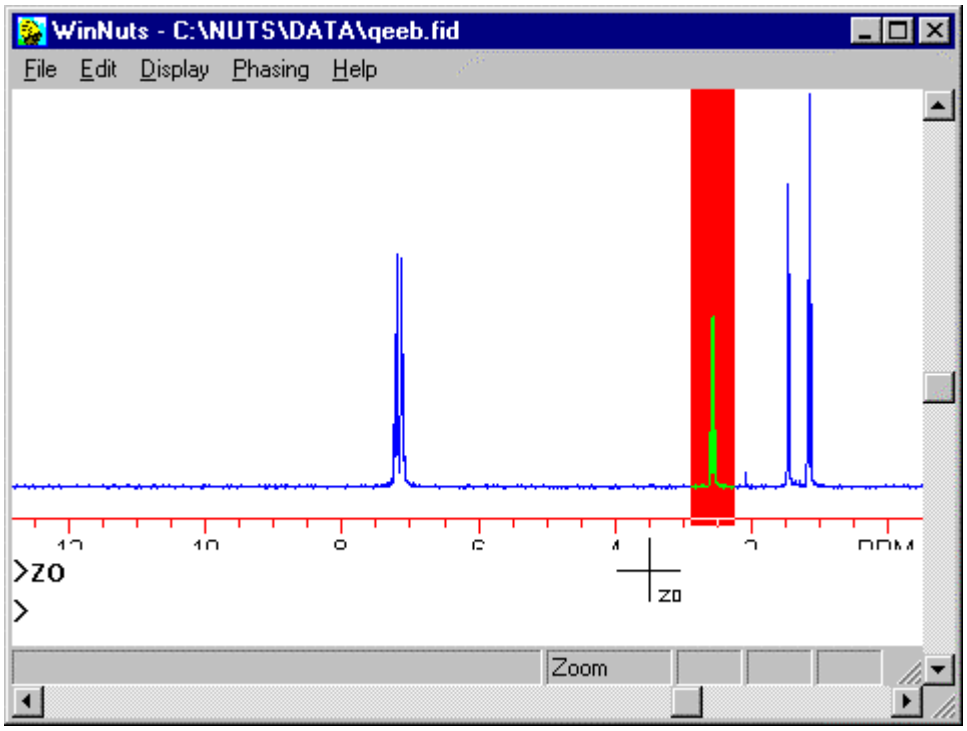
A -- Add new notes box
C -- Clear all notes boxes
P -- create new notes box containing acquisition Parameters
S -- toggle on/off display of all notes boxes
Ctrl-V -- Paste clipboard contents into an open text box (Command-V on the Mac)
click right mouse button on a notes box to edit
<**Enter**> to exit the subroutine

Inset plots (IS)

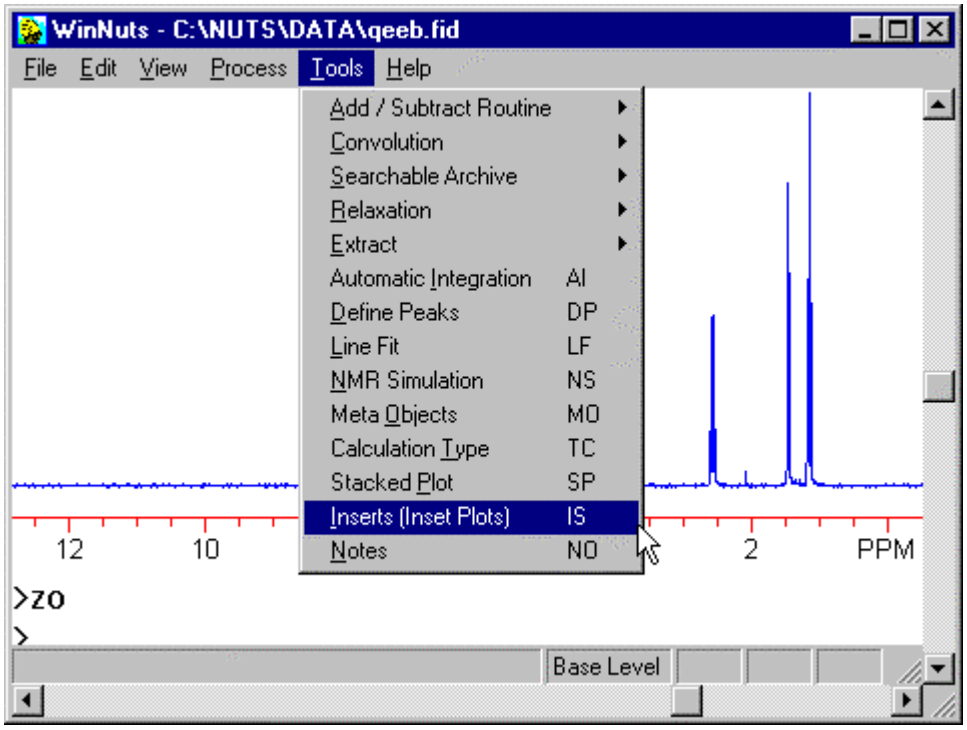
IS – Inset plots subroutine

Note that inset plots can be created only with 1D data.

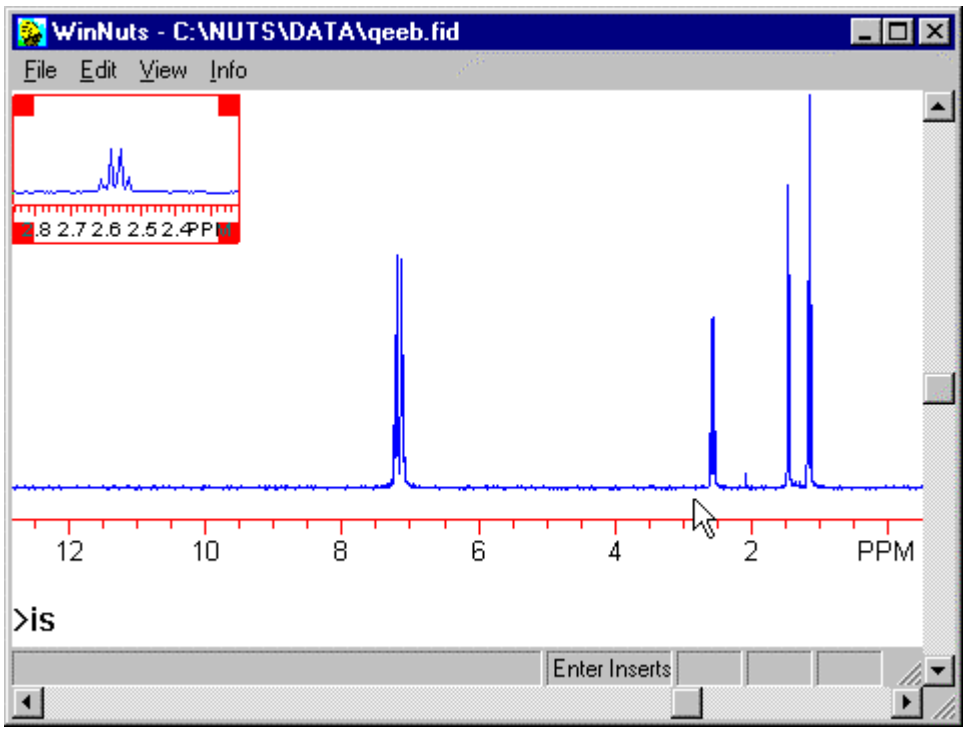
This subroutine allows the user to define multiple inset plots, which are "tied" to a point in the spectrum, but can be repositioned with the mouse. **IS** enters the routine (also from the Tools menu). Inset plots can also be accomplished from the MetaObjects routine, but the IS routine is more flexible and should work better for most situations.



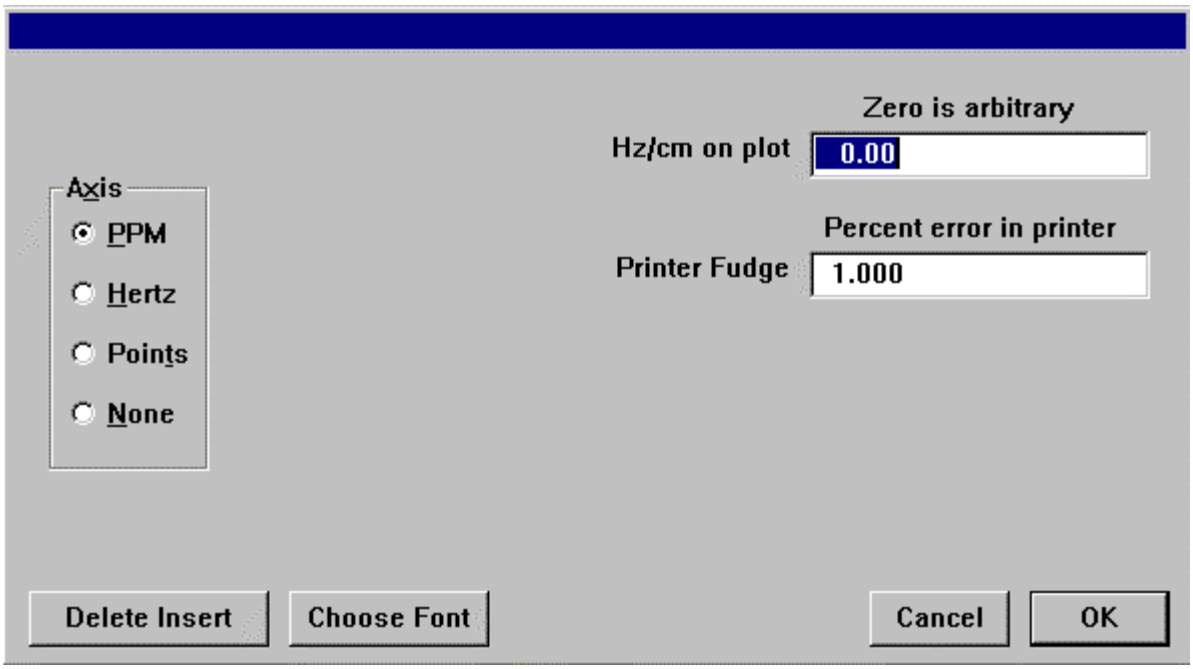
Begin by selecting, with Zoom, the region that will be the inset. It works best NOT to expand to these limits, so type **Ctrl-F** before entering the IS routine.



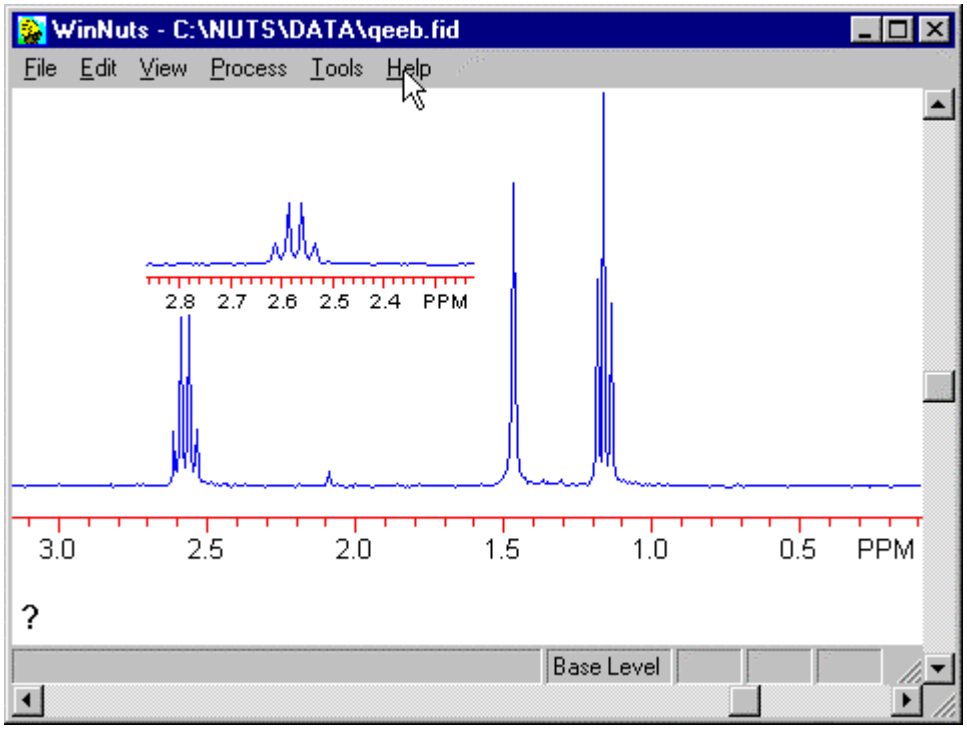
Enter the IS subroutine from the Tools menu or by typing **IS**.



Typing **A** (or selecting Add region from the Edit menu) creates the inset, which can be moved and resized with the mouse.
Display of all defined insets can be toggled on and off with **S** or from the View menu.



Clicking on a chosen inset with the right mouse button displays this box, allowing the properties of the inset to be set. The axis font can be set by clicking on Choose Font. The axis units can be selected independently for each inset.
The scale of the inset can be set to a specific Hz/cm. Since the frequency range of the inset has already been fixed, entering a value here will cause the size of the inset to be changed. This could result in the inset being displayed with an undersireable size (tiny, or off the screen), in which case, the scaling value needs to be reset.
The Printer Fudge box lets you enter a correction factor so that the inset plot is correctly scaled on your printer. Once this fudge factor is determined, it can be set in the nuts.ini file.



It is important to understand that each inset is "attached" to the data point corresponding to its left edge. Therefore, its position on the screen will change as the displayed region is changed. For the case shown above, if the zoom limits are changed to display from 2 to 0 ppm, the inset will not be displayed.

Any DC offset the displayed spectrum had when the inset was created is carried over to the inset. In addition, the offset (of the inset's spectrum relative to its axis) can be adjusted after the inset is created, using the "[" and "]" keys to increase and decrease the offset for the currently selected inset.

<**Enter**> exits the subroutine, leaving the insets displayed.

The insets remain defined when a new spectrum is opened. Each inset is dynamically created, so that it displays the data in the region selected. If the data in that region changes, so does the inset. For this reason, it is usually best to clear all insets before opening a new file.

The insets are saved when the file is saved, and can be recalled when the file is opened, using the TA command or by selecting Auto Tailer Read from the File menu.

Arguments for inset plots have been added to allow the user to change the default position and size of insets when they are added. The values for the arguments are fractions of the screen's size. For example, "IS xpos 0.01" would change the default position of the insert to be 1 percent of the screen from the left. The syntax is:

IS xpos *fraction*
IS ypos *fraction*
IS xscale *fraction*
IS yscale *fraction*

where "*fraction*" is entered as a number between 0 and 1. These commands set the size/position; they do not create an inset. (0,0) is the top, left corner of the screen.

These commands can be used in macros, for example, this section of a macro initializes insets to be positioned in the top, left corner of the screen and to be 50% of the screen size in each dimension:

```
; initialize size & position for IS inset
2f
is xsize .5,
is ysize .5,
is xpos 0,
is ypos 0,
2n,
```

Subcommands:

A -- Add new inset
C -- Clear all insets
S -- toggle on/off display of all insets
click right mouse button on an inset to edit its properties
<**Enter**> to exit the subroutine

Manual peak picking and labeling

DP -- Define Peaks

This subroutine allows manual peak picking using the cursor, and allows the user to place labels on the peaks. (Note that DP is not included in NUTS Lite.) A summary of the DP subcommands is given at the bottom of this section. See illustrated example of DP, below.

The DP routine operates similarly on both 1D and 2D files. For 1D files, DP is an alternative to the automatic peak picking (PP) command for creating a list of only selected peaks. Once peaks have been selected in DP, a chemical shift or other label can be placed above the peak by typing **Ctrl-P**. On entering the DP routine, the cursor changes to a small crosshair labeled DP and the menu selections change. Commands are single-letter commands which are executed immediately. With the exception of functions which require the mouse, all commands can be accessed via the menus.

Position of peak labels -- By default, peak labels for 1D spectra are displayed at the top of the screen, with short lines drawn to show which peak corresponds to each label. The labels can alternatively be displayed just above the top of each peak. This can be set for all peaks in the nuts.ini file, or for each peak from within the DP routine by bringing up the parameters of a selected peak (see editing labels, below). NUTS now has some "smarts" to attempt to avoid overlap of labels on adjacent peaks. Labels can also be moved using the mouse (see moving labels, below).

Selecting peaks -- When the DP routine is entered, peaks can be selected by clicking the left mouse button. For 1D spectra, a vertical red line is drawn showing the point that was selected. For a contour or intensity map of a 2D file, a small cross is placed on the peak. Once selected, the peak labels can be displayed by typing **Ctrl-P**. By default, NUTS chooses the nearest maximum point (positive or negative) which it can find. This "Snap to" feature can be turned off from the Edit menu to allow picking shoulders, etc.

Automatic peak picking -- Automatic peak picking of 1D spectra is also available within DP from the Edit menu or by typing **A**. This uses the minimum height (**MH**) and RMS noise factor (**RM**), in the same way as PP. Automatic peak picking of 2D spectra is only available with the new Arrayed Mode option, which allows the entire data set to be placed into memory. A set of peaks from one 2D spectrum can be overlaid on the display of a different spectrum. See 2D compare.

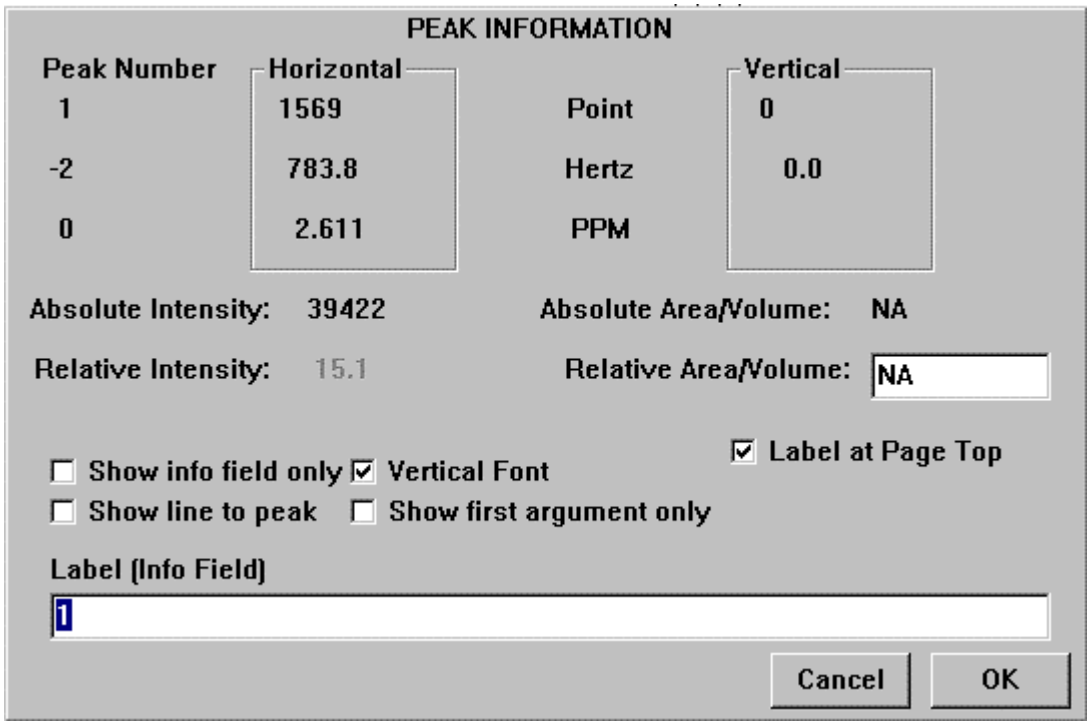
Deleting peaks -- Any peak can be deleted by placing the cursor on or near the peak to be deleted and typing **K** (Kill). (Note: Do NOT click the mouse button to do this, just position the cursor.) The K command will always delete a peak, whichever one is closest to the cursor at the time K is typed. All peaks can be deleted by typing **C** or choosing Clear Selected Peaks from the Edit menu.

Labels in Hz or ppm -- To display the peak position in Hz instead of ppm, type **H**. To display the text label instead of the chemical shift, type **I**. By default, the text label is the number of the peak, in the order selected. This is to allow correlation between peak labels and a peak list generated by **PP**. Both commands are also available from the Edit menu. Selected labels can be displayed as text even if all others are labeled with Hz or ppm. This is done by displaying the parameter dialog box for the selected peak (see editing labels, below) and checking the box labeled "Show Info label only". Note that this causes the text label even when other peak labels are toggled off with **Ctrl-P** or **PF**. This can be used to put multiple text labels on the screen, which need not be associated with a specific peak.

Creating a peak list -- The peaks selected are entered into a list containing shift, peak intensity and a peak label (eg., descriptive text) in the order the peaks were selected. The list can be reordered from largest to smallest chemical shift by selecting Reorder from the Edit menu or by typing **#** (number sign; shift-3 key). The list can be placed into the Windows clipboard for pasting into another document such as the Notepad by selecting Type List to Clipboard from the Edit menu or by typing **T**. If peaks are added, deleted or reordered, be sure to execute the **T** command again to update the clipboard contents

before pasting the list into another document. If the clipboard display feature is enabled (by typing **C** or **Ctrl-B** or from the Edit menu), the **T** command displays the list on the screen. Repeat the **T** command as peaks are added, deleted or reordered to see the current list.

Editing peak labels -- The information about each peak can be viewed by placing the cursor on or near the peak of interest and clicking the right mouse button. The dialog box shown below is displayed which shows the peak frequency, relative intensity, etc.



The bottom line is a label that can be edited for each peak. By default, the peak number is placed in the label box, but this can be edited to be any other text label. There is a check box which allows the user to choose to display the peak labels either horizontally or vertically for each peak independently. This preference can also be set in the nuts.ini file. There is a check box labeled "Show first argument only". This is for situations where extensive information has been input in the text label box, which can be too long to display above each peak. If this box is checked, only the characters up to the first space are used to label the peak. This could be the case if the user enters extensive comments such as substructure codes and assignments to be saved in the file's tailer.

This dialog box is the same whether the current spectrum is a 1D spectrum or a 2D contour map. (Slices from a 2D data set are treated the same as 1D spectra.) The box labeled "Relative Area" is used for volume integrals of 2D peaks. Enter a value for a chosen peak and the volume of all other peaks will be scale appropriately. See the description of volume integrals for details.

Font -- The font used for peak labels can be changed by typing **FV** or **FH** (for vertical and horizontal fonts, respectively) or selecting Set Fonts from the Edit menu.

Moving peak labels -- Beginning with version 5.09, it is now possible to adjust the position of each peak label using the mouse. Position the cursor on the label of interest, press and hold the left mouse button and drag the label to the desired position. The position of any peak label can also be changed in small steps up, down, right and left. To do this, place the cursor near the peak whose label will be moved and type **U** (up), **D** (down), **L** (left) or **R** (right). Do not click the mouse button to do this, just position the cursor. A label can be moved multiple steps in each direction. Note that the labels may look slightly different on the screen and on plots.

The list is not lost when the DP routine is exited, so it is possible to exit DP, choose a different region and re-enter DP to add more peaks to the list. Note that, for this reason, it is a good idea to delete all peaks (with **C**) on entering DP to operate on a new spectrum, so that peaks selected in the previous spectrum aren't carried over.

Saving peaks with the spectrum -- The list of selected peaks is saved with the file, and can be recalled at a later time with the TA command. The list of peaks can also be saved to a text file by choosing Save to File from the File menu or typing **S**. A peak list can be loaded from a previously saved file by choosing Get File from the File menu or by typing **G**. This command retrieves a set of chemical shifts from the file and applies those peak positions to the current spectrum, drawing red lines on the screen to indicate selected peaks.

Subcommands:

A -- Automatically pick peaks (Minimum Height must be set before entering DP)
C -- Display contents of clipboard on screen (same as Ctrl-B command)
G -- Get peak file. Recalls previously saved peak list and displays the corresponding peaks, deleting any peaks which had been selected.
H -- Label peaks with Hz, rather than PPM
I -- Label peaks with text label, rather than chemical shift.
K -- Delete the selected peak closest to the cursor location
S -- Save peak file.
s -- Save peak file in format consistent with SpecMan (from Spectrum Research)
T -- Copy peak list to the clipboard
Z -- Zero out all selected peaks.
-- Re-order the peak list, from highest to lowest chemical shift
Ctrl-C -- Copy screen to Windows clipboard as bitmap See copying spectra.
Alt-C -- Copy screen to Windows clipboard as Metafile

The need for the following 4 commands has been removed, now that peak labels can be moved with the mouse. However, at this time the commands still work. Each command operates on the selected peak closest to the cursor.

D -- Move peak label Down a small amount
L -- Move peak label Left a small amount
R -- Move peak label Right a small amount
U -- Move peak label Up a small amount

Volume Integrals of 2D peaks

The Define Peaks subroutine is used to select peaks whose information is entered into a list. This information includes chemical shift in both dimensions, an editable peak label and relative peak volumes.

Begin by entering DP and select peaks of interest by clicking with the left mouse button. Choose one peak whose volume is to be assigned and click with the right mouse button on or near that peak. Enter a value for volume in the box labeled Relative Area. Areas (volumes) of other peaks will be scaled to this value.

The Arrayed Mode allows automatic peak picking, including volume integrals.

Several points must be kept in mind when using this feature. The limits used for integration are determined by the minimum height (MH) command. The integral is the sum of all points in the vicinity of the peak whose intensity is greater than the minimum height value. This is done by starting at the peak maximum and "walking" in each direction, summing each point until the minimum height is reached. This summation is performed at the time the peak is selected, and is the value shown for Absolute Area. Setting the value of a chosen peak merely enters a scaling factor to give "nicer" numbers for the peak volumes. If a new value for MH is entered, the list of selected peaks must by cleared (by typing **Z**) and peaks reselected. Care must also be taken that a chosen peak is resolved from neighboring peaks at the level of the minimum height.

Control-P -- Toggle peak labels on/off

Peaks which have been selected in the Define Peaks subroutine can be labeled with their chemical shift or a text label by typing **Control-P**. This is a toggle, so typing it again removes the labels. In Links or macros, use " ^**P** ".

PN -- Turn peak labels on

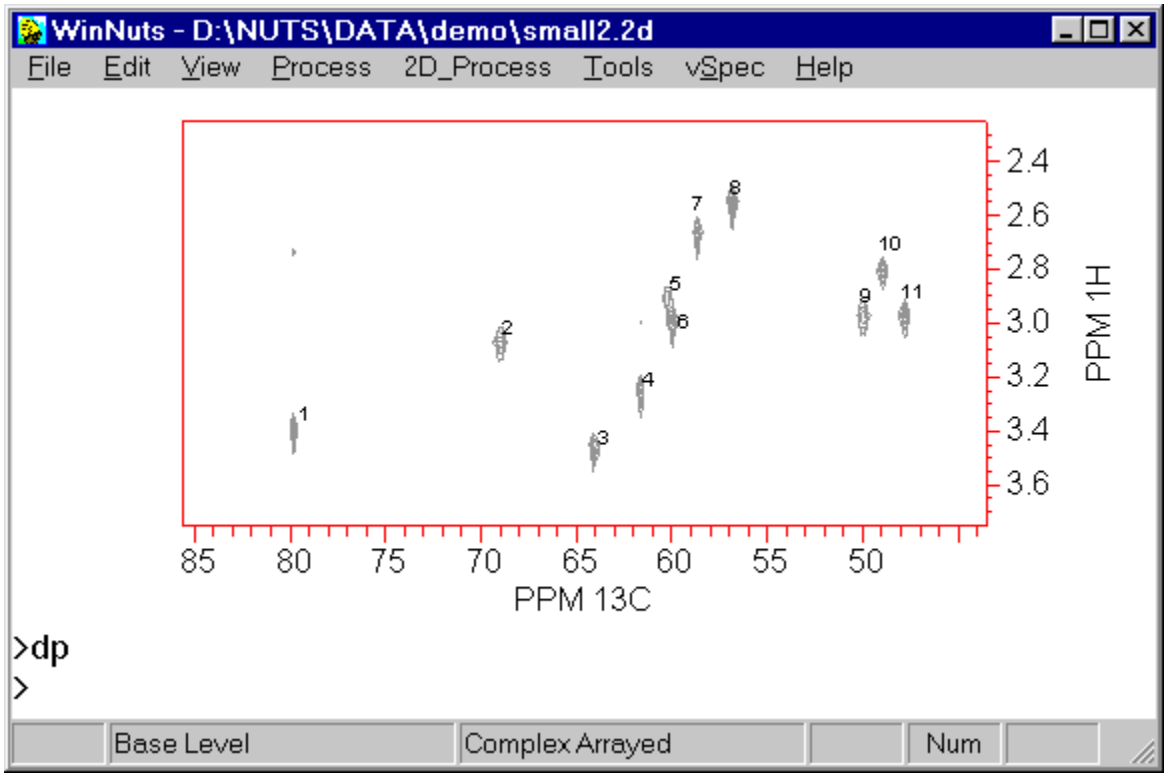
Peaks which have previously been selected in the Define Peaks subroutine can be labeled on the screen and plots by typing **PN**. Display of the labels can be turned off by **PF**. These commands perform the same function as **Ctrl-P**, which acts as a toggle.

PF -- Toggle peak labels off

Peaks which have previously been selected in the Define Peaks subroutine can be labeled on the screen and plots by typing **PN**. Display of the labels can be turned off by **PF**. These commands perform the same function as **Ctrl-P**, which acts as a toggle.

Automatic 2D Peak Picking (Arrayed Mode only)

Automatic peak picking of 2D data is now available in the Arrayed Mode. While in Arrayed Mode, with an intensity plot displayed, enter the **DP** routine and type **A** to perform automatic peak picking, shown below, including automatic volume integration.



List generated by automatic peak picking:

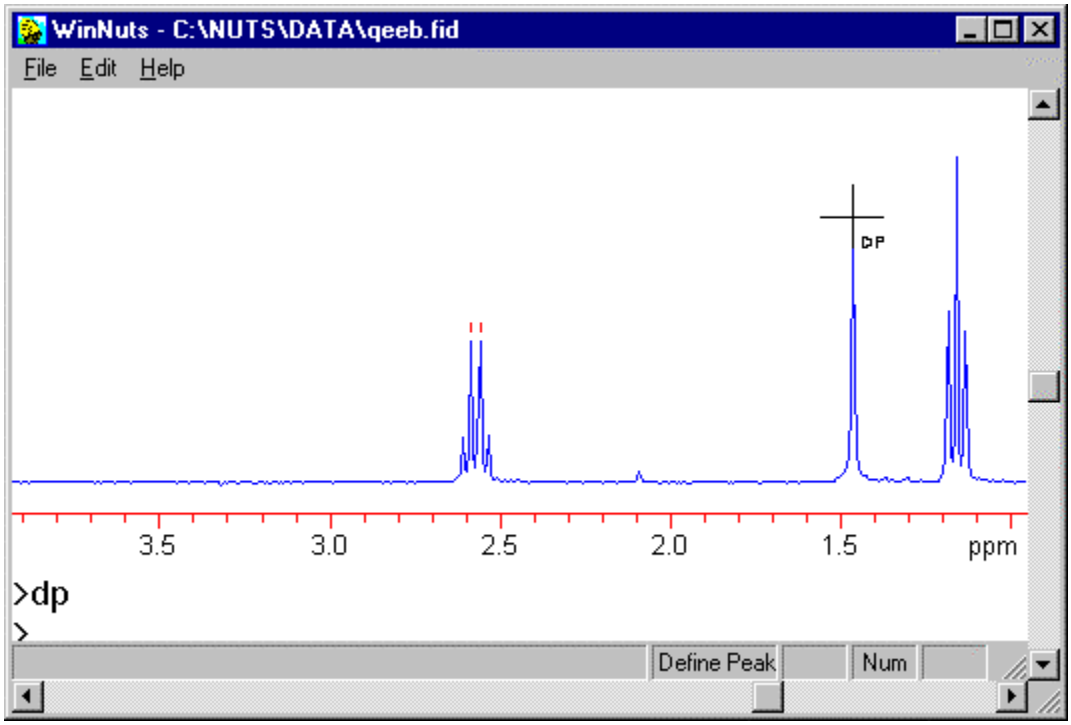
LINE LABEL	POINT	SLICE	PPM1	PPM2	INTENSITY	AREA	
1	36	15	79.876	3.401	96.783	0.77	1
2	101	29	68.982	3.074	79.186	1.22	2
3	131	13	64.077	3.439	60.209	1.02	3
4	146	21	61.703	3.254	79.742	0.97	4
5	155	35	60.209	2.920	55.281	1.21	5
6	156	32	60.043	2.997	112.151	2.15	6
7	164	46	58.733	2.666	79.937	0.82	7
8	175	51	56.891	2.538	92.602	1.50	8
9	217	33	49.866	2.977	79.109	1.17	9
10	223	40	48.950	2.811	78.639	1.00	10
11	230	33	47.787	2.982	84.700	1.26	11

Illustrated example of DP

Using the Define Peaks Subroutine (DP)

This subroutine allows peaks to be selected manually with the mouse, and provides options for displaying peak labels. See also Peak Picking (PP).

(Note that DP is not included in NUTS Lite. However, peaks can still be labeled using the PP command.)



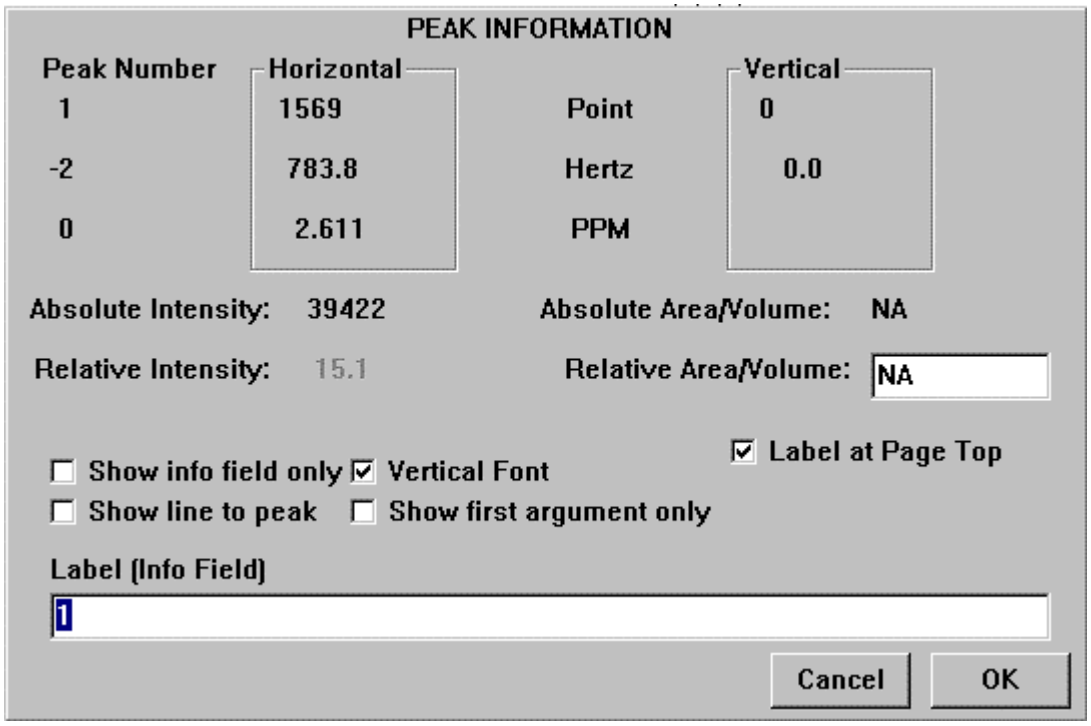
Enter the DP routine from the Tools menu or by typing **DP**.

To select peaks manually with the mouse, place the cursor at the top of the peak and click once with the left mouse button. By default, NUTS snaps to the closest peak maximum, but this can be turned off from the Edit menu.

Peaks can also be selected automatically by typing **A** or from the Edit menu. Peaks exceeding the Minimum Height threshold (**MH**) are selected in the same manner as for PP.

A small, red line shows each peak which has been selected.

All selected peaks can be cleared by typing **C** or from the Edit menu. A selected peak can be deleted by placing the cursor on the peak (don't press the mouse button) and typing **K** (kill).



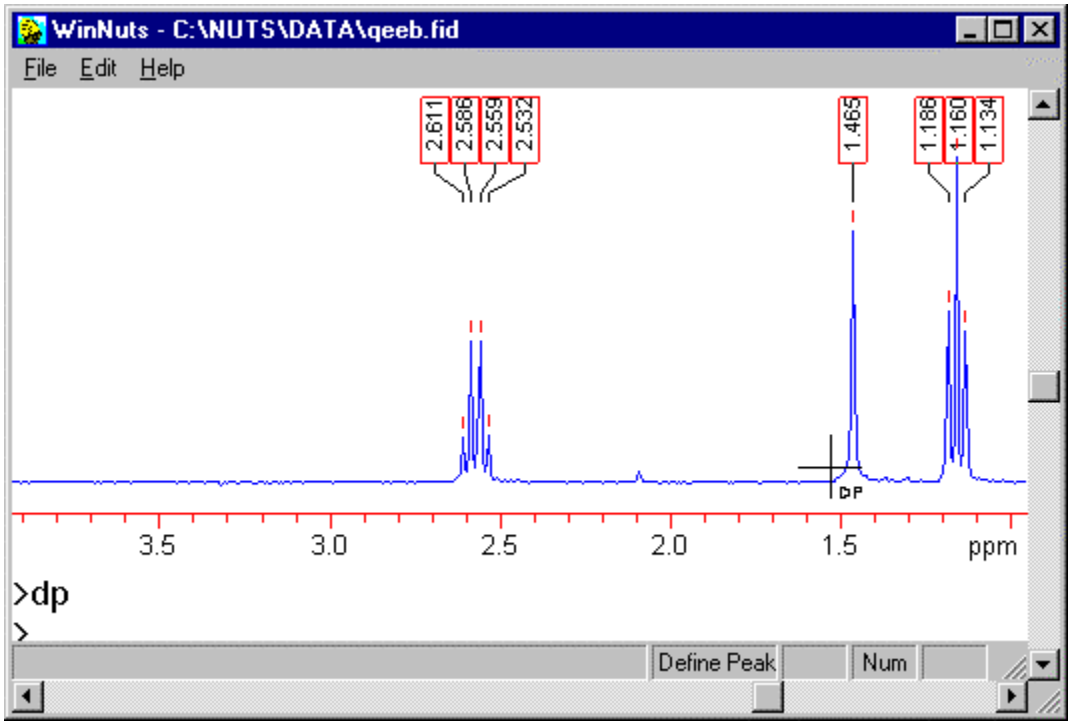
To view information for a selected peak, place the cursor near that peak and click with the right mouse button, which displays the box shown here. (Users with a single-button mouse should hold down the shift key and click the mouse button.)

The peak's frequency in Hz and ppm is displayed in the box labeled "Horizontal". (The box labeled "Vertical" is for the second dimension of 2D data. The values for Area/Volume refer to the volume of 2D crosspeaks. These items are not relevant in this case.)

By default, NUTS will label all peaks with chemical shift in ppm, but it is possible to display the text label instead for a chosen peak, by checking the "Show info field only" box. It is also possible to label all peaks with Hz or text -- see below.

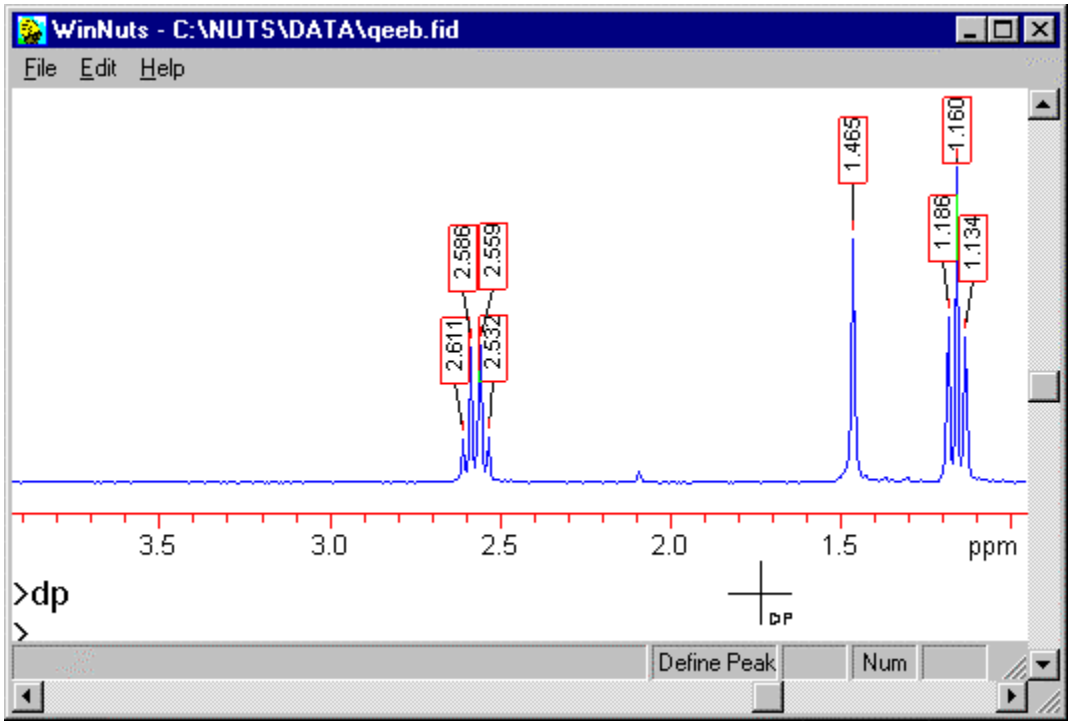
The peak label can be displayed either vertically or horizontally using the check box labeled Vertical Font. The default orientation of all labels can be set in the nuts.ini file. Note that Macs do not have the ability to display fonts vertically on the screen.

The list of selected peaks, including peak labels, is saved with the spectrum, in the file tailer. It is sometimes helpful to enter extra text in the text field for future reference, but not display all of that text on a plot. The check box labeled "Show first argument only" is used to display only the beginning of the text label, up to the first space encountered.



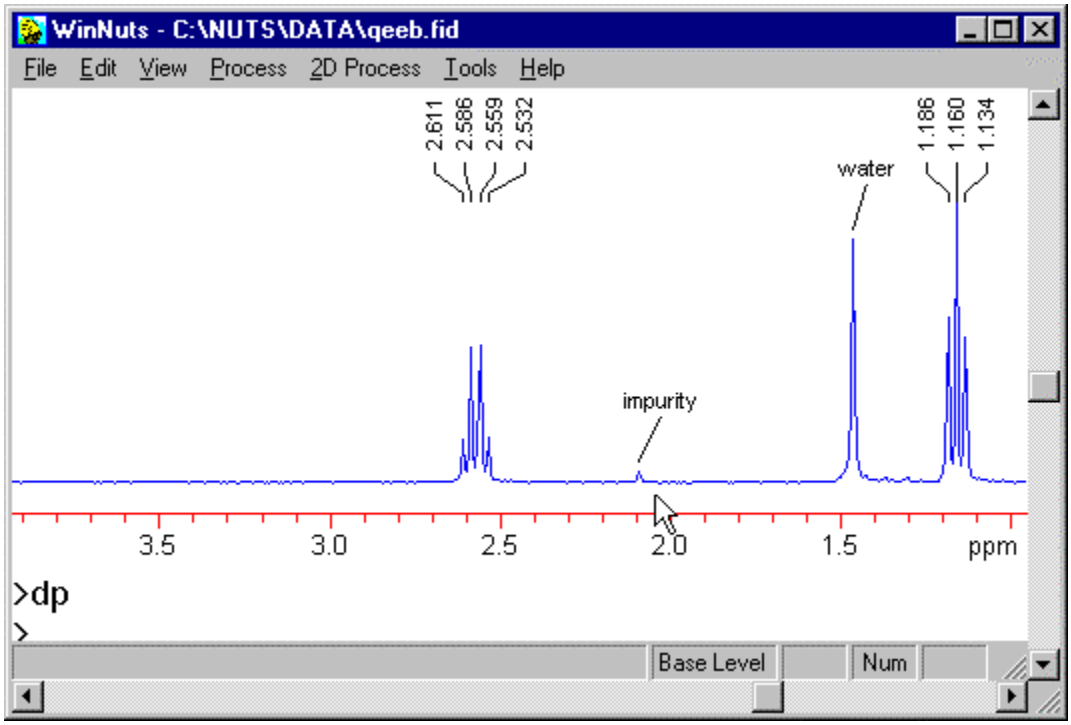
Display of peak labels is toggled on and off from the Edit menu or by typing **Ctrl-P** (which is active even after leaving the DP routine). By default, NUTS displays the chemical shift, in ppm, as the label. The other options are peak frequency in Hz (subcommand **H**, available from the Edit menu) or text label (subcommand **I**, also available from the Edit menu as Show Peak Information Label).

NUTS attempts to avoid overlap of peak labels, but this may not be possible for a very crowded spectrum. Labels can be placed either at the top of the page (shown above) or just above each peak, as shown below. This can be set as the default for all peaks in the nuts.ini file, or can be set for individual peaks by displaying the peak's information dialog box and selecting/unselecting "Labels at page top".



Individual labels can be moved by placing the cursor on the chosen label, holding down the left mouse button and dragging the label. Note that usually the label takes up less space when printed than on the screen, due to the increased resolution on the printer. Selecting a smaller font size, such as 6, will also help in avoiding overlap of labels.

A peak list can be placed into the clipboard by selecting Type Peak List to Clipboard from the Edit menu (subcommand **T**) from which it can be pasted into a text editor for printing or displayed on the screen by typing **Ctrl-B**. The peak list can also be saved to a text file from the File menu.



Exit the DP routine by typing <Enter>. The red lines and boxes around the labels are no longer displayed. Here, 2 of the peaks have text labels displayed, using horizontal font, positioned above the peaks rather than at the top of the page. Each of these options was selected from within the peak information dialog box.

After exiting DP, the set of peaks is still defined, and can be edited by re-entering the DP routine. They also still exist even if a new data file is opened, whether or not peaks exist at these frequencies in the new spectrum.

Display of the labels can be toggled on and off with **Ctrl-P**. Labels for peaks which have the "Show info field only" box selected will continue to be displayed even if display of labels is turned off.

Relaxation Analysis

See illustrated tutorial below showing analysis of T_1 data.

To use the automated relaxation data processing capability, the original 1D data files must have sequential file extensions to allow NUTS to process them using either a Link or a macro. Most of the useful tools for displaying and analyzing a series of 1D files require that they first be converted to a 2D data set.

This can be done as a 2-step process or all at once. For the 2-step process, execute a Link such as:

IM BC EM FT PS SB IN

This imports each FID, does an FT and phases the spectra, then saves them as a series of 1D spectra. NUTS will prompt for a file name for the spectra. Remember to set LB and phasing on one of the files before executing this Link. The IN command increments the file extension and loops back to the first command in the link. It will continue until it fails to find the next file. The spectra can then be converted to a 2D data set with a link such as:

GA SC IN

The SC command creates a 2D file.

To process the FIDs and save them as a 2D file all in one step, execute a link such as

IM BC EM FT PS SC IN

NUTS will prompt for the file name of the first file of the original data and the file name to save the data to, which will be a 2D file.

Use GA to read in the first slice of the 2D file, making it the current data set. The data can now be plotted as a stacked plot with SP or slices can be viewed with VW. This process is useful for any series of 1D files, such as kinetics data.

For a T_1 or T_2 experiment, NUTS also provides the capability of plotting the integral value of a chosen peak as a function of time, and also calculates T_1 or T_2. The time values are saved in a variable delay list and can be viewed and/or edited by typing D1. Be sure the time values are correctly entered in this list before attempting to calculate T1s. If the list is edited, type UH to update the file header and save the corrected values.

First, use Zoom to display just the peak of interest. Exit the Zoom subroutine and type GR (or choose Get Relaxation data from the Tools menu). This command measures the integral of the chosen region for each spectrum in the data set. Next type DR (or choose Data Reduction data from the Tools menu). NUTS displays a plot of integral value vs. time for the chosen peak. By default, Nuts assumes the data are T1 data, but this can be changed from the Fit menu. The curve displayed is a first guess, not a fit to the data. An exponential can be fit to the data by typing **O**. The resulting curve is displayed and the calculated T1 value printed on the screen. Note that good phasing and flat blaselines are necessary to get good integration and therefore, a good T1 recovery curve. The phasing and baseline of individual slices in a 2D data set can be corrected and the corrected slice saved back into the 2D data set with the S2 command.

For details of the T_1 calculation, see DR.

A sample set of T_1 data (^{13}C spectra of sucrose) is available on the Acorn NMR web site, and is called Sucrose.t1.

As an alternative to the automated approach, in which NUTS automatically integrates the peak for all time values, it is possible to create a table of time vs. amplitude values, saved as a file, and then fit these values to the T_1 equation. See description of RR command for details.

D1 -- Time values for arrayed experiment

Opens a dialog box for display and entry of a list of time values (in sec) used in an arrayed experiment, such as a T_1 experiment. If NUTS can identify a list of variable delay values in the data header, it will place the values in this list. Otherwise, the user can enter them manually. After entering a list of values, close the dialog box and type UH to update the file header, saving the list. Space is provided for up to 64 values.

These values are used in calculating T_1 . In the event that one or more spectra in a T_1 data set are corrupted, and the user would like to eliminate those values from the T_1 calculation, the corresponding delay value can simply be set to a negative number. NUTS will ignore the corresponding spectra for its calculation, although the values are still displayed on the plot, in a different color.

GR -- Get Relaxation data

Used in calculating T1 or T2 from a series of spectra. This command integrates the chosen region for each file in the data set and creates a list of time and area values which will be fit to a T1 equation. (The user does not have the ability to edit this list, but as an alternative, can create his/her own list of values saved as a file. To use this file to calculate T1 instead of the automated approach, use the RR command to read in the Relaxation data file.)

To use GR, the data must first be transformed, phased and saved as slices in a 2D data set. The user first selects a peak using Zoom to expand the spectrum so that only the peak of interest is displayed. Exit the Zoom subroutine using Enter. Type GR (or choose Get Relaxation data from the Tools menu). NUTS measures the integral of the displayed region for each spectrum in the data set. Note that the baseline and phasing in the expanded region must be good in order to obtain a realistic value for the integral. The baseline may need to be corrected for each spectrum in the data set and the corrected spectra saved. The simplest way to do this would be to execute a link such as the following, having already expanded the spectrum to display the region of interest:

```
GA BF SC IN
```

Be sure that the slice number (SL) is set to one before executing the link, so that the process is performed on the whole data set. The BF command removes DC and tilt for the currently displayed expanded region. The SC command saves the corrected data. A different name must be supplied for the corrected data set. See help on each command for more information.

To correct phasing or baseline of just single slices within the data set, make the changes and save the corrected slice with the S2 command, which saves the entire data set with only the currently displayed slice being changed.

The T_1 data can be displayed by typing **DR** (or choosing Display Relaxation data from the Tools menu). The entire process is repeated for each peak of interest.

DR -- Data Reduction

Plots integral vs. time for relaxation data on a chosen peak. The data can be fit using a choice of functions for T_1 or T_2 relaxation.

Three steps must precede the DR command:

1. The data must first be processed and saved as a 2D file (see Example).
2. A peak is selected by using Zoom to expand the spectrum so that only the chosen peak is displayed.
3. The GR command must be executed, which measures the integral of that peak in each of the spectra. (Alternatively, peak heights can be used; see below.) The GR command is also available from the Tools menu.

As an alternative, the data to be fit can be read in from a file consisting of time, amplitude data points. See Read Relaxation data file (RR) for details.

Typing DR (or choosing Data Reduction data from the Tools menu) displays a plot of integral vs. time with each data point represented by a small square. An initial guess at an exponential curve is displayed, but this does NOT represent a fit to the data.

By default, the data are assumed to be T1 data, and a 3-parameter fit is used. The fitting function can be changed from the Fit menu. Choosing Fit Function brings up a dialog box which allows the user to select the fitting function. The choices are

T_1 Inversion Recovery
T_1 Inversion Recovery fitting 3 parameters (default)
T_2

Equations are shown below.

Also from this dialog box, the user can choose to use peak heights rather than integrals. Peak height can be chosen with or without interpolation. If this option is changed, it is necessary to exit the DR routine and re-execute GR to make the new measurement.

To perform a fit, type **O** or select Optimize from the Fit menu. NUTS displays the curve determined by fitting the chosen equation to the data points, and also displays the values calculated in the fit process. A sample plot is shown below. If the integral for the longest delay value is set to 100, the integrals will be given as a percentage; otherwise, it is in the

absolute integral units internal to the program. The plot can be printed by typing **P** or selecting Print from the File menu. The whole process is repeated for each peak of interest.

A table of time and integral values can be placed into the Windows clipboard by typing T or selecting Data to Clipboard from the Edit menu. The integral values will be listed as relative values if the integral has been assigned a value; otherwise it is given in the absolute units internal to the program. This table can then be pasted into the Notepad or other document. Like any other text in the clipboard, this can also be displayed on the screen by toggling on clipboard display by typing **S** or selecting Show Clipboard from the Edit menu (equivalent to the CB command).

The list of delay values used in the experiment can be displayed and edited by typing **D1** from the NUTS base level or from within the DR subroutine by typing **D** or selecting Edit Time Data from the Edit menu. NUTS attempts to identify this list of values in the data header of the source data. If this attempt was unsuccessful, the user can simply enter the values. Executing a UH command (Update Header) saves the list in the header of the 2D data set. In the event that the user wishes to eliminate one or more spectra from the T_1 calculation, this can be done simply by changing the corresponding value in the **D1** list to a negative number, in which case NUTS ignores that point in the fit.

The equations used to fit the data are:

T13IR (3-parameter T_1-Inversion Recovery)

$$y = A * \{ 1 - [1 + W * (1 - \exp(-K/T))] * \exp(-x/T) \}$$

where

$T = T_1$ relaxation time
A = peak integral at time x >> T
K = total time between scans in the 180-t-90 sequence (equal to acquisition time plus relaxation delay time)
x = delay time t in the 180-t-90 pulse sequence
W = -(integral at time x=0 / A)

The parameter W is determined in the fitting process, as inversion in this experiment is not always perfect. This gives better results than assuming that the integral at time x=0 is simply the negative of the integral for infinitely long x.

ref: G.Levy and I.Peat, **J.Magn.Reson.**, **18**, 500 (1978).

T1IR (Inversion Recovery)

$$y = A * \{ 1 - [2 - \exp(-K/T)] * \exp(-x/T) \}$$

where

T = T_1 relaxation time
A = peak integral at time x >> T
K = total time between scans in the 180-t-90 sequence (equal to acquisition time plus relaxation delay time)
x = delay time t in the 180-t-90 pulse sequence

ref: Levy et al, **J.Magn.Reson.**, **11**, 58 (1973).

T2

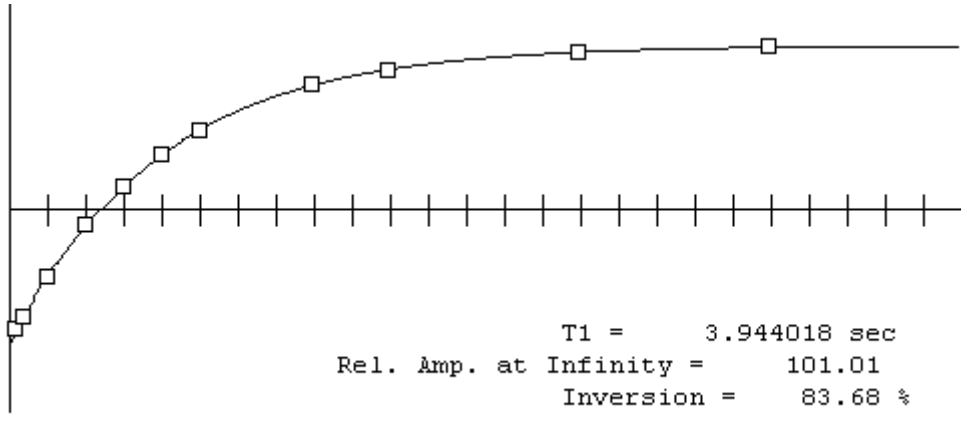
y = A * exp(-x/T)

where

T = T_2 relaxation time
A = peak integral at time x >> T
x = delay time t in the pulse sequence

Subcommands:

D Display/edit list of time values
F Choose fitting function
K Peak pick method: integrals, peak heights or interpolated peak heights
O Optimize fit to data points
P Print
S Show contents of clipboard on screen (must execute **T** first)
T Copy table of time and integral values to the clipboard
X Expand x-scale. Allows expansion to see details of early time points.
Ctrl-C Copy screen to clipboard for pasting into other applications
Enter Exit program



T1-Inversion Recovery plot of Integral vs time

RR -- Read Relaxation data file

Reads in a file containing time and amplitude values to be fit to the T_1 equation. This is an alternative to the Get Relaxation data (GR) command, which automatically measures integrals of the selected region for each file in the data set. After reading in a file with RR, the fit is performed with the Data Reduction (DR) command. These commands are also available from the Tools menu.

The format of the relaxation data file must be as follows:

```
Relaxation Data
time1 value1
time2 value2
...
time n value n
END
```

The file can be created with any text editor and must be saved before it can be read into NUTS. Extra spaces are ignored, as is any line beginning with a semi-colon, allowing comments to be inserted to identify the source of the data. The first line must be **Relaxation Data** and the last line must be **END**.

N.B. Be sure to read a spectrum into NUTS before executing RR and DR. This serves to initialize the display parameters. If RR and DR are executed immediately after the NUTS program is started, erroneous display and calculation result.

Illustrated example

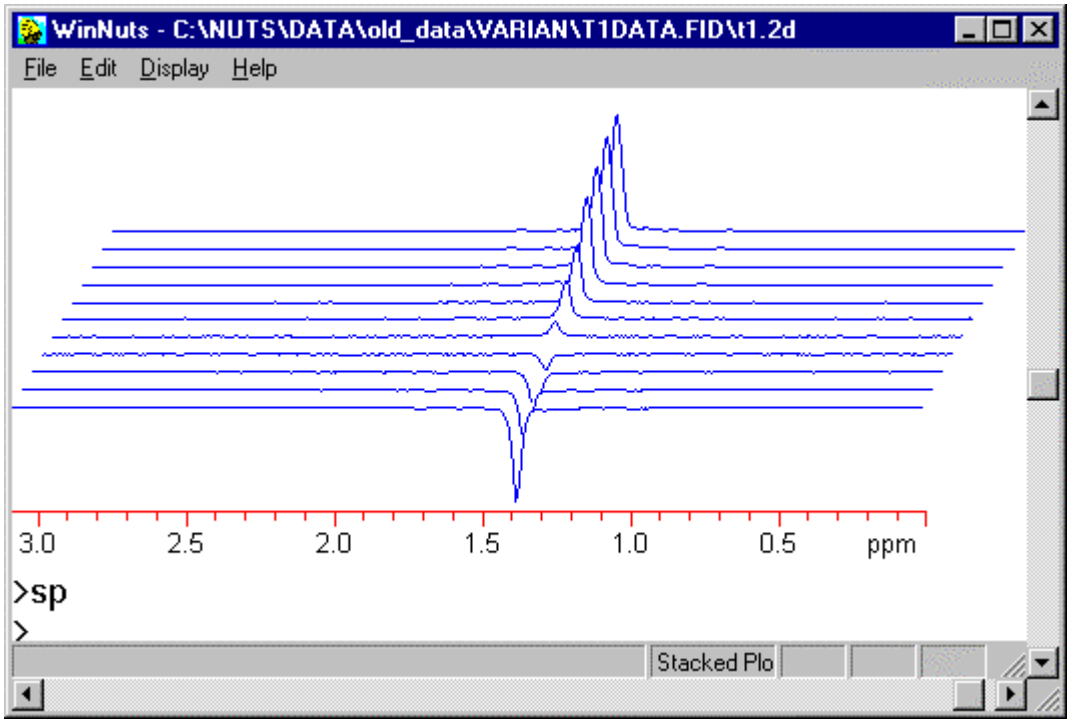
Analysis of Relaxation Data

The data must be saved as a NUTS 2D file. Data which consists of a series of 1D files can be converted to a 2D file using the following Link, provided the 1D file names have sequential file extensions (e.g., file.001, file.002, etc):

```
GA SC IN
```

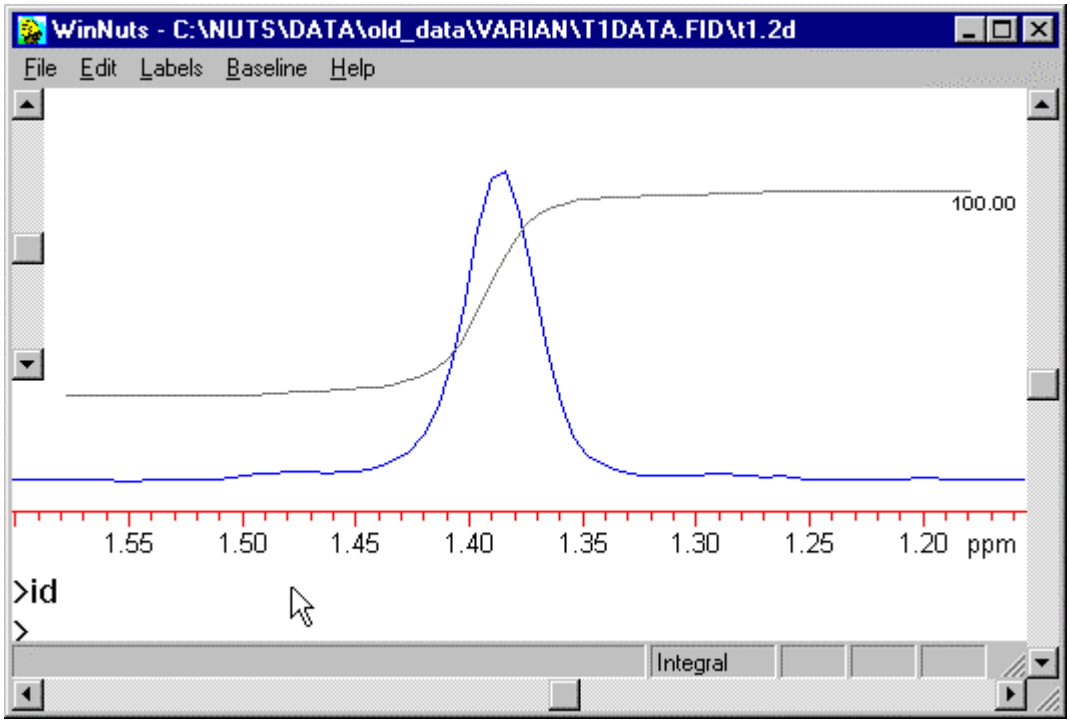
You will be prompted for the first 1D file and for a name for the newly created 2D file. When the Link finishes, read in the 2D file.

Note that this feature is not included in NUTS Lite.



The T_1 data has been saved as a 2D data set, shown here using a stacked plot display.

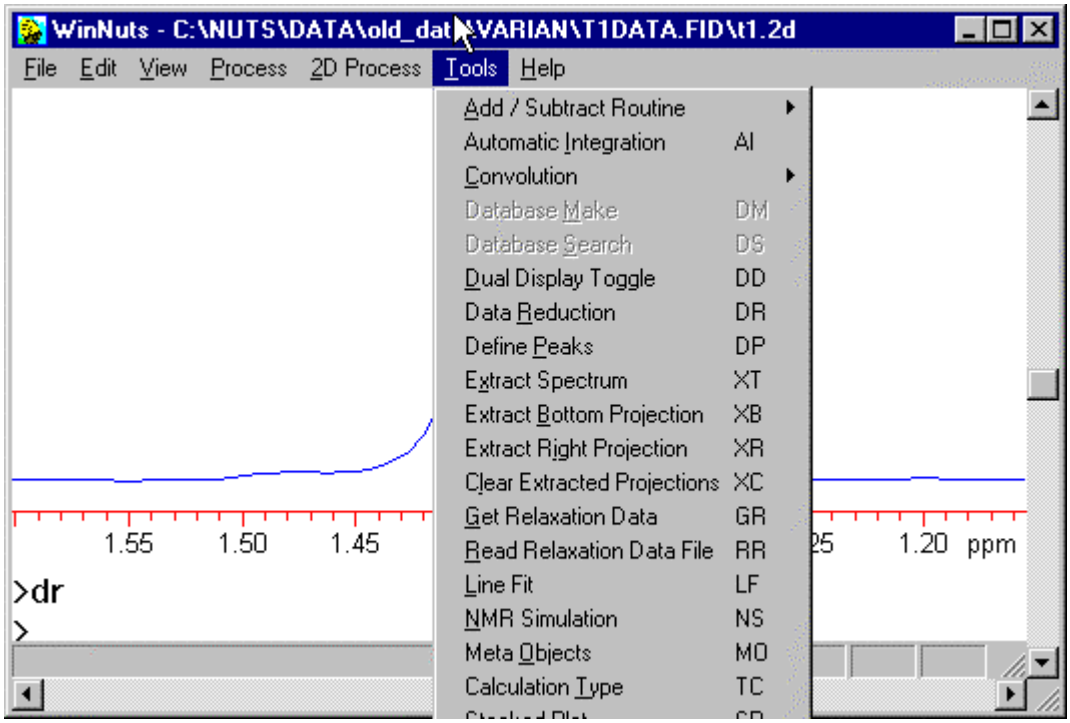
Be sure each slice is phased correctly and has a flat baseline.



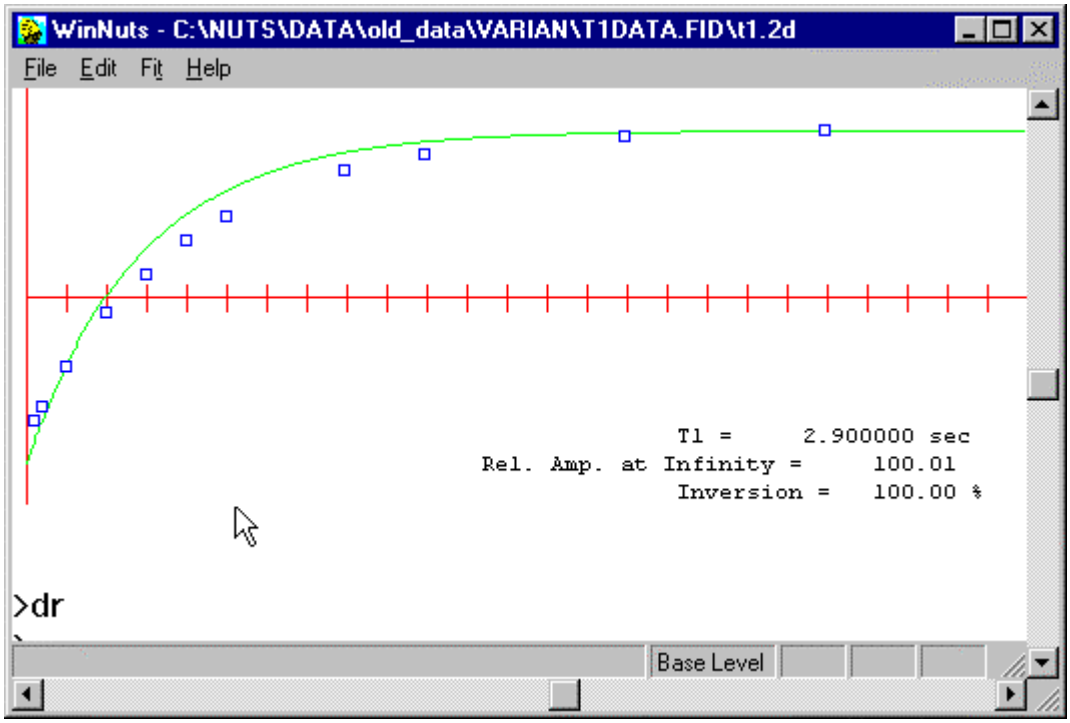
Select the peak whose T_1 is to be calculated, and use Zoom to expand so that only the peak is displayed with just a small amount of baseline on each side.

It will make the numbers easier to read if the last slice in the data set has its integral set to a convenient number, such as 100.

Exit the Iintegration and Zoom routines, leaving the expanded region displayed.



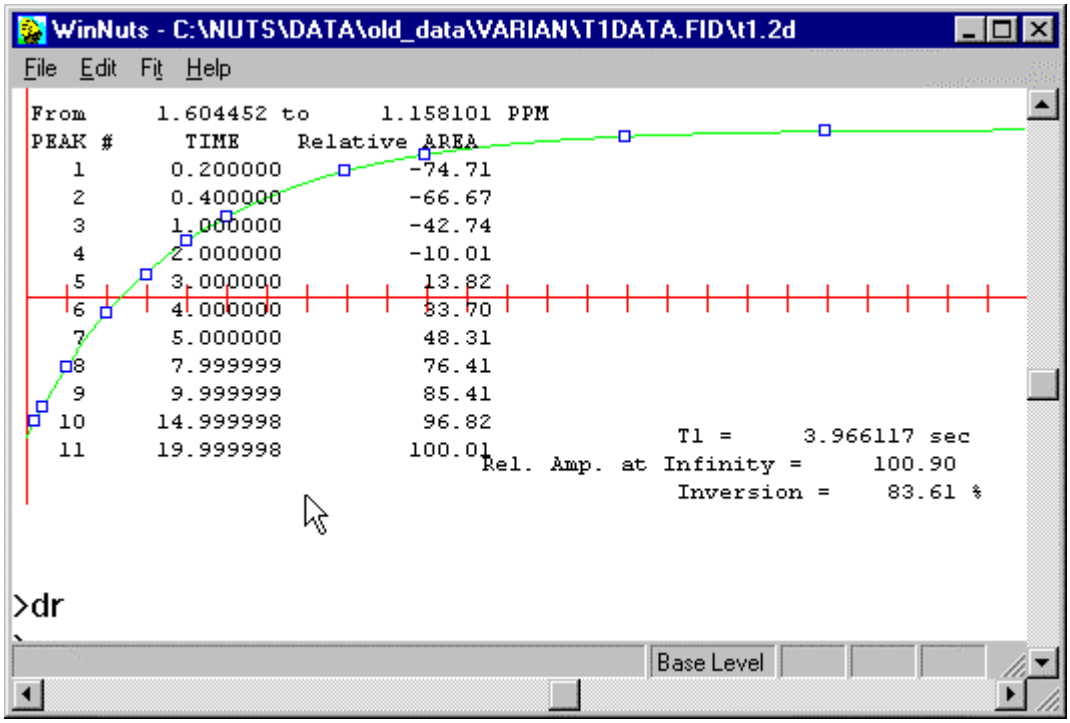
Choose Get Relaxation Data from the Tools menu (or type **GR**). This causes NUTS to read in each slice in turn and measure its integral for the expanded region. The display does not change when this operation is performed.



Choose Data Reduction from the Tools menu (or type **DR**). This enters the relaxation subroutine and displays a curve of area vs. time. The green curve does not represent a fit, just a quick first estimate.

To perform a Simplex fit, choose Optimize from the Fit menu (or type **O**).

See section on relaxation for equations and other options.



The fit has been performed.

To create a table of time vs. area, choose Data to Clipboard from the Edit menu (or type **T**). This list can be displayed on the screen by choosing Show Clipboard from the Edit menu (or type **S** or **Ctrl-B**).

Meta-objects (structures and other graphics) on plots

MO -- Meta Objects on plots

This is a subroutine for the addition and manipulation of graphical objects on the screen. (In the Windows version, these objects are Windows Metafiles, hence the name Meta Objects.) Graphical objects can be inset plots, chemical structures, logos or others. They can be pasted from the clipboard or imported from files. More than one object can be displayed on the screen, and each can be independently moved and resized using the mouse. The program keeps track of them in a linked list. To select which object to operate on, move through the list, forward or backward, until the desired object is indicated with a box with handles. See subcommands below. See illustrated example below of how to use MO.

Another subroutine (Insets) has been added for creating inset plots. The Insets routine creates the inset plots differently, and may work better.

A different subroutine has been added to display structures from previously saved molfiles.

As an experiment, MO subcommands are not presented in menus, but instead in a movable Helper window. The commands can be entered via the keyboard or via buttons in the Helper window. Display of Helper windows can be toggled on and off from the Help menu at the base program level, and can be set in the nuts.ini file.

Inset plot --To create an inset plot, first expand the spectrum to display just the region which will comprise the plot inset. Type **MO** to enter the subroutine, and then **A** to add this display to a linked list of graphical objects. The chosen region can be resized and moved with the mouse in a manner similar to standard graphics applications. It is possible to display multiple insets. Exit the MO subroutine, then select and expand a new region, and repeat as needed.

In the Helper window, you can set a multiplying factor for the axis font size, so that the numbers can be made more readable. This must be set before the inset is captured. Text such as the integral labels and peak labels may be too small to be read easily when the viewing area is reduced to form an inset plot. It may be helpful to increase the size of such text before capturing the inset. This can easily be done with the Windows version of NUTS using a macro such as the one shown below. (Changing font sizes in a macro does not appear to work at this time on the Mac).

Importing -- A graphical object can be read from a file which is an enhanced, standard or placeable metafile. Objects can also be copied to the clipboard from another application and pasted into NUTS while in the MO subroutine. (Note that enhanced metafiles are not supported under Win3.11, and metafiles are not supported on the Mac. The Mac version uses PICT files.) The objects are displayed until explicitly deleted or until Nuts is closed, even if another data file is opened.

When a file is saved, any objects which were pasted from the clipboard or created as inset plots are not saved with it, as these are memory-resident only. In the case of an object which was placed by reading a file, the path to that file is saved in the file's tailer, and can be recalled later using the TA command.

Subcommands:

A - Add currently displayed region as an inset plot
C - Paste the clipboard contents
D - Delete currently selected Meta Object
I - Import a graphical object from a file
N - Move to next Meta Object in linked list
R - Move to previous Meta Object in linked list
Shift-F1 - brings up Help for the MO subroutine
<Enter> - exit the MO subroutine

There is also the option of defining one or more graphical objects in the nuts.ini file (such as a company logo) which will be displayed on the screen automatically. This is demonstrated by the display of the acorn in the upper left corner of the screen. This can be removed by editing the nuts.ini file or, while Nuts is running, by deleting it from within the MO subroutine.

MetaObject macro

The following is a useful NUTS macro for adjusting font sizes for inset plots. The reason this becomes desirable is that font sizes chosen in Nuts assume a full-sized plot. When this is scaled down for an inset plot, the fonts can become too small to read. The strategy is to increase the font sizes, capture the plot, then reset the fonts. This is somewhat tedious to do manually, but can be done with a single command, using the following macro. See description of macros for a general explanation. This appears not to work on the Mac at this time.

```
NUTSMacro for adjusting font sizes for inset plots

; Assumes you have first chosen expansion for inset plot
; SET FONT commands increment/decrement font size
; increment must be set to 10x desired size change
; (+20 will increase font size by 2 points)
; font sizes are increased before capture of the inset plot,
; then reset afterwards

SET FONT_AXIS +40
SET FONT_integral +40

; enter MO subroutine, capture displayed region as inset, then exit

MO
A
^M

; reset font sizes, display full spectrum, reset vertical scale
SET FONT_AXIS -40
SET FONT_integral -40
^F
mf
end
```

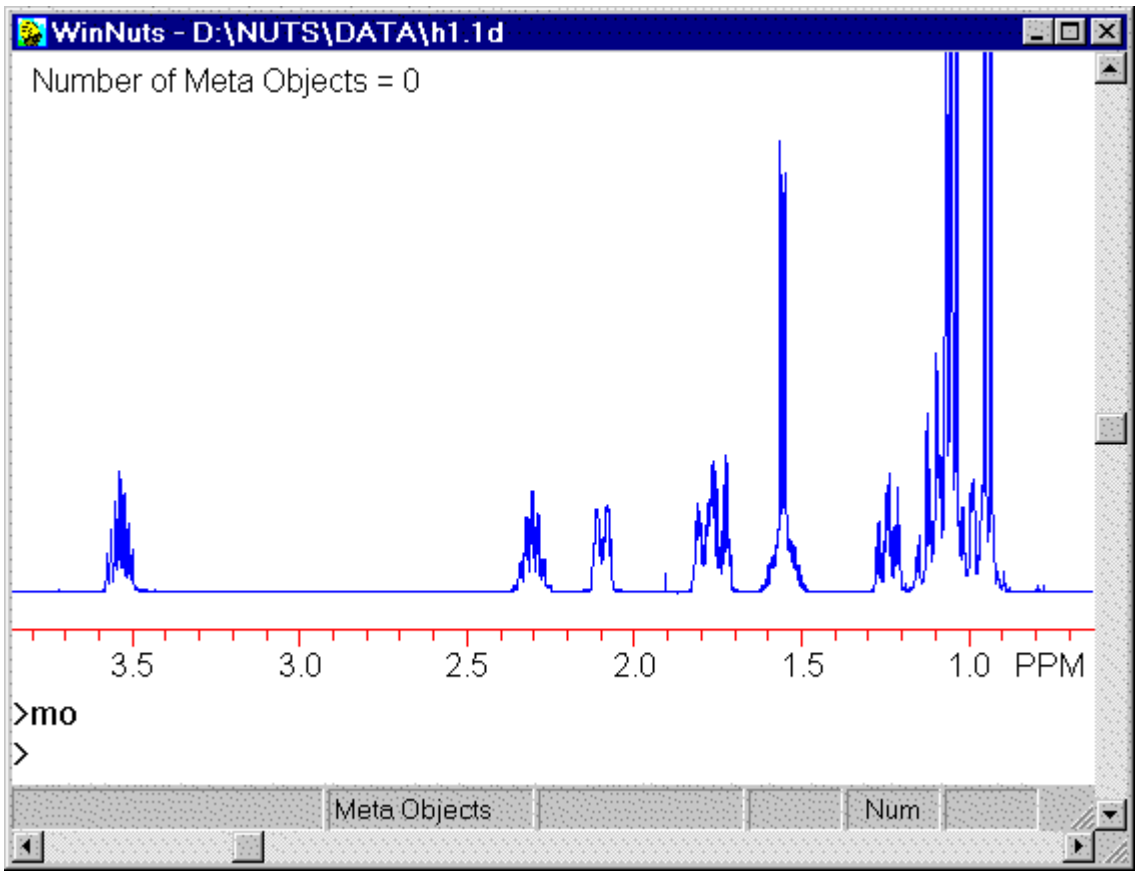
Illustrated example of MO

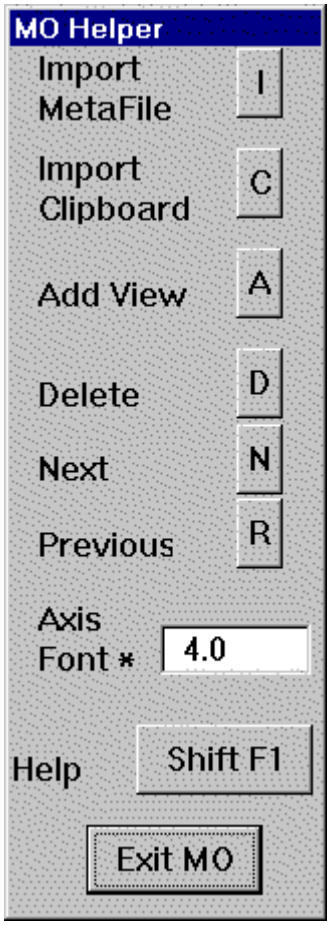
Using the MetaObjects (MO) subroutine

This subroutine is used for placing graphical objects on the screen, moving and resizing them. The graphical object can be imported from a file, pasted from the clipboard or can be an inset plot of the currently displayed spectral region.

There is also another subroutine for handling inset plots (IS, available from the Tools menu).

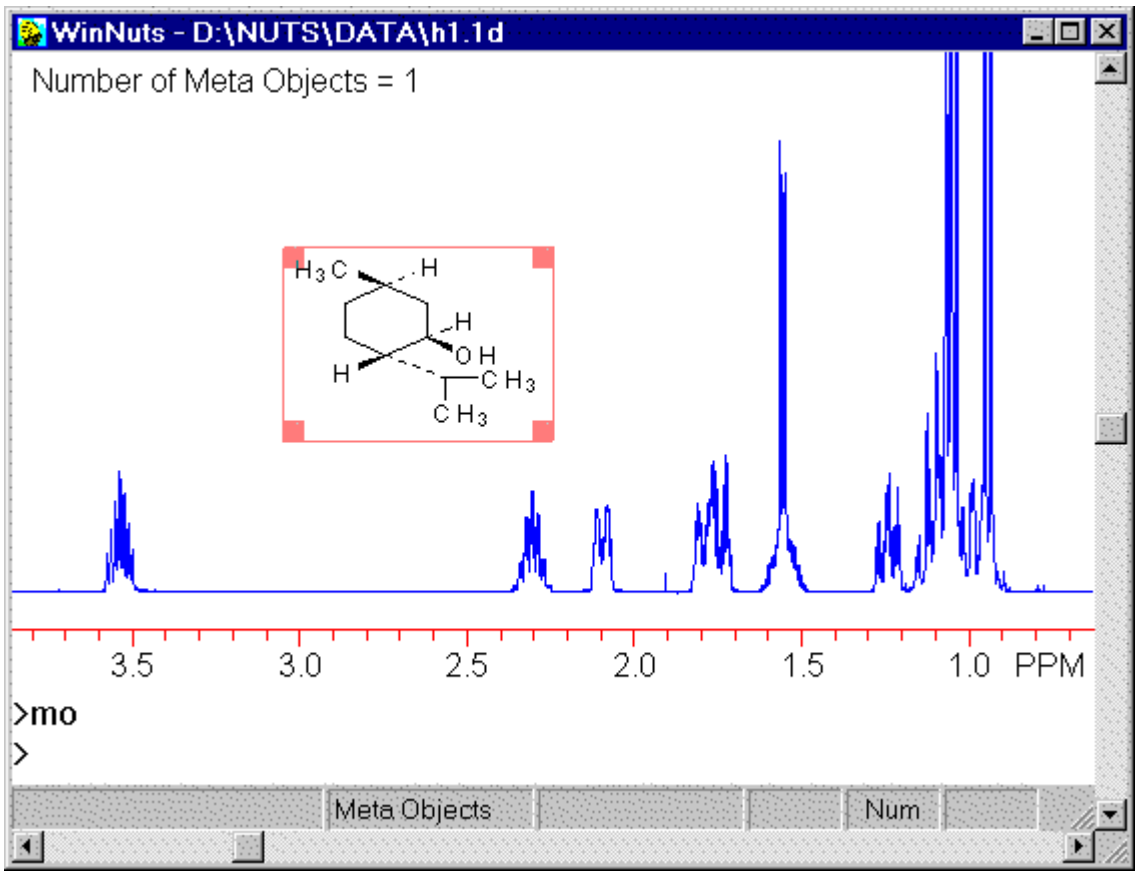
A different subroutine (ML) can be used to display a structure from a molfile.


WinNuts - D:\NUTS\DATA\h1.1d
Number of Meta Objects = 0
3.5
3.0
2.5
2.0
1.5
1.0
PPM
>mo
>
Meta Objects
Num



Enter the MO subroutine by typing **MO** or from the Tools menu.

Instead of menus, the MO routine uses a Helper window. Commands can be entered by clicking on buttons in the Helper window or by typing the corresponding commands from the keyboard. The Helper window can be moved on the screen by placing the cursor on the blue bar at the top and dragging. Note that "Meta Objects" appears on the gray status bar at the bottom of the screen to indicate that this subroutine is currently active.



Choose **I** to import a graphical object (such as a chemical structure) from a file. The file must be a Windows Metafile (or a PICT file on the Mac).

Choose **C** to paste a graphical object which has been copied to the clipboard.

The object can be moved with the mouse by placing the mouse within the red rectangle, holding down the left mouse button and dragging.

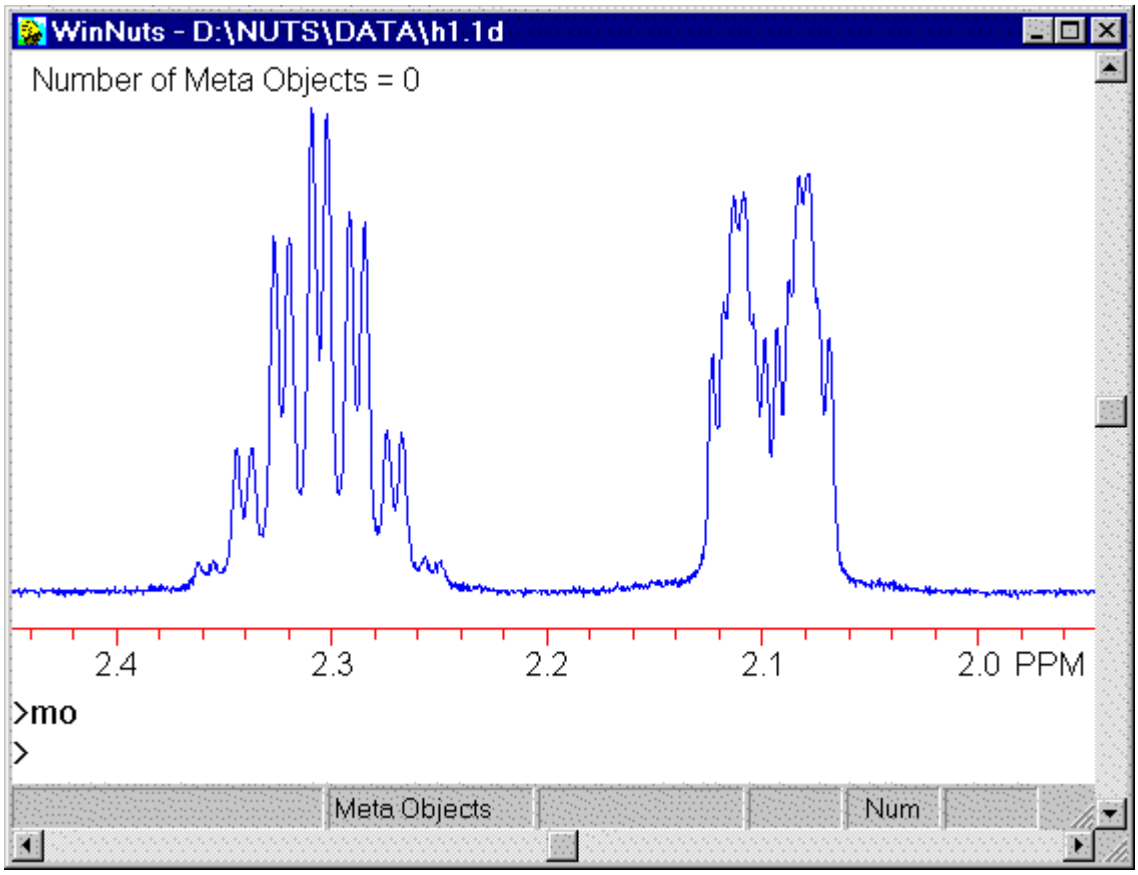
The object can be resized by placing the mouse on one of the "handles" on the edge of the red rectangle, holding down the left mose button and dragging

Several objects can be placed on the screen at one time. Nuts keeps track of them by placing them in a Linked List in the order in which they were created. To select which object to adjust, move backwards and forward through the list with the **N** and **R** buttons or keyboard commands.

Any selected object can be deleted with the button or keyboard command.

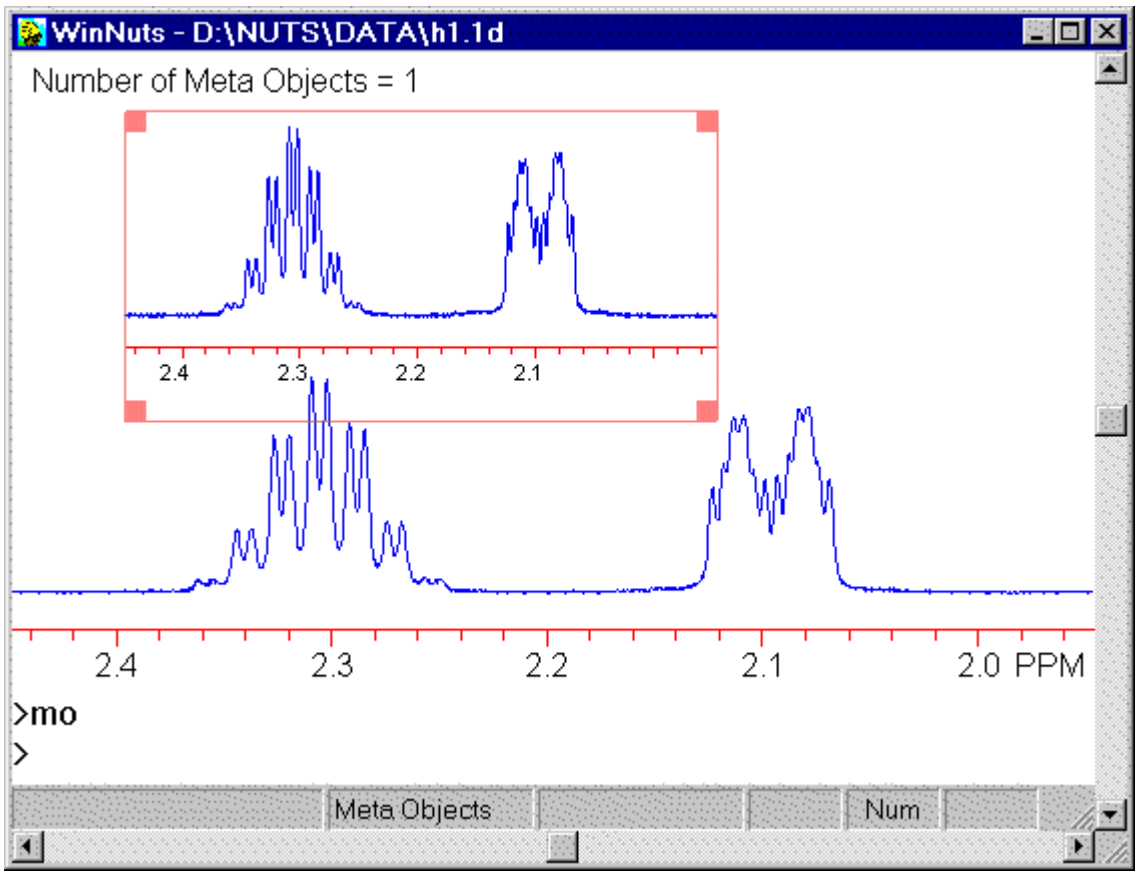
Exit the MO routine by clicking on the Exit MO button or by typing <ENTER>. The object which was added remains on the screen.

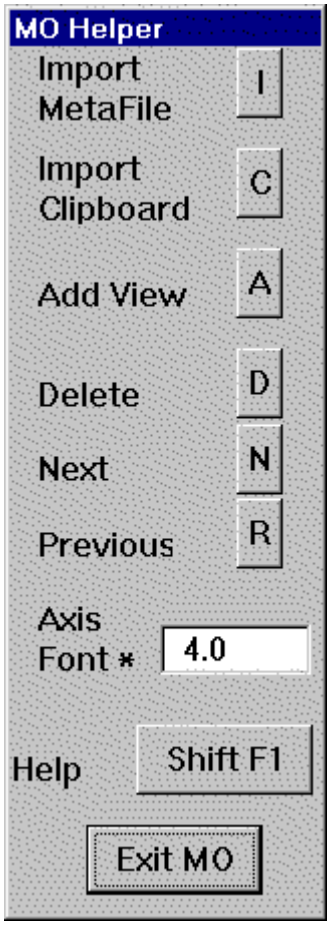
Inset plots



Use Zoom to display the region desired for the inset.

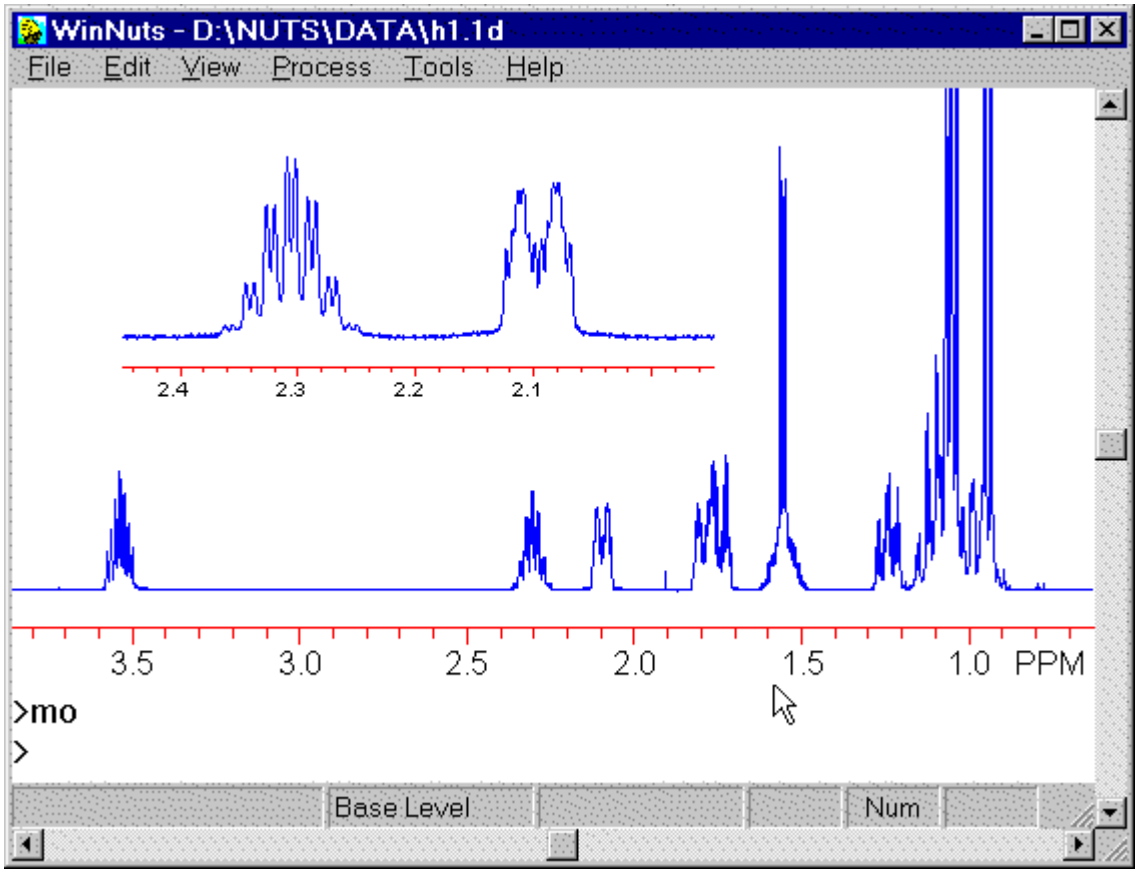
Enter the MO routine and select the Add View button or type A.


WinNuts - D:\NUTS\DATA\h1.1d
Number of Meta Objects = 1
2.4
2.3
2.2
2.1
2.4
2.3
2.2
2.1
2.0 PPM
>mo
>
Meta Objects
Num



The displayed region is captured and displayed bounded by the red rectangle, and can be moved and resized as above. The Helper Window includes a mutiplier for the font size of the axis labels. This can be increased to make the labels larger, but must be set before the inset is captured.

Exit the MO routine.



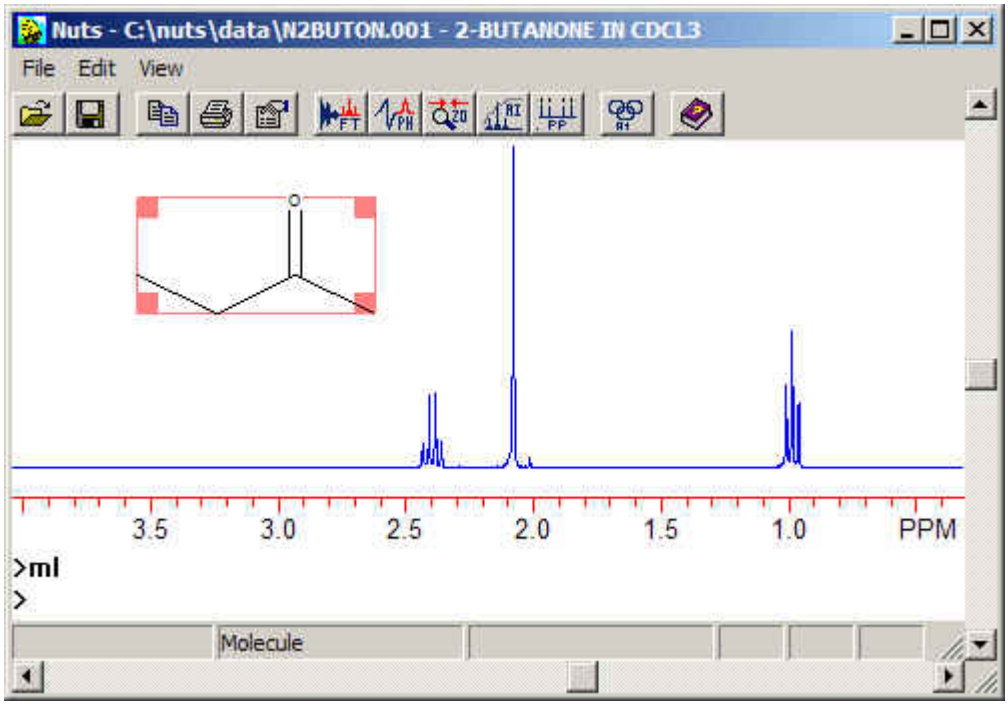
The inset is displayed here after typing Ctrl-F to display the entire spectrum.

Text, such as peak labels or integral values, may appear too small in the inset. To compensate for the font size being scaled down, try increasing the font size

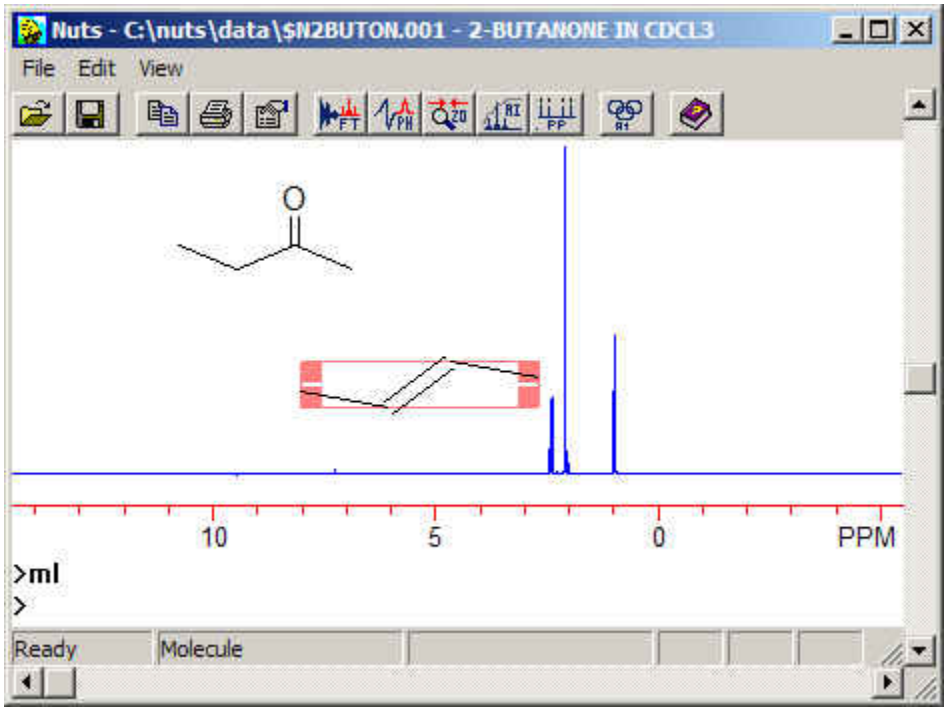
Molecule display from molfiles

ML – Molfile display

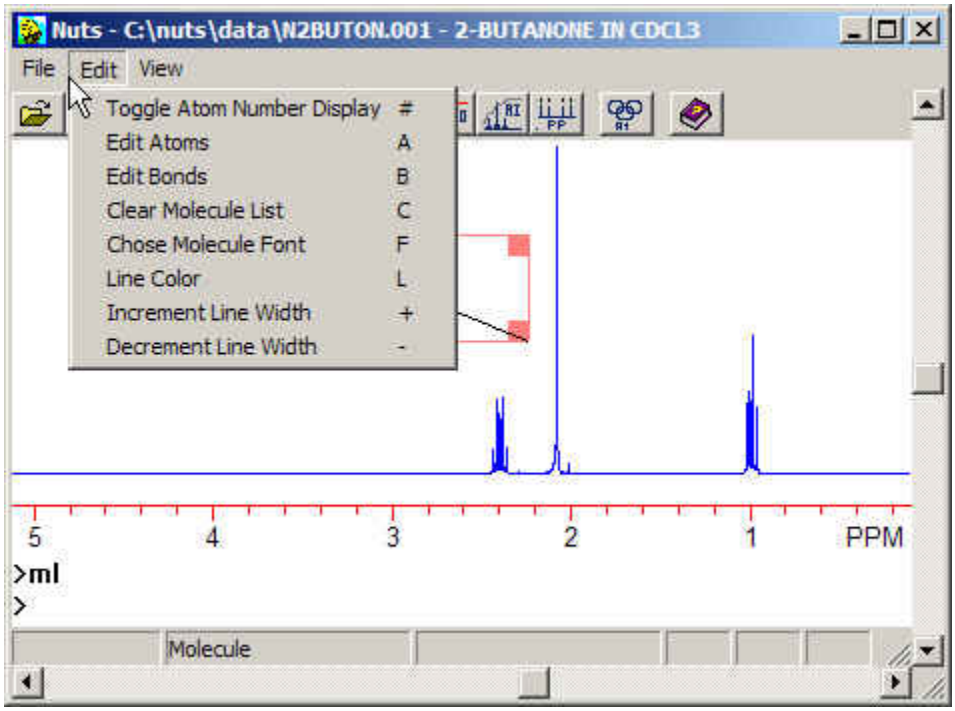
This is a subroutine for displaying structures based on a previously-saved molfile. Most drawing programs have the option of saving the structure as a molfile, which is a text file listing each atom and bond. A brief description of the molfile format is given below.



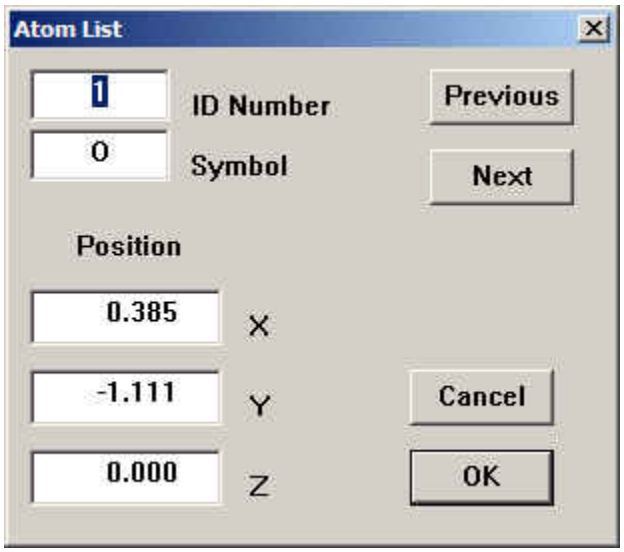
Typing **ML** enters the Molecule subroutine. Note that "Molecule" is displayed in the gray status bar, and the menus have changed. A File/Open dialog box is displayed, allowing the user to select a .mol file to be displayed. The molecule can be moved and resized in the usual way. As with all subroutines, typing <ENTER> or choosing Exit from the File menu will exit the subroutine back to the NUTS base level.



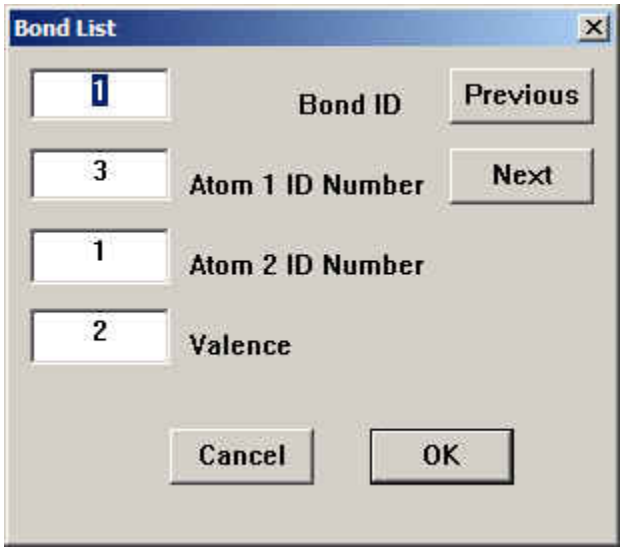
More than one molfile can be displayed. Typing **G** or choosing Get Molfile from the File menu allows additional files to be displayed. Typing **I** or choosing Increment from the File menu allows each structure, in turn, to be selected. The selected molecule can be deleted by typing **D**. All molecules can be cleared by typing **C** or choosing Clear Molecule List from the Edit menu.



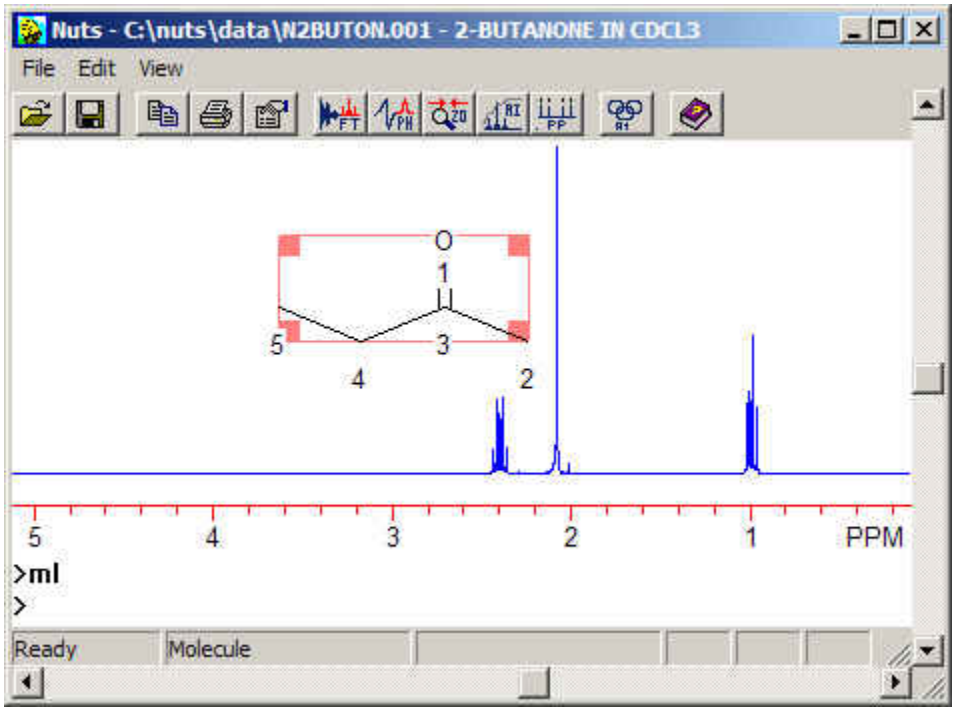
The Edit menu contains several options, which can also be entered from the keyboard. Commands operate on the currently selected molecule. The + and - keys allow the bond line width to be changed. The font can be changed by typing **F** or selecting Choose Molecule Font from the Edit menu.



The **A** command displays atom properties



The **B** command displays bond properties



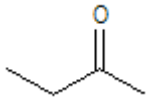
The # key toggles on/off display of the numeric atom labels.

The preliminary steps are in place to be able to search a chemical shift database by clicking on a specific carbon atom. This is done by generating a text-based code for the atom, and then searching a pre-existing text database for matches. Right click on a carbon atom to display matches found in a small database included with NUTS. The

code for the atom can be displayed by placing the cursor on the chosen atom and typing **?** Note that this feature is incomplete.

See also: Displaying a structure as a MetaObject, ^{13}C chemical shift searching, substructure coding

Sample molfile

A molfile for 2-butanone 

looks like:

```
ChemWindow
  5  4  0  0  0  0  0  0  0  0  1 V2000
    2.2994    2.0277    0.0000 O   0  0  0  0  0  0  0  0  0  0  0  0
    3.2617    0.3611    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
    2.2994    0.9166    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
    1.3372    0.3611    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
    0.3750    0.9166    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
  3  1  2  0
  3  2  1  0
  4  3  1  0
  5  4  1  0
M  END
```

The first 2 lines are unimportant for our purposes. The next 5 lines are the atom table, one line per atom. The first 4 columns are the atom's x, y and z coordinates and atom type. Because this was generated from a 2D drawing program (ChemWindow), all z coordinates are zero.

The next 4 lines comprise the bond table, 1 bond per line. The first 2 columns list which 2 atoms are connected by the bond. The third column indicates whether the bond is single (1), double (2), triple (3) or aromatic (4).

Details of the MolFile format

First line of table is the "Counts Line":

```
aaa bbb lll fff ccc sss xxx rrr ppp jjj mmm vvvvv
      aaa = number of atoms
      bbb = number of bonds
      lll = number of atom lists
      fff = obsolete
      ccc = chiral flag: 0=not chiral, 1=chiral
      sss = number of stext entries
      xxx = obsolete
      rrr = obsolete
```

```
      ppp = obsolete
      jjj = obsolete
      mmm = number of lines of additional properties,including
      the M END line.
      No longer supported, the default is set to 999.
      vvvvvv is the version number - this software accepts V2000
      or V3000
```

The next block of lines is the Atom Block:

```
xx.xx yy.yy zz.zz aa dd cc ss hh bb vv HH rr ii mm nn ee
      x = x atom coordinate
      y = y atom coordinate
      z = z atom coordinate
      a = atom symbol ( this software supports strings less than
      10 in length
      d = mass difference
      c = charge
      s = atom stereo parity - 0=not stereo, 1=odd, 2=even,
      3=either
      h = hydrogen count
      b = stereo care box
      v = valence
      H = H0 designator - 0=not specified, 1=no H atoms allowed
      r = Not Used
      i = Not Used
      m = atom-atom mapping number
      n = inversion/retention flag
      e = exact change flag - 0=property not applied,
      1=change on atom must be exactly as shown
```

The next block of lines is the Bond Block:

```
11 22 tt ss xx rr cc
      1 = First atom number
      2 = Second atom number
      t = Bond type - 1=Single, 2=Double, 3=Triple, 4=Aromatic
      s = Bond Stereo - 0=Not Stereo, 1=Up, 4=Either, 6=Down
      x = Not Used
      r = Bond Topology - 0=Either, 1=Ring, 2=Chain
      c = reacting center status
```

Detailed description of the molfile format can be found at http://www.mdli.com/downloads/public/ctfile/ctfile.jsp. Note that not all drawing programs adhere strictly to the published format.

Line-fitting (deconvolution)

LF – Line fit

This section describes the deconvolution subroutine. A summary of subcommands is given below. See example below of how to use LF.

Deconvolution, or line fitting, is a subroutine which adjusts parameters of a set of lines to fit peaks in a real spectrum. The parameters adjusted for each input peak are frequency, height, width at half height, and ratio of Lorentzian/Gaussian lineshape (expressed as percent Lorentzian). The user enters a set of initial peaks which will be varied in a Simplex optimization to find the best fit. Note that in more complex cases involving overlapping lines, this process may require an iterative approach with user input along the way.

The subcommands described below are single-letter commands which are executed immediately. All subcommands can be accessed via the menus, except those which require the mouse, and most can alternatively be executed from the command line. The menu selections list the single-letter commands for the user's information.

N.B. Before entering the LF subroutine, it is recommended to perform a Baseline Flatten (BF) operation on the displayed region of the spectrum, to be sure that the baseline in the region being fit is flat; otherwise, the fit will not be very good.

To begin, use Zoom to expand the spectrum so that only the peaks of interest are displayed, and then exit Zoom. Type **LF** or select Line Fit from the Tools menu to enter the fitting subroutine. Note that the menu bar has changed to display commands which are active in this subroutine. The first step is to pick a set of peaks as a starting point. Hold down the left mouse button, place the crosshair cursor at the top of one of the peaks and then release the mouse button. A red Lorentzian line will appear superimposed on the spectrum. To adjust its width, place the cursor at the peak's half height at the desired width of the line and click the right mouse button. To view the parameters for this line, type **I**, which brings up a dialog box containing all the information about the line. Any parameters except the absolute intensity and area can be adjusted, and the changes applied by clicking the Apply button. The areas are more easily compared by setting Relative Area of a chosen peak to a value such as 1. To do this, display the parameters for the chosen peak, then using the cursor, wipe across the box labeled Relative Area and type in a new value. All peaks will now have values expressed relative to this value. Close the dialog box to return to the spectrum.

To perform a Simplex fit to this line, type **O** (for Optimize). The red line will display the result of the fit. The new parameters can be viewed using the dialog box or they can be placed into the Windows clipboard by typing **T**. The parameters can then be pasted into the Windows or any other word processing program or text editor for editing, or can be displayed on the screen by typing Ctrl-B. Note that, to display the most recent parameters, not only must they be placed into the clipboard with T, but the screen display must be updated. This happens whenever some operation is performed which causes the screen to be re-drawn, but an update can be forced by typing Ctrl-B twice.

More lines can be added in the same manner as the first. Note that the current line is in red and all others are in green. A quicker way to enter the starting lines is to let the software find the peak maximum. Instead of placing the crosshair cursor on the peak maximum, place it directly below the peak, below the axis, and release the mouse button. NUTS find the peak maximum and positions the line at that frequency, provided the cursor is placed within +/- 2 data points of the position of the peak maximum. A still simpler method is to let the peakpicking function of NUTS find the peak positions and heights. Typing **P** performs peakpicking in the same manner as the PP command used at the base level of NUTS, and displays Lorentzian lines at the position of each peak. If not all peaks are selected, the user can adjust the parameters for minimum height (MH) and RMS noise factor (RM) by typing **M**, which brings up a dialog box for adjusting these 2 parameters. Lines can also be added or deleted manually.

As lines are entered, they are placed into a list. To move to the next line in the list, type **F** (for Forward) and to move to the previous line, type **B** (for Back). The easiest way to keep track of which line is which is to start with the peak farthest to the left and enter them in order from left to right. At any time, the peaks can be reordered from largest to smallest chemical shift by selecting Reorder form the Edit menu or by typing **R**. The current line (in red) can be deleted by typing **D**. Note that if the third line out of a list of 5, for example, is deleted and then a new line added in its place, the new line will be fifth and last in the list, until the list is Reordered. When the parameter dialog box is opened (with **I**), the parameters displayed correspond to the current line, shown in red. The parameters for any other line can be displayed by entering its line number or by clicking the Previous or Next buttons.

Starting with NUTS versions dated May, 2000, it is possible to "freeze" chosen parameters so they are not adjusted during the fit process. While the parameter dialog box is displayed (with command I), there is a check box labeled "Iterate" next to each of the 4 parameters. Removing the check mark will prevent the corresponding parameter from being adjusted during the fit.

Once all lines have been initialized and adjusted to some reasonable starting parameters, typing **A** initiates the Simplex fit on all lines. This can take a minute or more if the fit consists of several lines, as the dimensionality of the problem is large (4 times the number of lines). To fit a group of overlapping peaks, it works best if the starting point is as close as possible. Try optimizing each line individually (with **O**) before starting the complete fitting routine (**A**). The Simplex routine has been implemented to perform a fairly quick fit so that the user gets an indication of the progress. After one iteration, adjustments can be made to individual lines as needed and then the optimization performed again. Since the starting point for the second iteration is closer, the result will be better. During the optimization, NUTS displays a decreasing Error value as each Simplex loop is completed to allow the user to monitor how the optimization is proceeding.

There are several choices for viewing the calculated spectrum and comparing it to the real spectrum. Typing **S** displays the sum of the lines, which should overlay the real spectrum.

Typing the plus sign displays both the sum and the individual lines. Typing **L** displays the individual lines. Typing the period key toggles the display of individual lines between a solid and a dotted line. Typing the minus sign displays the difference between the real and calculated spectra. To perform the fit to the real spectrum, the LF routine first digitizes the theoretical line to match the real peak point-for-point. The digitized calculated spectrum can be viewed by typing **C**.

Exiting the line fitting subroutine (with Enter) does not delete the calculated lines. Therefore, LF can be exited and changes made to the frequency limits and then LF re-entered. The calculated lines will be adjusted to reflect the new display. Note that this could result in the fit operating on peaks which are not displayed.

NUTS provides the option of saving the calculated spectrum and/or the list of parameters which define the calculated peaks (position, height and width for each line). Once a calculated spectrum has been saved, it can be opened and manipulated exactly as any real spectrum. A previously saved list of parameters can be opened and the resulting calculated spectrum will be displayed. This allows the user to save a "template" for repeated use on a series of spectra. These options are available under the File menu.

New macro commands were added to set the file names for these operations:

SET LF_GET_FILENAME = xxxxxxx.yyy

SET LF_WRITE_FILENAME = xxxxxxx.yyy

Another set of Macro Commands were added to increment any filename extensions for these file names (xxx.001 to xxx.002):

DO INCREMENT_LF_GET_FILENAME

DO INCREMENT_LF_WRITE_FILENAME

Subcommands

A -- simplex fit to All lines
B -- move Back to previous line
C -- display digitized line Calculated from current Lorentzian (displayed in grey)
D -- Delete current line
F -- move Forward to next line
I -- display parameter Information dialog box
G -- Get (open) a lines information text file
L -- display individual Lines
M -- display Minimum height dialog box for peakpicking
O -- Optimize fit to current line
P -- Pick peaks for starting point
R -- Reorder peaks from low to high field. Remember to type T again to copy the

new list to the clipboard.
S -- display Sum of individual lines; this toggles between the sum and the individual lines
T -- Type parameter list into the Windows clipboard
W -- Write a lines information text file
Z -- Zero all lines
.(period key) -- Toggle display of individual lines between dotted and solid line.
+(Plus sign) -- Display both individual lines and sum of lines
-(Minus sign) -- Display difference between real and calculated spectra (displayed in grey)
% -- Set default percent Lorentzian lineshape (default is 1, or 100% Lorentzian).
ENTER -- exit LF subroutine

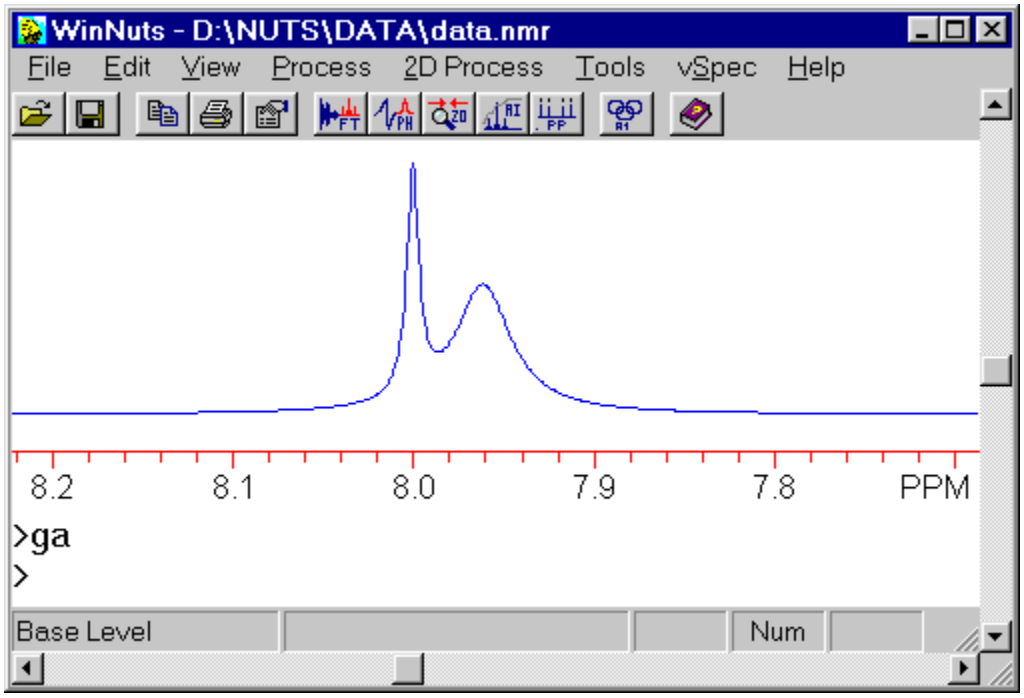
Illustrated example of LF

Using the Line Fitting (LF) subroutine.

This subroutine performs a Simplex fit to a set of peaks. The user must define a starting set of peaks before starting the fit process.

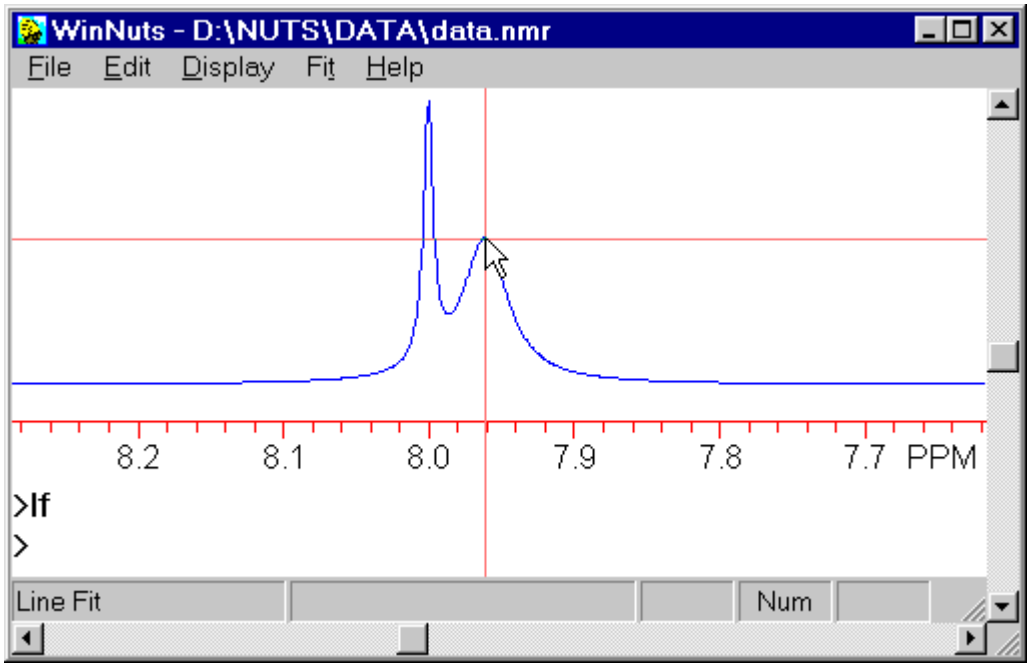
The parameters adjusted are each peak's frequency, intensity, width at half height and ratio of Lorentzian to Gaussian lineshape. Starting with NUTS versions dated May, 2000, it is possible to "freeze" chosen parameters so they are not adjusted during the fit process. See below.

Note that LF is not included in NUTS Lite.



Because these 2 peaks overlap, integrating does not give an accurate ratio of their areas.

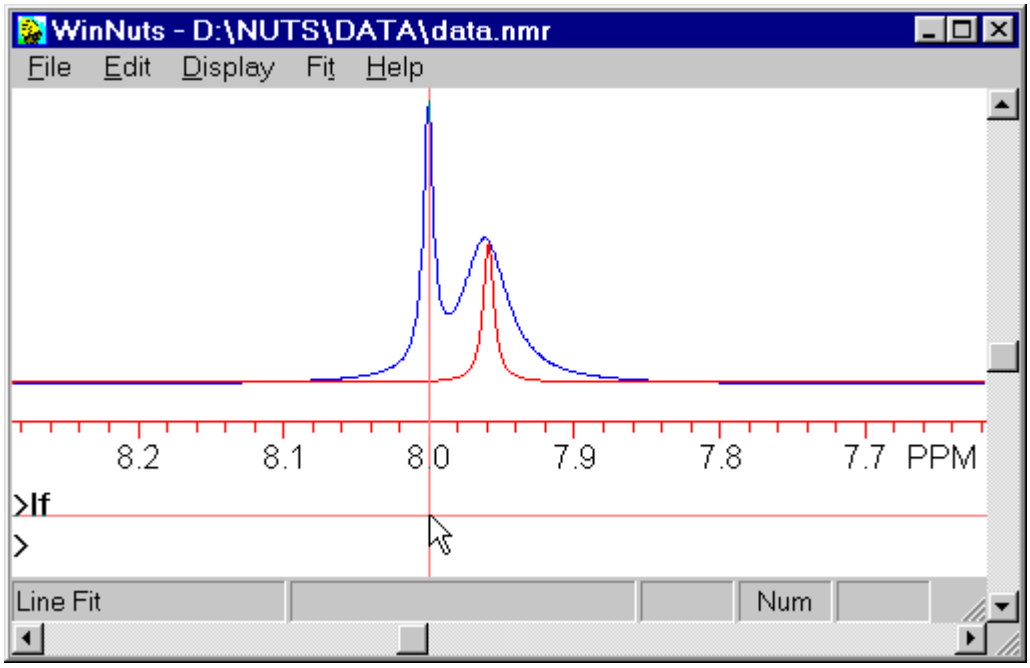
Start by expanding the spectrum to show just the region of interest. Enter the deconvolution routine by typing **LF** or by selecting Line Fit from the Tools menu.



Begin by creating a set of peaks that match the real spectrum reasonably well, as a starting point for the fitting routine.

There are 3 different ways to enter a set of peaks.

As shown above, place the cursor on the top of a peak and click once with the left mouse button.

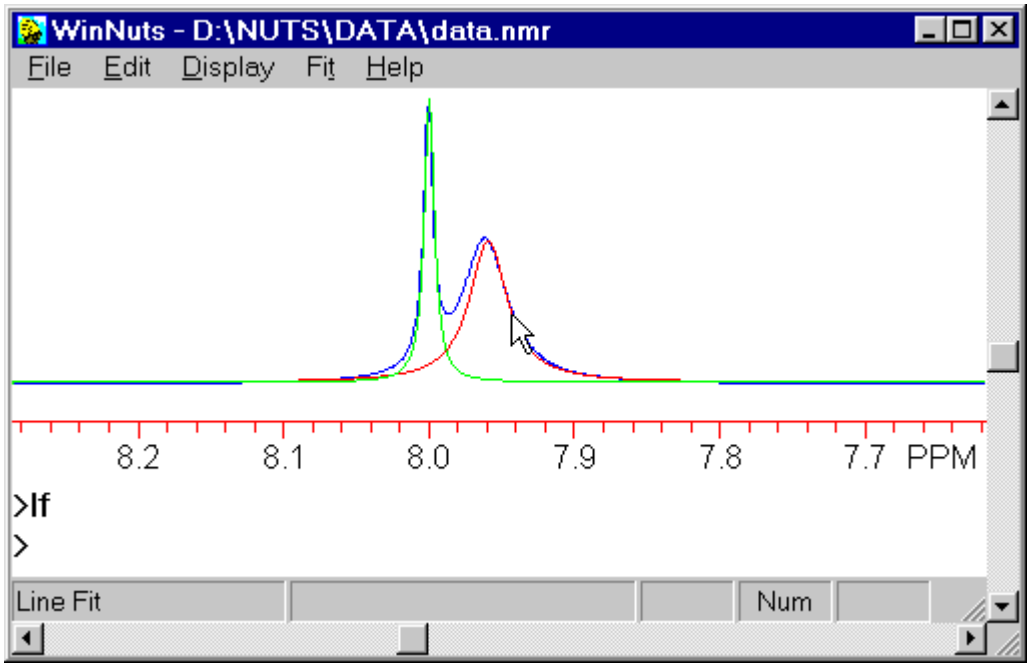


A red Lorentzian line is drawn at the location chosen in the previous step.

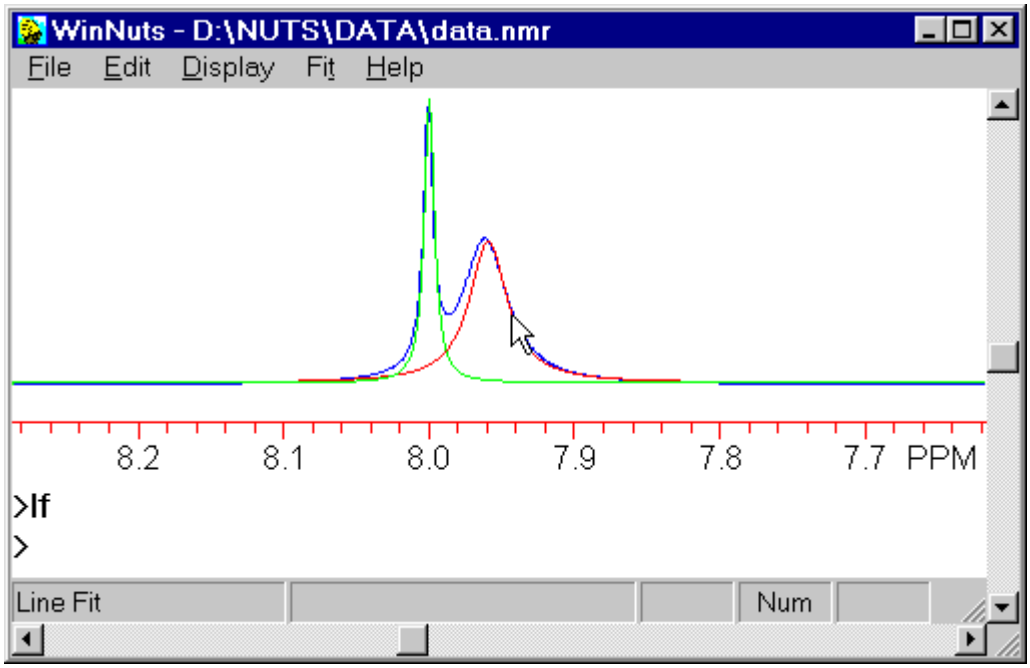
A second way to input a peak is to place the cursor below the axis at the chosen frequency. NUTS will find the closest peak maximum and put a peak there.

Notice that the second peak which was entered is red, and the first peak has changed to green. NUTS builds a list of peaks as they are entered, and the "current" peak is always in red.

A third method, not shown here, is to let NUTS select peaks using the same criteria as normal peak picking. To use this method, type **P** or choose Peak Pick from the Fit menu.



It is possible to select a different peak to be the current peak by moving through the list, by typing **F** (Forward) and **B** (Back) commands, which are also available from the Edit menu.



The broader peak has been selected as the current peak, so it is now shown in red.

The default peak width did not match the actual width of this peak. The fit will proceed better if its width is adjusted.

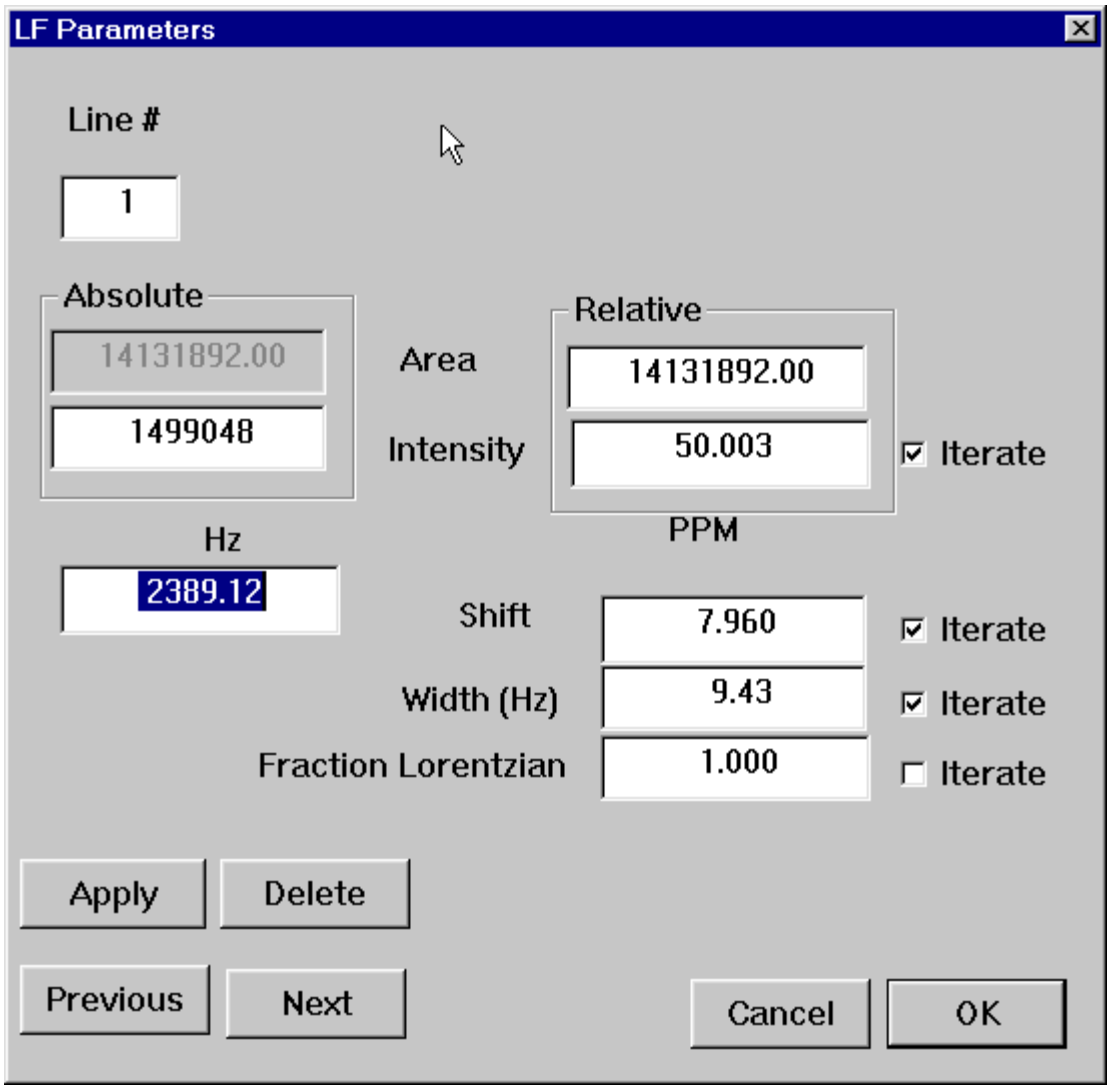
Place the cursor on the peak at half height, and click the right mouse button. The width of the red peak changes.

(Users with a single button mouse should hold down the shift key and use the mouse button when instructions call for use of the right mouse button.)

Once all starting peaks have been created, and line widths adjusted, we are ready to start the fitting process. By default, 4 parameters are adjusted during the fit for each peak: amplitude (intensity, or height), width, position (frequency) and ratio of Lorentzian to Gaussian lineshape. Any of these parameters may be "frozen", so that it is not adjusted during the fitting process (see below).

The fit is performed by typing **A** or selecting Optimize All Lines from the Fit menu. This is a Simplex optimization. The optimization can be aborted by typing **Q**

The parameters for each peak can be examined by typing **I** or selecting Information on Line from the Fit menu. This brings up the box shown below.



Intensity and Area are reported in two ways, as absolute and relative numbers. The relative intensity is relative to the tallest peak in the spectrum equal to 100, so is usually a more convenient number. The absolute numbers can be used to compare different spectra.

Shift is shown in both Hz and ppm.

Each peak is fit as a combination of Lorentzian and Gaussian lineshapes, expressed as Fraction Lorentzian, which is always a number between 0 and 1.

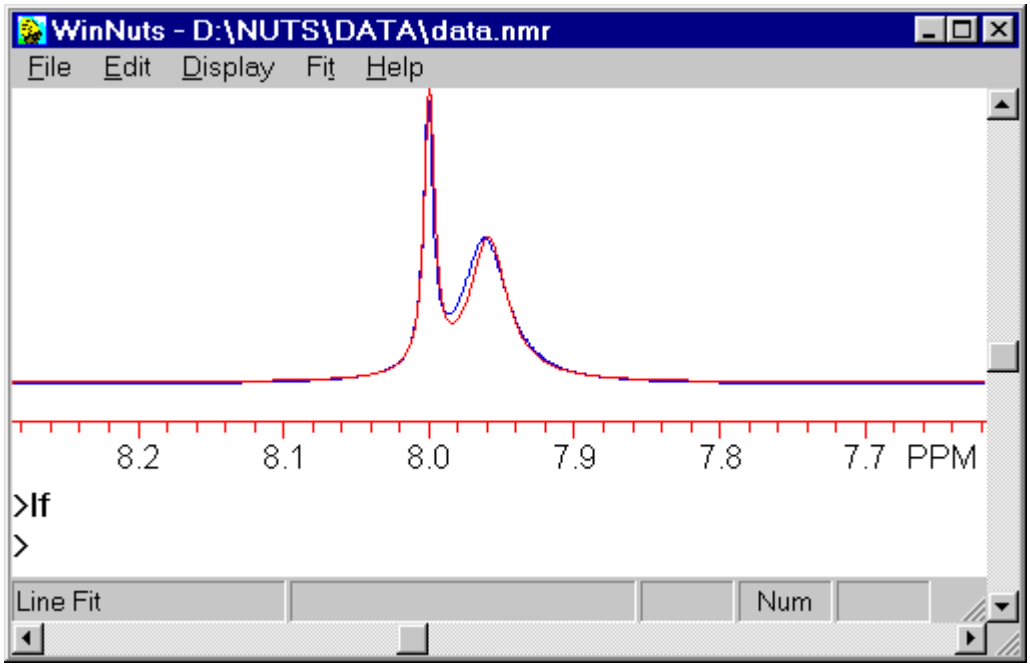
The user can change parameters by entering values in any field except Absolute Area. When a value is changed, and Apply or OK is clicked, related values are updated to reflect the change, and the peak is changed accordingly.

It is usually helpful to set the Relative Area of a chosen peak to a convenient number, which is done by entering the desired value in the box for Relative Area. All area values are then scaled relative to this.

Note the 4 check boxes labeled "Iterate", next to the Intensity, Shift, Width and Fraction Lorentzian values. A check mark in a box indicates that this parameter will be adjusted during the fit. To "freeze" a chosen parameter, remove the corresponding check mark.

The parameters for each peak can be placed into the clipboard by typing **T** or by selecting Type Peak List to Clipboard from the Edit menu. This can then be pasted into any text editor, or displayed on the screen using Ctrl-B. The resulting table looks like the following:

```
LINE     HZ     PPM    HEIGHT     REL_HT   WIDTH   AREA   REL_AREA
FRACTION LORENTZIAN
  1   2401.33   8.000  2716201    90.602   2.36  6408714      37.71
1.000
  2   2389.61   7.961  1521459    50.750  11.17 16996376     100.00
1.000
```



The sum of the calculated peaks can be displayed, rather than the individual peaks, by typing **S** or choosing Sum from the Display menu. The sum is shown above.

The Display menu provides other display options, including the difference between real and calculated peaks.

Spectrum simulation

NS - NMR Simulation

This NUTS subroutine calculates and displays a spectrum based on user-input values for chemical shifts and coupling constants. NUTS makes the assumption that in most cases, the user wants to fit a calculated spectrum to a real spectrum. Therefore, the NS routine uses the values of Spectrometer Frequency, Sweep Width, Spectrum Offset and Number of Points from the current spectrum for its calculation. (It is possible to change these values; see below.) Subcommands can be accessed via the menus or by typing single-letter commands which can be found next to the corresponding operation in the menu displays. See illustrated example below of how to use NS.

To begin, type **NS** or select Simulation from the Tools menu to enter the simulation subroutine. From the Edit menu, choose Add/Edit Simulation Data. This brings up a dialog box for entering data. First enter the number of spins, then enter chemical shifts (in either Hz or PPM; set units in lower left corner). To enter coupling constants, click on the button in the lower right corner. When finished, click on **Accept and Recalculate**, which closes the dialog box, performs the simulation and displays the resulting spectrum. Repeat this process to adjust the input parameters.

To enter data for degenerate spins (eg., a methyl group), enter each as a separate spin with the same value for chemical shift and couplings to other nuclei. (The couplings among degenerate spins are zero.)

Within the data input dialog box is a box labeled Nuclei. By default, all nuclei are taken to be H, on the assumption that this is the most common situation. The actual label is irrelevant; the only important point is whether or not all spins have the same label. If the Nuclei labels of 2 of the spins are set to be different, second order interactions between those 2 spins are ignored. (This can be used to compare the results obtained for a given set of input parameters with and without consideration of second order effects.) To simulate a heteronuclear case, simply make the chemical shift of the heteronucleus very different from the others, and the Nuclei labels need not be changed.

To compare real and simulated spectra, choose Both from the Display menu. The vertical scale of both spectra can be adjusted in the usual way, with either the right-hand scroll bar, Page UP/Page Down keys or the < > keys. To adjust the scale of just the simulated spectrum, type **L** or choose change LB/Amplitude from the Edit menu, which brings up a dialog box allowing the linewidth and/or scale of the simulated spectrum to be adjusted, without having to re-do the entire calculation. When the simulation subroutine is exited (with ENTER), the input data are not lost. This means the simulation routine can be exited and re-entered without losing data. It may be easier to view real and calculated spectra if the DC offset of the real spectrum is adjusted so that the 2 spectra do not overlap. It is possible to exit NS, change DC and re-enter NS. To change the

Spectrometer Frequency, exit NS, type SF, enter a new value, then re-enter NS. The input parameters are still active, but the spectrum is not automatically recalculated on re-entering NS. To recalculate the spectrum, choose Recalculate from the Edit menu or type **R**.

While in the simulation subroutine, the cursor readout works as usual. With both real and simulated spectra displayed, the cursor can be used to measure shifts and coupling constants from the real spectrum for input in the parameter input dialog box. This can be repeated until all starting parameters are input before investing time to calculate the spectrum by selecting Accept Changes in the dialog box.

Within the simulation subroutine, it is possible to enter Zoom to change the displayed region. This is done by typing **Z** or by choosing Enter Zoom from the Display menu. The cursor changes to the Zoom crosshair while in Zoom. Typing Enter exits the Zoom routine and returns to NS. Note that not all Zoom options are available. The calculated spectrum is not digitized in the way a real spectrum is, but the Zoom routine operates at the current digital resolution. This means that if the real spectrum being fit is poorly digitized, there may be limits on the ability to Zoom in on a narrow spectral region within NS.

To experiment with spectrum simulations not matching a real spectrum, it may be desirable to change the values of Spectrometer Frequency, Sweep Width, Spectrum Offset and Number of Points. With the exception of Number of Points, these parameters can simply be changed by bringing up the parameter dialog box from the Parameters menu and entering new values. To change Number of Points, choose File New from the File menu (or type **FN**), which brings up a dialog box allowing parameters to be set. On re-entering NS, any previously calculated spectrum is displayed, but this does not reflect the changes in parameters. Execute Recalculate to implement changes. Note that the time required to calculate a spectrum is independent of the Number of Points.

The simulation data can be saved as a text file containing both the input parameters and the calculated data as a list of frequencies and intensities. An example of such a text file is shown below. A previously saved simulation file can be opened from within the simulation subroutine and the resulting spectrum displayed. Note that if the current value for SF is different from that which was saved with the simulation file, the 2 spectra (real and simulated) cannot be compared until the simulation is recalculated. Regardless of whether the chemical shifts were entered as Hz or PPM, they are saved in the simulation file as PPM. This option is available from the File menu.

The simulated spectrum can also be saved as an FID. The resulting file can be manipulated in the same manner as real data. The FID is created from the calculated transition frequencies and intensities. The time required to generate the FID depends on the current Number of Points. Be sure that the current digital resolution (Number of Points/SW) is sufficient to adequately digitize the calculated spectrum, or artifacts may appear in the spectrum generated this way.

The spectrum can be printed by choosing Print from the File menu. Whatever is currently displayed (eg., the simulated spectrum or both real and simulated spectra) is what will appear on the plot. The currently displayed screen can also be copied to the Windows clipboard for pasting into a document using the Copy Screen option from the Edit menu.

The simulation subroutine includes the option of performing a Simplex optimization of spectral parameters to match a real spectrum. This is available from the Optimize menu or by typing **O**. Be advised that this process involves a huge number of calculations and so can be very time-consuming. It is best to adjust parameters manually first to be as close as possible before attempting optimization. Note that the optimization is not constrained to keeping shifts or coupling constants of degenerate spins the same, so that after optimization degenerate spins may have slightly different values. These can be adjusted manually and, if desired, the optimization repeated.

The time required to calculate a spectrum depends on the number of spins and on the speed of the computer. Calculations involving up to 8 spins take just a few seconds. A 10-spin simulation just under a minute on a reasonably fast Pentium. (We have not had the patience to time a 12-spin simulation!) Optimization is therefore practical only on smaller spin systems.

Subcommands:

The following subcommands are active within the NMR Simulation subroutine. They are single-letter commands which are executed immediately. All commands can also be accessed via the menus.

A Add/Edit simulation data; opens a dialog box for setting shift and coupling values
B Display Both simulated and real spectra
C Display Calculated spectrum only
D Display Digitized calculated spectrum
F Generate FID from calculated transition frequencies and intensities.
G Get simulation data from file
Q Quit drawing spectrum. Interrupts a slow drawing operation.
R Re-calculate spectrum based on input data
S Save simulated data to text file
Z Enter ZOOM subroutine to alter displayed frequency range. Not all ZOOM functions are active.
^C Copy currently displayed spectrum to Windows clipboard
- (minus sign) Display difference between real and calculated spectra

Sample Simulation File

When a simulation is saved from within the NMR Simulation subroutine, a text file is created which looks like the example below (which is for ODCB). This file can be read into the Windows Notepad or any word processor for editing and printing. It includes the

shifts and couplings from which the spectrum was calculated, and the transition frequencies and intensities which resulted from the calculation. Chemical shifts, labeled **V(i)** are in ppm. Coupling constants, labeled **J(i,j)**, are in Hz. The Spectrometer Frequency is included for reference only. When this file is read into the NS routine, SF is not changed. The SF value can be changed by exiting NS, resetting SF, re-entering NS and executing Recalculation.

```
NUTSsimulation
Scale =  10000.00
Spectrometer Frequency =   90.000000  MHz
LineWidth =     0.250  Hz
Spins = 4

V(1) =    7.23  PPM

V(2) =    7.23  PPM
J(1,2) =   0.30  Hz

V(3) =    6.97  PPM
J(1,3) =   1.50  Hz
J(2,3) =   8.10  Hz

V(4) =    6.97  PPM
J(1,4) =   8.10  Hz
J(2,4) =   1.50  Hz
J(3,4) =   7.50  Hz


Number   Transitions(Hz)  Transitions(PPM)    Intensities

    1       660.331          7.3370          0.121
    2       659.535          7.3282          0.137
    3       656.446          7.2938          1.241
    4       656.057          7.2895          1.281
    5       652.630          7.2514          2.886
    6       650.680          7.2298          1.659
    7       649.768          7.2196          1.863
    8       646.846          7.1872          2.759
    9       646.376          7.1820          2.978
   10       642.961          7.1440          0.501
   11       642.863          7.1429          0.572
   12       635.137          7.0571          0.572
   13       635.039          7.0560          0.501
   14       631.624          7.0180          2.978
   15       631.154          7.0128          2.759
   16       628.232          6.9804          1.863
   17       627.320          6.9702          1.659
   18       625.388          6.9488          2.886
   19       621.943          6.9105          1.281
   20       621.554          6.9062          1.241
   21       618.465          6.8718          0.137
   22       617.669          6.8630          0.121


End NUTS Simulation File
```

FN -- File New

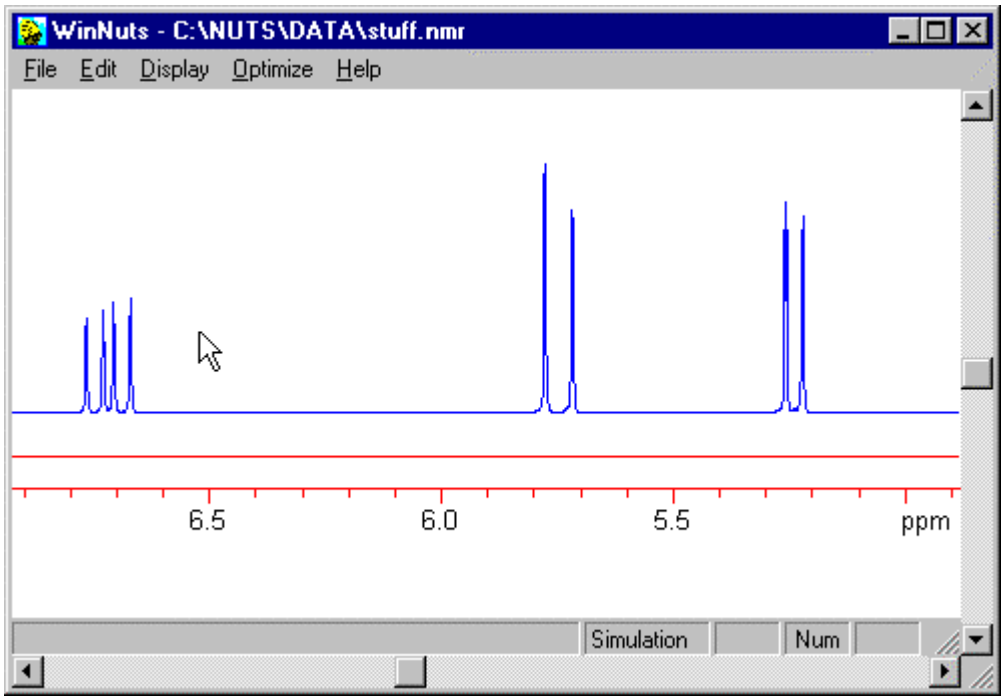
Used in conjunction with spectral simulation, this command allows the user to change the values of Spectrometer Frequency, Sweep Width, Spectrum Offset and Number of Points. Choosing File New from the File menu (or typing **FN**) brings up a dialog box allowing these parameters to be set.

Illustrated example of NS

Spectrum simulation from shifts and couplings (NS).

This subroutine calculates a spectrum, including second-order effects, from the user's input values of chemical shifts and coupling constants.

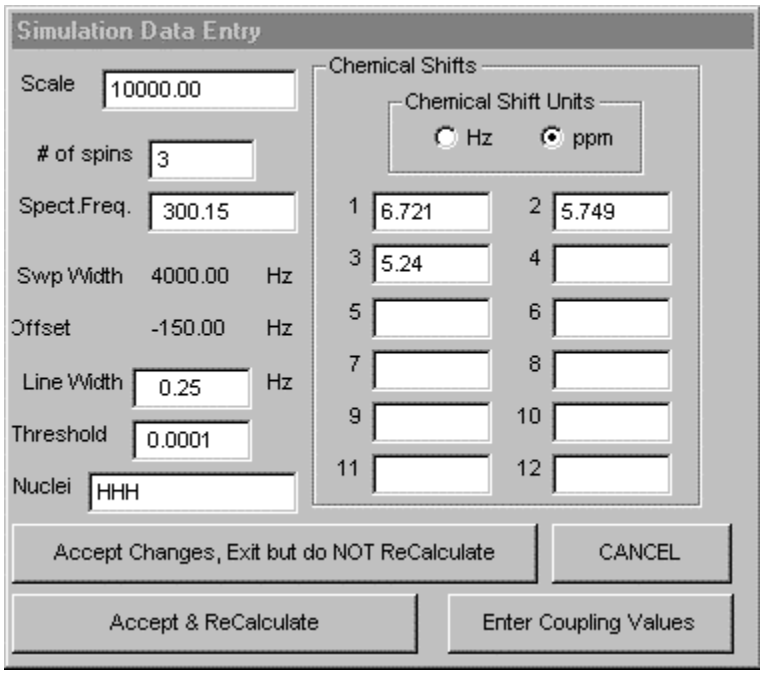
(Note that this is not included in NUTS Lite.)



On entering the NS routine, the calculated spectrum (which does not yet exist) is shown in red.

To display the real spectrum (in blue) also, select Both Spectrum and Calculation from the Display menu, or type **B**.

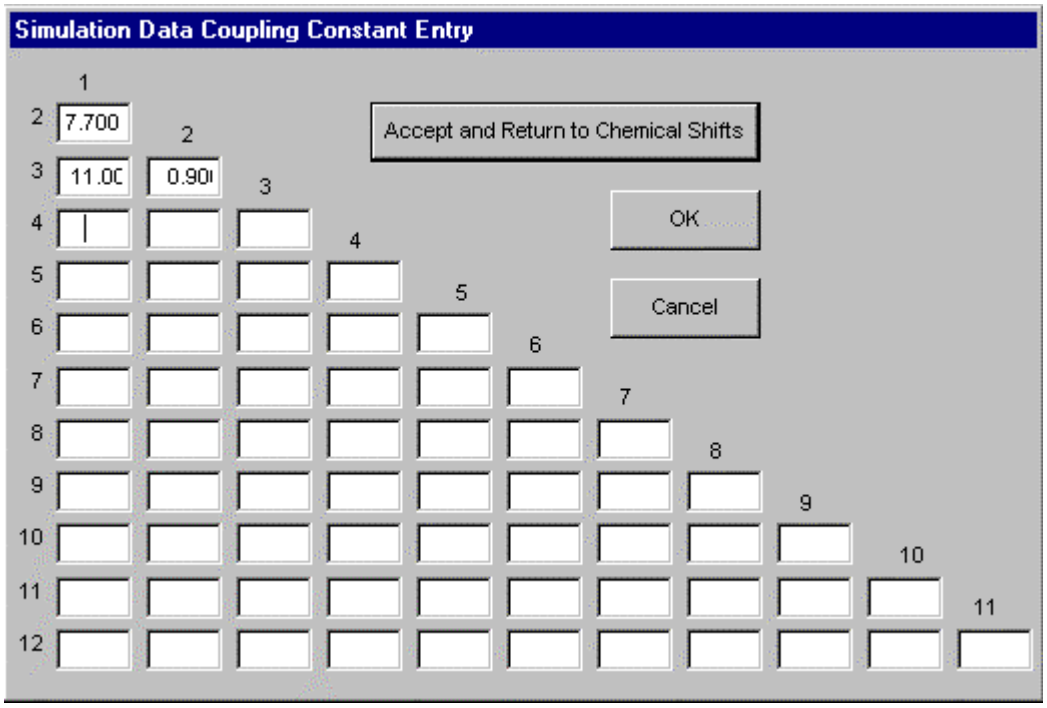
The cursor can be used to display chemical shifts and couplings, just as in the base level of NUTS.



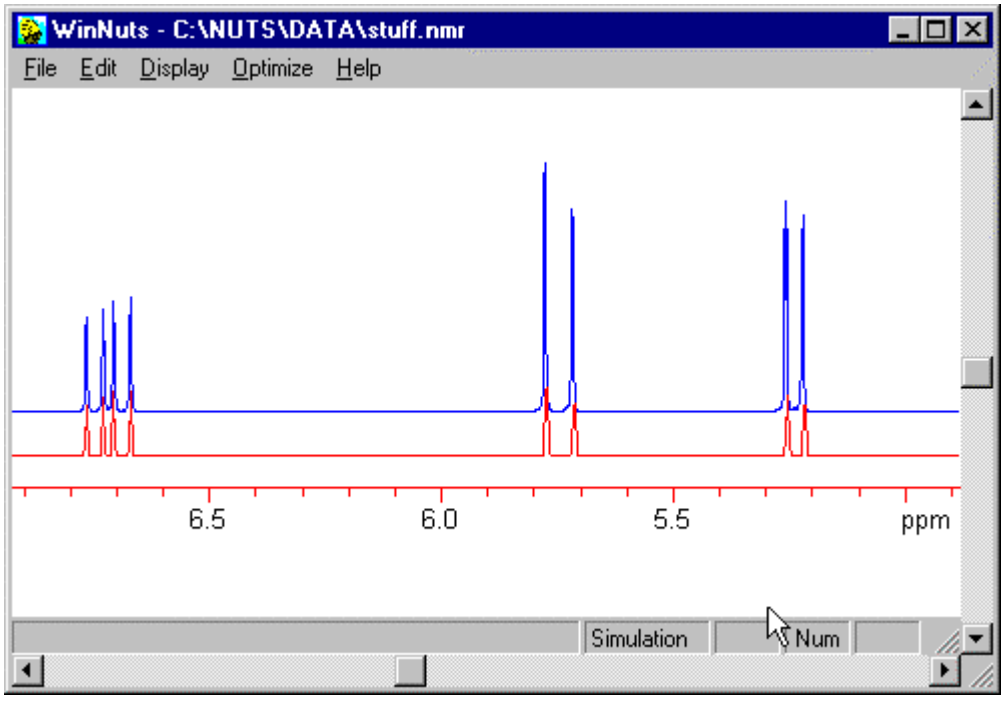
Typing **A,** or selecting Add/Edit Simulation Data from the Edit menu, brings up a dialog box allowing entry of chemical shifts and other parameters.

First, enter the number of nuclei in the spin system in the upper left corner.

You can go back and forth between this box and the spectrum by clicking on Accept Changes or Accept and Recalculate. Using Accept Changes does not calculate the spectrum, so is the quickest way to enter an initial set of values. Use Accept and Recalculate when you want to see the effect of newly entered values. To enter coupling constants, click on the button in the lower right corner.



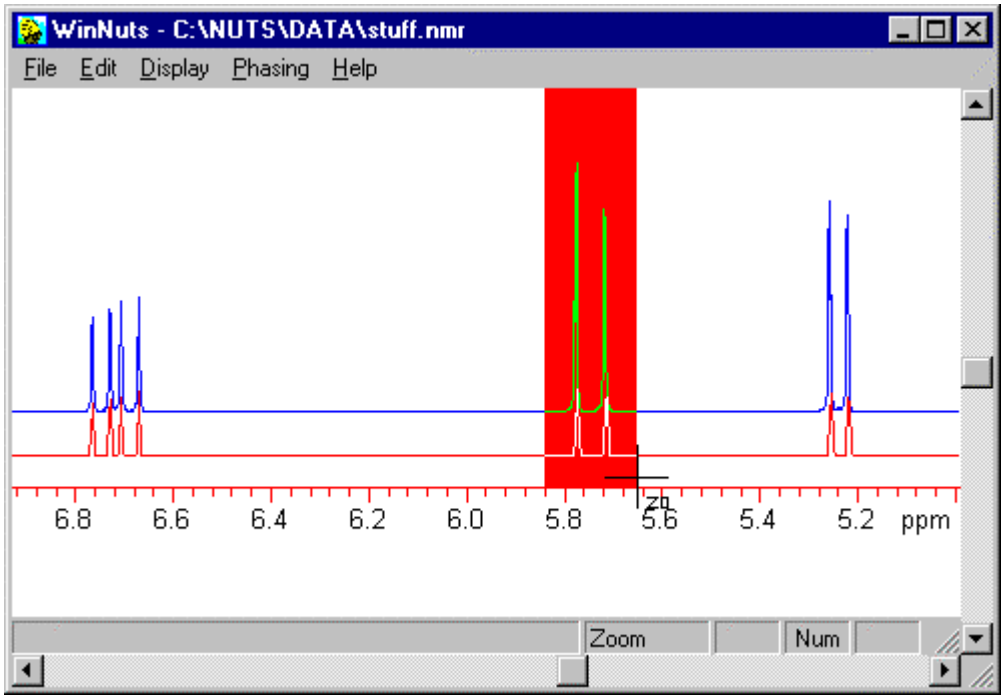
Fill in coupling constants, then click OK to return to display of the calculated spectrum or "Accept and Return to Chemical Shifts" to return to the previous parameter input box.



The calculated spectrum is shown in red.

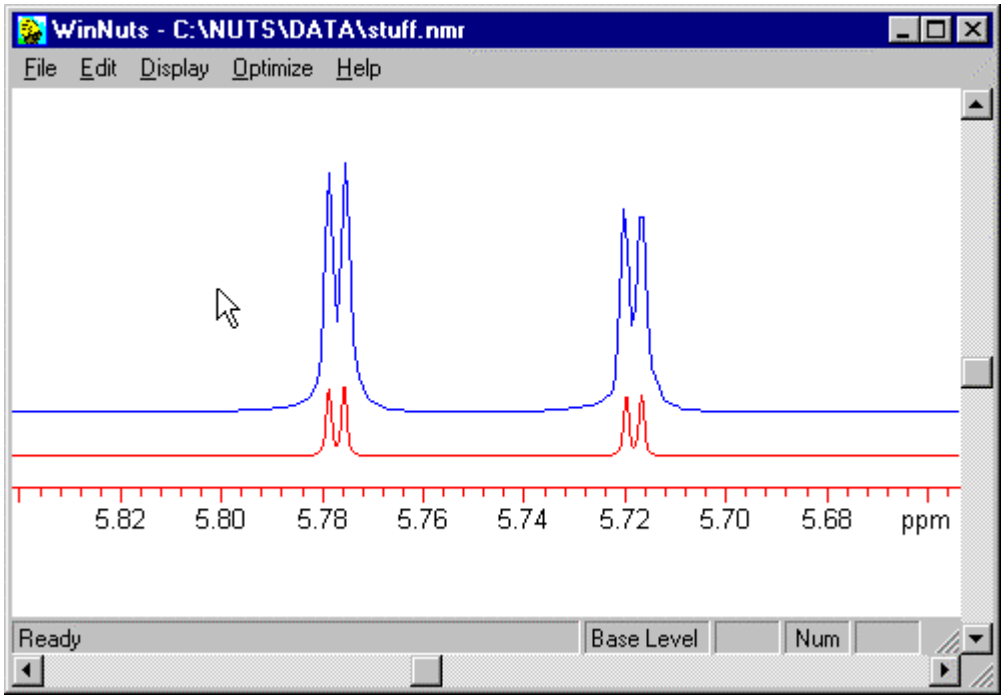
The scale of the calculated spectrum can be adjusted without recalculating by typing **L** or selecting Change LB/Amplitude from the Edit menu.

The normal scaling tools, such as the scroll bar, affect both spectra.



Zoom can be used to view details. Enter the Zoom routine by typing **Z** (***not*** ZO) or from the Display menu.

Select the region to expand as usual by holding down the left mouse button and dragging.....



… and type **Ctrl-E** to display the expanded region.

Values of shifts and couplings can be adjusted to optimize the match.

A Simplex fit can be performed, but due to the huge number of calculations involved, it is best to get close manually before attempting a fit.

Other options include displaying the difference between calculated and actual spectrum and saving the calculated spectrum as an FID.

Buffers subroutine

BU - Buffers

There are several methods within NUTS of placing data into a buffer for later use. Each was created to address a specific need, but they may be useful for other purposes. An effort has been made here to compare and contrast these tools to enable the user to apply them effectively.

> The **Add/Subtract buffer** (loaded with AL) is used with Dual Display as well as the Add/Subtract subroutine.
> The **Data-to-Buffer** (DB) and Buffer-to-Data (BD) commands were created to facilitate customized apodization functions.
> The **Combine Buffers** commands (B1, B2, B+ and B-) were created for use in processing echo-antiecho 2D data, where pairs of slices must be added and subtracted.

The **Buffers subroutine** allows several spectra or sections of spectra to be displayed on the same ppm axis even when acquisition parameters such as spectral width, spectrometer frequency and number of data points are not equal.

Buffers subroutine

This subroutine allows comparison of spectra in ways not permitted by the Dual Display, Add/Subtract and Stacked Plot routines (see comments below). Spectra can be displayed together even in cases where spectrometer frequency, sweep width and number of data points are not equal.

The subroutine allows spectra, or sections of spectra, to be placed into a series of buffers, each displayed at a specified vertical position on the screen. The buffer spectra are displayed above the current spectrum such that the ppm scale is the same for all. NUTS will adjust the display of each spectrum to compensate for different parameters.

The Buffers subroutine is entered by typing **BU** or from the View menu. The status notation in the gray Status Bar at the bottom of the NUTS window is changed to "BUffer Control". As with all subroutines, the Enter key will exit the routine. While in the Buffers subroutine, the displayed region of the displayed region of the current spectrum can be placed into a buffer using the subcommand **A** (or from the Edit menu). The buffers are numbered in the order in which they are added. An option in the View menu allows the buffer contents to be displayed after exiting the Buffers subroutine.

A chosen buffer can be selected, and its properties can be edited with the **E** command. By default, the most recently added buffer is the selected buffer. A different buffer can be selected by moving the mouse over the baseline of the buffer. When this is done, the buffer spectrum will blink and that buffer becomes selected. While the mouse is over that buffer's baseline, a right click or **E** command will display a dialog box showing that buffer's properties, such as amplitude, vertical position, color, pen width and identifying text.

The selected buffer can be moved vertically by pressing and holding the left mouse button. When this is done a red line is displayed and can be moved with the mouse. When the left mouse button is released, the buffer moves to that vertical position.

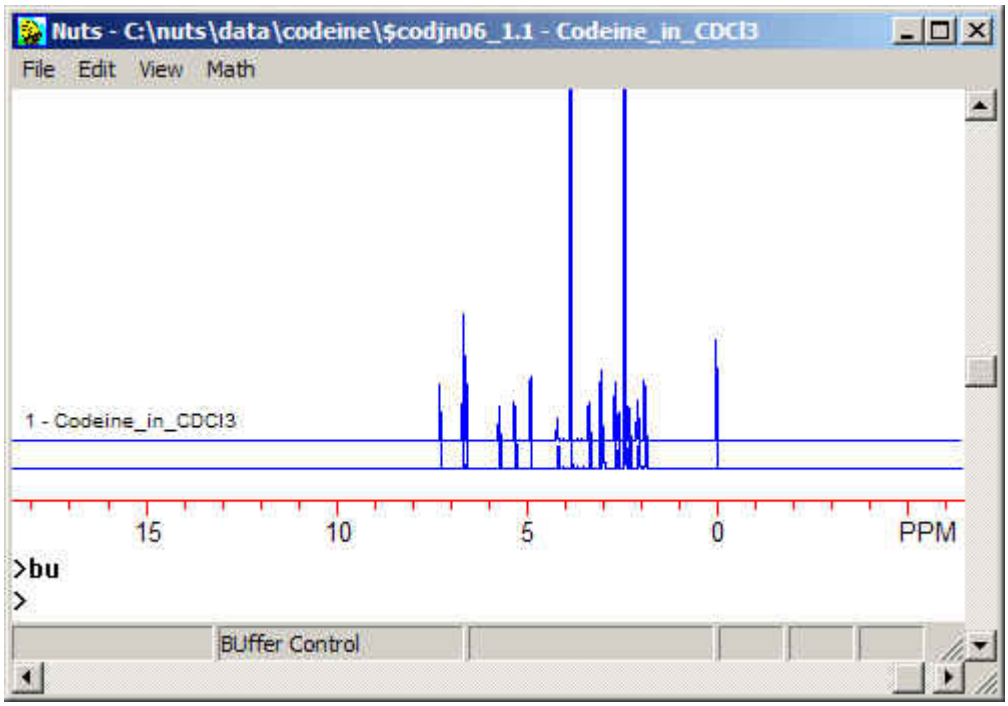
The selected buffer's vertical scale can be changed with the ">" and "<" keys. The Page Up/Down keys and the right-hand scroll bar adjust vertical scaling of all buffer spectra and the current spectrum simultaneously. Horizontal expansions (defined with the Zoom subroutine) affect the current spectrum and all buffers.

The selected buffer can be deleted with the **D** command. All buffers can be cleared with the **C** command. Both commands are also available from the Edit menu.

The Math menu allows addition, subtraction and multiplication of the selected buffer with the current spectrum.

The spectrum can be saved with all of the buffer spectra with command **S** or from the File menu. When later opened, all buffer spectra will be recalled with their associated properties. Saving from the NUTS base level (**SA** command) will not save the buffers.

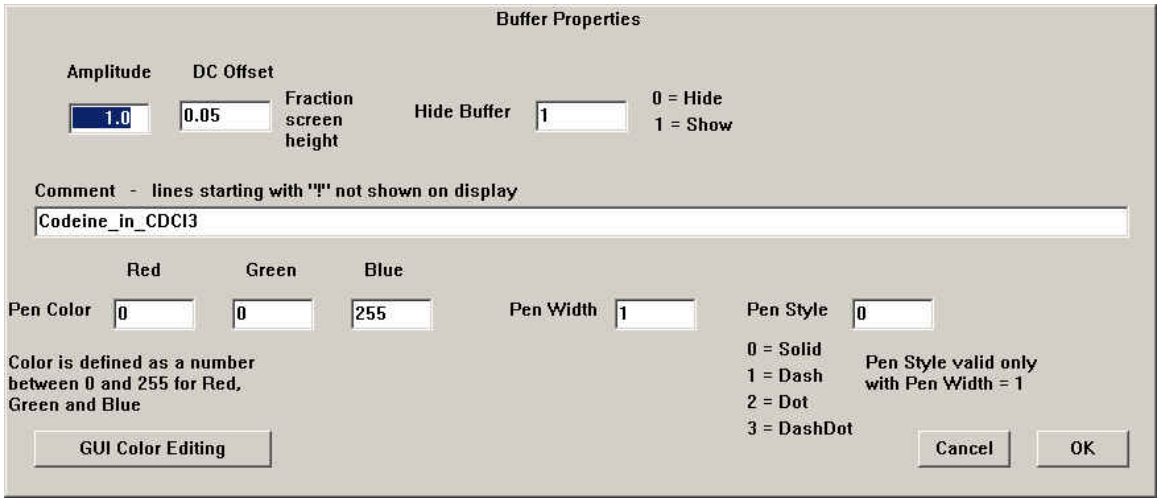
Using the Buffers subroutine



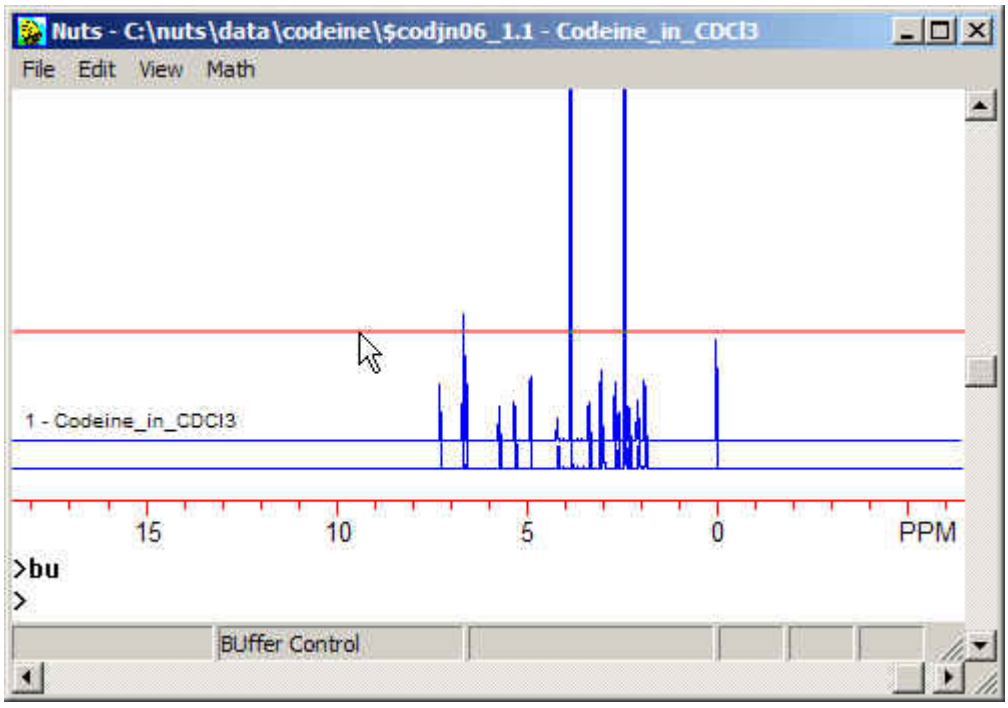
When the buffers subroutine is entered by typing **BU**, note that the menus change and the gray status bar at the bottom of the NUTS window shows "Buffer Control". The currently displayed spectrum or spectral region can be copied to a buffer with the **A** command, or from the Edit menu.

The number 1 (because this is the first buffer added) is shown at the left end of the buffer spectrum as an identifier, along with some text. By default, the comment line for the spectrum being displayed in the buffer is the displayed text, but that can be changed. The text remains displayed after exiting the BU subroutine, but the buffer number does not.

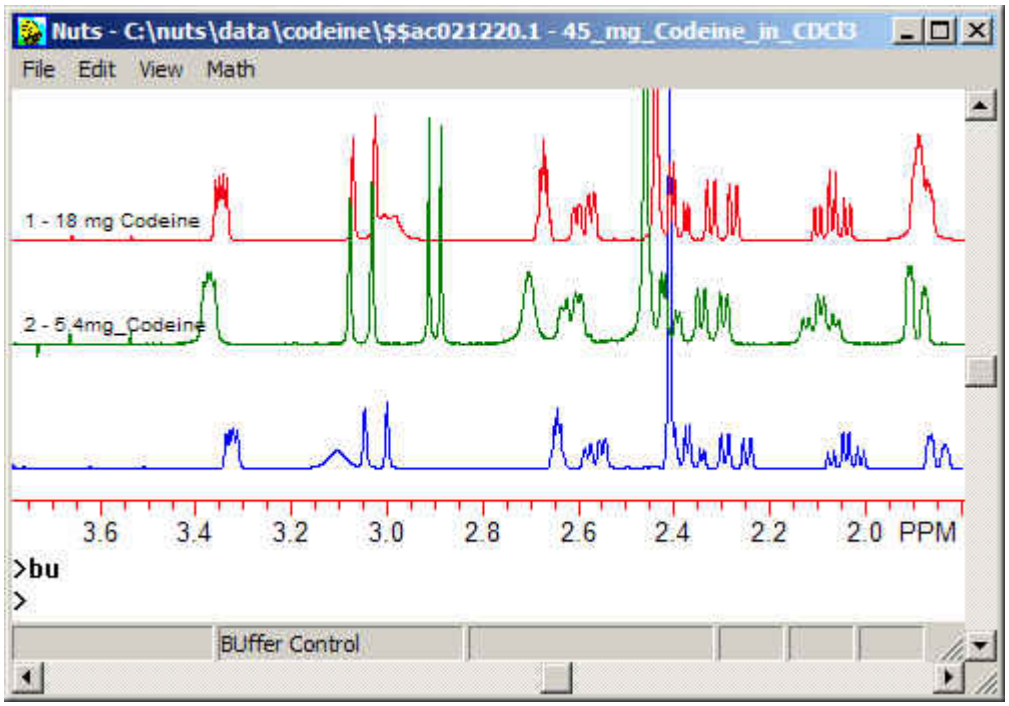
To edit a buffer's properties, move the mouse to the buffer's baseline and click the right mouse button (or type **E** on the keyboard). This dialog box is displayed:



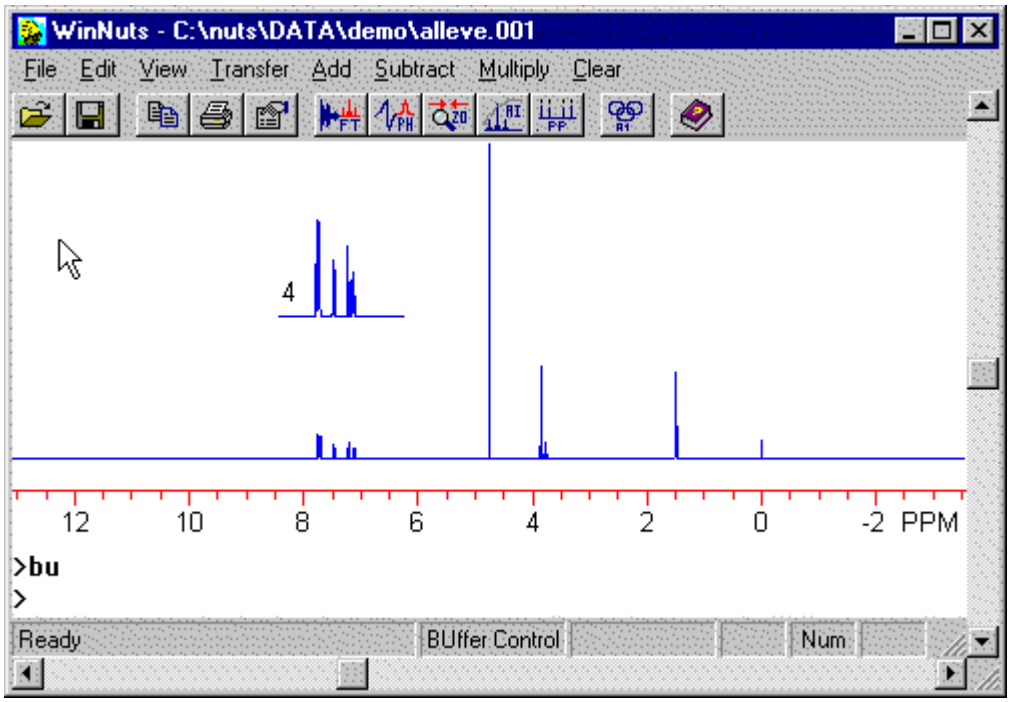
The vertical display (DC) offset and vertical scaling (amplitude) of the buffer can be set, as can the text of the comment line displayed with the buffer spectrum. The color of the spectrum can be set by entering RGB numbers or by clicking the Color Editing button. Pen width and style can also be set.



The vertical position of a buffer can be adjusted with the mouse. Place the cursor on the chosen buffer, at the level of its baseline. Press and hold the left mouse button. The red line indicates the position of the buffer spectrum. Move the red line to the desired position, and release the mouse button. The buffer spectrum will be re-drawn at that position.



As additional spectra are added to the buffers, each is assigned a sequential number.



If an expanded region of the spectrum is displayed when the buffer is loaded, the buffer will contain only that region.

Comparison of Dual Display, Add/Subtract, Stacked Plots and Buffers

Dual Display and Add/Subtract are limited to 2 spectra. There is no limit for the Buffers routine or for Stacked Plots.

Add/Subtract is the only choice that allows one spectrum to be shifted left and right relative to the other.

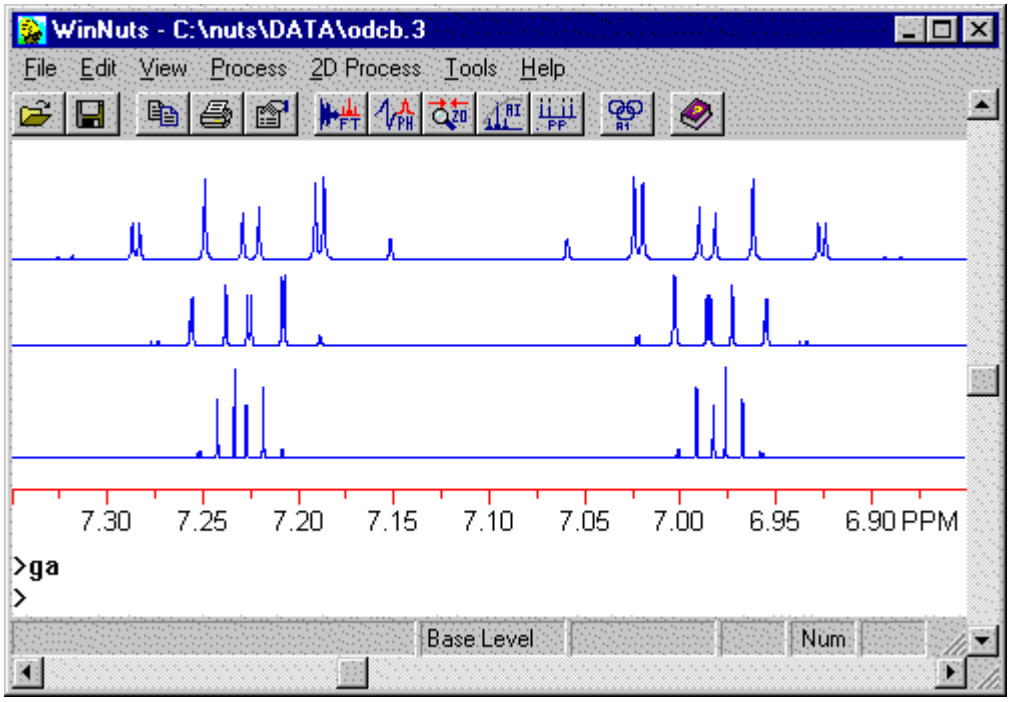
The Buffers routine is the only option in cases where the spectra differ in number of data points.

Add/Subtract and Dual Display will display spectra that differ in spectrometer frequency and/or spectral width, but the frequency scale will be nonsensical. This is because the spectra are aligned point by point, without regard to frequency. The Buffers routine is the only way to display spectra differing in spectrometer frequency or spectral width with correct frequency axis for all spectra.

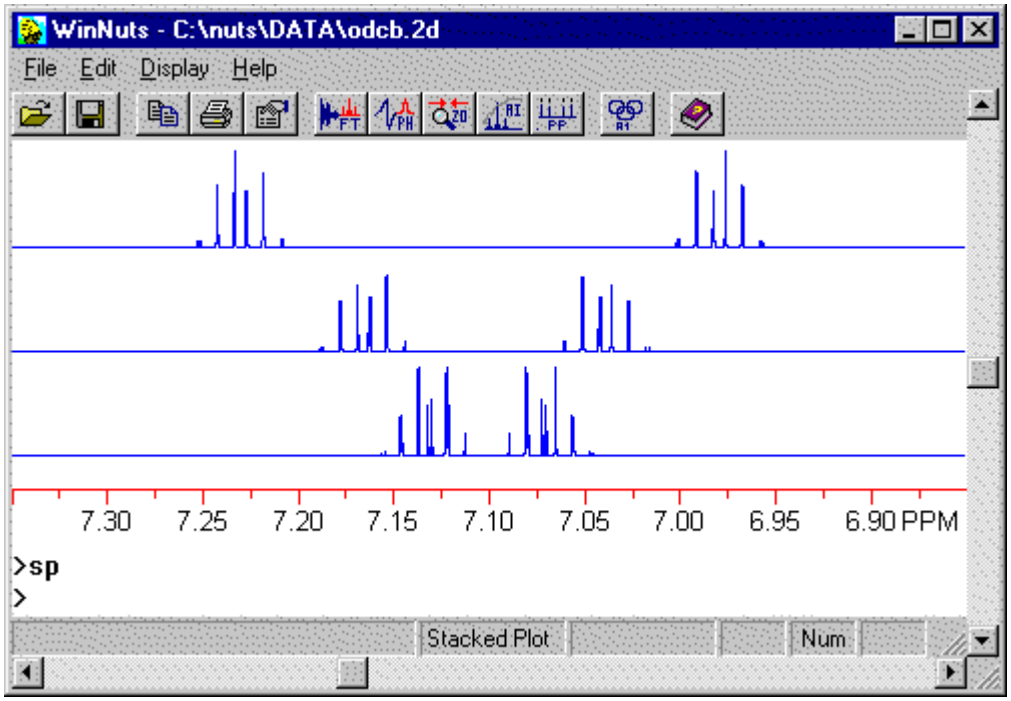
The Stacked Plot routine does not allow independent scaling or vertical repositioning of individual spectra.

Multiple spectra can remain displayed on the screen after exiting the Buffers subroutine (not true of the stacked plot routine). This makes it easier to add annotations (with the Notes subroutine) and otherwise compose a final presentation.

The 2 figures below show simulated spectra of ODCB at 100, 200 and 400 MHz using the buffers and stacked plot routines.



Using Buffers, the spectra are aligned by ppm, even though spectrometer frequencies and sweep widths differ. The ppm scale is correct for all.



Using a stacked plot, the spectra are aligned by points, not frequency, and the axis is correct only for the top spectrum.

For an example of a "creative" use of the Buffers routine, see Tricks with Buffers.

The following 4 commands are used to place data into buffers for later addition or subtraction. Do not confuse this with the Add/Subtract subroutine, which has its own buffer. This is used in some 2D processing, where pairs of slices must be added and subtracted. The following 4 commands ***do not*** work in Arrayed Mode; see below.

B1 -- Load Buffer 1

Load current data set into buffer 1. Used in conjunction with commands **B+** and **B-**, which add or subtract data loaded into buffers 1 and 2. These commands were added for use in processing gradient data. The contents of this buffer are not visible.

B2 -- Load Buffer 2

Load current data set into buffer 2. Used in conjunction with commands **B+** and **B-**, which add or subtract data loaded into buffers 1 and 2. The contents of this buffer are not visible.

B+ -- Add Buffers

Adds the contents of buffers 1 and 2. Used in conjunction with commands **B1** and **B2**, which load current data into buffers 1 and 2. The sum becomes the current data.

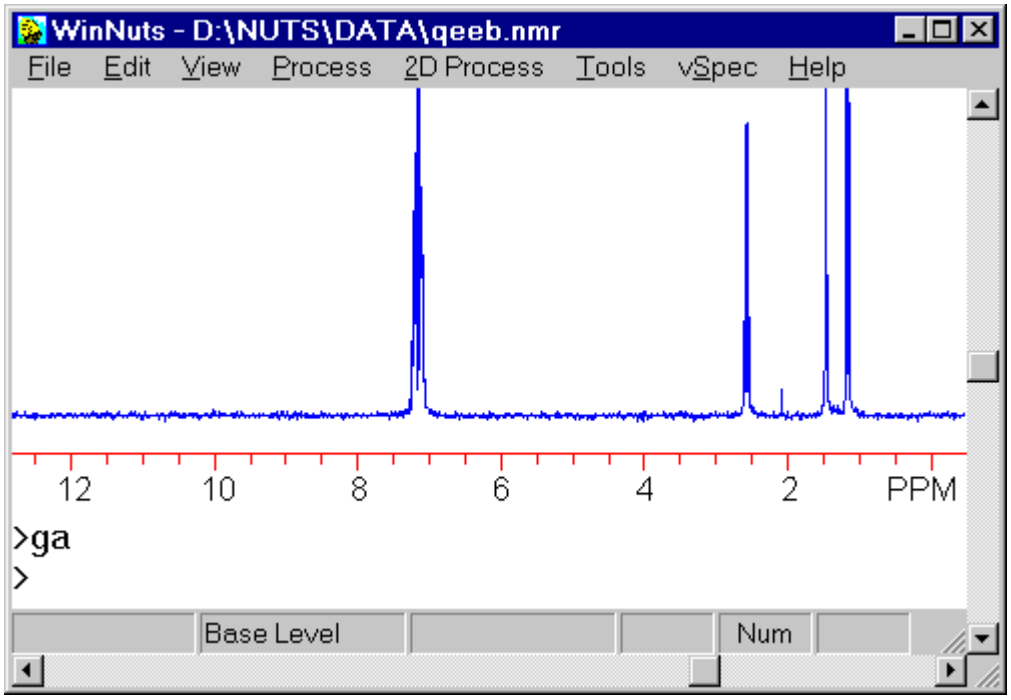
B- -- Subtract Buffers

Subtracts the contents of buffers 1 and 2 (B1 minus B2). Used in conjunction with commands **B1** and **B2**, which load current data into buffers 1 and 2. The difference becomes the current data.

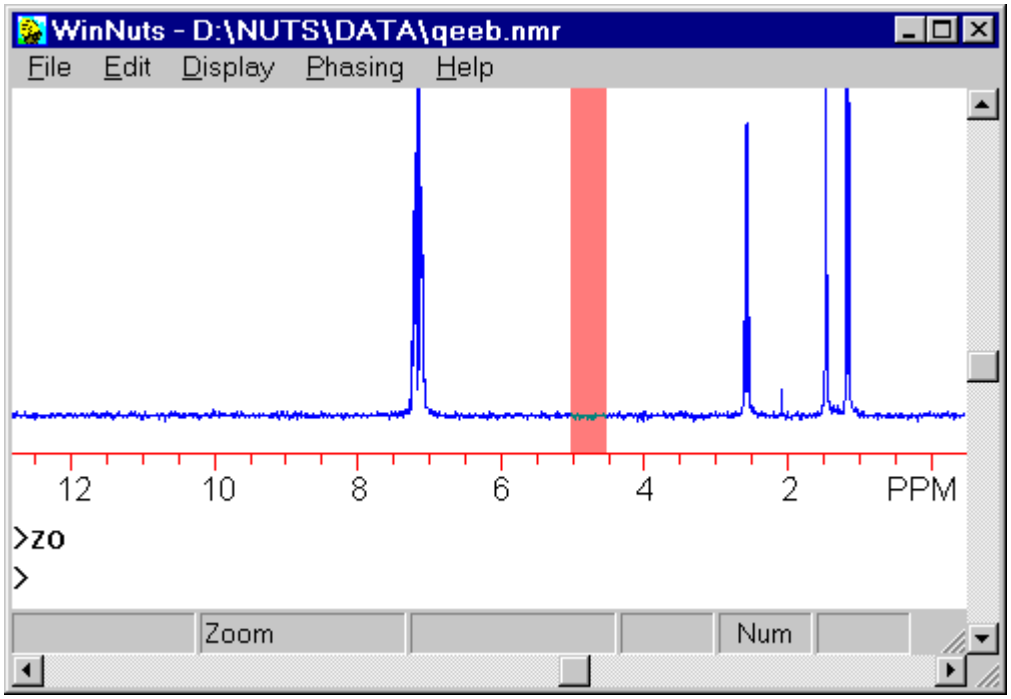
See also: DB and BD commands, described in the section on customized apodization functions.

Tricks with buffers

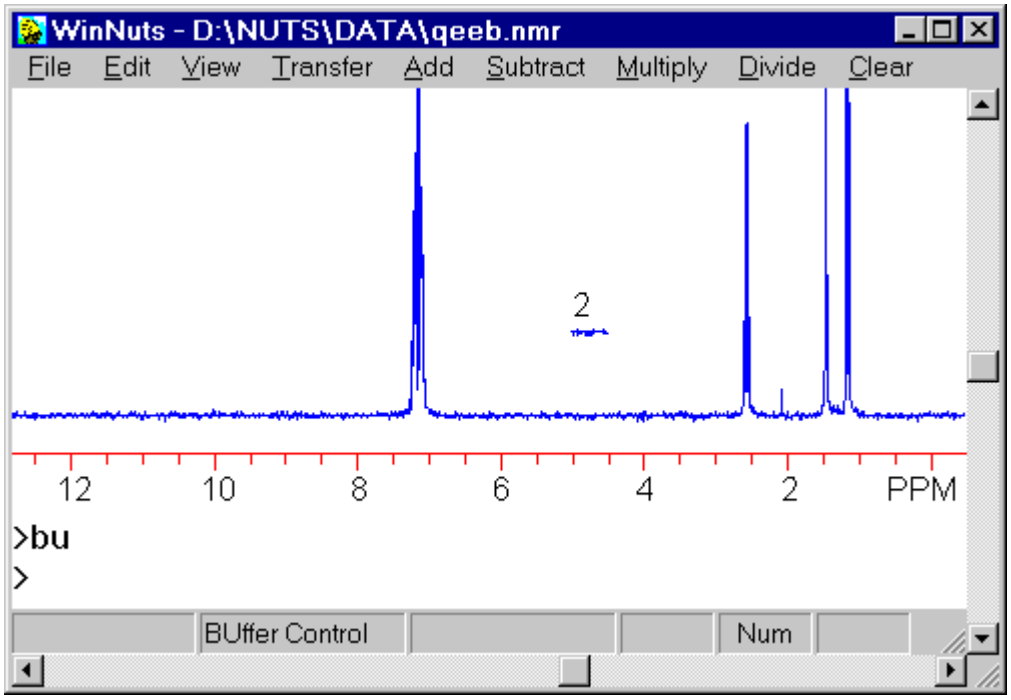
Here is an example of how the Buffers (BU) routine can be used to manipulate spectra.



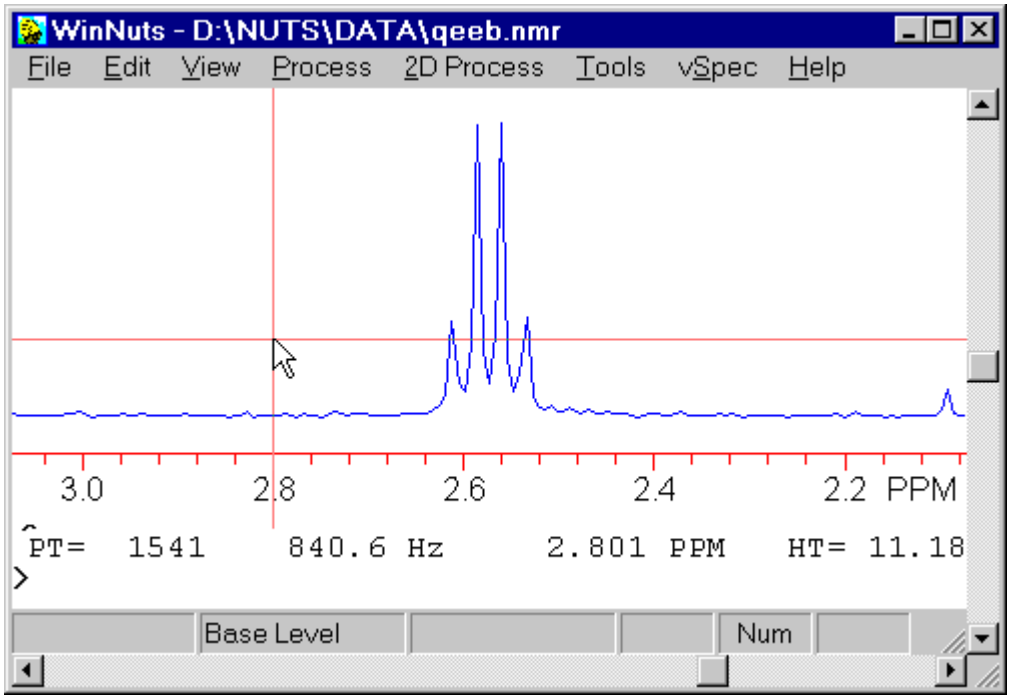
Let's say we want to "remove" the CH_2 peak from this spectrum of ethylbenzene, replacing the peak with noise.



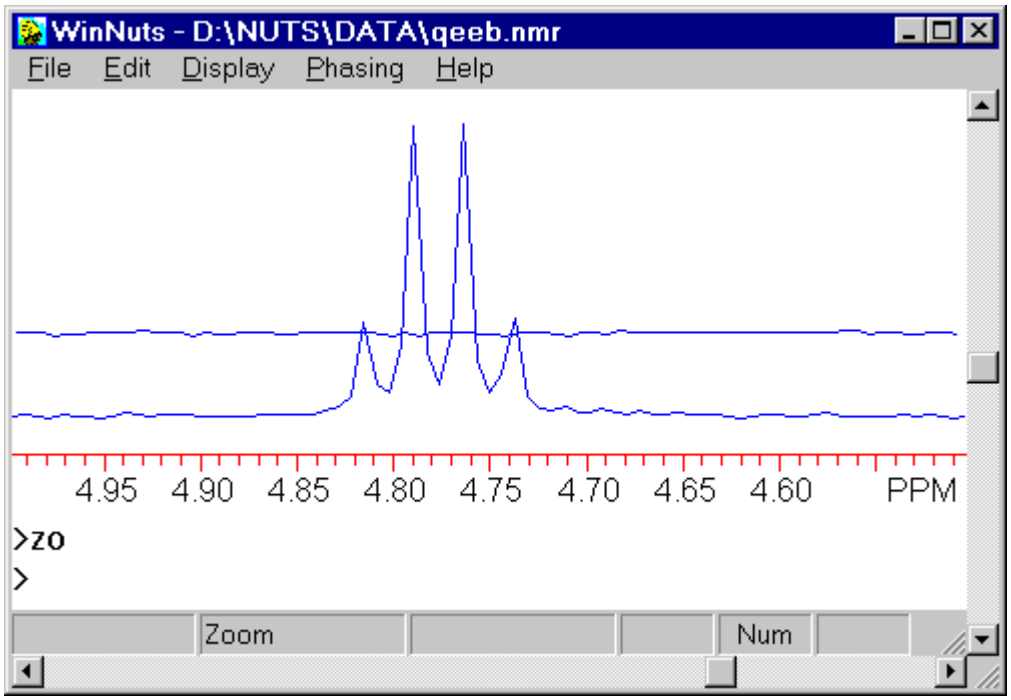
First select a suitable region of noise with which to replace the peak. Use the Zoom subcommand F to select a known spectral region, in this case 5.0 - 4.5 ppm.



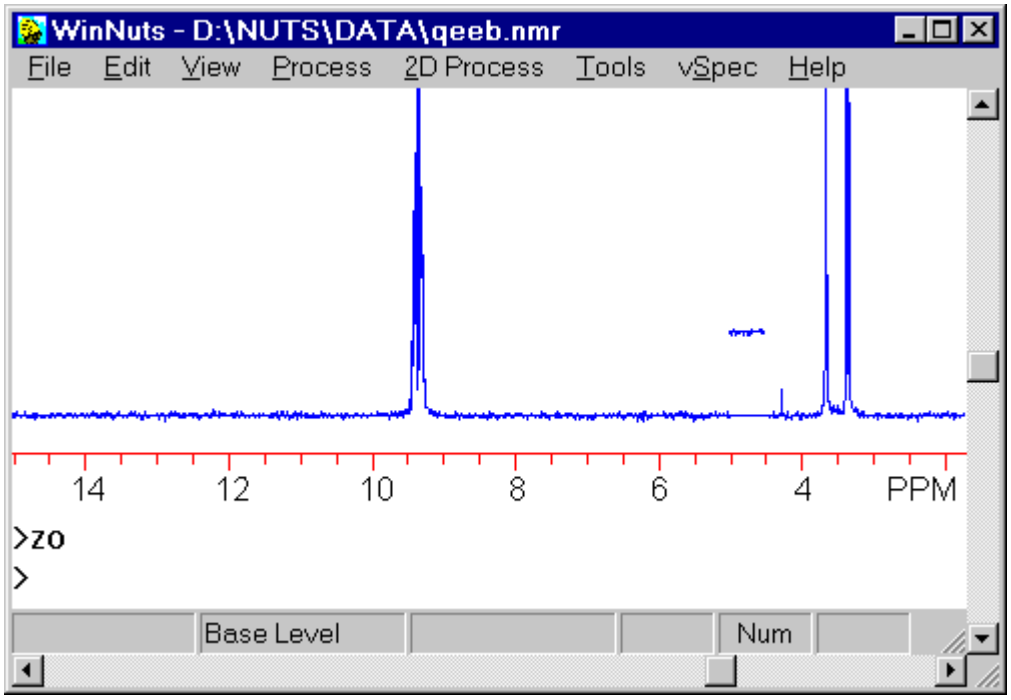
Expand to this region, exit Zoom, enter the Buffers routine (BU) and transfer the selected region to a Buffer (buffer #2 here). Enable viewing of buffers from outside the BU routine, from the View menu, so we can watch what we're doing. Exit Buffers.



Because buffers are always aligned by chemical shift, we need to change temporarily the shift reference of our spectrum, to line up the region around the CH_2 peak with the captured noise region. We need a 0.5ppm region in this example. Using the cursor, set a point to the left of CH_2 to be 5.0ppm.

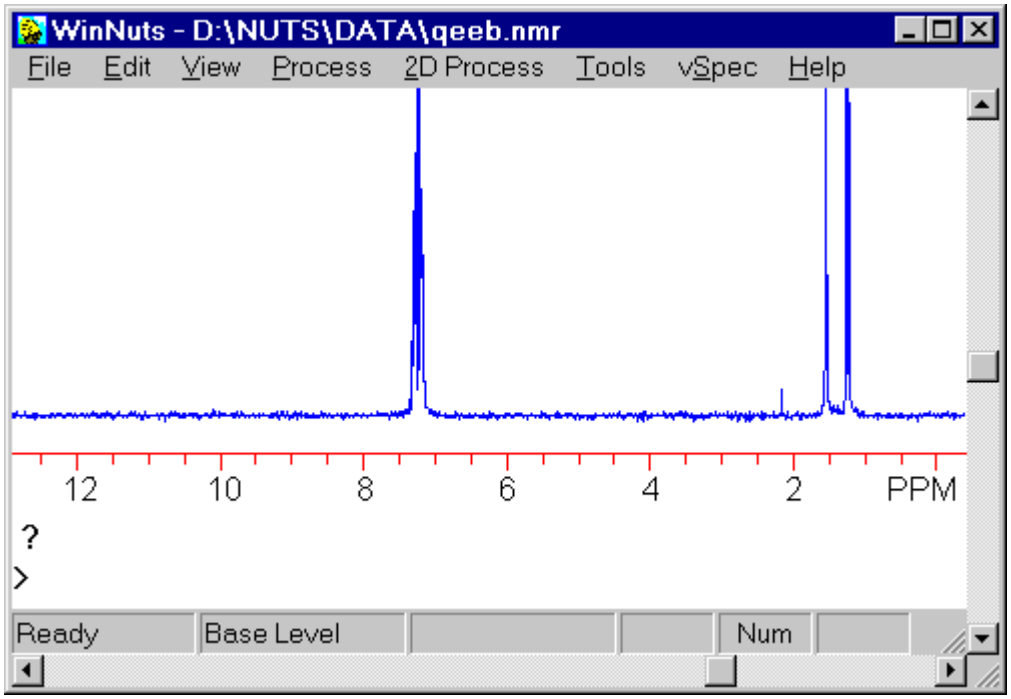


Then, use Zoom subcommand F to display 5.0 - 4.5 ppm. Use the Ctrl-Z command in Zoom to zero out this region.



Viewing the whole spectrum, it should look like this. Now, enter the Buffers routine and add buffer #2 to the current spectrum, and exit.

After resetting the chemical shift reference back where it belongs, we see the final result:



This is intended as an example of how NUTS tools can be combined to accomplish a task. Obviously, this sort of manipulation should be applied with care!

Linear Prediction (LN)

LN - Linear Prediction

This command performs either forward or backward linear prediction, depending on parameters set by the user. Backward prediction can be used to correct corrupted early data points, which cause rolling baselines. Forward prediction is used to predict data out to twice the actual acquisition time, and is used with severely truncated data, such as in the indirect dimension of 2D experiments as an alternative to zero-filling. See illustration of backward and forward linear prediction, below.

When the LN command is issued, a dialog box is displayed prompting for the following values:

Forward or backward prediction
Number of data points on which to base the prediction
Number of points to be predicted for back prediction
Maximum number of frequencies to predict.

For backward prediction (which is the default), the user must set the number of points to back predict, the number of data points upon which to base the prediction and the number of frequencies to predict. The default value for number of points to back predict is 2 and should usually be a small number (4 or less). Larger values may cause failure of the prediction algorithm. The number of data points on which the prediction is based must be less than half the total number of data points, or the algorithm fails. The larger this value, the longer the prediction process will take. The number of frequencies to predict is usually unknown, but usually a small value can be used. The larger the value, the higher the chances that the algorithm will fail. The option has been added of allowing the algorithm to determine the number of frequencies, by setting this parameter to -1.

For forward prediction, the user must select the Forward Prediction button at the bottom of the dialog box. Forward linear prediction always doubles the number of data points. The value for number of points to back predict is ignored.

LN can be used in Links and Macros, in which case the dialog box does not open. The parameters must be chosen before starting automated processing, and the last set of values will be used. It is advisable to experiment with parameters before initiating automated processing.

The values of all linear prediction parameters can be explicitly set in a macro, using SET commands, as shown in the examples below:

SET LNpts = 4
SET LNmdim = 64
SET LNnsig = 16
SET Lndirection = FORWARD
SET Lndirection = BACKWARD

Linear prediction takes considerable time (can take 20-30 min for a 2D data set, even on a fast computer).

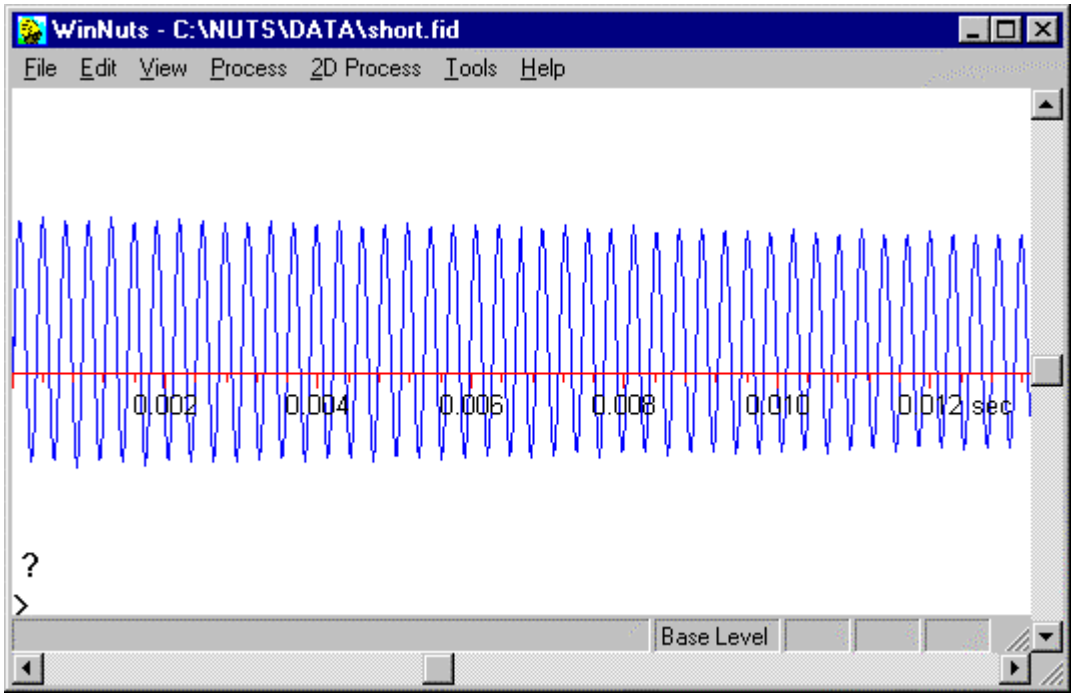
See also: Polynomial baseline correction

Illustration of forward prediction

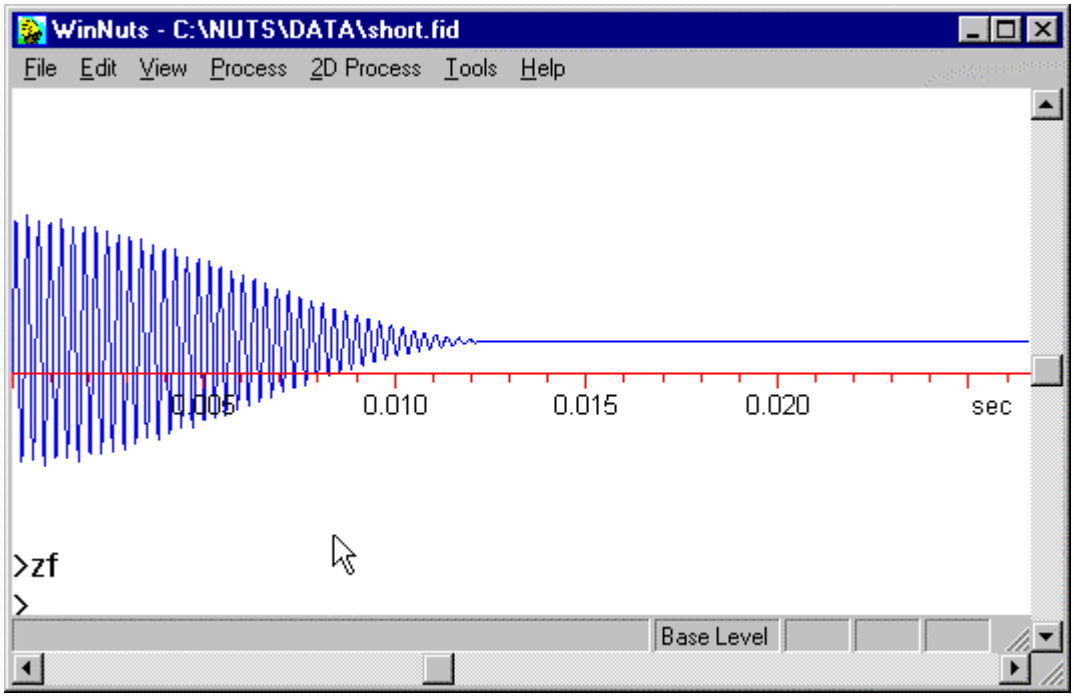
Forward Linear Prediction (LN)

This is used to improve resolution in cases where the FID is badly truncated. This occurs most often in 2D data, where time constraints limit the number of slices which are acquired. Data which is truncated must be severely apodized to bring the end of the FID to zero to avoid truncation artifacts. This amounts to throwing away data which was acquired at great expense of time. As an alternative, we can use Linear Prediction to generate additional data points and then apply a window function which acts mostly upon these predicted points to bring the end of the FID to zero, preserving the real data points. The result is prevention of the broadening and loss of signal that would otherwise result, giving a net improvement in resolution and signal-to-noise.

Note that linear prediction is not included in NUTS Lite.

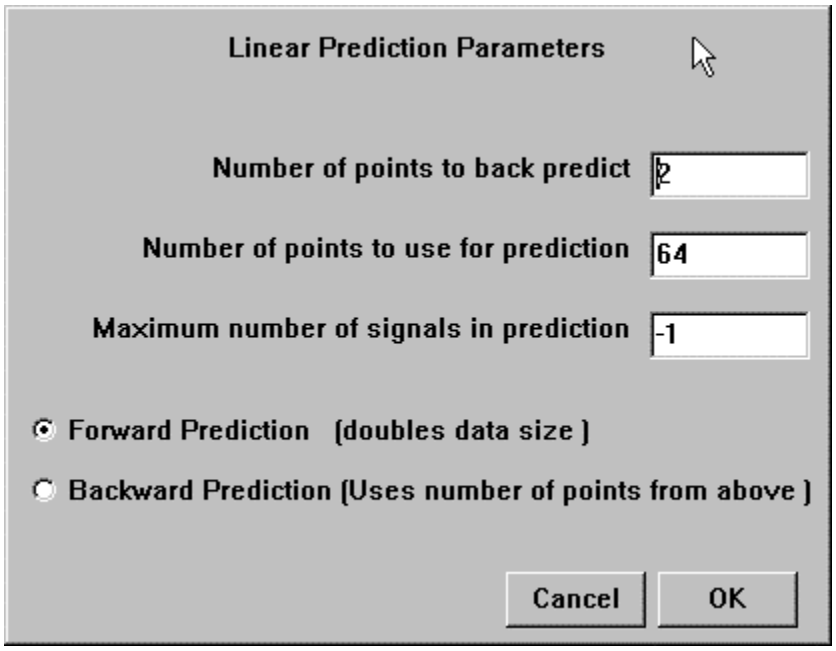


This FID has not decayed to zero, and must be apodized to prevent truncation artifacts, especially if zero-filling is desired.



The FID above has been apodized with cosine squared and zero-filled one time.

Note that we have thrown away good data.

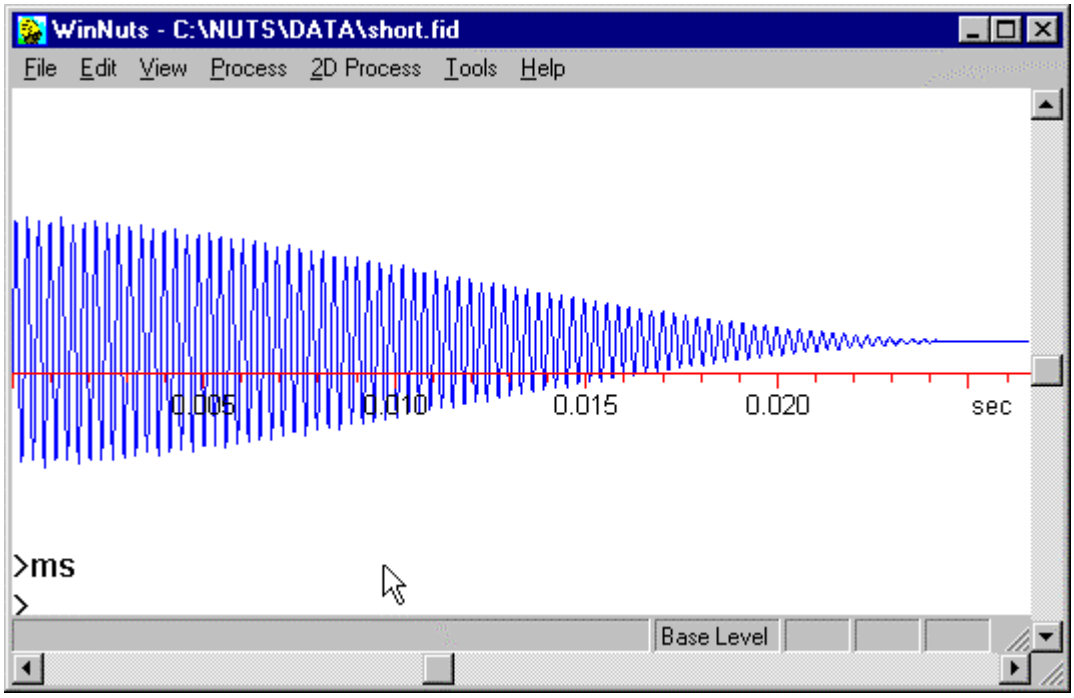


The original FID has been opened and LN typed.

The first item, number of points for back prediction, is irrelevant for the present case of forward prediction.

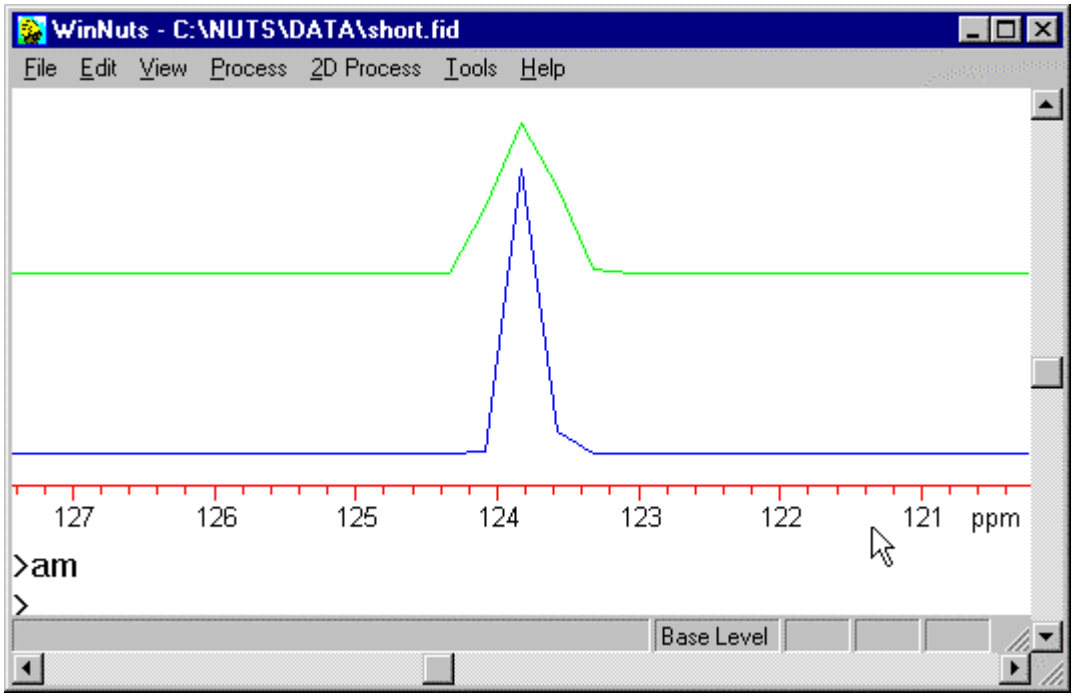
The prediction is based here on the last 64 points of the FID. This parameter cannot exceed one half of the number of data points in the FID, 512 in this case. Using larger numbers will make the calculation slower.

The LN operation will double the data size to 1024.



This is the same FID as above after forward linear prediction to double the number of points and then application of a cosine squared window function.

Note that there is much less loss of the original data points (the first half of the FID).



This dual display plot shows the improvement in the resulting spectrum.

The top spectrum results from apodization and zero-filling.

The bottom spectrum results from linear prediction followed by the same apodization

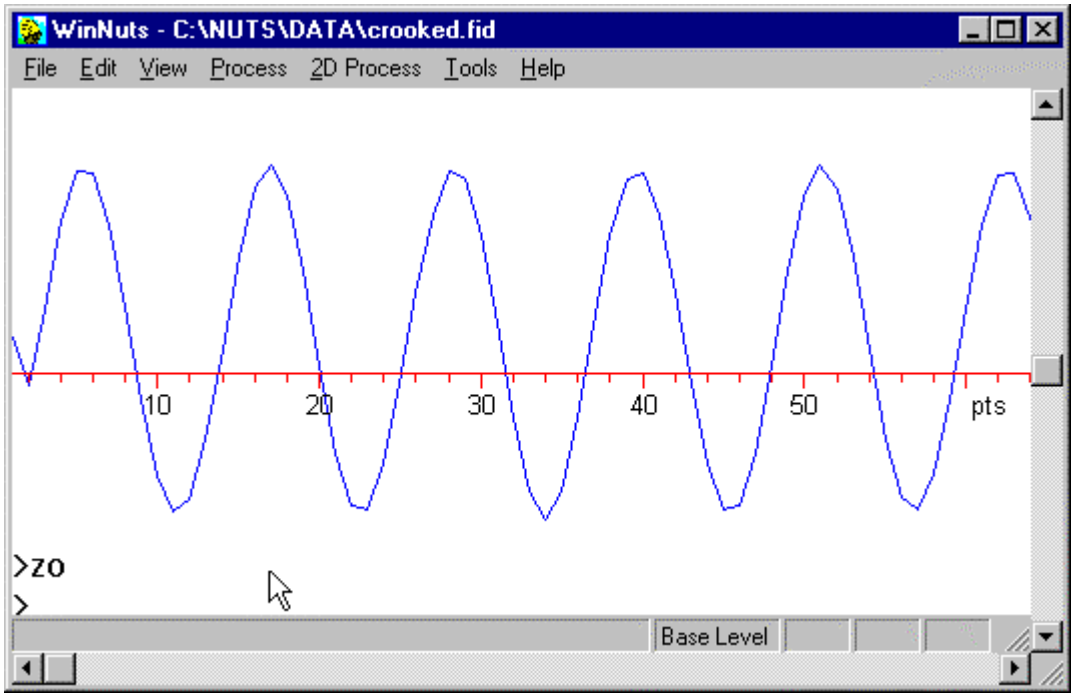
Illustration of backward prediction

Backward Linear Prediction (LN)

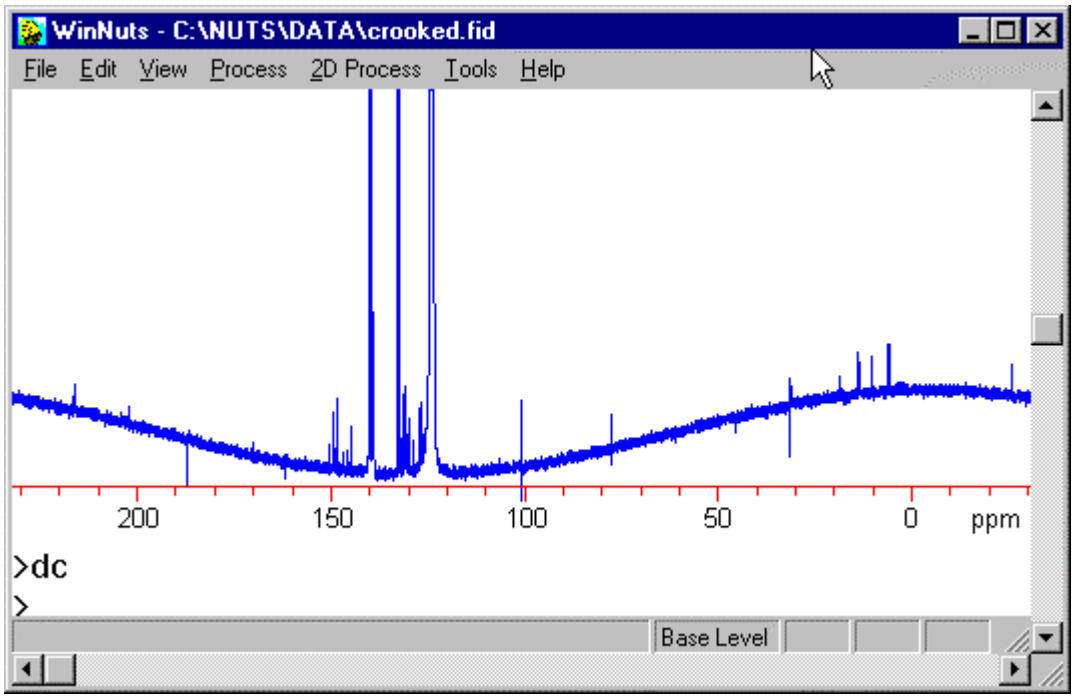
The first few data points in an FID can become corrupted due to such things as probe ring-down. This results in an undulating baseline. While this can be corrected using NUTS tools such as polynomial correction of the spectrum (FB), it can also be corrected using backward linear prediction.

See also: Forward Linear Prediction

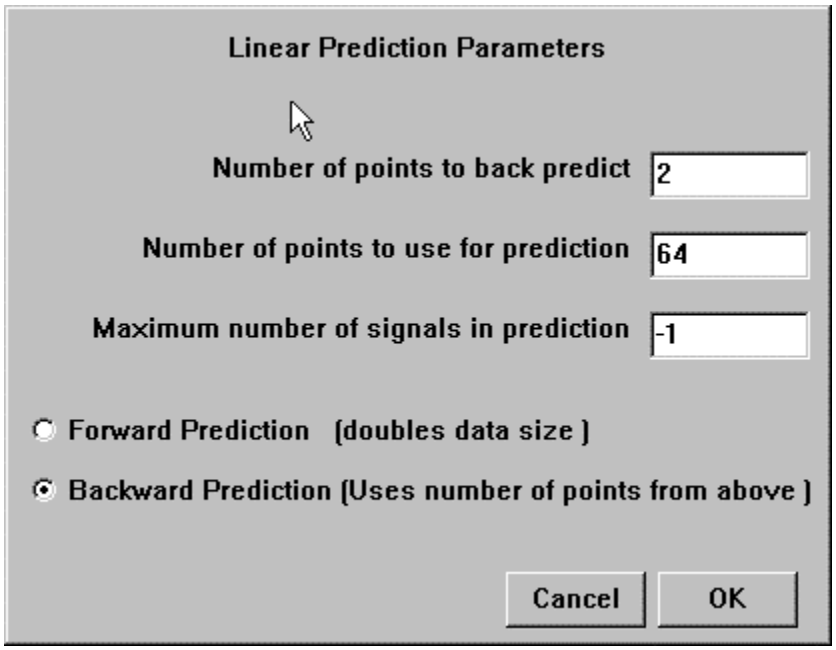
Note that linear prediction is not included in NUTS Lite.



Looking at the sinusoidal fluctuation of this FID, it is easy to see that the first point is not at the "correct" position.



After FT of the above FID, the baseline clearly needs to be corrected.

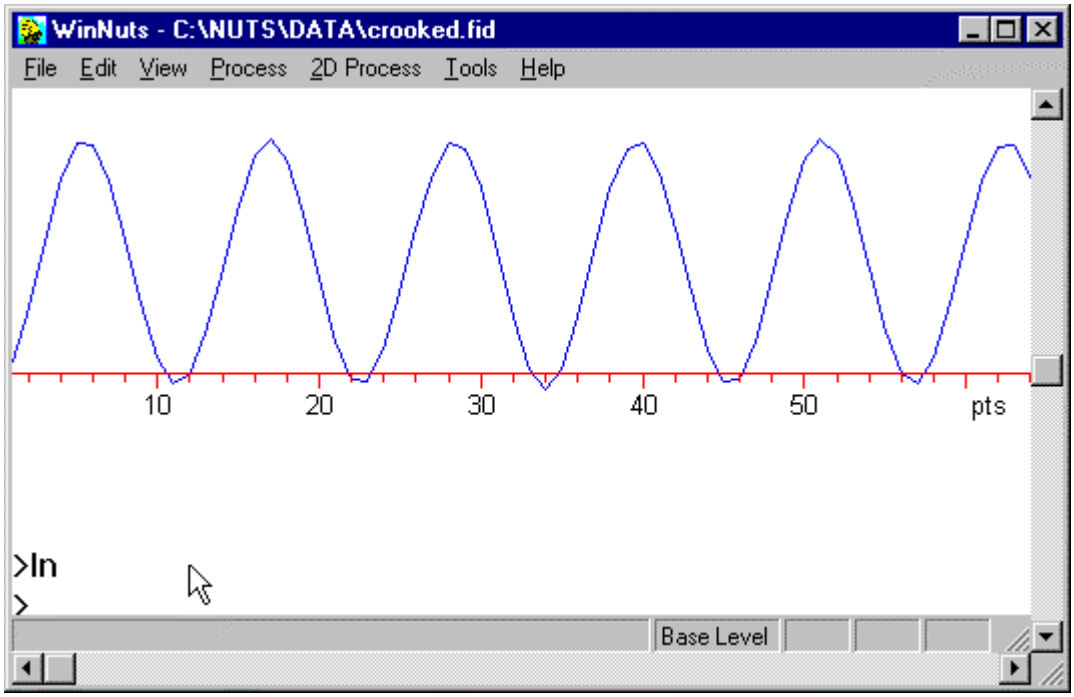


Taking the same FID, type **LN** (not available on menus) to bring up this screen for setting the necessary parameters.

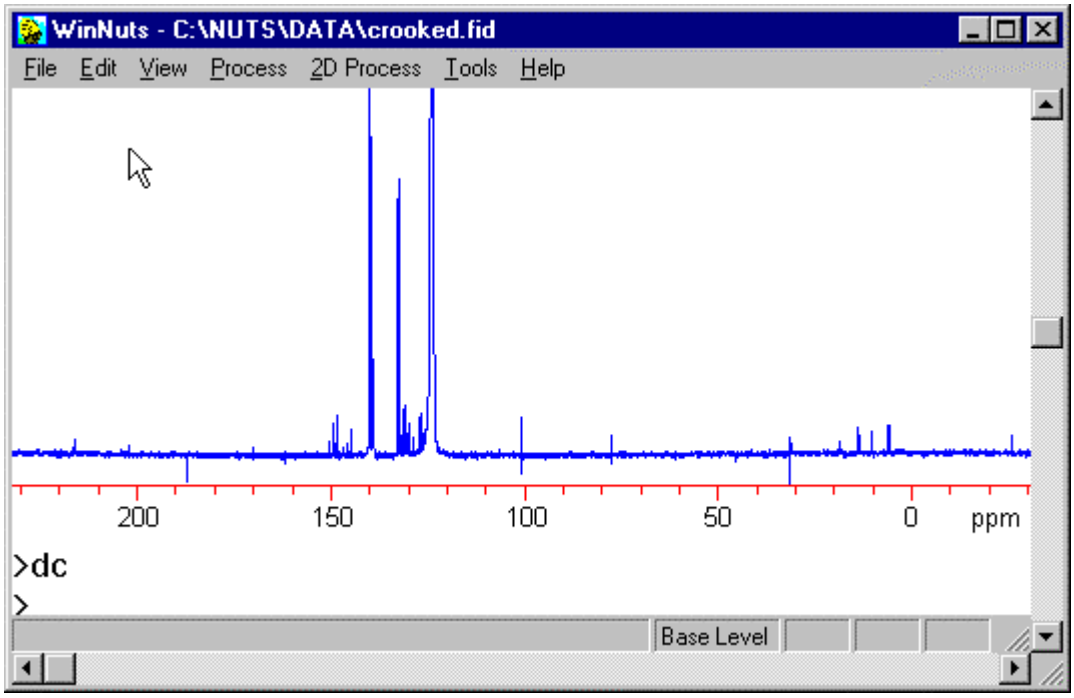
The values shown here are the default values and are usually appropriate for baseline correction.

We are going to correct the first 2 data points, based on the periodicity of the next 64 points. Most linear prediction algorithms need to know how many different frequencies are present in the spectrum, which is usually not known. By setting this value to -1, NUTS will determine this value.

We have chosen backward preciction.



After executing the backward linear prediction, the first data point is clearly in the correct place.



After FT, the baseline is much flatter.

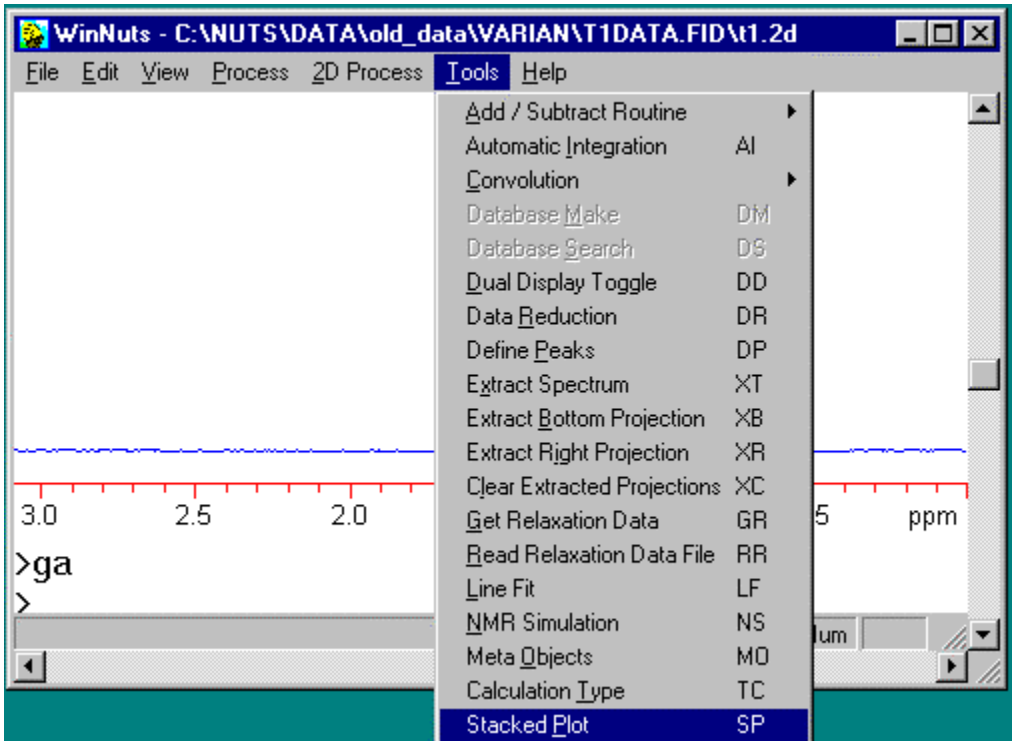
Stacked Plots (SP)

SP - Stacked plots

A stacked plot of 2D data or "arrayed" 1D data can be displayed. The spectra must first exist as a 2D data file in NUTS. (This is included in 1D versions of NUTS.) See below for instructions on creating a 2D file from separate 1D data.

To display just 2 spectra together, see also Dual Display. To display multiple spectra with differing spectrometer frequency, spectral width and/or number of data points, see Buffers subroutine.

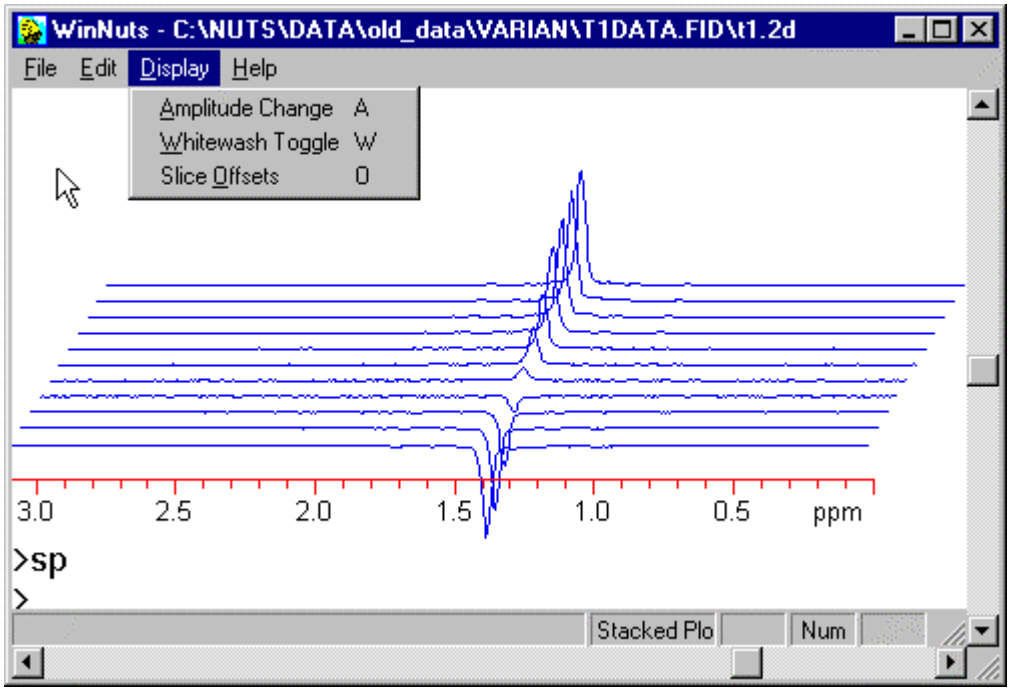
Note that stacked plots are not included in NUTS Lite.



Open the 2D data file. The first slice only is displayed.

Execute a Set Scale operation (keyboard command **SS** or from the 2D Process menu) which looks at all slices to find the tallest peak so that the display can be scaled appropriately.

Now, choose Stacked Plot from the Tools menu.

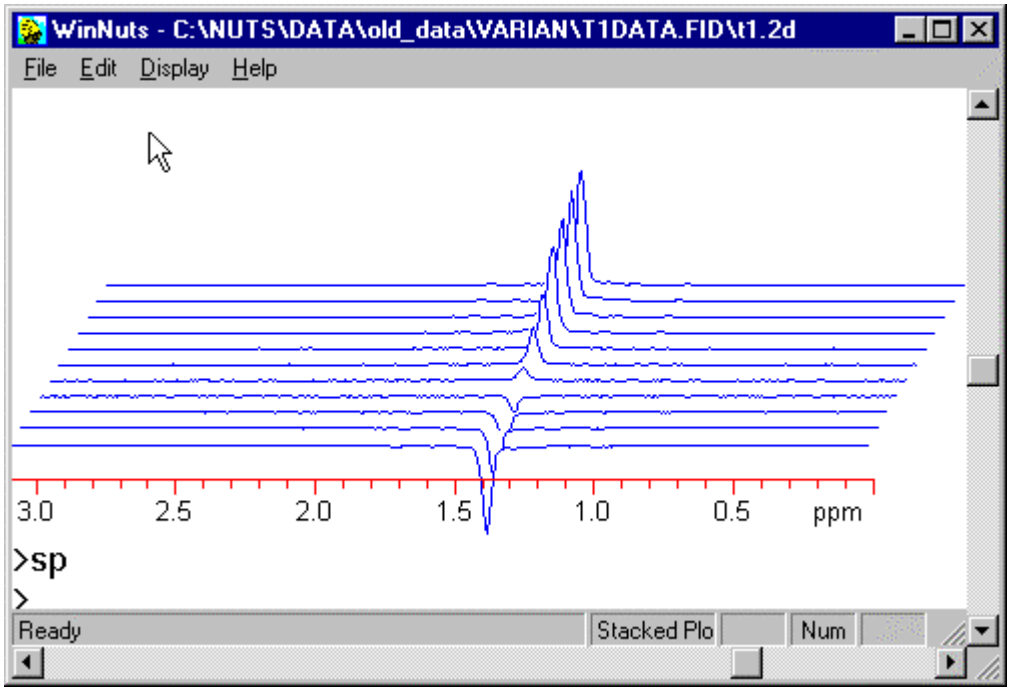


All slices are displayed and scaled to be on screen.

The default offsets are set in the Nuts.ini file, but can be edited by choosing Slice Offsets from the Display menu. The values used here are

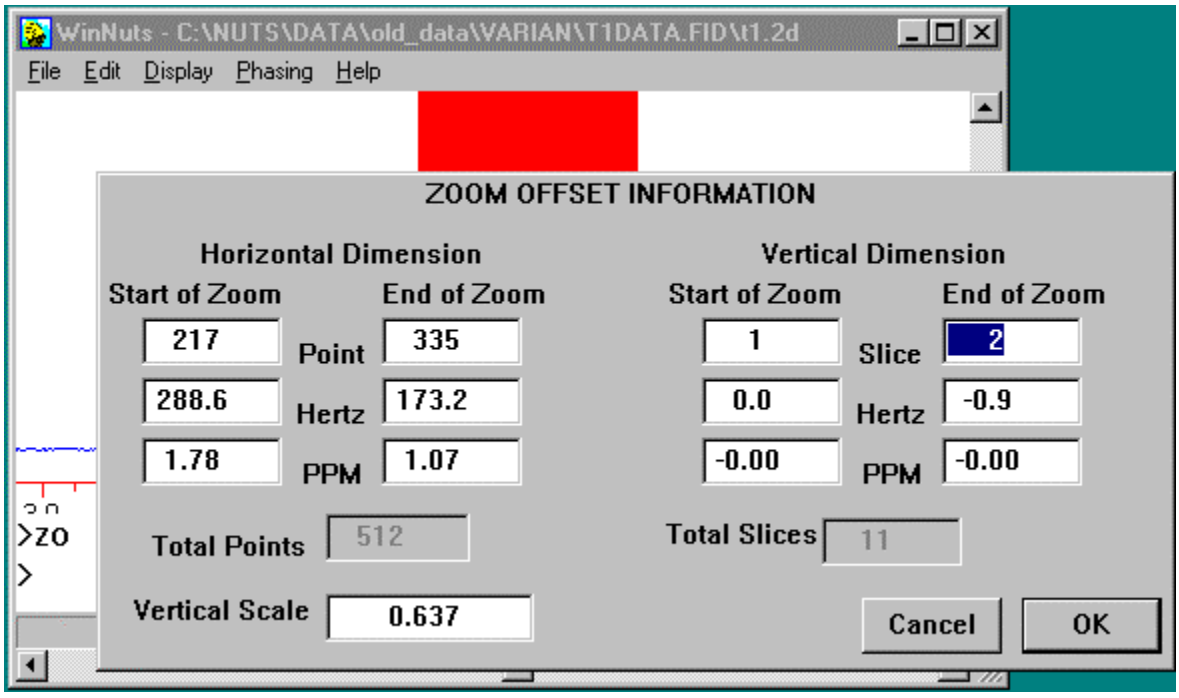
X-Offset = 10%
Y-Offset = 1 (on a scale of 1 to 10)

The vertical scale of all slices can be changed in the same way as 1D data.



The option of a "whitewashed" plot is available from the Display menu. In this mode, lines which are behind peaks are not displayed.

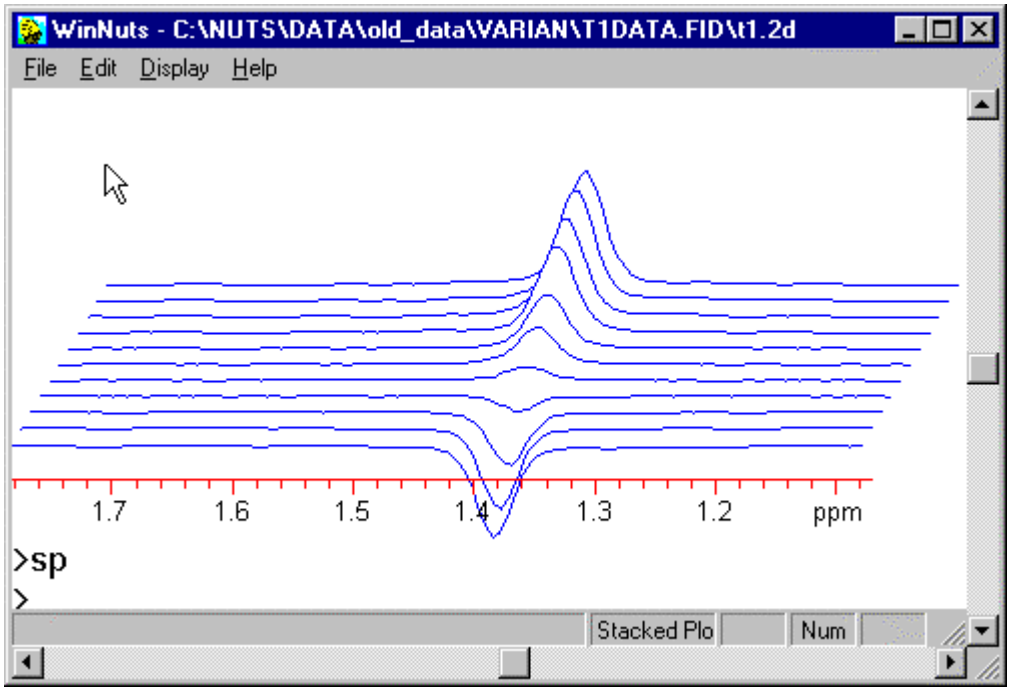
Compare this to the previous figure.



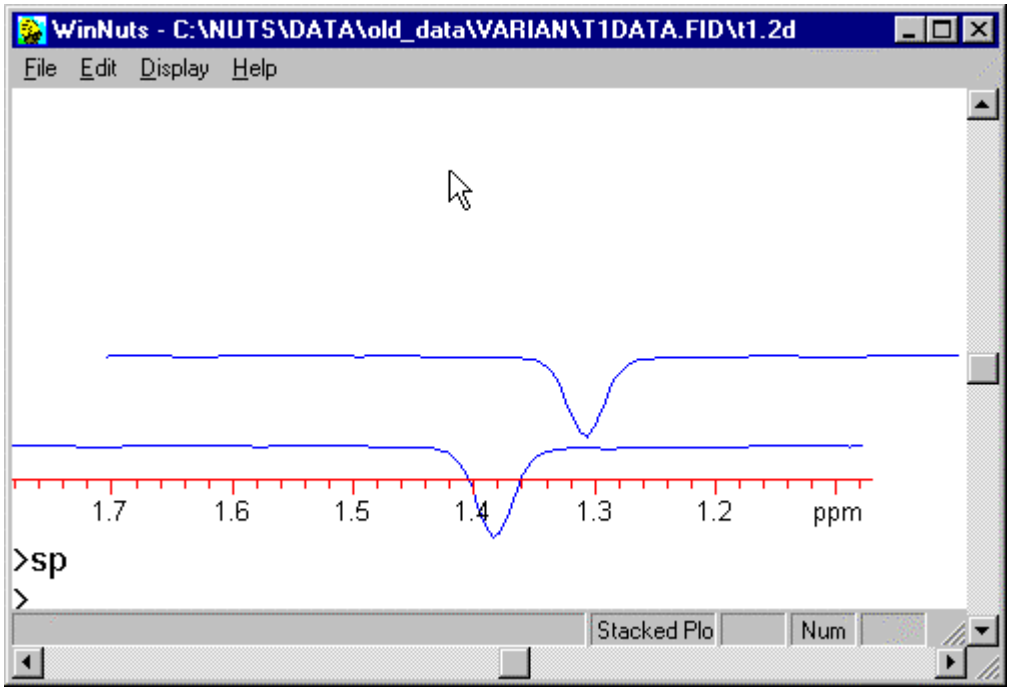
It is possible to construct a stacked plot of only a part of the total data set. The plot limits can be set in either of 2 ways:

For an actual 2D data set, display an intensity or contour plot and use 2D Zoom to select the region. Exit the contour plot and display the stacked plot.

Alternatively, the plot limits can be set explicitly using the Zoom subcommand **F**. (You must exit from the Stacked Plot display, with <Enter>, to enter Zoom.) The critical point to understand is that you ***must*** explicitly set the range of slices to be displayed on the right hand side of the screen (Vertical Dimension). Click on OK, then execute a Ctrl-E command to implement the chosen range.



The desired expanded region is displayed.



Failure to set the desired range of slices will result in display of only 2 slices, rather than the entire data set.

To build a 2D file from separate 1D spectra, the individual spectra must have sequential file extensions. Start by opening the first of the 1D files. A 2D data set can be created by running the following Link:

GA SC IN

NUTS will first ask for the name of the first file to open, and then for a name for the 2D file to be created. When the link has finished, open the 2D file.

Math functions (MA)

MA - Math functions in NUTS

(not included in NUTS Lite)

This is a subroutine for doing calculations. While the routine can be used as a calculator from the user interface (entered with command MA), its utility is in use within macros. The following definitions may make more sense if you examine the Math screen by typing MA.

There are 10 REGISTER, 10 MEMORY and 10 INTEGRAL locations. The REGISTERs act like a stack, similar to an RPN calculator. When a math operation is done, it is performed between REGISTER_0 and REGISTER_1 and the answer is put in REGISTER_0 and REGISTERs 2 thru 9 roll down one level. There is one comment field which can be placed into a Note, along with the contents of Register 0, so results are displayed on the screen. An example of a macro is shown below.

Related commands: Multiply and Divide data by a specified constant.

Macro commands

Use of the ENTER function puts the value in REGISTER_0 and rolls the stack up. For example,

```
DO MATH ENTER 1
```

Any location can be set to a specific value, such as

```
SET MATH REGISTER x value          Set Reg x to "value"
```

Clear all Registers, Memory and Integral values using

```
DO MATH CLEAR
```

The line below specifies the number of decimal places to show in calculations

```
SET MATH DECPLACES x
```

The math operations possible are:

add
subtract
multiply
divide
reciprocal
natural log
log base 10
exponential
power

The following operations are performed on Registers 0 and 1, and the result is then placed into Register 0.

```
DO MATH +
DO MATH -              Register 1 - Register 0
DO MATH *
DO MATH /              Register 1 / Register 0
DO MATH POWER      Reg0**Reg1
```

These operations are performed on the contents of Register 0:

DO MATH RECIPROCAL 1/Reg0
DO MATH EXP
DO MATH LN Natural Log
DO MATH LOG Log Base 10

The value of Register and Memory locations can be set from another location, using
DO MATH REGISTER x REGISTER y copy contents of Reg x to Reg y
DO MATH REGISTER x MEMORY y copy contents of Reg x to Mem y
DO MATH MEMORY x REGISTER y copy contents of Mem x to Reg y
DO MATH INTEGRAL x REGISTER y copy contents of Integral x to Reg y
DO MATH INTEGRAL x MEMORY y copy contents of Integral x to Mem y

Acquisition parameters can be set from Register 0, using
DO MATH SF1 Set SF1 to the value in Register 0
DO MATH SF2 Set SF2 to the value in Register 0
DO MATH SW1 Set SW1 to the value in Register 0
DO MATH SW2 Set SW2 to the value in Register 0
DO MATH OF1 Set OF1 to the value in Register 0
DO MATH OF2 Set OF2 to the value in Register 0

Acquisition parameters can be placed into Registers or Memory locations, using
DO MATH REGISTER x SF1 Put SF1 into Register x
DO MATH REGISTER x SF2 Put SF2 into Register x
DO MATH MEMORY x SF1 Put SF1 into Memory x
DO MATH MEMORY x SF2 Put SF2 into Memory x
DO MATH REGISTER x SW1 Put 1st Dimension sweep width into Register x
DO MATH REGISTER x SW2 Put 2nd Dimension sweep width into Register x
DO MATH MEMORY x SW1 Put 1st Dimension sweep width into Memory x
DO MATH MEMORY x SW2 Put 2nd Dimension sweep width into Memory x
DO MATH REGISTER x OF1 Put 1st Dim O1 into Reg x
DO MATH REGISTER x OF2 Put 2nd Dim O2 into Reg x
DO MATH MEMORY x OF1 Put 1st Dim O1 into Memory x
DO MATH MEMORY x OF2 Put 2nd Dim O2 into Memory x

The Zoom regions (Z0 - Z9) can be set from values in the Registers. The line below sets Zoom Region i. The value in register x becomes the left end of Zoom Region i (in ppm), and the value in register y becomes the right end (in ppm).
DO MATH ZOOM_REGION i x y

The following creates a Note (text annotation) consisting of the COMMENT and the contents of Register 0, and displays it at specified screen position (x_pos, y_pos) (in percent of display, a number between 0 and 100). The font used is the default Notes font, set in the nuts.ini file.

SET MATH COMMENT string	Make "string" a comment for output
DO MATH NOTE x_pos y_pos	

To automatically measure pre-defined integrals, the integral limits must first be defined using the Set Zoom Region command. (Do not confuse this with the Set Integral macro commands.) After all regions are defined, a single command, DO MATH GET_INT, measures all values and places them into the corresponding INTEGRAL locations. For example, The following sequence defines 2 integrals (7.4 - 7 ppm and 6.2 - 6.0 ppm), named as regions 1 and 2, and then the Get_Int command puts the integrals of those regions into Integral locations 1 and 2. It is important to understand that the DO MATH GET_INT command measures the integrals of ***all*** defined zoom regions.

```
set zoom_region 1 7.4 7
set zoom_region 2 6.2 6.0
do math get_int
```

It is also possible to load areas resulting from a line fit in the LF routine. The command

```
Do Math get_lf_areas
```

will insert the area values of peaks 1-10 from the LF calculation into the corresponding INTEGRAL locations.

These commands are used to request user to enter values at runtime. The entered value is placed in the specified location.

ASK MATH REGISTER x	Ask for a value for Reg x
ASK MATH MEMORY x	Ask for a value for Mem x

Example: The following is a macro which measures the mole% of 2 impurities, relative to the major component.

```
NutsMacro math test
; ask name of file to open
ask filea
ga
; clear any old Notes
no
c
^m

; integrals of 1 proton each from major component
set zoom_region 1 7.17 7.04
set zoom_region 2 7.27 7.18

; integral of impurity #1
```

```
set zoom_region 3 6.02 5.96

; integral of impurity #2
set zoom_region 4 2.72 2.68

; now get the integrals and place into Integral locations in Math routine
do math get_int

; average integrals of 2 protons from major component.  Move integrals from major
component
; into registers 0 and 1, add them, divide by 2.
do math integral 1 register 0
do math enter 1
do math integral 2 register 0
do math +
do math enter 2
do math /

; Put result into memory location for later retrieval
do math register 0 memory 7

; impurity peak #1 is from 2 protons, so move it to register 0, enter value of 2, divide,
then move to memory location

do math enter 1
do math integral 3 register 0
do math enter 2
do math /
do math register 0 memory 1

; calc mole % imp 1.  Divide previous result by integral of main component, mult by 100
do math enter 1
do math memory 7 register 0
do math /
do math enter 100
do math *

; Set comment, output result at specified screen location
set math comment Mole % Impurity #1
do math note 35 10

; now do same for impurity #2
do math integral 4 register 1
do math memory 7 register 0
do math /
do math enter 100
```

```
do math *
set math comment Mole % Impurity #2
do math note 35 20

end
```

Multiply

Multiply the current 1D data set or the currently displayed slice by a constant. This takes one argument, the factor by which the data is to be multiplied. In Arrayed Mode, only the current slice is multiplied, not the entire data set.

Divide

Divide the current 1D data set or the currently displayed slice by a constant. This takes one argument, the factor by which the data is to be divided. This can be helpful in cleaning up 2D data which has t_1 noise "stripes", which consist of a few data slices with high noise level. Display each slice and divide it to reduce the noise amplitude. This command is not array-aware, meaning that even in arrayed mode, it acts only on the displayed slice.

Copying spectra

Copying spectra to other applications

NUTS provides multiple options for copying spectra into other applications, either via the clipboard or by writing to a file. On the Mac, the options are to copy a bitmap (Ctrl-C) or PICT file (Alt-C) to the clipboard, also available from the Edit menu. In the Windows versions, the choices are bitmap or metaile, and there are variations in the type of metafile. The enhanced metafile is supported only on Windows.

The commands described below are

Control-C -- Copy to clipboard as bitmap

Alt-Shift-C -- Copy to clipboard as a standard metafile

Alt-Shift-E -- Copy to clipboard as an enhanced metafile

Alt-Shift-P -- Copy to clipboard as enhanced metafile with printer device context

Ctrl-Alt-C -- Copy to file as standard metafile

Ctrl-Alt-L -- Copy to file as placeable metafile

Ctrl-Alt-E -- Copy to file as enhanced metafile

Ctrl-Alt-P -- Copy to file as enhanced metafile with printer device context

Choosing **Copy Bitmap** from the Edit menu (or typing **Control-C**) copies the currently displayed screen to the clipboard, from which it can be pasted into other programs, such as word processing programs. The "picture" created this way is a bitmap, and can be edited with a Paint program. This is the quickest way to place spectra into reports and is often sufficient. The drawback to a bitmap is that the picture is created pixel by pixel and so is limited to screen resolution, whereas NMR data usually has much better inherent digital resolution. The spectra can end up looking coarse, similar to a FAXed image. Distortions can also result when the bitmap is re-sized. The quality of the final image will be affected by the size of the NUTS window before executing Copy. A large window will contain more pixels, so the resolution will be better. However, if the image is then reduced in size, some pixels can be "lost", and the image can have gaps. It may work better to set the NUTS window to the size of the final image desired, then Copy, and don't resize after pasting.

The second method for placing a spectrum into a report is to copy it as a Windows Metafile (or Mac PICT file), rather than a bitmap. A Metafile (or PICT) is a vector drawing and can be edited with Draw programs. This type of drawing preserves the digital resolution inherent in the data, so the spectrum looks as good as when printed directly out of NUTS. The disadvantage is that it is slow and the resulting picture when pasted into other programs can be very large. The more data points you have displayed, the slower the operation is.

The appearance of the image after pasting is affected not only by NUTS, but also by the target application (see comments below). NUTS offers multiple Metafile variants to give the user the ability to experiment with what works best with his/her preferences and other applications. Some trial and error is needed to determine the best way to paste spectra into a particular application.

The Enhanced Metafile is supported only under Windows. This seems to avoid some of the reported problems with printing from the target application, and seems to handle fonts somewhat better. Also, any inset plots which have been created (with MO) are copied along with the main plot, which is not true of standard metafiles. There are 2 types of Enhanced metafiles available from the Edit menu, one of which requires a printer "device context". Using a printer DC means that the image is generated with properties pertaining to a specific printer. If this is chosen, and a printer has not been defined in the current Nuts session, a print setup box will appear when a metafile is copied.

Why use a Printer Device Context?

When a metafile is copied to the clipboard, the coordinates are expressed as integers (because you can't have a fraction of a pixel) and this can cause roundoff errors if the number of points in the spectrum being copied exceeds the number of pixels. When the resulting metafile is pasted into the target application and stretched, distortion can result. The logical solution is to create the metafile at higher digital resolution. This is done by using a "Printer Device Context" which means that the metafile is composed at the digital resolution of the printer rather than the lower screen resolution. Most common printers are 300 or 600 dpi, but you can install a printer ***driver*** that has much higher resolution (> 2000 dpi), even though you don't actually have such a printer. Add a "printer" (such as a Linotronic) on your computer whose designated destination is FILE rather than a printer port, and select this printer from inside NUTS (File/Printer setup). Then choose "Copy Printer DC Enhanced Metafile to Clipboard" from the Edit menu. When pasted, the image should be essentially free of distortion. Note that many applications, such as Word, allow you to edit a pasted metafile, but to do this, the application converts it to its own internal graphics object, and this may re-create the very roundoff problem we had avoided.

We have recently (September, 2003) noticed that the procedure described above no longer yields high quality spectra in Word or PowerPoint. If you display the resulting spectrum at 500% scale, the distortion is obvious. We assume changes in newer versions of Office or perhaps Windows are responsible. There is a work-around. NUTS allows copy the metafile to a file, rather than to the clipboard. Save the spectrum to a file, then use *Insert/Picture from file* to place the spectrum into the document.

The added complication is that the target application into which the spectrum is pasted also affects the final image quality. Using the higher resolution printer device context solves the roundoff problem when pasting into Word or PageMaker. But pasting the very same copied spectrum into Publisher or PowerPoint still gives a spectrum distorted by roundoff.

A few additional points need to be noted regarding metafiles. Any text displayed on the screen (with Ctrl-B command) is not copied when copying as a standard metafile or as a PICT file, because placing the spectrum into the clipboard replaces the text that was there. Text on the screen is preserved when copying as an enhanced metafile or bitmap. Font sizes chosen in NUTS assume a full-page plot. If the spectrum is reduced when pasted into another application, the fonts are reduced proportionately, and may become too small. The font can first be changed in NUTS from the Edit/Fonts menu. This can easily be done with a macro which resets the font sizes. Whether or not the font can be changed after pasting into the target application depends on the specific application. PowerPoint, Publisher and Word have the ability to break the image down into its component parts, which allows you to edit parts of the spectrum (eg., change the font of the axis labels, remove a specific integral trace, change colors of any single item). However, this involves a conversion which can result in loss of image quality.

For documents which will ultimately be printed on a black and white printer, you may want to set all colors in NUTS to black (from the File / Page Setup menu, choose

monochrome display). Otherwise, from some applications (such as Microsoft Word), the laser printer will "dither" the colors and the lines will come out dashed, not solid. Figures for slides or posters may be more visible if the line thickness is increased before copying, which is also done from the NUTS File/Page Setup menu. Depending on the graphcis capabilities of the target application, annotations may be made after pasting. However, it may be simpler to add a structure or text annotations in NUTS before copying.

A Metafile can also be written directly to a file, rather than placed into the Windows clipboard, by typing **Ctrl+Alt+C** (for a Standard Metafile), **Ctrl+Alt+L** (for a Placeable Metafile) **Ctrl+Alt+E** (for Enhanced Metafile) **Ctrl+Alt+P** (for Enhanced Metafile with printer device context). These commands are also available from the Edit menu. The Metafile will consist of the currently displayed region. NUTS will prompt for a file name for the Metafile. (The file extension .WMF is suggested, as this will be recognized by many other Windows applications.)

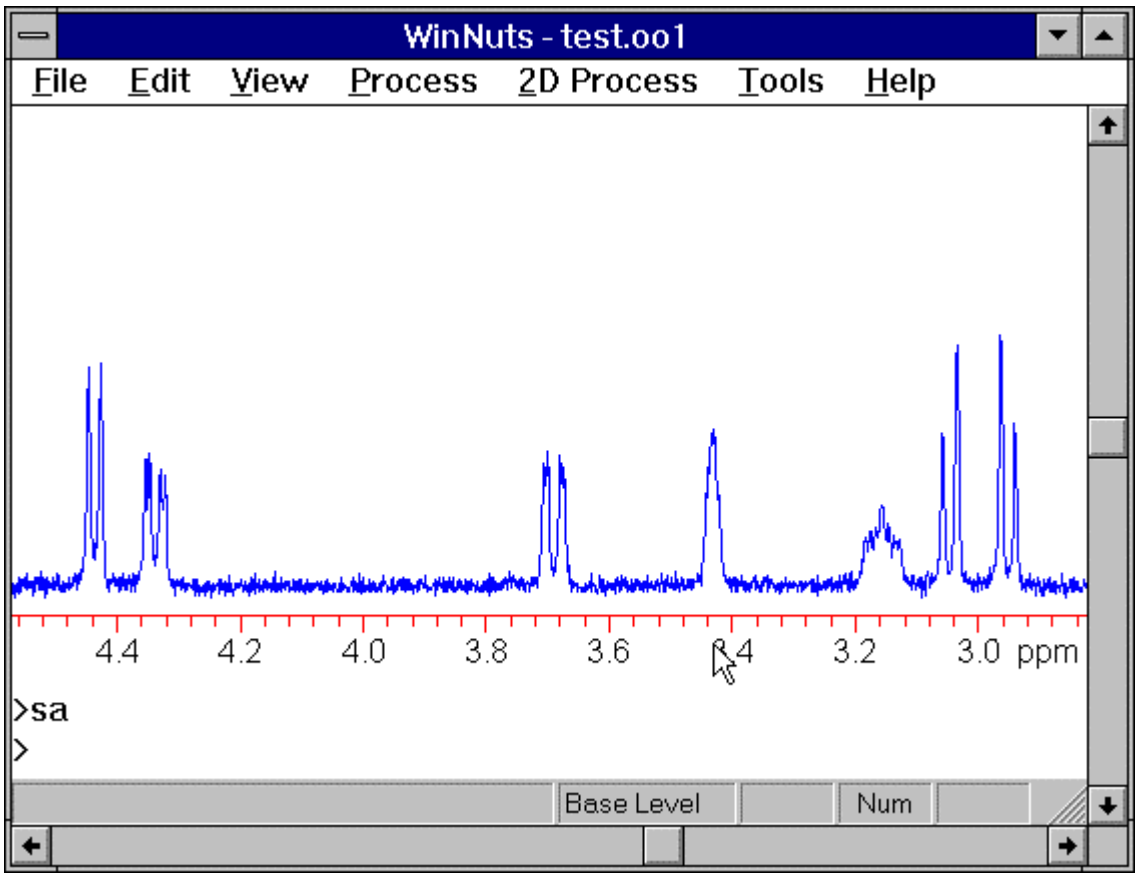
Another method of incorporating spectra into reports is to print to a file. Many applications, such asWord, have import filters for these files which can be used to insert a graphics object into the target application. Depending on the type of printer available and the import filters in the target application, it is possible to print to a file in either HPGL format or postscript format. To do this, choose Print Setup from the file menu and select "HPGL Plotter to File" or "Postscript Plotter to File" as the printer. (If this is not one of the printer options, the corresponding printer driver must first be installed. Then connect that printer to file using the Windows Control Panel.) Use Page Setup from the NUTS File menu to choose whether or not a box drawn will be drawn around the plot and whether or not parameters should be listed. Then choose Print from the File menu and supply a file name. The file can then be imported from within a word processing program or other application. How well this works is dependent on the import filter of the program into which the file is imported.

Window view for interactive apodization

WV - Window View

This subroutine allows experimentation with window functions while simultaneously viewing the apodization function, the FID and the resulting spectrum. The spectrum can be expanded with zoom so that the effects on lineshape can be more easily seen.

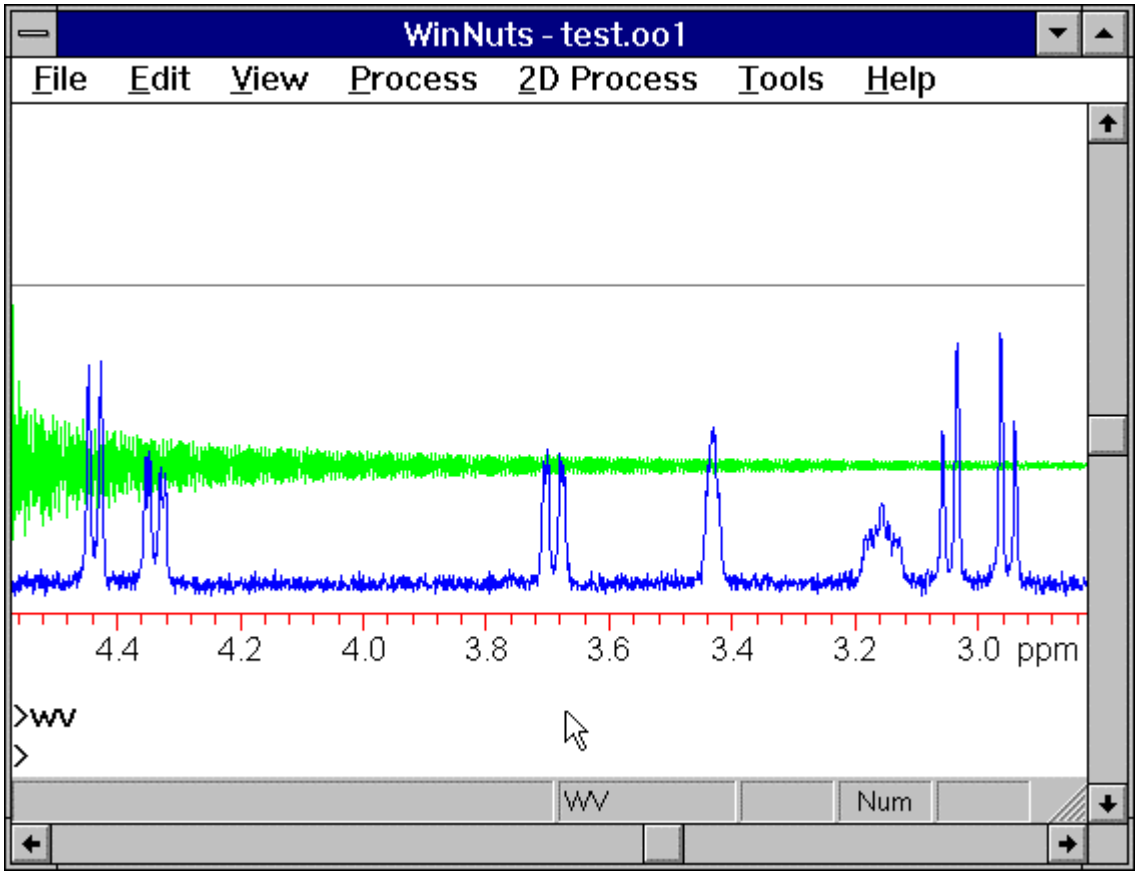
This subroutine is not included in NUTS Lite.



Start by processing the data with no apodization, FT and phase.

Before entering the WV subroutine, this has been expanded to view the region of interest.

Type **WV** to enter the subroutine.

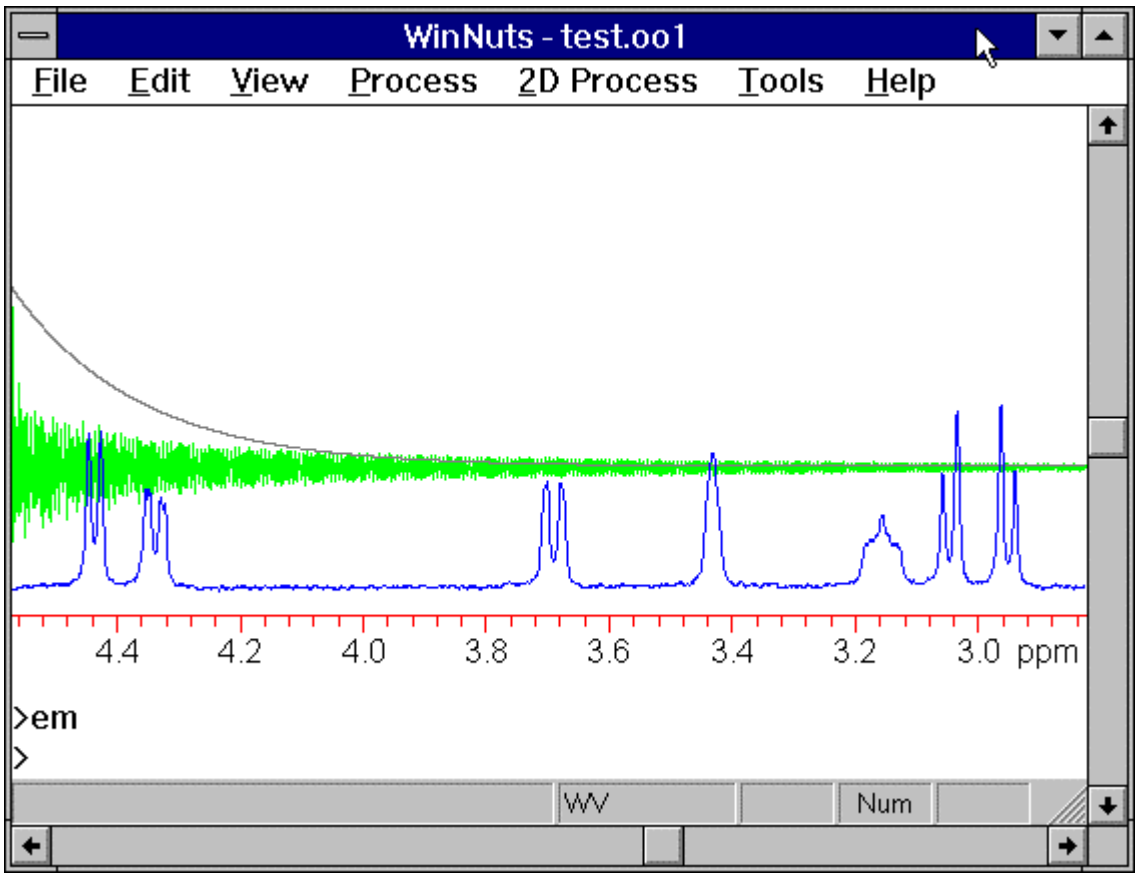


The grey line at the top is the apodization function.

The green trace is the entire FID, even when the spectrum is expanded. This allows adjustment of the apodization to match the shape of the FID.

Most Nuts commands are active while in the WV subroutine, allowing the user to set parameter values such as linebroadening (LB), gaussian factor (GF) and phase for sine function (S#).

No changes are made until the chosen window function is applied. (EM, LG, TF, MS or TM)

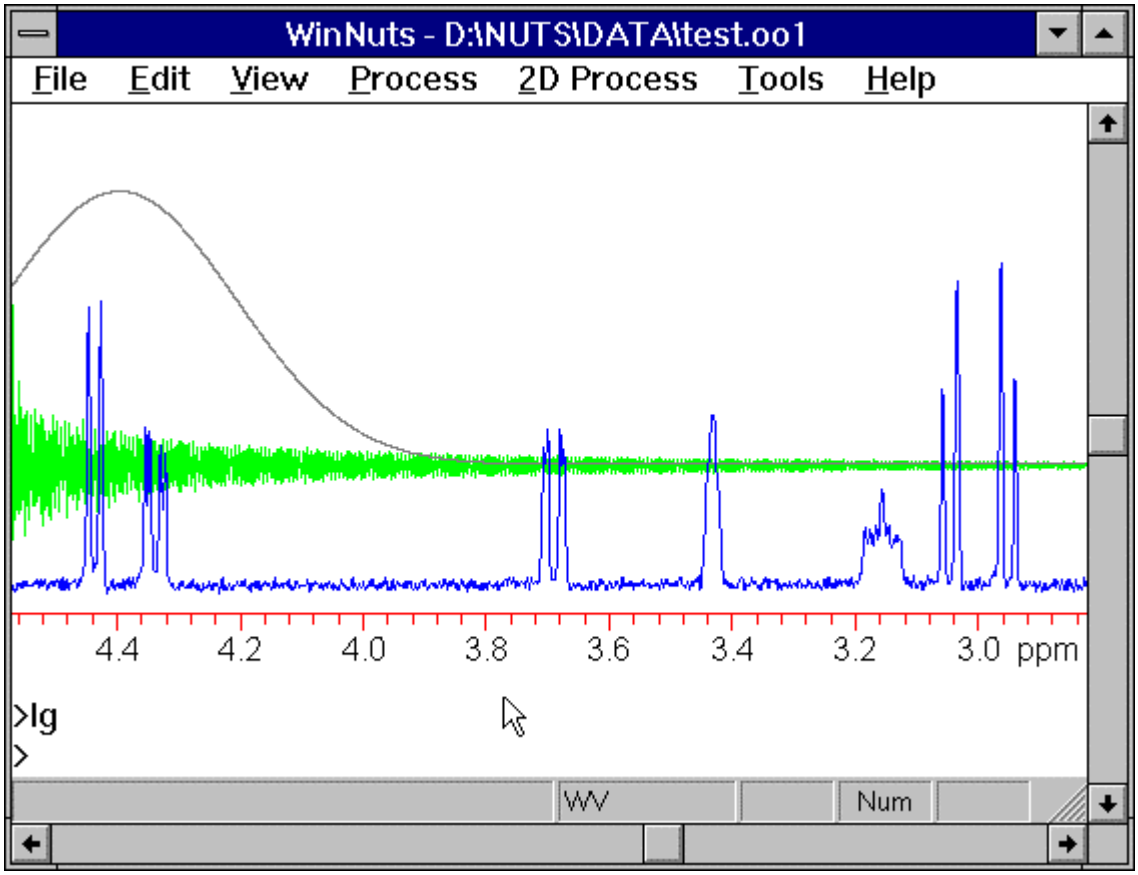


Here, LB was set to 1 and EM executed.

The grey line shows the shape of the resulting function. The FID does not change.

The spectrum has changed to reflect application of 1 Hz linebroadening.

This operation can be undone by typing Ctrl-Z, causing the apodization function and spectrum to be reset so that a different function can be applied.



Here, LB was set to -1, GF was set to 0.1 and LG was executed (Lorentz-Gauss resolution enhancement).

Exiting the routine with <ENTER> applies the current window function and displays the resulting spectrum.

Exiting the routine with <ESCAPE> aborts the operation, and reverts to the spectrum that was displayed before the WV routine was entered.

Note that commands ZF and SH cannot be executed while in the WV routine.

WV can be especially useful when optimizing window functions for 2D data.

Reference Deconvolution

We were intrigued by a poster presented at the 1994 ENC by Ken Metz (Poster # WP111) entitled "Simple Technique for Improving Resolution in Heteronuclear NMR Spectra by Deconvolution with the Measured B_0 Field Distribution". The poster demonstrated a processing technique for removing lineshape distortions, based on an earlier paper by

Morris (**J.Magn.Reson.**, **80**, *547*, 1988) and showed some impressive improvements in lineshape.

The basic idea is that if you know the shape of the distortion, you should be able to correct for it. To measure the distortion, you need a "reference" spectrum of a single, isolated peak whose ideal lineshape is known, eg., chloroform or water. A comparison of the reference spectrum and the ideal lineshape characterizes the shape of the distortion. Both ideal and reference peaks are mathematically adjusted to be at zero frequency and inverse FTed. An apodization function is created by dividing the ideal time-domain function by the reference FID. This apodization function is then applied to a real FID. The resulting lineshapes are substantially improved. The whole approach, of course, relies on the assumption that all peaks have the same distortion.

To implement Reference Deconvolution with NUTS:
The first step in creating the function is to have a spectrum containing an isolated singlet peak. This serves as a reference peak which characterizes the distorted lineshape. Use Zoom to expand the spectrum to a small region with only this singlet displayed. Estimate the real linewidth, in the absence of distortions, and set LB to this value (Type LB and enter the chosen value in the highlighted box.) Suggested values are 0.3 to 1 Hz. Typing CF creates the convolution function in 3 steps: It creates an FID from the displayed singlet via an inverse FT (removing all other peaks that were in the spectrum). It also creates an ideal FID corresponding to a Lorentzian line with width equal to LB. Finally, it creates the convolution function as the result of dividing the ideal FID by the "reference" FID created from the singlet peak in the real spectrum.

To apply this function, read in (with File/Open or GA) the FID to be corrected, and type CA (Convolution function Apply), then FT and proceed as usual. If the base of the peak appears distorted, it may help to repeat the entire process and use a larger value for LB. The convolution function has a shape somewhat like other resolution enhancement functions. It can be viewed (after being created by CF) by typing CV. The function can be saved by selecting the Save Convolution Filter option under the Tools/Convolution option. Similarly, a previously saved function can be recalled from the same menu.

Refs: K.Metz, Poster # WP111 presented at the 35th ENC, 1994
G.A.Morris, **J.Magn.Reson.**, **80**, *547* (1988).
A.Gibbs and G.A.Morris, **J.Magn.Reson.**, **91**, *71-83* (1991).

NUTS implementation of reference deconvolution

CF -- Create Convolution Function

Creates a convolution function relating observed lineshape to an ideal Lorentzian lineshape. When applied to an FID , the resulting lineshape is improved. Commands related to this operation can be found in the menus under Tools/Convolution.

Ref: K.Metz, Poster # WP111 presented at the 35th ENC, 1994
G.A.Morris, **J.Magn.Reson., 80**, 547 (1988).
A.Gibbs and G.A.Morris, **J.Magn.Reson., 91**, 71-83 (1991).

The basic idea is that if you know the shape of the distortion, you should be able to correct for it. To measure the distortion, you need a "reference" spectrum of a single, isolated peak whose ideal lineshape is known, eg., chloroform or water. A comparison of the reference spectrum and the ideal lineshape characterizes the shape of the distortion. Both ideal and reference peaks are mathematically adjusted to be at zero frequency and inverse FTed. An apodization function is created by dividing the ideal time-domain function by the reference FID. This apodization function is then applied to a real FID. The resulting lineshapes are substantially improved. The whole approach, of course, relies on the assumption that all peaks have the same distortion.
See step-by-step example below.

The first step in creating the function is to have a spectrum containing an isolated singlet peak. This serves as a reference peak which characterizes the distorted lineshape. Use Zoom to expand the spectrum to a small region with only this singlet displayed. Estimate the real linewidth, in the absence of distortions, and set **LB** to this value (Type LB and enter the chosen value in the highlighted box.) Suggested values are 0.3 to 1 Hz. Typing CF creates the convolution function in 3 steps: It creates an FID from the displayed singlet via an inverse FT (removing all other peaks that were in the spectrum). It also creates an ideal FID corresponding to a Lorentzian line with width equal to LB. Finally, it creates the convolution function as the result of dividing the ideal FID by the "reference" FID created from the singlet peak in the real spectrum.

To apply this function, read in (with File/Open or **GA**) the FID to be corrected, and type **CA** (Convolution function Apply), then FT and proceed as usual. If the base of the peak appears distorted, it may help to repeat the entire process and use a larger value for LB.

The convolution function has a shape somewhat like other resolution enhancement functions. It can be viewed (after being created by CF) by typing **CV**.

The function can be saved by selecting the Save Convolution Filter option under the Tools/Convolution option. Similarly, a previously saved function can be recalled from the same menu.

CV -- View Convolution function

Replaces the current spectrum with the calculated convolution function created with **CF**. This command is also available from the Tools/Convolution menu. A previously saved function can be recalled from the same menu.

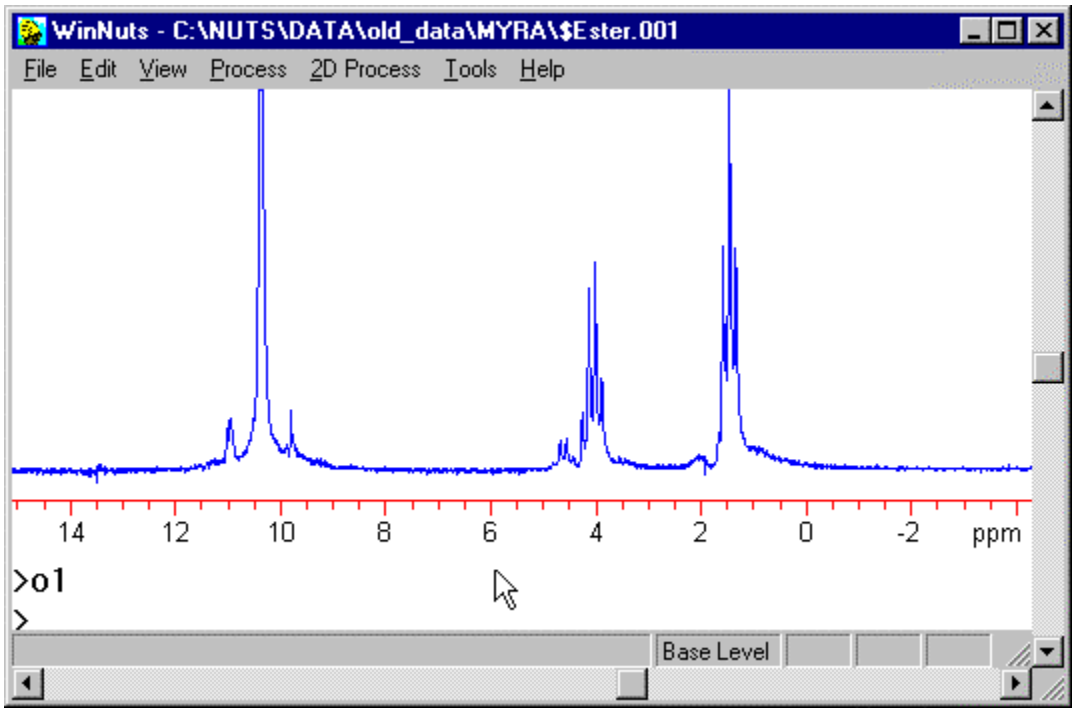
CA -- Apply Convolution function

Applies a convolution function created either using the **CF** command or placed into the convolution buffer using the DB command. This command is also available

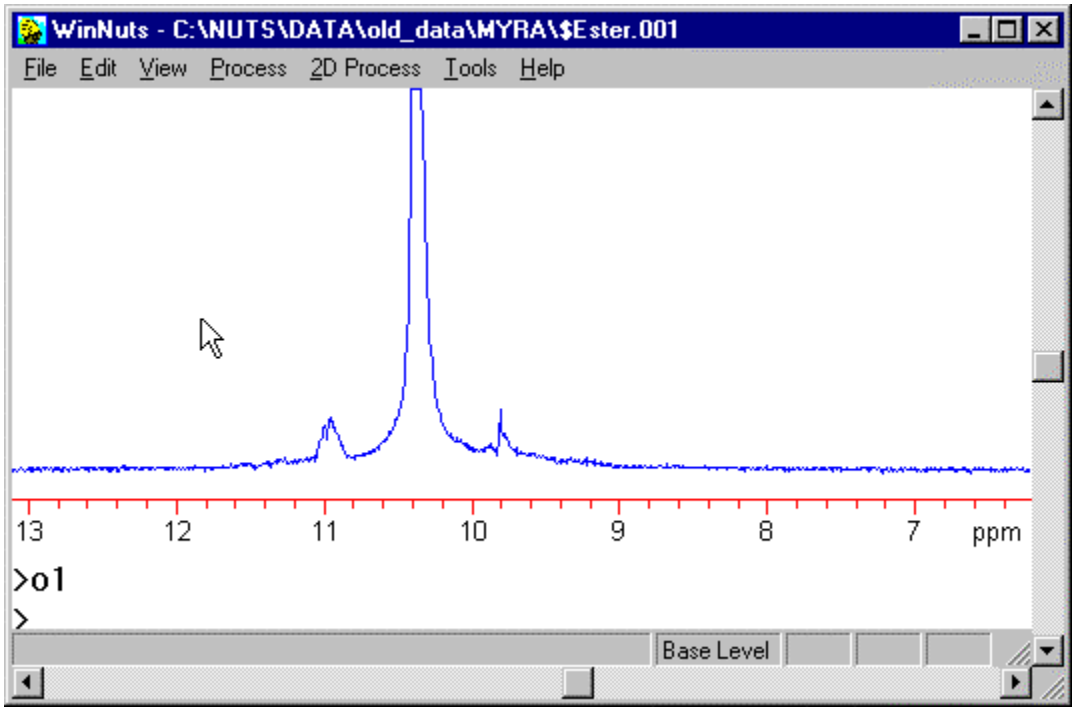
Illustrated example of reference deconvolution

This is a technique for correcting distorted lineshapes without loss of signal-to-noise. To do this, we need to be able to characterize the nature of the distortion, which is done using a reference spectrum. See description of reference deconvolution for details and references.

Note that this feature is not included in NUTS Lite.



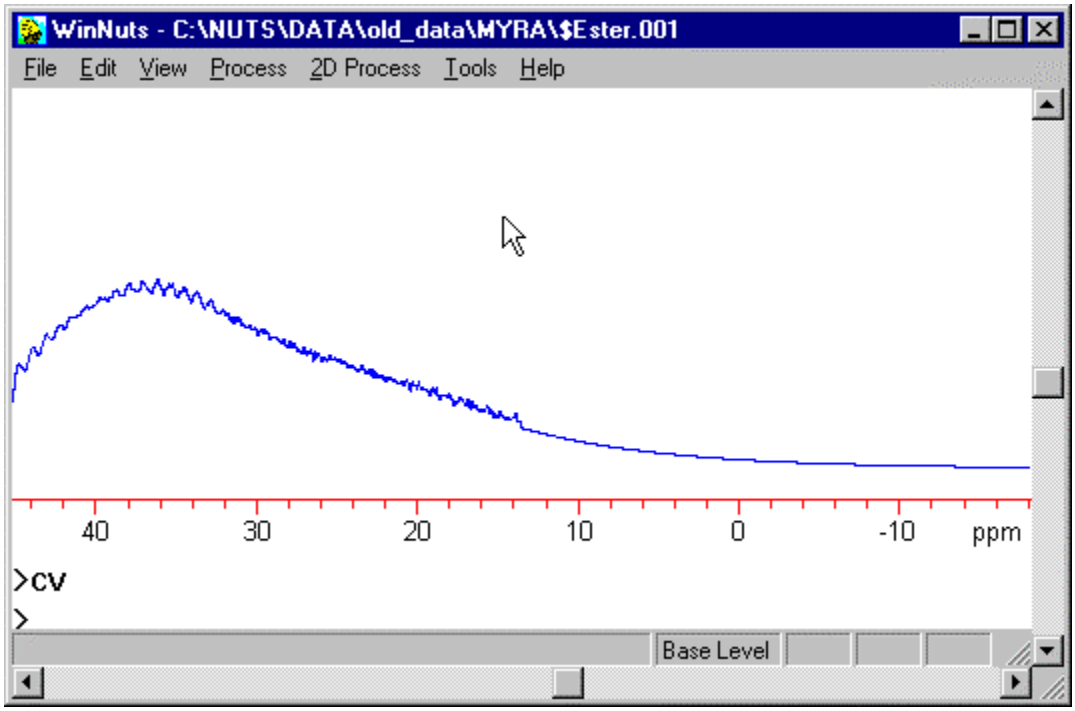
Start with a spectrum with poor lineshape.



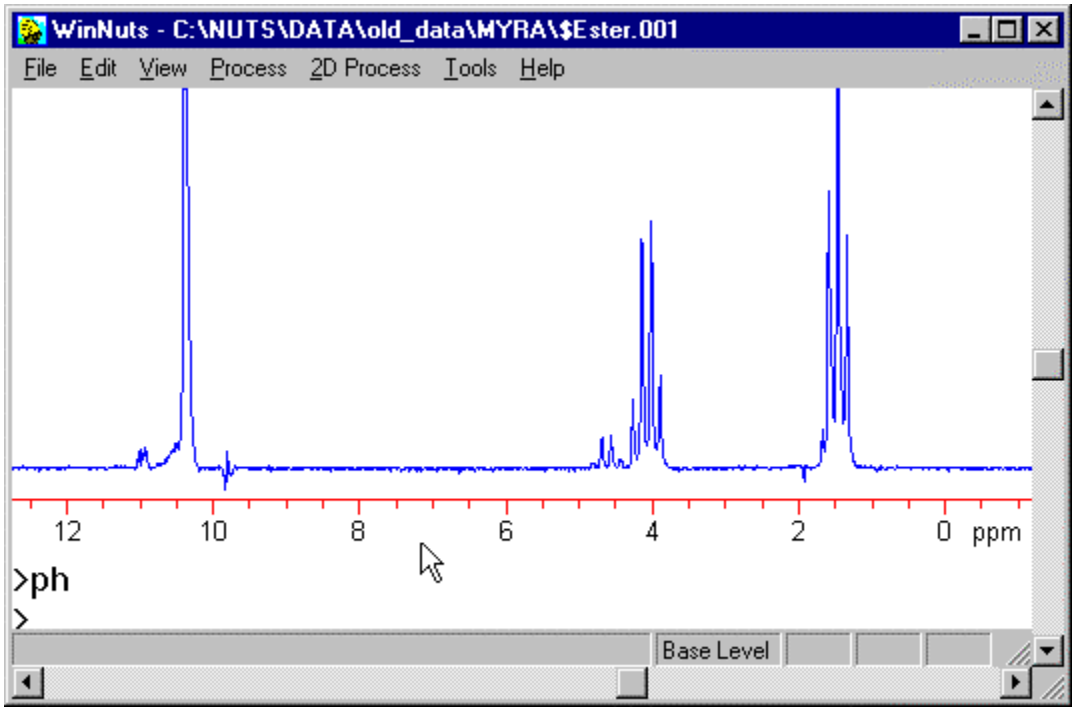
Expand to display just the isolated singlet. Enter a value for LB, the estimated linewidth in the absence of distortion. LB used here is 1 Hz.

Typing CF (also available from the Tools/Convolution menu) performs three steps:

- Creates a zero-frequency "reference" FID from the peak in the Zoom region
- Creates a zero-frequency FID from an ideal Lorentzian line with linewidth equal to LB.
- Creates a customized apodization function by dividing the ideal FID by the reference FID



The resulting function can be viewed with CV (or from the Tools/Convolution menu).



Read in the original FID again and apply the function with **CA** (or from the Tools/Convolution menu).

Compare the resulting lineshape with the first frame above.

Resonance Elimination (RE)

RE -- Resonance Elimination

This subroutine can be used to remove a single, dominant, low-frequency resonance from an FID. This is done with a fitting routine, in which amplitude, frequency, phase and Lorentzian/Gaussian linewidth are adjusted to match the actual data. This can be useful to remove a residual water line in the center of a spectrum. Results depend heavily on having a good, symmetrical lineshape, but can reduce the water resonance by more than 1000x in some examples. See details below on how to use RE.

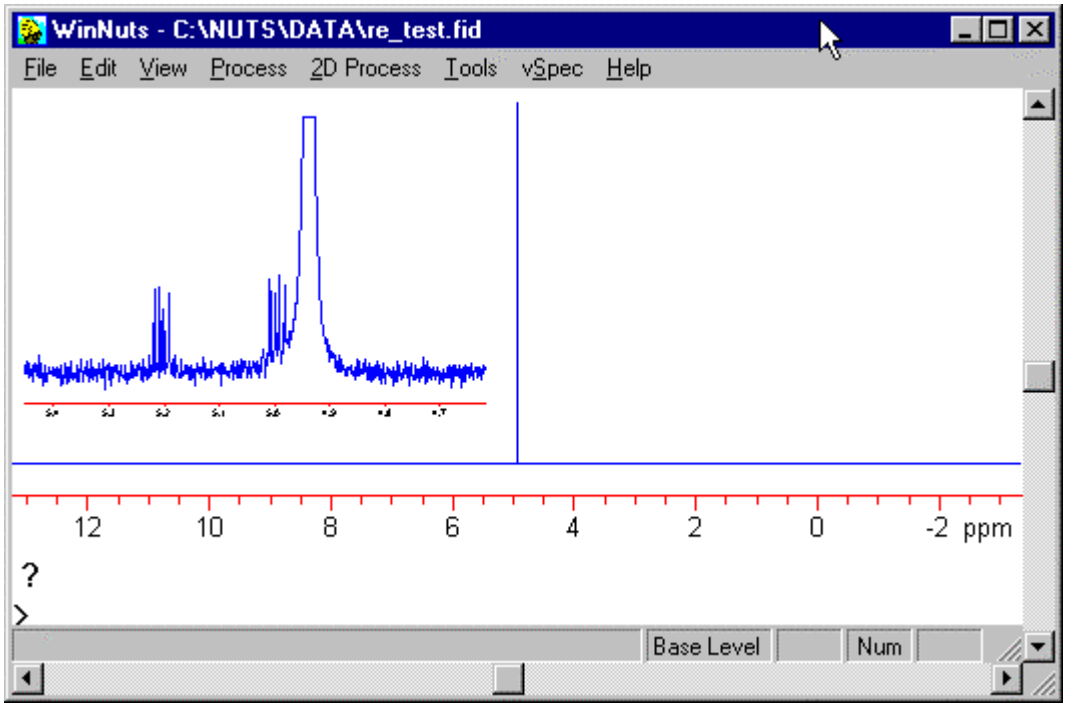
Subcommands, available from the Edit menu, are:

D Delete (subtract) the calculated FID from the data
E Edit the fit parameters
F Perform fit
R Reset parameters to default settings
<ENTER> Exit the RE subroutine

See also: Eliminate Dispersion component

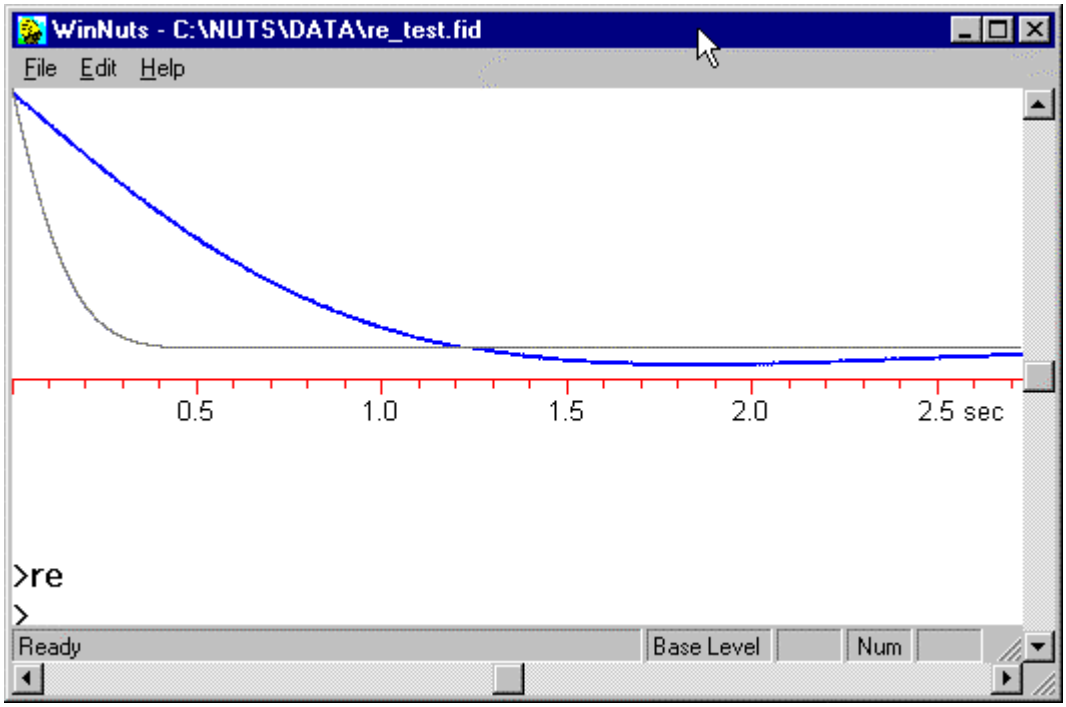
This subroutine fits an FID with a single frequency and then subtracts the calculated FID from the data. The FID must be dominated by a single resonant frequency near zero. Because the calculated FID will have ideal Lorentzian lineshape, the quality of the fit is critically dependent on the lineshape of the data.

Note that RE is not included in NUTS Lite.

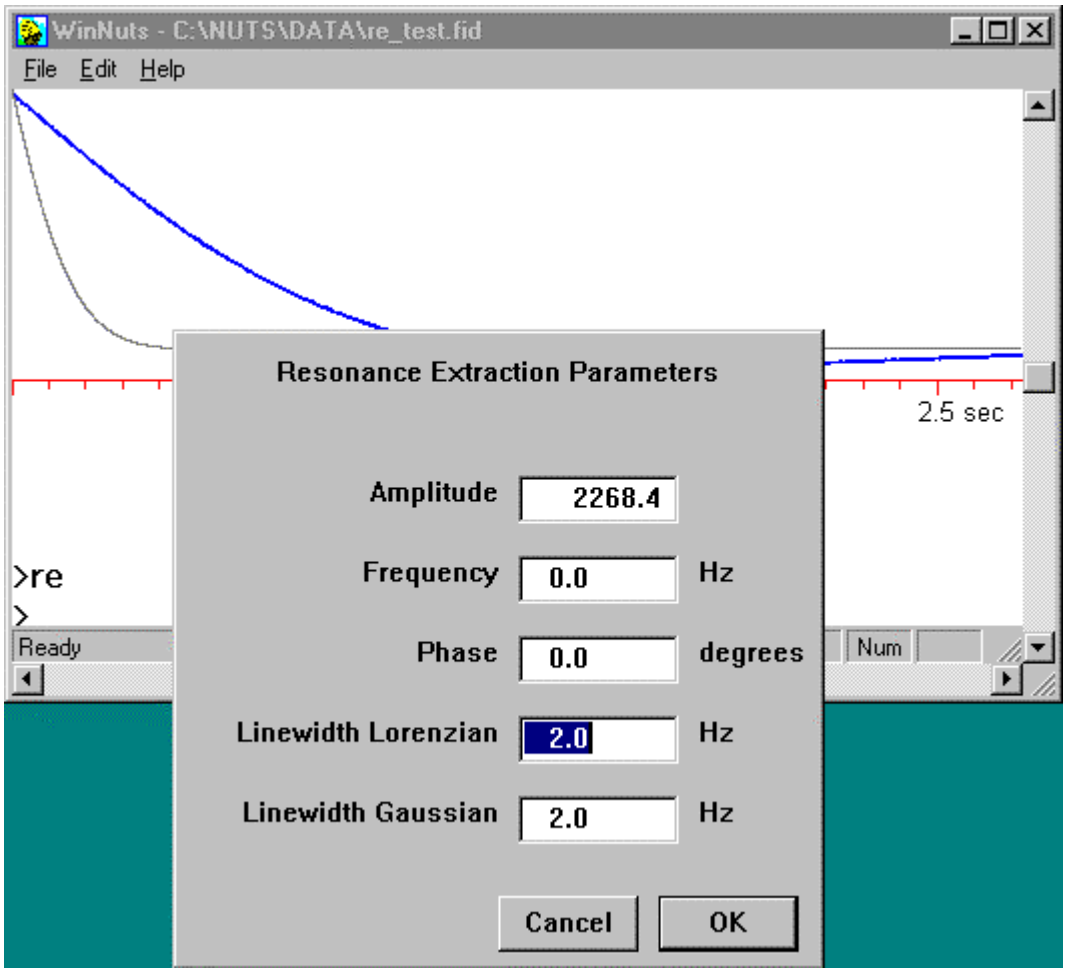


This spectrum has a very large peak at the center (0 frequency) and some small peaks very close to it. The goal is to remove the large peak without affecting the smaller ones.

(This is actually simulated data, with ideal lineshape.)

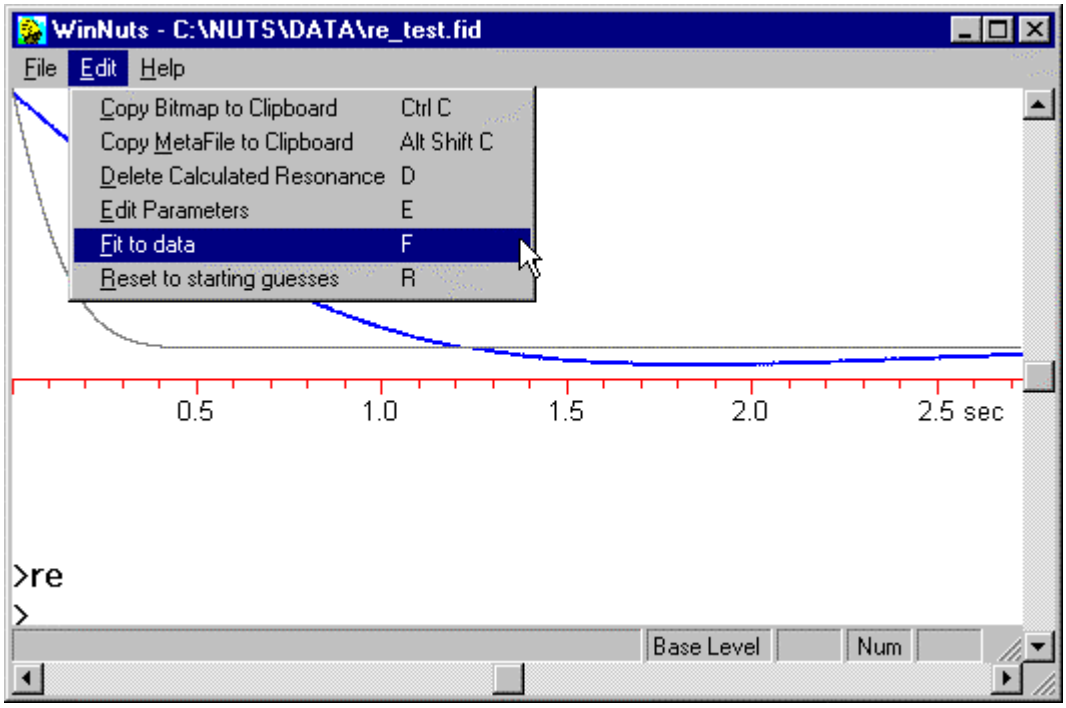


On entering the RE subroutine, a first guess at a matching FID is displayed



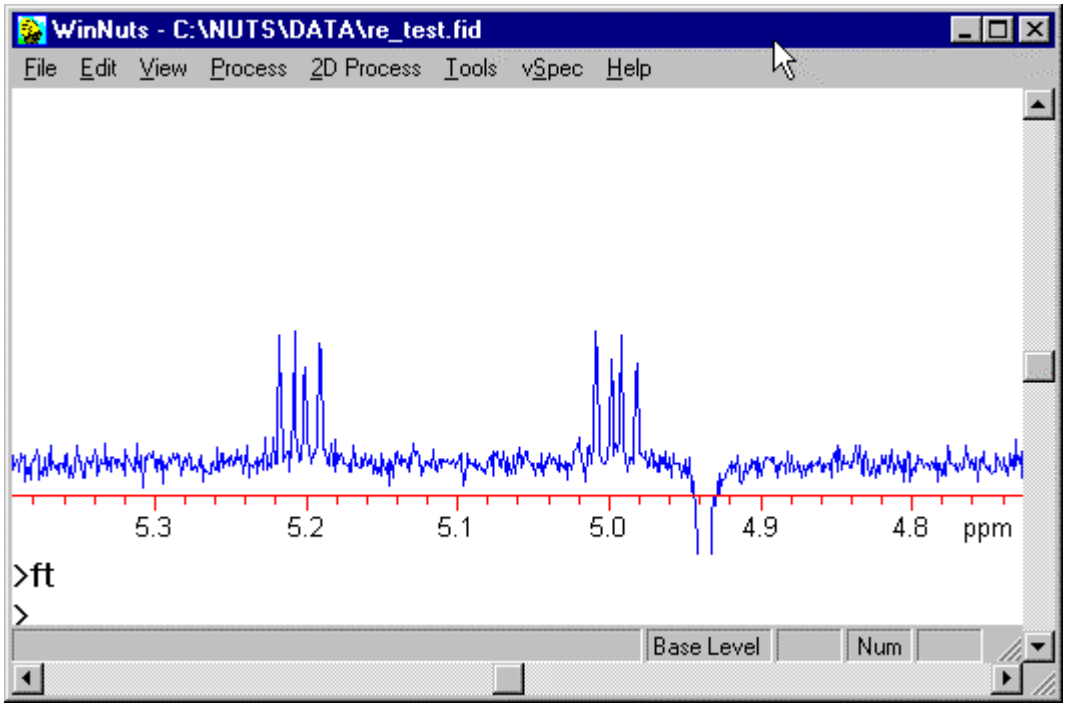
These parameters can be adjusted to give a closer starting point by selecting Edit Parameters from the Edit menu, or typing E.

This dialog box shows the parameters which are adjusted during the fit.

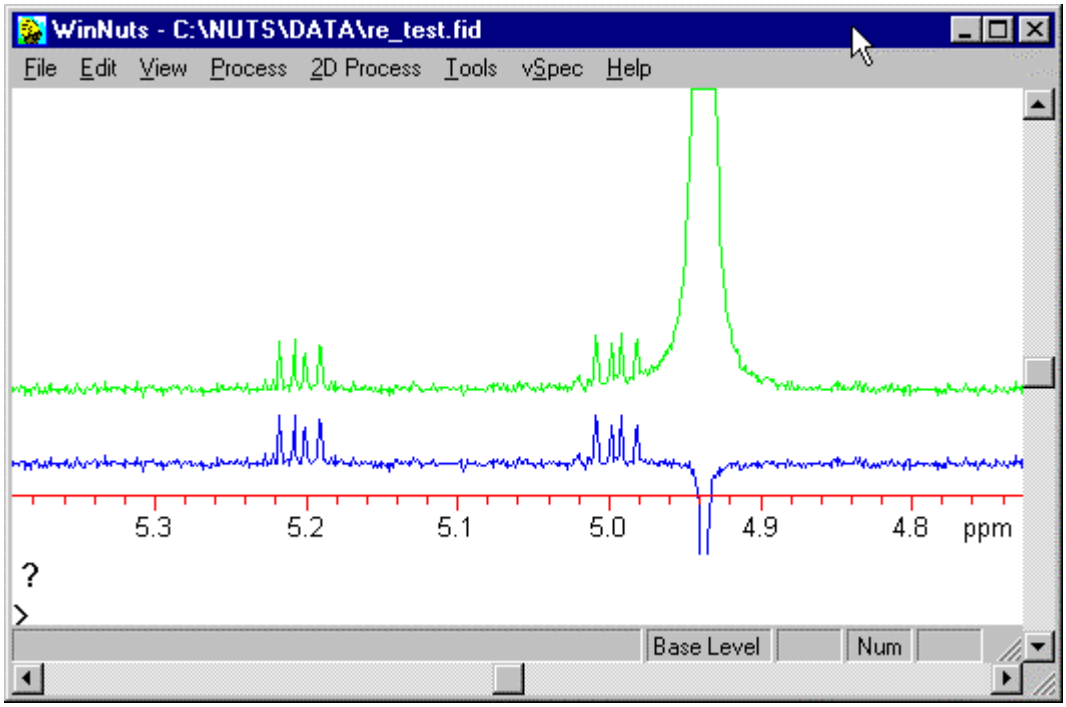


To initiate the fit, select Fit to Data from the Edit menu, or type **F**. The Simplex fit may take a couple of minutes.

When the fit is completed, select Delete Calculated Resonance from the Edit menu, or type **D**.



After FT, the major peak is removed. A small negative peak remains, because the fit was not perfect.



Comparison of spectra without (top) and with RE, using the Dual Display utility.

Eliminate Dispersion

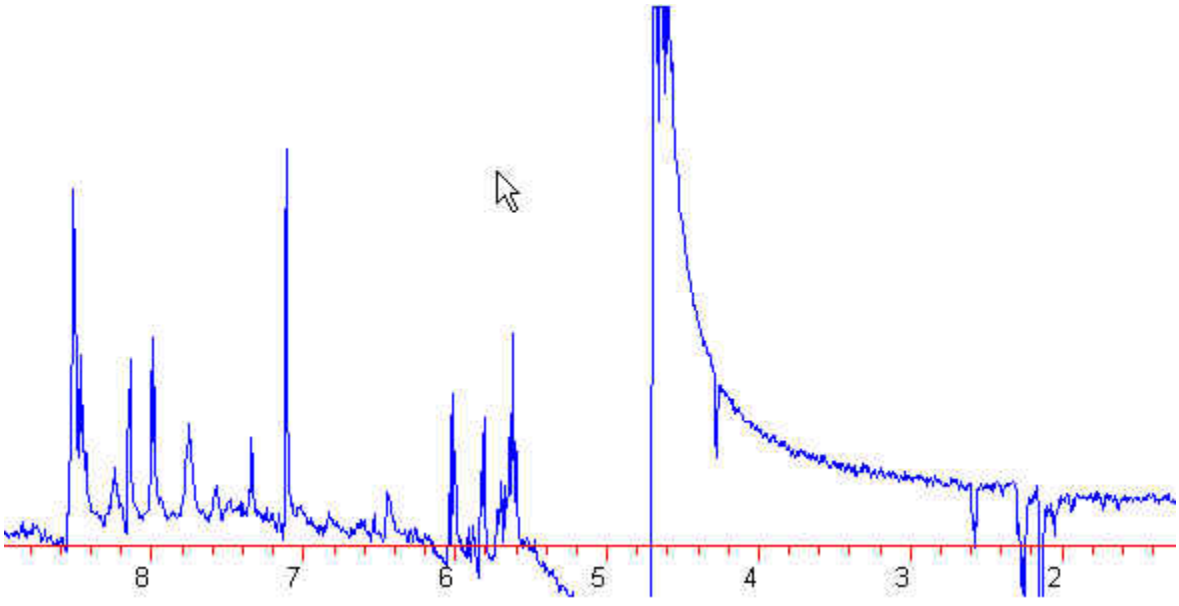
ED - Eliminate Dispersion

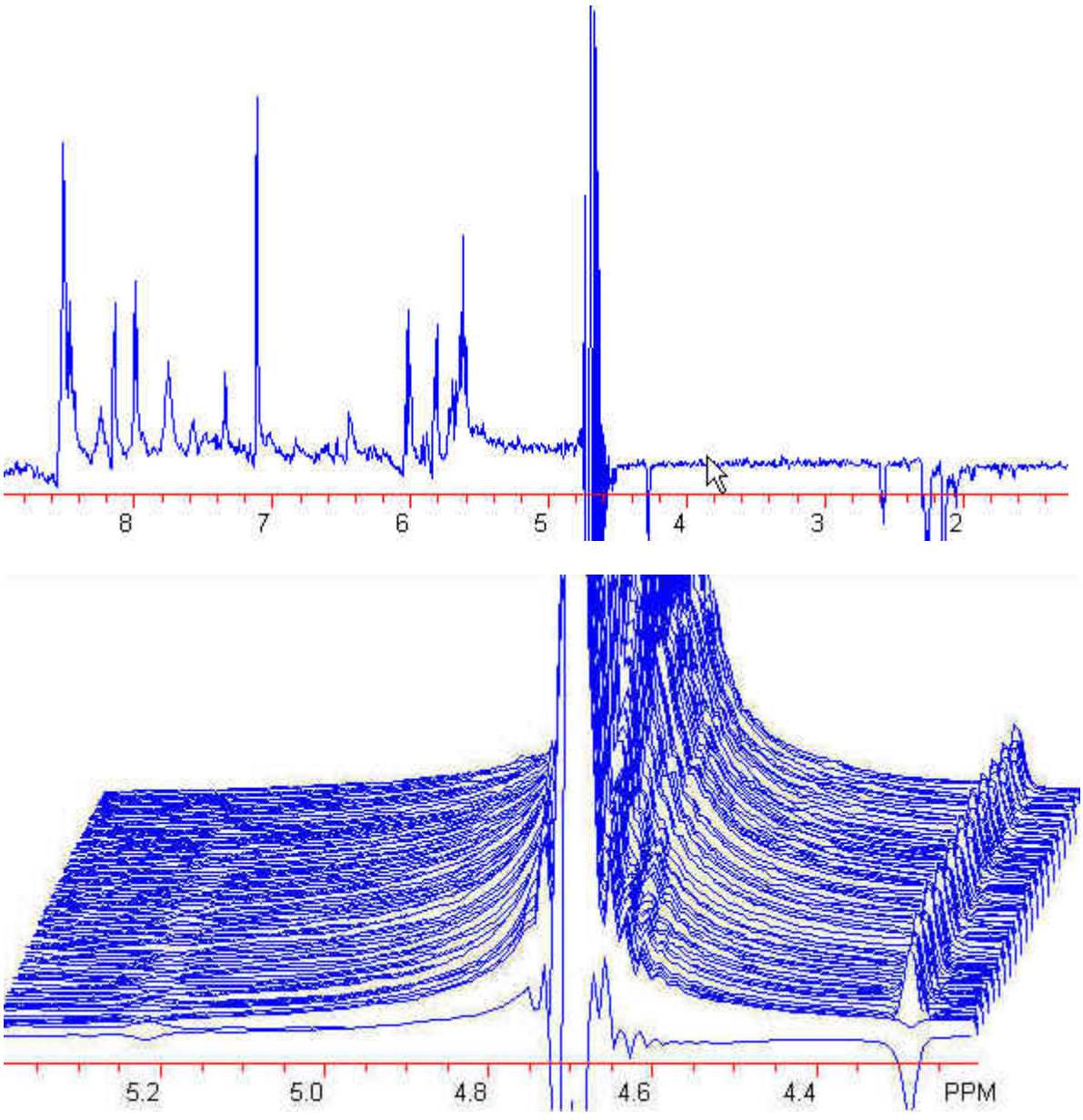
A routine to reduce residual dispersion lines at the center of a water suppressed spectrum was added at the request of Jerry Dallas and Marc Alder at Berlex per the reference:

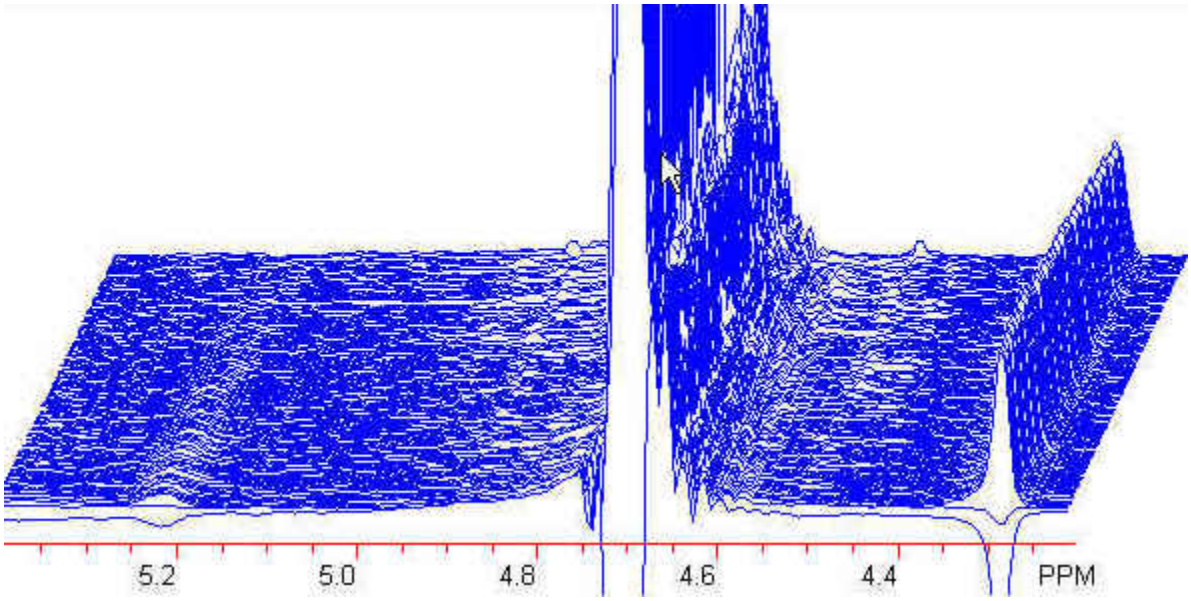
Adler M., and Wagner, G. "*A New Technique for the Removal of Baseline Distortions Caused*
by Strong Water Signals." (1991) **J. Magn. Reson. 91**, 450.

Often, 2D spectra acquired in H_2O using water suppression have a large dispersion component to the residual water peak. The dispersion line has large "wings" that extend on each side of the peak, and this can create substantial baseline distortions. This cannot be removed by digital filtering. By subtracting out the dispersion component, these broad wings are eliminated, giving a much flatter baseline and contour plots with less pronounced "zipper" appearance.

Compare the 2 pairs of plots below (single slice and stacked plots). The first plot shows the broad wings caused by the dispersion component of the residual water peak. The second one shows the much flatter baseline obtained after removing the dispersion component. In the stacked plot, notice also the modulation in the amplitude of the peak at 4.3 ppm caused by the variation in phase of the dispersion component of the water peak, which is eliminated when the dispersion component is removed.




8
7
6
5
4
3
2
5.2
5.0
4.8
4.6
4.4
PPM



To use this command, zoom in on the residual water peak. That expanded region will be used to fit a dispersion line:

$$I(w) = a*(w_o-w) / \{ (1/T_2)^2 + (w_o-w)^2 \}$$

using three parameters:

w_o - frequency of the center of the water peak. The peak must be close to zero frequency (center of the spectrum), as this parameter is adjusted over only a small range.

T_2 of the water peak

a - amplitude

This fitted calculated dispersion line is subtracted from the entire spectrum (all real data points).

The fit can be made faster by using default values of T_2 and/or w_o.

When ED is executed in the 2-lettered command mode, or in the non-2-lettered command mode with no arguments specified, NUTS uses the displayed zoom region to do a 1 parameter fit to amplitude using a fixed $T_2 = 10.0$ and $w_o = 0.0$

The command will take arguments when operating in the non-2-lettered command mode. Examples are:

ED 1 // same as default mode above
ED 1 20 // 1 parameter fit to amplitude using T_2 = 20.0 and w_o= zero.
ED 2 // 2 parameter fit to amplitude and T_2 with w_o= zero.
ED 3 // 3 parameter fit to amplitude, T_2 and w_o.

This can be used in Arrayed Mode to perform the fit on each slice with the single command.

Digital filtering

DH -- Digital High pass filter

This routine allows the user to define a frequency limit and apply it to an FID. Signals above that frequency limit will remain unchanged and signals at lower frequencies will be filtered out from the currently displayed FID.

This is done by creating a function in the frequency domain which is equal to one for all frequencies greater than the cut-off value and equal to zero for all frequencies less than the cut-off. The function is converted to the time domain using a Hilbert transform. This time domain function is then correlated with the FID to remove high frequency components.

The user can adjust the order of the function, which is the number of pts in the correlation function. The more points, the sharper the cutoff, but the operation also becomes slower. As the order approaches the number of points in the FID, the filter approaches being perfectly square.

If the number of data points is not equal to a power of 2, it is important to execute a zero-fill to next higher power of 2 **before** executing the digital filter. Failure to do this will hang the program.

DL -- Digital Low pass filter

This routine allows the user to define a frequency limit and apply it to an FID. Signals below that frequency limit will remain unchanged and signals at higher frequencies (measured from the center of the spectrum) will be filtered out from the currently displayed FID.

This is done by creating a function in the frequency domain which is equal to one for all frequencies less than the cut-off value and equal to zero for all frequencies greater than the cut-off. The function is converted to the time domain using a Hilbert transform. This time domain function is then correlated with the FID to remove high frequency components.

The user can adjust the order of the function, which is the number of points in the correlation function. The more points, the sharper the cutoff, but the operation also becomes slower. As the order approaches the number of points in the FID, the filter approaches being perfectly square.

If the number of data points is not equal to a power of 2, it is important to execute a zero-fill to next higher power of 2 **before** executing the digital filter. Failure to do this will hang the program.

D2 -- Decimate data by 2

This command is intended for use on an FID in conjunction with a digital low-pass filter. The D2 command "decimates" the data by a factor of 2, meaning that every other point is discarded. This reduces the data size by half and also reduces the spectral width by half (by effectively digitizing the data a factor of 2 slower). This only makes sense for use following application of a low pass filter set equal to half the spectral width.

See also: Extracting a spectral region (XT)

/2 -- Decimate FID

This command operates differently on time and frequency domain data.

When applied to an FID (time domain), this command reduces the data by half, similar to D2, but instead of simply deleting every other point, pairs of points are averaged. So each pair of points is replaced by their average.

When applied to a spectrum (frequency domain), one quarter of the spectral window on each end of the spectrum is discarded. Number of data points and SW are both reduced by a factor of 2.

The command will accept an argument (in the non-2-letter command mode) which is the number of points to eliminate at each end of the spectrum. The argument is ignored if the data is time domain.

Histograms (binning) (HI)

HI - Histogram

This is a new tool for reducing or digesting (also referred to as "binning" or "bucketing") a complicated spectrum for input into software that performs principal component analysis.

Background

Spectra such as ^{1}H spectra of biofluids are so complex that it is not possible to assign all peaks, yet they still contain valuable information. The spectra must be reduced to a simpler form that is more tractable to enable comparison of spectra and identification of correlations.

This is done by segmenting the spectrum into narrow frequency regions, usually 0.04 ppm wide, and summing all points in each region. Each region is thereby reduced to a single number, called a descriptor. This not only reduces the number of data points to a more manageable number, but also allows for small differences in chemical shifts, such as might be caused by variations in pH.

It may be desirable to eliminate some descriptors (such as the residual water peak, which varies from spectrum to spectrum). The values are normalized, to allow comparison of different spectra. The resulting data are output as an ascii text file consisting of 2 columns of numbers, chemical shift and intensity. This data can then be analyzed by other software.

Using the HI command in NUTS

6/19/2002
HI - (or equivalent, non-2-letter command **histogram**) This command generates a text file which is a list of intensity descriptors. The first item in each line of the descriptor file is the PPM value of the start of the descriptor. The second item is a relative sum of the intensity in the descriptor PPM range, which by default is 0.04 PPM wide. The descriptor file has the following characteristics:

All negative sums are zeroed
The sum of all descriptors is 1.0

By default, the size of each descriptor is 0.04 PPM. By default the total spectrum is used for the descriptor file and all data points in the file are used. It is possible to customize this by having NUTS read a file containing the relevant information. This is done with NUTS in the non-2-letter command mode. The command will take an argument which is the file name for this descriptor properties template file. A sample file is shown here:

```
Histogram_Template
Descriptor_Size 0.1
Include 10.0 0.0
Eliminate 9.50 9.0
Eliminate 5.00 4.00
```

The ends of the spectrum can be ignored by specifying a region to be digested, with the "Include" line. The regions specified by "Eliminate" are excluded from the descriptor file.

References

Metabolic profiling of chronic cadmium exposure in the rat, Griffin JL, Walker LA, Shore RF, Nicholson JK, CHEMICAL RESEARCH IN TOXICOLOGY, 14 (10): 1428-1434, Oct 2001

An NMR-based metabonomic approach to the investigation of coelomic fluid biochemistry in earthworms under toxic stress, Bundy JG, Osborn D, Weeks JM, Lindon JC, Nicholson JK, FEBS LETTERS, 500 (1-2): 31-35, Jun 29, 2001

Metabonomics: Metabolic processes studied by NMR spectroscopy of biofluids, Lindon JC, Nicholson JK, Holmes E, Everett JR, CONCEPTS IN MAGNETIC RESONANCE, 12 (5): 289-320, 2000

Pattern recognition methods and applications in biomedical magnetic resonance, Lindon JC, Holmes E, Nicholson JK, PROGRESS IN NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY, 39 (1): 1-40 Jul 2, 2001

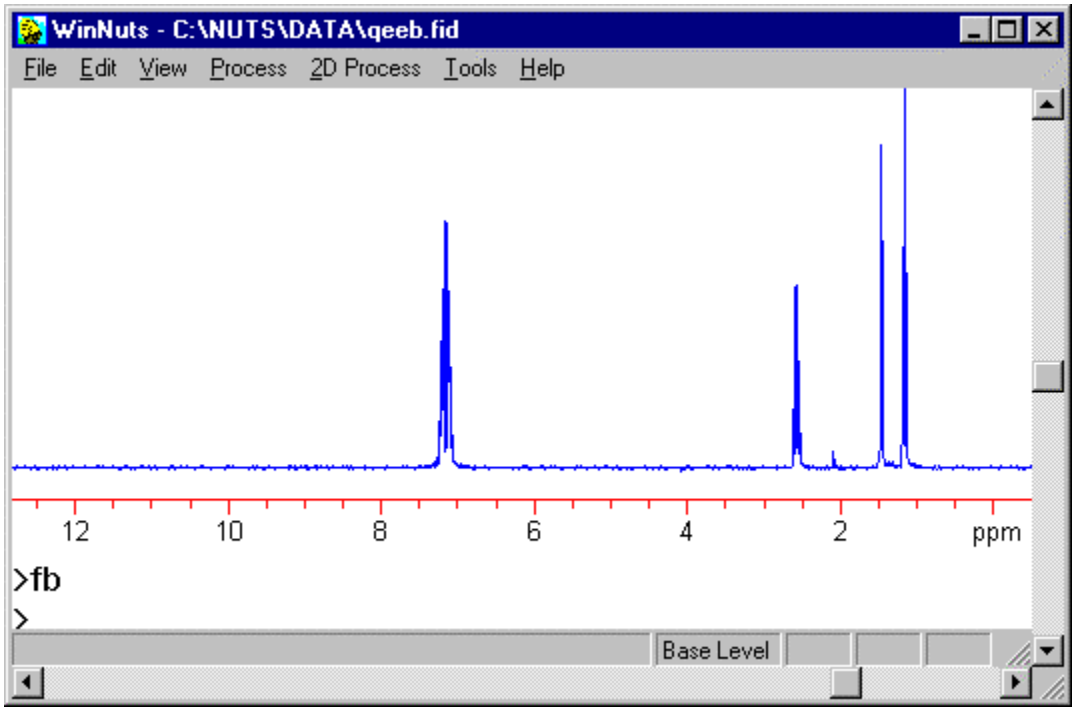
Extracting part of a spectrum

Extracting spectral regions (XL and XT)

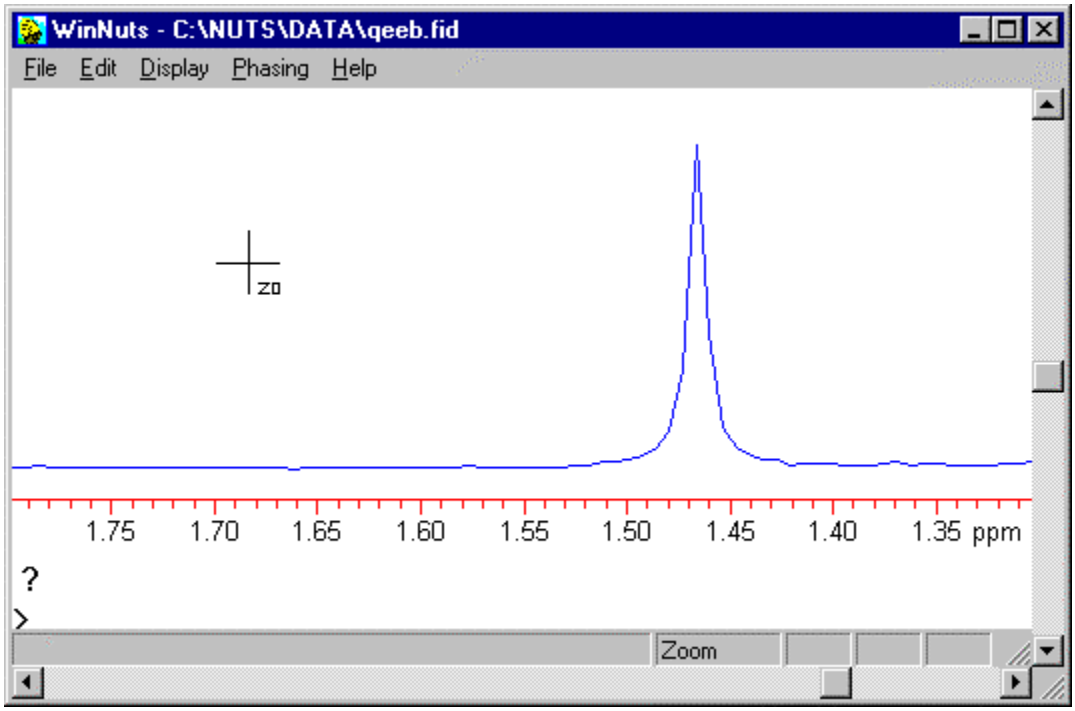
There are two different tools available in NUTS for extracting part of a spectrum.

Extract Line (XL) -- This command extracts just the displayed Zoom region, places the biggest peak in that region at the center of the extracted spectrum, and adds zeros to both ends of the spectrum so that the final size of the extracted spectrum is the same as the original spectrum. This was initially created for use with reference deconvolution.

Extract region (XT) -- This command extracts just the displayed Zoom region. The resulting spectrum has a data size equal to the number of points in the zoom region. This can be useful for overlaying spectra that were acquired with different offsets.

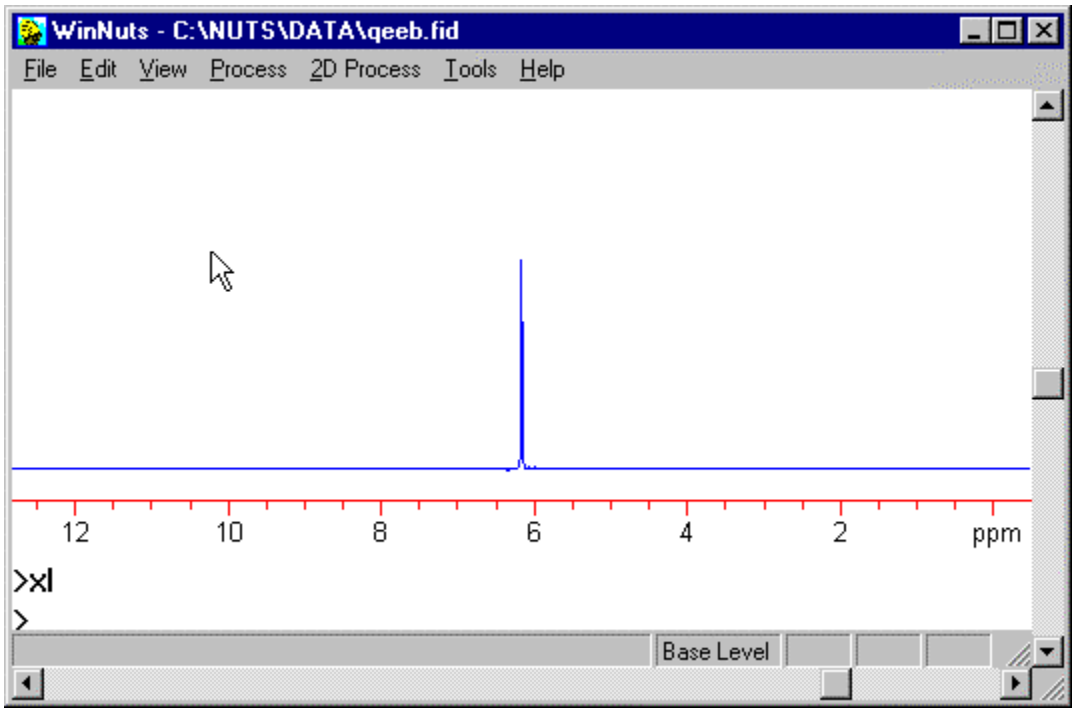


Begin with a spectrum of ethyl benzene.

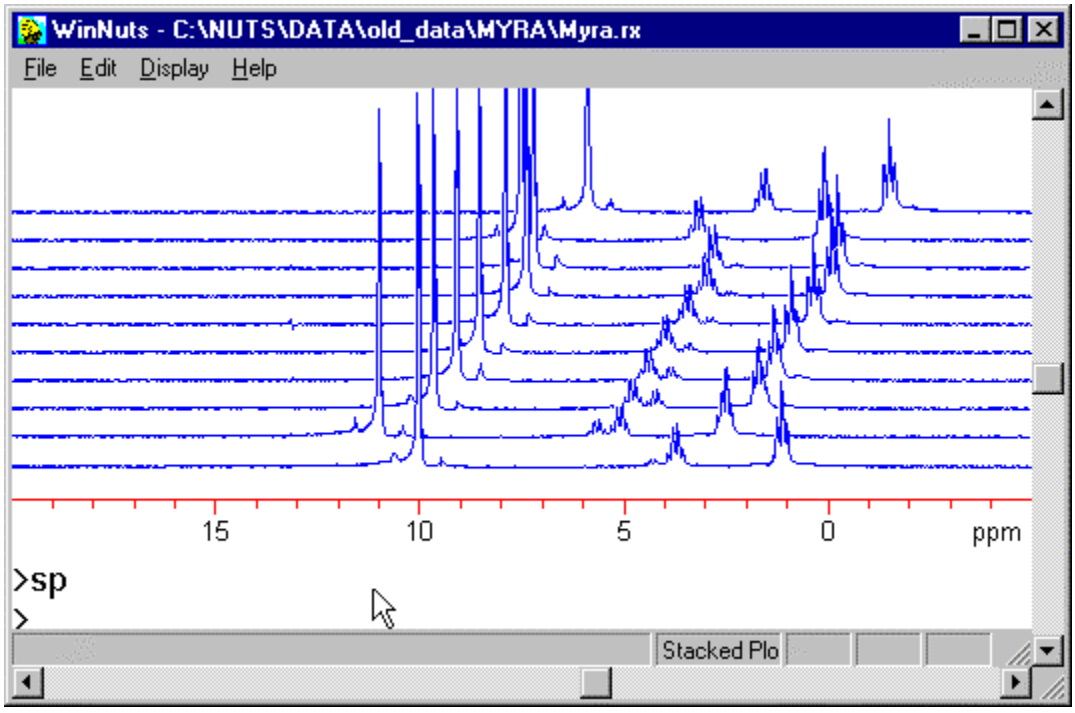


Use Zoom to expand so that just one peak is shown, in this case the water peak.

Exit zoom with <Enter>.

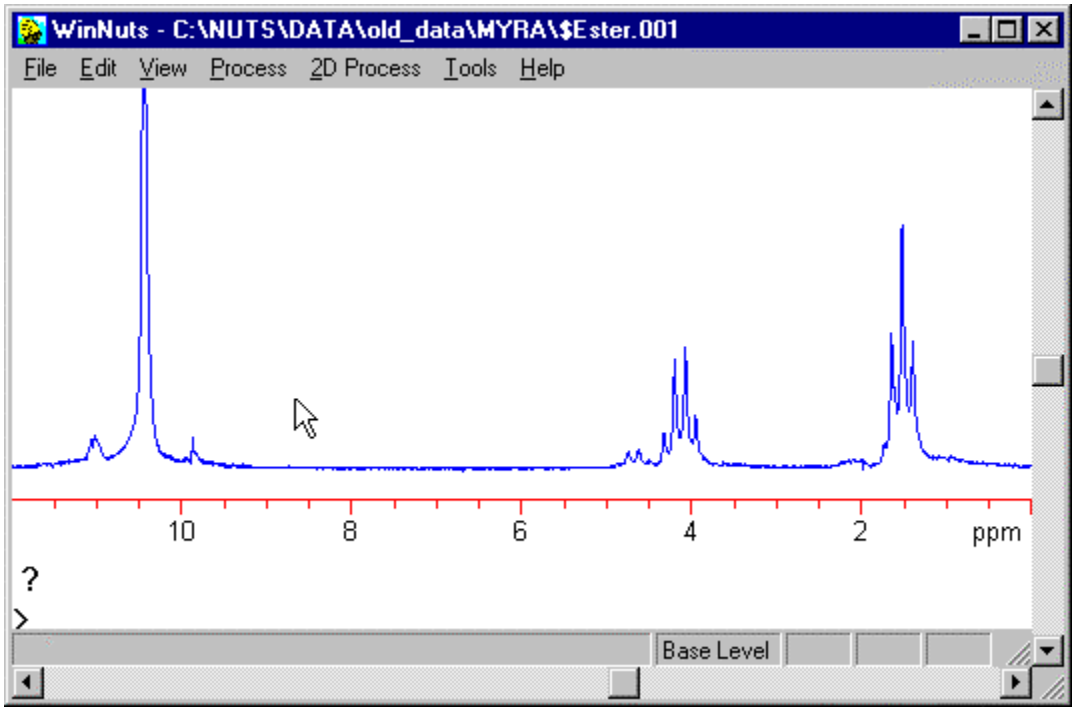


After executing **XL**, the spectrum consists of just the water peak, shifted to be in the center of the spectrum (zero frequency), and zeros are added to both ends so that the final data size is the same as the initial spectrum.



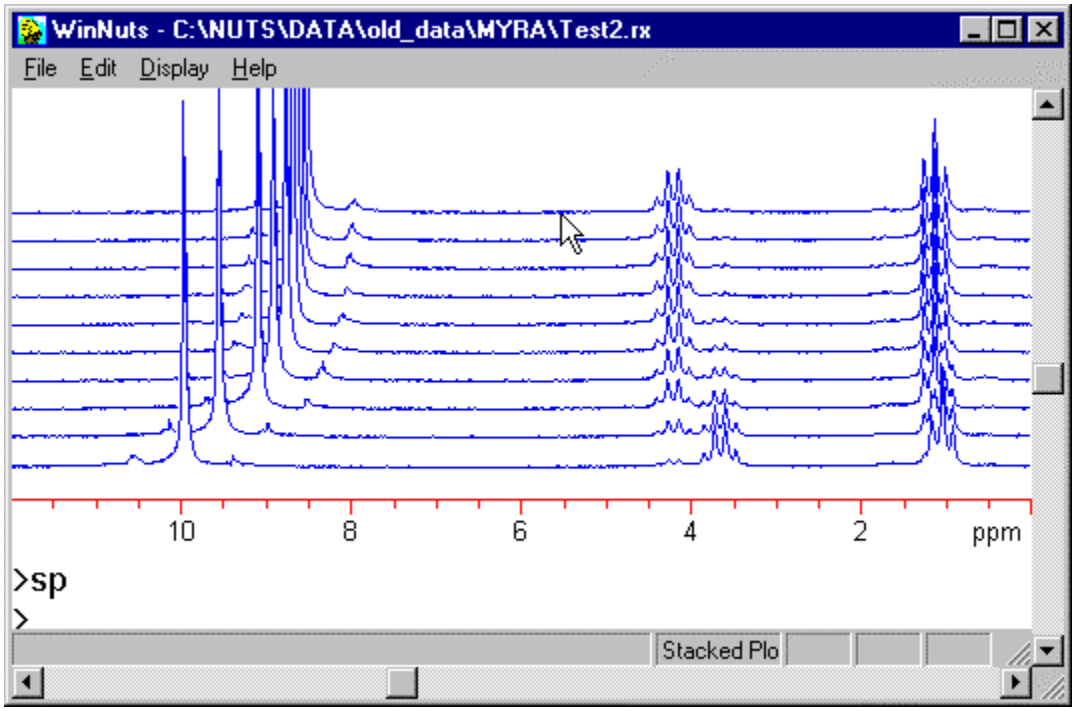
This is a kinetics experiment run on a spectrometer without a field/frequency lock, so the field moved during the course of the experiment.

The task was to create a display with the peaks correctly lined up.



Expand each spectrum to the same frequency limits, being sure that each spectrum is correctly referenced.

Execute a **XT** operation on each spectrum, saving the resulting files.



The extracted spectra were converted to a 2D file and now the peaks line up.

(To create a 2D file from a series of 1D spectra, run this Link:

GA SC IN

and supply the name of the 1D files and an name for the 2D file to be created.)

See also: X0 - X9 to extract pre-set spectral regions.

Searchable Archive

not included in NUTS Lite

Overview

This NUTS tool allows the user to create and search an archive file containing information about a collection of Nuts spectra. The file is a text file consisting of several fields, identified by keywords.

The information placed into the archive file is taken from the "tailer" of each file in a directory or folder. When a spectrum is processed with Nuts and then saved, several pieces of information are automatically saved in the file tailer. This can include:

a list of peaks defined by PP or within the DP subroutine
a list of integral regions defined via automatic integration (**AI**) or in the integration subroutine (ID)
a pointer to a metafile (or PICT file on the Mac) containing a molecular structure, imported into the MO routine
items such as multiple names, molecular formula and nucleus which are input via the IF command

Creation of an archive file involves first processing each spectrum and saving the processed data, all in the same directory or folder. The archive file is created within Nuts using the Database Make (**DM**) command, which reads the tailers of all files in the chosen directory and enters the information into a text file with appropriate keywords. This file could be searched with any search tool, but is most easily searched from within Nuts using the Database Search (**DS**) command. A dialog box is displayed allowing search criteria to be entered in several fields. With the exception of the chemical shifts, all searches are sub-string searches. For chemical shifts, a value is entered as well as a range. When an entry is made in the Name field, the search operation looks for matches in any of the 3 Name fields defined in the **IF** routine. Entries can be made in more than one field, which causes the search to find entries which meet all specified criteria (logical AND). Multiple entries can be made in the Name field, separated by a space, to search for a name containing multiple sub-strings. For example, entering "methyl phenyl" would search for occurrence of both methyl and phenyl in the name fields, and select only those containing both.

After the Search button is clicked, a box displays the number of hits found, and clicking on OK displays information about each file, in turn, that matched the search criteria. When the desired file is located, the corresponding spectrum is loaded into Nuts by clicking on the Load button. The information from the file tailer is automatically read, and peak labels, integrals and structures (if any) are displayed. To select another file from that search, use the View Search (**VS**) command. To perform another search, enter the DS command again.

These commands are available from the Tools menu.

DM -- Database Make

This command is active only with the optional Searchable Archive accessory, which allows the user to create and search a database composed of his own files.

Windows version -- This command takes all the NUTS files in the selected directory and constructs a text file from information saved in each file's tailer. This file can then be searched. This file includes pointers to the NUTS spectra, so that files matching search criteria can be loaded into Nuts. When the DM command is executed, a File Open dialog box is displayed. The user selects any file in the directory containing the Nuts data. All files to be included in the database must reside in that directory. The archive file has the default name _master.ndb, which can be renamed only after it is created.

Macintosh version -- Due to limitations of the Macintosh operating system and the Microsoft NT cross compiler for the Macintosh, the DM command does not operate in exactly the same way as the Windows version. The problem is that the Macintosh is not capable of creating a list of files in a folder in an automated way, which is used to create the searchable file. Instead, the user must first create a text file consisting of a list of the NUTS files, all saved in one folder, from which Nuts will construct the database. When the DM command is executed, a File Open dialog box is displayed. The user selects this text file, and then the command operates as for the Windows version. The default name for the archive file created by DM is [space]master.ndb (so that it will be displayed at the top of the list of files in that folder).

DS -- Database Search

This command is active only with the optional Searchable Archive accessory, which allows the user to create and search a database composed of his own files. The archive file is first created within Nuts using the Database Make (**DM**) command. Once the archive file has been created, it can be searched using **DS**. Show me how to search.

The DS command brings up a dialog box in which the user selects the database file created with the DM command. Then a screen is displayed in which the user can enter search criteria for searching that database file. It is possible to search by user name, date, compound name, comment line, molecular formula, nucleus, file name or chemical shift. With the exception of the chemical shifts, all searches are sub-string searches. For chemical shifts, a value is entered as well as a range. When an entry is made in the Name field, the search operation looks for matches in any of the 3 Name fields defined in the **IF** routine. Entries can be made in more than one field, which causes the search to find entries which meet all specified criteria (logical AND). Multiple entries can be made in the Name field, separated by a space, to search for a name containing multiple sub-strings. For example, entering "methyl phenyl" would search for occurrence of both methyl and phenyl in the name fields, and select only those containing both.

After the Search button is clicked, a box displays the number of hits found, and clicking on OK displays information about each file, in turn, that matched the search criteria. When the desired file is located, the corresponding spectrum is loaded into Nuts by clicking on the Load button. The information from the file tailer is automatically read, and peak labels, integrals and structures (if any) are displayed. To select another file meeting the search criteria, enter VS (View Search). To perform another search, enter the DS command again.

VS -- View Database Search

This command is active only with the optional Searchable Archive accessory, which allows the user to create and search a database composed of his own files. The archive file is first created within Nuts using the Database Make (**DM**) command. Once the archive file has been created, it can be searched using **DS**. VS is used to return to the list of files created by DS, without performing the search again.

Other Nuts commands which affect operation of the Searchable Archive include

Information Entry (IF) -- allows alternate sample or compound names and molecular formula to be entered, and subsequently saved in the file's tailer. This information is collected into the archive file by the DM command, and is searchable using the DS command.

MetaObjects subroutine (MO) -- This subroutine allows one or more graphical objects, such as a molecular structure, to be imported from a file (metafile for Windows, PICT file for Macintosh). A pointer to this file is subsequently saved in the file's tailer, and is incorporated into the archive file so that it is displayed when the spectrum is loaded following a search operation.

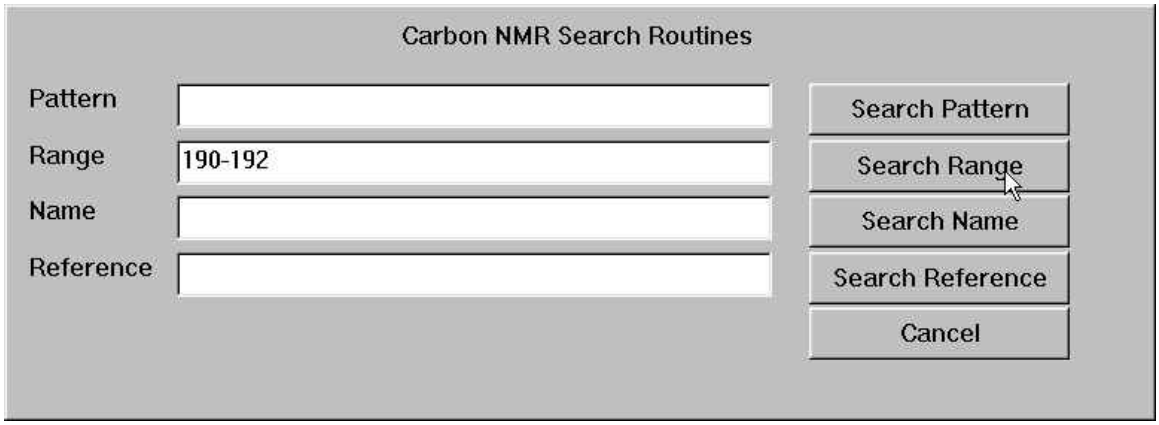
Define Peaks (DP) -- A list of peaks selected within the DP subroutine are saved in the file's tailer and is incorporated into the archive file. This allows searching by chemical shift. Note that peaks selected with the PP command are not saved in the file's tailer. Peaks must be selected using the DP subroutine.

Searching by chemical shift or substructure

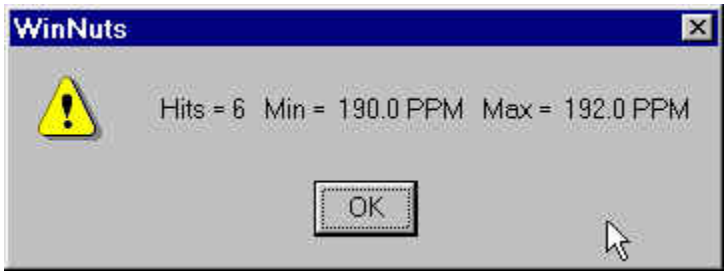
JJ - ^{13}C Chemical Shift Searching

This feature allows the user to search a small collection of ^{13}C spectral data based on substructure, chemical shift range, name (or part of a name) or reference number. The database resides in encrypted files installed with NUTS. The user can also create his/her own database which will also be searched with this command. See details below.

Typing **JJ** displays the following dialog box, allowing one of 4 different types of search criteria to be entered. The "pattern" option refers to a text-based system for describing substructure. The search is initiated by clicking on the corresponding button to the right. Only one criterion is searched, even if information is entered in other boxes. In the sample shown, we are searching for carbon peaks between 190 and 192 ppm, inclusive.



The following response is displayed, showing that the database contains 6 carbon peaks in this range:



Clicking on OK launches the web browser (Windows only) and displays the following report. (See below for how to configure NUTS to launch your browser.)

Carbon Chemical Shift Search Range Report

Carbon Shift Range is: **190.0 PPM - 192.0 PPM**

SHIFT	MOLECULAR FORMULA	REF #	NAME	PATTERN
191.4	C5H8O2	115	acetylacetone	5-APHJa
191.4	C5H8O2	115	acetylacetone	9-ALhl
190.0	C13H16O	454	2,2,3,3-tetramethyl-1-indanone	(9)-(D)aadVhd
191.3	C8H8O3	292	vanillin	9-HVhh
191.6	C9H10O3	346	3,5-dimethoxybenzaldehyde	9-HUhh
192.0	C7H6O	229	benzaldehyde	9-HVhh

Suppose we now want to know more about vanillin. We can perform another search, this time by reference number 292. The following report is generated.

Reference # Report

Reference # is: **292**

56.0	C8H8O3	292	vanillin	1-Ov

191.3	C8H8O3	292	vanillin	9-HVhh
127.4	C8H8O3	292	vanillin	10-HJaOa
109.4	C8H8O3	292	vanillin	10-HHPH
114.8	C8H8O3	292	vanillin	10-HHPH
109.4	C8H8O3	292	vanillin	10-HJhOaH
114.8	C8H8O3	292	vanillin	10-HJhOaH
129.5	C8H8O3	292	vanillin	10-JhHHP
147.5	C8H8O3	292	vanillin	10-OaHPH
152.3	C8H8O3	292	vanillin	10-PHOaJh

Vanillin has 8 carbons, but notice that the report has 10 entries. This is due to an ambiguity in the assignment of 2 of the peaks.

As an example of a substructure search, 3-AABc is entered and the Pattern Search button clicked. (3-AABc represents a methine carbon bearing 2 methyls and a CH_2-CH- group. See coding explanation.) The following response is displayed:

In this case, the database contains 5 examples of this substructure. NUTS displays the average shift of those 5 values, as well as the minimum and maximum values, giving the user an estimate of the possible shift range for this carbon. Clicking on OK displays the following report in the browser:

Carbon Pattern Report

Carbon Pattern is: **3-AABc**

SHIFT	MF	REF #	NAME	PATTERN
24.8	C6H13NO2	204	leucine (acidic)	3-AABc
24.8	C6H14O	214	4-methyl-2-pentanol	3-AABc
25.2	C6H13NO2	203	leucine (basic)	3-AABc
25.8	C60H92N12O10	499	gramicidin S	3-AABc
30.6	C12H24	445	7,9-dimethyl-1-decene	3-AABc

Searching by substructure can be done automatically if a molfile is displayed.

Configuring NUTS to work with a web browser (Windows only)

NUTS must first know where to find your computer's web browser. This is set in the nuts.ini file, in the [DIRECTORIES] section. Here are the default paths for WinNT and Win98. You must un-comment (remove semicolon from) the line corresponding to your OS, and verify that the path and file name are correct for your system.

```
; Default Directory for Window NT 4
```

```
BROWSER = C:\\Program Files\\Plus!\\Microsoft Internet\\iexplore.exe
; Default Directory for Windows 98
; BROWSER = C:\\Program Files\\Internet Explorer\\iexplore.exe
```

Creating a database of your spectral data

NUTS searches both the supplied, encrypted spectral data and also some user-editable files (with file names **!myshift.txt** and **!mynames.txt**). NUTS is supplied with sample files (shown here) that can be edited with any text editor. Simply follow the syntax shown. These files must be in the data subdirectory of the NUTS program.

```
MyShiftTable

; Users Carbon Pattern code and reference number list

;Code Shift Reference #
;1-Ban 23.8 515 ;This is a sample entry, remove seicolon
```

The syntax for the !myshifts.txt file is

`substructure_code shift reference#`
with each item separated by a space. The syntax for the !mynames.txt file is
`reference# molecular_formula name`

with each item separated by a space.

```
MyNamesTable

; User Reference Number and names list

;515 C6H15N diisopropylamine ;Sample entry, remove
semicolon
```

Substructure codes

^{13}C chemical shift searching is initiated with the command **JJ**. Searching from a displayed molfile is in development.

The substructure code is a simple, text-based system for classifying carbons by their type, nearest neighbors and next nearest neighbors. Each different type of carbon (methyl, carbonyl, phenyl, etc) is assigned a number. Its substituents are indicated by upper case letters, usually alphabetically. For each substituent, its substituents are, in turn, indicated by lower case letters, usually listed alphabetically.

For main groups and their substituents that are part of a ring, the number or upper-case letter is enclosed in parentheses. This does not apply to the ring systems designated explicitly (numbers 10-15). See examples below.

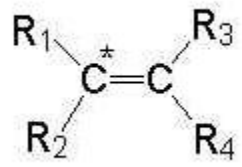
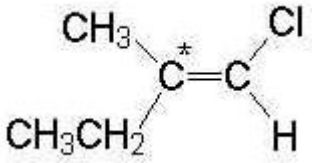
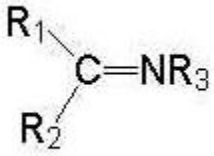
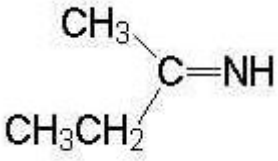
The following 2 tables show the numbers for each type of carbon, and the letters for each substituent.

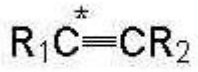
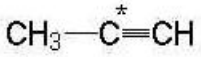
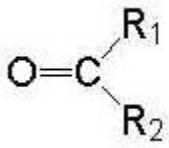
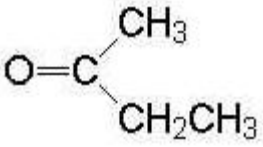
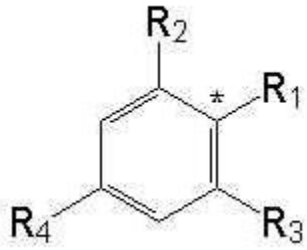
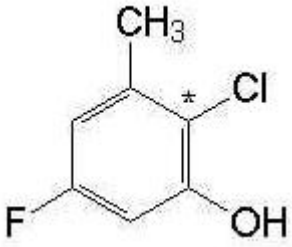
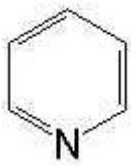
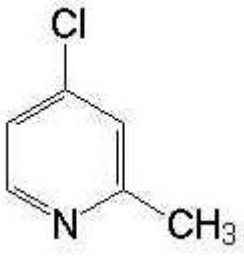
Partial codes can be entered for searching. For example, 2-B will return all CH_2s which have at least one CH_2 substituent.

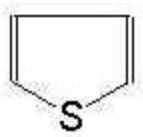
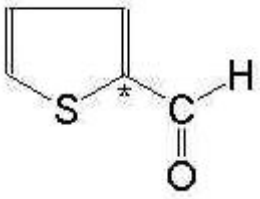
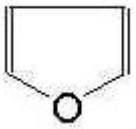
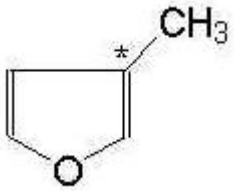
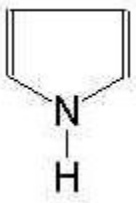
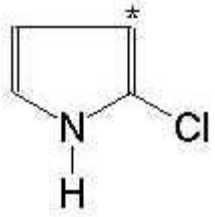
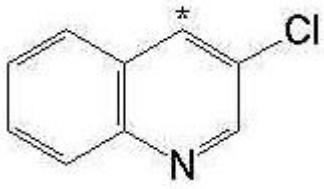
For olefin, phenyl and pyridine (types 5, 10 and 11, respectively), it is necessary to use * as a "wildcard". For example, 10-E*** will return all chloro-substituted phenyl carbons.

Try doing a Ref # or Range search first, and look at the codes returned to get a better idea of how the coding works.

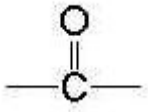
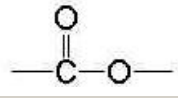
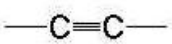
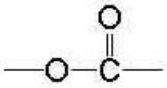
Main Groups

Group	Example	Code and Comments
1. CH_3--R_1	**CH_3**-CH_2-OH	1-Bp
2. CH_2--$R_{1,2}$	CH_3-**CH_2**-OH	2-AP list substituents alphabetically
3. CH--$R_{1,2,3}$	(CH_3-CH_2)$_2$-**CH**-OH	3-BaBaP list substituents alphabetically
4. C--$R_{1,2,3,4}$	(CH_3)$_3$-**C**-OH	4-AAAP list substituents alphabetically
5. 		List substituents starting with directly bonded groups, alphabetically, then gem, cis and trans to the first group, in that order *5-ABaEH and 5-EHABa
6. 		List directly bonded substituents first, alphabetically, then N substituent 6-ABaH for C=N carbon methyl: 1-Mb ethyl: 1-Bm and 2-AMa

7. 		List directly bonded substituent first *7-AH and 7-HA methyl: 1-Th
8. R--CN	CH_3-CH_2-**CN**	8-Ba for the cyano C ethyl: 1-Bm and 2-AM
9. 		9-ABa for the carbonyl methyl: 1-Jb ethyl: 1-Bj and 2-AJa
10. 		List directly bonded substituent, then ortho substituents, alphabetically, then para substituent *10-EAPF The other ring carbons are, clockwise: 10-PEHH 10-HFPA 10-FHHE 10-HAFP 10-AEHH
11. 		List directly bonded substituent first, followed by ortho substituents, alphabetically. (The report will include a lower case letter (a, b or g) following the number indicating alpha, beta or gamma. Don't use this a, b or g in searching.) 11-AH for C alpha to N 11-HAE for C beta to N 11-EHH for C gamma to N report will show 11-a-AH

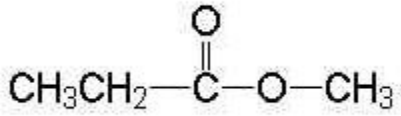
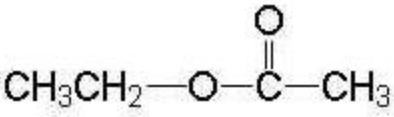
		11-b-HAE and 11-g-EHH
12. 		*12-JhH List directly bonded substituent first, followed by ortho substituents, alphabetically. The report will include lower case letter (a or b) following the number indicating alpha or beta (12-a-JhH in this case).
13. 		*13-AHH List directly bonded substituent first, followed by ortho substituents, alphabetically. The report will include lower case letter (a or b) following the number indicating alpha or beta (13-b-AHH in this case).
14. 		*14-HEH List directly bonded substituent first, followed by ortho substituents, alphabetically. The report will include lower case letter (a or b) following the number indicating alpha or beta (14-b-HEH in this case).
15. Miscellaneous aromatic		*15-(quinolyl-4)-H List directly bonded substituent only. Indicate ring system and position after number, in parentheses.

Substituent Groups

A. --CH_3	J. 	S. --S--
B. --CH_2--R_1	K. 	T. 
C. --CH--$R_{1,2}$	L. --C=	U. --CN
D. --C--$R_{1,2,3}$	M. --C=N	V. phenyl
E. --Cl	N. --N=	W. Misc. aromatic
F. --F	O. --O-- or --O-->	X. P
G. --Br	P. --OH	Y. Si
H. --H	Q. 	Z. Misc. atom
I. --I	R. --NO_2	

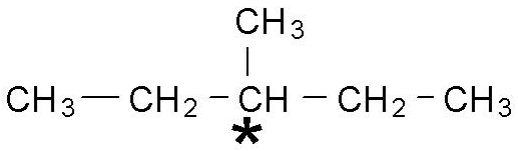
For a phenyl substituent, V, list its two ortho substituents in lower case letters, alphabetically.

Note that K and Q are different, as illustrated below:

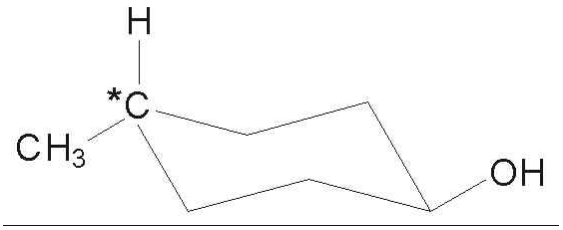
	Ethyl group codes are 1-Bk and 2-AKa Carbonyl code is 9-BaOa Methyl code is 1-Qb
	Ethyl group codes are 1-Bq and 2-AQa Carbonyl code is 9-AOb Methyl code is 1-Kb

Cyclic structures

Some rings are encoded explicitly (groups 10-15, above). Other rings are indicated using the codes described above, but using parentheses to indicate that the atom is part of a ring. Only main group numbers and nearest neighbors (upper case letters) are so indicated.



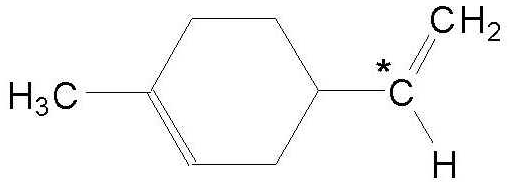
The CH is coded as 3-ABaBa (A for methly, Ba for each CH_2-CH_3)



The indicated CH has the similar substituents as above (one methyl, 2 methylenes) but they are now in a ring. The CH is encoded as (3)-A(B)b(B)b Note that lower case letters are not in parentheses.

If a methylene is in a ring, by definition, both of its substituents are in the ring. Methyls can never be part of a ring.

Another example:



The indicated carbon is coded as 5-(C)bbHHH because the attached CH is part of a ring. The olefinic CH_2 is 5-HHH(C)bb

The other carbons in this molecule are coded as:

methyl 1-(L)bl

Ring carbons, starting with C bearing methyl and proceeding clockwise:

(5)-A(B)bH(B)c olefinic Cs are coded with attached, gem, cis and trans, respectively

(2)-(B)c(L)al

(2)-(B)l(C)bl

(3)-(B)b(B)lLhl C bearing the olefinic substituent

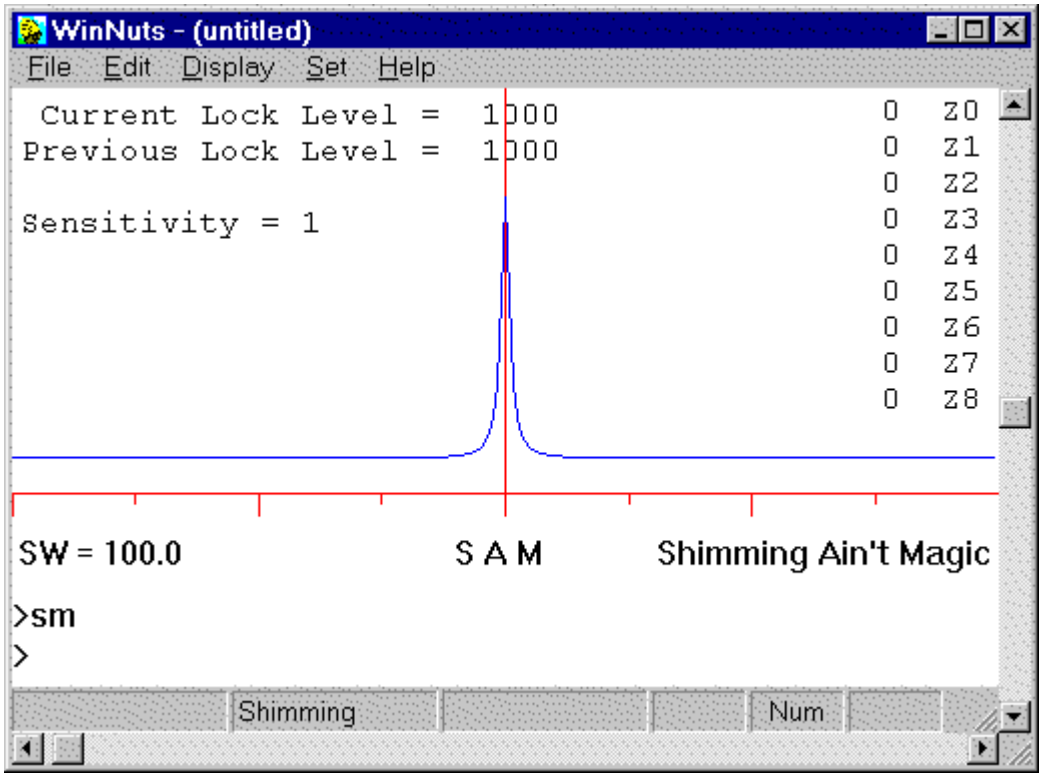
(2)-(C)bl(L)hl

(5)-(B)cH(B)bA

Shimming Simulation

SM - Shimming Simulation

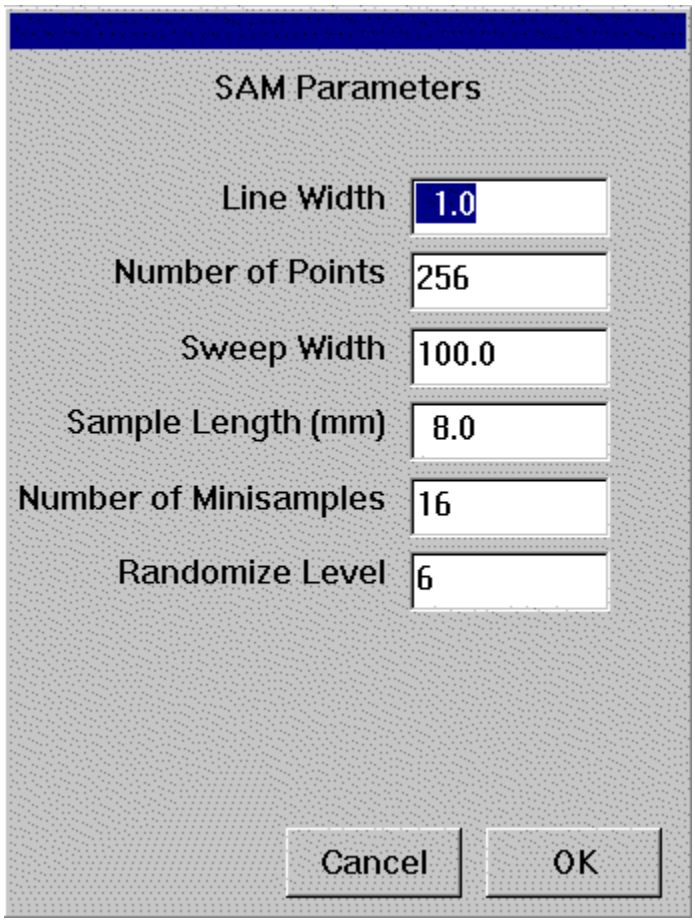
This command enters a subroutine which allows the user to practice adjusting the on-axis (Z) shims under ideal conditions. The "sample" consists of a single peak. By default, the peak is exactly on resonance, but this can be changed by adjusting the Z0 shim. The value of each shim gradient is displayed in the upper right portion of the screen. A value for the current lock level is displayed at the upper left, along with the lock level corresponding to the previous set of shims, so the user can easily gauge the effect of a shim adjustment. Perfect shims will give a lock level of 1000.



Each shim value is changed using the number keys on the keyboard -- each time a number between 0 and 8 is pressed, the corresponding shim is changed by a set increment. By default, the increment is 5 units. To change to larger step sizes, use the greater than key (>) to increase the Sensitivity, which applies a factor to the increment

size. (It is also possible to change the increment; see below). The number keys apply a positive increment. Holding down the shift key while typing a number applies a negative increment. These and other subcommands are described below.

Several parameters can be adjusted by selecting Edit Parameters from the Edit menu, which displays the screen shown below.

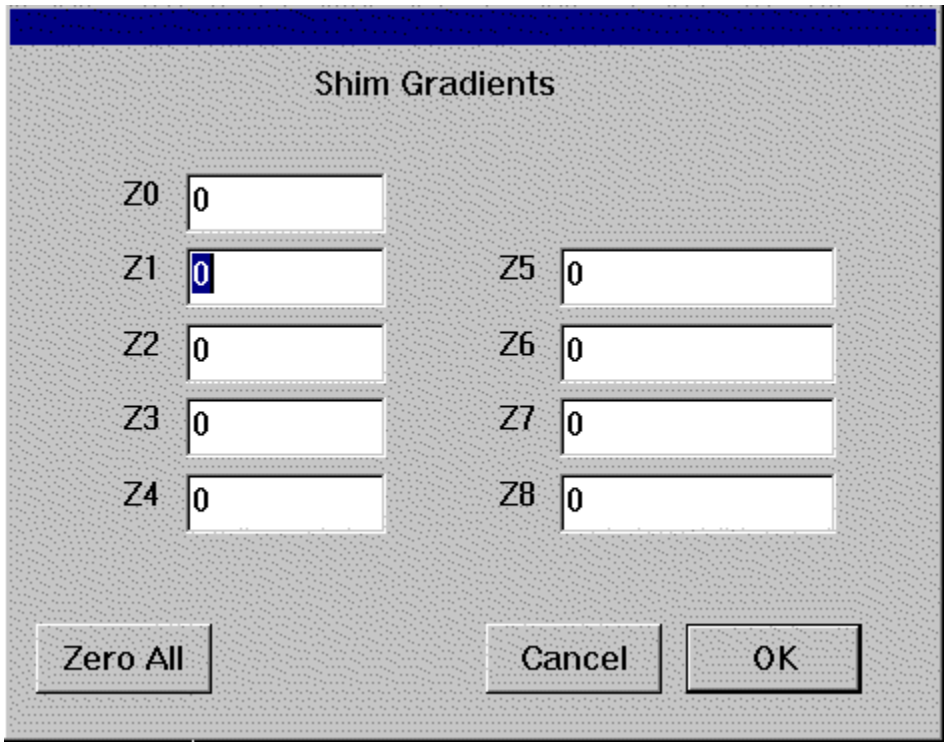


The lineshape is calculated as the superposition of "mini-samples" located along the length of the detector coil. In the case of a very broad resonance due to poor shims, the peak shape can become distorted because of this "discrete" approximation. A series of small, individual peaks can be seen when the signal should really be a continuous curve. This can be corrected by ssing a larger number of minisamples, but this will slow the calculation.

Number of points refers to the number of data points used in the Fourier Transform.

Randomize level refers to the highest order magnet gradient which is randomized by the ? command for practice shimming. By default, all gradients up to and including Z6 are randomized, but this can be changed to make the shimming problem easier (lower value) or harder (higher value).

A specific value for any shim can be entered directly, as an alternative to adjustment by increments. Choosing Set Gradients from the Set menu displays the screen below.



Test yourself with the Shimming Game

To simulate an imperfect magnet, whose gradients must be corrected using shims, select Randomize from the Set menu (or type ?). The resulting lineshape will be poor, and the lock level will be low. The goal is to obtain a narrow, symmetrical peak and a lock level of 1000, corresponding to a perfectly shimmed magnet. The values of the magnet gradients can be displayed by selecting Toggle Answer from the Display menu (or type :). The value for each shim needed to compensate perfectly for these gradients is the same absolute value as the corresponding magnet gradient, but the opposite sign. When the answer is displayed, a score is also shown, calculated from the elapsed time and percent lock level recovery, which are also displayed. Maximum possible score is 100,000.

A very useful tool for shimming under these conditions is the Z1 profile, described in the Hardware section, below.

Subcommands:

 # (0-8) Increment corresponding shim, Z0 - Z8
shift-# Decrement corresponding shim, Z0 - Z8

? Randomize magnet gradients
: Display magnet gradient values
F Display FID
S Display spectrum
E Edit parameters
G Set shim gradients
I Set increments for each shim adjustment
Z Zero magnet gradients
[Apply negative Z1 gradient
] Apply positive Z1 gradient
= Remove Z1 gradient
> Increase sensitivity -- increases multiplication factor for shim increments
< Decrease sensitivity -- decreases multiplication factor for shim increments
<ENTER> Exit shimming subroutine

A detailed description of shimming, symptoms of field inhomogeneity and factors that affect shimming can be found in the section on Shimming, below.

Virtual Spectrometer

Overview

The Virtual Spectrometer module in NUTS consists of a set of commands which simulate an FT-NMR spectrometer, producing realistic data corresponding to acquisition parameters set by the user. It is designed to mimic a "vanilla" NMR spectrometer, rather than imitating any actual manufacturer's instrument. In addition to the functions which are specific to the Virtual Spectrometer, all of the processing capabilities of NUTS are also available. Commands specific to the Virtual Spectrometer are available from the vSpec menu.

As with the rest of the NUTS program, most commands can be executed either from the menu or as keyboard commands, which are 2-letter commands executed without typing <Enter>.

The virtual NMR "sample" is a text file containing some parameters and a list of NMR frequencies and their intensities. Several sample files are provided (as a zip file for Windows and .sea file for Mac), and a sample file is shown below which includes an explanation of the different items in these files. The file must contain certain keywords which allow the Virtual Spectrometer to interpret correctly the data being read from the file. The file is most easily created from within the NUTS NMR simulation subroutine, but can also be created from scratch or edited using any text editor.

The user must set values for several NMR acquisition parameters as well as select the sample whose spectrum will be acquired. The sample is chosen using the **GS** command or by selecting Get Sample from the vSpec menu. This opens a standard dialog box for

specifying the name of a file to be opened. The file itself is not opened or shown at this time; this is simply the process used to retrieve the name of the file which will be read when acquisition is started. To set acquisition parameters, type **VP** or select Parameters from the vSpec menu. This opens a dialog box for input of parameters.

Once parameters are entered, the dialog box is closed and data acquisition initiated by typing **ZG** or selecting Zero and Go from the vSpec menu. Messages are displayed on the screen as acquisition proceeds, and the accumulating FID is displayed. If the user's PC is equipped for sound, the FID will be "played" through the PC's speakers (Windows only). Upon completion of data acquisition, the data can be processed using the NUTS processing capabilities.

A Tutorial is available as a Word document, and illustrates use of the Virtual Spectrometer.

Commands for the Virtual Spectrometer

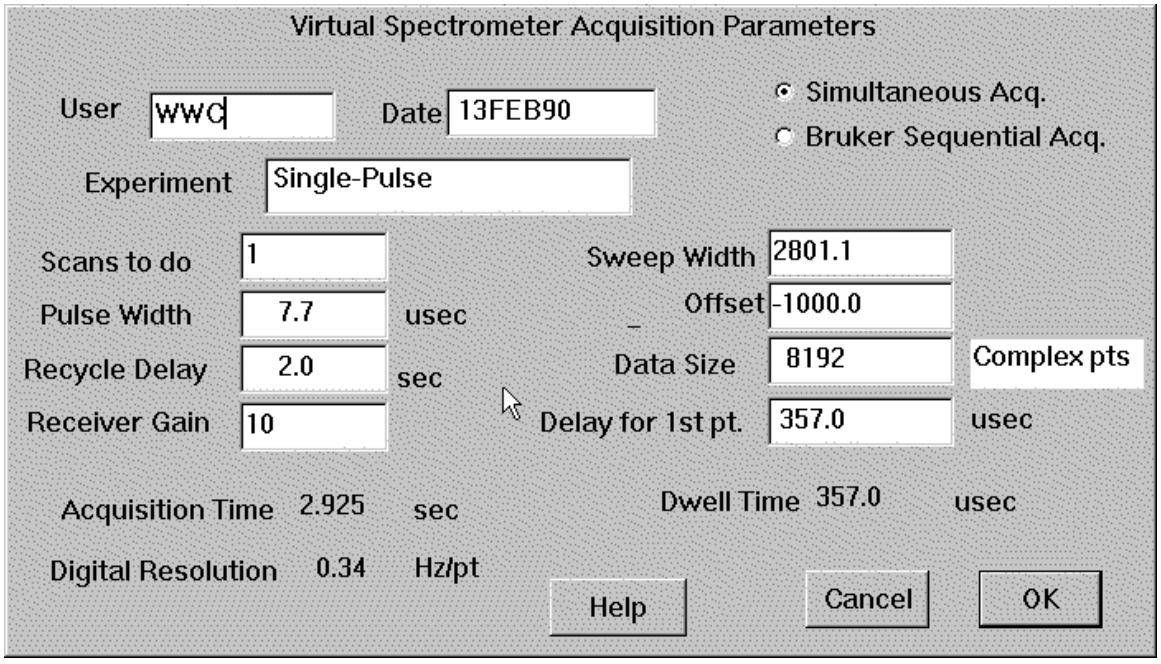
GS - Get Sample. Specifies the file from which frequencies will be read

ZG - Zero & GO. Reads file containing freqencies and generates the NMR data.

VP - Virtual Parameters. Opens a dialog box to set acquisition parameters.

Virtual Spectrometer Parameters

Acquisition parameters are input using the dialog box shown below, which is activated by typing VP or by selecting Parameters from the vSpec menu. A brief explanation of each item is shown below.



Descriptions below explain how the setting of each parameter will affect the spectrum.

User: Enter user's name or initials (for information only; does not affect data acquisition)
Date: for information only; does not affect data acquisition
Experiment: Select from the list of available experiments. Currently the only option is Single Pulse.
Scans to do: Enter the number of scans (acquisitions) which will be collected and added to form the final spectrum.
Pulse Width: The excitation pulse width in microseconds.
Recycle Delay: Delay between repeat scans to allow relaxation, in sec.
Receiver Gain: Adjusts the amplitude of the signal going into the receiver.
Spectral Width: Frequency range for the spectrum.
Spectrum Offset: Frequency of the "carrier", or center of the spectrum.
Data Size: Number of data points to be acquired.
Delay for first point: In microseconds.

The following values are displayed for information only, and cannot be changed directly.

Acquisition Time: The time required to complete a single scan, dependent on the Spectral Width and Data Size.
Digital Resolution: The number of data points per Hertz which will define the final spectrum.
Dwell Time: The time between data points, in microseconds. This is determined by the value chosen for Spectral Width.

Virtual Spectrometer Parameters

Pulse Width

Maximum signal is obtained when the excitation pulse is turned on for exactly the length of time necessary to rotate the net magnetization vector 90 degrees, referred to as a 90-degree pulse. This rotates the magnetization from its initial position aligned along the magnetic field (designated as the z-axis) to a position perpendicular to the z-axis, in the x-y plane. The pulse length necessary to do this is dependent on the each instrument's hardware, such as the transmitter power and the efficiency of the detector coil in the NMR probe. It must therefore be determined empirically and will vary slightly from day to day. Typical values are 5-15us.

The NMR operator must decide what fraction of the 90-degree pulse, commonly referred to as the "tip angle", will be used to generate the NMR signal. For a single-pulse experiment, as is used to acquire a basic spectrum, there is no value in exceeding a 90-degree pulse. So the choice for values of pulse width are from zero up to the 90-degree pulse.
The obvious question is: Why not always use a full 90-degree pulse? More is better, right? The answer is, not always. For a concentrated sample, a smaller pulse angle might

be necessary to keep the signal from overloading the receiver, which leads to artifacts in the spectrum. In addition, some of the most valuable information which can be obtained from NMR is quantitative. For this data to be reliable, the spectrum must be obtained under conditions which result in peaks whose integrated area is proportional to the number of nuclei which each peak represents. The complication is that different nuclei relax at different rates. Any nuclei which have not fully relaxed by the time the next excitation pulse is applied will give a signal with somewhat reduced intensity. The result is a spectrum with distorted integration. Therefore, the factors which must be considered are the concentration of the sample and the relaxation time of the nuclei under observation. A relaxation delay and/or smaller pulse angle may be necessary to ensure complete relaxation between scans. As with many aspects of NMR, the choice is a tradeoff and requires consideration of which factors are most important for the case at hand.

The easiest way to determine the 90 degree pulse length is to determine a 180 degree pulse length and divide by 2. A 180 pulse will give zero signal, and is very sensitive to small offsets from the exact value. It is much easier to determine zero signal than to determine the maximum, as it is a fairly broad maximum, making it difficult to distinguish among values that are close to the maximum. So, start with a small pulse length and increase it, viewing the signal. The signal amplitude will go through a maximum and back to zero as the pulse length is increased from 0 degrees to 180 degrees.

Carrier frequency

The center of the spectrum is determined by the carrier frequency, which is the sum of the Spectrometer Frequency (in MHz) and the Spectrum Offset (in Hz). On many spectrometers, the Spectrum Offset varies depending on the deuterated solvent being used for the Field-Frequency lock, and therefore changes from sample to sample. If the Spectrum Offset is set incorrectly, some peaks of interest may fall outside the the Spectral Window, which then appear "folded".
As the Offset value is increased, the spectral window is moved to lower field, which is to the left as the spectrum is normally viewed. Therefore, peaks will appear to move to the right.

Data Size

The number of data points which will be acquired. The algorithm used to perform a Fourier transform requires the number of data points in the time domain function (FID) to be a power of 2. For this reason, most spectrometers, including the Virtual Spectrometer, limit the choices for data size to be a power of 2. If a value is entered which is not a power of two, the value will be changed to the next higher power of 2.
To accomplish quadrature detection (which provides the ability to distinguish positive and negative frequencies), data are acquired in 2 channels, related by a 90 degree phase shift. Therefore, the data exist as 2 halves, usually referred to as real (in-phase) and imaginary (90 degree phase shifted) parts. Because these 2 halves are really complex

pairs of data points, the NUTS program expresses the number of data points as the number of complex pairs, but that is not true of all spectrometers. Some spectrometers give the data size as the total number of points (the sum of the number of points in the 2 channels). In that case, the number of points which will define the final spectrum will be half of the total data size. For example, starting with 4K (4096) total points (2K real and 2K imaginary) yields 2K points in the final spectrum. By contrast, NUTS considers this size of data to be 2K complex. This is of concern when calculating the data size required to yield the desired digital resolution.

DE delay

The digitizer, or Analog-to-Digital Converter (ADC), digitizes the signal coming from the NMR probe at a rate determined by the user's chosen value of Spectral Width. The time between data points is called the Dwell Time, equal to the reciprocal of the spectral width. However, signal acquisition is not started immediately after the excitation pulse, but after a small delay, necessary to allow the circuitry to recover from the effects of the intense excitation pulse. By default, most spectrometers (including the Virtual Spectrometer) set the DE value equal to the dwell time, meaning that a delay of 1 data point is used.
However, this is not usually the optimum DE setting. This is because the filters used to eliminate frequencies outside the chosen range cause a finite delay in the signal reaching the ADC. DE must be empirically chosen to match the delay introduced by the filters. When the DE value does not match the filter delay, baseline distortions (baseline "roll") are seen in the spectrum which make phasing and integration more difficult. If the DE value is too low, the baseline will have a hump ("frown") and the first-order phase value required to phase the spectrum will be negative. If the DE value is too high, the opposite is true: the baseline will have a dip ("smile") and the first-order phase value required to phase the spectrum will be positive. At the optimum DE value, the baseline will be flat and the first-order phase value required to phase the spectrum will be zero.

Number of Scans

The signal to noise ratio of a spectrum can be increased by repeating scans and adding the data. Signal from the sample will add with each repeat scan. Noise, which is random in phase, will add at a slower rate. Therefore the signal to noise ratio increases as the square root of the number of scans; eg, four times the number of scans is required to double the signal to noise ratio.

Receiver Gain

This controls an amplifier through which the signal passes just before it reaches the receiver (analog-to-digital converter, or ADC). This should be adjusted to the maximum amplitude that the ADC can handle without overloading it. If the signal amplitude is too low, small signals will not be discernible. If the ADC is overloaded, the signal will be truncated, or "clipped", resulting in distortion of the resulting spectrum. This can have the appearance of an undulating baseline, in the case of a small degree of clipping, or

spurious signals in the case of more severe clipping. The optimum setting can be determined by increasing the gain until clipping is observed and then reducing it from that value. In the case of NUTS, the cutoff occurs at about +/- half of full screen.
Note that receiver gain affects signal and noise equivalently, so will not affect the signal/noise ratio.

Relaxation Delay

This is the delay between the end of acquisition of each scan and the next excitation pulse. For nuclei with long relaxation times, more time must be allowed between successive excitations to permit the magnetization to return to equilibrium. Distortions of the spectrum, including distorted integration, results from too short a relaxation delay. On the other hand, one does not want to waste time in acquiring data. Another trade-off.

Spectral Width

This sets the range of frequencies which will be observed. In the case of NUTS, this is the size of the entire range, from one end to the other. (Note that some instruments set this parameter to be +/- on either side of center.) It must be set large enough to include all peaks of interest, but too large a setting reduces digital resolution and results in "wasting" data by acquiring regions which include only noise. Peaks which fall outside the spectral width, or "window", will be partially filtered out by the spectrometers filters, but not totally. They will appear "folded" or "aliased" into the spectrum, meaning that they appear at a frequency which is not their correct value. This can be detected by the appearance of peaks at anomalous frequencies and by the fact that folded peaks are often out of phase when all other peaks are phased correctly. The most foolproof way to determine the correct spectral width is to start with a value that is much larger than estimated, to determine where the peaks are, then reduce the value to encompass all peaks.
The spectral window is centered at the frequency of the NMR transmitter, commonly referred to as the "carrier" frequency.

Digital Resolution

This is the quotient: Spectral width / number of data points, expressed as Hz/pt. The digital resolution must be great enough (Hz/pt value small enough) compared to the width of the lines being observed, to define and resolve narrow peaks. Usually, the spectral width is fixed by the range of frequencies being observed. Therefore, the only parameter which can be varied is the number of data points. Acquiring more data points requires more time. We again have a trade-off between enough points to adequately digitize the NMR signal, but not so many points that time and disk space are wasted.

FID - Free Induction Decay

FT-NMR data is collected as a function of time following an excitation pulse. This time-domain data is referred to as a Free Induction Decay, or FID. It consists of a sum of

sinusoids oscillating at different frequencies, one for each peak in the spectrum. The signal decays to zero as a function of time, as the excited spins relax back to equilibrium.

Acquisition Time

The time required to collect data points for each FID. Acquisition time = (number of data pts) / spectral width.

Virtual samples

Virtual Spectrometer Sample File

The following is an example of a text file which is a "virtual NMR sample", which is read by Virtual Spectrometer when acquisition is initiated. It specifies several parameters of the sample and contains a list of NMR frequencies and their intensities.

Sample files for input into the Virtual Spectrometer are most easily created from within the NUTS simulation subroutine. This subroutine allows input of chemical shifts and coupling constants corresponding to real NMR data and calculates the NMR spectrum. The result of the calculation is a list of frequency and intensity values. This list can be saved as a text file which also includes several parameters which define characteristics of the sample and the spectrometer, specified with keywords, which the Virtual Spectrometer reads.

The components of the file that the Virtual Spectrometer reads are Spectrometer Frequency, PW90, Concentration, EBsensitivity, Intensity_Per_Spin (used to adjust for the arbitrary intensities created by the NS routine), plus frequency (in Hz) and intensity for each peak. If the parameter values are not present in the file, the program will use default settings.

```
NUTSsimulation
Sample Name = Unknown #1
Spectrometer Frequency = 300.149994 MHz
Concentration = 5.0
EBsensitivity = 120.0

PW90 = 10.0
Intensity_per_spin = 31.9870

Number Transitions(Hz) Transitions(PPM) Intensities
1       2101.0503      7.0000          32.000
2       786.4559       2.6202          3.779
3       786.2332       2.6195          3.785
4       778.6384       2.5942          3.918
5       778.5466       2.5939          3.921
6       778.4114       2.5934          7.773
7       778.3357       2.5932          7.852
8       770.7247       2.5678          4.067
9       770.6700       2.5676          4.069
10      770.5101       2.5671          8.147
```

```
11      770.4384        2.5668          8.151
12      762.7601        2.5413          4.227
13      762.5414        2.5405          4.234
14      359.1456        1.1966          6.234
15      359.0011        1.1961          12.454
16      358.8564        1.1956          6.221
17      351.1756        1.1700          23.999
18      351.0389        1.1695          23.974
19      343.3527        1.1439          5.785
20      343.2066        1.1435          11.558
21      343.0524        1.1429          5.773
End NUTS Simulation File
```

Importing data

Data translation

IM – Import data

Before a FID or spectrum can be processed by NUTS, it must be translated into the NUTS file format, called the Common Data File format. Most files can be translated from within NUTS with the Import command (IM). **IM** automatically detects the origin of the file and applies the appropriate translation. Import is also available from the File menu.

The What's New text file supplied with NUTS will list new and modified imports.

Nicolet 1180 and 1280 Dexter NT spectrometer data files
Nicolet/GE TMON data files from NT, QE and GN spectrometers
GE Omega spectrometer files exported with the GE Export command
Bruker Aspect 3000 data files
Bruker X32 and UNIX data files, including digitally filtered data
Varian VXR 5000, Unity and INOVA data
JEOL Delta, GX, EX, FX, Lambda, ALS and Alpha data files
Chemagnetics files
WinNMR files
Tecmag MacFID, MacNMR and NT-NMRfiles
ATI ASCII and binary files
SMIS data files
Analogic files
PC-NMR files in Lybrics format
Process Control Technology data files
Felix new format
NMRi format
ASCII format -- data should be in the form of (real, imaginary) pairs
JCAMP format
Galactic (Grams-32) files
AZARA
Elcint
Philips MRi Spectroscopy Data - Type II
Siemens

It is possible to bypass the automatic translation process and "force" a specific type of translation. This is not generally recommended because it is easy to select the wrong type, which can cause NUTS to hang or crash. In all but rare cases, it is best to do IM and let NUTS do its own auto identification process.

If you MUST force a type, the user can enter the non-2-letter command mode and enter a command like:

IM -type "force_type"

where allow words for "force_type" are:

NTDEXTER
NTTMON
GN
QE
NTDEXTER_NICNET
NTTMON_NICNET
GN_NICNET
QE_NICNET
OMEGA_ONE_FILE
OMEGA_TWO_FILE
BRUKERASPECT
FELIXNEW
ASC
CDFFDOS
CDFFUNIX
JEOL
JEOL_GENERIC
ATI
BRUKERASPECTBS
BRUKERETHERNET
SMIS
VARUNIX
BRUKERUNIX1
BRUKERUNIX2
BRUKERUNIXSGI1
BRUKERUNIXSGI2
BRUKERX32_1D
BRUKERX32_2D
BRUKNET
PCT
FXQ
ANALOGIC
LYBRICS
CHEMAGNETICS1
CHEMAGNETICS2
CHEMAGNETICS3
GALACTIC
IRIS_FELIX
CDFFINT
MACFID1
MACFID2
MACNMR1
MACNMR2
GEMS

FLXAII
NMRI
PHILIP_MR
JEOL_LAMBDA
WINNMR1DOF (Bruker WinNMR 1D Old Conversion FID)
WINNMR1DOS (Bruker WinNMR 1D Old Conversion Spectrum)
WINNMR2DOF (Bruker WinNMR 2D Old Conversion FID)
WINNMR2DOS (Bruker WinNMR 2D Old Conversion Spectrum)
WINNMR1DNF (Bruker WinNMR 1D New Conversion FID)
WINNMR1DNS (Bruker WinNMR 1D New Conversion Spectrum)
WINNMR2DNF (Bruker WinNMR 1D New Conversion)
WINNMR2DNS (Bruker WinNMR 1D New Conversion)
WINNMRAQS_FID (Bruker WinNMR using *.aqs file)
WINNMRAQS_R (Bruker WinNMR using *.aqs file)
WINNMRAQS_I (Bruker WinNMR using *.aqs file)
WINNMRAQS_SER (Bruker WinNMR using *.aqs file)
WINNMR1D97 (Bruker WinNMR 1D year 1997)
WINNMR2D97 (Bruker WinNMR 2D year 1997)
BRUKER_24_to_32 (Bruker Aspect transfers with LightNet to X32)
BRUKER_1R (Bruker Real Only data)
JEOL_ALS
JEOL_ALPHA
JEOL_DELTA
JCAMP
XWINNMR
TECMAG_NT
AZARA
ELCINT
PHILIPS2 (Philips MRi Spectroscopy Data - Type II)
SIEMENS

Varian data from XL and older Gemini spectrometers, based on the older computer, is translated into the NUTS format using a separate translation program called V_Helper, written by Steve Silber at Texas A&M. This program allows a PC to read a Varian floppy despite its foreign format.

It is usually best to use the auto-detect capability of the **IM** command and let NUTS identify the file type. It is possible to force NUTS to apply a specific translation, regardless of the file's origin, by selecting the desired file type from the File / Import menu. However, this can result in errors, including termination of the NUTS program, if the chosen format does not match the actual file type.

See details about how NUTS identifies different file types. Some spectrometers create multiple files for one data set, and the user must be careful to transfer all relevant files to the PC. In some cases, this involves creation of multiple layers of subdirectories, and it is

important to create the subdirectories correctly on the PC, or NUTS may fail to locate part of the data.

For detailed information about the file format, see CDFF.

Fortran code to write data in NUTS format (contributed by Todd Alam)

Data files can be displayed using the look (LO) command, useful for "hacking" apart data files.

IM -- Import File

Performs data translation "on the fly" and opens the translated file. NUTS automatically detects the source of the file and applies the appropriate translation.

For data from Unix computers, in most cases, multiple files are created when the spectrum is acquired. A directory is created bearing the file name entered by the user, into which all relevant files are placed. NUTS expects this file structure when it searches for the necessary files, so when files are transferred to the PC, the directory structure created on the PC must match that of the original data.

There are also some issues specific to Bruker data which may be important.

If a File Open (GA) command is attempted on a file which has not been translated into the NUTS format, a message appears asking if an auto-detect import should be attempted. If an import operation is to be included in a Link, use the GA command and not IM. The reason for this is when the GA command is used in a Link, the user is prompted for the file name only once. NUTS is smart enough to perform an import if necessary.

When IM is used, the original file remains unchanged with its original name. The translated file is given a file name consisting of a dollar sign ($) appended to the beginning of the original file name. This file is then loaded into NUTS and becomes File A. For 2D data, the translated file is always saved to the disk. For 1D files, whether or not the translated file is automatically saved to the disk depends on which version of NUTS is being used. It is saved in the Win95/NT version only. This file name is printed at the top of the NUTS screen and is printed on all plots. Because DOS can only handle files with file names up to 8 letters, appending an extra character to the file name can create problems for file names of greater than 7 letters (not including the extension). The user can always save the file under a new name with SB or chose Save As under the File menu.

The default directories for file importing (with IM) and for the file Open and Save operations (eg, GA and SA) can be different and can also be set in the NUTS.INI file. (Note that this does not work correctly on the Mac.) The translated file is saved in the directory currently being used for Open and Save operations. The current settings for these directories can be viewed with the WP command.

ZZ - Auto importing

This command imports a file with a default file name, as defined in the NUTS.ini file. This is useful in cases where the file to be opened always has the same file name and path name, as might be the case with some file transfer programs. The file name, with full path name, and the file type are defined in the NUTS.ini file. The relevant lines in the NUTS.ini file are shown in the examples below:

ZZ_FILE_PATH = C:\NUTS\DATA\FILE.QE

ZZ_FILE_TYPE = Lybrics

The path name must be the full path name. If ZZ_FILE_TYPE = Auto is used the imported file will be auto identified using NUTS standard file detection scheme.

BS -- Byte Swap

Used mainly for debugging data import operations. Reverses the byte order of floating point data. Data from some spectrometers requires this operation.

BV -- Byte Swap to VAX byte order

Used mainly for debugging data import operations. Reverses the byte order of floating point data. Data from some spectrometers requires this operation.

CX -- Convert data

Used mostly in the course of identifying and debugging data import problems. Allows selection of one of 3 options for imported data:

Byte swap for floating point data
Convert data from integer to floating point
Convert data from integer to floating point with byte swap

These can be useful to try when data does not appear to have imported correctly.

LO -- Look at data file

This is a keyboard command that displays the contents of a specified file, useful for "hacking" apart data file formats. The user specifies a format from the following choices, which controls how the file's contents will be displayed. These choices are available from the Display menu or by typing **T**.

Binary
CDFF (the format for NUTS files)
Unix

Macintosh
Aspect
1280

The default starting position for displaying the file is at zero, the beginning of the file. This can be changed from the Edit or Display menu, or by typing P. Scrolling up and down through the file is possible using the Page Up, Page Down and arrow keys. The currently displayed information can be copied to the Windows clipboard using Control-C or from the Edit menu. A new file can be opened from the File menu or by typing F. To exit this subroutine, type<Enter> or choose Exit from the File menu.

SE -- Visually inspect data file

This command was created to aid in the visual inspection of unknown data files. In the 2-lettered command mode, the command **SE** first brings up a dialog box and allows the user to select the file to look at. After this selection, another dialog box comes up which allows the user to specify how he wants to view the data. After selecting all the options and clicking OK, the data is imported without translation in that manner as a 1D file thereby allowing the user to visually "see". This can aid if finding actual NMR data in unknown files. Hint – It is often useful to first load an NMR file with NMR data of about the vertical scale size the user expects and then do an FS to fix the scale. This allows all the "junk" to be imported and go off-scale without shrinking smaller NMR data so small as not to be seen.

In the non-2-lettered command mode, the SEE command with no arguments behaves as above. The user can give arguments as shown below to directly import the specified file as instructed in the arguments:

SEE [Bytes2Skip] [WordType] [Complex] [Endian] [FileName]

where:
Bytes2Skip is the number of bytes to move into the file before starting to read the data
WordType is one of CHAR / SHORT / INT / FLOAT / DOUBLE
Complex is one of REAL / COMPLEX
Endian is one of BIG / LITTLE
FileName is the name of the file to import.

Importing details

Identifying foreign data files

The criteria by which NUTS identifies the origin of NMR data are as follows, ***although the list is not complete***, because new import types are added frequently. See also the section on data importing.

ATI Reads first line of an ASC header file. All characters are read in an ASCII text line until "\n" is found or the first 80 characters are read. If the first two words are FILE and VERSION (case insensitive) the file is identified as ATI.

Analogic data usually comes as two separate files, one with a base name and an extension of "SPC" and another with the same base name with the extension "ANM". The SPC file contains a 256 header and the binary data. This header contains some file information. If the second byte of the selected file (usually *.SPC) is 4D hex AND the second file (*.ANM) is present then identified as an Analogic file. Some additional parameters are obtained from the *.AMN file.

ASCII data is assumed to be in the format of (real, imaginary), with one pair of data points per line. A few basic parameters will be read if correct keywords are found. To import ASCII data, it is necessary to specify that the data is ascii, because there are no unique features in the file that NUTS can use to identify the data as ascii. This is done in the non-2-letter command mode with this series of commands:

2f
im -type asc <ENTER>
2n <ENTER>

NUTS expects the data to be in the form of (real, imaginary) pairs, one pair of numbers per line, but can handle other formats, as follows. If 3 numbers are found per line, NUTS will ignore the first number, and will import the second and third numbers as real and imaginary values, respectively. If only 1 number is found per line, NUTS assumes these are real data points and sets the imaginary points to zero.

Note that if your data is (x, y) pairs (in other words, (frequency, intensity) values), Nuts will "think" that the series of frequency values are the real data points, and the display will appear as a straight diagonal line. The actual data points are interpreted as being the imaginary data points. Execute an RI command to swap real and imaginary points, and you should see the data as expected.

NUTS can read a few basic parameters from the beginning of the file, provided the correct keyword is found. This is equivalent to the Full ASCII export format (E1) shown above. The allowed keywords are:

Spectrometer_Frequency or **SF** (in MHz)
domain (values are time or frequency)
comment: (note that colon must be included to be recognized)
user: (note that colon must be included to be recognized)
date: (note that colon must be included to be recognized)
scans
offset
SW or **Sweep_Width** (in Hz)

If no parameters are found, Nuts will import the data, but values for SF and SW must be entered manually.

Bruker ASPECT 3000 The first 3 bytes of the file are converted to a long word. If the word is 4687093 the file type is Bruker Aspect 3000. If the word is -687033 the file type is Bruker Aspect 3000 with the three bytes swapped. If the word is -4061952 the file type is Bruker Aspect 3000 transferred by BrukNet with packet information to be stripped. See also: Bruker data.

Bruker UNIX Bruker saves multiple files for each FID/spectrum, saved under a directory whose name is the name supplied by the user when the data were acquired. Within that directory is another directory whose name is an experiment number (usually 1). Within THAT directory are several files, including one called "fid" for 1D data or "ser" for 2D data which contain the actual data. Select "fid" or "ser" (as appropriate) for importing into NUTS. NUTS retrieves parameters from the "acqus" and "acqus2" files, which must be present. It is important to recreate the directories on the PC and that the file names remain unchanged, as this is how Nuts identifies the data as Bruker. The translated file is given a name corresponding to the top directory name with an extension of fid or ser, as appropriate. **NB:** During the import process a NUTS file will be created in the current NUTS data directory with the name of the directory two levels above the "fid" or "ser" file. If for some reason this is not a valid name the file will be given the name $BRUKER.IMP.

Bruker SGI -- Files from some Bruker systems with SGI computers create files which have 3 bytes of Aspect data stuffed into an SGI 4-byte word. The other byte is zeros with no attempt at sign extension. This type of translation can be forced with the File / Import / Bruker Unix SGI menu selections.

To attempt an autodetection on these files, the examples to date seem to have line 4 of the ACQUS file set to:

##ORIGIN=UXNMR/P, SPECTROSPIN AG

NUTS will search for the line starting ##ORIGIN and see if it contains the string UXNMR/P. If it does it will do a 24 bits stuffed into a 32 bit word type translation. Otherwise the auto-identification process is the same as the process for Bruker UNIX.

Chemagnetics -- If the file name is "d" with no extension and the same directory contains a file with the name "pg", then the file is identified as a Chemagnetics file. The imported file will be given the file name of the selected file's parent directory with a "$" appended to the front of the file name. Resulting file names with a base name greater than eight characters will truncated to eight characters.

Felix New Format If the first word in the file is in hexadecimal 0x01020304 the file is detected as Felix New UNIX format.

GE OMEGA If the file is a power of 2 in size, the current directory is examined for a file with the same base name but with the extension "HED". If found the file is identified as GE OMEGA. These are the files created by the Omega's Export routine. The data can also be exported as a single file, which can also be detected and imported by NUTS.

Hitachi files created on the Hitachi NMR systems and read into Labcalc GRAMS/386 DOS software for saving are detected by the second byte in the file header. If the second byte is 4D hex then the file is translated as "old style" GRAMS/386. If the second byte is 4B hex it is translated as "new style" GRAMS/386 with LSB 1st. If the second byte is 4C hex it is translated as "new style" GRAMS/386 with the MSB first. Files from an Hitachi instrument have some parameters like SF, SW and OF1 in the header area region identified as spare. All files identified as above are considered to be real (not complex).

JCAMP data is assumed to be in the format XYDATA= (X++(Y..Y)). Only real data can be imported at this time. Parameters are imported as desccribed in Davies & Lampen, **Applied Spectroscopy**, **47 (8)**, 1093-1099, 1993.

JEOL Delta (exported as JEOL Generic) These files are identified by their "BIN" extension. A second file should be present with the same name, but with a file extension "HDR", which contains parameters. These files are 64 bit IEEE double precision Floats. These are converted to 32 bit PC type floats in the CDFF file format.

JEOL GX and EX files -- If the file name extension is "GXD" the file is detected as JEOL GX or EX. A second file with the same name, but with the extension "GXP" contains parameter information. The parameter names are not the same for the 2 file types, so Nuts looks for and reads multiple key words for each parameter.

JEOL Lambda files -- If the first item in the file is "JEOLUSF001", the file is identified as JEOL Lambda format.

LYBRICS (PCNMR) files are detected by reading the beginning of the selected file and looking for the string "KEYS". If found, the file is assumed to be in the LYBRICS file format.

Nicolet 1280 The file is read as a packed 1280 word and the first Nicolet 20 bit word is read. If it is 11378 the Nicolet header size is subtracted from the filesize and the number of Nicolet 20 bit words is calculated. If this is a power of 2 then the file is an NT TMON file. If it is not a power of 2 the file is an NT Dexter file. If the first word is 12818, the file is a QE file. If the first word is 57923, the file is a GN file. If the first word is 11378, the file is a NT TMON file transferred by NicNet. If the first word is 12818, the file is a QE file transferred by NicNet. If the first word is 57923, the file is a GN file transferred by NicNet.

NMRi data is identified by detecting reasonably valid values at the expected locations corresponding to quadrature flag, FT flag and SF. Nuts will check for these values using byte order for either VAX or Unix systems.

SMIS If the file name extension is MRD the file is detected as SMIS type.

Tecmag MacFID and MacNMR files are identified by looking at the resource fork for valid values for Type and Creator, and also looks for a Version number in the range 9001 to 9712 at the appropriate location.

Varian UNIX Varian saves multiple files for each FID/spectrum. The files are in a directory whose name is the name supplied when the data were acquired. One of the files in that directory is called "fid" and is the actual data. If there is also a file with the name "procpar", Nuts identifies the file type as Varian Unix. To import the data, the user should select "fid". NUTS retrieves parameters from the other files. NUTS will save the translated data as a file whose name is the name of the directory and whose extension is fid.

Varian data from Gemini, XL and VXR 4000 spectrometers can be translated into the Common Data File format using a utility called V_Helper written by Steve Silber at Texas A&M University. This allows the PC to read Varian floppies and translates the data into various fomats. A version of V_Helper for use with NUTS is available through Acorn NMR for a small fee.

In a similar manner, **GE data from QE+** spectrometers can be transferred via floppy using a utility program available from Ken Ratzlaff at University of Kansas.

Troubleshooting import problems

The safest approach to importing data is to use the NUTS "auto-detect" function (IM command) to import all data. If you use the File/Import menu and select a specific file type, you may apply an incorrect data translation, with unpredictable (and probably undesirable!) results.

When data is transferred between computers via ftp, remember to explicitly set the transfer type to **binary**.

For spectrometer models that create multiple files, rather than a single file (e.g., Varian, Bruker), all files must be transferred and the file names must be unchanged. Failure to adhere to these guidelines will prevent NUTS from being able to locate the required information, and the import process will fail.

For Varian data, the data consists of 4 files (called fid, procpar, text and log). Use **IM** and select the file called **fid**. Data which has already been FT'd is saved with the name "phasefile".

For Bruker data (not including Aspect-based systems), the situation is much more complicated. Time-domain (fid) data is contained in the file called **fid** (1D) or **ser** (2D), and most parameters are contained in the files acqu and acqus (and acqu2 and acqu2s for

2D data). But additional files are needed - files called proc and procs (and proc2 and proc2s for 2D data) are saved

Debugging tools

See also: Look and See utilities for examining files.

NB - Nuts Bug flag

Used to provide additional information useful in determining the origin of problems in program operation. Turning this option on causes some status messages to be displayed in the course of normal program operation. This command is a toggle, so that typing it a second time turns the debugging option back off.

WP - Which Path

Displays a dialog box defining the path to the current import and data directories. This is for information only, and does not allow the user to change the paths. Added for diagnosing errors.

V_Helper program

This program is separate from NUTS. It allows a PC to read a floppy written by the older Varian Gemini, XL and series 4000 VXR spectrometers, and create a file in the NUTS (CDFF) format which can then be opened in NUTS. NUTS does not import these files Varian directly.

The program was written by Steve Silber at Texas A&M University. Some additional information about V_Helper can be found on his web site. Steve sells a full version with multiple options for output format, and sells V_Helper4Nuts for $25. All proceeds go to Texas A&M.

V_Helper is distributed on 5-1/4" diskette. There should be 4 programs on this diskette: DCT.EXE, VAR2CDFF.EXE, LIM2CDFF.EXE and ReadMe.1st.

DCT will do a diskette directory, similar to the dct command on the spectrometer systems. VAR2CDFF and LIM2CDFF will convert NMR data sets from Varian floppy disks or LimNet image files, respectively, to the Common Data File Format used by NUTS.

Using V_Helper

VAR2CDFF Version 1.6, S/N 1035.

Convert a Varian FID on floppy disk for use with NUTS program.

Usage: VAR2CDFF vdir.vname [dest_dir] [/Tn /Z /P /F /I]
vdir.vname is a Varian style directory and name.

dest_dir is the data file destination directory.

/Tn is a Truncate option. n=1, 2, 4, 8, 16, 32 or 64K complex points.
Default value for n is 8K for no value or invalid value.
/Z forces Zero fill to next power of two.
/I save a parameter Information file.
/P Print the spectral parameter summary to the standard printer.
/F Flip spectrum end for end. (Complex conjugate of fid data).
/B force use of B: (non-default) drive instead of A: for DSK5.

VAR2CDFF /I DSK5.TEST1 C:\FELIX will convert a NMR data set called test1 to a data file called c:\felix\test1.dat, and an information file called c:\felix\test1.inf. If no destination directory is specified, the current working directory is used.

LIM2CDFF Version 1.6, S/N 1035.

Convert a Varian FID from LimNet image for use with NUTS program.
Usage: LIM2CDFF limnet.img [dest_dir] [/Tn /Z /P /F /I]
limnet.img is the Varian limnet image, directory and name.

dest_dir is the data file destination directory.

/T is a Truncate option. n=1, 2, 4, 8, 16, 32 or 64K complex points.
Default value for n is 8K for no value or invalid value.
/Z forces extra Zero fill. Data is automatically zero filled to the next power of two. /Z forces fill to an extra power of 2.
/I saves parameter Information file.
/P Print the parameter information to the standard printer.
/F Flip spectrum end for end. (Complex conjugate of fid data).

LIM2CDFF g:\mydata.5 C:\NUTS will convert a NMR data set called mydata.5 to a data file called c:\NUTS\mydata.dat. An information file file called c:\NUTS\test1.inf may be generated by specifying the /I option, otherwise it is only displayed. If no destination directory is specified, the current working directory is used.

VAR2CDFF and LIM2CDFF have been modified to permit conversion of files containing more than 64K data points. Varian software normally limits NP to 60032, but

it is possible to collect larger data sets on a spectrometer with an acqisition processor memory expansion. These data sets will now be converted properly.

Troubleshooting

It is recommended to use Double Sided/Double Density diskettes, not High density.

V_HELPER uses its own floppy disk I/O instead of the ROM Bios routines. Errors can occur when running V_Helper in a DOS window under Win95, caused by Win95 interupting the disk read process. To test this, try booting your computer to a DOS floppy (running DOS without Win95) and try running the V_Helper utilities directly from DOS. If this eliminates the error, then the problem is with the Win95 background processes.

Oversampling and digital filters

NMR Data Oversampling and NUTS

It is the current trend for NMR spectrometers today to "oversample" the data during acquisition. Oversampling is the act of acquiring data at a faster rate than necessary for the desired spectral window. Since the rate the data is collected is inversely proportional to the observed spectral width after Fourier Transform, then oversampling is when data is acquired for a wider spectral width than necessary for desired spectral region. To make this description easier to follow, let's define a few shorthand terms:

- SW - the spectral region desired for observation.
- DW - the dwell time at which to acquire data for a spectral width of SW.
- ODW - oversampling dwell time which is greater than DW.
- OSW - oversampled sweep width before "decimation" which is greater than SW.

If the same number of data points were used, data collected at the ODW rate would have a lower digital resolution than would be obtained if the spectrum was collected at the DW rate.

One reason to oversample the data is that audio filters can have their cutoff frequencies set in the spectral region between SW and OSW. This leads to 1) less noise being folded back from frequencies greater than the Nyquist frequency, 2) less non-linear phase changes and 3) less amplitude distortion from filters in SW frequency range. Item 1 translates to increased signal to noise performance. Item 2 leads to spectra where all peaks can be phased with only a zero and first order phase correction. Item 3 leads to NMR spectra where integrals are less distorted.

Another more technical reason to oversample the NMR data is to "spread out" the digitizer quantization noise. When the digitizer samples a signal, it quantitates the signal in discreet steps (digitizer bits). The digitizer does this with some error often referred to

as digitizer noise. In 2x oversampled data, two adjacent data points can be averaged to produce a new data point. Averaging two points to get a new data point is the same as if the digitizer has sampled at a slower rate, but also has the effect of averaging the digitizer noise (a factor of 1.4 improvement in signal to noise) and is another bit of digitizer resolution thereby reducing the step quantization error by one bit.

With the availability of faster digitizers and with cheaper memory prices, the advantages for oversampling are cost effective and desirable. How oversampling is implemented by each NMR instrument manufacturer varies, but the key steps are to include some or all of the following to some degree:

1. Oversample data acquisition while applying sharp filtering at the Nyquist frequency.
2. Apply Digital Quadrature detection.
3. Apply digital filter.
4. Decimate the data.
5. Prepare the data for user consumption.

Step 1
Oversampling, as discussed above, is acquiring data at a faster rate than necessary for the desired spectral window. This can be done in quadrature and then Step 2 does not apply, or it can be done in the real mode (single channel) with the desired spectral window offset by some frequency, OF1. Then step 2 does apply.

Step 2
If the data is offset by some frequency OF1, then have the computer calculate to quadrature reference frequencies (sin OF1 and cos OF1) and "mix" them with the collected signal and low pass filter the resulting sum and difference frequencies to get the resulting "quadrature detected FID". This requires a much faster digitizer (which usually means fewer bits per sample) and is computationally intensive. Some manufacturers do this in a dedicated DSP so that the calculation speed issue is greatly reduced.

Step 3
Calculate and apply a lowpass filter such that when the data size is reduced (decimated) there will be no foldback of spectral noise.

Step 4
Reduce the data by the degree of oversampling. A decimation of 2 is done by averaging each two adjacent points and generating a new point. This doubles the dwell time and halves the sweep width.

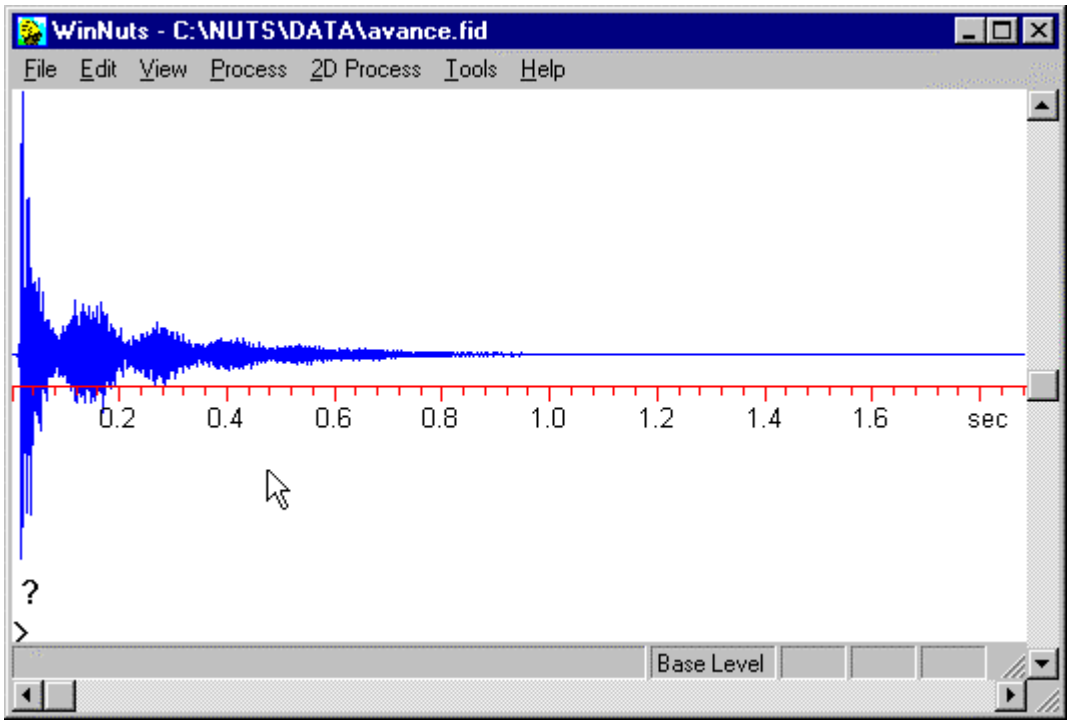
Step 5
Some DSP outputs are the correlation between a square wave filter and the NMR data, which is a method of doing the low pass filter. This data starts a zero and builds to a start of an FID at around 50 to 100 points. Other DSP outputs have further calculations to

present a more standard FID to the user.

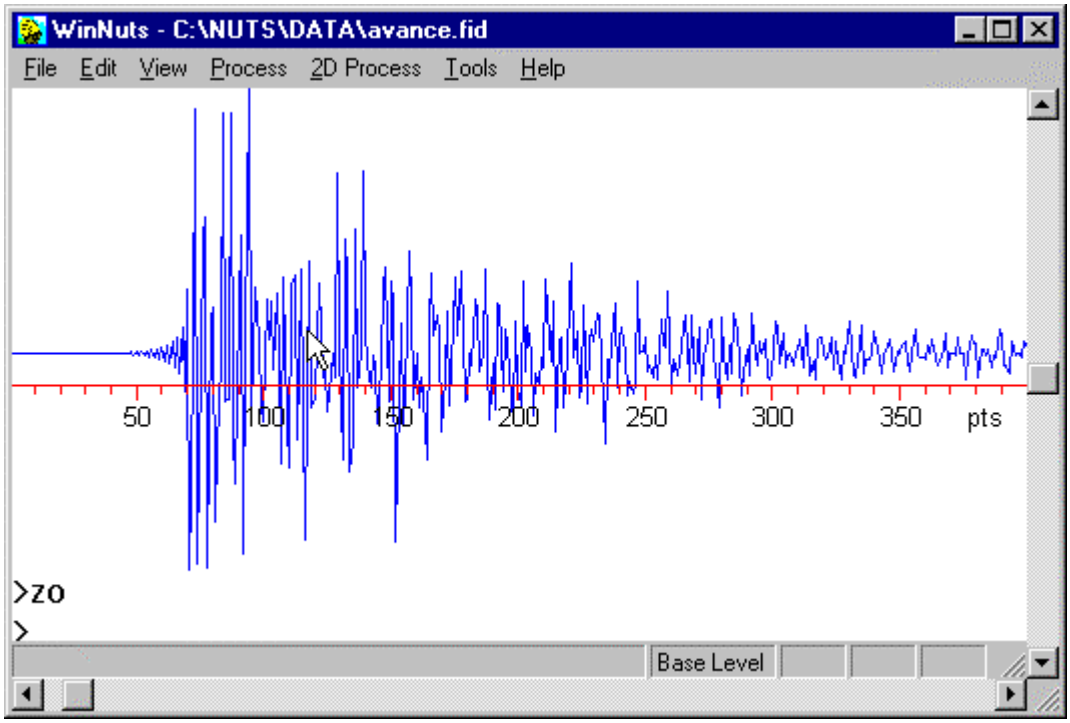
Processing digitally filtered data

An extra processing step is required before FT for digitally filtered data (Bruker data from the Avance series of spectrometers such as DRX and ARX, Tecmag NT-NMR data and some JEOL data). This is necessary because the data have been digitally filtered and "decimated" prior to saving the FID. The initial points of the FID are zero. A circular left shift must be performed before the FT. The number of points to shift is calculated from parameters found in the file header (the Decimation Number and the DSP firmware version found in the Bruker file header), but the user can change the number of points. (In a circular left shift, points are removed from the left end of the data and added to the right end.)

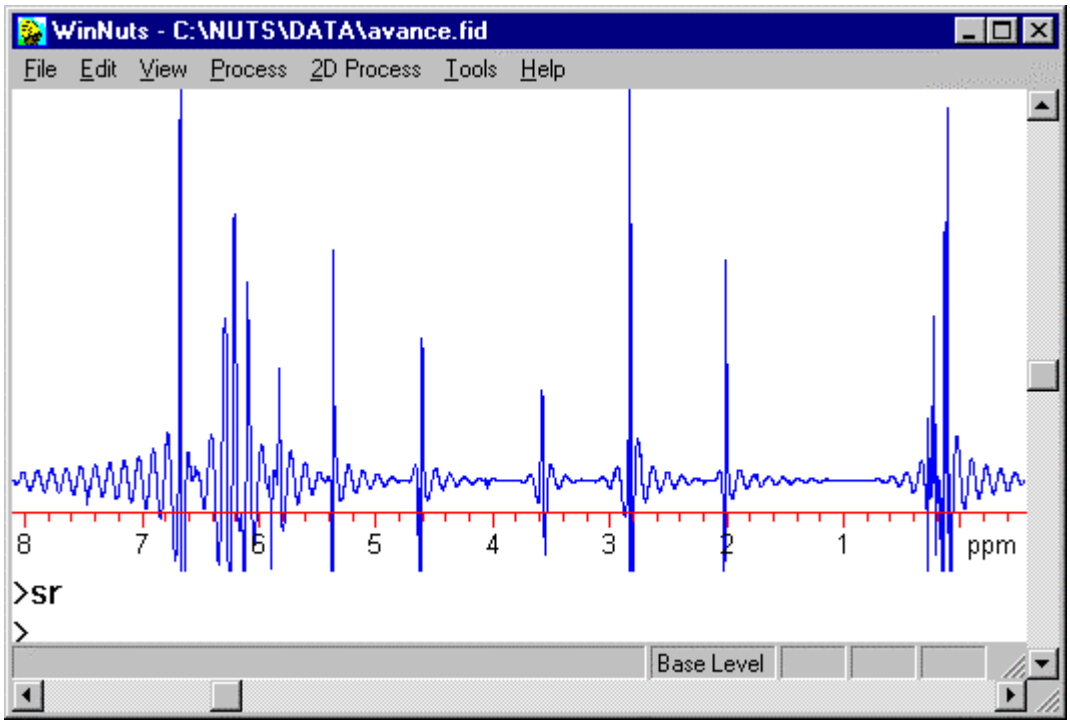
***** Note:*** NUTS attempts to perform the RD operation automatically when FT is executed. NUTS will use its calculated value for number of points to rotate. The value can be changed by typing RD. There are 2 ways to avoid having NUTS do automatically apply RD: Type RD and enter 0 for points to rotate, or use the command CT (complex FT) or BT (Bruker FT) instead of FT. The choice of CT or BT depends on whether your data was acquired using simultaneous or sequential acquisition.



This FID is typical of digitally filtered data. Note the odd appearance of the beginning of the FID.

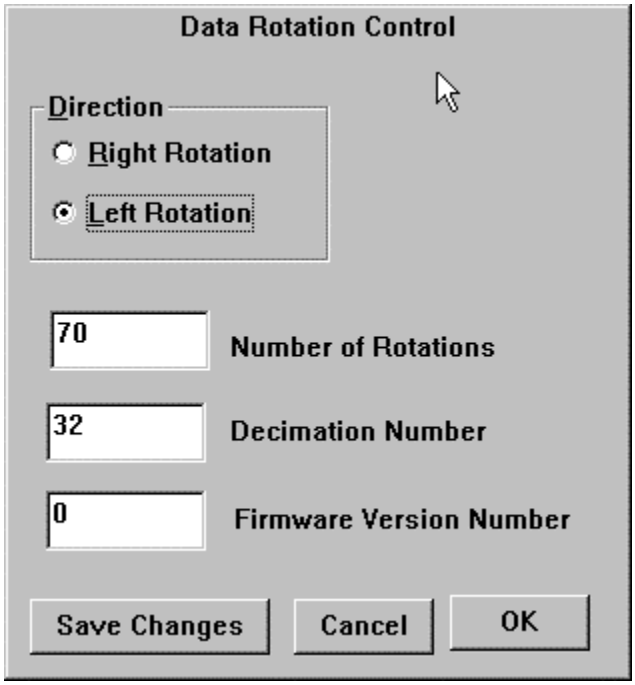


The FID above has been expanded to show the first 400 points, making it easier to see the beginning of the FID.



If an FT is performed on the above FID, a seriously distorted spectrum results. The "wiggles" can be removed with **very large** linear phase correction (many 100s of degrees) which is not very convenient.

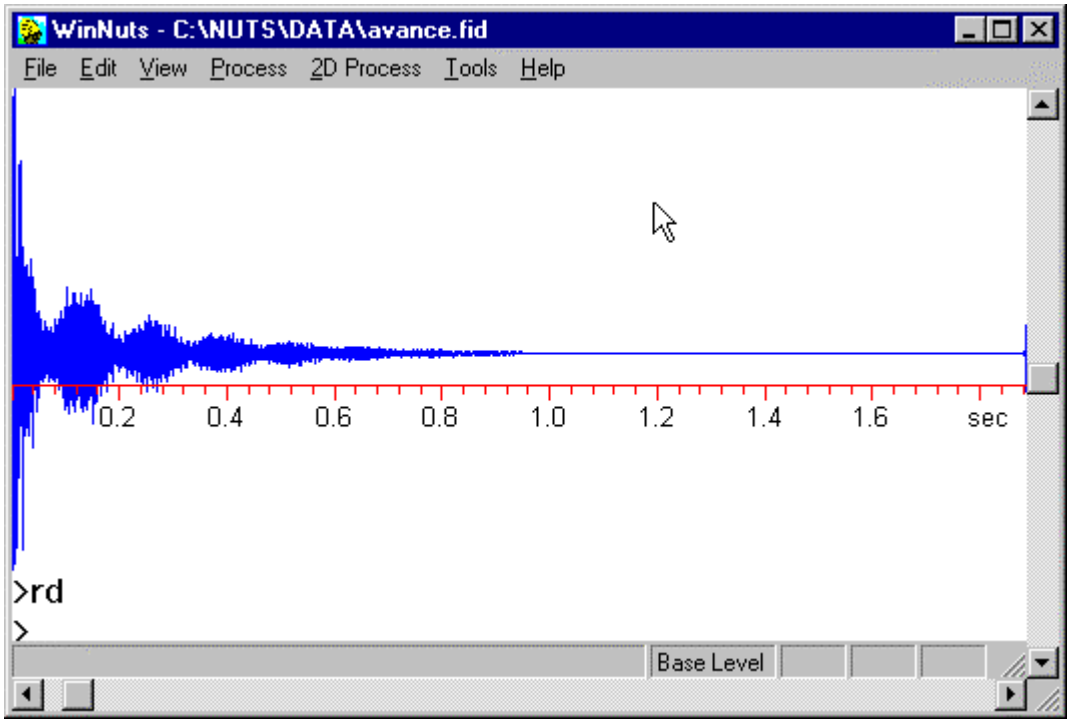
The first 70 points at the beginning of the FID need to be removed, which is done as a circular left shift, so that the points removed from the beginning of the FID are added onto the end. This is performed with the NUTS command RD.



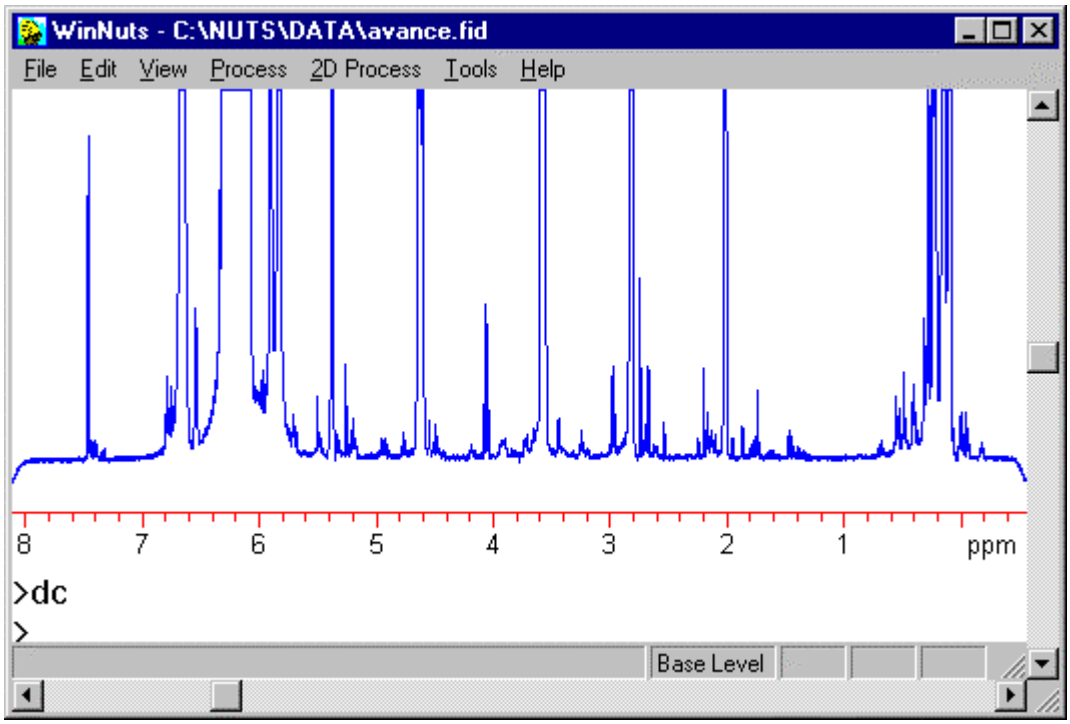
When RD is typed, this screen is displayed. NUTS determines the number of points to shift (Number of Rotations) from the Decimation Number and Firmware Version, which are imported with the data.

If NUTS has been able to determine a default value from parameters in the data header, a non-zero value will be displayed in the Number of Rotations box. Usually the user should just click on OK. If NUTS has not been able to determine the correct number, this value will be zero, and the user must input the appropriate number. This can be determined by examining the beginning of the FID.

The user can experiment with different values for Number of Rotations. After FT and phasing, look at the value for linear phase correction (TP command). When the correct value is used, the linear correction should be fairly small (less than 360 degrees).

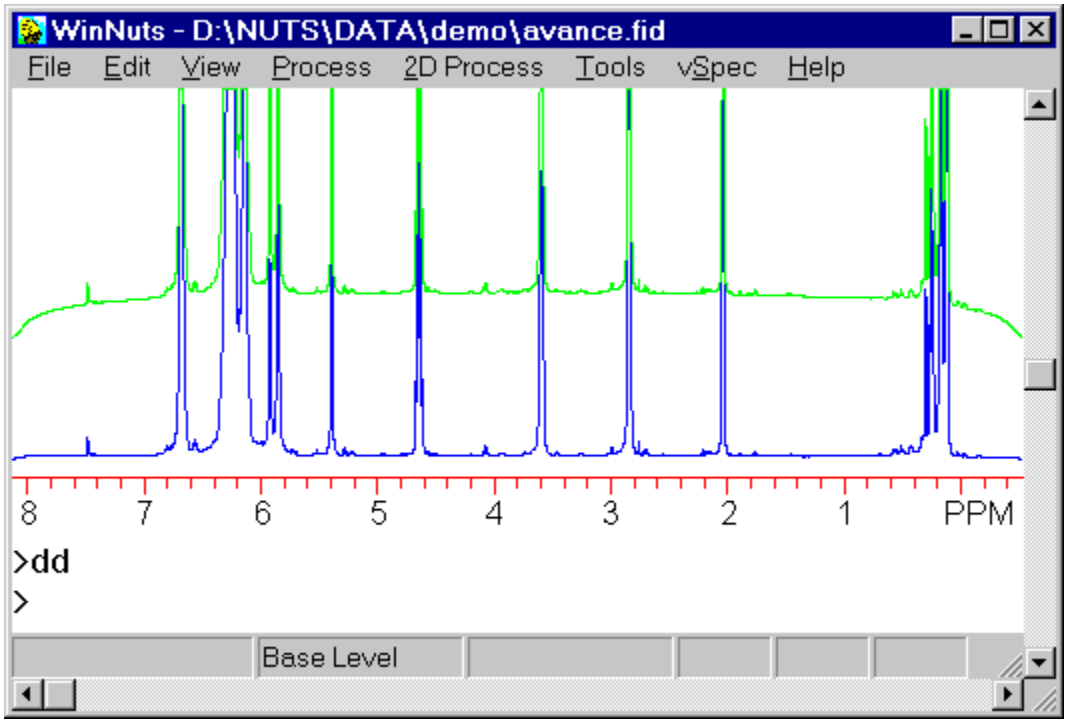


The resulting FID now has its maximum at the beginning of the FID. Note the non-zero points at the end of the FID.



FT of the FID after RD gives a reasonable looking spectrum. In some cases, as here, there is some roll-off at the ends of the spectrum. The normal BC command (to remove DC offset and tilt) does not work properly in this case. To compensate, either expand the spectrum so that the curved ends are not displayed and apply baseline correction, or use the BR command to ignore the ends of the spectrum while applying DC and tilt correction.

Any apodization should be applied before RD is executed. Below is a comparison of spectra resulting from exponential multiplication applied after (top) RD and before (bottom) RD. Note that the tailing off of the baseline is worse when the apodization is applied after RD. Retaining the non-zero points at the end of the FID is important for minimizing baseline distortion.



If the acquisition time was short enough that the FID has not decayed to zero, then doing an RD can result in a discontinuity, and the resulting spectrum will be distorted. (See description of zero-filling for an illustration.) Applying a window function before the RD operation, to bring the end of the FID smoothly to zero, will avoid this.

For Bruker data, NUTS determines how many points should be shifted by examining the parameters DSPFVS and DECIM found in the acqus file. Allowed DSPFVS values are 10, 11 and 12. DECIM can have any of several values. Milo Westler and coworkers at the University of Wisconsin generated a look-up table for the correct number of points to rotate, based on these 2 parameters. If either of these values is zero, NUTS concludes this is not digitally filtered data, and so zero points should be rotated.

However, sometimes the information found in the data header is incorrect or insufficient for NUTS to determine the correct number of points, so NUTS has an alternative approach. The **DF** command examines the beginning of the FID to determine where the "real" start of the data should be, and how may zeroed points precede it. It executes an FT, then attempts to undo the large linear phase correction by applying linear phase of -360 degrees for every zeroed point. This will not work properly for all data, so users are advised to experiment.

RD can be used for other purposes than just to correct digitally filtered data. See example in the previous section.

Artifacts seen in Bruker data

Special considerations for Bruker data

There are some peculiarities associated with Bruker data. Topics are

sequential data acquisition
byte swapping
directory structure
digitally filtered data
data transferred via Bruknet
2D Aspect data

See also: 2D processing, baseline correcting digitally filtered data

True quadruature (complex) detection is accomplished by splitting the signal from the probe into 2 channels, related by a 90-degree phase shift of the reference frequency. Bruker data is often acquired using "sequential" data acquisition, rather than true complex, or "simultaneous" data acquisition. To accomplish quadrature detection using this scheme, the data is digitized at twice the rate and points are placed into the 2 channels alternately. In other words, the n^{th} point placed into each channel are not acquired the same time. This requires that a special type of FT be performed (**BT**). Nuts should correctly identify sequential data, which is identified by setting the Domain parameter to TPPI, and executing an **FT** should automatically perform the appropriate type of FT. If a complex FT is performed on sequential data, artifacts are created which resemble severe quadrature images - every peak has an out-of-phase mirror image. See example below.

Sequential detection can cause a different artifact with similar appearance. If the 2 channels are switched, the data points become out of order in time, and following FT, each peak will have a large, out-of-phase mirror image. See example below. If the data is complex, switching the channels simply reverses the spectrum. We have been unable to identify any header information to detect which channel is real and which is

imaginary. In addition, we have some information that it depends on the method used to transfer the data from the Aspect to the PC. Executing an **RI** command (switching Real and Imaginary points) before the FT will fix the problem. An entry has been added to the NUTS.INI file which allows the user to automatically perform an RI on Bruker Aspect data at the time it is imported into NUTS, so that the user does not have to do this explicitly. The relevant entry in the NUTS.INI file is:

BRUKER_ASPECT_RI = TRUE

If the line is not present in the INI file then add it, left justified as typed above. There is also an entry in the nuts.ini file which is:

RI_ON_IMPORT = FALSE

This will apply an RI operation on all imported data, so will solve the problem only in cases where all data being imported into NUTS comes from an instrument that has this problem.

Some file transfer processes from Bruker Unix systems to the PC incorporate a "byte swap" in the file transfer process. The NUTS importing process wants the file to be an exact image of the Bruker Unix system fid and will get these files wrong on importing. This results in a corrupted file which does not resemble a normal FID. If the parameters of these files are imported correctly but the fid is wrong, set this parameter in the NUTS.INI file to TRUE.

BRUKER_UNIX_BS = TRUE

Note that changes made in the NUTS.INI file are not implemented until the next time the NUTS program is started.

The directory structure for Bruker Unix files must be exactly reproduced on the PC (or Mac) for NUTS to import the data successfully. The file name supplied by the user when the data are collected becomes the name of a directory. Within that is a subdirectory whose name is a number (1, 2, 3, etc.). The files associated with the data are in this subdirectory. This directory structure must be created on the PC (or Mac) and files placed in the correct directory. File names must be unchanged in moving data from the spectrometer, or NUTS will not be able to find the required information. When NUTS translates the data, it creates a file whose name is the name of the parent directory with a $ appended to the front.

Digital Filtering

Bruker data from Avance series spectrometers (DnX models) require special processing. The data have been digitally filtered and "decimated" prior to saving the FID. The FID, when first imported into NUTS, appears distorted because the initial points of the FID are zero. Before an FT can be performed, an **RD** (Rotate Data) operation must be performed.

This is a circular left shift of a specified number of points. NUTS calculates the appropriate number of points for the shift, or number of rotations, from the Decimation Number found in the Bruker header. Any apodization or zero-filling must be performed before the RD. See section above on how to process digitally filtered data.

Digitally filtered data have distorted baselines at the ends of the spectra, which can complicate baseline correction. This is illustrated in the section on baselines, above.

The FT command was recently modified to perform the RD operation automatically, using the values of DECIM and DSPFVS found in the Bruker header. If an RD is done manually, the FT will not perform the RD again. This is not fool-proof, but works in most cases.

Data transferred from the spectrometer to PC via Bruknet

This transfer process splits the data file into 2 parts. For an original file called **file.001**, the 2 resulting files are **file.001** and **p_file.001**. In this form, the data will not import correctly into NUTS. The solution is to re-combine the 2 files into a single file that NUTS will recognize and import. This is done on a PC with a simple batch file which is

```
copy p_%1 #%1
type %1 >> #%1
```

This batch file is placed into the directory containing the data files. Then execute the file with a single argument which is the name of the data file (e.g., file.001). The 2 files are combined and given the name **#file.001**, which can then be imported into NUTS.

2D data acquired on an Aspect-based spectrometer

The Aspect computer predates development of routine 2D spectroscopy. As a result, the Aspect data file format contains no provision for essential parameters for the indirect dimension. The missing parameters are essential to displaying a correct axis in the indirect dimension.

It is simple to enter values for the missing SF, SW and offset parameters, provided you know the correct values. It is suggested that SW and chemical shift information be placed into the Bruker TITLE when data are acquired, so they stay with the data.

Exporting data

NUTS allows data to be exported as ASCII and as JCAMP-DX5. Both options are available from the File menu. The ASCII export has some options, as described below.

Data can be exported in a macro using the following macro commands to set the filename to be used, and to increment the file extension of that file for subsequent exports:

Set ExportFile *filename*

Do increment_export_filename

See also criteria for ascii files to be imported.

EJ -- Export as JCAMP-DX (Real data points only)

This command exports only the real part of the data in the "XYDATA= (X++(Y..Y))" format, and does not use any of the compressed JCAMP formats. Only the displayed region of the spectrum is exported. See Davies & Lampen, **Applied Spectroscopy**, **47 (8)**, 1093-1099, 1993.

E6 -- Export as JCAMP-DX using NTUPLES format

The NTUPLES data format supports RI pairs. Bruker software can import and export data in this format using extensions which are Bruker specific. NUTS follows this technique by using extensions to the JCAMP-DX standard which are specific to NUTS. Efforts have been made to make these extensions the same as the Bruker extensions whenever possible. This means that NUTS can import Bruker exported JCAMP-DX spectra and, hopefully, Bruker can import NUTS exported JCAMP-DX spectra.

NUTS can also export 2D spectra in the JCAMP-DX style. This is outside the JCAMP-DX standard definitions and is not supported by Bruker. If we become aware of any further definitions to the JCAMP-DX standard, we will make efforts to keep our exports faithful to the defined standard.

Even though NUTS does export values for TD and TD_2D in the parameters for the number of points in both dimensions of a data set, they are ignored on import, with the actual number of points being determined as the data is being imported.

E1, E2, E3, E4 and E5 - Exporting Data

NUTS provides the capability of exporting data to a file in ASCII format. This is available from the File menu, as Export File, or via keyboard commands **E1**, **E2**, **E3**, **E4** and **E5**, which output the data in different formats. A list of options is provided which includes exporting header plus data points (Full; equivlaent to **E1**), just the header (**E2**), just the real points (**E3**), real and imaginary points (**E4**) or as PPM and Intensity (**E5**). The latter could then be imported into another application for line fitting or other analysis.

The output of the Full ASCII export (and the **E1** command) looks like the following:

```
COMMENT: Ethyl Benzene on a QE 300
DATE: 12/31/92
USER: WWC
Complex_Points = 2048
Spectrometer_Frequency = 300.152374 MHz
Sweep_Width = 4000.00 Hz
Offset = 1850.00 Hz
Domain = Time
Scans = 1
DATA Reals,Imaginaries
1528.104614,735.299377
425.757690,927.761475
76.757011,-2591.717285 etc.
```

The Header only option (E2) gives the following:

```
COMMENT: Ethyl Benzene on a QE 300
DATE: 12/31/92
USER: WWC
Complex_Points = 2048
Spectrometer_Frequency = 300.152374 MHz
Sweep_Width = 4000.00 Hz
Offset = 1850.00 Hz
Domain = Time
Scans = 1
```

The output of the Real Data Only ASCII export (E3) looks like the following:

```
DATA Reals Only
1528.104614
425.757690
76.757011
1459.461060
-1165.192627
-725.373047
-1354.193359 etc.
```

The output of the R&I Data Only ASCII export (E4) looks like the following:

```
DATA Reals,Imaginaries
1528.104614,735.299377
425.757690,927.761475
76.757011,-2591.717285
1459.461060,-1324.713379
-1165.192627,-843.652344
-725.373047,-1660.726563 etc.
```

The output of the PPM, Intensity ASCII Data Pairs export (E5) looks like the following:

```
12.826818,1528.104614
12.820312,425.757690
12.813805,76.757011
12.807298,1459.461060
12.800791,-1165.192627 etc.
```

See also: Importing ascii data, Data Translation.

NUTS file format

Common Data File Format

Header – Select which of 3 file formats to use

There are now 3 different NUTS file formats, referred to as Type 1 (original), Type 2 and Type 3. The changes have been made to add flexibility for future program modifications. The default format can be set in the NUTS.ini file, and can also be set while NUTS is running. To do this, enter the "non-2-letter" command mode (by typing **2F**), and type, for example,

header 2

which would set the header type to 2.

This command can also be used to determine the length of the header, in bytes:

header length

Type 1

The translated files have a 258 32bit word header which contains several pieces of information.

A list of file types which can be identified and imported into NUTS with the import (**IM**) command can be found in the section on data translation.

The general description of the created file is given below:

See also:

C structure definitions for NUTS file format
C code which defines the structure of the NUTS data file and includes routine for writing out a NUTS Type 1 data file
Looking at data files
FORTRAN program for converting data to CDFF Type 1

NOTE Word numbering starts at zero since that is the way computer addressing schemes would start.

Word Number	Variable Type	Description
0	int	Byte Key 04030201 Hexadecimal
1	int	Number of words in header (Usually has 256 here)
Start of general header information		
2	int	Number of Dimensions in data (1D, 2D, 3D etc. data)
NOTE - If data is written as integer NUTS will "import" the data as floats		
3	int	Data Format 0=IEEE float, 1=32 bit integer
4	int	Empty (Usually a 1 here)
5	int	Frame Size (Usually 32)
6	int	100 * Program Version Number
7	int	Number of points in second dimension
8	int	No Tailer = 0, Tailer present = 1
18	float	SW Sweep Width - NOT used by NUTS
19	float	SF Spectrometer Freq - Not used by NUTS
20 to 83	float	64 time values from an arrayed experiment
84-95	--	unassigned (95 is last word of general header information)
First Dimension Parameters		
96	int	pts1d - Data Points
97	int	complex1 - Data Type: 0 = Real; 1 = Complex; 2 = Bruker Interleaved
98	int	domain1 - Domain Type: 0 = Time; 1 = Frequency
99	int	axis1 - Axis Type: 0 = None; 1 = Points; 2 = Hz; 3 = PPM
100	long	decimation - Bruker decimation no. for digitally filtered data
101-111	--	unassigned
112	float	sw1 - Sweep Width
113	float	sf1 - Spectrometer Frequency
114	float	of1 - Reference Shift
115	float	Reference Point (unused by NUTS)
116	float	Phase Pivot (unused by NUTS)
117	float	tpa1 - Zero Order Phase
118	float	tpb1 - First Order Phase
119	float	tlb1 - LB -- Line Broadening by EM command
120-135	--	unassigned
Second Dimension Parameters.		
136	int	pts2d - Data Points
137	int	complex2 - Data Type: 0 = Real; 1 = Complex; 2 = Bruker Interleaved (TPPI)
138	int	domain2 - Domain Type: 0 = Time; 1 = Frequency
139	int	axis2 - Axis Type: 0 = None; 1 = Points; 2 = Hz 3 = PPM
140-151		unassigned
152	float	sw2 - Sweep Width

```
153         float       sf2 - Spectrometer Frequency
154         float       of2 - Reference Shift
155         float       Reference Point (unused by NUTS)
156         float       Phase Pivot (unused by NUTS)
157         float       tpa2 - Zero Order Phase
158         float       tpb2 - First Order Phase
159         float       tlb2 - LB -- Line Broadening by EM command
160-201      --          unassigned

General Parameters
204         float       Temperature
205-214     chars       EXperiment description string
215         float       90 Degree pulse length in usec
216         float       Recycle delay in seconds
217         long int    Number of Acquisitions
218 - 227     chars     USER name string
228 - 235     chars     DATE string
236 - 256     chars     COMMENT string

After the header comes the data as complex pairs.
If the data is real only then every other word must be
zero.

The first word of each slice of data is the size of that slice
in words. Therefore a 4096 Complex Pair data set would be
8192 words long.
NOTE: While NUTS writes this word into the data format it never
reads or uses this information.

The word numbers below assume the header is 256 Words long.
258         int         Data size of slice 1 in words
259...     float        Data

If multi-D then the next dimension.
... int                 Data Size of slice 2 in words
... float               Data

As many slices as needed.
```

Type 2

In addition to a larger header block (1026 rather than 258), the new format also eliminates the "spacer" between slices. In Type 1 format, the first word of each slice is an integer equal to the size of that slice. NUTS never used that value, it was included to make the format consistent with the Felix format from which it derived. To make things simpler, this spacer was eliminated in the Type 2 format.

Word Number	Variable Type	Description
0	int	04030201 Hexadecimal

```
1          int          Number of words in header, Usually 1024
2          int          1=1D, 2=2D, 3=3D etc
3          int          0 = float, 1 = integer
4          int          1 = NUTS 1st Header, 2 = NUTS 2nd Header,
5          int          Usually 32
6          int          Version Number of Program times 100
8          int          0 = No Tailer, 1 = Tailer
20-83      float        64 time values from an

1st Dimension Parameters
96         int          pts1d - Data Points
97         int          complex1 - Data Type: 0 = Real; 1 = Complex; 2 =
Bruker Interleaved
98         int          domain1 - Domain Type: 0 = Time; 1 = Frequency
99         int          axis1 - Axis Type: 0 = None; 1 = Points; 2 = Hz;
3 = PPM
100        long         decimation - Bruker decimation no. for digitally
filtered data
101-111     --          unassigned
112        float        sw1 - Sweep Width
113        float        sf1 - Spectrometer Frequency
114        float        of1 - Reference Shift
115        float        Reference Point (unused by NUTS)
116        float        Phase Pivot (unused by NUTS)
117        float        tpa1 - Zero Order Phase
118        float        tpb1 - First Order Phase
119        float        tlb1 - LB -- Line Broadening by EM command
120-135     --           unassigned
2nd Dimension Parameters
136         int         pts2d - Data Points
137         int         complex2 - Data Type: 0 = Real; 1 = Complex; 2 =
Bruker Interleaved (TPPI)
138         int         domain2 - Domain Type: 0 = Time; 1 = Frequency
139         int         axis2 - Axis Type: 0 = None; 1 = Points; 2 = Hz 3
= PPM
140-151      --         unassigned
152        float        sw2 - Sweep Width
153        float        sf2 - Spectrometer Frequency
154        float        of2 - Reference Shift
155        float        Reference Point (unused by NUTS)
156        float        Phase Pivot (unused by NUTS)
157        float        tpa2 - Zero Order Phase
158        float        tpb2 - First Order Phase
159        float        tlb2 - LB -- Line Broadening by EM command
160-201     --          unassigned

176-215 -- reserved for 3rd Dimension Parameters
216-255 -- reserved for 4th Dimension Parameters

General Parameters
256        float        Temperature for experiment
257        float        90 degree pulse length in usec
258        float        Recycle delay in seconds
259        int          Number of acquisitions
260-267    char         Name of pulse program
268-275    char         Name of nucleus
276-283    char         solvent
```

```
284-291    char        USER name string
292-299    char        DATE string
300-331    char        COMMENT string
332-1025    --         unassigned
After the header comes the data as complex pairs
(alternating real and imaginary pts).
If the data is real only then every other word must
be zero.  Subsequent slices follow immediately after the
preceeding slice.
```

Type 3

This format is based on the JCAMP specifications, but is not identical to JCAMP. The header is ASCII, with a keyword at the beginning of each line. This offers much more flexibility for future additions to information stored in the header, because the exact location of any item in the header and the order of items is not fixed. Some of the lines conform to the JCAMP-DX specification, others are NUTS-specific items and are in a proprietary section. Another advantage of this format is that the header can be viewed in any text editor.

The data is not stored as ASCII, as is the case for JCAMP files, because the file size would be much larger. The header is terminated with Ctrl-Z, and is followed by the data points, stored as 32-bit floating point numbers in Intel (little endian) byte order. The data are organized as Real, Imag, Real, Imag, etc. This is essentially the format proposed as JCAMP-DXB, which was not approved as a JCAMP standard.

An example is shown below, for a small ^{1}H spectrum.

```
##TITLE= Ethyl Benzene on a QE 300
##JCAMP-DXB $$JCAMPDX Header and Binary Data
##DATA TYPE= NMR SPECTRUM
##DATA Class= NTUPLES
##ORIGIN= NUTS NATIVE (RI)
##OWNER=
##SPECTROMETER/DATA SYSTEM= NUTS NATIVE (RI)
##INSTRUMENTAL PARAMETERS= H1
##.OBSERVE FREQUENCY= 300.152374
##.OBSERVE NUCLEUS= H1
##.DELAY= 0.000000
##.AVERAGES= 1
$$ NUTS specific parameters
##$AXIS_TYPE=3, 3, 0, 0
##$AQ_mod=1, 0, 0, 0
##$DATE= 12/31/92
##$USER= WWC
##$NAME1=ethylbenzene
##$NAME2=
##$NAME3=
```

```
##$FORMULA=C8H10
##$Nucleus1= H1
##$Nucleus2=
##$Nucleus3=
##$Nucleus4=
##$PATH= c:\nuts\data\test.nmr
##$DECIM=0
##$DOMAIN=1, 0, 0, 0
##$DSPFVS=0
##$POINTS=2048, 1, 1, 1
##$FREQUENCY=300.152374, 1.000000, 1.000000, 1.000000
##$SWEEP_WIDTH=4000.000000, 1.000000, 1.000000, 1.000000
##$FREQ_OFFSET=1850.000000, 0.000000, 0.000000, 0.000000
##$PULPROG= One Pulse
##$PULSE_LENGTH= 0.000000
$$ End of NUTS specific parameters
##NTUPLES=NMR SPECTRUM
##VAR_NAME= PPM, SPECTRUM/REAL, SPECTRUM/IMAG, PAGE NUMBER
##SYMBOL= X, R, I, N
##SYMBOL= INDEPENDENT, DEPENDENT, DEPENDENT, PAGE
##VAR_FORM= AFFN, ASDF, ASDF, AFFN
##VAR_DIM= 2048, 2048, 2048, 2
##UNITS= HZ, ARBITRARY UNITS, ARBITRARY UNITS
##FIRST= 3850.0000 , -1406.669434, -465.478027
##LAST= -150.0000 , -557.505615, -853.042786
##MIN= 3850.000 , -3443.531006, -100636.140625
##MAX= -150.000 , 257425.500000, 96545.257813
##FACTOR= 1.0, 1.000000, 1.000000
##YDATA=(Y..Y)
$$ Binary Data follows Control Z
$$ Binary( Total # Points) = Total # Bytes, Intel Little
Endian floats
$$ Complex Pairs packed RIRIRIRI...
##BINARY(2048)=16384,IEEE32L
```

followed by data

Customizing NUTS

nuts.ini configuration file

When the NUTS program is run, it automatically sets internal operational parameters to some default values. After setting these default values, the NUTS program looks for a configuration file called NUTS.INI. If it finds the file, NUTS looks through the file for keywords followed by a value. For every keyword found, the default value of the keyword parameter is changed to the value specified in the configuration file. This allows each user to customize NUTS to his/her own preferences. Use any text editor to make

changes in the NUTS.INI file which is supplied with the NUTS program. There are extensive comments included in the file, and it is suggested that users read through the file supplied with Nuts, to acquaint themselves with the options it provides.

Some of the parameters set by the NUTS.INI file can be overridden after the program is started. The reason for setting these parameters using the NUTS.INI file is that they remain set to the user's choice every time NUTS is run. These include the axis label, minimum height for peak picking, page setup for plots (whether or not to print parameters or draw a box around the plot, plot margins) and choice of font for the different types of text used in the program.

It is possible to use a different NUTS.INI file when NUTS starts, which would allow users sharing a computer to have personalized nuts.ini files. This is done by adding an argument to the command line when Nuts is started. For example, the following command would use a file called **fred.ini** in

```
nuts.exe -i fred.ini
```

A full path to the nuts.ini file can be specified using double or single quotes.

TB – Tabs

This command affects how integral and peak lists are generated. Separation between columns can be done as spaces or as Tabs. Spaces work best if the list is to be pasted into a text editor, such as notepad. Tabs work best if the list is to be pasted into a spreadsheet.

NU – Re-initialize NUTS from the nuts.ini file

See also: other startup options

A sample configuration file is shown below. It is divided into the following sections:

General Configuration - header version, UnDo, scrollbars
Directories - set defaults for working data directory, import directory, macro directory
Import - invoke automatic spectrum reverse on import of specific file types
Help - choices for Help files
Routines - set some initial values
Axis Labels
Phase - set mouse direction, sensitivity
FB - default baseline correction mode for FB command
Print - set pen width, choices for parameters on plots, box around plots, color, margins
Color - set colors for spectrum, axis, integrals, contour levels
Stacked Plot - X and Y offsets
Peak Picking - appearance of peak labels and peak lists

Offset Information - interpolation and snap-to-peak settings
Line Broadening
Line Fit - default lineshape for deconvolution
Macro - map function keys to specific macros
Links - define linked command lists
Font - set fonts for axis, peak labels, integral labels, etc.

```
;   NUTS.INI

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[GENERAL CONFIGURATION]


;  All blank lines and lines starting with a ";" are ignored.
;  The first word on other lines becomes the keyword.
;  The remaining part of the line in the value for the keyword
;  The keyword and values are case insensitive


; Over time, the file format for the NUTS header has changed
; to accommodate new features and capabilities.  The 3 versions
; are referred to as header types 1, 2 and 3.  All are described
; in the Help files.
NUTS_HEADER_VERSION = 3

;  Flag used to reduce the level of questions NUTS asks
;  is areas where the data could be destroyed if the program
;  continues.  Tends to make the command level operation
;  directly from the keyboard more like a Link or Macro was
;  controling NUTS operation.
;  If this term is not going to be used then leave a semicolon in front
;  of it so that the initialization part of NUTS will ignore it and
;  use its own internal default of EXPERT_MODE = FALSE.
;EXPERT_MODE = TRUE

; Nonsub-routine commands can have an undo buffer of 10.
; If the flag below is set to TRUE the undo buffers
; will save buffers to the disk with each two letter
; command.  This can take time depending on the size
; of the file and speed of the disk.  This is especially
; true of 2D files.
;UNDO = TRUE

; Nonsub-routine commands were originally two letter
; commands which automatically executed after entering
; the second character on the keyboard.
; If the flag below is set to TRUE then an <ENTER> is
; required before the command is executed by default when
; NUTS is started.  In this non two letter command mode
; commands can be longer than two letters.  This mode can
; be toggled on and off while NUTS is running with the
: 2N (2 letter command mode oN) and 2F (2 letter command
; mode oFf).
;CR_FOR_COMMANDS = TRUE
```

```
;  By default NUTS has a toolbar at the top of the window
;  below the menu.  If the semicoln is removed on the next
;  line, this feature will be disabled.
;Toolbar = FALSE

;  By default NUTS does not display Gridlines on the
;  display or plots.  If the parameter below is set to TRUE
;  Then gridlines will be displayed on NUTS startup.
;  This gridline display can be toggled on and off with ^G.
;GRIDLINES = TRUE

;  If a line is "MetaObjectFile" the next line is read
;  as a filename of a Windows Enhanced MetaFile on the
;  disk to be added to the MetaObjects for display and
;  printing.  The first item in the line is the file name.
;  The next 2 numbers define the x,y position of the upper
;  left corner of the object.  They are expressed as a
;  fraction of the total width and height of the screen
;  or plot.  The last 2 numbers define the object's
;  height and width, expressed as a fraction of the
;  height and width of the screen or plot.
;
;  NOTE:
;  Enhanced metafiles are not supported in Windows 3.1
;  under Win32s or on the Macintosh.  If they are called
;  the call will be ignored and no metaobject will be seen.
;
;  NOTE:
;  If the MetaFile is not in the same directory as the NUTS
;  program, then a full path must be included.  If a full
;  path is not included, then the display can come as go as
;  working directories are changed inside NUTS.
;
;  NOTE there can be more than one of these "MetaObjectFile"
;  line pairs in the INI file.
;
;  NOTE %NutsRoot% indicates the directory where the NUTS.INI
;  file is at
;MetaObjectFile
;%NutsRoot%acorn.emf 0.01 0.01 0.06 0.10


;  AXIS keyword has legal values of HEADER, NONE, HZ PPM, and POINTS
;  The keyword header means the file header value should be used.
;  Other keywords override the file header values.
AXIS = ppm

;  The default label for the main menu View / Spectral Parameters
;  and the 2D axis display can be set with the lines below.
DIMLABEL1 = Direct Dimension
DIMLABEL2 = Indirect Dimension 1
DIMLABEL3 = Indirect Dimension 2
DIMLABEL4 = Indirect Dimension 3

;  If this line contains a valid macro, the macro will
;  be executed when NUTS starts.
```

```
;AutoExecMacro = C:\NUTS\MACS\GET_EB.MAC


;  The default for NUTS is to NOT read a file tailer when
;  reading a file.  This is so information such as integral regions
;  and DP lists can be carried over from one spectrum to another.
;  The NUTS behavior can be modified by checking the option
;  under the FILE menu.
;  Setting the keyword below to TRUE makes the default behavior in
;  NUTS to be to read the tailer with each new file read.
AUTOTAILER = FALSE


;  When the HORIZONTAL_SCROLL_BARS flag is set to TRUE the
;  ZO and ID subroutines of the NUTS program will have
;  a bottom horizontal scroll bar for moving the displayed
;  region left and right.
HORIZONTAL_SCROLL_BARS = TRUE


;  When this flag is set to TRUE the NUTS program will
;  close Windows when NUTS is exited.
Exit_Windows = FALSE


;  CALCTYPE keyword allows the Macintosh version of NUTS to
;  set the floating point math calculations to the internal
;  math coprocessor (68881) on 68K Macintosh stystems.  Power
;  Macs should set this to WIN32 to allow floating point
;  math emulation.
;  For 68K macs with math coprocessor uncomment next line
;CALCTYPE = NATIVE


;  By default NUTS will set the initial windows position and size
;  to defalut sizes as determined by the operating system.
;  The values below can be set to override the default behavior
;  and set the X and Y upper left corner of the starting screen
;  and the X width and Y width of the starting screen.
;  All units are in screen pixels.
;  All four values must be set or this entry is ignored.
;X0_START = 3;
;Y0_START = 3;
;XW_START = 900;
;YW_START = 700;



;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[DIRECTORIES]


; NUTS can be configured to not overwite existing files.
; This requires all file saves to be to a non-exiting file name.
;NO_FILE_OVERWRITE = TRUE
```

```
;  DATADIR is the default path name for NUTS file open command.
;  If this term is used, it must be a full path name like
;  "C:\NUTS\DATA\"
;  If this term is not going to be used then leave a semicolon in front
;  of it so that the initialization part of NUTS will ignore it and
;  use its own internal defaults.
;DATADIR = C:\NUTS\DATA\

;  IMPORTDIR is the default path name for NUTS file import command.
;  If this term is used, it must be a full path name like
;  "C:\NUTS\DATA\"
;  If this term is not going to be used then leave a semicolon in front
;  of it so that the initialization part of NUTS will ignore it and
;  use its own internal defaults.
;IMPORTDIR = C:\NUTS\DATA\

;  If DEFAULT_IMPORT_OUT_DIR is set to a value below then that
directory
;  will be the default path where NUTS will put all imported files.
;  The default data directory will also be reset to this directory with
each import.
;  If DEFAULT_IMPORT_OUT_DIR is not set, then all imported files will
go
;  to the current Data Directory which is updated with each GA or GB
operation.
;DEFAULT_IMPORT_OUT_DIR = C:\NUTS\DATA\


;  MACRODIR is the default path name where NUTS will first look for
;  macros when using the run macro (RU) command.
;  If this term is used, it must be a full path name like
;  "C:\NUTS\DATA\"
;  If this term is not going to be used then leave a semicolon in front
;  of it so that the initialization part of NUTS will ignore it and
;  use its own internal defaults.
;MACRODIR = C:\NUTS\MAC\

;  ZZ_FILE_PATH is the default path name for NUTS ZZ auto-import
command.
;  ZZ_FILE_TYPE is the default import type for NUTS ZZ auto-import
command.
;  If these terms are used, it must be a full path and file name like
;     "C:\NUTS\DATA\FILE.QE"
;  and a legal import type like:
;     "ZZ_FILE_TYPE = LYBRICS"
;  If ZZ_FILE_TYPE = Auto  is used the imported file will be auto
;  identified using NUTS standard file detection scheme.
;  If these terms is not going to be used then put a semicolon in front
;  of it so that the initialization part of NUTS will ignore it and
;  bring a File Open Dialog inquiry the first time ZZ is used each
;  NUTS sesion.
ZZ_FILE_PATH = D:\PG\CODE\NUTS\DATA\QEEB.FID
ZZ_FILE_TYPE = Auto


;  Some reports and functions created or used by NUTS use
;  the system browser.  NUTS needs the full path name to
```

```
;  the desired browser to do this.
;  If the browser is "NONE" these the browser launch and report
;  are skipped.
;BROWSER = NONE
;  If the path has spaces the path needs to be enclosed in quotes.
;  If you prefer a different browser then prvide the full path to that
;  browser.
;  Default Directory for Window NT 4
;BROWSER = C:\Program Files\Plus!\Microsoft Internet\iexplore.exe
;  Default Directory for Windows 98 and Windows 2000
BROWSER = C:\Program Files\Internet Explorer\iexplore.exe

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[IMPORT]
;  Some file transfer processes from Bruker unix systems
;  to the PC world incorporate a "byte swap" in the file
;  transfer process. The NUTS importing process wants the
;  file to be an exact image of the Bruker Unix system fid
;  and will get these files wrong on importing.  If the
;  parameters of these files are imported correctly but
;  the fid is wrong, set the parameter below to TRUE.
BRUKER_UNIX_BS = false

;  Some file transfer processes result in the "real" fid and
;  "imaginary" fids being in either the opposite order or
;  the wrong relative phase direction.  NUTS makes a best
;  guess from the header information to correctly determine
;  this. The result of importing this kind of data is that
;  after FT the spectrum is backwards and requires an SR.
;  However, if with the data you use most this is consistently
;  required the flag below can be set to TRUE and NUTS will
;  swap the FIDs on all importing processes.
RI_ON_IMPORT = FALSE

;  Some file transfer processes from Bruker Aspect systems
;  have the "real" fid and "imaginary" fid in the
;  opposite order.  We have been unable to identify
;  any header information to detect this. In addition, we
;  have some information that it depends on the method the
;  data is transferred from the Aspect to the PC.
;  If Bruker Aspect data requires an RI to process without
;       bad "Quadrature" images set the parameter below to TRUE;
BRUKER_ASPECT_RI = FALSE

;  Some file transfer processes from Bruker XWinNMR systems
;  have the "real" fid and "imaginary" fid in the
;  opposite order.  We have been unable to identify
;  any header information to detect this, but this flag
;  will allow the default operation to be reversed.
BRUKER_XWIN_RI = false



;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[HELP]
```

```
;  WinHelp development has stopped for NUTS.  The new help is
;  HTML help.  For the time being both help files are being
distributed.
;  The flag below can be used to set the default help file type to
HTML.
;  The HTML help is more up to date and complete and is the recommended
;  help method for NUTS.  To use the HTML help on Win9x/NT IE 4.0 MUST
be
;  loaded on the computer.  It does NOT have to be the default browser.
;  To use HTML help on Windows 3.1 or Macintosh you must use browser
;  software such as IE 4.0 or Netscape 4.0
HTMLHELP = TRUE

;  The default for NUTS is to use helper dialog boxes where they
;  exist.
;  Setting the keyword below to FALSE configures NUTS to NOT use
;  helpers by default.
HELPERS = TRUE



;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[ROUTINES]


;  Intial values for the AS (Add/Subtract) routine
;  AS_Mult is the initial multiplier
;  AS_DC  is the initial percent vertical screen offset
AS_Mult = 1.0
AS_DC = 33.0

;  Flag used to set certain debug messages during NUTS operation.
;  This is used to help analyze problems remotely which cannot
;  be reproduced at Acorn NMR.
;  0 (zero) is off and 1 (one) is on.
NUTSBUG = 0


;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[AXIS LABELS]
;  The axis has different labels depending on how the data has been
;  processed and the users settings. By default these are:
;    PPM, Hz, sec, pts, slice
;  The user can change them here if desired.
AXIS_LABEL_PPM = PPM
AXIS_LABEL_HZ =  Hz
AXIS_LABEL_SEC = sec
AXIS_LABEL_PTS = pts
AXIS_LABEL_SLICE = slice


;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
```

```
[PHASE]
;  For the phasing operations PH and PE the mouse movement is
;  used to determine the amount of phasing to do.  By default
;  NUTS uses left and right mouse movement for these operations.
;  However, NUTS can be configured to use up and down mouse
;  movements for this operation or the sum of left and right
;  and up and down.
;  Left and Right  = 0
;  Up and Down     = 1
;  Sum L&R and U&D = 2
MOUSE_DIRECTION = 0;

;  Mouse movement step is scaled (multiplied by) the scaling number
;  below during PH and PE.
MOUSE_SCALING_PE = 10
MOUSE_SCALING_PH = 500

; DEFAULT_PA keyword sets the initial PA value used by the PC command

DEFAULT_PA = 0.0

; DEFAULT_PB keyword sets the initial PB value used by the PC command
DEFAULT_PB = 0.0

; DEFAULT_PC keyword sets the initial PB value used by the PC command
DEFAULT_PC = 0.0



;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[FB]
;  The baseline correction command "FB" fits a polynomial
;  to the baseline for the purpose of "straighting" the
;  baseline.  The Symplex method seems to work the best
;  in most cases, however the Least Squares method is
;  50 to 100 times faster and gives better than 90% of the
;  bang for the buck.  Both methods are available from the
;  command line by user choice.  However, when FB is used
;  in a Link or Macro, the flag below will set the method
;  used without user interaction.  The allowed values are
;  TRUE or 0 (for Simplex), FALSE or 1 (for least squares) and
;  2 for "fudge" method.  The latter is not a polynomial fit,
;  but rather removes DC and tilt separately for each segment of
;  the spectrum.  See Baselines in Nuts Help for details.
FB_SYMPLEX = FALSE

; FB and FX baseline correcting routines can be used while in a zoomed
;  display mode. When so used the baselines of the regions
;  outside the zoomed display region can be adjusted in DC value
;  to prevent a discontinuity in the total spectral baseline
;  by setting CONTINUOUS_BASELINE to TRUE.
;  Sometimes, the user would rather have the baseline become
;  discontinuous. One reason being to keep integral values
;  outside of the zoomed display region from being changed by
;  a DC baseline adjustment. To not do these DC baseline adjustments
;  outside of the displayed region set CONTINUOUS_BASELINE to FALSE.
```

```
CONTINUOUS_BASELINE = FALSE



;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[PRINT]
;  The default pen width is 1 (one).  If the line below in uncommented
;  it can be used to set the initial pen width to another value.
PEN_WIDTH_SCREEN  = 1;
PEN_WIDTH_PRINTER = 2;


;  PRINT_BOX keyword sets the default presentation of plots.
;       If TRUE then a box will be printed around the plot.
;       Otherwise no box will be printed.
PRINT_BOX = TRUE


;  PRINT_PARAMETERS keyword sets whether the spectral parameters
;  will be printed on each plot.
;       If TRUE then the parameters will be printed on each plot.
;       Otherwise they will not be printed.
PRINT_PARAMETERS = TRUE


;  SQUARE_2D keyword sets whether 2D contour and intensity plots
;  are forced to be square in the X and Y dimensions.
;       If TRUE then the 2D plots will be square.
;       Otherwise they will not be forced to be square.
SQUARE_2D = FALSE


;  PRINT_COLOR keyword sets whether the display colors will be
;  sent to the printer as displayed or remapped to black and white.
;       If TRUE then the colors will be printed as displayed.
;       Otherwise they will be converted to black and white.
PRINT_COLOR = FALSE


;  TOP_MARGIN keyword sets the printer's top margin in millimeters.
;  Devices such as laser printers often have a "dead" area
;       on the edges of the paper.  This margin is in addition
;       to the devices "dead" area.
TOP_MARGIN = 7


;  BOTTOM_MARGIN keyword sets the printer's bottom margin in
millimeters.
;  Devices such as laser printers often have a "dead" area
;       on the edges of the paper.  This margin is in addition
;       to the devices "dead" area.
BOTTOM_MARGIN = 7


;  LEFT_MARGIN keyword sets the printer's left margin in millimeters.
;  Devices such as laser printers often have a "dead" area
```

```
;       on the edges of the paper.  This margin is in addition
;       to the devices "dead" area.
LEFT_MARGIN = 1


;  RIGHT_MARGIN keyword sets the printer's right margin in millimeters.
;  Devices such as laser printers often have a "dead" area
;       on the edges of the paper.  This margin is in addition
;       to the devices "dead" area.
RIGHT_MARGIN = 1


;  ???_2D_MARGIN keyword sets the specified margin of a 2D IP or CP
;  display as a fraction of the current windows width.
TOP_2D_MARGIN = 0.10;
BOTTOM_2D_MARGIN = 0.10;
LEFT_2D_MARGIN = 0.10;
RIGHT_2D_MARGIN = 0.10;



;  DISPLAY_MONO keyword sets whether the display will be
;  in color or monochrome.
;       If FALSE then the display will be in color,
;       otherwise, the display will be monochrome.
DISPLAY_MONO = FALSE


;  When doing inserts (inset plots) the user can specify the
;  hertz per centimeter for the lenth of the insert when
;  plotted.  The device drivers for the printer devices sometimes
;  hav an error of a certain reproducible percentage.  The user
;  can enter that error here so it can be automatically set when
;  adding inserts.
INSERT_PRINTER_FUDGE = 1.000




;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[COLOR]


; Colors will vary with different types of Windows graphics drivers.
; They are defined here as the scale of the red, green and blue parts
; of the color spectrum on a scale between 0 and 255 intensity;
; Zero is no color such that if all three colors were a level 0 the
; color would be black.  If all three colors were defined to be 255
; the color would be white.
AXIS_RED   = 255
AXIS_BLUE  = 0
AXIS_GREEN = 0

REAL_RED   = 0
REAL_BLUE  = 255
REAL_GREEN = 0
```

```
IMAG_RED   = 0
IMAG_BLUE  = 0
IMAG_GREEN = 255

INTEGRAL_RED   = 128
INTEGRAL_BLUE  = 128
INTEGRAL_GREEN = 128


; The Contour Displays(CP) and Intensity Displays (IP) can be set to
use
; different number of levels (between 1 and 10) and each level can be
; displayed in a different color.

IP_LEVELS = 10

; Each level will be at the value set by MH times the values below.
; Care needs to be taken that the values always increase.
IP_LEVEL_1 = 1.0
IP_LEVEL_2 = 1.5
IP_LEVEL_3 = 2.3
IP_LEVEL_4 = 3.5
IP_LEVEL_5 = 5.2
IP_LEVEL_6 = 7.8
IP_LEVEL_7 = 11.6
IP_LEVEL_8 = 17.5
IP_LEVEL_9 = 26.2
IP_LEVEL_10 = 40.0


; The color will vary with different types of Windows graphics drivers.
; They are defined here as the scale of the red, green and blue parts
; of the color spectrum on a scale between 0 and 255 intensity;
; Zero is no color such that if all three colors were a level 0 the
; color would be black.  If all three colors were defined to be 255
; the color would be white.
IP_RED_1 = 150
IP_RED_2 = 150
IP_RED_3 = 150
IP_RED_4 = 150
IP_RED_5 = 125
IP_RED_6 = 100
IP_RED_7 = 75
IP_RED_8 = 50
IP_RED_9 = 25
IP_RED_10 = 0

IP_GREEN_1 = 150
IP_GREEN_2 = 150
IP_GREEN_3 = 150
IP_GREEN_4 = 150
IP_GREEN_5 = 125
IP_GREEN_6 = 100
IP_GREEN_7 = 75
IP_GREEN_8 = 50
IP_GREEN_9 = 25
```

```
IP_GREEN_10 =0

IP_BLUE_1 = 150
IP_BLUE_2 = 150
IP_BLUE_3 = 150
IP_BLUE_4 = 150
IP_BLUE_5 = 125
IP_BLUE_6 = 100
IP_BLUE_7 = 75
IP_BLUE_8 = 50
IP_BLUE_9 = 25
IP_BLUE_10 = 0

;  Now for the negative contour levels
IP_RED_MINUS_1 = 25
IP_RED_MINUS_2 = 50
IP_RED_MINUS_3 = 75
IP_RED_MINUS_4 = 100
IP_RED_MINUS_5 = 125
IP_RED_MINUS_6 = 150
IP_RED_MINUS_7 = 175
IP_RED_MINUS_8 = 200
IP_RED_MINUS_9 = 225
IP_RED_MINUS_10 = 255

IP_GREEN_MINUS_1 = 0
IP_GREEN_MINUS_2 = 0
IP_GREEN_MINUS_3 = 0
IP_GREEN_MINUS_4 = 0
IP_GREEN_MINUS_5 = 0
IP_GREEN_MINUS_6 = 0
IP_GREEN_MINUS_7 = 0
IP_GREEN_MINUS_8 = 0
IP_GREEN_MINUS_9 = 0
IP_GREEN_MINUS_10 = 0

IP_BLUE_MINUS_1 = 0
IP_BLUE_MINUS_2 = 0
IP_BLUE_MINUS_3 = 0
IP_BLUE_MINUS_4 = 0
IP_BLUE_MINUS_5 = 0
IP_BLUE_MINUS_6 = 0
IP_BLUE_MINUS_7 = 0
IP_BLUE_MINUS_8 = 0
IP_BLUE_MINUS_9 = 0
IP_BLUE_MINUS_10 = 0


;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[STACKED PLOT]


;  The keyword X_OFFSET allows the operator to set the default
;  stacked plot X offset values,  a typical value is 10.0 and
represents
;  the % the x width will be reduced on the first plot.
```

```
X_OFFSET = 10.0;

;  The keyword Y_OFFSET allows the operator to set the default
;  stacked plot Y offset values,  a typical value is 1.0 and represents
;  the Y offset which will put all the plots in the display.
;  A larger number will make the plots farther apart in the vertical
;  direction, while a smaller number moves them closer together.
Y_OFFSET = 1.0

;  The keyword DC_OFFSET allows the operator to make the default
;  screen and plot Y offset other than zero.
;  Start with numbers like 10 and 20 then adjust to desired value;
DC_OFFSET = 0



;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[PEAK PICKING]


; When the peakpicking operation is done, a copy of the peak pick table
; is placed into the clipboard. This table is done in two possible
ways:
; 1) space separated columns such that, when a fixed size font is used,
;    the columns line up. Best for pasting into Notepad. Set UseTabs to
FALSE.
; 2) tab separated columns. Best for pasting into a spreadsheet
program.
;    Set UseTabs to TRUE
; This column separation is also used for the Integral Tables.
UseTabs = TRUE

; MH keyword sets the initial Minimum Height for the Peakpicking
command
MH = 10.0


; RM keyword sets the multiple of the RMS noise level
; a peak must change after a maximum before it will be declared a real
; peak in the peakpicking command
RM = 3

;When NUTS is in its base level of operation and
;the right mouse button is pressed then a target crosshair is
;displayed on the screen. While the crosshair is being displayed,
;the "M" sub-command resets the MH value to the level of the
;horizontal crosshair. It is sometimes convenient to have NUTS
;automatically do a new PP command when the M sub-command is given.
;The default action can be set in the NUTS.INI with the line:
DO_PP_WITH_M = TRUE

; DP_PREVENT_OVERLAP keyword sets the initial mode for whether the DP
labels
; try to automatically avoid overlap
DP_PREVENT_OVERLAP = TRUE
```

```
; DP_ONTOP keyword sets the initial mode for the DP labels to be at the
; top of the display
;DP_ONTOP = TRUE
DP_ONTOP = TRUE


; DP_WITHLINE keyword sets the initial mode for the DP labels to have a
line
; drawn from label to indicate where the peak is at for that label
;DP_WITHLINE = TRUE
DP_WITHLINE = FALSE


; DP_ALWAYSINFO keyword sets the initial mode for the DP labels to be
showing
; the information field always even when peak labels are off
DP_ALWAYSINFO = FALSE


; DP_FIRSTARG keyword sets the initial mode for the DP labels to show
only
; the first argument (to a space) instead of the entire info field.
This is
; most useful in the searchable archive accessory
DP_FIRSTARG = FALSE


; default 1D DP peak label orientations are vertical.
; One of the following lines can be uncommented (remove the semicolon)
; to change this default.
1D_DP_LABELS = vertical
;1D_DP_LABELS = horizontal


; default 2D DP peak label orientations are horizontal.
; One of the following lines can be uncommented (remove the semicolon)
; to change this default.
;2D_DP_LABELS = vertical
;2D_DP_LABELS = horizontal



;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[OFFSET INFORMATION]
; When in the target display mode (crosshair on the screen) pressing
; "V" brings up the Offset Information dialog box. The user then enters
; the chemical shift information for the current crosshair position.
; By default the system uses a three point Peak Interpolation for
setting
; the chemical shift. Also by default the system will "snap to tallest
; nearby peak" mode when setting the chemical shift. The user can set
; the set the default modes below by removing the comment semi-colon
; and setting the argument to either TRUE or FALSE. The Peak
Interpolation
```

```
; is also used by the peak picking routine when reporting peak
positions.
;INTERPOLATE_PEAKS=FALSE
;SNAP_TO_PEAK=FALSE



;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[LINE BROADENING]

; LB keyword sets the default value for LB when the program is started.
LB = 0.2


;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[LINE FIT]

;  Fraction_Lorenztian keyword sets the default fraction for Lorenztian
lineshape
;  used by routines lile LF.  The fraction Gaussian lineshape id 1
minus this value.
Fraction_Lorentzian = 1.0



;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[INTEGRAL]
;  ID_DISPLAY sets the sub-integral value display to the
;  end of the sub-integral if it is set to END,
;  top of the display if it is TOP and
;  at the bottom just above the axis if it is BOTTOM
ID_DISPLAY = END

The default orientation for the integral labels can be set
by setting the following line to TRUE or FALSE
ID_HORIZONTAL_FONT=TRUE


;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[MACRO]
;  Specified macros can be run with the Ctrl-Fxx function keys.
;  To enable this feature enter the full path to the desired
;  macro and remove the semi-colon from the front of one of the
;  lines below.
;Macro_1 =  C:\NUTS\MAC\MAG_COSY.MAC
;Macro_2 =  C:\NUTS\MAC\MAG_COSY.MAC
;Macro_3 =  C:\NUTS\MAC\MAG_COSY.MAC
;Macro_4 =  C:\NUTS\MAC\MAG_COSY.MAC
;Macro_5 =  C:\NUTS\MAC\MAG_COSY.MAC
;Macro_6 =  C:\NUTS\MAC\MAG_COSY.MAC
;Macro_7 =  C:\NUTS\MAC\MAG_COSY.MAC
;Macro_8 =  C:\NUTS\MAC\MAG_COSY.MAC
;Macro_9 =  C:\NUTS\MAC\MAG_COSY.MAC
```

```
;Macro_10 = C:\NUTS\MAC\MAG_COSY.MAC
;Macro_11 = C:\NUTS\MAC\MAG_COSY.MAC
;Macro_12 = C:\NUTS\MAC\MAG_COSY.MAC



;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[LINKS]
;  LINKS can be set with the keyword LINK#
LINK1 IM BC EM FT PS
LINK2 BC EM FT AP
LINK3 EM FT IN
LINK4 EM FT MC IN
LINK5 GA BC EM FT PS TR IA GA BC EM FT PS TR ST IN
LINK6 GA EM FT SR SC IN
LINK7 BC EM FT PS
LINK8 GA BC EM FT IN
LINK9 GA MS FT SR MC SC IN
LINK0   GA BC EM FT PS SC IN




;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;
[FONT]
; The keyword "AxisFont" allows the default font for the axis to be
reset
; Its use is slightly different from other INI entries.
; The keyword alone appears on the first line.
; The next line MUST be the Font Name
; The third line MUST be 10 times the desired point size.
; The fourth line MUST be the weight of the font.
;      0 to 400 is normal weight.
;      401 to 900 is bold weight.
; The fifth line is a 1 for italic and 0 for normal.
;    If these terms are not going to be used then put a semicolon in
front of the
;    lines so that the initialization part of NUTS will ignore them and
;    use its own internal defaults.
AxisFont
Arial
100
400
0

; The keyword "CmdLine" allows the default font for the Command Line to
be reset.
; Its use is slightly different from other INI entries.
; The keyword alone appears on the first line.
; The next line MUST be the Font Name
; The third line MUST be 10 times the desired point size.
; The fourth line MUST be the weight of the font.
;      0 to 400 is normal weight.
;      401 to 900 is bold weight.
```

```
; The fifth line is a 1 for italic and 0 for normal.
;    If these terms are not going to be used then put a semicolon in
front of the
;    lines so that the initialization part of NUTS will ignore them and
;    use its own internal defaults.
CmdLine
SYSTEM_FIXED_FONT
90
400
0

; The keyword "IntegralFont" allows the default font for the integrals
to be reset
; Its use is slightly different from other INI entries.
; The keyword alone appears on the first line.
; The next line MUST be the Font Name
; The third line MUST be 10 times the desired point size.
; The fourth line MUST be the weight of the font.
;      0 to 400 is normal weight.
;      401 to 900 is bold weight.
; The fifth line is a 1 for italic and 0 for normal.
;    If these terms are not going to be used then put a semicolon in
front of the
;    lines so that the initialization part of NUTS will ignore them and
;    use its own internal defaults.
IntegralFont
Arial
100
400
0

; The keyword "NoteFont" allows the default font for the Notes to be
set.
; Its use is slightly different from other INI entries.
; The keyword alone appears on the first line.
; The next line MUST be the Font Name
; The third line MUST be 10 times the desired point size.
; The fourth line MUST be the weight of the font.
;      0 to 400 is normal weight.
;      401 to 900 is bold weight.
; The fifth line is a 1 for italic and 0 for normal.
;    If these terms are not going to be used then put a semicolon in
front of the
;    lines so that the initialization part of NUTS will ignore them and
;    use its own internal defaults.
NoteFont
Arial
100
400
0

; The keyword "ParmFont" allows the default font for the plots
parameter to be reset.
; Its use is slightly different from other INI entries.
; The keyword alone appears on the first line.
; The next line MUST be the Font Name
; The third line MUST be 10 times the desired point size.
```

```
; The fourth line MUST be the weight of the font.
;      0 to 400 is normal weight.
;      401 to 900 is bold weight.
; The fifth line is a 1 for italic and 0 for normal.
;  If these term is not going to be used then put a semicolon in front
of the
;  lines so that the initialization part of NUTS will ignore them and
;  use its own internal defaults.
ParmFont
Times New Roman
100
400
0

; The keyword "PeakFont" allows the default font for the peak values on
the screen
; to be reset.
; Its use is slightly different from other INI entries.
; The keyword alone appears on the first line.
; The next line MUST be the Font Name
; The third line MUST be 10 times the desired point size.
; The fourth line MUST be the weight of the font.
;      0 to 400 is normal weight.
;      401 to 900 is bold weight.
; The fifth line is a 1 for italic and 0 for normal.
;  If these term is not going to be used then put a semicolon in front
of the
;  lines so that the initialization part of NUTS will ignore them and
;  use its own internal defaults.
PeakFont
Arial
90
400
0

; The keyword "ClipFont" allows the default font for the clipboard font
to be reset.
; Its use is slightly different from other INI entries.
; The keyword alone appears on the first line.
; The next line MUST be the Font Name
; The third line MUST be 10 times the desired point size.
; The fourth line MUST be the weight of the font.
;      0 to 400 is normal weight.
;      401 to 900 is bold weight.
; The fifth line is a 1 for italic and 0 for normal.
;  If these term is not going to be used then put a semicolon in front
of the
;  lines so that the initialization part of NUTS will ignore them and
;  use its own internal defaults.
ClipFont
Courier New
100
400
0


;end INI file
```

Setting colors in NUTS

Colors for the spectrum, axis and integrals can be set in the nuts.ini file.

BW – Black and white display

CD – Color display

The display can be changed from color to black and white either from the File/Page Setup menu selection, or with the commands **BW** (changes all colors to black) and **CD** (color display on).

WC – Which color

The color of the spectrum can be changed while NUTS is running using the **WC** (which color) command.

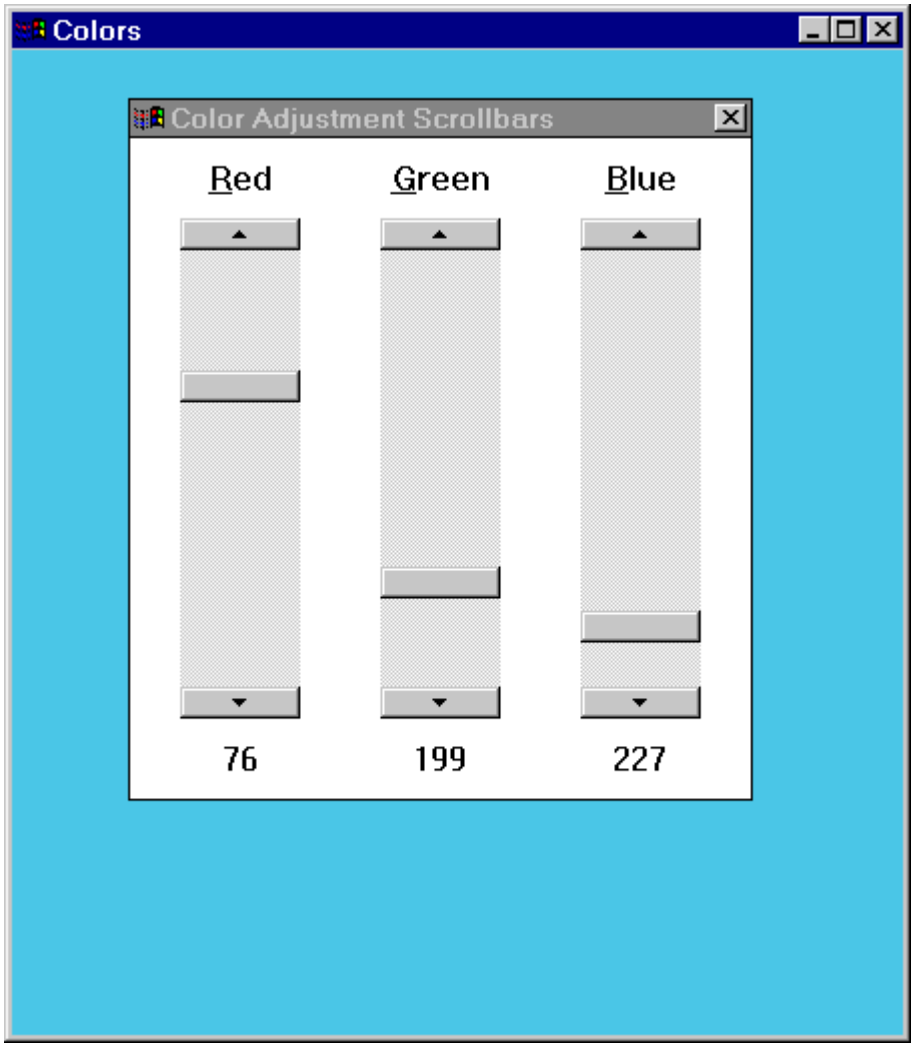
The color of each spectrum in the Buffers subroutine can be set from the Edit menu in that subroutine.

CR – Contour level colors

The colors for each level of a contour plot are set in the nuts.ini file or can be entered while NUTS is running using the **CR** command. Colors are set by entering numerical values for Red, Green and Blue.

In the dialog boxes to set fonts, there is an option for choosing the font color.

Included in the NUTS installation files for the Windows version is a useful program called colors.exe, which lets the user experiment with setting RGB values by showing the resulting colors, as shown below.



Setting fonts

The fonts used within NUTS can be set by the user either within the NUTS program or in the NUTS.INI file. If set in the NUTS.INI file, the selected fonts will be implemented each time NUTS is run. The font can be set independently for each use of text within NUTS: the axis label, the command line at the bottom of the screen, the parameters printed on the bottom of plots, the clipboard display on the screen, integral values and peak labels.

Within NUTS, fonts can be set by choosing Set Fonts from the Edit menu. A sub-menu allows selection of the text whose font is to be changed, and a typical Windows font dialog box is displayed. The font choices are all scalable fonts which are available on both the screen and printer, to make the operation as WYSIWYG (What You See Is What You Get) as possible. In practice, font sizes of 8-10 are recommended for best visibility. For the clipboard display (see CB command) of peak lists or integral lists, a fixed-space

font such as Courier is recommended so that the columns line up. Note that the amount of space taken up by the clipboard display is not the same on the screen and on plots, so it is best to test the font choice by printing.

The font for each type of text can also be set using the following 2-letter commands from the keyboard, by-passing the menus:

FA Select font parameters for axis.
FC Select font parameters for clipboard display using CB.
FI Select font parameters for integral labels.
FL Select font parameters for command line.
FM Select font parameters for parameters printed on plots.
FH Select font parameters for horizontal peak labels.
FV Select font parameters for vertical peak labels.

FA -- Set axis font

Brings up a Windows font dialog box allowing selection of font parameters for the axis label. This command is also available by choosing Set Fonts from the Edit menu. The font choices are all scalable fonts which are available on both the screen and printer, to make the operation as WYSIWYG (What You See Is What You Get) as possible. In practice, font sizes of 8-10 are suggested for best visibility. The text may look slightly different when printed, so it is best to test the choice of font by printing.

FM -- Set font for parameter list on plots

Brings up a Windows font dialog box allowing selection of font parameters for the list of spectral parameters printed on the bottom of plots. This command is also available by choosing Set Fonts from the Edit menu. In practice, font sizes of 8-10 are suggested for best visibility. It is best to test the choice of font by printing.

The user can choose whether or not spectral parameters should be printed on plots by choosing Page Setup from the File menu. This can also be set in the NUTS.INI file. An alternative to printing the parameters on the bottom of plots is to use the LP command to place the parameters into the Windows clipboard and then use CB to display the contents of the clipboard on the plot.

FC -- Set font for clipboard display

Brings up a Windows font dialog box allowing selection of font parameters for text display using the CB command. This command is also available by choosing Set Fonts from the Edit menu. The font choices are all scalable fonts which are available on both the screen and printer, to make the operation as WYSIWYG (What You See Is What You Get) as possible. In practice, font sizes of 8-10 are suggested for best visibility. For display of peak lists or integral lists, a fixed-space font such as Courier is recommended so that the columns line up. Note that the amount of space taken up by the clipboard

display is not the same on the screen and on plots, so it is best to test the font choice by printing.

FI -- Set font for Integral labels

Brings up a Windows font dialog box allowing selection of font parameters for labels on integral segments. This command is also available by choosing Set Fonts from the Edit menu. The font choices are all scalable fonts which are available on both the screen and printer, to make the operation as WYSIWYG (What You See Is What You Get) as possible. In practice, font sizes of 8-10 are suggested for best visibility. The text may look slightly different when printed, so it is best to test the choice of font by printing.

With addition of the option to display integral labels vertically (Dec 2002), the fonts for horizontal and vertical integral labels are set independently. This means the **FI** command displays the font dialog box twice, for the 2 different orientations.

FL -- Set font for command line

Brings up a Windows font dialog box allowing selection of font parameters for the command line at the bottom of the screen. This command is also available by choosing Set Fonts from the Edit menu. In practice, a font size of about 10 is suggested for best visibility.

FH -- Set font for horizontal peak labels

Brings up a Windows font dialog box allowing selection of font parameters for the chemical shift labels displayed above peaks. Each label can be oriented either horizontally or vertically. The labels are toggled on and off by typing **Ctrl-P**. This command is also available by choosing Set Fonts from the Edit menu. A font size of 8 is suggested. It is best to test the choice of font by printing because appearance on the screen and plots will usually differ.

FV -- Set font for vertical peak labels

Brings up a Windows font dialog box allowing selection of font parameters for the chemical shift labels displayed above peaks. Each label can be oriented either horizontally or vertically. The labels are toggled on and off by typing **Ctrl-P**. This command is also available by choosing Set Fonts from the Edit menu. A font size of 8 is suggested. It is best to test the choice of font by printing because appearance on the screen and plots will usually differ.

Page setup

There are several options which can be set from the File/Page Setup menu. Some can also be set in the NUTS.INI file, so that they are established each time the program is run.

Square 2D plots -- Check this box to force 2D plots to be square. Does not affect 1D plots.

Border box -- Refers to a border drawn around the entire plot.

Parameters on page -- 3 lines of acquisition and processing parameters printed below the spectrum.

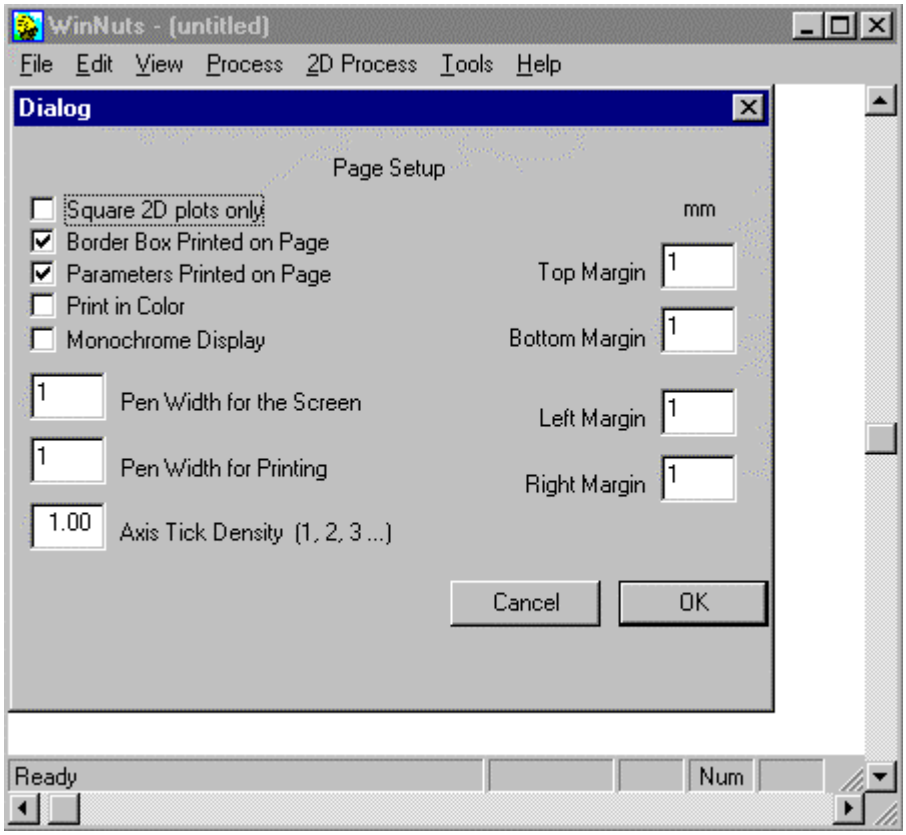
Print in color -- By default, Nuts converts all colors to black when printing. Check this box to print colors as shown on the screen. Note that colors of different objects can be set in the Nuts.ini file.

Monochrome display -- Turns all colors on the screen to black. This is most useful when copying spectra for pasting into other applications, which will then be printed on a black-and-white printer. In that situation, some applications "dither" the colors, and they end up as dotted lines. This can also be set with the BW command, and unset with CD.

Pen width on screen and on printer -- These 2 parameters allow independent adjustment of thickness of lines on the screen and on plots. In some cases, such as making transparencies, the thin lines printed on high resolution printers do not show up well, which can be solved by drawing the lines thicker. Starting with version 971106, this can also be set in the nuts.ini file.

Axis tick density -- Nuts makes its own decisions about the number of axis tick marks that will fit comfortably on a page. This allows the user to override the default settings, and draw more or fewer tick marks.

Margins -- The margins on all 4 sides of a plot can be set independently in millimeters. Note that this is in addition to the default, which is very narrow margins.



Startup options

NUTS can executed with various options by including arguments on the command line. The choices are indicated by a switch that tells NUTS what to do with the file name that follows. The switches are:

-f open file
-m run macro
-i start NUTS using a different nuts.ini file

A single argument is assumed to be a file that should be opened automatically as soon as NUTS starts, equivalent to the **-f** switch. The complete path can be specified using single or double quotes. These 2 commands are equivalent, and will open the file called **file.fid** as when NUTS starts:

```
nuts.exe "c:\data\file.fid"
nuts.exe -f "c:\data\file.fid"
```

A macro can be run immediately on startup in two different ways. If the same macro should be run every time, it can be specified in the nuts.ini file with the keyword

AutoExecMacro. If different macros should be run, the macro can be specified on the command line with the -m switch. For example:

```
nuts.exe -m "c:\macro\file.mac"
```

By default, when NUTS is started, it reads a file called nuts.ini, which must be in the same folder as the NUTS program. The nuts.ini file contains the user's preferences for many different parameters. If the user wants to use a different nuts.ini file when NUTS starts, it is signaled by a "**-i**" switch, where the next argument must be the path to the nuts.ini file, enclosed in double or single quotes. For example,

```
nuts.exe -i "c:\fred\nuts.ini"
```

Setting the computer time

The Windows version of NUTS can synchronize your computer's time to an Internet Time Server. This will only work if your computer is connected to the Internet when this operation is performed.

Choose Set NetTime from the Help menu. Click on the Set Correct Time button to synchronize. A list of Timer Service servers is displayed by clicking on the Select Server button.

Hardware considerations

Probe details

Probe construction and performance can seem like black magic because there are many details requiring close attention. Some of the details can be mutually exclusive, requiring some creative compromise. Since many of the "details" are VERY important, getting any one of them wrong may make the probe's performance suboptimal. Probe Q is discussed in a separate Note. Listed in the table below are other important probe performance parameters.

Sensitivity
90^o Pulse Width
Resolution and Lineshape
RF Pulse Homogeneity
VT Range and Regulation
Decoupler Gamma H_2

Sensitivity
The most important details which affect a probes sensitivity are:

Coil Length
Coil Filling Factor
Probe Q
Dielectric value of sample
Percentage of the overall coils inductance in the coil leads

The first and second items in the Sensitivity Parameters list reduce to how much sample can the probe coil see. It is not the first thing that comes to an NMR operator's mind when thinking of sensitivity, but it is one of the most commonly varied probe parameters in the vendors' war of sensitivity numbers. For a 5mm ^1H probe, the typical coil is between 10 and 20 mm long. Coils as long as 25 mm have been produced. At least three things limit the possible coil length:

1. **Length of magnet's high homogeneity magnetic field**
In general, the higher the magnetic field, the shorter the useful magnetic length. Also the more narrow the bore, the shorter the useful magnetic field. A perfect probe can never perform better than the magnet homogeneity allows. Many vendors use long coil lengths, which make magnet to magnet variations more important. Some vendors optimize probe coil lengths based on each magnet. If you are in the market to buy an instrument, this can be one of the potential reasons why the sales demonstrations can be better than the performance you get on delivery.

2. **Length of the room temperature shims' active region**
When the main magnetic field is long enough, the probe designer still needs to be concerned with the useful area over which the RT shims operate. If the main field is long enough and the shims don't operate with the necessary purity over the coil's length, the

probe's performance will be suboptimal. The first symptom that the coil is too long for the magnet and shim set is a tendency for the NMR signals to "split" or become doublets with VERY small changes in Z^2 or with small changes in room temperature.

3. **RF Homogeneity**
Solenoid coils can have good RF homogeneity over long lengths. However, most superconducting magnet probes must use some type of Helmholtz coils. As these coils get longer, they have lower RF homogeneity. One way to see this low RF homogeneity is to measure the ratio of the signal from a 90 degree pulse and a 180^o or 360^o pulse. The larger the ratio, the better the probe's RF homogeneity. This parameter can be very important in many 2D experiments even with compensated pulses.

The other dimension determining coil filling factor is off-axis. The filling factor in this dimension is limited by how close to the sample the coil can be placed. As the sample insert is brought in closer to the sample tube the tolerances on probe construction become tighter. The first symptom of these tolerances being wrong is trouble with sample spinning. As these tolerances get tighter, higher quality tubes are required. Another way to get a better filling factor is to use thinner wall tubes. The thinner walls allow more sample to be inside the probe's coil and therefore the probe gives higher sensitivity numbers. The sensitivity difference from very thin wall tubes is much larger with 5mm probes than larger diameter probes. When looking at probe performance specifications from NMR vendors be sure to know what sample tubes they are using.

Probe coils

One very critical part of a probe is the probe coil itself. Great care is taken to design probe coils for the best RF homogeneity and highest Q as well as many other important parameters. In this design process, the leads between the active probe coil and the probe's tuning and matching capacitors are often overlooked. These leads can play a very important role in the overall probe performance.

The first area of consideration is the length of the probe coil's leads. The longer the leads, the farther removed the capacitors and other probe components can be from the active area of interest. This is good because these components have a magnetic susceptibility which affects probe resolution, lineshape and shimability. It is bad because the leads add inductance to the total probe coil inductance AND are not surrounded by sample. In effect, this condition reduces the probe coil's filling factor. The maximum sensitivity would occur when all of the sample coil inductance was filled with sample. In probe design, the probe sensitivity can be improved when the ratio of active coil inductance to coil lead inductance is increased. This is one reason why multiple turn coils give better results than single turn coils. This holds true whether the multiple turns are in parallel or series.

One way to minimize the effect of the probe coil's leads is to get as much of the tuning capacitance near the active part of the coil as possible. Since the circulating current in a probe is proportional to the Q and impedance level of the coil, having most of the

capacitance at the bottom of the coil and before the leads would reduce the ability of the leads to pick up signal. However, it also reduces the tuning range from the variable capacitor at the end of the leads. This range is not only needed for tuning the probe to other nuclei but for samples of different ionic strength on the same nucleus.

Where the leads are located relative to the sample is also very important. If the probe coil's leads are run up the sample insert, then they will be near the sample in areas removed from the main active region of the probe coil. These removed areas have at least two major problems. The first is that they do not have good B1 homogeneity. Possible consequences of poor B1 homogeneity are poor inversion and poor water saturation. The second major problem area is that the sample near the leads experiences a different magnetic field value, resulting in poor spectral lineshape (hump). This phenomenon gives rise to a strange set of conditions with unexpected results. If the magnetic field were perfect in all areas where there is sample and coil inductance, then the lineshape would be perfect. If the magnetic field is perfect in the active area of the coil but of an extremely different value (ie., would give rise to an NMR signal outside the spectral window) in the area of the leads the lineshape would be okay. This means that often a very good magnet or a very poor magnet can give good lineshape results, but an in between magnet would give poor results.

To get around the "lead pickup" problem, the coil's leads must either not pick up sample signal or be shielded from the sample signal. This can be done in many ways:

Geometrically position the leads to minimize differential signal pickup by each lead. This works because if both leads see the same signal there is no induced voltage across the coil's output and therefore no signal from the leads.

- Shield the leads with a band of copper or another material (plating) in the area of the leads.
- Arrange a virtual ground area of the coil to shield the leads.
- Get as much of the coil's tune capacitance as close to the active coil as possible. This lowers the current in the leads and thereby the lead pickup.

One way to test your current ^{1}H probe's lead response is to take a very small drop of water in the bottom of a 5mm tube and place it in the center of the probe coil. Run a spectrum using a very wide sweep width, process and integrate the spectrum. Set the integral to a value such as 100. Now repeat the process changing the sample depth in 2 mm increments using the same normalization and processing constants. Move in both directions until the signal goes to zero. Be certain to go far enough since the signal often goes to zero and returns as the sample is moved from the coil to the lead area. Use these data to create a plot of integrated intensity versus sample depth. This plot has a wealth of information. It shows the proper sample depth, the length of the coil, and any lead pickup taking place. Sometimes lead pickup can be minimized by pulling the bottom of the sample up above the area of lead pickup. This makes shimming more difficult due to end effects of the sample, but should reduce lead pickup.

Probe Q

One parameter affecting sensitivity is the probe Q. Q is defined as the frequency of the resonant circuit divided by the half power bandwidth. Many of today's probes have unloaded Qs greater than 300. If all other parameters are the same, the higher the probe Q the greater the sensitivity.
What determines the probe Q? The AC resistance of the resonant circuit. The lower the AC resistance, the higher the probe Q. A very simple answer to a very complex question. The probe has two components which can limit the AC resistance: the coil and the capacitor of the resonant circuit.

The Coil
The resistance of the coil (wire) goes up as the frequency goes up by a phenomenon called "Skin Effect". The "Skin Effect" is caused by the magnetic field created by the current in the wire. The magnetic field forces the electrons to move in a curved path until they hit the surface of the wire. Therefore the current is forced to flow in a smaller part of the wire. Said in another way the, wire appears smaller to a high frequency current than a low frequency current. The smaller area for current flow raises the AC resistance to current flow and thereby lowers the Q of the resonant circuit. Some probe designers combat this by using foil, as opposed to wire, to increase the surface area. Other probe designs use separate coils in parallel. Since resistances in parallel are less than either individual resistance, the AC resistance of parallel coils is less than in either individual coil. Another advantage of parallel coils is that the total inductance of parallel coils is less than in either individual coil. Since the higher frequency probes tend to have very small capacitance values for resonance, the lower inductance makes it easier to reach higher frequencies with a given capacitance. Often the minimum value of this capacitance is limited by the stray capacitance. The parallel inductors lower the total inductance and therefore higher capacitor values can be used.
The AC resistance of the coil is also determined by its geometry. This can be a very complex issue, but some of its basic characteristics can be simplified. If the coil has any sharp turns, the electrons from the probe's current generate a magnetic field causing them to crowd to the inside edge. This crowding reduces the effective amount of conductor available to the current, thereby raising its resistance and lowering Q. This effect is very similar to the "Skin Depth" effect discussed before. The electron crowding in the corners also raises the inductance per unit distance of electron flow. Therefore, to get the lowest resistance and inductance per unit of length, the degree of sharpness at all corners of the probe coil should be limited as much as possible.

The Capacitor
The AC resistance of the capacitors is usually related to the materials from which they are constructed. Air capacitors formed by two pieces of conductor in parallel have a very low resistance and therefore can have a very high Q. To obtain a given capacitance value, this type of capacitor needs to be very big or have its parallel surfaces very close. Mechanical tolerances and electrical arcing under the high voltages of an RF pulse limit the use of this type of capacitor. To get the surfaces closer together and increase the capacitance of a given surface area, capacitors have a dielectric between the conductive

surfaces. This dielectric introduces higher resistance in the capacitor, which lowers the Q of the resonant circuit. The material from which the tunable capacitors are constructed is usually the most lossy and therefore lowers the Q the most. For this reason, a compromise between the desired tuning range and the probe Q is a problem many probe designers face.

The Sample

One component which limits probe Q is the sample. The sample increases losses in the resonant circuit by inducing eddy currents in the solvent. The more conductive the sample the more the losses and the lower the probe Q. This is often seen by the longer 90^{o} pulses with water samples than with organic solvent samples.

The losses in the sample are induced by the electric field values inside the probe. The more distributed capacitance a probe has in its resonant circuit, the lower the electric field values. Therefore, a probe with distributed capacitance is less affected by the sample. Unfortunately, the capacitor usually has a higher resistance than the coil. Therefore, the use of distributed capacitance lowers the probe's unloaded Q, but can raise the loaded Q. This statement reduces to the probe designer's need to compromise probe design between organic and water samples.

As was indicated before, probe design entails many details and compromises. These can often be between mutually exclusive requirements.

Noise factor

One important parameter which indicates how well a spectrometer is working is the noise figure of the preamp and total system. An inexpensive method of determining the system noise performance is the "hot/cold resistor" test. Done with care, this can provide valuable insight into spectrometer performance.

The first step is constructing the noise source. Take a piece of RG58 coax cable and carefully strip the cover back about 2 inches. Now slide the shield back as far as possible (about 1 inch), cut the exposed center conductor to a 1/2 inch in length and strip off the insulation on a 1/4 inch of the conductor. Solder a 1% 50 Ohm metal film resistor to the center lead. Cover with shrink tubing and shrink the cover tight leaving the unconnected end of the resistor exposed. Slide the shield down over the resistor and solder the shield and the unconnected end of the resistor together to make a shielded noise source. Take care to make all leads as short as possible. In most environments, it is very important to use a shielded noise source to help reduce outside RF noise. Interference renders this measurement useless.

Carbon resistors increase resistance as much as 20% when cooled in liquid nitrogen. For this reason, two sources need to be prepared if carbon resistors are to be used. One which measures 50 Ohm at 20oC and one which measures 50 Ohm in liquid nitrogen. Determine the values with an Ohm meter. The metal film resistors change only a few percent when cooled by liquid nitrogen and are therefore preferred.

Connect the shielded 50 Ohm noise source to the input of the preamp in place of the probe. If the entire system noise figure is to be determined, leave all devices for transmitter coupling in place. Set the spectrometer to operate on the frequency of interest with a large sweep width (+/- 10,000 Hz) and a gain level set to fill the digitizer more

than half way when acquiring a single scan. Collect a scan and determine the RMS of the noise after fourier transform with no line-broadening. This can be done with computerized routines or by plotting the noise and drawing a line on top and bottom of the noise such that about 10% of the maximum noise excursions fall outside the lines. Any obvious spikes in the noise spectrum can be ignored, but their presence indicates interference in the NMR system.
Repeat the above process with the 50 Ohm liquid nitrogen noise source in place of the probe while the source is being cooled by liquid nitrogen. Process the data such that the same normalization constants are used and determine the RMS value of the noise as above.
These two RMS noise values can be used to calculate the instrument noise figure as indicated by the equation below:

```
NF(dB) = -1.279 - 10log[1 - { (RMSc/RMSw) }**2]
```

where RMSc is the value of the RMS noise in liquid Nitrogen and RMSw is the value at room temperature (20 degC).
A table relating noise figure to the ratio of the cold RMS noise value to the warm RMS noise value (RMSc/RMSw) as calculated by the above equation is shown below

Ratio	NF(dB)	Ratio	NF(dB)
0.99	15.73	0.90	5.93
0.98	12.74	0.85	4.29
0.97	11.01	0.80	3.16
0.96	9.78	0.75	2.31
0.95	8.83	0.70	1.65
0.94	8.06	0.65	1.11
0.93	7.41	0.60	0.66
0.92	6.86	0.55	0.29
0.91	6.37		

This test is easy to do, but requires careful experimental technique. If a noise level difference cannot be observed between a hot and cold resistor, make sure the system can observe an NMR signal from a standard sample. If you are unable to obtain a reproducible system noise figure, problems in the system noise profile could be presenting limitations on spectrometer performance. A good procedure is to strip the spectrometer receiving system to the bare minimum. If possible take out any unnecessary lock filters (turn off the lock) and decoupler filters from the receiving system. Remove the transmitter coupler if possible. With this minimum system determine the system noise figure. Anything more than 3.0 dB needs to be improved. Now add back the removed components one by one. Repeat the test after the addition of each component to determine how they affect the system noise figure. Today's spectrometers typically give overall system noise figure of less than 2.0 dB.

To be most useful, these noise figure tests should be done routinely as part of preventive maintenance on the NMR instrument. This history of performance makes it easy to see when something goes wrong. They are still useful without this history, since most

working NMR systems have noise figures in the range range 1.0-2.5 dB. If the NMR system is performing outside this range, other noise tests can help determine which module of the system is at fault.
The system noise figure is an important factor in determining the NMR instrument's overall signal to noise. The NMR instrument is designed to have the preamplifier gain and noise figure determine the total system noise figure. As a rule of thumb, a 1 dB increase in the noise figure decreases the signal to noise by 10-15%. For adequate signal to noise, the overall system noise figure needs to be in the 1.0 to 2.5 dB range. If the system noise figure is outside that range, further tests are needed. Remember, if the signal to noise is low, then there can be at least three problems:

Not enough signal from the probe
Poor system noise figure
Noise from other sources

Typical Problem Areas:

Frequency dependent?
Change to another nucleus and determine the noise figure. It is best if the other nucleus uses a different preamp. If the noise figure then falls within the desirable range the preamp is suspect, but not proven guilty. Check the other tests described.
Transmitter Power Amplifier
In some NMR spectrometers the power amplifier is linear. These amplifiers can often emits RF noise at the observe frequency. If the noise blanking circuitry is defective or inadequate this will add noise to the system. With the noise meter measuring the system noise level, disconnect the transmitter cable. If the noise level (noise level not noise figure) drops there is noise coming from the power amplifier. This needs to be fixed before the NMR system will deliver optimal signal to noise.
Decoupler Power Amplifier
The phenomenon is the same as above except the amplifier to be checked is the decoupler power amplifier.
Lock
The lock transmitter can also add noise to the system. Observe the noise level on the noise meter with the preamp connected to the probe. If the noise level increases or decreases when the lock cable is disconnected from the probe, then the lock is adding noise (meter increases) to the overall system or the lock may be overloading the preamp (meter increases or decreases). Either way is not desirable. Further filtering of the lock and/or receiver system is required. Remember the lock can be putting noise in at the observe frequency AND/OR the lock transmitter can be overloading the preamp.
Transmitter Coupler and Directional Couplers
Most NMR systems have some circuitry in front of the preamp to couple on the transmitter and/or decoupler. Measure the system noise figure with and without this circuitry. The difference is the loss in this circuitry. It should be less than 0.5 dB and not more than 1.0 dB.
Filters
Most NMR systems have some circuitry in front of the preamp to filter out the lock

transmitter and/or decoupler. Once again, measure the system noise figure with and without this circuitry. The difference is the loss in this circuitry. It should be between 0.5 and 1.0 dB.

Gain Level

If the preamp's gain is not much larger than the console noise figure, then the system noise figure is not determined by the preamp. In many systems, the dynamic range of the NMR signal is very large. This is especially true for biological samples in water. With large signals the operating spectrometer gain settings are sometimes set low to keep the preamp and console from overloading. Overloading produces artifacts, lineshape distortion and baseline distortion in the NMR spectrum. Determine the NMR system noise figure at several setting ranging from very high to very low. If your system is forced to operate at a gain setting at which it has a poor noise figure, you need to improve the dynamic range of the preamp.

RF Interference

Sometimes the probe or console picks up RF interference. A good way to detect this interference is to connect an amplified speaker to the audio channel. You can hear the interference. The noise meter can also indicate the interference by a changing level reading. The speaker and noise level test needs to be done with and without the probe connected to determine the source of interference.

Magnetic Shielding

The *seat of the pants* guide to understanding the problems of shielding NMR magnets

One of the problems associated with working with NMR instruments is the stray magnet field surrounding the magnet. This stray magnetic field has grown to be a larger problem as the magnets had moved to higher fields and wider bores. The problems with stray magnet fields are multiple and include:

- Small metal objects can be attracted to the magnet. These objects can move at speeds which can harm objects which get in the way.
- Credit Cards with magnetic strips can be erased.
- Watches and heart pace makers can cease operation in the magnet field. They may not start working when removed from the magnetic field.
- Televisions and computer monitors are distorted in the fringe magnetic fields. These items can be affected for a considerable distance in the horizontal direction and for one or two floors above and below the magnet.

It should be noted that if the NMR magnet can affect objects at these distances, then movement of magnetic objects at these distances can affect the NMR magnet. This can cause field shifts larger than the NMR system's lock can correct and/or the loss of magnet homogeneity.

The problems these stray magnetic fields can cause are often made worse by the reactions of the people working in these stray fields. To my knowledge, no scientific studies have

shown any harm to biological objects caused by static stray magnetic fields, even very strong fields like inside an MRI unit. However, the emotional reaction of many people to finding out they are in a stray magnetic field can be "non-linear". Often these are the same people strapping magnets on their own or their horse's body for the "beneficial" and therapeutic effects of magnetic fields. Take this "non-linear" attitude and combine the litigious nature of today's society and people and organizations can tend to overreact.

The first approach many groups make to addressing the logical and illogical issues that the stray magnetic field can cause is to put a steel plate between the magnet and the area where they do not want any stray magnetic field. This can often make the stray magnetic field in the target area stronger. Below we will attempt to explain without rigorous mathematical treatment why this is. While making this explanation, we will cover topics which will lead the reader to a more complete understanding of the problems associated with magnetic shielding.

Flux Channel

When talking about magnetic field strength, it is helpful to think in the terms of magnetic flux lines. The more flux lines, the stronger the magnetic field. The straighter the flux lines, the more homogeneous the magnetic field. When we have a magnet, we have two magnetic poles -- the north pole and the south pole. With a super-conducting magnet, we will define of the top of the magnet as being the north pole and the bottom of the magnet as being the south pole. When we have a magnet, we have flux lines coming out of the north pole (top of the magnet) and moving through the channel of least resistance to the south pole of the magnet (bottom of the magnet). The path of least resistance can be a vacuum or in our case usually air. If the magnet is isolated with only air surrounding it, then the flux lines make large sweeping curves out the top of the magnet, arcing around the side of the magnet and back to the bottom of the magnet. All flux lines leaving the top of the magnet need to return to the bottom of the magnet.

Magnetic metals like steel have a lower resistance to the passage of flux lines than does air. Therefore, all the flux lines on their way from the top of the magnet will prefer to move through steel rather than through air. This means that any flux line which intersects a steel object will proceed through the steel object until it needs to exit the steel object to return to the bottom of the magnet. Since the flux lines like to move through the steel more than through the air, all the flux lines possible tend to collect in the steel object for as long as possible. This will be true as long as the steel remains out of magnetic saturation. The thicker the steel the more flux lines can be captured before the steel object becomes saturated. The steel object acts like a "flux channel", where it grabs all the flux lines it can and channels them down to where they need to exit to get back to the magnet.

Another factor complicating this process is somewhat counter intuitive. A piece of steel is attracted to the area where the magnetic field has the strongest gradient (most inhomogeneous), ***not*** the strongest absolute field value. An interesting example (don't try this at home!) is to drop a nail into a super-conducting magnet. The nail will be strongly pulled into the top of the magnet and will fall freely through the homogeneous region of

the magnet and stick near the bottom exit port of the magnet. If you take a wooden stick and push up on the nail it will move freely back through the homogeneous region of the magnet but will be very difficult to push out the top. This is because, even though the field in stronger in the center of the magnet, it has less magnetic gradient in the homogeneous center of the magnet than near the ends of the magnet where the flux lines spread out on their individual return path arcs.

So, in our example of placing a steel plate between the magnet and the area we desire to shield from the magnetic field, the ends of the steel plate will have all the flux lines possible trying to get into it, traveling down to where they must exit to get back to the magnet (the flux channel) and exiting in a big bunch. Remembering that the more flux lines, the stronger the field, what we have accomplished with our actions is to make the magnetic field near the ends of the steel plate stronger (collected flux lines) and more inhomogeneous (bent more). Both of these points make the stray magnetic field more of a problem near the ends of the steel plate. One way to overcome this problem is to make the steel plate much larger that the desired area to shield. As the dimensions of the steel plate get larger, the larger the area of the lowest magnetic field behind the steel plate.

A more practical way to address this issue is to make a steel box totally surrounding the magnet. Then the flux lines going out the top of the magnet would enter the steel flux channel on the top and move through the flux channel on the side to the flux channel on the bottom and back to the magnet. The area outside the steel box would have a very low residual magnetic field. One problem with this approach is that the smaller the box you build around the magnet, the thicker the steel has to be to stay out of magnetic saturation. Very close in, this can take tons of steel to shield a small magnet and lots more to shield a big magnet. So to avoid having to use such thick steel, you move farther away from the magnet and make the walls of the room into a magnetic shield. Of course, the smaller the room, the thicker the steel required and the more effect opening the steel door in such a room would have on the magnet itself.

Forces on the Magnet

At this point, it is appropriate to talk about the forces on the magnet itself. When a steel object is attracted to a magnet, we need to remember that the magnet itself is being attracted to the steel object with the same force. The force of this mutual attraction is proportional to the size of the steel object and its distance from the magnet. If a big steel object gets near a super-conducting magnet, it can pull the magnet over. Even if this is avoided, the magnet inside the dewar can have a lot of force on it. With many super-conducting magnets, the internal supports in the dewar are not very strong. The magnet is often hung from the top with the minimal amount of materials and held in vertical alignment with small struts. Both the material to hang the magnet and the struts are as small as possible to avoid thermal leaks to the outside world. These thermal leaks would lead to higher helium boil-off. Since the internal supporting material is so weak, a metal object nearby can cause enough force to alter or break the magnet supports. This is an irreversible process leading to much higher boil-off and the need to disassemble the magnet for repair.

So when shielding a magnet, do two things; 1) keep the steel as far from the magnet as practical and 2) keep the shielding as symmetrical around the magnet as possible. The reason for the symmetry is that a force from one side can be balanced by a force from the other side. This symmetry also makes it easier to shim the main magnetic field. When thinking about symmetry, don't forget the distance from the top of the magnet to steel objects above and the distance from the bottom of the magnet to similar steel objects below.

Monitor Shield

If one of the problems you are trying to deal with in an NMR facility is the distortion of televisions and computer monitors, then a much more cost effective way to deal with this issue than shielding the entire magnet room is to build a shielding box for the monitor. This can be a sheet metal box with five sides where the front of the monitor faces the open side of the box. Low cost steel plates can be bolted to angle iron and painted to construct the box (keep such a box from getting so close to the magnet that it gets pulled in). Keep at least one inch of space between the box and the monitor for ventilation. With some larger monitors a small ventilation fan is required to keep the monitor cool enough for operation. Boxes such as these are amazingly effective at keeping monitors working without distortion.

A company called Field Management Services sells a monitor shield called JitterBox.

Active Magnet Shielding

What we have discussed here so far has been passive shielding of magnetic fields. Today many NMR and magnet vendors are providing active magnet shielding. This is done by building a second magnet on the outside of the main magnet with a field of the opposite sign, thereby canceling the external magnetic field. Here the outside magnet acts as the other pole with the top of the outside magnet being the south pole and the bottom of the magnet being the north pole. This is a really good flux channel! Obviously, if the shielding magnet and the main magnet were of the same size and strength, the net field inside and outside the magnet would be zero. To avoid this problem the outside magnet is bigger in size and therefore can operate at a smaller field strength. This is because the field at some distance from the magnet is less than the maximum field strength and it is this field which needs to be cancelled. Using active shielding always means that some of the main magnet's field is being cancelled, so this technique can only be used when you have field strength to burn.

Shimming

Part I
Foreword In the olden days. Why is it called shimming?
Introduction What is shimming.

Equations for Common Room Temperature Shims Table showing field generated by common shims.
Sample Spinning Why samples are spun and the complications.

Part II
Basics What do we look at to shim.
Shim Interactions Procedure for adjusting shims with zero-, first- and second-order interactions.
A New Magnet Things which need to be checked when a magnet is installed.
Checking coil position A quick test for probe coil and shim coil alignment.
Before You Start To Shim Get off on the right foot.
Probe Coil Plot
A Shimming Procedure A systematic method of adjusting shims.

Part III
Symptoms of Inhomogeneity Lineshapes resulting from different axial gradients.

Part IV
The Z1 Profile Applying a gradient to display an axial "image" of the sample.
The Quickie Z1 Profile Approach to Shimming. Get Z3 and Z4 correct in a hurry.

Part V
The Sample and Shimming End effects, particulates, dissolved material, radiation damping.
Probes and Shimming Probe materials, magnetic susceptibility correction, size of the probe coil.
RF Homogeneity Origin of different types of spinning sidebands.
Computer Shimming Things to be aware of in using automatic shimming.

Foreword

In the beginning, the field homogeneity of large electromagnets was adjusted by mechanical alignment of the magnet pole faces. The more parallel the pole faces, the more homogeneous the magnetic field. The first step in the process of adjusting magnetic homogeneity was to adjust the position of the magnet's pole faces by turning three large bolts which held the pole faces. Adjusting these bolts tilted the pole faces relative to each other with the aim of making the pole faces more parallel. If the bolts ran out of range, thin pieces of brass were placed between the magnet yoke and the pole pieces to move the pole pieces as parallel as possible. These thin pieces of brass were also placed in other strategic locations to make the pole faces parallel in a manner not addressed by the three adjustment bolts. The metal pieces were called ***shim stock*** and the seemingly endless process of placing and removing pieces of shim stock acquired the name "***shimming***". Because tons of magnetic field pressure existed on the pole faces, the magnet had to be turned off to place and remove the shim stock. When the sample was spinning, the final part of adjusting magnetic homogeneity with these systems was to adjust a ratchet bolt which pulled together or pushed apart the tops of the magnet pole pieces to give a fine

adjustment of the Y gradient. All of these processes were mechanical in nature. After these adjustments, the NMR instruments were typically capable of giving better than 0.2 Hz resolution. This is rather impressive when you consider that 0.2 Hz out of 60 MHz represents 3 parts per billion field homogeneity over the volume of the sample.

To increase the performance, reduce the difficulty of adjusting magnetic homogeneity and reduce the manufacturing difficulty of the magnets, an electronic "shimming" process was developed which used a series of small electromagnets having very specific magnet field contours. These small electromagnets are placed around the sample area. Each small electromagnet can be used to adjust the field in the area of observation to create more of or counteract existing types of magnetic gradients. A complete series of these electromagnets can be used to adjust the magnetic field homogeneity to a given level of purity depending on how many types of adjustment electromagnets are used. The process of adjusting the magnetic field homogeneity by adjusting the current in each of the small electromagnets retained the name shimming and the small electromagnets assumed the name "shims".

At first only a few low order (X, Y, and Z) electrical shims were used. As the fields became higher, magnet production became more difficult, and more and higher order electrical shims were added to maintain the same level of performance. These electrical shims are not 100% pure and have interactions with shims of a similar nature (ZX creates some Z gradient and X gradient in addition to the intended ZX gradient). Because of these interactions, the number of adjustments necessary to shim the magnet increases geometrically with the number of shims, not just linearly. In addition, the raw field encountered in superconducting magnets is usually worse than in electromagnets, so larger corrections are required. These two facts make the process of shimming superconducting magnets more difficult and the shimming process more important to obtain useful NMR spectra.

To obtain 0.2 Hz resolution requires ten times greater magnetic field homogeneity at 600 MHz than at 60 MHz. Therefore, in addition to the higher field superconducting magnets being more difficult to shim, shimming becomes more important to obtain the same results as the magnetic fields increase. Other aspects of an NMR instrument's performance are also affected by shimming, such as the NMR signal's lineshape, which is critical for achieving good solvent suppression. So the necessary evil of adjusting the small electromagnets, called shimming, remains very important in today's NMR instrumentation.

Introduction

Shimming a magnet for use in NMR is similar to many sports: mental attitude is a key to success. Shimming is often made more difficult than necessary by the operator's belief that it is a "magic" process far too complex to understand. Successful shimming is a simple, but often very time-consuming, process. An organized and logical approach is key if the process is to be both fast and effective. Each shim or shim type generates symptoms in the NMR instrument's performance indicating its misadjustment. An

understanding of the relationship of each shim and these instrumental performance symptoms reduces shimming from a random knob turning task to a scientific procedure of adjustment and observation of effects.

Shimming is definitely a serial process. This means that things should be taken one step at a time; it does not mean, however, that there is one and only one defined process to be used. Instead of a "cookbook" approach to shimming where a defined stepwise procedure is followed, shimming should be approached like solving a murder mystery. In a murder mystery, there are many things which are common from one murder mystery to the next. The murderer needs a motive, the opportunity, etc. However, not every murder mystery is solved by the same stepwise procedure. In other words, the solution of a murder mystery has some generalized procedures and then becomes a search for clues. The process can be long and involved, but luckily the NMR instrument provides many clues to be followed. As will be discussed later, lineshape provides the clues to the solution of the mystery.

Shimming has some generalized procedures to be used when certain clues are observed. In addition to these procedures, there are tools available for use in the shimming process. Each tool is called upon as the conditions warrant, and each works in some cases, but not all. In addition, as the shimming process proceeds, additional clues suggest the use of other tools. The shimming process is a little like peeling an onion - removing one layer often reveals another layer underneath (and crying sometimes results).

The most common method of adjusting the homogeneity of an NMR magnet is the observation of an NMR signal. Since the natural line width of an NMR signal may be less than 0.1 Hz, then even for a 100 MHz NMR instrument a field homogeneity of one part per billion would be required to measure this line width. Very few test instruments have this precision. This means that the NMR instrument becomes the test instrument used to adjust itself.

An additional problem with the shimming process is that the observed NMR signal results from the integrated signal from the total volume of the observed sample, which may have many different resonant frequencies with different degrees of excitation arising from different positions in the sample. Visualize the NMR sample as a continuum of isolated mini-samples, each of which is infinitesimally small. Each mini-sample then generates a signal whose linewidth is determined by the T2 relaxation time of the sample and whose frequency results from the field value at that point. The intensity of the signal generated by each mini-sample would reflect the amount of excitation at that point. What the NMR operator observes is the sum of signals from all the mini-samples. In other words, the NMR signal is the total integrated signal over the total sample volume times each area's degree of excitation. It is the integration of the NMR signal response which leads to a major difficulty in the shimming process. Any knowledge as to which part of the NMR sample is experiencing the magnetic inhomogeneity is lost in this integration process.

The NMR operator adjusts the field homogeneity for the NMR instrument with a set of electronic shims, each of which has its effects over a complex geometry. If positional

information for each of the mini-samples were not lost in the integration process discussed above, the NMR operator would have much better clues as to which shim to adjust. There are, however, clues from the NMR signal which allow the NMR operator to narrow the selection of shims which probably need adjustment. These clues will be discussed later.

The shim gradients used to adjust the magnetic field homogeneity in superconducting magnets have the common names shown in the table on the next page. However, these shims have more complicated expanded equations describing their actions as indicated in the table. Although every different type of shim set has a slightly different set of expanded equations, the expanded equations for the shim gradients shown in the table are reasonable examples of the full gradient created by each shim coil. The equations are shown for interest and are not used in the shimming process to be described.

Equations for Common Room Temperature Shims

Shim	Equation for Field Generated	Interaction Type
Z0	1	0
Z1	z	1
Z2	2z2 - (x2 + y2)	1
Z3	z[2z2 - 3(x2 + y2)]	2
Z4	8z2[z2 - 3(x2 + y2)]+3(x2 + y2)2	2
Z5	48z3[z2 - 5(x2 + y2)]+90z(x2 + y2)2	2
X	x	0
Y	y	0
ZX	zx	2
ZY	zy	2
X2-Y2	x2-y2	1
XY	xy	1
Z2X	x[4z2 - (x2 + y2)]	2
Z2Y	y[4z2 - (x2 + y2)]	2
ZXY	zxy	2
Z(X2 - Y2)	z(x2 - y2)	2
X3	x(x2-3y2)	1
Y3	y(3x2-y2)	1

Sample Spinning

In the early days of NMR, Bloch suggested that the effective homogeneity of the magnetic field can be improved in a simple way by providing a motion of the molecules within the sample. The rate of molecular motion necessary to accomplish this task is on the order of 4 Hz. This speed is easily obtainable by mechanical motion of the sample. Anderson and Arnold demonstrated the improvement by spinning a water sample about an axis coincident with the axis of the receiver coil. At rotational speeds in excess of 10 Hz, they reduced the halfwidth of the NMR signal by a factor of 17 and increased the amplitude by a factor of 7. The size of the improvement is proportional to the size of the field inhomogeneity along the axes averaged by the spinning process. As the magnetic field homogeneity increases, the improvement obtained by sample spinning decreases.

Spinning the NMR sample tube averages the field inhomogeneities along two axes but not along the axis about which the sample is spun. If the NMR operator could make a spherical sample and spin about the X, Y and Z axes simultaneously, shimming might become a lot easier. However, no simple mechanical means of preparing and spinning the sample to average all three axes has yet been devised. The effect of spinning in all three axes simultaneously can be seen when starting and stopping spinning on large diameter tubes. The turbulence created by starting and stopping causes sample mixing along the spinning axis. If the NMR operator watches the lock level of a spinning sample and suddenly turns off the spinner, the lock level will actually rise while the sample is mixing from the turbulence. The same effect can be seen when starting to spin where the lock level goes up to a higher level and fades back down to a final value between the non-spin value and its highest level. This is because turbulence when the spinner starts causes mixing of the sample, thereby effectively spinning about all axes simultaneously. The effect is most visible on large diameter tubes.

The spinning process divides the shim set into two different types: the type not averaged by spinning, or "on-axis" shims, and the type which are averaged by spinning, or "off-axis" shims. Adjusting the on-axis shims while spinning and the off-axis shims while not spinning can be used to "decouple" the different types of shims and simplifies the process.

Under some conditions of field inhomogeneity, spinning the sample produces an amplitude modulation of the NMR signal. This process gives rise to "spinning sidebands" on either side of the resonance signal. The sidebands occur at integer multiples of the spinning rate and have a tendency to become smaller as the spinning rate is increased. These properties can be used to help identify these spurious signals. For a more thorough discussion of the origins of different types of spinning sidebands, see the section on RF Homogeneity.

Basics of shimming

Progress during shimming can be monitored in various ways, the most common being those listed below:

- Swept NMR resonance.

- Lock level.
- Free induction decay (FID).

Choice of the most useful method depends on the instrument and its current condition. The swept NMR resonance can be a signal from a proton, deuterium from the lock solvent, or another NMR-active nucleus which is swept by either field sweep or frequency sweep. To be used for shimming, the signal should have sufficient signal-to-noise (S/N) that the height and ringing pattern can be observed. The sweep repetition rate should be fast enough to give a "real time" response as the shims are adjusted. This method of homogeneity measurement is best used for initial adjustment of the shims from a raw magnetic field. It is also commonly used when the spectrometer has no internal lock. The swept response should be adjusted for pure absorption phase and the rf power should be adjusted to avoid saturation of the signal. The height of the initial signal response should be used to determine the best response while shimming. The ringdown pattern can be used for the final adjustment of Zl and Z2. Care should be taken not to overemphasize the ringdown pattern in the initial stages of shimming since varying lineshapes can produce better ringing, but less desirable lineshape and resolution.

The lock level is probably the most common method of adjusting homogeneity. The lock level can be displayed by an analog meter on the NMR spectrometer or as a level on a CRT display. The lock signal can be digitized and displayed as a number or level on a computer display. The NMR lock nucleus must not be partially saturated with rf power and the phase of the NMR signal MUST be adjusted carefully. Care should be taken if the lock phase is adjusted by maximizing the lock level. An asymmetric NMR lineshape leads to a lock maximum at a phase that is not at pure absorption. Therefore, when the lock phase is adjusted by maximizing the lock level, improper lock phase can lead to an asymmetric lineshape, or asymmetric lineshape can lead to an improper lock phase. This problem does not prevent the phase adjustment of the lock using the lock level, but care should be taken that whenever an even-order axial shim is significantly changed the lock phase is readjusted.

Another problem with using the lock level for homogeneity adjustment is the relative sensitivity to field inhomogeneities of the NMR lock signal and the signal under observation. Many spectrometers use an internal deuterium lock. If the nuclei under observation are protons, then the lock signal's sensitivity to inhomogeneities is one-sixth that of a proton (the ratio of the magnetogyric ratios of the nuclei). This is further complicated by a deuterium resonance usually being broader than its proton counterpart. Again, this does not mean that the lock signal cannot be used for homogeneity adjustment, but means that care should be taken not to be misled by the lock level. In general, good shimming results can be obtained by using a lock solvent with a sharp lock signal to do shimming for basic lineshape and minimum SSB (Spinning SideBands) followed by a touch up adjustment of Zl and Z2 on the FID signal.

Using the FID response is probably the most difficult method of shimming and should probably be left for the final "touch-up" of Zl and Z2. However, many spectroscopists prefer this method to the other two methods. To the trained eye, the FID yields

information about the lineshape and resolution similar to the ringdown pattern from a swept signal. Some excellent examples of FID shape and resulting lineshape are given in an article by Chmurny and Hoult. Briefly, the longer the FID rings, the better the resolution. The closer the FID's shape to a perfect exponential decay, the better the lineshape. A FID that falls sharply to another level and then decays more slowly either has poor lineshape displaying considerable hump or contains the resonances from two or more signals with different line widths. Some NMR computer systems can integrate the total area of the FID and yield a number indicative of the resolution similar to the lock level. FID shimming is the most sensitive method for shimming and has the advantage of using the observed nucleus as the shimming criterion. This means that shimming of the FID does not suffer from the loss of sensitivity to resolution caused by different magnetogyric ratios of the nuclei that is often seen when using a lock signal and it also removes all doubt that what the lock system "sees" is not what the observe system "sees".

All three of the above methods for measuring relative magnetic homogeneity of NMR instruments can be successfully used. Some areas to be concerned with in each method have been pointed out. The shimming sequences described later refer only to response. Except where otherwise indicated, the operator should choose his/her own method. It is probably best to use all of them in some combination during the shimming process.

Shim Interactions

If the shimming operation were a simple maximization of each shim gradient, then shimming would be quick and easy. However, several things prohibit this.

By their design some shims generate some gradients of other shims. This means that Z4 is expected to generate some Z2 and Z0.

Each shim has impurities of other shims. For example, when the Z4 gradient current is adjusted it not only generates a Z4 gradient but also some expected Z2 and some unexpected Z3 and Z1 gradients.

As the shimming process progresses, the response being used to adjust the shims becomes more sensitive. For example, if the magnetic field has a 20% Z2 gradient and an 80% Z4 gradient, adjustment of Z2 first will be insensitive. Then after Z4 is adjusted, Z2 will be more sensitive and might adjust to a different value. This is an over-simplified explanation and is used only as an example.

Shims have, by nature of their design, levels of interactions with other shims. See the table Equations for Common Room Temperature Shims.

Zero Order Shim Adjustment

Shims having no interactions (zero-order interaction) can be adjusted for best homogeneity by one simple adjustment. However, most shims have interactions that

complicate the process. The other two types of shim interaction are called first-order and second-order interactions.

First Order Shim Adjustment

First-order interactions are the type that allow a true minimization of magnetic inhomogeneity by repeated maximization of the individual shims. After the complete set of shims is adjusted, readjustment of the first shim of the set leads to a different optimum. Successive iterations then lead to less and less change on readjustment until finally no further change is observed.

An example of this first-order interaction is that of Z1 and Z2. If the Zl shim is adjusted for optimum response followed by adjustment of Z2, then Zl has a new optimum when readjusted. If the process is repeated, the amounts by which Z1 and Z2 change on each iteration decrease until the optimum values for Z1 and Z2 are found. The process can often be accomplished faster by noting the direction the Z1 shim is changing and moving slightly too far on the early corrections. The ability to make educated guesses based on a knowledge of the shims leads to a much faster shimming process.

Second Order Shim Adjustment

Second-order interactions are of the type where a given shim must first be moved and then others adjusted before any determination of improvement can be evaluated. Successive optimizations (first-order process) of this shim type and other shims do not necessarily lead to the best homogeneity. The process employed for second-order interactions is usually to change this shim a measured amount and reoptimize another set of shims. If this leads to a better response, then the shim is changed another measured amount and the process repeated until the response starts to decline. If the initial response is worse, the other direction is tried.

Which Shims are which Order

A classification of each shim as to the type of interactions to be expected is shown in the table. The interaction order assigned to each shim is a general description of the expected type of response. In some circumstances, a simpler type of interaction is observed. However, the operator should not expect a simpler interaction, but proceed on the worst case assumption. It can often be noted that shims displaying a complex type of interaction in the early stages of the shimming process display a much simpler type of interaction as the homogeneity improves. This simplification process often yields zero-order interactions for most shims in the later stages of shimming. Under these conditions, the shims appear to "drive" directly to their final positions with almost no interactions.

Open All Senses

As can be deduced from the summary just given, shimming can be a complex undertaking. It is important that the NMR operator use all tools and techniques available

to him/her in the process. In addition, he/she needs to be very observant. The NMR instrument is giving lots of feedback at all times. These should be taken as clues leading the operator to the successful conclusion of the shimming process. The operator should, however, be wary of drawing conclusions from too little information. He/she will often be receiving contradictory information. Tests should be formulated to expand and explore contradictory clues. The operator needs to avoid becoming frustrated by contradictory clues, but also not avoid frustration by ignoring some information. Note all information the NMR instrument is providing, even if it is not immediately understood. The entire shimming process has a lot in common with a detective story with the NMR operator being the detective and the NMR instrument providing the mystery of what are the best shim values.

A New Magnet

When an NMR instrument is first installed, the setup is usually done by a trained service engineer. Most do a good job, but turnover is high in the service area and sometimes mistakes are made. The ease which the NMR operator can operate his instrument over the instrument's lifetime is often directly related to the skill of the installation service engineer. If the superconducting magnet is properly setup and a set of shim libraries established for all the probes, routine operation of the instrument can be very easy. In fact, when problems are encountered with resolution or lineshape with the instrument even years after the installation, the problems can often be improved by going back to the shim values left by the installation engineer. A common problem in NMR laboratories is that these shim values are not kept or are stored where no one can find them. It's a little like computer disk backups: they are not needed until it's too late to do them.

Making sure the room temperature shim assembly is installed in the correct position in the magnet is very important. Often the best position for the room temperature shim assembly is with the center of the room temperature shim assembly at the center of the superconducting shims. The center of the superconducting shims is usually taken from the magnet manufacturer's data. This can be confirmed with a technique very similar to that used to establish the center of the room temperature shim assembly. There are conditions where the center of the RT shims should not be coincident with the center of the superconducting shims. In general, these arise when a different area of the magnet has a better overall field shape (on and off axis) than the area around the center of the superconducting shims. However, when the center of the RT shims is not the center of the superconducting shims it is more often than not a mistake.

Before You Start To Shim

One problem encountered in the superconducting magnet shimming process is the interaction of the various shims. When shims interact, shimming becomes a tedious process requiring many repetitive passes through the shimming sequence. Shim interactions become much greater when the center of the shim set and the center of the sample are not coincident. Since the Z1 gradient has a zero contribution to the field at its center ($z = 0$), it can be used to locate the center of the shim set. One or both of the

processes described below can be used to ensure the probe and shim alignment. If the sample and shim alignment is unknown and the alignment process is not followed, the shimming sequences described later still optimize the field homogeneity, but there are considerably more shim interactions. These interactions make the shimming process more difficult than necessary.

Most NMR systems do not allow the probe position and the room temperature shim assembly position to move independently. For this reason, if one of the following tests indicates that the probe center and the room temperature shim center are not the same, the problem probably resides in the placement of the probe coil in the probe. This is not usually an NMR operator adjustable parameter. If any of the tests reveal a misalignment, check several different probes and see if they are all misaligned. If the problem exists in only one probe, consider having the probe manufacturer check the coil placement. If all the probes are misaligned, something fundamental in the spectrometer installation or design is wrong.

Checking coil position

If the NMR spectrometer can display a swept lock signal, the easiest method to check the probe and room temperature assembly alignment is to observe the swept lock display while adjusting the Z1 shim control. The first step is to display the swept lock signal. Then while noting the lock signal display position, adjust the Z1 shim current in one direction and then the other. If the probe is in alignment with the room temperature shim assembly, the lock signal should not move, but should become wider and shorter while staying in the same position. If it does move while becoming broader and shorter, the direction should change as Z1 is moved in different directions. If the peak moves then there is some degree of misalignment between the probe position and the room temperature shim alignment.

A value judgment is called for if the lock signal does change position with Z1 current. In general, if the peak moves less than it broadens then the misalignment is small and is usually best ignored. If the peak moves more than it broadens then corrective action, if possible, would make shimming easier.

If a swept lock signal is not available, an alternative, albeit slightly more involved, technique for determining the relative probe position and room temperature shim alignment is the effect of Z1 current on a plot of probe coil response. While being more involved, this technique also gives more information which is useful for understanding the overall NMR instrument's operation. Place a drop of water in the bottom of an NMR tube. The smaller the drop the better. Also be as careful as possible to keep water from the sides of the NMR tube. Put this sample in the NMR spinner at a depth such that the drop of water is in the center of the probe's coil and insert the sample into the spectrometer. The sample should not be spinning. Set up the spectrometer for 1H observation and acquire a spectrum with a sweep width appropriate for the experiment. What is appropriate comes with experience and you will know more about how large a sweep width to use when the experiment is over, but for now use something like +/-2000

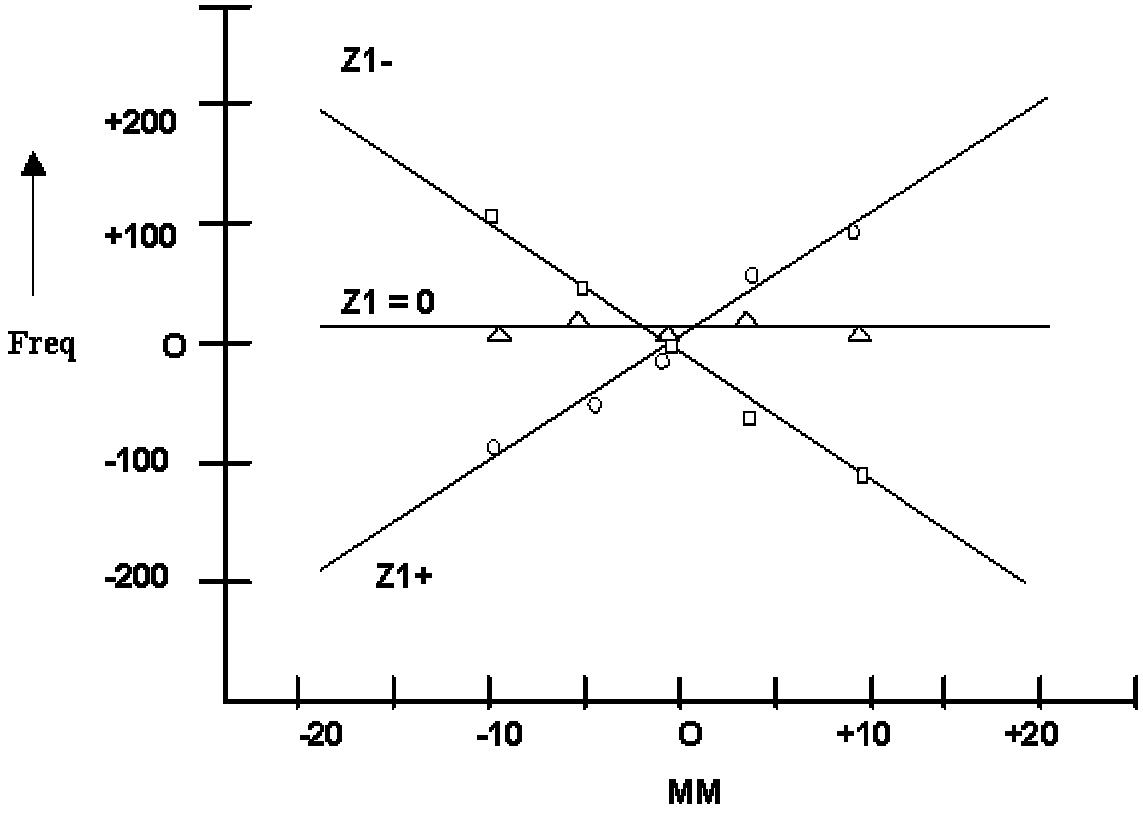
Hz. After doing the experiment you may want to go back and collect the data again using a different sweep width.

Process the spectrum using a magnitude calculation and whatever processing parameters are necessary to keep the computer scaling constant for the duration of this experiment (e.g., Bruker or Nicolet AI command). Take an integral of the entire sweep width and set up whatever integral corrections are necessary. If possible set this integral value to 100 and record all future integrals relative to this one. Record the integral value and the frequency position of half height on the integral line. An eyeball guess at the frequency position is usually more than good enough for this experiment. Now adjust the Z1 current in the positive direction an appropriate amount. What is appropriate again comes with experience, but it needs to be a large amount and you should note that you are going to change the Z1 current the same amount in the negative direction next, so do not change the current more than you have available in the opposite direction. Now record the integral value and the frequency position of the half height of the integral with the Z1 current change in the positive direction and again with the same Z1 current change in the negative direction. A table similar to the one below should result:

Z Position	Z1+ Frequency	Z1+ Integral	Z1=0 Frequency	Z1=0 Integral	Z1- Frequency	Z1- Integral
10 mm	+ 90 Hz	45	- 3 Hz	48	- 104 Hz	44
5 mm	+ 50 Hz	92	+ 2 Hz	96	- 65 Hz	93
0 mm	- 10 Hz	98	- 7 Hz	100	- 10 Hz	97
-5 mm	- 60 Hz	93	+ 3 Hz	95	+ 30 Hz	91
-10 mm	- 90 Hz	48	- 5 Hz	50	+ 102 Hz	40

Do not be concerned at this stage if the frequencies or integral values are changing by small amounts. If they are changing by large amounts, make sure you did this right by reproducing the values, but if they are changing by small amounts, complete the data collection.

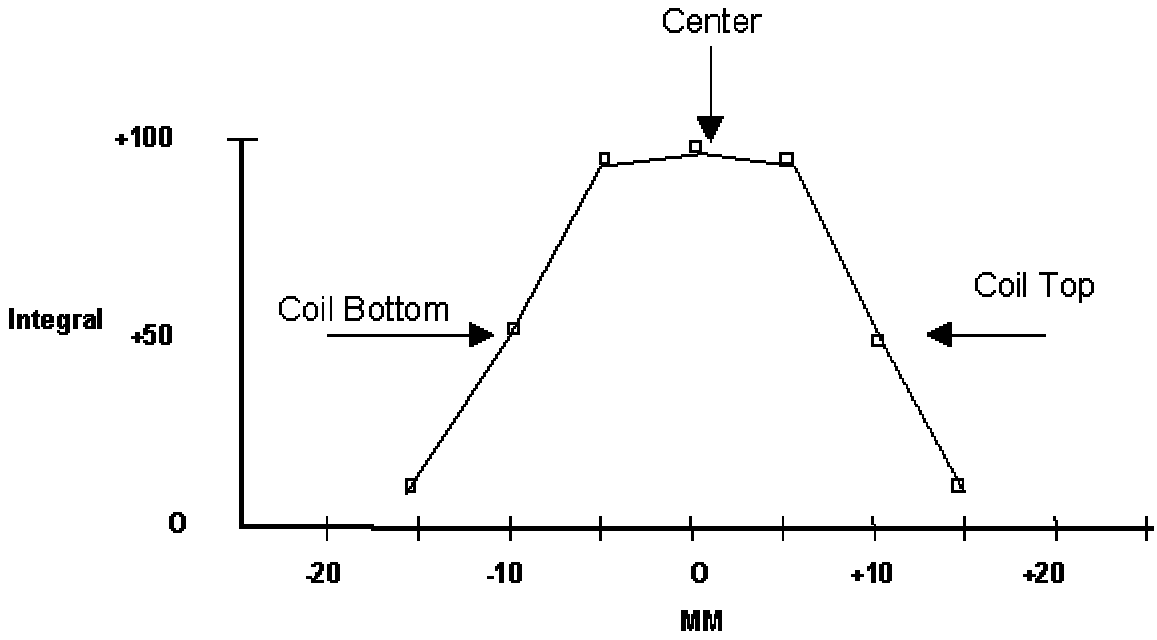
Now move the position of the water drop up 5mm in the spinner and repeat the above experiment. Record the data and move the water drop position up in 5mm steps until the water signal can no longer be observed. Then move the water drop 5mm below the original position. Record the data and move the water drop position down in 5mm steps until the water signal can no longer be observed. Plot the data of the frequency versus Z position of the water drop for the three values of Z1 current. You should get a graph something like the one shown below.



NMR Frequency Versus Position in the Probe for Different Values of Z1

The three lines should cross at one point, but usually you get a small triangle. For this purpose, the center of the triangle is the center of the room temperature shim assembly.

Now plot the data of the integral versus Z position when the water drop is at the Z1 = 0 current. NOTE: the Z1 = 0 current value is not zero current but the starting value for Z0 on your instrument. You should get a graph something like shown below:



NMR Signal Integral Versus Position in Probe

The plot of NMR signal versus water drop position lets you see over what height of sample the probe coil is active. This should describe roughly a rectangular region. The center of this rectangular region should be the region on the previous graph where the Z1 current lines crossed. This method tells the NMR operator where the active coil region is located relative to the room temperature shim assembly. In addition, it tells the NMR operator how long the probe coil is. This information is very useful when determining how long a sample is required in the NMR instrument for shimming. It also detects probe coil lead pickup which can make shimming more difficult. Techniques for shimming different sample lengths will be discussed in the chapter on The Sample and Shimming.

If the plot is roughly what is shown above with the ends approaching zero but with increased intensity further out, then you have detected coil lead pickup. This phenomenon comes from the probe coil's leads being at a high voltage potential and not being shielded from the sample. If this occurs, it is often the case that the lead pickup is occurring at a region of the RT shims that is not very homogeneous, which leads to lineshape problems.

The unshielded lead problem often reveals itself in another way. When you have a normal single resonance close to the carrier frequency and keep increasing the pulse length past a 90 degree flip to a 180 degree flip, the signal should pass through a null. With lead pickup, most of the signal goes through a null but a small portion of it remains positive even when the rest of the signal is becoming negative with increasing pulse length. The small positive portion of the signal is coming from lead pickup. The sample

seen by the leads is outside the main coil region is seeing a much smaller effective field than the main region of the sample. The effective 180 degree flip for this region can be many times that for sample in the main coil region. Often, since this sample can be in a region of different field homogeneity and therefore have a different field value, the small peak which remains positive will be to one side or another of the main peak which is going through a null with a 180 degree pulse.

If either of these symptoms of probe coil lead pickup are observed, the probe should be modified to have a lead shield. This will lead to better lineshapes, pulse homogeneity and solvent suppression experiments.

A Shimming Procedure

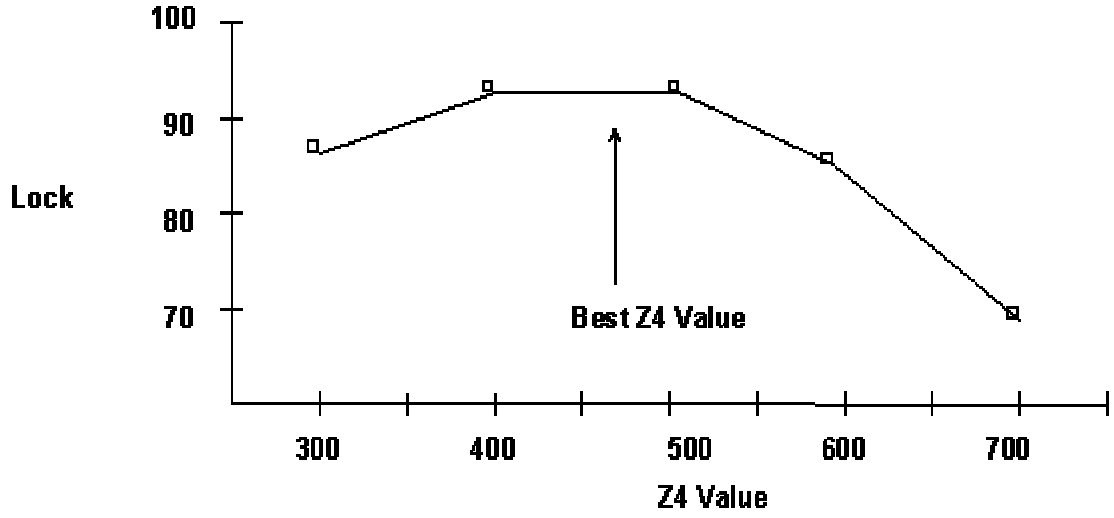
A shimming procedure follows. It should not be taken as the gospel, but as a suggestion of where to start the learning process about the relationship of shimming procedures, your samples, your instrument and the NMR data acquisition process in general as it relates to your laboratory. Not every instrument will shim the same, nor will every sample. This procedure is a starting place. Modify it at will for your environment. Later discussions will talk about what to look for and do when this process is done. For this procedure the optimization processes are:

Zero Order - This is a straightforward process of adjusting the control for the best response.

First Order - Adjust one control, then the next control, then the next until all controls in the set have been adjusted. Repeat the process until no further response improvement can be obtained.

Second Order - Note the current response level. Adjust a shim control from the current value a defined amount (a rule of thumb would be enough to change the response to between 50% and 75% of the original value) to a new value. Optimize all other shims in the set with a first order process. If the new response is better than the previous response, note the new response level and adjust the shim to a new value in the same direction and repeat the process. If the new response is less than the original response, adjust the shim control a defined amount in the opposite direction and repeat the process. Continue until the best value is clearly determined. This means it is necessary to go too far to make sure no further improvement can be made and then return to the optimum value. It is often useful in this process to plot the results of the shim of interest and the response value after the other shims have been optimized. The operator can then make sure that the changes being observed are real and significant and can better determine the optimum shim value. A typical plot where the Z4 shim is set to a series of values (at which Z1, Z2 and Z3 are optimized) versus the lock response is shown below. This plot gives the operator confidence that Z4 is at its optimal position.

Z4 Plot Technique



First Pass

If the NMR spectrometer is in a state of unknown homogeneity or is known to have poor homogeneity, a simple optimization of certain shims is the first step in the shimming sequence. A swept NMR signal is the recommended method of judging the response for this operation:

- Spin the sample (20-30 Hz) and adjust the Z1 and the Z2 shims interactively to produce the tallest swept signal response (first-order process).
- Stop the spinner and adjust X and Y for the tallest swept signal response (first-order process).
- Adjust X and ZX for the tallest swept signal response (second-order process).
- Adjust Y and ZY for the tallest swept signal response (second-order process).
- Adjust XY and X2-Y2 for the tallest swept signal response (first-order process).
- If any large shim changes were observed in the above process then the process should be repeated from Step 1.

After the above procedure, the NMR instrument should be capable of a field/frequency lock. Any one of the three methods described above for measuring homogeneity can be used in the following sequence steps.

Spinning Shims

The adjustment procedure for the spinning shims should be conducted with the sample spinning at more than 10 Hz. Care should be taken at all times to avoid a vortex. A vortex

leads to a false shim optimum, with Z2 usually being the most misadjusted. If the lock signal is being used for shimming, then ensure that the lock signal is not being partially saturated with rf power and that the lock phase is correctly adjusted. The lock phase should be reexamined each time a large change is made in an even-order Z shim.

In the adjustment of Z3, Z4, and Z5 described below (steps 2, 3, and 4), it is best to make a plot of the response level versus the shim under adjustment. If the operator is careful to proceed far enough past the maximum response position for the shim under adjustment, then the plot should reveal a broad curve. The best position for the shim can then be determined even if an interpolation between two sample positions is necessary. With experience, this plotting becomes an automatic mental process. Also, confidence is gained that all the shims were correctly optimized when a broad smooth curve is obtained as a result of this process.

- Use the first-order process to optimize Zl and Z2.
- Use the second-order process to optimize Z3. Note the position of Z3 and the response. Change Z3 enough to degrade the response by 20-30%. Repeat the process in Step 1. If the new position for Z3 has yielded a better response, then continue in the same direction. If the new response is poorer, then try the other direction for Z3.
- Use the second-order process to optimize Z4. Note the position of Z4 and the response. Change Z4 enough to change the response by 30-40%. Repeat the process in Step 1. Adjust Z3 to provide the optimum response. If the Z3 shim changes considerably, then repeat Step 1 again and readjust Z3 again for maximum response. If, after optimizing Z3, Z2, and Zl, the new response is better than the previous response, then continue in the same direction. If the response is worse, then try the other direction.

The Z5 shim is difficult to adjust for two reasons. First, only probes with longer coils give significant response change with Z5 owing to its strong dependence on distance. Second, the Z5 shim often has more Zl, Z2, Z3, and Z4 components than a Z5 component in its correction. The Z5 shim normally needs to be adjusted only with wide-bore magnet systems with large-diameter tubes or with longer coil probes. To adjust Z5, note its position and the response. Change Z5 enough to lower the response by 30-50%. Repeat Step 1. Adjust Z3 for the maximum response. Adjust Z4 for the maximum response. If either Z3 or Z4 changed a considerable amount, repeat Step 1 and reoptimize Z3 and Z4. If the new response obtained after this procedure is better than before, continue in the same direction. If the response is worse, try the other direction with Z5.

Nonspinning shims

The nonspin shim set should be adjusted while the sample is not spinning. Changing the nonspin shims which have Z components causes changes in the spinning shim set. If any of these shims change significantly, then the spinning shim sequence should be repeated after completion of the nonspinning sequence. With all shims involving a second-order

process, the technique described under the spinning shim sequence of plotting the result and interpolating the shim position should be followed either on paper or mentally.

- Adjust X and Y interactively using the first-order process for maximum response.
- Use a second-order process to adjust ZX. Note the position of ZX and the response. Change ZX by enough to lower the response 10% and adjust X for a maximum response. If the new response is better, continue in the same direction with ZX. If the response is less, try the opposite direction with ZX.
- Repeat Step 2 but using the Y and ZY shims.
- Adjust XY and X2-Y2 interactively using the first-order process for maximum response. If either XY or X2-Y2 changed significantly, then repeat Steps 2 and 3.
- Use a second-order process to adjust Z2X. Note the position of Z2X and the response. Change Z2X by enough to decrease the response 30%. Maximize the response with ZX. Maximize the response with X. If the new response is larger than the initial response, then continue with Z2X in the same direction. If the response is less, then try the opposite direction.
- Repeat Step 5 but using Z2Y, ZY, and Y.
- Use a second-order process to adjust ZXY. Note the position of ZXY and the response. Change ZXY enough to decrease the response by 20%. Maximize the response with XY. If the new response is larger than the initial response, continue with ZXY in the same direction. If the response is less, try the other direction.
- Repeat Step 7 but using Z(X2-Y2) and X2-Y2.
- Adjust X3 and X interactively for maximum response (first-order process).
- Adjust Y3 and Y interactively for maximum response (first-order process).

If the nonspin shim settings have significantly changed, then the spinning shim sequence should be repeated. If there are significant changes in the spin set after optimization, repeat the nonspin set also.

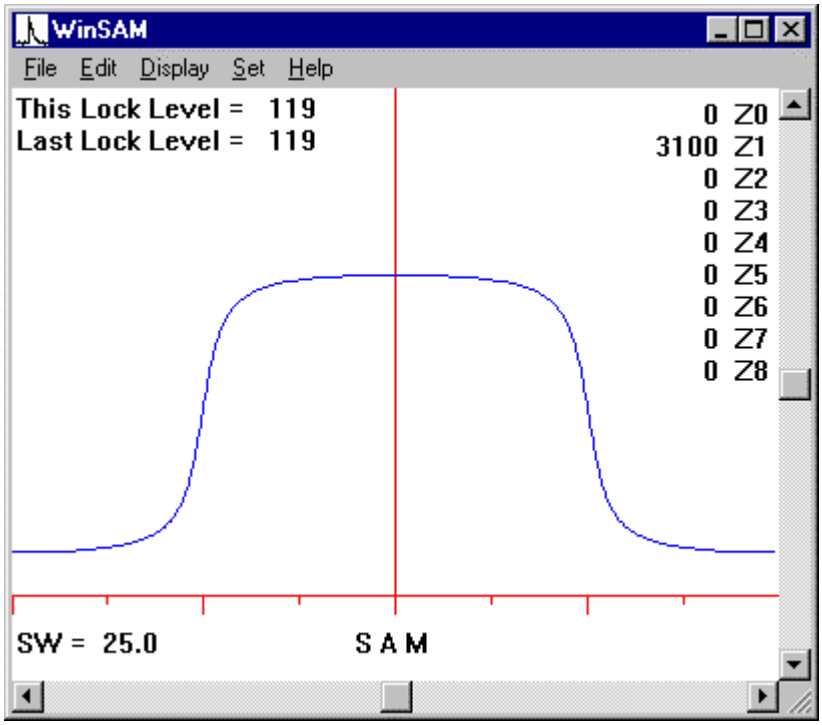
Final Pass

After the spinning and nonspinning sequences have been conducted, the NMR instrument should be delivering less than 0.5 Hz line width with good lineshape and minimum spinning sidebands. This is all that is required for most NMR experiments. If better resolution is desired, then a first-order adjustment of Zl and Z2 using the FID can be helpful. Maximize the response by adjusting Z1 and Z2 for maximum ringout of the FID. If the FID is not observable then the swept lock signal ringout is the best second choice. The ringout of the FID or the lock signal is a very sensitive measure of resolution. Adjusting only Zl and Z2 prevents changes in the lineshape.

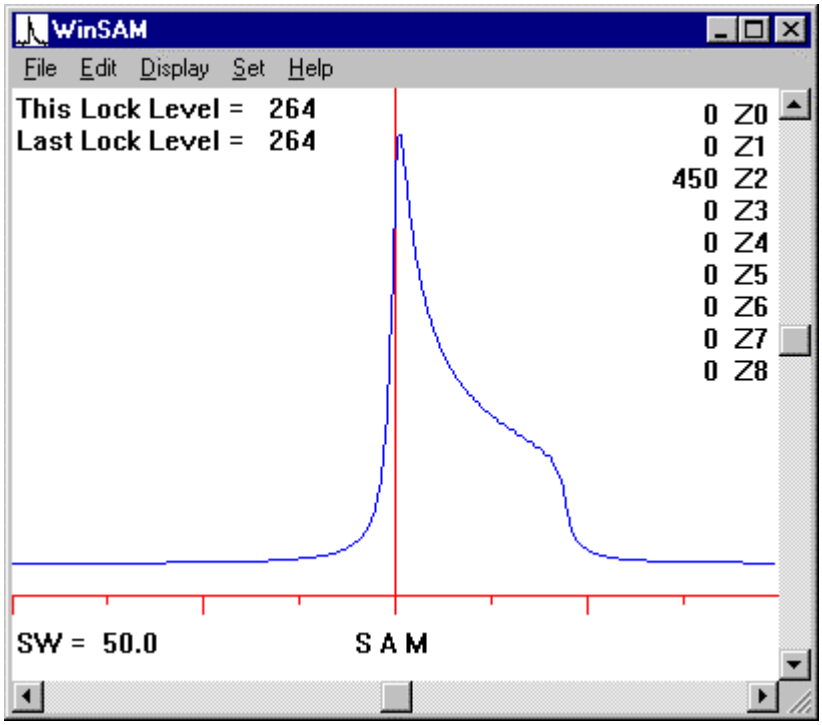
Symptoms of inhomogeneity

An examination of the NMR signal lineshape can often reveal which shim needs the most attention. In general, even-order spinning shims (Z2 and Z4) create asymmetric

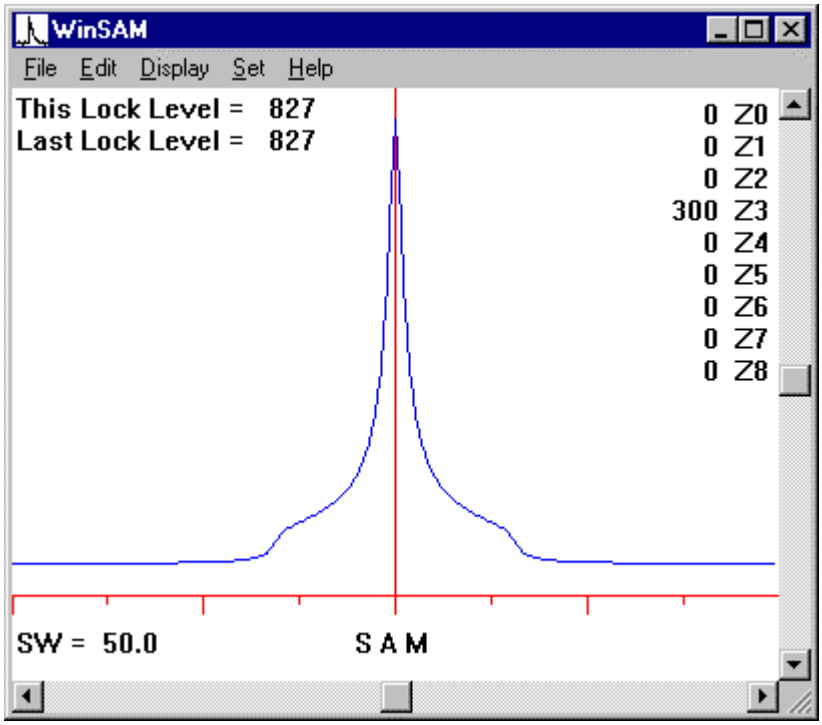
lineshapes when misadjusted. The odd-order spinning shims (Zl, Z3, and Z5) create symmetrical lineshape symptoms when misadjusted. Also, in general, the higher order the shim causing the problem, the farther down the peak the symptom will appear. The lineshape problems to be expected are summarized in the following figures, which were created using the SAM subroutine within NUTS.



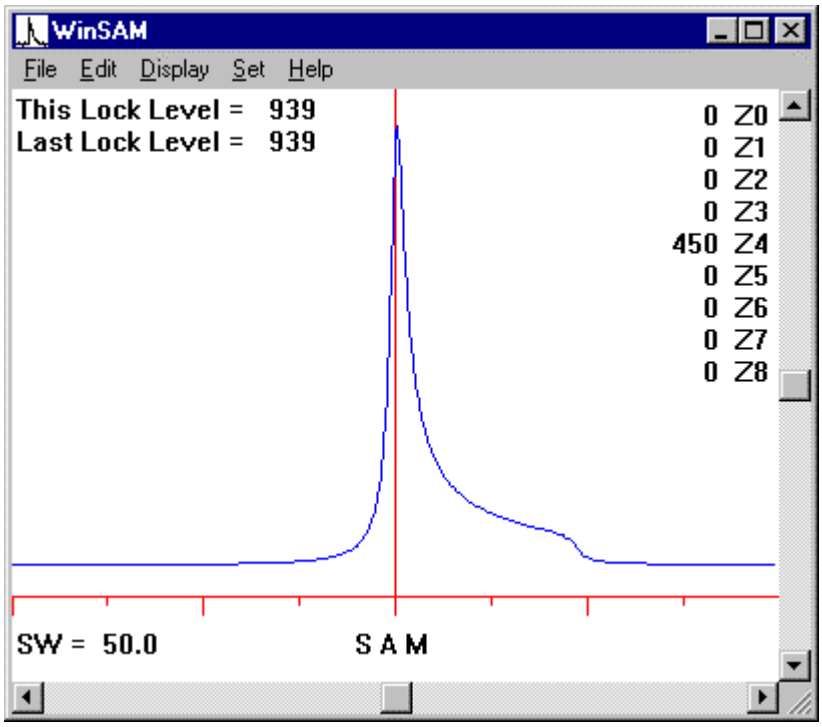
Z1 gradient



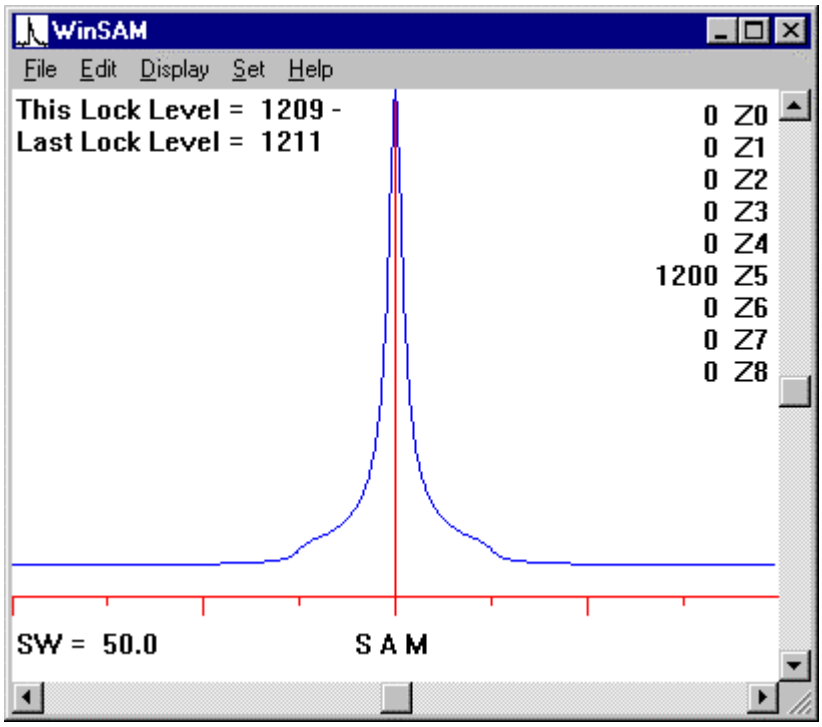
Z2 gradient



Z3 gradient


WinSAM
File Edit Display Set Help
This Lock Level = 939
Last Lock Level = 939
0 Z0
0 Z1
0 Z2
0 Z3
450 Z4
0 Z5
0 Z6
0 Z7
0 Z8
SW = 50.0
S A M

Z4 gradient


WinSAM
File Edit Display Set Help
This Lock Level = 1209 -
Last Lock Level = 1211
0 Z0
0 Z1
0 Z2
0 Z3
0 Z4
1200 Z5
0 Z6
0 Z7
0 Z8
SW = 50.0
S A M

Z5 gradient

The most common observable symptoms of misadjustment of the nonspin shims are spinning sidebands. First-order spinning sidebands are derived from field inhomogeneities the sample experiences once each rotation (X, Y, ZX, and ZY). Second-order spinning sidebands are derived from field inhomogeneities which the sample experiences twice per sample rotation (XY and X2-Y2).

The third-order nonspin shims, Z2X, Z2Y, ZXY, Z(X2-Y2), X3, and Y3, can create both spinning sideband problems and lineshape problems. The lineshape problems are usually very low at the base of the peak. An especially interesting symptom from two of these shims can be seen while adjusting the spinning shims. When the adjustment of Z3 changes the observed NMR signal from a broad-based signal with no spinning sidebands to a narrow-based signal with spinning sidebands, then the responsible shim is most likely Z2X or Z2Y.

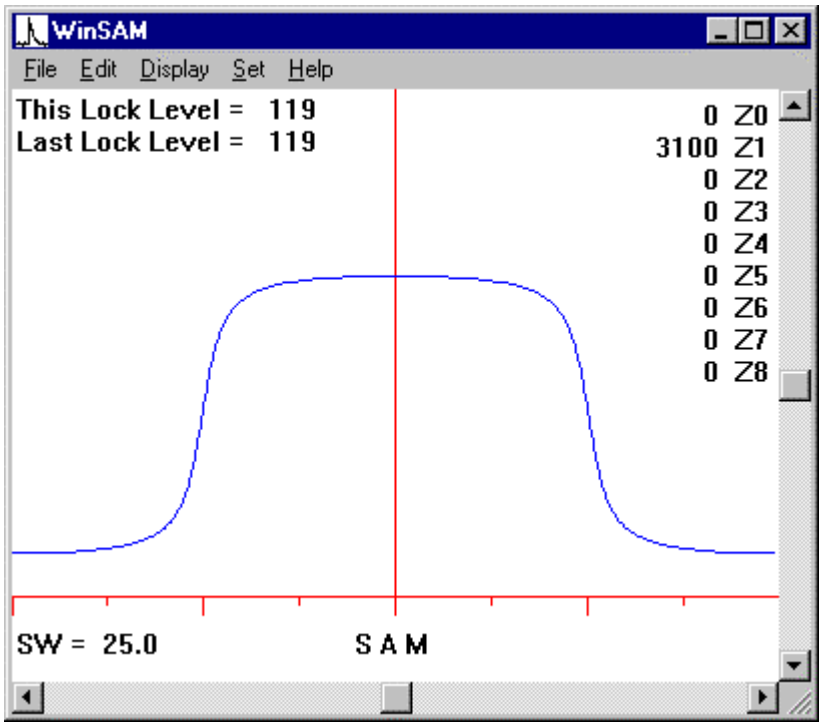
As the NMR spectroscopist endeavors to find more sensitivity, one method he has historically turned to is longer coils in the NMR probe. As the coils become longer and see more sample, it becomes much harder to optimize the field over the entire length of the coil. Also, the higher-order shim gradients become much more important with longer coils, making shimming more complex. Usually some degradation of lineshape is inevitable when longer coils are used. Conversely, if better resolution and lineshape are required, a probe with a shorter coil should be tried.

The probe coil also contributes to resolution and lineshape problems by means of the magnetic susceptibility of the materials of construction. All parts of the probe have a magnetic susceptibility to some degree. This magnetic susceptibility has a tendency to bend the magnetic field nearby. These bent field lines distort the observed lineshape and the effect is greatest close to the source of the magnetic susceptibility. As the probe filling factors become better and the coil parts move closer to the sample, the coil's magnetic susceptibility has a greater effect on lineshape and resolution. A typical probe induces magnetic gradients on the sample which look a lot like a Z4 inhomogeneity symptom. The process of creating these gradients and how to shim them is discussed in depth in the chapter about Probes and Shimming.

Z1 profile

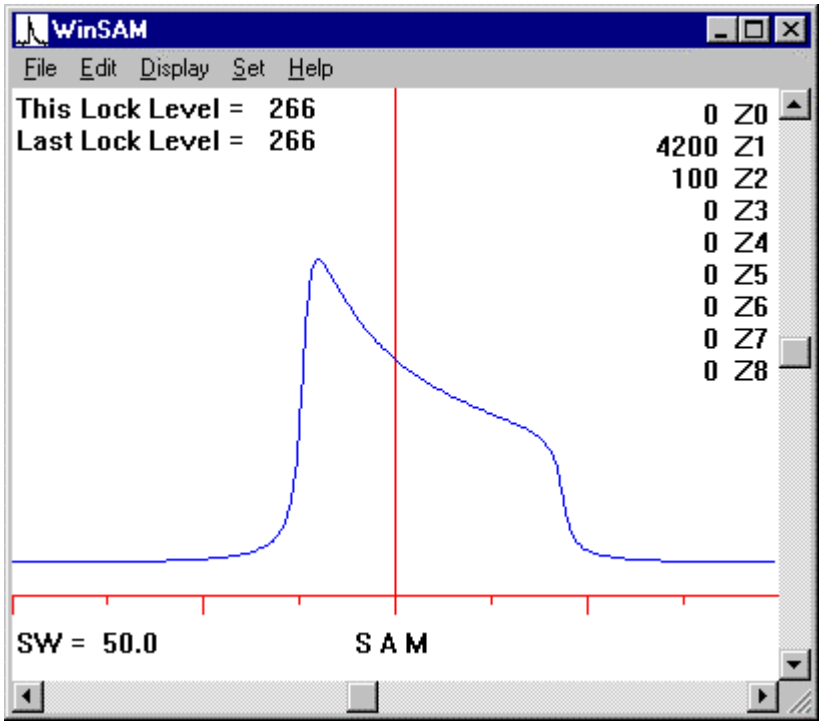
One tool which can be used to help the NMR operator in the shimming process is the "Z1 Profile". This can be thought of as an axial image of the sample in the probe. To take a Z1 profile, the NMR operator applies a large Z1 gradient and examines the resulting lineshape. If the probe had no magnetic susceptibility and the magnet's field were perfect,

then when a large Z1 gradient is applied the resulting lineshape is a rectangle as shown in the figure below:

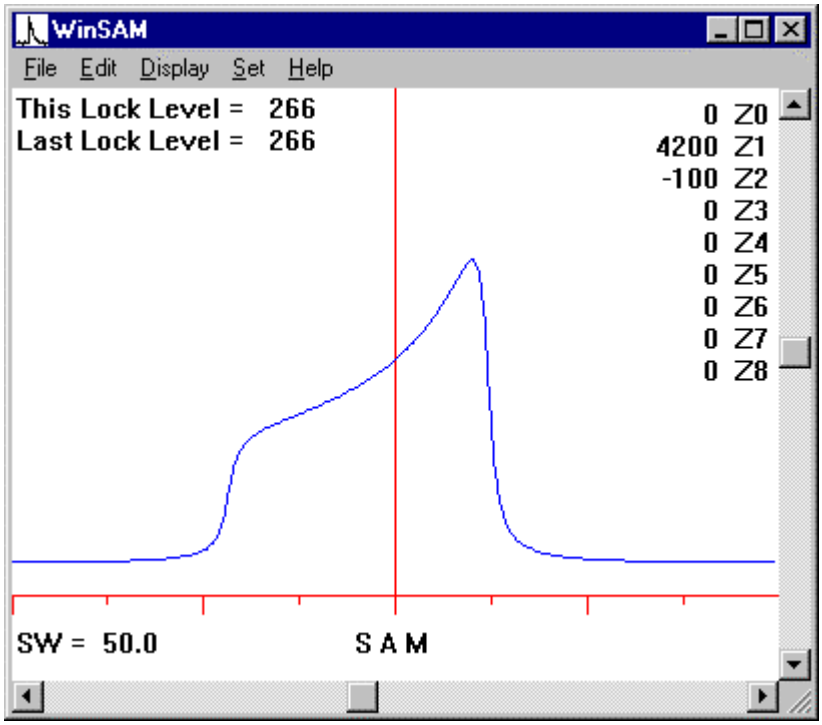


While this may seem a very uninteresting and undesired lineshape it contains a lot of useful information. Imagine the NMR sample being composed of many discrete finite samples placed along the axis of the NMR tube. When a Z1 gradient is applied to the sample, each of these finite samples would have a slightly different frequency based on the slightly different Z1 gradient value at each position along the Z axis. Under these conditions the left side of the lineshape spectrum above is one end of the NMR sample, the middle of the spectrum is the middle of the sample and the right end of the spectrum is the other end of the sample. There is no standard for NMR instruments as to which direction of Z1 gradient is generated with a positive current in the Z1 coil. For this reason, whether the left end is the top or the bottom of the sample depends on the polarity of the Z1 gradient applied in the NMR system when the Z1 gradient is adjusted in one specific direction. On some instruments the adjustment of the Z1 gradient in one direction (positive) creates the Z1 profile with the bottom of the sample to the low frequency side. The same adjustment on a different instrument could produce the opposite effect. No matter which direction is the top in this profile, the application of the Z1 gradient produces an "image" of the sample in the coil. This means that we now have some positional information to work with. Let's explore what we can do with this information.

While we have our Z1 profile image, let's apply a Z2 gradient to the shims. Depending on the direction, we get something like one of the two lineshape images below:


WinSAM
File Edit Display Set Help
This Lock Level = 266
Last Lock Level = 266
0 Z0
4200 Z1
100 Z2
0 Z3
0 Z4
0 Z5
0 Z6
0 Z7
0 Z8
SW = 50.0
S A M

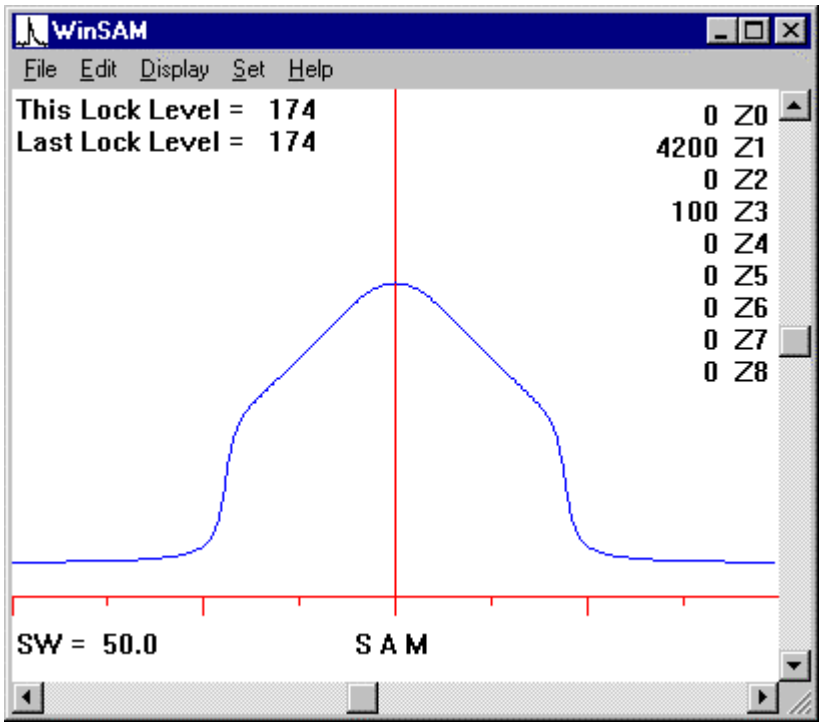
+ Z2


WinSAM
File Edit Display Set Help
This Lock Level = 266
Last Lock Level = 266
0 Z0
4200 Z1
-100 Z2
0 Z3
0 Z4
0 Z5
0 Z6
0 Z7
0 Z8
SW = 50.0
S A M

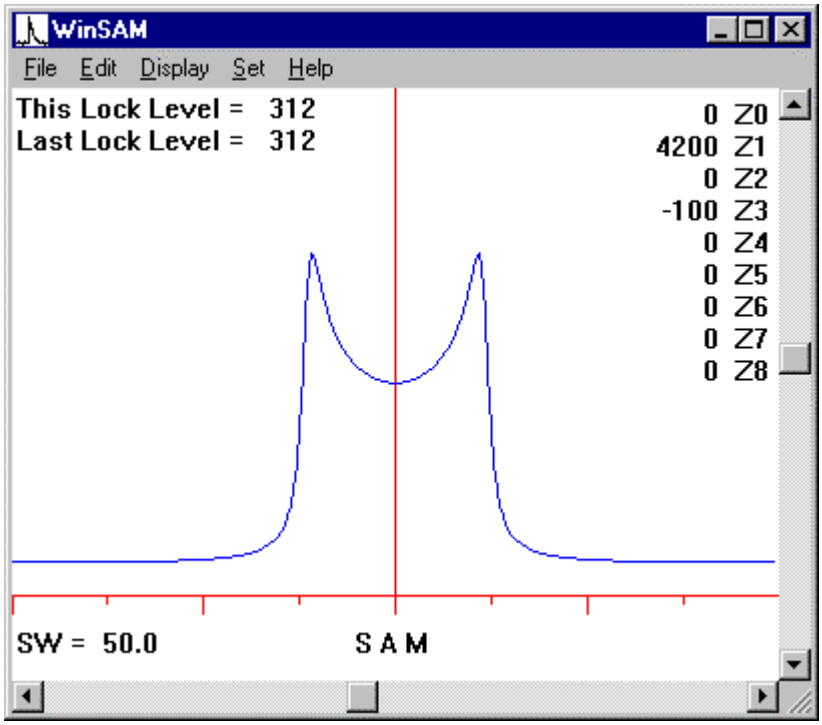
- Z2

It is easy to note that the shape is asymmetrical. It can also be noted that the top of the Z1 profile is a curve with the order Z2. With practice and when comparing to later examples, the reader will see that as the gradient order increases, the outside edges of the Z1 profile are affected more than the center. In fact, with the higher order gradients the center of the Z1 profile is relatively unaffected.

Starting from the basic Z1 profile again and applying a Z3 gradient would give one of the two pictures below:



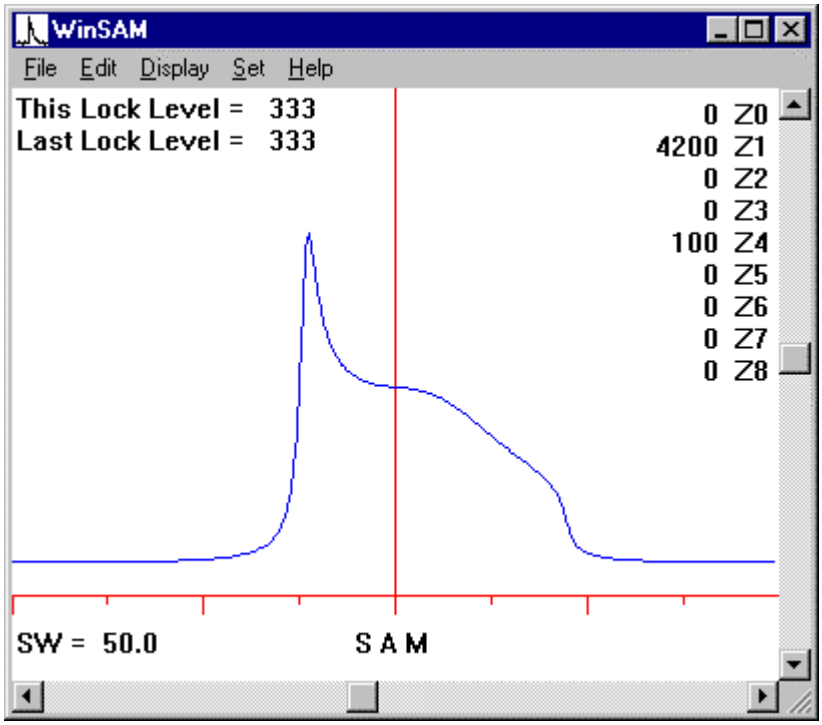
+ Z3



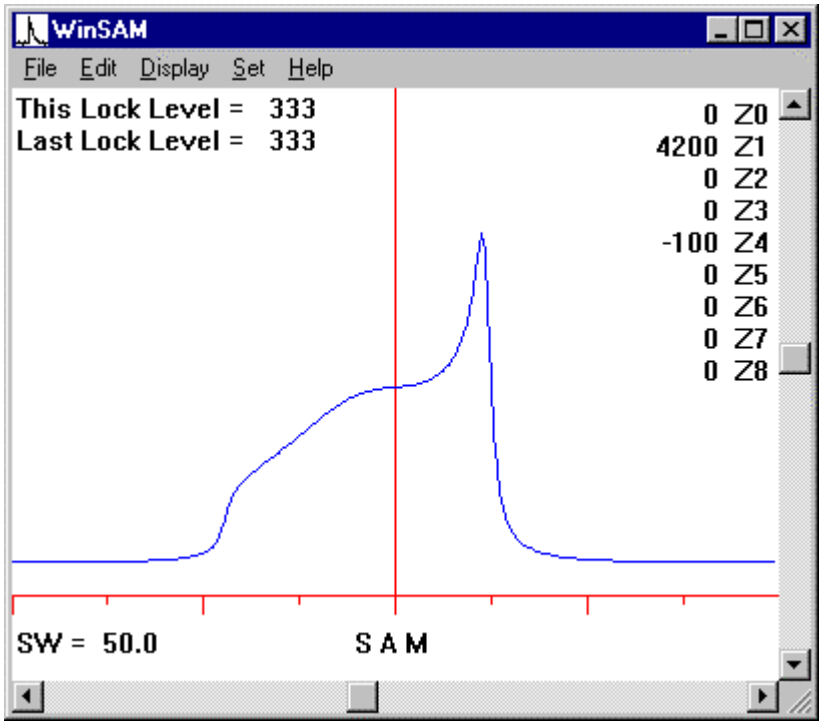
- Z3

Here it is easy to note that the Z1 profile changed shape symmetrically. For now the reader should note the drooping edges when Z3 is changed in one direction and the peaked edges when Z3 is changed in the opposite direction. Also note how far from the center the effects start taking place. As we move to higher order gradients, it will be easy to note that the effect takes place farther to the outside edges.

Starting once again from the basic Z1 profile and applying a Z4 gradient would give one of the two pictures below:


WinSAM
File Edit Display Set Help
This Lock Level = 333
Last Lock Level = 333
0 Z0
4200 Z1
0 Z2
0 Z3
100 Z4
0 Z5
0 Z6
0 Z7
0 Z8
SW = 50.0
S A M

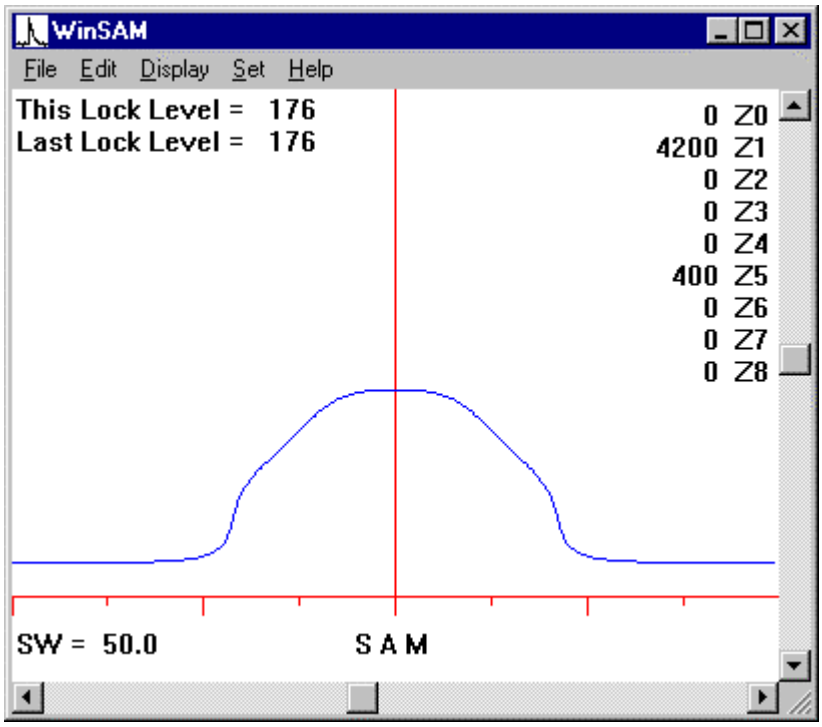
+ Z4


WinSAM
File Edit Display Set Help
This Lock Level = 333
Last Lock Level = 333
0 Z0
4200 Z1
0 Z2
0 Z3
-100 Z4
0 Z5
0 Z6
0 Z7
0 Z8
SW = 50.0
S A M

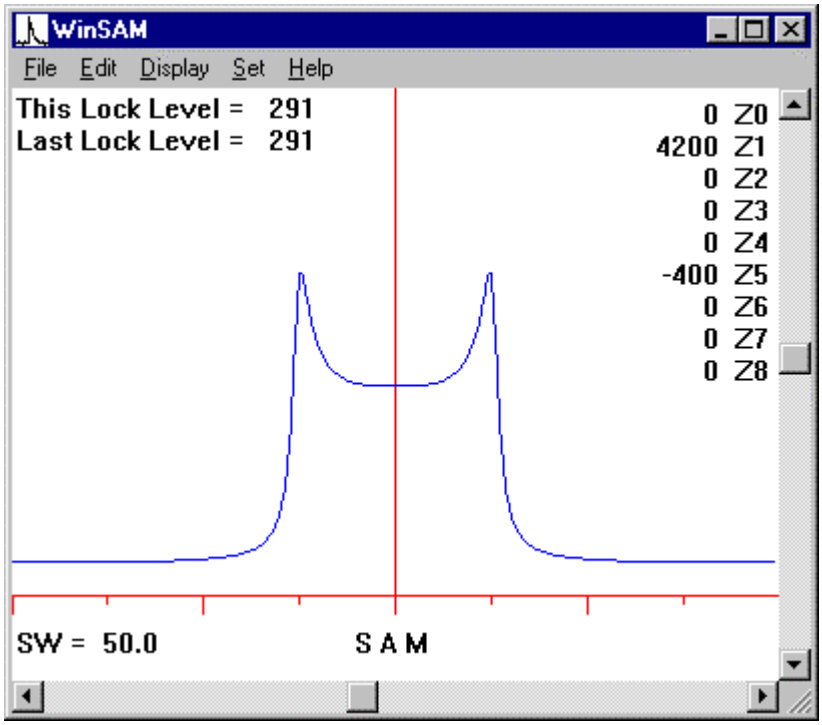
- Z4

It can be seen that the change in the Z1 profile is again asymmetrical, but now there is a flat region near the center that is relatively unaffected compared to the ends of the Z1 profile.

Starting again from the basic Z1 profile and applying a Z5 gradient would give one of the two pictures below:



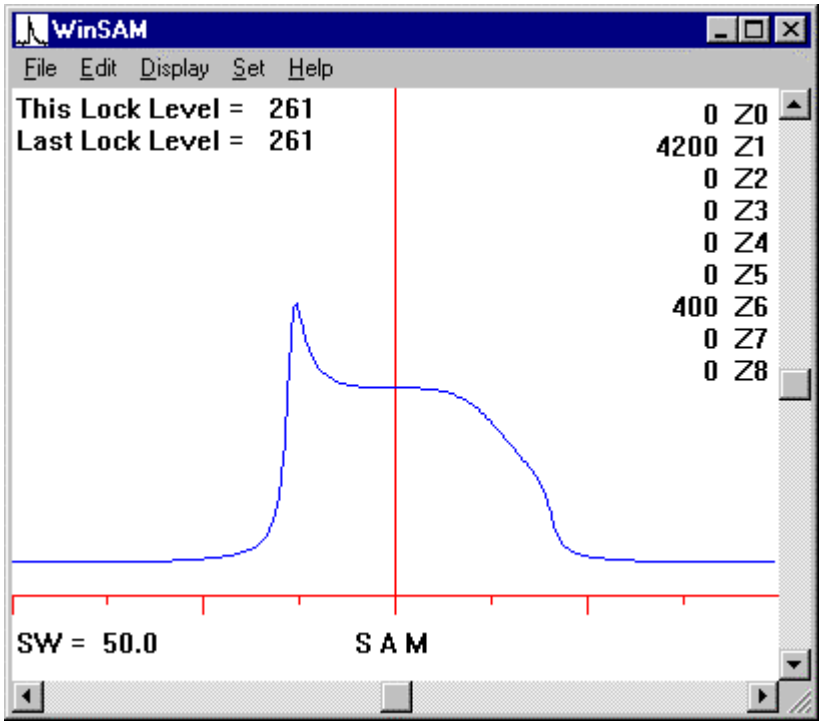
+ Z5



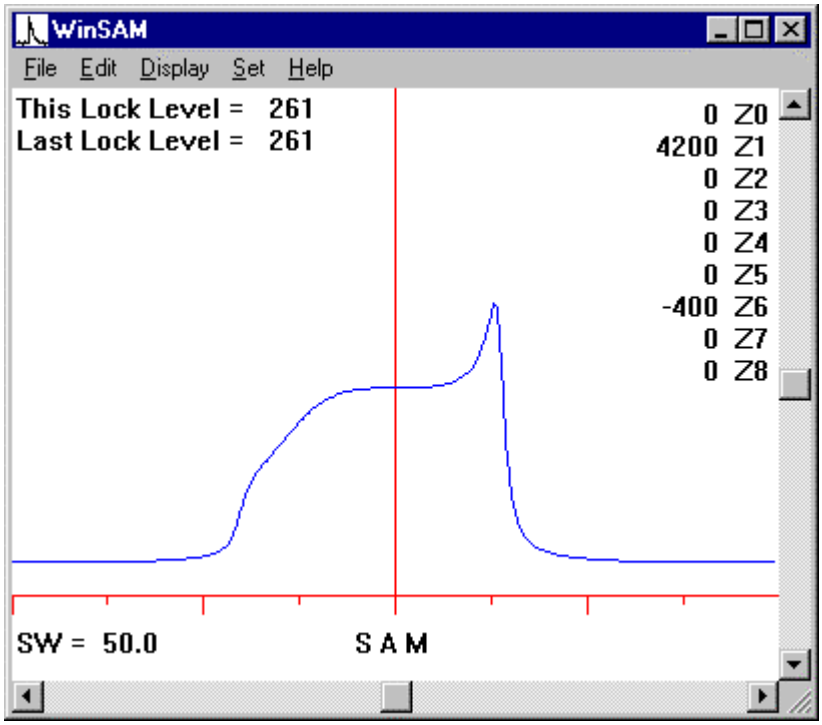
- Z5

It can be seen that the change in the Z1 profile is symmetrical. Again there is a flat region near the center that is relatively unaffected compared to the sides of the Z1 profile.

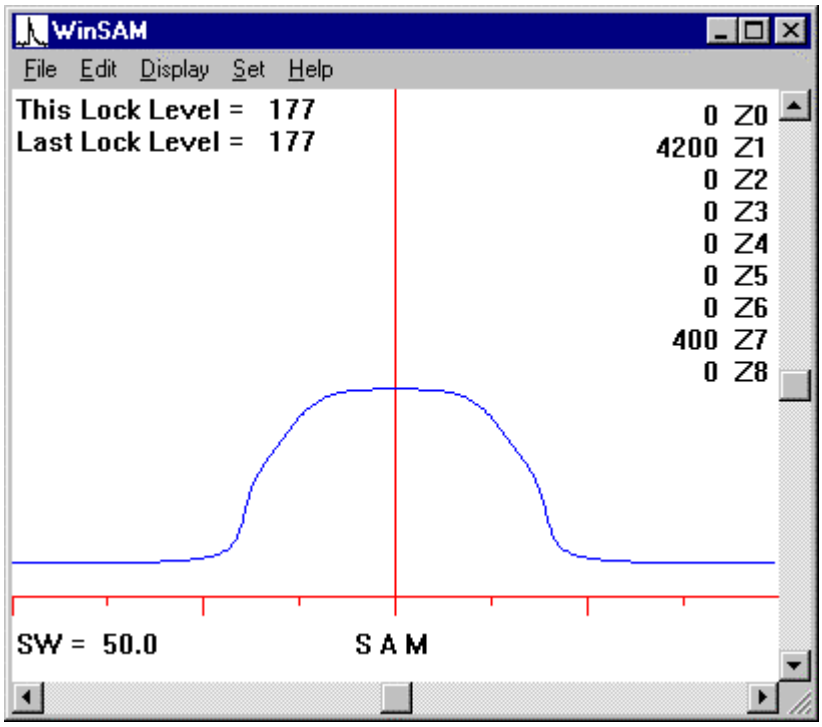
Starting from the basic Z1 profile and applying a Z6, Z7, and a Z8 gradient would give following set of pictures:


WinSAM
File Edit Display Set Help
This Lock Level = 261
Last Lock Level = 261
0 Z0
4200 Z1
0 Z2
0 Z3
0 Z4
0 Z5
400 Z6
0 Z7
0 Z8
SW = 50.0
S A M

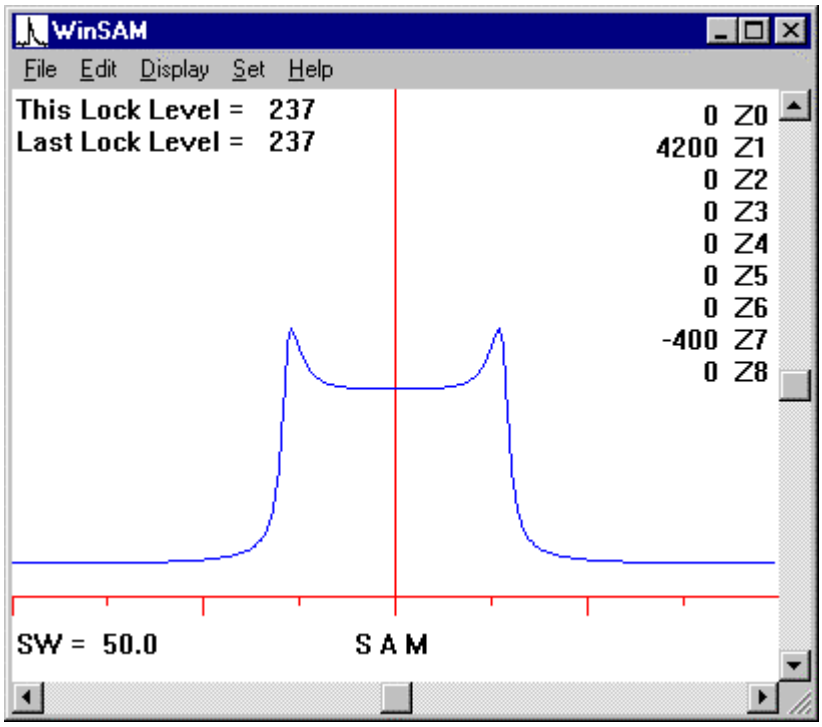
+ Z6


WinSAM
File Edit Display Set Help
This Lock Level = 261
Last Lock Level = 261
0 Z0
4200 Z1
0 Z2
0 Z3
0 Z4
0 Z5
-400 Z6
0 Z7
0 Z8
SW = 50.0
S A M

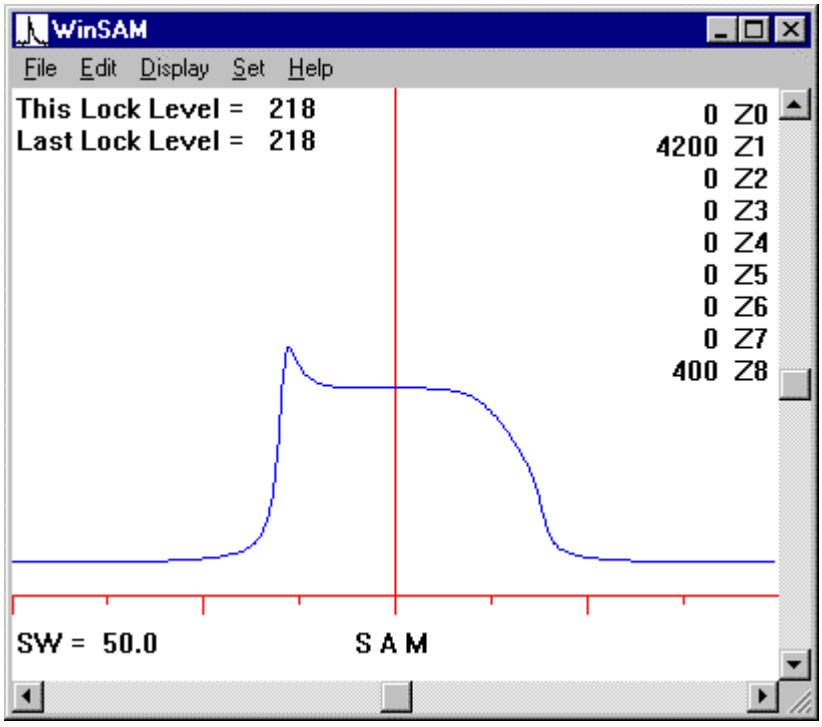
- Z6


WinSAM
File Edit Display Set Help
This Lock Level = 177
Last Lock Level = 177
0 Z0
4200 Z1
0 Z2
0 Z3
0 Z4
0 Z5
0 Z6
400 Z7
0 Z8
SW = 50.0
S A M

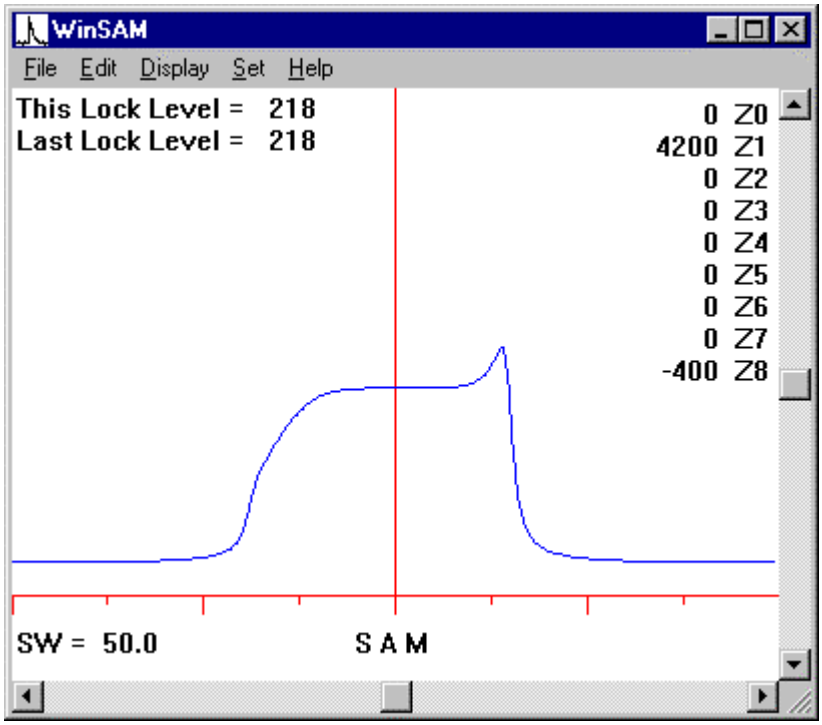
+ Z7


WinSAM
File Edit Display Set Help
This Lock Level = 237
Last Lock Level = 237
0 Z0
4200 Z1
0 Z2
0 Z3
0 Z4
0 Z5
0 Z6
-400 Z7
0 Z8
SW = 50.0
S A M

- Z7


WinSAM
File Edit Display Set Help
This Lock Level = 218
Last Lock Level = 218
0 Z0
4200 Z1
0 Z2
0 Z3
0 Z4
0 Z5
0 Z6
0 Z7
400 Z8
SW = 50.0
S A M

+ Z8



- Z8

A pattern emerges from the complete set of Z1 profiles:

1. Even order gradients (Z2, Z4, Z6, and Z8) produce asymmetrical changes in the Z1 profile. The plus and minus Z1 profiles have about the same width at half height.
2. Odd order gradients (Z1, Z3, Z5, and Z7) produce symmetrical changes in the Z1 profile. The plus and minus Z1 profiles have different width at half height.
3. The higher the order of shim gradient applied, the farther from the center the significant effects are observed in the Z1 profile.
4. Odd order gradients, particularly Z3, give different widths with opposite polarity Z1 profiles, while even order gradients are unchanged. Z3 should be adjusted to give the same width at half height in the presence of positive and negative Z1 gradients.

There are similar effects that can be observed when shimming while looking at normal lineshapes instead of the Z1 profile:

1. An asymmetrical change in lineshape is seen when even order Z shims are adjusted.
2. A symmetrical change in lineshape is seen when odd order Z shims are adjusted.
3. The higher the order of shim gradient adjusted, the lower down the peak the lineshape distortion is noticed.

From this study of Z1 profiles, a very different procedure of shim optimization emerges as a new shim tool. Normally the lowest order gradients (Z1, Z2 and Z3) are adjusted first, then based on lineshape criteria, the higher order gradients are adjusted. The low order gradients are re-adjusted as necessary in this process. However, if the NMR operator applies a Z1 gradient to get a "Z1 profile" of the sample, the highest order gradients are adjusted first to get the most square and symmetrical ends on the Z1 profile, then the center region is flattened with the lower order gradients. During the entire process odd order gradients are adjusted when a symmetrical change is desired. Even order gradients are adjusted when an asymmetrical change is desired.

In general, when trying to shim using this technique the goal is to make the squarest and flattest Z1 profile possible. This applies even when the NMR probe has magnetic susceptibility problems. When you shim for the squarest and flattest Z1 profile, you will get the best lineshape when the Z1 gradient is removed. If the NMR probe has very severe magnetic susceptibility problems, the Z1 profile will not be very flat at all. With probe magnetic susceptibility problems localized to specific regions of the probe's coil, the Z1 profile may have many peaks and valleys where it should be flat. Probes that have several regions of deep peaks and valleys will be very difficult to shim and probably not produce a good lineshape when done shimming.

This technique is not the "one and only, best way" to shim. It should be considered a tool and used when necessary. It does seem to offer some advantages when trying to adjust the higher order gradients. Use this technique as one of your many tools for shimming logically.

The Z1 profile can also be used to visualize the probe's B1 homogeneity. After shimming is complete, apply a Z1 gradient. Take a spectrum with a 90^{o} pulse and plot out the rectangular lineshape. This represents the probes coil's excitation picture. Now take another spectrum with a 180^{o} pulse and plot it out with the same scale and position as above. This plot will have a region near the center of the initial rectangular region which is near zero intensity. This shows the area of the sample which is experiencing the 180^{o} degree tip angle. The ends of the initial rectangular region represent the ends of the probe's coil. These regions will not be near zero because the ends of the coil receive less than a 180^{o} tip angle. In probes with a good B1 homogeneity the region near zero will be a significant portion of the initial rectangular region. In probes with poor B1 homogeneity, the center region will dip slightly below zero intensity with little or no flat region near zero. This is a picture of the sample receiving a 180^{o} tip angle versus position in the probe's coil.

The Quickie Z1 Profile Approach to Shimming.

1. Start with a first pass optimized shimmed instrument with at least Z1 and Z2 optimized using a sample with a single strong resonance.
2. Put on a Z1 gradient by the amount (MagZ1) to get a Z1 profile with a width at half height of at least 200 Hz.

3. Note the width at half height with both positive Z1 gradient (+MagZ1) and negative Z1 gradient (-MagZ1). If they are different, change Z3 by an incremental amount and re-measure. Continue adjusting Z3 until the width at half height of the positive and negative Z1 profiles are the same.
4. Adjust Z4 for the flattest top with the same symmetry with both the positive and negative Z1 profiles.
5. take off the Z1 gradient and optimize Z1 and Z2.

How the sample affects shimming

The NMR sample and its preparation have tremendous influence on the quality of the spectra and the shimming process. These areas include:

- End effects (magnetic susceptibility) of the sample.
- Particulate materials in the sample.
- Dissolved materials in the sample.
- Radiation damping.

End Effects

An often overlooked parameter in the shimming process is the magnetic susceptibility effect caused by the ends of the sample. All solvents have a very high magnetic susceptibility value, and water is higher than most. Unless the sample is infinitely long, the end effects of the solvent can be seen. Usually the NMR operator tries to shim out these effects without fully appreciating their origin. The NMR probe has an rf coil which is used to generate the excitation of the nucleus and to receive its signal. When properly constructed, the NMR probe's coil generates and receives a signal over a sample volume which is a cylinder with height equal to the height of the coil window. This region is referred to as the "active" region of the probe. An empirical method of determining the active region of the probe's receiver coil is discussed in the Before You Start To Shim chapter. If the ends of the sample are far removed from the active region, then the magnet field change caused by the ends of the sample and the solvent's magnetic susceptibility are also far removed from the active area of the probe's coil. Under this condition, there may be a change in the absolute value of the magnetic field in the active area of the probe's coil, but it is a constant change best described by the Z0 gradient.

The key question in this discussion is how far is "far enough removed" from the active area of the probe. The answer depends on many variables, a few important ones being the height of the receiver coil, the diameter of the receiver coil and the magnetic susceptibility of the solvent. The larger the diameter of the tube the farther the effect from the solvent ends is detectable. For a 5 mm tube, the effects are easy to measure when the sample extends 1 cm above and below the active region of the probe's coil. The effects are difficult to measure when the sample extends 2 cm above and below the active region of the coil. This is more sample length than most NMR operators want to use. The shorter the sample used, the more difficult the shimming process will be, but less sample is

required. This is another example of the trade-offs always being made when operating an NMR spectrometer.

As a general rule, if the sample length used in the NMR spectrometer's 5mm probes is going to be longer than 2 cm above and below the active region of the probe's coil, the sample-to-sample variations of the shim values derived from the end effects of the solvent will be negligible. If the typical sample to be run on the NMR spectrometer is going to be shorter than 1.5 cm above and below the active region of the coil, then the sample length should be tightly controlled and maintained the same for all samples. Then if the sample tube's position in the spinner is kept the same, a set of shim values can be developed and reused from sample to sample.

When a spectrum of a limited amount of sample is necessary, one can use a micro sample probe or constrain the sample in a spherical micro-cell. A spherical micro-cell works better than a cylindrical micro-cell. This is because the magnetic susceptibility influences on the sample caused by the glass of the micro-cell are only of the Z0 type when the sample is surrounded by a sphere or is part of an infinite cylinder. When the micro-cell is inserted in a 5mm NMR tube, it is usually best to surround the micro-cell by the same solvent as used in the micro-cell such that the ends of the surrounding solvent extend more than 2 cm above and below the coil's active area.

Since deuterochloroform is a common NMR solvent, another trick which has been used by many NMR laboratories is to take advantage of the fact that Teflon has about the same magnetic susceptibility as chloroform. The sample is then placed between two Teflon plugs. The total length of the sample and Teflon plugs is then maintained to be 2 cm above and below the probe's active region. This technique also minimizes the effects from the end of the solvent and makes shimming small samples easier.

Particulates

After much expense and time to obtain precious sample and put it into an NMR tube, many an NMR spectrum has been of much lower quality than necessary because of particulates in the sample. When the NMR sample is examined under a magnifying glass, one can often see little bits of this and that floating around. These particulates often have a very different magnetic susceptibility from the NMR sample. This influence on the magnetic homogeneity over the active volume of the probe cannot be compensated for by shimming since the particulates are moving about while the sample is spinning. These need to be removed by filtering through commercial sample filters or glass wool stuffed into the bottom of a pipette.

As surprising as it sounds, it is not at all uncommon for the particulates to be iron. Holding a small magnet to the side of the NMR sample can cause these iron particles to run to one side of the sample tube, as seen by examining the sample under a magnifying glass. Some samples with iron particles can be examined before putting them into the NMR spectrometer and appear clean. After shimming for a while with unsatisfactory results, the sample can be removed and examined under a magnifying glass and big

chunks can be seen. This comes about because the original sample had some very fine iron particles that could not be seen, but which coagulated (stuck together) after being magnetized in the magnet. It is often a good idea to look at the sample under a magnifying glass before expending a lot of time on a sample which seems difficult to shim.

Dissolved material

In addition to having macroscopic iron particles in the sample, it is possible to have dissolved paramagnetic material in the sample. This will not be visible to the eye, but can cause dramatic broadening of NMR lines due to paramagnetic relaxation, which is a very efficient mechanism of T2. relaxation. Even dissolved oxygen in the sample will cause broadening, which is why NMR resolution standard samples are always supplied degassed in sealed tubes.

Viscosity is also a cause of broadening of NMR lines because of the reduced correlation times for molecular tumbling. Slower motions are a more efficient mechanism of T2 relaxation.

Radiation Damping

Another broadening phenomenon which can be observed under some conditions is called "radiation damping". This phenomenon when used in conjunction with NMR is different from radiation damping when discussed by an electronics engineer. When discussed in relation to NMR, it is an additional relaxation mechanism for the nuclei arising from energy in the NMR sample being coupled into the probe's coil. Losses in the probe's circuit then provide a means for removing energy from the nuclei or "relaxing" the nuclear polarization. This relaxation process is proportional to the strength of the nuclear signal, the frequency of the nuclear signal, the sample coil filling factor and the Q of the probe's tuned circuit at the nuclear frequency. This means that it is most likely to be observed on high field instruments when observing proton in water solutions. The phenomenon can be seen with other samples such as CHCl3 on 500 MHz and 600 MHz systems if the CHCl3 sample is very concentrated (>5%).

The presence of radiation damping is easy to detect. Run a spectrum of the sample under conditions where you suspect radiation damping and measure the linewidth of the signal. Then tune the probe way off resonance and run the same spectrum again. You will of course have a reduced signal to noise, but if the line becomes narrower, then radiation damping was present. Since the degree of relaxation the nucleus receives by this mechanism is directly related to the strength of the NMR signal, it is possible that water of a protein sample could be broadened by this process without broadening the protein signals themselves.

Ideally, probes should have little or no influence on the shimming process. However, many probes have one or more characteristics that make them non-ideal. In general, the

"better" the probe, the easier the shimming process. Major areas where probes can influence shimming are:

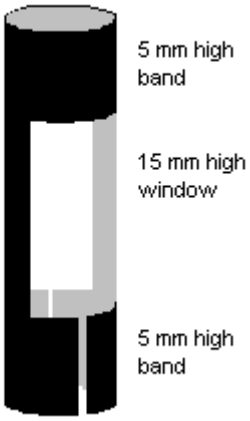
- The materials used in probe construction.
- Size (length and diameter) of the probe coil.
- The RF homogeneity generated by the probe coil.

Each of these areas produces constraints and/or influences on the shimming process. An understanding of these influences and what the NMR operator can and cannot do makes shimming less frustrating.

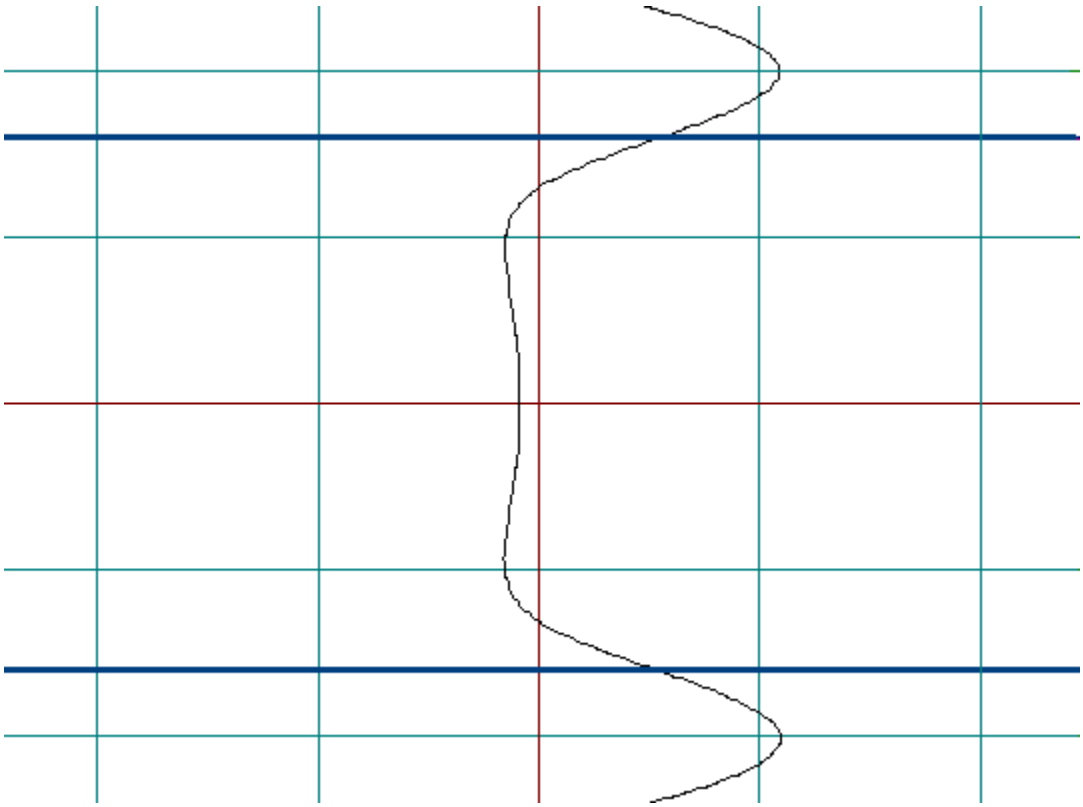
Probe Materials

The basic way probe materials can influence the shimming process is by the magnetic susceptibility of the probe materials. The area of the probe of major concern is the probe's coil because it is closest to the active region of the probe, the volume of sample which contributes to observed signal. Other parts of the probe can influence the shimming process, but they usually are farther away from the probe's active region and, because the effects fall off with distance, they have less influence. Because of the distance from the sample, the effect that these probe parts produce is a low order gradient. Low order gradients can cause the shim values of particular gradients to change, but they are usually straightforward to shim. The coil, however, is much closer to the probe's active region and has a complex geometric arrangement relative to the sample. Any magnetic susceptibility of the probe's coil changes the magnetic field profile in the active region in a complex manner related to the complex geometric pattern. The NMR operator is then faced with the prospect of trying to correct this influence with the shims.

The influence of the probe coil's magnetic susceptibility was mentioned in the chapter on Symptoms of Inhomogeneity. Here we will expand on this issue and explore in more depth a simple probe coil often referred to as a single turn Helmholtz or coaxial cavity.

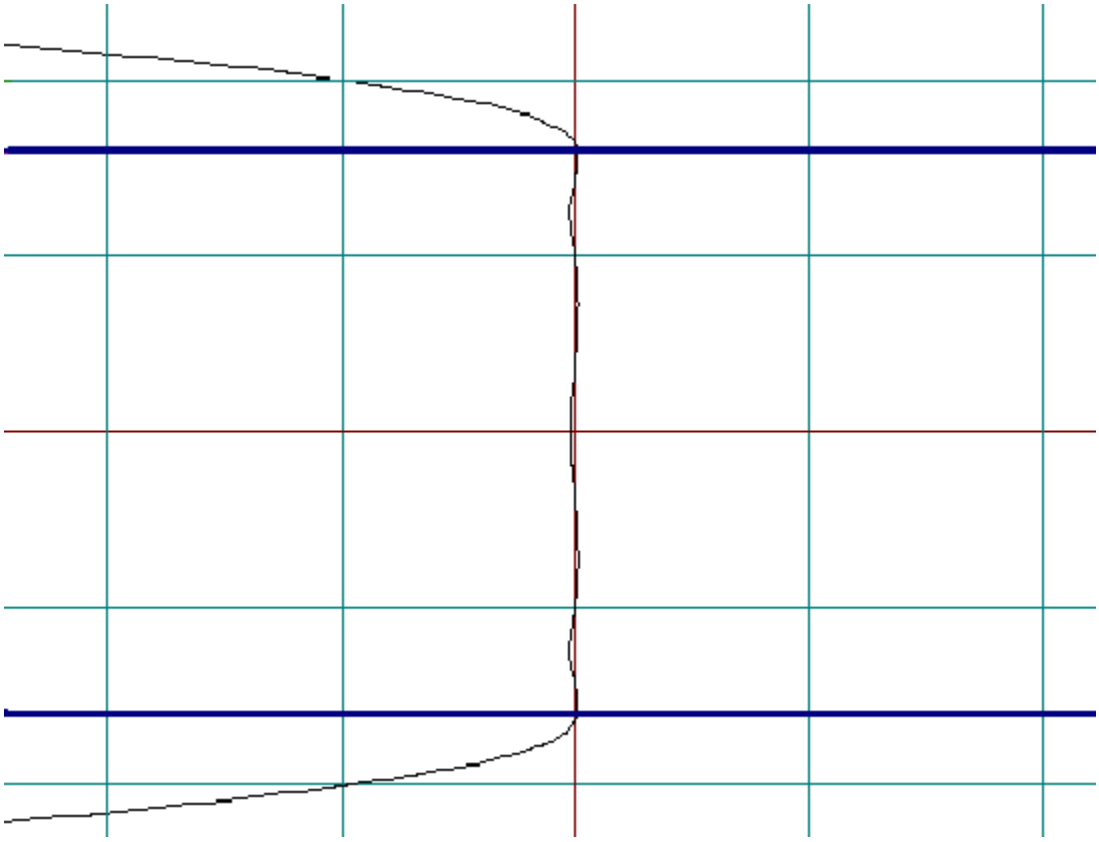


A simplified drawing of this probe design is shown on the left. If the coil material has a magnetic susceptibility, it perturbs the magnetic field producing a gradient whose geometry is related to the geometry of the coil.



If the coil described were made of material with non-zero magnetic susceptibility such as copper, the gradient profile generated along the Z axis through the center of the sample can be calculated and would look like the graph to the left. The vertical axis in this figure represents position along the sample. The heavier horizontal blue lines represent the ends of the coil window.

Gradients are displayed as horizontal deviations from zero at the center. A perfect field would have a profile which is a straight, vertical line at the center of the plot, showing no gradient over the active region of the probe, which is the area between the top and bottom edges of the window. The deviation from ideality of the gradient profile shown would result in a distorted lineshape. Note that the largest gradients are found at the ends of the coil window, where there is a sudden change in magnetic susceptibility.

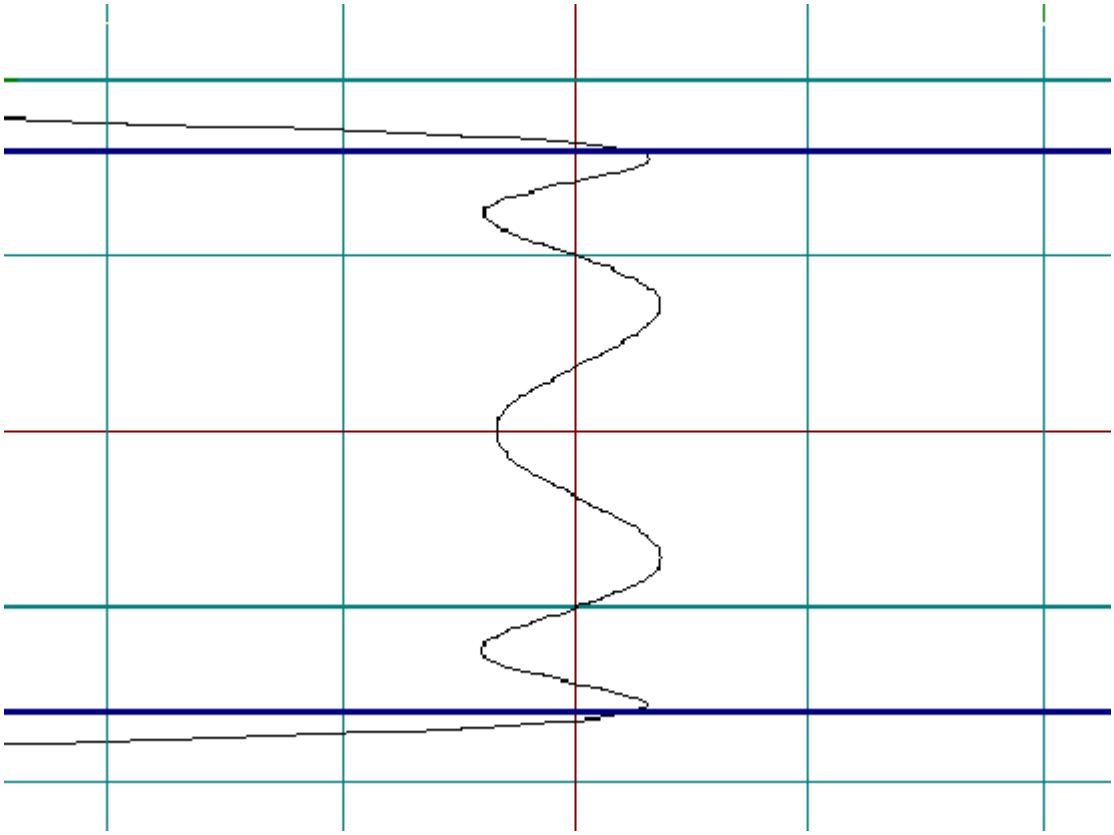


If it is assumed that only the sample within the window generates signal, the values of Z0 through Z4 gradients best able to correct this coil-induced gradient can be calculated. If we apply these gradients to the induced field to correct them (called shimming), the result is shown on the graph to the left. Notice that there are residual field gradients not correctable with Z0 - Z4.

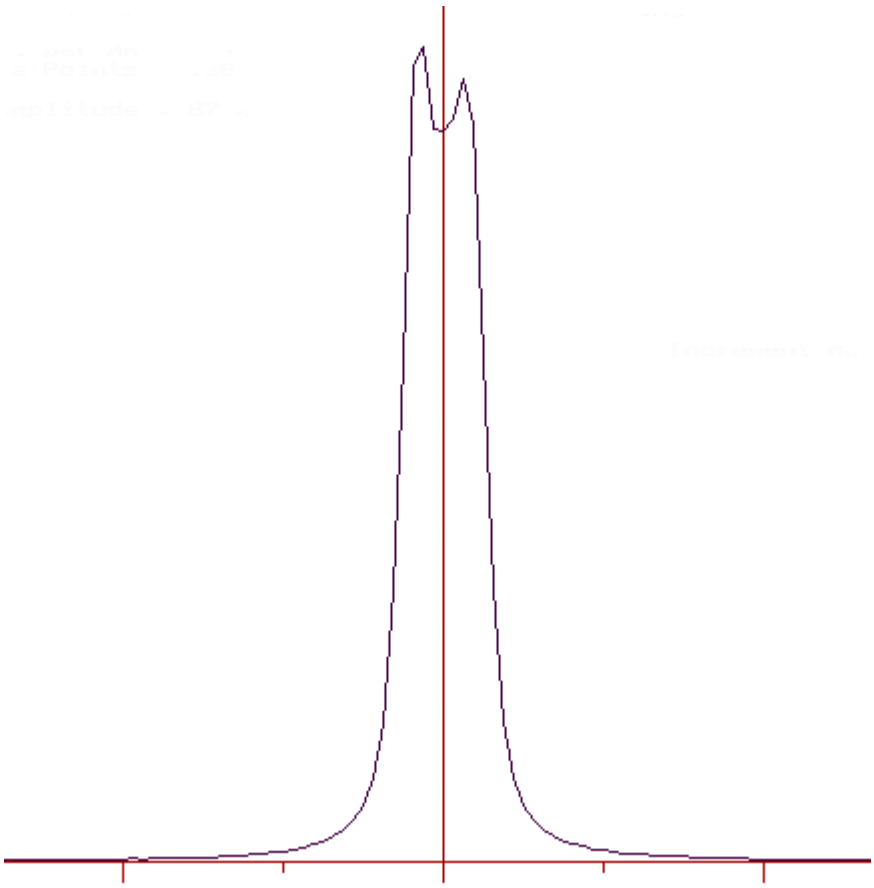
The corrections applied here (in arbitrary units) were:

Z0 -77
Z1 -85
Z2 -1155
Z3 454
Z4 324

Most of the distortion is 2nd order.



This is the same field profile as in the previous figure, with the horizontal scale increased to make the distortion more obvious.

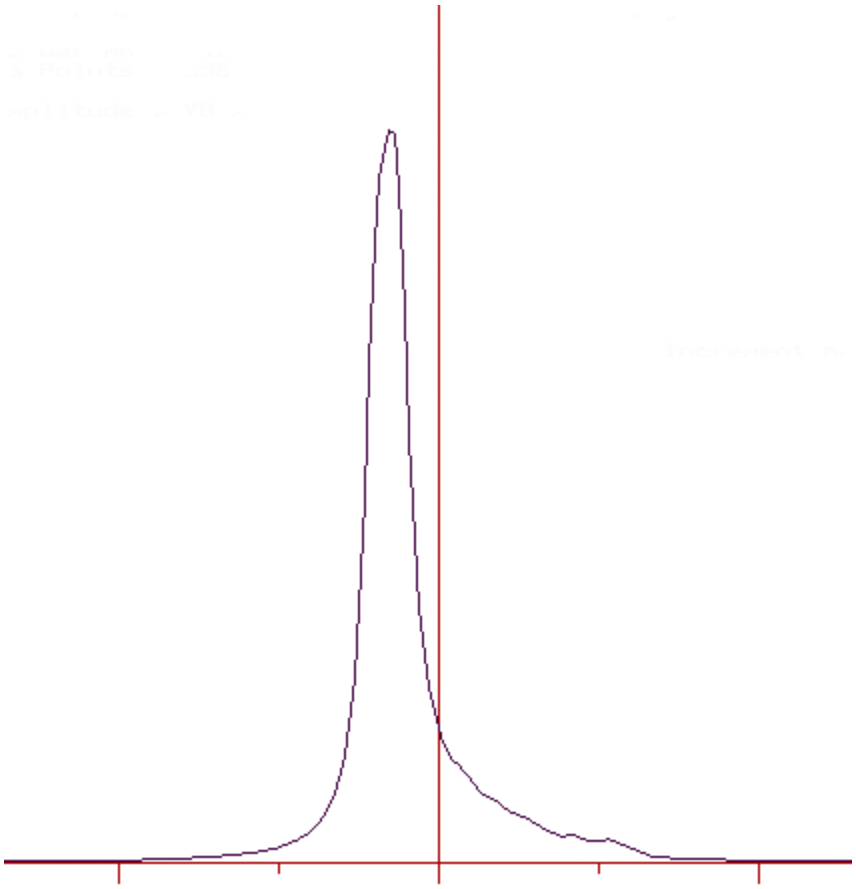


This is the lineshape corresponding to the gradient plot above. Even though the gradients have been corrected to the best extent possible using gradients Z0 through Z4, the result is a split line -- totally unacceptable.

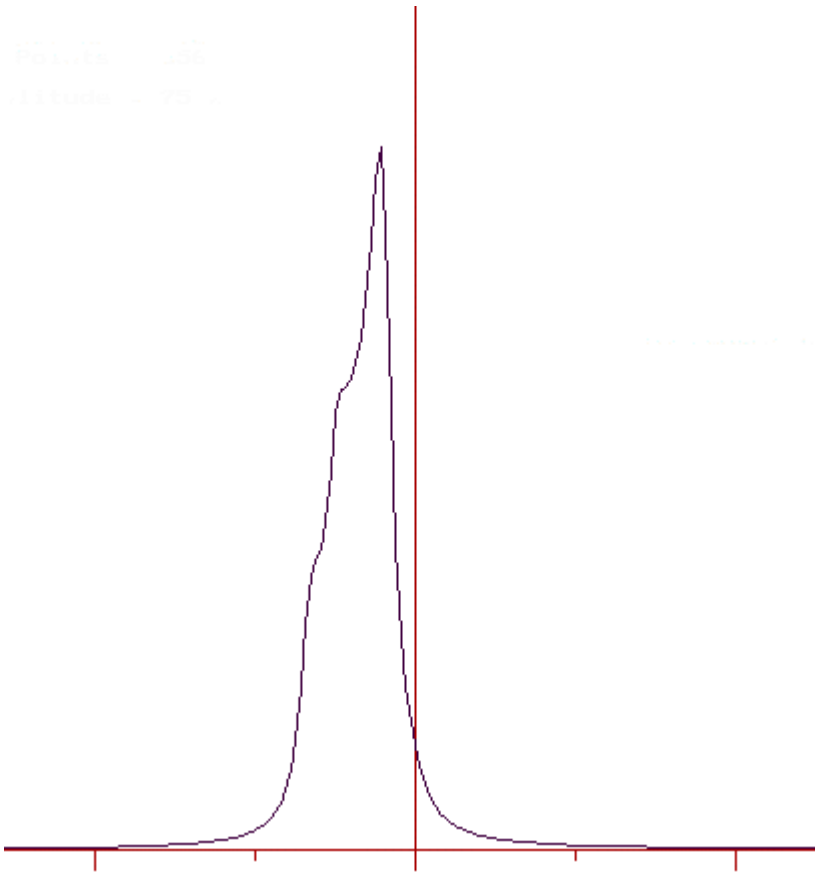
As can be seen in the graphs, substantial field gradients can be induced by the probe coil if the materials have non-zero magnetic susceptibility. If using such a probe, the NMR operator would adjust these shim gradients to correct the coil-induced gradients. Even after this is done, there are residual gradients that are not correctable by Z0 through Z4. These residual gradients oscillate between a slightly positive and a slightly negative field value. This field would generate the "split field" type of lineshape shown above, where the peaks would either be broader than necessary or split into a doublet. This type of lineshape would result from perfectly compensating for the coil-induced gradients up to 4th order. However, the NMR operator would be very unhappy with the resulting lineshape and might continue to shim, probably concentrating on the even order shims. In general, the operator would probably move Z2 a little one way and Z4 a little the other way and then adjust Z1 for the best lineshape. This process consists of misadjusting the shims to smear out the lineshape so that it is acceptable. This results in shimming

symptoms that many operators may have noticed: the resolution gets better while the lineshape distortions get worse. In extreme cases of this phenomenon, the NMR operator observes a very frustrating symptom. When the NMR instrument is shimmed the lines in the spectrum tend to split. About the only cure for such a problem is to de-shim the system to spread out the doublet to a broad singlet or use a different probe.

The actual shimming process to correct for the probe coil's magnetic susceptibility influence is more complicated from the NMR operator's point of view. Using the example of the typical single turn Helmholtz coil made from made from a material with magnetic susceptibility one fifth that of pure copper in a perfect magnetic field, the magnetic gradients induced by the probe on the sample would generate a lineshape as shown on the left below. The user would look at this and try to correct the lineshape problem using Z4. Using only pure Z4 the user would get the result shown the figure on the right below.. This generates a lineshape which looks like Z2 needs adjustment.

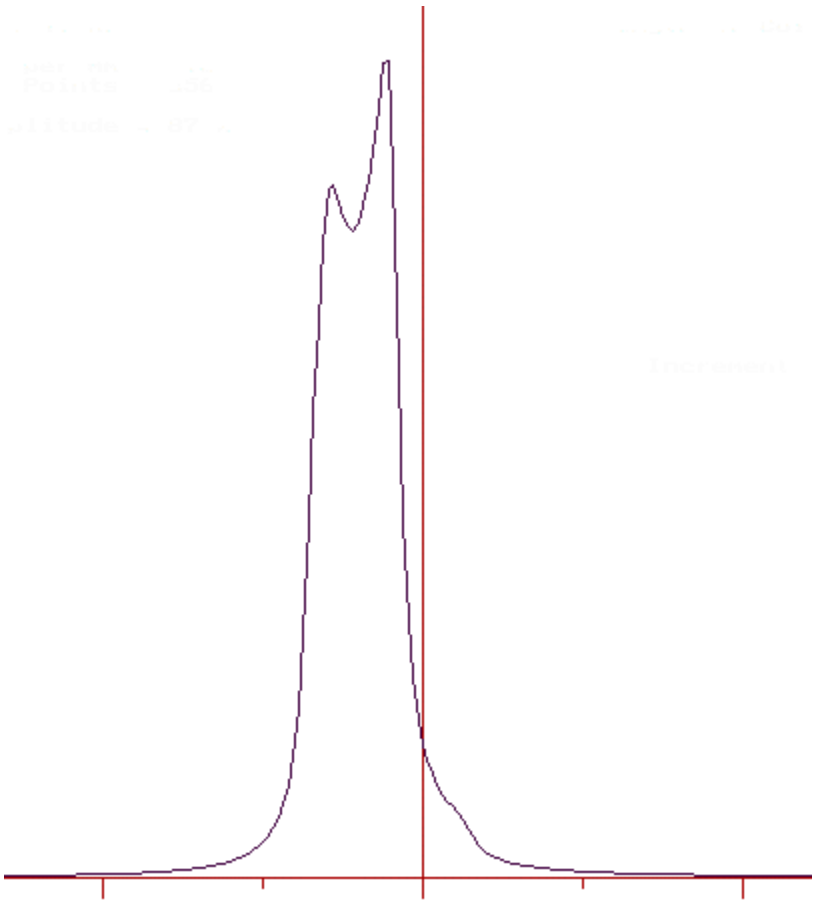


Effect of non-zero susceptibility on lineshape

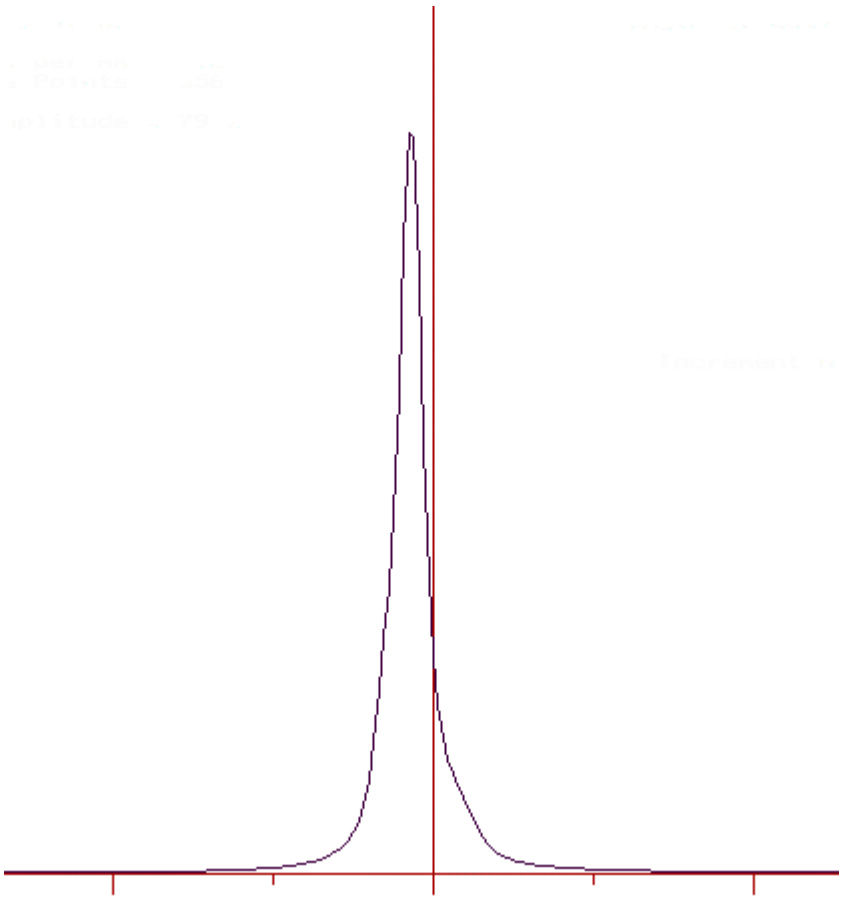


Attempt to correct using only Z4

Correcting with pure Z2 would give the result shown on the left below. This again looks like Z4 needs adjustment, but less than at the start shown in step 1. Iterating back and forth between Z2 and Z4 would correct the lineshape with the final result shown at the right below.



Attempt to correct using only Z2



Attempt to correct using Z2 and Z4.

While the result looks like an acceptable lineshape, its low order lineshape, width at half height and peak amplitude are all poorer than what would have been obtained with the same probe and sample in a probe with zero magnetic susceptibility components.

In the absence of magnetic susceptibility problems, as the operator changes the value of Z4, the hump in the base of the lineshape peak moves through the peak and appears on the opposite side. The time when the hump is totally under the peak results in the best resolution. In cases where the probe's magnetic susceptibility is a problem, the lineshape hump looks like a Z4 problem, but is a magnetic susceptibility problem. The gradients induced in the sample by magnetic susceptibility of the probe coil perturb the sample near the ends of the coil more than in the center of the coil. This creates a gradient profile that looks like Z4. The actual profile is a much more complex function which, in its simplest form, contains Z2, Z4, Z6, Z8 etc. Since most NMR spectrometers do not have these as shimable gradients, the best the operator can do is over-correct the sample near the ends

of the coil with Z4 and try to back-correct the sample near the middle of the coil with Z2. In process of doing this, when the small hump starts to move under the peak, the resolution degrades. It is impossible to get the best resolution and no Z4 hump in these cases.

The previous discussion can be simulated using the SAM program to simulate shimming. In doing so, it can be seen that the peak height obtained from a copper coil probe is about 80% that of a zero susceptibility probe. The use of Z6 as well as Z2 and Z4 brings this up to around 90%. More can be gained using Z8. The nice thing about the shimming simulator is that it can produce a known perfect magnet, with perfect non-interacting shims and a probe of known magnetic susceptibility. All these things are usually unknown in the real world.

The susceptibility phenomenon is usually present more in lower frequency probes than high frequency probes. This is because the lower frequency probes usually have more inductance in the probe coil. Therefore, the probe coil has more turns of wire, and therefore larger amounts of the magnetically imperfect material. The lower frequency probes are also more likely to have a separate decoupler coil which also adds more metal to the probe. However, the same PPM of magnetic susceptibility problems perturb high frequency nuclei more in Hertz. For this reason, lineshape problems produced by probe coil magnetic susceptibility are usually observed more with higher frequency probes such as proton. The addition of more probe coils and therefore more materials in reverse probes and dual probes where one of the nuclei is proton is one of the reasons that good lineshape performance in these probes is more difficult.

Today, all NMR probe manufacturers correct the probe coil's magnetic susceptibility to some degree. It is interesting to note that glass has a much greater magnetic susceptibility than the copper from which coils are commonly made. The insert and sample tube are effectively infinitely long uniform cylinders and, by virtue of this symmetry, generate no field gradients at the sample. When a hole is drilled in the center of an NMR probe insert (as in a CIDNP probe), this symmetry is lost and results in substantially degraded resolution. A cracked insert can also perturb lineshape. In these cases, continued shimming can be of little use. If the user can turn the probe upside down and bits of glass rattle or even fall out, then the cracked insert will make shimming very difficult. When constructing a probe, always make the material in the probe appear as a long cylinder to the sample area. This will ensure the minimum perturbation on lineshape and resolution.

Magnetic Susceptibility Correction

There are several techniques used by probe builders to reduce problems with gradients induced by probe coil materials. One way is to correct the magnetic susceptibility of the probe material. A common way to do this is to use a copper-aluminum sandwich. Good, oxygen-free copper is diamagnetic (negative magnetic susceptibility) and aluminum is paramagnetic (positive magnetic susceptibility). The goal is to make a composite with close to zero magnetic susceptibility by adjusting the amounts of the component materials. Other plating techniques are also commonly used. The difficulty is that the

magnetic susceptibility varies greatly with purity and measuring the magnetic susceptibility accurately for these materials is difficult.

Very few techniques can measure magnetic susceptibility at the absolute levels which cause very strong gradients in a probe. A common way is to measure a large amount of the probe coil material in a very strong, very inhomogeneous magnetic field either by a change in weight or a change in deflection when the magnetic field is present and absent. To have the necessary sensitivity, a large sample of the probe coil material is necessary. This presents the additional problem that small point-to-point fluctuations in the magnetic susceptibility within the large sample of material can average to zero. When these materials are used in a probe, point-to-point susceptibility fluctuations can be present all along the probe coil leading to field gradients and lineshape distortions.

Another method is to use the NMR spectrometer itself to measure the materials. This is usually done by placing a piece of the material to be tested at the top of a shimmed NMR system's probe coil. This will create a lineshape distortion which resembles a solids powder pattern. If the material is diamagnetic, the powder pattern is sloped in one direction and if the material is paramagnetic, the powder pattern is sloped in the opposite direction. If the material has no magnetic susceptibility, there is no lineshape distortion. This technique has the ability to test smaller samples more resembling the probe coil itself and in fact can be used on an actual probe coil. A limitation of the technique is that you need a good lineshape to do the test well. The better a lineshape is to start with, the more sensitive the technique becomes. This technique ends up being a bootstrapping technique where a good probe is used to make better materials, which are used to make better probes which allows one to make better materials...

Another technique to improve the situation of gradients induced by probe coil magnetic susceptibility is to change the shape of the coil window. Depending on several parameters such as the length of the coil and the height and width of the window, the shape of the window can change the type and magnitude of the gradients induced. This improvement in lineshape can be obtained just by changing the window shape even if the materials in the probe coil are not magnetically compensated. In the previous discussion of the single turn Helmholtz coil, the coil window was rectangular in shape. As the bands of copper at the coil ends become small compared to window height, the minimum influence from the coil's magnetic susceptibility will be observed with this rectangular shape. As the bands of copper at the coil ends become large compared to the coil window height, an ellipsoid window shape becomes better. The best shape depends on the ratio of the height and width of the window as well as the size of the bands at the top and bottom of the coil. The relationship is complex and can be explored in much greater detail with the SAM shimming simulator program.

Another common problem which comes from probe coil magnetic susceptibility arises when the susceptibility at the top and the bottom of the coil are different. In this situation, a high order even gradient, typically Z4, is used to correct the asymmetrical lineshape induced by the probe's materials. But the amount of correction required for the top region and the bottom region are different. Typically what happens is that, as the asymmetry is

improved with the change of Z4, a different asymmetry is produced on the other side of the lineshape. One of these situations can be demonstrated with the SAM shimming simulation program discussed later. With probes of this type the "best" lineshape does not occur at the settings which give best width at half height. This produces a situation where shimming for the best response produces an asymmetrical lineshape. The NMR operator is then forced to use Z4 to produce the best lineshape and the remaining controls to produce the best width at half height. Shimming for the best response with Z4 does produce the best width at half height but not the best lineshape. This is a case that many NMR operators will recognize and makes shimming Z4 very difficult. It is also a case where automatic computer shimming will lead to the "wrong" answer. What is happening is that Z4 is being used to destroy the field in the center of the probe's active region, but make the field values at the ends of the coil acceptable for lineshape. The NMR operator then tries to make the field in the center of the probe's active region as good as possible with lower order gradients. Here is an example of a situation where the use of a Z6 or Z8 shim would provide better results.

Size of the Probe Coil

The size of the probe coil, or its length and diameter, can affect the shimming process in three ways. The first way is by the magnetic susceptibility of the probe materials and the induced, non-correctable gradients as was just discussed. As the coil length becomes larger at a constant window width, the NMR lineshape tends to split. This aspect ratio of the coil is very important when shimming probes which have less than perfect magnetic susceptibility compensation for their materials. Having a longer receiver coil is also something many probe manufacturers try to do since longer coils "see" more sample and therefore have greater sensitivity.

If the probe's materials were perfect, then longer coils and larger diameter coils would still be more difficult to shim, but only because it is always more difficult to make a magnetic field perfect over a larger volume than a smaller volume. The case of longer and larger diameter probes is a situation where a "good" magnet is easier than a "bad" magnet to shim. This also means that wide bore magnets are better than narrow bore magnets at a given sample volume. As the amount of sample decreases relative to the probe materials, the probe materials become more important to the shimming process than good or bad magnets. This is one reason why micro-sample probes are difficult to shim and not commonly used. As probe material compensation becomes better, micro-sample probes will probably become more useful.

RF Homogeneity

RF homogeneity affects many areas of an NMR instrument's performance, but not usually the shimming process. One notable exception is spinning sidebands (SSB). An SSB is a small peak occurring at the spin rate on one or both sides of the main peak and is present only when spinning the sample. There are three kinds of SSBs listed below. Types 2 and 3 are not improved by shimming. Note their characteristics and avoid trying to improve them by shimming.

1. One kind of SSB is first- and second-order (sometimes more) sidebands which are in phase with, and usually present on both sides of, the main peak. These signal average with the main peak so do not tend to decrease with acquisition of more scans. These arise from off-axis gradients in the magnetic field and can be decreased or eliminated with shimming.

2. Another kind of SSB is a first-order (sometimes second) SSB present on both sides of the main peak and out of phase with the main peak. These will not have the same phase with each scan (not phase coherent) and do not signal average with the main peak. They arise from perturbations in the probe's tuning from irregularities in the sample tube and wobbling while spinning. As the probe's tuning is affected, the phase of the NMR signal is affected. Therefore, as the sample spins, the signal is phase modulated. These are not improved by shimming. Improving these requires either much better symmetry while spinning, better sample tubes or balancing the probe's electrical circuit.

3. Another kind of SSB occurs on one side only at either twice or four times the spin rate. This SSB is in phase with the main peak and signal averages with the main peak. It arises from the RF inhomogeneity of the probe. If the probe has two vertical bands on its coil, the SSB will be the second order (at twice the spin rate). If the probe coil has four vertical bands, the SSB will be a fourth order SSB. Which side of the main peak the offending SSB appears on is determined by the sign of the magnetogyric ratio of the NMR nucleus and its spinning direction. This SSB arises from the sample spinning through areas of stronger and weaker RF pickup by the coil, which takes place near the coil's bands when RF homogeneity is poor. Larger coils in both length and diameter tend to have lower RF homogeneity. This is not improved by shimming.

Computer shimming

Many instrumental approaches to automatic shimming have been used in the past. These include electrical feedback systems which maintain the Zl shim at optimum lock level while running for prolonged periods to the more recent computer methods for shimming. The most common computerized methods on the NMR market today use computer-driven DACs as one input to the shim power supplies and a Simplex computer optimization routine to adjust the shim settings. The Simplex routines do have some requirements. If these requirements are not met, then the results obtained will not be optimum. Some of the parameters to watch are listed below:

1. Each shim should have equal control of the homogeneity. This is best done by setting the voltage from the DACs to the shim power supply such that an equal DAC step for each shim produces an equal depression of the lock level.
2. The initial step size for the Simplex search should be large enough to include the best value for each shim to be optimized by the Simplex routine.
3. The lock phase must be correctly adjusted if the Simplex routine is using the lock signal for measuring homogeneity.
4. The waiting period between measurements of homogeneity must be long enough to allow the NMR system time to respond. This is especially true if the lock signal

is being used as a measure of homogeneity. The response time for a lock with deuteroacetone as a lock solvent can be several seconds as the homogeneity improves.

5. When the FID is used, a steady state must be established and maintained between the rf pulses before the FID can provide a valid measurement of the homogeneity.
6. The most sensitive method to adjust the final values for Zl and Z2 is by using the FID as the measurement of homogeneity.

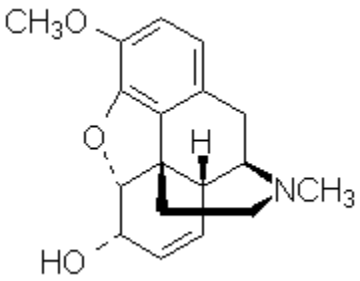
In theory, the Simplex routines should be able to optimize the homogeneity regardless of the shim interactions. However, experience has shown that with second-order shim interactions, Simplex often fails to find the best values. In these cases, slower and less elegant computer routines following a shimming sequence similar to the one described above give better results.

2D experiments on small molecules

Introduction

NMR Analysis of Codeine

Codeine was selected as a test sample to illustrate the application of different NMR experiments to structure determination and assignment, and to compare various versions of each experiment.



$C_{18}H_{21}NO_3$, MW= 299.4

NMR samples used were between 3 and 18 mg, in .65ml $CDCl_3$ with TMS, run on a JEOL Eclipse$^+$ 400 spectrometer.

Each experiment is described, along with acquisition and processing parameters, and spectra are shown with assignment of all peaks. For readers interested in the details of data acquisition, several good books are available.

See table of assignments and assignment strategy.

Experiments:

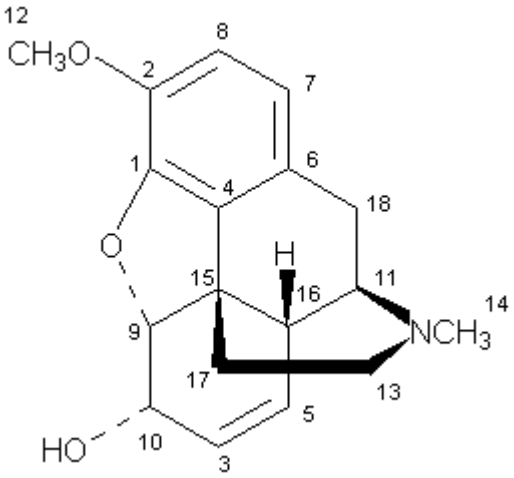
1D ^{1}H and ^{13}C
DEPT and APT
COSY
TOCSY
NOESY
HMQC
HMBC
HMQC-TOCSY
INADEQUATE
3D HMQC-TOCSY

In the course of acquiring and processing these data, some observations were made, including:

comparison of HMQC and HSQC
choice of processing parameters for HMQC (window function and magnitude vs. power spectrum)
comparison of HMBC spectra run on 3 mg sample and 18 mg sample

Codeine FIDs and spectra are available for download from
http://www.acornnmr.com/codeine/data

Assignments

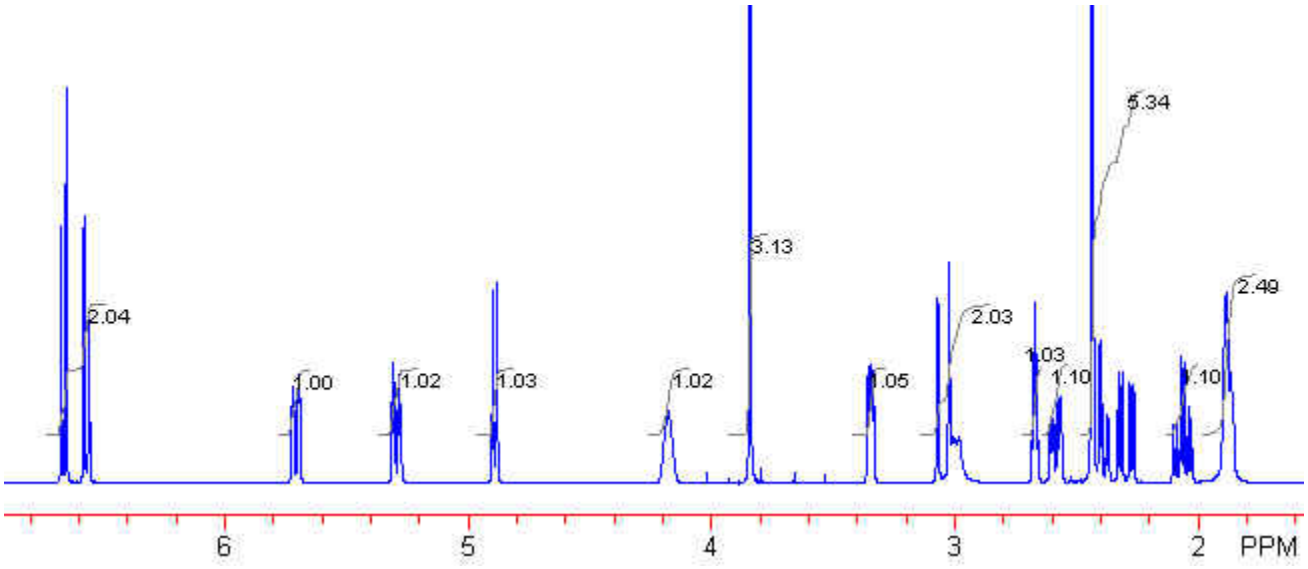


	^{13}C (ppm)		^{1}H(ppm)
1	146.38	q	
2	142.23	q	
3	133.43	CH	5.71
4	131.13	q	
5	128.30	CH	5.29
6	127.30	q	
7	119.58	CH	6.57
8	113.03	CH	6.66
9	91.39	CH	4.89
10	66.43	CH	4.18
11	58.92	CH	3.35
12	56.40	CH_3	3.84
13	46.47	CH_2	2.59, 2.40
14	43.12	CH_3	2.44
15	42.99	q	

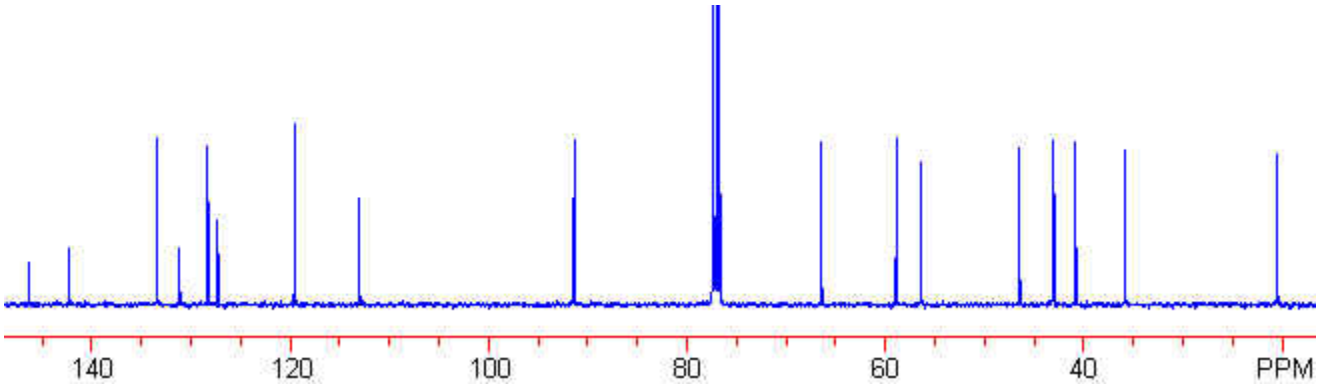
16	40.82	CH	2.67
17	35.85	CH_2	2.06, 1.88
18	20.46	CH_2	3.04, 2.30
		OH	2.99

Assignment strategy

Starting with the ^{1}H spectrum, some features are immediately obvious (2 aromatics, 2 methyl singlets). The rest of the spectrum consists of numerous multiplets that integrate to 1 H each. It is not clear how many of these might be non-equivalent CH_2s.



One of the first things we need to know is how many carbons are there, and how many of each multiplicity - quaternary, CH, CH_2 and CH_3. So the next step, assuming there is enough material, is a 1D ^{13}C spectrum, which shows we have 18 Cs. (There are 2 peaks at ~43 ppm).

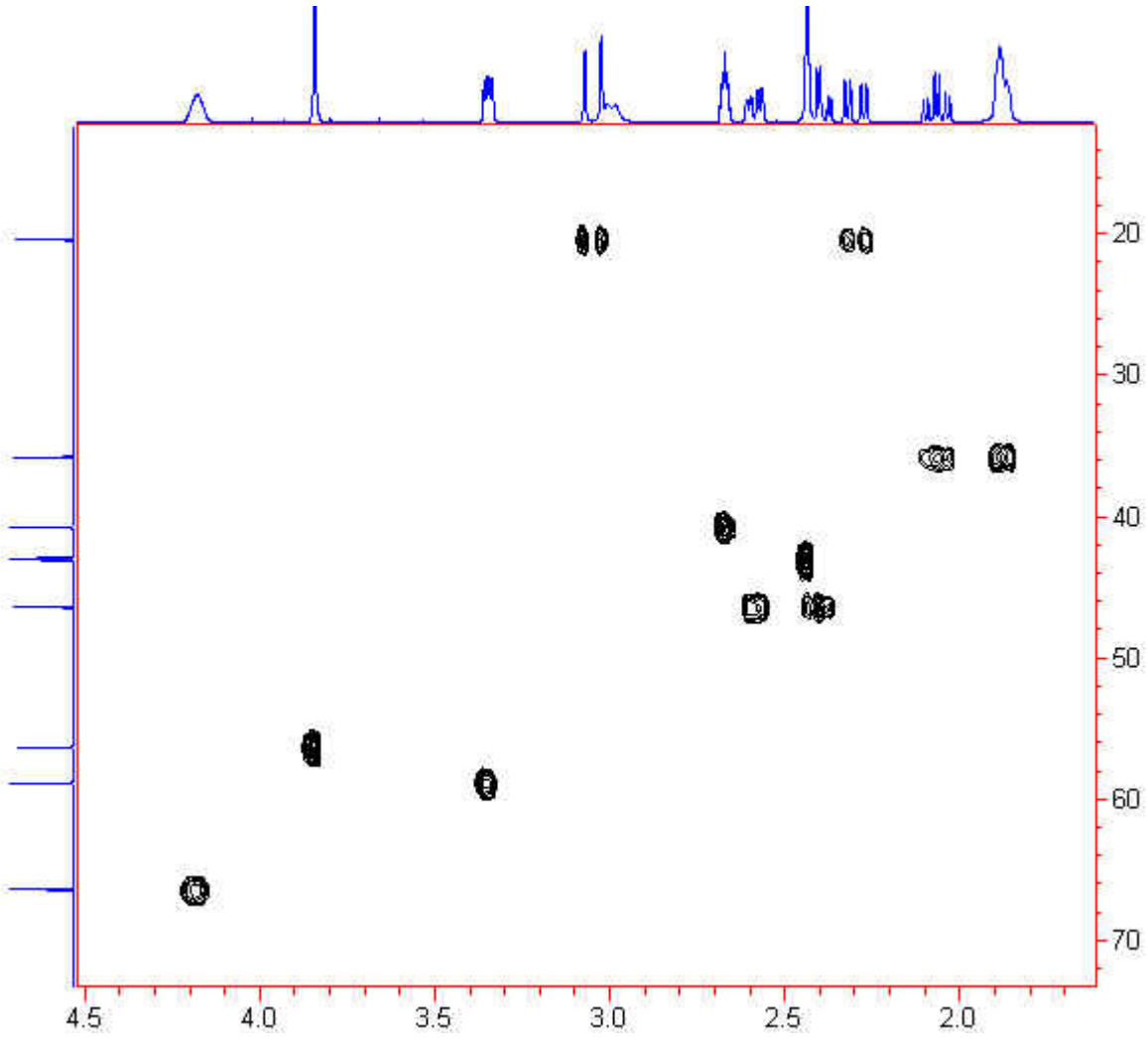


It is helpful to have an unambiguous label for each carbon. We find it convenient to number the carbons from 1 to n starting downfield.

There are 2 options for determining multiplicity - DEPT and HMQC (or the combined experiment, DEPT-HMQC). DEPT will unambiguously determine multiplicities, but HMQC has the advantage of also "connecting" ^{1}H and ^{13}C peaks, so information from each can be used to assign the other. For example, methyl peaks are often obvious in a ^{1}H spectrum, allowing us to identify methyls in the ^{13}C spectrum. The HMQC also allows us to pair up geminal CH_2 ^{1}H peaks, which show up in the HMQC as 2 peaks at the position of a single ^{13}C. 3 CH_2s are easily identified in the 2D plot below.

At this point, we can create the following table:

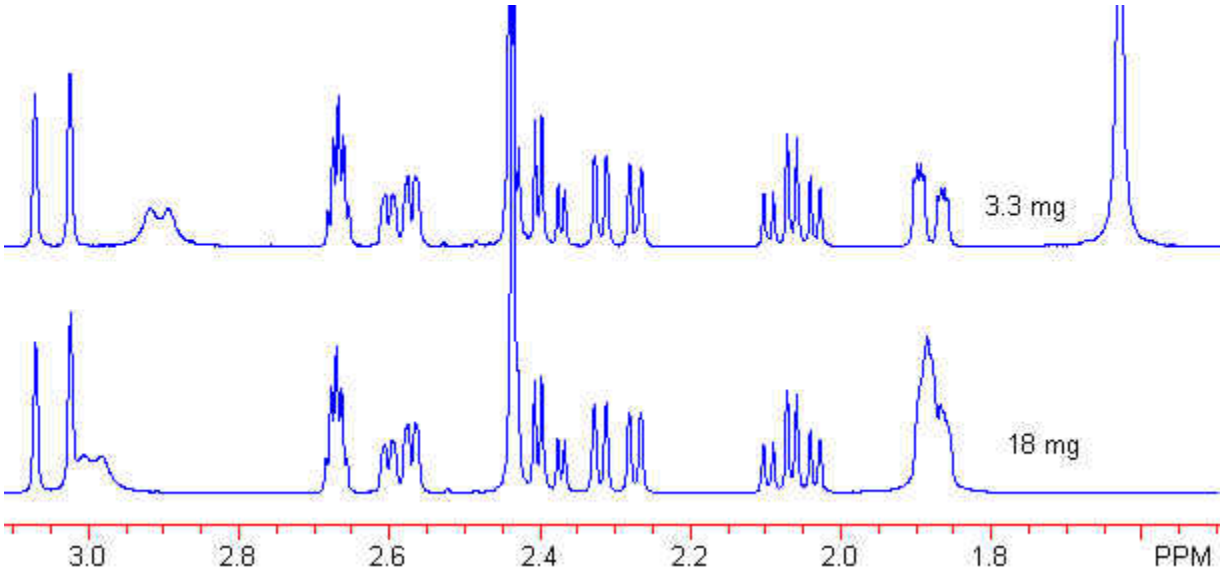
Peak	ppm	Mult	Peak	ppm	Mult
1	146.38	q	**10**	66.43	CH
2	142.23	q	**11**	58.92	CH
3	133.43	CH	**12**	56.40	CH_3
4	131.13	q	**13**	46.47	CH_2
5	128.30	CH	**14**	43.12	CH_3
6	127.30	q	**15**	42.99	q
7	119.58	CH	**16**	40.82	CH
8	113.03	CH	**17**	35.85	CH_2
9	91.39	CH	**18**	20.46	CH_2



From the ^{13}C and DEPT and/or HMQC, we determine the structure contains:

8 CHs
3 CH_2s
2 CH_3s
and the remaining 5 must be quaternary Cs.

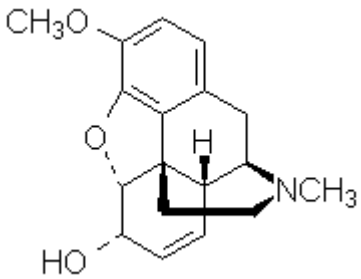
This gives a total of 20 Hs. There is also an OH. But this doesn't match the integration in the ^{1}H spectrum above. The peak at 1.87 ppm, with integral of 2.49, is actually just 1 H, overlapping with H_2O. Compare spectra at different concentrations, below. At lower concentration, water is shifted upfield approximately .5 ppm.



From the HMQC, we connect each ^{1}H peak to its ^{13}C peak, so we now have numerical labels for all Hs.

	^{13}C (ppm)		^{1}H(ppm)
1	146.38	q	
2	142.23	q	
3	133.43	CH	5.71
4	131.13	q	
5	128.30	CH	5.29
6	127.30	q	
7	119.58	CH	6.57
8	113.03	CH	6.66
9	91.39	CH	4.89
10	66.43	CH	4.18
11	58.92	CH	3.35
12	56.40	CH_3	3.84
13	46.47	CH_2	2.59, 2.40
14	43.12	CH_3	2.44
15	42.99	q	
16	40.82	CH	2.67
17	35.85	CH_2	2.06, 1.88
18	20.46	CH_2	3.04, 2.30
		OH	2.99

Assignments



The next step is examination of ^{1}H and ^{13}C spectra to identify those peaks whose assignment is obvious based on chemical shift.

We can identify one OMe (3.84 ppm) and one NMe (2.44 ppm) in the proton spectrum, and can connect them to carbons 56.4 ppm (#12) and 43.1 ppm (#14), respectively, from the HMQC spectrum.

The aromatic Hs are obvious as the 2 doublets at ~ 6.6 ppm with a mutual coupling of 8 Hz, but it is not clear which is which. The corresponding aromatic CH carbons are 119.6 (#7) and 113.0 ppm (#8). Both are either *ortho* or *para* to O substituents, so are upfield.

The olefinic Hs are at 5.71 and 5.29 ppm, with corresponding Cs at 133.4 and 128.3 ppm (#3 and 5), respectively, but again it is not clear which is which.

Carbon #9 at 91.4 ppm (H at 4.89 ppm) and carbon #10 at 66.4 ppm (H at 4.18 ppm) can be assigned to the epoxide and hydroxyl carbons, respectively.

The most downfield carbons (#1 and 2 at 146.4 and 142.2 ppm) are expected to be the O-substituted aromatics, but it is not clear which is which.

The 2 remaining quaternary aromatics must be #4 and 6 (127.3 and 131.1 ppm), but it is not obvious which is which.

The only aliphatic quaternary C is #15 at 43.0 ppm, so it is assigned by process of elimination.

Some additional assignments can be made from the COSY and NOESY spectra.

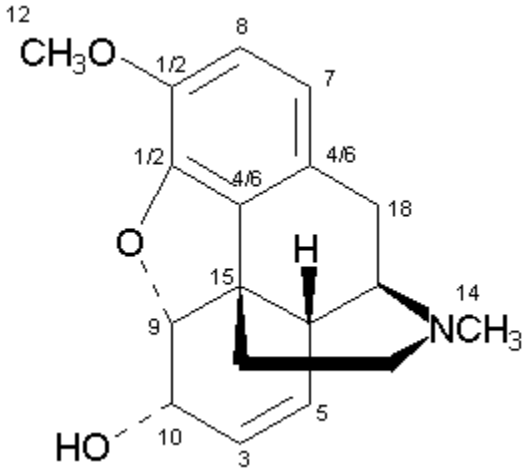
A COSY cross-peak from the OH (2.9 ppm) to 4.18 ppm confirms this as the CH bearing OH (#10).

A NOESY cross-peak from OMe to the aromatic H at 6.66 ppm identifies the latter as (#8) *ortho* to the methoxy, distinguishing between #7 and 8.

A NOESY cross-peak from H-3 to H-10 identifies the H at 5.71 ppm as adjacent to #10 and resolves the uncertainty of #3 and 5.

NOESY crosspeaks from H-7 to H-18 and H-18' allows #18 to be assigned.

Assignments so far are summarized in the structure below:



The HMBC (multiple-bond C-H correlation) spectrum will allow the remaining assignments to be made.

The methoxy protons have a strong 3-bond coupling, in the HMBC spectrum, to the aromatic carbon bearing OMe. This allows assignment of C-1 to carbon bearing OMe and, by elimination, C-2 is the C bearing the epoxide O.

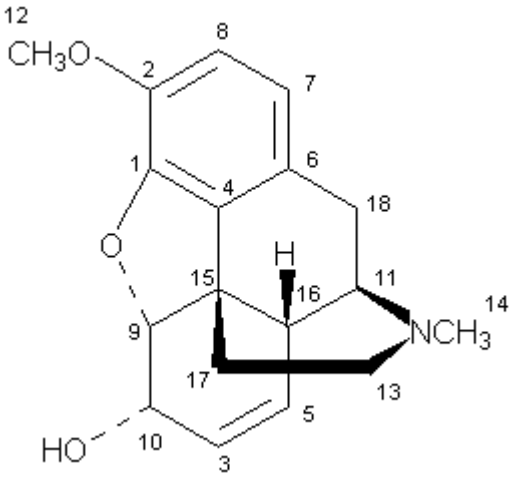
The HMBC is optimized for 8 Hz couplings, typical of aromatic 3-bond couplings. The HMBC shows correlations from H-8 to C-1 and C-6, and from H-7 to C-2 and C-4. This confirms assignment of C-1 and C-2, and allows assignment of C-4 and C-6.

The remaining ambiguities are distinguishing CHs #11 and 16, and CH_2s #13 and 17, plus stereo-specific assignments for Hs 13, 17 and 18.

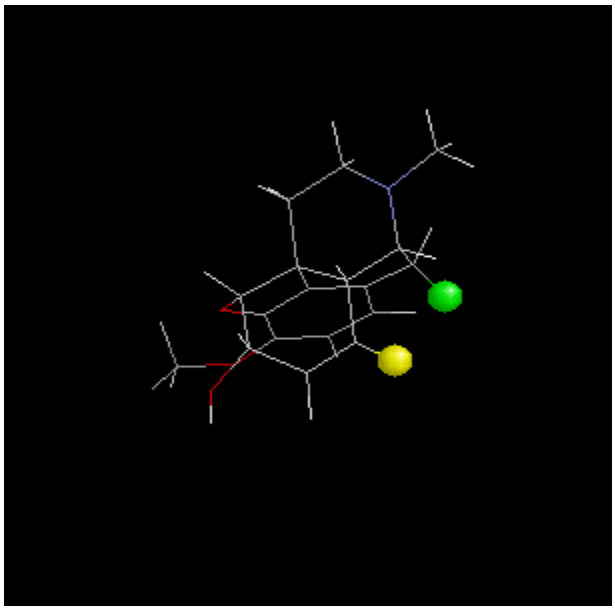
An HMBC peak from the N-Me protons (#14) to C-11 (3-bond) resolves the ambiguity between #11 and #16.

An HMBC peak from H-9 to C-17 (3-bond) resolves the ambiguity between #17 and 13.

The resulting assignments are:

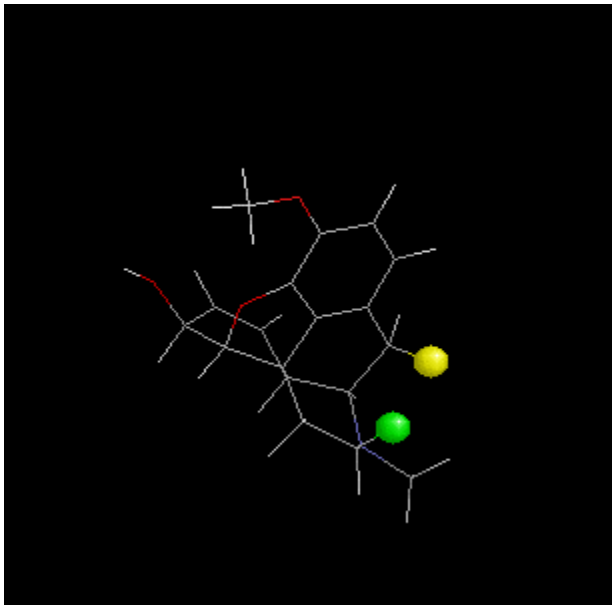


The NOESY spectrum provides stereo-specific assignments of the methylene Hs.



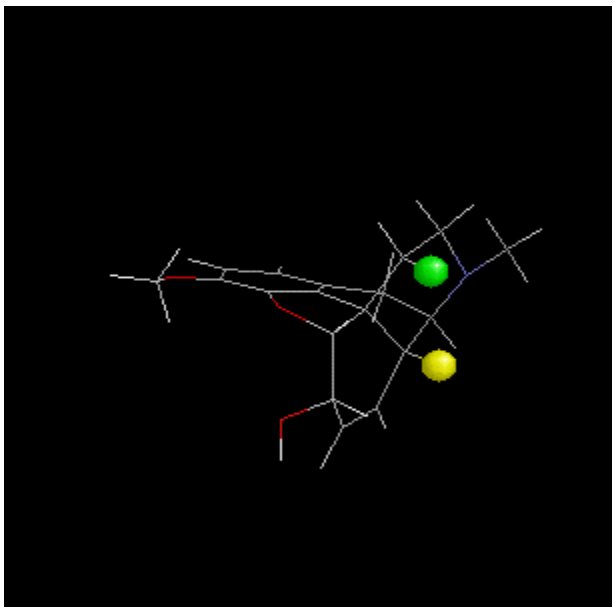
The NOESY spectrum shows an NOE between H-5 and H-18'.

H-5 is shown in yellow, H-18' is green.



The NOESY spectrum shows an NOE between H-18 and H-13.

H-18 is shown in yellow, H-13 is green.



The NOESY spectrum shows an NOE between H-16 and H-17.

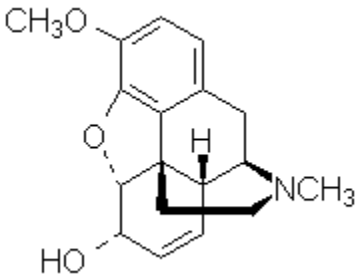
H-16 is shown in yellow, H-17 is green.

The final step is to identify all peaks observed in the HMBC spectrum, looking for any inconsistencies in the assignments. Any peak that would require an implausible coupling (too many bonds) suggests that an error has been made in the assignments.

C	H	# bonds	C	H	# bonds
1	8	3	11	18, 18'	2
1	9	3	11	13	3
2	8	2	11	14	3

2	7	3	13	17	2
2	12	3	14 and/or 15	11	3
3	9	3	14 and/or 15	13	3
4	7	3	15	17	2
4	18, 18'	3	16	18	3
4	17	3	17	9	3
4	9	3	17	13	2
6	8	3	18	7	3
6	18, 18'	2			
7	18, 18'	3			

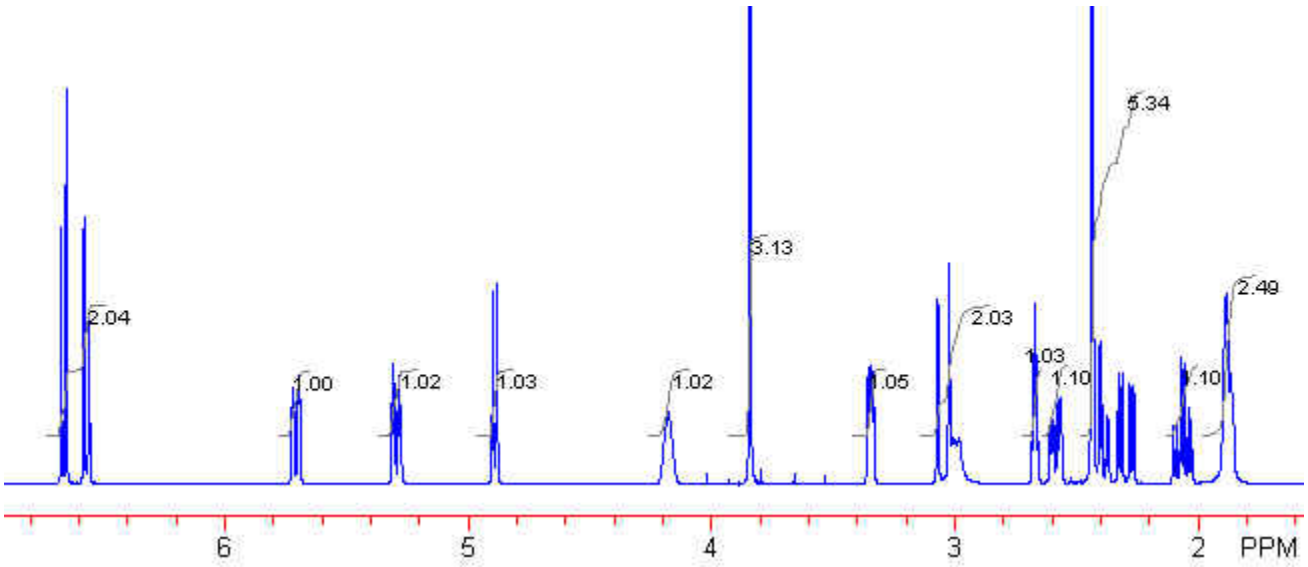
1D ^{1}H and ^{13}C



$C_{18}H_{21}NO_3$, MW= 299.4

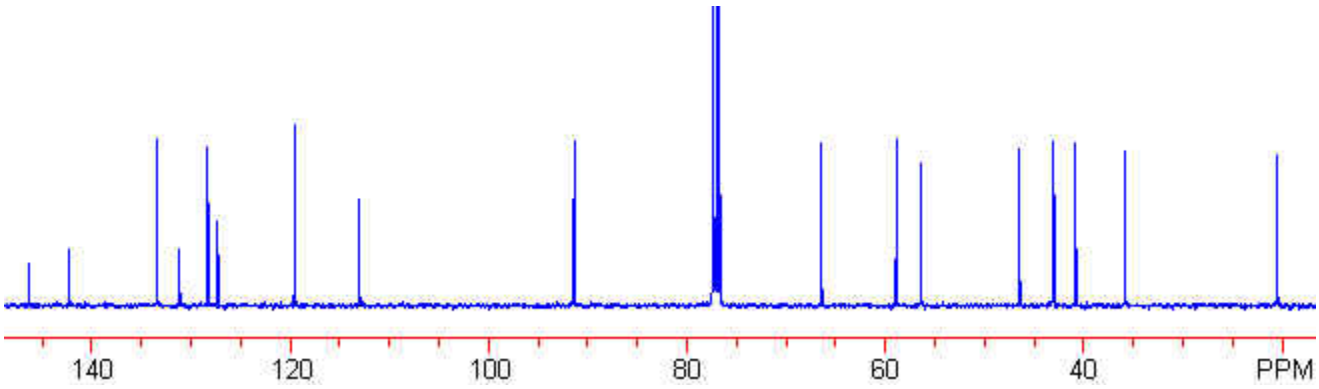
Data acquired on a JEOL Eclipse^{+} 400 spectrometer.

^{1}H spectrum

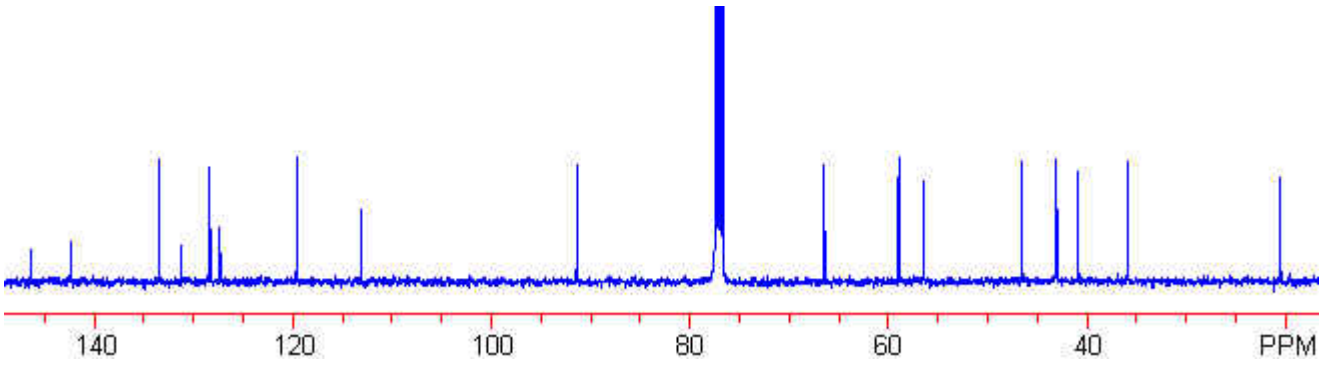


^{13}C spectra

18 mg sample, 1.3 hrs acquisition time

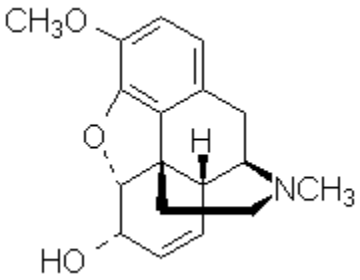


3.3 mg sample, 5 hrs acquisition time



DEPT and APT

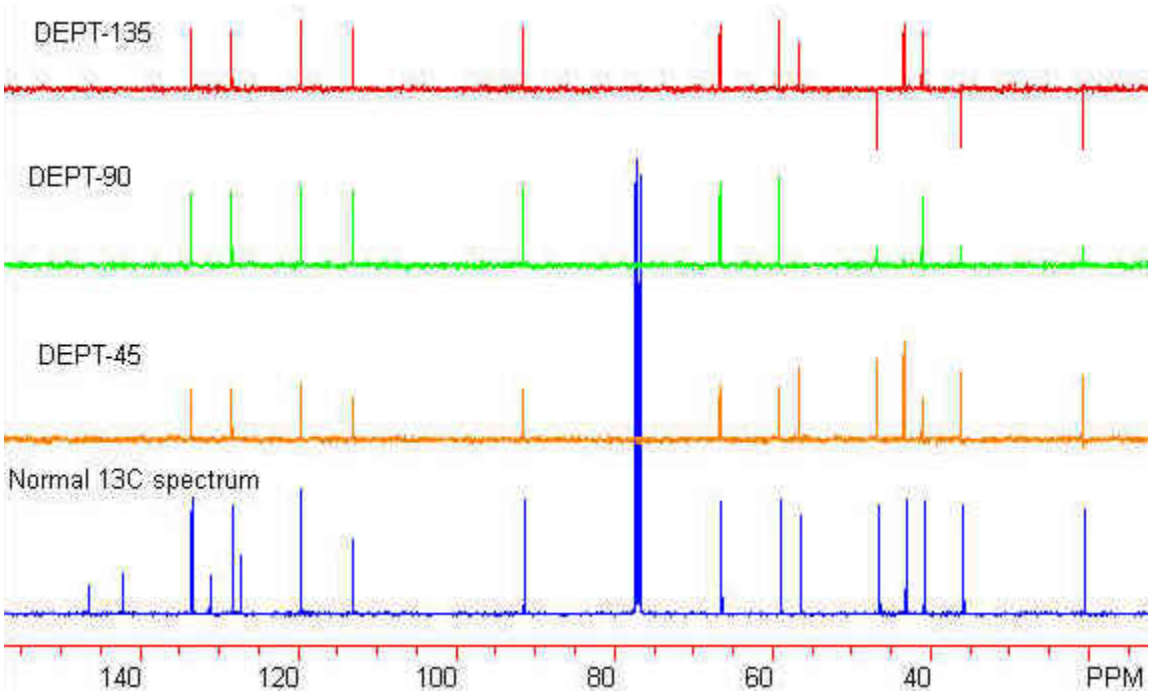
Both experiments are used to identify "multiplicity" (quaternary, CH, CH_2 or CH_3) of peaks in a ^{13}C spectrum. Usually, DEPT is preferred because much less time is required. For DEPT, ^{1}H magnetization is generated first, then transferred to ^{13}C. This "polarization transfer" enhances sensitivity. Also, the experiment repetition rate is dependent on relaxation of ^{1}H, rather than ^{13}C, so a shorter delay is needed. DEPT also can distinguish between CH and CH_3, unlike APT, although quaternary Cs are not observed in DEPT.



The sample is 18 mg of codeine in .65 ml $CDCl_3$

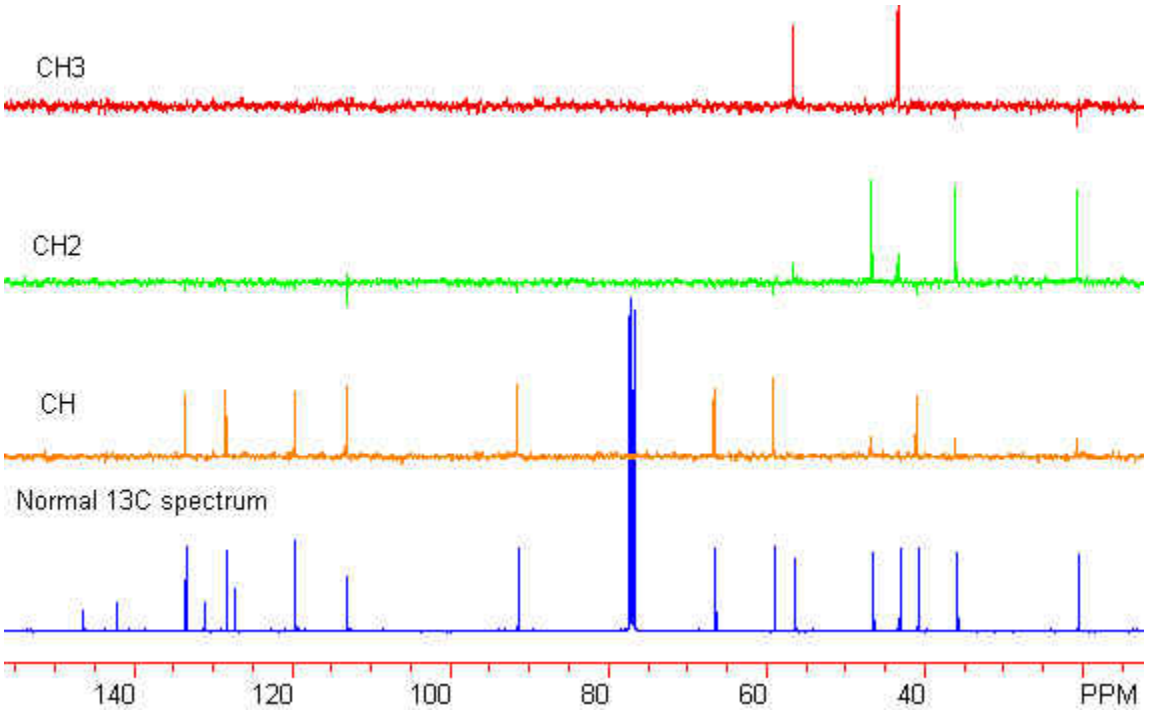
DEPT spectra shown in the figure below are, from top to bottom:

DEPT-135	CH and CH_3 peaks up, CH_2 peaks inverted
DEPT-90	CH peaks only
DEPT-45	all protonated carbons
normal ^{13}C spectrum	



The 3 DEPT spectra were acquired in less than 10 min each. From the DEPT-135, CH_2 peaks are identified as the 3 inverted peaks. DEPT-90 contains only CHs. Any positive peaks in DEPT-135 which don't appear in DEPT-90 are CH_3s.

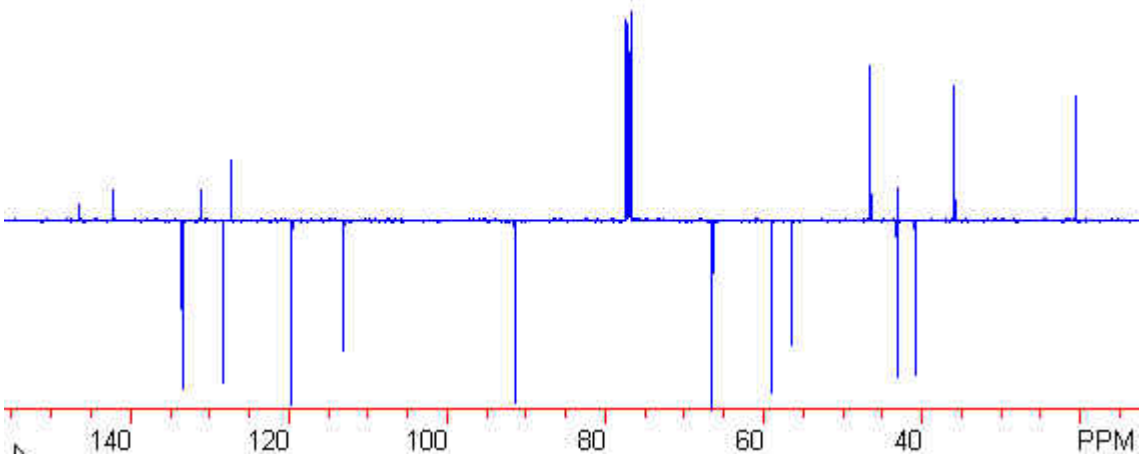
The 3 DEPT spectra can be combined by addition and subtraction with appropriate coefficients to yield "edited DEPT" spectra, in which each spectrum contains only peaks of one multiplicity (CH, CH_2 or CH_3), as shown below.



The subtraction is not perfect, most likely due to incomplete relaxation, but multiplicity of all peaks can be readily determined.

In practice, creation of the edited display is not necessary, as multiplicity of all peaks can be easily assigned from just a DEPT-135 and, if needed, a DEPT-90 to distinguish CH and CH_3.

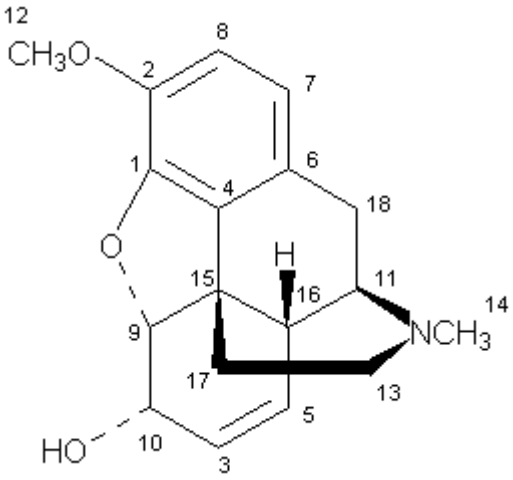
An APT spectrum provides nearly equivalent information. Quaternary and CH_2 peaks are positive, CH and CH_3 peaks are inverted. For codeine, the APT clearly resolves the 2 peaks at 43 ppm, a quaternary and a CH. The APT experiment is simpler to set up, as it does not require calibration of a proton 90 degree pulse. Also, many older generation spectrometers are not capable of accurate phase shifts of the decoupler channel, needed for DEPT. The disadvantages of APT are its lower sensitivity, the need to wait between scans for ^{13}C relaxation, and no means of distinguishing CH from CH_3 peaks.



This spectrum was acquired in 2.5 hrs.

COSY

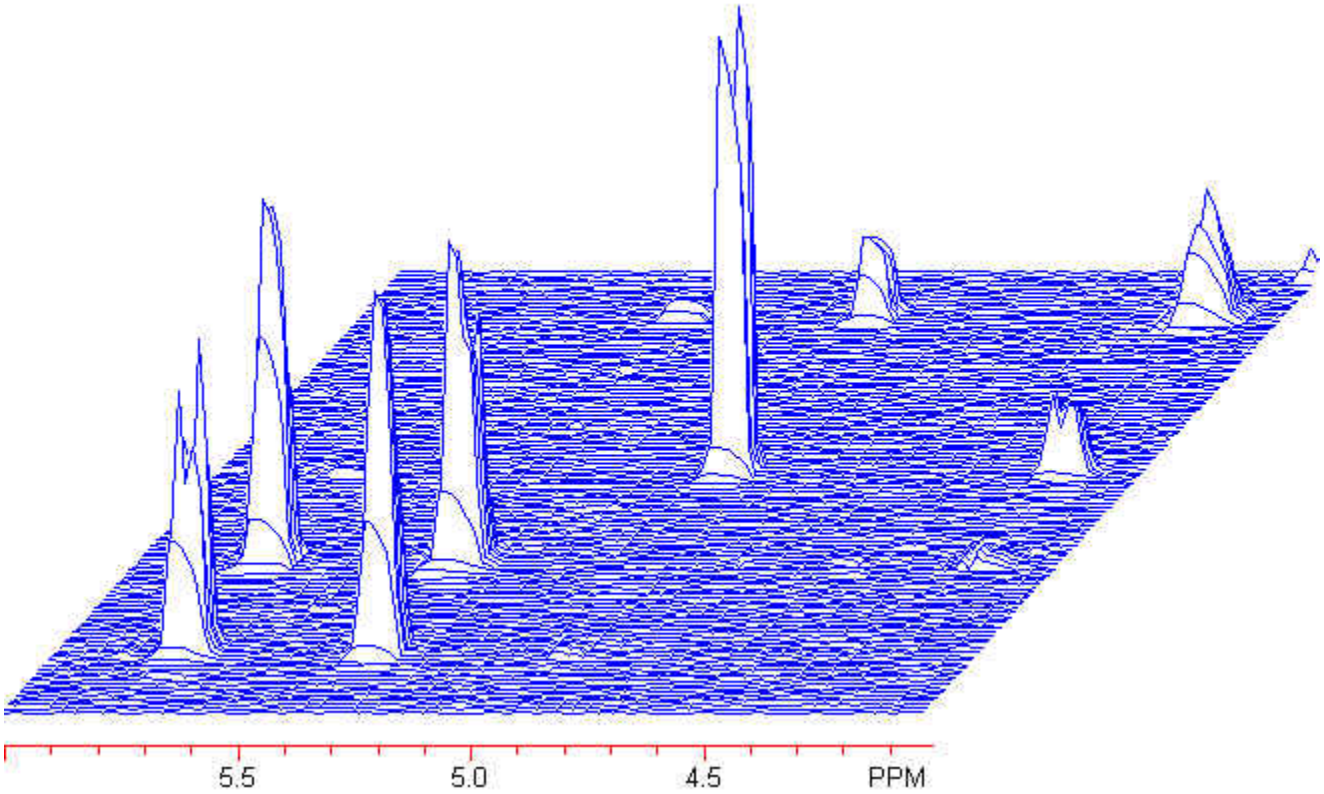
This is a 2D experiment that shows which Hs are coupled to each other. For simple spectra, this information can often be extracted by measuring and matching couplings, or by selective decoupling, but unless this can be done very quickly, a COSY is more efficient, because all coupling information is obtained in one experiment.

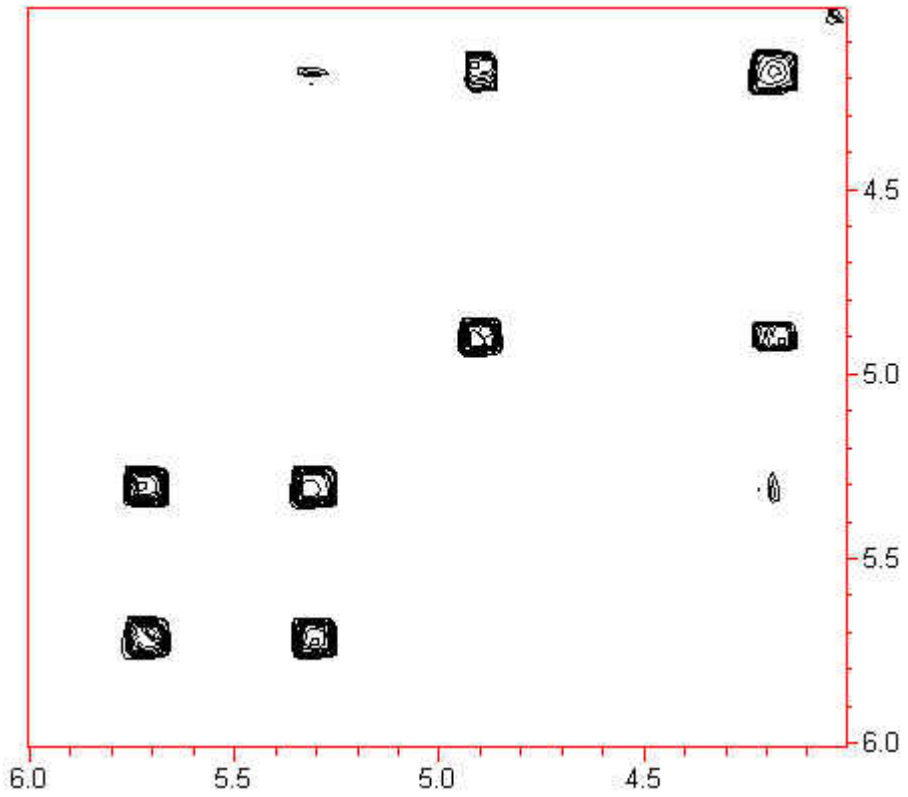


The sample was 3.3 mg of codeine in .65 ml $CDCl_3$

The spectrum was acquired in 5 minutes (not including set-up and processing)

One way to view a 2D spectrum is as a 3D, or "stacked", plot. However, it is difficult to measure peak frequencies using this type of plot, and many peaks are obscured behind larger peaks. The more useful representation is a contour plot, in which peaks are represented by a series of concentric rings (contour lines) drawn at vertical intervals, analogous to a topological map. Taller peaks are drawn with more contour lines. The 2 plots below illustrate these 2 view for the same region of the COSY spectrum.

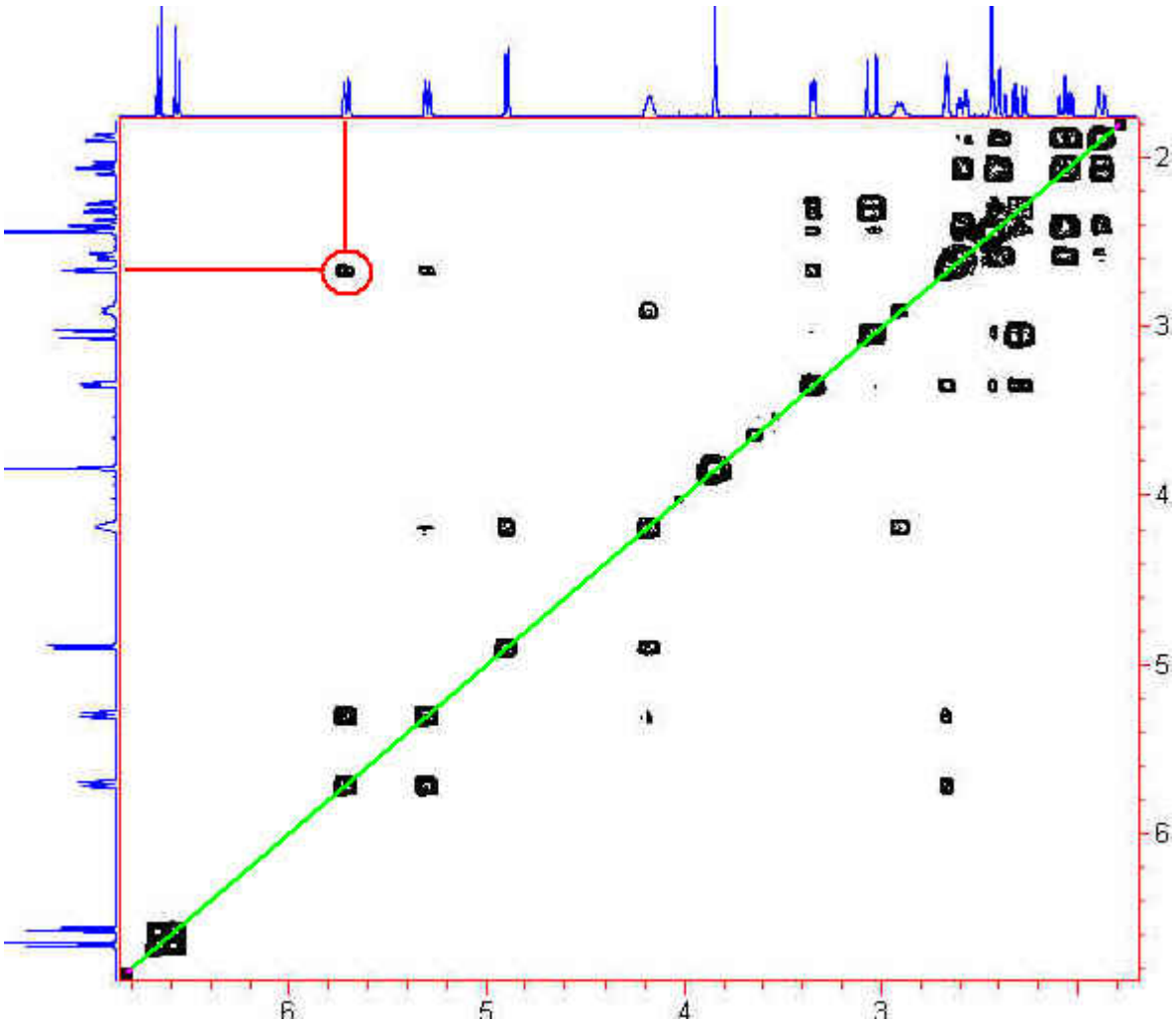




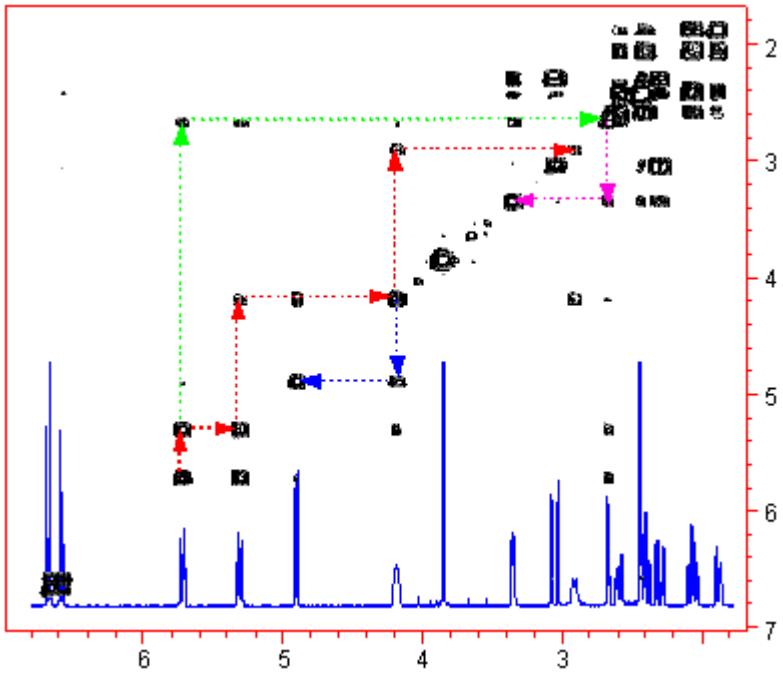
For COSY data (as with all homonuclear 2D plots), the diagonal (indicated by green line in the figure below) consists of intense peaks that match the normal spectrum, as do projections onto each axis.

The coupling information is contained in the off-diagonal peaks, called cross-peaks. The coordinates of each cross-peak are the chemical shifts of the 2 Hs that are coupled. The cross-peak shown in red "connects" protons at 2.7 ppm and 5.7 ppm (H-16 and H-3, referring to the numbering scheme shown above).

The spectrum is symmetrical about the diagonal - a peak located in the upper left triangle will have a corresponding peak in the lower right triangle of the plot.



Coupling "networks" can be traced out, as shown in the figure below.



The colored arrows trace out coupling networks, corresponding to:

H-5 –> H-3 –> H-10 –> OH

H-10 -> H-9

H-3 –> H-16

H-16 –> H-11

Table of COSY correlations

shift	shift	Assignments
6.6	6.7	7 - 8
5.7	5.3	3 - 5
5.7	2.7	3 - 16
5.7	4.9	3-9 weak
5.3	4.2	5 - 10
5.3	2.7	5 - 16
4.9	4.2	9 - 10
4.2	2.9	10 - OH
3.3	2.7	11- 16
3.3	2.4	11 - 14
3.3	2.3	11 - 18'
3.0	2.4	18 - 14
3.0	2.3	18 - 18'
2.6	2.4	13 - 13'
2.6	2.1	13 - 17
2.6	1.9	13 - 17'

2.4	2.1	13' - 17
2.4	1.9	13' - 17'
2.1	1.9	17 - 17'

Acquisition Parameters:

512 complex points in direct dimension
128 t_1 increments
2 scans
1 sec relaxation delay
Total acquisition time: 5 min

Including setup, processing and plotting, total time was 15-20 min.

Processing:

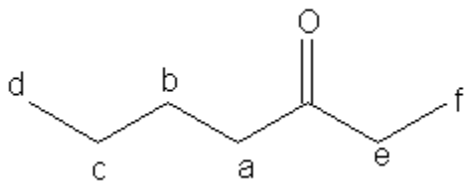
sine squared window function was applied in both dimensions (with 0 degree phase shift)
2x zero-fill was used in the indirect dimension, for a final data size of 512 x 512
magnitude calculation (no phasing is required)
symmetrization

For details on processing, see COSY processing in the 2D section

TOCSY

TOCSY (Total Correlation Spectroscopy) is similar to COSY, in that it maps out which Hs are coupled to each other, but in a TOCSY spectrum, correlations are seen between all Hs in a spin system, not just those directly coupled to each other.

For example, consider 3-heptanone:



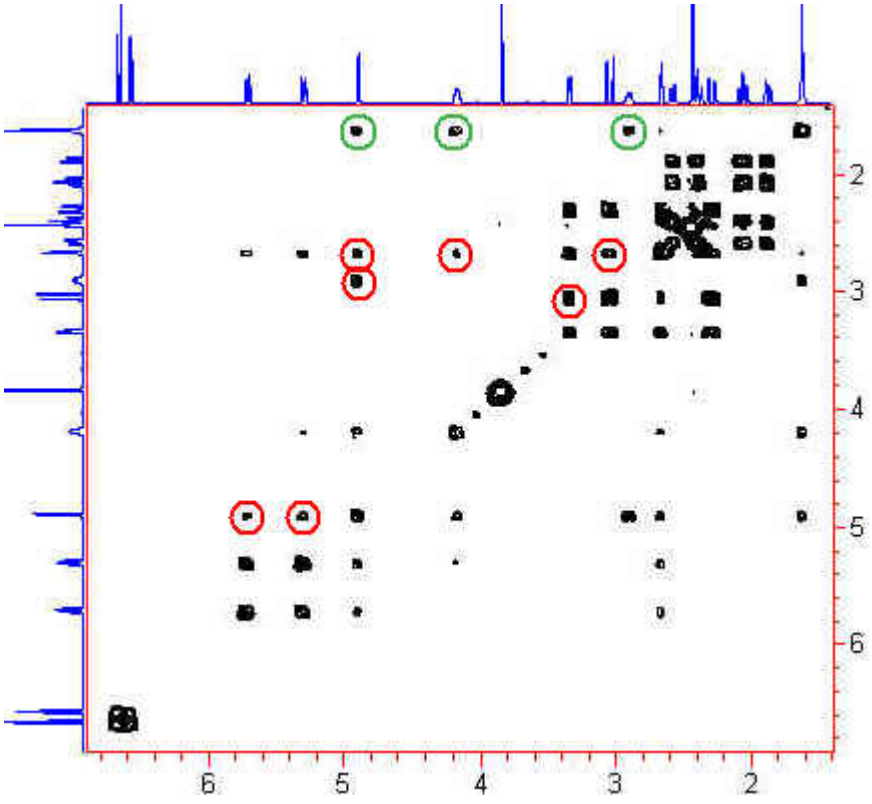
Protons **a**, **b**, **c** and **d** constitute one spin system, an unbroken network of coupled protons. The ethyl group, **e** and **f**, constitutes a second, separate spin system, because there is no coupling between **a** and **e**, across the carbonyl.

In a COSY spectrum, CH_2 **a** would show a correlation to CH_2 **b**. In a TOCSY spectrum, it would also show correlations to CH_2s **c** and **d**.

Codeine (3.3 mg in .65 ml $CDCl_3$)

The TOCSY spectrum of codeine is shown below. As with all homonuclear 2D plots, the diagonal (lower left corner to upper right corner) consists of intense peaks that match the normal spectrum, as do projections onto each axis. The interesting information is contained in the "cross-peaks", which appear at the coordinates of 2 protons which belong to the same spin system.

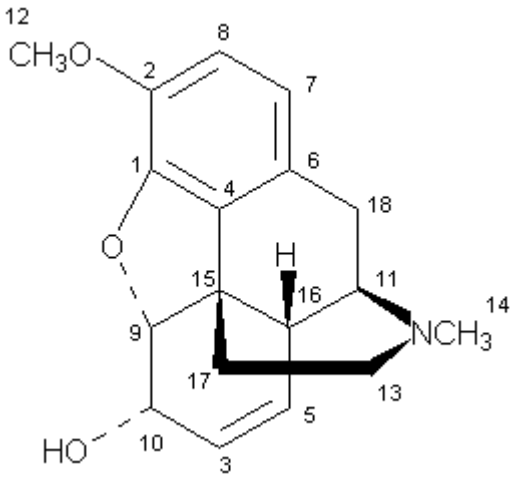
The cross-peaks marked with red and green circles are longer-range correlations, not observed in the COSY spectrum. The green circles mark cross-peaks to the water peak. Water in the solvent exchanges with the OH of codeine, which is coupled to H-10, which is, in turn, coupled to H-9.



It takes time for the longer-range correlations to develop (referred to as magnetization transfer), so the final spectrum is dependent on delay values in the pulse sequence. In practice, several spectra may be acquired using different delay values. The intensity of

each cross-peak will vary through the series of spectra - long range correlations will "grow in" as the delay value is increased, and shorter range couplings may disappear.

Table of TOCSY peaks:
(' indicates the more upfield of geminal CH_2 protons)



8 --> 7
3 --> 5, 9, 10, 16
5 --> 9, 10, 11, 16
9 --> 10, 16, OH, H_2O
10 --> 16, OH, H_2O
11 --> 16, 18, 18'
18 --> 16, 18'
16 --> 18'
13 --> 13', 17, 17'
13' --> 17, 17'
17 --> 17'

Acquisition parameters:

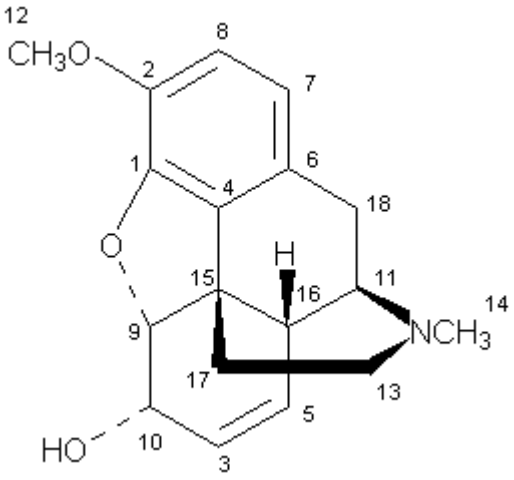
512 complex points in the direct dimension
128 t_1 increments
mixing time 70 ms
4 scans
2 sec relaxation delay
Total time: ~20 min.

Processing parameters:

sine squared window function (0 degree phase shift) in both dimensions
2x zero-fill in the indirect dimension
magnitude calculation (no phasing needed)
final size 512 x 512

This is a magnitude experiment, so is processed in the same way as COSY – see description in the 2D section.

NOESY

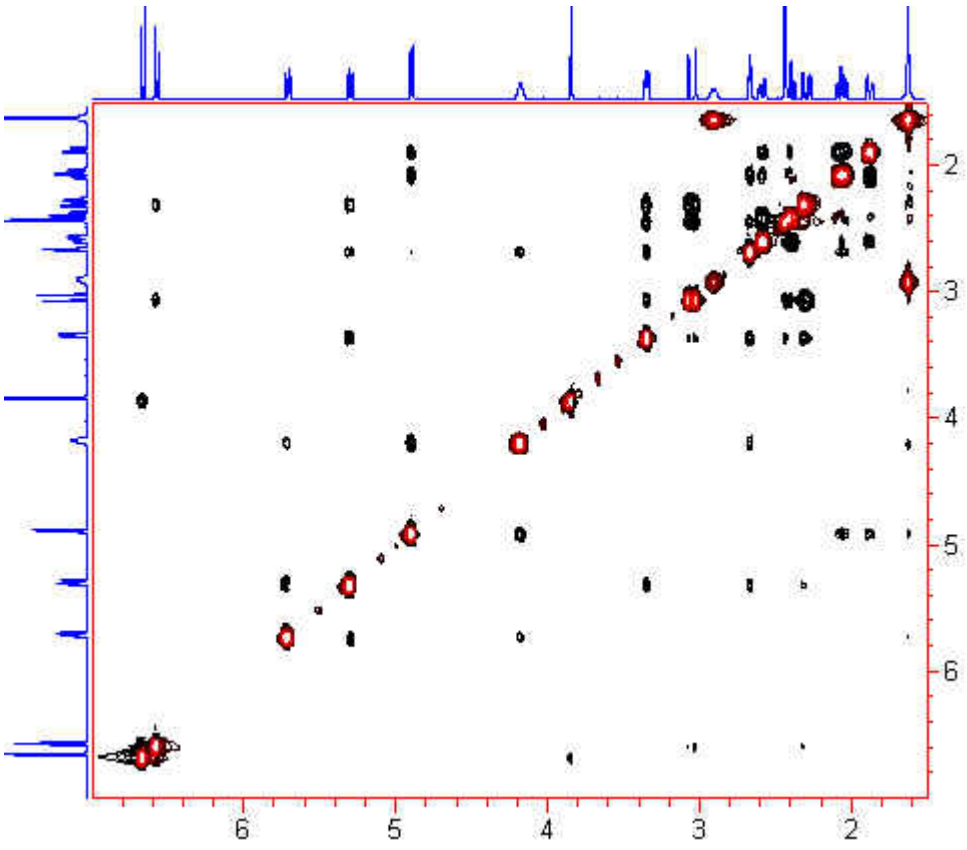


The sample is 3.3 mg of codeine in .65 ml $CDCl_3$

A contour plot of the NOESY spectrum is shown below. As with all homonuclear 2D plots, the diagonal consists of intense peaks that match the normal spectrum, as do projections onto each axis. The interesting information is contained in the "cross-peaks", which appear at the coordinates of 2 protons which have an NOE correlation.

For small molecules, the NOE is negative. Exchange peaks have the opposite sign from NOE peaks, making them easy to identify. The water peak at 1.5 ppm exchanges with the OH at 2.9 ppm, shown here in red.

The spectrum is phased with the large diagonal peaks inverted (shown in red here), so the NOE cross-peaks are positive.



Expansion of the upfield region:

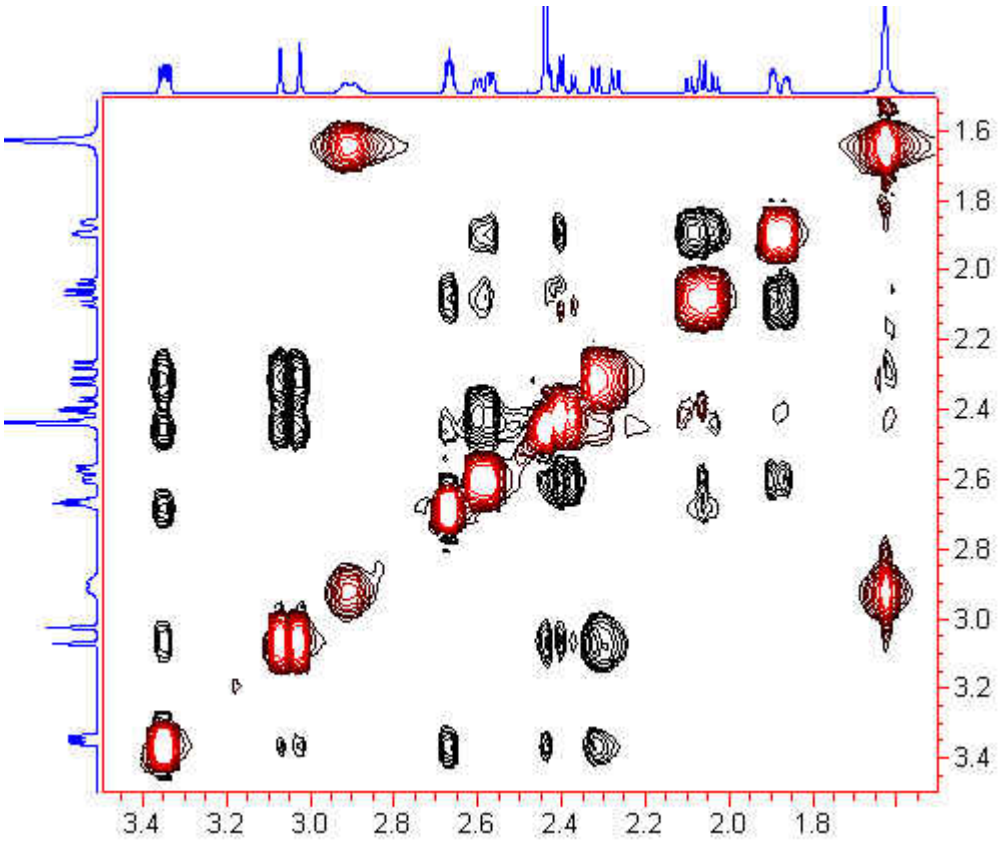
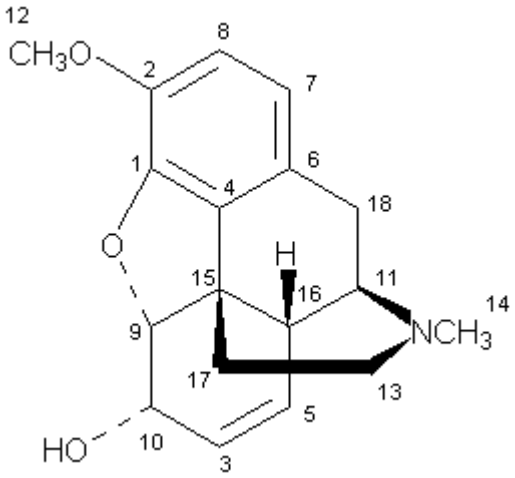


Table of NOEs: (' indicates the more upfield of geminal CH_2 protons)

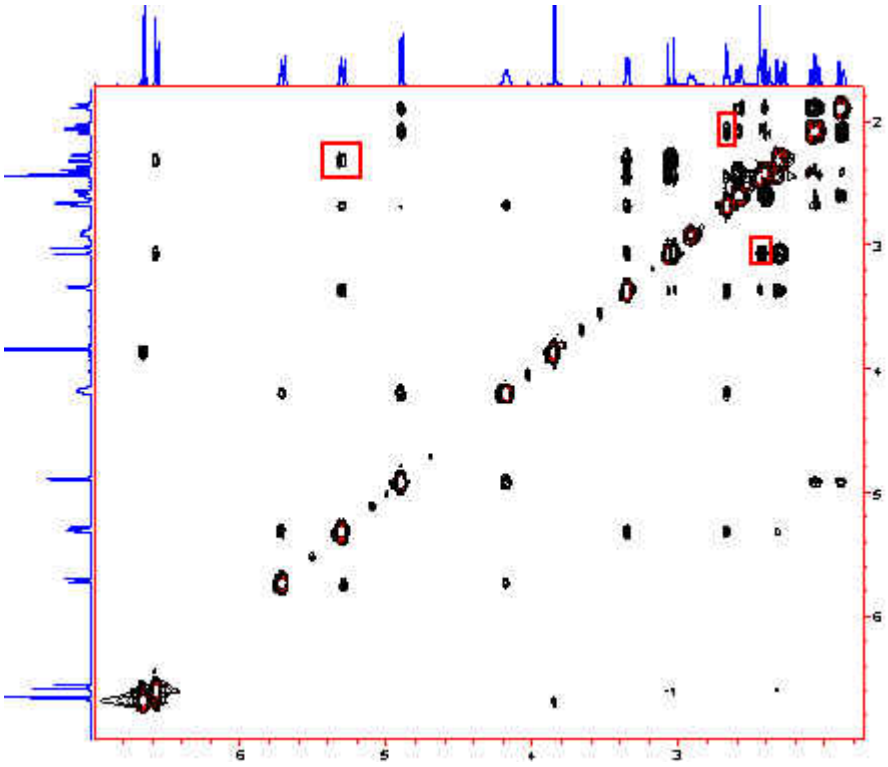
8 - 7, 12
7 - 18, 18'
3 - 5, 10
5 - 11, 16, 18'
9 - 10, 17, 17'
10 - 16
11 - 18, 16, 14, 18'
18 - 13, 18'
16 - 14, 17
13 - 14, 17, 17'
13' - 17, 17'
17 - 17'



In addition to confirming assignments, the NOESY spectrum allows stereospecific assignments of methylene Hs. The 3 cross-peaks indicated in red on the plot below distinguish between the 3 CH_2 pairs:

5 -18'
16 - 17
18 - 13

These NOEs can be viewed interactively in 3D using Chime.



Acquisition parameters:

512 complex points in the direct dimension
256 t_1 increments
mixing time: 0.8 sec
experiment is hypercomplex (method of States, et al) and phase sensitive
16 scans
2 sec relaxation delay
Total time: 5 hrs.

Processing parameters:

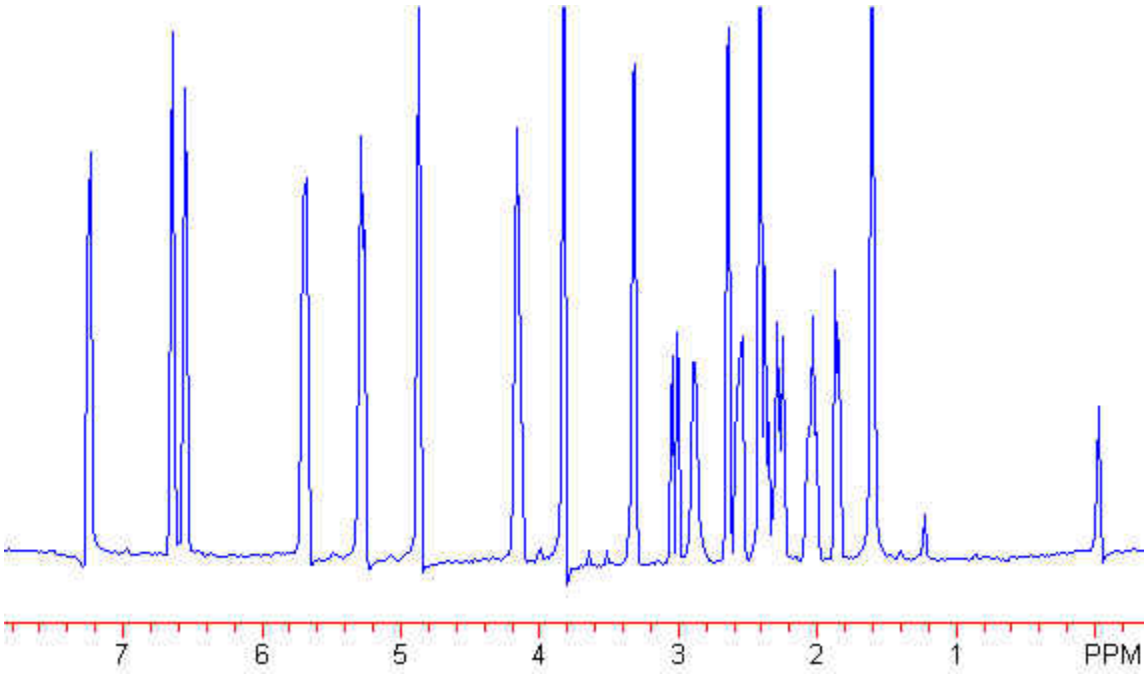
cosine squared window function (sine function with 90 degree phase shift) in both dimensions
phased so all peaks in first slice are inverted
2x zero-fill in the indirect dimension
final size 512 x 512

Noesy experiments are particularly susceptible to phase and baseline problems, as described below.

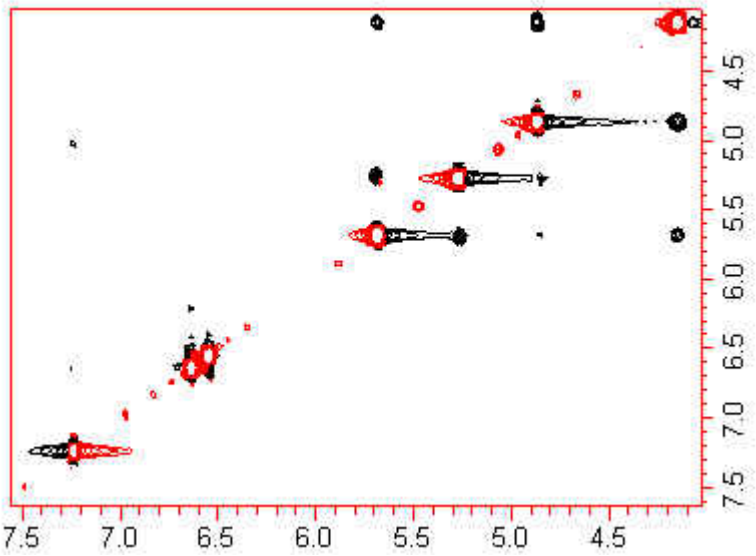
This is a hypercomplex phase-sensitive experiment (method of States, et al). For processing details, see hypercomplex processing in the 2D section.

Phasing problems in NOESY spectra

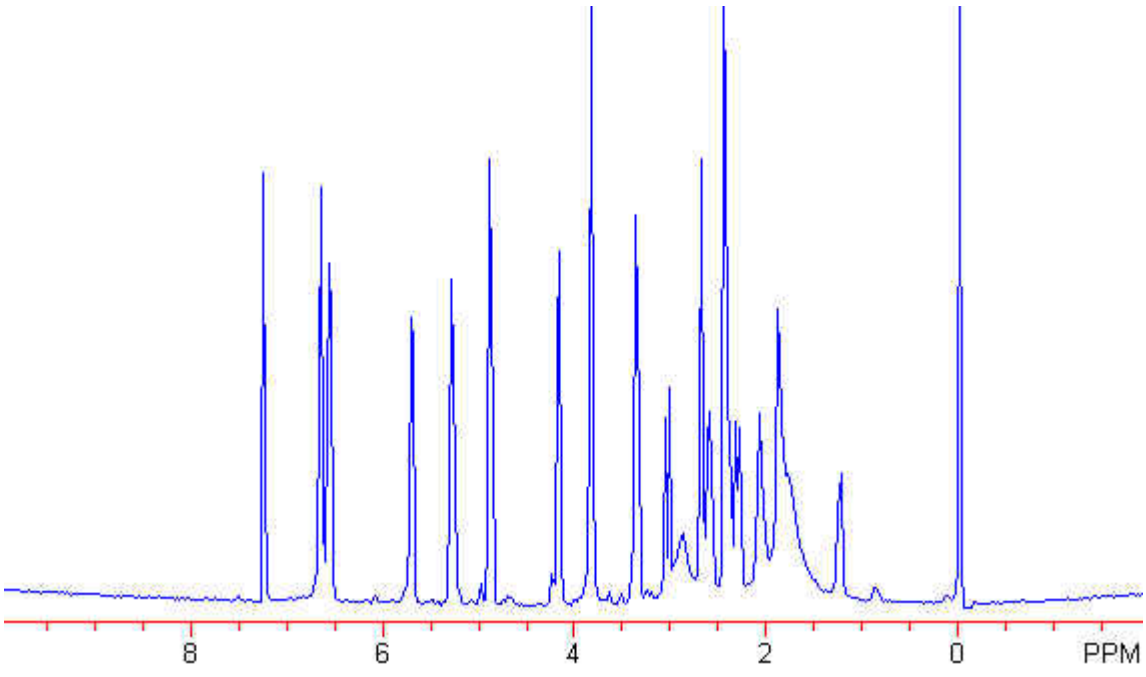
Below is the first slice of a NOESY spectrum of codeine. The sweep width is too narrow, resulting in phase distortions due to the filters (see more detailed explanation in the section on phasing above). It is not possible to get all peaks phased correctly.



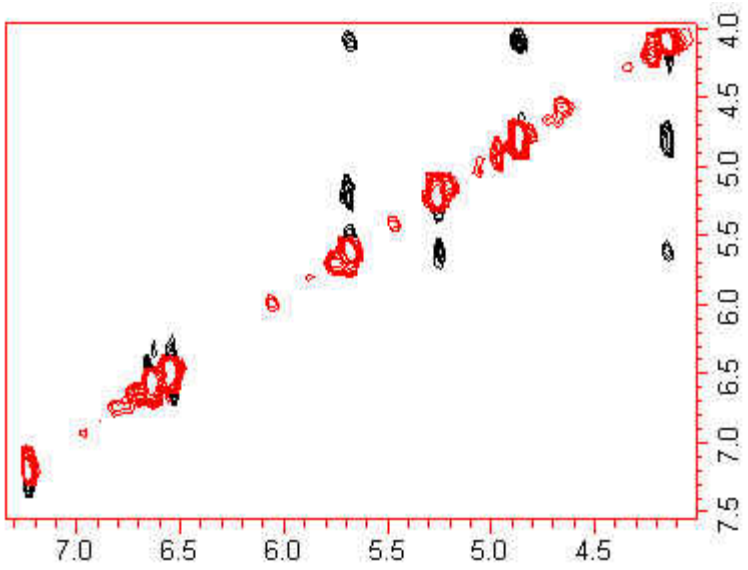
After processing, this phase "twist" causes streaks in the contour plot that can obscure peaks.



Below is the first slice of a NOESY spectrum of a different codeine sample, taken with larger sweep width. Notice that all peaks can be phased.



The resulting contour plot is free of the streaks seen above.

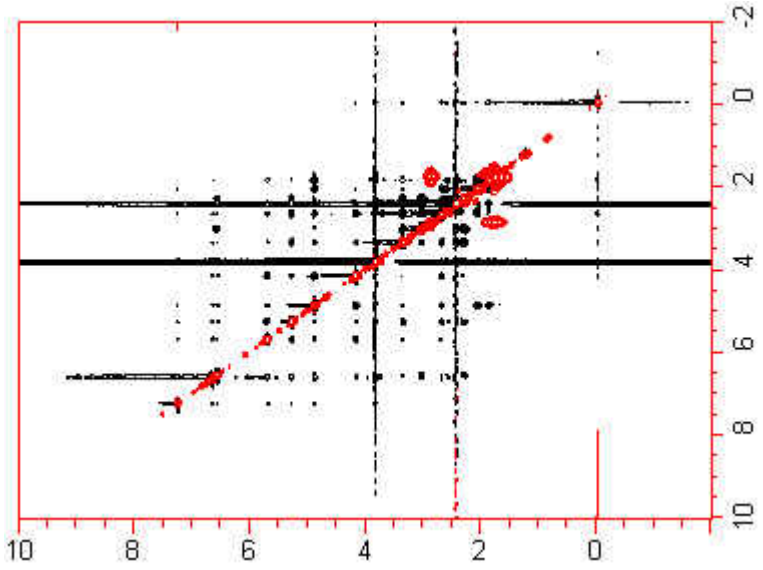


See also: baseline problems in noesy spectra, below

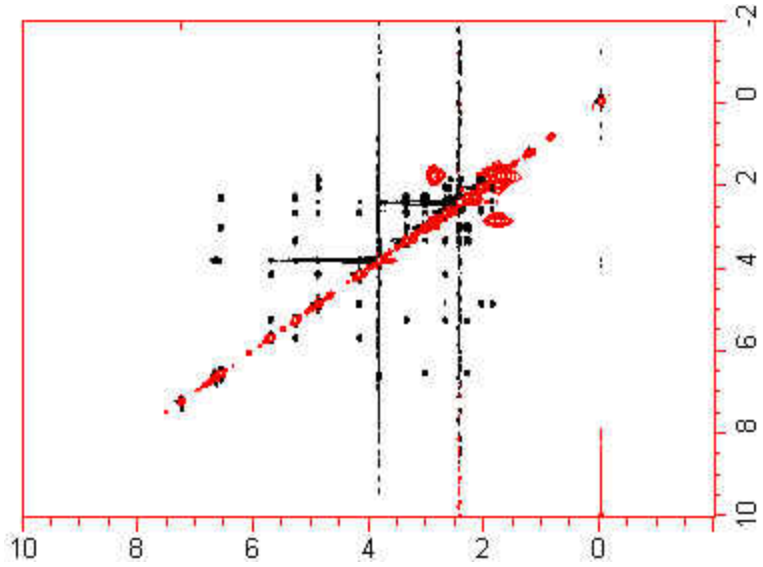
Baselines in NOESY spectra

NOESY spectra are particularly susceptible to bad baselines because the information of interest is contained in very small peaks. Baseline correction during processing can improve the results, but care to acquire data with flat baselines to begin with reduces the need for correction. Parameters to adjust are opening the sweep width far enough to avoid distortions due to filter cut-off, and adjustment of the pre-acquisition delay to minimize linear phase correction and baseline curvature. (See the section on phasing for details on audio filters and the section on baselines.)

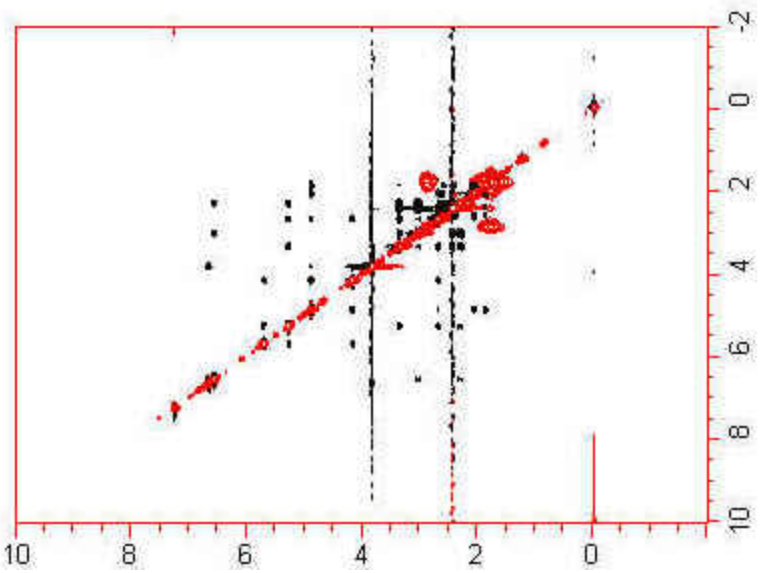
The 3 spectra below are from the same NOESY data set (3.5 mg of codeine in $CDCl_3$) which had substantial baseline curvature. The spectra were processed identically except for baseline correction.



The spectrum above was processed with no correction of baselines.

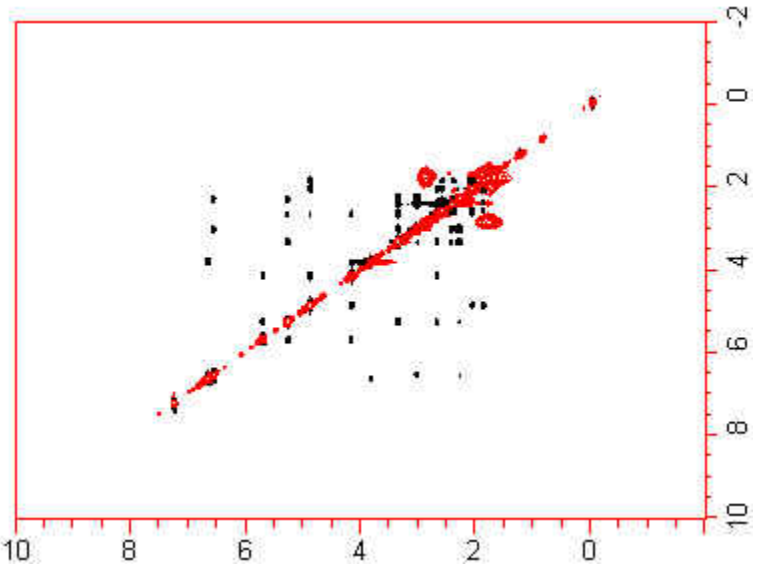


A BC was performed after FT in the direct dimension, removing DC offset and linear tilt.



A polynomial baseline correction was performed after FT in the direct dimension gives a slightly better result.

The vertical streaks are at the position of the 2 intense methyl singlets, commonly referred to as "t_1-noise" (where t_1 refers to the indirect dimension). (The above spectra are viewed with the direct dimension running horizontally.) Viewing individual slices, it is apparent that the noise level in these slices is 3-5x greater than in the rest of the spectrum, and some of the slices have severely curved baselines. The appearance of the spectrum can be improved by selectively dividing each of the offending slices by a constant, combined with careful polynomial correction of specific slices, as needed.



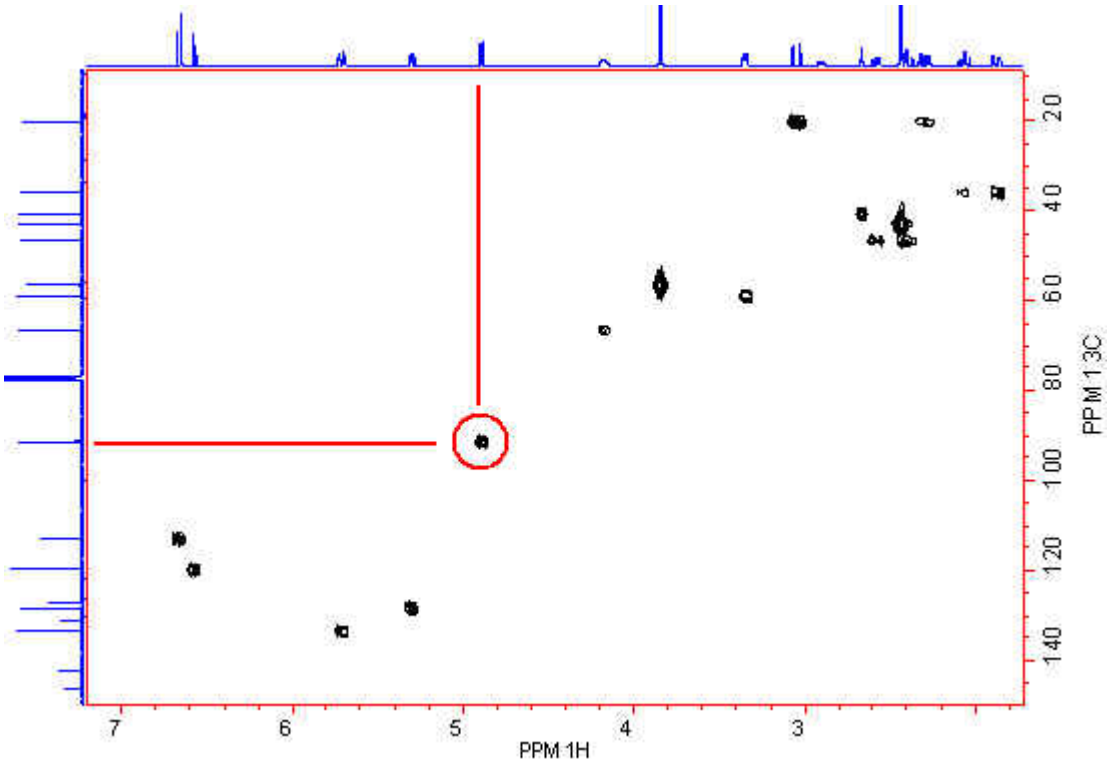
This is shown in more detail in the section on 2D editing, below.

HMQC

This is a 2D experiment used to correlate, or connect, ^{1}H and ^{13}C peaks for directly bonded C-H pairs. The coordinates of each peak seen in the contour plot are the ^{1}H and ^{13}C chemical shifts. This is helpful in making assignments by comparing ^{1}H and ^{13}C spectra.

This experiment yields the same information as the older "HETCOR" experiment, but is more sensitive, so can be done in less time and/or with less material. This is possible because in the HMQC experiment, the signal is detected by observing protons, rather than carbons, which is inherently more sensitive, and the relaxation time is shorter. This so-called "inverse detection" experiment is technically more difficult and is possible only on newer model spectrometers. Acorn NMR's new JEOL Eclipse^{+} 400 is equipped to perform inverse experiments, and uses Z-gradients for improved spectral quality.

The time required for an HMQC depends on the amount of material, but can be done in 1/2 hour or less, compared to several hours for a HETCOR spectrum.

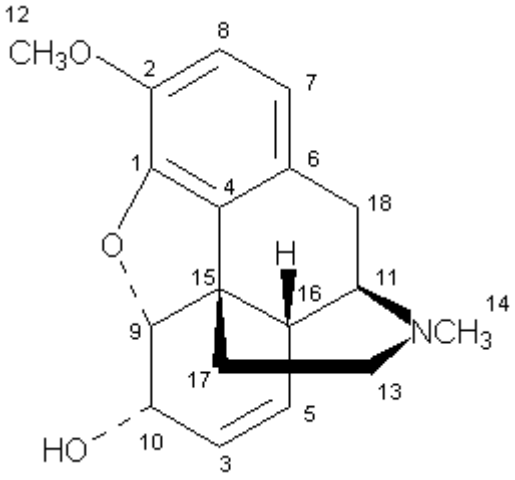


Contour plot of the HMQC spectrum. Because it is a heteronuclear experiment, the 2 axes are different, and the plot is not symmetrical. Unlike a COSY spectrum, there are no diagonal peaks.

Normal 1D ^{1}H and ^{13}C spectra are shown along the edges. Peaks occur at coordinates in the 2 dimensions corresponding to the chemical shifts of a carbon and its directly bonded proton(s). For example, the contour peak indicated in red shows that the ^{13}C with peak at 91.5 ppm is bonded to the ^{1}H with peak at 4.9 ppm.

Non-equivalent methylene protons are easily identified as 2 peaks located at the same ^{13}C position. There are 3 CH_2s in the codeine HMQC spectrum.

^{1}H	^{13}C	Assignment
6.6	113	8
6.5	120	7
5.7	133	3
5.3	128	5
4.8	91	9
4.2	66	10
3.8	56	12
3.3	59	11
3.0 & 2.3	20	18
2.6	40	16
2.6 & 2.4	46	13
2.4	43	14
2.0 & 1.8	36	17



The sample is 3.3 mg codeine in ~ .65 ml $CDCl_3$

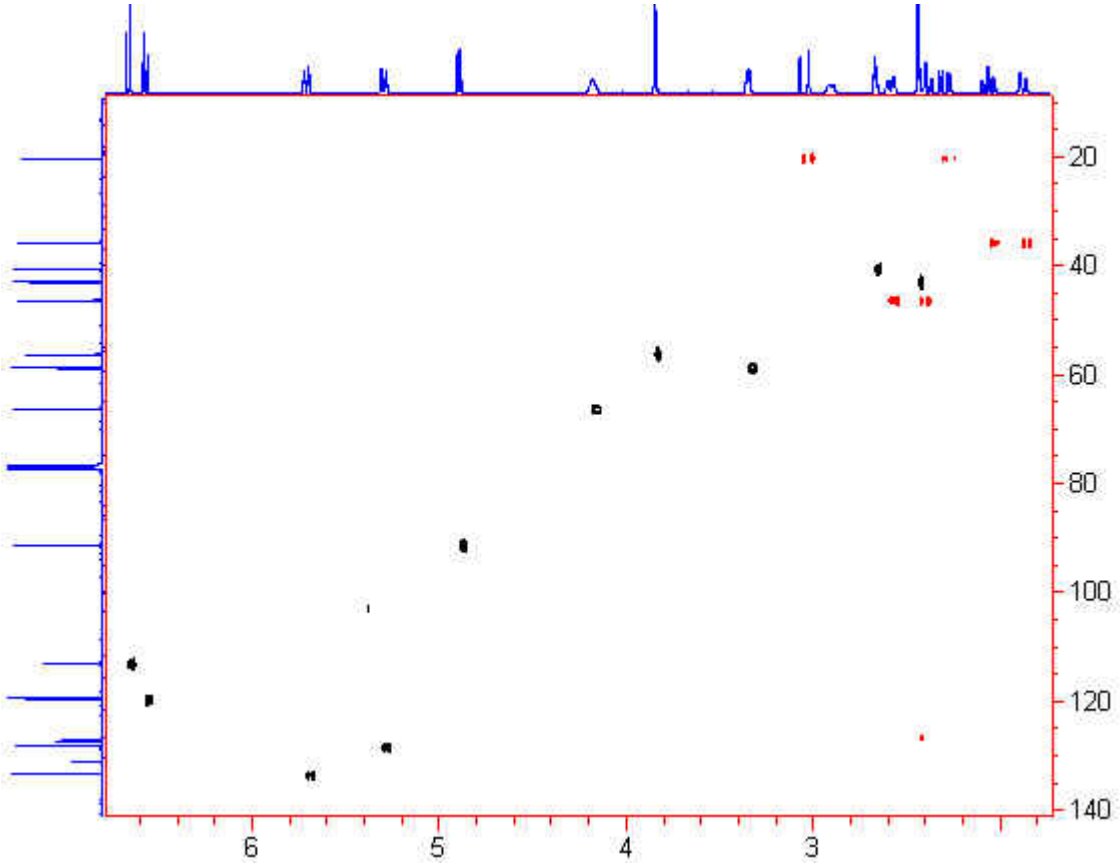
Acquisition Parameters:

512 complex points in direct dimension
128 t_1 increments
2 scans
2 sec. relaxation delay
Total acquisition time: ~ 10 min.

Processing:

sine squared window function in both dimensions with 45 degree phase shift
2x zero-fill in the indirect dimension
magnitude calculation (no phasing is required)
final data size 512 x 512

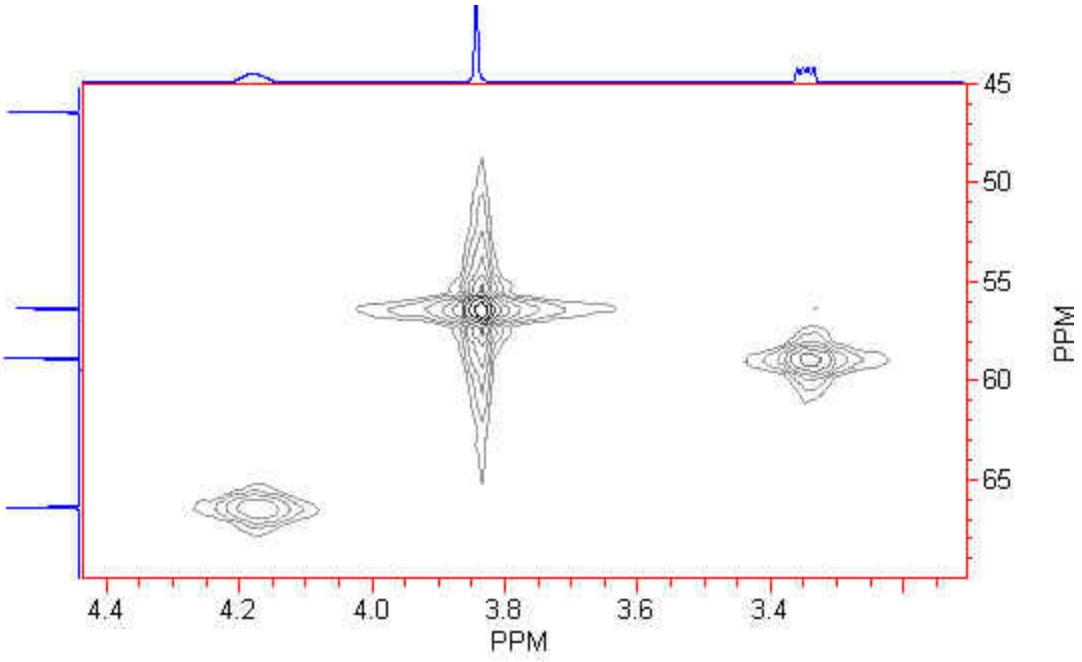
There are variations on this experiment, including a version in which CH_2s have phase opposite of that of CH and CH_3 peaks, called an HSQC-DEPT spectrum. Negative peaks are shown in red in the plot below, easily identifying the 3 CH_2s in codeine.



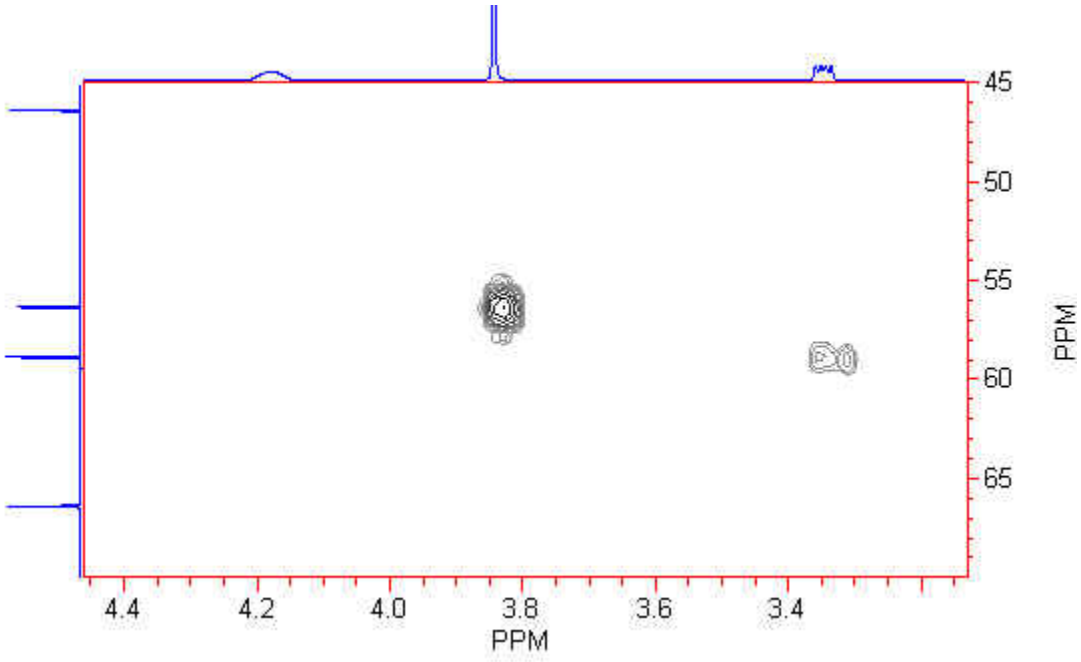
Processing HMQC data
HMQC spectra of codeine

There are several variations on ^{1}H-detected one-bond C-H correlation experiments. Magnitude mode experiments have the advantage of simplified processing, because no phase correction is required. The last step in processing is a magnitude calculation. Choice of window function dramatically affects the quality of the resulting spectrum.

The sample used here was 3.3 mg codeine in $CDCl_3$, acquisition time 2.5 hrs.

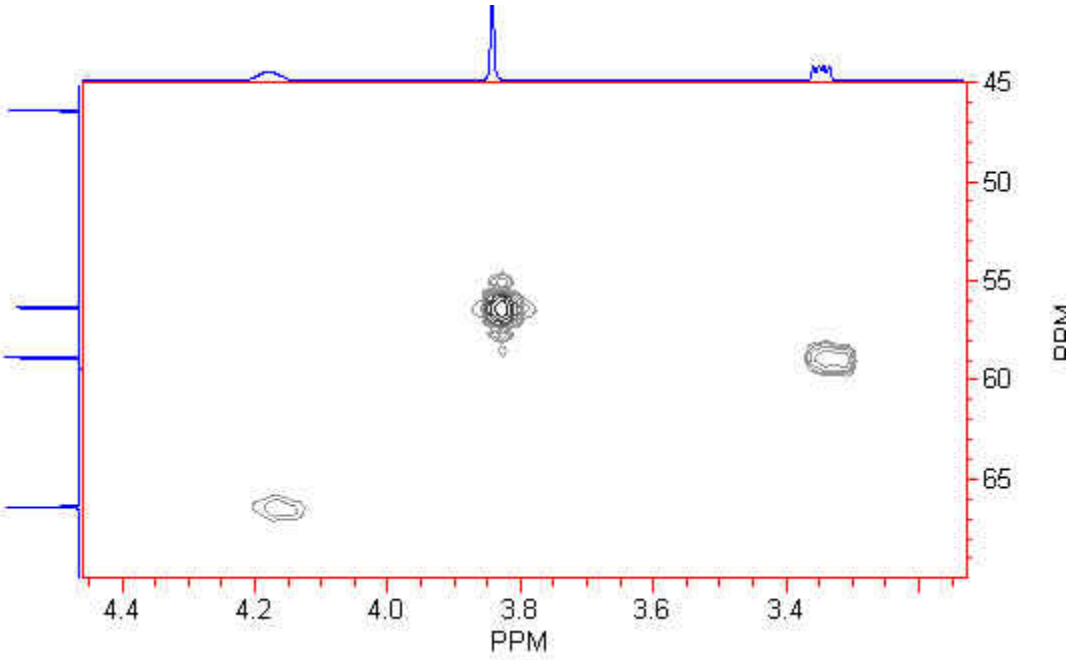


A 90-degree shifted sine squared function was used above. Note the broad "wings" on the peaks that result from the subsequent magnitude calculation. This is most severe for the intense methyl peak in the center of this plot.

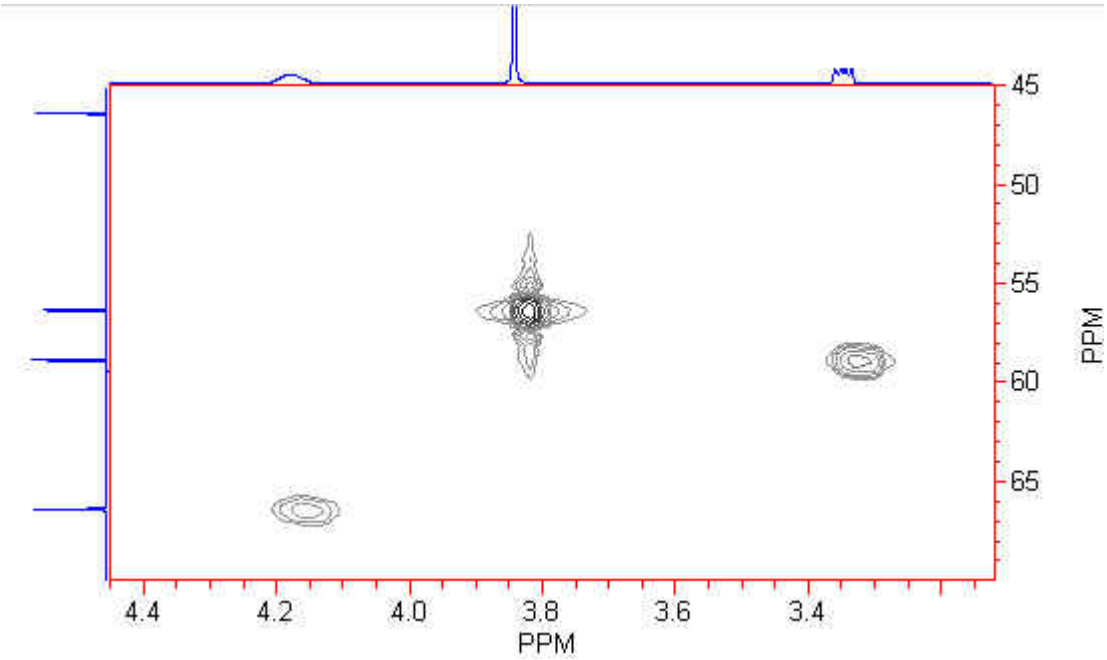


A 0-degree shifted sine squared function is commonly used to avoid the broad wings in magnitude spectra. However, in this case, use of this function led to complete loss of one

peak! The missing peak is broad in the ^{1}H spectrum, and is severely attenuated by the sine function.

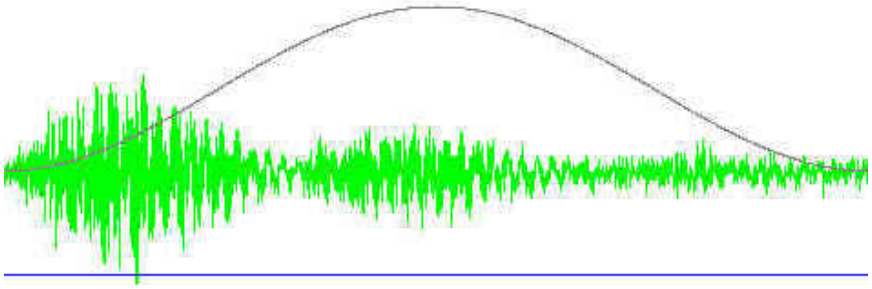


A 30-degree (above) or 45-degree (below) shifted sine squared function both seem to be good compromises.



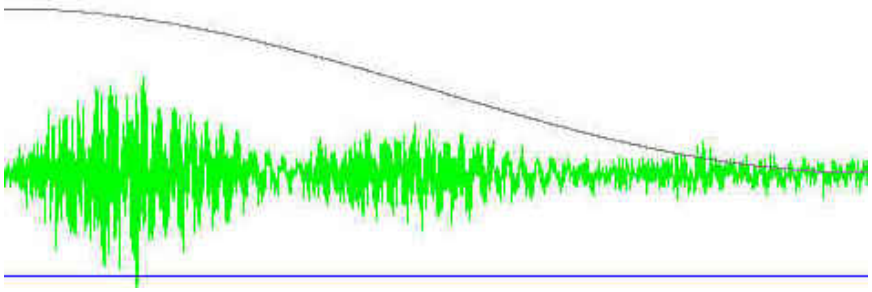
The bottom line is, you need to match the window function to the shape of the fid. This is a case where the NUTS "window view" command (WV) is helpful, because it allows you to compare the shape of the fid and window function.

For example, below is the first slice of an HMBC FID (green), with grey lines showing the shape of different window functions:



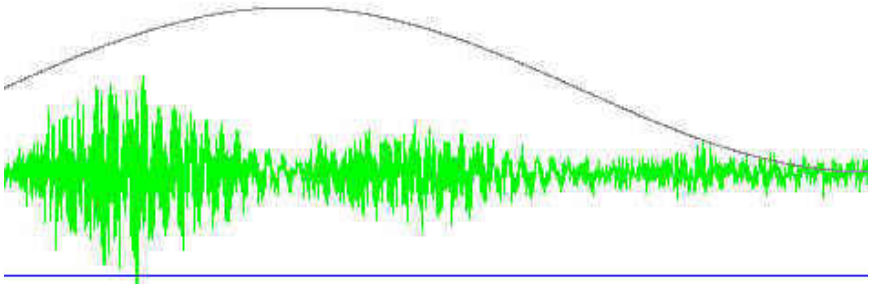
sine squared window function, 0 degree phase shift

It is clear that this results in loss of signal near the beginning of the FID. For a broad line, which decays quickly, signal can be lost.



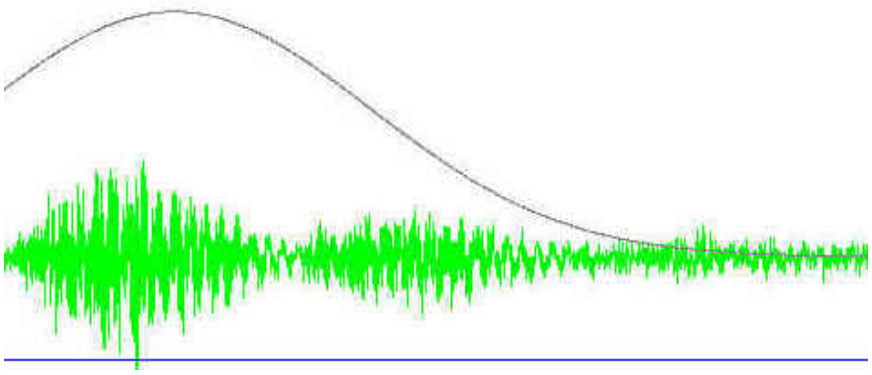
sine squared window function, 90 degree phase shift

This doesn't discard signal, but also does nothing to attenuate the early time points which give rise to the broad "wings"



sine squared window function, 45 degree phase shift

A reasonable compromise...



...but this might be the better choice.

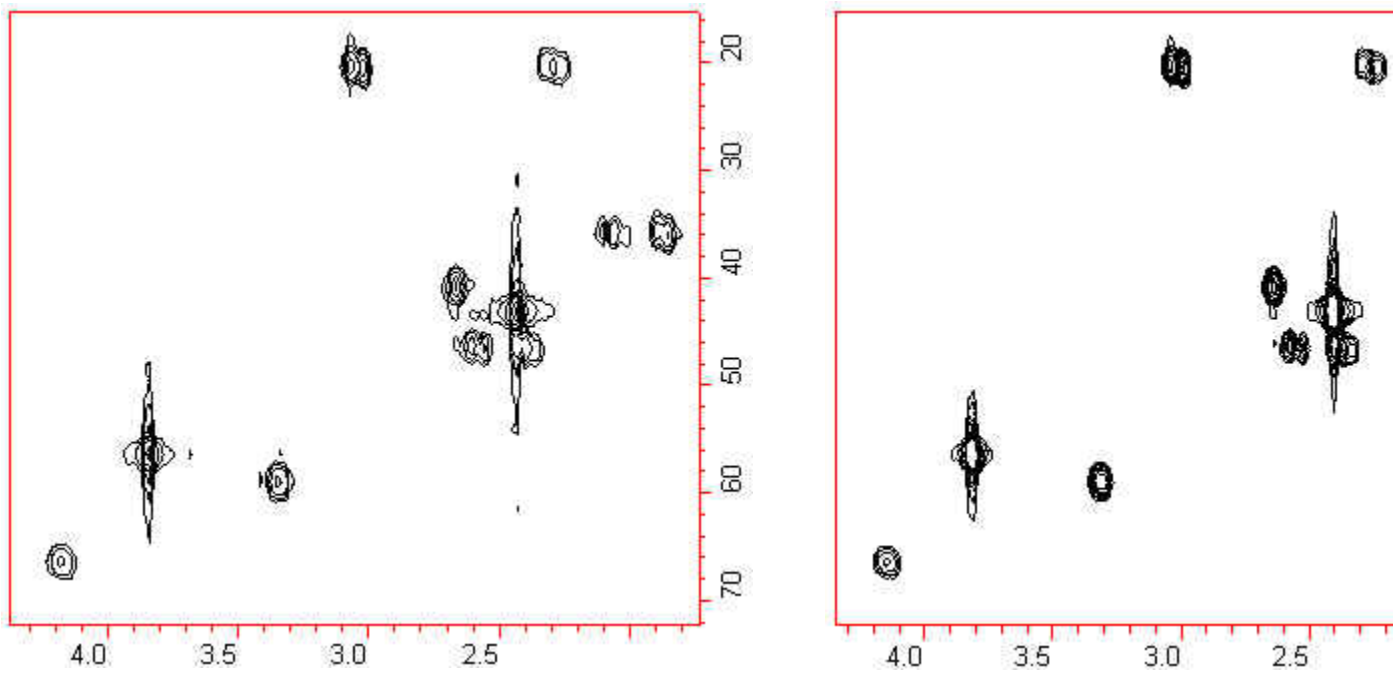
This is a Lorentz-Gauss window function applied twice, with

LB = -1
GF = 0.2

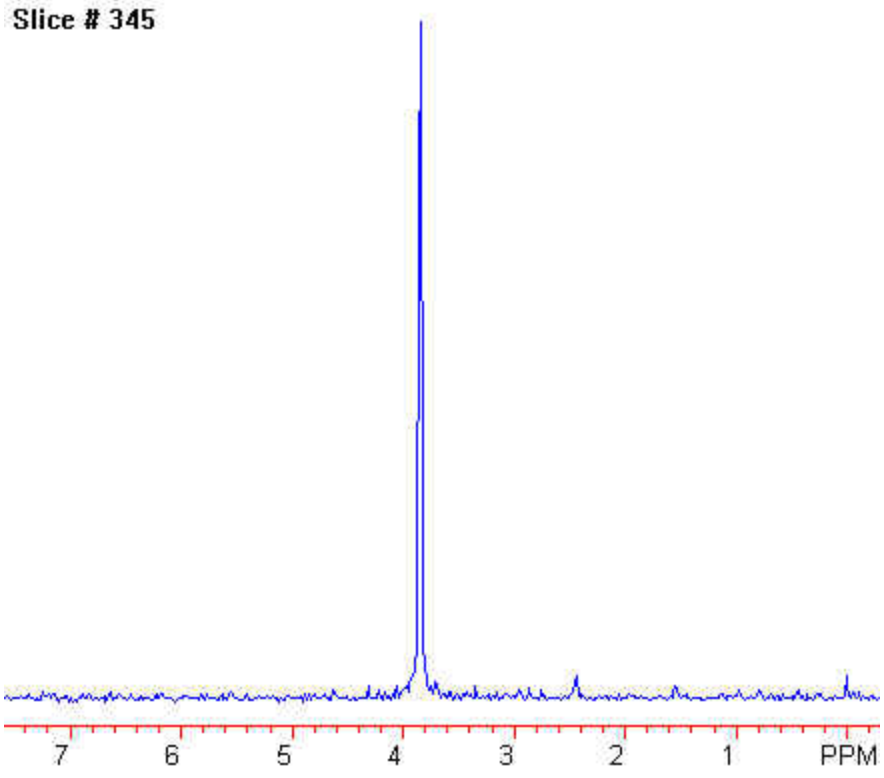
Magnitude vs. Power Spectrum

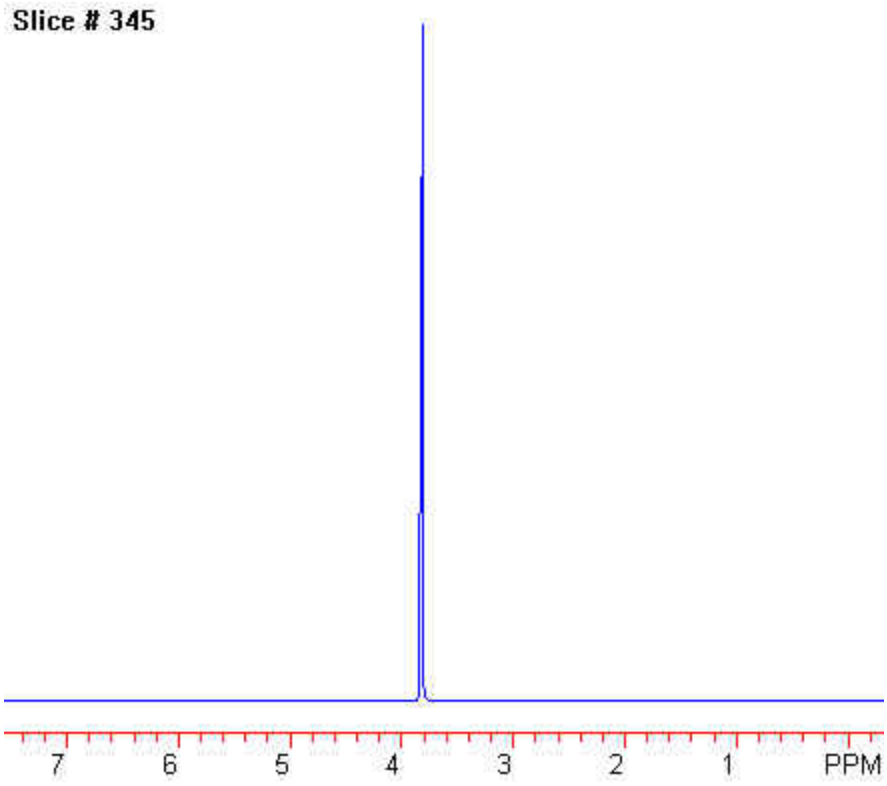
Below are expansions of the same codeine HMQC spectrum shown above, processed with 45 degree sine squared window function in both dimensions.

The plot on the left was processed using Magnitude Calculation (NUTS command MC) as the last step. For the plot on the right, a Power Spectrum (NUTS command M2) was used instead of MC.



Below are slices through the methoxy peak of the HMQC data shown above. MC used in the top spectrum, M2 used in the spectrum below it. Compare the signal-to-noise.


Slice # 345
7
6
5
4
3
2
1
PPM



MC uses this equation: SQRT(Re2 + Im2)

M2 uses: Re2 + Im2

HMBC

This is a 2D experiment used to correlate, or connect, ^{1}H and ^{13}C peaks for atoms separated by multiple bonds (usually 2 or 3). The coordinates of each peak seen in the contour plot are the ^{1}H and ^{13}C chemical shifts. This is extremely useful for making assignments and mapping out covalent structure.

The information obtained is an extension of that obtained from an HMQC spectrum, but is more complicated to analyze. Like HMQC, this is an "inverse detection" experiment, and is possible only on newer model spectrometers.

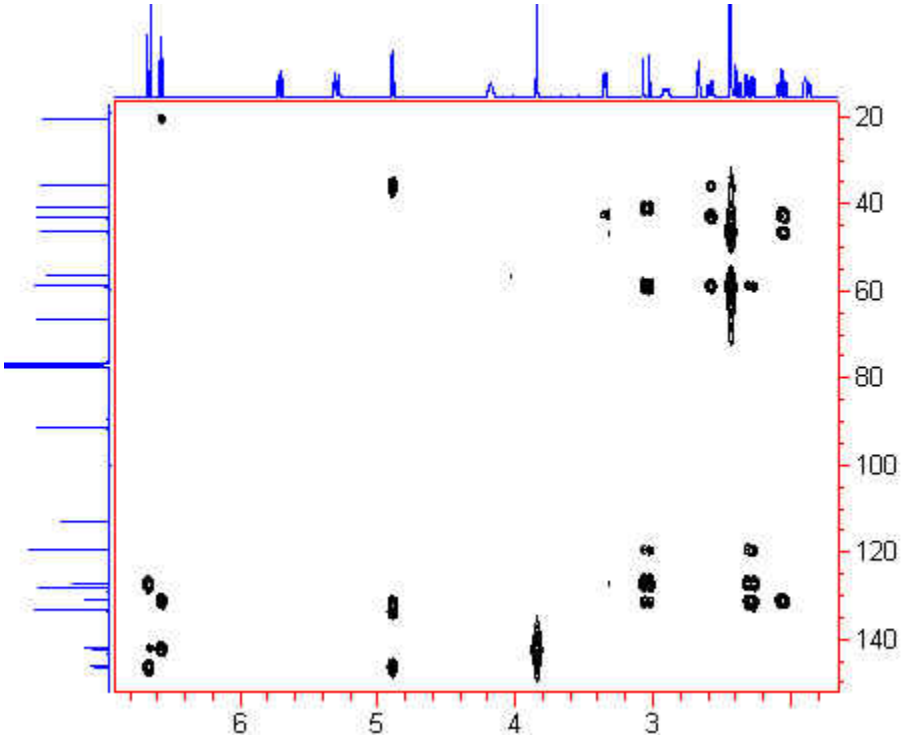
The time required for an HMBC depends on the amount of material, but is much greater than for HMQC, and can take from an hour to overnight.

The contour plot shown below is of 3.3 mg codeine in ~ .65 ml $CDCl_3$. See also comparison to the HMBC spectrum in the next section of an 18 mg sample.

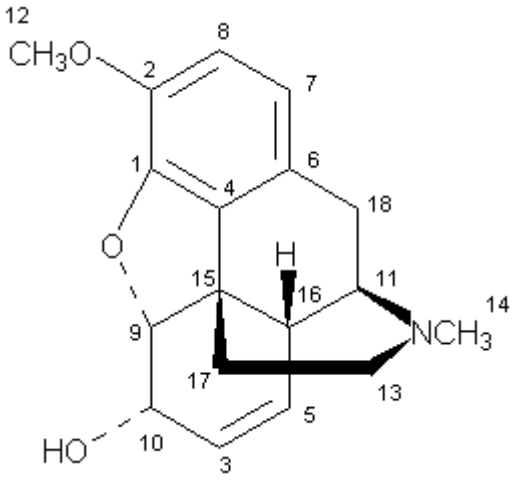
Normal 1D ^{1}H and ^{13}C spectra are shown along the edges. Peaks occur at coordinates in the 2 dimensions corresponding to the chemical shifts of a carbon and protons separated by (usually) 2 or 3 bonds. The experiment is optimized for couplings of ~8 Hz. Smaller couplings are observed, but their intensities are reduced. Compare to the spectrum in the next section, obtained when the experiment is optimized for 4 Hz.

The experiment is designed to suppress 1-bond correlations, but a few are observed in most spectra. In concentrated samples of conjugated systems, 4-bond correlations can be observed. There is no way to know how many bonds separate an H and C when a peak is observed, so analysis is a process of attempting to assign all observed peaks, testing for consistency and checking to be sure none of the assignments would require implausible or impossible couplings.

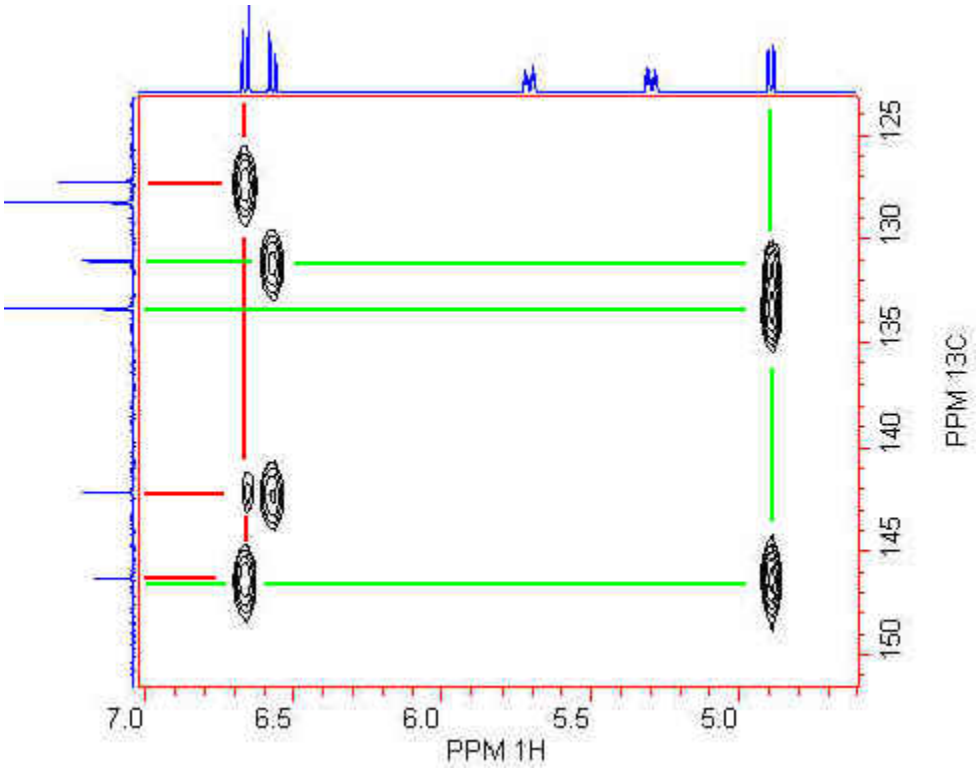
Because of the large number of peaks observed, analysis requires several expanded plots. In this case, the spectrum has been divided into 4 sections, each of which is discussed below.



The discussion below uses the numbering system shown at right.



The numbers were assigned to peaks in the 1D ^{13}C spectrum, starting downfield, moving upfield, and numbering each sequentially. This generates a unique identifier for each Carbon, even before knowing any assignments.

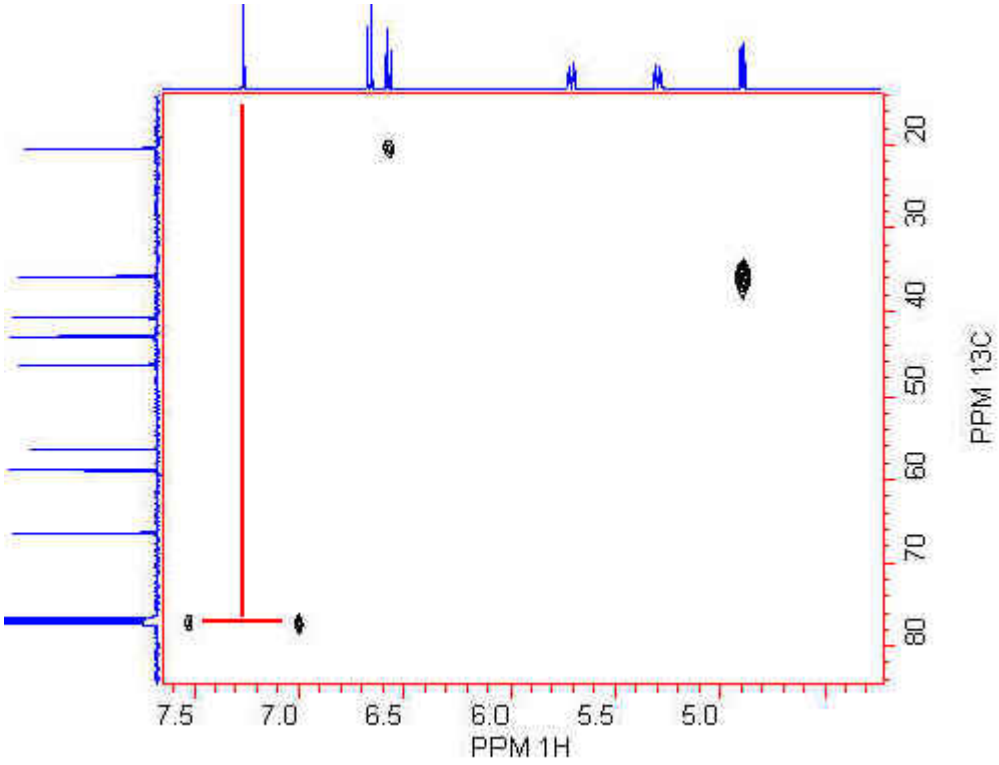


In aromatic rings, the most common correlations seen in HMBC spectra are 3-bond correlations because they are typically 7-8 Hz, which is the value for which the experiment is optimized. The coupling constant is affected by substituents, so 2-bond correlations are also sometimes observed.

The red lines in the plot above show correlations from aromatic proton H-8 to aromatic carbons C-1 and C-6 (both are 3-bond couplings) and a weak correlation to C-2, a 2-bond coupling.

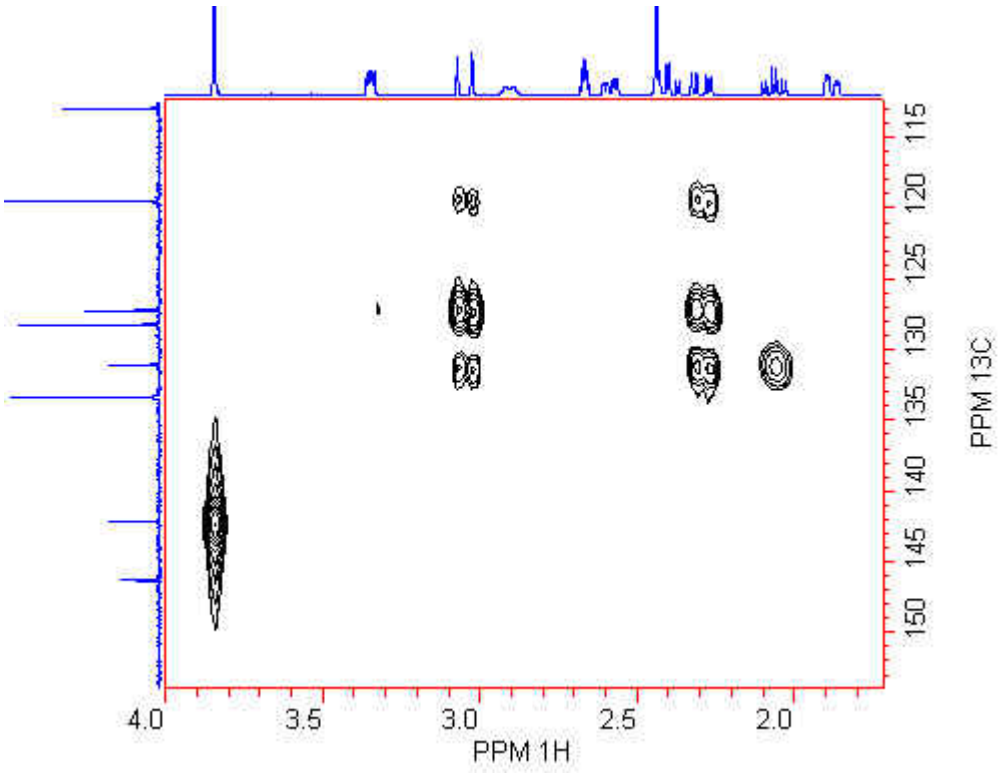
The other aromatic proton, H-7, has correlations to C-2 and C-4, both of which are 3-bond couplings.

The green lines in the plot above show correlations from proton H-9 to carbons C-1, C-3 and C-4 (all are 3-bound couplings). With the poor digital resolution of the spectrum in the carbon dimension (512 data points spread over 17kHz), the peaks for C-3 and C-4 run together because they are barely resolved.



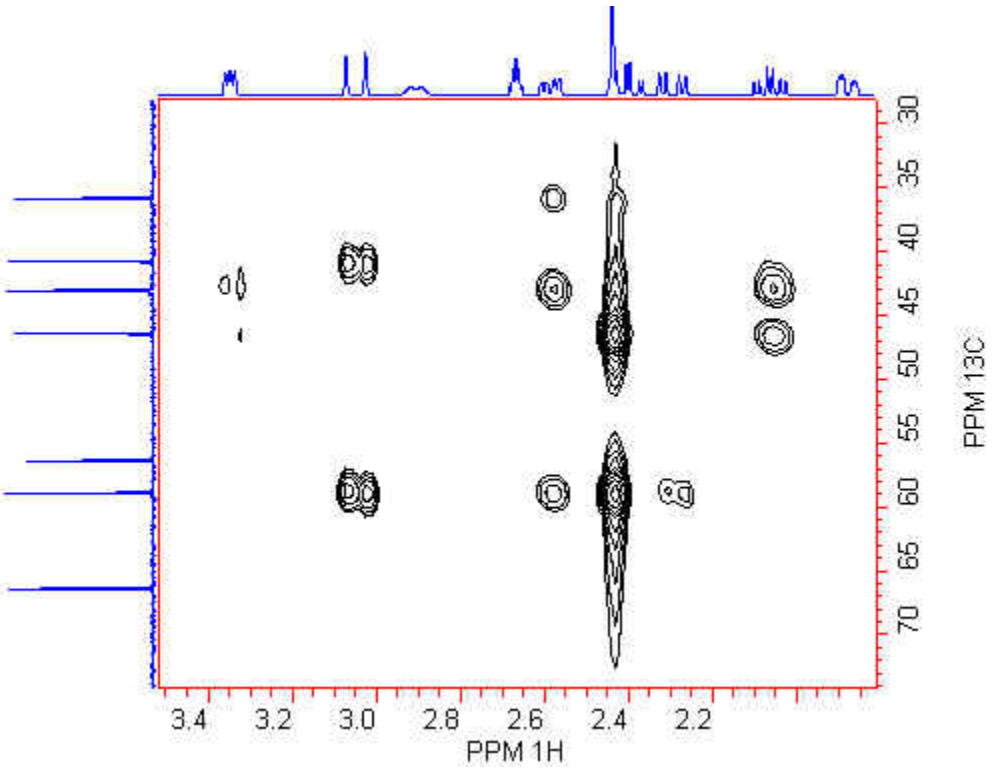
The peaks indicated in red above are due to 1-bond coupling in $CHCl_3$ solvent. Note that the pair of peaks don't line up with any H peaks, but are symmetrically located about the $CHCl_3$ peak, with a separation equal to the 1-bond C-H coupling constant.

The other 2 peaks in this plot are H-7 to C-18 and H-9 to C-17.



The region in the above plot shows correlations between aliphatic Hs and aromatic Carbons. In the lower left corner is a peak showing the 3-bond coupling between the methoxy Hs and C-2, the aromatic carbon bearing the methoxy.

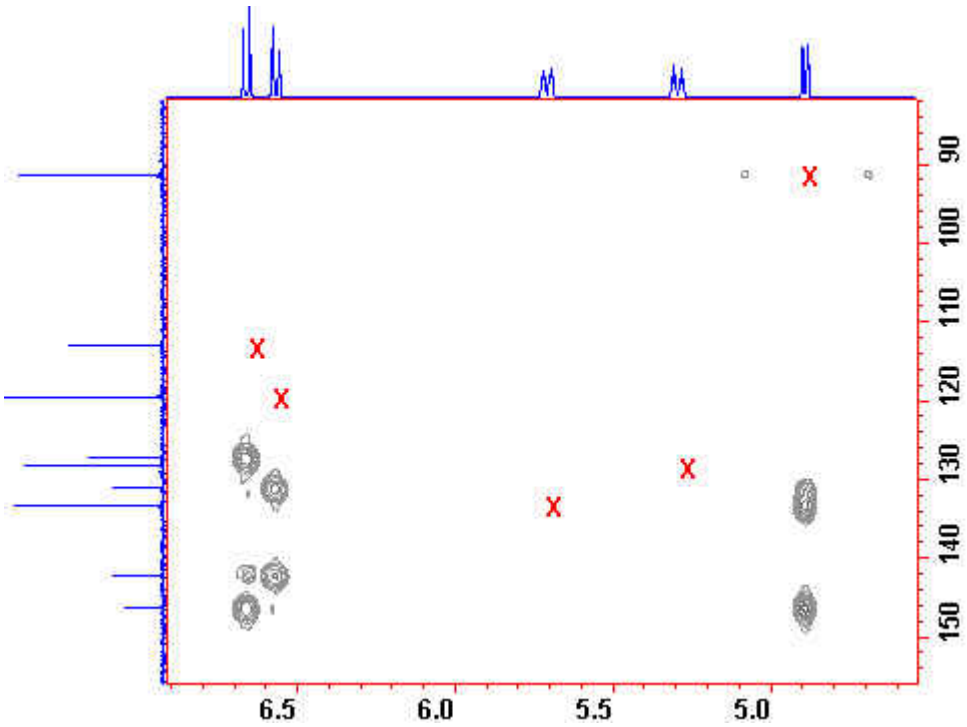
Two protons show correlations to the same 3 carbons. These are the geminal H-18 protons, showing coupling to C-7 and C-4 (both 3-bond couplings) and C-6 (2-bond coupling). The remaining peak is H-17 to C-4. Note that the corresponding coupling from H-17' is not observed. As with H-H couplings, the value of the 3-bond coupling constant is dependent on the dihedral angle.



The last quadrant is shown above, showing correlations between aliphatic Hs and Cs. The peaks are identified below, for each H, starting from the left. Carbons 14 and 15 are only 0.1 ppm apart, and are not resolved in the 2D spectrum.

H-11 to C-14 and/or C-15
H-18 to C-16, C-11
H-13 to C-17, C-14 and/or C-15, C-11
H-14 to C-13 and C-11
H-18' to C-11
H-17 to C14 and/or C-15, C-13

During the process of assigning HMBC peaks, it can be useful to indicate on the plot the positions of the 1-bond correlations. NUTS has the ability to do this, using the compare command (described in the NUTS 2D section). In the HMBC plot below, 1-bond HMQC peaks are indicated by **X**.



Acquisition Parameters:

512 complex points in direct dimension
128 t_1 increments
8 scans
2 sec. relaxation delay
Total acquisition time: 35 min

Processing:

sine squared window function in both dimensions with 0 degree phase shift in t_2 and 90 degree phase shift in t_1
2x zero-fill in the indirect dimension
magnitude calculation (no phasing is required)
final data size 512 x 512

HMBC optimized for 4 Hz couplings

See explanation of the HMBC experiment, above.

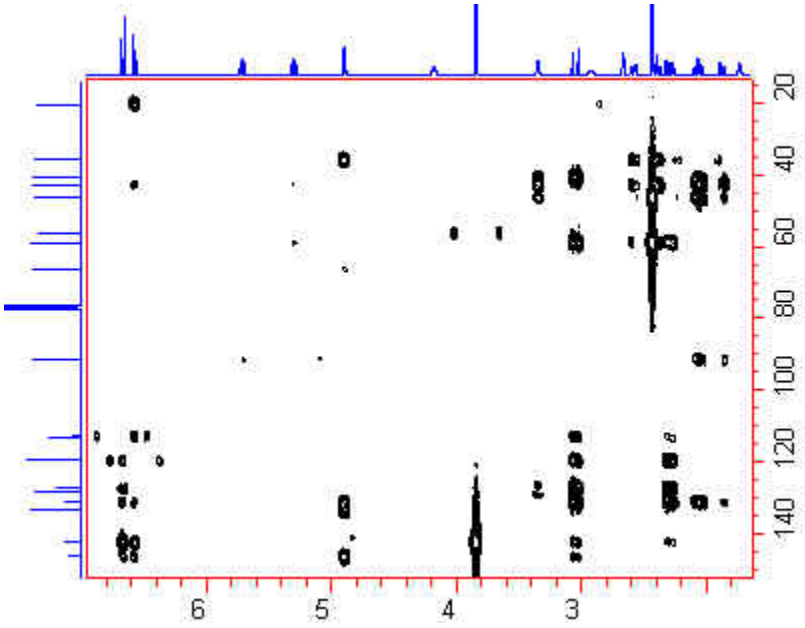
Long range C-H couplings cover a range of values, typically less than 10 Hz. The experiment is optimized for a specific coupling, and selection of that value is a trade-off. Correlations due to couplings of other values are reduced in intensity. To optimize for a specific J value, a delay in the pulse sequence is set to 1/(2J). For very small couplings,

this delay becomes so long that much of the magnetization is lost to relaxation. Typically, the delay is optimized for J of 7-8 Hz, the expected value for aromatic 3-bond couplings. However, longer-range couplings can be observed if the delay is optimized for smaller couplings.

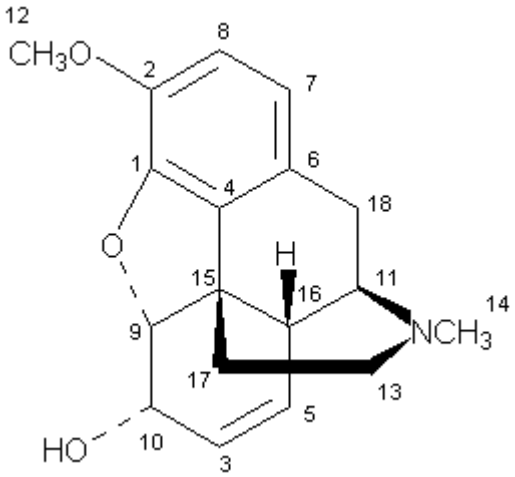
The contour plots shown below are of 8 mg codeine in ~ .65 ml $CDCl_3$, in which the experiment was optimized for couplings of 4 Hz.

Compare to the HMBC spectrum optimized for 8 Hz, above.

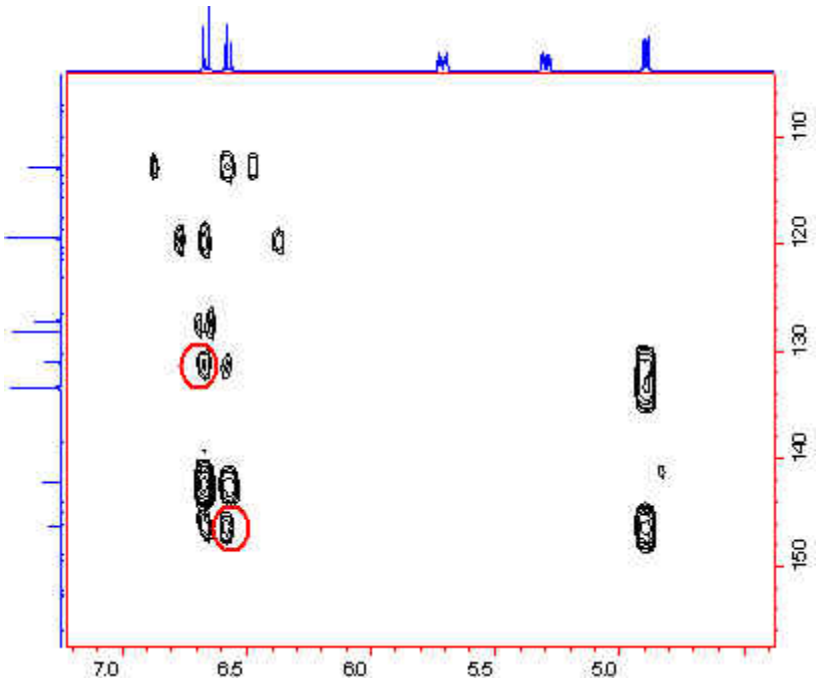
Because of the large number of peaks observed, analysis requires several expanded plots. In this case, the spectrum has been divided into 4 sections, each of which is discussed below.



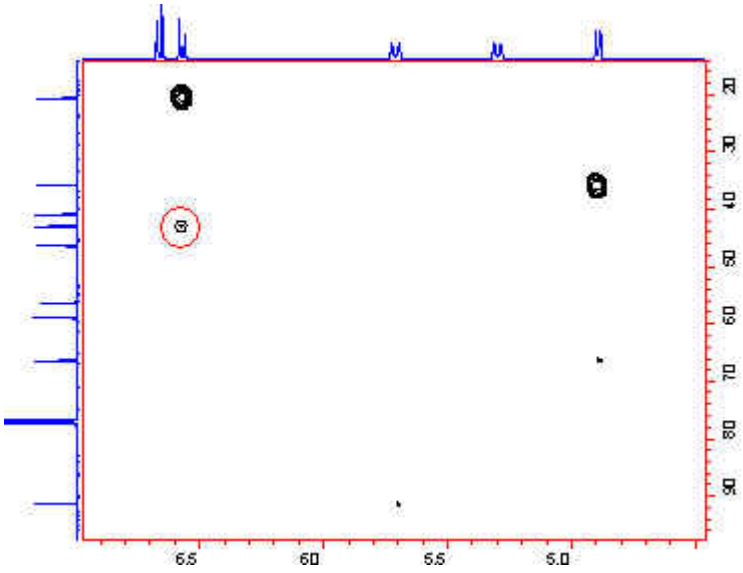
The discussion below uses the numbering system shown at right.



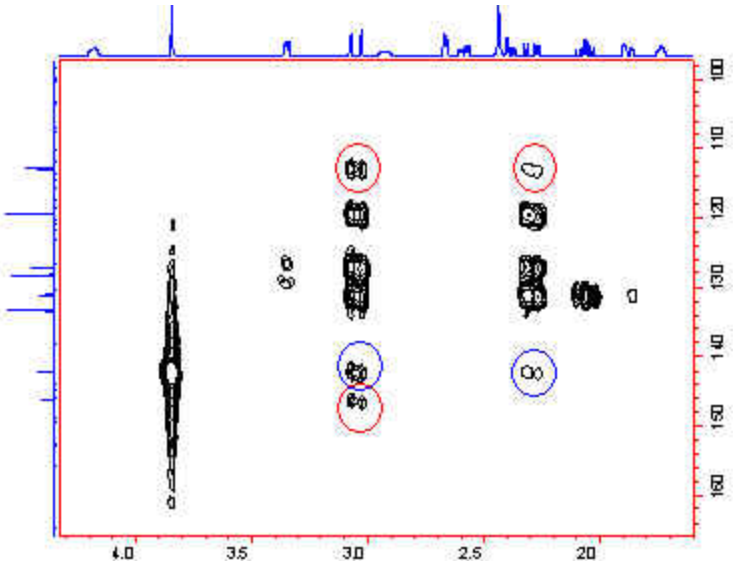
The numbers were assigned to peaks in the 1D ^{13}C spectrum, starting downfield, moving upfield, and numbering each sequentially. This generates a unique identifier for each Carbon, even before knowing any assignments.



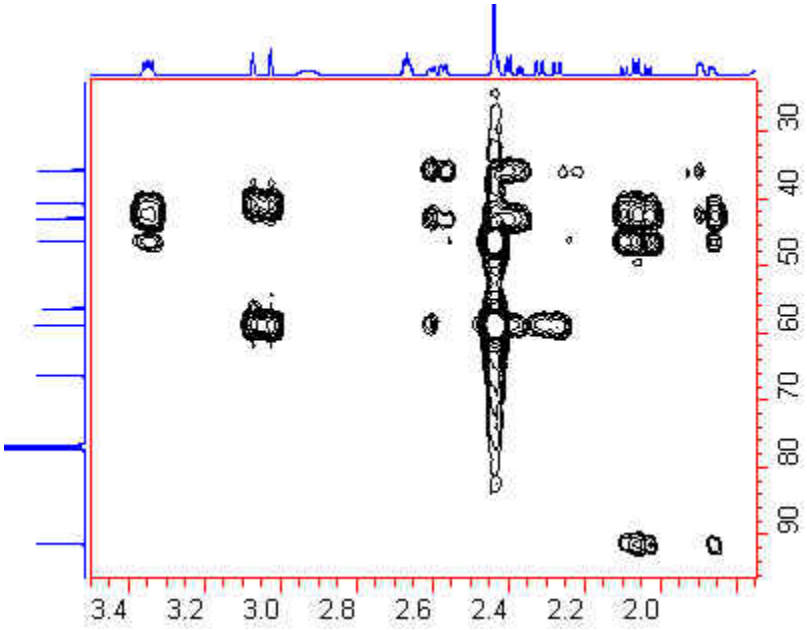
Compare to the same quadrant in the HMBC optimized for 8 Hz. Several additional 2-bond couplings are observed, as are 1-bond couplings for 7 and 8. The peaks indicated by red circles are 4-bond couplings: H-8 to C-4 and H-7 to C-1.



Compared to the 8 Hz-optimized spectrum, one additional peak is observed in this quadrant, indicated by the red circle. This is a 4-bond coupling from H-7 to C-15.



In this quadrant, three 4-bond peaks are indicated by red circles (H-18 to C-8 and C-1; H-18' to C-8). In addition, two **5-bond** couplings are indicated by blue circles. These are H-18 and H-18' to C-2.



The only additional correlations in the quadrant not seen in the 8 Hz-optimized spectrum are from H-13' (just upfield of the intense N-Me singlet) to carbons 17, 14/15 and 11.

Acquisition Parameters:

512 complex points in direct dimension
128 t_1 increments
64 scans

2 sec. relaxation delay
Total acquisition time: 4.5 hrs

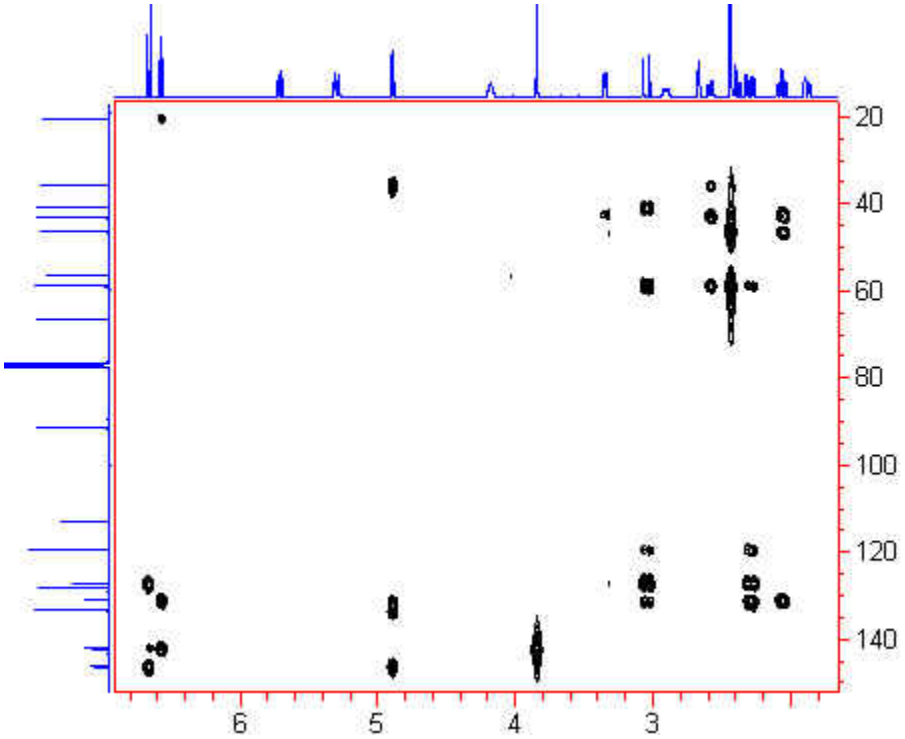
Processing:

sine squared window function in both dimensions with 0 degree phase shift in t_2 and 90 degree phase shift in t_1
2x zero-fill in the indirect dimension
magnitude calculation (no phasing is required)
final data size 512 x 512

Concentration needed for HMBC
How much is enough?

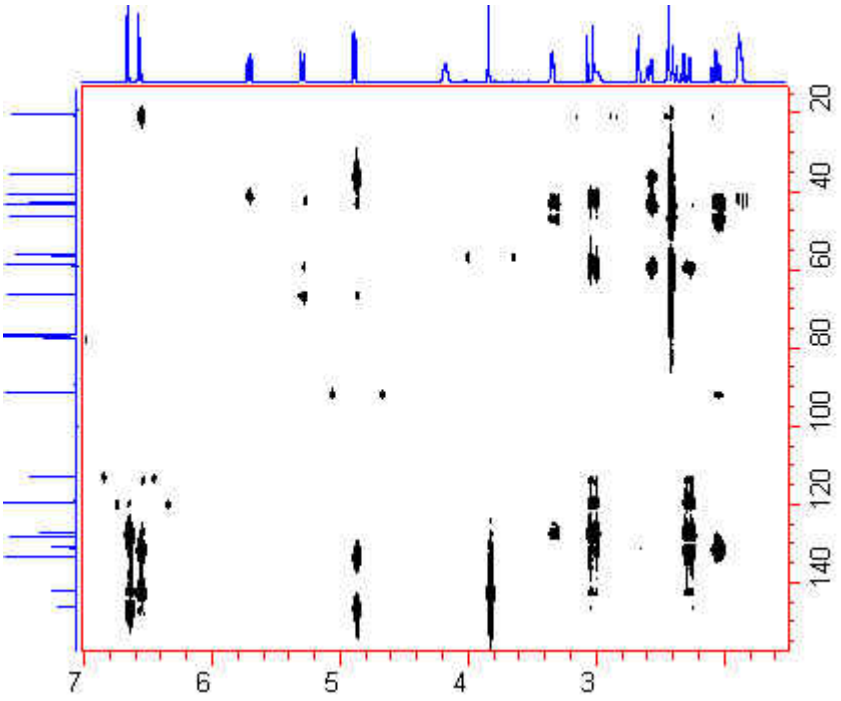
This isn't a black-and-white issue. In general, the answer is a few mg, assuming it's a small molecule. The higher the MW, the more mg we need. With less material, the experiment runs longer.

Below is a comparison of 2 samples of codeine, 3 mg and 18 mg. 3 mg yields a good spectrum in 1/2 hr. The more concentrated sample, run for longer time, shows several additional peaks. Remember that codeine is quite small, MW = 299.



3 mg of codeine

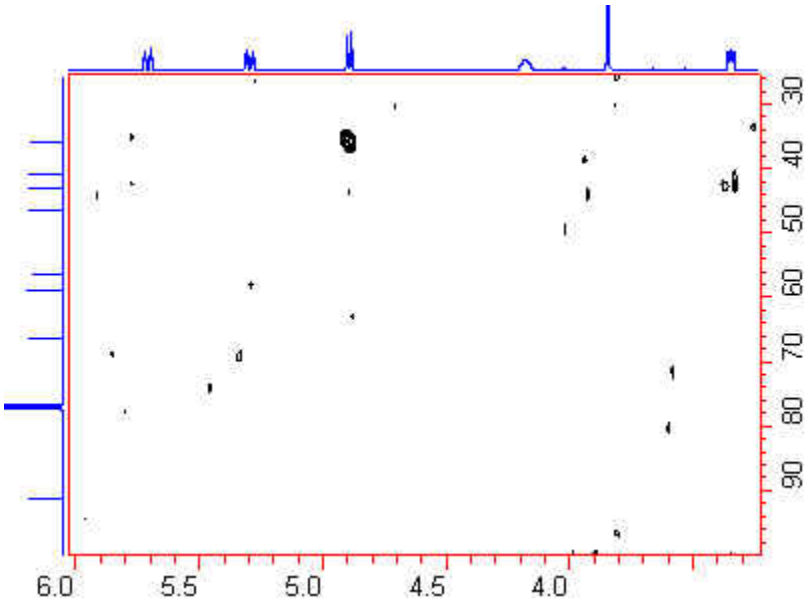
total acquisition time: 35 min

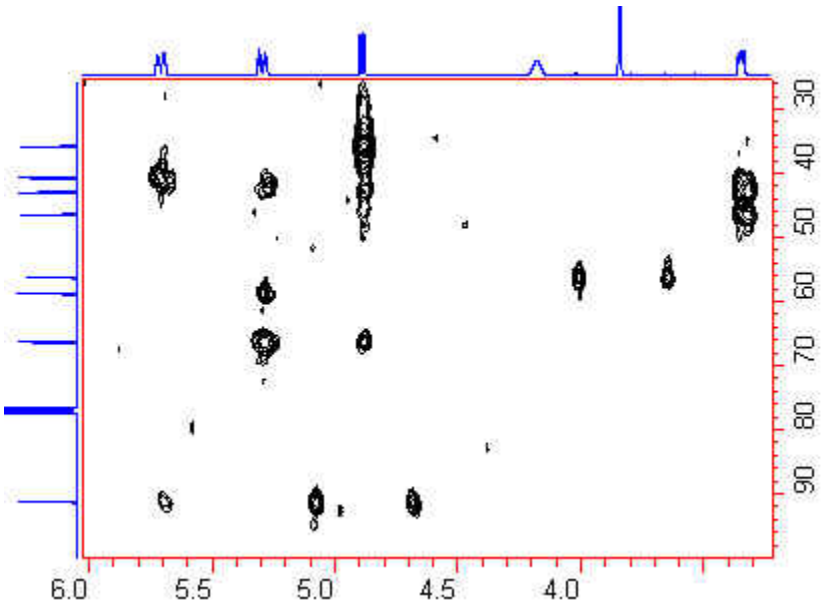


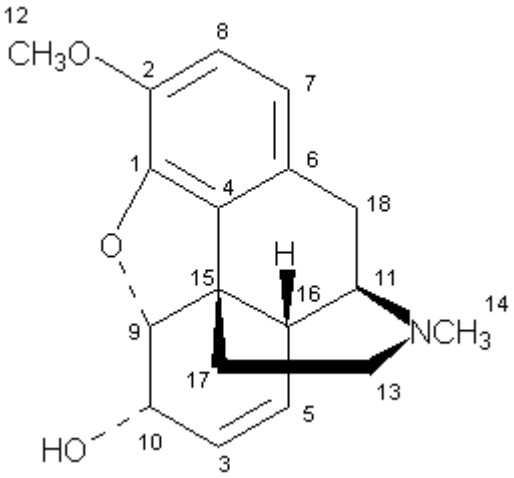
18 mg codeine

total acquisition time: 1 hr. 10 min.

The expanded plots below compare the same region of the 2 spectra. (Top: 3 mg sample Bottom: 18 mg sample) The concentrated sample shows eight additional 2- and 3-bond correlations, and two 1-bond correlations.







The additional correlations seen in this region of the spectrum of the more concentrated sample are:

H ----> C	
3	16 and 9
5	16, 11 and 10
9	15 and 10
11	13

1-bond correlations are also seen for 9 and 12.

HMQC-TOCSY

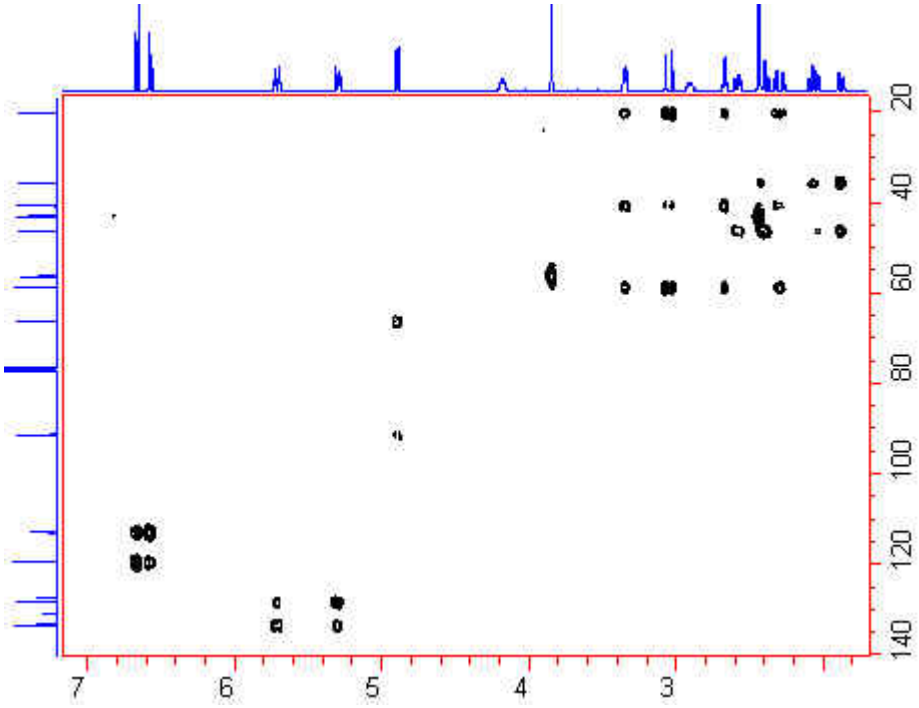
This is an experiment that can be useful for tracing out ^{1}H-^{1}H connectivity in crowded spectra with overlap of ^{1}H peaks.

The first step of the pulse sequence is an HMQC, in which ^{1}H magnetization is transferred to the directly-bonded ^{13}C. ^{13}C magnetization evolves during t_1 and then transferred back to the directly-bonded proton(s). The completion of the sequence is a TOCSY mixing period, which transfers magnetization from the "carbon spin-labeled" proton along a chain of coupled protons. Note that this experiment includes a TOCSY

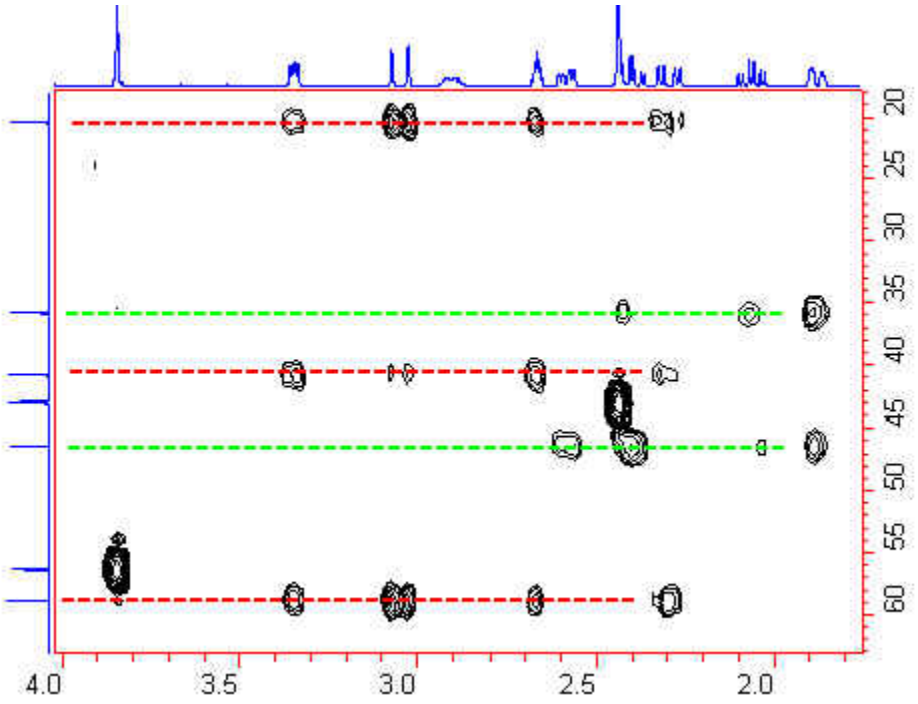
mixing period, but not the TOCSY ***evolution*** period. Compare to the 3D HMQC-TOCSY experiment in the section on 3D processing.

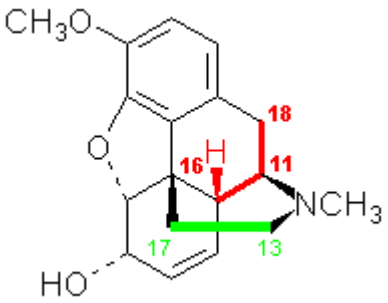
In the resulting spectrum, the carbon spectrum runs along the vertical dimension. At the chemical shift of each protonated ^{13}C, peaks appear at the chemical shifts of the directly bonded proton(s) and of other protons in the same spin system.

The experiment can be repeated using different mixing times in the TOCSY sequence. As with TOCSY, the intensity of each cross-peak will vary through the series of spectra - long range correlations will "grow in" as the delay value is increased, and shorter range couplings may disappear.



Expanded aliphatic region:



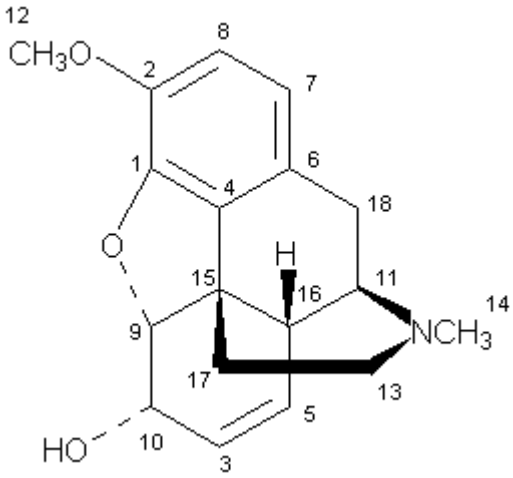


The red dotted lines show that 3 different carbons (11, 16 and 18) correlate to the same 4 Hs (11, 16, 18 and 18'). Those 4 Hs make up an isolated spin system.

The green dotted lines show that 2 carbons (13 and 17) connect to Hs 13', 17 and 17' (and C-13 also connects to H-13). CH_2s 13 and 17 are another isolated spin system.

The remaining 2 strong peaks are the methyl 1-bond correlations.

Assignment	**^{13}C**	**^{1}H**
3	133	3 and 5
5	128	3 and 5
7	120	7 and 8
8	113	7 and 8
9	91	9
10	66	9
11	59	11, 16, 18, 18'
12	56	none
13	46	13, 13', 17, 17'
14	43	none
16	40	11, 16, 18, 18'
17	36	13'. 17. 17'



18	20	11, 16, 18, 18'

The sample is 3.3 mg codeine in ~ .65 ml $CDCl_3$

Acquisition Parameters:

512 complex points in direct dimension
128 t_1 increments
16 scans
?ms mixing time
2 sec. relaxation delay
Total acquisition time: ~ 1.25 hrs

Processing:

sine squared window function in both dimensions with 0 degree phase shift
2x zero-fill in the indirect dimension
magnitude calculation (no phasing is required)
final data size 512 x 512

See also: detailed description of 2D processing, 3D HMQC-TOCSY

Interactive view - Those who have installed the Chime plug-in can view and manipulate the codeine structure in 3D, examine and expand the ^{1}H spectrum, and interactively correlate ^{1}H peaks with H atoms in the structure.

INADEQUATE

The acronym stands for Insensitive Natural Abundance DoublE QUAntum Transfer Experiment. (Not very informative!)

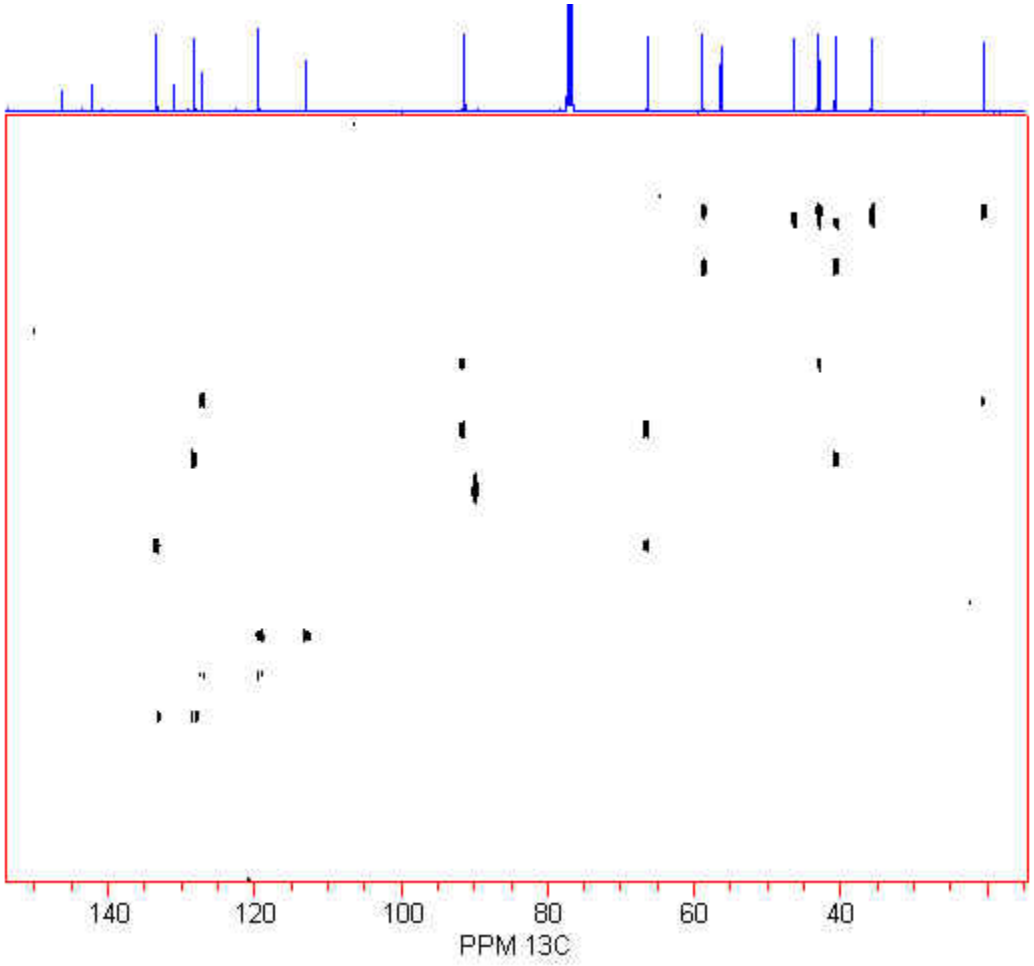
The experiment maps out the carbon-carbon connectivity via ^{13}C-^{13}C 1-bond coupling. With only 1% natural abundance, the probability of adjacent carbons being the ^{13}C isotope is 1% of 1%, or 10^{-4}.

The sample is 200 mg codeine in $CDCl_3$ with $Cr(acac)_3$ to reduce the relaxation time.

Connectivity is established by creating double-quantum (2Q) coherence from a pair of coupled carbons. The magnetization evolves during t_1 with frequency equal to the sum of the offset frequencies of 2 contributing carbons. The 2Q coherence is then mixed back into both of the 1Q transitions for detection, labeling both peaks in each pair with the

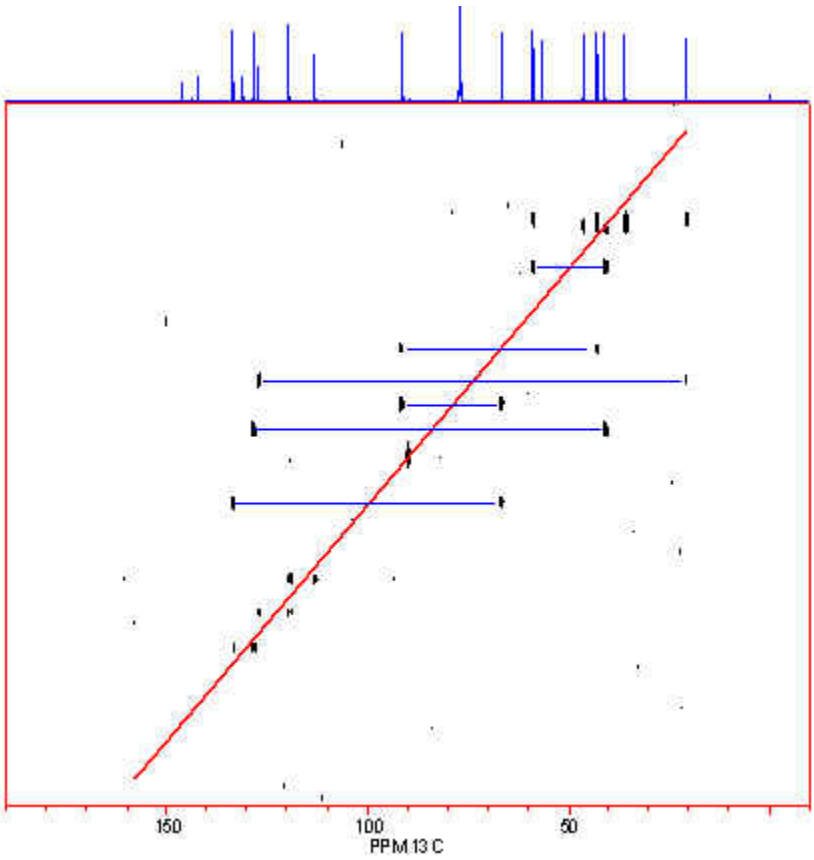
same 2Q frequency. Therefore, a pair of peaks appearing at the same 2Q frequency are coupled.

The 2D spectrum is plotted with the normal ^{13}C spectrum along the horizontal axis and the 2Q frequency along the vertical axis.



Each C-C connection results in a pair of peaks along a horizontal line. Pairs of C-C peaks are indicated by the blue lines. (Not all pairs are marked.)

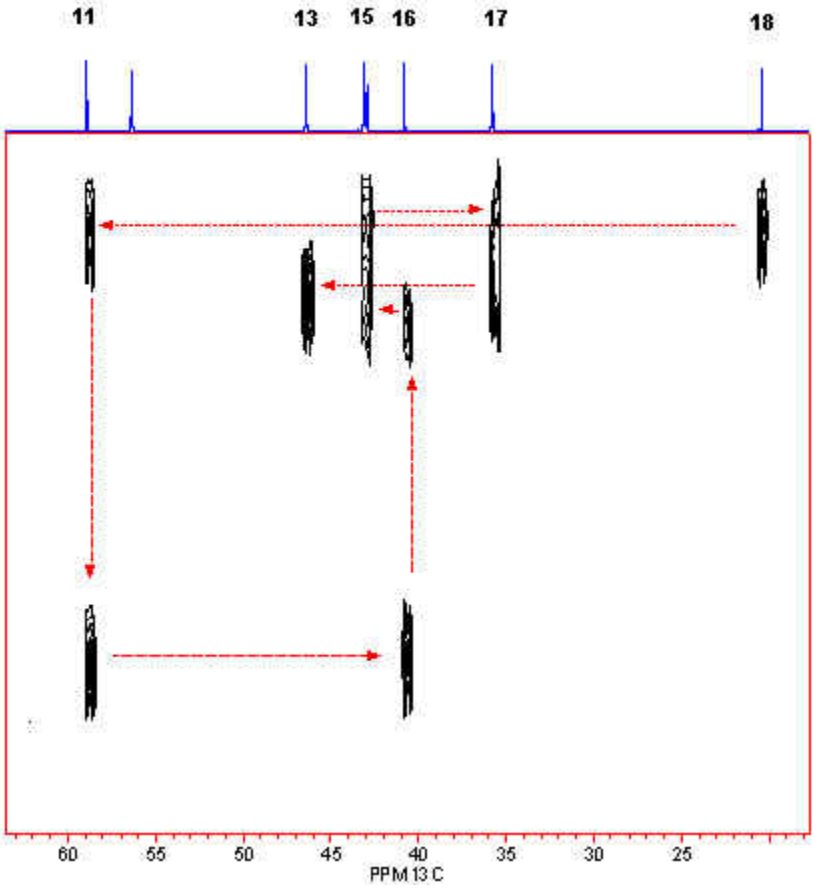
The center of each horizontal connecting line falls along a line, shown below in red, which is $f_1=2*f_2$ (where f_2 is the normal ^{13}C dimension, and f_1 is the 2Q dimension).



Because of the symmetry, connectivity can be established even if one of a pair of peaks is too weak to be observed. The signal-to-noise in this spectrum is not sufficient to see peaks from some of the low-intensity quaternary peaks. Missing are peaks 1-2, 1-4, 1-8, 4-6 and 4-15.

Note there is an artifact peak in the center of the spectrum, in both dimensions.

Part of the aliphatic region is shown below. The red arrows trace the carbon backbone, starting with C-18 in the upper right corner: 18 --> 11 --> 16 --> 15 --> 17 --> 13.



Acquisition Parameters:

2048 complex points in direct dimension
128 t_1 increments
350 scans
1 sec. relaxation delay
Total acquisition time: ~ 13.5 hrs

Processing:

window function was 10 Hz exponential linebroadening in both dimensions
1x zero-fill in the indirect dimension
magnitude calculation (no phasing is required)
final data size 2048x256

2D data processing and display

2D commands

This section describes the processing and display of 2D data. Topics and commands covered:

Quick intensity plots and contour plots
Setting the chemical shift reference
Setting contour levels
Setting colors for contour levels
Turning on/off gridlines
Selecting individual slices for display
Viewing slices
Summing slices
Creating projections
Placing projections or 1D spectra along the edges of a contour plot
Stacked plots
Symmetrize
Tilt
Transpose data
Save
Substitute one slice for another
Zero diagonal
Processing TPPI, hypercomplex and echo-antiecho data

See also:

Arrayed Mode processing with NUTS Professional version
Processing 2D data -- step-by-step processing descriptions for different types of 2D data
Examples of 2D spectra using codeine
Sample macros
Displaying 2D data
Setting chemical shift reference
Editing 2D data
comparing multiple 2D spectra
2D nomenclature

Macros have been supplied which perform the basic types of 2D processing (magnitude, TPPI and hypercomplex). These macros simply string together linked command sequences (Links) so that the entire processing can be performed with a single command. The user should have a basic understanding of the commands in the Links and the macros because some parameters vary from experiment to experiment or with different spectrometers. The macros have comments and, combined with the explanation below, should contain sufficient information.

IP -- Intensity Plot

If the currently displayed data is a 2D data set, this command displays the data as a two dimensional intensity map. NUTS can also draw contour plots (CP), which look nicer, but take longer to display. The intensity plot is faster because its speed is limited by the graphics display process, while the contour plot is calculation-limited. The intensity map is recommended for initial viewing of the data, determining the levels to be displayed, setting shift references and Zoom frequency limits, etc., leaving the contour plot for the final display, for viewing details and plotting. Display of 2D data is illustrated below.

The SS (set 2D scale) command should be executed before IP or CP so that the scale is reasonable for an initial display.

The levels of data represented by the different contour levels are determined as multiples of the MH parameter. For versions compiled after Dec, 2001, the contour threshold can be changed interactively in several ways. The coarsest adjustment is made using the scroll bar along the right edge of the screen. Page Up and Page Down keys, Arrow Up and Arrow Down keys and the "<" and ">" keys apply finer adjustments.

The MH value can also be set explicitly. To lower the first contour level to be displayed, type MH and set it to a smaller value.

The number of levels, the MH multiplier for each level and the color for each level are set in the NUTS.INI file. The maximum number of levels is 10. The multiplier for each level can be set while NUTS is running using the **LV** command. Contour level colors can be changed from within NUTS using the **CR** command. Changes made using LV and CR are not saved for future NUTS sessions. To make the changes persistent, edit the nuts.ini file.

NUTS provides the option of displaying horizontal and/or vertical projections or a 1D spectrum along each axis. These options are available from the Borders menu or by using the proj command. .

By default, both positive and negative contour levels are displayed. The user can switch to just positive by typing C+ (plus) or to just negative levels by typing C- (minus). To display both, type **C0** (zero). *(In versions of NUTS older than Sept, 2001, this is done with the single-letter commands +, - and 0, respectively.)* The colors for each level are set separately in the NUTS.INI file, or can be set within NUTS with the **CR** command.

The intensity map or contour plot can be interrupted before finishing drawing by typing **<escape>**. This command halts the Windows screen paint operation, allowing the user to change parameters such as frequency limits or minimum height, or to plot the spectrum, without having to wait for the 2D display to be completed. The mouse can be used to define a Zoom region even though the screen paint operation is incomplete, because NUTS "knows" where the peaks are. Once the parameter change is completed, the screen paint is re-started.

CP -- Contour Plot

If data set A is a 2D data set, this command displays the current data as a two dimensional contour plot. This is a nicer display than the intensity plot, but takes longer to draw. The intensity plot is recommended for initial viewing of the data, determining the levels to be displayed, setting shift references and Zoom frequency limits, etc., leaving the contour plot for the final display for viewing details and plotting. The colors and contour level spacing are set in the NUTS.INI file and cannot be changed after NUTS is started. See description of the NUTS.INI file for explanation of how these parameters are set.

Control-G – Display a grid on the contour plot

This command is a toggle, so executing it a second time will remove the grid. See also: Line List routine for putting lines on a contour plot.

2D – Enter 2D display mode (Intensity plot)

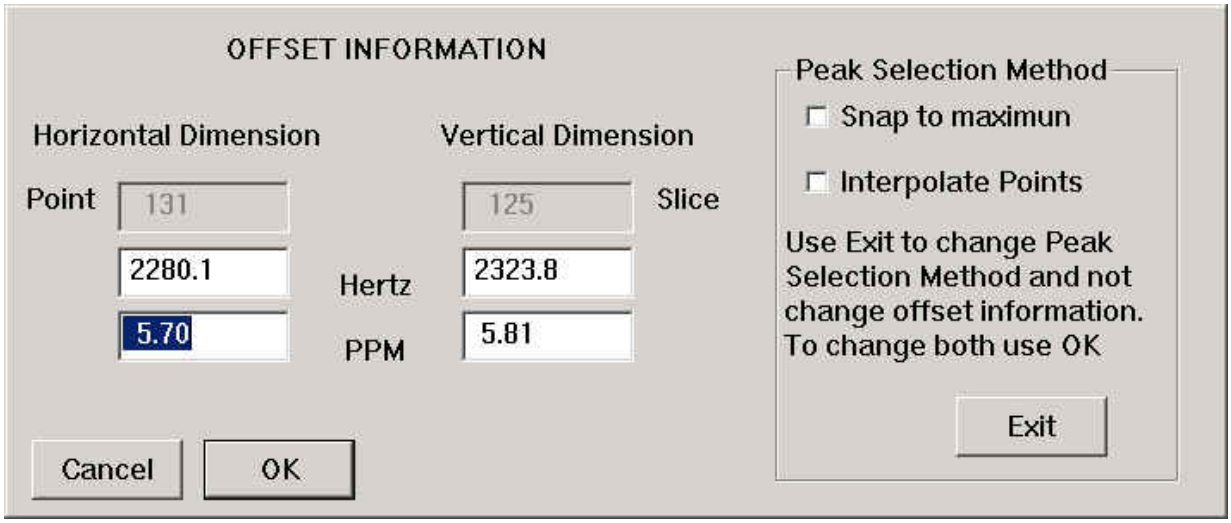
1D – Exit 2D display mode

C+, C- and C0 -- Display positive, negative or all contour levels

By default, both positive and negative contour levels are displayed. The user can switch to just positive by typing **C+** (plus) or to just negative levels by typing **C-** (minus). To display both, type **C0** (zero).

Setting the chemical shift reference

The coordinates of a peak in the 2D plot can be displayed by holding down the left mouse button, just as for 1D spectra. The cursor location is given in the lower right corner of the screen. The slice number and shift value in Hz and PPM is given for the vertical (2nd) dimension. The point number and shift value in Hz and PPM is given for the horizontal (1st) dimension. While holding down the left mouse button, typing **O** brings up a dialog box allowing the user to set the offset (chemical shift reference) in both dimensions.



Note that the 2 checkboxes on the right should ***not*** be checked when working with 2D plots.

Because of the poor digital resolution of most 2D data, and the fact that NUTS will read the cursor location as the nearest actual data point, setting chemical shift reference with the cursor will often result in the crosspeaks not lining up with the high resolution 1D spectra placed along the edges of the contour plot. The easiest way to fix this is to adjust the Offset parameters explicitly. The "offset" in each dimension is defined as the number of Hz from the center of the spectrum to 0 ppm. Using the cursor, you can determine the frequency difference, in Hz, between the crosspeak and the corresponding peak in the 1D "border" spectrum, best measured using an expanded display. Bring up the parameters dialog box by typing O1 (for horizontal dimension) or O2 (for vertical dimension). Change the appropriate offset parameter by the measured difference. If it is determined that the values of these corrections are always the same, this process can be automated using a macro.

After 2D parameters have been changed, use the **UH** (Update Header) command to save the changes. (**Caution**: When processing in Arrayed Mode, the UH command should NOT be used if any processing has been performed since the data set was saved, as the updated header will be written to the ***original*** file, and parameters may not be compatible.)

While holding down the left mouse button, press and hold the right mouse button. The slice corresponding to the cursor location is displayed. As the mouse is moved vertically, the slice is updated in real time. The Page Up and Page Down keys can be used to scale the slices. Users with a single-button mouse can press the period key in place of the right mouse button.

Two dimensional Zoom works in the same way as for 1D spectra. Typing **ZO** or double clicking the left mouse button enters the Zoom routine, indicated by the cursor changing to a small crosshair labeled "ZO". Hold down the left mouse button and drag the mouse

to highlight a region for expansion. Typing **control-E** or clicking the right mouse button jumps to expanded display of this region. **Control-F** and **control-E** toggle between fill and expanded display, as does clicking the right mouse button. Typing <Enter> exits the Zoom routine.

Exit the 2D display mode by typing **1D**. *(In versions older than Sept, 2001, 2D display is exited by typing <ENTER>.)*

Outside of the 2D display mode, individual slices can be displayed by specifying the slice number with SL, and the View (VW) command allows stepping through slices. To view slices in the second dimension requires that the data set first be transposed with the TD command so that the dimension of interest becomes the horizontal dimension.

2D Peak Picking and 2D Volume Integration are performed within the Define Peaks (DP) subroutine.

LV -- Set contour levels

This command allows the multiplier for each level to be set while NUTS is running. These changes are not saved when NUTS is closed. Permanent changes must be made in the nuts.ini file, along with the number of levels and the color for each level. Note that the maximum number of levels is 10. See also 2D display.

CR -- Set contour colors

This command allows the color for each level to be set while NUTS is running. Colors are set as numerical Red, Green and Blue values. See Setting Colors. Changes made with CR are not saved when NUTS is closed. Permanent changes must be made in the nuts.ini file, along with the level multipliers for each level.

Ctrl-G -- Toggle on/off display of grid lines

This command will display gridlines on an intensity or contour plot. The command is a toggle, so entering it a second time will remove the gridlines. The lines are drawn at the position of the major tick marks on the axis, and cannot be moved or their spacing changed by the user. Users who want gridlines displayed always can add a line to the nuts.ini file which is
GRIDLINES = TRUE
which makes display of gridlines the default condition. (Gridlines are shown on 1D data as well, but this is probably not very useful.)

SS -- Set 2D data Scale

Examines all slices of a 2D data set and sets the automatic scaling factor which is used in commands such as View data (VW), stacked plots (SP), intensity plots (IP) and contour plots (CP). SS should be performed before attempting to display 2D data to get

reasonable scaling. This command also identifies the largest positive and negative peaks in the data set and sets their difference to 100%. This value is then the basis for the Minimum Height (MH) parameter, which is used to set the scale for 2D Intensity Plots (IP).

SL -- Set Slice

This commands allows the user to select which slice of a 2D data set will be available for processing. When a 2D data set is read by NUTS, the first slice is displayed and is available for data processing. The user should avoid saving a slice as a file under the same name as the 2D file (Save command from menu or SA command) as the 1D file will replace the 2D data set. Use the Save As menu command or SB keyboard command to save the processed 1D file under a different name.

VW -- View 2D data slices

Steps through slices of a 2D data set. Set starting slice with SL. While in Arrayed Mode and in the View routine, subcommand **Z** will zero all data points in the currently displayed slice.

To view a series of 1D files, they must first be converted to a 2D file. See description under Stacked Plot command.

Subcommands:

I or **N** - Increment to next slice
D or **R**- Decrement to previous slice
Z - Zero the displayed slice.
<Enter> - exit VW

SU or sum -- Sum slices

Sums a specified range of slices of a 2D data set. By default, all slices are summed. If executed in the "non-2-letter" command mode, it is possible to specify a subset of slices. The command takes 2 arguments, which are the first and last slices to be included in the sum. For example, this command would sum slices 5 through 20, inclusive:

```
sum 5 20
```

Note that the sum becomes the current data (1D) set. If in arrayed mode, save the data before performing the sum. See sample macros in the section on Automated Processing.

PJ -- Projection

Calculate the "skyline" projection of the current 2D data set along the horizontal axis. The projection consists of the largest value in the 2D data set at each data point. When

the procedure is complete, the projection is displayed as a 1D file which can be saved with **SA**. To return to the 2D data set, it must be reopened with **GA**.

A projection can be created from a subset of the entire data set by first expanding the displayed region using Zoom. The desired limits can be entered using the F subcommand within Zoom or using the mouse. Use Ctrl-E to display the expanded region. PJ will create a projection of the displayed region.

Placing projections on contour plots

NUTS provides the option of displaying a horizontal and/or vertical projection along any side of a 2D intensity or contour map. These projections are automatically scaled such that the tallest peak in the displayed region is set to a height of one-tenth of the total display size. Display of projections along the Top, Right, Bottom and Left sides of the plot are toggled on/off with the commands **P1**, **P2**, **P3** and **P4**, respectively. *(Note that in versions of NUTS older than Sept, 2001, this is done with the single-letter commands **1, 2, 3** and **4**.)* These commands are also available from the Borders menu, or from the command line (see below).

The projections can be scaled independently by choosing Edit Display Parameters from the Edit menu, which allows a scaling factor to be entered for each projection. (Note that this same dialog box allows margins to be set for each side of the 2D plot. Values are entered as fraction of total display.) Each projection can also be clipped, allowing the vertical scale to be increased to show small peaks while limiting the height of larger peaks. This option is available from the Display menu. As always, what is displayed on the screen is what will be plotted.

Using high resolution spectra as projections

It is often preferable to use a high resolution 1D spectrum instead of the actual projections due to the better digital resolution. The 1D spectrum must already exist, saved to disk, and must have been processed. The file is selected from the Borders menu. The 1D file need not have exactly the same spectral window as the 2D plot. The appropriate region will be displayed, based on the chemical shift scale.

Scaling and clipping are performed in the same manner as for actual projections, described above.

Once a 1D file has been selected in this manner for use as a projection, the commands **P1**, **P2**, **P3** and **P4**, respectively will toggle on/off display of the projection on top, right, bottom and left sides of the plot. *(Note that in versions of NUTS older than Sept, 2001, this is done with the single-letter commands **1, 2, 3** and **4**.)* If it is desired to display instead the actual projection, rather than a separate spectrum, the file must be removed from this projection "buffer" with the XC command.

These can also be defined from the command line or in a macro.

PROJ (or PROJECTION) – specify projection file and position

This non-2-letter command allows display of projections on the edges of contour plots to be turned on and off, and also allows a file to be specified for use as each projection. The command takes 2 or 3 arguments. The first argument defines which side projection is the focus of the command. Allowed 1st arguments are:

Top Projection

-1
-top
-t

Right projection

-2
-right
-r

Bottom projection

-3
-bottom
-b

Left projection

-4
-left
-l

The second required argument determines whether or not the specified projection is shown:

-on
-off

A third optional argument defines a file name to use for the specified side projection spectrum.

XC -- Clear projections

Removes any file which has been defined for use as a projection on the edges of a 2D plot.

SP -- Stacked Plot

Plots slices of a 2D data set on the screen. A series of 1D spectra can be plotted if they are first stored as a 2D file (see instructions).

Before executing **SP**, read in the first slice of the 2D file with **GA** and use SS to set the scaling. The entire plot can be scaled vertically in the same ways as 1D spectra. The vertical scale can also be set by typing **A** or from the Display menu, which brings up the Amplitude Change dialog box (same as AC command). Press Enter to exit from the stacked plot.

The use of SP is illustrated in the section on Advanced 1D processing.

When SP is executed, a new set of menu choices is displayed. Under the Display menu is an option to whitewash the stacked plot. The command is a toggle, so executing it a second time undoes the whitewash. Equivalently, whitewash can be toggled by typing **W**. Depending on the type of data, whitewashing may or may not be desirable. The whitewashed spectrum contains many more "draw" operations, so will take longer to draw on the screen and to plot. In most cases, a whitewashed plot is too large to copy as a metafile to other applications, and it must be copied as a bitmap instead. (See copying spectra for further information).

The offsets in both x and y dimensions can be changed by typing **O** or choosing Offset from the Display menu. The X-offset can be changed from 0 to 100% and the Y-offset from 1 to 10. These offsets can also be set in the Nuts.ini file, so that the user's preference is always set as the default.

Because drawing the stacked plot can be slow, the draw operation can be terminated by typing **Q**.

A subset of the entire data set can be plotted by using 2D Zoom from an intensity plot (**IP**) or contour plot (**CP**) to select a region to be plotted. From the 1D display of a slice, type **ZO** to enter Zoom, then **F** to bring up the dialog which allows setting of frequency limits for expansion. The limits **must** be set in **both** dimensions by points, Hz or PPM. Failure to set the limits in the vertical dimension will result in only 2 slices being displayed. Click OK to close the dialog box. Type **Ctrl-E** to expand to the limits you have set. Now typing SP will display a stacked plot of the selected region. To plot the entire spectrum, first type Ctrl-F to select the full display.

Note that it is necessary to explicitly set values for the first and last slice to be displayed, followed by **Ctrl-E**, and that this operation needs to be repeated if the horizontal expansion is changed. Failure to do this will cause NUTS to display only 2 slices.

Once the stacked plot is displayed on the screen, it can be plotted with PL.

SY -- Symmetrize 2D data set

This is applied to symmetrical homonuclear 2D data, such as COSY experiments, in which the data are ideally symmetrical about the diagonal, f1=f2. Symmetrization is used to remove artifacts from the 2D spectrum to improve its appearance. Noise and other artifacts which do not occur symmetrically on both sides of the diagonal are eliminated. The data set must be square (that is, the same number of points in both dimensions).

The SY command examines the entire 2D data set, comparing each point to its symmetrically related partner across the diagonal. Then both of these points are replaced by the smaller (absolute value) of the 2 points.

This process can take a minute or more, depending on the size of the data set. It also involves multiple TD operations, saving temporary files in the process, so there must be sufficient disk space available.

Ref: R.Bauman, G.Wider, R.R.Ernst and K.Wuthrich, **J.Magn.Reson.**, **44**, 402-406, 1981.

TD -- Transpose Data

This command is available only in the 2D and Pro versions of NUTS. The TD command swaps the 1st and 2nd dimensions and their associated parameters. See also: processing 3D data.

SC -- Save dataset C

Used in macros or links. This command asks for a new file name for File C and saves the current data to File C as the first slice of a 2D data set. Subsequent SC commands save the current data set to File C as successive slices of a 2D data set. When this command is used in a Link (the preferable method), File C is closed when the link finishes. When this command is used manually, the operator must use the Close File C (CC) command when finished writing to file C. An opened File C will be closed automatically when the NUTS program is exited.

When used in a Link, the SC command asks for the name for File C on the first pass only. On each additional pass through the link, the SC command saves the current data set to the already opened File C as the next slice.

UH -- Update Header

Writes parameters into the header of a 2D file. This should be executed after parameters have been changed to insure the changes are saved with the file. For example, after setting the chemical shift reference or after editing the variable delay list for relaxation data, UH will save the changes.

Caution: When processing in Arrayed Mode, the UH command should NOT be used if any processing has been performed since the data set was saved, as the updated header will be written to the ***original*** file, and parameters may not be compatible.

S2 -- Save 2D data set

(Used in non-arrayed mode only)

This command can only be executed when the current data file A is a 2D data set. A dialog box prompts for a file name. The command functions differently depending on whether a new file name is supplied or the current file name is chosen. If a file name different from the current file A name is entered, the entire 2D data set is copied into the new file exactly as it currently exists on disk and the new file becomes data file A. If the file name supplied is the same as the current file name, the entire 2D data set is saved as it currently exists on disk EXCEPT for the currently displayed slice, which is saved as it is currently displayed. This allows modifications on individual slices to be saved permanently.

One situation for which this is useful is for touch-up phasing of a series of kinetics or relaxation data following automated processing. The 1D spectra must first be converted into a 2D data set.

Another use is when one slice of a 2D data set gets corrupted. A simple way to "fudge" the data so that it can be displayed without artifacts is to overwrite the corrupted slice with its neighboring slice, since adjacent slices should be similar. Let's assume that slice 10 is corrupted and we will replace it with a copy of slice 11. To do this, display slice 11 and place it in the Add / Subtract buffer by typing **AL**. Be sure that the Add / Subtract multiplier (**AM**) is set to 1, its default value. Next, display slice 10 and zero the entire slice by typing **ZE**. Then type **AS, +** and <Enter> to add the contents of the Add / Subtract buffer (which is slice 11). Slices 10 and 11 are now identical. With the modified slice 10 displayed, use the **S2** command to save the modification, specifying the file name to be the same as the current file name. This entire procedure must be repeated for each slice which needs to be modified, because the S2 command updates only the currently displayed slice.

In arrayed mode, simply use SA (or File/Save) to save the file.

Substitute -- Slice substitution

(Arrayed Mode only)

This command allows one slice of data to be copied over (replace) another slice. This is useful when a slice has been corrupted. Note that this requires NUTS to be placed into the "non-2-letter" command mode (with 2F).

For 2D data,

substitute target_slice source_slice

For 3D data, it is possible to perform this slice substitution either for a single plane of the 3D data, or for all planes. To operate on a single plane, the plane is specified as the first argument:

substitute plane target_slice source_slice

If the substitute command has just two arguments, then all planes of a 3D data set will have the source_slice copied to the target_slice.

ZeroDiagonal

A non-two-letter command which takes either one or two arguments. This is used with homonuclear 2D data to eliminate (zero out) all data points including and around the large diagonal peak.

If only one argument is given, then the command takes a 2D data set and zeros the diagonal over a range which is plus and minus the number of points specified by the argument. If two arguments are given, then the 2D data set's diagonal is zeroed plus the first argument number of points and minus the second argument number of points. Usage:

ZeroDiagonal [+ points] [-points]

See also: Removing the dispersion component of a residual solvent peak.

TL (or Tilt) -- Tilt of 2D data

(arrayed mode only)

TL (or Tilt) is used to rotate a 2D data set, such as J-resolved data.

The default rotation is a counter-clockwise rotation of 45 degrees, based on Hz, so NUTS first calculates the Hz/point in both dimensions.

In the non-2-letter command mode, arguments can be supplied to apply the tilt differently.

An argument of **C** or **CC** is used to define the direction of the tilt to be clockwise or counter-clockwise, respectively.

An second argument is taken as the number of points to tilt per slice (fractional points allowed).

If the TILT command is given with an argument which is not **C** or **CC** then the argument is taken as the number of points to tilt per slice (fractional points allowed) and the tilt will by default be in the counter-clockwise direction.

For example,

```
tilt c 0.1
```

would rotate clockwise 1 data point every 10th slice.

An invalid first argument is used as the number of points to shift and any second argument is ignored. The center slice (slice number_of_slices divided by 2) is taken as the center and is left unshifted.

TPPI -- Time Proportional Phase Incrementation

TPPI is a method for achieving quadrature detection (distinguishing positive and negative frequencies) in the indirect dimension of a 2D experiment. This allows data to be acquired as phase sensitive.

Processing TPPI data requires a ***Real*** Fourier transform (as opposed to the normal complex FT).

A detailed explanation of a macro for processing TPPI data is available.

Hypercomplex 2D data

(method of States, et. al.)

An example of processing hypercomplex data is available, including step-by-step explanation, and sample macros.

The alternative to TPPI for obtaining phase-sensitive 2D data is to acquire 2 FIDs (with a 90 degree phase shift; ie., a quadrature pair) for each t_1 point. After FT of each FID in the first (t_2) dimension, the imaginary part of each is discarded and the real parts are combined to form real and imaginary halves, and a complex FT is performed in the second (t_1) dimension.

NUTS uses the commands TR and TI ("tag" real and imaginary halves, respectively) to select which half of the data will be saved. (Usually, only TR will be used to save the real half of each spectrum. However, the TI command is provided in case the need arises to save the imaginary half. This could be the case depending on how the phase cycling was done in the experiment.) These commands are followed by the command ST (save "tagged" data) instead of the usual SC command. This is done in processing the first (t_2) dimension only. The Link for the first dimension processing then looks like

GA BC EM FT PS TR IA GA BC EM FT PS TR ST IN

The first time TR (or TI) is encountered, the selected part becomes the real half of the final complex pair. The second time one of these commands is encountered, the selected part becomes the imaginary half of the complex pair. The ST command then saves the data as a complex interferogram in t_1. The IA (Increment counter for file A) command must be included before reading in the second FID.

The above Link is appropriate for data which have been saved as a single file, with the pairs of FIDs occurring sequentially. Some spectrometers save the 2 halves of the data as 2 separate files. For this case, the Link for processing in the first dimension must be modified as follows:

GA BC EM FT PS BC TR GB BC EM FT PS BC TR ST IN

so that slices are read alternately from the 2 different files, designated as A and B. Or, use the interleave command to form a single file.

The second dimension processing is normal, using FT to perform a complex transform. If phasing is needed in the second dimension, PS can be included in the link or performed after, using a link such as

GA PS SC IN

performed after the appropriate phasing parameters are determined.

Interleave

(Arrayed Mode only)

This is a non-2-letter command which interleaves two data sets into a new data set. This is used for hypercomplex data that was saved on the spectrometer as 2 separate files, creating a single file containing all the data. This command works only for the Complex Arrayed Mode. The two data sets must have the same number of points and slices. The resulting data set is not automatically saved. Syntax:

Interleave FileName1 FileName2

TR -- Tag Reals

Used in processing hypercomplex (States type) 2D data.

The TR command is used to discard the imaginary half of each pair of spectra acquired for a given t1 value. The real halves are then used to construct a complex t1 interferogram. The ST command then stores the interferogram as complex data ready to be processed with a complex FT in the t1 dimension.

For complete description and examples of use, see hypercomplex (States type) 2D data.

TI -- Tag Imaginaries

Selects the Imaginary half of the data to be saved and discards the Real half. Intended for use in processing hypercomplex (States type) 2D data. (N.B. Normally, the TR (Tag Reals) command is used, but TI is provided to give the user maximum flexibility in data processing.)

ST -- Store Tagged data

Used in conjunction with the TR (tag reals) (or TI (tag imaginaries)) command to process hypercomplex (States type) 2D data. The TR command is used to construct a complex t1 interferogram. The ST command then stores the interferogram as complex data ready to be processed with a complex FT in the t1 dimension.

For a complete description and example of use, see hypercomplex (States type) 2D data.

The next 4 commands work only in Arrayed Mode, and on data sets having an even number of slices. These commands facilitate processing of echo-antiecho gradient data which must be processed by adding or subtracting pairs of slices. (An example is Varian g_hsqc data, which uses the C2 command.) Each command operates on a pair of slices, n and n+1. The real halves of the 2 slices are either added or subtracted, and the sum becomes the real half of a single processed slice. The imaginary halves of the 2 slices are either added or subtracted, and the sum becomes the imaginary half of the processed slice. So, in each case, the processed data has half as many slices as it started out with.

Echo-antiecho 2D data

These phase-sensitive gradient experiments are run such that each slice contains both sine and cosine terms in t_1. To process these data, we FT and phase slice 1 and slice 2, then calculate the sum and the difference of them. Because the 2 slices are, respectively, (cos t_1 + *i*sin t_1) and (cos t_1- *i*sin t_1), the sum gives you just cosine, and the difference gives you just sine. Together, they comprise a complex "FID" in the indirect dimension.

This add/subtract process is handled with command C2 which works in Arrayed Mode only, but can be accomplished in non-arrayed mode using a combination of commands. See details.

C1 -- Combine Mode #1

This command adds the reals and imaginaries of even and odd slices and gives back a data set with half the number of slices.

C2 -- Combine Mode #2

This command adds the reals and subtracts the imaginaries of even and odd slices and gives back a data set with half the number of slices. Used in processing echo-antiecho data.

C3 -- Combine Mode #3

This command subtracts the reals and adds the imaginaries of even and odd slices and gives back a data set with half the number of slices.

C4 -- Combine Mode #4

This command subtracts the reals and subtracts the imaginaries of even and odd slices and gives back a data set with half the number of slices.

GC -- Get data set C

Opens the dialog box for loading a file. The last name used for File C, if any, will be the default selected name. Data set C is used in 2D processing. This is rarely used, as GA is "smart" enough to detect when the file is 2D.

OC -- Open file C

(rarely used) This command allows the user to open a 2D file and move to the last slice of data. Further Save to file C (**SC**) commands add the currently displayed data set to the end of file C. When finished the user should close file C with the **CC** command.

The better way to accomplish the same thing is with a Link which contains SC and IN commands, and automatically takes care of opening and closing the 2D file.

CC -- Close file C

(rarely used) If a File C has been opened for writing data into, this command closes File C. This could be used to manually combine 1D files into a 2D data set, but this is more easily performed with a Link such as GA SC IN. When using such a Link, the IN command takes care of closing File C when the complete 2D data set has been processed.

Contour plot display

When a 2D file is opened (with GA or from the File/Open menu), the first slice is displayed. An intensity plot (IP) or contour plot (CP) can be displayed. IP is much faster and should be used for an overview of the data, and to set frequency limits and contour levels. CP is used to view the data in detail.

In versions of NUTS newer than Sept, 2001, the command **2D** is used to enter the 2D display mode, and displays an intensity plot. Exit back to 1D display by typing **1D**.

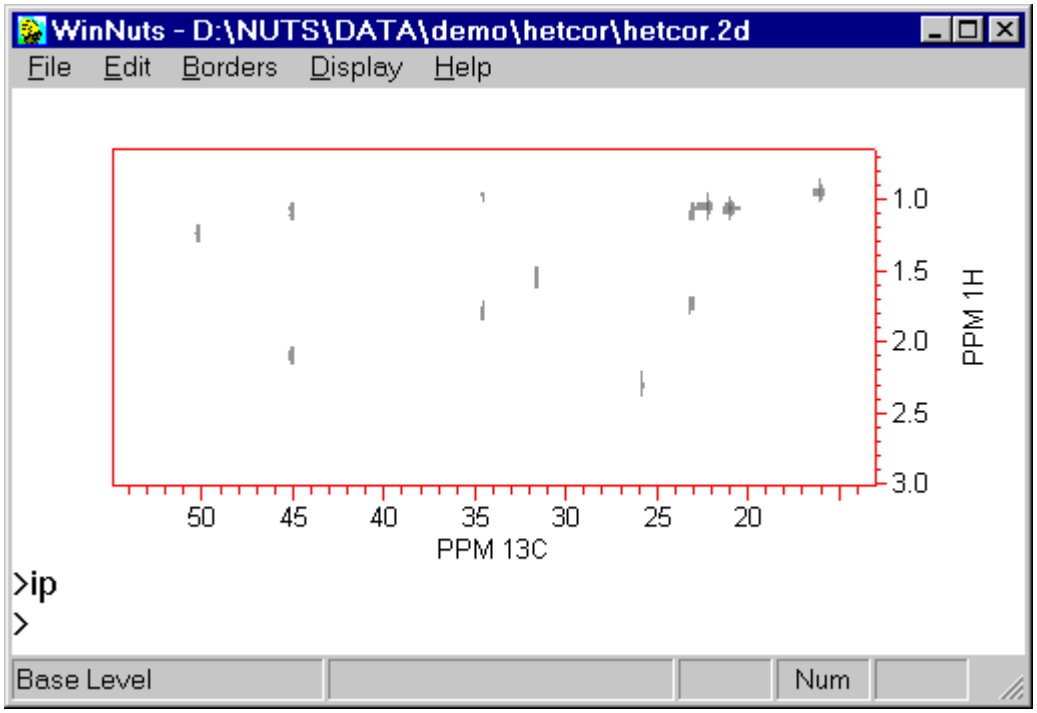
Contour levels are set as multiples of the Minimum Height (MH). The levels are set in the Nuts.ini file, but can be changed after starting Nuts using the **LV** (levels) command. The minimum height is expressed as a percentage of the tallest peak in the entire data set. So, the first step is to find the intensity of the largest peak and from that to set the display scale. This is done with **SS** (or Set Scale from the 2D menu).

Contour level colors are set in the nuts.ini file or can be set from within NUTS using the **CR** command.

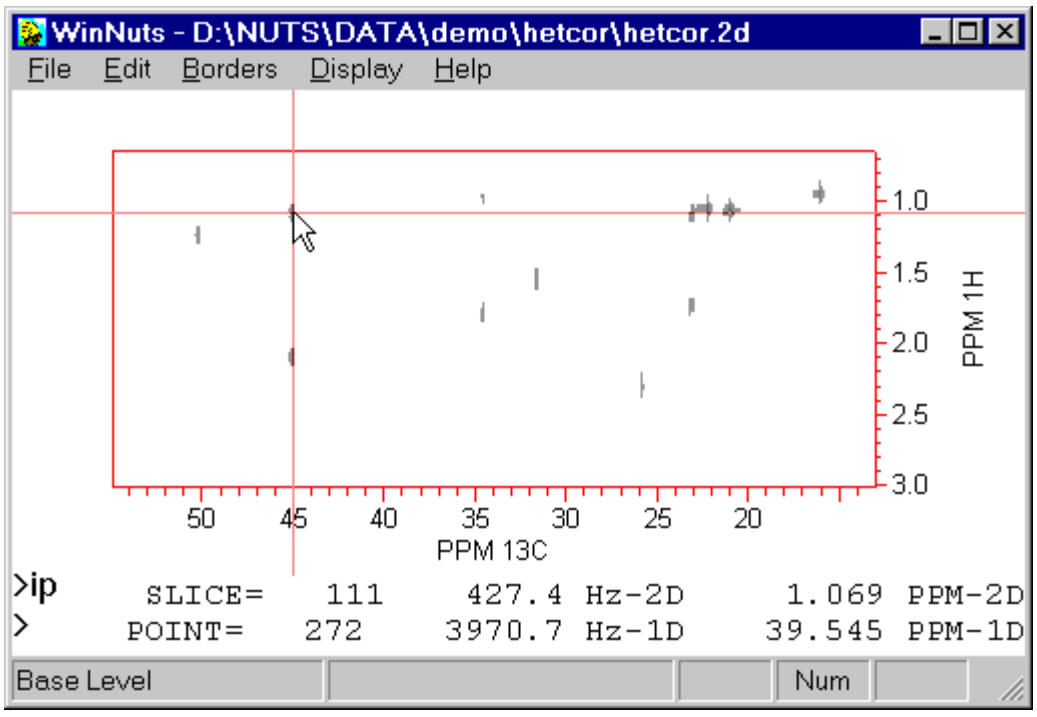
By default, the first contour level is at the minimum height, which the user can change by typing **MH**. The choice of this setting depends on the type of data being displayed. For HETCOR data, where all peaks are approximately the same intensity, a value of 10% is reasonable. For NOESY data, in which the crosspeaks are much smaller than the diagonal, the MH value can be as small as 0.1%.

Starting with December 2001, the MH value can be changed while the intensity or contour plot is displayed. This is done using a scroll bar displayed on the right edge of the screen, or with the PageUp, PageDown, Arrow Up, Arrow Down, "<" key and ">" keys. Due to the speed of contour plot recalculation, especially on older PCs or with large 2D data sets, this is best done in the intensity display mode or in a zoomed region if in the contour display mode.

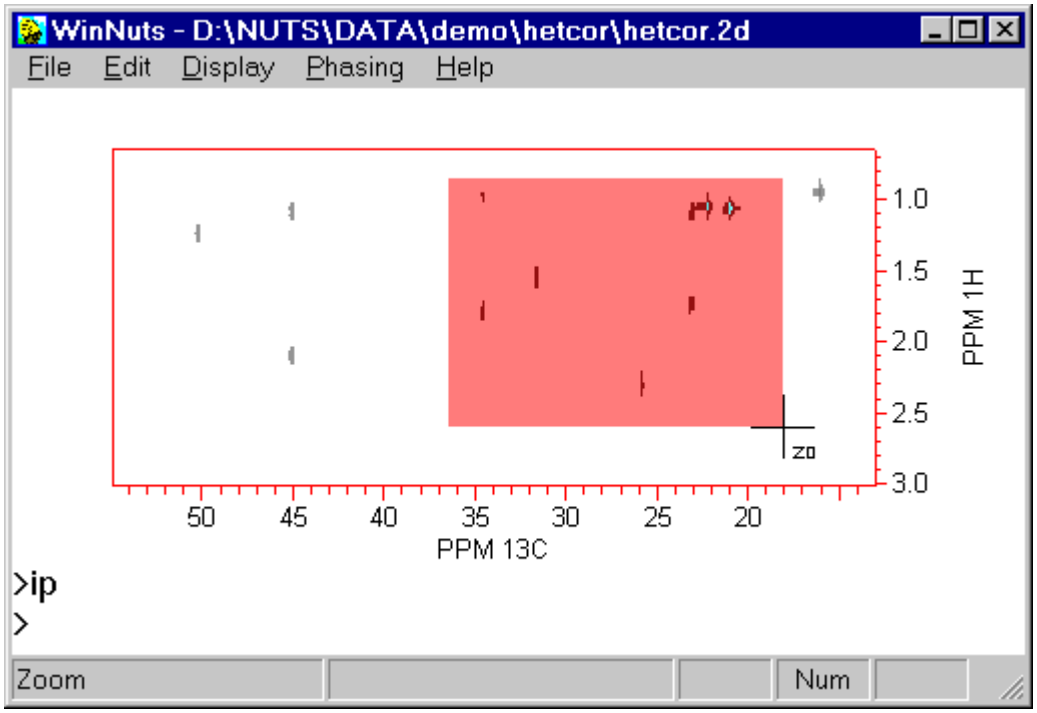
Text labels for the axes can be entered from the View/Spectral Parameters menu in the NUTS base level (not in the 2D display routine).



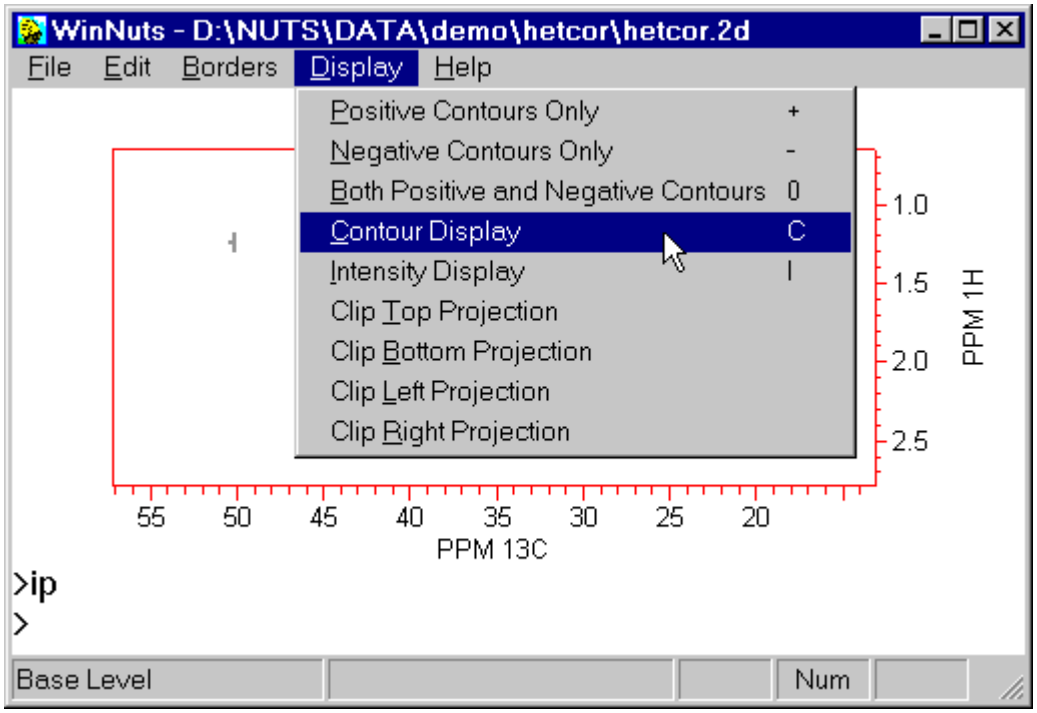
Display an intensity plot with the 2D or IP command (or from the 2D menu). Intensity plots are much faster than contour plots, so it makes sense to use that display mode for an initial look at the data. The size of the plot is adjusted by choosing Edit Display Parameters from the Edit menu, and entering a value for the margin on each side.



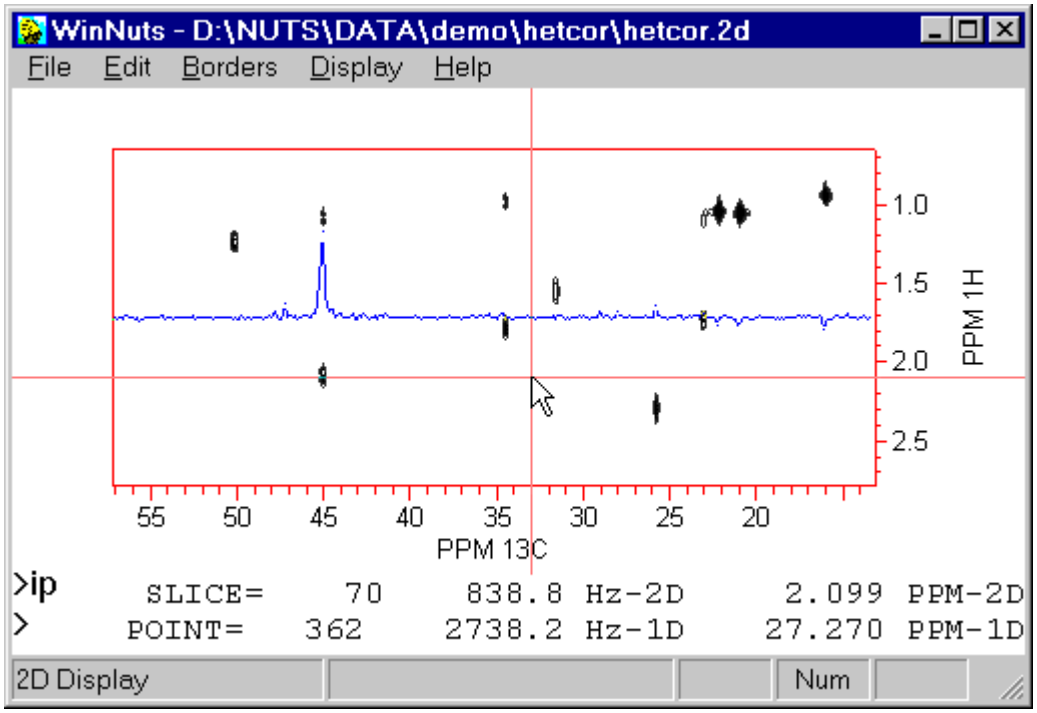
The chemical shifts in both dimensions can be displayed by pressing and holding the left mouse button.



The Zoom expansion routine can be used to display chosen regions. Type **ZO** to enter the Zoom routine, then press and hold the left mouse button and drag across the region of interest. Type **Ctrl-E** to expand to the chosen limits, or click the right mouse button.

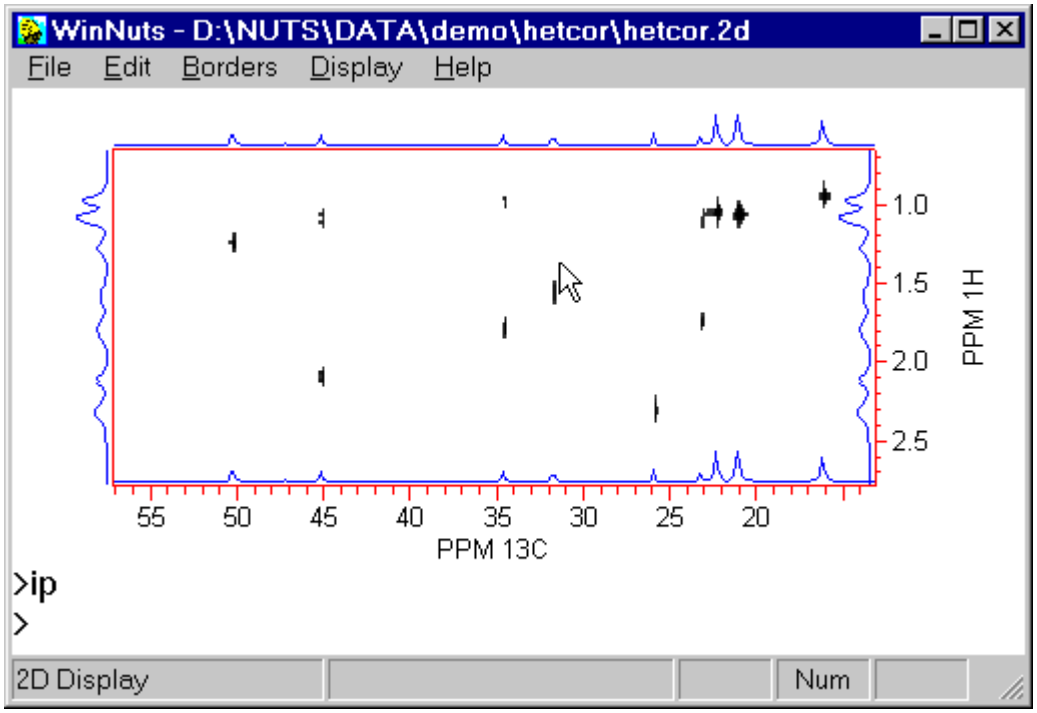


Change to contour plot, instead of intensity plot, by typing **CP** *(for versions of NUTS older than Sept, 2001, this is done with the single-letter command* ***C****).* Note the other options available from this menu.



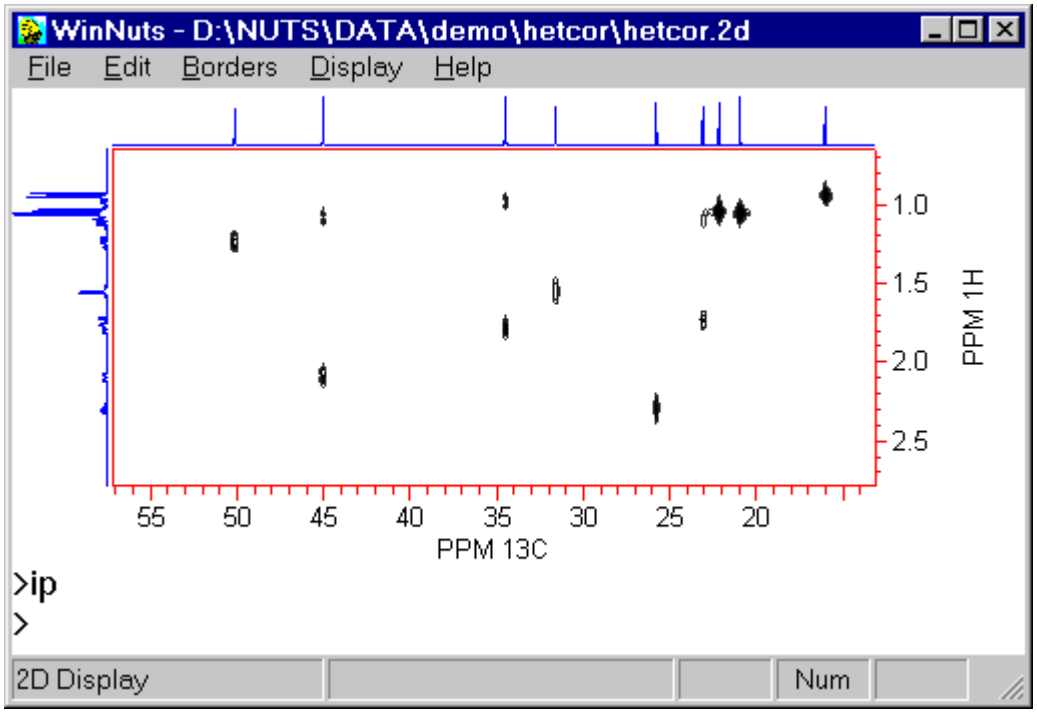
Slices can be displayed overlaid on the contour plot. Press and hold the left mouse button, place the cursor on a peak of interest and then press and hold the right mouse button also.

(Users with a single button mouse should press the period key on the keyboard instead of the right mouse button.)



Projections can be displayed along each axis from the Borders menu or using the commands P1, P2, P3 and P4 which toggle on/off display of projections along the top, right, bottom and/or left edges, respectively. *(In versions of NUTS older than Sept, 2001, this is done with the single-character commands 1, 2, 3 and 4.)* The vertical scale for each projection is set independently by choosing Edit Display Parameters from the Edit menu, and entering a multiplying factor for the chosen projection. If the projection contains both large and small peaks, it is possible to scale up the projection to see the small peaks, and then clip (truncate) the taller peaks. Clipping is turned on and off from the Display menu.

If high resolution 1D spectra exist, they can be displayed instead of the actual projection by selecting Pick the Bottom/Top/Left/Right Spectrum from the Borders menu. If such spectra have been defined, their display is automatically toggled on. As with the calculated projections, the vertical scale of high resolution "projection" can be adjusted by choosing Edit Display Parameters from the Edit menu. Either projection can be clipped, allowing small peaks to be seen while limiting the height of larger peaks. This is available from the Display menu.



The 2D plot can be resized by selecting Edit Display Parameters from the Edit menu. The Margin for each side is set independently, entered as fraction of total display.

Line list routine

LL – Line list

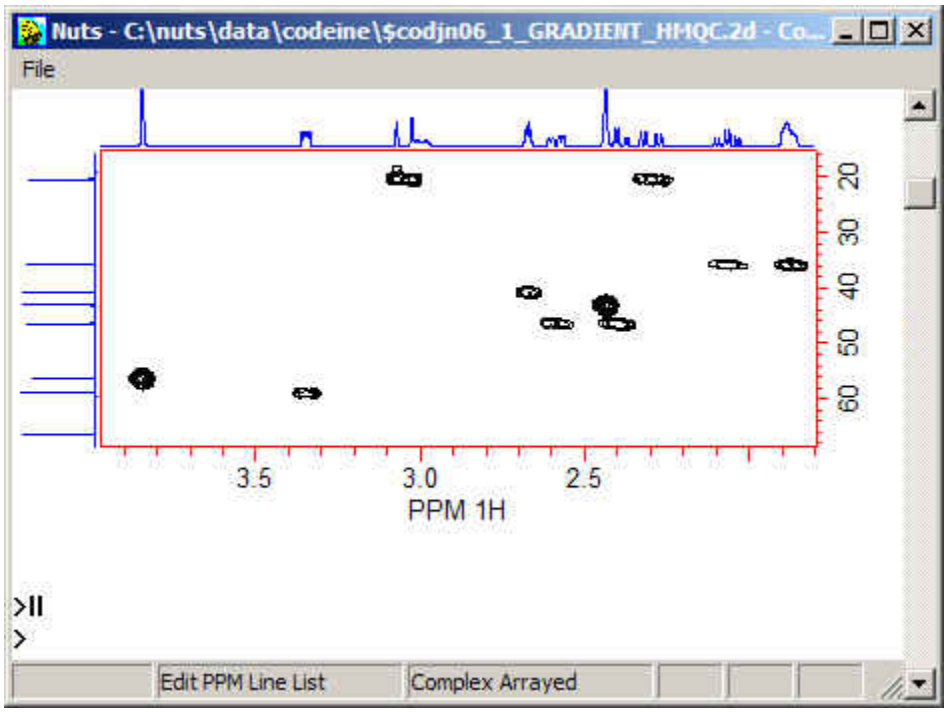
This is a subroutine within 2D display that allows vertical and horizontal lines to be drawn on the spectrum to aid in spectral interpretation. The lines are "attached" to the spectrum by ppm value, so the lines remain in place as the spectrum is zoomed in/out.

Lines can be placed on a 2D plot without entering the subroutine, but the subroutine allows the appearance of the line to be edited.

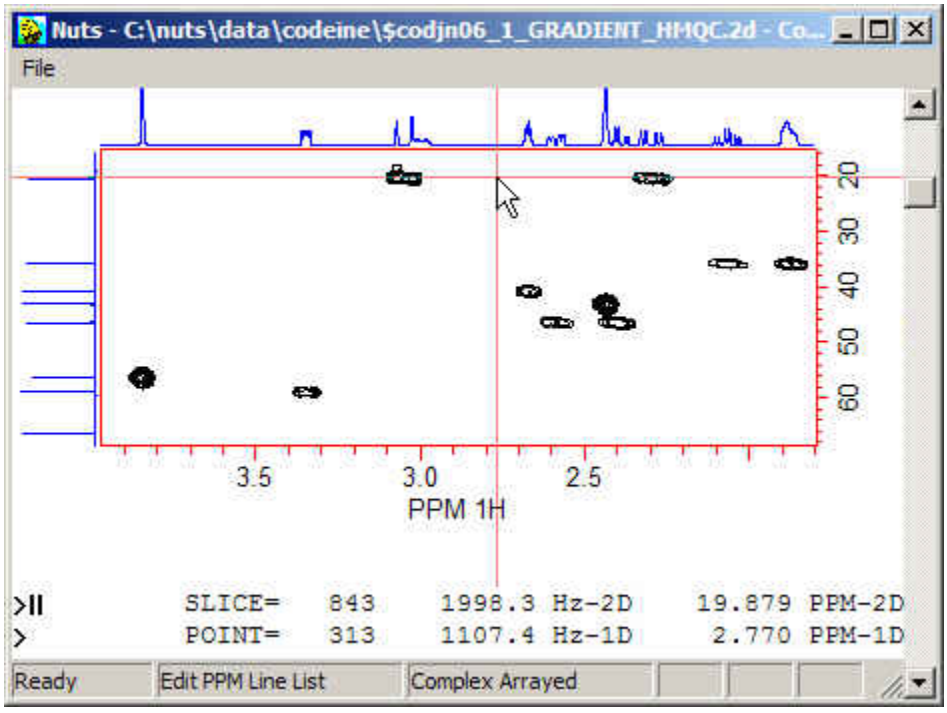
To add a line to a 2D plot, first press and hold the left mouse button to display a large red cross-hair cursor (referred to as the "target" cursor). While the target cursor is displayed, typing a **V** places a vertical line at the current target position, and typing an **H** places a horizontal line at the target position. **L** clears the last entered line and **C** clears all entered lines.

When the command **ppmlines** or **LL** is given from the 2D base level, the Edit Line List routine is entered. If the cursor is moved over a line, it will blink and that line becomes the selected as the current line. At this position, a right click or the key **E** will enter the mode to edit the properties of the current line. Typing the **D** key will delete the current

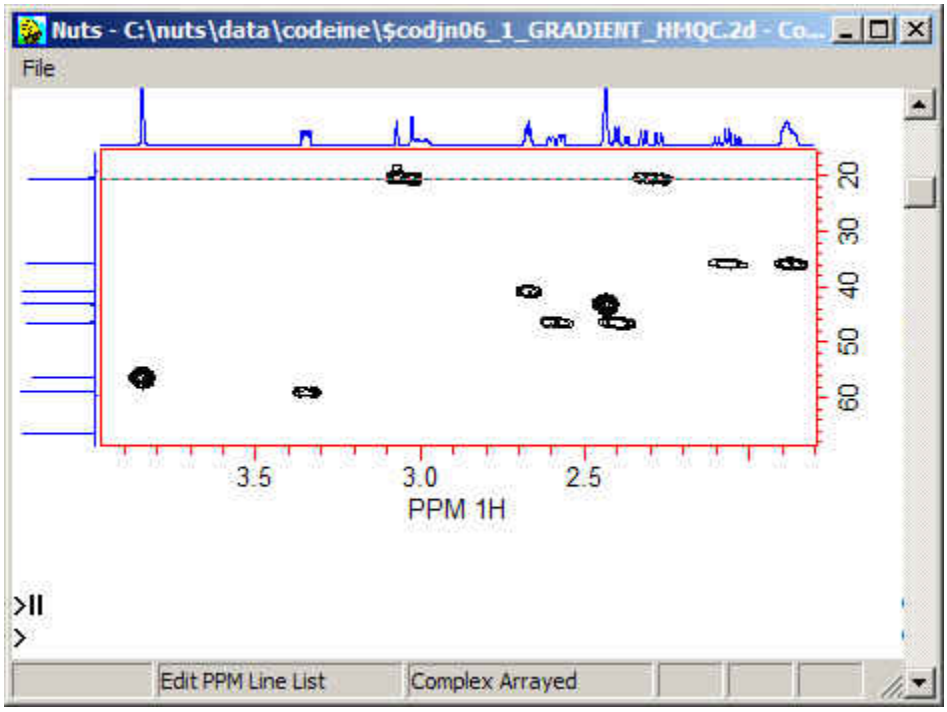
line and typing **C** will clear all current lines. To add more lines while in the LL subroutine, holding down the mouse button is optional, but the target cursor is helpful in choosing the desired line position. Place the cursor at the desired position, and type either **V** for a vertical line or **H** for a horizontal line.



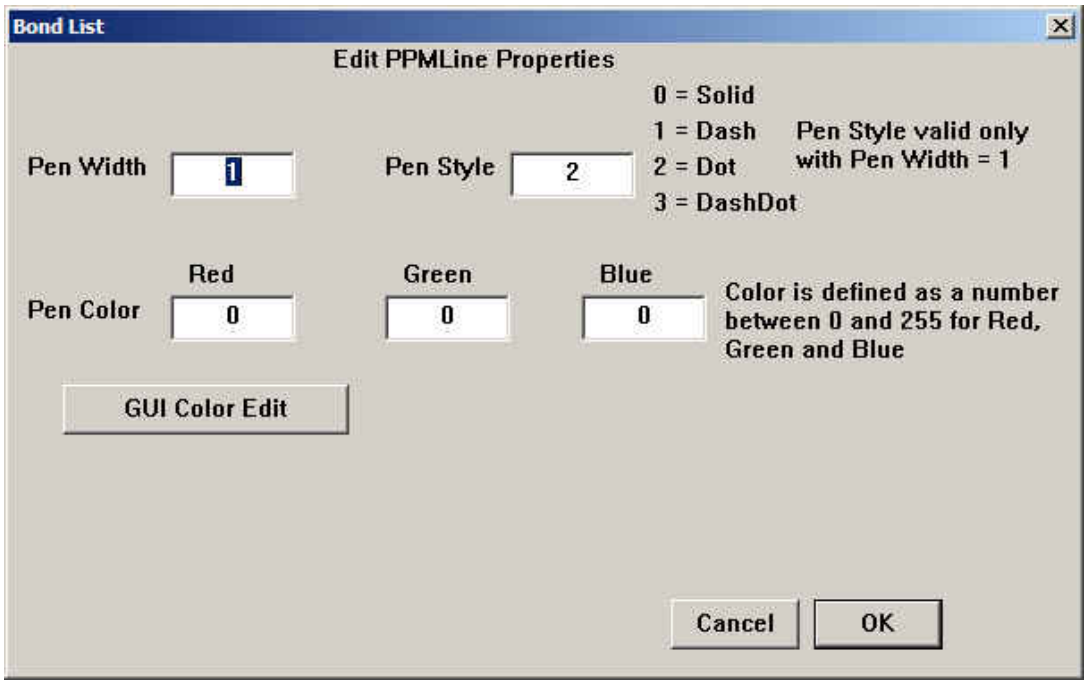
On entering the LL routine, note that the status bar shows Edit PPM Line List and the menus have changed. As with all subroutines, typing <ENTER> or choosing Exit from the File menu will exit the LL subroutine.



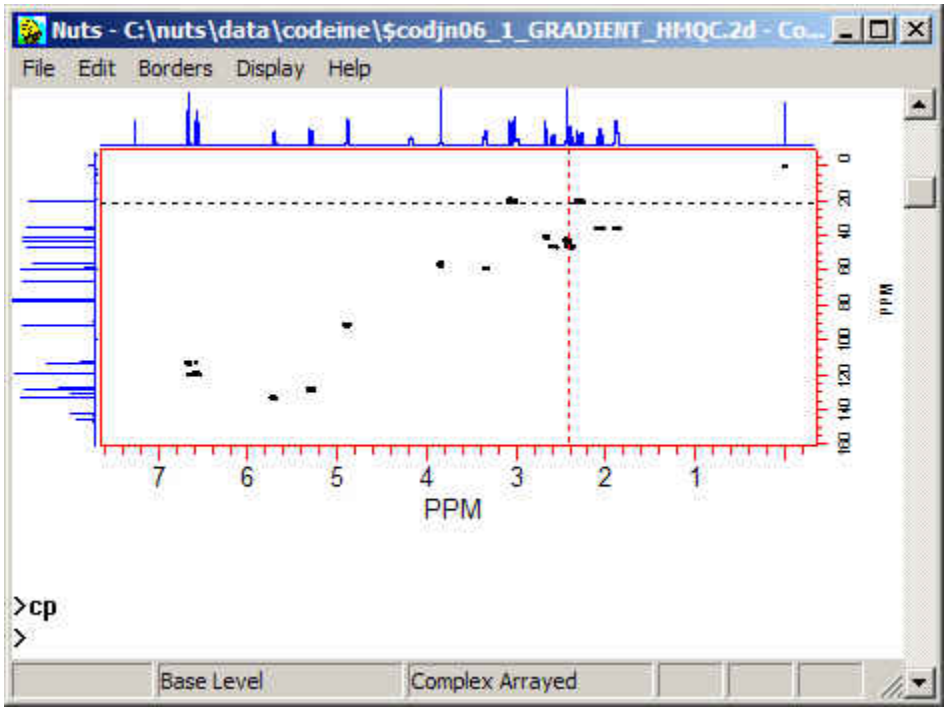
Use the "target" cursor to display a large cross-hair, to aid in positioning the line.



Typing **H** draws a horizontal line at the cursor position.



Selecting the line (by moving the mouse cursor across the line, causing the line to blink) and typing **E** displays a dialog box that allows the line's width, style and color to be set. Color is set using RGB values or by clicking the GUI Color button to select color.



The lines remain after exiting the LL routine, and continue to be displayed at the same ppm value even if the displayed region is changed.

Examples of processing

Processing 2D data

The user must have a basic understanding of how the data was collected to select the correct processing method. For example, just knowing that the experiment is COSY is not enough, because the data may or may not be phase-sensitive. The data types that can be processed with NUTS are magnitude, hypercomplex (method of States, et al), echo-antiecho, States-TPPI and TPPI. Each type must be processed differently.

Sample macros with detailed explanation is provided for those experiments for which we have sample data. We will create macros for the others if sample files can be supplied. Contact us for help in creating customized macros.

A recommended reference is T.D.W.Claridge, "*High-Resolution NMR Techniques in Organic Chemistry*", 1999.

Step-by-step instructions for:

Magnitude data - COSY or HETCOR
Hypercomplex data
TPPI data
Echo-antiecho data
States-TPPI data

****** NUTS-Pro versions newer than May, 2002, include a modified arrayed mode for improved processing of phase-sensitive data. See description of PT command.

See also:

arrayed mode processing (NUTS-Pro users only)
macros - commands used in macros for automated processing
2D processing - commands related to 2D
displaying 2D data - options for contour plots
phasing 2D data
symmetrizing
editing 2D data
sample data for downloading
Varian DEPT data - macros for processing and creating edited DEPT plots
comparing multiple 2D spectra

Varian 2D data

Processing description and macros are provided for the following Varian experiments. The same procedures are applicable to data from other spectrometers.

NOESY - nuclear Overhauser spectroscopy; hypercomplex, phase-sensitive using States method
The same processing procedure applies to tntocsy, tocsy, roesy, and hypercomplex cosy data.

g_hsqc - gradient heteronuclear single-quantum correlation; echo-antiecho, phase-sensitive

hsqc - heteronuclear single-quantum correlation; hypercomplex, phase-sensitive

hmbc - heteronuclear multiple bond correlation; hypercomplex, not phase-sensitive

g_hmbc - gradient heteronuclear multiple bond correlation; magnitude

cosy - magnitude COSY, not phase-sensitive

2dexch - 2D exchange experiment for solids; hypercomplex data in which the 2 halves of the data are scaled differently

Bruker 2D data

magnitude -- no phasing, a magnitude calculation is done at the end of processing; This is appropriate for experiments such as cosy, cosygs, cosygsmf, cosy45gs, inv4gs, invgslrlp. See also hetcor processing.

phase-sensitive experiments:

TPPI -- requires REAL ft in the indirect dimension

States (hypercomplex) -- pairs of slices are combined to generate complex "fids" in the indirect dimension

echo-antiecho -- gradient experiment, pairs of slices are added and subtracted to generate complex "fids" in the indirect dimension

states-tppi data -- experiments such as noesygpst and roesyprst

For Avance-series spectrometers, you can tell which kind of data you have by opening the file called pulseprogram using a text editor such as Word or WordPad (notepad

doesn't work well, due to the absence of carriage returns). Toward the end of this file is a parameter called MC2, which indicates the type of processing required.

MC2 values:

QF indicates magnitude data
QSEQ or TPPI indicates TPPI data
States indicates States-type hypercomplex data
States-TPPI indicates States-TPPI data
Echo-antiecho indicates echo-antiecho data

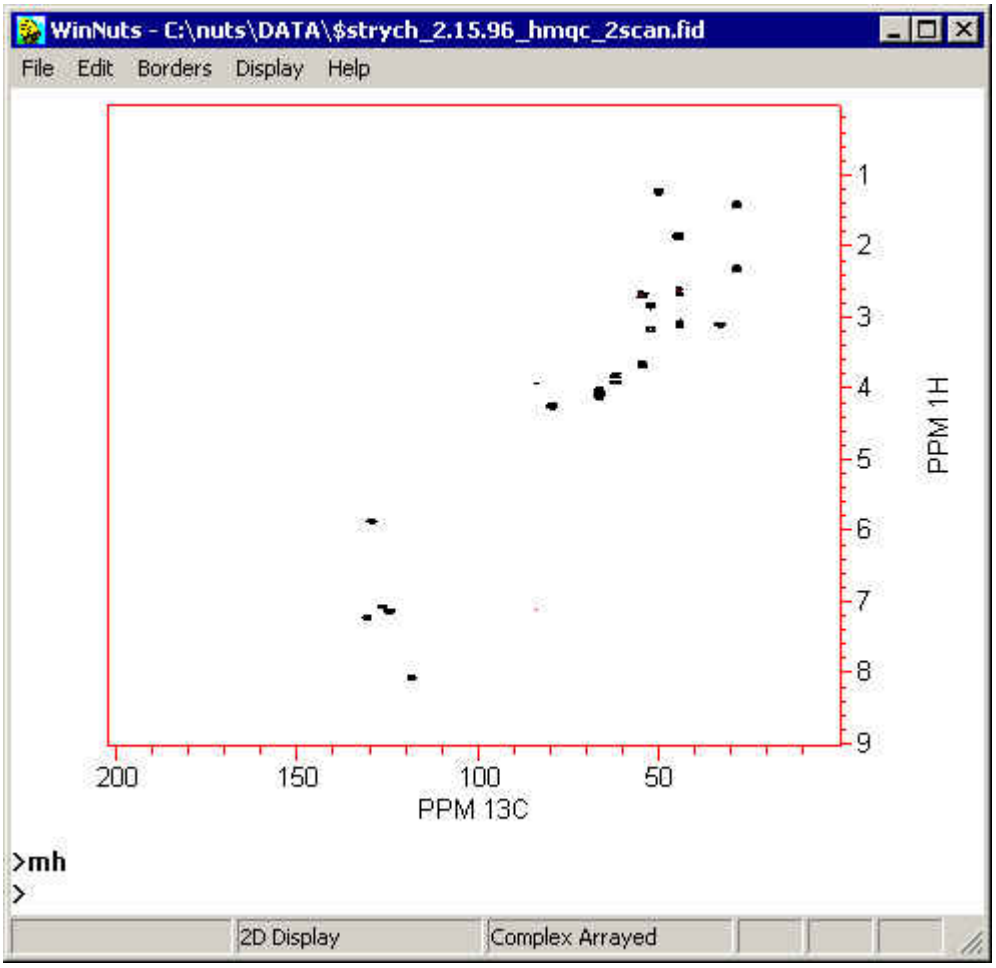
The name of the pulse program also indicates the data type. The names are concatenation of a base name (usually 4 letters) plus a series of 2-letter codes that are shorthand notation for various properties of the experiment. Codes of interest here are:

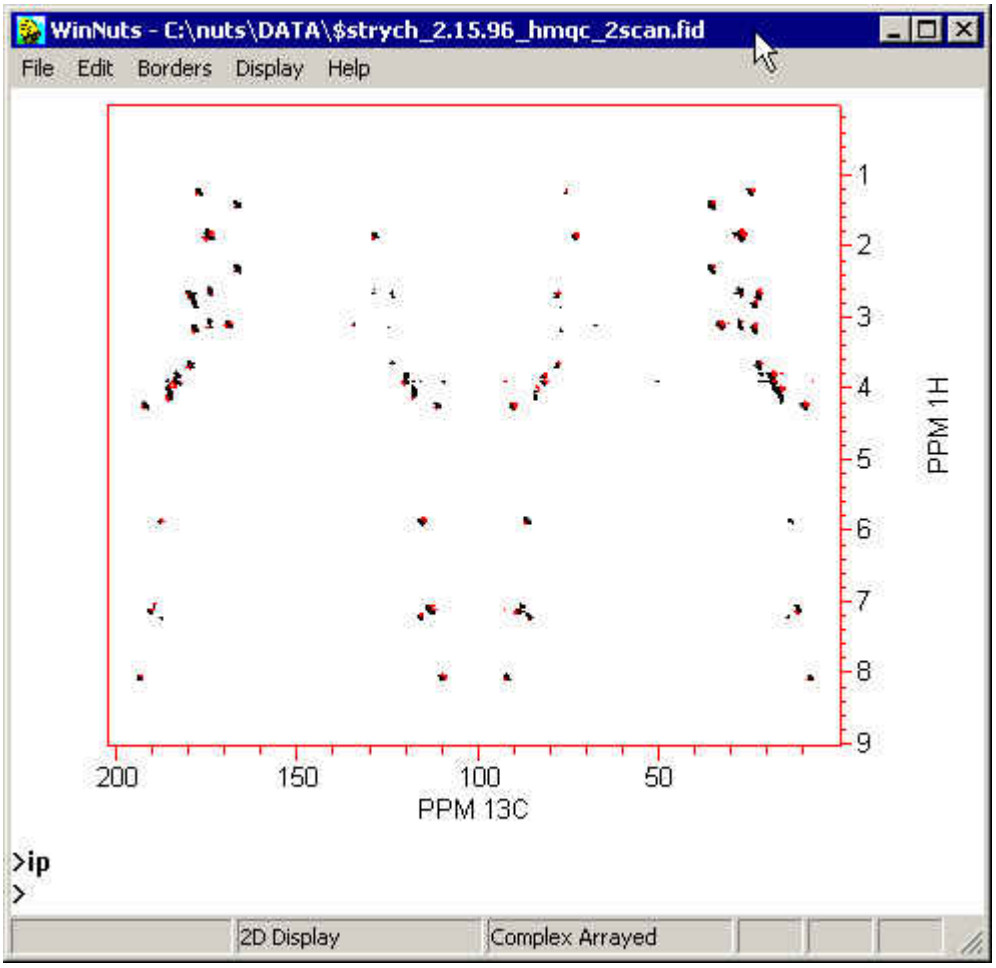
ea -- echo-antiecho
gs -- gradient selection (use magnitude processing)
sh -- use States-type processing
tp -- use TPPI processing
st -- use states-tppi processing

For Aspect-based spectrometers, data is most likely magnitude or TPPI, depending on whether or not it is phase-sensitive. Reading the .aur file on the spectrometer will usually tell you which. Or, process the first slice of the data and try to phase it; if it can't be phased, assume it's magnitude. If it can be phased, assume it's TPPI.

How to tell if something is wrong

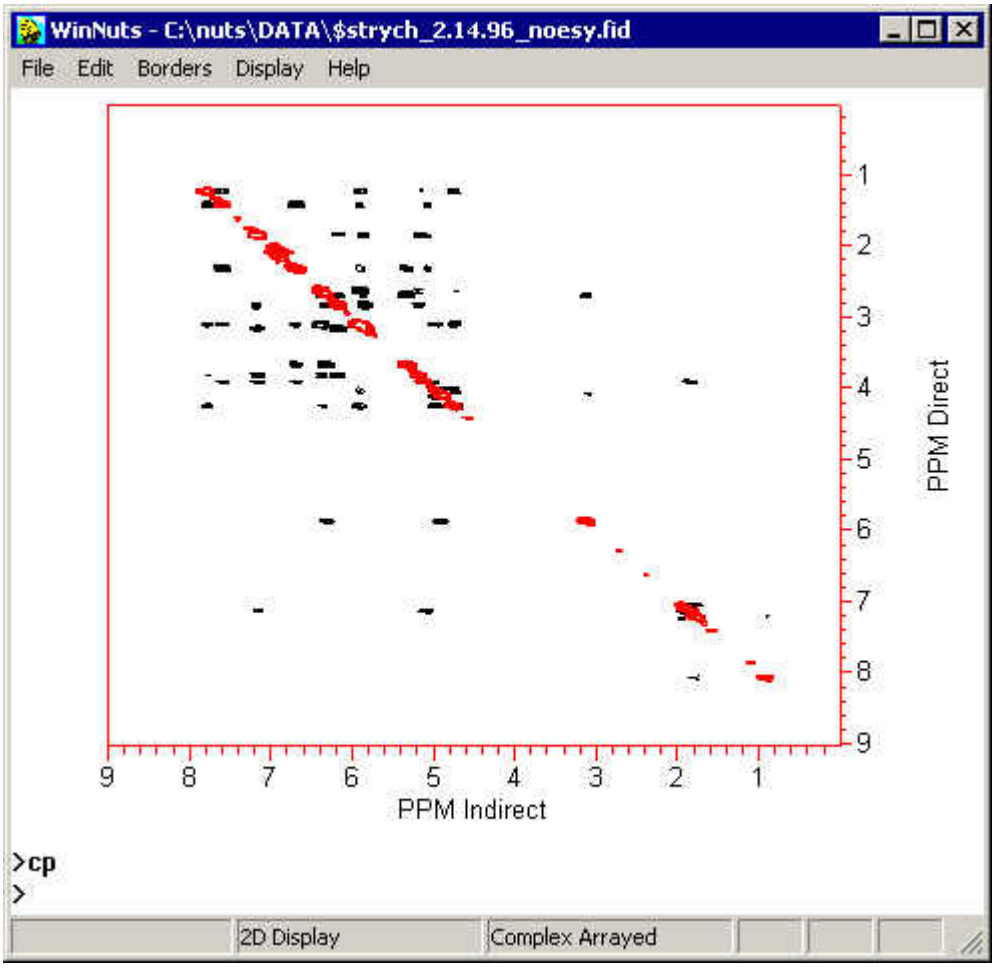
If the wrong type of processing is done, a set of mirror image peaks is often seen. Compare the plots shown below for an HMQC spectrum of strychnine. The top spectrum is correct. The bottom spectrum was obtained by failing to process the data as hypercomplex.


WinNuts - C:\nuts\DATA\$strych_2.15.96_hmqc_2scan.fid
File Edit Borders Display Help
1
2
3
4
5
6
7
8
9
PPM 1H
200
150
100
50
PPM 13C
>mh
>
2D Display
Complex Arrayed



Because there are so many variations in spectrometer models and pulse sequences, it is not possible to provide users with macros that will work correctly on 2D data from all spectrometers. It is recommended that users process a known data set first, for each 2D experiment commonly used on their spectrometers, to determine the correct processing commands under conditions where the correct final result is known.

Another, less serious, problem is that sometimes the final spectrum appears to have the diagonal going the wrong way, as shown below.



This is easily fixed by including a spectrum reverse (SR) in the processing for the second (indirect) dimension. Or, if there is already a SR in the processing list, remove it and re-process.

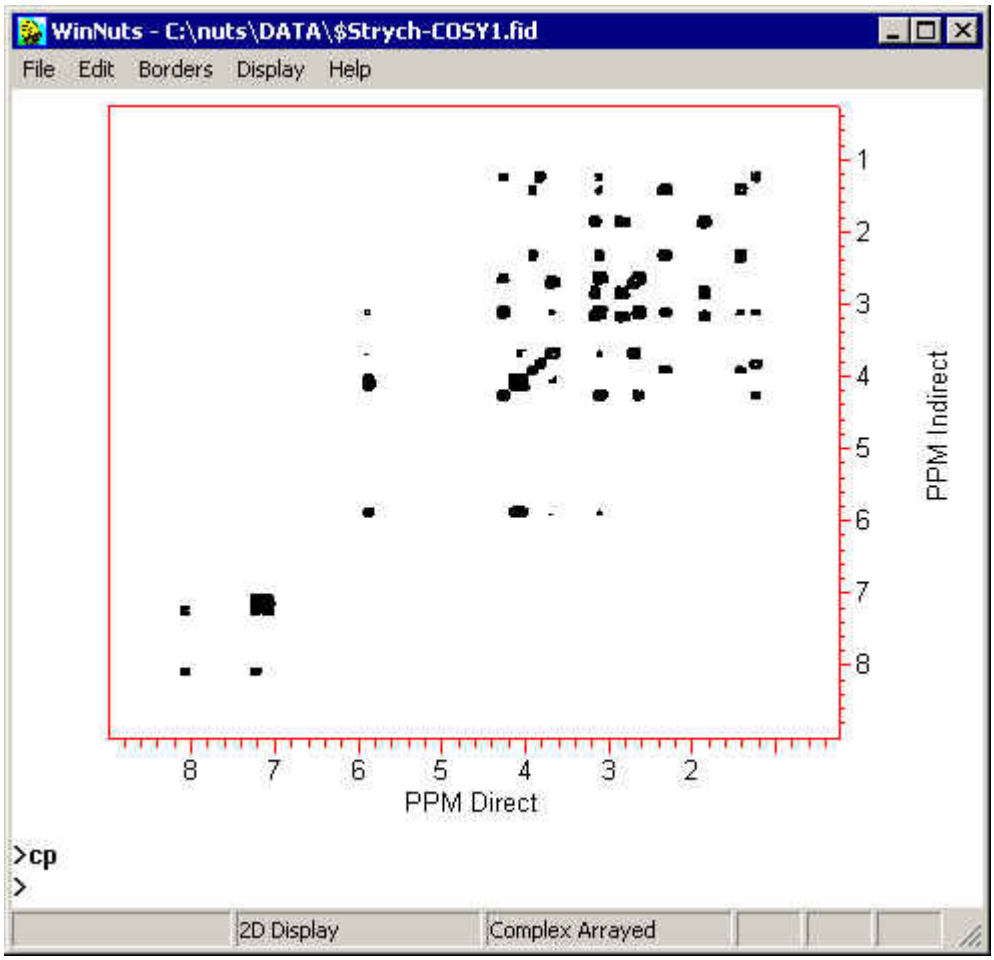
If the data is homonuclear and "square" (same number of data points in both dimensions), the spectrum can be symmetrized using the SY command. Choose Spectral Parameters from the View menu and check the Number of Points in the 2 dimensions. Additional zero-filling can be done during processing to make the data end up square, if symmetrizing is desired. Attempting to execute SY if the data set is not square will generate an error message.

Magnitude experiments

COSY

COSY data can be acquired in any of several "flavors" and each must be processed differently. This example applies to a magnitude COSY spectrum (not hypercomplex,

not phase-sensitive). Any magnitude data is processed in the same manner; see hetcor processing, below.



Varian COSY spectrum of strychnine.
This data set can be downloaded, along with processing macros.

Using Arrayed Mode, processing can be done using a macro (below) or simply by entering the following commands using the command line.

Users with the standard 2D version cannot use Arrayed Mode, and can process with a different macro (below).

See also: macros, 2D processing commands, displaying 2D data, processing 2D data

Arrayed Mode processing from the command line

IM	import file called fid
AR	enter arrayed mode
S#	set phase shift for sine multiplication to 0

MS MS	apply sine squared window function
FT	no phasing is done
TD	transpose data
MS MS	apply sine squared window function
ZF	zero-fill (may be repeated if needed)
FT	
SR	this spectrum needs spectrum reverse (may or may not be needed)
MC	magnitude calculation
BC	remove any DC offset and tilt in baseline
TD	to view data with direct dimension horizontal
MH	set minimum height to 1 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

If the data is "square" (same number of data points in both dimensions), the spectrum can be symmetrized using the SY command. Choose Spectral Parameters from the View menu and check the Number of Points in the 2 dimensions. Additional zero-filling can be done during processing to make the data end up square, if symmetrizing is desired.

Be sure to save the processed data (with **SA**).

Macro using Arrayed Mode

```
NUTSMACRO Varian magnitude cosy data for NUTS-Professional
ask filea
ga
set array_on
set s# 0
bc ms ms ft
;transpose data
td
ms ms zf ft sr mc bc
;transpose data
td
set mh 1
ss
ip
end
```

Note that NUTS remains in Arrayed Mode at the end of the macro. The data can be symmetrized, if desired, provided it has the same number of data points in both dimensions. Be sure to save the final data.

Macro without Arrayed Mode

Processing is done one 1 slice at a time, so the sequence of commands is done in a Link, which is repeated for each slice.

Temporary files are written at each stage of processing, so the macro must reset the file names at each stage. Be sure to save the final result, or it will be overwritten the next time the macro is run.

NUTSMacro Varian magnitude cosy	
ask filea ga	Macro asks for FID to open
set sl 1 set s# 0	window function used here is sine squared, applied with ms ms after setting s#=0
set filec ft1.2d	set name for temp file
ga bc ms ms ft bc sc in	1st dimension processing **GA** reads in the first slice **BC** removes DC offset of the 2 halves of the FID **MS MS** applies sine squared **BC** after FT removes DC and tilt in baseline **SC** saves the data **IN** increments the slice counter
set filec td.2d set filea ft1.2d set sl 1 ga td	reset file names transpose data
set filea td.2d set filec ft2.2d set sl 1 set lb 5	reset file names
ga ms ms zf ft mc sr bc sc in	2nd dimension processing this data requires spectrum reverse (SR) and magnitude calculation (MC)
set filea ft2.2d set filec final.2d set sl 1 ga td	reset file names transpose data
set filea final.2d ga ss set mh 1 ip end	open file data set set contour threshold (MH = 1) set scale, display intensity plot

The data can be symmetrized, if desired, provided it has the same number of data points in both dimensions.

HETCOR

Processing magnitude HETCOR data

This is among the simplest 2D experiments, as it requires no phasing. A sample HETCOR data file called small.2d (a ^{13}C-^{1}H spectrum of sucrose) can be downloaded. The resulting 2D plot is shown below.

This page shows how to process this data set with step-by-step explanation. 2 macros are described, one for NUTS-Professional users and one for NUTS-2D users. The goal with these examples is to describe the basic functions to allow users to customize them.

See also descriptions of Links and Macros, magnitude COSY processing, processing Varian and Bruker 2D data.

Users with the Professional version of NUTS can process the data using the following keyboard commands.

Arrayed Mode processing from the command line

IM	import file called fid (Varian) or ser (Bruker)
AR	enter arrayed mode
LB	set line broadening to 5
EM	apply exponential window function
FT	no phasing is done
BC	remove DC and tilt of baseline
TD	transpose data
MS	apply sine window function (could use EM here, if preferred)
ZF	zero-fill (may be repeated if needed)
FT	
SR	small.2d sample spectrum needs spectrum reverse; other data may or may not need this
MC	magnitude calculation
BC	remove any DC and tilt in baseline
TD	to view data with direct dimension horizontal
MH	set minimum height to 10 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

Be sure to save the processed data.

Macro using Arrayed Mode

```
NUTSMACRO   Magnitude HETCOR processing using NUTS-Professional
;macro prompts for name of data set
ask FileA
ga
set array_on
; 5 Hz linebroadening in 1st dimension
set LB 5
;link to process in 1st dimension
bc em ft bc
;transpose data
td
;link to process in 2nd dimension
;uses sine window function, spectrum reverse and magnitude calculation
ms ft sr mc bc
; transpose data
td
set mh 10
ss
ip
end
```

Note that NUTS remains in Arrayed Mode at the end of the macro. Be sure to save the final data.

Macro without Arrayed Mode

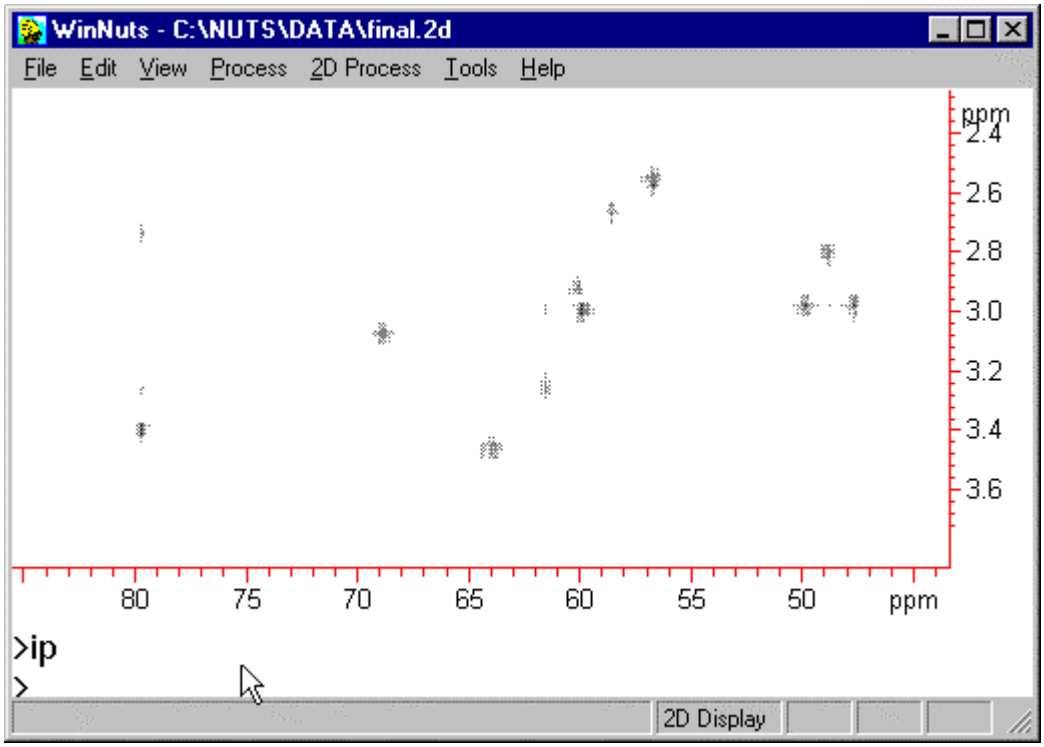
Processing is done one 1 slice at a time, so the sequence of commands is done in a Link, which is repeated for each slice.

Temporary files are written at each stage of processing, so the macro must reset the file names at each stage. Be sure to save the final result.

NUTSMACRO for magnitude HETCOR ask FileA ga set SL 1	The macro first asks for the name of the file to be processed, so that the macro does not need to be edited each time it is run. GA opens the file. The pointer is set to the first slice (just to be safe).
set FileC ft1.2d	The macro writes several temporary files during processing. A file created as output in one step becomes the starting point for the following step, which is why FileA and FileC keep being reset. The output of the first step will be a 2D file called **ft1.2d**.
set LB 5 ga bc em ft bc sc in	The linebroadening for the first (carbon) dimension is set to 5 Hz. The Link reads in each slice, baseline corrects to remove any DC offset of the 2 halves of the FID, applies the exponential multiplication and FT

	BC applied to the spectrum removes any DC and tilt in the baseline The resulting spectrum is saved as FileC with SC; the IN command increments the file pointers and loops to the beginning of the Link.
set FileA ft1.2d set FileC td.2d set SL 1	Now **ft1.2d**, the result of the first dimension FT, becomes the starting point for the next processing step, so is set to be FileA. The output of the next step will be a file called **td.2d** Again, to be safe, the file pointer is set to the first slice.
td	TD rotates the matrix so that slices in the other dimension can be read
set FileA td.2d set FileC ft2.2d set SL 1	The next step will operate on **td.2d**, which is now set to be FileA The result of the second dimension processing, FileC, will be called **ft2.2d** Again, to be safe, the file pointer is set to the first slice.
ga em ft sr mc bc sc in	This Link processes in the second (proton) dimension. Because the digital resolution is poor, we will use the same 5 Hz. linebroadening. Hetcor data from this particular instrument requires a spectrum reverse (SR) command in this dimension. That may need to be removed for data from other sources. MC performs a magnitude calculation and BC removes DC and tilt from the baseline.
set FileA ft2.2d ga set SL 1 set FileC final.2d	The data is best viewed with the ^{13}C dimension horizontally, as this is customary and is also the dimension with better digital resolution. So we again reset file names and set the slice pointer.
td	Transpose the data
set FileA final.2d ga set SL 1	Load the first slice of the final data set
ss set MH 10 ip end	SS normalizes the display scale. It looks through the entire data set to find the largest peak and sets it equal to 100%. Contour levels are defined in the nuts.ini file as mulitples of the Minimum Height (MH) which is expressed as a percent of the largest peak. So SS is needed to get the display correct. MH of 10 means that the first contour is drawn at 10% of the largest peak, appropriate for hetcor data, in which most peaks are similar in height. IP displays a quick intensity plot

The result should look like this:



HMQC

Example – Bruker HMQC

Macro using Arrayed Mode

```
NUTSMACRO B_inv4gs_pro
; Bruker Magnitude HMQC and HMBC data,
; applicable for inv4gs, invgslrlp
; arrayed mode processing - requires NUTS-Professional version
; this is magnitude data, no phasing done

; turn on arrayed mode
set array_on

; request name of file to open, and open it
ask FileA
ga

; set phase for sine mult to 0
set s# 0

; 1st dimension processing (F2 or t2)
; uses sine window function
; no Spectrum Reverse for ptype data
```

```
ga bc ms ft

;transpose data
td

; 2nd dimension processing (F1 or t1)
; uses sine window function and zero-fill
; Spectrum reverse (SR) included for Bruker-SGI-data
ms zf ft mc td sr

; set minimum height for intensity plot to 3%
set mh 3
ss
ip
end
```

Macro without Arrayed Mode

Processing is done one 1 slice at a time, so the sequence of commands is done in a Link, which is repeated for each slice.

Before running the macro, import the data, FT and phase. When the macro starts, it will ask for a file to open - select the translated file. Temporary files are written at each stage of processing, so the macro must reset the file names at each stage. Be sure to save the final result.

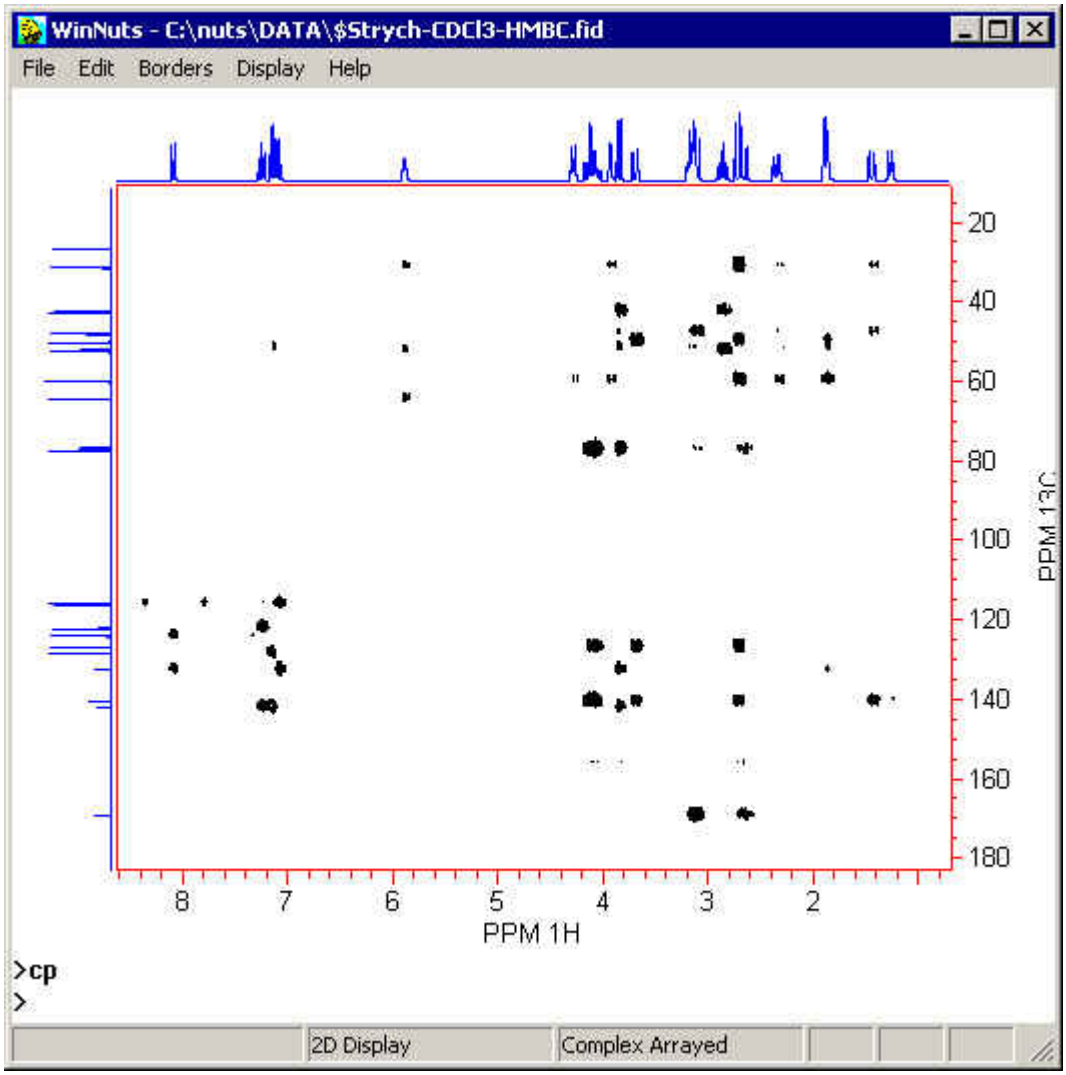
```
NUTSMACRO B_inv4gs_pro
; Bruker Magnitude HMQC and HMBC data,
; applicable for inv4gs, invgslrlp
; does not use arrayed mode processing
; this is magnitude data, no phasing done


; request name of file to open, and open it
ask FileA
ga

; set phase for sine mult to 0
set s# 0

set filec ft1.2d

; 1st dimension processing (F2 or t2)
; uses sine window function
; no Spectrum Reverse for ptype data
ga bc ms ft sc in

; reset file names
set filea ft1.2d
set filec td.2d
set sl 1
ga
;transpose data
td
```

```
set filea td.2d
set filec ft2.2d
set sl 1
; 2nd dimension processing (F1 or t1)
; uses sine window function and zero-fill
; Spectrum reverse (SR) included for Bruker-SGI-data
ga ms zf ft mc td sr sc in

set filea ft2.2d
set sl 1
ga

; set minimum height for intensity plot to 3%
set mh 3
ss
ip
end
```

HMBC

Example – Varian HMBC

Varian acquires HMBC data as hypercomplex, even though the data is not phase-sensitive, and a magnitude calculation is done at the end of processing. In a hypercomplex experiment, 2 FIDs are acquired for each t_1 time point, with a phase shift of one pulse. After FT in the direct dimension, the real halves of each pair of spectra are combined to create complex interferograms in the indirect dimension.



Varian HMBC spectrum of strychnine.
This data set can be downloaded, along with processing macros.

Using Arrayed Mode, processing can be done using a macro (below) or simply by entering the following commands using the command line.

Users with the standard 2D version cannot use Arrayed Mode, and can process with a different macro (below).

See also: macros, 2D processing, displaying 2D data

Arrayed Mode processing from the command line

AR	enter arrayed mode
IM	import file called fid
S#	set phase shift for sine multiplication to 45

MS MS	apply cosine squared window function
FT	
TR TR ST	"tag" real half of each slice and store
TD	transpose data
MS MS	
ZF ZF	zero-fill (experience has shown there is benefit from doing several ZFs, even 4 times)
FT	
MC	magnitude calculation
BC	remove any DC and tilt in baseline
TD	to view data with direct dimension horizontal
MH	set minimum height to 5 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

Be sure to save the processed data.

Macro using Arrayed Mode

```
NUTSMACRO Varian hmbc data (hypercomplex and magnitude) for NUTS-
Professional
set array_on
ask filea
ga
set s# 45
;window fcn is sine-squared
bc ms ms ft
; combine slices for hypercomplex data
tr tr st
;transpose data
td
;2nd dimension processing
;window fcn is sine-squared and we do one zero-fill
; magnitude calculation
ms ms zf ft mc bc
;transpose data
td
set mh 5
ss
ip
end
```

Note that NUTS remains in Arrayed Mode at the end of the macro. Be sure to save the final data.

Macro without Arrayed Mode

Processing is done one 1 slice at a time, so the sequence of commands is done in a Link, which is repeated for each slice.

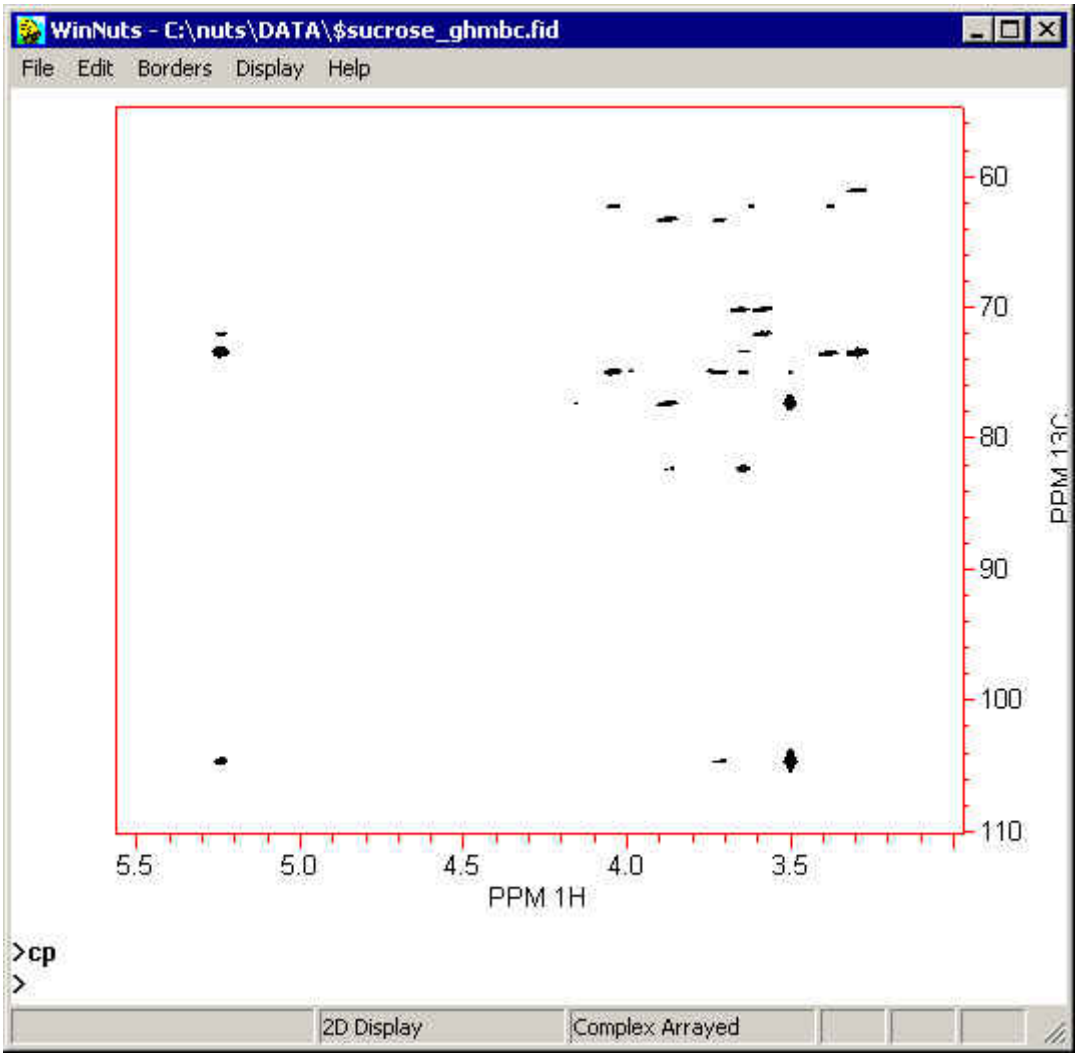
Temporary files are written at each stage of processing, so the macro must reset the file names at each stage. Be sure to save the final result.

NUTSMacro Varian HMBC, hypercomplex and magnitude	
ask filea ga	Macro asks for FID to open
set sl 1 set s# 45	window function used here is sine squared, applied with ms ms after setting s#=45
set filec ft1.2d	
ga bc ms ms ft bc tr ia ga bc ms ms ft bc tr st in	1st dimension processing **GA** reads in the first slice **BC** removes DC offset of the 2 halves of the FID **MS MS** applies cosine squared **BC** after FT removes DC and tilt in baseline **TR** "tags" this to be the real half of the complex FID in t_1 **IA** increments slice counter for File A **GA** reads in the next slice, which is processed same way **TR** "tags" this to be the imaginary half of the complex FID in t_1 **ST** saves the 2 halves, and **IN** increments the file pointers and loops to the beginning of the Link
set filec td.2d set filea ft1.2d set sl 1 ga td	reset file names transpose data
set filea td.2d set filec ft2.2d set sl 1	reset file names
ga ms ms zf ft mc bc sc in	2nd dimension processing
set filea ft2.2d set filec final.2d set sl 1 ga	reset file names transpose data

td	
set filea final.2d ga ss set mh 5 ip end	open file data set set contour threshold (MH = 5) set scale, display intensity plot

Example - Varian gHMBC

Varian g_hmbc data is a simple magnitude experiment, not phase sensitive and not hypercomplex.



Varian g_hmbc spectrum of sucrose.
This data set can be downloaded, along with processing macros.

Using Arrayed Mode, processing can be done using a macro (below) or simply by entering the following commands using the command line.

Users with the standard 2D version cannot use Arrayed Mode, and can process with a different macro (below).

See also: macros, 2D processing, displaying 2D data

Arrayed Mode processing from the command line

AR	enter arrayed mode
IM	import file called fid
S#	set phase shift for sine multiplication to 0
MS MS	apply sine squared window function
FT	
TD	transpose data
LB EM	set line broadening to an appropriate value, apply EM (alternatively, cosine squared can be used.
ZF ZF	zero-fill (experience has shown there is benefit from doing several ZFs, even 4 times)
FT	
MC	magnitude calculation
BC	remove any DC and tilt in baseline
TD	to view data with direct dimension horizontal
MH	set minimum height to 5 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

Be sure to save the processed data.

Macro using Arrayed Mode

```
NUTSMACRO Varian g_hmbc data (magnitude) for NUTS-Professional
set array_on
ask filea
ga
set s# 0
set LB 5
bc ms ms ft
;transpose data
td
em zf ft mc bc
;transpose data
```

```
td
set mh 5
ss
ip
end
```

Note that NUTS remains in Arrayed Mode at the end of the macro. Be sure to save the final data.

Macro without Arrayed Mode

Processing is done one 1 slice at a time, so the sequence of commands is done in a Link, which is repeated for each slice.

Temporary files are written at each stage of processing, so the macro must reset the file names at each stage. Be sure to save the final result.

NUTSMacro Varian g_hmbc, magnitude	
ask filea ga	Macro asks for FID to open
set sl 1 set s# 0	window function used here is sine squared, applied with ms ms after setting s#=0
set filec ft1.2d	
ga bc ms ms ft bc sc in	1st dimension processing **GA** reads in the first slice **BC** removes DC offset of the 2 halves of the FID **MS MS** applies sine squared **BC** after FT removes DC and tilt in baseline **SC** saves the data **IN** increments the slice counter
set filec td.2d set filea ft1.2d set sl 1 ga td	reset file names transpose data
set filea td.2d set filec ft2.2d set sl 1 set lb 5	reset file names
ga em zf ft mc bc sc in	2nd dimension processing
set filea ft2.2d set filec final.2d	reset file names

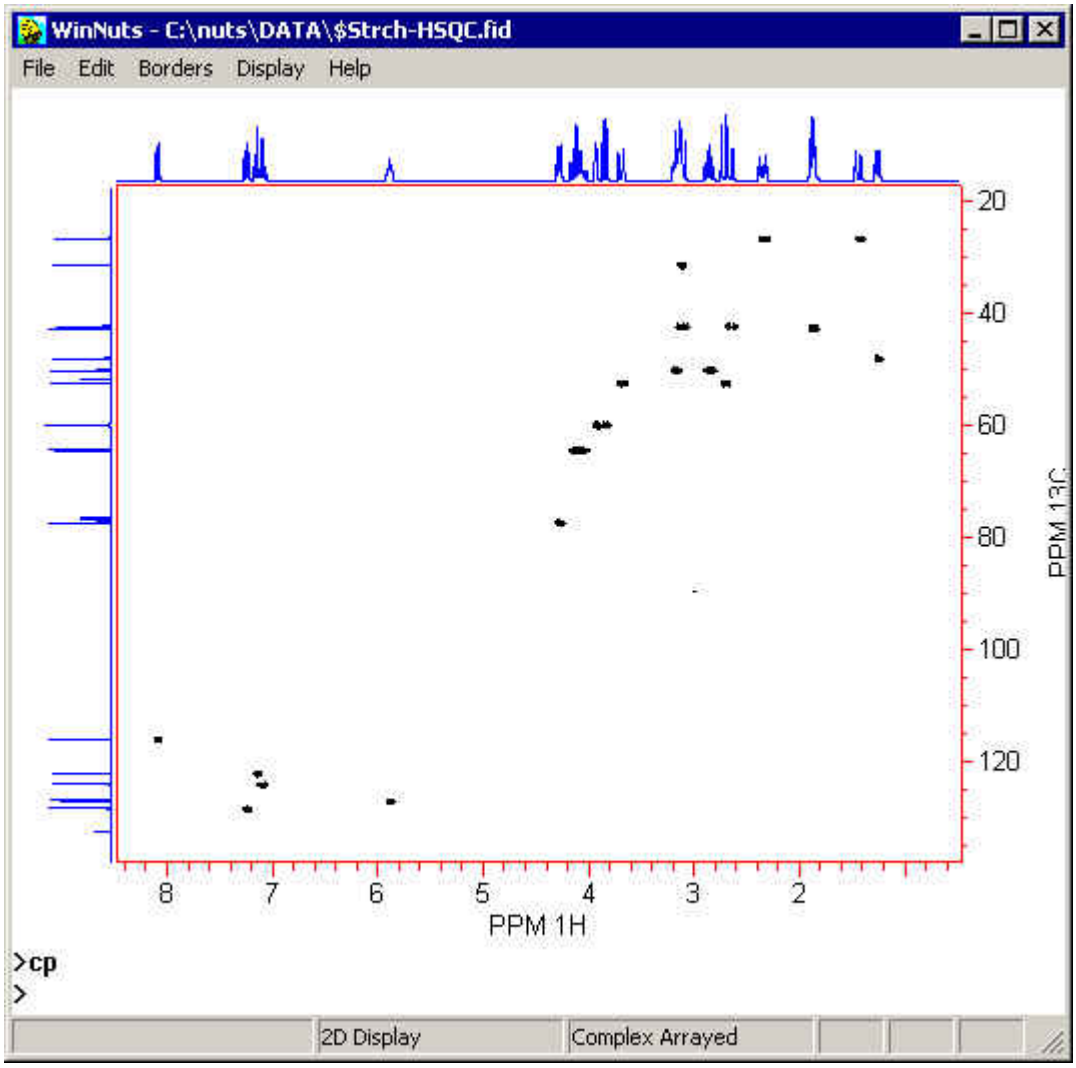
set sl 1 ga td	transpose data
set filea final.2d ga ss set mh 5 ip end	open file data set set contour threshold (MH = 5) set scale, display intensity plot

Hypercomplex experiments (method of States, et al)

Data in a single file – Varian HSQC

Varian acquires phase-sensitive HSQC data as hypercomplex, meaning that 2 FIDs are acquired for each t_1 time point, with a phase shift of one pulse. After FT in the direct dimension, the pairs of spectra are combined to create complex interferograms in the indirect dimension.

Note that Varian's g-hsqc experiment is echo-antiecho, not hypercomplex, and must be processed differently.



Varian phase-sensitive HSQC of strychnine.
This data set can be downloaded, along with processing macros.

Using Arrayed Mode, processing can be done using a macro (below) or simply by entering the following commands using the command line.

NUTS-Pro versions newer than May, 2002, include a modified arrayed mode for improved processing of hypercomplex data. See description of Arrayed Mode below.

Users with the standard 2D version cannot use Arrayed Mode, and can process with a different macro (below).

See also: macros, 2D processing, displaying 2D data, phasing 2D data

Arrayed Mode processing from the command line

AR	enter arrayed mode
IM	import file called fid
S#	set phase shift for sine multiplication to 90
MS MS	apply cosine squared window function
FT	
PH	phase while viewing the first slice; on exiting phase routine, the entire data set is phased
TR TR ST	"tag" real half of each slice and store
TD	transpose data
MS MS	
ZF	zero-fill (may be repeated if needed)
FT	may require phasing; see 2D phasing
TD	to view data with direct dimension horizontal
MH	set minimum height to 5 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

Be sure to save the processed data.

Arrayed Mode processing using new PT feature (May, 2002)

Be sure you are not in arrayed mode, and open the data to be processed.

PT	set processing type to *2D_HyperComplex*
AR	enter arrayed mode (note that status bar says "pairwise complex arrayed mode"
S#	set phase shift for sine multiplication to 90
MS MS	apply cosine squared window function
FT	
PH	phase while viewing the first slice; on exiting phase routine, the entire data set is phased
TD	transpose data
MS MS	
ZF	zero-fill (may be repeated if needed)
FT	may require phasing; see 2D phasing
(SR)	may be needed to make diagonal run in the conventional direction
TD	to view data with direct dimension horizontal
MH	set minimum height to 3 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

All quadrants of the data are saved, allowing phasing in both dimensions. Be sure to save the processed data. To open the processed data at a later date, enter AR first, so that the data is sorted correctly when opened.

Macro using Arrayed Mode (does not use the new modified arrayed mode)

```
NUTSMACRO Varian hypercomplex HSQC processing for Arrayed Mode
;macro prompts for name of data set
; first, import, process and phase 1st slice

set array_on
ask FileA
ga
set s# 90
bc ms ms ft ps bc
; combine hypercomplex slices
tr tr st
;transpose data
td
ms ms zf ft
td
set mh 4
ss
ip
end
```

Macro without Arrayed Mode

Processing is done one 1 slice at a time, so the sequence of commands is done in a Link, which is repeated for each slice.

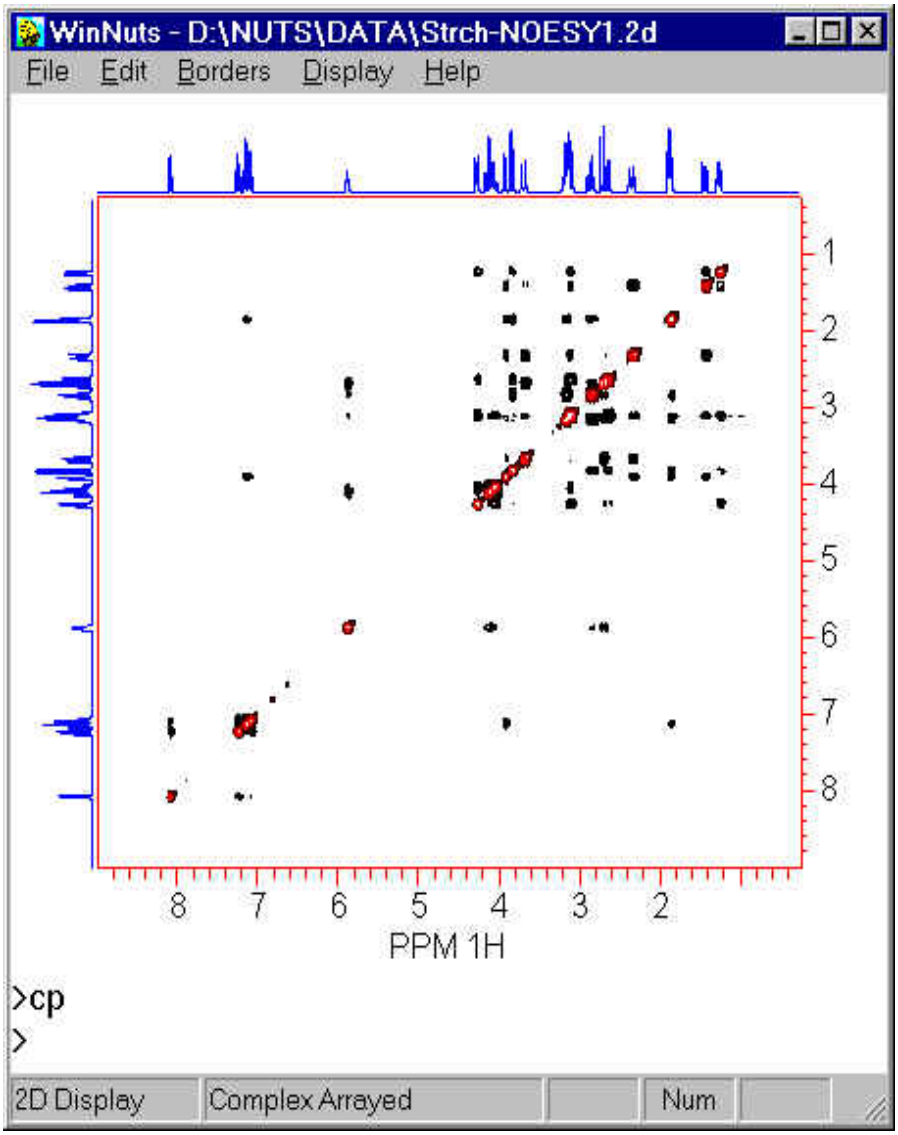
Before running the macro, import the data, FT and phase. When the macro starts, it will ask for a file to open - select the translated file. Temporary files are written at each stage of processing, so the macro must reset the file names at each stage. Be sure to save the final result.

NUTSMacro Varian Hypercomplex HSQC ask filea ga set sl 1	Before running macro, open data set, FT and phase 1st slice Macro asks for name of data set to open - supply translated file name
set filec ft1.2d set s# 90	window fcn will be cosine squared; phase is set with S#
ga bc ms ms ft ps bc tr ia ga bc ms ms ft ps bc tr st in	Link to process 1st dimension **GA** reads in the first slice **BC** removes DC offset of the 2 halves of the FID **MS MS** applies cosine squared **BC** after FT removes DC and tilt in baseline

	IA increments slice counter for File A **TR** "tags" this to be the real half of the complex FID in t_1 **GA** reads in the next slice, which is processed same way **TR** "tags" this to be the imaginary half of the complex FID in t_1 **ST** saves the 2 halves, and **IN** increments the file pointers and loops to the beginning of the Link
set filec td.2d set filea ft1.2d set sl 1 ga td	reset file names transpose data
set filea td.2d set filec ft2.2d	reset file names
set sl 1 ga ms ms zf ft sc in	2nd dimension processing
set filea ft2.2d set filec final.2d set sl 1 ga td	reset file names transpose data
set filea final.2d ga ss set mh 4 ip end	open final file set scale, set contour threshold (MH = 4)

Varian NOESY

Varian (and JEOL) acquires some phase-sensitive data (such as NOESY) as hypercomplex, meaning that 2 FIDs are acquired for each t_1 time point, with a phase shift of one pulse in the sequence. After FT in the direct dimension, the pairs of spectra are combined to create complex interferograms in the indirect dimension.



Varian phase-sensitive NOESY of strychnine.
This data set can be downloaded, along with processing macros.

Using Arrayed Mode, processing can be done using a macro (below) or simply by entering commands using the command line, shown below.

NUTS-Pro versions newer than May, 2002, include a modified arrayed mode for that allows phasing in both dimensions after processing. Commands for processing are listed below.

Users with the standard 2D version cannot use Arrayed Mode, and can process with a different macro (below).

See also: macros, 2D processing, displaying 2D data, phasing 2D data

Arrayed Mode processing using new PT feature (May, 2002)

Be sure you are not in arrayed mode, and open the data to be processed. (In most cases, NUTS will automatically exit arrayed mode when opening a new file.)

PT	set processing type to *2D_HyperComplex*
AR	enter arrayed mode (note that status bar says "pairwise complex arrayed mode"
S#	set phase shift for sine multiplication to 90
MS MS	apply cosine squared window function
FT	
PH	phase while viewing the first slice; on exiting phase routine, the entire data set is phased
TD	transpose data
MS MS	
ZF	zero-fill (may be repeated if needed)
FT	may require phasing; see 2D phasing
SR	may or may not be needed to make diagonal run in the conventional direction
TD	to view data with direct dimension horizontal
MH	set minimum height to 0.3 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

All quadrants of the data are saved, allowing phasing in both dimensions.

Be sure to save the processed data.

With NUTS versions dated May 2002 to Nov 2002, you must enter the pairwise arrayed mode before re-opening the processed data, so that the data is sorted correctly when opened. Versions newer than Nov 2002 will read the PT parameter and automatically start the pairwise arrayed mode when the file is opened.

Arrayed Mode processing from the command line

IM	import data file
AR	enter arrayed mode
S#	set phase shift for sine multiplication to 90
MS MS	apply cosine squared window function
FT	
PH	phase while viewing the first slice; on exiting phase routine, the entire data set is phased
TR TR ST	"tag" real half of each slice and store

TD	transpose data
MS MS	
ZF	zero-fill (may be repeated if needed)
FT	may require phasing; see 2D phasing
SR	may be needed to make diagonal run in the conventional direction
TD	to view data with direct dimension horizontal
MH	set minimum height to 0.3 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

Be sure to save the processed data.

Macro using Arrayed Mode (does not use the new modified arrayed mode)

```
NUTSMACRO Varian NOESY processing for Arrayed Mode
;macro prompts for name of data set
; first, import, process and phase 1st slice

ask FileA
ga
set array_on
set s# 90
bc ms ms ft ps bc
; combine hypercomplex slices
tr tr st
;transpose data
td
ms ms zf ft
td
set mh .3
ss
ip
end
```

This macro ends displaying data with the indirect dimension on the horizontal axis. This is because usually some phasing is needed. See 2D phasing.

Macro without Arrayed Mode

Processing is done one 1 slice at a time, so the sequence of commands is done in a Link, which is repeated for each slice.

Before running the macro, import the data, FT and phase. When the macro starts, it will ask for a file to open - select the translated file. Temporary files are written at each stage

of processing, so the macro must reset the file names at each stage. Be sure to save the final result.

NUTSMacro Hypercomplex ask filea ga set sl 1	Before running macro, open data set, FT and phase 1st slice Macro asks for name of data set to open - supply translated file name
set filec ft1.2d set s# 90	window fcn will be cosine squared; phase is set with S#
ga bc ms ms ft ps bc tr ia ga bc ms ms ft ps bc tr st in	Link to process 1st dimension **GA** reads in the first slice **BC** removes DC offset of the 2 halves of the FID **MS MS** applies cosine squared **BC** after FT removes DC and tilt in baseline **TR** "tags" this to be the real half of the complex FID in t_1 **IA** increments slice counter for File A **GA** reads in the next slice, which is processed same way **TR** "tags" this to be the imaginary half of the complex FID in t_1 **ST** saves the 2 halves, and **IN** increments the file pointers and loops to the beginning of the Link
set filec td.2d set filea ft1.2d set sl 1 ga td	reset file names transpose data
set filea td.2d set filec ft2.2d	reset file names
set sl 1 ga ms ms zf ft sc in	2nd dimension processing
set filea ft2.2d set sl 1 ga set mh .3 ip end	reset file names set contour threshold (MH = 0.3), set scale, display intensity plot

This macro ends displaying data with the indirect dimension on the horizontal axis. This is because usually some phasing is needed. See 2D phasing.

Data in a pair of files

The first section presents a pair of macros appropriate for data which are saved with the 2 halves contained in separate files. Processing has been split into 2 parts. The first part processes in the first dimension and performs a TD. This allows the user to examine the data and determine the phasing required in the second dimension. The second part processes the second dimension using that phasing.

Following this are macros appropriate for data which are saved with the 2 halves interleaved (Varian).

It is recommended that users begin by examining the explanation of HETCOR processing first, as many steps are the same and HETCOR is a simpler case.

Because this is a phase-sensitive experiment, begin by reading in the first slice of the data, apply window function, FT and phase. These phase parameters will be applied to the rest of the data with the PS command. Customization of the macros may be desirable to use different window functions or to incoporate a spectrum reverse (SR) if needed.

See also description of commands for processing hypercomplex data.

NUTSMacro Hypercomplex 2D, part I ask FileA ask FileB ga set SL 1 set FileC ft1.2d	The macro prompts for the names of the files containing the 2 halves of the complex data, called Files A and B. GA sets the data directory, and setting Slice to 1 makes sure the file pointer is set to the beginning of the file. File C, **ft1.2d**, will be the result of the first dimension FT.
set LB 1 ga bc em ft ps bc tr gb bc em ft ps bc tr st in	Processing in the first dimension: The first slice is read from File A, apodized, FT'd and phased. The real half of the resulting spectrum is "tagged" with TR to become half of the complex "FID" in t_1. The first slice from File B is treated similarly. The real halves from the 2 files are saved as a complex FT in t_1 with ST.
set FileC td.2d set FileA ft1.2d set SL 1	The file created in the first step becomes the input file (File A) for the next step.The file pointer is set to 1, just to be safe.

ga td	The TD operation rotates the matrix so that slices in the 2nd dimension can be accessed.
set FileA td.2d set SL 1 ga end	Reset file name, set Slice pointer to 1 and read in the first slice.

We stop the processing at this point, to examine the t_1 interferograms to determine the appropriate window function and phase parameters. This requires selecting slices that contain signal, most easily accomplished by viewing an intensity plot. Type SS to normalize the display scale, set MH to 5-10 and type IP to display an intensity plot. The cursor can be used to determine the slice numbers of slices that contain signal. Slices can be displayed overlaid on the intensity plot, which is the easiest way to scan through the data. Hold down left mouse button to display a horizontal cursor, then press the right mouse button simultaneously to display slices as the cursor is moved up and down through the data set. (Users with a single-button mouse should press the period key on the keyboard instead of the right mouse button.) Note slice numbers of slices with good signal-to-noise, then exit the IP routine with ENTER.

To determine phase parameters in the second dimension, we need a spectrum with peaks near both ends of the spectrum, which can be accomplished by adding together 2 slices with peaks at opposite ends of the spectrum. Read in the first slice of interest (with SL), apply an apppropriate window function and FT. Place this slice into the Add/Subtract buffer (with AL). Read in and process the second slice. Enter the Add/Subtract routine (AS), add the 2 spectra (with plus sign) and exit the subroutine with ENTER. Now phase this spectrum, then note the phasing applied (TP). Enter the values of zero- and first-order phasing into the PA and PB parameters. This phase correction will then be applied (with PC) to each slice.

NUTSMacro Hypercomplex 2D, part II set FileA td.2d set SL 1 ga set FileC ft2.2d	The second half of the processing begins by reading in the first slice of **td.2d** After processing, the file created is called **ft2.2d**.
set S# 90 ga bc ms ms zf ft pc bc sc in	Set the phase for sine multiplication to 90 degrees (cosine). Link to process in the second dimension includes a cosine squared apodization (MS MS) and one zero-fill, followed by FT and phasing using the PA and PB parameters determined above.
ss end	SS normalizes the display scale. It looks through the entire data set to find the largest peak and sets it equal to 100%. Contour levels are defined in the nuts.ini file as mulitples of the Minimum Height

	(MH) which is expressed as a percent of the largest peak. So SS is needed to get the display correct.

This macro ends with the second dimension displayed horizontally. It may be desirable to perform another TD operation to view the data from the better digitized dimension. It has not been done as part of the macro in case additional phasing is required. If additional phasing is required in the second dimension, determine the phase correction needed and set values for PA and PB. Phasing is accomplished with a Link such as

ga pc sc in

Interleaved hypercomplex data

This section describes processing which is appropriate for data which are saved with the 2 halves interleaved. As above, begin by reading in the first slice of the data, apply window function, FT and phase. These phase parameters will be applied to the rest of the data with the PS command. Customization of the macros may be desirable to use different window functions or to incoporate a spectrum reverse (SR) if needed.

NUTSMacro Hypercomplex 2D, part I ask FileA ga set SL 1 set FileC ft1.2d	The macro prompts for the file name of the FID, called File A. GA sets the data directory, and setting Slice to 1 makes sure the file pointer is set to the beginning of the file. File C, **ft1.2d**, will be the result of the first dimension FT.
set S# 90 ga bc ms ms ft ps bc tr ia ga bc ms ms ft ps bc tr st in	Processing in the first dimension: The window function used here is cosine squared, applied with ms ms after setting the phase S# = 90 The first slice is read from File A, apodized, FT'd and phased. The real half of the resulting spectrum is "tagged" with TR to become half of the complex "FID" in t_1. The slice pointer for File A is incremented (IA), so that the next slice is read and processed in the same manner. The real halves from the 2 files are saved as a complex FT in t_1 with ST. The IN command increments the slice pointer for both File A and File C.
set FileA ft1.2d set SL 1 ga set FileC td.2d td	The file created in the first step becomes the input file (File A) for the next step.The file pointer is set to 1, just to be safe. The TD operation rotates the matrix so that slices in the 2nd dimension can be accessed.

```
set FileA td.2d
set SL 1                    Reset file name, set Slice pointer to 1 and read in the
ga                          first slice.
end
```

As before, we stop the macro at this point to determine phase parameters for the second dimension. From this point, processing is the same as for the case above.

TPPI experiments

TPPI (Time Proportional Phase Incrementation) is one method for accomplishing phase-sensitive "quadrature detection" in the indirect dimension, most often used in Bruker experiments. The only "trick" to processing is that a ***real*** FT is required in the indirect dimension.

This should not be confused with States-TPPI data, which must be processed differently.

A recommended reference is T.D.W.Claridge, "*High-Resolution NMR Techniques in Organic Chemistry*", 1999.

Customization of the macros may be desirable to use different window functions or to incorporate a spectrum reverse (SR) if needed.

See also:

- Arrayed Mode (NUTS-Pro users only)
- Commands for processing 2D data
- 2D Processing details
- Processing States-TPPI data
- Processing hypercomplex data
- Processing echo-antiecho data
- Processing magnitude hetcor or COSY 2D data
- Displaying 2D data

Using Arrayed Mode, processing can be done using a macro (below) or simply by entering the following commands using the command line. NUTS-Pro users with NUTS versions newer than Nov 2002 can process data using the modified arrayed mode, such that both dimensions can be phased after processing. See below.

Users with the standard 2D version cannot use Arrayed Mode, and can process with a different macro (below).

Arrayed Mode processing from the command line

IM	import file called ser
AR	enter arrayed mode
S#	set phase shift for sine multiplication to 90
MS MS	apply cosine squared window function
FT	
PH	phase while viewing the first slice; on exiting phase routine, the entire data set is phased
TD	transpose data
MS MS	
ZF	zero-fill (may be repeated if needed)
RT	Performs a ***real*** FT. This is critical! may require phasing; see 2D phasing
TD	to view data with direct dimension horizontal
MH	set minimum height to 0.3 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

Be sure to save the processed data.

NUTS-Pro users with copies newer than Nov 2002 can process using the modified arrayed mode, allowing phasing in both dimensions after processing. Processing is as above, except that instead of the AR command, execute the following command (in non-2-letter command mode):

ar tppi2pairwise

This creates the required additional 2 quadrants of data, filled with zeroes, and enters the pairwise arrayed mode.

With NUTS versions dated May 2002 to Nov 2002, you must enter the pairwise arrayed mode before re-opening the processed data, so that the data is sorted correctly when opened. Versions newer than Nov 2002 will read the PT parameter and automatically start the pairwise arrayed mode when the file is opened.

Macro using Arrayed Mode

```
NUTSMacro TPPI 2D for NUTS Professional
; process and phase on 1st slice before starting macro
ask filea
ga
; turn on arrayed mode
set array_on
;window fcn is cosine squared, applied with ms ms
set s# 90
process 1st dimension, phase with previously determined values
bc ms ms ft ps
```

```
; transpose data
td
; 2nd dimension processing with cosine sq, zero-fill and real FT
ms ms zf rt bc
; set scale, set contour threshold (MH = .3) and display intensity plot
ss
set mh .3
ip
end
```

This macro ends displaying data with the indirect dimension on the horizontal axis. This is because usually some phasing is needed. See 2D phasing. Note that the processed data has not been saved.

Macro without Arrayed Mode

Processing is done one 1 slice at a time, so the sequence of commands is done in a Link, which is repeated for each slice.

Before running the macro, import the data, FT and phase. When the macro starts, it will ask for a file to open - select the translated file. Temporary files are written at each stage of processing, so the macro must reset the file names at each stage. Be sure to save the final result.

NUTSMacro phase-sensitive TPPI ask FileA ga set SL 1 set FileC ft1.2d	The macro prompts the user for the name of the data file to be read, designated File A. Temporary file names are used for intermediate stages of processing. The file after the first dimension FT is called **ft1.2d**.
set S# 90 ga ms ms ft ps sc in	The window function used here is cosine squared, accomplished with MS MS after setting the phase for sine multiplication to 90 degrees. The processing Link applies the window function, FT and phases with phase parameters determined on the first slice, before the macro was started. The FT'd data is saved as File C.
set FileA ft1.2d set FileC td.2d set SL 1 ga	The file created in the previous step becomes the input for the next step. File C, which will be the output of the next step, is defined as **td.2d**.
td	Transpose the data to access slices in the second dimension.
set fileA td.2d set fileC ft2.2d set SL 1 ga	The input data for the next processing step is **td.2d**. The slice pointer is set to slice 1 and the file opened. The file to be created in the next step is **ft2.2d**.

Set S# 90 ga ms ms zf rt sc in	Link to process in 2nd dimension uses cosine squared window function and one zero-fill. A real transform (RT) is used instead of a "normal" FT. No phase correction is applied.
set FileA ft2.2d ga set SL 1	The processed data, **ft2.2d**, is read in and the slice pointer set to 1.
ss end	SS normalizes the display scale. It looks through the entire data set to find the largest peak and sets it equal to 100%. Contour levels are defined in the nuts.ini file as mulitples of the Minimum Height (MH) which is expressed as a percent of the largest peak. So SS is needed to get the display correct.

This macro ends with the second dimension displayed horizontally. It may be desirable to perform another TD operation to view the data from the better digitized dimension. It has not been done as part of the macro in case additional phasing is required. Phasing can be done as described in the section on 2D phasing.

Forward linear prediction (LN) can be used in the second dimension processing instead of zero-filling. After the first dimension processing, determine the appropriate Linear Prediction parameters by testing on one or more slices. The most recent values for each parameter will be used when LN is used in a Link or macro. Then modify the macro for processing in the second dimension by replacing ZF with LN. Processing will require significantly more time when LN is included (For a 1K complex by 256 NOESY data set, processed on a Pentium 100, this macro takes about 20 min.)

States-TPPI experiments

The terms are confusing, as phase-sensitive data can be acquired as States (also called hypercomplex), as TPPI (Time Proportional Phase Incrementation) or as States-TPPI (in addition to other methods), each of which must be processed differently.

For States-TPPI, you need to invert every other data point in the indirect dimension "fid", or equivalently, every other slice in the direct dimension AFTER you have combined pairs of spectra to form the complex "fids" in the direct dimension.

With States (hypercomplex) data, 2 FIDs are acquired for each t_1 time point, with a phase shift of one pulse in the sequence. After FT in the direct dimension, the real halves of each pair of spectra are combined to create complex interferograms in the indirect dimension. So you end up with half as many slices as you had to start with.

For States-TPPI, after this data shuffling operation, you must also invert every other slice.

A new command, **invert**, has been added to NUTS for processing States-TPPI data, which works only in the Arrayed Mode, so requires NUTS-Professional version. See below for processing in non-arrayed mode.

NUTS-Pro versions newer than May, 2002, include a modified arrayed mode for improved processing of States-TPPI data. This mode allows phasing in both dimensions after processing.

Invert – invert every other slice, for processing States-TPPI data

This command inverts either the even or odd slices of a 2 or 3D dataset. This command works only in the non-two-letter command mode and only during arrayed mode operation.

Examples:

invert even - used for States-TPPI processing
The above command would invert every even numbered slice of the current dataset

Related commands:

invert odd
The above command would invert every odd numbered slice of the current dataset.

invert real
The above command would invert all reals for every slice of the current dataset.

invert imag
The above command would invert all imaginaries for every slice of the current dataset.

Arrayed Mode processing from the command line

AR	enter arrayed mode
IM	import data
S#	set phase shift for sine multiplication to 90
MS MS	apply cosine squared window function
FT	
PH	phase while viewing the first slice; on exiting phase routine, the entire data set is phased
TR TR ST	"tag" real half of each slice and store
2F	turn off 2-letter command mode
INVERT EVEN <enter>	invert command must be terminated with <ENTER>

2N <enter>	return to 2-letter command mode
TD	transpose data
MS MS	
ZF	zero-fill (may be repeated if needed)
FT	may require phasing; see 2D phasing
(SR)	may be needed to make diagonal run in the conventional direction
TD	to view data with direct dimension horizontal
MH	set minimum height to 0.3 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

Be sure to save the processed data.

Arrayed Mode processing using new PT feature (May, 2002)

Be sure you are not in arrayed mode, and open the data to be processed.

PT	set processing type to *2D_StatesTPPI*
AR	enter arrayed mode (note that status bar says "pairwise complex arrayed mode"
S#	set phase shift for sine multiplication to 90
MS MS	apply cosine squared window function
FT	
PH	phase while viewing the first slice; on exiting phase routine, the entire data set is phased
2F	turn off 2-letter command mode
INVERT EVEN <enter>	invert command must be terminated with <ENTER>
2N <enter>	return to 2-letter command mode
TD	transpose data
MS MS	
ZF	zero-fill (may be repeated if needed)
FT	may require phasing; see 2D phasing
(SR)	may be needed to make diagonal run in the conventional direction
PH	phase
TD	to view data with direct dimension horizontal
MH	set minimum height to 0.3 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

All quadrants of the data are saved, allowing phasing in both dimensions. Be sure to save the processed data. To open the processed data at a later date, enter AR first, so that the data is sorted correctly when opened.

Processing in non-arrayed mode

This can be done using the NUTS "phase incremented" (PI) command by setting PA = 180 and PB = 0.

Processing is done one 1 slice at a time, so the sequence of commands is done in a Link, which is repeated for each slice.

Before running the macro, import the data, FT and phase. When the macro starts, it will ask for a file to open - select the translated file. Temporary files are written at each stage of processing, so the macro must reset the file names at each stage. Be sure to save the final result.

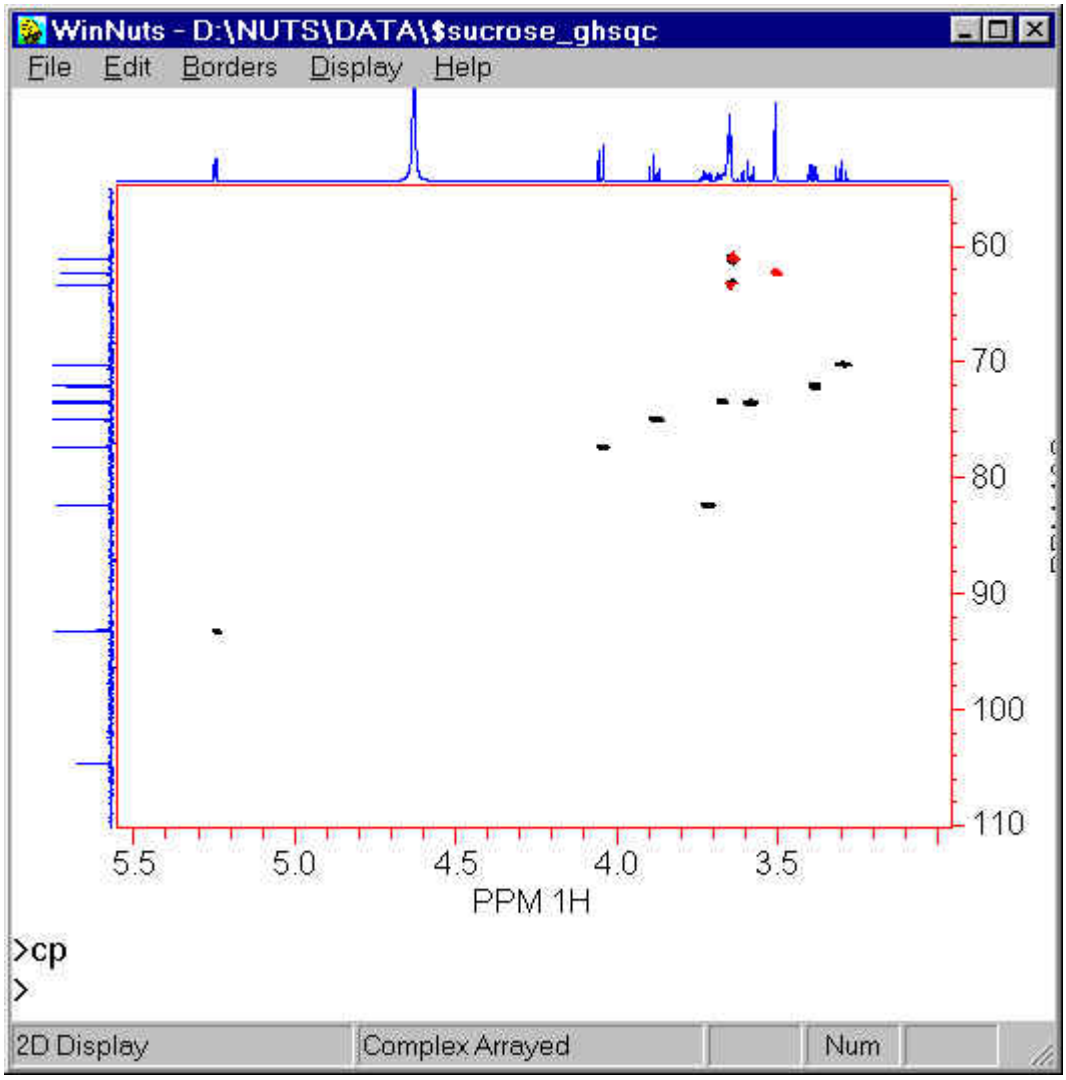
NUTSMacro States-TPPI ask filea ga set sl 1	Before running macro, open data set, FT and phase 1st slice Macro asks for name of data set to open - supply translated file name
set filec ft1.2d set s# 90	window fcn will be cosine squared; phase is set with S#
ga bc ms ms ft ps tr ia ga bc ms ms ft ps tr st in	Link to process 1st dimension **GA** reads in the first slice **BC** removes DC offset of the 2 halves of the FID **MS MS** applies cosine squared **TR** "tags" this to be the real half of the complex FID in t_1 **IA** increments slice counter for File A **GA** reads in the next slice, which is processed same way **TR** "tags" this to be the imaginary half of the complex FID in t_1 **ST** saves the 2 halves, and **IN** increments the file pointers and loops to the beginning of the Link
set filea ft1.2d set filec invert.2d set sl 1 ga set pa 180	reset file names **PI** (Phase Incremented) inverts every other slice

ga pi sc in	
set filec td.2d set filea ft1.2d set sl 1 ga td	reset file names transpose data
set filea td.2d set filec ft2.2d	reset file names
set sl 1 ga ms ms zf ft sc in	2nd dimension processing
set filea ft2.2d set sl 1 ga set mh .3 ip end	reset file names set contour threshold (MH = 0.3), set scale, display intensity plot

This macro ends displaying data with the indirect dimension on the horizontal axis. This is because usually some phasing is needed. See 2D phasing.

Echo-antiecho experiments

These phase-sensitive gradient experiments are run such that each slice contains both sine and cosine terms in t_1. This means that the data must be handled differently from hypercomplex data, in which odd numbered slices contain only cos t_1 terms, and even numbered slices contain only sin t_1 terms.



Varian g-hsqc spectrum of sucrose.
This data set can be downloaded, along with processing macros.

The data are processed differently depending on whether or not Arrayed Mode (NUTS-Professional version only) is used.

To process this data, we FT and phase slice 1 and slice 2, then calculate the sum and the difference of them. Because the 2 slices are, respectively, (cos t_1 + *i*sin t_1) and (cos t_1- *i*sin t_1), the sum gives you just cosine, and the difference gives you just sine. Together, they comprise a complex "FID" in the indirect dimension.

This add/subtract process is handled with command C2 which works in Arrayed Mode only, but can be accomplished in non-arrayed mode using a combination of commands. See below.

Phase correction in the direct dimension can be difficult, because the first slice may have little signal. If a 1D spectrum was acquired when the 2D experiment was done, the phase correction can be determined using the 1D spectrum, and then applied to the 2D data using PS. If not, phasing can be determined after processing.

Using Arrayed Mode, processing can be done using a macro (below) or simply by entering the following commands using the command line. NUTS-Pro versions newer than May, 2002, include a modified arrayed mode which allows processing in both dimensions after FT.

Users with the standard 2D version cannot use Arrayed Mode, and can process with a different macro (below).

See also: macros, 2D processing, displaying 2D data, phasing 2D data

Arrayed Mode processing from the command line

IM	import file called fid (for Varian data)
AR	enter arrayed mode
S#	set phase shift for sine multiplication to 90
BC MS MS	baseline correct & apply cosine squared window function
FT	
PS	phase if using pre-determined phase correction. Use PH instead to phase on first slice. If S/N is insufficient, skip phasing for now
C2	adds the reals and subtracts the imaginaries of pairs of slices
TD	transpose data
MS MS	
ZF	zero-fill (may be repeated if needed)
FT	may require phasing;
TD	to view data with direct dimension horizontal
MH	set minimum height to 2 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

Note that the processed data ***has not been saved*** at the end of this, and NUTS remains in Arrayed Mode.

Arrayed Mode processing using new PT feature (May, 2002)

Be sure you are not in arrayed mode, and open the data to be processed.

PT	set processing type to *2D_EchoAntiecho*
AR	enter arrayed mode (note that status bar says "pairwise complex arrayed

	mode"
S#	set phase shift for sine multiplication to 90
MS MS	apply cosine squared window function
FT	
PH	phase while viewing the first slice; on exiting phase routine, the entire data set is phased. If S/N is insufficient, skip phasing for now
C2	add/subtract pairs of spectra to create complex "fids" in indirect dimension
TD	transpose data
MS MS	
ZF	zero-fill (may be repeated if needed)
FT	may require phasing; see 2D phasing
(SR)	may be needed to make diagonal run in the conventional direction
PH	phase
TD	to view data with direct dimension horizontal
MH	set minimum height to 0.3 (good starting guess for contour threshold)
SS	set scale
IP	intensity plot

All quadrants of the data are saved, allowing phasing in both dimensions. Be sure to save the processed data.

With NUTS versions dated May 2002 to Nov 2002, you must enter the pairwise arrayed mode before re-opening the processed data, so that the data is sorted correctly when opened. Versions newer than Nov 2002 will read the PT parameter and automatically start the pairwise arrayed mode when the file is opened.

Macro for processing using Arrayed Mode

```
NUTSMACRO Varian g-hsqc in arrayed mode
; if 1st dimension phase correction is known, phasing should be added
after FT
; may need phase correction in indirect dimension after macro finishes
set array_on
set s# 90
bc ms ms ft
; combine data
c2
; transpose
td
; process using zero-fill and spectrum reverse
ms ms zf ft sr
td
set mh 2
ss
ip
end
```

Macro for processing without Arrayed Mode

Commands have been added to facilitate the addition/subtraction process. Two buffers, called B1 and B2, have been created. Typing B1 (or B2) copies the current data into Buffer 1 (or 2). Then typing B+ sums B1 + B2 and places the sum into the current data set. Similarly, B- does the same with the difference. However, by being a bit clever, it can be done without these commands.

It is recommended that users unfamiliar with 2D processing in NUTS begin by examining the explanation of HETCOR processing first, as many steps are the same and HETCOR is a simpler case. The processing required here differs from "normal" hypercomplex data only in the Link to process in the first dimension. It is recommended that users also read through the description of hypercomplex phase-sensitive 2D data.

NUTSMacro Varian echo-antiecho g_hsqc	determine phase correction before beginning
set s# 90 ask filea ga	window function used here is cosine squared, applied with **ms ms** after setting s#=90
set filec ft1.2d	macro requests name of data set to be processed
	data are saved as $\mathbf{I_x cos(wt_1)+I_y sin(wt_1)}$ and $\mathbf{I_x cos(wt_1)-I_y sin(wt_1)}$ We form the real half of the t_1 complex pair from (slice 1 + slice 2) and the imaginary half from (slice 1 - slice 2)
	(Note that this Link appears in the macro all on one line)
ga zf b1 ia ga zf b2 b+ ms ms ft ps tr b- ms ms ft ps ti st in	**GA** reads in the first slice **ZF** will zero-fill to the next higher power of 2 **B1** loads it into buffer 1 **IA** increments the extension for fileA **GA ZF B2** reads in the next slice, zerofills and loads it into buffer 2 **B+** adds the slices, then we apply window function, FT and phase **TR** "tags" this to be the real half of the complex FID in t_1 **B-** subtracts the contents of the buffers, then we apply window function, FT and phase **TI** "tags" this to be the imaginary half of the complex FID in t_1 **ST** saves the 2 halves, and **IN** increments the file pointers and loops to the beginning of the Link

set filec td.2d set filea ft1.2d set sl 1 ga td	set new file names and transpose data for processing in the second dimension
set filea td.2d set sl 1 ga set filec ft2.2d	read in transposed data, set file names
ga bc ms ms zf ft sr bc sc in	process 2nd dimension with cosine squared, 1x zerofill and spectrum reverse
set filea ft2.2d set sl 1 ga	reset file name, read in transformed data
set mh 4	set minimum height to 4 (good first guess)
ss ip	set scale, draw intensity plot
end	

Phasing can be done after running the macro. See 2D phasing.

2D Exchange experiments

This is a hypercomplex experiment in which the 2 halves of the data are scaled differently. See description of noesy processing for detailed description of hypercomplex data and how to process it in NUTS.

After the first dimension FT, the real half of each odd-numbered slice is combined with the real half of the following even-numbered slice, but the even numbered slices must first be scaled by a factor of -0.3. Except for this scaling, processing is essentially the same as other hypercomplex experiments.

Because the NUTS Arrayed Mode applies the same processing to all slices, Arrayed Mode cannot be used in processing this data.

The scaling is most simply accomplished by placing the data to be scaled into the add/subtract buffer with an appropriate factor, then adding the buffer to the current data.

To accomplish a scaling factor of -0.3, the multiplier (AM) is set to -1.3, and then the buffer is added to the current data (-1.3 + 1 = -.3) This is accomplished in a Link with

the ***al as*** commands. AL loads the buffer, using the value of AM as a multiplier, and AS adds the buffer to the data.

The Link for processing in the first dimension is:

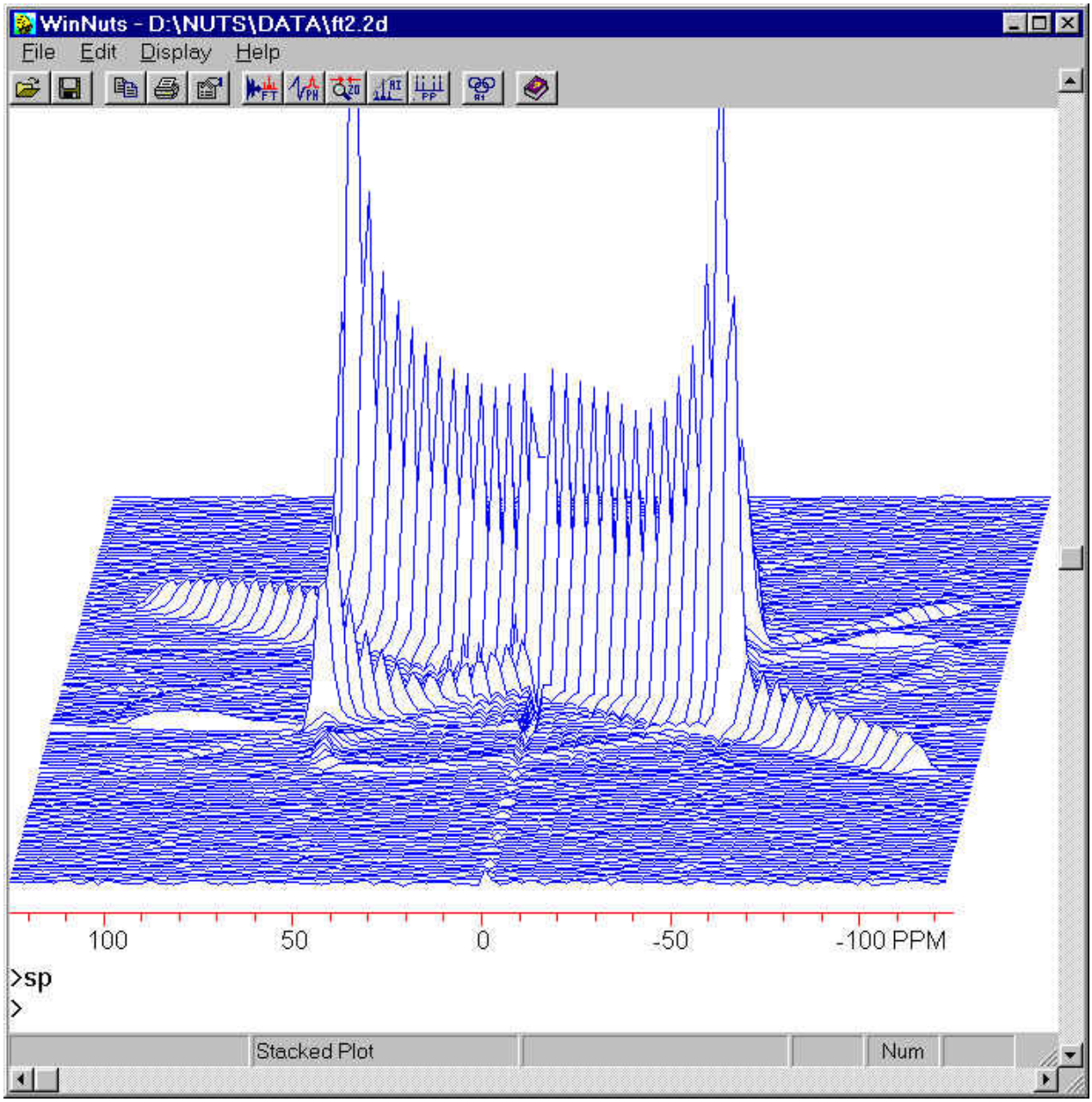
ga ls ls ls ls bc em ft ps bc tr ia ga ls ls ls ls bc em ft ps ***al as*** bc tr st in

Each cycle through the Link processes a pair of slices. Four left shifts (LS) are performed before the FT, because this is an echo experiment and data acquisition starts a bit before the echo.

The complete macro is shown here. Before beginning, open the data, set LB, FT and phase the first slice. At the end of the macro, phasing must be done in the indirect dimension. See phasing 2D data for description.

```
NutsMacro 2dexch
ask filea
set filec ft1.2d
set am -1.3
ga bc ls ls ls ls em ft ps bc tr ia ga bc ls ls ls ls em ft ps al as bc
tr st in

; reset file names and transpose data
set filea ft1.2d
set sl 1
ga
set filec td.2d
td

; reset file names and process 2nd dimension
set filea td.2d
set sl 1
ga
set filec ft2.2d
ga bc em ft sc in

; read in final data set and display stacked plot
set filea ft2.2d
set sl 1
ga
ss
sp
end
```

This is illustrated using a ^{2}H spectrum of d_6-dimethylsulfone (courtesy of Dr. Detlef Reichert, University of Halle, Germany).



For this spectrum, the direct dimension was processed using 100 Hz linebroadening and zero-order phase correction of 247.5 degrees. Phase correction in the indirect dimension was zero-order = -45.3 and 1st order = 89.2

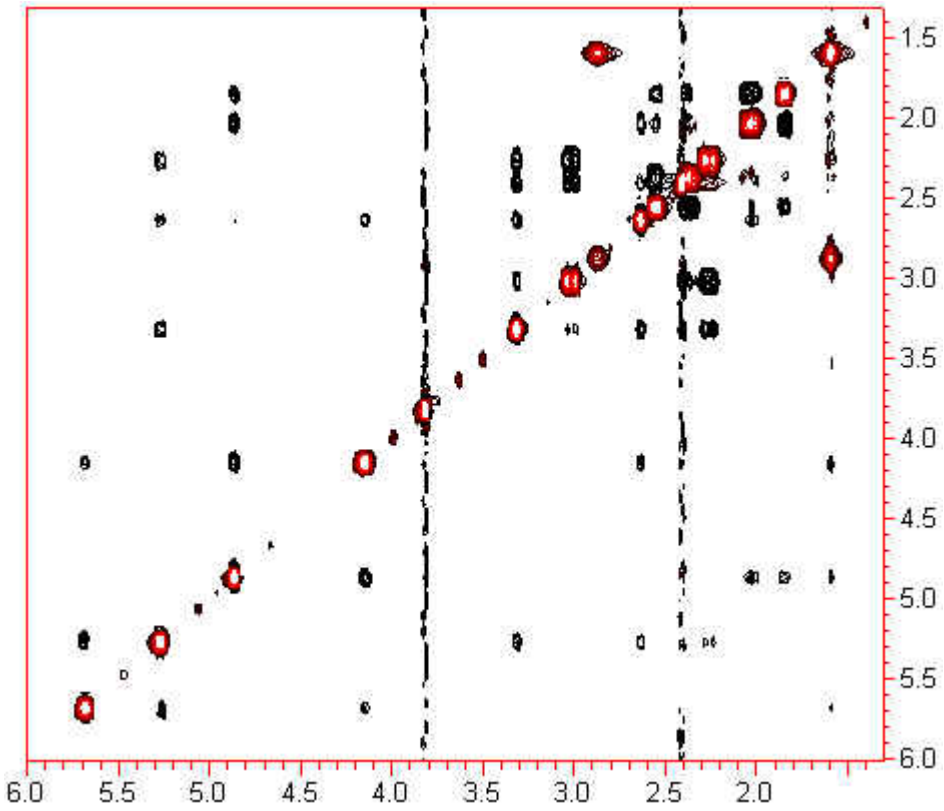
Note the low-intensity, elliptical exchange features. Dr. Reichert points out that ideally, the scaling eliminates the anti-diagonal, but some anti-diagonal remains, due to the anisotropy of the relaxation times T1Z and T1Q, preventing complete cancellation of the mirror images.

Reference: **J.Magn.Reson**, **79**, 269-290 (1988)

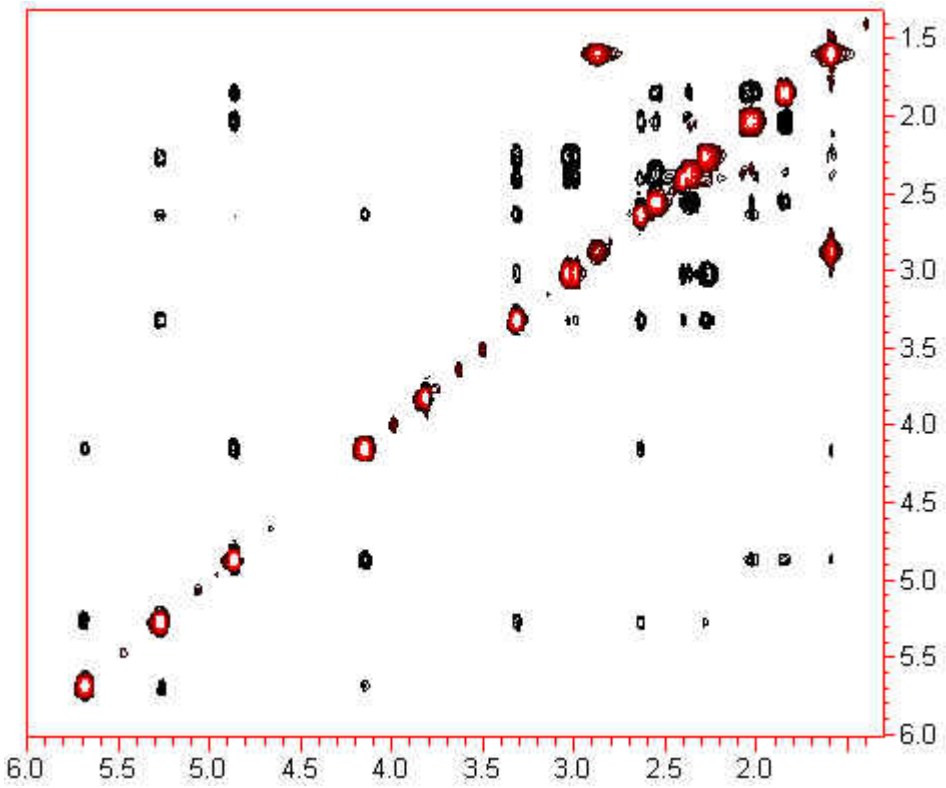
Editing 2D spectra

The data shown here is a phase-sensitive NOESY spectrum of codeine, displayed with the direct dimension horizontal.

Note the "t_1 noise" at the positions of the 2 methyls.

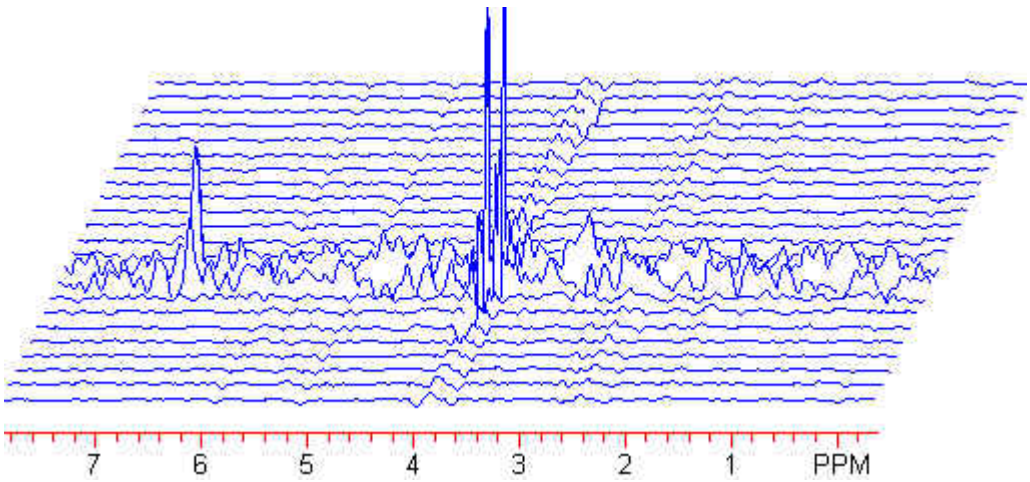


The noise causing the streaks exists in only 2 slices per methyl peak. The offending slices can be edited to give a much nicer plot.



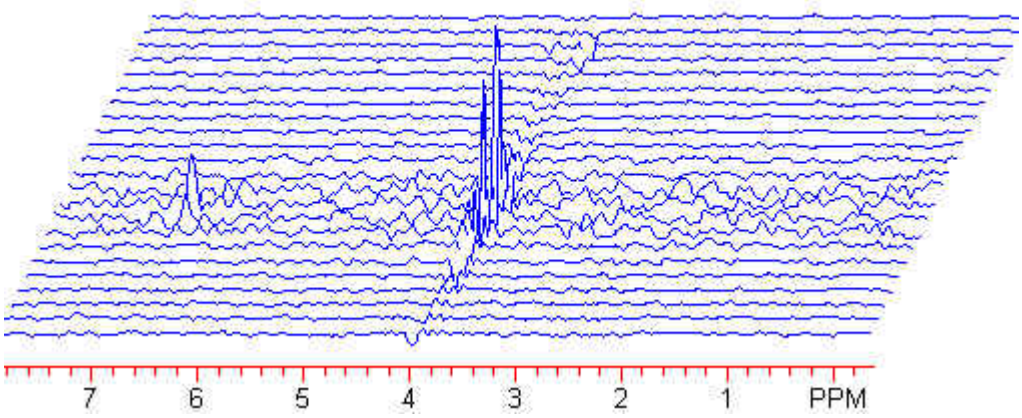
Because NUTS displays only horizontal slices, first transpose the data (TD) so the slices containing streaks can be accessed.

A stacked plot shows why we see streaks in the contour plot. The noise level in 2 slices is much greater than in the rest of the data. We don't want to zero out these slices, as they contain useful crosspeaks.



Use the View routine (VW) to step through slices and the Slice command (SL) to display a selected slice. Editing of selected slices is simpler to do in arrayed mode, because it is not necessary to save the data after editing each slice. (If not in arrayed mode, the S2 command is used to save after each modification - if you don't save, when you display a different slice, your changes will be lost.) As you look through the data, there may be slices with distorted baselines, which can be corrected with FB or QB.

To remove the streaks, we reduce the amplitude of all points in the 2 offending slices, and the noise level becomes more comparable to that of the rest of the data. This was done using the NUTS divide command, reducing the amplitude of all data points in the 2 selected slices by a factor of 4. This is sufficient to eliminate the streak from the contour plot without affecting the crosspeak.



In some cases, a single slice of a 2D data set may be corrupted. It may be possible to salvage the data by removing the offending slice. This can be done by zeroing all points in that slice (with ZE or, more conveniently, the View subcommand Z) or, usually better, substituting the adjacent slice for the one that is corrupted.

Arrayed Mode (NUTS-Pro only)

This is included in NUTS Professional version only.

May 2003 - A preliminary 4D arrayed mode was created.

May 2002 - arrayed mode was modified to allow phase correction of processed data in both dimension.

The entire 2D data set is placed into memory, resulting in much faster 2D processing (a factor of ~12 in our tests on Windows, and a factor of ~40 on Macs). The "normal" way

of processing 2D data is by looping through a command string, applying the commands to each slice in turn. With the Arrayed Mode, the entire data set is placed into memory, and each command operates on the entire 2D data set; *i.e.*, typing FT one time transforms the all slices.

The amount of available memory needed is ~2.5 times size of the 2D data set being processed. If sufficient memory is not available, NUTS will use virtual memory, which is very slow.

As new 2D processing features are added to NUTS, many function only in the arrayed mode.

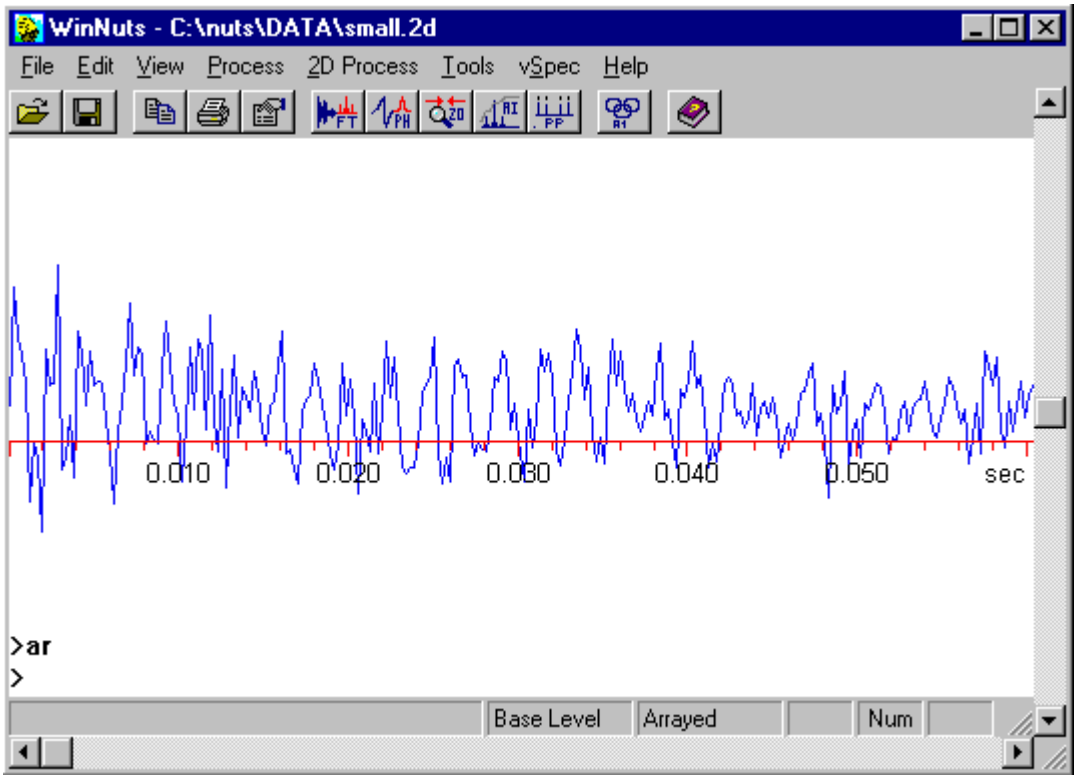
See step-by-step processing instructions for different types of 2D data, including sample macros.

AR – Arrayed Mode

The **AR** command (not yet in menus) is a toggle, and turns on/off arrayed mode. In a macro, you can also use

Set array_on
Set array_off

The only indication you will have that Arrayed Mode has successfully been entered is the appearance of the words "Complex Arrayed" or "Pairwise Complex Arrayed" on the gray status bar. On exiting the Arrayed Mode, this will change to say "Non-Arrayed".



Most commands are "array-aware", meaning that if in arrayed mode, NUTS will apply the command to all slices. However, some commands apply only to the displayed slice. It is important to keep track of when NUTS is in the arrayed mode, and understand whether a given operation affects the entire data set or a single slice.

Type **AR** to enter Arrayed Mode. Process in the first dimension with commands such as

GA BC MS FT

These commands can be typed directly, or can be executed as a Link or a Macro. Note that there is no SC command and no IN command.

Phasing using any of the phasing commands will display just one slice while phasing, but on exiting the phasing subroutine (PE or PH), the correction is applied to all slices. See phasing details.

Next, execute **TD** to rotate the matrix in preparation for processing in the second dimension. Execute an appropriate series of commands, such as

MS FT MC

Typing AR exits Arrayed Mode. You will be asked if you want to save the data before exiting Arrayed Mode, which you should do, or you will lose the processing just completed.

Note that you cannot go back to the previous step, as the data is not saved at each step, as is the case when processing in the "regular" mode. Of course, the original data is unchanged, so it is always possible to go back and start over. If UnDo is turned on, typing Ctrl-Z will undo the last command. However, it is not recommended to use UnDo in arrayed mode because of the large amount of memory required.

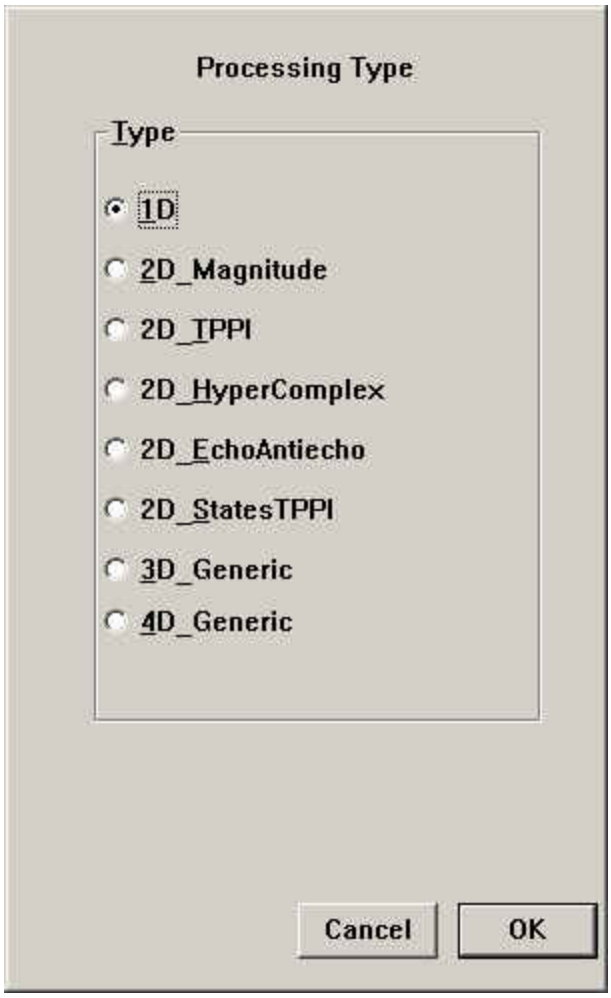
After processing the second dimension, you can execute another TD command to view the data with the first dimension displayed horizontally.

PT – Processing type

Starting in May, 2002, the Arrayed Mode can be configured for different Processing Types, depending on what kind of 2D data is to be processed. This allows phase-sensitive data acquired as TPPI, hypercomplex, echo-antiecho or States-TPPI to be processed while maintaining complex data correctly in both dimensions, so that the data can be phased in both dimensions after processing.

In the past, NUTS discarded the imaginary half of the data following FT in the direct dimension. This made it impossible to phase the data in the direct dimension after further processing. Ability to phase the frequency-domain data requires that the imaginary half of the data be carried through the entire processing operation, and that the Transpose operation correctly sort the data to maintain complex data pairs in each dimension.

For hypercomplex, echo-antiecho and States-TPPI data, before entering Arrayed Mode, type **PT** (Processing Type), which displays this dialog box:



The important changes involve setting Processing Type to either 2D_HyperComplex or 2D_EchoAntiecho, for processing the corresponding type of phase-sensitive data.

2D_HyperComplex is for processing hypercomplex data (method of States, et al).

2D_EchoAntiecho is for processing gradient data acquired as echo-antiecho (also referred to as pn data).

2D_StatesTPPI functions the same as 2D_HyperComplex.

By default, 1D is chosen if the data set consists of only 1 slice, or 2D_Magnitude if the data contains more than 1 slice. 2D Magnitude is identical to the Arrayed Mode in previous versions.

For 2D_TPPI mode, it is not necessary to set the Processing Type from this screen, because additional manipulations are needed. See details.

If one of the 3 modes, 2D_HyperComplex, 2D_EchoAntiecho or 2D_StatesTPPI, is selected, then when arrayed mode is entered, the data is sorted and displayed in "Pairwise Complex" arrayed mode.

If PT is not set, entering arrayed mode with the AR command will treat the data as magnitude data, and phasing after processing will not be possible.

Previously, the value NUTS displayed for data size in the indirect dimension (number of slices) was the total number of slices, not number of complex slices. Hypercomplex, echo-antiecho and States-TPPI data consist of pairs of FIDs (2 FIDs acquired for each t_1 value). For example, if 128 t_1 values were collected, NUTS would display the total number of slices as 256 (2 FIDs for each of the 128 values). In "Pairwise Complex" arrayed mode, NUTS reports the data size in the indirect dimension as 128 complex points, and would display only 128 points (the first of each pair of FIDs).

Important: As of Nov, 2002, Nuts will look at the PT value when a file is opened, and enter/exit arrayed mode, as appropriate.

With previous versions, to open a data set that had been saved while in the "Pairwise Complex" arrayed mode, you must first re-enter arrayed mode. This is because of the data sorting required to display the data properly. Similarly, you must exit "Pairwise Complex" arrayed mode before importing or opening data that has not been saved from within that routine. Exit AR, open the data set, choose the correct processing type using PT, then type AR to enter the correct arrayed mode.

The details of the data sorting that is performed in Pairwise Complex arrayed mode, and some additional commands, can be found here.

In macros, the Processing Type can be set to any of the values shown in the figure above. This must be done in the non-2-letter command mode. For example,

```
2f
pt 2D_hypercomplex,
2n,
```

The comma at the end of lines while in non-2-letter command mode are interpreted as an <ENTER>, and are required.

Processing instructions (see also: Processing 2D data)

Hypercomplex data

Import or open the 2D data file, type **TP** and select *2D_Hypercomplex*. Click on **OK** to close this box. Type **AR** to enter the "Pairwise Complex" arrayed mode. Apply an appropriate window function, FT and phase. Type **TD** to transpose the data. Apply an

appropriate window function, zerofill if desired, FT and phase. You can go back and forth between the dimensions with TD, and phase in each.

Note that the "tr tr st" series of commands is not used in this mode.

States-TPPI data

Import or open the 2D data file, type **TP** and select *2D_StatesTPPI*. Click on **OK** to close this box. Type **AR** to enter the "Pairwise Complex" arrayed mode. Apply an appropriate window function, FT and phase.

Before transposing, it is necessary to invert every other slice. To do this, type **2f** to exit the 2-letter command mode, and type **invert even <ENTER>**. Then return to 2-letter command mode by typing **2n <ENTER>**.

Type **TD** to transpose the data. Apply an appropriate window function, zerofill if desired, FT and phase. You can go back and forth between the dimensions with TD, and phase in each.

Note that the "tr tr st" series of commands is not used in this mode.

Echo-antiecho data

Import or open the 2D data file, type **TP** and select *2D_EchoAntiecho*. Click on **OK** to close this box. Type **AR** to enter the "Pairwise Complex" arrayed mode. Apply an appropriate window function, FT and phase.
Type **C2** to add and subtract each pair of spectra.
Type **TD** to transpose the data. Apply an appropriate window function, zerofill if desired, FT and phase. You can go back and forth between the dimensions with TD, and phase in each.

TPPI data

First be sure you are in non-arrayed mode, and open the unprocessed data. Then type (in the non-2-letter command mode):

ar tppi2pairwise

This does 2 things. The pairwise arrayed mode is entered, and zeroes are loaded into the "B" half of the data. The process type is automatically set to "twoD_TPPI", and the TD will do no swapping of data pairs.

Be sure to use RT (real transform) in the indirect dimension!

Phase-sensitive arrayed mode (NUTS-Pro only)

New commands created in association with modified arrayed mode, May 2002

These are non-2-letter commands that give the user control over data sorting and allow viewing of data quadrants not normally displayed. None of these commands is necessary for correct processing of data, but may be useful in some situations.

Hypercomplex, echo-antiecho and States-TPPI experiments all acquire 2 FIDs for each t_1 value, and hence are referred to here as "pairwise" data. A new command, **array 2** (also referred to as "Pairwise Complex Arrayed Mode") was created to work with data so as to preserve all data quadrants during processing, which is necessary to allow phasing in both dimensions after processing.

For the purposes of the following descriptions, a notation has been devised. The first FID of each pair is labeled as the **A** data set, the second is labeled as the **B** data set. Each has both real and imaginary halves, referred to as **A.r**, **A.i**, **B.r** and **B.i**. (The conventional notation of RR, IR, RI and II can become confusing when considering operations such as the pairwise adding and subtracting done when processing echo-antiecho data, and this notation seems easier to follow.)

When the Processing Type (**PT**) is set to 2D_Hypercomplex, 2D_EchoAntiecho or 2D_StatesTPPI, when arrayed mode is entered (**AR** command), NUTS actually enters the **array 2** mode. Data is sorted such that odd-numbered slices become **A** and even-numbered slices become **B**. Only the real half of the **A** data set is displayed (**A.r**).

Note that this means that, in Pairwise Complex arrayed mode, NUTS counts the data size in the indirect dimension (number of FIDs) as ***complex*** points, and not as total number of slices, as it has previously done.

The **RI** command can be used to swap the real and imaginary halves of the data, allowing **A.i** to be displayed.

AB – Swap quadrants in phase-sensitive arrayed mode

To view the **B** half of the data, it must first be swapped with the **A** half, as only the **A** half can be displayed. A new command called **AB** has been created. **AB** takes 2 arguments which are the labels of the 2 quadrants to be swapped. The labels are **ar**, **ai**, **br** and **bi**, corresponding to **A.r**, **A.i**, **B.r** and **B.i**, respectively. For example, to display the **B.r** data, type

ab arbr <enter> (note no space between ar and br)

It is important to keep track of what you've moved where!

The TD command (Transpose Data) can take one of 3 arguments to specify how the quadrants are sorted during the transpose. If the Processing Type has been set correctly, the argument is not needed; TD without any argument will execute the appropriate sorting.

TD hyper - for hypercomplex and States-TPPI data. This swaps **A.i** and **B.r** during the transpose.

TD echo - for echo-antiecho data. This swaps **A.i** and **B.i** during the transpose.

TD noswap - does no swapping, just transposes each quadrant separately.

Extension of the new arrayed mode to handle TPPI data was done in Nov, 2002.

TPPI data is phase-sensitive, but it is not "pairwise" data - only one FID is acquired per t_1 value - so we don't want to split the data into **A** and **B** halves. The FIDs comprise the **A** half of the data, and we want all zeroes in the **B** half. Nuts has been modified to do this with the command

ar tppi2pairwise

This fills out the **B** half of the data with zeroes, enters the pairwise arrayed mode, and sets process type to 2D_TPPI.

Process as usual (window fcn, FT, phase, BC). The transpose needs to be done without data sorting, which is handled automatically, based on the PT setting.

Process in the indirect dimension as usual for TPPI data (using Real FT !!).

2D phasing

Phasing is done differently depending on whether or not NUTS is in the Arrayed Mode. In Arrayed Mode, the entire data set is in memory, and commands are applied to all slices simultaneously. Arrayed Mode is available in NUTS Professional version.

Arrayed mode

Phasing in the first dimension can be done with either the PH or PE command. This is done while viewing the first slice. When the phase has been adjusted, exiting the phase routine applies that correction to all slices.

Phasing in the indirect dimension is a bit different, because usually any given slice has only one peak of significant intensity, so is not sufficient to determine 2 phase correction

parameters. Start by choosing a slice that has a peak with good signal-to-noise near one end of the spectrum.

(This is most simply done by displaying an intensity plot and viewing slices in real time. Hold down left mouse button to display a horizontal cursor, then press the right mouse button simultaneously (or type period key on keyboard) to display slices at the cursor position).

Once a slice is selected, exit the IP routine so that the chosen slice is displayed as a 1D spectrum. *Place the pivot point on the large peak in this slice.* (See PH for setting pivot point). Now enter PH and phase this peak using ***only*** zero-order (left mouse button) correction. Hit <Enter> to exit and apply this zero-order correction to the entire data set.

Now repeat the slice selection process, this time choosing a slice with a large peak near the opposite end of the spectrum. Enter PH and phase using ***only*** first-order (right mouse button) correction. Hit <Enter> to exit and apply this first-order correction to the entire data set. Now the whole data set should be correctly phased.

"Pairwise" Arrayed mode

NUTS-Pro versions dated May 2002 and newer allow processing of phase-sensitive data such that all quadrants of data are retained, and the data can be phased in both dimensions after processing. This is done as described above for the indirect dimension. See details of the pairwise arrayed mode.

Non-arrayed mode

Direct dimension - The first step in processing is to import the data and process the first slice, including phase correction. Then a macro (or link) is run that executes a series of commands on each slice, and the PS (phase same) command is used to apply this phase correction to all slices.

To determine phase parameters in the second dimension, we need a spectrum with peaks near both ends of the spectrum. This is usually accomplished by adding together 2 slices from opposite ends of the spectrum. To find which slices contain signal, display an intensity plot (IP), and view slices in real time (hold down left mouse button to display a horizontal cursor, then press the right mouse button simultaneously to display slices). Note slice numbers of slices with good signal-to-noise, then exit the IP routine with ENTER. Read in the first slice of interest (with SL) and place this slice into the Add/Subtract buffer (with AL). Read in the second slice. Enter the Add/Subtract routine (AS), add the 2 spectra (with plus sign) and exit the subroutine with ENTER. Now phase this spectrum, then note the phasing applied (TP). Enter the values of zero- and first-order phasing into the PA and PB parameters. Then execute this Link **ga pc sc in** to apply this phase correction.

Phasing the direct dimension after FT of both dimensions

Sometimes it is necessary or desirable to adjust phasing of the direct dimension after FT of both dimensions.

For NUTS-Pro versions older than May 2002, (Nov 2002 for TPPI data) and for NUTS-2D versions, phasing after processing poses a problem, because NUTS discards the imaginary half of the direct dimension spectra after processing. However, there is a procedure to accomplish this.

Make sure the data is displayed with the direct dimension on the horizontal axis. Use a Hilbert transform (HT) to generate a "FID" that has both real and imaginary parts. Then use FT to recreate a spectrum that has complementary real and complex parts, and can be phased in the usual way (as described above).

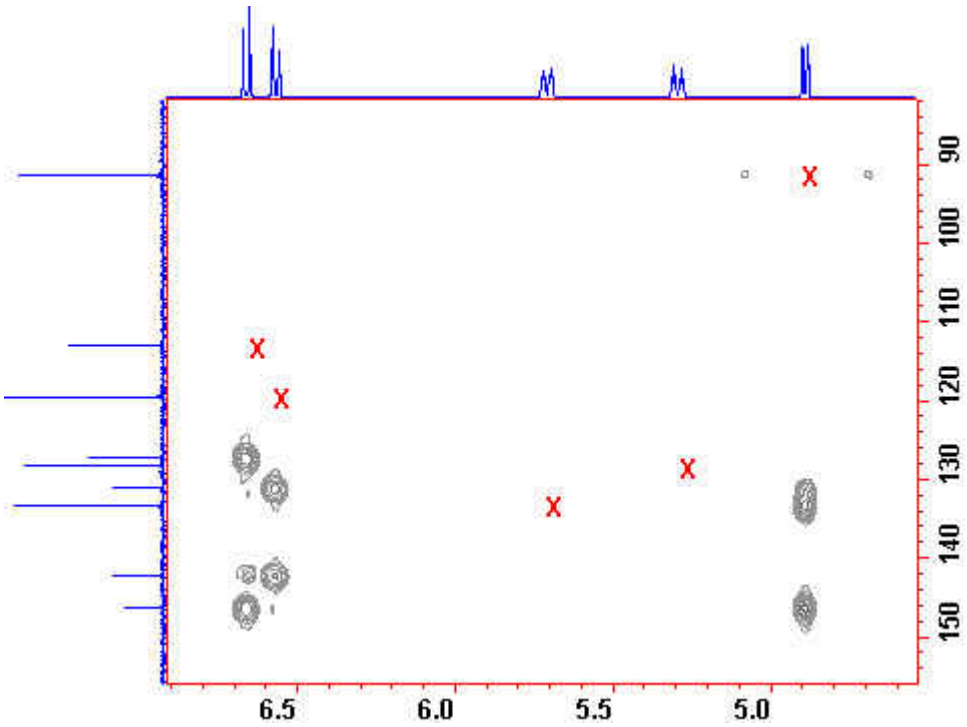
Comparing 2D spectra

Compare – Overlaying 2D data

NUTS has the ability to display a "mask" of peak positions overlaid on a 2D plot. This is a "non-2-letter" command and requires NUTS-Pro operating in the arrayed mode.

The "mask" is created by opening a dataset and picking peaks using the DP subroutine. After all peaks are picked, the file is saved. The peak list is automatically saved in the file.

Then the second file is opened, and 2D mode entered. Compare is used to display the peaks from the first dataset overlaid on the contour plot of the second dataset. This is illustrated below for HMQC and HMBC spectra of codeine. The HMQC data file was opened, peak-picked, and saved. Then the HMBC file was opened, the contour plot displayed, and **compare -m X** typed. The previously saved HMQC file was selected, and its peaks are indicated with X. (The font is set using a currently undocumented command, **J6**.)



The compare command can take one or more arguments, which are specified in a unix-like syntax.

compare ask for a filename for 2D comparison
compare <filename> compare to specified file
compare -on or **compare -n** turns on compare display when doing 2D display
compare -off or **compare -f** turns off compare display when doing 2D display
compare -clear or **compare -c** clears the 2D comparison list
compare -marker X or **compare -m X** set marker letter for this file to "X" and ask for a filename
compare -m X <filename> compare to filename using marker "X"

If no filenames are given in the command line, a dialog box will be presented to the user allowing selection of a filename for the compare operation. This is true regardless of whether a -m letter assignment is given. If a -m letter assignment is not given by the user, then the first comparison file uses the letter "A", the second comparison file uses letter "B" and so on.

3D Processing

Overview of 3D

We have tested this only on a single 3D data set from a Bruker spectrometer. Users with data from other systems are encouraged to supply sample data sets for us to test.

Processing of 3D data is done in a new *3D Arrayed Mode*. Many of the commands necessary for 3D processing are non-2-letter commands, so the user must enter this mode with the **2F** command. In this mode, commands are not executed until <Enter> is typed.

DIMS – Specify number of data points in each dimension

Because the import routine may not recognize 3D data , the data may be imported as though it were 2D data. Thus it is necessary to change parameters to define the number of data points in each dimension. This is done with the new **dims** command. Typing just dims <enter> will print out the number of data points in each dimension. To change the values, type

dims i j k l

where i=number of (complex) points in the first dimension, j=number of (complex) points in the second dimension, k=number of (complex) points in the third dimension and l=number of (complex) points in the fourth dimension (which for now is 1).

The total number of points (i x j x j x l) must remain unchanged, or the process will abort.

Acquisition parameters can be viewed for all 3 dimensions by selecting Spectral parameters from the View menu.

Processing is done in a manner very similar to 2D. The main difference is that when data are transposed for processing and display along a different dimension, it is now necessary to specify which dimension will be transposed. A general procedure is described below, and specific steps for one particular data set are described in the example below.

Processing normally begins by opening the data file and examining the first slice, setting parameters for window functions, doing FT and phasing. Then the 3D arrayed mode can be entered. To do this, first execute **2F** to change to non-2-letter command mode, then type

AR 3D <enter>

This will load the entire 3D data set, so will take several seconds. Now, each command will be executed on the entire data set, not just a single slice. For example,

MS
FT
PS

will apply sine apodization, FT and phase with previously determined phase correction. The appropriate steps needed will depend on the particular experiment, as it does with 2D.

Next, we need to transpose the data to process in the second dimension. The TD command now requires an argument to specify which of the other 2 dimensions will be transposed with the current dimension. So

TD 2

will display interferograms along the second dimension, and processing proceeds in essentially the same manner as 2D data.

As the different dimensions are transposed, it is necessary to keep track of which is which. View/Spectral Parameters will display 3 columns of parameters, one for each dimension. The user can change the label for each to something more meaningful. The first (left-hand) column always corresponds to the currently "active" dimension, whose points are displayed from left to right. The second (middle) column is dimension 2, which are the different slices. A contour plot or stacked plot will display dimension 1 horizontally and dimension 2 vertically on the screen. The third column of parameters is for dimension 3, which can be visualized as a stack of 2D planes.

Plane – Display a plane of a 2D data set

At the moment, Nuts has no way to display the third dimension. Instead, a particular plane is chosen and a 2D plot (contour or stacked plot) of dimensions 1 and 2 is displayed. While in the contour or stacked plot display mode, the sequence of planes can be stepped through using the [and] commands, which (like all commands in subroutines) are executed immediately, not requiring <enter>. A particular plane can also be specified with the **plane** command, for example:

plane 7

ACQORDER – Specify the acquisition order for multi-dimensional data

acqorder has a syntax similar to the **dims** command, and allows the user to rearrange the order of the different dimensions.

A step-by-step example of processing a 3D data set is given in the following example.

Example of 3D processing (ubiquitin)

Processing a 3D CBCA(CO)NH experiment

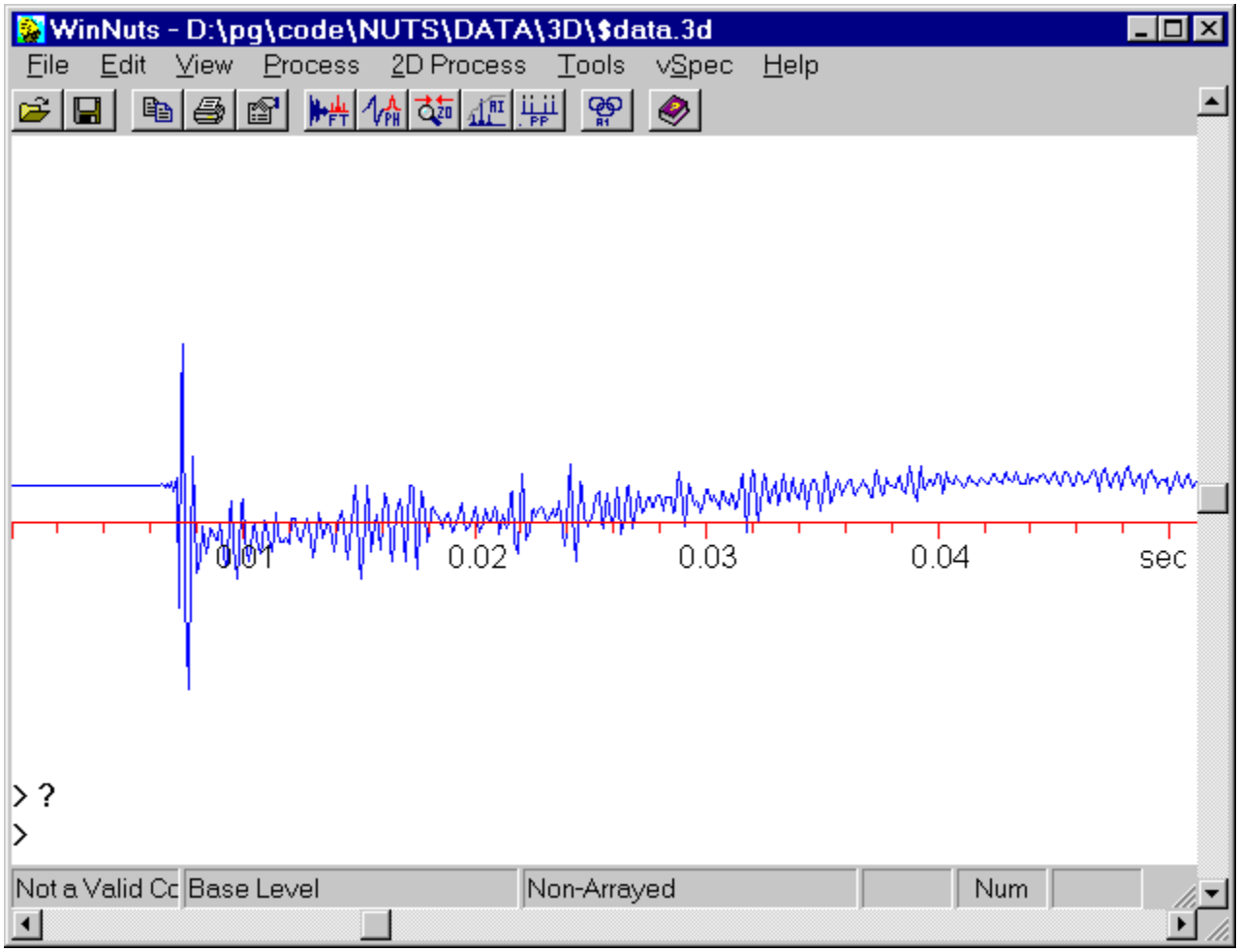
The sample was $^{13}C,^{15}N$-ubiquitin run on a Bruker 600. (Data kindly supplied by DuPont Pharmaceuticals.)

The data set is ^{1}H-detected (512 complex points), with ^{13}C in the second dimension (110 total slices, hypercomplex) and ^{15}N in the third dimension (96 total slices, hypercomplex) for a total size of 43 MBytes. It was acquired with digital filtering, so needs to be processed with RD. There is also a residual water signal which needs to be removed to avoid some truncation artifacts, so we use a digital high-pass filter (DH). An illustration of the truncation artifacts is shown below.

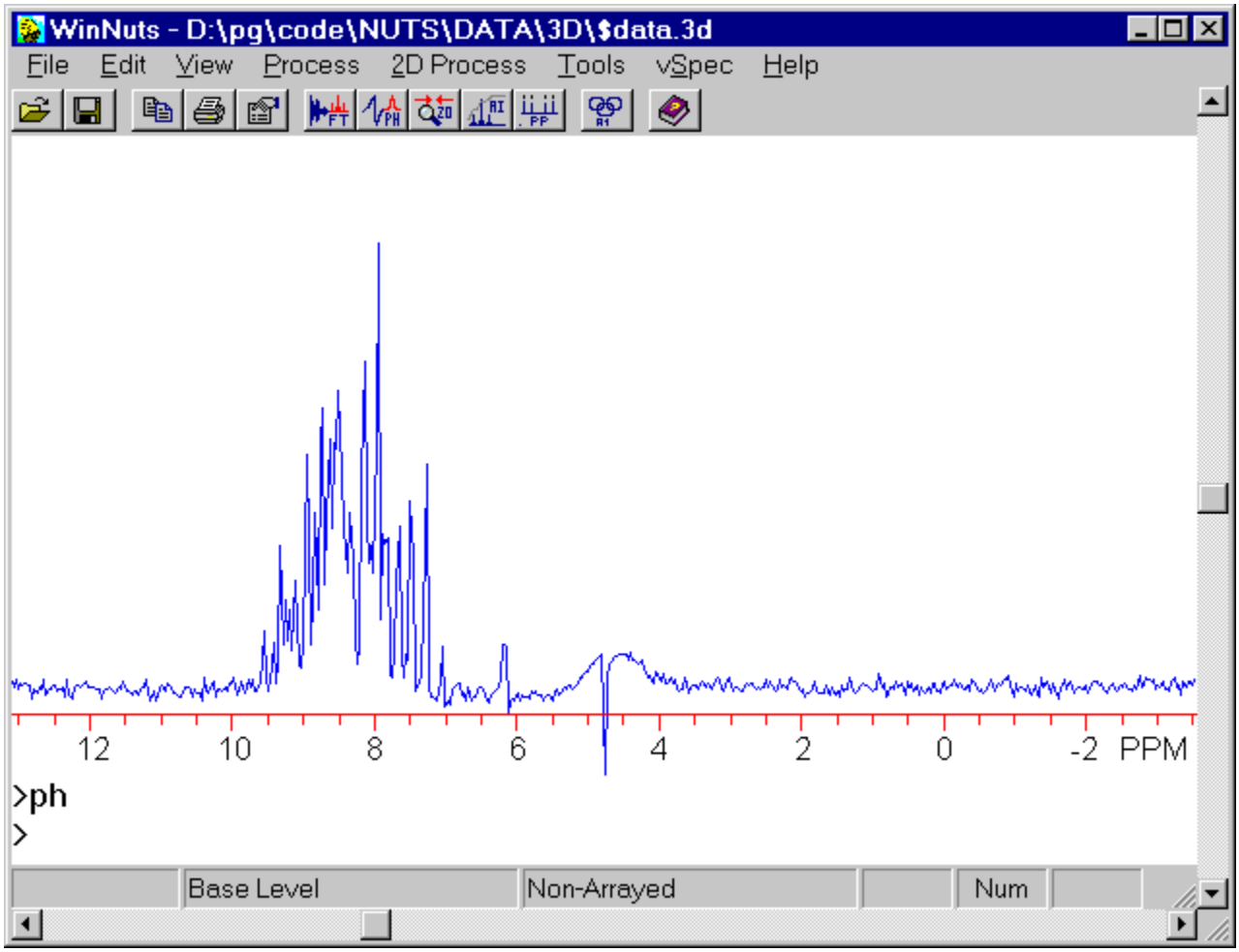
See macro for complete processing with a single command, but this can also be done from the keyboard, as described below.

Using a macro, processing took *42 seconds* on a Pentium II/400, including RD and DH operations.

The data was imported with IM, which finishes by displaying the first slice.



Processing parameters are determined by processing the first slice. The parameters used were cosine multiplication (set S# to 90, them MS), RD to correct for artifacts of the digital filter, DH (filter width of 250 Hz) to remove the residual water signal, FT and phase. It is important to examine the FIDs and tailor processing to avoid artifacts such as truncation. (See example below.)



The data was nominally a 2D data set, with 512 complex points and 10,560 slices (110 x 96) in a Bruker ser file. Nuts imported this as a 2D file, so the dimension sizes must be set correctly. To do this, enter non-2-letter command mode by typing 2F, and then use the dims command.

dims <enter>

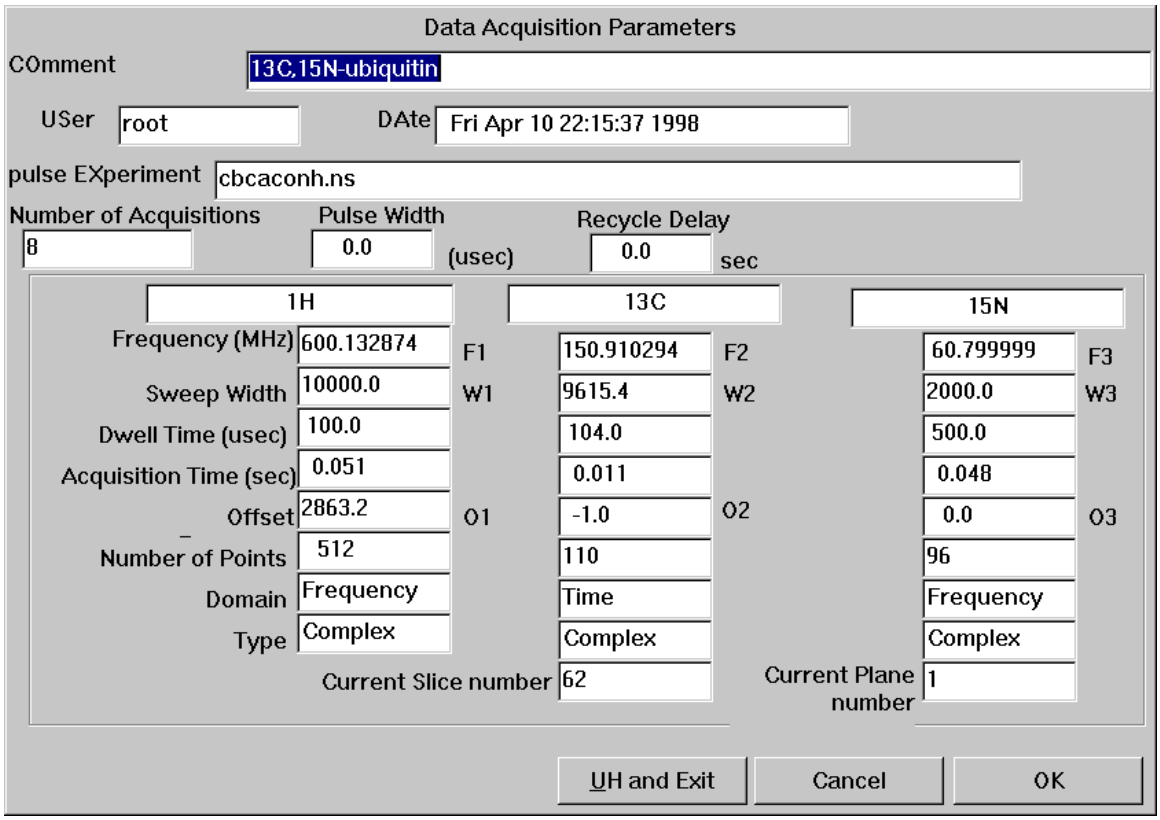
will print out the number of points in each dimension, which for this data set is

dims - Points 1D = 512 2D = 10560 3D = 1 4D = 1

To set these correctly, use dims and enter the 4 values, all on one line, separated by spaces:

dims 512 110 96 1

Once a value greater than 1 is entered for dimension 3, the View/Spectral Parameters menu will display parameters for all 3 dimensions:



Note that the 3rd dimension did not contain correct values for SF and SW. This is because the raw data did not include any 3D parameters. The correct values were entered using this screen.

We are now ready to process. The first step is to enter "threeD arrayed mode", by typing

ar 3d <enter>

The following series of commands is executed. While not specified, each command is terminated by <enter>. See Nuts Help for explanation of each command. (Remember that the data is hypercomplex in both indirect dimensions.)

ms
rd
dh
ft
ps
tr
tr
st

Now we are done with the proton dimension, and need to transpose the data to process the carbon dimension. Because we will need to "build" complex interferograms in the nitrogen dimension (using tr tr st commands), we need the carbon dimension to be dimension 1 and the nitrogen dimension to be dimension 2. This is accomplished by 2 successive TD commands. The TD command, when working with 3D data, takes an argument to specify which dimension will be transposed with the current dimension 1.

td 3

This puts the proton as dimension 3 and nitrogen as dimension 1.

td 2

This puts the carbon as dimension 1 and nitrogen as dimension 2. Now process with

ms
ft
tr
tr
st

This data was collected to require no phasing in the carbon and nitrogen dimensions. We have constructed our complex interferograms in the nitrogen dimension, so we transpose to process the nitrogen dimension.

td 2

And process with

ms
ft

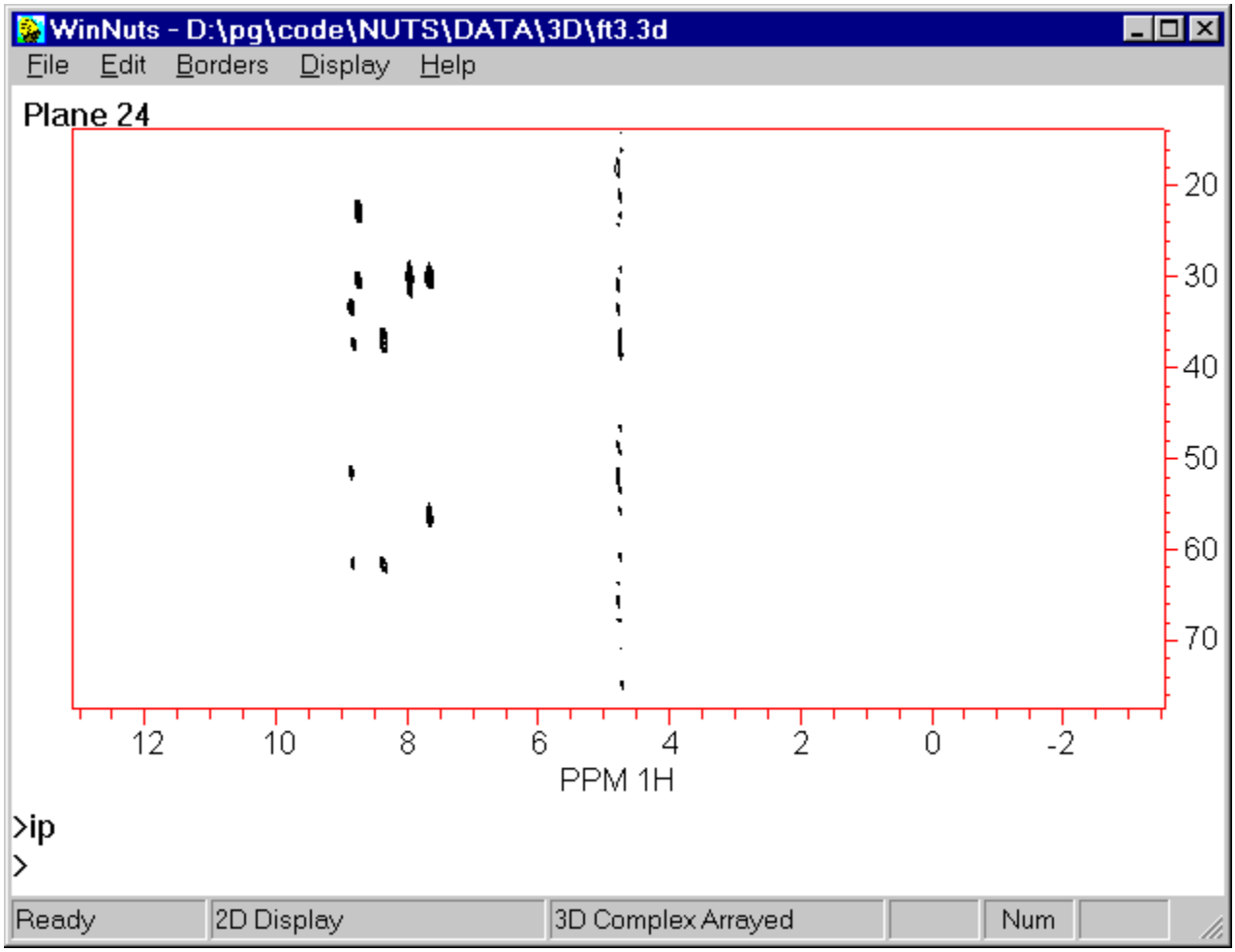
And a final TD to put the nitrogen back to dimension 3 and display protons as dimension 1:

td 3

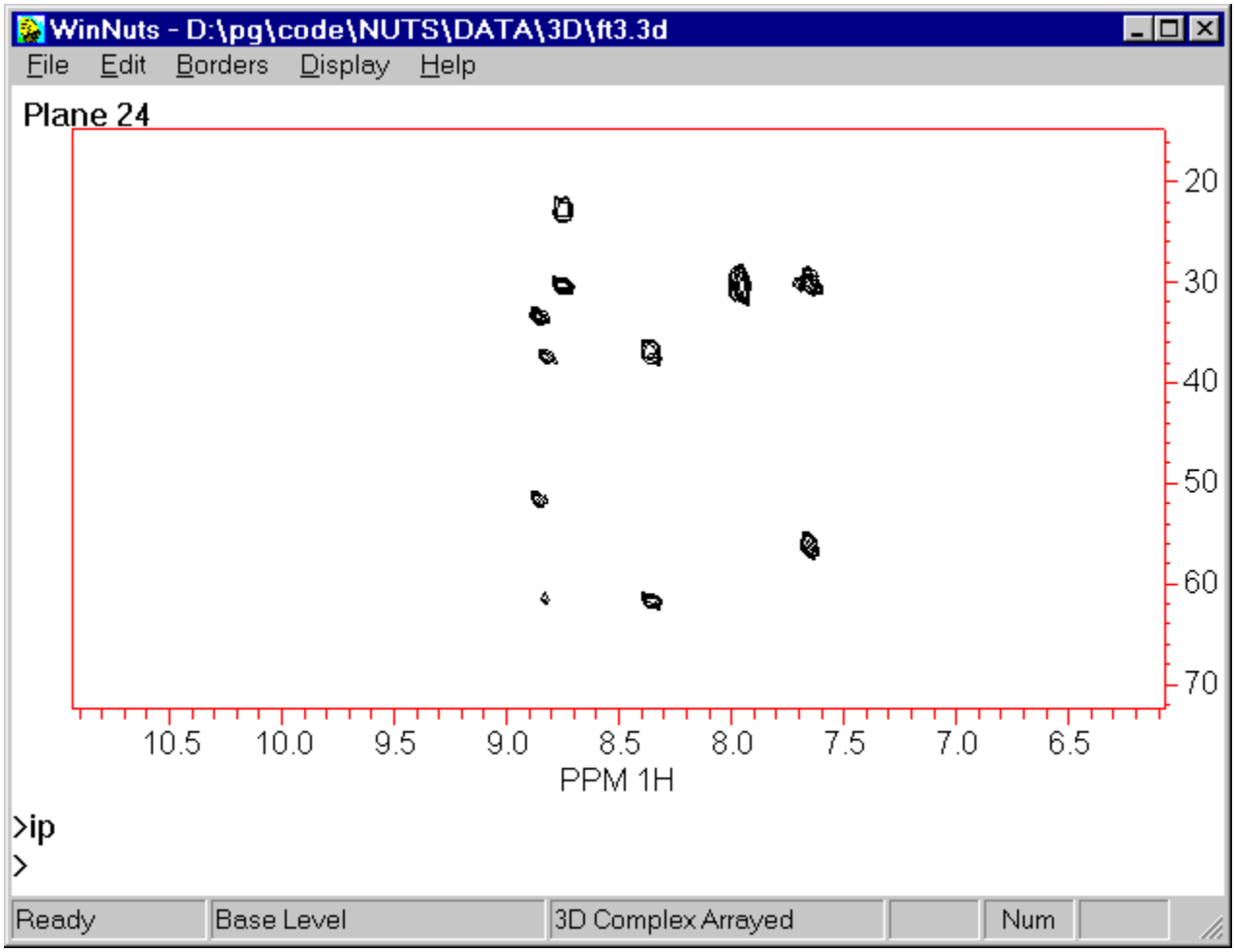
Now we are ready to view the data. IP will display the intensity plot, and the commands

plane +
plane -

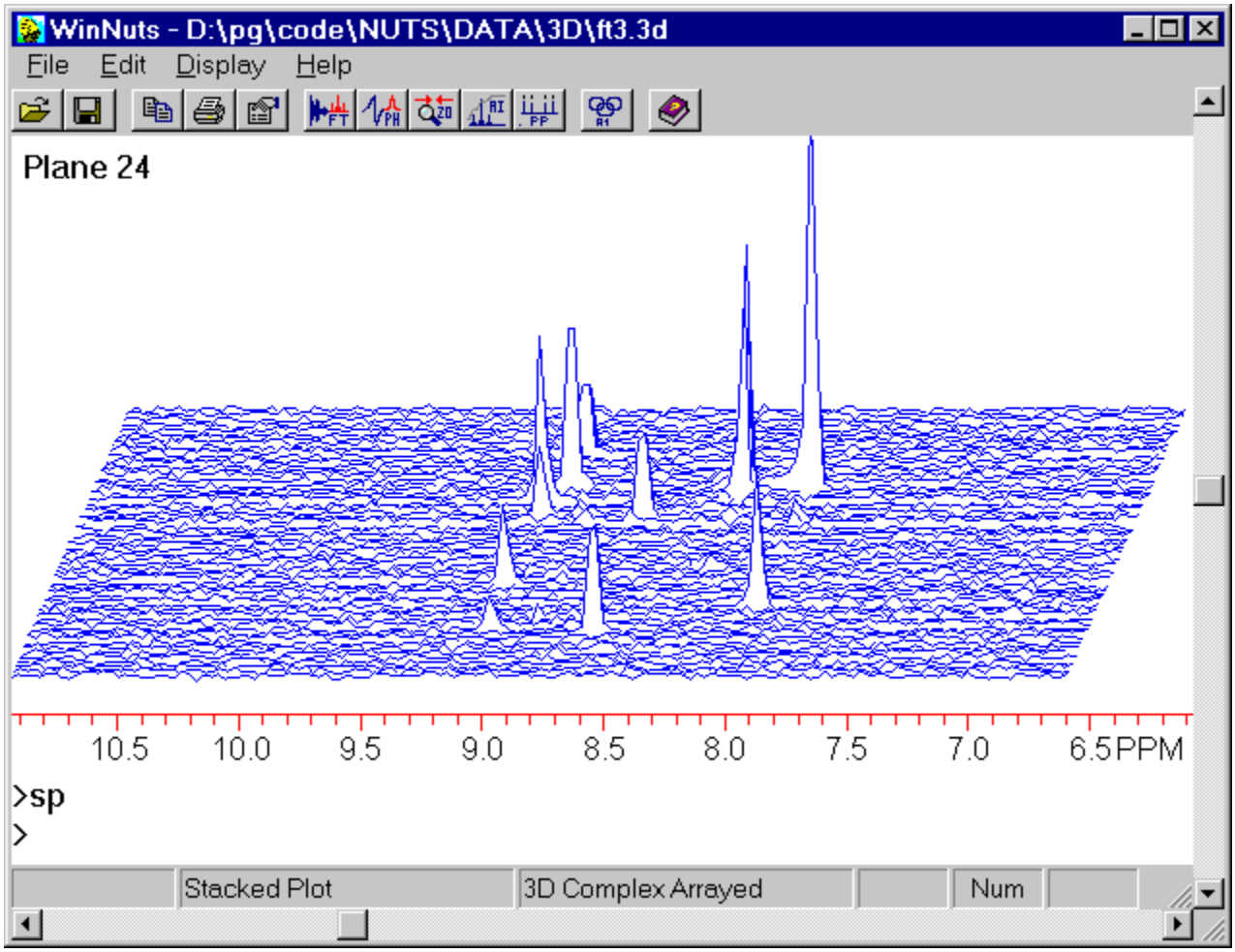
will step through ^{15}N planes. The number of the currently displayed plane is shown in the upper left corner of the screen.



The zoom subroutine works just as for 2D data to expand the display.



Exit from the IP routine and use SP to view stacked plots. The] and [keys will step through planes.

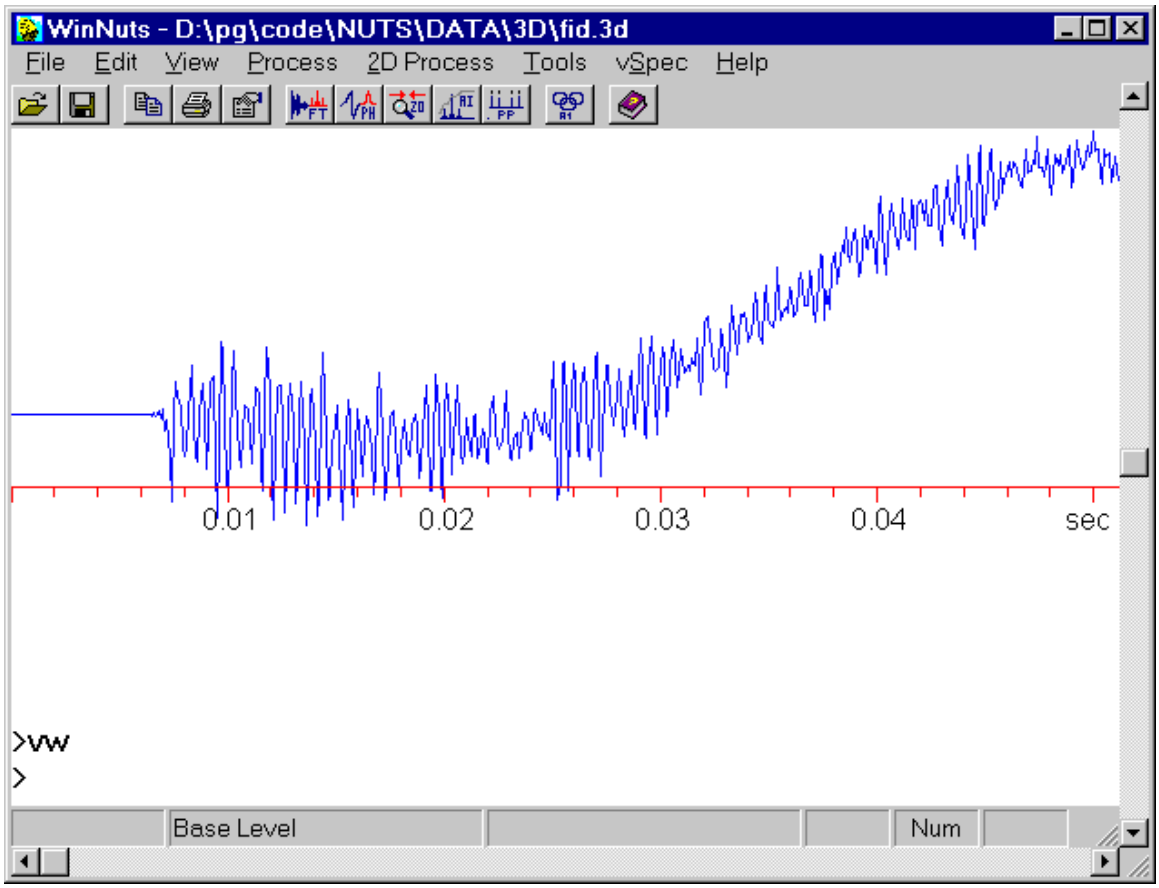


Truncation problems

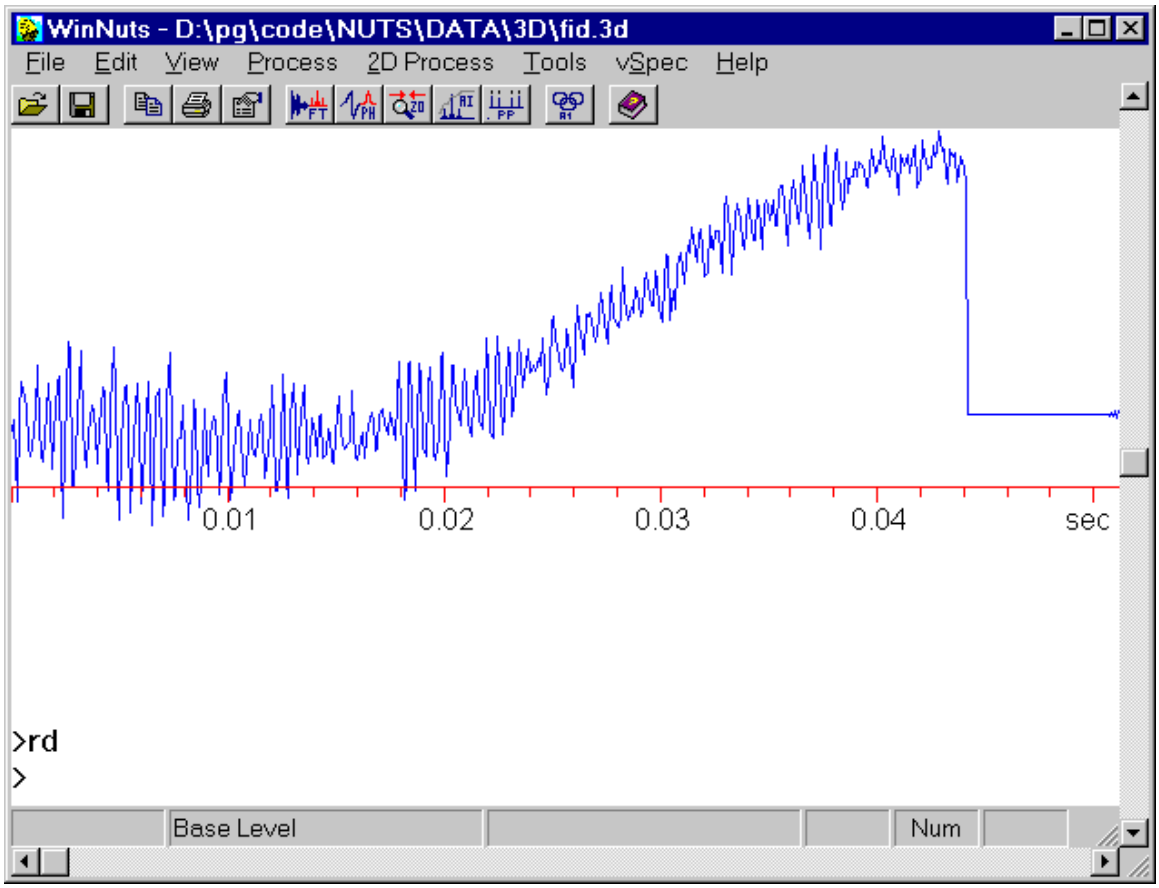
Preventing truncation artifacts

This 3D data set has only 512 complex points in the direct (^{1}H) dimension, and the data is digitally filtered, so the first ~70 points are zero. It is a good idea to step through the slices (with VW) and examine the data before beginning processing. This allows experimentation with processing parameters to determine which will give the best results.

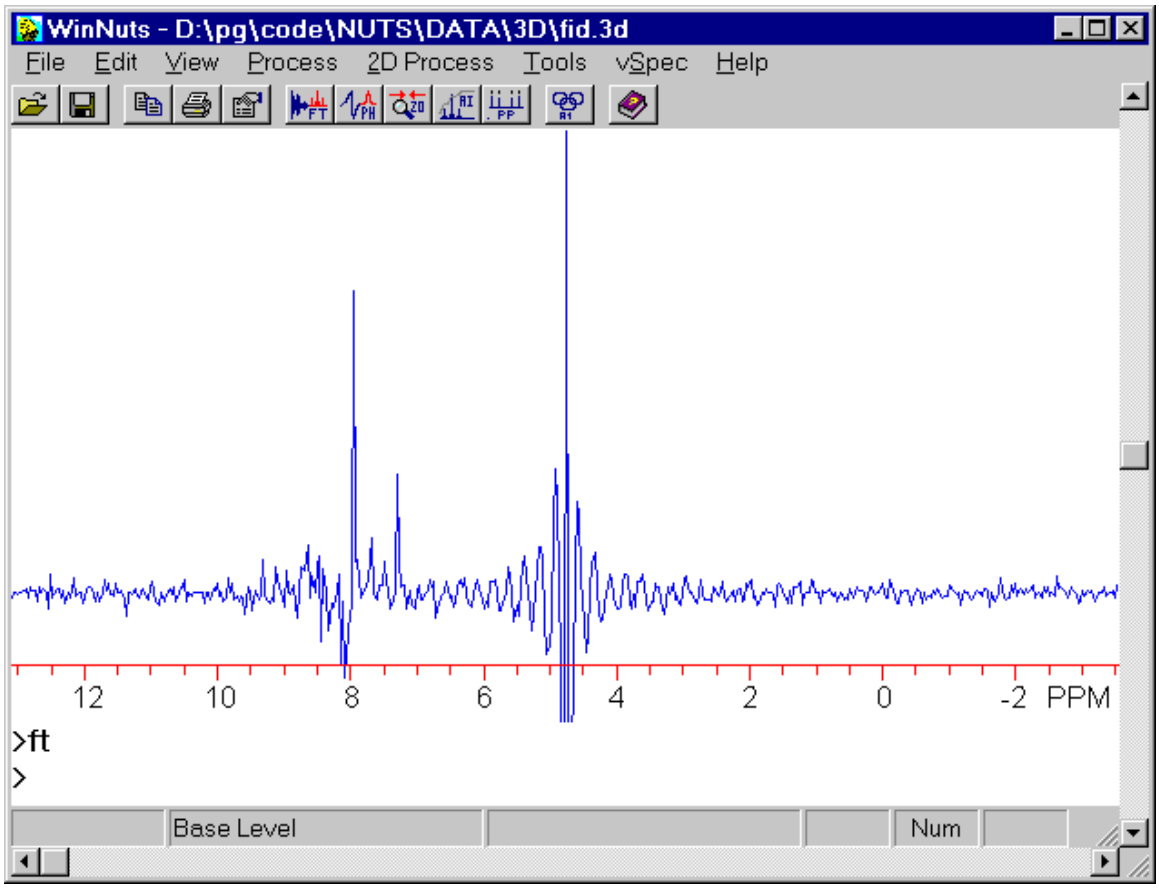
Here is a slice from the 3D data



The FID does not approach zero at the end of the acquisition time due to the large, low-frequency water signal.

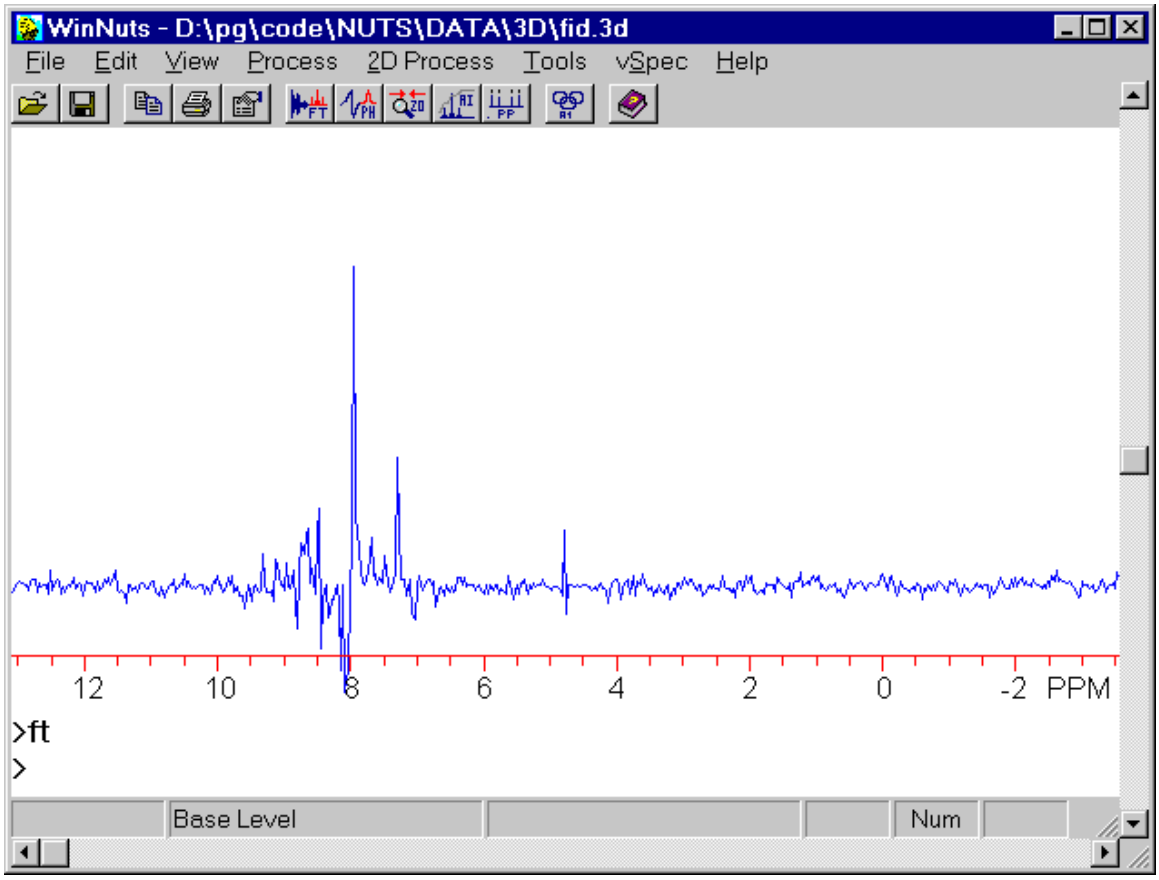


Following the RD operation, we have a large discontinuity.



After FT, this gives large "wiggles".

Here is the same slice, processed with cosine multiplication before RD, and then DH.



3D HMQC-TOCSY of codeine

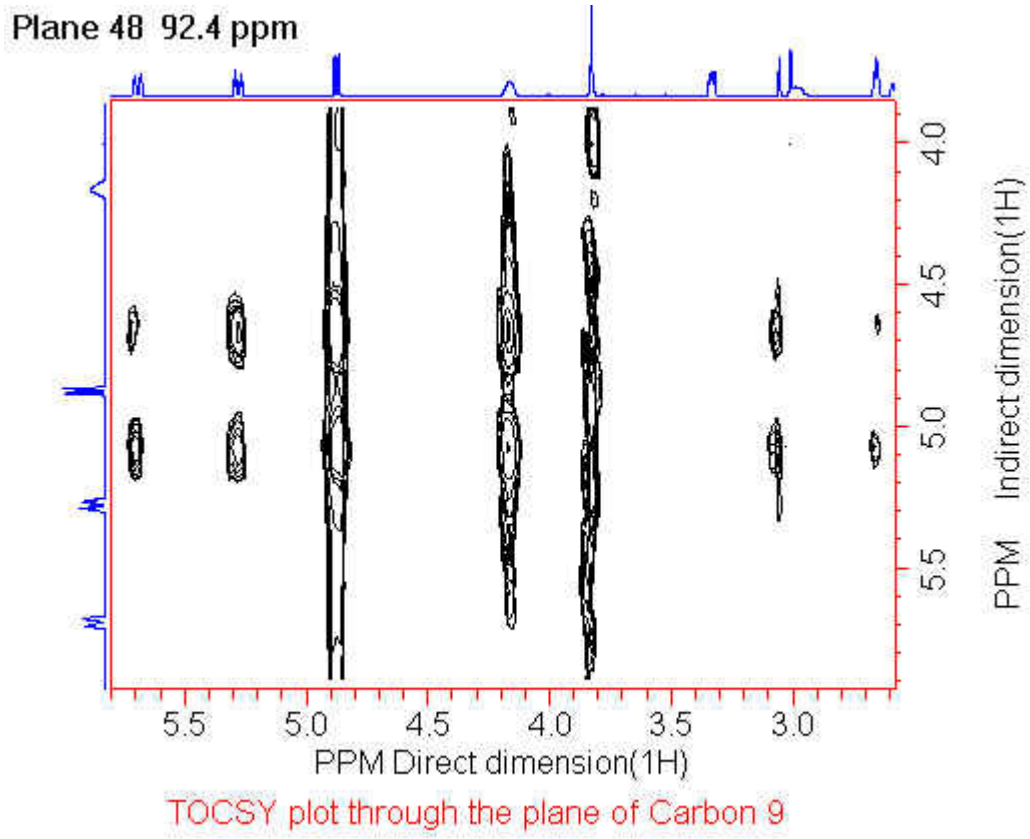
This is a 3D analog of the 2D HMQC-TOCSY, and can resolve connections that are ambiguous due to overlapping peaks. For codeine, this is not necessary because the spectra have minimal overlap, but it serves as an illustration of the experiment and its interpretation.

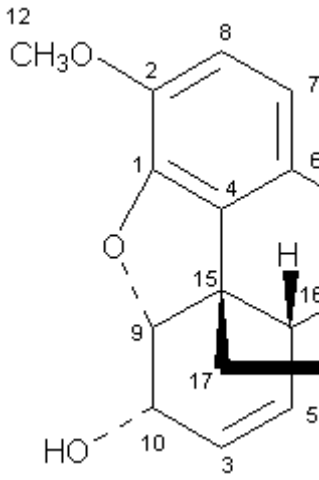
The first step of the pulse sequence is an HMQC, in which ^{1}H magnetization is transferred to the directly-bonded ^{13}C. ^{13}C magnetization evolves during t_1 as a function of ^{13}C chemical shift and ^{1}H-^{13}C coupling. The magnetization is then transferred back to the directly-bonded proton(s). The completion of the sequence is a TOCSY experiment, which transfers magnetization from the "carbon spin-labeled" proton along a chain of coupled protons.

The difference between the 2D and 3D experiments is that in the 2D version, the TOCSY evolution is eliminated, and all peaks related to a given carbon fall along along a single line. In the 3D version, the TOCSY magnetization transfer is "sampled" during the 3rd incremented time delay in the experiment, and the TOCSY information is spread out over the 3rd dimension, so can resolve ambiguities arising from peak overlap in the 2D spectrum.

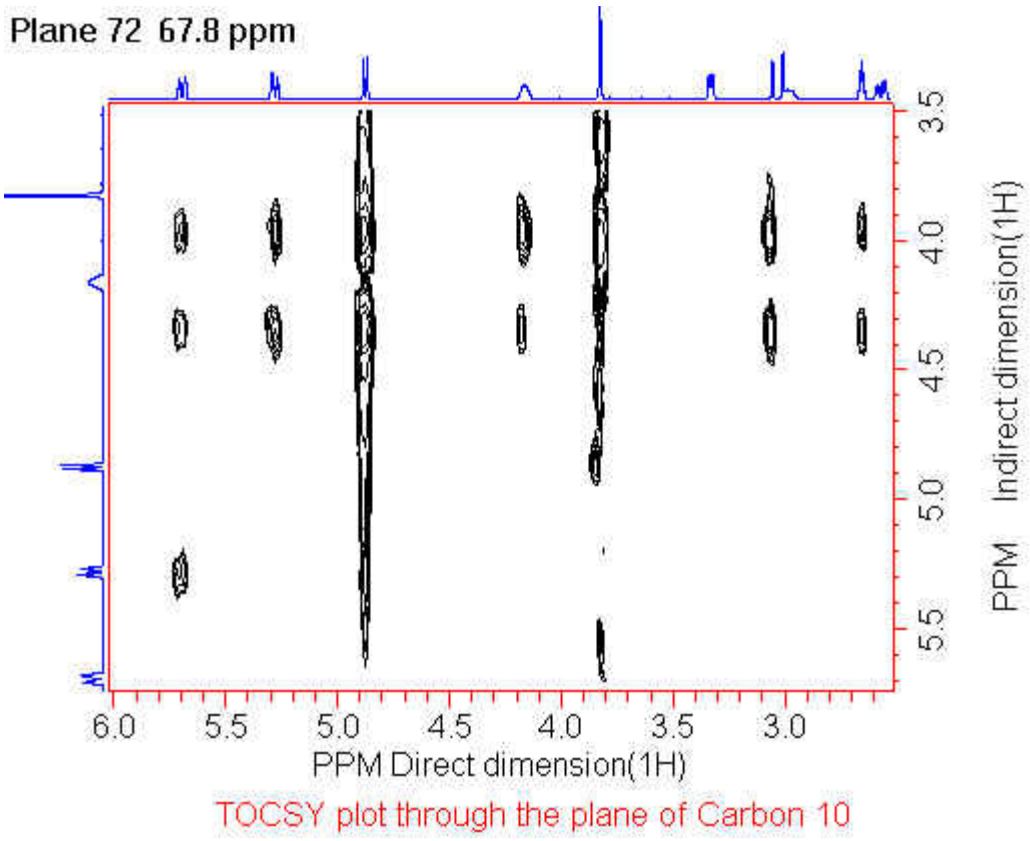
In the resulting 3D spectrum, the carbon spectrum runs along the Z dimension, perpendicular to the plane of the screen/plot. The data are viewed by stepping through planes of spectra, each plane corresponding to a different ^{13}C chemical shift. At the chemical shift of each protonated ^{13}C, a 2D TOCSY spectrum is observed containing proton peaks for the proton(s) directly bonded to that carbon, and correlated to other protons in its spin system.

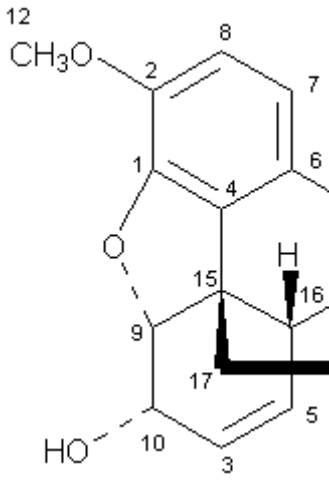
The experiment can be performed using different mixing times in the TOCSY sequence. As with TOCSY, the intensity of each cross-peak will vary with mixing time - long range correlations will "grow in" as the mixing time is increased, and shorter range couplings may disappear.



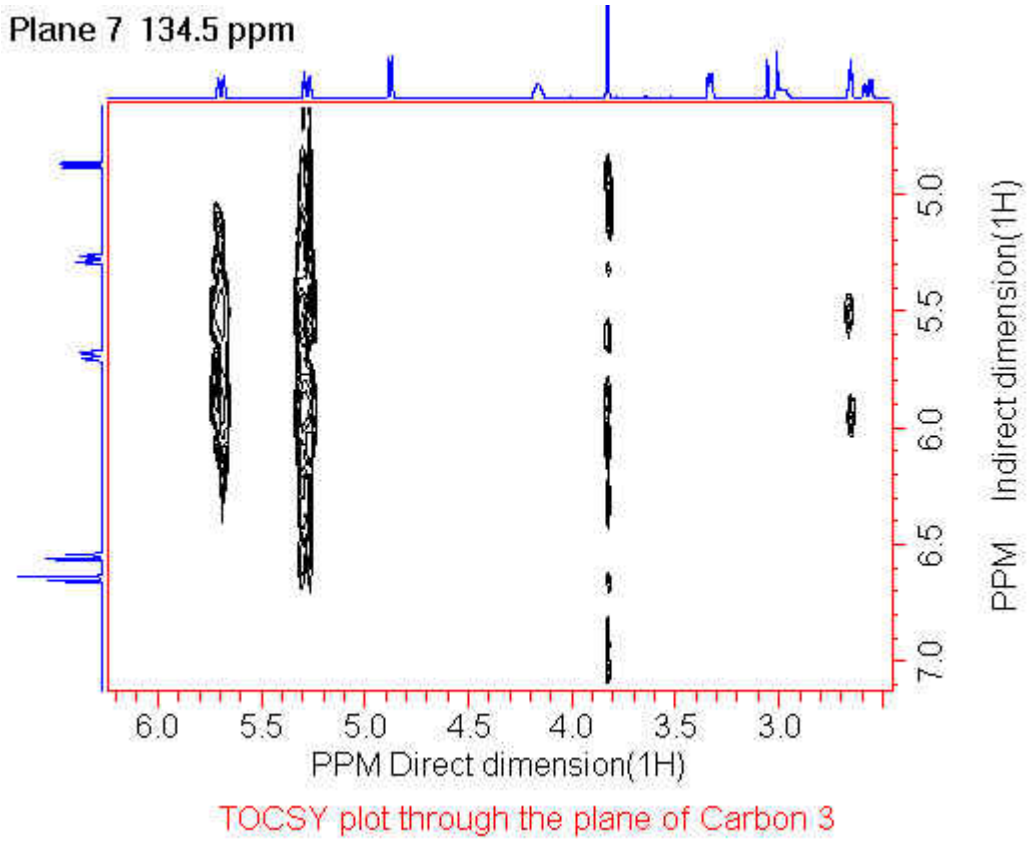


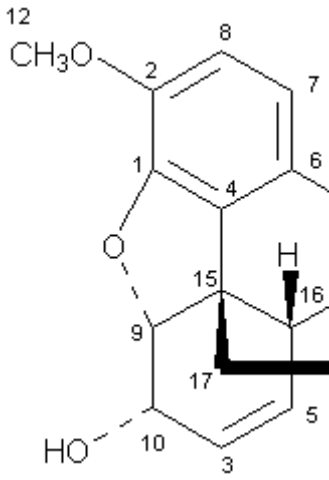
Pairs of vertically-spaced peaks are observed along the horizontal line at the chemical shift of H-9. The peaks occur in pairs due to ^{13}C-^{1}H 1-bond coupling of C-9 to H-9. A pair of peaks is observed at the ^{1}H shifts of (from left to right) Hs 3, 5, 9, 10, OH and 16, and arise from magnetization relayed through the spin system by scalar coupling.



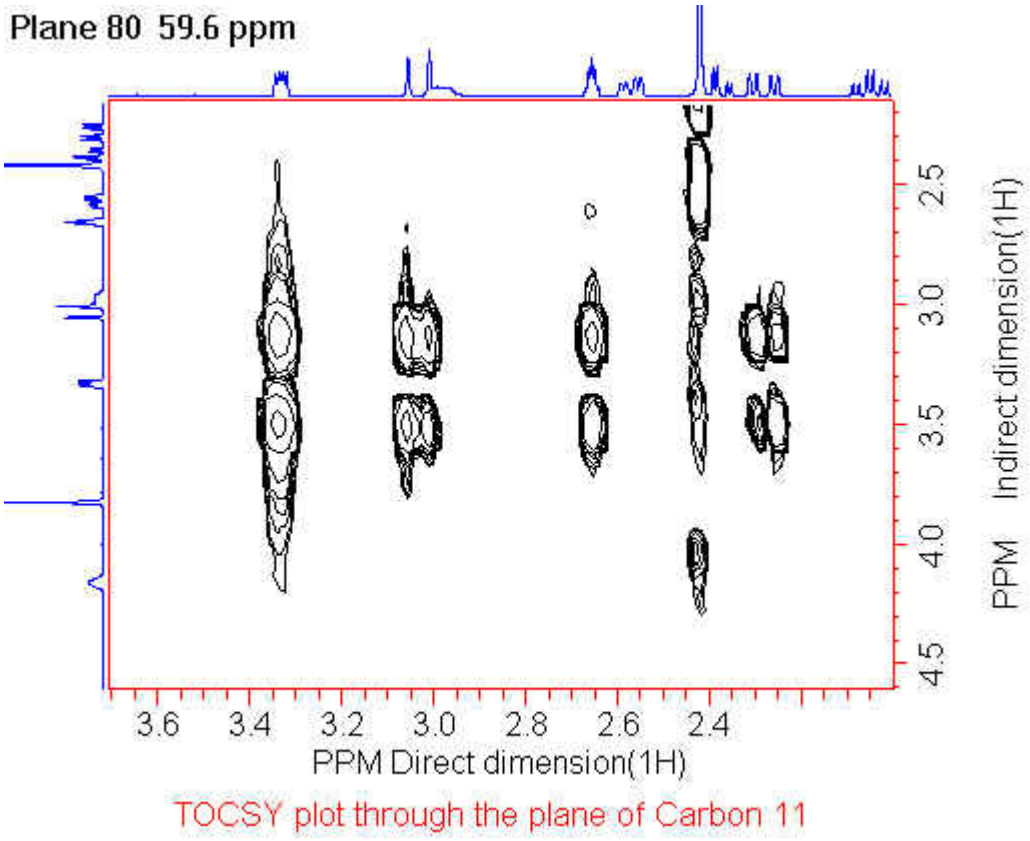


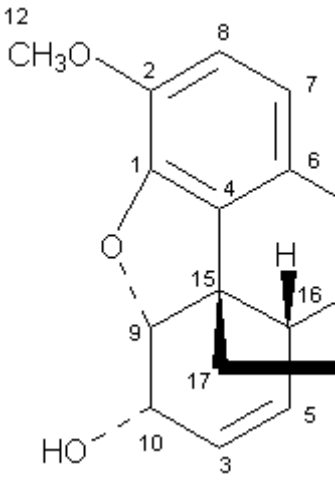
Pairs of vertically-spaced peaks are observed along the horizontal line at the chemical shift of H-10 (4.9 ppm). The TOCSY peaks are to Hs 3, 5, 9, 10, OH and 16 (from left to right). There is an intense vertical noise stripe at 3.8 ppm due to the large OMe singlet.



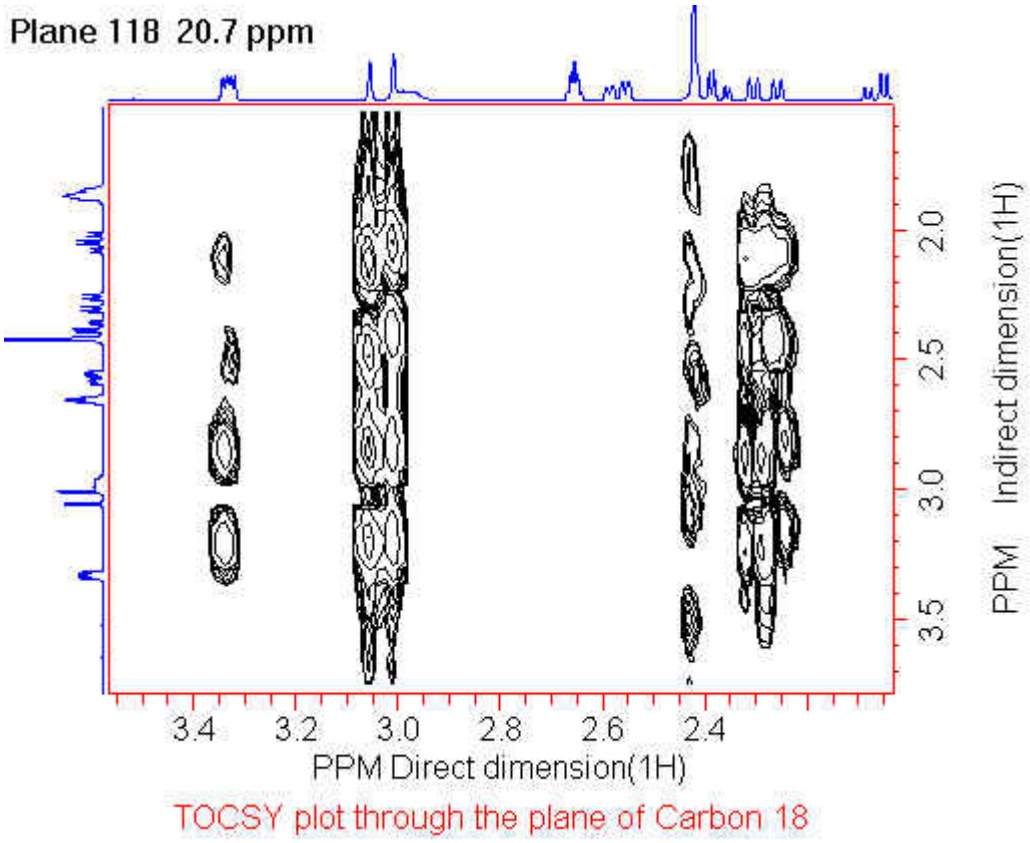


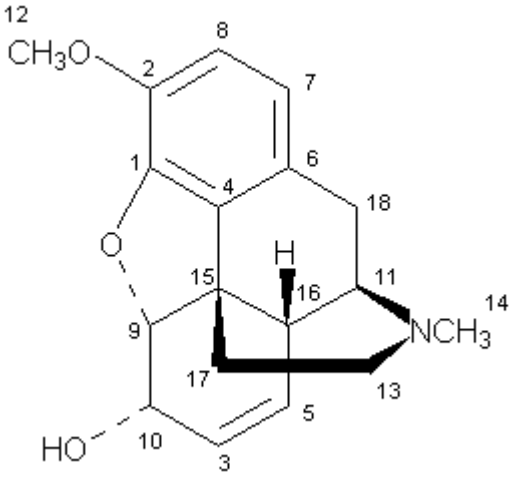
TOCSY peaks are seen from H-3 to H-5 and H-16.



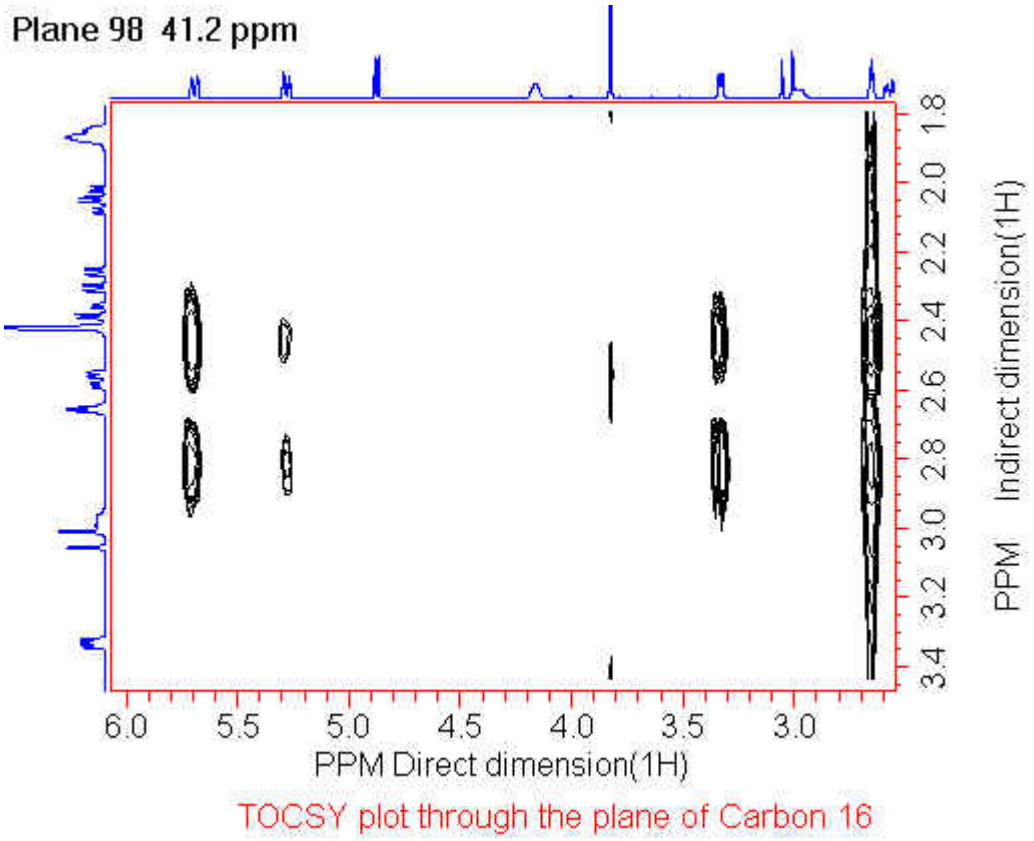


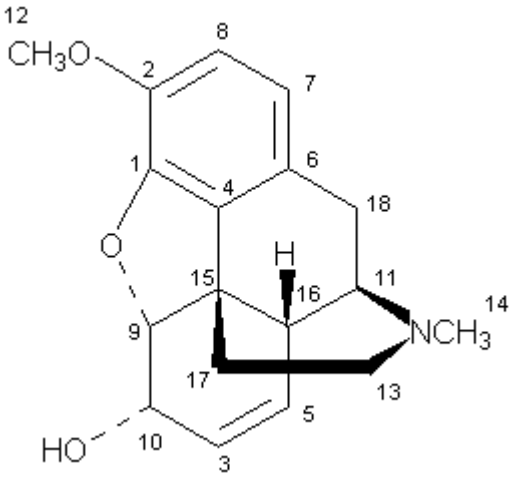
TOCSY peaks are seen from H-11 to H-18, H-16 and H-18'. Another vertical noise band is seen at 2.4 ppm, due to the intense singlet from N-Me.





TOCSY peaks are seen from both H-18 peaks to each other, and to H-11.





TOCSY peaks are seen to H-3, H-5 and H-11.

Note that there is one other difference in the appearance of the TOCSY spectrum in any given plane, as compared to a 2D ^{1}H-^{1}H TOCSY: The symmetry about the diagonal, normally seen for homonuclear spectra, is absent in the planes of the 3D HMQC-TOCSY.

In a homonuclear experiment such as COSY, the first 90º pulse excites all protons. During the evolution time, magnetization that started out on proton A is transferred to its coupled partner, proton B, and vice versa. A crosspeak appearing on one side of the diagonal is due to proton A magnetization transferring to proton B, and the symmetrical peak on the other side of the diagonal arises from proton B magnetization that transfers to proton A.

In the 3D HMQC-TOCSY sequence, the HMQC part serves as a "filter", meaning that only magnetization that started out on proton A exists at the start of the TOCSY part, and is then transferred to other protons in the spin system. The reverse process, involving transfer of proton B magnetization to proton A, gives contour peaks on a different plane of the 3D spectrum.

The sample is 45 mg codeine in ~ .65 ml $CDCl_3$

Acquisition Parameters:

512 complex points in direct dimension
128 t_1 increments in the ^{13}C dimension
64 t_3 increments in the ^{1}H dimension
8 scans
mixing time
2 sec. relaxation delay
Total acquisition time: ~ 40 hrs

Processing:

sine squared window function in the direct dimension with 0 degree phase shift
5 Hz exponential multiplication in the 13C dimension
exponential multiplication and 1x zero-fill in the ^{1}H indirect dimension
magnitude calculation (no phasing is required)
final data size 512 x 128 x 128 = 65 Mbytes

See also: detailed description of 3D processing

Interactive view - Those who have installed the Chime plug-in can view and manipulate the codeine structure in 3D, examine and expand the ^{1}H spectrum, and interactively correlate ^{1}H peaks with H atoms in the structure.

3D processing macro

```
nutsmacro 3D processing
; Bruker data from DuPont Pharmaceuticals
; data is States-type hypercomplex in both indirect dimensions
```

```
; macro uses long command mode
; data size is 512 x 55 x 48 (complex) (43 MByte file)
; with RD, DH and phasing in proton dimension
; total time, after data loaded into 3D mode, 42 sec (PII-400)
; without RD and DH, total time 18 sec

; before starting, open file, MS (S#=90) RD, DH (to set parameters)
; FT 1st slice and determing phasing

; note that spaces are *not ignored* in non-2-letter command mode!

2f,
ga,
set s# 90

; enter 3D arrayed mode
ar 3d,

; cosine mult, RD (this is Bruker digitally filtered data) and
; digital high-pass filter (this has residual water peak)
ms,
rd,
dh,

; ft and phase, then combine Real parts to form complex FT in
; second dimension (see Help on States)
ft,
ps,
tr,tr,st,

; transpose data to exchange 1st and 3rd dimensions
td 3,

; transpose data to exchange 2nd and 3rd dimensions, so
; we are ready to process 2nd dimension, similar to 1st
td 2,
ms,
ft,
tr,tr,st,

; transpose 2nd and 3rd dimensions so we can FT in 3rd dimension
td 2,
ms,
ft,

; transpose again so direct dimension is displayed horizontally
td 3,
ss,
ip,

end
```

4D Processing

A new arrayed mode and associated data structure was added. The new mode is 4D Complex Arrayed mode which packs the data into a 4 dimension data matrix.

It has its own ProcessType (PT) similar to the Generic 3D called Generic 4D. Once this mode has been set with the PT command, the AR command will automatically enter this mode when issued without arguments. The AR command with the argument "4" or "fourD" will force the arrayed mode to start in the 4D Complex Arrayed mode.

The FT and EM commands are now "array aware" of the 4D mode and the Acquisition Parameters Dialog box (available from the View menu) will display the parameters for all dimensions when there is a point count greater than 1 in the 4th dimension.

Truman Brown has supplied some CSI spectroscopy data which is a 4D data set (512 complex by 8 by 8 by 8) and an import filter for this data has been written and depends on the presence of two files: NAME.ddf and NAME.raw. The DDF file has the parameters in a specific format and the RAW file is the data points as 2 byte integers in big endian format.

This is a first pass at displaying 4D CSI data. The tools developed for displaying chosen planes and slices of 3D data work similarly in the 4D mode.

Image display

MR or MRI – Image display

IMAGE – Image display

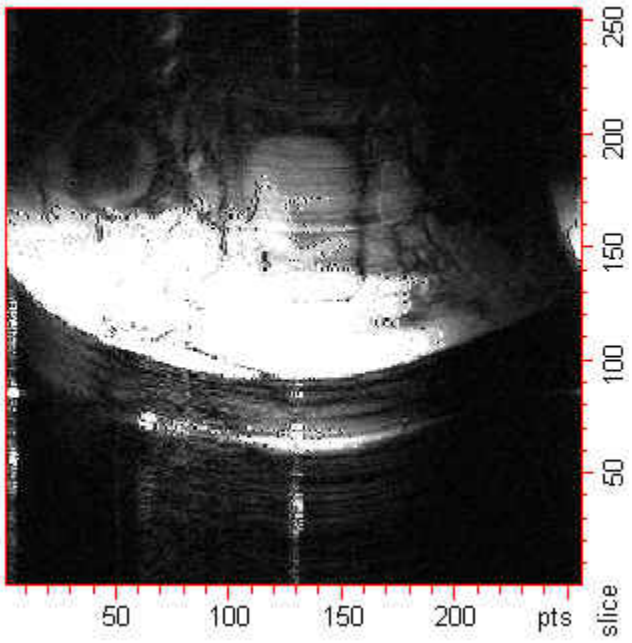
An import filter for images supplied by Truman Brown was added to the import function of NUTS, and a new routine to display these images was added.

The command has several optional arguments:

1. IMAGE (Actualsize or A) displays the image using the same number of pixels on the screen as the image has. Otherwise the image is scaled within NUTS.

2. IMAGE (NegativeImage or Negative or N) toggles the display of the negative of the image. Another argument is allowed which can be ON or OFF which allows setting this feature in an absolute manner.

3. IMAGE (SquareImage or Square or S) toggles the forcing of the image to be square; otherwise the image is scaled within NUTS. By default NUTS starts in the square image display mode.



FAQs

NUTS Frequently Asked Questions

General Topics:

Installation problems
Data importing - spectrum doesn't "look right"
Spectrum is backwards
Plotting
Integration

Q. There is a glitch in the center of my spectrum. How can I get rid of it?

A. A center glitch arises if there is a DC offset between the 2 channels. Both real and imaginary parts of the FID should decay to zero by the end of the acquisition, but sometimes that "zero" level is not truly zero, and the offset may not be the same for both channels. A BC command, issued before the FT, is used to adjust the DC level of each channel to real zero, and will eliminate the center glitch. See example in the section on apodization.
There is an exception to this - if the FID has NOT decayed to zero (e.g., truncated 2D data), applying a BC may *create* a DC offset, and cause a glitch to appear. See details.

Q. I have a spectrum with a folded peak. Is there a way to correct this with NUTS?

A. Yes, provided a few conditions are met. The RD command, which performs cyclic rotation, can be used to "fix" a spectrum with folded peaks. See details in the section on "tricks with RD".

Q. I have spectra with really bad baselines. Does NUTS have tools to flatten very distorted baselines?

A. NUTS has several tools for baseline correction, including solutions for very bad baselines.

Q. How can I most easily distribute spectra to NMR facility customers?

A. Data can be emailed or posted for download. The customer's computer is easily configured so that (s)he can just click on the file name or icon, which launches NUTS, loads the data and displays it according to the customer's preferences. See details in the section on "eliminating paper".

Q. How can I get the best quality image pasted into a report?

A. Very good images can be created from NUTS, but accomplishing this requires an understanding of how copying via the clipboard works. See Copying for an explanation.

Q. How do I put my own logo in place of the acorn on my screen and plots?

A. Edit the nuts.ini file. To remove the acorn, "comment out" (put a semicolon at the beginning of the line) the line that consists of **MetaObjectFile.** To replace the acorn with your own logo, put the file name for your logo in place of acorn.emf. (Your logo must be a metafile.) The purpose for displaying the acorn was to illustrate how users can insert a logo of their choice. Note that Nuts must be restarted for this change to take effect. To remove the logo while in Nuts, enter the MO (metaobjects) subroutine and use D to delete the image.

Q. On some files, when I do Automatic Integration, numeric values are not displayed. Why not?

A. When AI is executed, the smallest integral found is set to one. If your baseline is poor, this integral can have a negative value, so Nuts defaults to setting it to zero, and no labels are displayed. Try baseline correcting, then redo AI.

Q. I am having trouble with the integral values. I don't see any values associated with the integrals either at the top of the screen or under the integrals themselves.

A. As soon as you start defining integral regions for different peaks, you can assign a convenient value to a selected integral. Click the mouse button once to get a red vertical cursor. Place that cursor so that it is on top of the chosen integral. Type V (for value) which brings up a box in which you can enter a value. Until you do this, no values are defined, so none are displayed. The scaling factor you have now set stays the same until explicitly changed by you, regardless of the display scale of the spectrum or the integral trace, even if you leave the integration routine and re-enter.

See Integration for illustration of how to do this.

Q. My data was acquired with block averaging. Can NUTS sum the files automatically?

A. Yes, as long as the files have sequentially numbered file extensions (file.001, file.002...) Create and execute a Link which consists of commands:

GA AS AL IN

See Add/Subtract for details.

Q. The spectrum looks OK on the screen, but when I print, the axis and integrals are scrunched into the first 2 inches of the left side of the plot.

A. Modern applications do not communicate directly to the printer, the operating system takes care of that. Printing problems like this are generally caused by the printer driver. The first thing to try is to obtain the most recent version of the driver from the printer

manufacturer. It can be hard to tell which version you actually have, so downloading and installing the current version is the first step, even if it appears that this is not newer than what you are using. The other thing to try is a printer driver for a similar printer. Even though the driver is not for your specific model of printer, it may function just fine, and may not have the same error. If problems persist, there may be a conflict between the printer driver and something else that is installed.
Based on reports from customers, this particular problem occurs with HP 5 and 6 model printers. Sometimes this occurs only on the first plot after NUTS is started, but other users report it happening on all plots. This problem is usually addressed by getting a newer printer driver version. In one case, installing a driver for a 5N on a 5L printer (or vice versa?) fixed the problem. As a test, try installing the driver for an HP LaserJet Series II with the existing printer. If this driver works, then we can be reasonably sure the problem is due to the printer driver.

Q. After doing a fit in the line fit subroutine, I need to report the error for the fit to the actual data. There is an error value printed on the screen. What is the meaning of this error value?

A. The error reported during LF Simplex fit is calculated as described below. It has no statistical meaning but was developed empirically to perform the best guide for the Simplex routine.

error = 100.0 times (difference between actual points and calculated points, squared) divided by (square of all points)

See Line Fit routine for illustration of how to use this subroutine.

Q. My spectrum after FT appears to be backwards. What is wrong?

A. This is easily fixed with the SR (Spectrum Reverse) command. Why it happens is more involved. For GE Omega data, this can result from the way the frequencies are generated in the spectrometer. For example, proton spectra may be correct, but carbon spectra are reversed. There is no way for Nuts to know which is correct, so this must be corrected manually. At one time, it appeared that importing of Varian Unix data resulted in reversed spectra, which has since been corrected. You can configure your nuts.ini file to perform this correction automatically when data is imported, but be advised that this will then be done on every data set. If you process data from different sources, this may not be advisable.

Q. My Bruker data has huge quadrature images, even though the spectrum looks OK on the spectrometer.

A. This can result from either of 2 sources, both related to the fact that Bruker does "quadrature" detection in a strange way, which they call "sequential" acquisition. This means that they use a single A-to-D converter, and digitize the data at twice the normal rate, alternately placing points into the A and B channels. Therefore, the data does not

exist as true quadrature pairs because the real point and its corresponding imaginary point were not acquired at the same time. This necessitates a special type of FT. Nuts should correctly identify Bruker sequential data, and apply the "Bruker" transform when the FT command is executed. If your data type is incorrectly identified, executing an FT command will use the wrong type of FT, and cause large images. Check the Data Type parameter (View menu/ Spectral Parameters) to be sure it is correct. Bruker sequential data is identified as "TPPI", simultaneous data is identified as "Complex", non-quadrature data is identified as "Real".

These quad images can also result from swapping the real and imaginary channels, which can happen when the data is transferred to the PC. In this case, executing an RI command on the FID will swap the 2 halves of the data, and subsequent FT should produce a correct spectrum.

See description of Bruker artifacts for illustration.

Q. How do I process Bruker digitally filtered data?

A. Bruker does some pre-processing before saving the data, so that the FID looks strange. The initial points are zero, then build up to the beginning of the FID around 60-70 pts from the beginning. To process this data, you must first do a circular left shift so that the top of the FID is at time=0. The command is RD. Nuts should correctly calculate the number of points to shift. See Bruker digitally filtered data for illustration.

Q. How do I display a chemical structure on my plot?

A. This is done in a subroutine called MO (stands for Meta Objects). This routine is also one way to create inset plots. The Windows version now allows you to place a standard, placeable or enhanced Windows metafile onto the screen, either from a file or from the clipboard. This can be a chemical structure, a logo or other graphical object. (Note that Win 3.11 does not support enhanced metafiles.)
The Mac version uses PICT files instead of Windows metafiles. You can also copy a PICT file into the clipboard from some other application and paste it into Nuts. This works best if you make the object fill the screen before you copy. You can also import a PICT file from a file. However, this does not print properly. The size of the object when printed is much smaller than on the screen. This seems to be because PICT files don't keep track of the size as it relates to printer resolution. So, at this point, using the clipboard seems to be the better alternative.
More than one object can be displayed on the screen. The program keeps track of them in a linked list. Each object can be moved and re-sized using the mouse. To select which object to operate on, you move through the list, forward or backward, until the desired object is indicated with a box with handles for re-sizing (see below for command list).
In the Windows version, there is also the option of defining one or more graphical objects in the nuts.ini file which will be displayed on the screen automatically. (This does not currently work on the Mac.) This is demonstrated in the Windows version by the display of the acorn in the upper left corner of the screen. This can be removed by editing the

nuts.ini file or, while Nuts is running, by deleting it from within the MO subroutine.
The MO subroutine does not display menus, but instead has a "helper box" which has buttons for each operation. The buttons are labeled with the corresponding keyboard command. The helper box can be toggled on and off from the Help menu at the base level of the program.
The MO subcommands are:

A to add the currently displayed region as an inset plot
C to paste the contents of the clipboard
I to import a graphical object from a file
D to delete the selected object
N to move to the Next object in the list
R to move to the previous object in the list
Shift-F1 brings up Help for the MO subroutine
Enter exits the subroutine

See Meta Objects for illustration.

Q. How do I do a stacked plot of a series of 1D files (such as kinetics data)?

A. If the data exist as separate 1D files, they must be packed into a NUTS 2D file, which can be done with the following Link, assuming the files have sequential file extensions:
GA SC IN
When this is run, Nuts will prompt for the file to read (the first of the 1D files) and for a name for the 2D file to be created.
(For Varian arrayed data, the data is already a 2D file after importing into Nuts.)
Then open the 2D file, execute a SS command (Set Scale) and SP to perform the stacked plot. The offsets in both dimensions can be set using O or by selecting Slice Offsets from the Display menu.

See Stacked Plots for illustration.

Q. I want to create a field in my Access database containing the name of an NMR data file, so that clicking on it will launch NUTS and load my spectrum. Can this be done?

A. Yes. Create a field in an Access table as a HYPERLINK "" \l field. In that field, enter the full path and file name of the data file. You must associate the file extension(s) of the NMR data files with NUTS, so that clicking on the file name will launch NUTS. (You can use File Manager, and select File/Associate). Also, the Access .mdb file must reside in the NUTS directory, so that NUTS can find the other files it needs.

Q. I have a heteronuclear 2D data set, and want the labels on the 2 axes to be different (^{1}H and ^{13}C). But the axes always end up labeled the same. How can I do this?

A. Each dimension is labeled with its "acquisition order". Each dimension must have a different acquisition order label. Switch NUTS to the "non-2-letter" command mode

(**2F**), and type **acqorder** to see what the labels currently are. To change the labels, type **acqorder 1 2 3 4** (for example). Now that the different dimensions are labeled differently, you can change the axis labels from the View/Spectral Parameters screen.

Technical Support

Acorn NMR's technical support service is available to NUTS users free during the 90 days warranty period after initial purchase and by an annual subscription thereafter, for 15% of current purchase price.

Technical support is available by phone from Acorn NMR during normal business hours (9:00 AM to 5:00 PM Monday through Friday except holidays). The preferred method of technical support is by email.

Telephone support is available at:

(925) 456-1020
(925) 456-1024 FAX

Email support is available from support@acornnmr.com

Questions involving problems with data files are often best addressed by sending sample data files to Acorn NMR. Files can be sent by ftp to

ftp.acornnmr.com

Change directory to INCOMING, which is write-only for anonymous logons. You may get an "access denied" message, but that results from your ftp software trying to list the contents of the directory, which is not allowed. Ignore the error. It is still possible to upload files. It is important to email us at support@acornnmr.com after uploading a file with a description of the file and problems you are having.

Notice of New Features

The main Help page, www.acornnmr.com/NutsHelp, lists new features.

Upgrades

Users with active technical support service from Acorn NMR (90 warranty period or by subscription thereafter) can download a new copy of Nuts as often as desired. As long as the compile date of the Nuts version falls within the support date of the user's license, the Nuts version will be fully functional. If the user's support period has expired, the new version will function as a "demo" copy, although the older version will continue to work forever.

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Appendix I - License and support policy and disclaimer

NUTS is a software program for NMR data processing under Microsoft Windows and Macintosh.

The software is supplied with 90 days of telephone and e-mail support. The user receives a license "key" which contains encrypted information about the licensee and support status. At any time during the support period, the user may download a new copy of NUTS from the ftp or WWW site and the license "key" will convert it to a licensed and personalized copy. If the license has expired, the new copy will operate only as a demo, but earlier copies of NUTS will still function. Support can be extended for 15% of the purchase price per year, including discounted copies.

After 90 days, a 1-year support contract is 15% of current list price for the combined NUTS package owned. This entitles the user to download updated copies of the program at any time.

Users who choose not to purchase support can use their original copy of Nuts forever.

Obtaining an updated copy of Nuts, for users who are under support, simply consists of downloading a new copy from the web or ftp site, which is converted into a fully functional, personally licensed copy when combined with a license "key" file. A new copy may be downloaded at any time, and becomes a full copy, provided the support period has not expired.

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All other terms apply as for single-user licenses.

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Some states do not allow the exclusions of implied warranties, so the above exclusion may not apply to you. This warranty gives you specific legal rights and you may also have other rights which vary from state to state.

Acorn NMR does not warrant that the functions contained in the program will meet your requirements or that the operation of the program will be uninterrupted or error free.

LIMITATION OF REMEDIES

In the event that the computer media on which the program is provided is defective in materials or workmanship, your exclusive remedy is limited to its replacement, or a refund of the purchase price therefore, by Acorn NMR in accordance with its applicable product return and replacement policies.

In no event will Acorn NMR or its dealers be Liable to you for any damages, including lost profits, lost savings, or other incidental or consequential damages arising from the use or inability to use the program (whether or not due to any defects therein) even if Acorn NMR or its dealers have been advised of the possibility of such damages, or for any claim by any other party. (Some states do not allow the limitation or exclusion of liability for consequential damages so the above limitation may not apply to you.)

GENERAL TERMS

This Agreement can only be modified by a written agreement signed by you and Acorn NMR, and changes from the terms and conditions of this Agreement made in any other manner will be of no effect. If any portion of this Agreement shall be held invalid, illegal, or unenforceable, the validity, legality, and enforceability of the remainder of the Agreement shall not in any way be affected or impaired thereby. This Agreement shall be governed by the laws of the State of California.

ACKNOWLEDGMENT

Your use of the program acknowledges that you have read this agreement, understand it, and agree to be bound by its terms and conditions. You further agree that it is the complete and exclusive statement of the agreement between you and Acorn NMR which supersedes all proposals or prior agreements, oral or written, and all other communications between you and Acorn NMR relating to the subject matter of this agreement.