Eft

Spectrometer Guides

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PN 000103-000 Rev. Q

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About this Manual

This manual describes procedures called Spectrometer Guides to perform routine NMR experiments and calibrations on the Aii Eft NMR Spectrometer.

How this Manual is Organized

The Eft Spectrometer Guides manual includes three sections:

-Introduction to Eft NMR Software

Provides a brief description of the software programs used with the Eft spectrometer. These include basic information for Windows, NUTS, and WinPNMR.

-Experiment Guides

Experiment Guides are step-by-step procedures for performing basic Eft NMR procedures. There are ten Guides:

- Sample Preparation
- Spinner Types
- Instrument Setup
- ^{1}H 1D Spectra
- ^{13}C 1D Spectra
- DEPT
- Block Averaging with Peak Registration
- COSY and HETCOR
- XPT, X-Nucleus with Polarization Transfer
- Relaxation Time (T1)
- Kinetics
- Spin Simulation

-Appendix

There are Experiment Guides included for spectrometer maintenance.

- Calibration and Maintenance Schedule
- Update Phase Correction Parameters
- Proton Observe 90° Pulse Width
- High Power Decoupler 90° Pulse Width
- Decoupler Power for Broad Band Decoupling
- Carbon Observe 90° Pulse Width
- Adjust 3^{rd} and 4^{th} Order Shims
- Adjust Magnet Shunt
- Computer Maintenance
- Wide Band Probe Setup, Tune, Calibration

Conventions Used in this Manual

Fonts

The table below explains the various fonts and notations used in this manual.

If you see . . .	It means . . .
`Courier New Bold`	Text that is displayed on the computer screen. It is also used to designate file and directory names.
`Courier New`	Text the user types that appears on the screen.
`Courier New Italic`	Variable for which the user types the value or name, either numeric or text. For example, if the manual reads: **`Enter filename:`** *`filename`* ... the user might type ... **`Enter filename:`** `ethylacetate_dept`
Square brackets [ ] Bold	Text between brackets is an item or text in the Windows environment that the user selects with the mouse.
`Angle brackets < >`	A key or combination of keys on the keyboard, such as `<Enter>` or `<ESC>`, which do not appear on the screen. When two keys must be depressed simultaneously, action is indicated as `<Key+Key>`, for example: `<Alt+Tab>`.

Icons

This icon . . .	Indicates . . .
NB	Notes that are information, tips or suggestions.
	Important item to check carefully.
	Cautions indicate hazardous situations which, if not avoided, may result in property damage or injury.

Table of Contents

Introduction to the Eft NMR Software

General

The Eft NMR software consists of two programs: WinPNMR (PNMR) for spectrometer control, including data acquisition and WinNUTS (NUTS) for processing, data reduction, display, and plotting. Both programs operate within the Microsoft Windows environment and are opened and ready for use when the computer is booted.

Getting Around in Windows

As long as PNMR and NUTS are the only programs open, toggling between NUTS and PNMR is accomplished by pressing

<Alt+Tab> or by clicking on the appropriate button on the Task Bar.

If programs in addition to PNMR and NUTS are open, pressing <Alt+Tab> displays a box containing a row of icons. A blue square indicates the icon of the program that would come to the foreground upon releasing <Alt>. Pressing <Tab> while continuing to hold down <Alt> will move the blue square to the next icon. Release <Alt> to bring the selected program to the foreground.

NUTS and PNMR are closed by clicking on the close window box ☒ in the upper right corner of the display. Both can be opened by clicking **[Start],** then either the **[NUTS]** or **[PNMR]** icon in the Start Menu Box.

From their command lines:

NUTS is closed by typing

>xx

PNMR is closed by typing:

H1>ex<Enter>

You may have need for Windows Notepad (Notepad) to modify text files used by PNMR pulse programs. In a standard Windows configuration Notepad is opened by clicking

[Start] [Programs] [Windows Explorer]

Then double-click on the file to be edited. If necessary, select to open the file with Notepad.

For file management, use Windows Explorer by clicking

[Start] [Programs] [Windows Explorer]

One can then move, copy, rename or delete files in the windows fashion.

PNMR Basics

NB PNMR keyboard commands are not case sensitive. PNMR commands must be followed by <Enter> to cause execution. The only exceptions are the Ctrl+ one letter commands in the GS mode and the Ctrl+ one letter commands to halt acquisition.

The PNMR prompt indicates the nucleus you are set to observe. For example, the

H1> prompt indicates you are set to observe protons.

Changing the observe nucleus is done using the **nu** command, for example,

H1>nu C13<Enter> will switch the spectrometer from ^{1}H to ^{13}C observe and give you the C13 prompt

C13>

Upon switching to a nucleus, its default acquisition parameters are loaded and displayed in the PNMR window. To change a parameter value, type the two-letter parameter pneumonic, <space>, the new value, and <Enter>. For example,

H1>rg 10<Enter> changes the receiver gain value to 10.

To return to the default values, read the initialization file for that nucleus. For example,

H1>re H1.ini<Enter> loads the H1 default parameters.

Experiments on the Eft spectrometer are done using pulse programs, which are run by typing their names at the prompt. For example,

H1>cosy<Enter> will load the parameters for the COSY experiment and run it asking the operator for a filename and a few crucial parameters.

The Help file for PNMR (PNMR_help.txt) file is viewed in Notepad by clicking on the PNMR Help icon on the Start Menu. Notepad provides a vertical slider to scroll through the text and a search menu item to help locate particular key words.

NUTS Basics

NUTS commands are usually run from the pull down menus or commands typed at the prompt.

NB The two letter NUTS commands execute without the need for <Enter>. <Enter> ends various routines and subroutines.

Most data processing in NUTS is done with Links or Macros. A link is a defined series of NUTS commands done sequentially as a shorthand means of performing processing tasks that would otherwise involve typing several commands. Macros are used to perform more complex tasks where the alternative of typing individual commands would be impractical.

The Link menu is viewed by typing

>li at the prompt or selecting from the appropriate pull-down menu.

Links can either be run from the link menu, or by typing the appropriate letter-number combination, as for example:

>a1 to run Link 1.

The Macro menu is viewed by typing

>ru at the command line or selecting from a pull-down menu **[File][run macro].**

Macros may also be associated with function keys F1 through F12 and executed as in the example below:

><Ctrl+F1>

Data manipulation in NUTS relies on frequent use of the ZOOM routine, which one uses to view an expanded subsection of a spectrum. The ZOOM routine is entered by typing:

>zo or double clicking the left mouse button (left MB).

A crosshair cursor with a ZO label and either a vertical red line or vertical red rectangle both appears on the display. By holding the left MB down and dragging the mouse, the ZOOM region can be selected and sized on the display. Expansion to the ZOOM region is done by typing:

<Ctrl+E> or clicking the right MB.

Typing

<Enter> will leave the ZOOM routine which then redisplays the arrow cursor.

NB If NUTS does not respond to a command as expected, it may be in a subroutine, such as ZOOM. Press <Enter> one or two times to return to the base level prompt.

The full spectrum is displayed using

<Ctrl+F> whether in the ZOOM routine or not. Its counterpart <Ctrl+E> works the same way, showing the most recent expansion.

While expanded, the ZOOM window can be moved left or right with the scroll bar at the bottom of the screen. At the right hand side of the screen is a slider for adjusting the vertical amplitude. The amplitude can be adjusted other ways including using the arrow keys <↑><↓> or <Page Up>,<Page Down>.

NUTS links are assigned and their functions are described below:

Link	NUTS Commands	Function
a1	zz bc em ft ps ^f*	Process spectrum. Phase same as previous. Used for nuclei other than ^{1}H.
a2	zz bc ft pc ^f*	Same as above, except phase using default phase parameters. Used only for ^{1}H.
a3	ga sl bc em ft	Process stored spectrum no phase correction.
a4	ga bc ln em	Get stored spectrum with no processing.
a5	zz bc em ft ps	Process spectrum. Phase same as previous. Used for nuclei other than ^{1}H.
a6	zz bc ft pc	Same as above, except phase using stored parameters. Used only for ^{1}H.

* Use ^f to display the full spectrum.

NUTS macros are assigned to function keys and are described below:

Function	Function Key	Macro Name	Notes
Process and plot proton spectrum.	F1	aii_H1_auto.mac	Process, reference and plot with integration and peak picks.
Process proton spectrum.	F2	aii_H1_1D.mac	Process proton spectrum and set reference.
Process carbon spectrum.	F3	aii_C13_1D.mac	Process carbon spectrum with peak picking.
Process COSY spectrum.	F5	aii_cosy.mac	
Process HETCOR spectrum.	F6	aii_hetcor.mac	
Process T1.	F7	aii_t1.mac	Use for proton or carbon inversion recovery.
Process kinetics.	F9	aii_kinetic.mac	
Process DEPT.	F11	aii_dept.mac	Process DEPT 45, 90 and 135 spectra.
Process C13 BAPR.	F12	aii_C13_bapr.mac	

Online help for NUTS is available on the menu bar and on the start menu. In addition, information, help, and tutorials are available at the Acorn NMR Web site (http://www.acornnmr.com).

Experiment Guides

Experiment Guides are brief, step-by-step procedures for operating the Eft spectrometer. The Guides serve both as learning tools for the new user and as quick reference tools for the experienced user.

Sample Preparation Guide

The quality of results on the Eft NMR spectrometer is critically dependent on using appropriate sample preparation methods. For all samples:

1. Use a total sample solution volume of 0.7ml (4cm liquid height).
2. If the sample contains particulate matter, filter the solution through glass wool or cotton that is loosely packed in a Pasteur pipette.
3. Use 7 inch Wilmad 507-pp or Norell 502 or equivalent quality sample tubes.

^{1}H Samples

1. Sample concentration of about 5% or about 0.3M is generally appropriate.
2. TMS or HMDS at 0.5% is sufficient to reference a 0.3M solution.

^{13}C Samples

1. Neat (meaning 100% concentration) liquids will give good spectra in 1 minute and 1M samples will allow good spectra to be obtained in 5 minutes.
2. TMS at 2% should be adequate to reference a 1M solution.

Sample Spinners

The sample spinner required depends on the type of probe being used. All Eft Spectrometers (60 and 90MHz) with ^{13}C or broadband observe capabilities use an Aii probe and the Aii sample spinner (Fig. 1). Eft Spectrometers with only ^{1}H observe capability use the original magnet manufacturer's probe with the appropriate sample spinner. Spectrometer upgrades for Varian 360A or 360L systems use the spinner shown in Fig. 2. Spectrometer upgrades for Varian 360 (no type indicator) systems use the spinner in Fig. 3. Proton only Hitachi R-Series systems use the spinner in Fig. 4. Note the correct orientation of the spinner on the sample tube. The position of the spinner is set with the appropriate depth gauge.

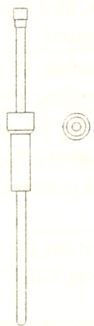

Fig. 1 Aii Probe

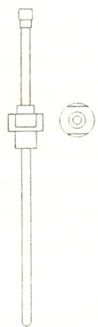

Fig. 2 Varian Types A and L

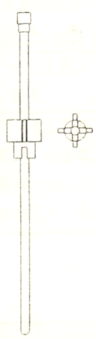

Fig. 3 Varian (No type)

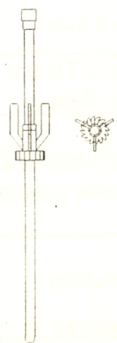

Fig. 4 Hitachi R-Series

Instrument Setup Guide

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
A	**PREPARE**		Purpose: adjust field and optimize shims. Run if instrument idle ≥ week otherwise skip to **B**.
1	Sample		Tap water, spinning. (Do not use the sealed Homo Adj. sample supplied with original CW instrument)
2	Enter PNMR.	<Alt+Tab>	(If necessary.)
3	Acquire data.	H1>prep<Enter>	Spectrometer enters GS mode.
4	Gain Adjusted		The spectrometer adjusts the receiver gain.
5	Spectrum acquisition.		Prep takes a spectrum
6	Enter NUTS and process data.	<Alt+Tab> >a2	Trim phase as required. Use cursor to determine peak position in ppm, including sign.
7	Enter PNMR and set field for shimming.	<Alt+Tab> *value*<Enter>	Change to PNMR and enter the peak position in the User Input box.
8	Adjust spinning speed.	**Adjust spin air flow** <Ctrl+Q>	Adjust spinning speed so the crest-to-crest spacing in the FID is about 5 horizontal divisions.
9	Minimize side bands.		Y, X, Z, etc shims optimized. Note *
10	Adjust spinning speed.	**Adjust spin air flow** <Ctrl+Q>	Adjust spinning speed so the crest-to-crest spacing in the FID is about 2 horizontal divisions.
11	Optimize resolution.		Spinning shims set, spectrum acquired. Note *
12	Enter NUTS and process data.	<Alt+Tab> >a2	(Trim phase as required.) Use cursor to determine peak position in ppm, including sign.
13	Enter PNMR and set field.	<Alt+Tab> *value* <Enter>	Change to PNMR and enter the peak position in the User Input box. (Value should be ≈ -4)
B	**SHIM THE MAGNET**		Purpose: Optimize resolution.
1	Sample		5% ethylbenzene or 5% ethylmethacrylate
2	Enter PNMR.	<Alt+Tab>	(If necessary.)
3	Enter shim routine.	H1>shim <Enter> or C13>shim <Enter>	The spectrometer automatically adjusts the gain before shimming.
4	Enter RD value	*value* <Enter>	Dilute sample RD=5; Conc. sample RD=2
5	Shim		Allow time to shim. Note *
C	**TMS REFERENCE**		Purpose: Correctly position the spectrum.
1	Sample		5% ethylbenzene or 5% ethylmethacrylate
2	Enter PNMR.	<Alt+Tab>	(If necessary.)
3	Verify parameters.		Verify that the parameters make sense. Otherwise H1>re h1.ini<Enter>
4	Acquire data.	H1>zg <Enter> <Enter> to use the default file name	If FID display is red, reduce RG, and repeat zg. Proceed to next step when prompted.
5	Enter NUTS and process data	<Alt+Tab> >a2	(Trim phase as required.) Use cursor to determine TMS peak position in ppm, including sign.
6	Enter PNMR and enter TMS peak position.	<Alt+Tab> H1>fo <Enter> *value* <Enter> 0 <Enter>	Switch back to PNMR, execute the fo command, enter TMS position determined above for current position, and 0 (zero) for desired position. Repeat to confirm.
7	Adjust offset.		*Manual shims only*: Set the offset on the shim unit to the value shown in the "Set Shim" dialog box.
D	**PHASE CHECK ^{1}H**		If Step 5 above does not give spectrum with correct phase, see "Update Phase Correction Parameters" in the Appendix.

* *Systems with manual shims only*: Carefully follow the screen prompts until the shim routine goes to next step or exits to PNMR.

^{1}H 1D Spectra Guide

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		See Sample Preparation Guide. Position sample spinner using the depth gauge, place in probe
2	Enter PNMR program.	<Alt+Tab>	(If necessary)
3	Select ^{1}H observe.	C13>nu H1<Enter>	Required only if the prompt is not "H1".
4	Verify parameters.		Verify that parameters make sense.
5	Acquire data.	H1>zg<Enter> then *filename*<Enter> or <Enter> for default	Enter filename if desired but it is usually better to use the default (pnmrfid) unless intending to save the data long term.
6	Enter NUTS.	<Alt+Tab>	
7a	Process data.	<Ctrl+F1>	Process and plot using aii_H1_auto.mac, which references TMS, peak picks, integrates, and accepts spectral information and plots.
	OR, preferably:		
7b	Process data.	<Ctrl+F2> then [filename] [Open] to select a file or [Open] for default filename	Process using aii_H1_1D.mac to show the entire spectrum referenced to TMS. Expansion zo, integration id, peak picking pp or dp, and plotting pl may be done manually.
8	Enter zoom routine.	>zo	Set up for phasing.
9	Select two regions of interest. (see comment)	<1> then <2> <Enter> to exit "zo"	Drag cursor over a **strong** peak on left. Press <1> to assign as region 1. Drag cursor over a **strong** peak on the right and press <2> to assign as region 2.
10	Trim phase.	>pe <Enter> to exit "pe"	Phase left side peak by pressing and holding left MB while dragging mouse side to side. Repeat using the right MB to adjust the right peak.
11	Fit baseline.	>fb <l> <Enter>	Enter fb subroutine, remove stripes on or too close to peaks, press the letter "l" for Least Squares fit, save result and exit fb with <Enter>.
12	Enter integral display.	>id	
13	Integrate data.	two clicks of left MB, then one left click <Enter> to exit "id"	For each broken integral, click left MB twice on left side of peak(s) then once on right side. To assign a relative integral value place cursor on integral, click left MB, press <v> and enter number. <Ctrl+I> toggles integrals on/off.
14	Pick Peaks a. Automatic or b. Manual	>pp or >dp <c> <Enter> to exit "dp"	a. Automatically picks peaks - Vertical red lines indicate selected peaks. Use MH and RM to change peaks selected. b. Manual peak pick -The cursor becomes a crosshair with a DP label. <a> automatically picks peak; <c> clears all peak picks; <k> removes a single peak pick nearest the cursor. Add peak by clicking the left MB near any peak. <t> writes peak list to the table. <Ctrl+B> toggles peak pick table on/off. <Ctrl+P> toggles the peak labels on/off.
15	Expand selected region.	>zo <Enter> to exit "zo"	Select expansion region with mouse or <f> to enter fixed offsets with information dialog box. <Ctrl+E> gives the expanded region <Ctrl+F> gives the full spectrum.
16	Plot Data.	>pl	

^{13}C 1D Spectra Guide

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		See Sample Preparation Guide. Position sample spinner using the depth gauge, place in probe.
2	Enter PNMR program.	<Alt+Tab>	(If necessary.)
3	Select ^{13}C observe.	H1>nu c13<Enter>	Required only if the prompt is not C13>.
	Optional: Shim sample.	C13>shim<Enter>	Follow on-screen directions. Shims are optimized. Dilute sample RD=5; Conc. sample RD=2
4	Acquire ^{1}H spectrum.	C13>zgh<Enter>	Obtain ^{1}H spectrum using default conditions
5	Enter NUTS and process data.	<Alt+Tab> >a2	Trim phase as required. Use cursor to determine TMS peak position in ppm, including sign.
6	Return to PNMR and enter TMS peak position.	<Alt+Tab> C13>fo<Enter> *value*<Enter> 0<Enter>	Enter the current position (in ppm) of the TMS peak to the first dialog box and 0 (zero) to the second dialog box. Repeat to confirm.
	Optional: Confirm field offset and save ^{1}H spectrum for border of HETCOR plot.	C13>zgh<Enter> <Alt+Tab>,>a2 >sa *filename*	Acquire proton spectrum. Switch to NUTS and process with a2 link. Enter filename for ^{1}H spectrum, for example *border_h1*. <*Alt+Tab*> to return to PNMR
7	Verify parameters.		Verify that parameters make sense: for neat samples ns=12, for 1M samples ns=60, for concentration <1M use the BAPR program.
8	Acquire data.	C13>zg<Enter> then *filename*<Enter> or <Enter> for default	Enter filename if desired but it is usually better to use the default (pnmrfid) unless intending to save the data long term.
9	Enter NUTS.	<Alt+Tab>	
10	Process data.	<Ctrl+F3> then [filename] [Open] to select a file or [Open] for default	Aii_C13_1D.mac provides a calculated chemical shift scale, does an automatic peak pick, and sets the display range from 220 to -10 ppm.
11	Enter line broadening.	*value* <Enter>	LB=0.5 Hz is a typical value.
	Optional: Pick peaks manually.	>dp <Enter>	The cursor becomes a crosshair with a Dp label. <c> clears all peak picks; <k> removes a single peak pick at the cursor location. Add peaks by aligning cursor on a peak and clicking the left MB. <t> to write peak list to the table.
12	Plot Data.	>pl	
	Optional: Save ^{13}C spectrum for border of HETCOR plot.	<Ctrl+B> <Ctrl+P> >sa *filename*	Remove peak labels and table displays. Enter filename for ^{13}C spectrum, for example *border_c13*.
	Optional: Add scans	C13>Go <Enter>	In case the signal to noise is too low, the acquisition can be extended.
	Number of added scans	*scans*<Enter>	For neat samples ns=12, for 1M samples ns=60, for concentration <1M use the BAPR program.
		filename <Enter>	The program will ask for a filename when finished. Use the same name as step 8.

NOTE: For weak samples use Block Averaging with Peak Registration (BAPR)

DEPT Experiment Guide

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		See Sample Preparation Guide. Position sample spinner using the depth gauge, place in probe.
2	Enter PNMR program.	<Alt+Tab>	(If necessary.)
3	Select ^{13}C observe.	**H1>**nu C13<Enter>	Required only if the prompt is not **C13>**.
	Optional: Shim sample.	**C13>**shim<Enter>	Follow on-screen directions. Shims are optimized.
4	Acquire ^{1}H spectrum.	**C13>**zgh<Enter>	
5	Enter NUTS and process data.	<Alt+Tab> >a2	Process ^{1}H spectrum with a2 link. Trim phase as required. Use cursor to determine TMS peak position in ppm, including sign.
6	Enter PNMR program.	<Alt+Tab>	
7	Enter TMS peak position.	**C13>**fo<Enter> *value*<Enter> 0<Enter>	In the first dialog box enter the current position (in ppm)of the TMS peak. In the second dialog box enter 0 (zero). Repeat to confirm.
8	Verify parameters.		Verify that the parameters make sense: for neat samples ns=12, for 1M samples ns=60.
9	Run DEPT program.	**C13>**dept<Enter> then *filename*<Enter> or <Enter> for default	The program requests a filename while providing the default name My_dept. Use the default unless planning to save the data long term.
10	Acquire data.		The pulse program runs DEPT45, DEPT90 and DEPT135, requiring about 3 min using the parameters specified in step 8.
11	Enter NUTS.	<Alt+Tab>	
12	Process data.	<Ctrl+F11> then **[filename] [Open]** to select a file or **[Open]** for default	Data is processed with macro aii_dept.mac. Use mouse to select default data file [my_dept] or named data file [filename].
13	Enter line broadening.	*value*<Enter>	LB = 0.5 Hz is a typical value.
14	Display Stacked Plot.	↑ and/or ↓	Macro applies an approximate chemical shift scale, sets display for 220 to -10 ppm window, and displays a stacked plot of the DEPT45, DEPT90, and DEPT135 spectra. Adjust amplitude with Page Up/Page Down, arrow keys or vertical slider
15	Print Stacked Plot.	<p>	
16	Exit Stacked Plot.	<Enter>	To redisplay stacked plot from the base level NUTS prompt, use the command "sp".

Block Averaging with Peak Registration Guide

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		See Sample Preparation Guide. Position sample spinner using the depth gauge, place in probe.
2	Enter PNMR program.	<Alt+Tab>	(If necessary.)
3	Select ^{13}C observe.	H1>nu C13<Enter>	Required only if the prompt is not C13>.
4	Shim.	C13>shim<Enter>	It is important to set parameters for shimming since the Y gradient is optimized between blocks.
5	Acquire proton spectrum and set field offset.	C13>zgh<Enter> C13>fo<Enter>	Process proton spectrum with a2 link, phase, determine TMS position, execute fo and enter value in ppm. Repeat to confirm.
6	Verify parameters and set number of scans.		Choose number of scans (ns) per block so registration peak is visible after ns scans.
7	Run BAPR program.	C13>bapr<Enter> then *filename*<Enter> or <Enter> for default	The program asks for the number of blocks of data to acquire and for a filename. Enter filename if desired but it is usually better to use the default (My_bapr) unless one intends to save the data long term.
8	Enter NUTS.	<Alt+Tab>	
9	Check LB parameter.	>lb	LB=1 Hz is a typical value.
10	Process first spectrum.	>a1	Execute processing commands in link a1. (Use <Ctrl+F3> if processing stored data from an earlier BAPR experiment.)
11	Trim phase.	>zo and >pe	Phase adjustment as necessary.
12	Define registration peak.	>zo and <0> <Enter>	Zoom to an isolated peak, well separated from other peaks, and define zoom region with <0>. The zoom region 0 must contain only one peak.
13	Run bapr processing macro.	<Ctrl+F12> then [filename] [Open] to select a file or [Open] for default	Process using aii_C13_bapr.mac, which sums the blocks, applies an approximate chemical shift scale, peak picks and sets display to 220 to -10 ppm window.
14	Plot data.	>pl	Note: Phase adjustment can still be made before plotting.
15	Optional: Save data.		In Windows Explorer, either rename My_bapr or create a new folder and move My_bapr file from c:\Eft\Data into the new folder.

H1 BAPR: Running an H1 BAPR experiment using a user-defined registration peak follows nearly the same procedure, except that at step 13 run the analogous proton macro as follows:

>ru and then choose **[aii_H1_bapr.mac] [open]**

To process data in which the TMS peak is suitable for automatic peak registration, simply follow the ^{1}H Survey Spectra Guide starting at step 7b on page 7.

C13 BAPR:

Use the following table to estimate the number of milligrams of sample needed in 0.7 ml of solvent to reach the desired concentration. Then, use the final two columns to estimate the number of scans needed per block.

	mg of sample required in 0.7 ml				60 MHz	90 MHz
Conc. (M)	MW 100	MW 200	MW 300	MW 400	scans per block	scans per block
0.1	3.5	7	10.5	14	480	320
0.2	7	14	21	28	120	80
0.4	14	28	42	56	32	20
0.8	28	56	84	112	8	4
1.6	56	112	168	224	2	1

COSY and HETCOR* Experiment Guide

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		See Sample Preparation Guide. Position sample spinner using the depth gauge, place in probe.
2	Enter PNMR program.	<Alt+Tab>	(If necessary.)
3	Set Experiment Parameters.	**H1>**cosy<Enter>	For HETCOR: **C13>**hetcor<Enter>.
4	Enter file name.	*filename*<Enter> or <Enter> for default	Enter filename if desired, but it is usually better to use the default (my_cosy or my_hetcor) unless one intends to save the data long term.
5	Set relaxation delay.	*value*<Enter>	2 seconds is a typical rd for COSY.
6	Set number of scans.	*value*<Enter>	*For COSY*: Optional: 4 scans to minimize artifacts. *For HETCOR*: increase NS by multiples of 4 for weak samples, <2M.
7	*COSY Only*: The gain is adjusted		The spectrometer adjusts the receiver gain.
8	Acquire data.		
9	Enter NUTS.	<Alt+Tab>	
10	Process data.	<Ctrl+F5> then **[filename] [Open]** or **[Open]** for default	Runs aii_cosy.mac. (<Ctrl+F6> runs aii_hetcor.mac in the case of hetcor.) Use mouse to select data file in dialog box.
11	Enter data acquisition parameters.	**[OK]**	Add name, date and experiment if desired. Macro then shows an intensity plot when done.
12	Add borders.	**[border] [Pick top spectrum] [filename] [open]**	Open border menu; scroll to pick top spectrum. Repeat the process for the left border.
13	Adjust data display.	>mh or use the slider at the right side of the screen	Adjust **M**inimum **H**eight for best display. (Hint: Look for off-diagonal peaks. **MH** value normally is between 0.5 and 2.0 for COSY.)
14	COSY only: Set plot limits.	>zo <f> <Ctrl+E>	Enter start and end of zoom for both dimensions. Exit zoom with <Enter><Enter>
15	Display contour plot.	<cp>	Change to contour display before plotting.
16	Print Contour Plot.	>pl	Type <1d> to exit 2D display mode. To redisplay intensity plot from the base level NUTS prompt, type the command "ip".

* Note that the HETCOR operating procedure is very similar to the steps used for COSY. Data acquisition is initiated with the PNMR pulse program **C13>**hetcor<Enter> and processed in NUTS with <Ctrl+F6>, the macro aii_hetcor.mac.

XPT, X-nucleus with Polarization Transfer.

It is frequently the case that one obtains a better result for an X-nucleus by acquiring the spectrum with magnetization transfer from H1. One way of transferring magnetization from H1 to another nucleus is the DEPT sequence. The advantages are due to the large gyromagnetic ratio of H1, the fact that H1 relaxation times are frequently much shorter than those of X-nuclei in the same molecule, and that one avoids the problem of having a NOE of near -1 for nuclei with a negative gyromagnetic ratio.

However, to run the polarization transfer experiment more knowledge is required. A reasonable guess at the largest coupling to H1 by the X-nucleus is required to set the timing for the transfer step. The optimum flip angle for the read pulse is dependent on the number of H1 so coupled to the X-nucleus. The XPT pulse program is designed to allow block averaging with peak registration as an option.

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		See Sample Preparation Guide. Position sample spinner using the depth gauge, place in probe.
2	Enter PNMR program.	<Alt+Tab>	(If necessary.)
3	Select X observe.	**H1>**nu X<Enter>	Si29 for example
	Optional: Shim sample.	**Si29>**shim<Enter>	Follow on-screen directions. Shims are optimized.
4	Acquire ^{1}H spectrum.	**Si29>**zgh<Enter>	
5	Enter NUTS and process data.	<Alt+Tab> >a2	Process ^{1}H spectrum with a2 link. Trim phase as required. Use cursor to determine TMS peak position in ppm, including sign.
6	Enter PNMR program.	<Alt+Tab>	
7	Enter TMS peak position.	**Si29>**fo<Enter> *value*<Enter> 0<Enter>	In the first dialog box enter the current position (in ppm)of the TMS peak. In the second dialog box enter 0 (zero). Repeat to confirm.
8	Verify parameters.		Verify that the parameters make sense. Particularly the number of scans.
9	Run XPT program.	**Si29>**xpt<Enter> then *filename*<Enter> or <Enter> for default	The program requests a filename while providing the default name my_xpt. Use the default unless planning to save the data long term.
10	Enter coupling constant	*value*<Enter>	At least, a guess at the largest X-H1 coupling.
11	Enter flip angle for read pulse, in degrees.	*value*<Enter>	90 degrees for 1 coupled H1 18 degrees for 9 coupled H1
12	Enter number of blocks to acquire and indicate if the program should shim between blocks	*value*<Enter> 1 or 0<Enter>	Set number of blocks for required s/n. If the acquisition is going to be more than an hour have the program shim between blocks.
13	Enter NUTS	<Alt+Tab>	
14	Process data.	>a1 or >ru	Use the appropriate link or macro for processing. 1 block data can be processed with the "a1" link. Multiblock data should be processed with the aii_Xbapr.mac. Set the LB before running the link or macro.
15	Expand display as required and reference.	>zo <f>	To reference a peak, put the vertical cursor on it and type <o> then enter the chemical shift value.
16	Print the spectrum	>pl	To redisplay stacked plot from the base level NUTS prompt, use the command "sp".

Relaxation Time (T1) Experiment Guide

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		See Sample Preparation Guide. Set Field, properly position the spectrum and adjust RG
2	Enter PNMR program.	<Alt+Tab>	(If necessary.)
3	Set parameters.		*For proton*: SI=16384 NS=1 RG=as appropriate *For carbon (4M solution)*: SI=16384 NS=4 RG=100
4	Acquire relaxation data.	**H1>**invrec<Enter> or **C13>**invrec<Enter>	For proton T1 measurement. For carbon-13 T1 measurement.
5	Enter filename.	*filename*<Enter> or <Enter> for default	Status window shows the default filename (my_t1).
6	Enter relaxation delay.	*value*<Enter>	RD must be at least six times the longest expected T1 to get an accurate measurement.
7	File containing delay times.	<Enter>	Use default: t1list. (Note: Values are used in calculation at step 19.)
8	List delay times.	<Enter>	Status window shows list of delays.
9	Acquire data.	<Enter>	If FID is red, or status window reports ADC overloaded during experiment, stop acquisition and reduce rg setting. When complete, status window shows instructions to process data.
10	Enter NUTS.	<Alt+Tab>	
11	Process data.	>a1	Execute processing commands in link a1. (Use link a3 and set s1 to the last one if processing stored spectra from an earlier experiment.) Use LB ~1 for C13.
12	Trim phase.	>zo and >pe	Fine-tune the phase adjustment as necessary.
13	Process data series.	<Ctrl+F7>	Runs NUTS macro: aii_t1.mac
14	Open data file.	**[filename] [Open]** or **[Open]** for default	Click on file name and open.
15	Enter data acquisition parameters.	[OK]	Enter your name, the date, experiment title and any additional information in appropriate cells, e.g., ***Comment***.
16	Display stacked plot.	↑ and/or ↓	Data displayed as a stacked plot. Adjust amplitude with arrow keys or vertical slider .
17	Print stacked plot.	<p>	
18	Exit stacked plot.	<Enter>	To redisplay stacked plot from the base level NUTS prompt, type the command "sp".
19	Calculate T1 value.	>zo	Enter zoom mode.
20	Select peak to be measured.		Click left MB to left of peak and drag to right. Selected peak is shown in red.
21	Expand selected peak.	<Ctrl+E>	
22	Get relaxation data.	>gr	Integrates selected peak for each spectrum in series and saves the results.
23	Reduce data.	>dr	Shows X-Y plot of peak integration vs. time.
24	Optimize fit.	<o>	Select **[T1 3IR]** method.
25	Print data.	<p>	Then <Enter> to exit data reduction mode.

Kinetics Experiment Guide

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		See Sample Preparation Guide. Run spectrum on reference sample and use `fo` command to properly position the spectrum. Prepare and equilibrate reactants to same temperature as magnet. Have NMR sample tube ready with spin collar.
2	Enter PNMR program.	<Alt+Tab>	(If necessary.)
3	Start kinetics program and set parameters.	**H1>**kinetic<Enter>	Verify: SI=16384 NS=1 Gain (`rg`) must be set based on the reactant with the largest signal, which should be half of full scale. Suggestion: Run preliminary spectrum of one reactant to set parameters.
4	Enter filename.	*filename*<Enter> or <Enter> for default	Status window shows the default filename (my_kinetic).
5	Enter first delay time.	*value*<Enter>	Use initial delay approx. equal to 1/5 the expected half-life of the reaction, so about half the spectra will be taken before the mid-point of the reaction. All times in seconds.
6	Enter number of experiments.	*value*<Enter>	Try 20.
7	Enter time between mixing & starting NMR acquisition.	*value*<Enter>	PNMR counts the time down, starting at the moment the mixing starts in step 10 below. 60 seconds to mix may be appropriate.
8	Note list of delays.	<Enter>	Press <Enter> until the end of list.
9	Set/Display pulse width.	*value*<Enter>	The program either requests the 90° pulse width or reads it from the pulses.txt file. The pulse width is divided by two to get a 45° flip angle.
10	Mix reactants.	<Enter>	Follow prompt. PNMR starts countdown and begins to acquire spectra at set delay intervals.
11	Switch to NUTS.	<Alt+Tab>	
12	Process first spectrum.	>a1	Execute processing commands with link `a1`.
13	Trim phase.	>zo and >pe or >ph	Fine-tune the phase adjustment as necessary.
14	Run kinetics macro.	<Ctrl+F9>	
15	Open data file.	**[filename] [Open]** or **[Open]** for default	Click on file name and open. Macro processes entire data series.
16	Enter data acquisition parameters	*sample name, experiment* <Tab> *initials* **[OK]**	Enter sample name, experiment, etc. and initials in the appropriate cells
17	Display stacked plot	↑ and/or ↓	Data displayed as a stacked plot. Adjust amplitude with Page Up/Page Down, arrow keys or vertical slider.
18	Print stacked plot	<p>	<Enter> to exit stacked plot mode.
19	Exit stacked plot	<Enter>	To redisplay stacked plot from the base level NUTS prompt, type the command "sp".
20	Determine reaction rate.	>zo	Enter zoom mode.

Kinetics Experiment Guide *(continued)*

21	Select peak to be measured.		Click left MB to left of peak and drag to right. Selected peak is shown in red. Press <Enter> to exit zoom routine.
22	Expand selected peak.	<Ctrl+E>	
23	Extract peak integrals.	>gr	Integrates selected peak for each spectrum in series and saves the results.
24	Reduce data and display	>dr	Shows X-Y plot of peak integration vs. time.
25	Choose fit function.	<f>	For reactant peaks select **[T2 single decay]**. For product peaks select **[T1IR]**.
26	Optimize fit.	<o>	Note T2/T1 values, which are = 1/reaction rate, if first order kinetics.
27	Print data.	<p>	Then <Enter> to exit data reduction mode.
28	Repeat.		Repeat steps 18 – 27 for each peak.
			Note: Use NUTS dl (letter "d" numeral "1") command to list the delay times.

Spin Simulation Guide

1	Enter **NUTS** program.	<Alt+Tab>	If necessary
2	Process experimental spectrum.		Creates simulation parameters, e.g. sweep width, to be identical to experimental parameters, and gives option to optimize fit to experimental spectrum.
3	Trim phase.	>zo and >pe	Fine-tune the phase adjustment as necessary.
4	Expand.	>zo	Zoom to show peaks of interest.
5	Measure chemical shifts and coupling constants.		With the mouse cursor, click the left MB and measure peak positions to determine chemical shifts and coupling constants.
6	Start spin simulation subroutine.	>ns	
7	Add simulation data.	<a>	
8	Enter number of spins and chemical shifts.		Fill in fields in dialog box. The number of spins is the total number of spins including those that are equivalent. Enter chemical shifts for each spin. Those that are equivalent have identical chemical shifts.
9	Enter coupling constants.	*values* **[Accept and Return to Chemical Shifts]**	Fill in matrix. Return to chemical shift data.
10	Calculates Spectrum.	**[Accept & Recalculate]**	The time to calculate may increase substantially with increasing number of spins. Repeat steps 8-10 to refine chemical shifts and coupling constants. <b> to show experimental and calculated spectra.
	Optional: Optimize fit.	<o> then <y>	Choose single simplex optimization. The calculation may be very time-consuming and often steps 8-10 produce a fairly good match to the experimental spectrum. <q> to abort.
11	Note results of fit.	<a> <b>	<a> Examine tables of chemical shifts and coupling constants showing the results of the fit done above. <b> Show both calculated (bottom) and experimental (top) spectra.

Appendix

Calibration and Maintenance Schedule

Procedure	Purpose	Approximate Frequency
Update phase correction Parameters.	Update PA and PB phase correction parameters in Nuts.ini file so "pc" command routinely produces a properly phased spectrum.	monthly
Proton observe 90° pulse width	Determine and store calibrated proton 90° pulse time for use by experiments like COSY, and T1.	quarterly
High power decoupler 90° pulse width.	Determine high power decoupler 90° pulse width. Values are automatically stored for use by experiments like DEPT and HETCOR.	quarterly
Decoupler power for H1 WB decoupling	Determine decoupler power with dlpcal pulse program.	quarterly
Carbon observe 90° pulse width.	Determine carbon 90° pulse width with c90cal pulse program. Values are automatically stored for use by experiments like DEPT and HETCOR.	quarterly
Adjust 3rd and 4th order shims	Reduce width and asymmetry near base of peaks.	quarterly
Adjust magnet shunt	Change the magnet's field so the field offset is near center – necessary only if the field offset is near the end of its range (± 2040).	as required
Software installation	Install PNMR and/or NUTS software	as required
Tune WB Probe. On WB Observe Only	Probe Setup, Tune, and Pulse Width Calibration	as required

Update Phase Correction Parameters

1	Sample		5% ethylbenzene or 5% ethylmethacrylate, spinning.
2	Enter PNMR.	<Alt+Tab>	(If necessary.)
3	Acquire data.	**H1>**zg<Enter>	
4	Enter NUTS.	<Alt+Tab>	
5	Process data.	>a2	
6	Trim phase.	>zo and >pe or >ph	Fine-tune the phase adjustment.
7	Record phase in the case of ^{1}H.	>tp <Enter> to close dialog box	Note total phase-correction applied and update the **DEFAULT_PA** and **DEFAULT_PB** values in the c:\nuts\Nuts.ini file. Save the modified file. Close and reopen NUTS.
8	Set field for acquisition	**H1>**prep<Enter>	Follow this guide (Part A on page 6) to set field

Proton Observe 90° Pulse Width (>H1)

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		Load 98% n-propylbenzoate + 2% TMS (with ^{1}H-only spectrometers use 5% ethylbenzene or 5% ethylmethacrylate) reference sample. Shim as required. Take a spectrum and use `fo` command to position the ^{1}H spectrum.
2	Select ^{1}H Observe.	`C13>nu H1<Enter>`	(If necessary.)
3	Determine 90° pulse width.	`H1>h90cal<Enter>`	All of the standard .ini files as well as the pulses file are automatically updated.

High Power Decoupler (HP Dec) 90° Pulse Width (>H1)

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		Load 98% n-propylbenzoate + 2% TMS reference sample. Shim as required.
2	Select ^{1}H Observe.	`C13>nu H1<Enter>`	(If necessary.)
3	Determine high power decoupler 90	`H1>dec90cal<Enter>`	All of the standard .ini files as well as the pulses file are automatically updated.

Decoupler Power for Broad Band Decoupling (>C13)

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		Load 98% n-propylbenzoate + 2% TMS reference sample. Shim as required. Take a spectrum and use `fo` command to position the ^{1}H spectrum.
2	Select ^{13}C observe.	`H1>nu C13<Enter>`	(If necessary.)
3	Determine decoupler 90° pulse width.	`C13>dlpcal<Enter>`	All of the standard .ini files as well as the pulses file are automatically updated.

Carbon Observe 90° Pulse Width (>C13)

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		Load 98% n-propylbenzoate + 2% TMS reference sample. Shim as required.
2	Select ^{13}C observe.	`H1>nu C13<Enter>`	(If necessary.)
3	Determine 90° pulse width.	`C13>c90cal<Enter>`	All of the standard .ini files as well as the pulses file are automatically updated.

Adjust the 3rd and 4th Order Shims

Step	Function or Dialog Box	<Keystroke>/[Select]/<*Data Entry*>	Comment
1	Sample		Tap water sample, spinning. (Do not use the sealed "Homo. Adj." sample supplied with the original CW spectrometer.)
2	Switch to maintenance module	X>nu mm<Enter>	
3	Run Shim routine.	MM>shim2<Enter>	Optimizes spinning resolution and lineshape.
			Manual shims only: Follow instructions displayed on monitor. Process may take about one hour. *Automatic shims*: May take about ten minutes.

Adjust Magnet Shunt

1	Load water sample		Tap water, spinning. (Do not use the "Homo. Adj." sample supplied with original CW instrument)
2	Enter PNMR.	<Alt+Tab>	(If necessary.)
3	Switch to maintenance module	X>nu mm<Enter>	(If necessary.)
4	Run Shunt.	MM>shunt<Enter>	Gain is automatically adjusted to correct value.
5	Switch to Spectrum	<Ctrl+S>	
6	Adjust shunt.	Center peak on screen.	Engage the shunt screw with the brass screwdriver. For Varian magnets turning the shunt screw CCW moves the signal to the left. For Hitachi magnets turning the shunt screw CW moves the signal to the left. It is normally difficult to get the shunt screw engaged …keep trying. Be brave, as long as you can see the signal it is hard to do any harm. If in doubt, call Aii (317) 783-4126.
7	Exit GS mode.	<Ctrl+Q>	<Ctrl+S> to switch back to FID

Computer Hard Disk Defragment

1	Start DiskDefragmenter	Click **[Start]** button, then **[Programs]** **[Accessories]** **[System Tools]** **[Disk Defragmenter]**	Follow the system specific directions below to defragment the local hard drive(s).
2a	For Win-9X	Choose Disk via Pulldown, then Click **[OK]** button	The time necessary to defragment will depend upon the level of fragmentation and the computer. Click **[Details]** to monitor progress
2b	For Win-2K/XP	Choose Disk, then Click **[Defragment]** button	Disk defragment will proceed. The time necessary for defragment will depend upon the level of fragmentation and the computer.

NMR Software/Data Backup

1	Data backup	Open **[WindowsExplorer]**	Within Windows Explorer, check to see if another hard disk is installed. If there is not a backup hard disk installed, use the Zip drive for the system/data backup.
2a	2nd hard disk drive	Highlight *C:\Eft* folder and the *C:\Nuts* folder	Copy <Ctrl+C> the C:\Eft and C:\Nuts folders Paste <Ctrl+V> them in the destination drive.
2b	Zip drive	Highlight *C:\Eft* folder and the *C:\Nuts* folder	Copy <Ctrl+C> the C:\Eft and C:\Nuts folders Paste <Ctrl+V> them on the destination disk.

Computer Disk Scan (Win-9X)

1	Open scandisk program	Click **[Start]** button, then **[Programs]** **[Accessories]** **[System Tools]** **[Scandisk]**	Follow the directions below to scan the disk for errors on the local hard drive(s)
2	Start disk scan	Choose Disk via Pulldown, then Click **[OK]** button	The time necessary to scan will depend upon the computer.

Computer Disk Scan (Win-2K/XP)

1	Administrator login	Logout NMR User, then Login as administrator	Must have administrator privileges to run scandisk on the local disks.
2	Setup disk scan	Open **[WindowsExplorer]**	
3	Choose local disk	RightClick *local disk C:* then choose **[Properties]**	Choose [properties] from the pulldown menu.
4	Open Tools tab	Click **[Tools]** tab	
5	Start disk scan setup	In *Error-checking* section click **[Check Now...]**	
6	Options	In the popup window, check both boxes, then click **[Start]**	This will give the most thorough error checking with prompts from the user.
7	Disk scan start	Click **[Yes]** to start scan upon next boot, Then **[OK]**	
8	Reboot	Reboot the computer	

Wide Band Only: Probe Setup, Tune, and PW Calibration

A	Set up New Nucleus		Install a new nucleus for observation
1	Enter the new observe nucleus.	H1>nu x<Enter>	X = a nucleus for which a folder does not exist
2	Create all necessary files	x>setup<Enter>	This command initializes the .ini files and the pulses files for the nucleus. Note: The .ini file will have to be customized for the proper acquisition values (w1,ns,rg,rd, etc)
3	Change observe parameters for new observe nucleus	x>w1 *value*<Enter> x>ns *value*<Enter> x>rd *value*<Enter> etc.	Suitable observe parameters may require experimentation.
	Store parameters	x>wr x.ini<Enter>	
B.	Tune WB Probe		Properly tune wide band or x channel of the probe.
1	***Important Note 1*** →		This guide describes a general procedure for any X nucleus including ^{13}C. Use h1tune or f19tune for ^{1}H and ^{19}F respectively. The screen dialog boxes contain additional information **specific** for the nucleus of interest.
2	***Important Note 2*** →		Tuning the ^{1}H channel of the probe affects the tune of the X channel. After tuning the ^{1}H, the X channel **must** be re-tuned before making observations.
3	Load Sample.		The probe should be tuned with a typical sample in the probe.
4	Enter observe nucleus	?>nu x<Enter>	Previously observed nucleus Go To step A1. New Observe Nucleus Go To step C1.
5	Initiate tuning process.	x>wbtune<Enter> **Follow Instructions on Screen**	x = specific nucleus being observed.
C.	Calibrate 90° Pulse Width		
1	Import starting parameters	x>re cal.ini<Enter>	Read starting parameters used for 90° pulse width calibration.
2	Adjust observe parameters	x>zg <Enter>	Process data in Nuts. Check that there is a good signal — observe within 1000Hz of center of screen.
3	Change observe parameters as necessary	x>w1 *value*<Enter> x>ns *value*<Enter> x>rd *value*<Enter> etc.	
4	Save parameters	x>wr cal.ini	
5	Determine pulse width.	x>x90cal<Enter> or for Carbon-13 C13>c90cal<Enter>	All of the standard .ini files as well as pulses.txt are automatically updated.