Standard Operation Procedures for

Anasazi EFT FT-NMR Spectrometer

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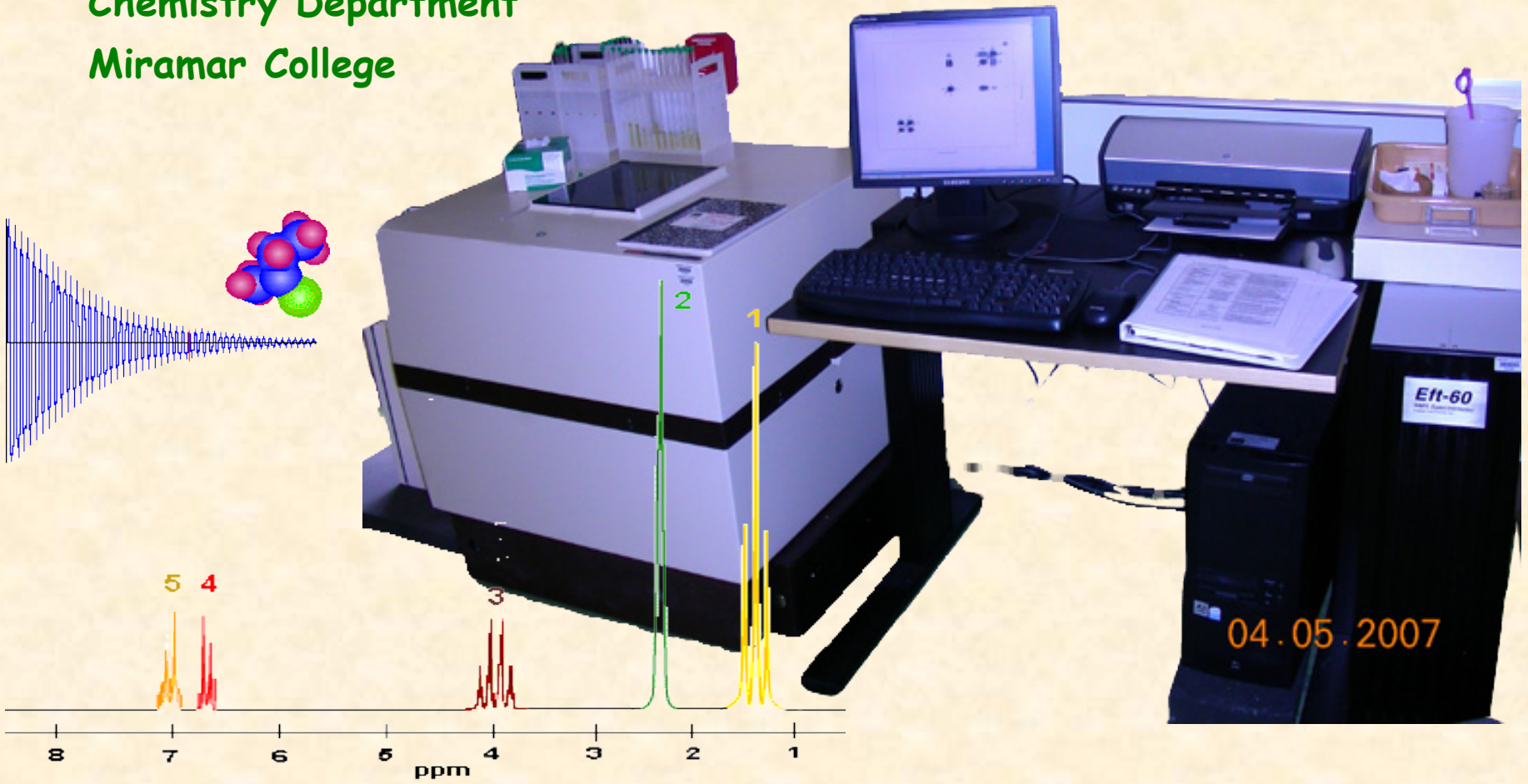


Precautions

- If you wear a pacemaker, **STOP**, you are not allowed to use this instrument. The magnet may affect your pacemaker.
- Remove watches or electronic devices on your person, these may get demagnetize.
- Do not handle spinner with your bare hands, always use Kimwipe® to handle the NMR spinner and clean any finger residue from NMR sample before inserting in magnet
- Clean the NMR area when you finish using the instrument.

Quick Overview

I. Prepare the sample

A. Use NMR tube with appropriate sample

II. Turn on Spectrometer and Inserting Sample

A. In PNMR, run D_2O sample to optimize spin rate and to shim magnet

III. Preparing Spectrometer to suppress spinning sidebands

A. Enter chemical shift of water
B. Adjust spin rate so FID shows crest-to-crest of 5 division
C. Adjust spin rate so FID shows crest-to-crest of 2 division

IV Acquire NMR spectrum

A. Adjust shim and gain to sample.
B. Set up field offset freq so TMS is at 0 PPM
C. Collect data for NMR sample, 1H or 13C.
Be sure number of scan (NS) is appropriate for good S/N

V. Process and plot the data

A. In NUTS zoom in to region of interest
B. Phase spectrum (AP) and apply base line correction (BC)
C. Integrate the 1H NMR spectrum and assign integration value.
D. Pick peaks and label chemical shift
E. Plot spectrum

I. Preparing the NMR Sample

1. Sign log book
2. Find an appropriate solvent to use for your sample. If your sample is a non-viscose liquid, then use a neat sample.

The quality of results is critically dependent on appropriate sample preparation methods.
For all samples:

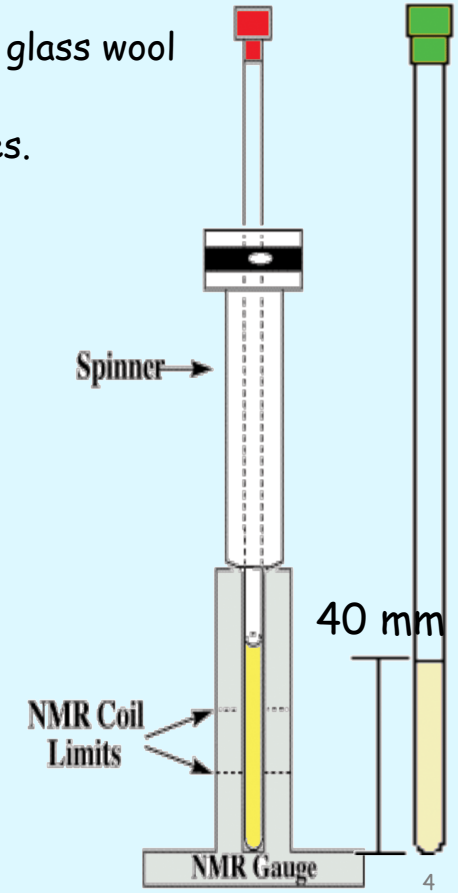
1. Use a total sample solution volume of 0. 7ml (4cm liquid height).
2. If the sample contains particulate matter, filter the solution through glass wool or cotton that is loosely packed in a Pasteur pipette.
3. Use 7" Wilmad 507-pp or Norell 502 or equivalent quality sample tubes.

^{1}H Samples

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

^{13}C Samples

- Neat (meaning 100% concentration) liquids will give good spectra in 1 minute and 1M samples will allow good spectra with ≈ 120 scans.
- TMS at 2% should be adequate to reference a 1M solution.

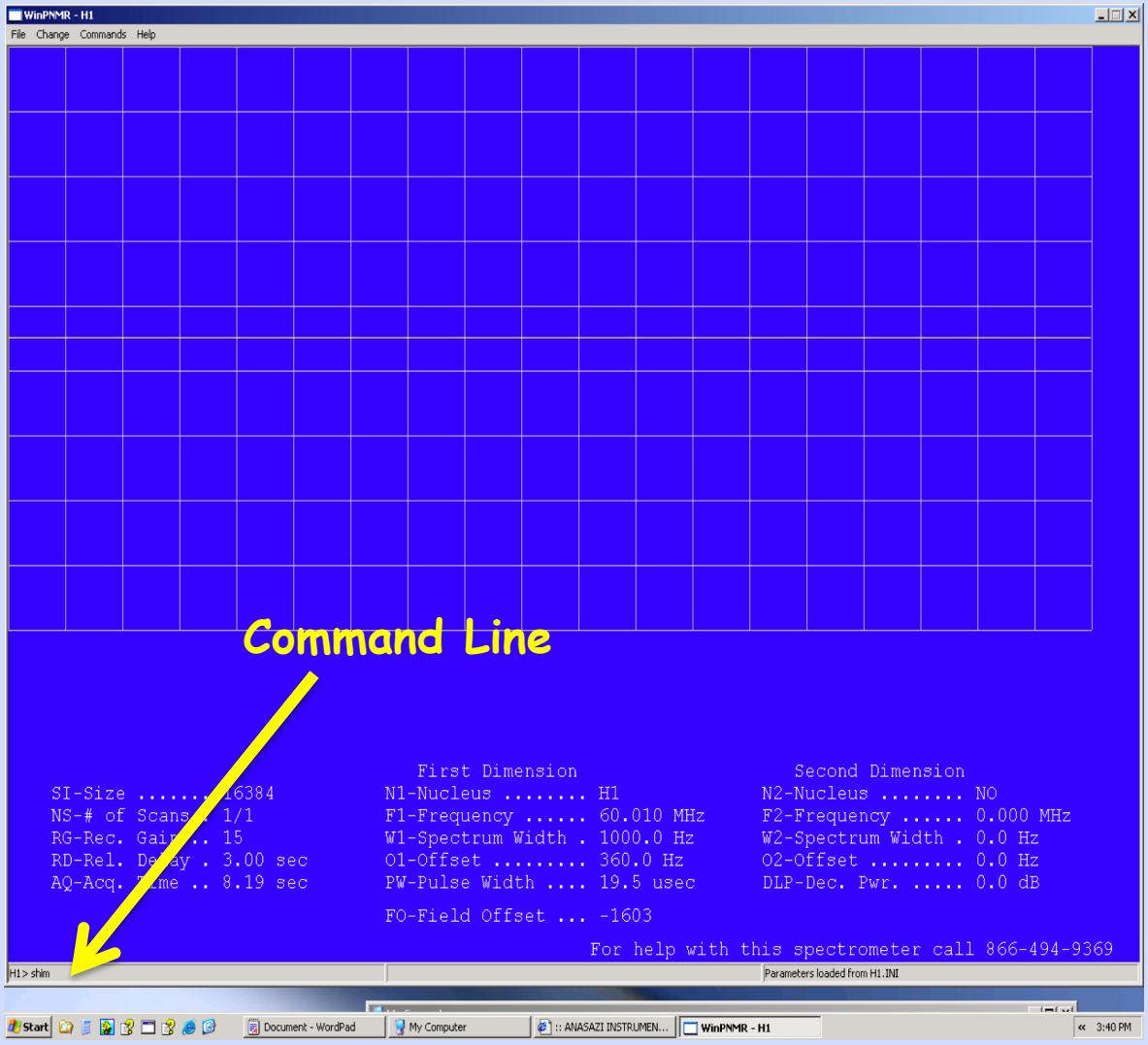


II. Turning on the Spectrometer and Checking Computer

1. Turn on the computer if it is not already on.
2. In the opening screen find PNMR icon and Nuts icon
3. Launch both PNMR and NUTS

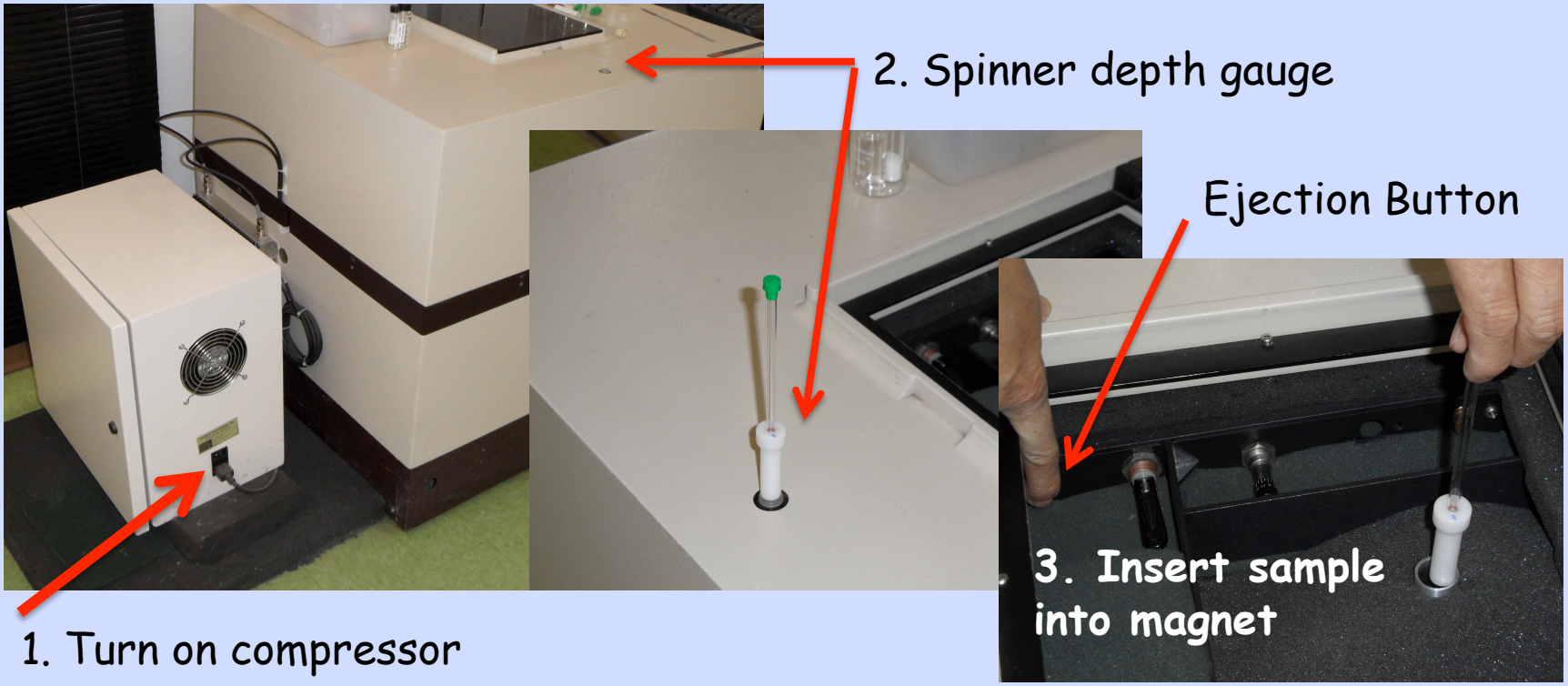
II. Turning on the Spectrometer and Checking Computer

1. Opening Screen for PNMR



II. Turning on the Spectrometer and insert sample

1. Turn on the air compressor.
2. Insert the NMR sample tube in the spinner collar. Position spinner collar using the dept gauge.
3. Depress the air eject button, place the NMR sample in the aperture and then depress the air ejection button so the sample drops slowly down the magnet. Look down the aperture and make sure the sample is spinning



III. Adjust Spin Rate Use D_2O/H_2O sample

This procedure is to adjust field and optimize shims.
If the shims are optimize, skip to step IV

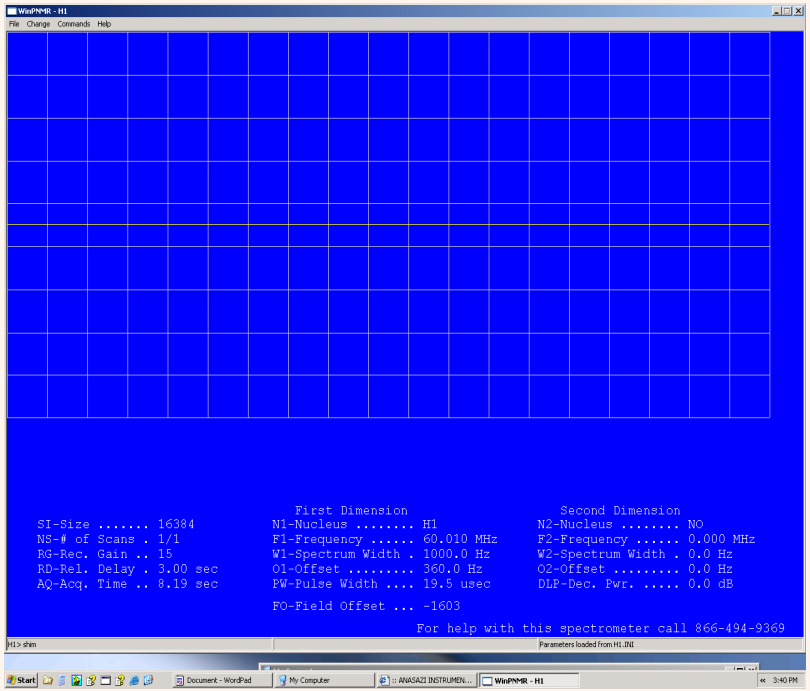
Brief summary of commands-

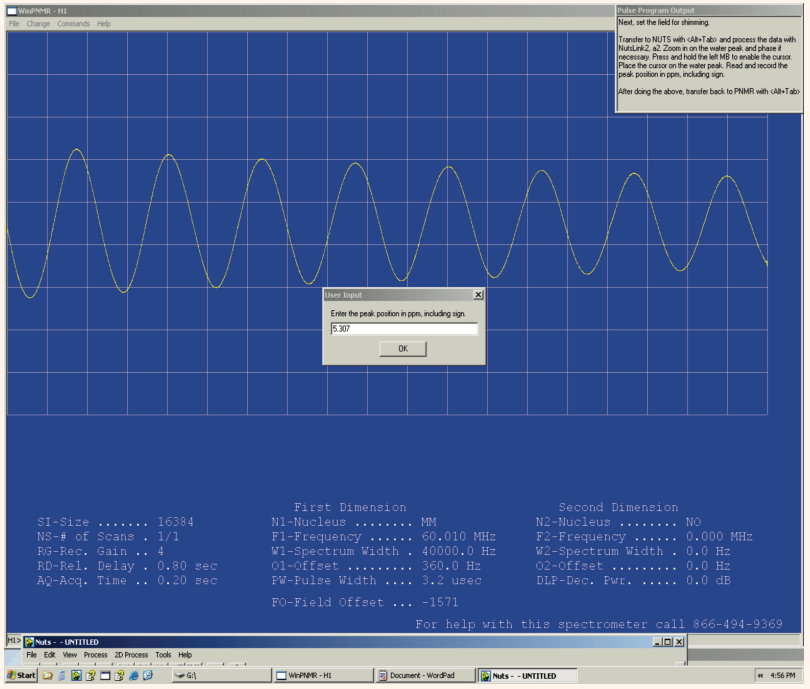
	Keystroke	Function	Comment
1		Sample to run	Use the NMR sample labeled D2O
2	<Alt +Tab>	Open PNMR	
3	H1> prep	Acquire data	Spectrometer enters GS mode.
4		Gain Adjusted	The spectrometer adjusts the receiver gain .
5		Spectrum acquisition.	Prep takes a spectrum
6	<Alt +Tab> >a2	Enter NUTS and process data	Trim phase as required. Use cursor to determine peak position in ppm, including sign.
7	<Alt +Tab> value <E>	Enter PNMR and set field for shimming	Change to PNMR and enter the peak position in the User Input box
8	Adj spin <Ctl+Q>	Adjust spinning speed.	Adj ust 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	Adj spin <Ctl+Q>	Adjust spinning speed.	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	<Alt +Tab> >a2	Enter NUTS and process data	(Trim phase as required .) Use cursor to determine peak position in ppm, including sign
13	<Alt +Tab> value <E>	Enter PNMR and set field	Change to PNMR and enter the peak position in the User Input box (Values should be ≈ -4)

III. Adjust Spin Rate Use D_2O/H_2O sample

This procedure is to adjust field and optimize shims.
If the shims are optimize, skip to step IV ... continue

	Keystroke	Function	Comment
1		Sample to run	Use the NMR sample labeled D_2O
2	<Alt +Tab>	Open PNMR	
3	H1> prep	Acquire data	Spectrometer enters GS mode.
4		Gain Adjusted	The spectrometer adjusts the receiver gain .
5		Spectrum acquisition.	Prep takes a spectrum
6	<Alt +Tab> >a2	Enter NUTS and process data	Trim phase as required. Use cursor to determine peak position in ppm, including sign.
7	<Alt +Tab> value <E>	Enter PNMR and set field for shimming	Change to PNMR and enter the peak position in the dialog box

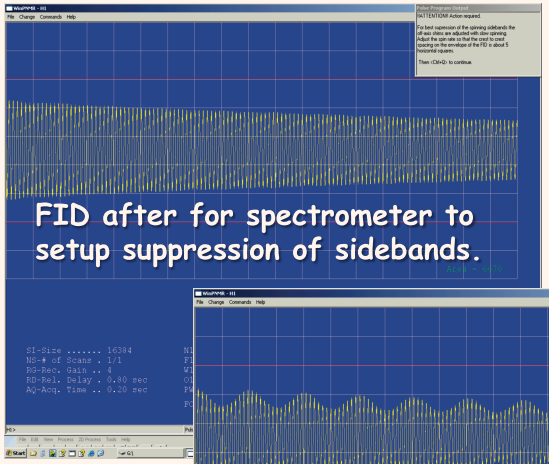




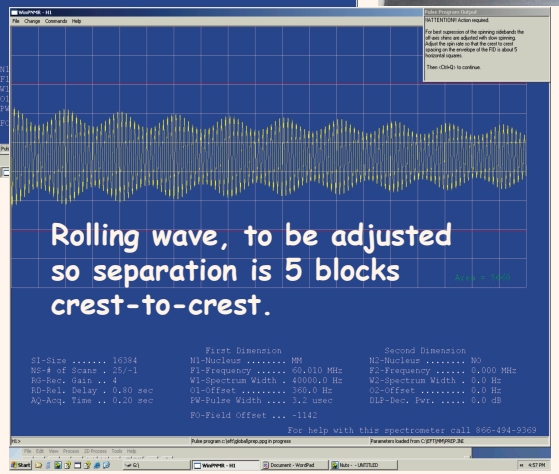
III. Adjust Spin Rate Use D_2O/H_2O sample

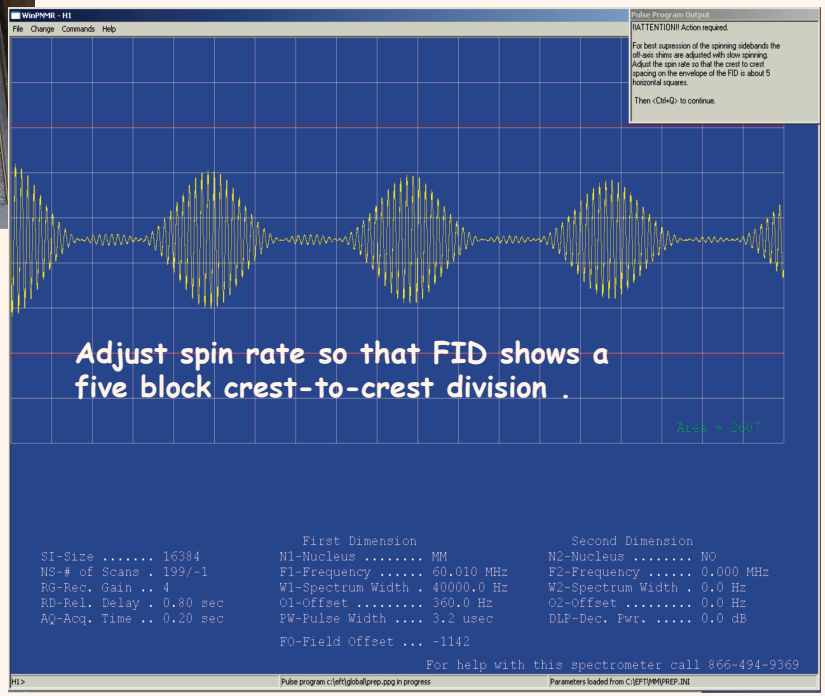
Adjust spin rate so rolling wave is separated by 5 division.

	Keystroke	Function	Comment
8	Adj spin <Ctl+Q>	Adjust spinning speed.	Adjust spinning speed so the crest-to-crest spacing in the FID is about 5 horizontal divisions.



Adjusts spin rate with this spinner control.
Increase spin rate by turning clockwise.





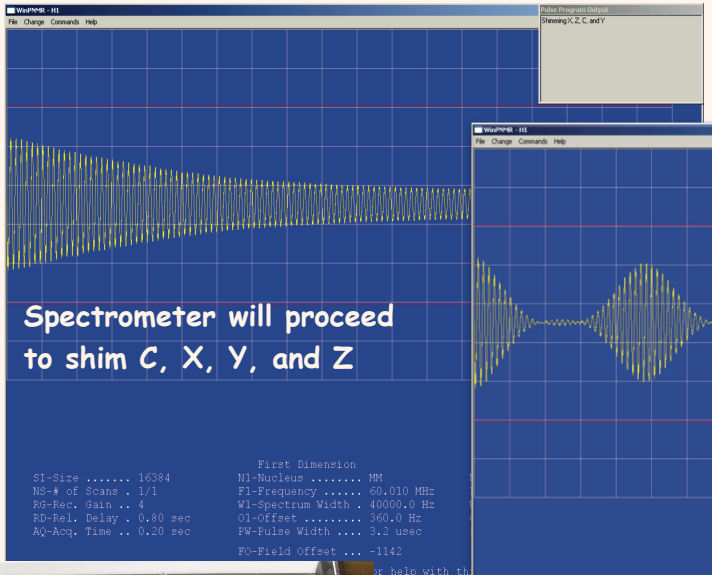
8 After assigning peak position, spectrometer goes through routine for suppression of spinning sideband.

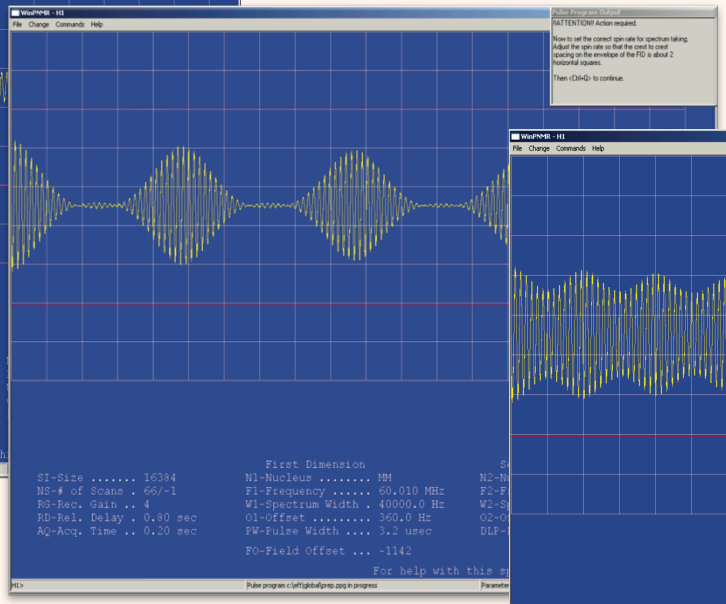
The display will show an FID with rolling waves. Adjust spin speed so that the crest-crest is separated by five divisions.

III. Adjust Spin Rate Use D_2O/H_2O sample

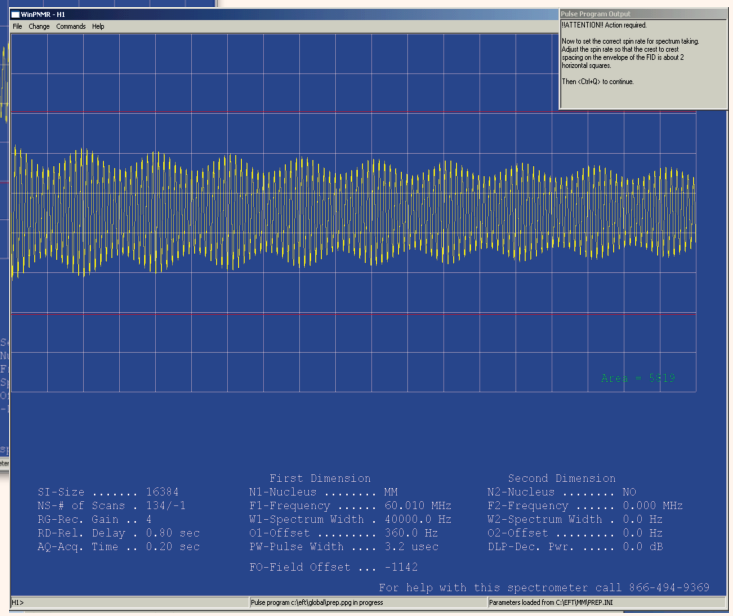
After adjusting spin speed so crest-to-crest is separated by two division.

	Keystroke	Function	Comment
9		Minimize side bands.	Y, X, Z, etc shims optimized. Note *
10	Adj spin <Ctl+Q>	Adjust spinning speed.	Adjust spinning speed so the crest-to-crest spacing in the FID is about 2 horizontal divisions





Decrease spin until crest to crest division changes to two block division.

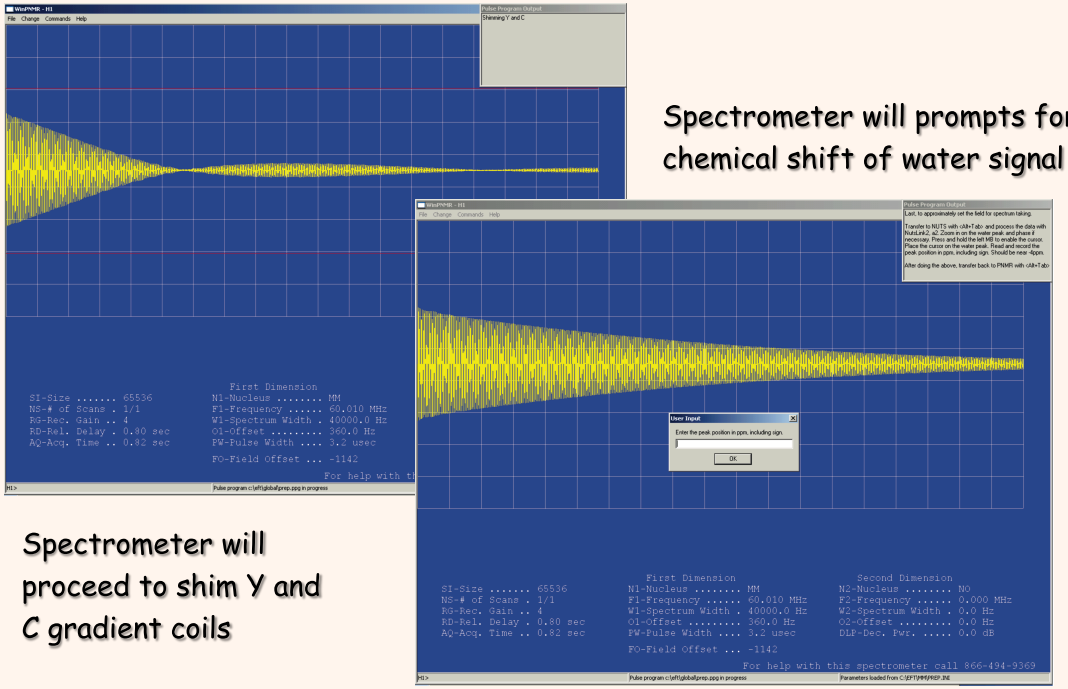


Adjusts spin rate with this spinner control. Decrease spin rate by turning counter clockwise.

III. Adjust Spin Rate Use D_2O/H_2O sample

This procedure is to adjust field and optimize shims.
If the shims are optimize, skip to step IV ... continue

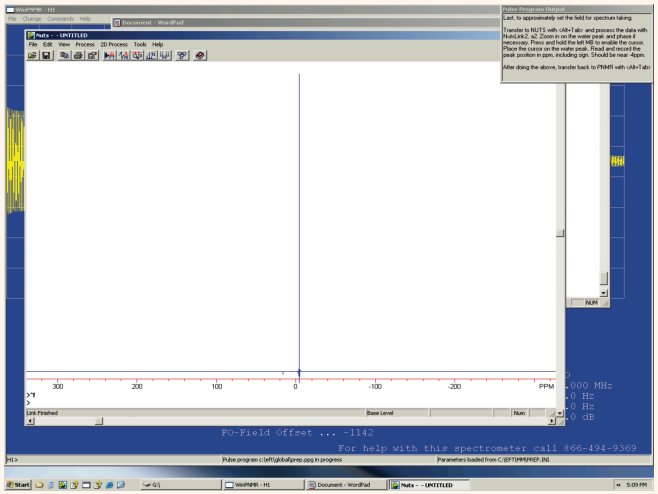
	Keystroke	Function	Comment
11		Optimize resolution.	Spinning shims set, spectrum acquired . Note *
12	<Alt +Tab> >a2	Enter NUTS and process data	(Trim phase as required .) Use cursor to determine peak position in ppm, including sign



Spectrometer will prompts for chemical shift of water signal

Spectrometer will proceed to shim Y and C gradient coils

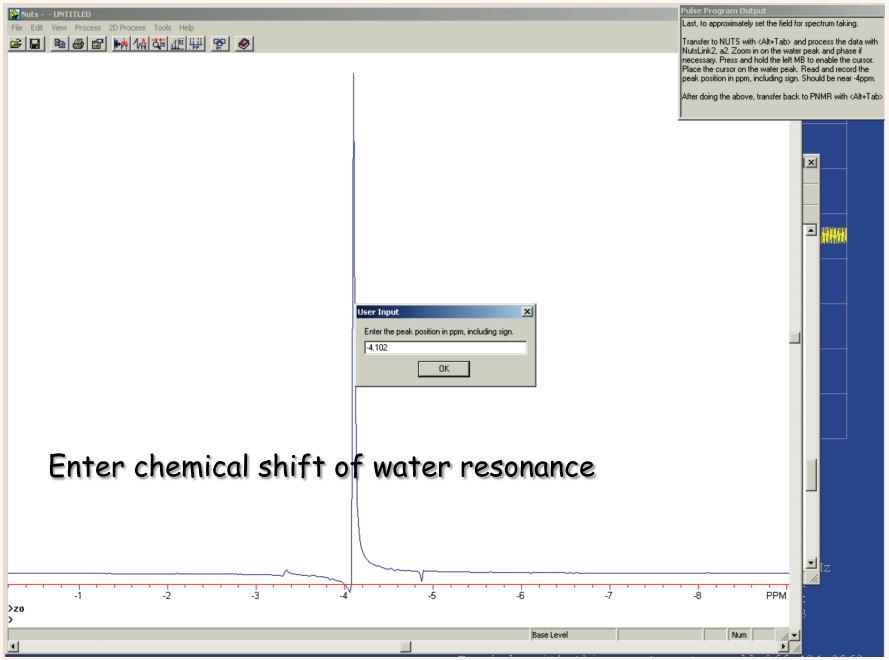
After <Alt+Tab> and a2 command zoom to water signal.



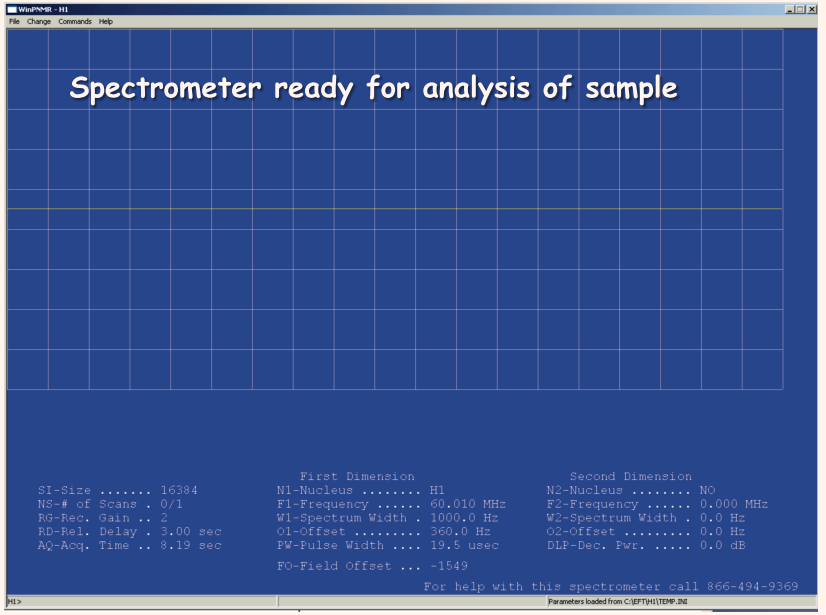
III. Adjust Spin Rate Use D_2O/H_2O sample

This procedure is to adjust field and optimize shims.
If the shims are optimize, skip to step IV ... continue

	Keystroke	Function	Comment
13	<Alt +Tab> value <E>	Enter PNMR and set field	Change to PNMR and enter the peak position in the User Dialog box (Values should be ≈ -4)
	Spectrometer is now ready for analysis of sample.		



Enter chemical shift of water resonance



IV. Acquiring ^{1}H NMR Spectrum

A. Shimming the magnet

This procedure to optimize the resolution

Summary of commands

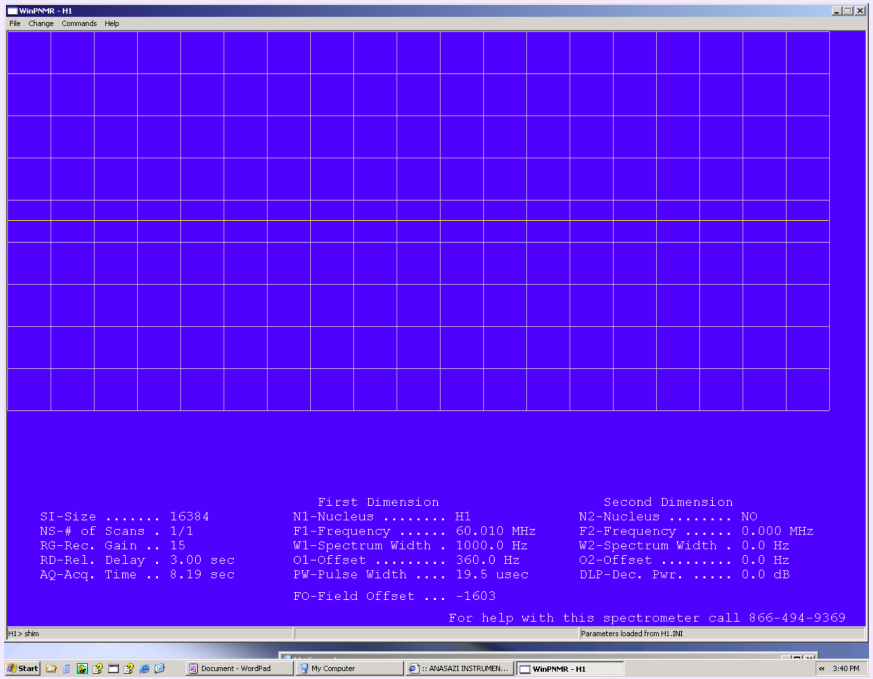
	Keystroke	Function	Comment
1		Sample to run	5% ethyl benzene or 5% ethyl methacrylate
2	<Alt +Tab>	Enter PNMR	
3	H1> shim <E> or C13 shim <E>	Enter shim routine	The spectrometer automatically adjusts the gain before shimming
4	value <E>	Enter RD value	Dilute sample RD=5; Conc. sample RD=2
5		Shim	Allow time to shim. Note *

IV. Acquiring ^{1}H NMR Spectrum

A. Shimming the magnet.

This procedure to optimize the resolution

	Keystroke	Function	Comment
1		Sample to run	5% ethyl benzene or 5% ethyl methacrylate
2	<Alt +Tab>	Enter PNMR	
3	H1> shim <E> or C13> shim <E>	Enter shim routine	The spectrometer automatically adjusts the gain before shimming



Insert sample to be analyze and prepare spectrometer to shim.

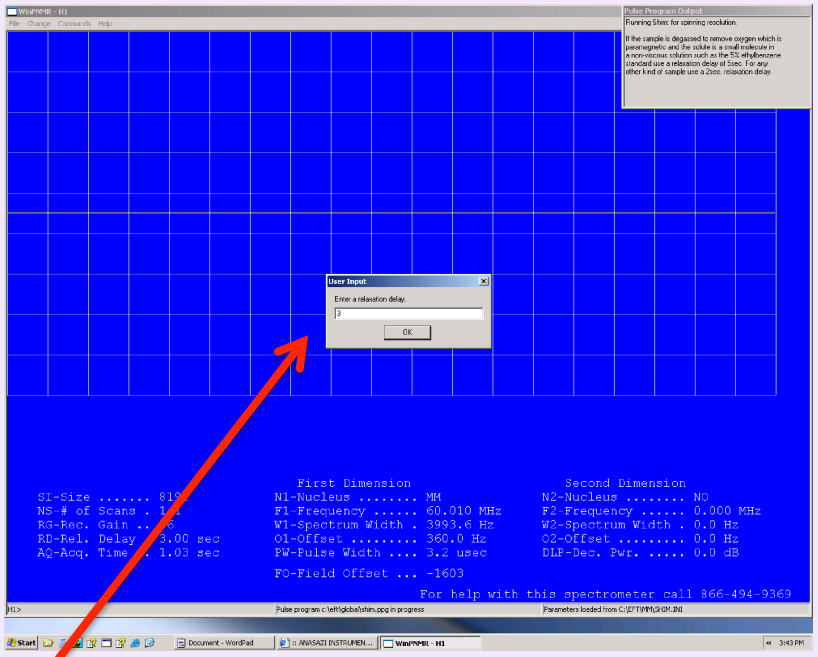
Enter shim command.

IV. Acquiring ^{1}H NMR Spectrum

A. Shimming the magnet.

This procedure to optimize the resolution

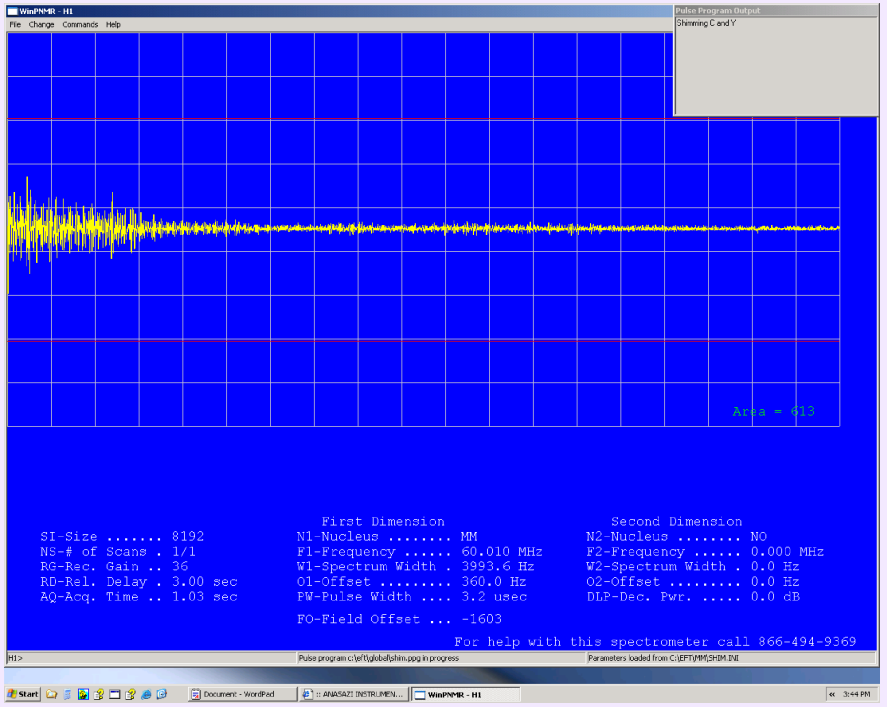
	Keystroke	Function	Comment
4	value <E>	Enter RD value	Dilute sample RD=5; Conc. sample RD=2
5		Shim	Allow time to shim. Note *



4 Enter Relaxation Delay in dialog box

5 PNMR continues to ship on sample.

6 Upon completion, spectrometer stops



IV. Acquiring ^{1}H NMR Spectrum

B Setting TMS to 0 ppm

This procedure to correctly position the spectrum relative to TMS

Summary of commands

	Keystroke	Function	Comment
1		Sample to run	5% ethyl benzene or 5% ethyl methacrylate or Analyte to be analyze
2	<Alt+Tab>	Enter PNMR	
3		Verify parameters	Verify that the parameters make sense. Otherwise Hl>re hl . ini <Enter>
4	Hl>zg <E> filename <E>	Acquire data.	If FID display is red, reduce RG, and repeat zg. Proceed to next step when prompted .
5	<Alt+Tab> >a2	Enter NUTS and process data	(Trim phase as required.) Use cursor to determine TMS peak position in ppm, including sign.
6	<Alt +Tab>	Enter PNMR	Switch back to PNMR,
7	H1>fo <E> value <E> 0 <E>	Enter present TMS peak position assign to 0 ppm	Execute the fo command, enter TMS position determine from Nuts and 0 PPM (zero) for desired position. Repeat to confirm.

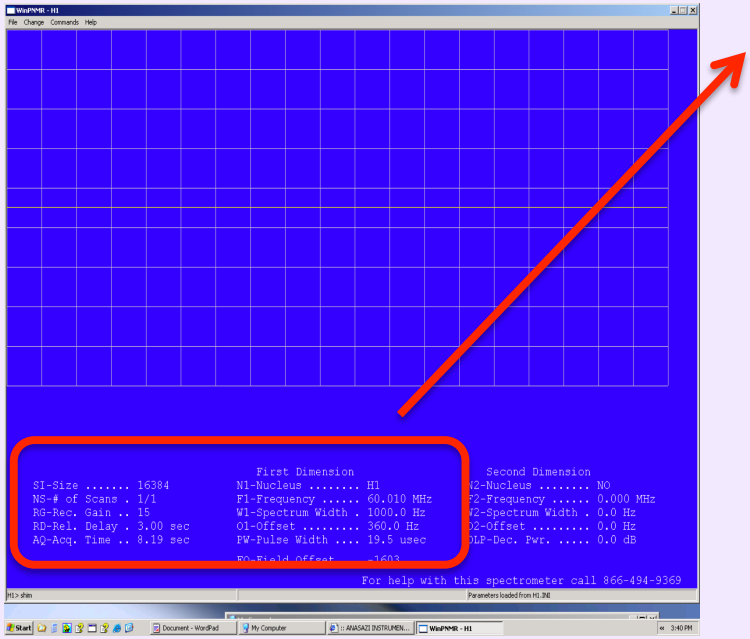
IV. Acquiring ^{1}H NMR Spectrum

B Setting TMS to 0 ppm

This procedure to correctly position the spectrum relative to TMS

Summary of commands

	Keystroke	Function	Comment
1		Sample to run	5% ethyl benzene or 5% ethyl methacrylate or Analyte to be analyze
2	<Alt+Tab>	Enter PNMR	
3		Verify parameters	Verify that the parameters make sense. Otherwise Hl>re h1.ini <Enter>



SI – Size.....16384
NS- # of Scans. 1/1
RG- Rec. Gain...15
RD- Rel Delay.. 3.00 sec
AQ- Acq Time .. 8.19 sec

First Dimension
N1 – Nucleus..... H1
F1- Frequency.....60.010 MHz
W1- Spectrum Width. 1000.0 Hz
O1- Offset.....360.0 Hz
PW- Pulse Width .....19.5 usec

FO- Field Offset.....-1603

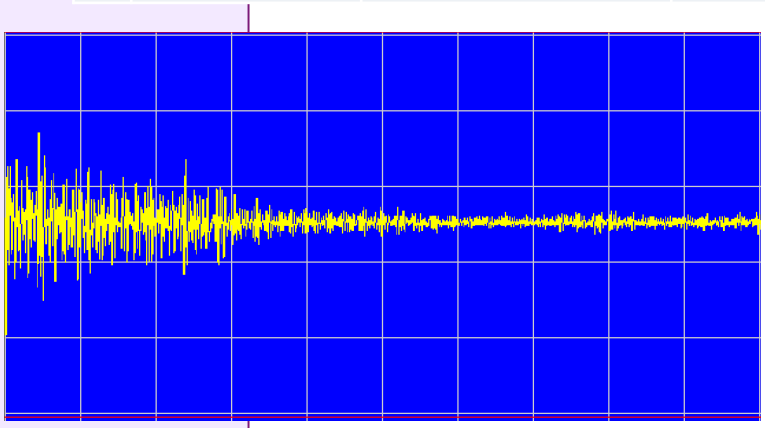
IV. Acquiring ^{1}H NMR Spectrum

B Setting TMS to 0 ppm

This procedure to correctly position the spectrum relative to TMS

Summary of commands

	Keystroke	Function	Comment
4	Hl>zg <E> filename <E>	Acquire data.	If FID display is red, reduce RG, and repeat zg. Proceed to next step when prompted .
5	<Alt+Tab> >a2	Enter NUTS and process data	(Trim phase as required.) Use cursor to determine TMS peak position in ppm, including sign.



4 Acquisition after zg command

5. <alt+Tab>, a2 and zo command to TMS

Move mouse cursor to center of peak, note chemical shift position

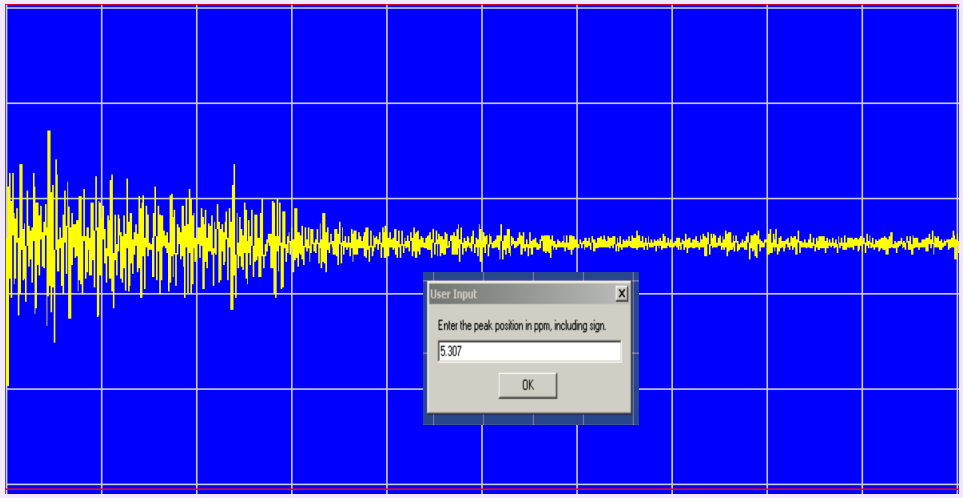
IV. Acquiring ^{1}H NMR Spectrum

B1 **Setting TMS to 0 ppm** through fo (field offset) command

This procedure to correctly position the spectrum relative to TMS

Summary of commands

	Keystroke	Function	Comment
6	<Alt +Tab>	Enter PNMR	Switch back to PNMR,
7	H1>fo <E> value <E> 0 <E>	Enter present TMS peak position assign to 0 ppm	Execute the fo command, enter TMS position determine from Nuts and 0 PPM (zero) for desired position. Repeat to confirm.



6. **<alt+Tab>** back to PNMR
7. H1> **fo**, type **<value>** TMS present chemical shift **<E>**
   type TMS assign value "**0**" **<E>**

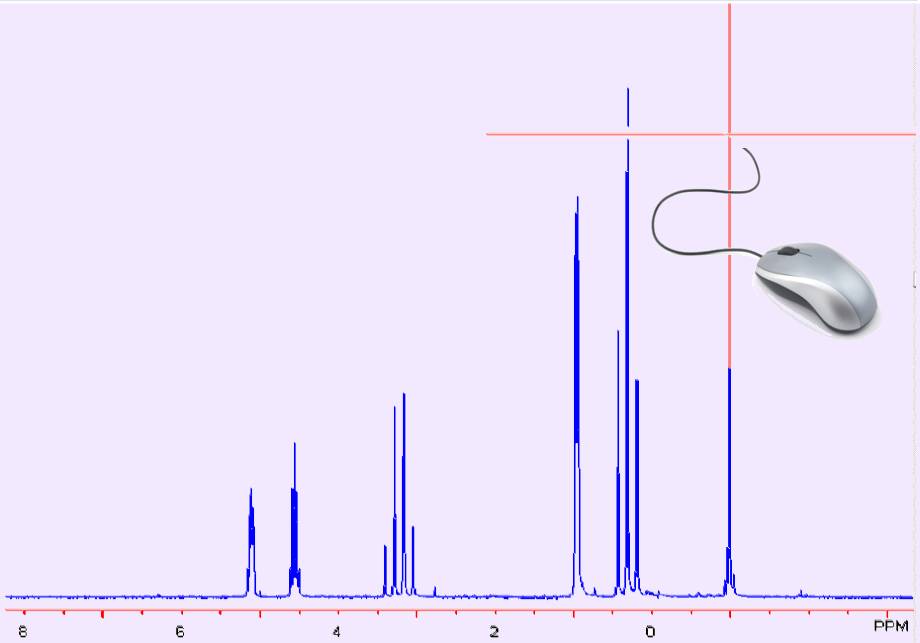
IV. Acquiring ^{1}H NMR Spectrum

B2 Setting TMS to 0 ppm after collecting acquisition

If this step is skipped, the TMS can still be assigned to 0 ppm manually by shifting the axis of the spectrum.

Summary of commands

	Keystroke	Function	Comment
1		Process the spectrum	Acquire, then process the data by phasing and applying baseline correction. Move cursor to the TMS signal
2	H> o	Assign TMS to zero ppm	Hold down left mouse button to TMS signal. While holding down button, type "o" letter-o
3	<value>	Confirm and assign	Dialog menu pops up. Confirm cursor present position in first set of data set, assign to 0 ppm in second column



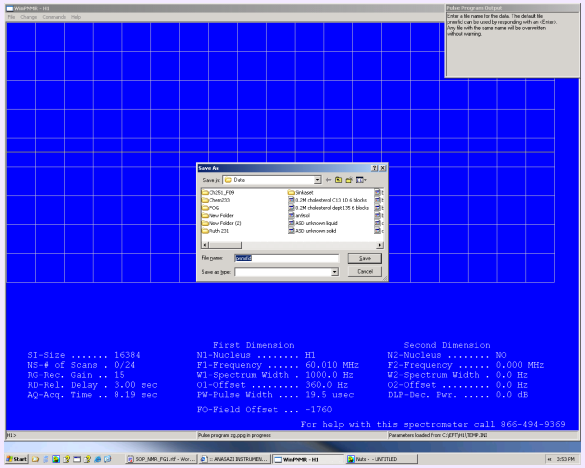
IV. Acquiring ^{1}H NMR Spectrum

C Acquiring the Spectrum

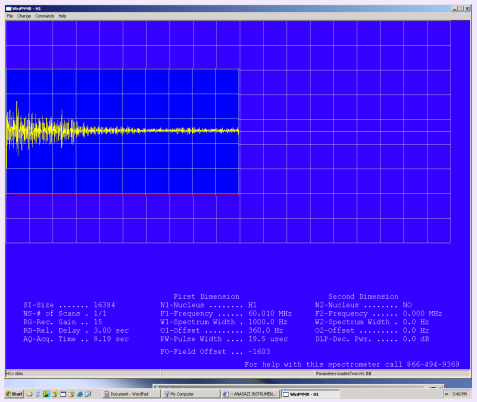
Set nu and ns value and begin acquiring FID

Summary of commands

	Keystroke	Function	Comment
1		Sample to run	Analyte, see sample preparation guide
2	<Alt +Tab>	Open PNMR	Switch from NUTS to PNMR
3	C13> nu H1<E>	Select 'H observe.	If the spectrometer is on another nuclei, then set it to 1H Required only if the prompt is not "H1 " .
4		Verify parameters.	Verify that parameters make sense.
5	H1> zg <E> filename <E>	Acquire data.	Enter filename if desired but it is usually better to use the default (pnmrfid) unless intending to save the data



2. <alt+Tab> back to PNMR
3. make sure nu is 1H



4. Verify parameters
5. H1> zg <E>, type <file name> and begin acquisition

IV. Acquiring ^{1}H NMR Spectrum

C Acquiring the Spectrum

Set nu and ns value and begin acquiring FID

Summary of commands

	Keystroke	Function	Comment
1		Sample to run	Analyte, see sample preparation guide
2	**<Alt +Tab>**	Open PNMR	Switch from NUTS to PNMR
3	C13> **nu H1**<E>	Select 'H observe.	If the spectrometer is on another nuclei, then set it to 1H Required only if the prompt is not "H1 " .
4		Verify parameters.	Verify that parameters make sense.
5	H1> **zg <E> filename <E>**	Acquire data.	Enter filename if desired but it is usually better to use the default (pnmrfid) unless intending to save the data
6	**<Alt +Tab>**	Enter NUTS.	
7	**<Ctrl+F1>**	Process data.	Process and plot using aii_H1 _auto.mac, which reference TMS, peak picks, integrate, and accepts spectral information and plots

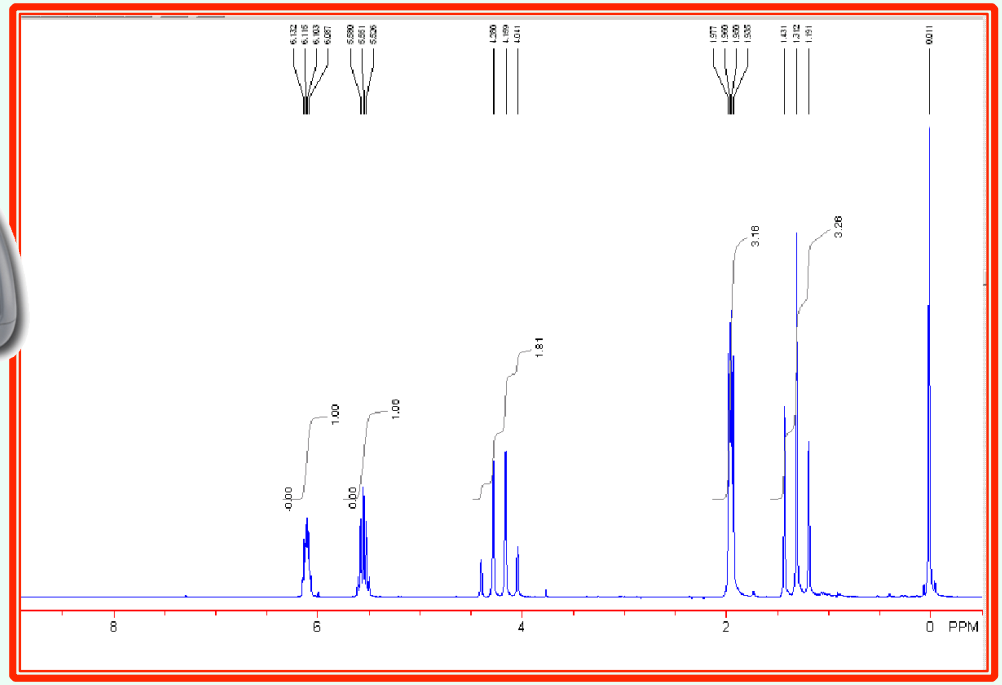
V. Processing the Data

A1. Processing the Data

Automatic processing

Summary of commands

	Keystroke	Function	Comment
1a	<Ctrl+F1>	Process data.	Process and plot using aii_H1 _auto.mac, which reference TMS, peak pics integrate, and accepts spectral information and plots
1b	<Ctrl+F2>	Process data	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.



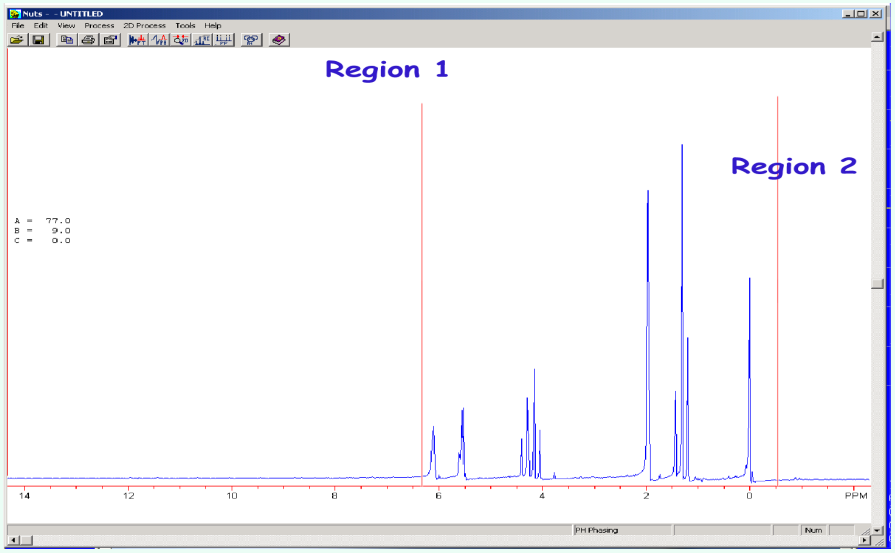
V. Processing the Data

B1. Phasing Method: Processing the Data

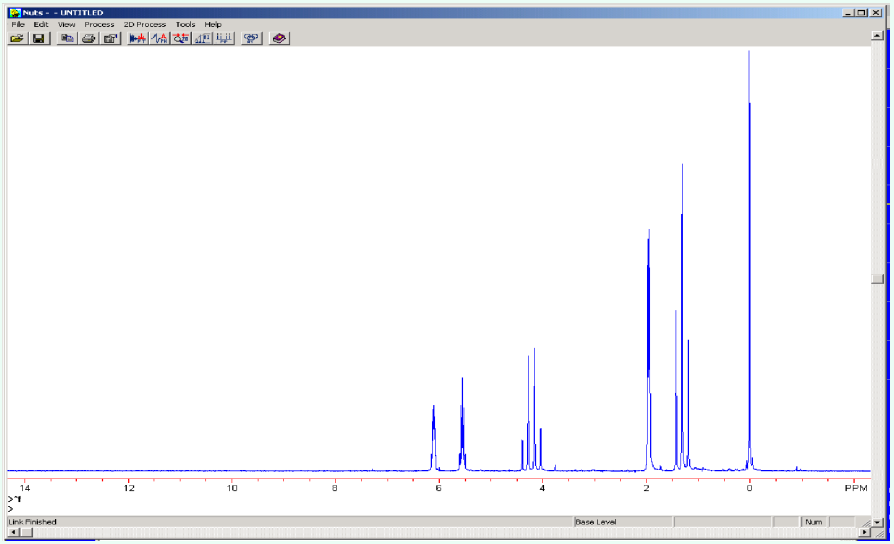
Phasing extreme ends of spectrum

Summary of commands

	Keystroke	Function	Comment
1	<Alt +Tab> H1>a2	Process data	Switch to NUTS and FT the FID
2	>zo <E> to exit	Enter zoom	
3	<1> <2>	Select two regions of interest	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.
4	>pe <E> to exit	Trim phase	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.



3 Setting up region to phase



4 After phasing routine

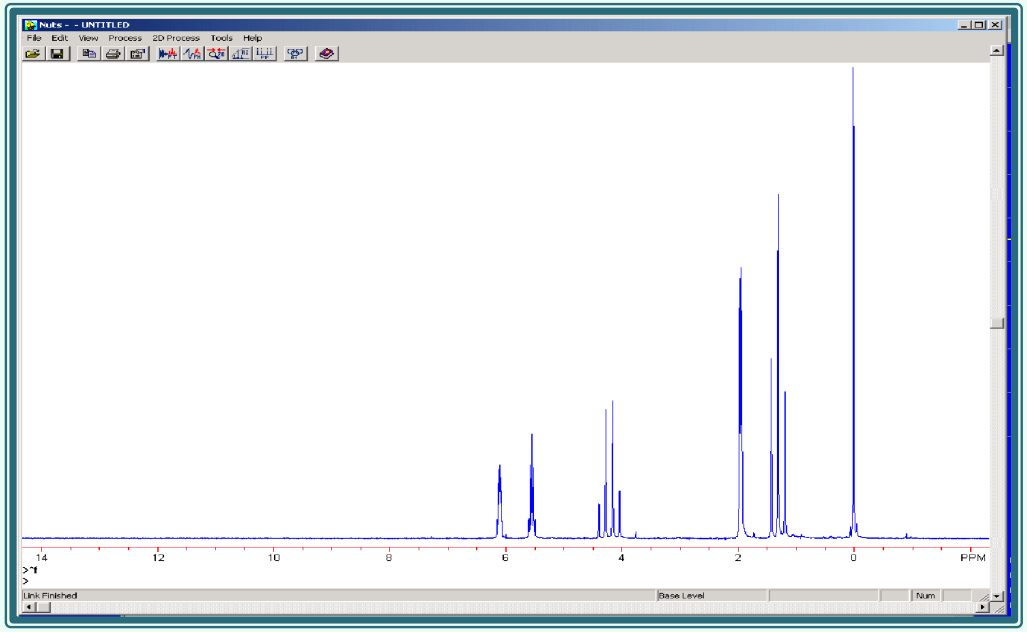
V. Processing the Data

B2. Phasing Method: Processing the Data

Manual Phasing

Summary of commands

	Keystroke	Function	Comment
1	<Alt +Tab> H1>a2	Process data	Switch to NUTS and FT the FID
2	>zo <E> to exit	Enters zoom	
3	>AP or >QP or >MP	Phase spectrum	AP will autophase the spectrum QP will apply quick phasing If these do not give satisfactory results then use mouse phasing- MP will allow manual phasing with the mouse. Click with left mouse button and drag cursor left and right until spectrum is in correctly phase.

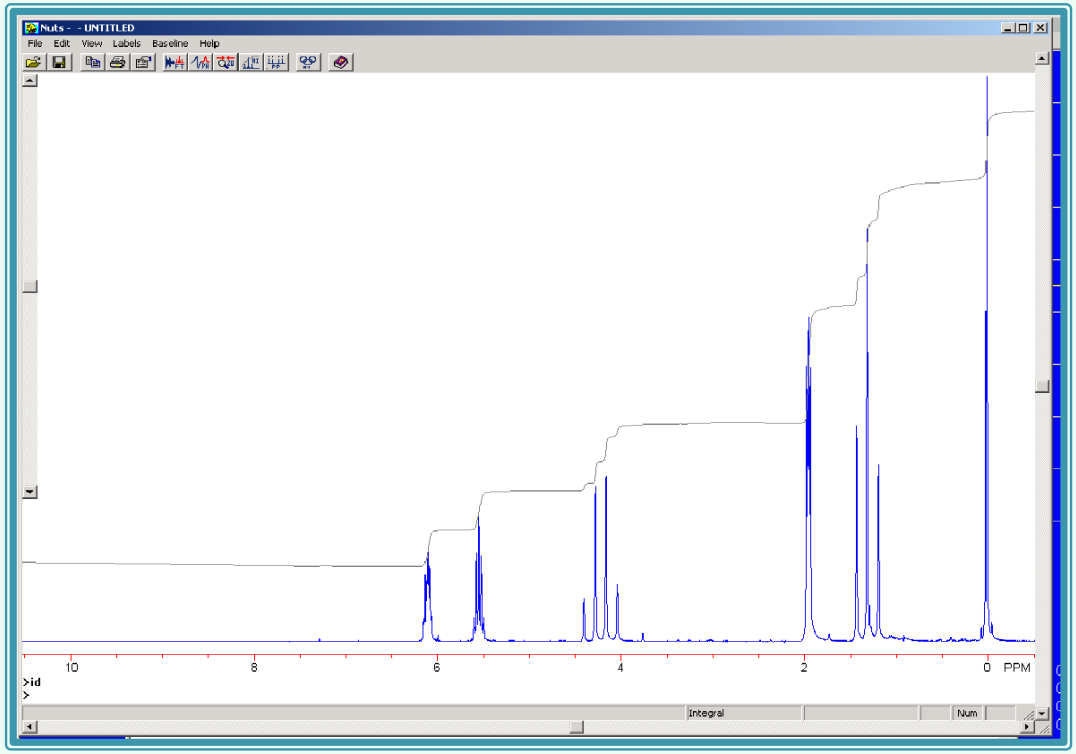


V. Processing the Data

C. Integration Processing the Manual data processing

Summary of commands

	Keystroke	Function	Comment
1	>BC	Base line correction	Applies baseline correction
2	>id	integrals	The integral will be displayed. If the intensity is too high, lower the intensity by moving slider to left down. Exit from id with <Enter>
3	>c		Clears integral memory: At this point the integration should step up from left to right after each peak.

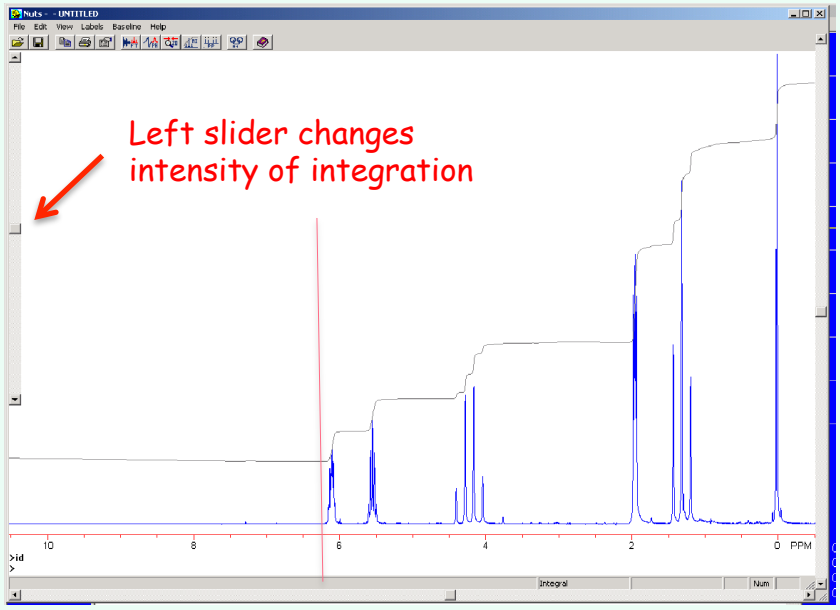


V. Processing the Data

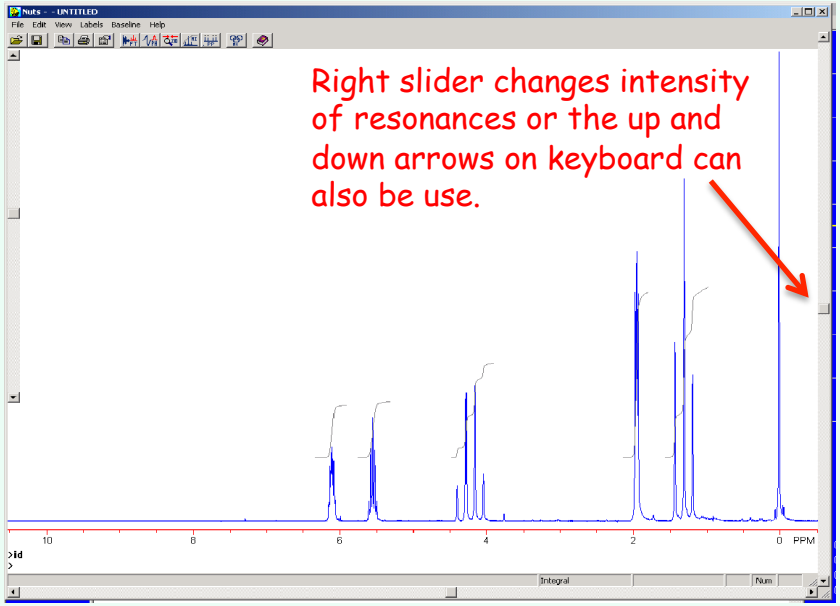
C. Integration Processing the Manual data processing

Summary of commands

	Keystroke	Function	Comment
4	Mouse button control	Mouse Button: Clk –Clk MB then one Clk after peak	Create broken integral so only peaks shows integration plot for each broken integral, click left MB twice on left side of peak(s) then once on right side.



4 Create broken line by clicking left of signal twice and then to the right of signal once



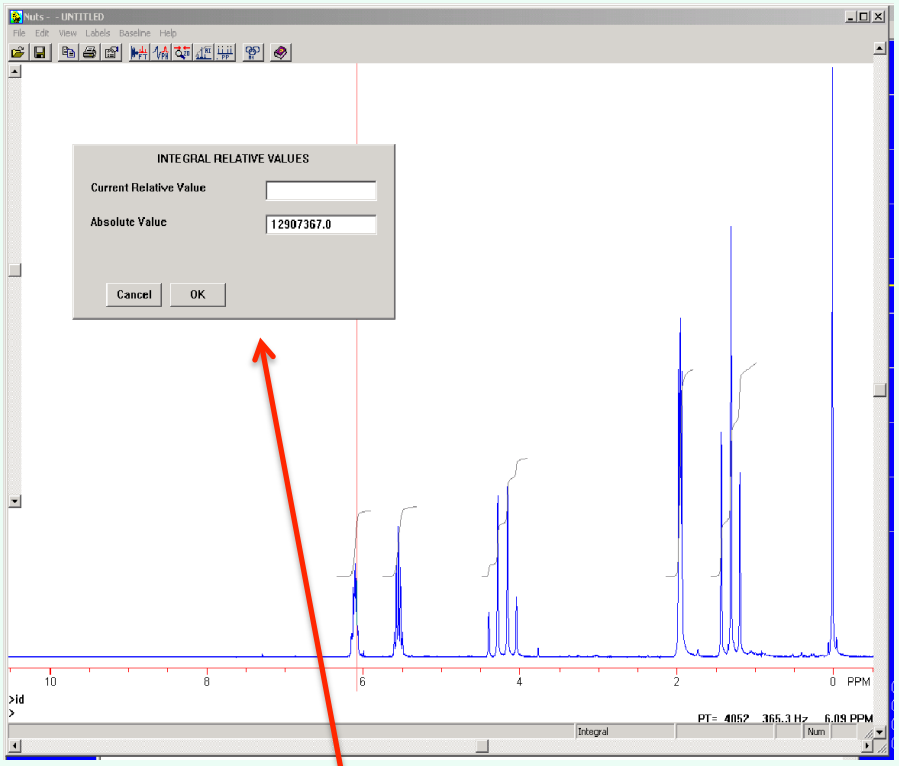
After selecting all resonances of interest, the broken line integration is shown.

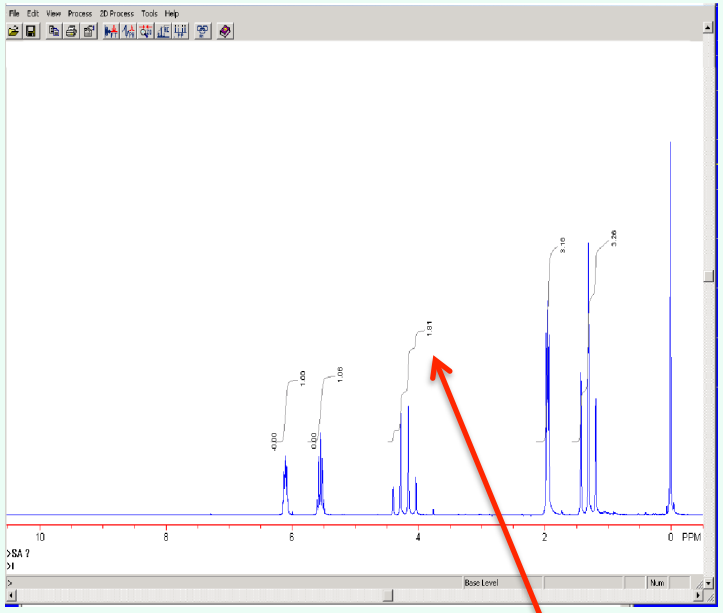
V. Processing the Data

C. Integration Processing the Manual data processing

Summary of commands

	Keystroke	Function	Comment
5	>v + Lf MB value <E>	Assign integral an integral value	To assign a relative integral value place cursor on integral, click left MB, press <v> and enter number. <Ctrl+I> toggles integrals on/off.





5. To assign specific value to integration, click near one of the integrals and while holding the left mouse button down, type "v". A dialog window shows. Assign the relative value of that integral.

The other integrals will be assigned an integration relative to the integral selected.

V. Processing the Data

D1. Pick Peak data processing

Auto Pick peaks

Summary of commands

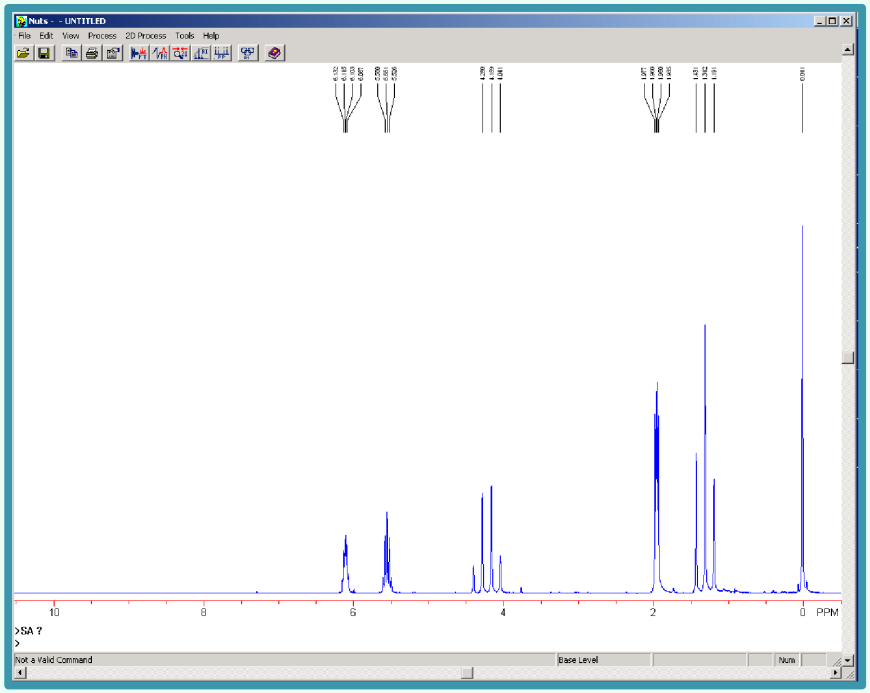
	Keys	Function	Comment
1	>pp		Automatically picks peaks - Vertical red lines indicate selected peaks. Use MH and RM to change peaks selected.

D2. Pick Peak data processing

Manual Pick peaks

Summary of commands

	Keys	Function	Comment
1	>dp >c <E> to exit		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*.



1a. PP command automatically pick peaks and labels the peak.

1b. If some of peaks are not labeled, then DP command can be used to label peaks of interest.

V. Processing the Data

E. Zoom and Print

Summary of commands

	Keystroke	Function	Comment
1	<Alt +Tab> H1>a2	Process data	Switch to NUTS and FT the FID
2	>zo <Ctrl+E> <Ctrl+F> <E> to exit	Enters zoom Expands and in Full display	Zoom into region of interest. For 1H NMR zoom between 9 and -0.5 ppm <Ctrl+E> Zooms to selected region <Ctrl+F> Expands from zoom region
3	<Ctrl+I>	Displays integration	
4	<Ctrl+P>	Display pick peaks	
5	pl		Plots the spectrum shown on display