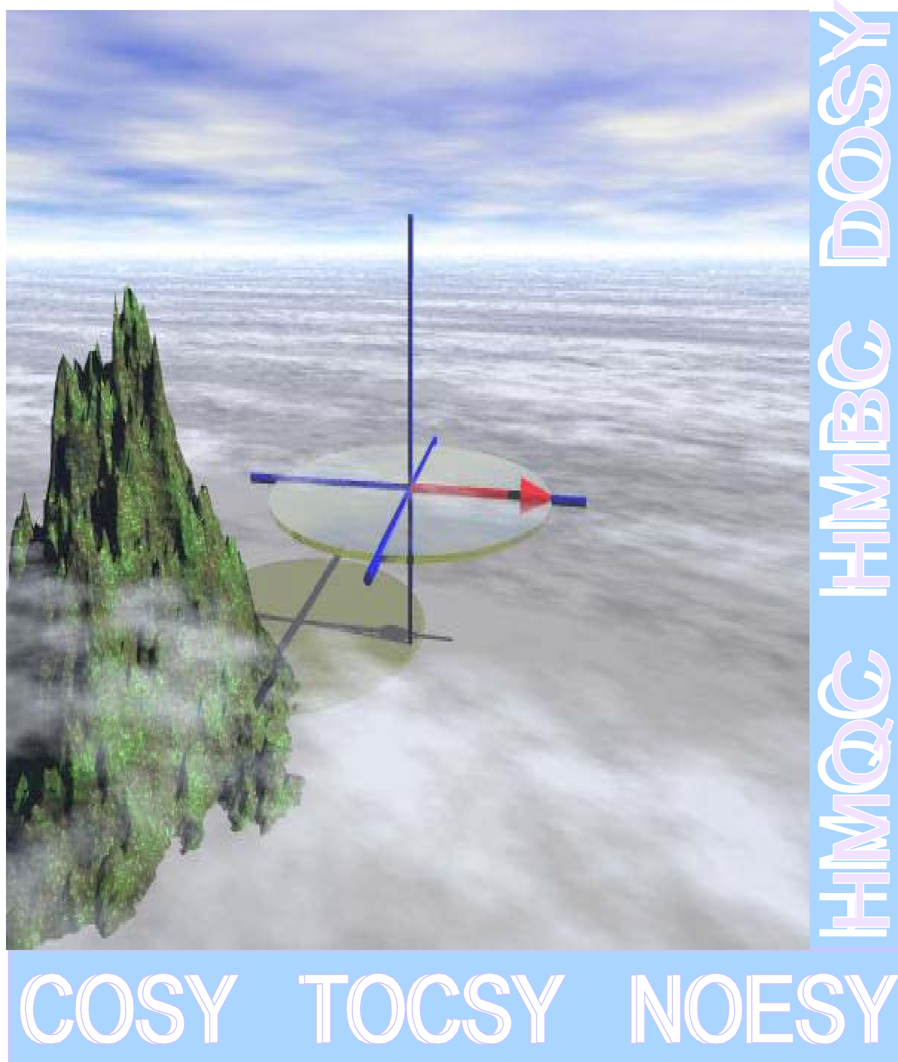
# NMR Experiment Guide

2011




May, 2011 Emory University

# Experimental Guide for Varian NMR Spectrometers

**June, 2011**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# Introduction:  NMR Experiment Procedures

**Step 1. Login**

Varian NMR spectrometers are controlled by a host Sun computer or PC. For security and administration reasons, all research groups at Emory University are issued their group usernames and passwords for the host computers. All authorized NMR users must use their group usernames and passwords to login to the host computers.

**Step 2. Change Sample**
A sealed $CDCl_3$ standard is always placed in the magnet to keep the instrument in working condition when not in use. Every user needs to replace the standard sample with your own sample at the beginning of each experiment and change back at the end of the experiment.

**Step 3. Lock and Shim the Magnet**
Locking the magnet keeps its field constant and stable during the data acquisition. Shimming the magnet makes it uniform and homogenous for a sample to get a reasonable resolution and line shape.

**Step 4. Setup Parameters and Acquire Data**
Retrieve default parameters that are setup and updated for you by the NMR Center so that you may collect your data efficiently. Change some parameters accordingly to meet your own experiment needs.

**Step 5.  Process Data, Plot Spectrum and Save the Spectrum**
This step converts data into NMR spectra. After proper processing, you could plot it on paper for your record. Also you should save the spectrum for your future review.

**Step 6. Logout**
Your mission is completed and you need to do a few things for the next user or leaving the instrument.

Step 1, 2, 3, and 6 are the same for all experiments. We will describe them in detail in Experiment One. Step 4 and 5 differ from experiment to experiment.

## Note on NMR Operating Software:

Varian INOVA600, INOVA400, UNITY Plus 600, UNITY400:
Solaris 8 and VNMR 6.1C on SUN Workstations

Varian Mercury 300, Mercury Vx 300 (at WW) VNMRS400 and Rollins V400:
RedHat Linux 4.0WS and VNMRJ 1.1D or VNMRJ2.1B on Dell 380N PC

Bruker Avance 600WB: IRIS 6.5 (SIG O2) and Xwin-NMR 3.5

Off-line data processing by PC or Mac.

NUTS from Acornnmr, Inc. www.acornnmr.com
MestReNova 5.01 from www.mestrec.com.

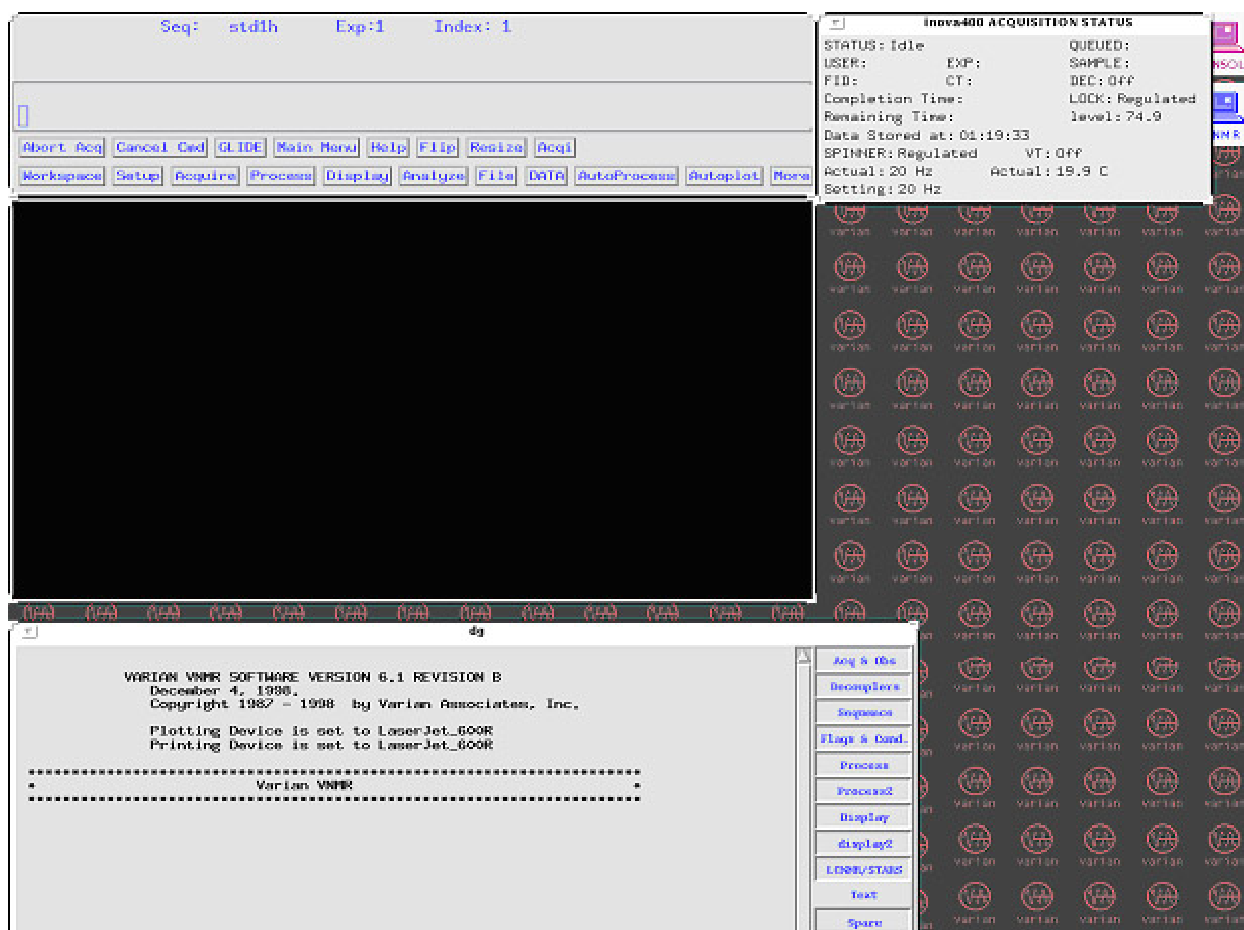
# Experiment 1: 1D Proton and Basic Operations

Standard Sample to be used: 10% Strychnine in $CDCl_3$

## Step 1. Login

1.1 Sign in NMR logbook.
Type in your username at login prompt on the screen.
Type in your account password as the screen prompts.
Please note both the username and password are case sensitive.
If you could not login, contact Dr. Wu.

The VNMR software will be loaded in several seconds with the display of four windows. The upper left window is used for entering commands and selecting menu buttons; the middle left for displaying NMR spectra and the lower left for displaying NMR parameters and other text messages. The upper right window is called "**ACQUISITION STATUS**" window, indicating the working status of the instrument, such as lock level, spin rate, temperature and acquisition time, etc.

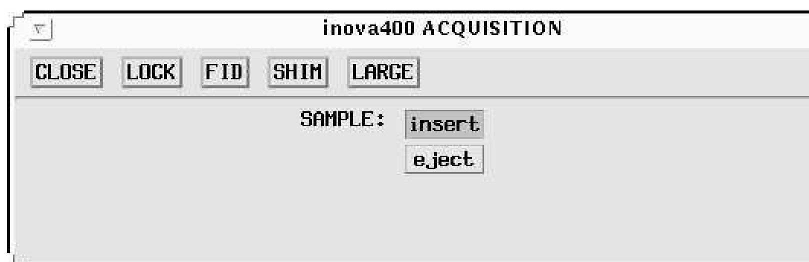



## Step 2. Load Standard Parameters and Change Sample

After VNMR software loaded as above, Type ***h1*** to load the standard parameter for 1D proton experiment with a default $CDCl_3$ solvent. If your solvent is not $CDCl_3$, you may

simply type ***h1('solvent')***, where the solvent may be one among acetone, DMSO, D2O, etc. You may also type ***h1*** and then type ***solvent='DMSO'*** etc. In this case, you may have to check the reference peak, then reset the correct chemical shift for your spectrum.

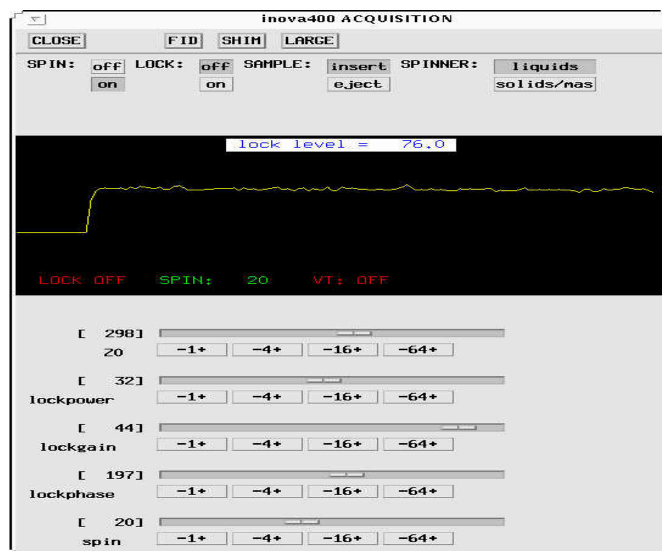
2.1 Click on Acqi or type **acqi** in the command line to display the fifth window at lower right, which is called "**ACQUISITION**" window. All the operations hereafter in **Step 2** and **Step 3** will be operated in this window. There are CLOSE, LOCK, FID, SHIM and LARGE popup menu buttons, and Sample eject and Sample insert buttons. We will be focusing on the LOCK and the SHIM buttons first.




2.2 Click on LOCK button to display lock sub-window then click SPIN: off button to turn off sample spinning; click LOCK: off to turn off the lock.

2.3 After a few seconds, the spin rate displays zero, then click on the SAMPLE: eject button to eject the standard sample to the top of the magnet; remove the spinner, replace the sample with your own, use sample depth gauge to measure the correct tube position, use kimwipes to clean the spinner and your sample, then put the spinner back onto the top of the magnet again. **Caution: place the spinner with a sample on top of the magnet only when ejecting air is on.**

2.4 Click on the SAMPLE: insert button to put the spinner back down inside the probe. Wait for 3 to 5 seconds, then click on the SPIN: on button to make the sample spin.



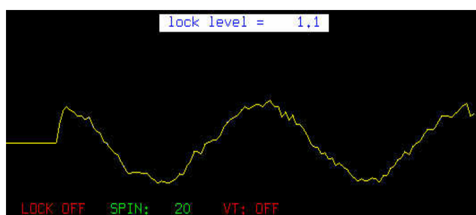

Lock sub-window

## Step 3. How to Lock and Shim the Magnet

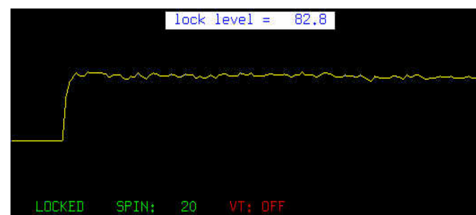
In this step, you need to adjust lock and shim parameters by using the mouse. Each parameter can be increased or decreased by 1, 4, 16 and 64 units as shown by corresponding –1+, -4+, -16+ and –64+ buttons. For example, when you place the cursor on a button, say –4+, if you click the left mouse button once, the corresponding parameter is decreased by 4 units; if you click the right mouse button twice, the parameter is increased by 8 units.

3.1 If you are using $CDCl_3$ solvent, the magnet could be locked almost instantly most of the time. This is indicated by the display of lock signal plateau and a green **LOCKED** message below it. Then you directly go to step 3.3.

3.2 If you don't see a plateau signal with a **LOCKED** message or you are using solvent other than CDCl3, click on LOCK: off button, adjust Z0 to make wave-like lock signal into plateau-like resonance, then click LOCK: on button. A green LOCKED should be shown. Then adjust the lock phase to make the lock level as high as possible. You need to reduce both the lock power and lock gain several units if the lock level exceeds 100. You may also check our record sheets on the desk. There are Z0, Lock power, Lock gain, and Lock phase values for different solvents recorded by previous users.




Not Locked

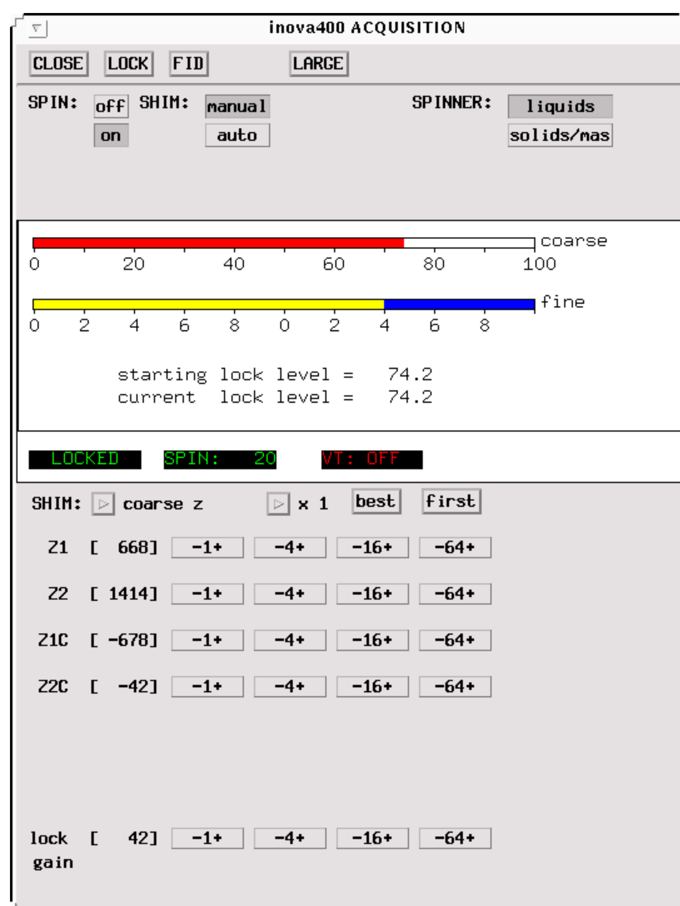



Locked

How to lock a sample having mixed solvents: Always select the strongest signal for your lock. For example, CD3OD, you have two lock frequencies, Use the CD3 resonance signal. If solvent is 10% D2O + 90% DMSO, then you use DMSO-D as lock.

3.3 Now it is shimming time. There are as many ways to shim the magnet. We suggest you shim it this way:

3.2.1 Click SHIM button to display shim sub-window. The lock level is shown in this window as two color bars and exact lock level is shown underneath the bars. In the middle of the window, there is one triangular button after '**SHIM:**' which let you switch from shim sub-windows and lock sub-window by just click on it. Now it should show **coarse z** by the button.




3.2.2 Adjust Z1C (Z1 coarse) and Z2C(Z2 coarse) by alternatively changing 1 or 4 units to increase the lock level as high as you can. If the lock level increases to 100, decrease lock gain on the bottom of the window and then continue to adjust Z1C and Z2C. Higher lock level indicates better homogeneity of the magnetic field.

3.2.3 Click on the triangular button to switch to **fine z** sub-window. Adjust Z1, Z2 and Z3 one after another to make the lock level as high as possible, then adjust Z3, Z2, Z1 as such back and forth a couple of times. We do not suggest you to adjust Z4 and Z5 at this stage. If you really want to adjust them, it is better write them down before you change them, so that you are able to go back in the case you get a worse shimming.

3.3 Finally click the CLOSE button to end the shimming procedure.

**Tip 1. Retrieve our default standard shimming file.**

If you have a very bad shim, type "**loadshim**" in the command window to reinstall our default shim file for the standard sample.
If you would to like to save your good shims, type svs('shim_name'). The file will be saved in: **/export/home/nmruser/your_ID/vnmrsys/shims.**
You could reload your shims by changing to the above file folder, select the shim file, then click **main→ file→data→** ( high light the shim file) then click **load shim**, then type **su** to use the new shim file.

**Step 4. Setup Parameters and Run 1D Proton Experiment**

4.1 Type ***dg*** to check the standard parameter for 1D proton experiment with a default $CDCl_3$ solvent. If your solvent is not $CDCl_3$, you may simply type ***h1('solvent')***, where the solvent may be one among acetone, DMSO, D2O, etc. You may also type ***h1*** and then type ***solvent='DMSO'*** etc. In this case, you may have to check the reference peak, then reset the correct chemical shift for your spectrum.

```
dg
  ACQUISITION            SAMPLE              PROCESSING           FLAGS
sfrq      399.941  date   May 30 2000  lb           0.20  il          n
tn             H1  solvent     cdcl13  sb       not used  in          n
at          2.499  file           exp  gf       not used  dp          y
np          25984      DECOUPLING      awc      not used  hs         nn
sw         5199.2  dn              H1  lsfid    not used      SPECIAL
fb           3000  dof              0  phfid    not used  temp   not used
bs             16  dm             nnn  wtfile
ss              0  dmm              c  proc           ft
tpwr           60  dmf            200  fn          32768
pw            6.0  dpwr            30  math            f
p1              0
d1          1.000                      werr        react
d2              0                      wexp     procplot
tof         199.6                      wbs
nt              1                      wnt
ct              0

Acq & Obs | Decouplers | Sequence | Flags & Cond. | Process | Process2 | Display | display2 | LCNMR/STARS | Text | Spare
```

Proton standard parameters.

The standard parameters will be shown in the left lower window.

**Tip 2. Check Current Parameters**

Type ***dg*** to display the current parameters for the loaded experiment. If you have to change parameters, make sure you know what it is for. Type ***su*** to set it up and type ***dg*** to look at them.

4.2 Type ***nt=8, 16*** or ***32***, according to sample concentration (or use a multiple of 8 for nt).

4.3 Type ***ga*** (submit to acquisition) to begin acquisition.

4.4 After acquisition is completed, a proton spectrum should be displayed on the screen now. If not, type ***wft*** (Weight and Fourier transform) to display the spectrum, then enter ***aph*** (automatic phasing) to phase the spectrum. Type ***vsadj*** (vertical scale adjustment) to adjust the vertical scale of the spectrum automatically. Type ***dscale*** or click on Dscale button to display chemical shift scale under the spectrum.

**Tip 3. Manual Phasing**

If you need to improve the phasing, you may manually phase the spectrum by first clicking on the Phase button, clicking on the region of interest, and moving the mouse up or down while pressing the LMB to adjust its phase. You may continue to click the cursor on the next region and adjust the phase same way. Clicking on the Box or Cursor button will exit the manual phase routine or type ds.

**Tip 4. Manual Vertical Scale Adjusting**

Place the cursor above the base line and click middle mouse button to increase the vertical scale. Place the cursor below the base line and click middle mouse button to decrease the vertical scale.

## Step 5. Data Processing

5.1 Zoom operation
Click the LMB on the left side and click the RMB on the right side of a peak. Two vertical red cursors will appear at the click points, then click the Expand button on the menu bar. The region between the red cursors will be expanded. Click on Full button to return to the full spectrum after expanding, or type **f** then press enter. You may do this zooming operation as many times as you like.

5.2 Set reference
After zooming the region containing the reference peak, click the LMB near the top of the peak, then type ***nl*** to place the cursor exactly on the top of the peak. Then type ***rl(x.xxp)*** to set its chemical shift. For example, ***rl(4.80p)*** will set a peak frequency to 4.80ppm.

5.3 Integral operation
Enter ***vp=12 or larger***. Type ***ds cz*** to clear all the previous integral values in the buffer. Click on the Part Integral button for the full spectrum or its expanded region to display an integral curve, then click on the Resets button. Now click the LMB at both sides of each peak or integral region to get separated integral lines of the spectrum.

If you know the number of protons, a specific peak represents, you may set the value for that integral as follows: place the cursor on the integral and then click on Set Int. The computer will then ask what value to assign that integral. After you input the value for a particular integral, the computer will recalculate the value of every other integral relatively. You are able to see these values by typing ***dpir***.

**Tip 5. Redo integral operation**
If you made a mistake during the integral operation, you can redo the integral operation by typing ***cz***. Or you could click RMB, to correct the last setting of the integral curve.

5.4 Peak picking operation
Type ***ds*** and click on Th to display a yellow horizontal threshold line. Every peak above this line will be assigned its chemical shift in ppm (axis='p') or hz (axis='h'). Press and hold the LMB to move the yellow cursor to the position you desire, then click on the Th button again. Type ***dpf*** to display peak frequencies above spectrum peaks.

**Tip 6. Returning to interactive adjustment**
During the above operations, if you are not able to find the button you need or you are unable to use the red cursor, simply click on Main menu > Display > Interactive

5.5 Label your experiment data

Type ***ctext*** to clear any text label for the present experimental data. Type ***text('Your sample name date\\solvent')*** to label the present experimental data. Command ***dtext*** will display the text on the top left corner of your spectrum.

5.6 Print and plot spectrum and related information

All printing procedure starts with ***pl*** and ends with ***page***. You can put other printing commands selectively between these two commands.

***ppa*---** to print select parameters with text labels.
***pl*---** to print the present spectrum;
***pap*---** to print all the parameters including the text labels, if any.
***ptext*---** to print text labels without other parameters.
***pscale*---** to print the spectrum scale in ppm or hz by ***axis="p"*** or ***axis="h"***.
***ppf*---** to print all the peak frequencies over the respective peaks.
***pll*---** to print a list of the frequencies and intensities.
***pirn*---** to print normalized integral values below the respective peaks.
***pir*---** to print proton numbers below the respective peaks.
***page*** --- to expel the paper from printer.

If you want to print integrals, set vp =12 first, or it wouldn't print.

5.7 Save your experimental data and reload your experimental data

You may save your spectrum data.

The directory path is /export/home/nmrusr/Login_ID/ when you login. It is your group directory. You should make a directory for your own files.

Type ***mkdir('yourname')*** to make a directory of your own. Type **pwd** to check the current directory. If it does not, click on Main menu > File then highlight your directory name and click on Set directory. Remember you can go back to your group directory at any time by typing ***cd***. To check the current directory, type ***pwd***. Type ***svf('file_name')*** to save your data in the form of FID.

5.8. To reload your file, go to your directory first as described above, then click on Main menu > File > highlight your file > click on load. Then type **wft**.

## Step 6. Logout

6.1 Retrieve standard file and replace your sample with the standard.

Type ***h1*** to set up standard proton file. This is especially important after you have done a carbon-13 experiment. It will turn off the decoupling channel automatically.

Then see **Step 2** for details about replacing NMR sample tubes.

6.2 Lock and shim the magnet

See **Step 3** for details. You are required to shim the magnet to the required level: **'loadshim'** will reload the current shims for the standard.

6.3 Exit the VNMR program

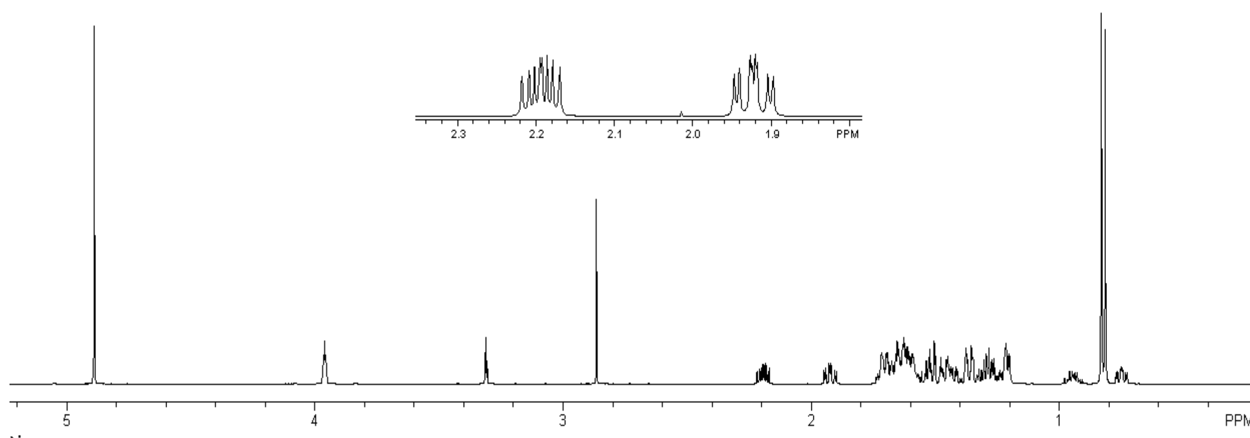
Type ***exit*** to close the VNMR program. Now you will see a full screen of Varian's logo.

6.4 Exit UNIX system

Press the RMB to select Exit item in the popped up menu, release RMB. You will be prompted to select one of two choices: **Exit** or **Cancel**. Click on **Exit** to exit UNIX system. The screen will become blank and a prompt appears.

**Caution: It is important to logout completely, otherwise your account will be still charged!**

**Tip:**
Insert a part of your spectrum on the top of your full spectrum:




1. After the processing completed, type **pl pscale** no page command, that will save the full spectrum in plotter queue;
2. Use both red cursors to select the area you want to inserted, then type **inset**, The part of the spectrum will be displayed in the middle of the spectrum. Use mouse to adjust the size and location. Then type **pl pscale page**.
3. After printing is completed, type: **ds full f and vs=0**, you will get the original spectrum back.
4. You could repeat these steps for few times and insert several spectra in the same plot before you type the **page** command.
5. If you want to print the spectrum in particular range: type cr=XX.Xp delta=XXp, two red cursors will be the in the positions as you specified, left cursor at XX.X ppm and the right cursor at XX ppm away from the left cursor. Then click expend and print the spectrum.

You can use the same way to plot two or more spectra together to compare the difference of these spectra by select the same region (use ***cr*** and ***delta*** command).

# Experiment 2: 1D Carbon-13 and Basic Operations

Sample to be used: 30% Strychnine in $CDCl_3$

**Step 1. Login**

(See Experiment One)

**Step 2. Change Sample**

(See Experiment One)

**Step 3. Lock and Shim The Magnet**

(See Experiment One)

**Step 4. Setup Parameters Carbon Experiment**

4.1 **For** Standard C-13 spectrum (Fully decoupled with NOE enhancement): Type ***c13 or c13('solvent')*** to call up the standard carbon-13 experiment parameters with $CDCl_3$ solvent or different solvent.

4.2 Enter ***nt=128, 256***, or more according to the nature of your sample. Type ***time*** to check the total time of the experiment.

4.3 Type ***ga*** to begin acquisition.

4.5 After acquisition is done, a carbon spectrum should be displayed. If not, enter ***wft***. Type ***aph*** for automatic phasing. Manual phasing is often necessary for a carbon-13 spectrum.

**Tip 7. Use large values for nt and aa Commands**

$^{13}C$ sensitivity is very low. A common practice is to set a very large number for **nt** (ex. nt=5000 etc.) to start the experiment. You can check the spectrum by typing **wft aph** and stop the experiment at any time by typing the **aa** command to abort acquisition. The **time** command can be used to estimate the total experiment time with a given **nt**.
If you want to observe some longer relaxation carbons, such as N-C=O's, you may set the d1 to 5 seconds or longer.

**Step 5. Data Processing**

5.1 Zoom operation

Click the LMB on the left side of a peak and the RMB on the right side. Two vertical red cursors will appear at the click points, then click the Expand button on the menu bar and the region between the red cursors will be expanded. You may do this zooming operation as many times as you like.

5.2 Set spectrum reference

After zooming the region containing the reference peak, click the LMB near the top of the peak, then type ***nl*** to place the cursor exactly on the top of the peak. Next, type ***rl(x.xxp)*** to set its chemical shift. For example, ***rl(77p)*** will set a peak frequency to 77.0 ppm.

5.3 Peak picking operation

Type ***ds*** and click the **next** and **th** buttons to display the horizontal threshold line. Press and hold the LMB to move the yellow cursor to the position you desire, then click on the **th** button again. Type ***dpf*** to display peak frequencies above spectrum peaks.

5.4 Label your experiment data

Type ***ctext*** to clear any text label for the present experimental data.
Type ***text("Your sample name, date\\solvent, etc")*** to label the present experimental data.
Type ***dtext*** to display the text label of present data on the upper left of the spectrum.

5.5 Print and plot spectrum and related information
***pl***----to begin print the present spectrum;
***pap***---to print all the parameters including the text labels, if any.
***ppa***---to print some parameters and text labels.
***pscale***---to print the spectrum scale in the unit of ppm or hz by ***axis="p"*** or ***axis="h".***
***pltext***--- to print the label.
***ppf***---to print all the peak frequencies over the respective peaks.
***page*** ---to expel the paper from printer.

**5.6 Save your experiment data**
You have to save your data in /export/home/nmrusr/Login_ID/yourname/
Type ***svf('file_name')*** to save your data in the form of FID.

**Step 6. Logout (You MUST type h1 before you logout)**

6.1 Retrieve the standard file and replace your sample with the standard sample.
Type ***h1*** to set up the standard proton file. This will also turn off the decoupler power.
Next, see **Step 2** for details about replacing NMR sample tubes.

6.2 Lock and shim the magnet
See **Step 3** for details. Before exiting, you are required to shim the magnet to the required level.

6.3 Exit the VNMR program
Type ***exit*** to close the VNMR program. Now you will see a full screen of Varian's logo.

6.4 Exit UNIX system
Press the RMB to select the Exit item in the popped up menu, then release the RMB. You will be prompted to select one of two choices: Exit or Cancel. Click on Exit to exit UNIX system. The screen will become blank and a prompt appears.

6.5 Logout
Type ***logout***. Sign off the logbook for the instrument.

**Congratulations!!! You have finished a complete 1D carbon-13 experiment. Make sure you type the h1 command before you logout.**

## Experiment 3: Proton 90° Pulse Width Calibration

Sample to be used: 10% Strychnine in $CDCl_3$

***Warning: You are not allowed to tune the probe without Dr. Wu's permission! If your experiment requires probe tuning, please contact our service instructors in advance.***

**Step 1.** Login

**Step 2.** Change Sample and type ***h1 su*** to load proton parameters before doing lock and shimming. Then call up an NMR staff to check the probe. Tuning the probe may be necessary for different solvents.

**Step 3. Lock and Shim the Magnet**

**Step 4. Setup Parameters and Run Experiment**

Run a 1D proton spectrum, set the cursor to a peak near the center of the spectrum, then type **movetof** to set the peak on-resonance for calibration.

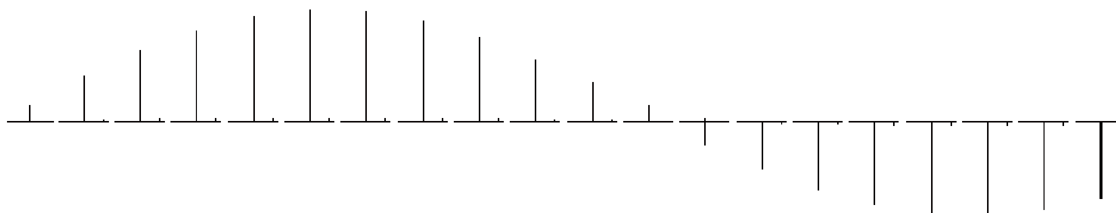
4.1 ***nt=1*** and ***d1=10*** or a value which is long enough to enable protons to relax back to thermal equilibrium status. (the default transmitter power: ***tpwr=56 the max is 60***)

4.2 Type ***array*** and input the following parameter and numbers as the computer requests:
**parameter to be arrayed:** ***pw***.
***enter number of steps in array: 20***
***enter starting value: 2***
***enter array increment: 2***
You are able to see the whole array parameters in the Text Window on the bottom by typing ***da***

4.3 After the acquisition is completed, save the data.

**Step 5. Data Processing**

5.1 Display arrayed spectrum
When the acquisition is finished, enter ***ds(1)*** for displaying the first spectrum of the array. Type ***aph*** for autophasing. Move the spectrum up a little bit by typing ***vp=50***. Choose a singlet or a doublet peak to expand and then enter ***ai dssh*** for displaying peaks at the same chemical shift range in all of the individual spectra horizontally.



5.2 Null point and 90° pulse width

From the arrayed spectrum, identify the first null point (where the peak intensity is 0), then check the pw value corresponding to it. This pw value is the 180 degree pulse width. The 90° pulse width is this pw value divided by 2 at given power (***tpwr***). In this case ***tpwr=56***. Type p***w90=xx*** to set up this parameter, where xx was obtained as above. Record this number for your future application. The 90 degree pulse for an NMR standard was calibrated by NMR center and it is recorded in the logbook, your 90 degree pulse should not very far from this number.

5.3 Label your experiment data

See Experiment One.

5.4 Print and plot spectrum and related information

Type ***plww pap page*** to print spectra in white wash mode (after the first spectrum, each spectrum is blanked out in regions in which it is behind an earlier spectrum).

5.6 Save your experimental data

See Experiment One

**Step 6. Exit and Logout**

See Experiment One

**Congratulations!!! You have finished a complete Proton 90° Pulse Width Calibration experiment.**

# Experiment 4: 90° Pulse Width Calibration for Carbon Channel

Sample to be used: 40% p-dioxane in benzene-$d^6$ (ASTM)

**Warning: You are not allowed to tune the probe without Dr. Wu's permission!**

**Step 1.** Login

**Step 2.** Change Sample as routine.
**Type *c13('Benzene') su* to load C13 parameters. Type *dm='nnn'* to turn off the decoupler.** Then call up a NMR staff person to check the probe. Probe tuning may be necessary for different solvents.

**Step 3. Lock and Shim the Magnet**

**Step 4. Setup Parameters**

4.1 Type ***dm='yyy' su*** to turn the decoupler back on.
***tpwr=xx (maximum 60), nt=1,*** then enter ***ga*** to begin acquisition.

4.2 After the acquisition is finished, phase the spectrum.

4.3 Set ***d1=20*** or a value which enables the carbon to relax back to thermal equilibrium status. (If you have added Cr(AcAc), then d1 can be set to 1 second).

4.4 Type ***array*** and input the following parameter and numbers as computer requests:
**parameter to be arrayed:** ***pw***.
***enter number of steps in array: 20***
***enter starting value: 2***
***enter array increment: 2***
You are able to see the whole array parameters in the Text Window on the bottom by typing ***da***

4.5 Type ***ai ga*** to begin acquisition. The ***ai*** means absolute intensity mode.

**Step 5. Data Processing**

**5.1 Display arrayed spectrum**
When the acquisition is finished, enter ***ds(1)*** for displaying the first spectrum of the array. Type ***aph*** for autophasing, then type ***vp=50*** to move the spectrum up. Choose a singlet peak to expend and then enter ***dssh*** for displaying peaks at the same chemical shift range in all of the individual spectra horizontally.

**5.2 Null point spectrum and 90° pulse width**
From the arrayed spectrum, identify the first null point (where the peak intensity is 0), then check the ***pw*** value corresponding to it. The 90° pulse width is this ***pw*** value divided by 2 at given power (***tpwr***). In this case, ***tpwr=60***.
Type ***pw90=xx*** to set up this parameter, where ***xx*** was obtained as above.

**5.3 Label your experiment data**

**5.4 Print the spectrum and related information**
Type ***plww pap page*** to print spectra in white wash mode (after the first spectrum, each spectrum is blanked out in regions in which it is behind an earlier spectrum).

**5.5 Save your experiment data**

**Step 6. Exit and Logout ( You must type h1 before you logout)**

# Experiment 5: APT

Sample to be used: 30% Menthol in $CDCl_3$ with 2% $Cr(AcAc)_3$

**Step 1. Login The Computer System**

See Experiment One.

**Step 2. Change Sample**

See Experiment One.

**Step 3. Lock And Shim The Magnet**

See Experiment One.

**Step 4. Setup Parameters and Run Experiment**

4.1 type ***c13*** to load standard carbon experiment parameters.

4.2 Set ***nt=8***. Type ***ga*** to begin acquisition for a common 1D-carbon spectrum.

4.3 After acquisition, type ***aph*** for automatic phasing and /or manual phasing and then set the sw if needed. Remember, APT will show all carbon signals, including the solvent peaks.

4.4 Type ***apt*** to load standard APT parameters. Double check following parameters:

***pw***----- Use 90 degree pulse or less for $^{13}C$

***p1***-----180° $^{13}C$ pulse width. (It is the double value of the 90° pulse width. Check the last page of the log book for 90° pulse width).

***tpwr*-----**transmitter power. It must be paired with 90° pulse width. (Check the last of the log book for 90° pulse width and transmitter power ***tpwr***)

***d2***-----the tau delay time. d2=0.007 (7ms) will make CH, CH3 peaks down and C, CH2 peak up. We assume the $^1J_{CH}$=140 Hz 1/140Hz = 7.14 ms.

***d1***-----the pulse delay. It is set to around 10 seconds.

***dm***----decouple mode. It is set as ***dm='yny'*** for APT.

***nt***------scan numbers depends on sample concentration (minimum 16 scans).

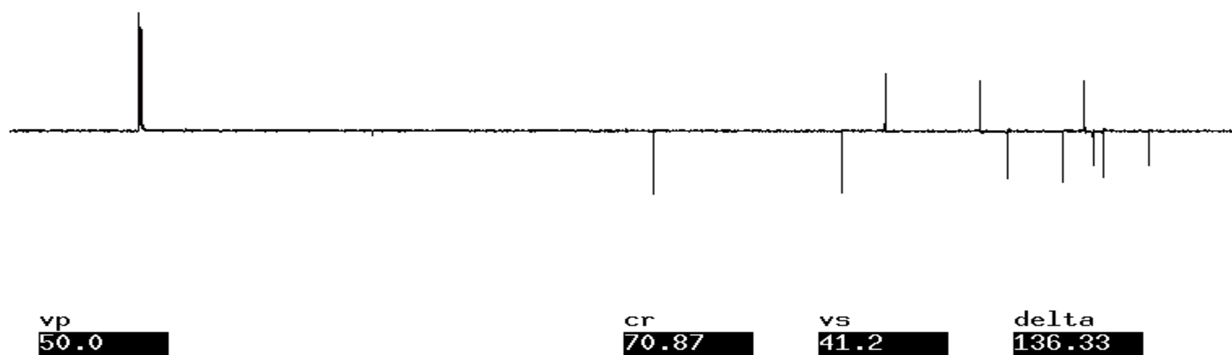
4.5 Type ***ga*** to acquire data.

**Step 5. Data Processing**

When the acquisition is finished, type ***aptaph*** macro to phase APT automatically. Or manually phase the spectrum.

Type ***pl pscale pap page*** to plot the spectrum.

**Step 6. Exit and Logout** See **Experiment One**




APT spectrum of Menthol in CDCl3 on INOVA600. pw=11, p1 =22 tpwr=60, d2=0.007, d3=0.001 dm='yny' dpwr=35 (Maximum 40) dmf=8000 decoupling modulation frequency (1/pw90 at power 35 dB).

# Experiment 6: DEPT

Sample to be used: 30% Menthol in CDCl3

**Step 1. Login The Computer System**

See Experiment One

**Step 2. Change Sample**

See Experiment One

**Step 3. Lock And Shim The Magnet**

See Experiment One

**Step 4. Setup Parameters And Run Experiment**

4.1 Type command ***c13*** to setup standard parameters for 1D carbon spectrum.

4.2 Set ***nt=4*** or 8 to acquire an 1D carbon spectrum, and set the chemical shift reference and spectral width, remember all carbons without proton attached, including the solvent peak will not show on the DEPT spectrum.

4.3 Type ***dept*** to setup standard parameters for DEPT experiment.

4.4 Check and/or change the following important parameters:

***ss=4*** steady-state pulse or dummy scans

***pw***=the value of 90° pulse width for C13 channel; Check the last page of the log book.

***tpwr***=the value transmitter power of C13 for $90^o$ pulse width; Check the last page of the log book.

***pp***=the value of proton 90°-pulse width from decoupler channel; Check the last page of the log book.

***pplvl***=the value of proton $90^o$-pulse transmitter power from decoupler channel; Check the last page of the log book.

| | |
|---|---|
| ***d1=5*** | relaxation time |
| ***j=140*** | average C-H coupling constant; |
| ***nt=32*** or more | number of scans, A multiple of 16 is suggested. |

4.5 Type command ***ga*** to acquire data.

**Step 5. Data Processing**

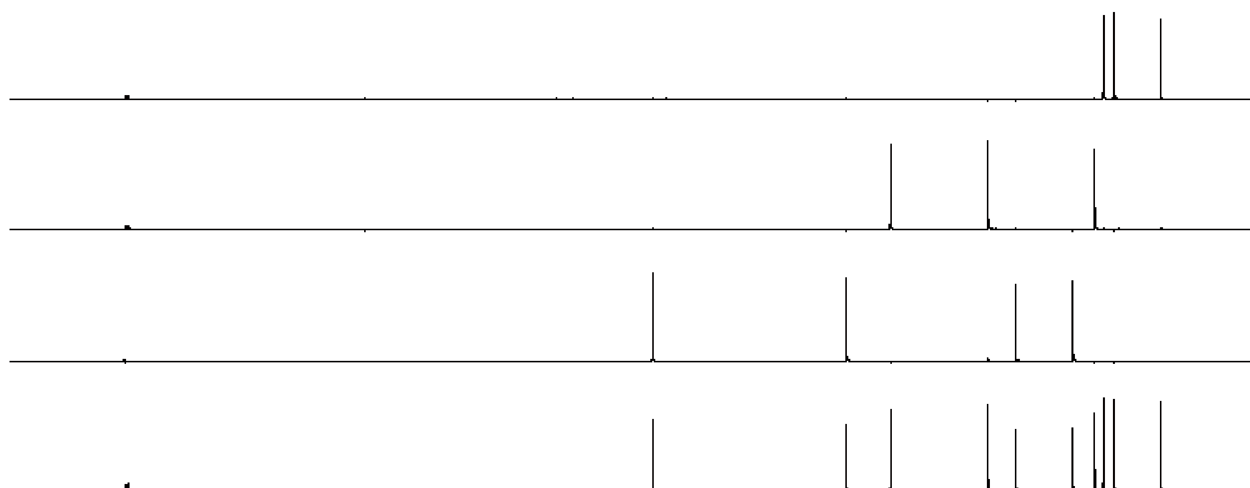
5.1 After the acquisition, type ***ds(1)*** to display the first spectrum.

5.2 Phase the spectrum and select a proper threshold by using menu button of **th.** Then type **fp.**

5.3 Enter ***autodept*** to analyze and display arrayed spectra of $CH_x$, CH, CH2 and CH3 and print out DEPT spectrum. There are several ways to process the arrayed spectra. You could use ***adept*** and ***dssa***, then ***pldept*** to plot the spectra.

**Step 6. Exit and Logout**

See Experiment One



| vs | sp(ppm) | wp(ppm) | first | last | step |
|---|---|---|---|---|---|
| 43.7 | 5.77 | 136.87 | 1 | 4 | 1 |

DEPT spectrum of Menthol in CDCl3 on INOVA600. pw=12 p1=22 tpwr=60 pp=12.2 pplvl=56 J=140 dm='nny' dmm='ccw' dmf=8000 dpwr=35 mult=arrayed (0.5,1,1,1.5).

Note:
This experiment takes four times of the acquisition time of your 1D $^{13}C$ spectrum that reaches a good enough S/N ratio. For example, your 1D carbon takes 256 scans (about 15 minutes) to get a good spectrum, then your DEPT needs about one hour (15 x 4=60 minutes).

# Experiment 7: INEPT

Sample to be used: 30% Menthol in CDCl3

**Step 1. Login The Computer System**

See Experiment One

**Step 2. Change Sample**

See Experiment One

**Step 3. Lock And Shim The Magnet**

See Experiment One

**Step 4. Setup Parameters And Run Experiment**

4.1 Type command ***c13*** to setup standard parameters for 1D carbon spectrum.

4.2 Set ***nt=4*** or 8 to acquire an 1D carbon spectrum, and set the chemical shift value.

4.3 Type ***inept*** to setup standard parameters for DEPT experiment.

4.4 Check the following important parameters:

***ss=4*** steady-state pulse or dummy scans

***pw=*** the value of 90° pulse for carbon-13; Check the last page of the log book.

***tpwr=*** the value of transmitter power of 90$^o$ pulse for C13 channel; Check the last page of the log book.

***pp=*** the value of proton 90° pulse width from decoupler channel; Check the last page of the log book.

***pplvl=*** the value of proton 90$^o$-pulse power from decoupler channel; Check the last page of the log book.

***d1=5*** relaxation time

***j=140*** average C-H coupling constant;

***mult=3*** multiplicity to make CH, CH3 up and CH2 down;

***nt=64*** number of scans, A multiple of 16 is suggested.

***dm='nny'*** setting for coupled spectrum;

***focus='y'*** refocusing for coupled spectrum;

***normal='y'*** normal multiplets in coupled spectrum

4.5 Type command ***ga*** to acquire data.

**Step 5. Data Processing**

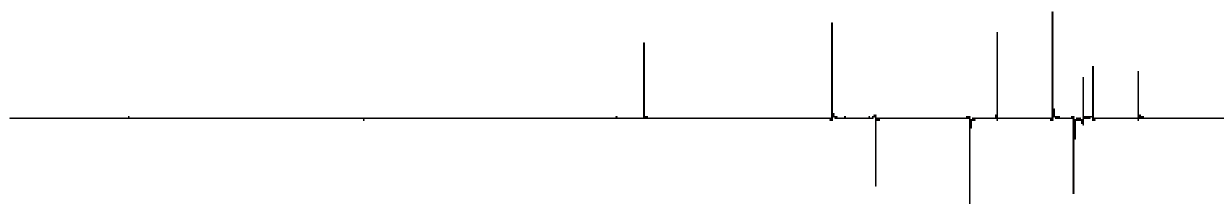
5.1 After the acquisition, the first spectrum will be displayed automatically.

5.2 Phase the spectrum, if necessary.

5.3 Type ***pl pscale pap page*** to plot the INEPT spectrum.

**Step 6. Exit and Logout**

See Experiment One



vp 50.0 cr 70.87 vs 50.0 delta

INEPT spectrum of Menthol in $CDCl_3$ on INOVA600. pw=11 p1=22 tpwr=60 pp=12.2 pplvl=56

# Experiment 8: Presaturation and Water Suppression

Sample to be used: 10% Strychnine in $CDCl_3$

**Step 1. Login The Computer System**

See Experiment One

**Step 2. Change Sample**

See Experiment One

**Step 3. Lock And Shim The Magnet**

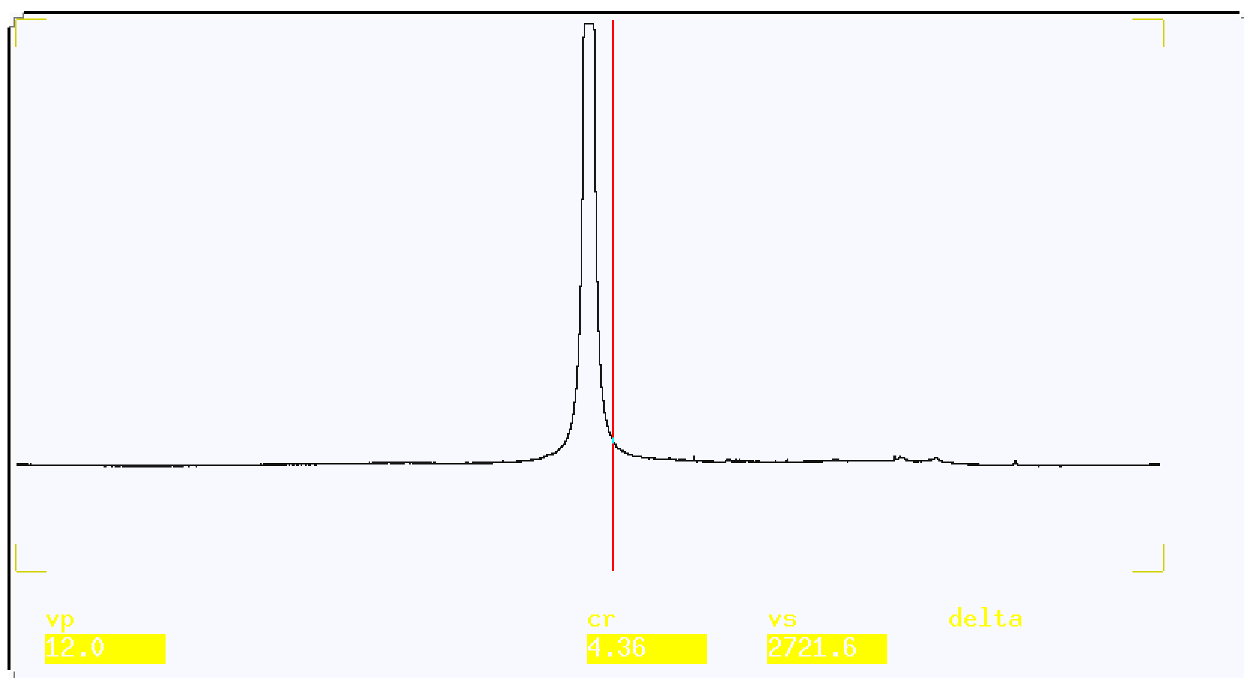
See Experiment One

**Note:** For the samples using $H_2O$ + $D_2O$ as solvent, the water peak is huge. To be able to see other signals the water peak need to be suppressed more efficiently. There are several aspects we need to emphasize here: 1) Tune the probe on your sample. 2) Use gradient shimming to get a good shimming. 3) Put the transmitter frequency on the water peak.

**Step 4. Setup Parameters and Run Experiment**

4.1 Load your sample into the probe as usual. Type ***h1('d2o') su*** to load the proton experiment parameters. Because of the huge water signal, the receiver will be overflowed if you start to acquire the data using the default value. To avoid the receiver overflow, do as follows: Type ***gain?*** to check the value of the ***gain***. Change the ***gain*** value to a smaller value. The ***gain*** value could be set as small as 1. When acquiring the data. If you still get an "ADC overflow" error message after you set ***gain=1,*** then the ***pw*** value needs to be set to a smaller value until no "ADC overflow" message is shown. If you have less than 2% water in the solvent, then you could use the standard proton parameters.

4.2 After acquisition completes, type ***aph*** for automatic phasing and do any necessary manual phasing. At this point spectrum is only the water peak.




Plasma sample in 90% $H_2O$ + 10% $D_2O$ without suppression.

4.3 Set the reference peak, then move the red cursor to the peak to be saturated and type the ***nl*** and ***sd*** commands to set the saturation peak.

4.4 Type ***presat*** to retrieve the standard file for the presaturation experiment. Check and change the following parameters: ***ss=4, nt=8, d1=0.2, satdly=1, tof=dof; satfrq=dof. satpwr=6*** (Min=1, Max=12 step by 1 unit), ***spin=0*** and finally, type ***su*** to set up the parameters. Reset the gain by typing gain=5 or 10, pw=5 etc..

```
  ACQUISITION              SAMPLE            PROCESSING             FLAGS                Acq & Obs
sfrq      599.738  date    Jun  3 2005  lb             0.20  composit          n        Decouplers
tn             H1  solvent       cdcl3  sb         not used  il                n        Sequence
at          1.598  temp       not used  gf         not used  in                n        Flags & Cond.
np          25570       DECOUPLING      fn         not used  dp                y        Process
sw         8000.0  dn               H1  math              f  hs               nn        Process2
fb       not used  dof           534.6                           SATURATION             Display
bs             16  dm              nnn  werr          react  sspul             n        display2
ss              4  dmm               w  wexp       procplot  satpwr            2        LCNMR/STARS
tpwr           56  dpwr              0  wbs          testsn  satfrq      534.597        Text
pw            5.0  homo              n  wnt                  satdly        1.000        Spare
p1              0                                            satmode         ynn        Setup EXP
d1          0.200
d2              0
tof         534.6
nt              4
ct              0
```

Presat parameters.

4.5 Type ***ga*** to acquire data. A spectrum will be displayed when acquisition is finished.

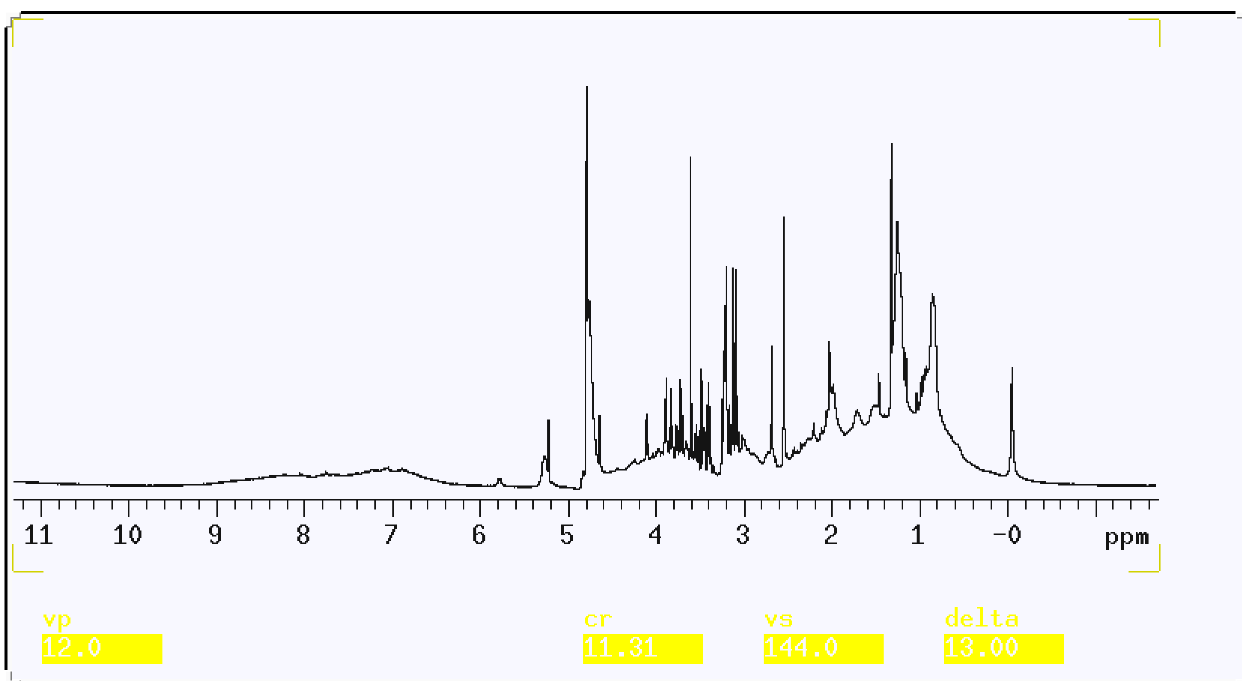
4.7 Check if the peak of interest is efficiently removed by the pulse in the resulting phased spectrum. If not, increase ***satpwr*** by 2 units and repeat the above experiment (The maximum satpwr 16). If the water peak is still unable to suppressed, please contact Dr. Wu for help. If yes, set **nt** between 64 and 128, then enter ***ga*** to acquire data.

## Step 5. Data Processing

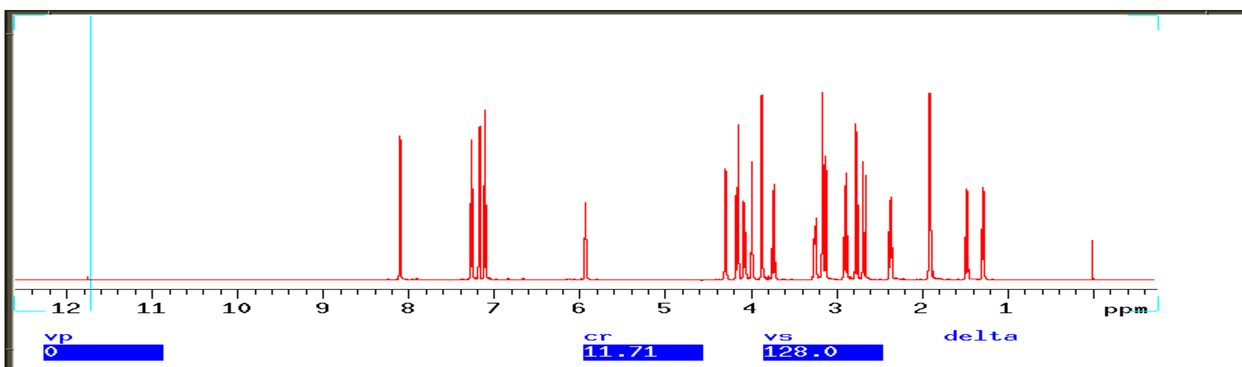
The data processing for the Presat experiment is same as that of 1D Proton experiment. See Experiment One.

## Step 6. Exit And Logout

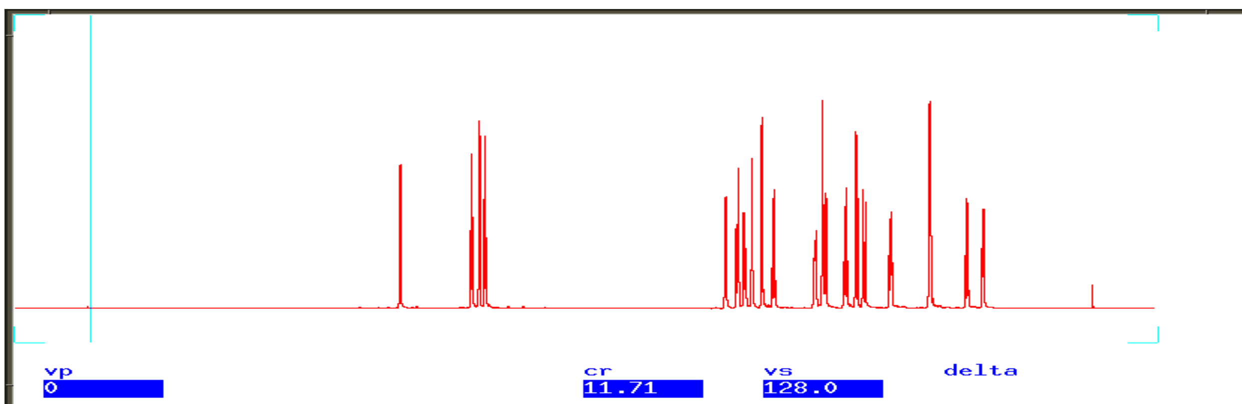
See Experiment One



Plasma sample in 90% $H_2O$ + 10% $D_2O$ after the water peak suppressed.



Normal 1D proton spectrum of strychnine in $CDCl_3$.



Same sample, but the peak at 6 ppm was suppressed.

## Experiment 9: Gradient Shimming

Sample to be used: 2% $H_2O$ in dopped $D_2O$

**Step 1.** Load your sample into the probe as usual. Type ***h1('d2o') su*** to load the proton experiment parameters. Ask an NMR staff person to help you tune the probe. Lock the machine and shim manually.

**Step 2.** Because of the huge water signal, the receiver will be overflowed if you start to acquire the data using the default value. To avoid the receiver overflow, do as follows:

Type ***gain?*** to check the value of the ***gain***. Change the ***gain*** value to a smaller value. The ***gain*** value could be set as small as 1. When acquiring the data. If you still get an "ADC overflow" error message after you set ***gain=1,*** then the ***pw*** value needs to be set to a smaller value until no "ADC overflow" message is shown. Save the spectrum and change to another experiment (***jexp2)*** to do gradient shimming.

**Step 3.** Turn on the Pulsed Gradient Driver module in the console (ask an NMR staff person for help if this is the first time you do this)

Type ***pfgon='nny'***

Type ***gmapsys*** and press return.

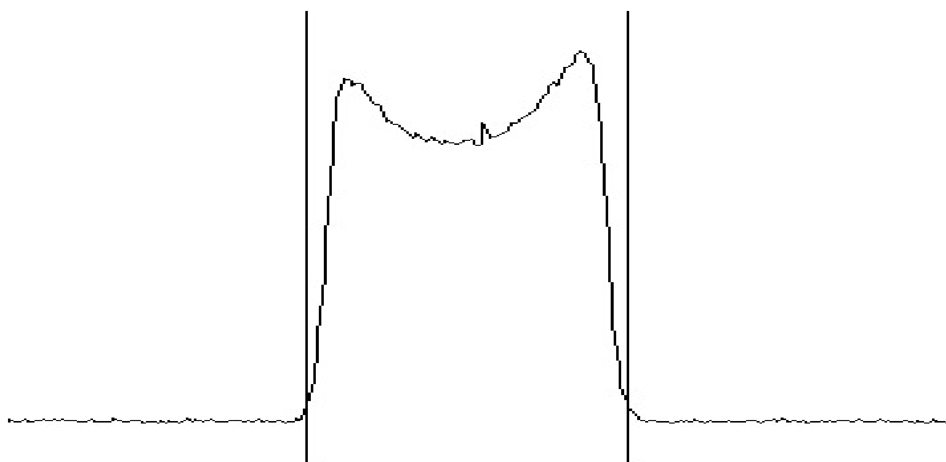
Click on **Set Params** > **Gradient, Nucleus** > **Pfg H1** > **Return** > **Go, dssh**

You may set the pw= "90 degree pulse" and p1= "180 degree pulse" these numbers are written on the log book of each instrument.

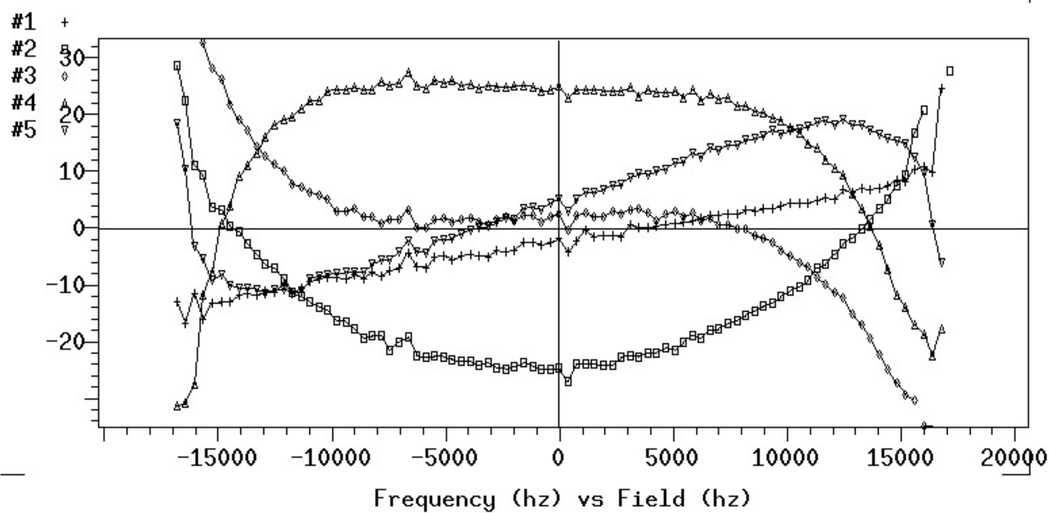
After acquisition completes, there are two broad peaks displayed. If the peaks do not look like as following, then there is a problem. You may have to talk to our NMR center staff.

| vs | sp(ppm) | wp(ppm) | first | last | step |
|---|---|---|---|---|---|
| 38880.4 | -78.66 | 166.74 | 1 | 2 | 1 |

The click **find gzwin,** computer will find the gradient shim region.

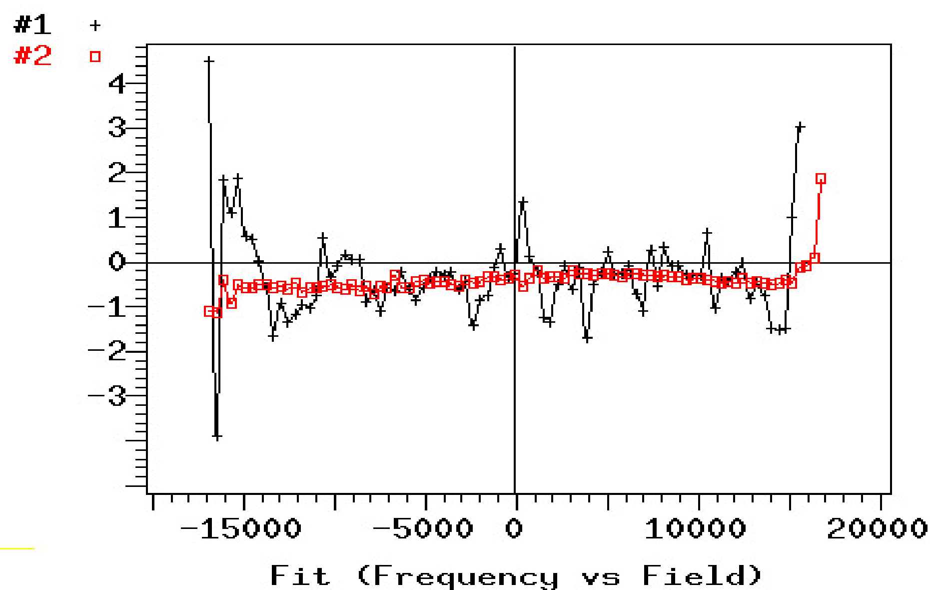


Then type gmapsys
(Shimming size can be changed now by typing **gzsize**=7)
Click on **Shim Maps** > **Make Shimmap**, then type a file name when prompted.
(file is saved in directory /vnmrsys/gshimlb/shimmaps)




After you finish mapping,
Click on **Return** > **Display** > **Display Shimmaps**
(lines should be smooth in the shimmap)

Click on **Return** > **Autoshim on Z**
The map rms err should be smaller than 1.00.
Click on **Set Shim**




Finishing:
Type **Pfgon=’nnn’ su**
Turn off the pfg module to standyby.
**Save the shim file svs(‘shim_021909’)**

**Gradient Shim On VNMRS System:**

Same concept, but there is a little difference. Type gmapsys, a new window will come up. Click Acquire Trial Spectrum, That is same as Go dssh icon as above. If everything is OK, then click Make Gradient map, you may have to input the map name. After completed, then click auto gradient shim on Z. Save the new shim file.

# Experiment 10: COSY

**Step 1. Login The Computer System**
See Experiment One

**Step 2. Change Sample**
See Experiment One

**Step 3. Lock And Shim The Magnet**
See Experiment One

**Step 4. Setup Parameters And Run Experiment**

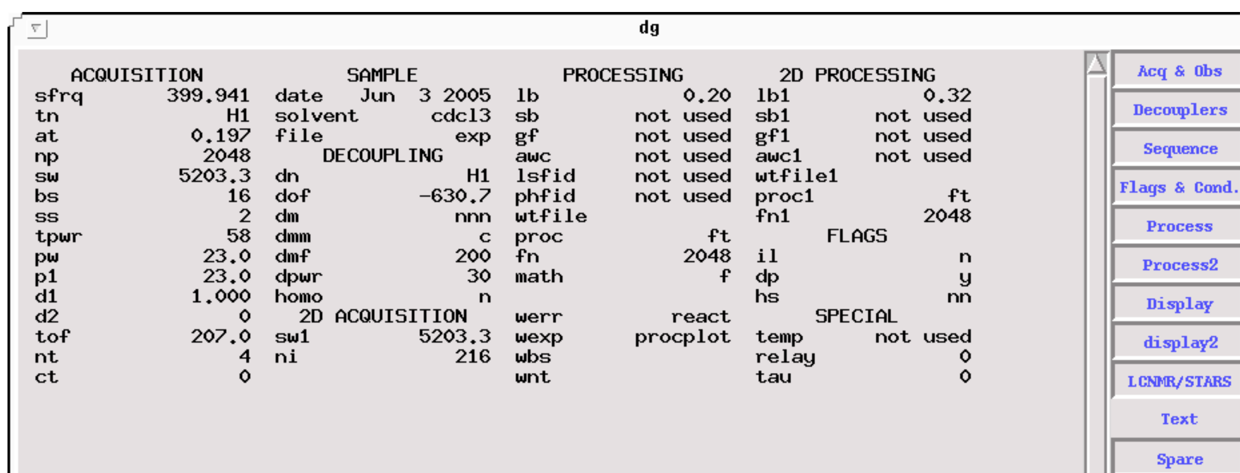
4.1 Run a proton 1D spectrum by using proton standard parameters.(See **Experiment One**)

4.2 Set the two red cursors to define the desired ***sw*** (spectrum width); type ***movesw*** then ***ga***, Check the new spectrum and make sure no peak is fold in. Save this spectrum in case for later reference. Type ***COSY*** on the command line and type ***dg*** to show the parameters. Check the following parameters:

| | |
|---|---|
| ***ss=4*** | dummy scans for COSY |
| ***d1=2*** | delay; set to 2 seconds. |
| ***fn1=2048*** | zero-fill the f1 dimension to 2K data points. |
| ***tpwr=xx*** | transmitter power of the $90^{o}$-pulse-width of proton channel: check the last page of the log book. |
| ***pw=xx.x*** | $90^{o}$ pulse width for proton: check the last page of the log book. |
| ***nt=8*** | minimum ***nt*** is 8 (16, 32). The larger the value, the better the spectrum. |
| ***ni=128, 256*** | number of FIDs, (128, 256,512). The larger the value, the better. |

Make sure ***sw = sw1*** and ***fn = fn1***

Type ***time*** to check the total experiment time. You can increase or decrease the value of ***nt*** or ***ni*** to fit your allotted time. **Turn the spin off** and type ***ga*** to start the experiment. After acquisition, save the data.



```
dg
  ACQUISITION           SAMPLE                PROCESSING              2D PROCESSING
sfrq      399.941  date    Jun  3 2005  lb               0.20  lb1               0.32
tn             H1  solvent       cdcl3  sb           not used  sb1           not used
at          0.197  file            exp  gf           not used  gf1           not used
np           2048      DECOUPLING       awc          not used  awc1          not used
sw         5203.3  dn               H1  lsfid        not used  wtfile1
bs             16  dof          -630.7  phfid        not used  proc1               ft
ss              2  dm              nnn  wtfile                 fn1               2048
tpwr           58  dmm               c  proc               ft        FLAGS
pw           23.0  dmf             200  fn               2048  il                   n
p1           23.0  dpwr             30  math                f  dp                   y
d1          1.000  homo              n                         hs                  nn
d2              0    2D ACQUISITION     werr            react        SPECIAL
tof         207.0  sw1          5203.3  wexp         procplot  temp          not used
nt              4  ni              216  wbs                    relay                0
ct              0                       wnt                    tau                  0
```



COSY parameters.

**Step 5. Data Processing and Spectrum Printing**

5.1. Reload the data:
Click on Main Menu and File , then highlight the file name and click on Load

5.2. See detailed processing instruction in **Experiment 17**. Alternately, you could type ***wft2d foldt*** to autoprocess the data by using default processing parameters.

5.3. Adjust the vertical scale of the 2-D spectrum using the vs+20 and vs-20 button to eliminate the unnecessary background noise.

5.3 Type ***pcon page*** to print the 2-D spectrum.

5.4. To print 2-D spectrum with 1-D projection spectrum:
Click on Return, Size and Center buttons to place the spectrum in the middle of the screen.

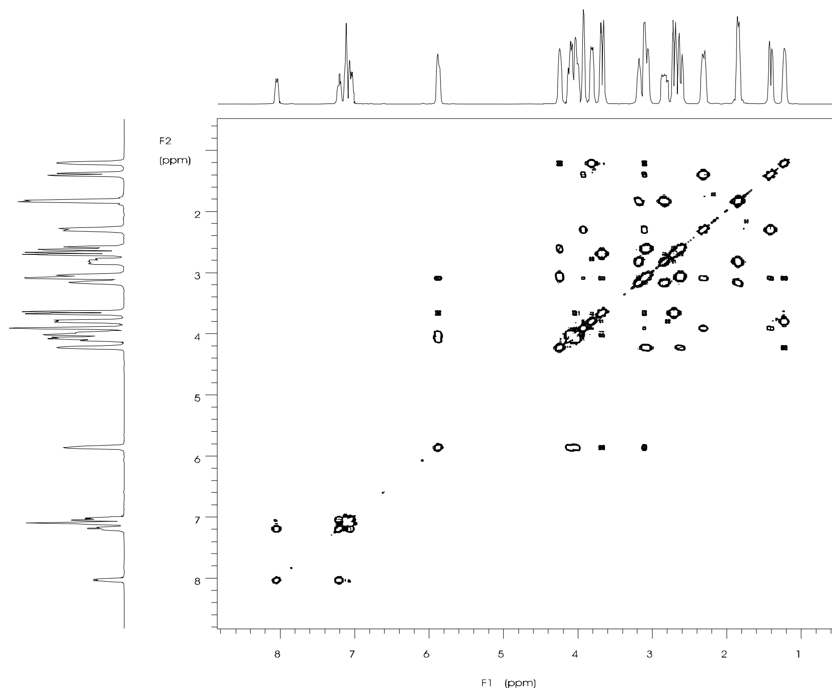
5.5. Click on Proj, Hproj(max) and Plot buttons to display the horizontal projection1-D spectrum on the top of the 2-D spectrum. Use the MMB to adjust the vertical scale of the 1-D projection spectrum.
Click on Vproj(max) and Plot buttons to display vertical projection 1-D spectrum on the left of the spectrum. Use the MMB to adjust the scale of the 1-D spectrum.

5.6. Type ***pcon page*** to print the 2-D spectrum with 1-D projection spectra.

5.7 To expend the area you are interested in:
Press and drag left mouse button to control a pair of the cursors (looks like a red-cross) and define the left and bottom edge of the spectrum. Use the right mouse button to control another pair of cursors to define the upper and right edge of the spectrum. Then click Expand to expand the spectrum.

**Step 6. Exit And Logout**

See Experiment One




COSY Spectrum of Strychnine in CDCl3.

# Experiment 11: HETCOR

**Step 1. Login The Computer System**

See Experiment One

**Step 2. Change Sample**

See Experiment One

**Step 3. Lock And Shim The Magnet**

See Experiment One

**Step 4. Setup Parameters And Run Experiment**

4.1. In exp1, run a 1D proton spectrum by using standard parameters. After phasing and setting reference, use the two red cursors to narrow the spectrum of interest by 1ppm upfield and 1ppm downfield. Type ***movesw*** to change the spectral width.

4.2. Type ***ga*** to run the 1D proton experiment with a modified **sw**. Phase the spectrum.

4.3. Type ***jexp2*** to joint exp2. Run a 1D-carbon experiment. After phasing and setting the reference, narrow the spectrum of interest using the two red cursors. Type ***movesw*** to change the ***sw***. Remember you will not see these carbons without proton attached on the HETCOR. So set the correct sw will increase your spectrum quality!

4.4 Type ***ga*** to run the 1D carbon experiment with a new **sw**. Phase the spectrum.

4.5 Type ***hetcor*** to set up a standard HETCOR experiment. Remember: your proton spectrum is in exp1.

4.6. Double-check the following two pairs of parameters (the correct values are listed on the last page of the log book):

***tpwr*** transmitter power for carbon
***pw*** 90° pulse for carbon

***pp*** 90° pulse for proton decoupler channel
***pplvl*** proton pulse power for decoupler channel
(***pp*** and ***pplvl*** should be as same as the ones in DEPT experiment in Experiment 6)

After entering correct values for the above parameters, check the following parameters:

***ss=4*** dummy scans
***sw=*** sw should equal to the ***sw*** value in exp2 for carbon domain
***sw1=*** sw1 should equal to the ***sw*** value in exp1 for proton domain;
***dof=*** ***dof*** should equal the ***tof*** value in exp1 for proton;
***nt=n*16*** acquisition scans of multiple of 16;
***ni=256*** number of increments in 1st dimension;
***np=2048*** number of points to record FID;
***d1=3*** carbon relaxation time

4.7. Type ***time*** to check the experiment time. ***d1*** can be adjusted to fill the allotted time.

4.8. Turn the spin off and do any needed minor shimming. Enter ***ga*** to acquire data.

4.9. Save the spectrum by typing ***svf('hetcor_mydata')***

**Step 5. Data Processing and Spectrum Printing**

5.1. Recall the data "hetcor_mydata.fid"
Click on Main Menu and File , then highlight the file name and click on Load

5.2. Type ***wft2da*** or follow the detailed processing method in experiment 17.

5.3 Click on Return, Size and Center buttons to place the spectrum in the middle of the screen.

5.4. Adjust the vertical scale of the 2-D spectrum using the vs+20 and vs-20 button to eliminate the unnecessary background color.

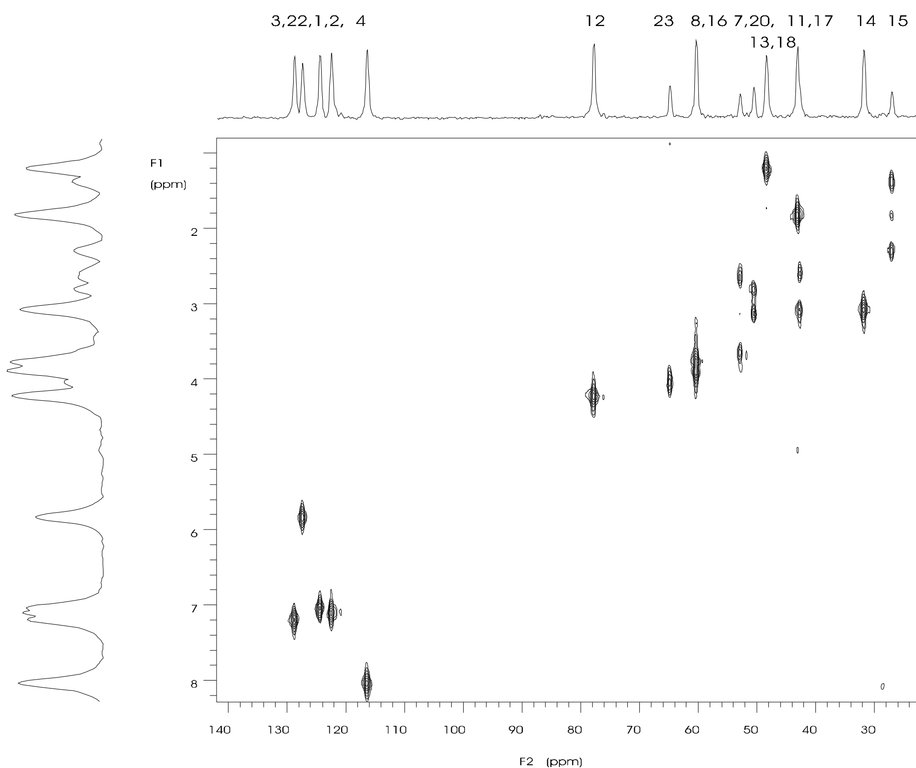
5.4.1. Click on Proj, Hproj(max) and Plot buttons to display the horizontal projection 1-D spectrum on the top of the 2-D spectrum. Use the MMB to adjust the vertical scale of the 1-D projection spectrum.

5.6. Click on Vproj(max) and Plot buttons to display vertical projection 1-D spectrum on the left of the spectrum. Use the MMB to adjust the scale of the 1-D spectrum.

5.7. Type ***pcon page*** to print the 2-D spectrum with 1-D projection spectra.

**Step 6. Exit And Logout**

See Experiment One




HETCOR Spectrum of strychnine in $CDCl_3$.

# Experiment 12: 1D NOE------Presat

Sample to be used: 10% Strychnine in $CDCl_3$

**Step 1. Login The Computer System**

See Experiment One

**Step 2. Change Sample**

See Experiment One

**Step 3. Lock And Shim The Magnet**

See Experiment One

**Step 4. Setup Parameters And Run Experiment**

4.1 Run a standard proton spectrum and save the file.

4.2 Put the cursor on the peak to be irradiated, then type ***nl sd*** to display the frequency, shown as ***dof=xxx.xx*** on the top of the screen. Write down this first ***dof*** value (If the peak is a multiplet, put the cursor in the middle of the multiplet and type ***sd***). Next, move the cursor to a no-peak region close to the peak to be irradiated, Type ***sd*** to display the second ***dof*** value. Make sure to record this value.

4.3 Type ***presat*** to load standard file for the presaturation experiment. Check and change the following parameters: ***ss=4, nt=8, gain=10, d1=2, satdly=1, satfrq=the first dof value written down***.

***satpwr=2*** (Min=1, Max=15 step by 1 unit), and type ***su***. Finally, turn off the ***spin***. Type ***ga*** to run a quick spectrum. Check if the irradiated peak has disappeared from the resulting spectrum. If not, repeatedly increase ***satpwr*** by 2 units and run the experiment again until the peak has disappeared.

4.4 Change the ***nt*** value to ***128*** or ***256*** and type ***ga*** to acquire data for the first spectrum. After the acquisition is finished, save the spectrum.

4.5.1 ***ONLY*** change the ***satfrq*** parameter to the second ***dof*** value recorded previously, then type ***ga*** to obtain a second spectrum. Save this new spectrum.

**Step 5. Data Processing**

5.1. Type ***jexp5*** to go to Exp5 and load the first spectrum by the following procedure (***you must use Exp5 to do this operation***): Click on Main Manu, File and highlight the first spectrum file, then click on load. Type ***lb=5 wft*** to show the spectrum.

5.2. Type ***jexp1*** to go to Exp1. Load and process the second spectrum by the procedure as described in step 5.1.

5.3. Type ***spsub*** to subtract the second spectrum (in Exp1) from the first (in Exp5) and then type ***jexp5*** and ***ds*** to show the resulting spectrum in Exp5. Type ***vp=50*** to move the spectrum to the middle of the screen.

5.4. Plot the spectrum.

**Step 6. Exit And Logout**

See **Experiment One**

## Experiment 12: 1D NOE------ NOEDIF

Sample to be used: 10% Strychnine in CDCl3

**(This method is for Mercury 300. Because of the hardware configuration, two NOE methods we introduced before (Experiment 11 and 18) are not available on Mercury 300)**

**Step 1.** Run a proton spectrum in experiment 1. then type ***mp*(1, 2)** to move this proton parameter to experiment 2. Type ***jexp*2** to go to experiment 2. Type ***wft*** to show the proton spectrum.

**Step 2.** Type ***noedif*** to bring up the NOEDIF parameters.
Find and expend the peak you want to irradiated. Now you need to set up some parameters:

***f0*** to ***f5*** are the irradiation points (frequencies).
For a singlet, use ***f0,f1*** and ***f2.***
Set up ***f0*** value: put the red cursor on the halfway-up point of the left side of the peak and type ***sd*** then type ***f0=dof***.
Use the same method to set up ***f1*** and ***f2***. ***f1*** is for the top of the peak and ***f2*** is for the halfway-up point of the right side of the peak.

For a doublet, use ***f0***, ***f1***, ***f2*** and ***f3***. ***f0*** is for left side of the first peak and ***f1*** is for the left side of the second peak. ***f2*** is for the right side of the first peak and ***f3*** is for the right side of the second peak.

For a triplet or higher multiplets, ***f0*** to ***f#*** (# is equal to the number of the multiplets) are used with each value is set on the top of the each peak.

***dofoff*** is off resonance frequency. Default value is -10000 Hz. You can also set it up to by putting the red cursor to where there is no peak and type ***sd*** and then type ***dofoff=dof***.

Check up following parameters:
***Ctrl='n'***
***ss=16***
***satpwr*** is the irradiate power from 0 to 10.
***tau=0.1***
***d1=4***
***d2=0***
***dm='ynn'***
***nt*** is a value of multiple of 16. Usually put a big number like 128 or more.
***bs='n'***
Turn off the spinner.
Type ***ga*** to start the acquiring the data.

**Step 3.** After fishing the acquisition, type ***wft*** to start to process the spectrum. (Note, this spectrum is already the result of the subtraction of two spectra. If you type ***aph*** to phase it, usually the peak irradiated is up and noe peaks are down. If you prefer the other way, phase the peaks manually.)

# Experiment 12: 1D NOE------ CYCLENOE

Sample to be used: 10% Strychnine in CDCl3

**Step 1.** Run a proton spectrum in experiment 1. Type ***mp***(1,n) to move this proton parameter to experiment n. Type ***jexp*n** to go to experiment n. Type ***wft*** to show the proton spectrum.

**Step 2.** Type ***cyclenoe*** to bring up the cyclenoe parameters.
Find and expend the peak you want to irradiated. Now you need to set up some parameters:

***satfrq*** is the frequency of the peak you want to irradiated. Put the red cursor on the middle of the peak, type ***nl*** and ***sd,*** and then type ***satfrq=dof***.
***control*** is the off-resonance frequency. Put the red cursor to where there is no peak (but close to the peak you want to irradiated) and type ***sd***, followed by typing ***control=dof***.

***pattern*** is the pattern type (1 for singlet, 2 for doublet, etc. If it is difficult to decide what a multiplet is, just guess one. Anyway, you need to set a number for *pattern*)
***spacing*** is the coupling constant, in Hz, for the multiplet you want to irradiate. Again, if it is difficult to measure a coupling constant, guess one.
Check up following parameters:
***cycle='y'***
***intsub='y'***
***satpwr*** is the irradiate power from -10 to 2. -10 usually is enough.
***tau=0.1***
***mix=0***
***nt*** is a value of multiple of 16. Usually put a big number like 128 or more.
Turn off the spinner.
Type ***ga*** to start the acquiring the data.

**Step 3.** After fishing the acquisition, type ***wft*** to start to process the spectrum. (Note, this spectrum is already the result of the subtraction of two spectra. If you type ***aph*** to phase it, usually the peak irradiated is up and NOE peaks are down. If you prefer the other way , phase the peaks manually.)

# Experiment 13: 1D Carbon with Complete Fluorine Decoupling by Using Wave Function Generator

Sample to be used: 20% Perfluoro-2-methyl-2-pentene

**Step 1. Login The Computer System**

(See Experiment One)

**Step 2. Change Sample**

(See Experiment One)

**Step 3. Lock And Shim The Magnet**

(See Experiment One)

**Step 4. Setup Parameters And Run Experiment**

4.1 type ***f19*** to load standard fluorine parameters with $CDCl_3$ as solvent. Run a F19 spectrum and save the file.

4.2 Find the value of ***tof*** by typing **dg** and record this value. ***tof*** is the center frequency of the observe transmitter.

4.3 Find the transmitter frequencies for each peaks as follows:

4.3.1 Put the cursor on a peak and type ***movetof***. Write down the new ***tof*** value after typing **dg**.

4.3.2 Load the original spectrum again and find the ***tof*** value for another peak as in step 4.3.1.

4.3.3 Repeat step 4.3.1 and 4.3.2 until all the frequency values for all peaks are recorded.

4.4 Calculate the ***offset*** value.

4.4.1 Subtract the transmitter frequency value of each peak recorded in step 4.3 from the ***tof*** value of the spectrum recorded in step 4.2. Record these values.

Remember, always subtract the frequency value of a peak from the ***tof*** value, so that when the frequency of a peak is higher than the ***tof*** value, you get a positive ***offset*** value and when the frequency of a peak is lower than ***tof***, you get a negtive ***offset*** value.

4.5 Use Waveform generator to generate a shaped decoupling sequence:

4.5.1 Load the F19 spectrum and type ***wft***

4.5.2 Click Pbox button on the menu bar

Click Het-dec

Click Adiabatic and then type 300 after J in Hz on the top of the screen for C-F coupling.

Click Options

Click Offset and input the ***offset*** value of the first (leftmost) peak which should be decoupled.

Click Bandwidth and input 4000------- the area will be decoupled.

Click Return.

Click WURST, three parameters will be displayed for the decoupling on the top.

4.5.3. Click Options and repeat the remaining steps in the step 4.5.2. until all the ***offset*** value are input.

Click Close.

4.5.4. Click Name and give a file name for the shaped pulse.

Click Close.

The computer will ask you to

**Enter reference 90 degree pulse width (μse):** (F19 90 degree pulse width)

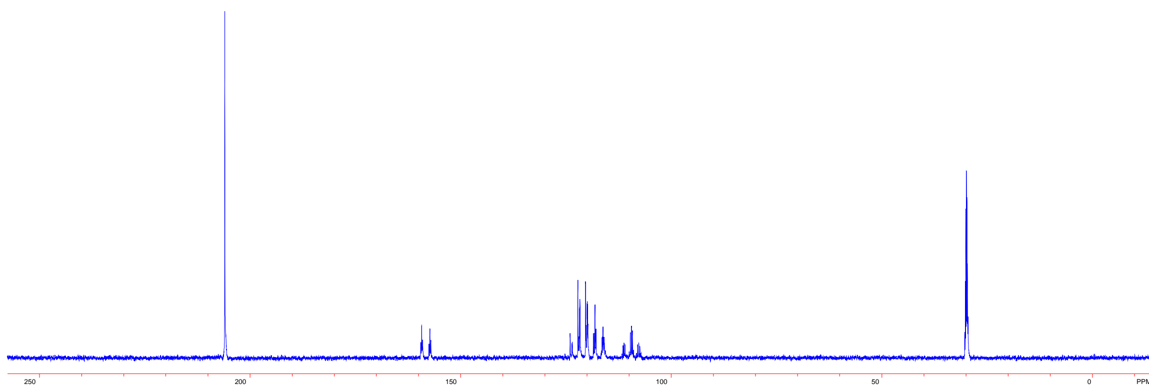
**Enter reference power level:** (power level when you measure the F19 90 degree)
A shaped pulse will be displayed on the screen.

Write down the following parameters:
**dres**=x
**dmf**=xxxxx
Remember you shaped pulse file name.

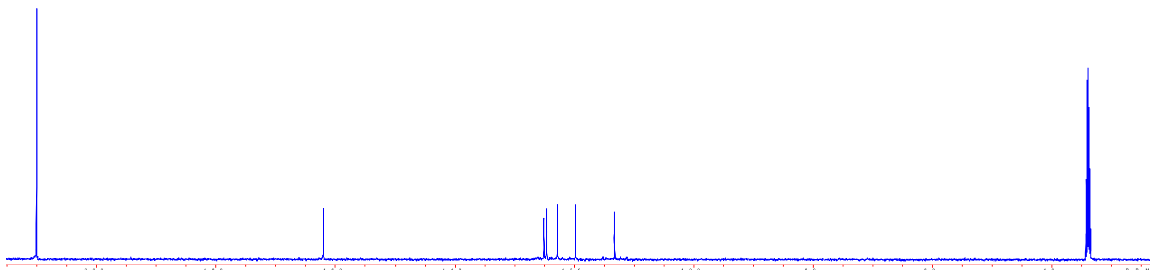
4.5.5 Load the standard C13 experiment parameters by typing ***c13***
Input following parameters:
***dres*** = x
***dmf*** = xxxxx
***dm*** = ‘nny’
***dmm*** = ‘ccp’
***dseq*** =’shaped pulse file name’
***dof*** = the ***tof*** of F19 spectrum
Type ***ga*** to acquire data.

**Step 5. Data Processing**
See steps in Experiment Two.

**Step 6. Exit And Logout**
See Experiment One.




$^{13}C$ spectrum of $(CF_3)_2CCFCF_2CF_3$ in Acetone_D6 was acquired on INOVA600 without $^{19}F$ decoupling.



Fully $^{19}F$ decoupled $^{13}C$ spectrum of $(CF_3)_2CCFCF_2CF_3$ acquired on INOVA600.

## Experiment 14: Variable Temperature Experiment (25 to 80 $^{o}$C)

Sample to be used: 10% Strychnine in $CDCl_3$

**Step 1. Login The Computer System**

See Experiment One

**Step 2. Change Sample**

See Experiment One

**Step 3. Lock And Shim The Magnet**

See Experiment One

Note: Double check your solvent boiling point or freezing point. If you need much higher temperature or much lower temperature experiments, please let us know. We could help you. You may have to order a high pressure Liquid Nitrogen tank for Low temperature Experiments. The temperature range we have done on the Unity plus -120 $^{o}$C – 120$^{o}$C with a Broad Band Probe.

**Step 4. Setup Parameters And Run Experiment**

4.1 type ***h1('solvent')*** to load standard proton parameters and run a normal 1D experiment.

4.2 For a single temperature:

type ***temp*** to open the temperature control box. Click on and drag the temperature bar to the desired temperature. Wait until the temperature reading on the remote display reaches the desired value, then for another 5 to 10 minutes to let the sample reach the equilibrium temperature. Shim the magnet again and type ***ga*** to start acquiring the data.

**Step 5. Data Processing**

See Experiment One.

**Step 6. Terminated the variable temperature function and exit**

Click and drag the temperature control bar to 25$^{o}$C. Wait until the temperature drops to around 25$^{o}$C, and click the temperature off button. Close the temperature control window. Exit and logout.

***Note: The operational temperature ranges are limited on INOVA600, INOVA400 and VNMRS400. The high temperature is set to 75$^{o}$C and low temperature is set to -10$^{o}$C. Beyond this range, you need to talk to Dr. Wu for help.***

# Experiment 15: NOESY

The procedure for NOESY experiment is quite similar to COSY experiment (see experiment 9)

**Step 1** Insert your sample and tune the probe on proton channel.

**Step 2** **Parameters Set-up and Run Experiment**

Run a proton 1D spectrum by using proton standard parameters (See **Experiment One**)
Using two cursors to define the ***sw*** (spectrum width) just wide enough to include all peaks and then type ***movesw*** and ***ga*** to run another proton experiment with new ***sw***. Save the spectrum.

**Step 3** Type ***NOESY*** to load NOESY parameters.

Check the parameters:
***pw***= 90$^o$ pulse width of proton channel.
***tpwr=*** transmitter power of the proton channel for 90$^o$ pulse width
***d1=2***
***nt=16, or 32***
***ni=128*** (or 256 or 512, depends on the machine time you have)
Type ***time*** to check how long the experiment it will take and adjust the ***nt*** and ***ni*** to fit your time signed up.
***mix*** (mixing time)=0.3 The value is ranging from 0.1 to 0.6(ms) depends on the molecular size of your sample. To obtain best result, it is better to do a set of experiments with different ***mix*** value and chose a best spectrum.
***fn1=2048; fn=2048 np=2048***
**Turn off spin, Double check the non-spin shims.**
Type ***ga*** to start acquisition.
After the acquisition, save the spectrum.

**Step 4** **Processing and Printing**

Reload your NOESY spectrum file by clicking on Main Menu and File , then highlight the file name and click on Load.
Type wft2da to do automatic weighting and Fourier transformation or follow the procedure in **Experiment 17** to process the data manually.

*For a good NOESY spectrum, you should deoxygenate your sample.*

## Experiment 16: TOCSY

Standard Sample to be used: 10% Strychnine in $CDCl_3$

**Step 1. Login The Computer System**

See Experiment One.

**Step 2. Change Sample**

See Experiment One.

**Step 3. Lock And Shim The Magnet**

See Experiment One.

**Step 4. Setup Parameters and Run Experiment**

4.1 type ***h1*** to load standard proton experiment parameters.

4.2 Set ***nt=1***. Type ***ga*** to begin acquisition for a common 1D-proton spectrum.

4.3 After acquisition, type ***aph*** for automatic phasing and /or manual phasing and then set the sw if needed.

4.4 Type ***tocsy*** to load standard tocsy parameters. Double check following parameters:

***P1*** *---- 90 degree of proton at the power of p1lvl (I600, p1level=58, p1=8.1)*
***P1lvl*** *---- Power level for p1 pulse (hard pulse 90 degree)*
***pw***----- spin lock 90 degree pulse (50us at tpwr=47 on INOVA600)
***tpwr-----*** Spin lock power level for pw.
***sw=sw1 ---- It is home nuclear, so both scales are the same.***
***Np=2048***
***Ss=8 dummy scans***
***Ni = 256 or more***
***Mix =0.3 – 0.7 seconds ----- Spin lock time***
***Trim=0.003 seconds ----- trim pulse***
***Hs='nn'***
***Fn=fn1=2048***
***d1***-----the pulse delay. It is set to around 1 second or longer.
***nt***------scan numbers depends on sample concentration (minimum 16 scans).
Turn spin off

4.5 Type ***ga*** to acquire data. After experiment complete, save the data.

**Step 5. Data Processing**

When the acquisition is finished, use the same way of COSY to process the data.

**Step 6. Exit and Logout** See **Experiment One**

# Experiment 17: HMQC/HMBC

HMQC experiment only available on INOVA600 since the default probe is an Inverse Detection probe. If it is the first time you try to do this experiment, please ask Dr. Wang or Dr. Wu to assist you to set it up to avoid hardware damage. You may have to reconfigure the hardware for proper setup.

**Step 1**: Sign-up your time (at least 2 hours depending on your sample concentration) and contact the NMR staff so that we can help you to tune the probes and connect a microwave filter etc.

**Step 2**: Run 1D proton spectrum in experiment 1. Using two red cursor to select a reasonable spectrum width (cutting off no-signal area in both high field and low field) and type **movesw**. Type **ga** to run another proton spectrum with new spectrum width. Write down the parameters **tof** and **sw**, This is for your Proton region with the correct chemical shift reference.

**Step 3**: Change to experiment 2 by typing **jexp2** to run 1D carbon spectrum in experiment 2. Select reasonable spectrum width and run a carbon experiment again as same as in step 2. You don't have to see all the carbon signals, the solvent peak is enough. You only need to collect one scan. Make sure the solvent peak is set to the correct chemical shift, as well as the region of the spectrum that covers all your protonated carbons. Remember, HMQC will not show the carbons without proton attached. Write down the parameters **tof** and **sw**. (remember you will only observe the protonated carbon for HMQC). This is for your Carbon region with the correct chemical shift reference.

**Step 4**. Return to experiment 1 (typing **jexp1**), and type **hmq**c. If presaturation is required, type **HMQC**. The computer will load the pulse sequence with default parameters. The **sw** will be the same as that in the proton spectrum. Manually change the **sw1** to the numbers of **sw** written down from the carbon experiment in step 3 and change **dof** to the number of **tof** in carbon spectrum.
Check the two pairs of 90$^{o}$ pulse parameters from the back of the log book:
**pw** is the proton 90$^{o}$ pulse width and **tpwr** is the power.
**pwx** is the **carbon decoupler** 90$^{o}$ pulse width and **pwxlvl** is the power.

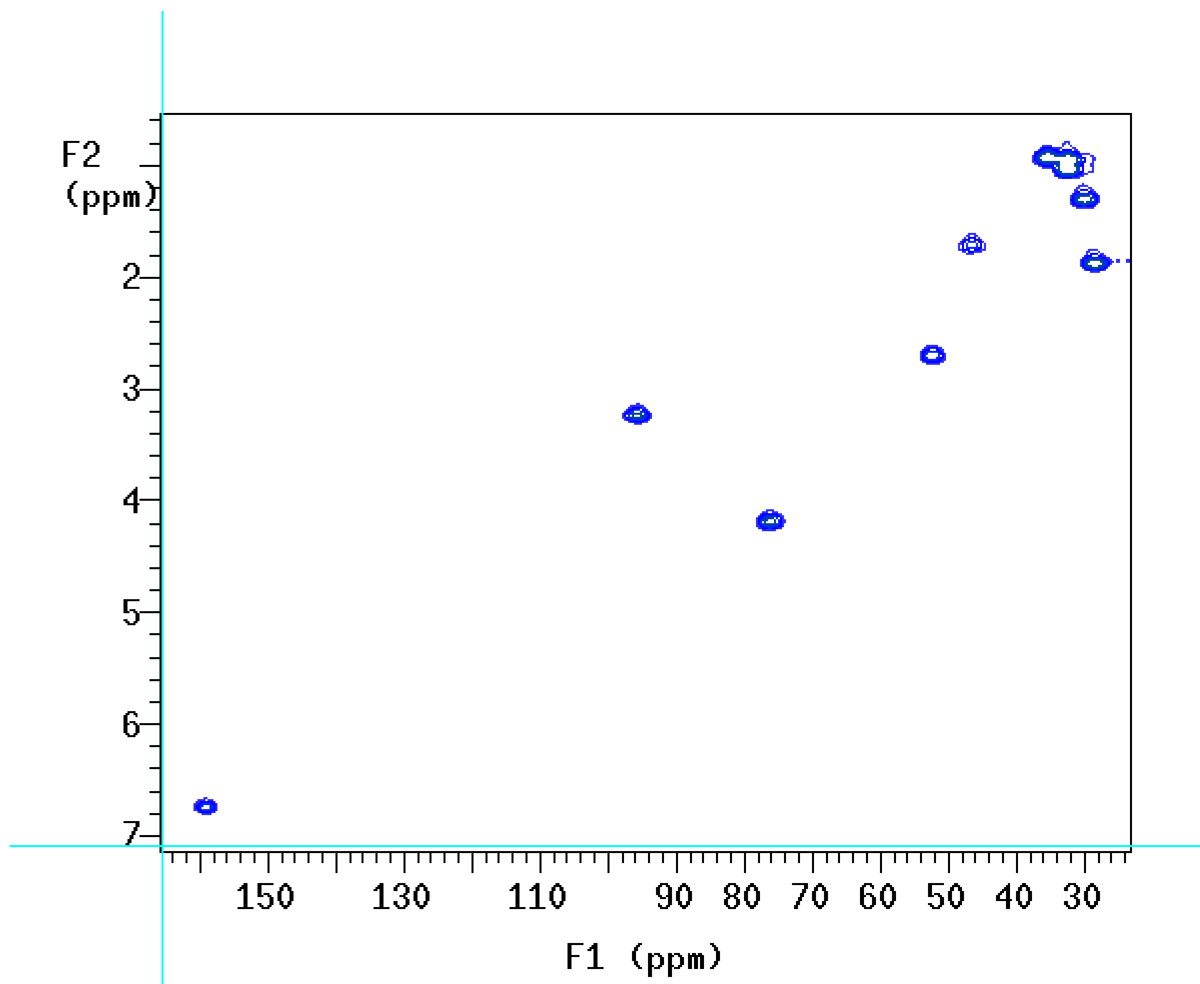
```
  ACQUISITION            SAMPLE          sw1        27285.1     2D PROCESSING
sfrq      599.737  date    Jun 16 2004   ni             256   lb1        210.50
tn             H1  solvent       cdcl3   phase      arrayed   sb1         0.002
at          0.236  file /export/home/~       PROCESSING       sbs1       -0.002
np           2048  vnmr1/vnmrsys/ID_p~   lb           59.24   gf1      not used
sw         4341.2  robe/HMQC_yu_good.~   sb           0.035   awc1     not used
bs       not used                  fid   sbs         -0.004   wtfile1
ss              4      DECOUPLING        gf        not used   fn1          2048
tpwr           56  dn              C13   awc       not used          FLAGS
pw            9.7  dof           105.7   wtfile               il              n
d1          0.900  dm              nny   proc            ft   dp              y
d2              0  dmm             ccg   fn            2048   hs             yy
tof        -591.5  dmf           20000   math             f   mbond           n
nt             16  dpwr             45         PRESAT                SPECIAL
ct              0  pwx            12.0   satflg          nn   temp     not used
                   pwxlvl           58   satdly           0   null          0.3
                   homo              n   satpwr           0   j           140.0
                     2D ACQUISITION      satfrq           0   taumb           0

Acq & Obs | Decouplers | Sequence | Flags & Cond. | Process | Process2 | Display | display2 | LCNMR/STARS | Text | Spare
```

Type **time** to check the total experiment time.

Finally type **ga** to start the experiment. After finish the experiment, save the data and report to the staff to help you reconnect the cable.

Using the same procedure for HECTOR to process the HMQC data.

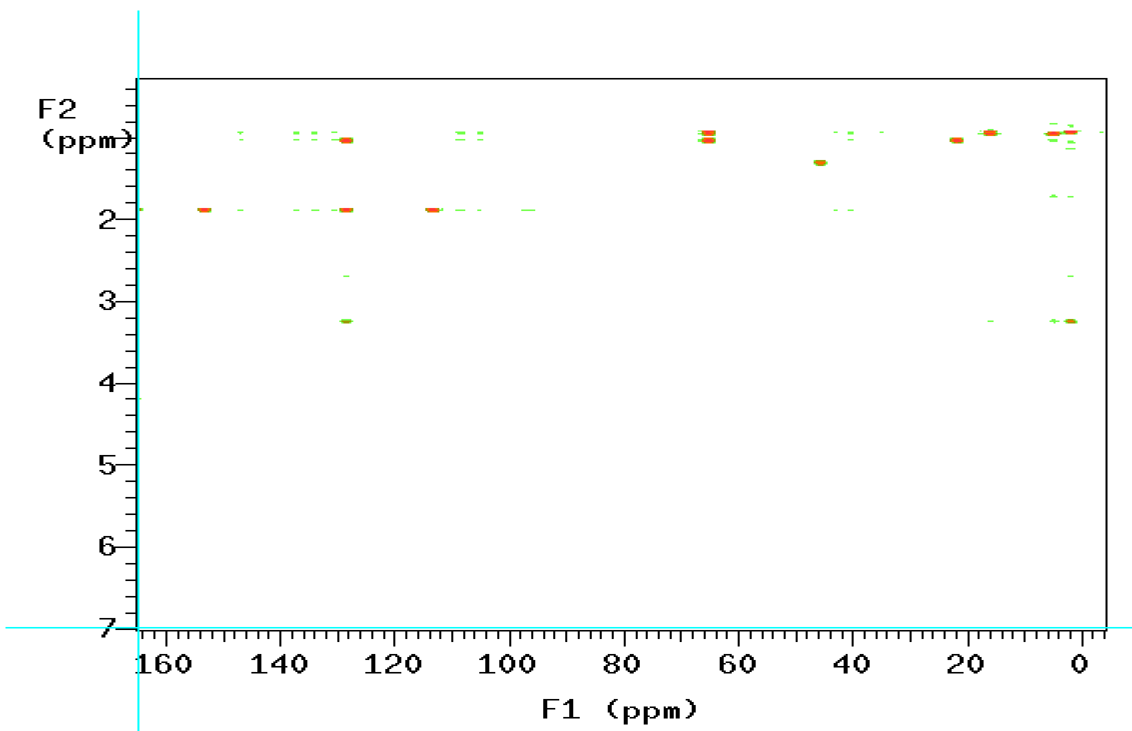



**HMBC** experiment is the same kind of experiment, the difference is you have to input the parameter of jnxh, that is the j coupling of three bond from proton to the carbon you are interested. The average number is 5 – 8 hz. Also you should double or triple of the nt scans, since HMBC is much less sensitive than the HMQC experiment. You may need little more samples.

## HMBC parameters:

| ACQUISITION | | TRANSMITTER | | HMBC | | PROCESSING | |
|---|---|---|---|---|---|---|---|
| sw | 4490.3 | tn | H1 | j1xh | 137.0 | sb | 0.077 |
| at | 0.228 | sfrq | 599.737 | jnxh | 5.0 | sbs | not used |
| np | 2048 | tof | -591.5 | GRADIENTS | | fn | 2048 |
| bs | 16 | tpwr | 56 | PFGflg | n | 2D PROCESSING | |
| ss | 8 | pw | 9.700 | hsglvl | 2000 | sb1 | 0.004 |
| d1 | 1.000 | DECOUPLER | | hsgt | 0.002000 | sbs1 | not used |
| nt | 32 | dn | C13 | SPECIAL | | fn1 | 2048 |
| ct | 0 | dof | 128.0 | temp | not used | SAMPLE | |
| 2D ACQUISITION | | pwxlvl | 61 | spin | not used | date | Jun 17 2004 |
| sw1 | 27155.5 | pwx | 12.000 | gain | 2 | solvent | cdcl3 |
| ni | 512 | dm | nnn | pw90 | 8.000 | sample | undefined |
| phase | arrayed | dmm | ccc | sspul | n | | |
| PRESATURATION | | dmf | 200 | | | | |
| satmode | n | dpwr | 0 | | | | |
| satfrq | 0 | | | | | | |
| satdly | 0 | | | | | | |
| satpwr | 0 | | | | | | |

Acq & Obs
Decouplers
Sequence
Flags & Cond.
Process
Process2
Display
display2
LCNMR/STARS
Text
Spare



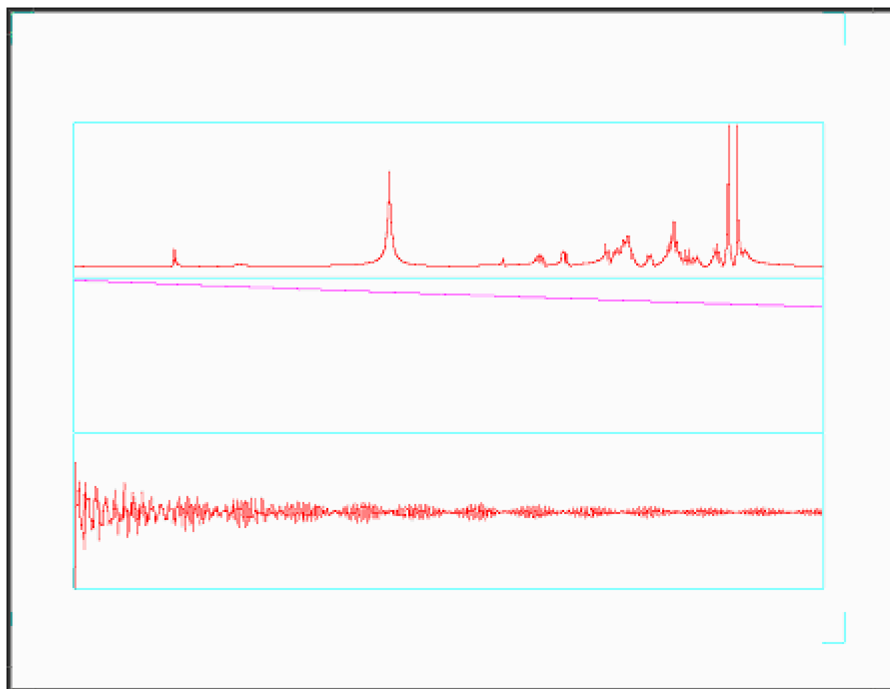

*HMBC spectrum of strychnine in CDCl3*

# Experiment 18: 2D Data Processing

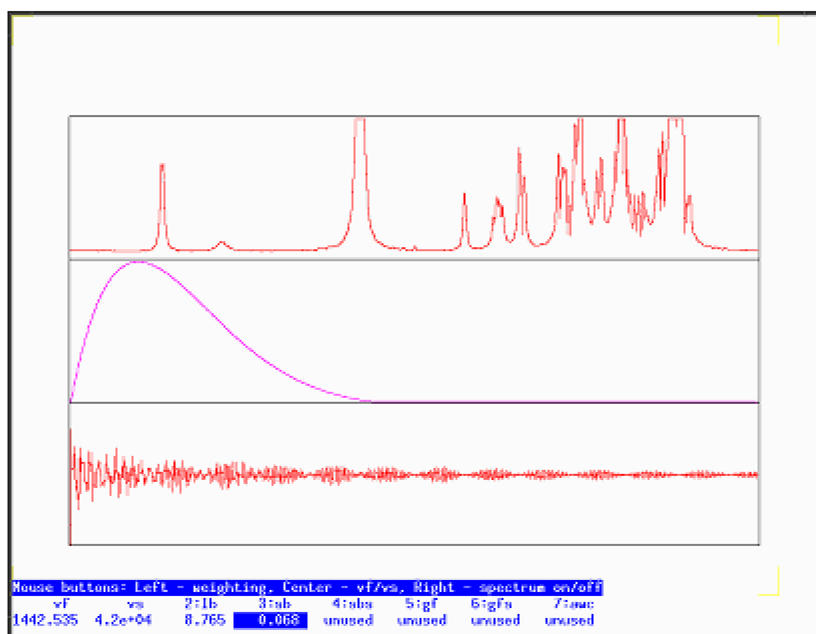
**Step 1.** Load the data

From the menu: **Main → File →** select the file you saved, then click **Load**.

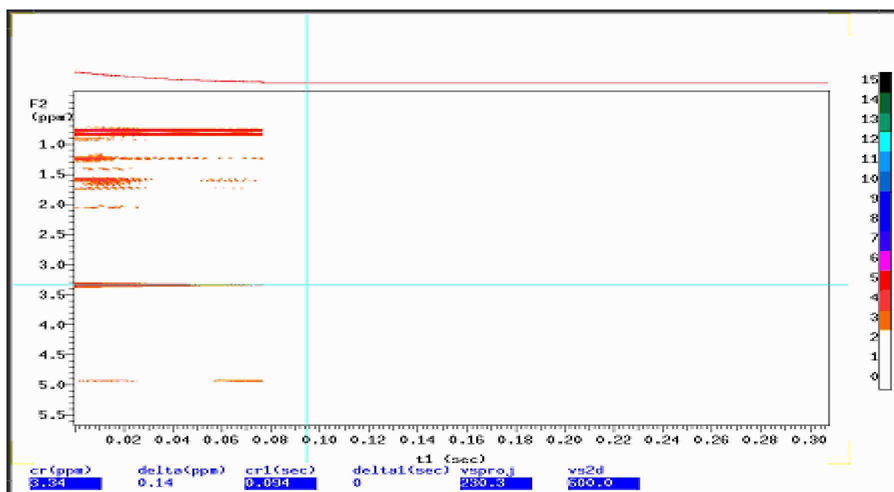
**Step 2:** Processing F2 dimension
From the menu: **Main → Process → Adj Weighting**
The following window will be displayed:



The top window is the spectrum that is the result after applying the window function and FT; the middle window is for the weighting function adjustment; the bottom window is the FID after applying the window function. From the menu, you could use **lb** (line broadening), **sb** (Sinebell weighting function), **sbs** (Sinebell squared weighting function), **gf** (Gaussian weighting function) and **gfs** Gaussian squared weighting function) functions by clicking the icon. Then move the mouse to the middle window to adjust the window function. The purpose of this operation is to make the peak shape as following:



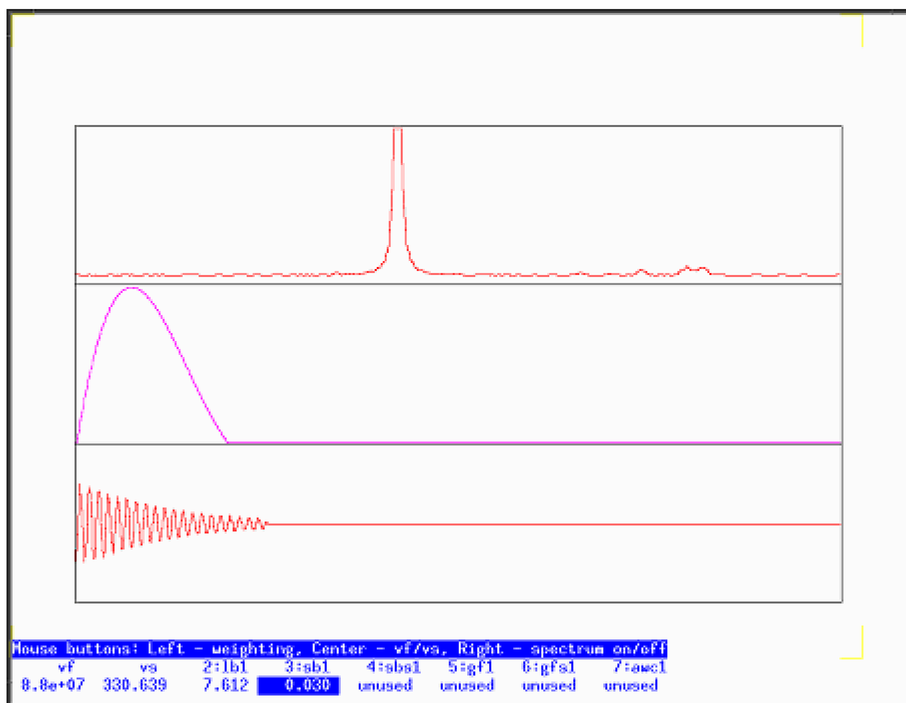

You could combine these window functions to change the shape of the peaks until you are satisfied with the spectrum. Then click **return**. If you acquired a phase sensitive 2D spectrum, there will be an icon "**phase F2**" on the menu, then you have to click **phase F2**. A 1D spectrum will be displayed. Phase it. If there is no "phase F2" icon, you acquired data is magnitude mode. You could click **Transform F2**. Computer will process the F2 dimension data. After it completes, a new window will be displayed.



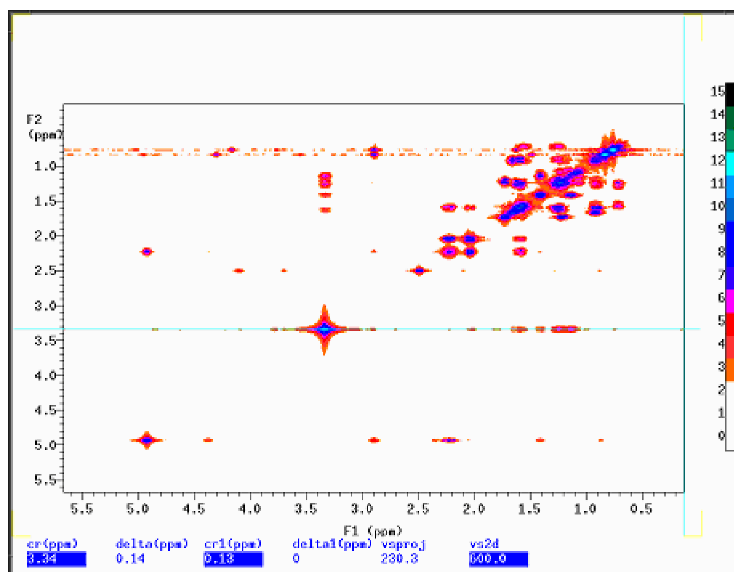

If there is nothing in the window, change **vs2d** parameter, by typing vs2d=5000 or bigger. This will increase the display intensity level. Then click **Redraw.** The red lines are your data, and white area is zero filling.

**Step 3.** Process the F1 dimension:

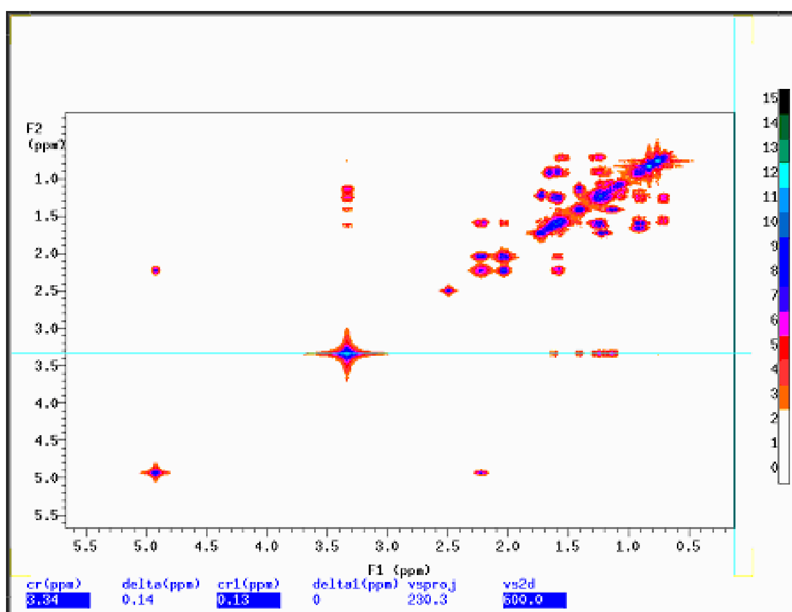
There will be red line in the middle of the spectrum, use mouse to move the line on a blue line, then click **Trace**. Then click **return** → **Adj Weighting**, A window will be displayed. Adjust the function as before.




Then click **return** → **F1 Transform**. The computer will process the F1 dimension. The 2D spectrum will be displayed on the screen after few seconds. If it is a COSY spectrum, you could type **foldt** to get rid of the unsymmetrical noise. Don't use **foldt** on NOESY!



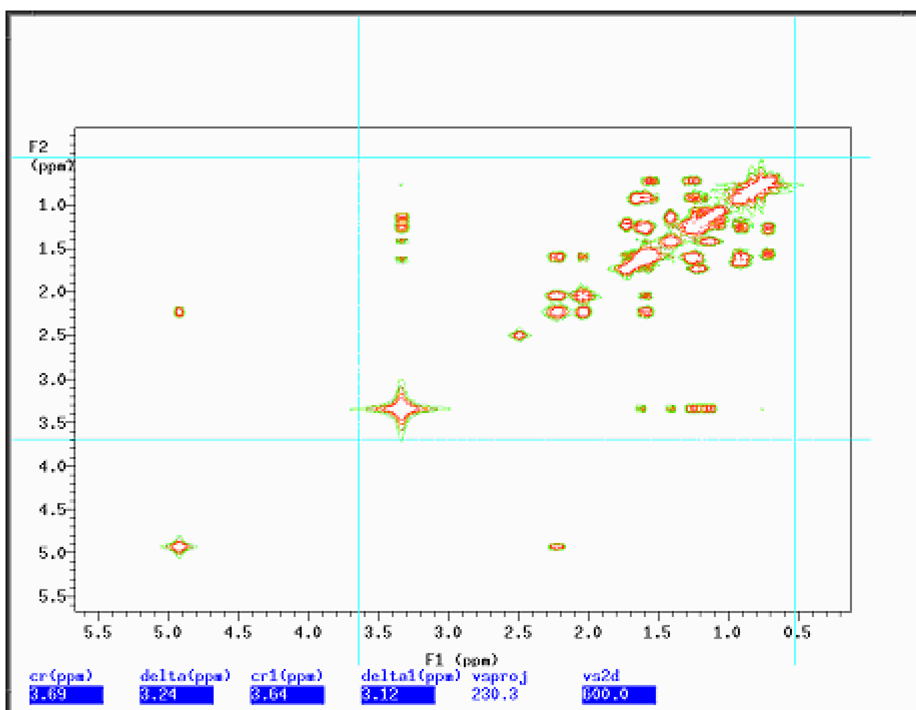
COSY spectrum before **foldt** operation



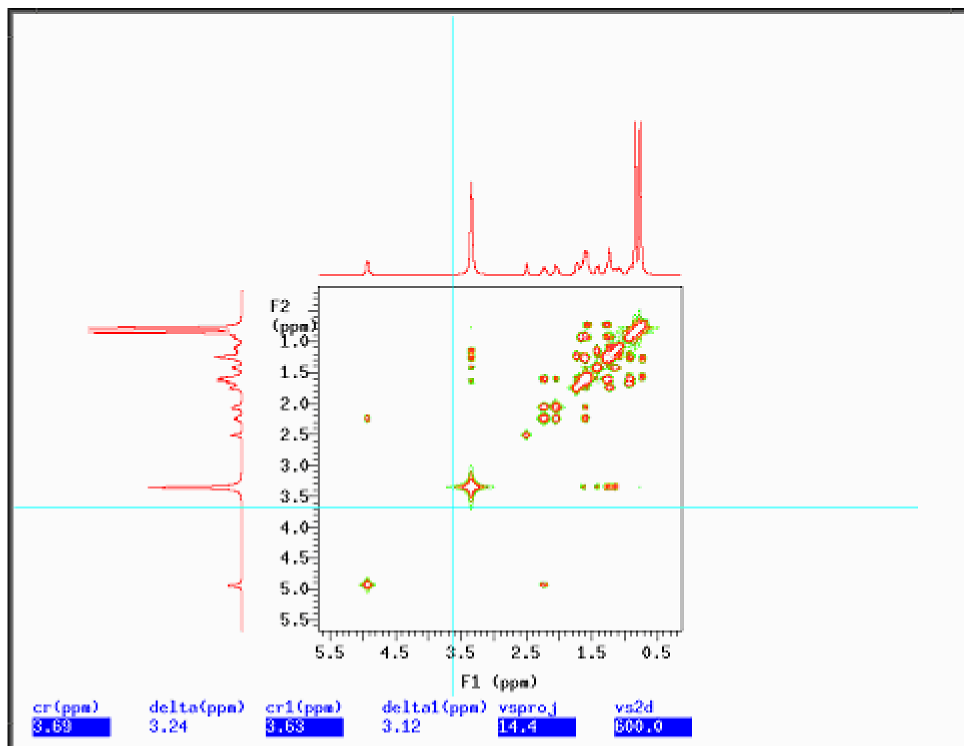

COSY spectrum after **foldt** operation

**Step 4**. Display and Printing:

On the menu, click **return→contour**, to display the spectrum as contour mode.
Click **return → size**, to change the window size.



Click **project→Hproj(max)** to display 1D project spectrum. Click **Vproj(max)** to display 1D project spectrum on the side. You want to plot them with the 2D spectrum, you have to click plot after you click **Hproj(max) or Vproj(max).**




To add text by typing text('your_text'). Tp print the text by typing pltext.
To print the spectrum, type **pcon(18,1.4) page**. The number 18 is the level, the 1.4 is spacing. If you have questions, please feel free to ask our service instructors.

## Experiment 19: Variable Temperature Experiment (25 to 80 $^{o}$C)

Sample to be used: 10% Strychnine in $CDCl_3$

***Note: Double check your solvent boiling point or freezing point. If you need much higher temperature or much lower temperature experiments, please let us know. We could help you.***

**Step 1. Login The Computer System**

See Experiment One

**Step 2. Change Sample**

See Experiment One

**Step 3. Lock And Shim The Magnet**

See Experiment One

**Step 4. Setup Parameters And Run Experiment**

4.1 type ***h1('solvent')*** to load standard proton parameters and run a normal 1D experiment.

4.3 For a single temperature:

type ***temp*** to open the temperature control box. Click on and drag the temperature bar to the desired temperature. Wait until the temperature reading on the remote display reaches the desired value, then for another 5 to 10 minutes to let the sample reach the equilibrium temperature. Shim the magnet again and type ***ga*** to start acquiring the data.

**Step 5. Data Processing**

See Experiment One.

**Step 6. Terminated the variable temperature function and exit**

6.1 Click and drag the temperature control bar to 25$^{o}$C. Wait until the temperature drops to around 25$^{o}$C, and click the temperature off button. Close the temperature control window. Exit and logout.

# Experiment 20: $^{19}$F $^{31}$P and Other Heteronuclear NMR: 1D Fluorine 19 Basic Operations

You must be an authorized user to run $^{19}$F experiment. Probe tune is not required on INOVA400. However, on other NMR systems, you have to tune the probe or exchange probe for $^{19}$F experiment.

Login and locking and shimming of your sample as usual.

Type ***f19*** to call up the standard fluorine experiment parameters with CDCl3 solvent.
Enter ***nt=64*** or other nt number according to the nature of your sample.
Type ***ga*** to begin acquisition. After acquisition is done, a fluorine spectrum will be displayed. If not, enter ***wft***. Type ***aph*** for automatic phasing. Other processing procedures are same as proton experiment.

Note: the standard fluorine 19 experiment parameters sets up a window width of 90 ppm and the chemical shifts shown on your spectrum have no meaning if you do not do referencing.
The common reference compounds for $^{19}$F experiment are $C_6F_6$, $CCl_3F$ and $CF_3OOH$. They can be used for both internal and external reference. The simple way to make an external reference is as follows:

> Dissolve small amount of a reference compound in same deuteriated solvent as your sample in a NMR tube. Run a quick $^{19}$F experiment with this solution after locking and shimming. Set reference compound peak to the correct chemical shift number. Then immediately run a $^{19}$F experiment with your sample without change any parameter except ***nt***. The chemical shift of resulting spectrum will not change much from the first experiment.
>
> To run a more accurate experiment with an external reference, you need to buy a special capillary tube called 'Stem Coaxial Insert' from WilMad (product No. WGS-5BL for 5 mm NMR tube), which allows you to put the solution of reference compound into the capillary then insert it into your sample tube. In this way, you run sample with the reference but without contamination by the reference compound and your reference insert can be used again and again.

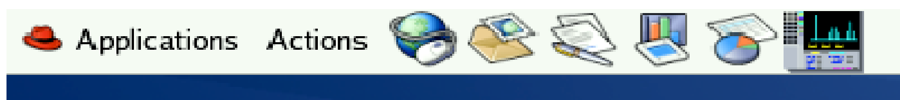
# Experiment 21: VNMRJ $^1$H Operating Instructions

***Note: All the commands you have learn from the VNMR (INOVA600, INOVA400) works on the VNMRJ.***

## *Login*

Login to the system by entering your group's user name and password.

## *Starting vnmrj*

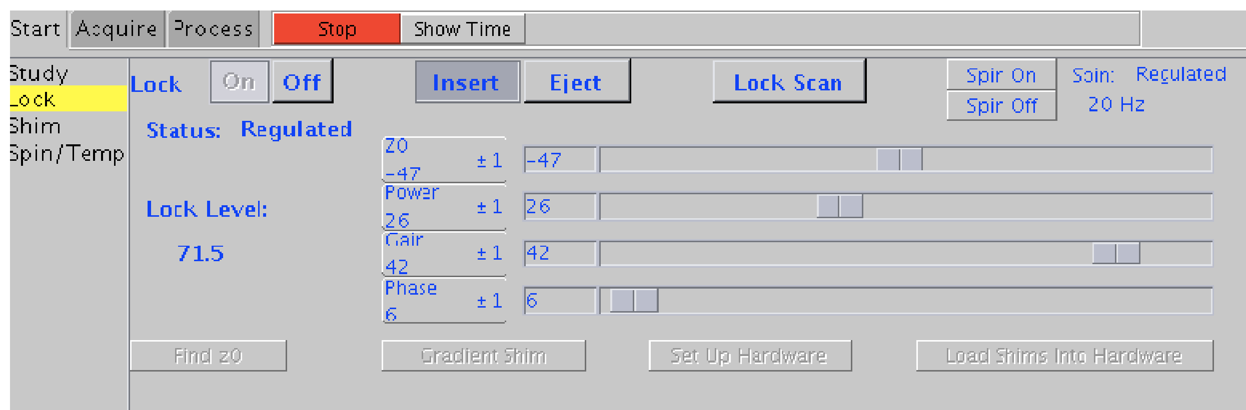



Single click this icon to start VNMRJ software.

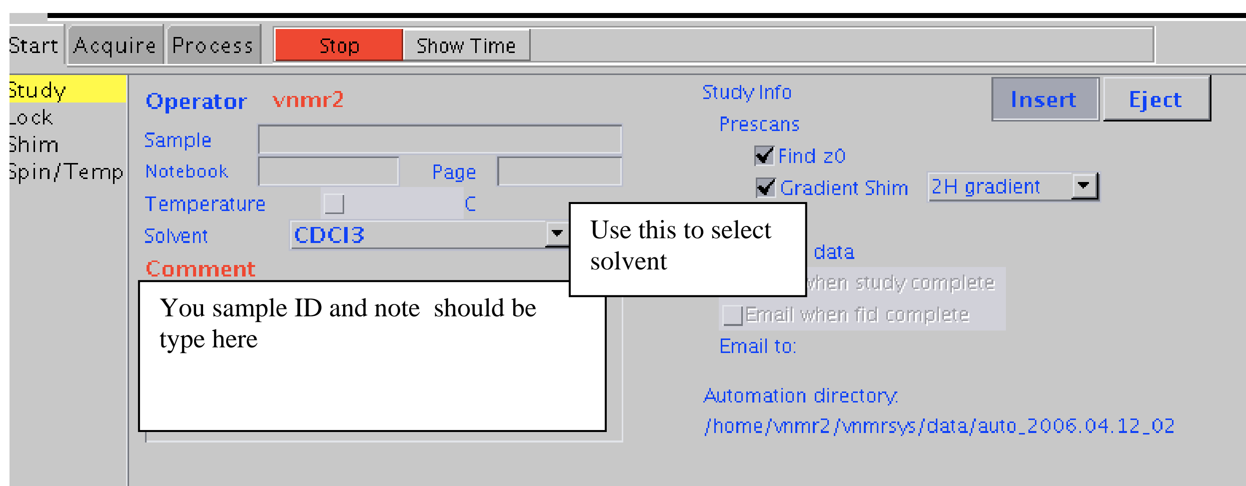
1. Start vnmrj by **single** clicking the vnmrj spectrum icon at the top of the screen.
2. Choose to run a $^1$H experiment by dragging the proton icon on the left of the screen to the black area or type h1('solvent'). The default solvent is CDCl3.

## *Inserting your sample*

1. Click **spin off**, and Click **lock off** and then Click ***eject*** to remove the standard from the magnet.

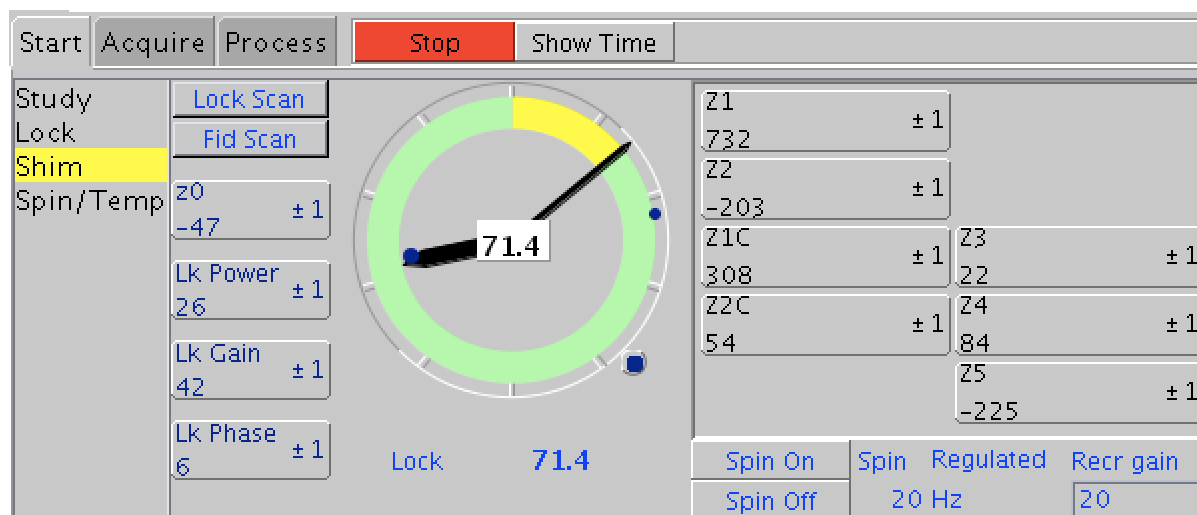



2. Replace the standard with your sample and click on ***insert***. After the sample is proper inserted, then click **spin on**. The spin rate is displayed at the bottom.
3. If you wish to use a solvent other than CDCl3, select which solvent you would like to use with the drag-down box on the following page. Click the **Study tab**.
4. If you would like to print a sample name/ID on your spectrum, insert this text into the comment box (The text will print later by the command **pap**).




### *Shimming and locking your sample*

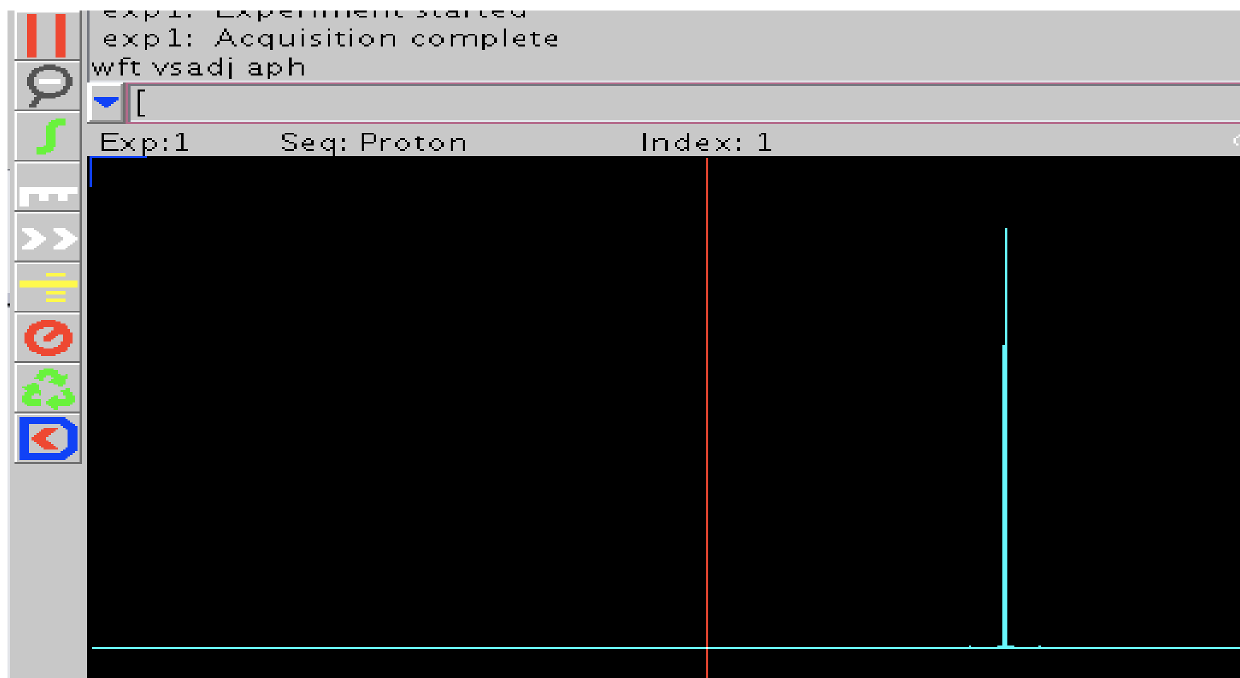
1. If you are using a solvent other than CDCl3, click on the lock tab and change Z0, and then Power, Gain, and Phase as necessary to lock your sample. The values can be adjusted with the left and right mouse buttons as in the case with the other instruments, or by typing in the desired value and pressing enter.
2. Shim your sample by first clicking on the shim tab, then by adjusting the Z1 Z2 and Z3 values. Use command "loadshim" to reload standard shim file for the standard sample.




### *Acquiring your $^{1}$H spectrum*

1. Check the current acquisition parameters by clicking on the process tag, then by typing **dg** in the input box above the black box.
2. To change any parameters, click the acquire tag and make adjustments as necessary by typing in the input box. Use the format **"parameter = value"** to make changes.

3. Alternatively, you may use the default parameters and simply change the number of scans by typing in the command input box (i.e., type **nt = 16** for 16 scans).
4. Start the experiment by typing **ga**.




## *Processing your spectrum*

1. After acquisition is complete, the spectrum should be displayed on the screen.
2. Apply the same commands as used on the other instruments to process the spectrum (i.e., type **wft vsadj aph** in the input box for Fourier Transform, vertical scaling of peaks, and autophasing, respectively).
3. ***Displaying the scale***: Note the set of processing icons to the left of the spectrum. Display the scale by clicking on the white ruler icon.
4. ***Expanding the spectrum***: To expand the spectrum, select the top icon containing 2 red lines. Place the cursors where desired, then expand the region by choosing the zoom in/out (magnifying glass) icon. Use this icon to expand the spectrum and also to return to normal size.
5. ***Setting the solvent reference peak***: As in the case with the other instruments, place the red cursor on your solvent peak, then type **rl(x.xxp)** in the input box to place the cursor directly on the peak and set the reference value (i.e., **rl(7.27p)** for $CDCl_3$).
6. ***Integration***: Click on the green integral icon, then take note of the two additional integration icons that appear just below it. Click the middle integration icon and use the mouse to cut the line, then return to the spectrum by typing **ds** in the input box. If necessary, you can also click on the top integral button or type **cz** to display the full integral.
7. ***Seting a peak integral reference value***: To set a specific peak integration value, click on the process tab, then the cursors/integration sub tab. Place the cursor on the desired

peak and type in the desired number of protons in the *normalization value* box. Save changes by clicking on the *set integral value* icon.

8. ***Set a precise spectrum width***: To set a specific numerical range for your spectrum, use the same commands as used previously on other instruments (i.e., typing **cr = 9.5p delta = 10p** then clicking the expand icon will set the window from -0.5 to 9.5 ppm).
9. ***Setting the peak threshold***: To choose the level of peaks for peak picking, press the icon labeled by the yellow threshold line and adjust the line accordingly.
10. ***Plotting and printing the spectrum*** To plot and print, type any combination of the following commands into the input box. Note that these are the same commands as the ones used for other instruments, but are listed here as a reminder.

pl : print spectrum
pir: print integrals
pscale: print scale
pltext: print text label (from the comment box)
pap: print acquisition parameters
pll: print peak locations (ppm and Hertz) and peak height
ppf: print peak frequencies above each peak
page: eject page from the printer

## *Saving the data*

1. If you do not yet have a directory in your group's folder, make a new directory by typing the command **mkdir('directory_name')**. Alternately, you can open your group's folder (top left of the desktop) and create a new directory by selecting *File Create folder*. Command "pwd" to check the current directory.
2. To save a file in an existing directory, first choose the directory by typing **cd('your_directory')**. Next, save the file by typing **svf('file_name')**.
3. To retrieve your data later, find your file in your group's folder on the desktop and drag it to the black processing window. Process as described previously.

## *Finishing the experiment*

1. Eject your sample and replace with the standard sample.
2. If you used a solvent other than CDCl3, adjust the lock parameters back to the required ones listed under the monitor.
3. Click on the *loadshim* icon at the top of the page, or type "loadshim" to reload the standard shim file.
4. Manually shim the sample to reach the required lock level.
5. Exit the system by choosing *File Exit vnmrj* or by typing **exit** into the input box.
6. Logout by choosing *Actions logout*, then click *OK*.

# VNMRJ $^{13}$C Operating Instructions

*Follow the VNMRJ $^{1}$H instructions to login to vnmrj and insert, shim, and lock your sample. Remember all the commands on the inova400 could be used on the VNMRS.*

### *Acquiring your $^{13}$C spectrum*

1. Choose to run a $^{13}$C experiment by dragging the carbon icon on the left of the screen to the black area or type **c13**
2. Check the current acquisition parameters by clicking on the process tag, then by typing **dg** in the input box above the black box.
3. To change any parameters, click the acquire tag and make adjustments as necessary by typing in the input box. Use the format **"parameter = value"** to make changes.
4. Alternatively, you may use the default parameters and simply change the number of scans by typing in the command input box (i.e., type **nt = 16** for 16 scans).
5. After typing in the number of scans, type **time** to find out how long the experiment will take. If you run an excess number of scans and would like to stop early, type **aa** to abort the experiment.
6. Start the experiment by typing **ga**.

### *Processing your spectrum*

1. Apply the same commands as used on the other instruments to process the spectrum (i.e., type **wft aph** in the input box for Fourier Transform and autophasing, respectively).
2. ***Displaying the scale***: As in the case for a $^{1}$H experiment, note the set of processing icons to the left of the spectrum. Display the scale by clicking on the white ruler icon.
3. ***Expanding the spectrum***: To expand the spectrum, select the top icon containing 2 red lines. Place the cursors where desired, then expand the region by choosing the zoom in/out (magnifying glass) icon. Use this icon to expand the spectrum and also to return to normal size.
4. ***Setting the solvent reference peak***: As in the case with the other instruments, place the red cursor on your solvent peak, then type **rl(xx.xxp)** in the input box to place the cursor directly on the peak and set the reference value (i.e., **rl(77.23p)** for $CDCl_3$).

*Follow the VNMRJ $^{1}$H instructions to plot and print the spectrum, save the data, and replace your sample with the standard sample.* **Before exiting the system, make sure to drag the proton icon to the black window then type su or type h1 to turn of the decoupler.**

# VNMRJ APT Operating Instructions

### *Preparing for the APT experiment*

1. Use the VNMR 1H and 13C instructions to shim and lock your sample. Change solvent settings as necessary. Drag the carbon icon to the black window and acquire a carbon spectrum using standard parameters.

2. Type **wft aph** to process the spectrum.

3. Use the toolbar to place the red cursors just to the left and right of the carbon peaks. Type **movesw** to select this as the new range for the experiment. Remember all carbons, include solvent carbons, will show up on the APT spectrum.

4. Click on the Process and Text Output tabs. Type **dg** to load the updated parameters. Record the values for sw and tof for use later in the experiment.

### *Acquiring the APT spectrum*

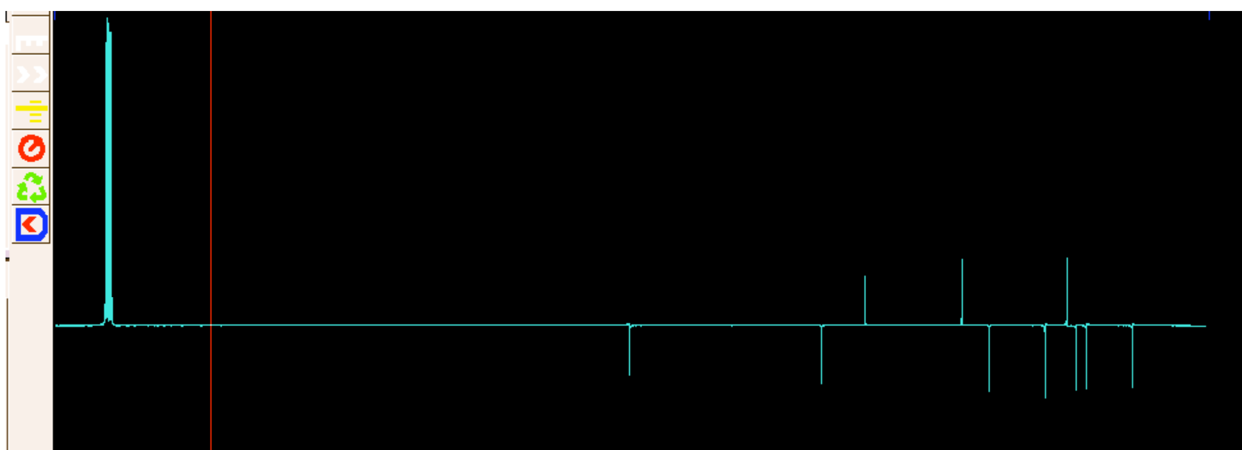
1. Drag the *APT* icon from the left of the screen to the black window (this will return the experiment to default parameters).

2. Using the values recorded previously in step 4, enter the new parameters for sw and tof by typing **sw = x** and **tof = x.** Type **dg** and verify that the parameters are correct.

3. Enter the number of scans by typing **nt = x**, then type **ga** to start the experiment.

4. After the experiment is finished, type **aptaph** to automatically phase the peaks. If the phasing is still off, you can adjust manually by choosing the red circle icon on the toolbar and then adjusting with the left and right mouse buttons.

5. Set the solvent reference peak by typing **rl(x.xxp)**.



APT spectrum of Menthol in CDCl3.  UP: CH2 and Cs and Down CH and CH3s.

6. Print the spectrum using standard processing commands.

### *Finishing the Experiment*

1. Remove your sample and replace with the standard sample, then turn on the spin and shim to the required lock level.

2. Drag the proton icon to the black window or type **h1** to load standard parameters.

3. Exit vnmrj and log out.

### Additional information:

These instructions are very generalized. If you cannot obtain an acceptable APT spectrum, please see Dr. Wu for assistance, as additional parameters may need to be adjusted or the probe needs to be re-tuned for your sample.

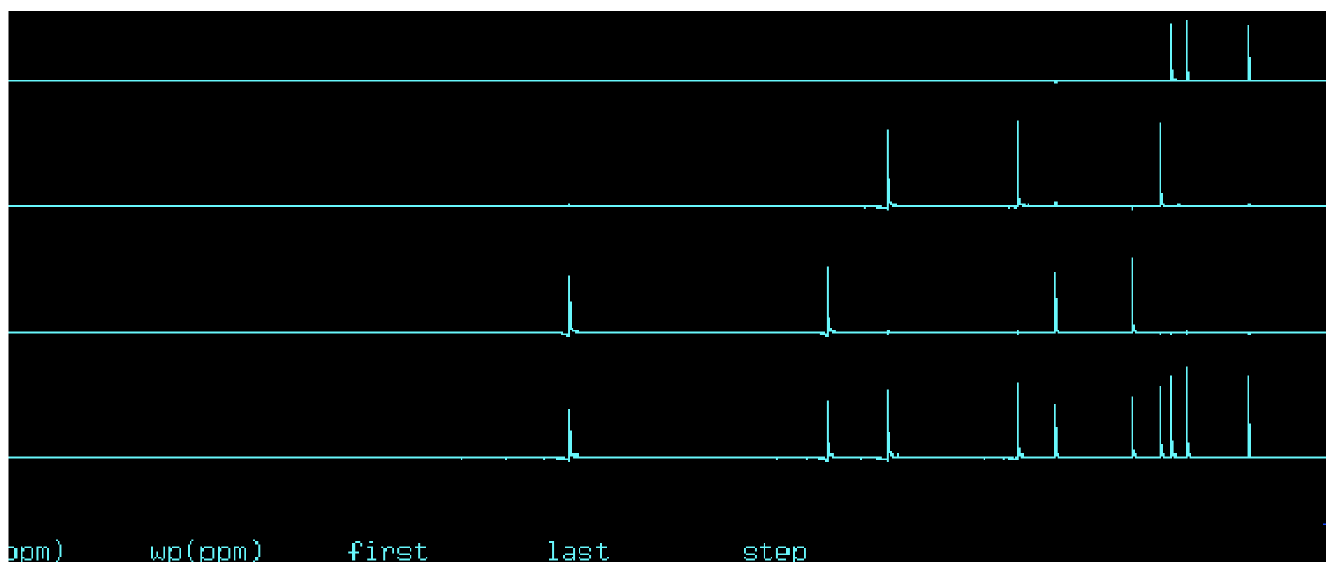
## VNMRJ DEPT Operating Instructions

### *Preparing for the DEPT experiment*

1. Use the VNMR 1H and 13C instructions to shim and lock your sample.
2. Change solvent settings as necessary. Drag the *carbon* icon to the black window and acquire a carbon spectrum using standard parameters.
3. Type **wft aph** to process the spectrum. Use the toolbar to place the red cursors just to the left and right of the carbon peaks. Type **movesw** to choose this as your range for the experiment.
4. Click on the Process and Text Output tabs. Type **dg** to load the updated parameters. Record the values for sw and tof for use later in the experiment.

## *Acquiring the DEPT spectrum*

1. Drag the *DEPT* icon from the left of the screen to the black window (this will return the experiment to default parameters).
2. Using the values recorded previously in step 4, enter the new parameters for sw and tof by typing **sw = x** and **tof = x**. Type **dg** to update the parameters.
3. Enter the number of scans by typing **nt = x**, then type **ga** to start the experiment.
4. After the experiment is finished, type **autodept** to automatically plot and print the DEPT spectrum.




## *Finishing the Experiment*

1. Remove your sample and replace with the standard sample, then turn on the spin and shim to the required lock level.
2. Drag the proton icon to the black window to load standard parameters.
3. Exit vnmrj and log out.

## Additional information:

These instructions are very generalized. If you cannot obtain an acceptable DEPT spectrum, please see Dr. Wu for assistance, as additional parameters may need to be adjusted.

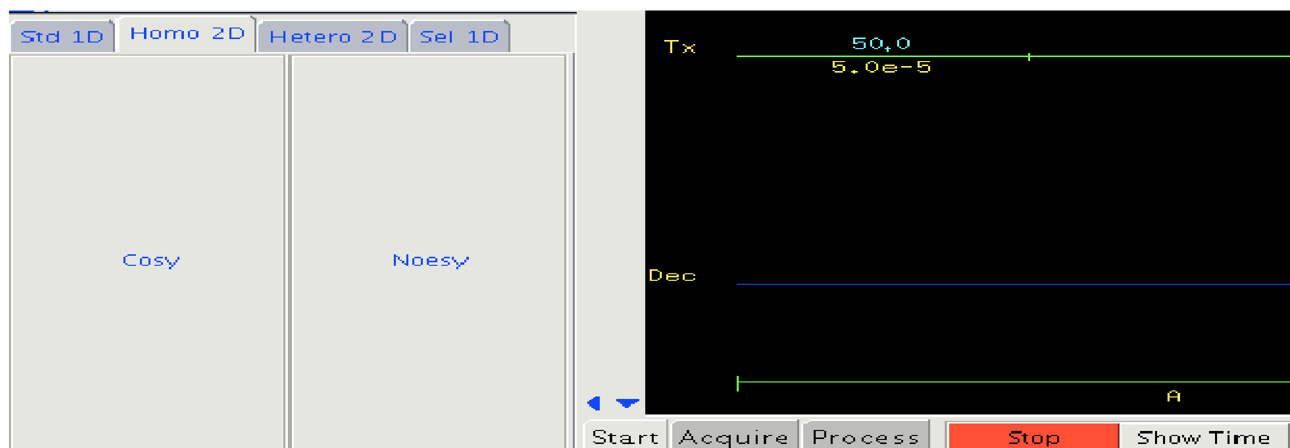
# VNMRJ COSY Operating Instructions

## *Preparing for the COSY experiment*

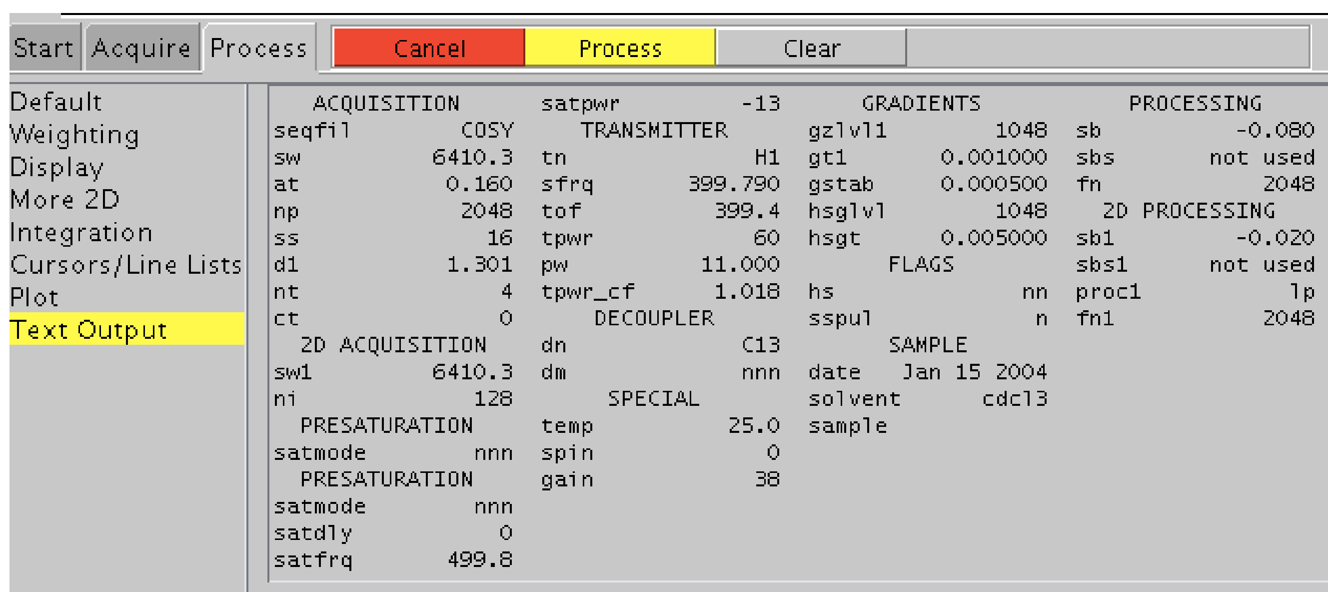
1. Use the VNMR $^{1}$H instructions to shim and lock your sample. Change solvent settings as necessary. Drag the proton icon to the black window and acquire the proton spectrum using standard parameters.
2. Process the $^{1}$H spectrum as listed in the instructions. Set the solvent reference peak, and save the file for later reference.
3. Type **gain?** in the command input box to determine the value to use for gain in the COSY experiment. For COSY or 2D experiments, you have set the gain, rather leave it as "autogain".
4. Use the $^{1}$H spectrum to determine the spectral width for the COSY. Do this by placing the red cursor on both sides of the spectrum and typing **movesw**.

## *Acquiring the COSY spectrum*

1. Click on the Homo 2D tab on the left of the screen.
2. Drag the COSY icon to the black window.




3. Type **dg** to view the current parameters. The parameters will be displayed after choosing the process tab (horizontal menu) and text output tab (vertical menu).
4. Change the gain value to the number used for the $^{1}$H experiment in the previous section. This can be done by typing **gain = x** in the command input box.
5. Change the number of scans by typing **nt = x** (where x = 16, 32, etc.). Note that the minimum is 4 scans, and a larger number of scans will lead to a better spectrum.
6. Change the number of FID's by typing **ni = x**. Typically the desired values for x are 128, 256, 512, etc. It is best to start with at least ni = 256, and a larger value is better.
7. Check the parameters again as explained in step 3. sw=sw1, np=1024 or 2048, fn=2048 fn1=2048,Type **time** to see the length of the experiment. Adjust nt and ni to fit your allotted time.



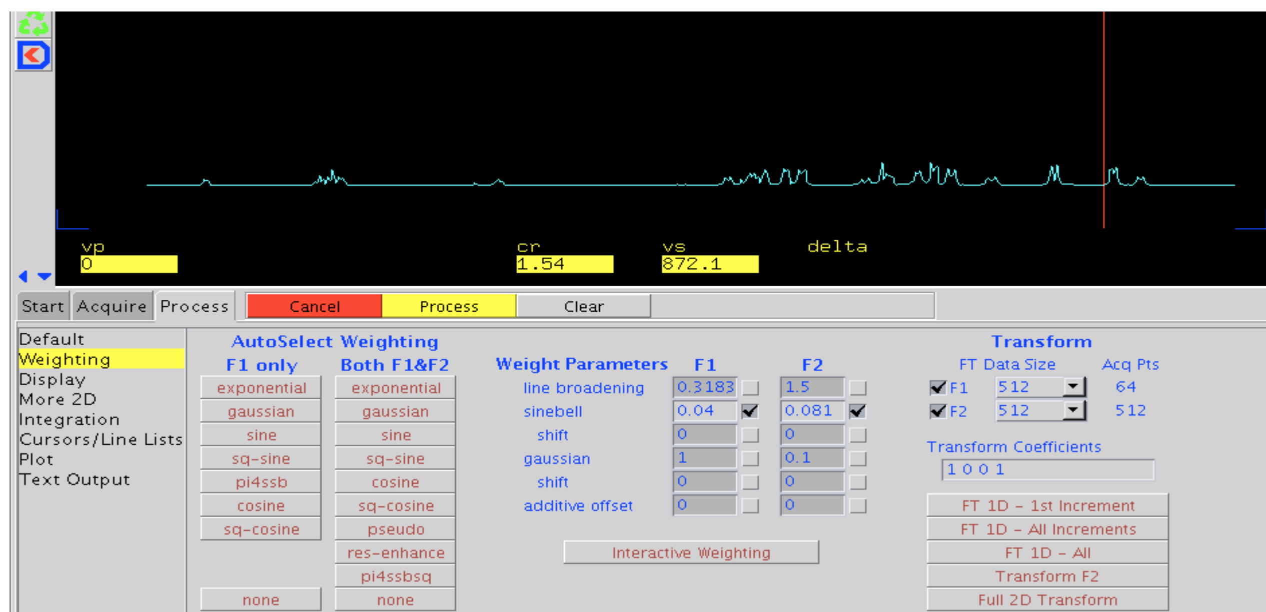

8. Turn off the spin (under the start and lock tabs), and type **ga** to begin the experiment.

### *Processing the spectrum*

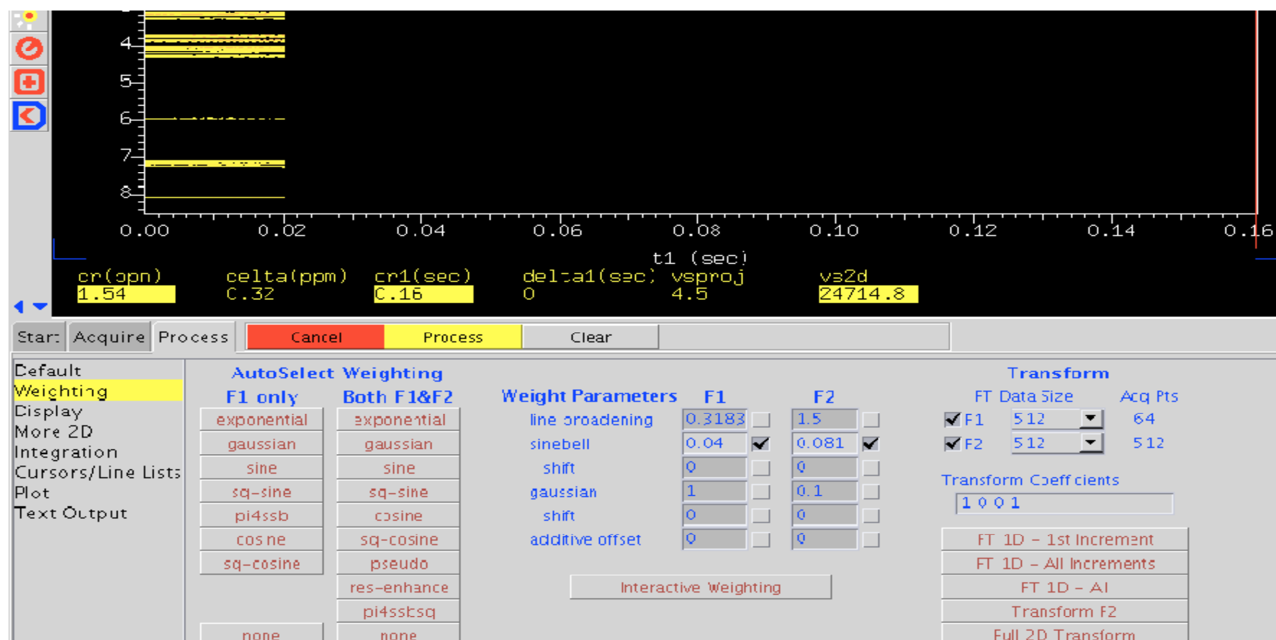
***1.*** If you would like to use the default processing/plotting method, you can print now by typing **pcon page** in the command input box. If you would like to manually process the spectrum, continue below.

2. ***Peak shape adjustment (F1 and F2):***

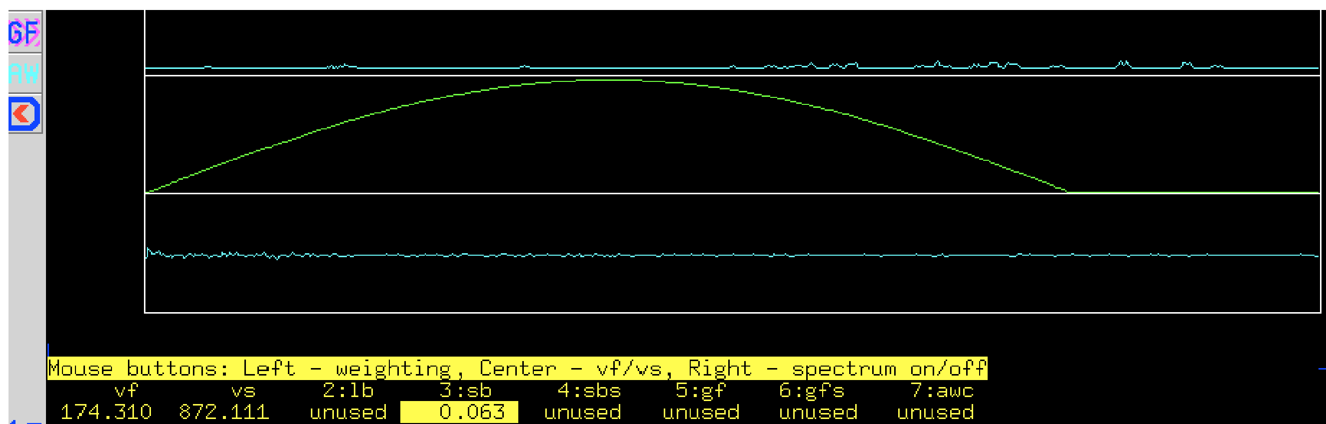
After selecting the process tab on the horizontal menu, choose the weighting tab on the vertical menu. To adjust F2, first click on *FT 1D - 1st increment*. If the full spectrum is not shown, type **full** followed by **f** to show the whole spectrum.



There are two options for adjusting the values for F2. You can simply input new values for F2 by entering numbers in the boxes under the weight parameters heading and F2 subheading, and then clicking the *Transform F2* icon.




Alternately, you can manually adjust F2 by clicking on *interactive weighting* and adjusting the Gaussian curve with the left mouse button until the desired peak shape is obtained. When adjustment is finished, click the *transform F2* icon.



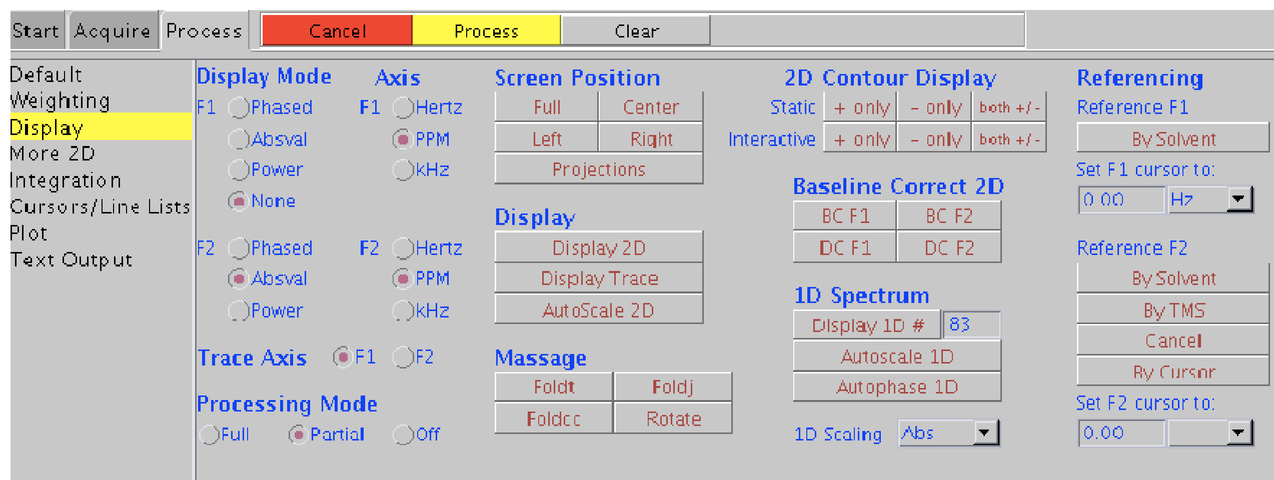

To adjust F1, choose any single line of the spectrum using the red cursor lines, then click on *interactive weighting* and manually adjust the curve as described in the last step. When adjustment is finished, click the *full 2D transform* icon.

3. ***Eliminating peak noise:*** To remove excess unsymmetrical noise, type **foldt** in the command input box.

4. ***Setting the solvent reference peak:*** Note that this step is unnecessary if you have already set the reference peak in your 1H NMR experiment at the beginning of these instructions. However, if necessary, you may set this value by using the cursor and zoom

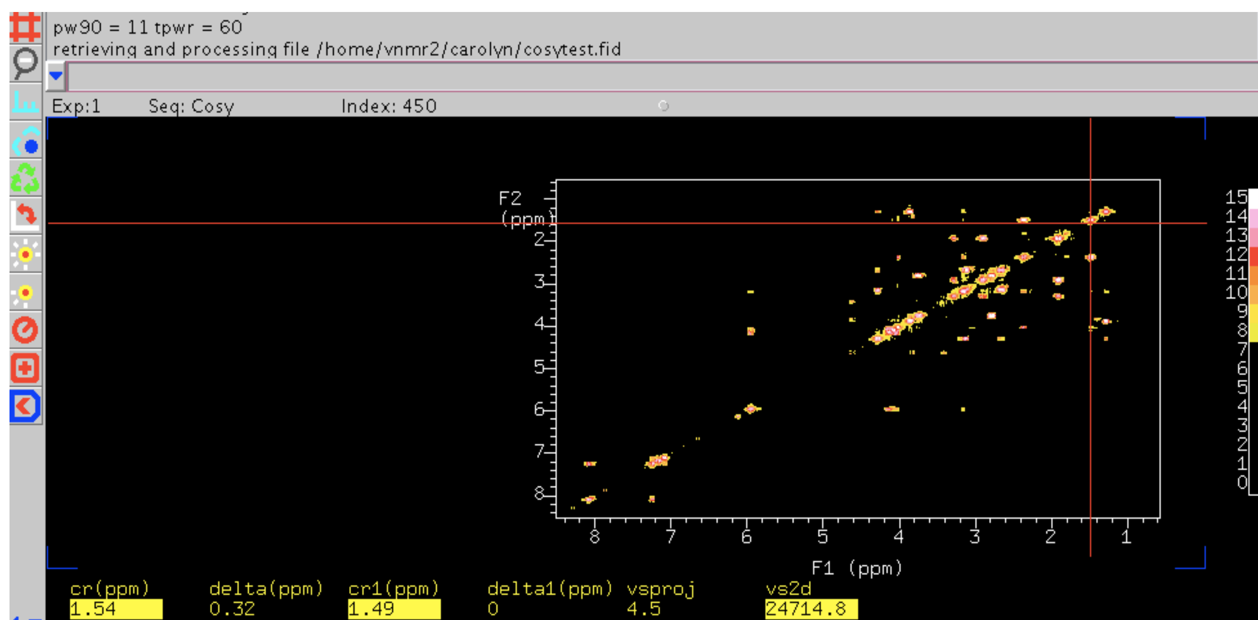
(magnifying glass) icons to expand the area containing the solvent peak. Place the cursor on the desired peak and type in the command **rl(x.xxp)** to set the reference value.

5. ***Decreasing static noise:*** Click on the display tab on the vertical menu. Check to make sure that "none" is selected under Display Mode, then look for the 2D Contour Display heading. Select the static *both +/-* icon to decrease static noise.




6. ***Changing the screen position of the spectrum:*** look for the Screen Position heading. You may choose from the *full*, *center, left*, and *right* icons, depending on how you would like to plot your spectrum. Typically it is best to choose the *center* option since it will allow for easier plotting of the peaks and spectrum later.

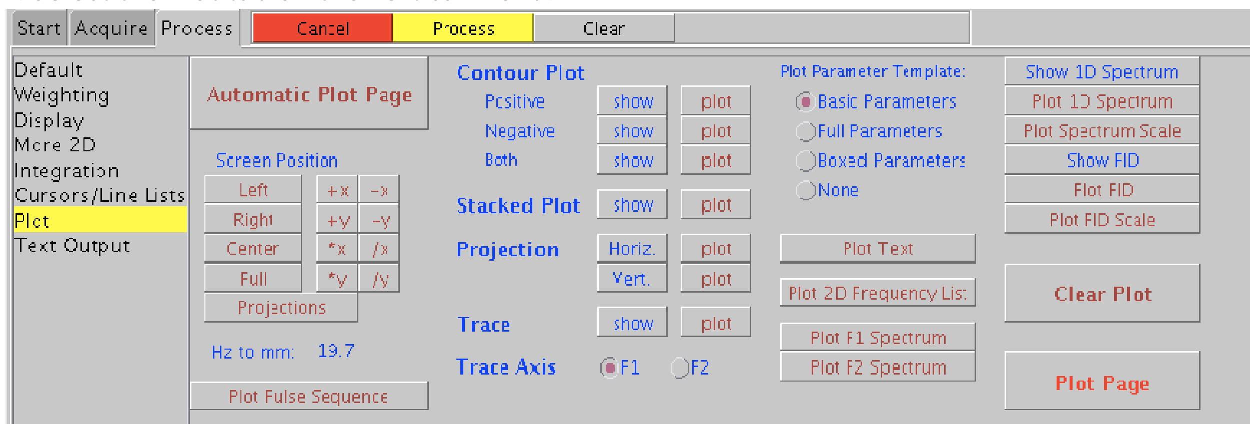
7. ***Increasing/decreasing intensity of peaks:*** Adjust intensity of peaks by using the yellow bulb icons on the toolbar.



***8. Expanding regions of the spectrum:*** Choose the red cursor icon on the toolbar. Use the left and right mouse buttons to select the area to be expanded and click on the zoom icon to expand. Also use this icon to return to normal size.

## *Plotting and printing the COSY spectrum*

1. Select the Plot tab on the vertical menu.




2. Look for the Contour Plot heading, then click on the *show* icon next to the *both* subheading. Click on the *plot* icon just to the right to mount the vertical and horizontal contour.
3. To add your proton peaks to the spectrum, look for the Projection heading, then click on *Horizontal* and *plot* (icon directly to the right) to add the horizontal peaks. Add the vertical peaks in the same manner, by clicking on *vertical* and then *plot*.
4. To print, click on the *plot page* icon.

## *Finishing the Experiment*

1. Remove your sample and replace with the standard sample, then turn on the spin and shim to the required lock level.
2. Under the Standard 1D tab, drag the proton icon to the black window to load standard parameters.
3. Exit vnmrj and log out.

# Experiment 22: Solid-State NMR CP-MAS Operations

Sample to be used: Uniformly 13C 15N labeled Glycine

## Step 1. Sample Preparations

**1.1** For Natural Abundance samples 100 mg of solid sample will be needed. For $^{13}C$ or $^{15}N$ enriched samples >30 mg of sample will be required.
**1.2** Pack sample into a Bruker solid-state NMR Rotor. Rotors can be obtained through Dr. Wu. Training on sample packing see solid-state NMR service instructor.
**1.3** After packing sample spin-test rotor using Bruker MAS Manual Controller-C. Set Bearing Pressure to 20 psi and slowly increase Drive pressure until a spin rate of ~10 kHz. Spinning should be stable at ~10 kHz for 30-45 seconds. Then slowly reduce drive and bearing pressure and eject sample. Consult Dr. Wu for detailed training on using spinning module.

## Step 2. Change Sample

2.3 Verify FTS System for temperature requirement.
2.4 Insert sample. Open the AVANCE 600 panel door and press **INSERT** on MAS Controller. Climb ladder and insert sample at top of magnet. At MAS Controller, press **SPIN RATE** and adjust with **up arrows** to 3500 kHz and press **GO**.
2.5 Once the temperature has reached 258K, press **SPIN RATE** and adjust with **up arrows** to 10000 kHz and press **GO**.
**2.4** Have Dr. Wu or solid-state NMR Instructor assist you with the following steps in preparing the equipment: (1) tune the probe, (2) measure $^{1}H$ and $^{13}C$ pulse widths.

## Step 3. Measure 1H T1

3.4 Type **ased** and set **PULPROG** to cpecho_1H_T1_adcaq.av.akm.
3.5 Set dipolar dephasing delay (**d8**) to 1μs and **ns** to 1. Type **za** then phase a negative carbon signal.
3.6 Try different values of **d8** to find the value of **d8** that eliminates all signal (i.e. This value is $T_{null}$).
3.7 Approximate T1 as $T1=T_{null}/\ln 2$. The recycle delay (**d1**) should be set to a value greater than 5*T1 (Typical T1 samples for peptides are between 0.2-1.5s).

### Step 4. Measure Cross-Polarization (CP) Build-up

**4.1** Type **ased** and verify **SPNAMO=ramp70** and **CPDRG2=spinal64.13.**
**4.2** Type **ns=128** (or scans with sufficient signal to noise). Type **gs** and Zoom in on peak of interest and set **dp1** and **dp2** to define ppm range for peak.
**4.3** Use **popt** program to array the contact time (**p15**) starting at 500 μs to 3000 μs in steps of 250 μs. Choose the contact time that yields the maximum signal.

### Step 5. Record 13C CP-MAS for Sample

**5.1** Type **ased** and setup the following parameters

| | | |
|---|---|---|
| p3 | 1H 90 pulse width | 1.9 μs |
| pl12 | 1H 90 power level | Obtain from Solid-State NMR Instructor |
| p15 | CP Contact Time | Measure in Step 4 |
| pl1 | 1H CP Power Level | Obtain from Solid-State NMR Instructor |
| pl13 | 1H Decoupling Power Level | Obtain from Solid-State NMR Instructor |
| pl1 | 13C Power Level | Obtain from Solid-State NMR Instructor |
| p2 | 13C Hahn echo pulse | Obtain from Solid-State NMR Instructor |
| d1 | Recycle Delay | Measure in Step 3 |
| pl11 | 13C CP pulse width | 10 μs |

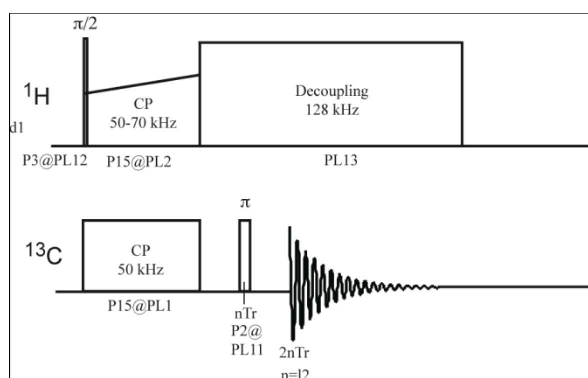



**Figure 1: CP-MAS Pulse Sequence**

**5.2** Set **ns=1** and type **gs.** Verify FID takes between ½-2/3 of the screen. If not adjust receiver gain (**rg).** Type **gs** and have solid-state NMR instructor verify probe is properly tuned.

**5.3** Set **ns=16-32k** of scans for natural abundance samples, and **ns=1-8k** for 13C enriched samples.

**Step 6. CP-MAS Data processing**

**6.1** Set **tdeff** then type **fp** to lowest value so that it does not attenuate signals of interest, while minimizing noise level.

**6.2** Determine type of processing needed (fp,efp, or gfp). If using **gfp** set **gb=0.0005.** Set lb (- value for gfp) and array until signal drops by ½. Compare **gfp** processing to **fp** to determine if any desired resolution has been lost (e.g. a shoulder in a carbonyl peak you wish to analyze). Array **lb** by decreasing the magnitude of the number until resolution has reached desired level.

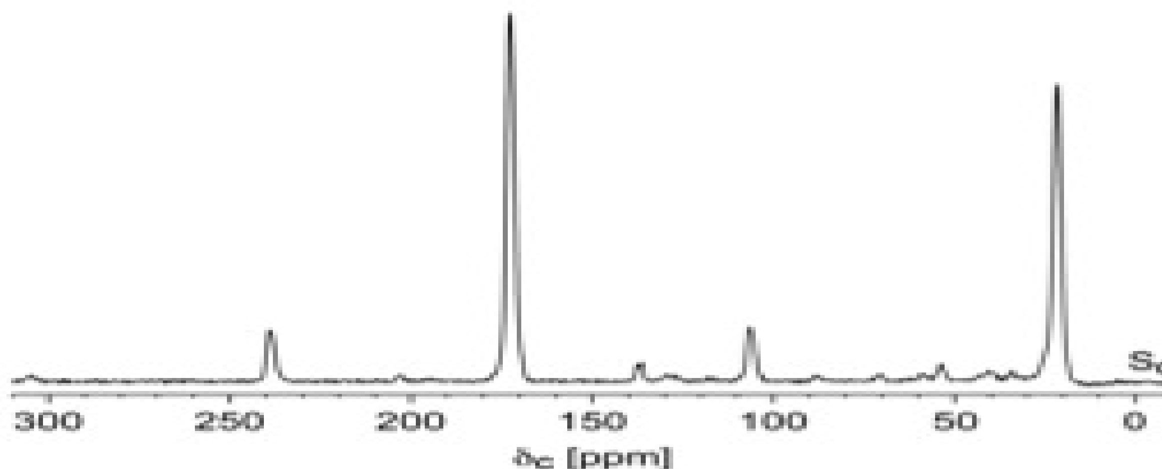



Figure 2: CP-MAS Spectra of peptide containing 13C carbonyl label (172 ppm) on leucine and 13C methyl label (23 ppm) on alanine collected at spinning speed=10 kHz. Note spinning side-bands from carbonyl peak at 238 ppm and 108 ppm – spaced 10 kHz from the center band. Remaining peaks represent natural abundance carbons from the peptide.

# Appendix A: Download Your Data from Host Computers to a PC

**NMR Center has an internal network that is not connected to outside network. To access the NMR data on all NMR host computers, you have to use a PC to connect the internal network and download the data. There is a PC available in the NMR lab. Bring your own USB, or portable hard disk.**

Login if screen is locked: username: nmr user
password: nmr4me

Double Click on “Shortcut to Smart FTP” to open the Smart FTP window.

Type the IP address of the host computer you want to download data from in ‘Address’ box.
IP addresses for each host computers:
**inova400: 170.140.188.70**
**inova600: 170.140.188.67**
**mercury300: 170.140.188.72**

Type your group username in ‘Login’ box.

Type the password of your group in ‘password’ box and press Enter.
You maybe need to wait a couple of minutes to establish the connection.
When you see the folders and files appear in the panels, the connection is established.

Find your folders and files and drag them to the desktop (or you may want to create a temporary folder) on this computer.

Copy the data to the media you like: USB flash drive (flash key), zip disk, floppy disk or burn your data on a CD.

Delete your data and folders you created from this computer.

## Appendix B: When and how to use command *su acqproc*

***su acqproc*** is a command to connect or disconnect the communication between Sun host computer and CPU board in the NMR console. There are several occasions users need to use it.

**When**: The communication is lost. The computer seems not to respond to any command you typed in and you are not able to open the 'acqi' window. A word **'inactive'** is usually shown after STATUS in the ACQUISTION STATUS window.

**How**: Open the Console window by double click on the Console icon on the up-right corner of the screen (Sometimes it is hiding behind ACQUISTION STATUS window). Type ***su acqproc*** and press **Return**. A message "Starting 'Expproc' will show up. At same time "Word '**inactive**' will change to '**idle**' in the ACQUISION STATUS window. You should be able to operate the instrument again.

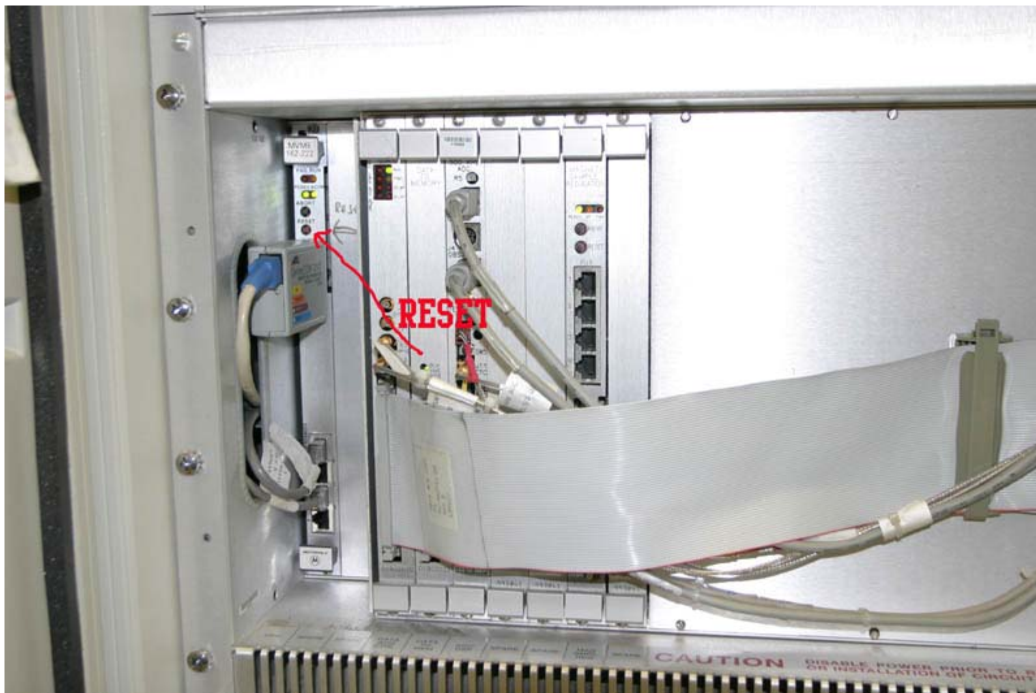
**When**: The Lock button is missing in the ACQUISION window. It happens very often when you type h1, then immediately you clicked acqi. After you type h1, you should wait until "idle", then click "acqi".

**How**: Open the Console window, type ***su acqproc*** and press **Return** to disconnect the communication. Type ***su acqproc*** and press **Return** again to establish the communication.

If above operation doesn't work, you should call Dr. Wu for help. Sometime you have to reset the console when he asks you to do so. The following pictures show the reset button on different console.

UNITY600 Plus Console RESET button position.

Mercury 300 RESET Button position.



INOVA400 and INOVA600 RESET Button position

## Appendix C: Most Useful Commands for VNMR and VNMRJ

aa—abort acquisition
ai—absolute intensity mode
alock='n' auto lock setting
alfa----Delay time before acquisition
aph—automatic phase correction
at—acquisition time (sec)
axis—scale units: axis='h' or axis='p'
bc—baseline correction
bs—block size
cd—change directory: cd('/data/nmryao')
cexp—create experiment: cexp(7)
cr—cursor value
ct—completed transients (scans)
cz—clear zeros (integral)
d1—first delay (relaxation delay in seconds)
d2—second delay in seconds
da—display array (parameters)
dc—drift correction
dconi—display contours interactively
delta—cursor difference
df—display fid
dfrq—decoupler frequency
dg—display group of parameters
dli—display integral list
dll—display line list
dlni—display normalized integral list
dm—decoupler mode: dm='nny'
dn—decoupler nucleus

dof—decoupler offset
dpcon—display plotted contours:
dpcon(10,1.2)
dpf—display peak frequencies
dpir—display integral regions
dpirn—display normalized integral regions
dps—display pulse sequence
dpwr—decoupler power
dres—digital resolution
ds—display spectrum
dscale—display scale descale(-10)
dssa—display stacked spectra
dssh—display stacked spectra horizontally
dtext---display text on the screen
f—display full spectrum
foldt—symmetrize 2D data (cosy)
fn—Fourier number (zerofill)
fn1—Fourier number in 2nd dimension
full—display spectrum in full window
ga—acquire and process
gain—receiver gain: gain='n' for autogain
go—acquire spectrum
ho—horizontal offset
ins—integral normalization scale
io—integral offset
isadj—adjust integral scaling
jexp—join experiment: jexp2
lb—line broadening
lp—left phase
movesw—move sweepwidth
movetof—move tof
mp—move parameters: mp(1,2)
nl—nearest line
nm—normalized mode
np—number of points
nt—number of transients (scans)
pad—preacquisition delay
page—send to plotter
pap—plot all parameters
pcon—plot contours: pcon(10,1.2)
phase(180)—phase spectrum 180°
pir—plot integral regions
pirn—plot normalized integral regions
pl—plot spectrum
pll—print line list
plot—plot everything
plww—print spectra whitewashed
ppa—plot partial parameters
ppf—plot peak frequencies
process—transform, phase, integrate
spectrum
spsub---Subtract current spectra from exp5.
pscale—plot scale
pw—pulse width
pwd—present working directory
ra—resume acquisition (stopped by sa)
rl—reference line: rl(7.27p)
rof2---Receiver gating time
rp—right phase
rt—retrieve FID
rtp—retrieve parameters

rts—retrieve shims
sa—stop acquisition
sc—start of chart (in mm)
sc2—start of chart in 2nd dimension (in mm)
sd—set decoupler frequency
sda—add another decoupler value
sfrq—spectrometer frequency
sp—start of chart (in ppm)
sp1—start of chart in 2nd dimension (in ppm)
ss—steady state scans or dummy scans
su—setup hardware parameters
svf—save FID
svs—save shims only
svp—save parameters only
sw—spectral width or sweep width
temp—set temperature: temp=’n’
tn—transmitter nucleus
tof—transmitter offset (middle of sweepwidth)
tpwr—transmitter power
text(‘your_ID\\date’)
unlock—unlock a locked experiment: unlock(2)
vo—vertical offset
vp—vertical position
vs—vertical scale
vsadj—vertical scale adjust
wc—width of chart (in mm)
wc2—width of chart in 2nd dimension (in mm)
wshim=’n’ start with auto shim
wp—width of chart (in ppm)
wp1—width of chart in 2nd dimension (in ppm)
wft—weighted Fourier transform
wft2d—transform 2D absolute value data
wft2da—transform 2D phase-sensitive data
wti—interactive weighting
z—cut integral reset

# Appendix D:  Processing NMR data with NUTS

The data from Varian NMR instrument consist four individual files: fid, log procpar and text. The fid is the spectrum data. The log file contains the date of the spectrum acquired, The text file contain the note that you input by the command text('your_ID\\date').  And the procpar file contains all the acquisition parameters, including the shim data.

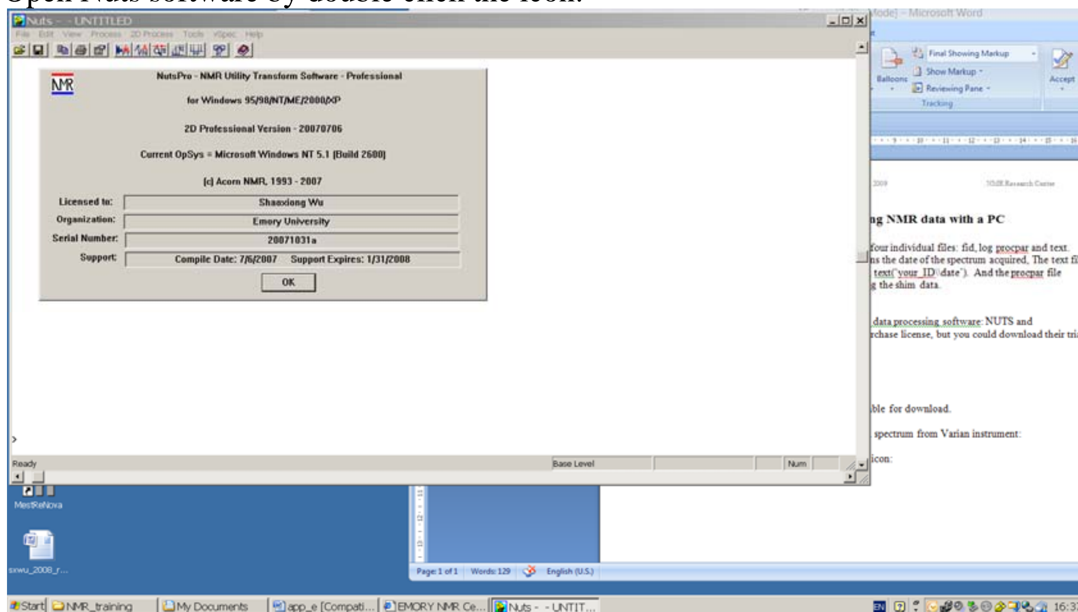
In our NMR center, we have two off-line NMR data processing software: NUTS and MestReNova. For both software you have to purchase license, but you could download their trial version.

http://www.acornnmr.com/
http://www.mestrec.com/

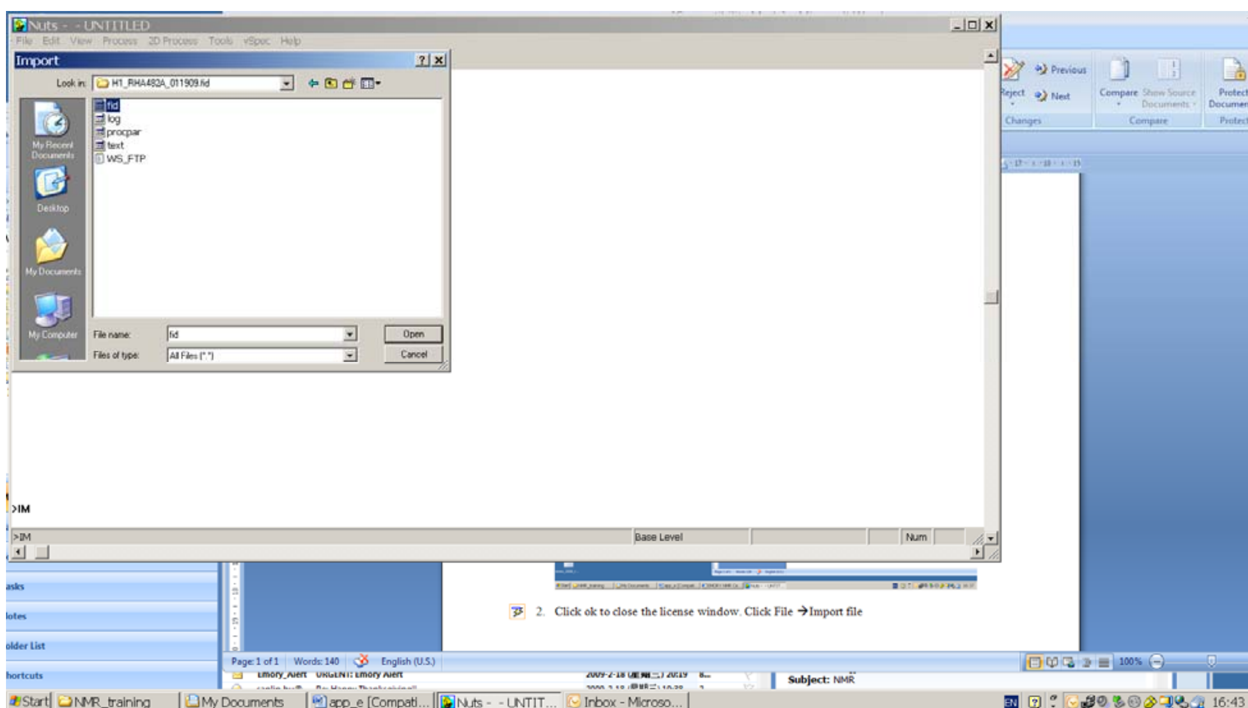
Also there are detailed operation manuals available for download.

Following is an example to use NUTs process a spectrum from Varian instrument:

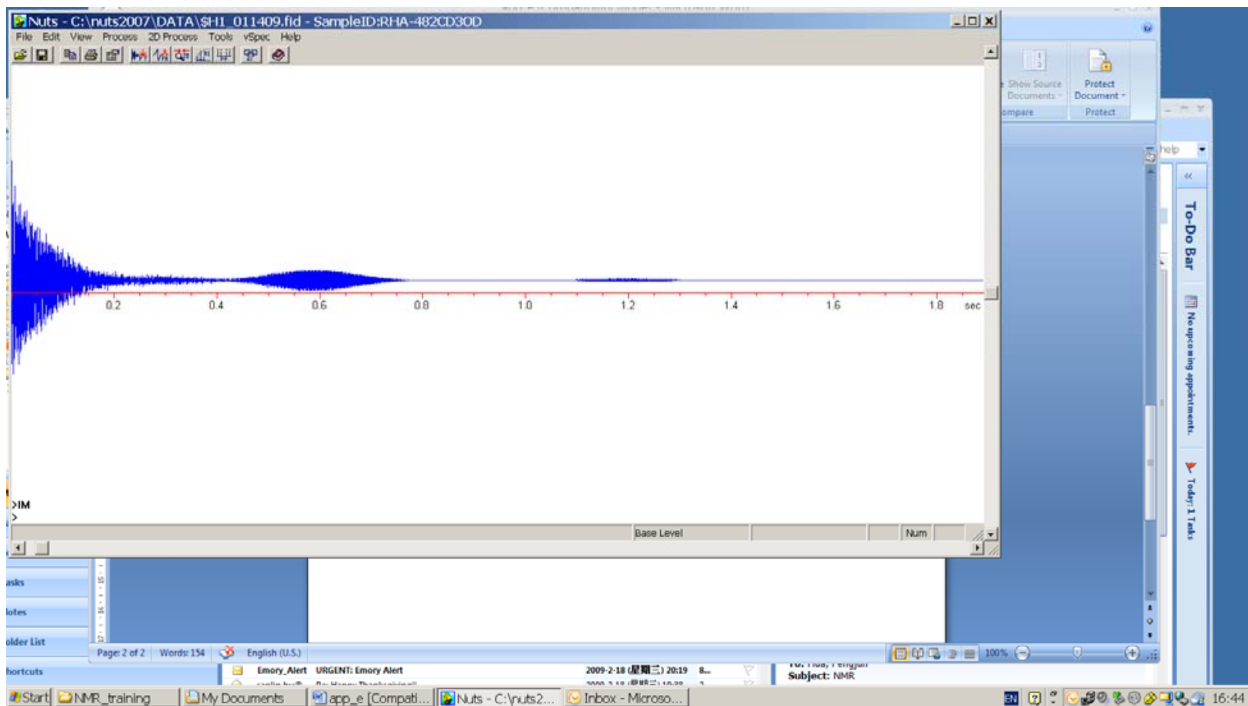
1. Open Nuts software by double click the icon:



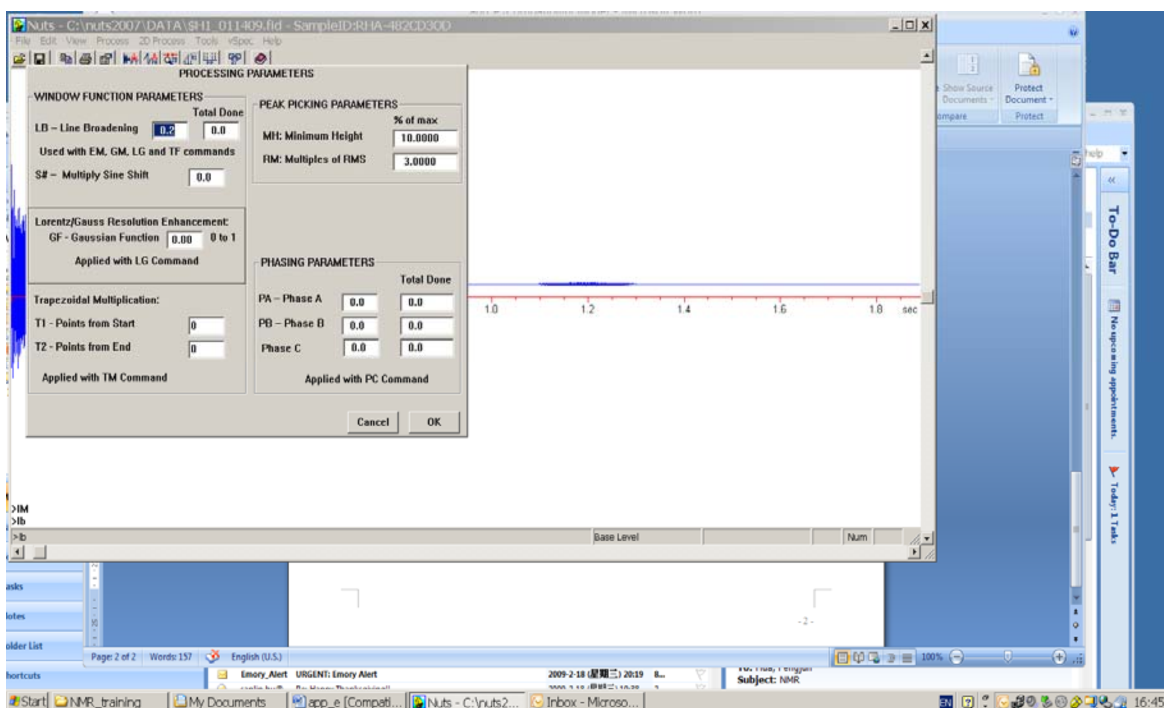

2. Click ok to close the license window. Click File →Import file → select the file and open fid and click ok.




After the fid loaded:

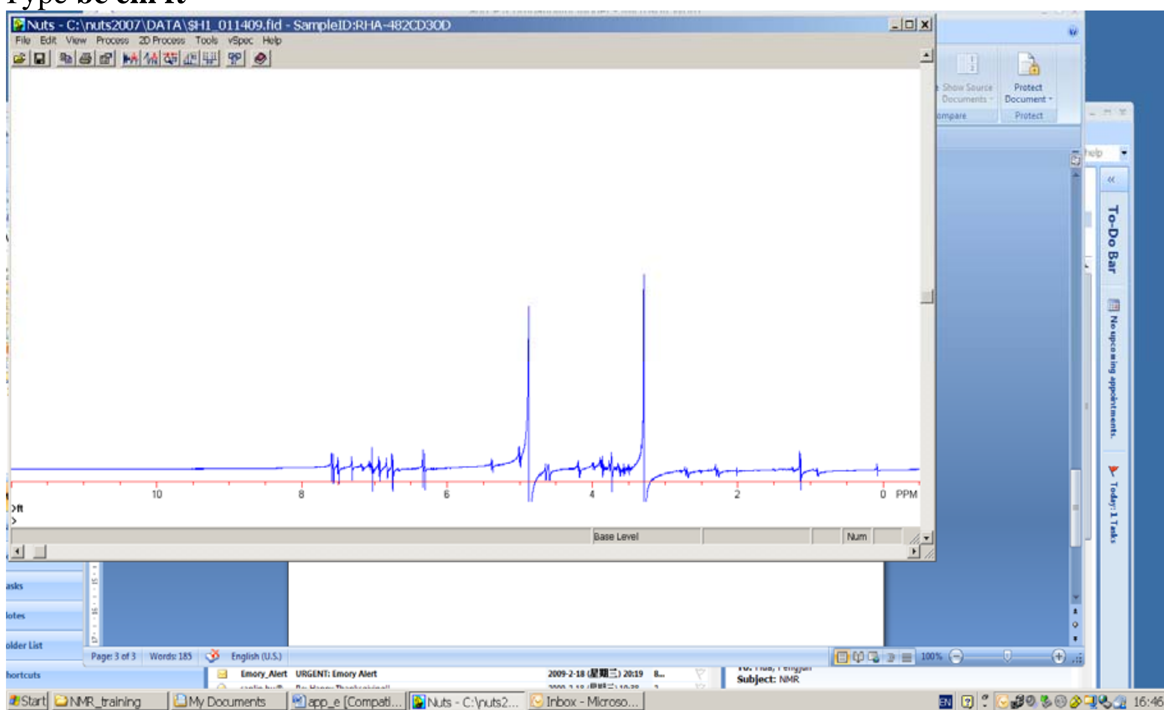



You type lb:

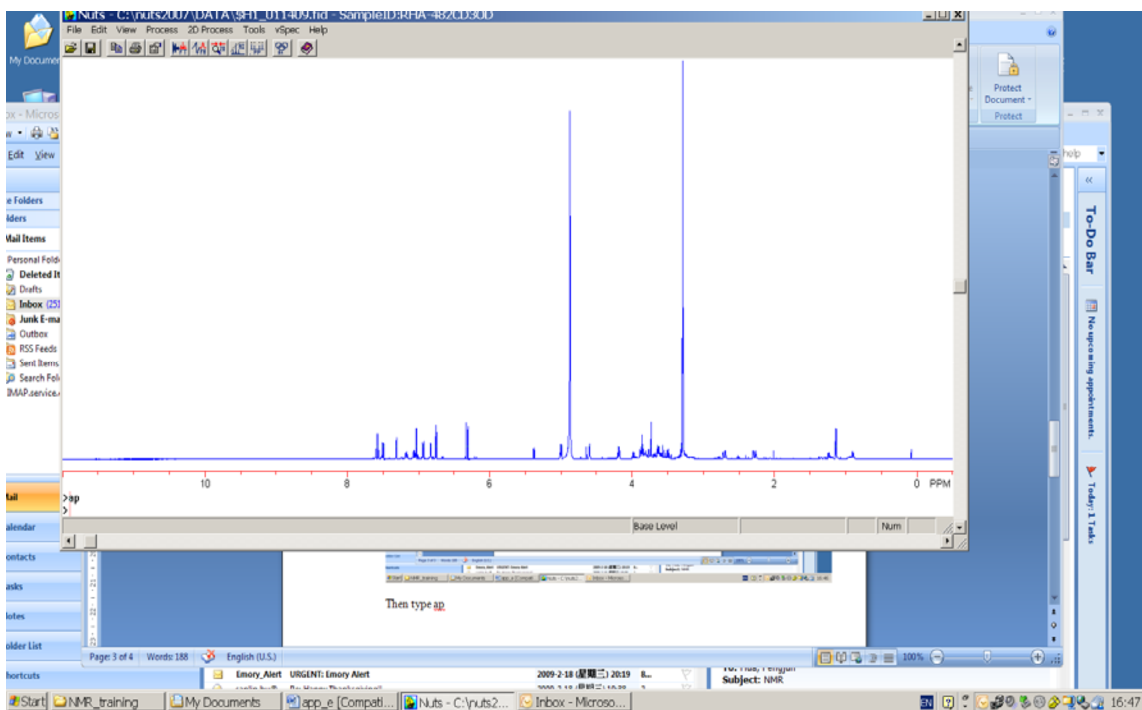



If it is proton spectrum, set it to 0.2 hz, if it is a carbon spectrum set it to 2 hz. Then click ok

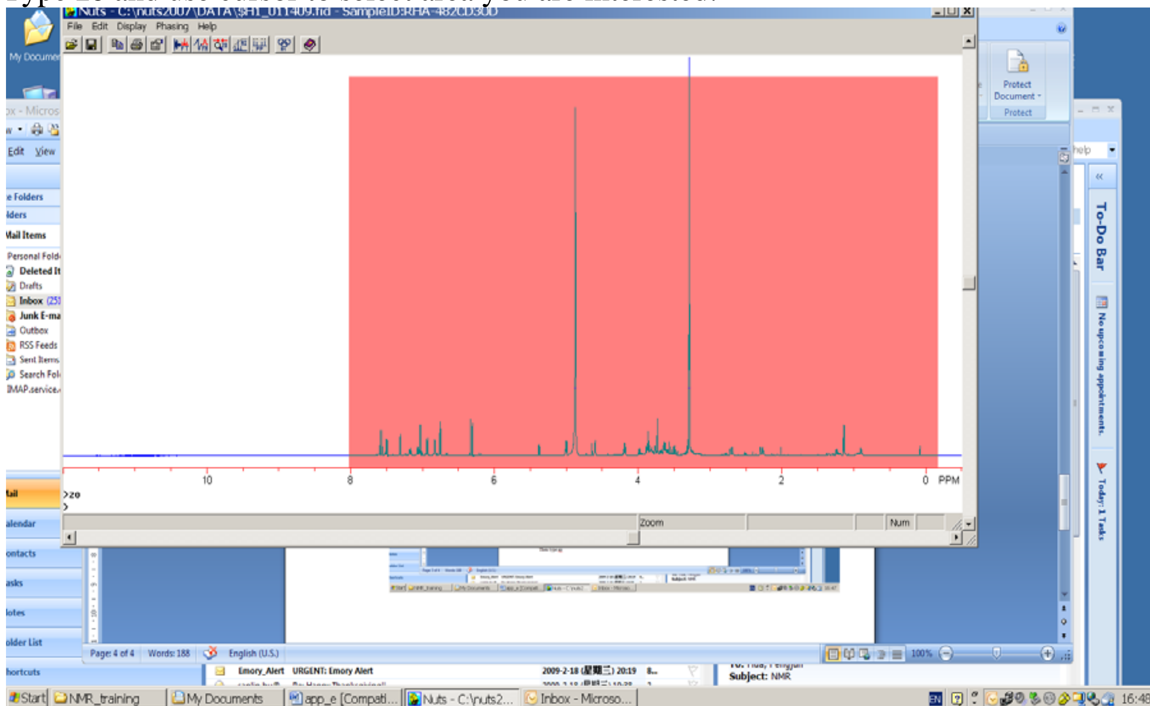
Type **bc em ft**



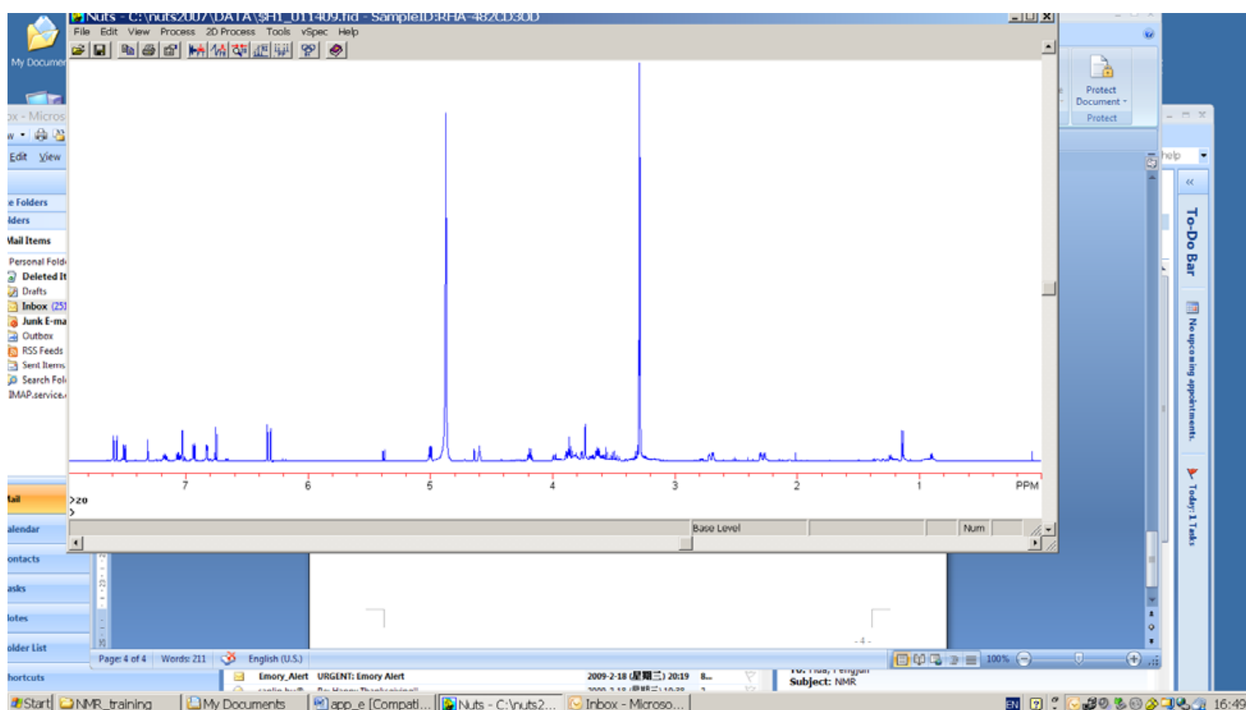

Then type **ap** for auto phase.



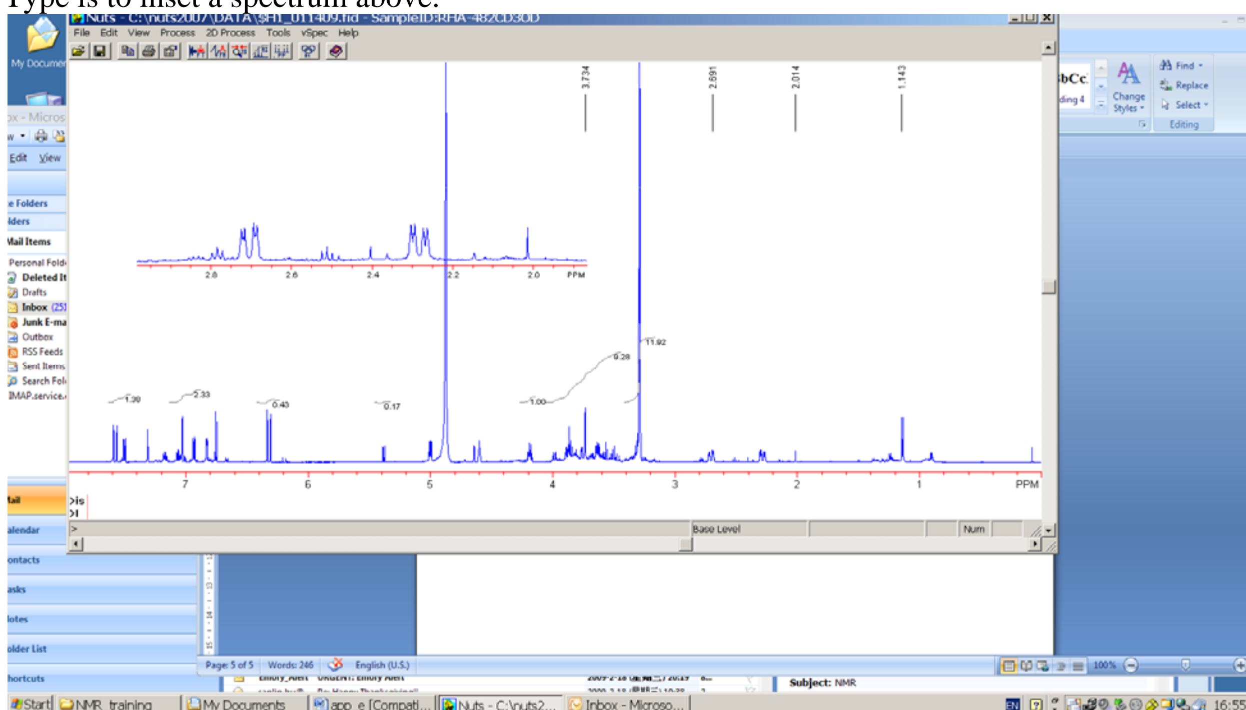

Type **zo** and use cursor to select area you are interested:




The put the mouse into red area and click right mouse button.




Use the right bar to increase the peak height. Type **id** to integrate the spectrum. Click help for more information. You could print the spectrum at this point. You could print it as a PDF file. Type is to inset a spectrum above.

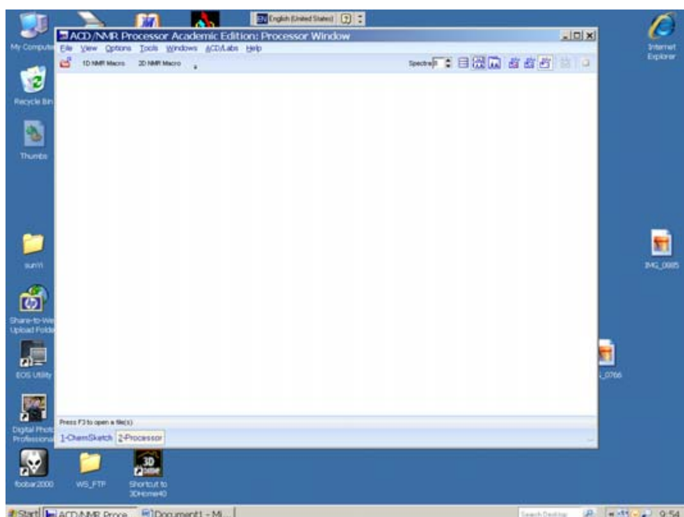



You could print the spectrum with the inset, integrals, and peak labels. Also you could save it a file that could use on the power point and Microsoft word. For further instruction, download the manual from their website.

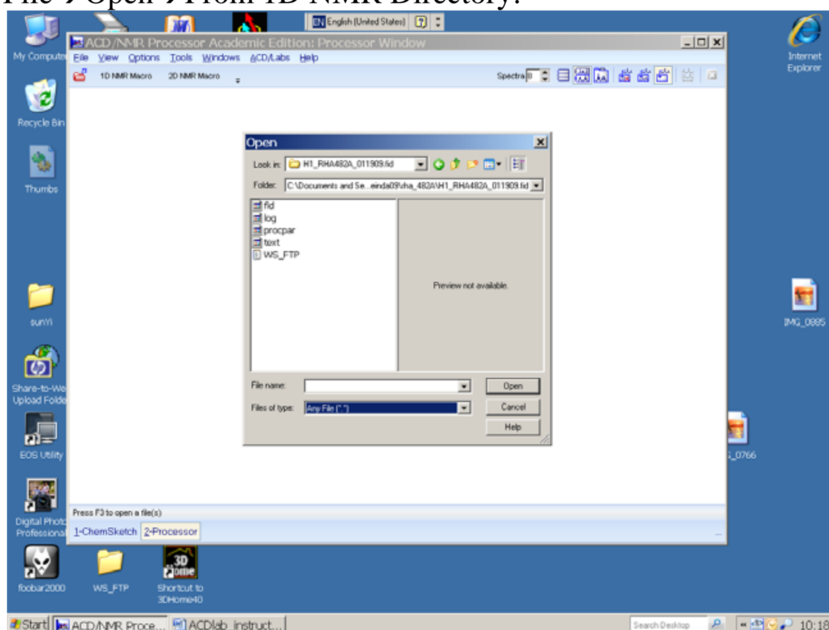
# Appendix D: Processing NMR data with ACDLABS

The software is free and you can download and install it on your personal computer. Detailed instruction: **http://www.emory.edu/NMR/docs/ACD_NMR_processing.pdf**

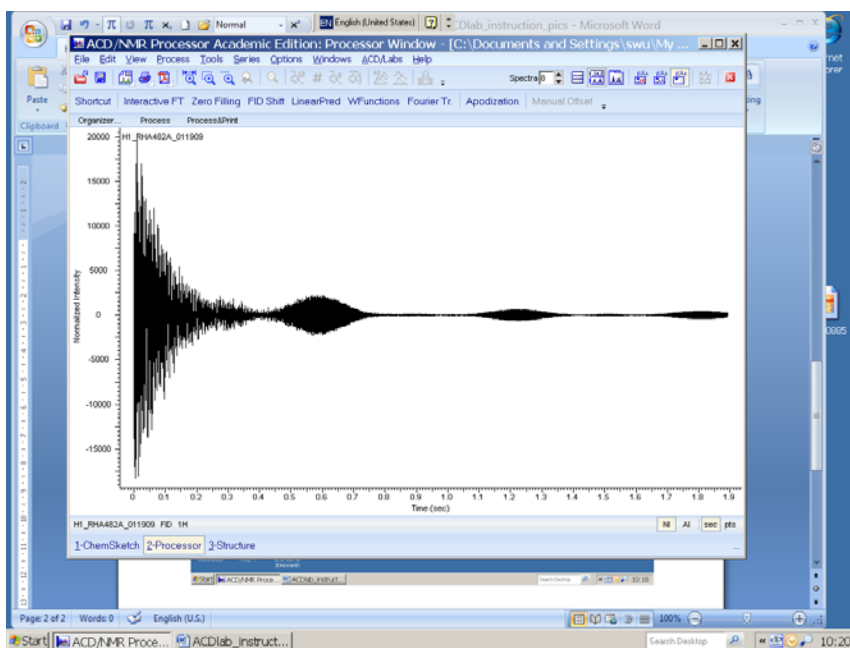
1. Open ACD/NMR Processor:



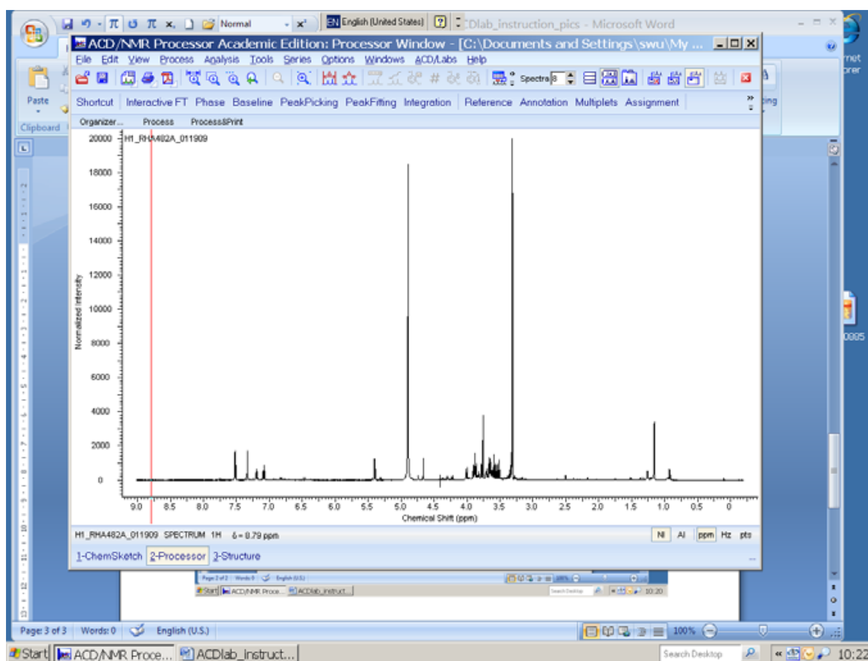

2. Open Varian NMR data:
   File→Open→From 1D NMR Directory:



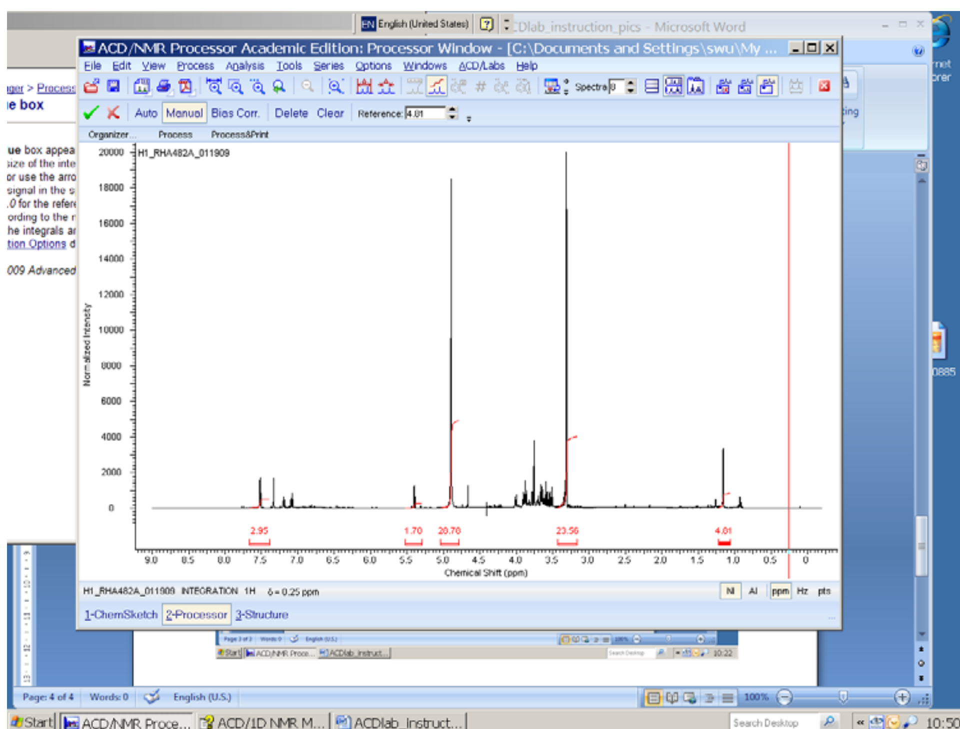

Select fid and click open:




Process→ Fourier Transform → Default Transform




Integration:
Analysis → Integration→ manual Integration → set the red cursor to the left of the peak, and hold the left mouse button to move the cursor to the right side of the peak. (That will be the integral area). The integral value will be displayed under the scale line.



There is a help manual. There are lots of options you can explore by click help manual.