Varian NMR Instructions - 1D

Department of Chemistry NMR Facility
University of Minnesota

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1.   General Information

The University of Minnesota Chemistry Department NMR Laboratory is located in S168 and S169 Kolthoff Hall and is operated by :

Dr. Letitia Yao   Dr. Steve Philson
Tel: 625-8374   Tel: 626-0297
yao@chem.umn.edu   philson@nmr.chem.umn.edu

Check out our website at: **http://nmr.chem.umn.edu**

The laboratory contains 6 NMR spectrometers:

Varian INOVA 500	(VI-500)
Varian INOVA 300	(VI-300)
Varian INOVA 300	(VXR-300)
Varian UNITY 300 with automatic sample changer	(VAC-300)
Varian UNITY 200 with automatic sample changer	(VAC-200)
Chemagnetics CMX-400 (solids only)	(CMX-400)

This document describes operation of the three hands-on Varian NMR spectrometers (VI-500, VI-300, and VXR-300).
- The standard probe for the VI-500 is a triple-resonance indirect detection PFG probe that is set up to observe ^1H, ^{13}C, and ^{31}P nuclei and is equipped with pulsed field gradients (PFG). Note that ^{31}P requires a cable change.
- The standard probe for the VXR-300 is a 4-nucleus probe equipped to observe ^1H, ^{13}C, ^{19}F, and ^{31}P and has gradients.
- The standard probe for the VI-300 is a broadband probe that is set up to observe ^1H and ^{13}C nuclei.
- All three spectrometers are equipped with additional probes that can be tuned to most other nuclei. Contact Letitia for information on scheduling time to do other nuclei.
- The VI-300 and VI-500 are equipped for temperature control. *Separate training is required for use of the variable temperature equipment.*
     The VT range of the standard PFG probe in the VI-500 is -20°C to +60°C.
     The VT range of the standard probe in the VI-300 is -100°C to +100°C.
     Other probes are available on these instruments that can go from -100°C to +100°C.
- Several Sun workstations are located in the NMR lab for off-line processing of data. Use these workstations for processing and plotting of data. Our goal is to maximize the amount of time the spectrometers are busy collecting data.
 --To run non-routine experiments (VT or non-standard nuclei), schedule time with Letitia one week in advance.

2.   References

This document is designed to briefly describe how to operate the Varian NMR spectrometers. For more information on operation of the spectrometers see the on-line versions of the Varian manuals:
     Getting Started – more details on general operating instructions
     User Guide: Liquids NMR – description of experiments and relevant parameters
     Command and Parameter Reference - alphabetical list of commands and parameters with
          a thorough description of what they do (also available on-line on the Sun computers).
For general information on NMR spectroscopy see:
     Modern NMR Techniques for Chemistry Research by Andrew E. Derome
          Good description of NMR fundamentals and tips on how to get good NMR spectra -
          everything from sample preparation to data acquisition to data processing and analysis.
     High-Resolution NMR Techniques in Organic Chemistry by Timothy D.W. Claridge
          Update of Derome, including current techniques such as gradients.
     Basic One- and Two-Dimensional NMR Spectroscopy by Horst Friebolin
          Good information on interpretation of NMR spectra.
     Structure Determination of Organic Compounds by Pretsch, Buhlmann, and Affolter
          Tables of spectral data

3.   Rules

- All users must be trained by the NMR lab personnel- *NO UNAUTHORIZED USERS*.
- Each authorized user is given a 6-letter code and computer account for scheduling time on the instruments and for using the instruments. *Use a secure password and do NOT let anyone else use your account. This is grounds for suspension.*
- No food or beverages in the lab.
- No chemistry in the NMR Lab. This includes adding solvent to your NMR tubes. We are not equipped with fume hoods and do not want to deal with spilled chemicals or solvents.
- Do NOT leave NMR tubes in the NMR Lab.
- No chipped or cracked NMR tubes.
- Use **507PP** or **528PP** NMR tubes on the Varian instruments to prevent damage to the probes.
- Always use *new* 528 NMR tubes for VT work. Old tubes may be scratched and more likely to break.
- All users must abide by the scheduling restrictions posted in the lab.
- All users must sign the spectrometer log book.
- *All users must lock and shim on the dummy sample when finished using the spectrometer.*

4.   Scheduling

Scheduling time on the hands-on NMR instruments is done through a computer scheduling program that is accessible from the scheduling terminal near the entrance to the NMR lab or from any Sun workstation in the NMR lab. To pull up the scheduling program, select **[Sched]** via the menu buttons. Scheduling restrictions are in effect from 8am to 6pm weekdays. It is also possible to access the scheduling computer from other computers on campus by telnetting to "nmr.chem.umn.edu" using your 6-letter NMR lab code. While scheduling time, if the computer program does not reserve the time you requested, chances are you are trying to circumvent the rules.

5.   Varian Software

The software that runs the Varian spectrometers and that is used to process data on the workstations is called VNMR. This program is automatically started when you log into any Sun computer in the NMR lab.

5.1.   Windows

Command and Status Window

The top window is where much of the interaction with the VNMR program takes place. This window contains menu buttons that can be used to execute most of the commands necessary to run the spectrometer and a command line on which these and other commands can be typed. Parameters can also be changed by typing on the command line.

Above the command line are the status and error lines. These give information about what command is being executed, what pulse sequence is being used, and any errors or warnings that occur.

The value of a parameter can be queried by typing the name of the parameter followed by a question mark: **nt?**. The value of a numeric parameter can be changed by typing the parameter name followed by = and the new value: **nt=64**.

The value of a string parameter can be changed in a similar fashion, except that the string must be enclosed in single quotes: **dm='nyn'**. Parameters can also be modified by typing directly in the (**dg**) window (see below).

Graphics Window

The middle window is the graphics window. This is where spectra and pulse sequences are displayed and manipulated.

Text Window

The bottom window is the text window, also called the **dg** window. It is used to display parameters used for acquisition and processing of data, output from certain commands, and help information. Values can be changed by typing directly into this window. The right-hand toolbar is used to select different sets of parameters (acquisition/observation, decoupler, processing, etc). The Text option will give the dg window that is most familiar to veteran users of vnmr.

Shown below is the **dg** window with Acq & Obs parameters selected from the right-hand toolbar. Note the interactive parameter settings.
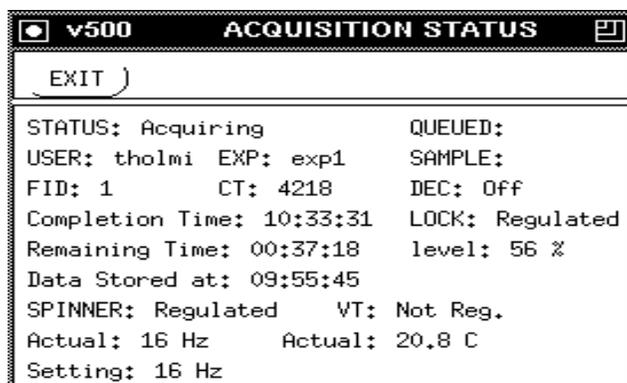
```
           Acquisition              Observe Channel                Sample              Acq & Obs

Spect width          5997.9   Hz  ▭   Nucleus    H1          Date      Mar 11 1998    Decouplers
Acq. time            2.0007   sec ▭   Spect Freq. 299.891  MHz  File      exp
Acquired complex pts 12000              offset      1321.9  Hz  ▭  Solvent   D20       Sequence
Recycle delay        1.5      sec ▭   obs pulse   12.1     us  ▭  temperature 29     C ▭  Flags & Cond.
Transients           4                  power       63
Steady state         0                                                                 Process

                                      Calibration:            Channel   : Nucl.  Status  Process2
                                      pi/2 pulse: 12.1   us    Observe   : H1             Display
                                            power     63       Decoupler : H1     —  nnn  display2
                                                              Decoupler2 :        —       LCNMR/STARS
                                                              Decoupler3 :        —       Text

                                                              Gradient Type: nnn          Spare
```

Shown below is the **dg** window with the Text option selected.

```
    ACQUISITION          SAMPLE            PROCESSING            FLAGS         ▲  Acq & Obs
sfrq      299.891  date   Feb 27 1998  lb        0.20   il              n
tn            H1   solvent      CDC13  sb    not used   in              n        Decouplers
at         2.001   file          exp  gf    not used   dp              y
np         24000        DECOUPLING      awc   not used   hs             nn       Sequence
sw        5997.9   dn            H1   lsfid  not used       SPECIAL
fb          3400   dof            0   phfid  not used   temp      not used       Flags & Cond.
bs            16   dm           nnn   wtfile
ss             0   dmm            c   proc          ft                            Process
tpwr          63   dmf          200   fn         32768
pw          12.1   dpwr          30   math           f                           Process2
p1             0
d1         1.500                       werr                                       Display
d2             0                       wexp
tof        1321.9                      wbs                                        display2
nt            16                       wnt
ct             0                                                                  LCNMR/STARS

                                                                                 Text

                                                                                 Spare
                                                                              ▼
```

Acqstat Window

On spectrometers, the Acqstat window appears alongside the three VNMR windows. It gives information about the spectrometer status, e.g., what experiment is running, how much longer it will run, the temperature, etc.:
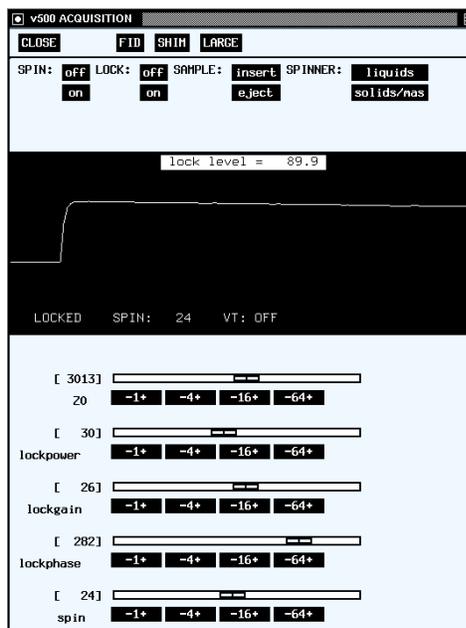
```
[●] v500        ACQUISITION STATUS    [凹]

  EXIT )

STATUS: Acquiring          QUEUED:
USER: tholmi  EXP: exp1    SAMPLE:
FID: 1          CT: 4218   DEC: Off
Completion Time: 10:33:31  LOCK: Regulated
Remaining Time: 00:37:18   level: 56 %
Data Stored at: 09:55:45
SPINNER: Regulated     VT: Not Reg.
Actual: 16 Hz      Actual: 20.8 C
Setting: 16 Hz
```

Acqi Window

The **[Acqi]** (acquisition interactive) window is used to load samples and to lock and shim on samples. Select the **[Acqi]** button from the upper menu line to access this window. The **[Acqi]** window is shown below, after the **[Lock]** option has been selected.

The Acqi window allows interactive adjustment of lock and shim parameters. This is done using the buttons like **[- 16 +]**. Use the *left* mouse button to decrement the parameter by the value on the button, or use the *right* mouse button to increment the parameter by the value on the button.

 [Acqi] window:

```
[●] v500 ACQUISITION                      [凹]
CLOSE        FID  SHIM  LARGE
SPIN: off LOCK: off SAMPLE: insert SPINNER:  liquids
      on        on          eject           solids/nas


              lock level =   89.9


    LOCKED    SPIN:   24    VT: OFF

 [ 3013] [          ══          ]
    Z0    -1+  -4+  -16+  -64+
 [   30] [        ══            ]
lockpower -1+  -4+  -16+  -64+
 [   26] [          ══          ]
 lockgain -1+  -4+  -16+  -64+
 [  282] [               ══     ]
lockphase -1+  -4+  -16+  -64+
 [   24] [        ══            ]
    spin  -1+  -4+  -16+  -64+
```

5.2.   Experiments: Creating, Joining, and Unlocking Them

The VNMR software is set up with 9999 different work areas called experiments. Each experiment is entirely separate from the others, so you can be acquiring a carbon spectrum in experiment 2 and processing and plotting a proton spectrum in experiment 1 while waiting for the carbon experiment to finish.   The experiment you are currently working in is displayed at the top of the command and status window of VNMR.

To create an experiment, use the command **cexp(*n*)** or the menu buttons: **[Main Menu]** - **[Workspace]** - **[Create New]**.

To change (join) from one experiment to another experiment, type **jexp*n*** (*n* is a number between 1 and 9) on the command line or use the menu buttons: **[Main Menu]** - **[Workspace]** - **[exp*n*]**. You can move parameters between experiments by typing **mp(1,3)** which moves parameters from experiment 1 to experiment 3.

To unlock experiments, type **unlock(*n*)** where *n* is the experiment number. If for some reason you are logged into more than one workstation at the same time, you must be in different experiments for each login.

5.3.   Exiting VNMR

To exit from the VNMR program and log out of a Sun computer, type **exit** on the command line of VNMR or use the permanent menu button: **[Exit VNMR]**. *Lock and shim on the dummy sample before exiting!*

5.4.   Running VNMR from a Remote Computer

It is possible to run VNMR and display it on a remote computer such as a Sun, SGI, or IBM UNIX workstations and Macintosh or IBM PC's with X-server software. This can be useful if you would like to process data remotely from your lab or office and plot spectra to your networked printer. If you would like to have this capability, contact Letitia or Steve. You need to supply us with the IP address of the computer that you are using and the name of your printer.

6.   Sample Preparation

Sample preparation is an important and often overlooked part of an NMR experiment. If the sample is not prepared properly, the resulting NMR spectrum may be of poor quality. It is important to make sure that the NMR tubes you are using are not scratched, cracked or chipped, and of sufficiently good quality (**507PP** or **528PP**). The NMR tube should then be filled to a height of 5cm with sample and deuterated solvent. If the height is less than 5cm, it will be more difficult to shim on the sample and the lines in the NMR spectrum may be broader than necessary. It is also important to make sure there is no precipitate in the tube and that the sample is mixed thoroughly - these can also affect shimming and spectral line width. For experiments where quantitative NOEs are desired, it is also important to be sure that no paramagnetic impurities get into the sample, and to filter and de-gas the sample. The book by Derome mentioned in the References section gives more details about sample preparation. There is also information on our web page.

7.   Setting Up an Experiment

When you walk up to one of the Varian spectrometers, the previous user will have put the dummy sample (D_2O) in the magnet and locked and shimmed on it (as you should also do after you are done using the instrument). After you log in you will need to load your sample and lock and shim on it prior to acquiring a spectrum.

EASY EXPERIMENTAL SETUP with the **[Change Sample]** menu button:

a. Select the **[Change Sample]** menu button.

b. **[Eject]** the dummy sample and **[Insert]** your sample. (See Section 7.1: Loading a Sample.)

c. The **[Set Std Shims]** button loads the standard shims file and executes the **su** command. (See Section 7.3: Loading Standard Shims.) Wait for the beep.

d. Choose **[Set Solvent]** and select the proper solvent for your sample.

e. The **[Autolock]** feature finds the **Z0** of your solvent and locks on it. The lock is set to very high values of lockpower and lockgain; you will need to reduce those after **[Autolock]**, so click on the **[Acqi]** button, select **[Lock]**, and reduce lockpower and lockgain so that the lock level falls below 100%. (See Section 7.2: Locking.)

f. You can then **[Auto-Shim]** or shim manually in the **[Acqi]** window (preferred method).   Typically, the autoshimming takes ~5-10 minutes, so you will save time by shimming the magnet yourself. (See Section 7.4: Shimming.)

g. Once the sample is locked and shimmed, you can **[Setup Exp]** and choose the nucleus you want to observe. Standard parameters for that nucleus in the desired solvent are then retrieved.

See the following sections for more detailed descriptions of these processes.

7.1.   Loading a Sample

a. To load a sample, select the **[Acqi]** button on the main menu line. Select **[Lock]** and turn the spinner **[OFF]**.

b. Select the **[eject]** button, and the air will turn on and lift the dummy sample to the top of the magnet.

The VI- 500 is on air shocks to damp vibrations and will rock if touched. DO NOT TOUCH THE MAGNET.

c. Remove the dummy sample from the sample spinner and place your sample into the spinner. Use the sample depth gauge to position your sample in the spinner properly. For normal height samples the bottom of the NMR tube will go to the bottom of the sample depth gauge, and for shorter samples the sample must be centered on the middle line of the depth gauge. This will make shimming on short samples easier.

d. Select **[insert]** to lower the sample into the magnet.
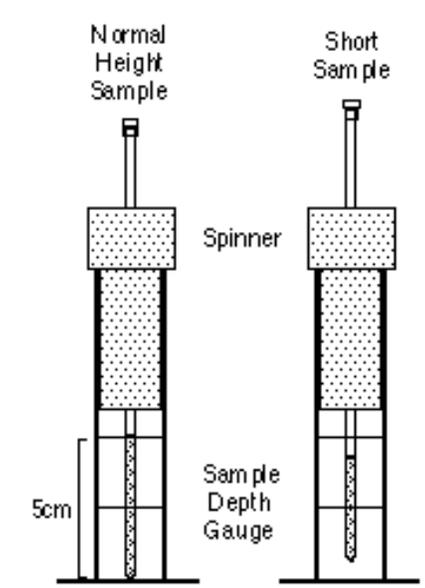
7.2.   Locking

The purpose of the lock is to maintain the magnetic field at a constant value throughout the experiment. The lock is essentially a deuterium NMR spectrometer that monitors the frequency of the solvent deuterium resonance and adjusts the magnetic field using the **Z0** shim to keep this frequency constant. The result is narrower lines in the spectrum than would be obtained without a lock. Locking is performed in the **[Acqi]** window.

Lock Power controls the power of the deuterium lock transmitter, and **Lock Gain** controls the amplification of the lock signal received from your sample. Using too much **Lock Power** (> 40 or so) will saturate the solvent deuterium resonance and lead to an unstable lock signal. Saturation occurs at different power levels for different solvents, e.g., acetone saturates at a much lower level (=10) than chloroform. Using larger values of **Lock Gain** may give a noisy lock signal. Keep the **Lock Gain** higher than the **Lock Power** to avoid saturation of your signal.

You can lock on your solvent, either manually or automatically.

MANUAL LOCK

In the [**Acqi**] window, select [**Lock**]. Set Lock to [**off**] and spin to [**on**].

a. Adjust **Z0** (in steps of 1 or 4 on the 300s and steps of 16 or 64 on the 500) using the left and right mouse buttons until a sine wave is seen. If no sine wave is seen, or if it is very low in amplitude, increase **Lock Gain** and, if necessary, **Lock Power**. Continue adjusting **Z0** until the wavelength increases and finally approximately one wavelength or less is seen:
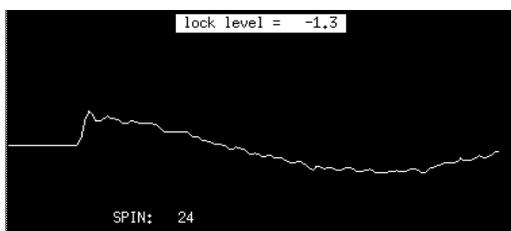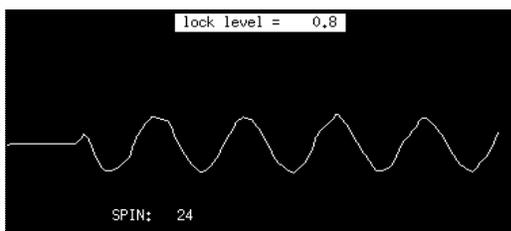
b. Turn the Lock [**on**] and you should see:
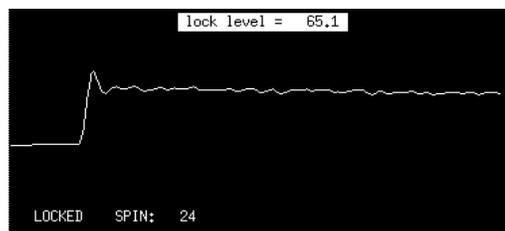
If you don't see this, turn Lock [**off**] and readjust **Z0** or increase **Lock Power** and/or **Lock Gain**.

c. Adjust **Lock Power** and **Lock Gain** until the lock signal is between 50 and 100%.

d. Adjust **Lock Phase** in steps of 1 to maximize the lock signal.

AUTOMATIC LOCK

Use the [**Change Sample**] - [**Set Solvent**] - [**Auto-Lock**] buttons or type **autolock('*solvent*')**. Then:

a. Open the lock display window with the buttons [**Acqi**] - [**Lock**].

b. If the autolock macro did not find the lock, turn the Lock [**off**] and spin to [**on**]. Adjust the value of Z0 until you find the correct frequency for your solvent (see manual locking).

c. Turn [**Lock**] to [**on**].

d. Reduce **Lock Power** and **Lock Gain** until the locklevel is between 50 and 100%.

e. Adjust **Lock Phase** in steps of 1 to maximize the lock signal.

*If you have difficulty locking, the problem is generally in the values of **Lock Power** and/or **Lock Phase** --the **Lock Power** may be too high and your signal is saturated or the **Lock Phase** value is incorrect. One or both of these are indicated if your lock level is very erratic or you lose the lock when you go to the Shim panel. Reduce the **Lock Power** and adjust **Lock Phase** so that you maximize the locklevel. Increase the **Lock Gain** as necessary. In extreme cases, the shims are also way off; reload the standard shims and try again.*

7.3.   Loading the Standard Shims

It is usually a good idea to load the standard shims, since the previous user may have made substantial changes to the shim values. To load the standard shims, use the menu buttons in the command and status window: **[Change Sample]** - **[Set Std Shims]**. Wait for the spectrometer to beep.

Alternatively, you can type **rts('STD_SHIMS_auto')**, followed by an **su** command. If you have a shim file for a particular sample, you can retrieve it this way, replacing STD_SHIMS_auto by your filename.

7.4.   Shimming

The purpose of shimming is to make the magnetic field homogeneous over the entire volume of the sample so that lines in the NMR spectrum will be as narrow as possible. Shimming is performed by adjusting the current flowing through a series of differently-shaped coils of wire surrounding the sample (current flowing through a coil of wire produces a magnetic field). Shims are divided into two categories, spinning (axial) shims and non-spinning (non-axial) shims. The spinning shims (**Z1 - Z7**) adjust the magnetic field along the length of the sample, and the non-spinning shims (everything else with an **X** or **Y**) have components perpendicular to this. As implied by the names, the spinning shims are adjusted with the sample spinning and the non-spinning shims are adjusted with the sample not spinning. Usually it is only necessary to adjust **Z1** and **Z2** (or **Z1C** and **Z2C**) for each new sample. For short samples it may also be necessary to adjust **Z3** and **Z4**. For experiments run under non-spinning conditions, you will need to adjust the non-spinning (X,Y) shims, especially if the lock level drops dramatically when you turn the spinner off.

a. Open the **[Acqi]** window and select **[Shim]**.

b. *Iteratively* adjust **Z1C** and **Z2C** (coarse adjustments) in steps of 1 or 4 to maximize the lock signal (300MHz). If the lock signal goes above 100%, reduce **Lock Gain** and, if necessary, **Lock Power** to bring the lock level to <100%. (N.B. The 500 does not have coarse adjustments for the shims. Use **Z1** and **Z2** in increments of 4 or 16.)

c. If necessary, *iteratively* adjust **Z1** and **Z2** (fine) in steps of 4 or 16 to maximize the lock signal.

d. When it appears that you can't make any more progress, adjust the **Lock Gain** so that the lock signal is between 50% and 100%. You may also want to check the **Lock Phase** to maximize the lock signal.

*e. Type **su** in the command line to setup the lock and shim parameters. This will also disconnect you from the Acqi window.*

f. If you want to save this shimset (perhaps you have a sample that you will need to run several times), type **svs('filename')**.

The best check of your shimming is the resultant peak lineshape: narrow and symmetric is the goal.
- In general, even numbered shims (Z2 and Z4) cause asymmetrical broadening, with misadjustments in Z4 being closer to the base of the peak. Odd numbered shims (Z1 and Z3) cause symmetrical broadening, again with misadjustments in Z3 being closer to the base.
- Acquire a quick spectrum (**nt=1**). (See Section 8 for detailed instructions on acquiring spectra.)
- Find a singlet line in your spectrum (the CDCl$_3$ peak at 7.24ppm or the TMS peak at 0ppm are good ones to use) and expand it.
- Place the cursor on the peak by clicking on it with the left mouse button and type **nl dres** to move to the top of the nearest line (**nl**) and display the digital resolution and linewidth (**dres**).

- The optimal linewidth will vary somewhat with molecular weight of the compound and the solvent, but for small organic molecules in non-viscous solvents like chloroform or acetone, the linewidth should usually be less than 1 Hz.
- If the lines in the spectrum are asymmetric or broader than you would like, shim on the sample some more and take another proton spectrum. Repeat until you are satisfied with the results.

8.   Acquiring a Proton Spectrum

8.1.   Setup and Acquisition

1a. If you have used the **[Change Sample]** menu button to lock on your solvent, you can continue with the **[Setup Exp]** option. Choose the appropriate nucleus and the standard parameters for that nucleus in your particular solvent will be chosen. Proceed to step 2.

OR

1b. Select **[Main Menu]** - **[Setup]** - **[Nucleus, Solvent]** - **[H1]** - **[D20]** (or whatever your nucleus and solvent are). If the solvent you are using is not listed on one of the menu buttons, select **[Other]** and type in the name of your solvent. To see a list of solvents recognized by the VNMR software type **cat('/vnmr/solvents')** and use the scroll bar on the text window of VNMR to scroll through the solvents.

2. Both options 1a and 1b will recall the standard parameters for acquisition. Change parameters as needed. To change the number of scans, set nt equal to the desired number, e.g., **nt=64**. See section 8.2 for more detailed descriptions of parameters.

3. To start acquisition of a standard proton experiment, type **ga** or select **[Main Menu]** - **[Acquire]** - **[Go, wft]**.

The command **ga** is equivalent to the **[Go, wft]** option which collects your spectrum and automatically processes it.

The command **go** or menu button **[Go]** will only acquire your spectrum; you must process it yourself by typing **wft**. This is a useful option for 2D experiments where you do not want to process your data on the spectrometer.

The menu button **[Go, periodic wft]** will acquire your spectrum and process it after every **bs** number of scans. This is equivalent to typing **go** or **ga**, then **wft** whenever you wish to update your spectrum. The **[Go, Periodic wft]** option, however, will slow you down if you are doing any data manipulation during the acquisition.
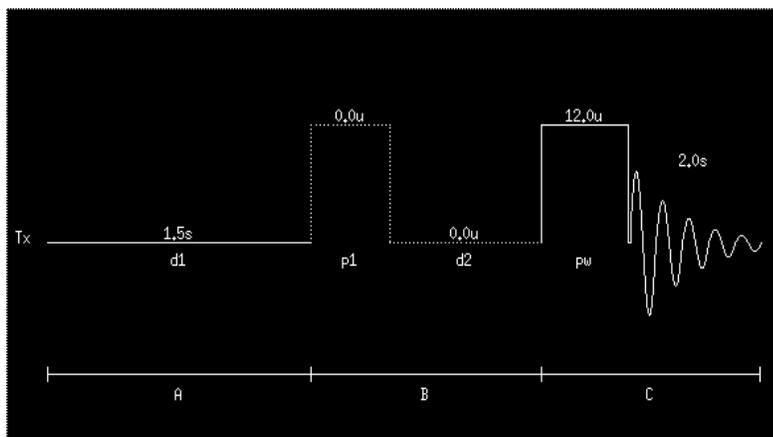
4. When the experiment is complete, the spectrum will be displayed in the graphics window. Once the spectrum appears, try to auto-phase it by typing **aph**. If it doesn't autophase correctly, you may need to manually phase the spectrum (see section 12.4).

5. You should also save your file: **svf('*filename'*)**. See section 11 for more details on saving and retrieving data.

8.2. Parameters

The standard parameters should be fine for most routine proton spectra, with the possible exception that the number of scans will need to be increased for dilute samples. The parameter **nt** controls the number of scans and can be set to a different value by typing **nt=64**, e.g. Remember that spectral signal-to-noise increases by the square-root of the number of scans -- to double the signal-to-noise, you must collect 4x as many scans.

The standard proton pulse sequence as displayed by the **dps** command is shown below. The Acquisition and Observation parameter page is also shown.

The relevant acquisition parameters are:

Spect. width (sw) - spectral width in Hz. Total width of spectrum. Data points are acquired at a rate of one complex point every 1/**sw** seconds. Use the command **movesw** to change **sw** after setting the cursors to the desired region. This command also automatically adjusts the transmitter offset, **tof**.

Acq. time (at) - acquisition time. **at** = **np**/(2x**sw**). 1/**at** is the best spectral resolution obtainable (assuming perfect shimming), e.g., with a 2 second acquisition time you will not be able to resolve peaks less than 0.5 Hz apart.

Acquired complex points (np) - number of data points acquired. Increase np to increase the resolution.

Recycle delay (d1) - relaxation delay. For most samples the default value of 1.5 seconds will be adequate, however, if the relaxation times (T1) of protons in the sample are known to be longer than a couple of seconds, this value should be increased.

Transients (nt) - number of transients (scans).

Steady state (ss) - steady-state scans. Dummy scans done at the beginning of the experiment to obtain

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steady-state magnetization - usually used in 2D experiments.

Nucleus (tn) - transmitter nucleus. The nucleus to be observed.

Spect Freq (sfrq) - spectrometer frequency in MHz. This is set automatically when a nucleus and solvent
are selected.

offset (tof) - transmitter offset in Hz. Determines the center of the spectral width acquired. To change this
value, use the command **movesw** (see above) or put the cursor on the desired frequency for **tof** and type
movetof.

obs pulse (pw) - pulse width. The length of the radio-frequency pulse used to excite the nuclear spins.
To obtain maximum signal from a single pulse, this should be set to a 90-degree pulse,
typically 7-15 microseconds (depends on instrument, probe, and tpwr value).

Power (tpwr) - transmitter power. A lower power results in a weaker pulse and
a longer 90 degree pulse width. Do not exceed 58 on the PFG probes.

pi/2 pulse (pw90) - 90-degree pulse width. Frequently calibrated and set by NMR Lab staff.

To change a numerical parameter simply type **nt=128** or **pw=pw90** or type the appropriate value into the dialog box.
To change a string parameter type **tn='H1'** (single quotes around the string value). To find out the value of any
parameter, you can query it, e.g., **pw?** will tell you the value of the pulsewidth.

There are also certain flags and conditions that can be set.

- Selecting the option "On Insert" for Autolock and Autoshim tells the instrument to autolock and autoshim
before the acquisition begins.

- With Autogain selected (gain='n'), the receiver gain is automatically adjusted. (N.B. This is not the same as
Lock Gain.)

- Blocksize (bs) - block size. Data is saved to disk and can be displayed every **bs** scans so that you can look
at the spectrum while still acquiring data--type **wft** to update the display.

- "When exp completes" or **wexp** tells the computer to execute a particular command at the end of the experiment.

- "At each blocksize" tells the computer to execute, for instance, a **wft** after every block (equivalent to the **[Go,
periodic wft]** option).

9.   Acquiring a Carbon Spectrum

9.1.   Setup and Acquisition

1a. If you have used the **[Change Sample]** menu button to lock on your solvent, you can continue with the **[Setup]** option. Choose C13 as the nucleus and the standard parameters in your particular solvent will be chosen. Proceed to step 2.

OR

1b. Select **[Main Menu]** - **[Setup]** - **[Nucleus, Solvent]** - **[C13]** - **[D20]** (or whatever your solvent is). If the solvent you're using is not listed on one of the menu buttons, select **[Other]** and type in the name of your solvent. To see a list of solvents recognized by the VNMR software type **cat('/vnmr/solvents')** and use the scroll bar on the text window of VNMR to scroll through the solvents.

2. Both options 1a and 1b will recall the standard parameters for acquisition. Change parameters as needed. To change the number of scans, set nt equal to the desired number, e.g., **nt=128**. See sections 8.2 and 9.2 for more detailed descriptions of parameters.

If you are unsure about how many scans will be needed to obtain sufficient signal-to-noise, set **nt** very large (**nt=100000**) and **bs=16** and run the experiment. Check the spectrum periodically by typing **wft** until the spectrum looks sufficiently good and abort the acquisition using the **[Abort Acq]** button or by typing **aa** and then save the FID. (See the **[Go, Periodic wft]** option in step 3.)

To determine how long an acquisition will take, type **time** on the command line. This will display the approximate length of time the currently set-up acquisition will take.

3. To start acquisition of a standard carbon experiment, type **ga** or select **[Main Menu]** - **[Acquire]** - **[Go, wft]**.

> The command **ga** is equivalent to the **[Go, wft]** option which collects your spectrum and automatically processes it.

> The command **go** or menu button **[Go]** will only acquire your spectrum; you must process it yourself by typing **wft**. This is a useful option for 2D experiments where you do not want to process your data on the spectrometer.

> The menu button **[Go, periodic wft]** will acquire your spectrum and process it after every **bs** number of scans. This is equivalent to typing **go** or **ga**, then **wft** whenever you wish to update your spectrum. The **[Go, Periodic wft]** option, however, will slow you down if you are doing any data manipulation during the acquisition.

4. When the experiment is complete, the spectrum will be displayed in the graphics window. Once the spectrum appears, try to auto-phase it by typing **aph**. If it doesn't autophase correctly, you may need to manually phase the spectrum (see section 12.4).

5. You should also save your file: **svf('*filename*')**. See section 11 for more details on saving and retrieving data.

9.2.   Parameters

The standard parameters should be fine for most routine carbon spectra, with the possible exception that the number of scans may need to be increased. The parameter **nt** controls the number of scans and can be set to a different value by typing **nt=512**. Remember that spectral signal-to-noise increases by the square-root of the number of scans -- to double the signal-to-noise, you must collect 4x as many scans. Increase **d1** to 3-5 seconds if you are missing quaternary or carbonyl carbons as these types of carbons have longer relaxation times.

The standard carbon pulse sequence as displayed by the **dps dg** commands is:
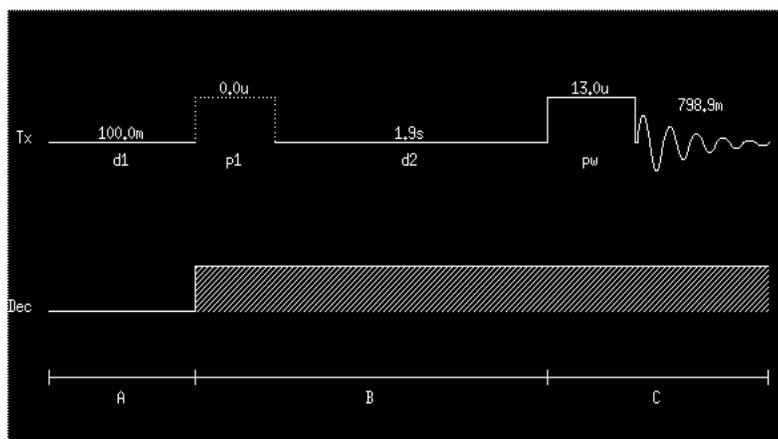

```
   ACQUISITION           SAMPLE            PROCESSING           FLAGS
sfrq    125.893  date    Jun 27 94  lb           1.20  il              n
tn          C13  solvent       D2O  sb       not used  in              n
at        0.800  file          exp  gf       not used  dp              y
np        48000       DECOUPLING     awc      not used  hs             ny
sw       30000.0  dn             H1  lsfid    not used  hst         0.015
fb        16500  dof          13.9  phfid    not used  alock           n
bs           32  dm            nyy  wtfile             gain           40
ss            0  dmm             w  proc           ft  spin     not used
tpwr         60  dmf          8800  fn       not used      SPECIAL
pw         13.0  dpwr           38  math            f  temp     not used
p1            0  homo            n                     priority        5
d1        0.100                     werr
d2        1.900                     wexp          wft
tof       657.2                     wbs
nt           64                     wnt
ct            0
```

The top line of the pulse sequence shows the pulses on carbon and the bottom line shows the proton decoupler. The relevant acquisition parameters are the same as those described above for proton spectra, except for:

   d2 - proton presaturation time for NOE enhancement of the carbon spectrum.

Additionally several parameters control the decoupler:

   nucleus (dn) - decoupler nucleus.
   Decplr Frq (dfrq) - decoupler frequency in MHz.
   Offset (dof) - decoupler offset in Hz. Determines the frequency of the decoupler. Larger values move
         the decoupler downfield. This is commonly set with the **sd** command.
   ON/OFF status (dm) - decoupler mode. The pulse sequence is divided into three status periods - A, B, and C,
         which correspond to the times **d1**, **d2**, and **at**. The parameter **dm** is set to a string of three
         characters giving the on/off status of the decoupler during each of these periods.
               For a standard carbon experiment: **dm='nyy'** (default setting)
               For quantitative carbon spectra with no NOE enhancement: **dm='nny'**
               For proton coupled carbon spectra: **dm='nyn'**
   Power (dpwr) - decoupler power. Never exceed 40 or you may damage the probe.
   pi/2 pulse (dmf) - decoupler modulation frequency. Do not change. Calibrated and set periodically by NMR
            Lab staff.
   Modulation (dmm) - decoupler modulation mode. Set to **w** (waltz decoupling) for efficient decoupling of
            protons.
   homo - homonuclear decoupling flag. **homo='n'** for proton decoupling of carbon spectra.

10.   Acquiring a DEPT Spectrum

DEPT (Distortionless Enhancement by Polarization Transfer) spectra are used to determine the number of protons bonded to each carbon atom. You must be able to collect a decent ^{13}C spectrum to get a good DEPT spectrum.

10.1.   Setup and Acquisition

1a. To set up a DEPT experiment, first set up a carbon experiment. Proceed to step 2.

OR

1b. If you have already run a carbon experiment *on the same instrument*, load the FID.

2. Type **dept**. The only parameter that may need to be changed is the number of scans, **nt**.

3. The **time** command will display how long the experiment will take.

4. Type **ga**.

5. Once the DEPT experiment is done, type **wft** to process the spectra.

6. Type **ds(1)** to display the first spectrum.

7. Phase this spectrum so that all peaks are positive.

8. Type **dssa** to display the two DEPT spectra. The bottom spectrum contains only CH carbons and the top spectrum contains CH and CH_3 carbons (none in this example) pointing up and CH_2 carbons pointing down:
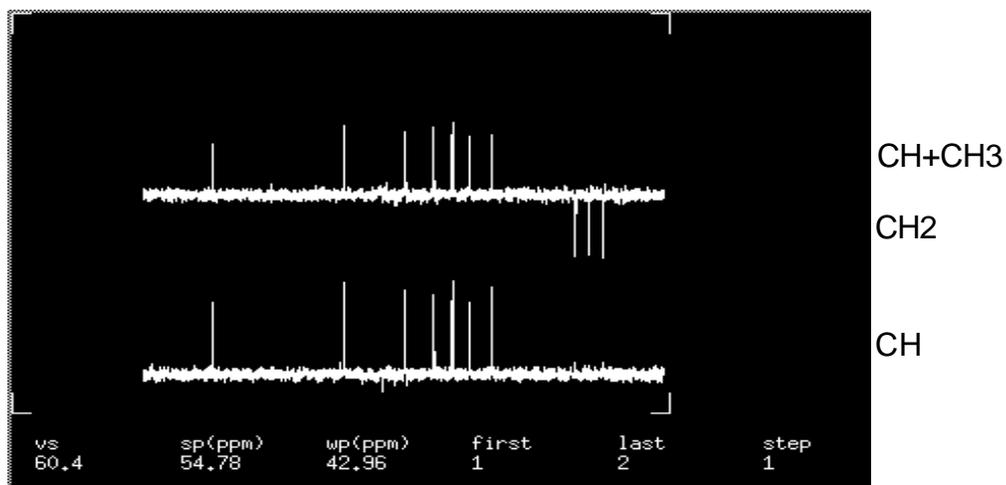
9. To plot dept spectra, use the following sequence of commands: **pl('all') pscale ppa page**.

11.   Saving and Loading Data

The VNMR program runs on Sun computers under the UNIX operating system. Therefore all files are stored in a UNIX filesystem and all file operations are actually done using UNIX commands. The VNMR program provides a simplified interface to the UNIX operating system, so understanding UNIX is not essential. All file operations in the VNMR program are done through the menu buttons under **[Main Menu]** - **[File]**. The buttons appearing at this point allow access to other menu buttons:

```
Set Directory  File Info  Tape  Load  Data  Delete  More  Return
```

If you prefer to use unix, open up a shell window using the left mouse button in the background to get a pull-down menu.

The off-line workstations have a slightly different menu structure:

```
Load FID   File Ops   DATA   VAC-200   VAC-300   Old fids   Other dirs   Return
```

[Load FID] - load your data (FID = Free Induction Decay)
[File Ops] - menu buttons for performing operations on files (copy, rename, delete, etc.)
[Data] - menu buttons for changing to your data directory and saving and loading data from the hands-on instruments
[VAC-200] - (data stations only) data collected on the 200 MHz autosampler, saved by date and sample number
[VAC-300] - (data stations only) data collected on the 300 MHz autosampler, saved by date and sample number
[Old fids] - (data stations only) menu buttons for working with old data that is on CD-ROMs
[Other dirs] - menu buttons for accessing other directories
[Return] - to go to the next upper menu line

11.1 Saving Data

Once a good spectrum is obtained, save it to disk for later processing and plotting. VNMR opens your data directory automatically when you log in. Filenames are essentially unrestricted in length and can consist of any alphanumeric string with no spaces in it and with no special characters except the dash "-" and underline "_" as separators. The **[Save FID]** button and the **svf** command prompt you for a filename and will save the data under that name with a '.fid' extension.

1a. Select the buttons: **[Main Menu]** - **[File]** - **[Data]** - **[Save FID]** to access your data directory. Enter a filename when prompted.

OR

1b. Type **svf** <return> and enter a filename when prompted. Or **svf(*'filename'*)**. Parameters only can be saved by the command **svp(*'filename'*)** and an extension .par will be appended. Shims only can be saved by the command **svs(*'filename'*)** and will be saved in your shim directory.

You can type **pwd** (a UNIX command that also works in VNMR) to print the current working directory. Your data directory as printed by **pwd** will be something like "/data/nmryao". After you change to your data directory, you won't have to hit the **[Data]** button again unless you first change to some other directory. Another way to change into your data directory is to type **cd(*'/data/6-letter code*)** on the command line to access data collected on the hands-on instruments. Type **cd(*'/v3data/6-letter code*)** for data from the VAC-300 or **cd(*'/v2data/6-letter code*)** for data from the VAC-200. This method can also be used to accessed other people's directories using their 6-letter code.

11.2 Retrieving (Loading) Data

1. To load data using the menu buttons, select: **[Main Menu]** - **[File]**. Your data directory opens by default. If you want to access autosampler data, select **[VAC-200]** or **[VAC-300]**.

2. Select the file you want to load by highlighting it with the left mouse button. (A second click will deselect the filename.)

3. Select the **[Load]** or **[Load FID]** button and the data set will be loaded into the VNMR program. As the name FID (Free Induction Decay) implies, only the time-domain data is saved.

> To retrieve parameters only, type **rtp(*'filename'*)** or highlight the parameter set and select **[Load]**.

> To retrieve shims only, type **rts(*'filename'*)** and type **su**.

4. After loading the data set, type **wft** to Fourier Transform the data and display the spectrum. Since all parameters are stored with the data at the time the **[Save FID]** is done, you should see the spectrum exactly as it was when you saved it.

5. (optional) After you do more data processing (phasing, setting the threshold for peak-picking, integration, etc.) you may want to save the data again under the same name, so the next time you load the data, you won't have to repeat all these steps. The easiest way to do that is to type **svf(*'filename'*)** in your *data* directory. N.B. You do not have "write" permission for the autosampler directories. If you use the same filename as the file you loaded, the program will prompt you for an answer to the question "File exists, overwrite [y/n]?" to which you type **y**.

Retrieving old data:

To recall old spectra that have been backed up and are no longer in your data directory, select **[Main Menu]** - **[File]** - **[Old fids]**. Select **[List]** to see a list of all your saved data. Find the file of interest in the second column , then select the CD-ROM location from the first column. Select the **[Select CD]** button to mount that CD. If your data is on a CD-ROM that is not loaded, ask Letitia or Steve to load the appropriate CD for you. This will then be accessible under the selection **[Special]**.

Retrieving other people's data:

To retrieve data from someone else's directory, you must know their 6-letter code.   Type **cd(*'/data/6-letter code'*)** to access data collected on the hands-on instruments. Type **cd(*'/v3data/6-letter code'*)** for data from the VAC-300 or **cd(*'/v2data/6-letter code'*)** for data from the VAC-200. Select **[File]** and those files will be displayed.

12.   Data Processing and Analysis

Once your data has been acquired on a spectrometer, save it and do all the processing and plotting on a data station, not the spectrometer (unless you are acquiring more data while you process and plot a previously acquired spectrum). Our goal is to maximize the amount of time the spectrometers are busy collecting data.

Data processing will generally consist of some or all of the following steps, each of which will be described in the following sections:
> Fourier Transform (section 12.1)
> Apodization (section 12.2)
> Interactive Display Mode (section 12.3)
> Phasing (section 12.4)
> Referencing (section 12.5)
> Peak Picking (section 12.6)
> Integration (section 12.7),

12.1.   Fourier Transform

The Fourier Transform provides a mathematical relationship between the time domain and the frequency domain. A typical NMR experiment is carried out by applying a short radio-frequency pulse at the appropriate frequency to excite the nuclear spins. This is followed by detection of the signal for a second or two as the spins relax back to equilibrium. The signal in an NMR experiment is thus acquired in the time-domain with the signal consisting of a number of damped sinusoidal signals oscillating at different frequencies corresponding to the different chemical shifts and J couplings present. The Fourier Transform takes a time-domain signal consisting of sums of sinusoidal signals and gives a frequency-domain spectrum with peaks at the frequencies of the sinusoidal oscillations in the time-domain data.

A Fourier Transform is performed in the VNMR program by typing **wft** (weighted Fourier Transform) or by using the menu buttons: **[Main Menu]** - **[Process]** - **[Weight, Transform]**. After data has been Fourier Transformed, the **ds** command is automatically executed to display the spectrum.
Default processing can be done by typing the command **process**. This command, however, may not always do a good job of processing, especially phasing and integrating, so it is useful to know how to do manual processing.

12.2.   Apodization (optional)

If the spectrum is not what you expected in terms of signal-to-noise or resolution, you can try processing your data further. Apodization is the process of multiplying the time-domain data by a weighting function prior to Fourier Transform of the data. The effect of the multiplication is to modify the lineshape of peaks in the spectrum. Two types of apodization are most common: sensitivity enhancement and resolution enhancement. The weighting function can be further customized using the interactive weighting program by typing **wti** or using the menu buttons **[Main Menu]** - **[Process]** - **[Adj Weighting]**. After a new apodization is selected, you must type **wft** to apply the apodization, do the Fourier Transform, and display the new spectrum.

*It is a good idea to first look at your data with the current parameters by typing **wft** before you apply further apodization. Your spectrum may not need further processing.*
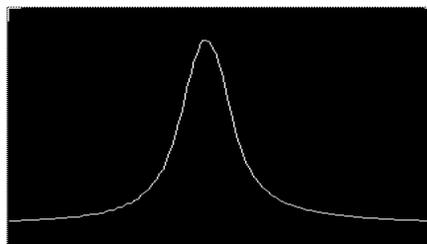
Zero-Filling

The main parameter related to the Fourier Transform is **fn**, the size of the Fourier Transform. This must have a value that is a power of 2 and determines how much zero-filling is done at the end of the FID prior to the Fourier Transform. The amount of zero-filling determines how well defined the peaks in the spectrum are.
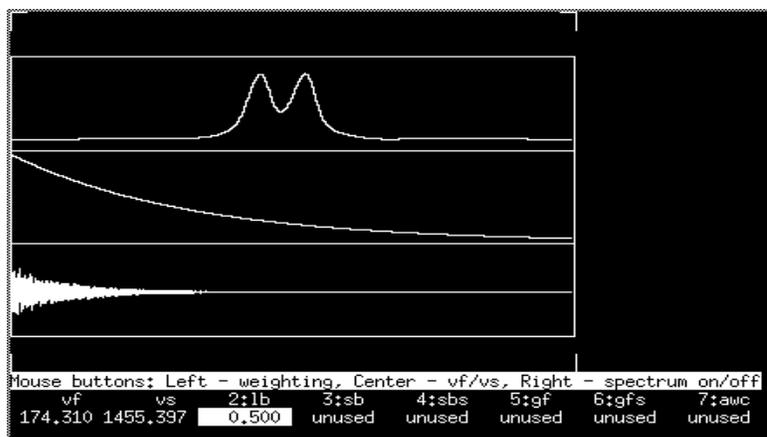
Small **fn**:
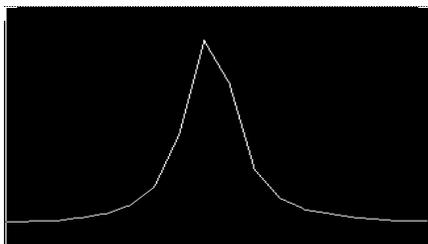
Large **fn**:

This also affects how accurately peaks are picked and integrals are calculated, so it is quite important to use a value of **fn** that is at least two times larger than **np** (the number of data points acquired) for quantitative work.

The interactive weighting program (**wti** command) displays the spectrum on the top, the FID on the bottom, and the weighting function in the center.

```
Mouse buttons: Left - weighting, Center - vf/vs, Right - spectrum on/off
      vf        vs       2:lb      3:sb      4:sbs     5:gf      6:gfs     7:awc
  174.310  1455.397    0.500    unused    unused    unused    unused    unused
```

The right mouse button turns the spectrum on and off. The vertical scale of the FID and spectrum can be adjusted using the middle mouse button. The value of the weighting function parameter (in this case **lb**) can be adjusted using the left mouse button.

The weighting function -- line broadening (**lb**), Gaussian (**gf**), shifted Gaussian (**gfs**), sine-bell (**sb**), shifted sine-bell (**sbs**), or additive weighting constant (**awc**) -- can be selected using the menu buttons that appear with the **wti** display.


```
 1:next fid )  2:lb )  3:sb )  4:sbs )  5:gf )  6:gfs )  7:awc )  8:return )
```

Alternatively, select **[Process]** from the right-hand toolbar in the **dg** window to display the processing parameters. You can change the parameters by typing in new numbers for linebroadening (**lb**), sinebell (**sb**), shifted sinebell (**sbs**), gaussian (**gf**) or shifted gaussian (**gfs**). Type **wft** to apply the apodization and return to the interactive display mode.

2 examples of common weighting functions are shown below:

Sensitivity enhancement

Sensitivity enhancement, also called line broadening, is typically performed with a decaying exponential weighting function. You can simply change the **lb** value to a higher number, e.g., **lb=3** to apply a line broadening of 3 Hz to your spectrum and type **wft** to apply this new function. You can also do this interactively using the menu buttons: **[Main Menu]** - **[Process]** - **[Select Params]** - **[Broaden]**, giving the weighting function shown here using the **wti** command:
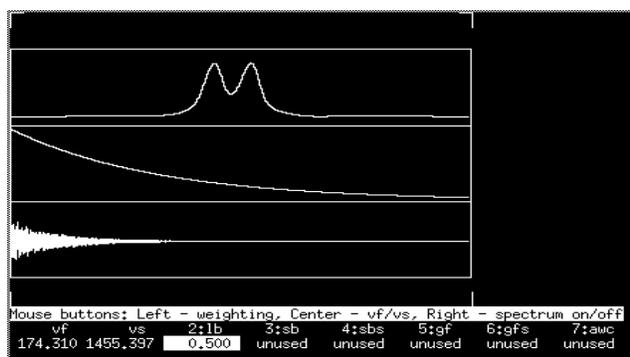
Adjust the value of **lb** by clicking the left mouse button in the middle window. The effect of multiplying the FID by a decaying exponential can be seen as preferentially weighting the early portion of the FID where most of the signal is present and attenuating the later portions of the FID where only noise is present. After Fourier Transform, the spectrum will appear to have better signal-to-noise, but the lines in the spectrum will be slightly broader.

Resolution Enhancement

Resolution enhancement is typically done using a Lorentz-to-Gauss weighting function, but can also be done with a sine-bell or shifted Gaussian. The Lorentz-to-Gauss function can be selected using the menu buttons: **[Main Menu]** - **[Process]** - **[Select Params]** - **[Resolve]**, giving a weighting function like this:
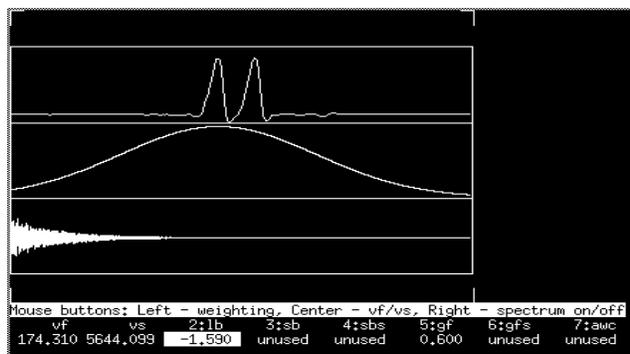
Note that the value for **lb** is negative. Adjust **lb** or **gf** by first selecting the appropriate option in the menu bar, then using the left mouse button in the middle window to change the value. The effect of multiplying the FID by this function prior to Fourier Transform is to preferentially weight the center portion of the FID and attenuate the beginning and end of the FID. This has the effect of removing the broad, fast-decaying tails of the lines which are present in the beginning portion of the FID, giving a narrower line with smaller tails, but with noticeably worse signal-to-noise. Resolution enhancement can be very useful when attempting to measure small J couplings in a spectrum.

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12.3.   Interactive Display Mode (ds command)

The **ds** command (also accessible through the menu buttons: **[Main Menu]** - **[Display]** - **[Interactive]**) displays your spectrum in the interactive mode. This command is also automatically executed whenever a Fourier Transform is done using the menu buttons or by typing **wft**.

It is possible to interact with the spectrum displayed in the graphics windows by a combination of menu and mouse buttons. When the interactive display mode is entered, the following row of menu buttons becomes available:

| Box | Int | Full | Mark | Phase | Th | Resets | Dscale | Lvl/Tlt | Set Int | Ref | Pbox | Return |

These buttons allow access to many commands needed when processing data.

The first and third menu buttons are toggled depending on whether one or two vertical cursors are displayed in the graphics window. If one cursor is displayed, the buttons are **[Box]** and **[Full]**, and if two cursors are displayed, the buttons are **[Cursor]** and **[Expand]**.

The **[Box/Cursor]** button is used to toggle between one and two cursors, and the **[Full/Expand]** button is used with two cursors displayed to expand the display to show only the region of the spectrum between the two cursors, or with one cursor displayed, to go back to displaying the entire spectrum.

The left and right mouse buttons are also used in several special modes for phasing, setting integral resets, adjusting the peak-picking threshold, and adjusting the integrals, each of which is described below. The middle mouse button is always used for adjusting vertical scales of the spectrum or the integration.

Expansions

The left and right mouse buttons control red vertical cursors and can be used to select regions for expansion display. To expand a region, place the left cursor (with the left mouse button) at the downfield end of the region of interest. Place the right cursor (with the right mouse button) on the upfield end of the region of interest. (With 2 cursors displayed, the left mouse button will move the positions of both of the cursors simultaneously. The right mouse button only adjusts the position of the right cursor.) Select **[Expand]** from the menu line.

To go back to a full spectrum, select **[Full]** from the menu line. The commands **f full** will also put you back to a full spectrum.

Vertical Scaling

The middle mouse button controls the vertical scale of the spectrum or, if displayed, the integral. (N.B. You cannot adjust the height of the spectrum with the mouse while the integral is displayed.) To increase the height of the spectrum, click with the middle mouse button above the peaks of interest. To decrease the scaling, click *below* the spectrum.

The spectrum vertical scale is determined by the parameter **vs** and can automatically be adjusted using the command **vsadj**. You can also change the scaling by typing a value for vs, e.g., **vs=1000**. You can decrease the height of the spectrum by half if you type **vs=vs/2**.

The integral vertical scale is determined by the parameter **is** and can be adjusted with the middle mouse button or by using the command **isadj** or type **is=#**.

The vertical position (**vp**) of the spectrum on the screen and plot can be adjusted by typing **vp=12**, where 12 is the minimum required for integration values to be printed below the spectrum.

12.4.   Phasing

Once the Fourier Transform has been done, the data needs to be phased to give pure absorption lineshapes. This can often be done using the **aph** command to auto-phase the spectrum. If auto-phasing does not work properly, manual phasing must be performed.

1. While in the interactive display mode (**ds**), click on the **[Phase]** button. At this point the cursor disappears from the graphics window.

2. To start phasing, click with the left mouse button above a peak near the upfield edge of the spectrum, and you will see:
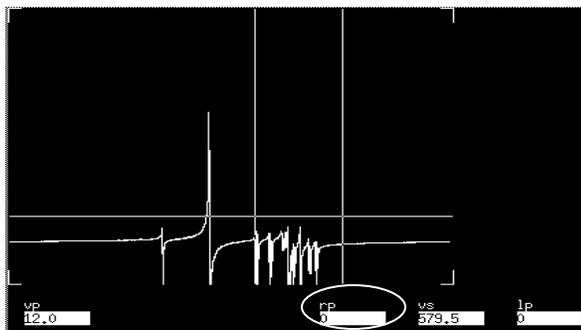
3. Adjust the phase of the peaks between the cursors by clicking and dragging up or down with the left (coarse) or right (fine) mouse button until the peaks look properly phased and the baseline is flat. If the cursor goes off the graphics window without the peak being properly phased, click on the **[Phase]** button again and start over. This part of the phasing process where you phase peaks on the right side of the spectrum changes the value of the VNMR parameter **rp**. This is a constant (zero-order) phase correction that is applied across the entire spectrum.

4. Once the peaks on the right side of the spectrum are properly phased, click with the left or right mouse button on a peak near the left edge of the spectrum, and drag up or down to phase these peaks properly. This part of the phasing process where you phase peaks on the left side of the spectrum changes the value of the VNMR parameter **lp**. This is linear (first-order) phase correction that is zero at the place where you did the zero-order phase correction and is linearly increasing across the spectrum. Usually the magnitude of **lp** should be less than 360. If it is larger, the baseline of the spectrum may not be flat, and/or it may be impossible to phase all peaks in the spectrum at the same time. If this occurs, it may be useful to type **lp=0**, and start over with the phasing process.

5. If the values of **lp** and **rp** get very large (> 500), you may want to set **lp=0** and **rp=0** and start over.
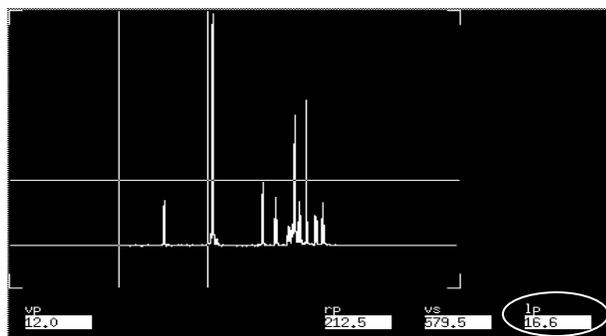
6. Once the spectrum is phased, click on the **[Box]** or **[Cursor]** button to exit phase mode. If the spectrum is still not phased properly, repeat these steps.


12.5.　　Referencing

The axis for the spectrum can be displayed by typing **dscale** or by clicking on the **[Dscale]** menu button. To display the scale in hertz, type **axis='h'** before the dscale command. To set the scale back to ppm, type **axis='p'**. If you selected the proper solvent when setting up a proton or carbon experiment, the axis should be very close to correct, however, if you want to make sure it is exactly correct, you should re-reference the spectrum using a reference line like TMS (0ppm) or the solvent line (since a small fraction of solvent molecules are protonated, you will usually see a solvent line in proton or carbon spectra).

1. To reference the spectrum, expand the display to show the reference line you want to use.

2. Click on the reference line with the left mouse button to move the cursor to that line and type **nl** which brings the cursor to the nearest line.

3a. Type **rl(0p)** (for TMS) or **rl(7.24p)** (for chloroform).

3b. Alternatively, use the menu button **[Ref]** and type the reference ppm value at the prompt.

12.6.　　Peak Picking

The frequencies of the peaks in the spectrum are typically the most useful information obtained from the spectrum.

1. To adjust the threshold for peak picking, select the **[Th]** menu button in the interactive display mode.

2. This will display a yellow horizontal line that can be dragged up and down using the left mouse button to set the appropriate level for peak picking.

3. Once you've set the threshold, click on the **[Th]** button again to exit the threshold adjustment mode.

4. To get a listing of peaks displayed on the spectrum, the command **dpf** or **dpf('top')** can be used:
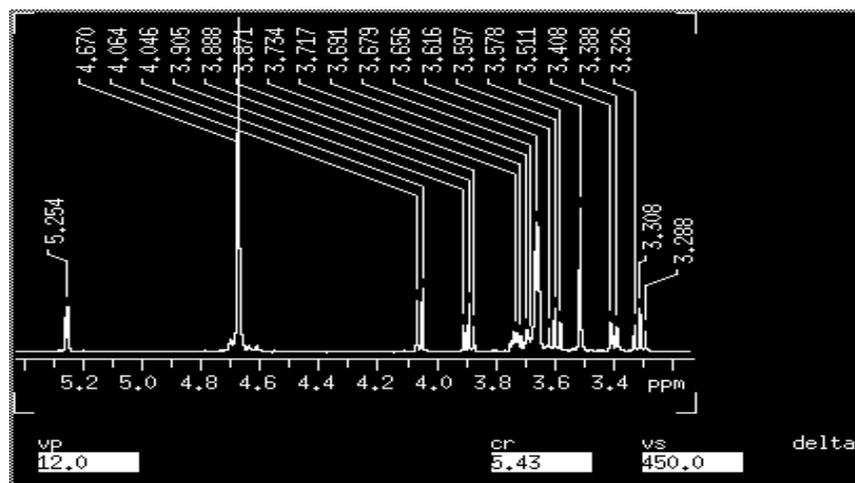
This command will pick all peaks above the threshold line and display those frequencies. Note that the **dpf** command takes you out of the interactive display mode. Type **ds** to return to it. A list of peaks can also be printed out to the VNMR text window using the **dll** command or directly to the plotter by the **pll** command.

12.7. Integration

1. The Integral options are **[Int]** and **[No Int]**. When in the **[Int]** mode, the green integral line is displayed.

2. With the integral displayed, the middle mouse button will adjust the vertical scale of the integral instead of the spectrum. (The vertical scale of the integral can also be adjusted automatically using the command **isadj**.)

3. If the integral line is not flat and horizontal, you can adjust the slope and bias of the integrals. Select the menu button **[lvl/tlt]**. This works much like phasing. First click on an integral region towards the right side of the spectrum and drag up or down with the left (coarse) or right (fine) mouse buttons until the integral looks like it is flat in the baseline regions to the left and right of the peak. Then click on an integral region towards the left side of the spectrum and drag up or down with the left or right mouse button until that integral region looks correct. Then click on the **[Box]** button to get you out of level-tilt mode. Repeat if necessary. The first time you click and adjust the integral you are adjusting the slope of the integral across the entire spectrum (the VNMR parameter **lvl**). The second time, you are adjusting the bias of the integral (the VNMR parameter **tlt**) - a linearly increasing correction to the slope across the spectrum.

4a. Then select **[resets]**. The left mouse button can now be used to select where to break the integral (set a zero) -- typically you will want to click with the left mouse button to the left and to the right of each peak in the spectrum that you want to integrate. If you make a mistake when setting the integral resets, you can click with the right mouse button to remove the nearest reset. Or click many times with the right mouse button to remove all resets or type **cz** (clear zeroes). *The integrated region is the solid green line; non-integrated regions are shown as a dotted line.*
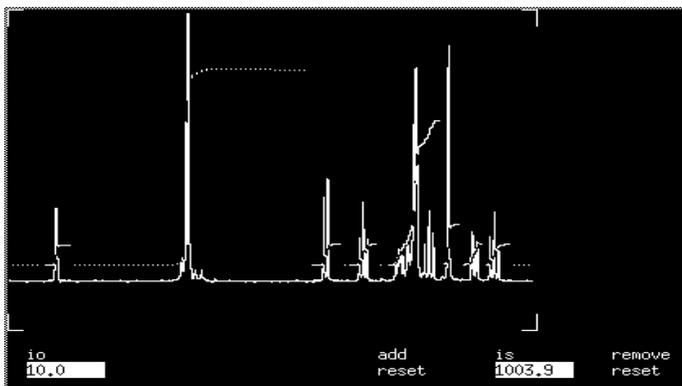
4b. You can also set the integrals by moving the cursor to each zero point and typing **z** followed by a return. This lets you expand regions without having to get out of the **[resets]** mode. The command **cz** also can be typed to clear all integral resets.

5. If you used step 4a, when you are done, click on the **[Box]** button to get you out of **[resets]** mode.

6. (optional) To display the integrals on the screen, make sure that **vp** is at least 12 (**vp=12**) and type **dpir** or **dpirn** (normalized).

7. (optional) To rescale the values displayed by either command, change the value of the parameter **ins**. For **dpirn**, set the values out of a possible 100 with **ins=100**. If you want a particular integral region to be equal to 3 (if it is a methyl group, for instance), place the cursor on that region and select the **[Set Int]** button, then type 3 at the prompt. In this case, use **dpir** to display the values.

8. (optional) Once the integrals have been divided into regions with *all* peaks under an integral, the command **bc** (baseline correct) will flatten all regions of the spectrum that aren't under an integral (baseline regions).

13. Plotting

Once the data has been processed, you will probably want to plot one or more views of the spectrum. The VNMR parameter **plotter** should be set to **'LaserJet_300R'** for landscape plots. To plot in portrait mode, type **plotter='LaserJet_300'**. The menu buttons can also be used to select the plotter. Select **[Main Menu]** - **[Change Plotter]**. Toggle this button until the desired plotter is displayed.

Landscape

Portrait

LaserJet_300R   LaserJet_300
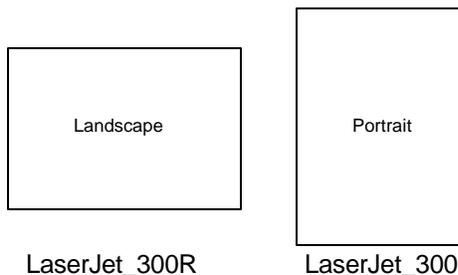
To put a title on your spectrum, type **text('title')** or **text('title of plot\\more text')** for multiple lines of text. The title will be plotted in the upper left of the page by the commands **ppa**, **pap**, or **pltext**. **pltext** is not necessary if you use **pap** or **ppa**.

The quick and dirty way to plot your spectrum is by typing **plot**.

For manual plotting, select one command from each group below:

For plotting the spectrum, choose one of the following:
> **pl** - plot spectrum and, if currently displayed, the integral.
> **plww** - plot a series of spectra taken in an arrayed experiment (DEPT, e.g.) in whitewash mode.
> **pl('all')** if you have an array of spectra (as with DEPT)

For plotting your scale:
> **pscale** - plot axis under spectrum. To change the units on the axis type **axis='h'** for Hertz or
>   **axis='p'** for ppm before typing **pscale**.

For listing parameters on your spectrum, choose one of the following:
> **pap** - plot all parameters in the upper left of the page.
> **ppa** - plot a short list of parameters in the upper left corner of the page.
> **pltext** - plot only the text; this is not necessary if you execute the **pap** or **ppa** commands.

For peak frequencies, choose one of the following:
> **ppf** - plot peak frequencies above spectrum with a line going down to the peak.
> **ppf('top')** - same, but peak frequencies are all plotted along the top of the plot.
> **ppf('pos', 'top')** - same, but plot only positive peaks.
> **vp=vp+80 ppf('pos', 'top') vp=vp-80** - same, but plot with short lines that don't go
>   all the way down to the top of the peak.

For integrations, choose one of the following:
> **pir** - plot integrals under the spectrum. **vp** (vertical position of the spectrum) must be at least 12 for
>   this to work. Use this if you have set the value of a region to a particular number.
> **pirn** - plot normalized integrals (out of 100%) under the spectrum. Again, **vp** must be at least 12.

To send your spectrum to the plotter (you *must* execute this command):
> **page** - send plot to the plotter. The output from all plotting commands go on the same plot until
>   the **page** command is typed.
> **page('clear')** - delete everything that has been plotted so far instead of sending it to the printer.

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For separate peak listings, choose one of the following:

> **pll** - plot a list of peak frequencies in ppm and in Hz. Do not do a **pap** or **ppa** or **page** if you do this
>   command.
> **pli** - plot a list of integrals. Do not do a **pap** or **ppa** or a **page** if you do this command.

A typical string of commands used to plot a view of the spectrum with integrals and peak picking might be:
pl pscale pap pirn vp=vp+80 ppf('pos','top') vp=vp-80 page.

The layout of the plot on the page is controlled by the following parameters:
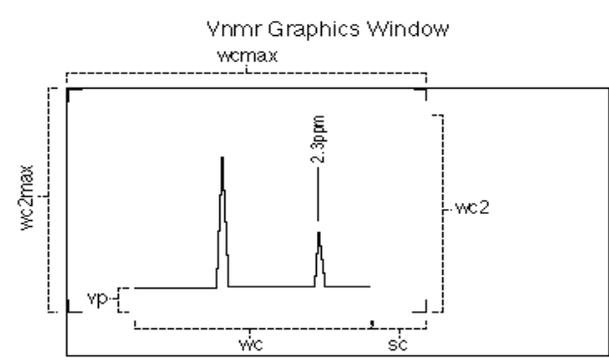
> **wcmax** - width of the page in mm. Do not change this value; it is set when the plotter is selected.
> **wc2max** - height of the page in mm. Do not change this value; it is set when the plotter is selected.
> **wc** - width of chart in mm. Must be less than or equal to **wcmax**. Determines the width of the spectrum
>   on the page.
> **sc** - start of chart in mm. Distance of right edge of the spectrum from the right edge of the page.
> **vp** - vertical position of spectrum in mm. Moves spectrum up and down on the page. Can be used
>   to plot spectrum further above the axis: **vp=12 pscale vp=vp+12 pl**.
> **wc2** - height of chart in mm. Usually set equal to **wc2max**. Determines how close to the top to
>   plot peak frequencies in the **ppf('top')** command.
> **wp** - ppm range of the plot. Input a number followed by "p".
> **sp** - start of the plot in ppm. Input a number followed by "p".
> **vs** - vertical scale of the spectrum.

14.   Printing

You may also want to print information from time to time. Make sure the VNMR parameter **printer** is set to **'LJ_printer'** before printing. Select **[Main Menu]** - **[Config]** and then select **[Select Printer]** repeatedly to cycle through all possible printers.

Anything that is printed to the VNMR text window can be sent to the printer using the **printon** and **printoff** commands. For example, to print the list of parameters in the text window that is displayed by the **dg** command, type **printon dg printoff**. You can print out a different format of peak listing with the commands **printon dll printoff**.

15.   Other Nuclei and Other Experiments

The VXR-300 is also set up to do ^{19}F and ^{31}P. Set up for these nuclei is the same as for ^{1}H or ^{13}C, except, of course, the nucleus. The VI-500 requires a cable change to run ^{31}P. The only parameters you should need to change are **d1** if the T_1 relaxation times of the nuclei in your compound are greater than a couple seconds and **dm** depending on whether or not you want proton decoupling. See the section on acquiring carbon spectra for more information on how the decoupler works in heteronuclear decoupling mode. *For all other nuclei, schedule time with Letitia a week in advance for a probe change.*