

# DCIF NMR TRAINING GUIDE

## 500 MHz

### VARIAN INOVA “502-CASPER”

### VARIAN INOVA “501-ROCKY”

### VARIAN INOVA “500-BULLWINKLE”

(last update 20080919)

The Varian Inova-500 has four RF channels (2 channels have waveform generation) and pulsed-field gradients (PFG). The waveform generators allow for shaped pulse experiments for improved frequency selection, reduced acquisition times, superior solvent suppression, and have numerous other advantages. Pulsed-field gradient enhanced experiments have fewer artifacts, suffer far less from solvent suppression problems, have reduced phase cycling requirements relative to the non-gradient experiments. Typically these experiments require far less time, provided the samples are of sufficient concentration.

The Varian Inova-501 has two RF channels (1 channel has waveform generation) and pulsed-field gradients (PFG).

The Varian Inova-502 has four RF channels (all four have waveform generators) and triple-axis PFGs.

Note that the variable temperature (VT) range of the Varian 500 and 502 is quite limited (a combination of probe and other hardware considerations). If you need to run NMR experiments at extreme temperatures (from -20° to -100° or from +80° to +150°C) you must use a switchable probe (normally in the Varian 501, occasionally in the 500 as well). Ask a DCIF member about VT training. Currently the facility has the following probes available for the Inova-500s:

- 5mm  $^1\text{H}\{^{15}\text{N}-^{31}\text{P}\}$  VT-PFG Indirect Detection Probe. Typically in the 502. Best for  $^1\text{H}$  detection
- 5mm  $^1\text{H}\{^{13}\text{C}, ^{15}\text{N}\}$  VT-PFG Triple Resonance Probe. Typically in the 500. Best for  $^1\text{H}$  detection
- 4mm VT nano-probe  $^1\text{H}\{^{15}\text{N}-^{31}\text{P}\}$  with PFG.
- 5mm  $^1\text{H}\{^{15}\text{N}-^{31}\text{P}\}$  VT-PFG Direct Detection Probe. Typically in the 501. X-Channel Sensitive
- Solid state CPMAS probe. Occasionally in the 500.

As with all of the other DCIF tutorials, the conventions used in this guide are as follows:

- **Boldface** type indicates commands that are typed into the VNMR input window or in a terminal window
- Italic [*Boldface*] type with square brackets indicates a button in the VNMR menu that is to be pushed.
- <**Boldface**> type surrounded by a bracket indicate keyboard strokes.
- **LMB** indicates the Left Mouse Button
- **MMB** indicates the Middle Mouse Button
- **RMB** indicates the Right Mouse Button
- Unless otherwise noted, all commands typed into the input window are followed by an <↵ Enter> keystroke.

1. Login as with the other Varian instruments and start VNMR. A login macro will automatically configure the plotter and printer (i.e., same as typing **setlj**) and change to your user directory according to your user ID (i.e., /data/group\_id/user\_id). You can create deeper nested directory structures using the make directory command.

Example: type **mkdir('book1')**. This will create a directory named *book1*  
type **cd('book1')**. This will change you to the directory *book1*  
(i.e. /data/group id/user id/book1)

Directory names *must not* contain characters such as \, ^, %, \$, blank spaces, etc. If you do use these characters you run the risk of losing your data.

### MIT Macros

**gohome:** If at any time you become lost in a directory nightmare, type **gohome** and you will return to *your* /data/<group\_id>/<user\_id>.

**restart:** If you login and find the VNMR program running, a message may indicate vp doesn't exist. The previous user from your group forgot to type **exit** before logging off. Type **restart** in VNMR to close the current program, then reopen VNMR.

**reset:** If the acqi button does not respond, type reset in VNMR to reestablish communication with the console. (reset is the equivalent of two su acqproc commands)

2. The Varian 500 NMR is ideally suited for the elucidation and characterization of not only small molecules but also many types of biomolecules. Many of the experiments (i.e., HSQC, HMBC, etc.) require the user to use multiple experiments. The VNMR software will allow for 9999 experiments numbered 1 through 9999. Some useful experiment access macros and commands are:
  - **explib** (macro). Displays a library of the currently available experiments in the “text window”.
  - **jexp#** (macro). Joins existing experiment number. Example: **jexp2** will join existing experiment number 2. If experiment number 2 does not exist a message ‘Cannot access...’ is displayed in the “status window”
  - **cexp(#)** (command). Create an experiment number. Example: **cexp(3)** will create *experiment 3*.
  - **delexp(#)** (command). Delete an experiment number. Example: **delexp(4)** will delete *experiment 4*.
  - **mp(from#, to#)** (command). Move parameters between experiments. This is useful when time is spent optimizing experimental parameters in one experiment and you would like to use them in another experiment. Example: **mp(1,2)** will move the experimental parameters from *experiment 1* to *experiment 2*. Note that mp does not move the FID.
  - **mf(from#, to#)** (command). Move FIDs between experiments. Example: **mf(1,2)** will move the currently loaded FID from *experiment 1* to *experiment 2*. The associated parameters are also moved.
  - **unlock(#)** (command). Will remove ‘locked’ or inactive experiment and join this same experiment. Example: **unlock(3)** will unlock and join *experiment 3*. This serves the same function as removing the lock\_#.primary in the group *vnmr* subdirectory.
3. Load a 1D parameter set. Click [*main menu*], [*setup*], [*nucleus, solvent*] and select the nucleus and solvent required. After making your selection type **su** to setup the system hardware to match the current parameters. For the following example we have chosen a typical 1D <sup>13</sup>C experiment with <sup>1</sup>H decoupled.

4. Load initial shims by typing **bestshim**.

This macro is the same as typing **rts('best') su**. If you would like to save your own shim file (say *my\_shims*), type **svs('my\_shims')**. This command will save a shim file called *my\_shims* in your *vnmr/sys/shims* directory. You can recall this file by typing **rts('my\_shims') su**. This shim file will be available to all members of your group.

5. Insert and lock your sample. Open the "Acquisition Window" by either typing **acqi** in the input window or click the **[Acqi]** menu button. Click the **[Lock]** button. Once the window opens click the **[Eject]** button, place the sample on the magnet, then click the **[Insert]** button. Bring the sample on resonance, set the lock, adjust the lock phase, and verify that the lock signal is not saturated. Decreasing the lock power by 6dB, which should in turn decrease the lock level by 1/2, will enable you to check for saturation. If the lock level doesn't drop 1/2 reduce the lock power and try again.

6. **Shim your sample.** Shim z1 and z2 to start. Shim z1 through z5 using gradient shimming; see instructions on the last page. Shim the low order x and y shims if not spinning the sample.

7. **The Tuning Stand.** The probe should be tuned before every experiment as a number of factors (including the tube, solvent, concentration, etc.) influence the environment surrounding the coil. Tuning, at the very least, will minimize the internal reflective losses. The following figure (Figure 1) was taken from the VNMR "Text window" (type **dg** if this information is not present). Many of the parameters you are undoubtedly familiar with such as *nt* (number of transients), *bs* (block size), *at* (acquisition time), and *pw* (pulse width). The important parameters of note here are *tn* (nucleus for observe transmitter) and *dn* (nucleus for first decoupler). Also note that *sfrq* (transmitter frequency of observed nucleus) is 125.676 (MHz). Figure 2, below, is a close-up of the tuning stand. There are several features to notice. The broadband amplifier (<sup>13</sup>C, <sup>31</sup>P, and all the other "X" nuclei) is located on the left

ACQUISITION		SAMPLE	
sfrq	125.676	date	Oct 30 1998
tn	C13	solvent	CDC13
at	0.869	file	exp
np	65536	DECOUPLING	
sw	37718.1	dn	H1
fb	not used	dof	0
bs	16	dm	yyy
ss	1	dmm	w
tpwr	58	dmf	10000
pw	7.5	dpwr	34
p1	0		
d1	3.000		
d2	0		
tof	615.5		
nt	256		
ct	0		

Figure 1: <sup>13</sup>C acquisition

while the high-band amplifier (<sup>1</sup>H and <sup>19</sup>F) is located on the right. Notice the channel selector (labeled CHAN vertically) in the upper central part of the figure is set to zero (0). Also note that the attenuator (labeled ATTEN vertically) is set to eight (8). This setting shouldn't need to be changed.



**Figure 2**

- Typically, **tn** (the observed nucleus) is tuned on channel 1 and **dn** (the decoupler nucleus) is tuned on channel 2.
- When tuning for  $^{13}\text{C}$  it is important to tune for both  $^{13}\text{C}$  and  $^1\text{H}$ . Otherwise you may have poor or incomplete decoupling.
- Note the position of the  $\frac{1}{4}$  wavelength cable on the broadband amplifier. The value of sfrq (see Figure1) will assist you in determining the correct cable.
- Many of the broadband nuclei require either a capacitor stick ( $^{15}\text{N}$  and  $^2\text{H}$  for example) or an inductor stick ( $^{31}\text{P}$  for example). If you have any doubts ask for assistance.

**8. Tuning the probe.** The probe is very delicate. Please exercise extreme caution when tuning or handling the probe. Never apply excessive force to the tuning rods. *Any* damage to the probe is not only costly but results in substantial down time. Figure 3 shows a close-up of the Varian 500 Inverse-Detection / Pulsed Field Gradient probe. The tuning rods extend directly out of the bottom of the probe. The highband channel is marked “*proton*” in red and the broadband channel is labeled “*x-channel*” in green. The tuning rods are made up of two parts: the upper (knurled portion) of the rod adjusts the tuning and the lower part (smooth barrel) adjusts the match.



Figure 3

- Verify that the Acquisition window is closed (this is the window in which you graphically lock / shim). If this window is not closed you will not be able to tune.
- Make sure that the channel selector is set to zero. Do not move the cables unless the channel selector is set to zero. Serious damage could result.
- Starting with the *observed nucleus*, move the cable from the “probe” connector on the amplifier to the “probe” connector next to the channel indicator. Move the filter(s) along with the cable. The red light next to the connector should flash.
- Next, connect the output cable from the same amplifier to the output connector at the bottom center of the tuning stand.
- Set the channel selector to “1”.
- Minimize the meter reading by adjusting the corresponding tune / match rod combination. You should be able to get the meter reading to be less than 5 with the attenuator set to 8.
- Set the channel selector back to “0” and return the cables back to their normal operating positions.
- Repeat for the decoupler channel (channel 2). Note that when using the inverse probe, the proton coil is most affected by sample changes. Note that when running a decoupled  $^{13}\text{C}$  experiment:  $^{13}\text{C} \Rightarrow$  channel 1 and  $^1\text{H} \Rightarrow$  channel 2.

9. (Optional) The Varian 500 has z-axis gradients. These gradients may be used for gradient assisted experiments (i.e., gCOSY, gHMQC, etc.) or for gradient shimming (see the attached tutorial). To query the status of the gradient amplifier type **pfgon?**. The status will be displayed in the status window. To turn the amplifier on *or* off, type **pfgon='nny' su** *or* **pfgon='nnn' su** respectively.
10. The acquisition of basic 1D  $^1\text{H}$  and  $^{13}\text{C}$  spectra is no different than the Mercury 300 instrument. Review the 300 Training guide, if necessary. The following is a list of basic 1D commands and parameters you should know. To get details about a specific command or parameter type **man('command')**.
- **np** (number of data points) Determined by *sw* and *at*. Total number of data points acquired is equal to **2\*sw\*at**.
  - **fn** (Fourier number in the *directly* detected dimension) If *fn* is greater than *np*, then *fn* minus *np* zeros are added to the data set (zero-filling).
  - **sw** (sweep width in *directly* detected dimension) To double the sweep width type **sw=sw\*2**. When changing the sweep width make sure that *all* peaks (including those you may not want) are included.
  - **at** (acquisition time in seconds)
  - **d1** (first delay). Allows the magnetization to equilibrate prior to initiating another pulse sequence. When quantifying an experiment, *d1* must be set to a *minimum* of  $5 \cdot T_1$  (the relaxation time of the nucleus of interest). To perform a  $T_1$  analysis see our **dot1** (do  $T_1$ ) tutorial.
  - **pw** (pulse width in  $\mu\text{s}$ )
  - **pw90** ( $90^\circ$  pulse width in  $\mu\text{s}$ ) Many 1- and 2D experiments require that the  $90^\circ$  pulse be calibrated prior to acquisition. See our “How to Calibrate a  $90^\circ$  Pulse” handout to learn how.
  - **p1** ( $1^{\text{st}}$  pulse width) Length of the first pulse in a standard two-pulse sequence.
  - **d2** length of the  $2^{\text{nd}}$  delay in a standard 2 pulse sequence. This delay is related to **ni** and **sw1** in 2D experiments.
  - **nt** (number of transients) Sets the number of transients to be acquired. To set *nt* automatically, say to fill a 45 minute time block, type **time(45)**.
  - **bs** (block size) Determines how often the acquisition computer stores data to disk.
  - **movesw** (move spectral window according to the cursor positions) When changing the spectral window it is important to include all peaks.
  - **movetof** (move transmitter offset) Moves the transmitter offset to the cursor position which now become the center of the spectrum. Note that previous referencing is maintained.
  - **ga / au / go**
  - **ft / wft / wti** (Fourier transform 1D data / weight and Fourier transform 1D data) **wti** opens a GUI allowing interactive weighting.

- **ffav** will automatically display the full spectrum, phase, adjust the vertical scale and display the horizontal scale for you. **ffav** is an 'in house' macro that consists of **f full aph vsadj dscale**.
- **aph** (automatic phase adjustment) Automatically calculates the phase parameters **lp** (left phase) and **rp** (right phase).
- **setref** (or **nl rl(#p)**) Sets the frequency reference to the currently defined solvent. An automatic version of **nl rl(#p)** (nearest line, reference line to # in ppm).
- **vsadj / isadj** (automatically adjusts the vertical scale / automatic integral scale adjustment)
- **wc / wp / sp** (width of chart (in mm) / width of plot (default in Hz, or use a p suffix) / start of plot (default in Hz, or use a p suffix)). Example **wp=10p sp=-0.5p**
- **ds / pl** (display a single spectrum in the graphics window (can also display an individual spectrum from within an array of spectra, i.e. **ds(5)**)/ plot spectrum (sends spectrum to the plot buffer)).
- **dscale/pscale** (display reference scale / plot reference scale)
- **dg / pap** (display group of acquisition and processing parameters / plots acquisition parameters)
- **dpf / dpfhz / ppf / pppfhz (th, axis)** (display peak frequencies (ppm) / display peak frequencies in Hz / plot peak frequencies (ppm) / plot peak frequencies in Hz). **[Th]** (threshold) interactively sets the peak threshold. Axis determines the reference axis label (i.e. **axis='p'** displays the axis in ppm or **axis='h'** displays the axis in Hz)
- **dpir / pir (vp)** (display peak integral regions in graphics window / plot integral regions) To plot the integral regions **vp** (vertical position) must be set to a minimum of 12 (**vp=12**).
- **getttext** Opens a crude text editor for adding text to your spectra (use the **pltext** command to plot the text).
- **page** Dumps the contents of the plot buffer to the printer / plotter. Can also dump the contents to a file: **page('my\_file')**. This is most useful when creating postscript files.
- **ll** Prints a list of line frequencies and intensities.
- **svs / rts** (save shim file / retrieve shim file) The command will query for a filename.

**11.** Type **svfz** save your data to a file. The **svfz** command will also save a copy of your data set to the workstation zippy in the sub-directory 'bullwinkle' of your home directory. If the computer slows down after the **svfz** command, zippy may have crashed or the network may be down; use **svf** to save your data on the spectrometer only. The command **rt** will allow you to retrieve a file. You should already be in your home directory, but if not, type **gohome**. If you have loaded a shim maps from the system directory that contains the shimmaps, you will not be able to save data in this directory (you will not have write permission here). Typing **cd** will take you to your group directory, and from there you can change to your personal subdirectory.

## 12. Simple DEPT Experiment:

- Setup a  $^{13}\text{C}$  observe (tune both channels),
- Collect the  $^{13}\text{C}$  1D and determine the minimum acceptable value for **nt**.
- Using the cursors to determine the desired spectral window then type **movesw** to reduce sweep width
- Type **dept** to load the dept parameters.
- Type time and adjust nt (in multiples of 4) to adjust your time accordingly.
- Type **mult=1.5** to run a DEPT135 (do this if nt is large)
- Type **au** to start the experiment.
- Plotting and processing commands:
  - **deptproc** (process an array of dept spectra). If the automatically phased spectra look poor, type **wft(1)**, phase the spectrum by hand, then type **deptproc1**.
  - **pldept** will plot the dept spectra.

Some basic 2D parameters to know:

- **sw** (sweep width in directly detected dimension)
- **sw1** (sweep width in 1<sup>st</sup> indirectly detected dimension)
- **tof** (frequency offset for observed transmitter). Can be moved using the cursor and the **movetof** macro. This new value of *tof* will define the center of the spectrum.
- **dof** (frequency offset for the 1<sup>st</sup> decoupler). May also be defined using the cursor and the **sd** (set decoupler frequency) macro but only if  $tn=dn$ .
- **fn** (Fourier number in the directly detected dimension – controls the size of the frequency array or matrix for 1D or 2D spectra, respectively)
- **fn1** (Fourier number in the 1<sup>st</sup> indirectly detected dimension)
- **lb / gf / sb / awc** (controlled with the interactive weighting window **wti**)
- **ss** (steady-state pulses) The number of complete pulse sequence executions without data acquisition. This allows the system to reach what is called the ‘NMR equilibrium’ prior to data acquisition.
- **d1** (first delay). Allows the magnetization to equilibrate prior to initiating another pulse sequence.

### 13. Simple gCOSY hints:

- Setup and collect a simple  $^1\text{H}$  observe experiment. Turn the autogain off by typing **gain='y'**
- Optimize (reduce as much as is possible) the spectral window with **movesw** then reacquire. Integrate every peak you observe, even those from impurities and the solvent (this defines the baseline by exclusion).
- Move the new parameters to a new experiment. Type **mp(X,Y) jexpY gCOSY** where X is, for example 1 and Y is, for example, 2.
- Adjust **nt** and **ni** as necessary, then type **au** (do *not* initiate the acquisition with the **ga** command). The parameter nt controls your signal to noise ratio, ni controls the resolution you will obtain in the  $f_1$  frequency dimension.
- When the experiment has completed, type **dolpav wft2da** (this may happen automatically if the **wexp** parameter is set to 'dolpav wft2da')
- Reduce the  $t_1$  noise ridges by typing **bc('f1')**
- To symmetrize the data set type **foldt**,
- To redraw the 2D spectrum, type **dconi** (click [*resize*] to toggle the size of the data display window; when the data display window is large, the [*flip*] button alternates whether the data display or parameter display window appears foremost).
- Expand a portion of the 2D spectrum using box mode (left mouse positions lower left corner of box, right mouse button controls the size of the box) and then [*expand*].
- To position a box so that its lower left and upper right corners appear exactly on the diagonal, type **even** (an MIT-only macro).
- Adjust vs2d with the middle mouse button or just type **vs2d=500** (or some other number)
- Use the middle mouse button to adjust the threshold th by clicking on the color bar on the right side of the 2D spectrum
- To draw the 2D spectrum with contour lines, type **dpcon(30,1.3)** (this will draw up to 30 contour lines with each subsequent line being 130% of the previous line's intensity). Type **dconi('restart')** to get the cursor back for the purposes of measuring the position of cross peaks.
- Typing **dpc** (MIT-only macro) is the same as typing **dpcon(30,1.3) dconi('restart')**
- To plot the spectrum, type **plcosy(20,1.4,X)** where the third argument (X) is the VNMR experiment containing the processed and phase 1D spectrum.
- Following plotting of the spectrum, you can return to devoting more of the data display window to the 2D spectrum by typing **big2d** (an MIT-only macro)

### 14. Finishing up and logging out:

- Open the acquisition interface (click [*acqi*]), then click [*lock*], then turn off the spinner (it may already be off if you have just collected a 2D spectrum), then turn off the lock, and then close the acqi window.
- Click [*main menu*] [*more*] [*exit VNMR*],
- Click [*EXIT*] sign on CDE toolbar.
- Sign the log book.

# VNMR GRADIENT SHIMMING INSTRUCTIONS

(9/19/2008)

All commands written in **bold type** are direct input at the input window prompt. Bracketed [**bold type**] represents a VNMR button.

Prior to starting, you should first load initial shims (type **bestshim**), lock, and shim on both z1 and z2 (use the +/- 16 button). You may also wish to adjust the first six xy shims: x1, y1, xz, yz, xy, and x2-y2. You may wish to iterate between x1 & xz the between y1 & yz as these are strongly coupled. Remember that when adjusting the 'xy' shims, the spinner must be off. Next, query the pulsed field gradient amplifier control by typing **pfgon?** Make sure this parameter is set to **pfgon='nny'**. If not, type **pfgon='nny'**. If you change it from 'nnn' to 'nny' you should go back and readjust the z1 shim (turning the gradient amp on makes a big difference in the optimal z1 shim current setting).

1. Type **jexp99** Join experiment 99; the recommended experiment for gradient shimming.
2. Type **gmapsys** in the Input Window (Run gradient auto-shimming, set parameters, map shims)
3. Click [**shim maps**], Click the [**Current Mapname**] A message "current mapname is: xxx.fid" will be displayed in the output window. If "xxx.fid" corresponds to the shimmap required for the currently installed probe (see the note taped to the monitor) skip to step number 6 below.
4. Click [**shimmap files**]
  - Click [**cd to userdir**] This will change you to *your group* "vnmr/sys/gshimlib/shimmaps" directory.
  - Click [**cd to systemdir**] This will change you to the "/export/home/vnmr/gshimlib/shimmaps" directory.
  - Highlight desired shimmap file, e.g., xxxx.fid. Again, there should be a note taped nearby telling you which shimmap to use. You must select the shimmap name posted nearby.
  - Click [**load shimmap**]
5. Type **gohome** to automatically change you back to your home directory. If you intend to save your data, you may still be in the "/export/home/vnmr/gshimlib/shimmaps" system directory and you will not be able to save data here.
6. Click [**return**]
7. Click [**set params**]
8. Click [**gradient, nucleus**]
9. Click [**pfg h2**] There is a reminder message to: check gradtype.
10. Type **nt=8** if using CDCl<sub>3</sub>, otherwise type **nt=4** (if using 501, double value for nt)
11. Click [**return**]
12. Click [**find gzwin**] as soon as the acquisition begins, you can type **sa('nt')** to avoid collecting the second FID which is not required for this calibration step.

When it finishes, the values of gzwin and tof will be displayed.
13. Type **gmapsys**
14. Enter the number of z shims to adjust by setting gzsize. Example, if you would like to adjust the first six shims, type **gzsize=5**. This will adjust z1 through z5.
15. Click [**autoshim on z**]
  - If the fitting step fails, check if gzwin value is equal to or less than 30, if it greater than 30, change it to 30 and try gradient shimming again; the fail often means that the sample volume is either too small and / or the sample is improperly positioned. It is recommended to use 0.7 mL total volume, although volumes as low as 0.4 to 0.5 mL often work.
16. When done, you may want to again tweak the "xy" shims, and possibly the z1 and z2 shims as well.
17. Type **jexp1** to join experiment 1, load a new set of parameters and restart spinner if desired. If you are performing a 2D experiment you should leave the spinner off.