

# Varian VNMR

## NMR Data Processing Software Instructions

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### Basic Program Layout

Upon opening the program, the VNMR software is composed of three basic windows: the **command window**, the **spectral window**, and the **parameter window**. As a user, it will be necessary to move freely between the windows for optimal operation of the spectrometer or for data processing. To “activate” the desired window, the mouse pointer must be in the window that is to be active for data manipulation. Clicking on the small border around each window will bring this active window to the front. At various times, other windows may appear and will be used for various functions.

The **command window** (small window at the top left of the screen) allows for direct entry of commands via typing of the appropriate text command or by using the cursor for selecting the appropriate command from the menu bar. A list of the most common commands can be found in the green binder on the table adjacent the Sun workstation. The menu bar has two rows of menu buttons. The top row is the main menu, and it will always consist of the same options. Clicking one of the buttons of the second row will change the command options available as evidenced by the appearance of a new set of buttons for this lower row of buttons. These options are dependant on the label on the menu button; *i.e.*, commands associated with *data files* will appear when the **[FILE]** button is selected. These commands will be discussed in detail later.

The **spectral window** (large window below the **command window** with a black or white background) is the area where the actual NMR spectra will be displayed. There are many interactive functions available that require spectrum manipulation from this window, such zooming, peak picking, insets, etc.

The **parameter window** (large window below the command window with a grey background and a series of tabs running along the right side) is used to display the instrument setting for the various experiments as well as other relevant information.

In the following directions, buttons are indicated by **[BUTTON TEXT]**, and commands that are to be typed on the command line are simply **bolded**.

### File Structure

The Unix file system is a rather complicated beast. Each user has a folder created in the file structure under `/export/home/“login name”`, for example `/export/home/autouser`. All data files created by a user are saved into this folder by default unless specifically told to do otherwise. NMR data files are further placed in `/export/home/“login name”/vnmrsys/data`. Avoid saving files into folders that are not “yours” at all costs!!

### Find and load your data file

Select **[Main Menu] ...[Data]**. At the prompt, enter your user ID. This should put you in the folder `/export/home/userdata/%YOUR USER NAME%/`. You can check what folder you are in at any time by entering the command **pwd** in the command window. The name of the directory you are now using will appear in the command window.

Your data files should be located in `/export/home/userdata/%YOUR USER NAME%/` folder. You can access this folder in two ways:

- 1) Click on the **[Main Menu]** button followed by the **[DATA]** button, and provide your user ID as requested.  
or
- 2) Select **[Main Menu] ...[File]**. Clicking once on the appropriate folder (for example, **vnmrsys**) then clicking **[Set Directory]** from the menubar (Repeat this process as necessary until you get to the desired folder). If you click the wrong folder name, it can be deselected by clicking on the name a second time. If you click **[Set Directory]** without a highlighted file name, you get a new series of menu commands. From this series, the **[change]** command can be used to change directories, and the **[return]** button will take you back to the old set of menu commands.

Locate the appropriate folder containing your NMR data, and select the appropriate filename (the filename assigned by you when you measured the spectra) by clicking on the name once, then clicking the **[Change]** button. You should get a list of your NMR data files (*i.e.*, proton.fid, carbon.fid, dept.fid, *etc*)

Select the appropriate NMR data file by clicking on the file name (*i.e.*, proton.fid). Click on the **[load]** button from the menu bar to *load* that datafile into the computer's memory. If you do not see the **[load]** button, then click the **[return]** button which will change the menu options.

## Data Processing

With the NMR data file loaded, press the **[AutoProcess]** button from the menu bar. This command will convert your raw fid data into the processed NMR spectrum.

After the **[AutoProcess]** command, the software will be in the **Interactive Display mode**. You can manually enter this mode at anytime by clicking the following keys (in sequence) **[Main Menu] - [Display] - [Interactive]**). It is possible to interact with the spectrum displayed in the graphics windows by a combination of menu and mouse buttons.

The first and third buttons in the **Interactive Display mode** 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 first button is used to toggle between one and two cursors, and the third 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. Generally, the left mouse button sets the left boundary, while the right boundary is set by the right mouse button. The middle mouse button is always used for adjusting vertical scales of the spectrum or the integration.

## Phasing

Once the Fourier Transform has been done, the spectrum needs to be phased to give pure absorption lineshapes. A properly phase NMR spectrum should have peaks that are symmetric. 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 done.

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. Two cursors will appear, and the section of the spectrum between these cursors will be highlighted (**ACTIVE**).
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. 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.

## Expansions (Zooming)

The left and right mouse buttons control 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 command **f full** typed and entered in the command window will also bring 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 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=some number**.

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

## Setting a Reference $\delta$ Value

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 (or  $\delta$ ), 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 (0 ppm) or a solvent line. (Since a small percentage of the solvent molecules are protonated, you will usually see a solvent line in proton spectra, and any carbons in the solvent will appear in the  $^{13}\text{C}$  spectra. Since the carbon is generally bonded to one or more deuterium atoms, the carbon signal will appear as a multiplet which can be predicted in each case).

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. **FOR  $^1\text{H-NMR}$**  -Type **rl(0.0p)** (for TMS) or **rl(7.24p)** (for chloroform).  
**FOR  $^{13}\text{C-NMR}$**  -Type **rl(0.0p)** (for TMS) or **rl(77.0p)** (for chloroform).
  - 3b. Alternatively, use the menu button [**Ref**] and type the reference ppm value at the prompt.

## Peak Picking

The frequencies of the peaks in the spectrum are typically the most useful information obtained from the spectrum, and are needed to determine coupling constants (**J** values) from  $^1\text{H}$  spectra.

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. (The integral display should be OFF at this point. See next section to turn off the integral display, if needed.)
3. Once you've set the threshold, click on the [**Mark**] button, and then the [**Th**] button again.
4. To get a listing of peaks displayed on the spectrum, the command **dpf** can be used:

This command will pick all peaks above the threshold line and display those frequencies which can be printed on the spectrum by including **ppf** in your plotting instructions (see **Plotting** section.). Note that the **dpf** command gets 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.

## Integration

1. The Integral options are [**Full Int**], [**Partial Int**] and [**No Int**]. When in the [**Full Int**] mode, the ENTIRE green integral line is displayed. When in the [**Partial Int**] mode, only the resets are visible, and [**No Int**] truns off the integral display.
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/tilt**]. 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 (flat in an area with no peaks to be integrated). Then click on the [**Box**] button to get 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 **tilt**) - 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 green 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 getting 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 instance, to set the values out of a possible 100, set **ins=100**. If you want a particular integral region to be equal to 3 (if you think 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.
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). Once you've finished all of the processing that you want to do to the spectrum, you can save it again as described in Section 11, so the next time you load the data you will not have to repeat all of these processing steps.

## Plotting

Once the data has been processed, you will probably want to plot one or more views of the spectrum. There are two methods for plotting

### Option 1) (recommended for basic users)

From the menu, select **[return]** which will take you to a new menu that has a **[plot]** button. Select the plot button to get a set of plot options. Simply click the button for the option(s) that you wish to have plotted; click all that apply:

**[Plot]** - You must click this button to for the spectrum(and integral if on the screen) to be plotted

**[Scale]** - This option will put the horizontal scale on the plot

**[PIR]** - This option will put the integral values on the plot, assuming that you did the integration as previously described.

**[Params]** - Puts a basic parameter set on the printout which includes the file name and type of NMR spectrum

**[All Params]** - Puts all instrument parameters on the printout

**[Peaks]** - Puts the peak labels on the printout, again assuming that you did the peak peaking as describe previously.

**[Page]** - Immediately sends all the data to the printer. **This button should be selected last!!**

**[Return]** - go back to the previous menu.

### Option 2)

To put a title on your spectrum, type **text('title')**. It will be plotted if you plot parameters with either the **ppa** or **pap** commands. Type **text('title of plot\more text')** for multiple lines of text. The title will be plotted in the upper left of the plot by the commands **pap**, **ppa**, 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 the 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.  
**pirn** - plot normalized integrals 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 is stored as part of 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.

For peak listings on your spectrum rather than listings at each peak, 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**.

**pli** - plot a list of integrals. Do not do a **pap** or **ppa** or a **page** if you do this command.

(The list of peak frequencies or integrals will print to the same location on the spectrum as the list of parameters. This is why you should not combine **pll** or **pli** with **pap** or **ppa**.)

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**.

These commands can be entered in any order, or on separate lines except that **page** must be last as it is the command that will send the information to the printer.

A simpler version used to print the spectrum with the scale and short parameter list would be:

**pl pscale ppa page**

If you also have an integral on the screen, and have assigned integral values,

**pl vp=12 pscale pirn ppa page**

References:

1. Dr. Letitia Yao Dr. Steve Philson, University of Minnesota NMR Facility, Instruction Manuals
2. VNMR Software Manuals for MercuryPlus, Varian. Inc.

# Troubleshooting

**Problem:** ASM puts data acquisition in the queue rather than starting the acquisition!

- Solution:**
- 1) Open the file manager (Third Icon from the left on the Solaris taskbar)
  - 2) Locate your AUTO directory, usually /export/home/*your\_user\_ID*/auto.
  - 3) Open the folder for today's date; auto\_19.02.04 would be Feb 19, 2004.
  - 4) Find the enterQ file and rename it enterQ.bak. Left click on the file icon to highlight the file, then from the menubar select **[Selected]...[Rename]**. Enter the new filename and hit **[enter]**.
  - 5) Close file manager
  - 6) Open a terminal window (Third arrow from the right on the Solaris taskbar, then select **this host**)
  - 7) Type su acqproc at the prompt and hit **Enter**; wait for the prompt
  - 8) Type su acqproc at the prompt and hit **Enter** (YES, repeat the same command twice)
  - 9) Close the terminal window
  - 10) Check the Acquisition status window in VNMR to ensure the instrument is *idle*.

**Problem:** The instrument is offline - indicated in the Acquisition Status window.

- Solution:**
- 1) Open a terminal window (Third arrow from the right on the Solaris taskbar, then select **this host**)
  - 2) Type su acqproc at the prompt and hit **Enter**; wait for the prompt
  - 3) Type su acqproc at the prompt and hit **Enter** (YES, repeat the same command twice)
  - 4) Close the terminal window
  - 5) Check the Acquisition status window in VNMR to ensure the instrument is *idle*.