

BARE BONES GUIDE TO Data Processing and Plotting on the Varian Inova-400, 500 and VXR-300 NMR Instruments

This guide is intended to accompany proper training and formal check-out by the NMR Facility Director, or the Director's Designee. You must be OK'd by Facility staff prior to using the instrument. This guide covers the most basic operational details for processing and plotting routine 1-dimensional NMR spectra.

August 28, 2007

Conventions in this manual:

Boldface text indicates commands to be typed at the computer

<angle brackets> are used to designate a key to be pressed (i.e. **<Ret>** for Return/Enter)

[square brackets] designate an icon/button in the VNMR menu to be *clicked*

Mouse Conventions: *click*, by default, refers to the Left Mouse Button.

LMB will be used to designate the Left Mouse Button

MMB will be used to designate the Middle Mouse Button

RMB will be used to designate the Right Mouse Button

Sometimes you will need to *hold*, rather than *click* the mouse button. This means that you should press and hold the button down throughout the operation.

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- 1) If the screensaver is active, *click* a mouse button, or hit **<Ret>**, then type the password to unlock the screen.
 - 2) Switch to your data directory in *rgroups*.
 - a) *Click* on **[rgroups]** in the menu, then select your group directory (LMB *click*), then *click* on **[Set Directory]**.
 - b) *Click* to select your directory, and then *click* on **[Set Directory]**.
 - c) You should notice the message displayed at the top of the screen, which indicates that the current directory is your directory.
 - 3) Load your spectrum (FID):
 - a) *Highlight* your file by *clicking* **LMB** on the filename (note all NMR files end with “.fid”).
 - b) *Click* on **[Load]**, and wait a few seconds for your file to load. You won't see your spectrum until you type **wft**.
 - 4) *Type* **wft <Ret>** to process and see your spectrum
 - a) If your spectrum doesn't cover the full width of the window, type **full <Ret>**.
 - 5) *Click* on **[Dscale]** (or type **dscale <Ret>**) to see the chemical shift scale.
 - 6) Zooming/Expanding a region:
 - a) *click* in the display, and you should see a red line (cursor)
 - b) move the line to the left side of your desired region
 - c) *click* the RightMouseButton (RMB), and you should see a second cursor.
 - i) if you *hold* **RMB**, you can drag the right cursor to the right side of the desired region.
 - d) *Click* on the **[Expand]** button (or...*press* **<F3>**)
 - e) *Click* on the **[Full]** button (or *press* **<F3>** again) to return to the full spectrum
 - 7) Vertical Expansion:
 - a) The MiddleMouseButton (**MMB**) is used to expand the spectrum (increase/decrease the intensity).

- b) Place the cursor directly over a peak, and *click* the left mouse button. The spectrum will adjust the scale, such that the peak below the cursor is at the level of the cursor
- c) If you *hold* the **MMB** over the peak, and move the mouse Up/Down, the spectrum will follow.

CAUTION: If you move the mouse from side-to-side (off of the peak maximum) the noise will rise to meet the cursor!!

- d) To reduce the intensity, place the cursor below the baseline, and *click* the **MMB**.

8) Note: You can totally reset the display by typing "**fdis <Return>**"

- 9) Phasing the Spectrum : type **aph <Ret>** to auto-phase the spectrum.
- a) Sometimes "aph" will introduce a badly rolling baseline (esp. in ¹⁹F NMR). If this happens do the following: type **lp=0<Ret>** then type **aph0<Ret>**. Use manual phasing to touch-up the phasing.
 - b) If the spectrum is still not properly phased you can adjust the phase manually:
 - i) *Click* on [**Phase**],
 - ii) Position the Pointer/Arrow over a peak near Left side of the spectrum.
 - iii) *Hold* the LeftMouseButton (**LMB**), and move the mouse forward/backward to phase this region, then *release* the mouse button.
 - iv) Position the arrow over a region on the Right side of the spectrum, *hold* the **RMB** (RightMouseButton) over this region, and move the mouse to phase this part of the spectrum.
 - v) *Type* **ds <Ret>**. (display spectrum)

10) Define the Chemical Shift Reference:

- a) Zoom-in (see step 6 above) on a peak of known chemical shift
- b) *Click* on the reference peak, (this puts the red cursor on the peak).
- c) *Type* **ref <ret>** , and enter the shift of the reference peak
 - i) Common Solvent Shifts are on charts posted all around the lab.

11) Baseline Correction (PROTON NMR ONLY):

- a) *Type* **abc<Ret>** to perform automatic baseline correction. This should flatten any slope in the integral baseline. (Note...if your data has too many points, you will get an error message. You will need to use alternative baseline correction methods in such cases).
- b) There are other ways to correct the baseline...please ask for a demonstration if needed.

12) Integration (usually only for proton or ¹⁹F, or ³¹P NMR):

- a) *Click* on [**Part Integrals**] or [**Part Int**] (if you don't see [Part Integrals], click on [No Integrals] or [Full Integrals], until you see [Part Integrals], then *click* on it).

Note: If you don't see any of these, *type* **ds <Ret>**..then try again.

Also: While an integral is displayed, the **MMB** will adjust the size of the integral line. If you wish to adjust the size of the spectrum, you have to turn off the integral display ([No Integral]), or manually enter new values for "vs" (i.e. vs=100).
- b) Zoom-in (Expand) a region to add/remove integral *resets*, then *click* on [**Resets**].
 - (1) Unselected regions will be "gray" (dotted lines), selected regions will be bright green.
- c) *Click* **LMB** before and after each peak/multiplet to select it for integration.
- d) To remove a reset, *click* **RMB** over the break in the integral line.
 - i) *Click* [**Return**] (or *type* **ds <Ret>**) to exit resets mode.
 - ii) *Click* [**Full**], and re-expand (zoom) on other regions as necessary to define your desired integrals.
 - iii) To clear all resets, and start over, *type* **cz <Ret>**.
- e) To Normalize the integral values to a pre-defined value (i.e. to fix a CH₂ for a value of 2.0):
 - i) Place the red cursor over the integral of known value.
 - ii) *Click* on the [**Set Int**] button, and enter the correct value, then **<Ret>**.
- f) To display the integral regions on the screen, *type* **dpir <Ret>**. To continue manipulating the spectrum, *click* [**Interactive**], or just *type* **ds <Ret>** again. (note "vs" must be at least 12 to display the integral values).

13) Peak-Picking, generating a peak list:

- a) Click on [**Th**] to display the yellow threshold line.
- b) Use the **LMB** to set the yellow line so that all desired peaks are bisected by the yellow line.
- c) Type **nll <Ret>** to see how many lines have been found, or **dpf** to display the peak frequencies on the screen.
 - i) Type **ds<Ret>** after doing "dpf" to continue working with your spectrum.

Important Note: If you change the vertical scale (vs), and don't change the threshold, you will change the number of lines that are found.

14) Entering Text:

- a) You can enter text for display or plotting, such as a description of the sample, reaction conditions, ...etc.
 - i) type **text(' your text here')** to enter the first line of text.
 - ii) type **atext(' more text ')** to add an additional line of text.
 - iii) type **dtext** to see your text on the screen (where it will come out on the plot). Type **ds** to re-display the spectrum, and clear the text from the display.
 - (1) Note: DO NOT use quotation marks, or apostrophes (') in your text...this will mess things up.
- b) If you know how to use the "vi" editor in Unix, you can type **textvi** and use the vi editor to edit your text. If the words "vi editor" mean nothing to you, then ignore this step completely.

15) Printing/Plotting the spectrum:

- a) Click on [**Select Plotter**] to select the paper size (the Kyocera can print 11x17" paper).
- b) Adjust the expanded region to show the range of peaks that you wish to plot. Adjust the vertical size appropriately because this will reflect the printout.
- c) If integral lines are displayed on the screen, they will also be plotted.
- d) Printer commands are typed on the command line, separated by <spaces>. You can type multiple printing/plotting commands on a line, or on separate lines.
- e) Nothing will be actually printed until the **page** command is typed. If you read the plotting descriptions below, you will see that you can combine multiple plotting commands on a single line, or on separate lines, and then type **page <Ret>** to send the final plot to the printer.
 - i) **pla** - *plot automatically*: plots spectrum, axis, integrals(if displayed), and parameters at the bottom.
 - (a) This is a macro that combines "pl pscale pir and ppab" in one command.
 - ii) **pl** - plots the spectrum trace, and integral lines if currently displayed ([Full Integral] or [Part Integral])
 - iii) **pscale** - plots the scale in either PPM or Hz (depends in "axis" parameter...axis='h' or axis='p' to change)
 - iv) **pir** - plots integral values (determined by [Set Int] in step 12.g.ii above).
 - (1) Note: the parameter "vp" must be ≥ 12 for pir to work.
 - v) **ppf** - plots peak frequencies above peaks (assuming that *th* was set properly in step 13.a earlier).
 - vi) **ppfh** - plots peak frequencies in Hz above peaks, axis remains in ppm.
 - vii) **pll(#)** - prints a line-list (Hz and PPM). # is optional, and represents an offset from the left edge.
 - (1) *hint*: # is a fraction of "wc". after selecting your printer/plotter, type "wc? <Return>" to see the width in plotter units. Divide that by 2, and that value should put the line list in the center. Divide by 4 and that value for # should put the list 1/4 of the page width from the left edge.
 - viii) **psp(#)** - prints *some* parameters, again offset from the left edge by # (see above).
 - ix) **pap(#)** - prints *all* parameters...more than psp, anyway.
 - x) **pltext** - prints text (if entered in step 15 above) on the plot in the upper-left corner of the display
 - xi) **ppab** - prints some parameters under the spectrum (requires vp = 18 or higher)
- f) Below you will see several printing commands with explanations of what will be printed:
 - i) **pla page <Ret>**.
 - prints spectrum, axis, integrals (if defined), and parameters at bottom (and top).
 - ii) **pla ppf page <Ret>**
 - prints spectrum, axis, integrals (if defined), peak-frequency labels, and parameters at bottom.
 - iii) **pl pscale psp page <Ret>**
 - This will print the spectrum, shift scale, and *some* parameters.

- iv) **pla pltext page <Ret>**
 - prints spectrum, axis, integrals (if defined), parameters at bottom(at top), and descriptive text in the upper-right corner of the plot window.
 - v) **pl pscale pir psp page <Ret>**
 - prints spectrum, scale, integral values, *some* parameters.
 - vi) **pl pscale ppf psp(75) page <Ret>**
 - prints spectrum, scale, peak-values above peaks, and parameters(offset from the left edge...near center).
 - vii) **pl pscale pir psp pll(75) page <Ret>**
 - prints spectrum, scale, integral values, parameters(left-justified), and a line-list near the center
 - viii) **pap pll(75) page<Ret>**
 - (1) prints *all* parameters on the left, and the line-list (based on *th*) near the center of the page.
- g) There are an infinite number of possibilities...feel free to experiment!
- h) Plotting to a PostScript File: You can re-direct your output to a file (for viewing in other applications like Adobe Acrobat, GhostView,...etc., and for pasting into other applications.
- i) After typing the plotting commands above, instead of typing *page*, type **page('filename.ps')**, and the output will go into *filename.ps* instead of the printer. The file (*filename.ps*) will be located in the current data directory.
 - ii) If using the auto-plot macro (*pla*), type **pla('filename.ps')** and the plot will go to *filename.ps*. If you want your peak-frequency labels to also appear in the PostScript file, type **pla('p', 'filename.ps')**.
- i) Inset Plots: Yes, you can plot your full spectrum, and place an expanded *inset* on the same page. READ CAREFULLY, and follow directions carefully.
- i) Setup your initial, full spectrum and type your plotting commands (i.e. pl, pscale, ...etc. don't type page).
 - ii) Type **s1 <Ret>**, this saves the display so that it may be recalled with "r1" later.
 - iii) If you want the scale plotted under your inset, do [dscale] now.
 - iv) Use the **LMB** and **RMB** to bracket/box the region desired for the inset with red cursors.
 - v) Type "**inset <Return>**", and you will see a new trace above...Now, **LMB** controls the position of the left-edge (start of chart or "sc", **MMB** controls vertical scale or "vs", and **RMB** controls the width the trace (width of chart or "wc").
 - (1) Note new buttons: [sc wc], [expand], [sp wp]
 - (2) Position the inset trace, and width, and intensity using the mouse buttons.
 - (3) You can change the vertical position ("vp", displayed in the lower-left corner) by typing "vp=# <Ret>", where a larger number will move the inset-trace up. Note, after doing this you need to *click* on [sc wc] to regain control of the positioning of the trace with the mouse buttons...you may need to experiment.
 - (4) Type **pl pscale <Ret>** to plot the inset trace and scale. You may also type additional commands, like ppf, pir, ...etc., to be operated on the inset plot region only.
 - (5) You can type **s2<Ret>** to save this inset region as "display 2", and recall it later using "r2<Ret>"
 - (6) type **page <Return>** to send the whole mess to the printer.
 - vi) You can return to your original display (the first trace plotted) by *typing* **r1<Ret>**. Then you can do another inset-plot if you wish.
 - vii) Type "**fdis <Return>**" to reset the display to normal.

16) When you are done:

Type **cd <Ret>** to return the current directory to *home*, so that the next user won't accidentally be working in your data directory.

17) If nobody is waiting to use the workstation, put the cursor on the CU wallpaper, **Hold the RMB**, and *select* [**lockscreen**] to activate the screensaver.

This is a work in progress. I welcome any comments/suggestions.

-Rich Shoemaker