

**Chem 2340**  
**Instruction Manual**  
**For**  
**NMR Spectroscopy**

**By**

**Charles L. Mayne**

**Revised 1/20/05**

## Chem 2340 NMR Spectroscopy

### Introduction.

**Please read this entire document before you begin submitting samples for NMR analysis.**

The NMR staff is always happy to assist you with problems you may have in operating the spectrometers or analyzing your data. They may be contacted as follows:

Charles L. Mayne	1-7413	B115N HEB	mayne@chem.utah.edu
Athena Webster	1-8582	1122N HEB	athenacw@chem.utah.edu
Dennis Edwards	1-4208	1428S HEB	edwards@chem.utah.edu

All of these people are frequently not in their offices, but if you call and leave voice mail, they will respond as soon as possible. Each of them carries a pager that alerts them when they have new voice mail. See Note 4) Where to go for help on page 15 for other sources of assistance with specific problems.

We are very interested in your feedback, either positive or negative. Written comments (by e-mail) to one of the NMR staff are preferred, but we are happy to have verbal ones as well.

What follows is a very abbreviated description of some of the capabilities of the NMR spectrometers. If you want to know more, full documentation is available online. Ask one of us to show you how to display it.

Items enclosed in angle brackets represent buttons to be pressed or menu items to be selected, e.g., <Button>. Click the item with the corresponding label using the left mouse button. Most of the time you will click with the left mouse button. If you are required to use the middle or right mouse buttons, the instructions will explicitly say so. Clicking a button means quickly press and release the mouse button with the cursor on the button. Pressing a button means press the mouse button with the cursor over the item and hold it down as you drag to the item you wish to select.

In Unix and vnmr almost everything you type is case sensitive, so pay attention to capital and lower case letters when you type.

## How to Acquire NMR Data on Your Sample.

### ***Setting Up Your Account.***

1. Before you submit your first sample or try to use your account, you must have a valid uNID and password. If you do not have one or don't remember the password, see Note 1) Username and Password on page 14.

- To verify that your uNID and password are working correctly, you must do the following. This procedure has to be done only once before you submit your first sample or try to use your account to analyze data.
- You can access your NMR account on [vnmr.chpc.utah.edu](http://vnmr.chpc.utah.edu) from the PC's located in a number of labs across campus that have the necessary installations of **PuTTY SSH** and **Xwin32**. All of the PC's in the fourth floor teaching laboratories and in 1100 HEB have the required software. All of the PC's in the Marriott Multimedia Center (MMC), in EMCB 130 (Engineering and Mines Classroom Building, and in the Peterson and Sage labs in the residence hall area also have the software.
- Xwin32 may already be running. If it is, its icon will appear at the right end of the Windows task bar. It will look something like this:

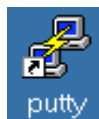


If this is the case, just go to the next step. Otherwise, to start Xwin32, simply double-click the corresponding shortcut on the desktop. The icon will look like this:



The icon may be a shortcut inside a folder on the desktop depending on which lab you are working in. If a shortcut is not present, ask an attendant how to find the program. When the window asking about the method to connect appears click <cancel>.

- Nothing will happen at this point. Xwin32 is waiting to display the windows from vnmr.
- Start PuTTY SSH using the shortcut on the desktop. The icon will look like this:

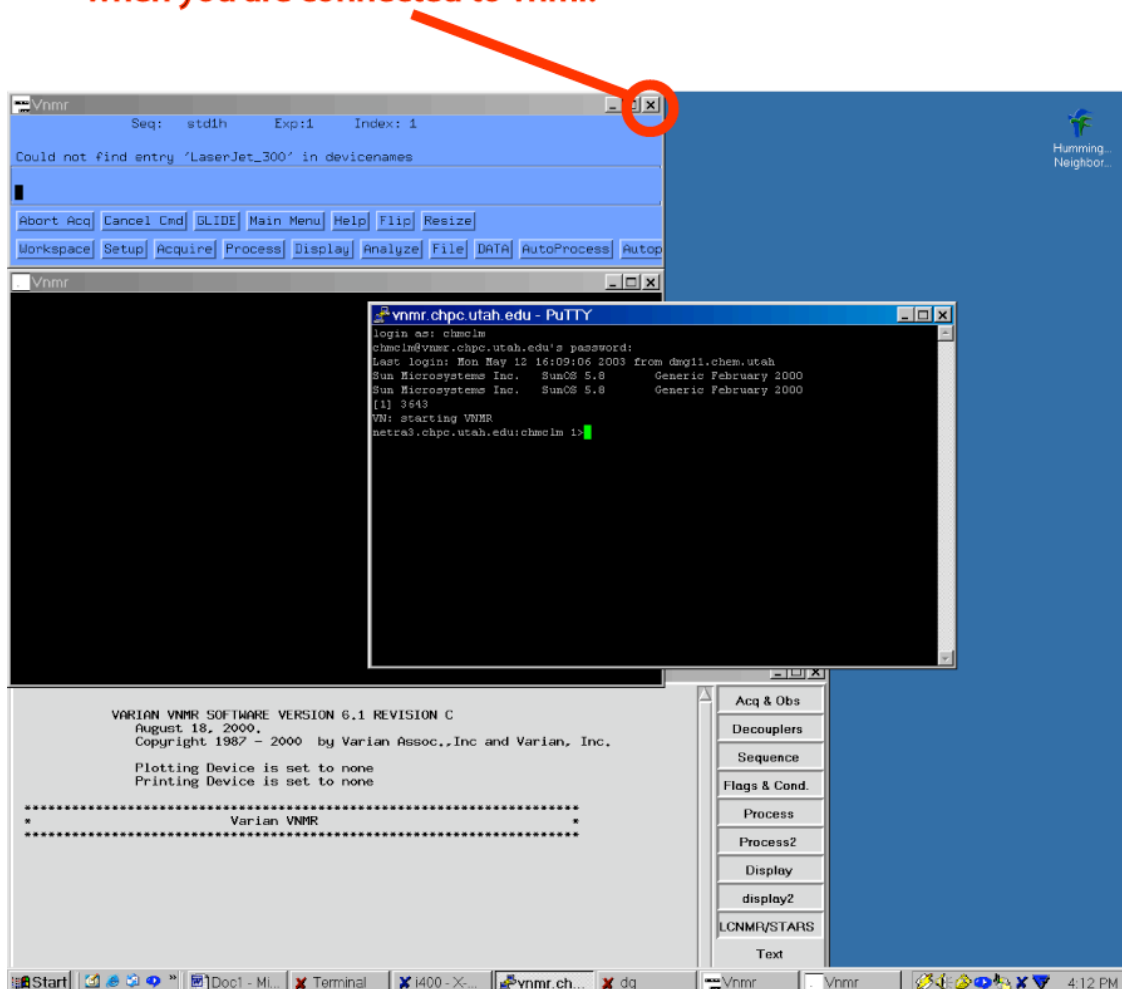


As before, if there is no shortcut, the program may be located in a folder on the desktop or ask an attendant.

- PuTTY will open a window in which you will see a list of sessions. Click on the VNMR session, then click <Load> and <Open>.
- If a PuTTY Security Alert message appears click <Yes>.
- A terminal window on one of three servers (netra1, netra2 or netra3) will open. You will be prompted for your username and password (Use your uNID and password. See Note 1) Username and Password on page 14 if you don't know your uNID and password.).

10. You should now see a screen that looks similar to this:

**Never use the "go away" box to close a window when you are connected to vnmr.**



11. If you see the above window, your account is configured correctly. If you cannot reach this point, you should ask for help. See Note 4) Where to go for help on page 15.
12. If you want to practice using the software, there is a subdirectory in your data directory called Test\_Data containing two data sets called 1H.fid and 13C.fid. Go to "Analyzing your NMR Data." on page 7 for instructions. Otherwise, exit the system as follows:

13. DO NOT USE THE X (“go away”) BOX IN THE UPPER RIGHT HAND CORNER OF THE WINDOW TO CLOSE A WINDOW BECAUSE THIS CLOSES THE WINDOW LOCALLY BUT DOES NOT ALLOW THE UNIX MACHINE TO WHICH YOU ARE CONNECTED TO PROPERLY TERMINATE THE PROGRAM RUNNING REMOTELY. To leave the system type “exit” on the command line or click <Main Menu><More><Exit VNMR>. If the <More> button is not visible, you may need to increase the size of the window by pressing the mouse button at its right edge and stretching the window out a little. Then close the PuTTY window by typing, “exit” at the prompt. You may have to type “exit” several times until the window disappears.

### Preparing a Sample.

1. Deuterated solvents are quite expensive. Use them as sparingly as possible.
2. Obtain a sample tube and septum cap from your TA or from the stockroom. Be sure they are clean and dry. Any residue will probably show up in your spectra. Do not use chipped or cracked tubes or damaged septa.
3. Your sample to be analyzed should be dried thoroughly. Residual solvents will show up in the NMR spectrum. Weigh 30 mg or pipet 40  $\mu\text{L}$  of your sample into the sample tube. Do not guess the amounts, weigh the material or measure the liquid with a calibrated pipet.
4. Add 0.5 mL of deuterated solvent to the sample tube. Normally you will use deuterated chloroform. Consult your TA about which solvent to use. Be sure the solvent has TMS (tetramethylsilane) or DSS (4,4-dimethyl 4-silapentane sodium sulfonate); the latter compound is used for  $\text{D}_2\text{O}$  where TMS is not soluble. Dissolve the sample in the deuterated solvent. Be sure the sample is completely dissolved. There must be no solid particles or undissolved liquid in the NMR sample. Samples that are not completely dissolved will be returned without NMR analysis.
5. Place the septum cap on the tube. The tube must be fully inserted into the cap to prevent solvent evaporation. Make sure the outside of the tube is clean and dry.
6. If the sample is completely dissolved, you are ready to submit it. Proceed to the next section, “Obtaining spectra.”
7. If you have made up your sample according to the instructions using the solvent specified by your TA, it should dissolve completely. If very little of the sample dissolves, you have probably made a mistake somewhere along the way. If most of the sample dissolves but some undissolved solid remains, you must filter out the undissolved solid before submitting the sample for NMR analysis. If undissolved liquid remains try using a different solvent. If undissolved solid remains, proceed as follows:
8. Obtain two disposable pipettes and enough glass wool to make a sphere about 1 cm in diameter when compressed.
9. Insert some of the glass wool into the large end of a disposable pipette.
10. Use the small end of the second pipette to tamp the glass wool firmly into the constriction of the first pipette.
11. Add more glass wool, if needed, until you have a tightly packed mass of glass wool in your pipette about 1 – 2 cm high. This will be your filter.

12. Rinse your filter by passing 0.5 mL of your deuterated solvent through it. Force all of the rinse solvent through the filter using a rubber bulb. Discard the rinse.
13. Obtain another NMR sample tube (you will not need another cap) and transfer your sample to it through the filter you have made using the second disposable pipette. Force the sample through the filter using a rubber bulb. Place the septum cap on the sample and submit it for analysis. Be sure to remove any solid clinging to the inside of the septum cap before placing it on the filtered sample.
14. Clean and dry the original NMR sample tube and either retain it for your next NMR sample or return it to where you got it.

### Obtaining spectra.

1. Before you submit your first sample you must have completed the steps on page 2 titled "Setting Up Your Account." This needs to be done only once.
2. Take your sample to room 4109 HEB where you will find a PC for submitting your sample.
3. You should find a sample submission form that looks something like the following on the screen of the computer. If not, ask the stockroom attendant or your TA to start the sample entry program for you.

The screenshot shows a window titled "Sample Entry Form". It contains several input fields and sections:

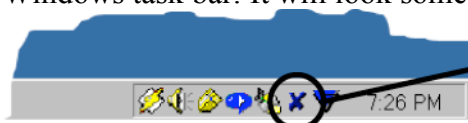
- "Insert Sample at location" with a large red number "1" in a box.
- "Enter your NID:" followed by a text input field.
- "Solvent Selection" section with dropdown menus for CDC13, D2O, Benzene, DMSO, Acetone, and CD2C12.
- "Experiments" section with checkboxes for H1 [1] and C13 [10].
- "Text" followed by a text input field.
- Buttons: "Customize Parameters", "Add Entry", "Exit and Save", and "Quit".
- "Number of samples submitted: 0" followed by a text input field.

4. The large number at the top of the screen is the number that will be assigned to your sample. Place your sample in the corresponding hole of the rack provided. If you do not place your sample in the correct hole of the rack, your data will not be accessible later.
5. Enter your uNID in the space provided. Be sure to enter your uNID correctly with no extraneous characters. The NMR spectrometer robot will use your uNID to place your NMR data in the correct directory on [vnmr.chpc.utah.edu](http://vnmr.chpc.utah.edu). A uNID consists of a lower case "u" followed by 7 digits.

6. Click the button next to the solvent used to make your sample. This will normally be  $\text{CDCl}_3$ .
7. Click the buttons next to the experiments you want to be run on this sample. Unless you have been instructed otherwise, choose both H1 and C13.
8. In the text box you must enter the name of the compound being analyzed, your name (not your uNID), and your lab section. You may also enter any other text you desire to identify your sample. This text will be stored with your NMR data on [vnmr.chpc.utah.edu](http://vnmr.chpc.utah.edu).
9. Press <Add Entry>. Be careful not to press any other buttons in this row. These are to be used only when everyone's samples have been submitted.
10. That's it! Your NMR data should be available on [vnmr.chpc.utah.edu](http://vnmr.chpc.utah.edu) within a few days. Write the date you submitted your sample in your lab notebook. A sheet will be posted on the bulletin board in the hall outside the lab indicating the submission dates of samples that have been completed.

### Analyzing your NMR Data.

1. You can access your NMR account on [vnmr.chpc.utah.edu](http://vnmr.chpc.utah.edu) from the PC's located in a number of labs across campus that have the necessary installations of **PuTTY SSH** and **Xwin32**. All of the PC's in the fourth floor teaching laboratories and in 1100 HEB have the required software. All of the PC's in the Marriott Multimedia Center (MMC), in EMCB 130 (Engineering and Mines Classroom Building, and in the Peterson and Sage labs in the residence hall area also have the software.
2. Xwin32 may already be running. If it is, its icon will appear at the right end of the Windows task bar. It will look something like this:



If this icon is present,  
Xwin32 is already running.

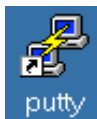
If this is the case, just go to the next step. Otherwise, to start Xwin32, simply double-click the corresponding shortcut on the desktop. The icon will look like this:



The icon may be a shortcut inside a folder on the desktop, depending on which lab you are working in. If a shortcut is not present, ask an attendant how to find the program. When the window asking about the method to connect appears click <cancel>.

3. Nothing will happen at this point. Xwin32 is waiting to display the windows from [vnmr](http://vnmr).

4. Start PuTTY SSH using the shortcut on the desktop. The icon will look like this:

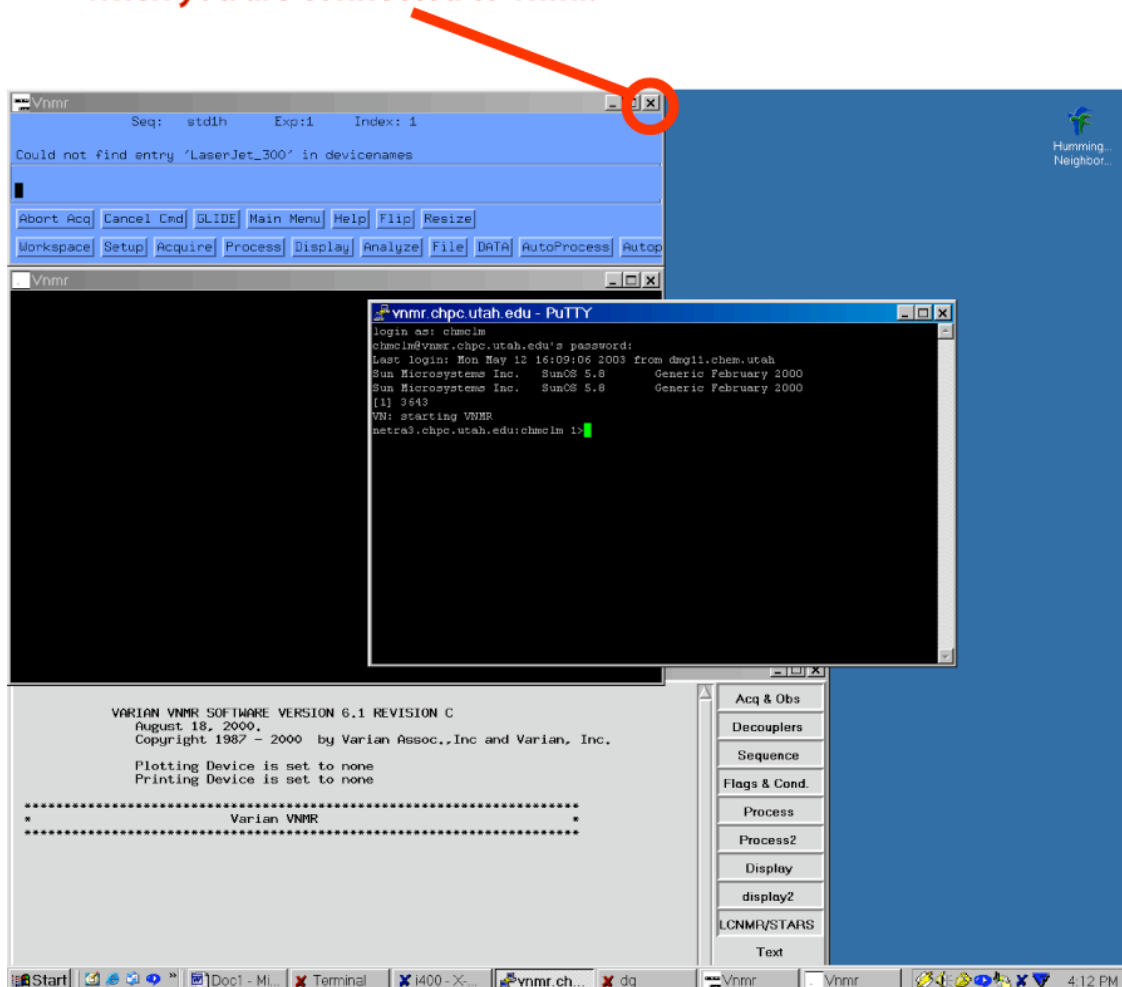


As before, if there is no shortcut, the program may be located in a folder on the desktop or ask an attendant.

5. PuTTY will open a window in which you will see a list of sessions. Click on the VNMR session, then click <Load> and <Open>.
6. If a PuTTY Security Alert message appears click <Yes>.
7. A terminal window on one of three servers (netra1, netra2 or netra3) will open. You will be prompted for your username and password (Use your uNID and password. See Note 1) Username and Password on page 14 if you don't know your uNID and password.).

8. You should now see a screen that looks similar to this:

**Never use the “go away” box to close a window when you are connected to vnmr.**



9. At this point your current directory should be your home directory. If you want to ensure that this is the case click <Main Menu><File><Set Directory><Home>.
10. Retrieve your data from vnmr.chpc.utah.edu by clicking <Main Menu><File>
11. You will see a list of subdirectories. Click on the one called “vnmrsys”. It will be highlighted. Now click <Set Directory>.
12. Again you will see a list of subdirectories. Click on the one called “data”. It will be highlighted. Click <Set Directory>.

13. You are now looking at the directory where your data is stored. If your sample has been analyzed you will see a directory named for the date when you submitted the sample. For example, if you submitted your sample on July 18, 2002, the name will be lab020718. You will also see a directory called Test\_Data. This directory was placed here in case you want to practice using the software before you have data on your own samples to analyze. Click on the directory you want. It will be highlighted. Click <Set Directory>.
14. You will now see a list of data files. For example, if your sample was assigned number 23 in the rack and if you asked for proton and carbon-13 spectra of your sample, you might see "23\_01.fid" and "23\_02.fid" for the proton and carbon spectra, respectively. If you entered the Test\_Data directory, you will see two data sets called 1H.fid and 13C.fid. Click on the one you want to view. It will be highlighted, then click <Load>. All NMR data files end with .fid; attempting to load a file that does not end in .fid will result in an error message. If you get lost in the directory structure, click <Main Menu><File><Set Directory><Home> and try again.
15. Typing text on the command line will display the text you entered when you submitted your sample.
16. Click <Main Menu> <AutoProcess>. The spectrum will appear on the screen.
17. If the spectrum is not occupying the full area within the blue corner markers, click <Main Menu> <Display> <Size> <Full Screen>.
18. Click <Full> to be sure that you are seeing the entire spectrum.
19. If desired, you can enlarge the graphics window by clicking <Resize>. Clicking <Resize> again will return to the smaller size graphics window.
20. You are now ready to analyze your NMR spectrum. The VNMR software has far too many features to discuss all of them here. A few examples of useful procedures will be given. The major headings below represent self-contained procedures that may be performed in any order as needed to extract the information you want from your spectra.

### **Expansions.**

21. Enter the interactive display mode by clicking <Main Menu><Display><Interactive>.
22. Note that there are three labels at the bottom of the graphics window showing the functions of the three mouse buttons. These labels will change depending on what modes you have selected.
23. You can now expand your spectrum to see details. Use the right and left mouse buttons to position the two red cursor bars so that they enclose a region you wish to expand, then click the <Expand> button.
24. The middle mouse button controls the vertical scale of the spectrum. Just point the cursor where you want the top of a resonance to be and click the middle button.
25. To return to the full spectrum click the <Full> button.

### **Setting Up the Chemical Shift Scale.**

26. Enter the interactive display mode by clicking <Main Menu><Display><Interactive>. If the chemical shift scale is not displayed click <dscale>.

27. The autoprocess procedure attempts to set the chemical shift of TMS to zero. However, for various reasons, the automatic procedure may not always give accurate results. You should always check to be sure that the chemical shifts are correctly referenced.
28. You can set the chemical shift of any resonance by placing the left cursor on the peak, then typing `rl(n)`, where *n* is the chemical shift you want that resonance to have. If the resonance is TMS, *n* can just be zero. But any peak of known chemical shift can be used to set the reference. For example, the proton chemical shift of  $\text{CHCl}_3$  is 7.27 ppm, so the command would be `rl(7.27p)`; the *p* is required to indicate that the value is given in ppm. All the resonances are now referenced to TMS indirectly through the known chemical shift of chloroform. This is not as accurate as referencing directly to TMS, because the presence of your solute molecule can change the chemical shift of the solvent slightly, but this procedure is adequate for many purposes. For carbon, the center peak of the  $\text{CDCl}_3$  triplet occurs at 77.23 ppm, and the command would be `rl(77.23p)`.

### **Integration.**

29. Enter the interactive display mode by clicking `<Main Menu><Display><Interactive>`.
30. The second button on the bottom row toggles between `<Part Integral>`, `<Full Integral>`, and `<No Integral>`. Click the button until you see the display you want. Partial integrals is generally the most useful. Set the display to this mode. When integrals are displayed, the middle mouse button controls the integral scale rather than the vertical scale of the spectrum.
31. The autoprocess macro attempts to establish points where the integral is reset to zero, but for many samples the automatic choices are not the most useful ones. You can remove all the resets by typing `cz` on the command line. This stands for clear zeros.
32. Click `<Resets>`. Notice that the mouse button definitions at the bottom of the graphics screen have changed. Point the cursor where you want a reset to be inserted and click the left mouse button. If you enter a reset that is incorrect, you can remove it by clicking the right mouse button. The middle mouse button will still control the integral scale.
33. Clicking any other menu button will turn off the reset entry mode.
34. If desired, you can expand a portion of the spectrum so that the resets can be placed more carefully between closely spaced multiplets.
35. Next the integrals must be scaled to some reasonable value. This is done by guessing the number of protons represented by a given integral. For example, suppose you have an upfield peak that may be a methyl. Place the left cursor within this integral region and click `<Set Int>`. You will be prompted for a value; type 3. The chosen integral will now be set to three protons, and all other integrals will be normalized to this one.

36. To display the numerical values of all the integrals beneath the spectrum on the screen, first type vp=12. This moves the spectrum up in the graphics window to make enough space to print the numerical values. Now type dpir; the numerical values will appear. If the integrals do not produce the integer ratios that you expect, try another normalization for one of the integrals until you get satisfactory results. Displaying the numerical values of the integrals takes you out of the interactive display mode. You can reenter the interactive display mode by clicking <Main Menu><Display><Interactive>. If the numerical values are too crowded, try displaying them using an expanded portion of the spectrum.

### **Labeling Resonances.**

37. Enter the interactive display mode by clicking <Main Menu><Display><Interactive>.
38. It is often convenient to label resonances with their chemical shifts or frequencies. This can be done by clicking <th>. A horizontal yellow cursor will appear. Press the left mouse button and drag the yellow cursor so that all the lines you wish to label protrude above the yellow line.
39. Click <Dscale>, and note whether the axis is in ppm or Hz. To change between the two type axis='p' for ppm or axis='h' for Hz. Remember that chemical shifts are always in ppm while coupling constants are in Hz.
40. Type dpf; each line will be labeled with its chemical shift or frequency.

### **Plotting.**

41. Before you can make printed copies of your spectra you must define the correct printer for the site where you are operating. Instructions for doing this can be found in Note 2) Printing and Plotting on page 15.
42. There are a wide variety of commands available to produce hard copy plots of your data. The basic idea is to issue a series of commands enumerating the items you want to appear on a single sheet of paper. When you have defined everything you want on the paper the page command is typed and the defined plot is sent to your currently selected printer. The table on page 13 gives some of the most useful plotting commands.
43. For example a basic plot of a spectrum with a chemical shift scale can be obtained by typing: pl pscale page. If analog integrals are displayed on the screen they will be plotted.
44. To add numerical values of the integrals to your plot one could type: vp=12 pl pscale pir page.

### **Viewing Other Data Sets.**

45. If you want to view another spectrum, click on <Main Menu><File>. If you have not executed any commands to change your current directory you should see the list of data files ending with ".fid" as before. Just return to step 14 choosing a different file.
46. If you do not see the expected list of data files or if you want to view files from a different submission date go back to step 9. Be sure to choose your home directory as directed in step 9.

47. You can alternatively navigate up one level of the directory hierarchy by clicking <Set Directory> <Parent> without highlighting any of the names in the list. Repeatedly clicking <Parent> will move you to successively higher levels of the directory tree. When you are in the directory you want, click <Return> to see the <Set Directory> and <Load> buttons as before. You can then navigate back down the directory structure to find the dataset that you want to process.

### Exiting the System.

48. To leave the system type "exit" on the command line or click <Main Menu><More><Exit VNMR>. If the <More> button is not visible, you may need to increase the size of the window by pressing the mouse button at the right edge of the window and stretching the window out a little. Then close the PuTTY terminal window by typing, "exit" at the prompt. You may have to type "exit" several times until the window disappears. DO NOT USE THE X BOX IN THE UPPER RIGHT HAND CORNER OF THE WINDOW TO CLOSE A WINDOW BECAUSE THIS CLOSES THE WINDOW LOCALLY BUT DOES NOT ALLOW THE UNIX SERVER MACHINE TO PROPERLY TERMINATE THE PROGRAM RUNNING REMOTELY.

### Some Useful Commands.

Here are a few useful commands. Some are typed on the command line while others use the menu buttons as indicated by the angle brackets.

<Main Menu> <File> <Set Directory> <Change>	This will prompt for the path to the desired directory. Type the path and press the enter key. The directory will become the new working directory.
ds(n)	Display the nth spectrum of a set. The default is the first one which is often the only one.
<Part Integral>	Display integrals with resets. This is the integral display mode you want to use for this class. The button cycles through two other modes (<Full Integral> and <No Integral>). Full Integrals should not be needed. No Integral can be selected when you want to remove integrals from the screen and from your plotted spectra.
cz	Clear all integral resets
<Resets>	Add (using the left mouse button) or remove (using the right mouse button) integral resets.
<Set Int>	The integral region selected by the cursor is set to a specified value entered at the prompt. All other integrals are then calculated relative to this one.
vp=12	Needed to move the spectrum up 12 mm to make room to display numerical integral values below the spectra on the screen or on paper.

dpir	Display numerical integrals below the spectrum on the screen.
dli	Display a list of integrals in the text window.
th	Displays a threshold bar horizontally on the screen. The bar may be adjusted using the left mouse button. All lines extending above the threshold will be labeled with their chemical shifts when the dpf or ppf commands are executed.
dpf	Label peaks with their chemical shifts on the screen.
ppf	When included in the list of plotting commands, the peaks are labeled with their chemical shifts or frequencies.
bc	Correct the baseline for drift. Do this only after you have defined all the integral resets. This improves the accuracy of your integrals.
dssh	This command displays all the spectra of a multiple FID experiment horizontally across the screen.
full	Return to using the full graphics window for a single spectrum.
page	Send a previously defined plot to the currently selected printer.
pl	Plot the currently displayed spectrum. Plot integrals if integrals are displayed.
pl('all')	Plot the spectra as seen on the screen with the dssh command and the parameter list.
pscale	Plot a scale beneath the spectrum.
ppa	Plot a list of parameters pertaining to the current spectrum.
pir	Plot numerical values of the integrals below the spectrum.

## NOTES

### Note 1) Username and Password

In order to submit samples and view the resultant spectra, you must have a uNID and password. This will be the same one you use to access the Campus Information System (CIS). You will not be able to use your old NID if you have one. You must use the new uNID that consists of your eight digit student ID with the first zero replaced by a lower case letter "u", for example, u1234567.

To ensure that your uNID and password work point a browser to <http://www.utah.edu>, choose Students, then Registration & Records, then Campus Information System. Try to sign on using your uNID and password. If the system accepts your sign on, your uNID and password are valid. If you fail, follow the directions on the sign on page to fix whatever problems you are having.

## Note 2) Printing and Plotting

Make sure you have selected the proper printer within VNMR for the computer lab in which you are located. See the options listed in the table below for the printer choices for each of the labs. If you select a printer in some other lab, your printed output will go there, not to the one located nearby. To select or change the printer: click <Main Menu> <More><Configure> <Show Output Devices>. If the current plotter is not the desired one, click <Select Plotter> until the displayed message shows that the proper one is set. Repeat using <Select Printer> until the proper printer selection is displayed. The printer and plotter are normally both set to the same device.

Lab	Printers
HEB 1100	chema
HEB 4209	chem4209
Peterson	peterson
EMCB 130	emcb1
MMC	mmc

At most of the open access labs there is a per page charge for printing. The charging procedure requires that you have money on your University ID card, just as is needed to use the photocopiers in the library. There is a machine near the consultant counter in the multimedia center to add money to your ID card. To print, submit your print job as usual; instead of the job printing it will go into a print queue. Go to the counter in the multimedia center with your ID and the PC# from which you are printing. The person at the counter will ask you how many pages you are printing, charge your card, and then release your job to the printer. In labs where there is no attendant, there are self-service machines. Just follow the instructions provided.

## Note 3) Computer Center Hours

The fourth floor teaching labs in Chemistry are open only when labs are in session. Other labs have varying hours. Be sure to find out the hours of the labs you want to use.

## Note 4) Where to go for help

If you are at the campus computer centers and PuTTY and/or Xwin32 are not working or you need help getting them started:

Go to the consultant counter/help desk of the computer center.

If you are in the chemistry computer labs and PuTTY and/or Xwin32 are not working or you need help getting them started:

Contact your TA or one of the NMR staff.

If no one is available, you may want to go to a campus computer center where there is a help desk consultant.

If you are having trouble connecting to [vnmr.chpc.utah.edu](http://vnmr.chpc.utah.edu) or getting VNMR started:

Contact the CHPC help desk at [problems@chpc.utah.edu](mailto:problems@chpc.utah.edu) or 1-4439 between the hours of 8:00 AM and 5:00 PM M-F.

If you are able to start VNMR successfully but have a question dealing with processing your data...

Contact your TA or one of the NMR staff. An NMR staff member will normally be available during lab times and will be willing to help you as other duties permit when labs are not in session.