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Varian Unity+ 400 MHz NMR
Routine Operating Procedures

Notes:
- Throughout this document commands that you type in are denoted in ***bold italics font***. All commands require a return.
- When typing commands remember that UNIX is "case sensitive", meaning that a capital letter is not equivalent to a lower case letter.
- The left mouse button is the normal operator, except in a few cases such as increasing lock or shim values, changing from axial z to z0, and manipulating the spectrum. During some operations you will see three yellow boxes lined up at the bottom right side of spectrum window. The codes in these three boxes indicate the functions of the left, middle, and right mouse buttons.
- In the VNMR program, to move a window to the "front" simply place the cursor on one of the edges of the window and click the left mouse button).
- Most of the commands you need for routine operation can be performed by clicking on a series of boxes in the menu bar above the spectrum window. However, all of these commands can also be entered directly by typing the command code into the cursor window above the menu bar. Sometimes, a particular operation can only be performed by typing in the command. If you are looking to do something different, check the "Command and Parameter Reference" book located by the instrument – chances are, you will find the command you are looking for. Also, it is possible to string commands together on one line, separated by spaces. Type return at the end of the line to execute your commands.

LOGIN

At the login prompt, login as ***advlab*** then enter the password at the prompt. This will automatically open the VNMR operating system. If the VNMR software is not loaded already, load it by clicking on the VNMR icon in the toolbar at the bottom of the screen.

Note: For more information about making your own directory within the advlab account see the appendix.

LOCKING AND SHIMMING

Note: There are two rows of menu buttons. The top row is a permanent one, but the bottom row changes as you move through menu options. The MAIN MENU button always brings you back to the highest level among the menu options.

1. Click on the ACQI button on the top menu, to open up the acquisition window. Occasionally the ACQI button is not available; in that case, type in the command ***acqi***.

2. Within the acquisition window, hit EJECT with the left mouse button to EJECT THE SAMPLE in the probe.

3. To insert a new sample, first carefully remove the spinner turbine from the top of the magnet. Please try to handle the turbine from the top part only; grease from your hands on the lower portion can interfere with sample spinning! Remove the tube from the turbine and place your CLEAN TUBE WITH SAMPLE AT 50 ± 1 MM HEIGHT in the spinner turbine to the correct depth using the plexiglass sample depth gauge. Place the tube/turbine in the top of the magnet. Make sure to give the sample a gentle push into the magnet, so that the turbine is not bouncing around on the cushion of air.

   Occasionally, the spinner turbine gets stuck at the top of the magnet and if it drops in suddenly your tube will break in the probe. This is VERY BAD! To avoid this, nudge the spinner turbine into the top of the magnet, as suggested in step 3, before clicking "insert" on the computer screen. If the sample does not start to move down immediately, hit "eject" again RIGHT AWAY! Then adjust the spinner and try again. If this happens more than once, find Dave!

4. Open the LOCK subwindow, within the Acquisition window. Often, the sample will "lock itself" if the solvent used is the same as that of the previous sample. If the sample is already locked you can skip to step 7. If the sample is not locked, turn off the lock by clicking LOCK: "off". Make sure your sample is spinning (check the spin value below the lock signal in the window; if it is colored green and the number is 20, your

sample is spinning properly). If it is not spinning, turn on the spinning by clicking the appropriate ON button.

5. Click Z0 to get only one sine wave on lock screen. You may need to first increase lock gain all the way, and lock power to >40 to see a strong sine wave. Probably start with the -16+ box of Z0. The left mouse button does -16 and the right does +16 when clicked in this box.

6. You want to move Z0 to get "right on top" of one sine wave. When you are "on one wave" it will appear as a straight line. Turn lock ON. If you see no sign waves, make sure lock is off and then move Z0 in one direction or the other until you do. Then move it in the direction that continues to give fewer and fewer waves, until you are "on top" of one sine wave.

7. Reduce lock gain and lock power to remove saturation in the lock signal. The spectrometer should be locked and the lock power should be set to around 22 for chloroform, and 15 for D_2O, acetone or benzene. Then set lock gain so that the lock level is about 80. If the lock level fluctuates in intensity, then the power is too high. Lower the power and increase gain until there is no longer any fluctuation.

8. Maximize lock signal using LOCK PHASE adjustment. Lower the gain as necessary to keep the lock signal in range.

9. Go into the SHIM subwindow. Shim using the Z1 and Z2 shims (**not** the Z1C or Z2C – C for coarse). Start with the Z1 -64+ button until signal is maximized (left mouse button decreases shim current, right mouse button increased the current). Once you are satisfied, do the same with Z2 -64+. Go back to Z1, but this time you might use finer adjustments, -16+. Go back and forth between Z1 and Z2 until you are satisfied.

10. You might want to readjust the LOCK PHASE to maximize lock signal, if the shimming was way off to begin with. If you change LOCK PHASE, you should probably go pack and touch up Z1 and Z2.

11. Check lock level. If it is between60 and 90 on the remote status module meter, it's fine. If not, lower or raise the lock gain.

12. Close the acquisition window by clicking on the CLOSE button at the upper right of the window.

EXPERIMENT SETUP AND DATA ACQUISITION

1. Set-up the proton parameters, with the proper solvent, by clicking the SETUP button in the command menu. Choose the 1H,CDCl3 parameter button if your solvent is $CDCl_3$. If you are using another solvent, click the NUCLEUS,SOLVENT button. The new menu will allow you to select H1 parameters and then the solvent of your choice. *If you are doing carbon NMR see the Carbon NMR section near the end of this document.*

2. To begin acquisition, type ***ga***, or use the ACQUIRE menu and click on the appropriate button.

3. The *Acquisition Status* window in the upper right corner of the monitor indicates what the spectrometer is doing.

4. After acquisition is complete, your spectrum should appear. Click the FULL button to display the full spectrum and phase it by typing ***aph***. Occasionally, you will need to manually phase your spectrum (this is often true for carbon spectra). Ask you instructor or lab asst. for help.

MANIPULATING YOUR SPECTRUM

Note: If you find that you can't manipulate a spectrum, click the following button sequence: MAIN MENU »» DISPLAY »» INTERACTIVE. The yellow boxes at the bottom of the spectrum, cr (cursor), vs (vertical scale), and delta (distance between cursors), refer to the function of the three mouse buttons. Also note that the middle mouse button controls the vertical scale.

Expanding around peaks of interest:

Many of the operations described below require that you expand around peaks. To expand, you set the cursor line (the red line) to the left side of the region you want to expand. Clicking and holding the left mouse button while you move the mouse allows you to position the red cursor. Once the red cursor is positioned, click and hold the right mouse button to move a *second* cursor to the right side of the area you want to expand. After you have defined the area to be expanded with the two red cursors, click the EXPAND.

When you have two red cursor lines on the spectrum, bear in mind that moving the left red cursor causes both cursors to move together. Only the right cursor moves independently. Therefore, if the right cursor is visible and positioned at the extreme far right side of the window, you will not be able to move the left cursor to the right. You either have to remove the right cursor from the window (click the CURSOR button) or move the right cursor close to the left cursor before moving the left cursor.

1. Set the position of the reference peak by placing the cursor on the peak of interest (e.g. TMS) then click the REF button (you may need to expand around the peak of interest). You will be prompted to enter a number for the desired ppm. Make sure you add a "p" after the number to designate ppm, rather than Hz. You can also set the reference by typing ***rl(x.xxp***) after you have placed the cursor on an expanded reference peak.

2. Click the DSCALE button to display the ppm scale at the bottom of your spectrum. Alternatively, you can type ***dscale***. You can switch from ppm to Hz by typing ***axis='h'*** or ***axis='p'***.

3. To integrate your spectrum, click the PART INTERGRAL button (this button toggles between PART INTEGRAL, FULL INTEGRAL, and NO INTEGRAL). Click the RESETS button to enter the mode that will allow you to "cut" the integral into segments for each peak of interest (the "PART INTEGRAL"). To do this, start from the left side of the spectrum and left-click just to the left of a peak, where you want the integral for that peak to start. This zeros the integral to begin an integral segment. You define the end of a segment by left-clicking to the right of the peak. Continue through your spectrum "chopping up" the integral into the integral segments of interest. If you make a mistake, a right-click undoes your last zeroing operation. You can clear all the "zeros" (segments) by typing ***cz***, (clear zeros).

   Important: when you are done selecting integral regions, click on the CURSOR or BOX button on the left side of the lower menu. This simply gets you out of the RESETS mode.

   Another option, if you are lucky to have very clearly defined, well separated peaks, is to have the computer select the integral segments. To do this type ***region*** Then click on the PART-INTEGRAL box to see the integral segments.

4. To set a nominal value for an integral segment, place the cursor on an integrated peak and click on SET INT button. You will be prompted to enter a numerical value for this integral segment. If you wish, typing ***dpir*** will display the results, but you must provide room under the spectrum so there is space to display the integer values (type ***vp=12*** to move the **v**ertical **p**osition of the spectrum up 12 mm).

5. To display the ppm or frequency of your peaks on the spectrum or in a table (much better) you will want to set the *peak threshold.* Click on the TH button and set the yellow line to the appropriate level. Any peak above the line will be recognized as a peak on your spectrum or in your line list. Any peak below this line is considered noise. Hitting the PEAKS box when sending your spectrum to the plotter will cause the peak frequencies to be plotted directly on your spectrum, above the individual peaks. Alternatively, you can print the peak frequencies on a separate sheet of paper by typing ***dll mull prul*** (each a separate command). Using the ***dll mull prul*** protocol is *highly* recommended for a proton spectrum and will provide a convenient list for determining coupling constants!

6. If you have displayed the integrals they will be plotted on your spectrum, but if you want to plot the integer values at the bottom of the spectrum as well, you need to allow space at the bottom of the spectrum. To do this type ***vp=12*** (**v**ertical **p**osition of spectrum up 12 mm from bottom of page).

7. To send a spectrum to the plotter, click MAIN MENU, DISPLAY, and PLOT boxes; then hit PLOT (again), SCALE, type ***pir*** (**p**rint **i**ntegral **r**egions - to plot the integral values of the integrals) and, perhaps, PEAKS.

8. To give your spectrum a title, type ***atext('xxxxxx')***.

9. To print what's been sent to the plotter, click the PAGE box, or type ***page***.

10. To save a FID/spectrum, type ***svf*** (you will be prompted for a file name), **but** you may have your own subdirectory for your files. To go to your subdirectory click on the FILE button to see the files/directories in the current directory. Select your directory if you have one, before saving an FID.

FINISHING UP

1. After you are through collecting data, remove your sample and insert the standard sample into the probe. LOCK & SHIM ON THE STANDARD SAMPLE!!

2. To LOGOUT, click on the desktop tool bar at the bottom of the screen to bring this window to the front. Find the EXIT button near the middle of this toolbar and click on it. You are done!

Acquiring Carbon NMR Spectra.

Carbon NMR spectra are even easier to acquire than proton spectra, because no integration need be done; however, you need a greater sample concentration than you do for a proton spectrum (>100mM).

1. Sample insertion and shimming is the same as that used in proton NMR.

2. Click the SET UP button, on the MAIN MENU.

3. Select the 13C,CDCl3 parameter button if your solvent is $CDCl_3$. If you are using another solvent, click the NUCLEUS,SOLVENT button. The new menu will allow you to select 13C parameters and the solvent of your choice.

4. To begin acquisition, type ***ga***, or use ACQUIRE menu and click on the appropriate box (e.g.GO, with FT).
5. Data is saved to the disk every 32 scans, anytime after 32 scans you can look at the data. Just type ***wft*** and the acquired data will be transformed. The default setting for the number of scans is 1024! You will probably not need this many. Once your data looks good, you can stop the acquisition with the ABORT ACQUISITION button.

7. AFTER ACQUISITION IS COMPLETE, transform the data using the ***wft*** command; use the FULL button to see the full spectrum and PHASE it by typing ***aph*** (although often carbon spectra require manual phasing).

8. Plotting procedures are the same as those used for proton spectra.

APPENDIX

FILES AND DIRECTORIES

UNIX files and directories are specified by paths in a way similar to DOS files and directories (actually DOS was patterned after UNIX). For instance, the file "junk" may be nested inside a number of directories and its complete pathname might be: **/export/home/alberg/nmrdata/junk**, which indicates that **junk** resides in the subdirectory **nmrdata**, which is in turn a subdirectory of **alberg**, etc. All of the user accounts are found in the **/export/home** directory. You can move up and down in the directory system in the VNMR program by clicking on the FILE box. To move down a directory click on the directory of interest and click on the CHANGE or SET DIRECTORY button. To move up a directory click on the PARENT button. The UNIX command ***pwd*** is useful to determine where you are.

As a member of the *nmr* group (a UNIX distinction) all nmr user accounts have permission to access and read anyone else's files (including shim files, which may be useful) however you cannot alter other user's files (of

course, this isn't true of the files of other members of your user account, so be careful). You can call up other user's files, save them in your directory and alter them as you like, but you cannot alter the original file. To look at other user's files simply move through the directories as discussed above.

To make a subdirectory within the **advlab** account, use the ***mkdir*** command as follows: type ***mkdir ('student')***. This can also be done via the menu buttons, under the FILE button on the MAIN MENU (see below). You probably want to do this when operating at your login level, so that your directory (we'll call it **'student'**) is directly under the **advlab** directory (i.e. its full pathname could be **/export/home/advlab/student**). After logging in, you should first change to your subdirectory. Then, if you save NMR data it will be saved in your subdirectory. You want to make sure you are operating in your subdirectory because a file gets saved to the ***pwd*** (present working directory) and if you don't know what the **pwd** is at the time you executed the save command, you may have trouble locating your file later. Of course, you cannot save files to the accounts of other users. If you try you will get an error message.

It is also possible to make a subdirectory using the command bar at the top of the spectrum window by going from MAIN MENU to FILE. Once you are in FILE, toggle through the boxes as follows: SET DIRECTORY-MORE-CREATE NEW. Also, to change the directory you are in, go from SET DIRECTORY to CHANGE.