

Instructions for Manual Acquisition of ^1H and ^{13}C NMR Spectra

(These instructions only work if you take the time to read them!)

This instruction sheet is for manual acquisition. The automation mode is much simpler, but the manual mode allows more control over the acquisition and processing of the spectrum. At some points in this sheet are hints for addressing likely problems that may arise. These are marked with reference numbers and are addressed at the end. Remember that this is LINUX software and no commands will work unless the cursor is over the window or blank that you are interested in.

I. Basic preparations

1. Make sure your sample is at least 1/3 full (but no more than 1/2 full). If the NMR tube is not at least this full, the instrument will have a hard time locking and shimming on your sample.
2. Make sure your NMR solvent is deuterated. Regular chloroform (CHCl_3) will not work; you must use CDCl_3 (or whatever the deuterated version of your solvent is).
3. Please have clean hands. Dirt on your hands will get into the magnet to some degree. Dirt in the magnet causes spinning problems.

II. Login and loading the sample into the magnet

1. The screen should show a login box. If you are using the instrument for a class, your class number is your ID (e.g. che381). Select your class from the scrolling list. Class passwords are all "chemistry". (If you have a faculty account and do not know your password, talk to Dave.)
2. Once logged in, you will see a set of icons to the right. Double click the "Delta" icon.
3. A window entitled "Delta" will open. This window has two rows of five icons toward the upper left. Click on icon four in the lower row. It looks like a metal can. [You *might* hear air hissing in the magnet at this point. This is the spinner turning on. Depending on your account settings, the sample may eject at this point. This has a fairly loud, sustained hiss to it.]
4. A window entitled "Spectrometer Control" will open. Click on the button at the bottom entitled "Sample". A new window will open.
5. In the "Sample: che-acq.davidson.edu" window in the upper left are the words "Sample State". Underneath are two buttons – an 'L' in a green box and an 'E' in a red box. If you heard an air hissing sound in step II.3., then the 'E' button will likely be depressed already. If so, the standard should be bobbing above the center of the magnet. If the standard has not automatically ejected already, press the 'E' button and wait (5 seconds?) for it to eject.
6. Go over to the magnet, step up on the riser (if necessary), and remove the standard from the magnet. Place the standard with its Teflon spinner in a safe place on the spectrometer cabinet.
7. On top of the spectrometer cabinet is a styrofoam holder full of spinners. Remove a spinner from the holder, and place it into the plastic depth gauge (also on the cabinet). *Carefully* insert your NMR sample into the hole in the top of the spinner and slide it down until the bottom of the tube hits the bottom of the depth gauge. Do not use too much force or your tube will break. [Spinners have two pieces – an outer sleeve and a narrower piece that holds the sample. Be sure that the narrow piece is fully depressed into the sleeve. When you push the NMR tube into the bottom of the depth gauge, the narrow piece should slide down with the tube (if it is not already all the way down).]
8. Place your sample/spinner into the top of the magnet. Oddly enough, you should put your sample into the slot that says not to insert a sample here. Go figure. Press the 'L' button in the

“Sample” window. The sample should drop down into the magnet and make a “clunk” sound when it hits bottom.

III. Locking and shimming

1. Pick the appropriate solvent from the solvent menu in the middle of the Sample window.
2. To the right of the solvent menu is the “Lock Control”. To the right of the “Level” setting are three buttons. Press the rightmost button with the picture of a lock and three lines with a coil around them. [This begins the autolock and shim procedure. This takes a couple minutes, and the magnet will lock, cancel the lock, and finally re-lock at the very end. You will also hear a lot of clicking in the magnet. At the end, the last line in the Delta window will be “Gradient Shim: Completed in 2 iterations”. Be patient.]
3. Once shimming has completed, confirm that the instrument is indeed locked. (Check for a green bar reading “LOCK ON” at the bottom of the “Lock Control” area. If it is not locked, there will be a red bar reading “LOCK OFF”.) If the instrument is not locked, click the rightmost button next to “Gain”. This button has a yellow lock with “AUTO” written on it. Wait a few seconds for the instrument to lock itself.

IV. Load Experiment and Acquisition

1. In the Spectrometer Control window click the “Expmnt” button at the bottom to open the Open Experiment window.
2. The directory at the top of the Open Experiment window should be “/usr/delta/global/experiments/”. If not, press the button with the globe on it to enter this directory.
3. For ^1H select “single_pulse.exp”, and for ^{13}C select “single_pulse_dec.exp”. To find these files, you can simply press ‘s’ to get to all the files that begin with ‘s’. The ^1H and ^{13}C files are the second and third ‘s’ files respectively. Alternatively, you can scroll through all the experiment files in the middle menu (starts with “apt.exp”).
4. With the correct experiment selected, click the OK button at the bottom, and a new window entitled “Experiment Tool: single_pulse.exp” will open (assuming ^1H). [Key differences between the ^1H and ^{13}C experiments will be addressed.]
5. This window has four parts: Header, Instrument, Acquisition, and Pulse. Each section may be accessed by pressed the corresponding button across the top of the window.
6. In the Header section enter your file name. This file will be saved into the main folder of the user who is logged onto the computer. For example, for the user CHE 202, simply entering “test” as the file name would save it into the directory “/home/CHE 202/file/data”. If you want to save the file into a subdirectory, specify that subdirectory in the file name. Typing “exp1/test” in file name would save the file “test” into the subdirectory “exp1” of “/home/CHE 202/file/data”. If a subdirectory does not exist, the computer will automatically create it. You may also enter an ID number for your sample. A number of default processing functions are listed. These are fairly standard and can be left intact. To finish the Header section, check the auto_gain box. [For most samples, you only need to mess with the Header section. All the other sections can be left alone with their default values.]
7. In the Instrument section pick your solvent. The recvr_gain field is ignored if you checked the auto_gain box in the Header section.
8. In the Acquisition section pick you nucleus in the x_domain. Set the x_offset and x_sweep to appropriate values. For ^1H the default values give a window of -2.5 to 12.5 ppm. For ^{13}C the range is -25 to 225 ppm. x_points gives the number of data points for the file. Set the number

of desired scans. $x_prescans$ can normally be set to 0. mod_return has no effect on the final spectrum, but it must be set to 1 to allow viewing of the spectrum as it is being collected.

9. In the Pulse section the default pulses are 45° for 1H and 30° for ^{13}C . These seem to work fine. For most 1H spectra the $relaxation_delay$ can be set down to 0.5[s] (do not use 0). A more typical number for ^{13}C would be 1 or 2[s].
10. With all the parameters entered, hit the “Submit” button at the bottom of the window.
11. Press the GO button in the alert window that popped up.
12. The FID and spectrum can be observed by pressing the “View” button in the Spectrometer Control window. The “View” button shows the FID after each scan and “Process” shows the spectrum (unphased). The bottom of the window shows the remaining time of the experiment.

V. Data Processing (setting the reference, peak picking, and integrating)

[It is very easy to make mistakes in the processing, and there are a couple ways to undo the mistakes. The first is to reload the data. Go to the Delta window and select “Auto” from the File. This will bring up the Open File window at the main directory for you as a user. Select your current file as specified in the Header section, and you will have a clean slate to work from. The second way to refresh your data is with the “Process” button in the upper left of the spectrum window. Delete any undesired processes from the listed in the middle left of the spectrum window and hit “Process”. The original data will be restored.]

Some handy commands for this section are...

Home	restores the spectrum to its original size and scale
End	sets the vertical scale to match the largest peak in the viewing region
Backspace	undoes the most recent resizing of the viewing region (very valuable)

1. At the end of the acquisition, a new window will open. This will have a number of buttons at the top, the FID in the middle, and the transformed and phased spectrum at the bottom. [Note that if you deleted the default processing functions in the Header section, the spectrum window may be blank.]
2. Setting the reference (both 1H and ^{13}C) – be sure you know your reference!: Select “Reference” followed by “Reference” from the Display pull-down menu. From the Zoom cursor menu pick the icon with a magnifying glass with a dot in the middle. Click and drag this cursor to get a very close view of the reference peak in the spectrum. Change to the Pick cursor menu and select the icon with the letter ‘C’ (second from left). Click on the peak to copy the ppm value into the buffer. Move the cursor to the “Position” box to the right, middle click (click the wheel) in that box to paste the ppm value, and scroll through the box to remove the default value (likely says 50[%]). Set the value in the “Reference” box to the desired ppm value (normally 0 ppm for TMS). [Remember that the cursor must be over the box for the keyboard to work in that box.] Press the “Process” button in the upper right to set the reference.
3. Peak picking (both 1H and ^{13}C): In the Zoom cursor menu pick the round magnifying glass icon. Click and drag with this cursor to enlarge the shortest peak of interest in the spectrum. In the Peak cursor menu pick the icon showing the diamond with a line above and below it (fourth from the left). Click at the baseline of the peak and raise the mouse to just below the top of the shortest peak. A green line should rise up with the mouse. With the baseline at the correct level, press the Auto Peak Pick button at the top of the window. This is the button with a white ‘X’ in an octagon. The correct peaks should be picked. Check the entire spectrum by pressing the “Home” key.

4. Integrating (generally ^1H only):
In the Zoom cursor menu pick the round magnifying glass icon. Click and drag to enlarge a peak or group of peaks of interest. In the Integral cursor menu pick the regular integral cursor (first on left). Beneath the x-axis (not in the actual spectrum window but in the grey area below it) click and drag to select a range of spectrum to integrate. Repeat for all the peaks on the screen. Change back to the Zoom cursor (magnifying glass icon) to get other peaks on the screen and repeat the integral cursor until all the peaks have been integrated. Set the peak area by getting a known integral on the screen. In the Integral cursor menu pick the integral/arrow icon (fourth from right). Click on the integral of interest. It should change from red to yellow. From the Options pull-down menu select "Show Options". Six boxes will appear at the top of the spectrum. In the "Normal" box, enter the desired integral value and press enter. The integrals should all scale to their proportional values. Re-select "Show Options" to get rid of the boxes.

VI. Printing

1. If parameters are desired on the spectrum, select "Plot Params" from under the Preferences pull-down menu.
2. With peak picking and integrals properly set, click the Print button (picture of a printer) toward the top of the window.
3. A new dialog "Print Options" will appear. Because of a bug, not all accounts can print properly to the printer (HP_LaserJet_2200). You may need to select Cups-PDF. This option "prints" the file to a PDF on the desktop. Open the PDF and print normally. Note that Cups-PDF will generally overwrite previous files, so you should print a file as soon as it is created.
4. Of course, if you want, you can expand regions of interest in your spectrum and print those out separately.

VII. Finishing up

1. Close the spectrum window.
2. Close the experiment set-up window.
3. In the Spectrometer Control window click on the "Sample" button at the bottom to bring the "Sample" window to the front.
4. Click the red 'E' button to eject your sample.
5. Take the standard (with spinner), double check the sample with the depth gauge, and exchange the sample and standard in the magnet.
6. Remove your sample from its spinner by holding the bottom, narrow part of the spinner and carefully pulling up on your sample.
7. Place the spinner in the holder.
8. Hit the 'L' button to drop the standard into the magnet.
9. Select the appropriate solvent of the standard (chloroform) from the scrolling menu on the left middle of the window.
10. On the right middle of the window are six buttons in two rows of three. Press the third button on the bottom row (Gradient Shim and Lock) and wait for the sample to shim and lock.
11. Raise the gain by three points. The gain controls are in the same larger box as the auto lock button.
12. Turn off the spinner by clicking the button with the red X in the spinner box in the upper middle of the window.
13. Close the Sample window.

14. In the Spectrometer Control window hit the red “Unlink” button and then close the entire window.
15. Close the “Delta” window and click “Exit” in the warning dialog box that will come up.
16. Log off the computer (System pull-down menu and Log out).

VIII. Leaving

1. Retrieve your sample from the autosampler.
2. Throw away any papers, kim-wipes, etc. that you have used.

Appendix A – Running a COSY – may be a bit dated

Running a COSY is no more difficult than either a ^1H or ^{13}C . To start, get your sample loaded into the instrument and locked and shimmed (sections I through III in this handout). Acquire a standard ^1H spectrum (section IV). Based on this spectrum, decide the x_sweep (the range of ppm values you want to observe) and x_offset (the middle of your ppm range) values for your COSY. Typically, you do not need to run a COSY on the full x_sweep value (12.5 ppm). Narrowing the window can increase the resolution of your COSY spectrum. At this point, you are ready to acquire a COSY spectrum.

1. In the Spectrometer Control window click the “Expmnt” button at the bottom to open the Open Experiment window.
2. Click on the global folder (folder with a globe on it) to get into the right experiment directory.
3. Scroll down through the experiments in the middle of the Open Experiment window, find “cosy.exp”, and select it.
4. A new window entitled “cosy_exp on host che-acq.davidson.edu” should open. In the Header section, select the checkbox for “autogain”.
5. Enter a title as desired.
6. If you want to narrow the width of the spectral window, enter the desired values into the x_sweep and x_offset fields (in the Experiment section). The x_offset is the middle of the window you want to examine, and x_sweep is the width of the window. Both values should be in the units of ppm.
7. Submit the experiment.
8. Once the experiment is complete (less than 30 minutes using the default values), a COSY spectrum will appear in an “nD Processor” window.
9. Click on the video camera button in the upper right to open the spectrum in a “2D Viewer” window.
10. From the Analysis menu select Symmetrize-Cosy. This will clean up any stray, off-diagonal peaks.
11. Play with the “Level Tool” if desired. This adjusts the dynamic range of the spectrum as well as where the “floor” of the spectrum is placed. It is a difficult tool to use, and honestly, I do not understand it well. To open the “Level Tool”, right click in the spectrum window and hold down the mouse button. A menu should appear with “Level Tool” being the first option. Select the “Level Tool” to get a display with a few slider controls. Raising the “Bias” slider amplifies the signal. Lowering the top “Bounds” slider has the same effect. After you move a slider, you must click “Apply” to see the end result. You can undo any damage by returning the slider. If you really mess up, close the Level Tool, close the spectrum, and go back to step 9 in the “nD Processor” for a new spectrum.

12. Normally, the projection spectra in a COSY are terrible. To get nice projection spectra, select Hi-Res-Load X-Projection from the Display menu. This brings up a file dialog. Find your nice ^1H spectrum that you ran in the opening of this COSY section. For the X-Projection, a nice spectrum should appear at the top of your 2-D COSY spectrum. Replace the Y-Projection as well.
13. If the spectrum window needs to be adjusted, use the Zoom cursor (magnifying glass) to shrink the COSY window as needed. Since the projection spectra are automatically linked to the central COSY spectrum, you should not need to adjust them. Note that the shortcut keys of "Home" and "End" work in all three spectra – COSY, X-, and Y-projects.
14. Print the spectrum.
15. Clean up as described above (sections VII and VIII).