

Varian (Agilent) 500 MHz NMR Operation Instructions (Haverford College)

Preparing your NMR Sample Tube

1. Dissolve (i.e. no particulates) your sample in 1 mL of deuterated NMR solvent (CDCl_3 , D_2O or $\text{d}^6\text{-DMSO}$ are recommended).
2. Place solution into a 9" long NMR tube and cap it.
 - 7" tubes are hard to remove from the carousel, and are older and of lower quality.
3. Find a gray spinner for your sample.
 - See figure 1.
 - These are usually kept in the top drawer right behind the NMR computer.
 - They should not be removed from E107.
4. Place the gray spinner into the depth gauge (Figure 2) and then carefully push the NMR tube into the opening at the top of the spinner.
 - It is a tight fit; hold the fragile glass NMR tube close to the bottom while pushing the tube straight down.
5. Push the tube down until it touches the bottom of the depth gauge while the spinner is still seated firmly at the top.
 - Be sure that the liquid sample covers the entire dotted rectangle marked on the inside of the depth gauge (if you used 1 mL of solvent, it will).
 - The rectangle indicates where the RF coil will fit around your sample
6. Holding either the top of the tube or the top (black) ring of the spinner, remove the tube and spinner from the depth gauge. Use a Kimwipe to remove fingerprints, etc. from the outside of the tube (below the spinner) and the spinner itself.
 - The NMR tube must extend below the spinner by the proper length, so don't let the tube move up or down (with respect to the spinner) after removing it from the depth gauge.
 - Inserting a tube or spinner that is dirty (on the outside) will degrade the NMR spectra of future samples (as well as your own).



Figure 1: spinners

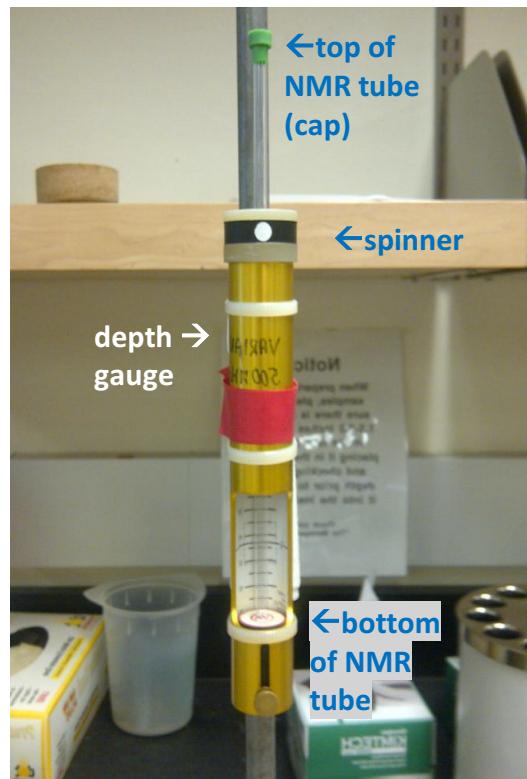


Figure 2: NMR tube in spinner, properly positioned in depth gauge

Placing your Sample in the Carousel and signing in

CAUTION: Do not approach the magnet if you have a pacemaker or other medical device that may be incompatible with strong magnetic fields. You may wish to remove cell phones and wallets (credit cards) from your pockets.

7. With the spinner in hand, climb the ladder and look at the top of the carousel. Select any free slot **except** for slot # 1 or the active sample slot (the one closest to you) (see Figure 3).
 - Explanation: there is already a sample in the magnet, and it needs to move up into this active sample slot before your tube can drop in.
8. Hold the top of the NMR tube and carefully lower the tube (+ spinner) straight down into the slot
 - Do not lean against the magnet – if you do, it will wobble back and forth since it is supported on a cushion of air, to shield against vibrations.
 - The NMR tube will stick up about 1.5" above the top of the carousel.
 - Note the number of the slot you used. Unfortunately, the sample numbers are at the bottom of the carousel.
9. Sign in to the log book with the date, your name, solvent, and type of experiment you plan to do. Also include the carousel slot that you are using.
 - That is so we can come find you if you forget to remove your tube from the carousel later on.



Figure 3: Never add a NMR tube+spinner to slot #1 or the carousel slot that is over the yellow caution triangle (the active sample slot). When this picture was taken, slot #1 WAS the active sample slot, but this is not always the case.

Starting the VnmrJ software with the correct operator name

10. On the NMR computer (you may need to move the mouse to wake it), if a user is already logged in and the VnmrJ software is running, click the Start tab (Figure 4, #6) to see the operator name (Figure 4, #7). If it is correct, you may skip to the next section ("Setting up a new Study").
 - It is important to be logged in as the correct user and to be using the correct operator name so that you can find your data file later.
11. To change operator name, exit from the VnmrJ software (using the File menu) and log out of the system (use the System: Log Out option from the linux desktop).
12. Login with the correct login name ("research" or "superlab" or "classes") and password (passwords can be found in the purple "run log" notebook).
13. Start the VnmrJ program (its icon will be on the desktop). You will be prompted to enter the operator name:
 - If you are a research student, enter your research group name for operator name

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- If you are in superlab or another class, choose your semester. Files associated with these operator names will generally disappear after the end of the semester (research group files may stay slightly longer, but don't count on long term storage)

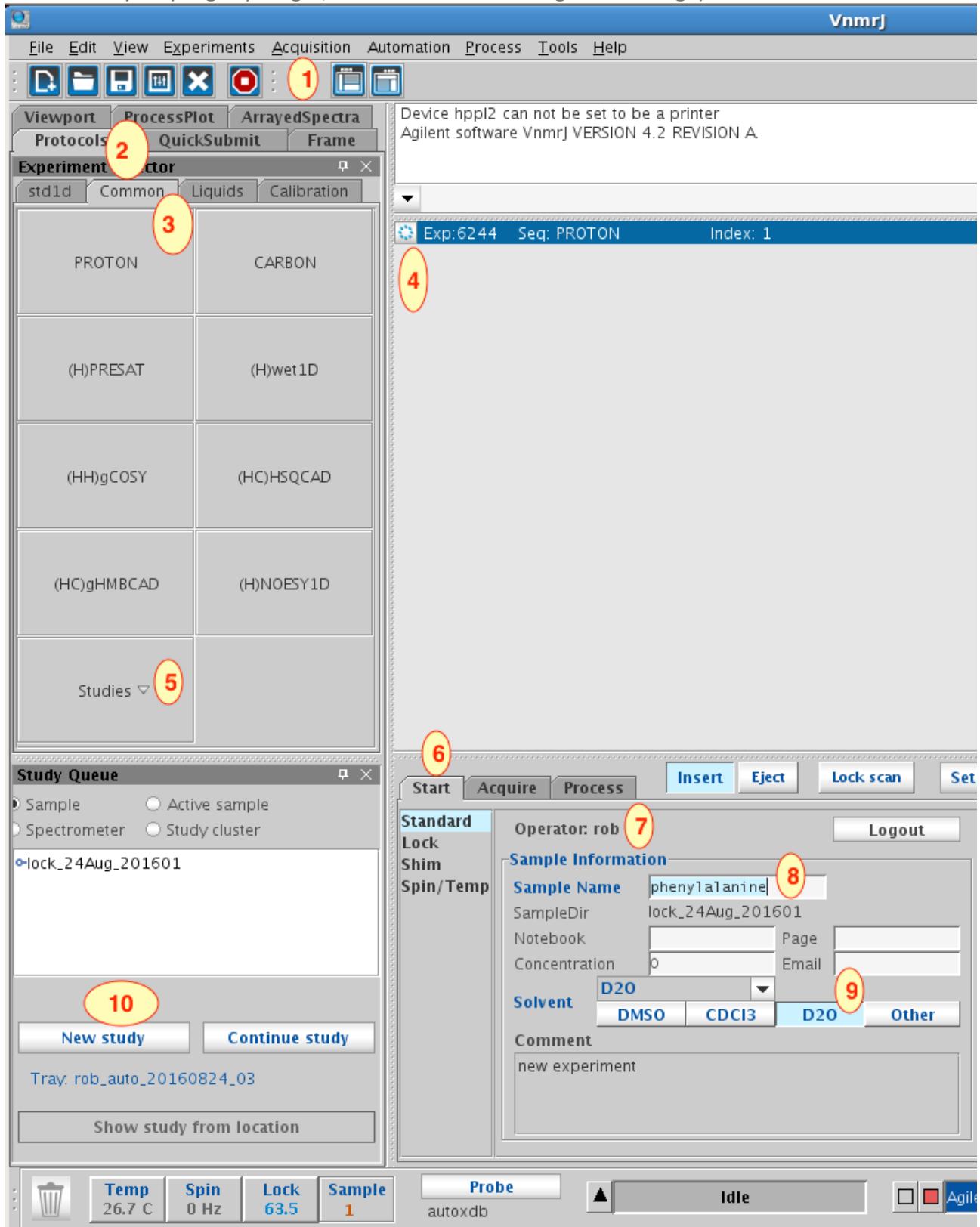


Figure 4. VnmrJ screenshot. Numbers are used in the instructions.

Setting up a new “Study” to run under Automation

The automation routine allows you to set up a “study” for each sample tube position in the carousel. Each “study” will consist of one or more NMR “experiments”; for instance, obtaining a 1-D proton NMR spectrum is an experiment, and obtaining a series of ^{13}C DEPT NMR spectra is another experiment.

14. Click on the small circle at position #4 (Figure 4) to display the sample slot numbers (a ring of 12 numbered dots – Figure 5).

15. If you are running the first sample of the day, or if your sample slot number is showing up as a circle with a color other than gray (indicating the slot number has already been used by someone else), choose “Automation Run [autodir] > New Automation Run” from the Automation menu (Figure 4, #1).

16. If they aren’t already selected, click on the Protocols and Common tabs (Figure 4, #2 and #3).
 - Do NOT use the Std1D tab

17. Click on ‘New Study’ in (Figure 4, #10) to create a new study.
 - A “study” is one or more experiments done back to back on the same sample

18. Click on the circle with the slot number for your sample (see Figure 5)

19. Type in a sample name and select the appropriate solvent (Figure 5, or Figure 4, #8 and #9).
 - It is useful to include your name or initials in the sample name, but you do not need to include the solvent, date or type of experiment.

20. Click on your experiment (i.e. ‘PROTON’; Figure 5 or Figure 4 #3).
 - If you wish, you may click on multiple experiments. A longer list of experiments is available under the “Liquids” tab.

Optional: Changing Acquisition Parameters for an Experiment

The first few times you collect NMR spectra, skip over this section. But by changing acquisition parameters, you may be able to get better quality spectra with more accurate integrations.

21. Double click the experiment where listed in the “to be submitted” study queue (it will have a name like “PROTON_001_day [0:24]” – upper left red circle in Figure 6). Wait about 10-15 seconds as the display on the right side changes to show the acquisition parameters.
22. If it isn’t already selected, click on the Acquire tab to the right of the Start tab (Figure 4, #6). Change parameters as desired for your spectrum. Some parameters you may wish to change for a proton spectrum are as follows:

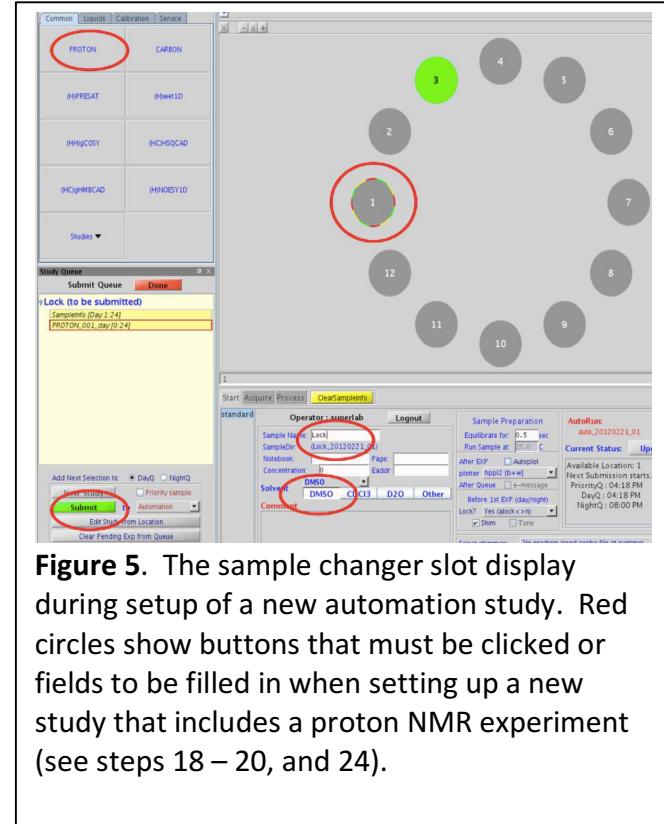


Figure 5. The sample changer slot display during setup of a new automation study. Red circles show buttons that must be clicked or fields to be filled in when setting up a new study that includes a proton NMR experiment (see steps 18 – 20, and 24).

- Spectral Width (in the “Default H1” list): By default this is -2 to 14 ppm, but if you know that all your sample’s signals are between 0 and 5 ppm, then a range of -1 to 6 ppm will suffice. Warning: do not attempt to exclude impurity peaks by cutting down the spectral range; if you do, those

peaks may show up as “Fourier fold-over” peaks at the wrong ppm.

- Acquisition time (on the left side of the “Acquisition” list): The default is 2-3 seconds (it will change if you change the spectral range), but to get the sharpest peaks, increase this to at least 5 seconds.
- Relaxation Delay (on the right side of the “Acquisition” list): The acquisition time + the relaxation delay should be at least 15 seconds to get the most accurate relative integrations.
- Number of scans (on the left side of the “Acquisition” list): By default this is 8, which is usually sufficient for proton spectra. But if you have a very weak sample, you can increase your signal to noise ratio by a factor of 4 by increasing the number of scans by a factor of $4^2 = 16$ (i.e. number of scans = 128). This of course will dramatically increase the time it takes to run your experiment (to at least 32 minutes, if you also increased the relaxation delay as described above).
- You may also, if you wish, change some of the processing parameters. But these can be changed after you collect your data, so it is not critical to change them now.

23. Once you are done editing the acquisition parameters, click ‘Save.’ on the line of buttons above the parameters. You may now add or change parameters for a second experiment if you wish.

Submitting your Study for Automation

- Click the green ‘Submit’ button (lower left of Figure 5). Your study has now been added to the automation queue. The sample slot number on the display should turn yellow.
- If yours is not the first sample of the day, data acquisition may still be in process on someone else’s sample. But otherwise, within a few seconds, expect to see and hear some activity:
 - After a few seconds, the sample changer should eject the sample in the magnet before rotating to your sample slot and dropping your tube into the magnet. The sample slot number on the display will turn purple to indicate it has become the active sample

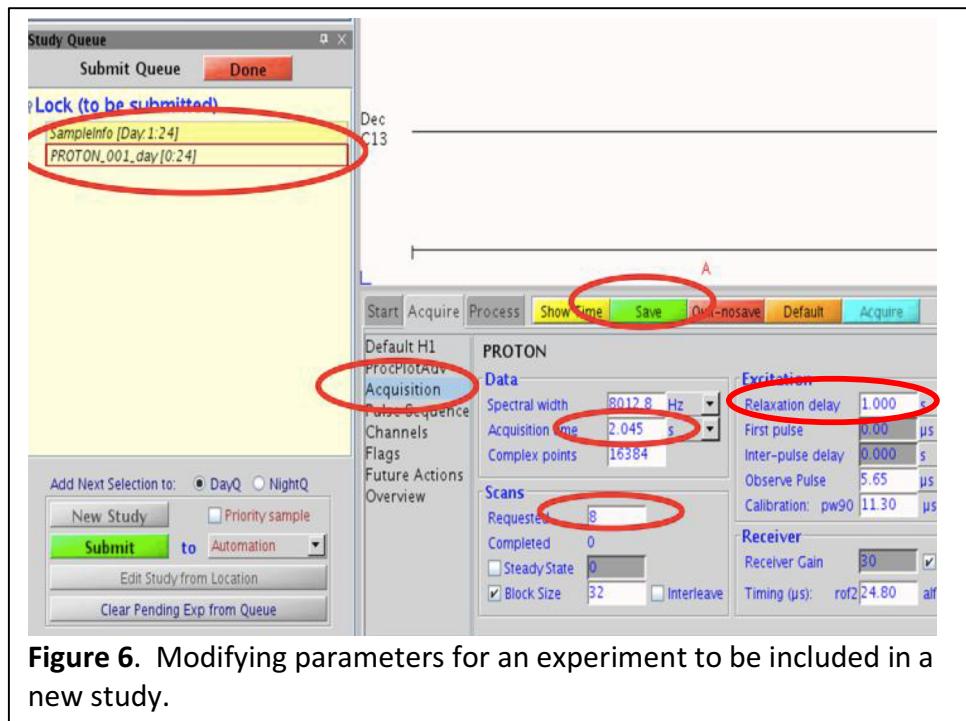


Figure 6. Modifying parameters for an experiment to be included in a new study.

- If nothing happens after 30 seconds (or error messages appear below), try right clicking on the sample slot number and select “Delete Study”. Then choose “Delete Day & Night Queue”. Then, make sure that the correct sample slot is still selected and submit your job again.
- If still nothing happens (and you are sure that nobody else is currently collecting data on their sample), you may try “Automation Controls > Abort Automation” at the bottom of the Automation menu. Then go back to step 15 and start a “New Automation Run”.
- If still nothing is happening, seek help.

After submitting your Study

The advantage of using the Automation Queue is that you can set up additional studies while data collection is in process.

26. To ensure efficient use of the NMR time, don't wait for data collection to be done before setting up the study for the next sample.
27. If another user is waiting to set up their sample, let them do so. If they are using a different operator name, they may close VnmrJ and restart it to change the operator name. Data collection will continue on your sample.
 - Unfortunately, if you change login names (research, classes, superlab), you cannot add new studies to the automation queue.
28. After adding studies for all samples to be run, add a study consisting of just a proton experiment for the lock sample, kept in sample slot #1. Use sample name “Lock” and solvent = D₂O.
 - The lock sample is just a tube containing D₂O. It is best to leave a sample in the magnet at all times (except during sample changes).
 - Putting the lock sample at the end of the queue ensures that your sample tubes will be ejected so you can get them back at the end of data collection.

Data Processing

When your study has completed, the position of your sample will turn green. At this point you can process your data by double-clicking on the data file (listed under completed studies at the lower left) and going to the Process tab. This gives you lots of options for integrals, plotting, etc.

29. VnmrJ will automatically integrate your sample. Go to the data processing toolbar and go to Integrals Delete Integrals.
30. If you cannot see the integrals go to Integrals Show Partial Integrals
31. Define the regions you would like to integrate with the define integrals scissors.
32. Go to Process Integration and select to integrate by ‘Single Peak.’
33. Move the cursor to a peak that represents a known number of protons.
34. Enter in the number of protons that corresponds to the chosen peak.
35. Click ‘Set Integral Value’ and then ‘Show Integral Values’ to display the integration.
36. To print your spectra go to Plot Automatic Plot Page.

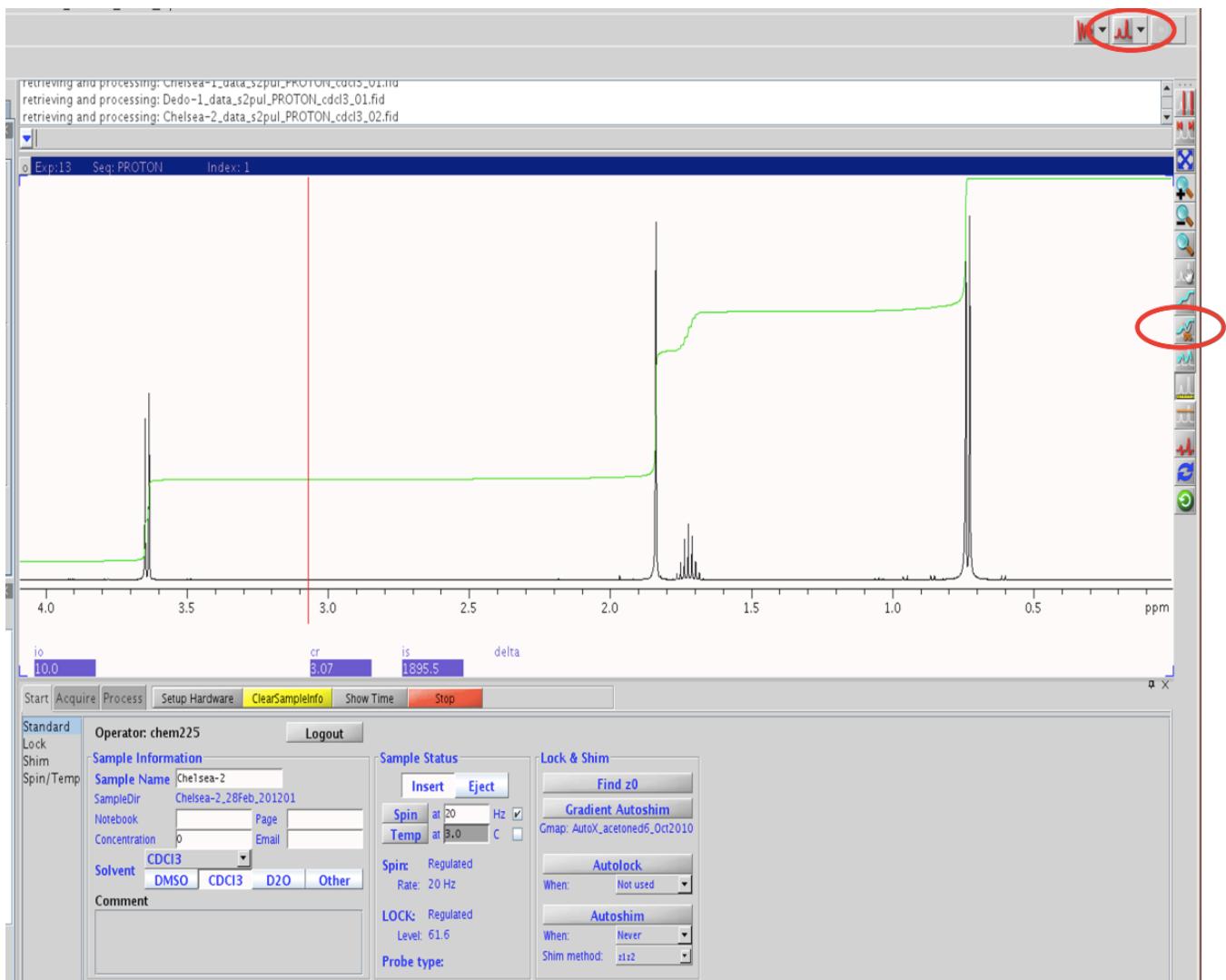


Figure 7. Data processing using VnmrJ software. Red circles indicate some of the icons that are used in setting up integration curves.

You may find it easier to use MestreNova software to process your data (see separate instructions). In order to find your data to use with MestreNova, you will need to know the login name, operator name, and the sample name.

When you are done ...

37. Carefully remove your sample(s) from the carousel. Be sure to lift the tube straight up so you don't snap off the bottom.
38. Remove the tube from the spinner and return the spinner to its box.
39. Ensure you've filled out the log book entry completely – add comments if anything unusual happened.
40. Take the NMR tube containing your sample with you – you are responsible for cleaning it.