

# Subsistence Instructions for the AV-250 NMR Spectrometer

or... Thirty One Steps to an Acceptable NMR Spectrum

1. Sign in the Log Book.
2. As you sit down to start, the computer should show the login screen – Welcome to BH053605. Enter your group's login name (*e.g.* Livant) followed by [return].
3. Type your password then [return].
4. Double left-click on the "Topsin 1.3" icon. The NMR software (which is called Topspin) starts. What appears is the last spectrum done by your group.
5. Right-click on a small black square region at the lower right of the screen, marked Lock. A pop-up menu appears. Left-click on Locksignal Display. The lock trace is displayed.
6. Some words about sample preparation: (*a*) You should use a high-quality NMR tube (*b*) if there is solid material floating around, you should filter the sample (*c*) use **EXACTLY 0.60 mL** of sample. Using **EXACTLY 0.60 mL** of sample will guarantee you get a good spectrum *quickly*, since the whole system is optimized for that sample size. If you use too much or too little sample, you can still get a spectrum, but it will be uglier and will take a lot longer to obtain.
7. It's time to put in the sample!! Note that the magnet will wipe out the magnetic strip on your credit card, and will affect wristwatches, so leave these on the desk. Take the cap off the magnet. Make sure the cursor is in the command line (the pink area below the spectrum window) and type "ej [return]" (eject); **WAIT ABOUT 10 SECONDS UNTIL THE AIR IS HISSING**; wipe the tube and spinner turbine with a Kimwipe; put your sample into the air stream; type "ij [return]" (inject) and the tube will be lowered down. Put the cap back on the magnet.



**MAKE SURE THAT THE AIR IS HISSING MAXIMALLY BEFORE PUTTING YOUR TUBE IN THE MAGNET!!!! YOU MUST WAIT ABOUT 10 SECONDS BEFORE THE AIR IS AT FULL TILT.**

8. Type "ro on [return]". This starts the spinning. (The [return] will be omitted from now on, but you should hit [return] after every command; also, unless otherwise specified all clicks will be with the left mouse button).
9. To create a new file, type either "edc" (edit dataset - current), or "new" (which is equivalent to edc). Choose a name for your dataset that identifies (*a*) you, and (*b*) the sample. For example "BD-II-43b" which might stand for compound b on page 43 of Ben Dover's second research notebook. Experiment number should be changed for each spectrum or NMR experiment which is done on this sample. For example experiment number 1 might be a proton spectrum, number 2 a carbon, number 3 a DEPT-135, and so on. Note the directory **must** always be /opt/topspin/. In setting up the new file, you should also specify the solvent used, and type in a title for the spectrum in the box marked Title. Under Experiment, you can choose to use the parameters of the spectrum which appeared when you started (see step 4) by choosing **Use current parameters**, or you can set up something different under Select. As in the past, PROTON means a proton spectrum with 16 pulses, and C13CPD means a carbon spectrum with composite pulse decoupling and 1024 pulses.
10. Type "gpro"
11. Type "lock", choose the solvent you are using, and execute. The lock display goes haywire for a little while, then settles down. The instrument adjusts lock gain and lock power, then says it's finished locking.
12. It's time to shim! Type "tune shimit". This starts an automated shimming routine which takes 3 or 4 minutes. If you have used **EXACTLY 0.60 mL** of sample, and if no one before you has monkeyed around with the shims, shimming will be very easy. While you are waiting for the shimming routine to end, proceed to the next step.
13. Did you remember to sign the Log Book? If not, do it now.
14. Type "ii" (initialize interface).
15. Type "rga", (set receiver gain automatically).
16. After rga is finished, type "zg" (zero memory and GO!!) and we're off and running!!
17. Once all the scans are collected, you can type "new" or "edc", change the EXPNO to the next number, and set up and begin a new experiment on this sample, *e.g.* a <sup>13</sup>C spectrum. (*i.e.* Re-do steps 9, 10, 14, 15, and 16). That new acquisition can be running while you are processing the spectrum you just collected. To get from experiment 2 to experiment 1, type "re 1". To get from experiment 1 to experiment 2, type "re 2". (Re means "read").

**18.** To process your data, check the LB parameter by typing "lb" and entering a new value if desired. If you want to do exponential multiplication using the LB parameter (typical for  $^{13}\text{C}$  spectra), followed by Fourier transformation, type "ef". If you don't want any line broadening, type "ft" (typical for  $^1\text{H}$  spectra).

**19.** You should see something which looks vaguely like an NMR spectrum, but it may be upside down or tiny or huge or whatever. You can affect the size of the display by clicking \*2 or \*8 or /2 or /8. While you are processing the spectrum, holding down the left mouse button and dragging it left or right causes the region you dragged over to expand to fill the screen.

**20.** To phase the spectrum the easy way, type "apks". This runs an automatic phasing routine that usually works nicely. If you'd rather phase by hand, click on the icon near the top of the screen which looks like an out-of-phase peak. Then click on "0" (zero) to do the zero-order phase, then on "1" to do the first order phase. Once satisfied with the phasing, click an icon which looks like an arrow going around a 90° corner pointing to a floppy disk (save-and-return). This saves the phasing parameters.

**21.** If your sample has TMS in it, type "sref" which will set TMS automatically to 0.00 ppm. If you want to use another peak as a chemical shift reference, put that peak in the spectrum window and broaden out the spectrum so that your peak is quite broad. Click on the calibration icon, which is just to the right of the phasing icon. Put the cursor on the center of the peak and click. Type in the chemical shift information. Save-and-Return icon.

**22.** Click the Integration icon, which is to the right of the Calibration icon. Then click an icon which looks roughly like a bracket (}) lying on its side. This enables you to define the integration regions, which you do by holding down the left mouse button and dragging horizontally. Once you're done, click the Save-and-Return icon.

**23.** Type "plot". If you like the way the spectrum looks, click Print in the File pull-down menu. If not, click Open in the File pull-down menu and click "general\_proton.xwp". This layout might look better to you. If so, print the spectrum.

**24.** Some tips: (i) to discontinue an acquisition and save the data, type "halt". If you click on the icon which looks like a stop sign, the acquisition will halt and your data will *not* be saved. (ii) The standard carbon procedure involves 1024 pulses, which takes over an hour. (There is a 30 minute time limit for using the 250 MHz NMR)! So, you can check on the progress of your  $^{13}\text{C}$  acquisition by typing "tr" in the window displaying the fid. Wait a few seconds and type "ef" (or "efp" if your phase parameters are OK), and inspect your spectrum. If it looks acceptable, type "halt". If not, let it acquire longer.

**25.** When you are all done, type "ro off" (stop the sample spinning) and "lock off" (turn off the lock).

**26.** Take the cap off the magnet.

**27.** Type "ej" (eject). Retrieve your sample from the top of the magnet. Put the black cap back on the magnet.

**28.** Type "ij" to turn off the air.

**29.** Type "exit". This will close the Topspin program.

**30.** In the main window, click on the red hat in the lower left corner, then Log Out. Ta da!! You've done it!!!

**31.** Record your ending time in the Log Book. Clean up any mess you have made, e.g. Kimwipes, scraps of paper. Please take your paper spectra with you. It is possible to transfer your files to CD, DVD, or "memory stick." We don't know how yet, but it's possible!