NUCLEAR MAGNETIC RESONANCE RESEARCH RESOURCE

Avance 300 Hands-On User Guide

Dr. Mike Lumsden
Coordinator, NMR-3
February, 2014: version 1.5

**** CAUTION ****

The wearing of safety glasses is mandatory whereas lab coats & protective gloves (latex, nitrile, etc) are prohibited at all times while working in NMR-3!

Before Starting…

- These instructions require NMR data to be open in the data window before starting (as shown in the yellow square in **Figure 1**). It doesn't matter what type of data (1D, 2D, ^{1}H, ^{13}C, etc) or who the owner is. If instead there is an empty blue screen in this area , use the data browser (blue rectangle in **Figure 1**) to open any dataset before proceeding.
- The EZ NMR Button Panel should be displayed along the right hand side of the TopSpin window (red rectangle in **Figure 1**). If it is not, click the yellow "EZ" icon (see the arrow in **Figure 1**) in the upper TopSpin toolbar to open it.
- The SampleXpress Lite indicator lamp (circled in yellow in **Figure 2**) should be green before beginning. If it is red or blue (indicating a problem), you will need to reset the autosampler. Instructions for this procedure are available in the AV-300 binder.
- The area enclosed by the green rectangle in **Figure 1** contains the TopSpin command line as well as a status area. The program uses the status bar to display information and messages about active TopSpin processes.

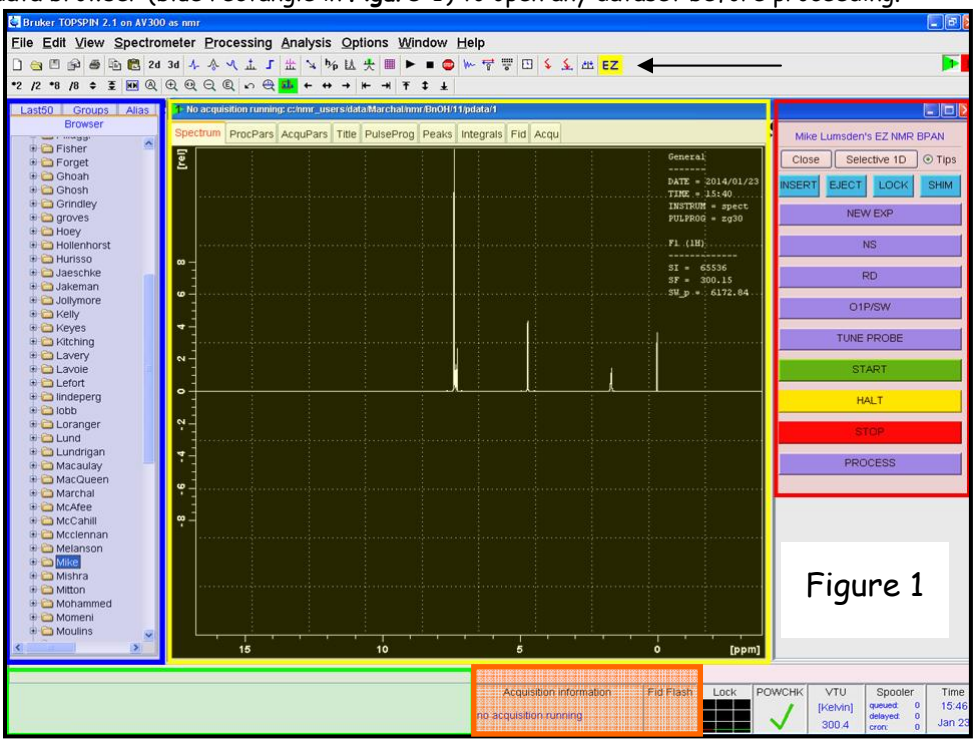

Figure 1

Preparing your NMR Sample…

1. Clean your NMR tube using a kimwipe. Then insert your NMR tube into one of the available blue spinners, making sure that you do not touch the spinner with your hands (avoids transferring grease from your fingers). If your tube is loose (moves easily) in the selected spinner, please choose a different one.
2. Adjust the position of the NMR tube in the spinner using the sample depth gauge provided.

3. Using isopropanol and a kimwipe, thoroughly clean your NMR tube in the area below the spinner where the sample resides. Be careful not get isopropanol on the blue spinner!

Getting Your First Sample in the NMR Magnet and Ready for NMR Experiments…

4. Place your sample(s) in any available position in the autosampler carousel, taking note of the position number(s) you choose. If you plan to study multiple samples during your FACES reservation, place them all in the carousel before proceeding.
5. Click the INSERT button. A window will open asking you for the position number of the sample you wish to place in the magnet. Type in the number (1 through 16) and click OK. The autosampler indicator lamp will turn white while your sample is inserted. Please wait for two things before proceeding to the next step: (1) the indicator lamp returns to green and (2) you hear a beep.
6. Proceed according to whether your solvent is deuterated or not:
    i. If your sample solvent is deuterated, click LOCK and select your solvent from the drop-down list. Wait for the beep.
    ii. If your sample is in protio solvent, DO NOT click LOCK. Instead turn the SWEEP button off (light NOT lit) on the BSMS keypad beside the computer monitor (location indicated by red circle in **Figure 3**).
7. This next step is also performed according to the nature of your solvent.
    iii. If your sample solvent is deuterated, click SHIM to perform an automatic topshim on your sample. This could take about 30 seconds or up to a couple of minutes depending upon the starting field homogeneity. Wait until you hear the beep before proceeding.
    iv. If your sample is in protio solvent, DO NOT click SHIM. Note that you may shim your sample using the shape of the fid (instructions for this procedure are beyond the scope of this document).

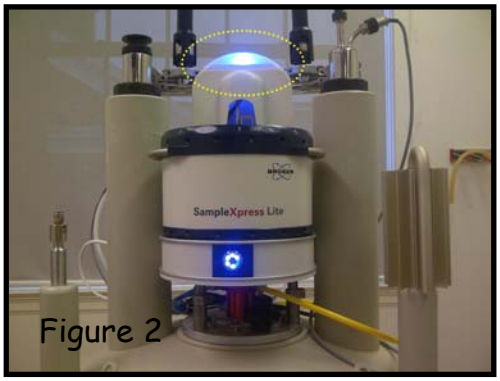

Figure 2

Running Your First Experiment…

8. Click NEW EXPERIMENT. The interface that appears is shown on the next page in **Figure 4**. Perform/take note of the following:
    v. The following 3 items should never change and should be set as follows: **PROCNO** = 1, **DIR** = c:\nmr_users, and **Experiment Dirs** = c:/Bruker/TOPSPIN/exp/stan/nmr/par/user
    vi. Enter information for the following 3 items as follows: **NAME** (a description of the sample you are running), **EXPNO** (an integer number; every experiment for a given sample NAME must have a unique EXPNO), and **USER** (your last name; keep this the same every time you use the spectrometer).
    vii. Click the following 2 drop-downs and select as follows: **Solvent** (select the solvent in your sample regardless if it is deuterated or not) and **Experiment** (select the experiment you wish to run.) Note that only experiments beginning with a 1d_ or 2d_ prefix can be performed on the AV-300.
    viii. When everything is entered/selected as above, click ok. A new dataset will open containing the default parameters for the experiment you selected. In the middle of the window you should see the text "No raw data available No processed data available".

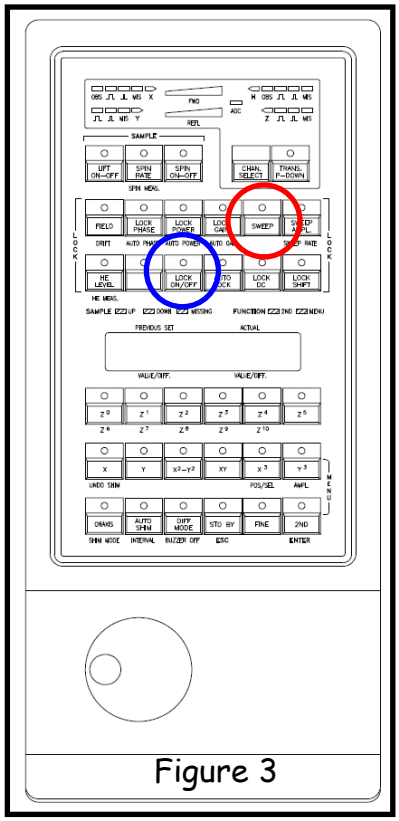
Figure 3

9. Change default parameters if you wish by clicking NS (number of scans), RD (relaxation delay), O1P/SW (middle of your spectrum and the sweep width in ppm) and entering the new values when prompted. Note that changing ns or rd produces a new window showing the new experiment time.
10. Click TUNE PROBE and wait for the beep before proceeding.
11. Start the experiment by clicking the green START button. Once the experiment begins, you can monitor the progress by checking the area in **Figure 1** indicated by the orange rectangle. In the "Acquisition Information" section, there should be a scan counter letting you know how many scans have been completed. In the "Fid Flash" box, a red fid flashes during your experiment acquisition and will be empty once the experiment completes.

Running Additional NMR Experiments on the Same Sample…

12. Return to step 8 above and enter/select the information for your next experiment. Most items will stay the same but you will have to change **EXPNO** to a new number (normally incremented by one from the previous experiment) and select the next experiment you want to run from the drop-down list in **Experiment**.
13. Perform steps 9, 10 and 11 to execute the additional experiment.
14. Repeat steps 12 and 13 for every additional experiment you wish to run on the sample in the magnet.

Running a Second Sample…

15. Click INSERT and type in the holder number for the next sample to be studied. The system will automatically eject the sample in the magnet and insert the next sample (there is no need to explicitly eject and re-insert).
16. Once the indicator lamp is green, return to step 6 and proceed as for the first sample by locking, shimming, etc.

Finishing Your Reservation…

17. Turn off the lock by pressing the LOCK ON/OFF button on the BSMS keypad beside the computer monitor (blue circle in **Fig 3**).
18. Click EJECT to remove your sample from the magnet. Please wait for two things before you remove your sample(s) from the carousel: (1) the indicator lamp is green and (2) you hear the beep. If you remove your sample before the light returns to green, you will create an error condition.
19. Return the blue spinner(s) to the plastic storage case (remember to use kimwipe and do not touch the spinners with your bare hands).
20. Your data should automatically be copied to the data server; there is no need to do a manual copy.

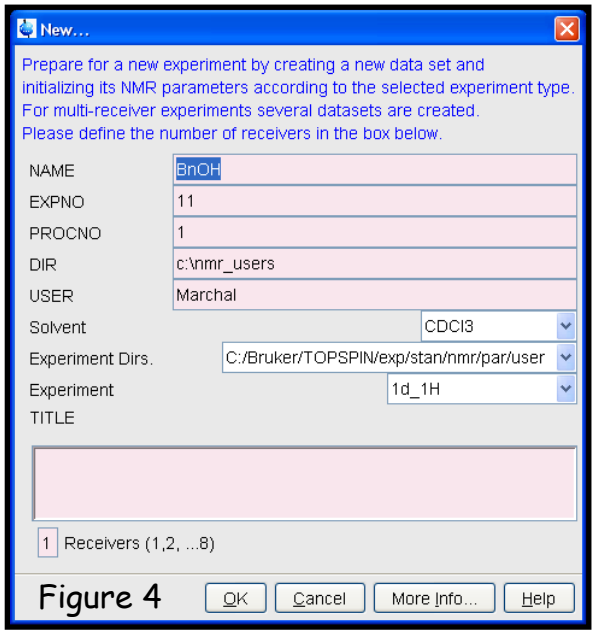

Figure 4

Section 2: Chaining Together Several Experiments to Run Unattended

If you plan to run a number of different experiments on a single sample, there is no requirement to stay at the spectrometer to manually start each one. Instead, they can be chained together such that when one experiment finishes, the next starts automatically. To accomplish this, first perform steps 4 through 7 above as normal (insert the same and lock and shim). Then proceed as follows:

- Click on NEW EXP and create an empty dataset for the first experiment you want to run (identical to number 8 above).
- Change any parameters as needed (identical to number 9 above).
- DO NOT click TUNE PROBE when chaining experiments together. The program that chains the experiments will perform the tuning step automatically for you.
- Click on NEW EXP again. Change the experiment number by +1 and select the second experiment in the Experiment drop-down list. Repeat for as many experiments as you wish to chain, **incrementing the experiment number each time by 1.**
- It is critical that you start the chain of experiments by returning to the first experiment you setup. To do this, in the TopSpin command line type "re #", where # in an integer corresponding to EXPNO for your first experiment.
- In the TopSpin command line, type "multizg2.ml". You will be prompted for the number of experiments you are chaining and then you will be given an estimate of the amount of time your queue will take.
- Once the first experiment starts, you may leave the NMR facility but please place the red "Experiment in Progress" sign in front of the keyboard.
- **Note** if you halt an experiment in a queue before NS scans have been reached, the multizg2.ml program will automatically move to the next experiment in the chain.

Section 3: Tips and Tricks

- You can watch your processed NMR spectrum accumulate in real time by selecting the Acqu tab and making sure the spectrum icon is selected while your experiment is running (as shown by the circled areas in **Figure 5**). After each scan, the spectrum is updated and thus this display allows you to gauge your signal-to-noise "on-the-fly". Please note that most times the spectrum is not phased properly and the chemical shift scale is not accurate in this particular display window. However, the display is sufficient to decide whether or not you have sufficient signal to perhaps stop the experiment early.

- You can stop an experiment early (before NS scans are acquired) by clicking the yellow HALT button. This is typically done when you have a stronger signal than you expected. Alternatively, experiments can be stopped immediately by clicking the STOP button (normally used when you don't see any signal at all and will likely need to start over)
- If your 1D spectrum displays a low signal-to-noise (S/N) ratio and you have more time remaining in your FACES reservation, you can improve things by typing "go" in the command line. "Go" will signal average NS additional scans to the raw data you just acquired (rather than clicking the green Start button which will erase the data already acquired).
- For any 1D experiment, the PROCESS button will not function properly until NS scans are completed or you press the HALT button. If you need to process a 1D spectrum prior to it finishing, you can type "tr" in the TopSpin command line and wait for the next scan to complete. After that, all processing commands will execute properly.

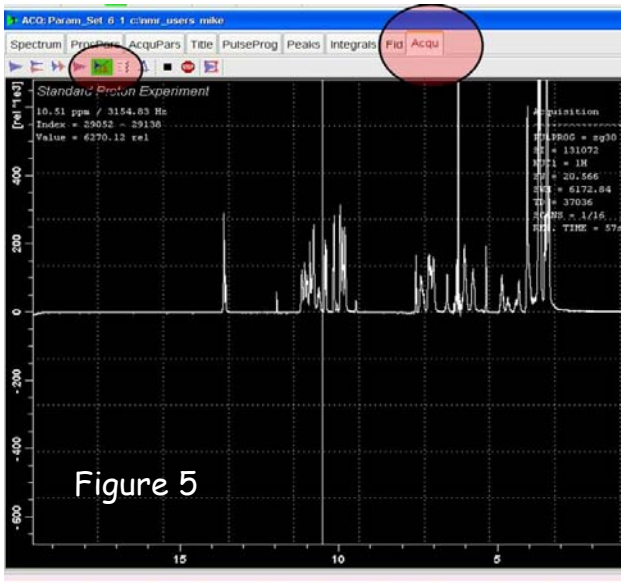

Figure 5

- For 2D experiments, processing can take place any time after the first row has been acquired. You can click PROCESS (it recognizes the 2D data set automatically) or issue any 2D processing commands you know once the first row is available.
- If you are running multiple ^{1}H samples during your reservation and each sample is in the same type of NMR tube and same deuterated solvent, it is not strictly necessary to perform the probe tuning step each time.