Manual Acquisition of NMR Spectra on the Avance II 400 Spectrometer

The Avance II 400 NMR spectrometer at Indiana State University is high-field nuclear magnetic resonance spectrometer with a 9.4 Tesla superconducting magnet operating at a nominal ^{1}H frequency of 400 MHz. In order to use it, you will need to be in a course or conducting research at Indiana State University, Rose-Hulman Institute of Technology, or Saint Mary-of-the-Woods College and be trained in its use. For training, see Dr. Richard Fitch (Rm. S35E, 237-2244) or Dr. Richard Kjonaas (Rm S51A, Phone 237-2237). Ordinarily students will run experiments in automation using the ICONNMR interface at the console or on the web (http://www.indstate.edu/chemistry/AVII-400.htm).

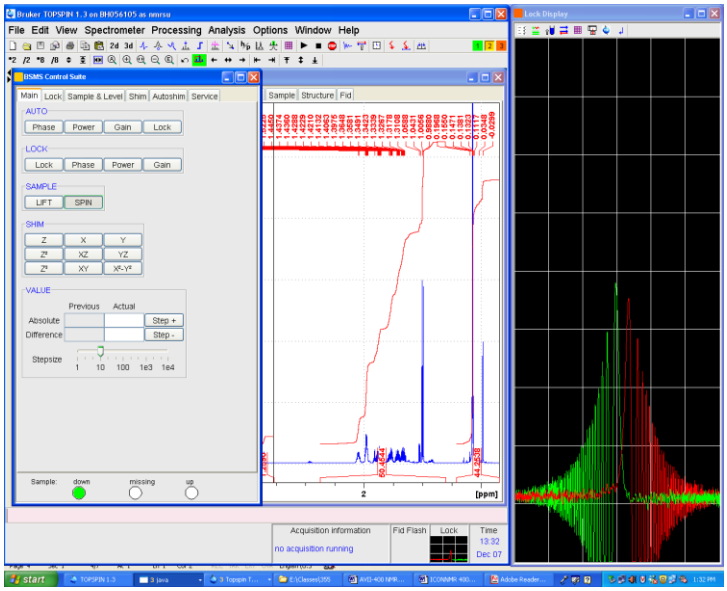
Manual operation is only to be used when ICONNMR is not active. Before running a sample manually, you must check to see that ICONNMR shows No Jobs at the bottom of the automation window and/or is paused or is not running. Do not attempt to run ICONNMR while running manually in Topspin. The two programs do not communicate well and you can damage the instrument and/or destroy your sample.

Prepare your sample

Wipe the outside of the NMR tube with a Kimwipe. Place the tube into one of the blue spinners in the blue suitcase storage rack on the counter. Only insert the sample an inch or two into the spinner and then remove the tube and spinner from the rack. Wipe the spinner off with the *same* Kimwipe and place the sample in the clear plastic depth gauge on the counter (this should be square in cross section, not round). Press the tube *gently* until it bottoms out in the gauge. Then remove the tube and spinner from the gauge and wipe the lower portion of the NMR tube with the *same* Kimwipe. From this point forward, only handle the tube by the upper part.
Note: once you have set the depth, do not touch the spinner. This can alter the depth of the sample and give you a poor spectrum, or (worse) cause the spinner not to spin, or (worst) bottom out the sample and break the tube in the NMR (very expensive).

Put the sample in the magnet

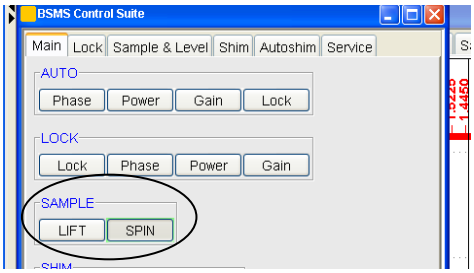
Place the sample in the magnet as follows. *If you are uncertain as to this operation or have forgotten, have someone assist you. You can damage the probe if you do not insert the sample properly.*

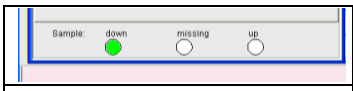
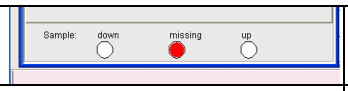


1. Go to the spectrometer workstation and bring up Topspin if it is not already running. When running, the typical appearance of the workstation is as shown at right. The main Topspin window is open and has an experiment open (note the

spectrum behind the BSMS Control Suite window). The BSMS panel controls the magnet and the lock signal is the dark window on the right with the green and red traces. The lock signal is the deuterium signal from the solvent used ($CDCl_3$ in this case).

2. In the BSMS Control Suite, make sure the Main tab is active and check the Status of the sample (3 indicator lights at bottom). A sample may or may not be present. If a sample is in the magnet, the leftmost indicator will be green as shown below. If not,the middle indicator will be red. In either event, press the LIFT button and wait for the sample to be ejected. Even if no sample is present, the indicator will still read "up" when the air pressure has reached the appropriate level.



		
Sample is inserted and set in the probe.	No sample or sample is in transit	Sample is up or airflow is OK for inserting a sample

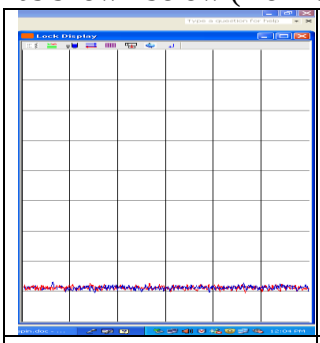
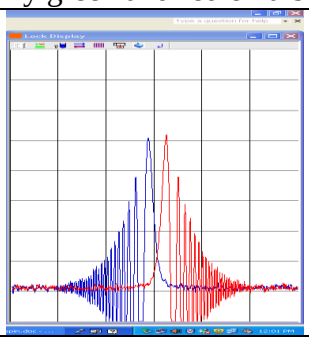
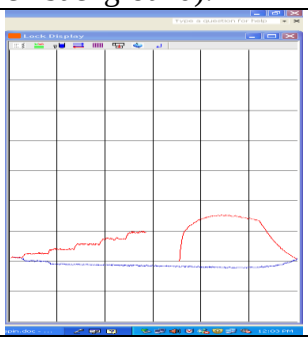
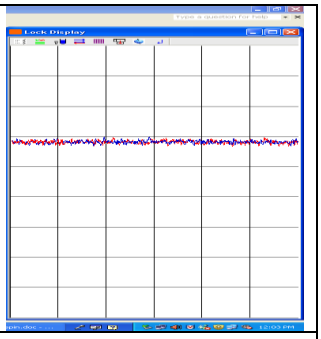
3. When the sample has ejected go to the NMR magnet and remove the sample from the bore as shown below. Remove the sample by lifting it out of the magnet straight up. The sample should be held as vertical as possible *Do not tilt the sample as it can cause the tube to catch on the edge of the bore and break. This is a very expensive mistake to make as it will require cleaning of the bore and the probe. Moreover, the solvents can damage the electronics in the probe, requiring an expensive repair (thousands of $$$).*

4. Place the existing sample in the rack on the bench and place your sample into the magnet. Again, keep the sample as vertical as possible. *Do not insert a sample into the magnet unless you can hear the air and the indicator on the BSMS panel reads "UP".* Otherwise the sample will fall to the bottom of the bore and break in the probe causing damage to the probe.

5. Return to the workstation and click on the LIFT button again. The air will decrease, allowing the sample to be inserted into the probe gently. The indicators will show when the sample is down.

Lock the sample

The spectrometer normally obtains its frequency reference from the deuterium signal from your solvent. The locksignal has four possible appearances depending on the condition of the sample as shown below (Normally green and red on a black background).

			
No Sample No D in solvent Solvent change.	Normal unlocked signal for a deuterated solvent.	Locking in progress. Gain is being adjusted.	Locked signal

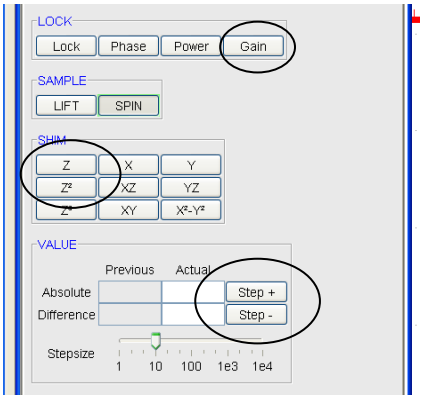
The trace on first panel on the left indicates a sample is either missing, has no deuterium or is a new solvent relative to the existing paramters. The most common reason for this with students is the use of a non-deuterated solvent ($CHCl_3$ for $CDCl_3$) or a neat sample, if it is a liquid. If you are certain that you have put the sample in and you are using $CDCl_3$, check the Sample indicator on the BSMS panel to see that the sample is DOWN. If not, try ejecting the sample and reinserting using the LIFT button to see if the sample got hung on something. Check the spinner for cleanliness or residues. If you are using any other solvent than CDCl3, you will not see the lock signal. This is normal and will correct itself when you lock the sample.

The second panel shows a sample containing deuterium, properly shimmed and unlocked with the proper parameters. The signal sweeps back and forth in green when sweeping left and in red when sweeping right. This is essentially a continuous wave spectrum of the deuterium resonance from your solvent.

In order to lock the sample, you should type LOCK on the command line. The system will ask you which solvent you are using and will then automatically begin locking the sample. During this period, the lock signal will do some gyrations and will look much like the third panel during the process. When finished, the lock signal will look like that in the fourth panel above. The line that is being swept is essentially the peak of the deuterium resonance. Note: you must have a file open in order to lock the sample. If there is no file open then you must either open an existing file or create a new one (see Creating a File, below).

Shim the sample

You will now need to shim the sample for optimum homogeneity of the magnetic field, which will give the strongest and sharpest signal. This is done within the BSMS panel. Click on the button marked Z in the SHIM box and then click on the STEP + button in the VALUE box. Continue clicking on the button until you see a deflection of the lock signal. If the signal improves (goes higher), continue

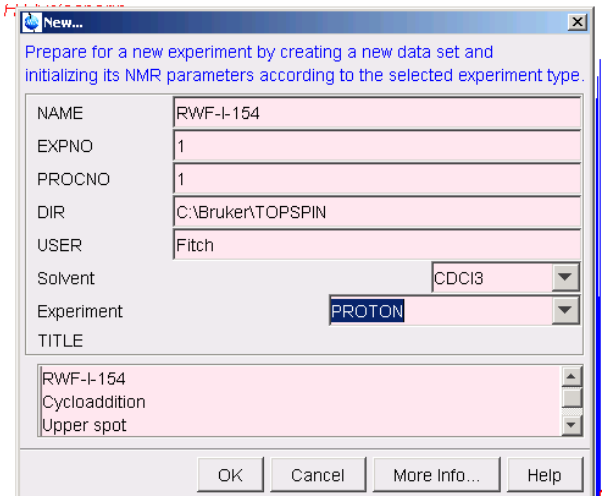


in that direction; if not, then click on the STEP – button in the same fashion. Once you are improving the signal, continue with that button until you reach a maximum or go off the top of the screen. If you go off the top, then click on the GAIN button in the LOCK box. Then click on STEP – to bring the signal back on screen and down to about the second line from the top. Then return to Z and continue shimming.

Once you have reached a maximum with Z, then click on Z2 and repeat the process until you have maximized the signal for Z2. Again, you can adjust the gain if you go off scale. Once you have maximized Z2, go back to Z1 and see if you have can improve the signal further. Then return to Z2 and do the same. Play the two against each other as long as you are improving the signal. Once you have reached a maximum for both Z and Z2, you will want to check to see if you have reached a local maximum. You are then properly shimmed. Use the GAIN to place the lock signal at the second line from the top and you are ready to begin acquisition.

Create an experiment

Before you can run a spectrum, you must create a file in which to put the data and parameters. If you have an existing file, you can simply open it and create a new file from that. Otherwise you simply click on File and New from the main menu (CTRL+N).



1. For the name use the one given to you by your instructor or research advisor for a given experiment. Typically this will be "Fitch-wintergreen", or something of the sort. For research students that may have multiple samples for a given experiment, I would recommend using your notebook number as the filename as shown at right and using the suffix A, B, C, etc for each sample from the experiment.

2. The experiment number will also be given to you for a class, but typically a proton experiment will be experiment number 1, ordinary carbon will be 2, etc.

3. The process number will normally be 1. The directory will always be C:\Bruker\TOPSPIN for this instrument unless there is reprocessing involved.

4. The user name is the one which you have been assigned (Chem 355, etc.). Research students are given their own usernames, but should keep spectra for courses with the other students for that course.

5. The solvent will typically be $CDCl_3$, but other solvents may be selected from the dropdown menu if needed.
6. The experiment is selected from the dropdown menu. Typical experiments are PROTON and C13CPD for carbon-13. Occasionally DEPT, APT, COSY, TOCSY and other one- and two-dimensional experiments need to be run. Your instructor or research advisor will tell you which experiments to run for a given sample on an as-needed basis.

7. The title should be appropriately descriptive. The following is suggested:
   Rick Fitch
   Chem355
   Elimination product *(do not identify the compound unless you know for sure what it is)*
   Chromatographed (*it is useful to include details as to the quality of the sample)*
   1H CDCl3
   10-22-06
   rwf *(these might be someone else's initials if they ran the experiment for you)*

8. When you have entered all of the required information, click on OK and a new experiment will be created with the parameters you have specified.

9. If you are running multiple experiments on the same sample there are a couple of things that are helpful.

   a. If you are incrementally running the same spectra with the same parameters on several samples from the same experiment (e.g. experiment 1), then you can type *iexpno* to increment the experiment number. This will create a new experiment (experiment 2) with exactly the same parameters. You will only need to change the title appropriately on the TITLE tab.

   b. If you are going from proton to carbon or other, simply click File -> New, or CTRL+N to create a new file. Topspin will remember the parameters for the file you are in. You need only change the experiment number, title, and parameters as appropriate.

Set up the spectrometer and run the experiment

Once you have an experiment set up and the sample inserted, locked and shimmed, you must set up the spectrometer.

1. Type *getprosol*. This reads the probe and solvent parameters into your experiment file.

2. Type *ii* to initialize the interface. This tells the spectrometer to set up the hardware (transmitter and receiver) for the experiment you specified.

3. Tune the probe if needed (see Tuning the probe, below). This is normally only needed when changing solvents or broadband nuclei (^{13}C to ^{31}P or vice versa). Check the logbook to see what was run last.

4. Type *rga* (receiver gain acquisition). This automatically determines the receiver gain so that your fid is not truncated and you have sufficient signal to produce a reasonable spectrum.

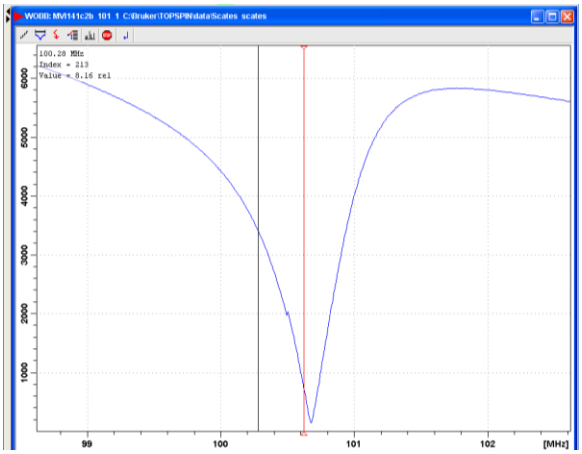
5. Once rga is finished, then type *zg* (zero and go) to start the experiment. The spectrometer will acquire the fid and store it. You can observe the acquisition while it is running. For certain experiments, you will need to use *xaua* (execute automatic acquisition) instead. This generally applies to 2D experiments.

6. This process applies to all nuclei except ^{19}F. For this nucleus, some cabling changes will also be required. See Dr. Fitch or Dr. Kjonaas for direction on how to do this if you need to run ^{19}F. Also note that fluorine cannot be run in automation.

7. Processing of the spectra may be done at the spectrometer or on the computers in S51J or your lab. Spectra may be copied to flash drives and taken with you, but do not delete your dataset on the workstation. The workstation is backed up weekly to the departmental server. Processing details are in the **Processing NMR data using Topspin** document on the chemistry department website at http://www.indstate.edu/chemistry/AVII-400.htm .

Tuning the probe

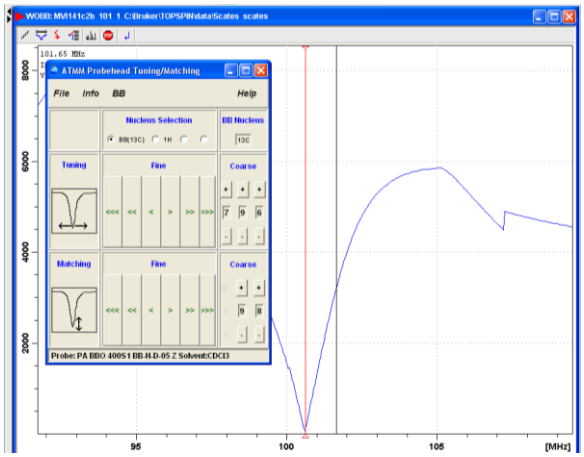
This only needs to be done when you are changing solvent or broadband nucleus. The probe is normally tuned for proton and carbon, but you should look at the logbook in the comment line to see what was run last. If ^{31}P was run last, then you should tune the carbon channel if you intend to run it. Likewise, if ^{19}F was run last, you will want to retune the proton channel.



Before you tune the nucleus, you will need to have the spectrometer set up properly. You should be in an experiment that is set up for the nucleus you wish to run, and you should already have done a *getprosol* and *ii*.

If you are uncertain if tuning is needed, simply type *wobb*. A window will appear (as shown for ^{13}C) displaying the wobble curve. This curve is a plot of reflected power versus frequency. The wobble curve appears as a dip, or inverted peak. Ideally this dip coincides with the frequency of the nucleus which you are going to run. In this case, you can see that the dip is to the right of the ^{13}C of 100.568 MHz. While this is close, it should be tuned for maximum sensitivity. To stop the wobble process, simply type *stop* or click on the stop button at the top of the window. *Note: performing a wobb requires pulsing the sample continuously. Make sure to stop the experiment as soon as possible to prevent probe heating and receiver damage if the wobble curve is well off resonance.*

If the ATM probe is in (normal), then tuning the probe is done by typing *atmm* (automatic tuning and matching in manual mode). This brings up the tuning window and the wobble curve for the nucleus you are set up to run. The same window will appear along with a control panel that is used to adjust tune and match values. The wobble curve moves in response to tuning and matching.



The Tune buttons move the curve left and right, and the match buttons move the curve up and down. The tune buttons move the curve to the left or right as marked. The match buttons may bring the curve down to the left or the right, dependin on which way you are off from the optimal match. However, the tune and match interact with one another, a bit like Z and Z2 when you shim. It is best to start with the tune and center the dip, then work on the matching. Adjust each in turn until you have the dip in the curve aligned with the red line and as low as possible. This will assure maximum sensitivity for your nucleus and avoid excessive reflected power, which could damage the probe. When you have finished, simply close the ATMM window and that will stop the process. At this point the probe is properly tuned for the nucleus you have chosen, as you can see to the right. The same procedures apply regardless of the nucleus you wish to tune. Simply make sure you are in the appropriate experiment for your nucleus, and that you have done *getprosol* and *ii*.