

Basic 1D NMR Training Manual for the Bruker 300 MHz NMR Spectrometer



Scheduling NMR Time:

Any use of the 300 MHz NMR spectrometer requires prior booking of a time slot using the faces scheduling system. After completion of the basic training course a user will be given a login name and password, via email, for the faces system. This login information can then be used to log in to the faces scheduling system at <http://faces.ccrc.uga.edu/> under the group name of ulethnmr. Due to the high demand for time on the 300 MHz NMR spectrometer, the instruments time has been broken down into peak and non-peak hours. Each of these time periods has a specific set of rules on how the instrument may be booked. Please consult the most recent version of the 300 MHz NMR policies for these rules and follow them at all times. If you have any questions regarding these policies please contact the NMR Facility manager for clarification. The most recent version of the 300 MHz policies, as well as this and other manuals, can be found at: <http://www.uleth.ca/artsci/magnetic-resonance/information-students.>

Safety Concerns:

(1) Magnet Awareness

The magnetic field of an NMR magnet is approximately 1 million times as intense as the earth's magnetic field - so treat the magnet with respect. Read and understand the warning notices posted at the entrance to the spectrometer room.

The magnet will attract objects containing steel, iron, or other ferromagnetic metals. Such items include tools, electronic equipment, gas cylinders, keys, and steel chairs. Never take these items near the magnet.

The magnet can corrupt the information contained on bank cards (or magnetic storage media) and may damage watches. Leave your wallet and watch outside the 5G line.

To prevent accidental damage to the magnet the probe should always remain in the magnet and students should not adjust or touch the probe.

Make sure any visitor or anyone carrying out maintenance or cleaning work in the spectrometer room is aware of the magnet and what it can do.

In the unlikely event of a magnet quench (or if the oxygen alarm sounds) leave the room immediately. Do not re-enter until permission to do so is given by the NMR Facility Manager.

(2) Electrical Safety

High voltages exist within the spectrometer. All maintenance of spectrometer equipment should be carried out by the NMR Facility Manager. Under no circumstances should you remove any spectrometer parts from the console. Do not adjust or touch the RF cabling on the 300 MHz instrument, as doing so while an experiment is running can result in severe injury or death.

(3) Handling cryogens

Be sure to follow the directions provided in the University of Lethbridge cryogen safety manual at all times. This document can be found at the facility website here:

<http://www.uleth.ca/artsci/magnetic-resonance/information-students>

Liquid nitrogen (and helium) can cause severe burns so use it with caution. Never directly handle anything that has been immersed in liquid nitrogen (use insulated gloves or tweezers). Always use eye and face protection when transferring liquid nitrogen under pressure (such as when filling the magnet). If the oxygen-level alarm sounds leave the room immediately. Do not re-enter until permission to do so is given by the NMR Facility Manager.

Step-by-Step Instructions on Using the Spectrometer:

(1) If the control computer is powered off or locked you should log on using the following account:

Username: nmr

Password: nmr



Figure 1 Windows Login screen for Spectrometer.

(2) If Topspin is not already open you can open it by double clicking on the topspin icon on the desktop.



Figure 2 Location of the Topspin icon on the desktop.

(3) The Topspin program window should now be open (figure 3). In order to display the data you will need to left click on the plus sign located next to the highlighted c: directory (shown with an arrow).

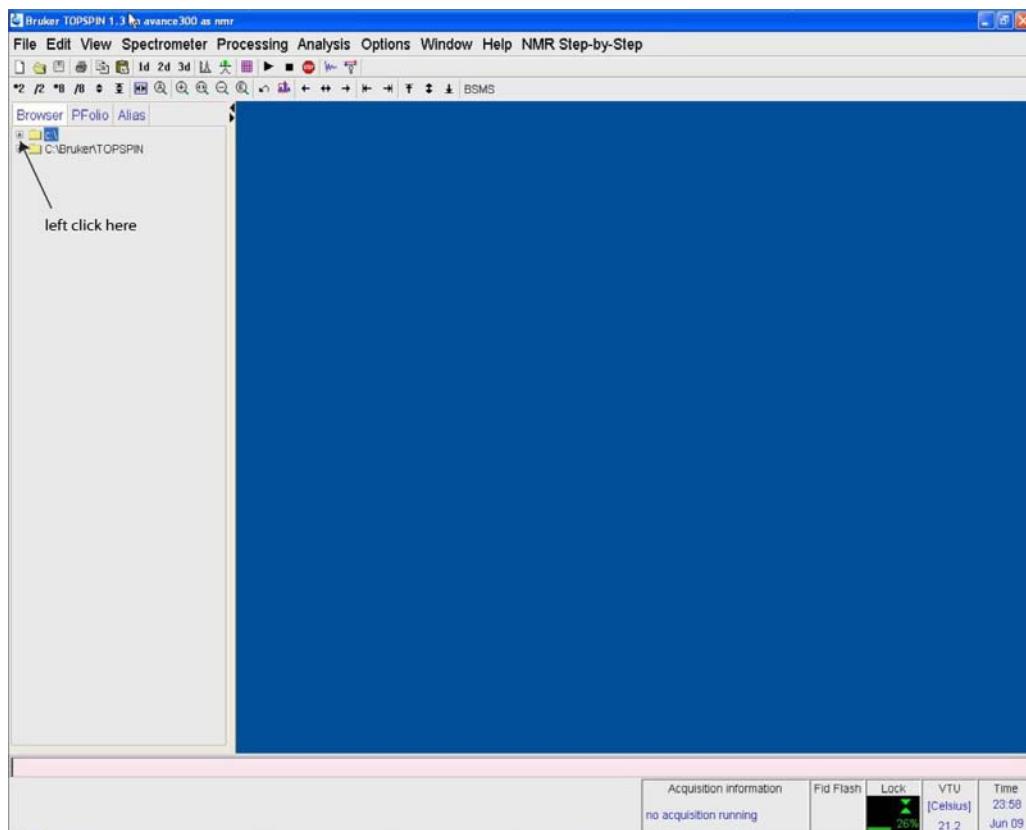


Figure 3 Main Topspin window.

(4) Topspin now shows the data structure for all users of the instrument. Navigate to your username and open up one of your old data sets. If you do not have any old data then you should open up the data set listed under the user Montina and called 1dProton (see figure 4 below). We will use this experiment to create a new experiment/dataset under your username.

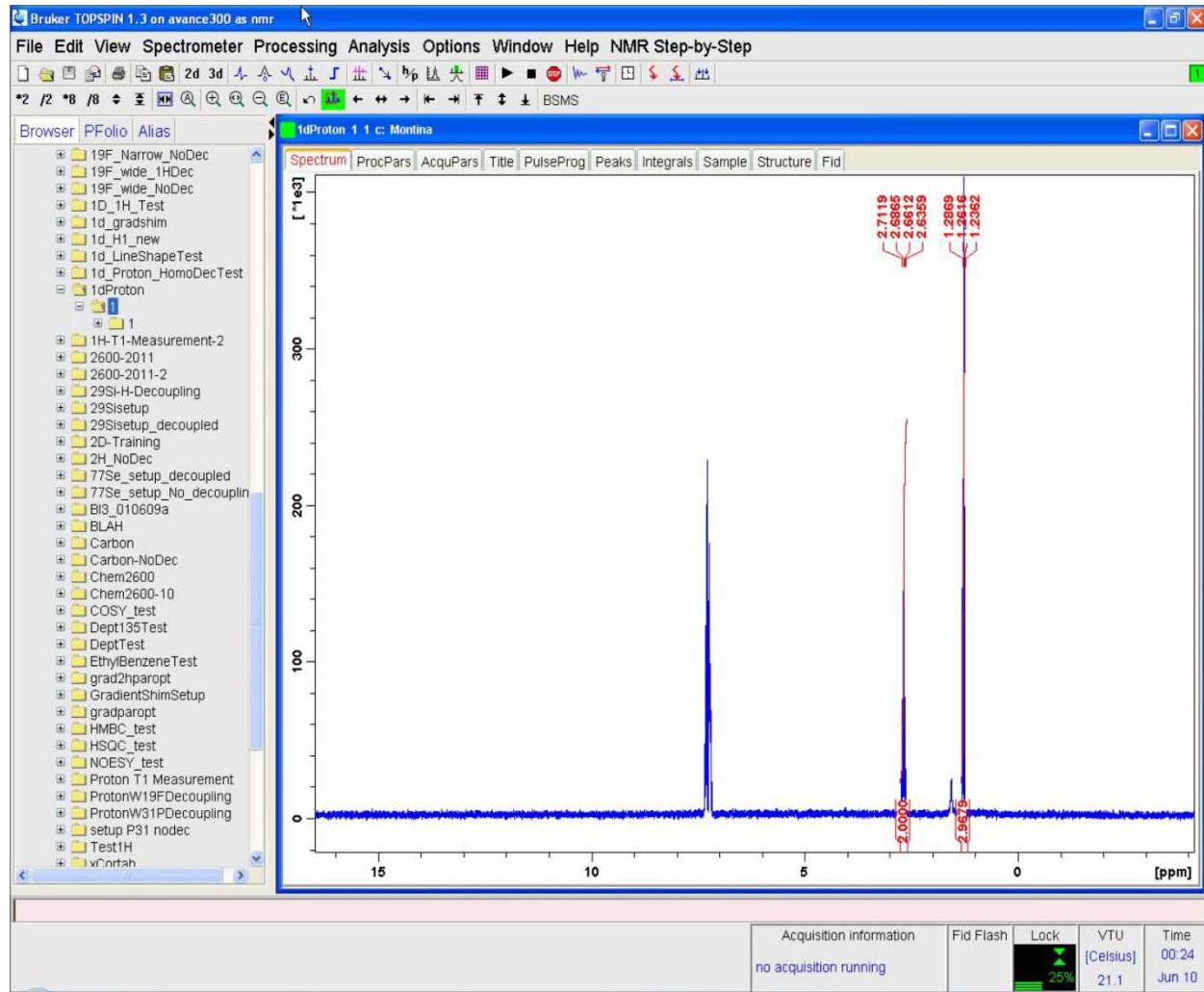


Figure 4 Topspin data window with Montina 1dProton data set showing.

(5) You will now begin using the NMR-Step-by-Step drop down menu. This menu contains all of the steps required to set up the spectrometer, as well as acquire and process your data. This training manual will also provide the commands associated with each of these steps, as well as point out where the corresponding icons are located in TopSpin (when possible). You should familiarize yourself with the NMR step by step menu now as it will be referred to but NOT shown throughout the rest of this manual.

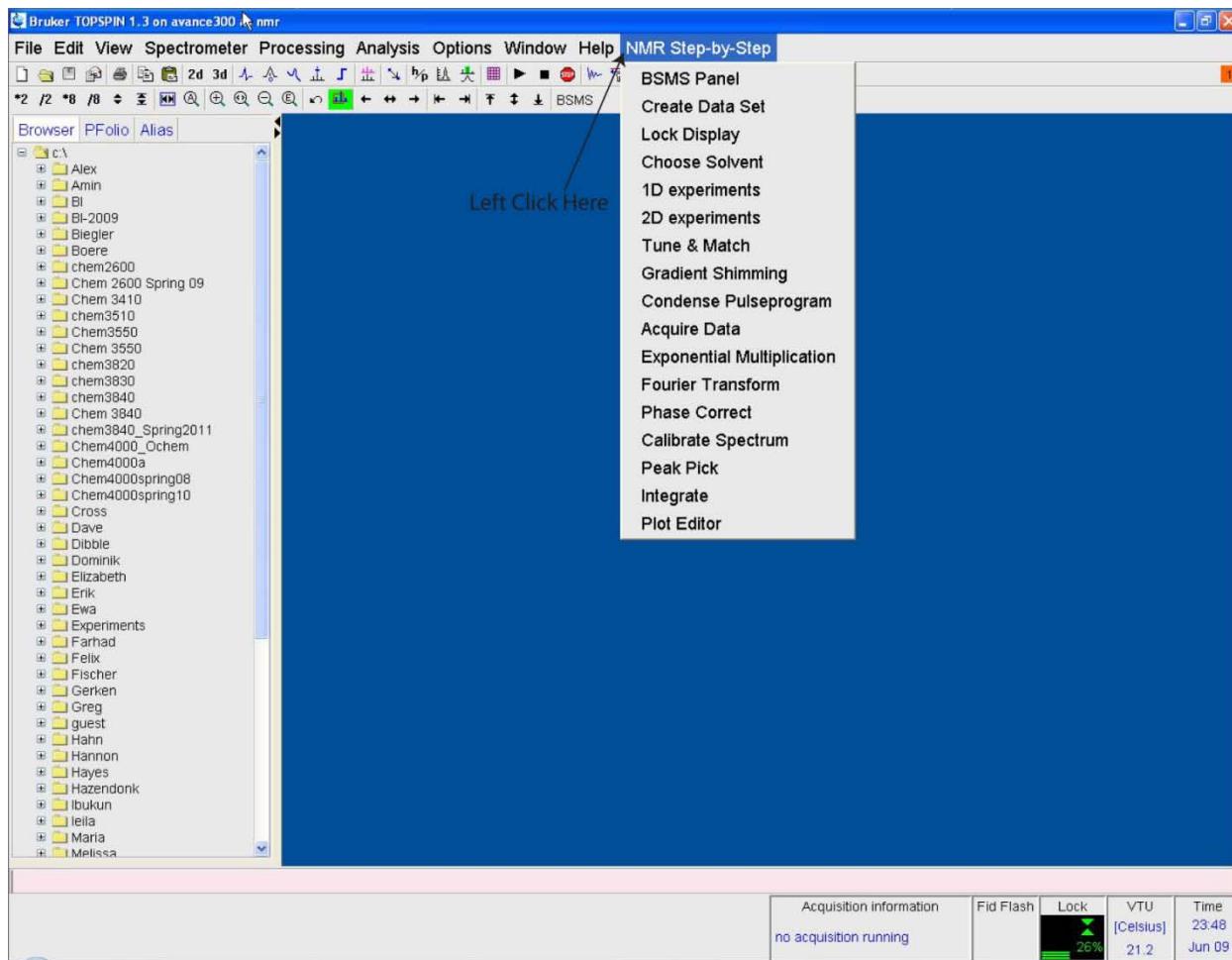


Figure 5 NMR step-by-step menu.

(6) You should now load your NMR sample into the magnet. This process is explained in the following steps:

(a) If the dust cap is in place on top of the magnet stack please remove it.
(b) First left click on the “BSMS Panel” option of the NMR step by step menu (Figure 5). Next left click on the lift button of the main tab of the BSMS interface (shown below in figure 6). This will cause air to flow out of the sample port (top of the stack) and the sample location indicator at the bottom of the BSMS should indicate that the sample is up.

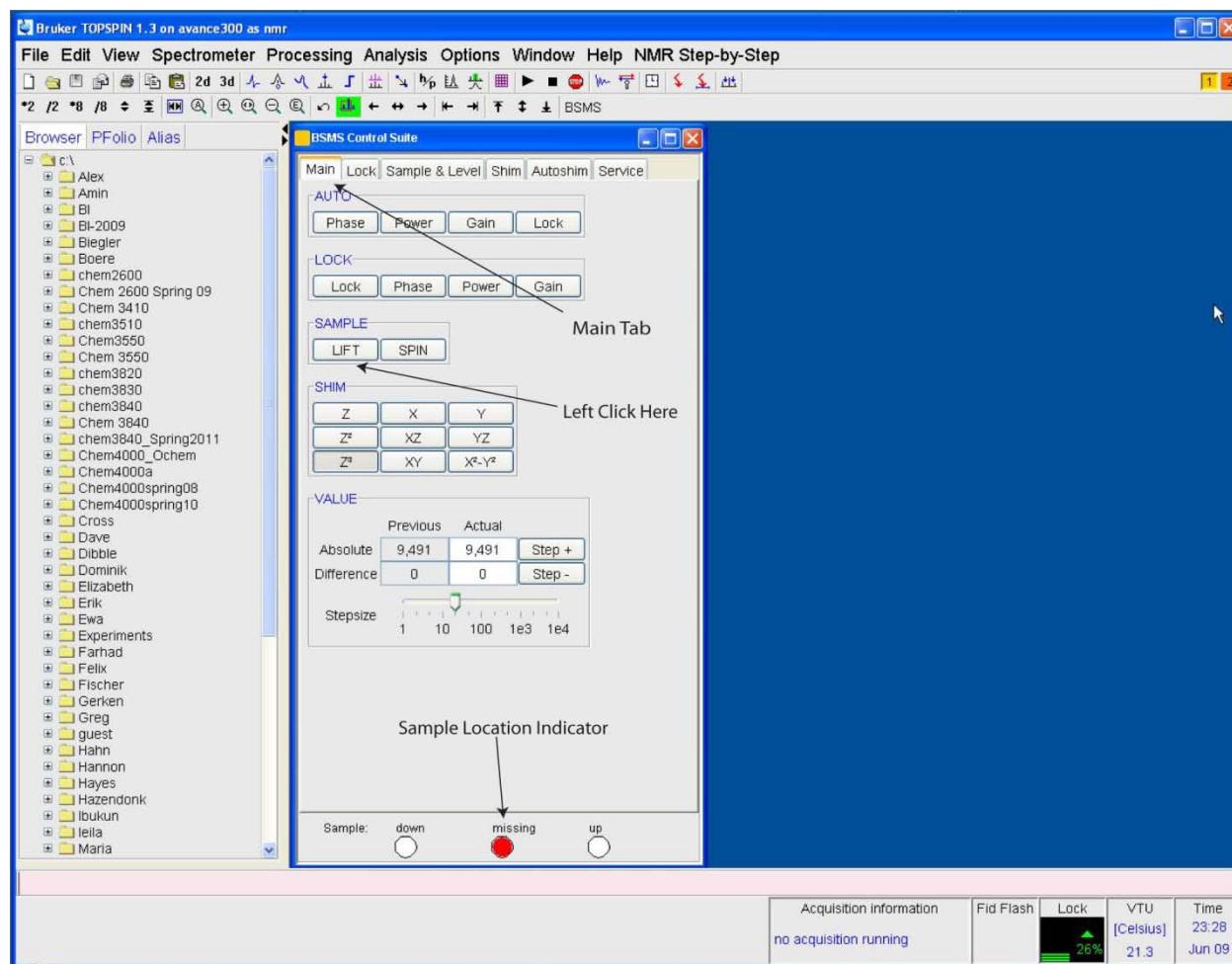


Figure 6 Main tab of the BSMS interface.

(c) The tube height guide (at right) has both a blue plastic component (the “spinner”) and a colourless glass component (the “guide”). Place the spinner into the top of the guide as shown, then carefully load your sample tube into the spinner so it touches (but is *no lower than*) the bottom of the glass guide. ***If your sample tube is too low when it is placed into the instrument, it will cause damage.***



(d) *Without* removing your NMR tube from the blue spinner, lift it out of the guide. Release your sample tube (along with the spinner) slowly over the top of the sample port *without* allowing it to drop. The sample should float on the stream of compressed air at the top of the sample port. If the flow rate is not high enough to support the sample without holding it, inform the facility manager so that the necessary adjustments can be made. **NEVER drop the spinner into the sample port without the presence of air flow, doing so will result in a costly repair.**

(e) Left click on the lift button of the main tab of the BSMS interface. This will cause air to slowly lower your sample into the probe. The sample location indicator should initially show missing, but will eventually show that the sample is down. If this sample indicator does not show that the sample is down please contact the facility manager before proceeding.

NOTE: A user should always handle the NMR tube by the top of the tube and not the body. Doing so prevents the grease and dirt from your hands getting into the instrument and probe. In addition, the spinner and NMR tube should be wiped clean with a Kimwipe before being placed into the sample port.

(7) Use the drop down menu to create a new experiment/dataset for acquiring the 1D proton spectra. This can be done by left clicking on the “Create Data Set” option in the NMR step-by-step menu. Each PI has their own preference for how students should name their data sets; be sure to name your data set according to your PI’s preference. The following is a general guideline:

- (a) The name is usually the name of the sample being run
- (b) The experiment number should correlate to different experiments on the same sample.
Ex. The 1D Proton, 1D Carbon, 2D COSY, and 2D HSQC spectra for the same sample can be labeled experiment number 1, 2, 3 and 4, respectively.
- (c) AVOID changing the procedure number (PROCNO), as this should usually stay set at 1.
- (d) The directory (DIR) should always remain set to c:
- (e) The user for the experiment should be your username for data storage
- (f) The title box should contain any title that you would like to appear on your printouts
- (g) The Solvent and Experiment boxes can be ignored as the next two steps of this manual explain how to set both of these parameters

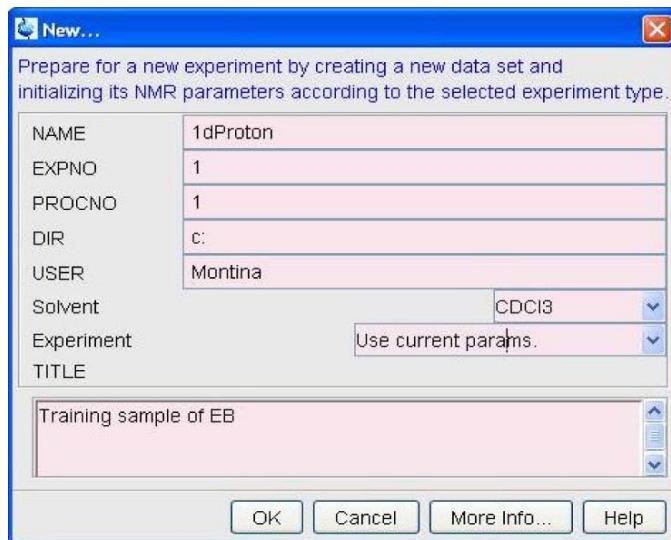


Figure 7 Create new dataset.

(8) You must now open up the lock screen and choose your deuterated solvent. This can be done using the following steps:

(a) If the lock display (Figure 8) is not open on the second monitor, you can open it by left clicking on “Lock Display” in the NMR step-by-step menu.

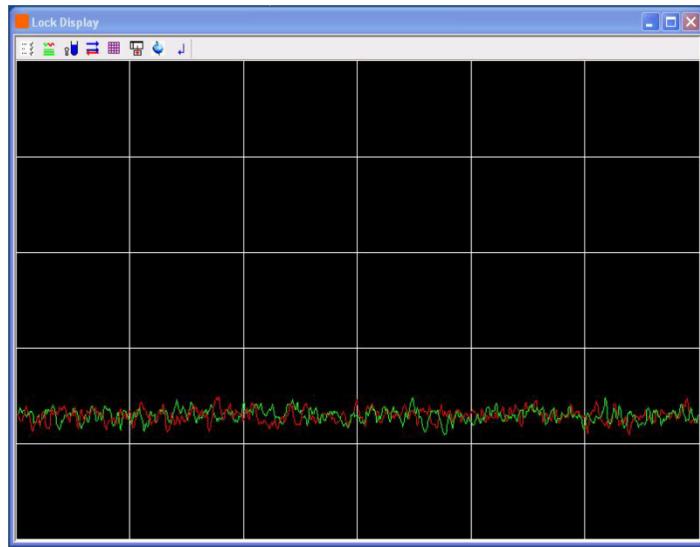


Figure 8 Lock Display (located on second monitor).

(b) Left click on “Choose Solvent” in the NMR step-by-step menu. This will bring up a list of common deuterated solvents and you should click on your solvent and click on ok (Figure 9).

Solvent	Description
Acetic	acetic acid-d4
Acetone	acetone-d6
C6D6	benzene-d6
CD2Cl2	methylenechloride-d2
CD3CN	acetonitrile-d3
CD3CN_SPE	LC-SPE Solvent (Acetonitrile)
CDC13	chlorform-d
CH3CN+D2O	HPLC Solvent (Acetonitrile/D2O)
CH3OH+D2O	HPLC Solvent (Methanol/D2O)
D2O	deuteriumoxide
DEE	diethylether-d10
Dioxane	dioxane-d8
DME	dimethylether-d6
DMF	dimethylformamide-d7
DMSO	dimethylsulfoxide-d6
EtOD	ethanol-d6
H2O+D2O	90%H2O and 10%D2O
MeOD	methanol-d4
Pyr	pyridine-d5
THF	tetrahydrofurane-d8
Tol	toluene-d8

Figure 9 Choose solvent menu.

If everything has worked correctly you should now have a lock on your solvent.

(9) You should now load the standard parameters for the 1D experiment you wish to run. This can be done by left clicking on the “1D Experiments” option in the NMR step-by-step menu. A list of available 1D experiments will appear in the box shown below. Select the experiment you wish to carry out and left click on copy all. This loads the correct parameters into the currently open data set. If you wish to run an experiment that is currently NOT in the 1D experiments menu please contact the facility manager and request that the experiment be implemented. There is a separate list that contains each of the 2D experiments that are currently implemented. If you wish to carry out 2D experiments please contact the facility manager so that he can arrange a 2D NMR training session for you.

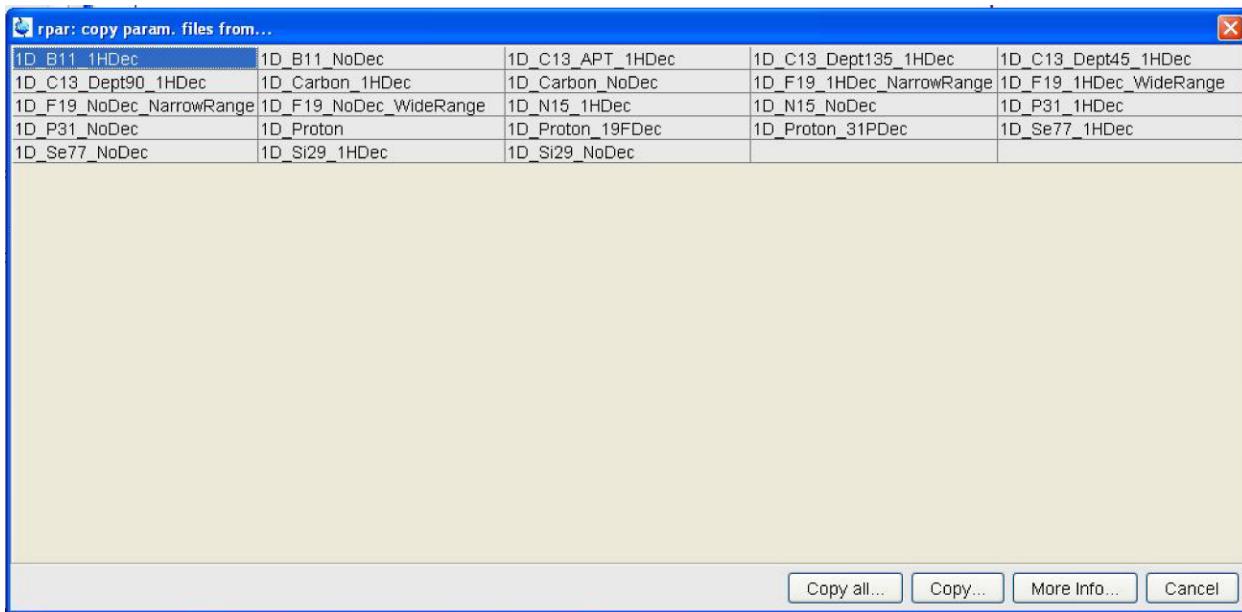


Figure 10 List of routine 1D experiments that are setup on the spectrometer.

(10) Now that your experiment has been created and your solvent is locked you are ready to prepare both the probe and magnet. The probe can be tuned by left clicking on “Tune and Match” in the NMR step-by-step menu. This will cause the WOBB interface to appear and the probe will be automatically tuned. This process will occur once if you are doing a single channel experiment and twice if you are doing a two channel experiment. When the tuning process is completed the bottom of the main topspin window should say “atma finished”.

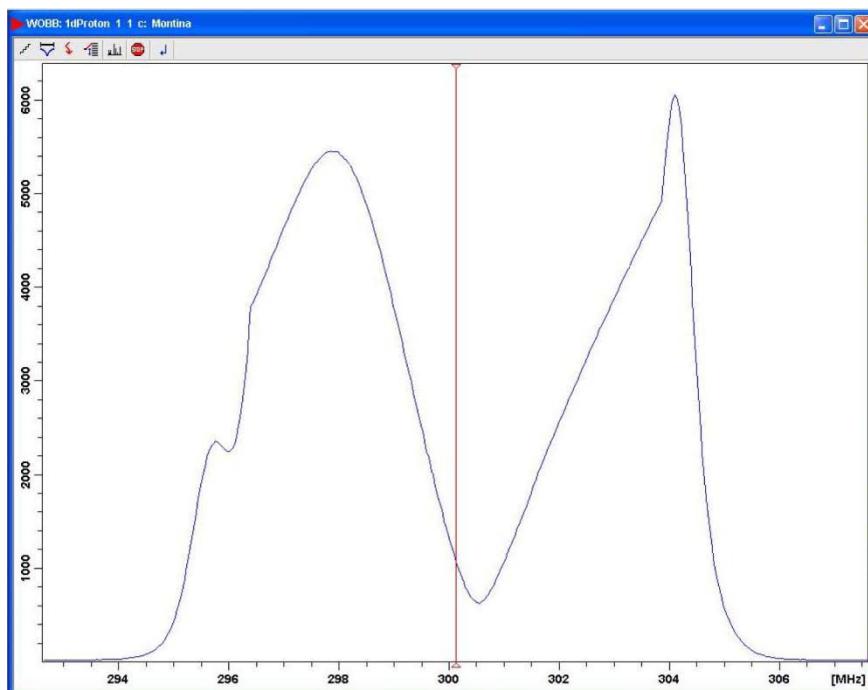


Figure 11 WOBB interface.

(11) Now that the probe is tuned the magnet can be shimmed. The automatic shimming process can be carried out by left clicking on “Gradient Shimming” in the NMR step-by-step menu. The process of auto-shimming can take a few minutes so please be patient. When the process is completed the bottom of the main topspin window should say “gradshimau finished”. If you are unhappy with the auto-shimming method you can use the “instructions for manual shimming” document which can be found at the following link: <http://www.uleth.ca/artsci/magnetic-resonance/information-students>.

(12) The auto-shimming process usually increases the lock signal to the point where it is greater than 100% and is no longer visible on the lock screen. When this occurs you should re-open the BSMS interface by left clicking on “BSMS Panel” in the NMR step-by-step menu. Inside the BSMS interface you should left click on the lock tab (see Figure 12). Now you should left click on the gain button as this will allow you to make adjustments to the gain. Lastly, you should left click on the “step –“ button until the lock signal re-appears on the screen and the lock signal strength is at ~80%. The step size ruler (shown in the highlighted box) can be used to increase or decrease the value by which the “step –“ and “step +” buttons change the lock gain.

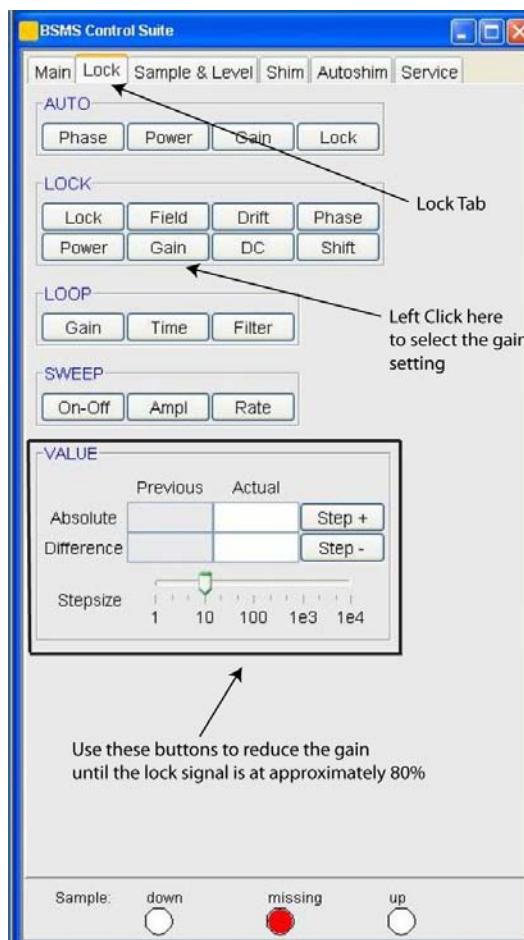


Figure 12 Lock tab of the BSMS interface.

(13) Now that the probe and magnet have been prepared you can adjust the acquisition parameters. NOTE: If you are going to use the standard acquisition parameter (these are loaded when you choose your 1D experiment) you can skip this step and continue with step 14. The full set of acquisition parameters are shown in Figure 13 below:

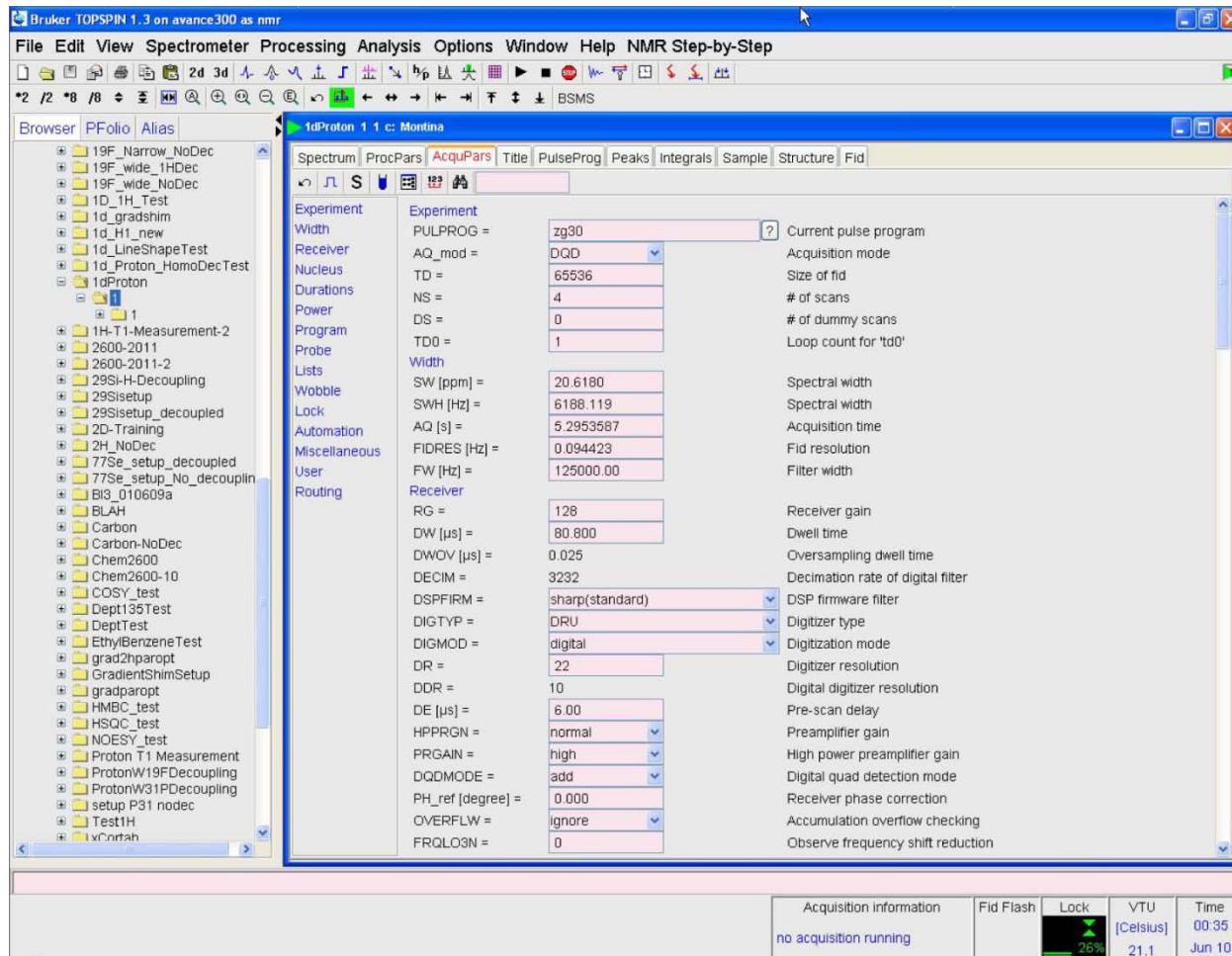


Figure 13 The Full list of acquisition parameters.

You should now simplify the parameters shown in the AcquPars tab by left clicking on “Condense Pulseprogram” in the NMR step-by-step menu. The new set of shortened parameters can be seen in Figure 14.

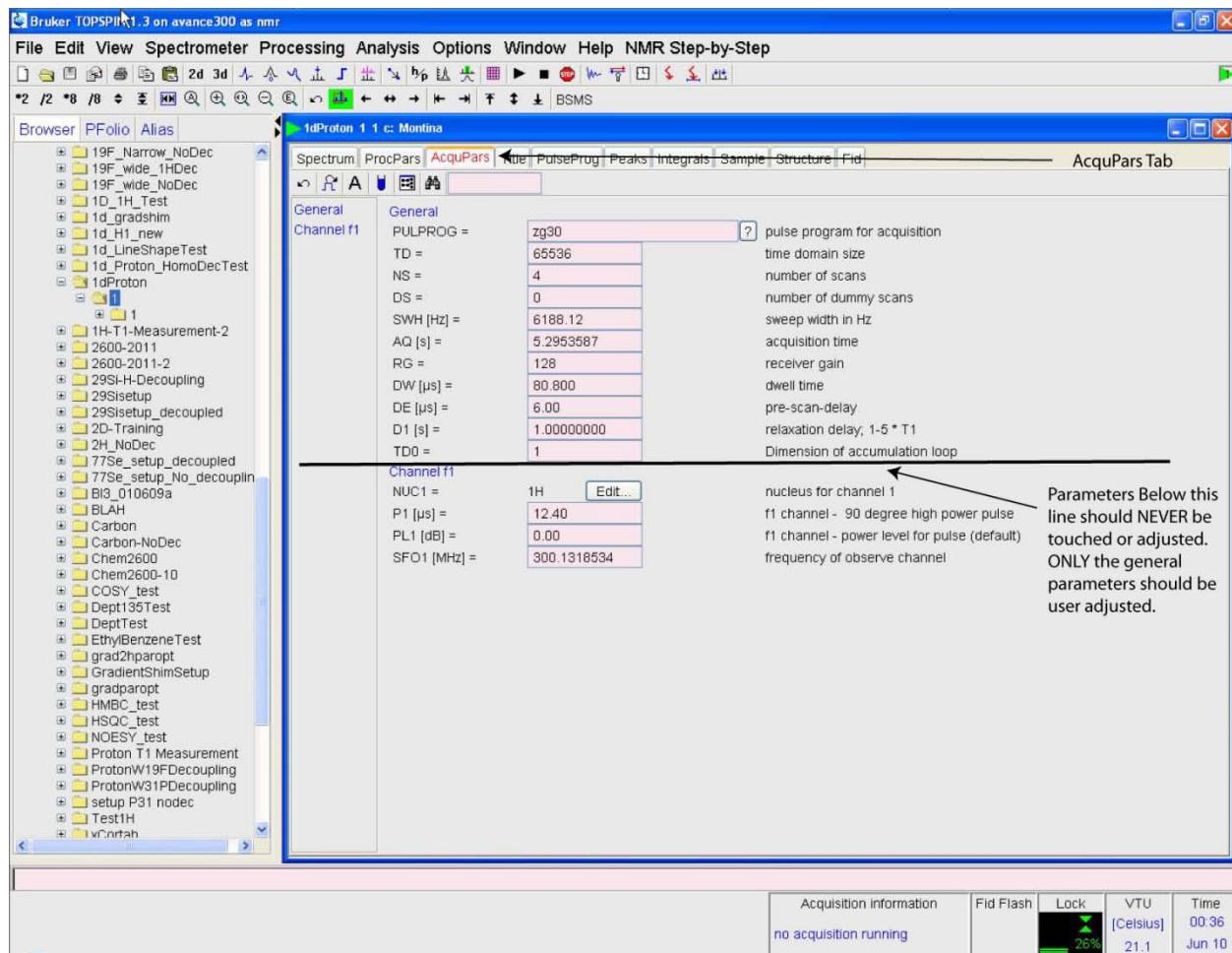


Figure 14 Condensed set of acquisition parameters.

Each parameter has a brief description located to the left of it. Users should **NEVER** adjust the Channel f1 or Channel f2 parameters located below the black line in the figure above. These are the pulse power and pulse duration parameters and adjusting them can result in **SEVERE** probe damage and a very costly repair. Furthermore, the TD0 and PULPROG parameters should never be adjusted by the user. The receiver gain (RG) parameter is automatically set when the user carries out an acquisition. The remaining parameters and how they apply to the acquisition of an FID is shown below (figure 15) in what is known as a pulse sequence diagram:

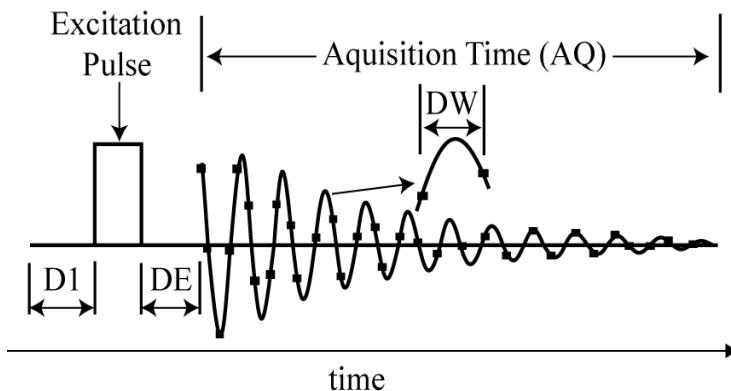


Figure 15 Pulse sequence diagram illustrating the acquisition parameters.

$$\text{Acquisition Time (AQ)} = \text{NumPoints (TD)} * \text{DwellTime (DW)}$$

$$\text{DwellTime (DW)} = 1 / \text{SpectralWidthInHertz (SWH)}$$

D1 = Recycle Delay DE = Pre-scan delay

NS = Number of scans DS = Number of dummy scans

(14) Now that the hardware is ready and the acquisition parameters have been set you can acquire your data. Acquisition can be started by typing “rga zg” into the command line and hitting enter or by left clicking on “Acquire Data” in the NMR step-by-step menu. When the acquisition is completed the bottom of the main topspin window should say “acquire macro finished” or “zg finished”. NOTE: the user can view the FID and spectra while the acquisition is running by left clicking on the FID icon shown below; furthermore, left clicking on the clock icon will tell a user the amount of time that an acquisition is going to take to complete.

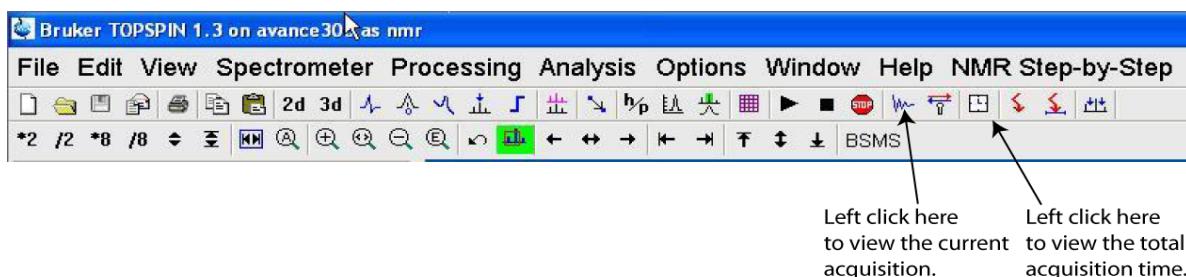


Figure 16 Topspin menu-bar.

(15) Now that your data has been acquired you can view the unprocessed FID by left clicking on the FID tab (shown below).

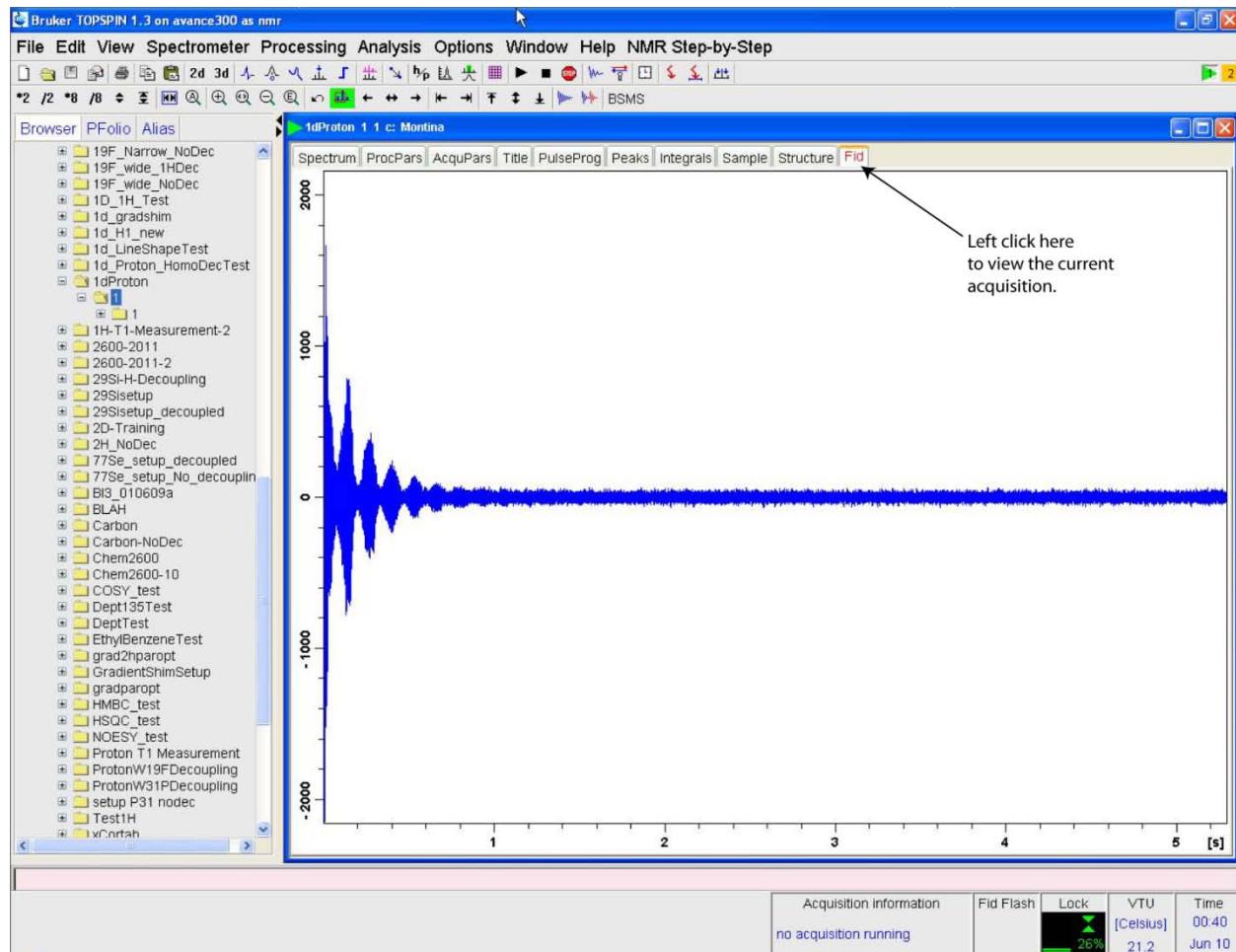


Figure 17 Unprocessed Free Induction Decay (FID)

In most cases you will want to apply exponential multiplication to the FID. This can be done by left clicking on “Exponential Multiplication” in the NMR step-by-step menu. Exponential multiplication is used to: (1) zero out the noise at the end of the data, (2) increase signal to noise, and (3) pull out additional features in the spectra (such as hard to resolve j-couplings). The figure below illustrates the effect of exponential multiplication on the FID seen above.

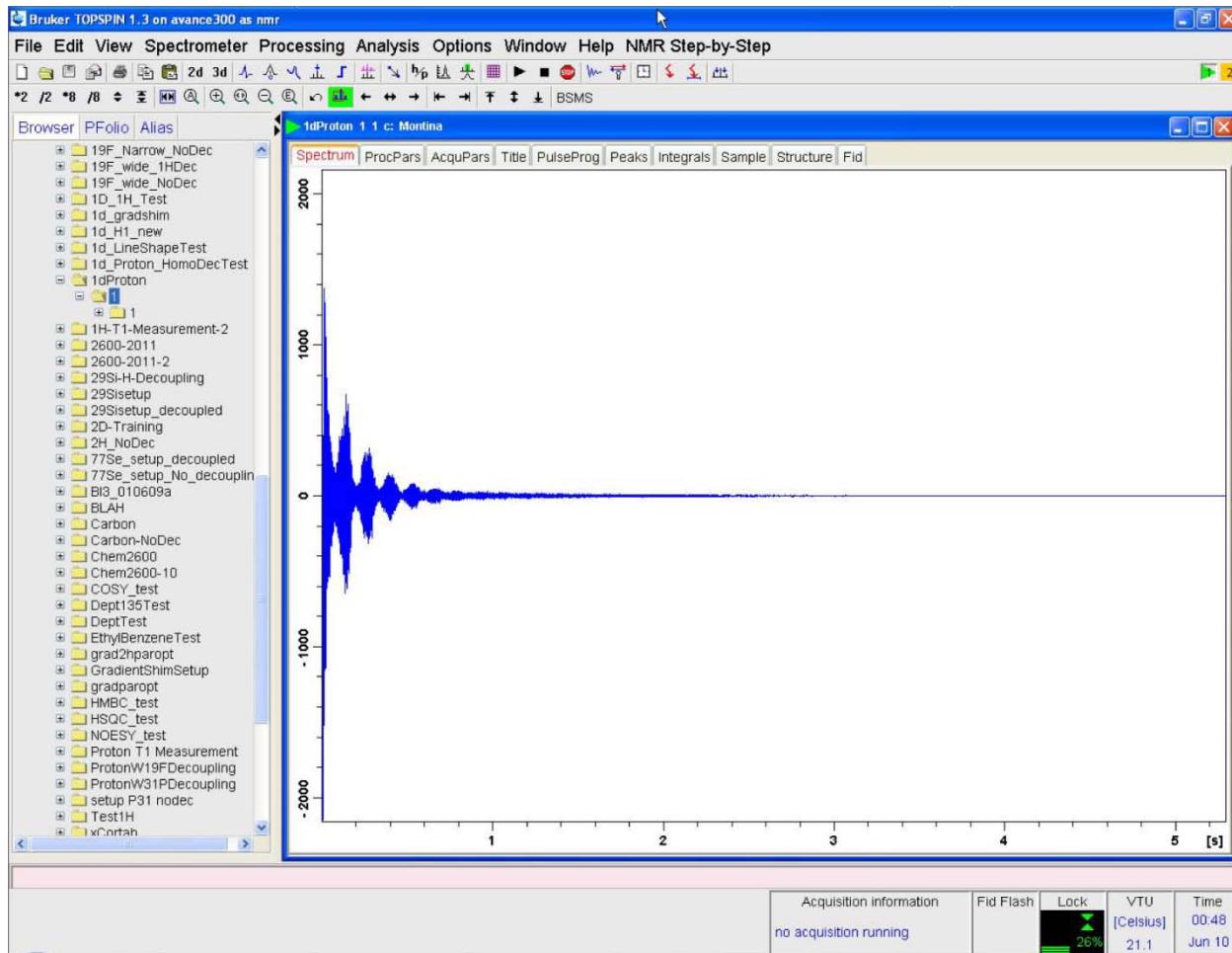


Figure 18 Processed Free Induction Decay (FID)

(16) Now that your data has been exponentially multiplied it is ready to be Fourier transformed. You can carry out a Fourier Transform (FT) by left clicking on “Fourier Transform” in the NMR step-by-step menu. The spectra resulting from the FT can be seen in the Spectrum tab (shown below in Figure 19).

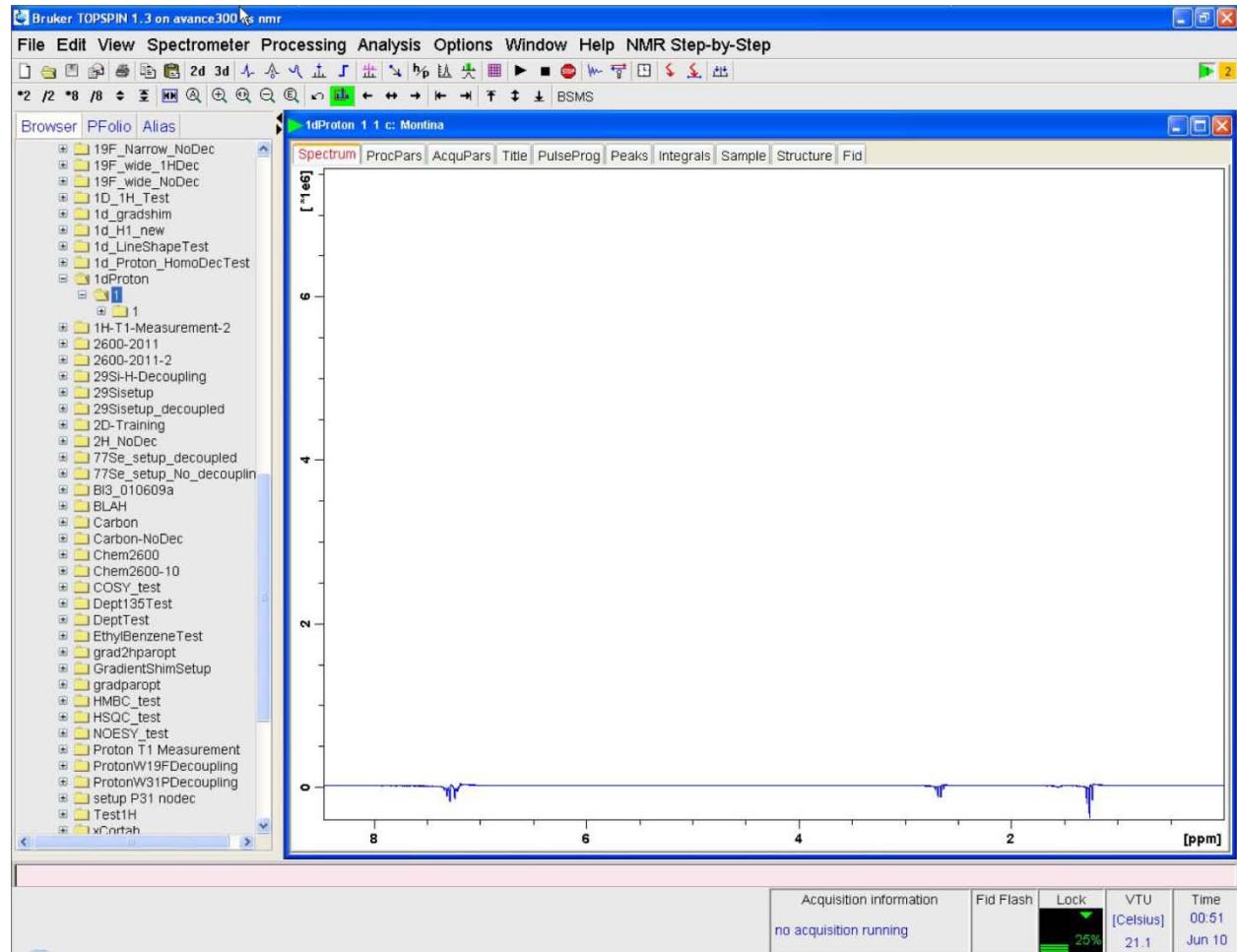


Figure 19 Spectra obtained following a FT of the FID shown in figure 19.

(17) Now you must apply automatic phase correction to the spectra. This can be done by left clicking on “Phase Correct” in the NMR step-by-step menu. The result of automatic phase correction for the spectra shown above can be seen in Figure 20.

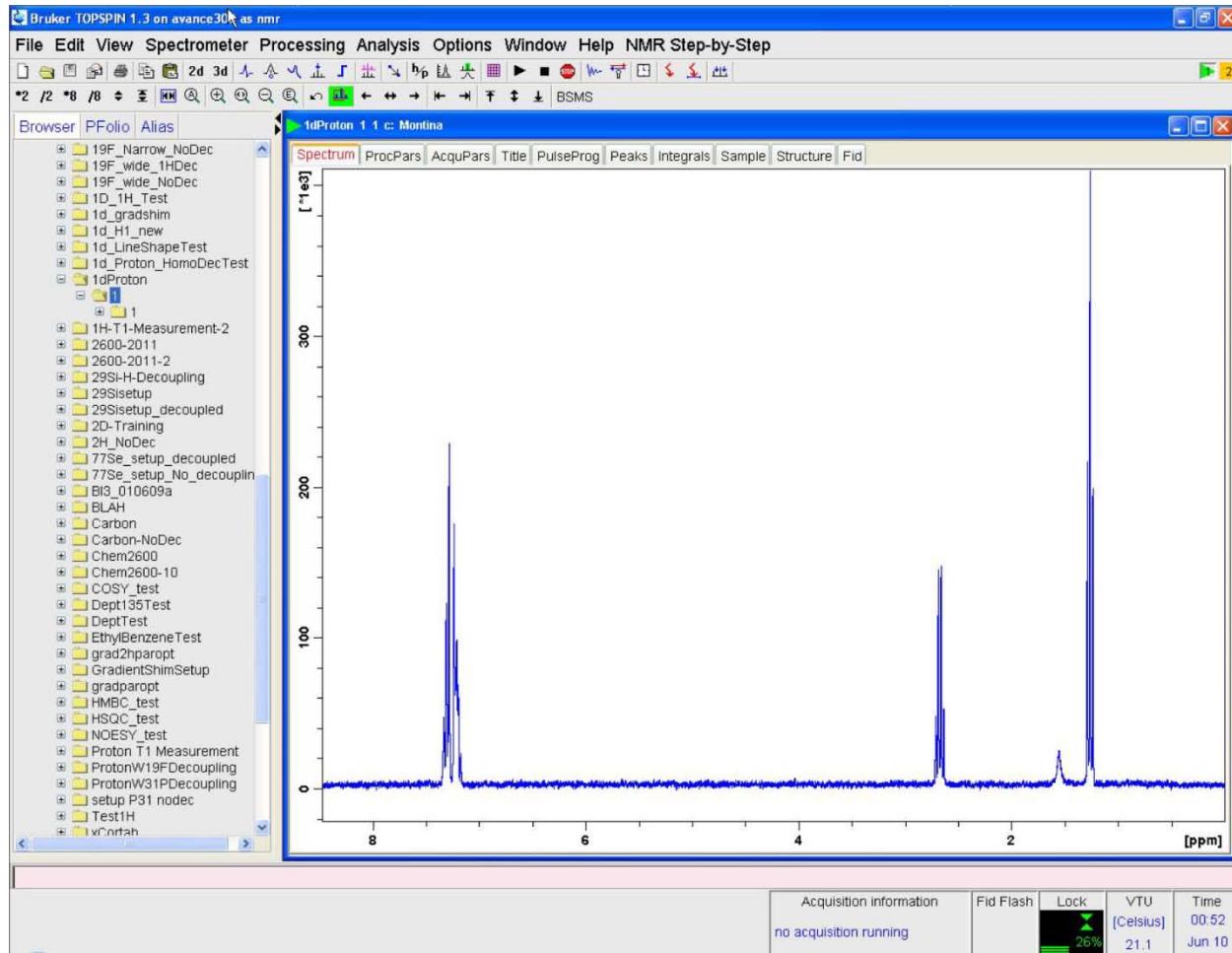


Figure 20 Spectra obtained after carrying out an automatic phase correction.

If you are unsatisfied with the automatic phase correction you can carry out manual phase correction. The process of manual phase correction will be shown in step 18.

(18) At this point it is important to note where the horizontal and vertical adjustments can be found in Topspin menu-bar. Figure 21 (below) illustrates where these buttons are found. All of the vertical scale adjustments are done with the buttons shown; however, the horizontal scale is adjusted by left clicking the mouse, dragging the cursor over the area of interest, and then left clicking one more time. The horizontal scale can be reset to the full window by left clicking on the icon indicated below. User should play with these buttons to get comfortable with their use.

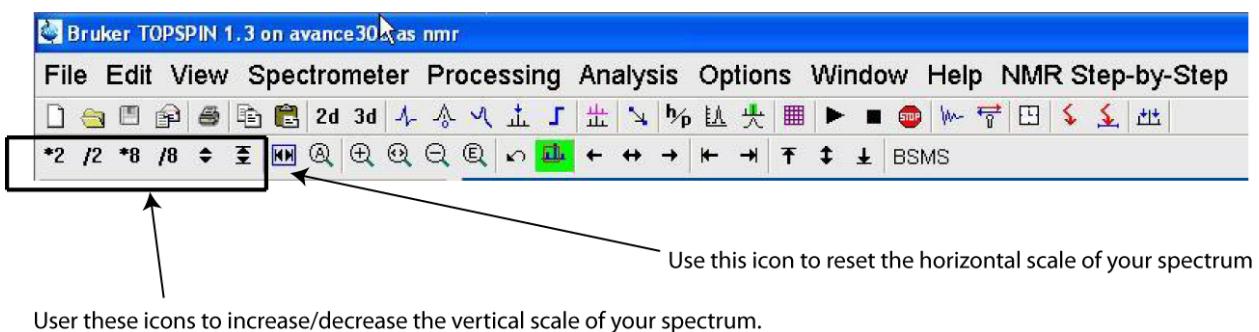


Figure 21 Buttons for vertically and horizontally adjusting the spectrum.

(19) The manual phase correction interface can be opened by left clicking on the icon shown in Figure 22.

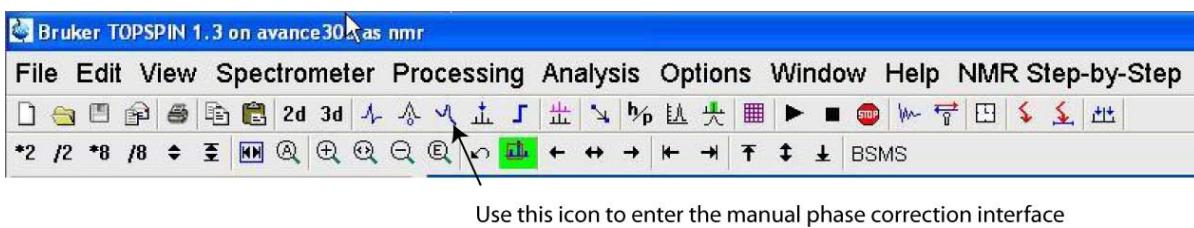


Figure 22 Topspin menu-bar icon for manual phase correction

You should Right click anywhere on the spectra to open a sub-menu and then left click on set pivot point in this menu. You can now set the pivot point by left clicking its location on the spectrum. Once you have set the pivot point you can carry out zero- and first-order phase correction by left clicking and holding down the mouse button on the either of the two icons shown below. Zero-order correction will phase correct the entire spectra while first-order correction will only phase regions of the spectra that are away from the pivot point. When you are done phasing you should left click on the save and return icon indicated below

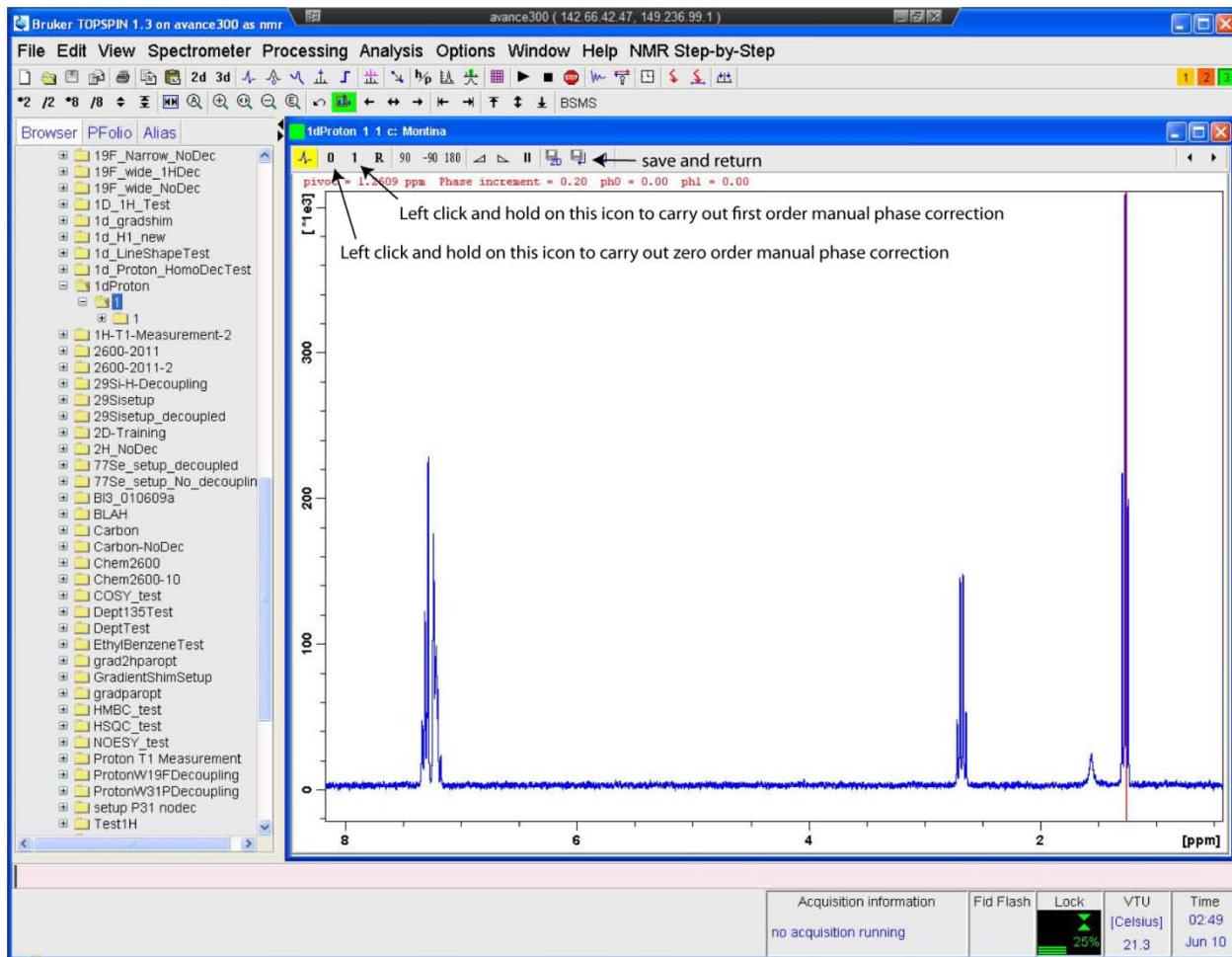


Figure 23 Interface for first- and second-order manual phase correction.

(20) Now that the spectrum has been phase corrected it can be referenced. For most proton and carbon spectra this can be done using the solvent as an internal reference. To reference the spectra the user should do the following:

- Left click on “Calibrate Spectrum” in the NMR step-by-step menu.
- Left click on the reference peak
- Enter the references chemical shift value (in ppm) in the box
- Left click on ok to complete the process.

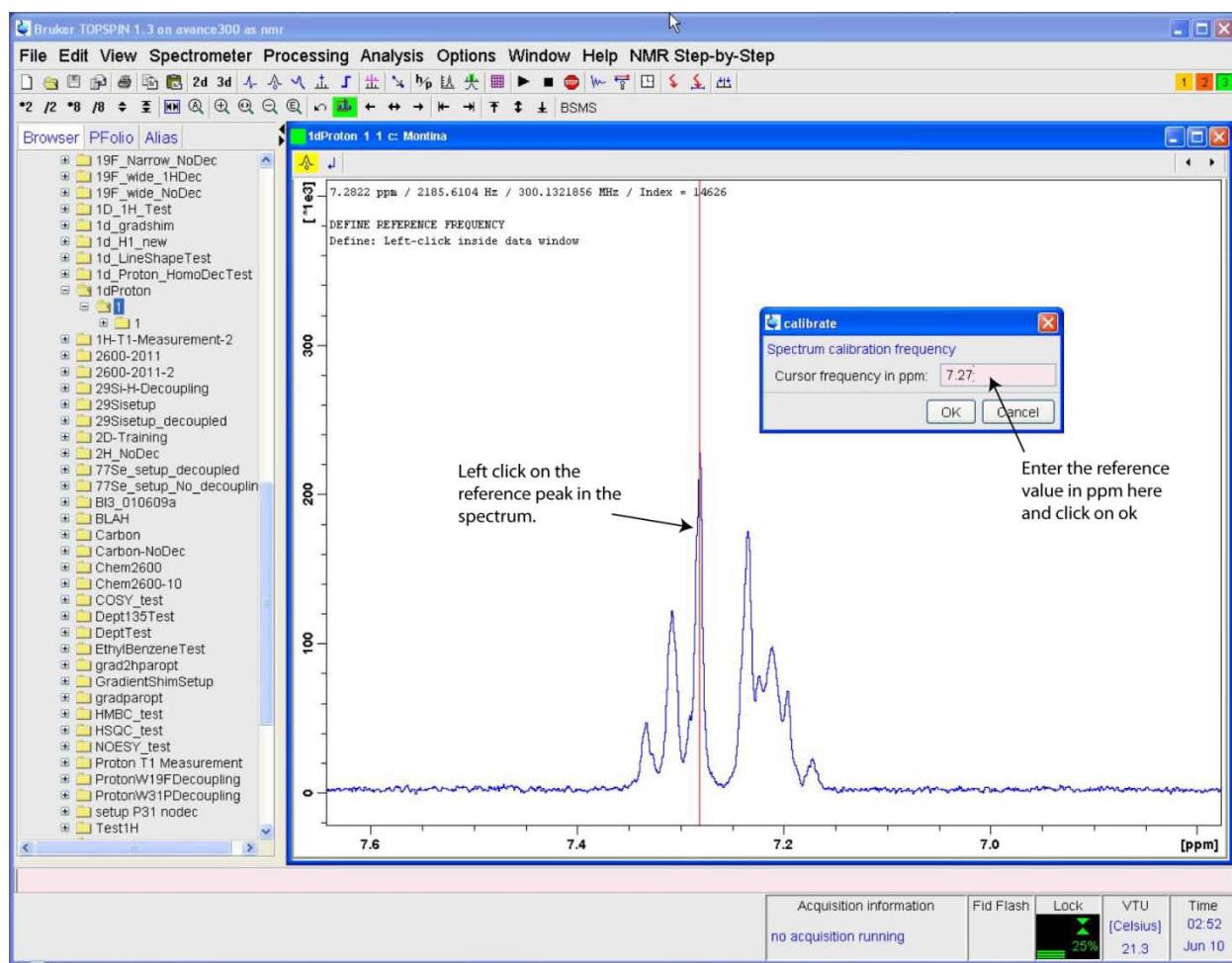


Figure 24 Interface for calibrating (referencing) the chemical shift scale using an internal solvent.

(21) The spectrum is now ready to be peak-picked. The peak-picking interface can be brought up by left clicking on “Peak Pick” in the NMR step-by-step menu or on the icon indicated in Figure 25. The select region button (indicated below) must be highlighted green in order to select the peaks of interest. Left clicking and holding down the mouse button allows the user to drag a green box over the peaks of interest. This box must be created over the top of the peaks for them to be picked properly. A user can right click anywhere on the spectrum to open the peak-picking sub-menu. This menu will allow the user to delete all regions or delete the region under the location that they right clicked. When all peak-picking is complete the user should left click on the save and return icon.

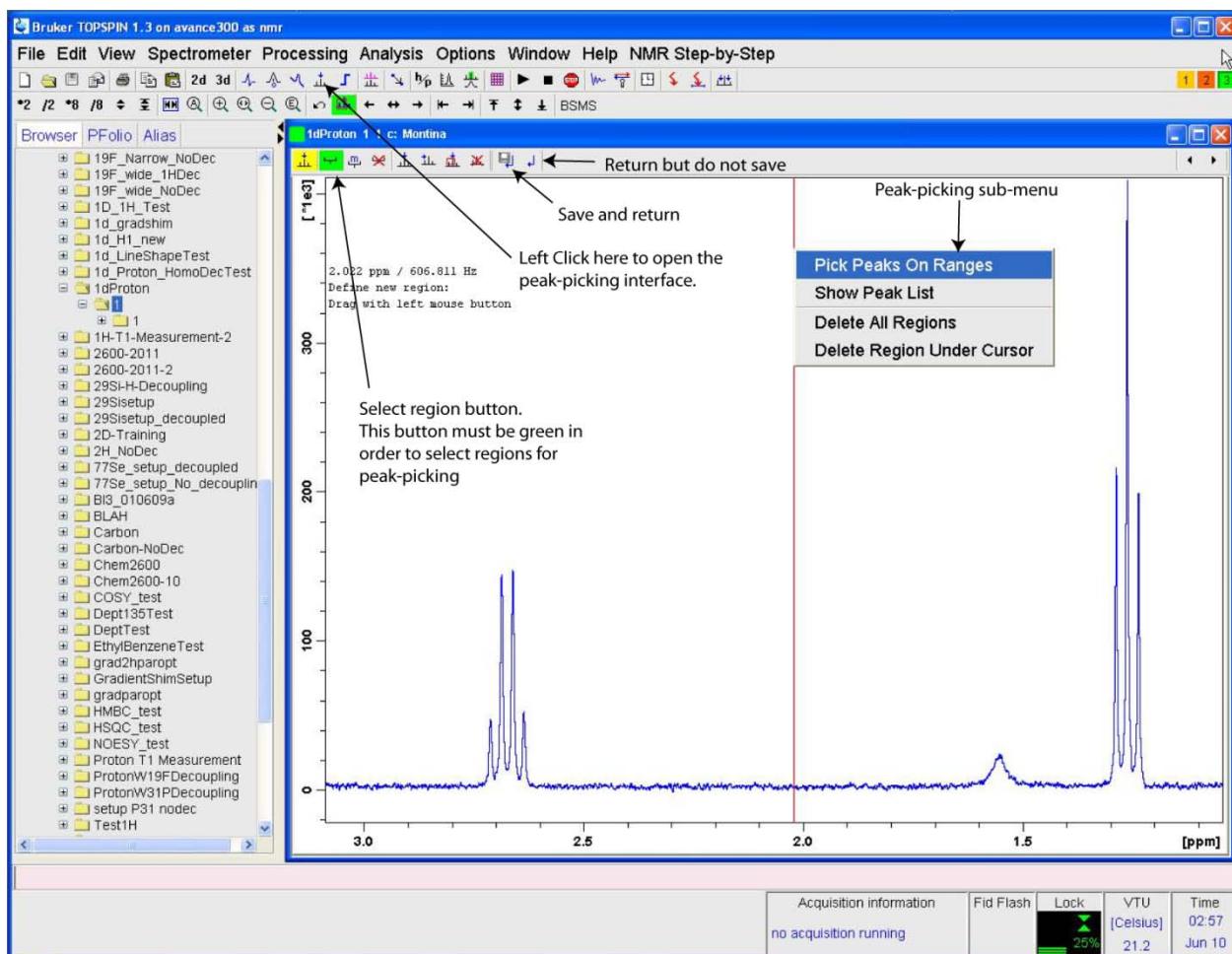


Figure 25 Topspin peak-picking interface.

(22) The spectrum is now ready to be integrated. The integration interface can be brought up by left clicking on “Integrate” in the NMR step-by-step menu or on the icon indicated in Figure 26. The select region button (indicated below) must be highlighted green in order to select the region for integration. The region for integration can be selected by left clicking the mouse, dragging the cursor over the area of interest, and then left clicking one more time. A user should right click over an integrated region to open the integration sub-menu. This menu will allow the user to calibrate and/or delete the integrated region. When all integration is complete the user should left click on the save and return icon.

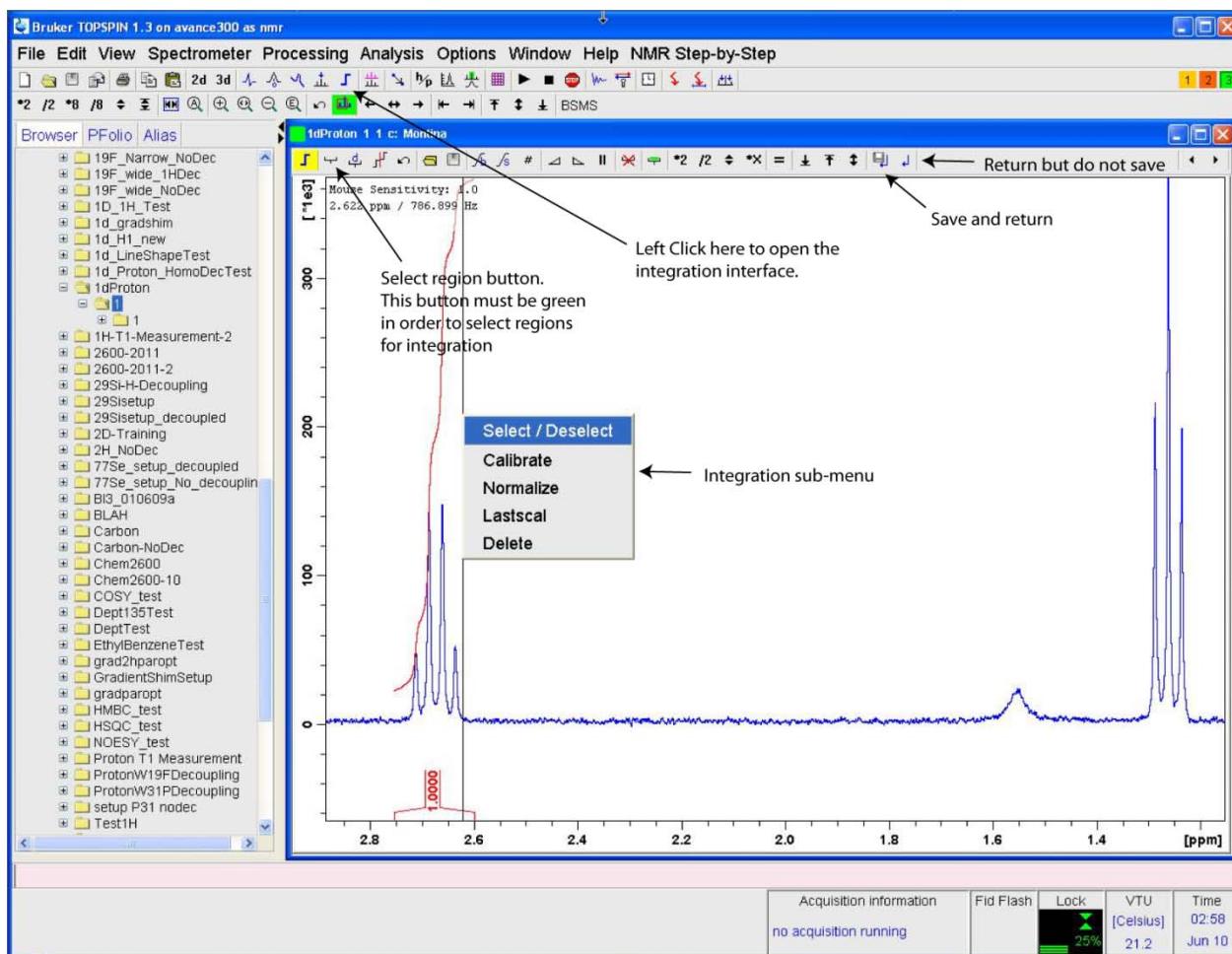


Figure 26 Topspin integration interface.

(23) Now that your spectrum has been acquired and processed it is time to prepare the spectrum for printing. Topspin uses a tool known as the plot editor to do this. The plot editor can be opened by left clicking on “Plot Editor” in the NMR step-by-step. Figure 27 shows the default layout that will open with the plot editor. This manual will provide very simple instructions for using the plot editor; however plot editor is a very powerful tool and users may wish to spend some additional time becoming more familiar with its use. The default layout contains several different boxes (shown below) including: the parameters box, title box, Bruker symbol box, and the main spectrum box.

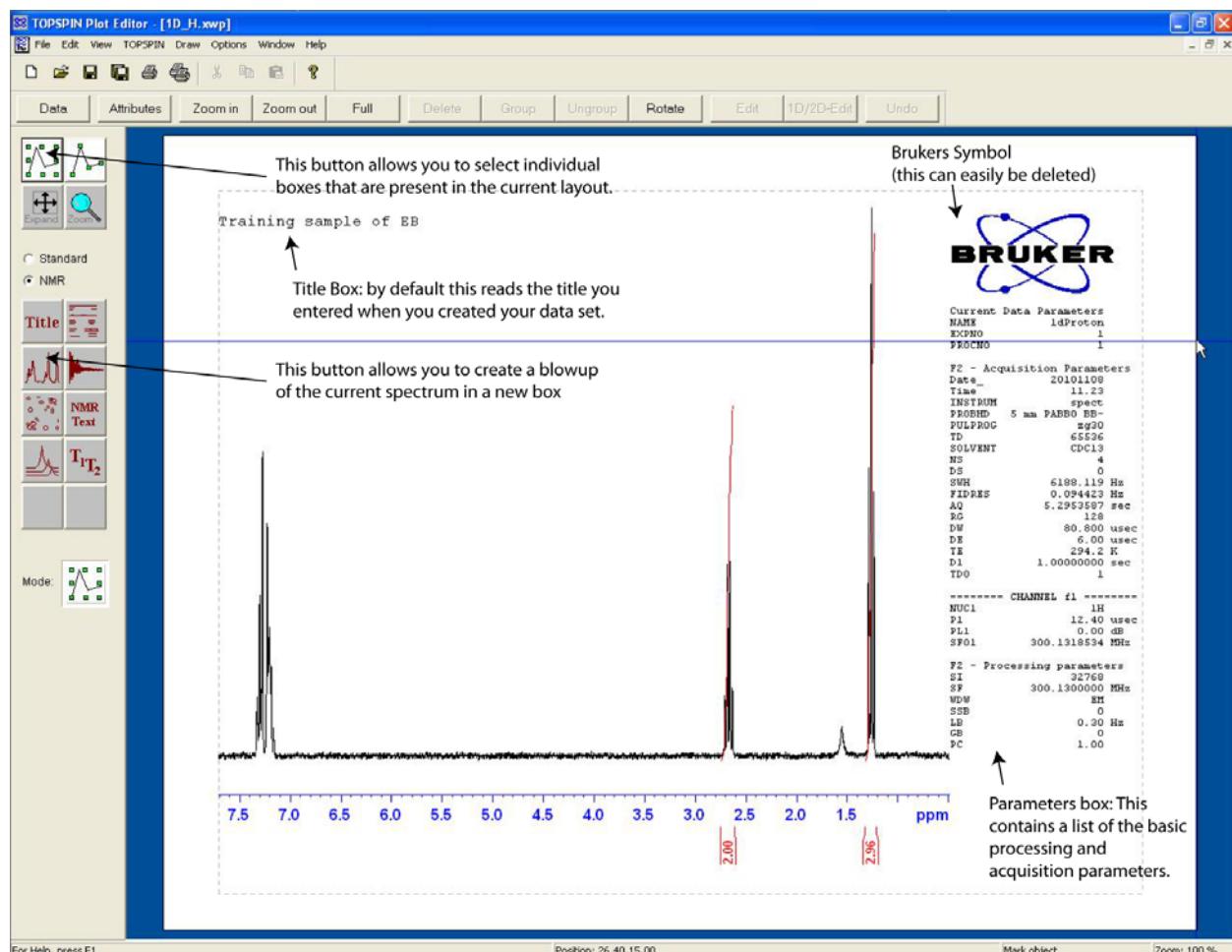


Figure 27 The default plot editor layout

Users can select any of the spectrum boxes and then right click to bring up a sub-menu that is very useful in manipulating how the plot layout and the spectrum are displayed. Figure 28 shows the main spectrum box selected and includes the right-click sub-menu.

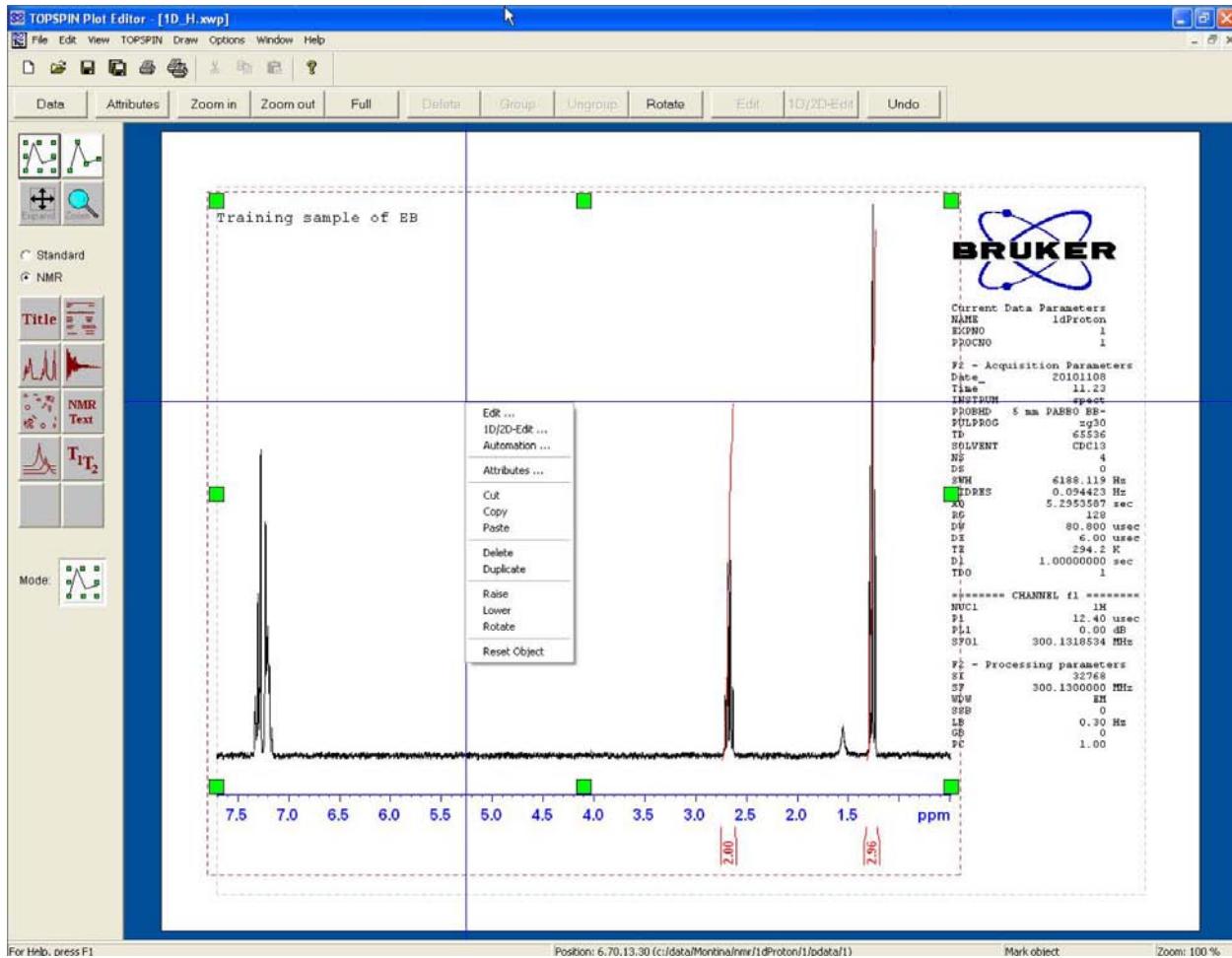


Figure 28 Right click sub-menu for the Plot Editor

Left clicking on edit in the sub-menu will open up a separate box that contains a Graph and 1DSpectrum tab. The graph tab (Figure 29 left) allows the user to manipulate properties of the scale and axis, while the 1DSpectrum tab (Figure 29 middle) allows users to change the way in which the integrals and peak labels are displayed. Left clicking on 1D/2D-Edit in the sub-menu will open up an “Edit Display Object” box (Figure 29 right) that will allow the user to manipulate and expand the actual spectra found within the selected box. User should spend time with each of these sub-menu options in order to become familiar with their capabilities.

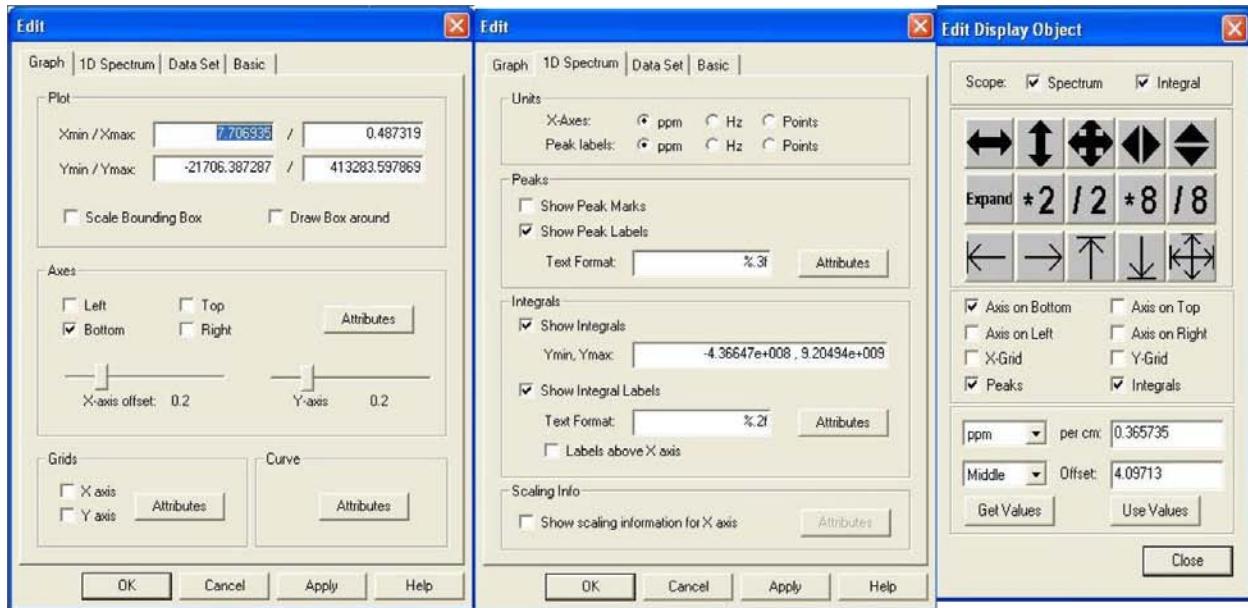


Figure 29 The Graph (left) and 1D Spectrum (middle) tabs of the Edit option from the sub-menu, as well as the Edit Display Object box (right) from the 1D/2D-Edit option of the sub-menu.

The four most important options of the file menu (Figure 30 A) of the Plot Editor are: “print”, “save as” (Figure 30 B), “open” (Figure 30 C), and “export”. These are summarized below:

Print: A user can print the spectrum layout shown in the Plot Editor at anytime by left clicking on the “print” option under the file menu.

Save as: Each user will spend a significant amount of time changing the default plot editor layout to their preference. The changes you have made to the layout can be saved by left clicking on the “save as” option under the file menu. This will bring up the save as box shown in Figure 30 B. This box allows users to save the changes they have made to the default layout to their own personal layout file. This personal layout file should be saved under the users own data directory. The data directory for each user can be found by navigating the menu to find the “C:\data\” folder and then opening up the folder corresponding to your user name. For example, I would save my new layout to “c:\data\Montina\” and give it whatever name I would like, such as 1H_layout.

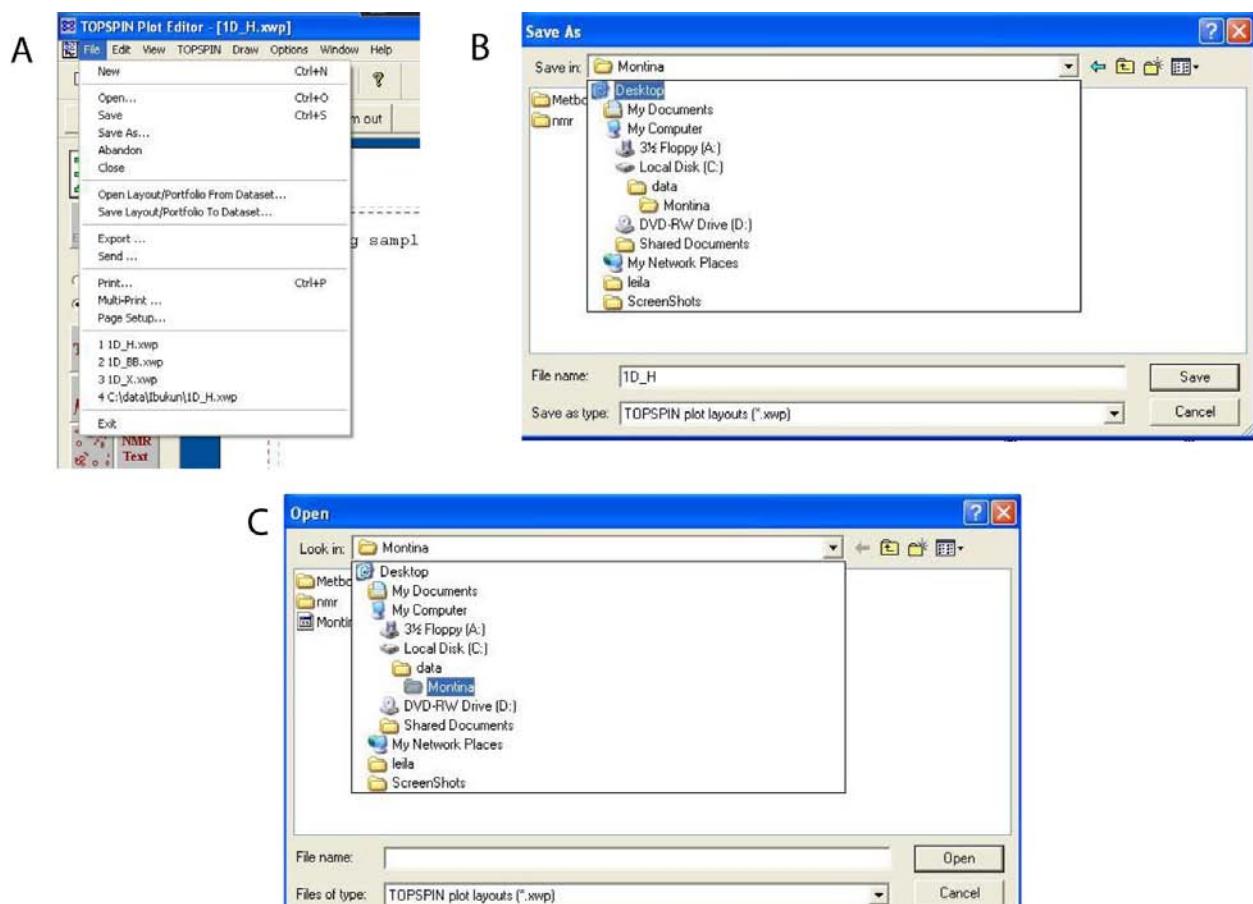


Figure 30 (A) The file menu of the plot editor; (B) The save as (layout) option of the file menu; (C) The open (layout) option of the file menu.

Open: The next time a user opens the plot editor they can left click on the “open” option of the file menu and navigate to their data directory and open their own personal layout (Figure 30 C).

This will save each user a significant amount of time when using the Plot Editor tool.

Export: Lastly, a user can save the Plot Editor output as a pdf, png, or ps file by left clicking on the “export” option in the Plot Editor File menu. Users should save the exported file under the dataset corresponding to the file. For example, I would save the Plot Editor pdf output of the 1dProton dataset show in Figure 5 to the folder: “c:\data\Montina\nmr\1dProton”.

(24) Now that you have processed, acquired, printed, and saved your data you can remove your sample from the magnet. Sample removal is explained in the following steps:

- (a) Re-open the BSMS panel by left clicking on “BSMS Panel” in the NMR step-by-step menu.
- (b) Left click on the lift button of the main tab in the BSMS panel (see Figure 6). This will cause a gentle stream of air to lift your sample out of the probe and your sample should re-appear at the sample port (top of the stack).
- (c) Remove the spinner and your sample from the sample port. You should hear a gentle stream of air coming from the sample port once your sample is removed
- (d) Left click on the lift button of the main tab of the BSMS panel. NOTE: After this step you should no longer hear a gentle stream of air coming from the sample port.
- (e) Remove your sample from the spinner and place the spinner back in the guide.
- (f) Place the dust cap back on top of the upper stack to prevent dust from entering the probe.

(25) Close your data set and leave Topspin open for the next user. NOTE: Your sample should leave the lab with you! No samples should be left in the 300 MHz NMR lab.