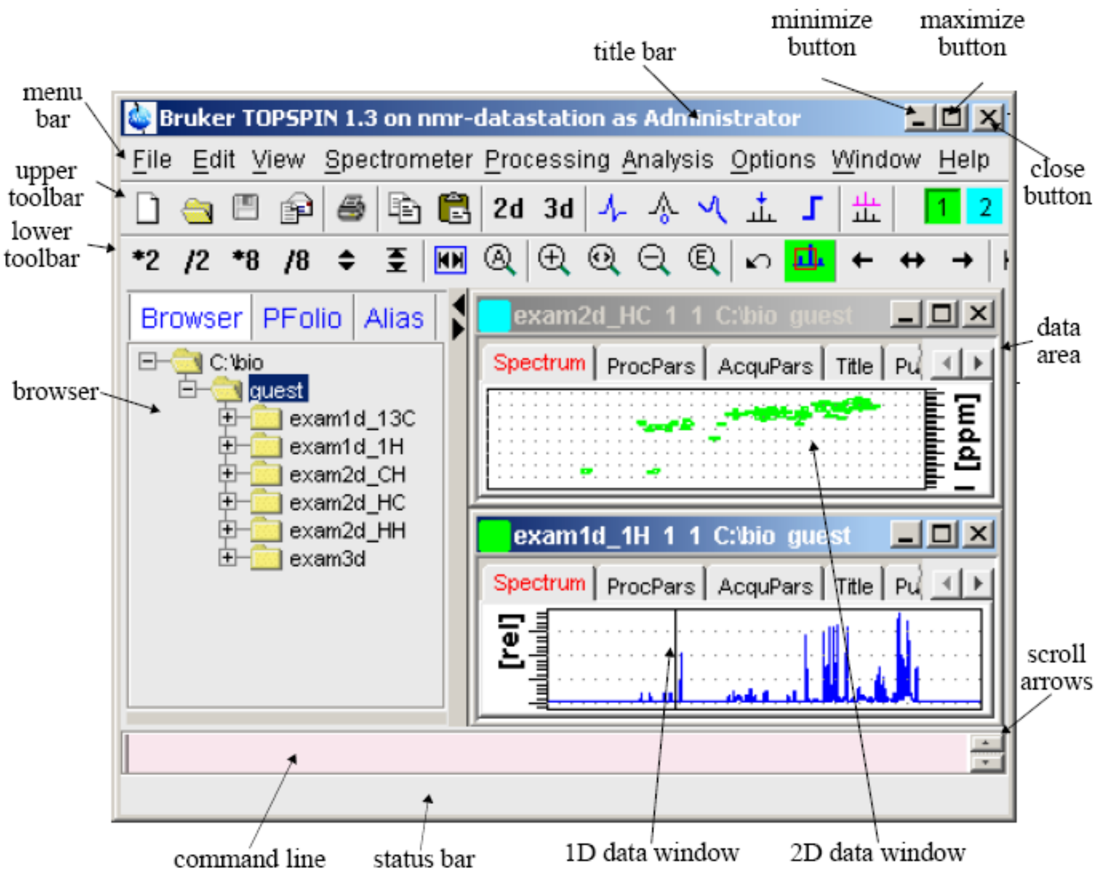
MRL Spectroscopy Facility



<u>500 MHz Solution-state NMR Procedure</u>

(Bruker AVANCE Machines running TopSpin under WINDOWS XP)

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Safety Issues

⚠ If you have metal implants, DO NOT do NMR yourself;

⚠ Take everything ferromagnetic or vulnerable to magnetic field, such as mechanic watches, cellular phones, keys, credit cards, bank cards, tapes, computer disks, etc., out of your pockets and put them somewhere away from magnets;

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I. Facilities Billing System (FBS)

To Schedule time and to use the instrument computer a FBS account is required. After training you will receive an invite for FBS or if you already have a FBS account the 400MHz DNP calendar will be added to it.

The instrument time is billed through FBS. Your FBS time = your recharged time. Recharge is calculated at an hourly rate. For current recharge rates go to the MRL website, http://www.mrl.ucsb.edu/sites/default/files/mrl_docs/rechargerate.pdf

- To begin an instrument session, you must log onto FBS first and either click on Start Timer if a reservation has already been made or selec Walk-Up .

  To do so use the FBS designated computer in the lab or any internet connected device, and navigate to http://ucsb.fbs.io

- Now you can log onto your account on the instrument computer. Remember to log off your computer account when finished.

- Once the session is finished you must log into your FBS account and click Stop Timer This will stop your FBS time. If you do not do this you could incur extra charges.

- The paper log sheet by the instrument is used as back up for the FBS system. Remember to mark you time, recharge number, and any notes or problems you would like to convey, feel free to use as many lines on the page as needed to be clear.

⚠ No shows will be charged **75%** of the scheduled time. If you cannot use your reserved time cancel it.

⚠ FBS records the billable time as the longer time between the scheduled time and the time used. So if your scheduled time is longer than the actual time on the instrument then the scheduled time will be charged.

II. Sample preparation

1. NMR tubes of **5mm** in OD (typically 7" long) are used and available from:
   i. Chemistry Department Stockroom, UCSB (Phone: x2107)
   ii. Aldrich (Phone: 800-558-9160)
   iii. Wilmad (Phone: 800-220-5171).

      Please get tubes for 500MHz or higher.

2. Samples are dissolved in **deuterated** solvents for three purposes:

      Preferred concentration: >0.1 mM and >50mM for ^{1}H and ^{13}C, respectively, and sample volume: > 0.5 ml or >4 cm in height for 5 mm tubes.

   i. Deuteration removes solvent ^{1}H signals which would otherwise dominate the ^{1}H spectrum.
   ii. Deuterons provide a lock signal.

      Lock is a deuterium NMR process that the spectrometer uses to prevent the magnetic field from changing during the course of nmr experiments, thus locking the spectrometer.

   iii. Deuterons provide an internal reference for the spectra of ^{1}H, ^{13}C, ^{29}Si, ^{31}P, etc., rendering addition of reference standards such as TMS unnecessary.

      Label your samples with your name and your advisor's name. This helps us take care of unknown samples.

III. Sign onto Logsheet

Enter

1. your name
2. your advisor's name and department
3. your recharge account number (in the format: 8-4xxxxx-xxxxx-3)
4. your start time
5. (**Do this at the end of experiment:** your stop time and duration of experiment)
6. (**Do this at the end of experiment:** Status of instrument and report problems if any)

IV. Start TopSpin Software

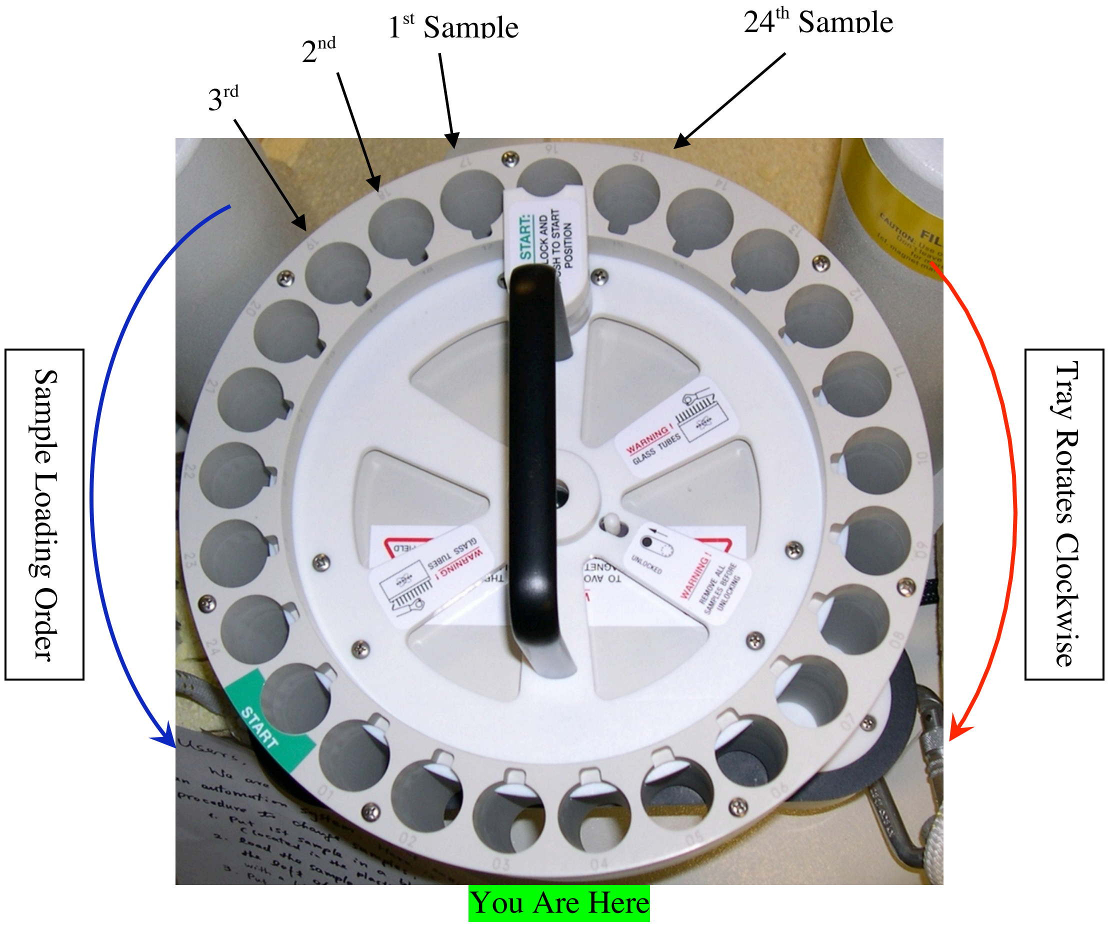
1. Make sure that the spectrometer is idle by looking at the computer. If yes, proceed to Step 2 below (if no, either wait, talk to the user on the machine, or do something else).
2. Login into the WINDOWS computer:

   Type your username, hit the Tab key (Don't use return here!)
   Type your password, hit return

3. Double click the TopSpin icon  on the desktop, the last dataset from your previous login session will appear.

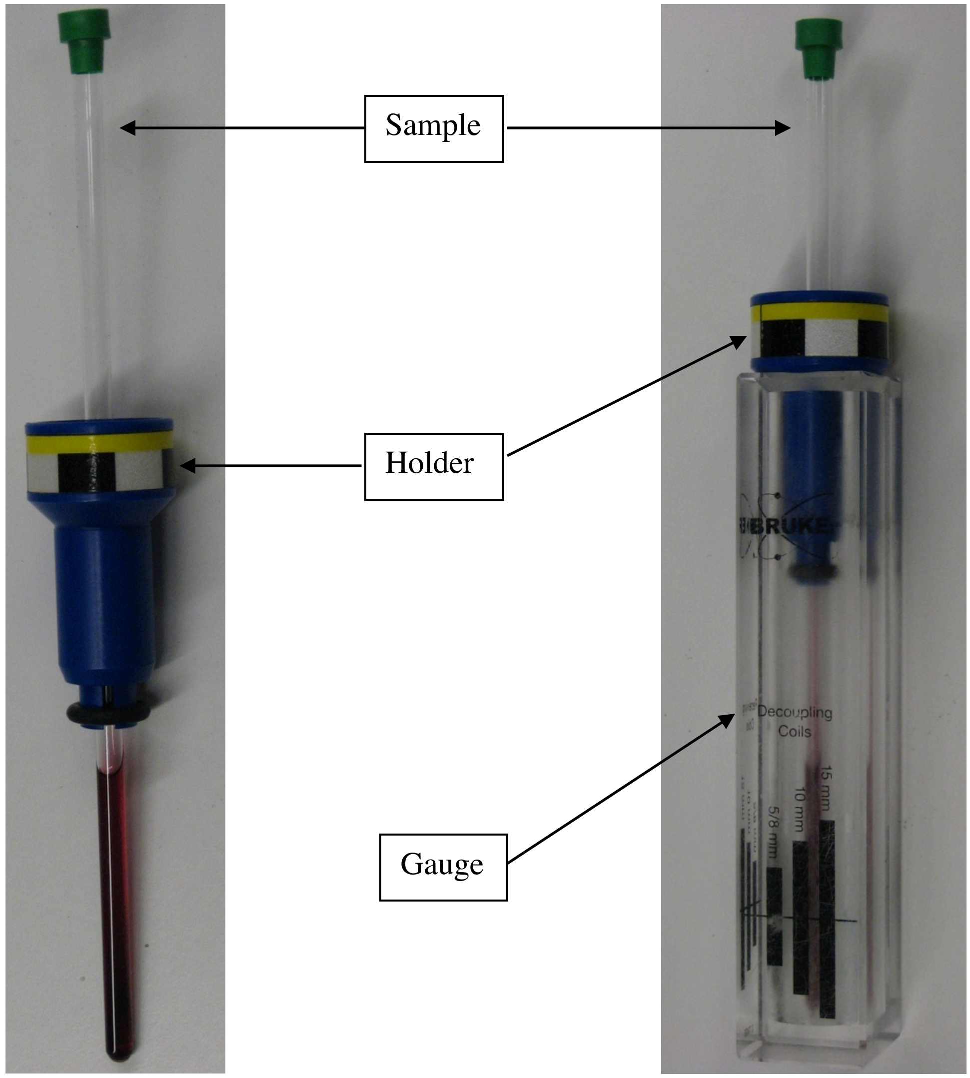
   Power of right-click provides more functions and options. If you cannot find something you want, try the right mouse button.

V. How to Load/Change Samples



The 24-slot sample tray

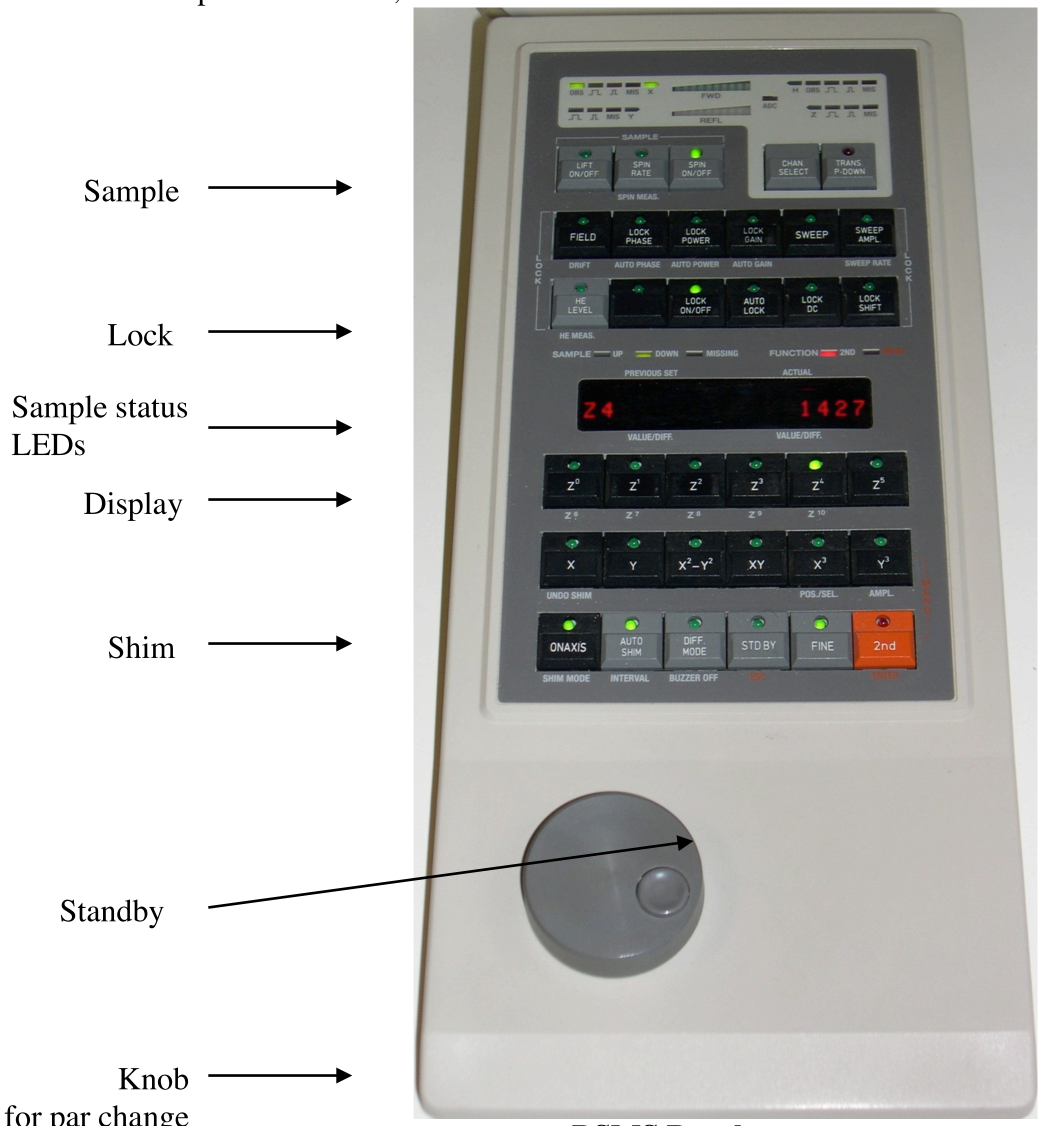
1. Load samples onto the sample changer as below:
   i. Put samples in the blue spinners, measure depth with the depth gauge.



ii. Clean the bottom half of sample tube with napkin while holding the top half;
iii. Load them in the slots sequentially to the left of the white plastic piece with words "TO START:", starting with the first slot.
iv. Please check that all samples have been correctly loaded and record the position of samples.

The rotation of sample tray is controlled by air either manually or automatically, depending on whichever mode is set and used. The rotation is clockwise when viewed from the top.

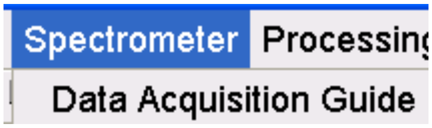
2. Push “Lift ON/OFF” button on the BSMS panel. The CDCl3 sample will come up and the sample tray will rotate. The 1st sample will be at the position to load and descend to the top of shim stack;



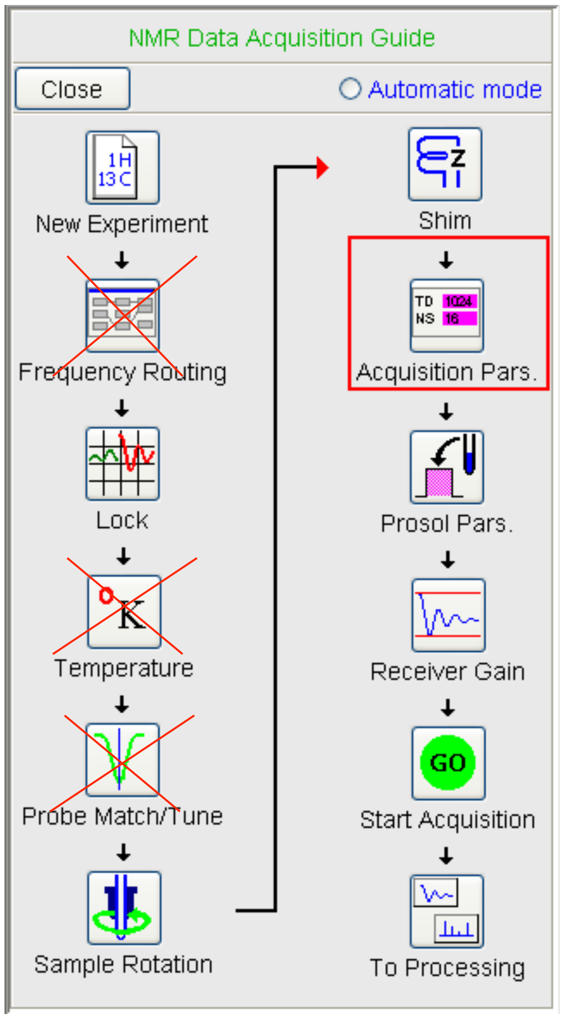
BSMS Panel

3. After ~10s (or when you don’t see the sample), press “Lift ON/OFF” button on the BSMS panel again to let the sample go down into the magnet. Wait for 10~15s for sample status LED to show DOWN (green);
4. Place the D_2O sample in the next available slot following your sample(s), which would be the 1st slot if you have only one sample to run;
5. Run experiments using the procedure below for the 1st sample;
6. Once done with the 1st sample, repeat steps 2, 3, and 5 for the next sample and other samples if available;
7. Once done with all samples, do steps 2 and 3 to load the D_2O sample, and lock the magnet for wrap-up as described in “Finishing up” at the end of the procedure.

V. ^{1}H-NMR Setup and Data Acquisition

Click 

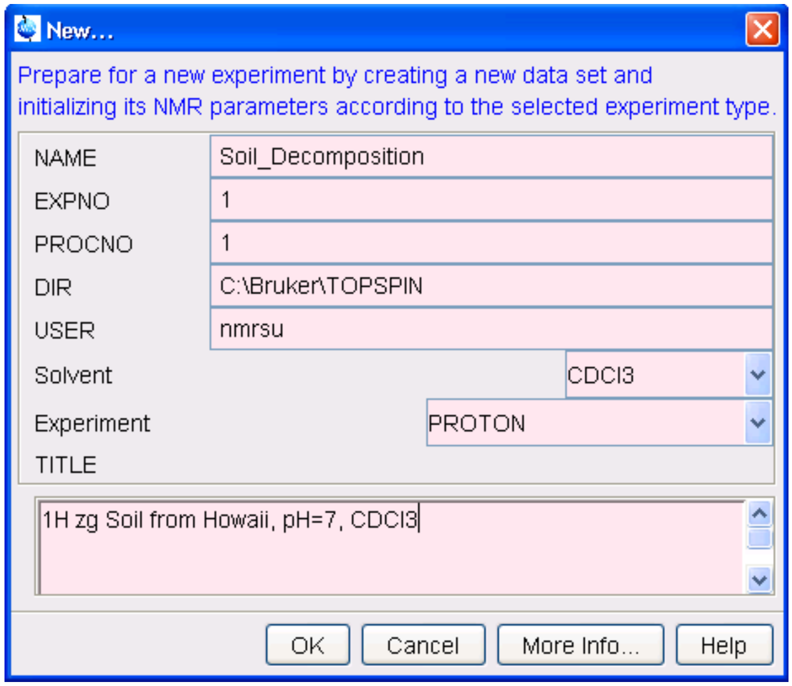
, the flow chart below appears at the right side of the data area:



The guide will walk you through the data acquisition process interactively.

1. Click on **New Experiment** 

(or *File* → *New*) [***New***] (Words in brackets are the corresponding commands): a window pops up where you can

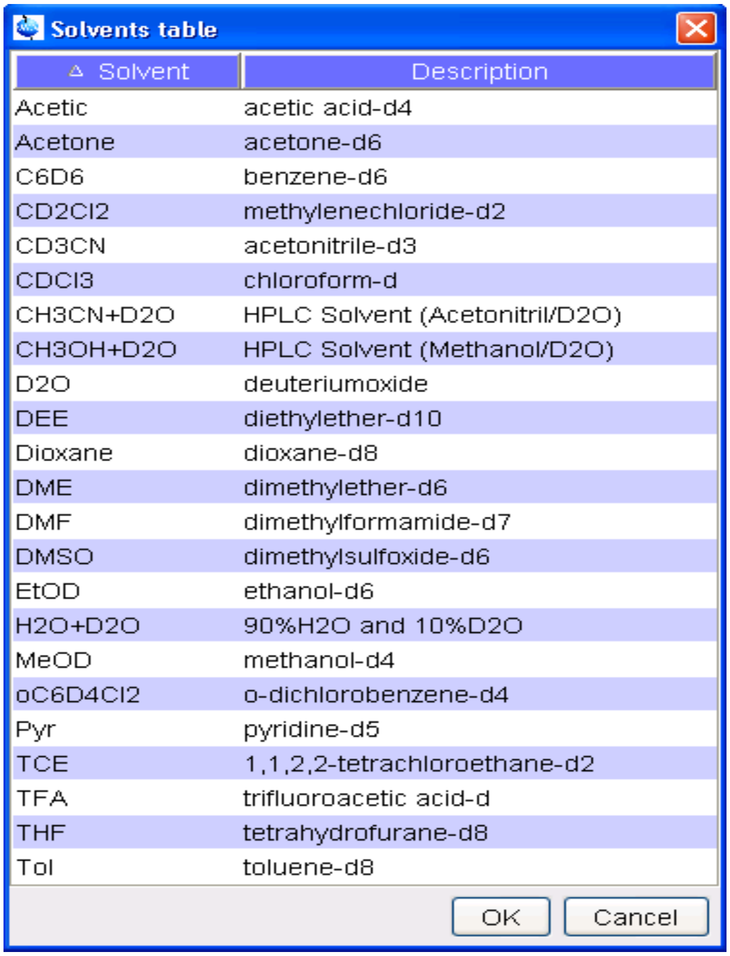


Name*:	Soil_Decomposition (e.g.)	(meaningful or descriptive)
EXPNO*:	1	(start with 1)
PROCNO:	1	(start with 1)
DU:	C:\Bruker\TOPSPIN	(don't change)
USER:	(your loginname)	(e.g. ssmith)
Solvent:	(leave it alone b/c it will be set when locking)	
Experiment:	e.g. PROTON or choose "Use Current Params" if you want to run the same experiment as the current data in display[$].	(experiment you want to do)
Title:	(any information useful for current sample, project, and/or experiment)	

*: IF YOU DO NOT CHANGE EITHER THE NAME OR THE EXPNO OF YOUR DATASET, YOU MAY OVERWRITE YOUR OLD DATA AND LOSE IT FOREVER.

$: To display an old dataset, go to the browser on the left, find and right-click on the desired dataset, and choose "Display" or "Display in a New Window".

2. Click on **Lock**, which opens a solvents table. Select the solvent for your sample followed by ***OK***. Wait for 1-2 min until you see that the Lock ON/OFF button on BSMS panel stays steady and a flat line (maybe noisy) sweeps back and forth in alternative red/green colors near the top of the lock window below. [See Appendix A: Lock troubleshooting].

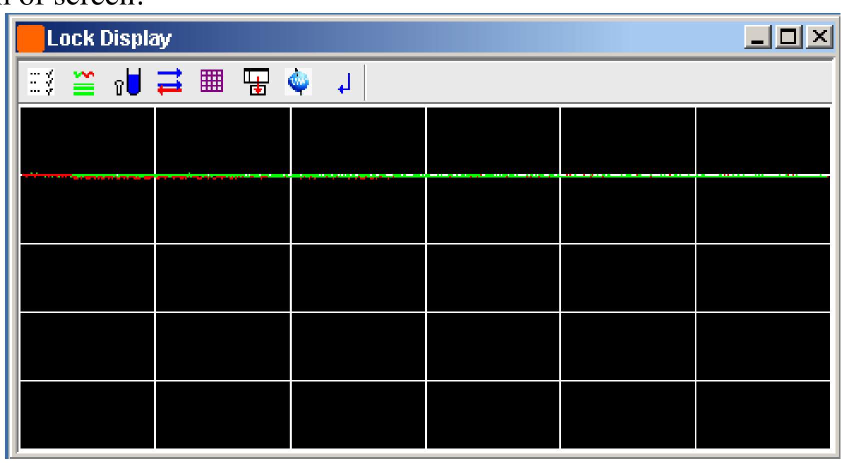


Solvents table

Solvent	Description
Acetic	acetic acid-d4
Acetone	acetone-d6
C6D6	benzene-d6
CD2Cl2	methylenechloride-d2
CD3CN	acetonitrile-d3
CDCl3	chloroform-d
CH3CN+D2O	HPLC Solvent (Acetonitril/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
oC6D4Cl2	o-dichlorobenzene-d4
Pyr	pyridine-d5
TCE	1,1,2,2-tetrachloroethane-d2
TFA	trifluoroacetic acid-d
THF	tetrahydrofurane-d8
Tol	toluene-d8

OK Cancel

3. Double click the small lock display screen to open the large lock display window. If it is not in the front, bring it up by clicking on the corresponding icon at the bottom of screen.

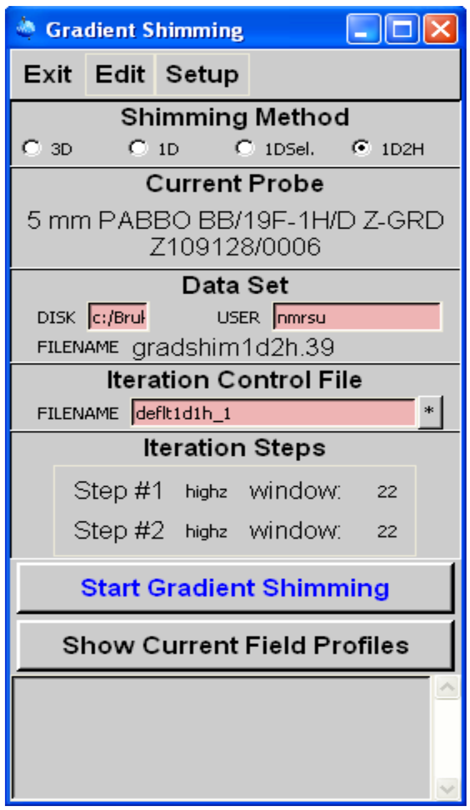


4. Check sample rotation (Don't Click on **Sample Rotation** , just consider it a checklist item): Make sure Sample Rotation is ON.

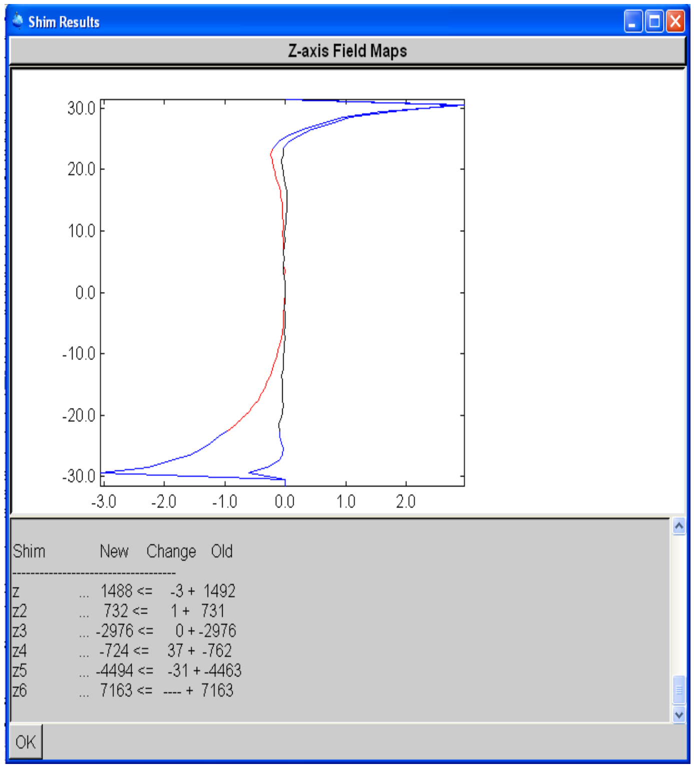
   Press the "**Spin ON/OFF**" button on the BSMS panel to toggle sample rotation ON and OFF. When turning on, the button will be lit flashing first and then becomes steady in about 15 s.

5. **Gradient shimming** (for manual shimming "by-hand" see Appendix B)

   a) Click on **Shim** **and choose "Gradient Shimming"**



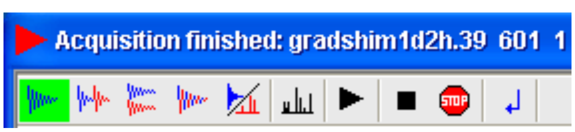
   b) Click on Start Gradient Shimming to start gradient shimming, which will shim the sample twice. Please wait for 2 – 3 min. until you see the shimming map below.



The X axis is the field strength felt by the subvolumes of sample at different positions along the tube axis. Only the bottom 40 mm of the sample is in effect, with -20 being the bottom, +20 the top, and 0 the center. Obviously, the more straight the vertical lines, the better the shimming. Normally the 2nd shimming improves the sample field homogeneity over the 1st one, as indicated by the grey curve and red curve, respectively.

c) Click on 

to close the “Shim results” window, on 

to close the “Gradient Shimming” window, and on the last button  on 

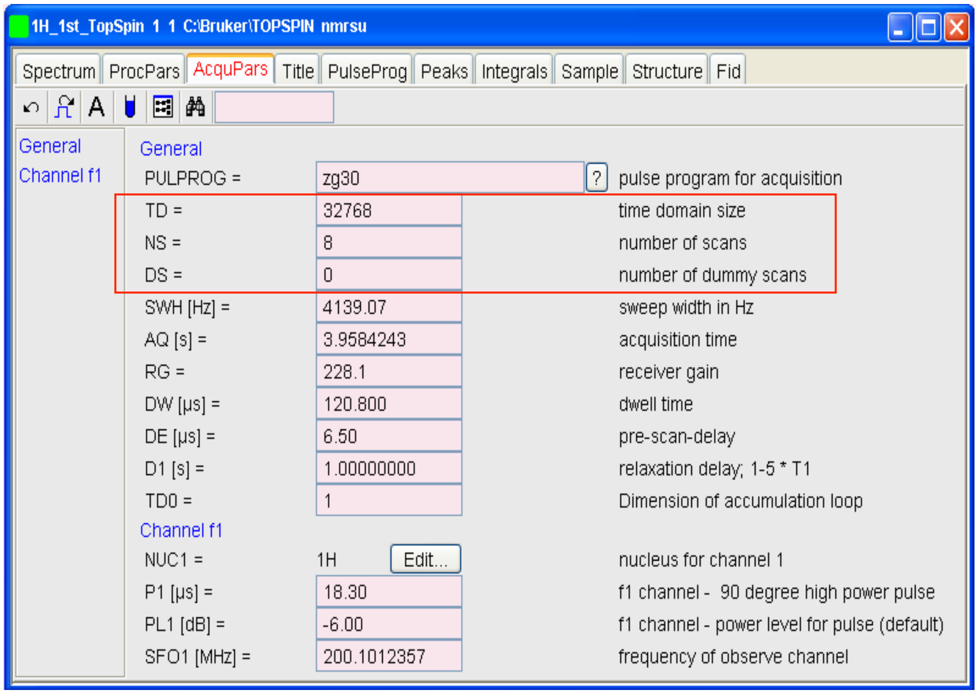
to close the gradient shimming dataset.

d) Now you’re done with shimming and it is time to proceed for experiments.

Please see Appendix B: Manual Shimming for how to shim manually..

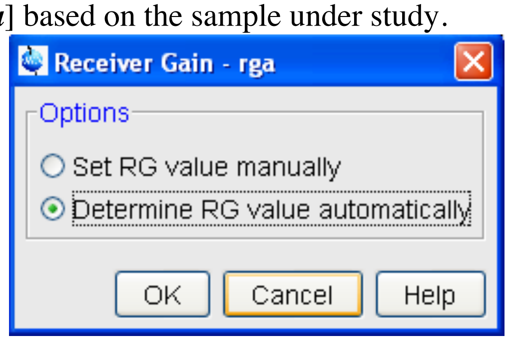
6. Click on **Acquisition Pars**  [***ased***] for modification of parameters. Set "*TD*" = 32k, "*NS*" = 8 and "*DS*" = 0 for simple ^{1}H experiments for practical samples.

    Please refer to [Appendix]() for simple spin dynamics of one-pulse FT-NMR.



7. Click on **Prosol Pars.**  to read the prosol (instrument dependent) parameters (Power levels, e.g. "*PL1*", and Pulse lengths, e.g. "*P1*").

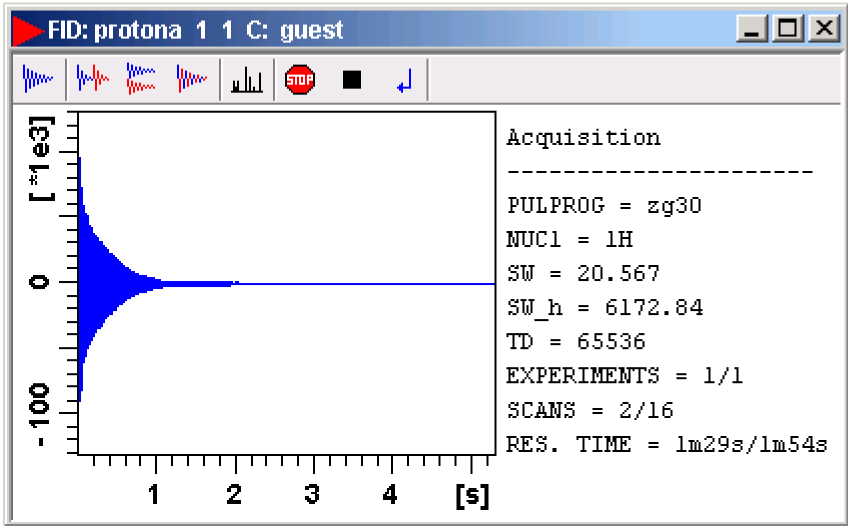
8. Click on **Receiver Gain**  and set Options to "Determine RG values automatically" [***rga***] based on the sample under study.



   After ***OK*** is clicked, wait for message "rga finished" in the status bar of TopSpin window to occur, indicating completion of rg setting.

9. Click on **Start Acquisition**  [*zg*]. (: If a warning message occurs, make sure to start acquisition in the right dataset). The FID display window below will appear along with some status parameters.

(If parameters are not shown, right-click in the FID window, choose "Display Properties", check "Status Parameters" and click on ***OK***).



Three of the buttons near the top of the window may be used to:

Stop the acquisition [***stop***] with the data in buffer discarded

Halt the acquisition [***halt***] with the data in buffer saved to disk

Close the FID window

To monitor the acquisition status, just look at the Status Parameters to the right of FID. When Res. Time = 0, acquisition is completed and it is time to process the raw data. Or look at the bottom right of the Topspin status bar.

Click to close the FID window once acquisition finishes.

VI. Finishing Up

YOU ARE NOT FINISHED WITH THE SPECTROMETER UNTIL YOU DO THE FOLLOWING.

a. Make sure the D_2O sample is in the first slot of sample changer.
b. Press "Lift ON/OFF" button on BSMS panel to eject your sample and rotate the D_2O sample to the loading position.
c. In ~10s, hit "Lift ON/OFF" button on BSMS panel again to load the D_2O sample.
d. Wait for ~15s, click on LOCKD2O to lock the spectrometer on the deuterated solvent and reset shim.

   To lock, a dataset has to be displayed.

e. Quit lock window by pressing "Quit" or clicking X of the lock window.
f. Exit TopSpin by clicking the X at the top-right corner of the software.
g. Logoff your account: click Start (bottom-left corner) and choose logoff followed by "Yes".
h. Important: On the logsheet, record your stop and duration times, and the spectrometer status. Report problems if any.
i. Important: remove all your samples from the tray and the NMR lab, and clean the lab space you have used.

VII. Data Processing Workstation

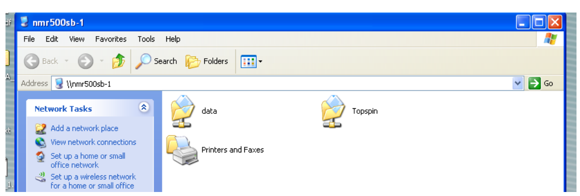
- Please go to NMR Processing Room (CNSI Room 1522) and use the computer denoted "Data Processing Workstation #1 or #2"

On the NMR Data Processing computers (WINDOWS 10, 128.111.243.67 or 68)

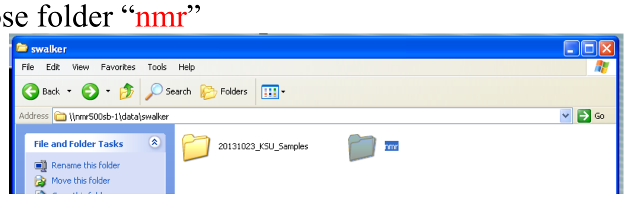
1. Login
   a. Type "nmr_user" as a username and input password "4epp967"
2. Connect to NMR Data
   a. Click on "Start" at bottom left corner
   b. Choose "RUN"
   c. Type the desired machine name:
      i. Choose "\\Nmr500sb-1" for solution state 500 MHz
      ii. Choose "\\Varian-nmr" for solution state 600 MHz
      iii. Choose "\\Nmr500wb-1" for solid state 500 MHz
      iv. Choose "\\Ipso400wb" for solid state 400 MHz
      v. Choose "\\Swbmri" for solid state 300 MHz
      vi. Choose "\\Nmr800sb" for 800 MHz

3. Login using your own NMR account

4. A window will pop up displaying your NMR data folder (indicating a successful connection)

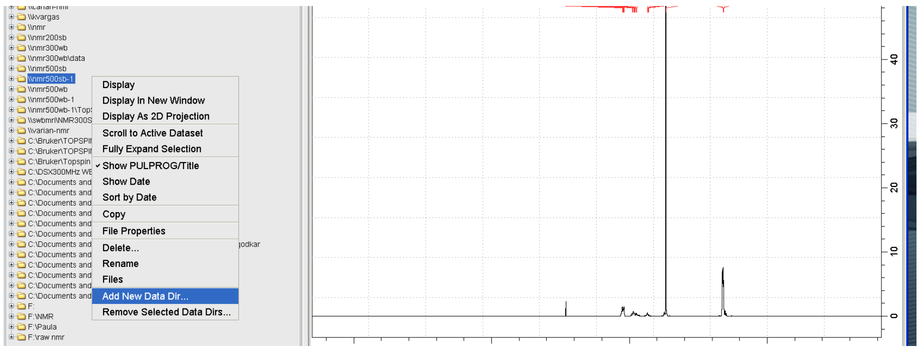


5. (OPTIONAL) Data Back-Up
   a. Go to folder: "Data"
   b. Find your personal directory and double click it
   c. Choose folder "nmr"

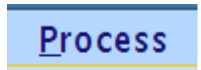


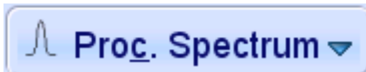
d. Now you can drag and drop the raw data files onto a flash/USB drive

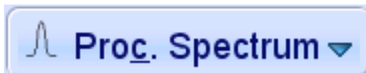
6. Data Processing using TopSpin™
   a. Double click on "TopSpin 2.1" on desktop
   b. Find your desired machine name (e.g. "\\nmr500sb-1" in the TopSpin™ browser panel on the left, expand it and navigate to your account folder
   c. (Optional) If you don't see your desired machine name (e.g. "\\nmr500sb-1" in the TopSpin™ browser panel on the left, add it yourself
      1. Right click in the browser panel area and choose "Add New Data Dir…"



      2. Type \\nmr500sb-1 or \\<IP address>
      3. Click "OK"
   d. Go to the dataset of interest by expanding \\nmr500sb-1 and find your account name
   e. Right click on the dataset and choose "Display"
   f. Begin processing your NMR Data – please refer to the relevant NMR Procedure Manual

VIII. NMR Data Processing 

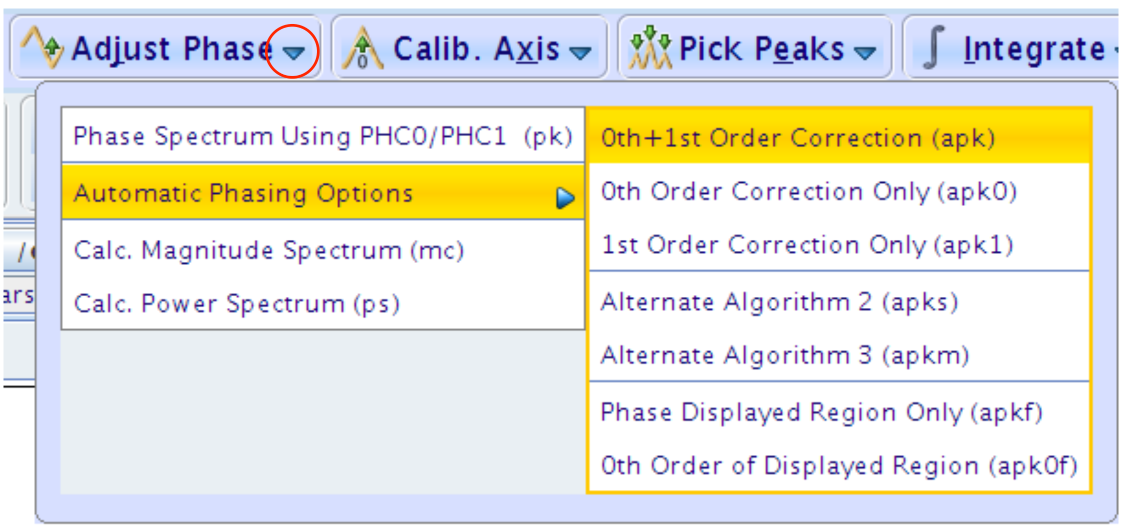
1.1. 

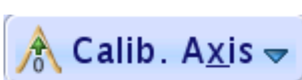
Click 

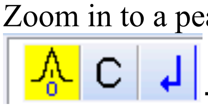
(this will execute window function, fourier transorm, and auto-phase)

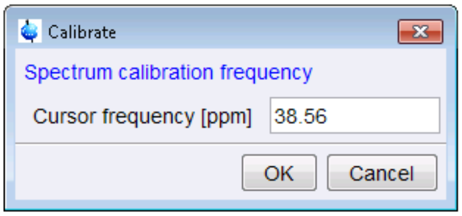
1.2. 

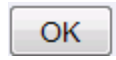
Auto-phase by clicking the drop down arrow → "Automatic Phasing Options" → "0th+1st Order Correction (apk)"



1.3. 

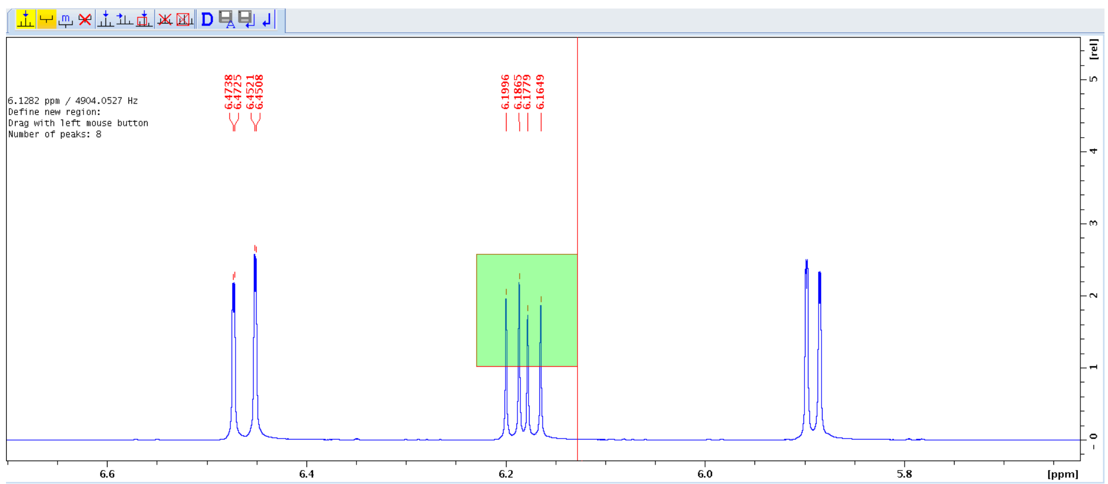
- Zoom in to a peak of known chemical shift →  →  → Click on the top of the peak →



→ 

1.4. 

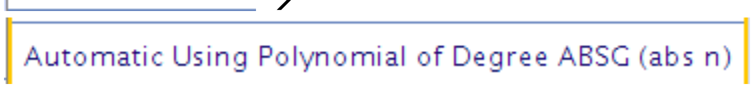
→ Activate  with LMB if necessary (Yellow means active) → Click-Hold-Drag LMB to pick peaks →



→ If necessary, use  to modify the green boxes →  to save the picked values.

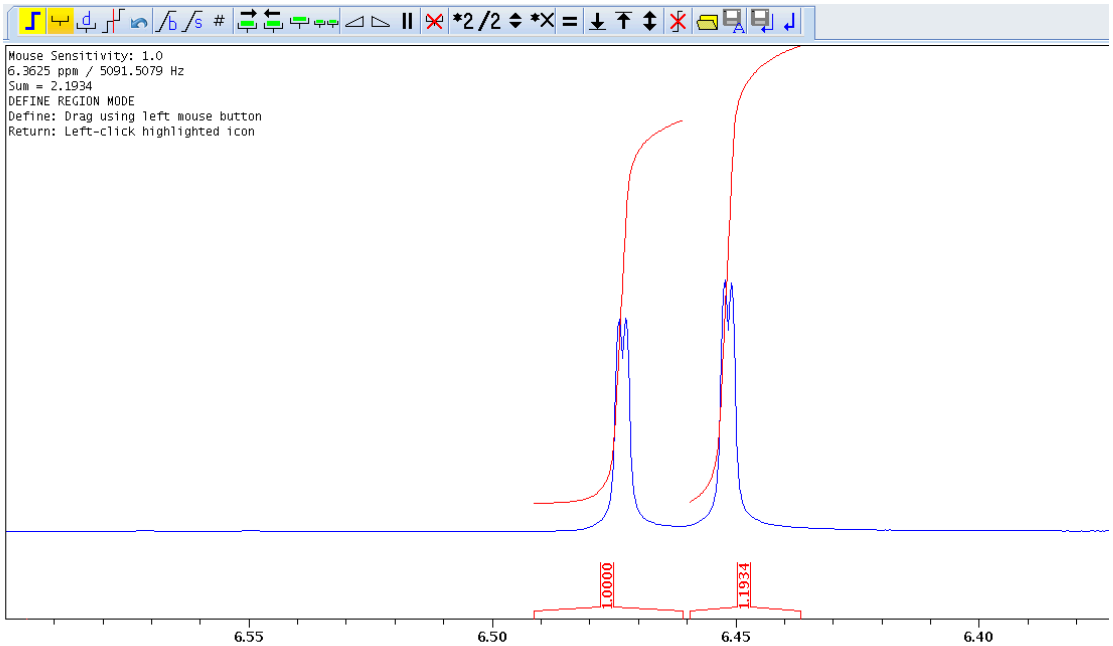
1.5. 

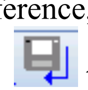
→



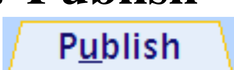
1.6. 

→ Activate  with LMB if necessary (Yellow means active) → Click-Hold-Drag LMB over regions to be integrated →



→ If necessary, calibrate integrals: right click on an integral to be used as reference, select Calibrate Current Integral, and input a calibration value →  to save the integral values.

IX. Publish



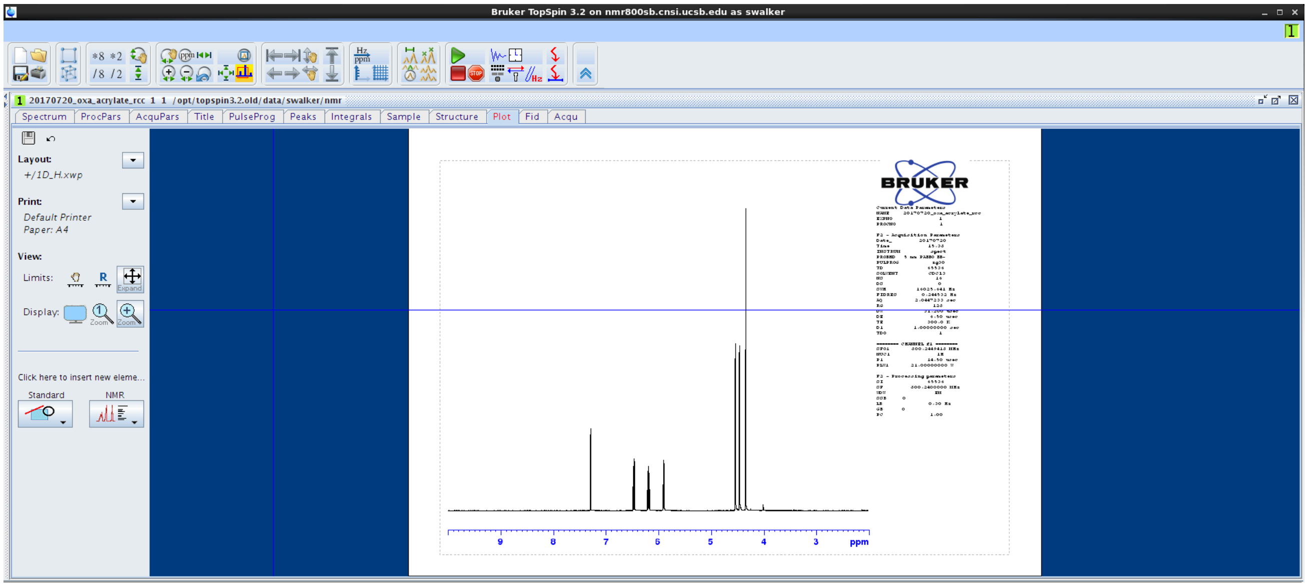
a. 

Print the active window.

b. 

Use the Plot Editor for more controlled printing.

→ Plot Editor opens:



**Click the drop-down arrow on “Layouts” and choose “Export”. Decide what file type you want to export as and add that at the end of your desired file name.

c. 

Save spectrum in .pdf, .png, and other formats.

*Click the TopSpin icon 

on the desktop to launch TopSpin
*Go to Menu Bar at the top and select: “Processing” → Choose: “Data Processing Guide”

**IMPORTANT: Wrap-Up

1. Quit TopSpin™ software
2. Logout of the “nmr_user” account

WARNING!! If you don’t logoff, your NMR data could be at risk!

X. Acquiring and Processing ^{13}C NMR (1H decoupled)

Sometimes, an 1D ^{13}C experiment may take hours or days depending on concentration (Recommended concentration is > 50 mM).

1. Go to *Spectrometer* → *Data Acquisition Guide* in the menu bar of TopSpin.
2. Click on **New Experiment** in the Guide window (see Procedure for ^{1}H NMR).
3. In the New … window, choose one of the standard parameter files for ^{13}C experiments below:

Par file	Spectral information
C13APT	CH+CH3 Positive, C+CH2 Negative (or vice versa), Intensity not quantitative
C^{13}C PD	all carbons POSITIVE, for quantitative analysis
C13DEPT135	CH+CH3 Positive, CH2 Negative, C Gone, Intensity not quantitative
C13DEPT45	CH+CH2+CH3 Positive, C Gone, Intensity not quantitative
C13DEPT90	CH+CH3 Positive, C+CH2 Gone, Intensity not quantitative

The first two experiments are the most popular ones. If only the carbon types are of interest to you, use C13APT. If carbon signals are going to be quantified, choose C13CPD.

4. Click on **Lock** in the Guide if you have not done so (see Procedure for ^{1}H NMR).
5. Click on **Probe Match/Tune** in the Guide, select "Automatic tuning / matching of ATM probe", followed by ***OK***. See Appendix I. Probe Tuning and Matching for Details.
6. Perform gradient shimming (see Procedure for ^{1}H NMR).
7. Click on **Acquisition Pars** (see Procedure for ^{1}H NMR) for modification of parameters. Set TD = 16k, NS = 64, DS = 0, and TD0 = 2000 for ^{13}C experiments.
8. Click on **Prosol Pars** (see Procedure for ^{1}H NMR).
9. Click on **Receiver Gain** (see Procedure for ^{1}H NMR) and set Options to "Set RG values manually". Input "16k" followed by return.

   To know how long the experiment lasts beforehand, click on in the upper toolbar.
10. Click on **Start Acquisition** (see Procedure for ^{1}H NMR).
11. Process data (see Procedure for ^{1}H NMR).

A few things to note:

- You don't have to wait until the experiment is done to process data. You can do processing as soon as the first NS scans are finished and saved to disk.
- A larger line broadening value is used for ^{13}C than for ^{1}H. Typically lb = 1 Hz.

12. Type "***halt***" if spectrum is satisfactory or let it continue if not. The acquisition will stop by itself after *NS*TD0* number of scans are completed.
13. For data processing of a ^{13}C spectrum, please refer to the procedure for ^{1}H NMR.
14. Finish up as for ^{1}H NMR.

XI. Appendices

A. Lock troubleshooting

Problem: "**Lock ON/OFF**" won't stop flashing and the lock signal stays at the bottom of the lock display window.

Possible reasons:

1. You started with a bad shimming file
2. Your sample is too concentrate, i.e. viscosity too high
3. Too little deuterons in your solvent

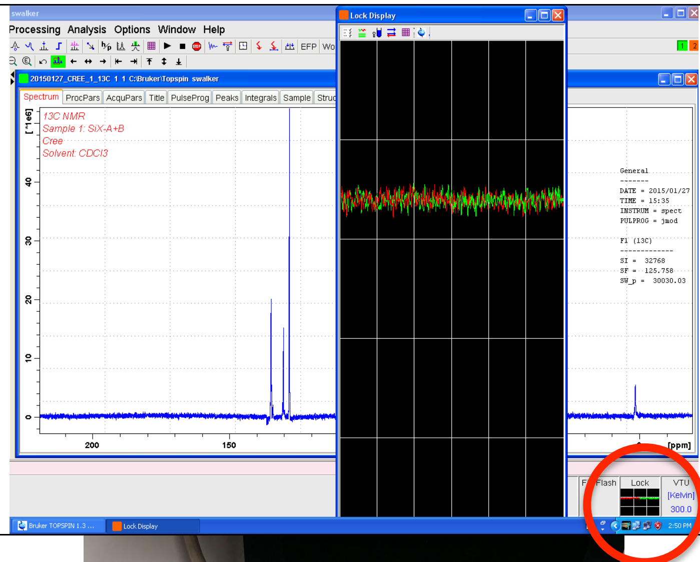
Solutions:

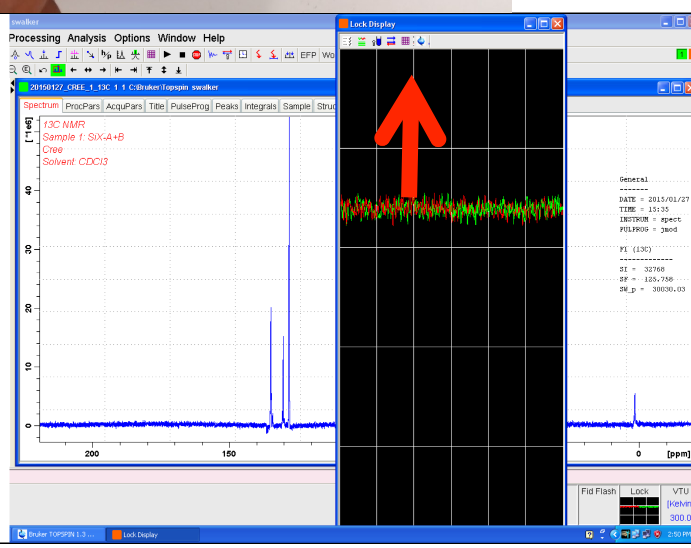
for 1. Type [***rsh***] in the command line to read in a good shimming file and lock again

for 2 & 3. Do manual locking as below:

- Press "**Lock ON/OFF**" to turn off lock (LED off). At this point, you may see a noisy and weak oscillatory lock signal.
- Hit "**Lock Gain**" and rotate the wheel on BSMS panel to increase lock signal amplitude until the signal fits the whole lock window.
- Then press "**Lock ON/OFF**" to try to lock spectrometer. The LED should stay solid after brief flashing if spectrometer locks (the oscillatory signal becomes a flat sweeping line). If not,
- Hit "**Lock Power**" and rotate the BSMS wheel to increase lock power. The lock signal will increase accordingly and may be saturated, i.e. the signal will not increase anymore with power. Remember: if the saturation happens, bring back the power by 2~3 units in reading.
- Then press "**Lock ON/OFF**" and immediately increase "**Lock Power**" until the spectrometer is locked, where the "**Lock ON/OFF**" LED should stay solid and the oscillatory signal becomes a flat sweeping line. Avoid saturation stated above.
- If you still have trouble, please ask Jerry/Jaya for help/debugging.

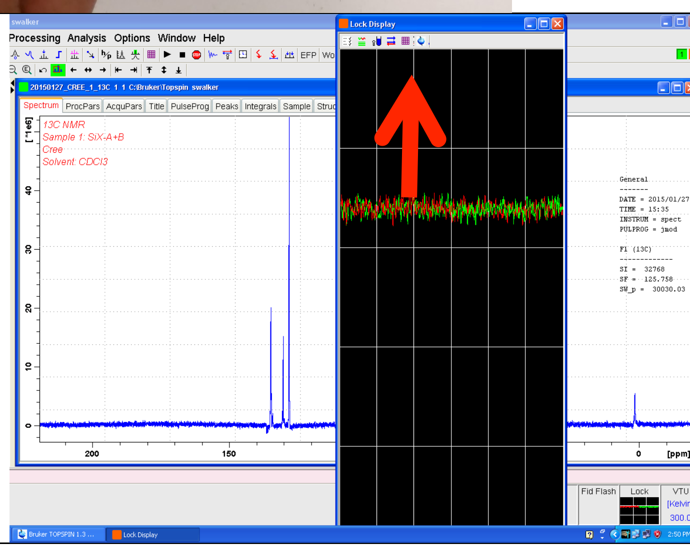
B. Manual Shimming

Click the small lock display (in bottom right corner of TopSpin) to open large lock display window	
Press Z1 on the BSMS panel to activate the Z1 shims	

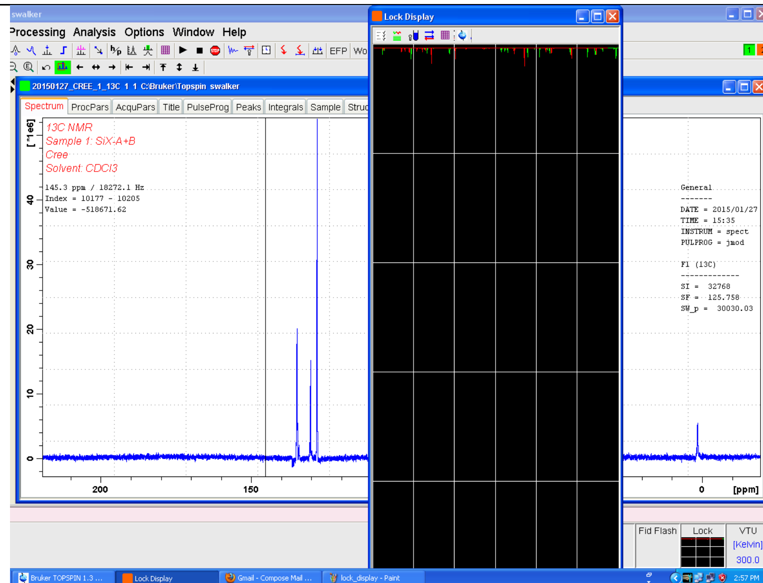
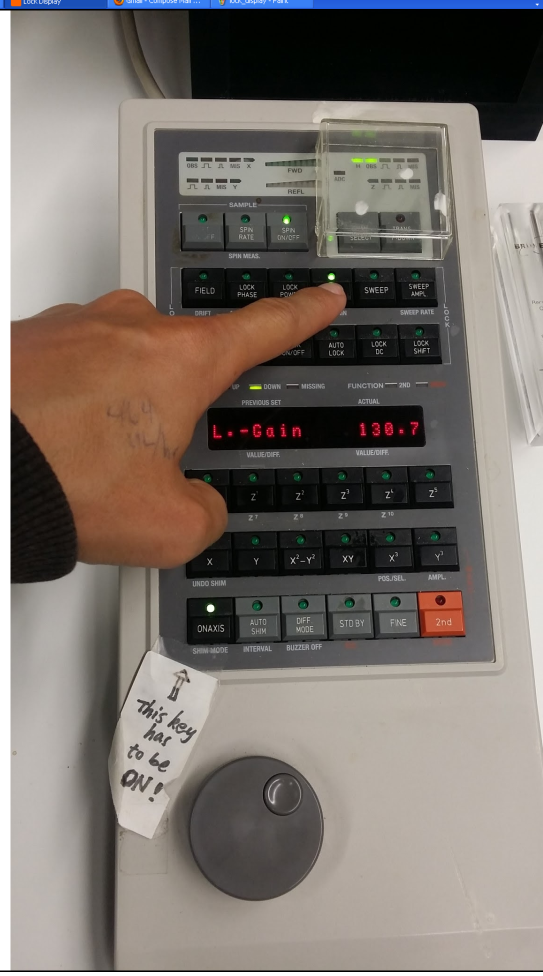
Turn the BSMS panel knob clockwise or counter-clockwise to increase the lock level.	
Turn the knob until you reach the maximum possible lock level.	

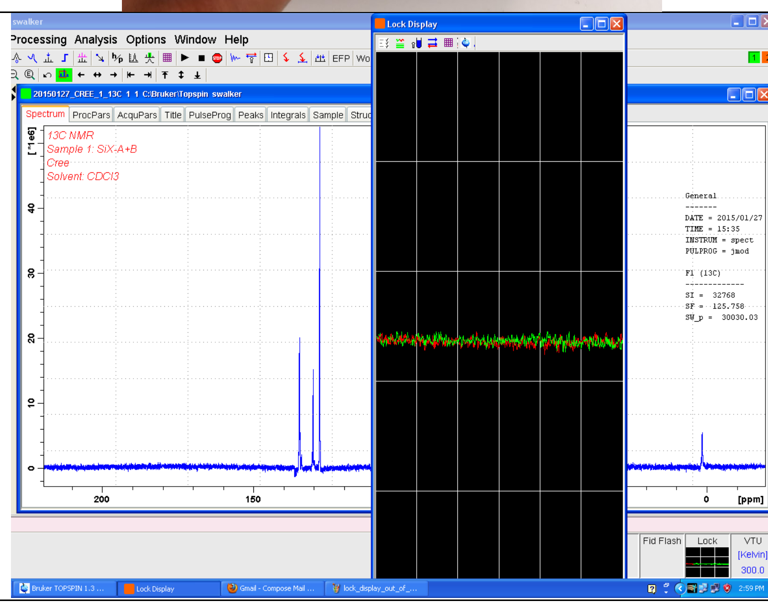
Then hit “Stand Bye” button to save values.	

Press the Z2 button on the BSMS panel to activate the Z2 shims	

Turn the BSMS panel knob clockwise or counter-clockwise to increase the lock level.	
Turn the knob until you reach the maximum possible lock level. (If the lock display goes out of view…see the last page of the manually shimming instruction)	

Then hit "Stand Bye" button to save values.	
NOW YOU ARE FINISHED SHIMMING! ☺	(please see next page if your lock value left the lock screen while shimming on Z1 or Z2)

<table>
<tr>
<td>Did your lock value leave the screen while shimming on either Z1 or Z2? If so, please look at the following directions:</td>
<td>
</td>
</tr>
<tr>
<td>Press the “lock gain” button on the BSMS panel</td>
<td>
</td>
</tr>
</table>

Turn knob COUNTER CLOCKWISE to decrease the lock gain and your lock value will reappear	
Continue with shimming of Z1 and/or Z2 if you have not maximized the lock value yet.	

C. Phase 1D Spectrum Manually

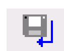
Phase the spectrum (make all peaks absorptive)

i. Click on  button (in the upper toolbar) and the toolbar above your spectrum should look like this:

ii. Move the red cursor line to the tallest peak, right-click there, and select Set Pivot Point to mark the peak with a vertical red line, indicating a pivot point to be used by Ph1. Right-click again and choose "Calculate Ph0". The tallest peak will now be phased roughly right.

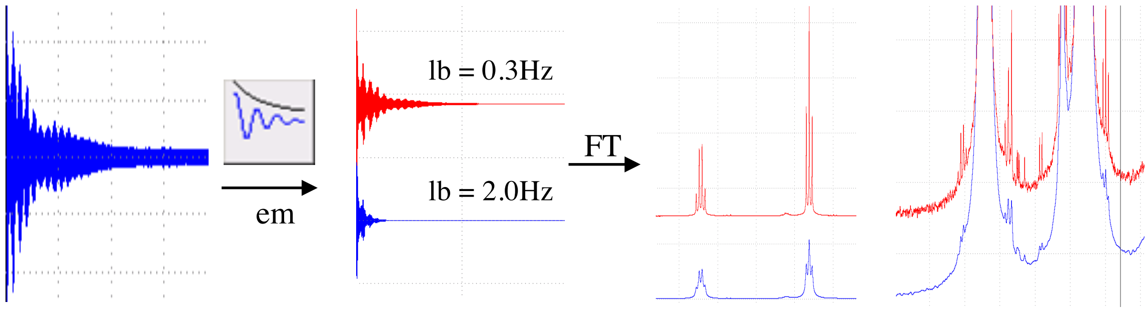
iii. Click and hold on  (zero order phase, used when the phase of peaks is frequency-independent). Dragging the mouse will change the zero order phase. Make the tallest peak exactly absorptive.

iv. If other peaks are still not leveled on both sides, click and hold on (first order phase, the phase of peaks is directly proportional to their frequency positions relative to the pivot point set above). Dragging the mouse to fix their phase. Many times this will also straighten out the baseline.

v. Click on  to save the phase values and leave the phase mode.

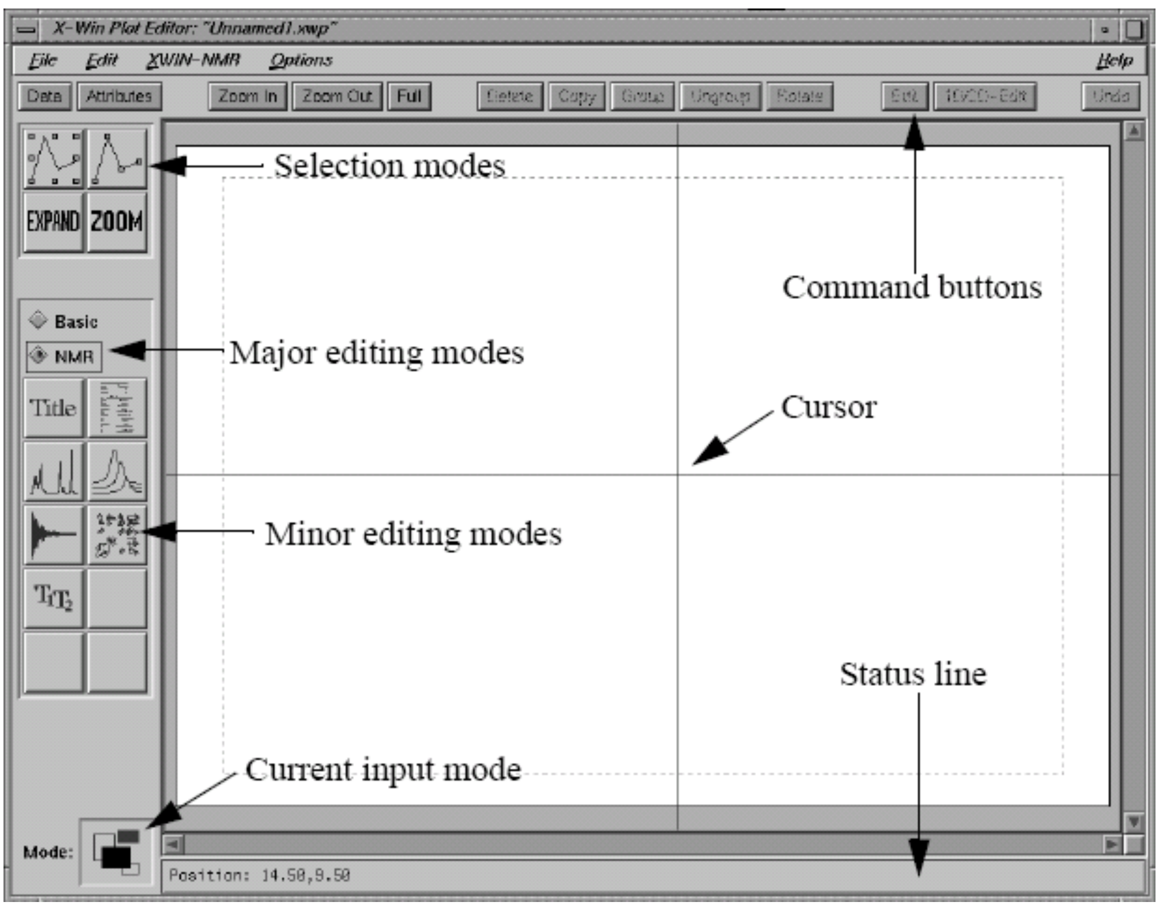
vi. If you want to process the same data again after you have done phasing, use "***efp***" (efp = em + ft + phase) instead of "***ef***", so you don't have to do "***phase***" again.

D. LB and Window Function



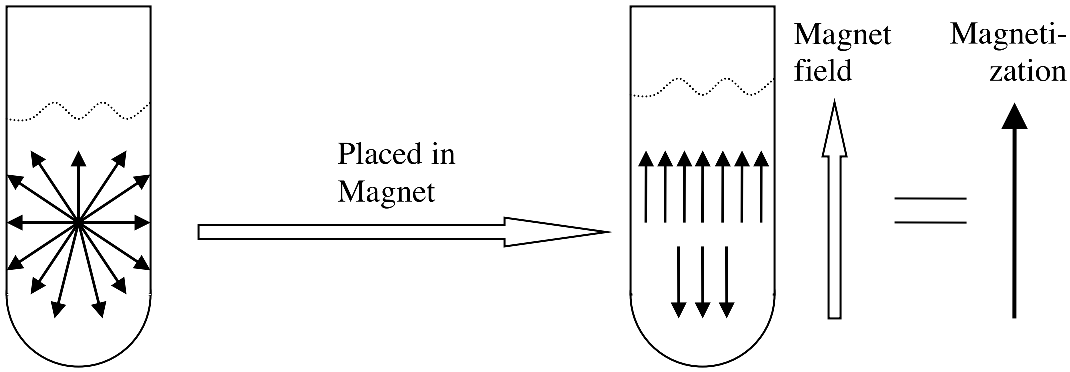
E. Plot Editor

This is a very nice and user-friendly program. You are referred to the Plot Editor manual of TopSpin for more details and encouraged to use it often for more controlled printouts.

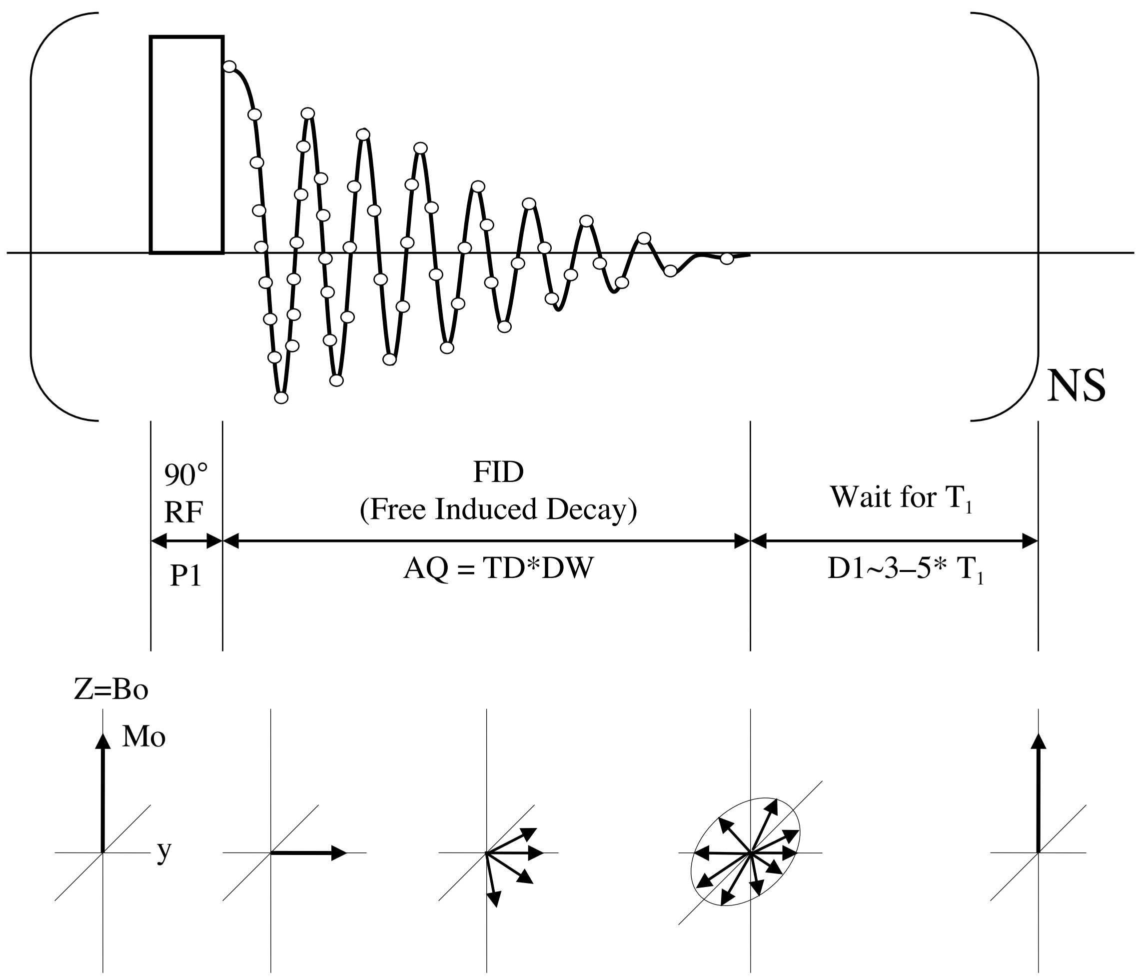


F. Spin Dynamics and Relaxation

Alignment of nuclei in magnetic field



Excitation, Acquisition and Relaxation

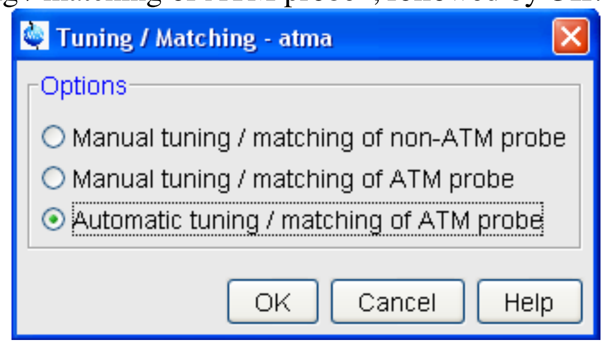


G. Probe Tuning and Matching

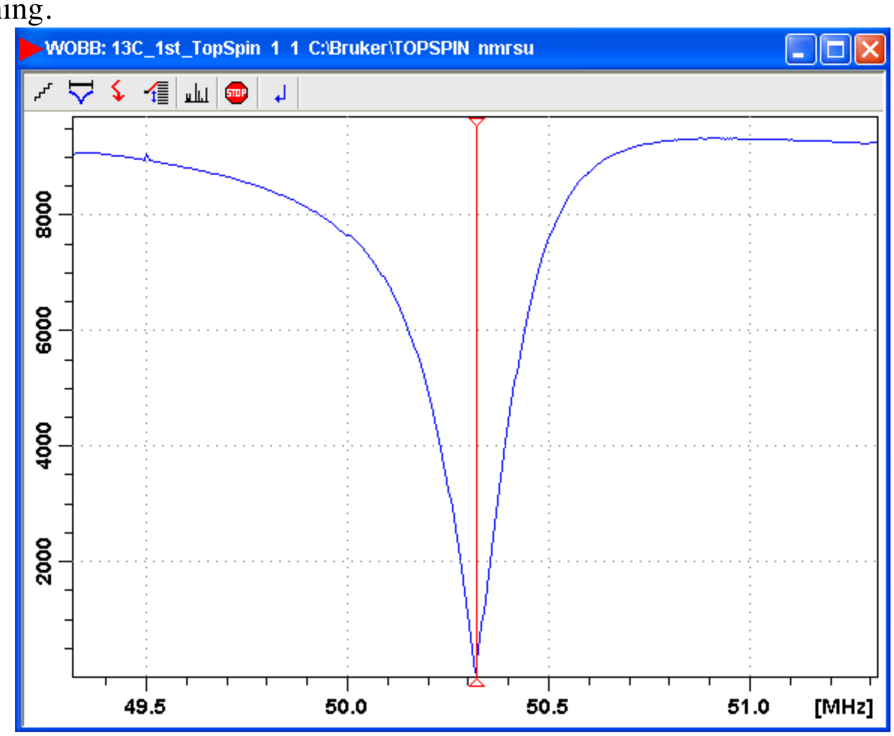
When you do NMR on a nucleus X other than ^{1}H and ^{13}C (e.g. X = ^{2}H, ^{19}F, ^{29}Si, ^{31}P, or …), you will need to tune the probe. You do not normally need to do this when performing ^{1}H or ^{13}C NMR.

<u>Steps</u>

Click on **Probe Tune/Match** in the **<u>Data Acquisition Guide</u>** and select "Automatic tuning / matching of ATM probe", followed by ***OK***.



After ~45s, you will see a WOBB window showing the tuning/matching curve, the horizontal position of which corresponds to tuning and the depth to matching.



The tuning curve should be aligned on the screen with the central redline and should reach all the way to the zero line of Y axis. If it doesn't look this way, then the probe isn't tuned.

The computer will tune and match the probe for you automatically. All you need to do at this point is to wait until you see the message "atma: finished" at the bottom left corner of Topspin.

H. Online NMR Book and Bruker NMR Encyclopedia

1) NMR Book: http://www.cis.rit.edu/htbooks/nmr/
Introduction to NMR concepts and practical issues.

2) NMR Guide & Encyclopedia: http://www.bruker.de/guide/
All you want to know about NMR.

I. Requirements for Access to the MRL NMR at CNSI

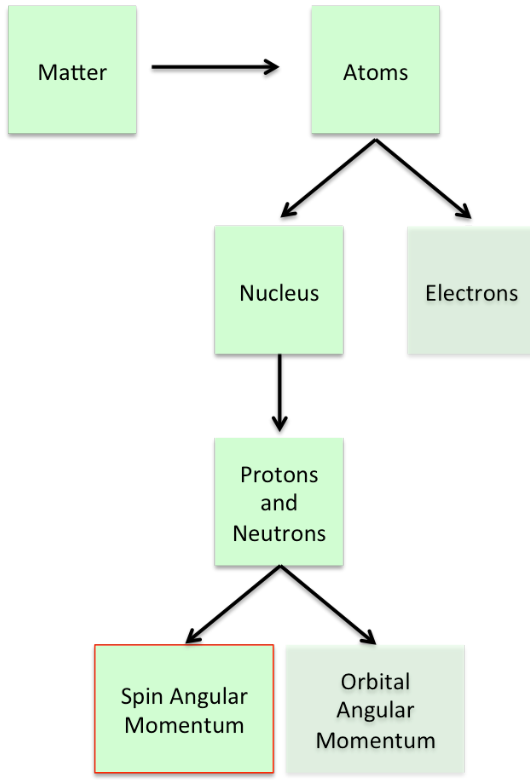
You have to pass the mini quiz within one month after training in order to be qualified for access to the NMR facility of MRL, which includes:

- Key Card for Lab & Building:
  1. Pass the MRL safety training;
  2. Fill out the CNSI access form: http://www.cnsi.ucsb.edu/facilities/building_services/access/access_application.pdf
  3. Take the form to Sylvia in 2066G, MRL
- Web Scheduling Account (email Jerry for a setup appointment)
- NMR Account (email Jerry for a setup appointment)

These requirements apply to both on- and off-campus users.

XII. NMR Basic Principles

1. Spin



*Spin is a quantum mechanical phenomena that has no physical analog in classical physics. However, it will be helpful to visualize it as a small bar magnet that precesses about an axis.

*The existence of spin angular momentum is inferred by experiments, such as the Stern-Gerlach experiment, in which particles are observed to have angular momentum that cannot be solely accounted for by orbital angular momentum alone.

*Electrons, protons, and neutrons all have a value of spin +/- ½.

2. Common NMR Nuclei

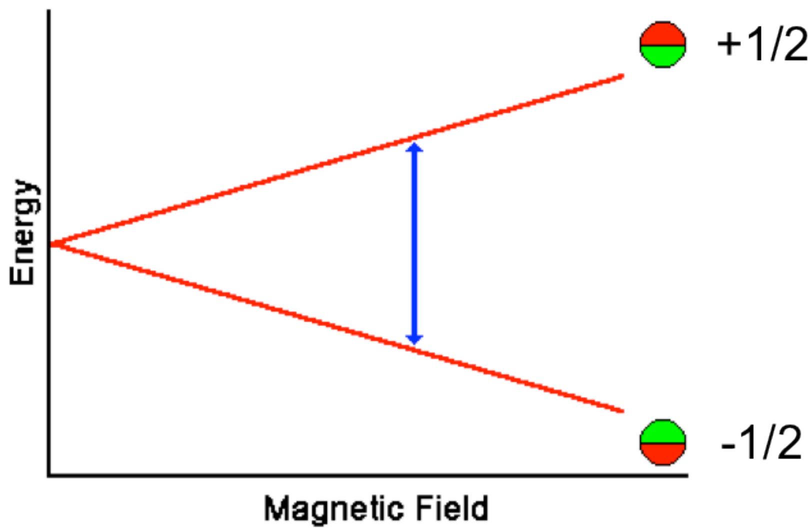
Nuclei	Unpaired Protons	Unpaired Neutrons	Net Spin	γ (MHz/T)
^{1}H	1	0	1/2	42.58
^{2}H	1	1	1	6.54
^{31}P	1	0	1/2	17.25
^{23}Na	1	2	3/2	11.27
^{14}N	1	1	1	3.08
^{13}C	0	1	1/2	10.71
^{19}F	1	0	1/2	40.08

Larmor Frequency Equation:

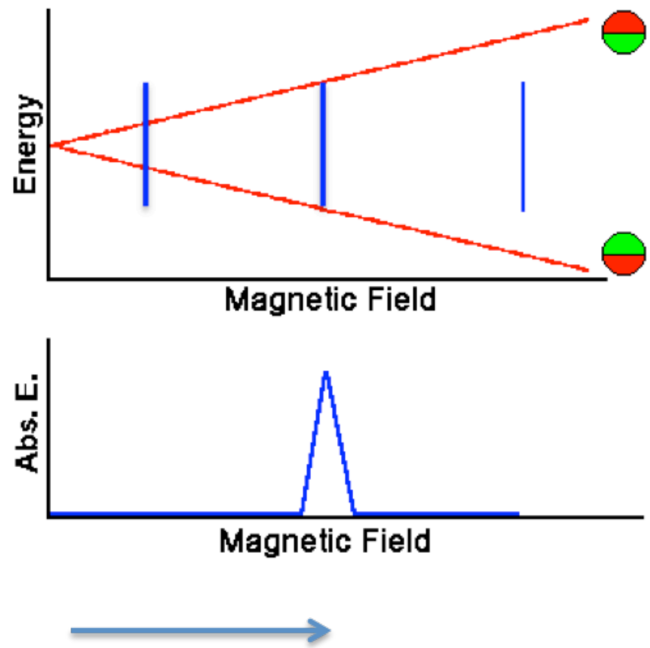
$$\nu = \gamma B_o$$

where γ is the gyromagnetic ratio (specific to each nuclei) and B_o is the magnetic field strength

3. Energy Level Diagram

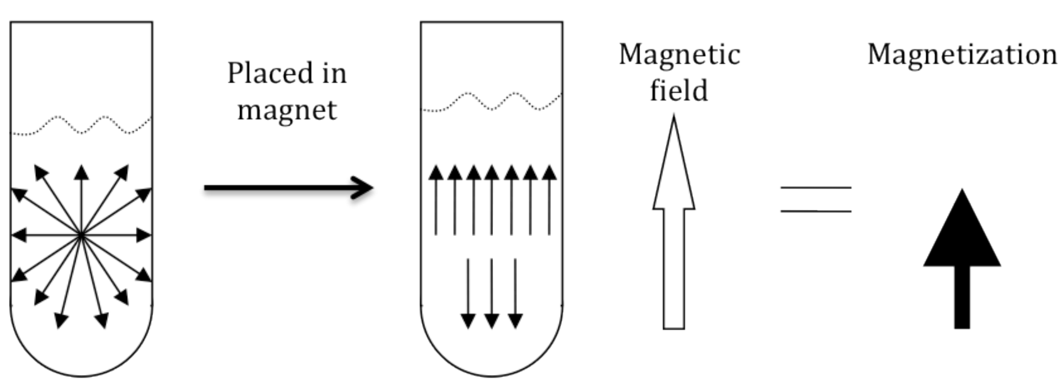


4. cw NMR



5. Magnetization

<u>Alignment of nuclei in a magnetic field</u>



6. Pulsed NMR, Relaxation, and Detection