2 NMR Training for the 600 MHz NMR with Chempack

INOVA 600 Tests and Assignment Certification

Student Name: _____

600-Test #1:
The student will be given a written test administered by Dr. Lee. This test will be similar to the test at the end of the appendix. Upon the successful completion of this written test the practical training sessions will begin.

Date Completed: _____Supervisor: _____

600-Test #2
The student will demonstrate the proper use of the instrument. This will include setting the depth of the sample and inserting the sample into the magnet, changing the probe file, setting up a ^{1}H experiment and tuning the probe. The student will be expected to properly identify all probe cables and connections and equipment components. At the successful completion of this test the student will be allowed to use the NMR from 8am – 5 pm, Monday –Friday.

Date Completed: _____Supervisor: _____

600 NMR Practical Assignment: Collect a 1D and 2D NMR Spectra
Collect a 1D ^{1}H and 2D COSY NMR spectra of Menthol. This sample is located in the NMR laboratory. Please shim the sample as well as you can. Follow the instructions located in Appendix A.2 of this manual.

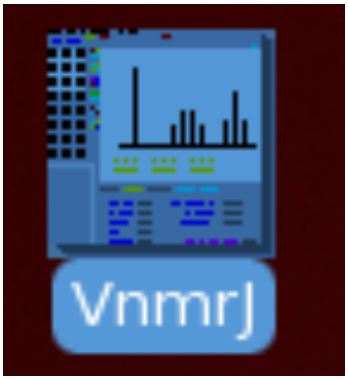
Date : _____ NMR Supervisor: _____

600-Test #3
Student gives demonstration of properly operating the instrument. The student must log into the spectrometer, insert sample, lock, shim, collect a ^{1}H experiment. The student will be expected to properly identify all probe cables and connections and equipment components. The test must be completed within 15 minutes. Upon completion of this test, the student is permitted to use the instrument at any time.

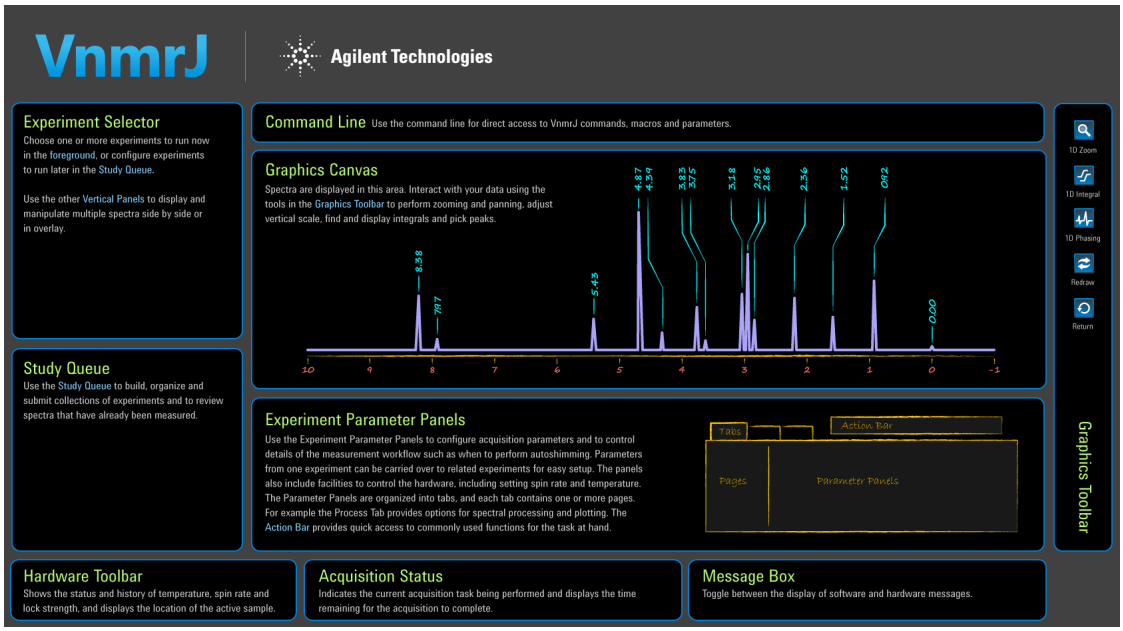
Date: _____ Research Advisor: _____

Getting started on the INOVA 600 MHz NMR Spectrometer using VnmrJ 4.2 with Redhat Linux 5.1

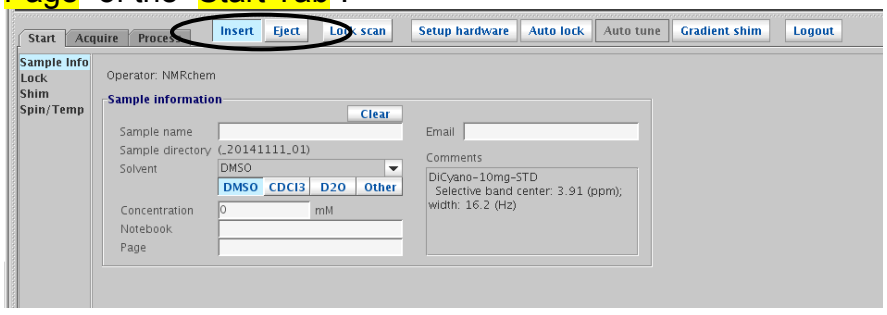
Log in and double click on the VNMRJ icon.



Overview of the VnmrJ 4.2 software



Insert your sample clicking the Eject and Insert buttons on the "Sample Info Page" of the "Start Tab".



Select the appropriate probe file for your experiment

Proper operation of both Chempack and BioPack within the VnmrJ 4.2 software requires the use of up-to-date and calibrated probe files. Setting up experiments from the software menu as described in the experimental instructions of this manual should result in the most recently calibrated values being automatically loaded into the experimental data sets.

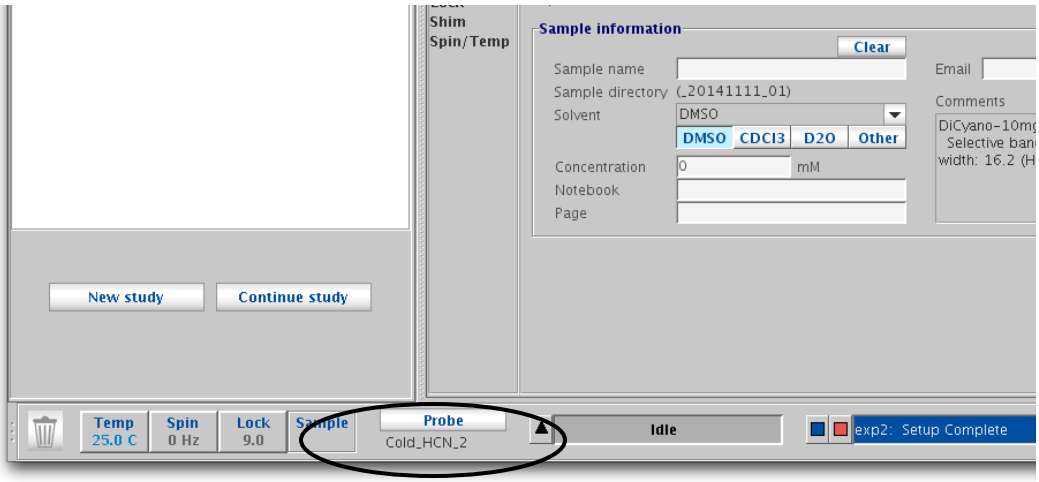
Probe Files

Probe files are specific to each instrument probe. They contain all of the necessary calibration information. The location of the system probe files is in the /vnmr/probes/ directory of each instrument and can be viewed by any user. The system probe file can only be changed by the system administrator. Groups may manage their own local probe files in desired, but be aware that only the system probe files will be updated with current calibration data. Groups that create their own probe files will be responsible for their maintenance.

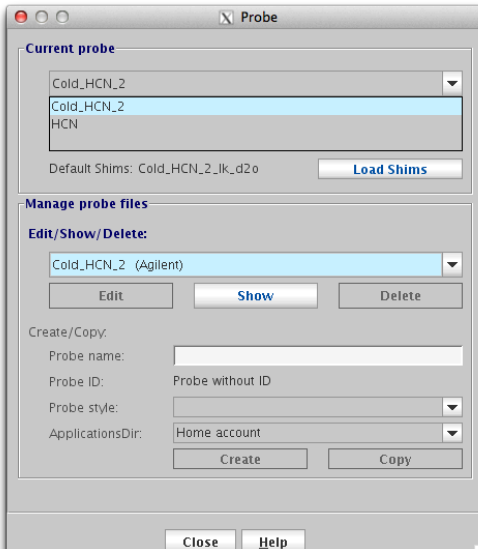
It is important for the user to be aware of which probe is on the instrument and which probe file is loaded in pulse sequence. The 600 MHz NMR spectrometer has two probe files, they are HCN and Cold_HCN_2. The HCN probe file contains the calibrated parameters for BioPack and the Cold_HCN_2 probe file contains the calibrated parameters for Chempack.

Changing the Probe File

The loaded probe file can be seen and accessed at the bottom of the VnmrJ window. Clicking on the Probe to access the probe files.



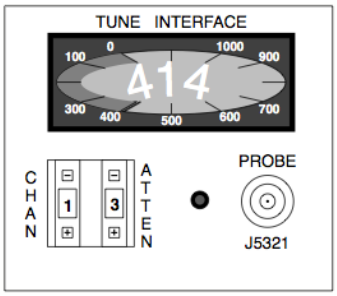
Click on the down arrow to view the probe file options. Select the desired file and close the box. For the Chempack training, use the Cold_HCN_2 probe file.



Tuning the Cold Probe on the INOVA 600 MHz NMR

WARNING: If you are a new user and you don't know how to do this or are unsure, **GET HELP**. Do not attempt to just "figure this out". Equipment can be damaged.

Probe tuning requires adjusting the tune wands very carefully to reduce the reflected power close to 0 range (for ^{1}H) on the tune interface panel located on the magnet console interface. Tuning the probe will insure that the 90° pulse width determination to be accurate and the pulse width will be as short as possible. If the sample is high in salt condition, the probe may not tune close to 0.

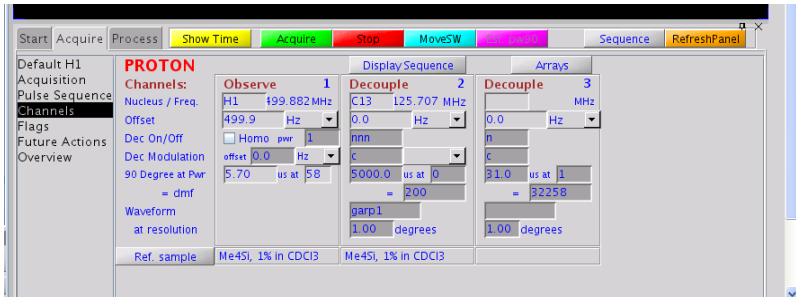


Tuning Tips: The wands can be very sensitive. Try to make small adjustments. If minimizing both the tune and the match and the reading doesn't go any lower, try moving the match part either clockwise or counter-clockwise to slightly increase the signal. Then try to minimize the signal again using the tune part. If the signal improves, continue to move the tune part in the same direction and repeat. If the signal does not improve, try adjusting the tune part slightly in the opposite direction to increase the signal and try to minimize again with the match part. This process should helps get out of any local minimum it is trapped in.

Do not force the tuning rods, if they feel "stuck" stop and ask for help.

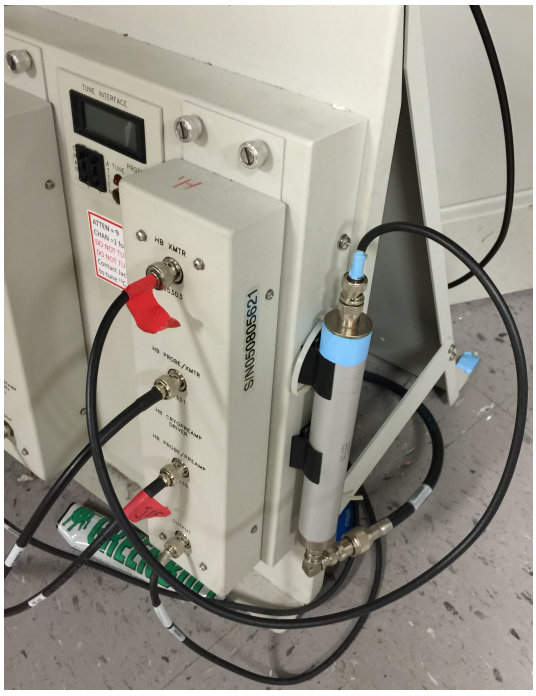
Tuning Step 1 – Setup Observing Channel

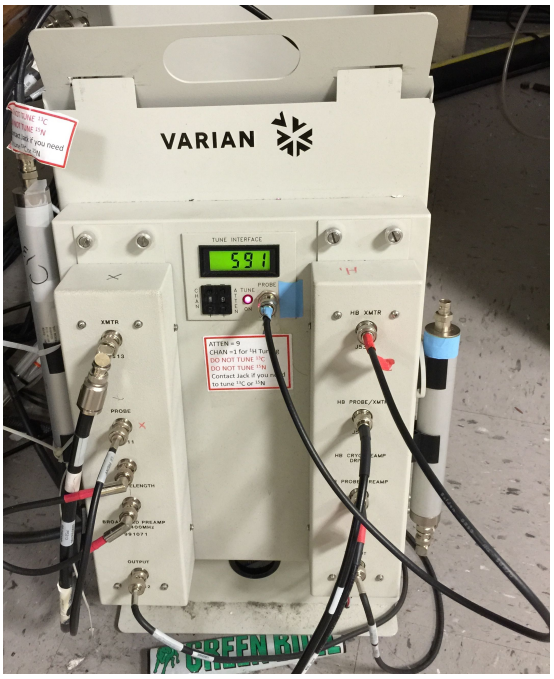
To tune the probe, first setup the correct frequencies for the console through the computer. To do this, load the experimental parameters, i.e. setup a proton experiment then type **su** on the command line. Look at the "Channels Page" of the "Acquire Tab" to see which nuclei is set up on each channel. Channel 1 is ^{1}H, and channel 2 is carbon.



Tuning Step 2 – Reconfigure Cable for Tuning

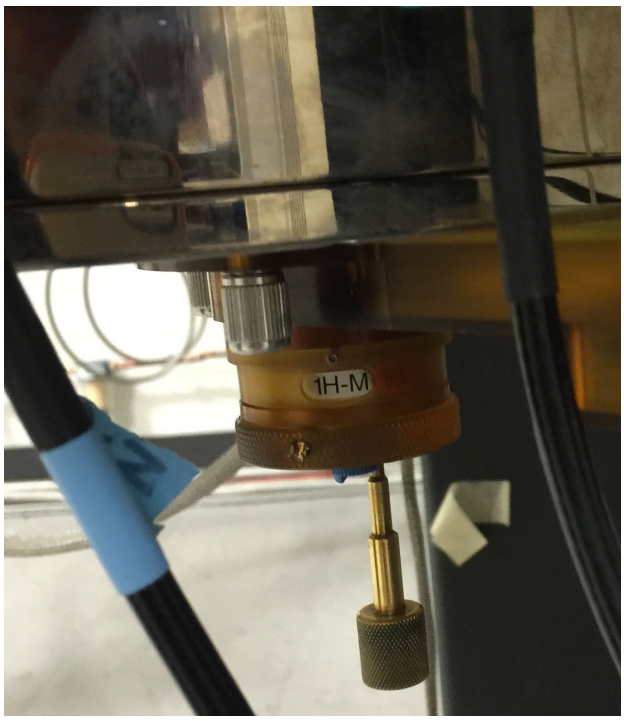
To tune ^{1}H channel, reconfigure the cable connection as shown on the image below and set CHAN to channel 1. The ATTEN always stays at 9 for the 600 MHz NMR. The bottom left image shows when the cable is connected as operating mode. Remove the cable from the top of the ^{1}H filter and connect the cable to the J5321 BNC port on the tune interface panel.





Tuning Setup 3 – Reconfigure Cable for Tuning

Turn the nuclei selection collar to the desire nuclei, for proton it would be 1H-T and 1H-M. Push the pin up into the probe to begin tuning. If the number on the tune interface display does not change while turning the tuning stick, STOP. Check Tuning Step 1 and 2 to make sure the console is setup to observe the correct channel (^{1}H channel for this exercise).

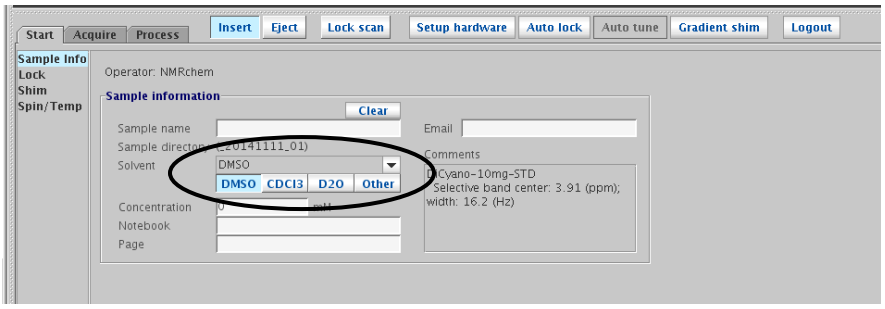


Start with 1H-T first, as the probe gets closer to being tuned, the number on the tune interface display will decrease. If the number stops decreasing, switch the nuclei selection collar to 1H-M to tune match. For NMR sample that is prepared in organic solvent with no salt, the reading can be tuning down to 0 or 1. Similarly, protein NMR sample with <200 mM NaCl or KCl, the reading can be tuning down to 0 or 1.

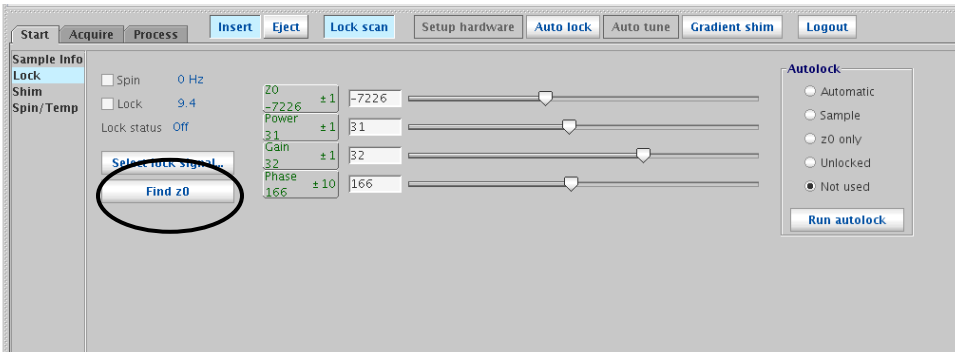
If you have accidently tuned the wrong nuclei such as ^{2}H, ^{13}C, or ^{15}N, PLEASE NOTICE Dr. Lee immediately.

Tuning Setup 4 – Reconnect cable back to operating mode
Remove the cable from the J5321 BNC port and reconnect the cable back to the top of the ^{1}H filter.

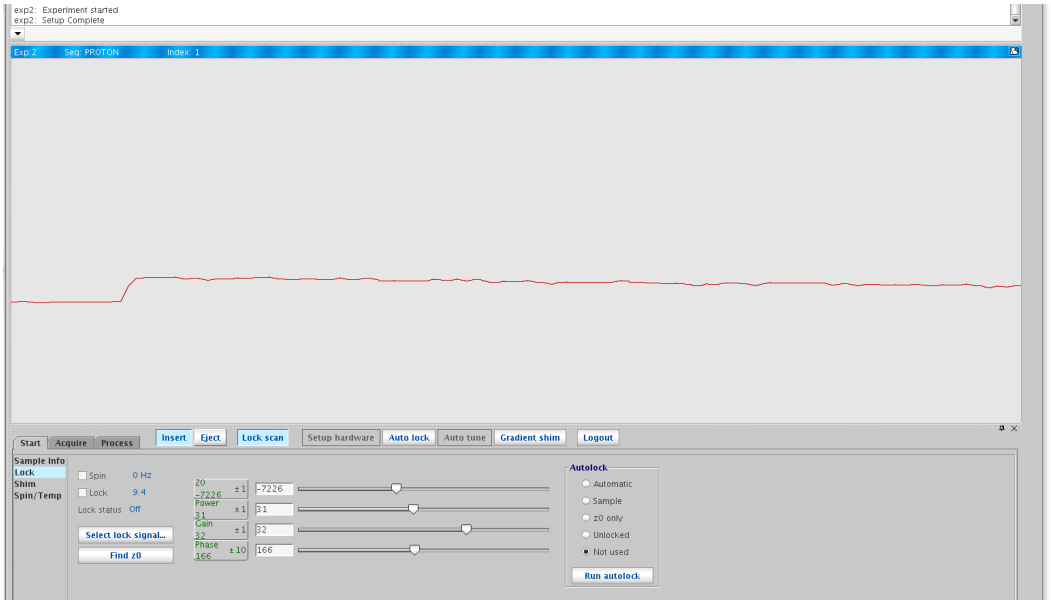
Select the desired solvent on the "Sample Info Page" of the "Start Tab".



Lock: Click the Find z0 button on the "Lock Page" of the "Start Tab"



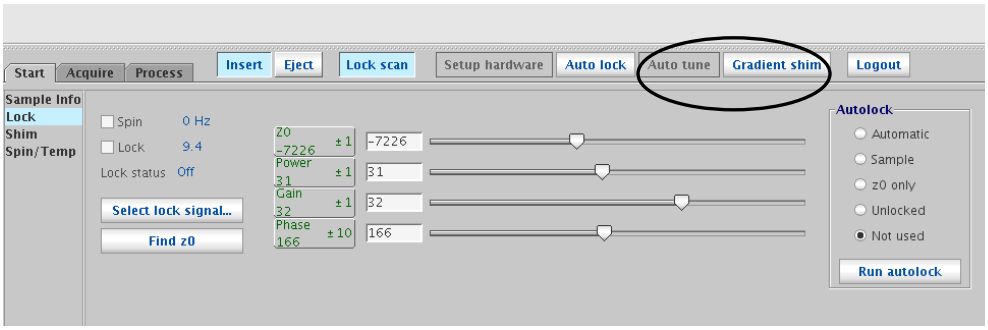
Click the "Lock Scan", uncheck the "Lock Box" to turn off the lock. Adjust (raise or lower as necessary) the Power and Gain until the signal completely appears on the screen.



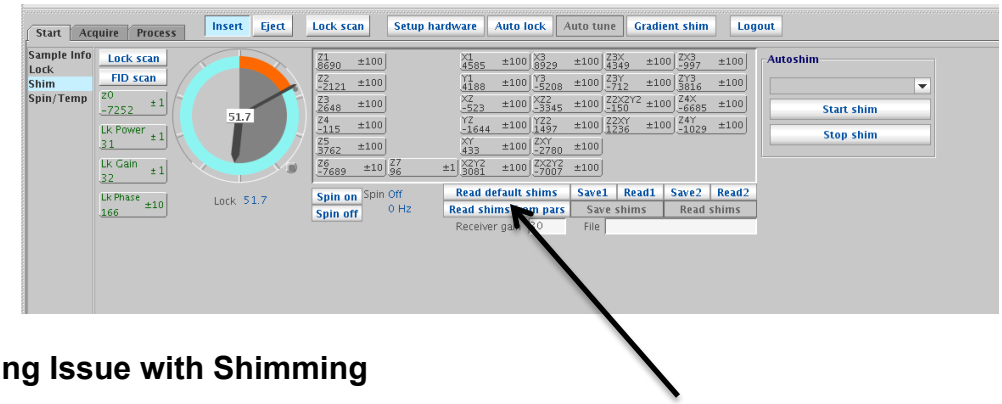
Adjust Z0 to maximize the lock signal. For best results adjust Z0 until signal is maximized, then adjust phase until the signal is maximized. Clicking the middle wheel of the mouse will change the steps of Z0, power, gain and phase to 1, 10 or 100. If the lock level is fluctuating at a low power level, then lower the gain.

Click Lock On when you are finished locking. Unclick the Lock Scan button so that the lock signal is no longer displayed on the screen. Failure to do this can result in the computer freezing.

Shim by clicking the "Gradient shim" button on the "Lock Page" of the "Start Tab". If you want to manually shim z1-z5, click the shim tab. It should not be necessary to shim the higher order shims.



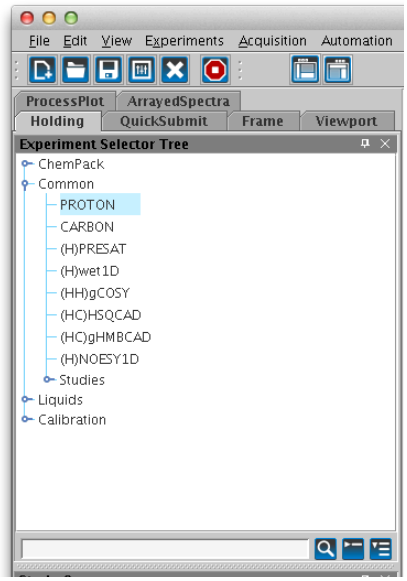
Alternatively, you may manually shim using the buttons on the "Shim Page" of the "Start Tab" to maximize the lock signal.



Having Issue with Shimming

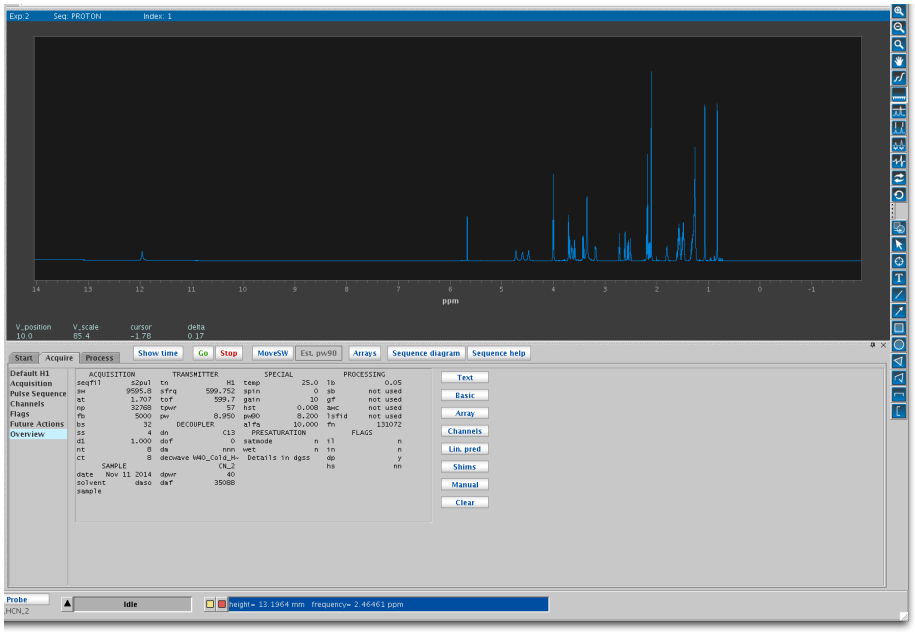
- Read in default shim by clicking on "Read default shims" button below the shim adjust button on the "Shim Page" of the "Start Tab". To restart the shimming process
- Adjust X1 and Y1 shims
- Change the gradient shimming parameters (please see xxx for instruction)
- Make sure you have enough volume in your sample tube to cover the coil.
- Visually inspect your sample to see if there are precipitations.

Load a Pulse Sequence Program Type *proton* on the command line to load the proton pulse sequence program or by clicking "PROTON" under the "Common" on the "Experiment Selector Tree".



Parameters may be accessed under the "Acquire Tab". Basic parameters such as sweep width, number of scans, relaxation delay, etc are found in the "Default H1 Page". Automatic plotting and integration can also be set to on or off here.

An "old style" parameter page can be accessed by typing *dg* in the command line and clicking on "Text Output Page" of the "Acquire Tab"



1D ^{1}H Experiment on the 600 MHz NMR

- Set *nt=16* and *ss=8*
- Type *ga* or *go* for acquisition
- Click File on the menu and Save As option to save your data

Changing Basic 1D Parameters

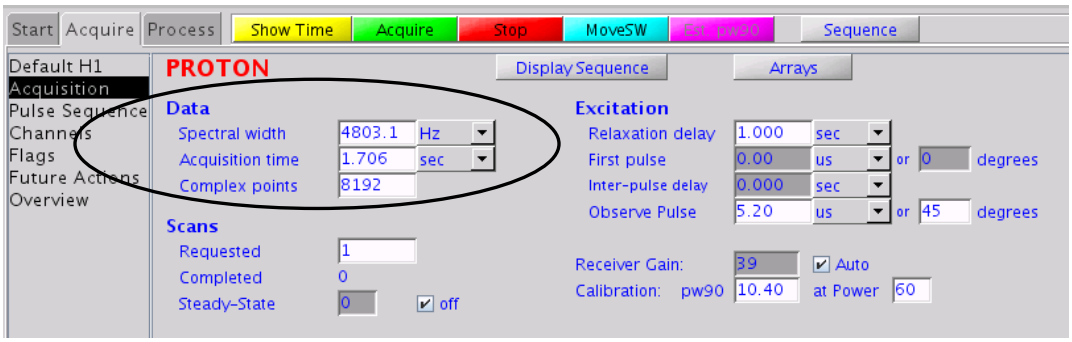
A list of parameters can be accessed on the text output page of the process panel after typing dg in the command window. A short description of each parameter listed can be found in the Command and Parameter Reference Manual. Each parameter can be changed by typing the abbreviation = value in the command window. For example to change the number of scans from 1 to 16, simply type nt=16 in the command window. It is necessary to type dg for the list to refresh, showing that the change has been made.

Selected parameter list

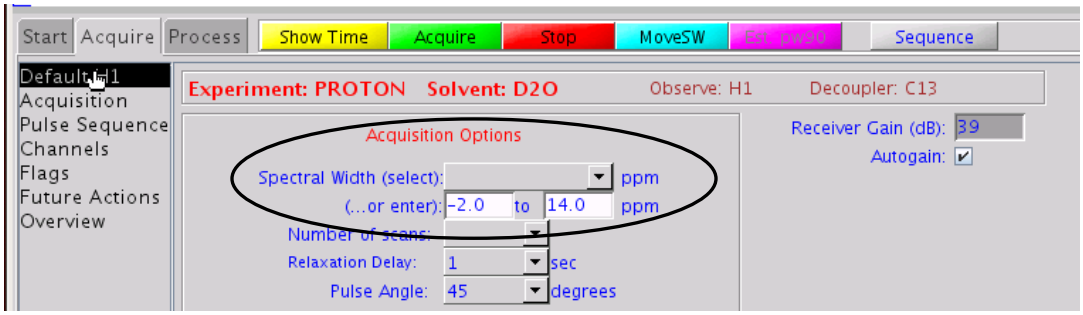
at	Acquisition time
d1	Delay time between scans in seconds –recycle delay
ni	Number of increments
np	Number of points
nt	Number of transients
pw	Pulse width
pw90	90^{o} pulse width
seqfil	Pulse sequence being used
sfrq	Observed frequency (frequency of tn)
sw	Sweep width (of tn)
tn	Transmitting nuclei (observed nuclei)
tof	Tuner offset frequency—center of the spectrum
tpwr	Transmitting nuclei power level
dfrq	Decoupling frequency
dmf	Decoupling Modulation Frequency
dn	Decoupling Nuclei
dof	Decoupling Offset Frequency
dpwr	Decoupler Power

Setting the Spectral Width

Setting the Spectral width involves both the *width* and the *center* of the frequency window. The two parameters which must be set correctly are sweepwith (sw) and tuner offset frquency (tof). Setting the Spectral Width using the fill in box on the "Acquire > Acquisition Page" will not change the center of the spectrum (tof) and it will remain at the default position of 6 ppm. An incorrect value for the center of the spectrum can result in a fold-over of the peaks.



The simplest way to set the Spectral Width is to type the ppm range that you want into the boxes on the "Acquire >Default H1 Page". This will set the center of the spectrum (tof) to the middle of the desired ppm range.

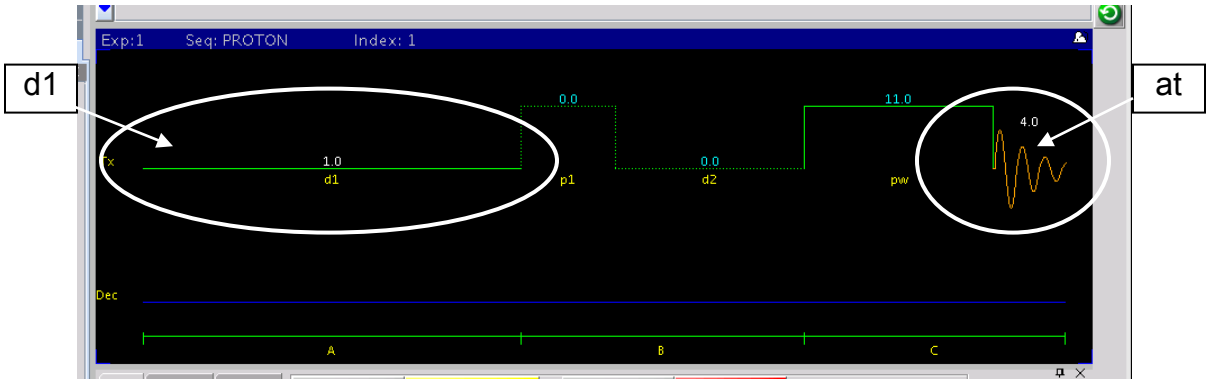


Alternately, the cursors and the movesw command can be used: Place the cursors around the peaks and type movesw. This will automatically change the sw to the position of the cursors and adjust tof to the halfway point between the cursors.

Setting the first delay (d1) and acquisition time (at).
The delay time, **d1**, is the time between the end of the acquisition time, **at**, and the next element in the pulse sequence. The **total recycle time** is the time between the last pulse and the next pulse at the beginning of the pulse sequence, typically is **d1** + **at**. The total recycle time usually reflects the T1, longitudinal relaxation time, of the sample. Typically, a value of 1-2 seconds will suffice. If the T1 is unknown, a quick T1 experiment should be done to estimate it. The T1 can be long especially in cases of heteronucleus detection such as carbon or other X nucleus. T1 values for quaternary carbons can be very long and the total recycle time should reflect this.

The acquisition time, **at**, is usually set based on a rough approximation of T2, transverse relaxation time. The T2 indicates how long the signal in the FID will last. As a general rule, large molecules such as proteins relax very quickly and require a short **at** (such as *at=0.5*). Small molecules relax much more slowly and the FID can last several seconds so often using a longer **at** is favorable (such as **at=3 to 10** seconds). If you type dps on the command line the pulse sequence

will be displayed. This is a graphical representation of the pulses that are taking place during each scan.

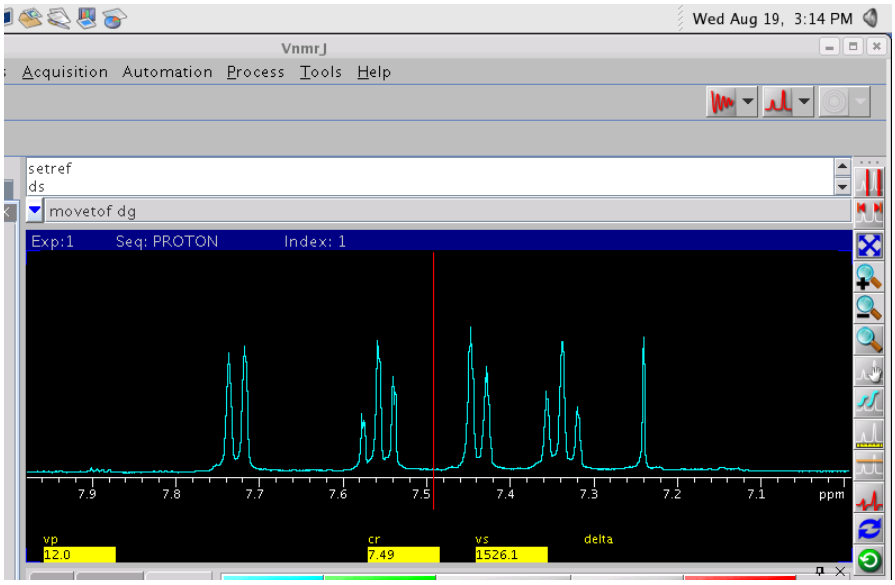


To change d1 or at, type d1=# and at=# on the command line. Type *dg* to refresh the parameter list and *dps* to refresh the pulse sequence.

Optimizing the ^{13}C experimental parameters

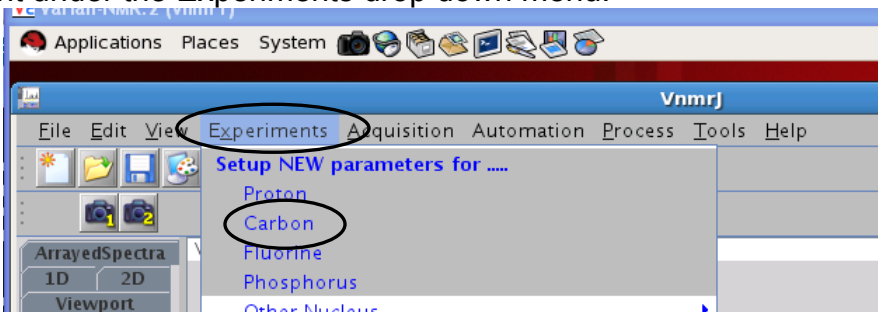
If the proton signals of your compound are either all aromatic or all aliphatic, the default parameters for ^{13}C 1-D NMR may not give good enough signal to noise. In this case, the instructions below can be followed to improve your data.

First acquire a proton spectrum of the sample in exp1. Move the transmitter offset to the center of the proton signals by placing the cursor in the middle of the peaks and typing **movetof** in the command window. This value may then be found by typing tof? in the command window.

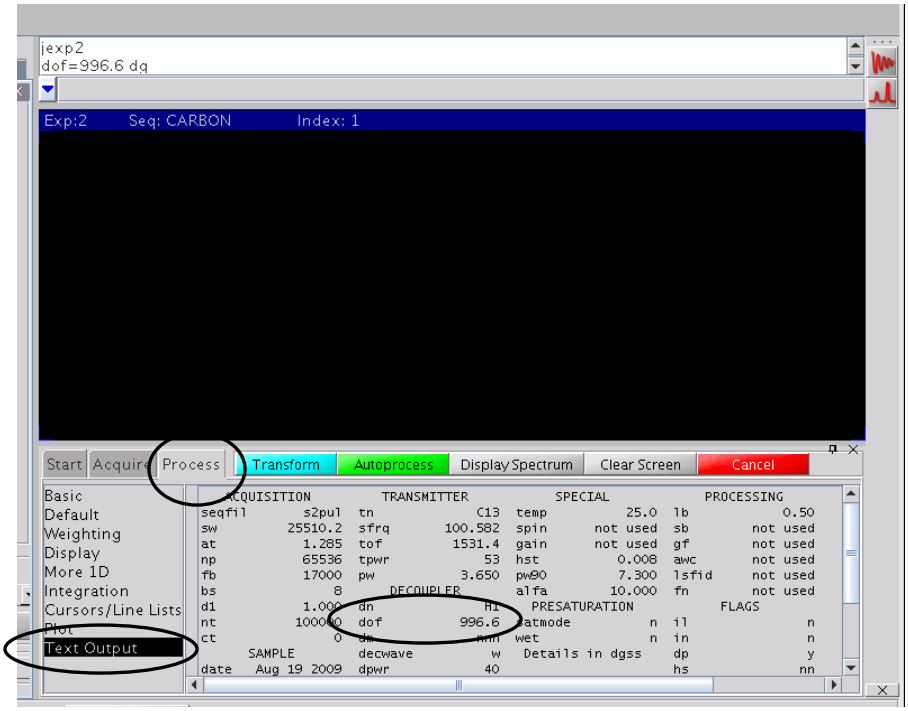


Note the **tof** (transmitter offset). This will become the set value of dof in the carbon experiment.

Join experiment 2 by typing jexp2 in the command window. If experiment 2 doesn't exist, create it by typing cexp(2). If experiment 2 is locked, unlock it by typing unlock(2). Set up the carbon experiment by choosing the carbon experiment under the Experiments drop down menu.



Set dof to the center of the proton peaks (tof) that was determined above. Type dg and the parameters will be shown in the text output (or overview) window of the process tab.

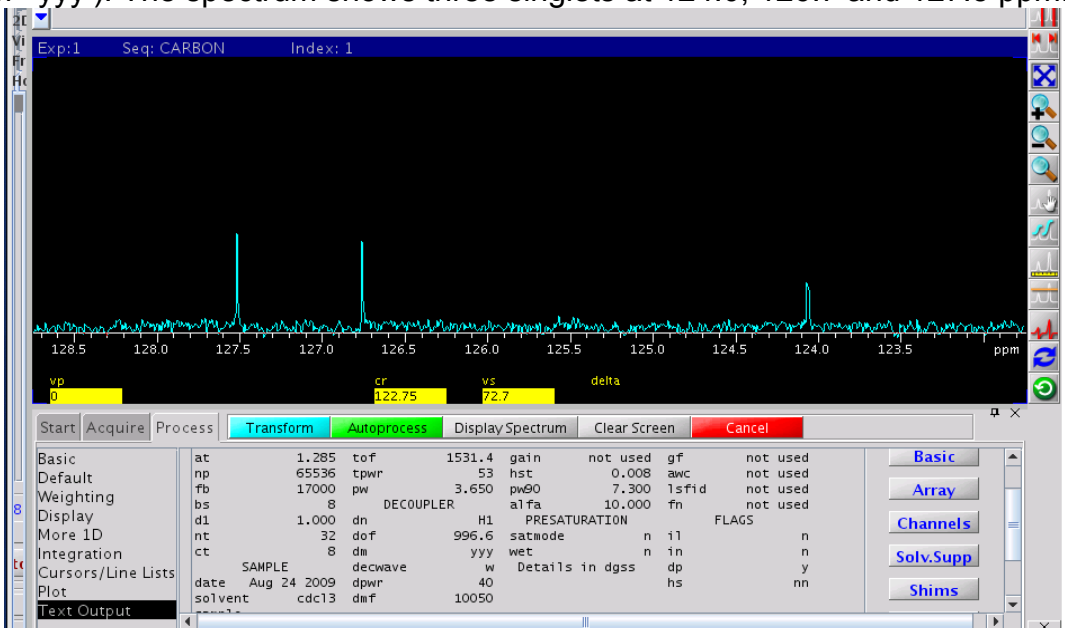


To determine the number of scans for your available time, type time(hours,minutes). For example, if I have 1 hour and 15 minutes, type time(1,15) dg. This will set the number of scans (nt) to the appropriate number. Type bs=16 ga wbs('wft') in the command window. This will start the experiment and show you the carbon experiment at the completion of every 16 scans.

If you would like to stop the experiment before it is complete, click the Stop button or type aa. **Save the data**, send it to the data station and print it.

Important Parameters:
The parameters dpwr, and dmf are essential for good results. These parameters are calibrated regularly and correct values should load into the parameter set when the experiment is set up. These parameter values along with their calibration dates can be found in the red calibration notebook located next to each spectrometer. The decoupler power (**dpwr**) should stay at or below 40db for safety. The decoupler modulation mode (**dmm**) is the mode the decoupler is using. To decouple protons, use waltz decoupling (**dmm='w'**). When using waltz or garp decoupling, the decoupler modulation frequency (**dmf**) is set to 4X γH2. The γH2 value is regularly calibrated and updated in the probe file.

The spectrum below was collected with 32 scans. The decoupler was on (dm='yyy'). The spectrum shows three singlets at 124.0, 126.7 and 127.5 ppm.



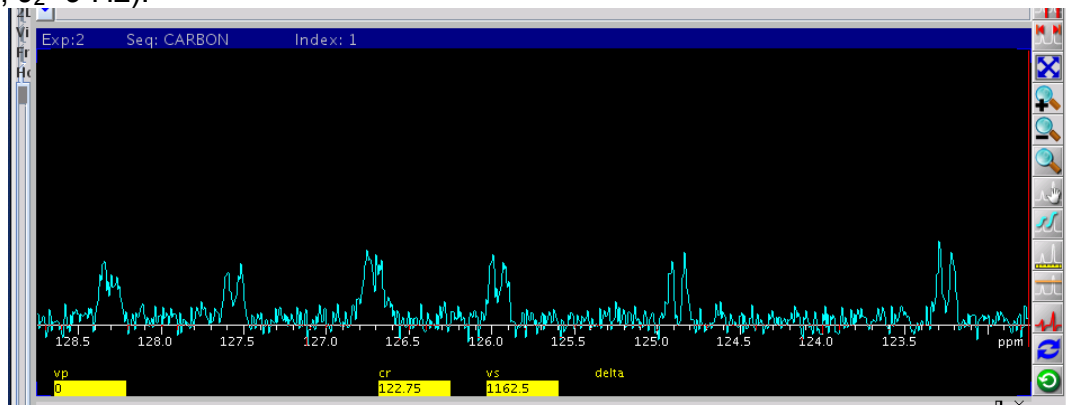
Integrating the Carbon Spectra
In a proton decoupled ^{13}C spectrum all the coupling information between the 1H and 13C has been removed and all the carbons peaks should be singlets. Integration of this spectrum may not be accurate due to the carbon-proton NOE enhancement. The default setting in the carbon pulse sequence accessible by the drop-down menu for the decoupler is "on" for the entire length of the experiment. This is designated by dm='yyy'. Use **dps** (display pulse sequence) to see when the decoupler is off and on. Decoupling the protons in the carbon experiment significantly increases signal intensity, but removes coupling information and the possibility of integrating the spectrum.

Proton Coupled ^{13}C Experiment

Carbon-13 1D NMR can also collected with the C-H coupling observed. This is referred to as a proton-coupled carbon-13 experiment: Different decoupling settings will allow for better or worse signal-to-noise and determines whether the spectrum can be accurately integrated and/or whether the coupling information (splitting) is retained.

Decoupling Mode	S/N	Integration	Splitting
'nnn'	poor	yes	yes
'nny'	fair	yes	no
'yyn'	fair	no	yes
'yyy'	best	no	no

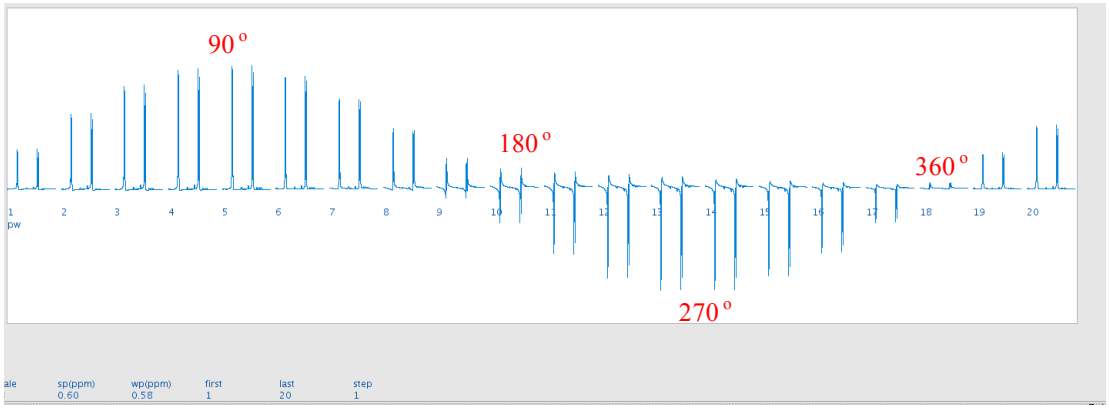
Set the decoupler to the desired value. The spectrum below was collected with the decoupler off (**dm='nnn').** This change in decoupler settings resulted in a large decrease in signal-to-noise but contains the coupling information. Many transients were needed to see carbon peaks (1798 scans). Carbon peaks from carbons attached to protons are split. This spectrum can be integrated. The spectrum shows three doublet of doubles at 124.0, 126.7 and 127.5 ppm (J_1=163 Hz, J_2=9 Hz).



^{1}H 90° Pulse Calibration

Introduction

What is the 90° pulse width? The radio frequency pulse is described by its power and duration (time). Before the pulse the proton "spin" is oriented in the z direction and it cannot be observed in the spectrum. The rf pulse "flips" the spin into the xy plane so that it can be observed. The maximum signal is seen when the spin is completely in the xy plane without any z component. The length of time that this takes is called the 90° pulse. In the spectra below the length of the pulse is varied from 1 to 45 microseconds. The most accurate way to determine the 90° pulse width is to find the 360° and divide it by 4. This value is directly affected by the pulse power. As you increase the power, then the 90° pulse will decrease. Normally the power (tpwr) for 600 MHz NMR is set to 57.



90° pw Calibration Step 1 – Collect a 1D ^{1}H spectrum

Collect a ^{1}D 1H spectrum using the default 90° pw and phase the spectrum. Expand the region around one peak, preferable a peak from a methyl group. Take note of the previously calibrated 90° pw and power level and record them below. These values can be found on the "Acquisition Page" of the "Acquire tab".

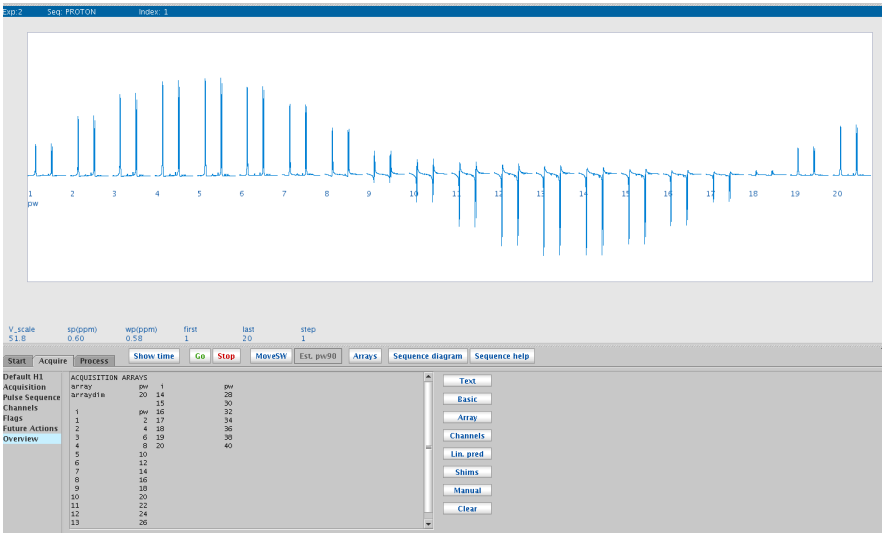
90° pw Calibration Step 2 – Collect Array (bigger increment) pw Spectra

The array needs to go past the 360° pw. This number can be estimated by taking the previously calibrated 90° pw value and multiplying it by 4. You will want your array to go past this point. In this example the 90° pw is 8.95 μs. The array must go past 8.95*4 (35.8 μseconds), therefore pw is arrayed from 3 to 45 μs in steps of 3 μs.

- Type *array* in the command line to start the arraying process.
- VnmrJ will ask which parameter to be array, enter *pw*
- VnmrJ will ask the array steps, enter *15* for running 15 spectra
- Enter the start point for the array, enter *3* to start at 3 μs
- Enter the increment size, enter 3 to increment each step by 3 μs

The array values will be displayed at the "Text Output Page" of the "Acquire Tab" (type *da* to show the array values), double check the values to make sure there are no typo. An appropriate delay, number of scans, absolute intensity must be now set and the experiment started. A delay of 2 seconds with 1 scan is chosen in the example. These parameters are set by typing, *d1=2 nt=1 ga* on the command line. To view the entire array and display the array values in the bottom text box, type *wft dssh dssl da* on the command line. It may be

necessary to adjust the vertical scale (vs) and the vertical position (vp) to make all spectra display completely on the screen.



The 90° pw is calculated by taking the 360° pw and dividing it by 4. The 360° pw is identified as the second null point in the curve. In this example, the 18th spectra is around the 360° pw. This corresponds to 36 μs.

90° pw Calibration Step 3 – Collect Array (0.1 μs increment) pw Spectra

To find a more accurate 90° pw create a second array that spans 4 μseconds around the 360° pw with 0.1 μseconds increments. In this example, the second array would be from 34 – 37.9 μs with an increment of 0.1 μseconds.
90° pw = 35.8 μs / 4 = 8.96 μs.