IOWA STATE UNIVERSITY

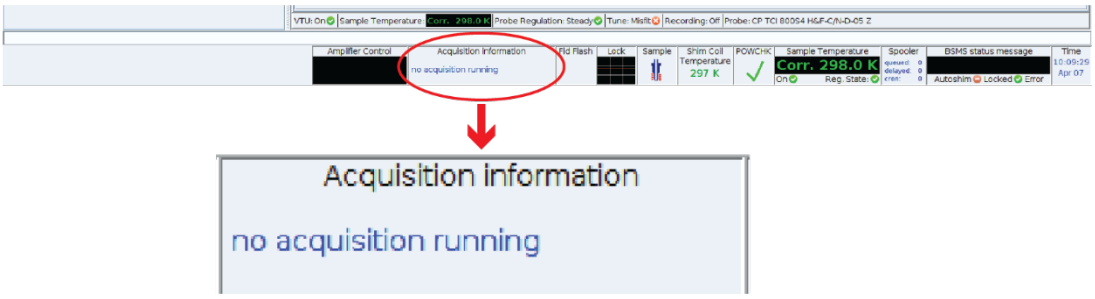
Biomolecular Nuclear Magnetic Resonance Facility

Initial Setup

STEP 1: EJECTING SAMPLES FROM THE NMR

BEFORE** starting, check to make sure the spectrometer is not currently running and that there is not a sample inside

1.1) Check that the spectrometer is not running by viewing the "Acquisition Information" box located at the bottom of the TopSpin window.



i. If the "Acquisition information" box reads, "no acquisition running", the spectrometer is not currently in use and you may move to **Step 1.2**.

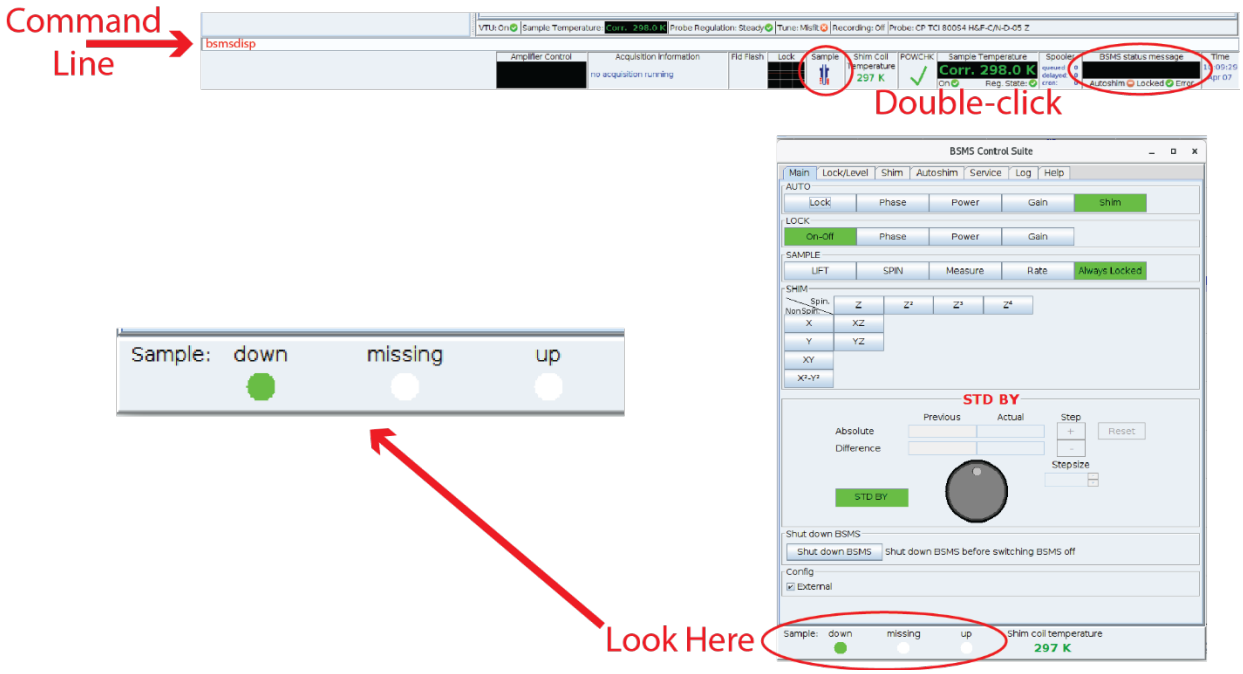
ii. If the "Acquisition information" box reads anything other than "no acquisition running" and you have the spectrometer booked, please contact an BNMRF staff member.

PLEASE DO NOT STOP SOMEONE ELSES EXPERIMENT IN PROGRESS!

1.2) Check that there is not currently a sample inside the spectrometer using one of the following methods below.

i. Open the BSMS Control Suite by double clicking on the "**Sample**" box or the "**BSMS status message**" located at the bottom of the TopSpin window.

ii. Alternatively, you can type "**bsmsdisp**" into the command prompt line and press enter.



a. If the "**down**" circle is filled, there is a sample in the spectrometer.

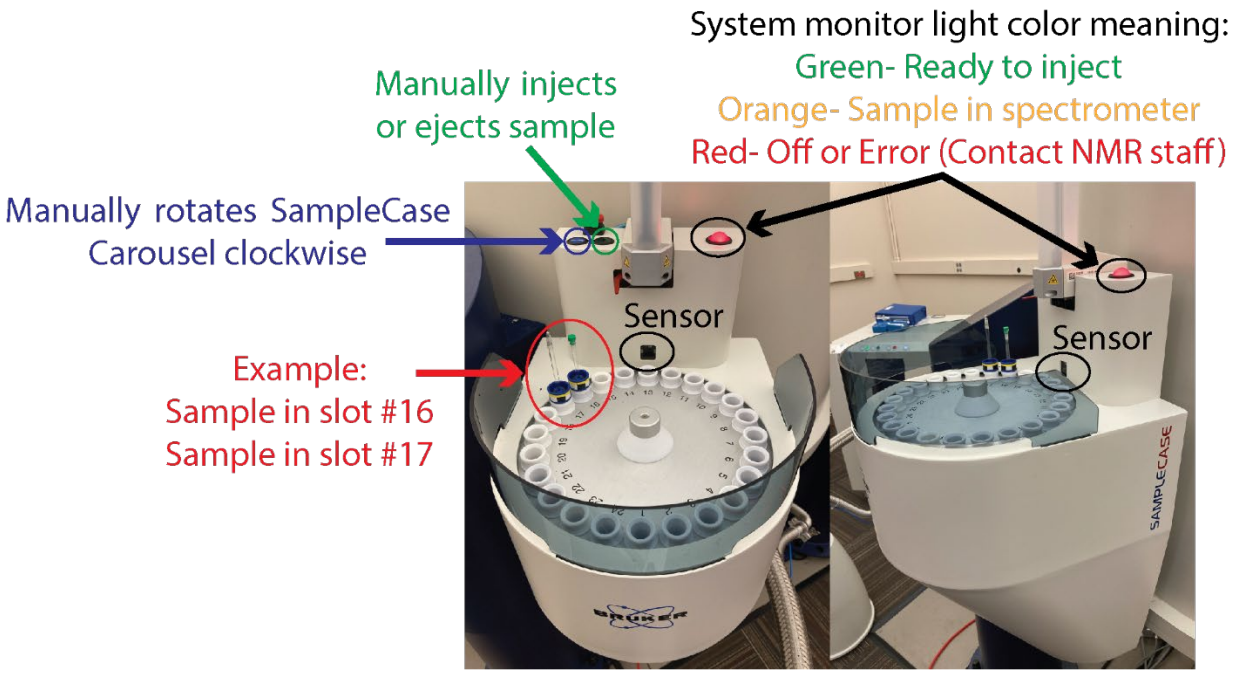
b. If the "**missing**"circle is filled, the LIFT is down but the spectroemter does not sense a sample. (CONTACT NMR STAFF)

c. If the "**up**" circl is filled, the LIFT is up and there is no sample inside.

At the BNMRF, we have SampleCase carousels for the magnets. This means if the "up" circle is filled, it is ready to receive a sample.

iii. Additionally, you can Check the system monitor light on the SampleCase carousel.

a. If **green**, there is no sample in the spectrometer.
b. If **orange**, there is a sample currently in the spectrometer.
c. If **red**, contact an NMR staff member.



1.3) If there is a sample in the spectrometer and there is no acquisition running, eject the sample using of the methods below.

If there is no sample in the spectrometer, proceed to **STEP 1.4.**

i. Type **ej** into the command prompt line and press enter.

Or

ii. Type **sx #** into the command prompt line and press enter (where # is the sample slot corresponding to your sample location in the SpinCase carousel, e.g., sx 16). **(SEE NOTE BELOW BEFORE USING THIS COMMAND)**

Or

iii. Manually eject the sample by pressing the **green** button located at the top left of the SpinCase Carousel (imaged above).

The **sx#** command will simultaneously eject any sample in the spectrometer and inject the sample located at the sample slot # used. Make sure your sample is in the correct spot before using this command. **(SEE STEP 4)**

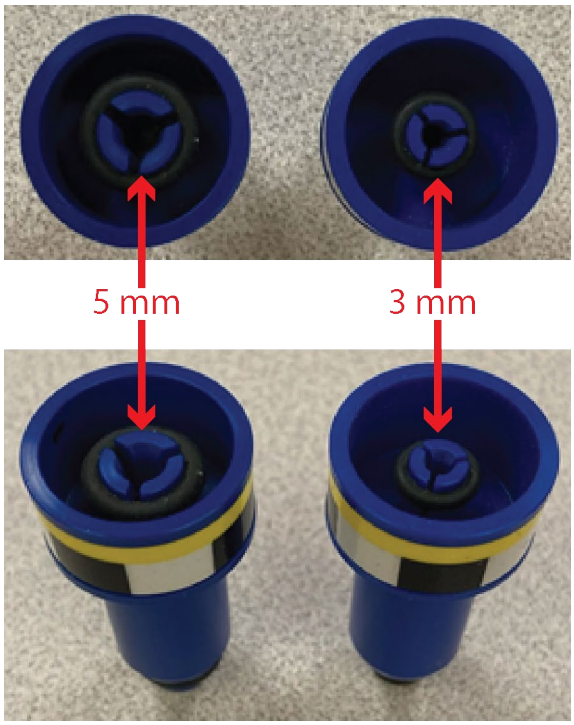
Once there is no sample in the NMR spectrometer, proceed to **STEP 2: INSERTING SAMPLE INTO SPINNER.**

STEP 2: INSERTING SAMPLE INTO SPINNER

Sample Volumes Required for Different NMR Tubes.

NMR Tube	Minimum Volume (µL)
5 mm Standard	500
5 mm Shigemi	260
3 mm Standard	160
3 mm Shigemi	70

2.1) Select the appropriate spinner based on the size of your NMR tube (3 or 5 mm).



Attempting to insert the wrong NMR tube can result in broken glass and injury!

2.2) Insert a spinner matching the dimensions of your NMR tube (3 mm or 5 mm) atop a depth gauge (imaged to the right).

ALL SPINNERS ARE NOT CREATED EQUAL!

If running experiments above 80°C or below 4°C, please consult with an NMR staff member before selecting a spinner.

IF YOU ARE UNSURE WHICH SPINNER TO USE, CONSULT AN NMR STAFF MEMBER!

2.3) Gently insert your sample into the spinner.

2.4) Set sample depth, referencing the depth gauge.

2.5) The sample should **FULLY** cover the **3/5/8 mm** marking.

(SEE IMAGE BELOW FOR EXAMPLE)

Samples that do not fully occupy the coil markings will result in poor shimming!!!

- DO NOT push with palm to insert NMR tube into spinner!
- DO NOT press the NMR tube hard against the base of the depth gauge (sample should make light contact).

Sample is *above* the 3/5/8 mm coil mark on the depth gauge when positioned correctly.

2.6) Your sample is now ready to be inserted into the NMR.

Be sure to set the sample temperature (STEP3) BEFORE injecting your sample!

STEP 3: SETTING SAMPLE TEMPERATURE

ALWAYS** check temperature is safe **BEFORE** inserting your sample

Maximum allowed range for the cryoprobe: ***-40°C to +80°C (233.15 K to 353.15 K)***

Use appropriate spinner.
(SEE "STEP 2: INSERTING SAMPLE INTO SPINNER")

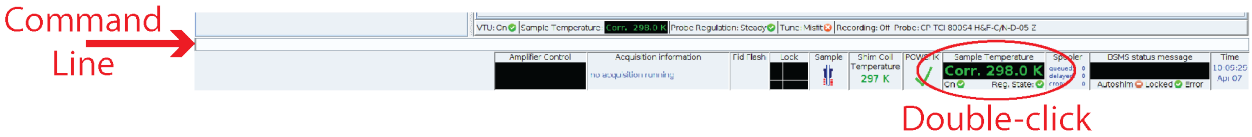
3.1) Set the sample temperature using one of the options below.

If using a temperature below 290 K *SEE "3.2) Setting BCU II"*

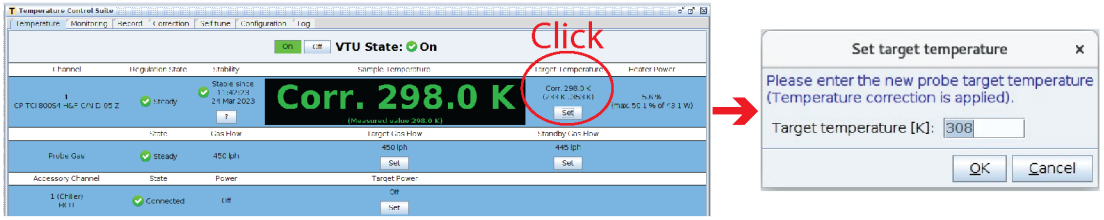
i. Type **teset n** into the command prompt line, (where **n** is desired temperature in K).

Or

ii. Type **edte** in the command line or **double-click** the "**Sample Temperature**" display icon at the bottom righthand side of the TopSpin window.



If using option **ii.**, the window below will appear.



ii$_a$. Click on the "**Set**" button below "**Target Temperature**" and the window box shown to the right (**Set target temperature**) will appear.

ii$_b$. Enter desired temperature and click "**OK**". (the color of the "**Sample Temperature**" will change. This is OK! Just wait for it to stabilize!) **(SEE COLORS BELOW)**

Corr. 298.0 K (warm), Corr. 298.0 K (cold), Corr. 298.0 K (stable)

- Make sure temperature is stable before injecting your sample.
- Allow sample to equilibrate for ~5 minutes inside the spectrometer before starting.

Once the "Sample Temperature" window returns to green, you are ready to insert your sample.

3.2) Setting BCU II

***For temperatures **below 290 K, activate the BCU II** using the protocol described below ***

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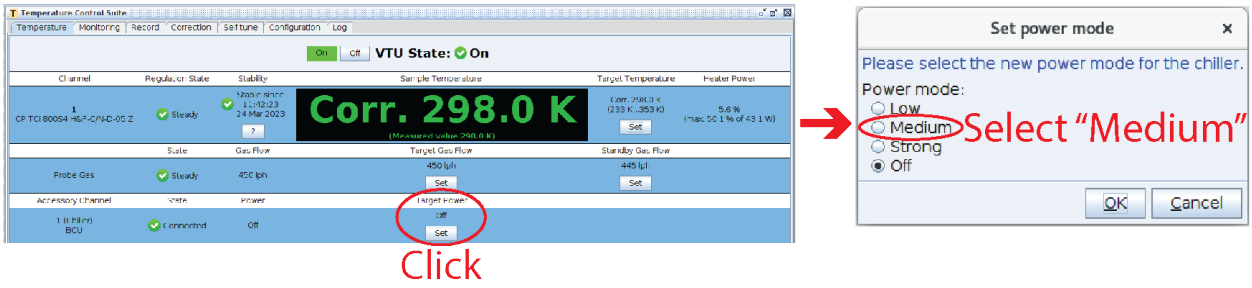
i. In the "Temperature Control Suite" window, click "Set" under "Target Power", which will open the "Set power mode" window.

If you've already closed the Temperature Control Suite Window, reopen it by typing **edte** into the command line or by double-clicking the temperature display icon.

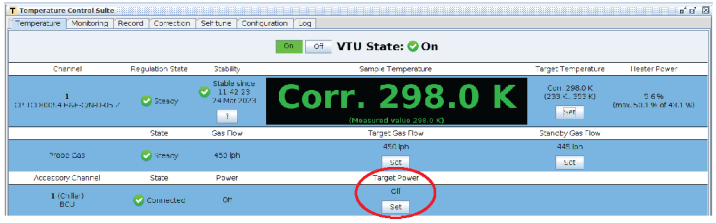
ii. Set power mode: **Medium** and click **OK**.



iii. Allow the BCU to run for several minutes (~15 minutes) to properly cool before setting up experiments.

iv. After the experiments are finished, TURN OFF the BCU II using the same protocol above, but Set power mode: **Off** and click **OK**.

Double check that the BCU II is off by referencing the "Target Power" in the Temperature Control Suite window.



Failure to turn off the BCU II can result in ice build-up and damage to the spectrometer!

STEP 4: INSERTING SAMPLE INTO NMR

4.1) Remove your sample with the spinner from the depth gauge and place it into an empty slot of the SampleCase carousel.

Record the slot number you have placed your sample in on the SampleCase Carousel.

4.2) Check that there is no sample currently in the spectrometer. If there is, remove it following the directions from **STEP 1** before injecting your sample.

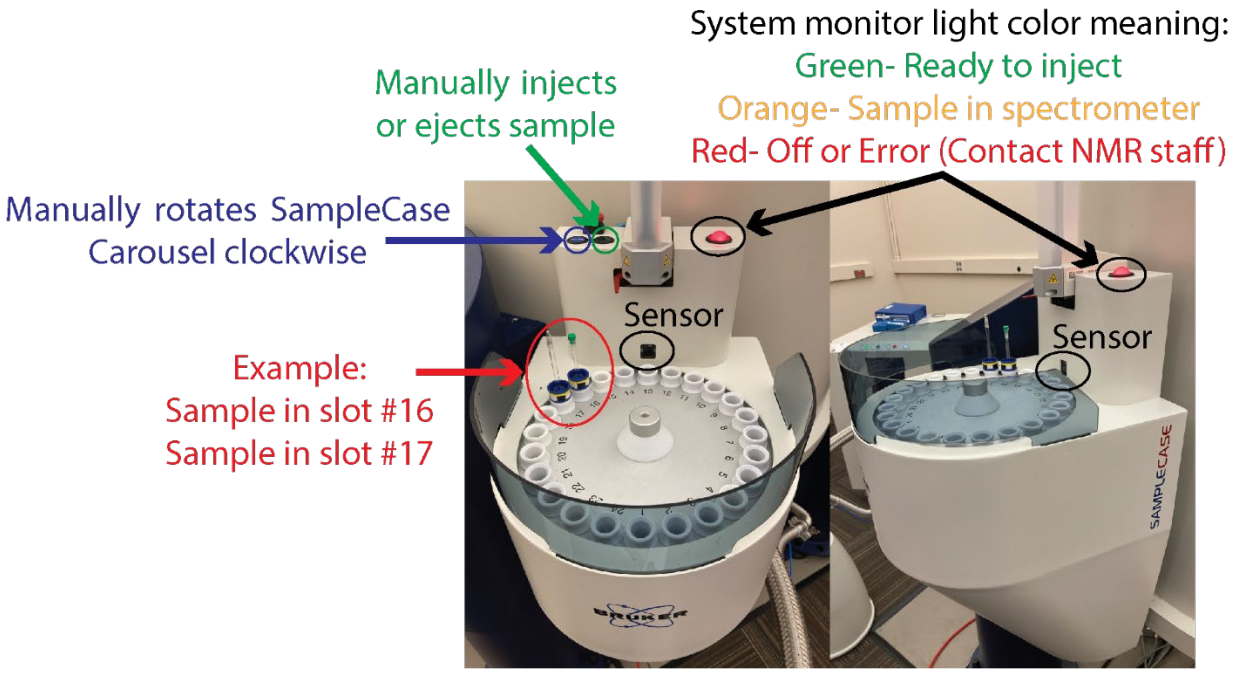
4.3) Inject your sample into the spectrometer using one of the three options provided below.

Option 1.

i. Use the Blue button (shown near the top left and next to the green button) to rotate the carousel clockwise until your sample is front of the sensor.

Each press of the blue button will rotate the carousel one position.

ii. Once your sample is in-line with the sensor a red dot will appear in the sensor (image below), at which point press the Green button to inject your sample.



Option 2.

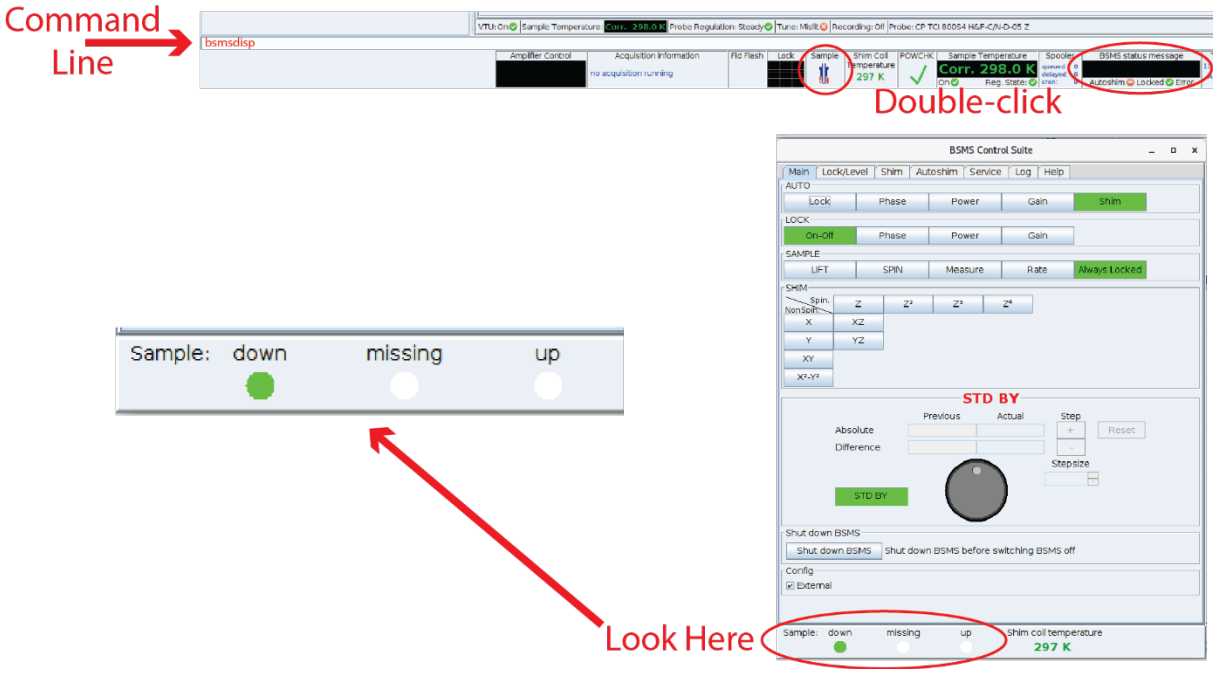
i. Use the Blue button to rotate the carousel clockwise until your sample is front of the sensor and the red sensor light comes on.

ii. Type **ij** into the command line and press enter. (**ij = inject current sample)**

Option 3.

i. Type **sx #** into the command prompt line and press enter (where # is the sample slot corresponding to your sample location in the SpinCase carousel, e.g., sx 16 in image above).

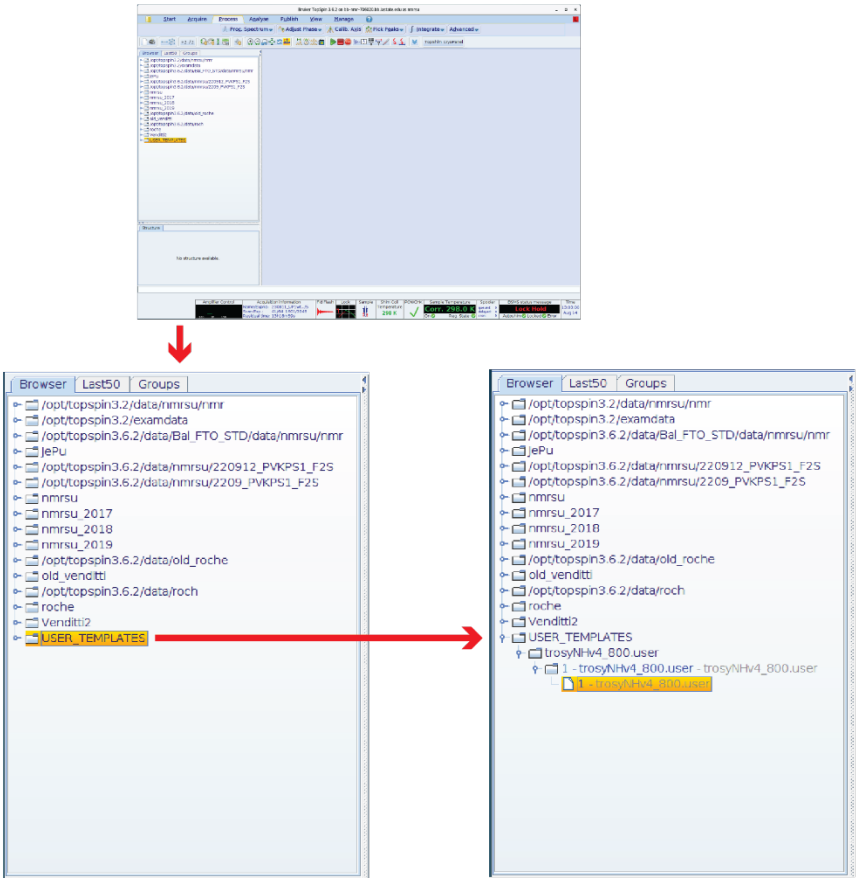
4.4) Once you have injected your sample, check that your sample is properly positioned in the spectrometer by confirming that the "down" circle is filled in the "BSMS Control Suite".



STEP 5: CREATE DATASET

There are two main methods on how to set up initial parameters. 1: Copy from a pre-existing dataset into a new one, or 2: Read in a generic parameter set. For this tutorial, we will focus on option 1. If you need an experiment that is not readily available under "USER_TEMPLATES", please contact an NMR staff member.

5.1) Begin by locating the "USER_TEMPLATES" folder under the browser section on the left-hand side of the TopSpin window (highlighted in yellow in image below).

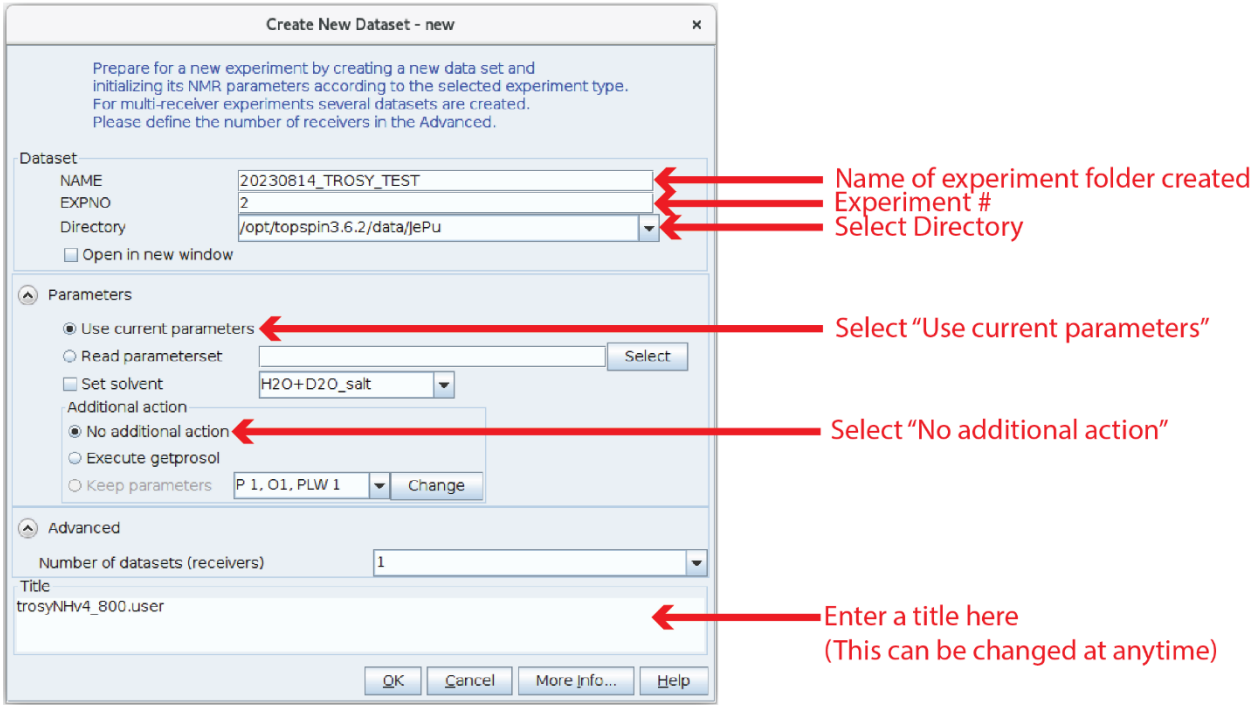


5.2) Double-click on the "**USER_TEMPLATES**" folder or click the small icon to the left of the folder. This expands the folder to show all available experiments.

For this example, only the trosyNHv4_800.user experiment is shown under "USER_TEMPLATES" to make the demonstration easier to follow.

5.3) Once "USER_TEMPLATES" is expanded, enter into the active dataset of the experiment you want by double-clicking the name of the experiment, then double-clicking the file that drops down under the experiment. (highlighted in yellow in the bottom right of image above, we have entered into the active dataset of the trosyNHv4_800.user experiment).

5.4) Once the active dataset is selected, type **edc** or **new** into the command line, or press **Ctrl+n** to open "Create New Dataset – new". (Image shown below)



5.5) Change the "NAME" to what you would like the experiment folder called.

It is best to use a format: YYYYMMDD_SampleInfo. This will organize your file tree by date and allow you to quickly find your experiment folder.

5.6) Change "EXPNO" to set the experiment # of the experiment you are creating inside the experiment folder you are creating.

5.7) Change "Directory" to store your new experiment folder in the selected directory.

For most users, this should be set to: /opt/topspin3.6.2/data/nmrsu for the 800 MHz or: /opt/topspin4.3/data/nmrsu for the 700 MHz.

5.8) Make sure "Use current parameters" is selected. (Shown in image above)

5.9) Make sure "No additional action" is selected. (Shown in image above)

5.10) Change "Title" to what you would like your experiment to be called.

The title can be changed any time by typing **edti** in the command line.

5.11) Once set correctly, click "**OK**" at the bottom of the "Create New Dataset – new".

5.12) Find your new data set via the directory selected under the file name chosen.

STEP 6: LOCKING

Now we need to lock the NMR to ensure that the strength of the magnetic field surrounding the sample does not change during the experiments.

6.1) To begin, type **lock** into the command line, which will pull up the "Solvents table".

Solvents table

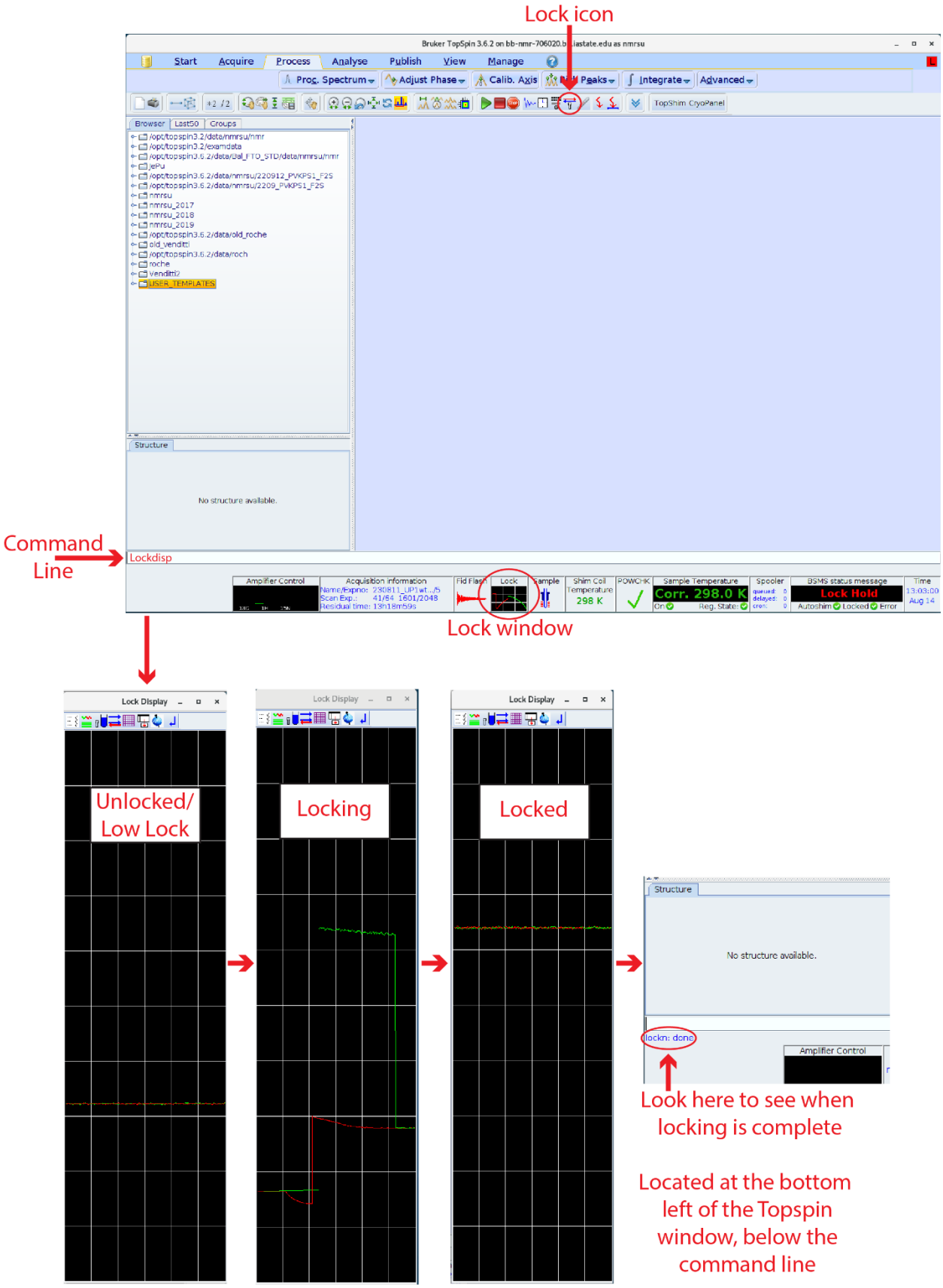
Solvent	Description
Acetic	acetic acid-d4
Acetone	acetone-d6
C6D6	benzene-d6
CD2Cl2	dichlormethane-d2
CD3CN	acetonitrile-d3
CD3CN_SPE	LC-SPE Solvent (Acetonitrile)
CD3OD_SPE	LC-SPE Solvent (Methanol-d4)
CDCl3	chloroform-d
CH3CN+D2O	HPLC Solvent (Acetonitril/D2O)
CH3OH+D2O	HPLC Solvent (Methanol/D2O)
D2O	deuteriumoxide
D2O_salt	deuteriumoxide with salt
Dioxane	dioxane-d8
DMF	N,N-dimethylformamide-d7
DMSO	dimethylsulfoxide-d6
EtOD	ethanol-d6
H2O+D2O	90%H2O and 10%D2O
H2O+D2O_salt	90%H2O and 10%D2O with salt
HDMSO	90%DMSO and 10%DMSO-d6
Juice	fruit juice
MeOD	methanol-d4
Plasma	blood plasma
Pyr	pyridine-d5
T_H2O+D2O+Me4NCl	(CD3)4NCl in 90%H2O and 10%D2O, for NMR thermometer
T_H2O+D2O+NaAc	sodium acetate in 90%H2O and 10%D2O, for NMR thermometer
T_H2O+D2O+Pivalate	pivalate-d9 in 90% H2O and 10% D2O, for NMR thermometer
T_MeOD	methanol-d4, for NMR thermometer
TFE	trifluroethanol-d3
THF	tetrahydrofuran-d8
Tol	toluene-d8
Urine	urine

OK Cancel

6.2) Click on the solvent in your sample and select "**OK**" at the bottom of the "Solvent table".

Here, we have selected "H2O+D2O_salt". This is a common selection for biological samples. However, you need to select the solvent that is appropriate for your sample.

6.3) Open the "Lock Display" window by typing **lockdisp** into the command line, Double-Clicking the "**Lock**" window at the bottom of the Topspin window, or click the **lock icon** near the top of the Topspin window.


Lock icon
Command Line
Lockdisp
Lock window
Unlocked/ Low Lock
Locking
Locked
No structure available.
lockn: done
Look here to see when locking is complete
Located at the bottom left of the Topspin window, below the command line

6.4) Use the lock display (imaged above) to follow along while the locking is performed.

6.5) Once the locking it complete, "lockn: done" will appear in the status line at the bottom left-hand side of the Topspin window. You can now move to Step 7.

If the **lock fails**, please follow the procedure below.

Unable to Lock:

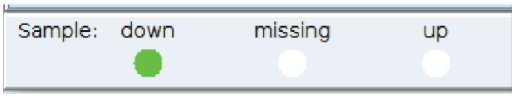
6.6) If you run into issues locking on your sample, first confirm:

i) You are using a deuterated solvent.

ii) You selected the correct solvent when locking.

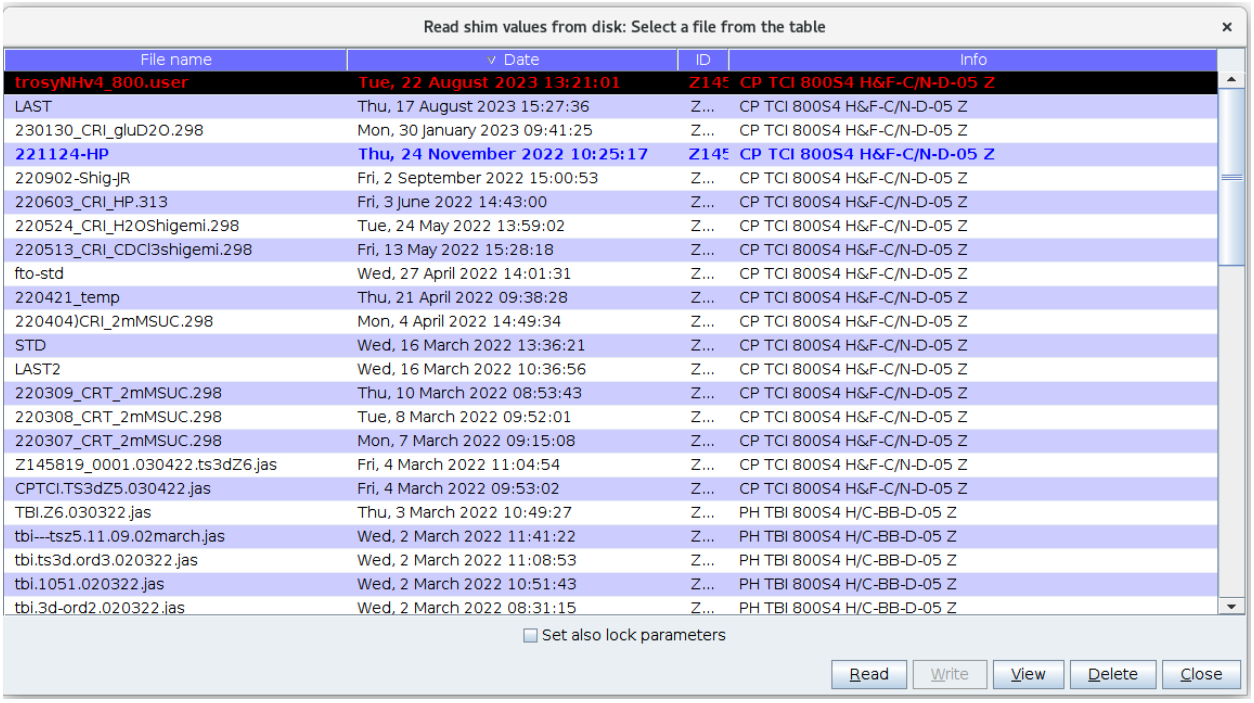
iii) The sample is properly centered using the depth gauge.

6.7) If the all answers are yes, type **bsmsdisp** in the command line to open the bsms display window and confirm that the sample is in the proper "down" position. If the sample is missing, try ejecting the sample, check the position in the depth gauge, and re-inject.



6.8) Try typing **lock off** in the command line, followed by **lock**.

6.9) Read in a previously saved shim file by typing **rsh** into the command line.



6.10) Select the file name “trosyNHv4_800.user by clicking on the file and then clicking read at the bottom. (Image above)

6.11) After reading in the “trosyNHv4_800.user” shim file, try relocking following **steps 6.1** through **6.5**.

6.12) If none of the above suggestions work, contact an NMR staff member.

STEP 7: MATCHING & TUNING

The quality of matching and tuning has a significant impact on the sensitivity. We must make sure to tune the probe circuity such that frequency at which it is most sensitive is the relevant transmission frequency. Matching will allow us to ensure the maximum amount of power arriving at the probe base is transmitted up the coil, while minimizing the amount of power that is reflected back towards the amplifiers and consequently lost.

7.1) First, you must identify what RF channels you need matched and tuned for your experiment. If you are running an experiment that uses multiple RF channels (e.g., ^{1}H-^{15}N TROSY), you must make sure the corresponding channels are matched and tuned.

7.2) The easiest way to determine this is by double-clicking into the active dataset within the directory that you created in **STEP 5** (imaged below). This will allow you to match and tune all the RF channels that are actively in the experiment you plan to run.

7.3) Once in your active dataset, the easiest way to match and tune is by typing **atma** into the command line and pressing enter.

This will perform an automated matching and tuning for all RF channels in the active dataset. However, the **matching and tuning can often be improved manually!**

7.4) To manually match and tune, type **atmm** into the command line and press enter. This will open the "AtmaControl" and the matching and tuning window in Topspin (imaged below).

7.5) Within the "AtmaControl" window, click the circle under "Nucleus" labeled "1H"

7.6) Use the arrow keys under "Tuning" and "Matching" within the "AtmaControl" window to manually match and tune. (Please refence the images two pages down).

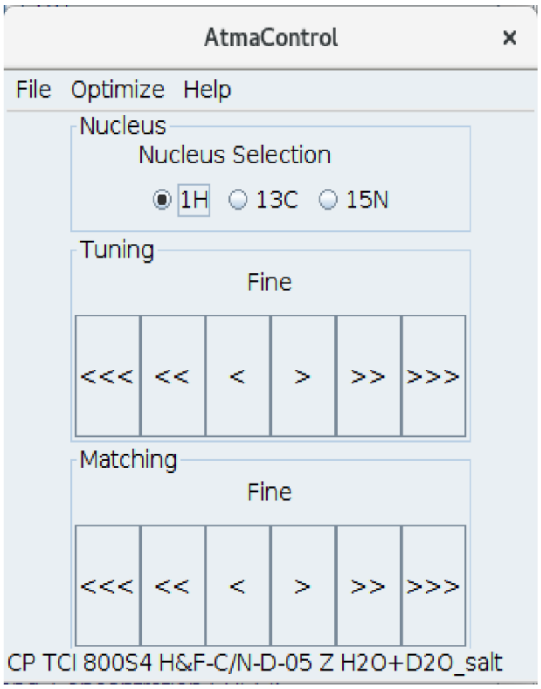
7.7) After matching and tuning, click "File" in the "AtmaControl" window and select "Save Position".

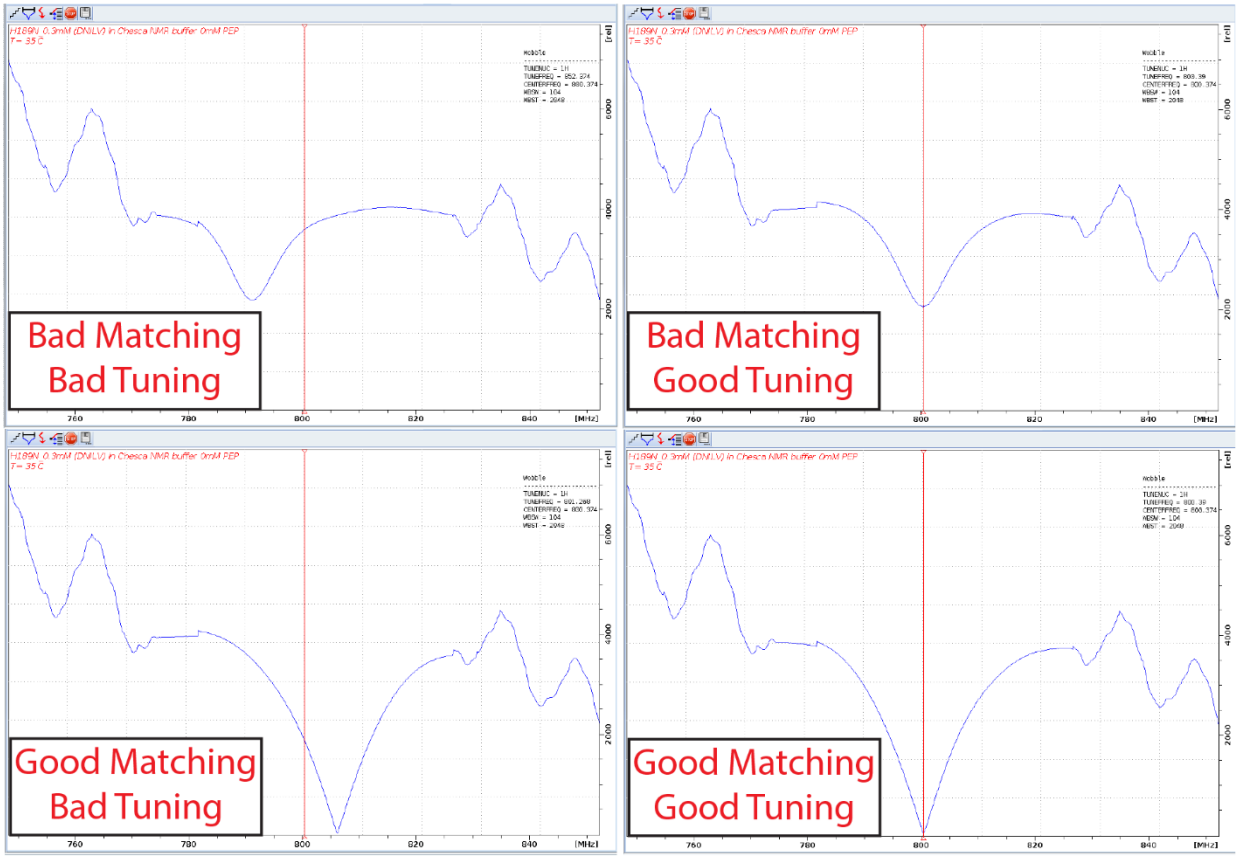
7.8) Repeat Steps 7.5 to 7.7 for each Nucleus in the "AtmaControl" window.

7.9) After all nuclei have be manually matched and tuned, go back through each nucleus. The matching and tuning adjustments of a nucleus interact with the matching and tuning of the other nuclei. You may need to repeat steps 7.5 to 7.7 two or three times for perfect matching and tuning of each nucleus.

7.10) Please refence the image below to see what a combination of good and bad matching and tuning looks like. The goal is to make the matching and tuning of all nuclei similar to the bottom right display of the image below.

7.11) Once all Nuclei positions are saved, you may close the atmm function by clicking the "x" in the top right corner of the "AtmaControl" window.

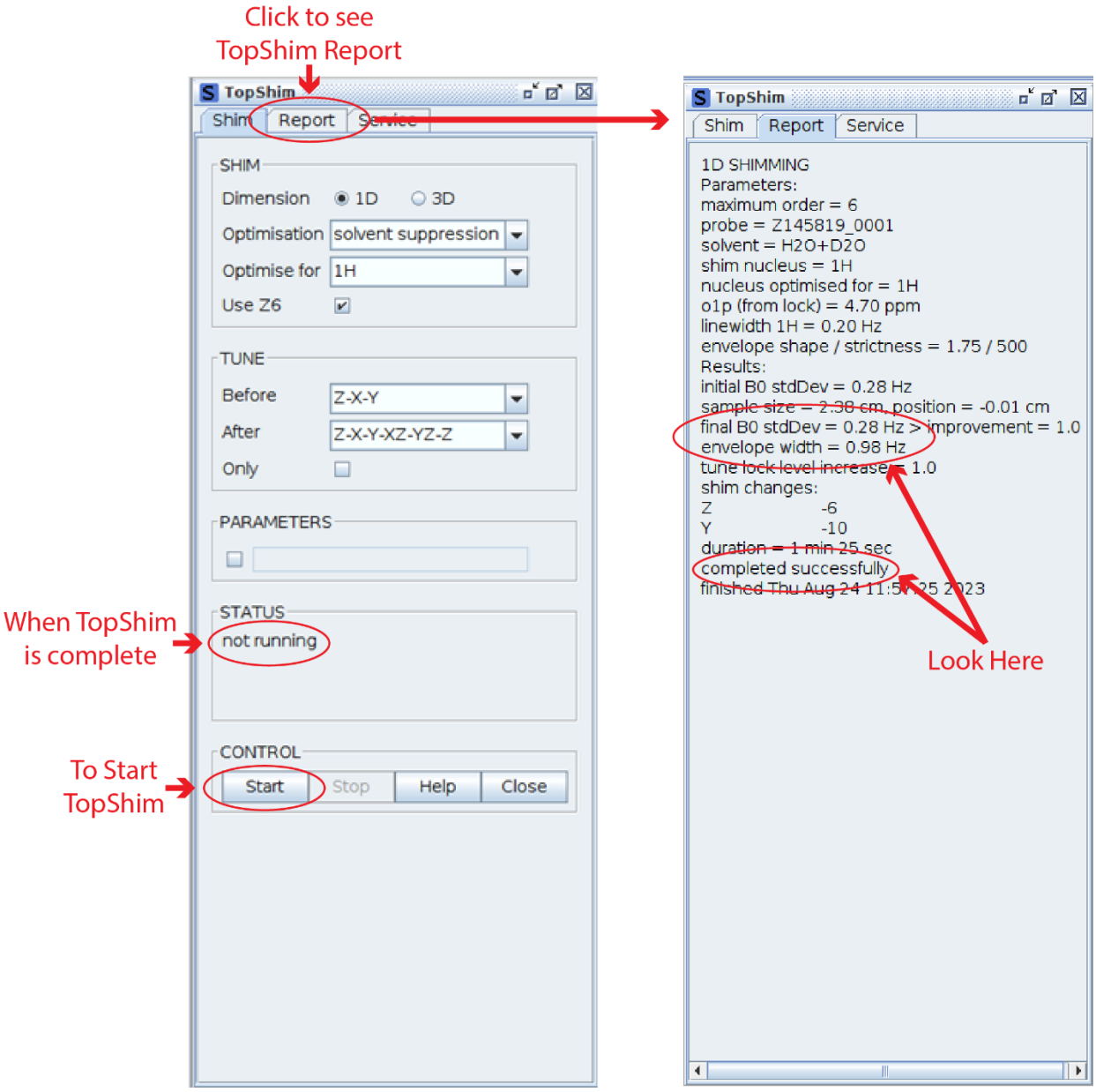

AtmaControl
File Optimize Help
Nucleus
Nucleus Selection
1H 13C 15N
Tuning
Fine
<<< << < > >> >>>
Matching
Fine
<<< << < > >> >>>
CP TCI 800S4 H&F-C/N-D-05 Z H2O+D2O_salt


Bad Matching
Bad Tuning
Bad Matching
Good Tuning
Good Matching
Bad Tuning
Good Matching
Good Tuning

STEP 8: SHIMMING

Next you must shim the sample in order to produce a homogenous magnetic field along the sample volume to obtain Lorentzian/Gaussian line shapes of different resonance in the spectrum you will be acquiring.

8.1) Type **topshim gui** into the command line. This will open the "TopShim" window shown in the left image below. The TopShim report (right image) will be discussed below.

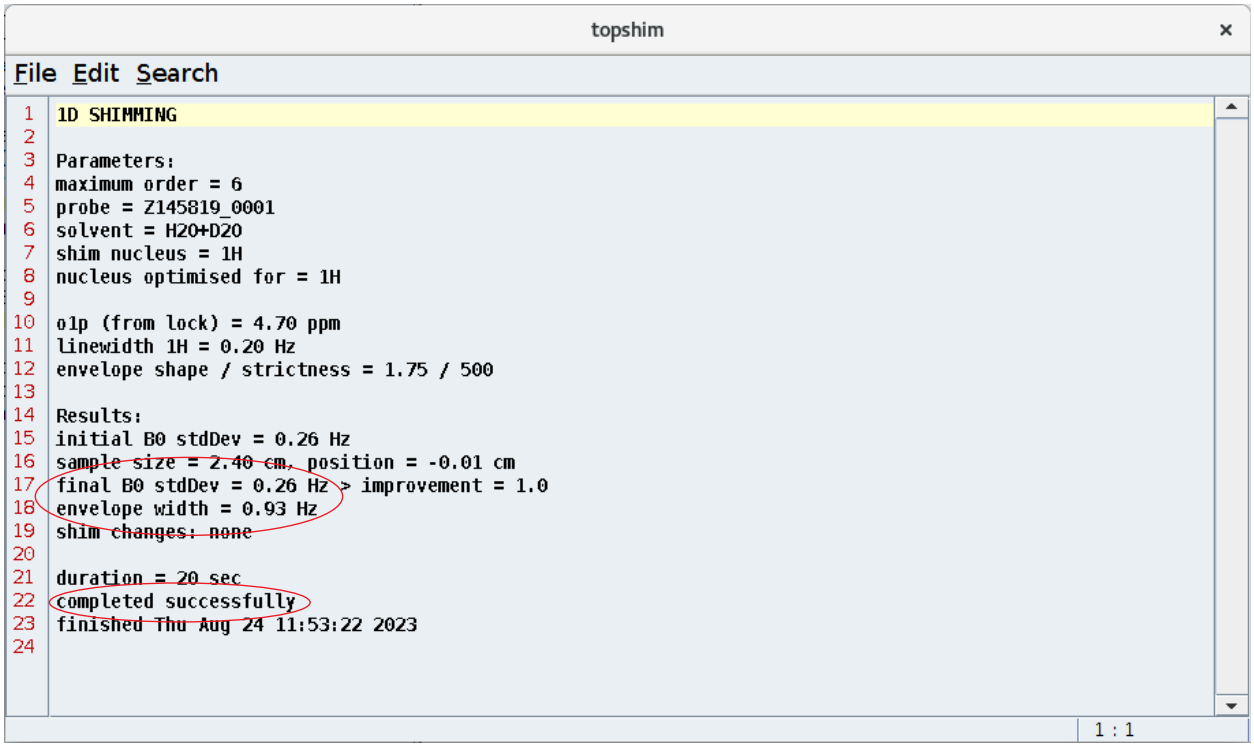


8.2) Check that your "TopShim" window matches that of the image shown above and press "**Start**" located at the bottom of the "TopShim" window.

8.3) When TopShim is complete you will see "not running" under "STATUS" in the "TopShim" window. (Do not close the window yet)

8.4) Check the results of the shimming by clicking the "Report" tab at the top of the "TopShim" window (report image shown above).

8.5) Alternatively, you can type **topshim report** into the command line to open the report file for the last shim (image below).



8.6) In either of the shim reports, look at and confirm:

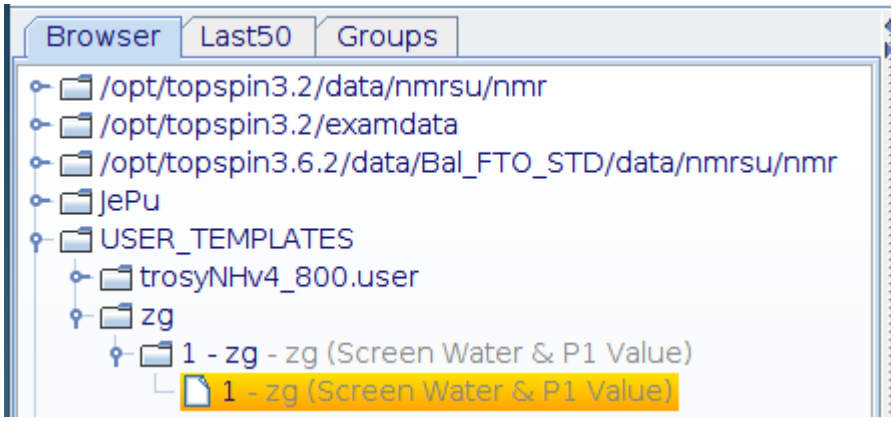
- **i.)** Final B0 stdDev ≤ ~0.50 Hz
- **ii.)** Envelope width ≤ ~1.00 Hz
- **iii.)** "completed successfully" (near the bottom).

8.7) If your final B0 stdDev or envelope width are not within the above recommended values, try rerunning topshim using **Tips to Improve Bad Shimming** (**Step 8.16**) found below.

8.8) If your final B0 stdDev and envelope width are within the recommended values, proceed to Step 8.9.

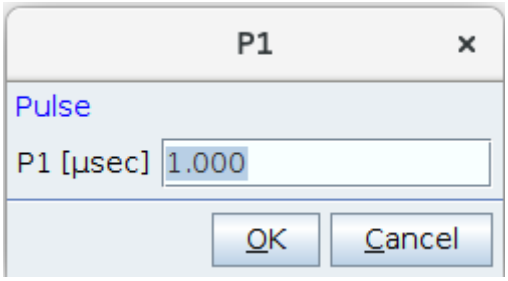
8.9) In additional to checking the final B0 stdDev and envelope width, it is a good idea to look at peak profile of water to evaluate the shim quality.

8.10) Referencing steps 5.1 to 5.12, copy the "zg" experiment from "USER_TEMPLATES" into your working directory.



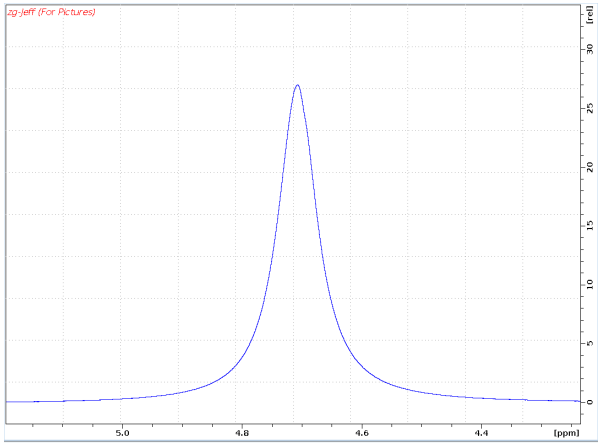
8.11) In your directory, find the newly created "zg" experiment and double-click into the experiment to make it the active dataset.

8.12) Type **p1** into the command prompt line and make sure it is set to 1 µs.



8.13) Once the p1 is set to 1 us, type **zg** into the command line to collect a single scan proton 1D.

8.14) Once zg is complete, type **fp** into the command line, then **apk** to get the image below.



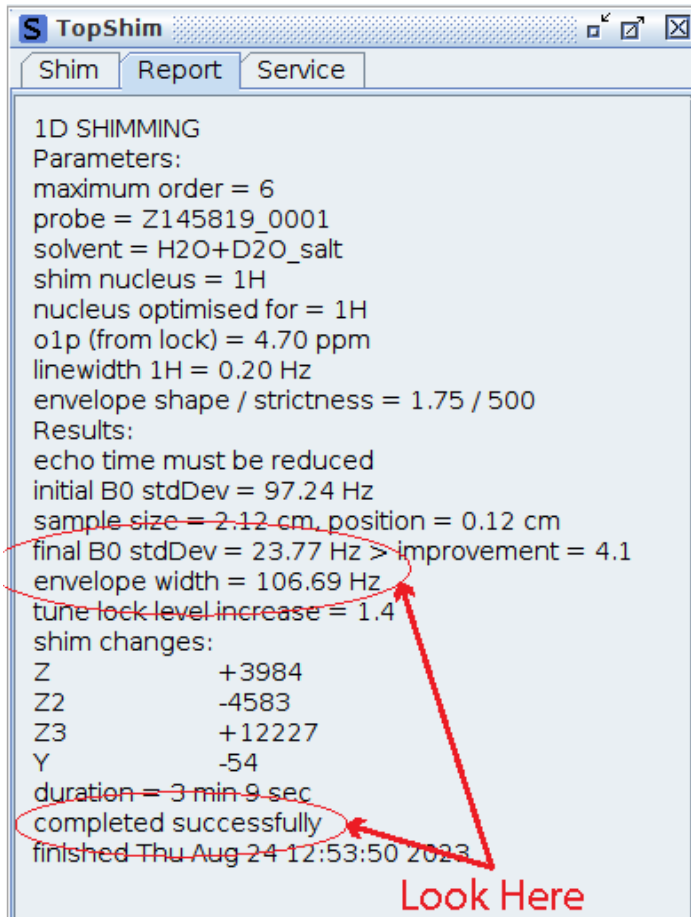
8.15) Note how the water peak is symmetrical, indicated a good shim. If you water peak looks similar to the image above, you are finished shimming and ready to proceed to **Step 9**.

Tips to Improve Bad Shimming:

Sometimes it can be challenging to get good shimming. Let's look at an example of bad shimming.

Here, a sample was intentionally positioned incorrectly into the depth gauge such that the sample did not cover the **3/5/8 mm** marking (reference Step 2.5).

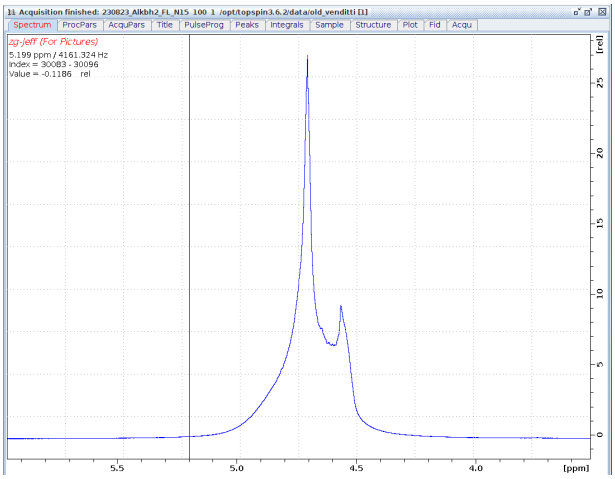
When TopShim was performed, the results below were seen.



Even though TopShim "completed successfully", we can see by looking at the final B0 StdDev and envelope width that we have very poor shimming.

Sometimes TopShim will fail altogether, this will show in place of "completed successfully".

If we follow **Steps 8.9** to **8.14** to look at the water peak profile, we get the image below.



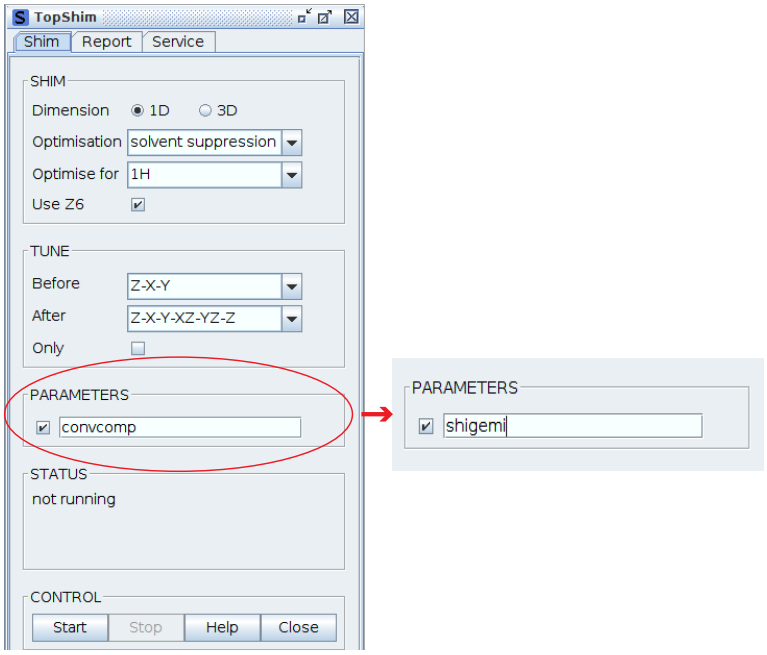
We see an asymmetric peak profile that tells us that the shimming is very poor.

This example is dramatic and you likely won't see a peak this distorted, but it is good practice to evaluate the B0 StdDev, envelope width, and the water peak profile.

Please see steps below for suggestions on how to improve samples with poor shimming.

8.16) Double check that the sample is correctly positioned in the depth gauge. From the above example, the importance of the sample covering the coil markings on the depth gauge can be seen.

8.17) If this doesn't help, you can try to use arguments in the "PARAMETERS" box within the TopShim Window.

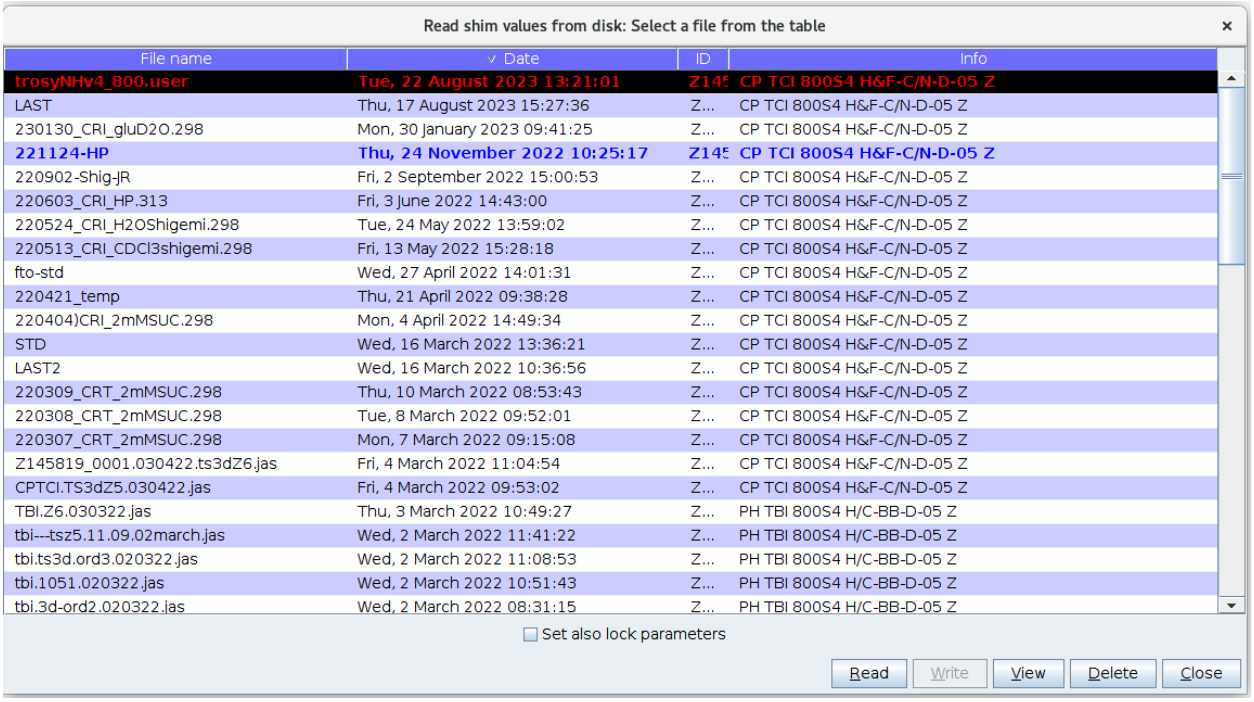


8.18) In the image above, the argument "**CONVCOMP**" or "**shigemi**" was used.

8.19) Try rerunning TopShim using **CONVCOMP**. If you are using a shigemi tube, then the **shigemi** argument may be better.

8.20) If you B0 final StdDev and envelope width are still not in the ideal range, you can try loading in a previous shim set (much like we discussed for locking, **Step 6.9**.

8.21) Type **rsh** into the command line.



8.22) Select the file name "trosyNHv4_800.user by clicking on the file and then clicking read at the bottom. (Image above)

8.23) After reading in the "trosyNHv4_800.user" shim file, try rerunning TopShim following **steps 8.1** through **8.8**.

8.24) If none of the above suggestions work, contact an NMR staff member.

Experiments can still be run with "non-ideal" shimming. If you are having trouble shimming but still want to acquire the experiment, an NMR staff member can help you manually shim your sample to help achieve the best results.

STEP 9: CALIBRATING O1

The O1 (Hz) or O1P (ppm) is important because it is the offset frequency (middle of the spectrum in the proton dimension) and is used to determine the spectral width (SW). Additionally, the O1/O1P is used for solvent suppression. For example, water is the most common solvent for protein NMR and as such the O1P is set to ~4.7 ppm to effectively suppress the water signal.

9.1) You should already have a "zg" experiment copied into your directory and STEPS 8.10 to 8.14 completed. You can use the water profile from this to determine your O1/O1P.

9.2) Start by doubling-clicking into your previously collected "zg" experiment to make it the active data-set and to open the image profile of the water peak.

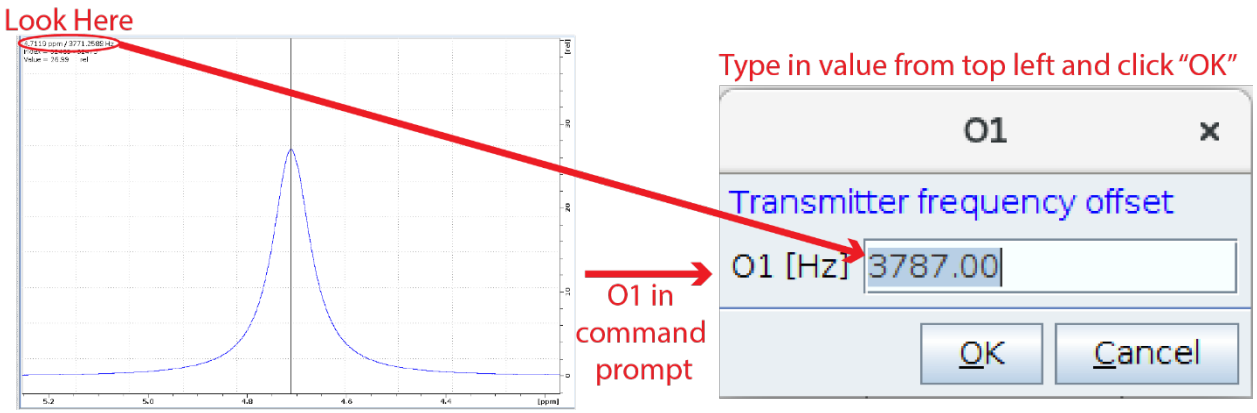
9.3) Using your mouse click and drag from one side of the water peak to the other to zoom in.

9.4) Now determine the O1/O1P using one of the methods below.

OPTION 1:

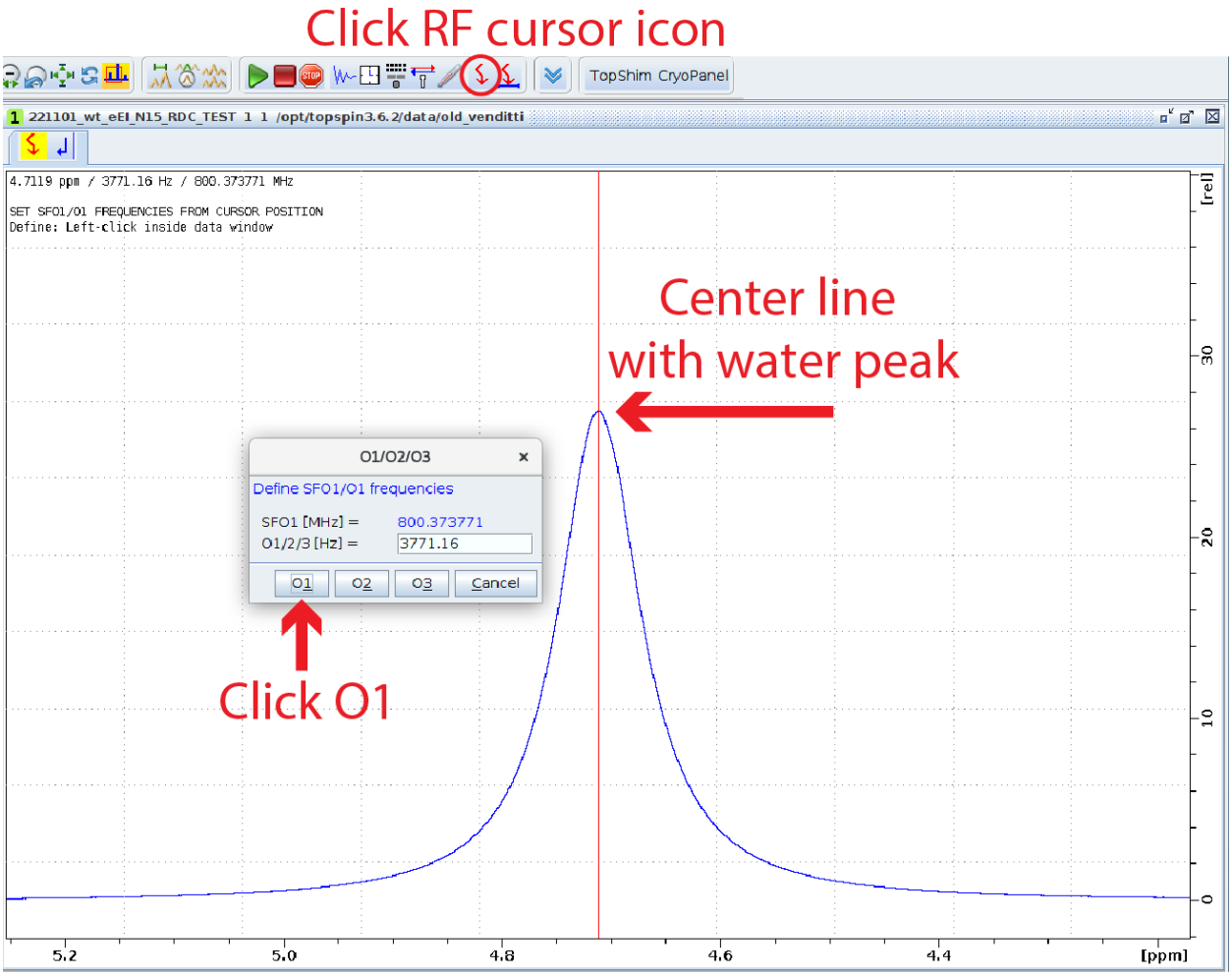
i. Place your mouse cursor over the center of the water peak (image shown below).
ii. Identify the O1/O1P value located at the top left of the image when the mouse is centered on water.
iii. Type "**O1**" or "**O1P**" into the command prompt line.
iv. Enter your recorded value and press "OK"

Take caution to correctly enter your value. If you type O1 into the command prompt, make sure to use the value in Hz. If using O1P, use ppm.



OPTION 2:

i. Click on the icon located at the top of the TopSpin window.
ii. Place your mouse over the center of the water peak and click.
iii. Once the "O1/O2/O3" window opens, click "O1" to set the O1.



9.5) Once you have recorded the O1 value in the "zg" experiment, copy the O1 value into the experiment you wish to run within your directory

9.6) Double click into the desired experiment to activate the dataset

9.7) Type "O1" into the command prompt line

9.8) Enter the new O1 value and click "**OK**".

Take caution to correctly enter your value. If you type O1 into the command prompt, make sure to use the value in Hz. If using O1P, use ppm.

STEP 9: ^{1}H PULSE CALIBRATION (P1)

The P1 represents the pulse length for the proton 90° pulse. Setting a correct P1 is important for maximum excitation/signal. This value is mainly dependent on the effective salt concentration in your sample. This can be done by allowing TopSpin to calculate it for us or by doing it manually using the water resonance at the O1P value determined above.

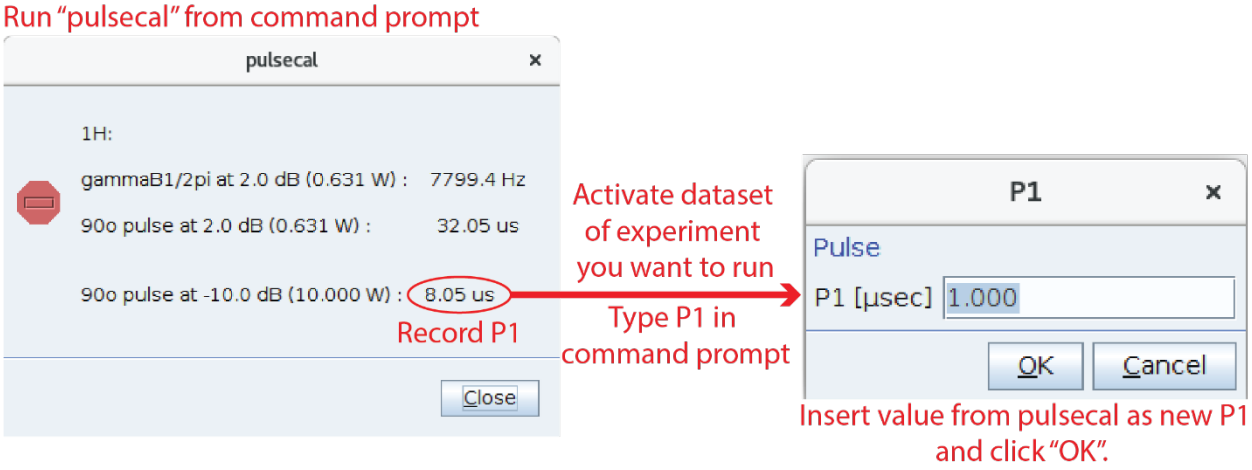
10.1) Calibrate p1 using one of the methods below.

OPTION 1:

i. Following the directions provided in **STEP 5**, create a new "zg" experiment in your directory with an arbitrary experiment number (e.g., 1000).
ii. Activate the new dataset by double clicking into the new "zg" experiment.
iii. Type "**pulsecal**" into the command prompt and hit "Enter".
iv. When pulsecal is complete, a window named "pulsecal" will appear.
v. Record the duration at the bottom of the pulsecal window for "90° pulse at -10.0 dB (10.000 W).

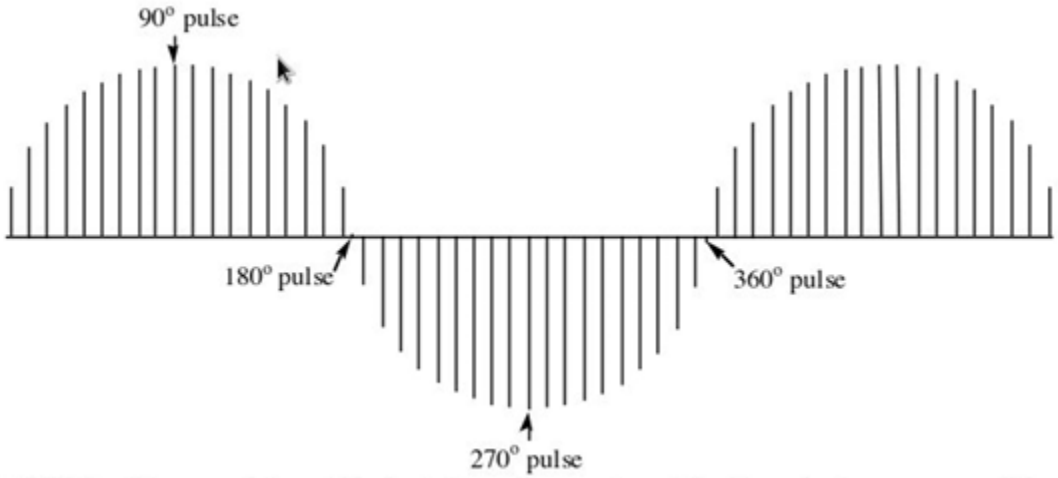
The value -10.0 dB is spectrometer dependent. The power for the 90° proton pulse could be different for different spectrometers.

vi. Activate the dataset of the experiment you want to run by double-clicking into it.
vii. Type "**p1**" into the command prompt line and hit enter.
viii. Enter the recorded P1 value from pulsecal as the new p1 value and click "**OK**".



OPTION 2:

Here we will calibrate the P1 using the 360° pulse duration of the water resonance at the recorded O1 in **STEP 9**.



There are two advantages determining the 360° pulse over a 90° pulse.

1) Once the P1 is close to the 360, the water magnetization returns to the positive z-axis, requiring only a short equilibration delay.

2) Looking at the image above, it can be seen that it is visually more straightforward to identify a zero transition (360°), than a maximum (90°) in a sine curve.

For the reasons above, we will determine the 360° pulse and then divide by 4 to get the P1.

i. Start by double-clicking into the "zg" experiment in your directory to activate the dataset.
ii. Type "**p1**" in the command prompt line and press enter
iii. Enter a starting value for a 360° pulse length in the P1 window and press "OK".

The 360° pulse length is dependent on the size of the NMR tube (3mm or 5 mm) you are using and the salt concentration of your sample. You can use a starting value of 32 us or 48 us for a 3mm or 5 mm NMR tube, respectively.

iv. Type "**zg**" into the command prompt line and press enter to run the experiment.
v. Type "**fp**" into the command prompt line and evaluate the water peak.

If the P1 value is too high, this will result in a positive water signal. If the P1 value is too low, this will result in a negative water signal. For a perfect 360° pulse, the spectrum will look almost like a flat line with some wiggles at the position of the water signal. (reference image above, and look at image below for an example of a calibrated 360° pulse).

vi. If the water signal is positive, type "**p1**" into the command prompt line.

vii. Lower the value of p1, press "OK".

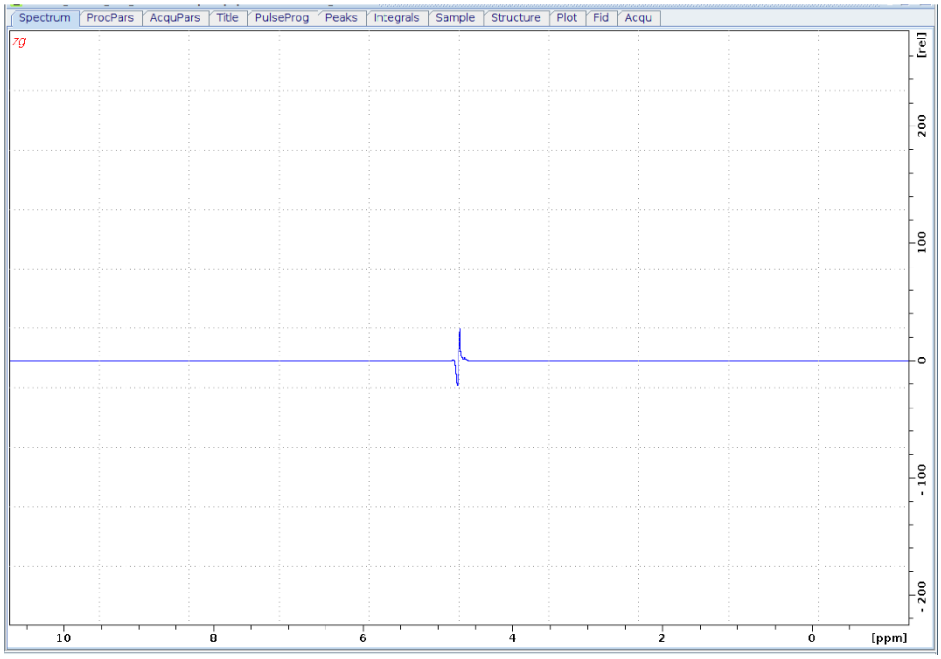
viii. Rerun the experiment by entering "**zg**" into the command prompt line.

ix. Once "**zg**" is complete, type "**fp**" into the command prompt line and press enter to reevaluate the water peak at the new 360° pulse length.

x. If the water signal is negative, complete steps vii. to ix., but increasing the p1 value.

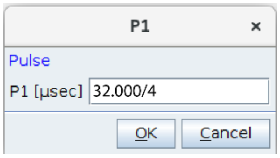
xi. Repeat steps vi. to x. until you see something to the image provided below.

When adjusting the p1 value, start by changing the value by 1-2 us. Once the p1 is getting close to a 360° pulse, you can perfect the pulse length by changing the p1 by 0.1 to 0.5 us steps.



xii. Once the p1 is set to a perfect 360° pulse, type "**p1**" into the command prompt line and press enter.

xiii. Divide the p1 value by 4 by adding "**/4**" in the P1 window display after the value of you 360° pulse and pressing "**OK**". (see image below for example)



xiv. Reenter "**p1**" into the command prompt line and record the P1 value.

xv. Activate the dataset of the experiment you want to run by double-clicking into it.

xvi. In the experiment dataset, enter "**p1**" into the command prompt and enter.

xvii. Enter the p1 value obtained from calibration and press "**OK**".

xviii. You now have a calibrated P1 and are ready to move on.

(To continue, find the corresponding document for your experiment on the BNMRF website)

Please direct any questions, concerns, or comments to bnmrf@iowastate.onmicrosoft.com.