How to Process T_1 Relaxation Measurement Data and Obtain T_1 Values Using Topspin 3

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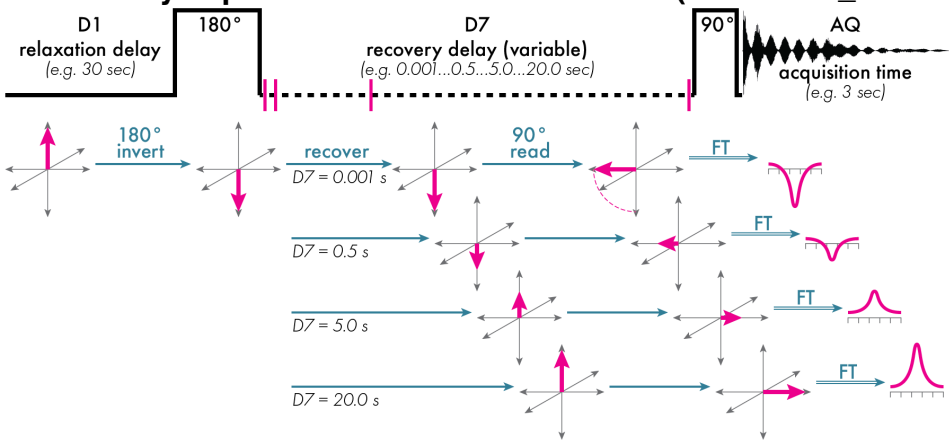
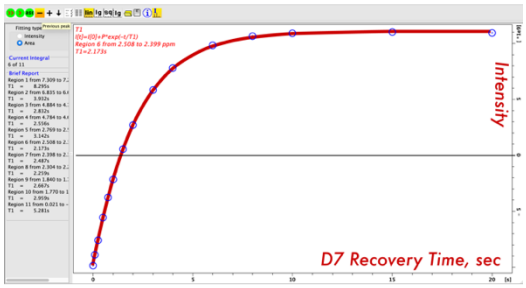
Motivation

The accuracy of all quantitative NMR methods, including simple integration, relies on full relaxation of all analyzed spins between scans. Your D1 value should be set to approximately FIVE times the longest T_1 of interest, but to do that you must know your T_1 values. You may *acquire* data automatically, but you must *process* it manually. Here is the preferred UChicago processing method using Topspin version 3. ^{1}H data is shown here, but the same procedure is used for data from other nuclei. Data acquisition is not covered here.

The Inversion-Recovery experiment for T_1 measurement ("PROTON_T1" for ^{1}H)



Experiment Description



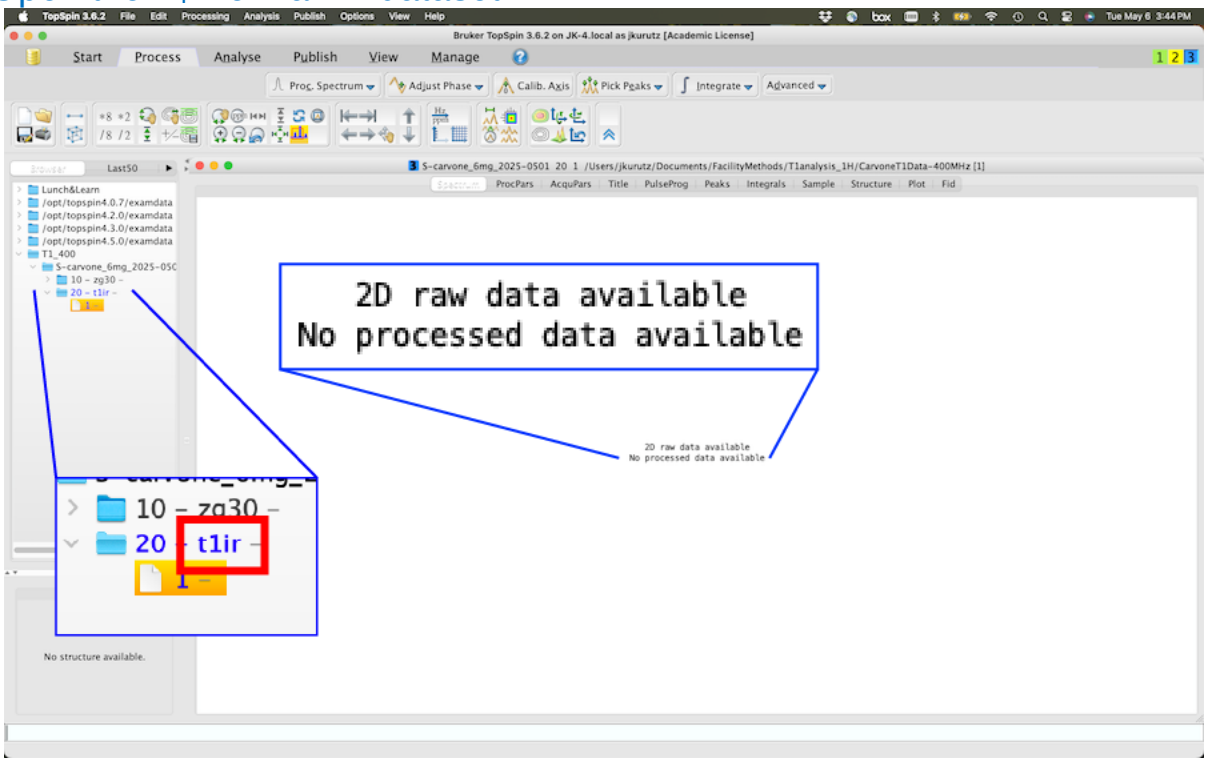
With all spins fully relaxed along the +Z axis, a 180° pulse ("inversion") is applied, aligning them along -Z. In the recovery period, they relax so their intensities gradually return to the +Z axis. The signs and intensities of their magnetizations at the end of recovery depend on the length of the recovery delay, D7, AND the rate of relaxation, R_1 ($1/T_1$). A 90° pulse is then applied to put the magnetizations in the X-Y plane, where they can be observed. Data are acquired with a small array of D7 values, and after Fourier transform, spectra are phased so the spectrum with the smallest D7 has negative peaks, and the one with largest D7 has positive peaks. Integrals of all peaks of interest are plotted as a function of D7, then fitted to obtain the T_1 relaxation times of all integrated peaks.

Summary of steps to process data and make measurements

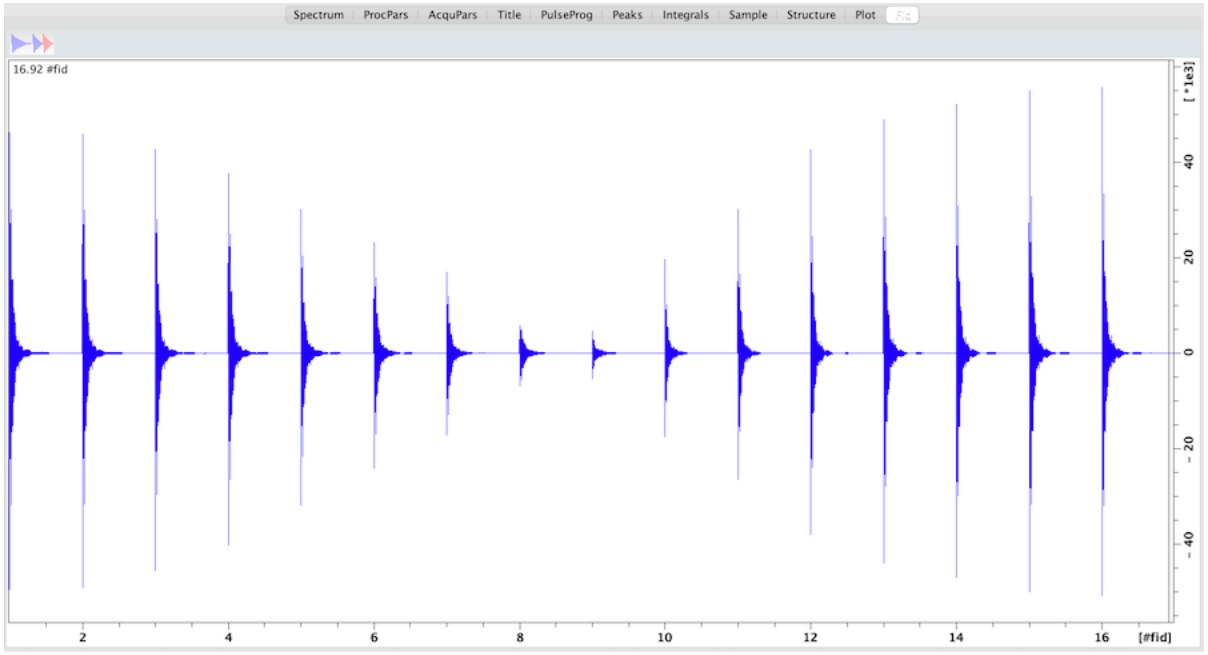
1. Open the t1ir dataset and check the number of FIDs
2. Perform Fourier transform in the F2 dimension
3. Perform manual 2D phase correction in F2
4. Perform automatic baseline correction in F2
5. Calibrate/reference your chemical shifts
6. Use the T1/T2 analysis tool to measure T_1 values
    a. Select the last spectrum in the series (longest D7)
    b. Define integral regions manually
    c. Enter the Relaxation Window and confirm Relaxation parameters
    d. Check "Fitting", parameters, specify "Area" for measurement
    e. Perform the data fitting with the Calculate tools
    f. Check T_1 values for reasonableness, inspect quality of data fitting
7. Generate a report and save it as a text file

VIDEO: These instructions are also described in our YouTube video: https://youtu.be/gAdC_n_pryo

1. Open the T_1 file – a 2D dataset

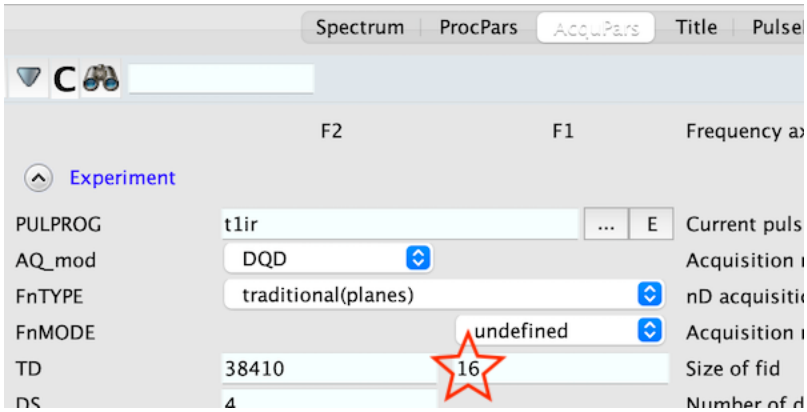


a. Notice in the data browser that your T_1 experiment is annotated with “t1ir”, and is colored blue to indicate that it is 2D data
b. Notice the text “2D raw data available”
c. Click the “FID” tab to see the FIDs



d. Notice the intensity shrinks and grows. This is normal. You may not see a common “null” in the middle if your peaks have a wide variety of T_1 relaxation rates.
e. Count the number of FIDs to be sure. The default at UChicago is **16**.

f. In the Acqupars tab, check the TD value in the F1 column. This is the number of FIDs that was intended to be acquired. It should match the number of FIDs you actually acquired. It should be **16** at UChicago, and we will assume it is **16** for the rest of this protocol.



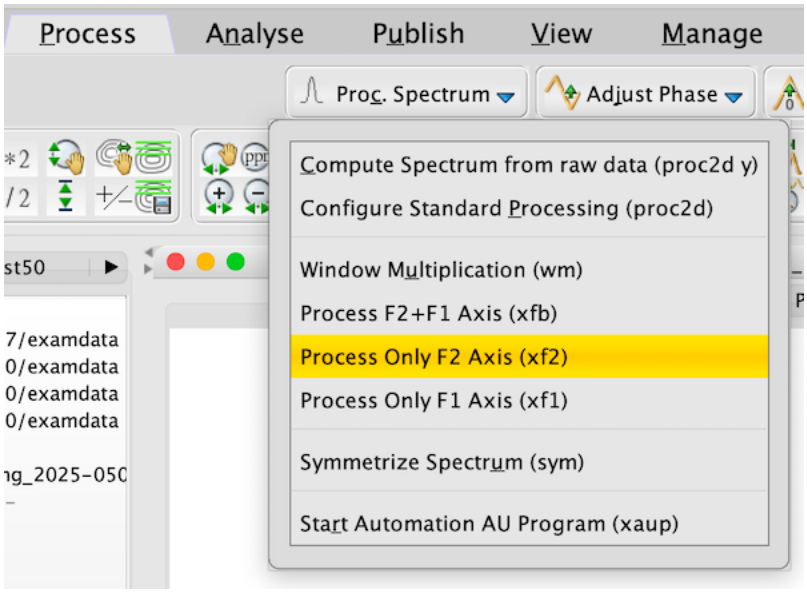
2. Fourier transform the 2D dataset

a. Specially process the 2D so ONLY the directly-detected dimension ("F2") is Fourier-transformed. CHOOSE i or ii:

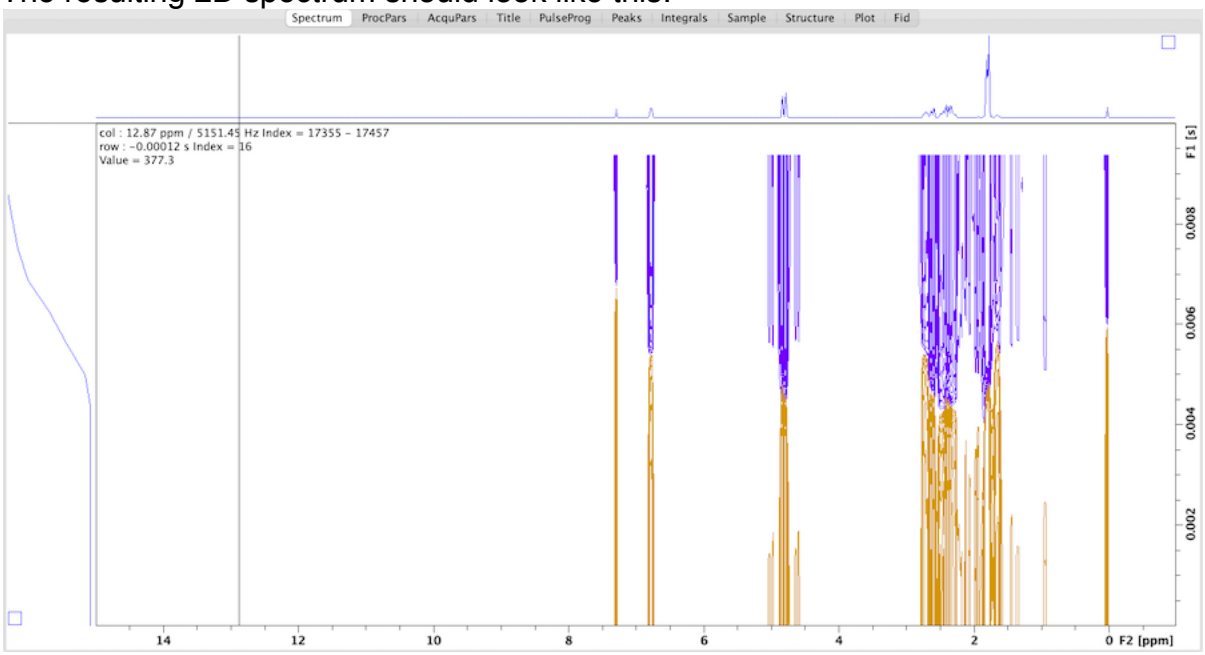
i. Pull down the "Process" button menu to "Process Only F2 Axis (xf2)"

OR

ii. Type "xf2" on the command line



b. The resulting 2D spectrum should look like this:



c. Key features to notice about this view:

i. ***AXES:*** *The X axis is chemical shift, but the Y axis units are not meaningful. Imagine you've stacked all 16 1D spectra (each with its own D7 recovery delay) along the Y axis, and you're looking at the assembly from above.*

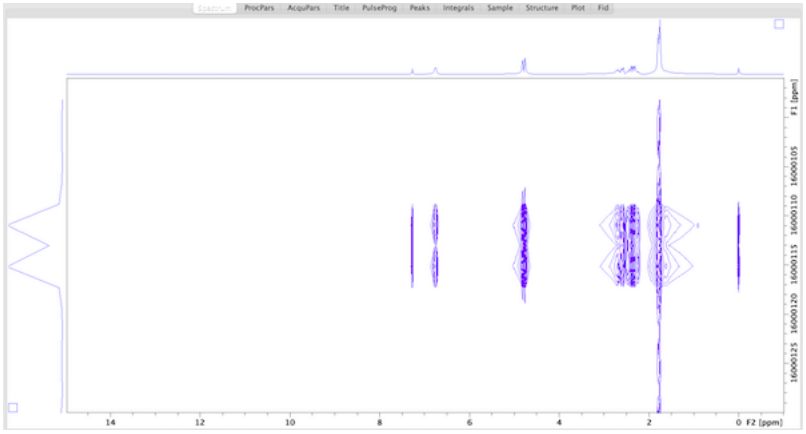
ii. ***TOP PROJECTION:*** *The projection at the top of the display is something like the sum of all your individual 1D peaks' positive intensities, not your hi-res 1D.*

iii. ***SIDE PROJECTION:*** *The projection at the left is something like the sum of all peaks' recovery curves, but only positive intensities are represented.*

iv. ***SIGN/COLOR CONVENTION:*** *In this 2D plot gold = negative intensity, and purple = positive. (Bruker's default is normally red = negative and blue = positive.) With UChicago default parameters, the first D7 delays (the bottom rows) are short (< 0.5 sec), which should give rise to negative intensity in the T_1 inversion-recovery experiment. Likewise, the last few D7 delays are long (> 5 sec), and their intensities should be positive.*

v. ***NULLS:*** *In the middle, as you step from small D7 values to larger ones, you should observe nulls where negative intensities transition to positive. You can expect the nulls for different peaks to be different.*

d. CHECK: If you processed by clicking the “Process” button or typing “xfb”, you’ll get an unusable spectrum that looks like this:

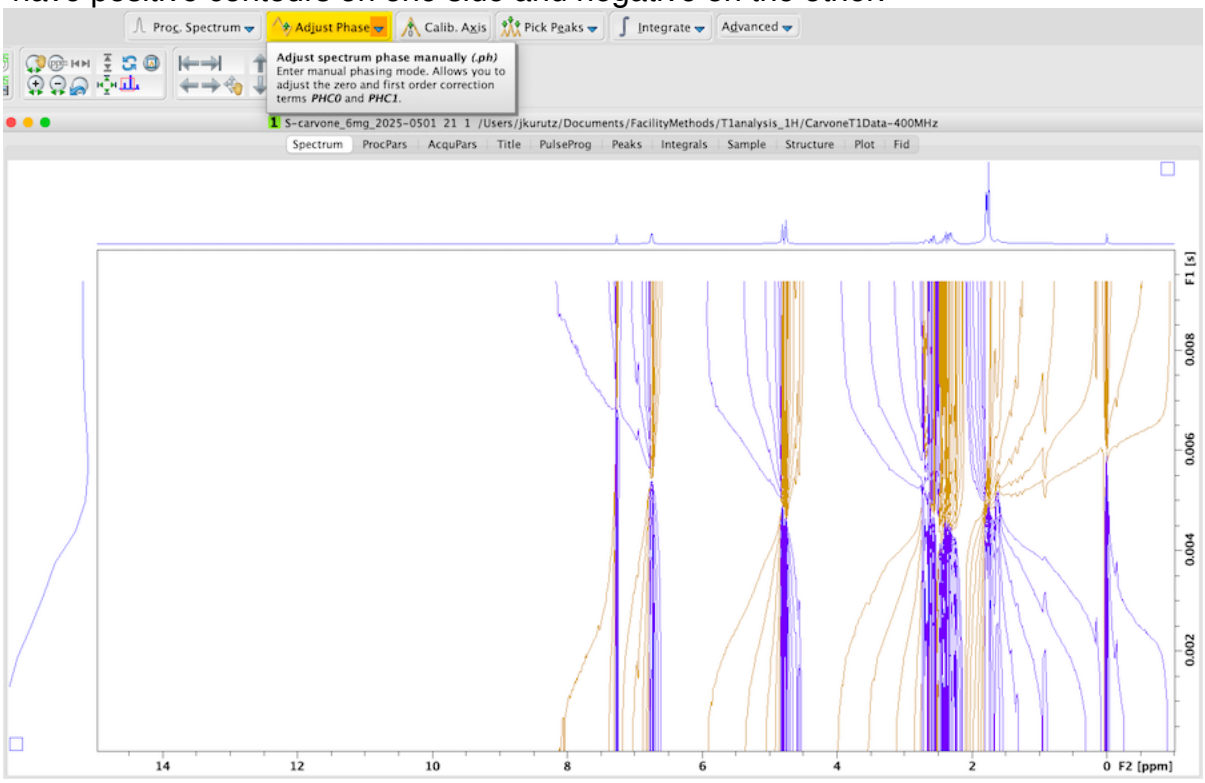


If your spectrum looks like this (peaks bunched up in the vertical center), you need to go back and perform the “xf2” Fourier transformation.

3. Perform manual 2D phase correction in F2

This is the recommended method for phasing T_1 datasets. It’s convenient to treat the 2D T_1 data like any other phase-sensitive 2D data and correct its phase accordingly. Bruker’s method, described at the end of this document, works, but it is complex and unique to processing relaxation data.

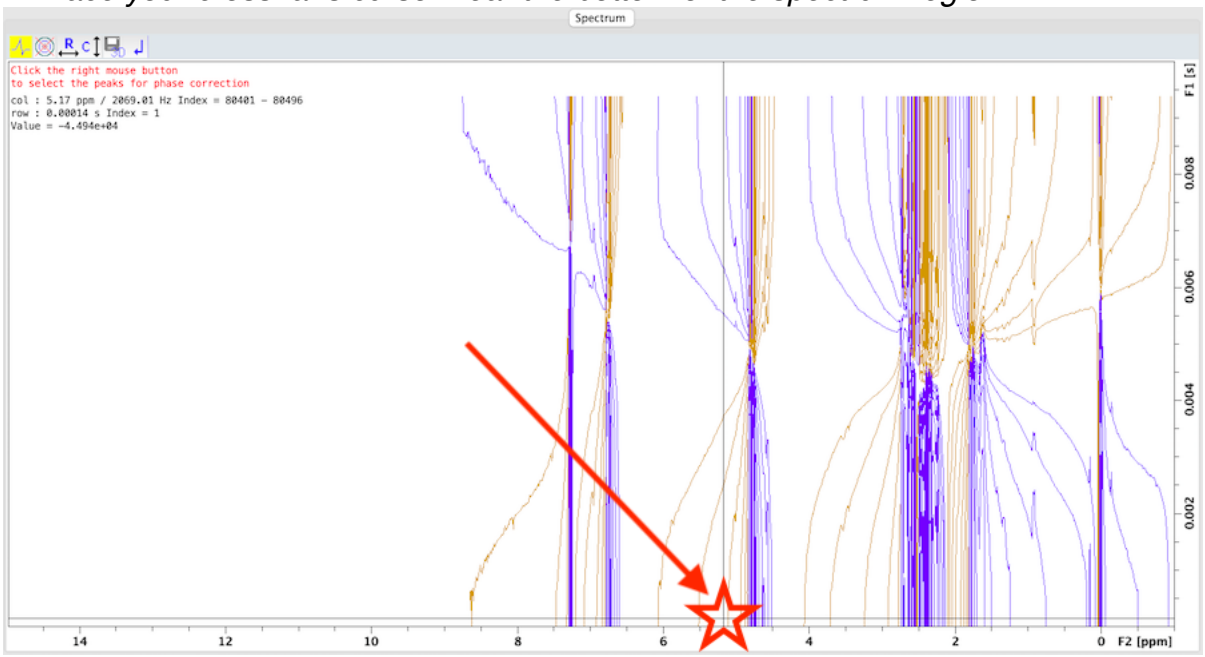
a. Scroll up to increase your 2D intensity until you see some noise and other contours around your peaks. You can tell this needs phase correction because most of the peaks have positive contours on one side and negative on the other.



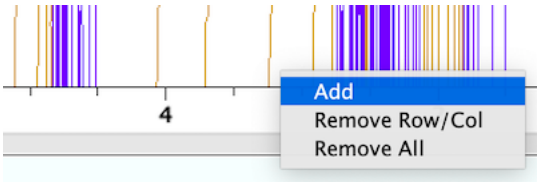
Click the “Adjust Phase” button to enter the phase adjustment tool.

b. Unlike the familiar 1D manual phasing tool, the 2D tool requires a two step process:
   i. *Selecting one or more horizontal slices of your 2D spectrum*
   ii. *Performing the familiar manual 1D phasing, but doing so on all selected 1D spectra simultaneously. For T_1 analysis, however, we only need one slice/row.*

c. Select the first 1D spectrum of the dataset. With UChicago defaults, the first 1D should be all negative peaks, and the last should be all positive. You may choose the last and ensure the peaks are positive, but here we choose the first.
   i. *Place your crosshairs cursor near the bottom of the spectrum region:*

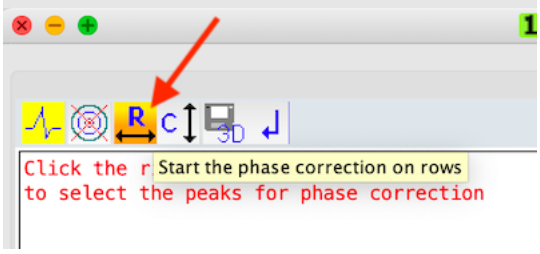


   ii. *Next, right-click your mouse and select "Add" to add that position to the list for phasing:*

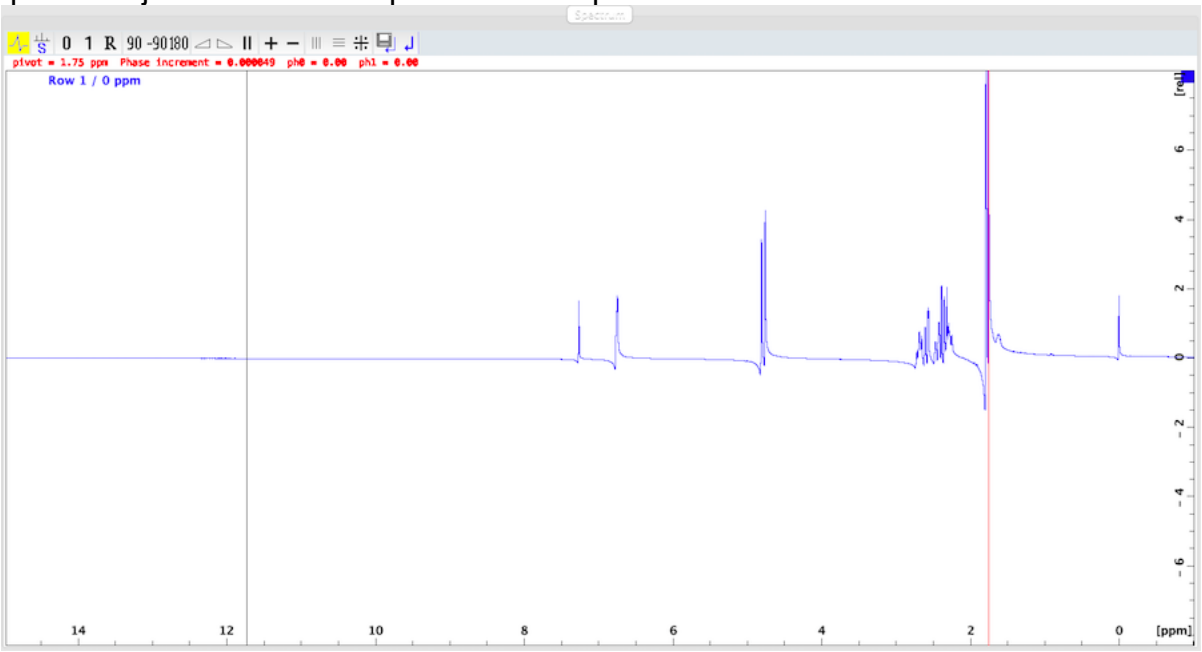


   iii. *For T_1 analysis, you just need the one 1D slice. If you were phasing a 2D HSQC or NOESY spectrum, however, you would be placing your crosshair at the centers of two or three peaks, total. Same routine for each: position the cursor, right-click…Add, repeat.*

d. Click the "R" button in the phasing tool to specify you want to phase the ROW (horizontal) corresponding to your "added" cursor position(s).

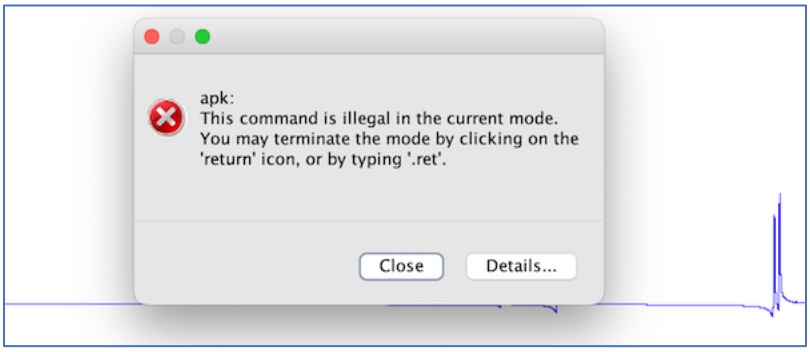


e. Now you'll enter the usual 1D phasing tool. Proceed normally with manual phasing. Starting on the next page, this protocol includes a refresher on manual 1D phasing in Topspin. If you are already comfortable with manual phasing in Topspin, just make your phase adjustments and skip ahead to step *h*.



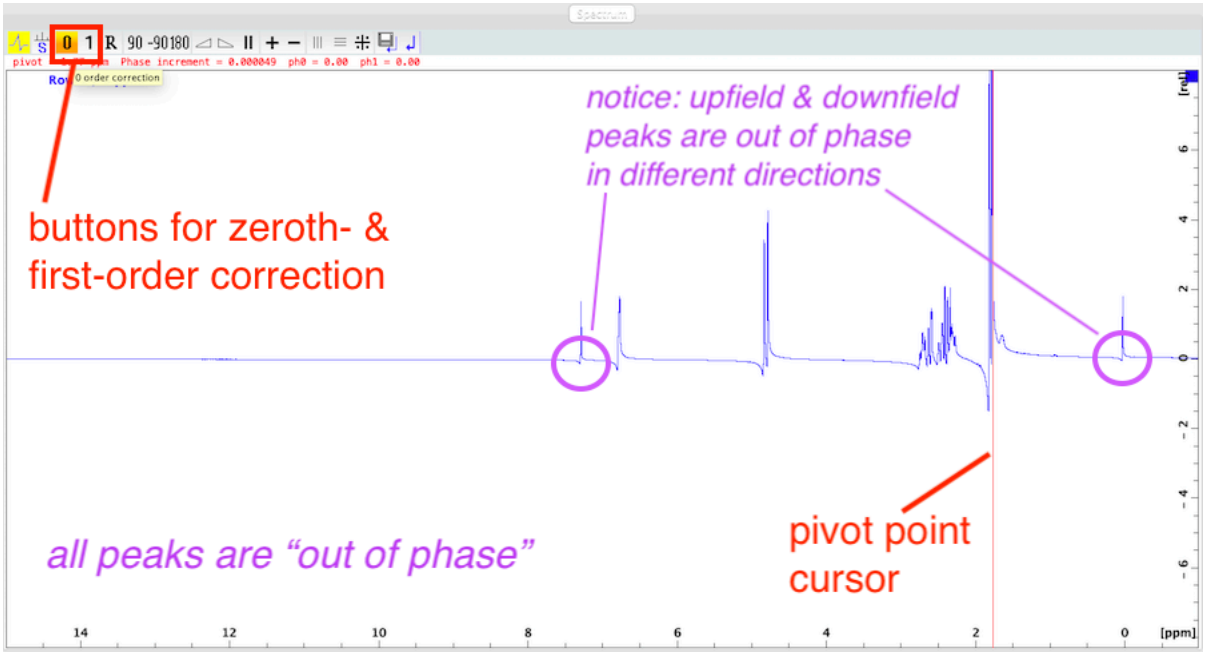
i. (If you had selected/added two or three spectra in the previous step, you'll see two or three 1D spectra. Applied phase adjustments will apply equally to all spectra simultaneously.)

f. Don't try to phase the spectrum automatically here ("apk" command). You'll see this error message:



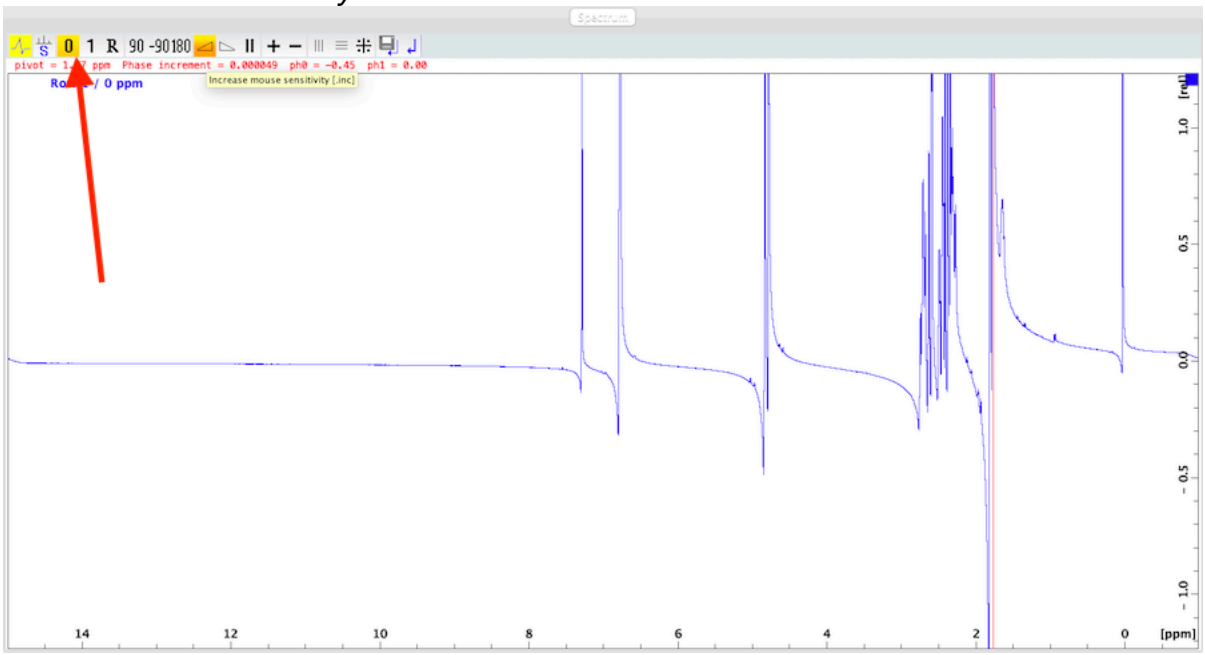
g. OPTIONAL REFRESHER ON MANUAL PHASING:

i. Orientation:

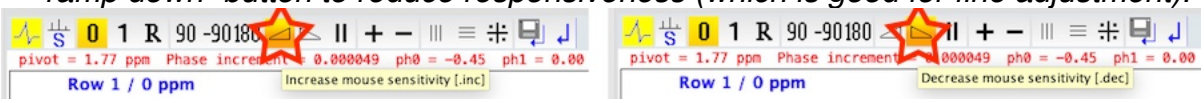


- Notice all the peaks look twisted at the base. This is called being "out of phase". Also notice that the direction of the "twist" here is different on opposite sides.
- The main buttons to adjust phasing are the "0" and "1" highlighted in this figure. They correspond to making "zeroth-" and "first-order" phase corrections, respectively.
- The vertical red cursor is called the "pivot point" and is special.

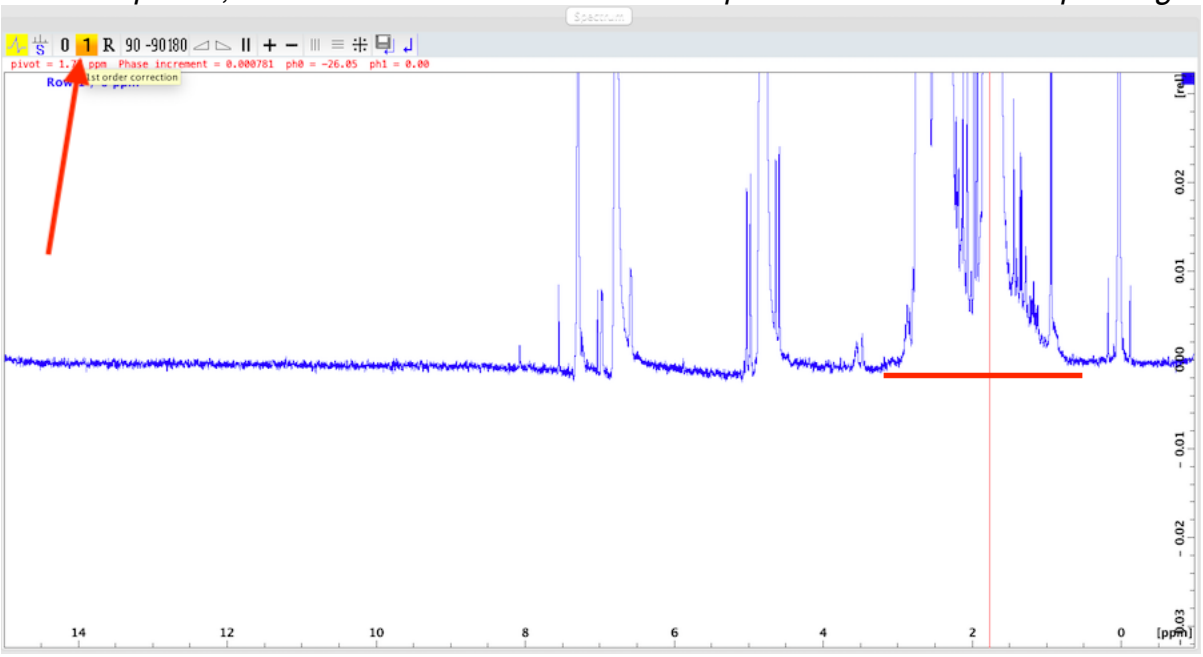
ii. Scroll up to observe the baseline more closely and try adjusting the zeroth-order phase. Place your cursor over the "0" button, click-and-hold on it, and move your mouse up and down WHILE HOLDING THE LEFT BUTTON DOWN. This is an unusual physical move, and you'll notice the cursor jiggle instead of relocating, which is weird the first time you see it.



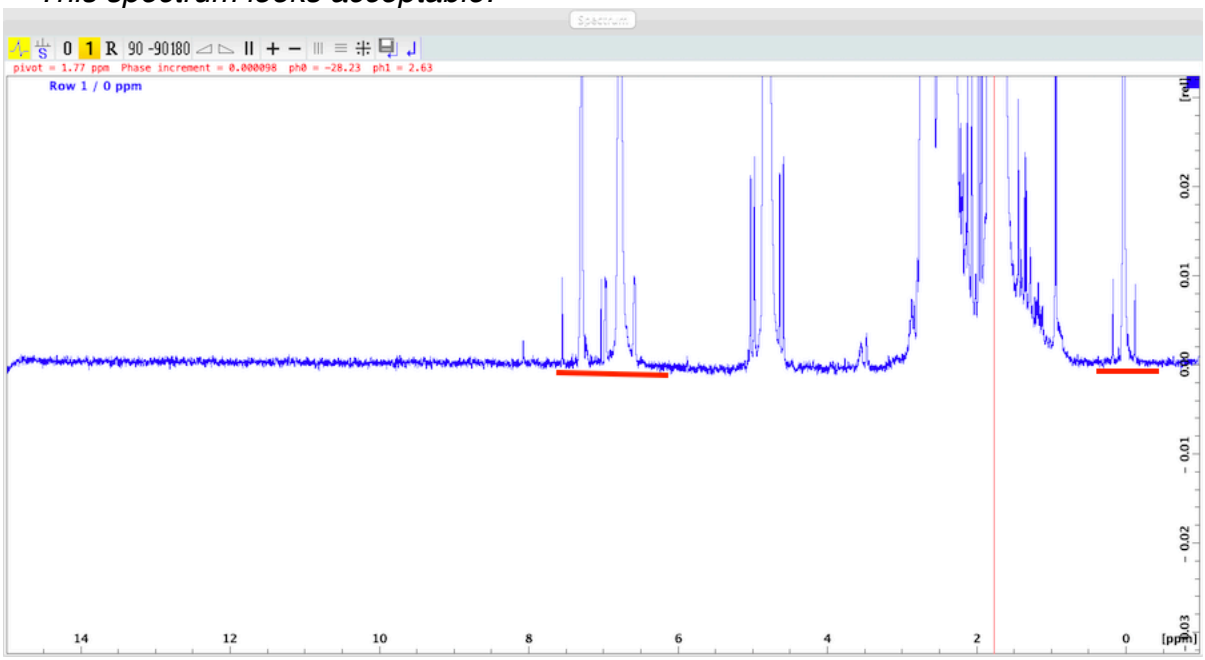
iii. *If not much happened to your spectrum when you moved your mouse, you probably just need to click the "ramp up" button a few times to increase the mouse responsiveness. Likewise, if you encountered too much adjustment, then click the "ramp down" button to reduce responsiveness (which is good for fine adjustment).*



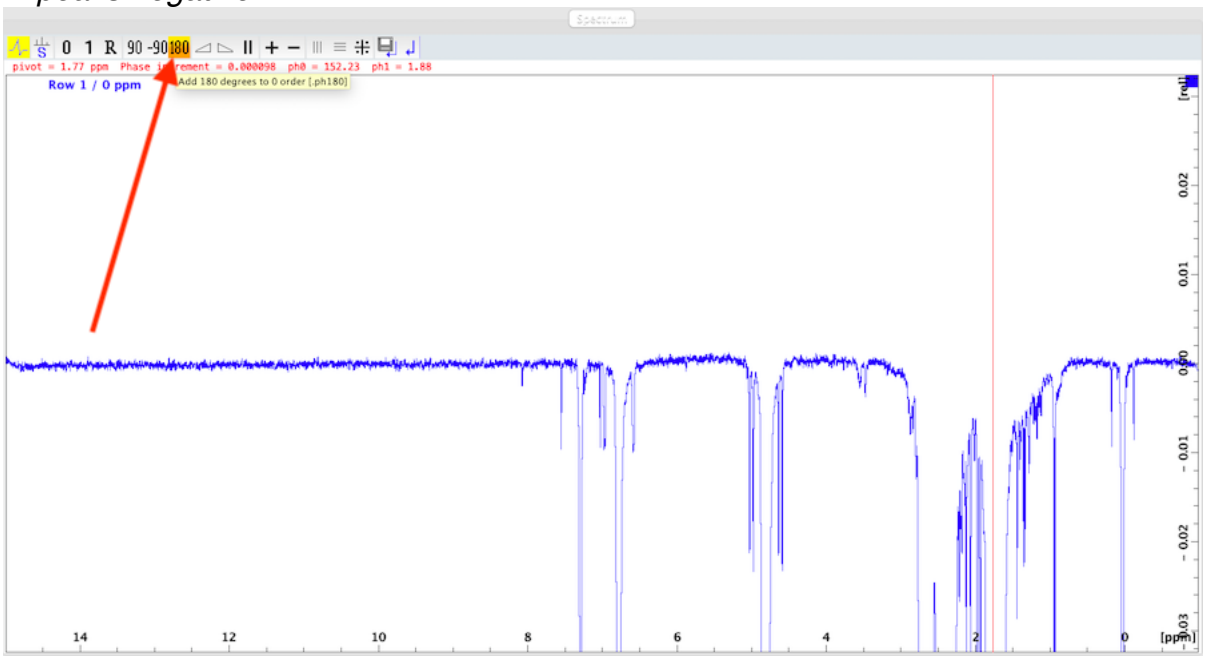
iv. *Adjust your zeroth-order phase until the peak region under the pivot point looks properly phased (or close to it). The baseline on one side of the peak should basically match up with the baseline on the other side. Do not worry about the phasing of peaks away from the peak under the pivot point. Here we see the peak under the pivot point is acceptable, so we can click the "1" button and proceed with first-order phasing.*



v. *Adjust your first-order phasing until the peaks at the right and left edges of your spectrum look properly phased. Don't worry if the overall baseline looks a little tilted or curved as long as the baseline on either side of every peak matches up. You may need to iterate back and forth between zeroth and first order phasing a couple times. This spectrum looks acceptable:*



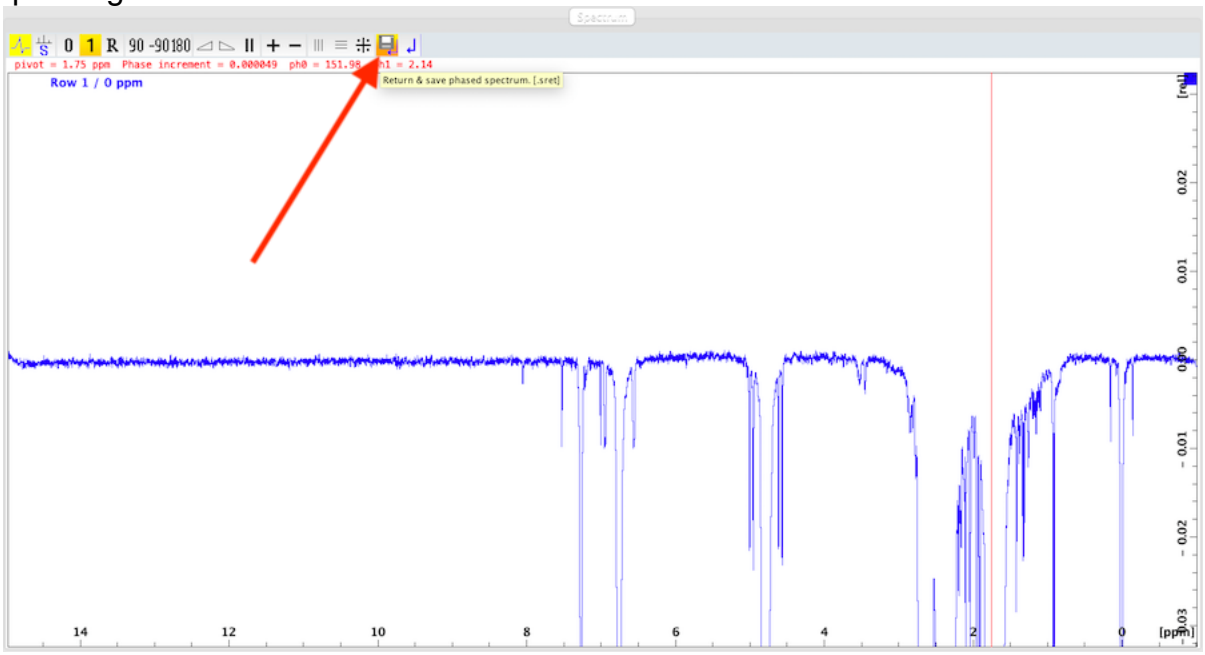
vi. *Use the "180" button to flip the spectrum over so all the peaks are negative. Recall we chose this spectrum because it corresponded to the first one in our 2D, so with UChicago defaults, it should be using a D7 delay of 0.001 sec, which should make the peaks negative.*



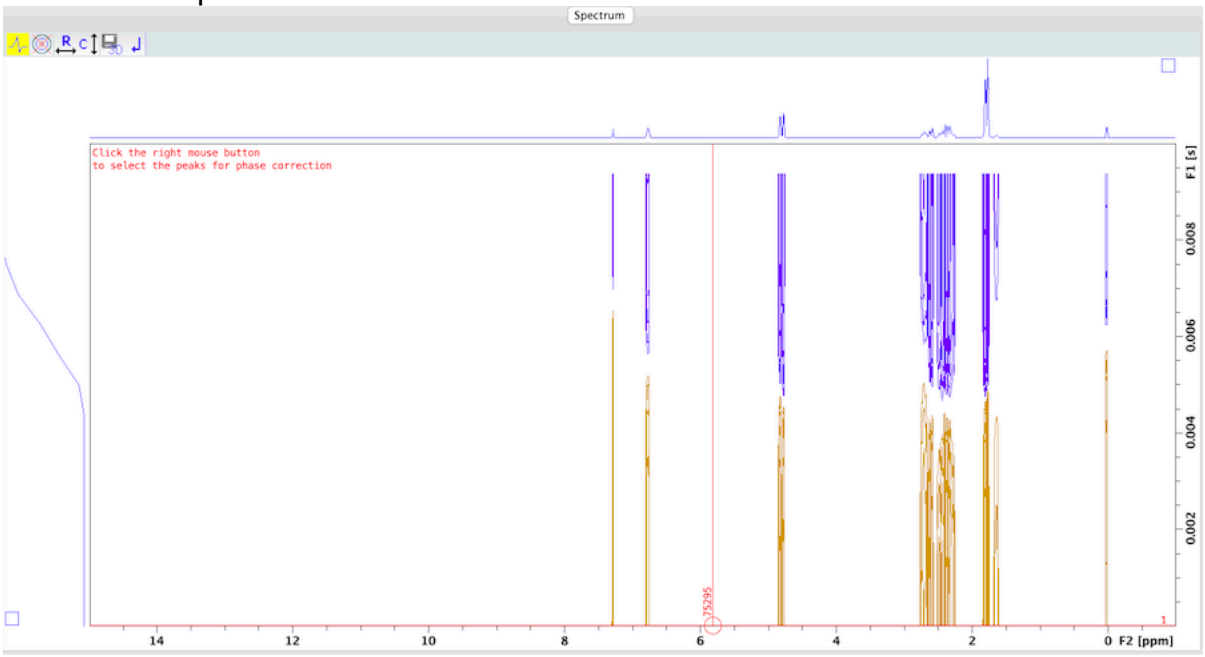
Note that you could have phased the peaks to be negative with just the zeroth- and first-order correction if you wanted. Continue tweaking phase if you want.

** THIS ENDS THE PHASING REFRESHER **

h. When your peaks are phased and negative, click the Save-and-Return button in the phasing tool:

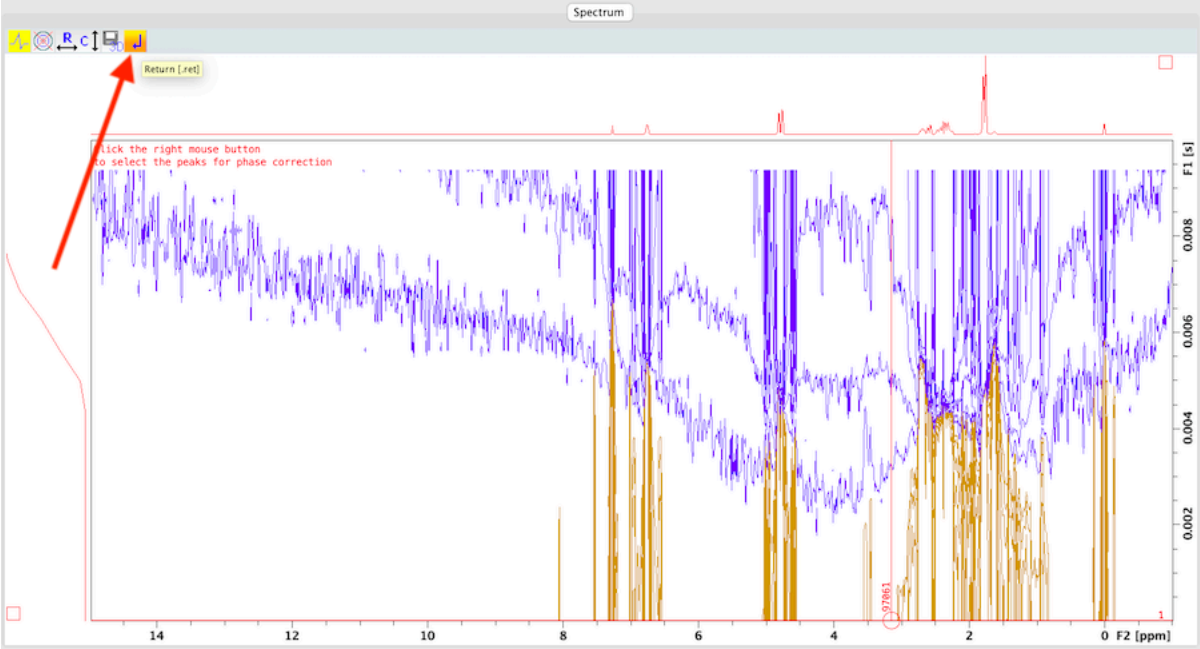


i. You'll return to the 2D phasing tool, and your 2D peaks should looks phased. Critically, the stripes should be negative (gold here) toward the bottom and positive (purple here) toward the top.



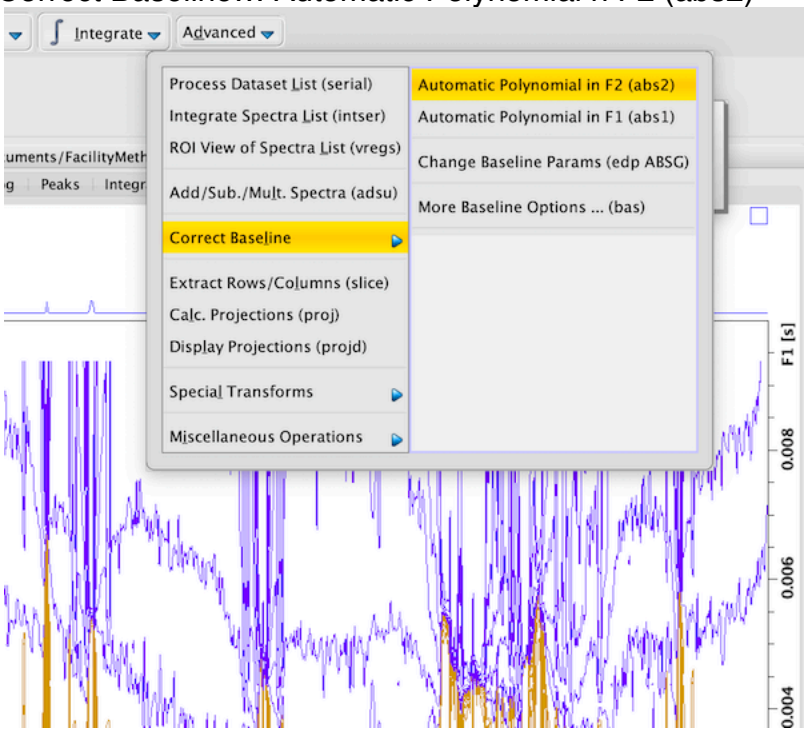
j. Scroll up the intensity until you see the noise floor and verify that the phasing looks good (baseline noise being equally positive or negative on either side of every peak. The example here looks good. If it didn't look good enough for you, click the R/Row button again and make phase adjustments.

   Click the Return button when done looking at the spectrum.

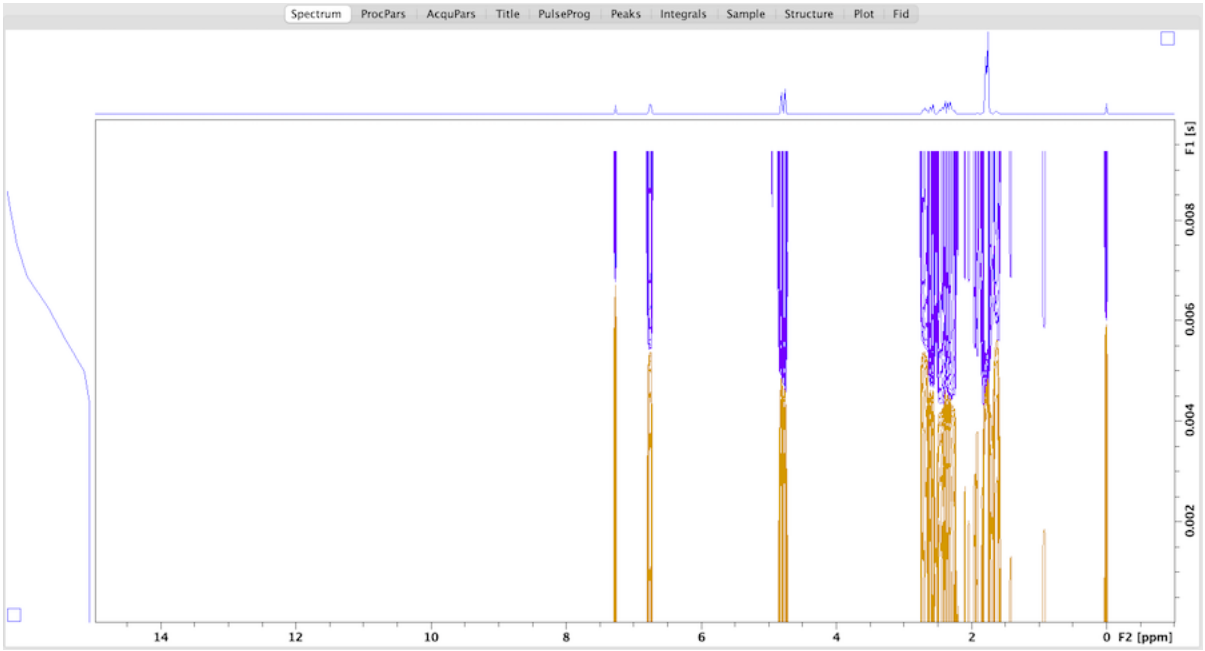


4. Correct the baseline in the F2 dimension only

a. In the Processing tab, navigate to
b. Advanced… Correct Baseline… Automatic Polynomial n F2 (abs2)



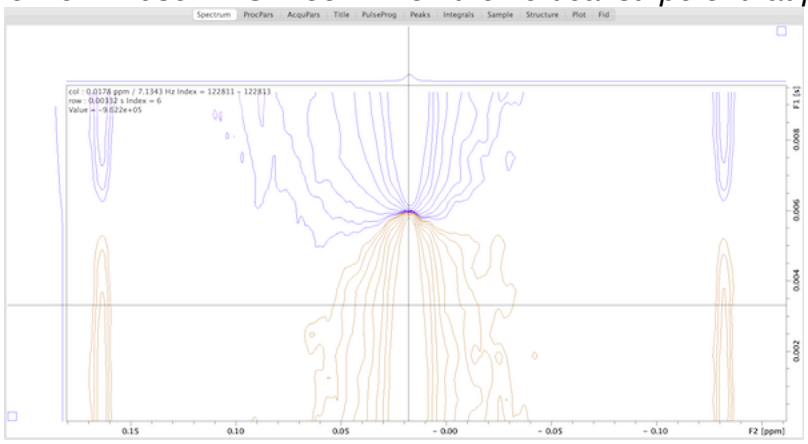
c. You should see the noise floor reduced:



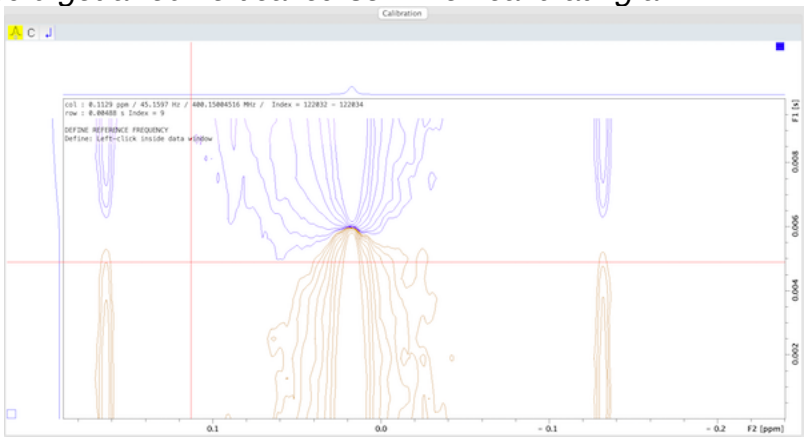
5. Calibrate/reference your chemical shifts

a. Calibrate the chemical shift of the X axis of your 2D spectrum. It's not much different from referencing a 1D. (If you don't do chemical shift referencing at this stage, you won't be able to correct it later in the T_1 analysis, and the peak frequencies reported in your table of T_1 relaxation times will be suboptimally referenced.)

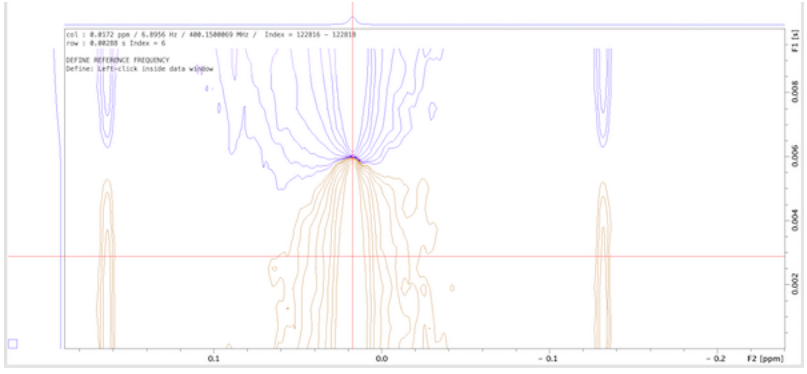
i. *Choose the peak you will use for chemical shift referencing, such as TMS or your solvent. Here we will use TMS. Zoom in on the vertical stripe of that peak in your 2D:*



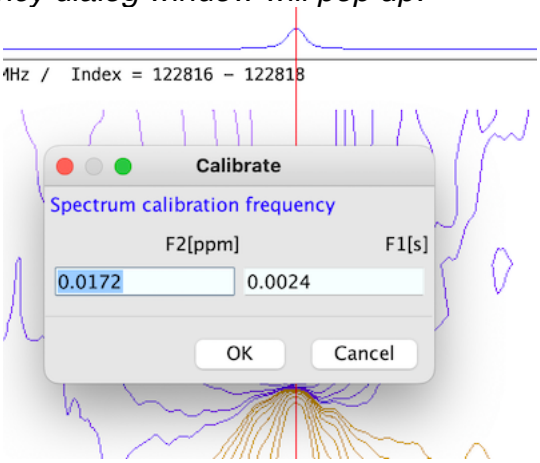
ii. *Click the Calibrate button under the Process tab. You should get a red crosshairs, like how you would get a red vertical cursor when calibrating a 1D.*



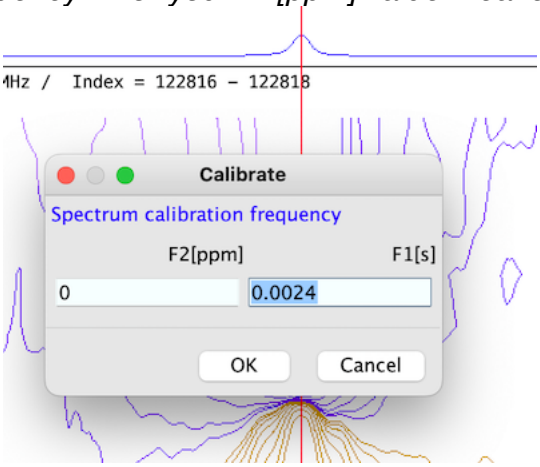
iii. *Place your crosshair so the vertical red cursor is precisely over your peak/stripe. Placement of the horizontal red cursor is irrelevant as long as you can see it somewhere.*



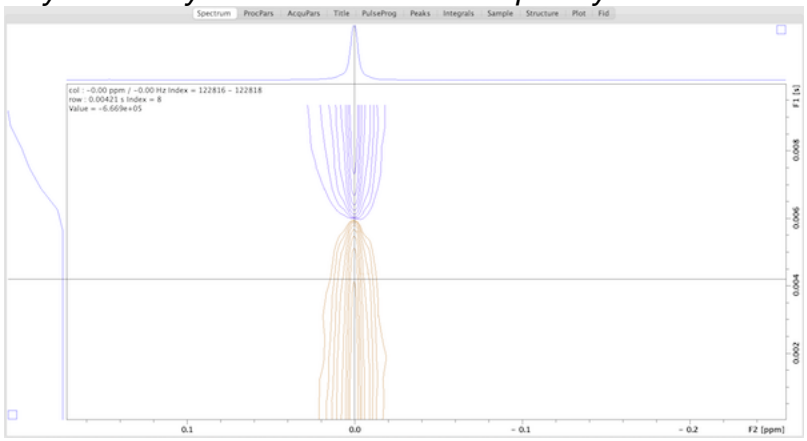
iv. *Click when your vertical crosshair is precisely in the center of your reference peak. The Calibrate frequency dialog window will pop up:*



v. *Type the proper frequency in for your F2[ppm] value. Leave the F1 field alone.*



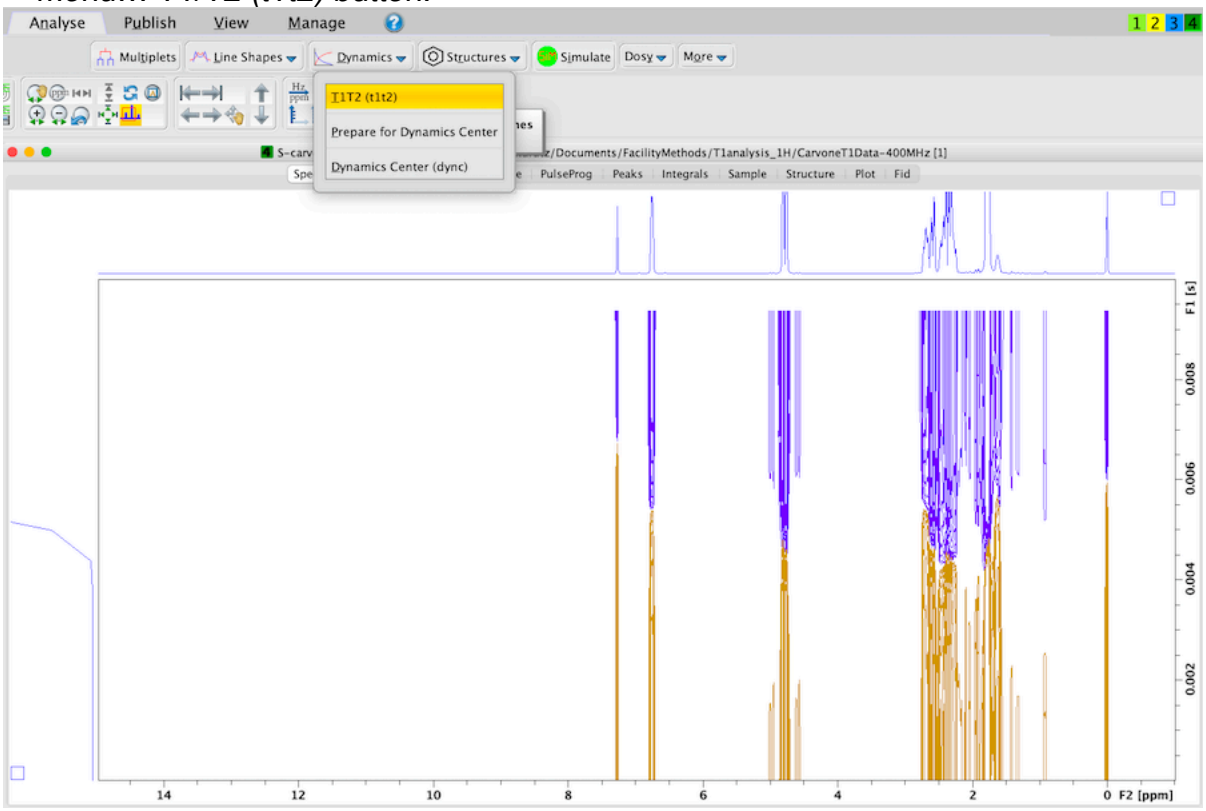
vi. *Click OK, and you'll see your new reference frequency reflected in the new 2D display:*



6. Use the T_1/T2 analysis tool to measure T_1 values

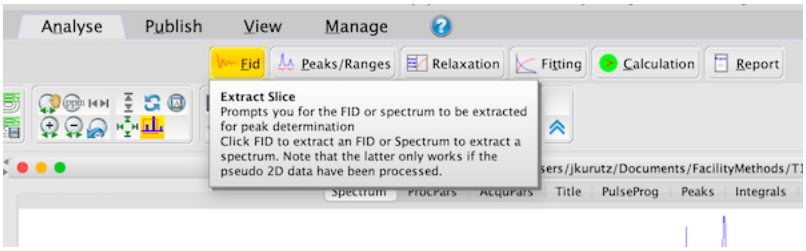
a. Start the analysis tool and select the final spectrum

i. While looking at your 2D processed data, navigate to the Analyse tab… Dynamics menu… T1/T2 (t1t2) button.

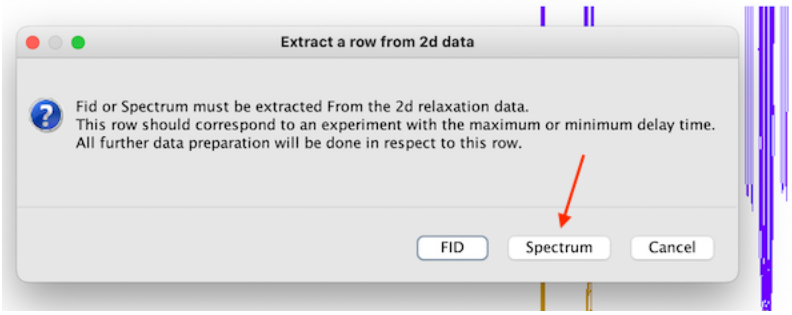


After selecting T1/T2 analysis, you should see a set of new buttons below the tabs.

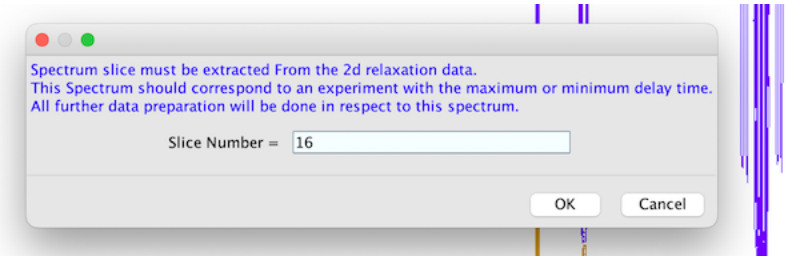
ii. To select the 1D spectrum you'll use for defining integral regions, click the FID / Extract Slice button.



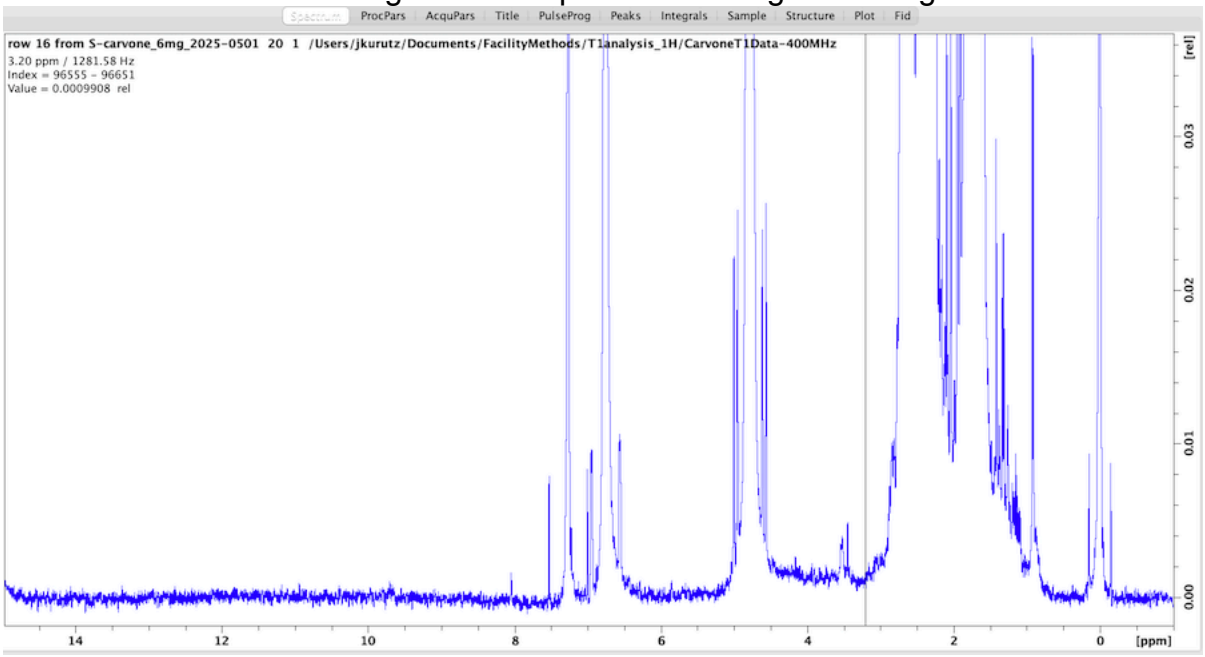
iii. You'll see a popup prompting you to select FID, Spectrum, or Cancel. Select Spectrum. This choice affects how you'll be defining your integral regions, so it's unclear why FID is even an option here.



iv. Select the number corresponding to the slice/row of the 2D you wish to use when defining your integral regions. To work with high-intensity, positive peaks, you need to select the 1D slice corresponding to your longest D7 recovery delay time. When using UChicago default parameters, this is slice #16. Change the default "1" to "16", then click "OK".

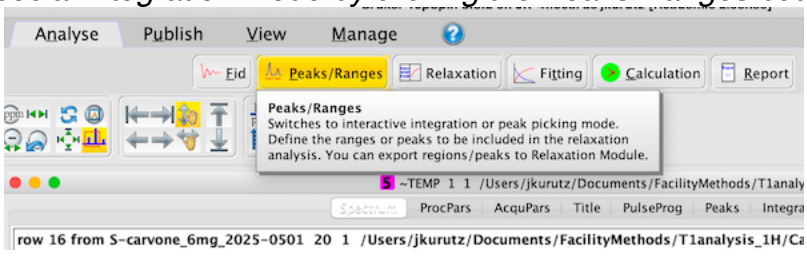


You'll see the 1D slice you chose. Scroll up the intensity to check that the phasing and baseline corrections are good. This spectrum looks good enough.

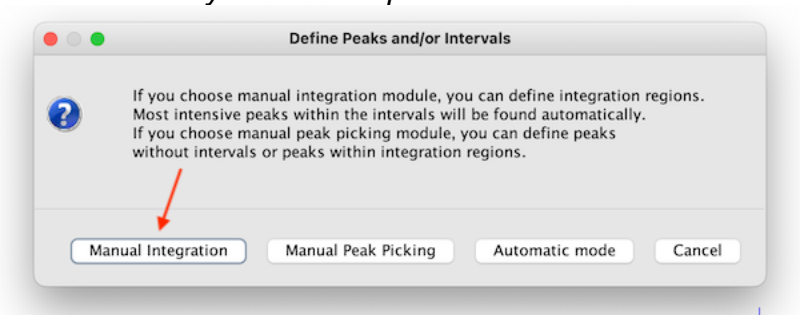


b. Define your integration regions

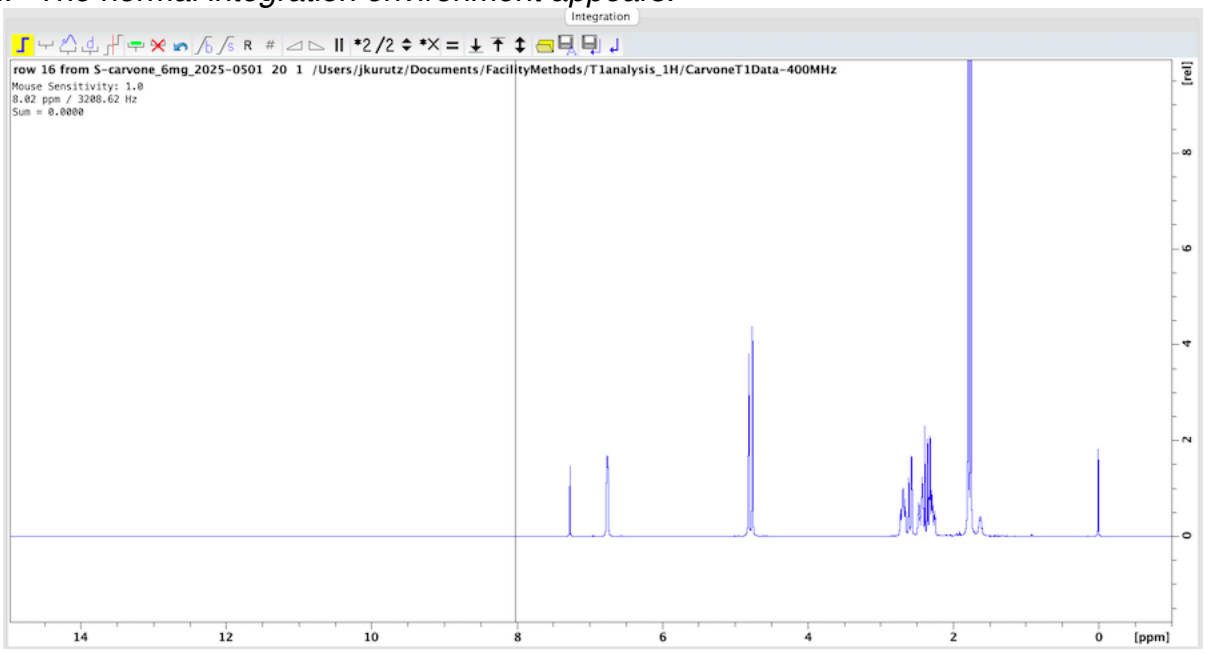
i. Enter the special integration mode by clicking the Peaks/Ranges button:



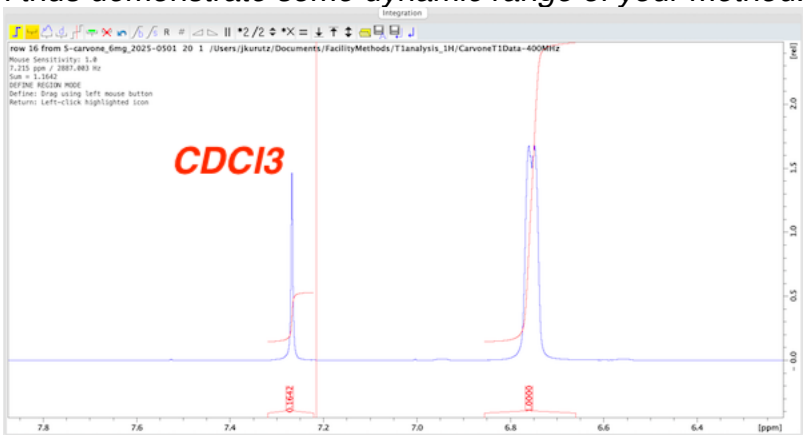
ii. *You'll need to choose which mechanism you want for peak quantitation. "Manual Integration" is almost always the best option.*



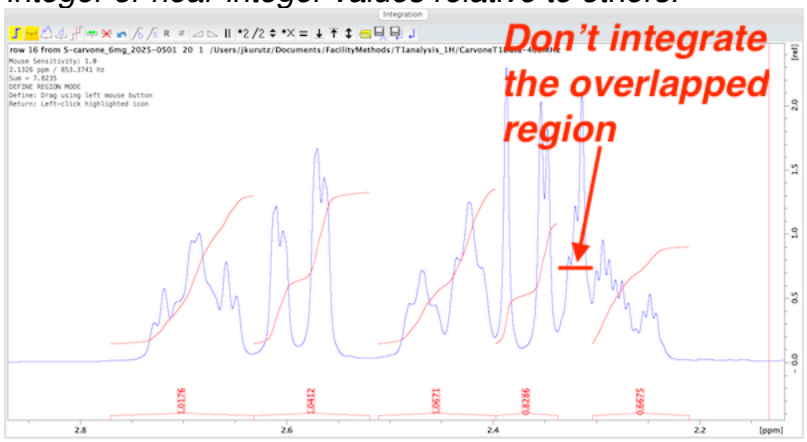
iii. *The normal integration environment appears.*



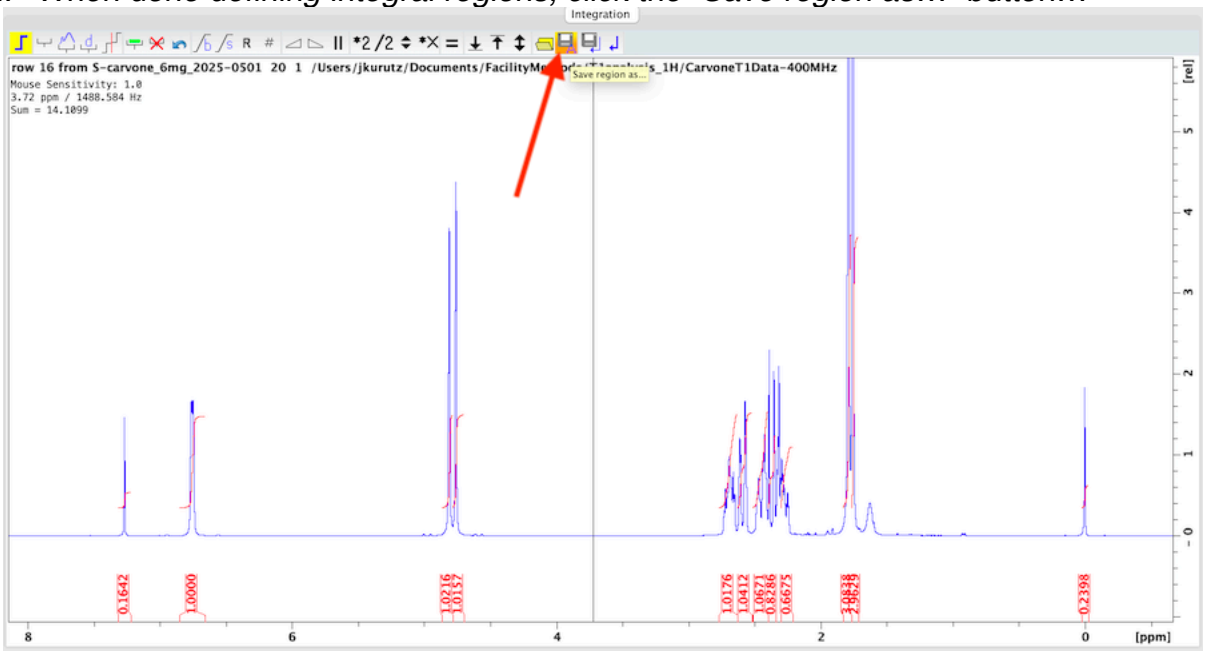
iv. *Tip #1 for integrating for T_1 measurement: include your solvent peak when integrating. Though you normally wouldn't do this, measuring T_1 of your solvent can be useful because it usually has a T_1 significantly longer than the resonances of interest. Inclusion can thus demonstrate some dynamic range of your method.*



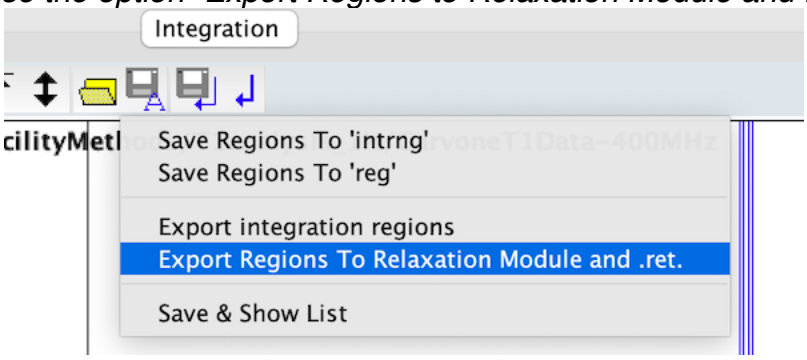
v. *Tip #2 for integrating for T_1 measurement: Don't bother integrating regions of overlapped peaks. But DO integrate portions of peaks that are not overlapped. The goal here is to determine the rate at which peaks are relaxing by measuring an area as a function of D7 recovery delay time. There is no requirement that peaks need to integrate to integer or near-integer values relative to others.*



vi. *When done defining integral regions, click the "Save region as…" button…*



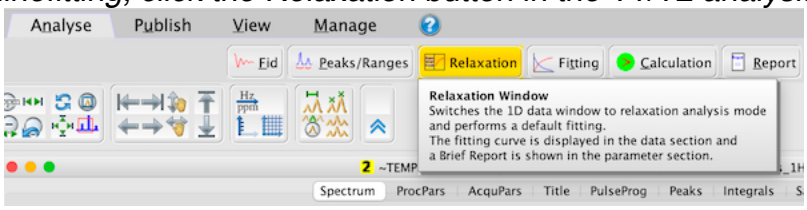
vii. *…and choose the option "Export Regions to Relaxation Module and .ret[urn]."*



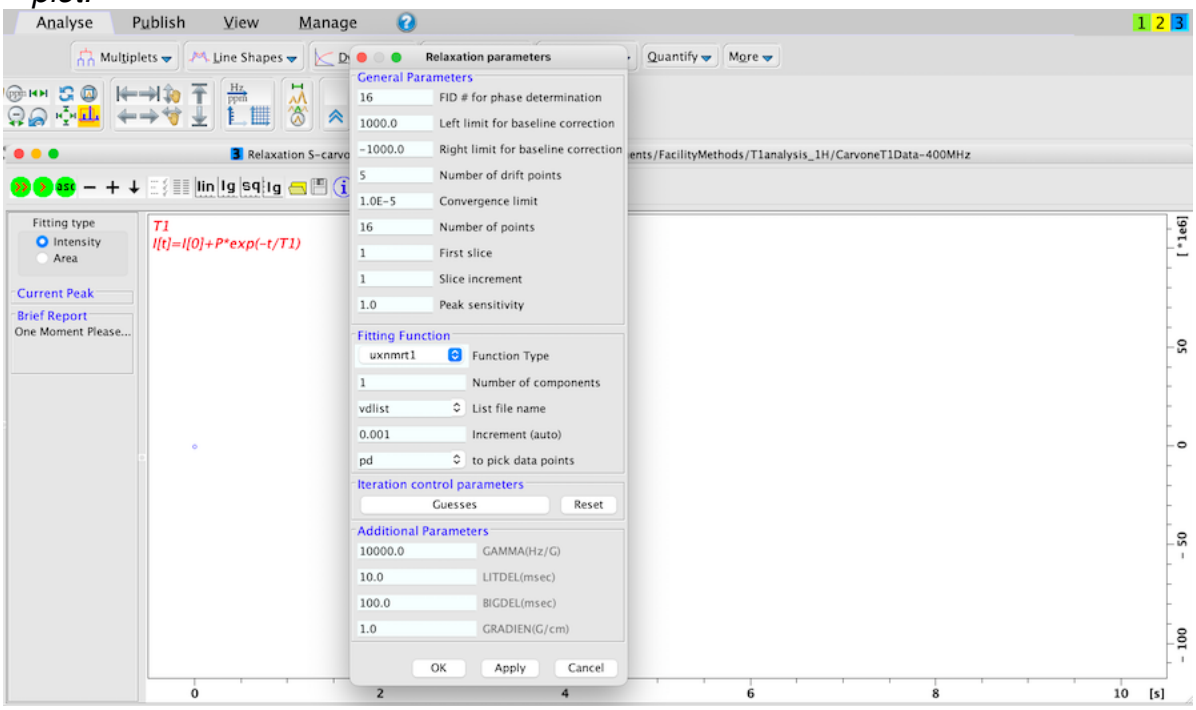
viii. *When done, you will exit the Integration tool and you see 1D spectrum #16.*

c. Enter the Relaxation Window and confirm Relaxation parameters

i. At this stage, you're almost ready to fit your data. To enter the Relaxation Window for appropriate linefitting, click the Relaxation button in the T1/T2 analysis toolbar:

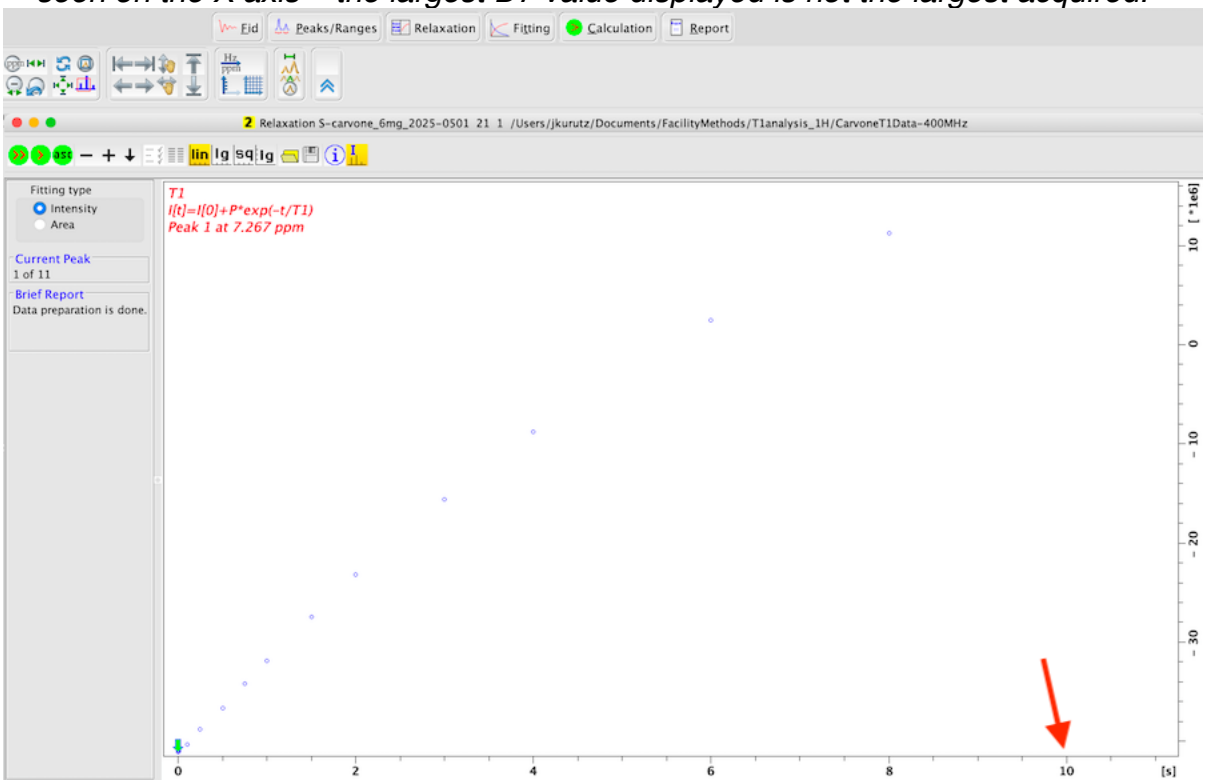


ii. Clicking Relaxation will close the display of the 1D spectrum and open a panel entitled "Relaxation parameters" over a new display of datapoints on an intensity vs D7 value plot.

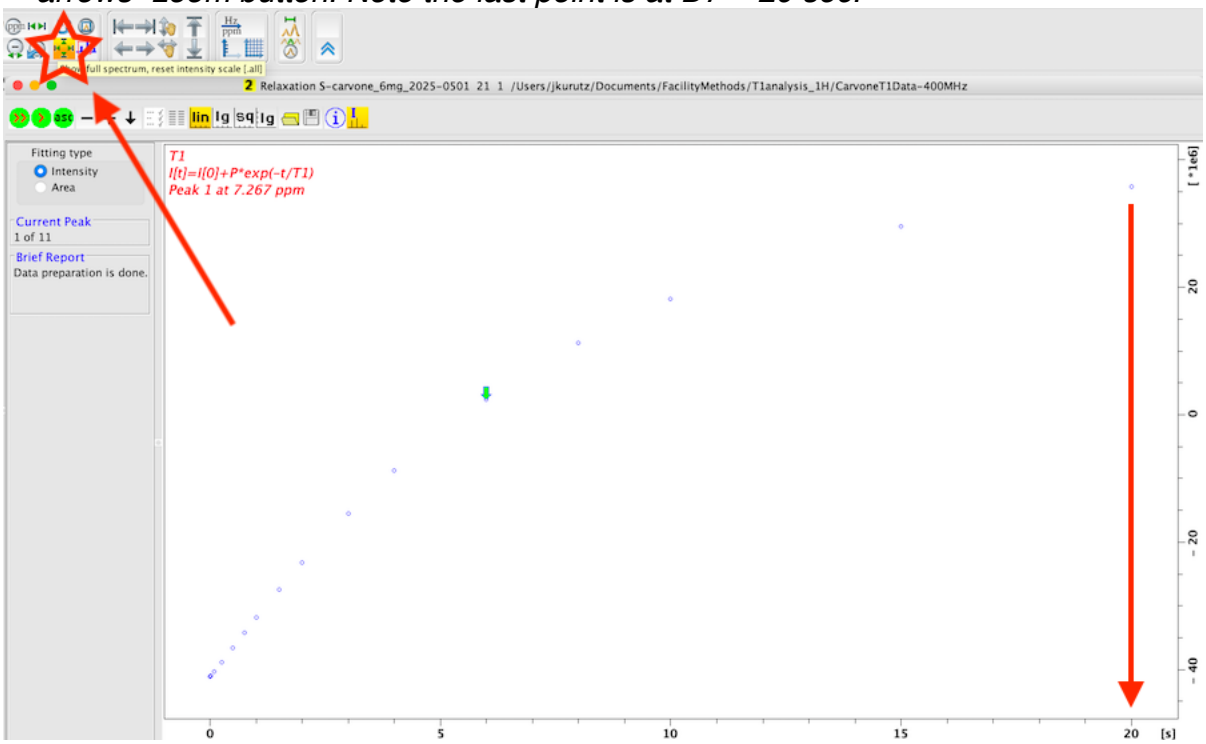


Generally, the values for the fields in the Relaxation parameters panel do not need to be changed, and one simply clicks the "Apply" button, then the "OK" button at the bottom. The system assumes you are trying to fit data to a curve consistent with T_1 relaxation rate measurement. You can see under the Fitting Function header, the Function type is set to "uxnmrt1".

iii. *Here you see datapoints representing intensity of the first (highest frequency) peak as a function of D7 delay time, in seconds. Here, the peak is designated as "Peak 1 at 7.267 ppm" in the red title. Not all the points are presented right away all the time, as seen on the X axis – the largest D7 value displayed is not the largest acquired.*



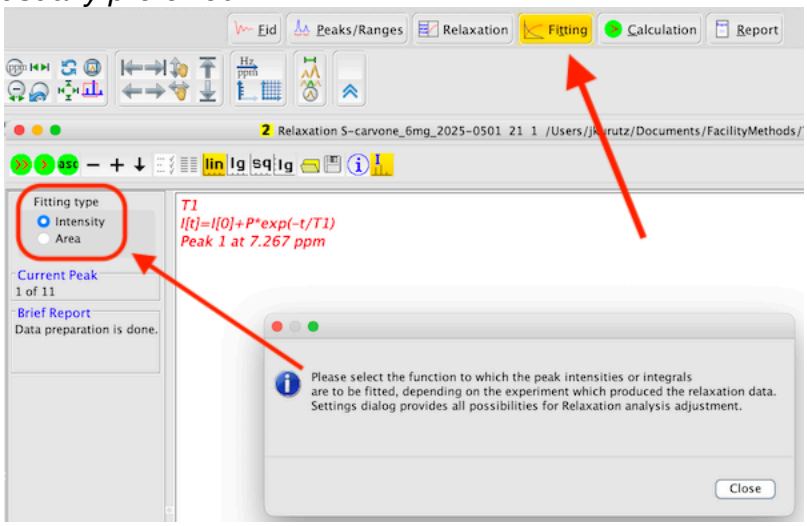
iv. *To display all data points for Peak 1, scaled vertically, click the usual "four-green-arrows" zoom button. Note the last point is at D7 = 20 sec.*



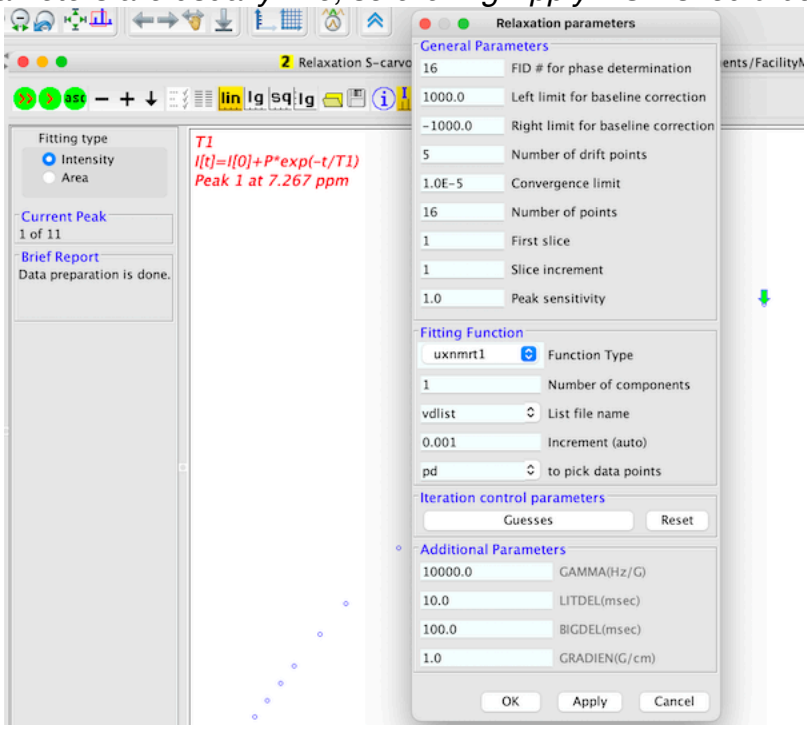
v. *PRACTICAL TIP: Sometimes the software removes the T1/T2 analysis buttons (FID, Peaks/Ranges, etc.) To get them back, simply re-navigate to the Analysis… Dynamics… pulldown menu and select T1/T2 analysis.*

d. Check Fitting parameters and specify that you want "Area" as the basis for measurement.

i. *The Fitting button reminds you that you have a choice to fit either Intensity data (peak height) or Area data (integrated area values). The system defaults to using Intensity, but Area is usually preferred.*

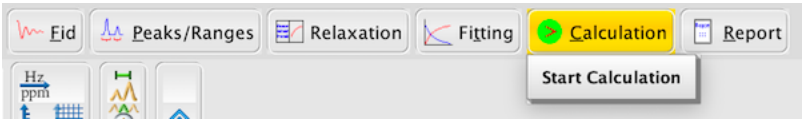


ii. *Clicking "Close" reopens the Relaxation fitting parameters if you want to adjust them. Default parameters are usually fine, so clicking Apply…OK should be OK.*

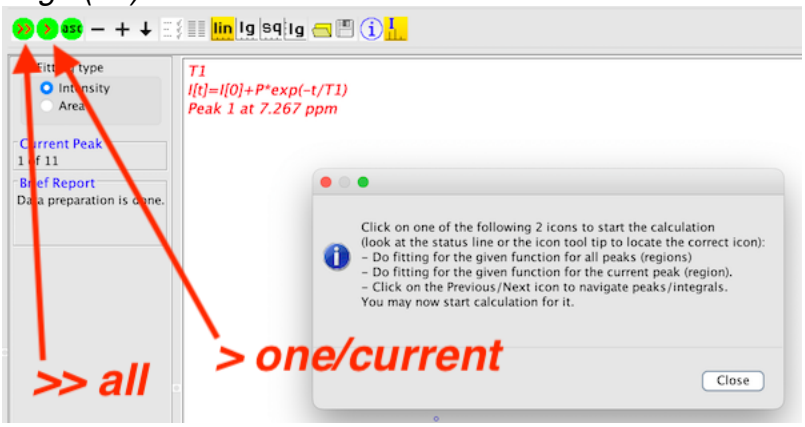


e. Perform the data fitting with the Calculate tools

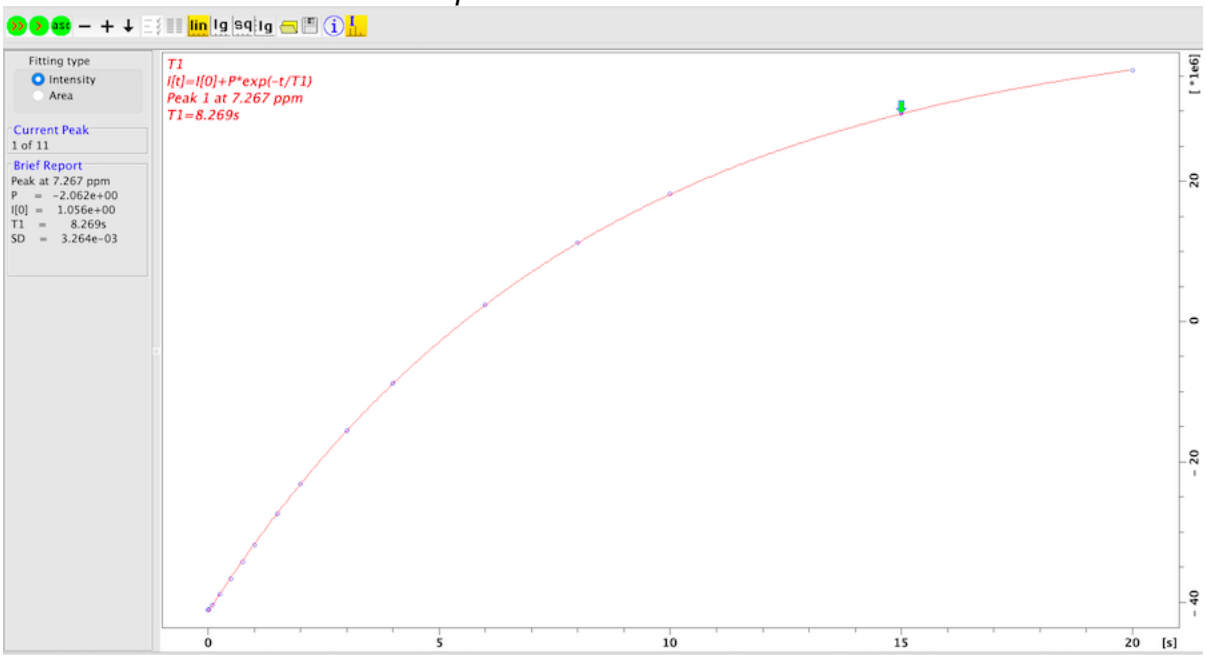
i. Click the Calculation button.



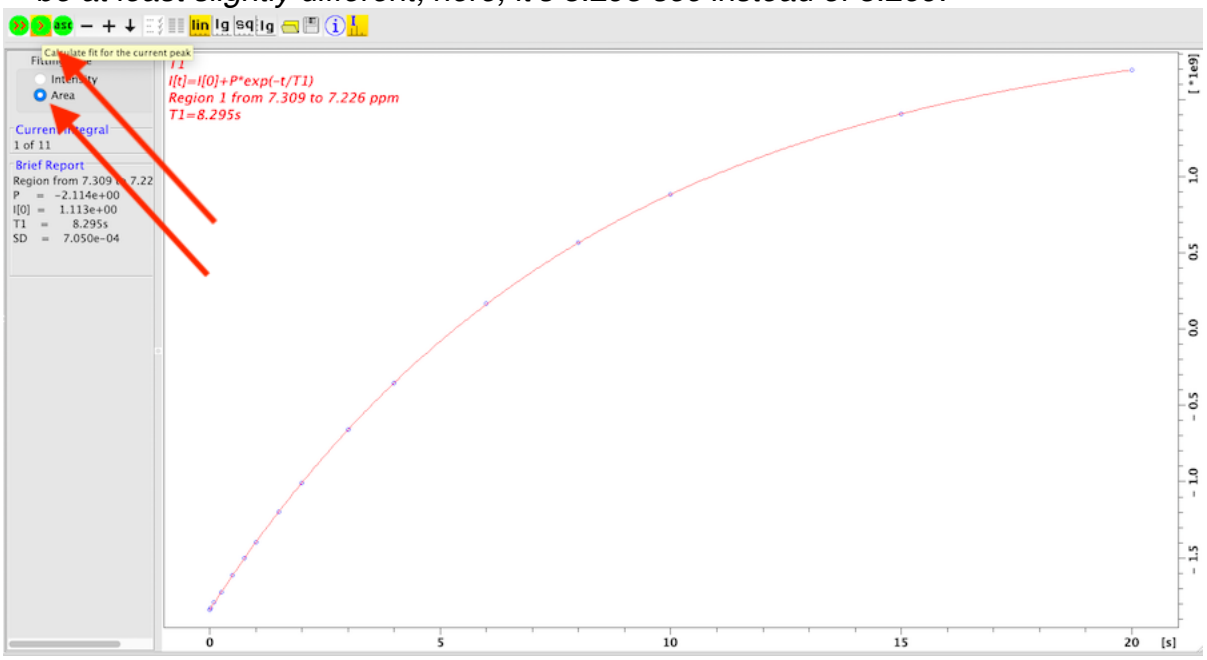
ii. The calculation window that pops up provides some helpful instruction to use the two buttons at the left to either calculate fits for ALL peaks/peak ranges (>>) or just one peak/peak range (>).



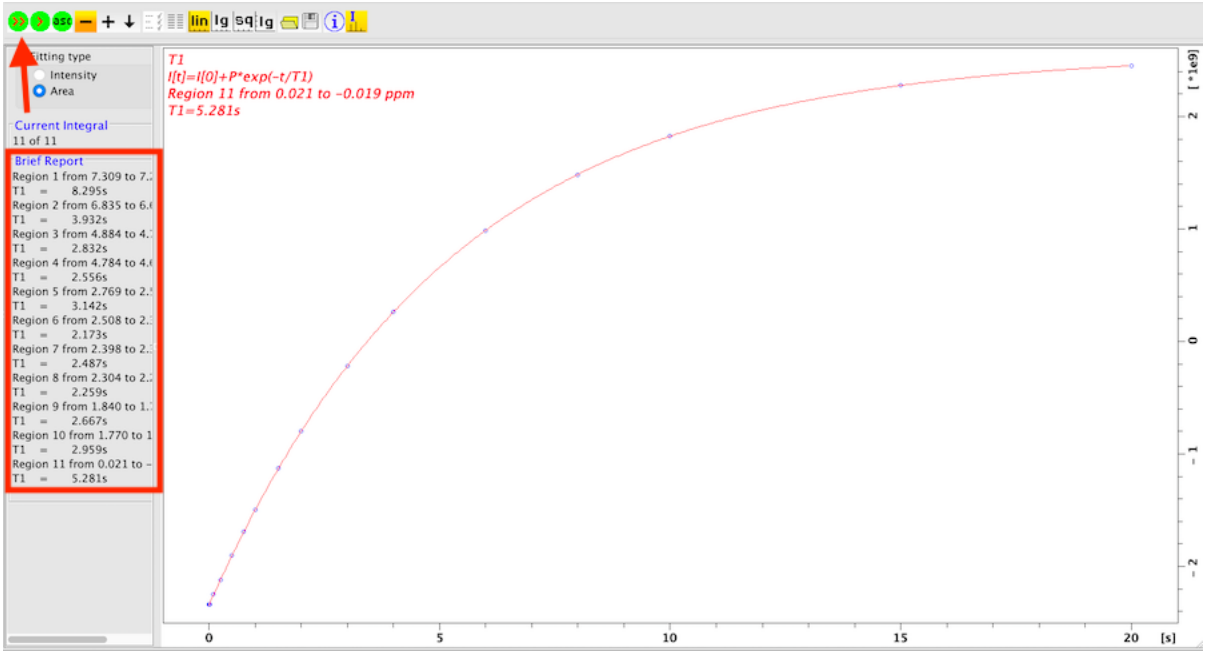
iii. Clicking the Close button on the popup window will get the system to fit the data for the first peak in the spectrum (in this case, the $CHCl_3$ peak at 7.267 ppm). Here you see the fit curve drawn in red and the calculated T_1 relaxation time, 8.269 sec, in the red title and in the left Brief Report area.



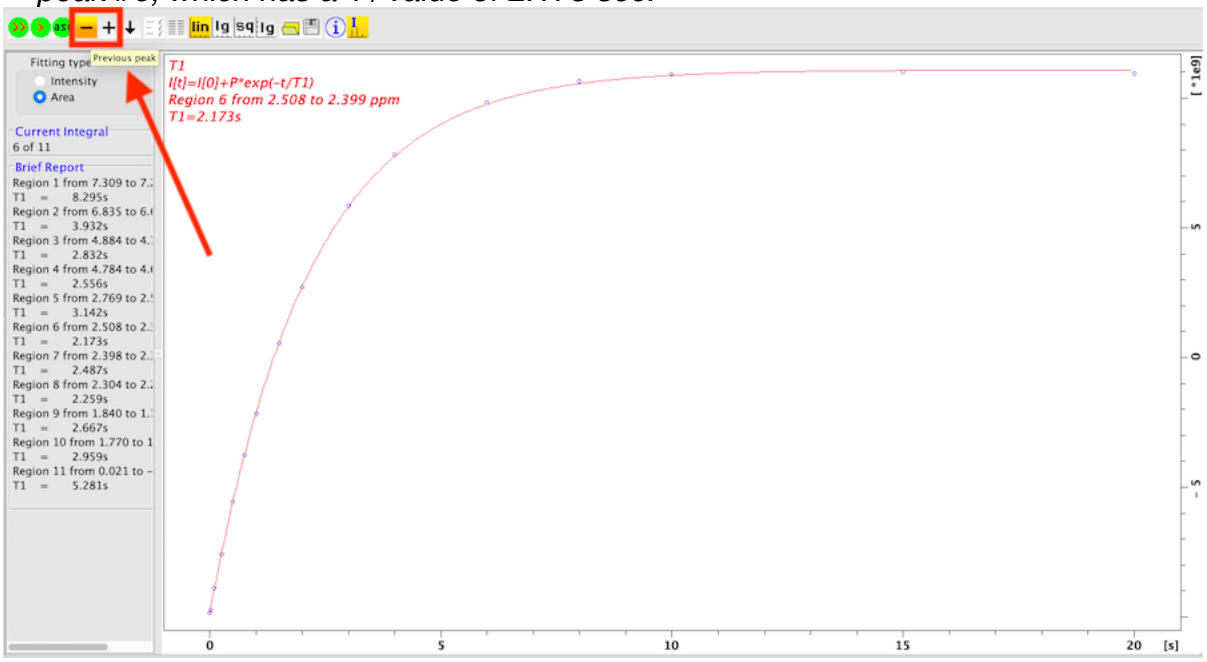
iv. *Instead of using the default choice, "Intensity", as the basis for the T_1 calculation, change it to "Area" and click the ">" to recalculate. The new calculated T_1 value should be at least slightly different; here, it's 8.295 sec instead of 8.269.*



v. *Clicking the green-circle " >> " button calculates T_1 values for ALL the peaks and displays them in the Brief Report area. The fit curve for the last integral region should be displayed. In this example, the last peak, #11, corresponds to TMS, which has a measured T_1 value of 5.281 sec.*



vi. *Use the "+" and "-" buttons to explore results for other integrated regions. Here we see peak #6, which has a T_1 value of 2.173 sec.*



f. Take a moment for evaluation. For this example:

i. *Residual $CHCl_3$, whose data are plotted immediate above, has a T_1 of approximately 8.3 sec. Note that at D7 = 20 seconds, the $CHCl_3$ has not plateaued – its area is still dependent on D7, and you can imagine employing longer D7 values would give rise to stronger $CHCl_3$ peaks. Thus, 20 seconds is not enough time for it to relax fully.*

The rule of thumb for setting recovery times for high-quality spectra is to allow 5 times T_1. 5 X T_1 here would be 41.5 seconds, and we can clearly see that 20 seconds is not long enough. Indeed, the D1 value in the default T_1 measurement parameter set is 30 seconds, which is probably insufficient to provide a good T_1 measurement for $CHCl_3$. However, solvent is expected to take a very long time to relax, and we are usually uninterested in precise measurement of its T_1.

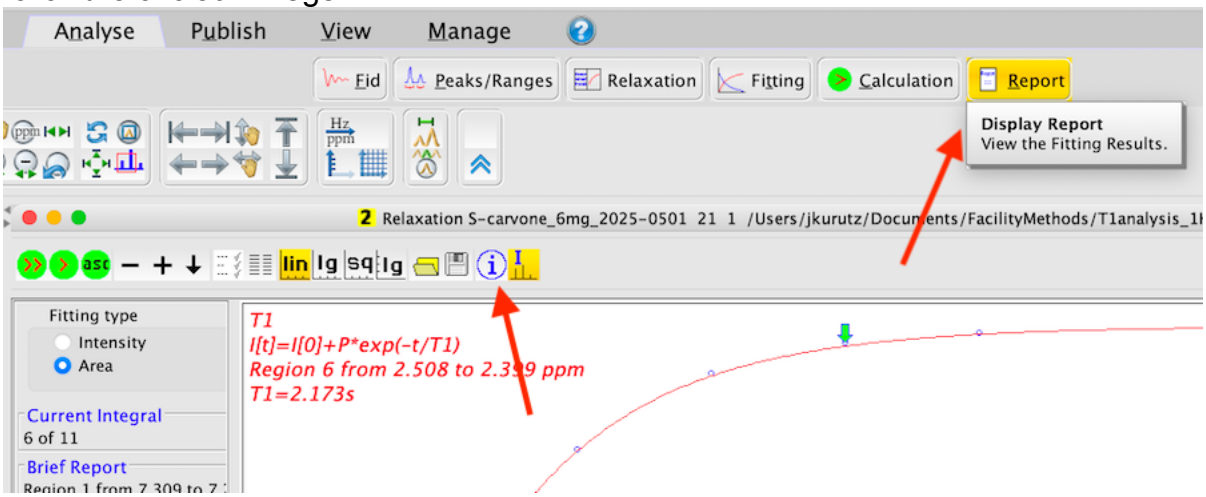
ii. *TMS has a T_1 of approximately 5.3 sec here. It has not plateaued in this experiment, but it is closer to doing so than $CHCl_3$.*

iii. *Carvone ^1H T_1 values range from 2.2 to 4.0 sec. Peak #6's relaxation behavior shows that its area is independent of D7 above D7 = 10 sec, indicating it has plateaued at about 10 sec or a little above. Peak #6's T_1 is 2.176, so 5 X T_1 = 10.9 sec, and we can conclude our 30 sec D1 value supports good T_1 measurement of this peak.*

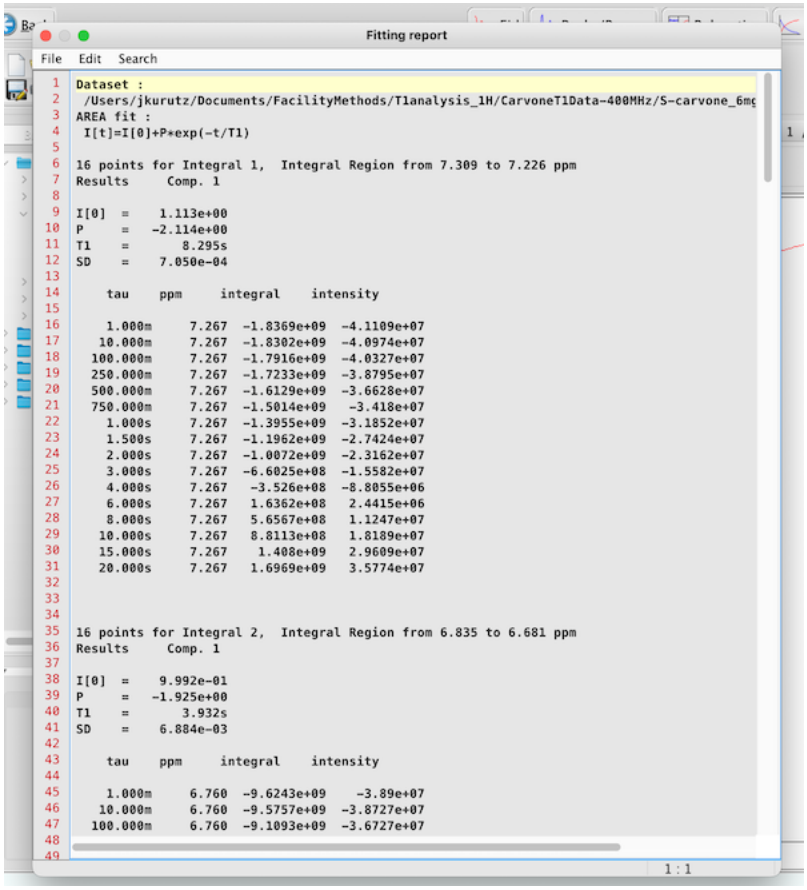
7. Generating a report and exporting your results

a. To export your results to a text file, it is recommended that you…
   i. *Open up a report in Topspin's T1/T2 analysis tool*
   ii. *Select-all and copy its contents*
   iii. *Paste into a new text file in Word or some other text editor.*
   (The Report panel's menu DOES have a "Save as…" tool, but I've found it strangely is unable to save to all locations. The copy/paste method is robust.)

b. Click either the Report button in the main T1/T2 Analysis toolbar (next to Calculate) or click the circled "**i**" logo.

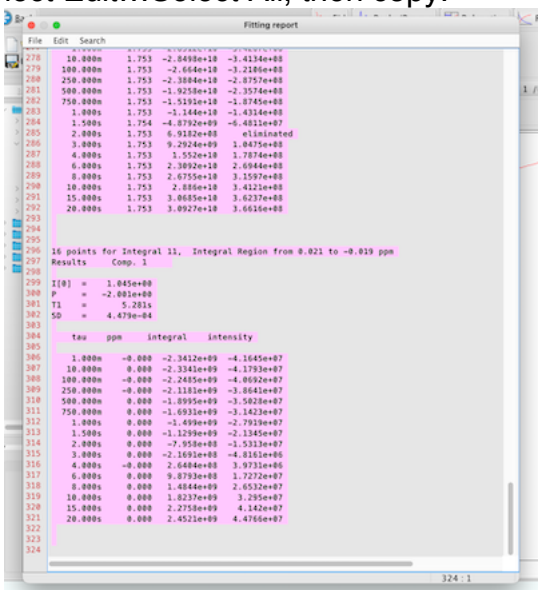


c. View the report:

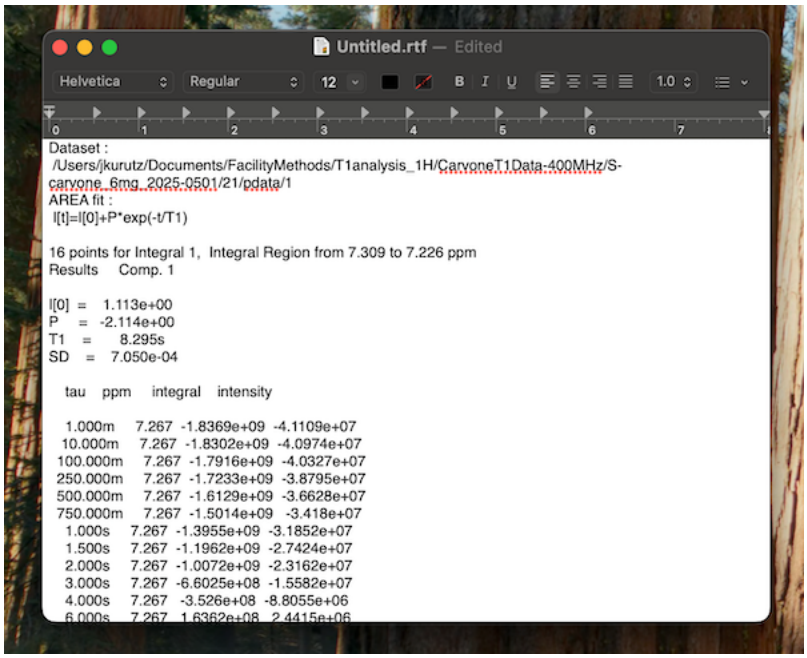


Note that this report is formatted to include ALL the data for each peak (integral and intensity at each D7 value). The calculated T_1 value and standard deviation of the fit are presented in the header information for each peak.

d. In the Report panel, select Edit…Select All, then copy.



e. Outside Topspin, open up a text editing tool (Word, TextEdit, etc.), create a new file, and past the contents into it.



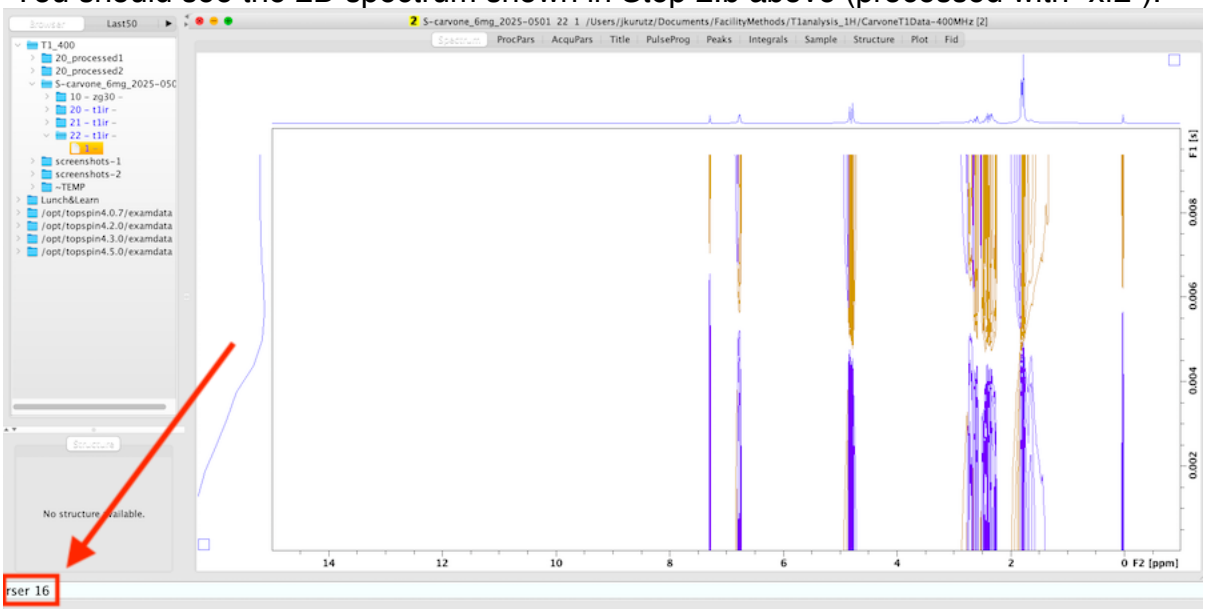
f. Save it like you would any other text file.

g. YOU'RE DONE!

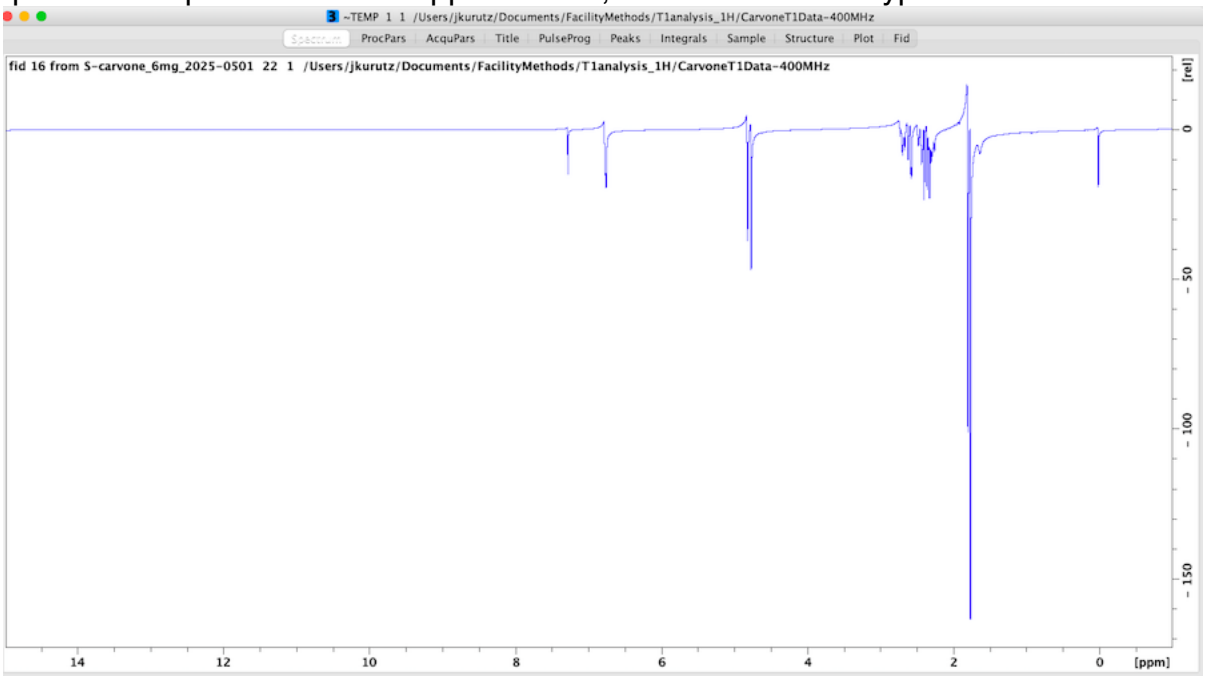
8. Appendix 1: Alternative 2D phasing method – Bruker's

This is the methods Bruker provides in their manual. It involves extracting 1D spectra from 2D data and using specialized tools for saving the resulting phase parameters and switching between 1D and 2D representations. You can avoid these complications by applying the general method for phasing 2D presented above.

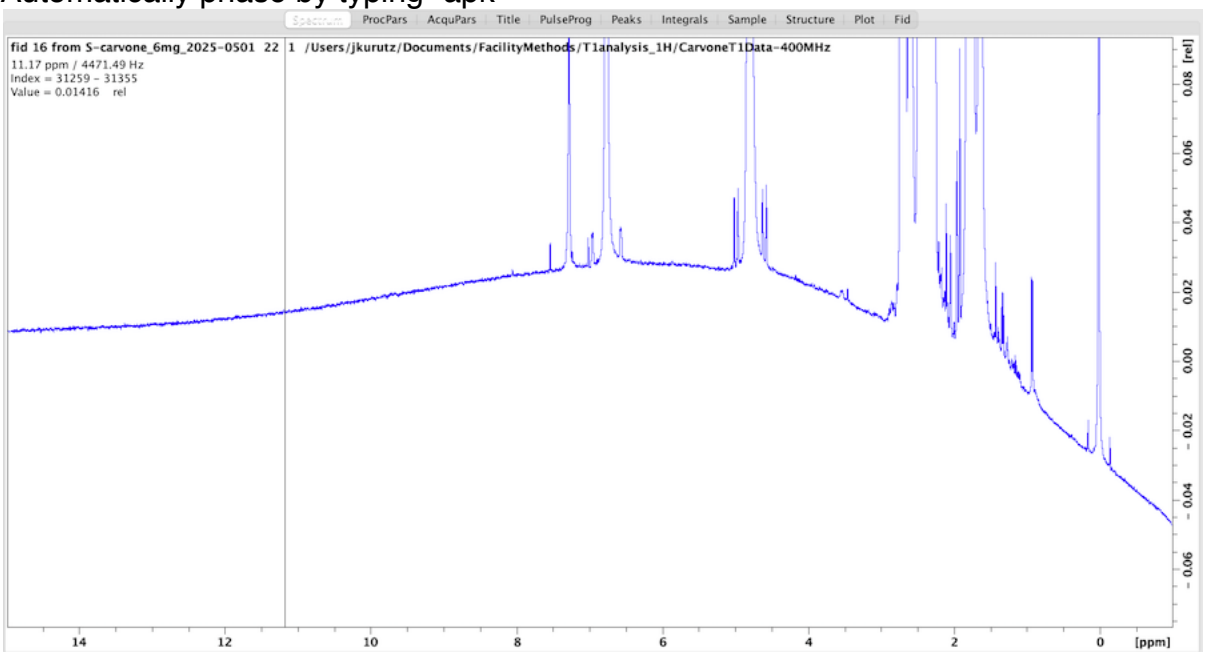
a. You should see the 2D spectrum shown in Step 2.b above (processed with "xf2").



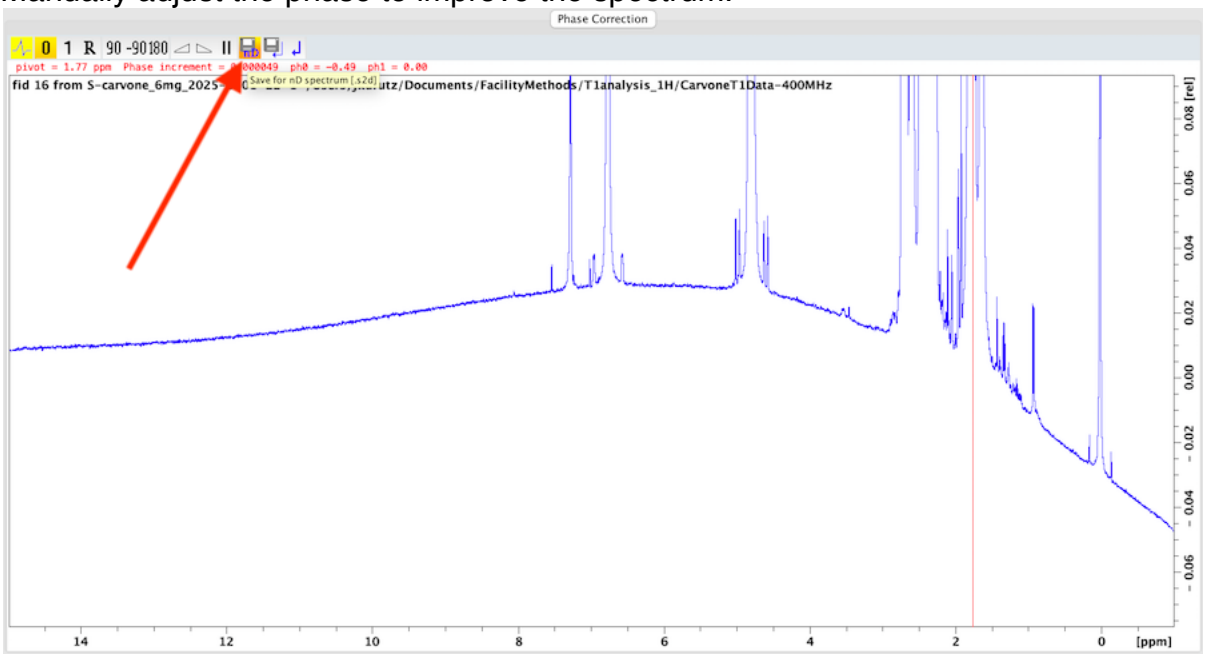
b. Extract the spectrum with the longest D7 value. As in Step 1.e above, identify the value for TD in the F1 column. It should be 16 for default UChicago acquisition parameters. Type "rser 16" (or substitute the accurate F1 TD value for your spectrum). A partially processed spectrum should appear. If not, click "Process" or type "ef".



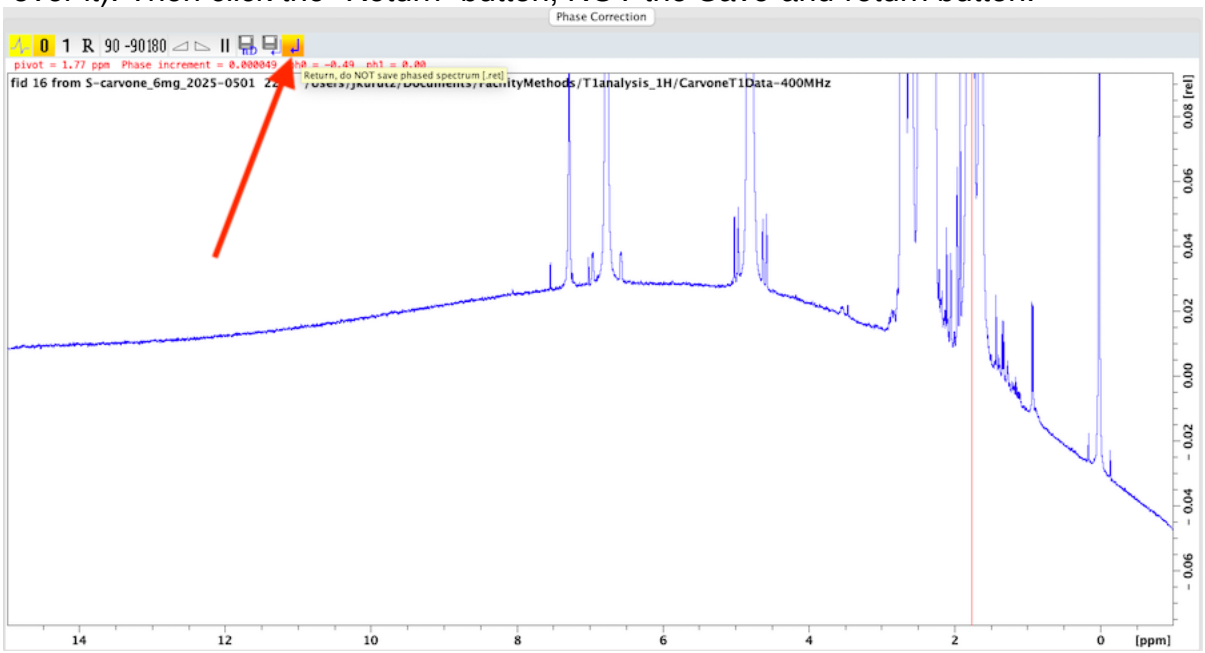
c. Automatically phase by typing “apk”



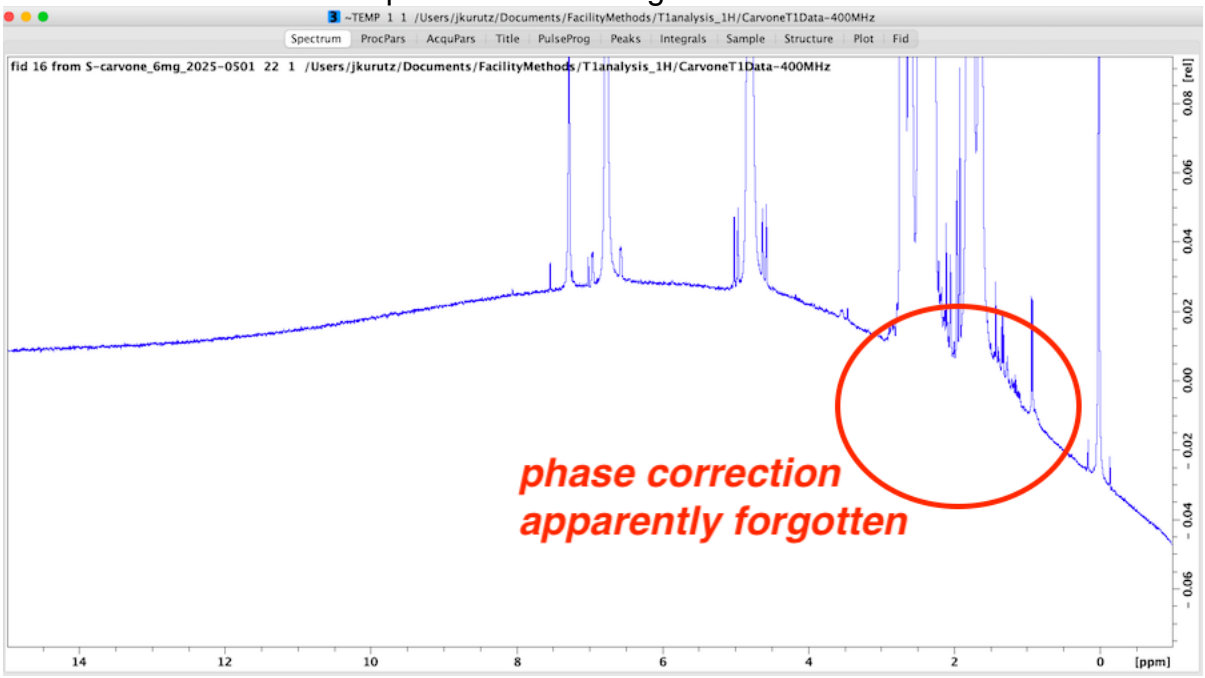
d. Manually adjust the phase to improve the spectrum.



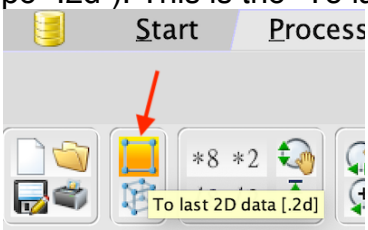
e. Save the phase by clicking on the “Save for nD spectrum” button (a floppy disk with “nD” over it). Then click the “Return” button, NOT the Save-and-return button.



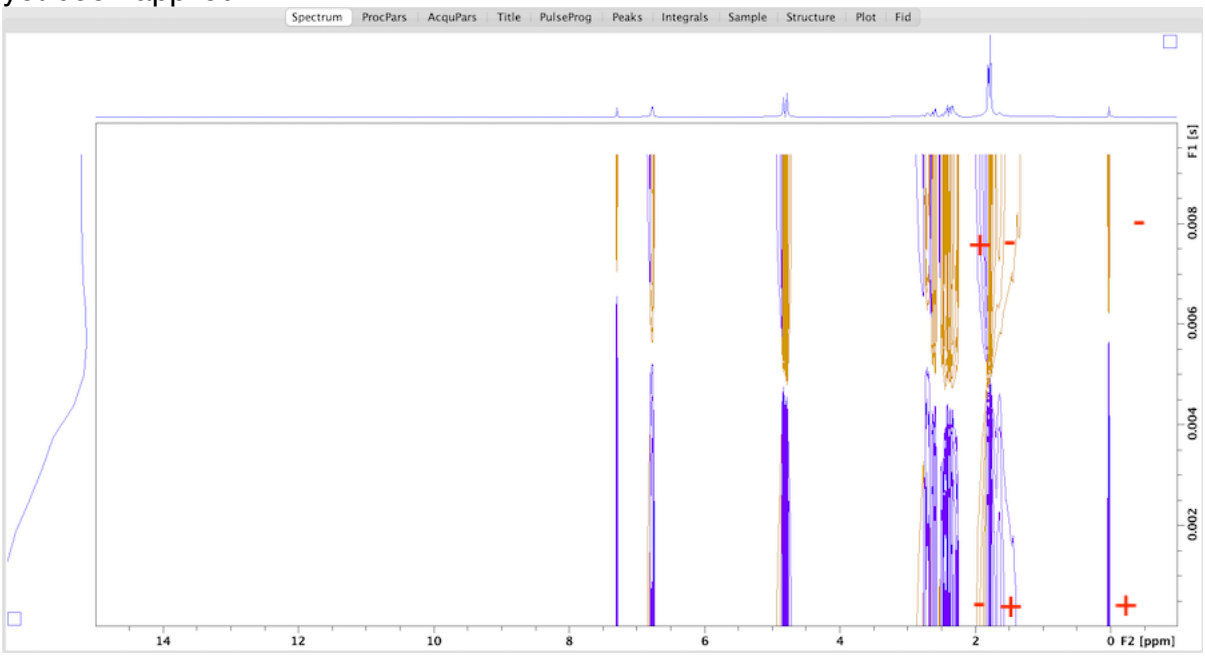
f. A quirk of this routine is that the phase correction is apparently forgotten after you exit the phasing routine. Part of Topspin does remember the proper phasing values… but that’s not reflected in the spectrum at this stage.



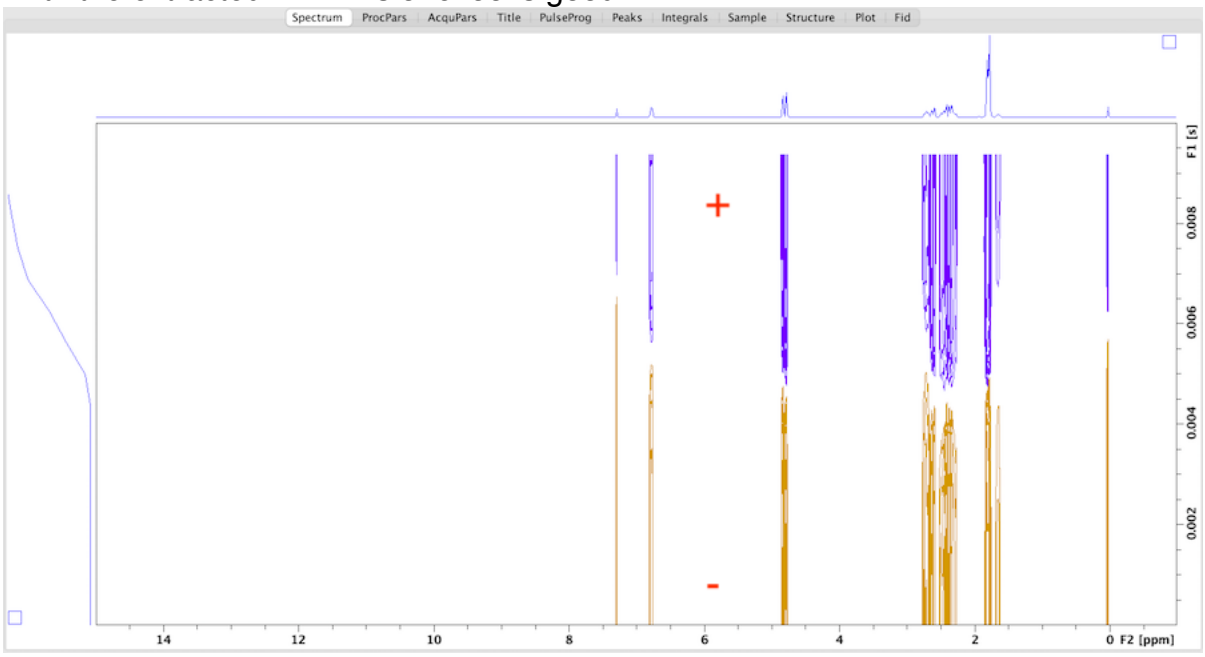
g. Switch back to the 2D display by clicking the odd “square with dots at the corners” button in the upper left (OR type “.2d”). This is the “To last 2D data” button.



h. Once you're looking at the 2D plot again, inspect the phasing. Curiously, no phase correction has been applied yet. If phasing were proper here, bottom rows should be negative (gold) and upper rows should be positive (purple). That is not the case here, indicating that phase correction has not been applied yet. Furthermore, inspection the aliphatic peaks, especially the one near 1.75 ppm, shows a twist to the peaks – baseline on one side is higher than the one on the other side. This clearly still needs phase correction, indicating that the correction we just developed in the extracted 1D has not yet been applied.



i. Type "xf2" or pull down the menu selection Process (tab)…Proc Spectrum…"Process Only F2 Axis (xf2). This reprocesses the spectrum with the phase correction developed with the extracted 1D. This one looks good:



j. Proceed to Step 4.a for Baseline Correction.