Step By Step Instructions for 2D NMR Experiments on the Bruker 300 MHz Spectrometer

(1) Use the drop down menu to create a new experiment/dataset for acquiring the 1D proton spectra. The name of this data set should be used for the rest of the experiments. Be sure to set the experiment number to 1.

(2) Go through the normal steps for acquiring the 1D proton spectra and acquire this spectra

(3) Ensure that the shims are sufficient for the resolution you would like in your 2D spectra. If you are unhappy with the shims you can either manually shim or go through another auto-shim process.

(4) Reference your proton spectra and then write down the value for the SR parameter. Referencing can be done by clicking on the calibrate spectrum option in the NMR step by step drop down menu. The SR parameter can be found in the ProcPars tab or by simply typing SR in the command line and pressing enter.

(5) Examine your referenced spectra to determine the required sweep width (SW). By default the proton SW is much larger than required and you will want to make sure that you use the smallest possible SW in your 2D acquisition. Remember that the SW must contain all peaks in the spectra in order to avoid folding. Make sure that you write down the value for the SW as you will need this value later when setting up the 2D experiment.

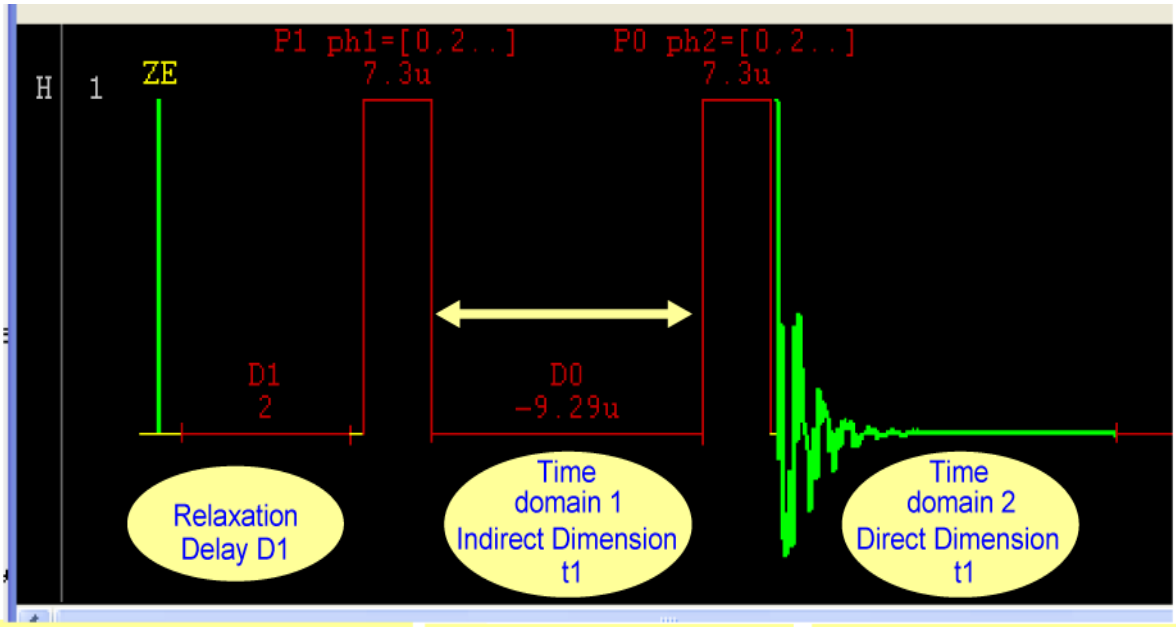
(6) Center the rf transmitter in the middle of the required sweep width. MAKE SURE that you are doing this after referencing your spectra. The rf transmitter can be centered by typing o1p into the command line and pressing enter.This will cause a box to appear and you can enter the frequency of the transmitter, in ppm, directly into this box. This value should be the middle of your spectra. Make sure that you write down the value for 01p as this will be used later when setting up the 2D experiment.

(7) If you are carrying out a hetero-nuclear 2D experiment, such as HSQC or HMBC, you must repeat steps 1-6 for the carbon nucleus. Be sure to use the same name for your data set but enter the experiment number as 2. You will also need to write down the sweep width (SW), o1p, and SR values for the carbon experiment.

(8) Create a new data set using the NMR step by step drop down menu. Again, use the same name for the experiment as you did in the previous steps but use a different experiment number (ex. 3 or 4 or 5).

(9) Load the 2D experiment of your choice from the 2D experiments list in the drop down menu.

A summary of the basic acquisition parameters for a 2D NMR experiment can be seen below:



Acquisition Time (AQ):
=TD*DW
=(NumPoints*DwellTime)

DwellTime (DW):
=1 / [SpectralWidth (SW)]

Digital Resolution (FIDRES):
=1 / [Acquisition Time (AQ)]

TD1 (F_1):
Number of data points in second time domain typically 128 to 512

SW (F_1):
Spectral width to be sampled in F1 domain e.g. H-1 10ppm or C13 200ppm.

TD2 (F_2):
Number of data points in second time domain typically 2048 to 4096

SW (F_2):
Spectral width to be sampled in F1 domain e.g. H-1 10ppm or C13 200ppm.

(10) Enter the sweep width for each dimension.

This can be done by clicking on the AcquPars tab and entering the SW for each nucleus into the F2 and F1 columns. If you are carrying out a COSY experiment then the values for SW in F1 and F2 should be equal to the SW value from your 1D proton experiment. If you are carrying out an HSQC or HMBC then the value for SW in F2 and F1 should correspond to the SW value you recorded earlier for the 1D proton and 1D carbon experiments, respectively (see figure below).

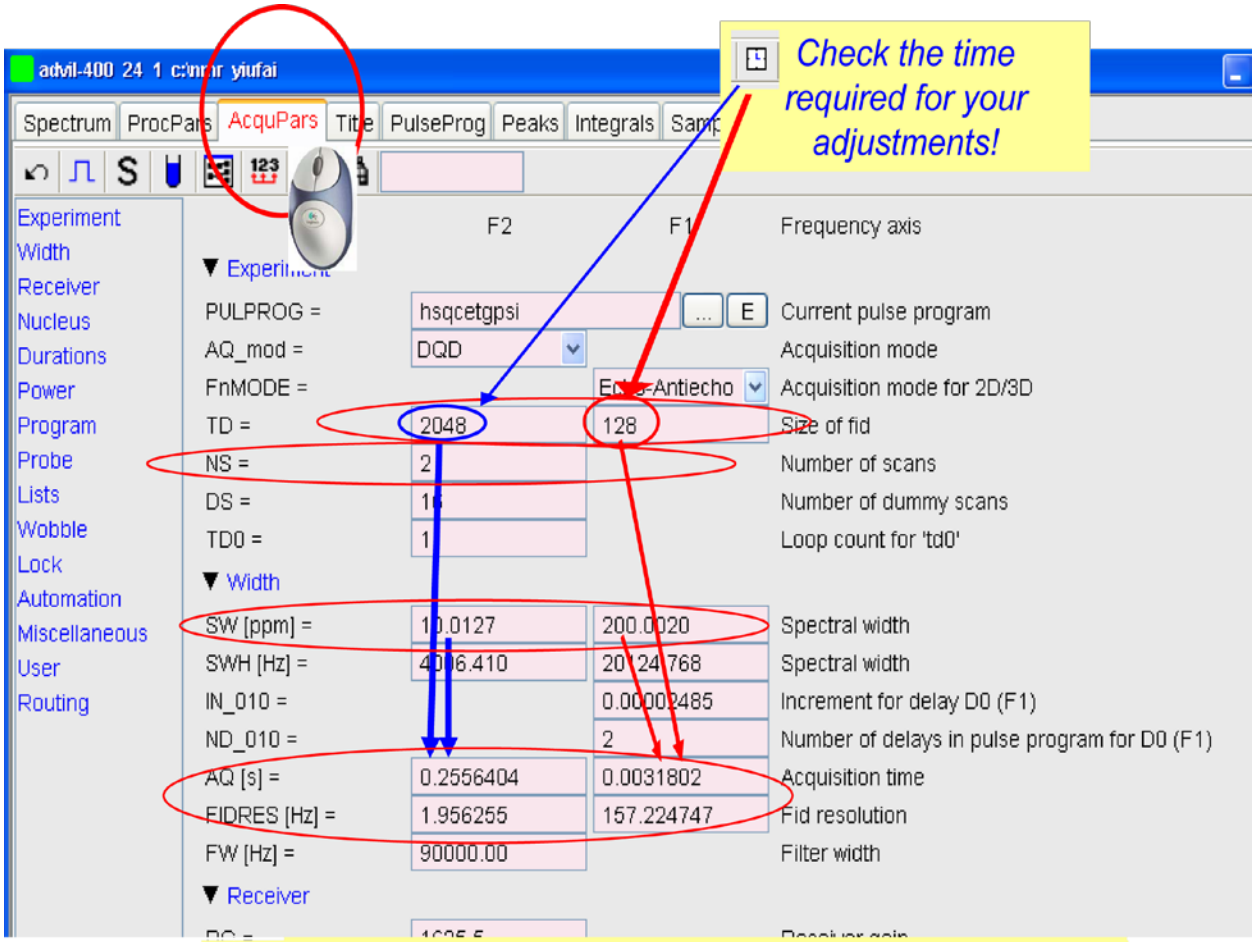
(11) Center the RF transmitter in the middle of your sweep width for both dimensions. This can be done by typing o1p into the command line and hitting enter. A box will appear and you can enter the value for o1p recorded earlier. Repeat this step for o2p by typing o2p into the command line and hitting enter. Note if you are running a COSY then the value of o1p and o2p will be the same. If you are running a HSQC or HMBC then the value of o1p and o2p correspond to the o1p value from the 1D proton and 1D carbon experiments, respectively (see figure below).

(12) Optimize the digital resolution of your direct and indirect dimensions/FID.

Remember that the digital resolutions (FIDRES) is equal to 1/[acquisition time (AQ)] and a smaller number for the digital resolution corresponds to better resolution. This corresponds to increasing the acquisition time (AQ). Recall that the acquisition time is equal to the number of points (TD) multiplied by the dwell time (DW). Since the dwell time is equal to 1/[sweep width (SW)], and the SW value has been determined, the only way to enhance the digital resolution is to increase the number of points (TD). **NOTE:** The number or points (TD) in the indirect dimension (F1) is more commonly referred to as the number of Increments (see figure below).

(13) Set the number or scans (NS) to be carried out.

In the step 12 you optimized the digital resolution based on the acquisition parameters. In this step you will vary the number of scans (NS) for each increment in order to increase/optimize the signal to noise ratio for the experiment. Remember that a high quality HSQC and HMBC will have a balance of both good digital resolution and a high signal to noise ratio (see figure below).



Set SW from 1D experiments and adjust TD (Num Points) for better digital resolution on both F2 and F1

Set the Number of Scans (NS) for each increment: a larger number of scans will increase the signal to noise ratio

Adjust o1p and o2p to center transmitter in the right place:

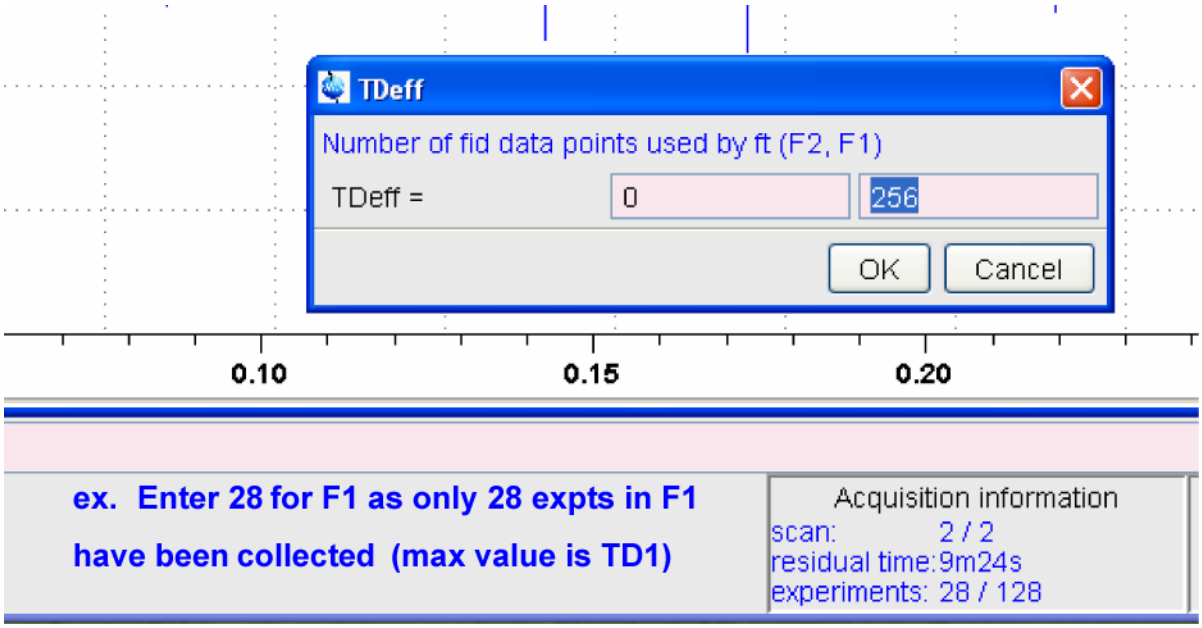
O1p*: *offset for* F_2 e.g. 5 ppm for H-1

O2p: *offset for* F_1 e.g. 100ppm for C-13

(14) Acquire your 2D data set.

This can be done by clicking on the acquire option in the NMR step by step drop down menu.

Note: You can preview the currently running experiment by typing “1 TDeff” into the command line and hitting enter. This will bring up a box labeled TDeff and you must enter the number of F1 increments that have been completed. Once this is done you can type xfb into the command line and hit enter. This will bring up the currently completed 2D spectra.

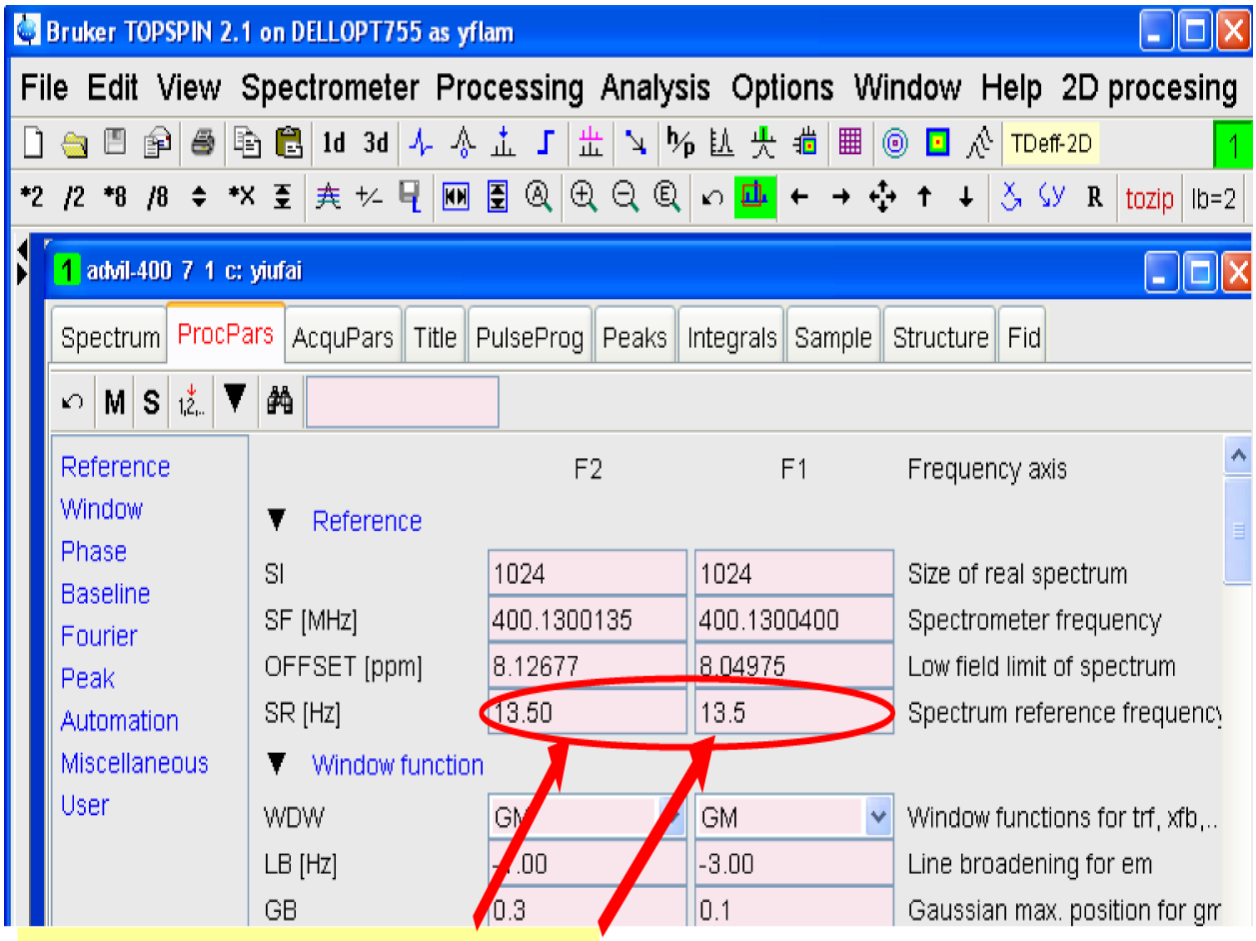


(16) Auto process your 2D data set.

First click on the process 2D option in the NMR step by step drop down menu to FT your 2D data set .Second click on the Phase 2D option in the NMR step by step drop down menu to automatically phase your 2D data set.

(17) Enter the SR values, or reference values, for your 2D experiment.

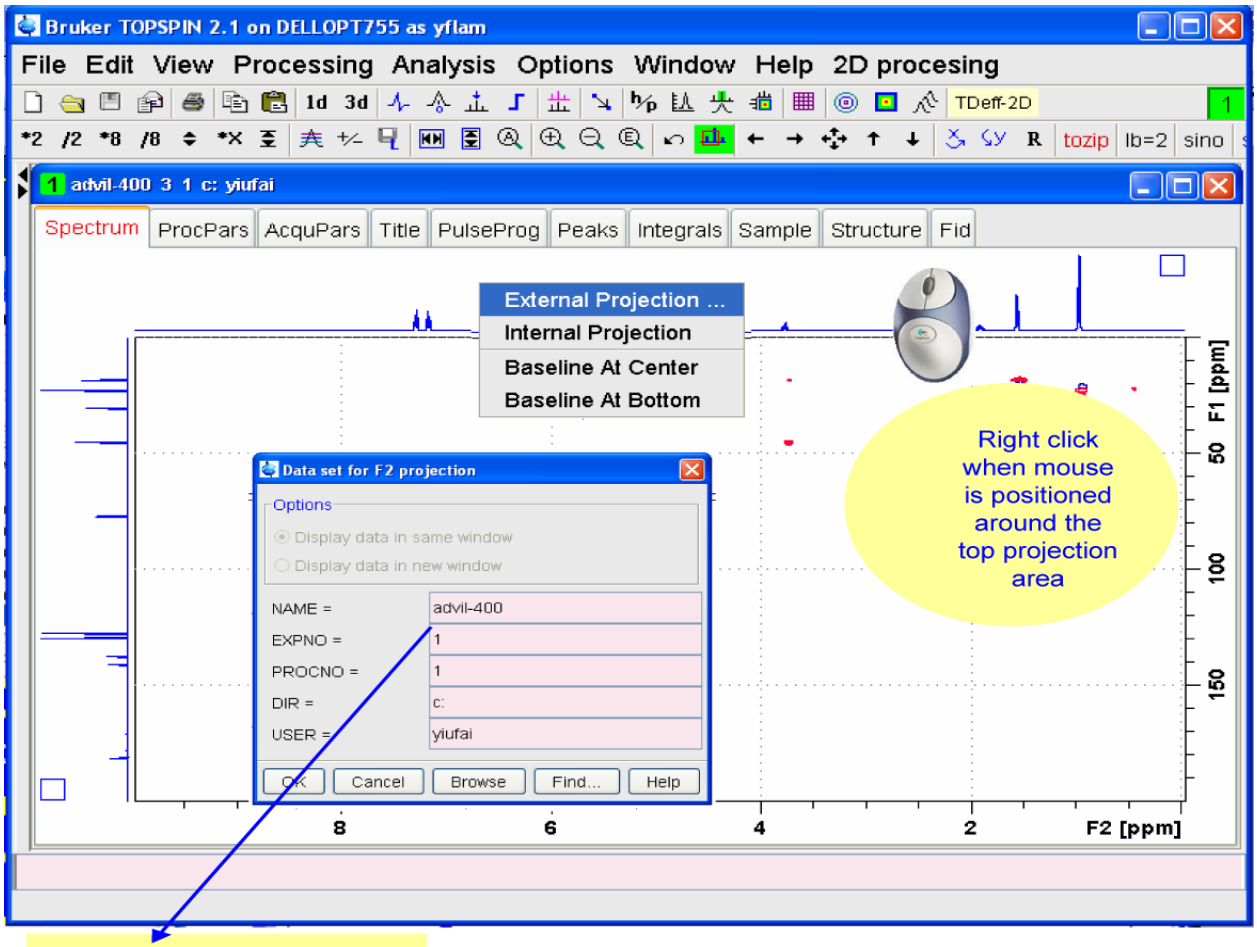
The SR values can be found in the ProcPars tab of the experiment. If you are carrying out a COSY experiment then the values for SR in F1 and F2 should be equal to the SR value from your 1D proton experiment. If you are carrying out an HSQC or HMBC then the value for SR in F2 and F1 should correspond to the SR value you recorded earlier for the 1D proton and 1D carbon experiments, respectively. This step ensures that both of your dimensions are properly referenced in chemical shift.



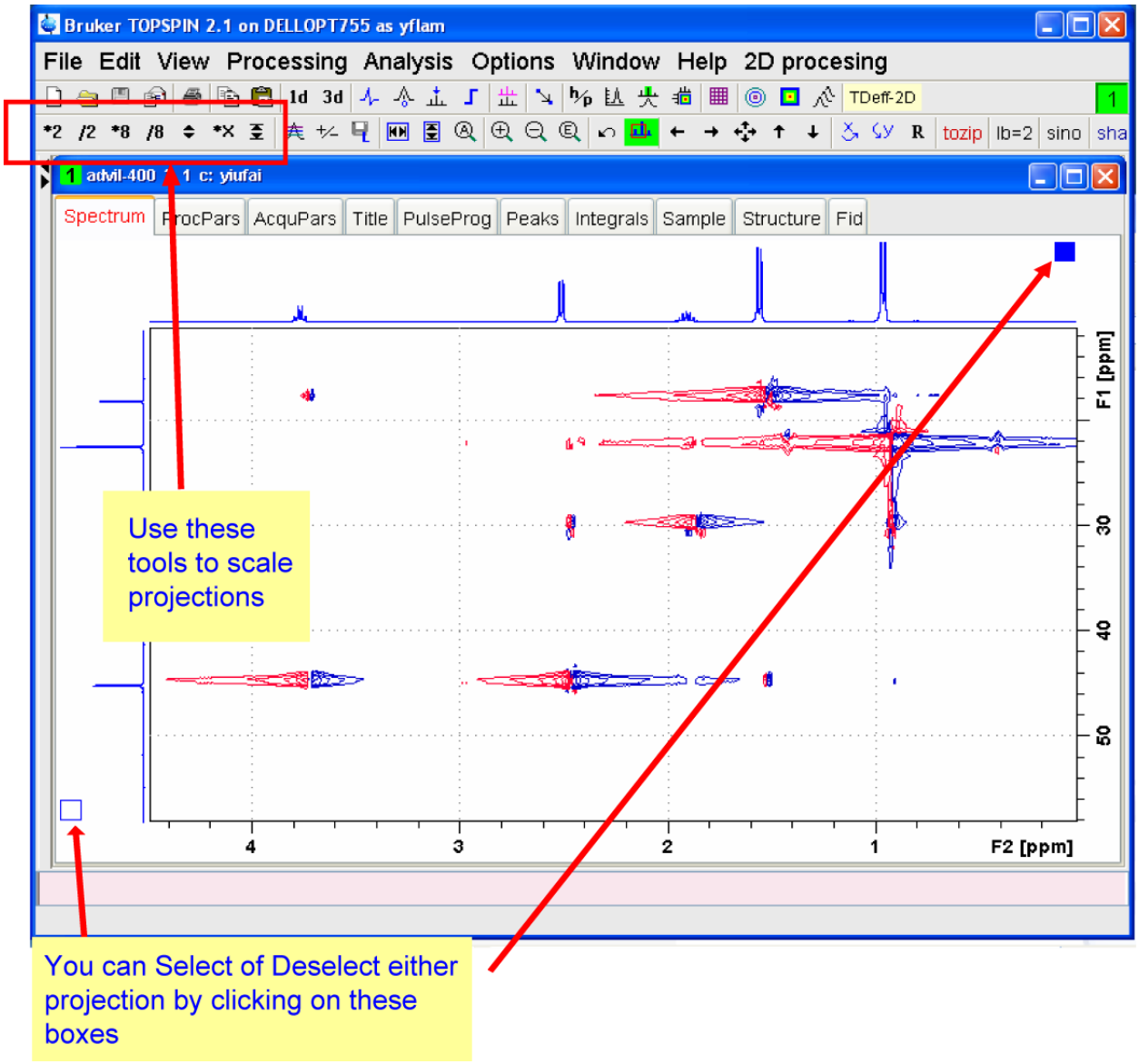
Obtain the values of SR from the 1D data sets, Import the values into the 2D ProcPars entries

(18) Import the F2 and F1 projections from the 1D carbon and proton experiments.

This can be done by positioning the mouse over the area where the projections appear and right clicking. After right clicking you can left click on the external projection option. This will bring up a box where you can specify the location, filename, and experiment number of the 1D data set being used for the projection. If you have followed the protocols above for creating these experiments then you should only need to enter the experiment number. Remember that in a HSQC or HMBC the F2 and F1 dimensions corresponds to the 1D proton and carbon experiments, respectively (See figure below).



(19) Scale your projections by selecting them individually and using the normal scaling tools for TopSpin (see figure below).



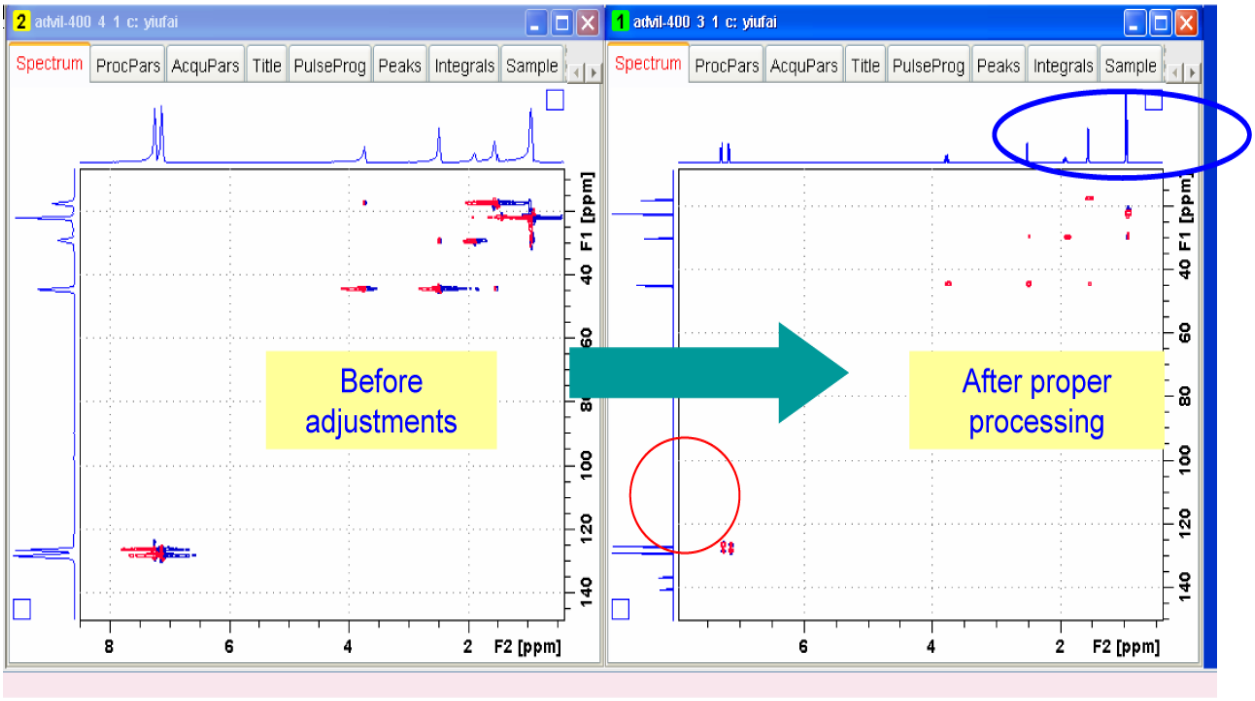
(20) **PRINT YOUR SPECTRA AND YOU ARE DONE!**

NOTE: In most cases the auto processing routines used above will be all that is required.

If the auto-processing done by TopSpin is not satisfactory then you must further process your 2D data. The next few Pages present a couple of screen shots on manually processing your data in Topspin; however, detailed instructions, such as those above, are not provided.

Manual Processing Instructions for 2D NMR Experiments

Advanced 2D NMR data processing with TOPSPIN

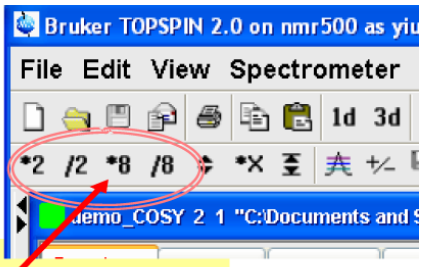


Flow Chart:

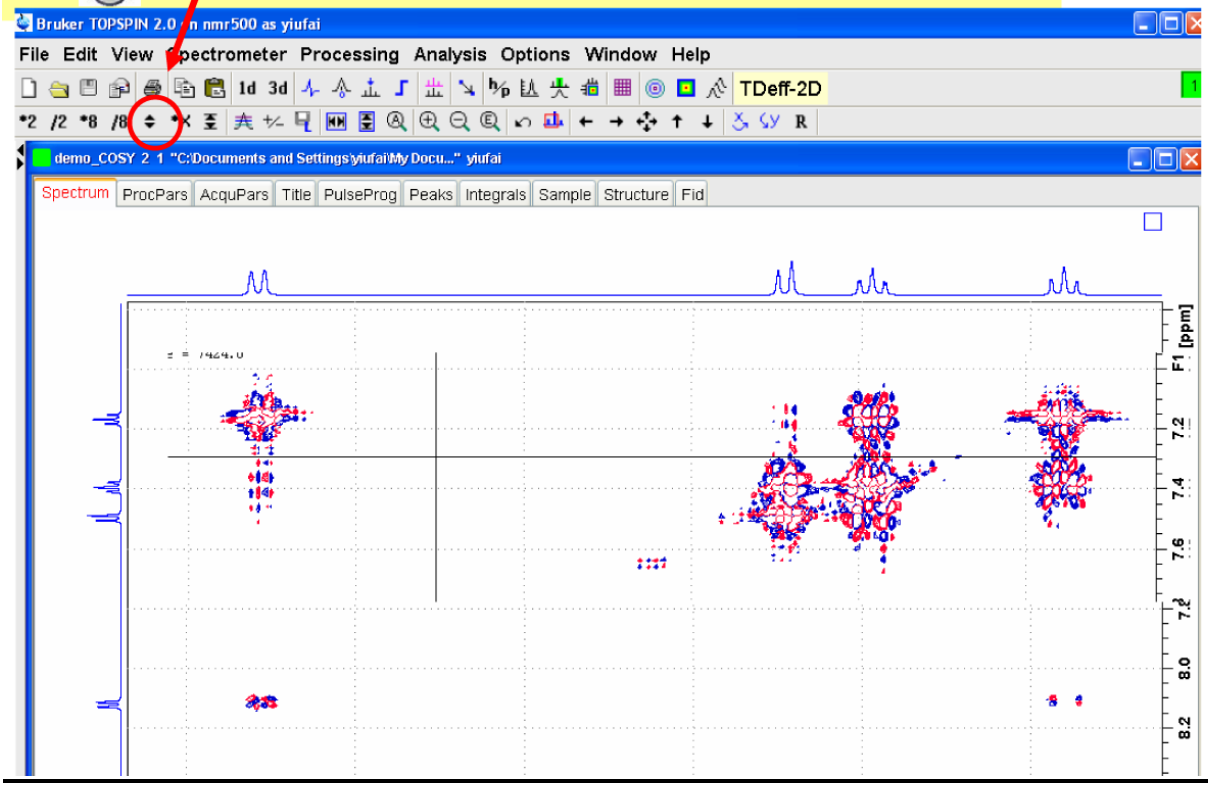
(1) Proper contour Adjustment
(2) Proper Phase Adjustment
(3) Plotting with TopSpin Plot Editor

(1) Contour Adjustment

Graphic Adjustments of 2D Contour

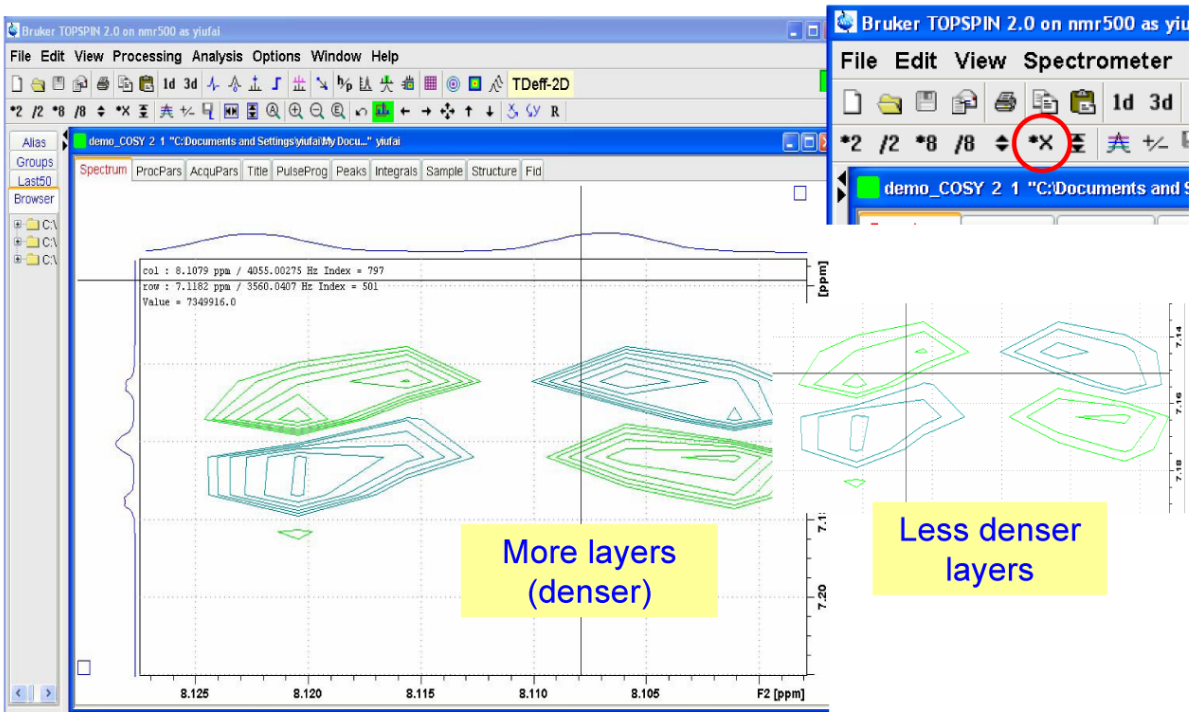


- **After Fourier Transform in both dimension (xfb):**
  - **Use ICONs in 2nd menu row to adjust in multiple of 2 or 8**
    - **OR**
    - **Click and drag to adjust the threshold (minimum level) for contour display..**

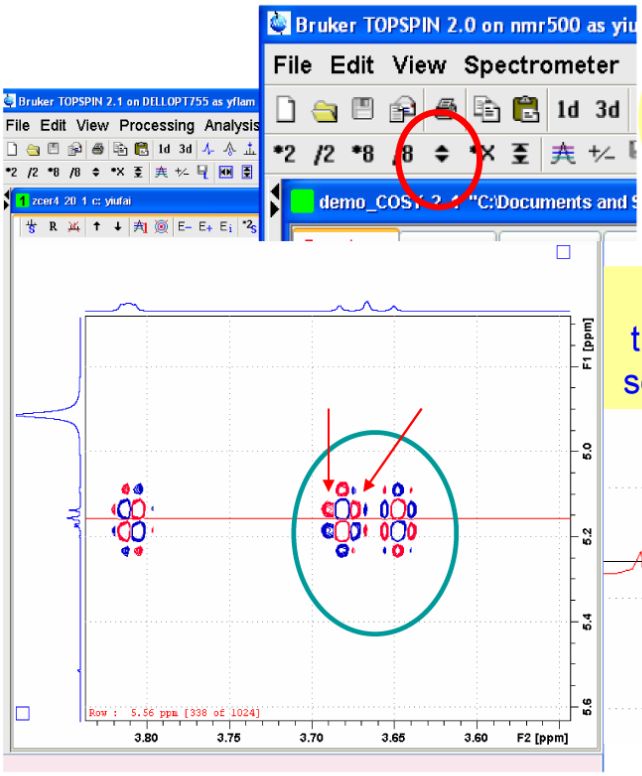


Adjust distance between contour levels:

- Click and drag to change the inter layers distance:



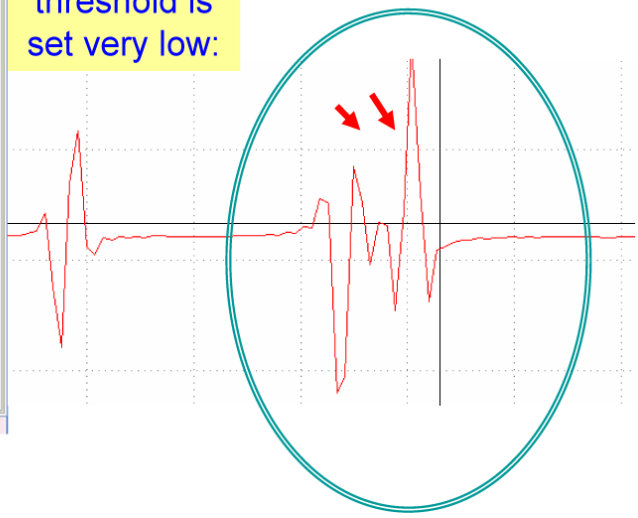
Example of Excessive threshold level adjustment:



Proper level depends on the S/N ratio, the intensities of the global and local cross-peaks;

In some 2D with large differences cross-peaks intensities, separate 2D plot is preferred.

When threshold is set very low:



(2) Phase Adjustment

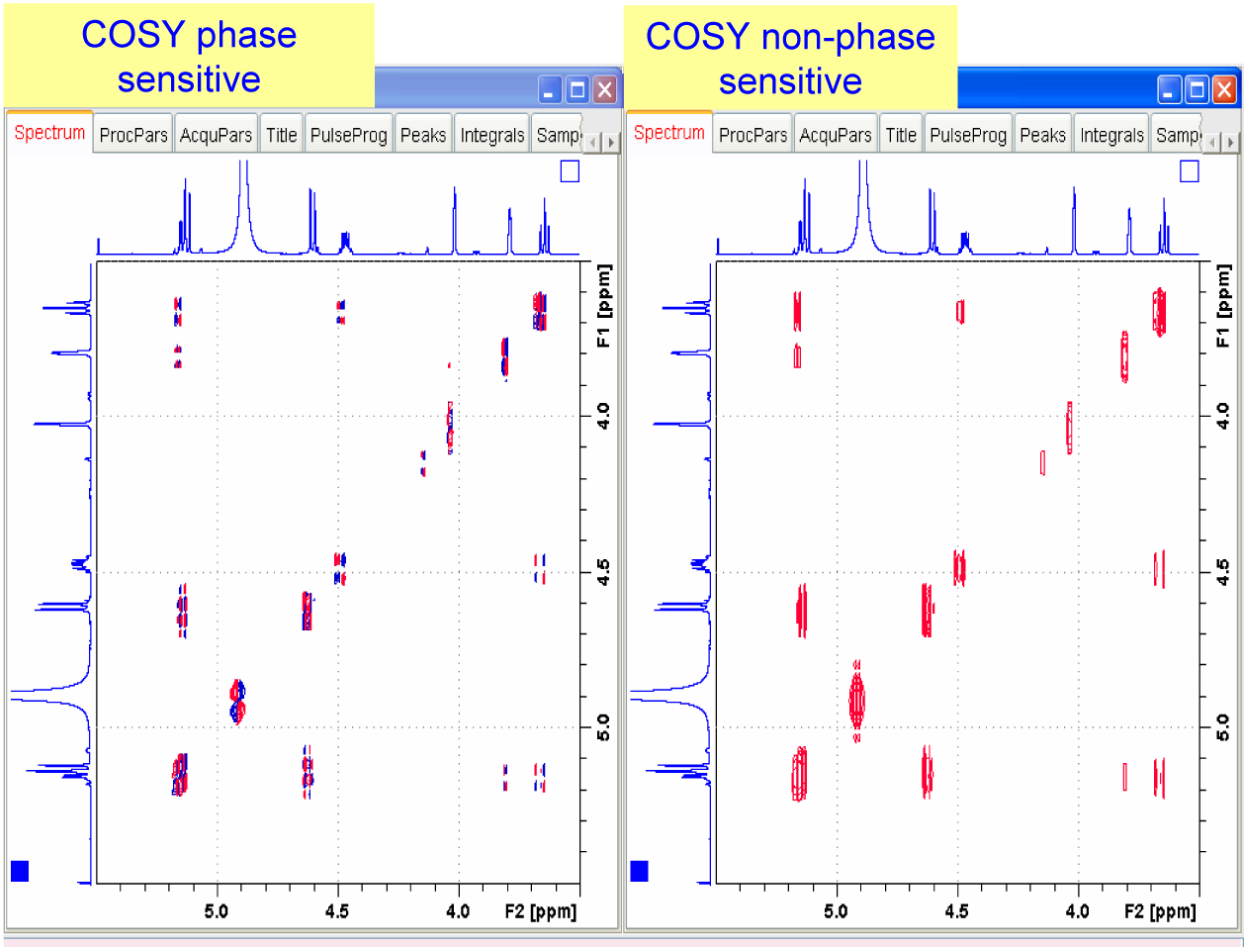
2D Phase adjustment:

- Phase properties:
  - 2D that yields magnitudes and no phase information:
    - Basic COSYqf
    - HMBC
  - 2D that yields phase sensitive correlations:
    - COSY, -- correlation peaks are dispersive.
    - HSQC – correlation peaks are absorptive, all positive.
    - NOESY – correlation peaks are absorptive, could be positive or negative.

Options of phasing:

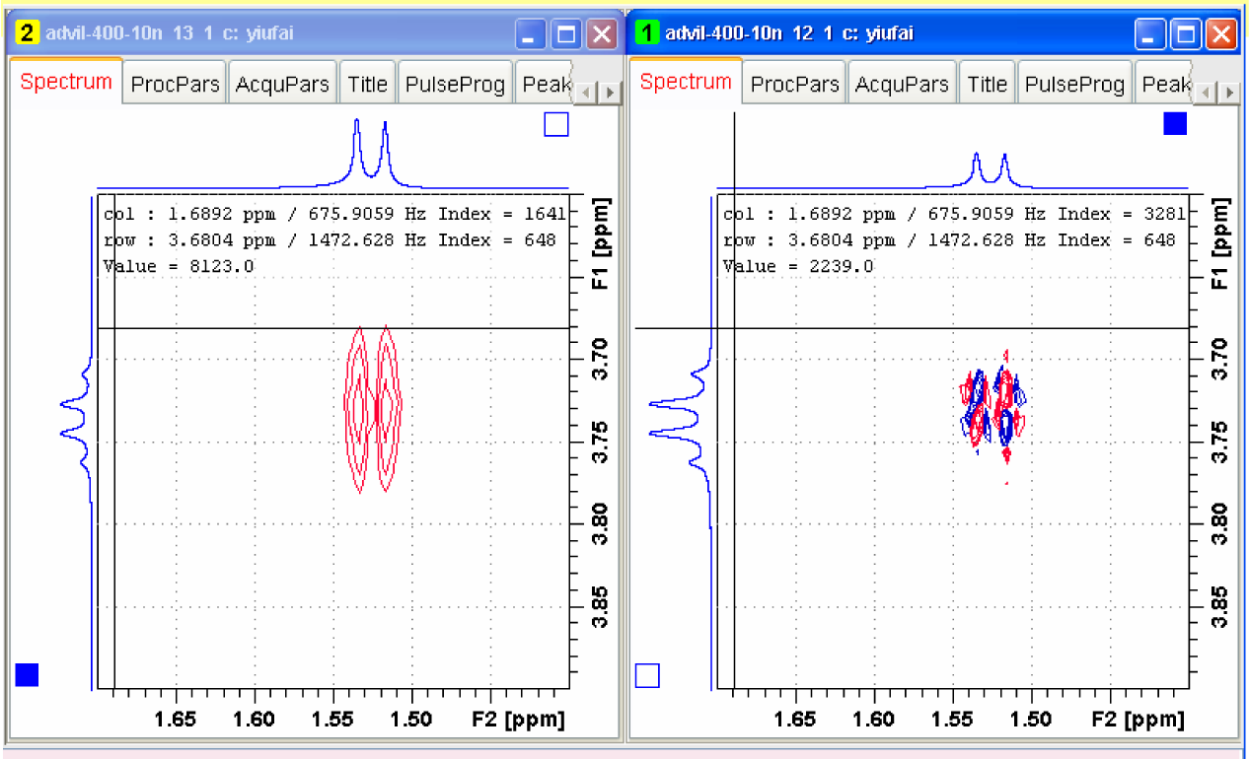
1. Automatic: For simple 2D data sets (with no cluster signals).
2. Manual phasing: for complex data set (takes time!).
3. Just skip phasing: type xfbm for magnitude conversion.

Difference between phased and magnitude contours:



Magnitude mode and phased 2D contours:

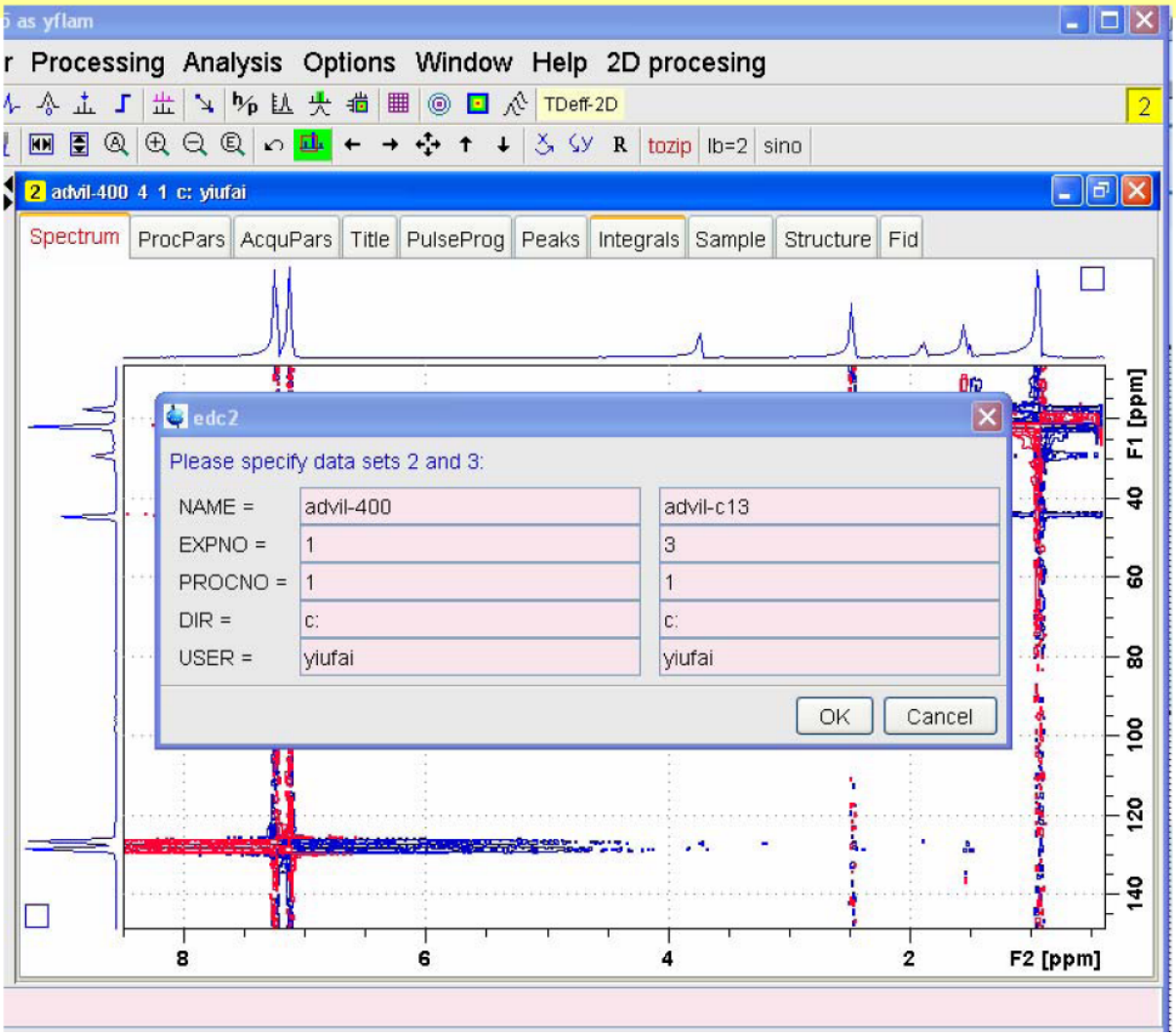
Enlarged comparison



Auto 2D phasing:

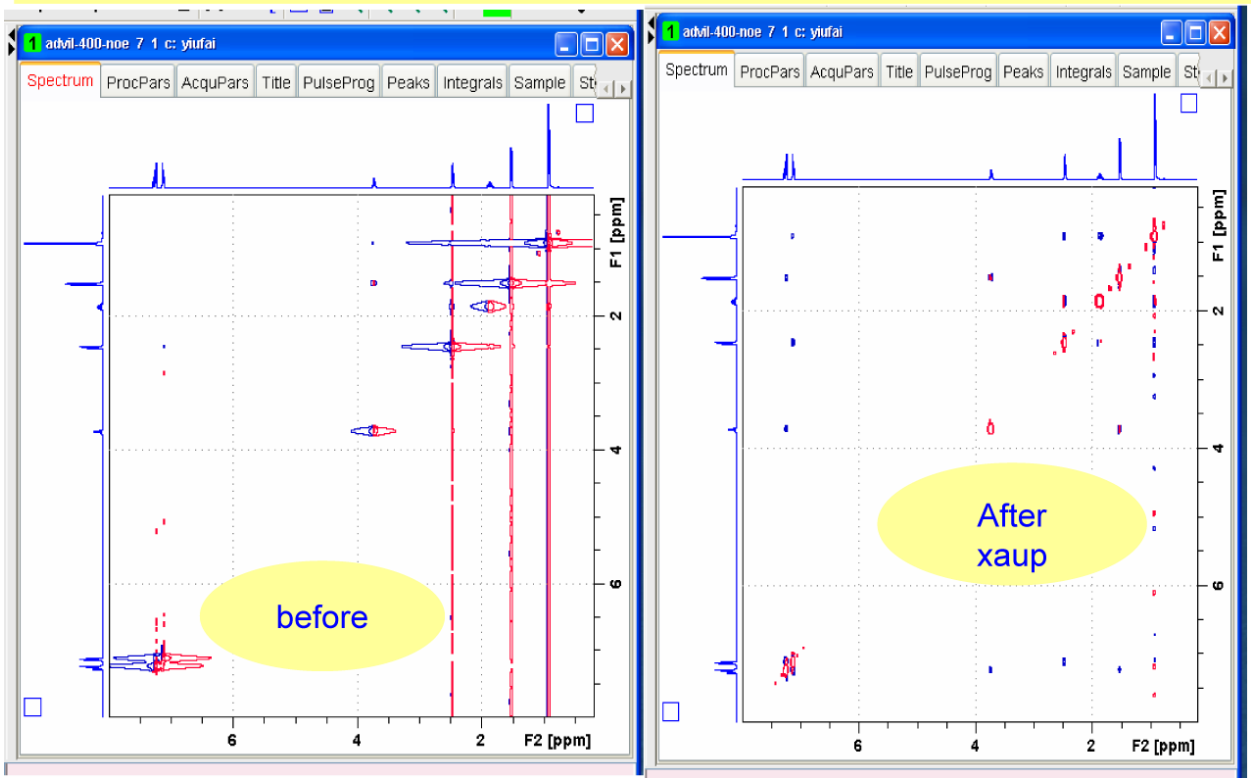
TOPSPIN software command for phase adjustments:

- Use automation:
  - Type edc2 and define projection on both F2 (set2) and F1 (set 3)
  - Type xaup
  - This is the method used by the drop down menu



Example of 2D NOESY phasing: using automation

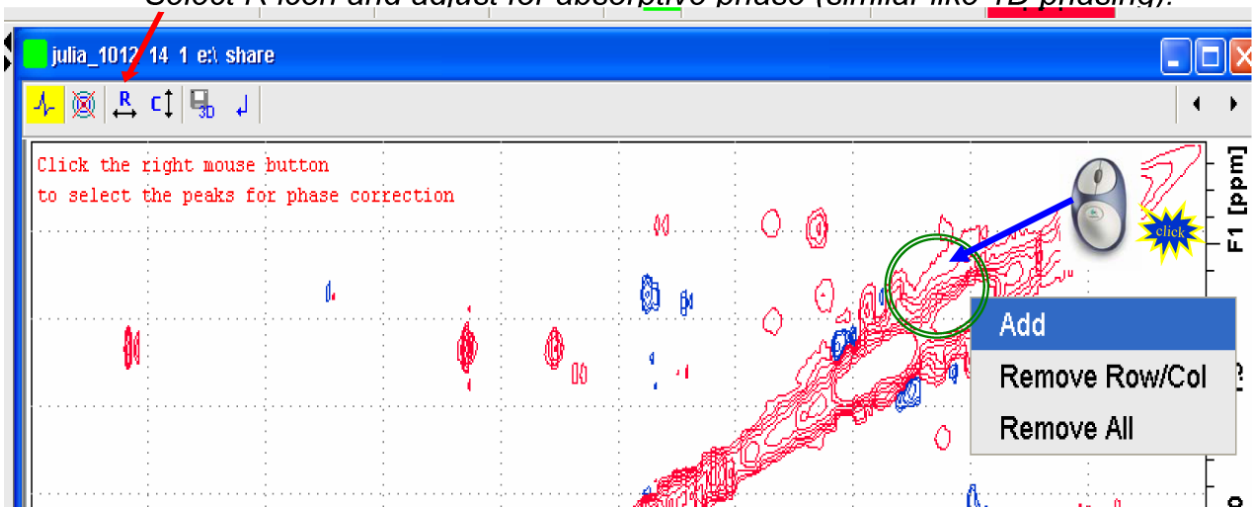
Remark: ***Auto-correlation diagonal peaks red, correlation peaks blue and opposite phase in this example shown. In general, NOE correlation peaks can be positive or negative, indicating it is a positive NOE, or negative NOE , or chemical exchange.***



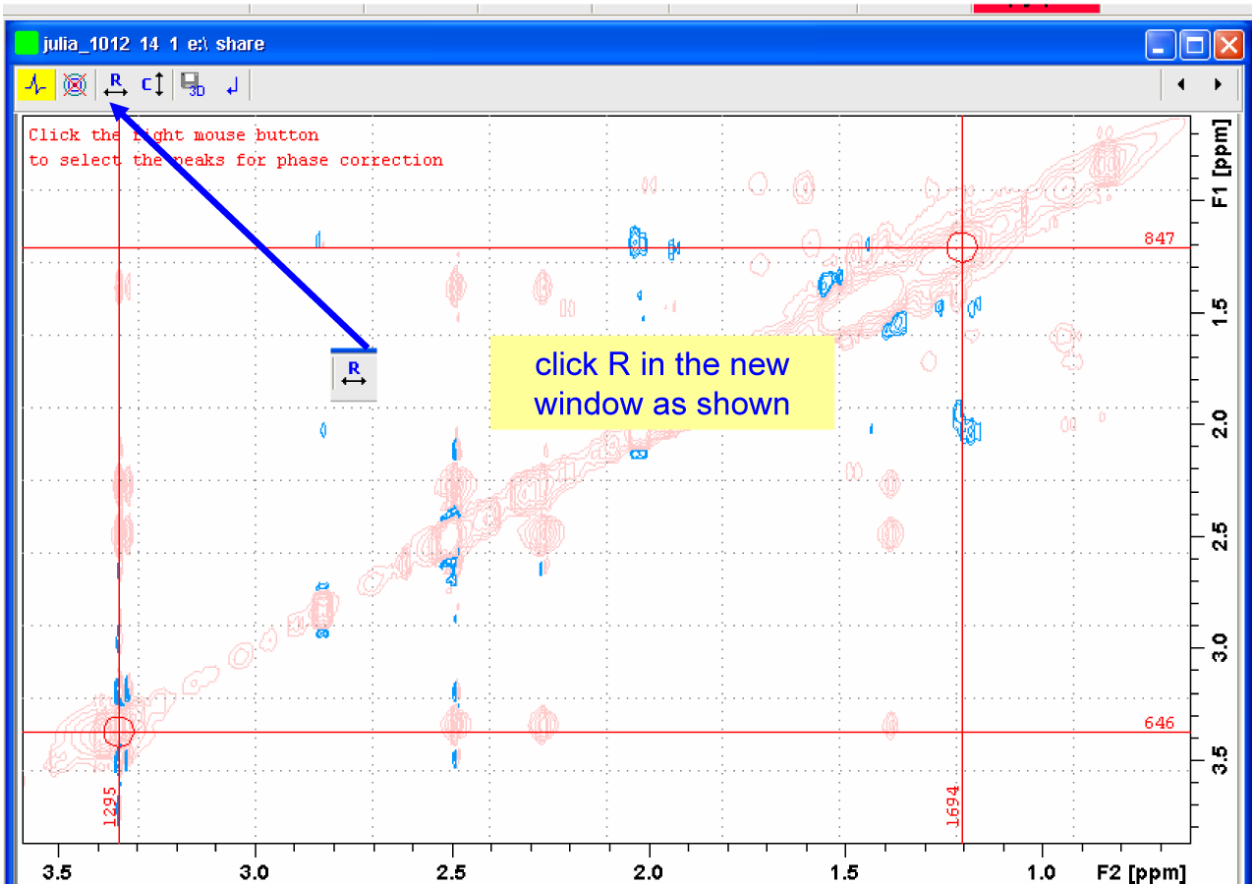
Manual and refined 2D Phasing :

Step 1 select cross peaks

- *Click phase* 
- *In the new window:*
  - *Place the mouse on one of the major auto-correlation signal, right click and "Add".*
  - *Repeat the same procedure to select another correlation peaks far away from the previous one, right click and " Add" again.*
  - *Repeat the same procedure if additional 3rd slides is desirable.*
- *Select R icon and adjust for absorptive phase (similar like 1D phasing).* 



2D Phasing: After two slides are selected

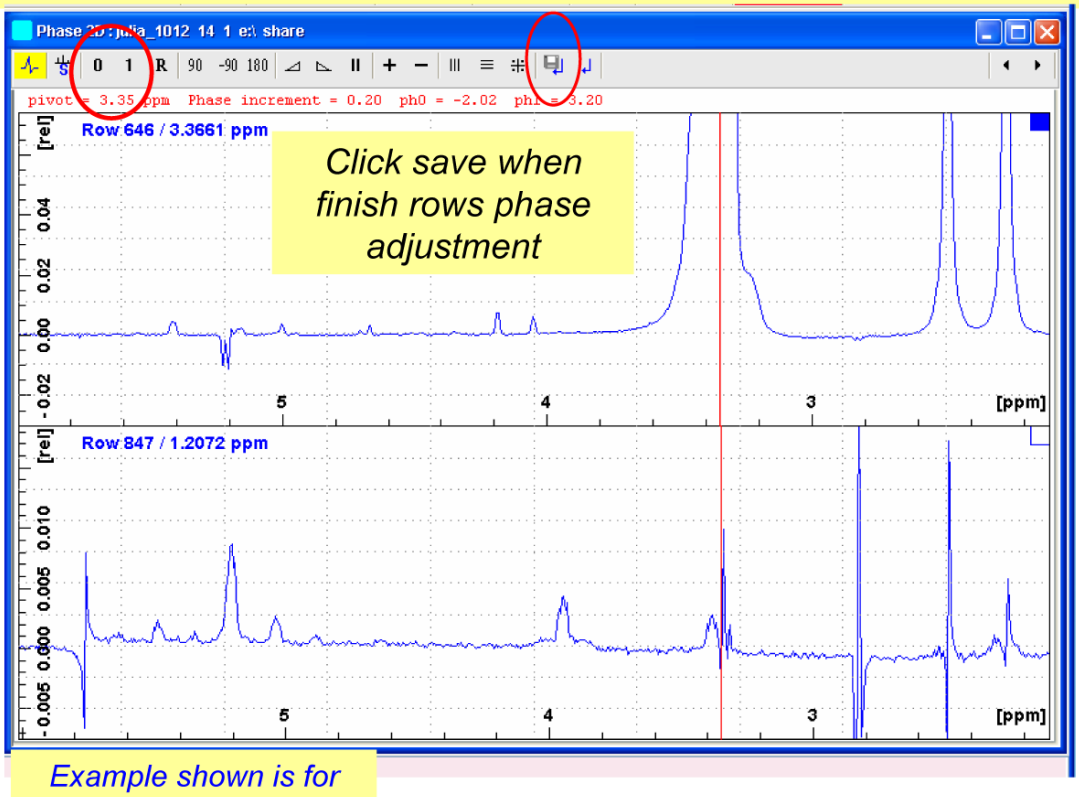


2D phase adjust: rows

- *Once R icon is click*

Use the O and 1 for phase adjustment for pivot and off pivot signals

Remark: Phase of correlation peaks depend on the nature of the 2D pulse sequences.

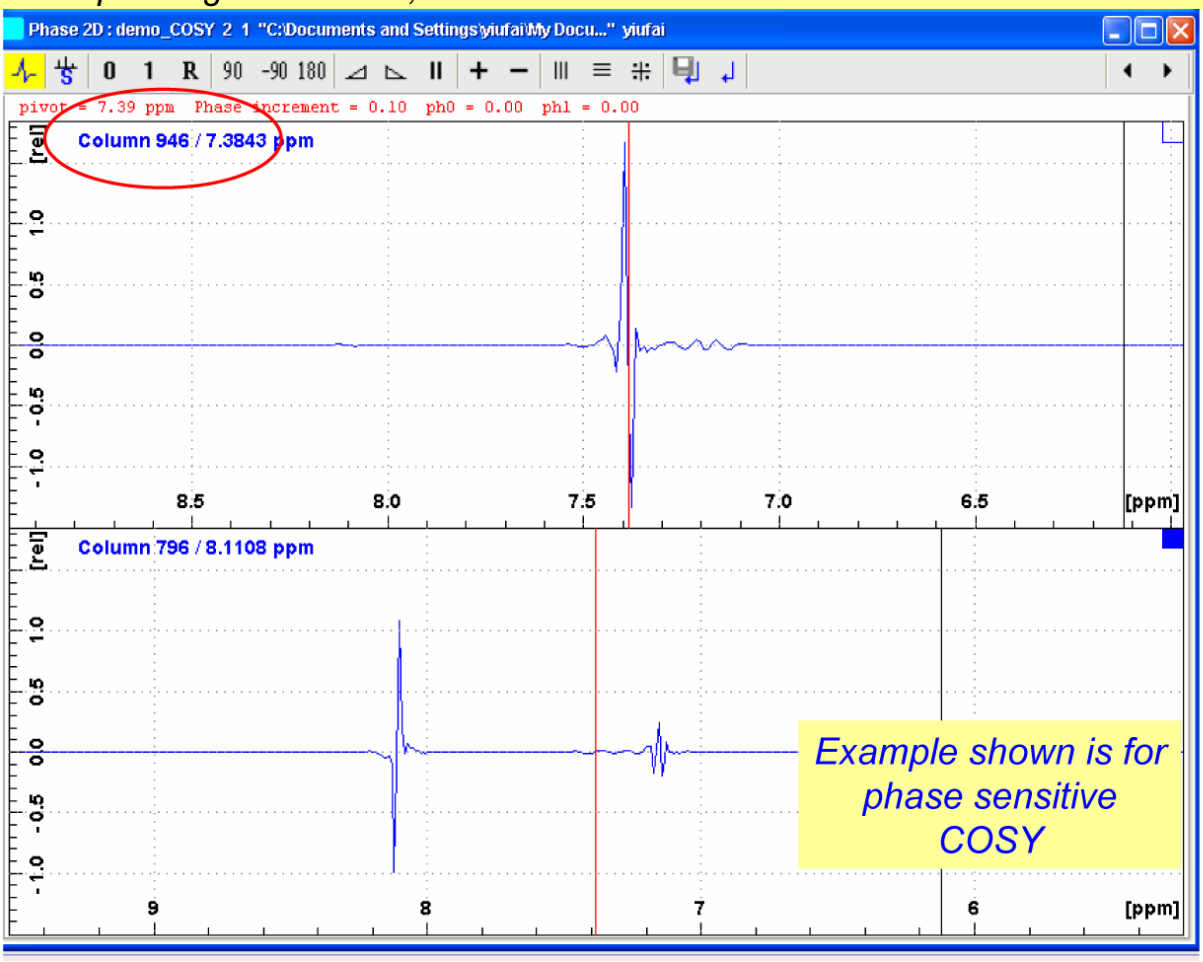


Click save when finish rows phase adjustment

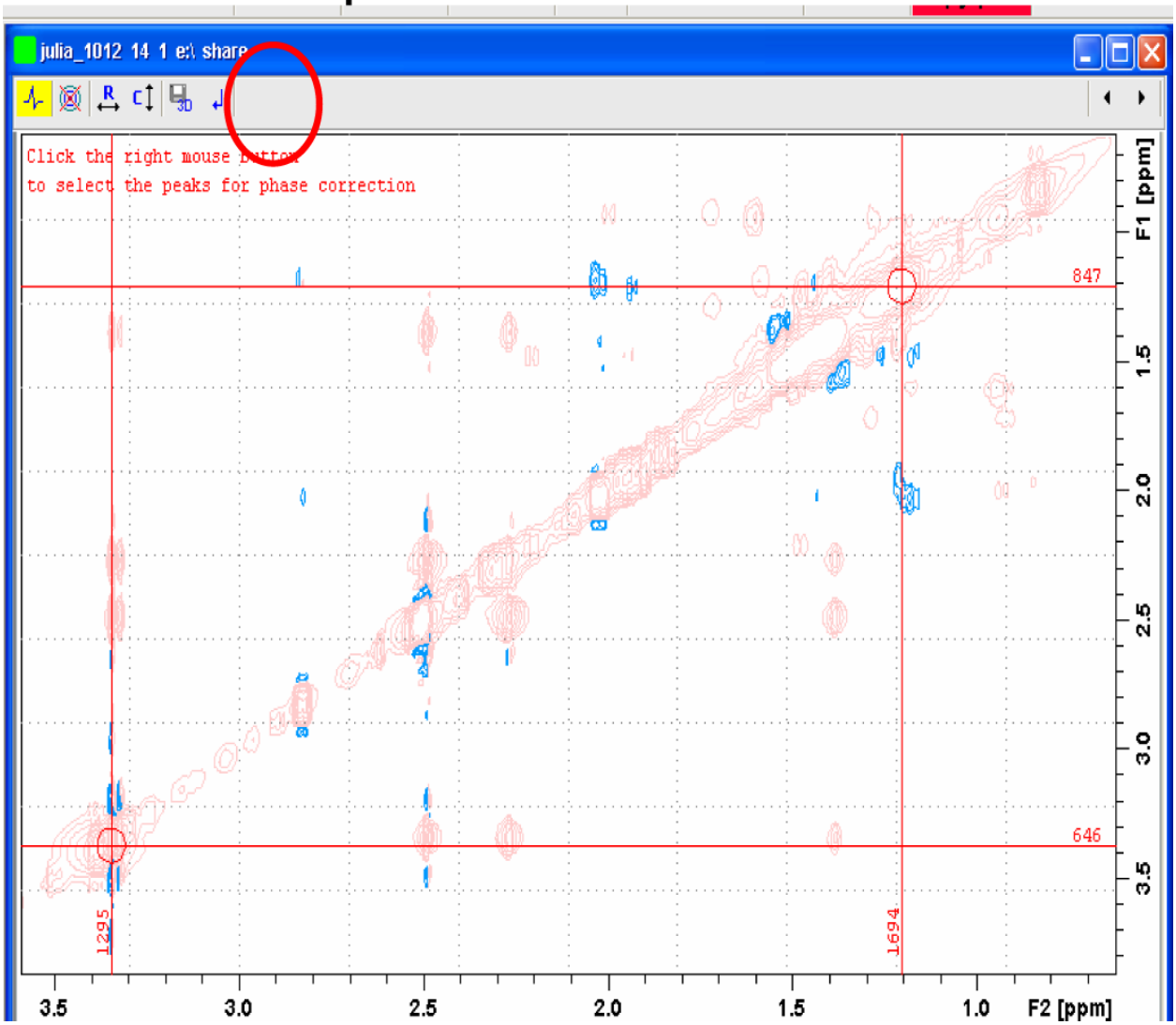
Example shown is for HSQC in this slide

Phasing: Column slides 2D

Repeat same phasing procedure for column, after C is icon is selected --click
After phasing the column, click save and return.

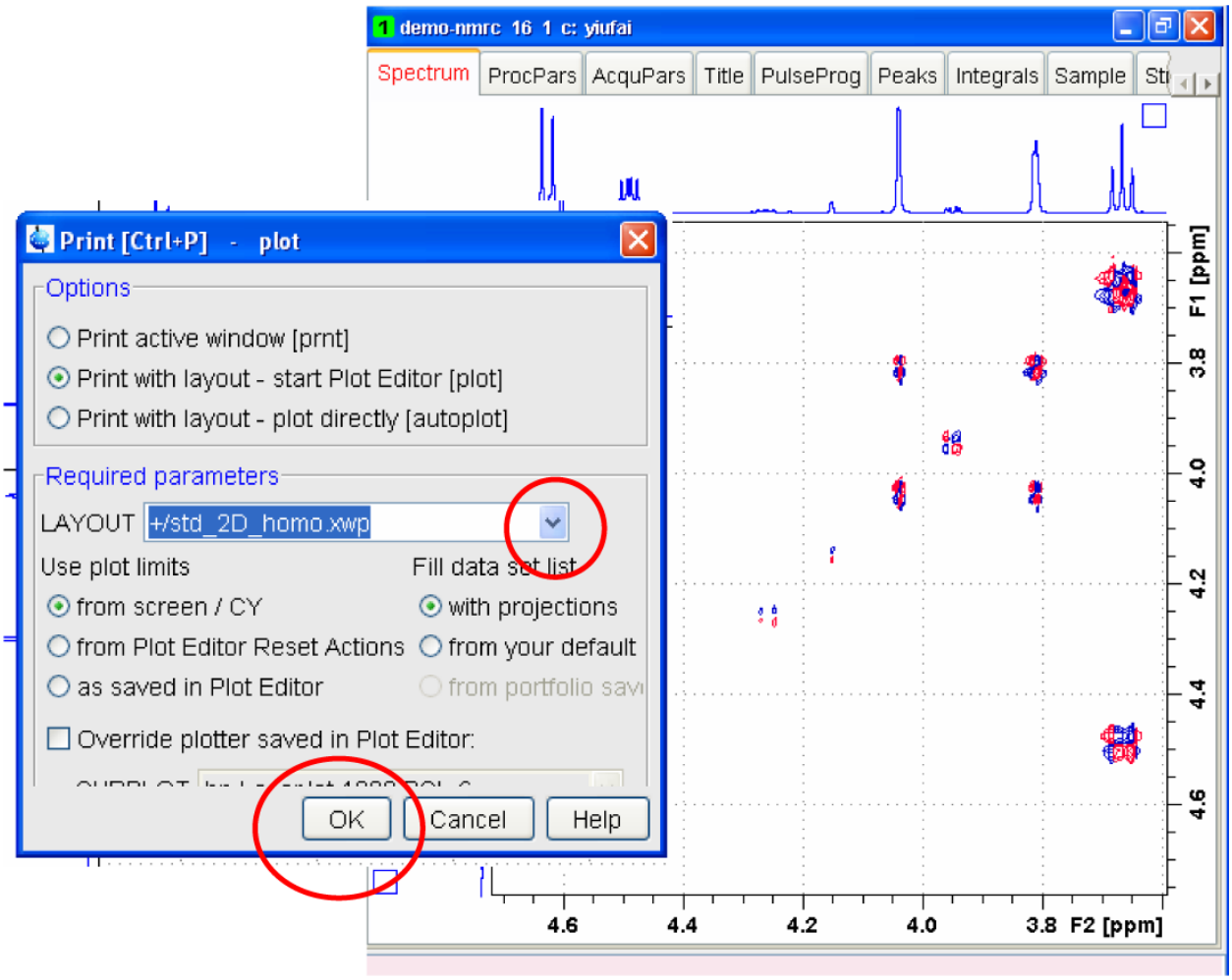


Finalize the phase correction:

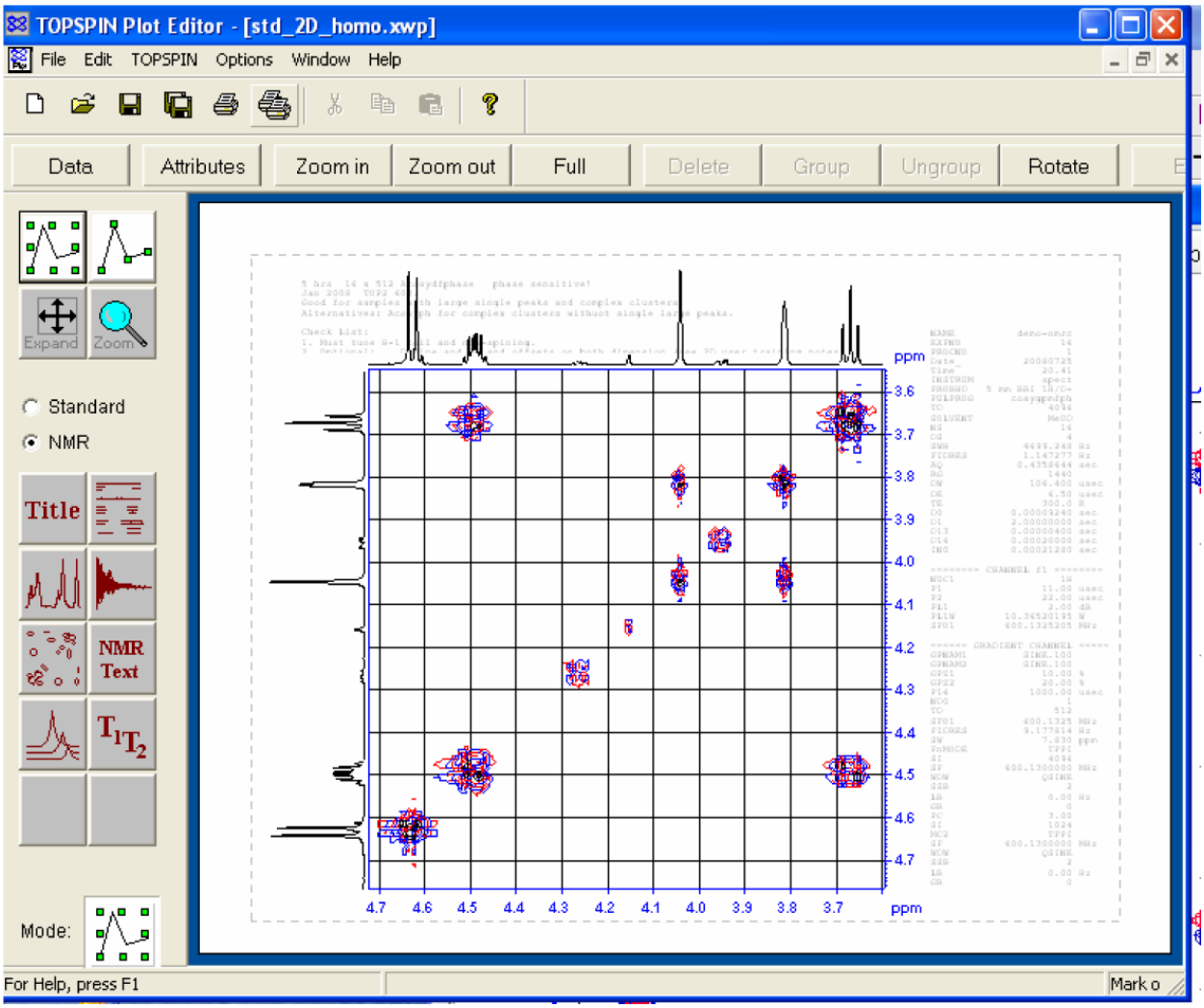


(3) Plotting with TopSpin Plot Editor

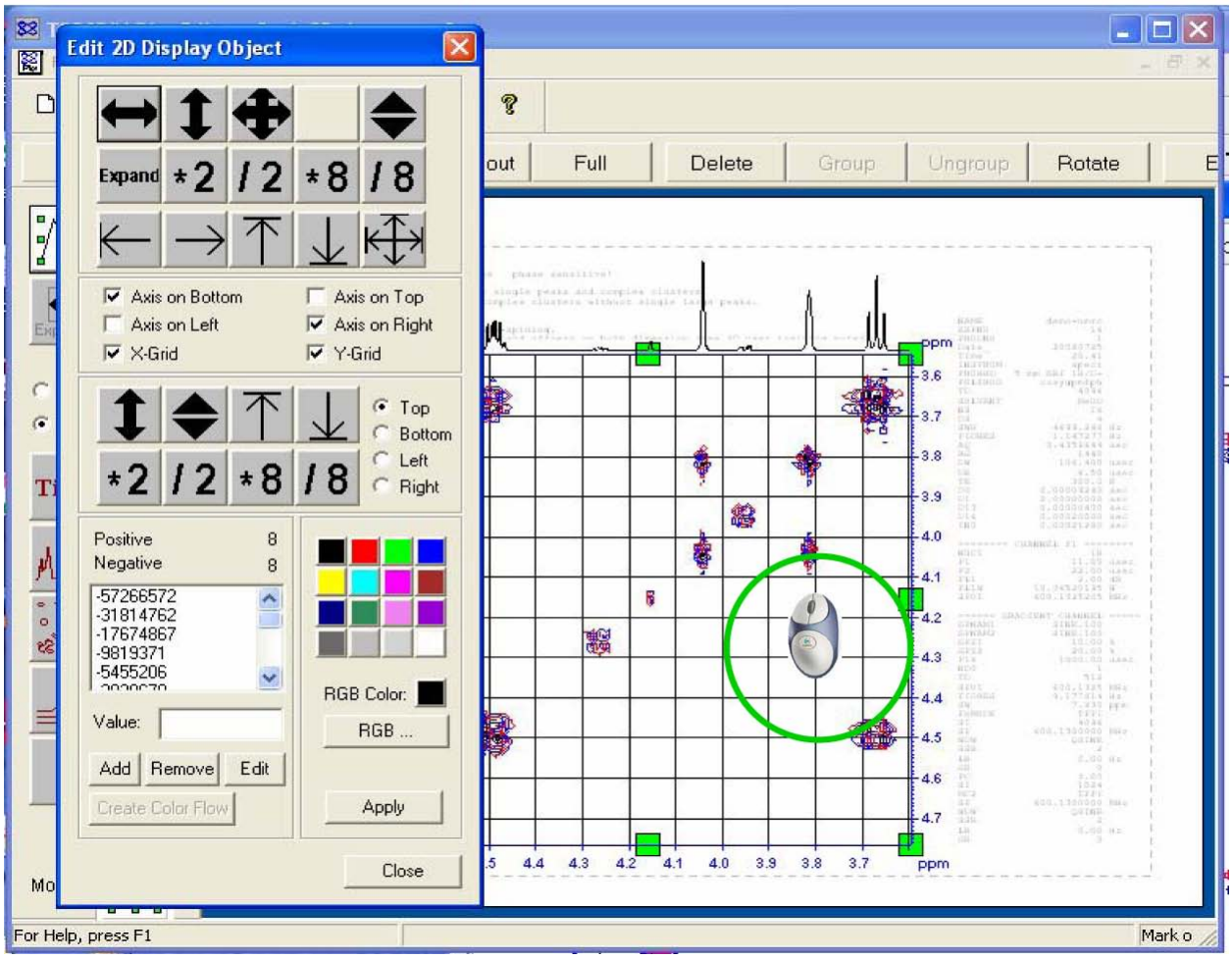
Select layout and call up plot-editor:



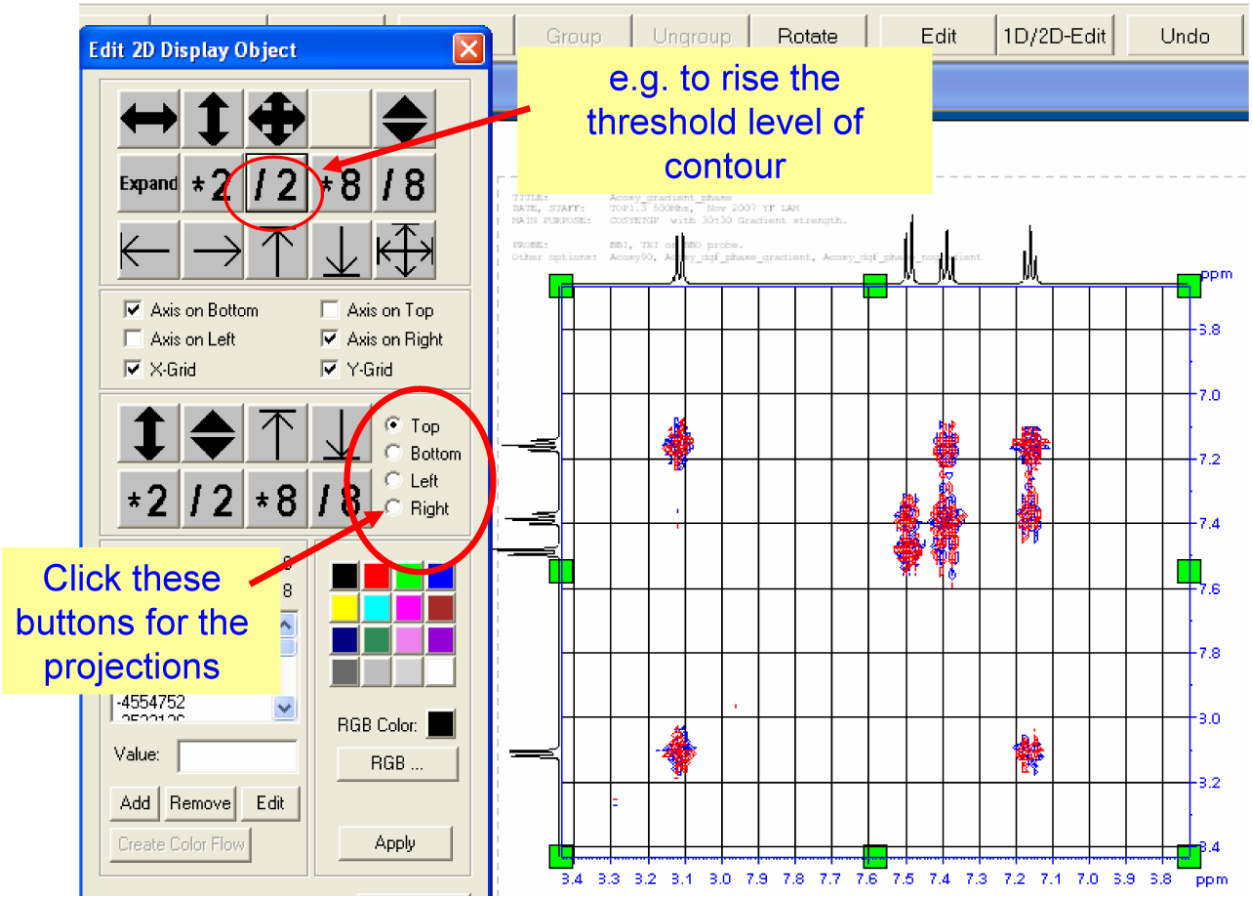
Plot-editor: For additional refine adjustments



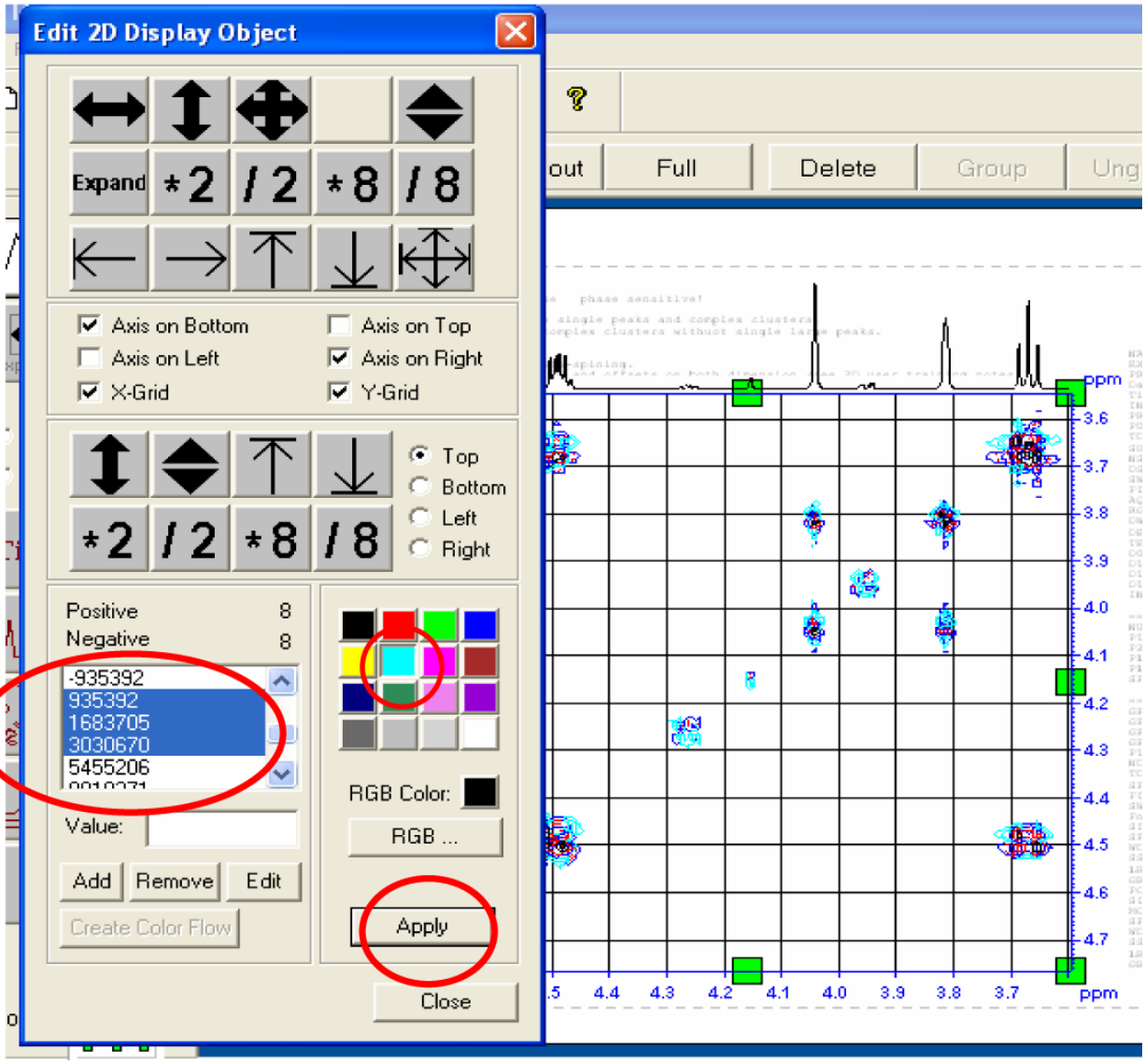
Activate 2D contour object and select 1D/2D Editor:



Use 1D/2D editor: adjust threshold level of contour



Modify colors for various contour layers:



Final outputs: