Common Acquisition Concepts and Problems for 1D/2D NMR

(1) Acquisition Parameters and Sampling the FID

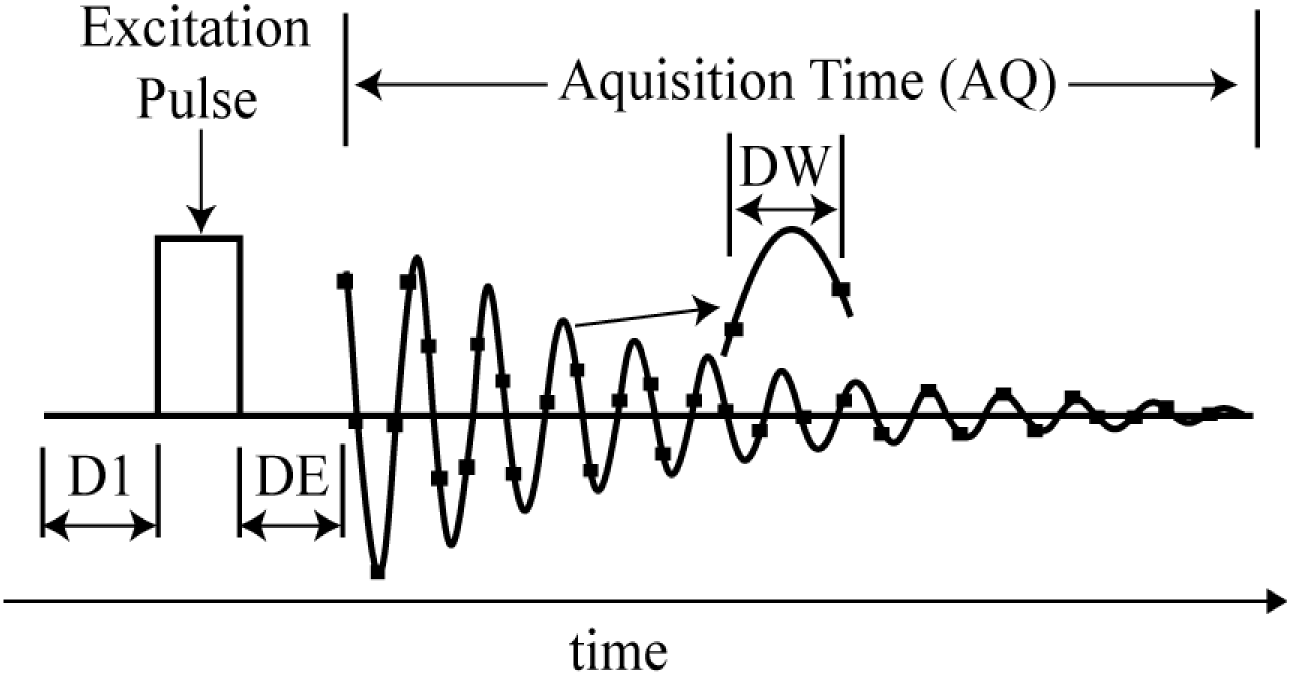
Acquisition Time (AQ) = NumPoints (TD) * DwellTime (DW)

DwellTime (DW) = 1 / SpectralWidth (SW)

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

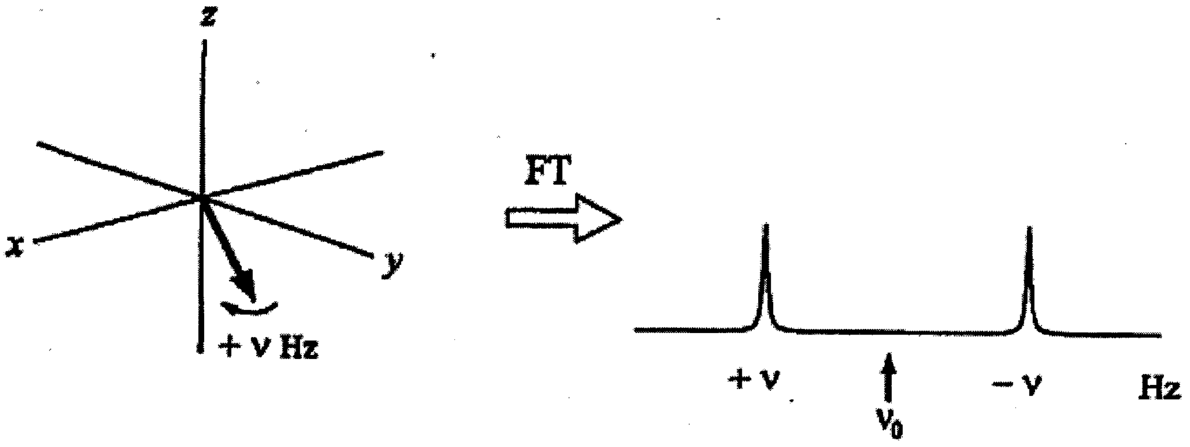
D1 = Recycle Delay   DE = Pre-scan delay

NS = Number or scans   DS = Number of dummy scans

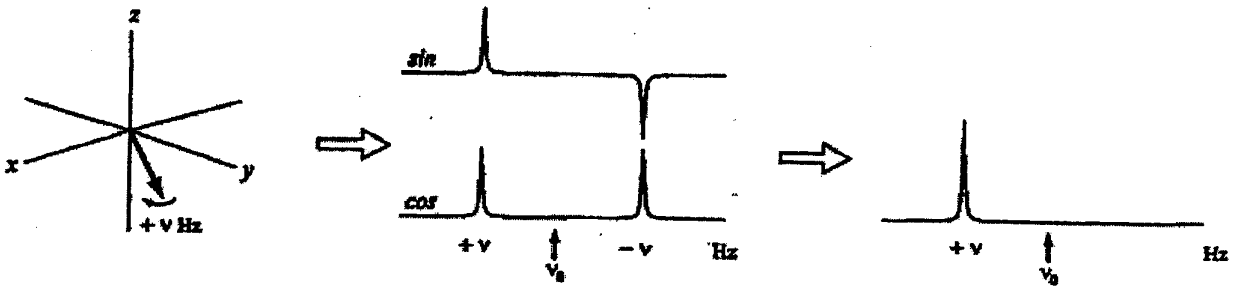


(2) Quadrature Detection

The spectrum corresponding to a positive precessing magnetization using single channel detection



Detection of a positive rotating magnetization using a two-channel scheme.



(3) Phasing

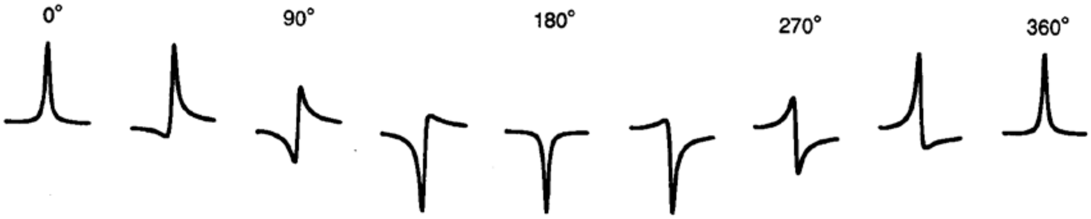
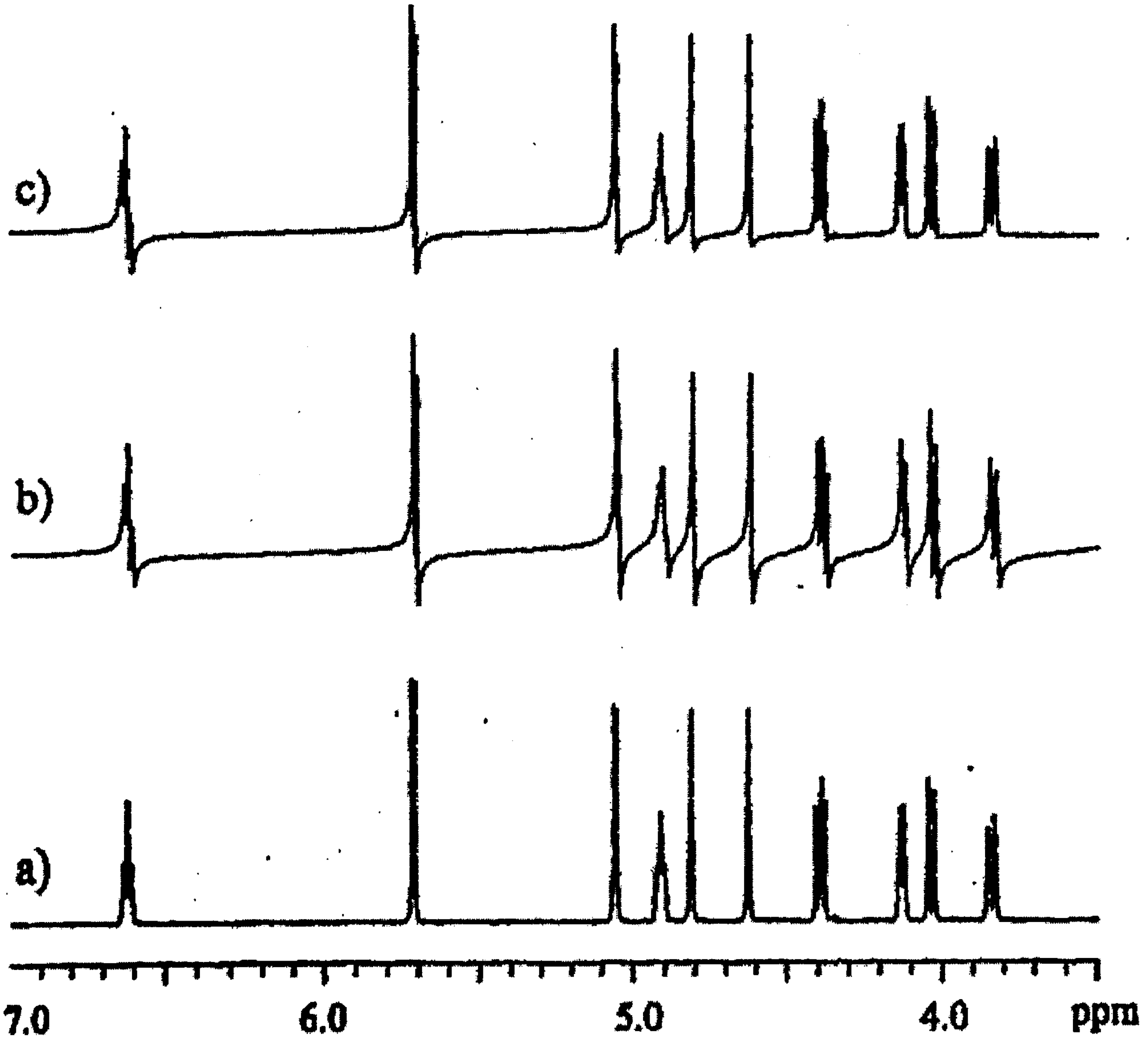
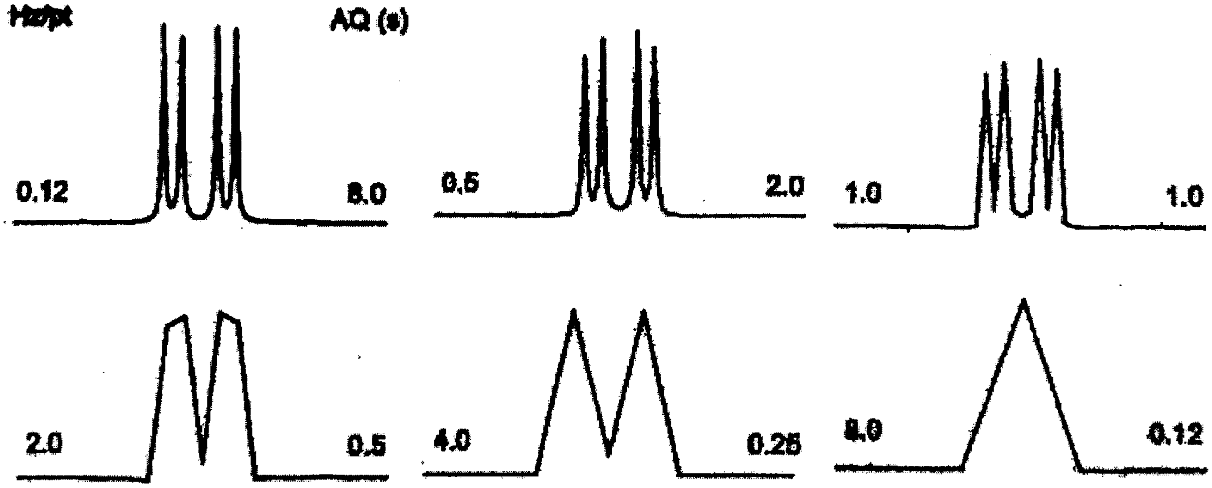


Fig. 1.15. The effect of a change in phase on the shape of a resonance signal; each step in phase is 45°.

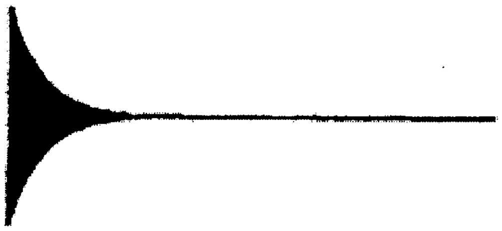
Phase Correcting a Spectrum, a) the initial spectrum, b) after zero-order correction, c) after first-order correction

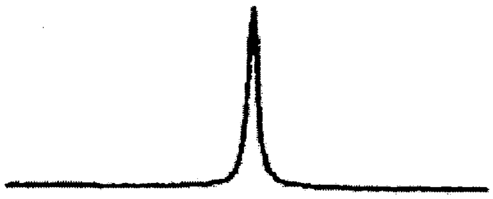


(4) Digital resolution

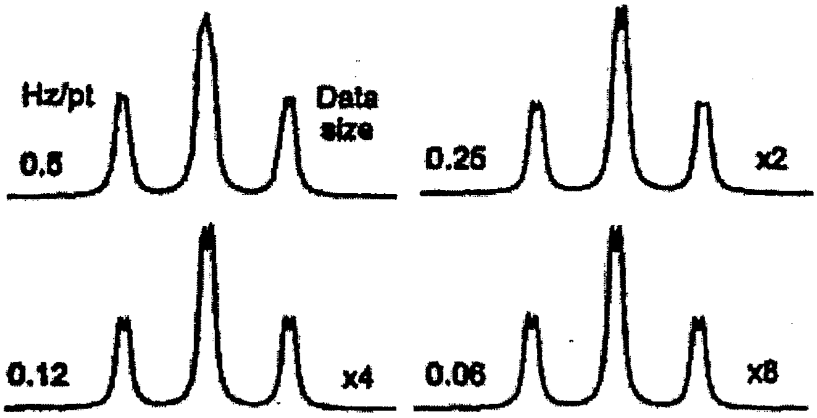


(5) Zero filling the FID





Zero filling may reveal fine structure that would otherwise be overlooked



(6) Exponential Multiplication

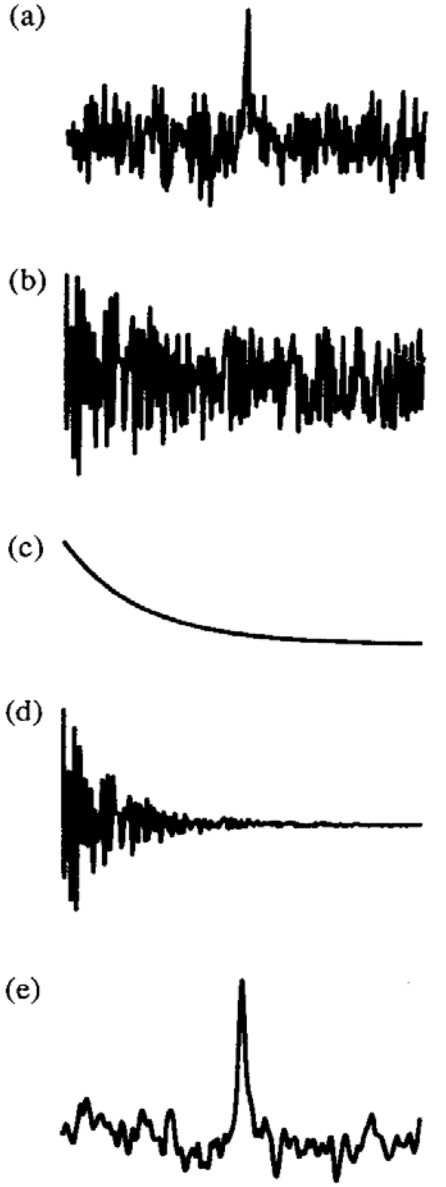


Fig. 1.23. Computer simulation of the effect of exponential multiplication: (a) signal from Fourier transformation of (b); (b) the 'untreated' FID; (c) the exponential decay function; (d) the FID after exponential multiplication; (e) signal from Fourier transformation of (d).

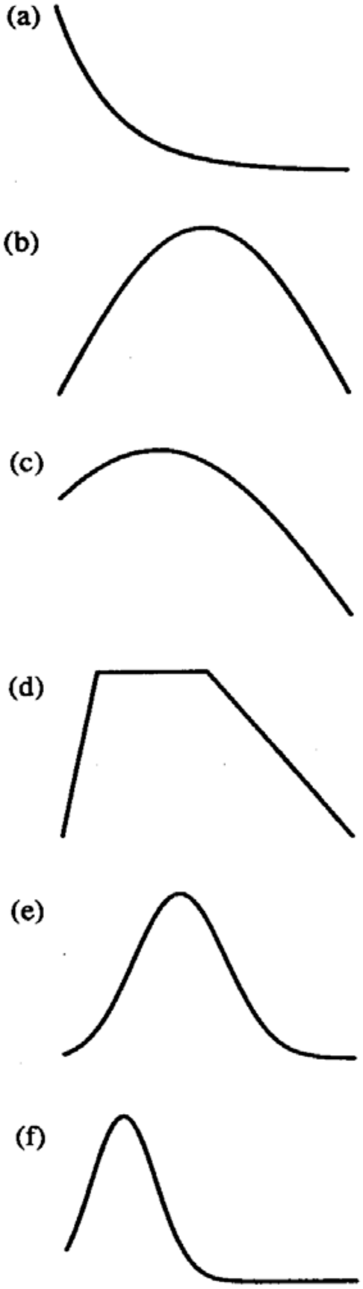
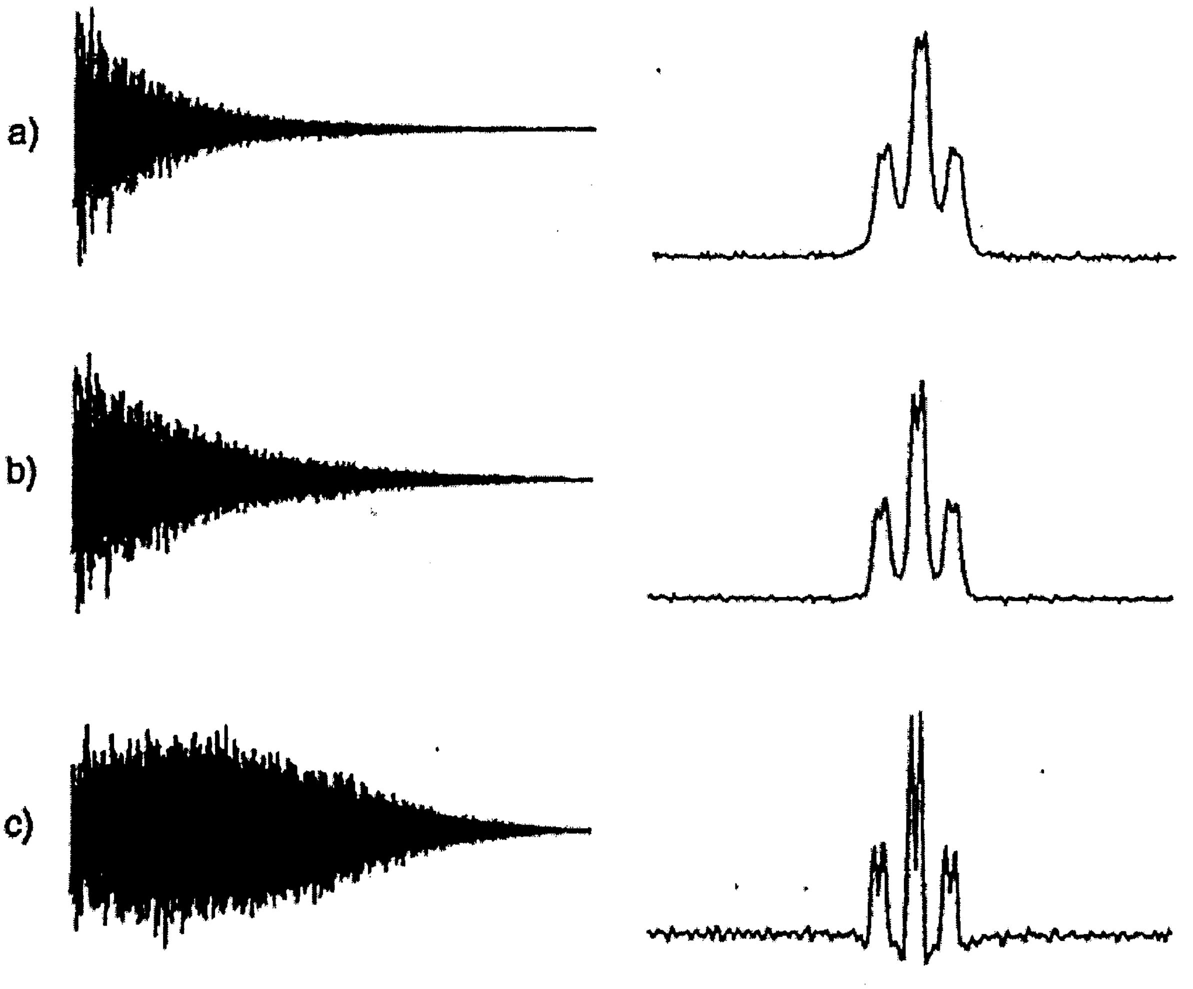


Fig. 1.24. Selected apodization window functions: (a) exponential multiplication; (b) sine-bell; (c) phase-shifted sine-bell; (d) trapezoidal multiplication; (e) Gaussian multiplication; (f) Gaussian multiplication with different parameters.

(7) Lorentz-Gauss Transformation

a) Unadulterated FID b) Exponential Multiplication of FID
c) Lorentz-Gauss Transformation of FID



(8) Folding

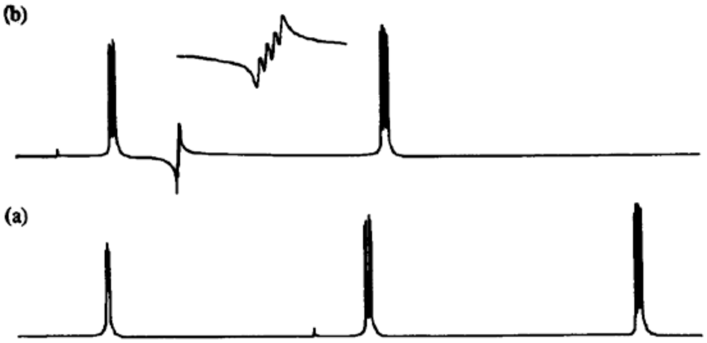
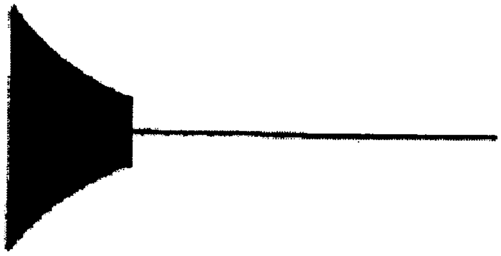
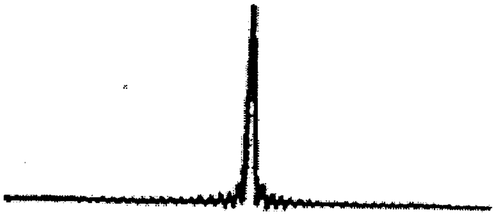


Fig. 1.19. ^{1}H spectra of furoic acid-d_1, **1.1**. (a) Acquired with the spectral width and reference frequency set correctly; (b) acquired with the reference incorrectly set. The inset shows the 'folded' multiplet.

(9) Truncation

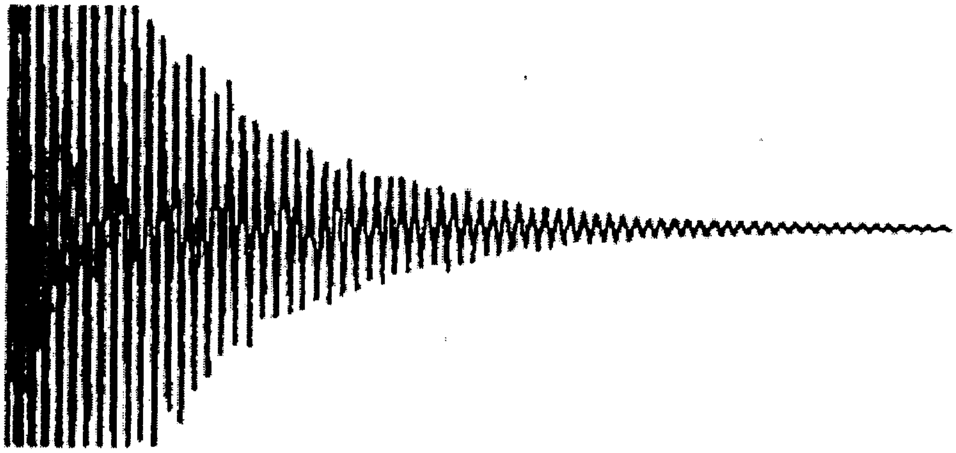
Truncation with zero filling



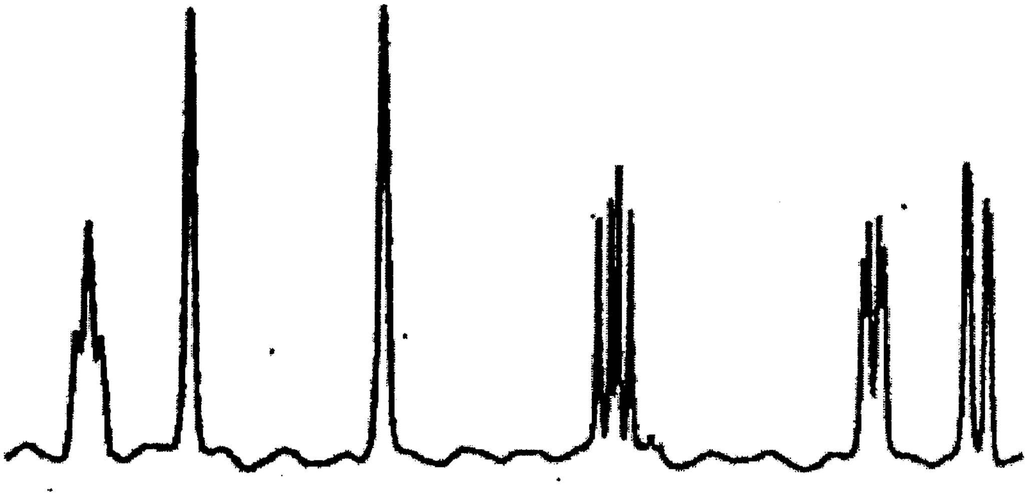


(10) Clipping

When the signal is too large to digitize the resulting FID is clipped.



Baseline Distortions caused by Clipping the FID



(11) 2D NMR

All of the NMR experiments that you have seen up to this point are one-dimensional (1D) techniques where the detected signal is a function of only one time variable. This results in a spectrum that gives the signal intensity as a function of only one frequency axis as seen in figure 29.

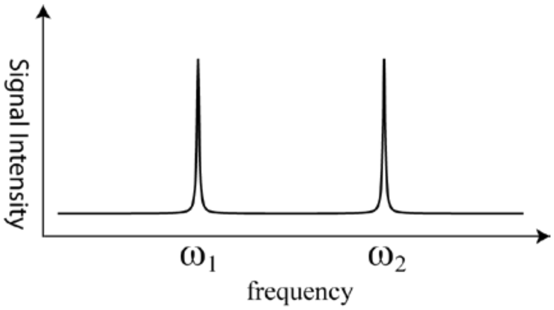


Figure 1A 1D NMR spectra containing to isotropic lines.

In complex molecules a 1D spectrum can become very crowded, due to multiple chemical shifts, and difficult to interpret; therefore, it is difficult to extract information about the connectivity, or interactions, of the various spin networks. In multi-dimensional NMR spectroscopy the detected signal is a function of several time variables and this provides a tool for analyzing the connectivity of these complex systems.

In two-dimensional (2D) NMR spectroscopy the signal is detected as a function of two time variables, thus providing a way to make observations of the spin-spin interactions directly. The general pulse sequence for a 2D NMR experiment is shown in figure 30 and is composed of four time periods known as preparation, evolution, mixing, and detection.

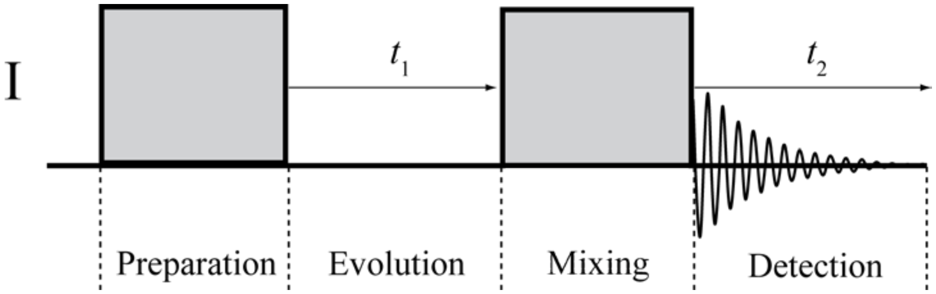


Figure 2 The general pulse sequence of a 2D NMR experiment.

The preparation period uses RF pulses to carefully manipulate the spin system and create a desired state, or coherence order, of the spin system. This state then evolves freely for the duration of the evolution period (t_1). It is important to note that the magnetization is not directly detected during the evolution period. The mixing period then uses RF pulses to re-convert the state of the spin system to a form in which it can be detected during the detection period (t_2). The resulting FID is now a function of both the evolution (t_1) and detection (t_2) time periods. The application of a Fourier transform in both time variables results in a 2D spectrum (figure 31) that contains the signal as a function of frequency along two axes, F_1 and F_2, and these axes correspond to evolution during the time periods of t_1 and t_2, respectively. The cross-peaks in figure 31 indicate correlations between spins due to coupling.

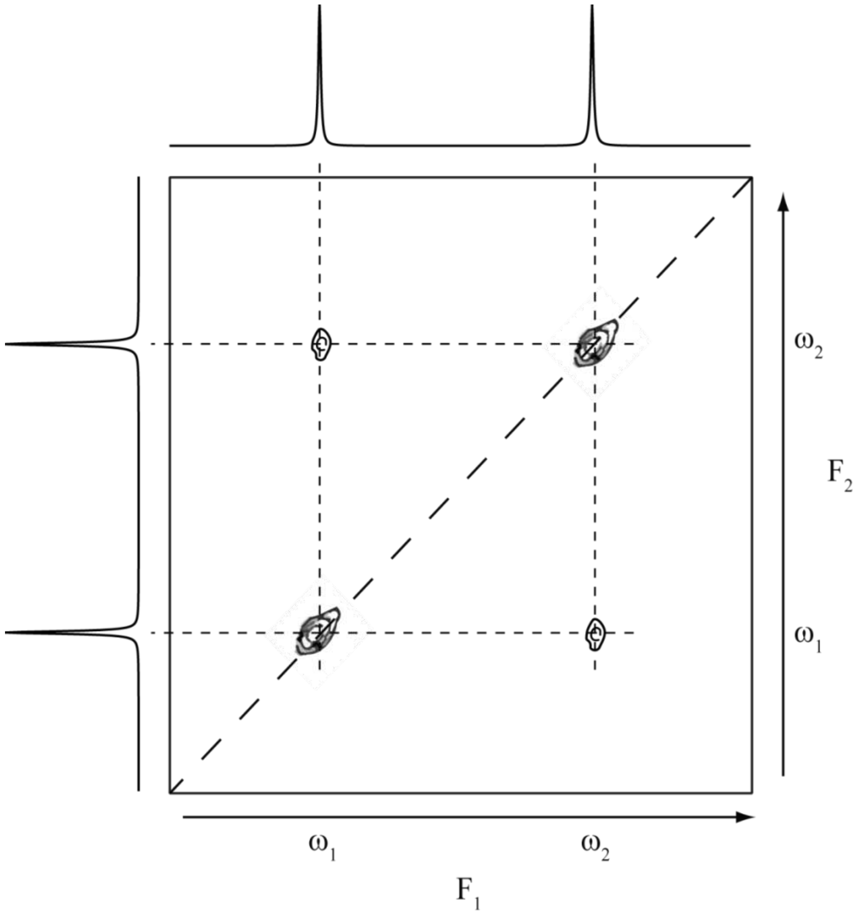


Figure 3A general 2D NMR spectra.

(12) T1 Evolution

The value of t_1 is incremented, and the sequence is repeated for each point in the indirect time dimension, thereby creating an array of FID's that constitute a data set that is two-dimensional in time S (t_1, t_2). This signal is then converted from time domains to the corresponding frequency domains, F_1 and F_2, via double Fourier transformation, as illustrated in figure 32. Finally, the 2D spectrum is displayed as a contour map plot with frequency axes labeled F_1 and F_2, correlations between the spins shown as a vertical projection of signal intensities, and the peak coordinates reflecting respective frequencies.

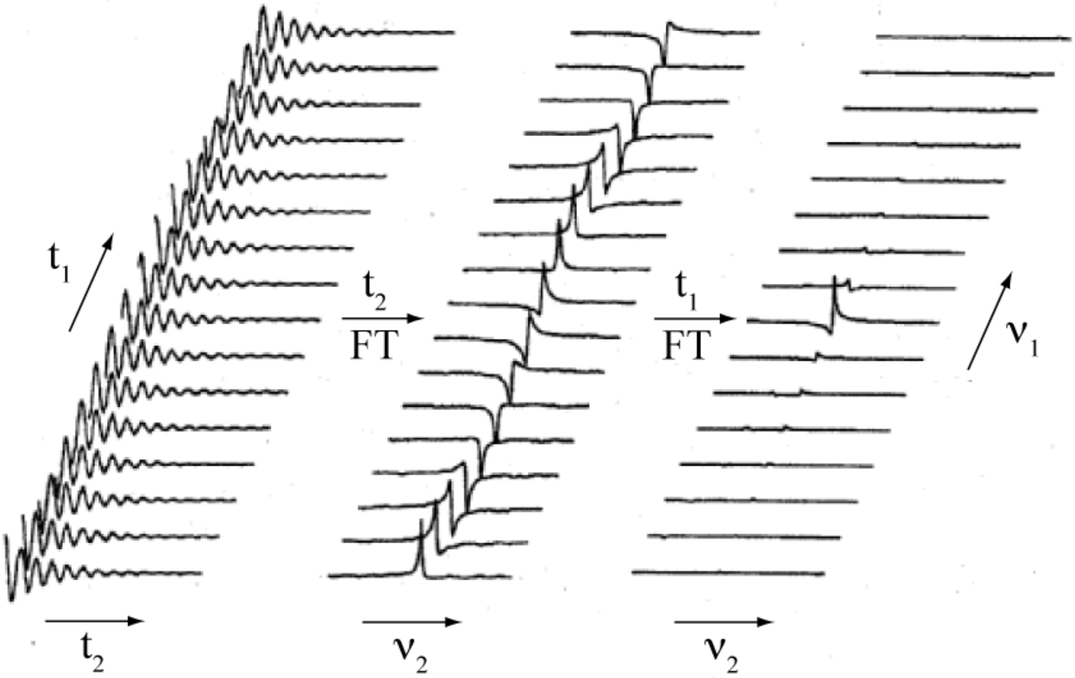


Figure 32 Structure of a two-dimensional NMR experiment.

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.

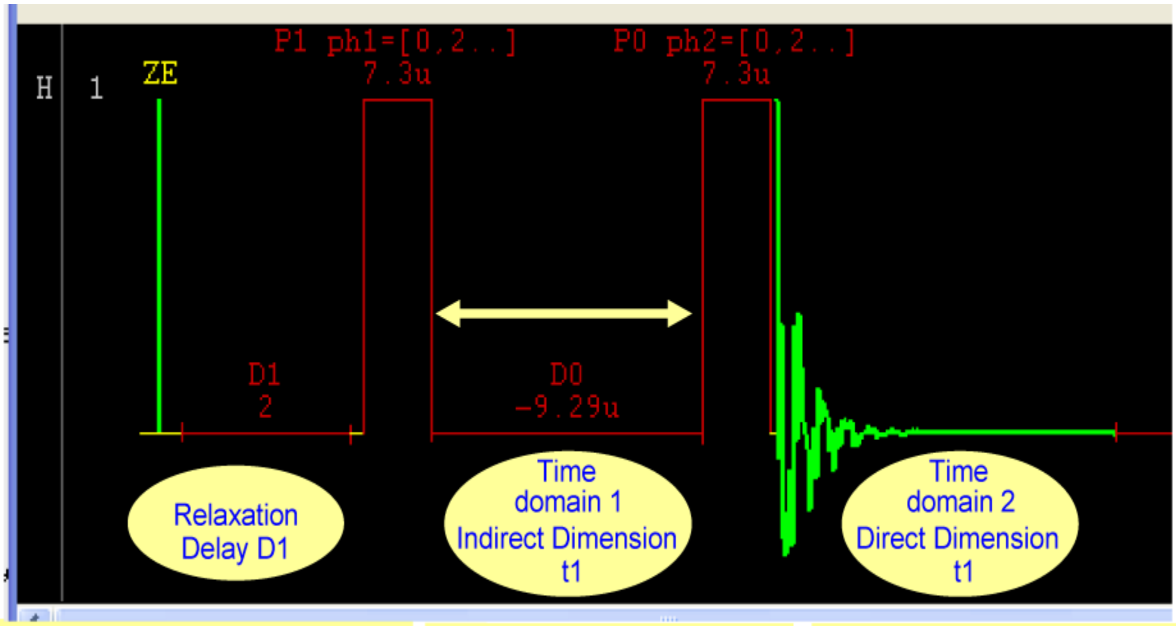
(6) Center the rf transmitter in the middle of the required sweep width. MAKE SURE that you are doing this after referencing your spectra. The rf transmitter can be centered by typing o1p into the command line and pressing enter.This will cause a box to appear and you can enter the frequency of the transmitter, 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).

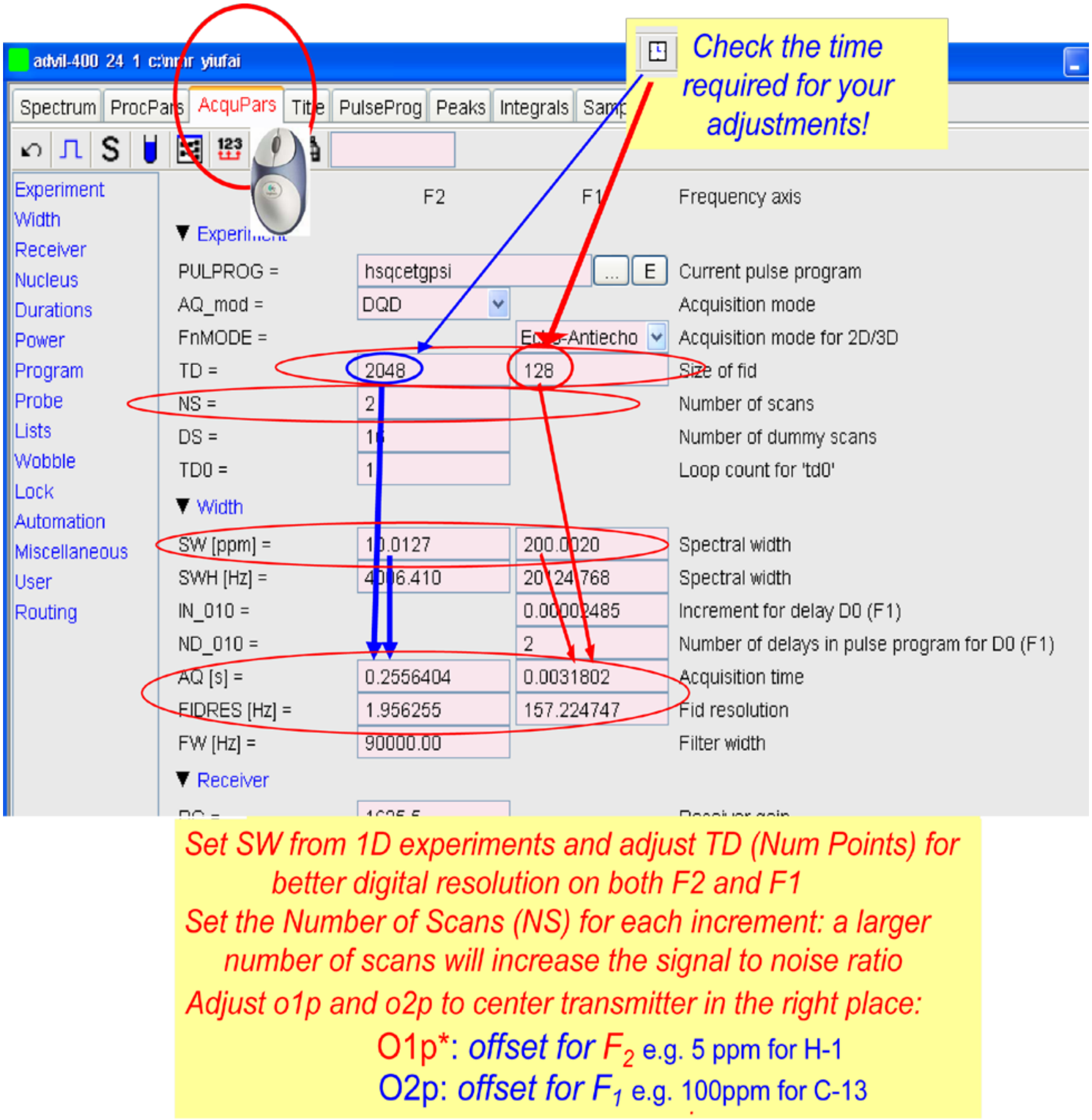
(11) Center the RF transmitter in the middle of your sweep width for both dimensions. This can be done by typing o1p into the command line and hitting enter. A box will appear and you can enter the value for o1p recorded earlier. Repeat this step for o2p by typing o2p into the command line and hitting 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).



Check the time required for your adjustments!

	F2	F1	Frequency axis
PULPROG =	hsqcetgpsi		Current pulse program
AQ_mod =	DQD		Acquisition mode
FnMODE =		Echo-Antiecho	Acquisition mode for 2D/3D
TD =	2048	128	Size of fid
NS =	2		Number of scans
DS =	16		Number of dummy scans
TD0 =	1		Loop count for 'td0'
SW [ppm] =	10.0127	200.0020	Spectral width
SWH [Hz] =	4006.410	20124.768	Spectral width
IN_010 =		0.00002485	Increment for delay D0 (F1)
ND_010 =		2	Number of delays in pulse program for D0 (F1)
AQ [s] =	0.2556404	0.0031802	Acquisition time
FIDRES [Hz] =	1.956255	157.224747	Fid resolution
FW [Hz] =	90000.00		Filter width

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:

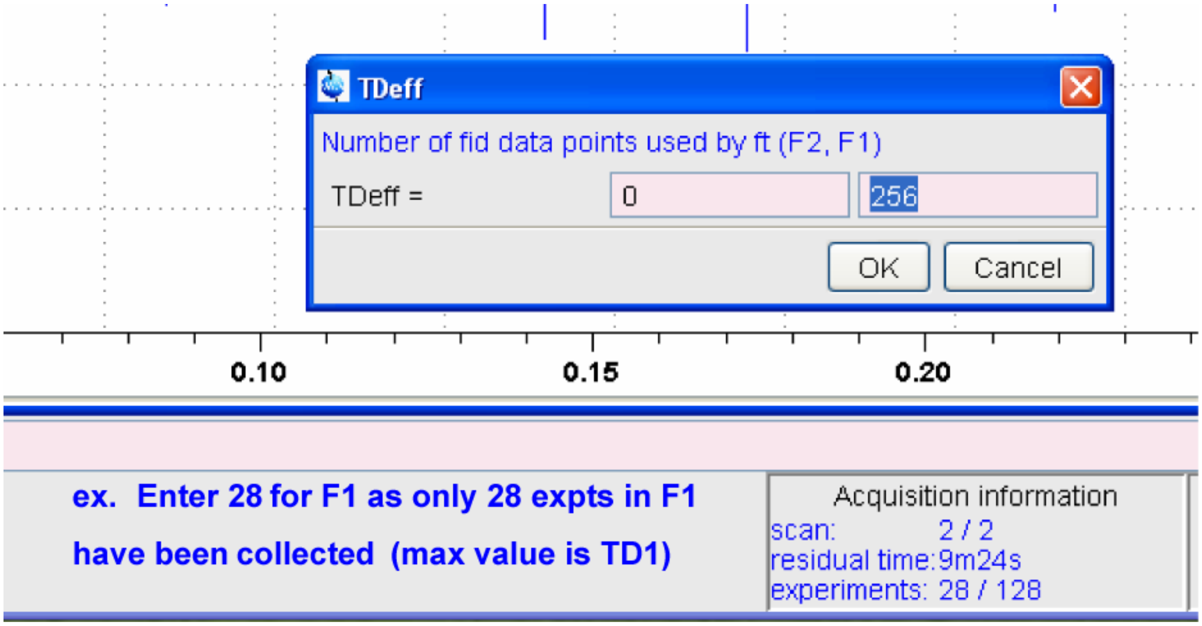
O1p*: *offset for F_2* e.g. 5 ppm for H-1

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.



Number of fid data points used by ft (F2, F1)

TDeff = 0 256

ex. Enter 28 for F1 as only 28 expts in F1 have been collected (max value is TD1)

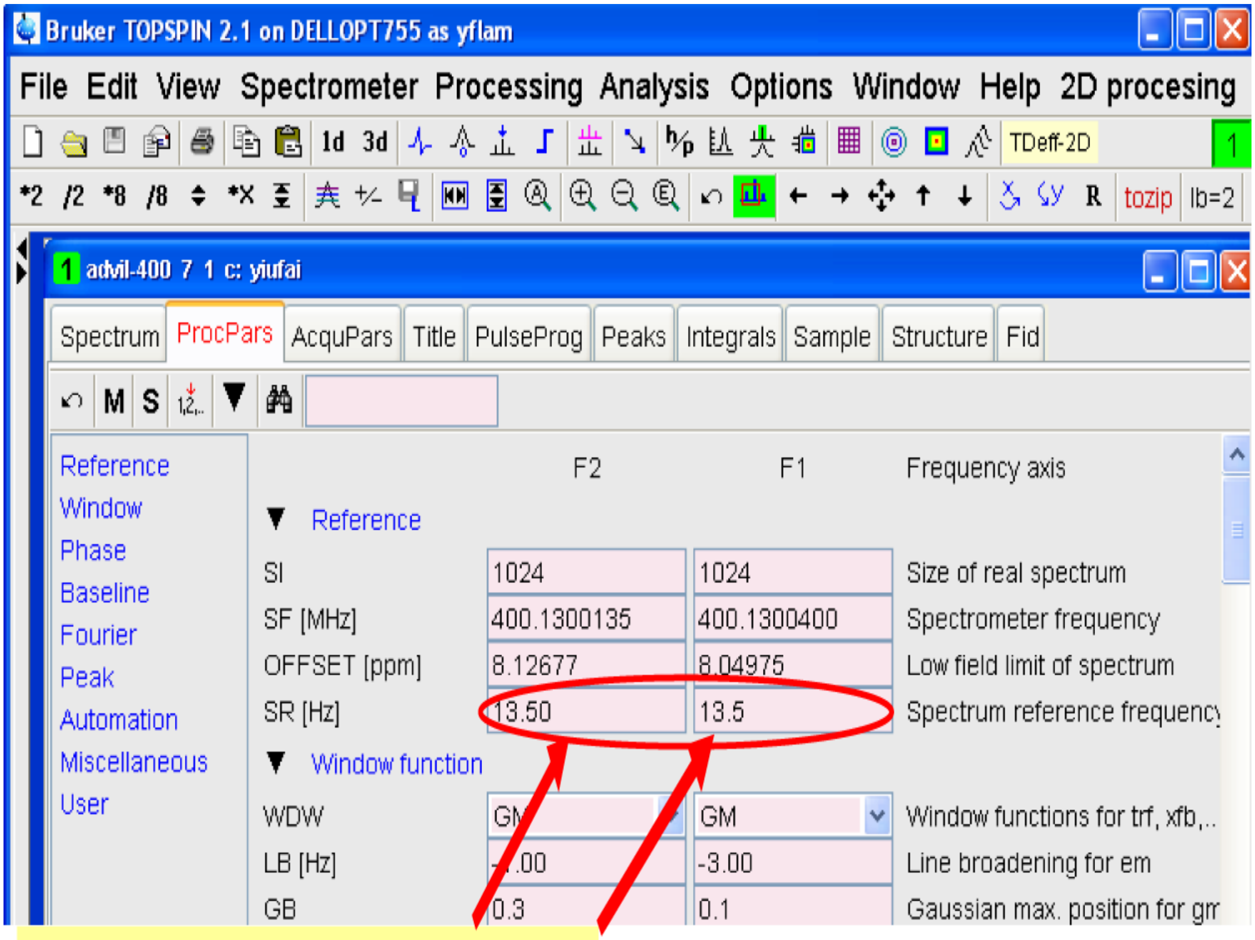
Acquisition information
scan: 2 / 2
residual time:9m24s
experiments: 28 / 128

(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.



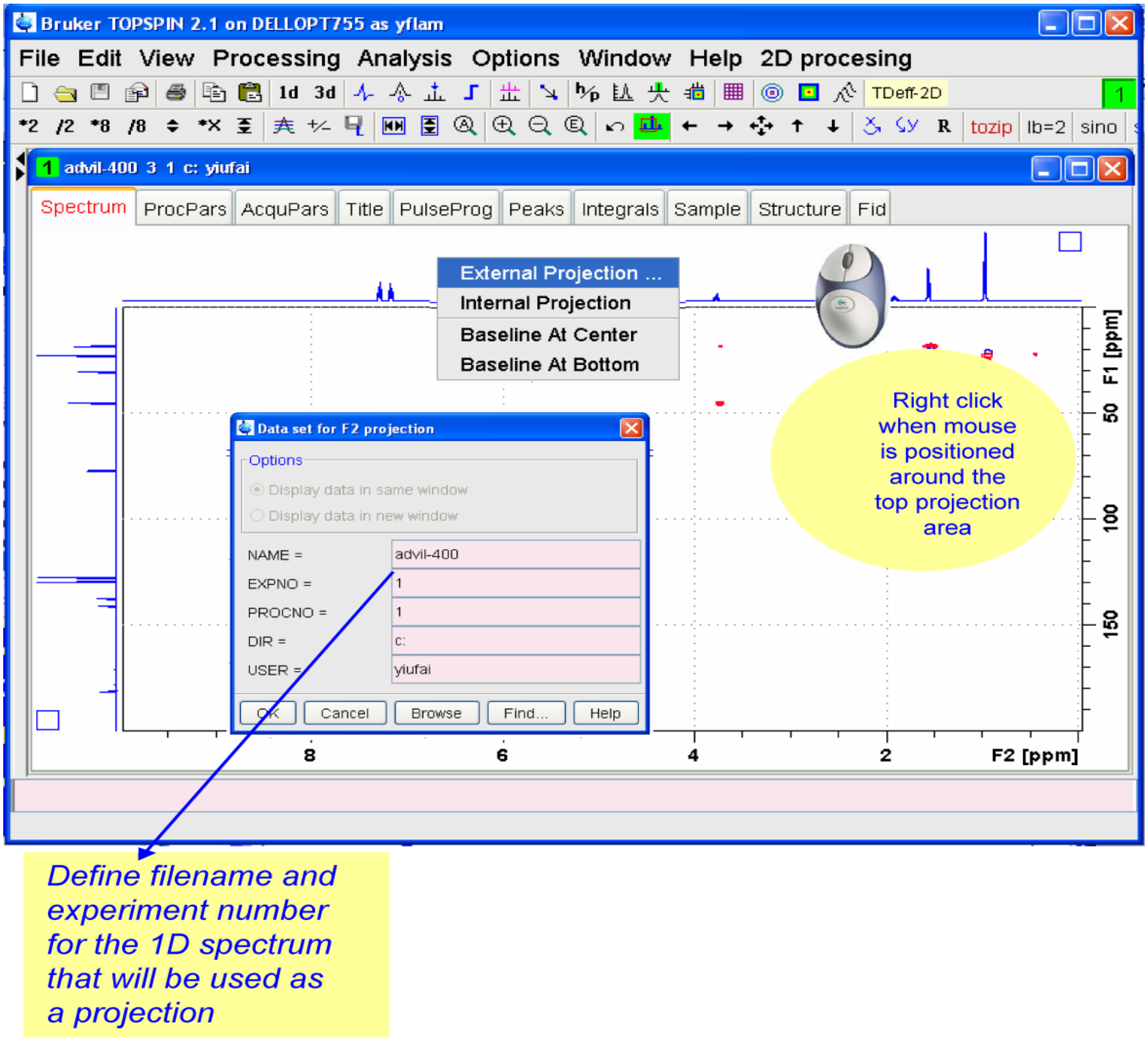
	F2	F1	Frequency axis
▼ Reference			
SI	1024	1024	Size of real spectrum
SF [MHz]	400.1300135	400.1300400	Spectrometer frequency
OFFSET [ppm]	8.12677	8.04975	Low field limit of spectrum
SR [Hz]	13.50	13.5	Spectrum reference frequenc
▼ Window function			
WDW	GM	GM	Window functions for trf, xfb,..
LB [Hz]	[illegible].00	-3.00	Line broadening for em
GB	0.3	0.1	Gaussian max. position for gm

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).

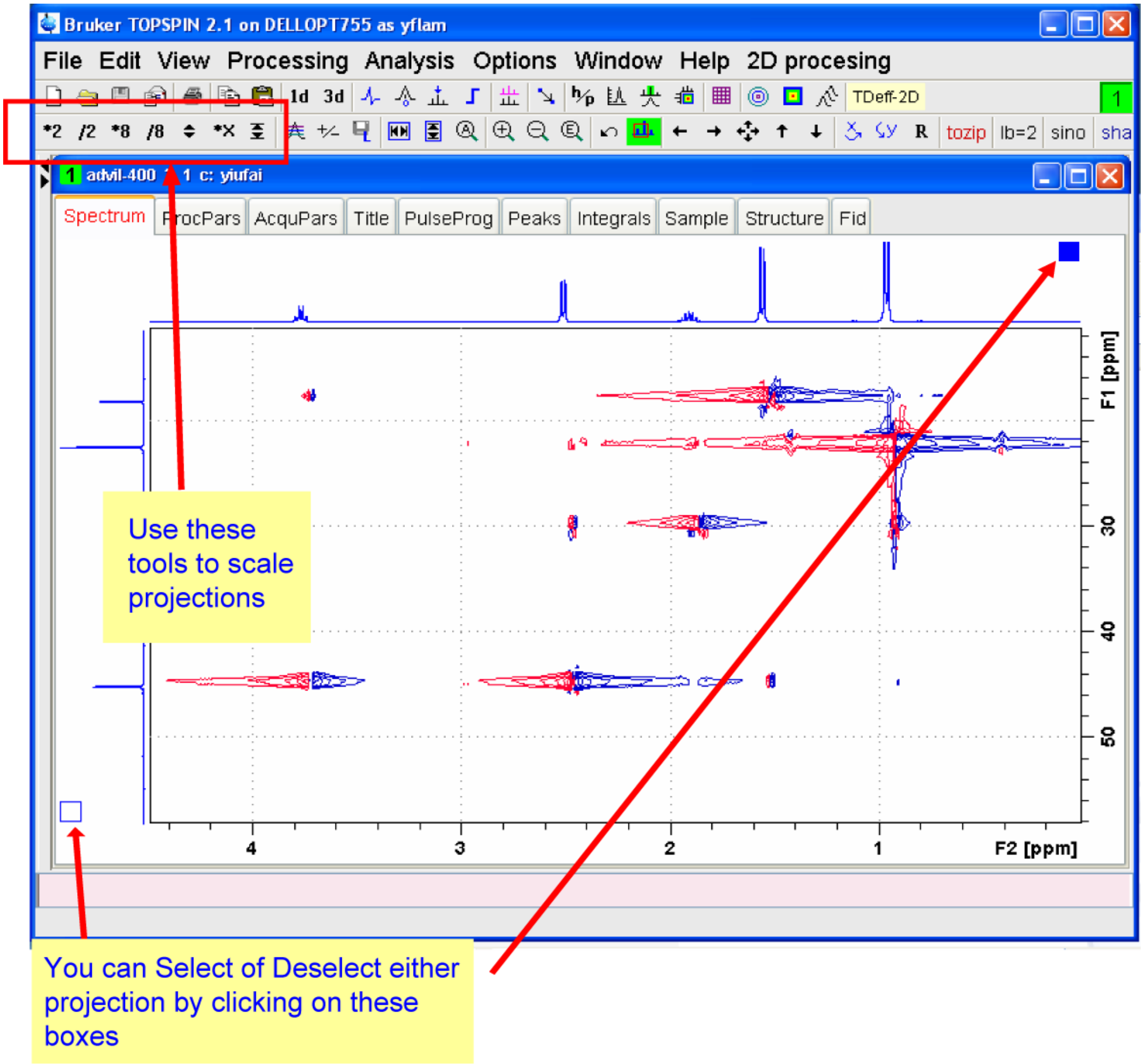


NAME = advil-400
EXPNO = 1
PROCNO = 1
DIR = c:
USER = yiufai

Right click when mouse is positioned around the top projection area

Define filename and experiment number for the 1D spectrum that will be used as a projection

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



Use these tools to scale projections

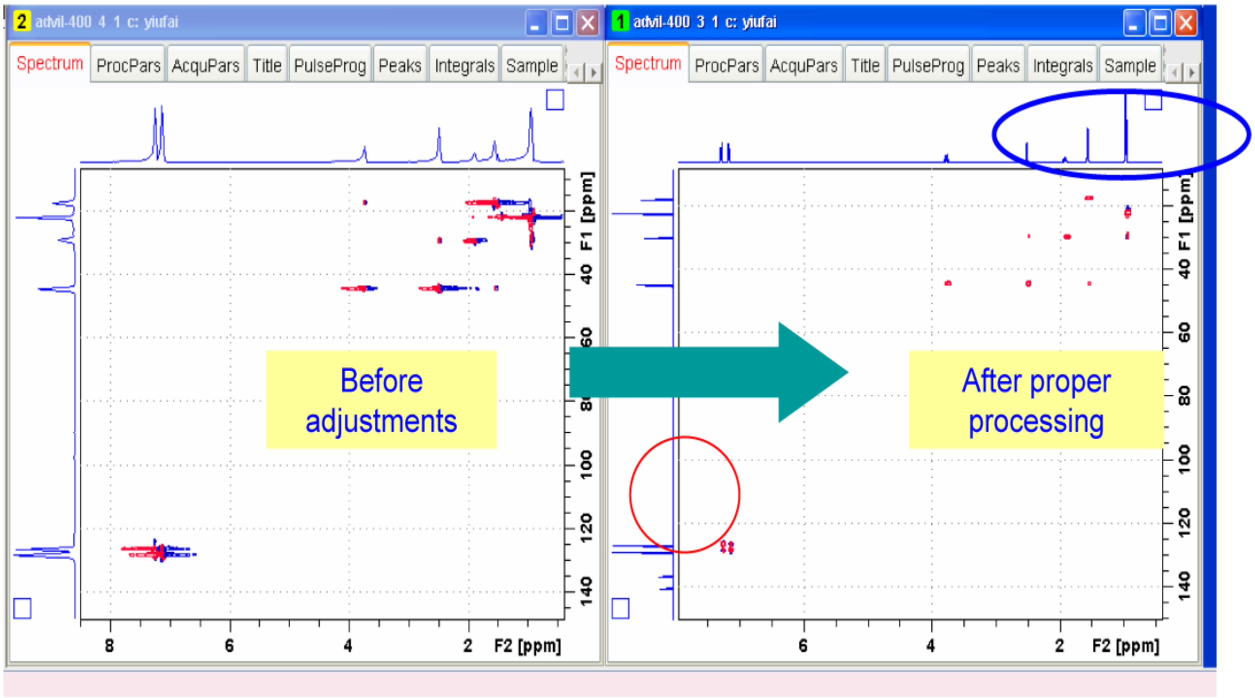
You can Select of Deselect either projection by clicking on these boxes

(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 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

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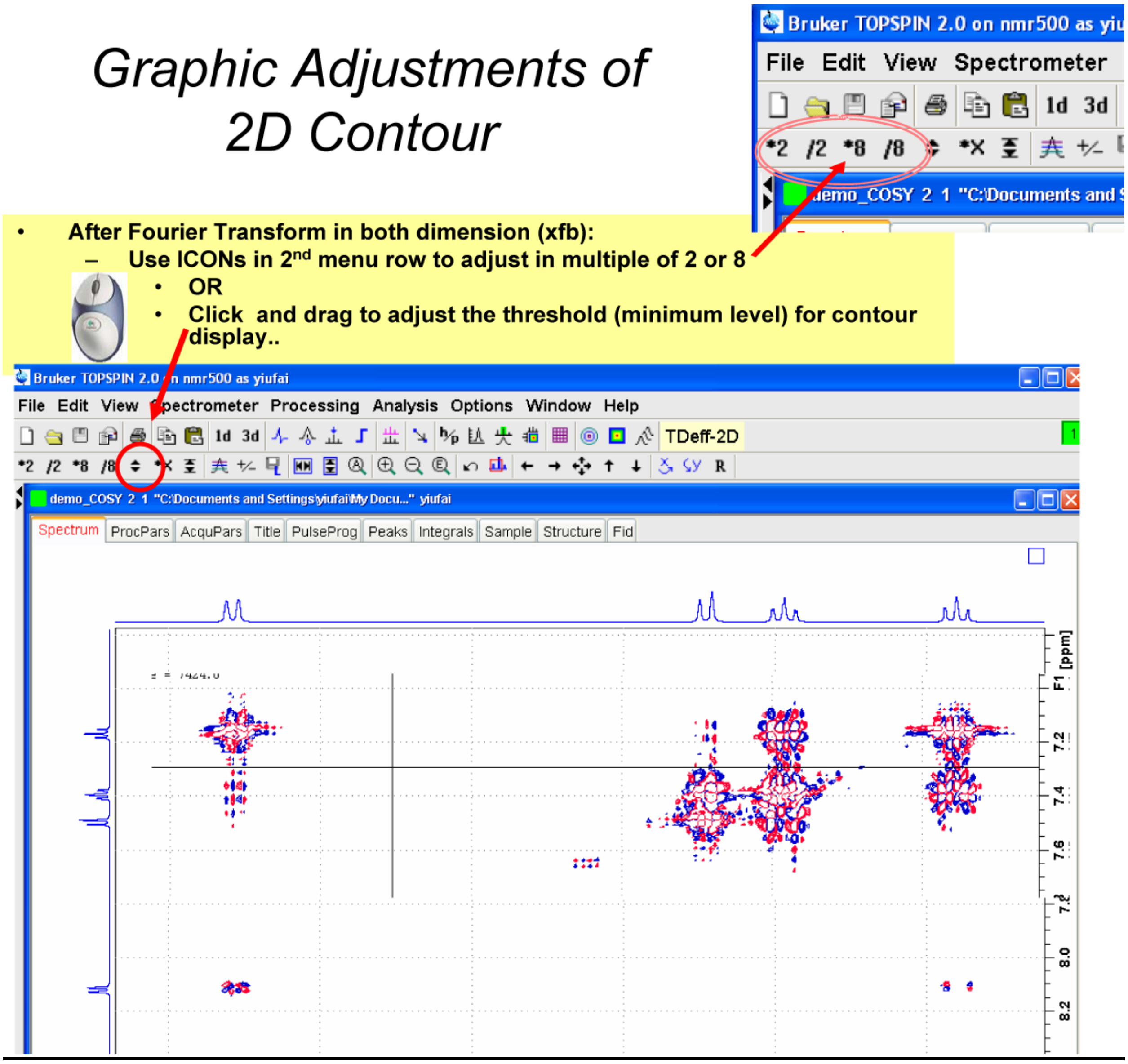
(1) Contour Adjustment

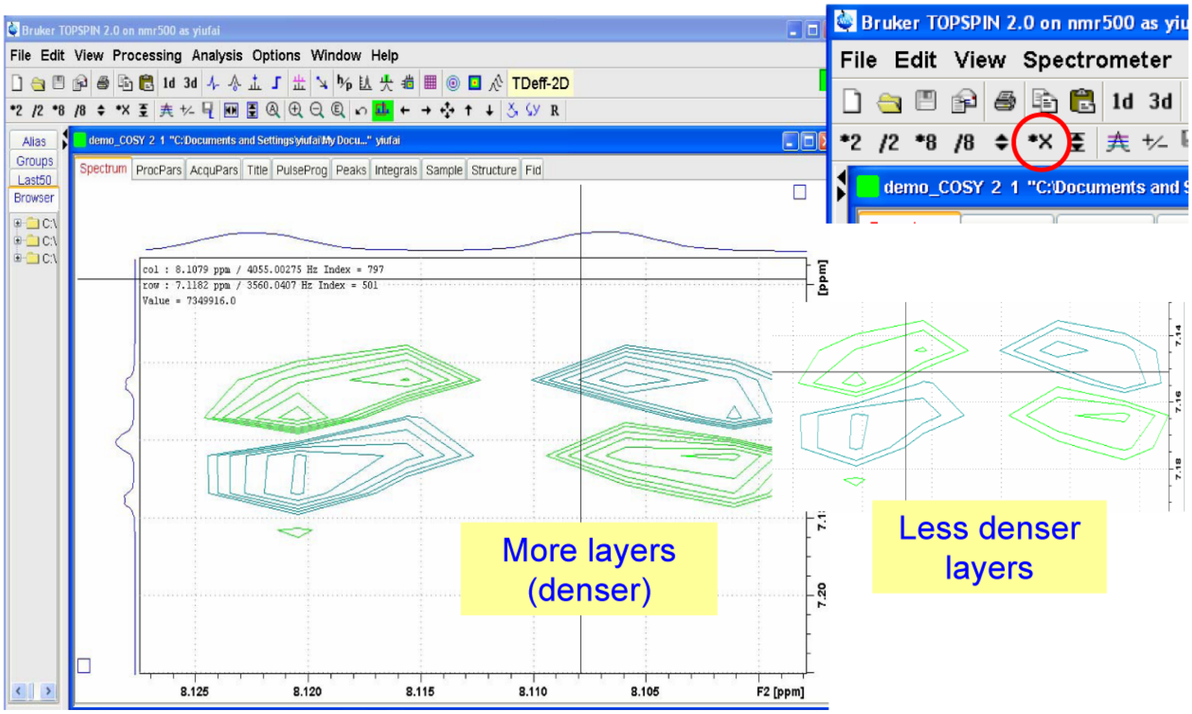


Graphic Adjustments of 2D Contour

- 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:

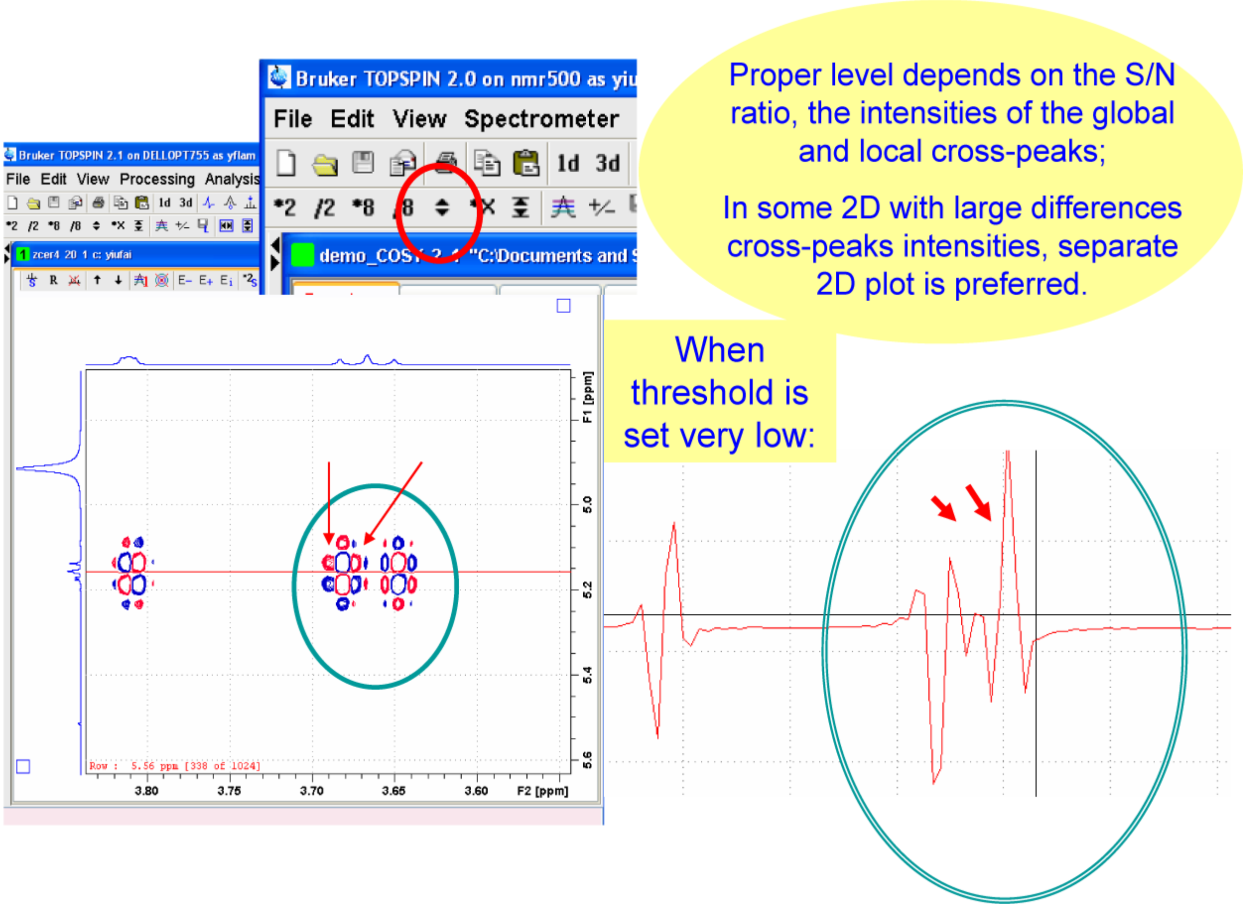


```
col : 8.1079 ppm / 4055.00275 Hz Index = 797
row : 7.1182 ppm / 3560.0407 Hz Index = 501
Value = 7349916.0
```

More layers (denser)

Less denser layers

Example of Excessive threshold level adjustment:



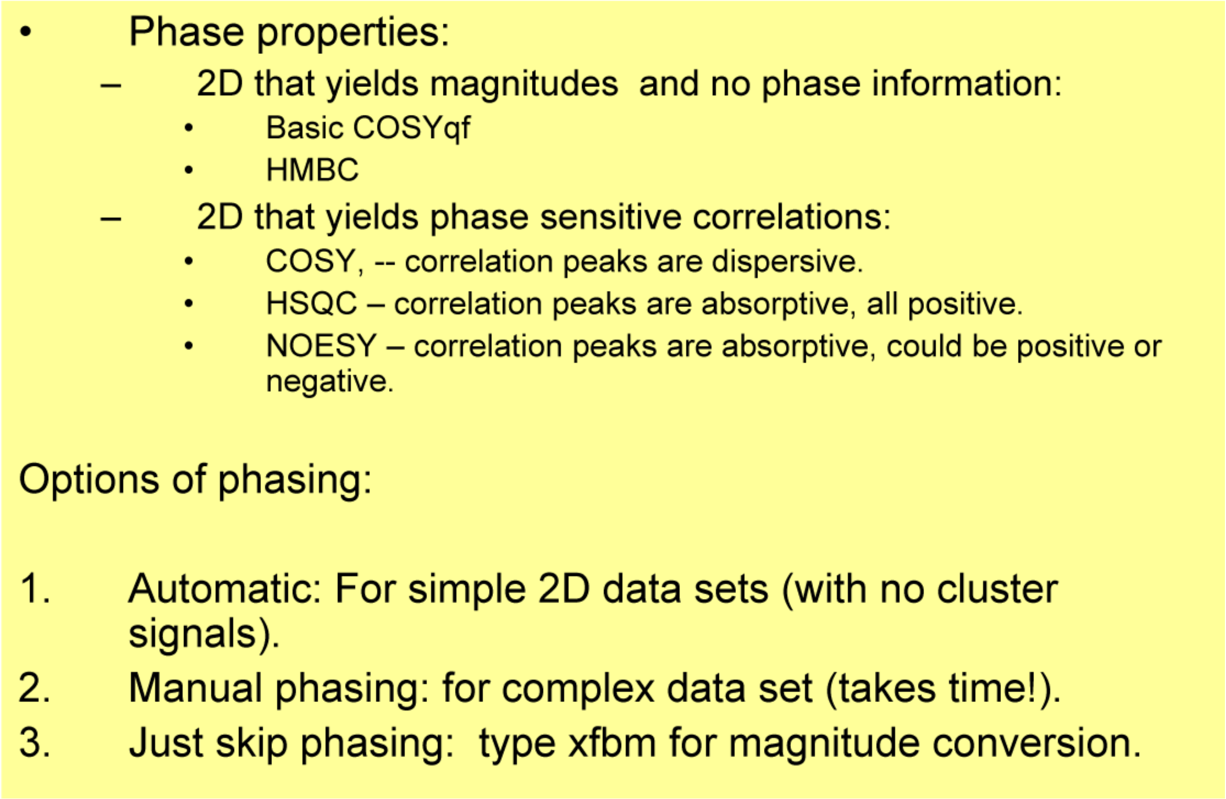
Proper level depends on the S/N ratio, the intensities of the global and local cross-peaks;

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:



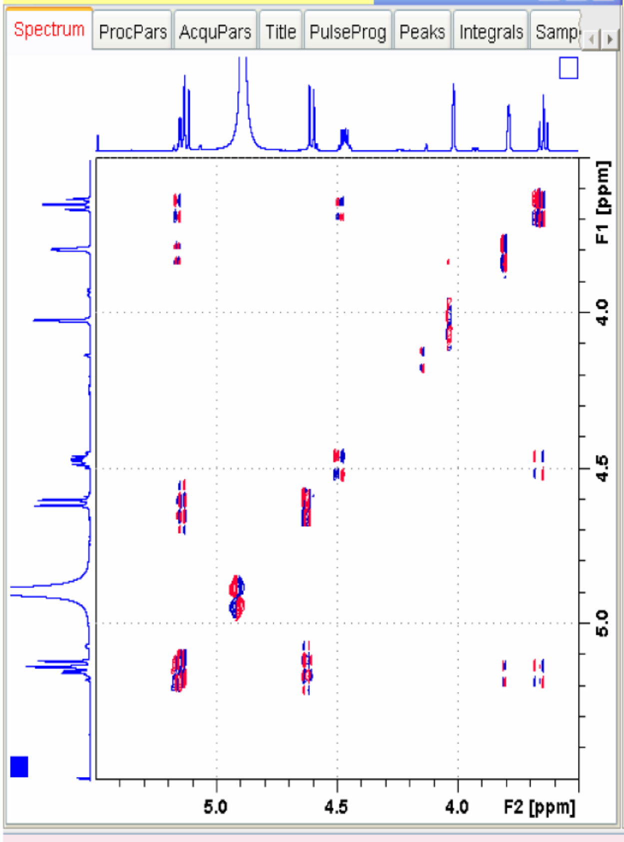
- Phase properties:
  - 2D that yields magnitudes and no phase information:
    - Basic COSYqf
    - HMBC
  - 2D that yields phase sensitive correlations:
    - COSY, -- correlation peaks are dispersive.
    - HSQC – correlation peaks are absorptive, all positive.
    - NOESY – correlation peaks are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

Options of phasing:

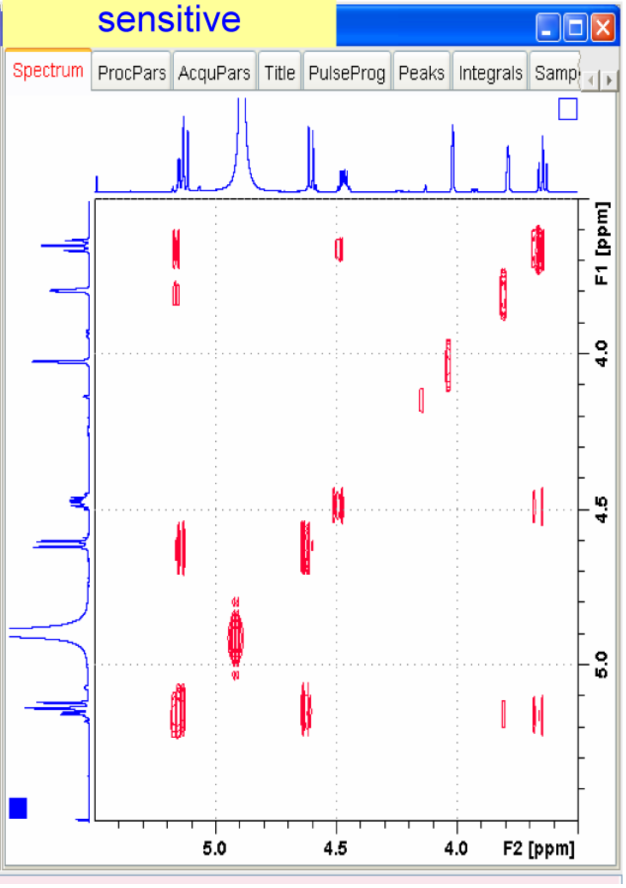
1. Automatic: For simple 2D data sets (with no cluster signals).
2. Manual phasing: for complex data set (takes time!).
3. Just skip phasing: type xfbm for magnitude conversion.

Difference between phased and magnitude contours:

COSY phase sensitive

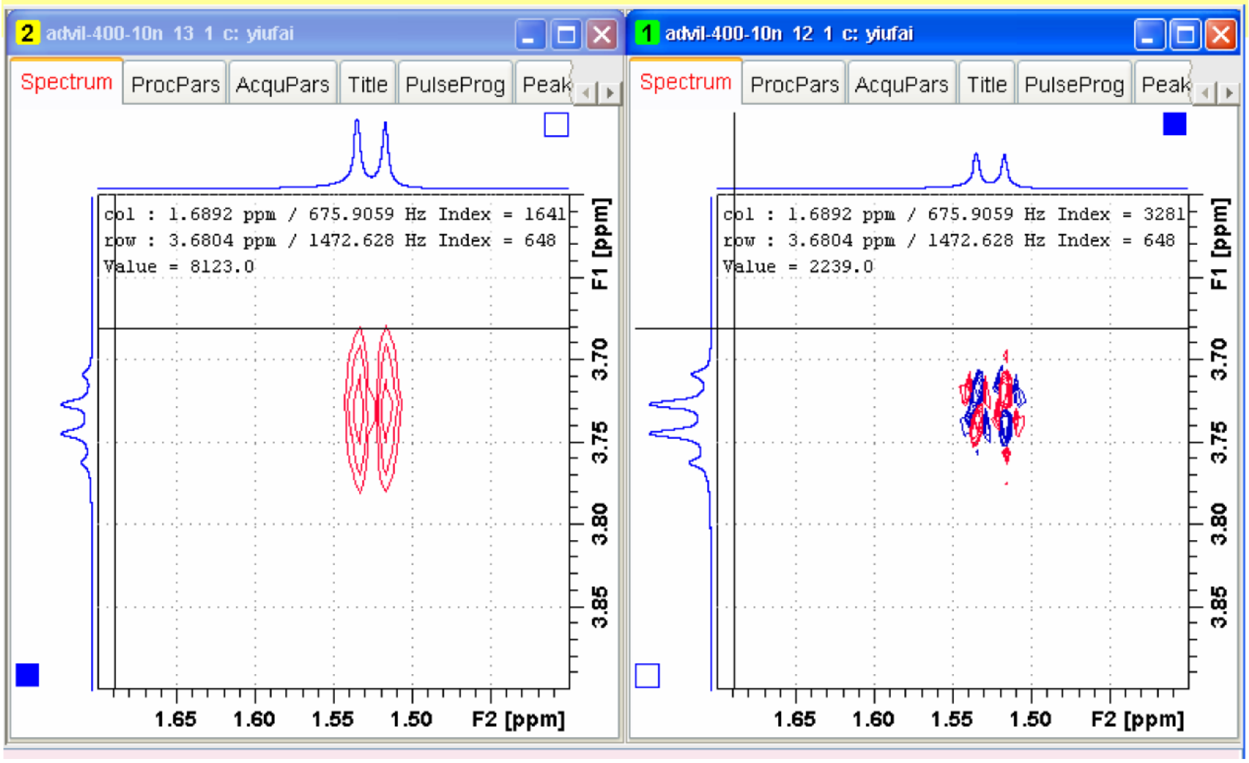


COSY non-phase sensitive



Magnitude mode and phased 2D contours:

Enlarged comparison



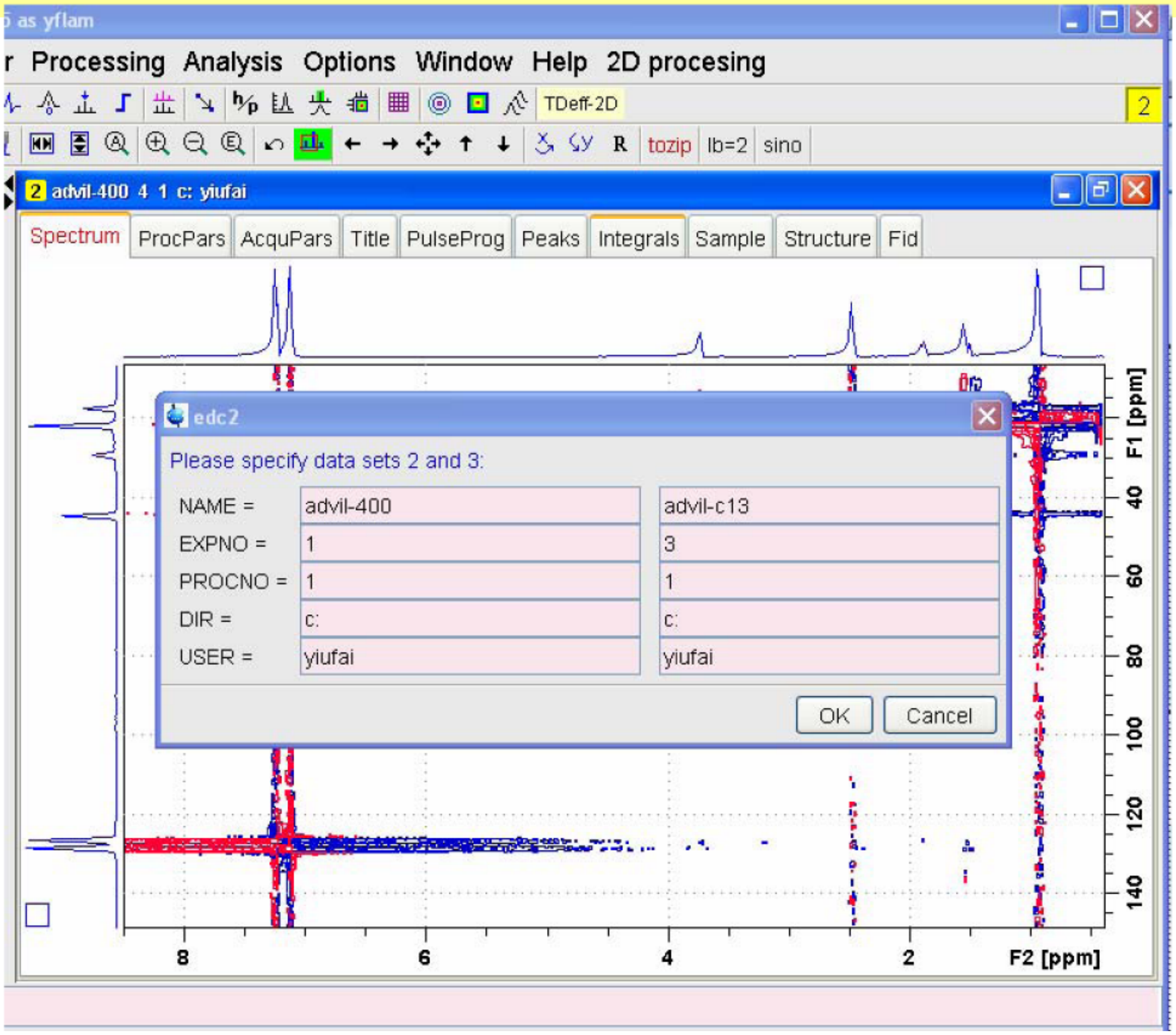
```
col : 1.6892 ppm / 675.9059 Hz Index = 1641
row : 3.6804 ppm / 1472.628 Hz Index = 648
Value = 8123.0
```

```
col : 1.6892 ppm / 675.9059 Hz Index = 3281
row : 3.6804 ppm / 1472.628 Hz Index = 648
Value = 2239.0
```

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

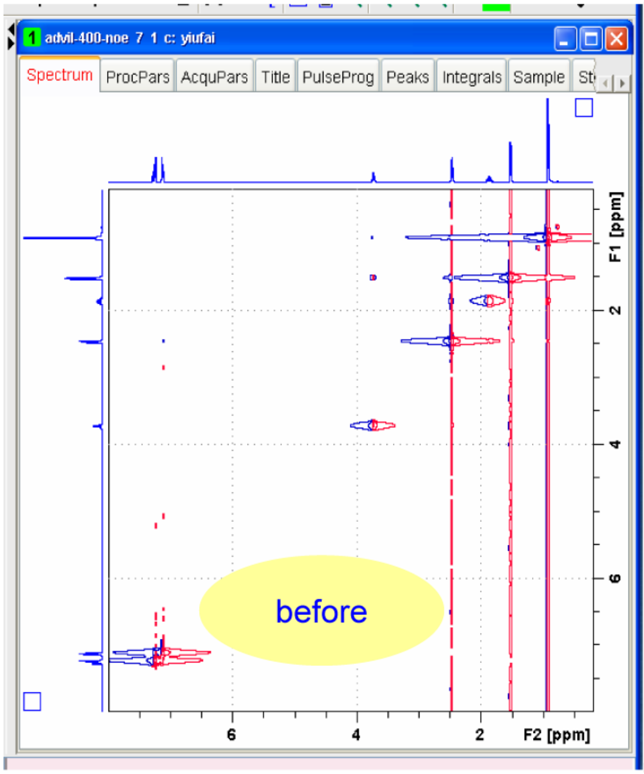


Please specify data sets 2 and 3:

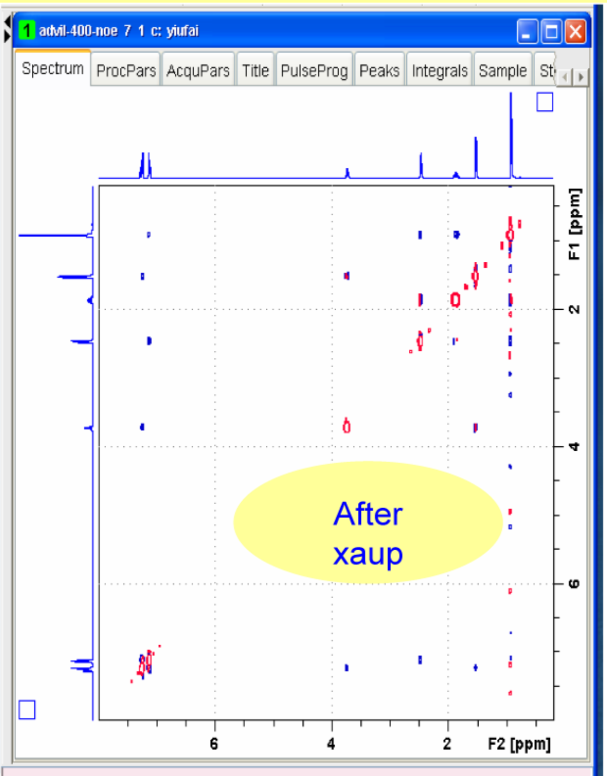
NAME =	advil-400	advil-c13
EXPNO =	1	3
PROCNO =	1	1
DIR =	c:	c:
USER =	yiufai	yiufai

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.***



before

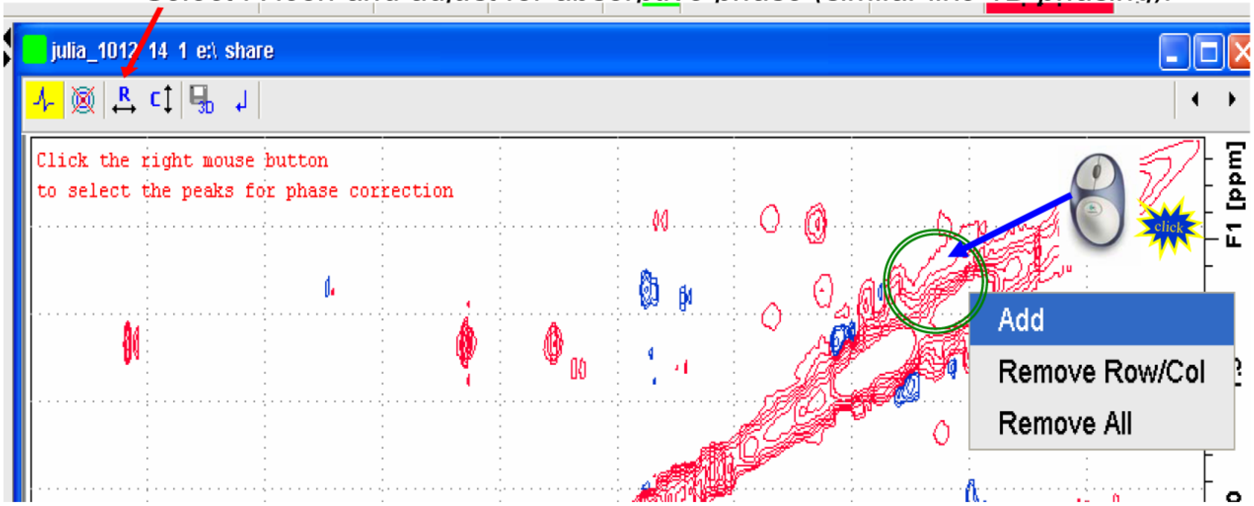


After xaup

Manual and refined 2D Phasing :

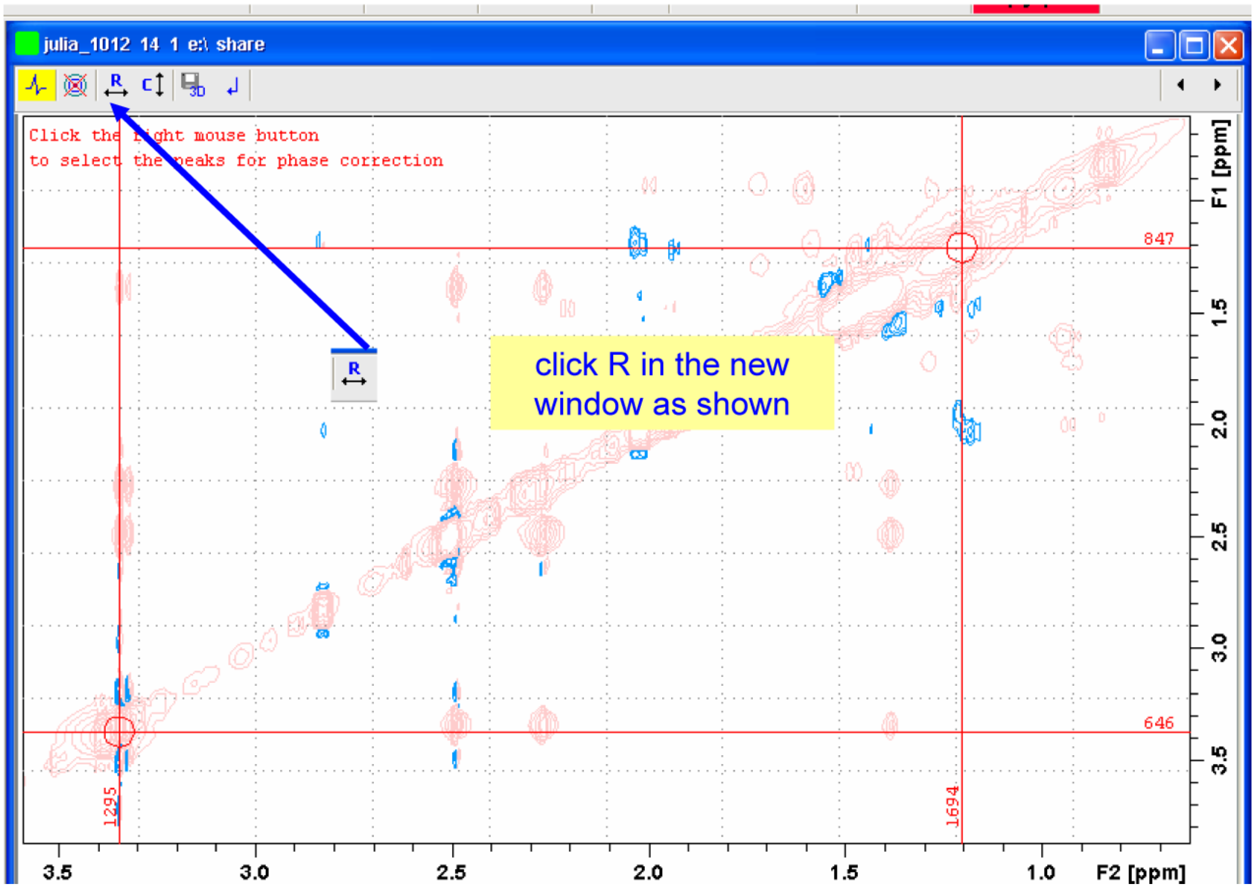
Step 1 select cross peaks

- *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).*



Click the right mouse button
to select the peaks for phase correction

2D Phasing: After two slides are selected



```
Click the right mouse button
to select the peaks for phase correction
```

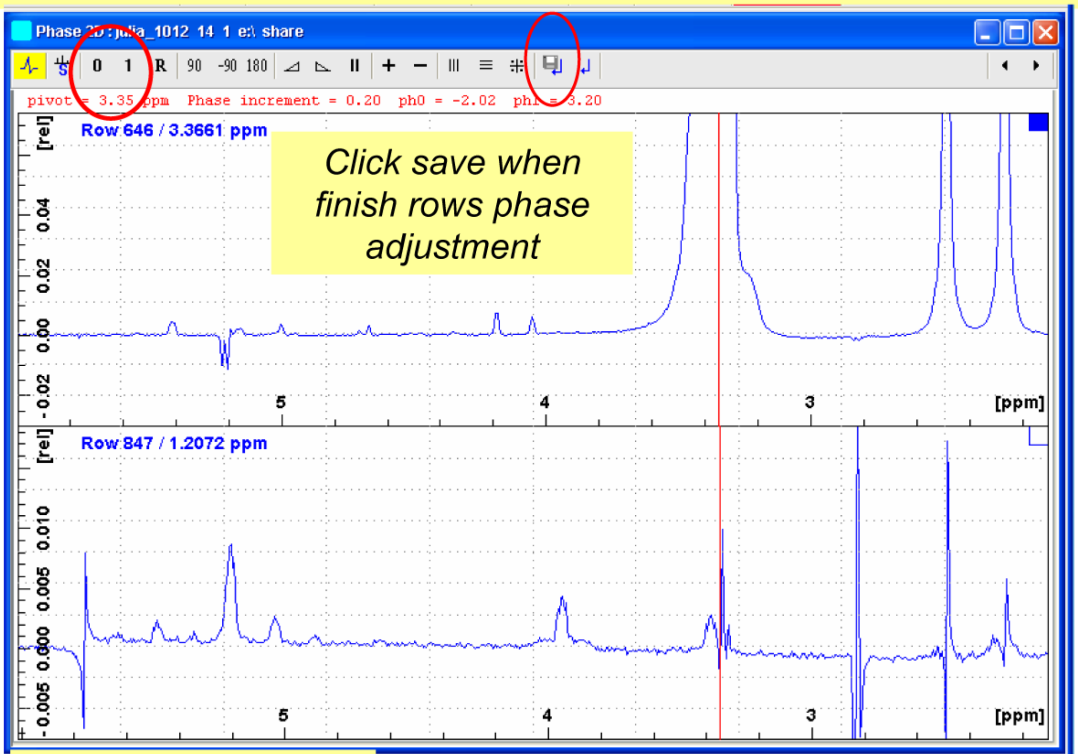
click R in the new window as shown

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.



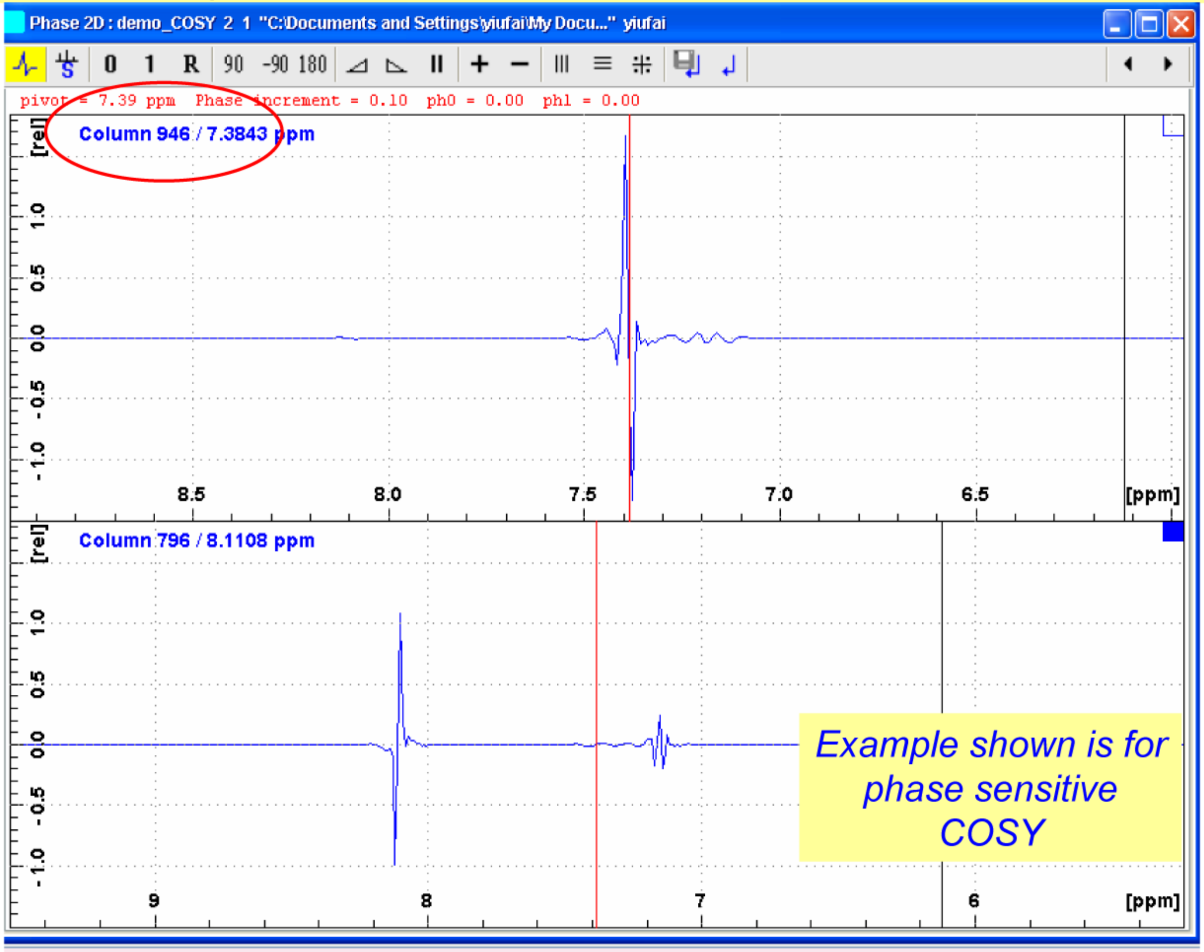
pivot = 3.35 ppm Phase increment = 0.20 ph0 = -2.02 ph1 = 3.20

Click save when finish rows phase adjustment

Example shown is for HSQC in this slide

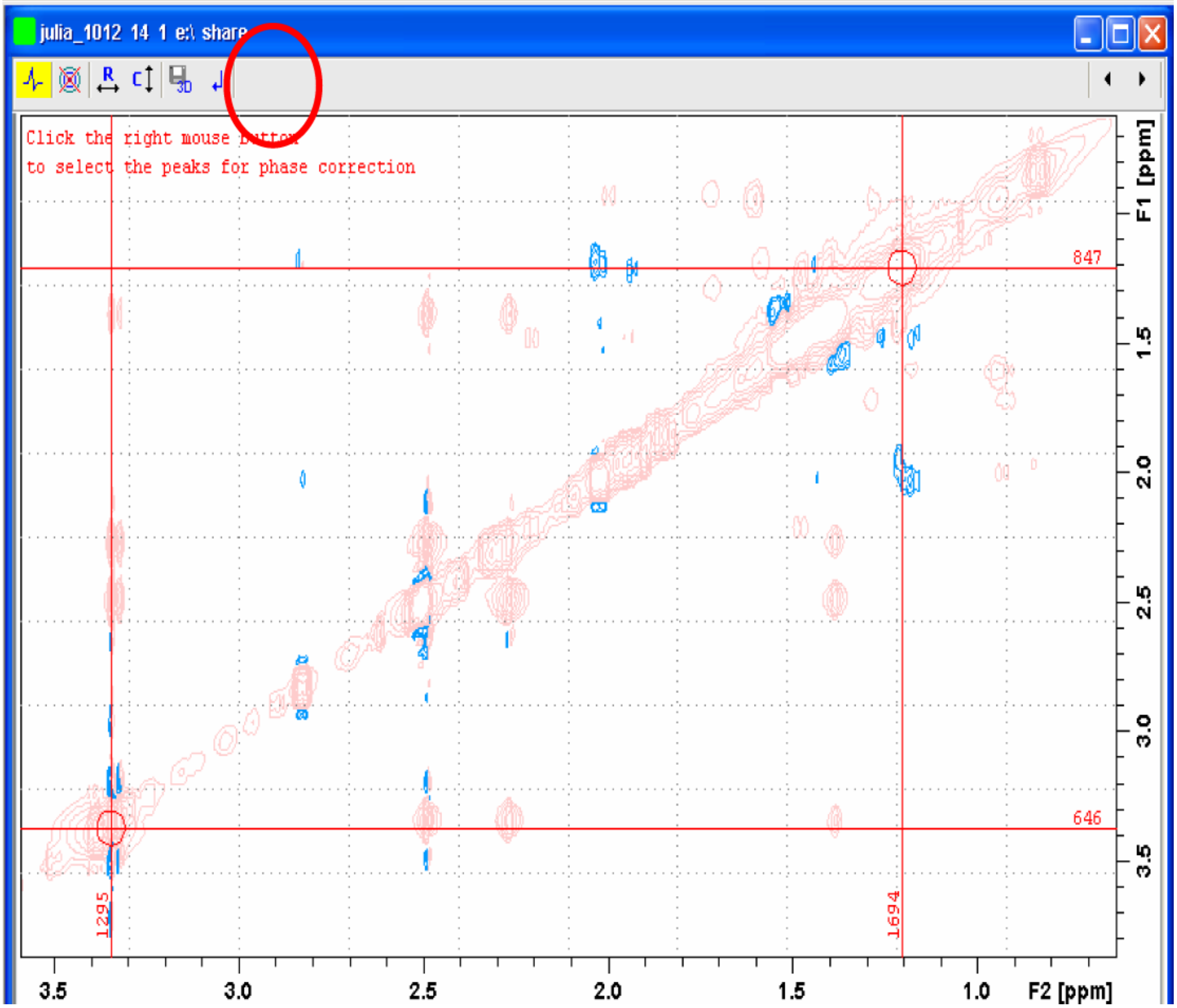
Phasing: Column slides 2D

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



Example shown is for phase sensitive COSY

Finalize the phase correction:



Click the right mouse button
to select the peaks for phase correction

(3) Plotting with TopSpin Plot Editor

Select layout and call up plot-editor:

Print [Ctrl+P] - plot

Options

○ Print active window [prnt]

● Print with layout - start Plot Editor [plot]

○ Print with layout - plot directly [autoplot]

Required parameters

LAYOUT +/std_2D_homo.xwp

Use plot limits

● from screen / CY

○ from Plot Editor Reset Actions

○ as saved in Plot Editor

Fill data set list

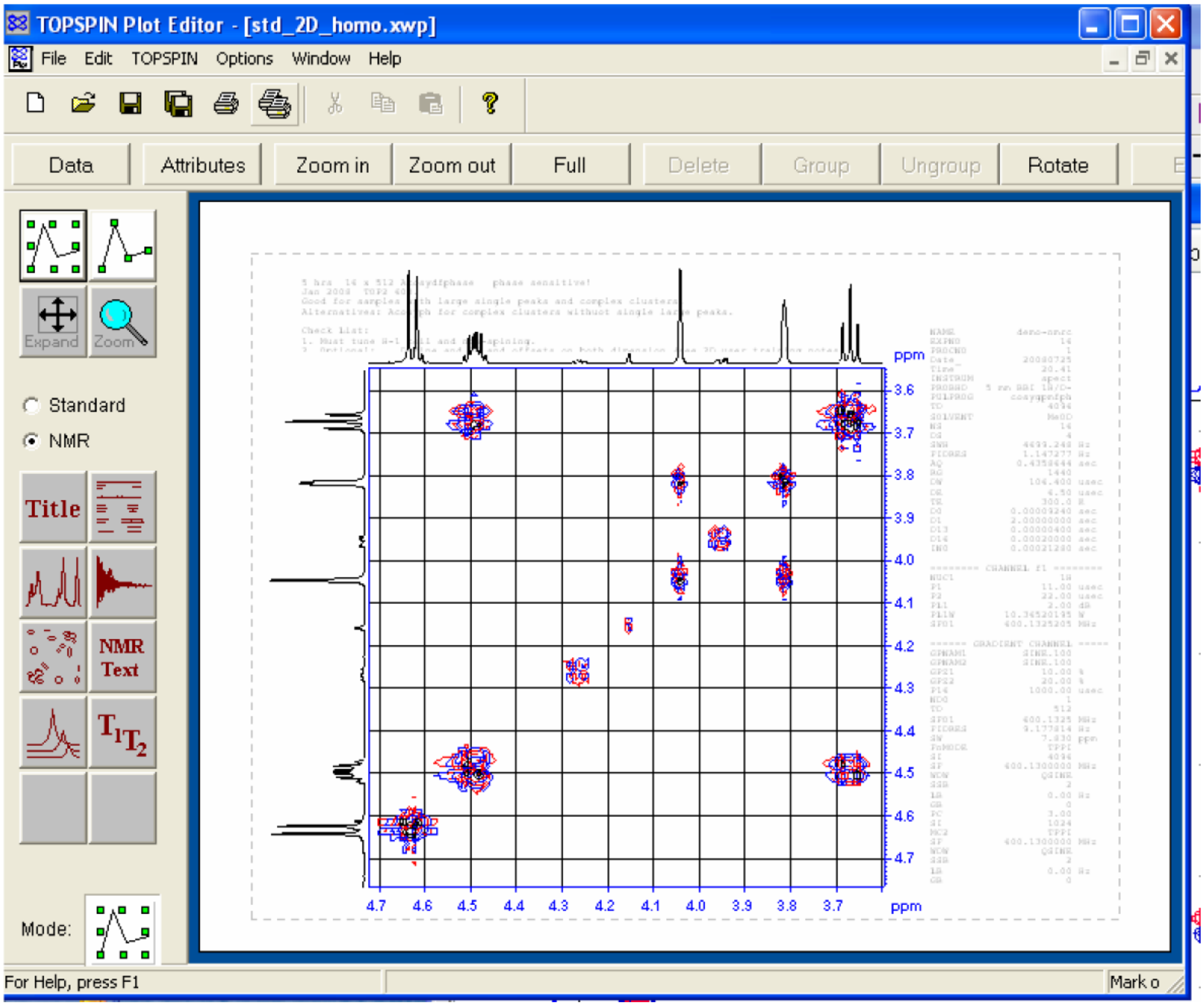
● with projections

○ from your default

○ from portfolio sav

☐ Override plotter saved in Plot Editor:

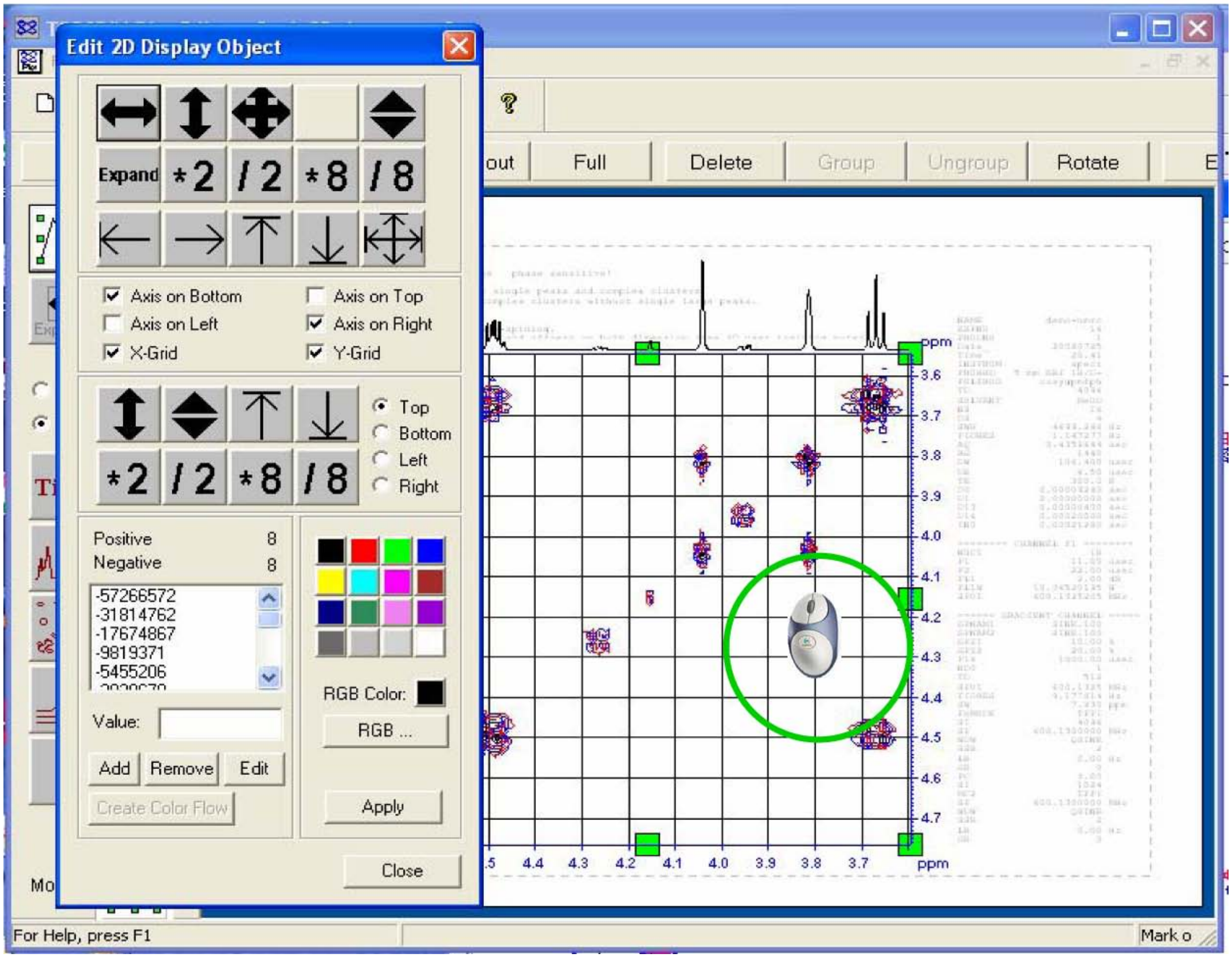
Plot-editor: For additional refine adjustments



○ Standard

● NMR

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



☑ Axis on Bottom
☐ Axis on Top
☐ Axis on Left
☑ Axis on Right
☑ X-Grid
☑ Y-Grid

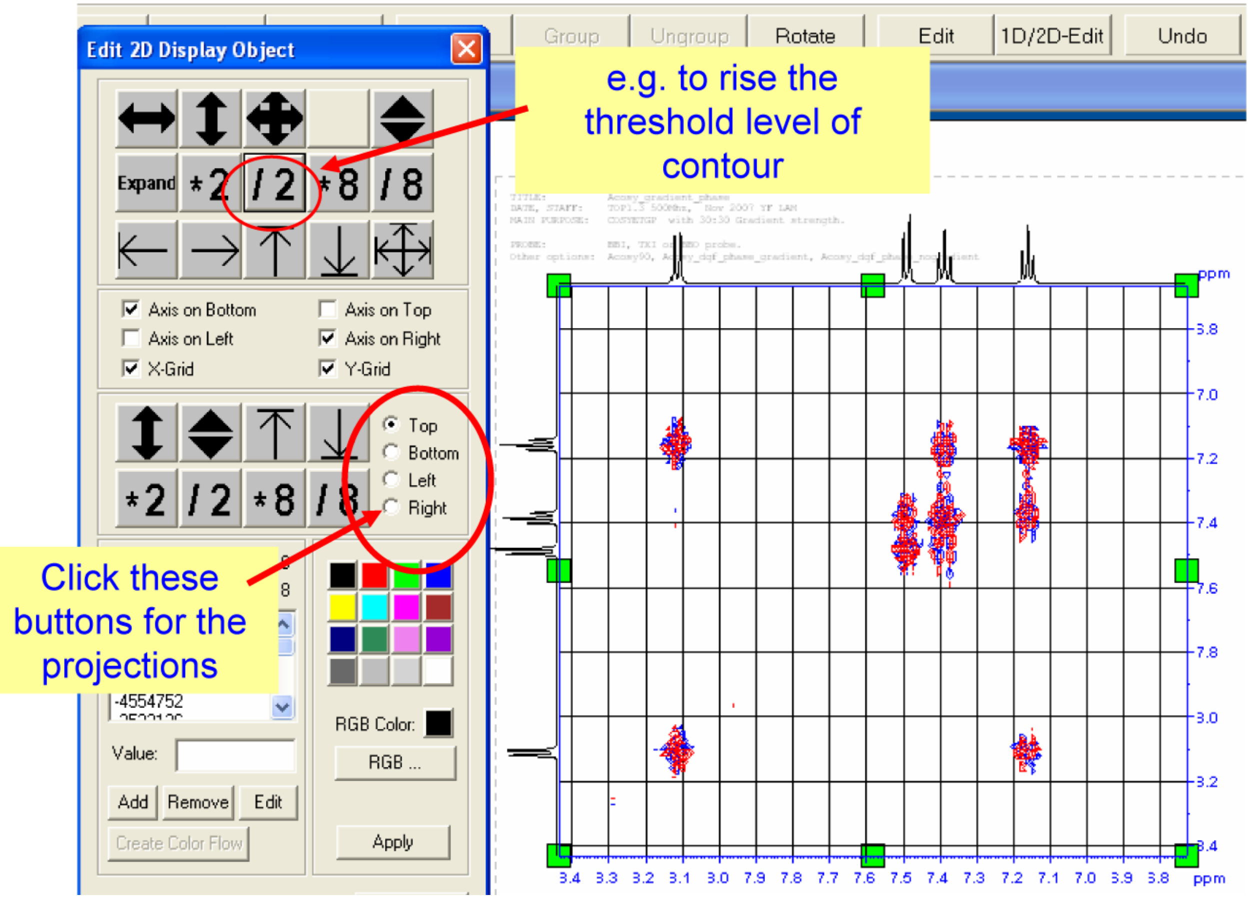
● Top
○ Bottom
○ Left
○ Right

Positive 8
Negative 8

-57266572
-31814762
-17674867
-9819371
-5455206

Value:

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



e.g. to rise the threshold level of contour

Click these buttons for the projections

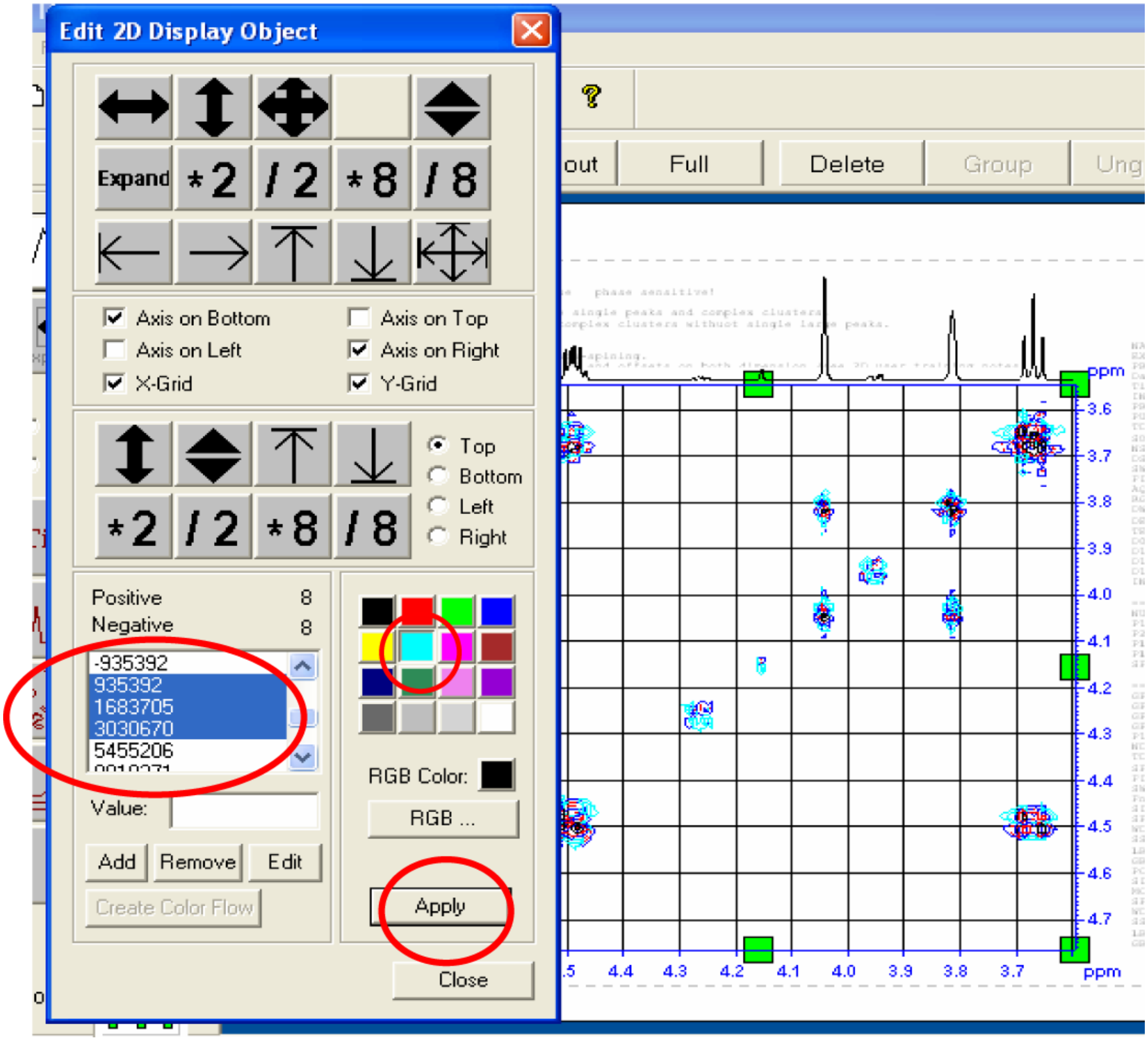
Edit 2D Display Object

☑ Axis on Bottom
☐ Axis on Top
☐ Axis on Left
☑ Axis on Right
☑ X-Grid
☑ Y-Grid

● Top
○ Bottom
○ Left
○ Right

-4554752

Modify colors for various contour layers:



Edit 2D Display Object

☑ Axis on Bottom
☐ Axis on Top
☐ Axis on Left
☑ Axis on Right
☑ X-Grid
☑ Y-Grid

● Top
○ Bottom
○ Left
○ Right

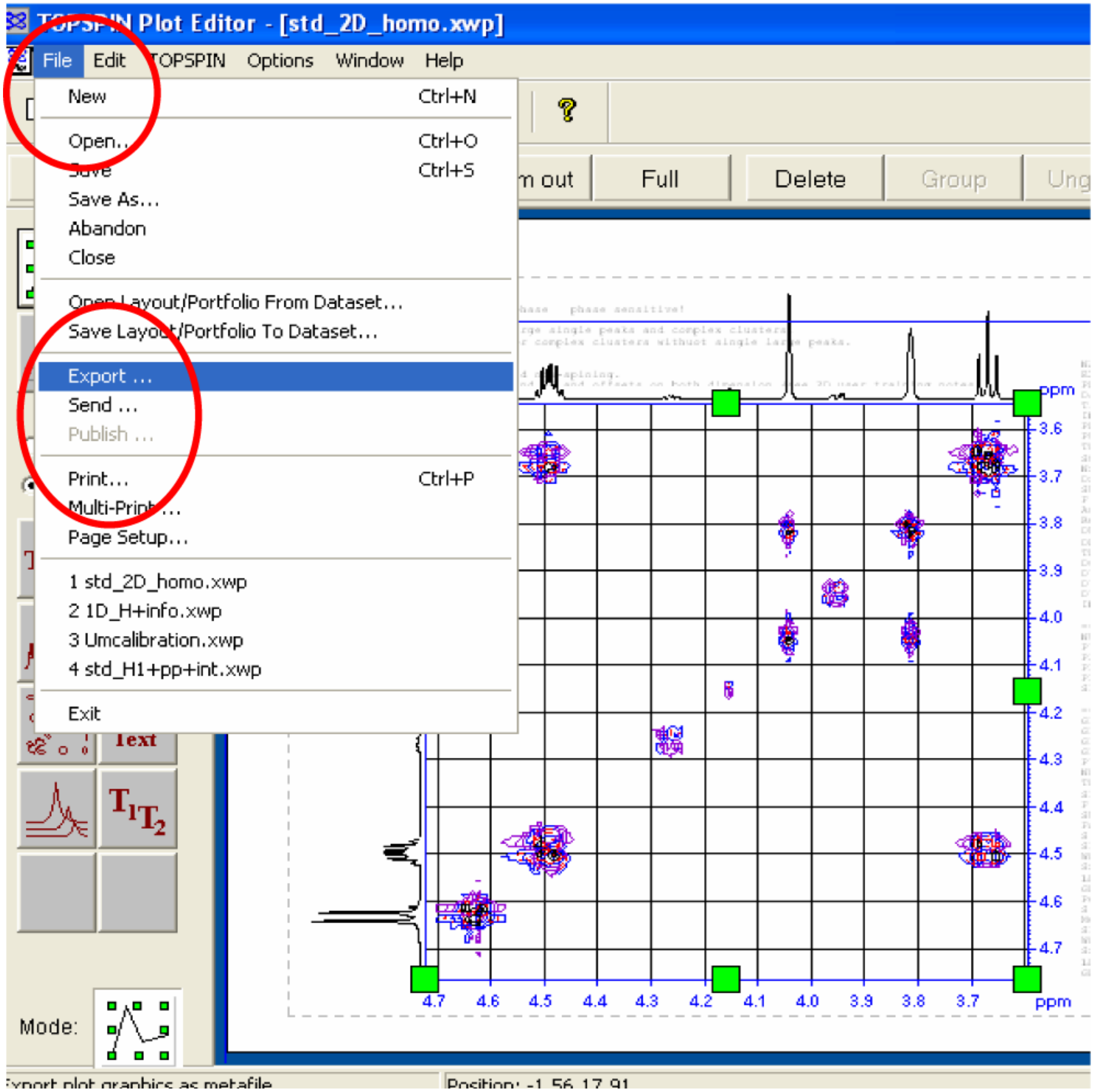
Positive 8
Negative 8

-935392
935392
1683705
3030670
5455206

Value:

RGB Color: ■

Final outputs:



Export plot graphics as metafile

Position: -1.56 17.91