2D NMR Spectrum Processing with Mnova

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General NMR Data Processing

NMR data processing is typically done in the following order in all processing programs:

- Read in raw FID
- Select and apply apodization (also called window or weighting) function
- Zero fill (**ZF**) the FID, usually at least doubling the original data size
- Fourier transform (**FT**)
- Phase spectrum
- Baseline correction

Some of these are done automatically by Mnova when data are read in.

NOTE:

- **Phasing** means, in most cases, to adjust the spectrum so that peaks across the spectrum are in-phase (absorptive mode) where all peaks point up (or down) with the peak bases look even on both sides.
- **Zero-order phase** (PH0) is a constant phase across the spectrum and is applied first. Next, set a **pivot** point for **1st-order phase** (PH1) adjustment. The **pivot** should be ideally set to a strong peak at either end of the spectrum. Then, adjust 1st-order phase which linearly changes across the spectrum from the "pivot" point. Watch the peaks at the other end of the spectrum away from the pivot while adjusting 1st-order phase.
- Baseline correction should be applied AFTER phasing. If a small phase adjustment is still needed after baseline correction, adjust the phases after baseline correction.

2D NMR Spectrum Processing with Mnova

The two dimensions in a 2D spectrum is specified as **t2** or **f2** (horizontal) and **t1** or **f1** (vertical) dimensions, respectively. t1 and t2 refer to time-domain data; f1 and f2 refer to frequency domain data after FT of t1 and t2 dimensions. The processing command below is often applied to f2 and f1 (or t2 and t1) separately.

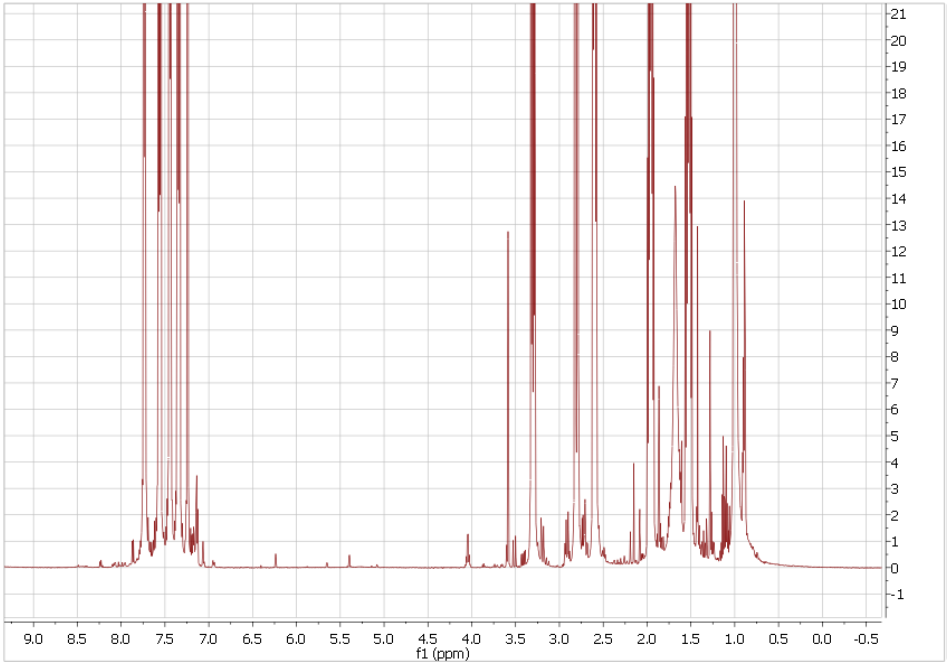
Select either **f2** or **f1** from the top menu buttons before applying the processing command.

2D spectrum should be processed in the following order in Mnova:

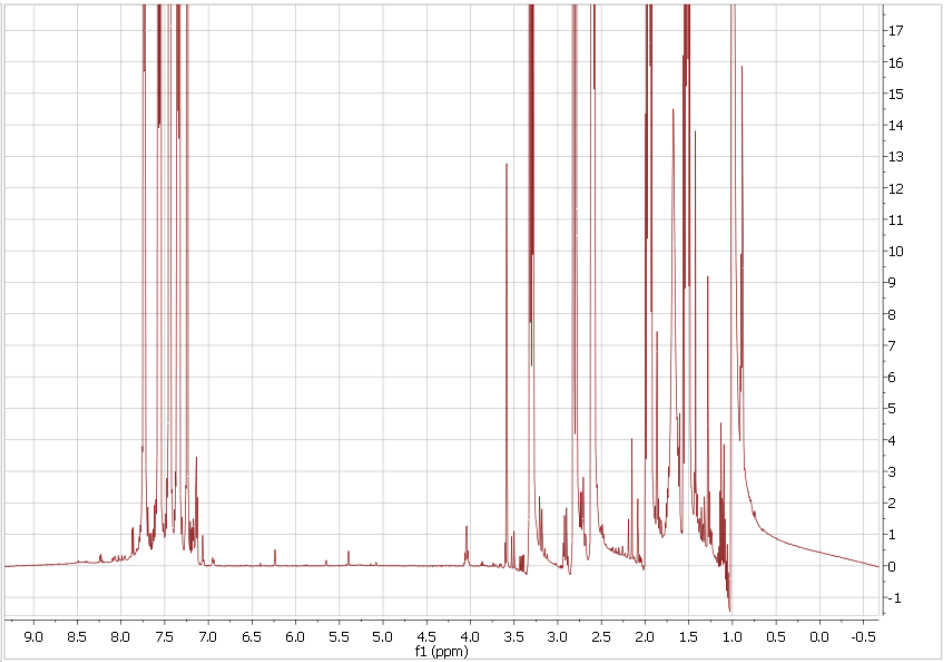
1. Open *.fid data
2. Check & Select appropriate window functions for t2 and t1 under ***Processing->Apodization***
3. Decide whether the spectrum needs phase adjustment
    1. For all **absolute-value** (or **magnitude**) mode spectra, such as typical COSY and HMBC, **DO NOT** apply phase correction.
    2. For **phase-sensitive** spectra, such as typical HSQC/HMQC/NOESY etc., phase both **f2** and **f1** dimensions. Apply both zero-order (**ph0**) and 1st-order (**ph1**) phases if necessary.
4. Apply baseline correction to **f2** and **f1** dimensions.
5. Play with other optional processing tricks such as t1 noise reduction or symmetrization, etc. **Be cautious that these routines may introduce artifacts or reduce signal/noise.** Always compare results with those from simple processing. As a rule of thumb, unless really necessary, do not apply these additional tricks.

When 1st-Order Phase Is Needed

Two spectra after zero-order phase correction

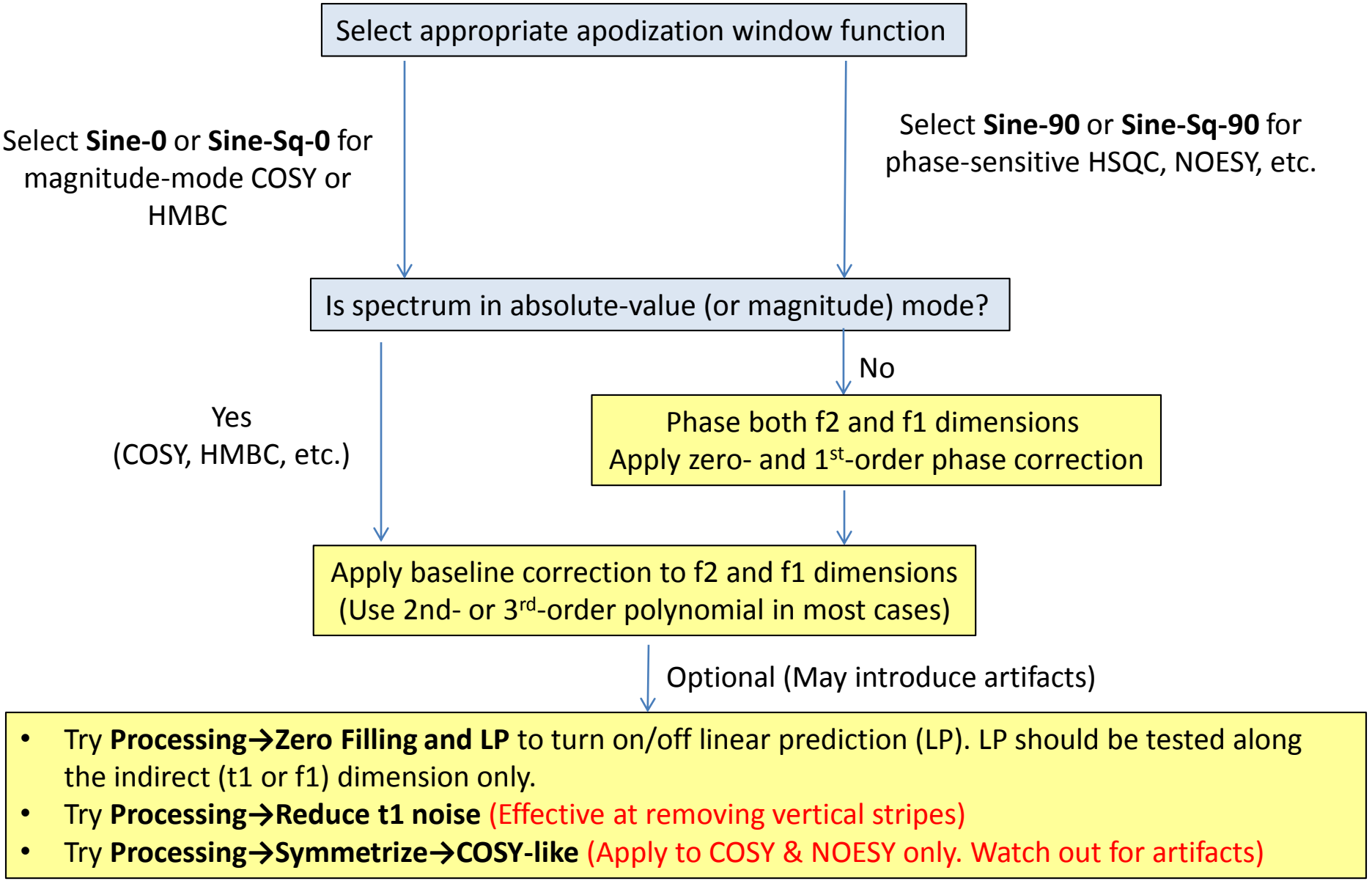


Phase is good across spectrum
1st-order phase is NOT needed



1st-order phase is needed.
Note the linear change in phase from left to right.

2D Processing Adjustment in Mnova



- Try **Processing→Zero Filling and LP** to turn on/off linear prediction (LP). LP should be tested along the indirect (t1 or f1) dimension only.
- Try **Processing→Reduce t1 noise** (Effective at removing vertical stripes)
- Try **Processing→Symmetrize→COSY-like** (Apply to COSY & NOESY only. Watch out for artifacts)

Zero Filling (ZF) and Linear Prediction (LP)

ZF and LP are two separate techniques to enhance the resolution of the original data.

- ZF simply appends the original FID with zeros to at least double the data size.
- Linear prediction is to predict additional FID data points using existing FID data. It is an alternative, sometimes more effective method at resolution enhancement.
- Both techniques offers better resolution, but LP may introduce artifacts (see below) and significantly reduce signal-to-noise of the spectrum.

NOTE:

- ZF is almost always advantageous to apply after FID weighting. It is safe in nearly all cases.
- LP is an optional trick. Use it only when extra resolution is desired. Always compare its result with that from normal processing (without LP). Pay attention to resolution enhancement and additional artifacts in the spectrum when LP is used.
- Typically, LP is only used for the indirect dimension (t1 or f1) where only a small number of data points are collected. The direct detect dimension (t2 or f2) usually has sufficient data points and LP is not necessary.

Magnitude-mode COSY Processing

NOTE: No phase correction should be applied.

Check apodization/window function along f2 and f1 to select **Sine 0.0** or **Sine Square 0.0** **(Processing→Apodization …)**

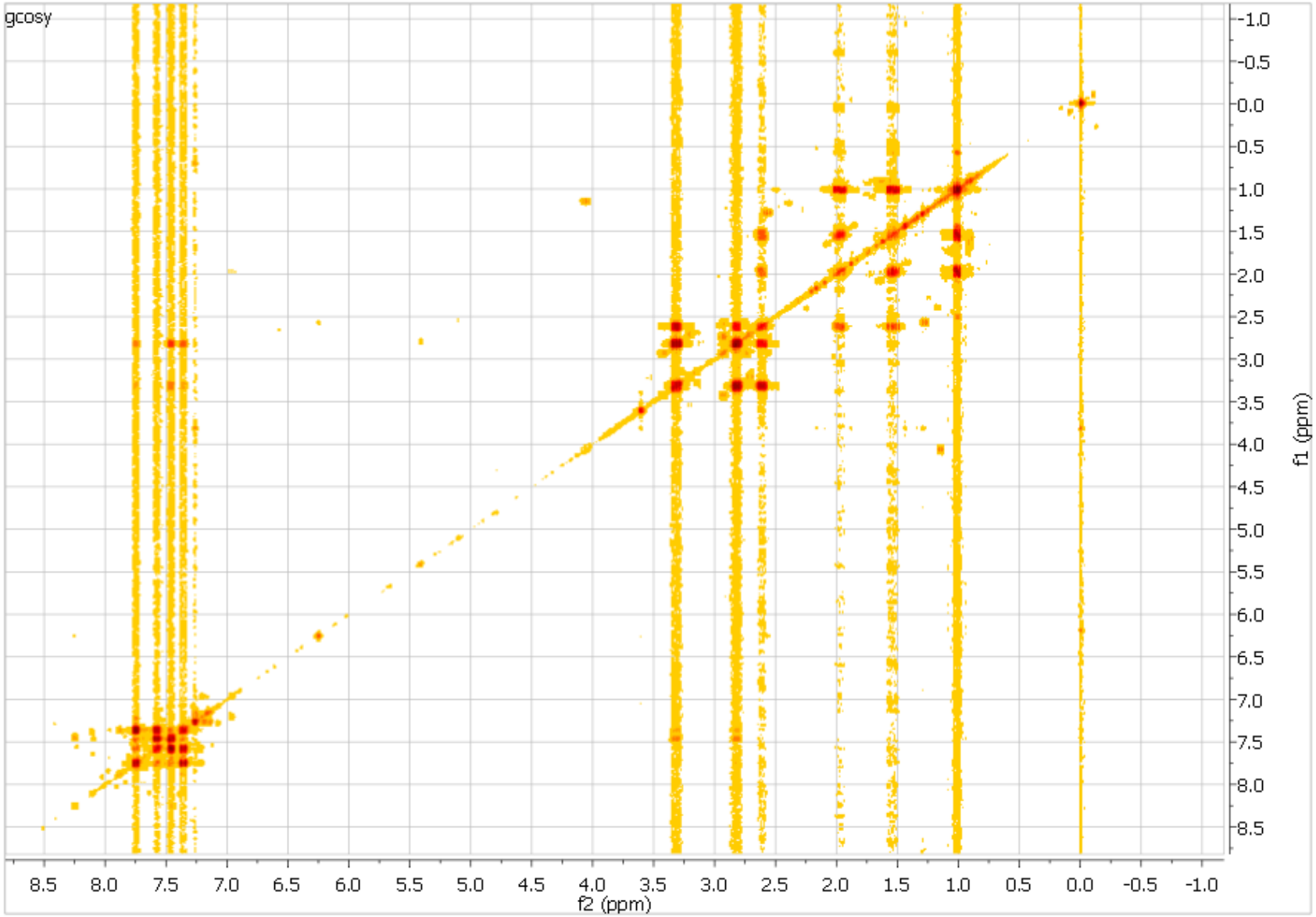
Apply baseline correction to f2 and f1 dimensions (Use 2- or 3-order polynomial)

Optional

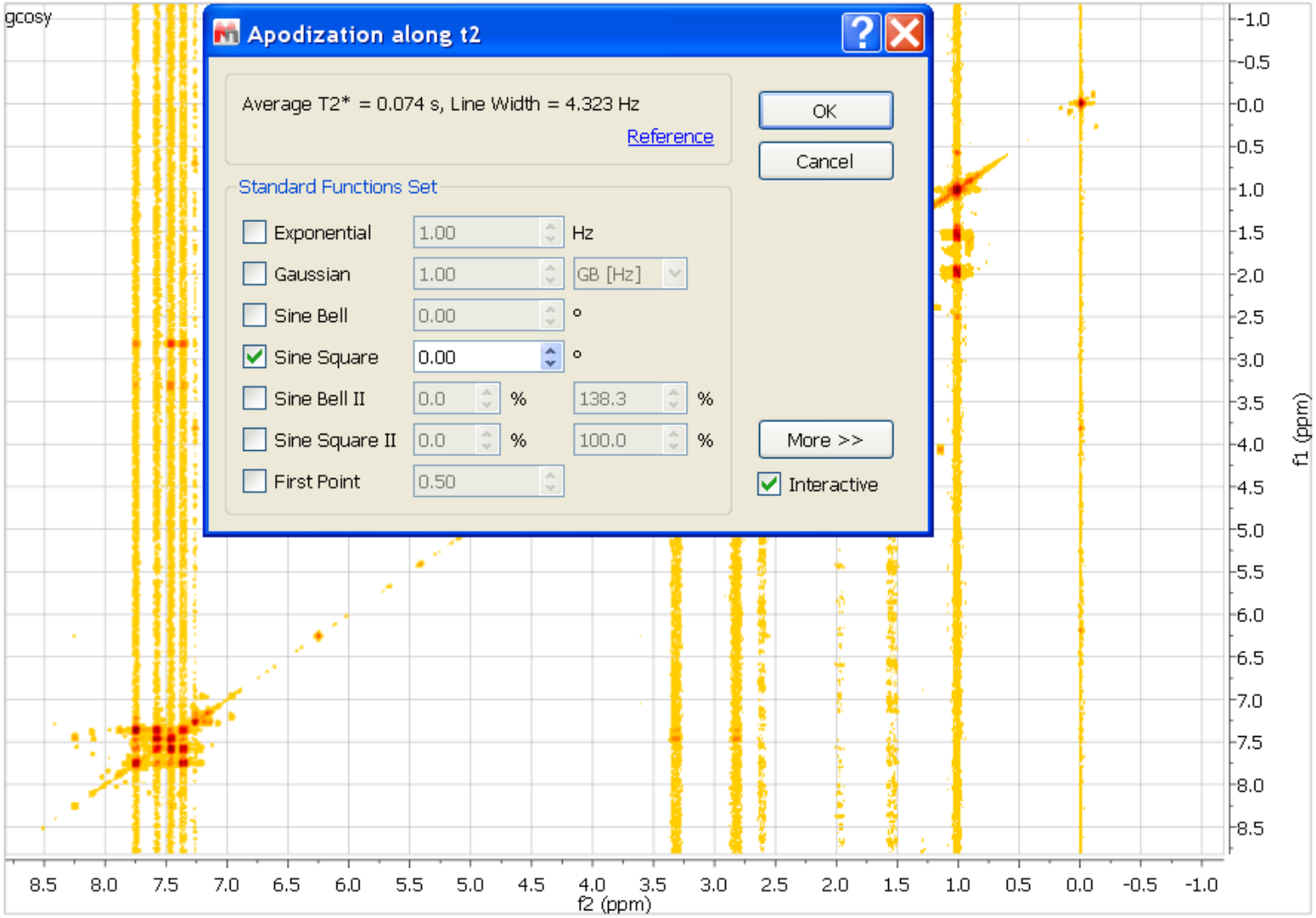
- Try **Processing→Zero Filling and LP** to turn on linear prediction (LP)
- Try **Processing→Reduce t1 noise** (Effective at removing vertical stripes)
- Try **Processing→Symmetrize→COSY-like** (Watch out for artifacts)

gCOSY: Initial look after automatic processing

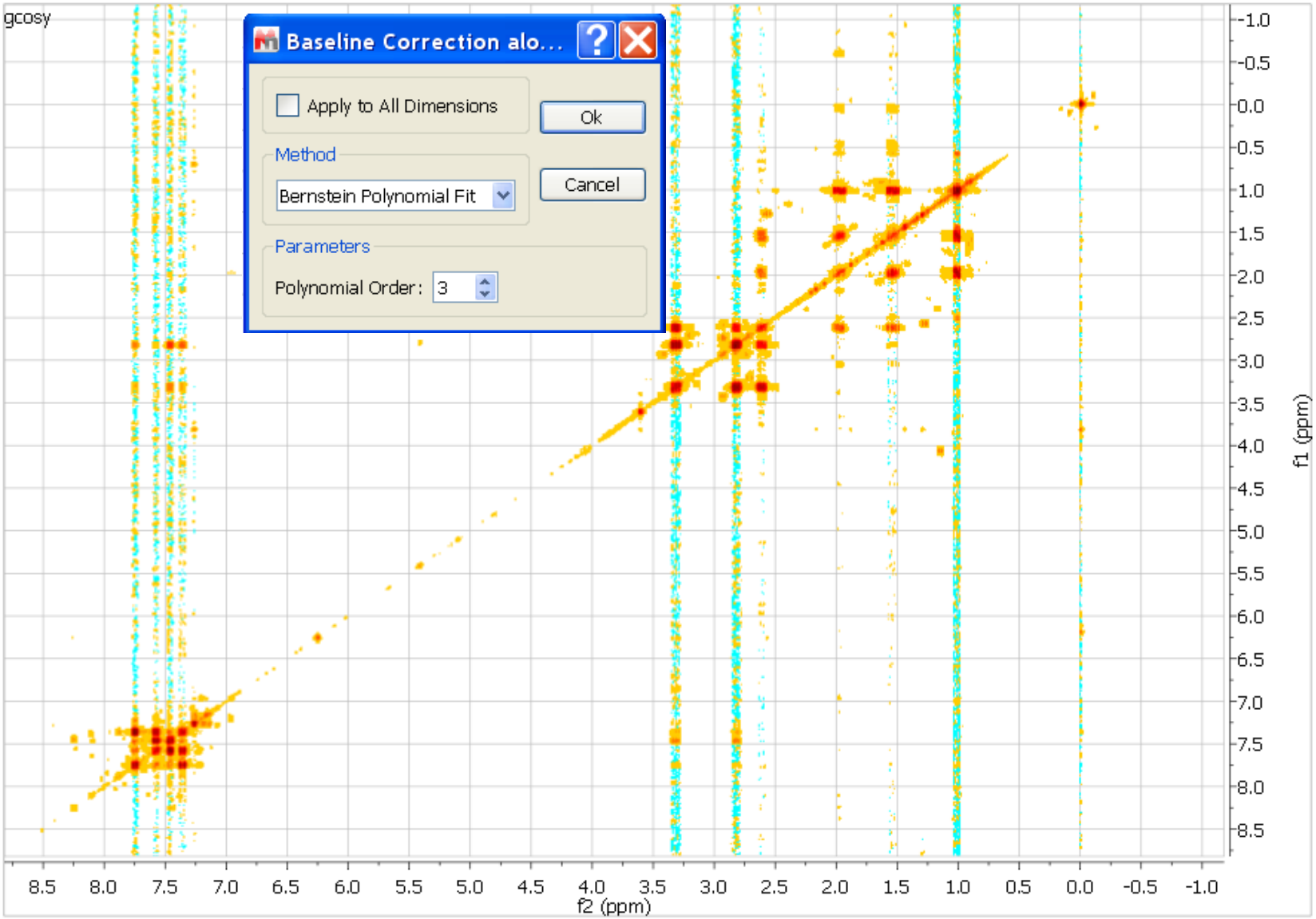
- Vertical stripes are called t1 noise



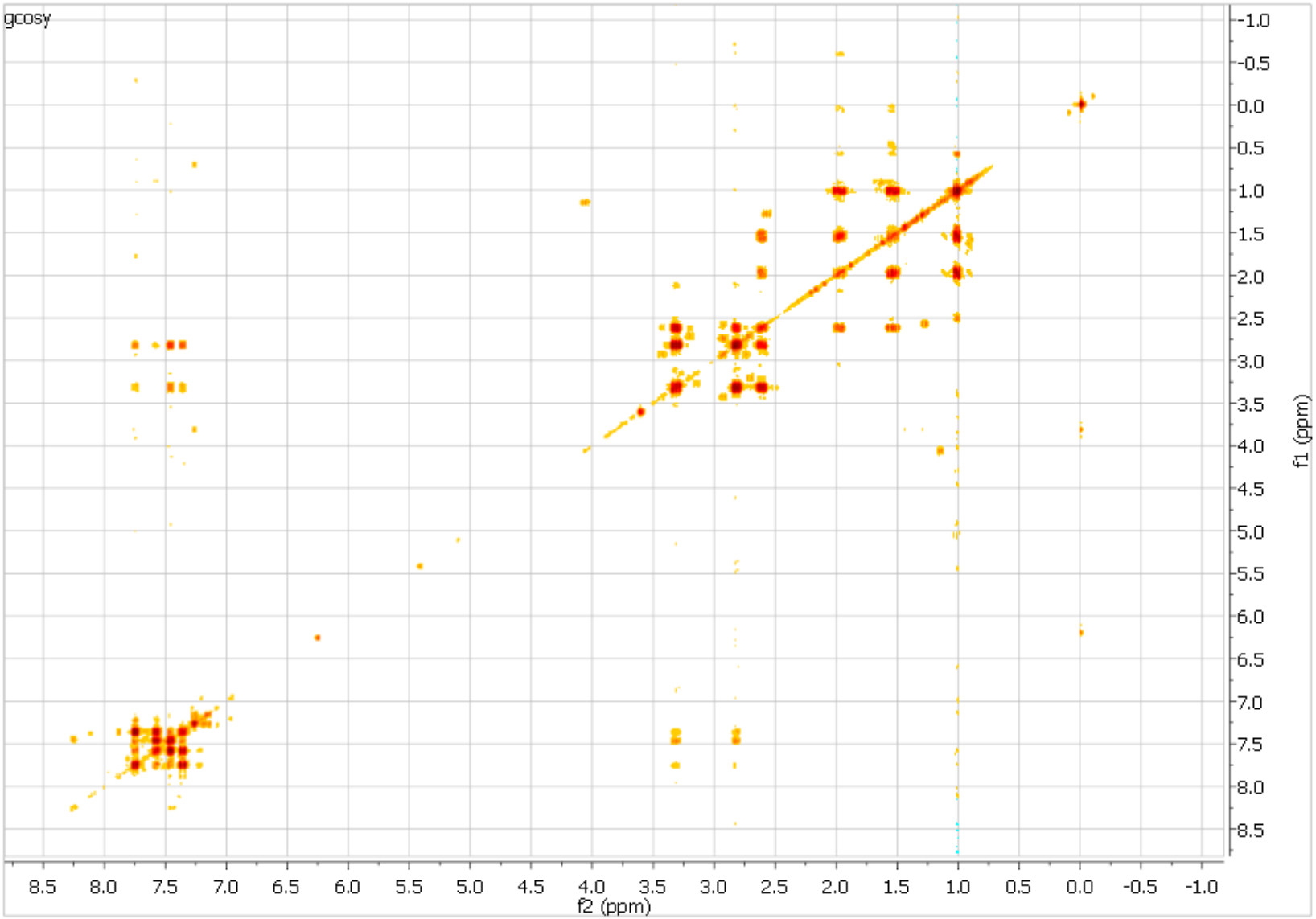
Check and set ***Processing→apodization*** function to Sine Square 0.0 along t2 and t1



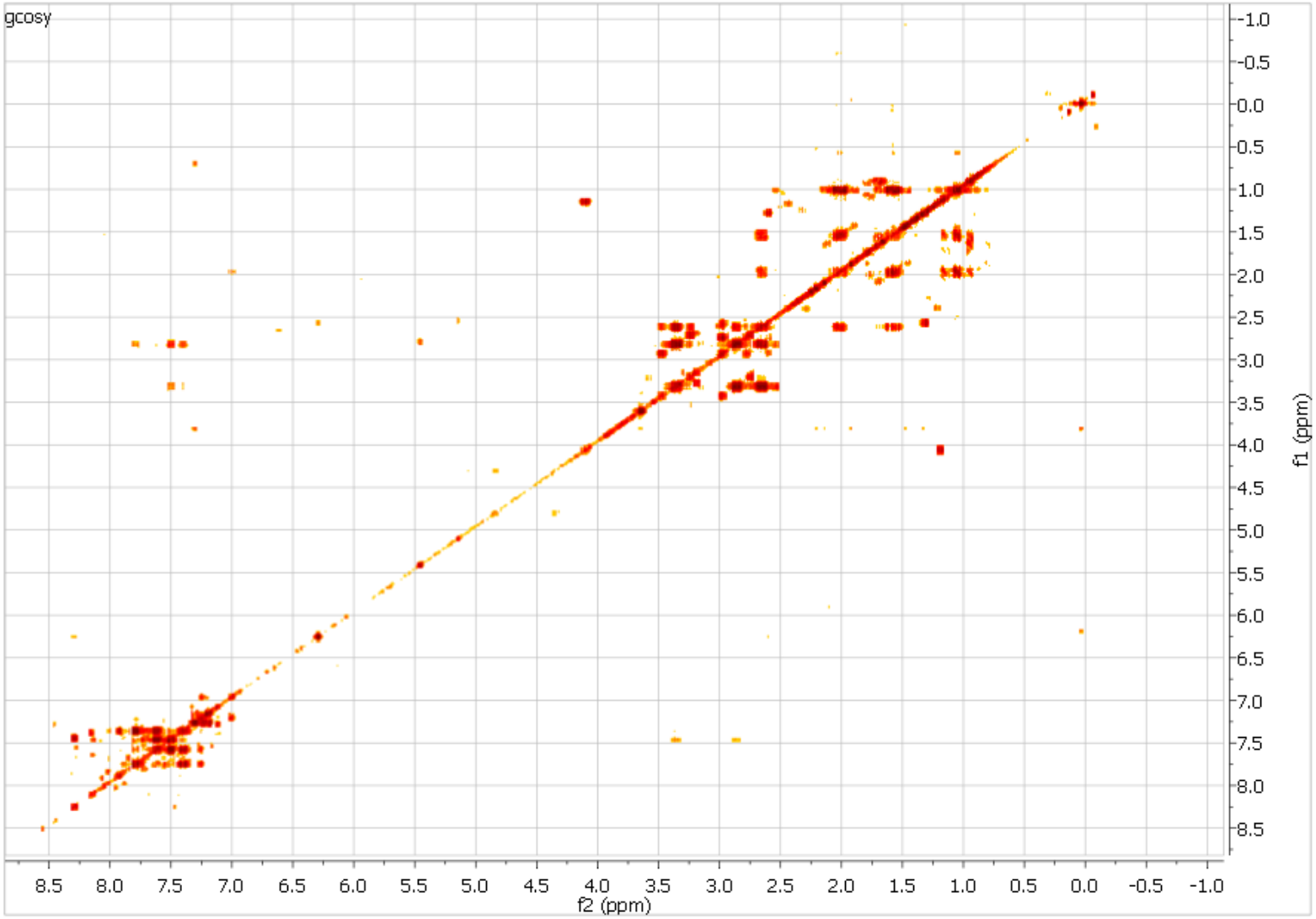
Apply baseline correction along f1 (vertical)



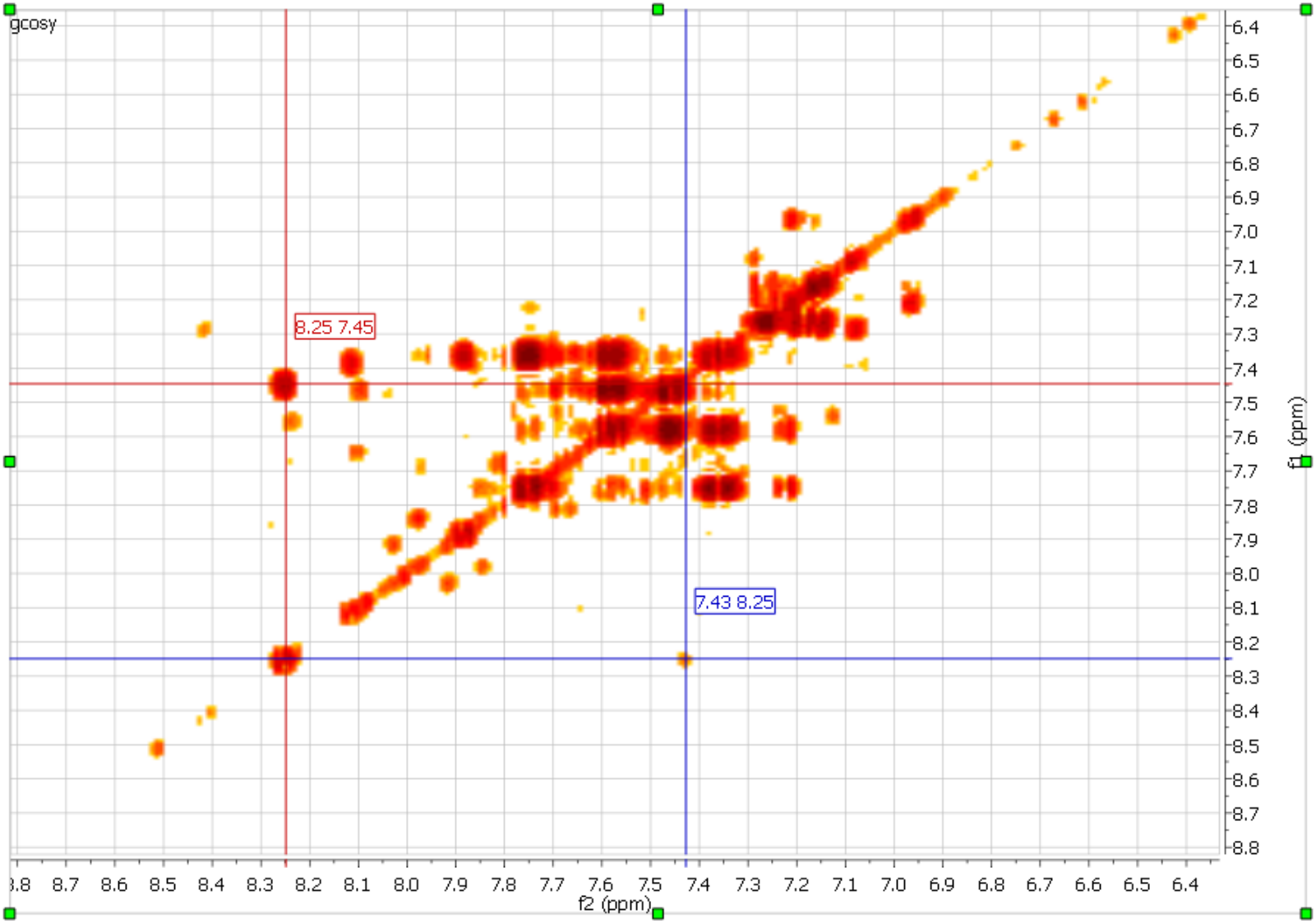
Raise the intensity threshold with middle mouse scroll button



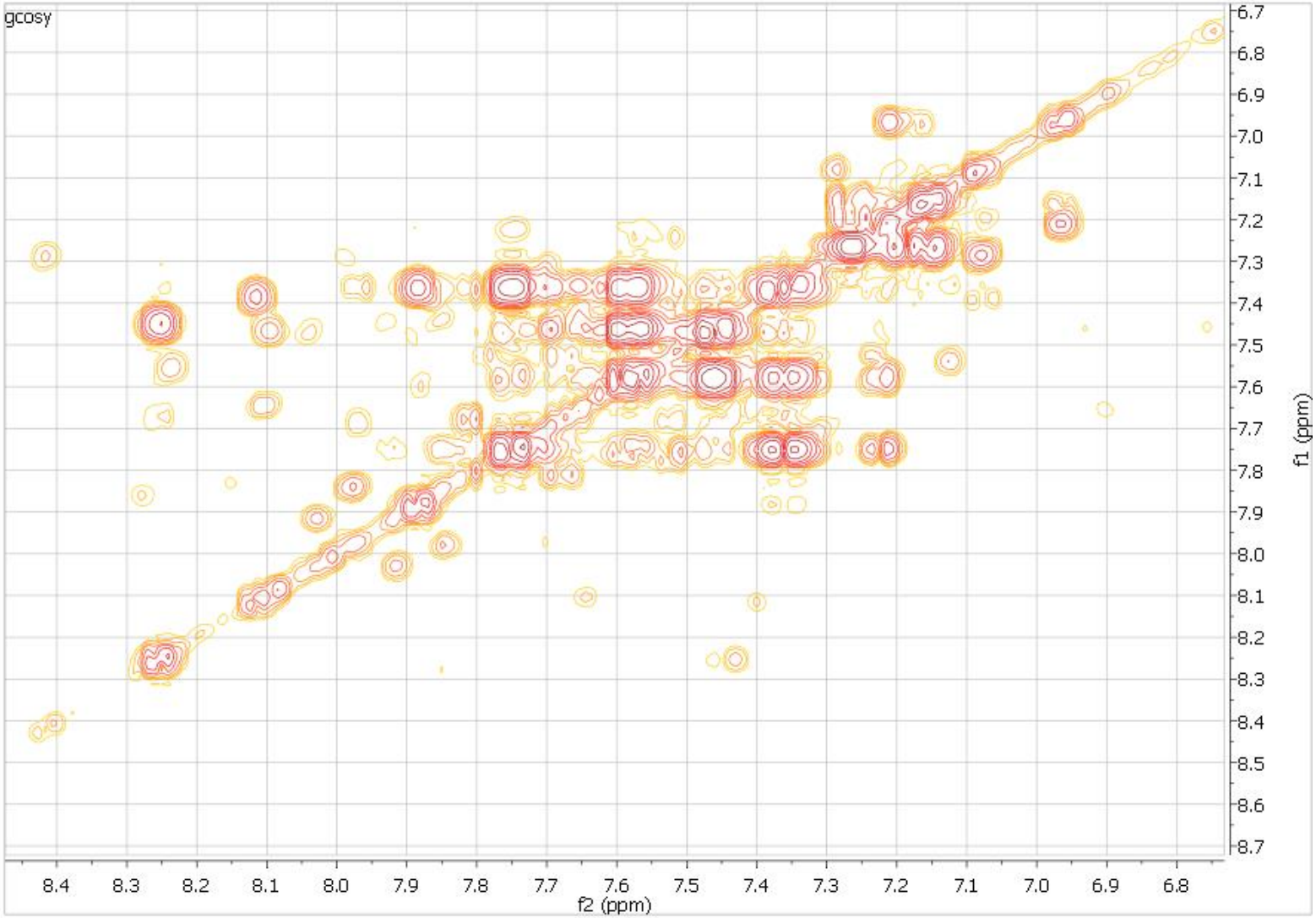
Turn on ***Processing→Reduce t1 noise.*** In this case, the improvement is significant, but be cautious with artifacts. To turn off t1 noise correction, toggle off ***Processing→Reduce t1 noise***



Use ***View→Crosshair (***or press ***C)*** to check symmetry of COSY crosspeaks
Hold the left mouse button and drag to show a box

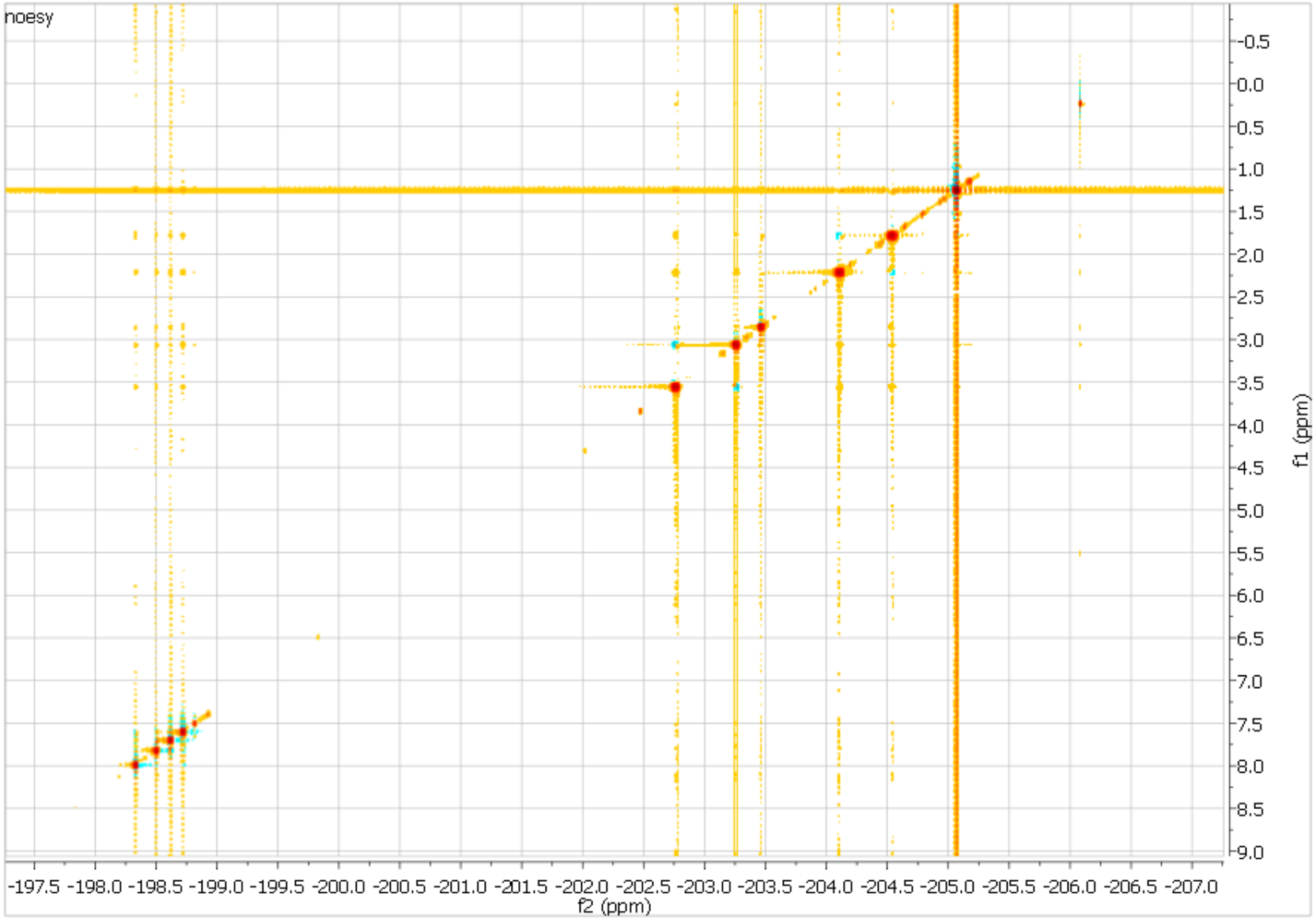


Select ***View→2D Plotting Method→Contour Plot*** or right mouse button click. Contour drawing on the fly is slower. Do this after data processing.

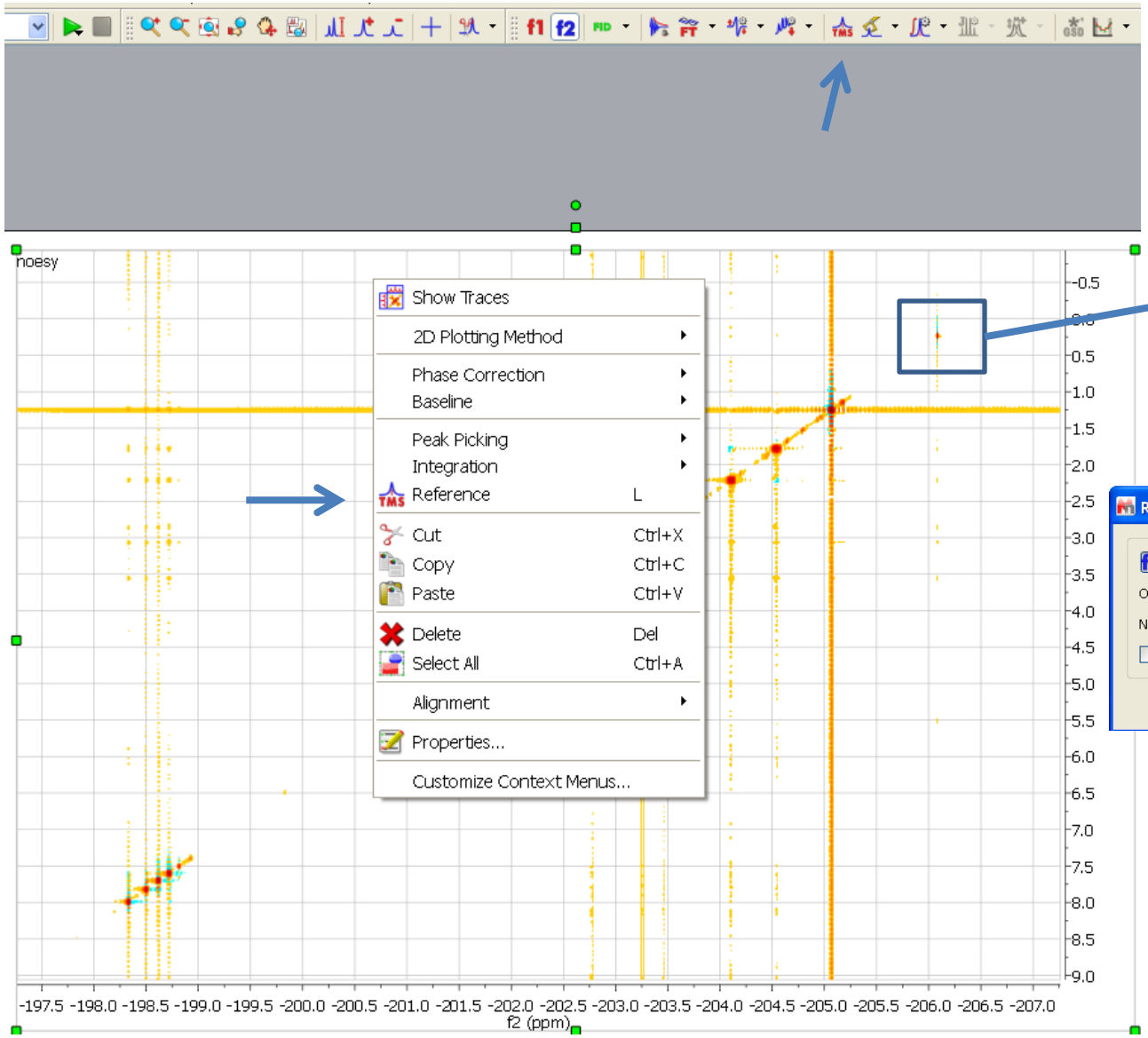


NOESY Processing: Initial look

- Reference is wrong
- Apodization/window function need to change

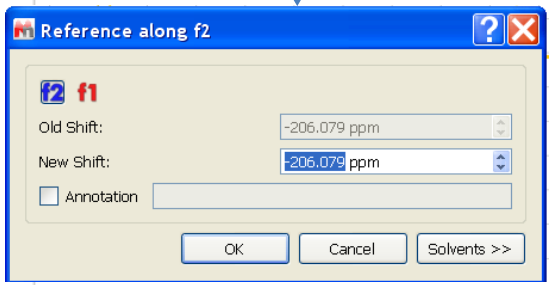


Referencing



Set crosshair over the reference peak

Click

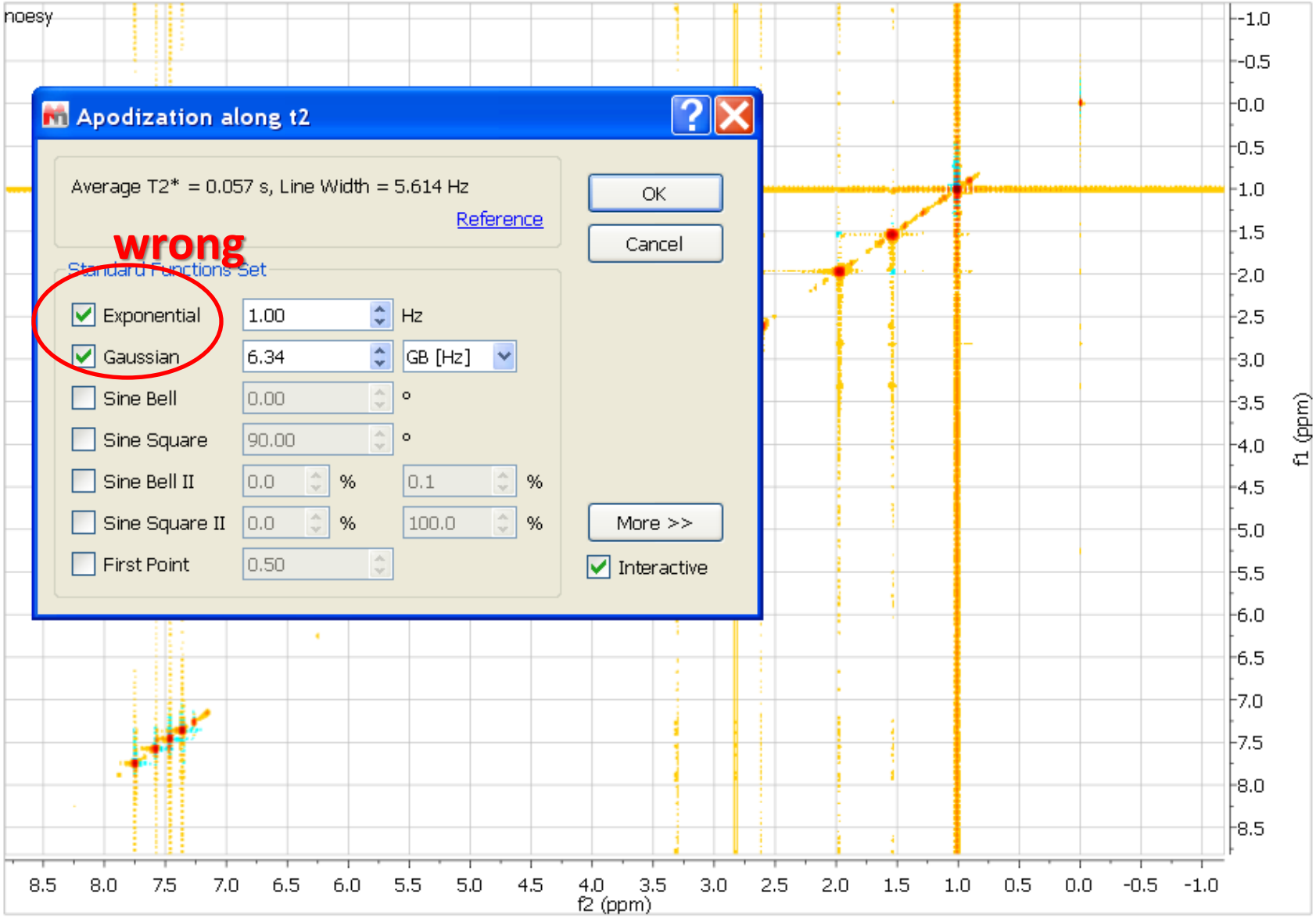


Change reference ppm for both f1 and f2

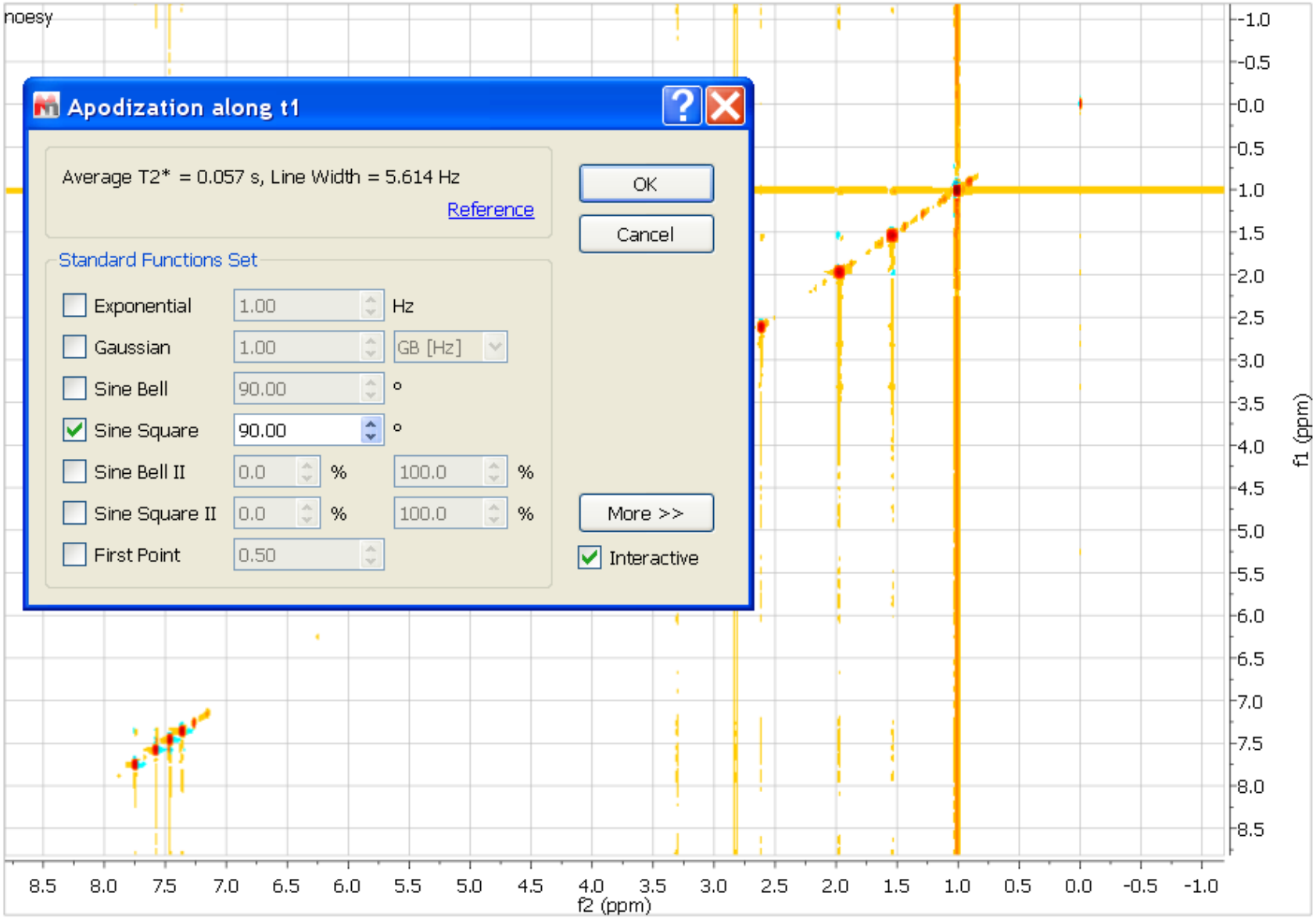
Select f1 or f2 from menu buttons and change ***Processing→Apodization*** to:

Sine Square 90.0 along t2

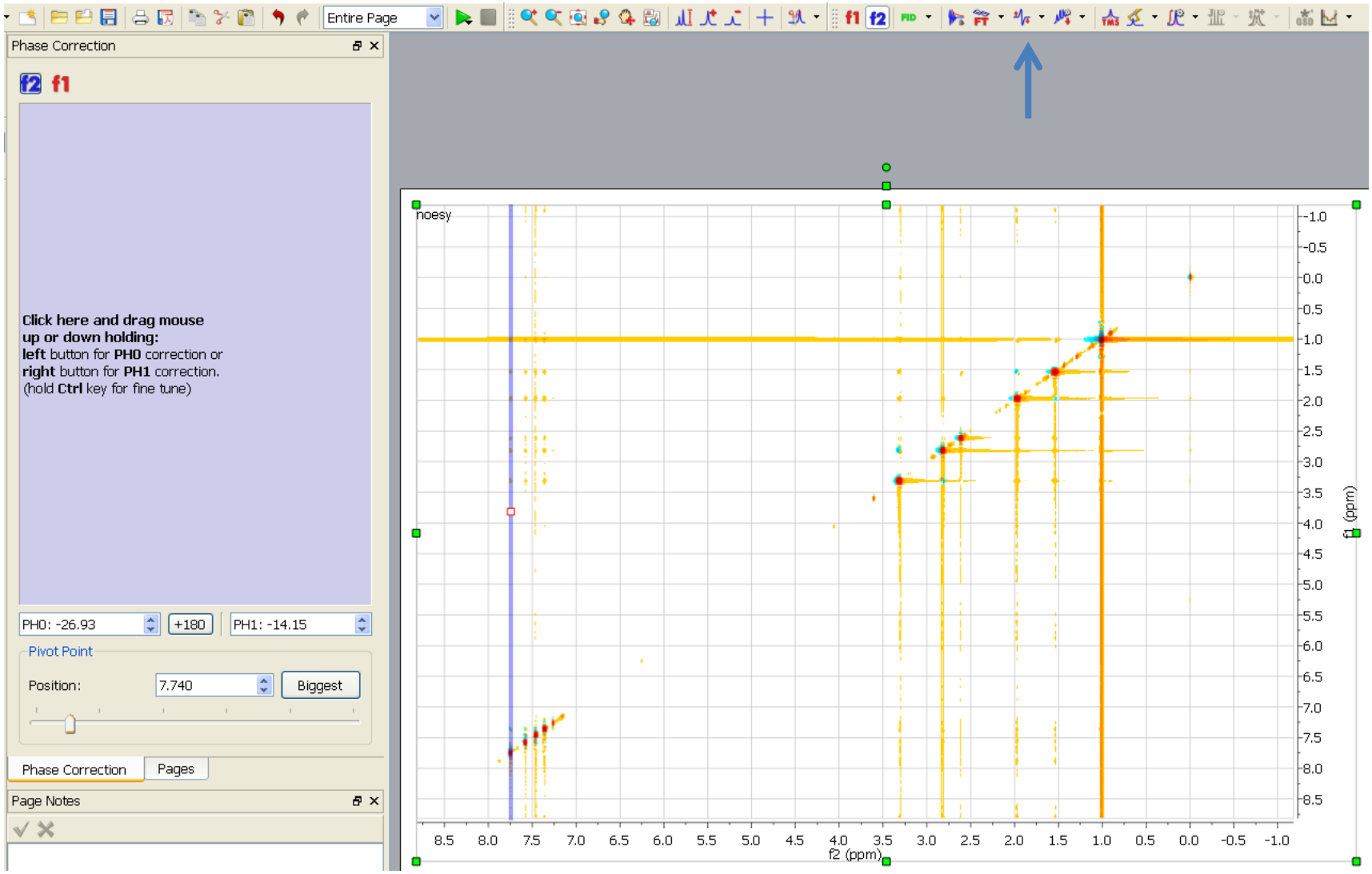
Sine Bell 90.0 along t1



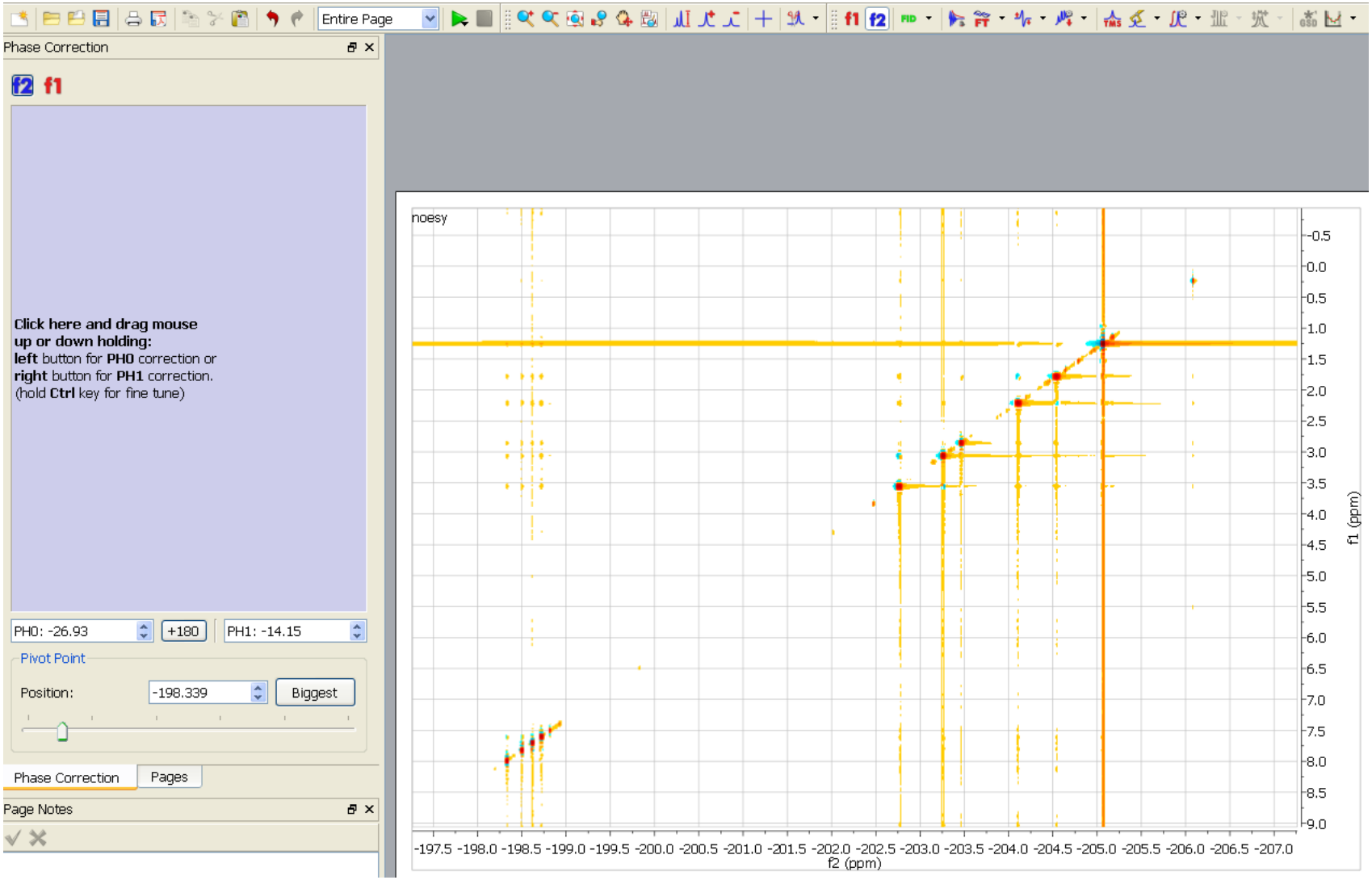
The 2D peaks will look smoother and the baseline noise is less with the new window function



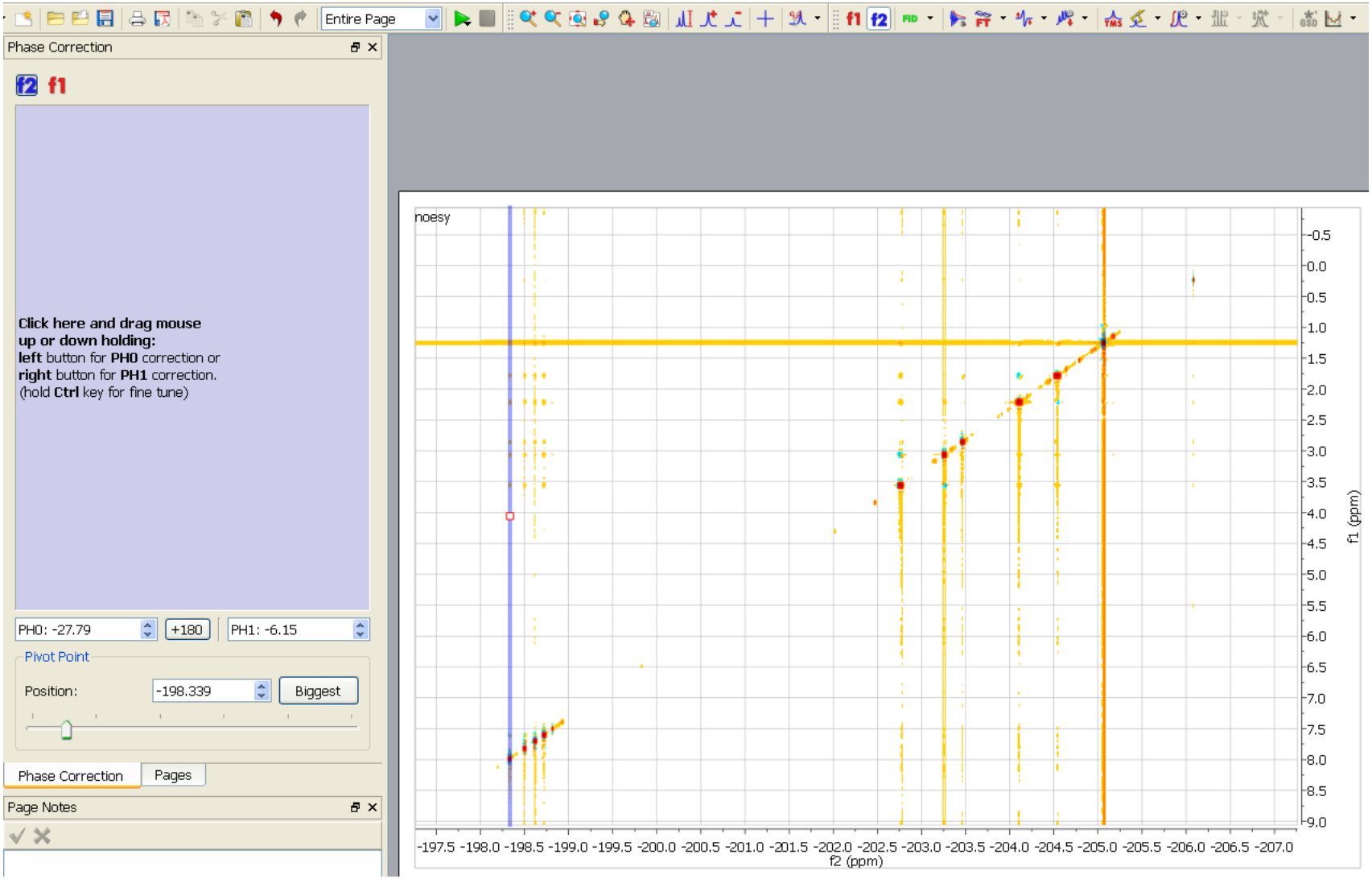
- Select manual phase correction
- Select f2. Drag pivot line (vertical blue line) to line up with a strong peak on left
- Adjust PH0 so that the selected peak is perfectly in phase
- Adjust PH1 so that the peaks on right also come into phase



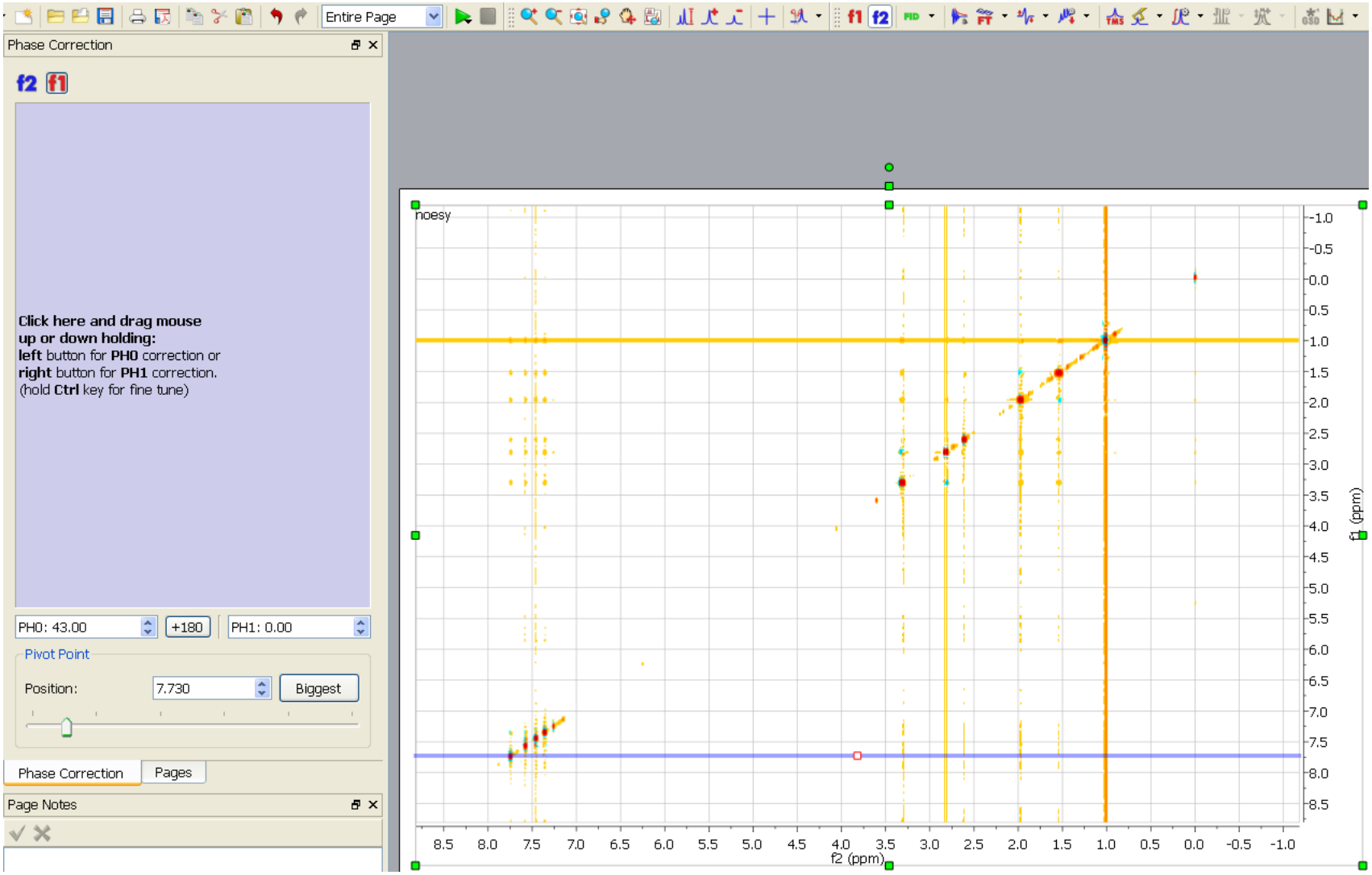
After PH0 adjustment along f2



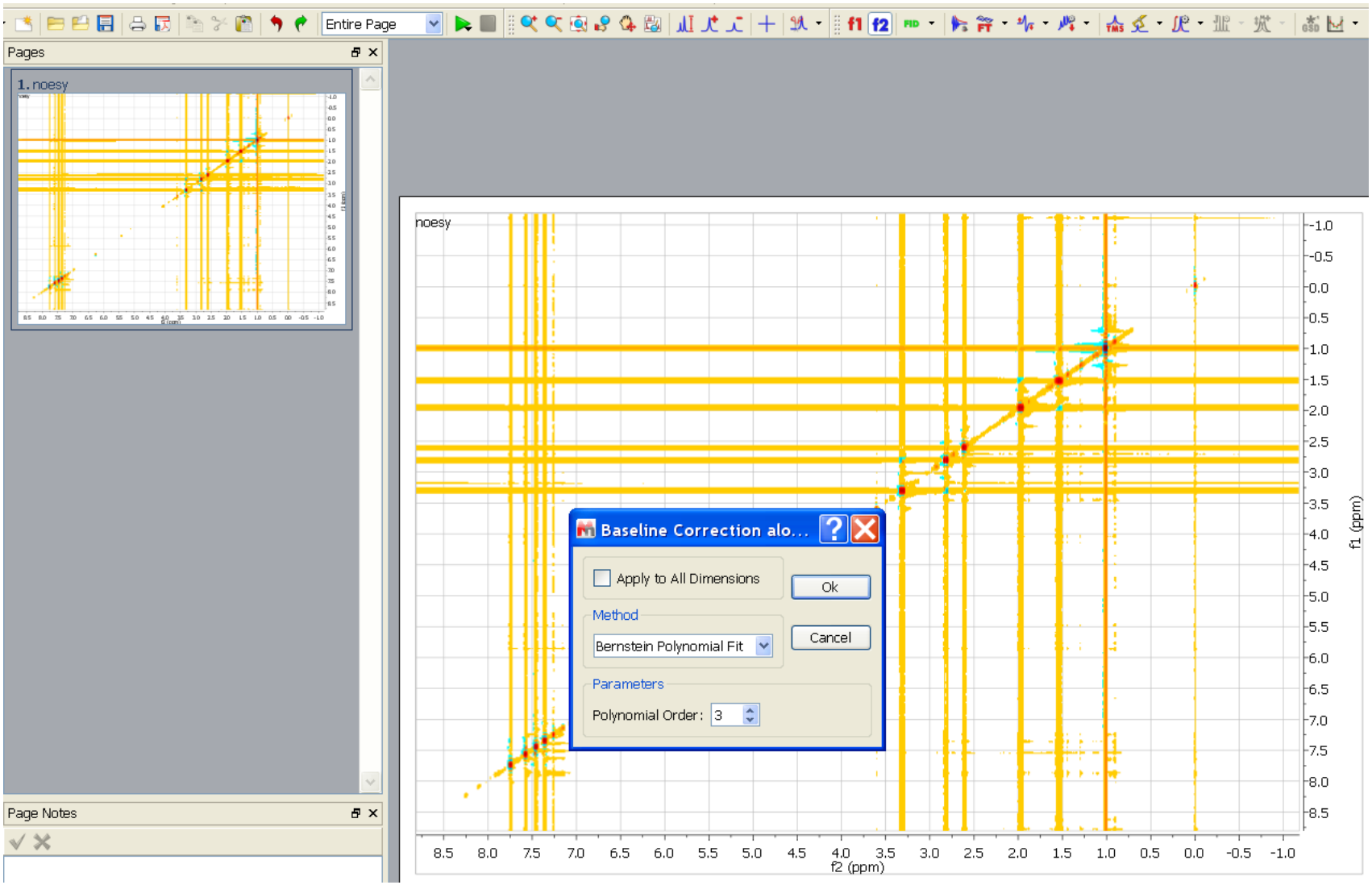
After PH1 adjustment along f2. Note improvement to peaks away from pivot



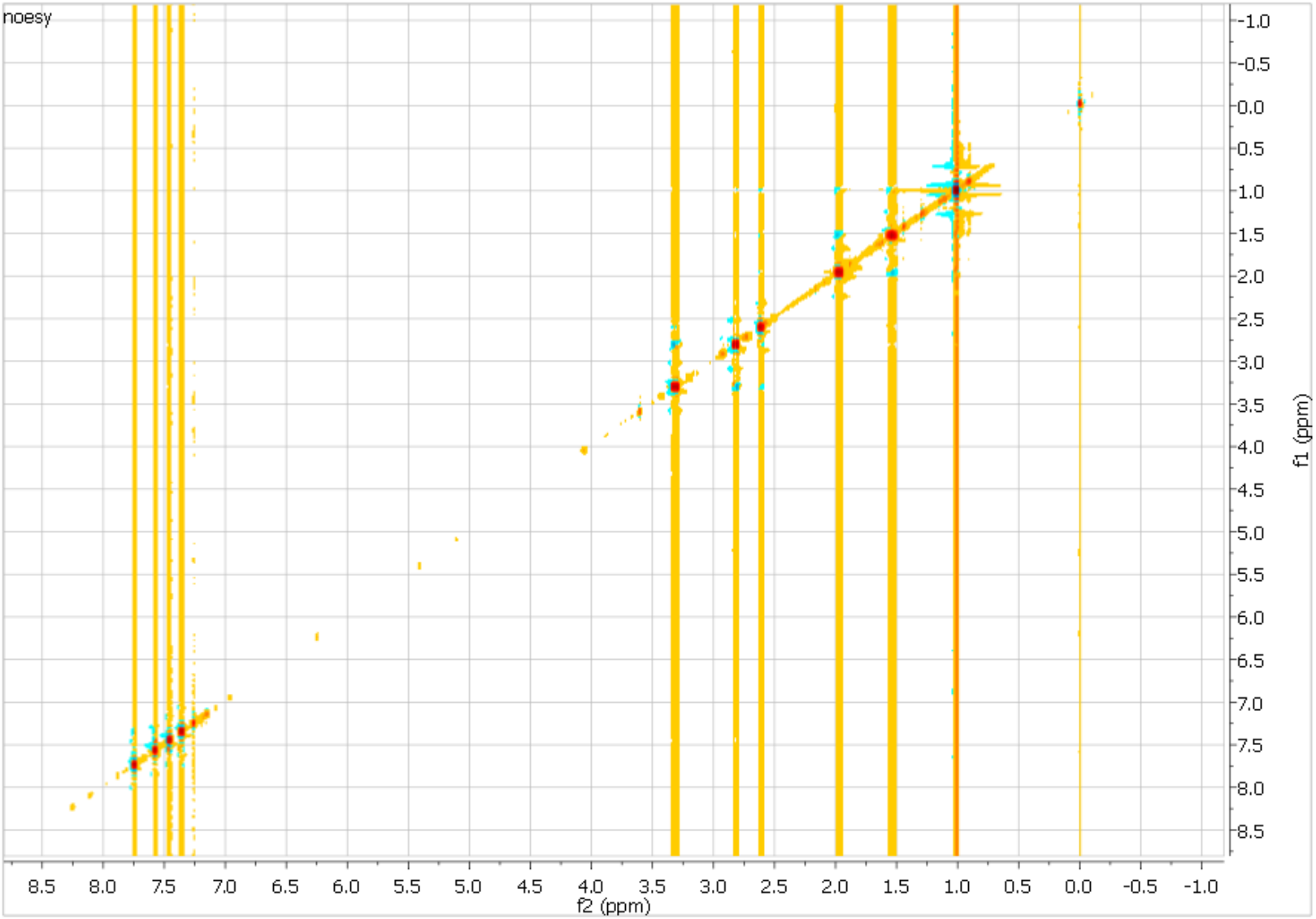
- For f1, select a peak near the bottom (or top) as the pivot
- Spectrum after PH0 adjustment
- PH1 seems OK. NO linear phase correction necessary.



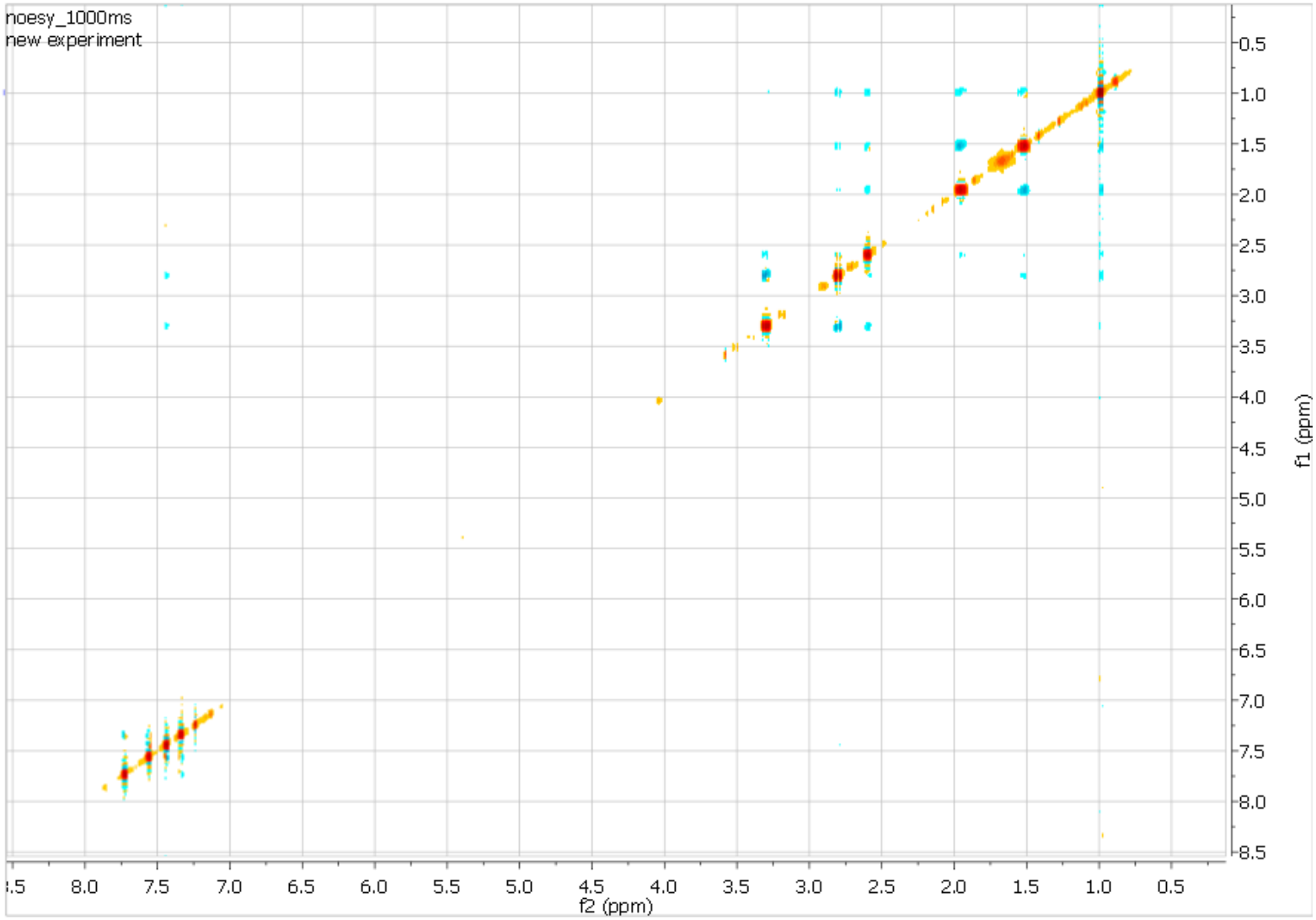
- Lower threshold so that baseline defects are seen
- Apply Polynomial baseline correction order 2 or 3 to f2 and f1 separately



After f2 baseline correction



After both f2 and f1 baseline correction

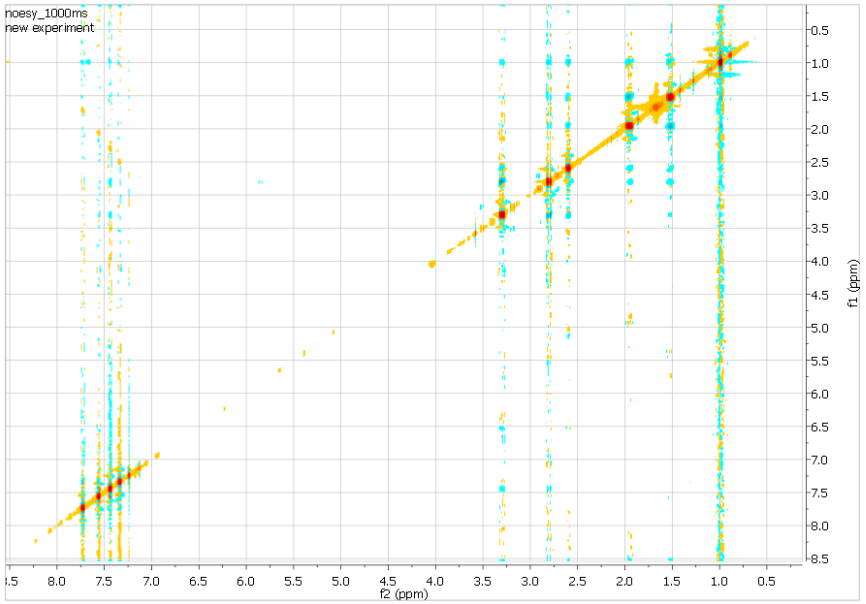


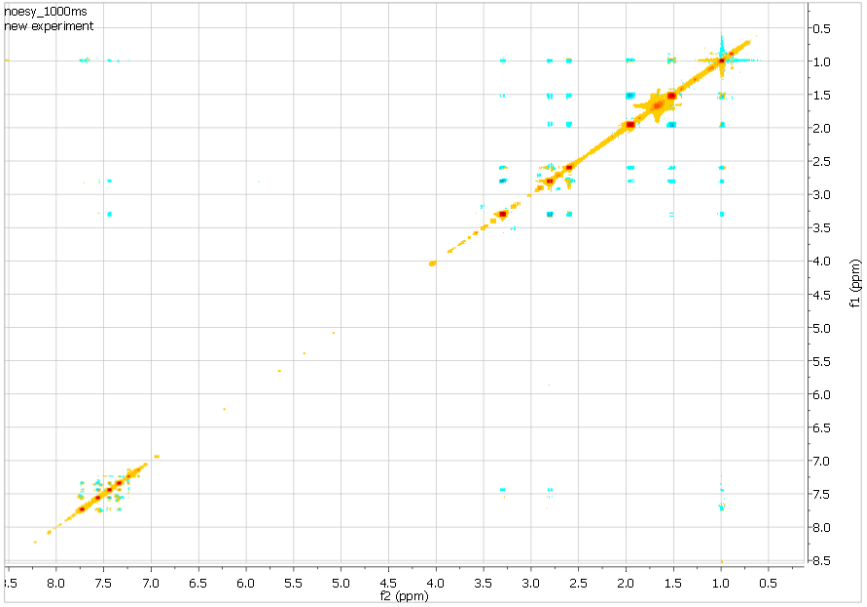
Additional processing

- ***Processing→Symmetrize→COSY-like***
- Spectrum quality improves.

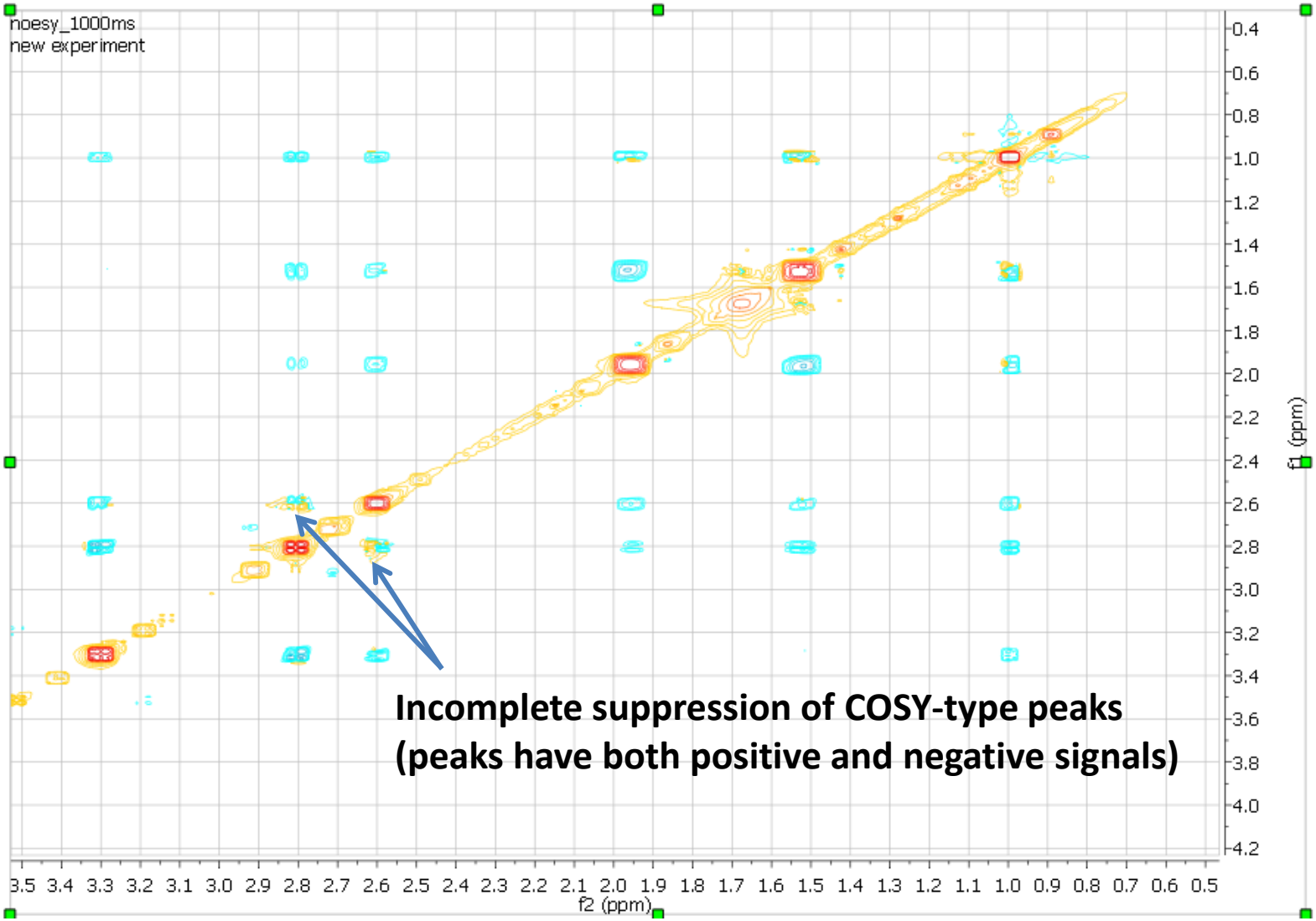
NOTE:

- Be careful with the “reduce t1 noise” or symmetrization procedure. Artificial crosspeaks may be created in the process if the background noise is strong.

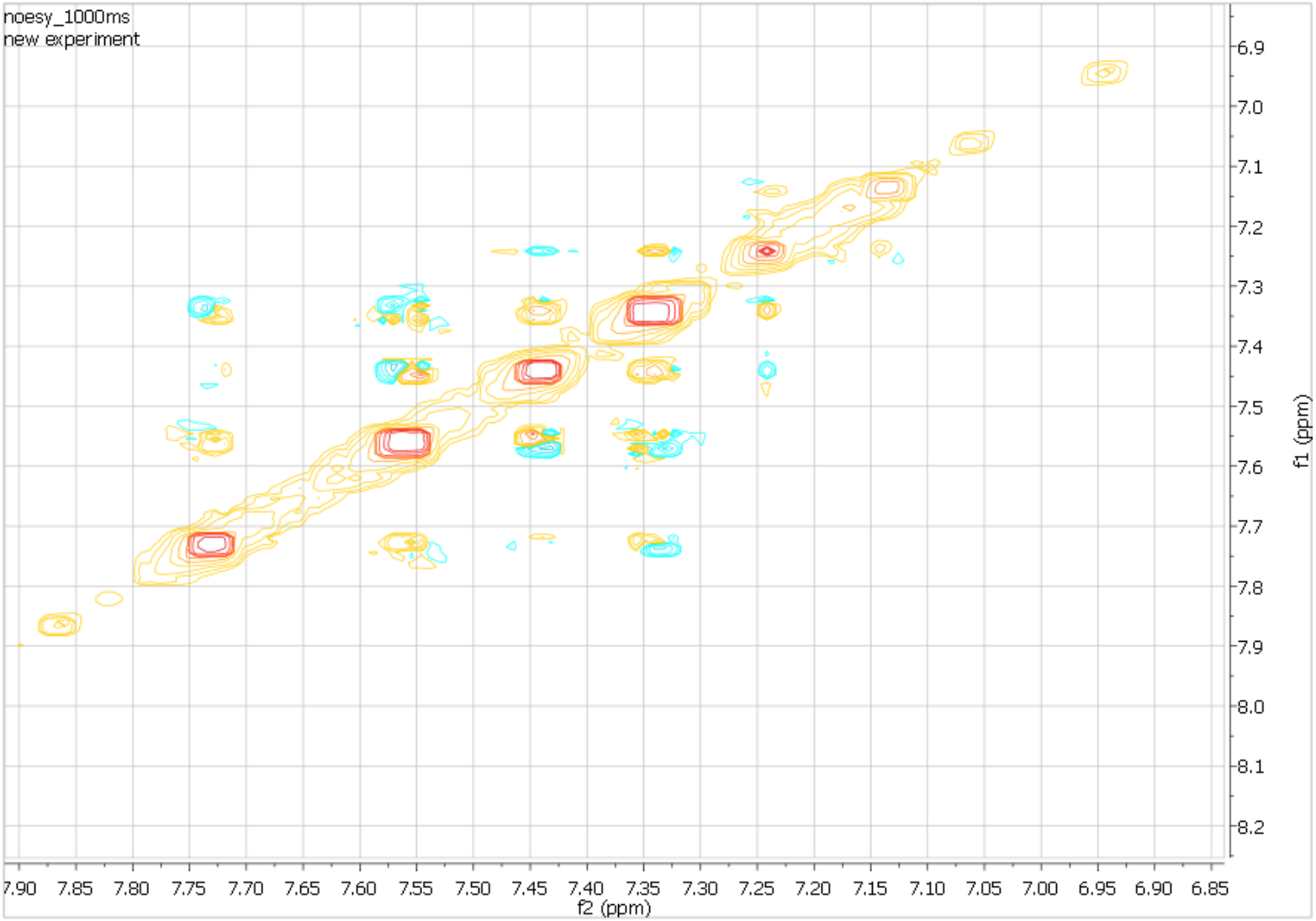




- **For small molecules, NOEs mostly have opposite signs with the diagonal peaks.**
- Typical artifacts are:
  - **dispersive peaks** (with mixed up/down signals) that come from through-bond COSY-correlations. These are suppressed but not completely.
  - **exchange crosspeaks** having same sign with diagonal peaks



These through-bond COSY-type peaks tend to cancel some or all through-space NOEs because they have opposite signs in some overlapping areas. Here, more COSY-type peaks are seen between the aromatic ring protons.



Exchange crosspeaks in NOESY are shown as in-phase peaks having the same sign as the diagonal peaks but opposite to NOESY crosspeaks. They can result from:

- chemical exchange, such as exchange of ^{1}H in H_2O with an amide ^{1}H.
- exchange among multiple conformers where a ^{1}H has a distinct chemical shift in each conformer.