2D NOESY

This experiment identifies resonances of protons 5Å or less from one-another and thus allows one to begin inferring the structure of a complex molecule, and to piece together adjacent spin systems. Magnetization is encoded with the chemical shift of the source proton during d2 and then allowed to transfer through space to protons nearby during a mix time. Short mix times allow only direct A → B transfer and emphasize shorter distances. Longer mix times approaching the T1 allow transfer over longer distances that may be critical in establishing isomer identity and global fold, however long mix times also permit multistep transfer (A → B → C) that may confuse interpretation. Plus/minus results can be obtained with a first-guess of mix=T1, but extraction of numerical distances is best performed with shorter mix times or better yet comparison of results obtained with a series of mix times.

Collecting a NOESY

Begin with your beautiful calibrated 1d.

On T2J, the pw90 at 55 dB is 51/4 = 12.75 us.

tpwr=61 gives pw90=27/4 = 6.75, half of 55, so we are linear. Also, note that this probe does not require nearly as much power as does Gort's probe. We will use lower values of tpwr.

phase it up (lp=-33) type **setlp0** , acquire again and rephase. lp is now -17.9 (better but not superb).

Convert current parameters to do ...> Homonuclear Correlations Experiments > NOESY. **Page 2** presents the NOESY pulse sequence with the Acquire>Pulse Sequence panel displayed. In the default implementation, a shaped pulse [1] helps to filter out artifacts that represent undesired COSY (through-bond) magnetization transfer mechanism. This is called a 'Z' filter [2]. It will not decrease the performance of your NOESy, but it will make the resulting spectrum a little easier to interpret. If you want to use it, be sure that you have a correct pw90 in your parameter set prior to converting it to a NOESY experiment because the software will use this to calculate the duration and power for the Z filter pulse. (We will not manipulate these values ourselves.)

You see the Grad-90-Grad crusher up front [3]. This serves to eliminate any left-over net magnetization from the preceding iteration of the sequence and start each scan with the same amount of magnetization, depending on the choice of d1 [4]. Note that the software carried in our calibrated pw90 and is using it for all the hard (non-selective) pulses [5].

The mix time is only 0.2 s [6]. This is much shorter than my sample's T1, so I will want to increase it if I want to see *all* my ^{1}Hs within 5 Å (even mix=T1 is not an absolute guarantee). However if you want to be able to infer distances from the cross-peak strengths, then a mix<<T_1 is the perfect choice.

In Acquire>Acquisition, I like to double at to 0.3 s [1], as for the TOCSY (**Page 3**). Similarly, it is still wise to run the first increment as a test of your gain and stability [2]. For your cleanest NOESY, d1 should be longer than T1. Ideally, several times longer. For the sake of speed we'll run at a minimal value of d1=T1 [3]. Better to use d1=3*T1 if you want quantitative distances.

Alternately, use the 'find gain' option [4]. This is only active when Full 2d is active, however what happens when you ask the instrument to 'Find Gain' is that it changes to 1D mode and also omits the steady-state scans. When it finishes however, it resets to 128 increments (change them back to 200 [5]). Note that the software has preserved our optimized sweep width and used it for both the F2 (directly-detected) and the F1 (indirectly-detected) dimensions [6]. (The parameter names for these are sw and sw1, respectively, with the '1' add-on indicating that it pertains to the first indirect dimension.

As for TOCSY, the software tries to get away with 128 increments when we reactivate 'Full 2D'. Enter 200 to get a resolution of 20 Hz per point in the indirect dimension (3,913.5 Hz wide divided by 200 points).

'Show Time '[7] says this will take 2 hours and 45 minutes. Because we have a strong sample, we can opt for 2 sans instead of 16. This cuts the experiment time to 21 minutes. As a rule of thumb, if you have a signal-to-noise level you like after n scans in an ordinary ^{1}H 1d, then using 4x that many scans should be adequate in a TOCSY.

Now click Acquire [9].

Once the 2D begins (**Page 4**), the spinner is turned off [1], to prevent artifacts due to fluctuations in spinner speed. (This is a good incentive to be sure that the XY shims are in decent shape.)

To see the individual 1Ds being collected, you can use the 'Arrayed Spectra' tab of the left-hand panel [2]. However you will only be able to display spectra after they have been Fourier transformed. Thus you will need to type **wft** (weighted Fourier transform). This will perform a 1D Fourier transform on all the FIDs collected up to that time. The software will tell you how many FIDs were transformed [3]. Alternately, because of the need to reset the vertical offset repeatedly when using the Arrayed Spectra panel, it may be easier to use commands.

wft dssa will Fourier transform all the FIDs collected so far and then display them as a vertical stack (dssa = display stacked spectra automatically). The screen gets crowded with more than ≈ 10 spectra on it, so when you have more, you way want to see only every 8th (for example). When I could see that there were >70 spectra collected, I typed **wft** and **dssa(1,70,8)** to display spectra 1 to 70 showing only every eighth spectrum (**Page 5**).

If the individual spectra are too tall, you can make them half as tall by typing **vs=vs/2** (new vertical scale set to half what it used to be, vs is the vertical scale).

Then (again) type **wft dssa(1,70,8)** [1]. Each time you type **wft** you will update the list of spectra that have been Fourier transformed, allowing yourself to see more of them, as you increase the 70 in the above to 80, and then 90, and so on. Later in data collection, you could type **wft dssa(1,270,16)** once you have 270 FIDs collected. The number of FIDs collected is continuously updated in the status line in the centre of the bottom line of the VnmrJ screen [2]. Alternately you can monitor your experiment in more detail in the Acquisition Status window [3], which is activated by clicking on the small triangle to the left of the status line [4].

When the data acquisition is complete, the software automatically processes it and displays a spectrum (**Page 6**). The automatic processing is very good. Red is positive and blue is negative intensity.* Negative intensity can be imagined to be upside down peaks.... Negative crosspeaks with a positive diagonal are expected for small molecules. The contours button [1] gives options for manipulating the display. Useful features are the possibility of increasing the vertical scale by 20% (making peaks 20% stronger [2], or decreasing the vertical scale by 20% (making peaks 20% weaker) [3].

*If you had the Z-filter activated, then the spectrum you get will be 'inverted' (negative diagonal and positive crosspeaks). Type rp=rp+180 to flip your spectrum[1] and click 'Display Spectrum'[1] to view the result, now with the 'official' positive diagonal and negative crosspeaks.

Manual processing

In Process>Weighting [1] (**Page 7**), click on 'FT 1st Increment' [2]. This Fourier transforms the first of you stack of 1Ds, the one for which d2 is zero and signal intensity is strongest. Click on Interactive weighting [3]. Adjust the Gaussian to your liking by clicking inside the middle panel of the display [4]. The mid-point of the Gaussian will move to where you clicked. Alternately, in the column of options for F2 processing [5], type in a number for the length of the Gaussian, or an expression such as 0.6*at [6] . Then click Transform F2 [7]. This will now apply your chosen weighting to all the 1Ds and Fourier transform them all.

The interferogram appears (**Page 8**). Just as for 1D spectra, you can use the 2D cursors to zoom in on the time points that have spectra associated with them (d2 values actually used). Place the left cursor at the bottom left using the left mouse button [1]. Then use the right mouse button to place the opposite corner of a box around the area containing data [2]. Click the magnifying glass action button to the right of the graphical display window to zoom in [3].

---

[1] If you like numerical economy and want to keep the right phase (rp) between -180° and +180°, you can first type **rp?** to find out what rp is currently. If it is already negative, execute the flip as above with **rp=rp+180**. However if rp is currently positive you can execute the flip with **rp=rp-180** (flipping in the opposite direction) and keeping the value of rp small.

On **Page 9** you can see the alternation of red and blue along the horizontal streaks . Each horizontal line is the magnetization that ends up at the chemical shift labelling the vertical axis at the chemical shift point where the line begins. You see that the amplitude of the magnetization detected at that chemical shift alternates between positive (red) and negative (blue) depending on how long a time d2 was allowed for the corresponding 1D spectrum (increment). The X-axis of this plot is duration of d2. Moreover the frequency characterizing the oscillations is different for different horizontal streaks (different detected chemical shifts).

Place the cursor on one of the streaks, at a chemical shift that is a signal of interest (not a contaminant). Click on 'Interactive Weighting' [1].

When the three-panel display initially appears (**page 10**), you may see nothing in the bottom panel that is supposed to contain a FID [1]. Click in that panel near the left-hand edge with the middle mouse, to enlarge the vertical scale. You see that the Gaussian is longer than the 'FID', because the software anticipates that you will double the 'FID'. You can just accept the default choice of Gaussian, which is usually very good. Alternately you could use the mouse (left button), clicking in the middle panel to shorten the Gaussian to match the duration of the 'FID'. Recall that this is not an actual FID, this is the amplitude of a detected peak, as a function of d2, an interferrogram. If you set the Gaussian to fit the observed interferrogram (**Page 11**), then you will need to double it (or triple it) if you double (or tripple) your data set size in F1.

Go to Process>More 2D (**Page 12**) and look at the F1 linear prediction. We collected 200 increments, so the values provided, that will add 200 new points [1], will double our data in F1. If you set the Gaussian to match the interferrogram in the step above, now type gf=2*gf. Doubling our interferrogram is sufficient in this case, because the actual data have already decayed to1/3 their starting strength by the use of 200 d2 values. If you don't remember the significance or setting of the linear prediction parameters, refer to the demonstration on processing 2D-TOCSY data.

Now click on 'Full 2D transform' [2].

Page 13 shows that the spectrum is displayed with too large a vertical scale (streaks normally below the display threshold are obvious), but also not perfectly phased, as there is more red on one side of the diagonal and more blue on the other [1].

Currently 'trace' is F1, we see the F1 label on the X-axis [2]. This means that any traces we select out of this will be spectra vs. F1 (indirectly detected chemical shift of source ^{1}Hs).

Go to Process>Display (**Page 14**). Place the cursor across a diagonal peak that is in the top right of the spectrum [1]. Under 1D spectrum click the Display ID# button [2].

The 1D slice that appears can be manipulated just as a 1D spectrum (**Page 15**). Adjust the right phase (rp) ONLY: just select phasing and place the mouse just ONCE,

adjusting the phase of the strong positive diagonal signal you have selected with the criterion that the baselines on either side of it should be horizontal and be level with one-another at the location of the peak [2]. Then Click 'Display Spectrum' [3].

The 2D looks more balanced now with respect to symmetry of the red smears to either side of the diagonal (**Page 16**, [1]). (There is still vertical smearing, but since this is in F2, we cannot fix it just now, see later.) Now put the cursor on a bottom-left diagonal peak [2] . Click on Display 1D # again [3].

On the 1D that appears activate phasing [1] (**Page 17**). Click once in the right side of the spectrum but MAKE NO ADJUSTMENT to the phase there (the right phase was already adjusted on the first diagonal peak, above). Move the cursor to the left edge of the spectrum where your current strong positive diagonal peak is. Activate the cursor again and here adjust the left phase [2] (lp, along with compensations in rp). Now click on 'Display Spectrum' [3]. The resulting 2D is much better balanced in the horizontal direction (**Page 18**).

To correct the phasing in F2 you need to swap the presentations of F2 and F1 in our display, by setting the trace to be F2 (horizontal F2) [1] in **page 18** to produce the result in **page 19**. Now the remaining imbalance in the red diagonal is horizontal, where we can deal with it exactly as above (pages 14-18) The result is shown in **Page 20**.

Finally, you can clean up the baseline streaks in F2 by clicking 'BC F2' under Baseline Correct 2D [1] of **Page 20**. The result is shown on **Page 21** where only the F1 streaks remain.

The F1 streaks are substantially the result of truncation and first point issues, rather than baseline problems. Moreover they are actually quite small. We see them in these figures primarily because the figures use over-large vertical scale to make it easy to see phase imperfections. Decrease the 2D vertical scale so as to focus on the peaks and crosspeaks. Do this either by clicking with the middle mouse on a point you would like to be barely visible (eg. the edge of a peak) or manually decrease the vertical scale by a factor of two by typing **vs2d=vs2d/2**. To implement this change, click on 'Display Spectrum' [1].

For output, we don't have plot previews, or a plotter yet, so use the screenshot. In the Linux bar, at the left-hand edge under Applications, select 'Accessories' (top option) and then 'Take screenshot' (third from the bottom).

Page 22 shows that indeed, the spectrum is very nice.

Plotting to Files

For plots to paper, T2J allows the standard automatic plot button to send your spectrum to the networked plotter. However for figures you can use in papers,

presentations and grant applications, go to the 2D tab on the left-hand side [2] and open the 'Print Screen' [3] (**Page 22**).

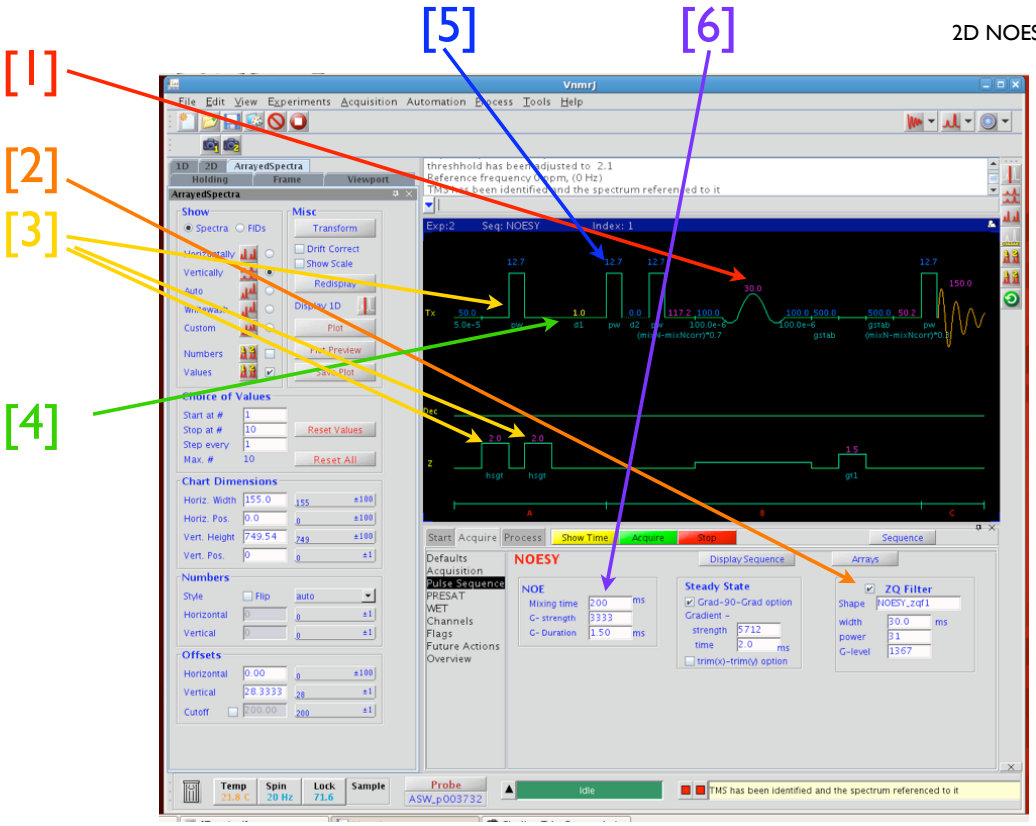
This allows you to choose a 'File' instead of a printer (**Page 23**). After choosing your desired file format [2] and specifying color [3] and the desired resolution, orientation and size, click save [4] .

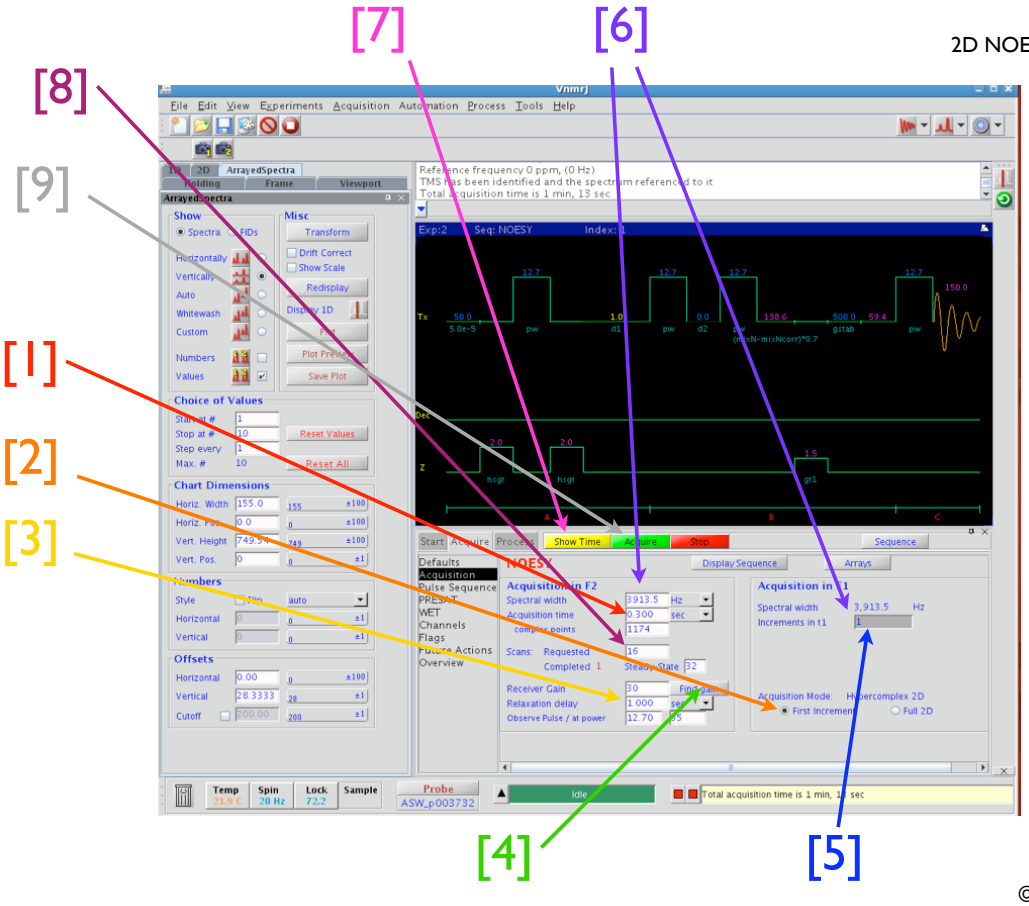
For a colour contour plot that you will include in printed documents, you may want to change the background colour to white, and the frame to black, to do this and more (*with great caution !!*), go to the menu at the top of the screen called 'Edit' [5] and draw down to select 'Display Options.....'

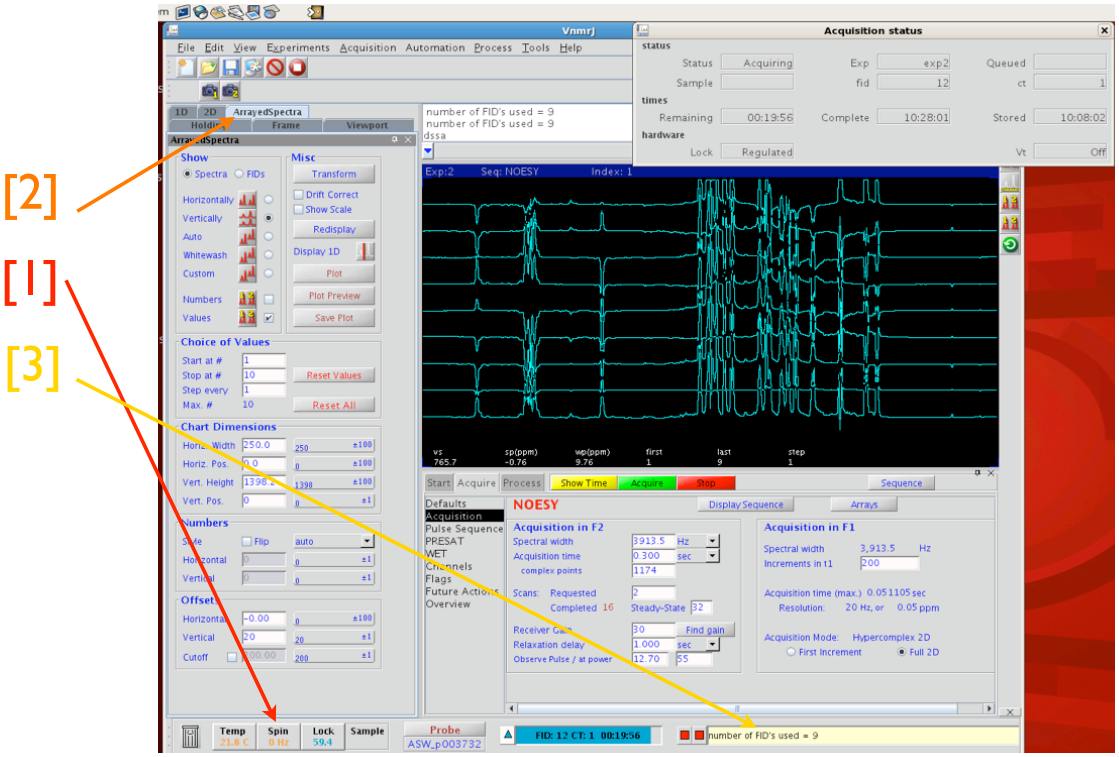
As shown on **page 24**, choose 'Display Colors' [1] and make changes you want, making sure to keep notes of what you changed and being sure to change these all back to their initial settings before logging off !!! What I changed was Background [2] (from black to white), Scale [3] (from white to black) and Cursor [4] (from red to white). In each case, click on the little box showing the current colour, to reveal a whole palette of choices. Take note of the current setting so that you can return to it before logging off !! (repeat).

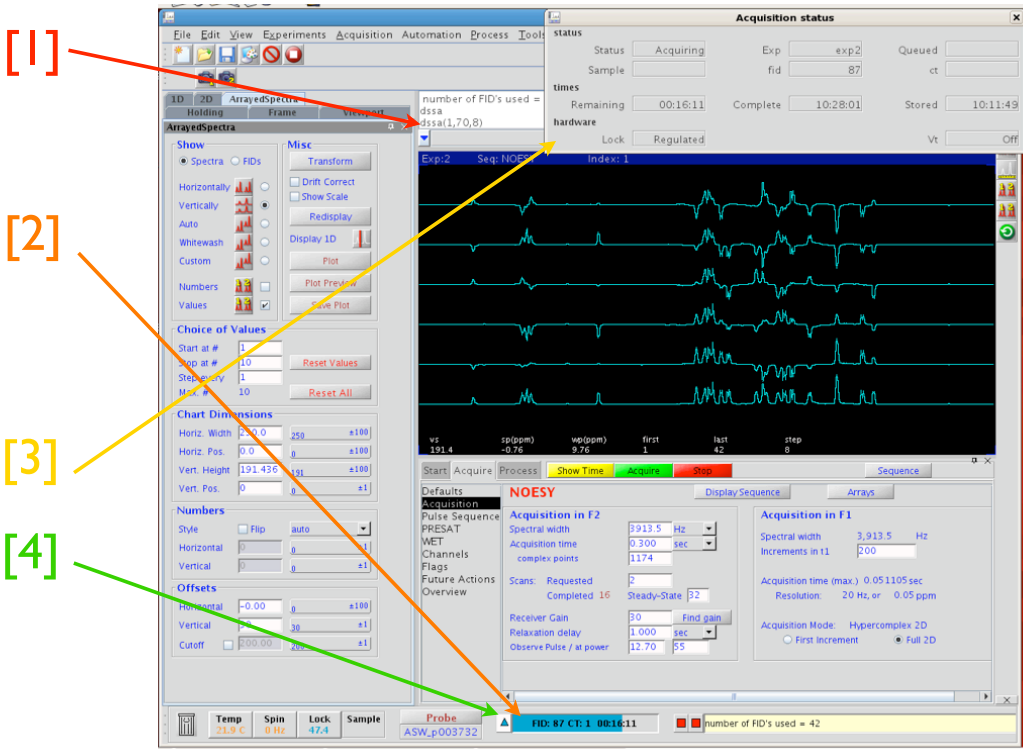
.

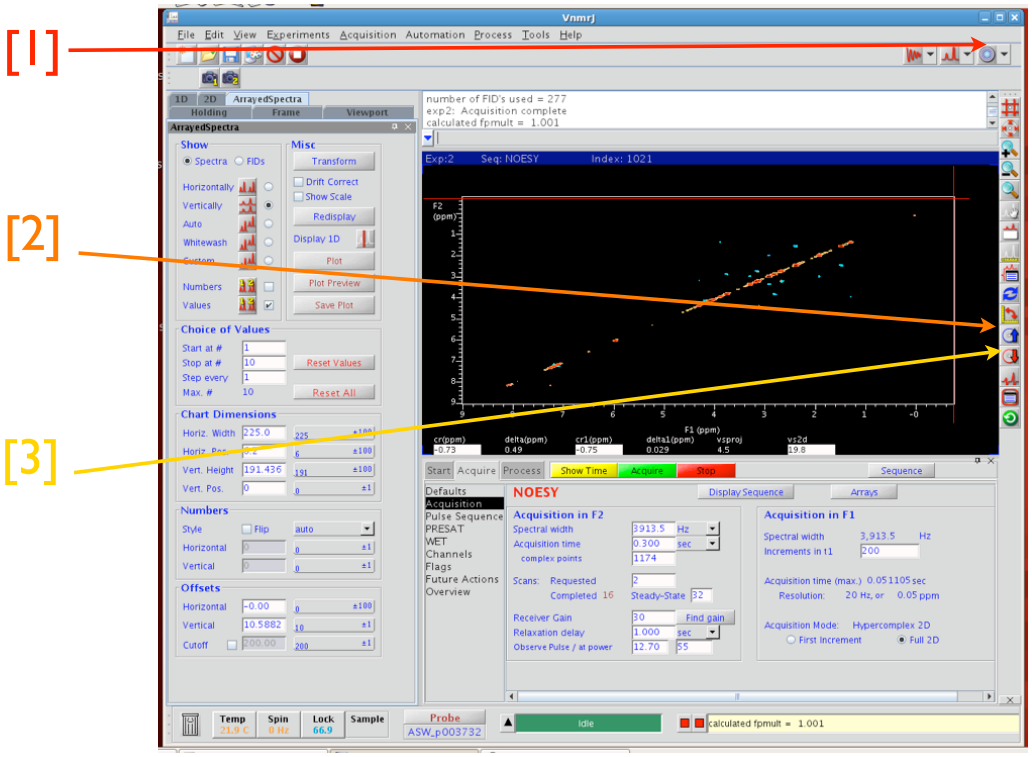
2D NOESY

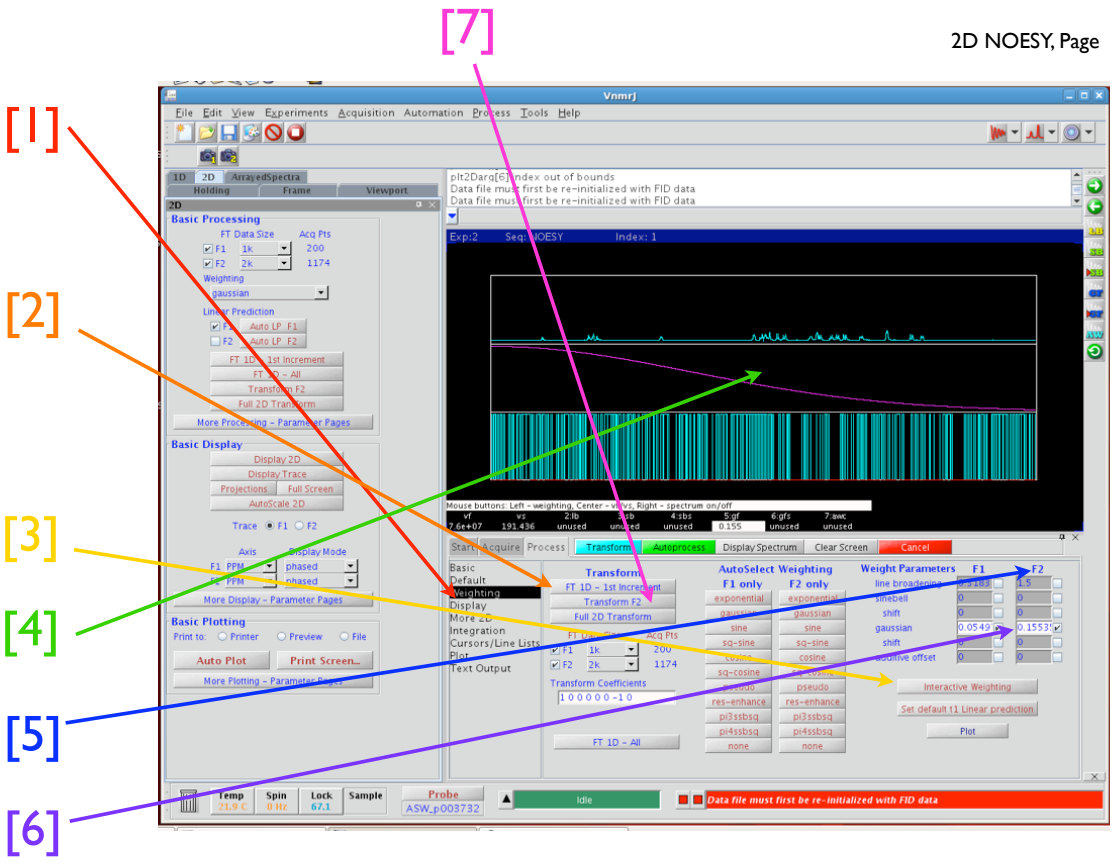


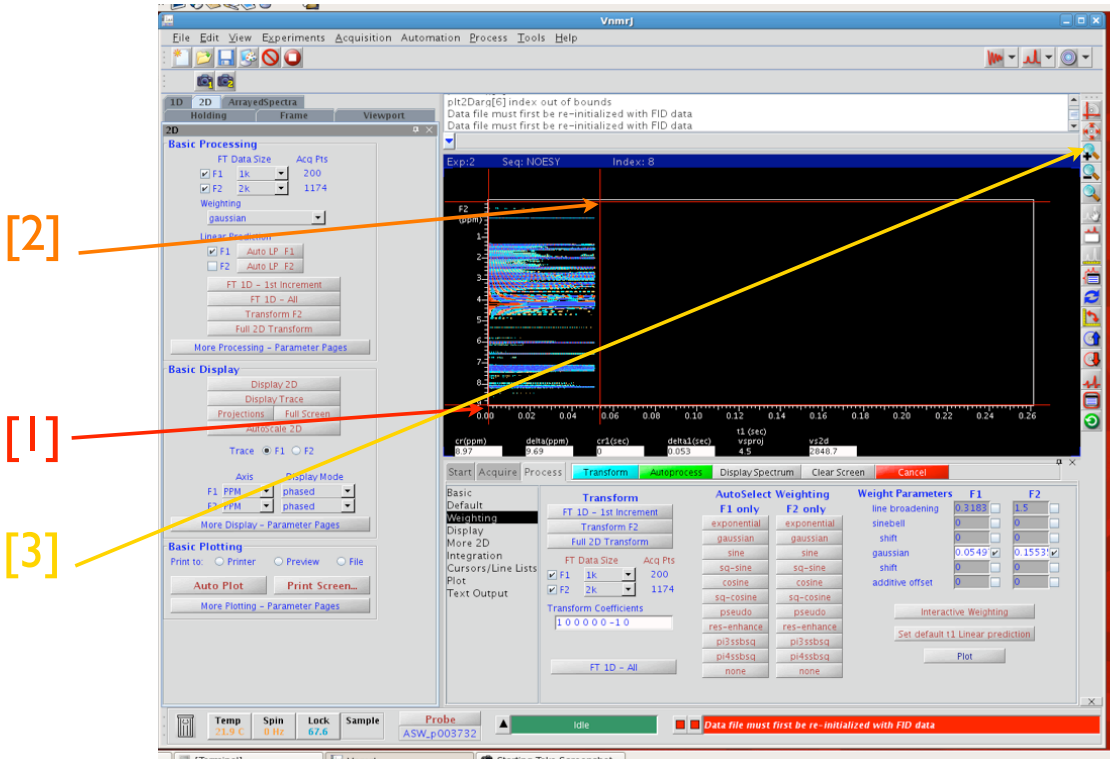


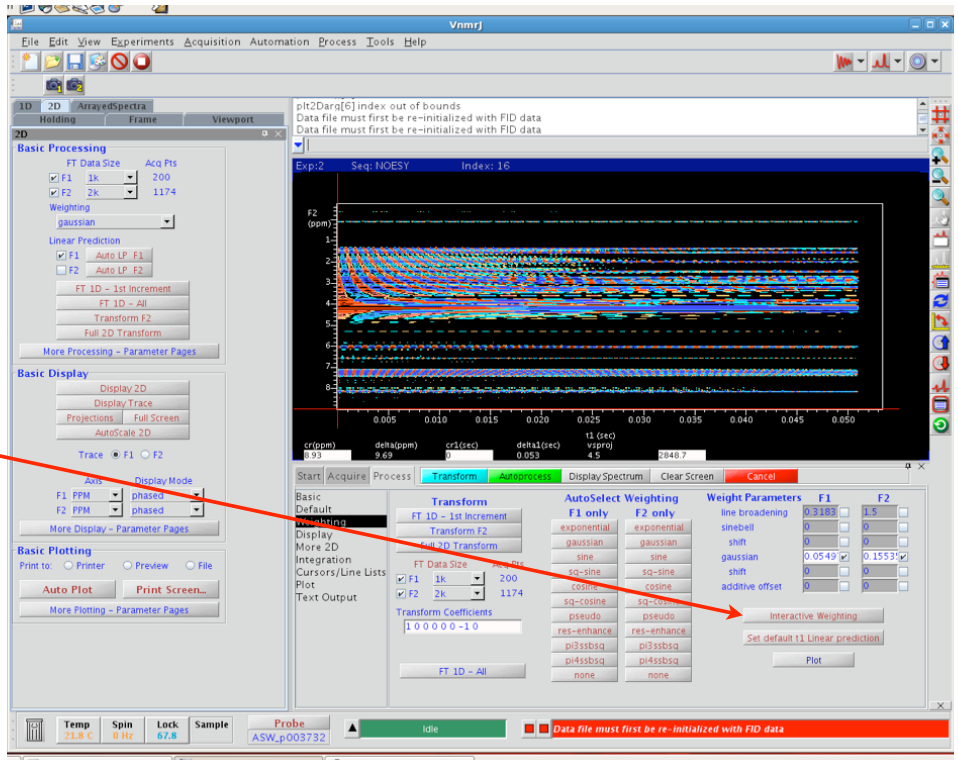


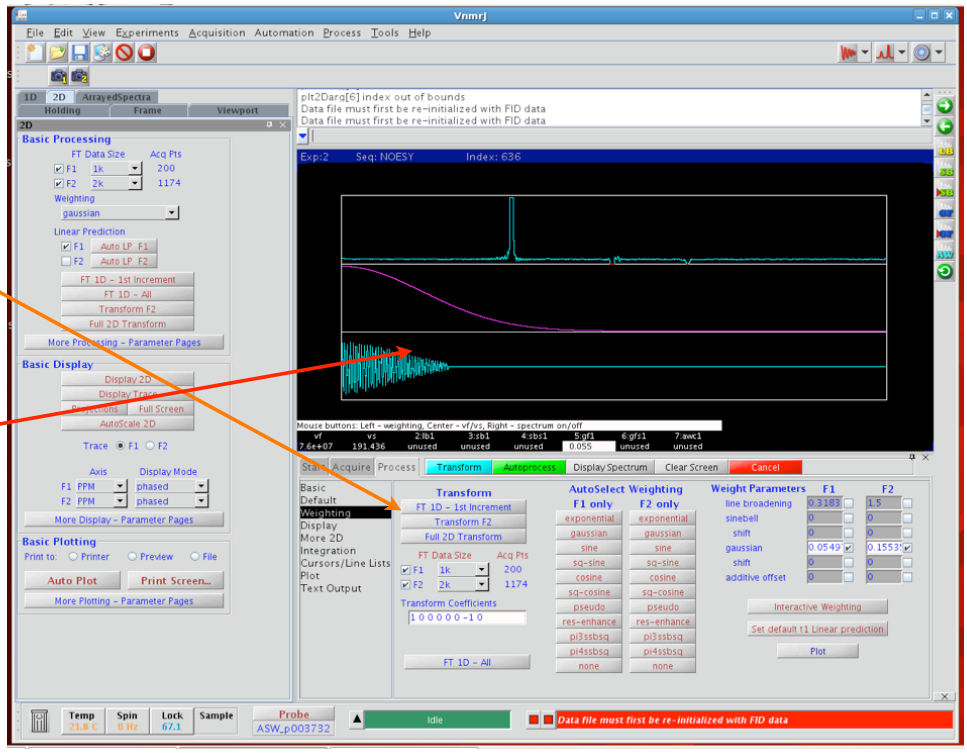


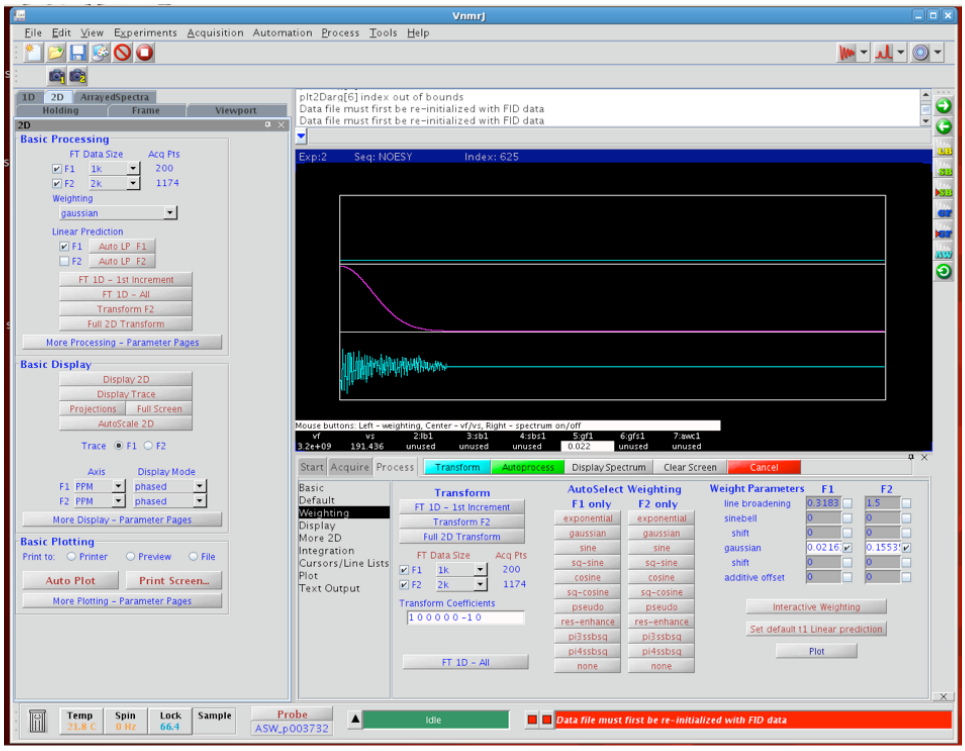


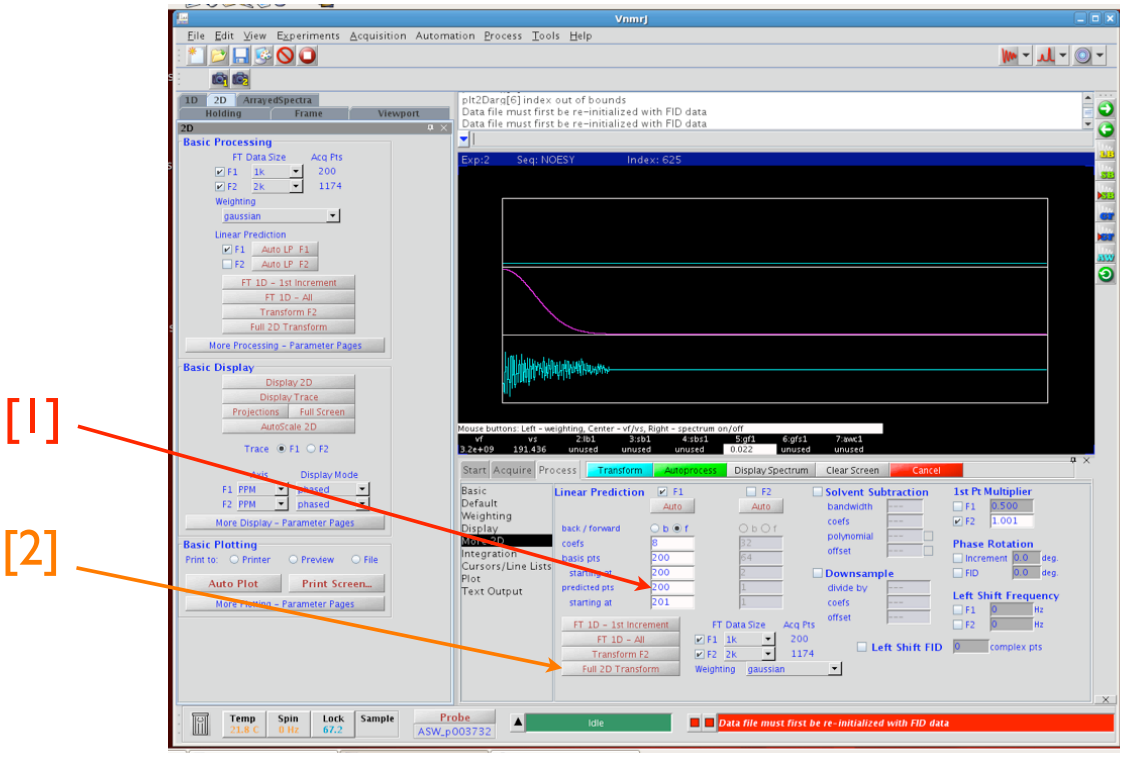


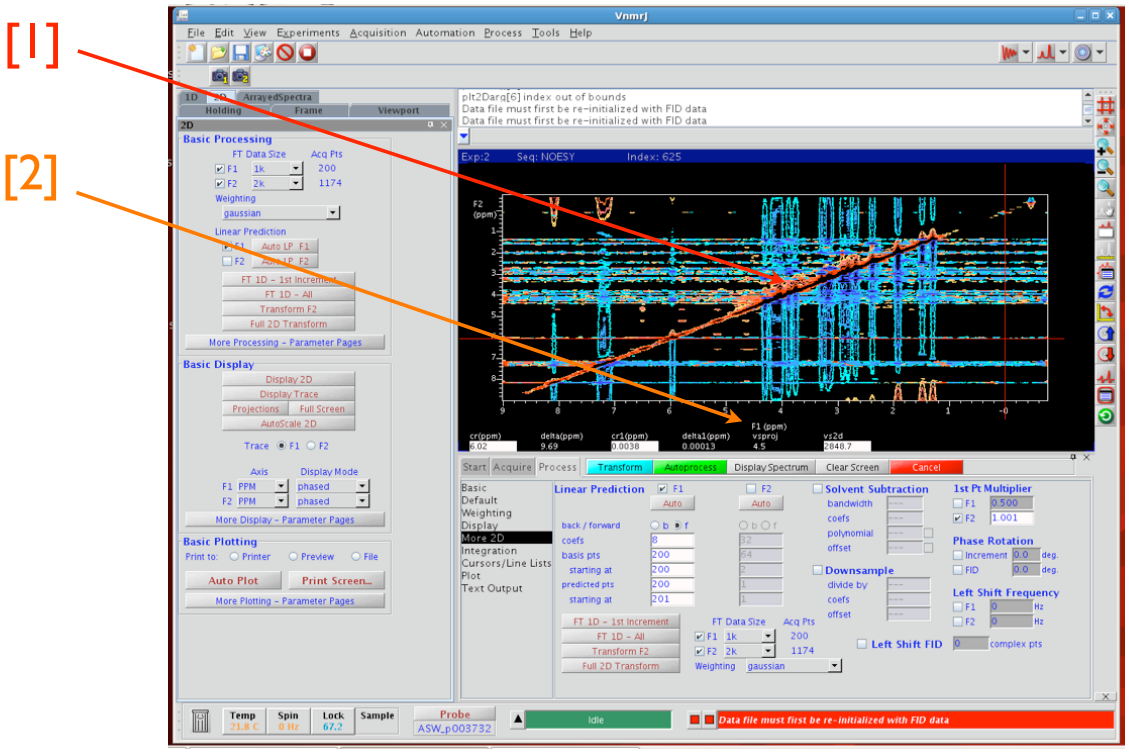


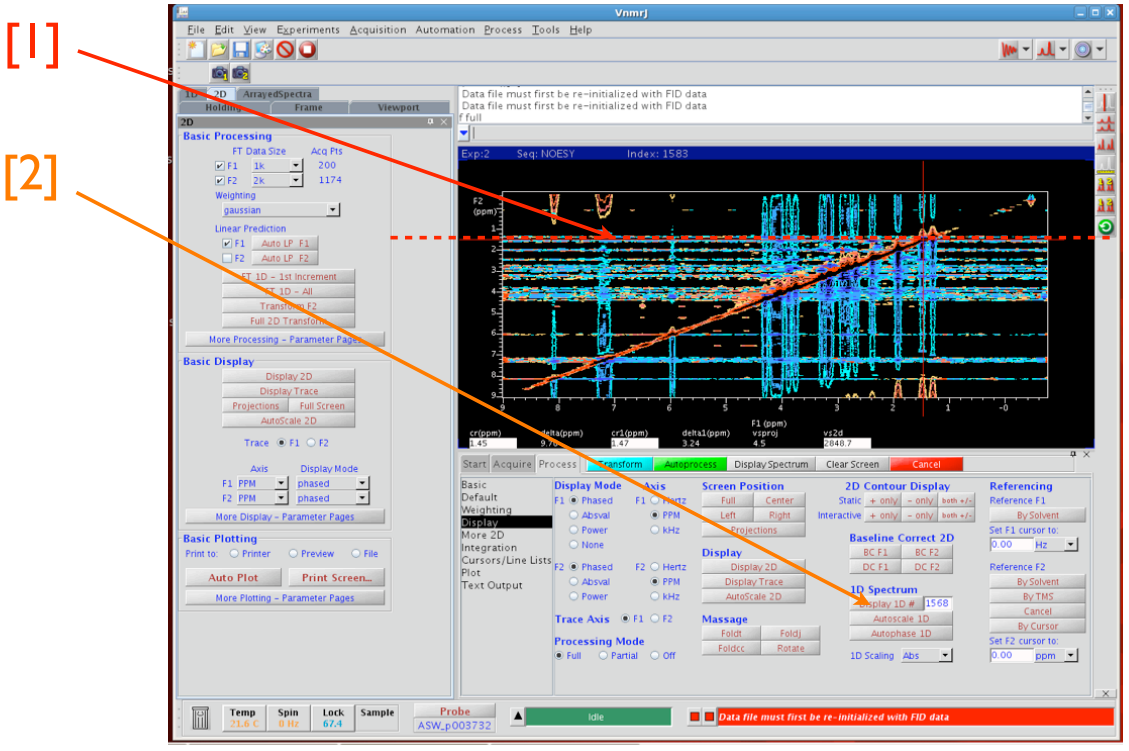


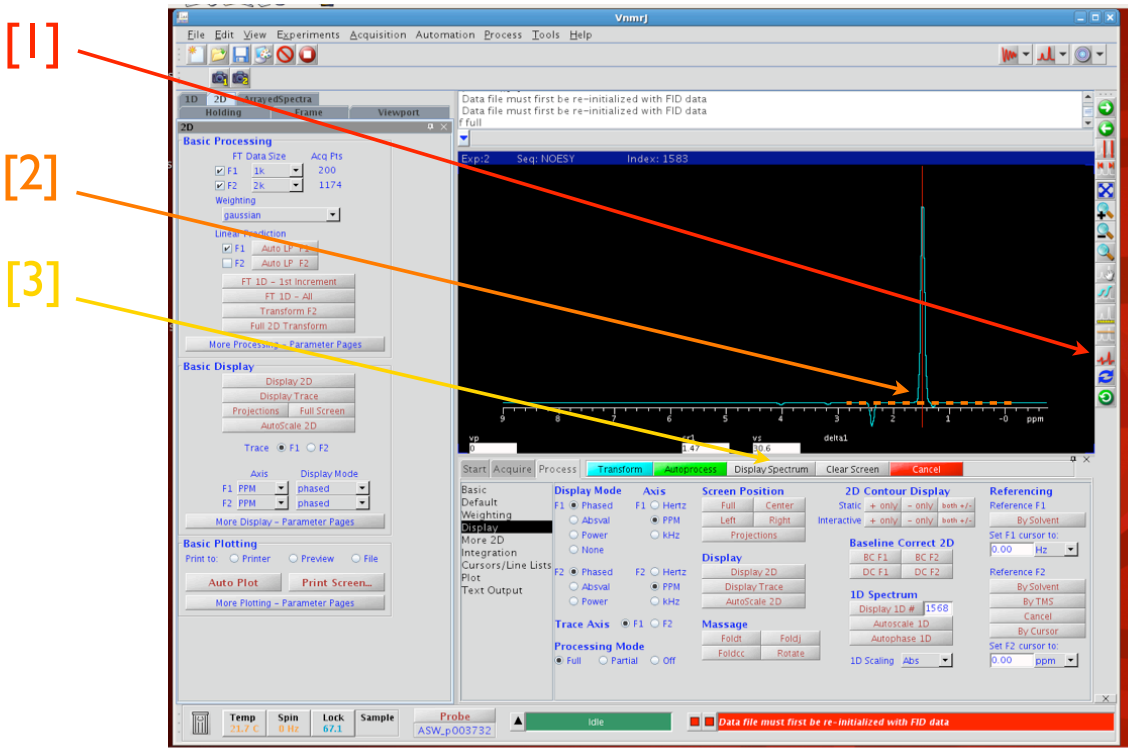


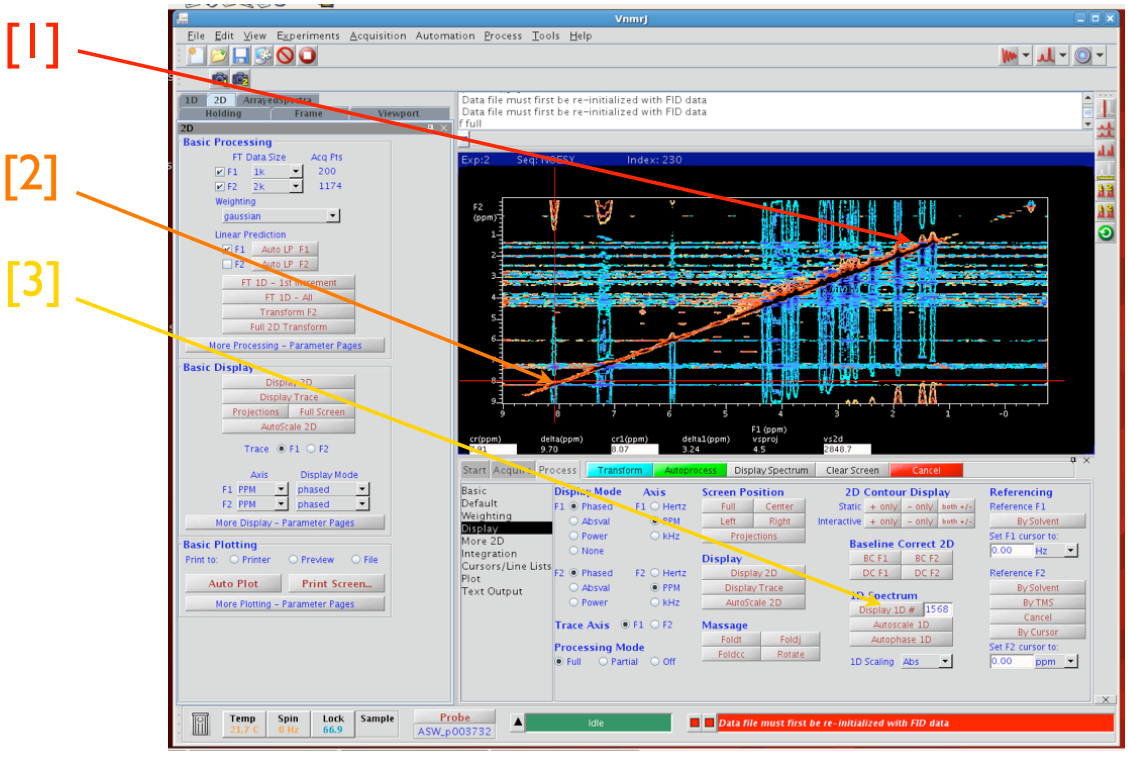


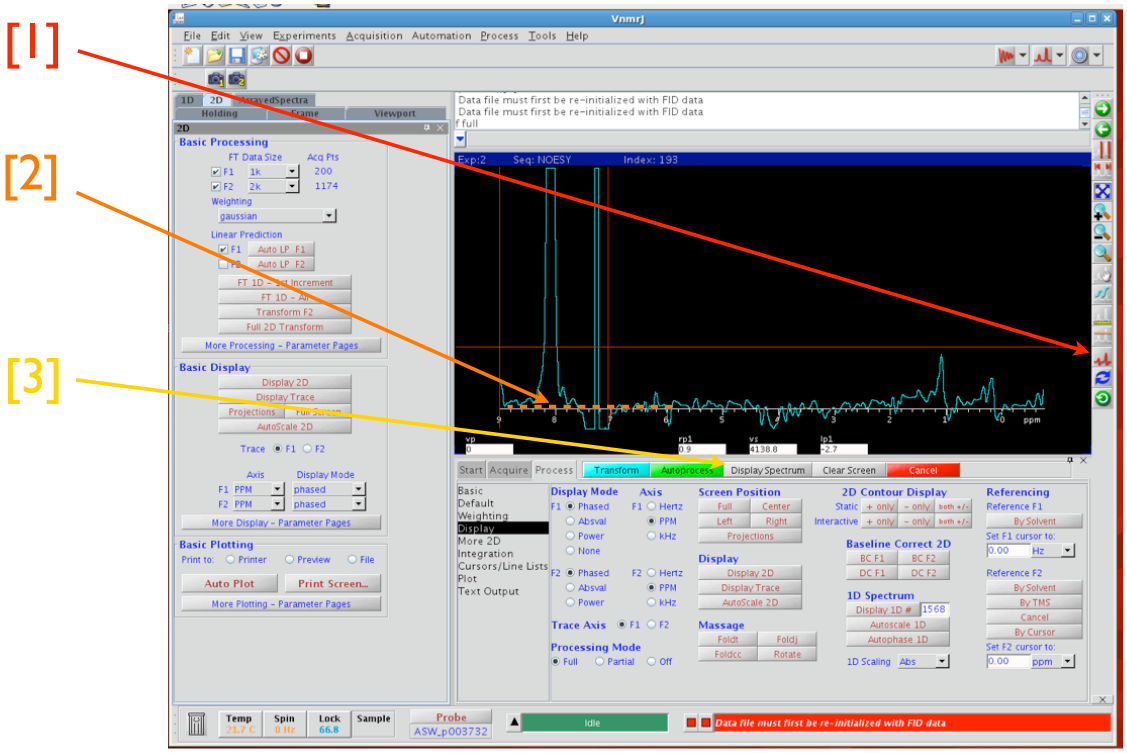


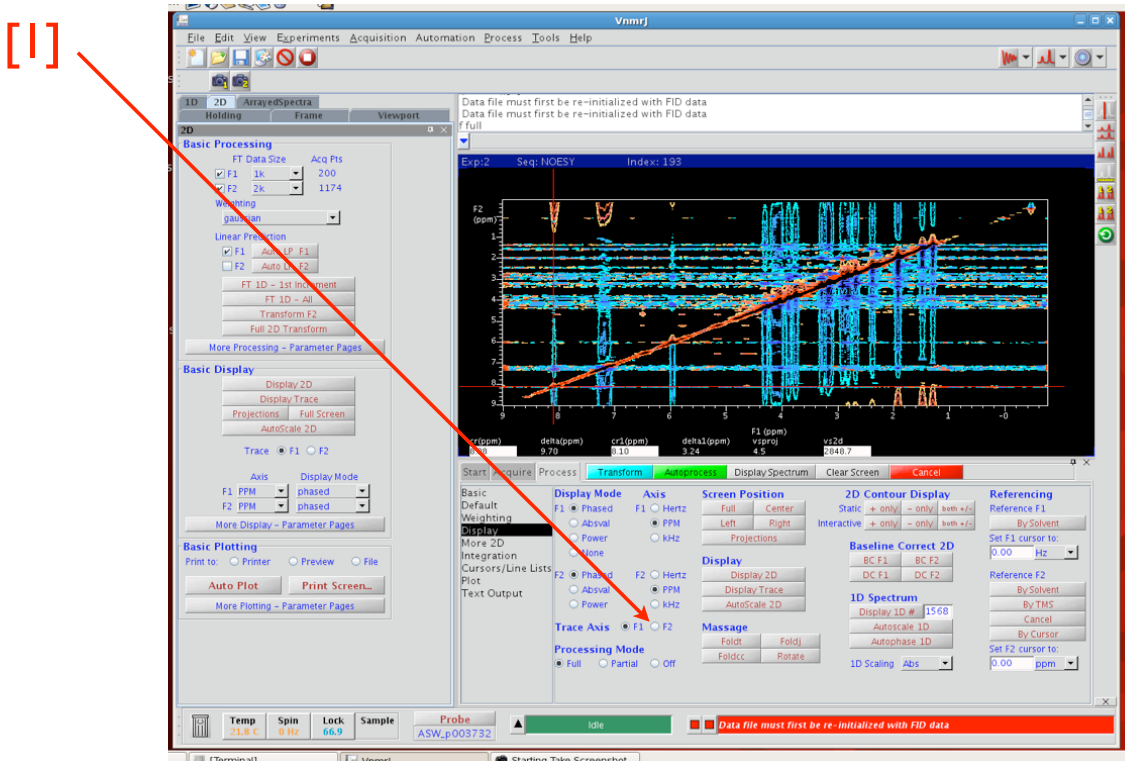




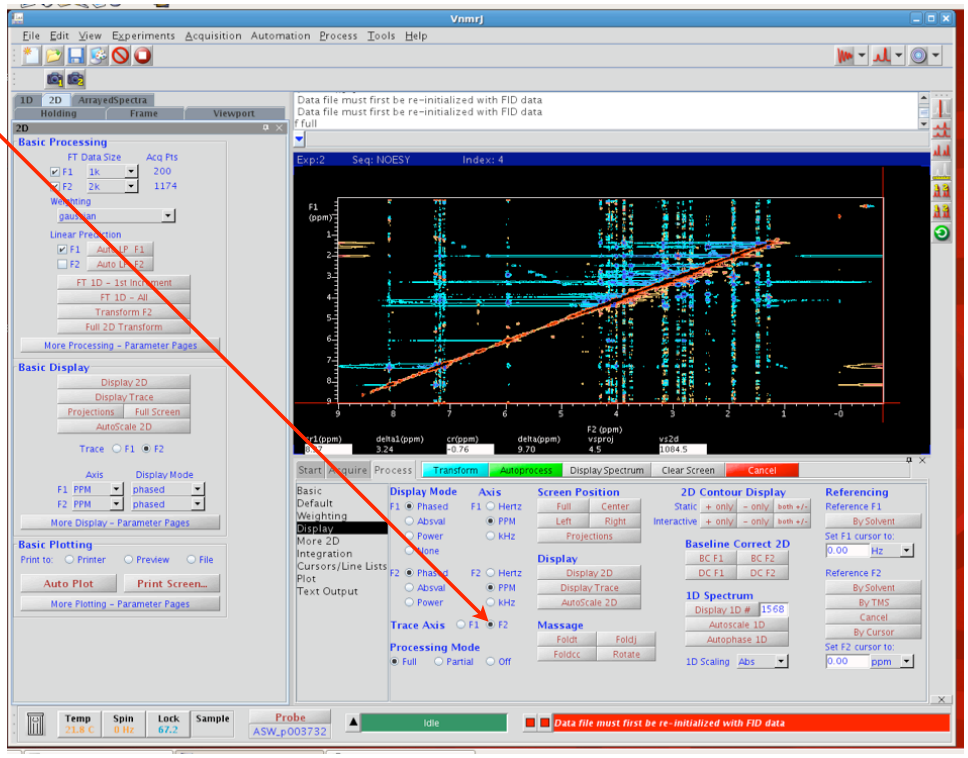








[1]



[1]