

## Practicum 7

## 2D TOCSY

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Typed commands are in bold, parameters are underlined.

### 2D TOCSY

Take your beautiful 1d, phase it perfectly and then calculate a pre-acquisition delay that will make left phase correction (lp) unnecessary. To do this, type 'setlp0' (numeral 0, not letter O). The **setlp0** macro uses your current lp, rof2 and alfa values to calculate new rof2 and alfa values that will make lp zero. rof2 and alfa are two very short delays after the last pulse that precede data acquisition.

Now collect a new 1d to confirm that the best value of lp really is approximately 0. Note the current values of rof2 and alfa that achieve this.

Under Experiments choose 'Convert current parameters to do .... > Homonuclear Correlation Experiments > TOCSY

In Acquire>Pulse Sequence we see the pulse sequence for this simple TOCSY (**Figure 2 (=page 2)**).

In this experiment, the initial pre-sequence of {gradient-pw90-gradient} serves to crush transverse magnetization that remains from the previous scan, take all longitudinal relaxation and tip it into the XY plane and then crush that too [1]. The result is zero net magnetization at the start of every scan, regardless of what scan had preceded it and also without requiring delays of  $3 \cdot T_1$  between scans.

Next we see the expected relaxation delay d1 during which magnetization recovers to Z [2]. Then the pulse sequence begins in earnest with a pw90 [3] to bring magnetization into the XY plane where it is allowed to precess for an interval d2 [4]. d2 will be varied stepwise in order to make the 'indirect' dimension of this 2d spectrum and reveal what the chemical shifts are of the 'source protons'. We use a 'trim' pulse [5] to select one component of the magnetization and then this component is subjected to a 'spin lock' [6] which in-essence over-rides the  $B_0$  field, gives all protons essentially identical frequencies and allows protons coupled to one-another directly or indirectly to exchange magnetization.

If A is coupled to B and B is coupled to C, a short spin lock allows A and B to exchange magnetization as well as B and C.<sup>1</sup> However during long spin locks we magnetization travels from A to B to C. Thus, long spin locks allow all the spins with direct or indirect coupling connections to one-another to exchange magnetization. The duration of the spin lock is the 'mix' time [7], and it should not be allowed to exceed 0.1 sec (80 ms is very good).

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<sup>1</sup> Protons connected by direct or multiple coupling connections are said to occur within a single spin system.

The spin lock has to have a field strength [8] comparable to the frequency range over which the protons are spread, ie  $\Delta\nu$ . The field strength is expressed in Hz according to the usual conventions. It is related to a  $\text{pw90}$  because the frequency in Hz is  $1/\text{pw360}$  and  $\text{pw360} = 4 \cdot \text{pw90}$ . Thus the calculated field strength of 4422 Hz was derived from our preset  $\Delta\nu$  of 4252 (an approximate match). From this field strength the  $\text{pw360}$  corresponding is calculated to be 226  $\mu\text{s}$  ( $=1/4422 \text{ Hz}$ ), producing a  $\text{pw90}$  of 56.5 ( $=226/4$ ). 56.5 is  $4 \cdot 14.125$ , so we expect the associated power to be 12 dB lower than the power associated with a  $\text{pw90}$  of 14.125. 14.125 is just over our  $\text{pw90}$  of 13.6 (for  $\text{tpwr}=62$ ), so we can agree that it corresponds to a power of 61 dB.<sup>2</sup> Thus a  $\text{pw90}$  of 56.5 can be associated with a power of  $61-12=49$  dB.

The trim pulse [5] is a routine value, just leave it at 2 ms [9].

Under Acquire> Flags make sure that your  $\text{rof2}$  and  $\alpha$  values are those that produce  $\text{lp}\approx 0$  (see above)

In Acquire>Acquisition (**Figure 3**), notice that 2 scans are being suggested per increment [1] but 32 steady-state scans are suggested up front [2]. The 80 ms spin locks will cause slight sample heating, which needs to stabilize before we begin actually collecting data. The 32 'stead-state' scans allow time for this.

In the full TOCSY there will be 200 increments [3]. Each of these is a 1D spectrum in its own right, and each uses an increasingly longer value for  $d2$ .<sup>3</sup> This explains why it will take 17 minutes to collect the data.

To test-drive your experiment, go to Acquire>Acquisition (**Figure 4**) and select (•) First Increment [1]. This causes the experiment to only collect one increment, employing a single value of  $d2$  [2]. [Show Time] [3] now yields 43 seconds. [Acquire] [4]. IMMEDIATELY upon completion go to Start>Shim and touch up Z1, FAST. Z1 is sensitive to temperature. You have to do this before the sample cools.

The first increment 1d allows you to be sure that the receiver gain will not overload. You also see that the resulting lines are broad. This is because the default acquisition time ( $\text{at}$ ) for 2D spectra is short [5], in order to keep data sets small. However this hurts resolution.

For good data, I prefer to double  $\text{at}$ . Doubling the acquisition time costs almost nothing in terms of the duration of the experiment (it adds .15 sec per 1.1 sec scan,

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<sup>2</sup> 1.12 is the sixth root of 2. Since 6 dB corresponds to a factor of 2, 1 dB corresponds to a factor of 1.12

<sup>3</sup> Thus magnetization will have evolved a bit longer before the X-component ( $\alpha \cos(\omega d2)$ ) is collected and input into the spin lock. Because the  $\cos$  will not tell us whether  $\omega$  is positive or negative, we also collect an analogous series of 1ds where the  $\sin(\omega d2)$  is collected by using a trim pulse that is on the Y axis (instead of the X-axis) (This assumes that magnetization began along the X-axis at  $d2=0$ ).

or  $\approx 10\%$ ), but it gives you one higher-resolution dimension, the directly-detected dimension. Doubling the indirect dimension would double the duration of the experiment because you would need to collect more increments each of which is a 1d spectrum, to get more acquisition points in that dimension. In **Figure 5**, when you now re-activate (•) Full 2D [1], you may find that Varian has chosen a new shorter value for the number of increments (eg. 128 [2]) for a 4252 Hz-wide spectrum this will mean 33 Hz per point [3] (not good resolution). Consistent with the short indirect Acquisition time of .030 sec., show time yields 13 minutes. Choose 200 increments in t1 and now show time yields 19 minutes, but we will get 21 Hz resolution in the indirect dimension (which we can improve upon using linear prediction).<sup>4</sup>

Upon clicking 'acquire' data collection will begin. The spinner will turn off, in order to prevent production of side-stripes due to spinning irregularities.

It is fun to see the individual 1Ds as they are collected. \*\*In Acquire>Future actions, try typing wft dssa in the line next to 'when block finishes'.\*\*

You can also see the incoming 1Ds in the display panel on the left-hand side, under arrayed spectra. (**Figure 6**). Choose the highest-number increment you'd like to see, and a step size that is an even number and roughly one tenth of the total number of spectra you have (in order to obtain 10 spectra on the screen). Then click on the button to get a vertical stack [3].

Now adjust the vertical height of the spectra [4] and the vertical position of the lowest one [5] to get them all on scale and non-overlapping. The bottom spectrum is the one collected with no chemical shift evolution between the excitation pulse and the beginning of the trim/spin-lock. This one is indeed an ordinary-looking 1H 1D. Looking up at the spectra above it, collected with increasingly long delays d2, see that signals are present always in the same place with respect to the horizontal axis: signals do not move around. Second, see that individual signals (eg [6] and [7]) oscillate with respect to their amplitude. They all start positive, but then they shrink, invert, grow negative and then shrink again from the negative and grow back to the positive. The frequency characterizing the amplitude oscillation for each peak can actually be quite complex. However to first-order, the peaks far from the center of the spectrum oscillate faster than the peaks in the centre. The frequency of the oscillation constitutes a second frequency for each peak, with the 'first' frequency being its chemical shift, evident on the horizontal axis of the display.

When the experiment finishes, the data set is automatically processed, and WOW the results are really very good (**Figure 7**). You can see a diagonal line of resonances from bottom left to top right. Each of these diagonal peaks occurs at a directly observed chemical shift exactly corresponding to that H's chemical shift in an ordinary 1H-1d. For reasons best known to Varian, the directly-observed

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<sup>4</sup> You can see that the number of increments

dimension is now the vertical axis. These are the chemical shifts of detected magnetization, that were on the horizontal axis in Figure 6. At each of these frequencies however, you can look along the 'row' of the spectrum (or ask the software to display a trace). Each of the dots you see also occurs at the chemical shift of a resonance in your simple 1D. Whenever you see a spot, you can conclude that the proton with the X-axis chemical shift is in the same spin system as the proton with the Y-chemical shift. Dots that are not on the diagonal are called 'cross peaks'. \*

If you request a 'trace' display, and move your cursor to a vertical position corresponding to a resonance, you will see what looks like a partial NMR spectrum. It is an NMR spectrum of the spin system of the proton you chose on the vertical axis. It gives you a 'list' of the protons that were the sources of the magnetization you finally detected on the chosen protons. These are called subspectra.

The default processing employs weighting as well as zero-filling (**Figure 8**, Process>Weighting). For weighting, processing defaults are a Gaussian which decays to half-height 60% of the way through your FID [1] ( $gf=0.6*at$ ) [2]. The indirect dimension is handled in an exactly analogous way [3], to be discussed in more detail below.  $gf1=0.6*at1 = 0.6*(ni-1)/sw1$ .

Zero-filling corresponds to making the FID longer than the number of data points collected by adding a string of zeros. This is because the Fourier transformation operation requires that the number of numbers used as input be a power of 2 (eg. 512 or 1024 ...). The Fourier transform then produces an output of the same size (eg. 512 numbers long). We know we will get the best resolution from a long FID (large number of data points), up to a point. However the mathematical operations slow down if too many data points are used. As a compromise, we at least double our data size, and usually quadruple it. Thus, with 1276 data points as input, we should select 4k as the Fourier number [4]. For our 200 points in the indirect dimension, we will choose at least 512, but more likely 1k as the Fourier transform size [5].

### **Custom-processing**

The processing steps provide you with A LOT of control over the final appearance and utility of the spectrum. If you had a small number of increments, to save time, you can recover a surprising amount of resolution by applying linear prediction, provided your data are good. If your data are not perfect, or you want to increase the emphasis on some features of the data (at the expense of others), you may want to manipulate the weighting.

We will do this in two phases, one that concentrates on the directly-detected FIDs, and the second of which focusses on the amplitude oscillations we saw resulting from evolution of magnetization during d2.

### **Direct dimension, F2**

The direct dimension is called the 'F2' dimension, because it is collected during the second detection interval, at. (The first detection interval is d2, and the corresponding dimension is called F1.)

Click on [FT 1D - 1st increment] and adjust the phase of the resulting 1d.

For speed, you may set the size of the F2 Fourier transform [4] a little small (eg. 2k) and then click [transform F2] [7]. This transforms all the increments as a series of 1Ds, and applies the same phase to all of them.

A new '2D' is made, called an interferogram (**Figure 9**). To bring the vertical scale to the point where you can see something, click in the box with the middle mouse. Time is the X-axis and there are a whole book of 1Ds all on their ends. The time in question is the duration of d2. Thus, each of the 1Ds we collected is standing on its end, at a horizontal position corresponding to the length of d2 delay used in the collection of that 1D.

In Process>Display, select Trace axis as F1 to get a stack of 1D spectra with time as the horizontal axis. Place the cursor on a strong resonance [2] (chemical shift is on vertical axis).

Click on 'Display Trace' [3] and then go to Process>Weighting (**Figure 10**). Varian will apply default weighting of various sorts, if you click on the corresponding button, [3] for example.

In Process>Weighting (**Figure 10**), click on 'Interactive Weighting. In the lower of the three panels, you are now looking at the amplitude of the peak you selected as a function of d2. You can see the peak's amplitude oscillation and that it decays slightly, like an FID. If we collected a larger number of increments, the decay would have been more obvious. Just like an FID, we can apply window functions and Fourier transformation to extract the frequencies of the oscillations present. This is valuable because it is evident that we do not have a single pure frequency governing this oscillation (look at the TMS peak though).

Normally, the weighting function is used to bring the FID to zero at its end, however this Gaussian has not dropped to zero at the end of the data. The long Gaussian reflects the fact that we will use linear prediction to double the length of this 'indirect FID'. You can get Varian to take care of linear prediction for you by clicking 'Set Default Linear Prediction' [2].

To control the linear prediction, go to Process>More 2D (**Figure 11**) Linear prediction is being used only for the indirect (F1) dimension [1], and it is being used to double the duration of the indirect FID ('forward' direction in time [2]) from 200 points (acquired) by adding an additional 200 points [3](calculated). The calculation is assuming some 8 signals per row [4] and is going to simulate those based on all 200 acquired data points (point number 200 [5] and previous). The 200 calculated points will begin at position 201 [6], constituting points 201-400 of the new and extended F1 FID.

Use of these parameters will cause the FID to be twice as long as was shown in the interactive weighting window (**Figure 10**). With the doubling in mind, you can see that the Gaussian suggested is in fact just fine. If you are concerned, you can always use the interactive weighting features to tailor the window function to the actual data FID that you see. Then you can account for the doubling produced by the linear prediction by doubling the parameters of your Gaussian. To do this type **gf1=2\*gf1**. Note that the parameter **gf** is used for the direct dimension but **gf1** plays exactly the same role for the F1 dimension. Similarly, a shifted Gaussian in the F1 dimension would be implemented with the parameter **gfs1**. Which you could also adjust based on the visible 'indirect FID' and then double mathematically to account for the linear prediction.

With your weighting functions and linear prediction set, go to Process>More 2D (**Figure 12**). Click Full 2D Transform [1]. This figure has a too-large vertical scale left over from our large value used to view the interferogram. Place the cursors just off-centre on one of the cross peaks (a peak that is not a diagonal), and click using the middle mouse button. This will cause the intensity at that point to act as a threshold for visibility, with adjustments of vertical scale and threshold. Alternately, use the pull-downs under the contour button to decrease vertical scale by  $\pm 20\%$  [2], to obtain **Figure 13**.

**\*\*If phase correction is required, \*\***

#### Damage control:

Sometimes you may have unattractive horizontal streaks extending from the cross-peaks and diagonal peaks. These can be ameliorated by setting the first-point-multiplier, **fpmult**. This is a value that will be multiplied with the first point of each FID prior to Fourier transformation. Under Processing> More 2D (**Figure 14**), look at the first-point multiplier, which is identified in the panel as 'F2' [1].<sup>5</sup> To adjust the F2 value (**fpmult**) click on FT 1D - 1st Increment [2] and then type **dc**. Place the pointer at the right-edge of the corrected 1d to mark it [3] and then remove the dc correction with **cdc**. If the new spectrum is below the drift-corrected one, increase the F2 first-point multiplier. If the spectrum is too high, decrease the F2 first-point multiplier.<sup>6</sup>

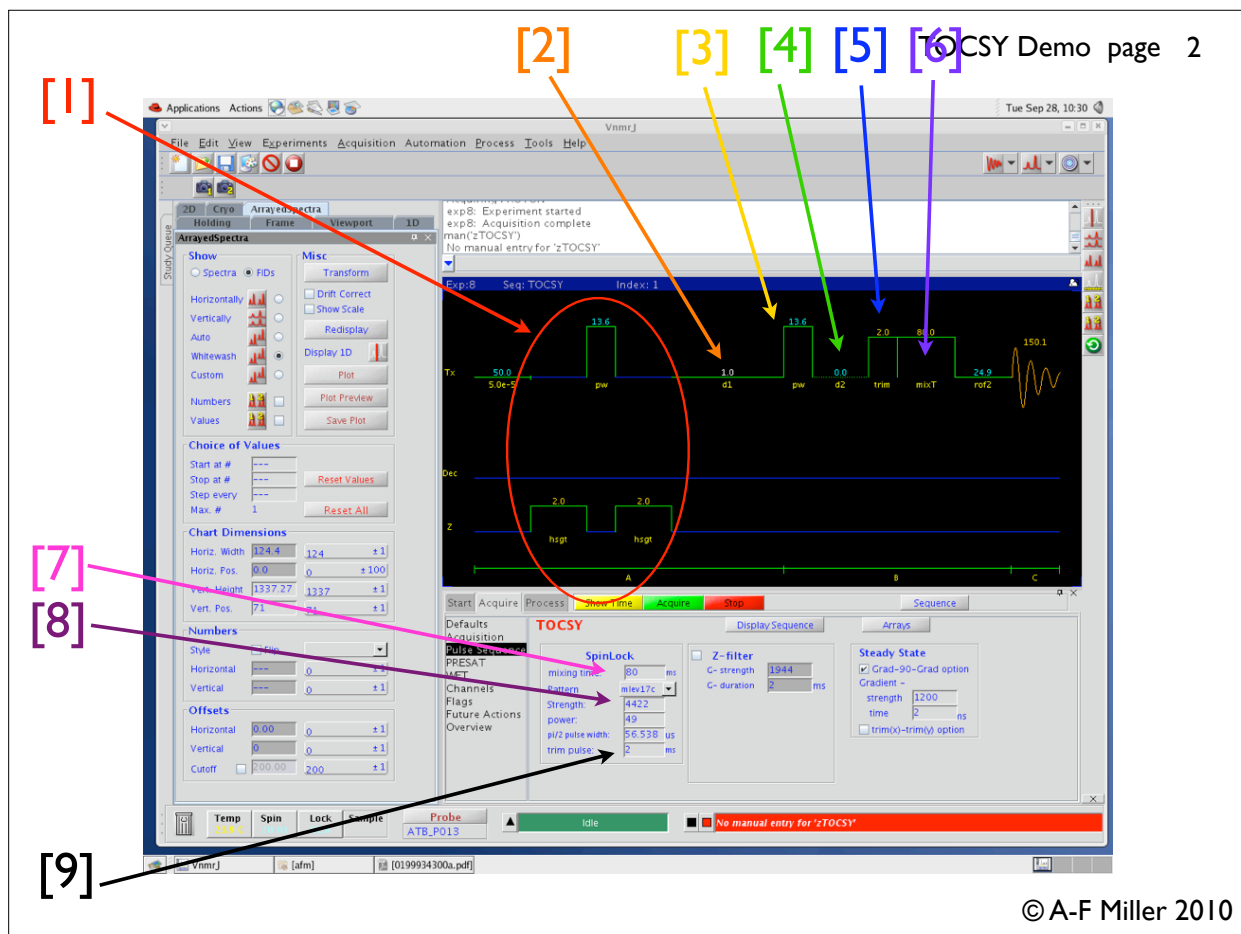
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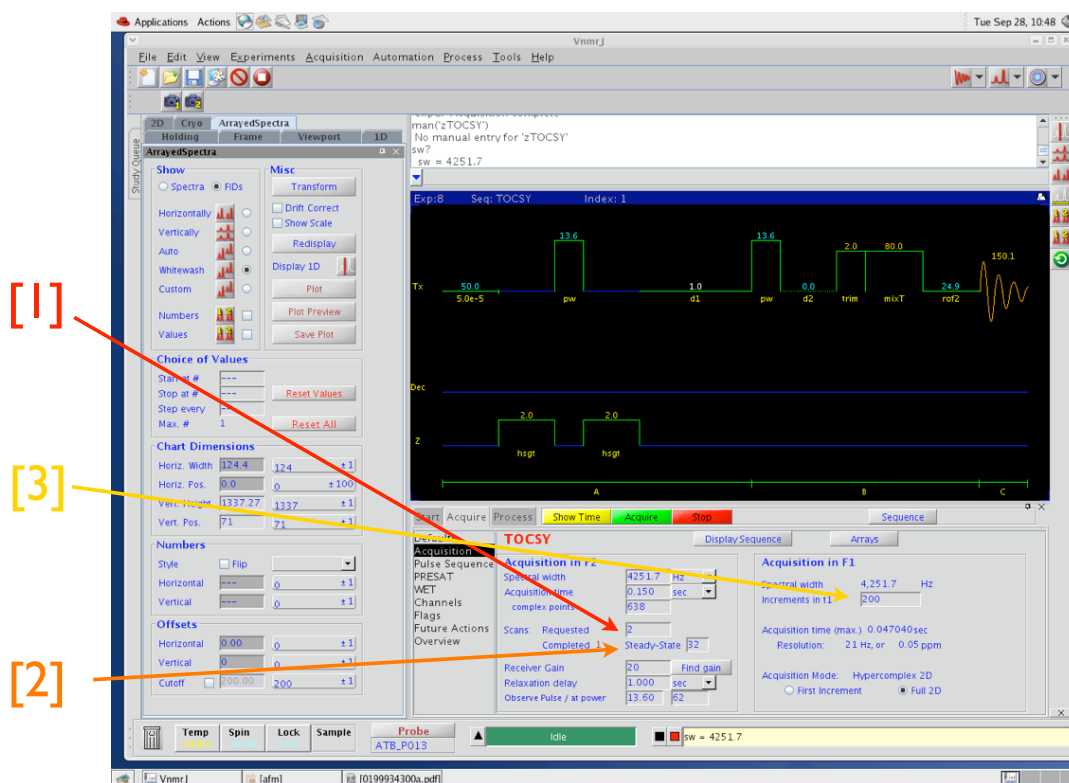
<sup>5</sup> A first-point multiplier is not needed for COSY, whose FID starts at zero

<sup>6</sup> Choosing a value near .5 helps to set the time-domain data used (F2) to a average level of zero. This is equivalent to a drift correction. Varian can set it for you.

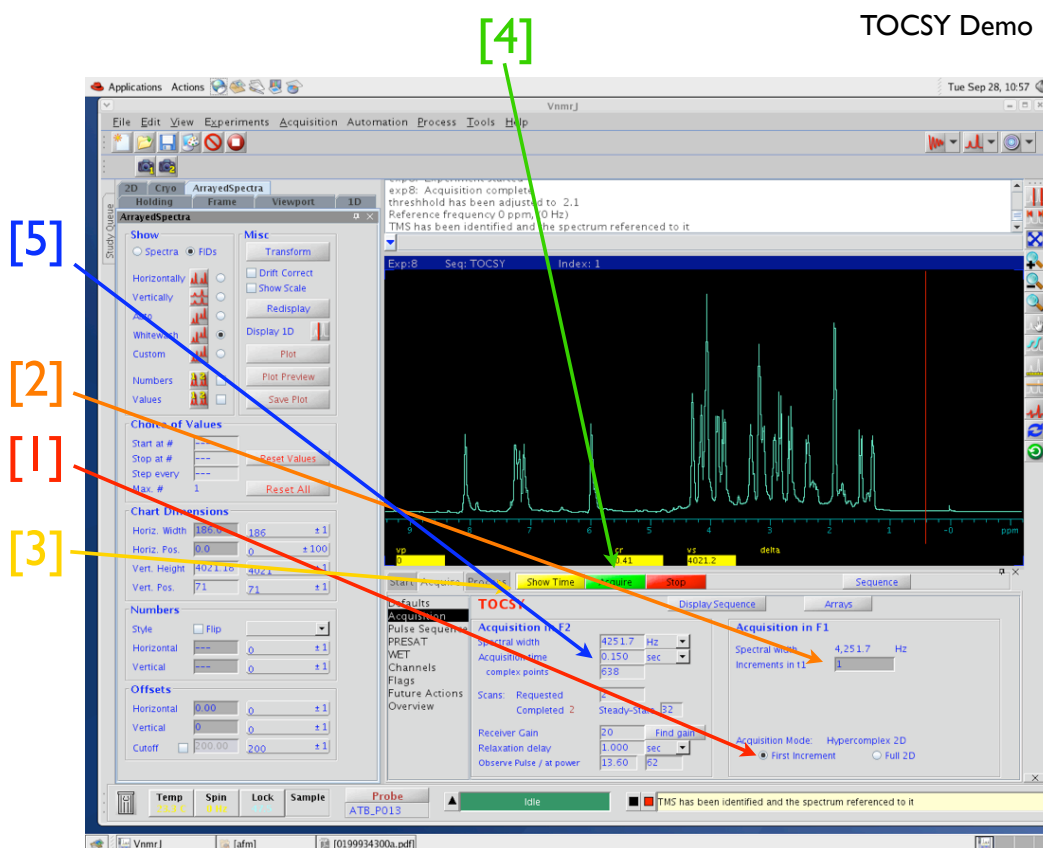
# 2D-TOCSY

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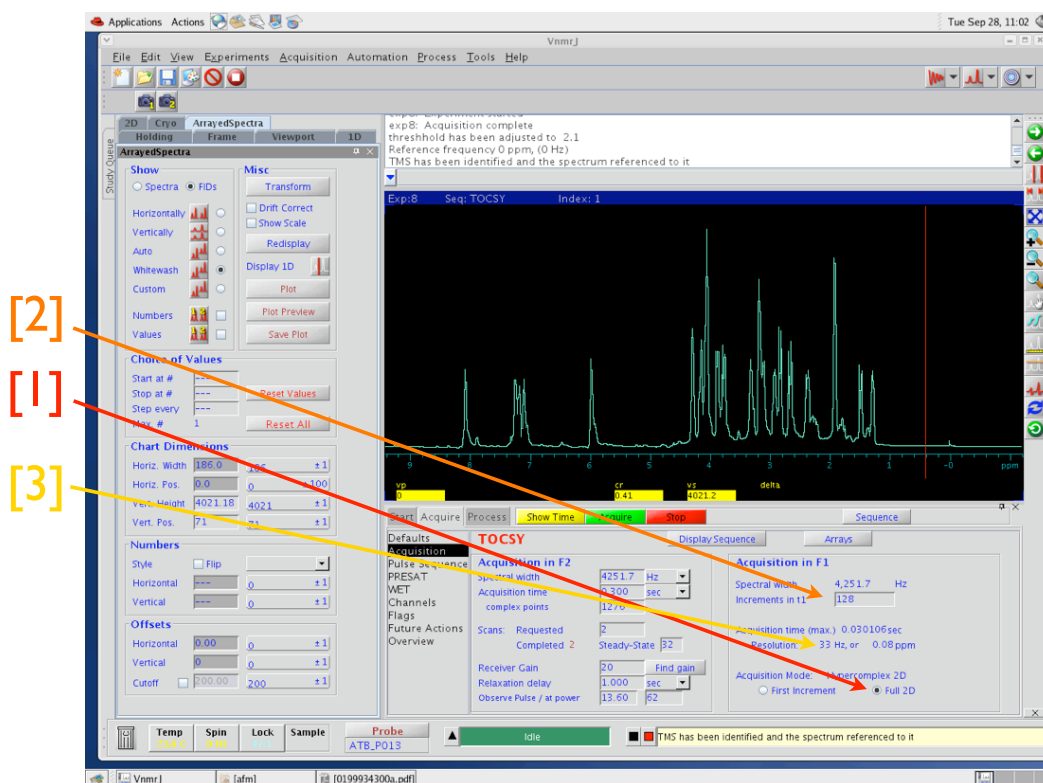


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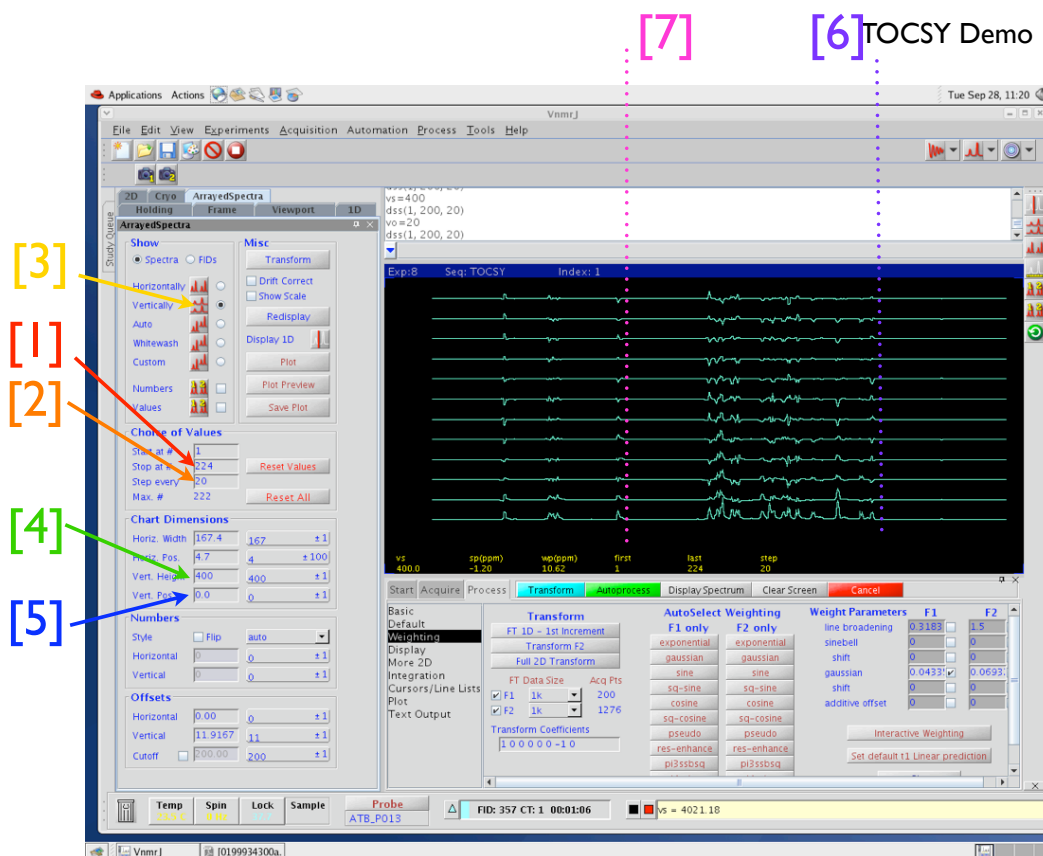


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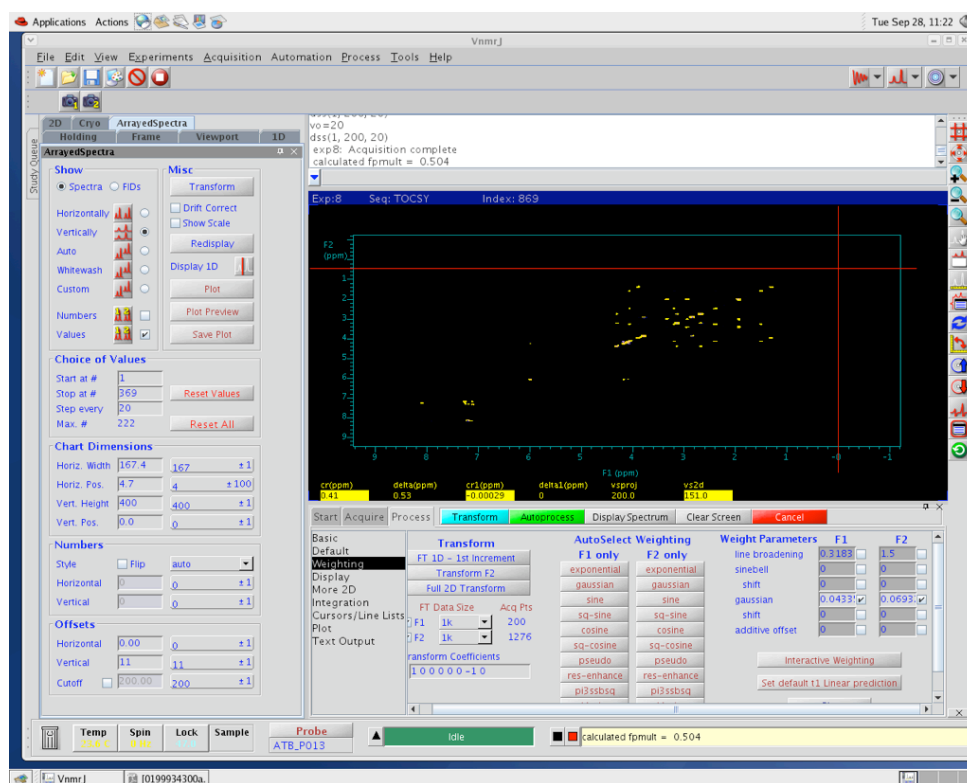




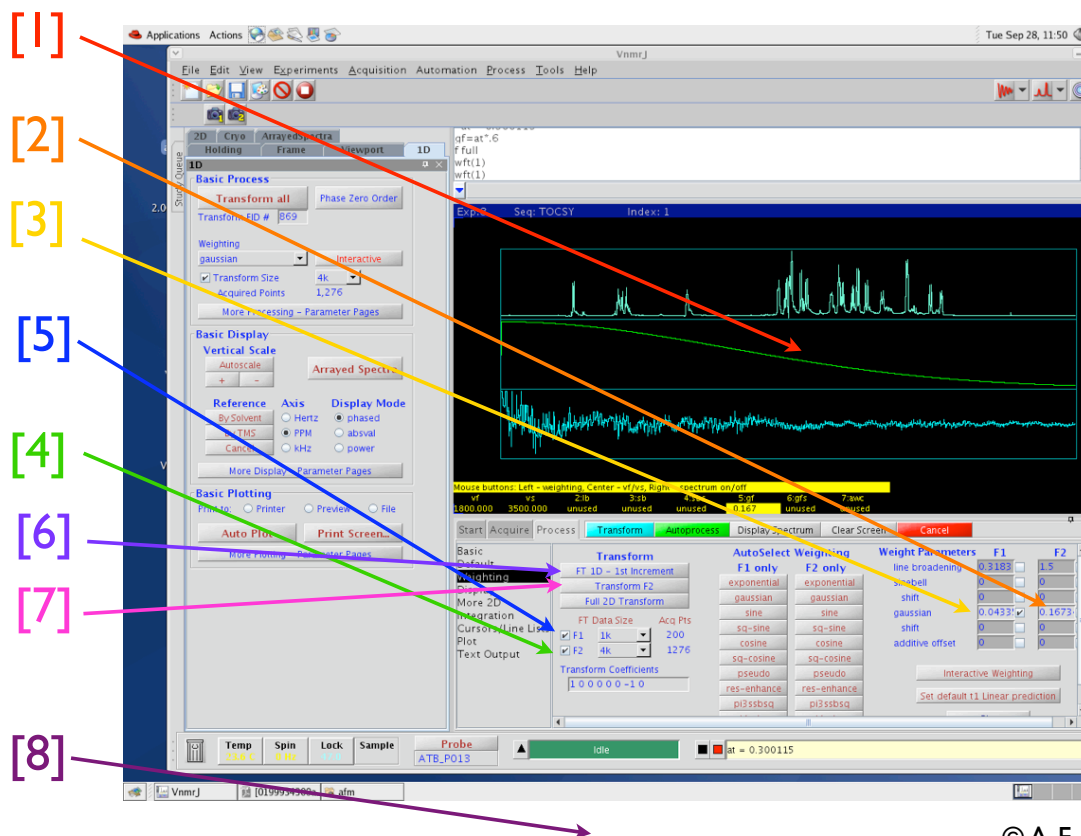
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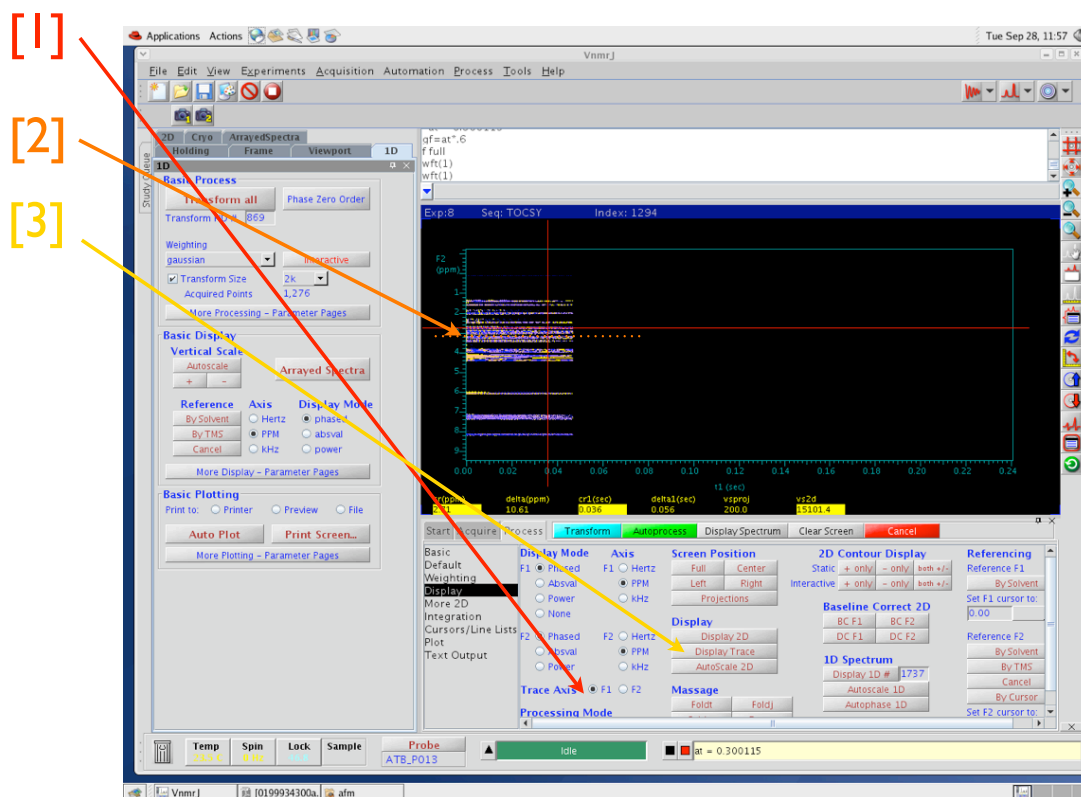
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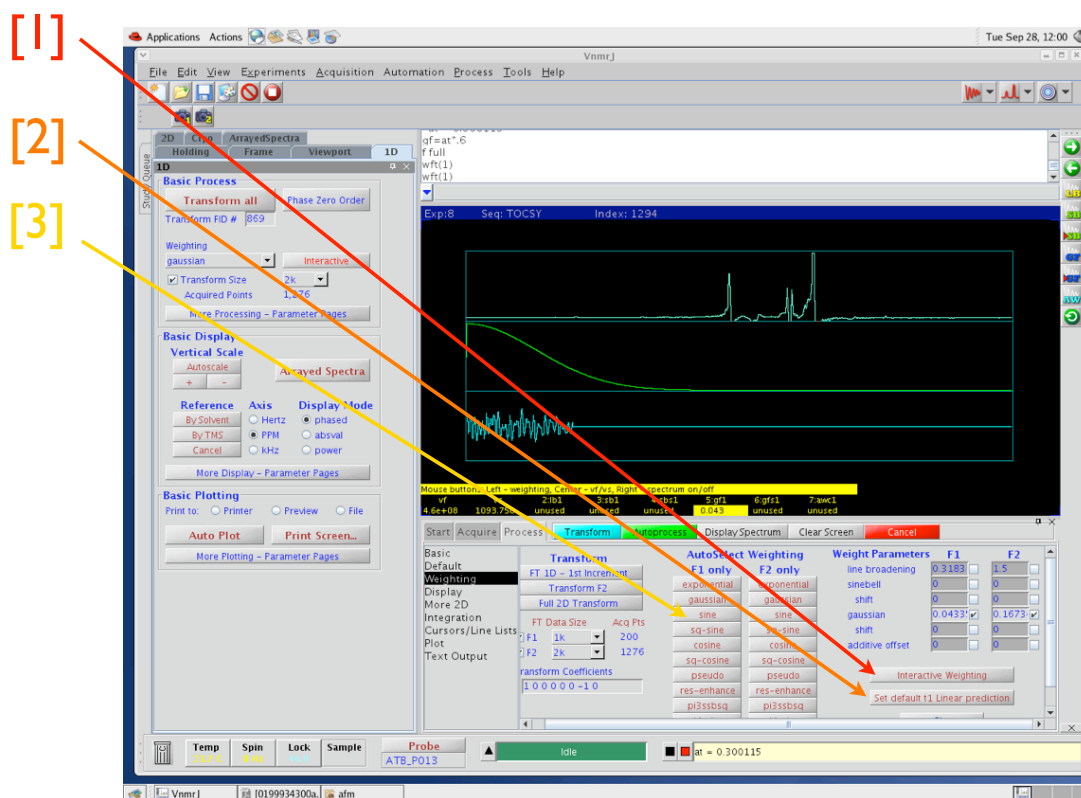
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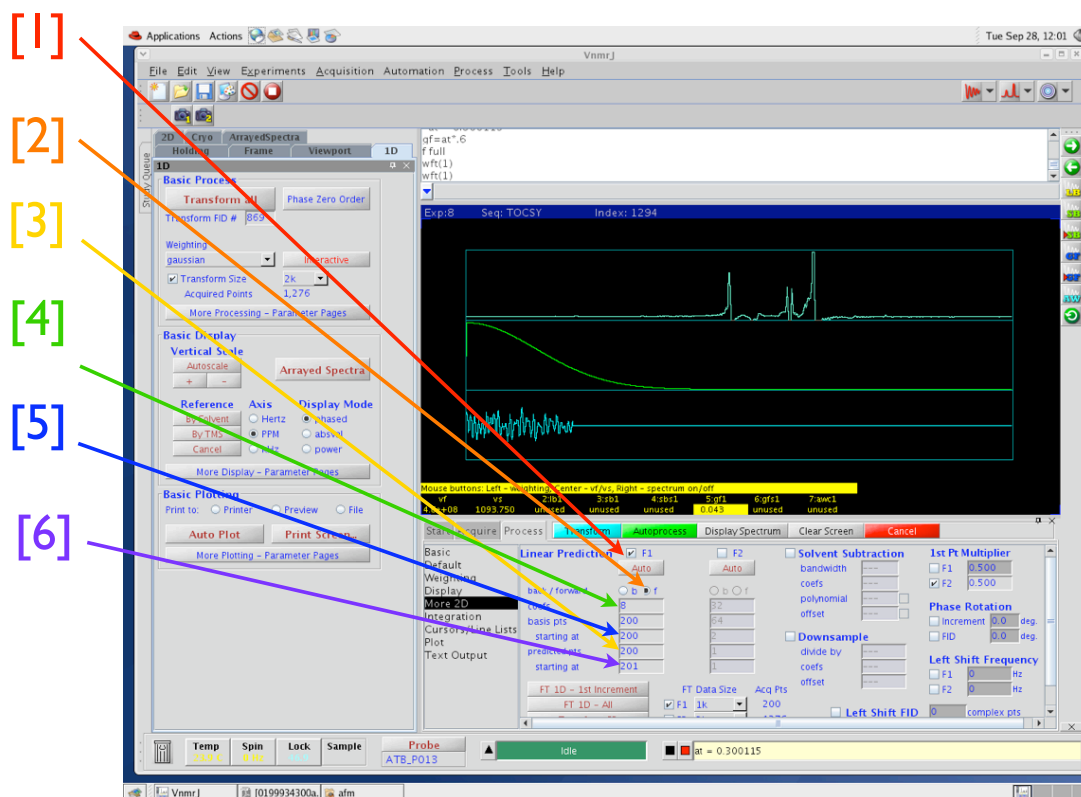
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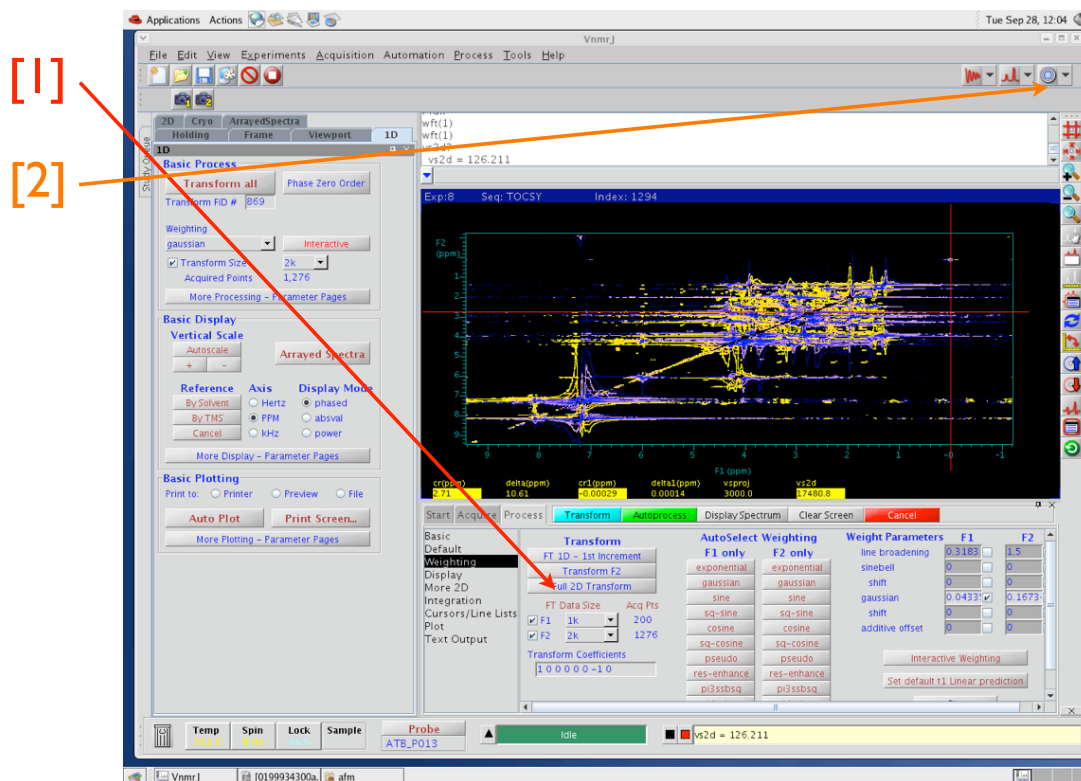
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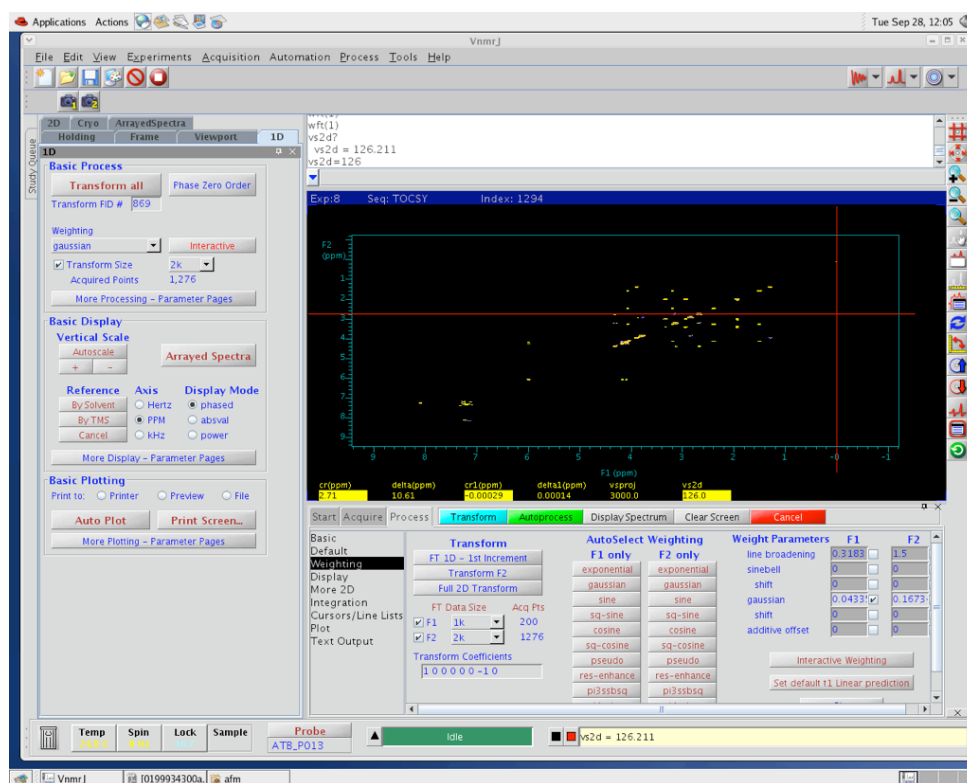
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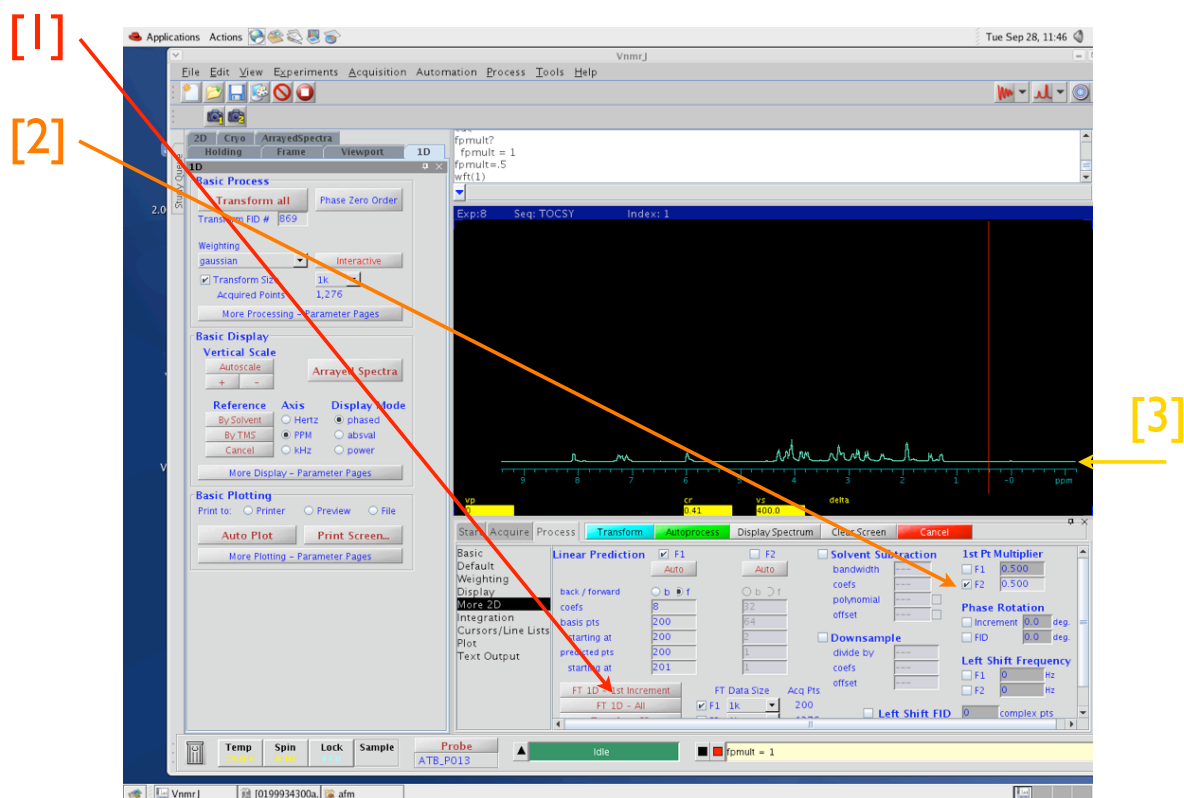
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