gradient Double-Quantum-Filtered COSY (gDQF-COSY)

This spectrum produces cross peaks exclusively between ^{1}Hs that are connected through bonds, usually 3 or less. (Exceptions are a few aromatic systems where double bonds permit observation of cross-peaks between ^{1}Hs 4 bounds apart.) Thus, once spin systems have been identified by a TOCSY< the COSY allows us to determine who are the nearest-neighbors in the spin system, and thus the order in which ^{1}Hs are connected.

Collecting the data

Begin with a calibrated 1H1d and a gain that is good for it. (For T2J I had pw90 = 12.5 at tpwr=55 sw=4011.4 and tof=-396.)

After phasing the spectrum beautifully, type **crof2**. This accomplishes the same thing that **setlp0** is supposed to, but I found **crof2** to work better.

Under experiments: Convert current parameters to do .... >Homonuclear Correlation Experiments >Gradient DQF COSY

Page 2 shows the pulse sequence including a gradient-90-gradient pre-sequence [1] to ensure that each scan begins with the same amount of magnetization. The two pulses that are the essence of a COSY are next, separated by d2 [2]. The 180° pulse that follows is in the center of a spin echo [3] that buys time for a gradient pulse. This gradient in combination with the one that follows the last 90° pulse work together to select only magnetization that was two-spin magnetization during the spin echo. This is the gradient '**d**ouble-**q**uantum **f**ilter' in the name DQF-COSY. For our purposes, what this accomplishes is to select for magnetization associated with pairs of ^{1}Hs, not single ^{1}Hs. This means we will get high-intensity off-diagonal peaks relative to diagonal peaks.

Note that a single scan is planned (!!) [4], along with receiver gain of 60 dB ! [5]. Only one scan is needed because the gradient double quantum filter is extremely good at eliminating artifacts. Show time gives 9 minutes (because this experiment requires only one scan). You may need more than one scan to get the signal-to-noise that you need. As a first guess, the number of scans (nt) that gives a good signal to noise in a 1-dimensional spectrum will be adequate for the DQF-COSY as well, but if the 1D is border-line, double nt.

In Acquire>Acquisition, change at from 0.15 to 0.3 s (**Page 3** [1])

'Find Gain' yielded 60 dB. However, the first increment of a COSY is naturally very weak (see page 4), whereas signal strength is stronger later. Therefore, I put gain to 30 **[2]**, consistent with the gain I used for my beautiful 1D.

Click on Acquire !!! **[3]**. This will get you a quick COSY that be very good 90% of the time.

Intelligent detection

In the COSY, the 'mix' is built right into the chemical shift evolution time d2 **[4]**. Spins are precessing at the frequencies corresponding to their chemical shifts with the result that these chemical shifts will be encoded in the amplitude collected by the second pulse, just as in NOESY and TOCSY. However in addition the development of J-coupling produces another kind of magnetization (called 'antiphase' magnetization) that is not directly detectable, but which incorporates information about both coupled spins. In this case it will have chemical shift encoding from the source ^{1}H but it will develop a sign that depends on the spin of another ^{1}H to which the source ^{1}H is coupled. This will happen in proportion to $\sin(\pi Jt)$. Thus the effect is maximized when the sine has its maximum value, which occurs when $\pi Jt = \pi/2$. Thus, for maximum COSY transfer, we'd like to have d2 values close to $t=1/2J$. J-values depend on the geometry and natures of the bonds linking the two ^{1}Hs in question, so you will be emphasizing one or another population of ^{1}H pairs when you choose which d2 values to cover. For the example of J=7 Hz, $1/2J \approx 70$ ms. Thus, although we have to start with d2 = 0, we want to increment it enough times, based on a large enough ni, that d2 reaches 70 ms.

To be comprehensive in favouring a range of ^{1}H pairs, I would recommend covering from 35 ms to 105 ms (from 50% under to 50% over the 'theoretical value' of 70 ms, above). Thus, I would choose an ni value to obtain a maximum d2 value of at1 ≈ 105 ms.

We have the formula that (ni-1)/sw = at1 which we can use to calculate an ni value. However the Varian software calculates at1 for you **[5]**, so you can do this by trial and error by inputting different ni values. You can also use the formula at1=(ni-1)/sw.

Now you can do the usual: 'Show Time' and 'Acquire'.

Page 4: You can monitor progress by typing wft dc dssa(1,200,10) (This string of commands performs a weighted Fourier transform, drift correction (simple baseline correction) and then displays a stack of spectra automatically for spectra number 1-200 showing only every 10th one.) You see that the first increment contains very little signal [1], but that signal 'grows in', becoming stronger as d2 gets longer [2]. This is consistent with the $\sin(\pi Jt)$ relationship.

COSY processing

Default processing employs weighting functions that are very slightly shifted cosines (sine-bell functions with an almost 90° shift). This produces stronger spectra, however the emphasis does not match the information content.

For the strongest emphasis on off-diagonal cross peaks with less emphasis on diagonals, we would like weighting functions that emphasize the magnetization that grows in as $\sin(\pi Jt)$. Go to Process>Weighting and click FT 1D - 1st Increment [1] (**page 5**). As advertised, the first increment is essentially empty. To get a much

later increment type wft(199) [1], to see the 199th 1D spectrum ([2] **Page 6**). (Stick to odd-numbered spectra, as these correspond to the real component of the complex ni increments.)

Click on 'Interactive Weighting' [3].

The display on **Page 7** appears. What we just transformed was a directly-detected FID (number 199), so we have F2 data. Therefore we will restrict ourselves to the F2 column of weighting functions [1]. Adjust the F2 shift [2] under the sine to make an almost unshifted sinebell in the centre of the window. The minus sign in front of the sine width (sb) makes it a *squared* sine bell [3]. Note that the F2 dimension is not extended by linear-prediction, so we can take the weighting as we see it. The goal here is to make a weighting function that goes to zero at the end of the FID but that peaks in the middle.

Click 'Transform F2' [4]. This will apply your chosen weighting function to all the F2 FIDs and transform them all.

The interferrogram that results on **Page 8** shows intensity oscillating at various F2 frequencies [1] . Place the cursor on one of these streaks [2] and click "Display 1D #' [3].

The indirect FID (**Page 9**) shows how intensity at 7 ppm starts at zero but grows in, with longer d2 values [1] (the horizontal axis here) and then shrinks again consistent with sin(πJt). Go to 'Process>Weighting' [2].

Click 'Interactive weighting' (**page 10** [1]) and match the sine bell to the intensity in the indirect FID using weighting parameters in the F1 column [2]. The two we will manipulate are sinebell [3] (sb1) and shift [4] (keep this at zero, sbs1)

Click on Full 2D Transform [5] to apply your weighting function to the F1 dimension and Fourier transform it.

To adjust the vertical scale of the resulting 2D, use middle mouse button, clicking near a peak, to decrease vertical scale to the point where peaks show but background is minimized (**Page 11**). Also you can type in new values for vs2d, based on the value given in the right-most box in the graphical display window [1]. For example, to decrease the vertical scale of the 2D to 80% of its current value, type **vs2d=0.8*vs2d** or you could type **vs2d=1000** (for example). Each time you enter a new value of vs2d, you will have to click on 'Display Spectrum' [2] again to implement your change.

COSY cross peaks have unusual characteristic phase: they are 'dispersive' in both dimensions. This means that they have a positive and a negative lobe, and the null between lobes is actually the centre frequency for the peak. This is true in both dimensions, so you see the sign (color) switch between the left and the right side of a peak, you also see the sign (color) switch between the bottom and the top of a peak. (**Page 12**)

To tweak the phase, go to Process>Display place a cursor across the lobes (not the center) of a *simple* cross-peak [1]. Click on 'Display 1D #' [2].

In this case, we have perfect phasing with equally large positive and negative lobes at rp1=0 and lp1=0 (**Page 13**, note the 1 indicates that these are the F1 analogs of rp and lp) . Phasing can be difficult to evaluate if you choose a complicated cross-peak. Avoid diagonal peaks for this. Click on Display Spectrum [1] to recover the 2D.

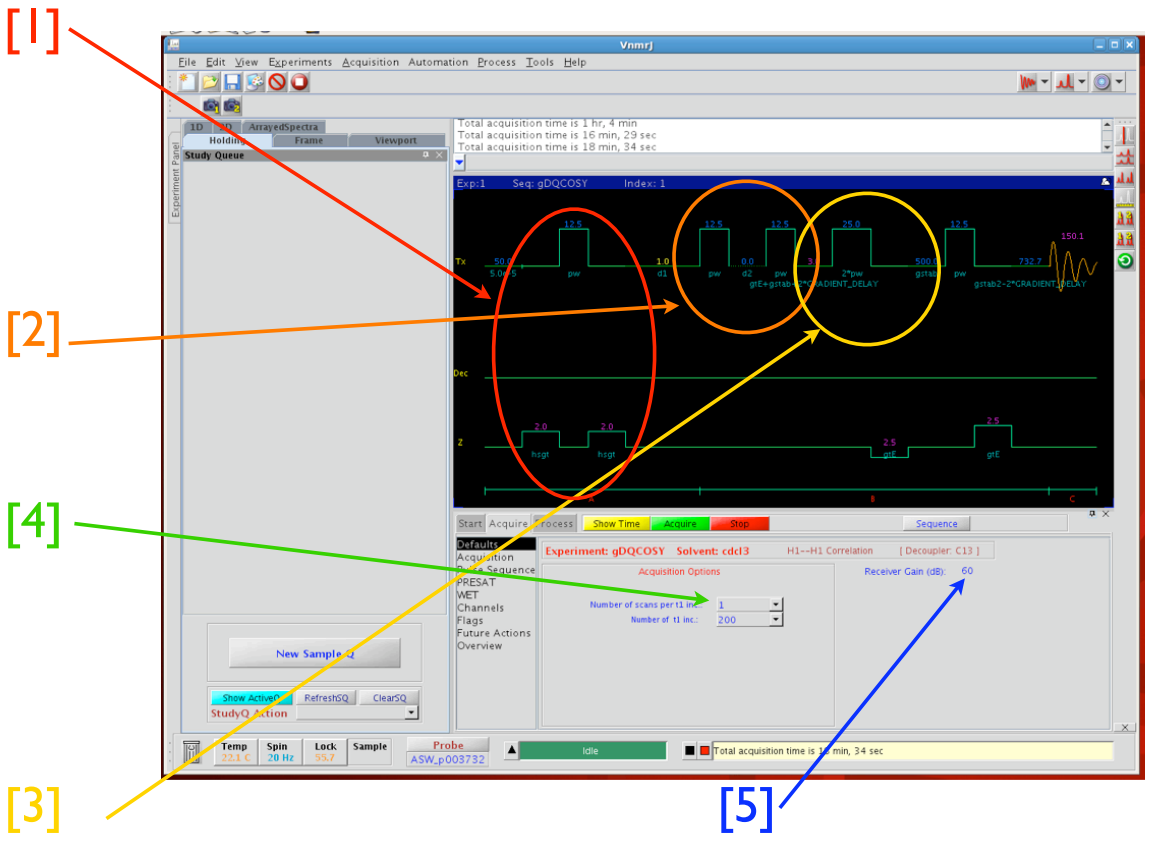
Select 'Trace Axis' = F2 [1] to make the F2 axis the horizontal [2] (**Page 14**). Again place a cursor across the lobes of a peak [3] and click on 'Display 1D' [4].
The phasing is perfect (rp=82.6 and lp=-16, ie close to theoretical values of 90 and 0) (**Page 15**). To obtain values for the phases you can either query them by typing **rp?** or click on the phasing icon to make the two values visible along the bottom edge of the graphical display window.

Note active and passive couplings can both be seen in the F2 dimension of our COSY, because the F2 dimension is the high-resolution dimension 9([5] on **page 14**). looking at the trace through the lobes of this cross-peak (**Page 15**) reveals both the 'active coupling' [1] that is responsible for this cross peak as well as an additional 'passive' coupling [2] affecting the ^{1}H responsible for the F2 chemical shift of this crosspeak.

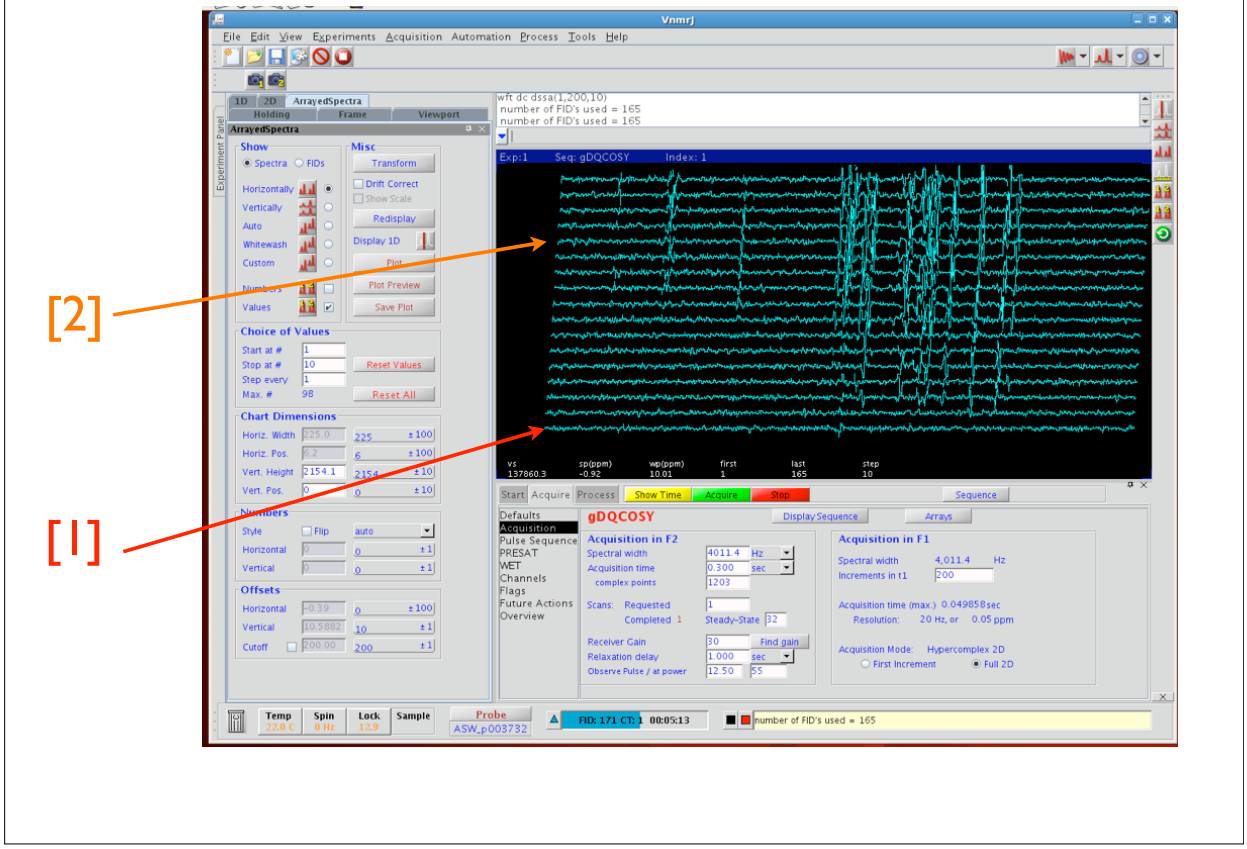
Page 16 shows my ps file output of a zoom into the region of the DQF-COSY with most of the signals. The strength as well as the disadvantage of the COSY is that it is such a strong spectrum that even the crosspeaks of impurities can be seen. I prefer to interpret the spectrum beginning with the strongest crosspeaks and only assimilating the weaker ones into the interpretation later. Looking at too many of them right from the start can be confusing. However if you have a short-lived or sparingly-soluble compound that you need to assign, a COSY can give you crosspeaks when no other experiment will.

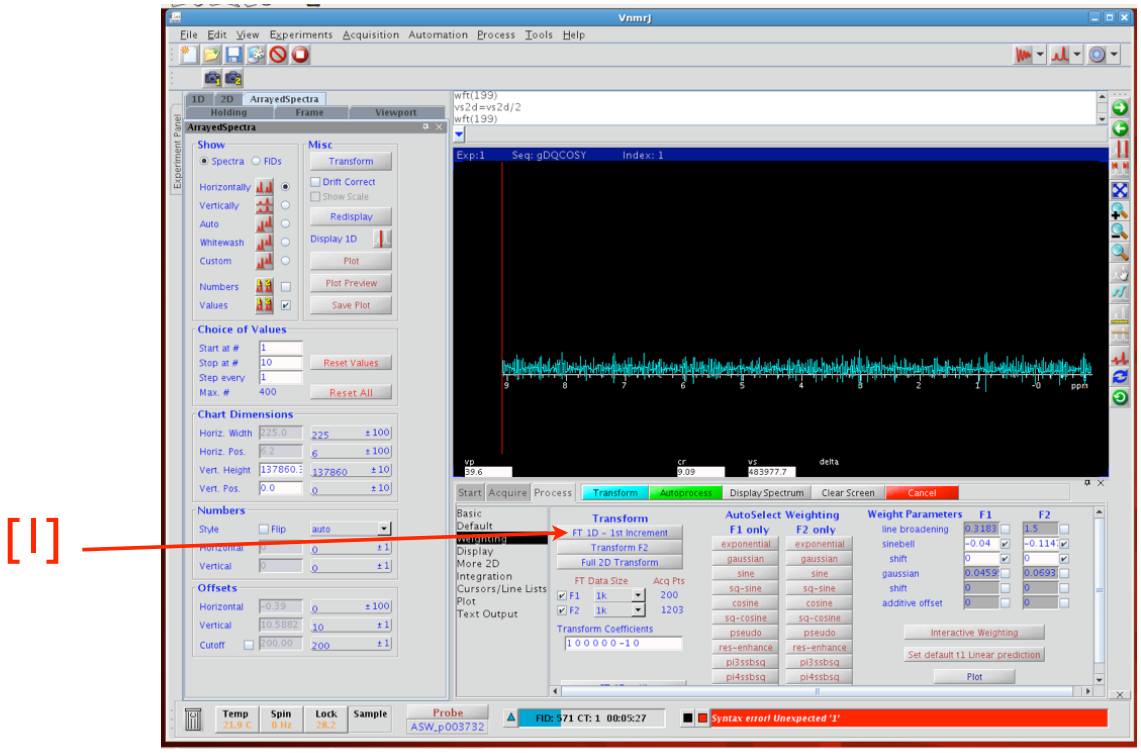
See the lecture notes for a walk and interpretation of this COSY.

COSY
(gradient-Double Quantum filtered COSY)




[1]
[2]
[3]
[4]
[5]


[2]
[1]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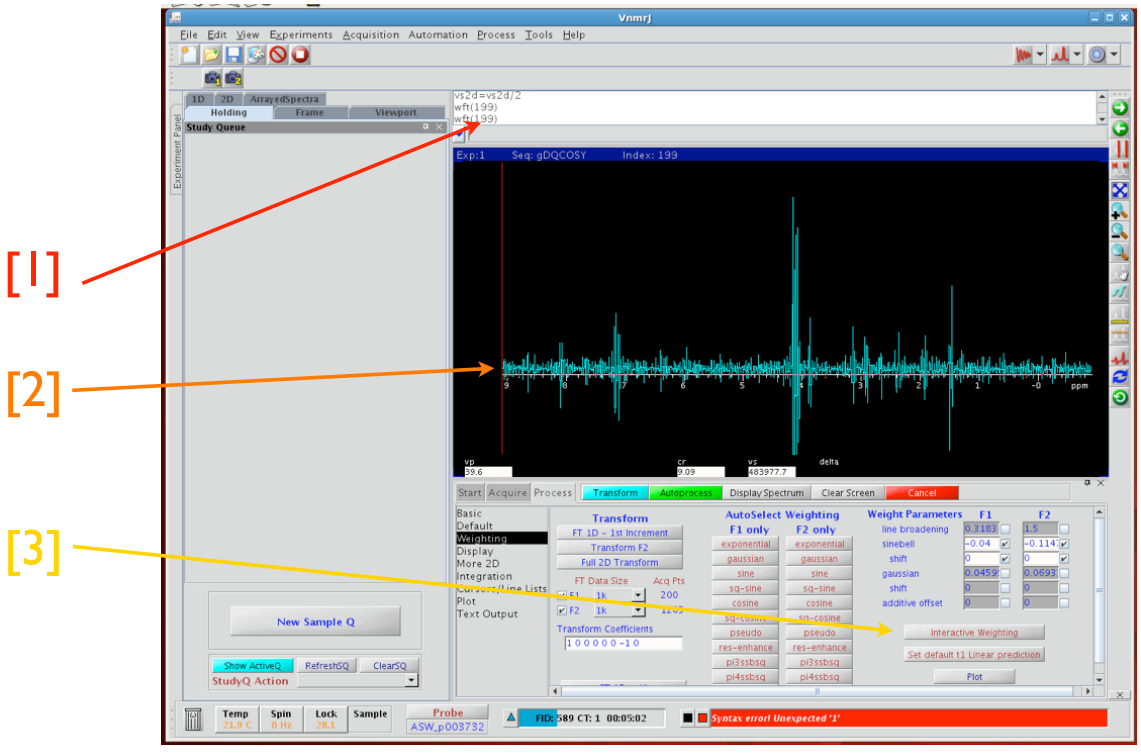
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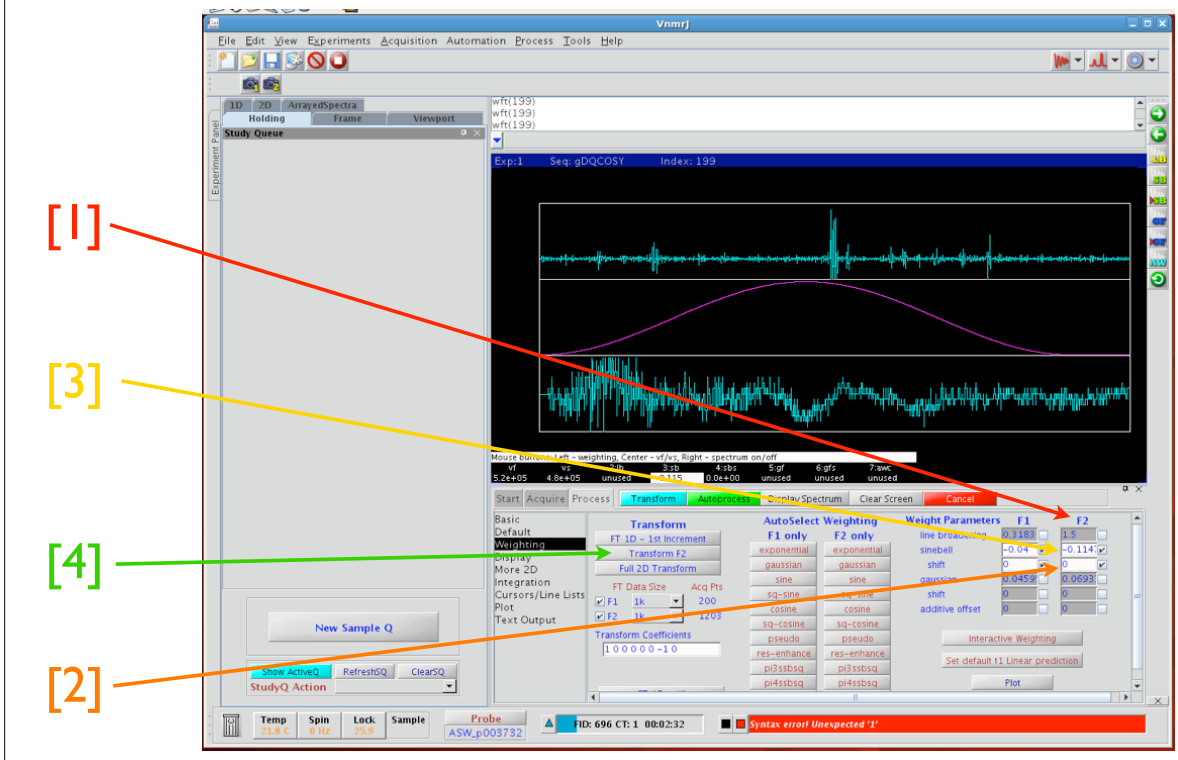
[2]
[3]


[1]
[3]
[4]
[2]
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Study Queue
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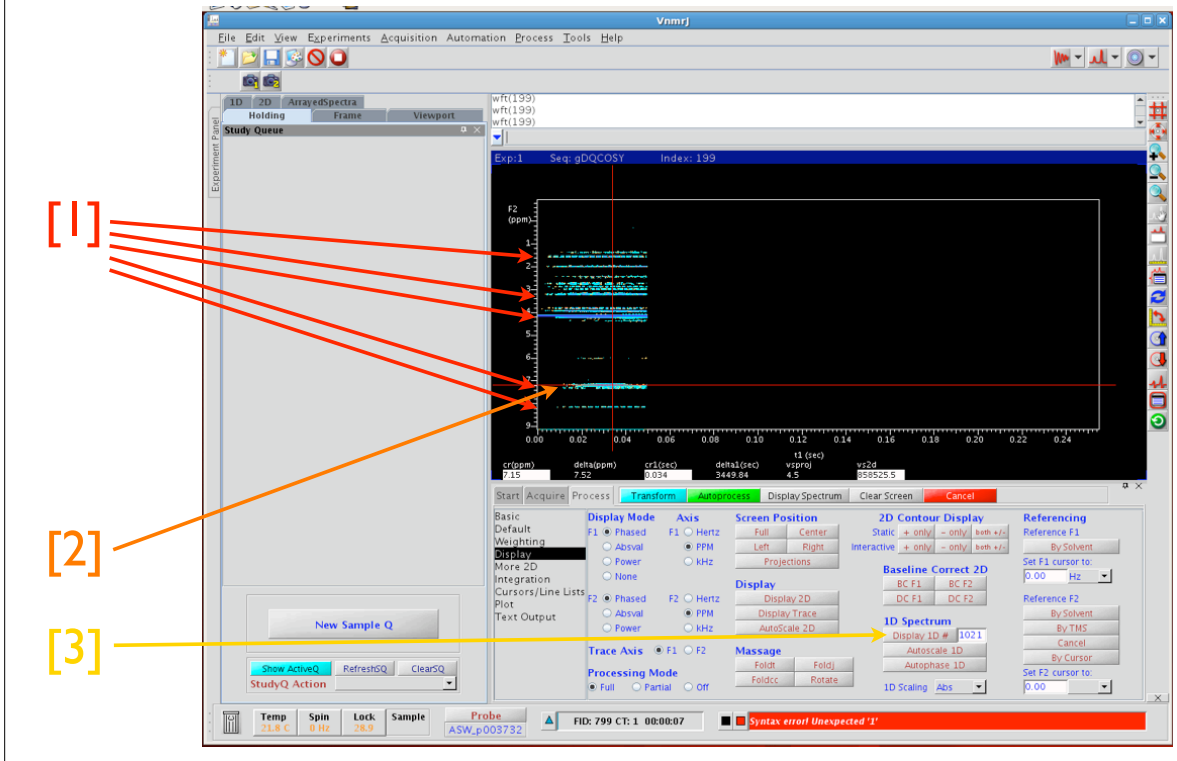
Start Acquire Process Transform Autoprocess Display Spectrum Clear Screen Cancel
Transform
FT 1D - 1st Increment
Transform F2
Full 2D Transform
AutoSelect Weighting
Weight Parameters
New Sample Q
Show ActiveQ RefreshSQ ClearSQ
StudyQ Action
Temp Spin Lock Sample Probe
FID: 696 CT: 1 00:02:32
Syntax error! Unexpected '1'


[1]
[2]
[3]
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Study Queue
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Start Acquire Process Transform Autoprocess Display Spectrum Clear Screen Cancel
Display Mode
Axis
Screen Position
2D Contour Display
Referencing
Baseline Correct 2D
1D Spectrum
Trace Axis
Processing Mode
Massage
New Sample Q
Show ActiveQ RefreshSQ ClearSQ
StudyQ Action
Temp Spin Lock Sample Probe
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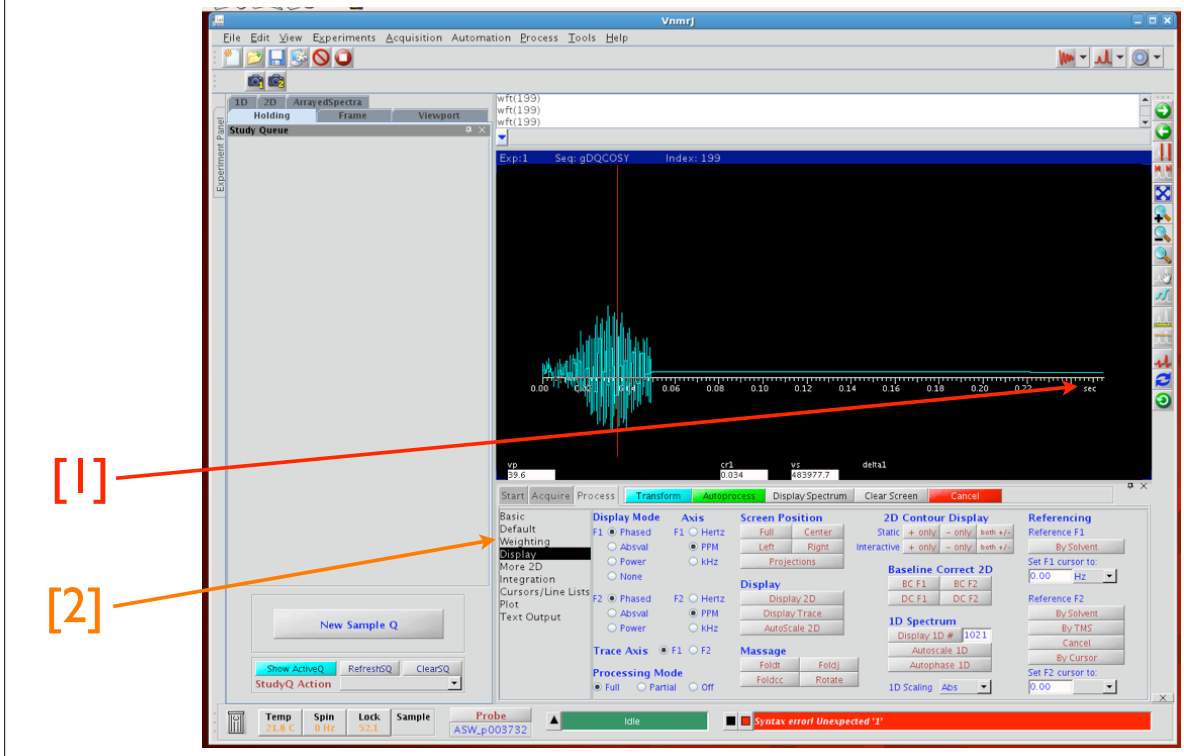
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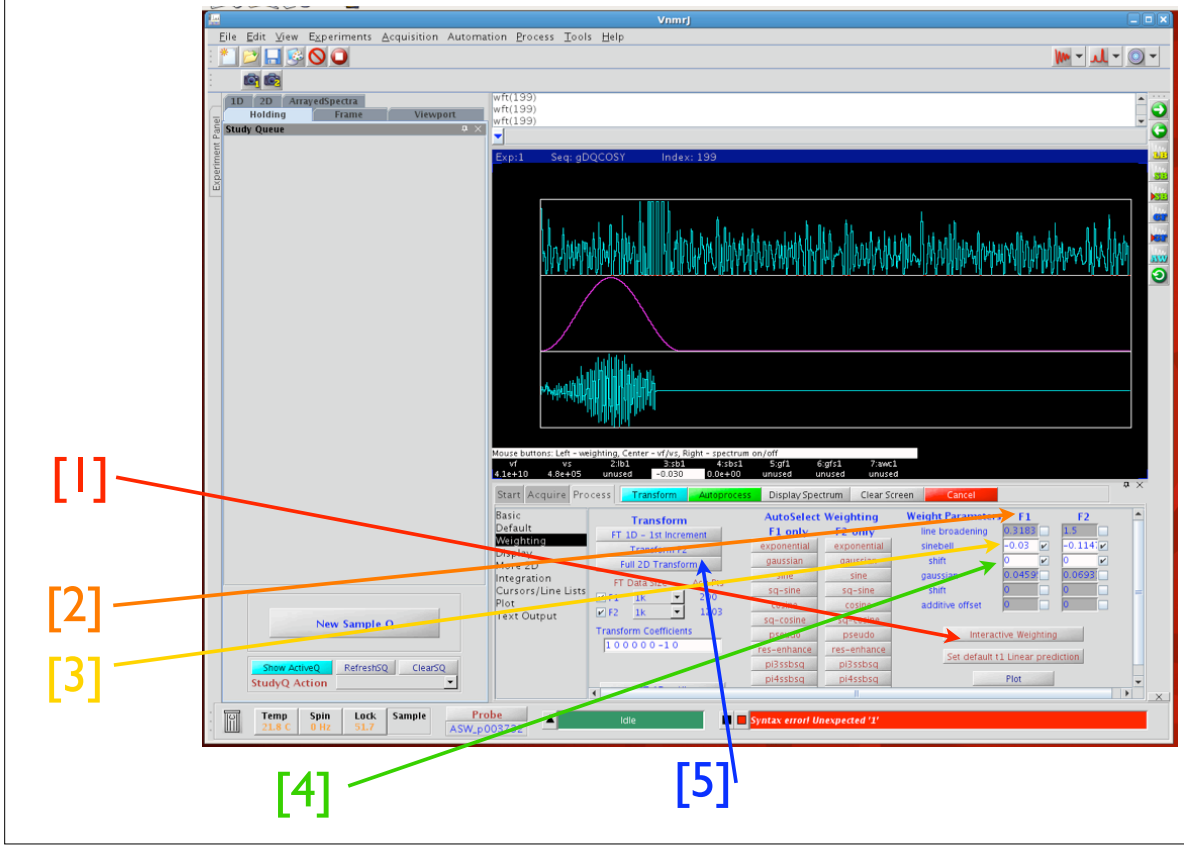
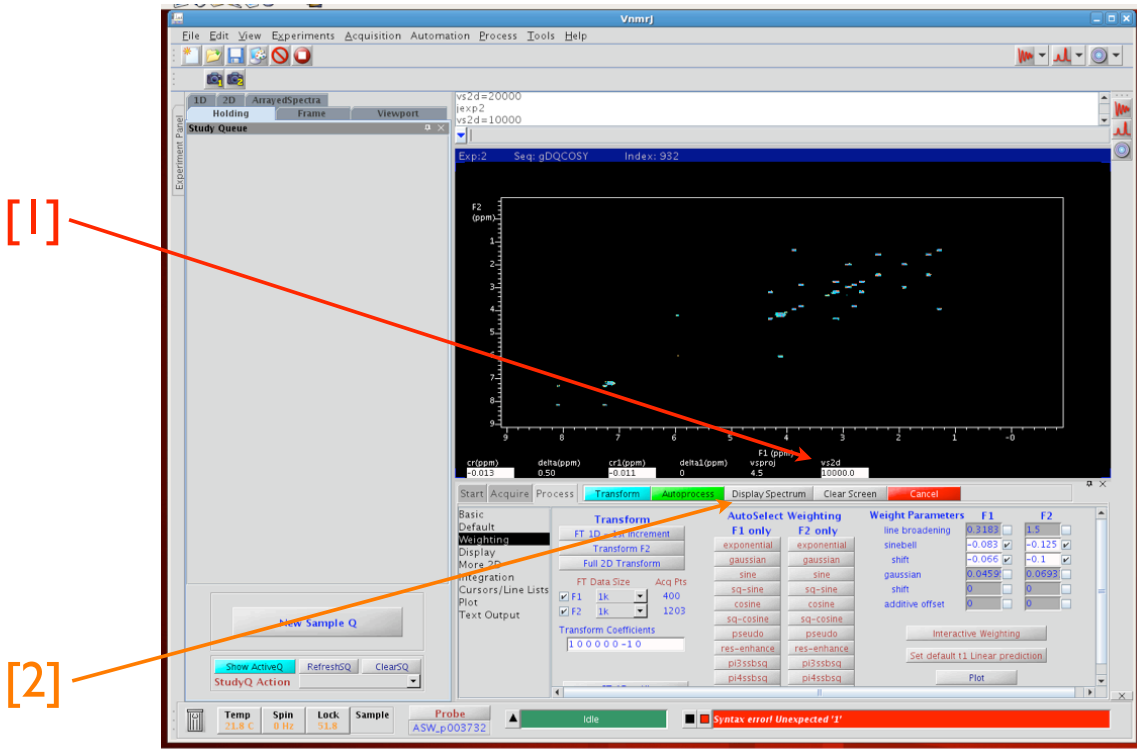
[2]


[1]
[2]
[3]
[4]
[5]


[1]
[2]
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F2 (ppm)
F1 (ppm)
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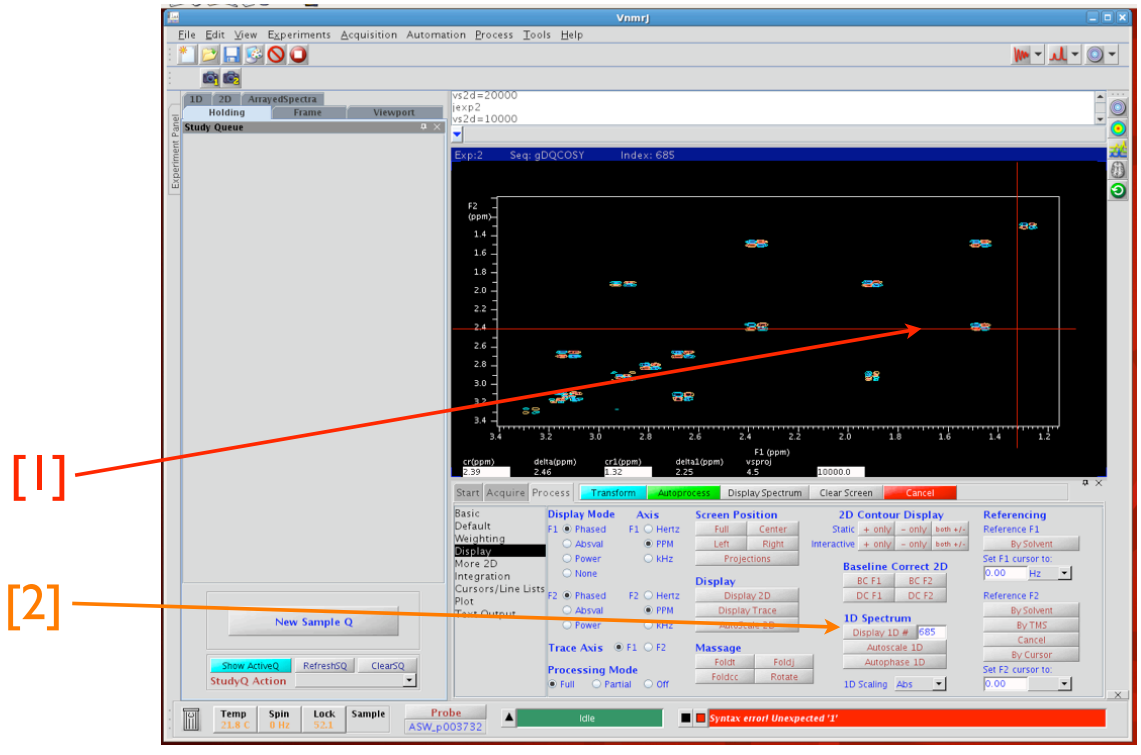
Basic
Default
Weighting
Display
More 2D
Integration
Cursors/Line Lists
Plot
Text Output
Transform
AutoSelect Weighting
F1 only
F2 only
Weight Parameters
Transform F2
Full 2D Transform
Transform Coefficients
Interactive Weighting
Set default t1 Linear prediction
Plot
New Sample Q
Show ActiveQ
RefreshSQ
ClearSQ
StudyQ Action
Temp
Spin
Lock
Sample
Probe
ASW_p003732
Idle
Syntax error! Unexpected '1'


[1]
[2]
Vnmrj
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Study Queue
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F2 (ppm)
F1 (ppm)
Start Acquire Process Transform Autoprocess Display Spectrum Clear Screen Cancel
Basic
Default
Weighting
Display
More 2D
Integration
Cursors/Line Lists
Plot
Text Output
Display Mode
Axis
Screen Position
2D Contour Display
Referencing
Display
Baseline Correct 2D
1D Spectrum
Trace Axis
Massage
Processing Mode
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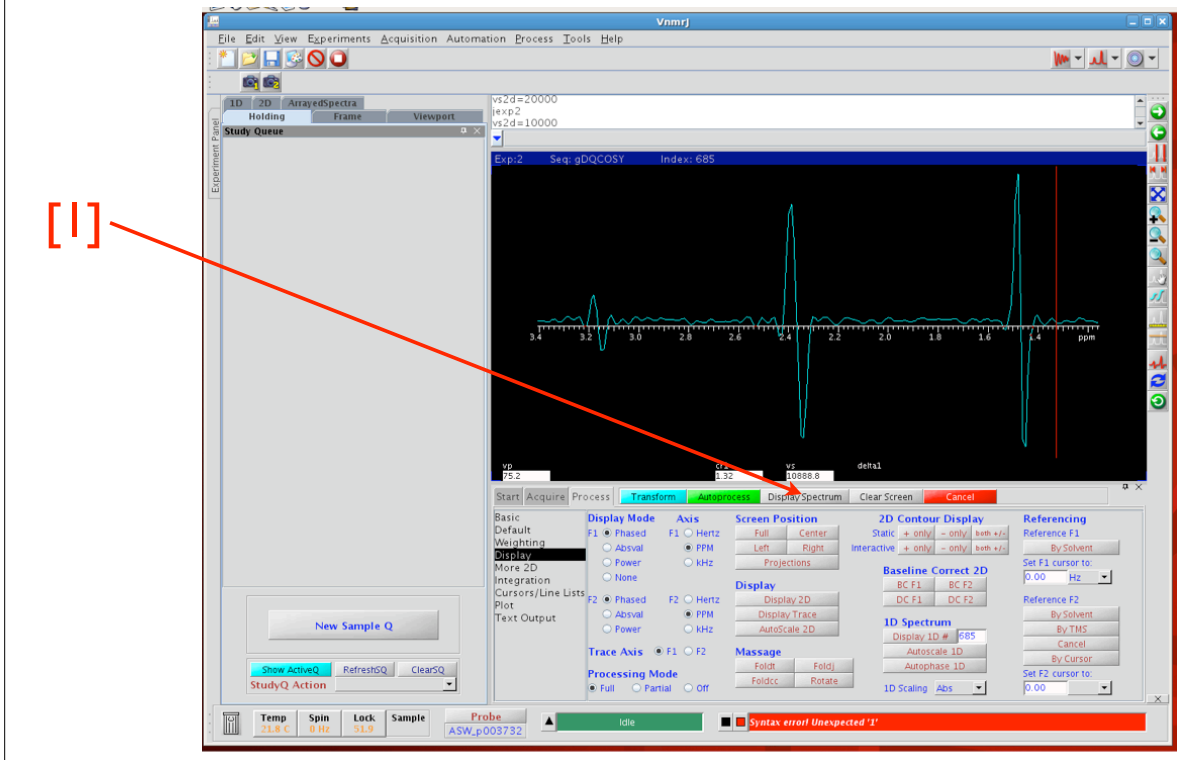
[1]
Vnmrj
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ppm
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Display Mode
Axis
Screen Position
2D Contour Display
Referencing
Baseline Correct 2D
1D Spectrum
Trace Axis
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Show ActiveQ RefreshSQ ClearSQ
StudyQ Action
Temp Spin Lock Sample Probe
ASW_p003732
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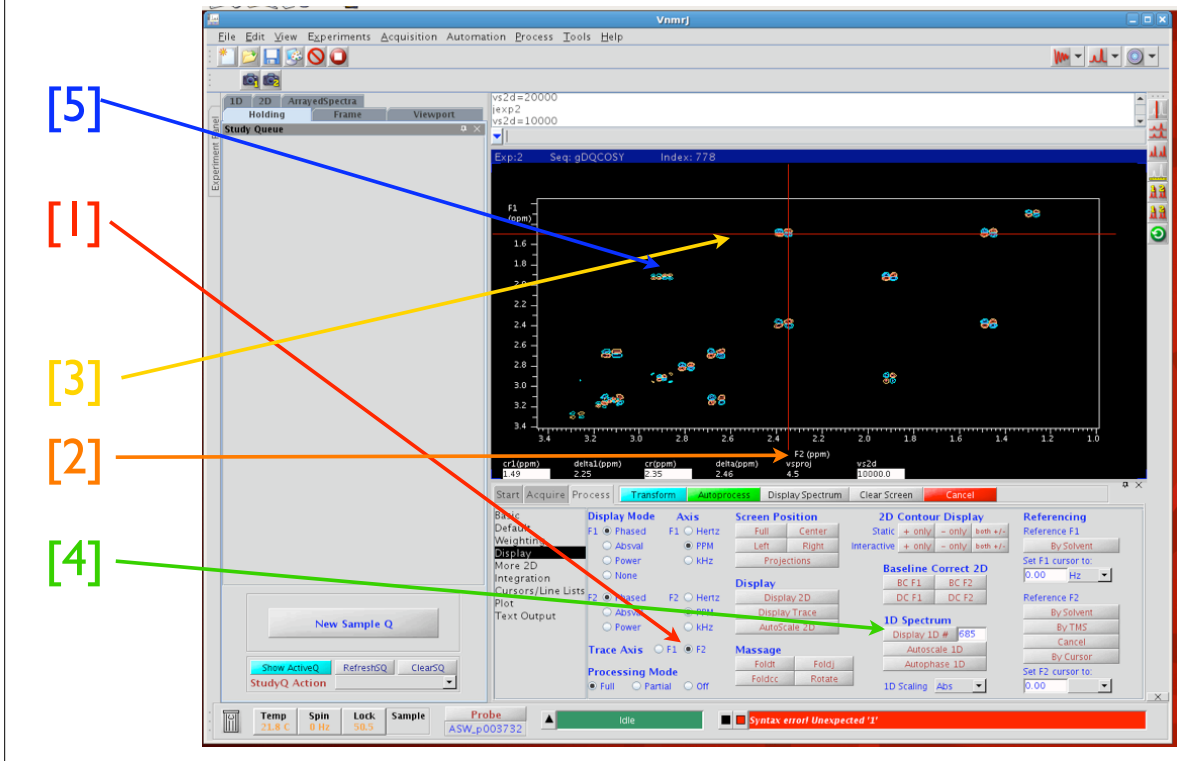
[5]
[1]
[3]
[2]
[4]
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F1 (ppm)
F2 (ppm)
Start Acquire Process Transform Autoprocess Display Spectrum Clear Screen Cancel
Display Mode
Axis
Screen Position
2D Contour Display
Referencing
Baseline Correct 2D
1D Spectrum
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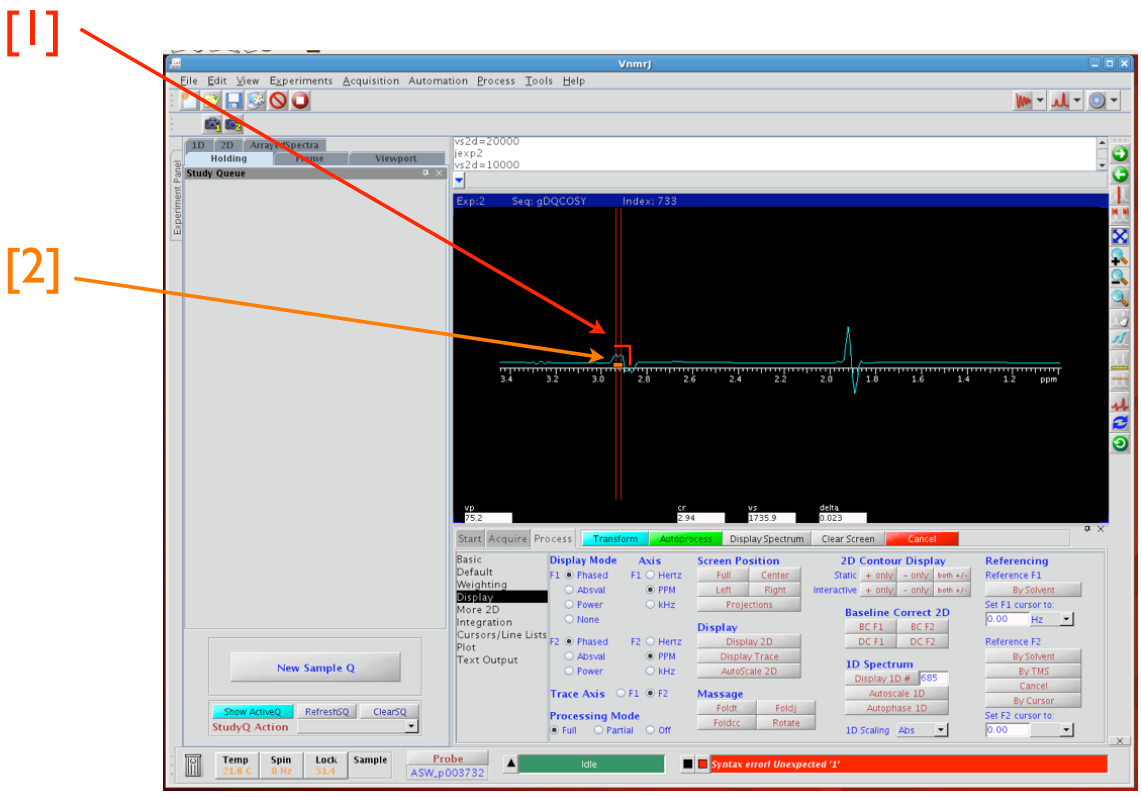

gDQF-COSY zoom