Wet 1d

A sample in 90% H_2O and 10 % D_2O.

Water sample has a lock at Z0 = 1956 (vs. -1100 for CDCl3). A basic proton experiment.

Collect a wide scan with gain = 0 and a 45 ° read pulse, because 100% water is 55 M and can saturate the receiver.

Indeed, the spectrum is dominated by a single resonance, that of water (**Figure 2**).

The automatic processing is best suited to samples in deuterated chloroform that contain TMS. Because we are not working in water, and we will soon suppress the water line, the software will not have access to a recognizable line for automated chemical shift referencing. Therefore we will turn the automatic processing off.

In Acquire>Future Actions look at 'When experiment Finishes' = 'process' (**Figure 3**). Replace this with 'wft dc vsadj' [1]. The foregoing will weight and Fourier transform (wft), apply a drift correction (dc raises or lowers the FID to place the long-time end of the FID at a vertical position of zero) and adjust the vertical scale such that the tallest peak will fill the vertical dimension to 90% (vsadj). This is relatively un-intrusive processing and will not change your phase values or chemical shift referencing, one you have them set.

Now acquire your spectrum again, and phase it up.

Calibrate the chemical shift axis on water, at 4.7 ppm (In Process>Display type 4.7 ppm into 'Reference cursor to' .... and select ppm. Place the cursor on the water line. Click on 'By Cursor'.)

Increase the vertical size of the spectrum by a factor of 100 by typing **vs=vs*100**. vs is the 'vertical scale'.

Our spectrometer is incredibly good (**Figure 4**). Despite the fact that the water line is off-centre, I do not see a significant quadrature artifact at a mirror-image offset down-field of the carrier [2].

Nonetheless, it is good form to place the carrier right on the solvent if the solvent line is very large (**Figure 5**). In Process>Cursors/Line Lists, place the cursor on the water line [1] and click 'Place on nearest line [2] (type 'nl', for nearest line). Then click on 'Move transmitter' [3]. In Acquire>Channels you have a new Offset value near -149 Hz. (**Figure 6**)

In any Acquisition panel, [Acquire] again.

Do not set the sweep width yet, because in the presence of the strong solvent line, we are not yet in a position to know the width of the ^1H spectrum of interest.

Suppressing the water line

In order to see the molecules of interest, present at mM concentrations, in the presence of 50 M water (90% x 55M), we need to suppress the water line. However the solvent suppression needs to be highly selective. Based on vs=100*vs the are other signals near water (**Figure 7**).

Under Experiments>Convert current parameters to do ....> Solvent Suppression - Select peaks choose 'WET'. A number of new options become available and the pulse sequence appears (**Figure 8**). In the top line, you see four shaped pulses. These are four repeats of a 90° pulse that is selective for water. Immediately after the first [1], when water has been selectively moved to the XY plane but all other magnetization remains along Z, we apply a large field gradient [2]. The gradient scrambles ('dephases') all XY magnetization, and thus substantially eliminates signal from water. This combination of excite-and-crush is repeated four times until the water magnetization has a net value of ≈ zero. Then the surviving magnetization, remaining along Z, is ecited as usual with a 90° pulse [3].

Make sure that the 'C13 Decoupling ...' is not active. [4]

Turn off Composite observe pulse [5]. In place of the default name 'wetshp' [6] we will make our own selective excitation pulse using Pbox, as we did last time.[1]

The value of the gradient strength [7] does not alter performance so long as the value is reasonable, and something near 2000 is.

Making water-selective our shaped pulse

To make a custom pulse, go to Edit>New Pulse Shapes (Pbox), select the 'New Waveform' tab and click 'New Waveform' (**Figure 9**). This time we'll use the boxes to establish the properties of our pulse. Choose excitation [1], I chose 'esnob' [2][2], then choose bandwidth 500 Hz [3] and leave the pulse length blank (to be calculated based on bandwidth). Make sure that Frequency offset is 0 [4] (in resonance = on water in this case). Confirm that the correct values of Reference pw90 and Reference power are in place [5]. Do NOT click on 'select from spectrum'. Just click on 'Add Waveform' [6] ('Wave #1 appears'). Enter a name (exc500HzOn_24Sep10) [7] and click on 'Make It !' [8].

Simulation reveals that an 800 Hz excitation bandwidth would probably be bad for the two signals nearest water.

---

[1] it is also fine to use wetshp and optimize it, since wetshp is a default on-resonance excitation pulse and we have placed water on-resonance.

[2] I got better performance with esnob,, but the slide was made when I was trying eburb1. I recommend esnob.

Note the pulse width and power recommended for your pulse by Pbox. In Acquire>WET, enter your pulse name [1], its width [2] and its recommended power [3]. (**Figure 10**). These are a great place to start.

For best water suppression, you will want to tweak the power used for the wet pulse. In Acquire>Acquisition, activate 'Arrays'. Under parameter name wetpwr [1] (**Figure 11**). This is the power given for the selective pulse. Create a linear array [2] with small step size [3] (eg. 0.5) that explores values both above and below the recommended wet pulse power. Note that this is one of the very few cases in which non-integer values are allowed for a pulse power. Acquire your series of spectra.

In Process>Display select Display Mode absval [1] (**Figure 12**). Display a single spectrum first [2]. Select and zoom in on the solvent line. Then use the left-hand panel to display the whole set of spectra side by side [3] with the wetpwr value displayed [4]. Choose the power that produces the best suppression (smallest water line). Reset the display to 'phased' [5]. Enter the optimum power for the wet pulse power (see Figure 21 [3]).

Collect a new spectrum using just the best power from your array. In Acquire>Acquisition set 2 scans [1] and activate 2 steady-state scans [2], with autogain turned on [3]. If autogain is able to use a power of 30 or higher, this is a sign that the water is well suppressed (**Figure 13**).

Processing to remove residual signal from water: Solvent subtraction

Processing can implement solvent suppression too. Because solvent is on resonance, its contribution to the spectrum is at very low relative frequencies (low relative to the carrier). The software simulates the FID to identify low-frequency components and can then subtract these out.

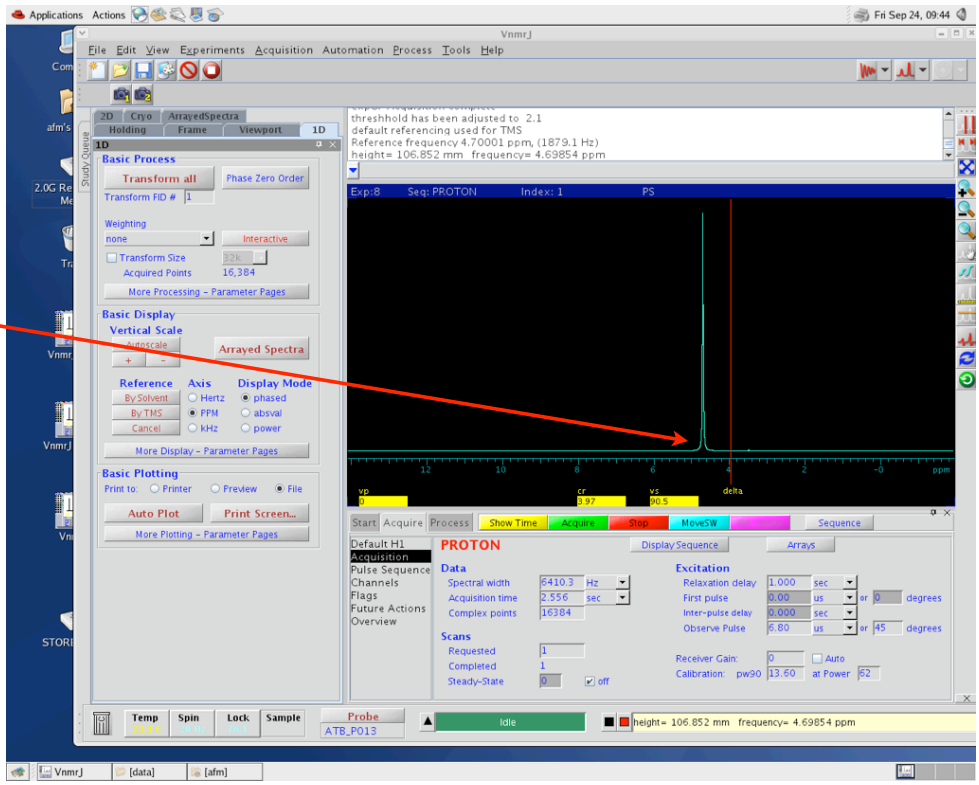
Without solvent subtraction, the solvent is still the largest single signal (**Figure 14**).

In Process>More1D activate Solvent Subtraction (**Figure 15** [1]). Apply this new processing component by retransforming (click on 'Transform' [2]). In this case frequencies of 50 Hz and lower are subtracted from the FID, essentially blanking the spectral region within 50 Hz [3] of the carrier frequency. You will have to tweak the phase again [4].

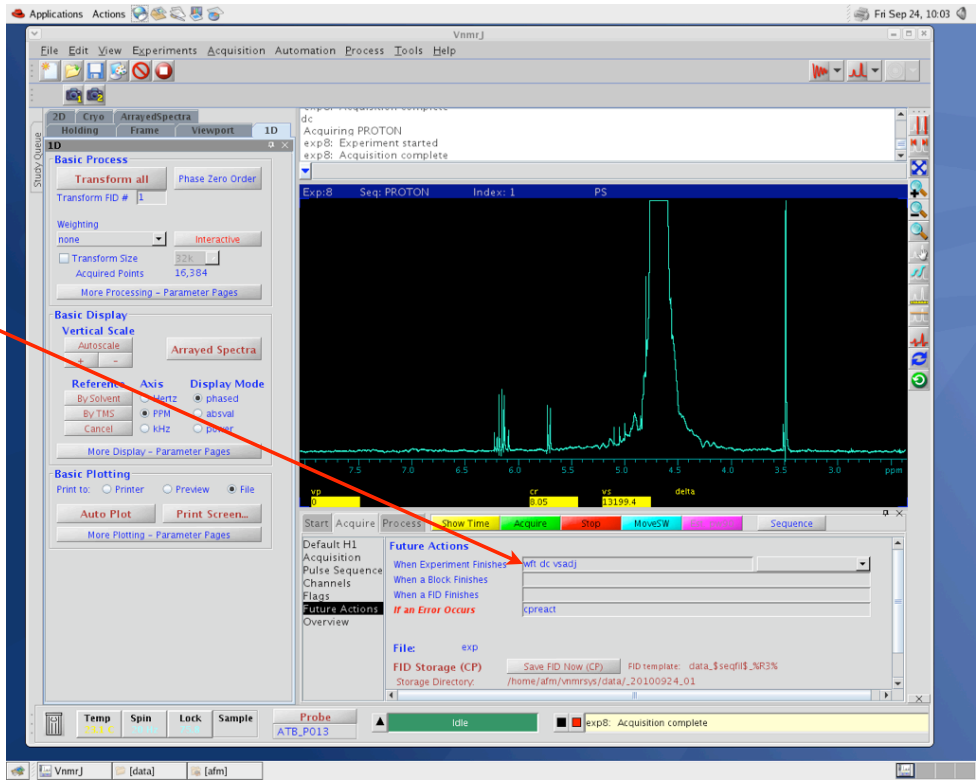
Figure 16 shows that you can now easily see even short lines, but the suppression of solvent has been sufficiently fast that exchanging resonances survive.

Solvent Suppression: wet 1D

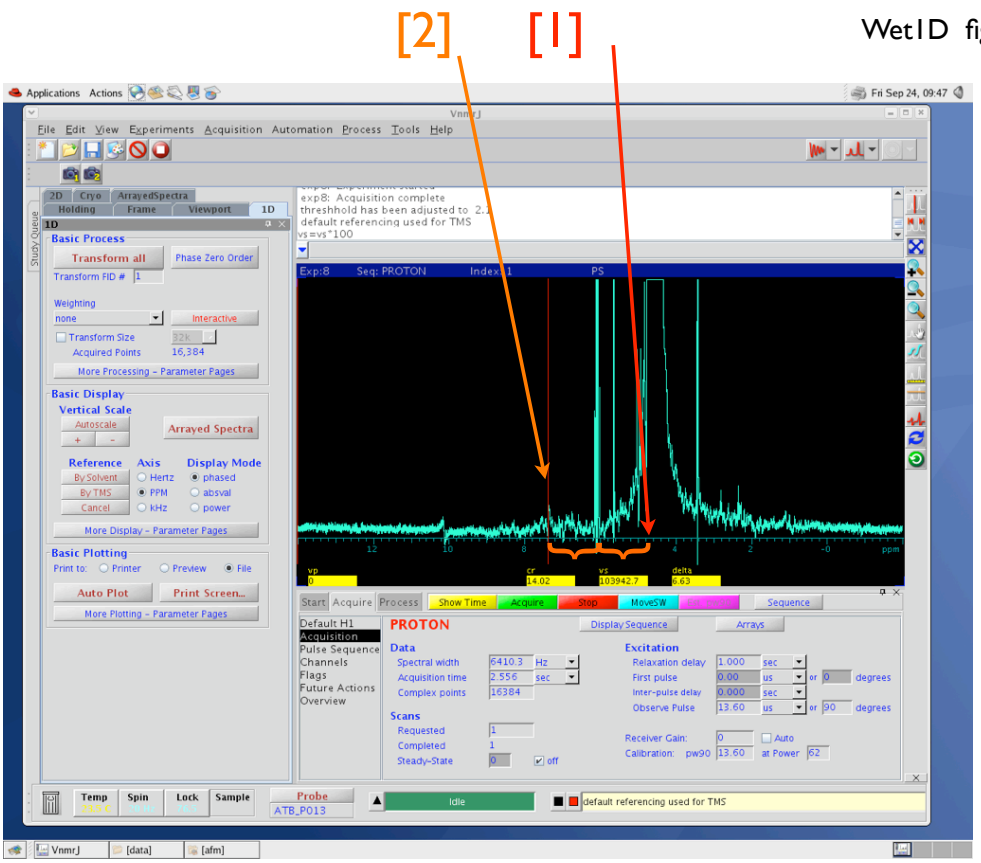
[1]



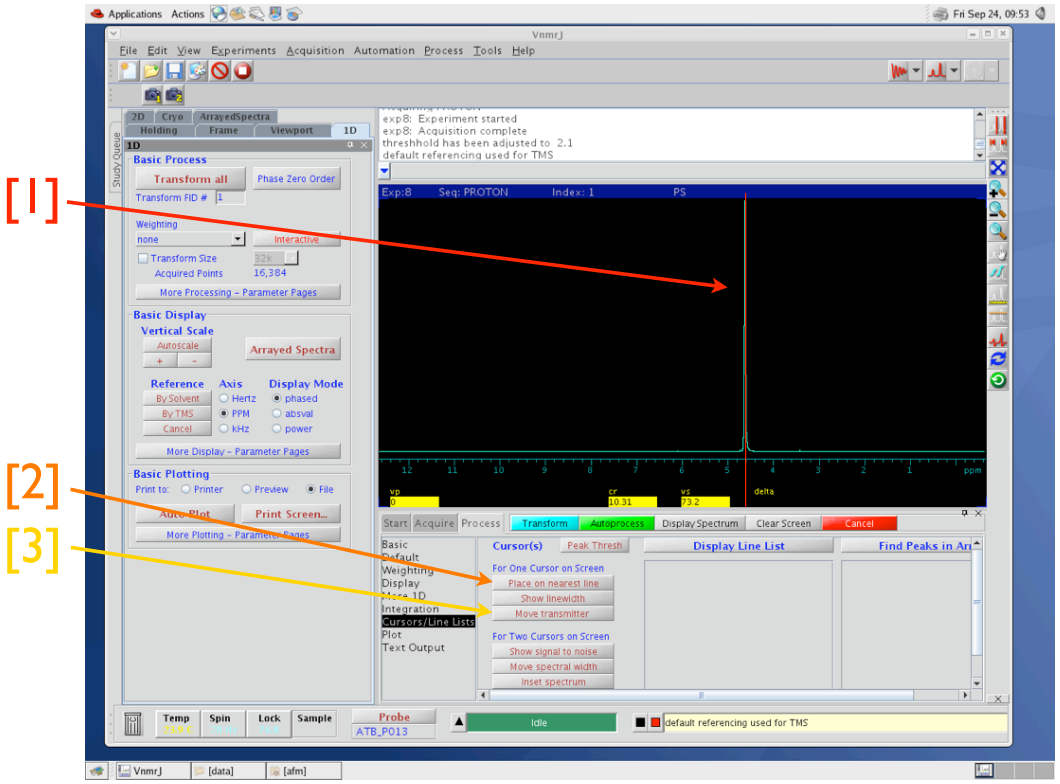
Wet1D figure 3



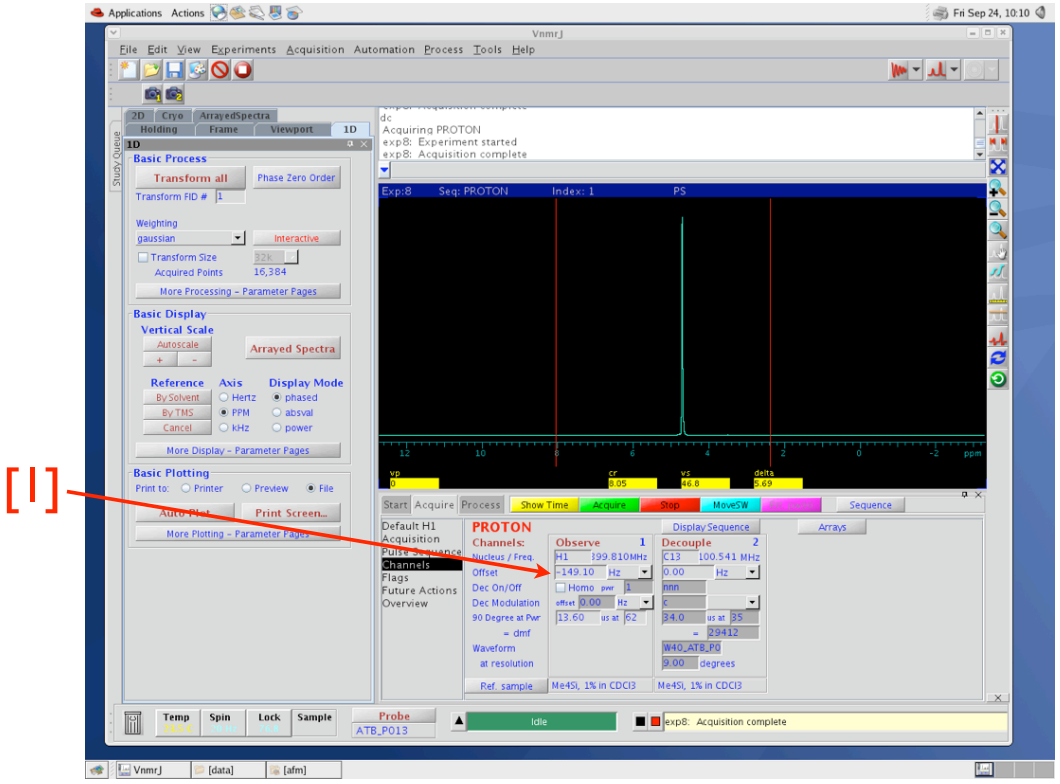
Wet1D figure 4



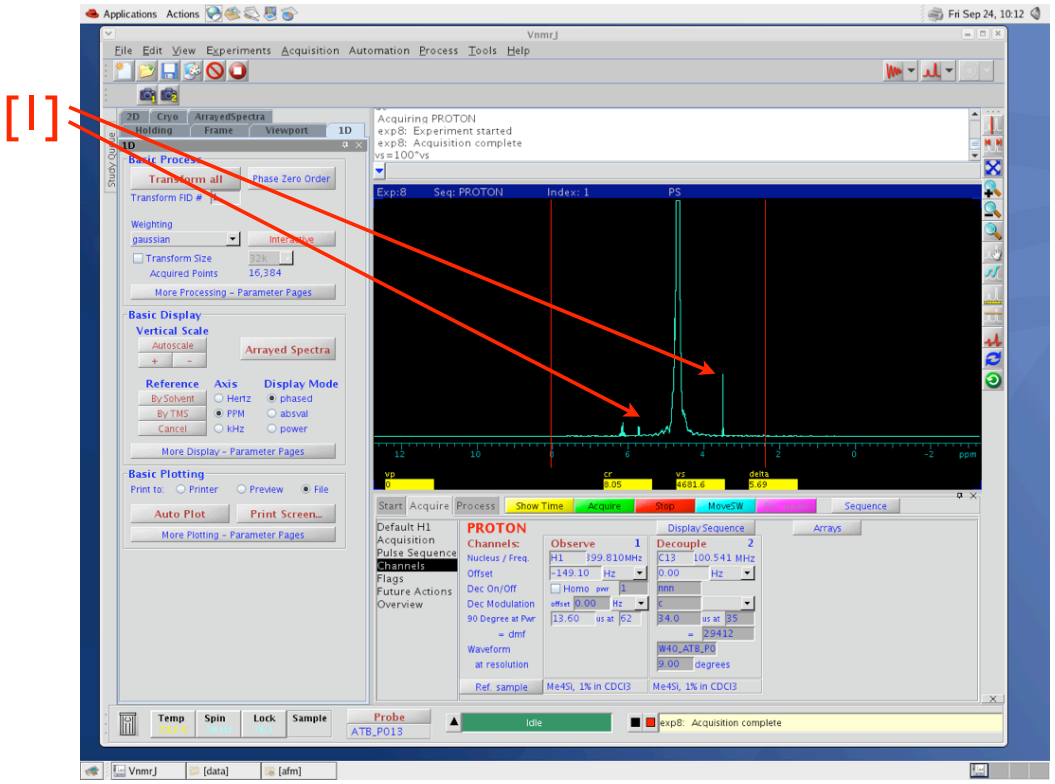
Wet1D figure 5



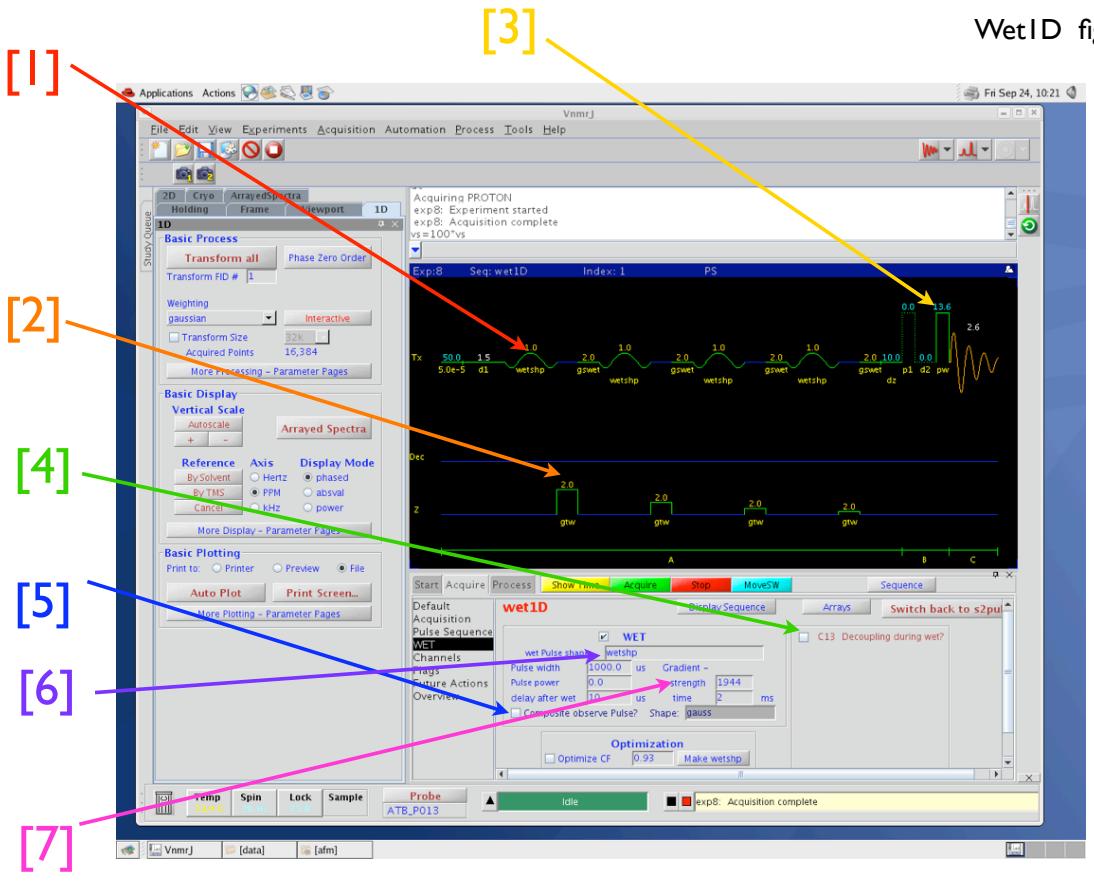
Wet1D figure 6



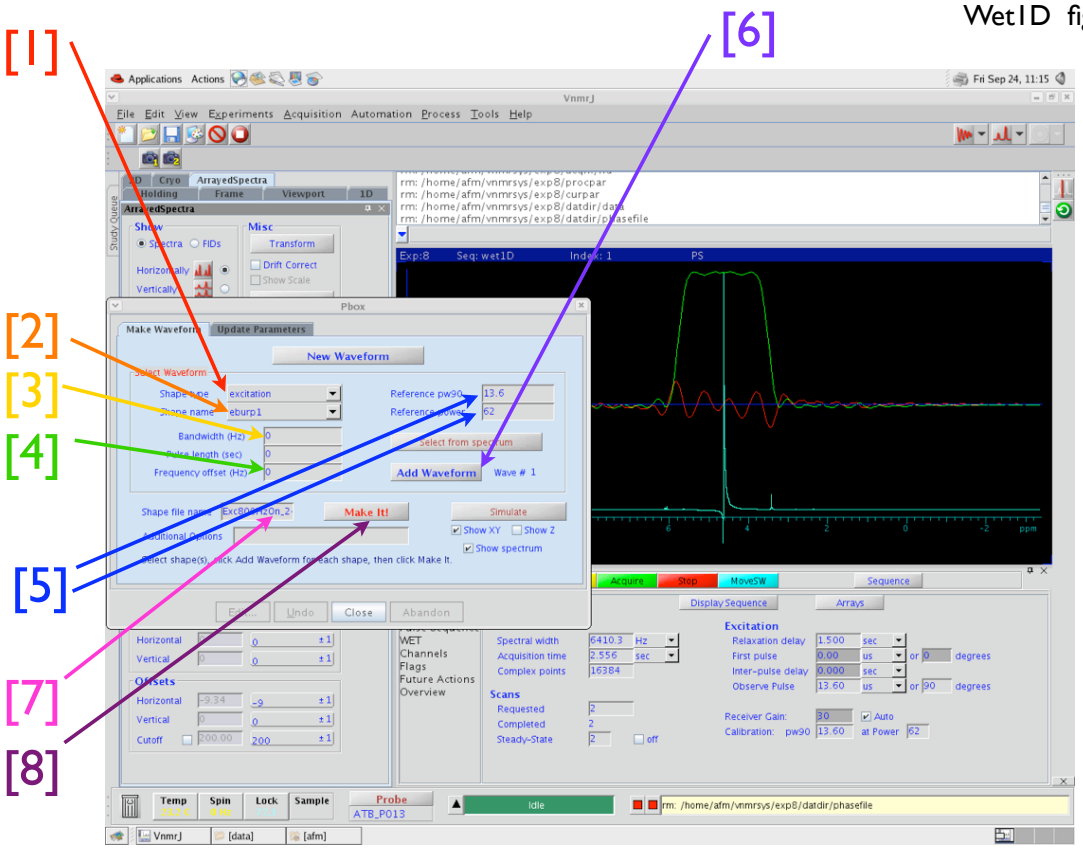
Wet1D figure 7



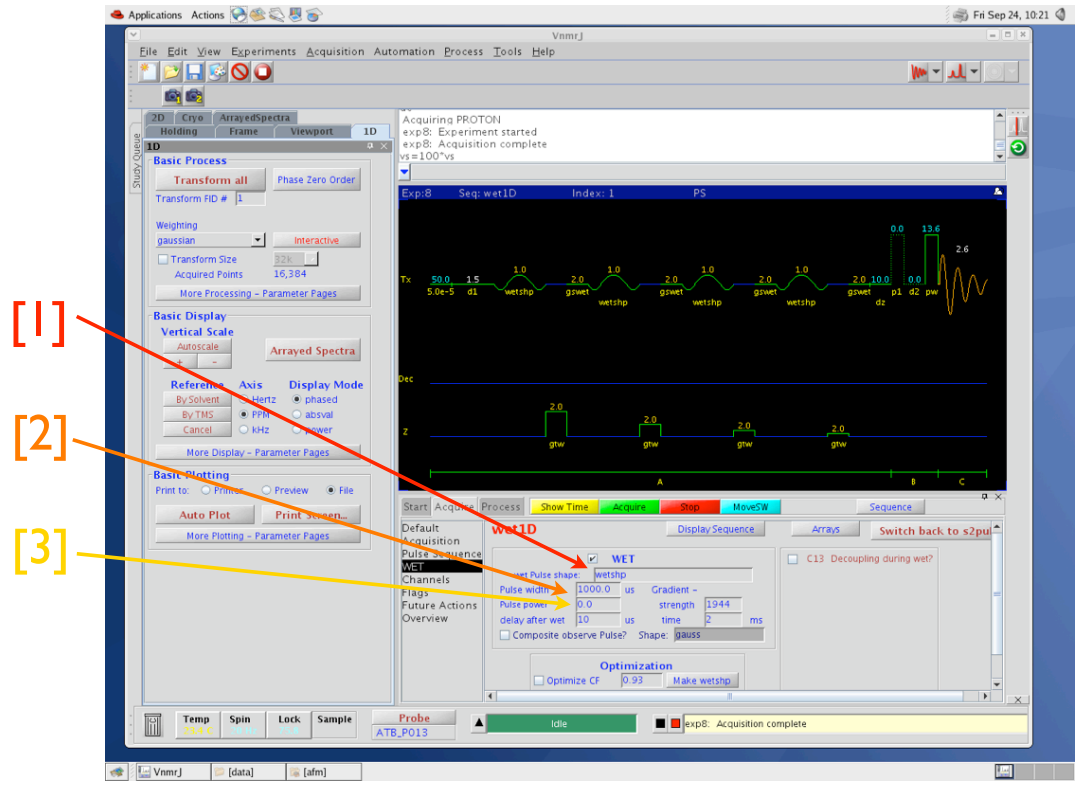
Wet1D figure 8



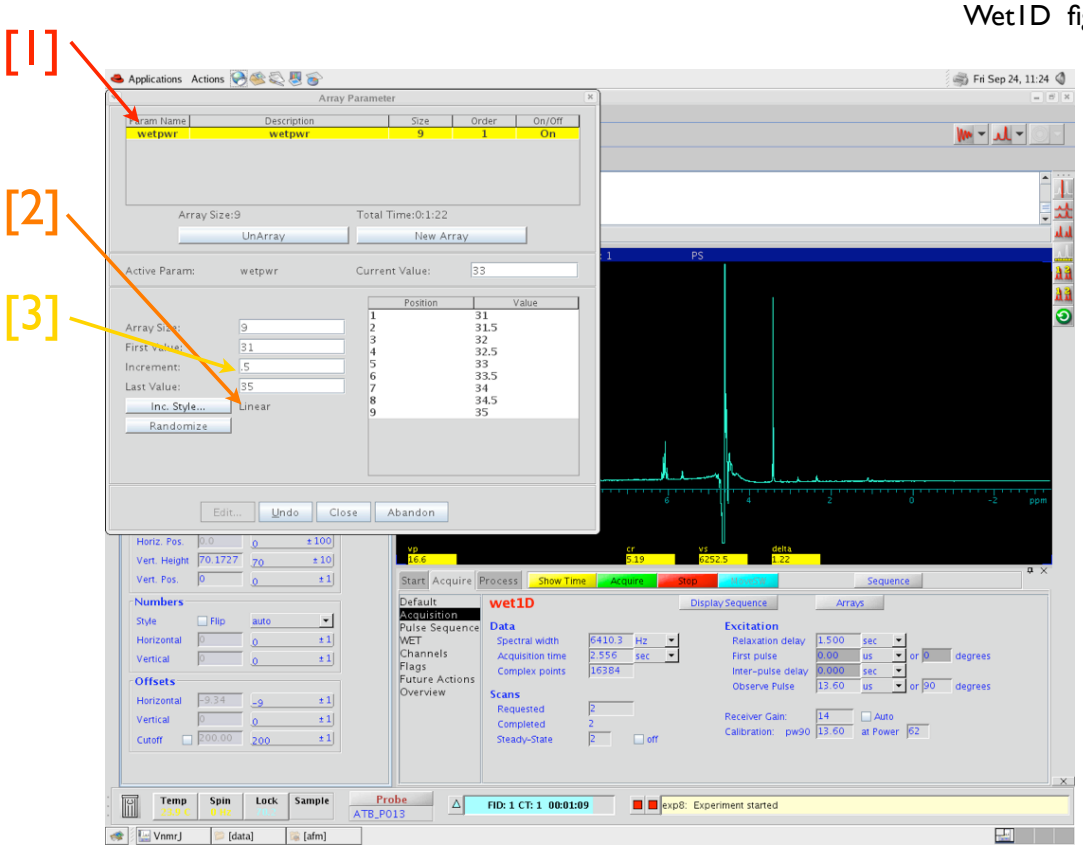
Wet1D figure 9



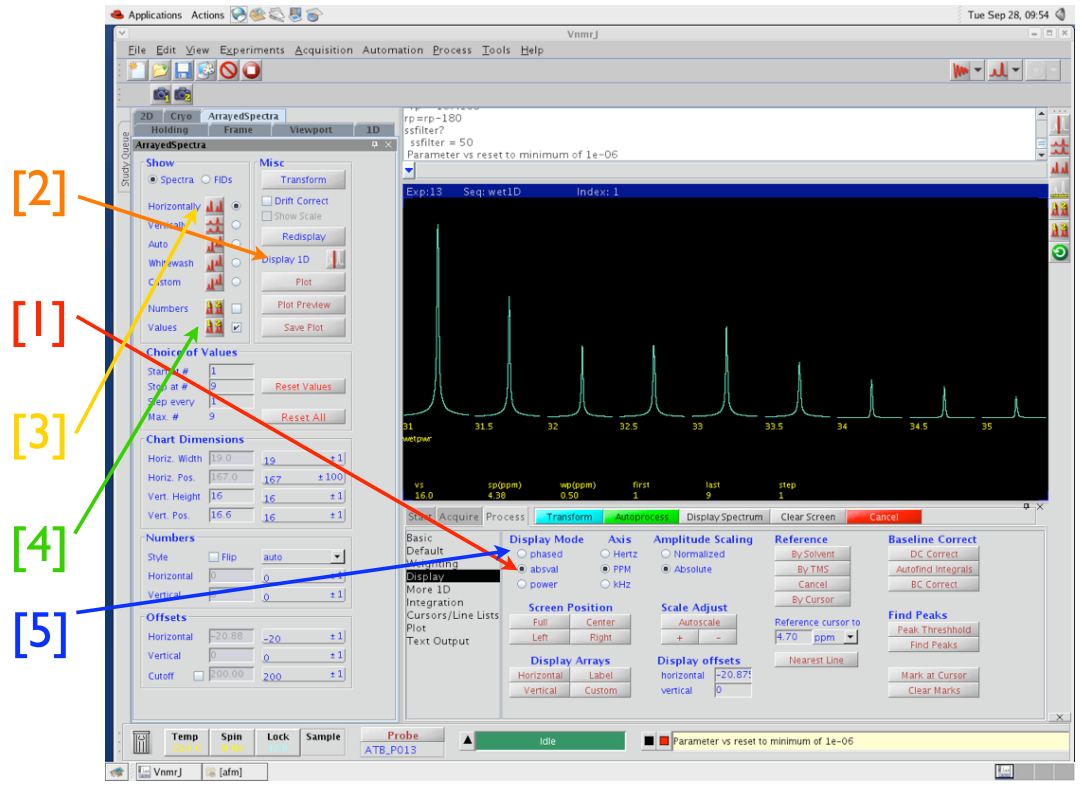
Wet1D figure 10



Wet1D figure 11

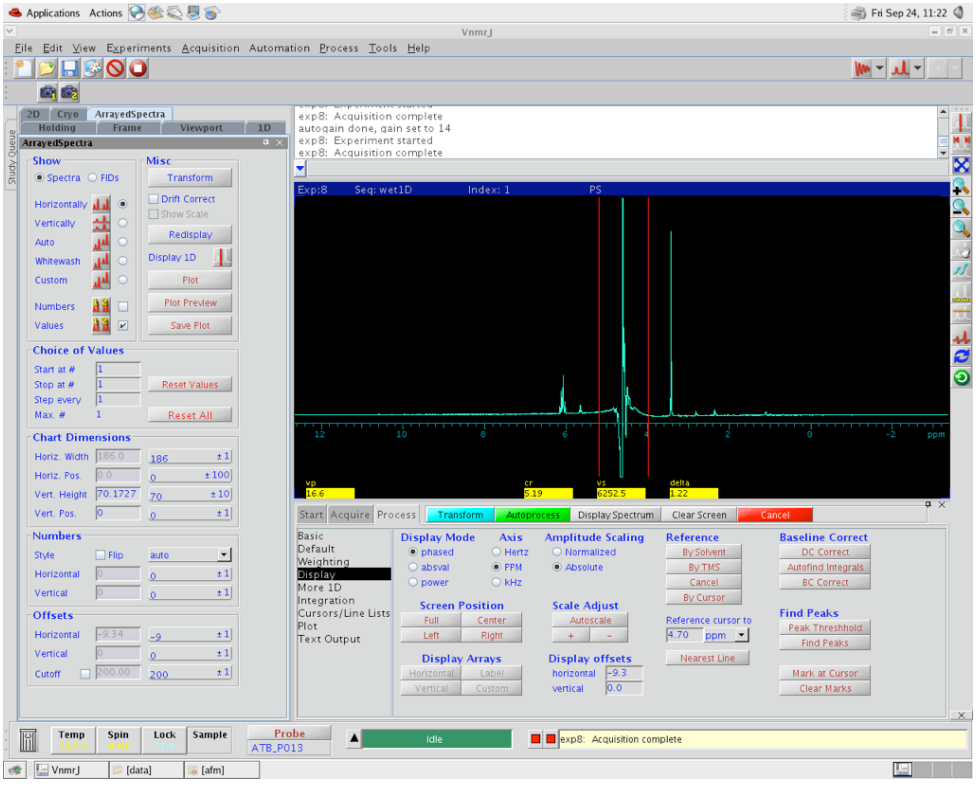


Wet1D figure 12

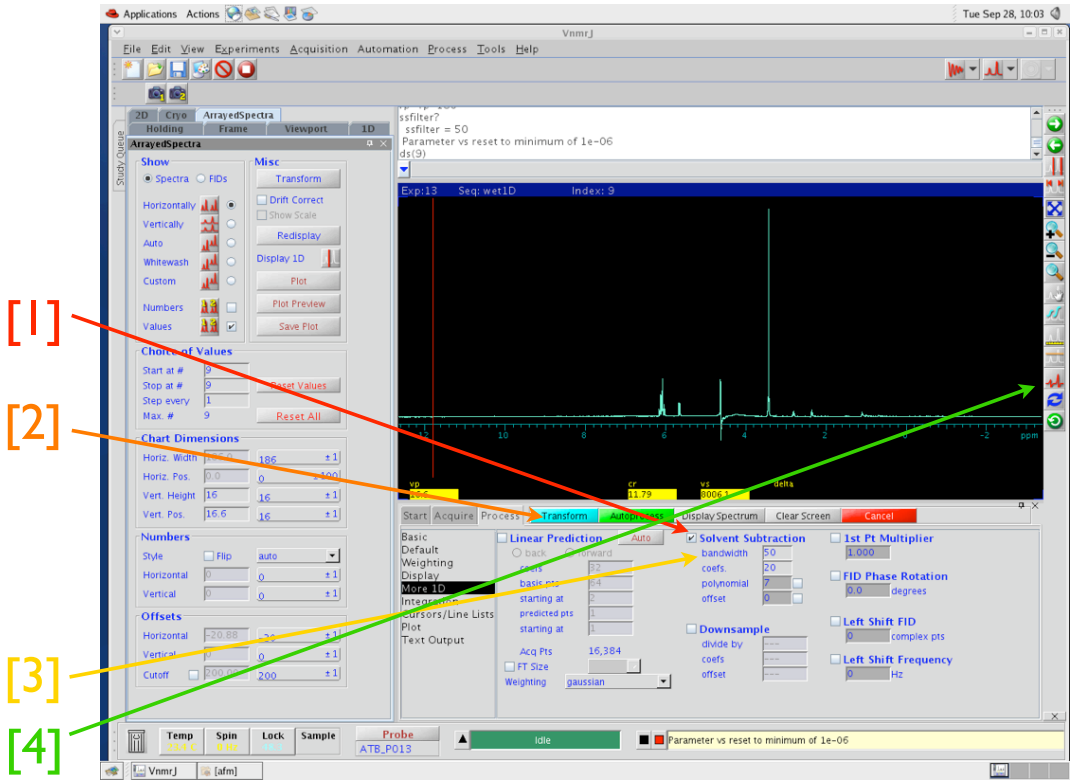


Wet1D figure 13

Wet1D figure 14



Wet1D figure 15



Wet1D figure 16