

## **Practicum 3, Fall 2010**

### **Measuring the longitudinal relaxation time: T<sub>1</sub>.**

Strychnine, dissolved CDCl<sub>3</sub>

The T<sub>1</sub> is the characteristic time of relaxation of Z-magnetization

Knowledge of T<sub>1</sub> enables you to make an informed choice of the duration of delay between scans. If too short a relaxation delay is chosen, then the peaks of slowly recovering protons will be smaller than they should be, and it will not be possible to compare peak areas to learn about proton numbers. Also, we use the T<sub>1</sub> as our first estimate for choosing mix times in NOESY spectra, and 3xT<sub>1</sub> as our relaxation delay in NOESY spectra. Hence the need to know T<sub>1</sub>.

Start with a beautiful 1d spectrum, and a calibrated pw90. (It turns out that the default value in the probe file is a good compromise for people in a rush or not bothered about perfection.)

Under the Experiments choose 'convert current parameters to do... > Relax. Measurements > T1'

This will retain your current sw, tof and pw90, but it will add a few features to the experiment to support measurement of the magnetization's recovery after inversion. To see the pulse sequence click Sequence [1] in **Figure 1**.

The first pulse is a 180 ° pulse (p1=2\*pw90) that will invert magnetization [2]. This places it in a high-energy state with respect to the field, so it will 'decay' back to the resting state. Time is allowed for this process, in the form of a delay called d2 [3]. So far all magnetization is still along Z, but its amplitude is recovering with time. To determine what the amplitude is after a given time d2, we use a 90 ° pulse (pw) [4] to 'read' our magnetization by rotating it into the XY plane where our receiver coils detect it [5]. We cannot monitor recovery continuously, instead we repeat the experiment 6 or more times, waiting a different amount of time d2 in each repeat before executing the read pulse. Thus we end up with a series of 1d spectra, each representing a different duration of recovery after the inversion pulse.

In Acquire>Defaults, a set of three boxes invites you to enter first-guesses of the T<sub>1</sub>s you sample may have, and Varian uses these to set the delays in the experiment to perform a measurement (you will have to use the scroll bar on the RHS of the panel to reveal these) [6]. Set T1 Mode to inversion recovery [7] (don't worry if the bar stays blank), then set T1 min to the shortest T1 you expect to be present among your resonances (say 0.2 sec.: enter 0.2) [8], the longest T1 you anticipate (err on the long side here, say 3 (seconds)) and the time you are willing to devote to the experiment (eg. 0.1 (hours)).

The min. and especially the max times will be used to choose an array of d2 values ranging from very short to something on the order of your maximum estimate. You can see these by clicking on 'Arrays'. [9]. The experiment takes a long time in part

due to the long relaxation time allowed between scans [10]. This is chosen to be  $\approx 5$  times your estimated longest  $T_1$ . The duration of the experiment also increases in proportion to the number of scans collected for each value of the delay  $d_2$ . Therefore, we will cut back on the number of scans if possible (I will change 4 to 1 [11]).

You may also want to go into the 'Array' panel and decrease the number of values of  $d_2$  used. Because we are expecting an exponential recovery, we anticipate that an exponential array [1] will be a better choice than a linear array (**Figure 2**). Thus in this case successive values of  $d_2$  are 2x the previous value, rather than being some value plus the previous value. The increment is the  $\ln(2)$ . If you want to triple each value to get the next one, use an increment of  $\ln(3)=1.1$ . If I am rushing I choose an array size of 6 (the minimum allowed) and an increment 1.1. The resulting array covers a factor of approximately 700. Thus for a longest estimate of 3 seconds I want a shortest value of 0.004. I go back and make adjustments to the first value and increment as needed to get the last value I want.

Make sure that Gain is a number, not autogain (Acquire>Acquisition)

As always, close the Array box, check Sequence, Show Time and then Acquire.

The result should be a series of spectra in which we see that spins are initially inverted and then gradually recover as the delay  $d_2$  is made longer (**Figure 3**).

In the LHS panel, with the 'Arrayed Spectra' tab active, change values in 'Chart Dimensions'. For Vert. Pos. use 20 [1] (from 90) Vert Height 300 [2] (from 900). Under Offset Vertical change this interactively (step size of 10) [3] with +10, it gives 17.7 (a good display) and in "Show" check the 'values' box [4].

You see that different peaks recover in different times. The null represents approx  $T_1 * 0.7$ . Divide null time by 0.7 to estimate  $T_1 \approx 1 \text{ sec} / .7 = 1.4 \text{ sec}$  for peak at 6 ppm.  $0.5 \text{ s} / .7 = 0.7 \text{ S}$  for aliphatics.

In Process>T1 Analysis (**Figure 4** [1]), click on Display Last Spectrum [2]. It should be upright as shown. If it is not, you will have to phase it manually (after activating the additional options on the right-hand-side of the graphical display window [3]). **Figure 4** shows a case where the software picked a ridiculous number of lines. Activate the action buttons on the RHS of the graphics display window [3] and activate threshold (**Figure 5** [1]). Move the threshold up to be more selective [2]. You can also use the cursors to restrict your analysis to one region of the spectrum by placing cursors around it and clicking the '+' magnifying glass [3]. Then click on 'Do T1 Analysis' [4]. All the lines the instrument picked will be in the table in the RHS box [5]. If you scroll all the way to the right edge of the box with the numbers in it [6], a second vertical scroll bar will appear inside the one visible in the figure. You may only be able to see the small down arrowhead at the bottom. Click on that to scroll up and down. First, all the picked peaks are listed and each is assigned an 'index' number, which will be used below to provide the  $T_1$  for the corresponding peak. Next there is a summary of the analysis in which each peak's  $T_1$  is provided

along with the associated error. Finally a detailed analysis follows for each picked peak.

Using the chemical shifts provided with the spectrum (**Figure 6** [1]) in conjunction with the numerical output telling you which index number corresponds to each chemical shift value [2] you can select a few lines and for them plot the peak amplitude vs. duration of recovery  $d_2$ . Next to 'Display Selected Fits' enter the line numbers for the lines you would like information on, for example "1, 10, 36" [3]. Then <return>.

A time course of magnetization recovery is produced (**Figure 7**).

Don't believe  $T_1$  values derived from curves that don't fit the data well. Don't believe any values whose errors are more than 10% the value (read the numerical output in the summary of analysis section). Don't believe a  $T_1$  that are more than one third of the recovery time you used between scans ( $=d_1+at$ ). If the  $T_1$ s you get are longer than  $(d_1+at)/3$ , repeat the experiment with a longer  $d_1$ , roughly 5 times the longest  $T_1$  you got.

To get a copy of the graphical output, use the LHpanel's 1D tab (**Figure 8** [1]), click 'Print Screen ...' [2].

In window that opens (**Figure 9**) select PSland, (•) file, give name with path (eg. che555/data/yourname/yourfile (omit the .ps), POSTSCRIPT, Mono/Color . . . Save, then Close

As usual, you can save your data set as a .fid file using the diskette icon in upper left. (**Figure 10**)

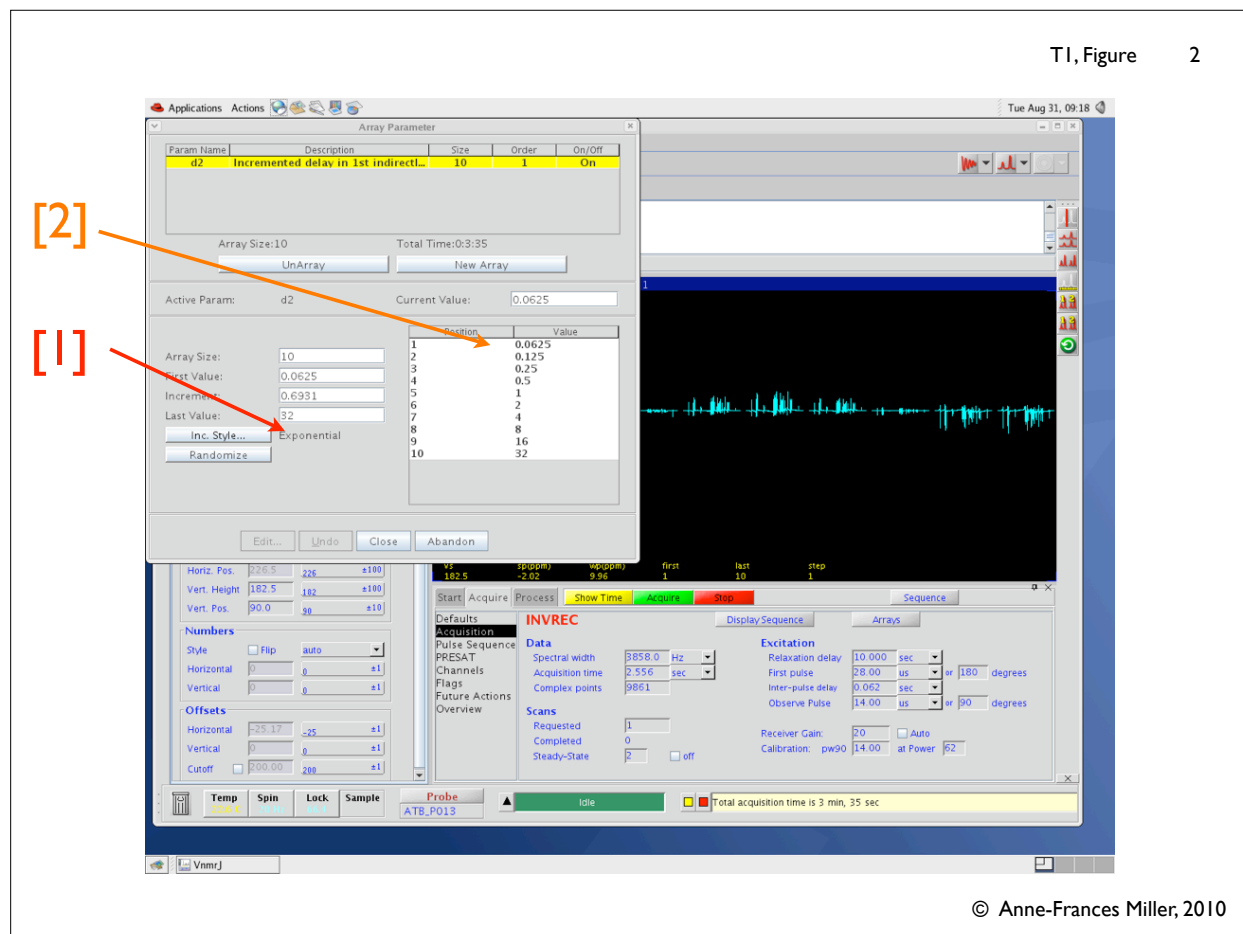
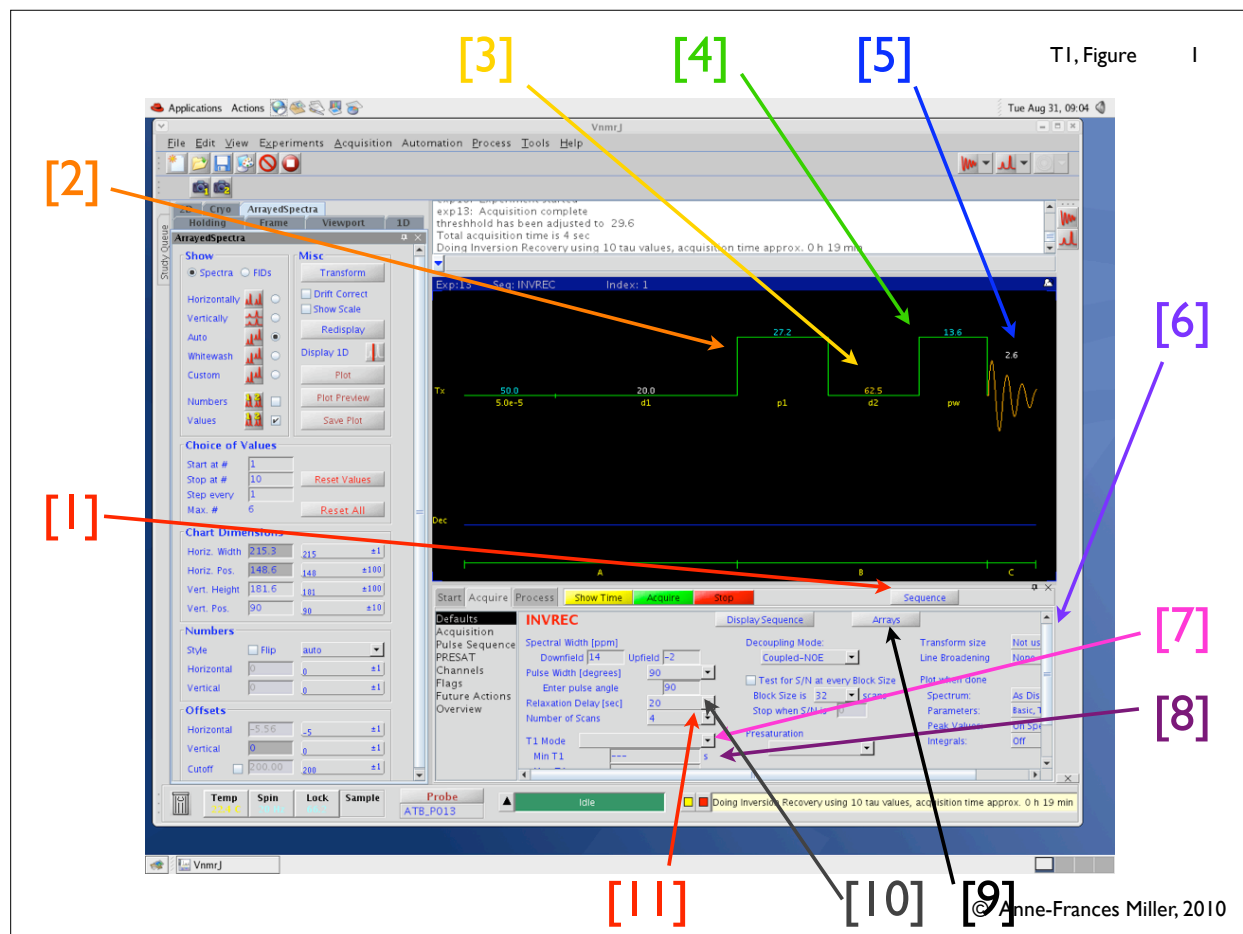
### **Illustration of why the relaxation delay must be longer than $T_1$**

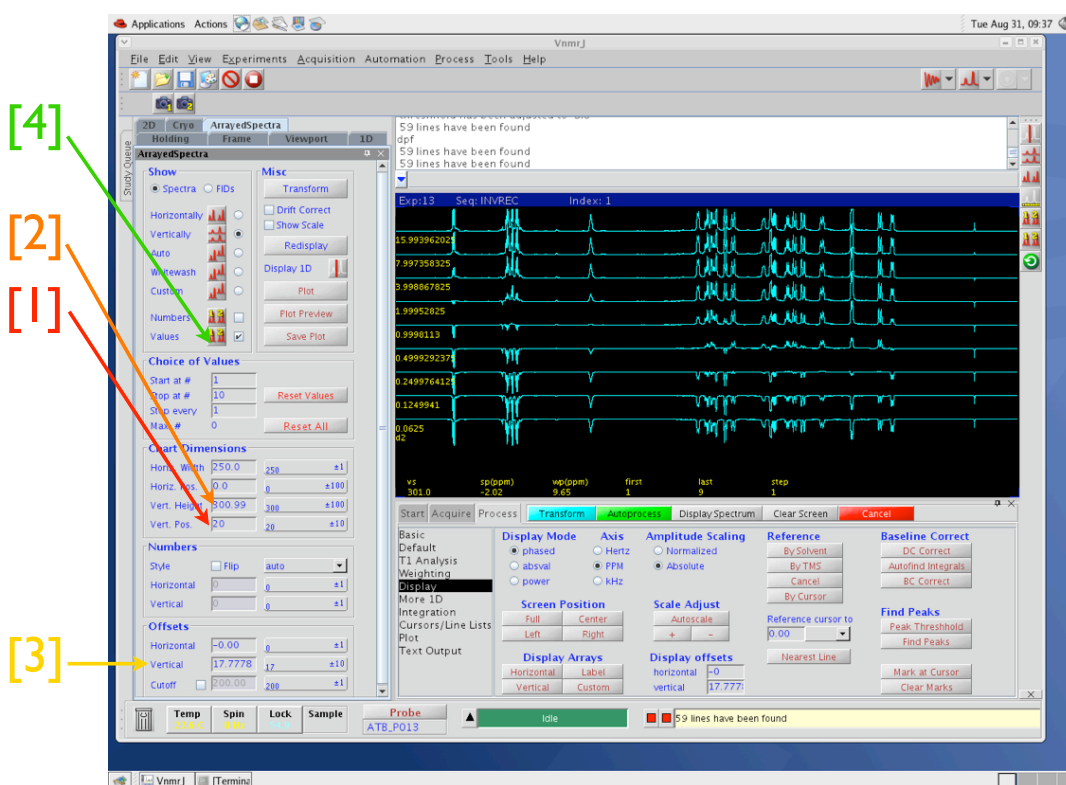
If you do not allow sufficient time between scans for resonances to recover, they will be smaller in subsequent scans. If the total recycle time ( $d_1+at$ ) is shorter than the  $T_1$ s of some resonances but longer than the  $T_1$ s of others, then the former resonances will be shrunk more than the others, in your spectra. Thus, peak areas will no longer be proportional to the numbers of protons associated with each peak. **Figure 11** compares the results of using four different  $d_1$  values all in combination with an  $at$  of 0.5. Thus, the total recycle times were 11.5, 3.8, 1.5 and 0.8 s (for  $d_1$  values of 11, 3.3, 1.0 and 0.3 s). The results show that the 0.8 s recycle time causes aromatic resonances, in particular, to be proportionately smaller (**Figure 12**). Note that the TMS line is also much smaller compared to the other lines when recycling is fast.

The above partial saturation of slow-relaxing resonances can be prevented either by choosing a longer ( $d_1 + at$ ) or by using a smaller tip-angle pulse. If you use a  $45^\circ$  pulse then you do not have to wait as long between scans, because magnetization does not need to recover as much.

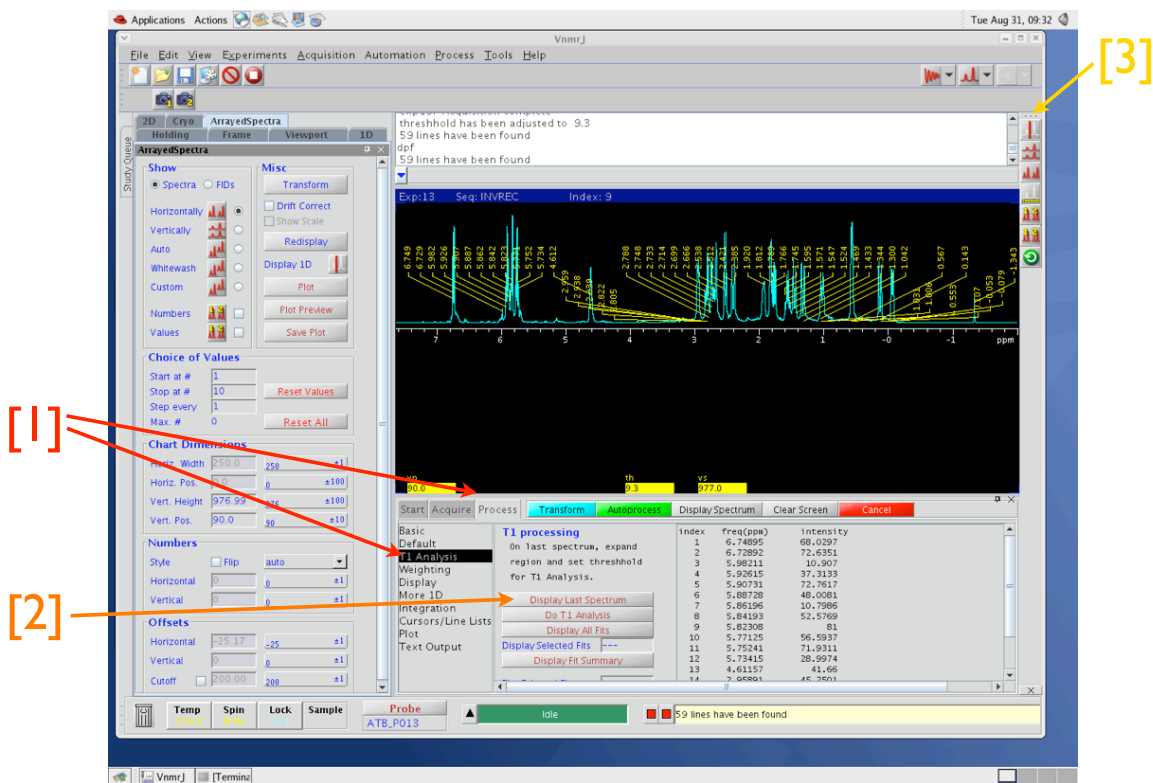
Using a smaller tip angle in combination with a shorter recycle time allows you to get the best data per hour of spectrometer time. This is invaluable for weak spectra such as  $^{13}\text{C}$  1ds, or samples that are not stable.

Once you know  $T_1$  the software can calculate the optimal pulse width and tip angle given a choice of d1. In the command line type '**ernst(3.4, 13.6)**' for the example of a case where the  $T_1$  is 3.4 s. and the pw90 is 13.6 us. Thus the general form of the command is '**ernst(t1\_est, pw90)**'. This causes the software will update the pulse width and tip angle of the experiment for maximal sensitivity per hour of spectrometer time, given the d1 value in your parameter set.





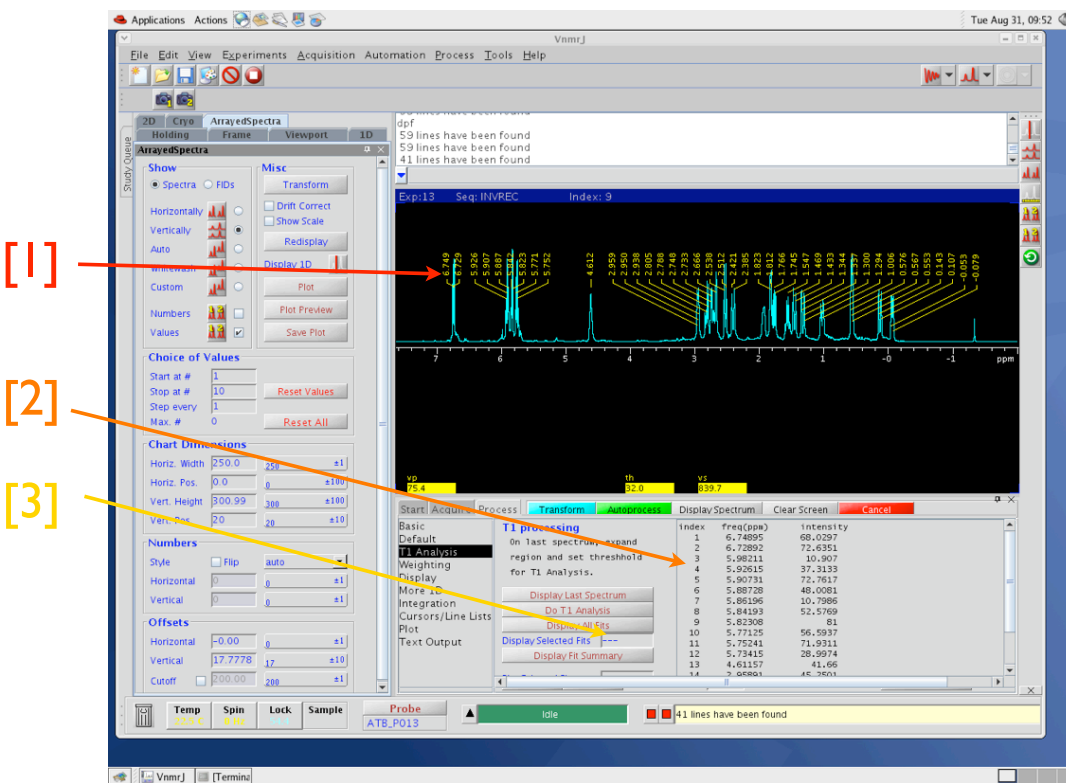
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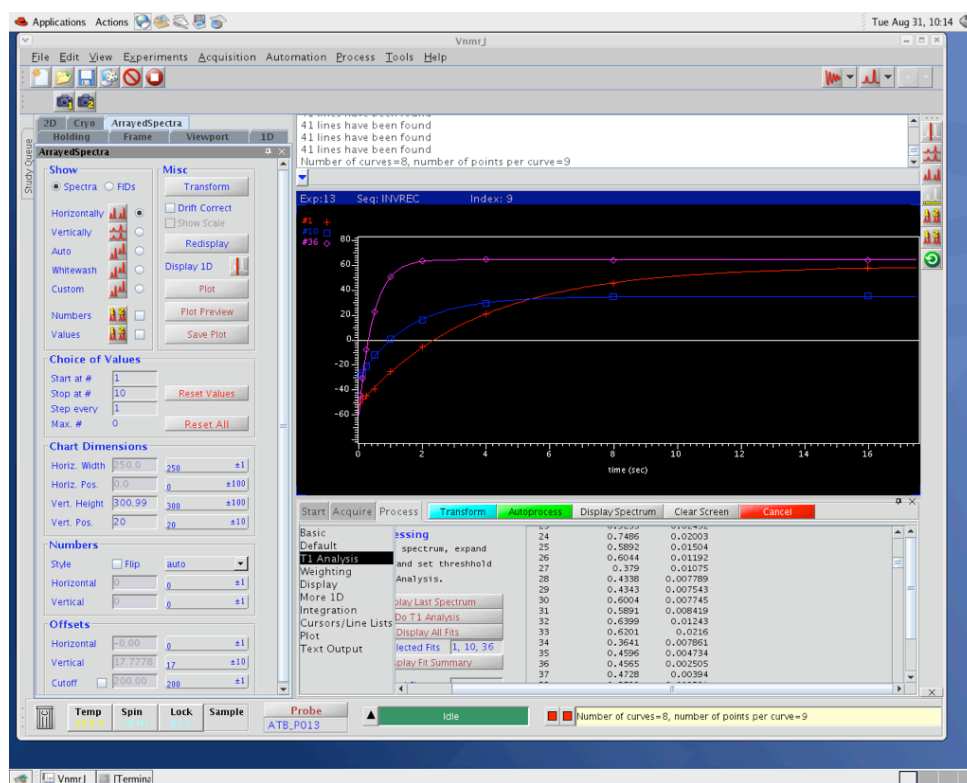
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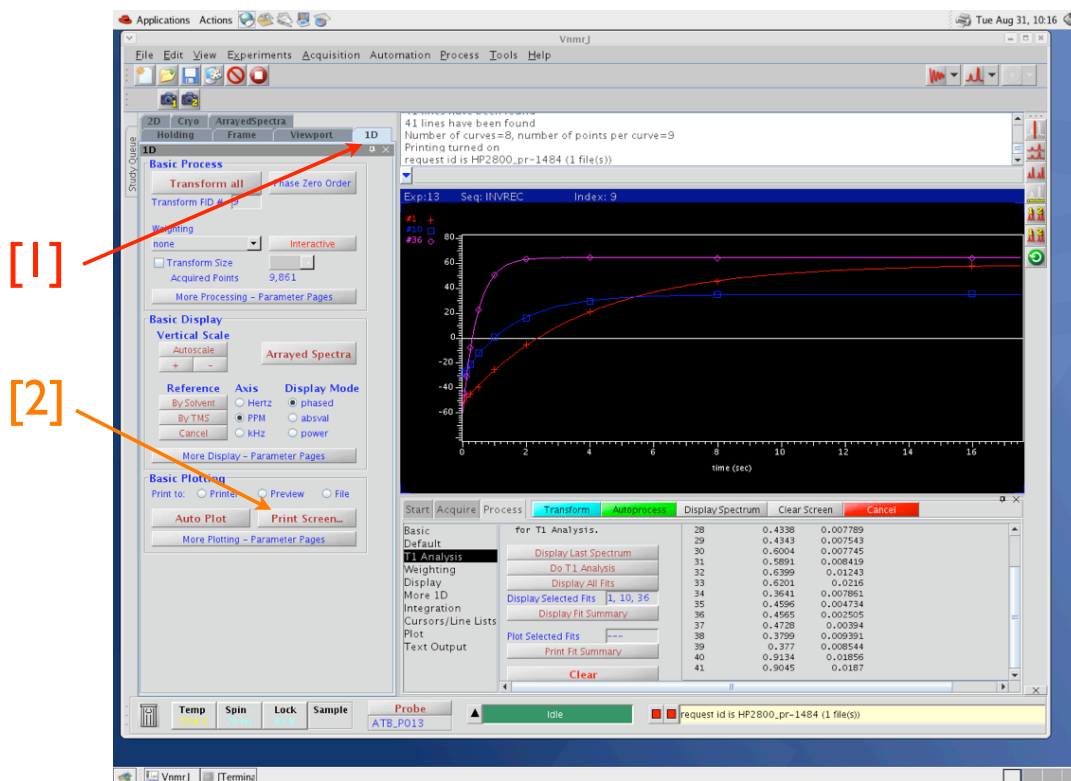
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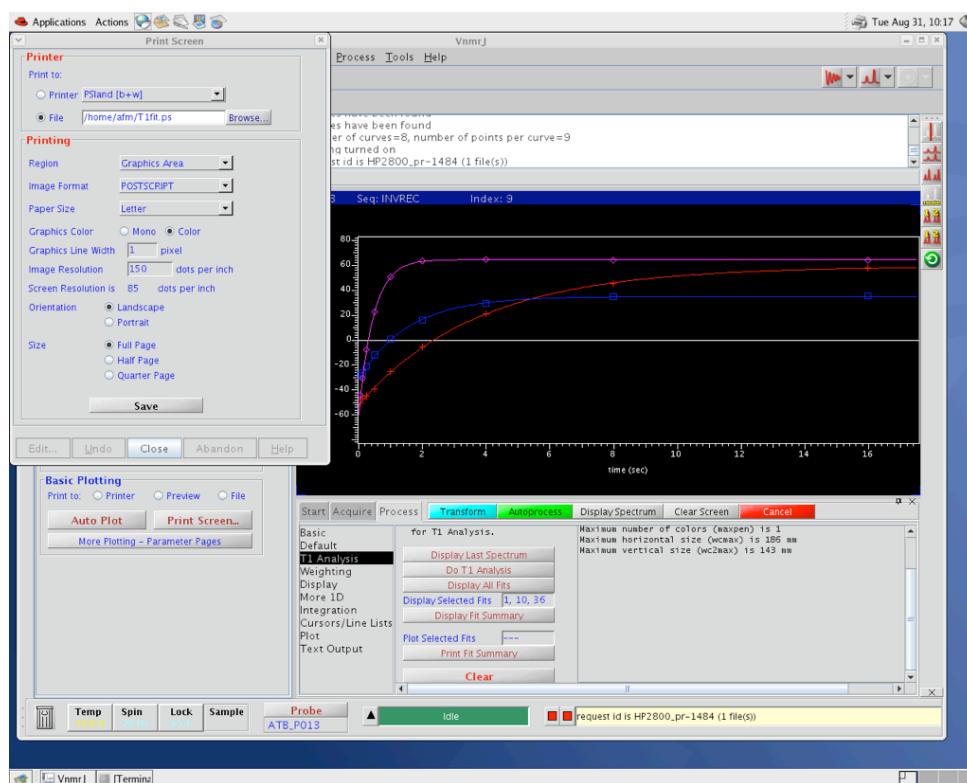


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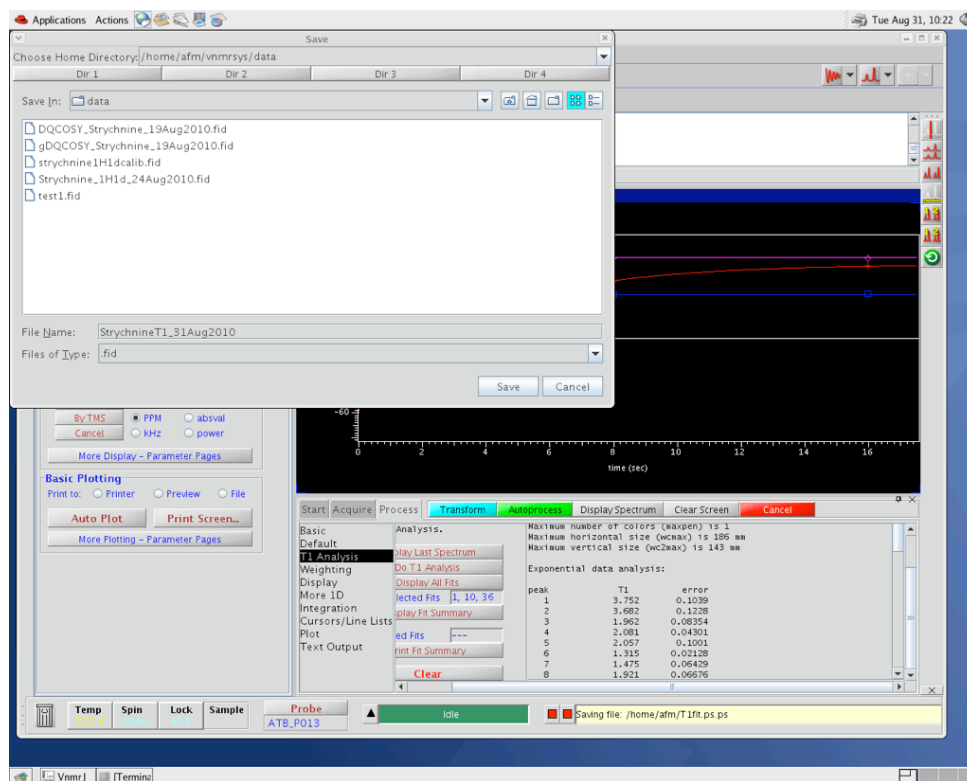


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