9. SPECTRAL PROCESSING

This video is about spectral processing. We've already acquired an FID, which is what we measure by pulsed FT NMR spectroscopy, and we no longer need the spectrometer; I may go to any computer with any up-to-date NMR processing software to continue with the next steps. As with all the videos and instructions in this series, what is demonstrated is neither the only way nor is it comprehensive – but it is sufficient to get you going. For this video, I will stay here at the DPX200 spectrometer. This is running Topspin 1.3 on a CentOS 5 operating system. Nonetheless, the ideas behind the spectral processing steps are generalizable to any software.

Are you ready? We're on step #9, processing. The Acqu tab is highlighted in the menu. This is our acquisition window. To go from the time-domain to the frequency-domain, we'll type “ft.” And the program has automatically changed to the Spectrum tab, which is now highlighted. I want to give a nod here to window functions. I like this interactive window display, where I can see the effect of enhancing the sensitivity at the expense of the resolution with this exponential function or enhancing resolution at the expense of the SNR with this Gaussian function. If my sample is still in the magnet, I usually skip the window functions, so that if I need to enhance sensitivity, I'll add a few more transients with the command “go”. Or, if I need to improve resolution, I'll try to shim even better and then start a new acquisition from scratch. Of course, if I'm processing at another date, after the sample is long gone, these window functions can still be used to improve the presentation of the spectrum.

Now we'd like to display the spectral lines as pure, positive absorption peaks. This is called the phase correction. We can type “apk” for an automatic phase correction. If we're still not satisfied, we can go into the interactive phase correction mode, with the first of these five processing buttons here. We know we're in phase correction mode, since we have this yellow icon here. We can't go into another mode until we leave this one. I will click and hold down the left mouse button on the zero. As I move the mouse forward and back, while still holding down the left-mouse button, it will affect the largest peak in the window around the red line. Once I'm satisfied with the phase. I'll mouse-over the one and hold down the left mouse button, and move my hand back and forth until the signal furthest away from the red line is well phased. These arrow will increase and decrease the sensitivity of the mouse. Once I'm satisfied, I'll click on this button decorated with an image of a floppydisk and return arrow to save the changes and to leave the phase correction mode. There, no more yellow icon in the corner. Where were the zero-order and first-order phase corrections saved ? Here in the ProcPar tab we've got two parameters: phc0 and phc1.

We have some other interesting parameters here. One is si, the number of points in the frequency domain. Do you remember the Topspin parameter name for the number of points in the time domain? It was td. We set si larger than td, for zero-filling, remember? This adds extra points, spaced dw apart, to the tail end of our acquired signal. After FT, those extra points intercalate between the experimental points, which can give a smoother appearance to the peaks of the spectrum, since the program is drawing lines between each of the points.

Over here are window function parameters. So to enhance the apparent sensitivity at the expense of resolution, I can multiply the FID by an exponential function before the Fourier transform. Let's choose something subtle, lb 0.3 Hz. Now how do I implement it? I'll type “efp” and the computer will first multiply the FID by and exponent, that's the “e”, then Fourier transform the FID, that's the “f” and then phase the spectrum using the phc0 and phc1 values, that's the “p”. Here we go: “efp” enter.

If I want to see the si points, I can right-click to pull up this menu and left-click to choose the display

properties. There we go. To zoom in: I place the arrow on one side of a peak, hold-down the left mouse button and move to the other side and then release.

One last processing parameter that I want to show you is this SR value, or signal reference. It's where the calibration of the chemical shift is stored. By using the lock interface inside Topspin, the chemical shift of chloroform should be pretty close to its literature value. Let's see how to set the chloroform to an exact reference value.

The second of the five processing buttons is the chemical shift calibration. First I'll zoom in on the chloroform peak. We'll take one of its literature values relative to TMS to calibrate the x-axis. Let's take a moment to see what hese buttons along the top do: *2, /2, *8, /8. This is an interactive scale button. I put the mouse arrow on top of the icon, hold down the left-mouse button, and then move my hand back and forth. There are three more interactive mouse buttons: this one for expansion, and I move my hand to the left and right with the mouse button depressed, this one for left and right shifting, and this one for up and down. These two buttons automatically give a full y- and a full x- display, respectively, one, two, to do the same as those two clicks together there's this button. And here we have, expand or zoom-in, and here zoom-out, and here exact zoom. What I use the most is the mouse itself. Again: the arrow on one side of a peak, hold-down the left mouse button and move to the other side and then release.

So, we were on calibration, I'll calibrate the chloroform signal to 7.26 ppm, according to one of the literature values. And when I write up the experimental section of my thesis, my poster, and my article, I will write that I calibrated the residual proton signal of the deuterated chloroform to 7.26 ppm. Let's check back on our SR value in the processing parameters tab. That's where the calibration is stored. Here's our new value.

The next processing step is to correct the baseline. The baseline is straight and is at zero, so the correction won't have much of an impact. I'll check that I'm displaying the full spectrum, here. And the automatic correction is done by typing “abs” and enter. There – and now the baseline is still straight and on zero. A well defined baseline is particularly important for integration of the peaks.

The next thing I want to do is called peak picking. I will type ppf and each peak now has a label with it's chemical shift. I can have more control by entering peak picking mode. I can draw a box. Modify the box. Manually peak a peak. Save and exit.

Now I want to know the relative areas of the two different peaks. What I haven't done is to make sure that each of the two peaks fully relaxed before acquiring the next FID, in order that there won't be some unknown scaling factor between the two proton signals. That's fine for a standard NMR spectrum. But if I need to quantify the relative peaks with any certainty, I will need to measure the T_1 relaxation time constant for the slowest relaxing peak of interest and then be sure to set my acquisition parameters so that aq + d1 is greater than or equal to 5 times T_1. Let's see how we integrate, regardless.

To integrate I should type “abs,” but we already did that for the baseline correction. To see the integration, I can go into integration mode with this button. There we are, yellow icon indicating that I am indeed in integration mode. If I right-click, I have some options, here. Save and exit, there.

Let me print a hard copy. I'll right click to display the title, integrals, peak picking. I'll arrange it on the screen how I want. And now I type “prnt” enter. That was the word print without the i.

In the next and final short video we'll use a different sample to quickly recap, plus a few bonuses.