# 3.1: Virtual Laboratory

This virtual laboratory makes use of the FTNMR simulation program written by Harold Bell to explore the effects of the parameters discussed in the Practical Aspects section of this module on the results of simulated NMR data. Although you are encouraged to explore this program more fully, the focus here is on the effects of applied magnetic field strength, signal averaging, relaxation times, repetition times, the number of data points and the receiver gain.

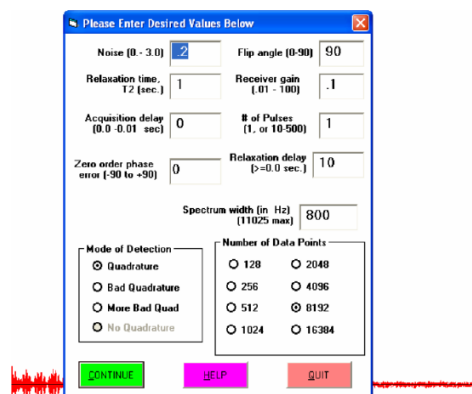1. Run the simulation program and select "Proton or Carbon System from the Menu" and click "Continue".
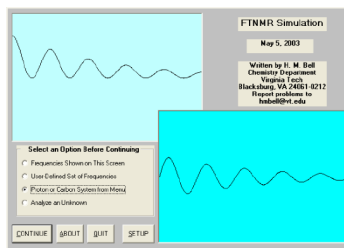
Choose one of the molecules from the proton menu. Compounds with a range of complexity are available and you may wish to investigate more than one molecule. In the new menu that appears, click the box that says "TMS in Sample?" Choose a NMR Frequency (magnetic field strength) from the list and click continue.
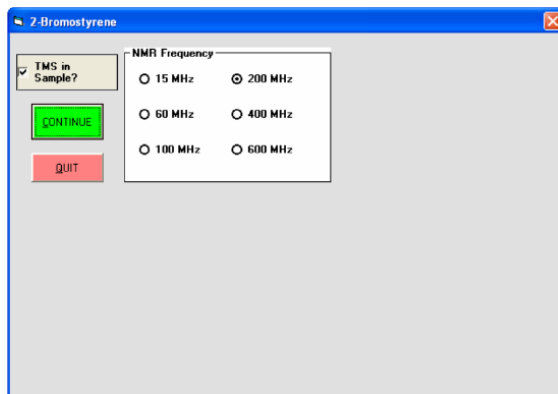
A new menu will appear (see figure below) that contains the resonance frequencies for the compound you selected. Click "Continue" and the coupling constants for these nuclei will appear. Clicking "Done" generates a list of the spectral frequencies. Click "DO FID" button to begin the process of simulating the FID.
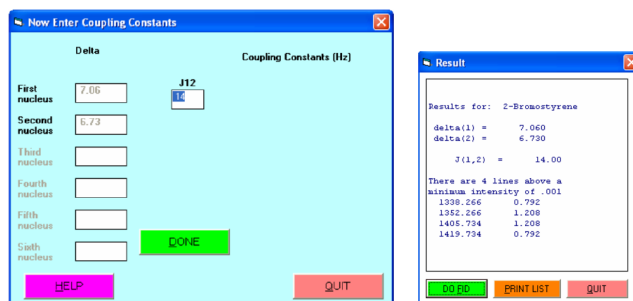
2. A new menu should appear that contains many of the acquisition parameters that we would like to investigate.

At this point there are some notable differences between the simulation and real data acquisition. This program allows you to add noise; this is not a feature of real spectrometers. Another difference here is that you can select the pulse width in degrees. If you were using a real spectrometer you would select the pulse power and calibrate the pulse width in μs that corresponded to the tip angle you desire. Start with these initial default parameters:

Default Parameters

- Noise 0.2
- T_2 Relaxation time 1.0 s
- Acquisition delay 0.0 s
- Zero order phase error 0.0
- Under "Mode of Detection", Click the box that says "Quadrature"
- Flip angle 90º
- Receiver gain 0.1
- Number of pulses 1
- Relaxation delay 10 s
- Use the default spectral window selected for your compound
- Number of data points 8192

Click the box at the bottom of the page to "Continue" and simulate the FID

3. A new menu should appear along the top of the page.
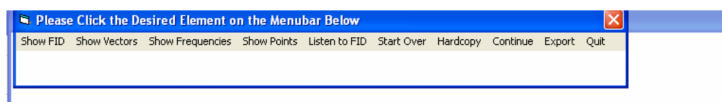
Click "Show FID" to display the FID you simulated. You will see that there are choices to show the whole FID or expansions of the FID with real or imaginary data points. If you click on "Show Points" you will see the individual data points that would have been acquired. Although the pulses we apply in NMR are in the radio frequency range, the digitized signals are on the order of a few thousand Hz. This places them in the audio range and you can hear your FID by clicking on "Listen to FID" button.

4. Click Continue and select "zero fill" and a new menu will appear.
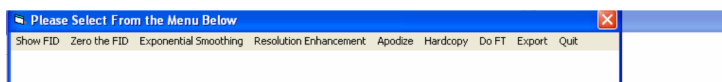
This menu accesses the data processing part of the simulation program. Choose "Exponential Smoothing" and use the value of 0.5 Hz line broadening and choose "Done". Answer "YES" to accept the smoothing function and apodize the FID. Select "Do FT" to Fourier transform the FID and generate the spectrum.

5. Now let's investigate the effect of the receiver gain. Choose "Start Over" and choose "Same Frequencies, Intensities and T_1's". Select gain values of 0.01, 0.1, 1, 10 and 100 and examine the effect on the FID and on the Fourier transformed spectrum. What is the optimum value of the gain? Can the gain be set too high? What is the effect of too high of gain on the FID? On the spectrum?

6. Using the gain you determined to be the optimum value, let's explore the effects of the number of pulses. Set the noise to 3.0 and repeat the simulation for 1 pulse. Now compare this result with 100 pulses. After selecting "100 pulses" and "Continue", a dialog box called "Pause" will pop up showing the FID after 1 pulse, select continue. You will now get more dialog boxes showing you how the FID looks after 10, 50 and 100 pulses. Do you see how the signal-to-noise ratio (S/N) improves with the number of FIDs coadded? How does the Fourier transformed spectrum compare with the one you measured using a single pulse? In this simulation, it is nearly just as rapid to coadd 100 FIDs as to measure 1, but with a real measurement it would take 100 times as long. In this case do you think that the S/N improvement would be worth the extra time? How would the S/N improvement for quantitative data affect the quality of the results you would obtain?

7. Now go back to 1 pulse leaving all the other parameters the same including a noise level of 3.0 and let's evaluate the effect of the exponential smoothing function. This time when you choose "Start Over" just select "Same FID" since this is a post-acquisition processing parameter and does not affect the saved data. Evaluate the transformed spectra using values of 0. 0.5, 1, 5 and 10 Hz line broadening. Which value do you think is the optimum? Why?

8. Click on "Start Over" and select "Complete Restart". This time choose "User-Defined Set of Frequencies" and press "Continue". A new menu should appear. Select 1-5 Single Frequencies". Enter a single frequency of 1.28 Hz. Choose a T_1 of 2 and intensity of 2, and press "Continue" and enter the default parameters from question #2 above. Click "Continue" to simulate the FID and then click "Show Vectors" and examine how the vectors relate to the FID. You may want to click "slow" on the vector speed bar to slow down the motion of the vector and the evolution of the FID. Fourier transform the FID and you should see a single resonance at 1.28 Hz.

9. Choose "Start Over" with the same frequencies, intensities and T_1's. Change the T_2 to lower and higher values. Examine the effect of T_2 on both the FID and the Fourier transformed spectrum.

10. In this exercise, we will explore the effects of the flip angle. Use the Default FID Parameters from question #2 above, except select a T_2 of 2 sec. Now investigate the following flip angle values: 30, 45, 60 and 90°. What is the effect of flip angle on the intensity of the FID and in the Fourier transformed spectrum? Can you use the vector model of NMR to explain the effect of flip angle on resonance intensity?

11. Now let's explore the effect the number of data points (this is related to the acquisition time in a real spectrometer). Choose "Start Over" with the same frequencies, intensities and T_1's. Use the Default FID Parameters except select a T_2 of 2 sec and a noise value of 0.1. Investigate the effect of the number of data points by choosing values less than and greater than 1024. What is the effect on the transformed spectrum? Why does the use of too few data points produce artifacts in the transformed spectrum?

12. With quadrature detection, the analog signal is split into two FIDs that differ by a 90° phase shift. These FIDs form the sine and cosine inputs to the complex Fourier Transform. Choose 512 points and click on "Show FID". You should have two possibilities, real and imaginary. How does the real FID differ from the imaginary one?

13. Now let's see what happens if we have more than one resonance in our FID. Click on "Start Over" and select "Complete Restart". This time enter two frequencies 1.28 Hz, and 2.56 Hz choose an intensity of 1 and a T_1 of 2 sec for each resonance. Click "Continue". Follow the instructions above for "Default FID Parameters" except choose 2048 data points and generate a new FID. Click on "Show Frequencies". You should see the individual components of the FID. Can you see how the two waves add to produce the FID? Now click on "show vectors" and examine how the vectors relate to the FID. Perform the Fourier transform and examine the spectrum. Does this spectrum make sense to you? Start over and choose some other frequencies. How does the choice of frequency affect the FID, vectors and the transformed spectrum?

14. Now let's explore the effect of Spectral Width. Choose "Start Over" and "Complete Restart". Choose a single frequency of 2.56 Hz, intensity of 2 and a T_1 of 2. Begin by using the "Default FID Parameters" listed above with a 100 Hz Spectrum Width to generate the FID. Now click on "Show Points". At this level of expansion, it is not possible to really see the individual data points. Select "Show FID" and choose "0.8 sec". Now you can better see that there are discrete data points that sample the FID. Now choose "Start Over" and choose "Same Frequencies, Intensities, T_1" and examine the effect the spectral width has on the FID. Reduce the value of the "Spectrum Width" to 20, 10, 5, 2 and 1 Hz expanding the FID (show the first 0.8 sec) to show the individual data points. What is the effect of changing the spectral width on the transformed spectrum? What happens if you choose a spectral width of 2 or 1 Hz? Is the frequency of the peak the expected value of 2.56 Hz?

15. Now let's examine what happens if our acquisition parameters do not allow for complete relaxation of the magnetization. Start completely over and again let's use 3 resonances with frequencies of 0 Hz (20sec), 5 Hz (5sec), and 10 Hz (1sec) all with intensities of 0.5 and with the T_1 values given in parentheses after the frequency. Use the "Default FID Parameters" except choose a relaxation delay of 100 sec, 10 pulses, a T_2 of 0.3, a spectrum width of 100 Hz and 1024 points. Fourier transform the FID to examine the relative intensities of the resonances in the NMR spectrum. Choose "Start Over" selecting the "Same

Frequencies, Intensities and T1's" and examine the effect of signal averaging on the intensity of the resonances by reducing the relaxation delay to 20, 10, 5, 2, and 0 sec, and Fourier transforming each FID (remember that in each case, the magnetization does relax during the acquisition time). What is the effect of reducing the relaxation delay on the relative intensities and on the overall S/N ratio.