


Module 1 Experimental Procedures

Experiment 1. What's in the Spectrometer?





- Identify elements of the spectrometer shown in Fig. 9 in the open spectrometer unit and identify the path of the light.

Experiment 2. Lambert-Beer's Law

- Prepare 0.01, 0.02, 0.03, 0.04 and 0.05 M aqueous solutions of CoCl_2 in volumetric flasks. When making up your solutions, note that you will be using cobalt chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) and be sure to use the proper molecular mass in your calculations. Your TA will prepare a solution with unknown concentration, between 0.01 and 0.05 M.
- Make sure that the absorbance spectrometer is plugged in and connected to the computer via USB port—you should be able to hear its fan running—and open the SpectraSuite program. Fill a cuvette with water, wipe the sides of the cuvette with a Kimwipe, and place the cuvette in the holder
- Under the Spectrometer menu select Rescan Devices. Find the absorbance spectrometer picture, expand menu [+], and expand Properties [+] to check that the serial number corresponds to the spectrometer. Right click on any other spectrometers and select Remove Spectrometer. If there is no graph displayed on the screen, right click the spectrometer picture and choose Show Graph.
- Click the Pause [II] button and change the parameters at the top of the screen to Integration Time = 10 ms, Average = 10, and Boxcar = 10. Check the Electric Dark Correction box. This box should remain checked throughout all experiments.
- Click Play [▶] to begin the acquisition. When you no longer see major fluctuations in the spectrum over time, click on the dark light bulb [💡] to store the dark reference spectrum. Check the Strobe/Lamp Enable box and repeat, clicking on the yellow light bulb [💡] to store reference spectrum. Now the Absorbance [A] icon should be accessible. Click it to enter absorbance mode and then click Pause.
- Remove and empty your cuvette. Wash with your first sample solution, empty, and then fill with the solution. Place in holder.
- Take a spectrum by clicking Play, then clicking Pause when you feel you have a good spectrum. You can change the viewing parameters of the graph using [⊞] to auto-scale, [↕] to vertically scale the spectrum, [🔍] to manually set the zoom parameters, and [↕] to toggle and pan the spectrum. You can also zoom in and out of the graph using the scrolling wheel on the mouse.
- Click the Save [💾] icon to save this spectrum. **Don't access this function through the File menu.** Under Desired Spectrum, select Processed Spectrum and make sure that the file type is ".001" and the appendage to the file name is ".ProcSpec" before pressing Save. If the Save button isn't activated, try clicking elsewhere in the Save window first.

- Remove, empty, and rinse the cuvette with your next sample. Fill the cuvette with the new solution, acquire a new spectrum, and save. Continue for all samples of solution.
- After all spectra have been taken, click Overlay Spectral Data [] icon and select one of your saved spectra. Select Processed and then click . Repeat for all of your processed spectra. This should overlay all of your spectra onto the same graph.
- Click on the spectrum graph area, then type “510” into the Wavelength (nm) text window at the bottom of the screen and hit enter. The absorbance values of your overlaid spectra at 510 nm will appear to the right of the text window in their respective colors. Record these values in your notebook.
- Do a linear fit to the function $A = f(c)$. Read off the value of the absorbance for the unknown sample and using the fit calculate its concentration. What is the approximate uncertainty of the measurement? What do you think the biggest sources of error are?

Experiment 3. Aromatic Hydrocarbons

- Prepare a cuvette half to two-thirds full of hexane, wipe the sides with a Kimwipe and place in holder. Set up the instrument using the parameters in Experiment 2. Obtain and store the dark spectrum and the reference spectrum of hexane.
- Add a drop of saturated naphthacene to the hexane cuvette and acquire a spectrum. Scale the graph such that you can resolve peaks, and continue to add naphthacene dropwise until peaks are very prominent. Save the spectrum and repeat the procedure for anthracene and naphthalene. It is not necessary to take a reference spectrum each time, but be sure to rinse the cuvette with hexane each time.
- Open your saved spectra. Right click on spectrum graph and select “Graph Layer Options”. Under the Peaks tab, select your spectral line from the Spectrum Source Selection window. Select the Show Peak Baseline Layer and press OK.
- At the bottom right corner of the graph, select the Peaks [] icon. The Peak Finding toolbar should appear directly above. Select the Configure [] icon in the Peak Finding toolbar to open the Peak Properties window. Set the Baseline absorbance somewhat below the peaks of interest and increase the Minimum Peak Width until the number of peaks found approaches the number of significant peaks you see on the graph. Click “Close” to exit the window.
- Using the Previous and Next Peak [ ] buttons, position the green cursor on each of the significant peaks and record the wavelengths (from the Wavelength box in the Peak Finding toolbar) in your notebook.
- Use the wavelength of the peak with the highest wavelength as the photon energy for the $n=1 \rightarrow n=2$ transition. Using the one-dimensional Particle-in-a-Box model, estimate the length, l , of each compound. Plot the length as a function of the number of coordinated hydrocarbon rings.

Experiment 4. Fluorescence of Quantum Dots

- Take absorption spectra of three samples of quantum dots using the procedure described earlier.
- Locate the fluorescence spectrometer. Check that the Xe light source is plugged into an electrical outlet, in pulsed flash mode and on. Place a cuvette with four clear windows containing quantum dots in a stand-alone cuvette holder. The source and detector should be plugged in to the holder at 90° angle.
- As before, make sure that SpectraSuite is recognizing the right device by using Rescan Devices, checking that the serial number matches, and removing any other spectrometers from the menu.
- Click the spectrometer picture and under the expanded menu [+], select Acquisition and expand [+]. Be sure that these fluorescence wavelength parameters are in the range of the wavelengths obtained from your absorbance measurements. If not, contact your TA.
- Make sure that the acquisition is paused, and then change acquisition parameters to a 1 second integration time, 1 scan to average, and a boxcar of 10. Also, check [✓] the Strobe/Lamp Enable box. You should hear an electrical whine from the lamp.
- Acquire spectra for each sample, recording the color and the wavelength of the maximum peak. Save each spectrum.

$$h\nu = E_{gap} + \frac{h^2}{8a^2} \left(\frac{1}{m_c^*} + \frac{1}{m_v^*} \right)$$

- Use the equation to estimate the size a of the quantum dots. For CdSe, $E_{gap} = 1.70$ eV and $m_c^* = 0.13m_e$ and $m_v^* = 0.45m_e$ are the effective masses in the conduction and valence bands respectively (electron and hole effective masses, which account approximately for the fact that the electrons in either band do not move freely) where m_e is the mass of an electron. How well do your results agree with the particle in a box expression? What do you think might be sources of discrepancy?

Experiment 5. Chloroform

- Move to the Nicolet 380 FT-IR and open the OMNIC software program on the computer interface. Under the **Window** top menu, click on New Window.
- Click on the **Exp Set** icon to open a dialogue window that allows you to configure the IR scan. Under the Collect header, set the number of scans to 16 and “Final format” to Interferogram. Click OK to exit.

- Put an empty thin cuvette in the holder and flush with nitrogen for 60 seconds, and perform a background scan. Fill the cuvette with chloroform and then perform a scan of your sample. What can be concluded from the observed interferogram? What do you expect to see in the spectrum?
- Set the spectrometer to the transmission mode via clicking the **Exp Set** icon again and making the appropriate change, and repeat the procedure to verify your conclusions.
- Where do you expect the peaks to appear if the sample is deuterated (CDCl_3)? Pick several peaks from the CHCl_3 data and calculate where the deuterated peaks should

$$\omega_0 = \sqrt{\frac{K}{\mu}}$$

be. Remember the relation from our discussion of harmonic oscillators. What does K represent? Would its values be different for CHCl_3 and CDCl_3 ? What about μ ?

- Repeat the experiment with deuterated chloroform and compare your results to prediction. What are some possible sources of any discrepancies?

Experiment 6. Essential Oils

- Put an IR card in the card holder in the FTIR instrument, close the cover and flush with nitrogen for 60 seconds to remove water and CO_2 vapors.
- Record the background spectrum. Put several drops of limonene on the window of an IR card, repeat the procedure and take the spectrum of the sample.
- Repeat the procedure with the carvone sample
- Try to assign as many functional groups in the spectrum as possible using literature or internet sources. Why are some vibrational frequencies relatively high and some relatively low? Recall the expression for the vibrational resonance frequency ω_0 .
- What are the differences between the spectra of the two compounds? Can you differentiate between S and R enantiomers using FTIR absorption spectroscopy?

Experiment 7. Protein Secondary Structure Determination

- Before starting, set the temperature on the heating bath to 50°C and turn on the peristaltic pump. While you are taking room temperature spectra, the bath will heat up.
- Using D_2O as the solvent, make three 100 μL solutions with 20 mg/mL concentrations of the three proteins you'll be studying: one for Lysozyme, one for Myoglobin, and one of Ribonuclease A.
- Purge the spectrometer with nitrogen for 4 minutes and collect a background spectrum of the empty spectrometer with 2 cm^{-1} resolution and 16 scans. Save the spectrum. In experimental setup, set this background as the default background.
- Clean the CaF_2 windows and Teflon spacer with water. Dry windows with lens paper. Dry Teflon spacer with a kimwipe.
- Load the CaF_2 cell with 30 μL of D_2O . Do not use the heating coil. The cell should be assembled in this order: tapered metal ring, tapered metal ring (opposite orientation), thick flat metal ring, lead gasket, CaF_2 window, 50 μm Teflon spacer, CaF_2 window, lead gasket, thin flat metal ring, threaded sealing cap. To load the cell, carefully slide in cell

components up to and including the Teflon spacer. Drop 30 μL of sample into center of window. Gently lower remaining stacked components onto sample drop and screw the threaded sealing cap down. Tighten firmly by hand until you encounter resistance. Verify that there are no bubbles in your clear aperture.

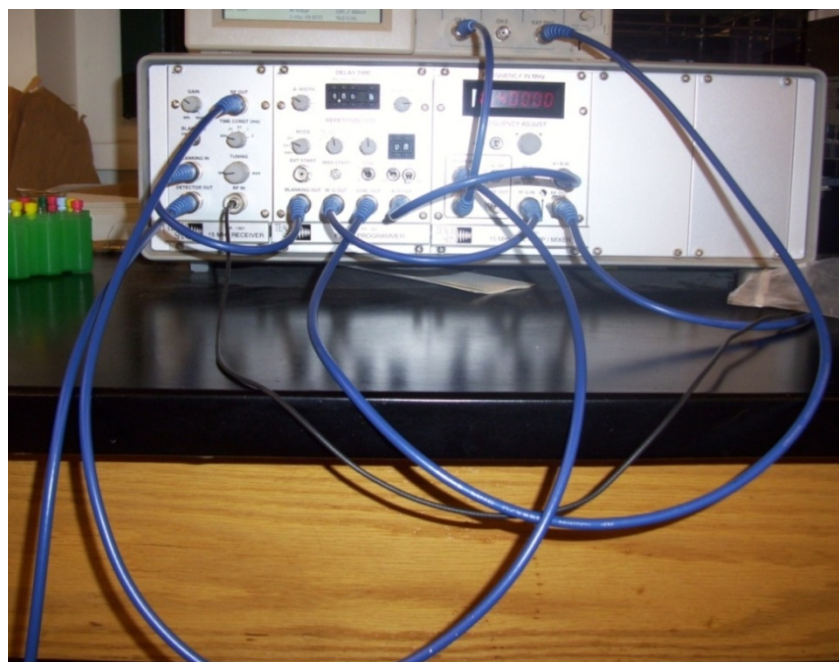
- Place the entire cell in the Nicolet spectrometer and purge with nitrogen for 4 minutes.
- Collect a D_2O spectrum with 2 cm^{-1} resolution and 16 scans. Save spectrum. Collect a D_2O background spectrum, save, and set it as the default background.
- Remove cell, disassemble, clean windows with water, and load Lysozyme.
- Collect a 16 scan, 2 cm^{-1} resolution spectrum of Lysozyme. Save the spectrum.
- Remove cell, disassemble, clean windows with water, and load Myoglobin.
- Collect a spectrum after purging.
- Repeat for RNase A.
- After collecting a room temperature RNase A FTIR spectrum, unscrew sealing cap and slide on heating coil. Retighten. Make sure the heating coil orientation is correct such that it will stick out of the top window of the spectrometer when the cell is loaded.
- After purging, set the temperature of the bath to 100°C .
- Collect spectra of RNase A as the temperature rises at $\sim 7^\circ\text{C}$ increments. Save the spectra and record the temperature. The maximum temperature should be $\sim 81^\circ\text{C}$.
- Turn off heater and allow to cool. When the cell is cool enough to handle, disassemble and clean.
- Compare Lysozyme, Myoglobin, and RNase A room temperature spectra in the 1600-1700 and 1900-1300 cm^{-1} ranges. Can you distinguish different secondary structures from the Amide I spectra?
- Compare the RNase A temperature-dependent spectra over the same ranges. To observe changes, subtract the room temperature spectrum from the others (control, right click selects both and then select the subtract button). What changes? What is happening to the protein as you heat it?
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Experiment 8. 15-MHz NMR

- Appendix B contains some useful information and photographs to help you through this experiment.
- Fill a sample vial about $\frac{3}{4}$ full of ethanol. Place an O-ring about half a centimeter from the top of the vial, then place a plastic cap on the top, lift the top panel on the magnet and insert the sample.



- Turn on the 15 MHz spectrometer and the oscilloscope. Verify that the blue cables on the spectrometer are connected as shown in the figure below.



- **Step 1: View pulse.** Make sure that the A pulse switch is on and the B pulse switch is off. These switches let us send electronic signals (pulses) to the magnet. Next, disconnect the blue cable from the A-B IN port on the spectrometer and use it to connect A-B OUT to Channel 1 on the oscilloscope, letting the MIXER OUT cable hang loose. Remember this configuration; every time you want to view the pulse (the pulse generator's input into the magnet) you will connect the cables in this fashion. Change the parameters on the oscilloscope (vertical and horizontal zoom and pan) until you are able to view the pulse. Measure and record the length, or duration, of the pulse using the cursors on the oscilloscope. What is the purpose of the RF pulse? Why would we want to use a square pulse for this purpose?

- **Step 2: View FID.** Set up the oscilloscope to view the free induction decay (FID) by returning the cables to the original setup (A-B IN and A-B OUT reconnected, MIXER OUT to the oscilloscope). This is the configuration you will use throughout the experiment to observe the FID, the time-domain response of the sample after receiving the pulse. Adjust the oscilloscope so that you're able to observe an oscillating signal; this is the FID. Measure the frequency of the FID. What is the significance of this value? How is the FID signal produced? Why is it an oscillating signal? What information does it give us? Why do we have to mix the signal coming from the sample with a reference signal?
- **Step 3: Tune to resonant frequency and optimize sample height.** With the frequency adjust set to coarse, turn the tuning knob on the pulse generator to adjust the frequency and watch the oscilloscope. The intensity of the FID and number of oscillations in the FID should change as you adjust the tuning. The number of oscillations should decrease as you get closer to the resonant frequency. When you've found the resonant frequency, you should see an exponential decay without oscillations. Now adjust the height of the sample in the magnet and watch the intensity of the FID as you do. Note the peak that changes the most dramatically and remember it—later, when you need to “reach a maximum in the intensity of the FID”, this is the peak you'll be monitoring. Adjust the rubber o-ring so that the sample is at the optimum height and you see the largest FID.
- **Concept check:** Before proceeding, check that you understand the three processes that cause the FID signal to decay. What are their relative time frames? What does it mean to have a 90 degree or a 180 degree ($\pi/2$ or π) pulse? What exactly is being rotated? Why does finding the 90 degree pulse maximize our signal? Are there any other angles that would achieve the same result?
- **Step 4: Create a $\pi/2$ pulse.** Turn the A-Width knob to adjust the pulse length. Watch the scope as you do this. We know that length of the pulse is $\pi/2$ when we reach a maximum in the intensity of the FID (the peak you noticed in Step 3). There are several possible maxima—you should make sure that the one you use doesn't move the A-Width knob more than halfway. Next, change instrument setup to observe your 90 degree pulse. Measure and record the length of the pulse.
- **Step 5: Create a π pulse.** Double the width of pulse to a π pulse (180). What should the corresponding FID look like? Consider whether you expect the signal to be at a maximum, and how the time delay of the response should compare to the FID of Step 4. Reconnect AB OUT to AB IN and MIXER OUT to Chn 1 on the scope without changing any other settings. Observe the FID and compare with your predictions. The FID should appear to be flat. If your FID is not entirely flat, what would cause it to have nonzero oscillations in intensity? Explain the state of the system right after the FID signal decays. What is the net magnetization of the sample at this time?
- **Step 6: Create two $\pi/2$ pulses using different channels.** Turn on the B Channel—having both A and B on will allow us to send more complicated pulse signals to the instrument. Set the delay time to 10 ms on the pulse generator. Be careful to note the units on the pulse programmer display and especially the white dot that represents a decimal point. Switch to FID view and adjust B-WIDTH to find a $\pi/2$ pulse using the same technique as before. What do you expect the corresponding

FID to look like? Why is the FID not zero after sending two $\pi/2$ pulses, one on the A channel and one on the B channel?

- **Step 7: Create a $\pi/2$ - π sequence.** As in Step 5, double the width of your B pulse, forming a 180 degree pulse with a delay time of 10 ms. **This is the setup for a spin echo experiment.** What do you observe? Set up the scope to view the Fourier Transform, and adjust the frequency of the spectrometer until you observe a peak. Record the location of this peak and the peak width (estimate the full width at half maximum, FWHM, as best you can) and note the units. Now set up the scope to view the FID again. Measure the frequency of the FID and compare this to the FT. We would expect to see multiple peaks for ethanol (if you're not sure why, revisit this point after Expt 11). Why don't we have these multiple peaks in a 15 MHz experiment? Calculate the energy difference (in Hz) that we would expect to see using this instrument between 2 peaks with a 2 ppm difference from each other. Compare this to the line width you observe in the FT.
- **Step 8: Dephasing and Lifetime in glycerol.** *Here we will study the dephasing and lifetime of glycerol in water. We will focus on the rates due to dynamical dephasing (T_2) due to evolving interactions (e.g. "collisions") within or among molecules, the lifetime of our nuclear spins (T_1), and inhomogeneous dephasing (Δ) due to static variation in the environments around different glycerol protons. Because solutions with different glycerol concentrations have different environments around the protons, the extent of inhomogeneity and the rates of dynamical dephasing processes may vary from sample to sample.*
 - ❖ To save time, each group will conduct this experiment using just one concentration of glycerol, and the data from different groups will be combined to yield concentration-dependent results.
 - ❖ Δ : Find the pulsewidth of pulse A which creates a $\pi/2$ pulse in your sample. Your FID will decay approximately exponentially. Measure the time delay that results in a drop in your FID intensity to $1/e$ (37%) of its maximum, initial value. In general the decay rate includes contributions from inhomogeneity, dephasing, and lifetime, i.e. Δ , T_2 , and T_1 .
 - ❖ T_2 : Find the pulsewidth of pulse B which creates a π pulse in your sample. You are now set up to perform a spin echo experiment. Use a delay time of 2.5 ms initially. Record the intensity of the echo using horizontal cursors. The most accurate way to do this is to measure the distance from the highest to the lowest points rather than trying to measure the distance between the highest point and the baseline. The echo will always appear at twice the delay time—why? Repeat this procedure, increasing the delay time for each measurement by 2.5 ms until no echo can be measured, recording the delay time and the intensity for each point. If the echo hasn't decayed by 50 ms, increase the step size to 10 ms.
 - ❖ T_1 : Set up your pulsewidths such that pulse A is a π pulse and pulse B is a $\pi/2$ pulse ("inversion recovery" measurement). To begin, measure the maximum (initial) amplitude of the FID following pulse B without pulse A on. Next, turn on pulse A and B and measure the maximum (initial) amplitude of the FID following pulse B. As before, start at a time delay of 2.5 ms, and continue to make

measurements in 2.5 ms steps. If the echo hasn't decayed by 50 ms, increase the step size to 10 ms.

- ❖ What processes and measurements do Δ , T_2 , and T_1 correspond to and which would you expect to change with glycerol concentration? Plot intensity versus delay time for the spin echo and inversion recovery at each concentration. What values of Δ , T_2 , and T_1 were found in your experiments? Do your results match your expectations? Explain any discrepancies.

Experiment 9. 300-MHz NMR

The detailed operating procedures are presented in Appendix C. It is essential that you read them before conducting experiments, for the safety of the NMR machine and for the success of your measurements. Make sure that you have thoroughly read all the NMR material in this manual and the Appendix before beginning.

Spectrum Interpretation and Chemical Determination

- Samples A, B, and C contain acetone, ethanol, and methanol in some order. Sample D is a mixture. Take spectra of all four samples to determine the identity of the first three samples and the identity and mole fractions of the mixture in Sample D. Rationalize the line positions, integrals, and splitting patterns that you observe.
- For the sample containing methanol calculate the chemical shift in ppm from the oscillation frequency determined in the previous experimental section and compare this value to the spectrum in the frequency domain obtained in this section, in which the units are ppm.
- For the sample containing ethanol, calculate the J-coupling parameter values for protons on adjacent atoms in ethanol using your spectrum.

Time Domain Measurements

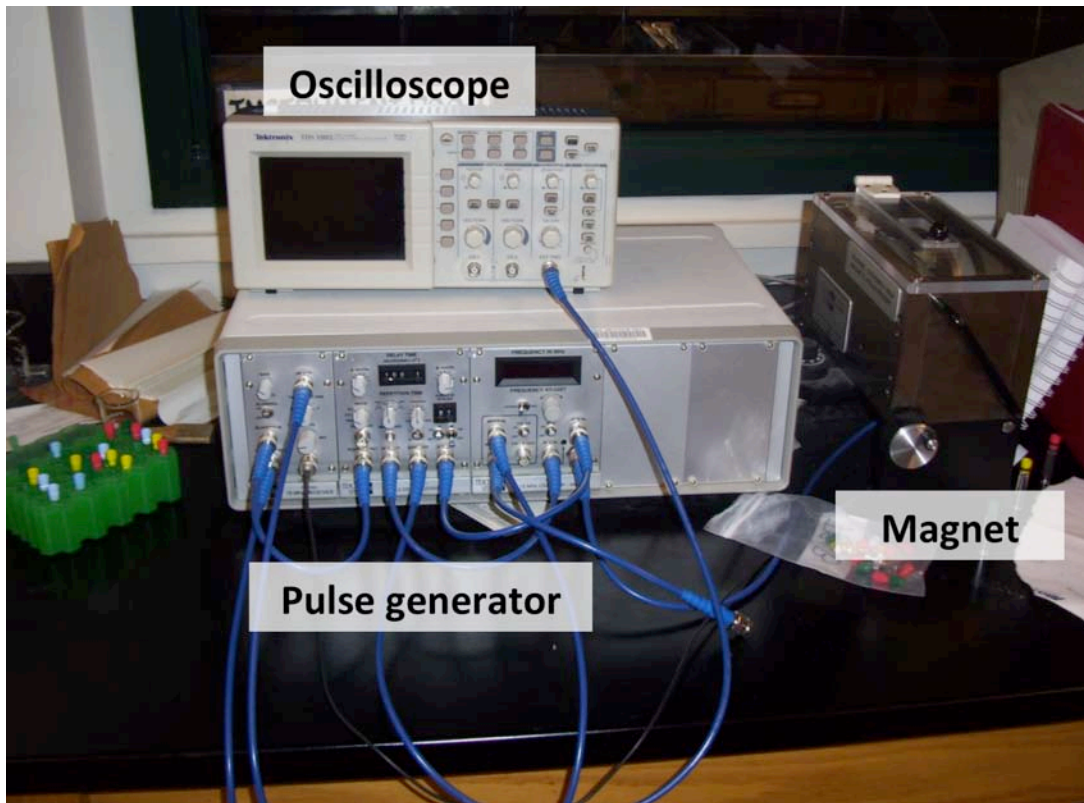
- *Observe the FID:* Enter the 'acqi' window, hit 'FID', and adjust the vertical scale until the FID is visible. For solution 'A' you should see two oscillation frequencies. By moving the cursor between the peaks of the two oscillations; calculate the two oscillation frequencies in Hertz.
- *Examine the linewidth:* Bring the cursor close to a peak. Enter **nl dres** (nearest line display resolution). Compare this value to the linewidth from the TeachSpin experiment.
- *Integrate your spectrum:* Click **[partial integral]** to display the integral line. Type **cz** to clear all resets (zeroes) already in memory. Click **[resets]** and use the left mouse button to define those regions you wish to integrate. The right mouse button can be used to undo mistakes. The parameter **is** sets the integrals scale and can be adjusted using the middle mouse button while in integral mode. Type **ds** to regain cursor control. Place the cursor over the desired region and click the **[Set int]** button. You will now be queried for an integral value. Type **ds dpir** (display peak integral regions) to get the integral values displayed underneath the spectrum.
- *Print the spectrum:* Type **pl pscale pap page** into the command line.

Appendix A: Electromagnetic Spectrum

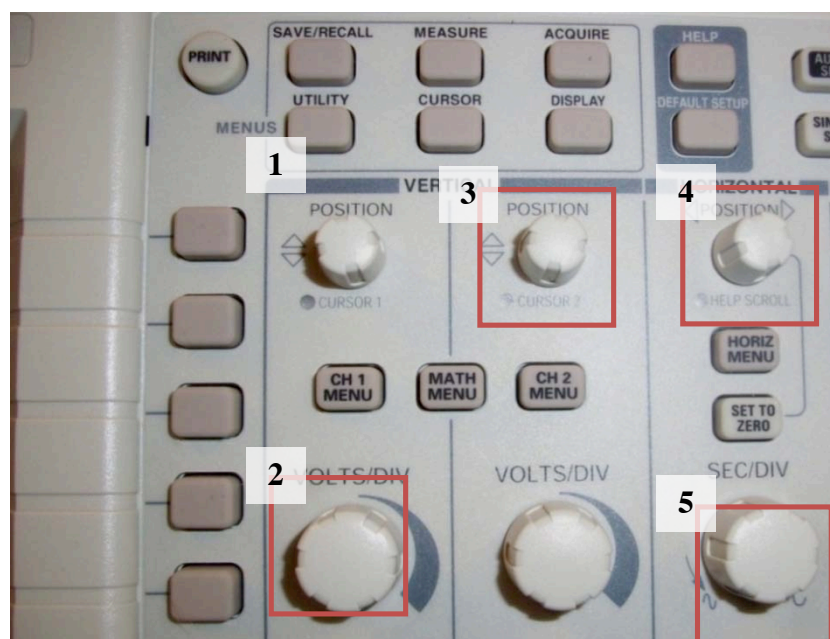
<http://lasp.colorado.edu/cassini/education/>

Please see: [The Electromagnetic Spectrum](#)

Appendix B: Helpful Hints for the 15 MHz

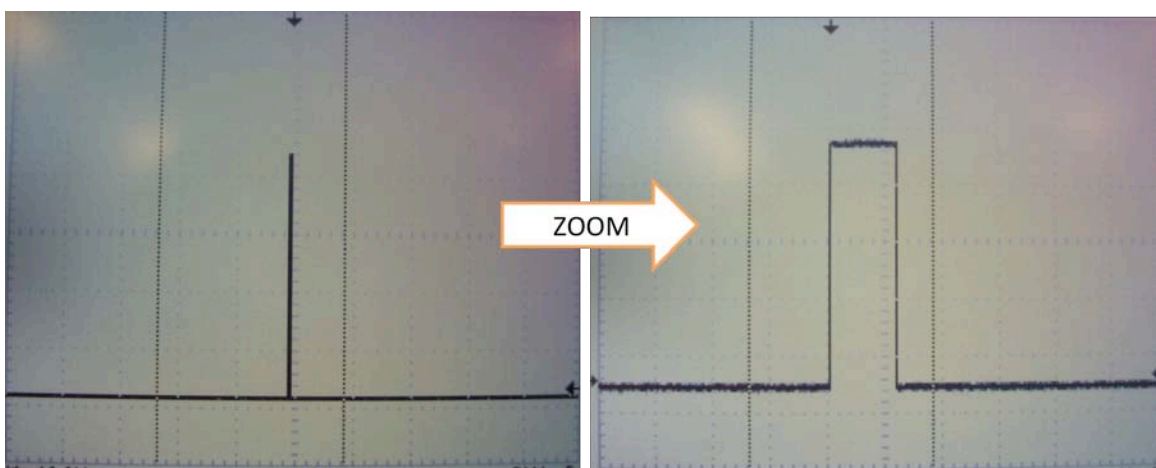


The picture above shows the three main components of the instrument setup. The **pulse generator** is where you will program the pulses that get sent to the magnet: the duration of each pulse, the frequency, the delay time between pulses, and the pulse patterns. The **magnet** consists of coils of wire which create a magnetic field and a place to put a sample. The **oscilloscope** is where you will view both the input of the pulse generator into the magnet and the output of the sample after receiving pulses. Its controls are shown below:

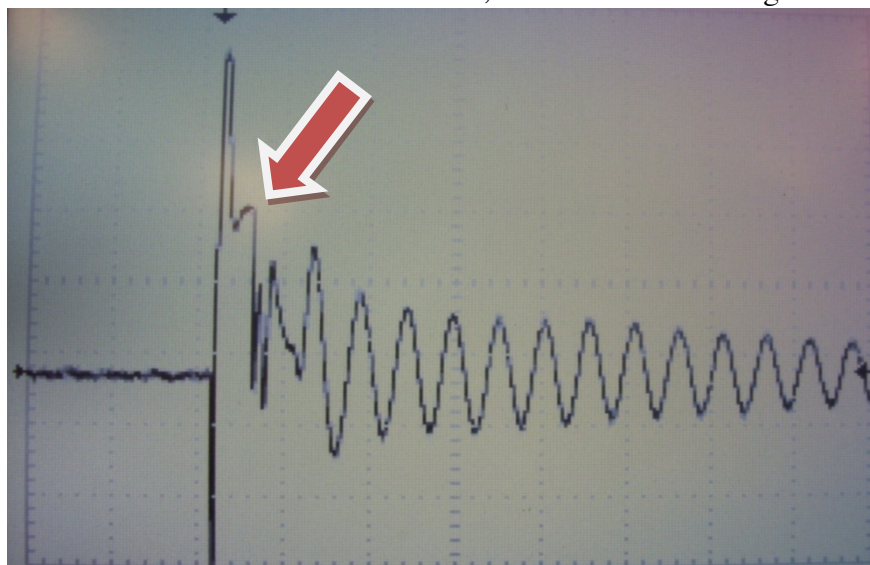


The five most important knobs are outlined in solid red. In display mode, **1**, **2**, **4**, and **5** control the panning (POSITION) and zooming (VOLTS/DIV or SEC/DIV) of the display. In cursor mode, **1** and **3** move the cursors. To change between modes, use the buttons above the vertical and horizontal controls. The five buttons in a column left of the vertical and horizontal controls allow you to change the options displayed on the right-hand side of the screen. They allow you to change between vertical and horizontal cursors when you're in cursor mode, and when you're in MATH MENU (in the center of the picture), they will allow you to do to cycle through operation options (add, subtract, etc.) until you find "FT".

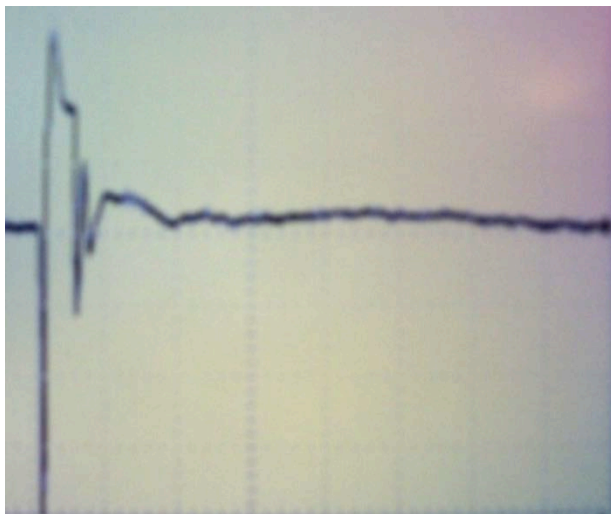
The pictures below show a pulse, which is what the pulse programmer sends to the magnet. When you first turn on the oscilloscope, it may take some adjusting to get the view on the left, but just keep fiddling with the zoom and panning until you see a single solid line. Zooming in even further (center it before zooming to avoid losing it off screen) will give you the image on the left, which is your pulse. The pulse duration is the width of this square pulse (time is x-axis).



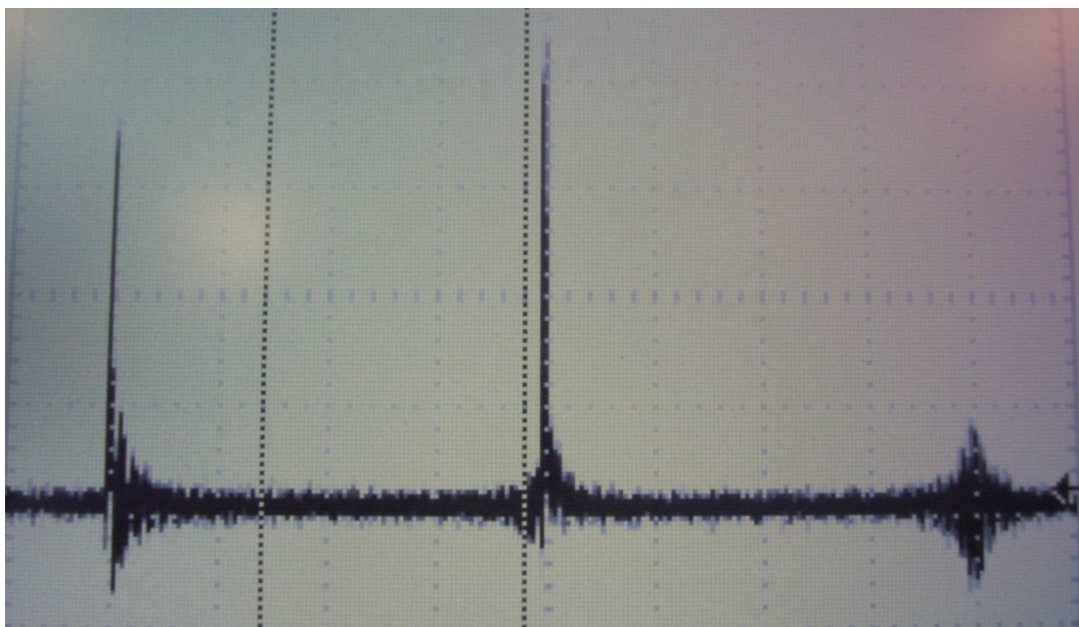
When you switch the cables around to view the FID, it will look something like this:



The flat part to the far left is the baseline before the pulse was applied. The first large spike is mostly noise—it contains the pulse you applied, and it overwhelms your signal response until a short time later, when you see the second smaller peak next to it (see arrow). This is the signal you will monitor when trying to maximize the FID. The long oscillation afterwards is the difference between your reference pulse and the natural frequency of the proton precessions, and is what you will minimize by adjusting the frequency on the pulse generator. Afterwards your signal should look something like this:

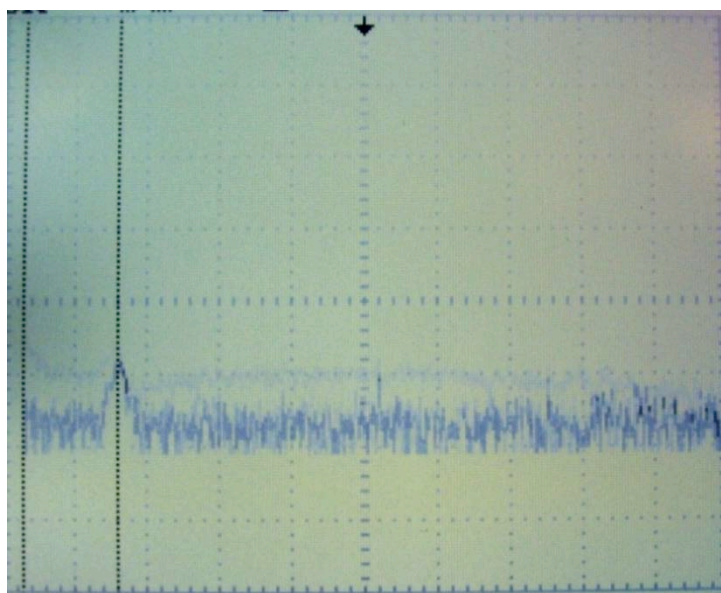


Note that the oscillations after the signal are almost completely gone. Another helpful visual is a spin echo:



At this point in the experiment you will have set up two pulses, which are the first two FIDs you see here. The last signal, which lacks the spike characteristic of a pulse sent to the instrument, is your echo! The echo can be much less pronounced than what you see here, but you will know to always look for it at the same distance from the second pulse that the second pulse is from the first. If that doesn't seem obvious, review the process that causes the echo.

Finally, this is what a FT of your signal should look like:

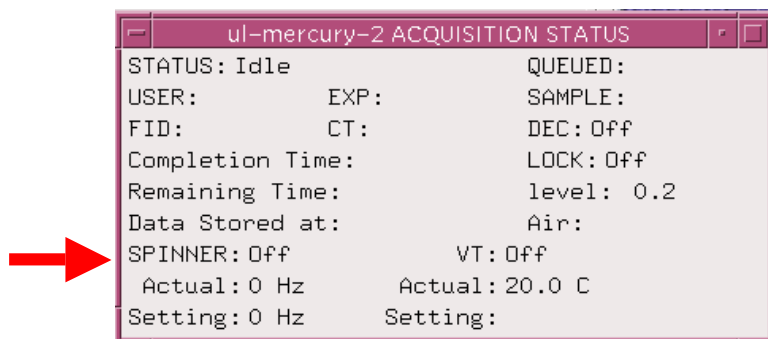


Note how broad this peak is (and how poor the signal-to-noise is) in comparison to the nice sharp peaks you see in the 300 MHz experiment!

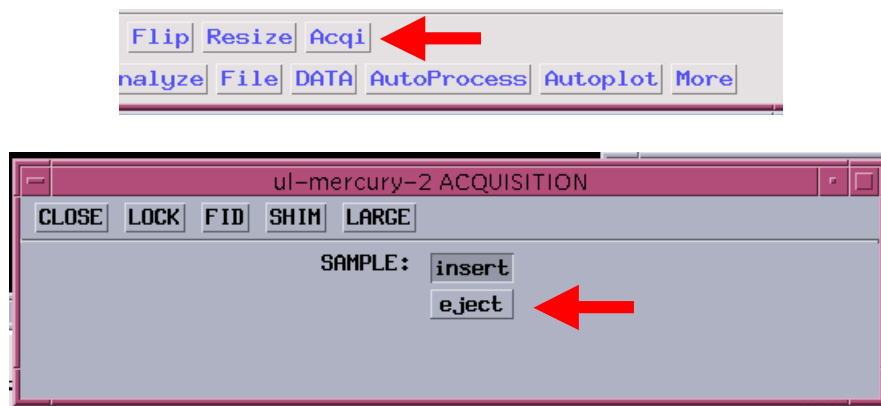
Appendix C: How to Obtain a ^1H NMR

Step 1: Get a lock signal and shim

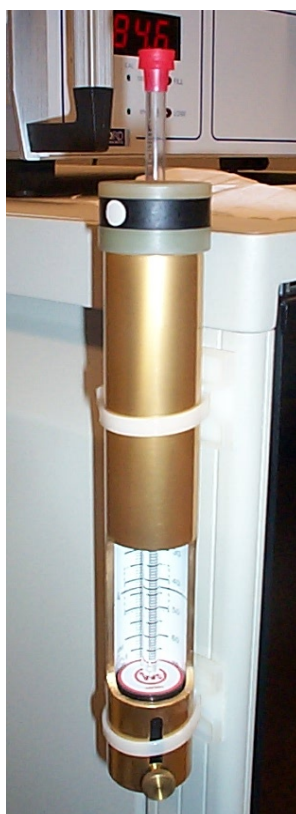
- Before ejecting the previous sample that has been left into the instrument (*there should always be a sample in the probe*) make sure that the **spinner is off** by checking the **Acquisition Status** window.



- To eject the sample and to recover the spinner (turbine), click the **Acqi** button in the Menu buttons window and click on **eject**.



- Remove the sample that is currently in the probe. Carefully insert your sample tube into the spinner and clean the outside of the NMR tube with a KimWipe. Position the tube into the spinner with the depth gauge. The purpose of the spinner is to allow the sample to be rotated at a frequency of 20 Hz inside the magnet, increasing the magnetic homogeneity of the sample.



- Climb the ladder to reach the top of the magnet and carefully position the tube and spinner on the air cushion that flows through the top NMR probe. Return to the computer and click **insert**.
- Set the spinner rate to **20 Hz** by clicking the **Lock** button in the Acqi window and adjusting the bottom parameter.
- In the command line at the top of the main window, type '**rts**' and enter '**best**'. This should set the shim-coils to reasonably good values to start with.
- From here, you have two choices. Option 1 will automatically lock, shim, and perform the selected experiment, which means you can **go straight to mid-Step 3**. Option 2 allows for manual shimming and locking.

Option 1: Automatic

- Click on **Walkup** tab in the Tcl/dg window, which is present on the bottom left area of the monitor. If you do not see the Walkup button, expand the window.

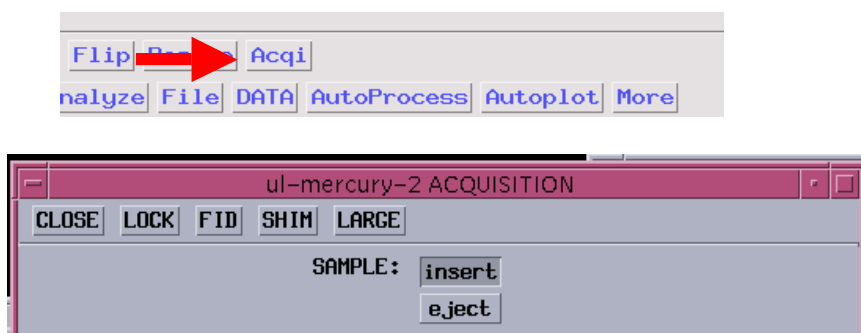


- Select the appropriate solvent from the dropdown Solvent menu, then click on the type of experiment you would like to run (in our case, ^1H). If the experiment does not start automatically, click the **Start ACQ** button.



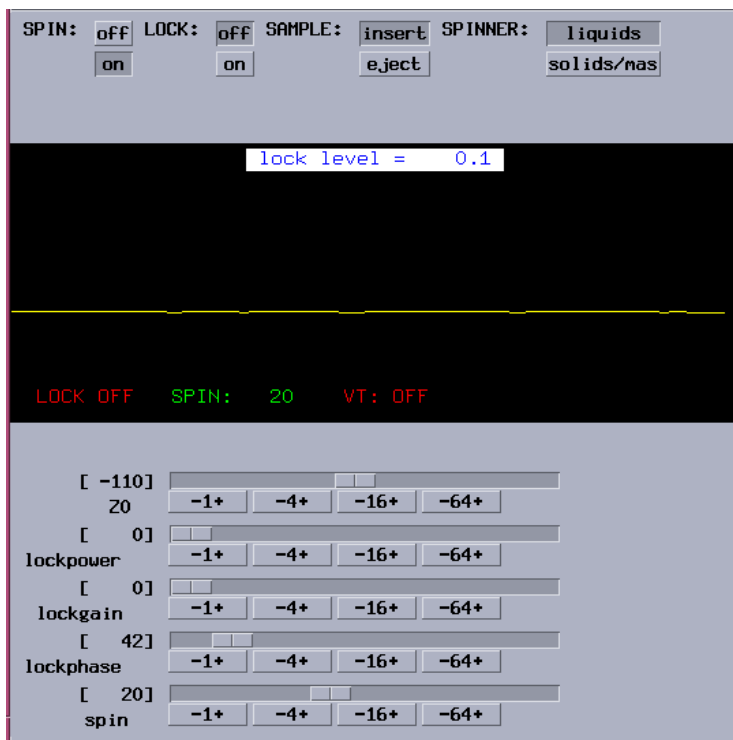
Option 2: Manual

- Click on the **Acqi** Tab and the Acquisition window will appear.

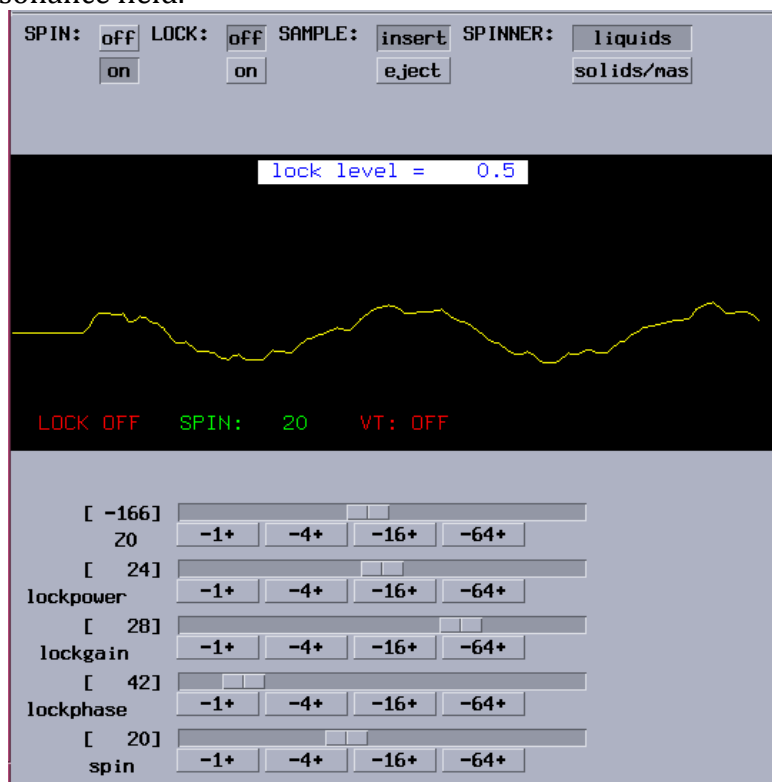


- Click on **Lock** button. Check that the **LOCK** is **off**.
- Initially the spinner is off. Set the spin to 20 Hz (left-clicking a button decreases the quantity, while the right increases). The spinner should now be on. Also increase the **lockpower**¹ to 20 and the **lockgain** to 28.

¹ The **lock power** and **lock gain** levels depends on the concentration of the deuterated solvent, the number of deuterium atoms in the solvent and the relaxation time of the deuterium in that particular solvent.



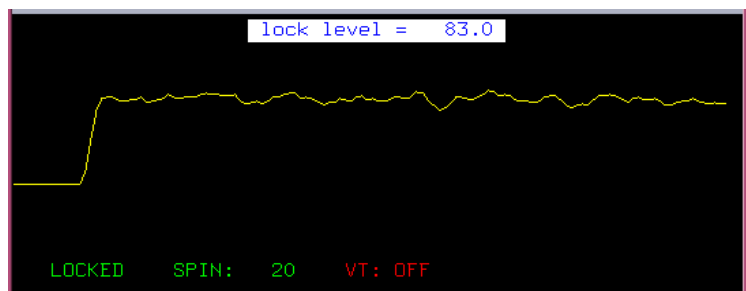
- Adjust the magnetic field frequency **Z0** shim (units are Hz), until a sine wave is seen. The greater the number of sine waves, the poorer the match of **Z0** with the deuterium resonance field.



- If the lock is near resonance, the wave looks like:



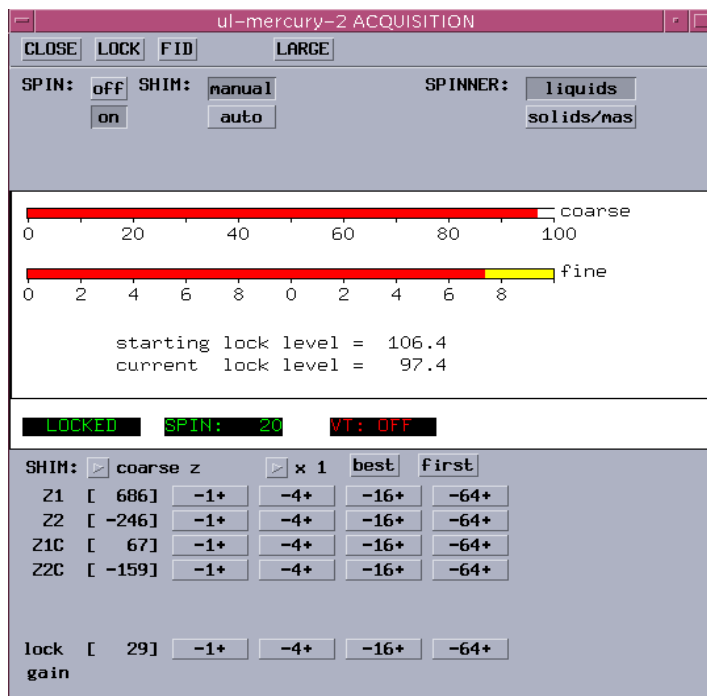
- If the amplitude is too low, increase the **Lock Gain**, and, if necessary the **Lock Power**², until the signal is between 50% and 100%. Continue to adjust **Z0** until one wavelength is seen, and then click the **Lock on** button. The lock step function shown below should appear.



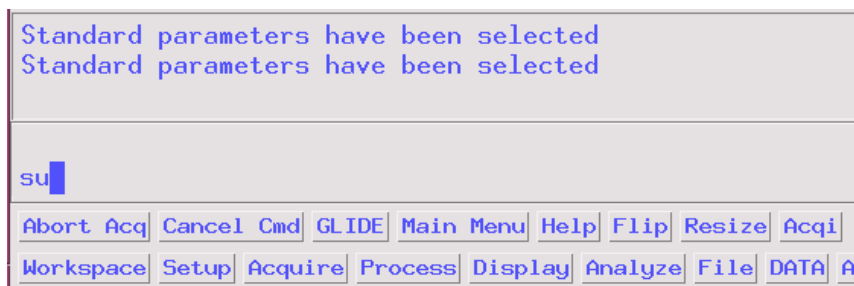
- If phase is not set properly, adjust the **lockphase** in steps of -1 or +1. You will notice that the lock signal is maximized. If the signal appears to be saturated (≥ 100), decrease the Lock Gain.

² A too high **Lock Power** (>40) is saturating the lock signal. Saturation is signaled by the fact that **the lock level is very erratic**. Large values for **Lock Gain** give a noisy lock signal. Reduce **Lock Power** and adjust **Lock Gain** to maximize the lock level.

- Now, to shim. Shimming adjusts the current flow through a series of differently shaped coils that surround the sample. Here, only the “spinning” (e.g. the sample-spinning) shims **Z1 - Z7** will be adjusted.
- In the **ACQUISITION** window click on **SHIM**. Start making small changes to the values of **Z1C**, **Z2C**, **Z1** and **Z2** in order to maximize the lock signal.

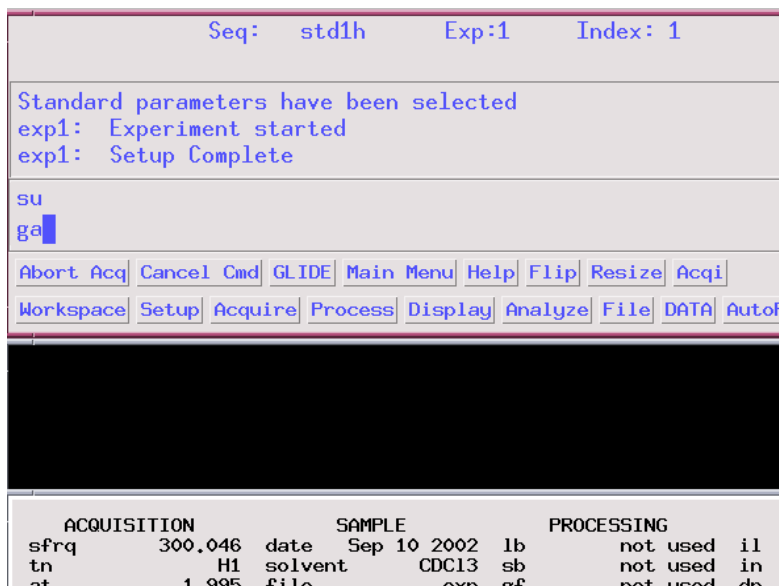


- When the lock signal goes above 100%, reduce the **Lock Gain** in the same window (or, if necessary, the **Lock Power** in the **Lock** window).
- In the command line type **su**, which sets up the lock and shim parameters and disconnects from the **Acqi** window.



Step 3: Acquire spectra

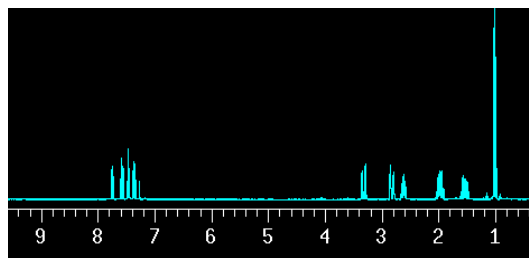
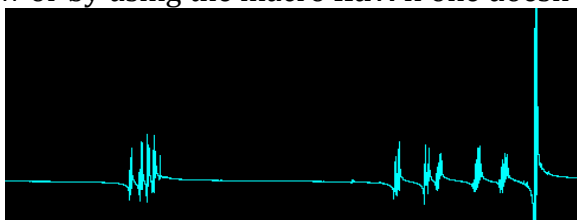
- Select **Main Menu**, **Setup**, and the appropriate **Nucleus** and **Solvent**. Now, type in **nt=8** to set the number of transients (acquisitions) to eight, which is a good choice for proton NMR. Then type **ga** to acquire and automatically process your spectrum.



The screenshot shows the NMR software interface. At the top, it displays 'Seq: std1h Exp:1 Index: 1'. Below this, a message states 'Standard parameters have been selected' and 'exp1: Experiment started' and 'exp1: Setup Complete'. The command window shows 'su' and 'ga'. A menu bar includes 'Abort Acq', 'Cancel Cmd', 'GLIDE', 'Main Menu', 'Help', 'Flip', 'Resize', 'Acqi', 'Workspace', 'Setup', 'Acquire', 'Process', 'Display', 'Analyze', 'File', 'DATA', and 'Autof'. At the bottom, a table lists acquisition parameters:

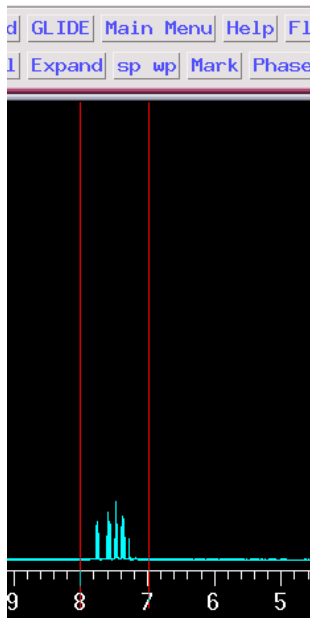
ACQUISITION	SAMPLE	PROCESSING
sfrq 300.046	date Sep 10 2002	1b not used
tn H1	solvent CDC13	sb not used
at 1.995	file exp of	not used

- After the transients are completed, the spectrum will be displayed on screen. Usually, the spectrum is not properly phased; the baseline isn't flat and isn't symmetric around the base of a peak. Auto-phase the spectrum by typing **aph** into the command window or by using the macro **ffav**. If one doesn't work, try the other.

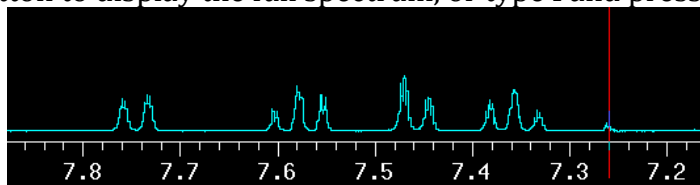


- If the x-axis disappears at any point, type the command **dscale** to retrieve it.
- Reference the spectrum either to your solvent or to TMS. In this example, the proton that is used comes from CHCl_3 , which is in small amounts in the CDCl_3 solvent.

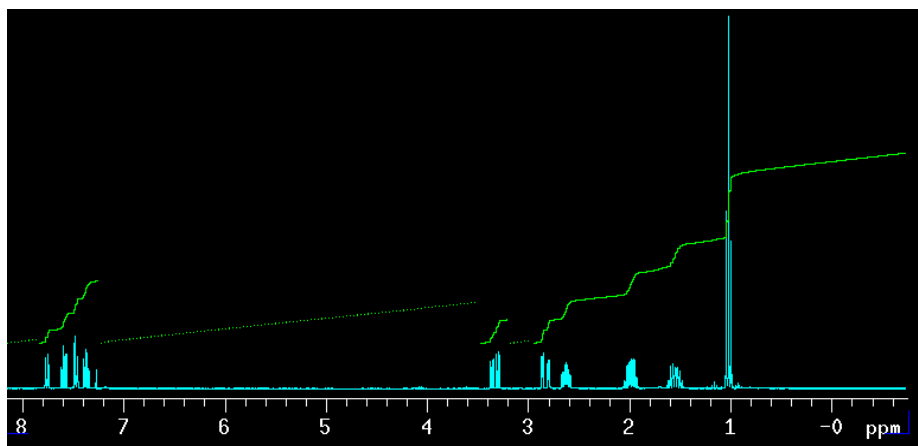
Expand the NMR scale around 7- 8 ppm: click on the **Box** button. Click on the **right button** after the arrow is close to 7 ppm. Click with the **left button** around 8.2 ppm.



- Click on the **Expand** button. With the mouse, bring the red cursor very close to the chloroform peak, then type the command **nl** (stands for nearest line), or **nl dres** to display digital resolution, which for a well shimmed probe should be less than 1 Hz. This will place the red cursor on top of the nearest line. Type the command **rl(7.27p)**. If you decide to use the **TMS** signal, type (**rl=0.0p**).
- Click the **full** button to display the full spectrum, or type **f** and press enter.



- *Integrate the spectrum:* Click **part integral**. The integral line will be displayed continuously. To cut the integral above each peak, type **cz** (clears all resets) and click **resets**. Left click on the integral slightly before the signal and then slightly after the signal. Repeat for each peak.



- To display the values of the integrals underneath the spectrum type **ds dpir**. If necessary make **vp=12** and type ds dpir again.
- Finally, save your file with the **svf('filename')** command.
- To get a hard copy with your ^1H NMR, plot the spectrum typing **pl pscale pir pap page** (meaning: plot, plot the ppm scale, plot the integral resets, plot some acquisition parameters)
- To see coupling patterns, you can expand regions of spectrum with the **box** and **expand** buttons. In order to get the peak frequency, first set the peak picking threshold parameter by clicking on **th**. With the left mouse, set the height of the yellow line such as to include all the peaks with significance (avoid the noise level signals). Type **dpf** to display the peak frequencies.
- You also can print the expanded region by typing **pl ppfhz pscale page**. Macro **ppfhz** provides peak position in hertz, which is very useful to extract the coupling constant values.

When you have finished

- Click **acqi**, **lock off**, **spin off**, make **lockpower = 0**, make **lock gain = 0**.
- Eject
- Substitute your sample with a sealed sample. Insert the sealed sample in the turbine. Wipe the sample with KimWipe, and **insert**.

Troubleshooting

- Disable auto-locking and auto-shimming features: Hit 'flags and conditions,' toggle 'autolock' to no and 'autoshim' to no.
- If the software is not responding: Close and open the program. Open a command prompt by right-clicking on the desktop background, paging to 'tools', and then 'terminal'. Enter 'su acqproc' into the command line to kill the current acquisition, then enter 'su acqproc' to re-initialize the spectrometer. If the software still does not respond, turn off the spectrometer (white switch around back), wait a few seconds, then turn it back on before re-opening the software.

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