APPENDIX 2

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Guidelines to operate Varian Mercury Plus for getting a ^{1}H NMR

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Please note that a **GOOD** sample will produce a **good** lock signal and **good ^{1}H and ^{13}C** NMR spectra. If the sample is bad, the instrument is unable to do miracles.

There are 4 steps in order to get an NMR spectrum.

1. Making a **good** NMR sample.
2. Getting a **good** lock signal and shimming the instrument.
3. Acquiring a ^{1}H and ^{13}C NMR.
4. Printing your results.

STEP 1: MAKING A PROFESSIONAL NMR SAMPLE:

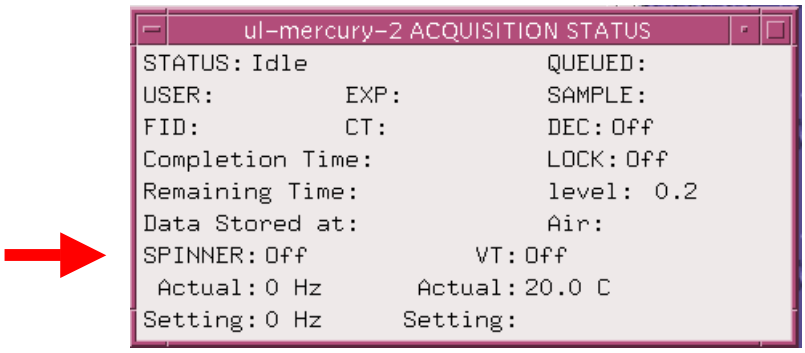
- In a small vial or test tube check if your compound dissolves in a non deuterated solvent (for example $CHCl_3$).
- If the preliminary test is satisfactory, dissolve **10-20 mg** of your compound in **0.5 – 0.7 mL** NMR solvent (the amount in the ampoule) in a separate vial. NMR solvents are deuterated (for example $CDCl_3$) and very expensive.
- If the sample is clear (homogeneous) after some swirling (agitation), transfer the it to a clean and dried NMR tube (without traces of washing solvents like acetone, ethanol, etc).
- Make sure that the NMR tube is not scratched, cracked or chipped.
- If the solution is **not clear**, filter it through a pipet filter. Insert a small wad of glass wool or a small piece of crumpled of a crumpled KimWipe into the neck of a Pasteur pipet. Place the narrow end of the pipet into the NMR tube. Be careful not to break the upper part of the NMR tube. Transfer the unfiltered solution into the pipet filter with a second Pasteur pipet. If necessary, a bulb to force the solution into the NMR tube. Carefully cap the tube.

STEP2: GET A LOCK SIGNAL.

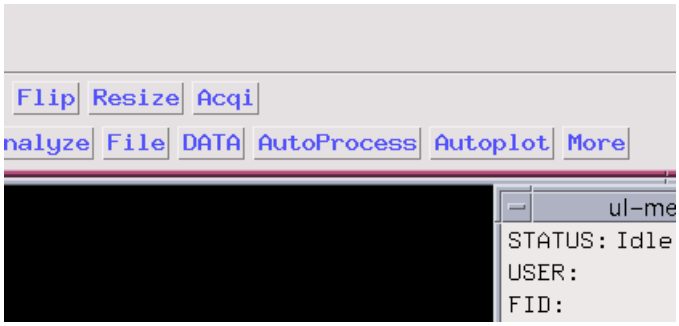
A. Inserting the NMR sample.

The sample is rotated at a frequency of 20 Hz inside the magnet. The rotation brings about extra magnetic homogeneity to the sample.

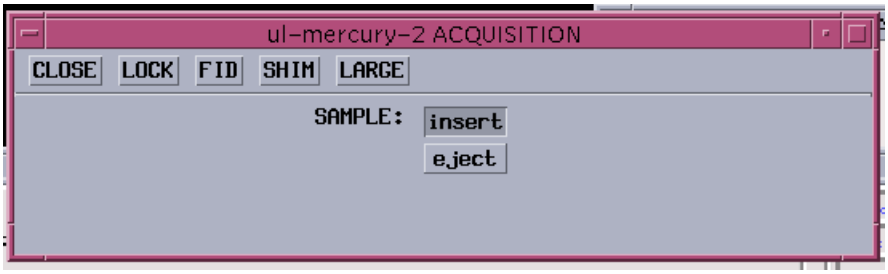
- Before ejecting the previous sample that has been left into the instrument (**there is always a sample in the NMR probe**) make sure that the **spinner is off** by checking the **Acquisition Status** window.



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



Then click on **SAMPLE eject**:



- Remove the sample that is currently in the probe.
- Carefully insert your sample tube into the spinner.

- Clean the outside of the NMR tube with a KimWipe.
- Position the tube into the spinner with the depth gauge.

- Climb the ladder to reach the top of the magnet and carefully position the tube & spinner on the air cushion that flows through the top NMR probe.

- Click on **insert**.

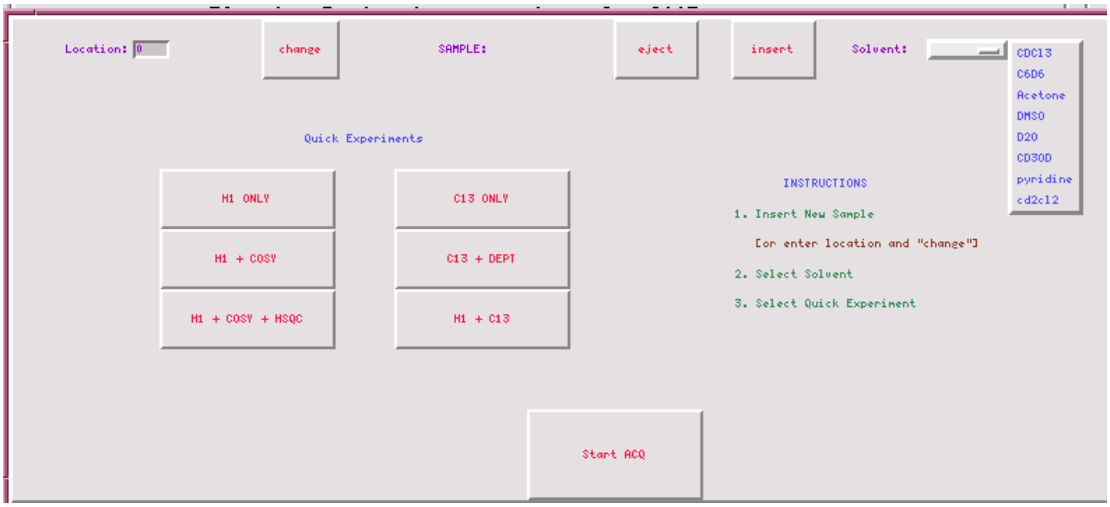
- Set the spinner rate to 20 Hz by clicking the Lock button in the Acqi window and adjusting the bottom parameter.

B. Getting the Lock Signal.

Option #1 will automatically lock and shim, and run the experiment to produce the[1]H NMR spectrum (e.g. STEP #3 inclusive!):

To obtain a 1H NMR spectrum by this methodClick on **Walkup** tab in the Tcl/dg window, which is present on the bottom left area of the monitor. If you do not seethe Walkup button, then expand the window.

- Select the appropriate (lock) **Solvent**. From the drop down **Solvent** menu (for example, **CDCl3**).



- Click on **H NMR** if you want to run a proton NMR.
- If the experiment does not start automatically, click the **Start ACQ** button.

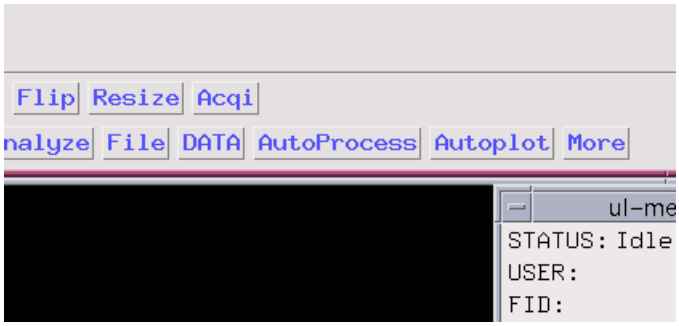
[1] Tcl: Tool Command Language, version of the dg program. (that displays group of parameters)

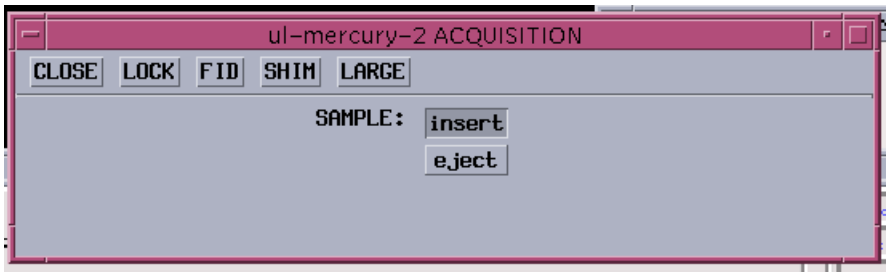
- Shimming, spectra acquisition, and plotting will be done automatically To obtain the spectra manually, use **Option #2.**

Option #2 in which you will learn manual locking[2] and shimming[3] and ^{1}H NMR.

A. To lock:

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



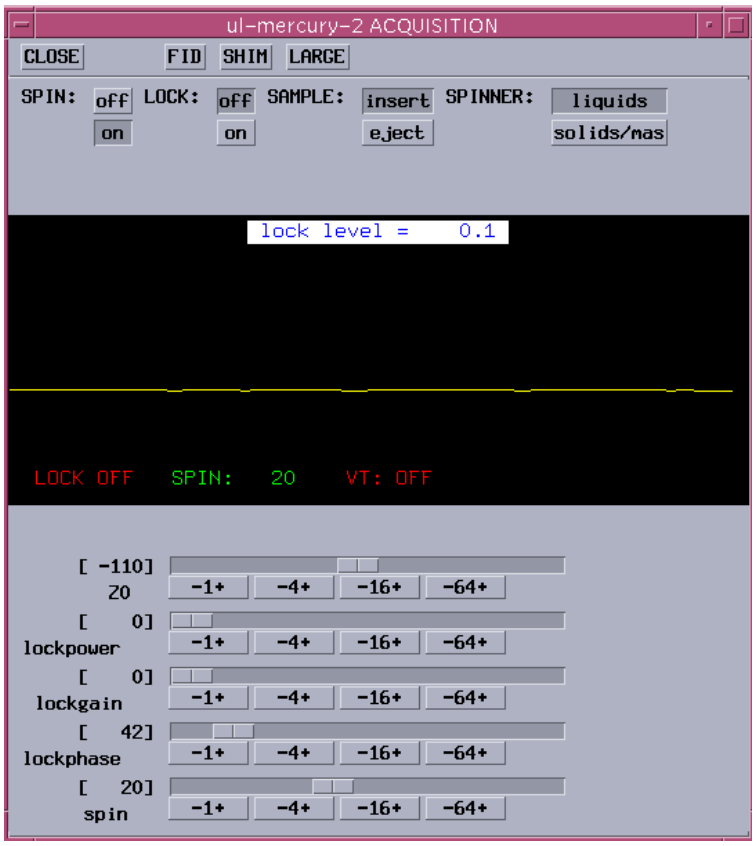


- Click on **Lock** button. Check that the **LOCK** is **off**.

  Initially the spinner is off. Set the spin to 20 Hz by clicking on 16 with the **right mouse button** (to increment the parameter) and then on 4 with the right mouse button. The **SPIN** is now **on.** The left mouse button is used to decrement the parameter

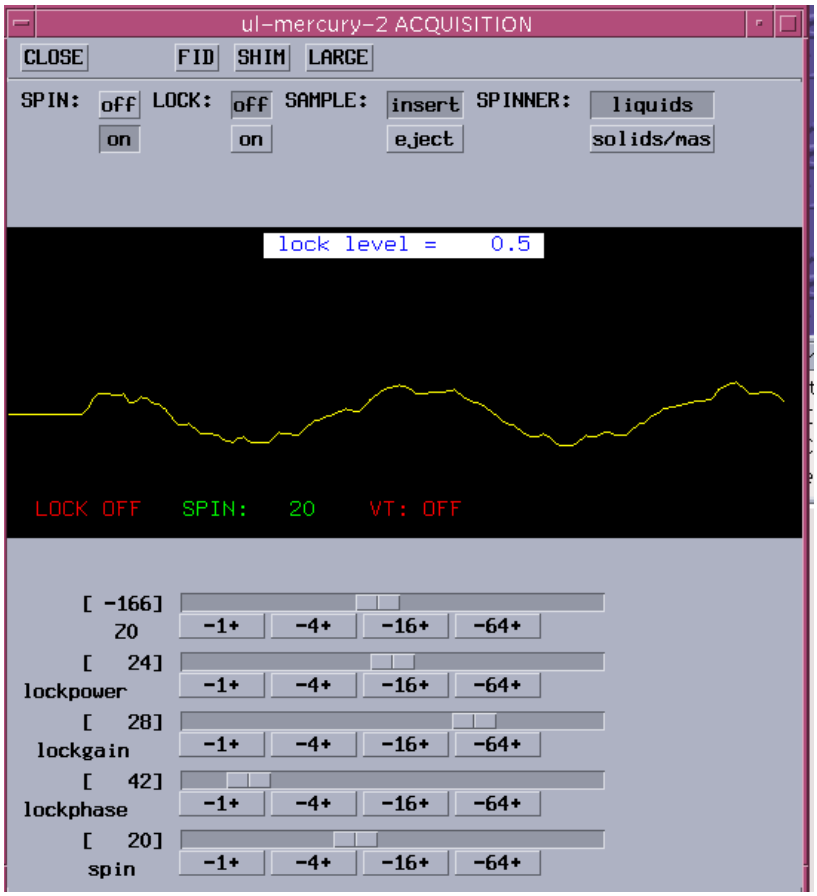
[2] In order to maintain the magnetic field at a constant value during the experiment, one must lock the instrument.

[3] A good shimming is making the magnetic field as homogeneous as possible. You will get very narrow lines in the NMR spectrum.



- Increase the **lockpower**[4] to 20 (+16+4) and the **lockgain** to 28 (+16+16-4).
- Adjust the magnetic field frequency **Z0** shim (buttons are in Hz units), until a sine wave is seen. The greater the number of sine waves, the poorer the match of **Z0** with the deuterium resonance field.

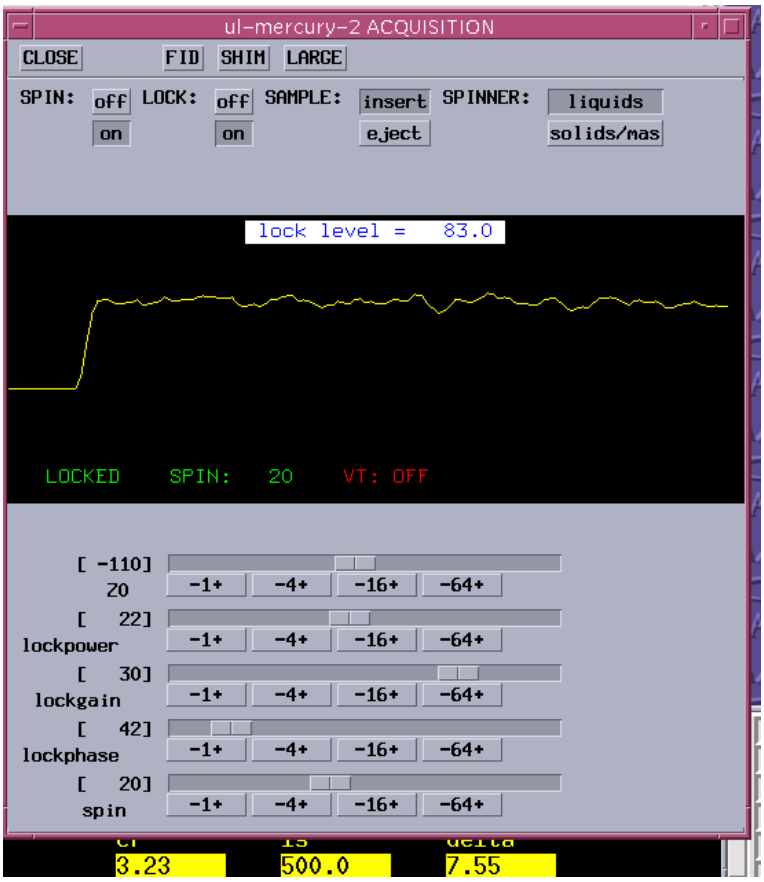
[4] The **lock power** and **lock gain** levels depends on the concentration of the deuterated solvent, the number of deuterium atoms in the solvent and the relaxation time of the deuterium in that particular solvent.



- 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 Powder**[5]**,** until the signal is between 50% and 100%.
- Continue to adjust **Z0** until one wave wavelength is seen:
- Click the **Lock on** button and the lock step function appears.

[5] 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** gives a noisy lock signal. Reduce **Lock Powder** and adjust **Lock Gain** to maximize the lock level.

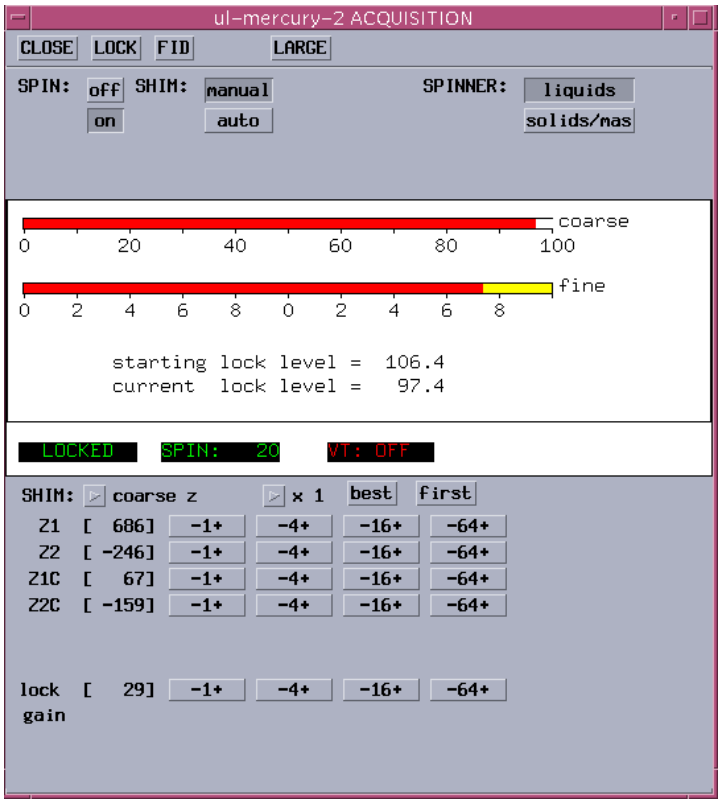


- If phase is not set properly, adjust the **lockphase** in steps of –1 or +1. You will notice that the lock signal is maximized.
- If the signal appears to be saturated (>= 100), decrease the Lock Gain.

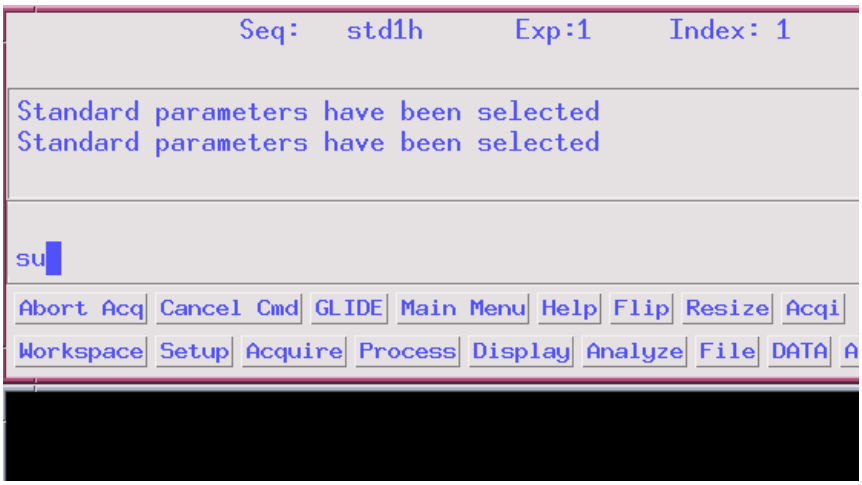
B. Shimming.

Shimming adjusts the current flow through a series of coils (differently shaped) that surround the NMR sample. Here, only the "spinning" (e.g. the sample is spinning) shims, **Z1 – Z7** will be adjusted. In the **ACQUISITION** window click on **SHIM**. The following window pops up.

- Start increasing (or decreasing) in steps of 1 or 4 the values of **Z1C**, **Z2C**, **Z1** and **Z2** such as *to maximize* the lock signal.

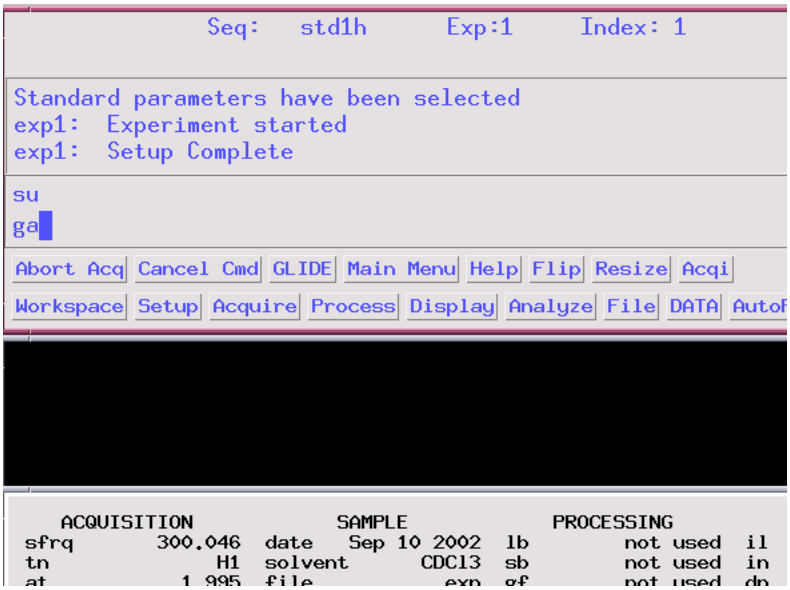


- When the lock signal goes above 100%, reduce the **Lock Gain** in the same window (or if necessary the **Lock Power** in the **Lock** window).
- In the command line type **su,** which sets up the lock and shim parameters and disconnects from the **Acqi** window.

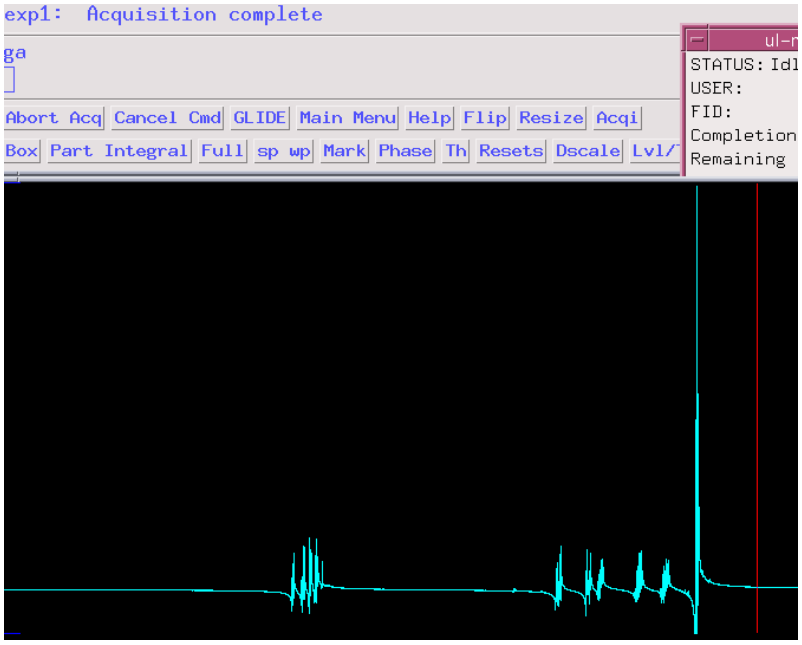


STEP 3: ACQUIRING A ^{1}H (AND ^{13}C NMR).

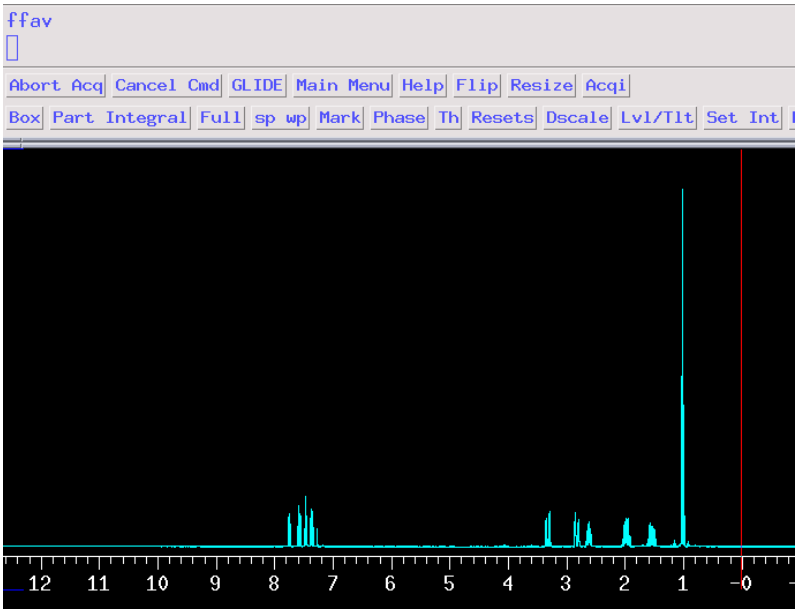
- Select **Main Menu**, **Setup**, the appropriate **Nucleus** and **Solvent**.
- For the ^{1}H NMR, **nt=8**, is a good choice (**nt** stands for number of transients (or acquisitions)).
- Type **ga** to acquire and automatically process your spectrum.



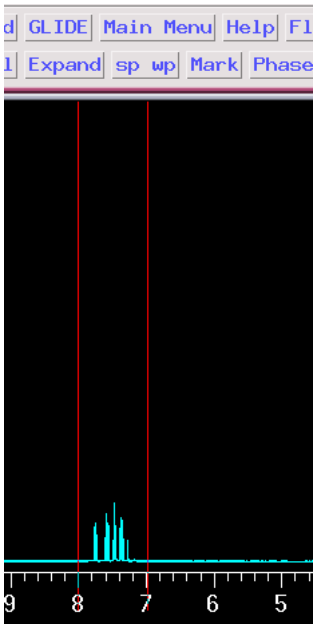
- After the transitions are completed, the spectrum will be displayed on screen. Usually, the spectrum is not properly phased.



- Auto-phase the spectrum by typing **aph** into the command window or by using the macro **ffav.**

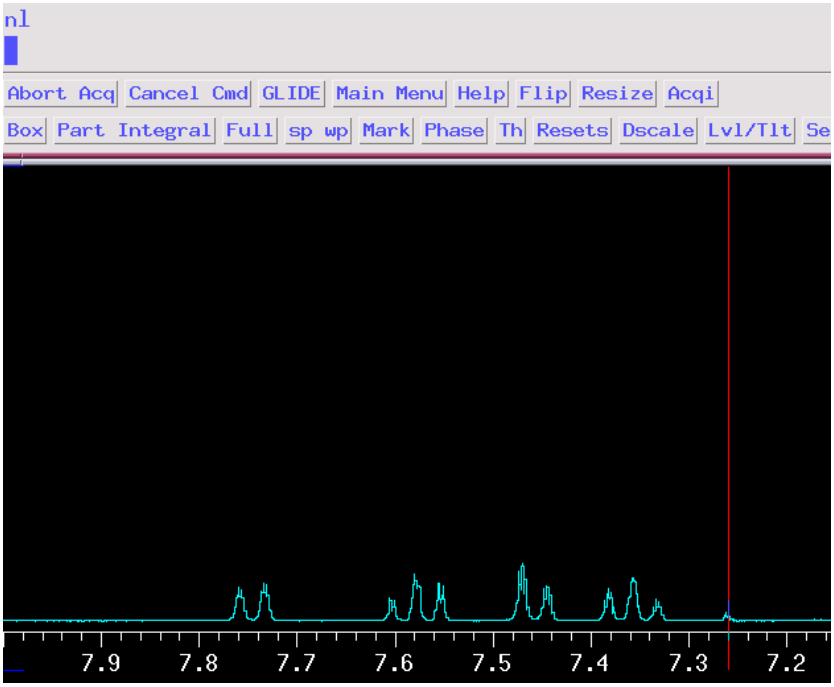


- In case that autophase does not work, you must phase the spectrum manually (ask the help of your TA).
- If the numbers on the bottom of the spectrum disappear, type the command **dscale.**
- Reference the spectrum either to your solvent or to TMS, In this example, the proton that is used comes from$CHCl_3$ (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.

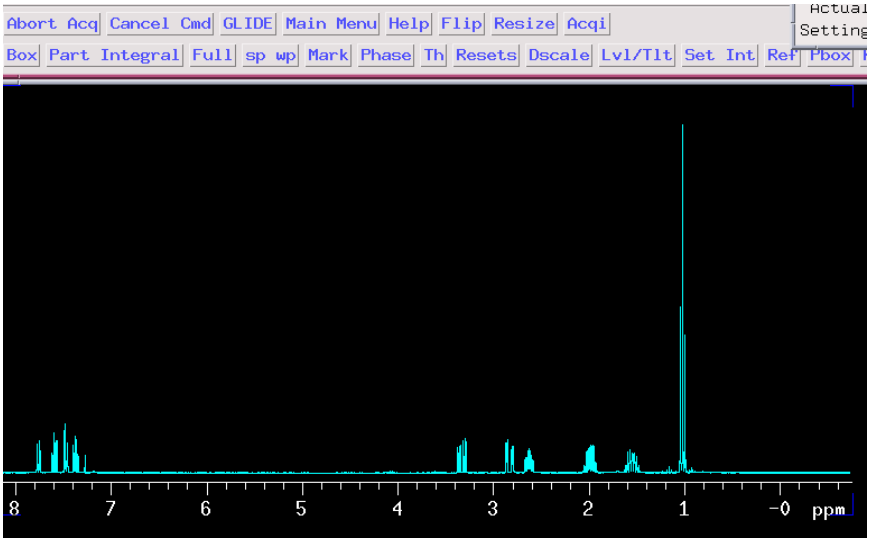


- Click on the **Expand** button.
- With the mouse, bring the red cursor very close to the chloroform peak, then type the command **nl** (stands for nearest line), or **nl dres** to display digital resolution, which for a well shimmed probe should be les than 1 Hz. This will place the red cursor on top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

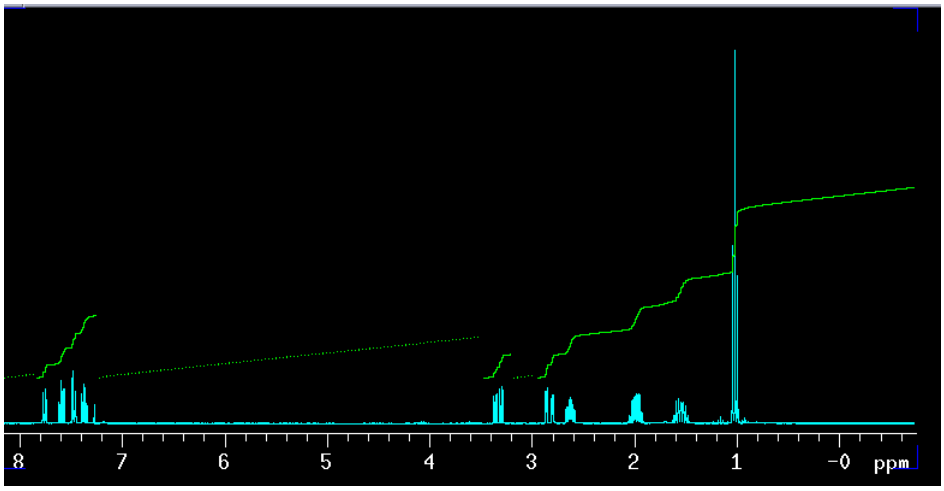
Click the **full** button to display the full spectrum, or type **f** and press enter.



- ***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. The integral Keep doing the cut on each signal.



- To display the values of the integrals underneath the spectrum type **ds dpir**. If necessary make **vp=12** and type ds dpir again.
- Save your file with the **svf**(‘*filename*’) command.

STEP 4: PRINTING YOUR RESULTS.

- To get a hard copy with your ^{1}H 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 high 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 on **acqi** button,
- Click **lock off**,
- Click **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**.

Glosary:

CDE Common Desktop environment; includes a toolbar, file manager, icons and point-and-click capabities.
VNMR Varian NMR software