9.1 NMR Operation Guide

Robert Kennedy Ph.D. and Anne Gorham M.S.   **20101117**

Objective: Obtain a well shimmed NMR spectra to determine: chemical shifts, integration(1H only), splitting patterns, coupling constants.

Contents:

Appendices:

This document is intended as an introductory guide to the Varian VNMR NMR acquisition and processing software catering to the needs of the MIT Undergraduate Lab. For those interested, a large amount of additional NMR related information can be found on the DCIF website at http://web.mit.edu/speclab/www/ulab.html.

Conventions used in this guide are as follows:
* **Boldface** type indicates commands that are typed into the VNMR input window or in a terminal window
* Italic **[Boldface]** type with square brackets indicates a button in the VNMR menu that is to be pushed.
* **<Boldface>** type surrounded by a bracket indicate keyboard strokes.
* **LMB** indicates the Left Mouse Button
* **MMB** indicates the Middle Mouse Button
* **RMB** indicates the Right Mouse Button
* Unless otherwise noted, all commands typed into the input window are be followed by an <↵ Enter> keystroke.

Magnet Safety:

- **CAUTION**: NMR Superconducting Magnets are ALWAYS ON!
- People with pacemakers, cochlear and other medical implants should not enter the NMR room without a doctor's permission.
- Keep all metal objects, electronic and magnetic devices, at least 5 feet from the magnet. This includes cell phones, iPods, jump drives, chairs, wallets, keys, hair clips and pins.
- If a tube breaks inside the magnet, contact the Undergraduate Lab Staff <u>immediately</u>.

Figure 1

NMR Equipment: (A) Computer Workstation, (B) NMR Console, generates and receives RF, (C) NMR Magnet.

Sample Preparation:

- Use a clean and dry 5 mm NMR tube that is free of cracks and chips.
- Choose an NMR solvent by testing the solubility of the compound in the non-deuterated solvent.
- Dissolve 20-100 mg of the compound in 0.7 mL of the chosen deuterated[1] solvent.
- If the solution is clear, add the solution to the NMR tube.
- If the solution is cloudy, filter using a small piece if a Kim Wipe inserted into a glass Pasteur pipette.
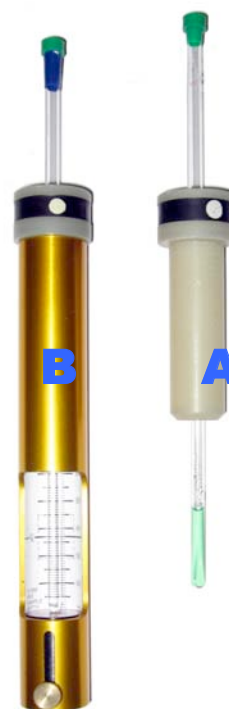- Cap and label the NMR tube.

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[1] Deuterated solvent is used for two purposes: 1) Serve as internal reference (lock) for the spectrometer to correct for magnetic field drift. 2) Prevent large solvent 1H signals.
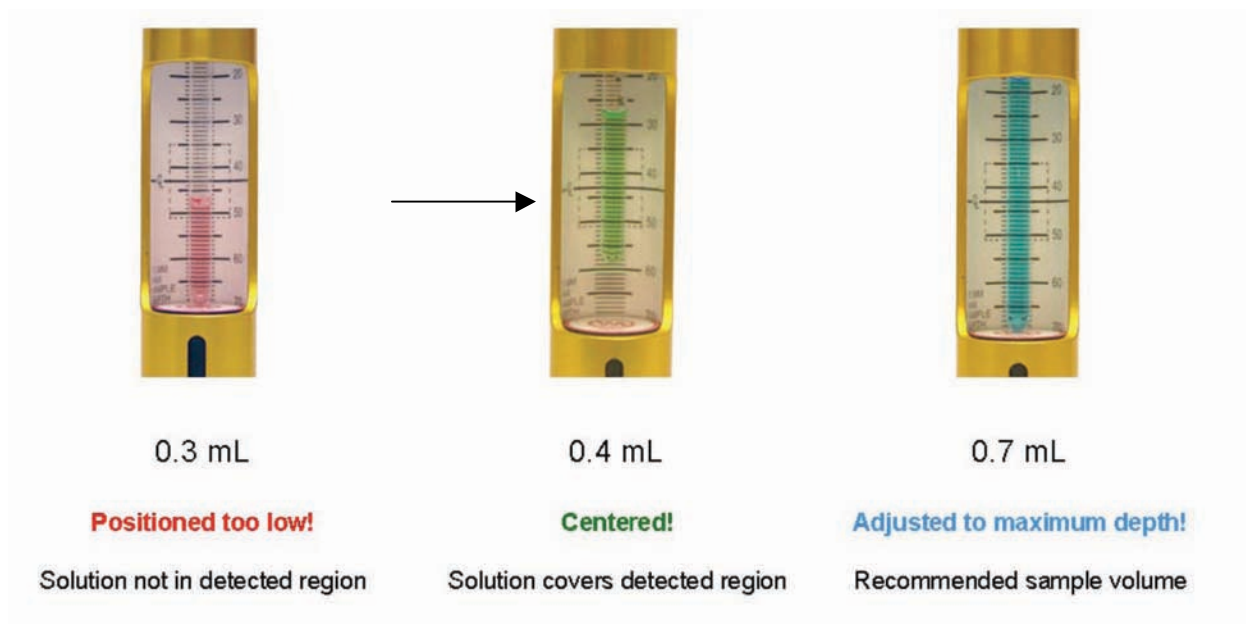
Set Sample Depth in Spinner:

Using the depth gauge prevents the NMR tube from hitting the bottom of the probe and helps center the sample inside the region of detection, minimizing perturbations to the magnetic field.

- Insert the spinner[2] (A) into the depth gauge (B).
- Wipe the NMR tube with a Kim Wipe.
- Carefully insert the NMR tube into the top of the spinner.
- With >0.7 mL of solvent push the tube to the bottom of the gauge. Samples with 0.7 mL of solvent will require less shimming.
- With < 0.5 mL of solvent center the solution on the center line CL (region of NMR detection).
- Remove the depth gauge and wipe the NMR tube a second time with a Kim Wipe.
- **CAUTION**: <u>WAIT</u> until the <u>EJECT AIR IS ON</u> to insert the sample into the magnet.

**Figure 2: Spinner and Tube (A)
Spinner and tube inside a depth gauge (B)**

0.3 mL	0.4 mL	0.7 mL
Positioned too low!	**Centered!**	**Adjusted to maximum depth!**
Solution not in detected region	Solution covers detected region	Recommended sample volume

Figure 3:

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[2] The spinner rotates the tube along the vertical axis within the magnet creating a more homogenous magnetic field. The white dot on the spinner is used to measure how fast the spinner is rotating.

VNMR Software Overview

Before we get started, we must first introduce some of the general features of the VNMR software interface. Upon initially opening the program (done by **single** clicking the VNMR application icon in the Toolbar shown at the right), the VNMR software display screen is divided into four distinct windows. These windows are called:
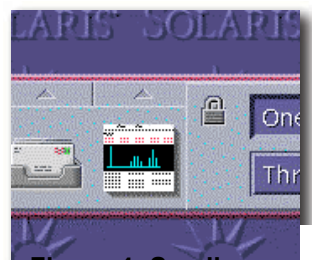

Figure 4: Small section of the toolbar showing the VNMR

1.) The Input Window
2.) The Graphics Window
3.) The Text Window
4.) The Acquisition Status Window.

The significance of each of these windows will be discussed.

1. The Input Window:

The Input Window is typically located at the top of the display and is sub-divided into three subsections. A graphics representation can be seen in Figure 5 below. VNMR system messages will be displayed in the Status Window (topmost portion), commands and parameters are typically input into the Input Window (middlemost portion), and menu selections can be made through the Menu Button Window (bottommost portion of the window).
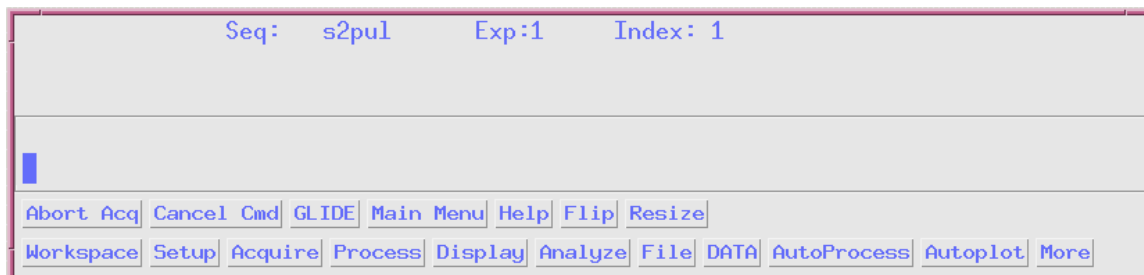
Figure 5: The VNMR Input Window. This is divided into the Status Window (Top), the Input Window (Middle), and the Menu Button Window (Bottom).

Most of the user input in this tutorial will be made through the Input Window and the Menu Button Window.

2. The Graphics Window:
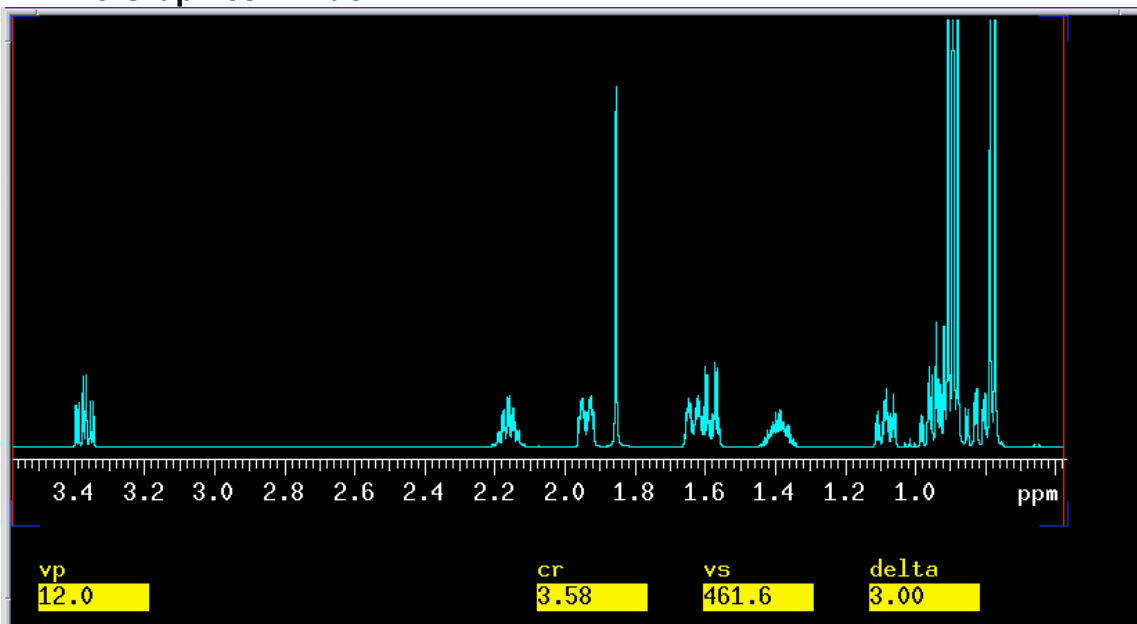
Figure 6: The VNMR Graphics Window

Here the user can graphically process visualize the newly acquired data. As an example we have shown here a small portion of a simple 1D ^{1}H spectrum of menthol ($C_{10}H_{20}O$) in deuterated chloroform ($CDCl_3$). Included in the window is the reference scale, vertical position (vp in mm) with respect to the bottom of the display, cursor position (cr in ppm), vertical scale (vs arbitrary units), and distance between the cursors (delta in ppm). This window can be thought of as a WYSIWYG (what you see is what you get) type of interface. Under normal circumstances most plots are created just as they are seen in the Graphics Window. Here the user can process, zoom, expand, and compare the data they've acquired.

3. The Text Window:

The Text Window can have many different faces. Here text messages, manual pages, and certain analysis output can be displayed.
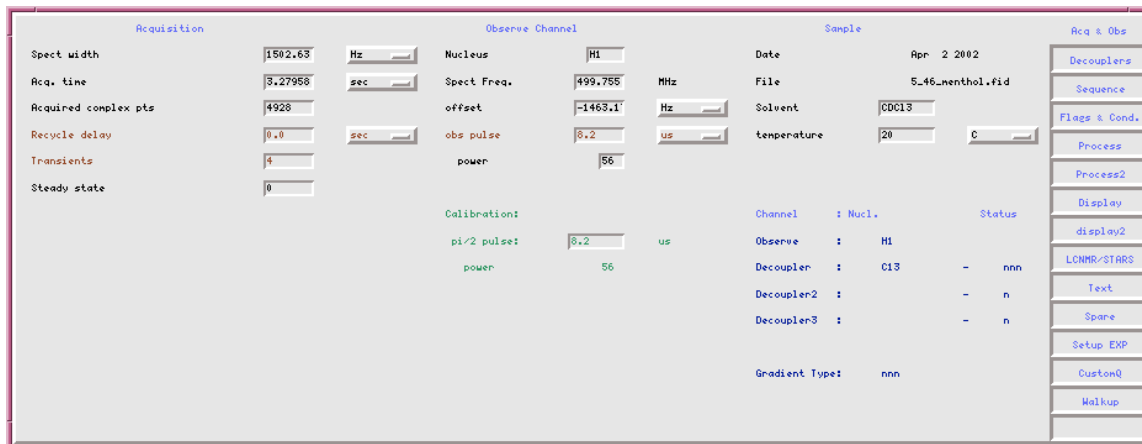

Figure 7: The Text Window. Note the buttons on the right-hand side. These allow for the easy visualization and changing of various parameters.

4. The Acquisition Status Window:

The VNMR software also has a small window called the Acquisition Status window, which is only available, when the workstation is connected to a spectrometer. The Acquisition Status window is shown in Figure 8 and displays information about the experiment being acquired.
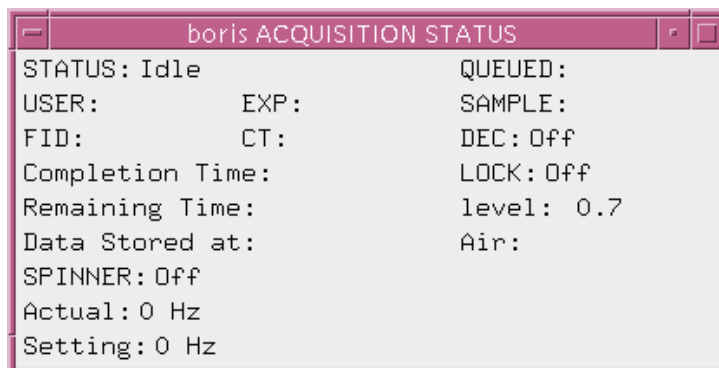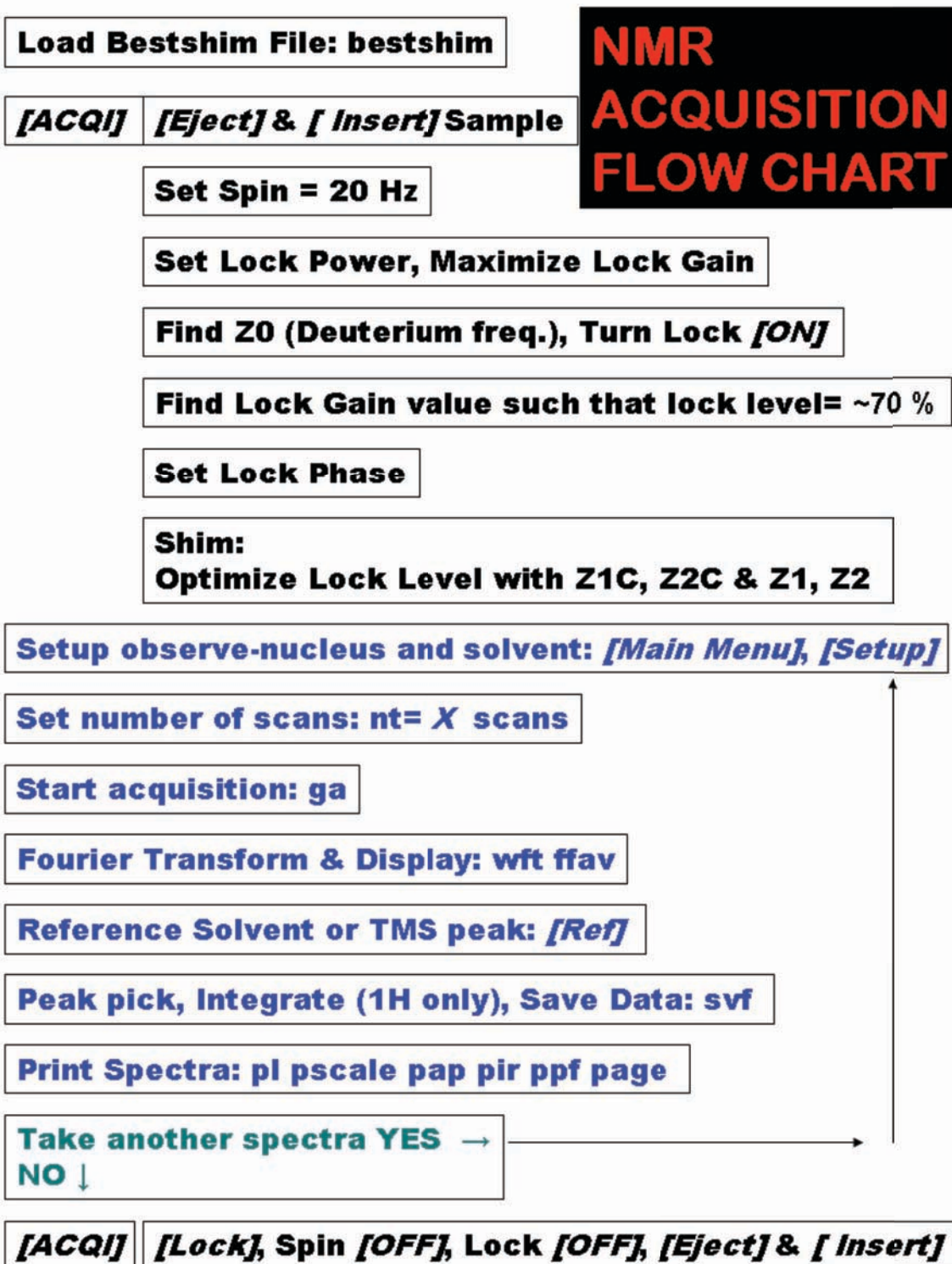

Figure 8: The Acquisition Status window. Note that the status here is 'idle'.

Acquisition Flow Chart

I.   Instrument Setup
II. Experiment Setup & Execution (repeat II if desired)
III. Sample removal

NMR ACQUISITION FLOW CHART

Load Bestshim File: bestshim

[ACQI] *[Eject]* & *[Insert]* Sample

Set Spin = 20 Hz

Set Lock Power, Maximize Lock Gain

Find Z0 (Deuterium freq.), Turn Lock *[ON]*

Find Lock Gain value such that lock level= ~70 %

Set Lock Phase

Shim:
Optimize Lock Level with Z1C, Z2C & Z1, Z2

Setup observe-nucleus and solvent: *[Main Menu]*, *[Setup]*

Set number of scans: nt= X scans

Start acquisition: ga

Fourier Transform & Display: wft ffav

Reference Solvent or TMS peak: *[Ref]*

Peak pick, Integrate (1H only), Save Data: svf

Print Spectra: pl pscale pap pir ppf page

Take another spectra YES →
NO ↓

[ACQI] *[Lock]*, Spin *[OFF]*, Lock *[OFF]*, *[Eject]* & *[Insert]*

I. Instrument Setup: Bestshim, Insert, Spin, Lock & Shim

Log onto the spectrometer workstation

Open the VNMR program by either clicking on the VNMR icon in the CDE toolbar.

1. Load Bestshim File

These Staff assigned shim values are only intended as a reasonable starting place to lock and shim.

Type **bestshim** in the input window. The **bestshim** command is a combination of two commands, **rts('best')** and **su**. You can also load other shim files using **rts('*file name*')**. Your TA may suggest other shim files to use. Remember to type **su** after you have loaded a shim file.

2. Set Sample Depth in Spinner (as directed above)and Insert Sample

Click *[acqi]* in the Menu Button window to connect to the acquisition interface. The Interactive Acquisition window will open, Figure 9.
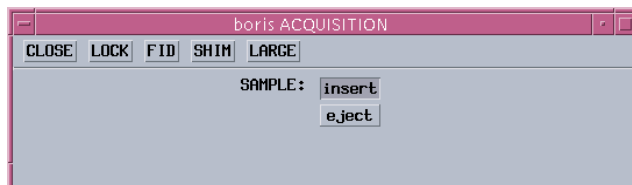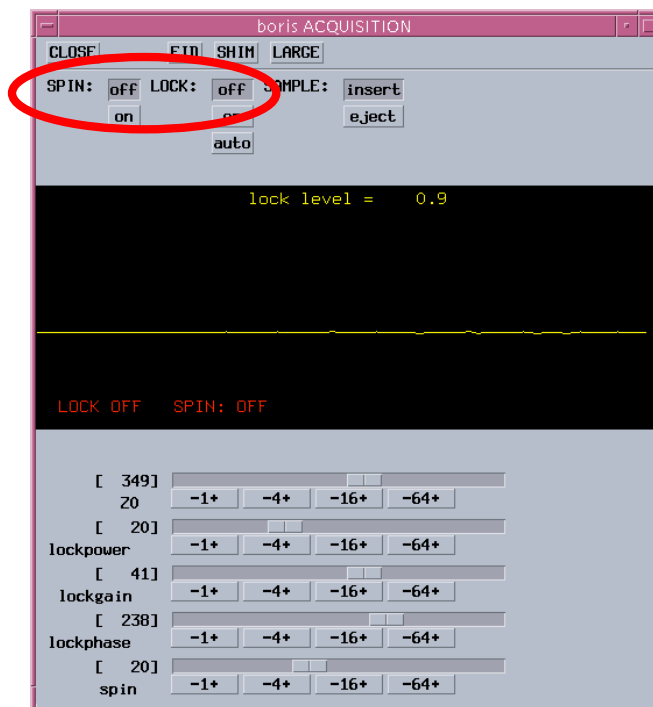

Figure 9: The Interactive Acquisition Window.

Click *[lock]* and make sure that the <u>spin</u> is set to zero or turned *[off]* and lock is turned *[off]*. A recessed button indicates the command or parameter is selected. A graphic representation is shown in Figure 10 below with spin set to 20 but turned off and the lock turned off.

Figure 10: The 'Lock' window within the acquisition interface. The adjustable parameters here are Z0 (magnetic field offset), lock power, lock gain, lock phase, and spin. Each of these may be adjusted by using either the slider bar (very coarse) of stepwise in increments of *[±1], [±4], [±16],* or *[±64].* (LMB: moves −, RMB: moves +)

3. Set Spin

Spinning the sample improves the effective magnetic field homogeneity for the bulk of the spins in the sample. Narrower line widths result. If the sample spin is not stable or does not spin at all contact a staff member for assistance.

Use the slider bar to set the spin rate to 20 Hz and Click spin *[on]*.

4. Set Lock Power

The magnetic field will drift and shift during data collection. A good experiment must compensate for these instabilities and this is why we lock. The "lock" may be considered a separate referencing experiment (in this case, deuterium). This deuterium experiment serves as a feedback loop to repetitively compensate for the magnetic field drift to keep the field constant (and keep good line resolution).

Be sure the lock is clicked *[off].*
Use the slider bar to set the lock power to the solvent dependent value listed below

These values have been determined sufficient for the Mercury UL1 and UL2.[3]

- CDCl$_3$ (chloroform)   use a lock power of 27
- D$_2$O   (water)   use a lock power of 18
- CD$_3$OD (methanol)   use a lock power of 12
- C$_2$D$_6$O (acetone)   use a lock power of 12

For solvents not listed, ask a Staff member for assistance.

## 5. Find On-Resonance Z0, the Deuterium Resonance Frequency

Use the slider bar to set the lock gain to maximum value.

Use the slider bar to set Z0 to the solvent dependent[4] approximate value listed on the chart on the spectrometer.[3]

Increase (**RMB)** or decrease (**LMB)** the *[-4+]* button until the frequency of the sine wave decreases and then becomes zero (looks like a step function or a square wave) as shown in Figure 11. Use the *[-1+]* button to optimize Z0 until the maximum lock level (square wave) is obtained. See Figure 11
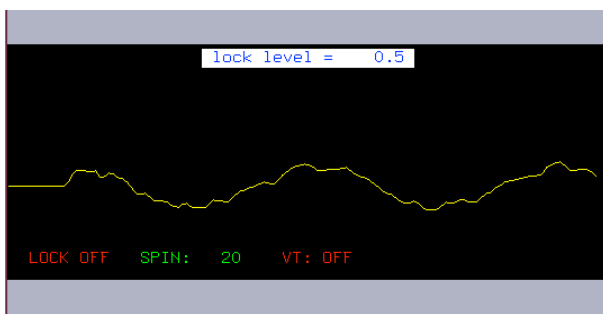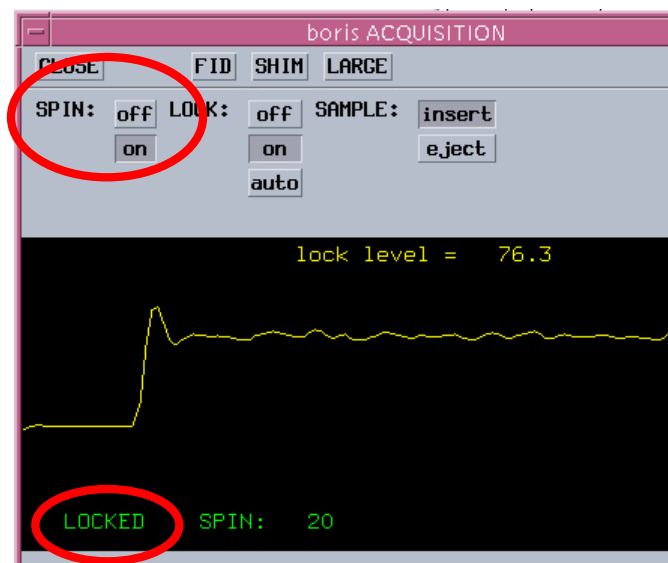

Z0 off resonance
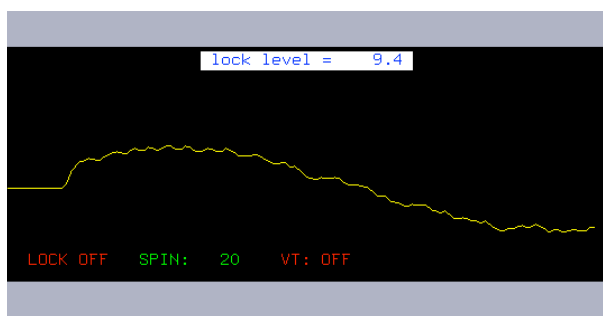
Z0 approaching resonance

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[3] The following variables are instrument dependent: Z0, lock power, lock gain, lock phase. Z0 and lock phase also vary over time. The spectrometer chart will be updated periodically by the Staff.
[4] Z0 is directly related to the chemical shift of protonated solvent.

Figure 11: Sample pictures of the lock signal as Z0 approaches the on-resonance step-function.

After Z0 is optimized (see a step signal with the maximum lock level), turn the lock: *[on]*.

6. Find a Lock Gain

Adjust the lock gain using *[-1+]* until the lock level is ~70 %.

7. Set Lock Phase Value

Use the *[-16+]* to adjust the lock phase to produce the maximum lock level.[3]

Click *[Close]* to exit the acquisition interface window.

8. Shim Sample:

Each sample perturbs the magnetic field differently. Shim adjustments are required to change the currents in 'corrective' coils surrounding the sample. These coils then perturb the magnetic field in such a way to (hopefully) make the field more homogeneous, producing better spectra. These coils are wrapped in such a way that they each affect the magnetic field *reasonably* independently, Unfortunately, physical constraints introduce "impurities" where the shim wires are physically positioned close together and consequently are influenced by the currents flowing through neighboring coils. Recall the importance of positioning the sample in the spinner. Correct and consistent volumes in the NMR tubes will be easier to shim.

Shimming can be performed by observing the lock level (higher ≡ more homogeneous) or shimming on the FID or spectrum. For now we will concentrate on shimming to increase the lock level. The lock level can be thought of as the *height* of the deuterium peak on which you are locked. The more homogeneous the magnetic field, the narrower the peak becomes. Since the area under the peak is constant, the peak height and lock level increase, as the field becomes more homogeneous.

The UL Mercury NMRs have 13 shims that adjust the magnetic field within the X, Y, Z, vector space. Many shims that have already been optimized for you when you type **bestshim** (assuming your sample volume (0.7 mL), tube quality, etc. are correct). Most of the shims should not be adjusted for typical samples. If you are spinning, the shims with x- and y-axis components must not be touched; only the z-axis shims should be adjusted. How many of the z-axis shims you should adjust depend on the sample's specific needs.

Everyone should probably touch up the Z1C (course Z1) and Z2C (course Z2) shims. For some synthetic chemists with non-crowded spectra, this may be enough to see what is needed. If the spectrum is crowded or if the line shape is important, Z1 (Z1 fine) and Z2 (Z2 fine) should be adjusted very carefully. Then if the spectrum indicates that more shimming is needed, the higher-order Z-axis shims may need to be carefully adjusted in a systematic fashion. Ask for Staff assistance if you need to shim to shim Z3-Z5. [5]

$$\Delta v = large \qquad \Delta v = small$$

Bad! ☹
NOT well shimmed

Good! ☺
Well shimmed
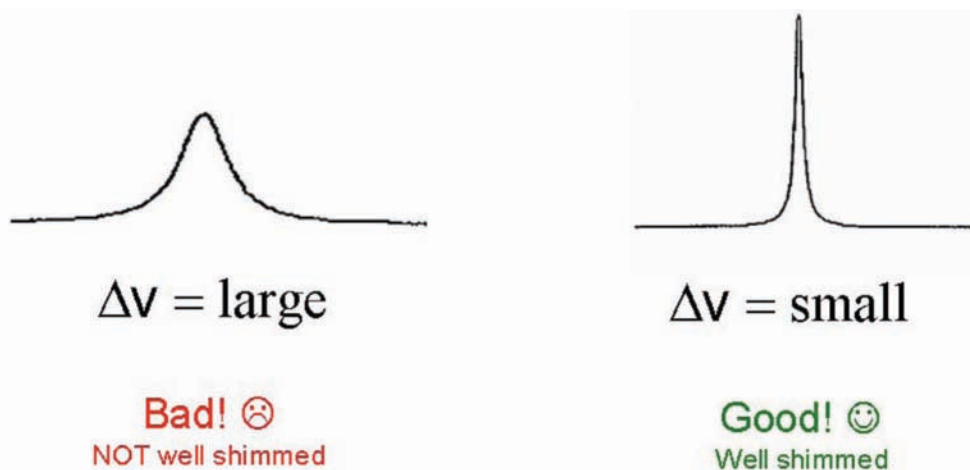
Figure 12 When am I finished shimming? Well optimized shims will produce sharp, symmetrical peaks.[6]

[5] If you would simply like more information about NMR spectroscopy and shimming check out the Acorn NMR website at http://www.acornnmr.com.

[6] Notice that a well shimmed peak is more intense, illustrating why lock level intensity can be used to measure magnetic field homogeneity.

Directions for shimming:

From the Interactive Acquisition window press *[shim]*

The Interactive Acquisition window will now be displayed in 'shim mode'. See Figure 13. The window displays two bar graphs labeled 'coarse' and 'fine'.
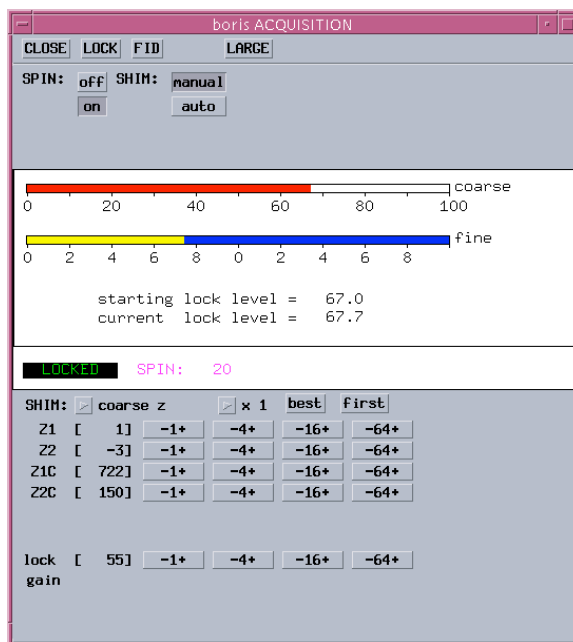
Figure 13: The 'shim' window of the Interactive Acquisition window. Close inspection shows that the 'fine' scale is nothing more than an expansion of the twenty-unit range where the current lock level resides. The name of the game now is to adjust the shims in order to maximize the lock level.

Start by clicking the *[-1+]* button of Z1C until the lock level is maximized.

The lock gain may need to be decreased (any time the lock level approaches 100, decrease the lock gain until the lock level is ~70 using the *[-1+]* button).

Maximize the lock level with the *[-1+]* button of Z2C and repeat Z1C.

Next, use the *[-64+]* button of Z1 until the lock level is maximized. Do the same with the *[-64+]* button of Z2. As these two shims are coupled, iterate back and forth between Z1 and Z2 using the *[-16+]* button, until a global maximum is achieved.

After shimming, adjust the lock gain until the lock level is ~70.

Click *[close]* in the Interactive Acquisition window.

NOTE: If the NMR spectrum does not have good line shape, start over: type **bestshim** in the input window and re-optimize the Z1C, Z2C, Z1, and Z2 shims as directed.

II. Proton Experiment Setup, Execution and Printing

1. Setup observe nucleus (pulse program) and solvent

Click *[main menu]*, click *[setup]* and then *[Nucleus, Solvent]*

Click *[1H]*

Click a *[solvent] button*

2. Set the Number of Scans

Type **nt=8** for 1H.

Type **time** in the input window to calculate the acquisition time required.

Type **su** to initialize the parameters with the spectrometer.

3. Start Acquisition, Stop

Type **ga** to start the acquisition

At any time, you can stop the acquisition by typing **sa** (**s**top **a**cquisition after next transient is collected) or **aa** (**a**bort **a**cquisition, now!).

4. Fourier Transform and Display

Type **wft ffav** in the Input window

Type **vp=12** in the Input window

If the baseline is not flat on both sides of each peak, seek help from your TA.

To Zoom-In:
Select a region in the Graphics Window by defining the region with the **LMB** and then the **RMB** (two red cursors will define the region), then click *[expand]*. The vertical scale can be adjusted, by dragging the **MMB** on top of a peak. Type **ffav** to return to the full view.

5. Reference Solvent Peak

To reference the spectrum, zoom-in on the solvent peak.

Click the spectrum with the **LMB** near the solvent peak.

Type **nl** (**n**earest **l**ine), then click **[Ref]** and enter the reference value from the CIL NMR Solvent data chart
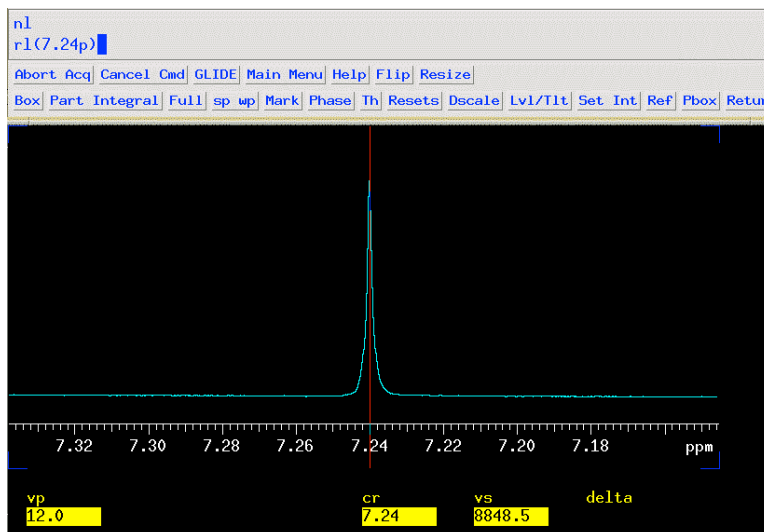

Figure 14: Referencing residual CHCl₃

6. Peak Pick
Zoom in on each peak

Click the threshold button *[Th]* and use the **MMB** to set the line at the lowest point for peak picking.

Print the peak expansions with chemical shift values in ppm.
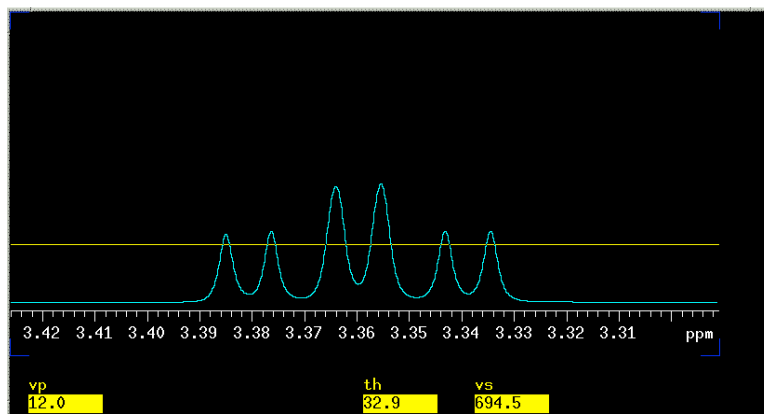
Type: **pl pscale ppf page**

Figure 15: Setting threshold for peak picking

Print the peak expansions with chemical shift values in Hz

Type: **pl pscale ppf page**

Use these values to calculate the coupling constants.

<u>Optional</u>: To view the peak picking on the monitor use the command **dpf** or **dpfhz**. Then type **ds** to return to the spectrum.
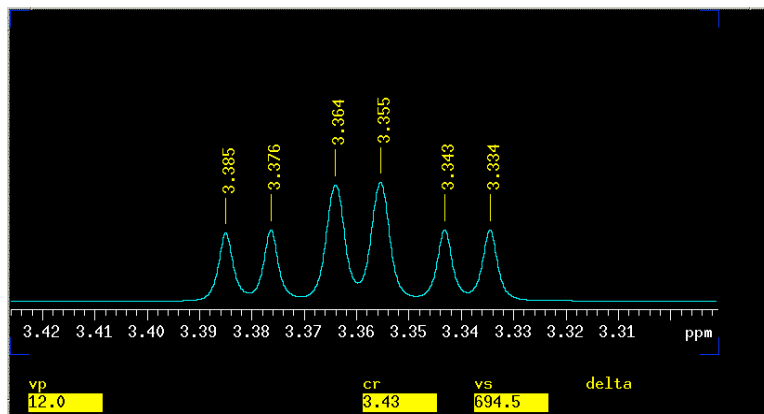
7. Integrate (Proton Data Only)

Type **intmod='partial'** to display the green integral line.

If the integral is not flat where the baseline is flat, use the *[Lvl/Tlt]* button and the **LMB** and **RMB** to adjust the integration.

Type **cz** (clear zeros from memory), then click *[resets]*. Use the **LMB** to define those regions you wish to integrate by clicking on both sides a peak. The **RMB** can be used to undo mistakes. Use the **MMB** to adjust the **is** (integral scale).

To assign an integral value to a particular region, type **ds** to regain cursor control then use the **LMB** to 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 display the integral values under the spectrum.

Print your spectrum with integration values:
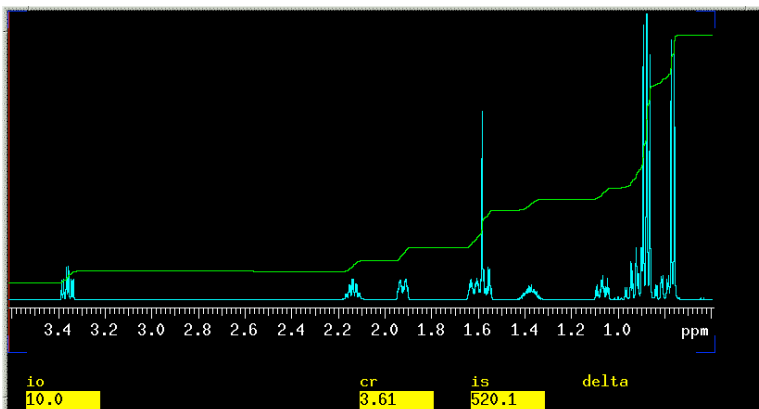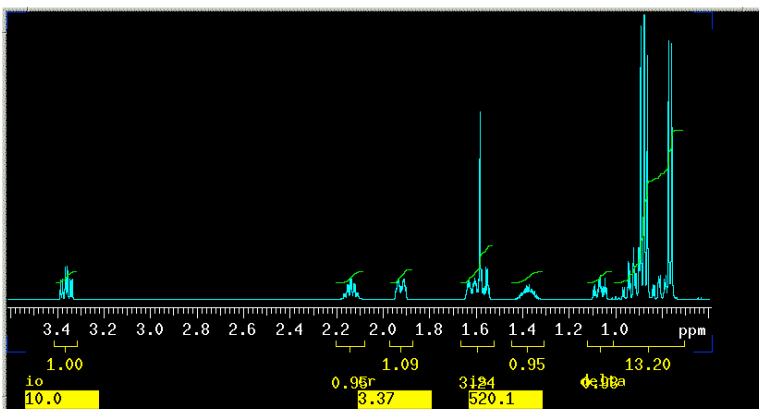**pl pscale pir page** (Zoom in and repeat printing if necessary)

Figure 17: 1H spectra of menthol with intmod='partial' and using the *[Lvl/Tlt]* button to level the integration.

Figure 18: 1H spectra of menthol after setting the *[resets]* and calibrating with *[Set int]* the peak at 3.36 ppm as equivalent to 1 proton. The command dpir was used to display the integral values below the x-axis.

8. Save Data

Type **svf < enter> ,** then type a ***filename*** at the prompt to save the fid to the disk. Do not use spaces or punctuation in the file name.

9. Review of Print Commands

To print your results, create the desired view of the spectrum within the Graphics Window. Then select which print commands you desire.

Type **pl pscale page**
This basic print command will print the spectrum (**pl**), scale (**pscale**) and **page** sends the plot to the printer.

Type **pl pscale pir ppfhz pap page**
Will print the spectrum (**pl**), the scale (**pscale**), the integral resets (**pir**), the peak picking in Hz (**ppfhz**) and the acquisition parameters (**pap**); **page** sends the plot to the printer.

Omit any command that is not desired. Add additional commands (ie **ppf** to print peak frequencies in ppm) before the **page** command.

III. Sample Removal

1. Stop acquiring data **sa**
2. Open *[ACQI]* choose the *[Lock]* Menu
3. Turn Spin *[Off]*
4. Turn Lock *[Off]*
5. *[Eject]* to turn on air and remove sample
6. *[Insert]* to shut off air
7. Click *[Close]* to exit the acquisition interface window.
8. Type **exit** in the command window to exit VNMR
9. Log off the computer **RMB**, Select logout
10. Leave at login screen for next user. DO NOT POWER OFF the workstation.

Appendix 1

Quick guide to performing a 1D 13C spectrum and a DEPT (Distortionless Enhancement by Polarization Transfer) experiment.

Experiment Setup and Acquisition

The basis for the DEPT experiment is simple. The transfer of polarization from protons to carbon enhances the carbon signal strength. By carefully calibrating the decoupler power, it is possible to distinguish CH_3 (methyl), CH_2 (methylene), and CH (methine) groups using three pulse widths of 45, 90, and 135 degrees. Since quaternary carbons have no attached protons, they are not detected.

- Use all of your of material (100-200 mg) in 0.7mL of deuterated solvent. If you have a low amount (less then 75mg), you may want to increase **nt** to 64 or 128 for the DEPT experiment. *Remember that you will need to recover your material from solution AFTER you have finished your DEPT.*
- Follow the NMR Instrument Setup guidelines to <u>insert, lock and shim</u> the sample.
- Turn spin off.

- Acquire a 13C spectrum, reference the solvent, peak pick and print the spectrum.

  1. *Click [main menu]*, then *[setup], then [C13,CDCl3]*
  2. Type: **nt= 64**   (nt = 64 should take about 3 minutes)
  3. Type: **su**
  4. Type: **ga**
  5. Type: **ffav**
  6. Reference the spectrum, zoom-in on the solvent peak. Click the spectrum with the **LMB** near the solvent peak. Type **nl** (**n**earest **l**ine), then click **[Ref]** and enter the reference value from the CIL NMR Solvent data chart
  7. Type **ffav**
  8. Click the threshold button *[Th]* and use the **MMB** to set the line at the lowest point for peak picking.
  9. Plot your carbon spectra **pl pscale ppf page**

- Acquire a DEPT experiment

  1. Type: **dept su** which will load the 'dept' parameters.
  2. Set **nt=32** to this value (or a multiple of 8). (nt=32 should take 10 minutes, nt=64 about 18 minutes, and nt=128 about 34 minutes).
  3. Type **time**, if you wish to see how long this experiment will take.
  4. Type **au** (submit experiment to acquisition and process data). Once completed, the processed data will automatically be sent to the printer.

- If the automatic processing fails, type **ds(1)** to display the first spectrum. Rephase and expand region of interest. Place a threshold line just below the lowest peak.
- Type **adept dssa** to analyze the data
- Type **pldept** to plot the DEPT spectra

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