OVERVIEW

The UNITY300 Spectrometer System

Our UNITY300 spectrometer system consist of four major parts:

- **Host computer** – UNIX computer from which the spectrometer is controlled and which processes the NMR data and prints out the spectra.

- **NMR console** – a two-bay cabinet housing radio frequency transmitters and data acquisition electronics; access is for facility personnel only.

- **Remote status unit** – a display with a meter and lights which sits to the right of the computer monitor and shows directly what is going on in the NMR: pulses, decoupling, acquisition of the FID and the lock level, all coming directly from the console.

- **Magnet leg** - a metal stand which distributes radio frequency cables from the console to the probe. The sample depth gauge is mounted on the magnet leg.

- **Superconducting magnet –** an Oxford Instruments 300 MHz (7.05 Tesla) magnet with superconducting coil cooled with liquid helium and liquid nitrogen. NEVER BRING METAL OBJECTS WITHIN 15 FEET OF THE MAGNET. Contact with tools, metal chairs, bicycles, etc., can severely damage the magnet and require repairs costing up to $40,000. A "quench" – catastrophic boiling of liquid helium – can happen at any time and will be obvious due to the sound of rapidly escaping He gas. In the event of a magnet quench, LEAVE THE ROOM IMMEDIATELY. Helium gas is deadly because it displaces oxygen quickly and leaves the room without breathable air!

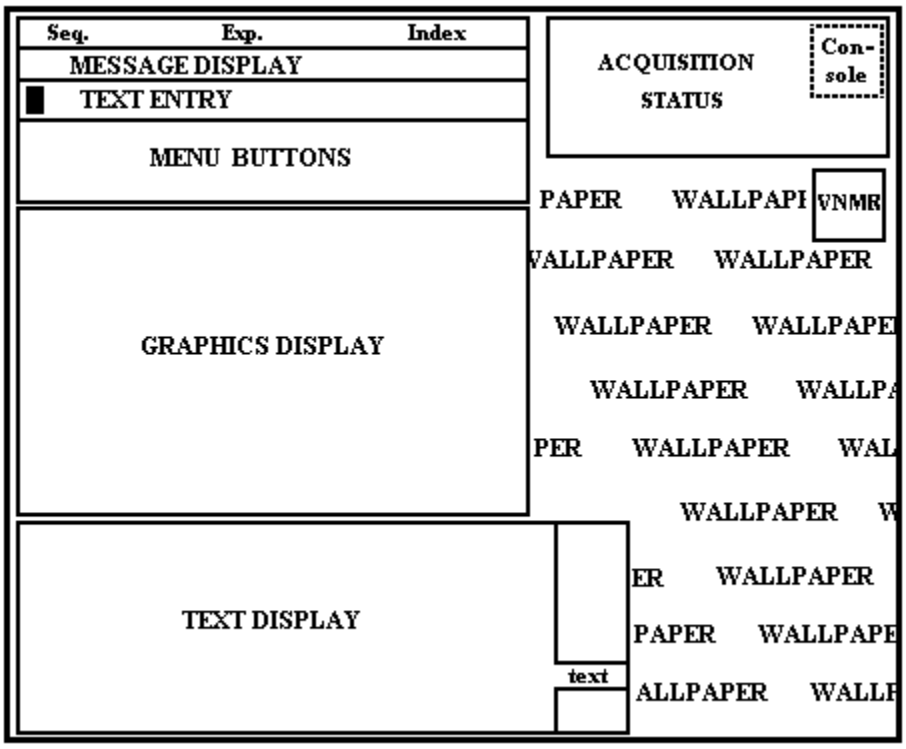
- **Probe –** the probe inserts into the magnet from below and the sample inserts into the probe from above, being gently lowered on a cushion of air. Surrounding the sample solution in the probe is a transmit – receive radio frequency coil which is used to excite the sample nuclei (transmit) and receive the FID signal (receive). We have two different probes for the Unity-300: a **Four-Nucleus Probe** which is the default probe and is optimized for ^{13}C and ^{31}P observation; and an **Inverse Probe** which is optimized for ^{1}H observation and has a second coil that can be tuned to any other nucleus.

Host computer

The spectrometer is controlled from a UNIX-based Sun Microsystems workstation ("unity300") running a program called VNMR that allows users to interact with the spectrometer using the OpenWindows Desktop environment. You must log in to the Sun computer with a login name and password, and the VNMR software will start up automatically. The workstation is a Sun Microsystems Ultra 10 with a flat-screen monitor. We also have two satellite workstations, "ernst" and "bloch", both of them Sun Microsystems SunBlade 100, which are available at no hourly cost for processing NMR data and printing out spectra. The **unity300** computer has one disk, called "/export", which contains all of the user's NMR data. This disk is available to **ernst** and **bloch**, so that using the satellite workstations is exactly the same as using unity300, except that there is no charge and you can't run the spectrometer from these workstations.

VNMR Display

The VNMR display screen is divided into a number of windows showing the user what is happening with the instrument and data. Like most windowing systems, these windows can be moved around and even closed by the user, but during routine use, the standard positions of the windows should be perfectly acceptable and we recommend users **DO NOT close or move them**. The diagram below shows the initial configuration of the display screen.



Windows

Status Window. The status window, at the top left of the VNMR display, shows the current pulse sequence and the experiment number (top line) and any short messages or responses from the software (bottom line.

Text Entry Window. Below the status window is a prompt

for entry of text commands. This prompt appears as a filled rectangle if it is active, and an open rectangle if it is not. To enter text commands, the window must be activated by clicking the left mouse button within it.

All text input from the keyboard is done in this window. Commands are entered by simply typing the name of the command followed by the **Return** key. Some commands require that you include additional information (an "argument") in parentheses. There should never be a space between the command name and the left parenthesis of a command argument. More than one command can be entered at the same time for sequential execution by separating the commands with spaces: for example, *pl pscale page* will execute all three of these commands in sequence.

Arguments for commands come in two types: *numbers* and *text*. Text must be enclosed in single quote marks ('...'), such as *'current'*. For example, by using the command *rts*, you can recall the current best shims from the disk: *rts('current')* .

Menu Buttons. Below the text entry window are two rows of menu buttons. All commands can be entered as text commands at the text entry prompt, or more conveniently they can be selected by clicking on a menu button with the left mouse button.

When you first start the VNMR program, the menu choices look like this:

[Abort Acq] [Cancel Cmd] [GLIDE] [Main Menu] [Help] [Flip] [Resize] [Acqi]
[Workspace][Setup][Acquire][Process][Display][Analyze][File][DATA][AutoProcess][AutoPlot][More]

The top menu is permanent but the bottom one changes depending on your selections. The bottom row shown above is the **Main Menu** which can be called at any time by clicking on the [Main Menu] button in the top row.

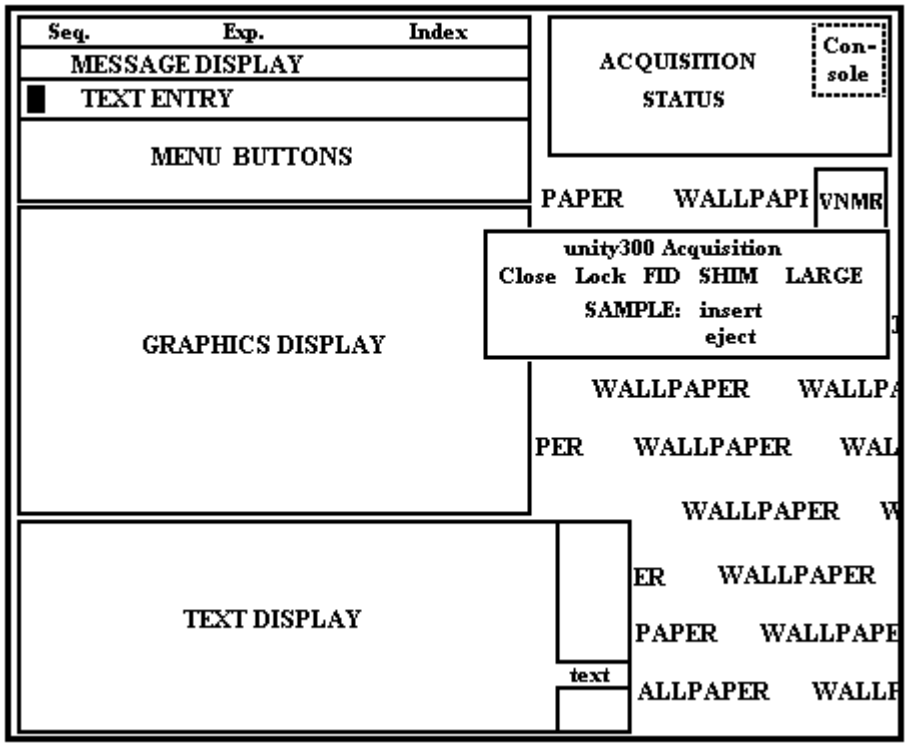
Graphics Display Window. Beneath the menu buttons is the main VNMR Graphics Display window, which has a solid black background. FIDs and spectra are displayed in this window. The window has two default sizes - small, in which the Graphics Display and Text Display windows can be viewed at the same time, and large, in which the graphics window covers the full width of the screen. You can change the size through the menu system by selecting the [Resize] button, which toggles (switches back and forth) between the *Large* Graphics Display and the *Small* Graphics Display. In the Large display, the Graphics Display window may cover the Text Display window, or the Text Display window may cover the Graphics Display window. By selecting the [Flip] button in the Main Menu, you can toggle between these two arrangements.

Text Display Window. At the bottom left of the screen is the Text Diplay window, which is used to display parameter lists, help files, peak lists and other text information. This is strictly an **output** window.

Acquisition Status Window. The acquisition status window, in the upper right of the monitor screen, displays information about the status of acquisition: is data being acquired or is the system idle? How many transients have been completed (`CT`), and what

is the estimated completion time of the experiment? What is the spinning speed? What is the lock level? This is also strictly an output window.

Interactive Acquisition Window. The last button in the top row of menu buttons – [Acqi] – opens the Interactive Acquisition Window. This window is used for sample eject and insert, locking and shimming. All of these are the first thing you must do before beginning to acquire data from your sample. This window appears on the right side of the screen, partially covering the Graphics Display window. The monitor display now looks like this:



There are five buttons at the top of the window: **[Close]** inactivates the window and replaces it with the **[Acqi]** button in the top row of the menu buttons. **[Lock]** enlarges the window and displays the deuterium (^{2}H) lock signal and the lock parameters, which can be interactively adjusted. **[FID]** allows you to shim while observing the effect on the FID or the spectrum. **[SHIM]** is for normal shimming where the lock level (height of solvent peak in the ^{2}H spectrum) is used as the criterion. [LARGE] toggles between the normal (small) display and a larger display of the Interactive Acquisition Window. Below these five buttons are the sample [insert] and [eject] buttons, which are used to lift the sample out of the probe on a cushion of air (eject) and to gently lower a new sample into the probe on a cushion of air (insert).

Unix Shells. The small square icon labeled "VNMR" at the right of the screen below the Acquisition Status window is the UNIX shell (window) in which the VNMR software is running. If you exit the VNMR program by typing "exit" at the Text Entry prompt, all windows will disappear except the VNMR Shell icon and the Console icon (which is normally hidden behind the Acquisition Status window). If you click (with the left mouse button) on this icon it will open an operating system (UNIX) window with a text entry prompt. You may have to click in the window and hit Return to see the prompt. To re-start the VNMR program, enter "vn" at the prompt. You can shrink this window back into an icon by clicking on top bar with the right mouse button and dragging down to select

"Close". The Console Window (UNIX shell) can be accessed by moving the Acquisition Status window (click right mouse on the top bar of the window, hold the mouse button down and "drag" the window out of the way) and clicking on the icon. You can re-size this window by clicking on one of the corners and dragging to a new size and shape. This window allows you to look around at your files, delete files, move files, send files to other computers, and in short do everything that is possible with UNIX commands. To reduce this window to an icon again, click on the upper bar with the right mouse button and drag down to select "Close".

***WARNING*: SHOULD THE HOST COMPUTER CRASH ON YOU OR SIMPLY QUIT RESPONDING, *DO NOT* UNDER ANY CIRCUMSTANCES REBOOT OR TURN OFF THE COMPUTER! THE UNIX OPERATING SYSTEM REQUIRES FOLLOWING A CERTAIN SHUTDOWN PROTOCOL BEFORE THE COMPUTER CAN BE RESET OR TURNED OFF.**

INSTEAD, ASK SOMEONE FROM THE NMR FACILITY TO HELP YOU! USUALLY THE PROBLEM CAN BE SOLVED WITHOUT REBOOTING THE COMPUTER.

Detailed Instructions for Routine Use of the Varian Unity-300

Sample Insertion, Lock, and Shimming

Inserting the Sample

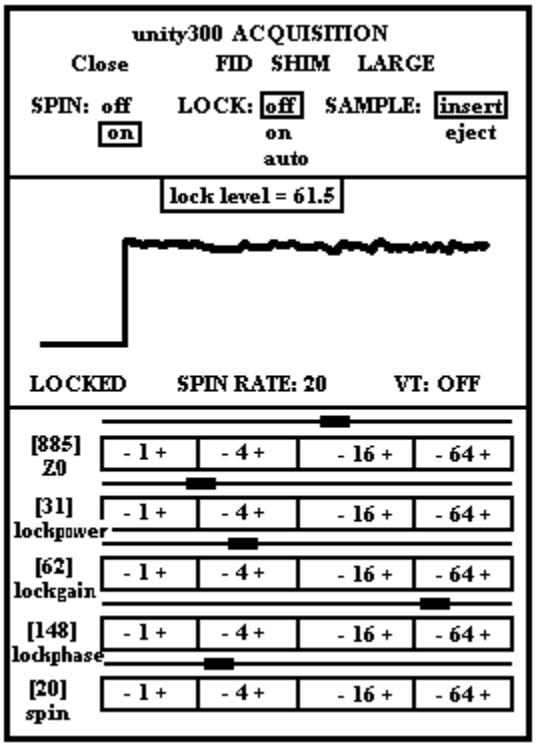
Activate the Interactive Acquisition window by selecting the **[Acqi]** menu button.

In the Interactive Acquisition window, click on **[eject]**. Pick up the sample and spinner turbine at the top of the magnet bore, remove the sample from the spinner (*careful!!! it's very easy to break the NMR tube when removing it from Varian's spinners!!!*) and insert your sample in the spinner. Also, be careful not to drop the spinner turbine – they are carefully balanced and very expensive to replace. Adjust the height of the sample tube in the spinner turbine using the gauge on the magnet leg. Using the squirt bottle put some ethanol on a Kim Wipe, clean the lower part of your tube and the narrow portion of the spinner turbine and set the sample and spinner on the air cushion at the top of the magnet bore. Make sure the sample is "bobbing" freely on the air cushion and not stuck on the top of the bore. If the sample is rocking violently in the eject air stream, check with the NMR facility personnel to make sure the eject air pressure is not set too high. Incorrect air pressure can break samples in the probe, leading to costly repairs and lengthy down time.

Click on **[insert]** and the sample will be gently lowered into magnet and inserted into the top of the probe.

Locking

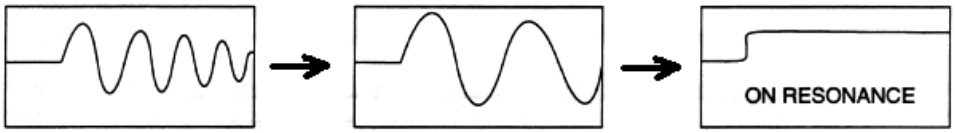
Click on [Lock] in the Interactive Acquisition window. The **LOCK** display will appear as shown below:



The top part of the window has the menu buttons for turning the spinner on and off, the lock on and off, and the sample eject and insert. The central part of the window displays the lock signal and shows the current status for lock level and spin rate. The lower section of the **LOCK** display window contains controls for changing the values of the lock parameters ***Z0***, ***lockpower***, ***lockgain***, ***lockphase***, and ***spin***. The current value of each parameter will be displayed as a number in brackets and as a slider control for making big changes. The value of the parameter can be changed by clicking on the buttons [- 1 +], [- 4 +], [- 16 +] and [- 64 +]. Each click with the ***left*** mouse button on one of these buttons ***subtracts*** the value shown; each click with the ***right*** mouse button ***adds*** the value shown to the appropriate parameter.

Start the sample spinning: Click **[on]** for SPIN and make sure that your sample spins at 20 rps. If the sample does not spin, eject it and check the sample depth. Clean the tube and the narrow (bottom) part of the spinner turbine thoroughly with ethanol, insert and try again. If it still doesn't spin, try a different spinner turbine, again adjusting the sample depth and cleaning with ethanol. If this doesn't work get help from the NMR facility personnel. Keep in mind that you CAN get a decent spectrum without spinning, particularly for ^{13}C spectra. To do this, enter **spinner=0** at the text entry prompt after running Setup (see below).

Set the Solvent Deuterium Signal to Zero Frequency: Click [off] next to LOCK to disable the deuterium lock feedback loop. Consult the card attached to the bottom of the monitor to find the optimal values for lock parameters *for your lock solvent* (e.g., CDCl3, DMSO-d6, etc.). Set ***Z0***, ***lockpower***, ***lockgain*** and ***lockphase*** to the values on the card. Adjust ***Z0*** (field) to the "on resonance" condition using the **[- 4 +]** button:



As you approach resonance (solvent ^{2}H peak in the center of the ^{2}H spectrum) you will see fewer and fewer oscillations or "wiggles" in the lock display, until you get a flat line (zero audio frequency). If you continue to change ***Z0*** in the same direction, you will again begin to see wiggles with more and more cycles displayed on the screen. If that happens reverse the direction of adjustment of ***Z0*** until you return to the "flat line" or resonance condition. If you cannot find any lock signal ("wiggles"):

Try increasing the **lockpower** (set it back to the card value when locked).

Make sure your solvent is deuterated.

Make sure the sample depth in the spinner is correct.

Make sure the shims are reasonably good by entering: **rts('current') su**

Explore higher and lower ***Z0*** values – the magnet drifts and the resonance value changes for each solvent. When you are far from resonance, not only are there many cycles displayed on the screen, but the signal gets weaker and weaker.

Lock on the Solvent Deuterium Signal. Click **[on]** in the **LOCK** menu to activate the deuterium lock feedback loop. The lock signal (flat line) will rise and be stable at a level above the reference line (left side) on the display (see diagram on previous page). The height of this flat line is the lock level, which corresponds to the height of the solvent deuterium peak in the ^{2}H spectrum.

Adjust the ***lockphase*** to get maximum lock level. Click on the **[- 16 +]** button and watch the numerical lock level display above the graphics display of the lock signal. If lockphase is not set right, the best effort at shimming will still not give you sharp peaks and a good spectrum.

Adjust the ***lockpower*** and ***lockgain*** to the card values if you have changed them to get the sample to lock.

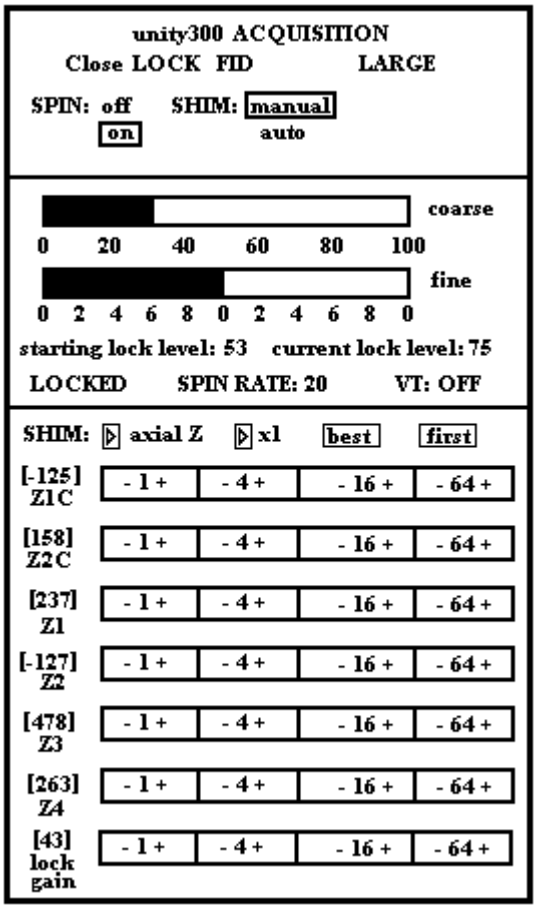
Note: *Both lockpower and lockgain will change the lock level, but it is important to understand the difference. Lockpower is the amount of excitation given to the deuterium nuclei in the sample, and lockgain is the amplification of the lock signal coming out of the sample before it is displayed. Too much lock power will saturate the deuterium nuclei in the sample, leading to an unstable or "breathing" lock signal. It is very difficult to shim using an unstable lock signal. Lockpower should be set to the card value, and lockgain should be adjusted to get a lock level of 60-80. If the lock level ever goes above 100 (off scale), simply decrease the lockgain to get a reading in the range of 60-80.*

Shimming

Click on the **[SHIM]** button to exit the **LOCK** display and open the **SHIM** display. The upper panel of the display should show that **SPIN** is **on** and **SHIM** is set to **manual**. The lock level (height of the solvent peak in the ^{2}H spectrum) is displayed as a horizontal "thermometer" or bar graph, running from 0 to 100 and labeled "coarse". An enlargement

of the region around the current lock level is shown below this, covering a range of 20 units and labeled "fine". Your goal is to increase the lock level as much as possible (move the lines to the right in the bar graphs) by adjusting the ***Z1C*** and ***Z2C*** shim values.

For each of these two shims, click with the left or right mouse button on the **[- 4 +]** button repeatedly and try to bring the lock signal to a maximum value and then keep going until it goes down again. Then go back until you reach the maximum again. Go back and forth between the ***Z1C*** and ***Z2C*** shims until you no longer get any improvement in the lock level. If the lock level reaches 100, click the left mouse on the **[- 1 +]** button of the **lockgain** row of buttons to bring the lock level down to about 80, then continue shimming. Don't be shy when adjusting the shims - be sure you have seen a real effect and not just a random fluctuation of the lock level. After you have adjusted ***Z1C*** and ***Z2C***, using the **[- 4 +]** button, try fine-tuning with the **[- 1 +]** button on ***Z1C*** and ***Z2C***.



When you are finished shimming, click the **Close** button to leave Interactive Acquisition.

Setting Up for Data Acquisition

An **experiment** is a set of files on disk, in which parameters and data are stored; it has room simultaneously for both raw data (FID) and processed data (the transformed spectrum). It is not necessary to remember to "save data" before performing an FT or other processing; the original FID data exists on the disk and is unaffected.

At any given time, you are always "joined" to an "experiment". Each experiment has a number (the current experiment number is shown by "Exp:" on the first line of the status window), which can be 1, 2, on up to 9. You can join any existing experiment by selecting the **Workspace** button in the **Main Menu**. This will diplay the menu options: **[Library][Exp 2][Exp 3][Create New][Delete]** if you are currently in Exp 1 and you have defined experiments 2 and 3. Simply click on **[Exp 2]** to join experiment 2. If you click on **[Create New]**, the next experiment number after the highest existing one will be created – in this case experiment 4. If you click on **[Delete]**, you will be asked to enter the number of the experiment to delete, and this experiment will disappear. If you click on **[Library]**, the text window will display the experiment library - a list of experiments that exist, their size, and information about the current data contained in each experiment. The most useful application of experiments is

to process data in one experiment while you are acquiring data in another. For example, you could set up a ^{1}H spectrum in Exp 1, and when the acquisition is complete go to Exp 2 and set up a ^{13}C experiment. While that experiment is running, you can go back to Exp 1 and process and plot out the ^{1}H spectrum. By the time you are done processing the ^{1}H spectrum, the ^{13}C acquisition may be finished.

The parameters in the current experiment remain active until they are changed. Thus, to repeat an experiment you have just performed, no "setting up" of parameters is necessary - simply start the acquisition. If you just need to change one or two parameters in order to set up the next experiment, that can be done by typing in those parameters, e.g. ***pw=6 nt=16***.

To set up an experiment from scratch, click on **Main Menu** and **Setup**. For routine experiments in $CDCl_3$, the most commonly used solvent, click on **[H1, CDCl3]** for a ^{1}H experiment and **[C13, CDCl3]** for a ^{13}C spectrum. For other nuclei or other solvents, click on **[Nucleus,Solvent]** and then choose from a list of nuclei (H1, H2, C13, N15, F19, P31, Other, Return) and a list of solvents (CDCl3, D2O, Benzene, DMSO, Acetone, Other, Return). If you select **[Other]** you will be prompted to enter the nucleus or solvent.

Enter **su** (setup) to send the parameters to the hardware (the spectrometer). Make sure that the temperature (VT) is not regulated (unless you are doing a variable temperature experiment) and check the lock level (Remote Status Display meter) to make sure it has not changed.

You can display the parameters in the text display window using the ***dg*** and ***dg1*** commands. ***dg*** shows the acquisition and processing parameters and ***dg1*** shows the display parameters. You can also check the current value of a parameter by simply entering the parameter name followed by a question mark, *e.g.* ***nt?*** **<Return>** or ***sw?*** **<Return>** . The parameter value will be displayed in the text message window. You can change the value of any parameter by entering the parameter name followed by the equal sign and the new value: for example, **nt=64**.

NOTE*: VNMR is case sensitive, so* ***nt*** *and* ***NT*** *are not the same!!! Remember, all acquisition parameters have lower case names!*

Selected Acquisition Parameters Displayed by dg

sfrq	exact spectrometer frequency used
tn	nucleus for observe transmitter
at	FID acquisition time in seconds
np	total number of data points being acquired (=2 * number of complex pairs)
sw	spectral width (Hz) in the observe dimension
bs	block size, see "Processing Data While Acquiring"
fb	filter bandwidth (set automatically from **sw**)
ss	number of dummy (steady-state) scans
tpwr	transmitter power in dB (logarithmic scale, 63 is maximum)
pw	pulse width for simple one-pulse experiment
d1	relaxation delay for simple one-pulse experiment
tof	transmitter offset (Hz) – sets the center of the spectral window

ct number of completed transients (scans)
lb line broadening (Hz) parameter for exponential multiplier
fn number of data points after zero filling

Acquiring Data

Starting the Acquisition

For ^{1}H experiments, use ***gain='n'*** (should be the default value) to activate autogain, in which the gain is automatically adjusted to an optimum value at the start of acquisition. If you repeat an acquisition for a particular sample, you can save time by first entering ***gain='y'*** so that the autogain procedure will be skipped. If you enter **gain?** The actual gain setting (0 to 63) will be displayed in the Message window.

Enter ***nt=number_of_scans***, where *number_of_scans* is a multiple of 8 (to allow for the complete phase cycle). Reasonably concentrated samples (> 1 mg) require only 16 scans to get a good quality ^{1}H spectrum, but typically 1024 scans or more may be required for a carbon spectrum. In theory, to get the same signal-to-noise ratio (S/N) as a proton spectrum, you would need about 4 million times as many scans! Usually we settle for poorer S/N in the carbon spectrum than we have in the proton spectrum. After you have set **nt**, you can check the time required for the experiment by entering **time** (no question mark). You can also simply enter **time(***hours,mins***)** where *hours : mins* is the amount of time you have available; the software will set **nt** to give approximately that length of experiment. For ^{13}C acquisitions it's better to set up for a longer acquisition (larger **nt** value) than the time available, and then check the spectrum (**wft** command) from time to time to see if there is sufficient S/N. If the parameter **bs** (block size) is set to 64, for example, the computer stores the accumulated FID data up to that point to the disk every 64 scans. Thus, every **bs** scans, an updated FID is available for Fourier transform by the user with the **wft** command.

Enter ***go*** to start the acquisition. This command will delete all of the old data in the current experiment, so if you want to save the old data you must first use ***svf('filename')***. In the Acquisition Status window you will see **Status: Auto Set Gain** while the spectrometer experiments with different values of gain (amplification of the FID signal) to get the largest signal without overflowing the ADC. Then you will see **Status: Acquiring** while the FID data is acquired. During this time the Acquisition Status window will display the value of **ct** (completed transients) until it reaches **nt** and the acquisition is complete. At this time the computer will beep and show **exp#: Acquisition Complete** in the Message window, where # is the experiment number where the acquisition was done. The Acquisition Status window will display **Status: Idle**. For a ^{13}C acquisition you can stop the acquisition whenever you are satisfied with the S/N: click on **[Abort Acq]** in the upper row of menu buttons to stop acquiring.

Enter ***df*** to display the FID when the acquisition is complete.

Processing the Data

Transforming the FID. After the acquisition is complete, you should save the raw data (FID) to disk. The FID is saved along with the parameters, the title (text) and the shim values in your local directory. This data belongs to you and cannot be removed or changed by anyone else. It resides in the directory **/export/home/<group>/<login>/vnmrsys/data**, where <group> is your research group and <login> is your login ID.

Use **svf('*filename*')** to save your FID; *filename* can be any combination of letters and numbers, but only the underscore (_) should be used for punctuation. The data will be saved as a directory called *filename.fid*. In this directory, four files are created: **fid**, which contains the actual binary data; **procpar**, which contains all of the parameters used; **text**, which contains any text you have entered to describe the sample and experiment; and **log**, which is a log of the exact times of acquisition. You can retrieve the FID later by using the **rt*('filename')*** command. You can also retrieve an FID by using the **[Data]** menu button in the Main Menu. A list of **.fid** files will be displayed, and you can highlight one of them and click on **[Load]** to bring this data into the current experiment. Then process the FID data as indicated below.

> Note: Whenever you are working in a particular experiment on the Unity-300, the VNMR software creates a file called **lock_#.primary**, where # is the number of the experiment you are using. The purpose of this file is to prevent you from changing the data in this experiment when you are working on a satellite station (such as **ernst** or **bloch**). If you try to load data into this experiment on **ernst** or **bloch** using the **rt** command or the **[Load]** button, it will fail to load the data. When you exit from VNMR on the Unity-300, the lock files are deleted and you can now load data into these experiments on the satellite stations. The only problem that can occur is if you don't first exit VNMR before you log out on the Unity-300 – the lock files will not be deleted and now you are permanently blocked from loading data into that experiment. To fix this problem you will have to use the UNIX **rm** command to remove the lock files from your login directory. Just be sure to always type **exit** at the text input prompt of VNMR before you log out of the UNIX computers **unity300**, **ernst** or **bloch**.

Check the parameter **lb** (type **lb?**) to assure that the correct exponential multiplier will be used: typical values are 0.2 Hz for proton spectra and 1.0 Hz for carbon spectra.

Enter **wft** to "weight" (exponentially multiply) and Fourier transform your FID. It will automatically display your transformed spectrum on the screen: you can redisplay your

spectrum at any time using the **ds** command. This not only displays the spectrum, but it puts you in "interactive" display mode, the equivalent of clicking on **[Display]** and **[Interactive]** from the Main Menu. Sometimes you may find that the spectrum display seems to be frozen and doesn't allow you to change things; just enter **ds** or click on **[Display]** and **[Interactive]** to return to the interactive display mode.

Vertical Scale. Use the middle mouse button to adjust the vertical scale of the displayed spectrum. Pressing this button causes the vertical scale to be adjusted so that the intensity at the position of the mouse arrow is equal to the vertical position of the mouse arrow. ***NOTE****: depressing the middle mouse button at the left edge of the spectrum will move the whole spectrum up or down instead of changing the vertical scale.* You can also use the typed command **vsadj** to adjust the vertical scale so that the largest peak is about 90% of the maximum height.

Horizontal Expansion. With the spectrum displayed on the screen, enter **f** to display the full spectrum. Click on **[Dscale]** to display the ppm scale under the spectrum. Using the *left* mouse button, place the vertical cursor line to the left of the *leftmost* peak. Then click with the *right* mouse button to the right of the *rightmost* peak. Click on **[Expand]** to horizontally expand the spectrum so that these two cursor positions are now the left and right edges of the displayed spectrum. The red vertical cursors can be displayed at any time by clicking on the **[Cursor]** button in the Interactive Display menu. When one cursor is displayed, this button says **[Box]**, meaning that if you select it you will get two cursors (enclosing a "box", or region of the spectrum).

Phase Correction. Your spectrum will look funny at first, with peak intensity extending both below and above the baseline (line of noise which contains no peaks). Enter **aph** to do an automatic phase correction. Unfortunately, in some cases (noisy spectrum or very wide spectral window) the **aph** routine does not work properly. In that case you have to do a manual phase correction. Expand your spectrum to include all of the peaks as described above. Enter **rp=0 lp=0** to set the zero-order and first-order phase parameters back to zero. Click on [Phase] to enter the interactive phase correction mode. Position the mouse arrow on a major peak toward the *right side* of the spectrum, about halfway vertically up the screen, and click the *left* mouse button. A horizontal cursor will be displayed at the level of the mouse arrow and two vertical cursors will be placed on either side of the mouse arrow. A region in between the vertical cursors will be displayed in a different color and *only this region* will be interactively updated. Now by holding down the left mouse button and dragging the mouse up or down within the two vertical cursors, phase the peaks in the region. Use the *left* mouse button for "coarse" adjustments and the *right* button for "fine" ones. This sets the zero-order phase correction (**rp**). Now move the mouse arrow to another major peak near the *left edge* of the display and click the left mouse button again. A new horizontal cursor will be displayed at the mouse arrow, and two new vertical cursors will be displayed on either side of the mouse arrow. Phase the region in between the vertical cursors as described above. This sets the slope or first-order phase correction (**lp**). Enter **ds** to redisplay the spectrum and exit the interactive phasing routine.

Referencing. The ppm scale is arbitrary and must be adjusted left and right by setting a peak of known chemical shift to its ppm value. Typically, we use TMS (0 ppm) for ^{1}H spectra and the center component of the solvent peak in ^{13}C spectra. Make sure the horizontal scale is in units of ppm; if not, enter **axis='p'**. If you don't have a horizontal scale, enter **dscale** or click on **[Dscale]** to display the scale below the spectrum. Expand the region around the reference peak and position the vertical cursor line on top of the reference peak. Enter **nl** (**n**earest **l**ine) to let the computer position the cursor precisely on the top of the peak. This doesn't always work, so you can position it manually if you wish. Enter **rl(#p)** (**r**eference **l**ine) to reference the peak, where # is the chemical shift of the peak in ppm. For example, to reference a ^{13}C spectrum acquired in CDCl3 solvent, position the cursor on the center of the three solvent peaks and enter **rl(77p)**. DO NOT use the single quote marks! If you expanded your spectrum to see the reference line better, enter **f ds** to display the full spectrum again.

Peak Peaking Sometimes you want a text list of the peaks in your spectrum with ppm values and intensities for all of the peaks. Alternatively, you can print the peak ppm values on the spectrum on top of each peak. In either case, you need to tell the program what intensity level corresponds to actual peaks and not noise. With the spectrum displayed on the screen, click on **[Th]**. This will display a horizontal cursor for setting the minimum intensity or "threshold". Use the left mouse button to position this cursor well above the noise level and below the top of the smallest peak you wish to have appear in the list. Enter **dpf** (**d**isplay **p**eak **f**requencies) to make sure that all peaks of interest are above the threshold. This will display the frequencies for those lines that are taller then the minimum intensity. Readjust the threshold if needed. ***NOTE:*** *after using* ***dpf*** *enter* ***ds*** *to get back to the interactive display mode*. If you want to print out the line listing, first make sure that the parameter **printer** is set to **hplj1200t** (enter **printer='hplj1200t'**). Then enter **printon dll printoff**. This will print out the line frequencies and intensities that are above the threshold. Actually, any command which generates text output in the Text Display window can be sandwiched between the **printon** and **printoff** commands so that the text output goes to the printer. For example, the command **printon dg dll printoff** will generate a printout of the parameters and the peak chemical shifts.

Integration. Integration allows you to print out the graphical integration lines over each peak, as well as to print under each peak an integral area, normalized so that it gives the number of protons directly. In the **Interactive Display** mode, there is a menu button which toggles between three labels: **[Part Integral]**, **[Full Integral]** and **[No Integral]**. Each time you click on this button its label changes to the next one in this list. Full integral displays the integral in a continuous line from one side of the spectrum to the other; Part Integral displays the individual integrals on each peak, with the regions between peaks "blanked" so they don't show up; No Integral displays the spectrum without the integrals. Click on the button until [Part Integral] is displayed on the button, and then click once more to display the integrals in this partial mode. Enter **cz** (clear zeroes). Any existing breaks separating the integrals (integral "zeros") are cleared. Click on **[Resets]**. Click the left

mouse button at the left edge of the leftmost peak of interest, then move the mouse arrow to the right edge of the same peak and click the left mouse button again. Repeat for each peak in the spectrum. Leave a little bit of space on either side of the peak so that the integral curve will give an indication of "drift". If the baseline (noise areas between peaks) is not flat (horizontal at zero intensity), the integral will drift up or down even in the area outside the peak. The integral line should be horizontal before and after the peak, indicating that there is no drift and therefore that the integral area is accurate. ***HINT:*** *If two peaks are adjacent to each other but you want a reset between them, click the button* ***twice*** *at the same place. This starts the next integral exactly at the end of the previous one.* Do not reset the integral in the middle of an overlap region; intensity should go down to the baseline level between peaks if you wish to integrate them separately. When you have "cut" all of the integral regions, enter **ds** to return to interactive display mode.

To get more accurate integral values you can "flatten" the baseline of the spectrum. After "cutting" the integrals around each peak, enter **bc(1)** to "baseline correct" the spectrum. The method assumes that anything that is not integrated is noise (baseline) and will attempt to fit the baseline to a smooth curve and then subtract out this curve from the spectrum. If ANY peak is not integrated, this will distort the baseline because the bc command will assume that the peak is baseline and try to "flatten" it.

Enter **vp=12**. The spectrum will move up to allow space for a numerical display of the integral areas. Click the middle mouse button above the right end of any displayed integral. This adjusts the integral vertical scale. Bring the vertical scale of the highest integral to near the top of the graphics display window. Enter **dpirn** to display the numerical values of the integrals on the screen. Note the integral value of any well-resolved single peak and normalize to the desired integral intensity (number of protons): enter **ins=ins * <desired value> / <observed value>**. For example, if a CH_3 peak in the ^1H spectrum has an integral value (displayed below the peak) of 0.06, enter **ins=ins*3/0.6**. Then enter **ds dpirn** to re-display the integrals. Often because of round-off error the new value will be close to the desired value, but not exactly correct. For example, if the same CH_3 peak now has an integral area of 2.79, enter **ins=ins*3/2.79**. Now enter **ds dpirn** and you should get a normalized area of 3.00 for the peak. ***NOTE****: if you change the region of the spectrum displayed on the screen, the normalization will have to be repeated. The parameter* **ins** *represents the* ***total*** *integral area of all integrals* ***displayed*** *on the screen.*

Plotting the Spectrum First of all, you need to select the plotter. Enter **plotter='hp7550a'** to use the Hewlett-Packard pen plotter, or **plotter='hplj1200'** to use the laserjet printer. Readjust the vertical scale and expansion if needed. NOTE: if you want to readjust the vertical scale while the integrals are displayed on the screen, click on [No Integrals] to turn them off in order to be able to change the vertical scale of the spectrum using the middle mouse button. Plotting the spectrum with the **pl** command is a "what you see is what you get" situation, for the spectrum and integrals; other frills must be requested specifically. If you want to plot the integrals, click on [Part Integrals] if they are not displayed. Enter

text('<title>'), where <title> is a short phrase, to give your spectrum a title. Now that you have titled, integrated and phased your spectrum, you might want to save your data again so that the optimal processing parameters are saved with the FID (**svf('<filename>')** and answer "y" to overwrite). To plot, enter the following commands:

pl -- to plot the spectrum and the integrals
pscale -- to plot the scale
ppf -- if you want to plot the peak frequencies
pirn -- if you want to plot the numerical values of the integrals on you spectrum
pap -- to plot the title and ***all*** parameters
- OR -
ppa -- to plot the title and the most important parameters
- OR -
pltext -- to plot the title only

page -- to start the plotting job

Note that all of the plot commands start with the letter "p". Similar commands which display things on the screen start with the letter "d" for display. For example, **ds, dscale, dpf**, and **dpirn** will all change the display on the screen in the same way that **pl, pscale, ppf** and **pirn**, respectively, plot on paper. You can put all of the plot commands on a single line of text at the command prompt, separated by spaces like this:

pl pscale ppf pirn pltext page

HINT: *you don't have to wait until the plotting is complete before sending the next spectrum to the plotter. The plotter will automatically queue the data and change the paper before starting the next spectrum.*

Removing Your sample and Inserting the Standard. Eject your sample using the Interactive Acquisition Window as you did before. Insert the $CHCl_3/CDCl_3$ standard and adjust the lock parameters. Activate the lock and make sure the lock phase is correct and the shims are roughly correct. You should be able to get a lock level of 80 to 90.

Leaving the Instrument. Enter the command **exit** in the VNMR text entry window. **NOTE: Failure to properly exit the VNMR software before logging out will cause problems for you when using the satellite stations and during your next session on the Unity-300. Always exit VNMR before logging out!!!!** See the discussion of "lock" files above for a detailed explanation.

Press and hold the right mouse button on the wallpaper with the Varian logo. This will bring a popup menu. Select **Exit** (the last choice) and confirm by clicking on **Exit** again to exit the window system and log out of your UNIX session.

Have a nice day. Come again soon.