

The Varian Unity-300 NMR Spectrometer

The Varian Unity-300, purchased in 1992, is a “modern” spectrometer with many advanced features:

1. Separation of Host and Acquisition computers.
2. Arrayed Experiments
3. Computer-controlled sample temperature
4. 4-Nucleus Probe
5. Inverse capability and Broad-Band Inverse probe

There are also some advanced features which new spectrometers routinely include which are not available on the Unity-300:

1. Pulsed-Field Gradients
2. Shaped (Selective) Pulses
3. Oversampling and Digital Filtering
4. A third channel or triple-resonance capability

These newer features will not be discussed here, but the advanced features which are available will be described in detail.

Separation of Host and Acquisition Computers

Older spectrometers such as the Varian Gemini-200 and the Bruker AM-250 have a single computer which is manufactured by the NMR manufacturer (Varian or Bruker) and which not only controls the sequence of pulses, delays, and data acquisition of the spectrometer but also processes the data (weighting function, Fourier transform, phasing) and displays, analyzes and plots it. These non-standard computers have their own operating systems and have great difficulty communicating with other computers. Varian pioneered the idea of attaching an “industry standard” UNIX workstation to the NMR spectrometer, using the Varian computer only for acquisition of data (control of pulses, timing of delays, sum to memory). The UNIX workstation (the “host” computer) is connected to the acquisition computer by an SCSI (“scuzzi”) communications link. Through this communication, the host computer controls all operations of the spectrometer hardware: initiation of pulse sequences, lock, shimming, temperature control, etc. (Fig. 1). The acquisition computer sends the acquired FID data back to the host computer via this same SCSI link. In this way the user deals with the spectrometer through a standardized and familiar computer which is identical to other graphics workstations used for any general purpose data processing and graphics display. The computer manufacturer (in this case, Sun Microsystems) is far better equipped than the NMR spectrometer manufacturer (Varian) to solve the problems of data processing, communication and graphics display. The host computer with its standard (UNIX) operating system runs the NMR software package provided by the spectrometer

manufacturer: in this case VNMR is the software written by Varian. All data processing from the FID to the final 1D or 2D spectrum display or plot is carried out by the host computer. In fact, there is no need to do any data processing at the spectrometer since we have two satellite Sun workstations running the same VNMR software. As soon as FID data is saved on the Unity-300 instrument, it is immediately available on a local network to the satellite computers ("bloch" and "ernst") located in the same room. Processing data on the satellite computers is free, so that in addition to making spectrometer time available for your colleagues, you save money!

To operate the Unity-300 you sit in front of the Sun Ultra-10 computer and use the keyboard and mouse (Fig. 2). The computer has an internal disk called **/export** that is used for NMR data storage. The "differential box" is a device operated by the Sun computer which controls all hardware in the spectrometer and sends FID data back to the Sun computer. On top of this box sits the Remote Status Unit, which directly indicates the lock level, whether the sample is locked and/or spinning, and the status of the RF pulses (transmitter or observe and decoupler) and the receiver (showing when data is being acquired and whether the digitizer is overflowing).

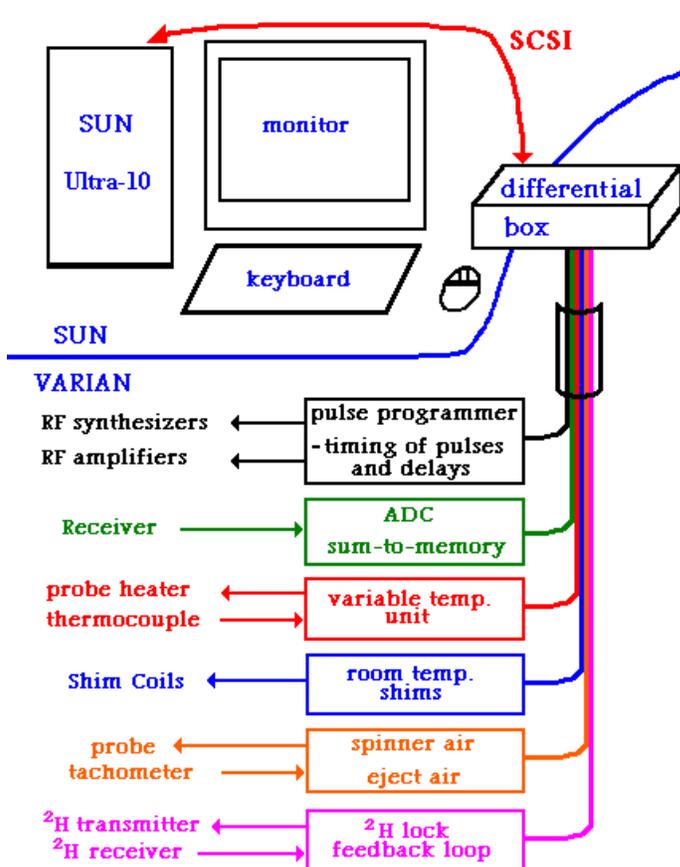


Figure 1. Block diagram of the Varian Unity-300 NMR spectrometer. The "differential box" is treated by the Sun (UNIX) computer as a peripheral device just like a printer, disk drive, or tape drive, and is connected to the Sun by a SCSI cable. The differential box, in turn, controls all of the hardware devices of the spectrometer, including the acquisition computer which consists of the pulse programmer, ADC and sum-to-memory. The blue line divides the Sun (host computer) portion of the spectrometer from the Varian (acquisition computer and hardware devices) portion.

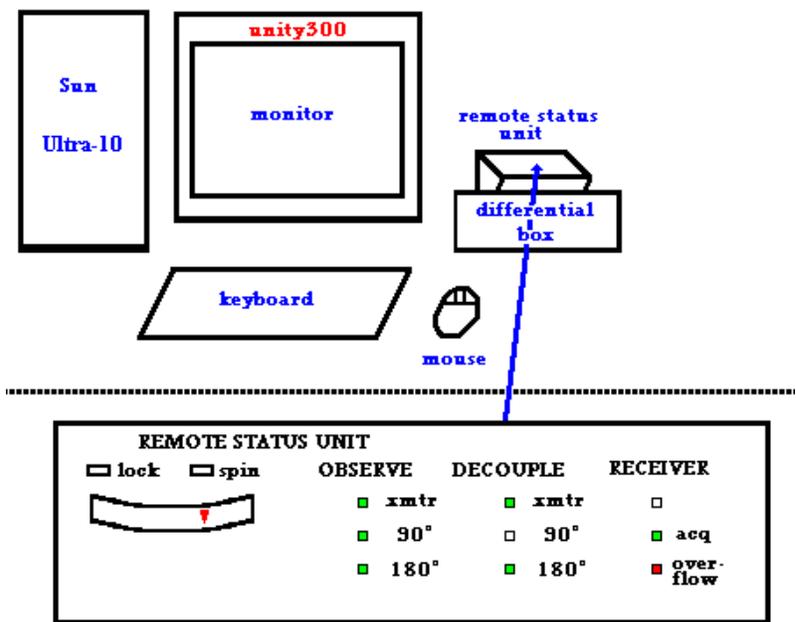


Figure 2. Diagram of the "cockpit" of the Unity-300 spectrometer. The CPU and internal disk ("/export") are located to the left of the monitor, and the differential box is located to the right. On top of the differential box is the remote status unit, which tells you exactly what is happening to the sample. Lights flash when an RF pulse is delivered either from the transmitter ("observe") or the decoupler ("decouple") and the 90° and 180° lights combine to indicate the phase of the pulse.

The VNMR software runs in a windows-like environment with different windows for lock, shimming, text entry, text output, and spectrum (graphics) display (Fig. 3). At any time you can also bring up a UNIX window (called a "shell") which displays the UNIX prompt and allows direct entry of UNIX commands for managing data files (move, rename, delete, etc.). Although there are many advantages to this division of labor between host and acquisition computer (Bruker quickly followed suit and now uses the same system with SGI workstations), there are a few disadvantages. First, the host-acquisition SCSI link sometimes crashes and needs to be re-established. When this happens, you will be unable to open the lock and shim windows that allow control of the spectrometer. There are UNIX commands that can be used to try to re-establish the link, but sometimes it is necessary to re-boot the entire UNIX system. Re-booting is not a simple task, and must be performed by facility personnel. A UNIX system is not like a PC: it cannot be simply turned off and turned on again. **NEVER TURN OFF THE POWER TO A UNIX WORKSTATION!!!** The second disadvantage of the host-acquisition system is that another layer of complexity has been placed between you, the user, and the spectrometer. For example, it is not possible to see the FID data on the screen as it is acquired since the data must first be transferred via the SCSI link. This is a minor inconvenience, similar to the complaint of many drivers that it is difficult to "feel the road" with power steering and automatic transmission in an automobile.

VNMR SCREEN LAYOUT

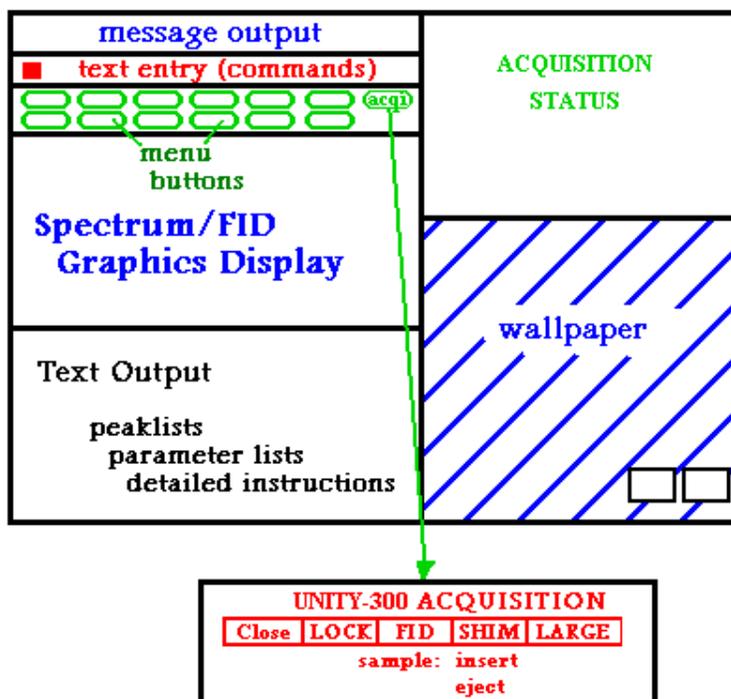


Figure 3. Diagram of the VNMR screen showing various windows. Commands can be entered in the text entry window as text, or menu buttons can be used to select options. The FID or spectrum data is displayed in the graphics window, and lists of text output appear below this in the text output window. The upper right window shows the status of the current acquisition, including scan number, temperature, spin rate, time remaining in the experiment, etc. Clicking the **acq** button in the acquisition window gives access to the interactive lock and shim displays. The background or "wallpaper" behind the windows is used to log out.

Each authorized user of the Unity-300 has a UNIX account on all three of the Sun workstations (bloch, ernst and unity300) with the same login ID and password. Logging in automatically starts the VNMR software on any of the workstations. Logging in on the Unity-300 instrument (unity300) also starts a billing clock which ends when you log out. The NMR billing is charged to your research director's account. Your login directory ("home directory") will be located on the internal hard disk "/export" under a directory for your research group. For example, if your login ID is "jones" and your research director is "hruby", you would have **/export/home/hruby/jones** as your home directory. Anything in your home directory is protected from being altered or erased by anyone else, but it can be copied by anyone. Presently there are no disk quotas, so you are on your "honor" not to clutter the disk too much. You can access your data over the internet using **ftp** (file transfer protocol) from any PC with a CD burner to archive and then delete your data. All data are accessible from any of the three workstations, without any need to transfer data from one workstation to another. NMR data files created by the VNMR software consist of a directory (e.g., myfile.fid) and four files within that directory: **text** (any text you want to write describing the sample and NMR experiment), **log** (a record of the exact time the experiment was started and completed), **procp** (a list of all NMR parameters with their values used in the experiment), and **fid** (the binary file containing the actual FID data). This file structure is the same for arrayed and 2D files, except that the **fid** file is much larger. VNMR does not store processed spectra, only the FID.

Arrayed Experiments and Other Neat Software Tricks

This is not really an advance in NMR hardware, but rather a software trick that is extremely powerful and convenient. In fact, arrayed experiments are also available on the Gemini-200 instrument. Most parameters can have multiple values in an array, so that the experiment is repeated for each value and the spectra can be displayed together to see the effect of changing the parameter. For example, the pulse width can be set to a list of six different values by entering **pw=10,11,12,13,14,15**. The **go** command will then repeat the current experiment six times with the value of the pulse width set to 10 microseconds, 11, 12, etc. in each successive experiment. The six FIDs are stored together as a single binary file and all six are processed together with the command **wft**. The command **dssh** (display stacked spectra horizontally) will display all six spectra side-by-side. All of the spectra are processed in an identical fashion, with the same phase and display parameters.

Arrayed experiments can be used for many purposes. In an inversion-recovery experiment, which is used to measure T_1 , the delay **d2** in the pulse sequence:

180° pulse - **d2** - 90° pulse - Acquisition

is arrayed to include a variety of times both greater than and less than the expected T_1 value. Then the resulting array of 1D spectra is analyzed to determine T_1 . In this case, the array might be entered as **d2=0,0.1,0.2,0.4,0.8,1.6,3.2** to give seven 1D experiments with delay values ranging from zero to 3.2 seconds. The binary data file would contain seven FIDs in order of the d2 values in the array. Another example is an NOE difference experiment where you might want to try irradiating a number of different peaks in the 1D ^1H spectrum to see if there are any NOE enhancements of other peaks in the spectrum. After determining the absolute frequencies of each peak you wish to irradiate, these values are entered into an array of **dof** (decoupler offset) values. The NOE experiment will then be performed a number of times, each time irradiating a different peak in the spectrum. If a “control” frequency (representing a position in the spectrum where there are no peaks) is included as the last entry in the array, the last spectrum can be used for subtraction as the control spectrum.

To check the values of an arrayed parameter, just enter **da** (display array) and the arrayed parameter will be displayed with a list of its values. Note that the receiver gain (**gain**) parameter must be set to a definite value (e.g., **gain=32**) for arrayed experiments. If **gain** is set to ‘**n**’ (not used), you will get the error message “autogain cannot be used in arrayed experiments” and the experiment will not be run. This is because the receiver gain would be adjusted automatically for each value of the arrayed parameter, making it impossible to quantitatively compare the peak intensities in the array of spectra. Instead, you must set the arrayed parameter to the value that is likely to result in the most intense FID and run a single spectrum. Then set **gain='y'** to fix the gain to the value automatically determined for that single parameter value. With the gain “locked in”, you can now enter the array values for the parameter of interest. Another feature that is useful in comparing arrayed spectra is the “absolute intensity mode”. Enter **ai** before performing the Fourier transform (**wft**) on an array of FIDs to make sure all of the spectra are scaled the same way. Otherwise, more intense spectra may be scaled down so that all

of the peaks fit nicely on the screen. This makes comparison of peak intensities difficult since the spectra are not presented with the same vertical scale. A common symptom of this mistake is the appearance of different noise levels in a stacked plot of arrayed spectra; if the spectra are viewed in absolute intensity mode the noise levels should all be the same.

Interleaved Acquisition. A series of spectra using an arrayed parameter can be acquired in an “interleaved” mode. Rather than complete all of the scans (or “transients”) of an experiment using one value of the arrayed parameter before starting the next experiment, you can complete a smaller number of transients on each FID and then start over with the first FID, adding in the new transients. For example, consider an NOE difference experiment using **il=‘y’** (interleave on) and **bs=8** (block size 8) with **nt=24** (number of transients 24). With the arrayed parameter **dof** set for four different frequencies, the NOE experiment would be carried out with 8 scans at the first **dof** value to give the first FID, 8 scans at the second frequency to give the second FID, and so on to the fourth and final FID. Then the first FID is retrieved from the disk and transients 9-16 are completed and added in to this FID using the first **dof** value in the array. The second FID is retrieved and transients 9-16 are completed using the second **dof** value, and so on. There are two advantages to this mode of acquisition: first, the data can be analyzed and compared as soon as one round (**bs** transients for all of the FIDs) has been completed - subsequent rounds simply improve the signal-to-noise ratio; second, for difference experiments such as NOE difference it is important that the FIDs to be compared be acquired as close to simultaneously as possible so that small changes in temperature, magnetic field strength, RF power, etc., do not affect the individual spectra to be compared. The interleaved mode with a small block size (**bs**) assures that the spectra are acquired at essentially the same time.

Stacked Plots. An array of spectra can be viewed as a “stacked” plot, with all of the spectra displayed together. The simplest way to do this is with the command **dssh** (display stacked spectra horizontally). For example, if your array contains four spectra the width of each spectrum is divided by four and they are all displayed side-by-side on the screen with the first spectrum (corresponding to the first value in the array) on the left. To go back to a single spectrum display, enter **ds** (display spectrum) and **fd** (full display, to reverse the effect of dividing the horizontal scale by four) to display the first spectrum in the array. To view the n^{th} spectrum in the array, enter **ds(n)** instead of **ds**. To plot the horizontal stacked plot of arrayed spectra, use the plot command **pl(‘all’)**. If you forget to specify “all” you will get only the first spectrum of the array, or the last one to be displayed individually. The second way to display an array is a vertical stacked spectrum. Set the desired vertical scale on the first spectrum, either using the mouse or with the command **vsadj** (vertical scale adjust). Then divide this value by the number (n) of spectra in the array (**vs=vs/n**) and set the horizontal offset to zero (**ho=0**). Then the command **dss** (display stacked spectra) will stack the array spectra up from bottom (first spectrum) to top (last spectrum) of the screen. You can also offset the spectra horizontally as they stack up by setting **ho** to a non-zero value. As before, use **pl(‘all’)** to

plot all of the spectra. Both horizontal stacked spectra (**dssh**) and vertical stacked spectra (**dss**) commands can be used either with the full spectrum or with an expanded region.

Macros and dps. Two other convenient features of the VNMR software are the use of macros and the display pulse sequence (**dps**) command. In VNMR you can enter the name of an experiment (e.g. "**dept**") and a series of operations (a "macro") are performed to set up the experiment and adjust the parameters to optimal values for that experiment. Text will appear describing the experiment and listing parameters that you will have to optimize. Sometimes this option is a little bit too powerful because it makes decisions for you and makes assumptions about what you have done before which may not be correct. But when it works it makes life very simple. The **dps** command displays a diagram of the current pulse sequence and even shows the values of various pulse lengths and delays. For complicated pulse programs this makes it easy to visualize what is happening and to check for errors in parameter values. It is also a great educational tool.

Computer-controlled sample temperature

There are many instances in which it is necessary to control the sample temperature during an NMR experiment. In some cases, an important chemical phenomenon such as reaction rate or exchange rate can be studied at a number of different temperatures. In other applications it is important that small temperature variations be avoided over a long 2D acquisition. The Unity-300 Variable Temperature (VT) Unit can control sample temperature precisely for any set temperature from -150°C to +200°C with a temperature variation of less than 0.1°C. Temperature control is achieved by passing a stream of gas (air or dry nitrogen) through the probe and heating it with a heating element inside the probe. The gas passes a thermocouple which monitors its temperature and then surrounds the sample tube and passes out the top of the magnet bore. The temperature measured at the thermocouple, which is very close to the sample tube, is displayed in digital form on the panel of the VT controller which is located at the upper left corner of the Unity-300 console. When the thermocouple temperature falls below the set temperature (defined by the variable **temp** in degrees Celsius), the heater coil is activated and the VT gas is heated until it reaches the set temperature. When the set temperature is reached, current to the heater coil reduced until a stable temperature at the thermocouple is achieved.

During routine operation at ambient (room) temperature, the variable **temp** is set to 'n' (not used), and the VT gas (air) is passed through the probe at room temperature without heating. This results in a sample temperature of about 21°C, which can vary with daily changes in building temperature. To operate at a temperature above ambient temperature, for example 30°C, set the variable **temp** to 30 (**temp=30**) and enter **su** (setup) to start controlling the temperature. Until either **su** is entered or an acquisition is started (**go**), the VT unit will not update its settings and will remain in its previous status. The temperature display should begin increasing from ambient temperature towards the set temperature. Stabilization at the set temperature requires about 5 minutes. To actively regulate temperature, the VT unit must have a gas supply which is about 10°C or

more colder than the desired set temperature. This means that attempting to regulate the sample temperature at 20°C or 25°C using ambient air as the VT gas will result in no regulation or poor regulation of temperature. It is the continual process of the probe heater fighting the colder supply gas which leads to precise temperature regulation. When you are finished with an experiment at elevated temperature, always set temp='n' and enter su to turn off the temperature regulation, and make sure that the sample returns to ambient (~21°C) temperature before logging off and leaving the instrument.

CAUTION: The VT unit must not be operated at a temperature higher than the boiling point of the sample solvent! Further, any VT operation above 100°C must use nitrogen gas rather than air as the VT gas to avoid destructive oxidation of the heater element and the thermocouple!

The convenience of temperature control by computer is offset somewhat by the ease of making mistakes. Normally it is difficult to damage the spectrometer hardware while sitting at the computer keyboard, but the Varian VT operation makes it easy! There are many different **experiments** in VNMR, each with its own FID and spectral data and its own parameters. If, for example, you join a experiment 3 (**jexp3**) and start acquisition without calling up a set of standard parameters, the value of temp may be set to some very high temperature. Let's say that someone has left **temp=150** in experiment 3, and you look over the parameters and decide that you don't need to re-load the standard parameter set. Starting the acquisition with **go** will activate the VT unit with a set temperature of 150°C and the probe will immediately start heating rapidly towards that temperature. If you don't notice the change in the displayed temperature at the VT controller (upper left corner of console), the sample will heat rapidly until the solvent boils, ejecting the cap from the sample tube and spewing the contents of your sample tube out into the probe. Further heating will cook the solute residue from your sample onto the probe coil and electronics, leading to a \$4,000-\$10,000 repair bill and a week or two of down time. How can this potential accident be prevented?

1. Always load standard parameters before starting an experiment.
2. Check the value of **temp** (should be 'n') before entering **su** or starting acquisition.
3. Keep an eye on the sample temperature display, especially after starting acquisition and before leaving the instrument.

Achieving sample temperatures at or below ambient temperature requires some means of cooling the VT gas. If the value of the variable **temp** is less than a cutoff value determined by the parameter **vtc** (variable temperature cutoff, usually 25°C), the VT gas is routed through a *heat exchanger* before entering the probe. This plumbing change is accomplished automatically by computer control of the spectrometer hardware. The heat exchanger is simply an insulated bucket with a coil of aluminum tubing inside it. Filling the bucket with a cold liquid (liquid nitrogen) causes the VT gas to cool as it passes through the coil of aluminum tubing. The VT gas must be manually switched from air to dry nitrogen to avoid condensation of water vapor and oxygen from air in the exchanger

coil. With liquid nitrogen in the heat exchanger bucket, even “house” nitrogen gas is too “wet” and ice will eventually block the flow of gas through the exchanger tubing. We have arranged a convenient source of dry nitrogen gas supplied by a liquid nitrogen tank in the adjacent room, room 118 (Gemini-200 room). The tank provides a continuous supply of room temperature dry nitrogen gas at low pressure, as long as there is liquid nitrogen in the tank. A gas regulator mounted on the tank provides a constant gas pressure (20 p.s.i.) which is routed through the wall in copper tubing to a flow regulator on the wall next to the Unity-300 magnet. Tygon tubing carries the dry nitrogen gas from the flow regulator to the heat exchanger bucket.

The bucket will hold liquid nitrogen for only about 30 minutes and must be regularly re-filled for longer experiments. An alternative arrangement involves a large liquid nitrogen dewar with a coil of copper tubing inserted in the dewar as a heat exchanger; this system can operate up to 8 hours without re-filling of liquid nitrogen.

Low temperature operation is a critical operation and requires supervision by facility staff until you are confident that you can perform all of the steps correctly.

- a. Fill the portable liquid nitrogen dewar with enough liquid nitrogen for your entire VT experiment.
- b. At the console enter **temp=20 su** to route the VT gas flow through the heat exchanger.
- c. In room 118, check that there is liquid nitrogen in the evaporator tank. Open the main regulator valve and set the reducer valve to 20 p.s.i. pressure.
- d. At the heat exchanger bucket, turn the black valve handle by 180 degrees to switch the VT gas supply from air to dry nitrogen.
- e. Adjust the flow meter mounted on the wall to a gas flow of 10 lpm (liters per minute). Leave the system alone for 10 minutes to allow dry nitrogen gas to flush the air out of the entire VT system.
- f. Fill the heat exchanger bucket about 3 inches deep with liquid nitrogen and connect it to the heat exchanger. The aluminum tabs on the sides of the bucket fit into slots in the heat exchanger, and the bucket is secured by turning 90 degrees. Add liquid nitrogen from the fill hole at the top of the heat exchanger using a plastic funnel, and replace the large cork in the fill hole. A full bucket of liquid nitrogen will last about 1.5-2 hours, so that for long runs it is best to refill it every hour.
- g. Lower the sample temperature in steps of 20°C, starting with **temp=10 su**. With each new value of **temp**, wait until the temperature is regulated at the new value (“VT:regulated” will be displayed in the acquisition status window of VNMR) and then wait an additional 5 minutes before entering the next value of **temp** followed by **su**. This wait is tedious but necessary in order to avoid a

temperature gradient in the sample which would result in a large difference between the actual temperature of the sample and the temperature display. When the desired final temperature is reached, wait another 15-20 min. for the temperature to equilibrate in the probe.

h. When finished, raise the value of **temp** in steps of 20°C. Wait an additional 5 minutes after each time the new temperature is established (VT:regulated) before further increasing the temperature setting. When the temperature is stable and regulated at 0°C or more, lower the bucket to expose the heat exchanger coils to room temperature air and set **temp=20 su**. Initially ice (frost) will form on the heat exchanger coils; when this has completely melted, the VT supply gas can be switched from dry nitrogen back to air by turning the black valve handle at the heat exchanger bucket 180 degrees. Close the flow meter valve on the wall and the reducer and master valves at the nitrogen evaporator tank. At this point you can enter **temp='n' su** and leave the instrument.

i. If you want accurate sample temperature, you should calibrate the VT temperature using either a temperature calibration sample (methanol, in which the difference in chemical shift between the CH₃ and OH peaks depends predictably on temperature) or a thermocouple (an nmr tube containing a temperature sensor - the combination can be lowered into the probe using the temperature sensor wire). Sometimes there are very large differences between the temperature displayed on the VT unit front panel and the actual sample temperature.

Another common error which is more frustrating than dangerous occurs when you change to another experiment to calibrate pulses, check a 1D spectrum, etc. If the variable **temp** is set to **'n'** in the new experiment, starting the acquisition (**go**) will immediately shut off the VT heater and send the temperature rapidly falling (or rising) toward ambient temperature. If you move to another experiment (or try to shim on the FID), always set the variable temp to the desired temperature before starting any acquisition.

The Four-Nucleus Probe

The standard probe on the Unity-300 is called the “four-nucleus” probe because it can detect ¹H, ¹³C, ¹⁹F, or ³¹P without any hardware changes or probe tuning. The probe has only two RF coils: one is double-tuned to ¹H and ¹⁹F frequencies, and the other is double-tuned to ¹³C and ³¹P frequencies. This tuning is very precise and delicate, and can only be adjusted at the Varian factory in Palo Alto, CA. Any of these four nuclei can be observed quickly and easily simply by calling up standard parameter sets in VNMR, except for ³¹P which requires changing an RF filter at the probe. This probe is a “normal” probe, meaning that the ¹³C/³¹P coil is closer to the sample tube than the ¹H/¹⁹F coil (the coils are arranged in concentric circles when viewed from above). This is a “normal” configuration because it places the coil of the less sensitive nuclei (¹³C and ³¹P) on the inside, closest to the sample. For experiments which detect ¹³C (or ³¹P), this is the best

arrangement: 1D ^{13}C , DEPT, APT, and HETCOR. For experiments which detect protons (^1H), however, sensitivity will be much greater with an inverse probe (see below). In any experiment where proton sensitivity is limiting due to low sample concentration or inherent insensitivity of the experiment (HMQC, HSQC, HMBC, NOESY) the inverse probe is preferred.

Inverse Capability and the Broad-Band Inverse Probe

A “normal mode” heteronuclear NMR experiment is any experiment which observes an X-nucleus (any nucleus other than proton) and involves pulses on either the X-nucleus alone or on both proton and X-nucleus. An example would be a simple 1D ^1H -decoupled ^{13}C spectrum, in which the sample ^{13}C nuclei are excited by a ^{13}C pulse and the ^{13}C magnetization is recorded as an FID while a series of ^1H pulses is used to decouple the ^1H - ^{13}C splittings. A more complex “normal mode” experiment is DEPT: pulses are delivered to ^1H and ^{13}C at various times during the pulse sequence, and the ^{13}C FID is recorded with simultaneous ^1H decoupling. An inverse experiment has excitation pulses on both the protons and the X nuclei, but the FID is recorded from the ^1H coil; that is, the ^1H spectrum is observed. This might seem like a trivial distinction, but until recently spectrometers were built so that any experiment which included X-nucleus pulses had to be observed on the X-nucleus. When so-called inverse experiments began to be used (primarily HMQC, HSQC, and HMBC) it was necessary to modify the hardware on older instruments to allow for this previously unanticipated application.

Such a modification is not necessary on the Unity-300 because it has a “dual-broadband” configuration (see Fig. 8, p. 41). Older instruments have a single broad-band amplifier which can produce pulses at any frequency, including ^{13}C and ^1H . This amplifier is called the “transmitter”. A second amplifier, fixed at the proton frequency, is used for proton decoupling (low power) and for normal-mode heteronuclear experiments (high power pulses). This amplifier is called the “decoupler”. On these older instruments, the observe frequency is fixed at the frequency of the transmitter. Using this terminology, an inverse experiment is one in which the transmitter is set at the ^1H frequency and the decoupler is used to generate X-nucleus pulses. Inverse experiments are thus impossible on unmodified older (“single-broadband”) instruments since the decoupler cannot operate at the X-nucleus frequency. The Unity-300 can easily perform inverse experiments by simply setting the transmitter frequency to ^1H and the (broad-band) decoupler frequency to the X-nucleus (e.g., ^{13}C) frequency. The importance of all of this will be evident when you see the advantages of proton-detected 2D ^1H - ^{13}C correlation experiments, which are far more sensitive than HETCOR.

The inverse broad-band probe has a ^1H transmit-receive RF coil on the inside, next to the sample tube, and an X-nucleus coil on the outside, farther away from the sample. The X-nucleus coil is tunable over a very wide range of frequencies, so it can be used for just about any nucleus except ^{19}F and ^1H . We normally leave it tuned to ^{13}C because the most common inverse experiments are ^1H - ^{13}C correlation 2D experiments. If you need to have the probe changed, get help from your ever-courteous and helpful NMR facility staff - don't attempt it on your own!!! A probe is a very delicate and precisely constructed \$15,000 device. The default probe for the Unity-300 is the four-

nucleus (“normal”) probe, so if you use the inverse probe you must arrange to change back to the default probe at the end of your reserved time.

All of the sensitivity advantages of the inverse probe will not be realized unless the probe is tuned with the sample in the probe. Tuning is the process of adjusting a capacitor in the tuned circuit, which includes the probe coil, so that the circuit resonates at precisely the frequency of the pulse. This not only maximizes the efficiency of transfer of RF pulse power to the sample (thus minimizing the 90 degree pulse width), but it also maximizes the sensitivity of detecting the FID during acquisition. For heteronuclear experiments it is necessary to tune both the ^1H coil and the X-nucleus coil in separate steps. Consult the NMR facility staff for help with probe tuning - you can damage the probe very easily if you don't know what you're doing!

Because of its “inverse” construction, the inverse probe is exquisitely sensitive for proton-detected experiments, but relatively insensitive for X-nucleus detected experiments. You would not want to use the inverse probe to carry out a DEPT experiment, for example, because DEPT is a ^{13}C -observe experiment and the ^{13}C sensitivity would be very low. Pulse widths are different on an inverse probe: the ^1H 90 degree pulse width will be shorter since the power of the RF pulse is more efficiently delivered to the sample from the (inner) ^1H coil, requiring less time to tip the magnetization by 90 degrees. For the same reasons the ^{13}C pulse width will be longer, because the more distant X-nucleus coil is less efficient at delivering pulse power to the sample. In general, you will have to use pulse widths determined on the same probe as starting points for pulse calibration or for “quickie” experiments without pulse calibration. Shims also depend on the probe, so changing probes will involve a certain amount of re-shimming to obtain optimal field homogeneity and peak shape.