

Vnmrj Training Guide

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This manual describes the most basic operation of our Varian NMR spectrometers with Vnmrj 2.2D and above and Chempack 4.1. More advanced techniques, like two-dimensional spectroscopy, are described in separate documents in the NMR Facility's web site. Full documentation for Vnmrj is available from its help menu and on printed copies of the manuals located the lab.

Please note that **reading all the information in the Training page of our web site is a requirement before you start using our Facility**. You are also required to read and understand our instrument's capabilities and limitations. This information can be found in the Instruments page of our web site. There is also a training video available in one of our workstations (*Krypton*).

To reserve instrument time, we use an online scheduler which is accessed from our web site at the bottom of the News page. On that page, we regularly publish important news and information about the NMR Facility. Please check the news every time you want to make a new reservation for current news and information.

Currently, the lab is equipped with five Varian NMR spectrometers: two 400MR (named *Gallium* and *Cobalt*), one Inova 500 (*Tin*), one VNMR5 500 (*Tellurium*) and one VNMR5 700 (*Ytterbium*). These have a variety of capabilities and each is best suited for some particular experiments. For example, *Cobalt* has better sensitivity for carbon-13 than *Tin*, but the latter has better sensitivity for protons. For a complete list of capabilities and limitations, consult our web site or ask the staff.

The lab also has five workstations where users can process and plot their spectra. One of them (*copper*) can also operate with the Windows operating system and has available a copy of MestReLab's MestReNova for NMR processing and some other chemistry-related software. Spectra stored in the spectrometers can be accessed from the workstations via a local network. Use of the workstations is free of charge.

If you encounter a problem when using Vnmrj or one of the spectrometers, read "*Common Problems and Solutions*" in the Documentation section of our web site or ask the staff for help.

Vnmrj overview

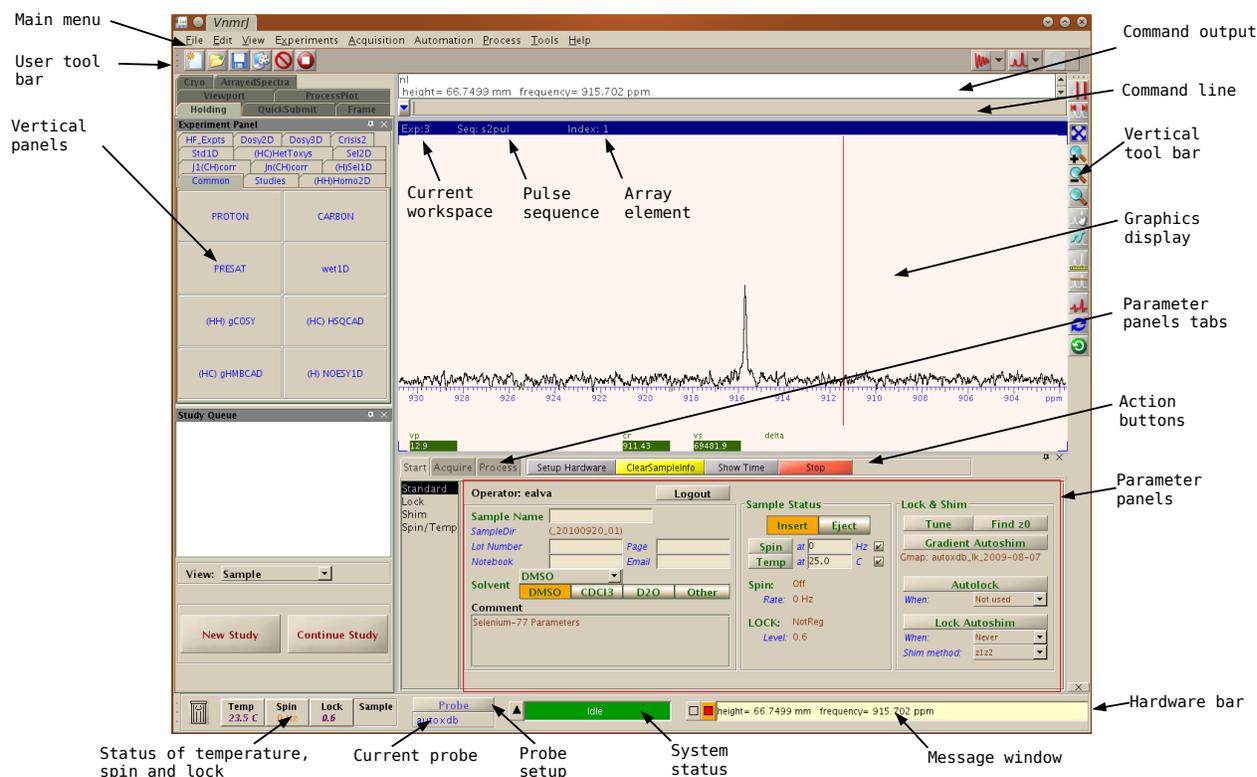
Vnmrj runs on computers under the Linux operating system. Users in our facility receive an account on each computer in the lab which can be accessed with their UM unique name (user name) and password. Each user has a home directory or folder located in `/home/username`. Inside that folder, Vnmrj stores its user-related files in a folder named `vnmrsys` and data files (spectra) in `vnmrsys/data` (`/home/username/vnmrsys/data`).

Vnmrj can operate in three modes: experimental, walkup and imaging. Because we use only the experimental interface by default in our Facility, this manual will cover only that interface. The walkup interface is a simplified mode of operation with many limitations and is meant mostly for automation.

A data set in Vnmrj consists of the NMR data itself (FID) and its associated parameters for acquisition and processing; and it is stored not as a single file but as a folder with an .fid extension containing several files. Data sets are loaded from the **File** menu. In addition, Vnmrj uses the concept of “workspaces” (analogous to opening several files in a word processing program) to perform several tasks simultaneously. Workspaces (also called *experiments*) are separate regions of computer memory independent of each other where spectra and parameters are kept. For example, you can be performing a proton acquisition in workspace 1, while setting up acquisition parameters for a carbon-13 in workspace 2, or plotting a two-dimensional spectrum in workspace 3. The number of available workspaces is essentially unlimited, but they must be created once before they can be used. Only workspace 1 already exists in any user account by default. Once created, data in any workspace is persistent between user logins. To create an additional workspace, select from the main menu: **File > Create Workspace**. To move between workspaces, type a command `jexp` (“join *experiment*”) immediately followed by a number; for example `jexp3` will take you to workspace 3, if it already exists. Or you can also select one from the menu **Tools > Workspace Information....** The workspace you are working on is displayed in the blue bar on top of the graphics display. The dynamic use of workspaces increases the efficiency when using Vnmrj and is highly recommended. For example, after acquiring a proton spectrum in experiment 1, you can copy the FID to experiment 3 with the command `mf(1,3)` (move fid from 1 to 3) and setup a 2D experiment based on the parameters you selected for the 1D spectrum.

Please refer to the figure below for a brief description of the layout of the graphical interface.

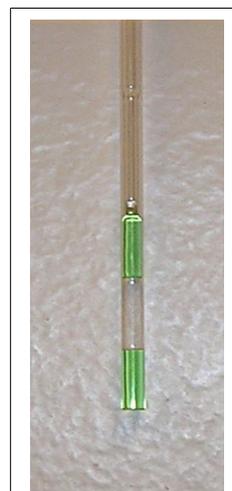
Vnmrj experimental interface:



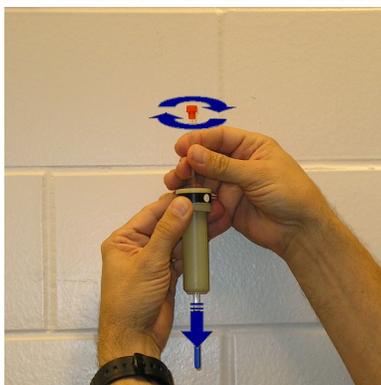
Sample preparation

Please use high quality, 5 mm NMR tubes like the Wilmad 528-PP-7, available from Aldrich. Cheap tubes may not spin properly and they may be more difficult to shim. The tubes should be at least 7 inches long. Do not use disposable tubes or broken/cracked tubes. The solvent volume should be about 0.6 mL (about 4 cm in the tube). Using less volume is possible but you will have to center the sample volume with the coil location shown in the depth gauge, and shimming may be difficult. For very small sample quantities, using a Shigemi tube matched to your solvent's magnetic susceptibility is recommended. For maximum resolution, the solution should be free from insoluble materials.

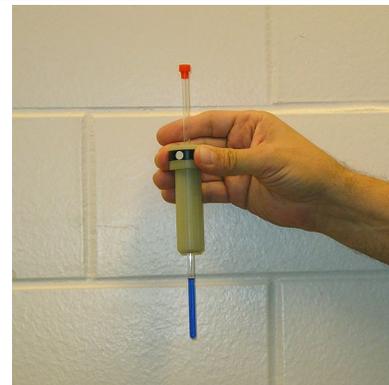
To position the tube in the magnet and spin it, a turbine (the "spinner") and depth gauge are used. Insert the sample tube in the spinner by pushing it gently along the hole while applying a little back and forth rotation until the tube goes all the way to the bottom of the depth gauge as shown below. Be careful: most tube breakages occur when inserting the tube in the spinner. Do not force it if it feels like a very tight fit. Do not insert the tube with the spinner already inside the depth gauge: insert it outside and then adjust the depth (if the tube breaks inside the gauge, the gauge will need to be cleaned). Do not insert the tube in the spinner over the keyboard or desk (if the tube breaks, you will contaminate and damage the keyboard and working surface). When inserting or retrieving the sample from the magnet, hold the spinner from the top rim (the black stripe with white dots). Avoid touching the lower part (the shaft).



For better sensitivity when using limited amounts of sample, a Shigemi tube is recommended.

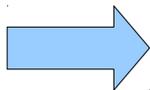


Insert the tube in the spinner by pushing it gently along the hole while rotating it back and forth.

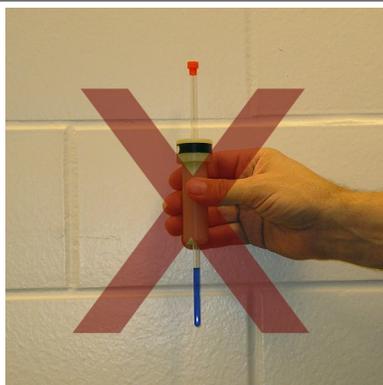


Hold the spinner from the top rim.

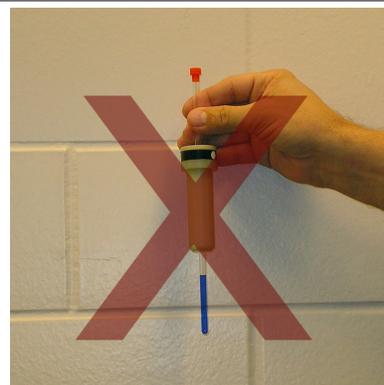
To insert the sample in the magnet, type `e` on the command line or click the **Eject** button. Position the spinner and sample on the stream of nitrogen on top of the magnet and type `i` or click the **Insert** button.



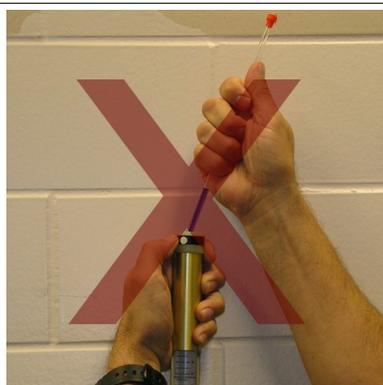
Do not lean against the magnet when loading your sample! And never, ever, insert an empty spinner or a tube without spinner in the magnet.



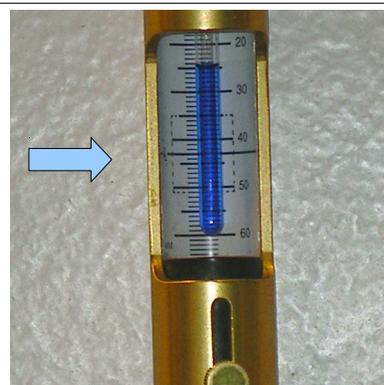
Do not hold or touch the spinner on the shaft



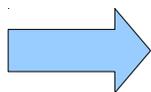
Do not hold the spinner from the NMR tube.



Do not insert the tube by forcing it in the spinner's hole, or when the spinner is mounted in the depth gauge.



If less than the recommended volume is used, center the solution's column with the center black line on the gauge's meter.



If you break your tube and spill the solution, you are responsible to thoroughly clean everything up and make sure no hazard remains to other users or the instruments. You should also let us know immediately to make sure everything is satisfactory. Users who don't show consideration for fellow users or our instruments will be banished from the NMR Lab.

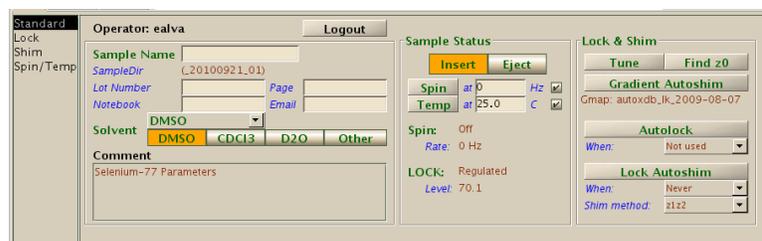
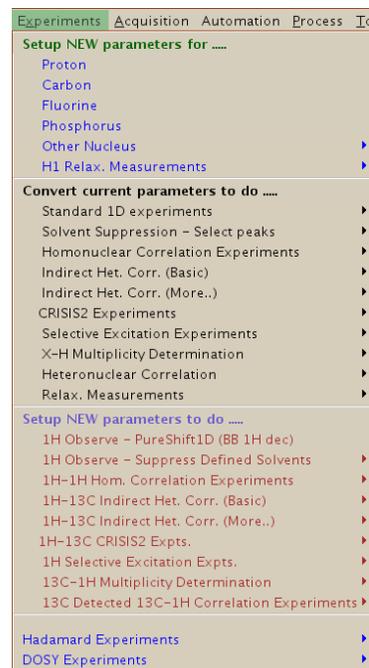
Preparing for acquisition

If you absolutely don't need more than simple, routine spectra; don't want to learn to optimize your experiments; or don't even care about the details of NMR spectroscopy but need some NMR spectra, there is an automation mode that can do everything for you except for loading the sample in the magnet. If that is what you want, go to the section *Automated Acquisitions*; otherwise keep reading.

Before you can run any NMR experiment you will need to specify a pulse sequence to be executed and several acquisition parameters like pulse durations, frequencies and powers; delays; the number of data points; and many others. Additionally, many of these parameters require proper calibration. All these parameters have been calibrated and given default values by the staff, and are stored on files in the computer's hard disk. To run an NMR experiment, you must first load a set of parameters. The parameters are then kept in portions of memory called workspaces where acquisition and processing will take place. The value of the parameters can be changed on the command line by typing the parameter's name followed by the equal sign and the new value, for example `sw=5000`. If the value is alphanumeric, it must be enclosed in single quotation marks, for example `gain='n'`. To find the current value of a parameter, type its name followed by a question mark, for example `nt?`. You can also inspect and change most common parameters from the appropriate parameter panel.

To load a parameter set, select an experiment from the **Experiments** menu. Notice that the **Experiments** menu is divided in 3 parts. The sections named **Setup NEW parameters for...** and **Setup NEW parameters to do...** will replace all current parameters with default values for the experiment requested; while **Convert current parameters to do...** will use the existing parameters in the current workspace and add any additional parameters needed for the experiment. For example, before doing a 2D experiment, it is a good practice to first measure a 1D proton spectrum, check on the quality of the shimming, and optimize parameters (e.g. spectral width, transmitter offset, relaxation delay). To do this, first select **Setup NEW parameters for... > Proton**, modify the parameters, take a spectrum and save it; and then select the 2D experiment from **Convert current parameters to do...** But if you don't care to optimize anything and just want your 2D spectrum with default parameters, you can choose it directly from **Setup NEW parameters to do...**

After the sample has been inserted but before the acquisition is started, the spectrometer must be prepared. This involves enabling the lock, tuning the probe and shimming the magnet. First, go to the **Start, Standard** parameter panel and choose a solvent. At this point you can enter a **Comment** to identify your sample, and choose a different spinning rate or temperature. We will deal with variable temperature operation later. The information in **Sample Name**, **Lot Number**, **Notebook**, **Page** and **Email** is used by vnmrj's database to locate spectra and is optional for normal operation.



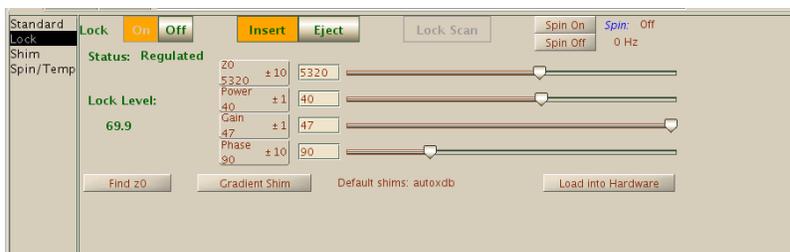
Locking the sample

In order to take a high resolution spectrum, the magnetic field has to be stable and homogeneous. If the magnetic field changes during the course of the acquisition, the signals will broaden and the spectrum will have poor resolution. To keep the field stable, the spectrometer has a mechanism (the lock system) that corrects the field's drift as it occurs. The drift is monitored using the absorption frequency of the solvent's deuterium resonance (the lock signal). As this frequency changes due to changes in the field's strength, an electric current is applied to a coil in the magnet that increases or decreases the field's strength to keep the field steady and the signal in the same position. To continuously monitor the deuterium signal, the spectrometer has a dedicated deuterium channel. When you "lock" your sample, the lock system starts monitoring the solvent's deuterium frequency (if it can find it).

But before you attempt to lock your sample, it is first necessary to set the appropriate lock parameters: Z0, lock power, lock gain and lock phase. All these parameters are found on the **Standard, Lock** parameter panel shown below and they vary from solvent to solvent and from spectrometer to spectrometer. To simplify the operation of our spectrometers, so the user doesn't have to remember the appropriate lock parameters, a macro called `setlock` was created that loads all lock parameters for the most common solvents (CDCl₃, C₆D₆, CD₂Cl₂, CD₃COCD₃, CD₃CN, THF-d₈, CD₃OD, DMSO-d₆ and D₂O; other solvents may need manual setup). After choosing your solvent in the **Start, Standard** panel, type `setlock`. This loads the lock parameters and also the current shims for the probe and solvent in use. But you still need to check that the lock has been established and, if needed, fine tune Z0.

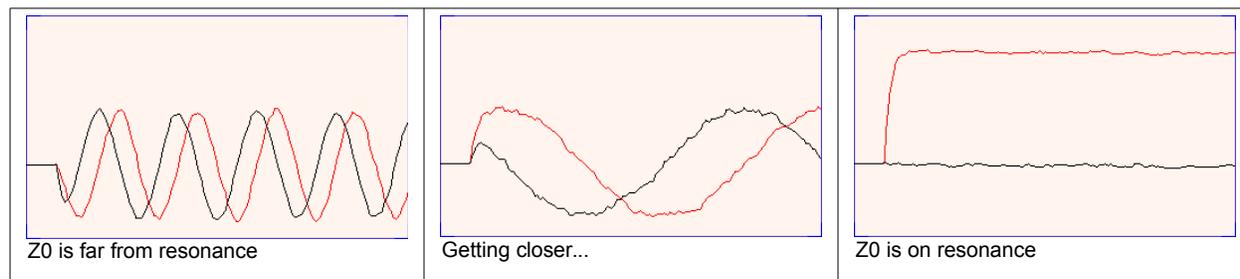
Before proceeding with the locking procedure, we will describe the operation of the buttons in the **Lock** and **Shim** panels. These buttons work in a different manner than all other buttons on vnmrj's interface. When you click one of them, the value of the parameter it controls increases or decreases by the amount indicated on the right side of the button; the value increases when the right mouse button is used and decreases when the left mouse button is used. To make fine or coarse adjustments, the increment/decrement amount can be changed between values of 1, 10 and 100 units by clicking with the middle button.

Z0
5320 ± 10



The lock field, **Z0**, controls the magnetic field strength and consequently, the frequency at which the signal from the deuterated solvent resonates. The value of Z0 is fairly constant for a particular solvent and magnet, but the value changes slowly over long periods of time (weeks or months) as the magnet's field drifts. It is adjusted so the lock frequency is in resonance with the solvent's absorption frequency. To do this, press the **Lock Scan** button on the **Start, Lock** panel and change **Z0** (left or right click the **Z0** button) until the lock signal appears on screen as a flat, zero-frequency sinusoidal signal. The figure below shows the lock scan when **Z0** is far from resonance (left), when it is close (middle), and when it is on resonance (right). Usually, it is not necessary to be exactly on resonance, but you should be close. You can also press the **Find z0** button to

have vnmrj find it for you, but be aware that **Find z0** may reset the lock power to a saturating value.



The **lock power** controls the RF radiation's power that is applied to the solvent's deuterium signal. Low powers give very weak signals and a poor, noisy lock while too high powers produce signal saturation leading to lock instability. The optimum power is determined mainly by the relaxation time of the solvent and probe design. For example, in the Inova 400 the optimum power for CDCl_3 is about 24 and that for acetone- d_6 is about 6; using a power of 24 for acetone will certainly lead to severe saturation and instability, and shimming will be impossible.

The **lock gain** provides amplification to the lock signal. Its value is not really important as long as a lock intensity of at least 60% is maintained during acquisitions.

The **lock phase** is mainly determined by the electronics and to a lesser extent by the solvent. Usually it is not critical to optimize it (the default parameters already have a good value) as long as an intense lock is produced. However, if you are unable to lock, make large changes (of ~30 units) and see if it helps. Optimize it if needed.

Clicking the **On** button in the **Start, Lock** panel engages the lock system. If the lock system didn't engage (the lock level is less than 10%), try increasing *temporarily* the power or the gain to kick it in, but once the lock is set, turn the power back to its previous value or you may saturate the lock signal.

Tuning the probe

In a typical NMR experiment, the nuclear spins in the sample are excited by the application of one or more short pulses of radio-frequency (RF) radiation, and eventually they precess freely across the axis of the external magnetic field, thus inducing an electric current in a coil located close by. The coil is located in the probe, whose main purposes are to hold the coil(s) close to the sample and to generate the strongest possible signal from the weak fluctuating currents induced by the nuclear spins as they precess. For the coils to be able to transmit the RF pulse to the sample and to detect the induced NMR signal efficiently, the coil circuitry should be optimized. The process is known as "tuning", and it is analogous to tuning your radio receiver to a particular radio station: if not properly tuned, your music will be noisy. Proper tuning is essential to obtain high quality NMR data. The exact frequency depends of course on the nucleus to be measured, but also on the solvent. The adjustments are made by turning the *tuning* and *matching* rods located on the bottom of the probe, which in turn adjust variable capacitors inside the probe body.

In our lab, the procedures for tuning are different depending on the probe used:

- The probes in the *Inova-400* and in *Cobalt* are permanently tuned to ^1H , ^{19}F , ^{13}C and ^{31}P and do not require additional tuning.
- The default probe in the *Inova-500* is always tuned to ^1H and ^{13}C and does not require further tuning by the user but can be tuned upon request for special

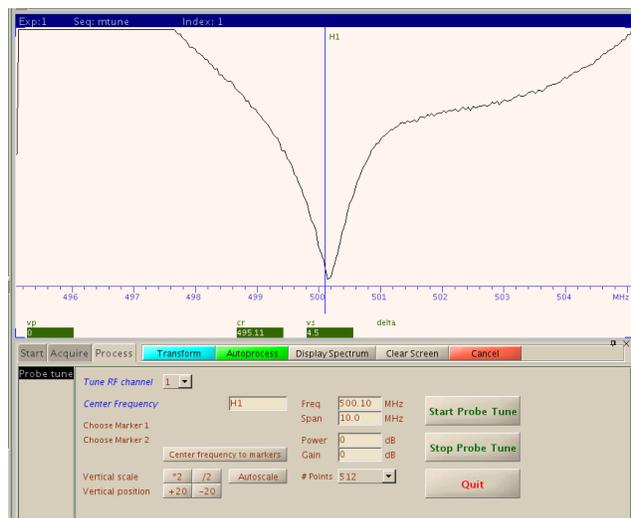
experiments. This instrument has another probe that can be tuned to any nucleus from ^{31}P to ^{15}N . If needed, the staff will install and tune it; users are not allowed to do this except when properly trained.

- The other MR400 instrument (*Gallium*) is equipped with an automatic tuning device (protune unit) that can tune the probe for the user. The software is set up to do automatic tuning before acquisition when a new sample is inserted in the magnet or when the nucleus or the solvent is changed. All you have to do is start the acquisition by clicking the **Acquire** button (typing *go* or *ga* will not do the tuning). Alternatively, you can request the tuning to be done at any time by clicking the **Tune** button on the **Start, Standard** panel and then click the appropriate button for your nucleus; or type in any desired nucleus (letters first, numbers last: eg. *Pb207*) followed by clicking **Tune to Criterion**.
- The VNMRs instruments (*Tellurium* and *Ytterbium*) are also equipped with protune units that can tune the probe for the user as explained above. Additionally, these instruments require a “quarter wavelength cable”, matched to the frequency of the nucleus, to be changed at the preamp interface before acquisition of heteronuclei other than ^{13}C . Ask the staff for details.



Unfortunately, the protune unit is notoriously unreliable. It usually does a good job, especially for ^1H , ^{19}F and ^{13}C , but for other nuclei it may fail. This is especially true for some nuclei like ^{11}B and ^{199}Hg in our 400-MR spectrometers *Cobalt* and *Gallium*. If automatic tuning fails

(characterized by the motors in the unit moving up and down for more than 1 minute), you will have to tune the probe manually as described in a separate writeup (*Probe tuning with the protune modules*). For this reason it is always a good idea to verify if tuning was successful, especially before starting a long acquisition and it will only take a few seconds. From the main menu choose **Tools > Probe Tuning > Manually Tune Probe**. Then select **Tune RF channel** (1 for ^1H or ^{19}F ; 2 for anything else) and enter a nucleus in the **Center Frequency**



field (letters first, numbers last: eg. *Pb207*). After clicking on **Start Probe Tune** the display should show a V-shaped dip centered with the blue line; if not, tuning was not successful and you will need to manually tune the probe or try automatic tuning again. Click **Stop Probe Tune** followed by **Quit** to return to normal operation.

Shimming

One of the most important factors to get a good quality NMR spectrum is shimming. NMR spectroscopy demands a homogeneous magnetic field over the whole of the sample volume located within the probe's detection coil. If the field is not homogeneous,

the resulting spectra will suffer from distorted line shape which in turn leads to poor resolution and sensitivity. For this reason, the field has to be optimized to every sample that is introduced in the magnet. The process of adjusting the field's homogeneity is called "shimming". Shimming is achieved with the help of coils (called shims) located at strategic places around the sample in the magnet. By applying different electrical currents to these coils, it is possible to compensate for local deficiencies in the field homogeneity. There can be from 12 to 32 or even more different shims depending on magnet design. These have names related to the axis of the field component they affect the most. For routine operation it is usually necessary to shim only the first two or three Z shims (Z1, Z2, Z3).

To shim the magnet you will need an indicator of field homogeneity. This can be provided by the lock signal, whose intensity is proportional to the field's homogeneity. To shim, you make changes to the shims and observe the lock intensity. Continue making changes until a maximum intensity is reached. The actual value is not important, only that you cannot improve it any more. Unfortunately, shimming is not as simple as adjusting each shim in sequence because they interact with each other. That is, changes in one shim may induce a change in another. Fortunately, the interactions are not very large and are well understood. For example, odd order shims (Z1, Z3, Z5) interact mainly with each other and only slightly with even order shims (Z2, Z4); and the other way around. There are several procedures for shimming that take into account these interactions. You should start by loading a set of standard shims from the computer (this is accomplished with the `setlock` macro). These were determined by the Facility staff using a standard sample in a high quality tube. Once loaded, the magnet is already pre-shimmed and requires only fine tuning of a subset of all its shims. Here, a very simple procedure for routine applications is described.

Go to the **Start, Shim** parameter panel. The lock strength, shown in the "clock" display, should be at least 10-20% before starting or shimming will probably be a useless exercise. If it is less than that, check the position of the tube on the spinner; make sure that you have at least 4 cm of solvent in the tube and that the lock has indeed "engaged". Then, turn the lock off, fine tune **Z0** to give a flat, near zero frequency signal, increase the gain to the maximum and the lock power by 10 units and turn the lock on again. The lock signal should go up indicating that it has engaged. Reduce the lock power to its previous value. Make a small change to **Z1** (**Z1C**, if present, is the the same as **Z1** but the adjustments are Coarse) and observe the lock signal. If the signal increases, continue making changes until it begins to decrease. If it decreases, change the shim in the opposite direction. After **Z1** is optimized, proceed in the same manner with **Z2** (or **Z2C**). Now, because **Z2** interacts with **Z1**, the latter must be readjusted. Repeat the whole procedure several times until no further improvement is possible. Usually, it is only necessary to adjust **Z1** and **Z2** but you can also adjust **Z3** and **Z4** to get better line shape. If you want to do it, change **Z3**, then readjust **Z2** and **Z1** as described and repeat until you reach the maximum. Then proceed with **Z4**, change it and readjust **Z1**, **Z2** and **Z3** as described.



Broad, symmetrical peaks indicate that Z1 and Z3 need optimization while asymmetrical peaks with long tails or shoulders on either side indicate faulty Z2 or Z4 settings. The

higher the order of the shim, the closer to the peak's base its effect will be. Large spinning side bands indicate poor non-spinning shims. These are shims with components in the X or Y axes and as the name implies, they must be adjusted with the spinning turned off. If you observe large spinning side bands, turn the spinning off and adjust X and Y interactively using the procedure for Z1-Z2.

Keep in mind that some solvents, particularly acetone and methanol, react more slowly to changes in the shims due to their typically long relaxation times. With these solvents, shimming has to be done with *low lock powers, slowly and patiently*.

You can also use automatic gradient shimming on all our instruments. This procedure shims Z1 to Z5 simultaneously and usually achieves satisfactory results. To use it, click the **Gradient Shim** button in the **Start, Standard** panel and wait until it finishes. If your tube requires more than the normal shimming, it may be necessary to run it twice. Give it a try, it may even give better results than your manual shimming! At the very least, it will provide a much better starting point to start manual shimming and doesn't even require the sample to be locked. Also, if you are unable to find the lock signal, try gradient shimming first.

It is also possible to shim *non deuterated* solvents. To do it, select the equivalent deuterated solvent from the list, turn the lock off and select from the main menu: **Acquisition > Go Gradient Shimming > Use 1H/gradient map**. Locking is not possible, of course, but this is not a problem with short term acquisitions, like routine 1D spectra, as the magnetic field in modern superconducting magnets is very stable. Gradient shimming of non deuterated solvents, and manual shimming using the FID is described in more detail in a separate writeup.

A special situation may arise when acquiring a fluorine spectrum in a *non deuterated* solvent. On spectrometers with automatic tuning capability, it is important to first take a proton spectrum to ensure the probe is tuned to proton, then do gradient shimming with the proton gradient map and then load parameters for fluorine and tune the probe to fluorine. If you don't follow this order but start by taking a fluorine spectrum, automatic shimming will fail because the probe will be tuned to fluorine before shimming with the proton gradient map is performed.

Variable temperature operation

For temperatures above room temperature, all you need to do is enter a value in the **Start, Spin/Temp** panel and click **Regulate Temp**.

For low temperature operation we have two choices. All spectrometers are equipped with an FTS cooling unit that can be used to cool the probe to temperatures up to about -20 °C. For lower temperatures, a bucket filled with liquid nitrogen is used.

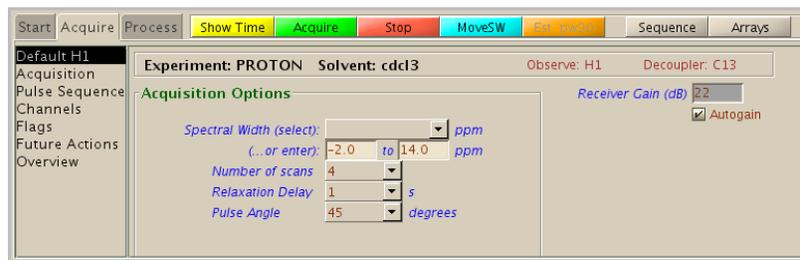
Ask the staff for further instructions before using low temperature for the first time.

In either case, be careful not to exceed the limits of the installed probe or the boiling or freezing points of your solvent. Make incremental changes to the temperature not larger than 30 °C until you reach the desired temperature. Keep in mind that the sample will require at least 5 to 10 minutes to fully equilibrate to the temperature in the probe, and you may need to re-shim or re-tune after large changes in temperature.

When you are finished, bring the temperature back to room temperature, again in steps of 30 °C, and finally click on **Temp Off** in the **Spin/Temp** panel to turn off the temperature controller. Allow enough time after your experiments for the temperature to recover completely before your time is up. The next user should find the probe at room temperature!

Modifying spectral parameters

Before starting the acquisition, you may want to adjust the most common spectral parameters. These are found in the **Acquire, Defaults** parameter panel.



Select a different **Spectral Width** from the list or type in the chemical shift range. The default values for some nuclei can be very far from optimum for your sample or may not even encompass the values you expect. Narrowing the range will increase the digital resolution.

Select a **Number of scans** (number of transients: parameter nt) or type any number you want in the command line. For example, low sensitivity nuclei or diluted samples may require a large unknown number of scans. In such cases, enter a large number like $nt=100000$ and stop the acquisition later when enough signal has been accumulated.

To get a more accurate integration you may need to increase the **Relaxation Delay** (parameter $d1$).

A **Pulse Angle** of 45 or 60 degrees is usually adequate for protons, but in order to get quaternary carbons in a carbon-13 spectrum it may help to lower the pulse angle to 30 degrees.

Other common parameters may be changed from the **Acquisition** panel or typed directly. For example, the acquisition time (at) determines the digital resolution of the spectrum; if you need high resolution fluorine spectra to accurately measure coupling constants, you should reduce the default spectral width and increase the default acquisition time. Do not change parameters that you don't understand and never increase pulse or decoupling powers.

When doing experiments other than simple 1D spectra, it is important, and sometimes necessary, to review and perhaps change the parameters in the **Pulse Sequence** panel, where the parameters pertinent to the chosen pulse sequence are accessible. Consult the experiment's documentation.

A note about spinning. Although the default parameters for common nuclei set a spinning rate of 20 (**Start, Standard** or **Spin/Temp** panels), this is not really necessary. The resolution in a well shimmed, modern superconducting magnet is usually such that you may not even notice a difference in the line widths with and without spinning, but you may be able to see spinning side bands when the sample is spinning and the non-axial shims are far from optimum. In fact, most 2D spectra are recorded with spinning off. Sometimes, the sample will not spin because of dirt accumulated inside the magnet. Please let us know if this happens so we can clean the magnet but don't worry about your spectrum and just turn spinning off.

Before starting the acquisition, you can check the time required by the experiment by clicking **Show Time**.

Click **Acquire** or type go , or ga to start the acquisition. After it has started, it can be stopped at any time by clicking **Stop** or typing aa (abort acquisition).

Summary

1. Insert the sample
2. Select an experiment
3. Enter solvent and sample information
4. Lock the magnet
5. Tune the probe
6. Shim the magnet
7. Adjust temperature and other spectral parameters
8. Start the acquisition

Acquisition

As previously mentioned, after an acquisition has been started, you can go to another workspace and do a different task, like reading a previous spectrum from the hard disk and process it or plot it. Or you can set up another experiment and start it. Once the undergoing experiment finishes, the new experiment will start. You can set up and start as many experiments as you want. At the end, you will have your spectra in separate workspaces; don't forget to save them!

Another way to create a queue of experiments on the same sample is to click **New Study** in the **Holding** vertical panel. You can then select several experiments from the buttons on the top of the vertical panel; customize them and run them with a single click on the **Submit** button. The sample will be automatically locked and shimmed, and the spectra will be transformed, plotted and saved. Read the section *Automated Acquisitions* further ahead for more information.

After the acquisition is started, the FIDs (free induction decays) resulting from each pulse sequence, are digitized and temporarily stored in the spectrometer's console (the electronics box near the magnet). The result of each transient or scan is added to the previous one to increase the signal to noise. It is only until a certain number of transients have been added (determined by the bs parameter, the block size) that the result is sent to the host computer. From this point on, the data can be transformed and examined: you don't have to wait until the experiment finishes (at least for 1D data; 2D data require readjusting linear prediction parameters before processing). Thus you can also monitor an acquisition in progress and stop it when you have collected enough scans, or stop it if you think your experiment is not going to work. The status of the acquisition (the number of scans collected so far and the time remaining to completion) is displayed in the status field in the lower part of vnmrj's window. To stop an acquisition, select from the menu **Acquisition > Abort Acquisition**; or type `aa`; or click **Stop**. Quitting vnmrj while an acquisition is in progress does not stop the acquisition.

Spectra can be saved from the **File > Save as...** menu; by typing `svf`; or by clicking the  icon on the user toolbar. Use only alphanumeric characters (a-z A-Z 0-9), dashes (-), or underline characters (_) in your file names: vnmrj does not like any other characters (including spaces).

To eject the sample once you are finished, click the **Eject** button (**Start, Standard** panel) or type `e`. Retrieve the sample from the top of the magnet and click **Insert** or type `i`. We do not insert a "standard sample" in the magnets when not in use. Do not insert the empty spinner in the magnet! And remember to log out before you leave.

Arrayed acquisitions

Some experiments, like a kinetics or a diffusion experiment, actually consist of collecting not one, but a series of spectra. The set is acquired as a single experiment and stored as a single file. To perform the acquisition, one of the parameter's is set up as an array of values. Vnmrj then acquires the first spectrum with the parameter set to the first value in the array, then the second spectrum is collected with the parameter set to the second value and so on. For example, to quickly determine the 90 degree pulse duration, you can set up an array of p_w values (the pulse width, in microseconds). You can do it by typing the parameter followed by the values separated by an equal sign and commas, with no spaces: $p_w=2, 4, 6, 8, 10, 12, 14$; or you can setup the array from the menu **Acquisition > Parameter Arrays...** The instructions for the experiment you want to run will normally tell you what parameters to array and how.

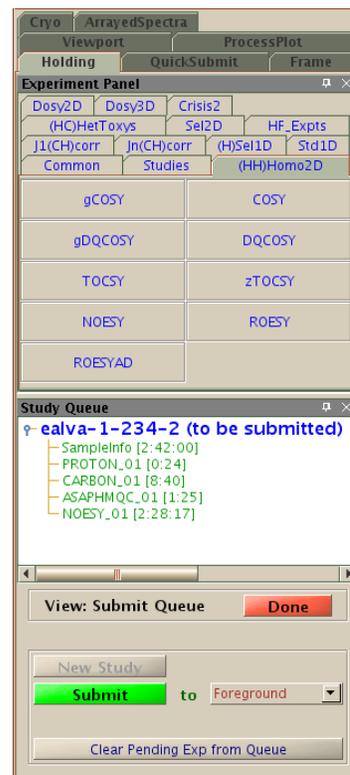
Automated Acquisitions

If you want a completely automated acquisition, proceed as follows:

1. Insert the sample in the magnet as usual.
2. Click **New Study** in the lower part of the **Holding** vertical panel (shown below), or select from the menu **Automation > New Automation**.
3. Enter a **Sample Name** in the **Start, Standard** panel, and select the solvent and other options. You can select **Autoplot** to have each spectrum plotted; autolock (**Lock? Yes**) to capture the lock automatically, **Shim** to shim the sample and **Tune** to tune the probe if you haven't done it yourself.



4. In the **Experiment Panel**, click the buttons for the desired experiments (some, like noesy1D require further customization).
5. Click **Submit**.



The experiments selected in step 4 will have default acquisition parameters that may or may not be suitable for you. Typically, you may need to change at least the number of transients or the spectral width. The experiments may be customized by double clicking the experiment name (in green) in the **Study Queue** window (not the experiment button). The parameter panels for that experiment will open up to allow you to make modifications as usual. Click **Save** to save any modified parameter and return to the

queue entry panel. Also on the **Study Queue** window, notice the acquisition times for the individual experiments displayed inside brackets to the right of each experiment. The total time for the queue is displayed at the bottom of the **Start, Standard** panel.

After each acquisition, the spectrum will be processed and plotted automatically (if requested) and the group of experiments (the queue) will be saved in a folder with a name consisting of the sample name, followed by the date and a sequence number separated by underlines; for example: ealva-1-234-2_20101026_01. Each FID will be saved inside that folder with a name consisting of the sample name followed by the experiment name followed by a sequence number; for example: ealva-1-234-2_HMQC_01.fid. If you need expansions of your spectra, you will have to open each spectrum and plot them manually yourself.

Processing

When you save a spectrum, the file is stored in the spectrometer's computer, usually in your vnmrj folder in `/home/username/vnmrj/sys/data`. Your files will be accessible from any workstation in the lab.

To load a spectrum stored on disk into the current workspace, select **Open...** from the **File** menu, or click the **Open...** icon  in the user tool bar just below the main menu (hover the mouse cursor over the icons to display their function). When you use this utility on any workstation in the lab, you will see links to all spectrometers. Clicking a link allows you to browse the files and load an FID stored in that instrument. If **Process data on drag-and-drop** is enabled (**Edit > System settings** menu), then the data will be Fourier transformed and processed automatically for you and it will be *almost* ready to plot: you may still need to check the referencing and phase as described below. If automatic processing is not enabled, you can do automatic processing from the menu **Process > Full Process**.

Processing an NMR spectrum generally involves applying a weighing function to the FID; applying linear prediction if needed; doing a Fourier transformation; phase correction; adjusting the chemical shift reference; selecting integral regions; baseline correction if needed; and possibly getting a list of peak positions. Most of these tasks are done during automatic processing. However, it is important to understand how to do them manually because the automatic algorithms may not be completely accurate for all spectra.

The parameters for processing can be adjusted from the **Process** panels. Many of these parameters can be found in more than one panel and also in some of the vertical panels on the left hand side of vnmrj. The repetition is meant for ease of use but sometimes can be confusing. *If you are not sure about a parameter or its value, don't change it.*

Weighting function

Select a weighting function and its parameter(s) on the **Weighting** panel to be applied before Fourier transformation. The parameter for zero-filling is also found here.

Linear prediction

Linear prediction is also applied before Fourier transformation. Its parameters are located in the **More 1D** or **More 2D** panels. There is more information on linear prediction in a separate writeup.

Fourier transformation

Fourier transformation of 1D FIDs can be performed by typing `wft`, or by clicking **Transform All** in the **Default** or **Weighting** panels. You can also click **Transform** in the action bar. For 2D data, the appropriate button is called **Full 2D Transform**.

Interactive Display manipulation

Once transformed, the spectrum will be shown in the graphics window where it can be manipulated with the mouse. To this effect, there is a tool bar with drop down menus in the user tool bar where you can access all mouse functions, and there is also a vertical tool bar, shown below, on the right hand side of vnmrj with equivalent buttons. You can display the function of the buttons by hovering the mouse cursor over them.

If you don't see a vertical red line on the spectrum, click the top button  to get it. This



line is the vnmrj cursor and you are now in what we may call “normal display mode”. Clicking with the mouse left button (left clicking) and dragging on the spectrum will move the cursor horizontally; middle clicking and dragging changes the intensity of the spectrum at the cursor position; and right clicking and dragging displays and moves a second cursor that can be used for zooming into a spectral region. You can also get a second cursor by clicking on the “Display one or two cursors” button  on the toolbar. The middle button has another, special function: if you click on a region very close to the left edge of the display, dragging up and down with the middle button will move the vertical position of the spectrum instead of affecting its intensity.

To zoom into a region, first enclose it with the two cursors and then click . Or you can click  and drag the mouse across the display to zoom in. If you choose this last button, you will enter into “zoom mode” (the mouse cursor changes into a magnifying lens) and you will have to click any other button, like the top button, to get out to normal display mode. Once you zoom into a region, clicking the pan and stretch button  will take you to another operating mode where the mouse buttons have reassigned functions, but unfortunately in this case the mouse cursor doesn't change shape to alert you. In this mode, dragging the left mouse button pans the spectrum left or right; the middle button changes the intensity of the spectrum; and the right button expands the spectrum but in a bizarre way that is difficult to control: after you click the spectrum with the right button,

any movement in any direction above the point where you clicked stretches the spectrum, and to compress it you have to move the mouse to a position below the point you initially clicked, possibly stretching it even more as you move the mouse down. Click the top button  in the toolbar to exit this mode.

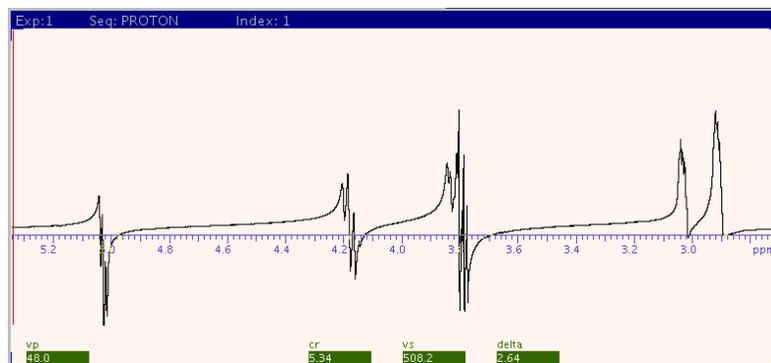
Phase correction

The Free Induction Decay (FID) data set is a function of a complex number, the intensity, versus time. During Fourier transformation, this function is converted to a function of intensity (but still a complex number) versus frequency. The spectrum displayed on the screen should correspond to the real or absorptive portion of the function, but it may contain “imaginary”

or dispersive contributions as shown in the spectrum below. If you chose automatic processing or automatic phase correction

(**Autophase** button in **Process, Default panel**), the spectrum will probably be

phased correctly. But on occasions, you will need to fine adjust it manually.



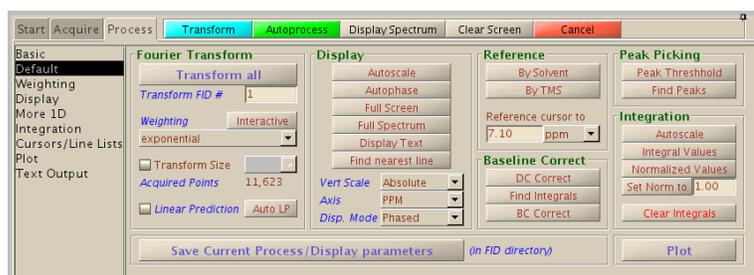
To phase a spectrum, first click the Phase button  to enter into phase correction mode. Left click on any portion of the spectrum close to its right edge and drag the mouse up or down until the rightmost peaks in the spectrum have only positive components; don't worry about the peaks on the left side of the spectrum yet. The right mouse button can also be used for fine adjustments. Then, left click close to the left edge of the spectrum and drag the mouse up or down until the peaks on the left side of the spectrum are completely in phase (positive intensity). Repeat the procedure if necessary until the full spectrum is properly phased. Click the top button on the toolbar to get out of phase correction mode.

Chemical shift referencing

Generally, the chemical shifts in the spectrum should be acceptable. By default, the chemical shifts are referenced using the solvent peak's *deuterium* signal. However, if you want to be more accurate, or if you have an unusual or non deuterated solvent, you may need to reference the chemical shifts manually using an internal standard like TMS.

The reference can be set on the **Process, Default** or **Display** panels. Expand a small region around your internal standard, place the cursor in its center and enter the standard's chemical shift in the **Reference cursor to** field.

After acquisition, the chemical shift of *any*



other nucleus will also be automatically referenced using the solvent's *deuterium* peak. But IUPAC recommends using the *proton* peak of the internal standards TMS for organic solvents and DSS for H₂O or D₂O. To reference *any* other nucleus in this manner, proceed as follows:

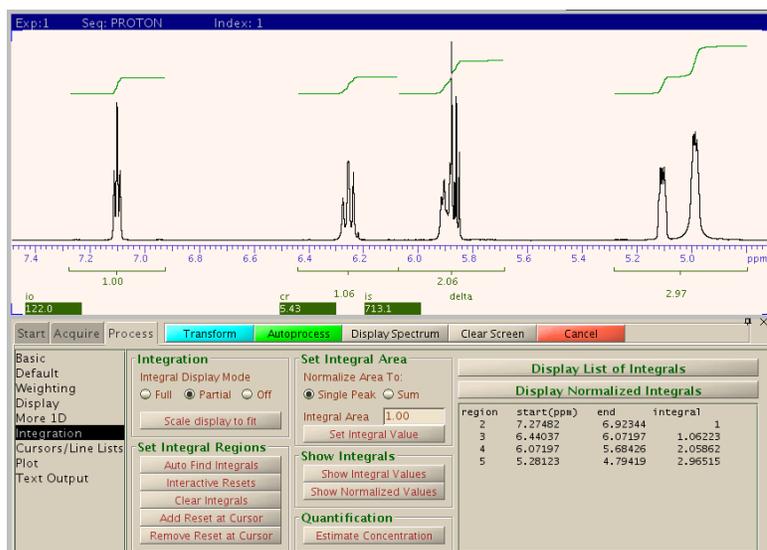
1. take a proton spectrum in workspace 1 and set the chemical shift of the internal standard (0 ppm for TMS or DSS)
2. take the spectrum of the nucleus of interest in another workspace, for example 2
3. type `mref(1,2)` in the command line to “move the reference” from workspace 1 to workspace 2.

Your spectrum is now referenced according to IUPAC without the need for cumbersome external references! (*Pure Appl. Chem.* 2001;73:1795-1818).

Integrating the spectrum

As a part of autoprocessing, integral regions are selected and the integrals' level and tilt are corrected. You may only need to normalize the areas by assigning a value to one of the integrals. To do

it, first click the  button in the vertical toolbar to display the integrals; place the cursor over one of the integrals, and in the **Process, Integration** panel select **Normalize Area To: Single Peak**; enter a value in the **Integral Area** field and click the **Set Integral Value** button. The integrals' values can be displayed with the button **Display List of Integrals** or with **Show Integral Values**.



If you want to select integral regions manually, first reset the regions previously defined, if any, with the **Clear Integrals** button. Then, select **Partial** under **Integral Display Mode**, click on **Interactive Resets** and define the regions by clicking on the spectrum over the left and right limits of each region that you want to create. Always define the areas so they enclose some empty baseline on both sides. When you are done, click the top button on the vertical toolbar to get out of this “region selection” mode.

It is also common to find that the whole integral “baseline” is not straight and horizontal: it may be tilted and may show a curvature. If that is the case, to get accurate integrations you will need to correct it manually. Select **Full** under **Integral Display Mode** and click the **Integral Lvl/Tilt** button  in the toolbar. Next, click on the *left* side of the spectrum and drag the mouse up and down until the integral baseline on that side of the spectrum is horizontal, then click on the *right* side of the spectrum and drag the mouse until the integral baseline is straight and horizontal across the spectrum. You may need to repeat the procedure to obtain optimum results. As usual, click the top toolbar button to get out of this operation mode. Notice that it is common to see some curvature or tilt

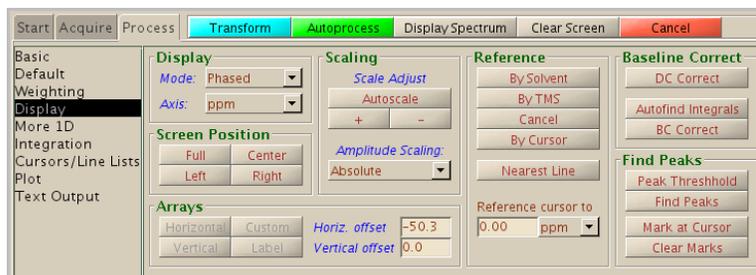
close to one or both edges of the spectrum: this is usually unavoidable.

Selecting **Partial**, **Full**, or **Off** under **Integral Display Mode** is equivalent to clicking the Show Integral Regions button , the Show Full Integral , or the Hide Integrals  button on the toolbar. Also notice that while the integrals are being displayed, the middle button will act on their intensity and not the spectrum's.

Baseline correction

There are two types of baseline correction available in vnmrj. The first one is a drift correction that only rectifies spectra with a spectral baseline that is straight but tilted or that has non zero intensity. The

second one can correct a spectrum with a curved baseline. Both can be applied from the **Process, Display** panel. Click **DC Correct** to apply a drift correction, or **BC Correct** to



apply a curvature correction. In the latter case, you need to have defined integral regions previously; click **Autofind Integrals** if you haven't defined regions before clicking **BC Correct**.

Getting a list of peak shifts

A list of peaks can be easily obtained with vnmrj. In the **Process, Cursors/Line Lists** click **Peak Threshold** or the Threshold button on the toolbar ; move the horizontal line to the lowest position in the spectrum where you want peaks to be listed, and click **Display Line List**.

Processing of arrays and 2D spectra

The processing of arrays of spectra is usually done in essentially the same way as with a simple 1D spectrum, but there may be additional processing required depending on the data to be extracted from the experiment; consult the documentation for instructions. Phase correction, integration and referencing are usually done on the first spectrum in the array. We have created macros to allow you to apply a baseline correction to all spectra in an array (UMbc) and to get a listing of all integrals in the array (UMdli). Read *Kinetics Experiments with Vnmrj* for more information.

Two more buttons will appear at the top of the tool bar to allow you to navigate between the spectra in the array. In addition, the **Arrayed Spectra vertical panel** contains buttons and parameters to display and plot stacks of spectra in many different ways. And you can also try our custom macro, UMdsarray, which will attempt to display the array with a nice slanted stack layout.

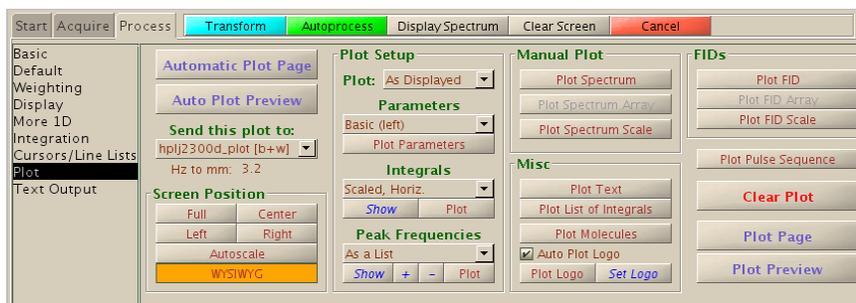
Processing of 2D spectra is described in a separate writeup.



Plotting

In vnmrj, a plot is created by choosing (adding together) its components, like the scale, the spectrum or the parameters and finally sending the result to the printer. Each

component is selected by pushing a button on the **Process, Plot** panel shown below.



First, choose the plotting options for the spectrum, parameters, integrals and peak frequencies. These are selected from the drop down menus in the **Plot Setup** section of the panel. For the Plot (spectrum) you usually want to select **As Displayed** to plot only the region currently displayed on the screen. For the parameters, **Basic (left)** prints only the most important parameters, and **Full (left)** prints almost all acquisition and processing parameters. To plot the integrals, they must first be displayed on screen, and **Scaled, Horiz.** is the most common option. You can choose any of the options and click on **Show** to preview your selection on the screen. For the peak frequencies, there are quite a few choices suitable to different spectra; the most common are **On Peaks, top, ppm** and **As a List**. Other options, like the width or height of the plot can be controlled from the command line or from the **Arrayed Spectra** vertical panel.

Once the layout is chosen, the easiest way to plot, as usual, is to select an automatic procedure. You can either click **Automatic Plot Page** to have a plot with the spectrum, scale and parameters sent directly to the printer, or click **Auto Plot Preview** to send it to an *Adobe Acrobat Reader* window as a pdf image for your review. Or you can create the plot manually by clicking the elements that you want, like **Plot Spectrum**, **Plot Spectrum Scale**, **Plot Parameters**, **Plot Integrals**, etc. Once you clicked the elements, click on **Plot Page** or **Plot Preview** to send your composition to the printer or to Acrobat Reader respectively. **Clear Plot** will remove your composition from memory to start all over; and our custom macro, *UMmailpage*, will send you the plot via email as an encapsulated postscript file and a high resolution image file (png format) ready to be inserted into word processing or presentation documents.

Although our laser printer prints in black and white, plotting in color is possible if you create an image or plot file as described below and print it on your own color printer. The options or colors are selected in the **Styles and Themes** window which is displayed when you select **Display options..** from the **Edit** menu. Two themes have been preconfigured in our lab: *UM_color* and *UM_gray*. The first plots the elements of the plot in different colors, and the second uses only black and different shades of gray. Choose a theme on the drop down menu of that window and click **Load**. The colors can also be changed, if needed as follows. For 1D plots, select the **DisplayColors** tab and **Plot Postscript** in the drop down menu. Then click on the color button or on the drop down menu to the right of each element and select or create a color. For 2D spectra, select the **2DColors** tab and **Plot Postscript** from the drop down menu. Then click on either **Absolute** or **Phase** for the kind of 2D spectrum to plot



(for example, a cosy or an hmbc are usually displayed in absolute mode and an hsqc or noesy in phase sensitive mode). Select a color for each of the contour levels on the plot as described above for the 1D elements. Click **Close** to accept the changes or **Abandon** to discard them and finish.

To plot with a different line width, create the plot manually as described above and then type `page1w(x)`, where x is the desired linewidth in points. The default width is 3: choose 1 for the finest and 5 to 9 for thicker lines.

Miscellaneous

There is more information about doing other common tasks, like transferring files via ftp or flash drives, or burning DVDs in the *Frequently Asked Questions* writeup available in our web page.

Recovering from problems

Problems arising from the hardware or software will inevitably occur. The most common problems and their solutions have been compiled in the document *Common Problems and Solutions* which is available in our web site and also in a white binder in the lab. Of these, possibly the most common is due to disrupted communications between the host computer and the spectrometer's console. This one can manifest itself as an unresponsive vnmrj or in many other ways, and is usually fixed by typing `abortallacqs` in the command line. Please consult *Common Problems and Solutions* for details.

Never power cycle the computers. You will risk a real probability of corrupting the file system beyond recovery.

Our instruments

Currently we have six Varian (now Agilent) spectrometers in our facility. Additional technical details can be found in our web page as well as current charge rates..

Gallium is a 400MR spectrometer equipped with an *AutoX-DB* direct-detection probe and automatic tuning. The temperature range is -80 to +130 °C. This probe is capable of detecting ^1H , ^{19}F and any nucleus with a frequency between those of ^{31}P and ^{15}N . It is more efficient for X-nuclei detection experiments (nuclei other than ^1H or ^{19}F).

Cobalt is a 400MR spectrometer equipped with a auto-switchable probe that is permanently tuned to ^1H , ^{19}F , ^{13}C and ^{31}P . The temperature range is -20 to +80 °C. This instrument is currently in walk-on mode of operation (no previous reservation is required) and is meant for quick routine analyses of synthesis products.

Tin is an Inova 500 spectrometer. It has two probes available: the *id* probe (the default) is an indirect detection probe without fluorine capability, temperature range -20 to 50 °C optimized for proton detection; the *sw* probe is an indirect detection probe, temperature range -100 to 150 °C, optimized for heteronuclear detection. The probes are switched and manually tuned by the staff upon request.

Zirconium is an Inova 400 spectrometer. It has only one probe that is permanently tuned to ^1H , ^{19}F , ^{13}C and ^{31}P . The temperature range is -20 to 80 °C. It is currently installed in room 2407 and is used for undergraduate teaching.

Tellurium is a VNMRS 500 spectrometer with automatic tuning, three RF channels and two probes. The *OneProbe* (the default) has a temperature range of -80 to 130 °C and excellent sensitivity for ^1H , ^{19}F , ^{13}C and other heteronuclei and is suitable for all kinds of experiments. The *HFX* probe is tuned to ^1H , ^{19}F and X, with a temperature range of -80

to 130 °C and is used for triple resonance experiments involving fluorine; for example proton acquisitions with fluorine decoupling and viceversa, or HF heteronuclear Overhauser experiments (HOESY). This instrument is typically used for long term experiments.

Ytterbium is a VNMRs 700 spectrometer with automatic tuning, three RF channels and two probes. The *AutoX-BB* probe (the default) can be tuned to ^1H and any nucleus from ^{31}P to ^{15}N . This probe has a temperature range of -80 to 130 °C and is used for the detection of proton and heteronuclei but cannot detect fluorine. The sensitivity for carbon-13 is the best in the lab. The *HCN* probe is a triple resonance probe tuned to ^1H , ^{13}C and ^{15}N , with a temperature range of -20 to 80 °C and is used for proton detection and triple resonance experiments. The sensitivity for proton with this probe is the best in our lab. This instrument is typically used for long term chemical and biomolecular experiments.