Location: 1232 Hach Hall
Contact: Shu Xu or Sarah Cady, 1234 Hach Hall

Safety

All researchers working in 1232 Hach Hall must complete the EH&S Course “Fire Safety and Extinguisher Training.” Please do not prepare samples directly in 1232 Hach Hall. Aprons, safety glasses, and rubber gloves are available in 1238A Hach Hall if you wish to conduct your sample preparation in CIF facilities. Researchers may wear lab coats and safety glasses in the 1232 Hach Hall, but please remove all gloves before handling NMR equipment or computers. Please do not bring any large ferromagnetic objects into the lab without permission. This includes certain chairs, bicycles, gas cylinders and tools.

Properly dispose of waste solvents and glass pipettes in the containers provided in 1238A. There is a broken glass container located in 1232 Hach in case of broken NMR tubes or other glassware. All of the computers in this lab have direct links from the desktop to MSDS sheets, the EH&S Laboratory Safety Manual and to the CIF Safety Manual.

Some safety concerns specific to high-field cryogenic superconducting magnets include:

- Users should remove credit cards, cell phones, mp3 players, keys and other ferromagnetic objects from pockets before approaching a magnet.
- Users with pacemakers or joint replacements should have a staff member assist them with the insertion of samples into the magnet as to prevent serious harm or injury.
- In case of magnet quench, the room will be filled with gaseous helium, which will be evident from a white cloud and an alarm sounding on the oxygen sensor. If you are in the room during a magnet quench, please exit as soon as possible – crawling on the floor if need be to reduce helium inhalation. If you are outside the room during a magnet quench, do not enter the room without proper breathing apparatus until the oxygen sensor alarm has stopped sounding.
Introduction

You must receive training before using this piece of equipment. The Varian MR-400 features a narrow bore 9.4 tesla/400 MHz magnet equipped with a OneNMR pulse-field-gradient probe. VNMRIJ 3.0 is used for data acquisition, and the MNova software is typically used for data processing. This instrument is used for routine $^1H/^13C$ characterization experiments, routine X nucleus detection, 1D selective experiments (APT, DEPT, NOESY1D) and 2D experiments.

Overview

The MR400 is controlled by a RedHat Linux PC communicating via two Ethernet ports – one to the instrument and one to the internet. The computer is part of a local area network in 1232 Hach Hall and is safeguarded behind a firewall. In order to minimize instrument time when users are waiting, the data acquired is immediately accessible on one of four data stations in 1232 Hach. The data is also accessible via the CIF Research Files Cloud Storage. When you log out, your data is instantly uploaded to the storage cloud. More information regarding Remote Data Access is available on the CIF website: http://dev.cif.iastate.edu/remote-data-access
Start up

After logging in, VNMRJ will start automatically.

- Slide the sample into the spinner and adjust the depth using the depth gauge.
- Wipe both the sample and the spinner with a Kimwipe.
- Approach the magnet and flip up the switch at the top to start the **EJECT** air. When you can hear the air, place the sample in the spinner into the bore at the top of the magnet. Flip the switch down to **INSERT** the sample.

![VNMRJ Interface](image)

Automatically Lock and Shim

- In the **[START]** tab.
  - Make sure you are in the **[Standard]** window (as shown above). Select solvent from drop-down menu, or click one of the common solvent buttons.
  - Auto-lock – Click **[FIND Z0]**. Process will take 1-2 minutes.
  - Auto-shim – Click **[GRADIENT AUTOSHIM]**. Process will take 2-3 minutes, **adapts shims Z1-Z5**.
  - After **[Gradient Shim]** has finished, click **[Shim]** below **[Standard]** and **[Lock]** in the **[START]** tab. Adjust X1, Y1, XZ and YZ manually to obtain the highest Lock Signal. These non-Z shims are **not** changed during the gradient shim routine and must be adjusted manually.

![VNMRJ Interface](image)

- Check **[LOCK LEVEL]** in bottom left corner of the screen after auto-lock & auto-shim.
  - **[LOCK LEVEL]** should be greater than 1%
  - If it is less than 1%, type `rts('standard.shim') <enter> load='y' <enter> su <enter>` and try **[GRADIENT AUTOSHIM]** again or proceed to the manual locking instructions.
Manually Lock

- In the [START] tab.
  - Select solvent from drop-down menu, or click one of the common solvent buttons.
  - Click [Lock] below the [Standard] heading to take you to the lock window.
  - Click [LOCK SCAN]
  - The three parameters you will need to adjust are \( Z0 \), [POWER], and [GAIN]

- Click [Lock OFF] and adjust [POWER] and [GAIN] to a high enough value until you can see a sine wave like the one below:

![Example of Unlocked Signal](image)

- Adjust \( Z0 \) by clicking the left or right mouse button (left for decrease, right for increase). You can also change the increment from ±100 to ±10 or ±1 by clicking \( Z0 \) using the middle mouse button. Increase or decrease \( Z0 \) until the sine waves get further apart, until eventually you have “one” continuous sine wave. If the sine waves are getting closer together, you are going the wrong direction. Once you get very close to one sine wave, VNMRJ will usually sense this and automatically switch to [Lock ON] and produce the following result:

![Example of Locked Signal](image)

- If the Lock Level is 100%, reduce [POWER] and [GAIN] slightly until the Lock Level is 60-70%. You do not want 100% Lock Level before proceeding with shimming.

- If the [LOCK LEVEL] is fluctuating wildly, you have saturated your signal and the [LOCK POWER] needs to be reduced.
**Manually Shim**

- In the [START] tab.
  - Click [Shim] below the [Standard] heading to take you to the lock window.
  - Start with a Lock Level that is less than 70%. Manually adjust Z1-Z4 until maximum Lock Signal is reached. Clicking on each shim with the left mouse button will DECREASE, right mouse button will INCREASE and the middle button will change the increment from ±100 to ±10 or ±1.
    - Alternatively, clicking [Gradient Shim] will adjust Z1-Z5 automatically, finding the best Z shims.
  - After adjusting Z1-Z5 or after [Gradient Shim] has finished, adjust X1, Y1, XZ and YZ manually to obtain the highest Lock Signal. These non-Z shims are **not changed during the gradient shim routine and must be adjusted manually.**

**Load an Experiment**

- The parameters for any experiment can be loaded by clicking one of the experiment buttons in any one of the tabs in the upper left corner of VNMRJ.
- Experiments can also be loaded from the Experiments drop down menu as shown to the left. There are additional experiments/nuclei that do not appear in the tabs that are available in the drop down menu.
- Load an experiment before proceeding to tune the probe.
- Once an experiment has been loaded, parameters such as “nt” (number of transients/scans), “bs” (block size), relaxation delay and pulse angle can be modified from the drop down menus in the Acquire tab.
Tuning

- The MR400 features two channels — a high frequency channel for $^1$H (400 MHz) and $^{19}$F (376 MHz), and a low frequency broadband channel for all other nuclei ($^{31}$P being the highest at 162 MHz and $^{15}$N being the lowest at 40 MHz).
- Typically the probe is tuned to $^1$H and some other nucleus on the broadband channel.
- The easiest way to tune the probe is to pre-load the experiment of interest (PROTON, CARBON, PHOSPHORUS, HMBC, etc.) so the computer knows which nuclei are required for the experiment. Then go to Automation -> Tune Probe in the drop down menu at the top of the screen.
- **DO NOT ATTEMPT TO TUNE THE PROBE WHILE AN ACQUISITION IS RUNNING!!** Type “halt” in the command line to stop any acquisition before proceeding.

Study Runs

- VNMRJ has a software feature called a “study run”. This feature allows the user to set up a queue of several experiments for a single sample.
- Thanks to the auto-tune capabilities of the probe, experiments with different nuclei ($^{13}$C, $^{31}$P, $^{15}$N, $^{11}$B, etc.) can be selected to run in sequence and the software will autotune the probe in between each experiment.

To Set Up a Study Run

- The Study Queue window is in the lower left-hand corner of the VNMRJ window. Select “New Study” in order to begin your study.
- Once “New Study” has been clicked, experiments can be added to the queue by clicking on their buttons on the respective tabs.
- Only experiments with buttons can be added to the queue. (Not experiments from drop-down menu.)
• There must be an entry in the Sample Name field, as VNMRJ will auto-save the Study Run in a folder with the name “test_sample_YYYYMMDD_##”
• The software can lock, shim and tune before the first experiment if you wish. (For example, you can insert your sample, start a study, select experiments, select lock & shim, and click submit and you will not have to do anything manually.)
• Double click on each separate experiment in the queue to change the number of scans, relaxation delay, or other associated parameters within the run.
• Click “Submit” to start the Study Queue. While the queue is running, you can type “halt” during a particular experiment, which will stop that experiment and continue to the next experiment in the queue.
• The total time for the study run is displayed at the bottom of the Start/Standard Tab and the “Sample Info” row of the Study Queue.

Tips and Tricks

Measuring a peak’s full-width half-max and signal to noise:

• Place the cursor on the peak of interest. Type `res` and the peak width at various points will appear on the screen.
• Signal to noise: Place the cursor on the peak of interest. Type `dsn(freq1,freq2)` where `freq1` and `freq2` are values in Hz that define the noise region. VNMRJ will give you a signal to noise value in the text box.

Creating a kinetic run or timed array:

• Acquire ¹H experiment as normal, making sure locking, shimming, temperature, etc. are all set as you would like them.
• Type `nt=1` since we want each time point to only have one scan to prevent any averaging of intensities over multiple scans.
• Type `pad=0, 3600, 3600`... or whatever time increments you desire, in seconds. The number of increments you type will be the number of experiments.
Troubleshooting

**Issue:** The acquisition window is grayed out and you cannot interact with any buttons. The status bar may have a message that says “Unable to open Viewport, may be in use by another process.”

**Solution:** Exit VNMRJ by typing `exit` in the command bar, or going to **File -> Exit**. Open your home folder (on the desktop) and go to the folder “vnmrsys” Scroll down and there should be a file called “lock1_primary” (sometimes there are multiple files that say lock#_primary). Delete the file(s) by dragging to the trash, and empty the trash. Reopen VNMRJ by double-clicking the icon on the desktop.

**Issue:** An acquisition is still running when VNMRJ opens after you log in.

**Solution:** Type `abortacq` in the command line of VNMRJ. If this does not work, follow the procedure for restarting the spectrometer as listed above.

**Issue:** The status bar says “Inactive” and an orange window is displayed. You cannot start the lock or shim or any acquisition. Various buttons may be grayed out, and VNMRJ does not return the message “Setup Complete” after typing the command **su**

**Solution:** The computer and the spectrometer have stopped communicating. Open the spectrometer cabinet and look at the board on the far right. If the lights have stopped moving up and down, this is indicative of the issue.

1. Exit VNMRJ by typing `exit` in the command bar, or going to **File -> Exit**.
2. Right-click on the desktop and click “Terminal” to open a new command prompt.
3. Type `su acqproc` and the computer should return the line “Stopping Acquisition Processes”. If the computer returns several errors, type `su acqproc` again, you want to see the line “Stopping Acquisition Processes”.
4. Open the spectrometer cabinet and hit the “RESET” button on the board on the far right hand side.
5. Type `su acqproc` again and the computer should return “Starting Acquisition Processes”.

⇒ CONTINUED ON NEXT PAGE
6. Open VNMRJ by double clicking on the icon. Reload the shims by going into the Shims window under the Start Tab. In the bottom right corner where it says “File” enter “standard.shim” hit Enter and click “Read Shims”:
   a. Alternatively, in the command line, you can type:
      
      rts('standard.shim') <enter> load='y' <enter> su <enter>

7. After reloading the shims, make sure all of the shims now have non-zero values. Experiments should run normally.
**Experiment Guide**

Please refer to the subsequent manual pages for a more in-depth explanation of how to set up each experiment. The extended guide has not been printed for each and every experiment, but if you would like an experiment to be added to the guide, just ask for the complete PDF or it can be printed off.

- Frequently used experiments appear in the [COMMON] tab.

- **Proton 1D** and **Carbon 1D, PRESAT** and **WET** used for $^1$H solvent suppression, **gCOSY** 2D used for 2-4 bond J-correlation, **HSQCAD** is 2D for 1-bond J-correlates protons with the directly attached carbons, **gHMBCAC** is 2D for multiple-bond J-correlation, **NOESY1D** is 1D for selecting $^1$H and finding through-space $^1$H correlations in a quick 1D experiment.
• The [Std1D] tab has many of the same experiments as the [COMMON] tab, plus a few different nuclei and some additional carbon experiments.

![Experiment Selector]

- Proton 1D, Carbon 1D, Fluorine 1D, and Phosphorus 1D are standard 1D experiments, PRESAT and WET used for 1H solvent suppression, T1 MEASURE and T2 MEASURE used to measure T1 and T2 relaxation times, APT 1D carbon experiment resulting in 13C spectrum where the methyl and methine peaks have opposite phase with respect to methylene and quaternary carbons, DEPT another 13C experiment where you can select 13C based on 1H multiplicity, PureShift 1D can simplify complicated 1H spectra with many overlapping multiplets by invoking 1H decoupling, HomoDec allows you to irradiate a 1H at a specific frequency which will result in a collapse of coupled multiplets, CARBONecho is not needed on this spectrometer since 1D Carbon is sufficient, BilevelDec is used to acquire a 13C-decoupled 1H spectrum (for 13C labeled samples).
The **[(HH)Homo2D]** tab contains H-H correlation experiments, both through-bond (COSY, TOCSY) and through-space (ROESY, NOESY).

- **gCOSY & COSY & zCOSY** are all 2D $^1$H-$^1$H correlation experiments that produce an absolute value spectrum with peaks along the diagonal corresponding to a 1D $^1$H experiment, and cross peaks between protons that have a non-zero J-coupling. **gCOSY** has a gradient which will reduce artifacts, but also reduces signal slightly. **zCOSY** produces cross peaks 2-4 bond coupled $^1$H and is used to determine homonuclear coupling constants. **gDQ COSY & DQ COSY** produce a 2D spectrum where peaks with no double-quantum transitions (singlets) are suppressed, producing a cleaner spectrum than traditional **COSY**. Again, the gradient and non-gradient versions trade reduction in artifacts for reduction in signal. **TOCSY & zTOCSY** produces a 2D spectrum with cross peaks between protons that are scalar coupled (through space). **zTOCSY** will filter out an zero-quantum peaks (singlets) to produce a cleaner spectrum. **ROESY & ROESYAD** will produce a 2D spectrum with cross peaks correlating $^1$H that are close together in space ($<4$ Å). Used for intermediate-sized molecules (MW=800-1000) where NOE may be zero. **ROESYAD** uses a low-power adiabatic spinlock for better signal. **NOESY** produces a 2D spectrum with cross peaks between $^1$H that are close in space ($<5$ Å), typically between proximal non-coupled portions of a molecule.
• The [J1(CH)corr] tab contains 2D C-H (or X-H) experiments for correlating directly attached protons to carbons.

• 2D CH one-bond correlation experiments are contained in this tab. Several of these experiments have a traditional and gradient version (e.g. HSQC versus gHSQC). The gradient selection will provide better artifact suppression with some loss in sensitivity (a factor of ~1.4). The adiabatic pulse sequences HSQCAD & gHSQCAD are the most recommended out of this series since they will more uniformly excite a broad $^{13}$C region. The other pulse sequences include HSQC & gHSQC in addition to HMQC & gHMQC, HETCOR & gHETCOR which support multiplicity editing that will cause the methylene groups to appear in the opposite phase to methine groups. ASAPHMQC produces the same type of spectrum as HMQC but with rapid recycle delays to shorten acquisition time.
The **Jn(CH)corr** tab contains 2D C-H (or X-H) experiments for correlating long-range protons to carbons.

These types of experiments produce 2D spectra showing long-range couplings from $^1$H to X nuclei (typically $^{13}$C or $^{15}$N). Adiabatic **gHMBCAD** is the most recommended of this set of experiments, as it provides the most uniform $^{13}$C excitation. **HMBC** and **gHMBC** are the non-adiabatic versions of this experiment. The **CIGAR** and **CIGARAD** experiments allow for a range of the Jn(XH) couplings, which is particularly useful for $^{15}$N correlation experiments where Jn(NH) can vary widely. To differentiate between 2-bond and >2-bond couplings the **gHMBCMmeAD** and **gHMBCRELAY** are used. The **gH2BC** and **gH2BCAD** experiments will show 2-bond $^1$H-X correlations involving non-quaternary carbons.
The [(H)Sel1D] tab contains 1D $^1$H experiments performed on a small, user-defined band of resonances, used to obtain targeted information about single resonances or small areas of complicated spectra.

These types of experiments allow for a very quick 1D correlation of NOE, ROE (through-space) or TOCSY and zTOCSY (through-bond). The experiments are very quick and a series of experiments can be run in a short period of time to get some initial structural information. All of these experiments involve first acquiring a 1D $^1$H experiment, loading the correlation experiment and selecting the region desired for correlation. **NOESY1D** and **ROESY1D** can be used to correlate protons that are close in space, but not necessarily coupled through-bond, <4-5 Å through-space. **TOCSY1D** and **zTOCSY1D** will correlate J-coupled, through-bond peaks. **zTOCSY1D** will contain only resonances within this spin system.
• The **[Sel2D]** tab contains selective 2D $^1$H-$^1$H and $^1$H-X correlation experiments that can improve resolution in a 2D experiment by reducing the number of increments needed in that dimension while allowing the user to select a region of interest. Useful for resolving nearly-degenerate $^1$H-$^{13}$C resonances or a crowded aromatic region of a NOESY.
  
  o Please contact a Facility staff member for additional training and information on these experiments.

• Other Tabs (Please contact a staff member for assistance):
  
  o **[(HC)Crisis2]**
  o **[(HC)HetToxys]**
  o **[(HC)CCcorr]**
  o Proton-flourine – HF expts
  o J-resolved – Jspectra
  o Hadamard encoded - Hadamard
Varian MR400 Experiment Guide

Common VNMRJ Commands
- **aa** – abort acquisition
- **aph** – auto phase correction
- **bs** – block size (number of scans until you can view a spectrum)
- **cexp** – create experiment to have simultaneous windows: `cexp(7)`
- **cr** – display cursor value
- **df** – display FID
- **dres** – digital resolution (peak width)
- **ds** – display spectrum
- **dscale** – display scale
- **f** – display full spectrum
- **ga** – acquire a spectrum and process
- **gain** – gain value (too high = artifacts): `gain?` will return a #, usually 20-30
- **halt** – stop acquisition before the experiment is finished
- **jexp** – join experiment: `jexp(7)`
- **nt** – number of transients (scans)
- **pad** – pre acquisition delay, used for timed array
- **ra** – resume acquisition (stopped with **sa**)
- **rl** – calibrate ppm value by placing cursor on a peak: `rl(7.27)`
- **rts** – read shim set. To reset shims: `rts('standard.shim')` followed by `load='y'`
- **sa** – stop acquisition (can be resumed with **ra**)
- **su** – setup hardware parameters (type before an experiment)
- **svs** – save shim set: `svs('myshims.shim')`
- **wft** – weighted Fourier transform