

Basic NMR training

(I) Spectrometers

The School of Pharmacy's NMR facility has two NMR spectrometers: (1) a Bruker Avance III HD 400 MHz instrument (AV-400), installed in 2017, and (2) a Varian ^{UNITY}INOVA 500 MHz instrument (UI-500), installed in 2001.

The AV-400 is equipped with a 60-sample autosampler for automated sample handling, with data acquisition controlled by IconNMR automation software. The BBO SmartProbe with automatic tuning provides excellent sensitivity for both ¹H (or ¹⁹F) and ¹³C (or other nuclei) experiments. Users usually will run a set of pre-defined experiments on AV-400, without needs to change a lot of parameters.

The UI-500 is running VNMRJ 4.2 on a CentOS 6 linux computer. Without a robot, it is typically used in manual or semi-automated mode. User can run basic routine experiments on it like AV-400. It is also well suited for experiments which require a greater level of user interaction, such as relaxation, diffusion, kinetics and variable temperature experiments, etc. Users will have full control over all parameters.

(II) NMR Facility Polices and Lab Safety

Access to the NMR laboratory (room 1411) is restricted to only those individuals who have either (1) successfully completed an NMR training course by Facility staff, which includes discussing and completing a safety checklist, or (2) have otherwise received training, from Facility staff, regarding the potential dangers inherent in a magnetic resonance facility. Taking or allowing *guests* into the laboratory is not permitted without prior approval by Facility staff. **Sharing of computer accounts and/or key access is explicitly prohibited.** Requests for access authorization must be made through the NMR Facility Director. These restrictions apply to all personnel: NMR users, custodians, maintenance workers, etc.

The potential dangers inherent in a magnetic resonance facility involve the presence of strong magnetic and radio-frequency fields and cryogenic fluids (liquid nitrogen and helium), plus the general hazards of handling chemicals and glassware (primarily NMR tubes). Because only Facility personnel handle cryogenics in the NMR lab, related precautions are not discussed further in this document; common hazards regarding other topics are described below. Note, however, that the following examples are in no way all-inclusive; it is always the responsibility of each individual to ensure that safe practices are followed. When in doubt, consult with the NMR Facility Director before proceeding.

Preliminary Considerations

Authorized Access Only The NMR Facility (room 1411) is a restricted-access laboratory. Only those directly authorized are allowed into the lab, and the doors are to be shut and locked at all times except during entry and exit.

Food and Drink Neither food nor drink is allowed in the NMR laboratory. Period!

Proper Attire Loose-fitting or high-heeled shoes should not be worn in the NMR laboratory. Such footwear greatly increases the risk of losing balance or falling when using the step platforms to insert or remove sample tubes from the magnets. Open-toed shoes of any kind are prohibited in laboratories by campus policy. You are responsible: Be safe, not sorry.

Reporting an Incident If you experience an incident during normal working days and hours, please contact directly either the NMR Facility Director or the Project Assistant (PA). For non-emergency incidents outside normal working hours, please report it using the “AIC Incident Report Form” on the Pharmacy AIC website. Do not assume that a particular incident you experience has already been reported by someone else; this is a corollary to the preceding point. For emergency, call the number on the emergency contact sheet.

Hazards Related to Super-Conducting Magnets

WARNING: Persons with implanted or attached medical devices such as pacemakers or prostheses are not allowed to enter the NMR Facility (room 1411) without authorization from a physician.

WARNING: High-field super-conducting magnets produce very strong, fringe magnetic fields that extend **in all directions** beyond the magnet canister, presenting invisible yet very real dangers related to the forceful attraction of ferromagnetic objects. These magnets are always on and cannot simply be turned off. The UI-500 magnet has its radial 5-Gauss perimeter marked out on the floor with red tape, and the AV-400 magnet’s 5-Gauss perimeter falls within an imaginary circle circumscribing the magnet legs. Ferromagnetic objects must be kept outside these 5-Gauss perimeters at all times.

WARNING: Although fairly common during the initial energization of super-conducting magnets, the violent quench of a stable magnet does occasionally occur. Violent quenches can cause the liquid helium (e.g., 120 L in the UI-500’s Oxford AS500 magnet when full) to boil off in a matter of seconds, venting spectacularly through safety check valves at the top of the magnet canister. If this happens, evacuate the lab immediately — after recovering from the initial scare. The very real danger associated with a violent quench lies in the risk of asphyxiation due to the displacement of oxygen in the room. Normal building ventilation will flush the helium gas out of the room after some time (about 15 minutes); there is no other danger and no real need to evacuate the building (although it would be okay to do so). Inform the NMR Facility Director of the news.

CAUTION: Magnetically encoded media (e.g., ATM cards), mechanical watches and some electronic devices may be damaged or destroyed if subjected to strong magnetic fields; keep such items outside the 5-Gauss perimeters.

Fire Extinguisher A non-ferromagnetic, CO₂ fire extinguisher is located at the right-hand end of the laboratory bench in room 1411.

Chemical and Glassware Hazards

Chemical Hazards NMR Facility users are responsible for knowing the chemical hazards of their compounds, and for taking proper steps to ensure their own and others' safety at all times, e.g., in the event of sample tube breakage and subsequent spill. It is the user's responsibility to completely clean up any spill, broken glass, etc., to the extent possible.

Radio-Nuclides No. Samples containing enriched quantities of radio-nuclides are not permitted in the NMR Facility.

Sample Preparation Whenever possible, NMR samples should be prepared in advance in the user's laboratory. If sample preparation must be done in the NMR Facility (as is typically the case for kinetics studies, for example), it is to be done only on the laboratory bench in room 1411; the computer desks or spectrometer consoles are never to be used for such purposes.

Toxic or Unpleasant Substances Such substances shall be addressed responsibly according to their nature. For example, flame sealing a sample within an NMR tube may be required to contain toxic vapors or an offensive smell.

Sample Disposal Facility users must promptly remove their samples and related materials from the laboratory when their experiments are completed. Arrangements can be made for those with special needs to store samples/tubes in the lab to facilitate their work; however, unlabeled or un-claimed NMR sample tubes or related goods persisting in the laboratory will be discarded.

Gloves If needed for extra protection, gloves (e.g., latex, nitrile) may be worn only while preparing or handling NMR samples. Gloves are **never** to be worn while operating computers or handling other community property.

Glassware Hazards Routine precautions should be observed when handling glassware, especially when inserting and withdrawing NMR tubes into and from the spinner turbine. Some spinner turbines use rubber O-rings to grip the NMR tubes, and the fit can be quite snug, depending upon the condition of the O-ring and the specific NMR tube used. Grip the tube firmly near the spinner and use a twisting motion while inserting or withdrawing the tube. Carelessness has resulted in puncture wounds.

Miscellaneous Considerations

Be Careful! Users must carefully insert and remove their NMR samples into/from the magnets, positioning themselves to maneuver the glass tube straight up or down — not at an angle — out of, or into, the upper barrel. Glass does not bend well at room temperature, and we have had far too many users snap a sample tube by catching it at an angle at the top of the upper barrel. These events are distracting and time-consuming to deal with, are potentially damaging and costly to the equipment, and **are easily and completely preventable**. If you think you're in a hurry in the NMR lab, go away and come back after you've adjusted your attitude; this is no place for reckless or irresponsible behavior!

Hands Off! Please keep your hands off the magnet canisters. If you feel compelled to support yourself while inserting or removing samples from the magnets, then you are probably

doing something else wrong.

Common Sense It is apparently necessary to remind some users to wash their hands and wipe their feet. Come on folks, this is a research laboratory, not kindergarten! Winter in Wisconsin involves snow and ice and sand and salt; these all belong outside, not in the NMR lab, so please do not track this crap into the lab. Let's keep our laboratory space and community property — keyboards, mice, work desks, floor, etc. — clean.

Temperature Control Variable-temperature (VT) work may be performed **only** after an individual has completed specific, on-site training by NMR Facility staff. Users are responsible for knowing and observing the temperature limitations of both their NMR samples and the Facility instrumentation, and must work safely within these limitations. Facility personnel are available for consultation and other assistance in these matters.

AV-400 Autosampler Policy It is the responsibility of individual users to remove their finished NMR samples from the 400 MHz robot and delete the experiments from the IconNMR menu in a timely manner. If necessary, individual users may remove others' completed samples to make holders available for incoming samples. Users are also responsible to label their NMR tubes correctly. If left in the NMR lab, all flasks or other containers for transporting NMR samples must be clearly and legibly labeled with the user's full name (not initials, etc.) so that ownership can be determined. Any such item that is not clearly identified will be discarded. Users may, if desired, temporarily leave their clearly labeled container on the NMR lab bench if they have one or more samples in the sample changer. There is a designated area for this purpose. It is occasionally necessary for Facility staff to remove all the NMR samples from the robot for system maintenance or repair. Users must therefore be able to identify their own samples from a collection of several. The worktable with the spinner and the depth gauge on provides a temporary place for users to add/remove multiple samples from the robot. No container should be left on the workplace after adding/removing samples --- no exceptions.

Eye Protection Users must provide and use their own eye protection as needed.

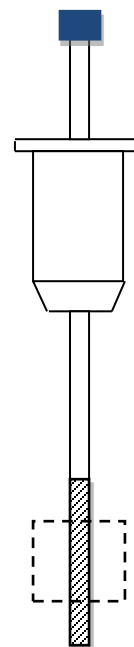
Consequences Unsafe, irresponsible or otherwise inappropriate use of the NMR Facility may result in sanctions up to and including loss of access privileges.

(III) Sample preparation

You need to dissolve your sample in a deuterated solvent, such as CDCl_3 . The optimal sample concentration depends on the nucleus: for ^1H NMR, a few mg is enough; for ^{13}C NMR, 20-30 mg of sample is recommended. The sample volume is usually around 0.6 ml, which gives you a 4-5 cm depth in a 5-mm NMR tube. Shorter sample is very hard to shim properly. The sample must be clear and homogeneous. Cloudy samples, gels, samples that have precipitate in them, and inhomogeneous samples will not give good results. If you see any solid particles in your sample, filter them out before you put the sample inside the NMR tube.

Sample position relative to the spinner is very important. The dash box (for UI-500) or the black bar (for AV-400) in the gauge is the position of the detector. Adjust the NMR tube so that the dash box is in the center of your sample (shaded). You need at least 1 cm solvent above and 1 cm below the dashed box. Shorter sample height will give you poor results.

**Spinner turbine =>
(keep clean!)**



Your sample tube is held by a spinner turbine. Please adjust the position of the NMR tube in the spinner turbine carefully to ensure the best result.

For the best results, please use the NMR tubes with high quality. Use of a particular quality NMR tube in a higher field instrument than it was designed for (for example, use an NMR tube designed for 200 MHz on AV400) may result in sub-optimal resolution, manifested by shimming difficulties and/or poor line shapes.

NMR tubes **MUST BE CLEAN** on the outside. Any grease, dirt, oil, compound, etc. will affect the spinner holder and will cause the tube to slip out of the spinner and shatter in the probe. Clean them gently with a Kimwipe and iPrOH (isopropanol) or acetone prior to insertion.

For 1D proton experiments, a few mg of compound is enough. If you want to get a carbon within a short period of time (for example, 10 minutes), 20-30 mg sample is recommended. Increase the number of scans if your concentration is low. Please note that the sensitivity is proportional to square root of scans. For example, you need 4 times of scans (hence 4 times of acquisition time) if you want to double your sensitivity, while 5 times of S/N needs 25 times of experimental time. For the quantity-limited sample, a Shigemi NMR tube is recommended. Do not use Shigemi tubes in AV400. Please use them in UI500 without spinning.

(IV) General procedure to run NMR experiments

(a) Step-by-step instruction on running experiments on AV-400

- Click “change user” and login to IconNMR with your username and password.

- Identify an empty sample holder in the IconNMR menu and make sure this position is also available in the autosampler. Insert your sample into the blue spinner.
- Press “Add” then “Go to position” on the autosampler control panel, put your sample into your desired holder.
- In the IconNMR menu, double click on your sample holder to add an experiment. You can select your experiment type, solvent and set a title here.
- If desired, hit the equals button to edit standard acquisition parameters including number of scans, spectral width, acquisition time, offset frequency, etc.
- If you would like to also collect another experiment on this sample, you can highlight the current experiment and then hit the Add button. Another experiment will appear below with an empty experiment type. Select the appropriate experiment and edit acquisition parameters if necessary.
- Highlight the appropriate holder directory, and hit the Submit icon.
- After all your experiments are finished, press “Remove” then “Go to position” on the autosampler control panel and take your sample out. Login to IconNMR again, select your finished holder and hit delete. This will make this position available again.

(b) Step-by-step instruction on running experiments on UI-500

- Login and open VNMRJ software by left double click the VNMRJ icon on the screen.
- Click “eject” and put your sample on the top of the magnet, and click “insert”.
- Click the “New study” button (left bottom corner of the screen) below “Study Queue”. Then double-click the experiment you like to run (for example, “PROTON” in the “Common” family) in the “Experiment Selection Tree” window.
- Choose your solvent. Type your sample name in the “sample name” field. This name is not your file name. It is not important and for your reference only.
- Below “New study”, a “submit” button will show up after you set up your experiments. Right to the “submit” button, make sure it is “Foreground”. If you see “Background”, change it to “Foreground”.
- If you want to change some parameters for your experiment, double click on your selected experiment (for example, “PROTON”) and make change under “Acquire”.
- Click “Submit” to start your experiments. Wait for a couple of minutes for the experiment to finish. You will see “DONE” on the screen when it finishes. Type “wft” if you want to see the spectrum now.
- Save you data in your desired folder now by clicking “File” then “Save as” at top left. Please remember no space and other special characters allowed in your file name. But underscore and dash (- and _) are OK.
- After everything done, eject your sample and insert again, then close VNMRJ.
- To logout, click on your login name at top-right and choose “quit”, then select “logout”.

Warning

You need to be *very careful* when you insert your sample. This is the moment when you can do great damage to the spectrometer, so pay attention!!! The sample is ejected and inserted slowly by compressed air from the upper barrel of the magnet. You should be able to hear a loud sound of air pressure when the sample is in “eject” position. Gently put your sample on the air cushion at the top of the magnet. DO NOT let go of the sample until you feel the air cushion is supporting it. Once the sample is resting on the air cushion, deactivate the eject air (“insert”) and the sample will gradually descend into the magnet. Never insert a spinner without a sample tube. Make sure your tube is not cracked, damaged or leaking before introducing it into the magnet. Any careless at this moment (for example, letting the sample go without the supporting air) will cause the sample to crash in the magnet and lead to thousands of dollars of damage.

(c) Run long carbon and any other more complicated experiment on 500

Now you finish your first 1D proton on 500. Your sample has been shimmed and locked. You are ready to run (other) longer experiments manually. Please DO NOT click “New Study” and “submit” again to run your second experiment on this sample, because you do not need to shim and lock again. Just select the experiment you like to run from the top “Experiments” menu, change the parameters if you want to, and type “go” to start. I recommend to use this way to run your long carbon experiments (rather than clicking “new study” and “submit” a long carbon), since you won’t waste a lot time in case when the shimming and locking procedures fail.

(d) Some Useful Commands on 500

The following are some the common commands you can type in the command window. Please note all commands are followed by the “enter” key.

- (1) e: eject
- (2) i: insert
- (3) go: start acquisition
- (4) aa: abort acquisition
- (5) wft: Fourier transformation with window function
- (6) ga: = go + wft, start acquisition and process data automatically after acquisition
- (7) aph: automatic phasing
- (8) time: show experiment time
- (9) fixit: load a saved shim file. If you have problems to shim, use this home-made macro to load a good shim file to start.
- (10) jexp1, jexp2...: join experiment #1, #2, etc. If you wish to open two or more data file at the same time, use this command to switch between different spectra.

{Note: “experiments” are like different “windows”; each has its own data set in it}

(V) Transferring data to your own computer

Your data will be saved on the Linux computers as a folder. We use the Samba file sharing tool to transfer your data to your own computer. To connect your computer to the Samba server, on your windows computer, open a file browser, right click on “Network” and select “Map network drive”. Type in [\\128.104.115.234\av400](smb://128.104.115.234/av400) for 400 (or [\\128.104.115.234\inova500](smb://128.104.115.234/inova500) for 500) in the field “Folder” and click “Finish”. Use the login and password you received during hands-on training. Please note that this Samba tool only works inside School of Pharmacy. The “nmr05” PC in the NMR lab has been connected to the Samba server already.

BACKUP YOUR DATA! It is highly recommended that you backup all your NMR data onto a CD or DVD frequently. NMR facility is not responsible for data storage. Sometimes all data on the Linux computers will be lost due to hard drive failure. Burning your data to a CD is a good way to prevent losing any data later.

(VI) NMR time reservation for 500 online

You do not need to book time to use AV400. If you only have a short carbon (10 minutes) or a few proton experiments (less than 15 minutes totally) to run on UI500, you don’t need reserve time in advance either. The online “Faces scheduling system” (<https://faces.ccrc.uga.edu/>) is for long experiments on UI500 only. It is a website-based application which you can access it from a computer, a tablet and even a phone. Please contact the Facility Director for an account if interested. You will receive an email with your login name and temporary password. Please click the link to finish setting up your account and password. After login (always use “UW_SOP” in “Group”), click “choose a schedule” and select “UI500”. The first click on an empty slot is the starting time, the second click on a later empty slot is the ending time. Click “OK” to finish your booking. You cannot book more than 2 hours during daytime (8:00 am to 6:00 pm). There is no limit for night and weekends.