7 Equipments

Spectrometers

There are three spectrometers located in the NMR laboratory: Varian UNITYplus 500 MHz (NMR500), Varian UNITYplus 500 MHz (Nightmare) and Varian INOVA 600 MHz. All spectrometers are equipped with waveform generators and pulse field gradients. The frequency at which protons precess in the particular strength of magnetic field is used to designate the magnets. For example, protons precess at 500 MHz in the 500 MHz magnet, but the magnet strength is actually 11.7 Tesla. Further, carbon precesses at about 125 MHz in a 500 MHz magnet. Nuclei such as carbon, nitrogen and phosphorous resonate at much lower frequencies than proton. Often, nuclei that resonant at the higher frequencies such as proton and fluorine are called "high band" and other nuclei like carbon and nitrogen are called "low band". The basic components of the spectrometer include: workstation, console, magnet and probe.

Workstation

The computer workstation is where most of the operation of the instrument occurs including data collection and simple processing. Extensive processing should be done on offline workstations that do not absorb instrument time. The workstation communicates with the console that, in turn, controls the console and the probe in the magnet. The workstations are Dell PC's running under Red Hat Linux 5.1 for 600 MHz and Sun Solaris 8 for both 500 MHz NMRs.

Console

The console contains the radio frequency generators, amplifiers, a variable temperature controller, pulsed-field gradient generator, waveform generators, and other computer components. In typical operation, a user will very rarely, if ever, need to interact with the console.

Magnet

The magnetic field in all of the instruments is generated by a current flowing through a solenoid of superconducting wire. For the wire to be superconducting, the wire must stay at liquid helium temperature (4 K) or below. Therefore, the cryostats are filled with liquid helium and outer liquid nitrogen to keep the magnets cold. If the magnets warm up above that temperature, a quench can occur. A quench is when the current in the magnet coil is lost. If a quench occurs, it is usually accompanied by a loud noise followed by fast release of helium gas from the cryostat. If this occurs, please leave the lab as quickly as possible. The magnet is contained inside the silver Dewar. The magnets are mounted on vibration legs. The air legs maintain level and stable against small vibrations by air pressure. Therefore, do not lean against the magnets because the magnet will rock. Try to avoid walking around near the magnet during an experiment because it can contribute to vibrations. Typically, the only time a user needs to go near the magnets is to insert the sample and tune the probe. **Never** take metal or magnetic objects near the magnets. **Always** check pockets and person for these things before approaching the magnet. Non-digital watches, cards with magnetic strips, and magnetic media (such as disks) will also be affected by the magnetic field. Also inside the magnet are the shim coils. Shim coils are a collection of electrical coils used to remove residual magnet field inhomogeneities. The temperature in the bore of the magnet, where the sample will sit, is controlled by a VT controller and is typically set at 25° C.

Probes

The probe is inside the bore of the magnet. The probe contains the transmitter/receiver coils on where pulses go into the sample and RF frequencies come out. The room temperature probes can be changed in a few minutes by facility personnel and have different configurations depending on the application intended. Probes are only changed by facility personnel or by specially trained users. The type of probe selected is determined by the nucleus to be detected and the specific experiment. The Nightmare and Plumeria spectrometers have indirect detection probes. These probes are the best choice for direct proton detection or indirect detect experiments. This probe has the proton transmitter/receiver coil closest to the sample and is, therefore, most sensitive for proton detection. The NMR500 spectrometer have broadband probe which typically have the X-nucleus coil closer and are, therefore, more sensitive for nuclei like carbon. Carbon can still be detected directly using an indirect detection probe, but it will have a much lower signal-to-noise. Broadband probes or probes that include a X-nucleus can be tuned to a different nucleus depending on the tuning range of the probe. Direct detection of an X-nucleus is best done with a broadband probe.

Choosing the Spectrometer

Instrument Specifications and Set-up

It is important to choose the appropriate instrument in the laboratory to answer the relevant experimental questions. Important things to consider include type of probe on the magnet, sensitivity of the system in regards to sample concentration, variable temperature set up, and magnetic field strength.

Varian UNITYplus 500 MHz NMR Spectrometer (Nightmare)

The 500 NMR is especially easy to use. The spectrometer has a 2-channel broadband probe. The proton channel (high band) can be tune to either ^{1}H or ^{19}F. The broadband channel is doubly tuned to ^{13}C, and ^{11}B and experiments can be collected without changing cables or tuning the probe. This instrument is also capable of running more advanced 1D and 2D experiments, but the sensitivity is less than on the other spectrometers and therefore requires higher sample concentration. This spectrometer has variable temperature accessory and can conveniently collect variable temperature experiments. The spectrometer is running Vnmr 6.1C software.

Varian UNITYplus 500 MHz NMR Spectrometer (Nightmare)

It is more sensitive than the NMR500 and requires less sample concentration. It is equipped with indirect detect ^{1}H probe for running indirect detection experiments with lower sample concentration. The spectrometer is running Vnmr 6.1C software.

Varian INOVA 600 MHz NMR Spectrometer

The 600 NMR is a three-channel system with a triple resonance cryogenic probe. This system has the highest resolution, best sensitivity in the laboratory. Longer 2D and 3D experiments have preference on this instrument over short 1D ^{1}H experiments. The spectrometer is running VNMRJ 4.2 software with Chempack 6.2 and BioPack options.

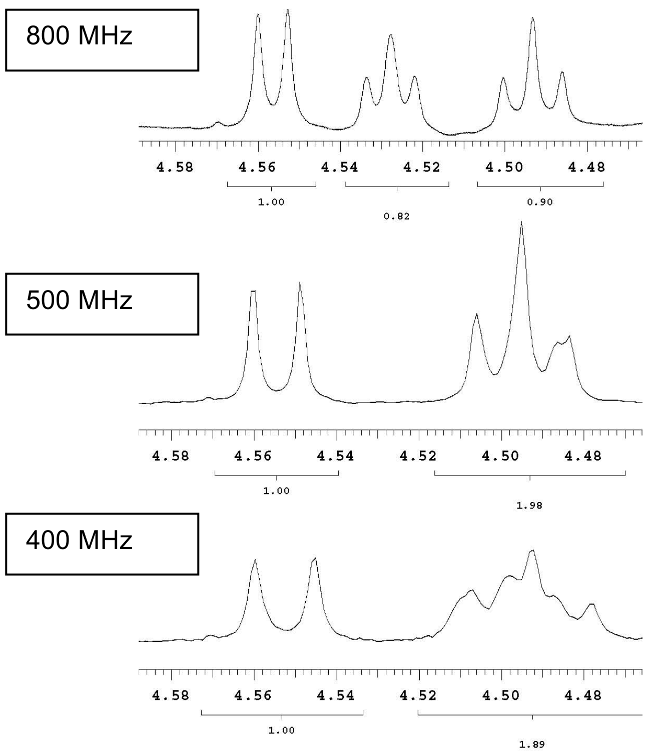
Sensitivity

Signal to noise increases as field strength is increased. Signal to noise is also dependent on the probe. Indirect detection probes are constructed to maximize the proton sensitivity, while direct detect probes are constructed to maximize broadband signal intensity. The table below lists the signal to noise values for the NMR instruments based on the manufacture specification.

	1H	F19	13C	P31	N15
NMR500, 500 MHz					
Broad-band probe	250:1	-	200:1	-	-
Nightmare, 500 MHz					
Indirect Detection Probe	500:1	-	-	-	-
Plumeria, 600 MHz					
Triple Res. Cold Probe	5000:1	-	-	--	--

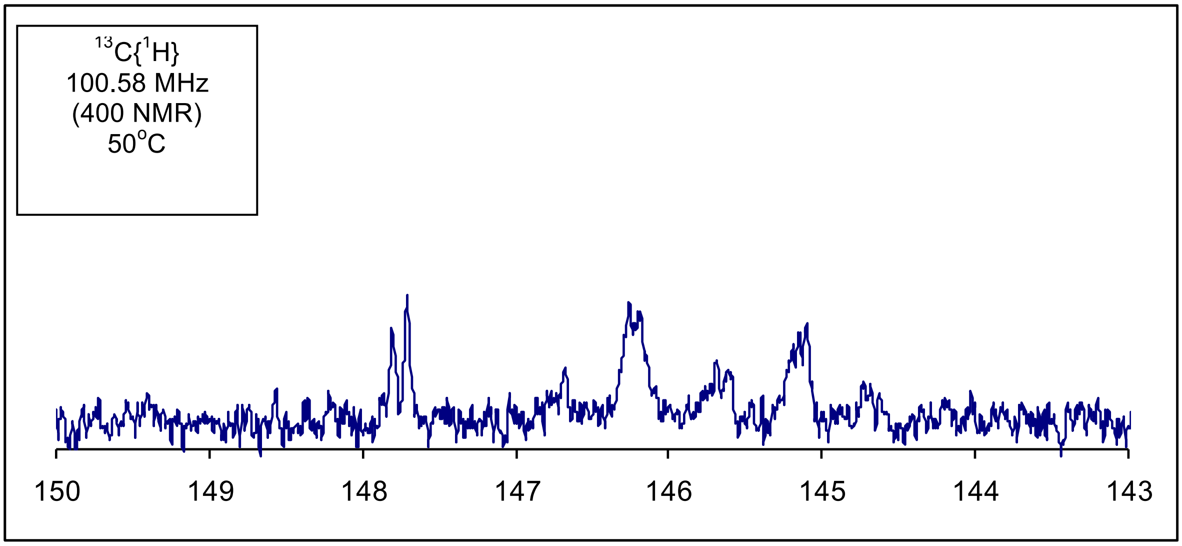
Resolution

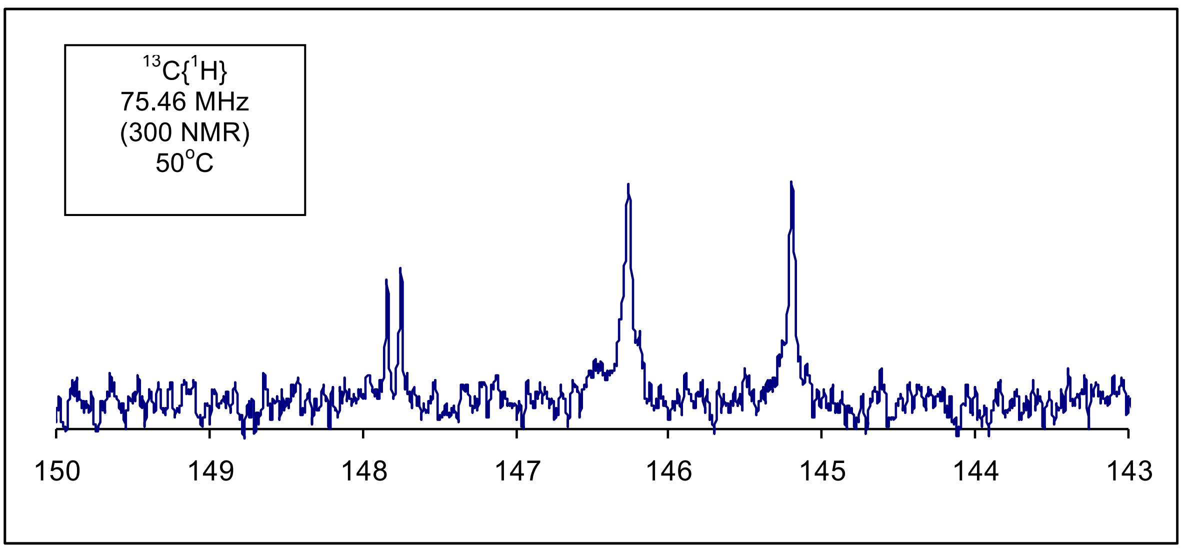
Resolution is normally increased as the field strength of the magnet is increased. Overlapped peaks on the 300 NMR may be resolved at higher field strength. The partial ^1H spectra of a natural product are shown below. The spectra were collected on the 400 and 500 spectrometers. The three peaks are completely resolved from each other at 800 MHz.



Dynamic Molecules

Molecules which are dynamic on the NMR time scale have a lower coalescence temperature on a lower field magnet. A single set of sharp lines is observed above the coalescence temperature. In the example below, the dynamic catenane compound had broad lines in the ^{13}C spectra at room temperature. On the 400 MHz NMR spectrometer, this temperature was not far enough above the coalescence temperature to result in sharp lines. However, considerably sharper lines were observed on the 300 MHz NMR spectrometer at 50°C due to the lower coalescence temperature.





Software

Intro to Vnmr and VnmrJ
Both of the 500 MHz NMR spectrometers are running Vnmr 6.1C. The computer of the 600 MHz NMR spectrometer is runn VnmrJ 4.2A.

The program that communicates between the workstation and console is called the “acqproc”. Occasionally this program loses communication between the console and workstation and needs to be re-started. Please ask facility personnel to do this if they are available. If no one is available, instructions are posted in the facility for re-setting the console.

Every user has a login account. They are responsible for remembering their user name and password. **Do not allow others to use the account or give out the password to anyone.** If the facility determines that a user is allowing unauthorized users to use the account, privileges *will* be suspended.

The Vnmr software currently uses UNIX for many things. Knowing a few simple UNIX commands can be very helpful (See Appendix for useful UNIX commands).

In a user account, there is a directory called "vnmrsys". This is a Vnmr system directory. In this directory are many subdirectories. These directories are probably empty unless someone has put in a new macro, pulse sequence, shim file, parameter file, etc.

The most significant of the subdirectories:

psglib - pulse sequence library, contains uncompiled pulse sequences
seqlib - sequence library, contains the compiled, executable versions of pulse sequences
maclib - macro library
shims - stores personal saved shim files
parlib - parameter files
probes – probe files (created with an **addprobe** command)
shapelib – shaped pulse files (*filename*.RF)
gshimlib – gradient shimming maps and files (created from **gmapsys**)

Global directories are found in the **/vnmr** directory (a few directories up from the personal directories). The global directories, like **psglib**, contain all the pulse sequences, macros, parameters, shims etc. available to all users. To see the pulse sequences available on a specific machine, look in the **psglib** in the VNMR directory. The main VNMR subdirectories can be altered by facility staff only. Files may be copied into a personal **vnmrsys** and altered there. Any macros, pulse sequences, parameter files, etc. in a personal **vnmrsys** will be accessed preferentially to the global **vnmrsys** during operation.

To put a new pulse sequence into the **vnmrsys**, copy the uncompiled sequence (*seqencename*.c) into the **psglib**. Be sure to copy any necessary macros into the **maclib**, any parameters into the **parlib**, shaped pulses into **shapelib** etc.

To compile a sequence copied into the **psglib**,
>**seqgen *pulseq.c***
This will compile the sequence and put the executable into the **seqlib**.

Pulse sequences can also be compiled from the Vnmr command line as
>**seqgen('*sequencename*').**
Vnmr also has various packages for specific applications. The most common one in the facility is called BioPack and includes many pulse sequences for applications to biomolecules. These packages can be installed or activated in individual user accounts.