

IMSERC NMR BASICS

Purpose of this manual

This manual is intended to provide the background knowledge necessary to effectively and efficiently operate NMR spectrometers at IMSERC.

Relation to certification

To make sure that all NMR users are familiar with IMSERC safety and procedural policies, and that they know enough to reliably acquire good-quality data without assistance, IMSERC requires all users to pass a certification test before they get full access to the NMR spectrometers. This document should help prepare users to pass the written portion of the certification test.

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General Lab Safety

Safety is paramount in a large multi-user environment such as IMSERC. In both the main lab of the BG75 in Tech building, and in room B530 in Silverman Hall, everyone must adhere to general lab safety practices:

- Must wear glasses or safety glasses. Normal glasses are OK only if you are not handling liquids or working with pressurized vessels.
- Must wear clothing that covers from shoulders to knees. Tank tops and shorts are not allowed.
- Must wear closed-toed shoes. Sandals and covered-toe sandals are not allowed.
- Must NOT bring or eat food or drink, including bottled water or covered coffee.

Please see the policies from the Chemistry Department concerning the importance Northwestern places on laboratory safety:

<http://www.chemistry.northwestern.edu/documents/department-resources/SafetyStandardsChemistry.pdf>

IMSERC staff are more than happy to answer any questions you have regarding safety. If you have any questions, please contact IMSERC staff.

NMR Lab Safety

Practicing NMR exposes one to some specific hazards not found in other environments. The primary danger arises from working around large magnets that are full of cryogenics (liquid nitrogen and liquid helium).

Magnet hazards

When working around large magnets, you must be aware of the metal objects on you – in your pockets, pinned to your clothes, parts of your clothes, piercings, jewelry, eyeglasses, etc. Most are nonmagnetic and won't pose a problem, but the ones that do can injure you and damage the instrument. Here are some general guidelines as to what is usually safe and what is not; *these are NOT complete lists!*

- **Common magnetic metals:** iron, steel, nickel
- **Common nonmagnetic metals:** copper, brass, aluminum, gold, silver, platinum, titanium, stainless steel, surgical stainless steel, magnesium, lead, tin, solder
- **Generally unsafe or problematic items:** hammers, screwdrivers, pocket knives, and other hand tools; steel-toed shoes, paper clips, staples, pins, computer equipment, three-ring binders, key rings, pacemakers, cochlear implants, hearing aids
- **Generally ok:** fine jewelry; surgical stainless steel pins, plates, and other implants; keys, coins, belt buckles, pens, most eyeglasses
- Inconvenient, but not technically hazardous: magnetic strips on many credit cards and identification cards can be erased if placed too close to an NMR magnet

When do you start worrying about the magnetic fields? People with pacemakers are usually prohibited from entering rooms with NMR magnets in them, though exceptions may be made for specific shielded modern magnets. The usual boundary is the “**five-gauss line**”, which is marked in tape on the floor around every magnet in IMSERC. Magnetic field strength is measured in units of “gauss”, and five gauss is an accepted limit above which you should be concerned about magnetic strips becoming erased (such as those on credit cards) and stray metal objects on you becoming attracted to the magnet. For reference, the Earth's magnetic field is approximately 0.5 gauss outside in Chicago, and approximately 117,000 gauss inside a 500 MHz NMR magnet.

IF you should find a magnetic object stuck to one of the IMSERC NMR magnets, DO NOT TRY AND PULL IT OFF! Contact staff immediately and put a **STOP sign** on the instrument's keyboard

so no one else uses the instrument until staff can check it out. If a large object such as a chair or a gas cylinder is stuck on a magnet, pulling on it exerts force on the magnet inside its large container and can permanently damage the delicate instrument. If a small object is stuck to the outside of the magnet, one risks it flying to the inside of the magnet, where it may be impossible to dislodge without deenergizing the magnet.

Cryogen hazards

NMR magnets contain large amounts of liquid nitrogen (boiling at 77 K) and liquid helium (boiling at 4.2 K) to keep the superconducting electromagnet wires cold. Each magnet is capable of a catastrophic failure known as a “**quench**”, in which all the cryogens boil off suddenly. This happens very rarely, but when it does happen, it poses a risk to everyone nearby. Besides the potential exposure to ultra-cold liquids and gases, the sudden appearance of hundreds of liters of inert gas displaces the oxygen around the magnet and creates an **asphyxiation hazard** (i.e., you cannot breathe).

A quench is usually preceded by a hissing noise and/or a plume of cold vapor coming out of the top of the magnet. If you see or hear such a thing, **GET AWAY FROM THE MAGNET AND CONTACT STAFF IMMEDIATELY (DAY OR NIGHT)!**

Reporting broken glass

To ensure that the NMR's remain available and performing at a high level, users must report to IMSERC staff any incident involving broken glass around NMR instruments, whether you broke something yourself or observe broken glass in the NMR area. For the manually run instruments, if you realize that the lock sample is not in the magnet with the instrument locked when you arrive, you must report the issue immediately so we can determine proper next steps to avoid long service interruptions. The policies ensure that the delicate glass pieces inside the spectrometer are not damaged and that users are leaving spectrometers ready for the next experiment. Failure to follow these policies can result in days to weeks of downtime as probes must be inspected for contamination, or worse yet, sent back to the vendor for repair.

If you break glass and report to us, you are helping us to bring the instrument to service at earliest possible time and there will never be any punishment for making a human mistake. Failure to report issues like the ones listed above are one of the few policy breaches that can result in suspension of NMR privileges. We prefer that you call an NMR staff member or Director to set up a response after a problem, but email is acceptable if you cannot reach someone.

When broken glass is not reported, we assume there is glass pieces inside probe. To check and clean up the probe, we have to take down the system. The first unpleasant consequence of doing so is the instrument down time, which could easily be a few days for system with cryoprobe. In addition, we take increased risk of damaging parts or possibly not being able to bring it back online. In these cases, IMSERC staff will be following up with users who either broke the glassware originally or subsequent users who fail to report an issue with lock samples or visible glass in the NMR area.

General NMR Knowledge

Resonance frequencies of different nuclei

• The **resonance frequency**, aka “**Larmor frequency**”, of a given nucleus depends on the field strength of the magnet it’s in AND its **gyromagnetic ratio** (aka magnetogyric ratio):

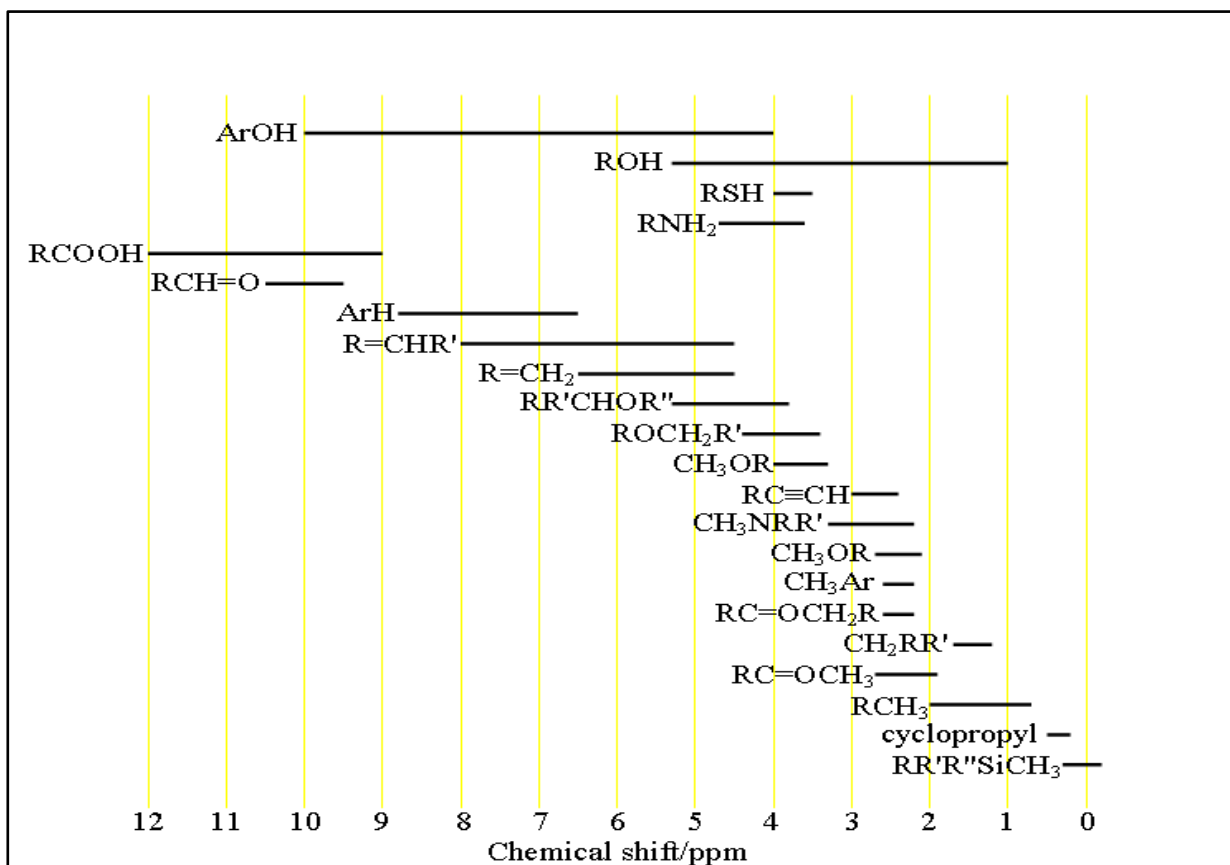
http://en.wikipedia.org/wiki/Gyromagnetic_ratio

• We generally describe NMR systems by the approximate resonance frequency of ^1H nuclei when placed in them. Thus, a “500” NMR system will cause ^1H atoms to resonate at approximately 500 MHz; on the same system, ^{13}C atoms will resonate at 125 MHz, ^{15}N atoms at 50 MHz and ^{31}P atoms at 203 MHz, according to their different gyromagnetic ratios. You can check out gyromagnetic ratios for all the NMR active nuclei from “NMR Periodic Table”:

<http://imsercdata.northwestern.edu/guide/eNMR/chem/NMRnuclei.html>

Chemical shifts and ppm

Chemists are usually most interested in the slight frequency differences between chemically different nuclei of the same type – different kinds of hydrogen in one molecule, for example. These small differences based on local chemical environment are called “**chemical shifts**.” For each nucleus type, such as ^1H , NMR spectroscopists define all chemical shifts relative to a standard reference that’s agreed to be defined as “0.000 ppm”. For ^1H , the frequency is that for the methyl ^1H ’s of tetramethyl silane (TMS). The chemical shifts of ^1H ’s in different functional groups have been well-characterized and are tabulated in many places. Here is a diagram showing the ^1H chemical shift ranges of different functional groups:

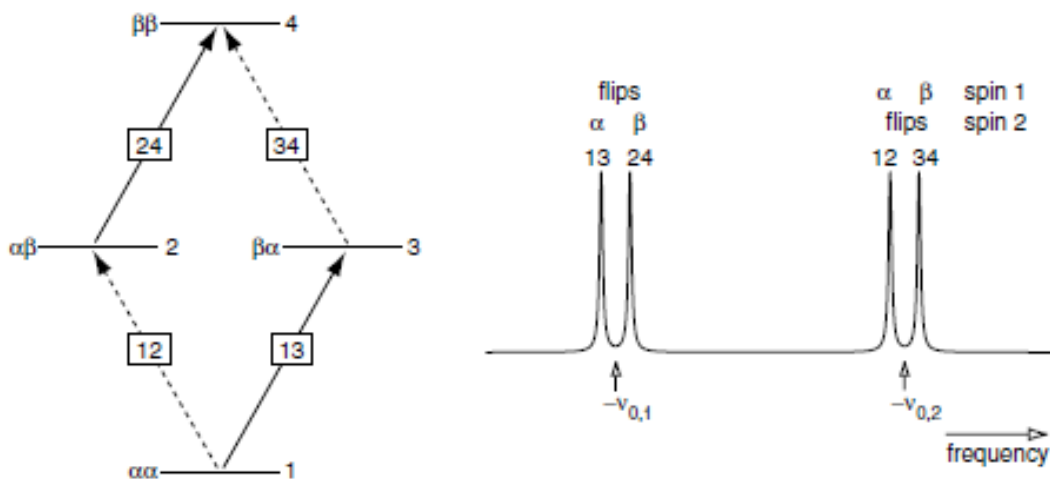


- It is sometimes handy to know how to approximately convert ppm to Hz. For ^1H nuclei on a 400 MHz instrument, the conversion is 400 Hz/ppm. On a 500, it's 500 Hz/ppm. For ^{13}C on a 400 MHz instrument, for which ^{13}C 's are resonating at 100 MHz, it's 100 Hz/ppm. You get the idea.

Scalar coupling (or J coupling)

Usually the NMR chemical shift for one proton comes as the multiplet structure resulted from the scalar coupling. It is originated from through-bond interaction mediated by bonding electrons between coupled spins. Critical molecular structure information can be obtained by examining J coupling constants. For a coupled two spin system, the different spin state (α or β) of spin 1 perturbs the spin 2 electrons differently. The energy levels of spin 2 are in turn affected differently by the polarized electrons at spin 2. The same is true for the spin 2 to spin 1 perturbation. The J coupling is always reported in Hz and field-independent. The following table and figure are taken (<http://www-keeler.ch.cam.ac.uk>) to illustrate how spin 1 and spin 2 of the coupled system are split to two lines by each other. Most of our NMR experiments transfer magnetization through scalar coupling.

transition	spin states	frequency
1 \rightarrow 2	$\alpha\alpha \rightarrow \alpha\beta$	$-\nu_{0,2} - \frac{1}{2}J_{12}$
3 \rightarrow 4	$\beta\alpha \rightarrow \beta\beta$	$-\nu_{0,2} + \frac{1}{2}J_{12}$
1 \rightarrow 3	$\alpha\alpha \rightarrow \beta\alpha$	$-\nu_{0,1} - \frac{1}{2}J_{12}$
2 \rightarrow 4	$\alpha\beta \rightarrow \beta\beta$	$-\nu_{0,1} + \frac{1}{2}J_{12}$



On the left, the energy levels of a two-spin system; the arrows show the allowed transitions: solid lines for transitions in which spin 1 flips and dotted for those in which spin 2 flips. On the right, the corresponding spectrum; it is assumed that the Larmor frequency of spin 2 is greater in magnitude than that of spin 1 and that the coupling J_{12} is positive.

Data Collection and the Fourier Transform

- Modern NMR data are collected by applying a **pulse** or series of pulses of radio-frequency light to the sample, then recording the RF signals given off by the sample. The resulting intensity-versus-time signal is basically a mixture of sine waves corresponding to all the frequencies emitted by the sample. These signals decay exponentially within a few seconds.
- The raw “**time-domain**” signal is called the “**free induction decay**”, or “**FID**”.
- To get the normal intensity-versus-frequency spectrum (“**frequency domain**”), we must apply a **Fourier transform**. All NMR software is equipped to perform Fourier transforms with the click of a single button or typing a single command, usually “ft”.

Sensitivity, S/N and experiment time

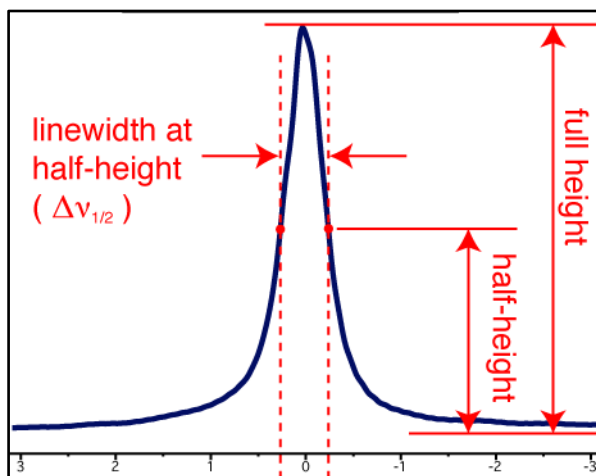
- NMR is an intrinsically insensitive technique, detecting magnetization from only millionths of a sample’s nuclei. It is useful to keep in mind the following:
- Sensitivity is gauged by a spectrum’s “**signal-to-noise ratio**”, abbreviated **S/N**. A peak with a S/N of 1.0 is indistinguishable from noise. One generally needs a S/N of approximately 5 to 10 to be satisfied that a spectral feature is actually a peak.
- A spectrum’s S/N can be improved by adding spectra together. Usually, one acquires a spectrum using several “**scans**”, aka “**transients**”, that are added together by the software. Doubling the number of scans of a spectrum improves its S/N by a factor of $\sqrt{2}$ (~1.4). Quadrupling the number of scans improves S/N by a factor of 2.0. So, ideally, the S/N of a spectrum using 64 scans is twice that of a spectrum using 16 scans.
- An experiment’s duration is directly related to the number of scans used. In general, a spectrum employing 64 scans takes four times as long to run as one that takes 16 scans.

Sensitivity, natural abundance, and receptivity

- For many elements, the most common NMR-active isotope is not its most abundant one. For carbon, only the isotope ^{13}C is NMR-active, but only 1.1% of carbon atoms are the ^{13}C isotope. The “**natural abundance**” of ^{13}C is therefore said to be 1.1%. Thus, the expected S/N of a carbon spectrum of a normal, non-enriched compound is only 1.1% that of the same, “enriched” compound in which all carbon atoms are ^{13}C .
- The S/N of a given nucleus also depends on its “**receptivity**”, which incorporates the intrinsic S/N expected relative to ^1H based on the different resonance frequency of the atom. ^{13}C , for instance, has a receptivity of only 0.0159 relative to ^1H when enriched, and just 1.7×10^{-4} at natural abundance.

Spectrum quality: Resolution

- One of the measures of a spectrum’s quality is its “**resolution**” – the ease with which an observer can distinguish two peaks that are close to one another. If each peak is very broad, the neighbors will blend together, and we say the peaks are poorly resolved.
- Resolution is generally assessed by measuring the “**linewidth at half height**” of a given peak, usually a solvent peak (e.g., the residual 0.1% CHCl_3 from a bottle of 99.9% CDCl_3):
- For everyday purposes, we generally say that a spectrum is acceptable if its CHCl_3 line at 7.24 ppm is less than 2.0 Hz wide at its half-height.
- For publication, it should be less than 1.0 Hz wide, which usually requires spending additional time adjusting the shims.



Locking

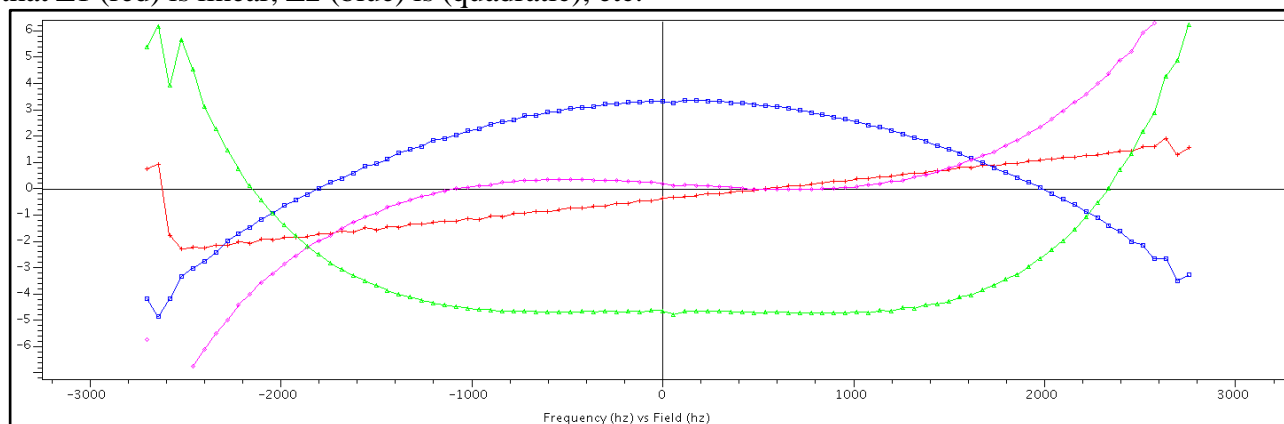
- Though NMR magnets are superconducting, which leads one to suspect their field remains constant over time, they do lose very small amounts of field. Specification for “**field drift**” on most modern magnets is on the order of 10 Hz lost per hour. This is a very small percentage of the total field, so a “500” MHz magnet will remain a 500 for decades. However, our peaks are ~ 1 Hz wide, so 10 Hz/hr is not constant enough for use to use in experiments lasting more than a few minutes.
- NMR spectrometers have a mechanism to correct for drift called a “lock circuit” – it “**locks**” the field so it is constant.
- The lock system is basically a spectrometer-within-a-spectrometer, constantly exciting and detecting signals from **deuterium** (^2H) in the sample. When it measures a drop in the deuterium signal intensity (called the “**lock level**”), the system interprets it as slight loss in B_0 field, and adds a small amount of magnetic field to compensate.
- The sample must have deuterium in it for locking to work. Using a deuterated solvent achieves this aim handily and also vastly reduces the ^1H signal that would otherwise appear from solvent in the ^1H spectrum.
- Locking also enables the software to reference the spectrum reasonably accurately. If the user tells the software that solvent is DMSO- d_6 , for instance, the software can calculate where 0.000 ppm is in the ^1H spectrum based on the ^2H resonance frequency of the solvent.
- For short experiments, locking is not necessary and deuterated solvent is not required for operation. Indeed, it can be very useful to monitor reactions by analyzing NMR spectra of undeuterated reaction mixtures.

Tuning/Matching

When you listen to NPR around Chicago, you need tune your FM radio to 91.5 MHz. Similarly if you want to collect C^{13} NMR spectrum on a 500 MHz system, you need tune the probe to 125 MHz. The NMR probe has at least two RF (radio frequency) coil circuits, one for high band and one for low band nucleus. The coils are used to efficiently transmit the RF pulses to the sample to manipulate the magnetization transfers before acquisition and to pick up the NMR signals during acquisition. The tuning is to adjust probe circuit to the desired frequency. The electrical properties of the coil circuit needs be optimized for each sample because the sample conditions, including solvents, concentration, pH, and salt, could change the frequency by up to a few MHz for same nucleus. The matching is to adjust probe circuit to meet impedance requirement (50 Ohm). Probe tuning and matching will ensure you get best sensitivity possible from your sample.

Shimming

- NMR detects signals from a section of the middle of the sample. If the magnetic field strength in one part of that section is even slightly different from that of another part, the resonance frequencies of the molecules from the different parts will be slightly different. This affects the spectrum by making every peak broad and misshapen, in proportion to the **inhomogeneity** of the magnetic field.
- To make the field useful for chemists, we use hardware called “**shims**” to improve the field’s homogeneity, i.e., make it more uniform.
- Shims are electromagnets that apply small corrective magnetic fields with well-defined inhomogeneities to the sample.
- The act of optimizing lineshape by adjusting the corrective magnetic field from shim coils by adjusting the electrical current through the shim coils is called “**shimming**.”
- Shims are named after the direction and polynomial order of the spatial dependence of the applied inhomogeneity. For example, the “Z1” shim applies a field along the “Z” direction of the magnet (along the magnet bore) that has a linear gradient (the “1” in Z1 stands for “first order polynomial”) Z1 may thus add field to the top of the sample and subtract field from the bottom of the sample, the strength of the field depending linearly on Z position. The field added by the “Z2” shim depends quadratically on Z (“2” for “second order”).
- Here is a diagram showing the extent of magnetic field added (positive along the chart’s Y axis) as a function of position along the magnet’s main field direction, the Z axis (the chart’s X-axis). Note that Z1 (red) is linear, Z2 (blue) is (quadratic), etc.



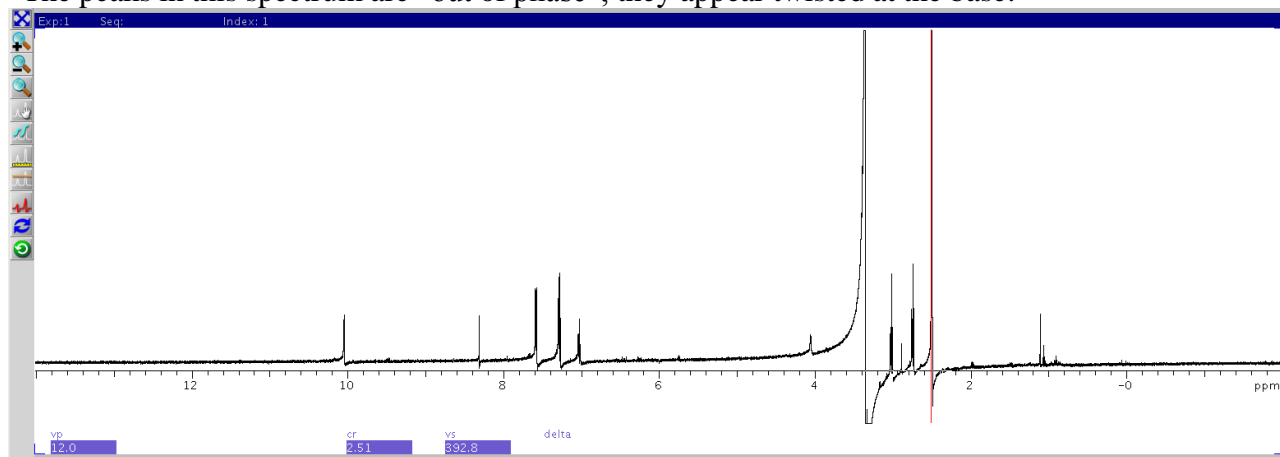
- Z0 is a special shim that adds a magnetic field that is constant across the sample - a “zeroth-order” field gradient. Z0 is used by the lock system.
- Shims are also applied in the X and Y direction, and are named similarly.
- To obtain good spectra, *every* sample must be shimmed shortly after being placed in the magnet – after it has equilibrated to a constant temperature.
- Usually, one can achieve good shimming using a routine called “gradient shimming”, which applies imaging technology to analytically calculate shim corrections in the Z direction. Most of the time, the user simply clicks the “Gradient Shim” button to achieve lineshape quality that is suitable for their purposes.
- Shims can also be manually adjusted to achieve better lineshape. Usually, the user adjusts a shim to higher or lower value and determines whether this improves the lock level; improvement in the lock level is taken to mean the field homogeneity is getting better, thus strengthening the signals. One usually adjusts one shim to give the best lock level, then proceed to optimize another shim, then return to the first, and continue adjusting to give the strongest lock level.
- There is no indicator that one is done with shimming – the user can continue shimming to improve lineshape forever. One simply has to stop when one suspects the shims are good enough for the purpose at hand.

Spinning

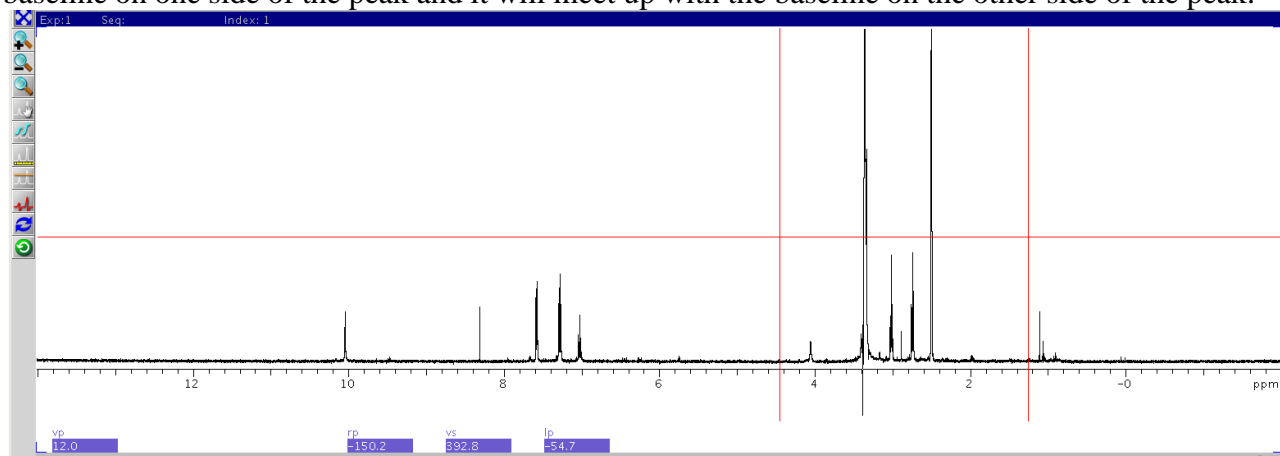
- In many NMR facilities, people improve their linewidths by spinning their samples. This averages away any magnetic inhomogeneities in X and Y directions across the sample that would otherwise make the spectral lines broader.
- In IMSERC, we DO NOT SPIN SAMPLES. Spinning a tube that is bent can severely damage the probe. There are enough people who use poor-quality tubes in our large working environment that spinning poses a large risk; a broken probe can make an instrument unavailable for weeks and cost thousands of dollars to repair.
- In some very special circumstances, such as running a reaction in a spectrometer and wishing to keep the solution well-mixed, when spinning may be allowed. In these cases, the user **MUST** present the sample to NMR staff for inspection before use.

Phasing

- For reasons having to do with digital sampling and the Fourier transform, we acquire analog NMR signals in two channels, which are 90° out of phase with one another. This effectively makes every point in the spectrum a combination of **“real” and “imaginary” points**.
- **“Phasing”** is the act of combining the real and imaginary data to yield a good-quality spectrum.
- The peaks in this spectrum are “out of phase”; they appear twisted at the base:



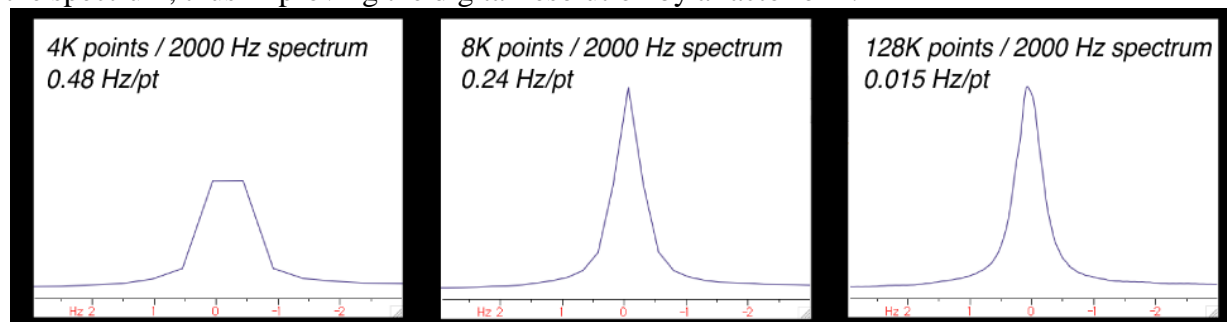
- The peaks in this spectrum are “properly phased”; you can draw an imaginary line through the baseline on one side of the peak and it will meet up with the baseline on the other side of the peak:



- The procedure for performing “phase-correction” depends on the software used for processing. Please consult the manual for each program for these procedures.

Zero-filling and digital resolution

- When a Fourier transform is performed, the number of points is conserved. Thus, FT of an FID with 32K complex time-domain points yields a spectrum with 32K complex frequency-domain points.
- **“Digital resolution”** is simply how many Hz are represented by each point in the frequency-domain spectrum. If one has a 10,000 Hz-wide spectrum described by 16K complex points, the digital resolution is $10,000/16,384 = 0.61$ Hz/pt.
- A peak that is 2.0 Hz wide would be represented by only 3.3 points, which is not very good. To obtain better digital resolution, one can acquire data for a longer period. Acquiring 64K points will yield 4 times better digital resolution than acquiring 16K points. However, the additional points will be acquired by extending the acquisition time, and because the signals are decaying with time, the S/N of the additional points will be low. Thus, improving digital resolution by acquiring for longer time decreases the spectrum’s S/N.
- One can improve the digital resolution of a spectrum without compromising S/N by adding “zeroes” to the end of the fid. Thus, if one acquires 16K actual points and adds 48K of zeroes to the end, the Fourier-transformed spectrum will have the same S/N but will use 64K points to describe the spectrum, thus improving the digital resolution by a factor of 4.



- One caveat to zero-filling: If an FID is too short, it will give rise to “wiggles” at the base of the peaks, the sharpest peaks being most affected. Zero-filling does not fix this problem, and it is usually best to fix the “wiggle” problem by increasing the acquisition time.

FID Weighting/Apodization

- S/N and linewidth can be adjusted by judicious **multiplication of the fid** by another function. Without going into the mathematical details of the Fourier transform and convolution, one usually multiplies one's FID by a simple exponential decay ($y=e^{-t}$) to yield a spectrum with improved S/N.

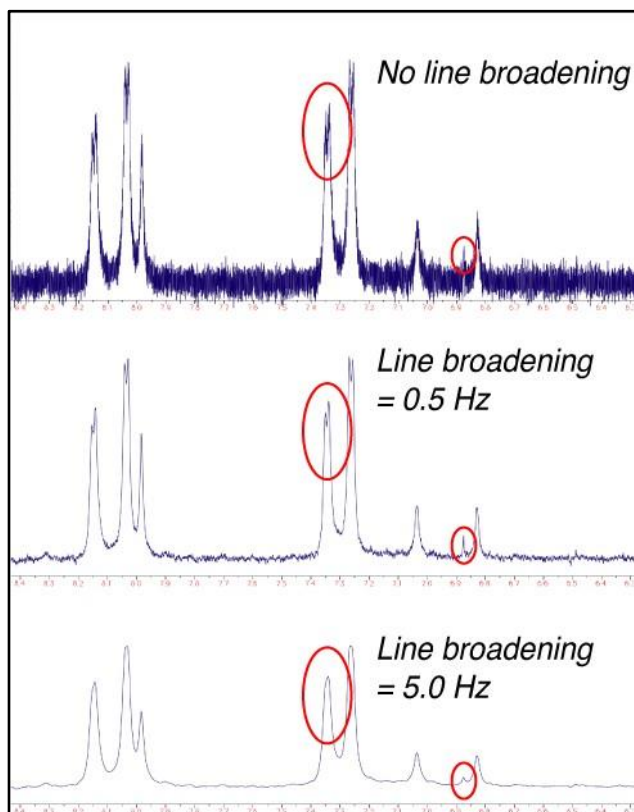
- The process of multiplying a signal by a mathematical weighting function is also known as “**apodization.**”

- Both Bruker and Varian apply weighting functions by default. If you wish to see your spectrum without weighting of the FID, you must deliberately change the weighting parameters.

- Essentially, one is multiplying the signal at the beginning of the FID, where the signal has not decayed significantly, by numbers close to 1, and multiplying the signal at the end of the FID, which has much less signal, by numbers close to zero. By emphasizing the region of the FID with more signal and deemphasizing the region with more noise, one thus improves S/N.

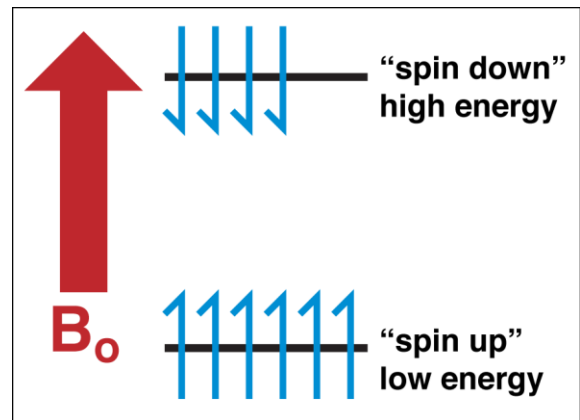
- The tradeoff in this multiplication is that all lines in the spectrum get broader. With an exponential decay weighting function, the degree of weighting is described by how much the function broadens the lines. E.g., Varian's 1D proton spectrum parameters usually default to a “0.5 Hz” line-broadening.

- If you wish to know a peak's true linewidth, you must FT your FID with NO weighting applied.



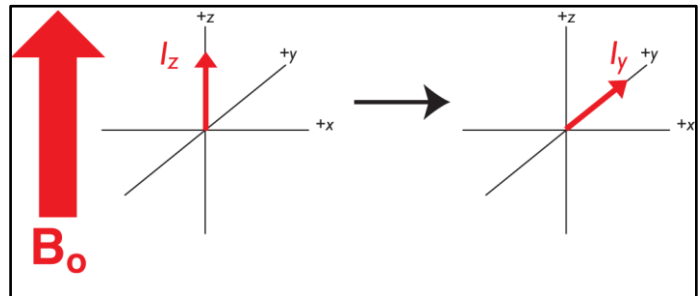
The “two-state” model of NMR

- The most basic description of NMR theory holds that placing nuclei in a magnetic field makes the nuclear spins align along the direction of the main magnetic field, B_0 . Spins align either “with the field” (“**spin up**”), a state which has lower energy, and “against the field” (“**spin down**”), a state which has higher energy – thus, “**two states**”.
- To get signals from our sample when it’s in the magnet, we shine light at it to promote spins from the low-energy state to the high-energy state. The light must have the same energy as the difference in energy levels, and in NMR this corresponds to radio-frequency light.
- After the RF irradiation is turned off, the spins relax back to their equilibrium state and emit RF light in the process. These are the signals we detect.



Vector model

- The “**vector model**” is a more nuanced description of NMR that includes three spatial dimensions to describe the orientation of nuclear spins. Here, we add up all the nuclear spin vectors with the same frequency in the sample, and treat the sum as a net magnetic moment.
- At equilibrium the net magnetic moment aligns along the **+Z axis**, in the same direction as the main magnetic field, B_0 .
- Application of RF light to the sample tips the magnetization away from +z, giving it a component in the XY-plane.
- All the signal we detect comes from magnetization in the XY-plane. This works because magnetization in the XY-plane will **precess** around B_0 like a gyroscope, and the oscillating variation in XY-magnetization induces a current in the detector that we amplify into a useful signal.
- Tipping the magnetization from +Z by 90° into the XY-plane with a single pulse yields signal with the greatest intensity. This is referred to as a “ **90° pulse**”.
- Continuing to apply RF light after the magnetization has been tipped 90° results in reduced signal. Tipping by 180° places magnetization along the $-Z$ axis, which has no XY-component, and provides no signal at all.

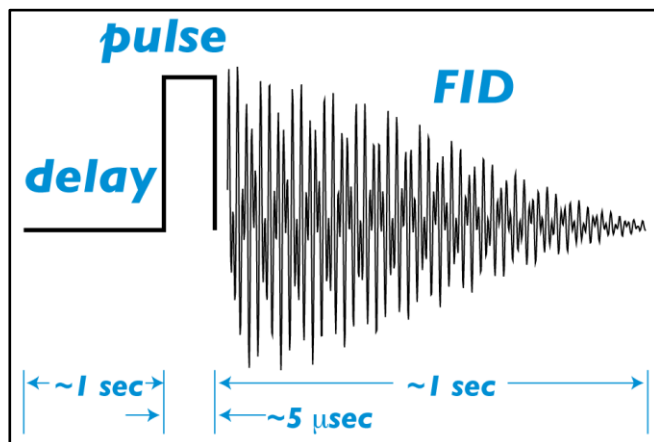


Relaxation

- After excitation, the nuclear magnetization begins to return to equilibrium. This process typically takes 0.2 to 5.0 seconds, and is called “**relaxation**”.
- In the two-state model, the excess high-energy “down” spins return to the “up” state, giving off RF light.
- In the vector model, net magnetization gradually returns to the +Z-axis, and the magnitude of the component in the XY-plane decays to zero exponentially.
- There are two major types of relaxation, referred to as “ **T_1** ” and “ **T_2** ”, or “spin-lattice” and “spin-spin” relaxation. Relaxation that restores magnetization along the +Z axis is called “ **T_1 relaxation**”, and is the type we must pay more attention to on a day-to-day basis. “ T_2 relaxation” refers to the decay of signal in the XY plane due to processes that reduce the additivity of individual spins to produce a strong net magnetization.

Pulse-and-Acquire Experiment

- Modern NMR spectrometers all excite nuclei by applying **pulses** of RF light and quickly follow up by detecting the resulting signal.
- By using short pulses of light, we can excite a broad range of frequencies, the breadth of the range being inversely dependent on the length of the pulse. We normally don't need to think about this for ^1H spectra, but ^{13}C spectra are somewhat affected, where the signals at the edges of the spectrum don't get excited efficiently. Some nuclei, like ^{195}Pt , have potential chemical shift ranges much greater than that of the broadest excitation we can manage, so it's important to know approximately where to expect a ^{195}Pt signal when setting up data collection.
- For simple 1D experiments, we often describe the excitation in terms of tip angle. A "45° pulse" is typical, indicating that, in the vector model, the net magnetization has been tipped 45° away from the +Z-axis. A **90° pulse** provides maximum excitation because with this tip angle, the net magnetization presents its maximum projection in the X-Y plane.



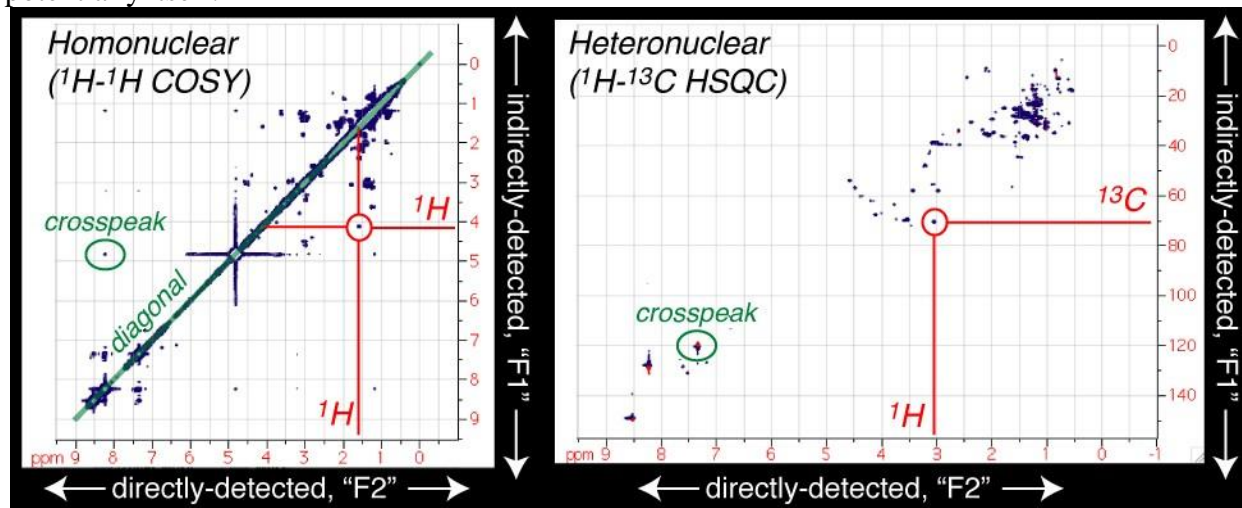
- With almost all NMR experiments, we include a delay period prior to the first pulse of an experiment that allows spins to return to equilibrium before being excited again. This is referred to as a "**relaxation delay**", and is always specified by the parameter "d1".
- To get the most accurate intensities and integrals in a spectrum, one should use a d1 delay longer than approximately five times the longest T_1 value in the sample.
- The program including delays, pulses, and acquisition period that is executed by the hardware is called a "**pulse sequence**", or "**pulse program**." Different kinds of chemical information require different pulse sequences, so the user must choose the sequence appropriate for the task at hand.

Steady-state/"dummy" scans

- The d1 delay in the pulse sequence is usually too short to allow for full relaxation between scans, and this haste gives rise to artifacts in the spectra. Most of the problems result from spins that are not excited to the same direction from one scan to another. After a number of scans, however, the net magnetization usually reaches an imperfect but consistent "steady-state" that adds relatively well from scan to scan.
- Many artifacts can be reduced by preceding the experiment with a number of experimental scans/transients that "pulse-and-acquire" on the sample, but do not record any data. These are called "**steady state scans**" (ss) on Varian/Agilent systems, and "**dummy scans**" (DS) on Bruker systems.
- One typically applies 4 to 16 steady state scans when aiming for high-quality data.

2D NMR – very brief

- Most 1D NMR data contain information that describes correlation between nuclear spins. For example, a 1D ^1H spectrum may exhibit many signals that are “split” due to ^1H - ^1H scalar coupling (one type of correlation). In a 1D ^{13}C spectrum acquired with ^1H decoupling off, one observes ^{13}C resonances split by resonances from the ^1H atoms bound to them.
- In 2D NMR spectra, one observes correlations between peaks directly. The x-axis frequency of a 2D peak, which resembles a mountain on a contour map, represents the frequency of one nucleus, and the y-axis frequency of the peak represents the frequency of a nucleus it is interacting with, potentially itself.



- In “homonuclear” 2D NMR, the two axes arise from the same nuclear type (virtually always ^1H). One observes a “diagonal” in these spectra that corresponds to nuclei interacting with themselves – the peaks on the diagonal have the same frequency in both dimensions. The off-diagonal “crosspeaks” represent interactions between different nuclei. The nature of the interaction depends on the pulse sequence applied. Here are the most common homonuclear 2D NMR experiments and their applications:

Experiment	What the name stands for	What the experiment does
COSY	Correlation Spectroscopy	Shows crosspeaks between ^1H signals are scalar-coupled to one another
TOCSY	Total Correlation Spectroscopy	Shows crosspeaks between all ^1H signals in a system of signals that are scalar-coupled to one or more of the system’s members.
NOESY	Nuclear Overhauser Effect Spectroscopy	Shows crosspeaks between signals from ^1H atoms that are within 5 Å of one another, regardless of scalar coupling
ROESY	Rotating-frame nOESY	Similar to NOESY, but provides more intensity for molecules with MW 800-1500.

• In “heteronuclear” 2D NMR, the two axes arise from different types of nuclei. Usually one axis is ^1H and the other axis is for a heteronucleus such as ^{13}C , ^{15}N , or ^{31}P . There is no “diagonal” in a heteronuclear 2D spectrum. The crosspeaks arise from interaction between the nuclei, and the nature of the interaction depends on the pulse sequence used. Here are the most common heteronuclear 2D NMR experiments and their applications:

Experiment	What the name stands for	What the experiment does
HSQC	Heteronuclear Single Quantum Coherence	Shows crosspeaks between ^1H signals and heteronuclear atoms (^{13}C , ^{15}N , ^{31}P , and ^{29}Si , usually) that are scalar-coupled to one another via short contacts, usually one covalent bond
HMQC	Heteronuclear Multiple Quantum Coherence	Practically the same as HSQC, but uses fewer pulses, thus is more robust when not tuning a probe or calibrating pulse widths
HMBC	Heteronuclear Multiple Bond Coherence	Shows crosspeaks between ^1H signals and heteronuclear atoms (^{13}C , ^{15}N , ^{31}P , and ^{29}Si , usually) that are scalar-coupled to one another via long contacts, usually two to five covalent bonds separating them. One-bond contacts appear as a pair of peaks: two peaks in the ^1H dimension at the same ^{13}C frequency, centered on the ^1H frequency and separated by the ^1H - ^{13}C coupling constant.

- A 2D NMR dataset is collected as a series of 1D spectra. There is a delay period in each 2D pulse sequence that encodes the second dimension.
- In the first 1D spectrum, the delay is zero. In the next the delay is incremented by a fixed amount. In the third, it is incremented further. Once all the 1D spectra are acquired and Fourier transformed, yielding the “direct dimension” of the spectrum, the intensity of each peak can be seen to oscillate as a function of the incremented delay time. Then this set of intensity-versus-time points is Fourier transformed to yield the “indirect dimension”.
- The resolution of the indirect dimension is therefore set by the number of “increments”. The number of increments is a very important parameter in collecting a 2D spectrum. It is usually set to between 32 and 256, depending on how broad you desire the peaks to be. More increments = skinnier peaks in the indirect dimension, but this takes more time.
- A 2D spectrum with 64 increments takes twice as long to acquire as one with 32.
- The “number of increments” should not be confused with the “number of transients”, which is the number of scans added to one another to yield a single FID.

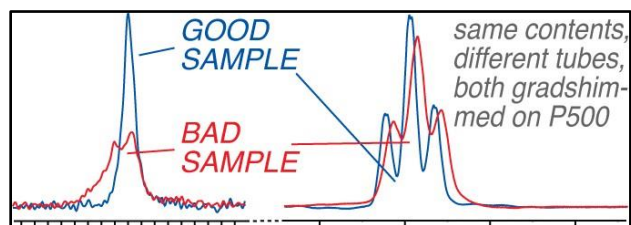
Further details for NMR general knowledge can be found by reading chapter 1-3 of:

Organic Structure Determination Using 2-D NMR Spectroscopy by Jeffrey H. Simpson
<http://www.sciencedirect.com.turing.library.northwestern.edu/science/book/9780123849700>

Sample preparation

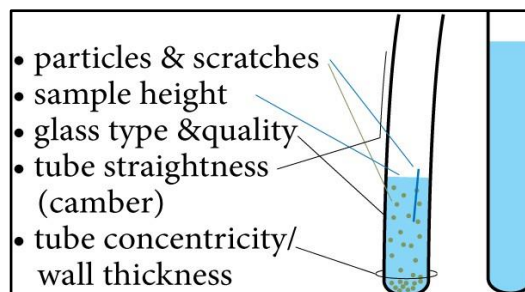
Sample quality affects spectra

- Low NMR sample quality increases every peak's width, making it hard to resolve small couplings and frequency differences.



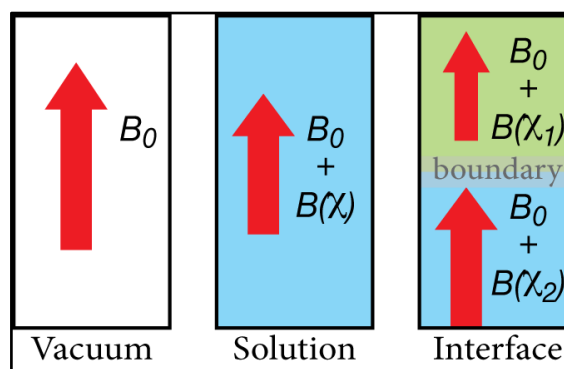
Factors affecting sample quality

- NMR spectra are strongly affected by both the sample contents and the NMR tube. These factors include:



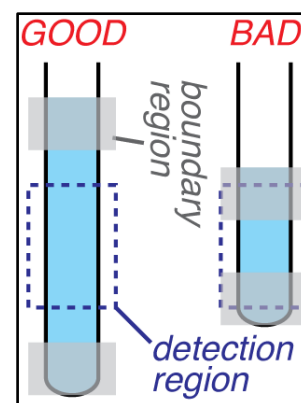
Boundaries Between Materials Cause Problems

- We shim to make the field more uniform across the sample, but sample factors can limit shimming's effectiveness.
- Every material gets magnetized in a magnetic field, and the strength of its response is its magnetic susceptibility (χ). Near the boundary between materials of different χ , the magnetic field changes strength, so nuclear resonance frequencies near boundaries are different than those in the bulk. To ensure field uniformity, one must minimize such interfaces.

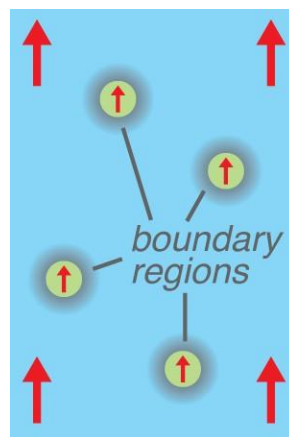


Sample Height

- To keep the solvent/air boundary far from the sensitive region of the probe, the sample must be fairly tall. In a 5mm tube, the sample should be ≥ 0.7 mL for best results. Use of 0.6 mL is usually acceptable, but samples less than 0.5 mL often present shimming problems.
- Many practicing chemists employ the "three-finger rule" for routine samples: they add their analyte to the NMR tube, then add enough solvent so the sample height is approximately the width of three fingers.

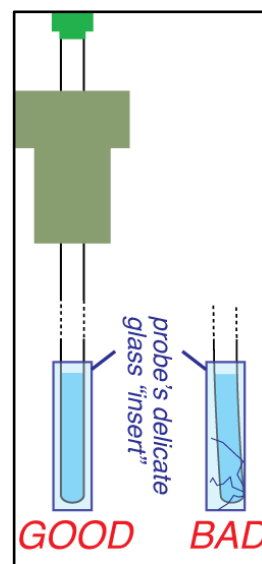


- Solid particles in a liquid sample present huge areas of solid/liquid interface that disrupt B_0 homogeneity. To get good data quality, particles must be removed, usually by filtering.



NMR Tube Straightness (Camber) and Concentricity

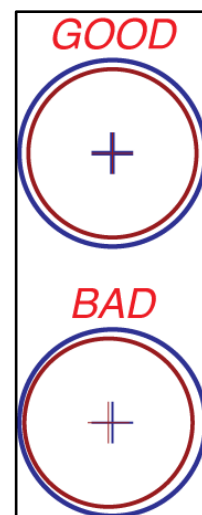
- A tube is held at the top, but the sample must be aligned precisely in the center of the probe for maximum performance. Tubes must therefore be precisely straight (i.e., low “**camber**”). Baking tubes can bend them, which risks breaking them and the probe when inserted or spun.



- The centers of the inner and outer surfaces of the tube may not coincide well. Poor positioning of the sample in the coil and nonuniformity of the glass wall thickness create shimming problems. “**Concentricity**” is the measure of the difference between the two circles’ centers. Tubes with higher “MHz” ratings have better concentricity.

NMR Tube Cleaning

- Rinse 1x with sample’s solvent
- Rinse 5-10x with non-chlorinated solvent, e.g. acetone &/or H_2O *
- Rinse 1x with D_2O **
- Store inverted on a lab wipe
- Dry with stream of N_2
- If absolutely necessary, dry flat in oven $\leq 125\text{ }^\circ\text{C}$, $\leq 45\text{ min}$ ***
- **NEVER STORE IN AN OVEN!**



*reduces solvent disposal hazard and cost

**replaces chemisorbed water on the glass with D_2O , reducing the residual water peak

***Baking tubes $>125\text{ }^\circ\text{C}$ upright in an Erlenmeyer flask can bend them, which **MAY DESTROY THE TUBE AND NMR PROBE!!**

IMSERC NMR Policies

- Users may only use spectrometers for which they are trained.
 - Except for use of spectrometers with automatic sample changers, all users must log in to the FOM calendar to activate the spectrometer's monitor and keep track of time used.
 - The user who is listed on the FOM as having reserved time must be using the instrument during that time; while collaborations are permitted, users are prohibited from reserving time for others.
 - All samples must be labeled so their owners can be clearly identified.
 - Samples may not be spun, except in unusual circumstances approved in person by facility staff.
 - When finished with manually-operated spectrometers, users must insert a "lock standard" in the magnet and make sure that the system is locked on it. The lock standard is a flame-sealed sample of CDCl₃, and is identified by having a painted red top.
 - Users working at temperatures other than 25 °C are responsible for making sure the instrument is re-equilibrated to 25 °C before they log out. This may take up to three hours, and it is important to factor in reequilibration time when making reservations.
 - On spectrometer computers, no web browsing or USB thumb/flash/jump drives are allowed; this reduces the risk of computer virus infection.
- Day/night policies, please see: <http://imserc.facilities.northwestern.edu/nmr/nmr-reservation-policy/>

IMSERC NMR Spectrometers

Different IMSERC NMR spectrometers are aimed at satisfying different experimental needs. For general principles on how to choose the NMR instrument for your specific experiment, please see: <http://imserc.facilities.northwestern.edu/nmr-chooser/>

Troubleshooting

IMSERC users are encouraged to troubleshoot and fix their own everyday problems. Most software problems can be resolved by closing and restarting the software. Hardware problems can often be resolved by rebooting the console. Please refer to the IMSERC bug report page to find troubleshooting solutions: <http://imserc.facilities.northwestern.edu/bugissue-reporting/>. If you don't find the solution, please log your problem there and the NMR staff will be responding as quickly as possible.