The instruction for obtaining a user account for the Inova500 spectrometer is outlined below. The course consists of two classes given by Dr. Joseph Vaughn. It is generally given twice per year, once in the early spring semester and again in the summer semester. Each class lasts about 2 – 3 hours. There is a required qualifying performance test after the first presentation (Advanced Mercury 300 MHz NMR Instruction Course), which must be completed using the Mercury 300 by each student within two weeks. After the second class (INTRODUCTORY NMR INSTRUCTION) there is a series of instructions given at the Inova 500 console for two students at a time. This lasts from 3 to 5 days. At the end of the console instruction the student must take a written multiple choice quiz. If the quiz is passed there is a one hour performance test given at the console.

Advanced Mercury 300 MHz NMR Instruction Course
(The Pre-Inova 500 MHz NMR Sessions)

by Joseph Vaughn

Course Description and Objectives
This course is designed specifically for Mercury 300 MHz NMR spectrometer users who will subsequently be instructed on proper use of the Inova 500 MHz NMR spectrometer. The course aims to instill in the student a better understanding of the theoretical aspects and pertinent parameters of certain NMR experiments, since knowledge of basic NMR theory and application is required to gain access to the Inova 500 MHz NMR.

Prerequisites
Students should be proficient users of the Mercury 300 MHz NMR (or some other equivalent spectrometer). Experience in running 13C experiments (including the DEPT experiment), decoupling and nOe experiments is helpful.

Course Content
The course will involve the setting-up of two calibration tests, 90° pulse measurement (or pulse width calibration) and T₁ measurement (inversion-recovery experiment).

One short-course presentation will be given by Dr. Joseph Vaughn to provide the background for completing the assignments described above. After completing Tests A and B below, Dr. Vaughn will present a second short-course to provide adequate basic NMR theory and application background so that the student can learn to use the Inova 500 MHz spectrometer properly.

Course Outline
A. **Test:** 90° Pulse Measurement (Pulse Width Calibration)

**Sample:** any desired sample  

**References:** VNMR Guide to NMR Experiments  

B. **Test:** $T_1$ Determination (Inversion-Recovery)

**Sample:** any desired sample  

**References:** VNMR Systems Operation Manual  

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### PLOTTING SPECTRA FOR 90 DEGREE PULSE CALIBRATION AND $T_1$ INVERSION RECOVERY MEASUREMENT

**Mac G5 commands:**

- `getspec` respond with appropriate number used to acquire data, e.g. gs1.
- `f full dc` no aph
- `dssa` displays a vertically stacked array
- `dssh` displays a horizontally stacked array
- `dssa dssh` displays a horizontally and vertically stacked array

To plot spectra enter: `pl(1,x) pscale page`. Type `pap page` to print acquisition parameters.

At any time if you want a full spectrum, you may type `ds(1) f full`.

If spectra overlap, you may plot them in sections by:

- `dssa(1,x) or dssa(1,x) dssh(1,x)`  
- `pl(1,x) ptxt pscale page`
- `dssa(x+1,n) or dssa(x+1,n) dssh(x+1,n)`  
- `pl(x+1,n) ptxt pscale page`
pap page plots acquisition parameters on a separate page.

In order to plot spectra stacked diagonally and full scale on the page enter:

- `f full ds` This displays a full spectrum.
- `dssh` This displays a horizontal array of spectra.
- `wc?` This should show a value of ~40.
- `wc=200` This sets the width of the plot to 200 mm.
- `dssa` This will display spectra stacked diagonally on the screen.
- `pl('all') pscale pap page` This will plot a stacked diagonal array of spectra a chemical shift scale, parameters as shown in the handout.
INTRODUCTORY NMR INSTRUCTION

by

Dr. JOSEPH VAUGHN
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   1) Sample preparation
   2) Shimming, resolution, lineshape
   3) Signal-to-noise
   4) Integration
   5) Proton homonuclear decoupling

V. Test
I. Introduction

This short course is available for anyone who is interested in obtaining introductory level knowledge of NMR spectroscopy and spectrometer design. It is required for those who need to obtain an account for using the Inova 500 NMR spectrometer. This handout (and class) is intended to supplement the other handouts which instruct you as to how to obtain an NMR spectrum. Its purpose is to provide you with a rudimentary understanding of the basic principles of pulsed excitation FT-NMR beyond the acquisition by rote method provided in the earlier handouts. You will be given a written test as well as a performance exam before you are authorized to have an account on the spectrometer. You must be able to:

1) Pass a written exam (sans notes) taken from material in this handout, class presentations and console sessions.

2) Obtain a properly optimized proton spectrum with a linewidth at half height of ≤ 0.5 Hz (digital resolution ≤0.1 Hz and non-spinning lineshape ≤ 5/10).

A sample in CDCl$_3$ will be provided. From an expansion of the CHCl$_3$ peak show a measurement of the linewidth at half height and lineshape.

2) Integrate a proton spectrum.

3) Obtain a properly optimized proton spectrum with homonuclear decoupling.

4) Measure signal-to-noise (S/N) of a proton spectrum.

5) Obtain a properly optimized NOE difference spectrum without artifacts.

It is the goal of these classes to teach you how to operate the spectrometer properly and to provide you with the necessary knowledge and skills to be able to obtain routine proton spectra with optimum efficiency and quality.
II. An NMR Spectrometer

A modern NMR spectrometer is really two spectrometers; an observe unit capable of observing many nuclei and a lock unit, which usually observes only $^2$H. Each of these consists of a radio frequency (rf) transmitter and receiver. That's it. It is very similar to your home stereo receiver and your favorite radio station. The transmitter transmits a selected rf signal to the probe (where the sample resides) in the magnet, Figures 1, 2, 3. The probe is just a tuned antenna. The magnetic nuclear moments of the sample aligned with the external field at equilibrium are perturbed from equilibrium and the magnetization couples to the probe as they return to equilibrium. The induced radio frequency voltage change in the probe is amplified and then detected by the receiver.

Optimal use of such an instrument requires considerable familiarity and understanding of its hardware components and the software which controls them and of NMR spectroscopy.

After logging on to the computer and inserting the sample, it is typical to find a $^2$H lock signal and "lock" the spectrometer. If a $^2$H containing solvent is used, the lock unit will observe the signal and lock the spectrometer provided certain conditions are met.

1) The Z0 current must be adjusted to the proper value to observe and center the $^2$H resonance for a given solvent. That is, the field is changed to meet the resonance condition for the solvent, e.g. CDCl$_3$, C$_6$D$_6$, D$_2$O, etc. For a given solvent, the Z0 of the Inova500 does not change very rapidly. However, it does change due to magnet drift. Magnet drift and other short term perturbations (e.g. movement of a metal cart or change in temperature, application of a transmitter pulse) are reasons it is necessary to lock the spectrometer.

2) Once Z0 is properly adjusted, the rf power level must be adjusted to give an adequate signal without saturation. If too much power is applied, the signal gradually begins to grow smaller and even disappear. This is because the population difference between the energy levels is reduced.

3) Next, the lock gain should be adjusted to provide an adequately strong signal for lock.

4) Then if the magnet field homogeneity (shimming) is adequate the spectrometer can be locked.

5) Finally, the lock phase needs to be adjusted to provide maximum lock signal intensity.

Very simply described a lock unit is just a continuous wave (CW) spectrometer which monitors and maintains zero voltage. That is: A CW sweep of the $^2$H signal produces an NMR signal consisting of an absorption and a dispersive component. The procedure
described above centers the lock on the dispersive signal. When the spectrometer is locked it monitors and maintains zero voltage at the center of the dispersive signal. For example if the field drifts upfield the signal produces a $+\Delta V$ and the lock responds by producing an equal $-\Delta V$, thus holding the field constant, Figure 4.

6) At this time the homogeneity should be adjusted with the interactive shim modes to increase the lock signal to its maximum. This usually corresponds to optimal conditions for the best lineshape, resolution and sensitivity. In homogeneity adjustment (shimming), it is usually necessary to adjust only Z1, Z2, Z3 and sometimes Z4 while spinning the sample. If spinning sidebands are severe ($\geq1\%$) or the non-spinning lineshape/resolution are poor, then it is necessary to adjust the non-spinning shims; X, Y, X2, Y2, ZX, ZY, XY, X2Y2. All of these shims are interactive. So - shimming is by necessity an iterative procedure. The skills necessary to perform all of these procedures will require some time to develop and will improve with experience.

There are several published articles describing shimming techniques and homogeneity evaluation based on lineshape, sensitivity (signal/noise, S/N), resolution and FID.
shape. One recent article, which I recommend is "SHIMMING PROCEDURES" by W.W. Conover and V. W. Miner.

III. Basics of FTNMR

The purpose of this section is to provide enough information to enable you to set up an FTNMR experiment properly and avoid the most common errors. It will cover the most important parameters in experimental setup. The parameters are:

- Spectrometer Frequency: \texttt{sfrq}
- Transmitter Power: \texttt{tpwr}
- Pulse Width: \texttt{pw}
- Acquisition Time: \texttt{at}
- Number of Data Points: \texttt{np}
- Sweep Width: \texttt{sw}
- Relaxation Delay: \texttt{d1}
- Transmitter offset frequency: \texttt{tof}

A one pulse NMR experiment can be represented as shown in Figure 5.

![Figure 5](image-url)

In this experiment one radio frequency (RF) pulse is applied to the sample. Some nuclei in the sample are excited at this characteristic resonance frequency and they respond by radiating RF energy during the acquisition time. This resonance frequency is dependent upon the strength of the external magnetic field and the nucleus you wish to observe. The spectrometer frequency of protons on the INOVA500 is approximately 499.843 MHz. This defines the center of the spectrum (\texttt{sfrq}) you acquire.

The radio frequency pulse is applied for a chosen duration, typically a few \(\mu\text{sec}\), usually described in terms of a flip angle in degrees. As shown in Figure 6, this is because of its effect on the bulk magnetization vector of the nuclear spins in the sample.
A 90° pulse width is the amount of time necessary to apply the pulse of rf energy in order to flip the bulk magnetization (\(M_z\)) away from its equilibrium position along the Z axis (i.e. aligned along the external magnetic field) and into the X-Y plane, i.e. the condition shown in Figure 6b. The 90° pulse width is about 10 µs on the VXR500 spectrometer. This value varies from one spectrometer and/or probe to another. It is also dependent upon the sample, solvent, nucleus, etc. For a given sample and probe and application of a rf pulse of constant power the following relationship exists.

\[
\varnothing = f(\gamma_1 \beta, pW)
\]

After applying a rf pulse to the sample, the nuclei which resonate at that frequency respond to this excitation by emitting rf energy at their resonant frequency. This is detected by the NMR probe and can be displayed, as shown in Figure 5, as a decaying cos wave, i.e. a cos function whose intensity decays as a function of time. It is called a Free Induction Decay or FID. The time required to record the FID is called the acquisition time(at). The emitted rf response is recorded and digitized by the console computer. That is, the FID is stored as a series of points by the computer. The number of points used to store the FID is called np. np can be changed explicitly by typing np=XXX. Of course, this will change the acquisition time. For a constant sw, larger np will produce higher digital resolution (see below).

The FID is a time domain spectrum, which can be Fourier transformed (FT) to produce a frequency domain spectrum. The frequency domain spectrum has several important parameters associated with it, e.g., the spectrometer frequency, sfrq 300.1, 499.843, etc.; the sweep width, sw; and the transmitter offset frequency, tof, Figure 7.
In order to insure the minimum number of points are used to properly digitize the spectrum, the following relationships exist:

\[
\text{at} = \frac{\text{np}}{2\text{sw}} \quad \text{dres} = \frac{1}{\text{at}} = \frac{2\text{sw}}{\text{np}}
\]

Equation 1 \hspace{2cm} \text{Equation 2}

The digital resolution (dres) is in Hz/point, and should normally be less than one half the peak width at half height. This ensures that the peak is defined by at least 3 data points and reduces peak truncation, Figure 8.

**Example:** The Mercury300 sfrq is 300.1 MHz, if your \text{sw} = 3600 \text{ Hz} (12 \text{ ppm}) and your required dres is 0.25 Hz/pt, from equation 2 the np required is:

\[
np = \frac{2\text{sw}}{\text{dres}} = 28800
\]

Because the computer processes in powers of 2, the next higher power of 2 should be used, 32768 points (easily entered as np=1024*32). The importance of insuring adequate digital resolution is illustrated in Figure 8.
Figure 8. The importance of adequate digital resolution is illustrated. Much important detail is lost if insufficient np is used. Top: np=32768, Bottom: np=4096

Once you set sw the Inova500 software will keep sw constant and allow changes in at which produce changes in np and vice versa.

The total time (TT) for an acquisition is the sum of at, PW and the recycle delay (d1). A recycle delay is necessary in order to insure complete relaxation of the nuclear spins between scans; i.e. total recovery of $M_z$ to equilibrium conditions. The total time is:

$$TT = PW + at + d1$$

PW can be ignored because it is very short (μsec) compared to at and d1, therefore:

$$TT = at + d1$$

Incomplete relaxation causes loss of signal and is one of the causes of inaccurate integrations. The relaxation time along the z axis for a nucleus is called T1 (longitudinal relaxation time) and normally, when a 90° pulse is used for excitation, a TT of 3xT1 is necessary for complete relaxation. For example, if T1 is 600 msec, then the TT should be 1.8 s. A longer or shorter pulse may require proportionally more or less TT.
INOVA 500 INSTRUCTION OUTLINE

AND

EXPECTED PERFORMANCE CAPABILITIES

FOR

OBTAINING AN ACCOUNT

by Dr. Joseph Vaughn

1. dg parameter set and dps:
   Tour and explain

2. Lock:
   Tour and explain lock display features.
   Z0, POWER, GAIN, PHASE

3. Shim:
   Tour and explain shim display features.
   Spinning modes (Z1 – Z7)
   Non-Spinning modes (X1 - ZX2)

4. Shim Methods:
   How to shim handout, explain interactive behaviour of shim
   modes

5. Acquisition and processing:
   ga, go, ft, wft

6. Foldover:
   Provide example and explain.
   Relevant parameters considerations; sfrq, sw, tof, solvent,
   phase(rp, lp), Nyquist frequency, np, dres, lineshape, res,
   resonance clipping, fn

7. ADC overflow:
   Provide example and explain.
   Relevant parameters and considerations; 16 bit ADCs,
   adc, clipping, ss, pw, gain, tpwr, attenuation, relaxation
8. Truncation:
Provide example and explain.
Relevant parameters and considerations; FID (Free Induction Decay) time domain spectrum, at, noise, sinc wiggles, dres, wti, fn

9. Quadrature Detection:
Provide example and explain.
Quadrature images, quadrature channel imbalance, real and imaginary FIDs, phase cycling (CYCLOPS),

10. Decoupling:
Provide example and explain:
Relevant parameters and considerations; dn, dof, dm, dmm, dmf, dpwr (CRITICAL CARE NEEDED), homo, dps

11. Difference NOE
Provide example and explain:
Relevant parameters and considerations; seqfil='cycledof', Cycledof macro, nt, dpwr, satfreq, control, intsub, cycle, pattern, splitting, sn, spt

12. 2D COSY, NOESY, HSQC, HMBC
Provide example and explain for appropriate users.

In order to qualify for an account to use the Inova 500, the student must pass a 15 question multiple choice quiz and pass a performance test. Performance evaluation will be based upon the student’s ability to efficiently setup and obtain an optimal 1D spectrum, decoupled spectra of two or more decoupled frequencies, and difference NOE spectra of two or more irradiated frequencies. These should be properly obtained based upon the instructions provided throughout the course with due consideration for all of the above parameters, i.e. flat baseline, no first order phase correction, maximum signal intensity, reduced or eliminated artifacts such as quadrature images and selective population transfer, etc.