

NMR FACILITY NEWSLETTER

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Upcoming Workshops

An Introductory NMR Workshop will be offered on May 20 and 21, those interested can sign up through the NMR Facility website. An Advanced Workshop will be offered later in the summer.

In addition, if any users of the NMR facility want to learn about specific NMR experiments or techniques (ie NOESY or DOSY etc) I would be willing to do a presentation and training session upon request.

Changes to NMR Facility Network Practices

Three out of four of the department's solution NMRs run Topspin version 1 or 2 which run on the Windows XP operating system. XP is no longer supported and will not run the Universities current virus and malware protection software. In order to protect our instruments and user's data our departmental IT person (and Employee of the Year) Joe Lichaa is installing an updated firewall on the NMR computers. In addition there is the possibility of viruses bypassing the firewall through use of USB drives by individuals backing up their data so all USB ports on the NMR computers are going to be deactivated. The computers will be set up to allow individuals to FTP their data sets back to designated computers or lab servers(if available) this change will be implemented in the near future.

On a personal note I would like to thank Stas Veinberg of the Schurko Lab for his excellent work in running the NMR Facility this Winter while I stayed home and watched Judge Judy reruns.

Newly Available Experiments


When the 500 MHz NMR was updated to the current generation of Bruker hardware (Avance III) and the latest version of the Bruker Software (Topspin 3) was installed last year, some new capabilities were added for solution NMR that many users are not aware of, I will list a few of them here. If you are interested in any of them please come and speak to me.

1) **Non-Uniform Sampling (NUS)**

Topspin 3, allows for much faster acquisition of 2 dimensional spectra such as the HMQC and HMBC experiments. This is possible due the implementation of Non-Uniform Sampling (NUS) data acquisition and processing. Data acquired using NUS can achieve the same resolution as a standard 2D experiment in about 1/3 to 1/4 the time than was required previously. In practice, this means that a 2D HMQC with good resolution can be acquired in 5-10 minutes and an HMBC in 20-40 minutes (for a fairly concentrated sample). In the Advanced Techniques section of this newsletter I will give more details of 2D NMR data collection and the use of NUS.

2) **¹⁹F Heteronuclear Experiments**

Previously on the 500 MHz only ¹⁹F heteronuclear experiments that correlated ¹H-¹⁹F nuclei could be collected. Those experiments are still available and are routine to run, however, with the upgrade we can now also run experiments such as 2D HMQC, HMBC with nuclei combinations such as ¹⁹F-¹³C, ¹⁹F-³¹P and ¹⁹F-¹¹B. These additional sets of experiments do however require a probe change so you have to consult with the facility coordinator to arrange a



time before running them.

3) 1D ^{19}F HOESY

HOESY experiments are heteronuclear NOESY experiments where the interactions of interest are between ^1H 's and other types of nuclei and can be used to identify the position of heteronuclei relative to the ^1H s in a molecule. In our present setup on the 500 MHz we can run selective HOESY experiments for receptive nuclei such as ^{31}P and ^{19}F . At present the ^{19}F HOESY has been set up. In this experiment you would first run a 1D ^{19}F experiment and identify the frequencies of your ^{19}F signals. The frequency of the signal of interest could then be put into ^{19}F HOESY parameters and any ^1H 's close to that ^{19}F nucleus would produce signals in the spectrum, while ^1H 's farther away would not produce a signal.


4) Accurate Concentration Determination

The concentration of compounds in your NMR sample can now be measured on the 500 MHz using the ERECTIC pulse sequence parameter set (Electronic REference To access In vivo Concentrations). The "in vivo" refers to the fact that this technique was originally developed for Magnetic Resonance Imaging (MRI) instruments and now has become available to in standard NMR instruments. This experiment has been calibrated by the facility coordinator using a Bruker standard sample of known concentration and the integrated value of that peak has been stored electronically. The concentration of any sample can now be determined by running the same experiment and comparing with the stored value, the literature suggests that this should be accurate to within a few %. At present it is available for ^1H but upon request it may be

set up for other nuclei if a good reference sample is available.

5) ^1H decoupled 1D ^1H Spectra (Pure Shift)

Scalar (or J) coupling of nuclei often is a source of information in NMR spectra but also complicates spectra and reduces the signal to noise and resolution of spectra by splitting single resonance signals into multiple, smaller, overlapping peaks. This has led to the development of techniques that remove that splitting (termed decoupling). These decoupling techniques have come into common use in removing heteronuclear scalar couplings (ie. removing ^1H couplings to ^{13}C from ^{13}C spectra) however it has remained difficult for technical reasons to remove homonuclear scalar couplings from 1D ^1H spectra,. This means the coupling produced by ^1H 's located within 2 or 3 bonds of each other results in peaks being split into doublets, triplets, etc in most spectra. For simple molecules with only a few ^1H s this is not an issue however for NMR spectra with many ^1H s the spectrum can become crowded and the scalar couplings increases the complexity and overlap of the spectrum making interpretation difficult. Recently a new technique has been developed called Pure Shift NMR, this is essentially a new way of collecting the 1D data as a pseudo-2D experiment that can then be processed to produce a 1D homonuclear decoupled spectrum. Since this data is collected as a 2D it takes 10 to 20 times longer to get a decoupled spectrum than the regular coupled 1D but that is still just 10 to 20 minutes for most samples. The facility coordinator can show users how to run and process this experiment upon request.



6) Deuterium NMR

The 500 MHz has greatly simplified the acquisition of ^2H spectra. Previously ^2H NMR required rerouting of cables between the amplifier, preamp and probe. On the AvanceIII 500 MHz the lock channel's deuterium amplifier is used to pulse the sample so that no external recabling is necessary and the experiment can be run with no more difficulty than a ^1H 1D.

7) UDEFT (Uniform Driven Equilibrium Fourier Transform) 1D ^{13}C NMR

This experiment uses special pulses to increase the rate of return of nuclei to their equilibrium state through a series of special pulses. This driven equilibrium allows the shortening of the re-equilibration time between scans and therefore allowing the collection of more scans and more signal in a shorter time period. This is especially beneficial for quaternary nuclei that tend to have very long re-equilibration time and show low signal to noise values in the standard experiments.

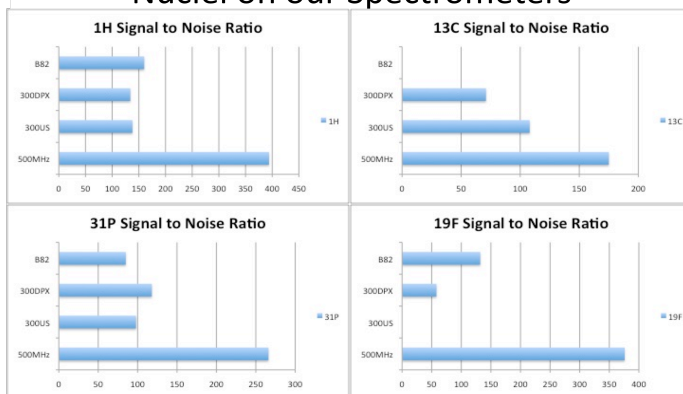
Issues in the NMR room

1) Which NMR should I use for a particular nucleus?

In Figure 1 I have plotted the signal to noise ratio for standard samples for ^1H , ^{13}C , ^{31}P and ^{19}F 1D experiments. In general, everything is better on the 500 MHz and there are some minor variations in sensitivities between the three 300 MHz instruments. Please note that it is not possible to run ^{19}F experiments on the 300US (blue) instrument. ^{19}F experiments are no longer to be run with on the older 300DPX (yellow) instrument due to the fragility of the tuning and

to the much lower sensitivity than the B82 300MHz and the 500 MHz for that nucleus.

Signal to Noise Ratios for Common Nuclei on our Spectrometers



2) Rm B82 EH, 300 MHz

The probe in the NMR in B82 has a history of tuning problems. At present the probe is fully operational and all 3 X channels (^{13}C , ^{31}P and ^{19}F) can now be efficiently tuned by the pneumatic, semi-automated switching method, users should never try to tune that instrument manually. The ^1H channel should not be tuned by anyone other than the Facility Coordinator due to the fragility of the apparatus. If the tuning for any nucleus appears to be off for this NMR or if problems are noted with any of the NMRs please contact the Facility Coordinator as soon as possible.

Contact: Ext 3997,

email: mrevingt@uwindsor.ca.

4) DPX300 Rm 394-5 EH, (Yellow)

For the time being no one is to tune the ^1H channel on this instrument because of the fragility of the tuning mechanism on the probe, if the tuning appears to far off please ask the Facility Manager to have a look at it. Tuning of nuclei other than ^1H is permitted as usual.

Advanced Techniques

2D NMR Experiments

In addition to the standard NMR experiments collected with one frequency dimension it is possible and relatively simple to collect NMR spectra with multiple frequency dimensions. The ability to collect these 2 or 3D data sets has allowed the development of NMR techniques that give information on interactions between various nuclei even for molecules even as large as proteins without overlap of signals. For most organic molecules 2 frequency dimensions are sufficient to prevent overlap and 2D NMR has become a useful everyday tool for chemists. Setting up 2D NMR experiments that produce good quality data is easy on modern spectrometers but an understanding of the basic ideas behind producing a 2D spectrum helps to avoid many common pitfalls. To discuss 2D NMR it will help to have a quick reminder about how 1D pulse sequences work. Figure 1 shows the standard 1D pulse and detect sequence with a 90° pulse followed by an observation period where the free induction decay (fid) signal is observed.

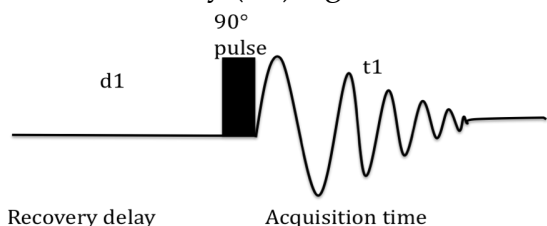


Figure 1: The standard 1D pulse sequence.

The fid is digitally sampled so that the stored data file is a set of intensity versus time points (often 16k to 64k points) that can then be processed by a Fourier Transform algorithm to give the familiar 1D intensity versus frequency NMR spectrum, Figure 2. The time between sampled points (dw or dwell time) depends on the spectral width observed and upon signaling theory.

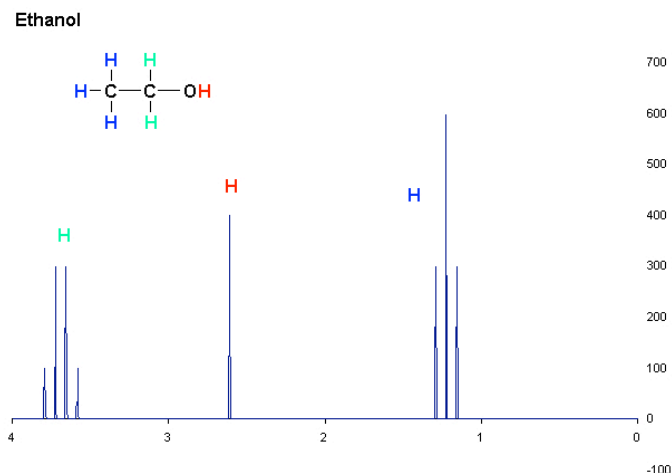


Figure 2: A 1D ^1H spectrum of ethanol

2D experiments use a more complex set of pulses and delays before the fid acquisition begins. Figure 3 shows the simplest 2D pulse sequence, the homonuclear COSY (COrrrelation Spectroscopy) experiment that shows interactions between nuclei that are J-coupled to each other.

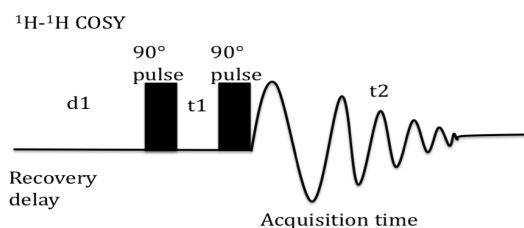


Figure 3: Pulse sequence for 2D COSY

This COSY pulse sequence consists of two 90° pulses (labeled P1 and P2) separated by a delay, t1. To collect a 2D data this sequence is run like the 1D experiment with t1 initially set to a small value, ie 4 usec and a 1D FID (the acquisition period for this fid is called t2) is collected after the second pulse. Then the t1 delay value is increased by a set amount, dw1, which is the dwell time of the second dimension and another 1D fid is collected. The second fid differs from the first because scalar coupling interactions are time dependent and evolve to a different degree depending on the length of t1. This increase of t1 by dw1 and collection of a 1D FID is repeated multiple times to form a 2D time domain data array.

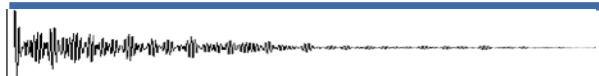


Figure 4 shows an array of unprocessed FIDs for a 2D experiment on the far left each collected with an incremented t_1 period.

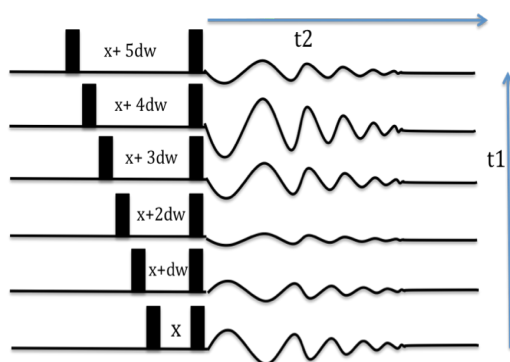


Figure 4: An array of fids collected with increasing t_1 periods

The horizontal t_2 dimension is then Fourier Transformed to give a frequency dimension F_2 (Figure 5).

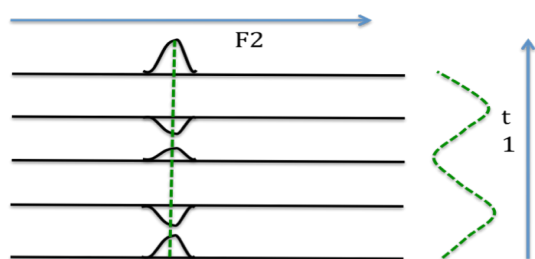
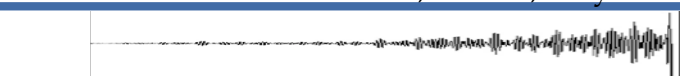


Figure 5: The array of fids after fourier transformation in the t_2 direction. The dotted green line at the right indicates variations in intensity of the peak in each fid.

The FID array is then a series of 1D spectra identical except for the peak intensities. If you trace one of the peaks through the set of F_2 spectra, as with the green dotted line in the F_2 - t_1 plot, you will see that the intensity of the peak oscillates like the signal in the 1D fid. When a large set of 1Ds (ie 256) has been collected there be enough values that trace out a digitized fid in the t_1 dimension that can be Fourier transformed to give a F_1 frequency spectrum. The resulting 2D spectrum can plotted as a contour map where the peaks appear at the



intersection of their chemical shifts on the F_1 and F_2 frequency axes (Figure 6).

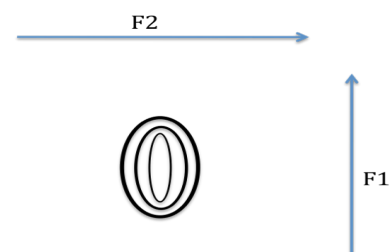


Figure 6 The fid array after fourier transformation in the t_1 dimension

An example ^1H - ^1H COSY spectrum (Figure 7) shows a diagonal line of large peaks that arise from auto-correlation of the nuclei being observed and many smaller off-diagonal cross peaks that arise from scalar coupling interactions between different ^1H s. 2D spectra are therefore actually collected as a series of 1Ds (usually 128-256 1D FIDs). This set of FIDs is then saved as a 2D matrix and processed to give the 2D spectral map. These spectra would take approximately 128 to 256 times as long to collect as a 1D spectrum. ^1H 1D spectra usually take 30-45 seconds to acquire so ^1H - ^1H 2D spectra commonly require 1-2 hours to collect.

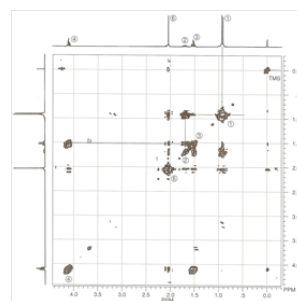
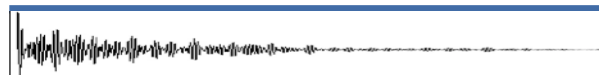



Figure 7: 2D ^1H - ^1H COSY spectrum

^1H - ^{13}C 2D spectra using 128 fids, that give peaks at the chemical shifts of the ^1H and ^{13}C nuclei that are attached by a single bond, take about as long to collect as a single, standard ^{13}C 1D because the 2D experiment takes advantage of better NMR characteristics of the ^1H to increase the sensitivity for ^{13}C . Therefore dilute samples





take overnight and more concentrated samples can produce ^{13}C 1D or ^1H - ^{13}C 2D data sets in an hour or so.

The time limiting factor for 2D NMR is the number of 1D fids that need to be collected to give sufficient resolution after Fourier transformation in the indirectly detected, t1 dimension. In the directly detected t2 dimension several thousand points can be collected in a second so that getting sufficient digital resolution in that dimension is not a problem. For the indirectly detected t1 dimension each point requires the collection of a separate 1D spectrum, which can take from 30 seconds to a couple of minutes depending on the number of scans required to give good signal to noise data. Good resolution often requires the acquisition of several hundred fids for a total experiment time of several hours. For this reason 2D experiments are often too lengthy to be run during the more heavily used daytime periods and are less used in our facility than they should be given the amount of information that can be derived from them. Therefore there has been a strong push by NMR spectroscopists to find ways of shortening the acquisition time for multidimensional experiments.


Linear Prediction of the FID


Linear prediction (lp) is a computational tool that has been available for many years which allows a researcher to acquire a FID consisting of X points and then to mathematically extrapolate the data to a total of 2X points before FT. Linear prediction, by extending the FID by a factor of up to 2 fold ideally allows a decrease in data collection time by a factor of up to 2 without sacrificing resolution. Linear prediction increases computer processing time for data roughly by an order of

magnitude, however, on the current generation of computers this is a just a few seconds for a 2D data set. The upper limit for accurate results using lp is a 2X increase in points and to be useful the signal to noise ratio of the data must be very good to prevent spectral noise from contributing enough signal to produce artifacts, so in practice it requires the 2 fold reduction in data collection is often not achieved. Linear prediction does allow a reduction in data acquisition time but often still leave 2D's too lengthy.

Non-Uniform Sampling

As a way of more substantially shortening data acquisition times many researchers have proposed alternate methods to the Fourier Transform of converting time domain NMR data to frequency spectra. The Fourier Transform (FT) is a very robust, computationally efficient algorithm and therefore was well matched to slower, older computers, however, the FT requires a large number of sequential time points which are very time consuming to collect. Several useful alternatives to the Fourier Transform exist that have the advantage of requiring only a random, non-sequential subset of the t1 data points. The use of these approaches can reduce the data collection time by 70-80% in some cases. In the literature many non-uniform sampling (NUS) techniques have been tested, they include; maximum, entropy processing, Hadamard transforms, and multi-dimensional decomposition (MDD). In practice hybrid processing schemes are usually used where the standard Fourier Transform is used to convert F2 (the directly detected dimension) while the indirectly detected dimensions are processed using the NUS approach. All of the NUS techniques require much more computational time than the Fourier Transform, however, on modern computers 2D experiments can be Fourier Transformed in a few seconds while





using the MDD NUS algorithm, that is a part of TOPSPIN3 takes just 3-4 minutes. Given that the NUS data collection approach can save several hours of data collection time for less sensitive 2D experiments this extra processing time is negligible. The limitation of the NUS approaches currently in use is that they don't handle large differences in peak intensity well. For example, NUS processing of homonuclear 2D experiments like the NOESY and COSY will reproduce the large diagonal peaks well but will do a poor job with the much smaller cross peaks that are the useful information produced by the experiment. NUS does work very well for most heteronuclear 2D experiments such as the HMQC and HMBC since most peaks are within the same order of magnitude. Running and processing using the NUS techniques is simple to do on the 500 MHz NMR that uses TOPSPIN3.

