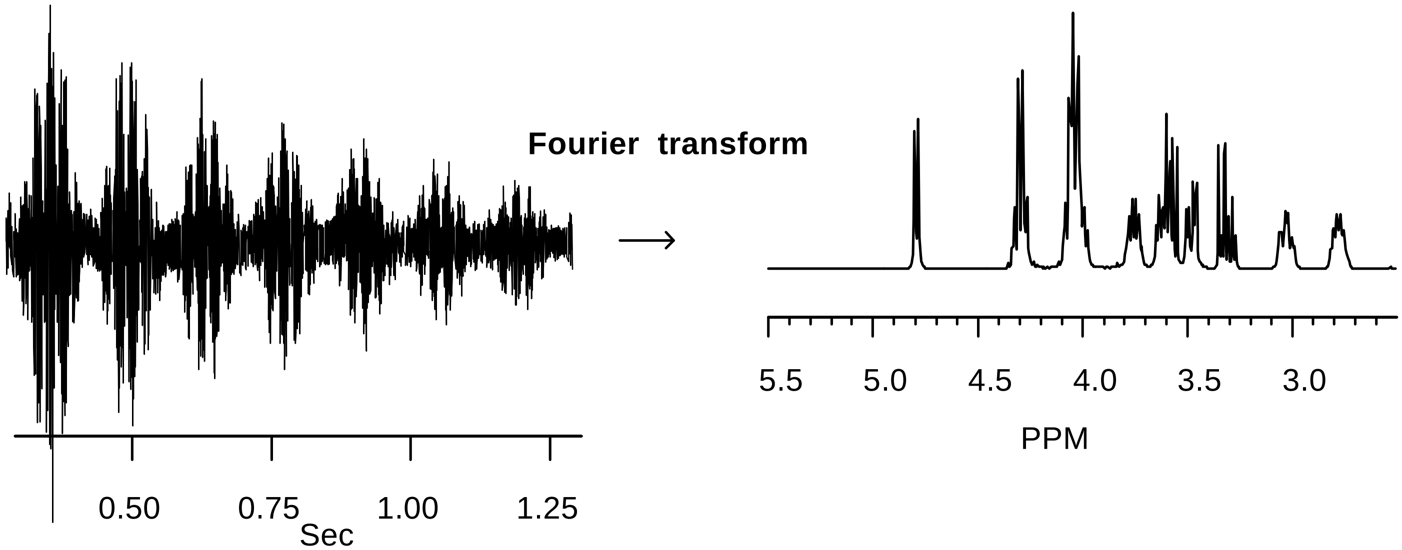
I. Zero-Filling Data

A. Review of Fourier Transformation in NMR

Very briefly, the actual NMR spectrum acquired by a modern pulse fourier transform NMR spectrometer is called a free-induction decay or fid. This spectrum results when a sample in the presence of a large external magnetic field is subjected to a short (several microseconds), high-power pulse (50-1,000W) of radio-frequency energy at the resonance frequency of the nuclei of interest. This burst of energy is released by the sample over a much longer period of time (typically seconds) as the nuclear spins return to their equilibrium energy states. The released energy is emitted as a radio wave. The frequency of this wave is dependent upon the local magnetic environment of the nuclei. If the excited nuclei in a sample are all in the same magnetic environment, the observed signal will consist of a single decaying radio frequency (sine wave). If there are several magnetically inequivalent nuclei, each will release its absorbed energy at a slightly different frequency. The observed signal will consist of a decaying waveform which is the sum of the individual decaying sine waves from each of the inequivalent nuclei. This signal *induces* a current in the nmr probe and the signal *decays* as the nuclei freely release their absorbed energy, hence the term free-induction decay.

The actual spectral data acquired by the NMR is the free-induction decay, or fid. The "spectrum" we always plot and interpret results from a mathematical manipulation (ft) of the acquired spectral data. In pulse ft-NMR, the fid is fourier transformed. The ft converts the AMPLITUDE vs. TIME domain information in the fid to the AMPLITUDE vs. FREQUENCY domain seen in the typical nmr "spectrum".



The continuous NMR radio-frequency signal (fid) emitted by the sample (for ^{1}H on the Gemini-300, the NMR signals occur at 300MHz, +/-several kilohertz) is reduced to the audio-frequency range by mixing out the high-frequency component. The low-frequency audio spectrum containing the NMR signals is then converted into a discrete series of data points by an analog-to-digital converter. The number of points that you acquire can be controlled with the parameter **np**. Alternatively, the acquisition time **at** can also be used to set the number of points. At a given sweep width, a longer value for **at** will always result in a greater number of data points **np**.

B. Digital Resolution in 1D NMR Spectra

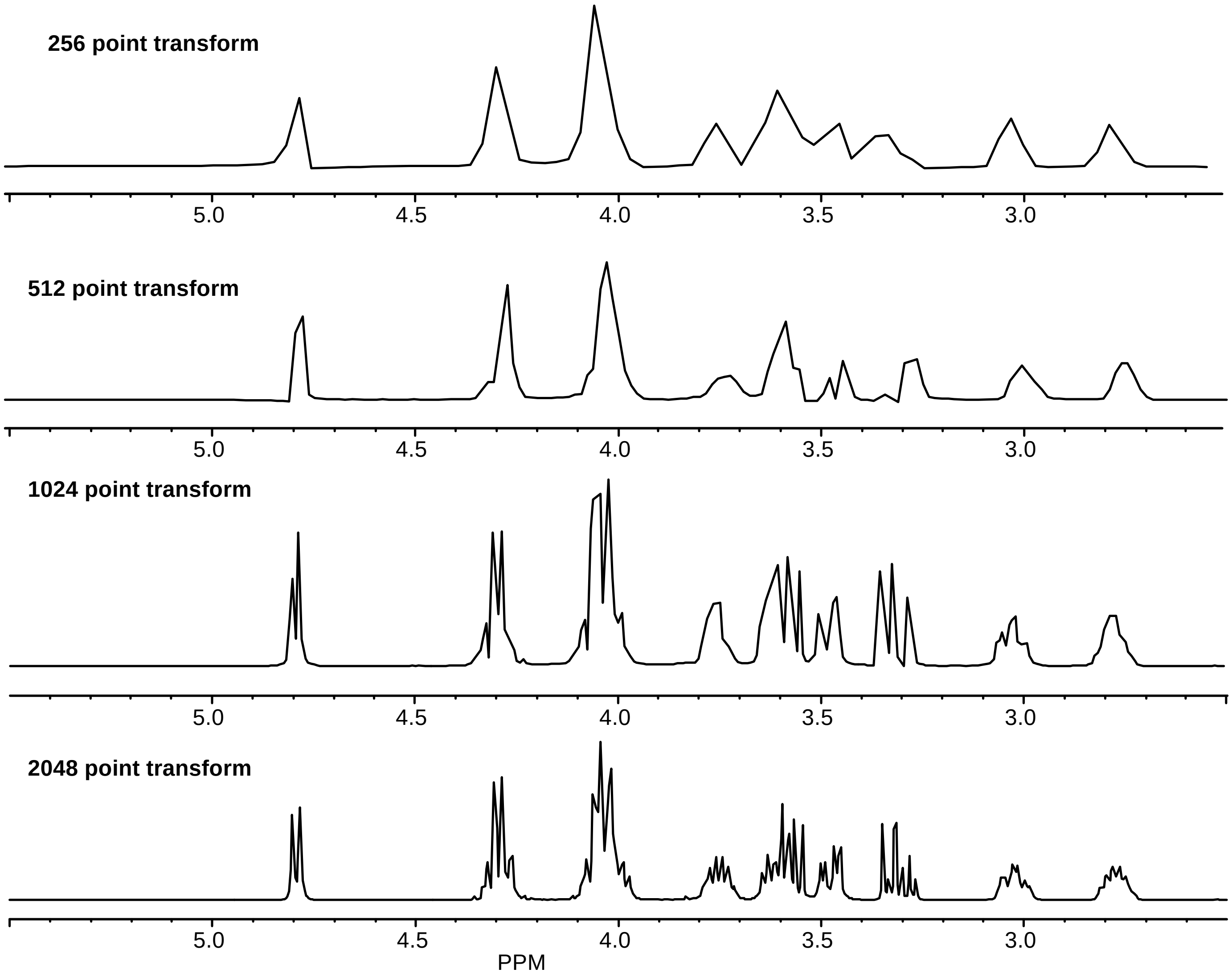
The fast fourier-transform algorithm used on all modern NMR spectrometers requires that the fourier transformation be carried out on a spectrum consisting of a power of 2 number of data-points (determined by the fourier number, **fn**). Ft of **fn** number of points will result in two spectra consisting of the sine and cosine components in the original fid. Each of the spectra contain **fn/2** points, and can be added to each other to yield the final, pure absorption (Lorentzian) spectrum of **fn/2** points. If the number of points **np** acquired is less than the nearest power of two (**fn**), then zeros will automatically be added to the fid prior to the ft to obtain a new fid with a power of 2 number of points. This process is called zero-filling. Zero-filling is automatically performed when necessary within VNMR and will always occur whenever **np<fn**. As **fn** can be set to any desired power of two, fids can be extensively zero-filled when necessary.

The *digital resolution* of the final spectrum in Hertz/point is equal to the sweep width divided by the number of points, or **sw/(fn/2)**. Typical digital resolutions are shown in the following table:

Instrument	Number of points **np**	fourier number **fn**	sweep width (Hz)	digital resolution (Hz/pt)
Gemini-300 ^{1}H ^{13}C	16384 32768	16384 32768	4551 17250	0.56 1.05
UnityPlus400 ^{1}H ^{13}C	16384 32768	32768 32768 (65536)	6000 23000	0.37 1.4 (0.7)
Unity 500 ^{1}H ^{13}C	32768 32768	32768 65536	8000 28750	0.49 0.88

Even with large values of **fn**, the digital resolution in basic spectra can be surprisingly low. In order to improve the digital resolution to the level required by any given experiment (careful measurement of coupling constants, for example), either **fn** can be increased or the acquisition sweep width **sw** reduced. The value of **fn** can often be easily increased. Although **fn** is typically equal to **np**, this is not required. Since the fid decays to noise over time, a large **np** will often simply lead to the acquisition of noise, resulting in a loss of sensitivity in the transformed spectrum. Hence **np** can often be less than **fn**, with no detrimental effects.

The effect of zero-filling and digital resolution can be seen in the figure on the next page. In this example, the same fid (**np**=8196 points) was transformed with **fn**=256, 512, 1024 and 2048. This example intentionally exagerates the effect of fourier number on the digital resolution of the result. However, :it clearly demonstrates that the transformed spectrum must contain a sufficient number of data points for fine structure is to be observed. Even with simple ^{1}H NMR spectra, the default values for **np** and **fn** can make it impossible to accurately measure simple effects, such as coupling constants. The table shows that typical default value for digital resolution for a standard ^{1}H NMR spectrum on the Unity 500 is only 0.49 Hz/point. Hence, any measurement of coupling constants can only be carried out to an accuracy of ±0.49Hz. Simply zero-filling by changing the default **fn** from 32768 (32K) to 131072 (128K) would increase the digital resolution to 0.12Hz/point. Further increases in digital resolution could be obtained by substantially narrowing the sweep width, perhaps by using selective excitation or shaped pulses.



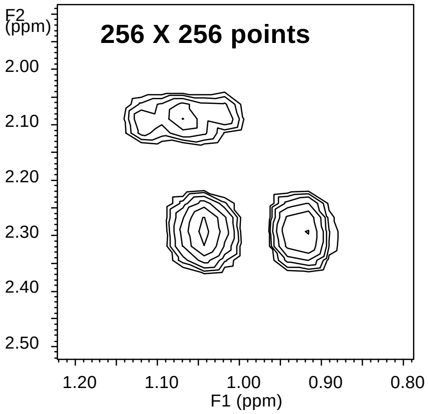
B. Digital Resolution in 2D NMR Spectra

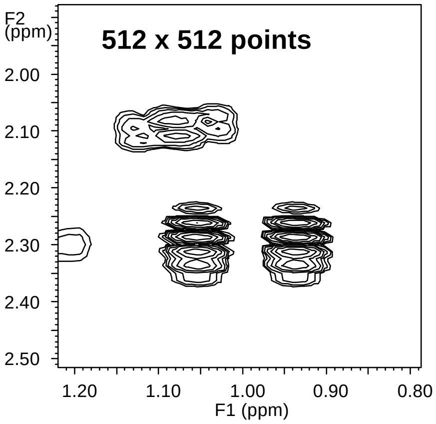
The problem of digital resolution in 1D spectra is usually easy to solve. In 2D spectra, digital resolution often becomes the most critical aspect of 2D nmr spectroscopy. Large values of **fn** and **fn1** can lead to extremely large data sets and long transform, display and plotting times. The parameters **fn** and **fn1** determine the digital resolution of the acquisition and time-incremented dimensions, called **f2** and **f1**, respectively. The following table shows some typical examples of digital resolution in 2D spectra, and some instances of effects of large data fourier numbers.

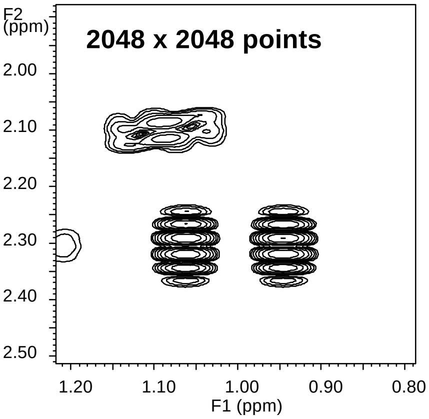
Experiment	**sweep width (f2) (f1)**	**fourier number (fn) (fn1)**	**digital res. (f2) (f1)**	**data size (Bytes) Fids / Spectrum**
1). COSY, 500 MHz ^{1}H 256 fids	8000 8000	1024 1024	15.6 15.6	1,048,576 1,0485,76
2). COSY, 500 MHz ^{1}H 512 fids	4000 4000	4096 4096	1.95 1.95	8,388,608 16,777,216
3). phase-sensitive NOESY, 500, 1200 fids	4000 4000	8192 4096	0.98 1.95	39,321,600 33,554,432
4). 3D NOESY-TOCSY, 500 MHz, 128 x 128 fids	4000 500 4000	1024 512 512	7.81 1.95 15.63	67,108,864 134,217,728

The table shows that typical quick 2D spectra (example 1), have a reasonably small overall data size (1 MByte), at very low digital resolution (15.6 Hz/point). This resolution would make it impossible to observe most proton-proton splittings. A more realistic COSY spectrum is given by example 2, a typical medium resolution 2d data set. The digital resolution is high enough to see larger couplings (1.95 Hz/point). However, the transformed data set is now 16 times larger than the quick, low resolution COSY of example 1. Processing, display and plotting will all take 16 times longer. Typical phase-sensitive 2D spectra will generally result in a doubling of these numbers (example 3) and data sizes can quickly get very large for even small 3D data sets (example 4, 134 MBytes).

The following figure shows some examples of the same 2D data set, transformed with diffferent digital resolutions.







These spectra have all been symmetrized. In the first example, fine couplings are simply not observed. Couplings begin to appear in the second case, but become increasingly well-defined as the digital resolution of the final transformed spectrum is increased. Remember that this increase in resolution is at the expense of a dramatic increase in time required for processing, display and plotting.