Multidimensional NMR

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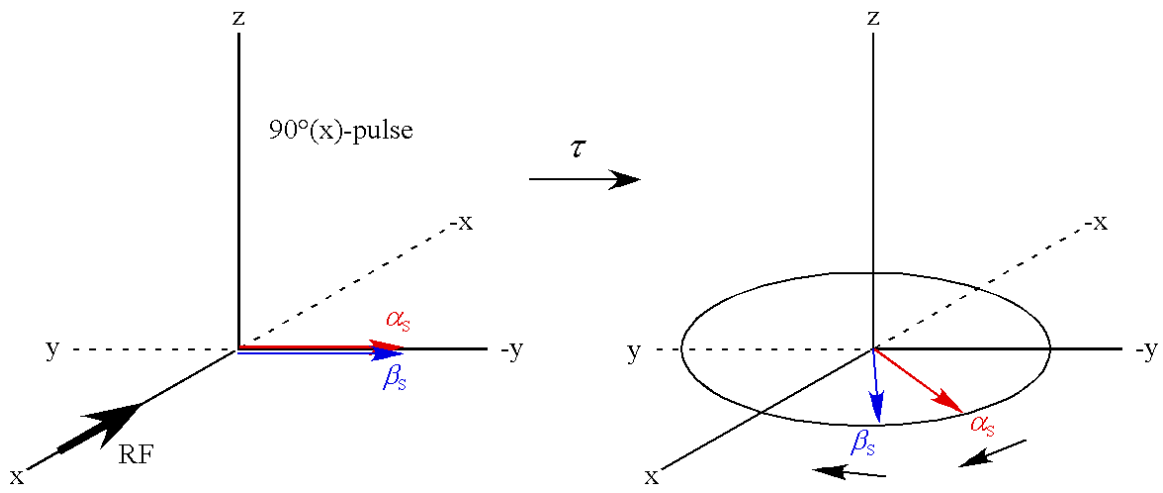
Thus far we have considered one-dimensional (1D) spectra – a set of intensities in a single frequency domain. We now consider additional time/frequency dimensions.

Before discussing multidimensional methods, it may be valuable to review the essential phenomenology associated with the pulsed NMR experiment.

In the most basic form, the NMR experiment consists of a single RF pulse followed by detection of the time-domain response (free-induction decay):



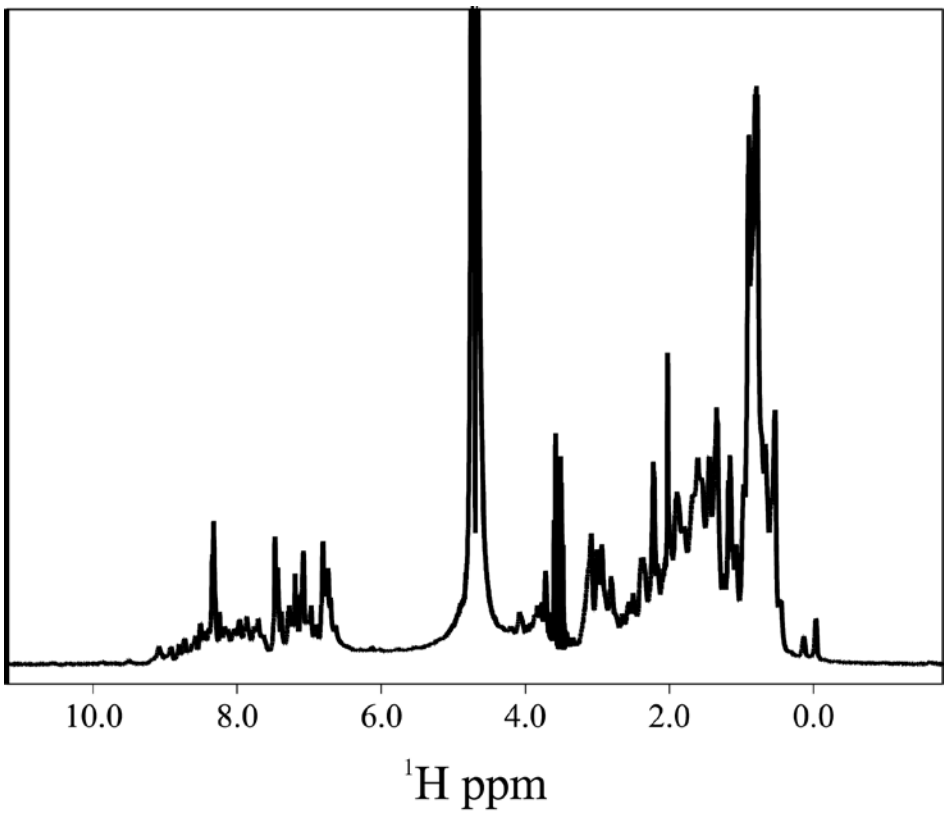
In the general case, during the detection interval the spins will precess about the z-axis according to their chemical shifts, with further development due to *J* coupling effects.

The Larmor frequencies of nuclei of interest fall in the MHz (radio) range, but remember that we detect precession in the xy-plane relative to the carrier frequency. The frequencies relative to the carrier frequency are in the kHz (audio) range.

The carrier frequency is also the central frequency of the RF pulse – the precise value of the carrier frequency is shared between the transmitter and receiver components (heterodyning).

The spectra we have considered up to this point have been relatively simple, and have generally consisted of a small number of well-separated resonances. This is unfortunately not the typical situation.

Generally, spectra may contain numerous resonances, many of which may have very similar chemical shifts. Natural products, heteropolymers and biological macromolecules are examples of molecules in which spectral resolution is limited by resonances that partially and/or completely overlap one another:



^{1}H NMR spectrum of a 14 kD protein. Note that in the chemical shift region between about -0.2 ppm and 0.7 ppm there are 73 methyl resonances.

As we have already noted, higher applied static magnetic fields produce intrinsically higher resolutions spectra.

For example at 2.35 T the consensus resonance frequency of ^{1}H nuclei is about 100 MHz (100 Hz/ppm) and for ^{13}C nuclei the consensus frequency is about 25 MHz (25 Hz/ppm), whereas at 11.74 T the resolution for protons is about 500 Hz/ppm while for ^{13}C it is 125 Hz/ppm.

It turns out that field strength alone will not be capable of producing the improvement in resolution that will be required to effectively study more complex molecules.

A New Dimension

The basic approach involves producing two time/frequency-domain spectra, one recorded in real-time, as in a normal spectrum and another spectrum that is linked to the first that is acquired in an indirect or virtual sense.

The essential requirement for producing multidimensional data is that the time/frequency domain in one dimension is linked through some sort of spin interaction to another dimension.

This linkage is named *mixing* and a so-called *mixing interval* is a component of all multidimensional NMR spectra.

In addition to a mixing interval, we will also expect to find a *detection interval*, which is completely analogous to the detection component of 1D spectra.

We also need another time/frequency domain. During detection, spin interactions *evolve* while they are being detected and so this reason, the additional time/frequency domain is named the *evolution interval*.

Finally, we need to initiate the experiment, and specifically we need to *prepare* the spins in some state so as to probe some specific effect. Often, this *preparation interval* will consist of a simple 90°-pulse, but it may be arbitrarily complex.

Thus overall our schematic form for a multidimensional experiment is as shown below:

Preparation	Evolution	Mixing	Detection

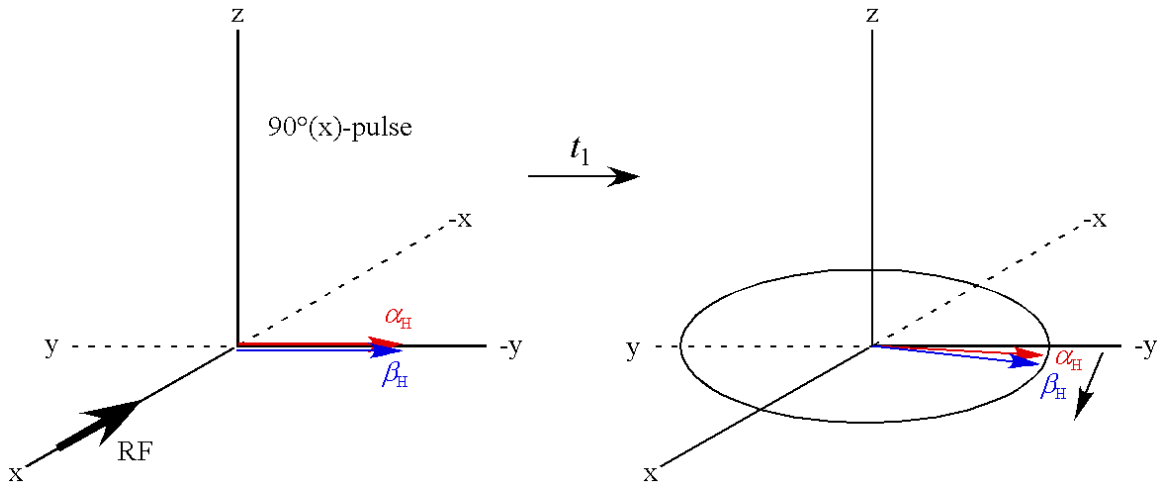
The scheme shown above specifically suggests a 2D experiment, two time/frequency domains. The concept is entirely general however and a 3D scheme could be represented as shown below:

Preparation	Evolution	Mixing	Evolution	Mixing	Detection

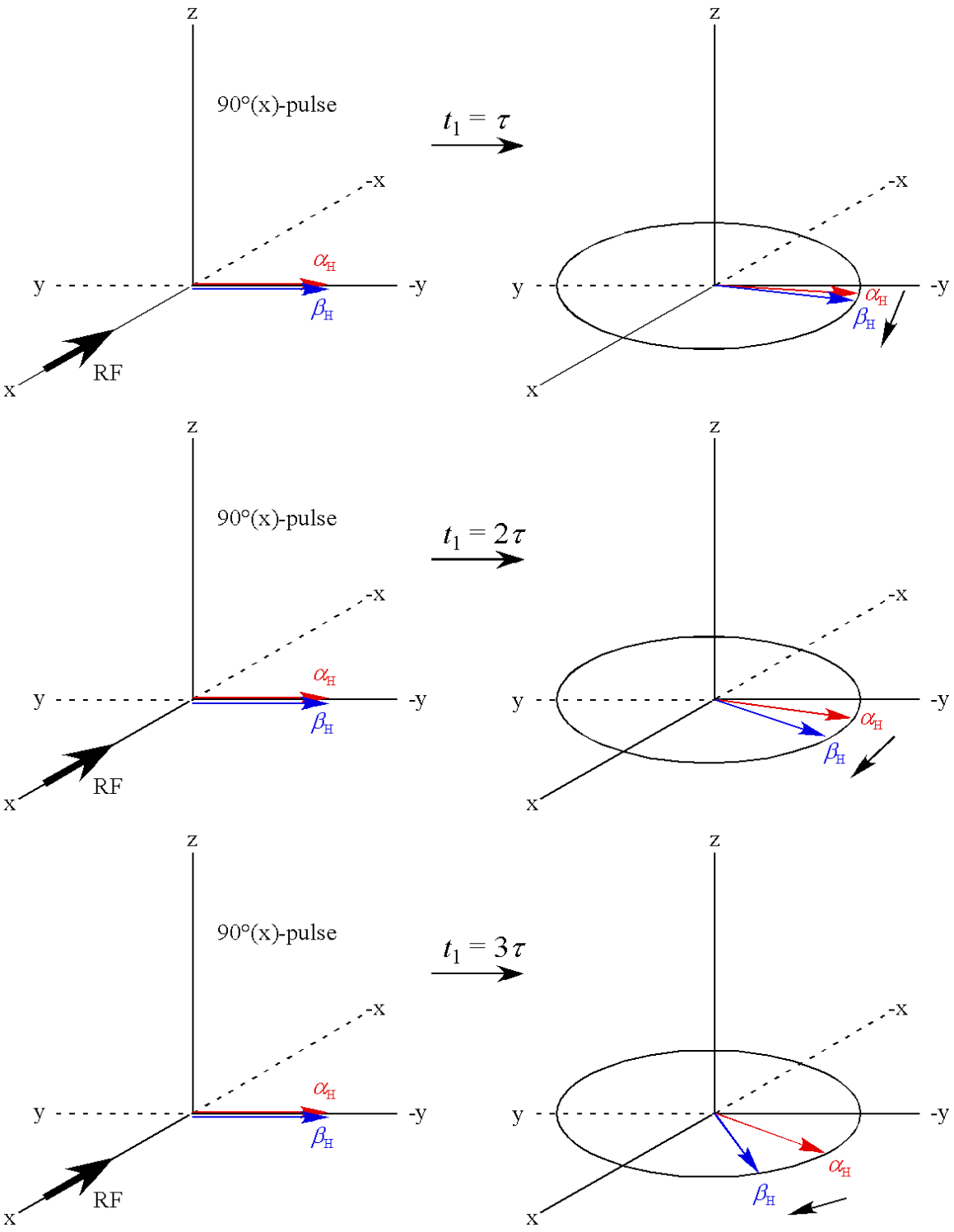
The mixing intervals link evolution intervals to one another and the result is detected during the final component of the experiment.

To develop this idea further, let's consider a specific example. For simplicity we will consider a simple AX ^{1}H spin system. Imagine we commence with the simplest nontrivial preparation, a ^{1}H 90°-pulse.

If we allow evolution to occur for some time, call it t_1, interactions will evolve and the effects will be transferred during mixing to cause a modification or modulation of the detected signal:



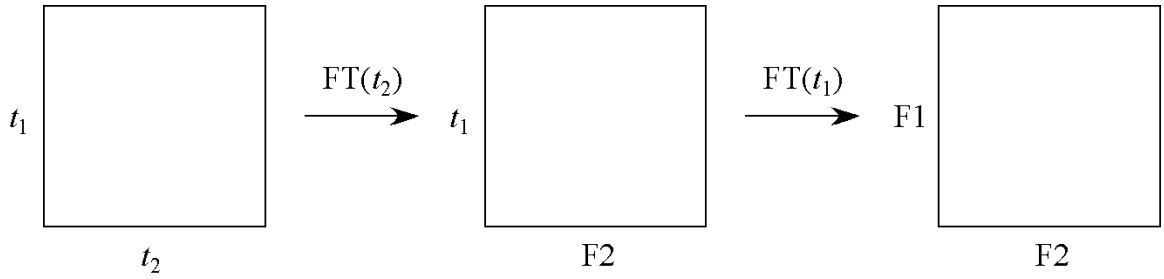
We can continue to increment the evolution time, with the effect of producing a continuously developing modulation in the detection interval:



We have complete freedom to design our evolution schedule, one simple version of which will be to reproduce the exact schedule employed to record the directly detected spectrum.

If we record 1024 complex points at 200 μs intervals – according to the Nyquist theorem, we can accurately detect frequencies from 2500 Hz below to 2500 Hz above the carrier frequency with a total acquisition time of 1024 $\times$ 200 ms = 204.4 ms. We can reproduce this effect in t_1 by recording a spectrum with t_1 set to 0, then 200 μs then 400 μs, then 600 μs and so on until we have recorded 1024 such experiments.

It will be most efficient to record both the real-time, t_2, and indirect spectra in the time-domain, t_1, and conduct a Fourier transform of the data to produce a two-dimensional (2D) frequency-domain spectrum:



The F1 and F2 domains will represent a correlation map of spin interactions that existed during the mixing interval.

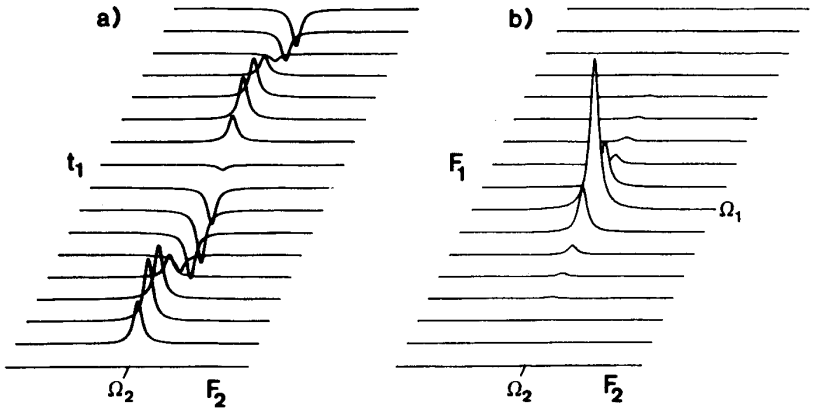
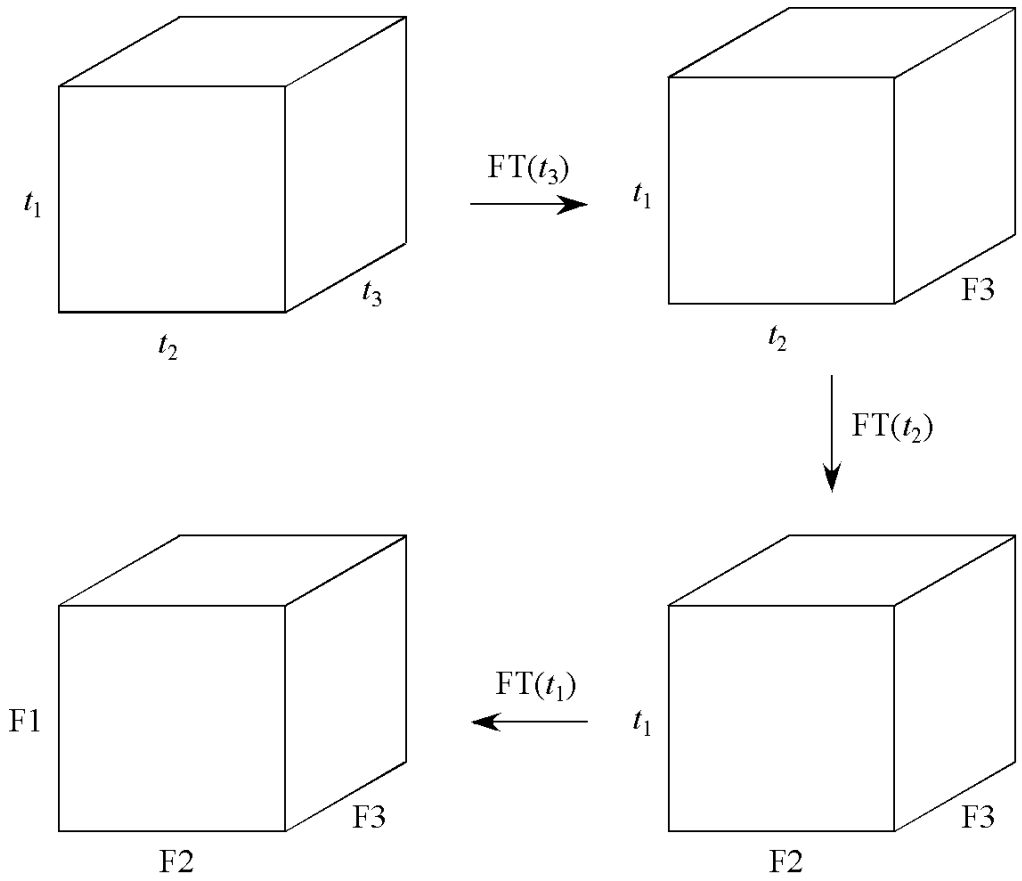


Figure 1.4 from Bax, A. (1984) *Two-Dimensional Nuclear Magnetic Resonance in Liquids*. D. Reidel Publishing, Boston: a) The absorptive component of a set of spectra which are modulated as a function of t_1. b) A two-dimensional spectrum obtained from (a) by Fourier transformation of cross-sections parallel to the t_1-axis.

The procedure is general, and conducting Fourier transformation of ND data may be accomplished as a series of sequential steps:



An important feature of multidimensional data is that regardless of the number of evolution-mixing interval sets we may install, there is only one direct detection interval. All other time-domains are detected as a modulation of the signal observed during direct detection.

If we conduct a Fourier transform of the real-time domain, i.e, and FT on the t_2 data, we would observe a spectrum; call it the F2 domain (*t* for time, F for frequency) that has been modulated with the incremented signal that persisted in the t_1 domain. We can then apply a second FT, this time over the t_1 data to produce an F1 domain.