SSSC Discovery Series
NMR2
Multidimensional NMR Spectroscopy

Topics:

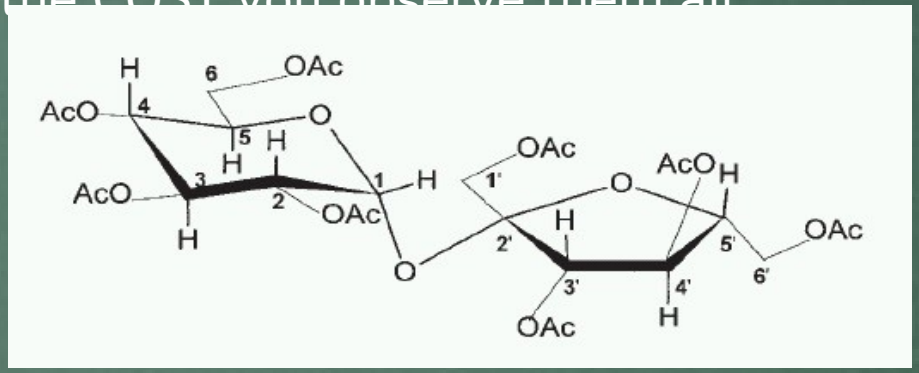
1. Some Common Experiments
2. Anatomy of a 2D experiment
3. 3D NMR spectroscopy

no quantum mechanic

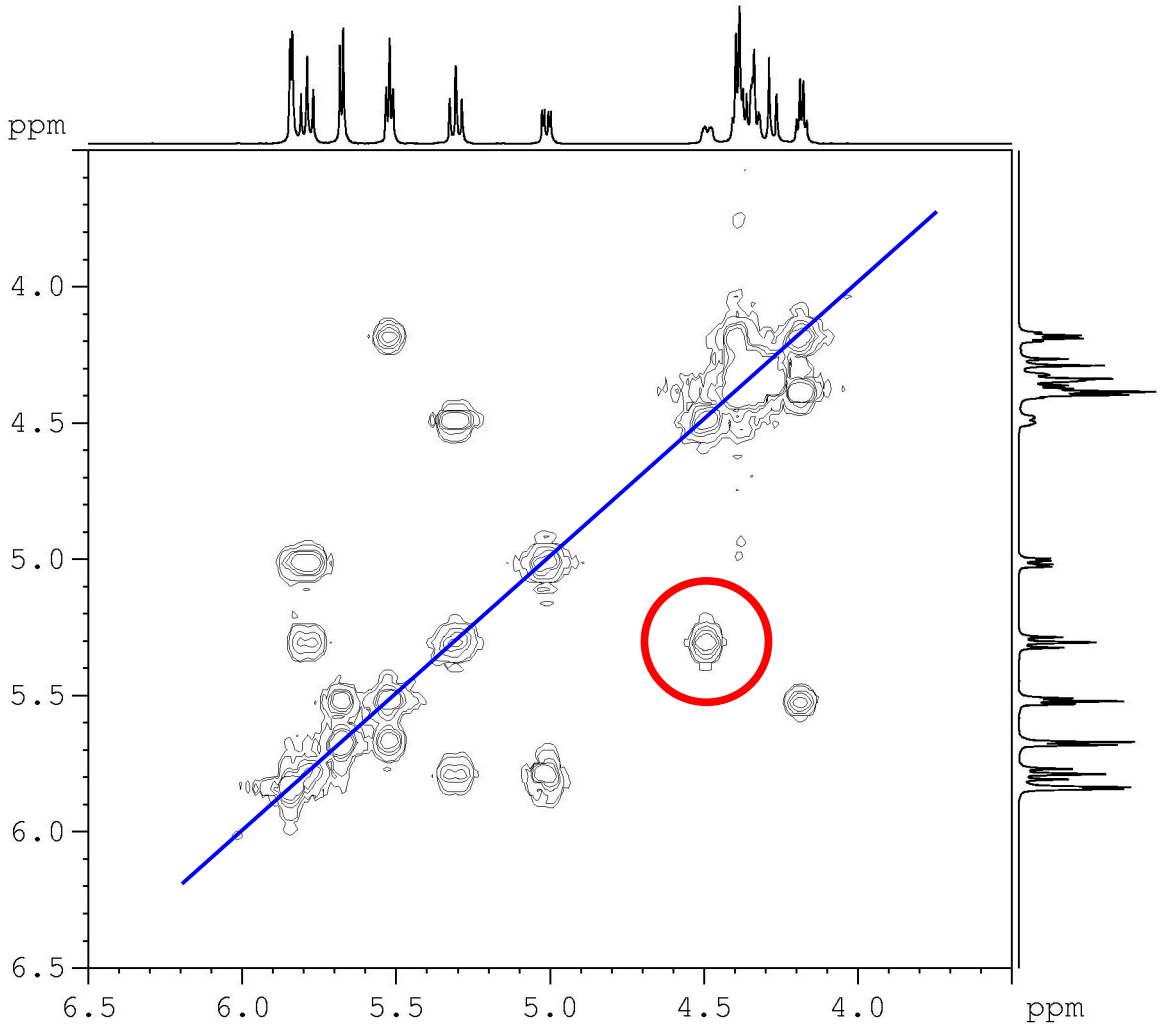
Some Common 2D Experiments

Very often, one will want to know which protons are J-coupled to which other protons in a spectrum to get an idea of the structure of the molecule of interest. One way to get this information is to do a series of decoupling experiments .. or .. a 2D COSY experiment can be performed. COSY is an acronym for COrrelation SpectroscopY .. nmr pulse programmers love acronyms.

Which experiment you choose depends on how many correlations you want observe and how much time you have. The decoupling experiment is a bit more difficult to set up but runs very quickly. The COSY sets up very quickly but takes more time to run. In the decoupling experiment you can choose how many correlations to observe. In the COSY you observe them all.



Some Common 2D Experiments

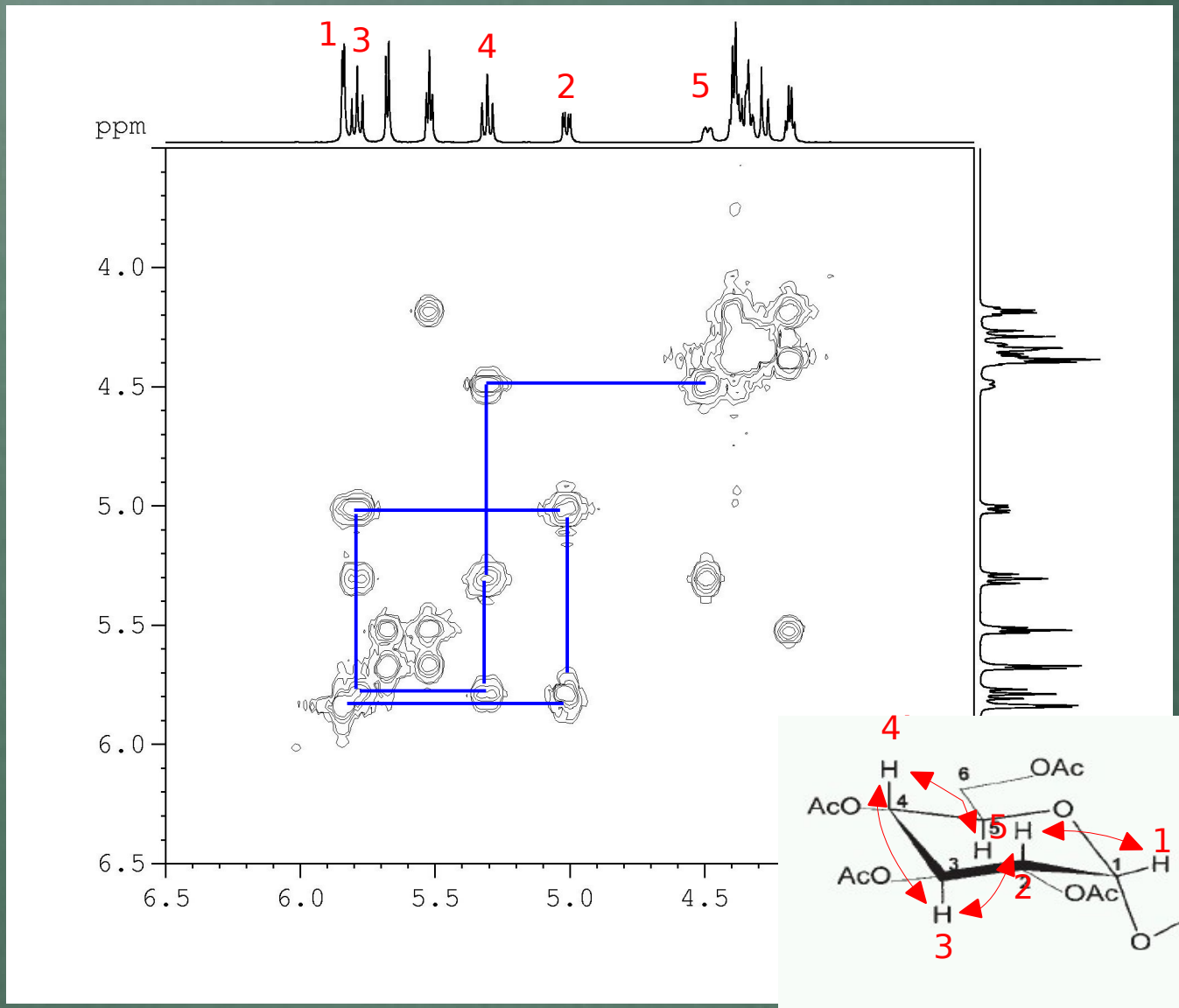


The COSY experiment.

The diagonal corresponds to the 1d spectrum

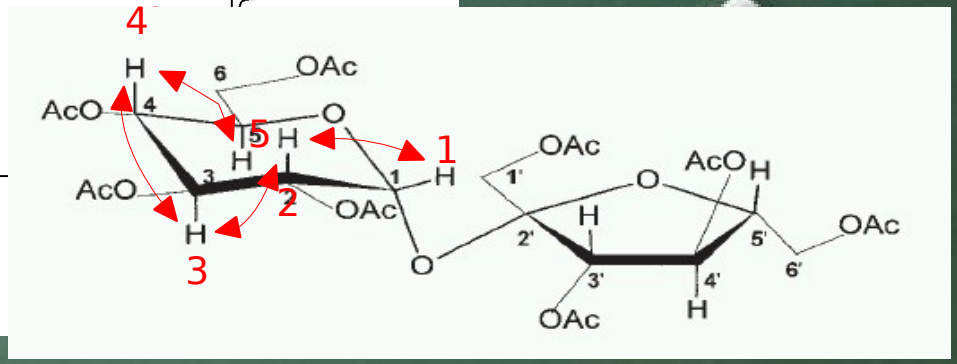
The off-diagonal or cross peaks represent the J-couplings between protons

Some Common 2D Experiments

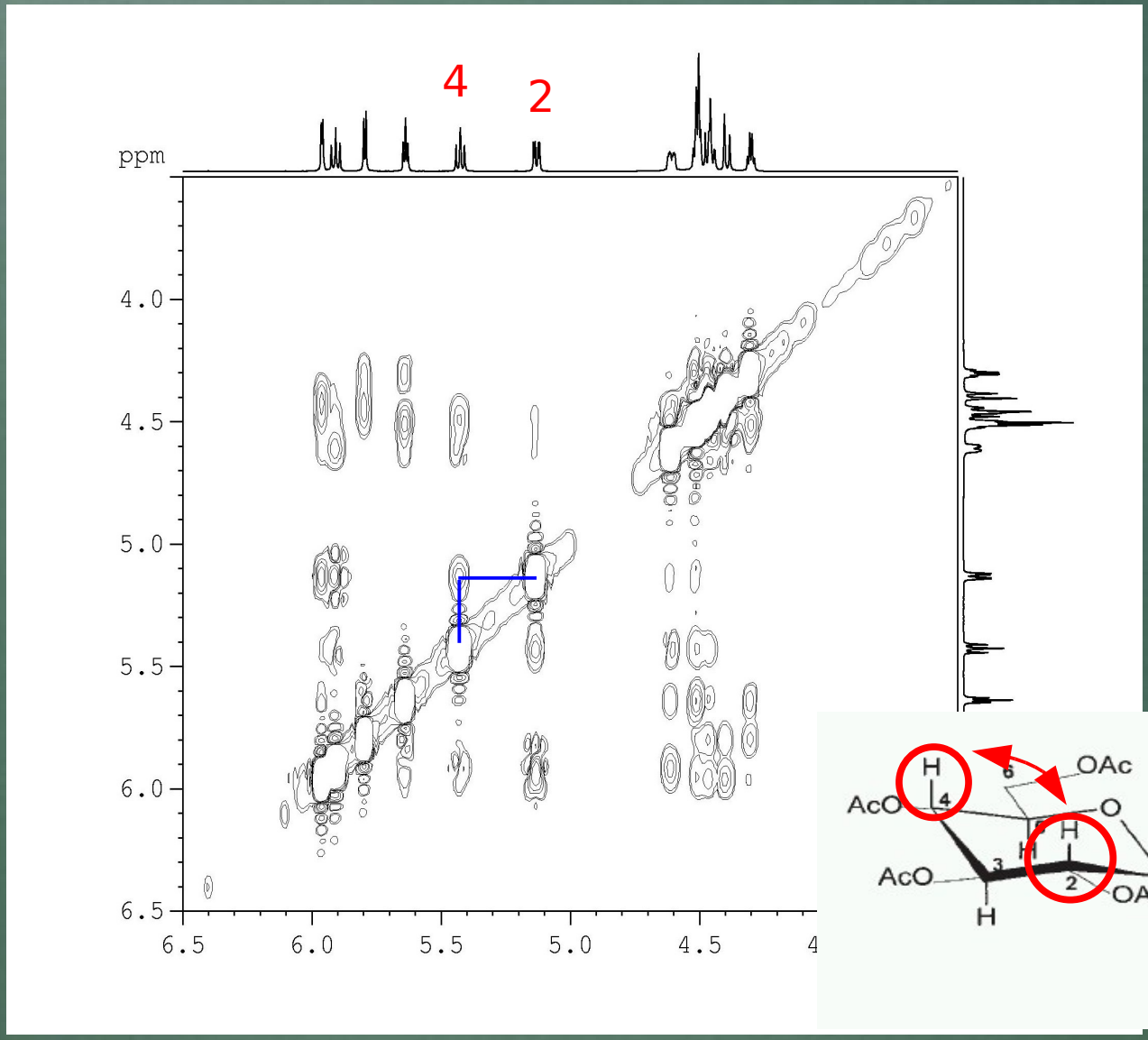


The COSY experiment.

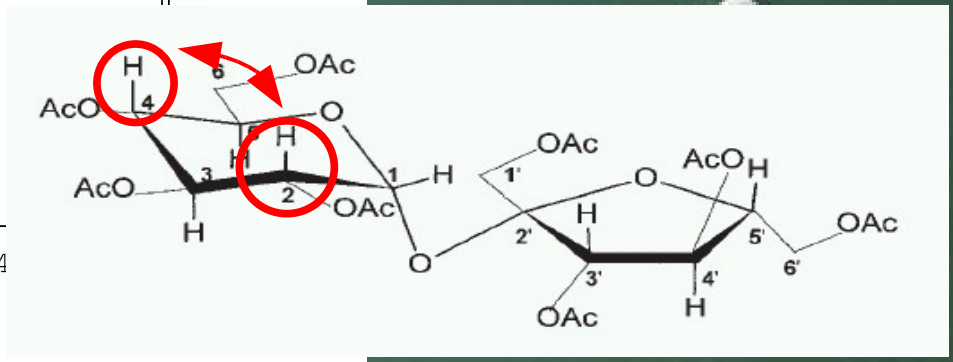
This set of correlations shows the coupling network formed by protons 1 to 5



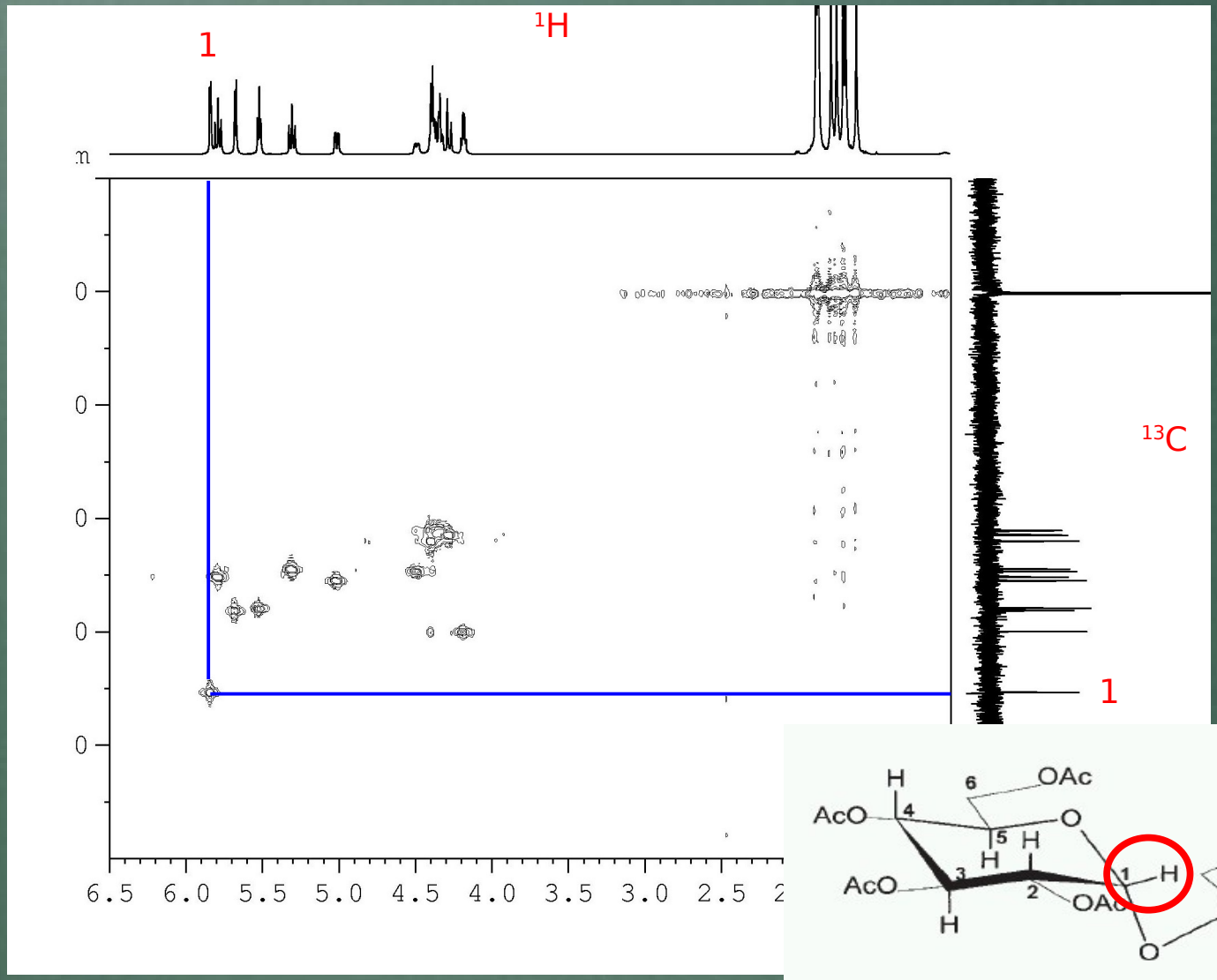
Some Common 2D Experiments



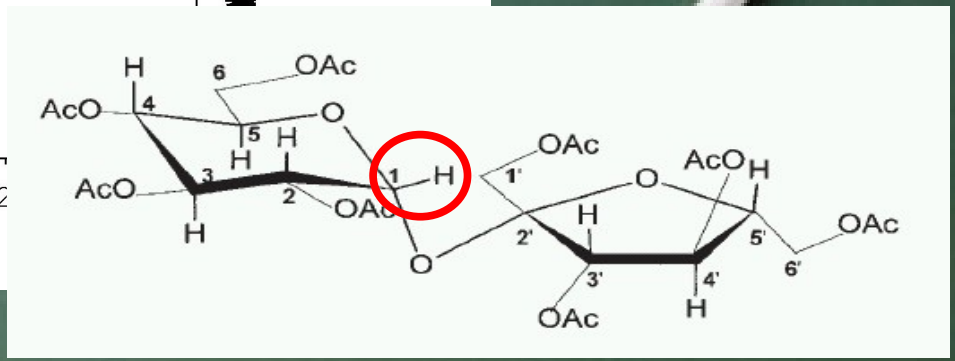
The NOESY experiment shows through-space dipolar couplings in the cross peaks.



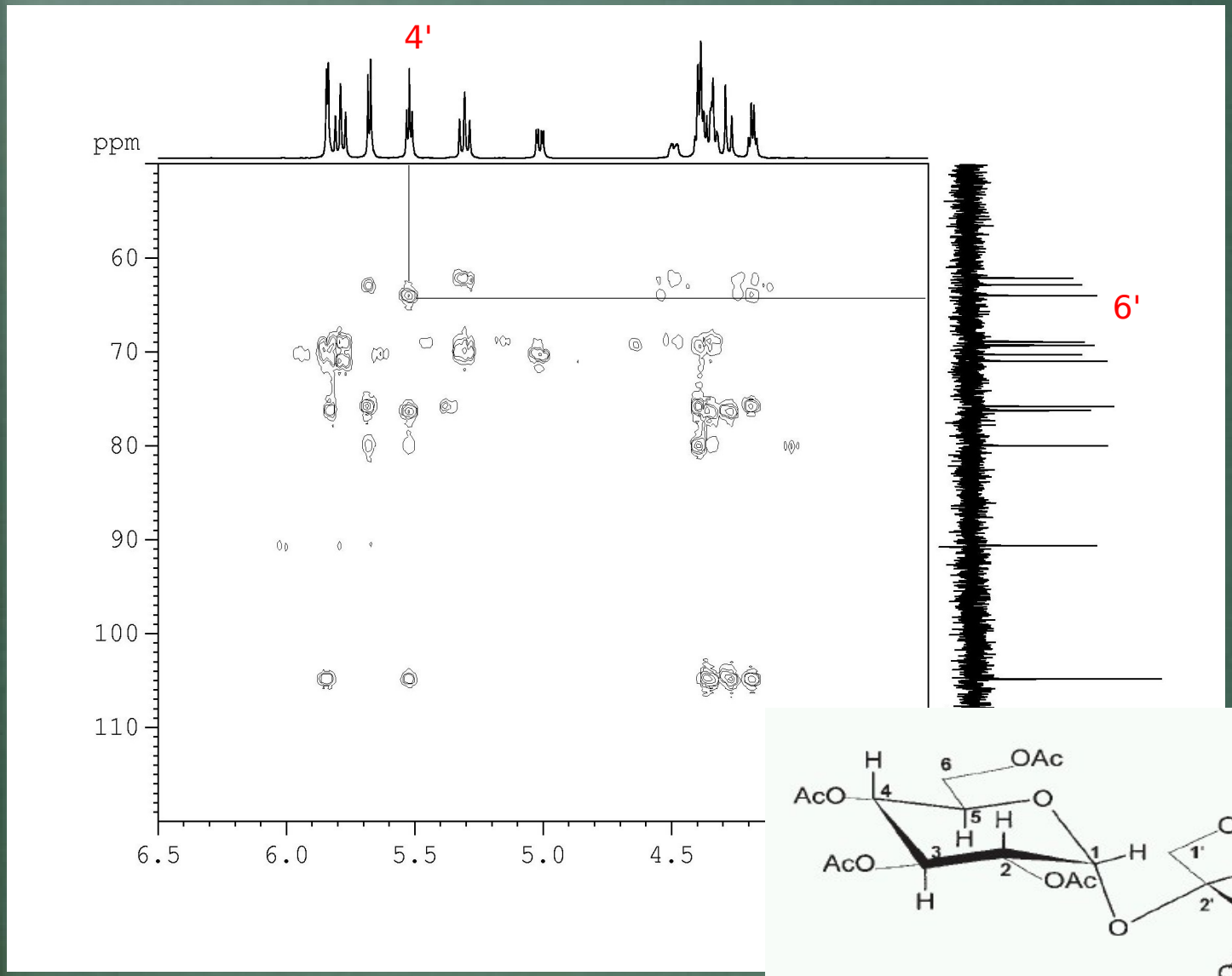
Some Common 2D Experiments



The HMQC experiment gives one-bond ^{1}H-^{13}C correlations



Some Common 2D Experiments



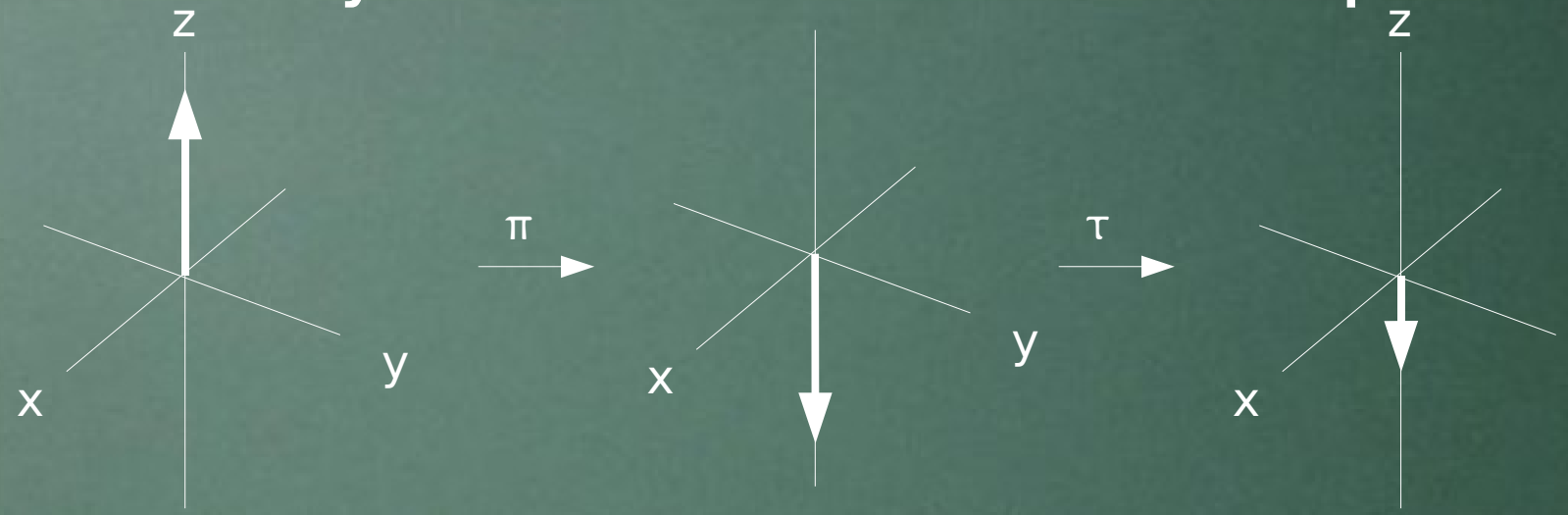
The HMBC experiment shows us 2 and 3 bond ^{1}H-^{13}C correlations.

Anatomy of a Two Dimensional NMR Experiment

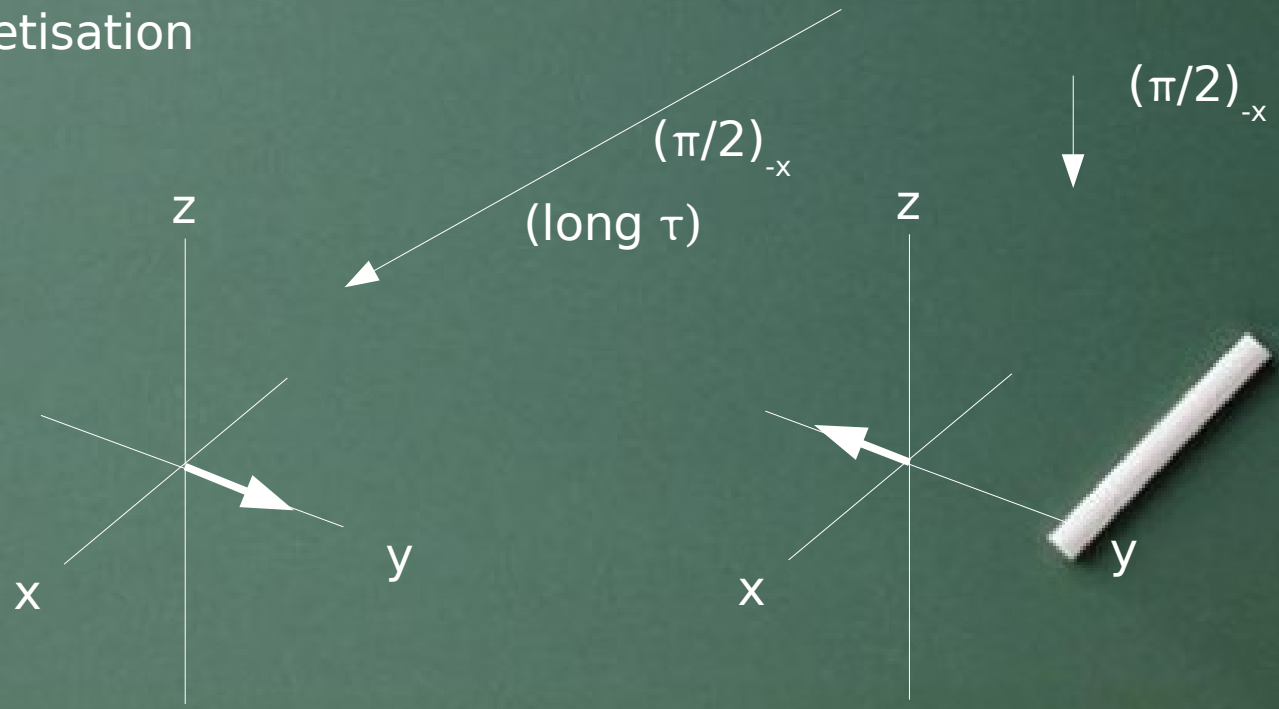
The inversion recovery experiment provides a good place to start looking at the second dimension.

τ is a *variable* time delay

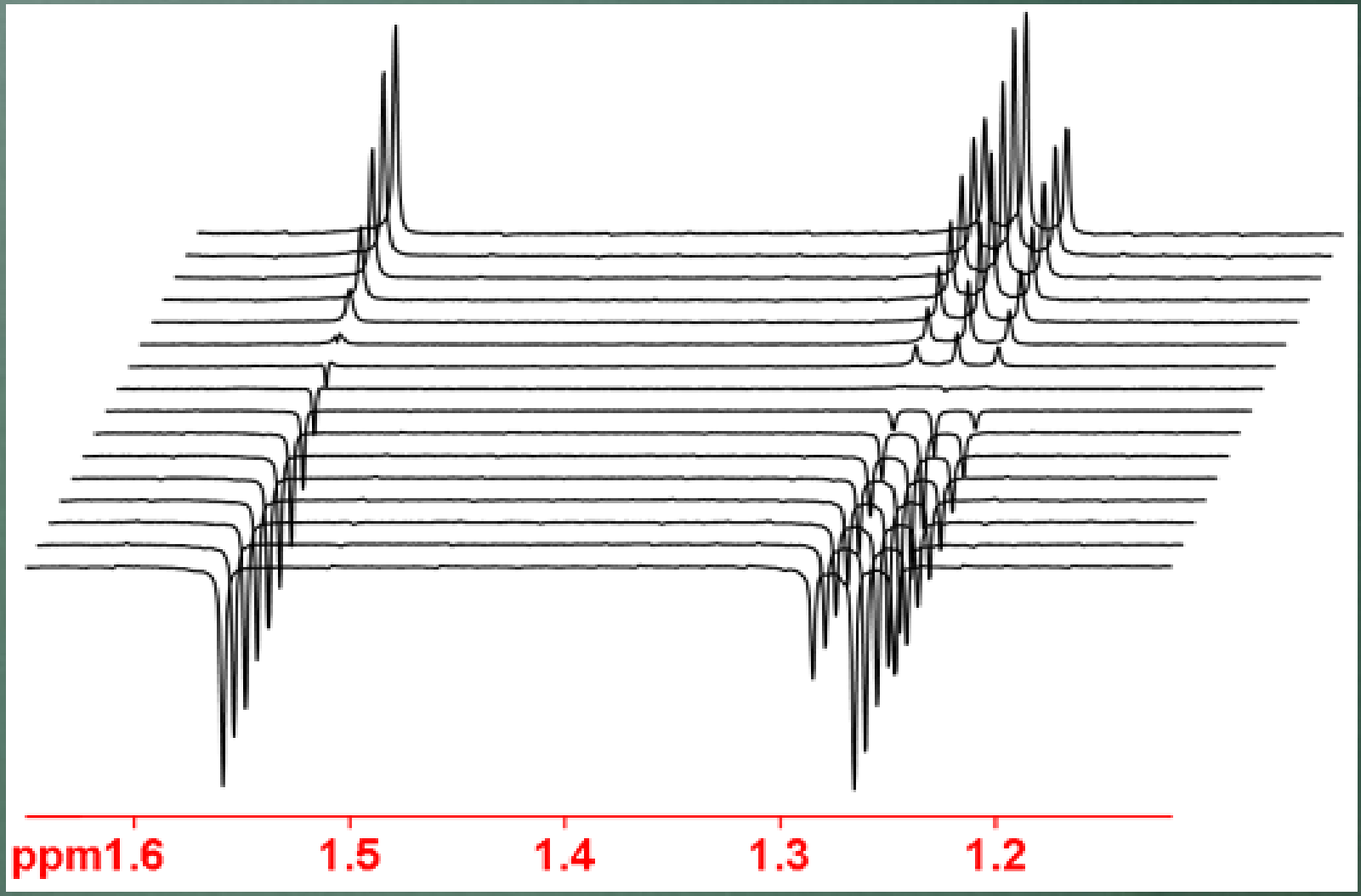
Anatomy of a Two Dimensional NMR Experiment



Equilibrium magnetisation



Anatomy of a Two Dimensional NMR Experiment



Anatomy of a Two Dimensional NMR Experiment

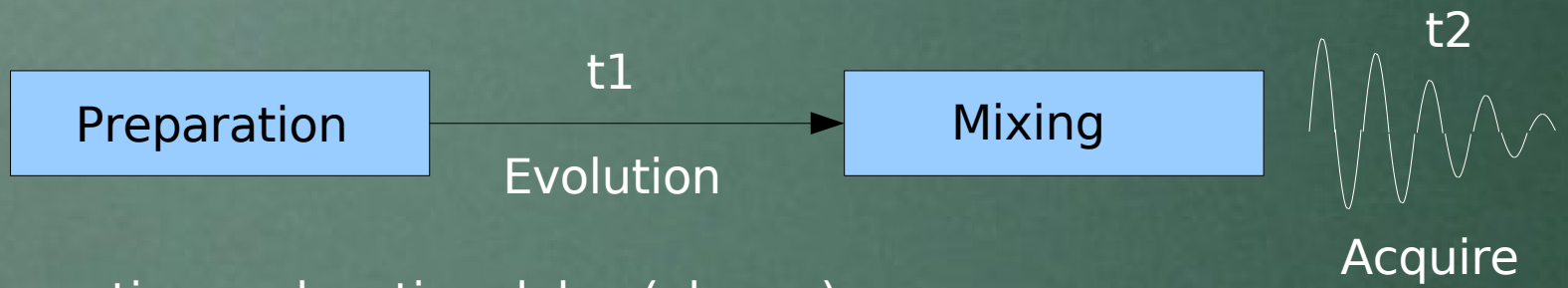
The 1st dimension of the plot corresponds to the usual 1 dimensional spectrum. In the 2nd dimension of the plot the peaks become less negative, go through zero and become positive. A plot of a peak's intensity would give an exponential plot:

Anatomy of a Two Dimensional NMR Experiment

Curve fitting gives the constant, T1. Thus, we say the 2nd dimension has T1 information “encoded” in it. This is the fundamental idea behind 2D NMR. One sets up the pulse sequence such that the desired information is encoded in the second dimension.

Anatomy of a Two Dimensional NMR Experiment

ALL two dimensional experiments have the same four basic pulse "blocks":



Preparation - relaxation delay (always)
- pulse(s) and delays to prepare the spin system for the evolution period

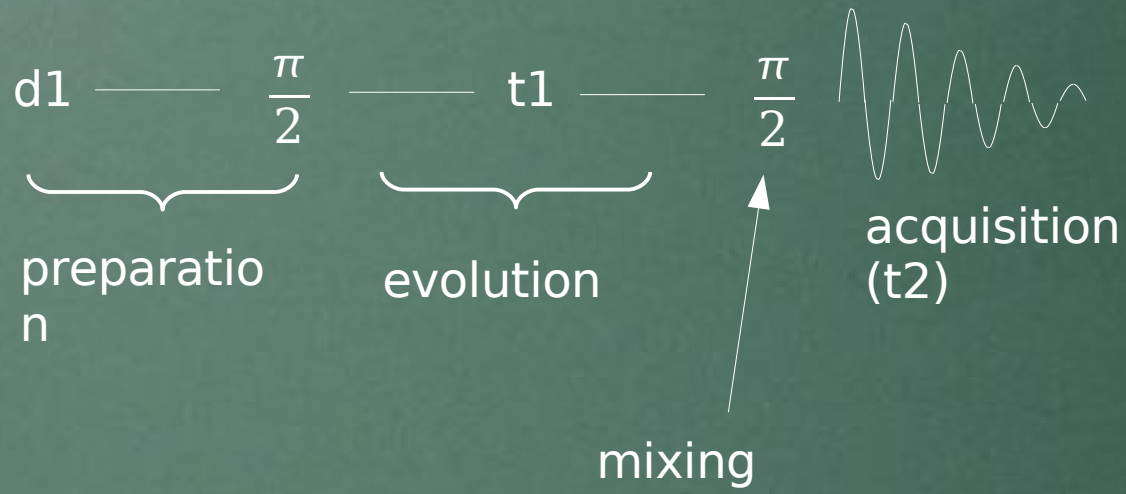
Evolution – a variable time period during which evolution of the spin state occurs. Starts at zero and increased in regular increments. FID recorded for each increment and stored in a 'serial' file. Time period referred to as 't1' ... corresponding frequency domain is 'f1'.

Mixing – pulse(s) to prepare spin system for acquisition

Acquisition – signal detection time period, referred to as 't2' and corresponding frequency domain referred to as 'f2'

Anatomy of a Two Dimensional NMR Experiment

The simplest (and the first) example of a true 2D experiment is the COSY (COrelation SpectroscopY) experiment:



Anatomy of a Two Dimensional NMR Experiment

The technique is very simple. Start the experiment with a very short delay and collect the data in a fid. Next, increment the t1 delay and redo the experiment, saving the new data at the end of the old data. Repeat for the required number of times.

1st experiment

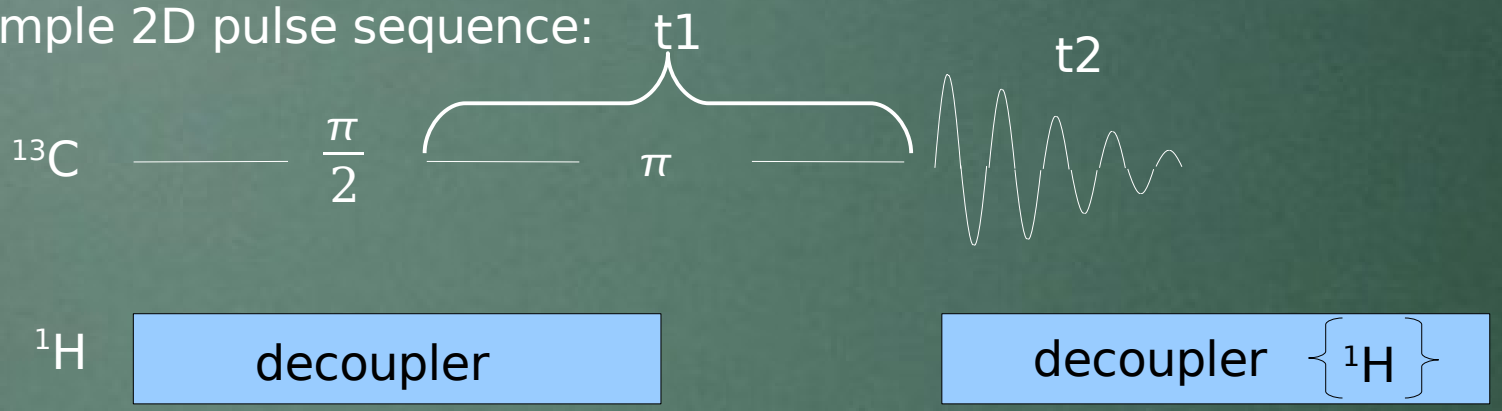
2nd experiment

etc.

The data file is called a 'serial' file ... because the fid's are sequentially or serially arranged. Each fid is slightly different from the previous one as a result of the incremented delay in the evolution block.

Anatomy of a Two Dimensional NMR Experiment

A simple 2D pulse sequence:

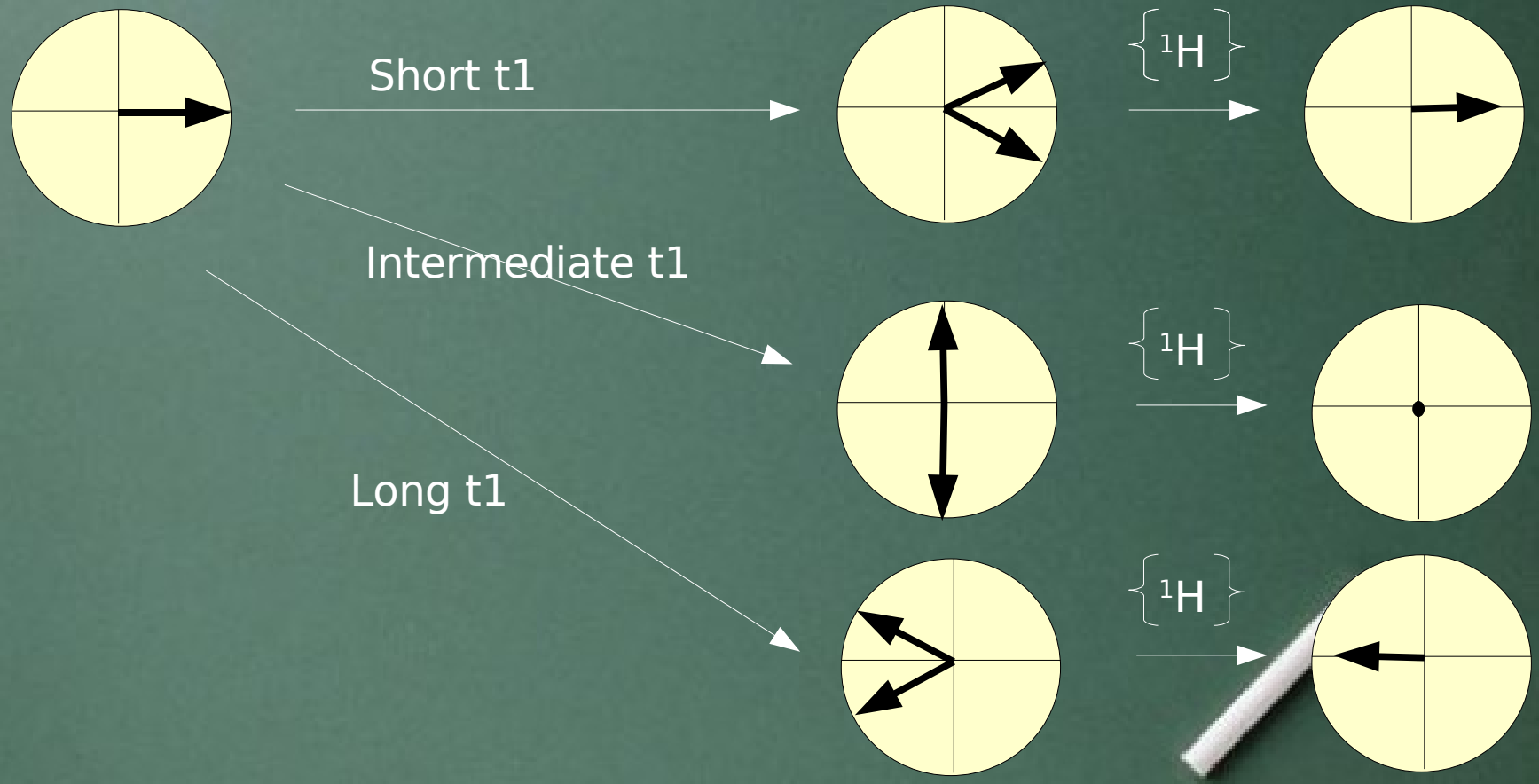


You would be correct in thinking that this pulse sequence looks familiar ... it is exactly the same as the APT or J-modulation pulse sequence. The only difference is that here, we are varying the time between the initial π/2 pulse and the beginning of acquisition. This is the ^{13}C J-spectrum experiment, useful for obtaining ^{1}H-^{13}C coupling constants.

During the t1 time interval, chemical shift is refocussed so that no net shift evolution occurs .. coupling evolution, however, does occur. During the t2 period, coupling does not occur but shift evolution does. Thus, when the data are transformed, f2 will contain chemical shifts and f1 will contain couplings.

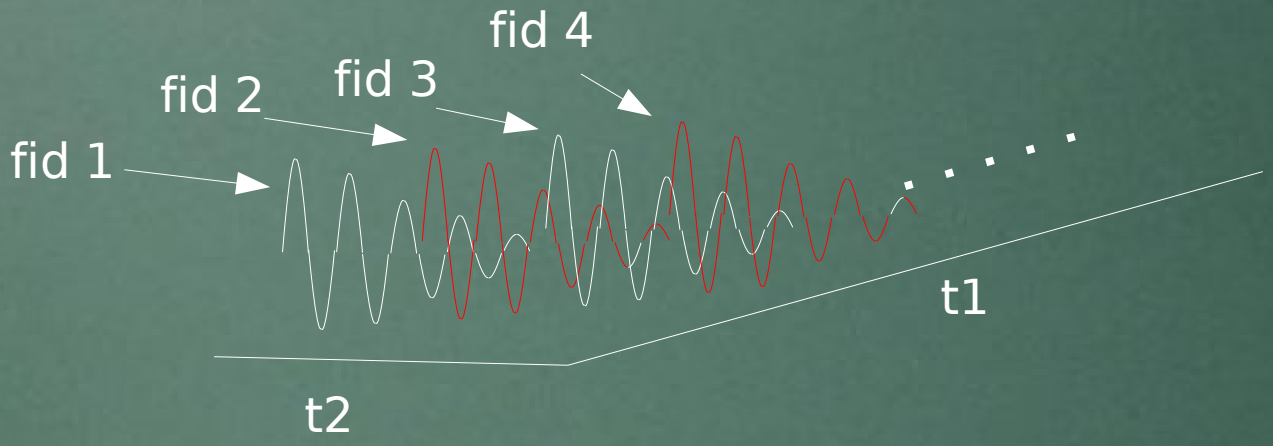
Anatomy of a Two Dimensional NMR Experiment

For CH (on-resonance pulse):



Anatomy of a Two Dimensional NMR Experiment

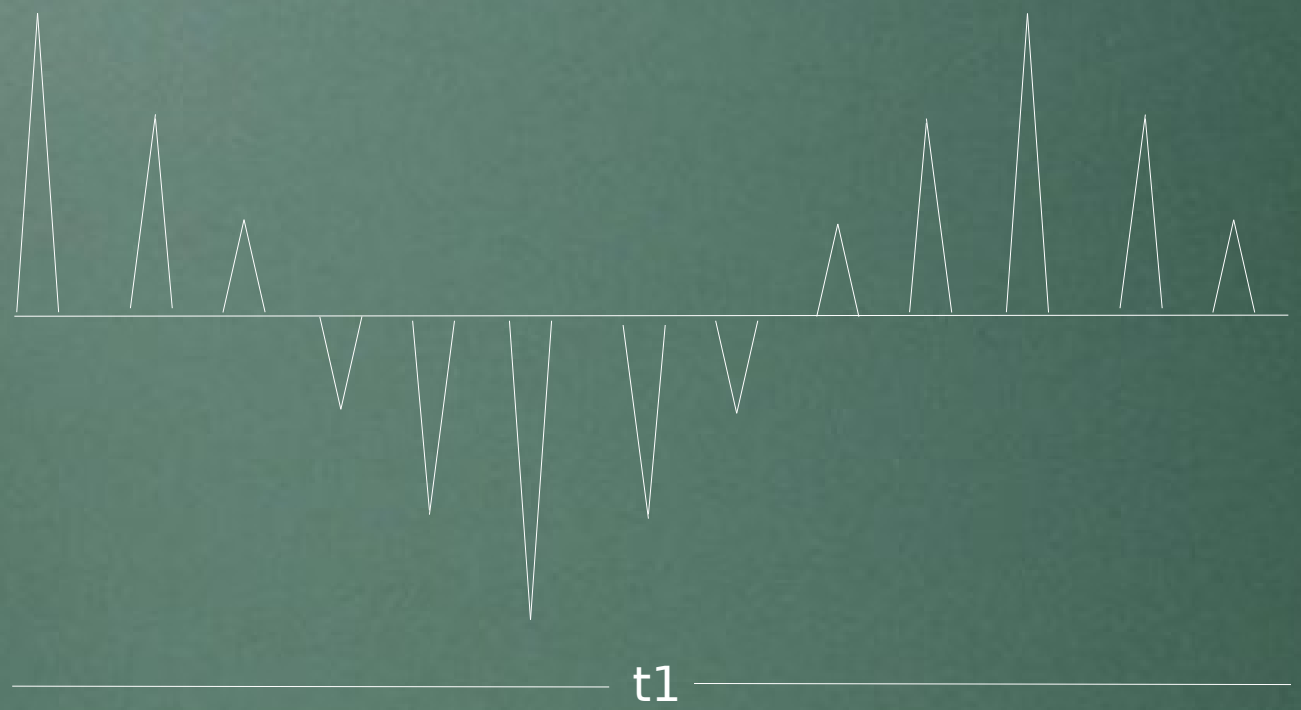
In our mind's eye we can change the way that we view the fid's:



Now fourier transform (and phase correct) each fid:

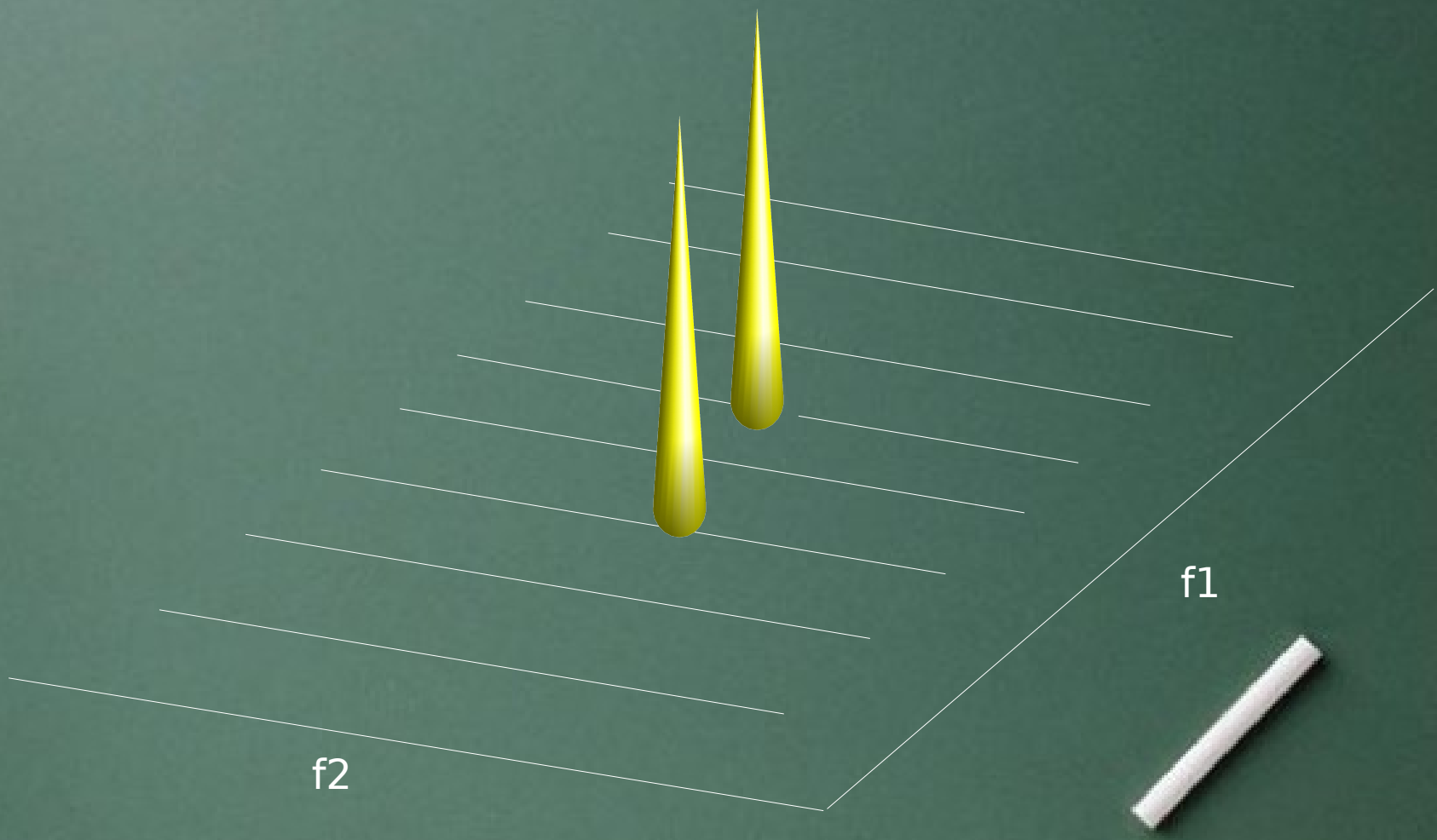
Anatomy of a Two Dimensional NMR Experiment

Anatomy of a Two Dimensional NMR Experiment

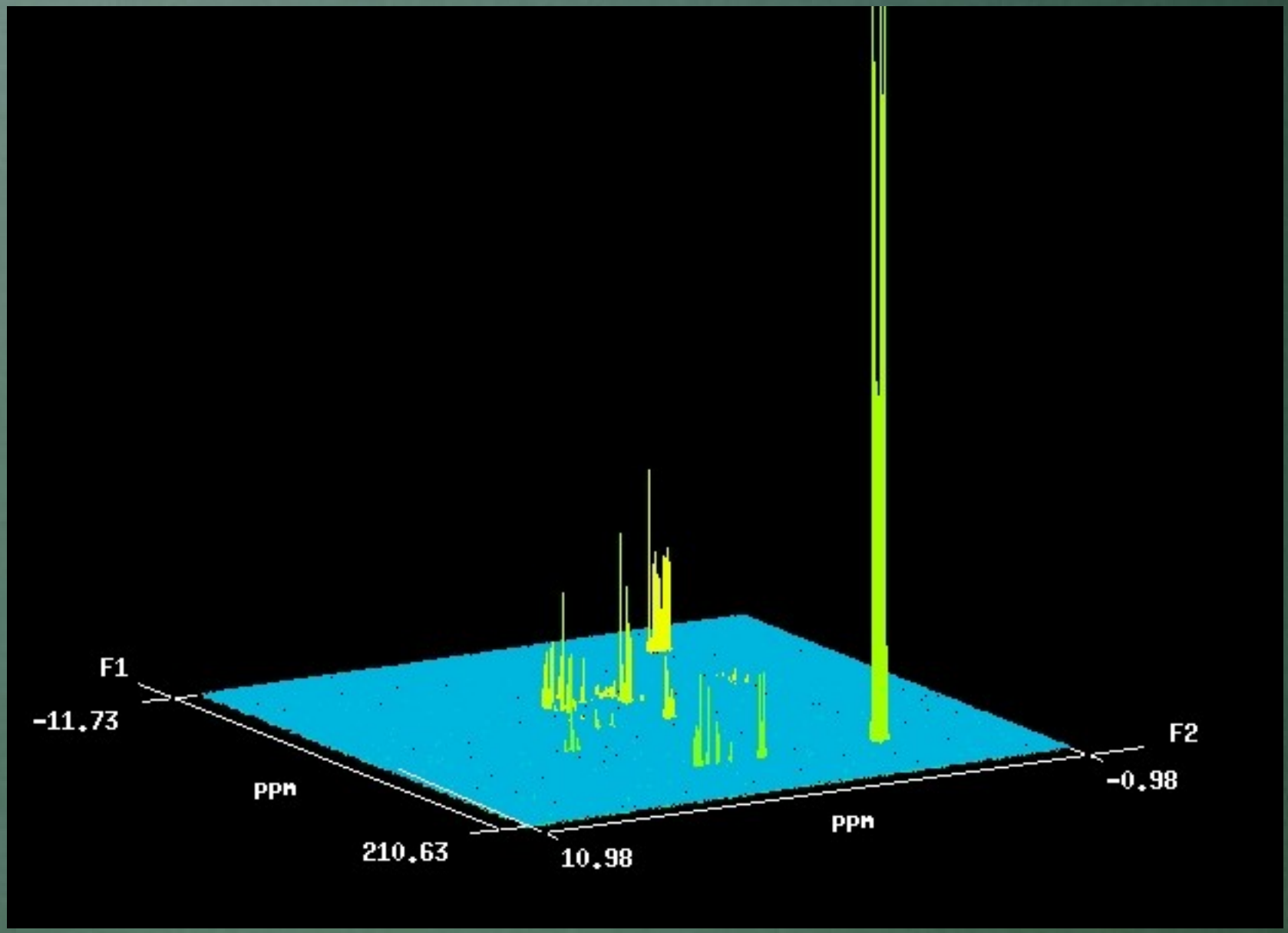


The peak amplitude points look like the data points associated with an fid but is called an *interferogram*. Can be fourier transformed just like an fid.

Anatomy of a Two Dimensional NMR Experiment



Anatomy of a Two Dimensional NMR Experiment



Anatomy of a Two Dimensional NMR Experiment

So .. how do we set up a 2D experiment? Remember that the time domain data are digitized for the duration of the acquisition time and that a certain number of data points are generated. Typically for ^{1}H this might be 16k points and be acquired in 8 scans for a total experiment time of say, two minutes. For a 10 ppm spectral width on a 500 Mhz spectrometer this would give a digital resolution of 5000 Hz/16384 points or 0.305 Hz/point.

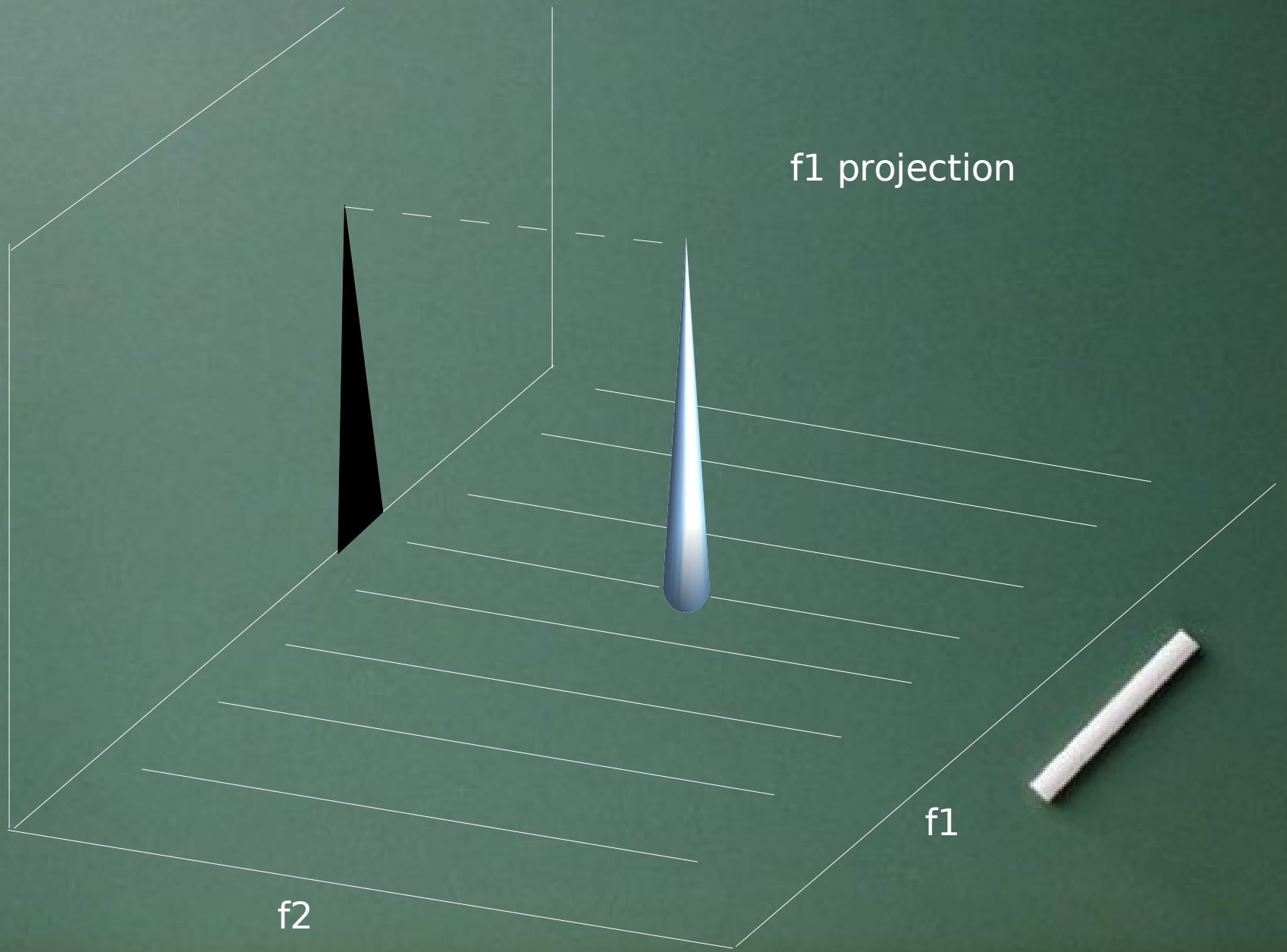
If we set our 2D experiment up so that we get the same resolution in each dimension as our 1D ^{1}H experiment that means each fid would contain 16K data points and we would have to aquire 16K fids! The total time required to do the experiment would be (assuming average time per fid acquisition of two minutes) 2x16384 = 32768 minutes or 546 hours or 22.75 days! Also the file size would be huge .. at least 16K x 16K x 2 (larger actually, since real and imaginary data stored simultaneously) or approximately 540 MB. (the 2 is there because a 16-bit data point consists of two 8-bit bytes).

Anatomy of a Two Dimensional NMR Experiment

Fortunately, it is not necessary to have the same resolution in a 2D experiment in either dimension as in a 1D experiment. We can generally get away with much coarser digital resolution since our peaks are being spread out over a second dimension and can usually be resolved.

Typically the t2 dimension will have 1k or 2k data points and the t1 dimension will have from 128 to 512 data points (not k!). This means that we can do the experiment in a much shorter time and use much less disk space.

Anatomy of a Two Dimensional NMR Experiment



Anatomy of a Two Dimensional NMR Experiment

This is an unusual way to get the chemical shift of very insensitive nuclei such as ^{15}N. You would do an HMBC with ^{15}N as the heteronucleus and then make a F1 projection and save it as a 1d file. From this you can get the chemical shifts of the ^{15}N nuclei in the sample. The spectrum will not be as well resolved as a normal 1D spectrum since we don't generally use high resolution in the F1 dimension but .. you can't have everything!

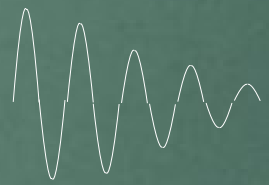
Setting Up a 2D Experiment

Setting up a 2D acquisition is similar to setting up a 1D experiment except that parameters for two dimensions must now be considered. The major considerations are the number of data points in each dimension, the nucleus associated with each dimension, the offset frequency for each dimension and the spectral width for each dimension.

It is not necessary to have the same digital resolution in a 2D spectrum as in a 1D spectrum since nmr responses are now being spread out in two dimensions. Therefore instead of having a TD2 of, say, 32k points we would generally use 1k-2k data points per fid. This will also have the happy consequence of saving much disk space and acquisition time ... it takes 32 times as long to fill 32k compared to 1k data points. There is also a (minor) problem associated with this. The fid in 1k data points will generally be truncated or cut off before it has fully decayed.

Setting Up a 2D Experiment

Instead of :

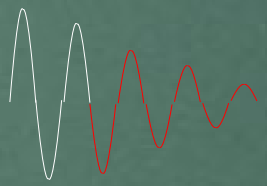


We generally see something like :

Setting Up a 2D Experiment

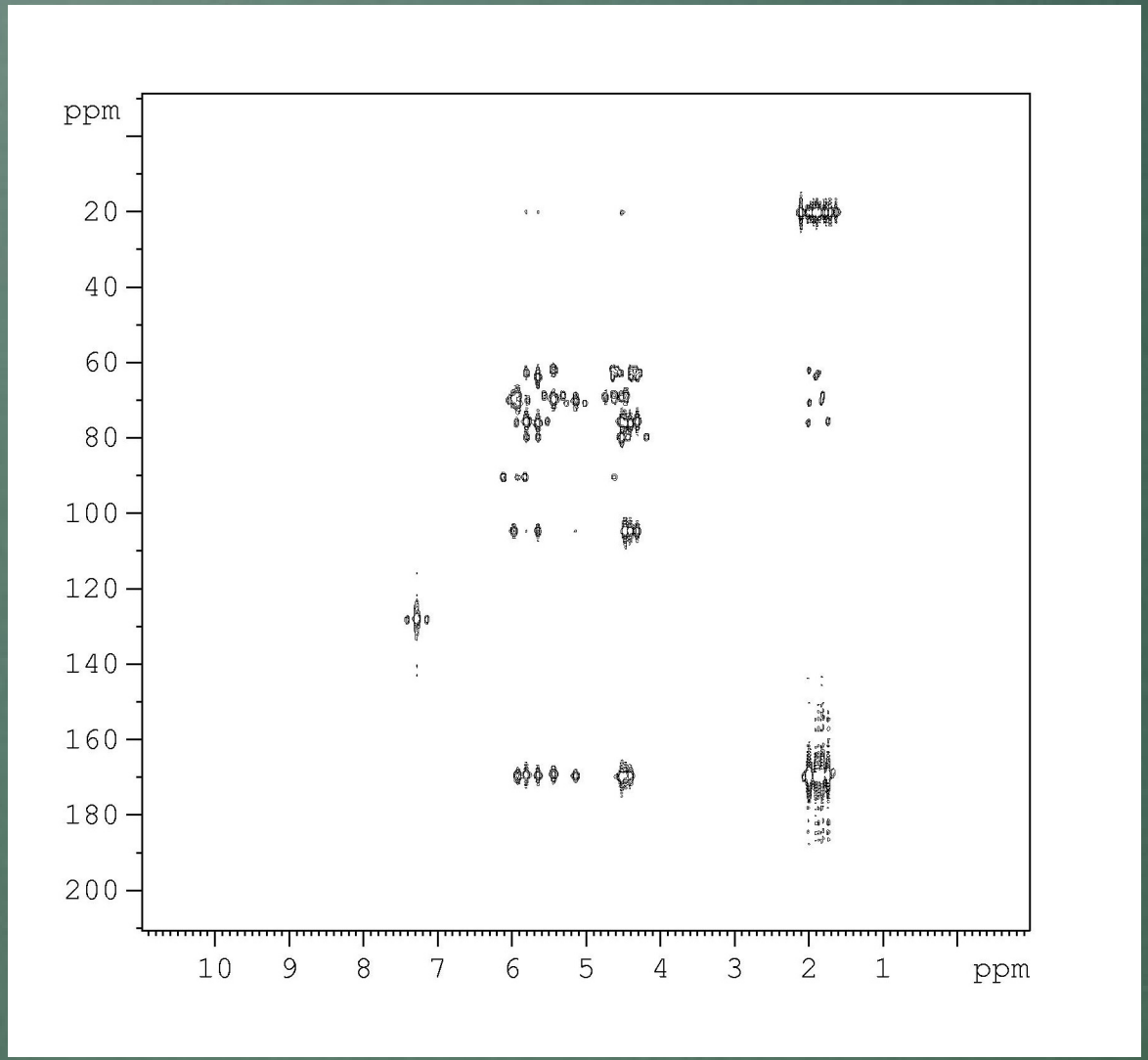
In 1D spectroscopy this would lead to a truncation artifact called 'sincing' in which there will be small 'squiggles' or oscillations at the base of the nmr peak in the transformed spectrum.

In 2D this shows up as symmetric 'trails' around the peaks in the spectrum. It is easy to get rid of, however, by using linear prediction when processing the data. This simply predicts what the fid would have looked like if it had been allowed to decay fully:



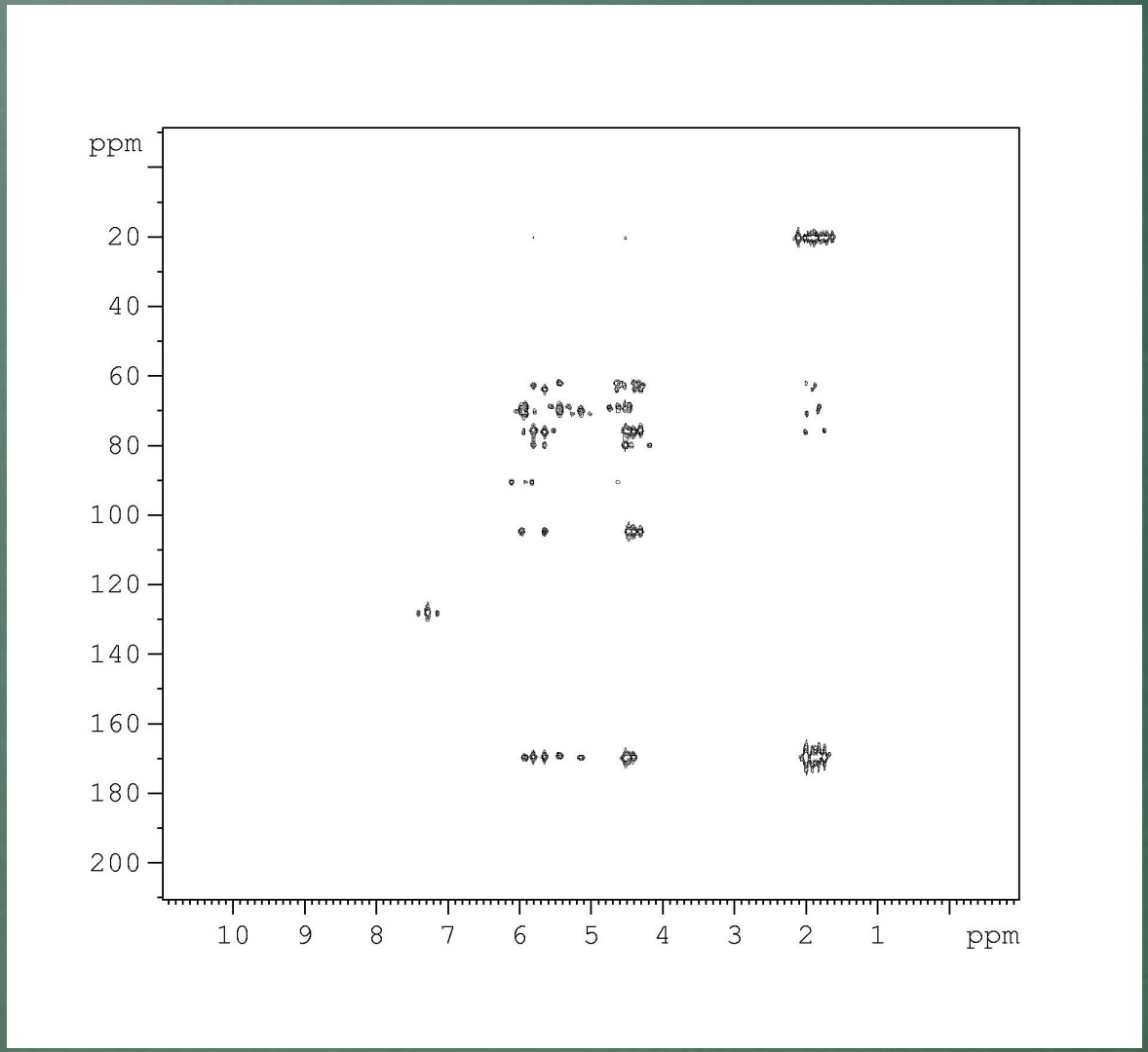
Setting Up a 2D Experiment

Without linear prediction:



Setting Up a 2D Experiment

With linear prediction:



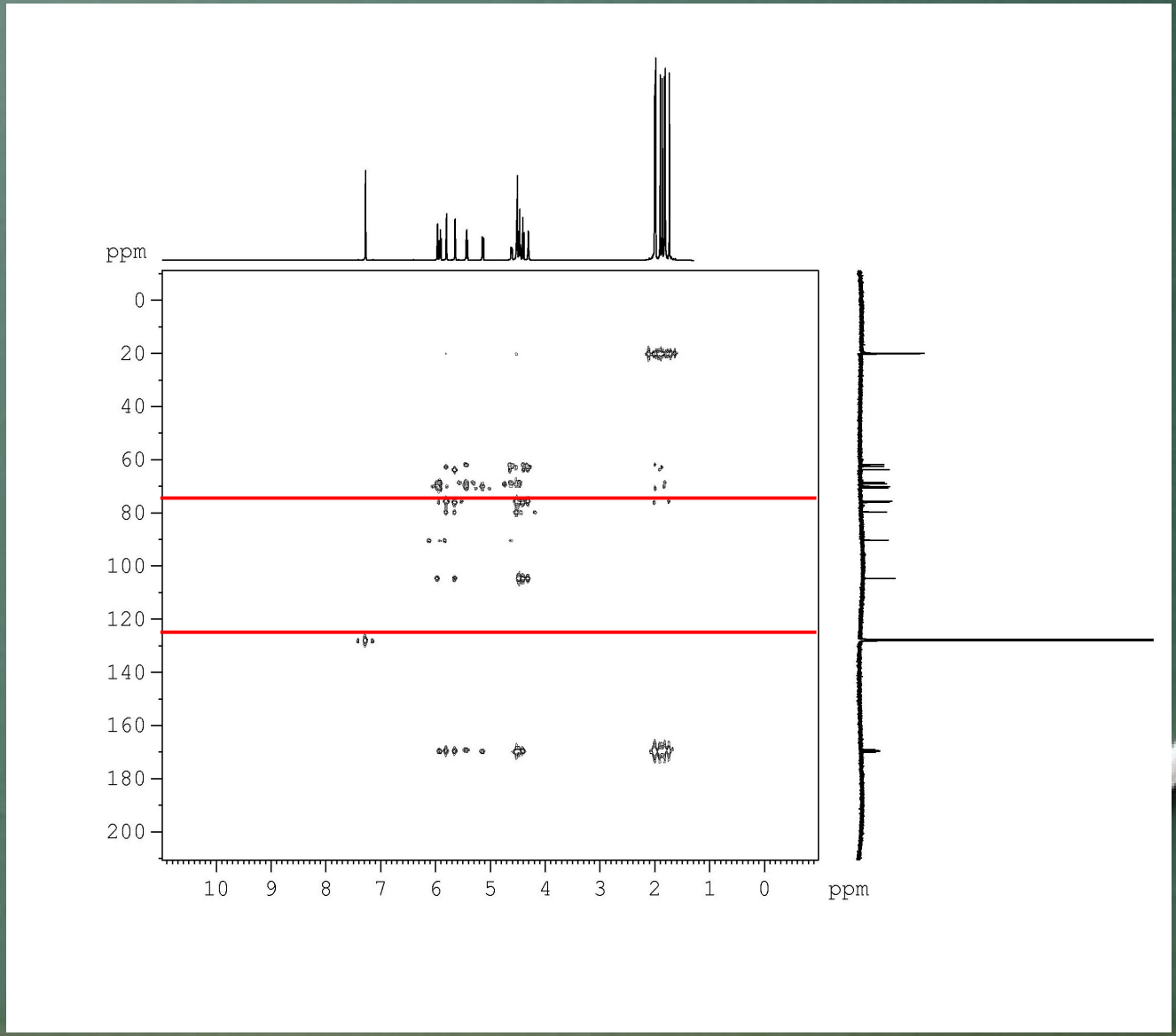
Setting Up a 2D Experiment

Something to be aware of when doing 2D nmr spectroscopy is that you can and usually do have digital filtering on during t2, the acquistion period. This means that there will be no folding of peaks outside of the spectral window in f2. This is **not** the case in f1 however. There is ***no*** digital filter applied in this dimension during acquistion.

When setting an experiment up from scratch it is necessary to take this into account by setting the transmitter frequency offset (O1) and the spectral window (SW) appropriately so as to avoid folding in f1.

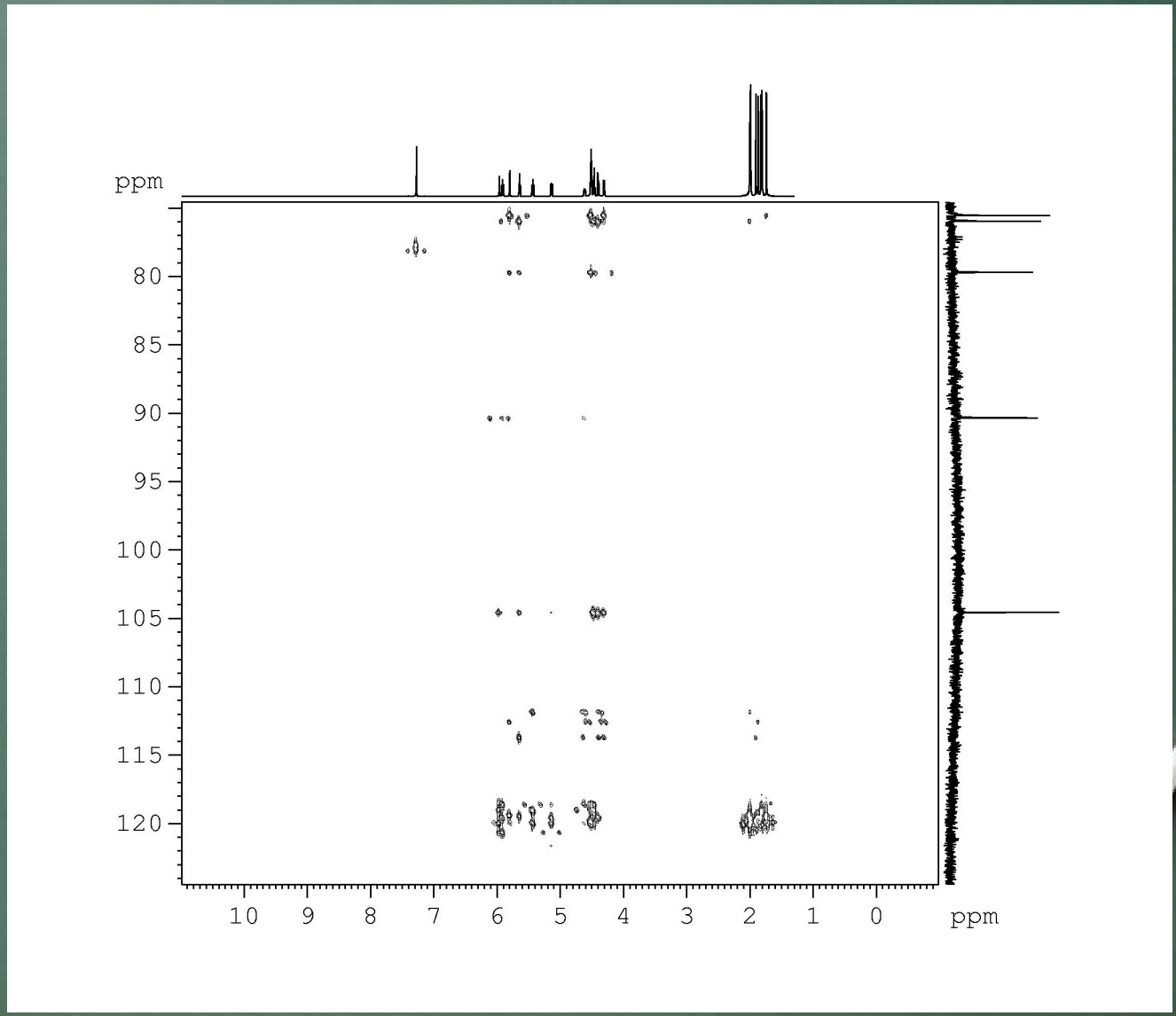
Setting Up a 2D Experiment

Normal HMBC:



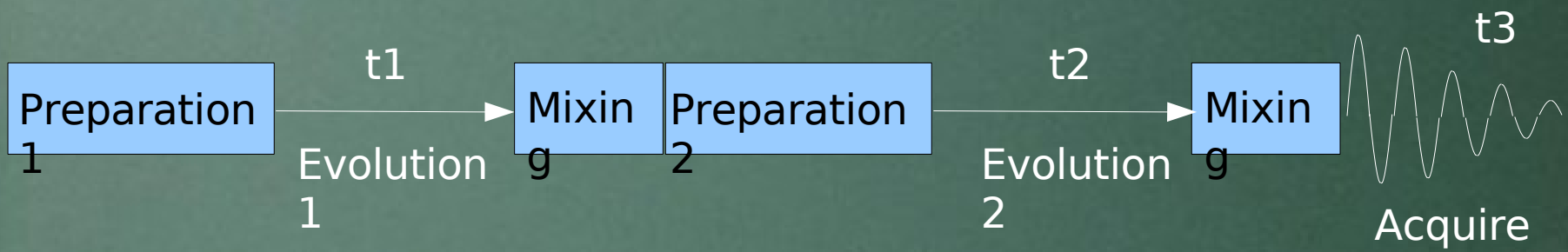
Setting Up a 2D Experiment

Folded HMBC:



3D!! Experiments

We can take the basic ideas behind the 2D nmr experiment and extend it to three dimensions:

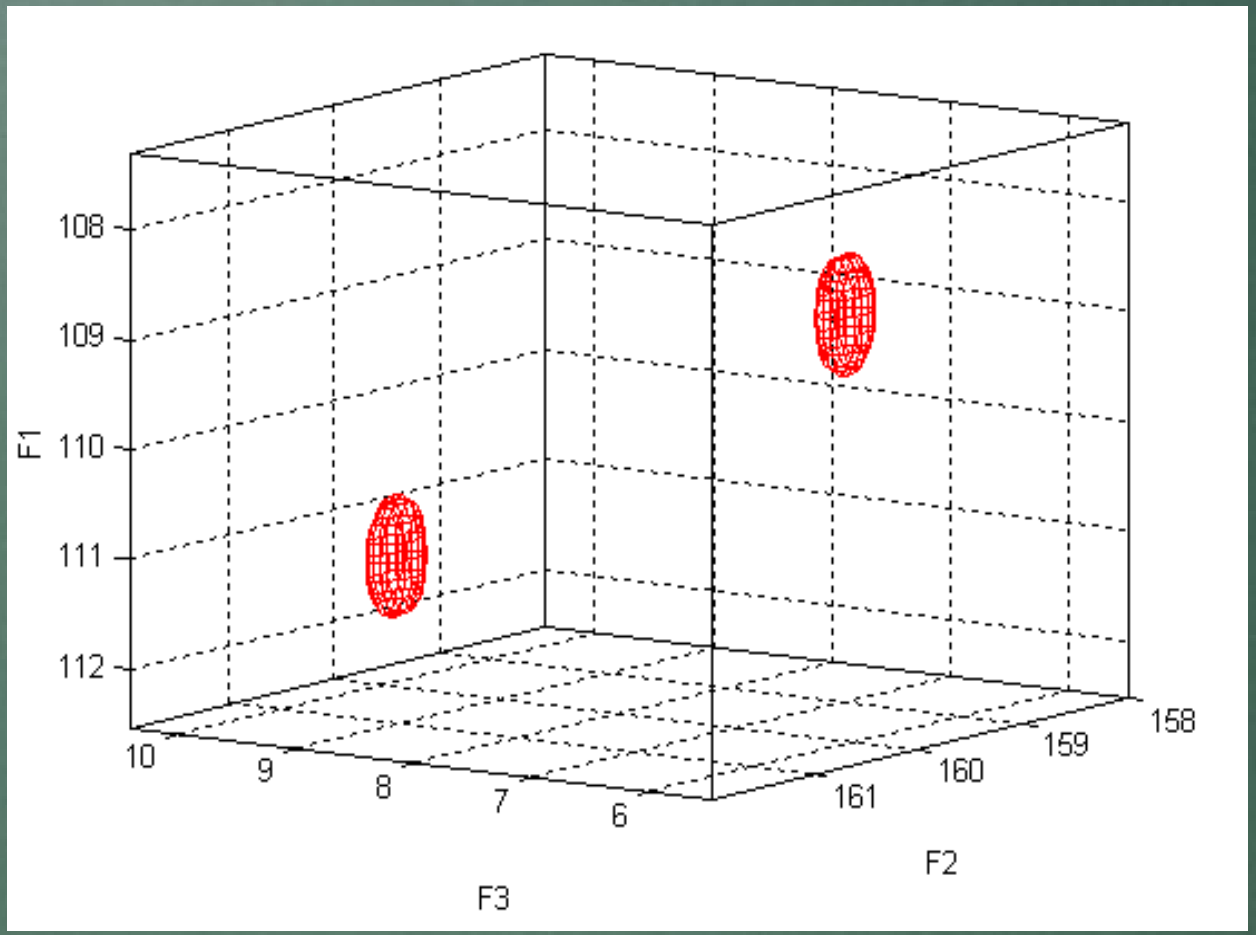


We now have ***two*** variable evolution periods, **t1** and **t2** followed by the mixing period and acquisition times. As in 2D nmr spectroscopy, information is encoded in t1 and t2. In this way we can incorporate two 2D experiments into one pulse sequence. We may, perhaps do a NOESY-HSQC experiment in which the two pulse sequences are incorporated into one.

The advantage of doing this is that very crowded areas of the spectrum can be spread out in three dimensions rather than two.

3D!! Experiments

3D!! Experiments

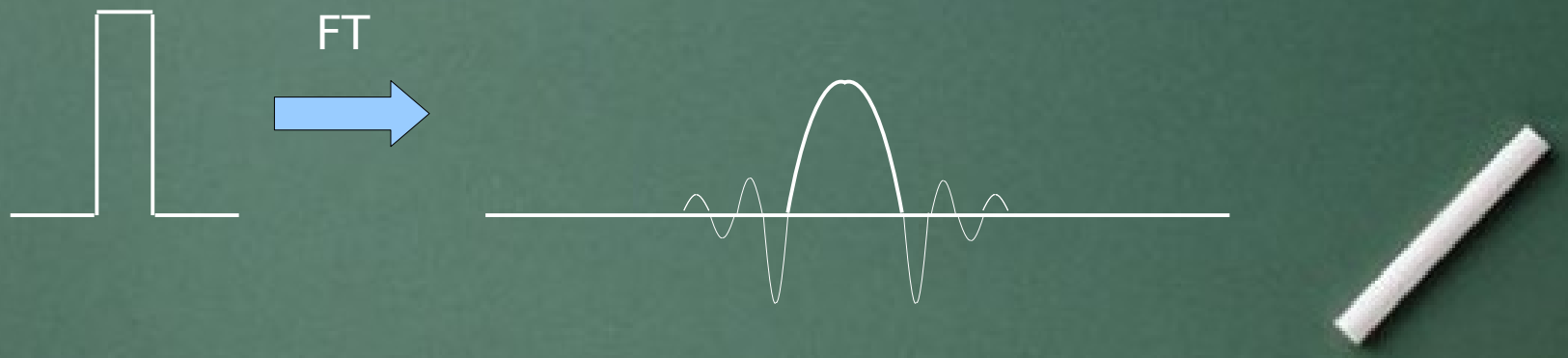


3D!! Experiments

Selective 2D Experiments

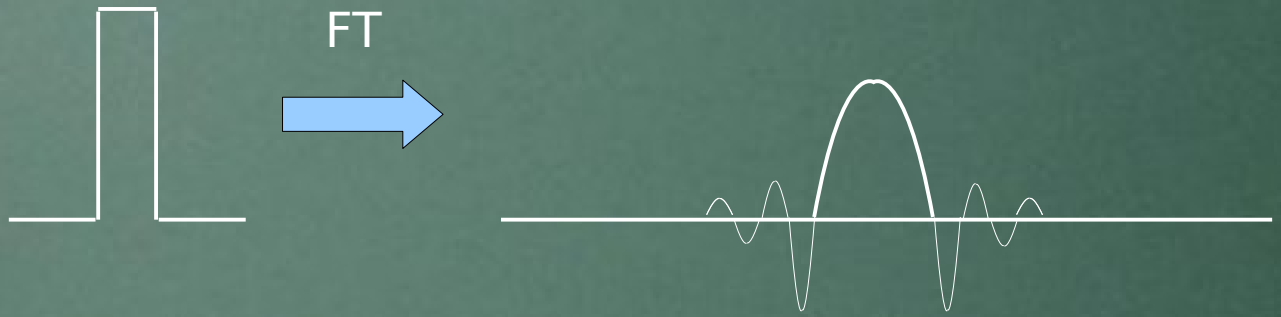
These experiment use the same pulse sequences as their corresponding 2D 'cousins' but use shaped pulses instead of rectangular, high-power pulses.

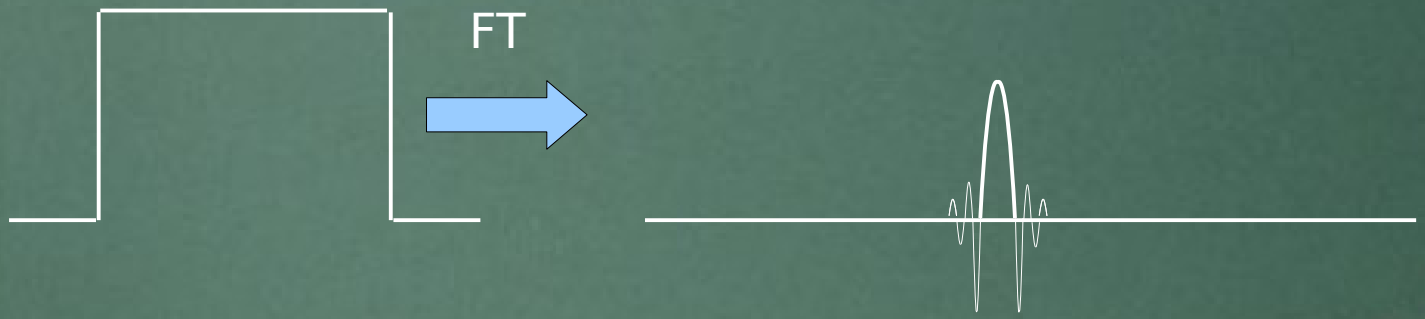
A shaped pulse will be much more selective in which nuclei are irradiated than a rectangular pulse will be. A short, high-power rectangular pulse will irradiate a wide frequency range because the fourier transform of the rectangle is a 'sinc' function:



Selective 2D Experiments

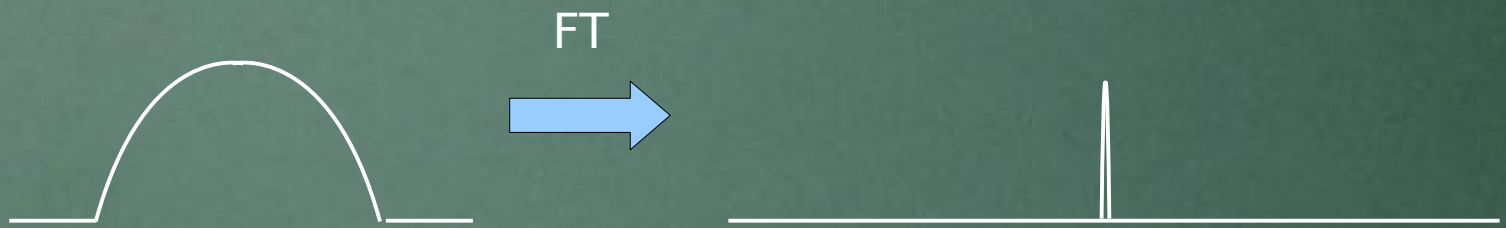
Also, the narrower the pulse the wider the excitation bandwidth:





Selective 2D Experiments

The sinc 'squiggles' at the sides can be greatly reduced by using shaped pulses. Also, if the pulse length is long the excitation bandwidth will be very small:



What does this mean for us? It means that if the offset frequency of the transmitter is exactly on the resonance of a particular nucleus, ***only*** that nucleus will be excited. If we were to do a normal 1d proton spectrum with a shaped pulse substituted for the regular rectangular pulse we would observe only one peak in the spectrum and we would have done a 'selective' experiment by selecting only that peak.

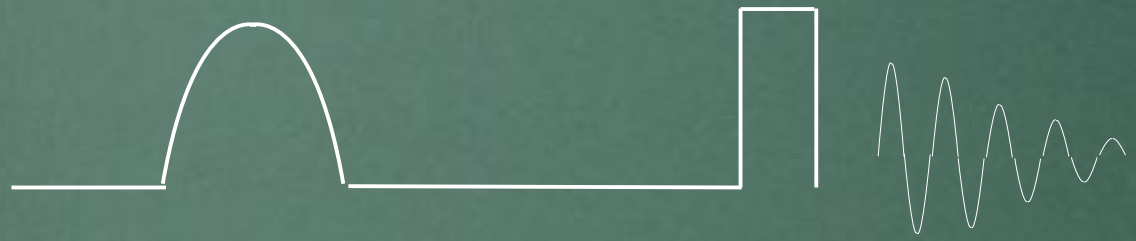
Selective 2D Experiments

What do we mean by a long pulse and a short pulse? Generally we measure pulse times in microseconds and a typical high power proton rectangular pulse may be on the order of 10 μsec in length. Shaped pulses on the other hand are usually at least 2000 μsec or longer depending on how selective we want them to be. They will, of course be at a lower power than the regular rectangular pulse.

What will happen if we replace the appropriate rectangular pulse in a 2D experiment with a shaped pulse?

Selective 2D Experiments

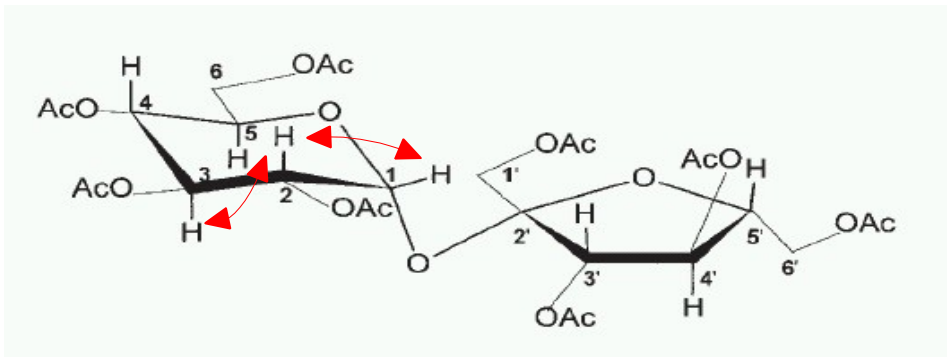
Let's try it with the COSY experiment:



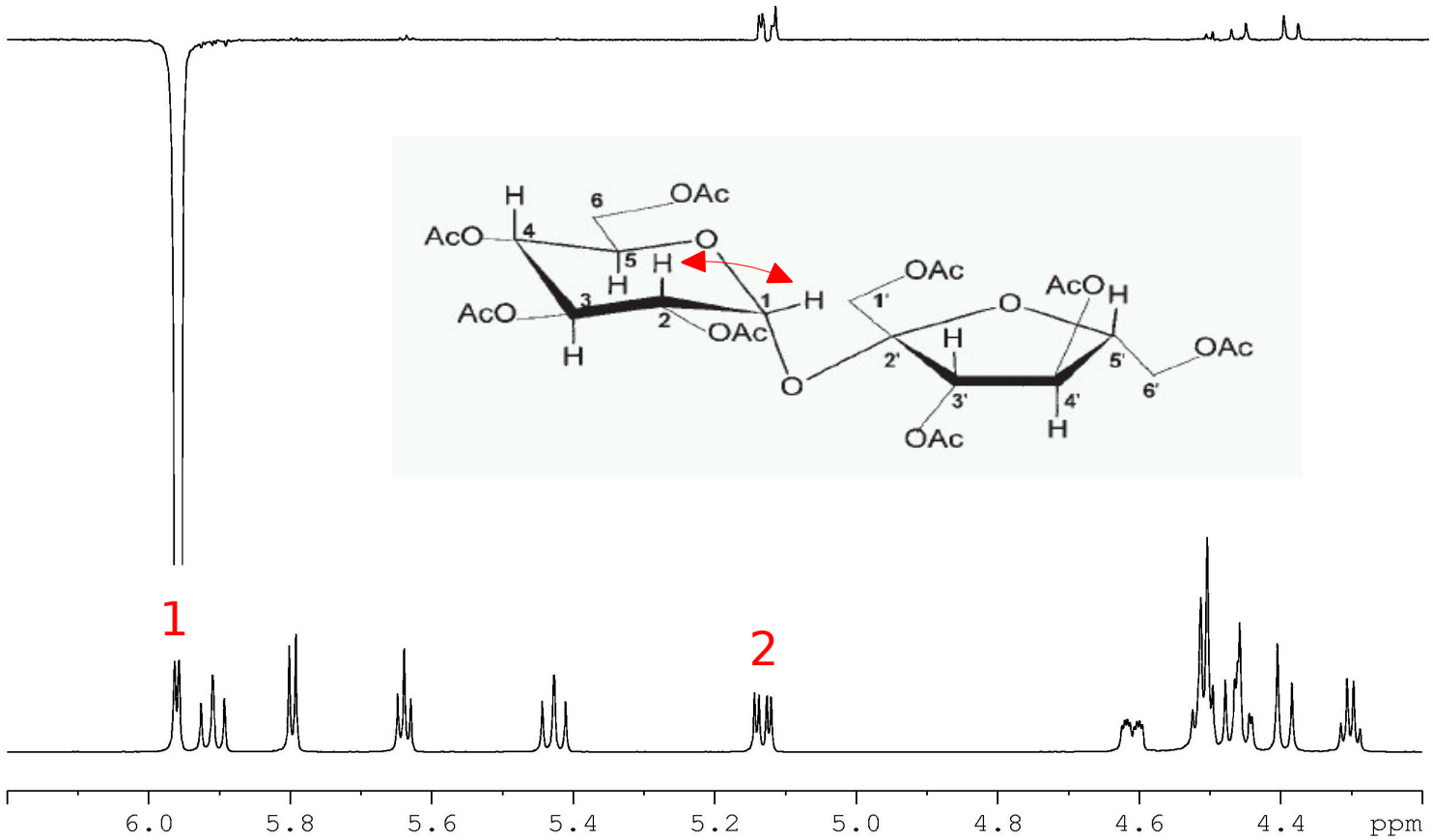
Selective 2D Experiments

The first is the 'normal' COSY pulse sequence with the variable t1 delay between the two 90 degree pulses. The second has a shaped pulse in place of the first rectangular pulse which means that only a vary small range of frequencies in the spectrum will be excited ... on resonance in fact. This is equivalent to looking at a 'slice' of the regular 2D spectrum. In other words, it is a one dimensional version of the two dimensional spectrum!

Selective 2D Experiments



Selective 2D Experiments



References
(all available in the Natural Sciences library except *)

Introductory with little math:

Sanders and Hunter, *Modern NMR Spectroscopy: A Guide for Chemists*.

Derome, *Modern NMR Techniques for chemistry research*.

Intermediate with math:

Levitt, *Spin Dynamics.*

Keeler, *Understanding NMR Spectroscopy.*

Advanced .. *lots* of math:

Slichter, *Principles of Magnetic Resonance*.

Ernst, Bodenhausen, Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*.

Practical information:

*Braun, Kalinowski, Berger, *200 and More Basic NMR Experiments*.

Fukushima, Roeder, *Experimental Pulse NMR; A Nuts and Bolts Approach*.

My Website:

http://chem4823.usask.ca/nmr/practical_nmr.html