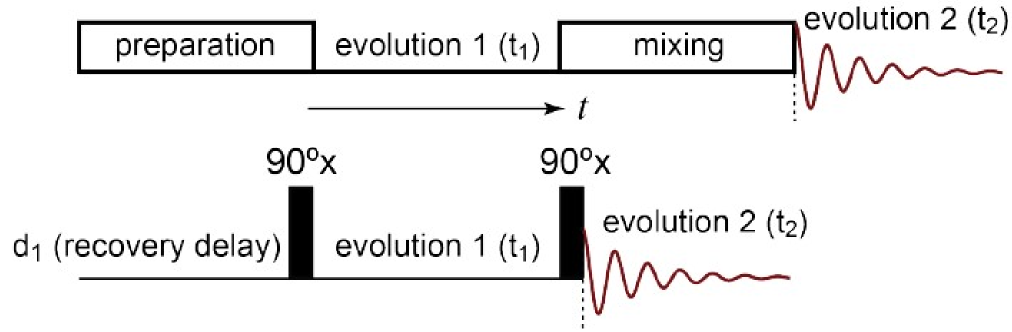
A brief history of 2D NMR!

- In 1971, the idea of 2D NMR was proposed by Jean Jeener.
- The 1975 Ernst paper “Two-dimensional spectroscopy, application to nuclear magnetic resonance” utilized Jeener’s idea to produce spectra.
- 1980s-present: application of NMR to protein structures
- In 1991 Ernst won a Nobel Prize in Chemistry for his contributions to Fourier Transform NMR

2D ^1H-^1H COrrelation SpectroscopY (COSY)

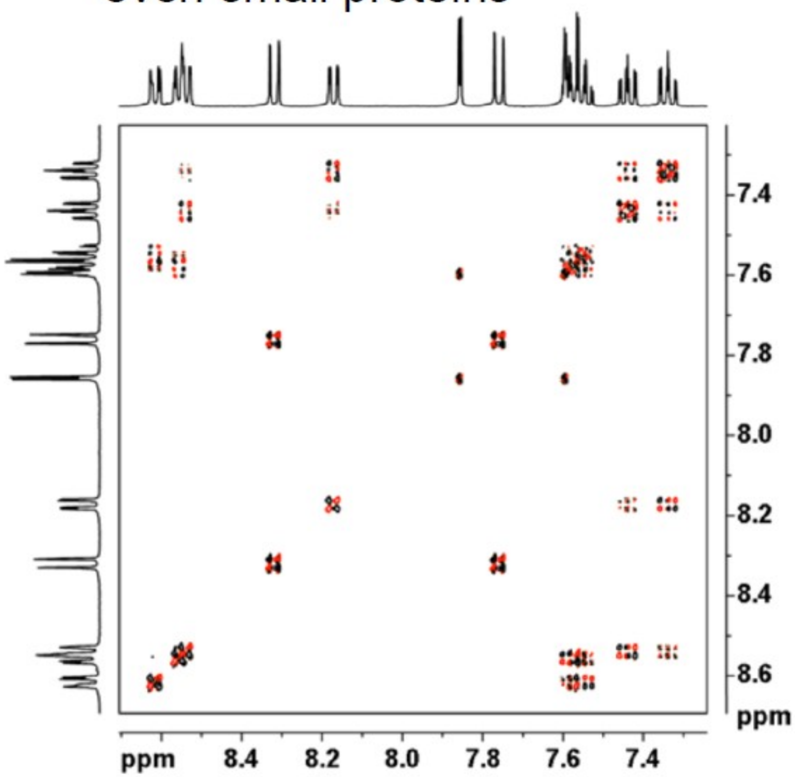
- One of the first, and most useful 2D experiments
  - one of the most common homonuclear experiments
  - workhorse experiment for small molecules to correlate coupled ^1H nuclei
  - 2- and 3-bond couplings mostly, but possible for longer range
  - good for correlating nuclei in small spin systems
- Deceptively simple looking experiment
  - preparation period: relaxation delay (d_1), then 90° *x* nonselective pulse to generate transverse magnetization for all ^1H nuclei
  - evolution period (t_1), all evolve according to chemical shifts and *J* couplings

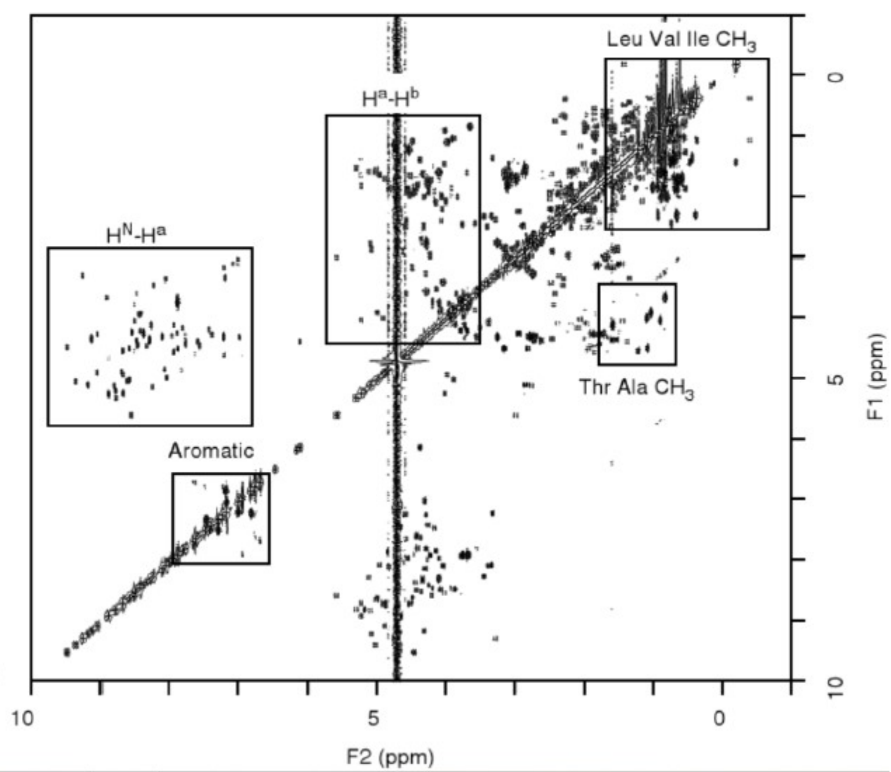


  - coherence transfer resulting from the mixing period (second 90° *x* nonselective pulse) causes a portion of the magnetization for each spin to precess at the frequencies of coupled spins!
  - crosspeaks (off-diagonal peaks) then identify frequencies of coupled spins

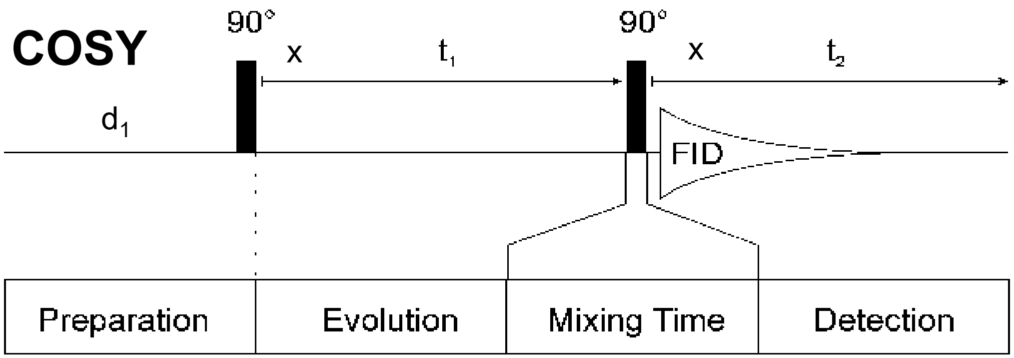
2D ^{1}H-^{1}H COrrelation SpectroscopY (COSY)

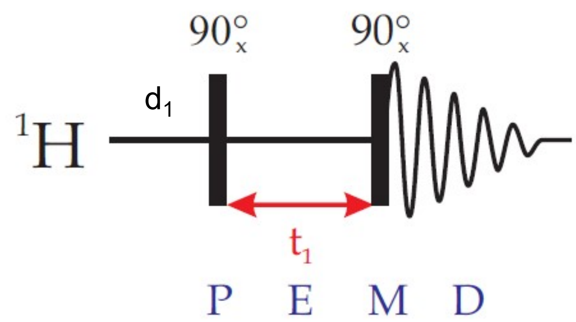
- Correlate chemical shifts of coupled nuclei in two dimensions
  - signals on diagonal are autocorrelated
  - signals off of the diagonal, crosspeaks, correlate chemical shifts of coupled nuclei (spectrum is symmetrical, each crosspeak appearing twice)
  - for proteins, is challenging to analyze some regions of COSY spectra for even small proteins

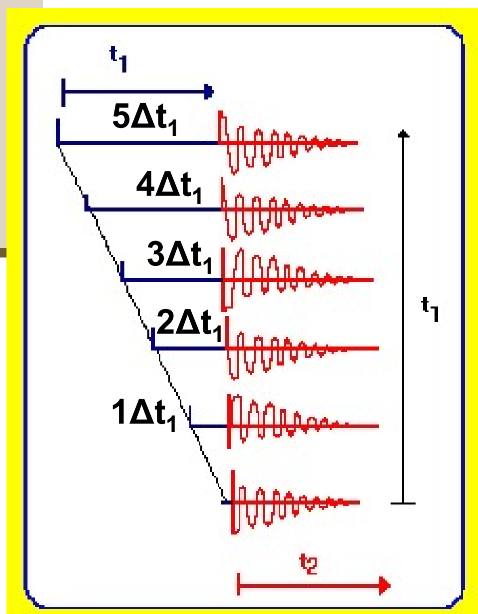


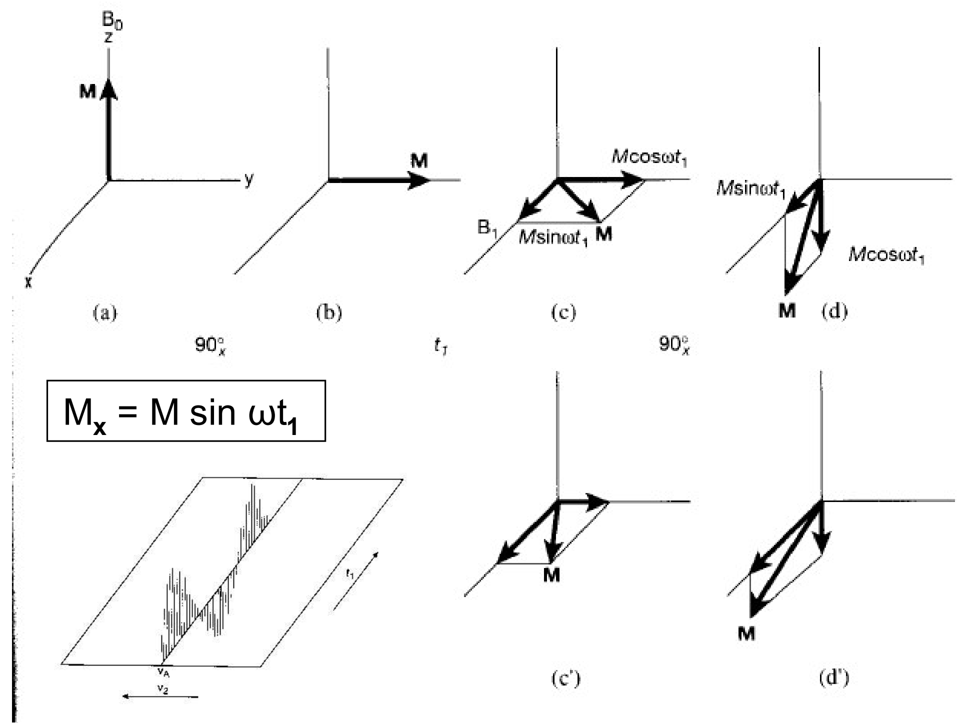


^{1}H-^{1}H COSY (90COSY, gCOSY, 45COSY, eCOSY, DQF-COSY)

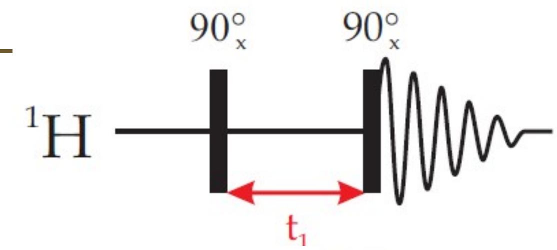


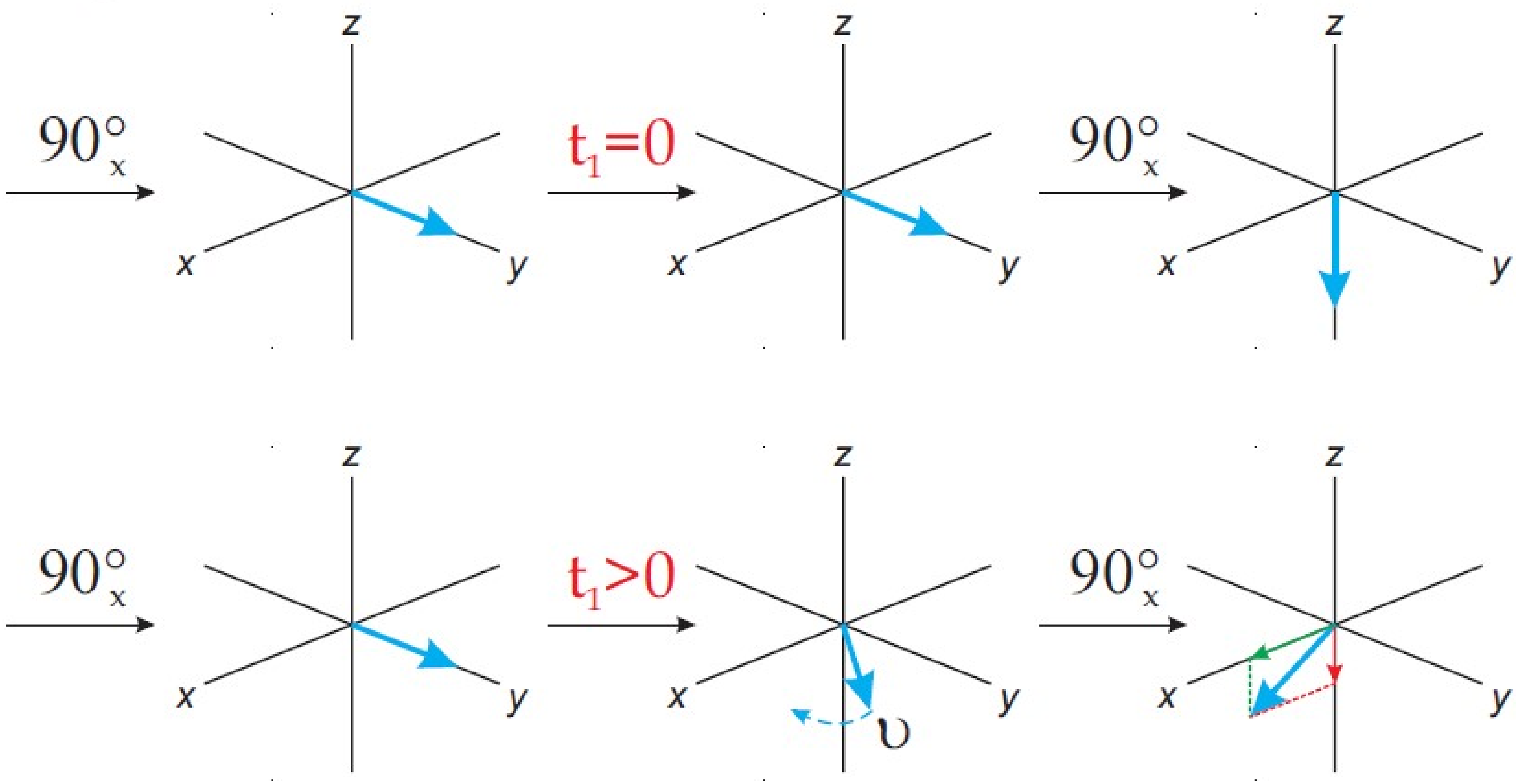






^1H-^1H COSY (spin precession with increments)



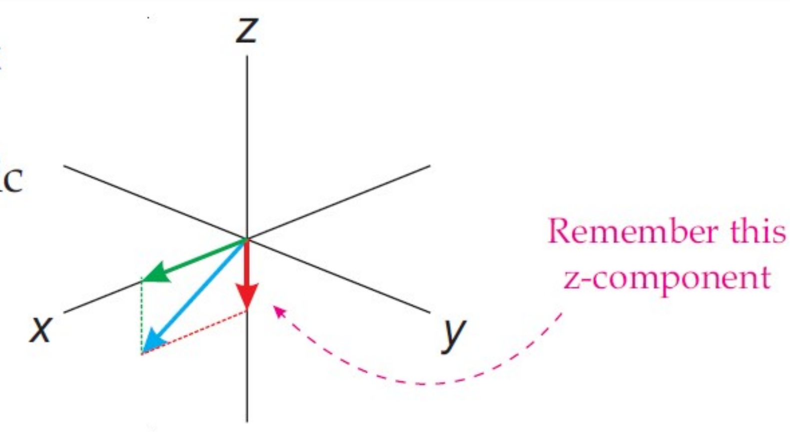


^{1}H-^{1}H COSY (f2 and t1 interferogram)

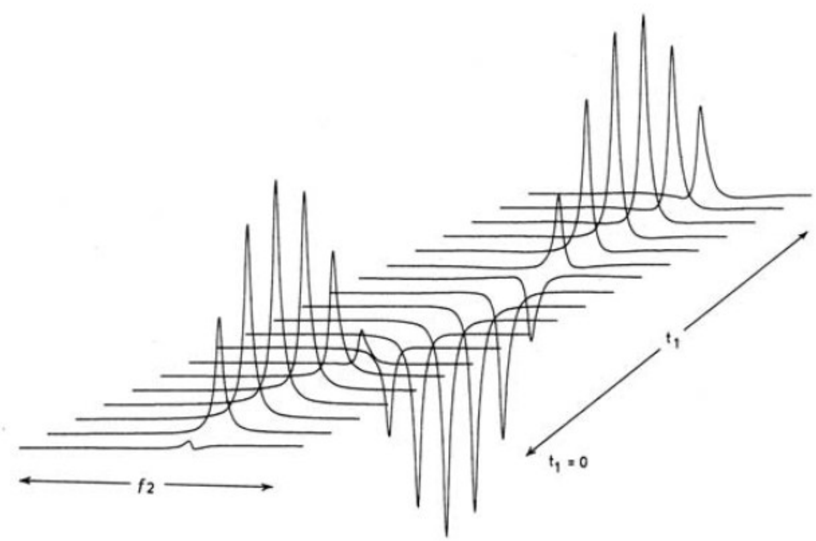
As the time t_1 is increased the x and z component of the magnetization follows a specific function.

$$M_x = M_0 \sin (360\, \upsilon\, t_1)$$

$$M_z = M_0 \cos (360\, \upsilon\, t_1)$$

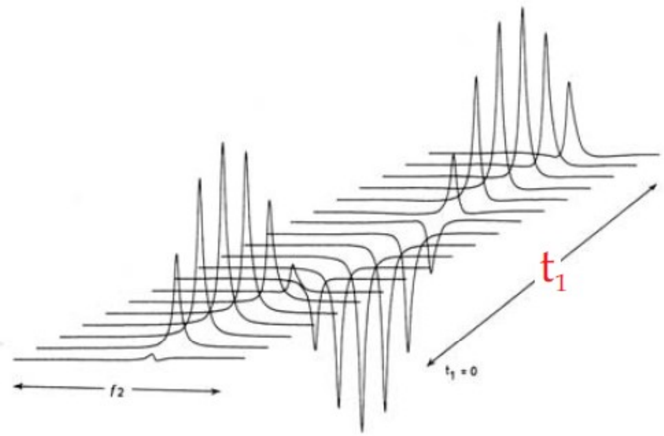


For our simple example we are only interested in the x component because data collection happens immediately after the second pulse. The FIDs at the different t_1's give spectra like this:

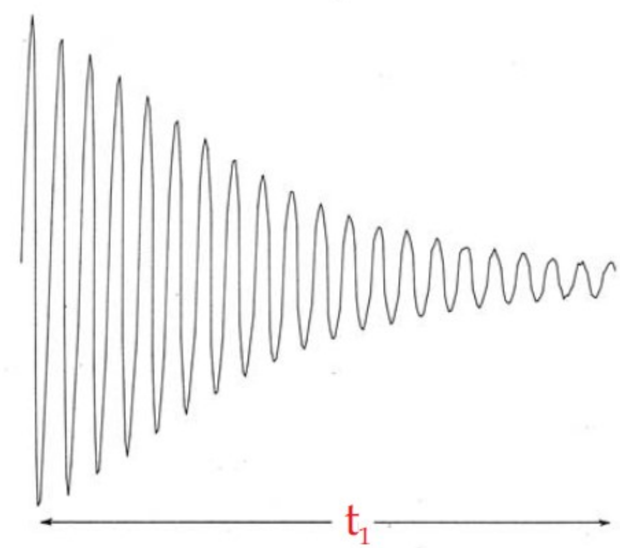


^1H-^1H COSY (f2 x t1 - interferogram)

To achieve reasonable resolution and f_1 bandwidths there are many (50-1,000) t_1 increments taken. The longer t_1 lengths show considerable T_2 (T_2^*)relaxation.



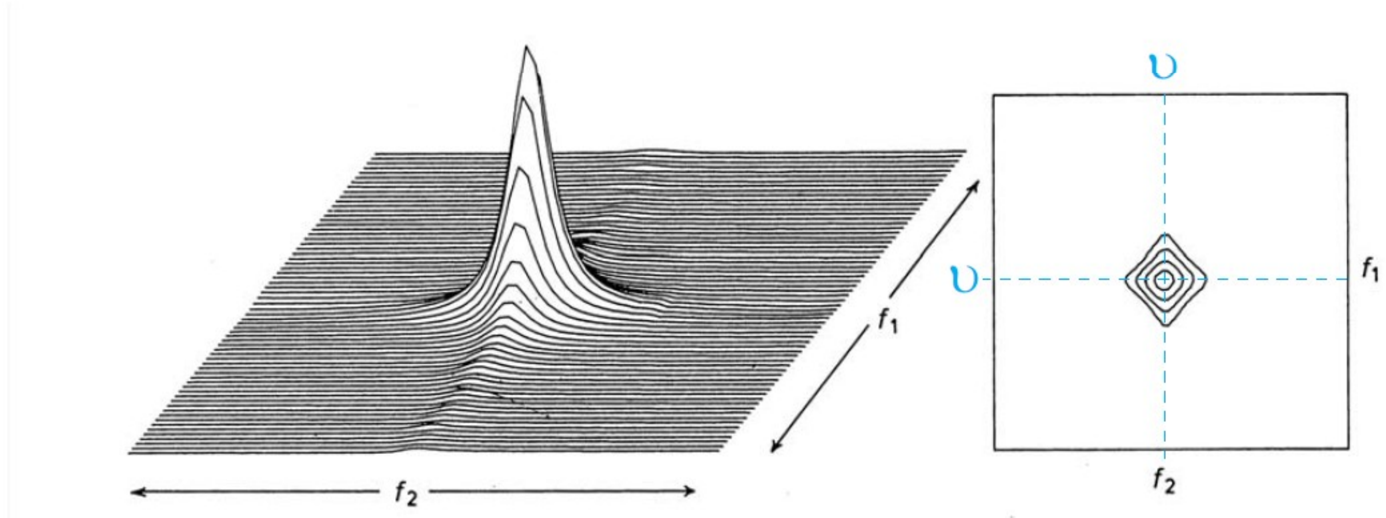
Plotting the resulting peak amplitude versus t_1 yields a wave form that looks exactly like a FID. It is commonly called an ***interferogram.***



The f_2 spectra are converted to t_1 domain data points in a process called ***transposing***. Every data point across f_2 is included, not just the peak centers. Because of this requirement, the f_2 domain is collected with a minimum number of points (short AQ and narrow SW).

^1H-^1H COSY (producing 2D peaks)

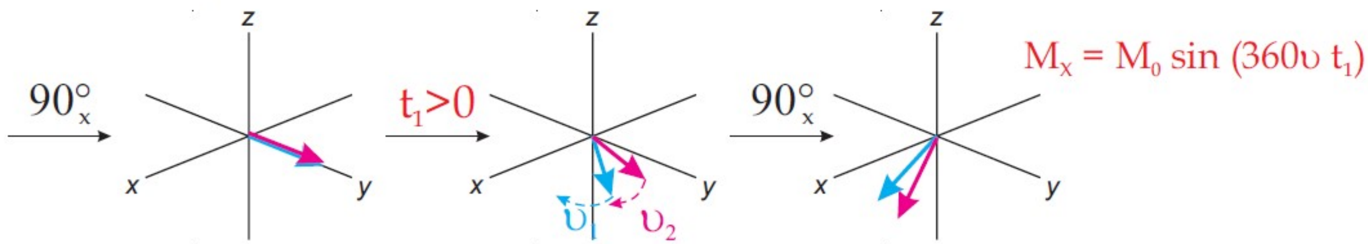
FT'ing the complete transposed data set yields a series of 1D spectra. Aligning the resulting f_1 frequency domain spectra yields a ***2D stacked spectrum*** with a single peak at the shift frequency υ in both dimensions. The data can also be represented by using a topography map style ***contour plot***.



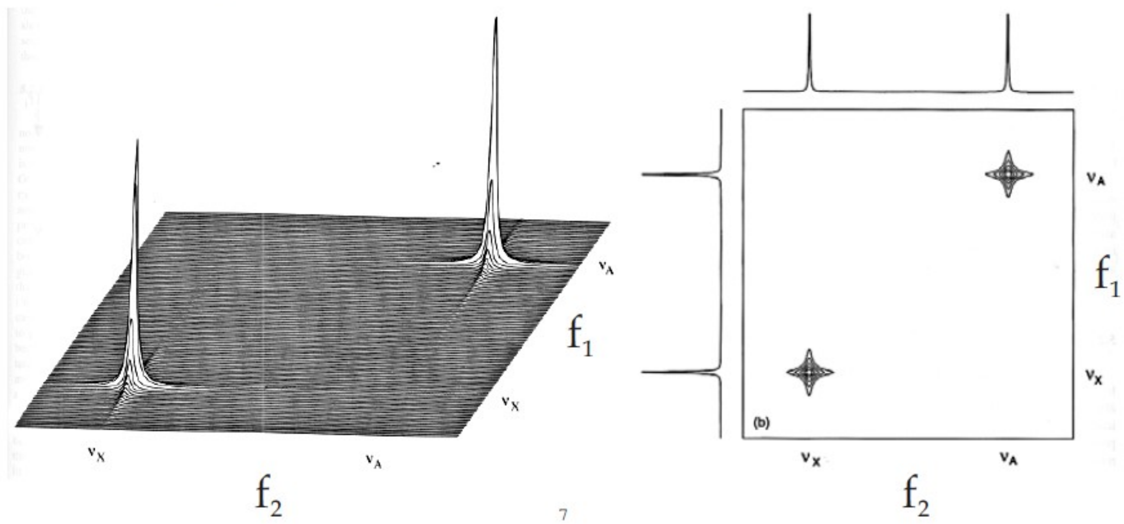
The outer ring of a contour plot represents the width of the peak at lower heights. The inner rings show how the pear narrows at higher slices.

^{1}H-^{1}H COSY (producing 2D diagonal peaks)

If we have two uncoupled resonances, both off resonance, the vector diagram will look like:

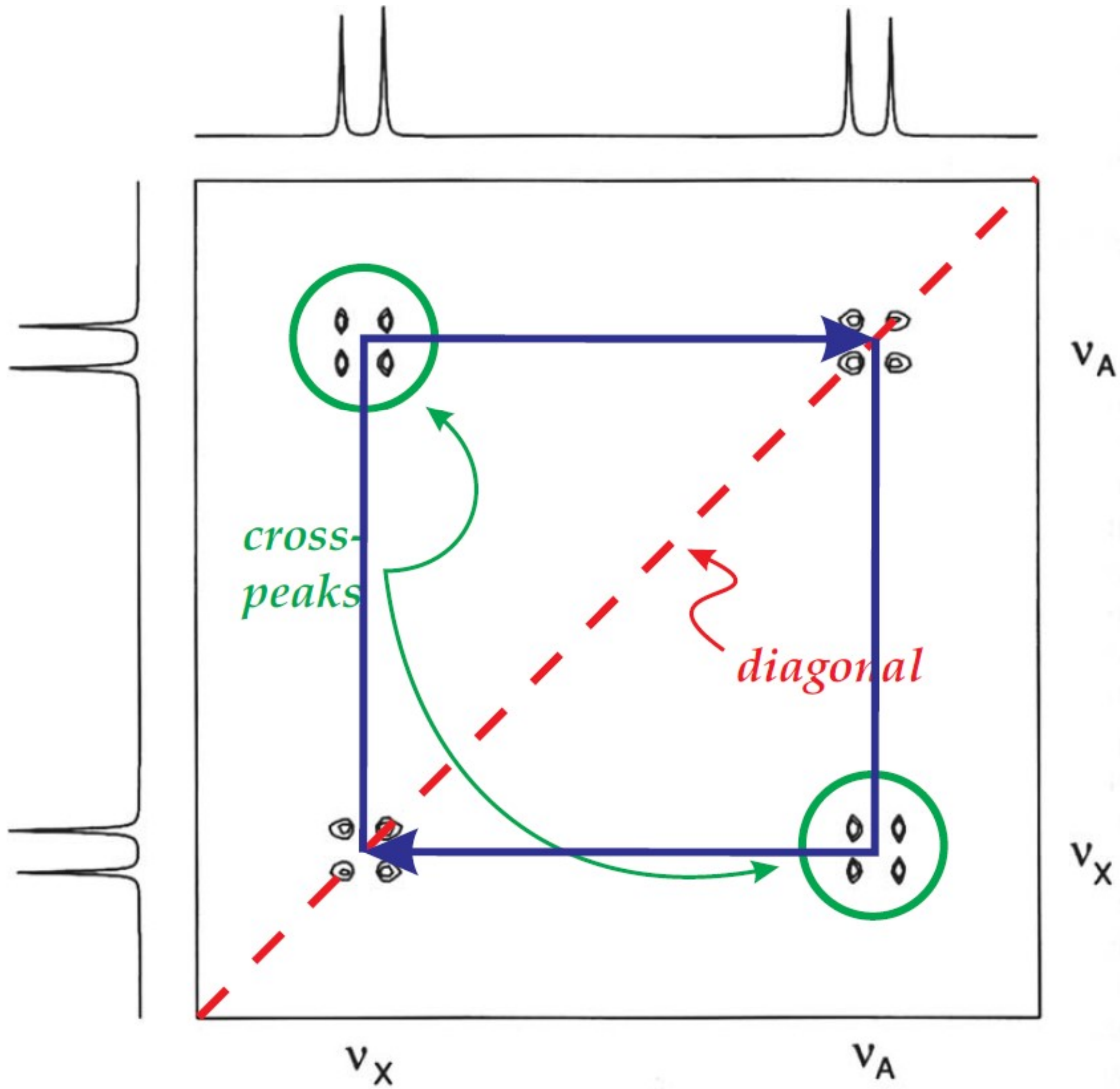


The resulting 2D transformation would give two resonances that fall on the diagonal. This concept is called ***chemical shift evolution***. Almost all 2D sequences have evolution based on chemical shifts.

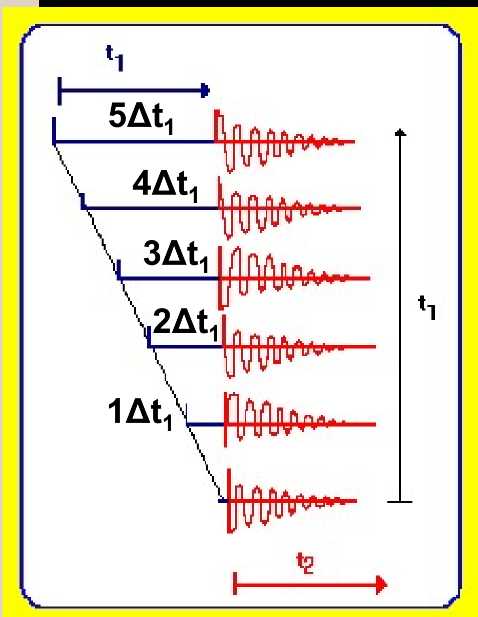


^1H-^1H COSY (peak pattern)

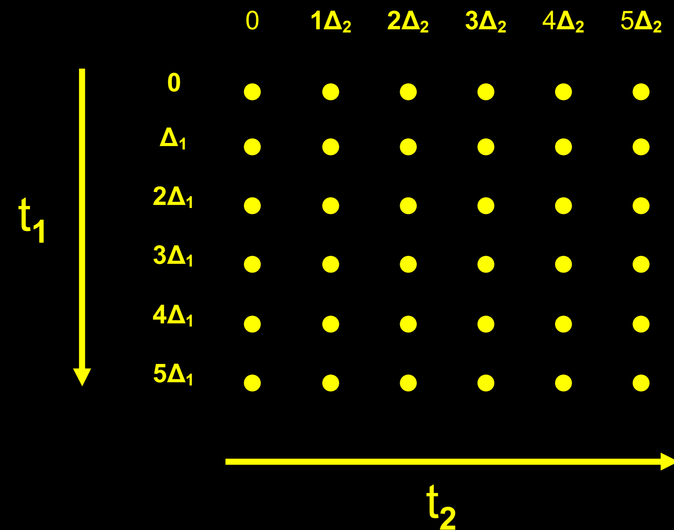
The cross-peaks *"connect"* the two resonances that couple each other.

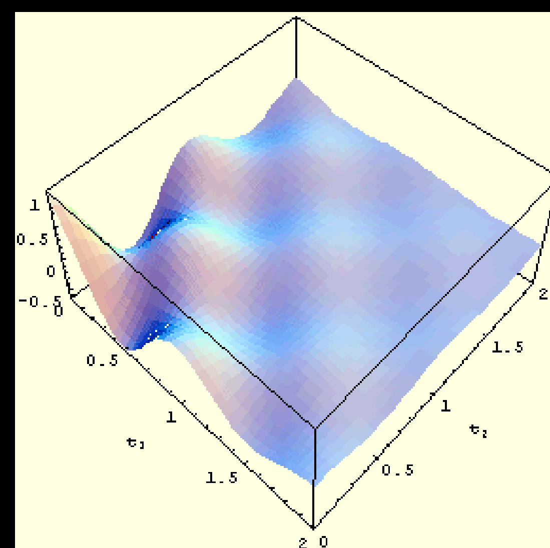


COSY



time domain

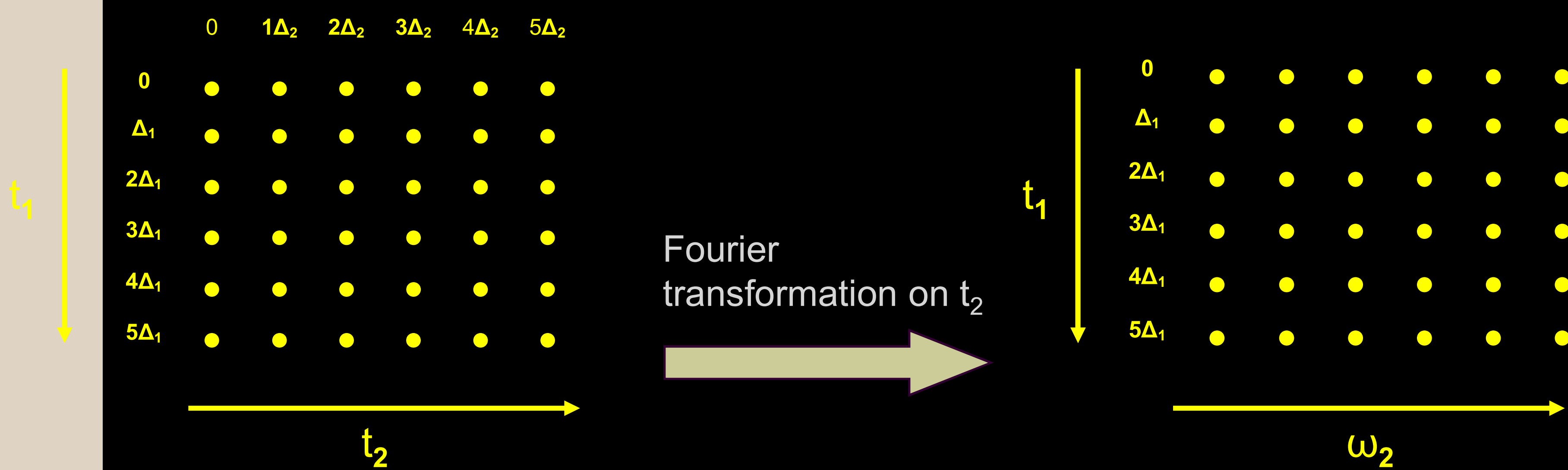


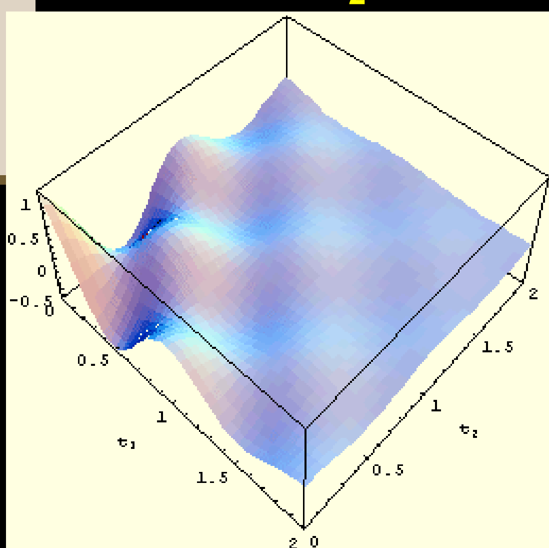


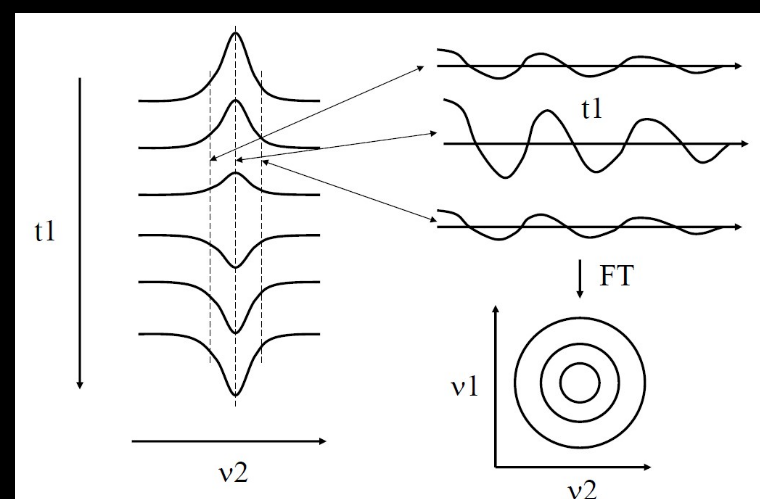
COSY

time domain

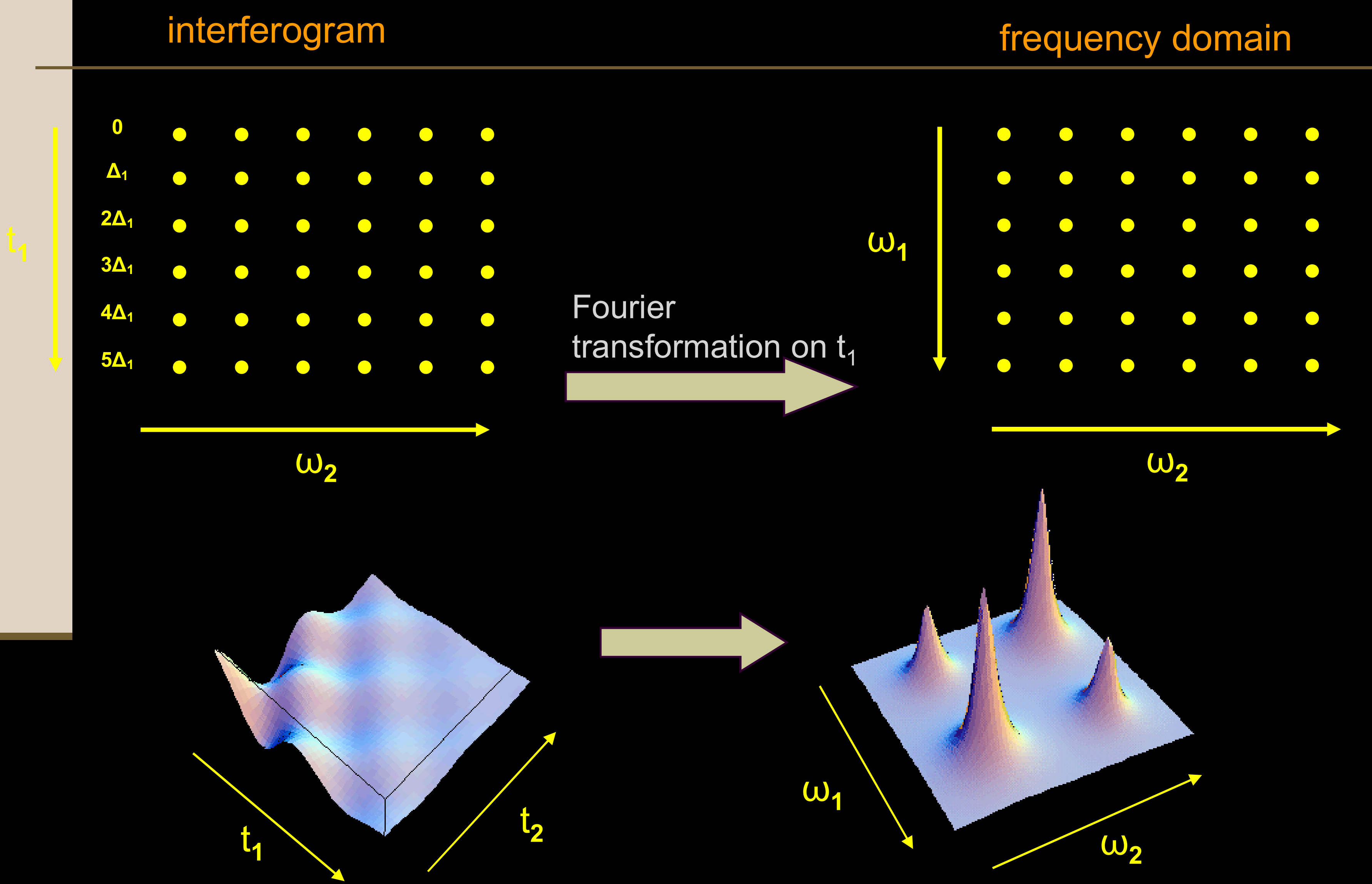
interferograms





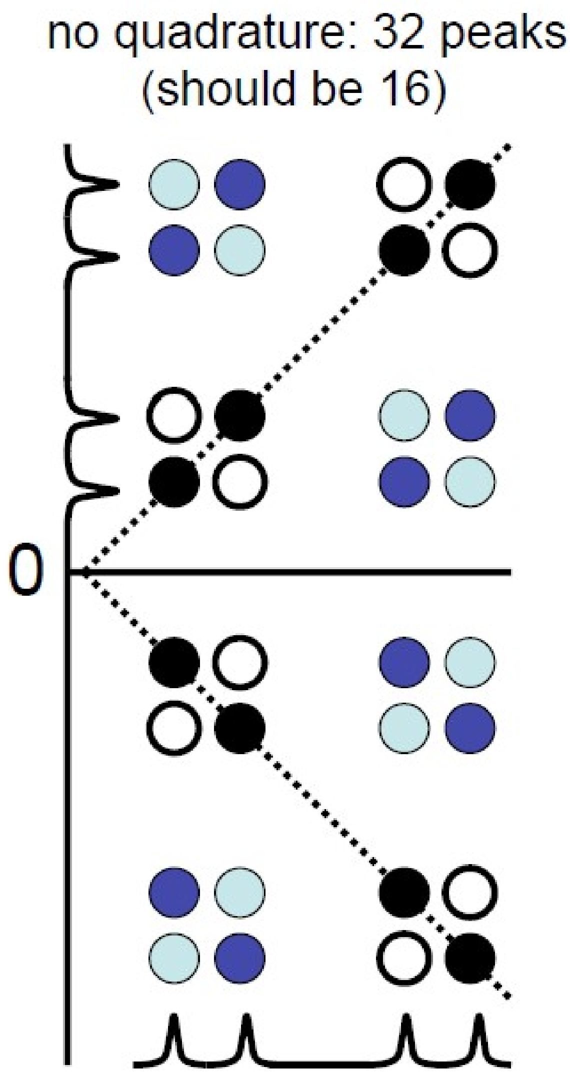


COSY



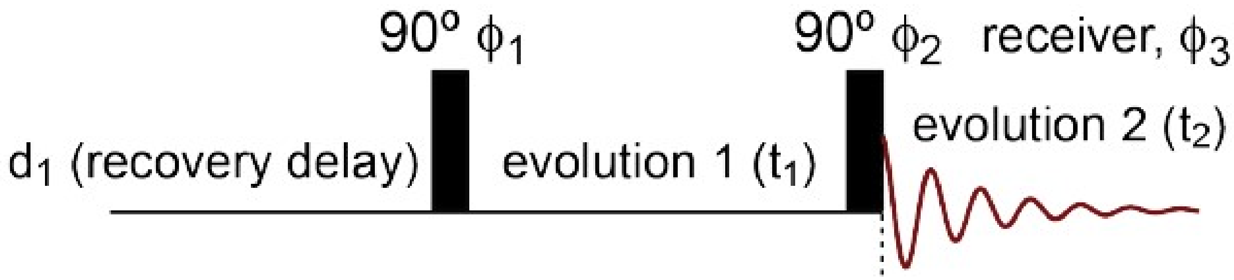
interferogram
frequency domain
0
Δ1
2Δ1
3Δ1
4Δ1
5Δ1
t1
ω2
Fourier
transformation on t1
ω1
ω2
t1
t2
ω1
ω2

Necessity of quadrature detection on t_1



- Without quadrature detection during t_1, the resultant COSY spectra after processing will lead to 2x actual number of peaks.
- The duplicated amount of peaks will be displayed either side of the RF transmiter (reference/carrier) frequency.
- Setting up quadrature detection as alternating phase of pulses (phase cycles), artifactual peaks can be eliminated.

“Phase Cycle” for COSY method



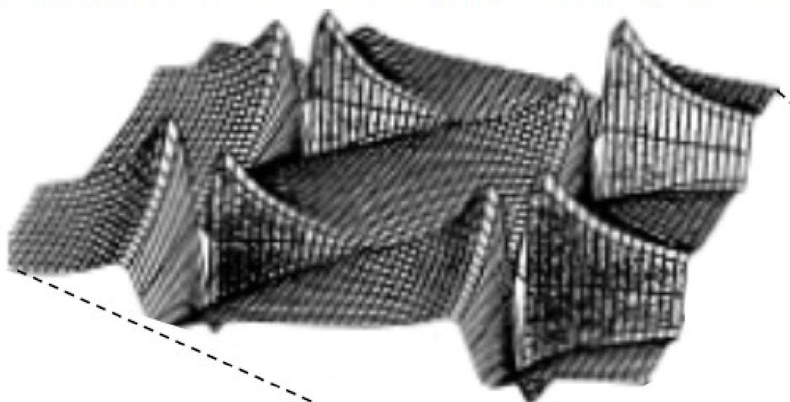
- Alternating phase of pulses on x- and y-axes will also alternate the functions as real and imaginary.
- Data will be stored as real and imaginary and phase cycles must be considering during data processing.
- Number of scans must be integral multiple numbers of steps used in phase cycle.

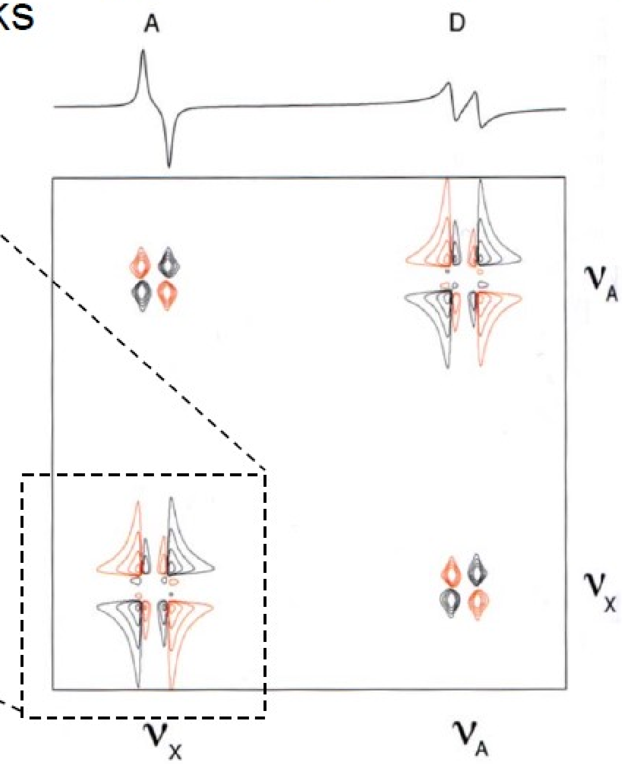
Phase cycle for COSY:

ϕ_1	ϕ_2	ϕ_3	memory
x	x	+	real, imag
x	y	+	imag, real
-x	x	-	real, imag
-x	y	-	imag, real

Another issue in COSY: dispersive and twisted auto-peaks (or cross-peaks)

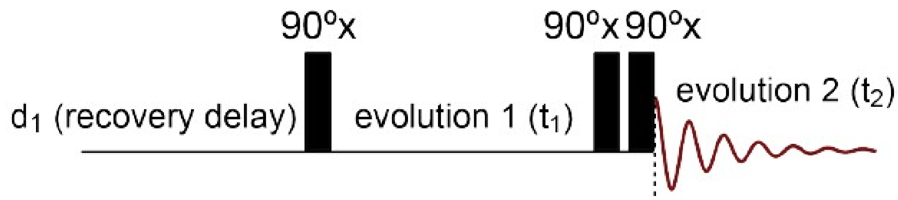
- Diagonal signals are products of dispersive components
  - they give a twisted, dispersive lineshape
  - these are problematic, especially in crowded regions of spectra, as they overlap and obscure (important) crosspeaks





  - crosspeaks are phased to give absorptive, antiphase signals, rendering diagonal signals with poor lineshape
  - can plot as magnitude (absolute value) signals, but these are broad (poor resolution)
  - still, the long "tails" of the diagonal peaks remain (crosspeaks obscured)
  - are many ways to collect COSY spectra, and some alleviate this problem

One solution: Double-Quantum Filtered COSY



- Double-quantum filtered COSY is one variation on the COSY method that helps to alleviate the diagonal peak problem
  - consider what happens to the multiple quantum terms that follow the second 90°*x* pulse when the third 90°*x* pulse is applied:

$$-2I_{xA}I_{yX} \xrightarrow{\pi/2\, I1x\ \ \pi/2\, I2x} -2I_{xA}I_{zX} \qquad -2I_{xX}I_{yA} \xrightarrow{\pi/2\, I1x\ \ \pi/2\, I2x} -2I_{xX}I_{zA}$$

  - during the evolution period, these multiple quantum terms were precessing with the frequencies of A and X, and now have been converted back to observable single quantum magnetization
- With appropriate phase cycling, other terms are removed, so only those terms that precessed as multiple quantum survive
- The advantage is that all terms (those governing both diagonal and cross peaks) are pure absorptive (antiphase)
- Other types of signals are also filtered out by the double-quantum filter (contaminants, spurious peaks, solvent, any single line signals)

Coherence Transfer Pathways in 1D and DQF-COSY

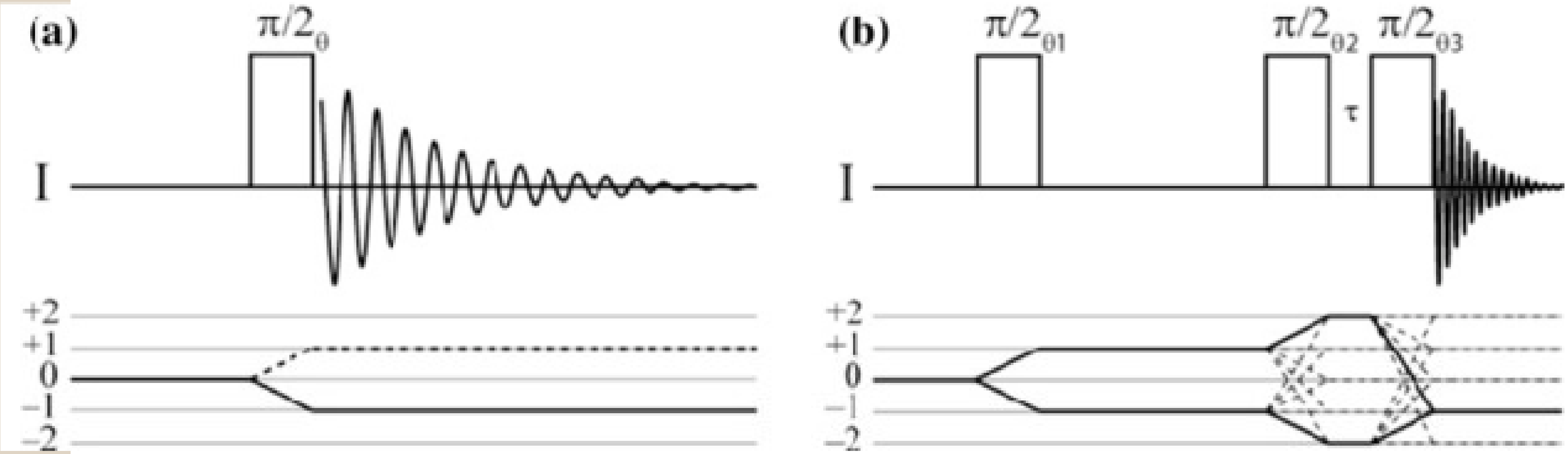
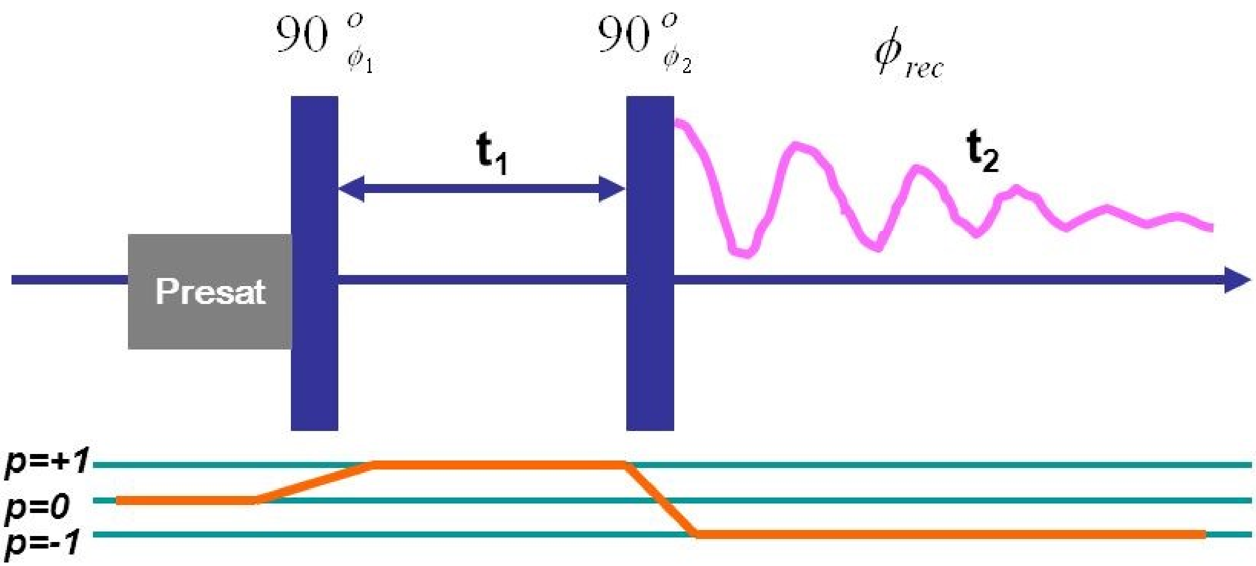


Fig. 14 Coherence pathway diagram for an arbitrary one-pulse experiment (**a**) and DQF COSY experiment (**b**)

Coherence Transfer Pathways in 2D COSY with phase cycling



$$\phi_1 = x, \phi_2 = x, \phi_{rec} = x$$

COSY pulse sequence (top) with a presaturation pulse to suppress water signal. Also shown in the diagram are the coherence transfer pathway (middle) and phase cycling scheme (bottom).