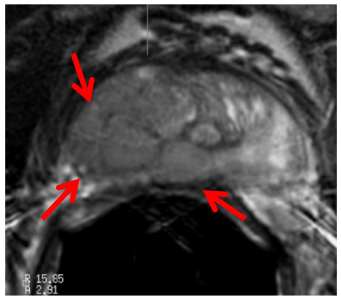
Lectures 1 - Basic Concepts of NMR Spectroscopy

- Physical Concepts Behind Magnetic Resonance Spectroscopy
- Instrumentation and RF coils
- Basic Spectral Acquisition and Pulse Sequences
- Spectral Acquisition –Gradients and Selective pulses

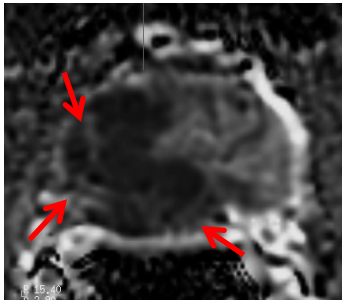
Magnetic Resonance Applications

- **Anatomic MRI** – structure, size and shape – water



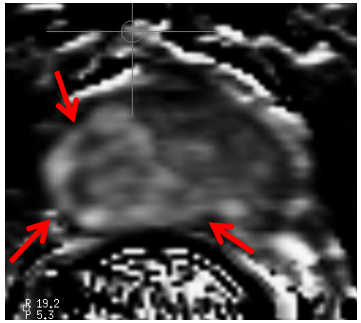
T_2 Weighted Image

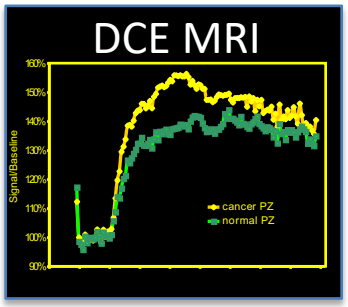
- **Diffusion weighted MRI** – micro-structure and cellularity – water



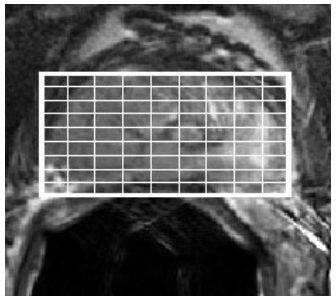
Apparent Diffusion Coefficient (ADC) Image

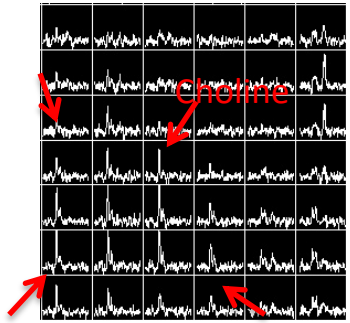
- **Contrast enhanced MRI -** perfusion – permeability of blood vessels, micro-vascularity - water





- **Magnetic Resonance Spectroscopy -** chemistry/biochemistry – chemical structure, metabolism

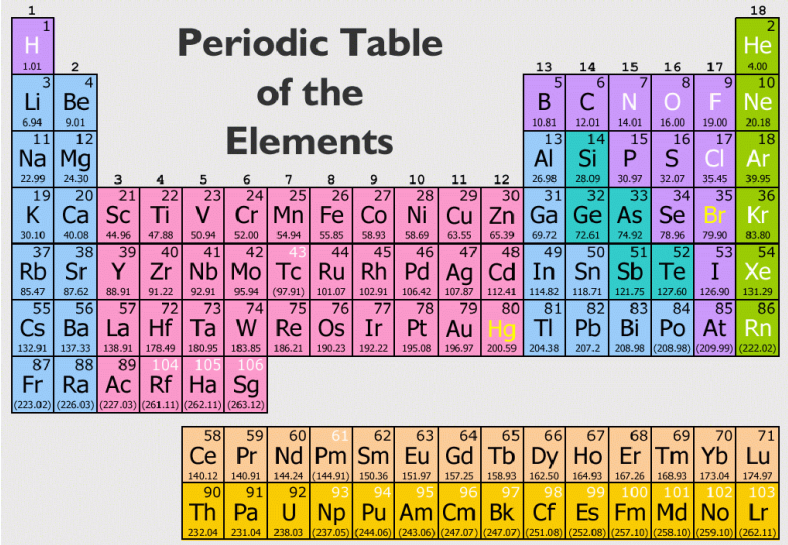




Nuclear Spin and NMR Active Nuclei

- Spin is a fundamental property of nature like electrical charge or mass. Spin comes in multiples of 1/2 and can be + or -. Individual unpaired electrons, protons, and neutrons each possesses a spin of 1/2.

- The shell model for the nucleus tells us that nucleons, just like electrons, fill orbitals.
- When the number of protons or neutrons equals 2, 8, 20, 28, 50, 82, and 126, orbitals are filled. Nucleons have spin, just like electrons do, and their spin can pair up when the orbitals are filled and cancel out.



- Almost every element in the periodic table has an isotope with a non zero nuclear spin.
- NMR can only be performed on isotopes with non zero nuclear spin and whose natural abundance is high enough to be detected.

Nuclei routinely used in NMR

	Unpaired protons	Unpaired neutrons	Net spin	(MHz/T)
^{1}H	1	0	1/2	42.58
^{2}H	1	1	1	6.54
^{31}P	1	0	1/2	17.25
^{23}Na	1	2	3/2	11.27
^{14}N	1	1	1	3.08
^{13}C	0	1	1/2	10.71
^{19}F	1	0	1/2	40.08

When placed in a magnetic field of strength B_0, a particle with a net spin can absorb a photon, of frequency, υ_o. The frequency depends on the gyromagnetic ratio, of the particle.

$$\upsilon_o = \gamma B_o$$

For hydrogen, = 42.58 MHz / T.

(≈ 63MHz for 1.5T, 128 MHz for 3T, 300 MHz for 7T, 500 MHz for 11.7T, and 600 MHz for 14T).

Magnetic Resonance?

- *Nuclear magnetic dipole* – charged particle spinning around an axis => small circular current…faster the nucleus spins => bigger the current

$$\vec{\mu} = \gamma \vec{I}$$

  - Magnetic moment μ, spin angular momentum I, gyromagnetic ratio γ (radians/s/T) inherent to a given nuclei
- When you put the nucleus in a field B, since the nucleus is spinning you have Larmor precession…
  - Spins precess perpendicular to the plane

$$\frac{d\vec{\mu}}{dt} = -\gamma \vec{B} \wedge \vec{\mu}$$

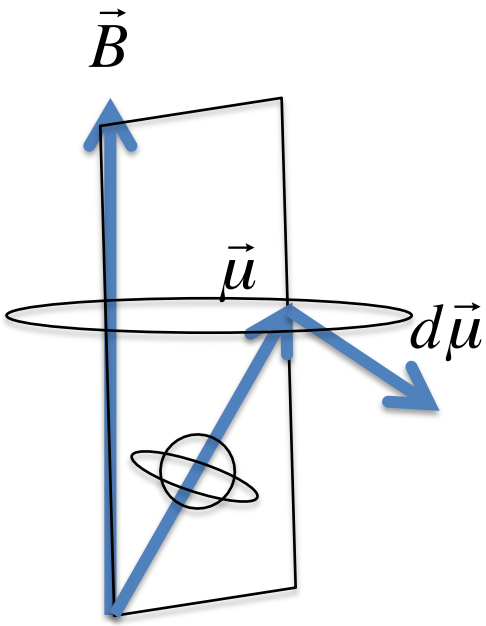
- Typically we have a static field B_0 and define angular velocity of the precession and frequency (*Larmor freq*)…

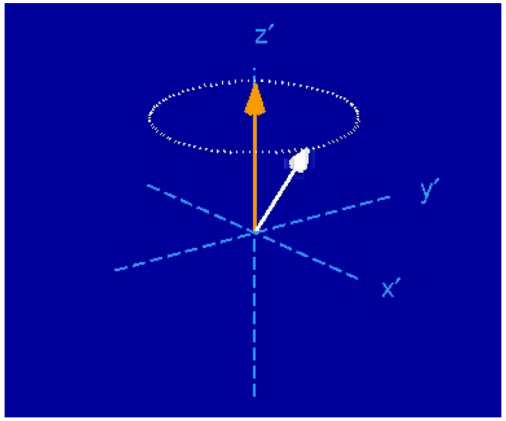
$$\omega_0 = \gamma B_0 \qquad \nu_0 = \frac{\omega_0}{2\pi}$$

velocity(radians/s)

Magnetic field (T)

Frequency (/s)





Magnetic Resonance

- *Since we care about a lot of spins in a sample…*the ensemble is M and precesses…

$$\frac{d\overrightarrow{M}}{dt} = -\gamma \overrightarrow{B} \wedge \overrightarrow{M}$$

  - Cross product implies you can only see magnetization when not aligned…
- At equilibrium, the net magnetization vector lies along the direction of the applied magnetic field Bo and is called the equilibrium magnetization M_o. In this configuration, the Z component of magnetization M_Z equals Mo. M_Z is referred to as the longitudinal magnetization. There is no transverse (M_X or M_Y) magnetization here.

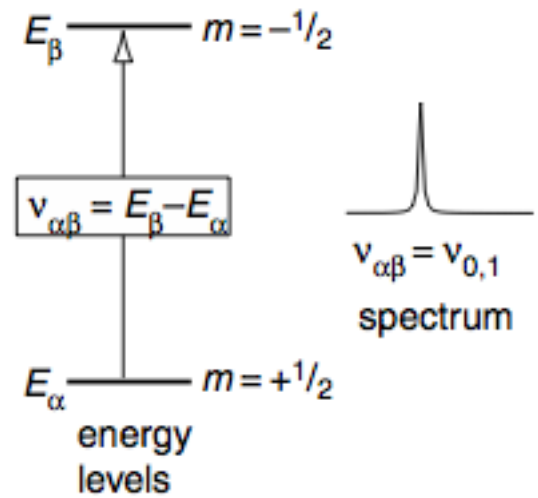
$$M_0 = \frac{N\gamma^2\hbar^2 I(I+1)}{3kT} B_0$$

  - N – number of spins, h is Planck's constant/2pi, k is boltzmann, T is temperature, I is the total magnetic quantum number

Magnetic Resonance?

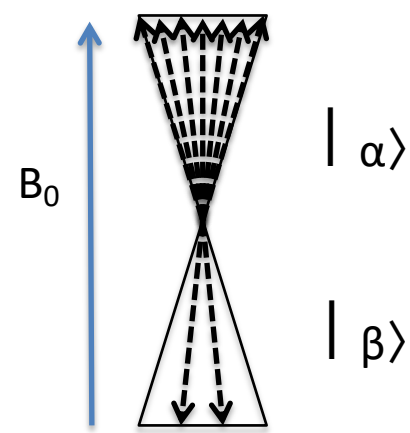
- The system is quantized and we can talk about transitions between energy states.
  - For spin ½ these are $|\alpha\rangle$ and $|\beta\rangle$ with energies E_α and E_β

$$E_i = -m_i \frac{\gamma h B_0}{2\pi}$$



$$\frac{N_\alpha}{N_\beta} = e^{\frac{(E_\beta - E_\alpha)}{kT}} = e^{\frac{\gamma \hbar B_0}{kT}}$$

Boltzmann



Energy Levels and Possible Transitions in a Weakly Coupled AX Spin 1/2 System

M	Level	Energy[a]	ΔM	Transition	Frequency
-1	1 ββ	$(f_A + f_X) + J/4$	0	$2 \rightarrow 3$	$f_A - f_X$
0	2 βα	$(f_A - f_X) - J/4$	1	$4 \rightarrow 2$	$f_X - J/2$
0	3 αβ	$-(f_A - f_X) - J/4$	1	$3 \rightarrow 1$	$f_X + J/2$
+1	4 αα	$-(f_A + f_X) + J/4$	1	$4 \rightarrow 3$	$f_A - J/2$
			1	$2 \rightarrow 1$	$f_A + J/2$
			2	$4 \rightarrow 1$	$f_A + f_X$

[a] Expressed in frequency units.

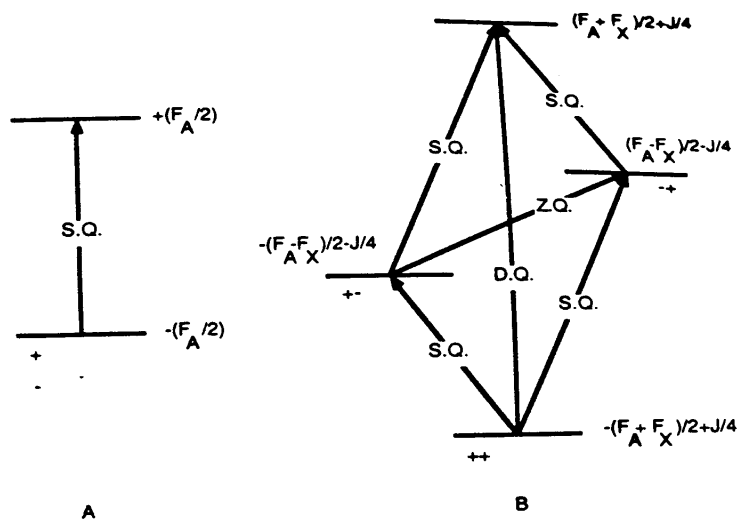
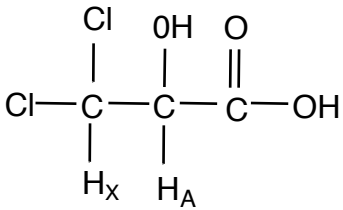
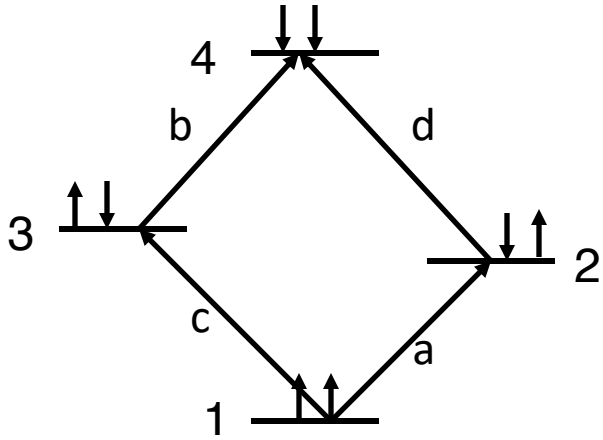


FIG. 1. (A) The energy level diagram for an isolated spin-½ system in a magnetic field. (B) The energy level diagram for a coupled AX spin system in a magnetic field. Four single-quantum transitions ($\Delta M = \pm 1$) occur at $F_A + J/2$, $F_A - J/2$, $F_X + J/2$, and $F_X - J/2$. One double-quantum transition ($\Delta M = \pm 2$) occurs at $F_A + F_X$ and one zero-quantum transition ($\Delta M = 0$) occurs at $F_A - F_X$.

Resulting NMR Spectrum

Compound

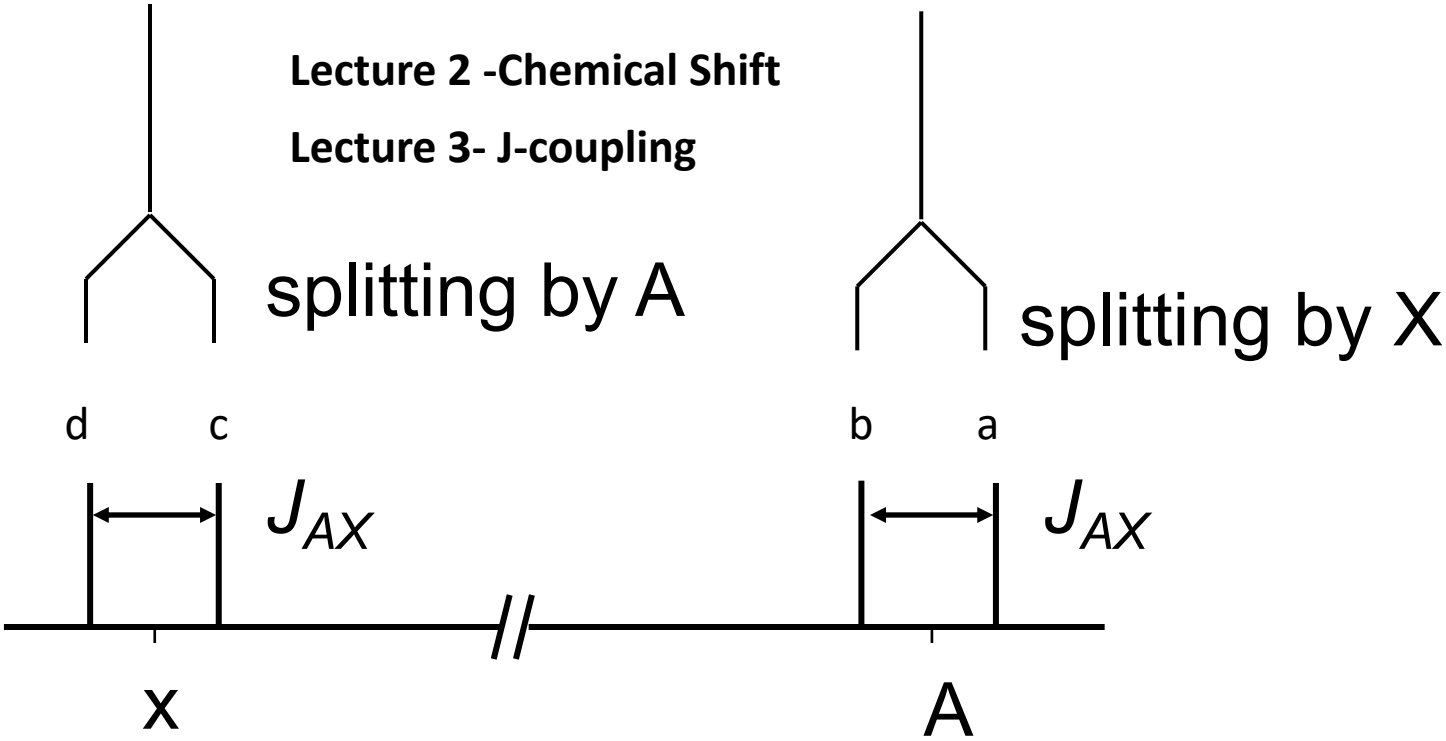




Energy level diagram demonstrating the spin states of a weakly coupled AX spin system and the transitions producing the peaks in the corresponding NMR spectrum.

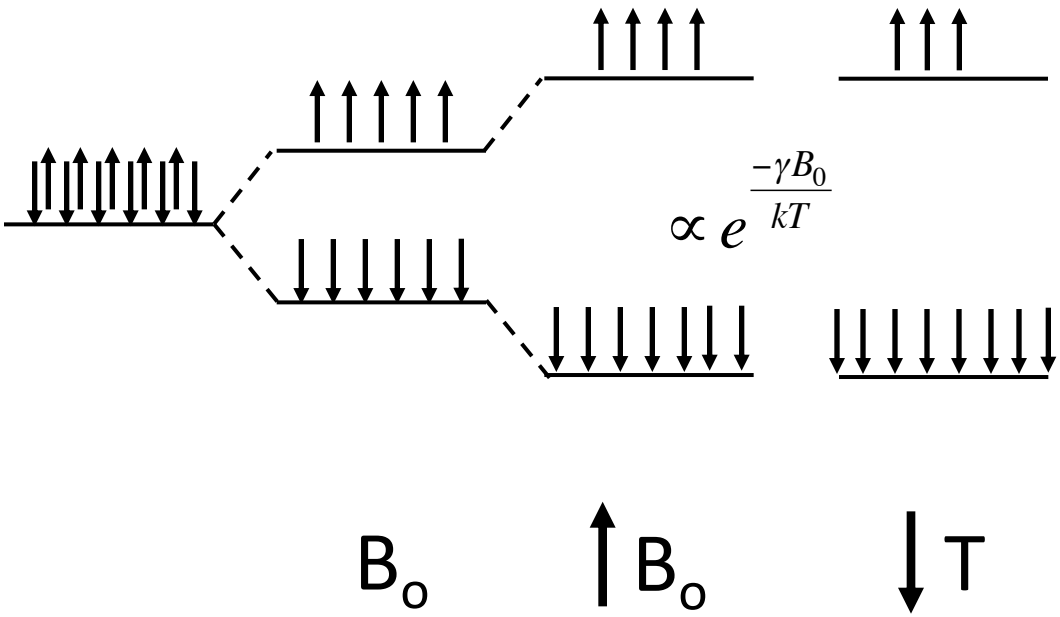
Lecture 2 -Chemical Shift

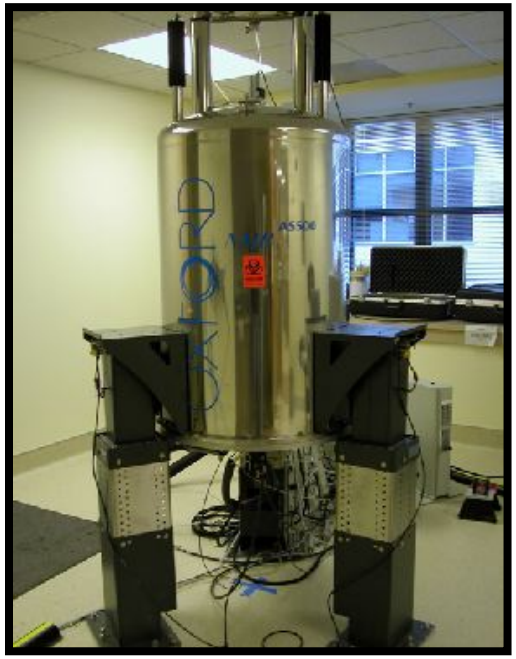
Lecture 3- J-coupling



Magnetic Resonance?

- Signal depends on the Boltzmann Distribution (k), gamma, field, and temperature

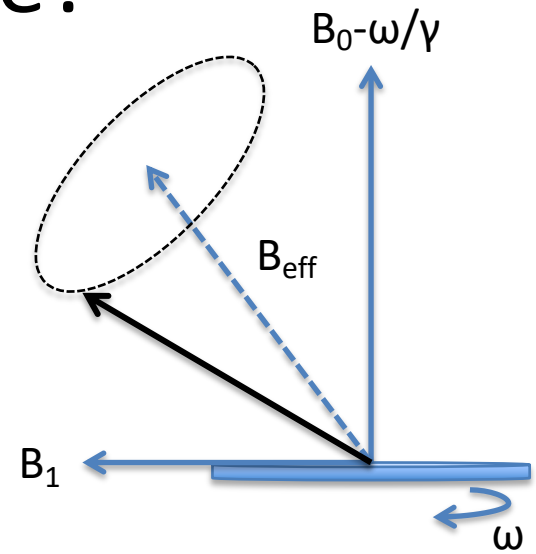




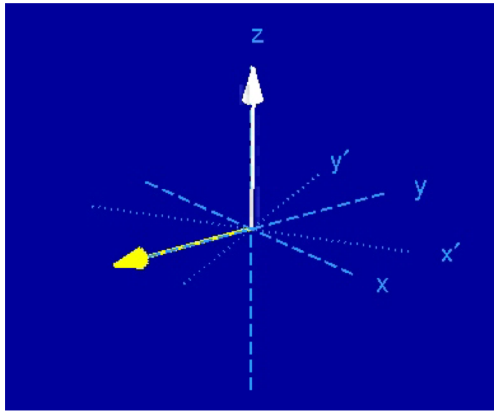
At 3T and 37ºc , there is less than a 0.000247% difference between the populations of the high and low energy ^{13}C spin states that yield the NMR signal.

$$P = (N_+ - N_-)/ (N_+ + N_-) = \tanh(g(h_{/2p})B_0/2kT)\ 2.47E^{-6}$$

Magnetic Resonance?



- Problem…if the magnetization is on Z (main field) we can't observe it…
- RF Excitation
  - Tip M away from Z with B_1
  - Then M precesses around the new field (B_0+B_1)
  - Since we would need a lot of power to generate a static B_1 that would actually influence the overall B field…
  - We let the B_1 field rotate around B_0 with the same frequency as M…=> we excite at the frequency of M
- Flip angle $$\alpha = \gamma B_1 t_p$$



Acquiring the Spectrum

Continuous Wave (CW) vs. Pulsed (FT) NMR

Prior to 1963, all NMR spectrometers were "continuous wave" instruments.

CW instruments worked by "sweeping" a frequency range and recording the response at each frequency. Literally, a piece of paper was taped to the console and a pen moved along with the frequency sweep and traced the spectrum.

CW spectroscopy is <u>exactly</u> analogous to striking every key on a piano, one at a time, from one end to the other, and recording the sounds generated.

The problem with CW spectrometers was that it took too long to acquire a spectrum and signal averaging was difficult.

Pulsed – Fourier Transform (FT) NMR

Richard Ernst won the Nobel Prize in 1991 for the development of FT-NMR, which made 2D and multinuclear NMR possible.

In FT-NMR, a broad range of frequencies are excited at once and the signal generated is recorded versus time.

FT-NMR is <u>exactly</u> analogous to striking all of the keys on a piano at the same time, except now you hear all of the notes superimposed rather than individually.

The Fourier Transform converts signal versus time into signal versus frequency:

$$f(\omega) = \int_{-\infty}^{+\infty} f(t)\exp\{i\omega t\}dt$$

In practice, computers perform a “discrete” FT using the Cooley-Tukey algorithm.

Magnetic Resonance

- Immediately after excitation, precession can't go on forever…
- Relaxation
  - T_1 – *spin-lattice*, restores Boltzmann => M_z regrows
  - T_2 – *spin-spin*, a result of an ensemble of different magnetic dipoles => M_{xy} decays
- The Bloch equations are a set of coupled differential equations which can be used to describe the behavior of a magnetization vector under any conditions. When properly integrated, the Bloch equations will yield the X', Y', and Z components of magnetization as a function of time.

Bloch Equations

$$\frac{d}{dt}M_x = -\gamma(B_y M_z - B_z M_y)$$

$$\frac{d}{dt}M_y = -\gamma(B_z M_x - B_x M_z)$$

$$\frac{d}{dt}M_z = -\gamma(B_x M_y - B_y M_x)$$

$$\xrightarrow{\vec{B} = \left[B_1, 0, B_0 - \frac{\omega}{\gamma}\right]}$$

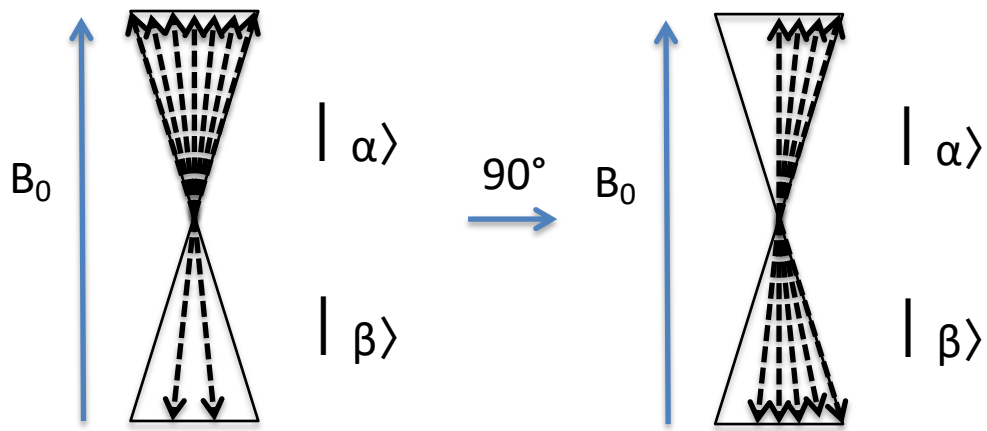
$$\frac{d}{dt}M_x = (\omega_0 - \omega)M_y - \frac{1}{T_2}M_x$$

$$\frac{d}{dt}M_y = -(\omega_0 - \omega)M_x - \frac{1}{T_2}M_y + \omega_1 M_z$$

$$\frac{d}{dt}M_z = -\omega_1 M_y - \frac{1}{T_1}(M_z - M_0)$$

Magnetic Resonance - T_1 relaxation – spin-lattice

- T_1 relaxation – spin-lattice
  - Spins exchange energy via "on resonance" fluctuations with the lattice
  - More fundamental… $|\alpha\rangle$ and $|\beta\rangle$ need to go through transitions to re-establish equilibrium



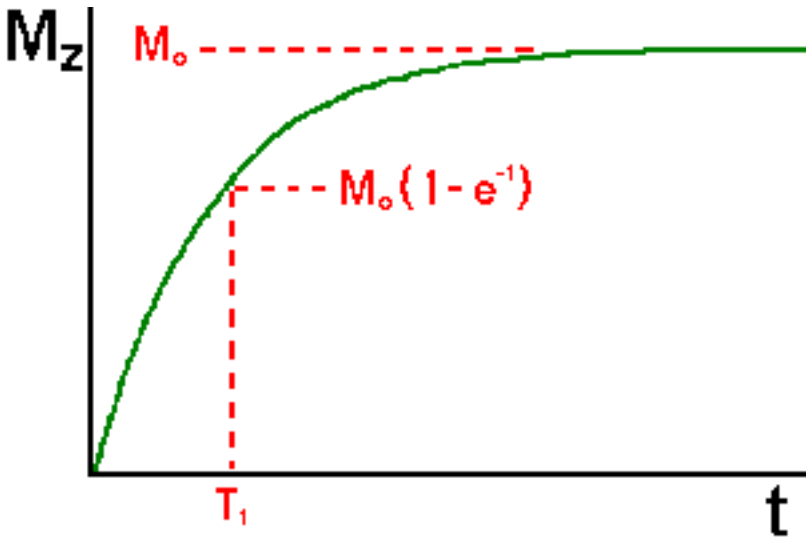
  - For this to happen, spins exchange energy with the surroundings…*lattice*
  - This is dependent on the local field the spin experiences

$$\frac{d}{dt}M_z = -\frac{1}{T_1}(M_z - M_0) \xrightarrow{\text{ode}} M_z(t) = M_0[1 - e^{-\frac{t}{T_1}}]$$

Ode – ordinary differential equation - is an equation containing a function of one independent variable and its derivatives.

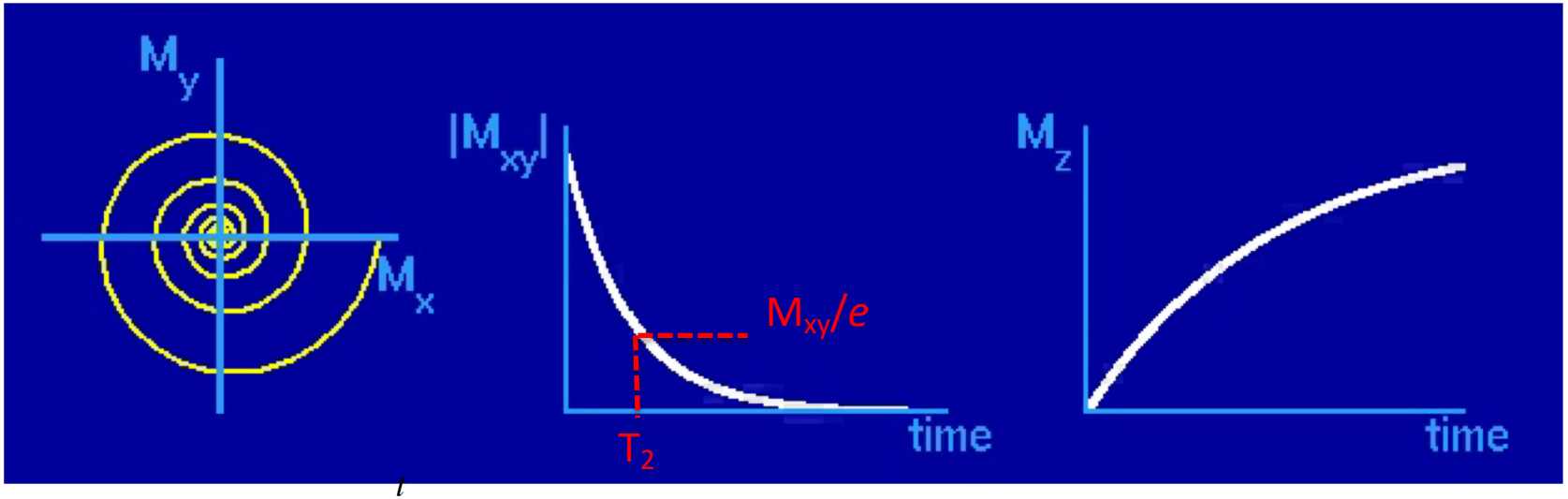
Magnetic Resonance - T_1 relaxation – spin-lattice

- The time constant which describes how M_Z returns to its equilibrium value is called the spin lattice relaxation time (T_1). The equation governing this behavior as a function of the time t after its displacement is:
- $M_z = M_o (1 - e^{-t/T1})$
- T_1 defined as the time required to change the Z component of magnetization by a factor of e.



Magnetic Resonance - T_2 relaxation – spin-spin

- T_2 relaxation – spin-spin
  - Spins "feel" magnetic fields from other molecules and become off frequency with respect to B_0 and other spins
  - …dephasing of spins from the Larmor Frequency, resulting in loss of transverse component



$$M_{xy}(t) = M_{xy} e^{-\frac{t}{T_2}}$$

The time constant which describes the return to equilibrium of the transverse magnetization, M_{XY}, is called the spin-spin relaxation time, T_2.

T_2 is always less than or equal to T_1. The net magnetization in the XY plane goes to zero and then the longitudinal magnetization grows in until we have M_o along Z.

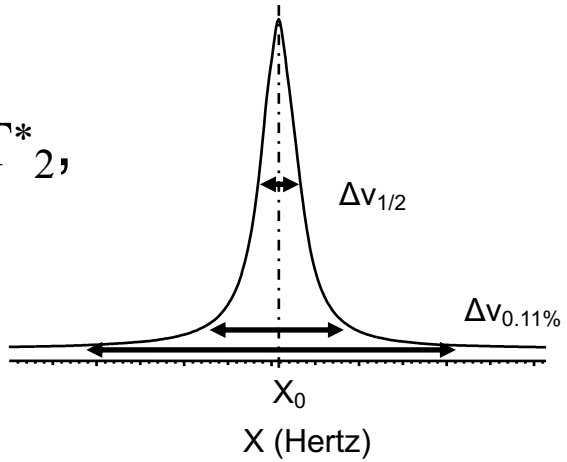
Magnetic Resonance T_2* - relaxation from magnetic field inhomogeneity –Lecture 3

- T_2* - relaxation from magnetic field inhomogeneity
  - In an ideal system line-widths represent pure T_2, effectively the decay of the FID
  - Observed T_2 is a convolution

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \gamma \Delta B_0$$

  - Shimming – homogenize the magnetic field using external coils
  - Other ways to get rid of T_2* - pulse sequences ?

Observed line-widths - $\Delta\nu_{1/2} = 1/\pi\ T^*_2$,
where $\pi = 3.146$



Magnetic Resonance- Correlation Time

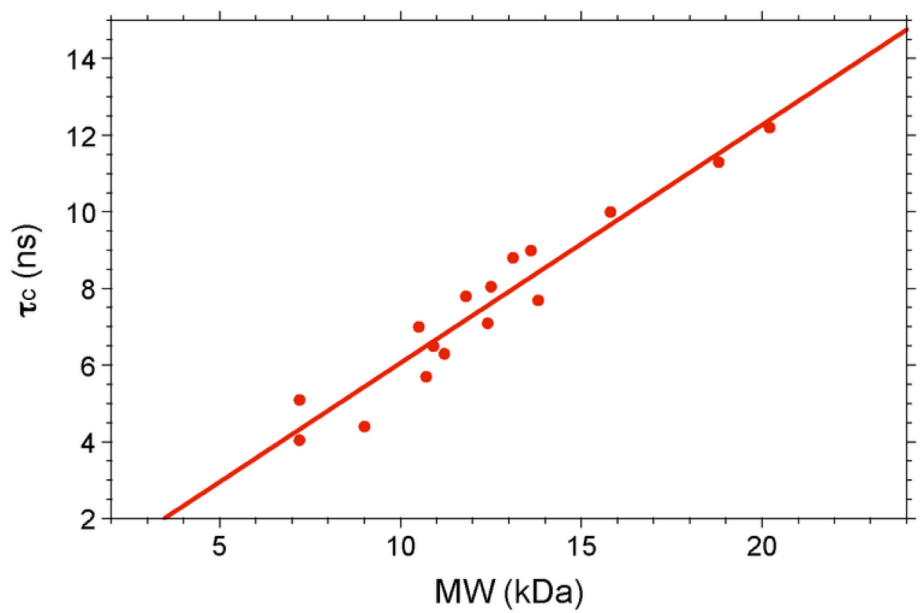
- Rotational correlation time
  - Characteristic tumbling rate of a molecule, Brownian rotation diffusion of a particle
  - Defined as time it takes a particle to rotate 1 radian
  - Stoke's approx…

$$\tau_c = \frac{4\pi\eta r^3}{3kT} \qquad r \approx \sqrt[3]{\frac{3M}{4\pi\rho N_a}} + r_w$$

η is the viscosity, r eff hydrodynamic radius

M, mass in kDa, ρ density,

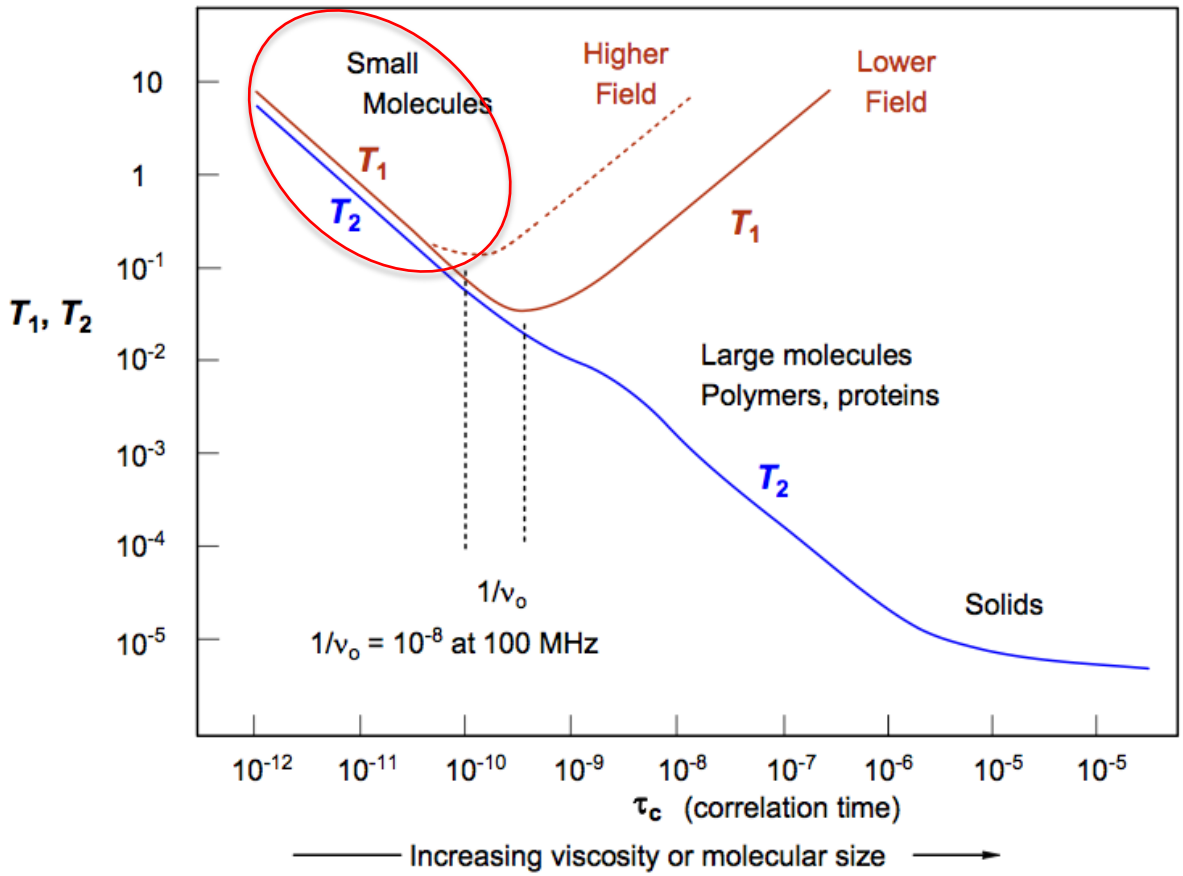
N_a avagadro, r_w = hydration (1-3A)



Magnetic Resonance – Correlation Time and T_1 and T_2 Relaxation

- What does this have to do with NMR?

$$\frac{T_1}{T_2} = 1 + \frac{1}{2}\omega_0^2\tau_c^2$$

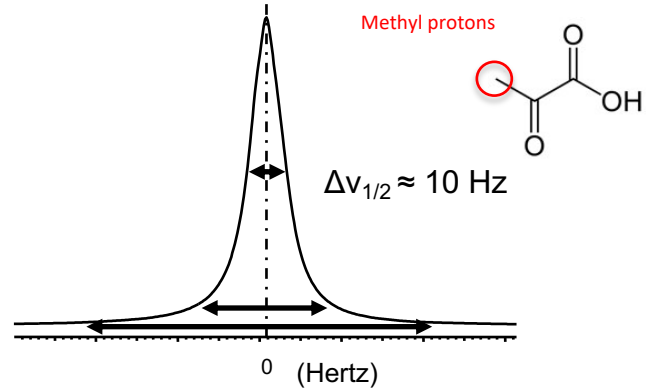


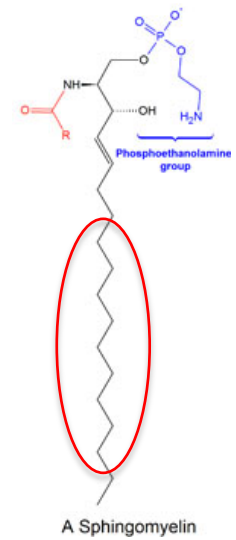
Magnetic Resonance – Correlation Time and NMR Line-width

The spectral linewidth ($\Delta\nu$) at half peak height ($\Delta\nu_{1/2}$) is described by the following equation.

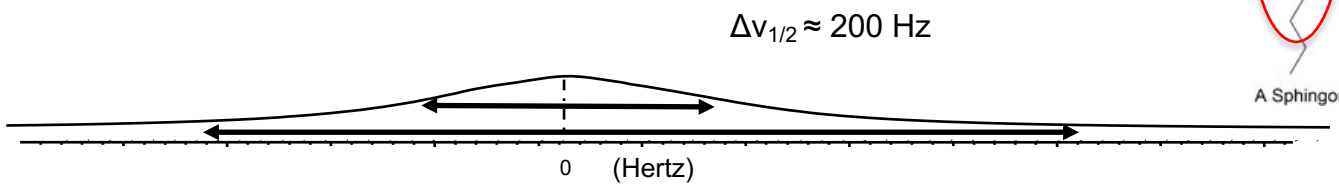
$$\Delta\nu_{1/2} = 1/\pi\, T^*_2, \text{ where } \pi = 3.146$$

MW≈ 88 g/mol, i.e. pyruvic acid



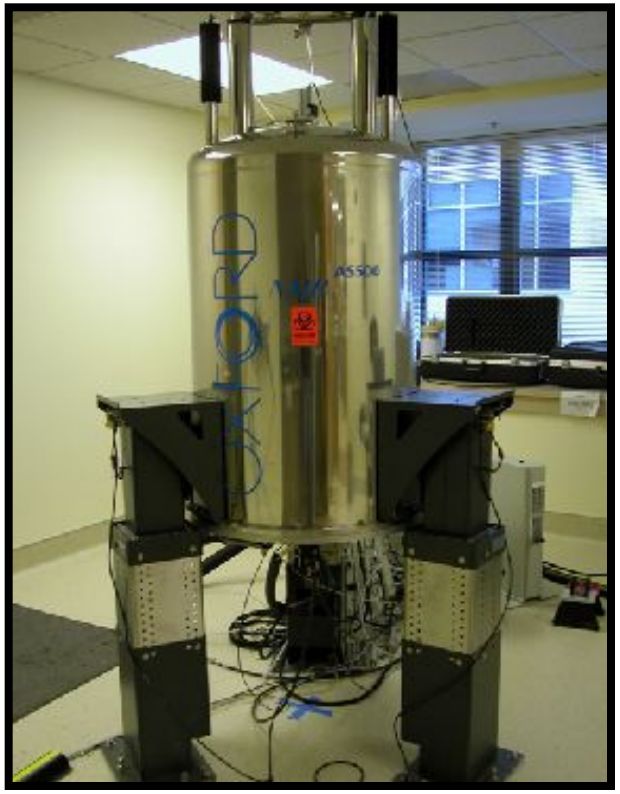


MW≈ 1,337 g/mol, i.e. Sphingomyelin



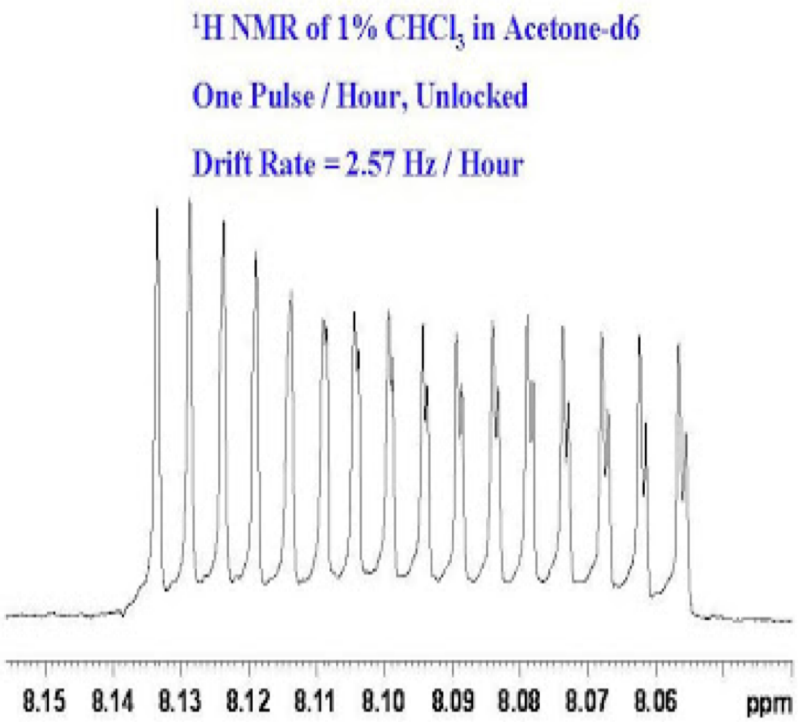
Acquire the spectrum (Hardware)?

- First, the magnet…
  - Generally superconducting to achieve greater electric currents…and thus higher magnetic fields (>2T)
  - Requires cryogenically cooled systems (He and N_2)
  - Why go to higher fields?
    - Sensitivity $\propto B_0^{3/2}$
    - Resolution $\propto B_0$
  - Demands of higher field
    - Better shimming to produce homogeneity – equivalent spins with same Larmor frequencies
    - Stability – requirement for field locking



Effects of magnetic field drifts over time

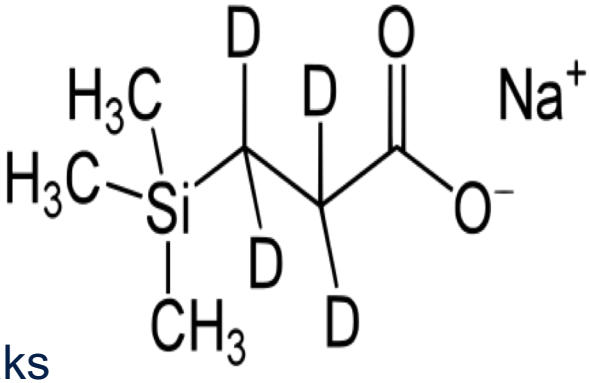
- Magnetic field strength can slowly decreases over time
- For long acquisitions, typically lock sample to D2O to compensate for magnetic drift



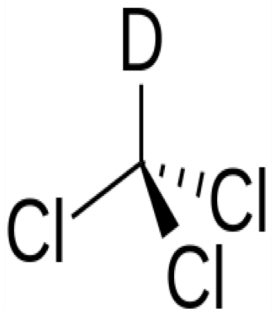
Magnet | Sample | Tune & match | Lock & Shim | Acquisition | Processing

Preparing your sample

1. Reference compound
   - Want compounds with a nice singlet peak that doesn't overlap with your peaks of interest
   - For metabolites, typically use TSP
2. Deuterated solvents
   - Prevent water signal from oversaturating other peaks
   - Used to lock field frequency



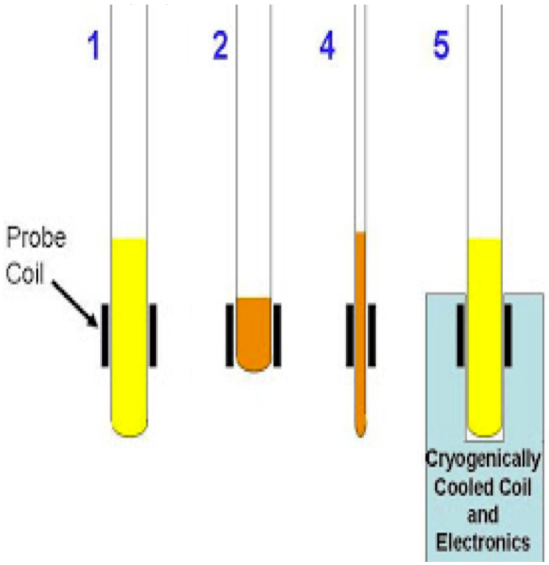
D_2O



Preparing your sample

3. Sample Volume is determined based off of:
   - "Sweet spot," or homogenous region of the magnetic field
   - Sensitive region of the RF Coil

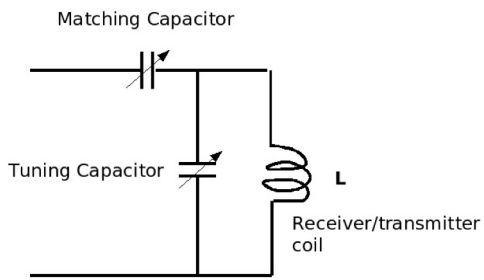
What if your sample volume is too little?

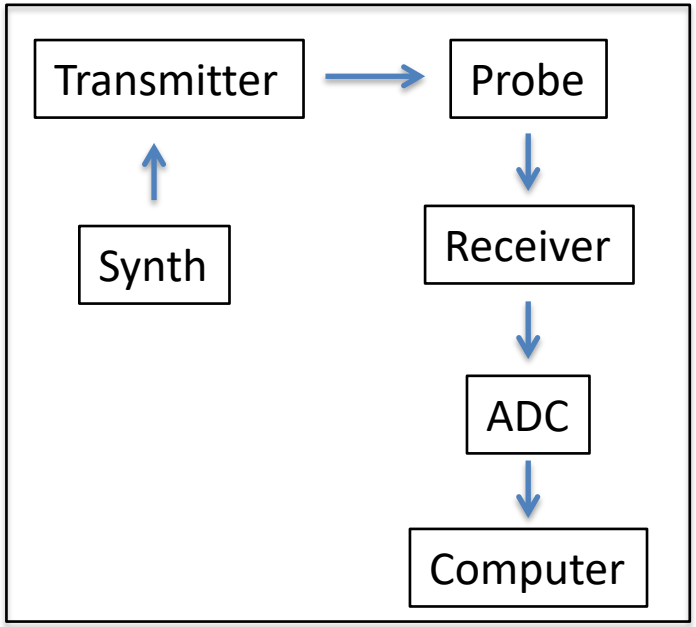


Acquire the spectrum?

- Probe
  - Typically transmit/receive - generate the B_1 field during the RF pulse and detect current induced
  - Coil must be tuned to resonance freq
    - i.e. the electric circuit is *resonant*
    - Easiest way to accomplish this is by putting a cap (C) in a parallel with a coil with inductance (L)

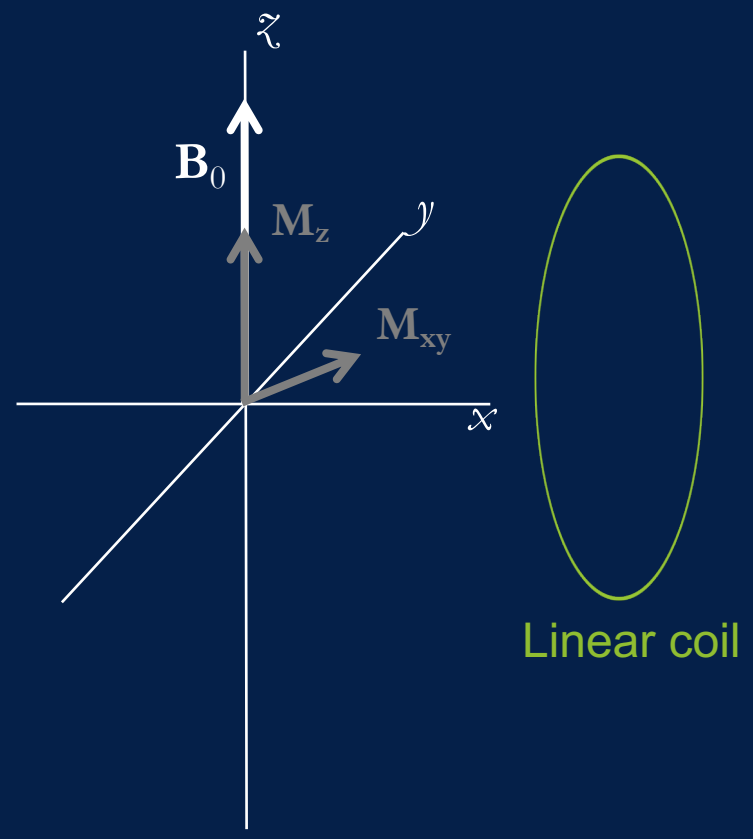
$$\omega = \sqrt{\frac{1}{LC}}$$



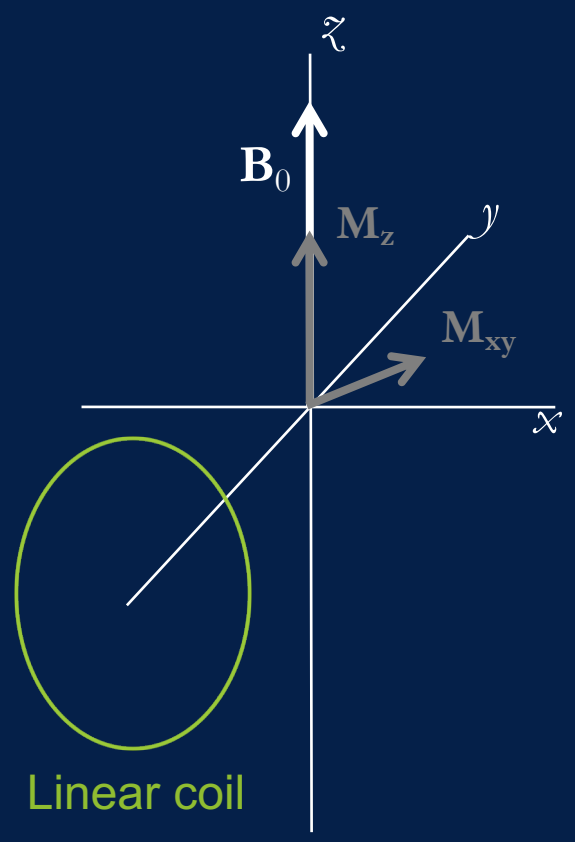


  - Decoupling?
  - Broadband
  - Q – quality factor, depends on the geometry
  - Filling factor

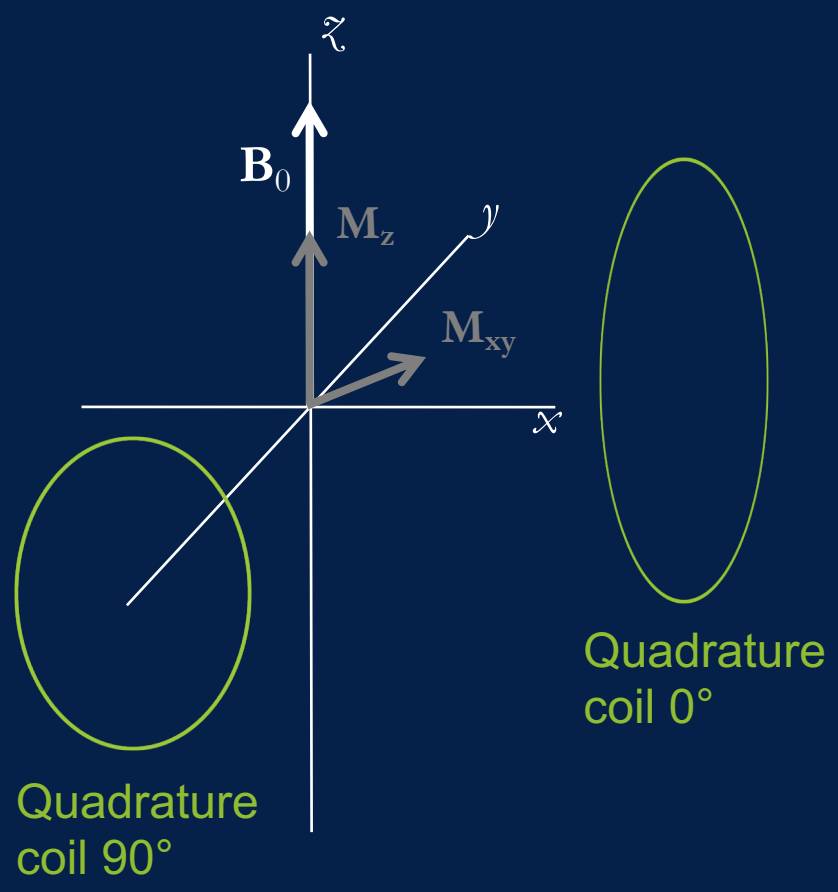
Linear Coil – $\mathbf{B}_1$ in x-Direction



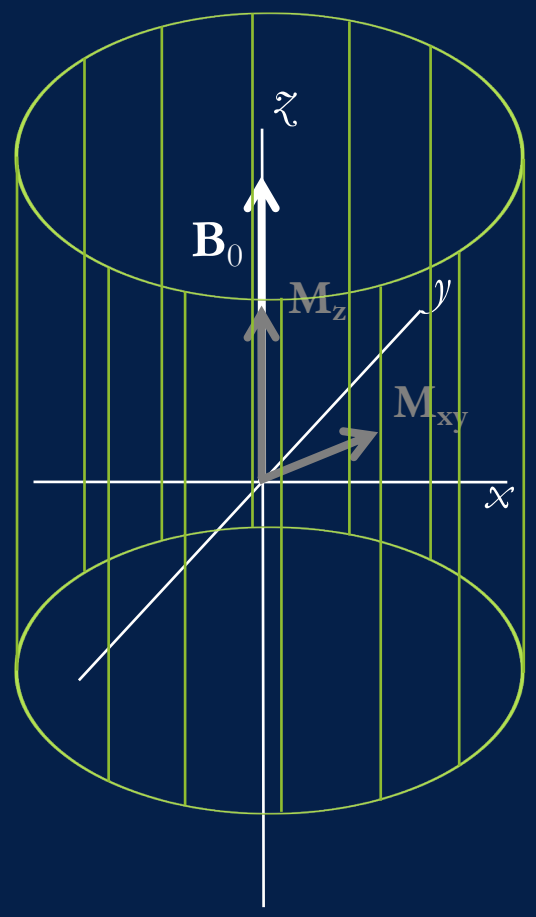
Linear Coil – $\mathbf{B}_1$ in y-Direction



Quadrature Coil



Birdcage Coil

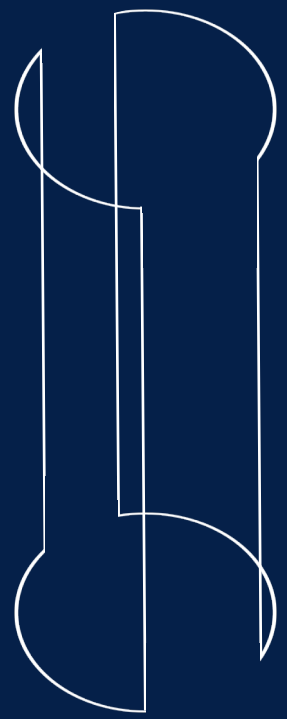


Birdcage coil

Spatially Uniform $\mathbf{B}_1$ Field

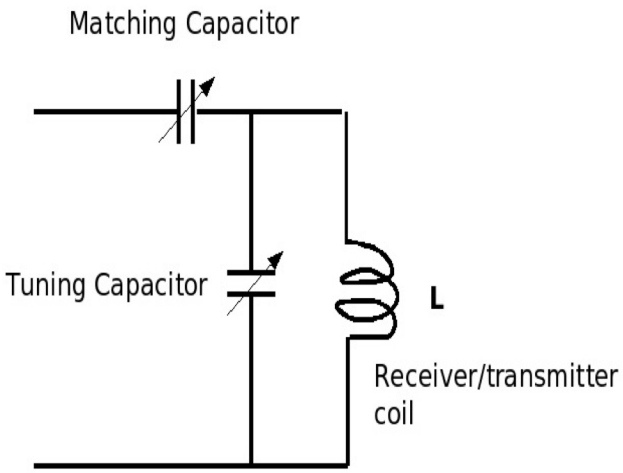
Design considerations…

- Solenoid has least uniform $\mathbf{B}_1$
- Saddle $\mathbf{B}_1$ is more uniform
- Birdcage $\mathbf{B}_1$ is more uniform
- Wavelength effects can be challenging
  - Humans at 7.4 T



Tune and Match your probe

Quality factor: $Q = \frac{L\omega_0}{r}$

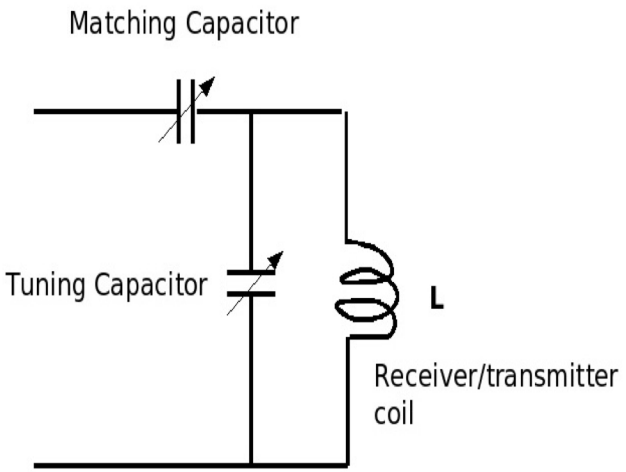


- Cryo probes have a high Q factor
  - Low resistance (r) = low power loss
  - High inductance

Tune and Match is unique to each sample

Each sample has a unique conductivity and dielectric constant.

- Conductive solutions act as resistors across the RF coil
- Thus, must tune and match every sample

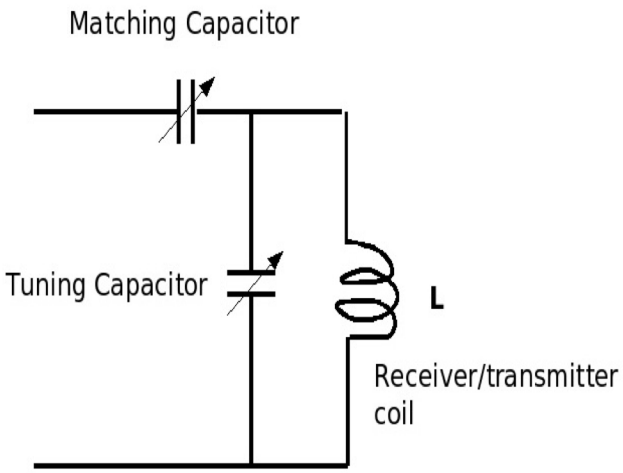


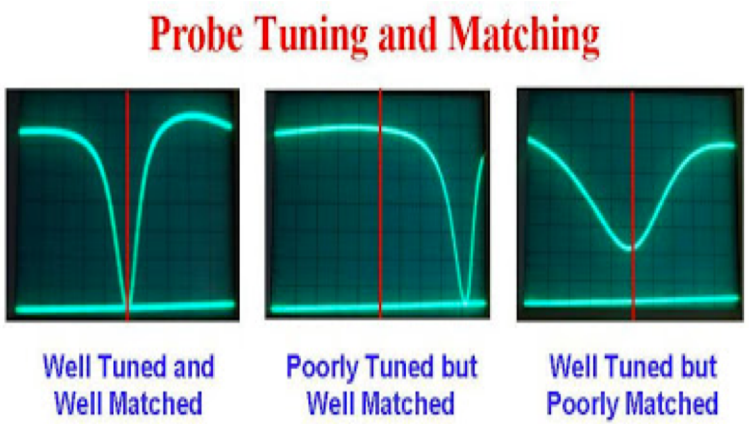
Tune and Match your probe

Tuning capacitor shifts the resonance frequency

What if the sample is not properly tuned?

- B1 field not as optimized
- Less transverse magnetization
- Less signal





Tune and Match your probe

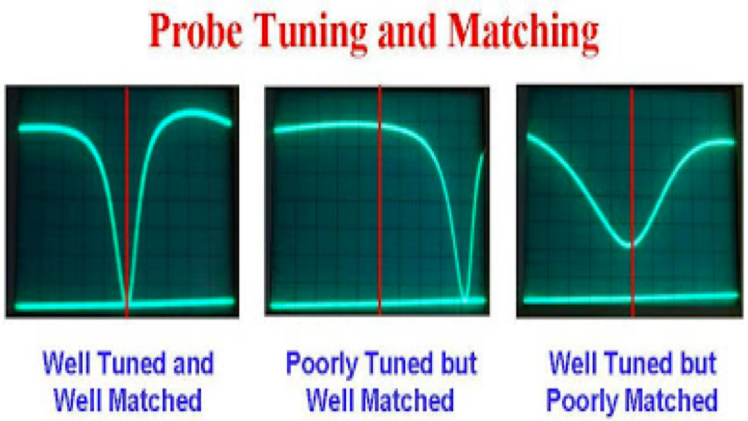
Matching capacitor changes the efficiency of the band pass filter

What happens when the probe is not matched?

RF power is not efficiently transferred and results in

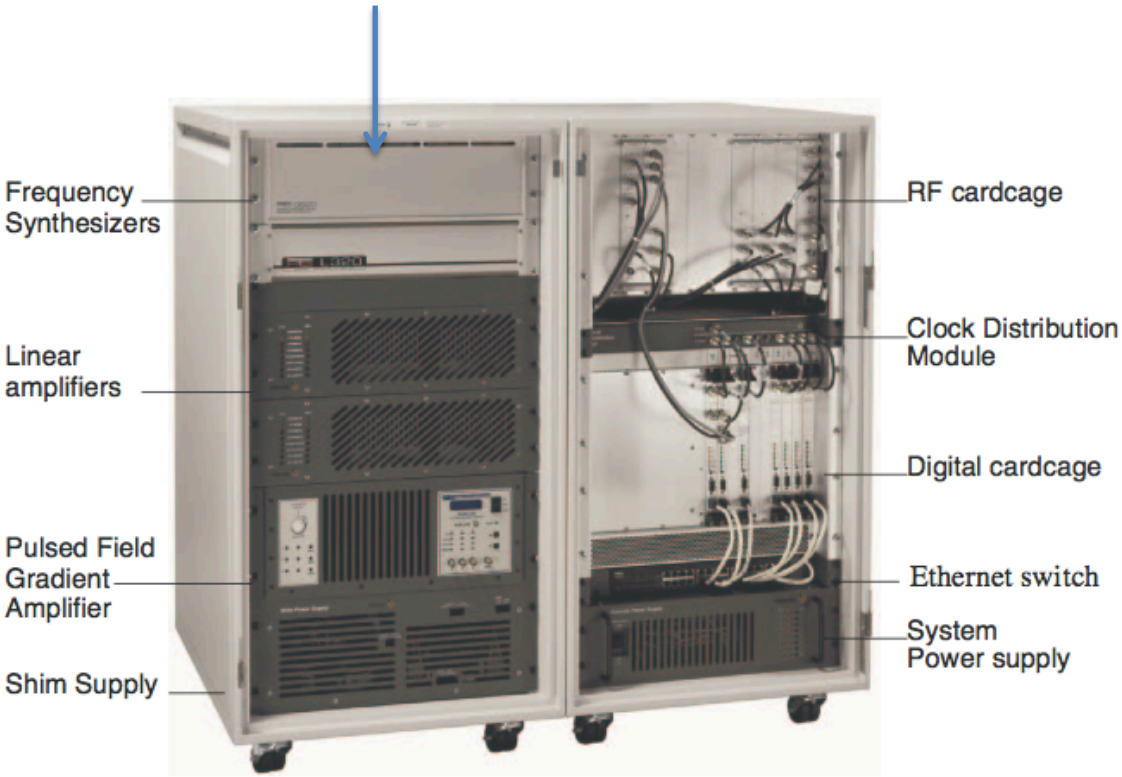
1. longer 90 pulses
2. loss of signal (→ lower SNR)
3. insufficient broadband decoupling (→ increased residual line broadening)

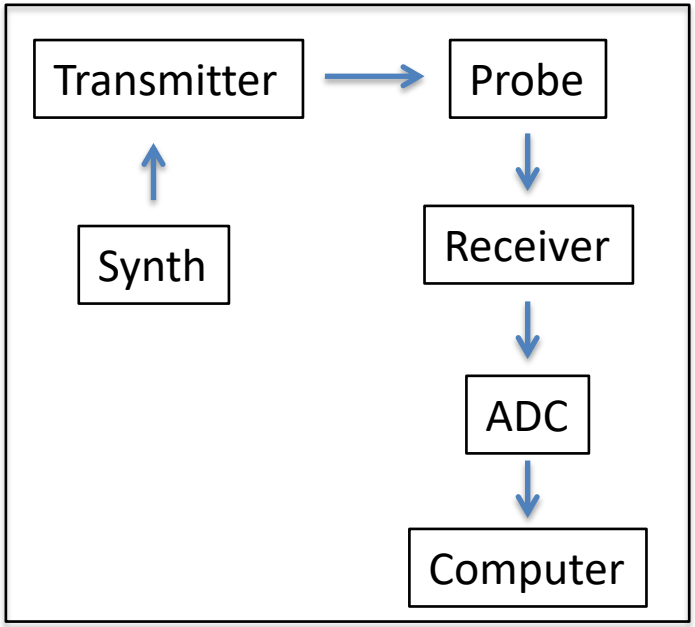
Can also burn your power amplifier and preamplifier since RF power isn't fully transmitted



Acquire the spectrum?

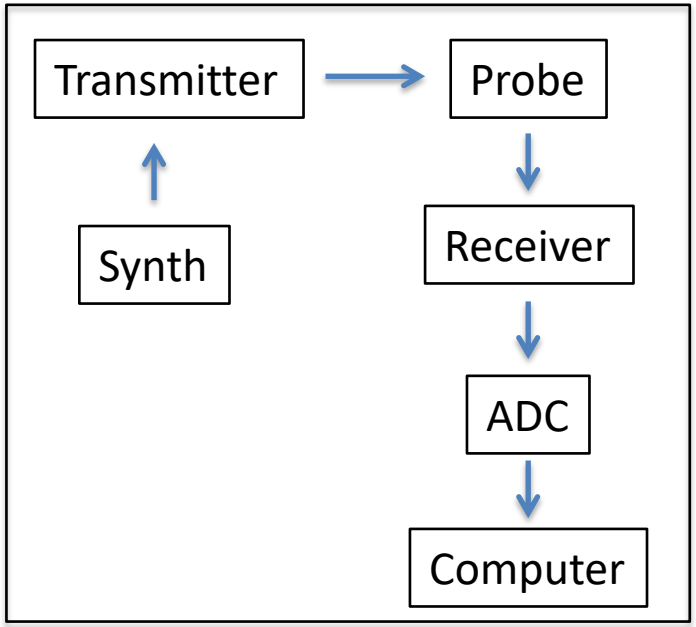
- Synthesizer
  - Need to generate RF at multiple frequencies
  - Multiple “channels”
  - Phases of the RF must also be defined and controlled





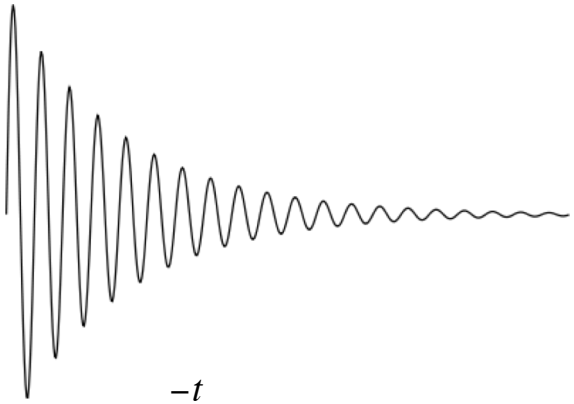
Acquire the spectrum

- Transmitter
  - Gates the RF output in the form of pulse shapes
  - Includes RF Amplifier => produces pulses on the order of a few watts to 100Ws, *in vivo* requires 1kWs, for durations of µs to ms
    - 50W yields a 90 of 20µs, if we want a 10µs we need to double B_1 => quadruple power => 200W
  - Usually expressed in dB attenuation of max
    - Double power => reduce the attenuation 3dB
    - To double the B_1 => 6dB



Acquire the spectrum

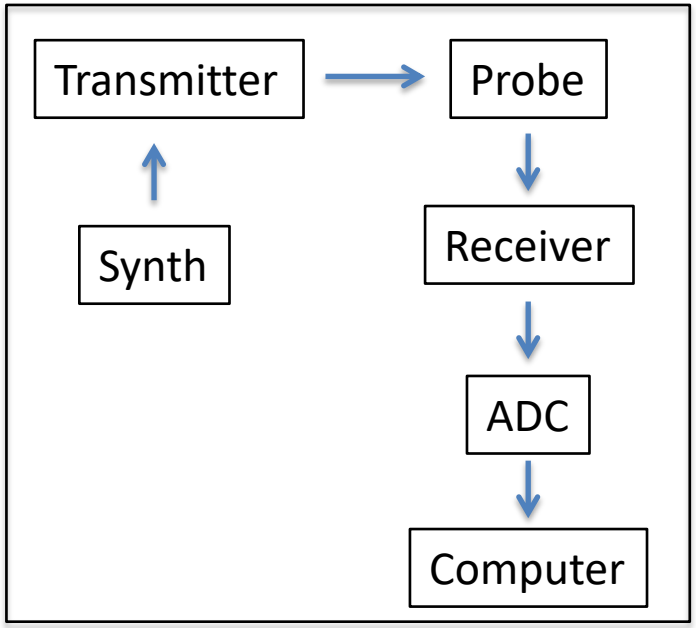
- Receiver
  - Signal induced into the coil
    - With a signal coil we acquire X or Y

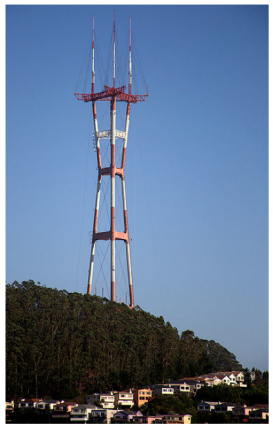


$$S(t) = Ce^{\frac{-t}{T_2}} \cos(\omega_0 t + \phi)$$

C is a constant, ϕ phase relative to synth

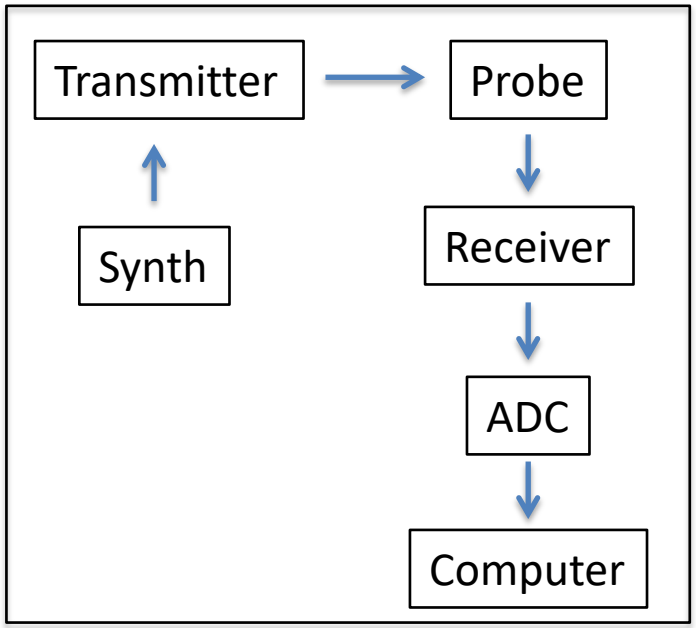
  - Carrier freq also fed into the receiver as a reference wave (to modulate the signal), this is later removed to result in the detection of the signal relative to the rotating frame = audio signal
    - Similar to the way we receive a radio station, it is broadcast superimposed on a carrier signal, we modulate the signal from the antenna of our radio and then remove the rest with low pass filtering…

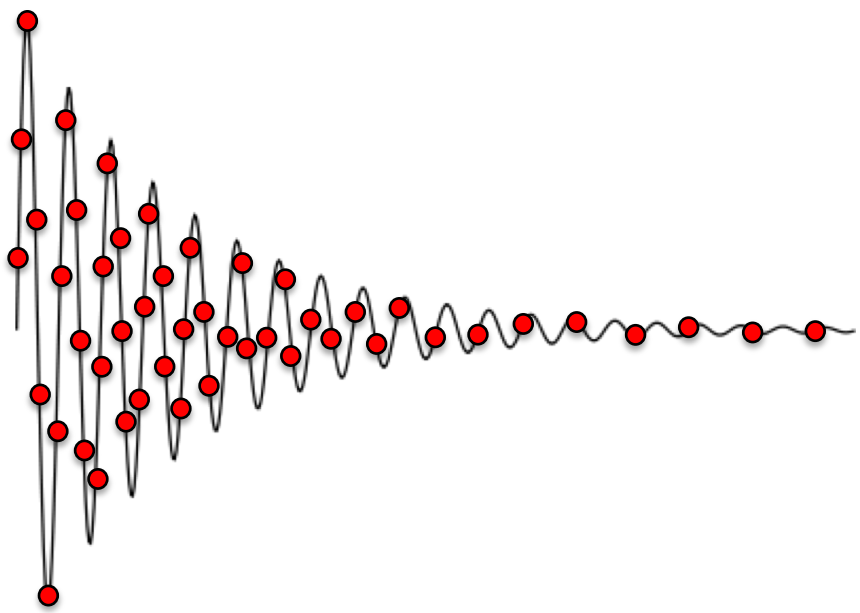




Acquire the spectrum?

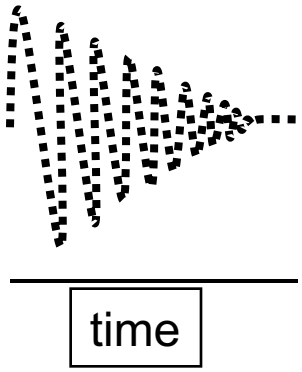
- Analog-to-Digital Converter (ADC)
  - Aka the digitizer, discretizes the FID
  - Time between points is the dwell time
  - Acquisition time = N points x dw
  - Gain is typically adjusted to make sure the signal range match the ADC





Nyquist frequency amounts to half the sampling rate
- Thus the max frequency detectable = 0.5dw

Dwell Time, Spectral Width, Acquisition Time



The Free Induction Decay (FID) is an analog signal that is digitized using an Analog-to-Digital Converter (ADC). The rate at which the FID is sampled by the ADC is very important!

The Nyquist sampling theorem states that in order to represent the frequency of a signal correctly, the sampling rate must be ≥ twice the signal frequency.

$$DW = 1/(2SW)$$

The dwell time (DW) = the reciprocal of twice the spectral width (SW) in Hz.

$$AT = NP \times DW$$

The acquisition time (AT) = number of points (NP) × DW.

$$\text{Digital Resolution} = 1/AT$$

Acquire the spectrum?

- Last but not least, the computer and Fourier
- Signal comes in…

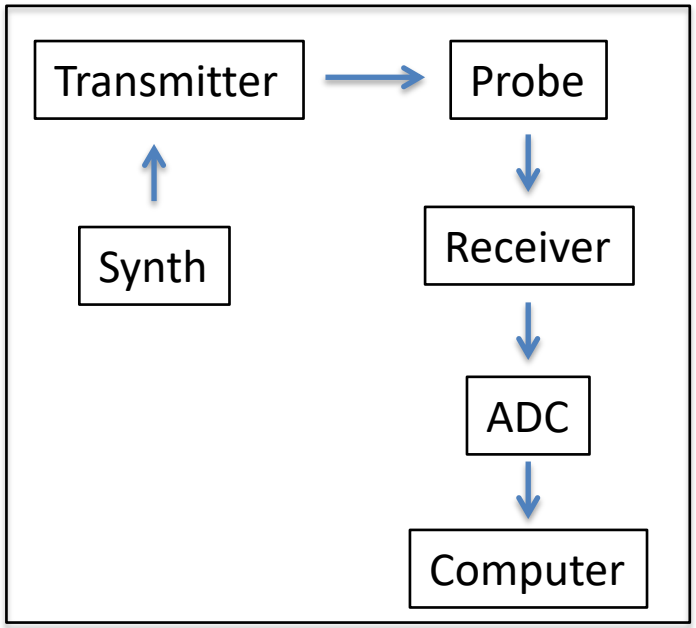
$$S(t) = e^{\frac{-t}{T_2}} e^{2\pi i \nu t}$$

- Fourier Transform

$$S(\nu) = \int_0^\infty \exp\left[-\left(\frac{1}{T_2} + 2\pi i(\nu - \nu_a)\right)t\right]dt$$

- Integrate

$$S(\nu) = \frac{1}{\frac{1}{T_2} + 2\pi i(\nu - \nu_a)}$$



$$S(\nu) = A(\nu) + iD(\nu)$$

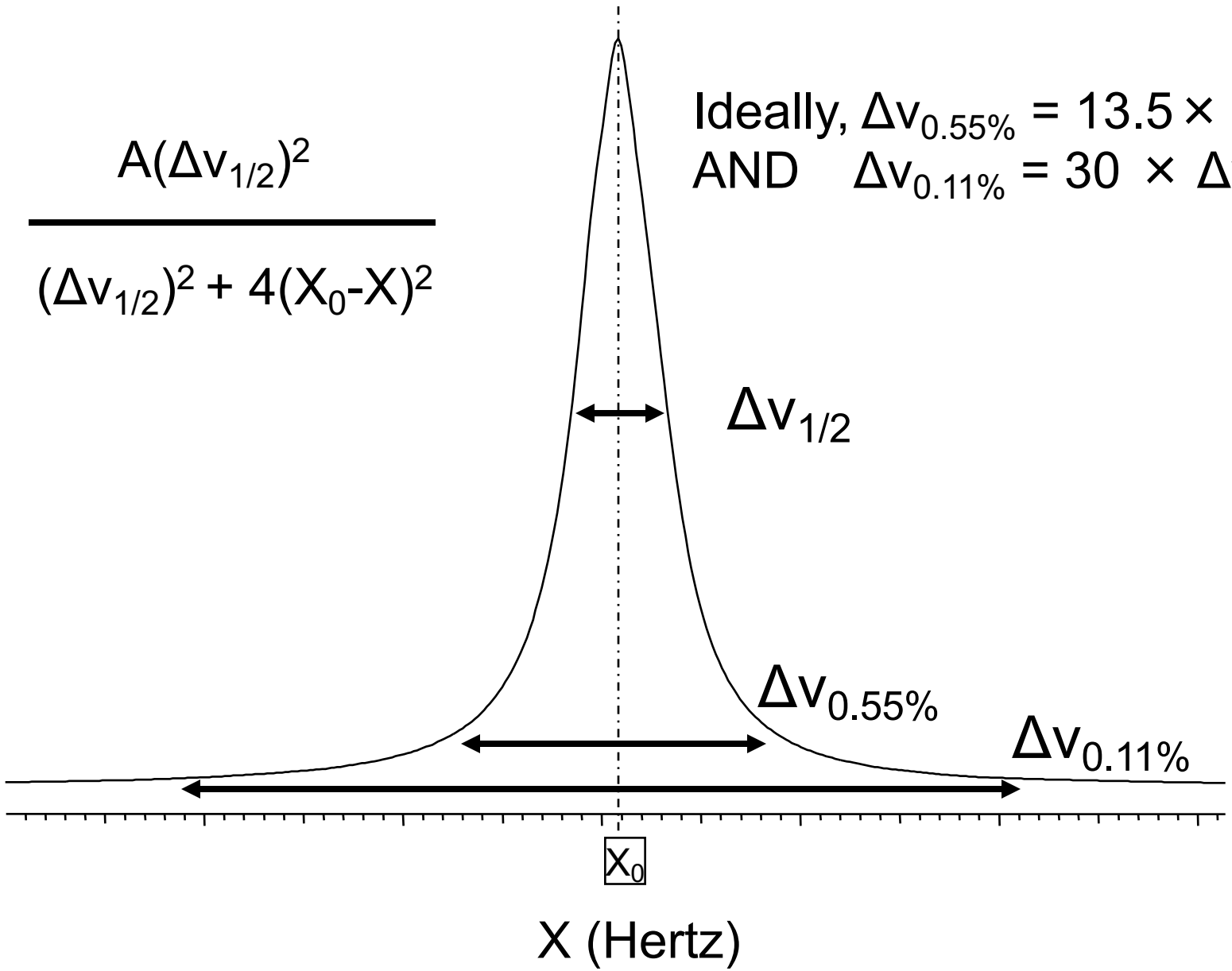
$$A(\nu) = \frac{\frac{1}{T_2}}{\frac{1}{T_2} + 4\pi^2(\nu - \nu_a)^2}$$

ν_a =larmor frequency relative to carrier

The "Ideal" NMR lineshape is called "Lorentzian"

$$Y = \frac{A(\Delta v_{1/2})^2}{(\Delta v_{1/2})^2 + 4(X_0 - X)^2}$$

Ideally, $\Delta v_{0.55\%} = 13.5 \times \Delta v_{1/2}$
AND $\Delta v_{0.11\%} = 30 \times \Delta v_{1/2}$



Acquire the spectrum

- Shimming?
  - Utilize shim coils to homogenize magnetic field
    - Similar in a sense to constant gradients
  - High order shimming?

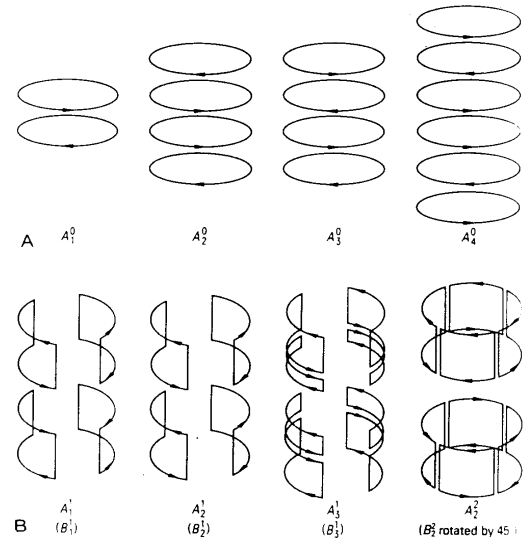
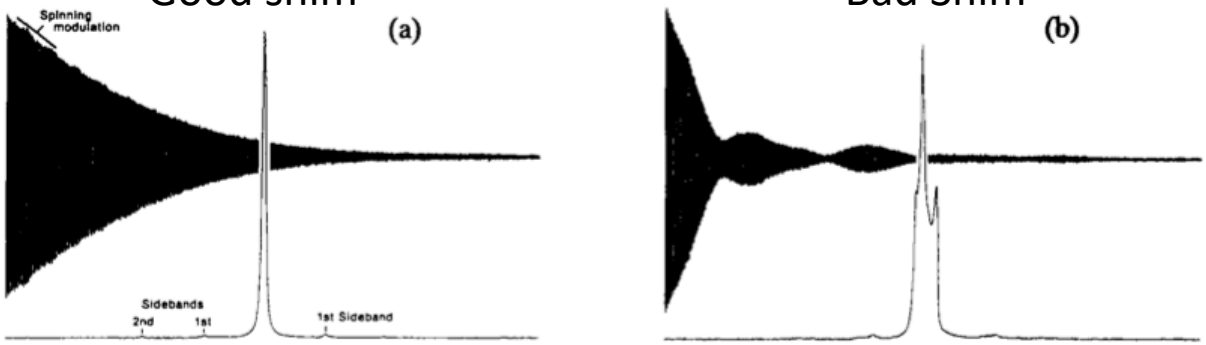


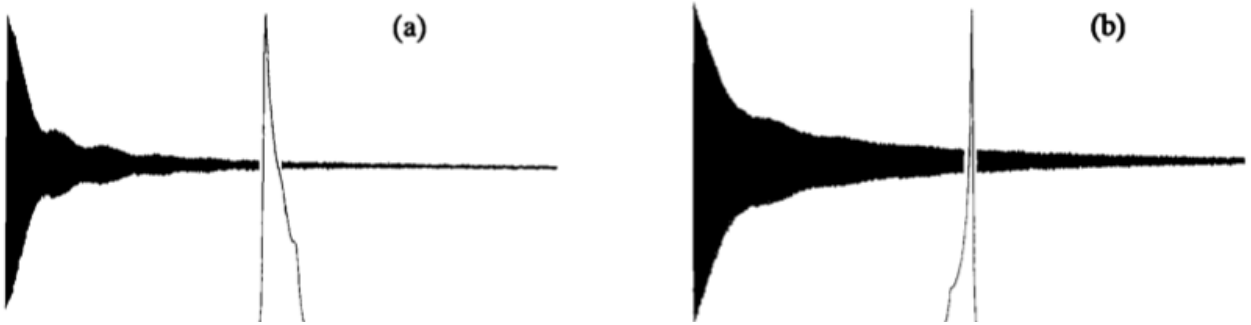
FIGURE 12–6. Schematic drawing of shim coil windings. *A*, Zonal windings. *B*. Tesseral windings.

Good shim

Bad Shim



Problem?....z shim



Problem?....z^2 shim

Order n	Degree m	Function	Common Name
1	0	z	z
2	0	$2z^2 - (x^2 + y^2)$	z^2

Manual shimming: shim coil design

Manual shimming alternates the current through several shim coils

- Axial shims: Z, Z^2, Z^3, Z^4, etc.
- Radial shims: X, Y, XY, XZ, YZ, X-Y, etc.

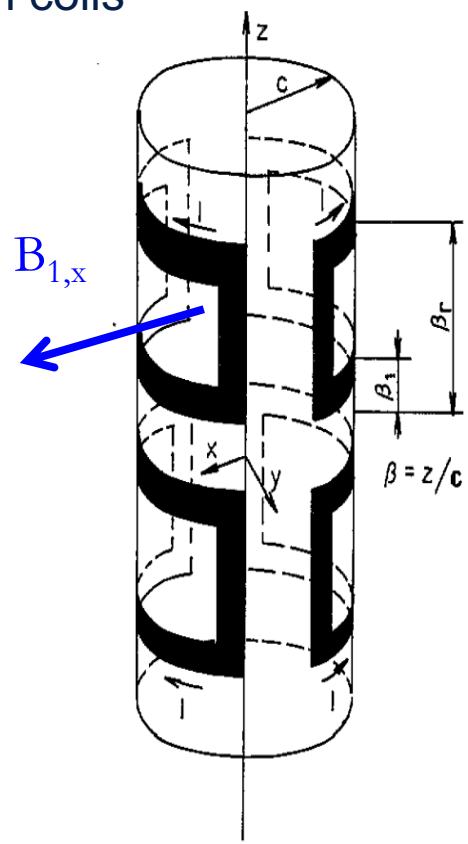
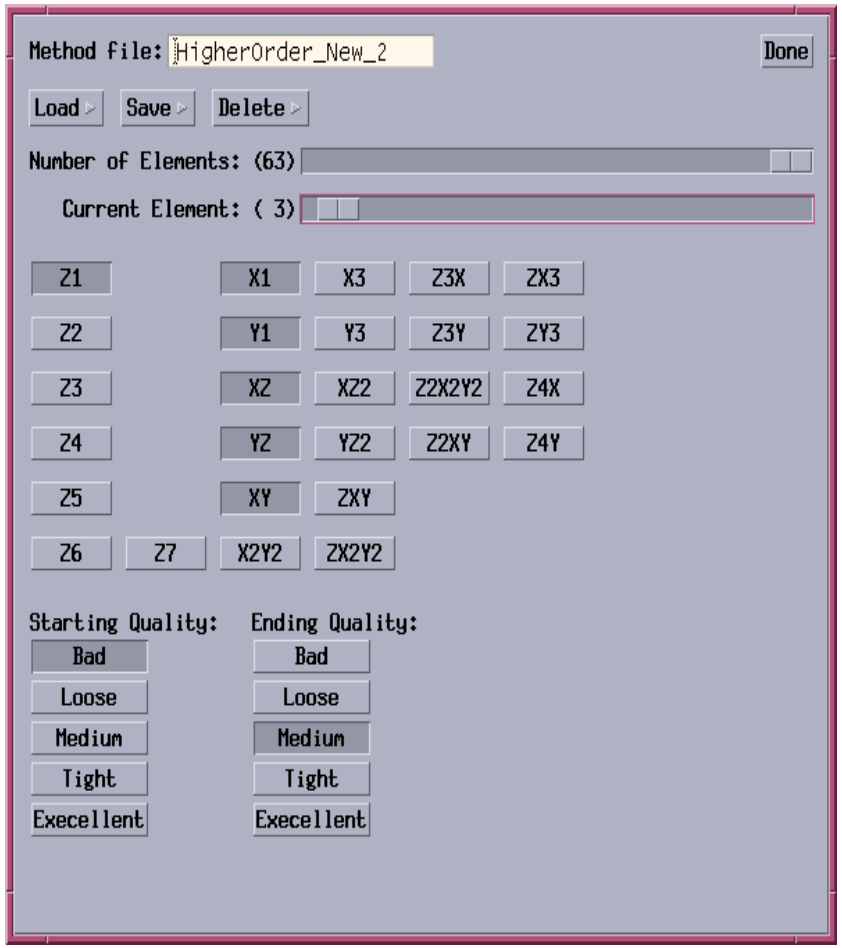


Figure 2. Saddle coils of a simple X-shim.

If the lineshape is not “Lorentzian” you need to shim!

Lorentzian lineshape is directly related to your ability to suppress water and acquire pretty-looking spectra

Shim adjustment Order
Z1 X1 XZ
Z1 Y1 YZ
Z1 X1 XZ Y1 YZ
X1 X3
Y1 Y3
Z1 Z3 X1 Z3X
Z1 Z3 Y1 Z3Y
Z1 X1 X3 ZX3
Z1 Y1 Y3 ZY3
Z1 Z2 Z4 X1 Z4X
Z1 Z2 Z4 Y1 Z4Y
Z1 Z2 X2Y2 Z2X2Y2
Z1 Z2 X1 XZ2
Z1 Z2 Y1 YZ2
Z1 Z2 X1 Y1 XY Z2XY
X1 Y1 ZXY
Z1 X2Y2 ZX2Y2
Z1 XY ZXY

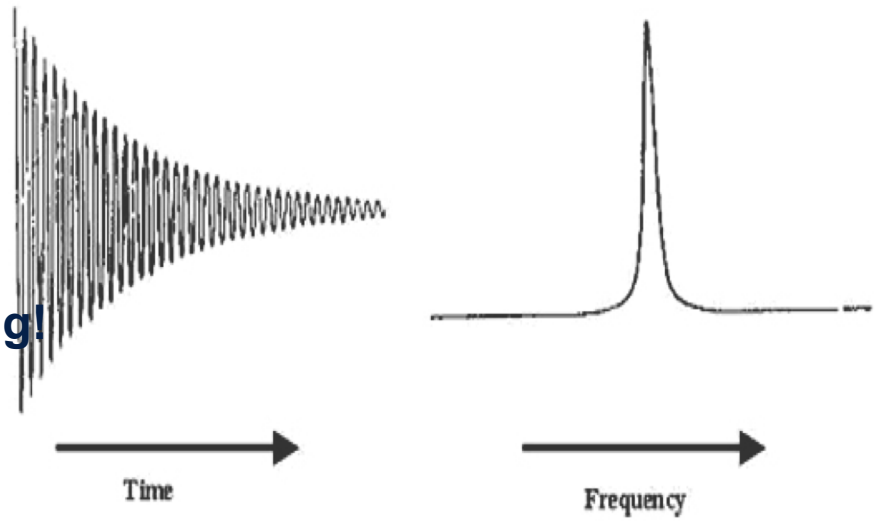


Shimming by hand ≠ Fun

Automated shimming much better!

Manual shimming methods

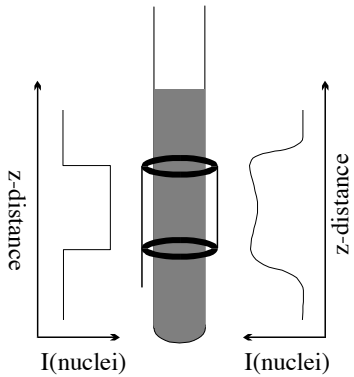
1. Shimming on the deuterium lock signal
2. Shimming on the FID
   - For samples with no lock solvent
3. Shimming on the FT line shape

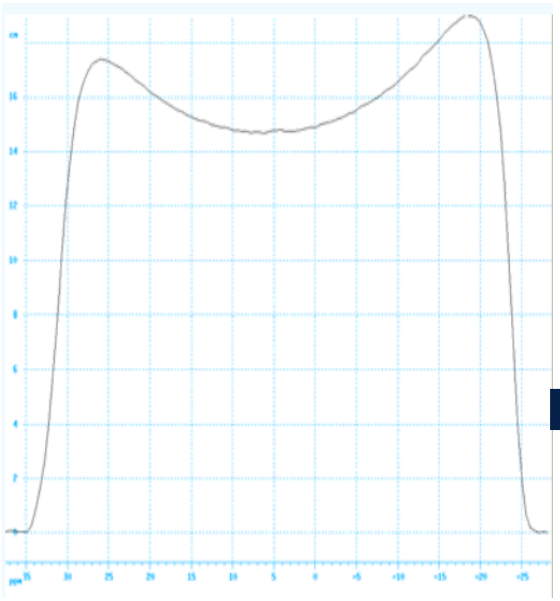


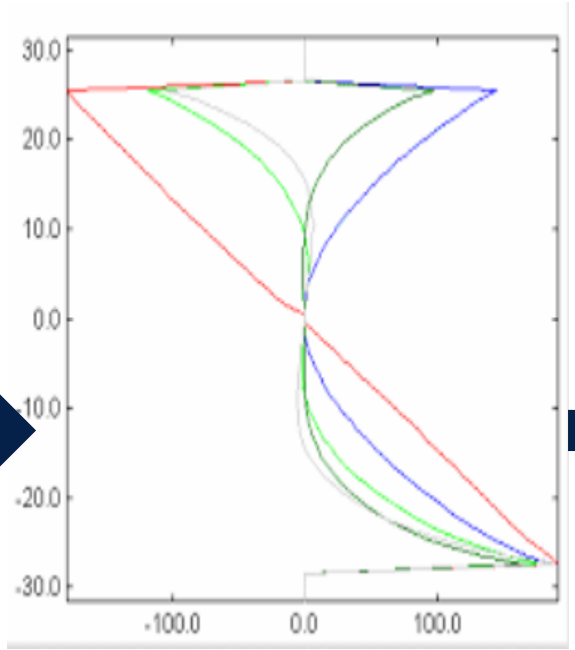
But, manual shimming is time consuming!

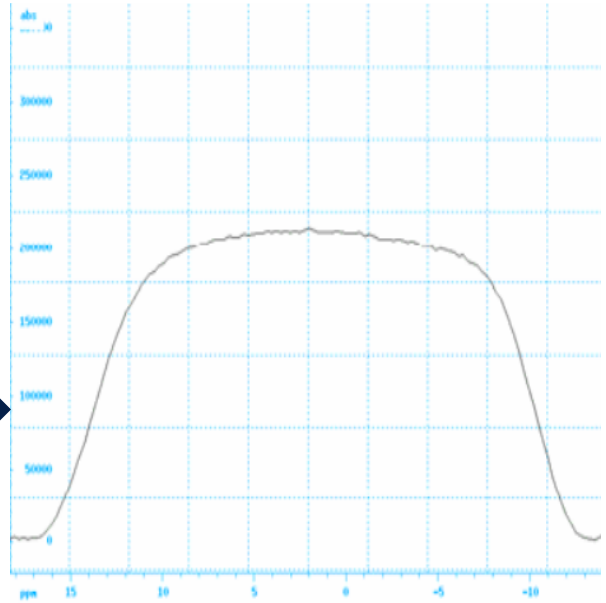
Gradient shimming

1. Shim mapping
2. Auto shimming based on shim map



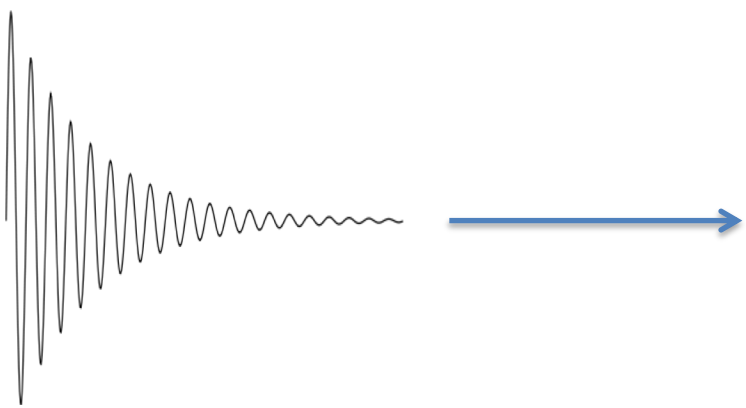


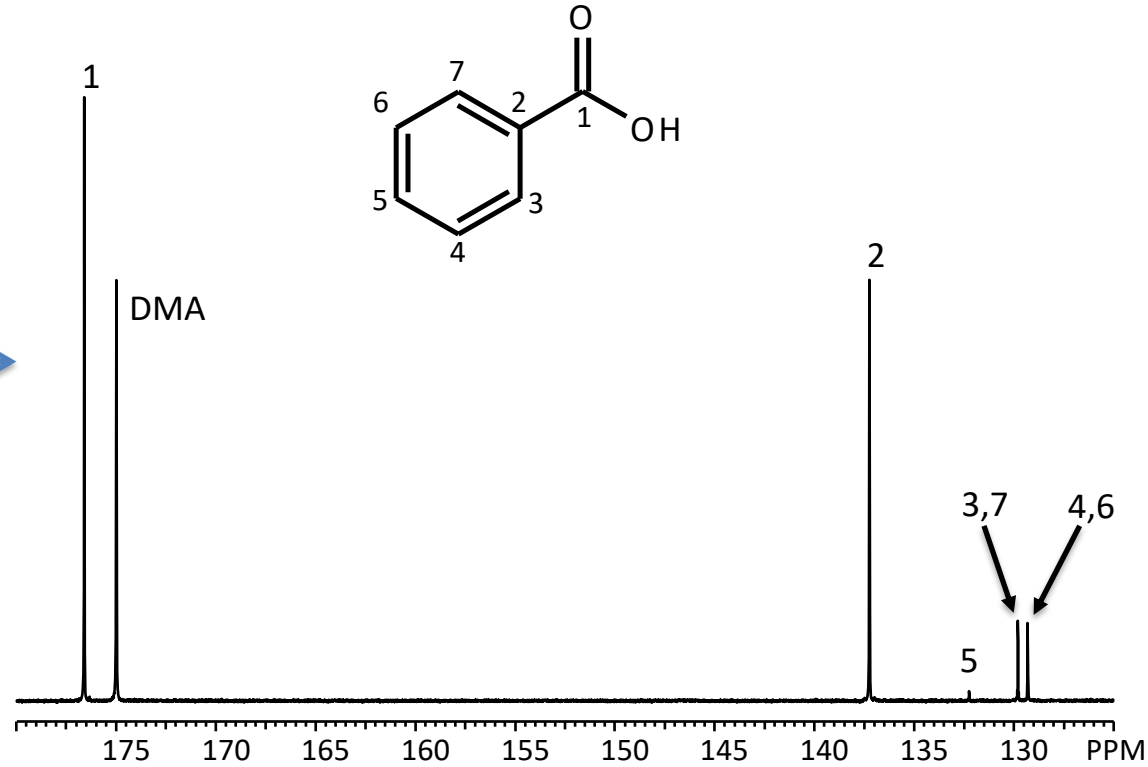




Acquire the spectrum?

- So ultimately…





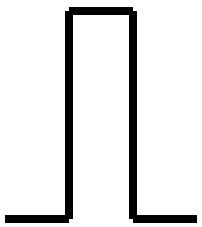
Which has some other features:

Chemical shift
J-coupling
Dipolar-Dipolar
Ring effects
CSA

Information content of an NMR Spectra
- Discussed in lectures 2 and 3

NMR Pulse Sequences

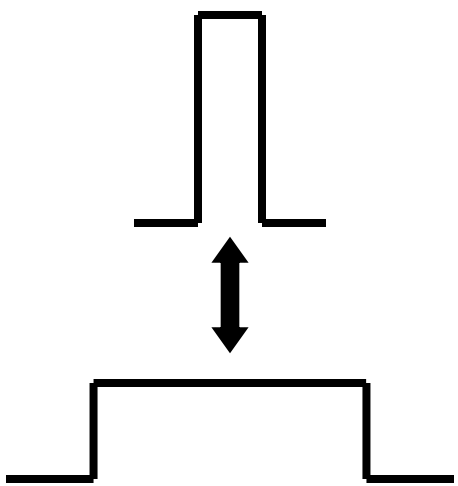
A pulse sequence is a set of RF pulses applied to a sample to produce a specific form of NMR signal.



In NMR, pulse sequences are described using basic symbols:

The height describes the power (in dB) and the width describes the duration of the pulse (μs – ms)

A 90º (π/2) pulse <u>equalizes</u> the spin populations given by the Boltzmann distribution, whereas a 180º (π) pulse <u>inverts</u> the populations.



The same flip angle can be achieved by applying a high power pulse for a short duration or a low power pulse for a longer duration.

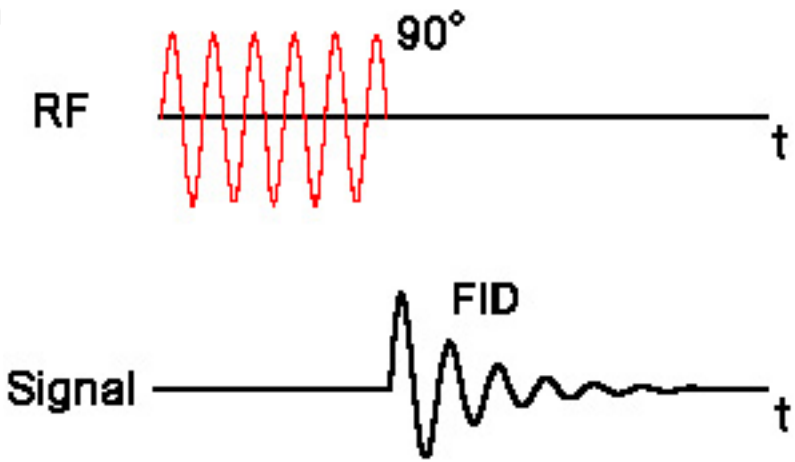
$$\Theta = \gamma B_1 t$$

However, the excitation bandwidth is ~1/t, so shorter pulses yield higher bandwidths.

NMR Pulse Sequences – Pulse and Acquire

In the 90-FID pulse sequence, net magnetization is rotated down into the X'Y' plane with a 90° pulse.

A timing diagram for a 90-FID pulse sequence has a plot of RF energy versus time and another for signal versus time.



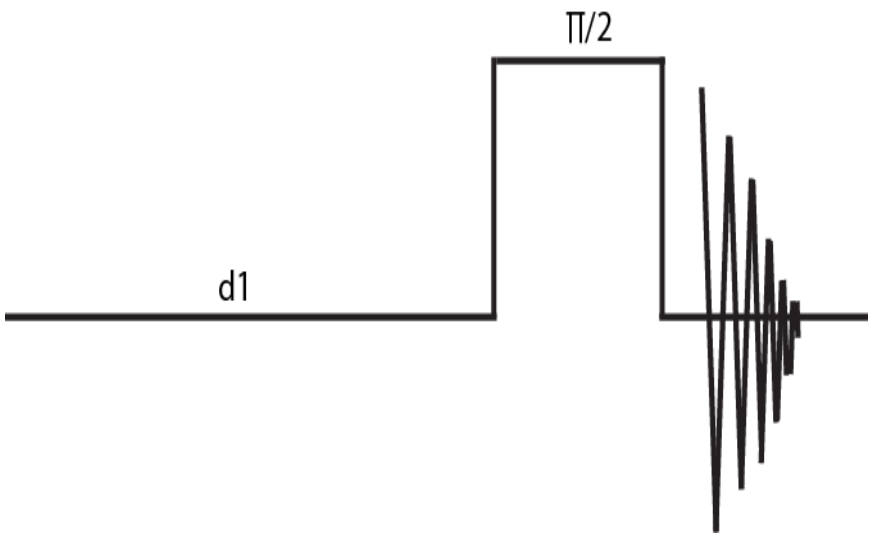
When this sequence is repeated, for example when signal-to-noise improvement is needed, the amplitude of the signal after being Fourier transformed (S) will depend on T_1 and the time between repetitions, called the repetition time (TR), of the sequence. In the signal equation below, *k* is a proportionality constant and is the density of spins (the number of nuclear dipoles per unit volume) in the sample.

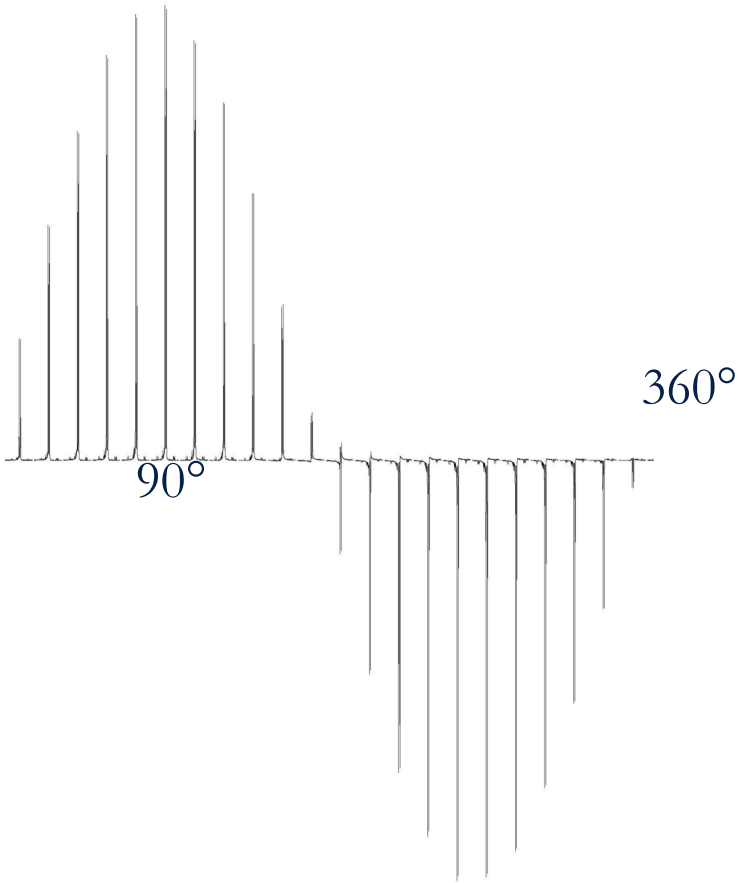
$$S = k\ (1 - e^{-TR/T1})$$

T.C. Farrar *An Introduction To Pulse NMR Spectroscopy* Farragut Press, Chicago 1987.

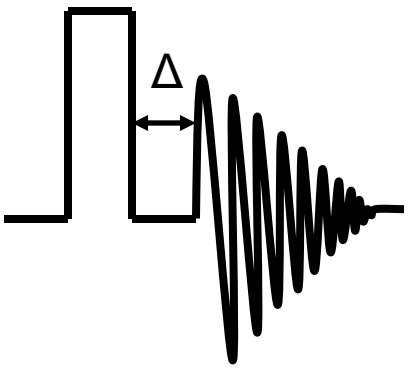
T.C. Farrar, E.D. Becker *Pulse And Fourier Transform NMR* Academic Press, New York 1971

Calibrating the 90° Pulse

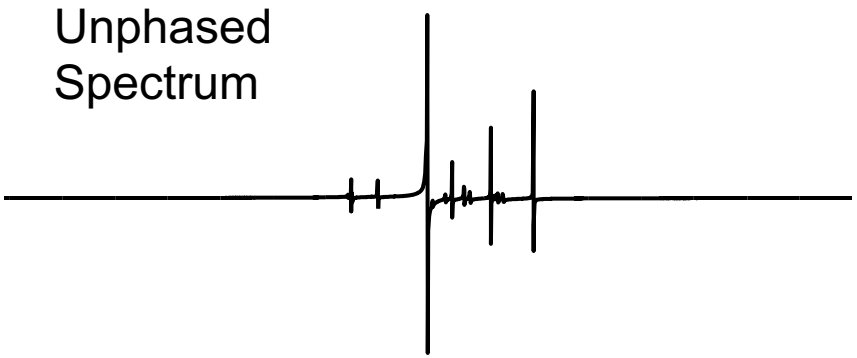


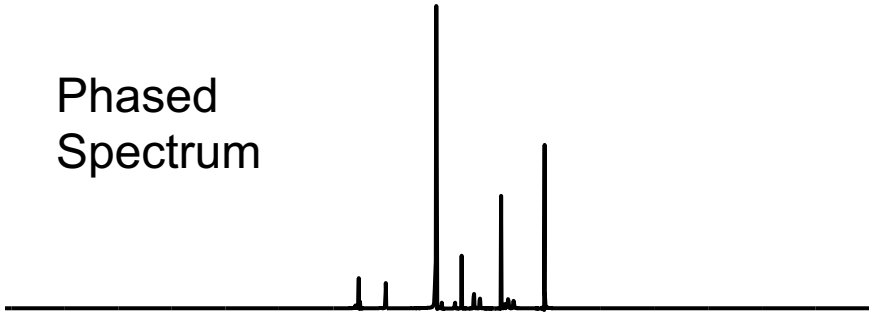


Phasing the Spectrum



A finite amount of time (<1 ms), referred to as the "dead time" (Δ), is required for the spectro-meter electronics to recover following a pulse before the receiver can be turned on. This delay imparts a phase shift on the data.





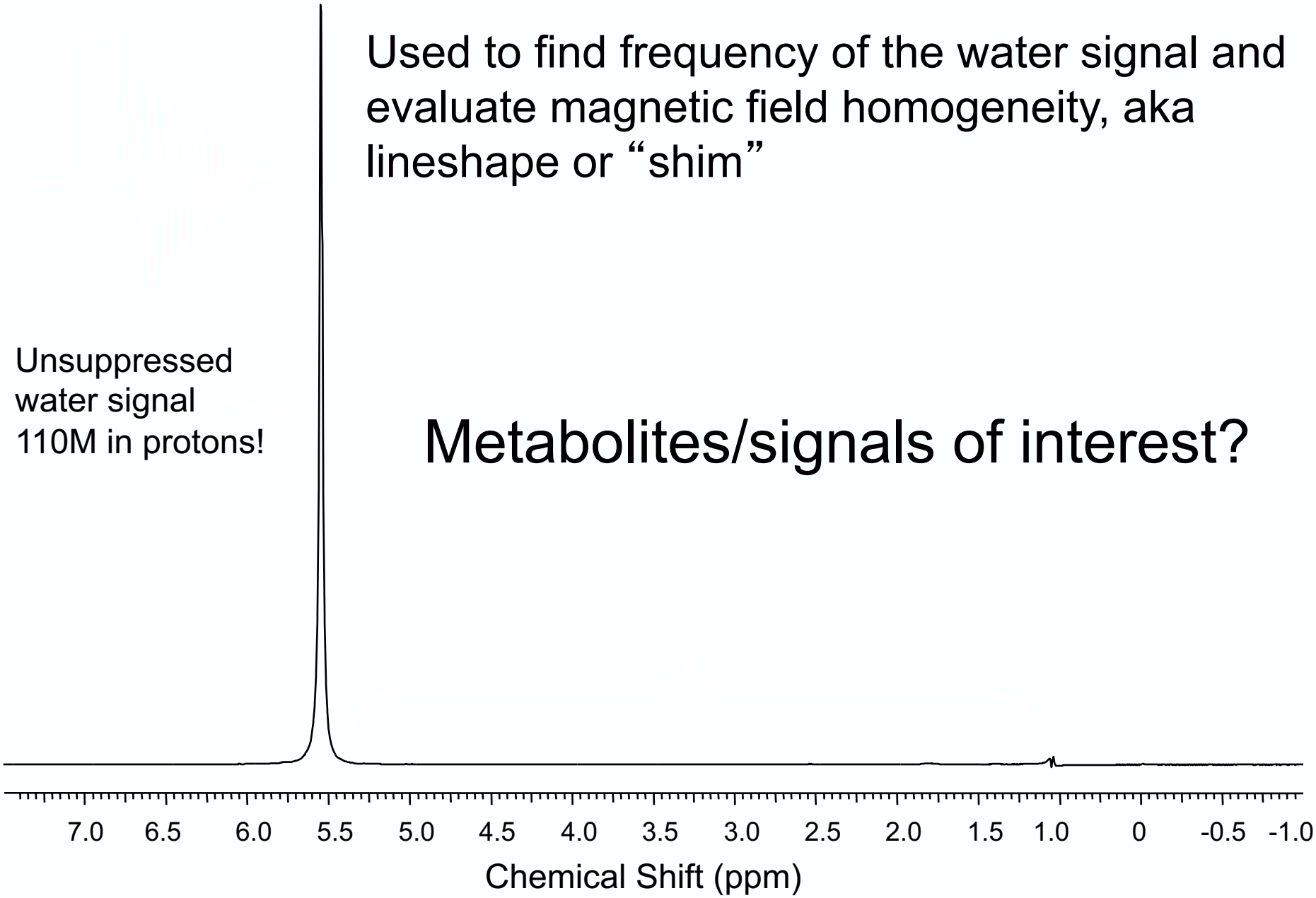
"zero order" phase shifts are caused by differences in the phase of the transmitter and the receiver. "First order" phase shifts are caused by Δ. If Δ is not too long, the phase error is roughly a linear function of the frequency.

1D pulse-acquire spectrum of human liver tissue

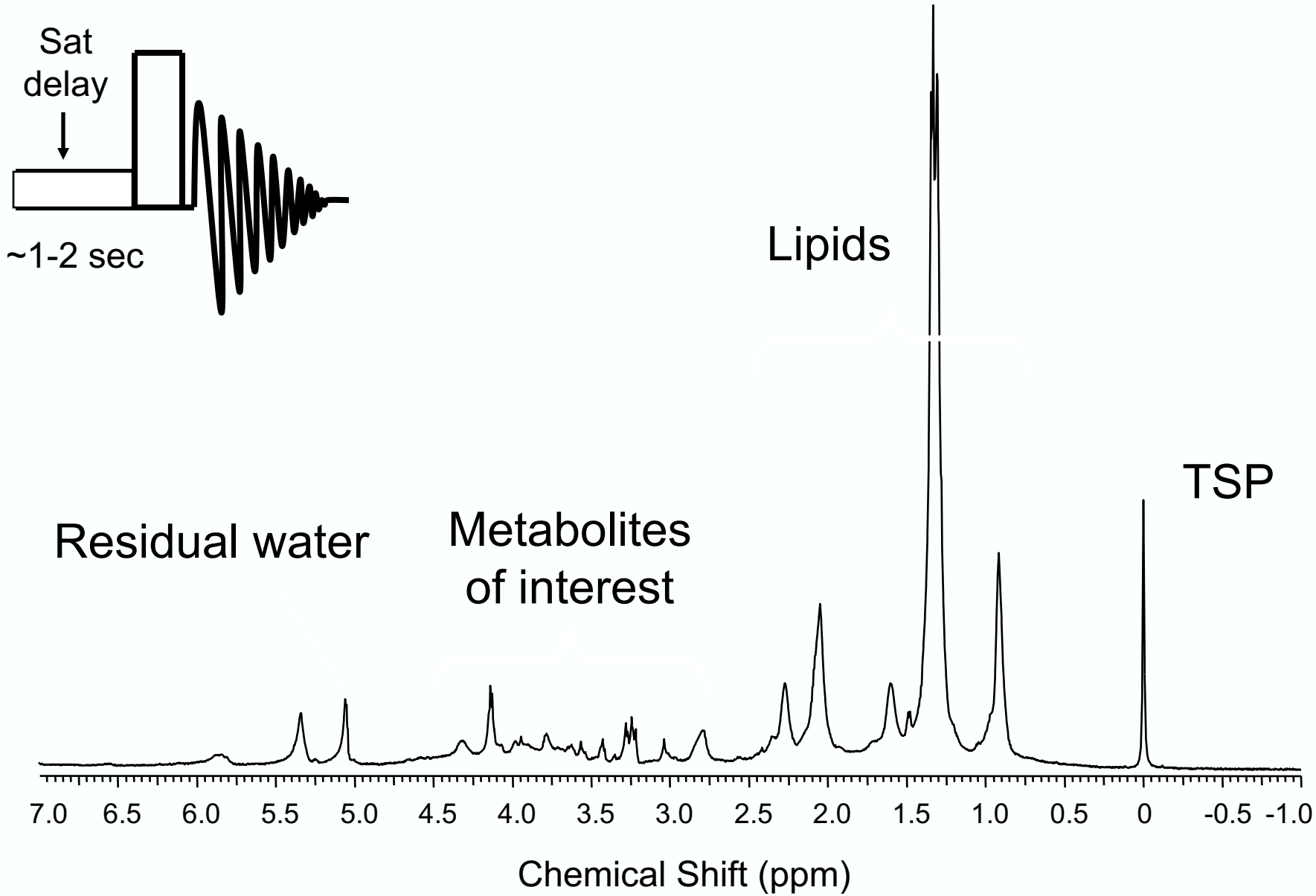
Used to find frequency of the water signal and evaluate magnetic field homogeneity, aka lineshape or “shim”

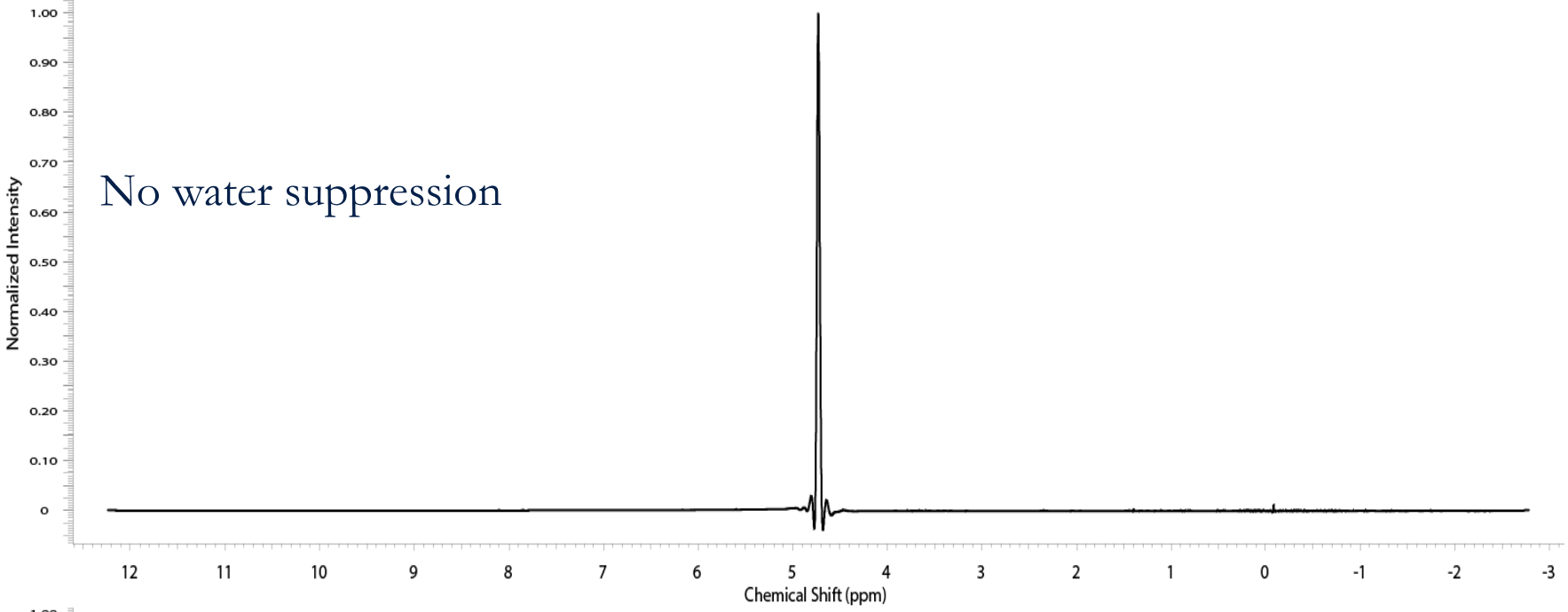
Unsuppressed water signal 110M in protons!

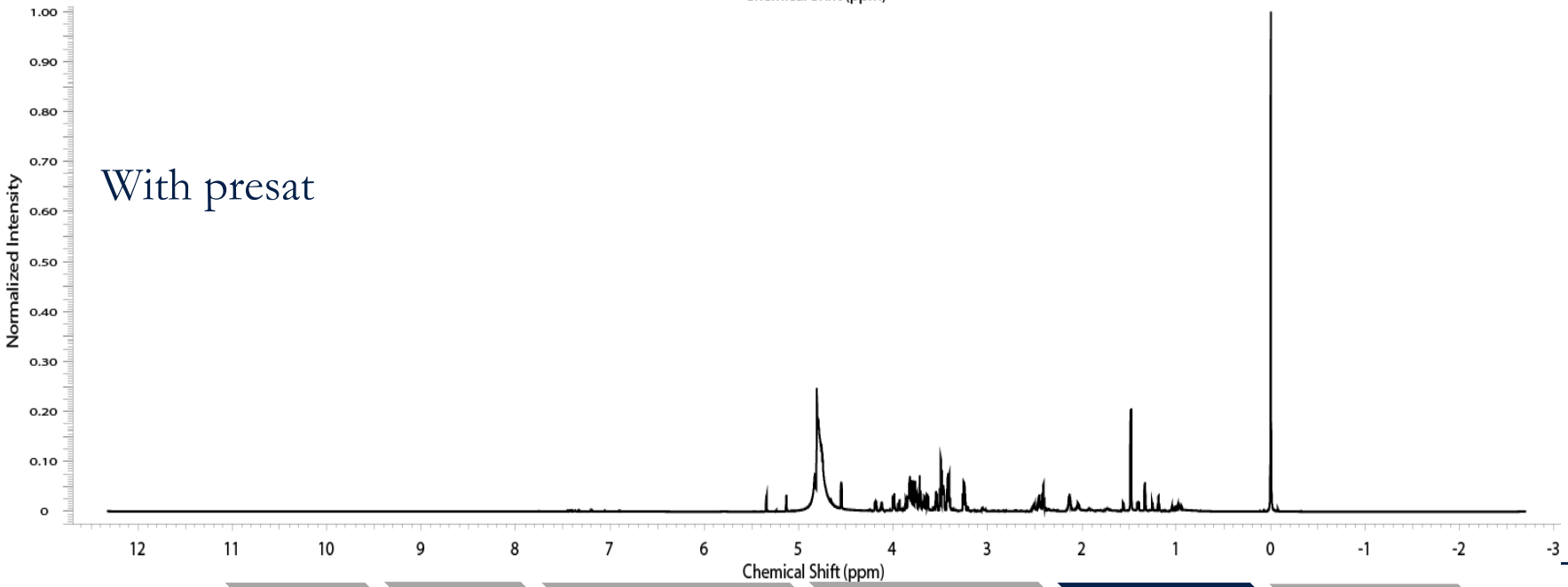
Metabolites/signals of interest?



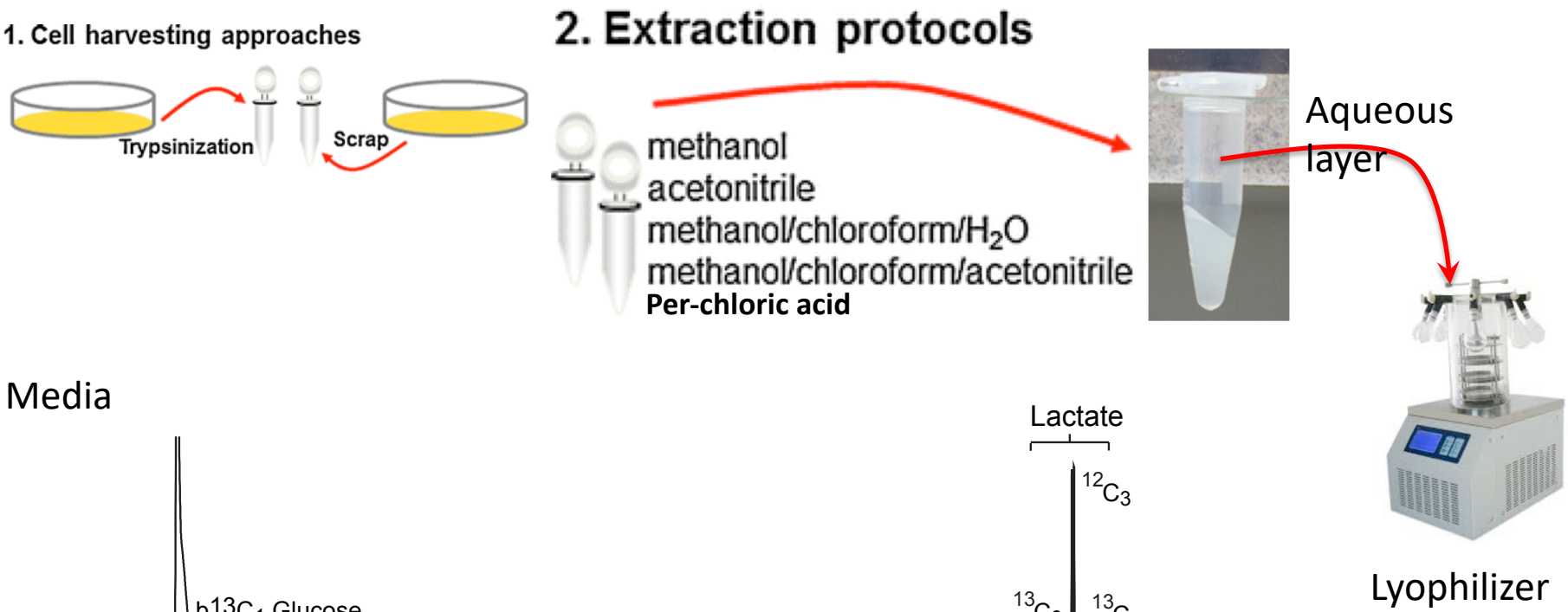
1D "presat" spectrum of human liver tissue

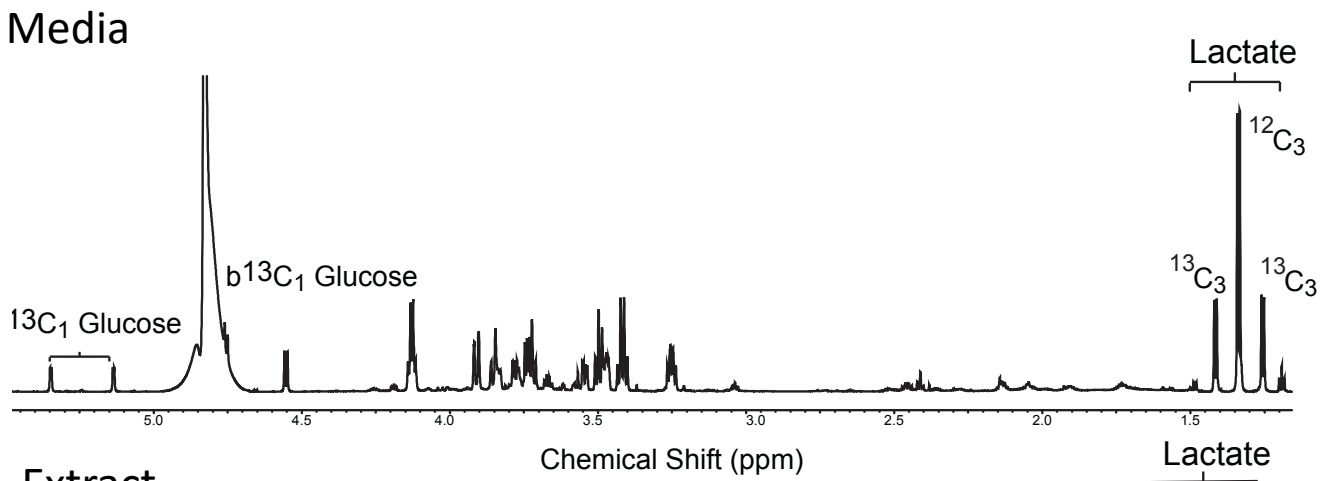


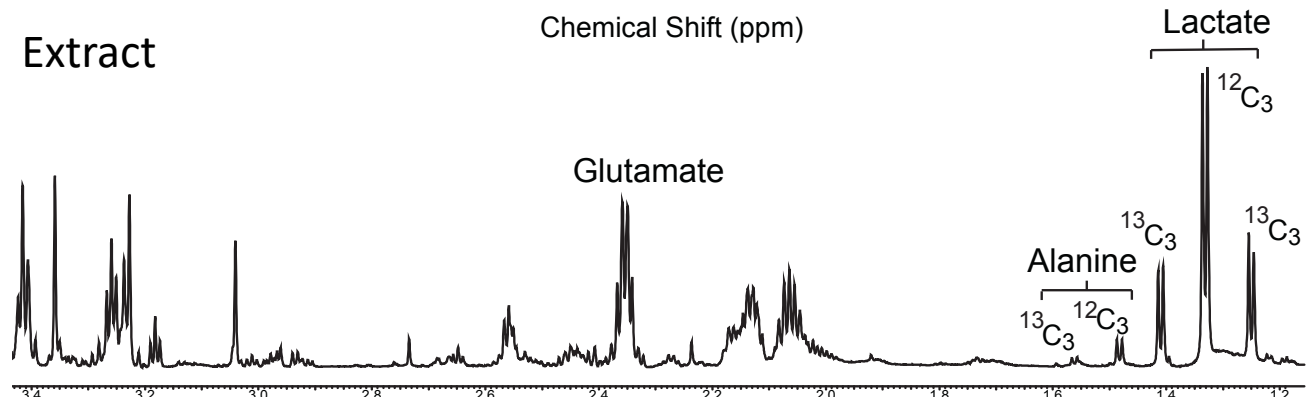

No water suppression
Normalized Intensity
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
Chemical Shift (ppm)


With presat
Normalized Intensity
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
Chemical Shift (ppm)

Cell/Tissue extraction

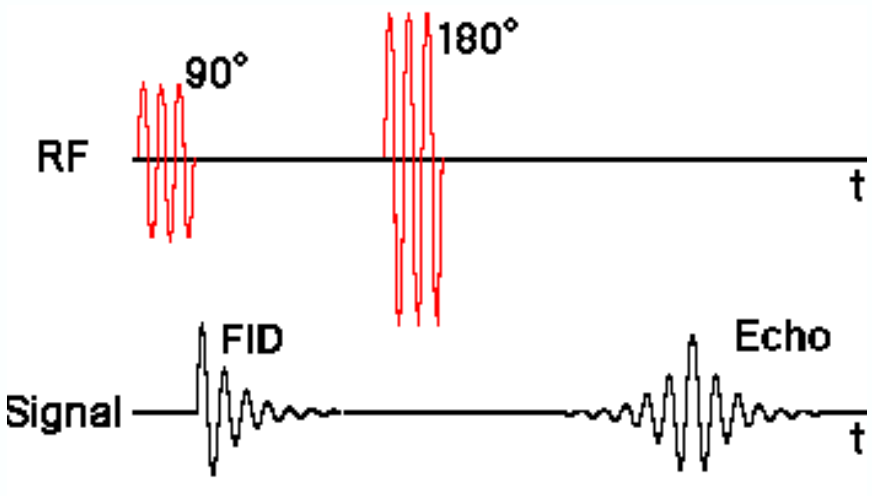






NMR Pulse Sequences – Spin Echo

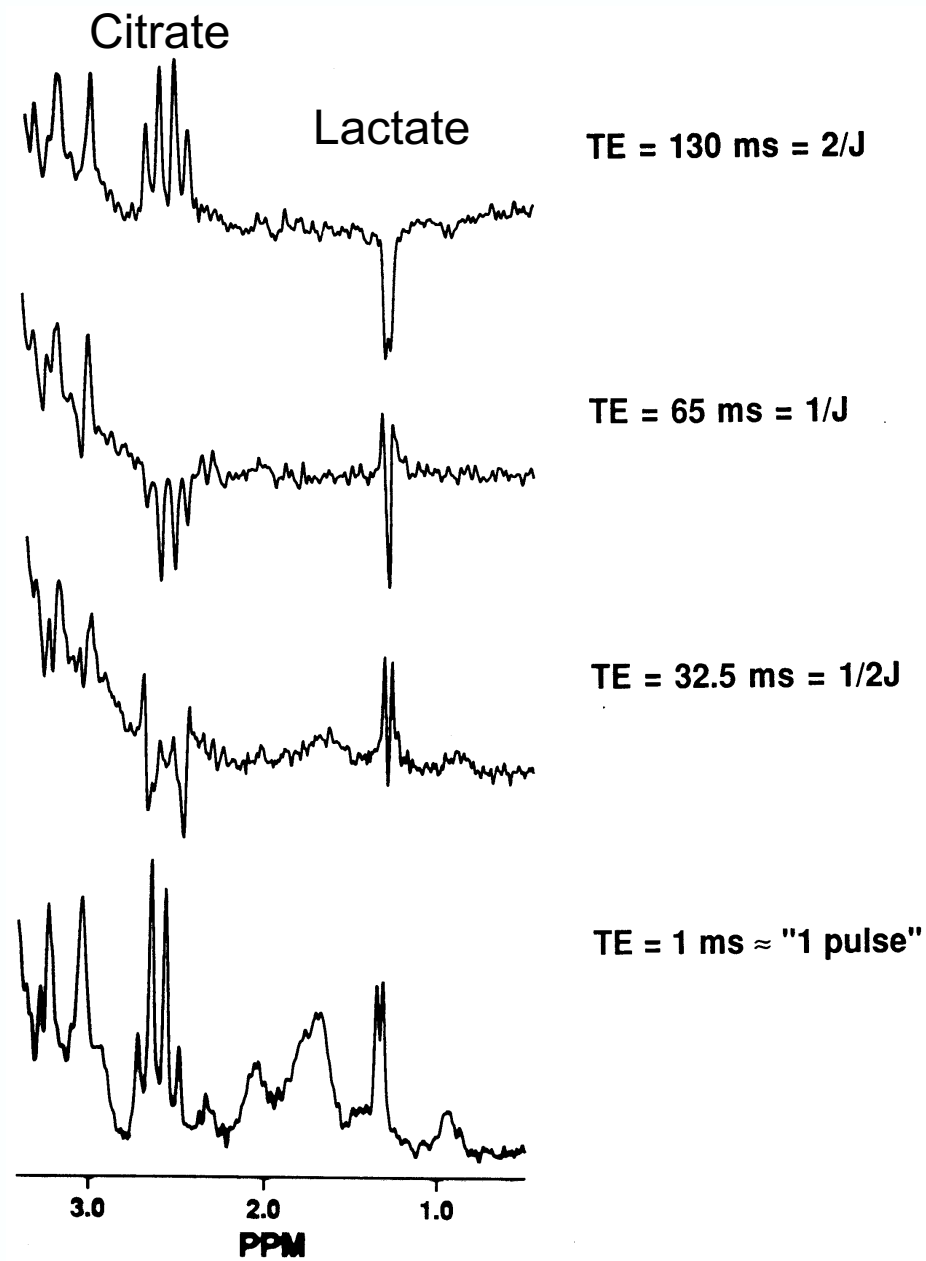
Another commonly used pulse sequence is the spin-echo pulse sequence. Here a 90° pulse is first applied to the spin system. The 90° degree pulse rotates the magnetization down into the X'Y' plane. The transverse magnetization begins to dephase. At some time in time after the 90° pulse, a 180° pulse is applied. This pulse rotates the magnetization by 180° about the X' axis. The 180° pulse causes the magnetization to at least partially rephase and to produce a signal called an echo.



The signal equation for a repeated spin echo sequence as a function of the repetition time, TR, and the echo time (TE) defined as the time between the 90° pulse and the maximum amplitude in the echo is

$$S = k\ (1 - e^{-TR/T1})\ e^{-TE/T2}$$

Spin echo - What is and what is not refocused?



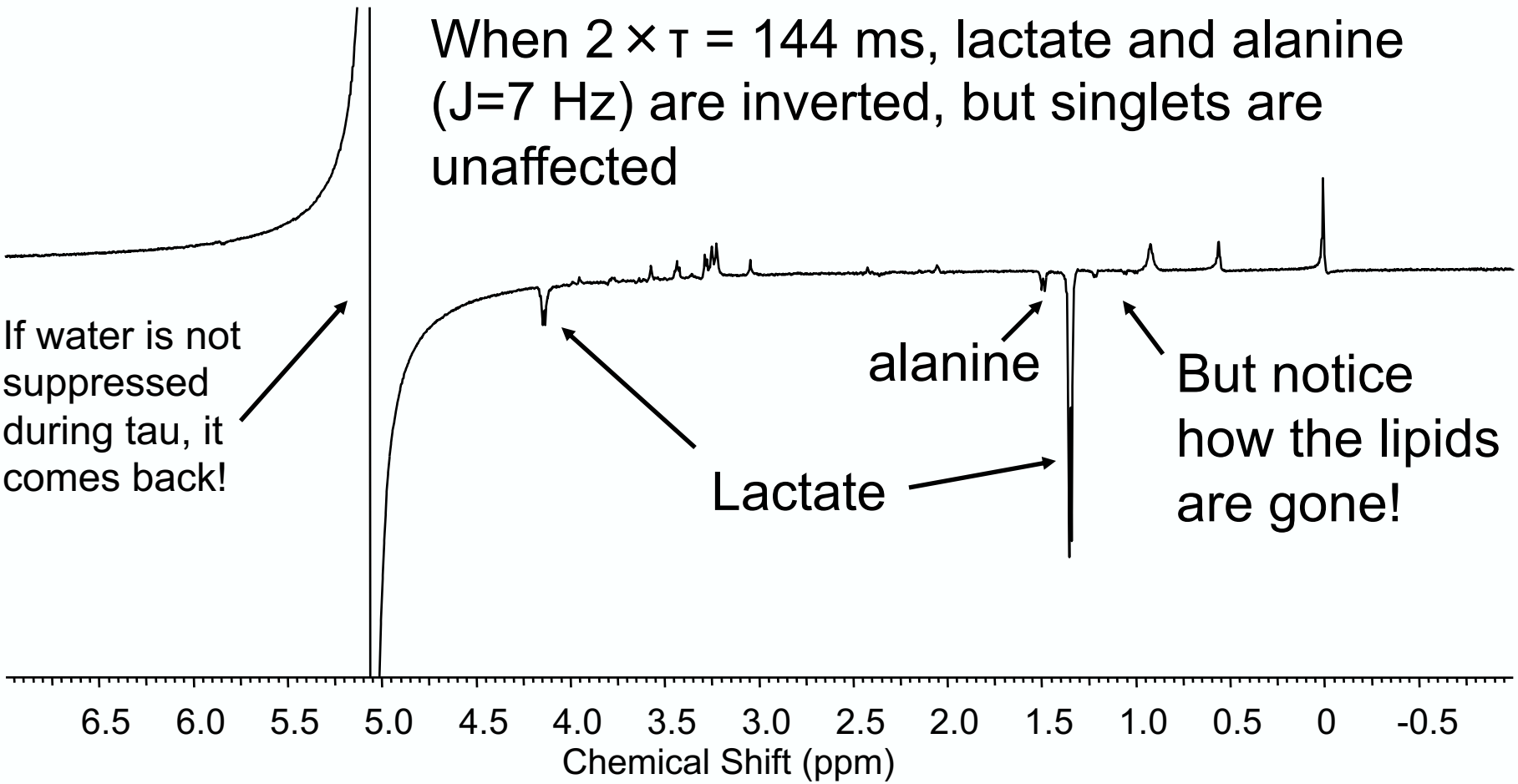
- T_2* is refocused – yielding the natural T_2
- J-modulation may or may not be completely refocused depending on the echo time

  This J-modulation results in a inverted doublet at TE = 1/2 * 1/(J/2) = 1/J = 1/7Hz = 144 ms, and returning to a positive doublet at TE= 1/(J/2) = 2/J = 288 ms.

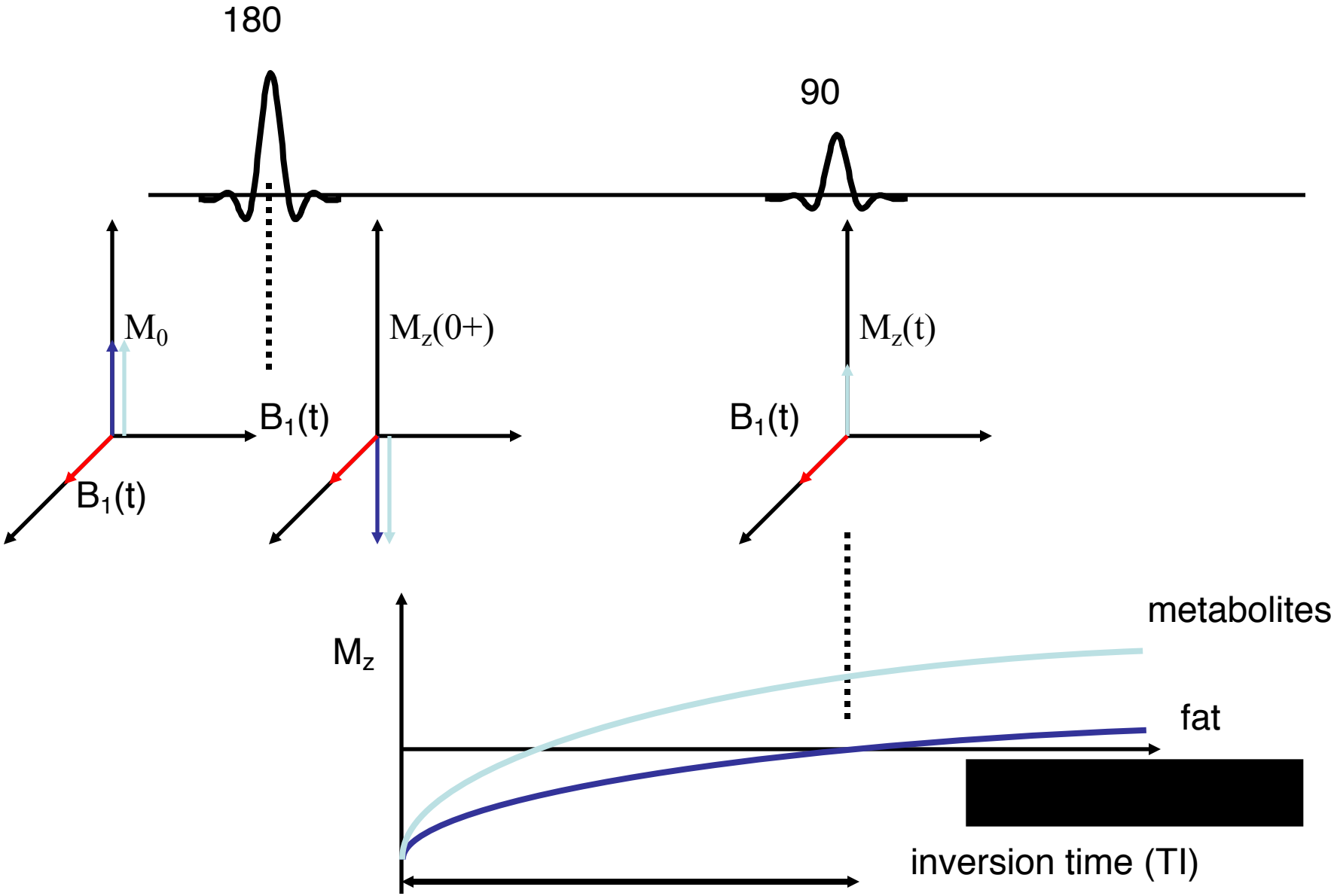
- Will get relaxation based signal losses with increasing echo time

1D “spin echo” spectrum of human liver tissue

When $2 \times \tau = 1/J$, coupled resonances are inverted



Inversion recovery



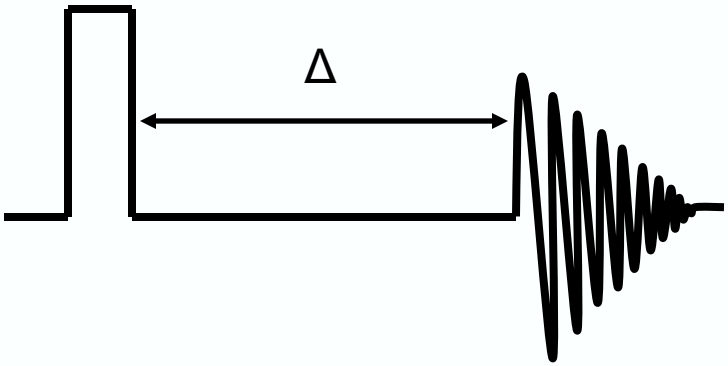
Inversion recovery

- $S = k\ (1 - 2e^{-TI/T1})$

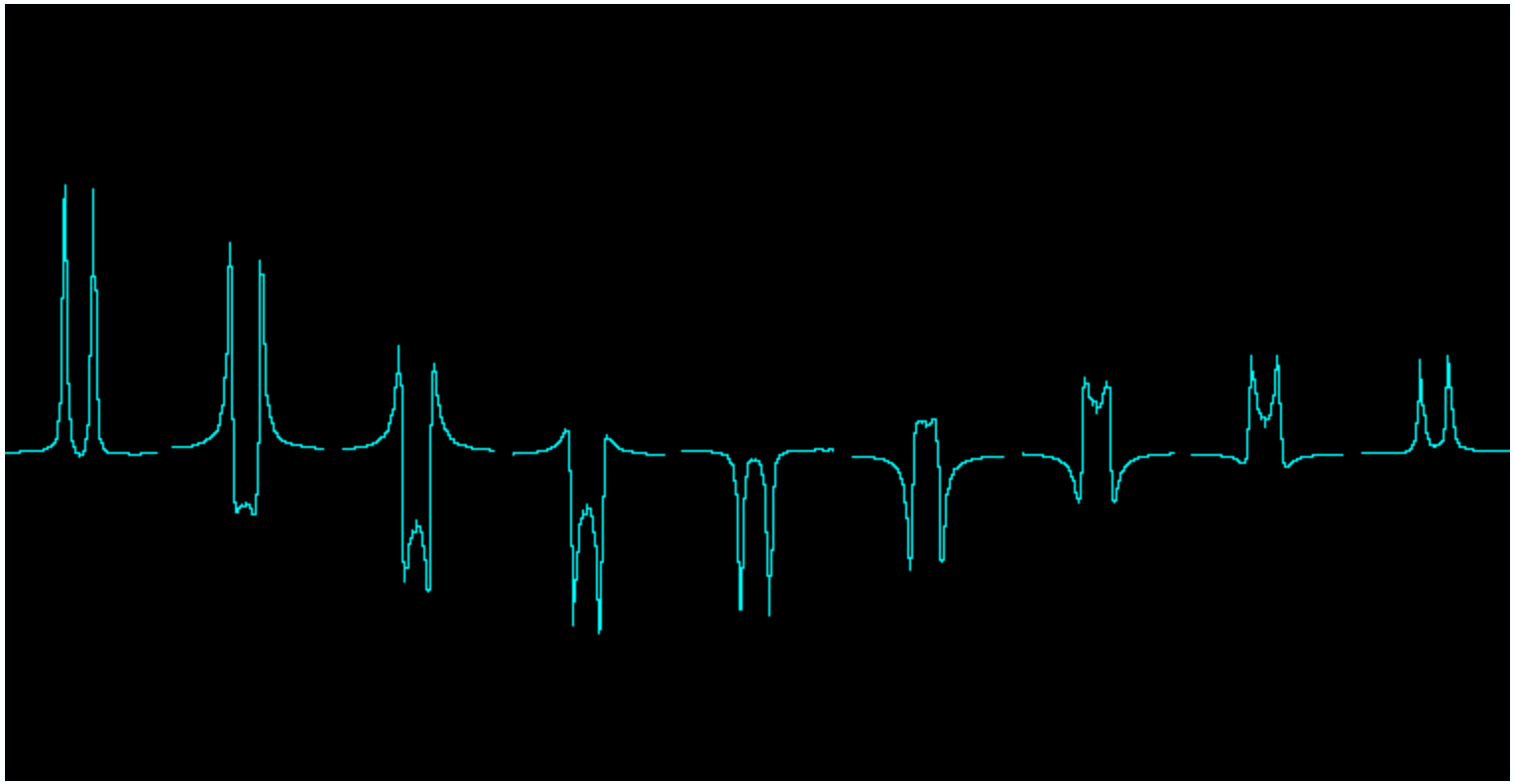
- It should be noted at this time that the zero crossing of this function occurs for $TI = T_1\ ln2$.

- T1 of fat = 260ms (@1.5T), T1 of metabolites = 1000ms (@1.5T)
  - TI (inversion time) = T1 * ln(2)
- Not all metabolites have recovered, inversion recovery results in 20-30% SNR loss

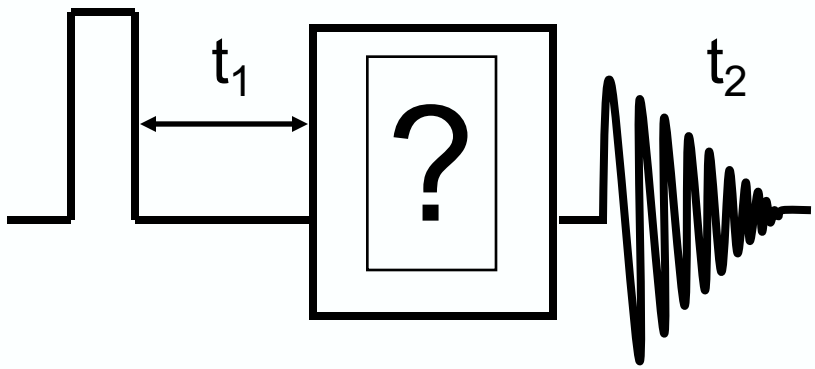
2D NMR Sequence



What if we increase the delay time between the pulse and the acquisition in a simple pulse and acquire sequence?

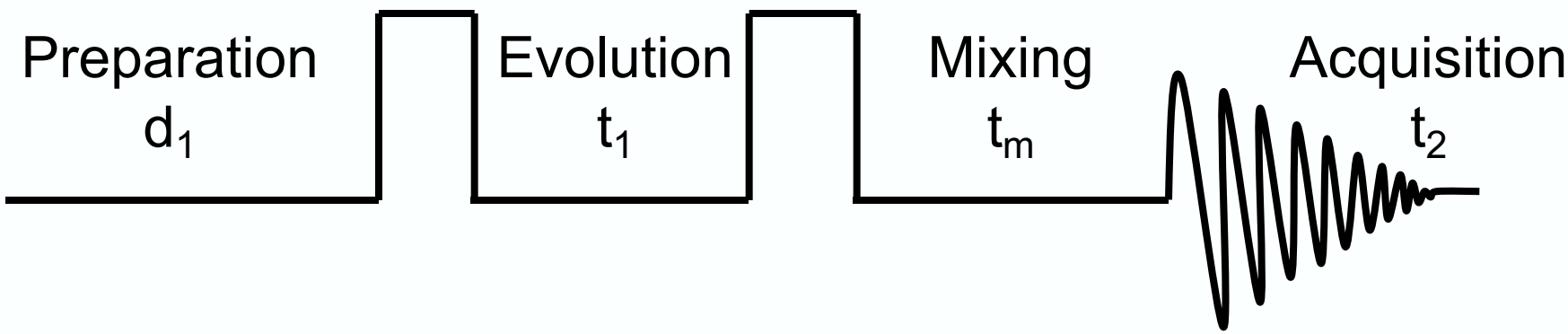


What is 2D NMR?



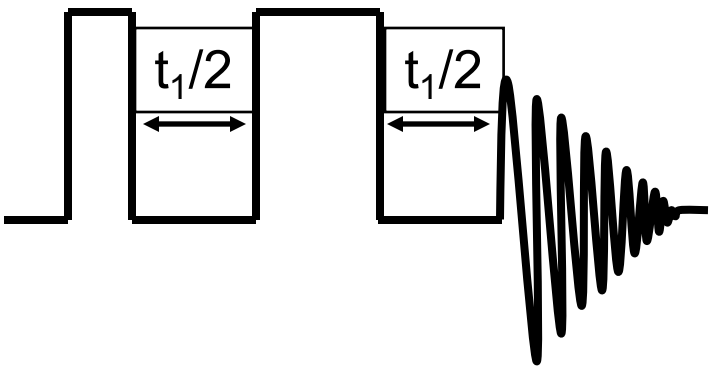
$$S(t_1,t_2) \xrightarrow{\text{2D FT}} S(F_1,F_2)$$

In 2D NMR, a series of FIDs are collected as t_1 is systematically incremented at intervals equal to DW. The NMR signal is now a function of the conventional acquisition time t_2 and the incremented delay time t_1. The INFORMATION CONTENT in a 2D NMR experiment depends on what ? Is.

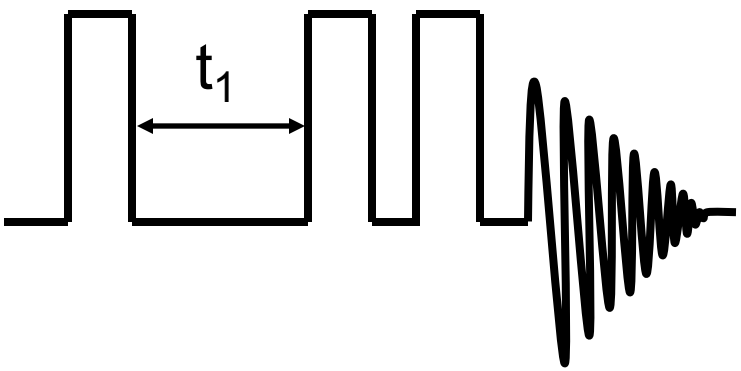


More generally, 2D NMR experiments are made up of 4 parts: preparation (relaxation delay – d_1), evolution (t_1), mixing (t_m), and acquisition (t_2) times. t_m is optional, but the rest are mandatory.

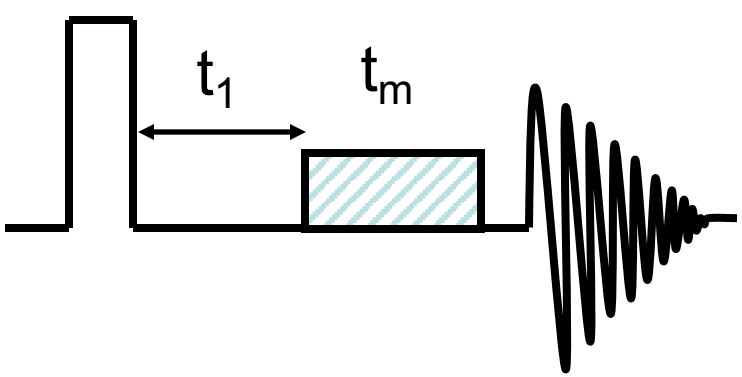
The major "Homonuclear" 2D NMR experiments: Through bond (scalar) interactions



J-Resolved Spectroscopy (JRES): Plots chemical shift vs. coupling pattern of the same multiplet. Not a very useful experiment because it doesn't resolve overlap. JRES has been done in vivo.

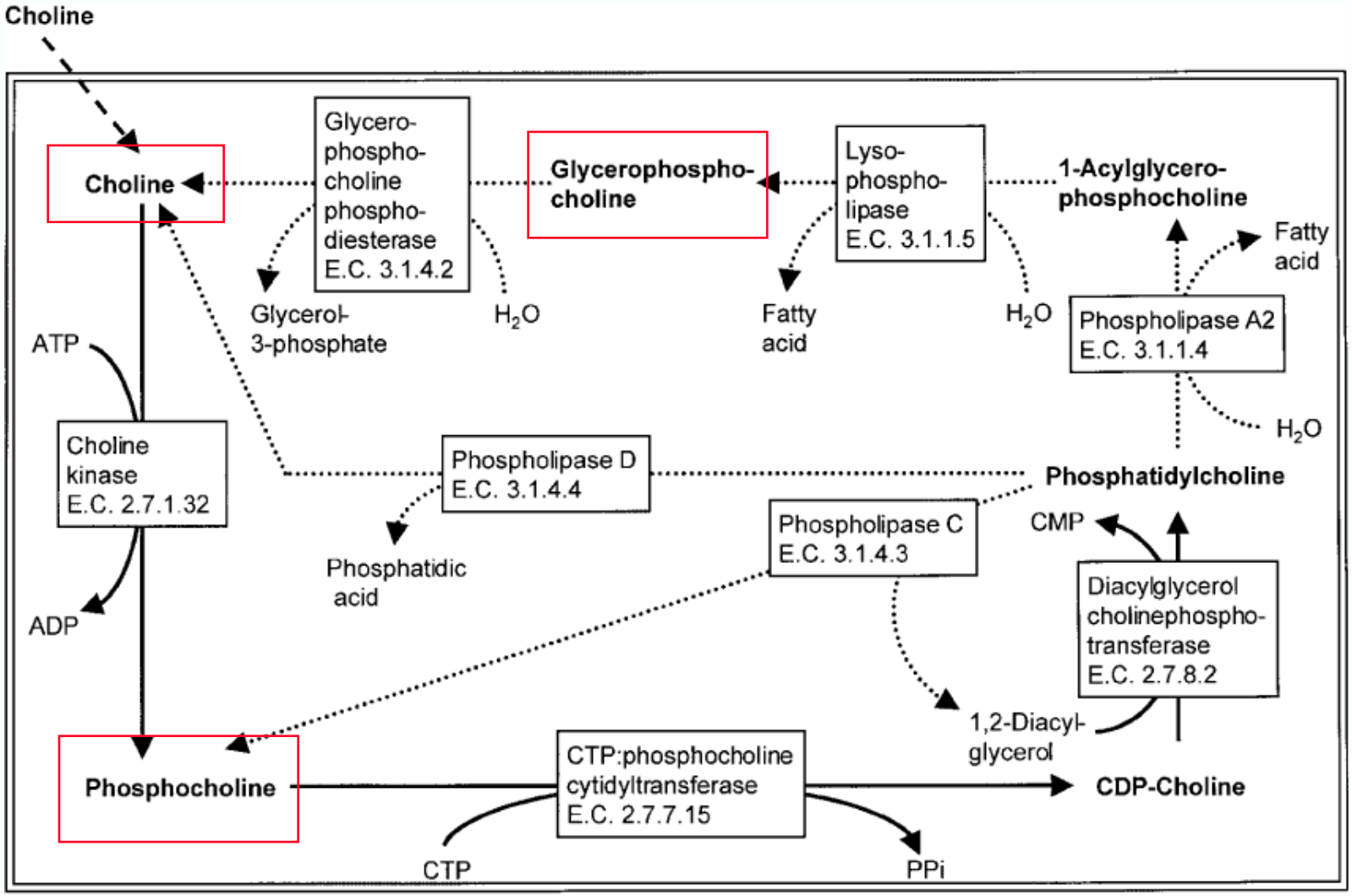


Correlation Spectroscopy (COSY): Plots chemical shift vs. chemical shift of coupling partner. Crosspeaks exhibit the "fine structure" of the multiplet. COSY has been done in vivo.



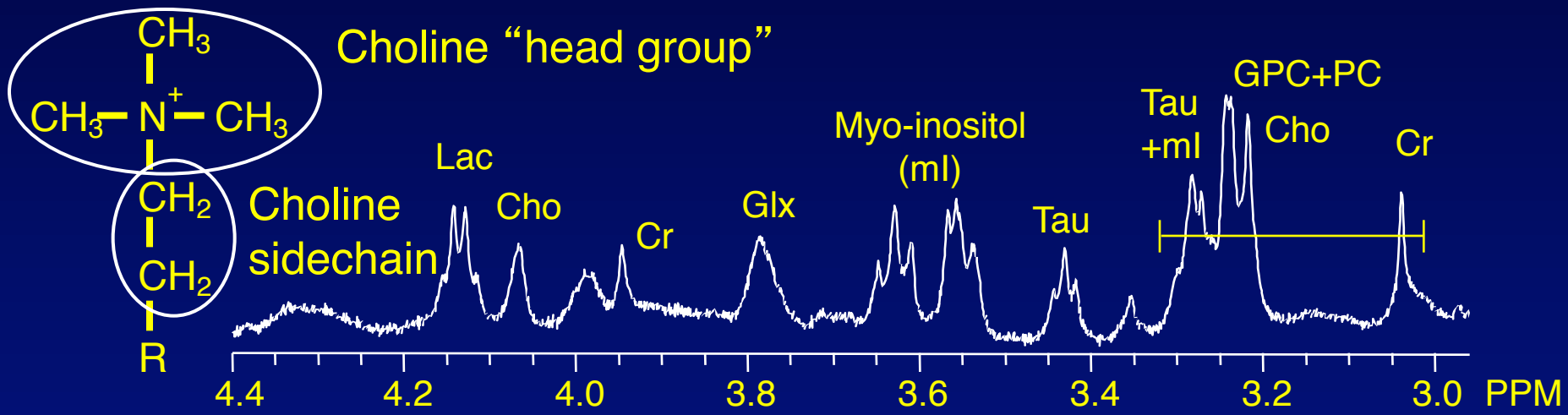
Total Correlation Spectroscopy (TOCSY): Plots chemical shift vs. chemical shifts of all protons in a spin system. Uses a "spin lock" to transfer magnetization during mixing period (t_m). Cross peaks usually don't display "fine structure." Advantage is that cross peaks can be quantified. TOCSY has not been done in vivo but should be!

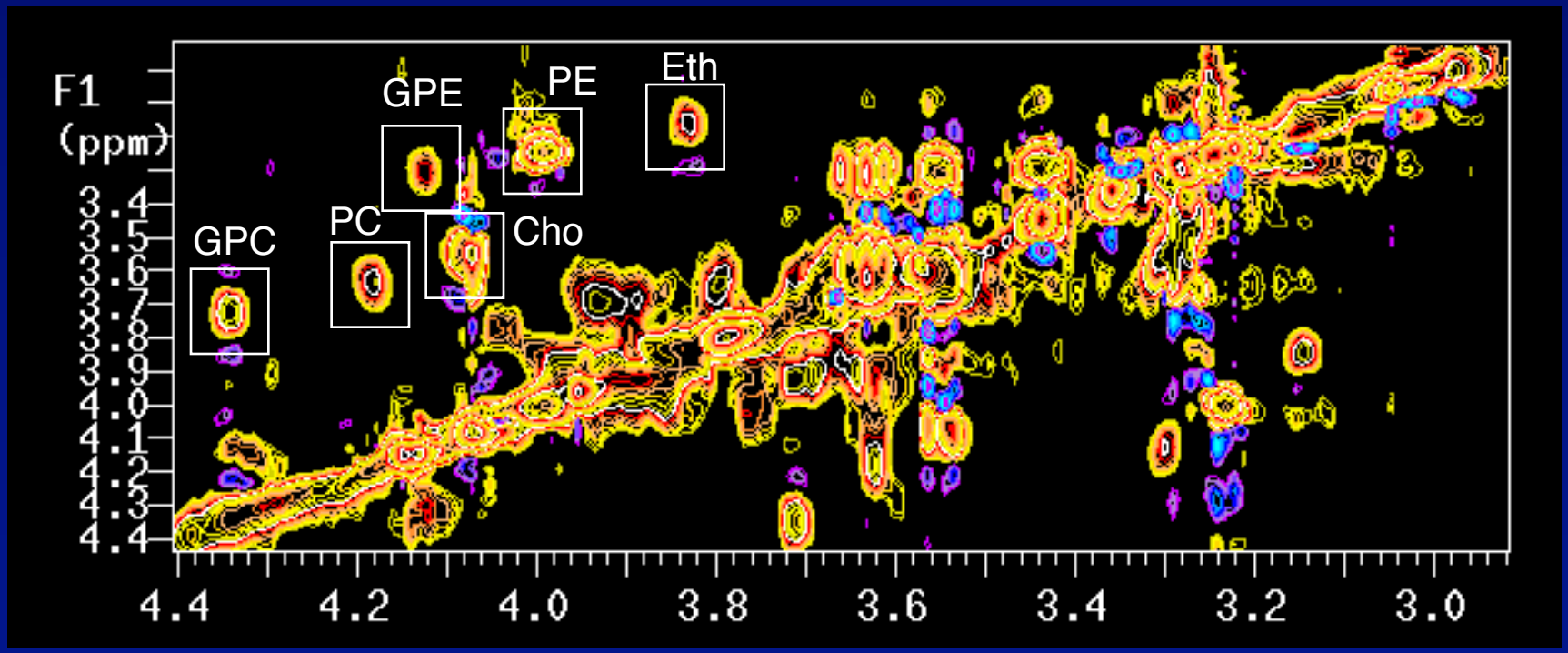
Choline Compounds: Building Blocks of Cells



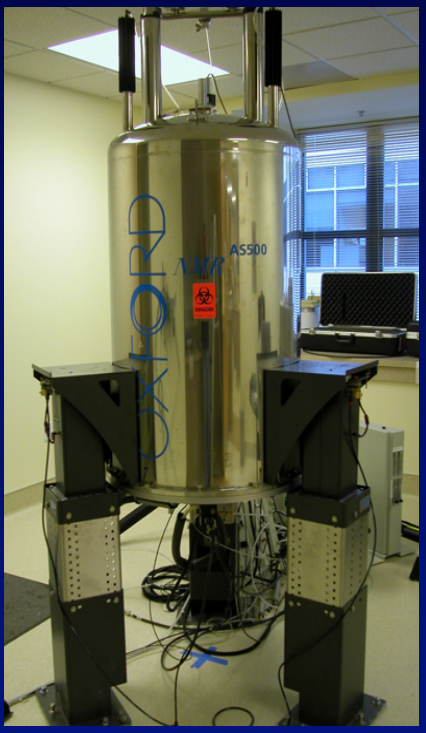
Bhujwalla - *Cancer Research - 61, 3599-3603 (2001) and 59, 80-89 (1999)*

Quantification of choline and ethanolamine metabolites by 2D Total Correlation Spectroscopy (TOCSY)



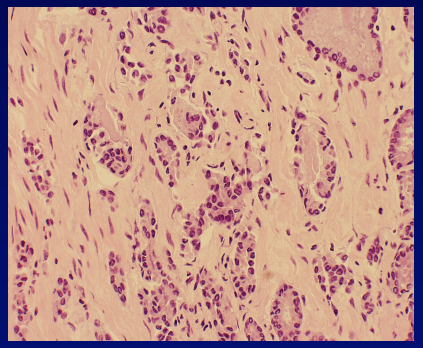


Ex Vivo Spectroscopy – HR-MAS Spectrosopy

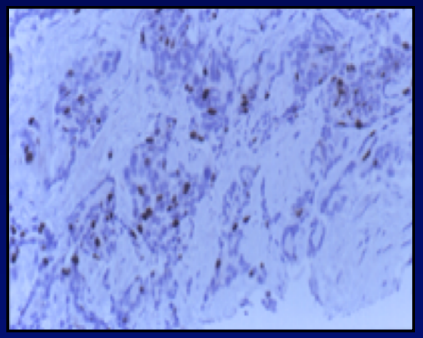


11.4 Tesla
(500 MHz) Varian NMR spectrometer for biopsy and cell culture studies.

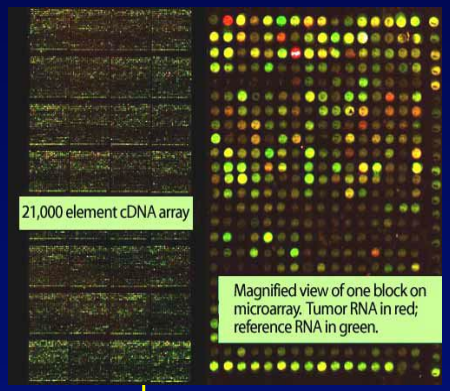
Quantitative Pathology



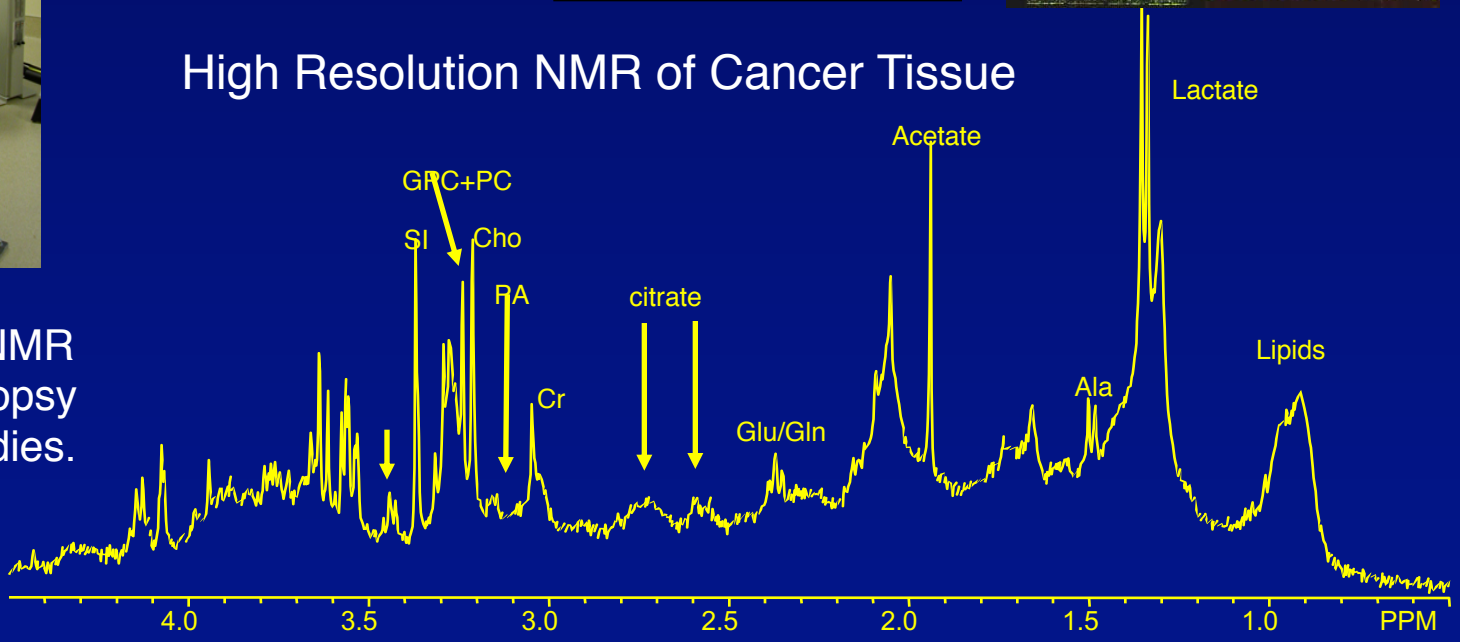
Immunohistochemistry
MIB-1-Proliferation



Gene Array Analysis



High Resolution NMR of Cancer Tissue



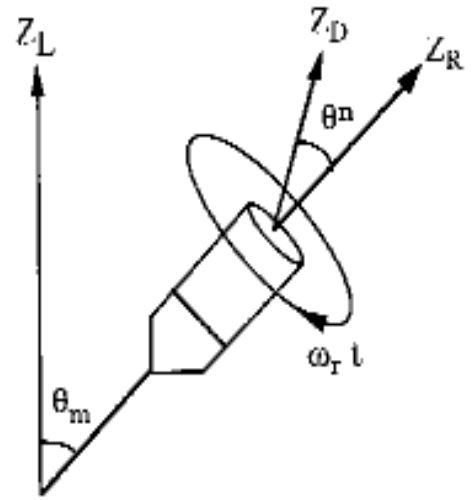
1-D High resolution Magic Angle Spectroscopy of image targeted human biopsy sample, ≈ By spinning at the magic angle (54.7°) and using moderate spin rates (2,250 Hz), solution-like spectra can be acquired prior to pathologic, immunohistochemical, proteomic, and/or genetic analysis of the same samples.

High Resolution Magic Angle Spinning

Dipolar coupling depends on orientation

$$D_{n,m} = \frac{\gamma_n \gamma_m h}{2\pi^2 \langle r_{n,m}^3 \rangle} \left\langle \frac{3\cos^2\theta - 1}{2} \right\rangle$$

γ_n and γ_m are the magnetogyric ratios of N and M, h is Planck's constant, $r_{n,m}$ is the distance between N and M, and θ is the angle between the internuclear vector and the external magnetic field



HR-MAS spectroscopy produces "solution like" spectra by eliminating (reducing) dipolar coupling and chemical shift anisotropy (CSA) effects:

- Dipolar coupling is cancelled by spinning at 54.7° ($3\cos^2\theta - 1 = 0$), and high spin rate needed to average the spin orientations.

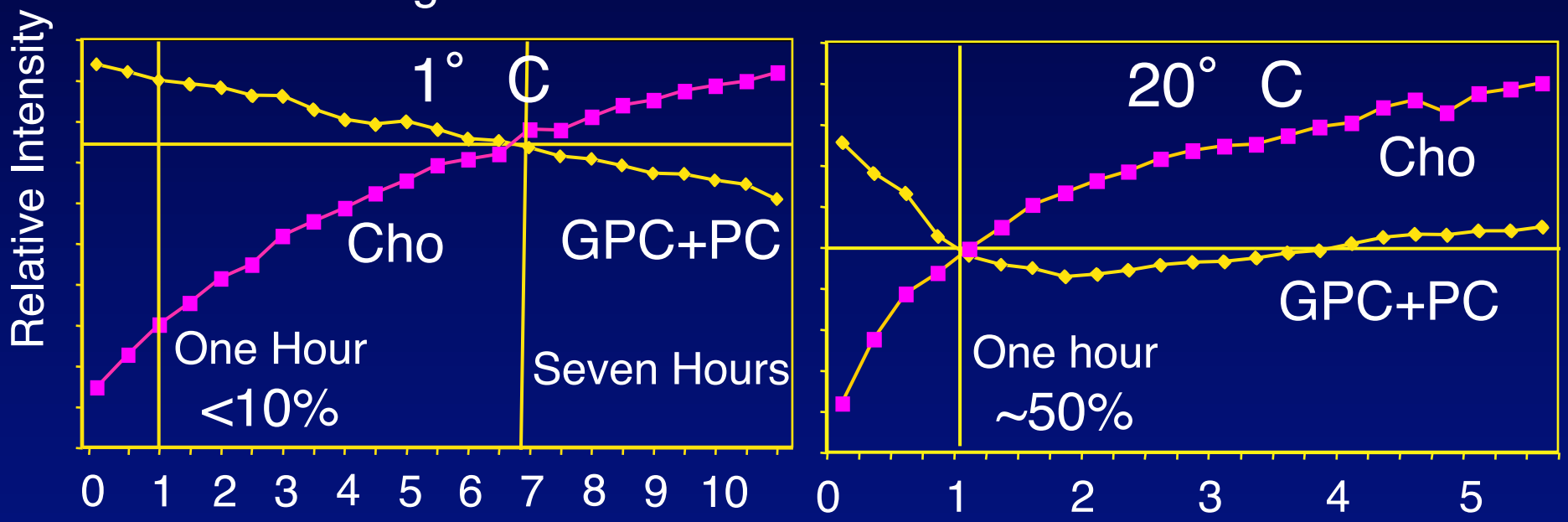
- CSA is averaged to zero when spin rate (ω_r) > width ($\Delta\nu$) of static line.

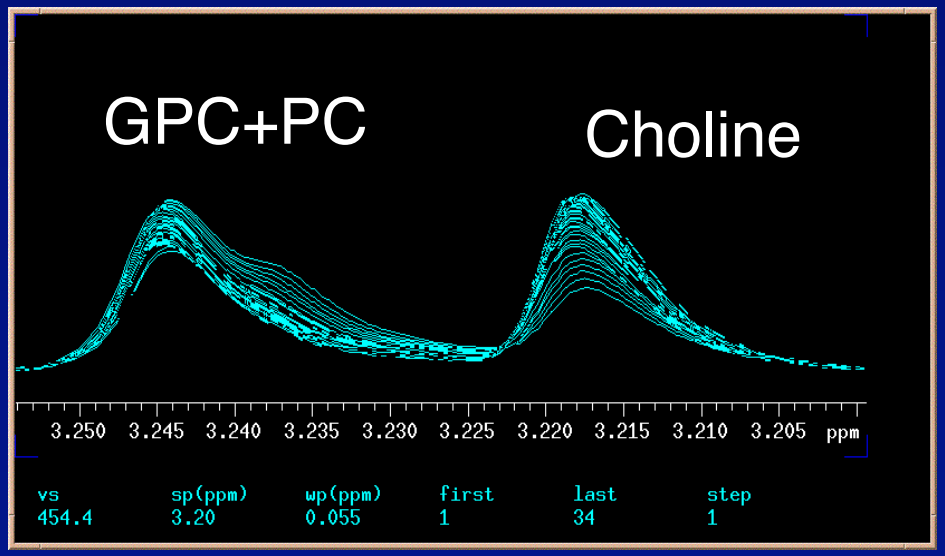
Varian INOVA 500 MHz HR-MAS Spectrometer. The high field (11.7T) spectrometer used for ex vivo studies provides the increased spectral resolution needed for the identification of new markers.

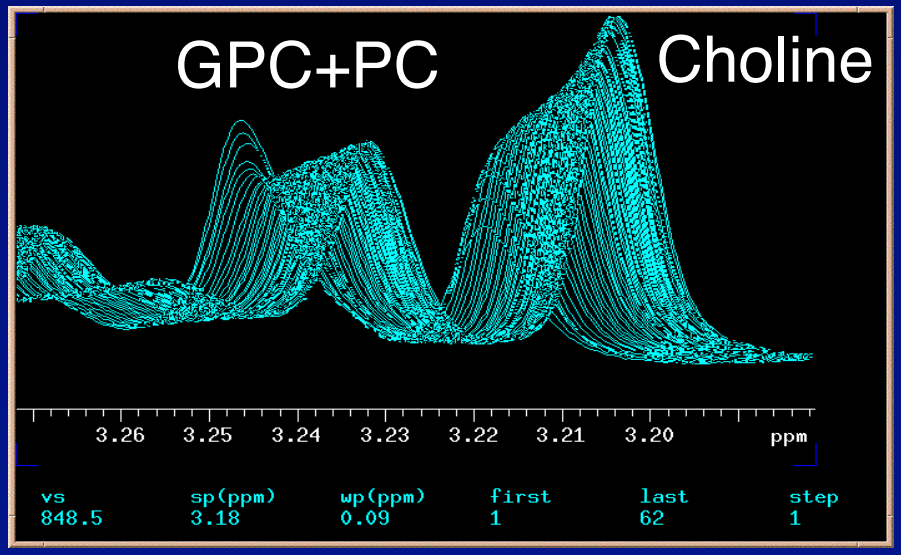
Leak proof zirconium rotors developed in 10, 20, and 35 µl volumes. Samples ~3 to 30 mg.

Data Acquisition - Low Temperature is Critical for Metabolite Preservation

5-fold less degradation at 1° C vs 20° C at 1 hour



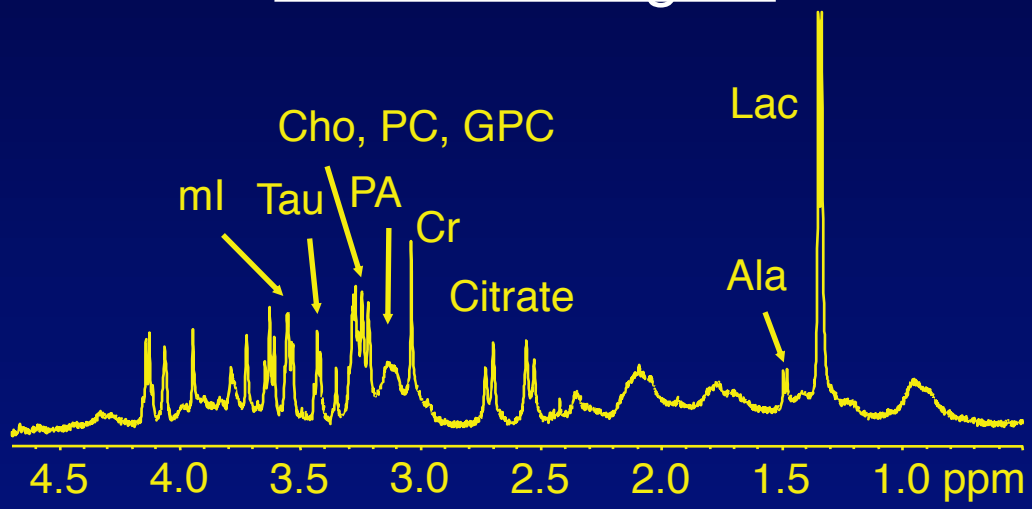




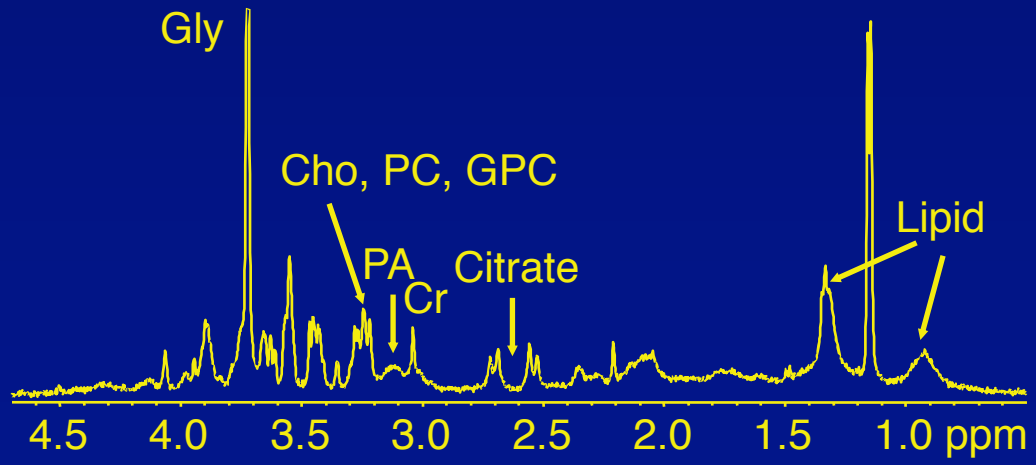
Swanson MG, Magn Reson Med, Jul/2008;60(1):33-4

No Significant Change in Pathology or RNA Integrity with HR-MAS

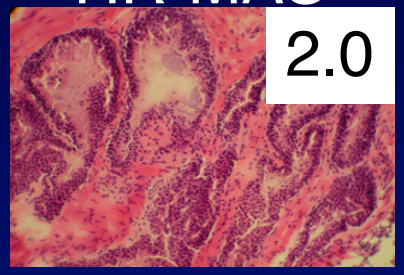
HR-MAS Surgical

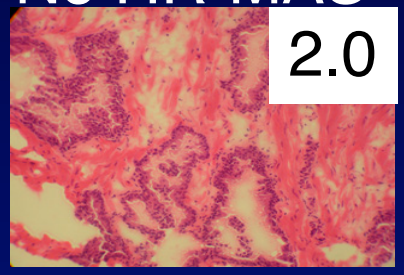


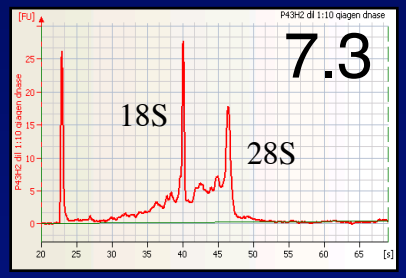
HR-MAS Biopsy

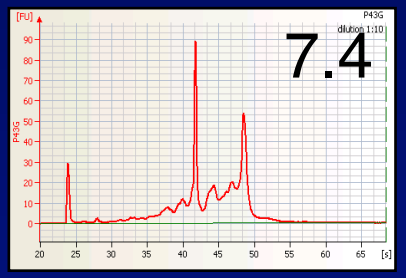


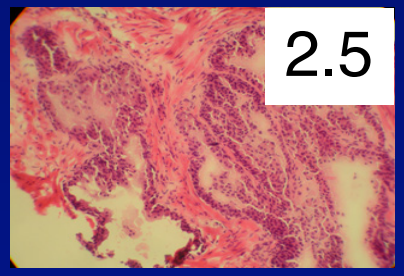
HR-MAS	No HR-MAS

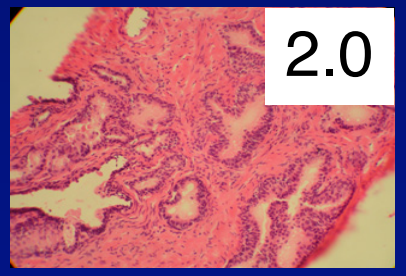


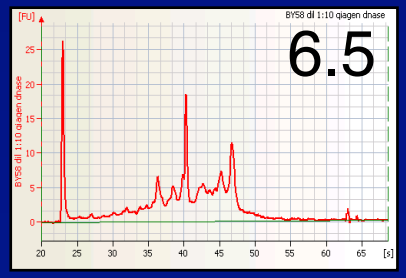


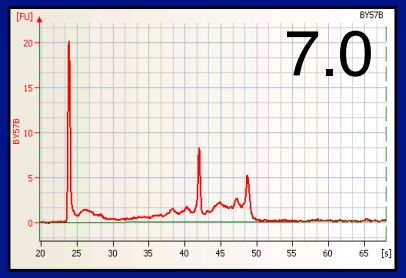








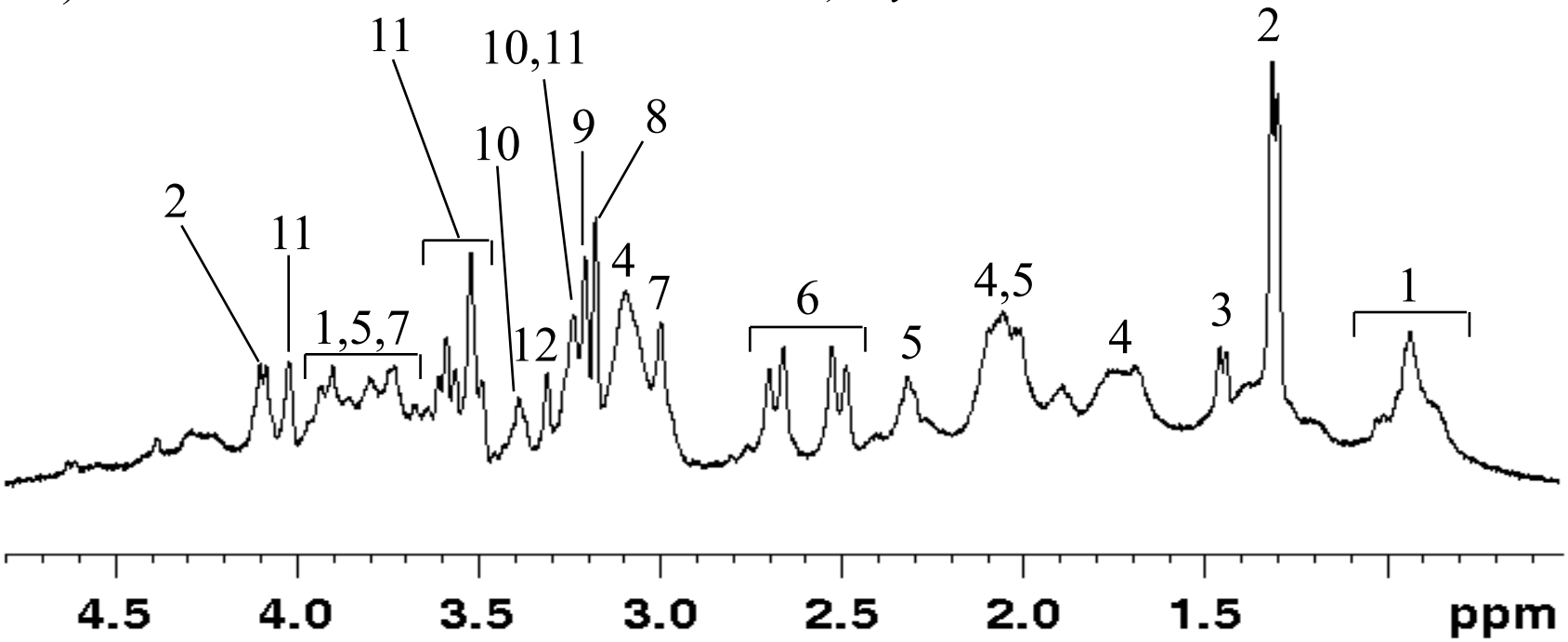




Santos CF, NMR Biomed. 2009 Dec 23, 391-

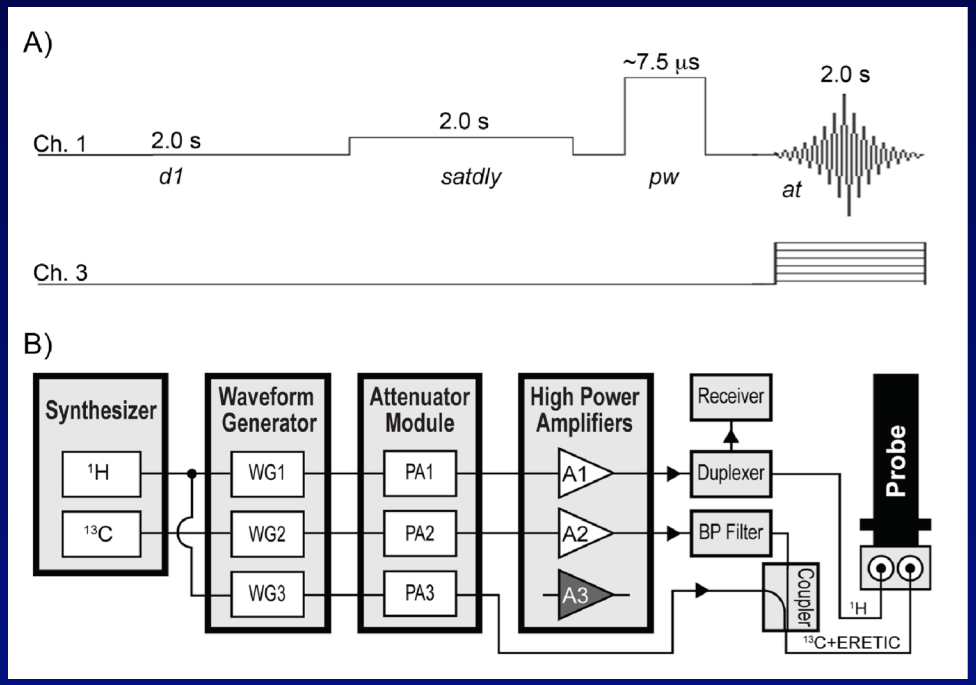
Qunatification of *In Vivo* Metabolites: A Mixture

1) leucine, isoleucine, valine
2) lactate
3) alanine
4) spermine, spermidine
5) glutamine, glutamate
6) citrate
7) creatine, phosphocreatine
8) choline, PC
9) GPC
10) taurine
11) *myo*-inositol
12) *scyllo*-inositol

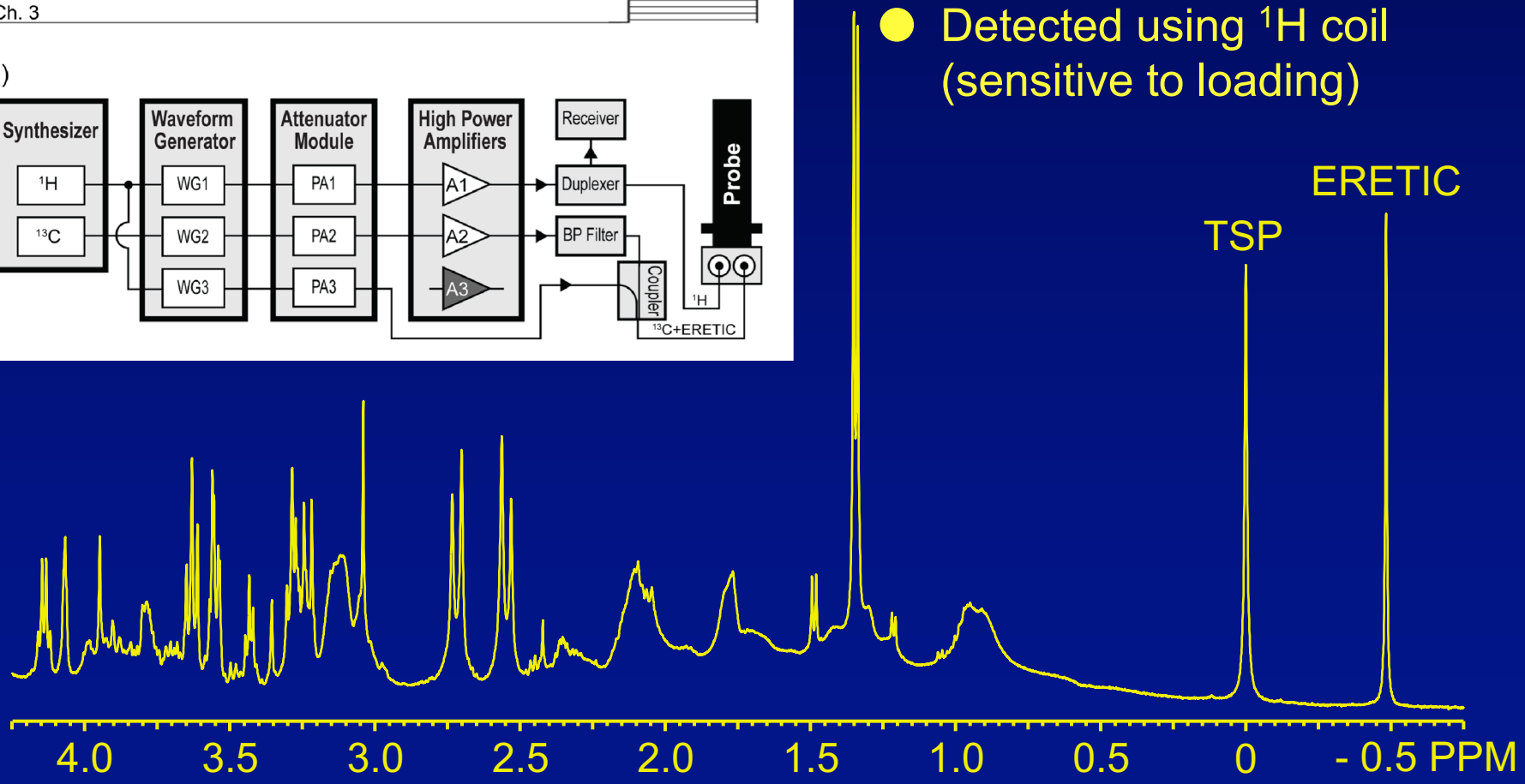


11.7 T MAS-NMR spectrum of approx. 6 mg of healthy prostate tissue

Determination of Absolute Metabolite Concentrations

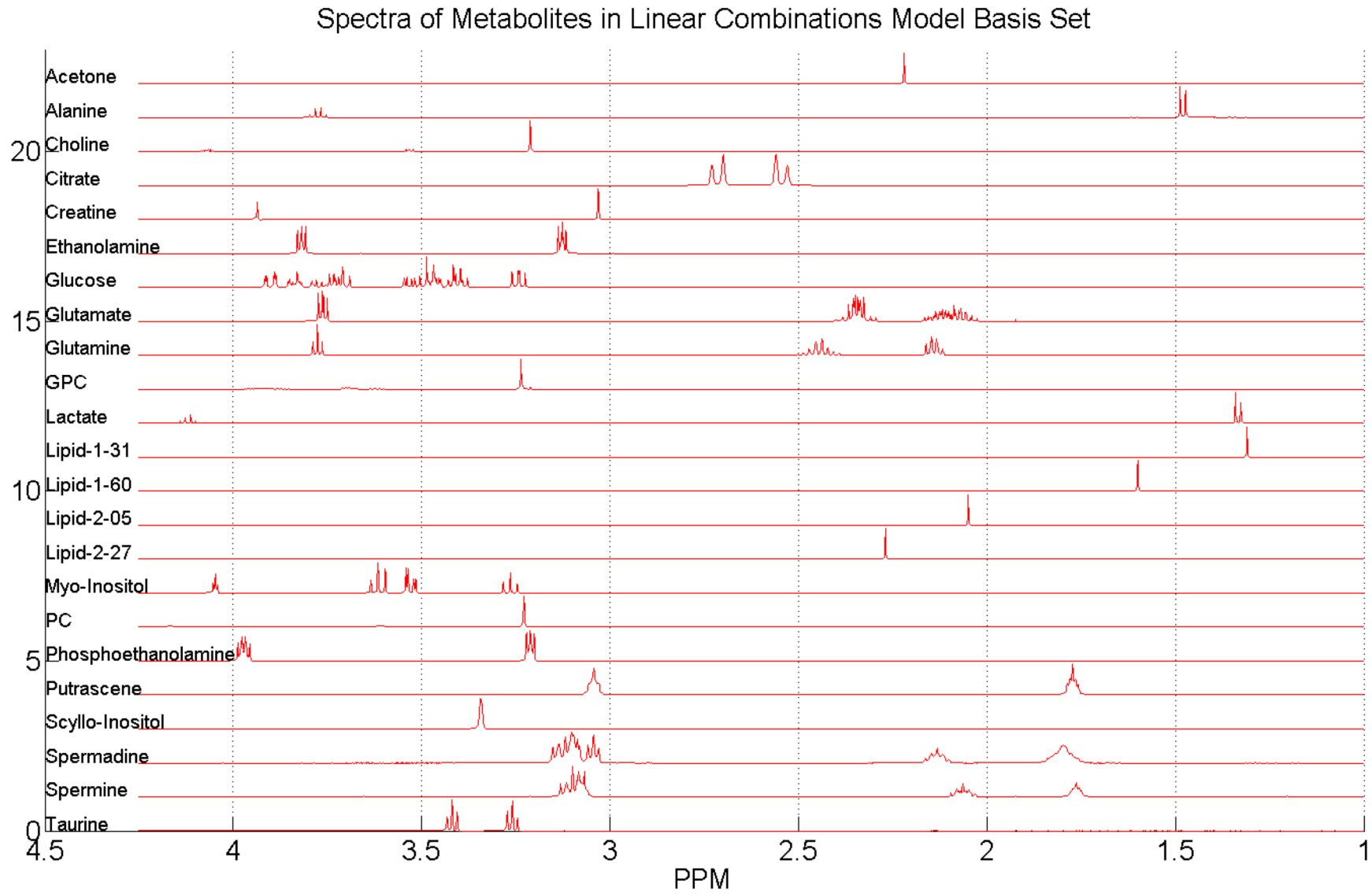


- ERETIC transmitted using X-coil tuned to ^{13}C (insensitive to loading)
- Detected using ^{1}H coil (sensitive to loading)



Albers MJ, et. al. Magn Reson Med, Mar/2009;61(3):525-32.

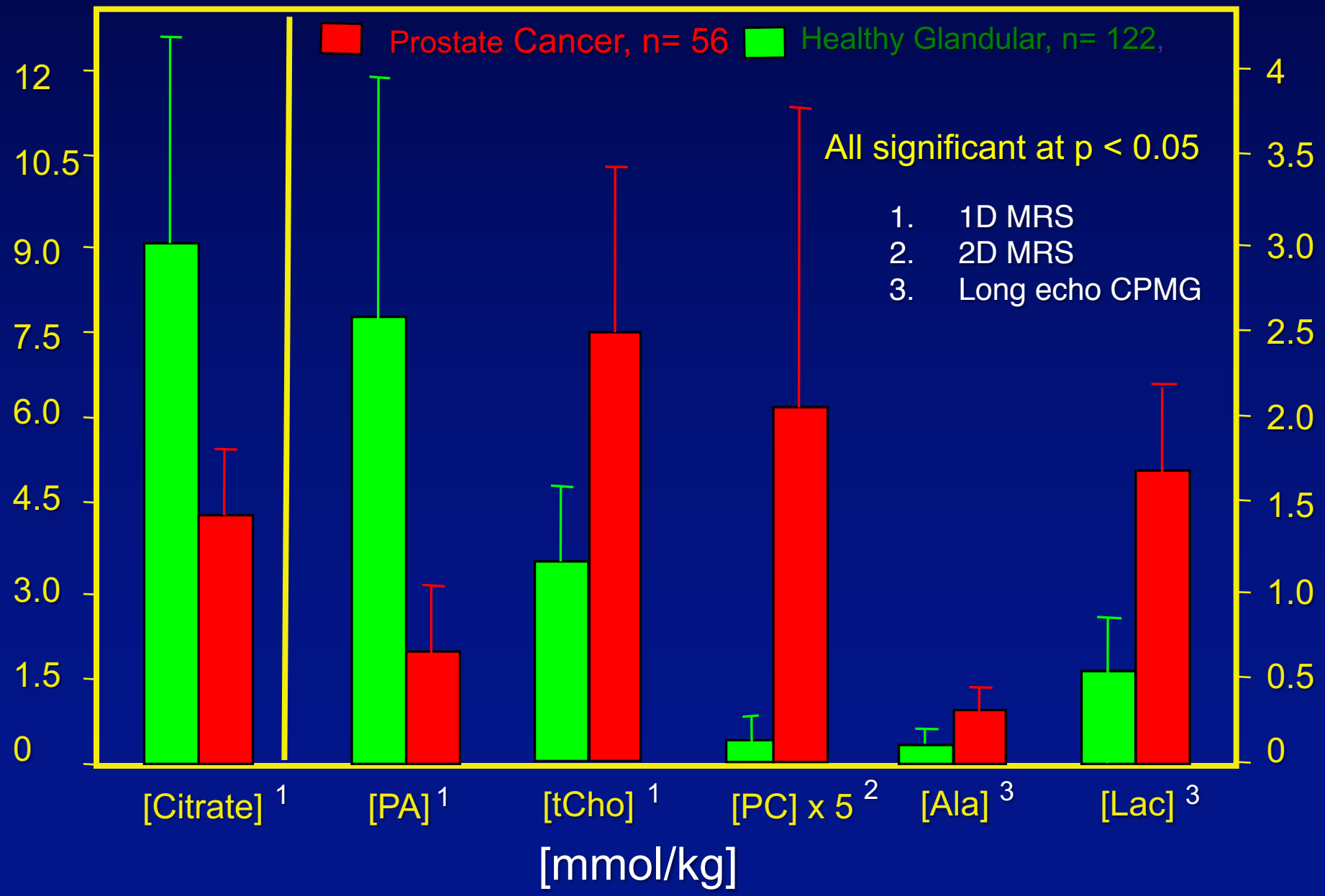
Quantifying Metabolites in Tissue - HR-QUEST

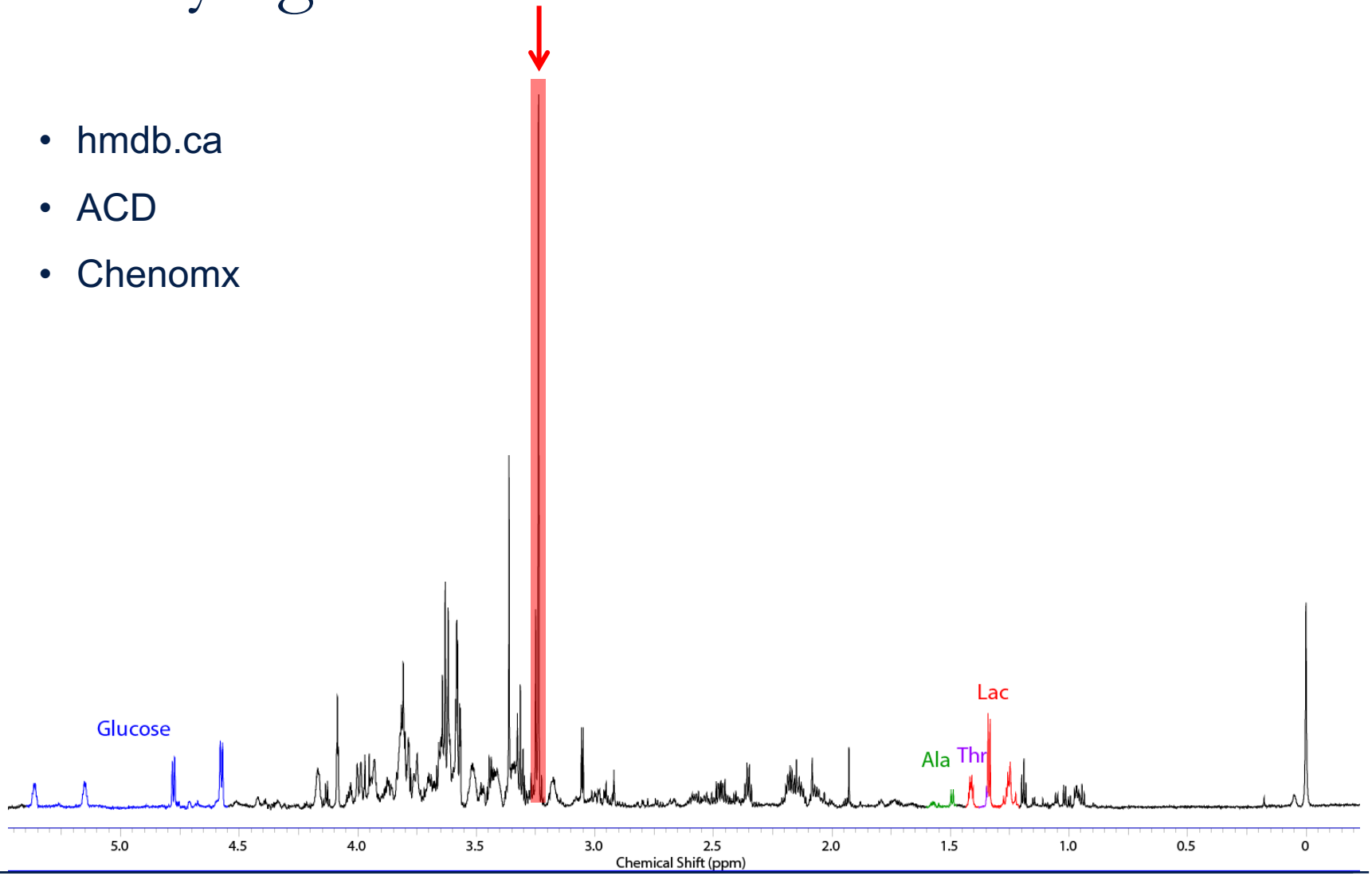


Ratiney, H, et. al. NMR in Biomed. In Press, 4/2010

Metabolic Biomarkers of Prostate Cancer

HR-MAS spectroscopy of 98 snap frozen Patient Prostate biopsies



Identifying metabolites

- hmdb.ca
- ACD
- Chenomx