What is an NMR?

- Niobium-tin-copper clad coil wound like a spool of thread. The current runs through this coil, creating the magnetic field.
- This coil is submerged in liquid helium (boiling point -452.1°F, -268.9°C, 4ºK – Brrr!!!!) .
- Liquid He chamber surrounded by liquid nitrogen (boiling point -320.5°F, -195.8°C, 77ºK).
- Sample and spinner lowered using air from the top, down through the bore, until it nests in the top of the probe.

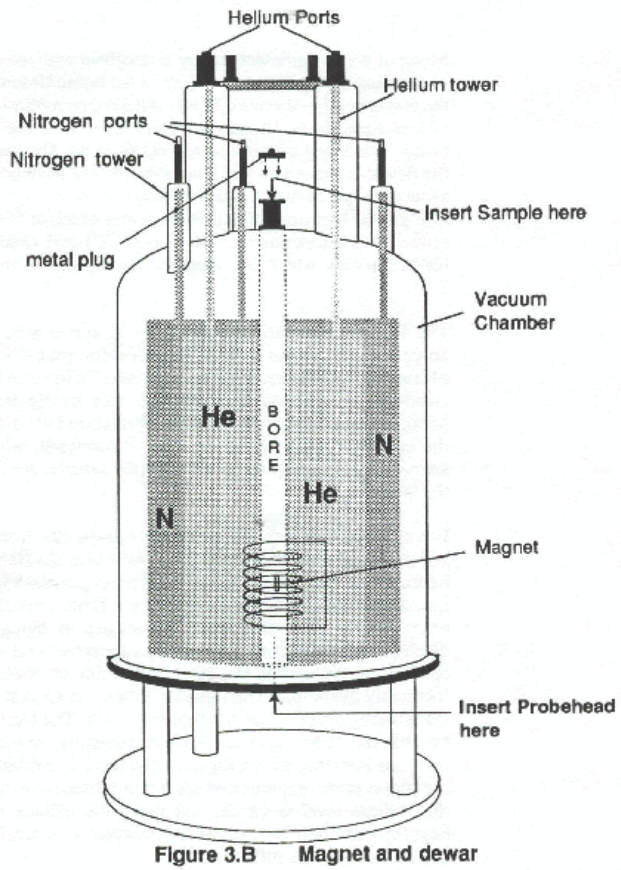


Figure 3.B Magnet and dewar

NMR Safety

- NMR magnets are ***ALWAYS*** live!!! You cannot turn off an NMR like you would a light switch.
- Nothing ferromagnetic allowed in the magnet's field! This includes most tools (hammers, wrenches, screwdrivers) and many metal items like paper clips, staples, bobby pins, barrettes, costume jewelry, wallet chains, metal buckets, floor buffers, and metal chairs.
- No watches, cellphones, iPods, or other digital media. These items can be damaged by the magnetic field.
- No credit and ATM cards.
- No pacemakers or metallic implants within 15 feet of the NMR.
- If you have a question about an item, please ask a DCIF staff member.

Be smart and be safe!

Where does the NMR signal come from?

Quantum Spin Number

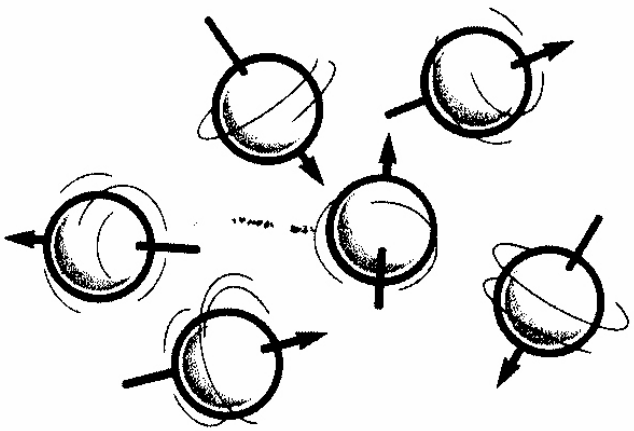
- Only nuclei that posses a property called **spin** can be “seen” by a NMR.
  - If a nucleus can have more than one energy state in a magnetic field, the **quantum spin number** (**I**) is not 0, and energy transitions for this nucleus are possible.
  - **I** depends on the number of protons (Z) and neutrons (n) in a nucleus.

I=0	**#Z is even**	**#n is even**	**mass # even**	**NO NMR SIGNAL!!!**
				^{12}C and ^{16}O are not NMR active!
I=1	**#Z is odd**	**#n is odd**	**mass # is even**	**SIGNAL!!**
				^{2}H and ^{14}N
I=1/2, 3/2, 5/2…..			**mass # is odd**	**SIGNAL!!**
				^{1}H, ^{13}C, ^{15}N

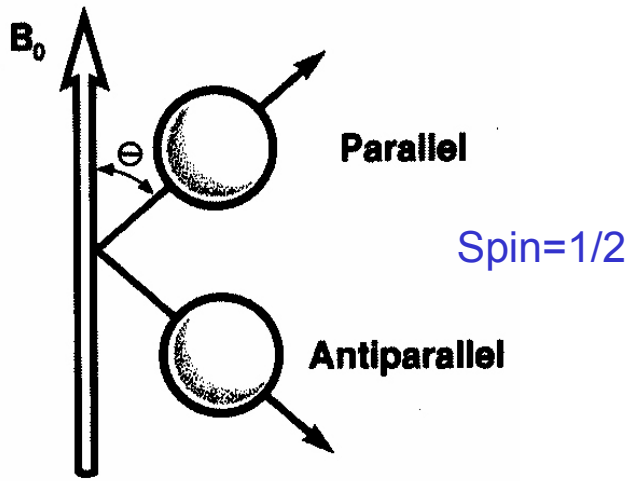
Where does the NMR signal come from?

The Zeeman Effect

- When inserted in a magnetic field (B_0) nuclei that possess *spin* align themselves according to their energy states.
  - This effect on their alignment is called the *Zeeman Effect*.



No B_0

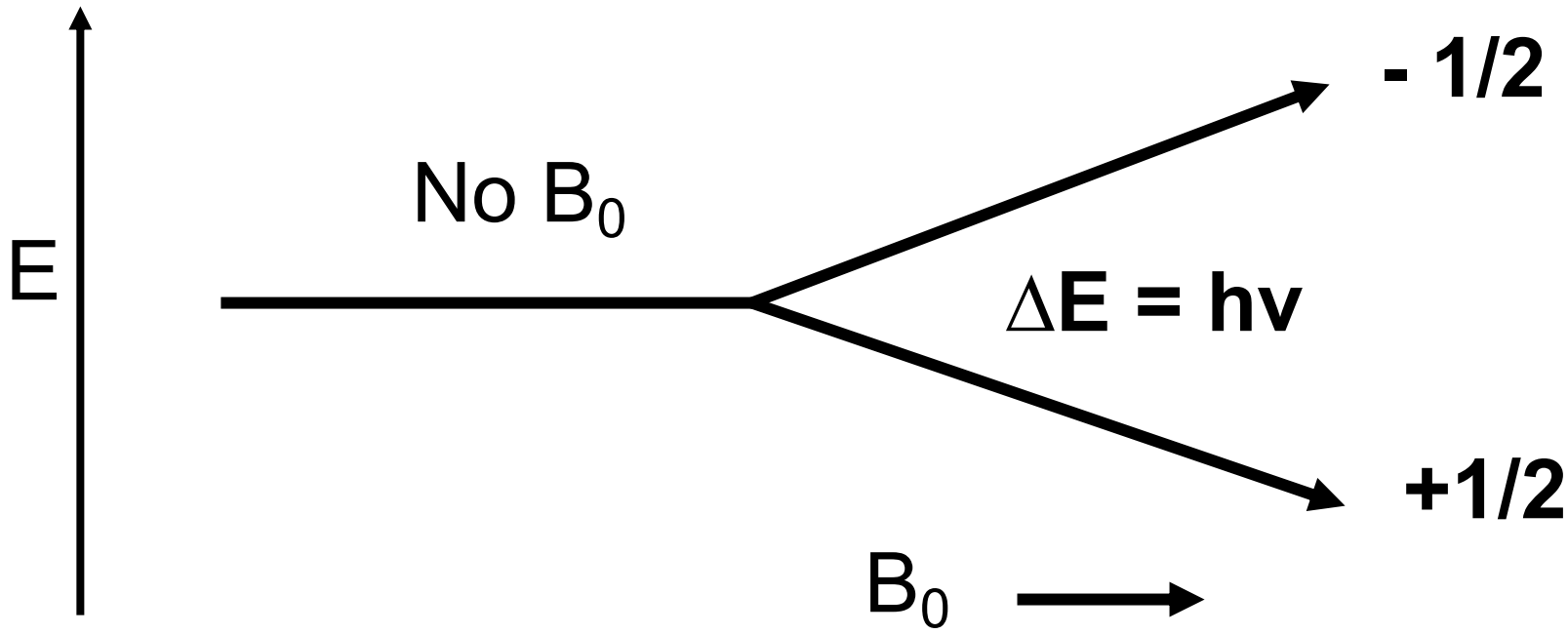


B_0

The Zeeman Effect

Energy levels if I=1/2

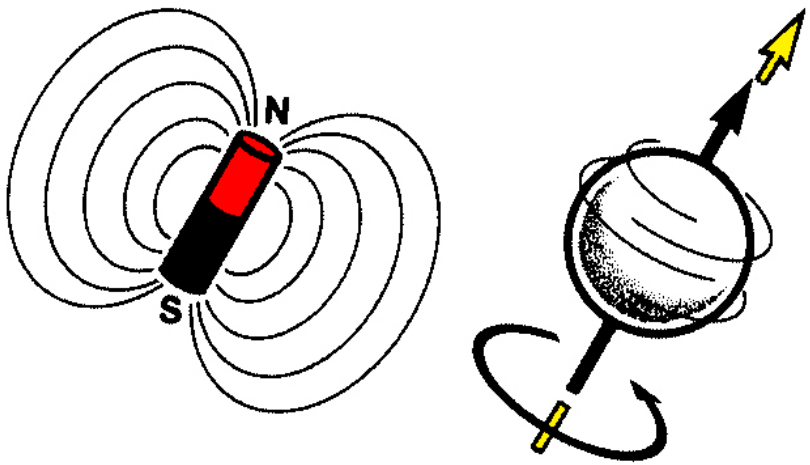
- The spins are said to be split into two populations, -1/2 (anti-parallel) and +1/2 (parallel), by B_0.
  - +1/2 is the lower energy state.



Where does the NMR signal come from?

Magnetic Moments

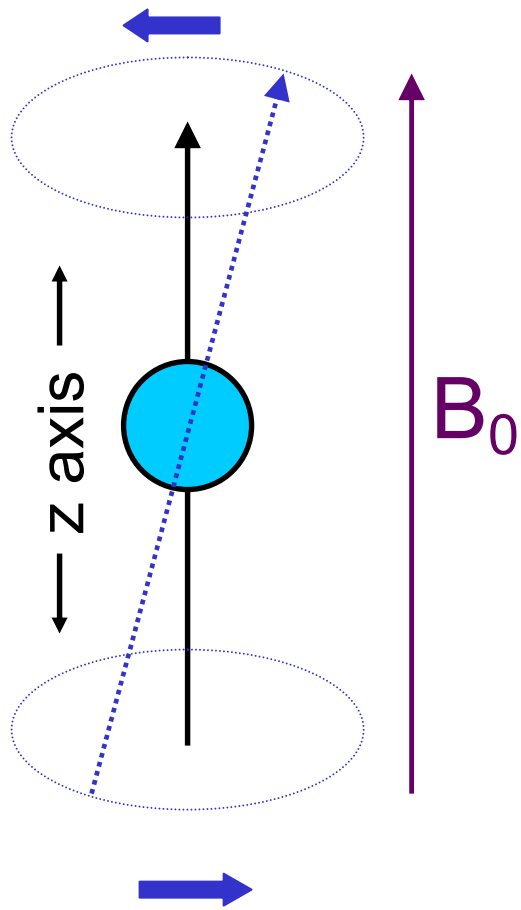
- Spinning, charged nuclei generate a magnetic field and possess a *magnetic moment* (μ).
  - This magnetic moment is a vector, and is partially aligned along the magnetic field (B_0) axis (the z axis).



Where does the NMR signal come from?

Larmor Precession

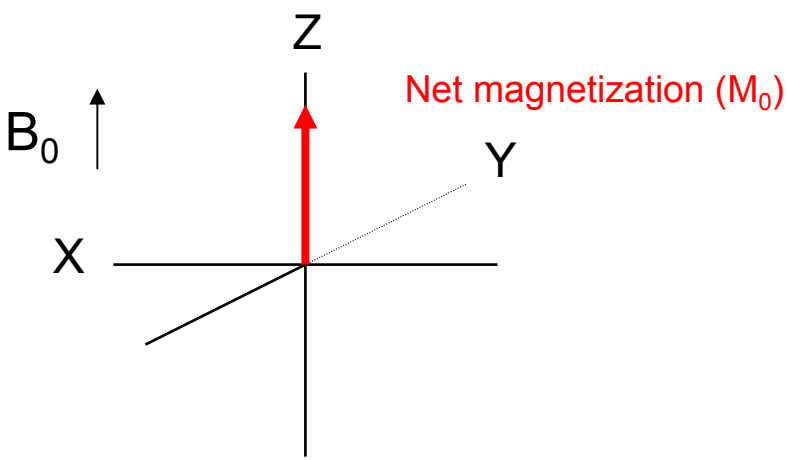
- These vectors are not exactly parallel to the z axis, they *precess*, much like a spinning top does.
- Within B_0, there is tendency for more spins to precess with a component aligned with the +z (+1/2 or parallel) direction.
  - Boltzmann! There are more spins in the lower energy state (+z direction or N+).
  - This population difference results in a net magnetization (M_0) vector in the +z direction.
  - There is a small component in the x and y direction, but this cancels out.

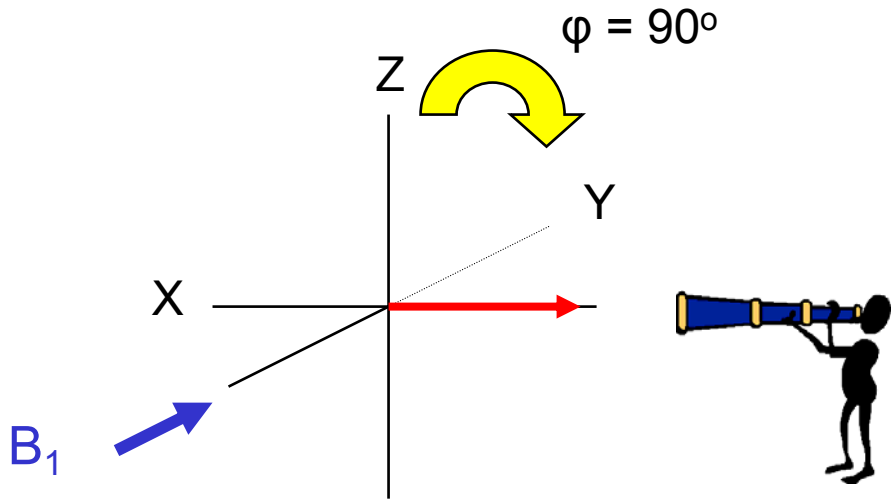


Where does the NMR signal come from?

Tip me over!

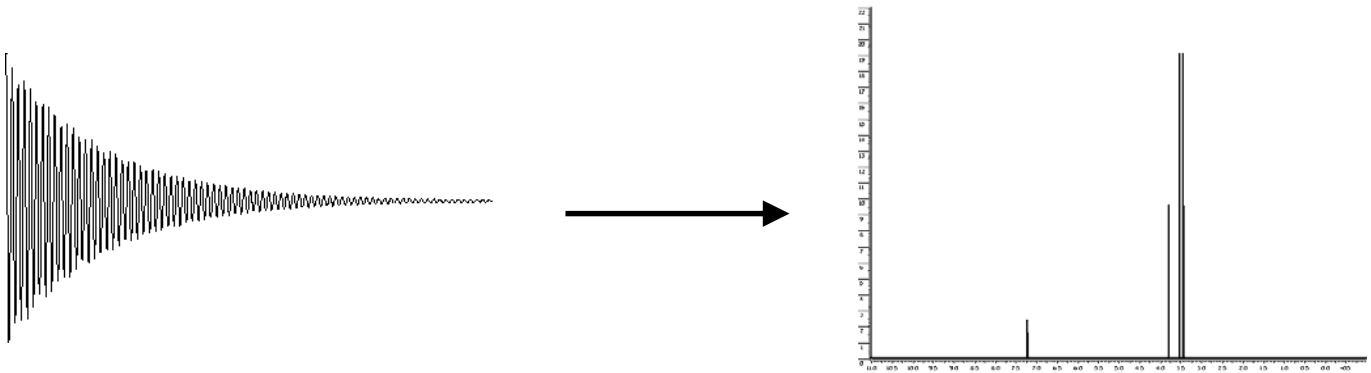
- Nuclei in B_0.
  - Zeeman effect → Boltzmann distribution.
- A second magnetic field is applied (B_1) at radio frequencies (a *pulse*).
  - The length and amplitude of the pulse determine how much the net magnetization vector is tipped from the z direction into the xy plane.
    - A 90^0 pulse tips M_0 fully into the xy plane.
- We "see" only what is tipped into the xy plane.
  - This is collected as a FID.
  - Relaxes back to ground state (along z-axis) and awaits next pulse.





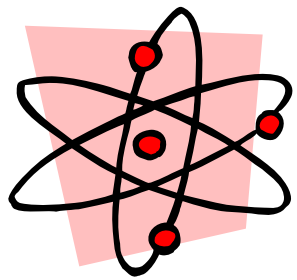
Fourier Transform
Time → Frequency

- FID (free induction decay) – sinusoidal, exponential function modulated by a decay function.
  - Net magnetization (M_0) was tipped into xy plane, and allowed to relax back to the z direction. FID collected during this time.
  - Must translate the time domain into the frequency domain
    - Amplitude vs. time → amplitude vs. frequency
    - A computer *Fourier transforms* (lots of math!) the FID into what we know as a NMR spectrum.



What kind of Information does a 1D spectrum give us?

- **Chemical shift** (δ) – chemical environment.
- **Scalar coupling** (J) – through bond coupling, provides conformational info. Good shimming allows measurement of small J-couplings.
- **Integration** – area under a curve, provides ratio of number of atoms in a particular chemical environment. Flat baseline + well phased spectrum = good integration (assuming sufficient relaxation delay).
- **NOE** (nuclear Overhauser effect) – through space coupling – who is next to who? Spatial orientation of groups. NOE experiments are experiments in which 'cross relaxation' occurs due to dipole-dipole interaction.
  - In decoupled carbon spectra, the NOE 'enhances' the carbon signal through the irradiation of protons near to carbons, and 'collapsing' multiplets into singlets. The enhancement varies from carbon to carbon, and thus decoupled carbon spectra are NOT quantitative.

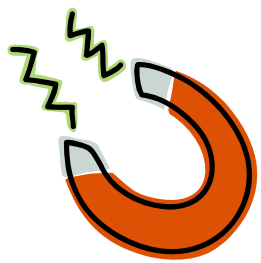


Important Relationships

Remember this!!!

$E=h\nu$	E=energy h=Planck's Constant ν=frequency (Nν)
$\Delta E=hB_0\gamma$	γ=gamma (gyromagnetic ratio)
$\nu/B_0 = \gamma$	Field and frequency are **directly proportional**!

As you increase the field of your magnet, you get a greater resonance frequency. This is why larger magnets are often more desirable.



Changes in the Resonance Frequency

Why everyone resonates at a different frequency

- Which nucleus
- Field of magnet
- Electron Density
- Scalar coupling

Varian 900 MHz NMR

Which Nucleus

Sensitivity and the Gyromagnetic Ratio

I = n (1) mass #	is even	I		abundance	γ (10^7 rad $T^{-1}s^{-1}$)
H – 2	*2*	*1*	*Q*	*0 .015 %*	*4.11*
N – 14	*14*	*1*	*Q*	*99.63*	*1.93*
I = n (1/2) mass #	is odd	I			
H – 1	*1*	*½*		*99.98*	*26.76*
C – 13	*13*	*½*		*1.11*	*6.73*
N – 15	*15*	*½*		*0.37*	*-2.71*
F – 19	*19*	*½*		*100*	*25.18*
P – 31	*31*	*½*		*100*	*10.84*
Si – 29	*29*	*½*		*4.7*	*-5.32*
V – 51	*51*	*7/2*		*99.76*	*7*
Sn – 117	*117*	*½*		*7.61*	*-9.5*

Q – Quadrupole moment. Happens when the charge not evenly distributed across nucleus.
γ (gamma) is a set value.

** You can see why ^1H easy to “see” in NMR – high abundance, and large γ **

Which Nucleus

Sensitivity of ^{1}H vs ^{13}C

$$\frac{^{1}H}{^{13}C} = \frac{\gamma^{3}H}{\gamma^{3}C} = \frac{(26.7519)^{3}}{(6.7283)^{3}} = 63$$

Factor in abundance….

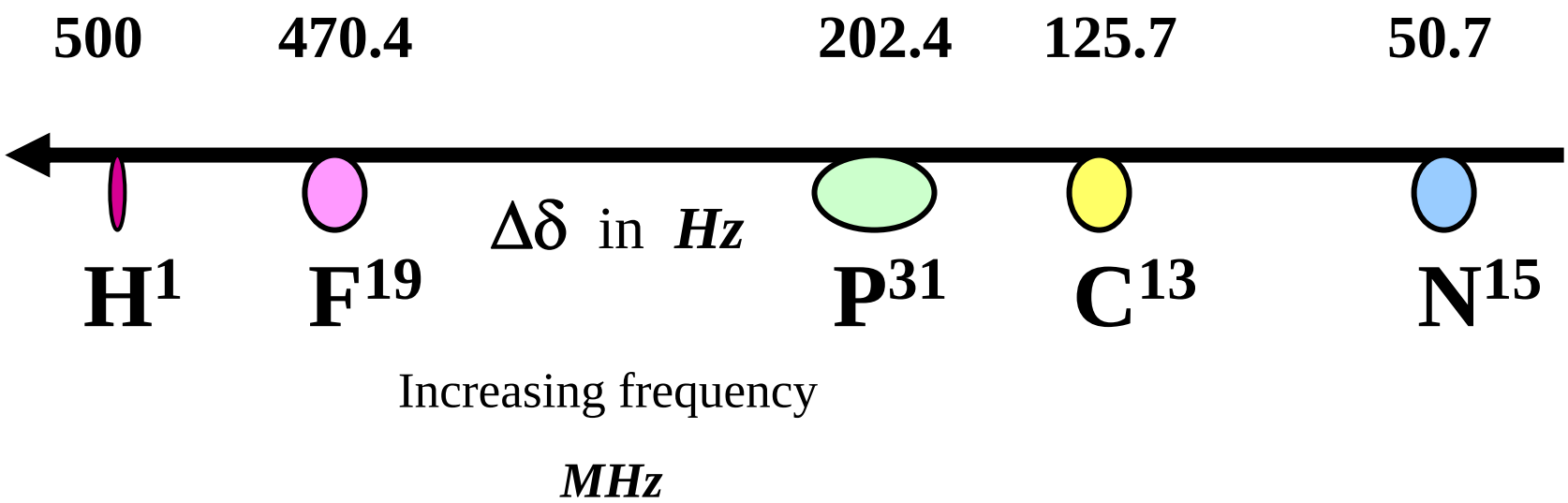
$$\frac{^{1}H}{^{13}C} = \frac{(99.98)}{(1.108)} \times 63 = 5672$$

^{1}H is 5672 times more sensitive than ^{13}C. That's why you need many more ^{13}C scans to get a decent spectrum.

Which Nucleus

Different nucleus means different frequency

- Different nuclei resonate at different frequencies in MHz.
  - When talking about 500 MHz NMR, we are referring to the resonance frequency of proton in that particular strength magnetic field.
  - This is why probes need to be tuned in MHz when switching between certain nuclei. ^{1}H and ^{19}F are typically tuned on one channel, while ^{31}P, ^{13}C, and ^{15}N on another.



Field of Magnet

Boltzmann revisited

- If spin = ½, then each spin will align itself in one of 2 possible orientations (Remember Zeeman???).
- At room temp, the number of spins in the lower energy level (N+) is slightly greater than the number in the upper level (N-).

$$N-/N+ = e^{-\Delta E/kT}$$ k is Boltzmann's Constant

- The NMR signal depends on this difference in population.

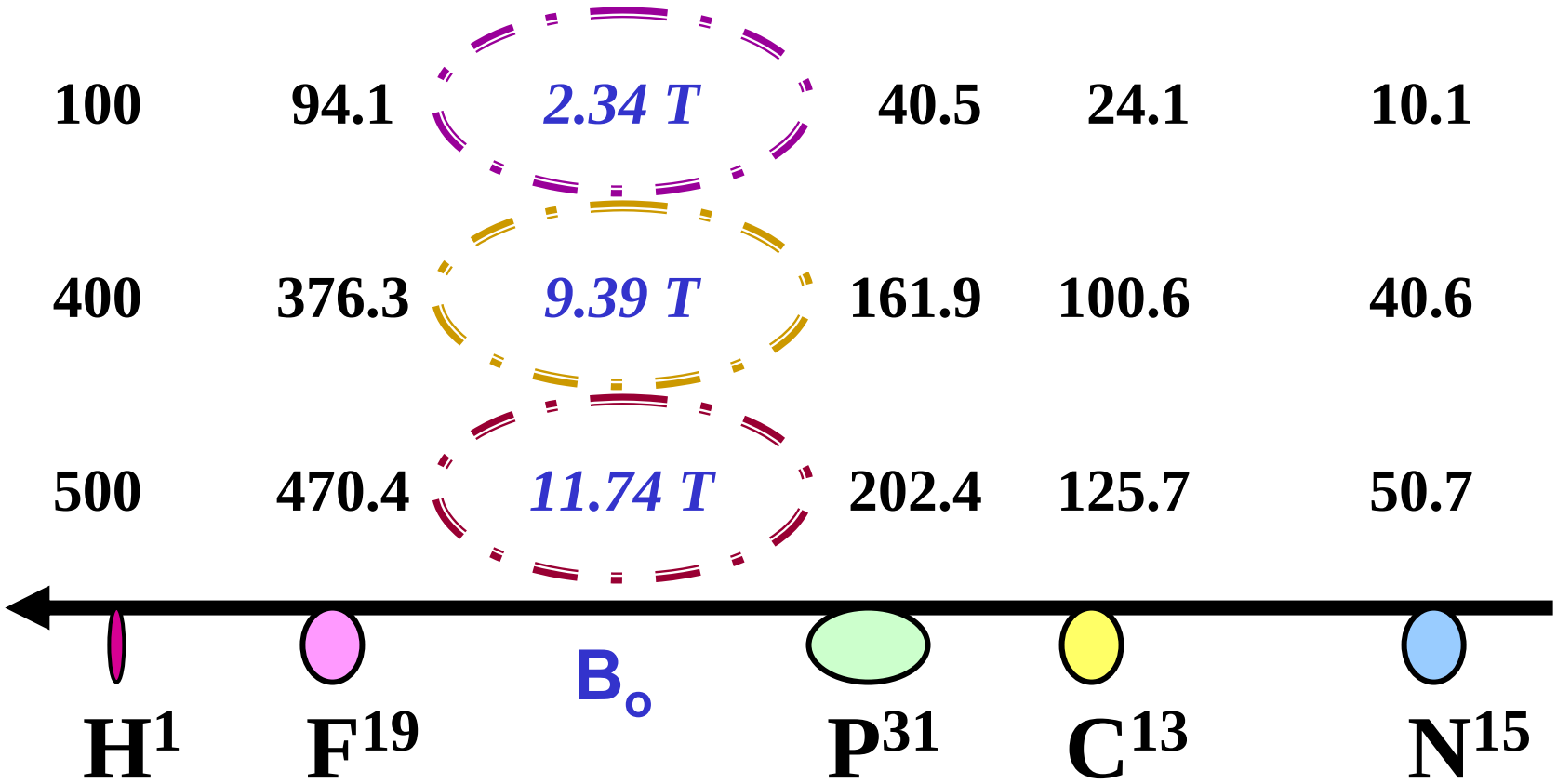
The greater the field B_0, the greater the population difference.

Greater difference = more signal

Field of Magnet

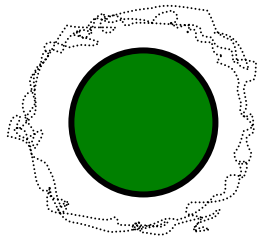
Changing the field of the magnet

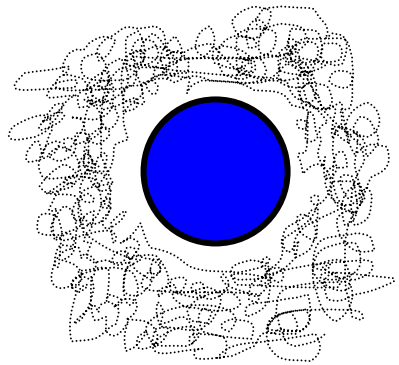
Different strength fields (in *Telsa*) mean different resonance frequencies.



Electron Density

How chemical shift works





Carbonyl

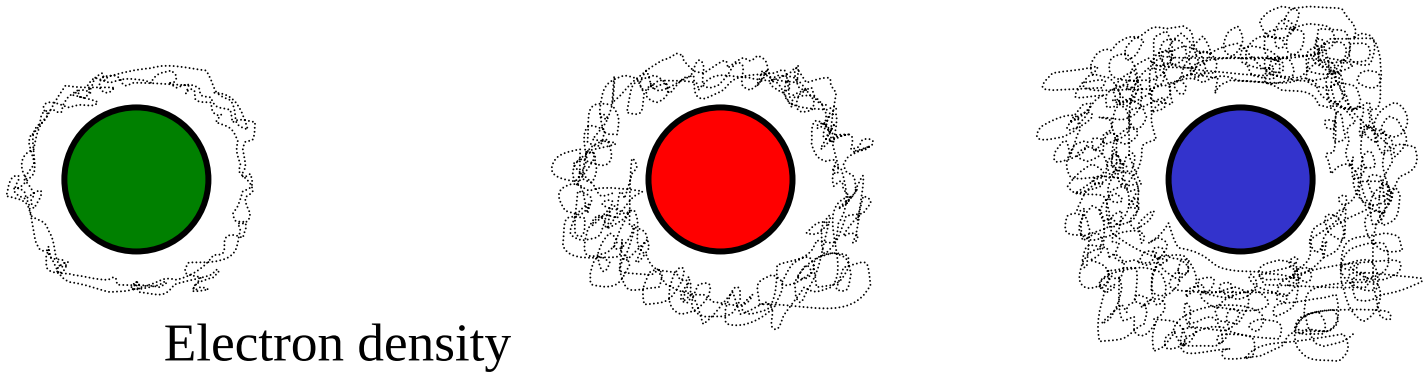
- Less e^- density
- Nucleus 'deshielded' from B_0
- Feels the field (B_{eff}) more

Methyl

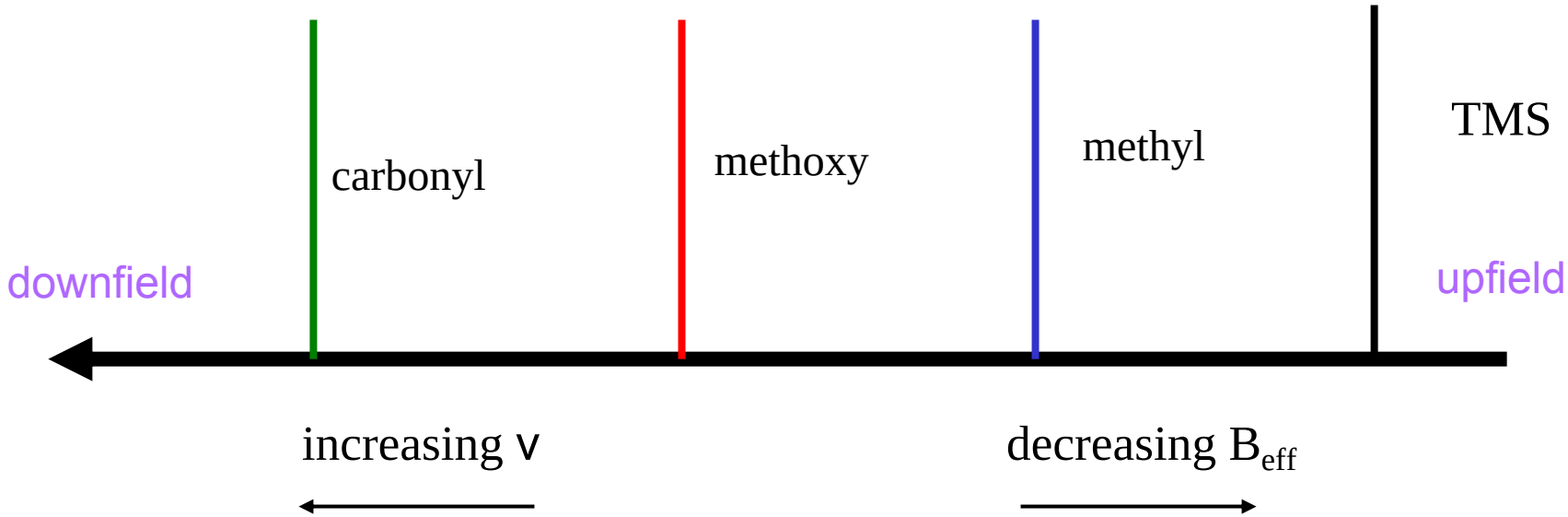
- More e^- density
- Nucleus 'shielded' from B_0 by e^-
- Feels the field (B_{eff}) less

Electron Density

How chemical shift works



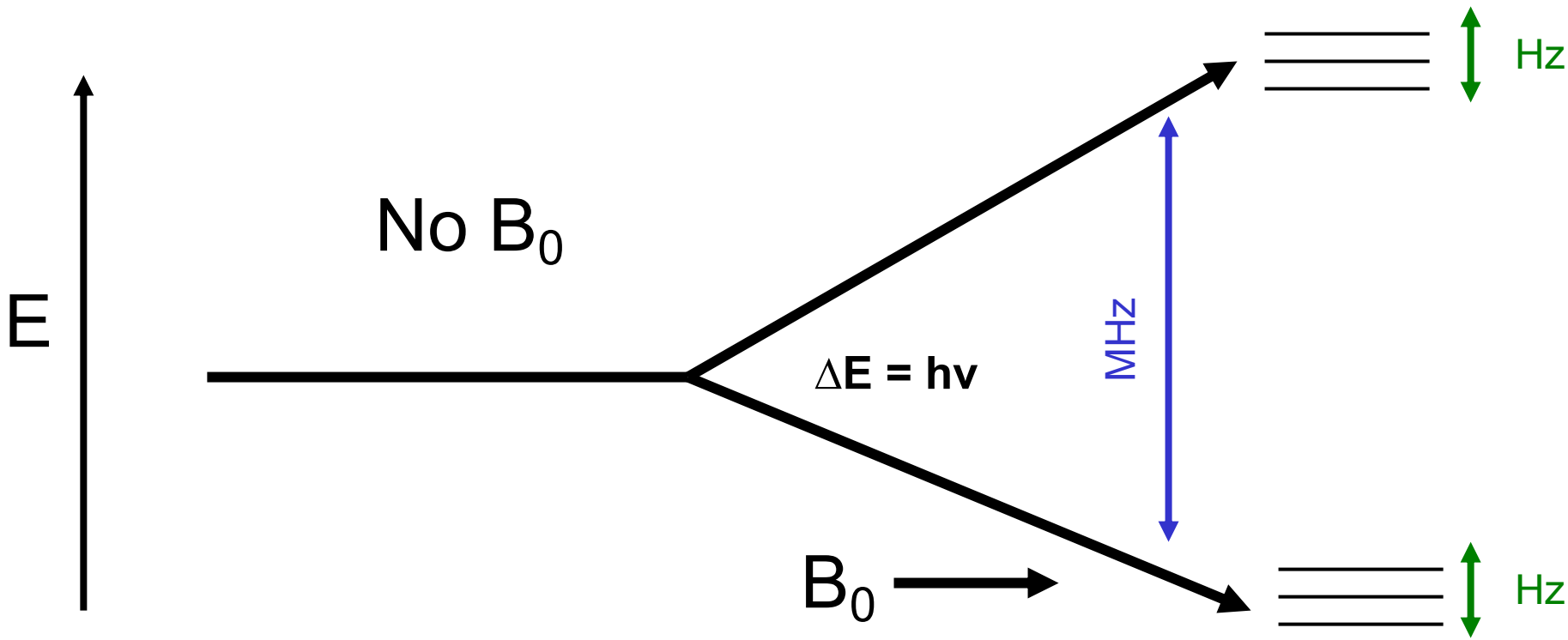
Electron density



Electron Density

How chemical shift works

- Electron density gives us small (0-20,000 Hz) changes in energy levels.
  - Gives us chemical shift (δ) in Hz.

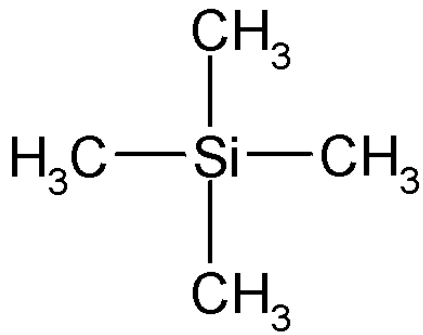


Electron Density

ppm and Hz

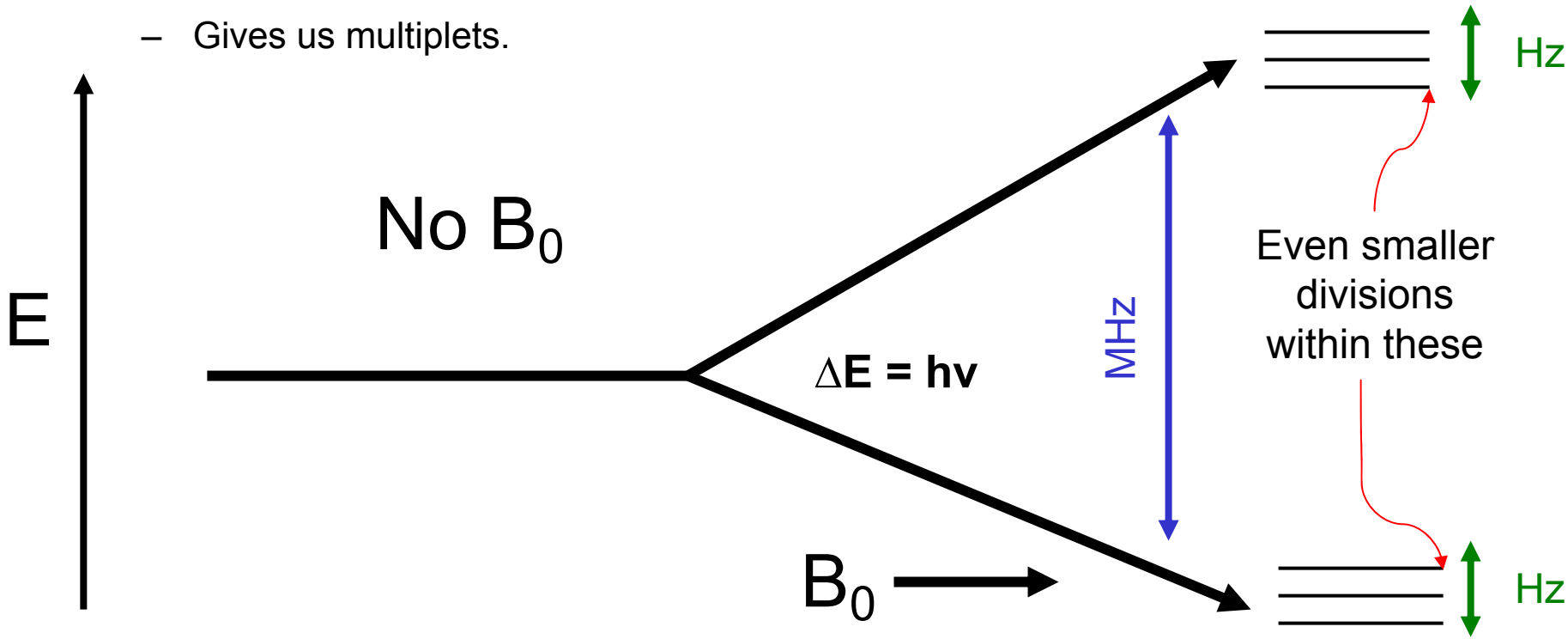
- Chemical shift in Hz is field dependent.
  - Coupling is always in Hz and is NOT field dependent. Therefore, the larger the field, the clearer the coupling, as δ >> J.
- Chemical shift in ppm is NOT field dependent.
  - ppm is parts per million. It is a ratio, so no units.
  - δ Hz / v MHz = ppm. Field of magnet does not matter.

- ^{1}H and ^{13}C referenced to TMS (tetramethylsilane) = 0
  - In D_2O, TMSP used instead of TMS.
- ^{31}P generally references to phosphoric acid (85%) = 0
- ^{15}N generally referenced to nitric acid = 0
- ^{19}F generally referenced to CCl_3F = 0



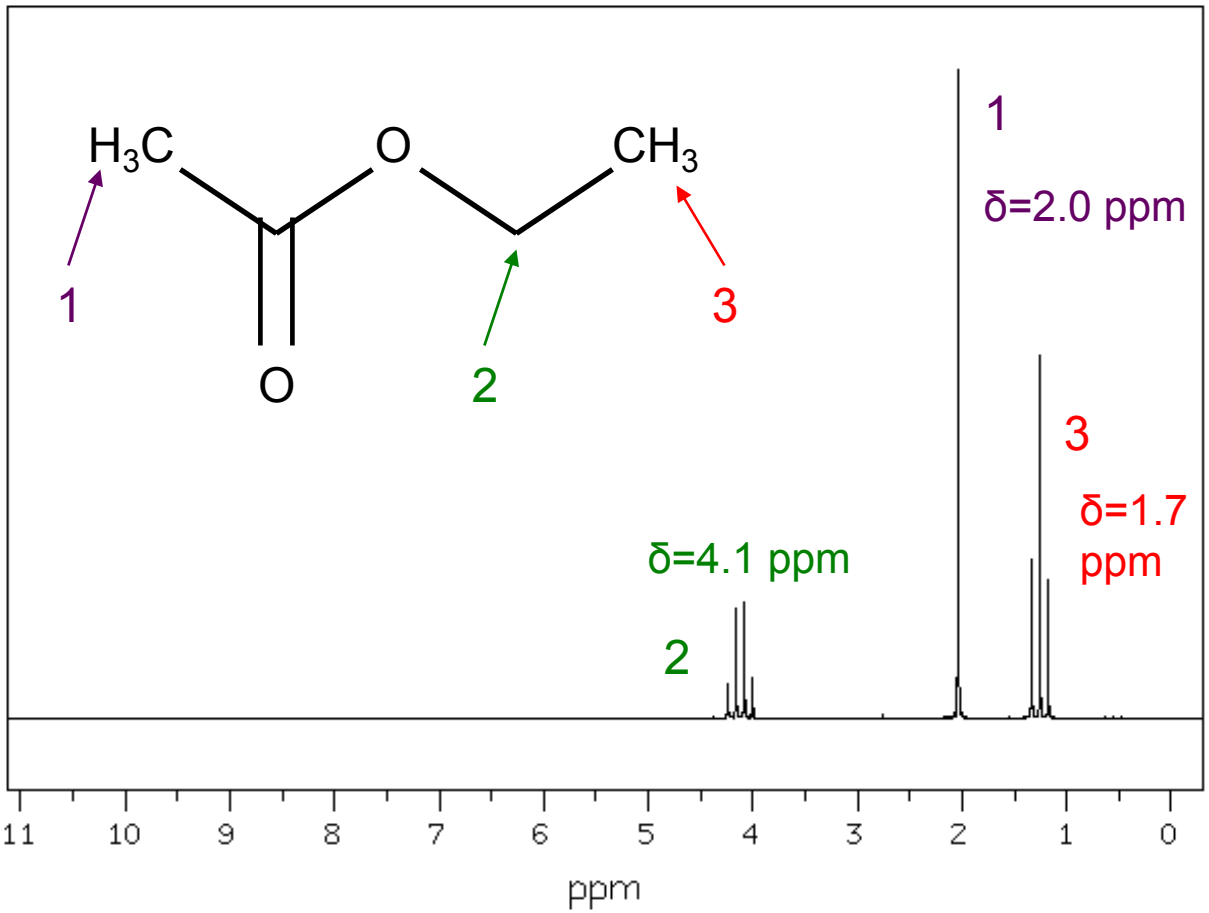
Coupling
Scalar (J) Couplings

- J couplings – through bond coupling, gives us small changes (0-500 Hz) in energy levels.
  - The spin state of one nucleus can create a slightly different B_{eff} in a neighboring nucleus.
  - Gives us multiplets.



Coupling

Ethyl acetate



Spin-Spin Splitting patterns

1. Singlet. 3 equivalent protons. Not coupled to any neighboring protons.

2. Quartet. 2 equivalent protons. Split (1:3:3:1) because coupled to the 3 ^{1}Hs at the 2 position.

3. Triplet. 3 equivalent protons. Split (1:2:1) because coupled to the 2 ^{1}Hs at the 3 position.

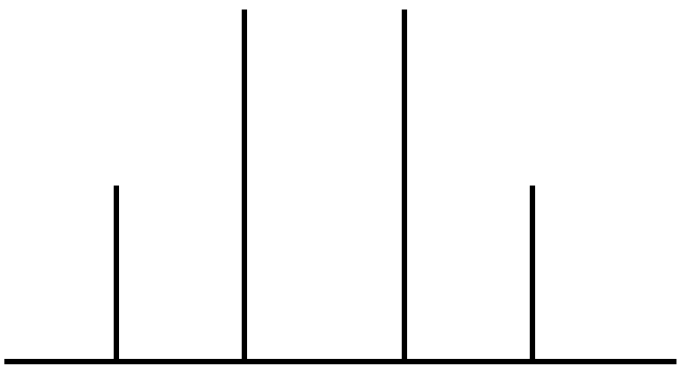
Multiplicity = N (# of neighbors) + 1

Coupling

Spin-Spin Splitting

—— CH_2 —— CH_3

CH_2 Resonance
Quartet (1:3:3:1)

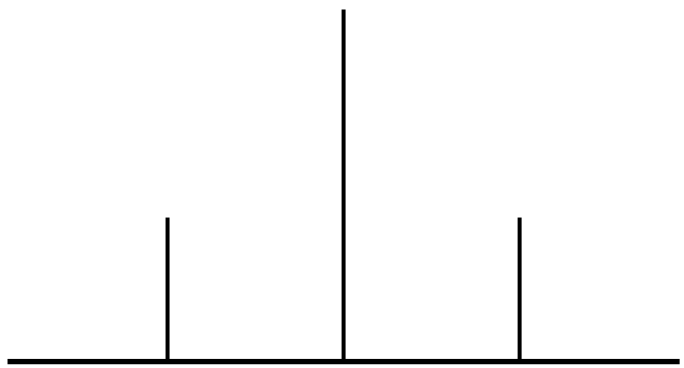


- - - - - + - + + + + +
- + - + - +
+ - - + + -

Spin states of neighboring

CH_3 protons

CH_3 Resonance
Triplet (1:2:1)



- - + - + +
- +

Spin states of neighboring

CH_2 protons

Credits

Special thanks to…..

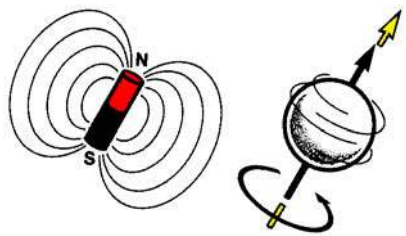
Kathleen Gallagher, University of New Hamsphire.

Jeremy N. S. Evans - BIOMOLECULAR NMR SPECTROSCOPY, Oxford Press, 1995

Varian Associates, Inc.

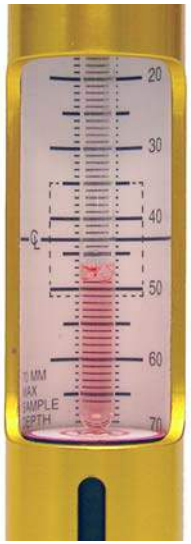
Joseph P. Hornak – Rensallear Institute of Technology

http://www.cis.rit.edu/htbooks/nmr/bnmr.htm



Setting Tube Depth

Varian



0.3 mL

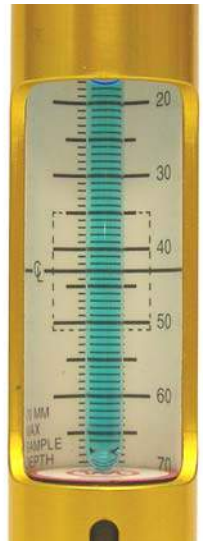
Positioned too low!

Solution not in detected region

0.4 mL

Centered!

Solution covers detected region



0.7 mL

Adjusted to maximum depth!

Recommended sample volume

Setting Tube Depth

Bruker

0.3 mL

Positioned too low!

Solution not in detected region

0.4 mL

Centered!

Solution covers detected region

0.7 mL

Adjusted to maximum depth!

Recommended sample volume

Locking

- Compensates for the transient variations in magnetic field strength.
- The locking circuit keeps the field B_0 at a constant value, so that Δv is constant, and the peaks are narrow.
  - Remember this? Field and frequency are directly proportional!

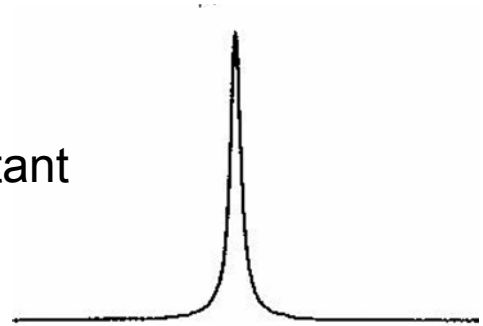
$$\frac{v}{B_0} = \gamma$$

- Typically lock on ^2H signal. With that frequency regulated, all others get regulated as well.

Locking

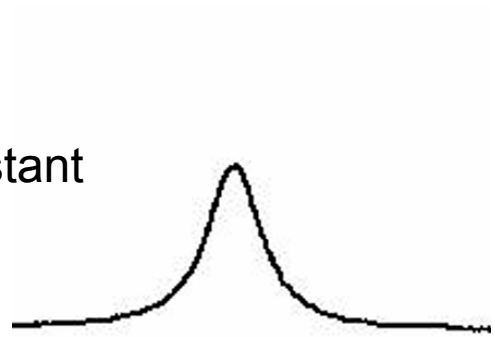
Accumulation over time

B_0 constant



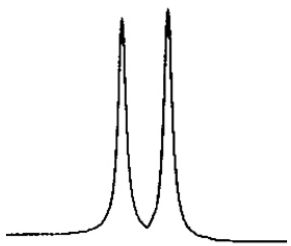
$\Delta\nu$ = small

B_0 not constant



$\Delta\nu$ = large

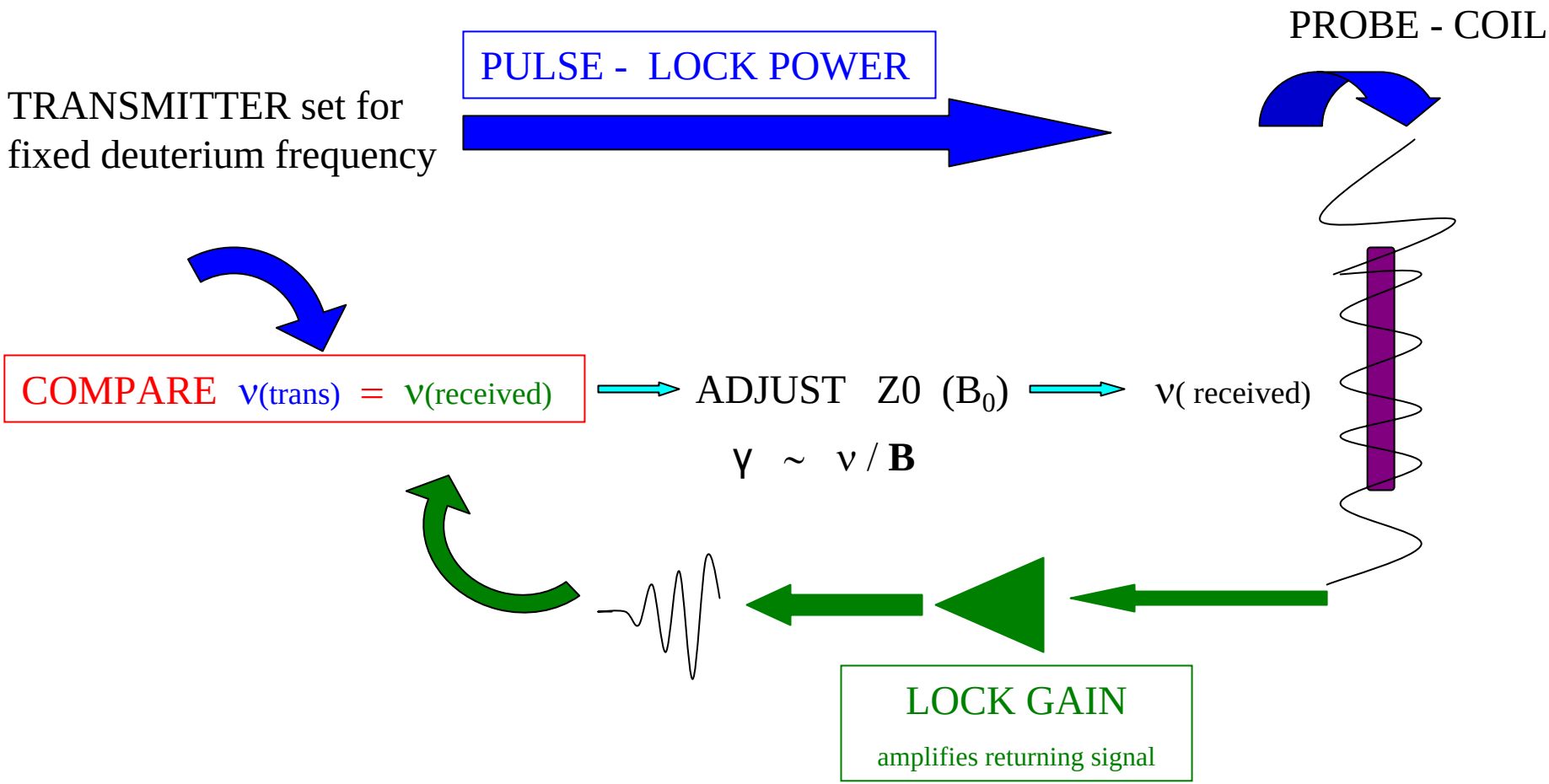
Can run unlocked, but run risk of getting broad peaks.



Locks and then re-locks – lock exactly on resonance or this could happen.

Locking

How does it work?

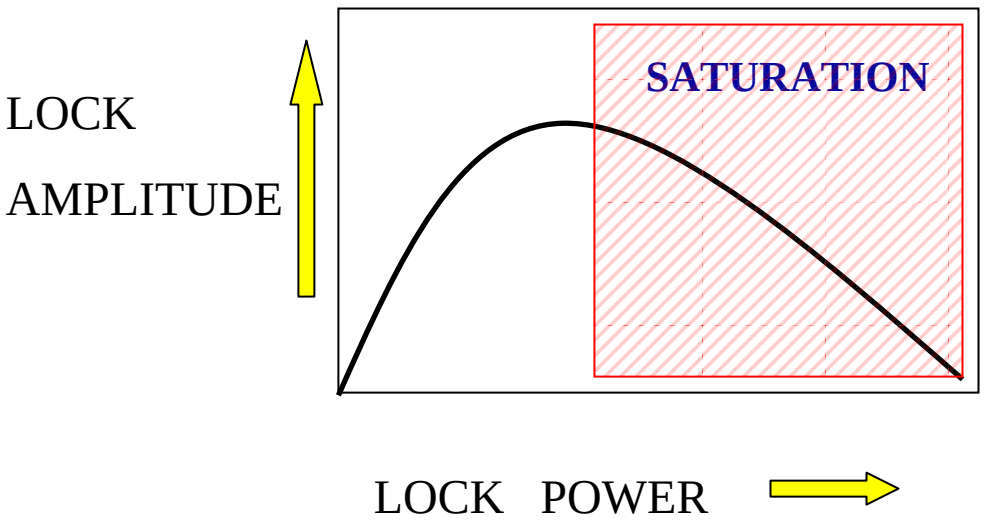


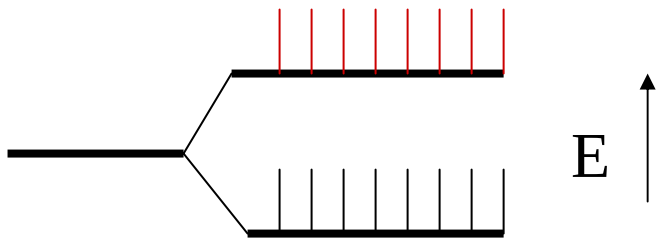
Goal of locking – match transmitted frequency (v trans) to the received frequency (v received). Once matched, said to be *on resonance*. Feedback loop is engaged (lock turned ON), and the instrument will "follow" any drift in the magnetic field.

Locking

Saturation

- Too much power!
  - Get no signal or an oscillating signal because there is an equal populations of spins in all energy states (remember Boltzmann??)





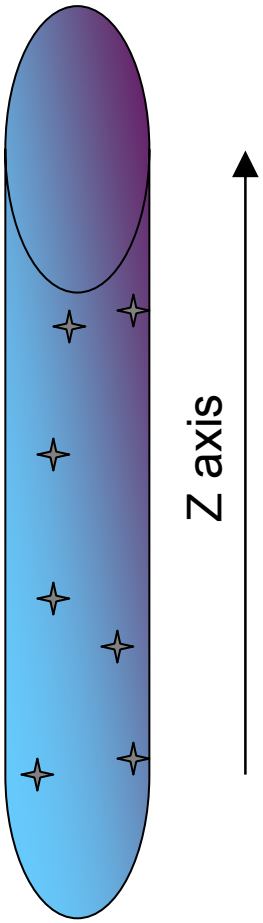
POPULATIONS EQUAL

SATURATION - NO SIGNAL

TOO MUCH POWER !!!

Shimming

ΔB_0 large



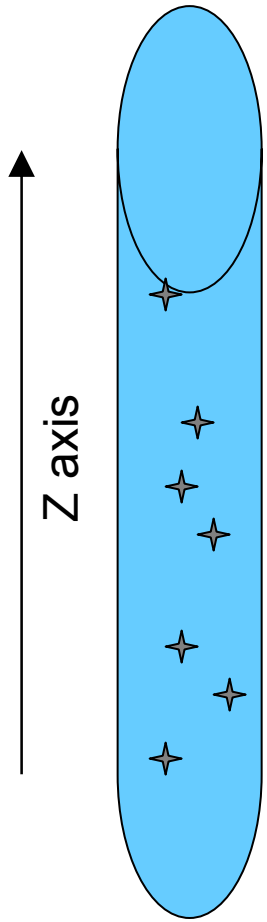
Z axis

SHIMMING is to make the field more HOMOGENEOUS

ΔB small → Δv small

$$\frac{v}{B_o} \sim \gamma$$

ΔB_0 small



Z axis

An aldehyde ^1H at the top of the tube will resonate
at the same frequency as an aldehyde ^1H at the bottom of the tube.

Bad Shimming

Wide, asymmetrical lines

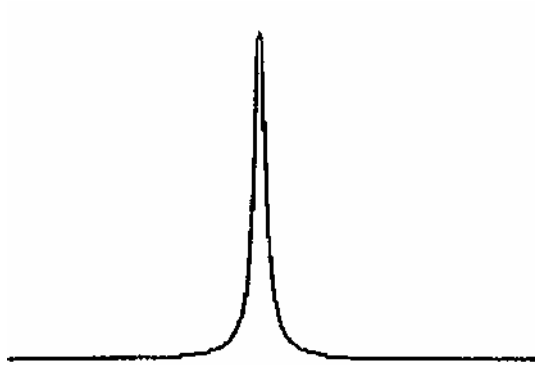
ΔB_0 large

Δv = large

Bad! ☹

NOT well shimmed

ΔB_0 small



Δv = small

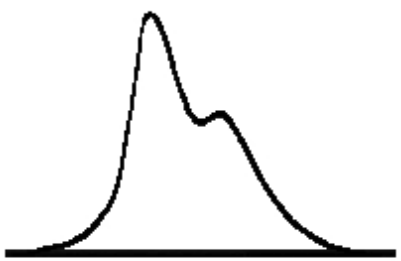
Good! ☺

Well shimmed

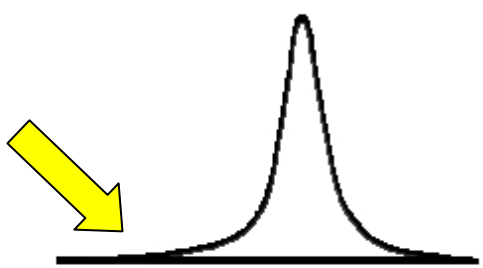
Bad Shimming

Wide, asymmetrical lines

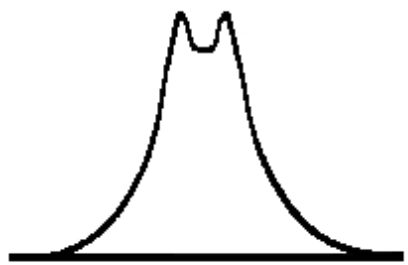
- How do we know when the shimming is good?
  - Amplitude of the lock
    - Have you adjusted the shims so that you have maximized your lock level?
  - Decay of the FID
    - Short, stubby FID = bad shimming
  - Examine actual peaks in finished spectrum
    - Are they all symmetrical? No tails or bumps?



Shoulder



Tail to the left



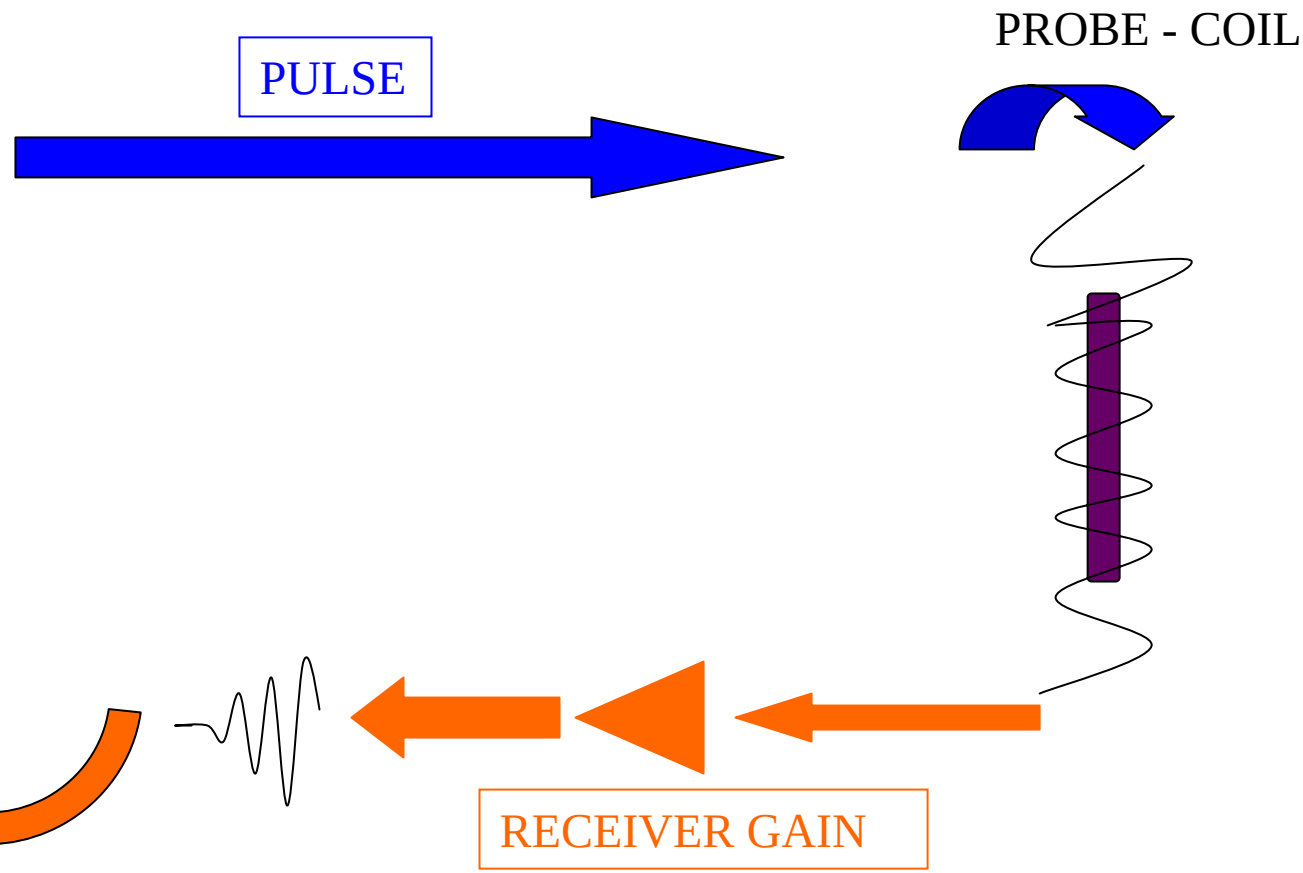
Splitting

Observe

What nucleus we are detecting

TRANSMITTER set for frequency of observed nucleus (in MHz).

- PROTON = 400 MHz
- CARBON = 100 MHz



Stored every BS (block size in Varian)

Typically, 2 observe channels – High frequency (^{1}H, ^{19}F) and Low frequency (^{13}C, ^{15}N, ^{31}P).

Observe

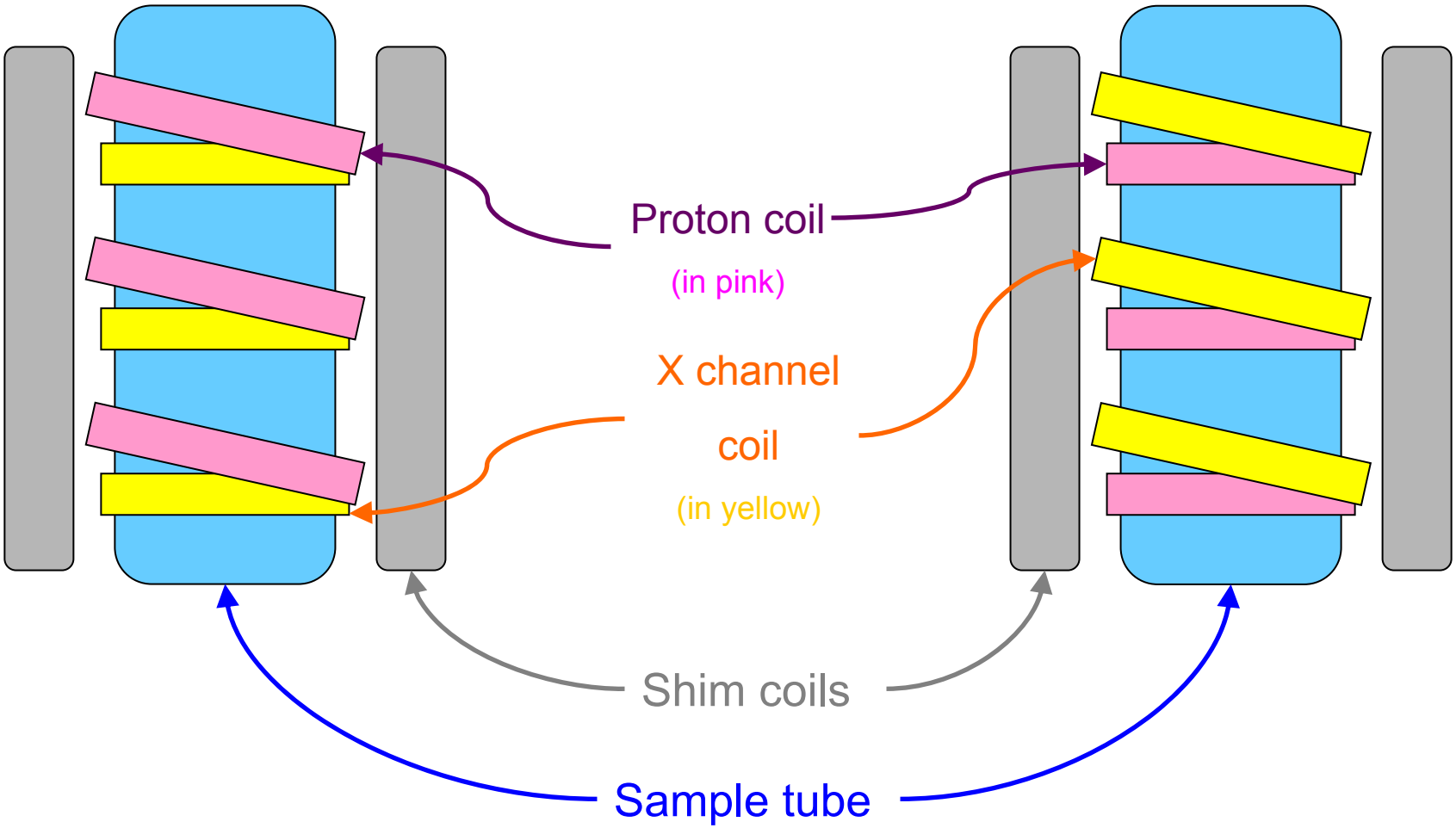
Which probe should I use?

Standard Broadband

X channel (usually Carbon) coil inside

Standard Inverse

Proton coil inside



Various Things to be Aware of

Common Problems and Things to Think About

- Phasing
- Baseline Correction
- Foldover
- Clipping and Truncation
- Signal to Noise
- Relaxation
  - T1
  - T2
- Apodization

Phasing

Zero Order vs. First Order

BAD First Order Phasing

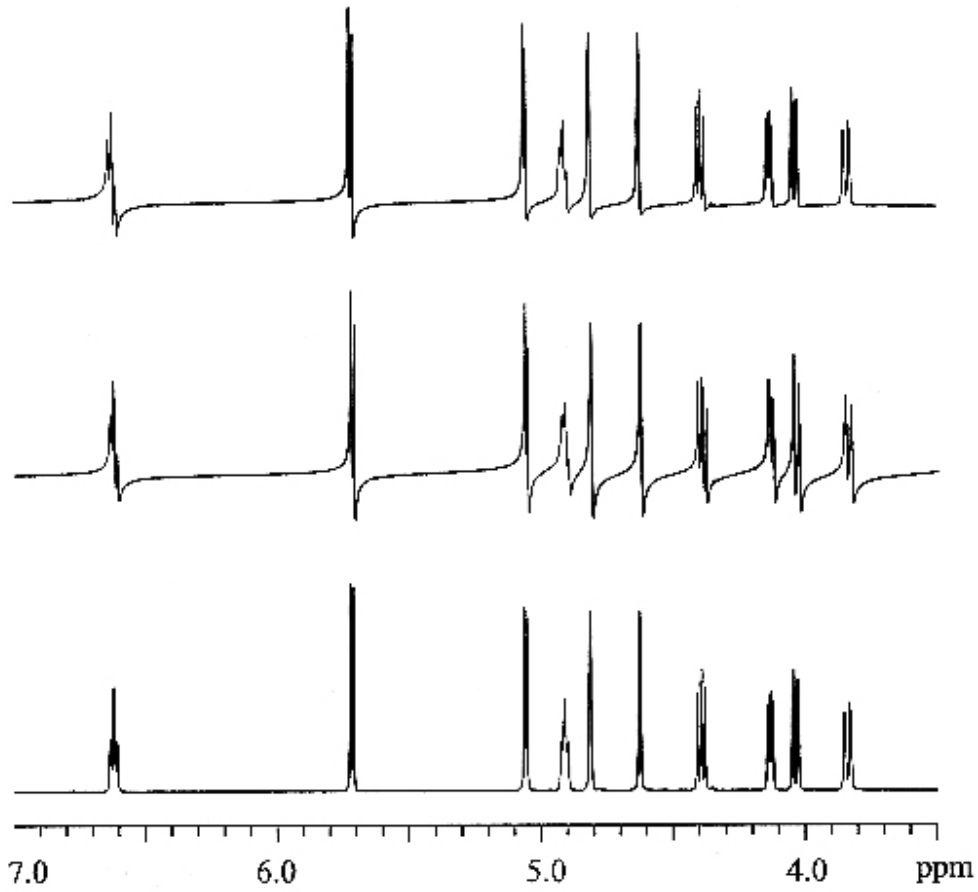
frequency dependent

BAD Zero Order Phasing

Not frequency dependent

Nicely phased!!!

Baseline is even on both sides of the peak.



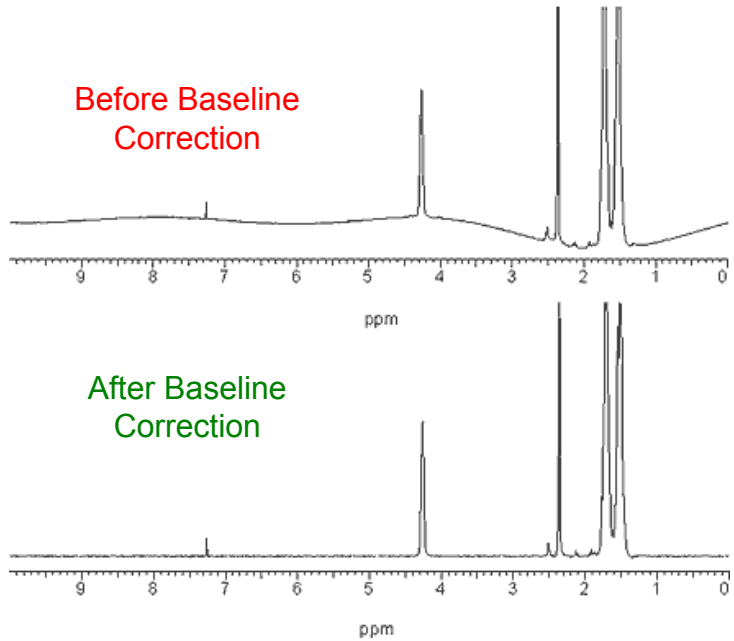
Baseline Correction

Why we want a flat baseline

- Easier to differentiate between noise and signal.
- Integration
  - Flat baseline = accurate integrations
- Presaturation – irradiates a selected frequency.
  - Solvent suppression – enables us to suppress an especially strong signal, allowing us to 'see' weaker signals better.
- 2D NMR! Now we have a flat 'surface' to work with.

Rolls and waves in baseline can be caused by receiver overload/especially concentrated sample.

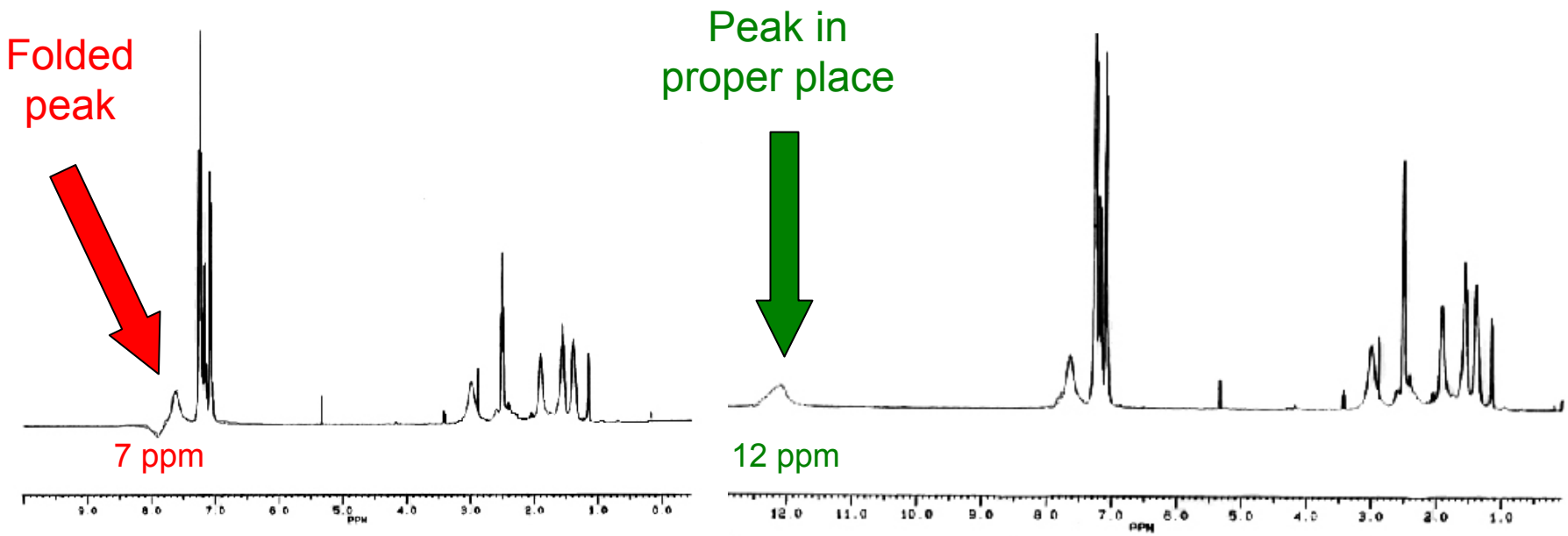
Reducing the pulse width and increasing the acquisition time can fix this.



Foldover

aka Aliasing

- Spectral window too small.
  - Any peak outside the detected range gets ‘folded’ back into the spectrum.
    - Bruker folds peaks in on same side.
    - Varian folds peaks in on the opposite side.
  - Impossible to phase!

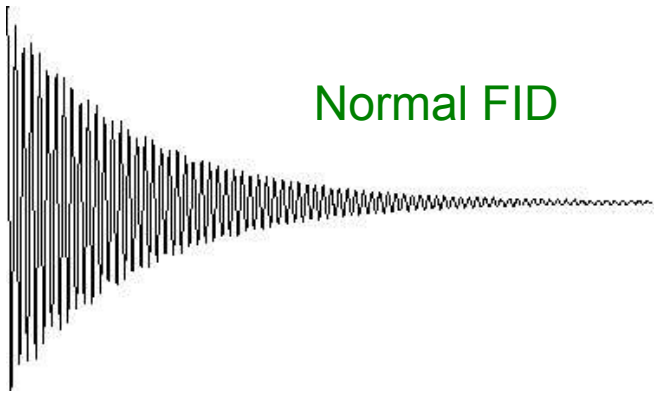


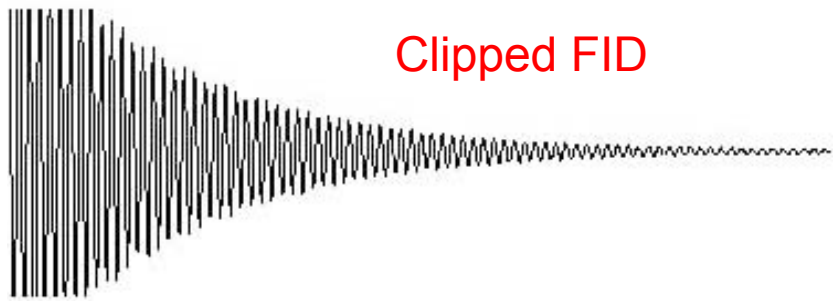
Bruker example

Clipping and Truncation

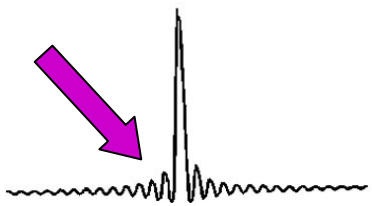
Cutting it short

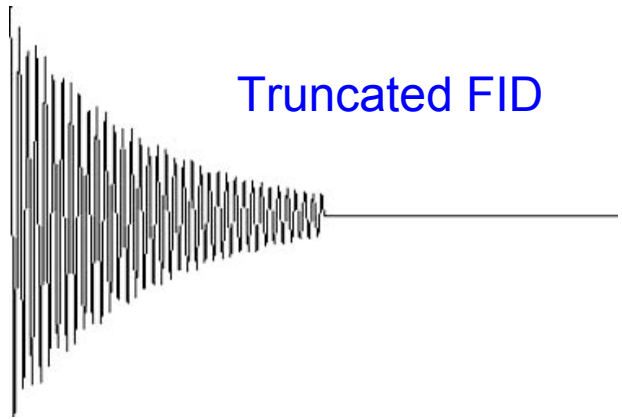
- Clipping – when the receiver gain is too high, and the top of the FID is clipped off. Produces broad peaks.





- Truncation – when the acquisition time is too short, and the end of the FID is cut off. FID not allowed to decay to zero.
  - Produces *Fourier ripples* on either side of your peak.





Signal to Noise

S/N ratio and Number of Scans

- S/N ratio increases with the number of scans.
  - In order to double the current S/N, you need to quadruple the number of scans.

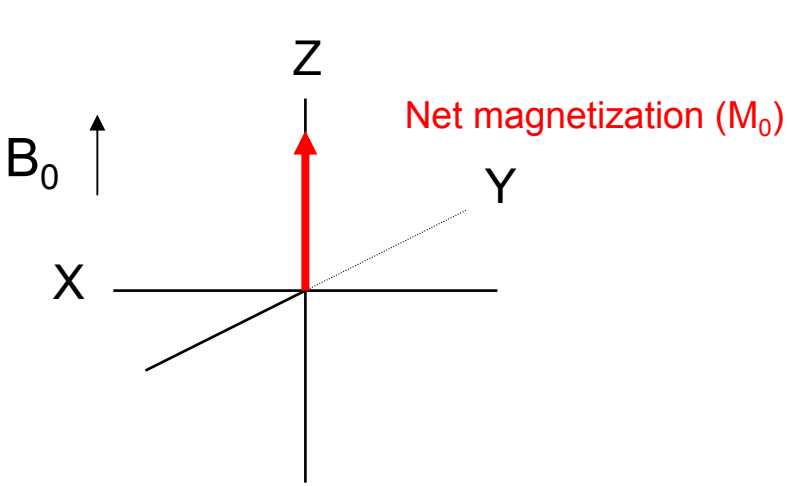
8 scans	S/N=10/1
32 scans	S/N=20/1
128 scans	S/N=40/1

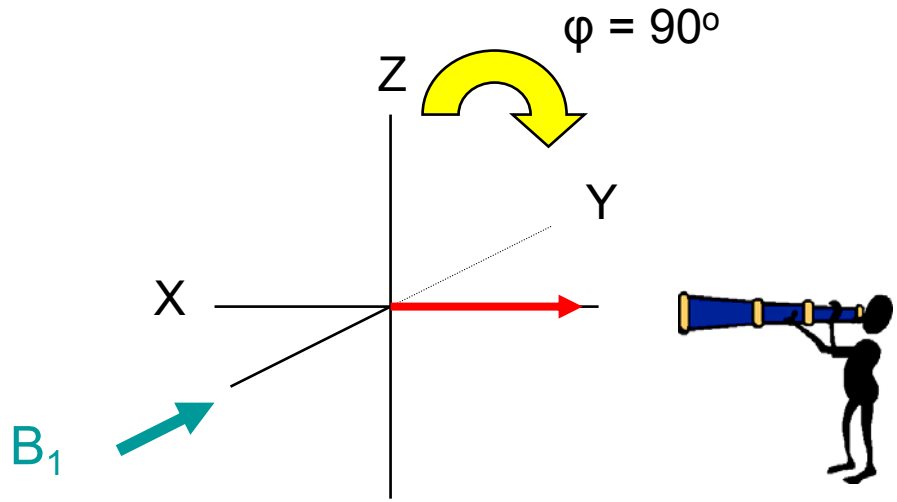
Notice that the longer you run, the less you gain over time. For example, there is not a huge gain in S/N between 3000 and 4000 scans.

Relaxation

T1 and T2

- Remember what happens when a pulse is applied?
  - A second magnetic field is applied (B_1) at radio frequencies (a *pulse*).
  - The length of the pulse (in microseconds) determines how much of the net magnetization is tipped from the z direction into the xy plane.
- We only detect what is tipped into the xy plane.
  - Relaxes back to ground state (along z-axis) and awaits next pulse.
    - Two types of relaxation – T1 and T2.

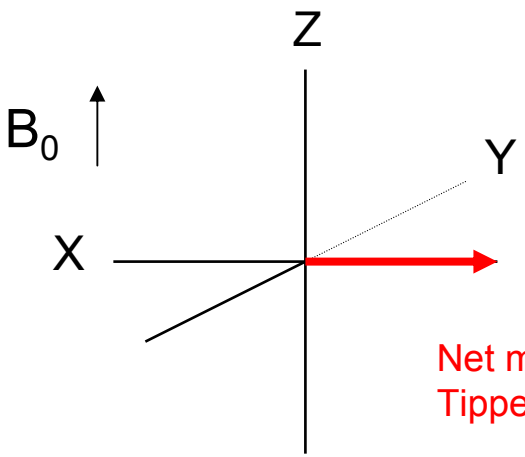




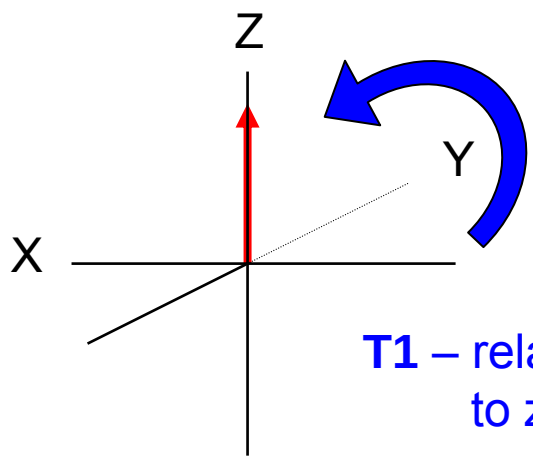
Relaxation

Spin-Lattice or T1

- T1 relaxation – how long it takes for the net magnetization to return to the ground state (z axis).
  - Different nuclei relax at different rates
  - Dipole-dipole interaction help with relaxation. The more neighbors, the faster the relaxation.
    - Smaller, organic molecules tend to be slow relaxers and have long T1s.
  - The same nuclei in different magnetic environments also relax at different rates.
    - Carbons are relaxed by neighboring protons. The more protons, the shorter the T1. Thus, quaternary carbons have long T1s because they have no attached protons.
  - Must wait 5 T1s between pulses to ensure that M_0 has fully relaxed.
    - We usually pulse less than 90^0,so that we do not have to wait that long.



Net magnetization (M_0)
Tipped by a 90^0 pulse

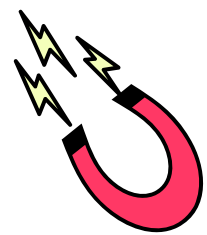


T1 – relaxing back to z axis

Relaxation

Why do we care about T1?

- If you do not wait the right amount of time between pulses, you are not allowing your net magnetization vector to return to the ground state before you pulse again.
  - You will basically 'beat down' your signal by pushing it more and more *past* the xy plane. The less signal in the xy plane, the less you will detect.
    - If you suspect that you are losing some of your signal, increase the delay, take a few scans, and check your spectrum.
  - Aromatics especially sensitive to being 'beaten down'.
  - In order to avoid having to wait 5 T1s (this can be long for certain carbons), we rarely use a full 90^0 pulse.
    - 45^0 and 33^0 pulses tip less M_0 into the xy plane, so we don't have as long for it to relax back to equilibrium.
    - Sacrifice some signal so that we can take more scans in less time.
- This is how solvent suppression (presaturation) is done! The delay between pulses (*tau* or τ) is set so that the selected peak is 'beaten down.'



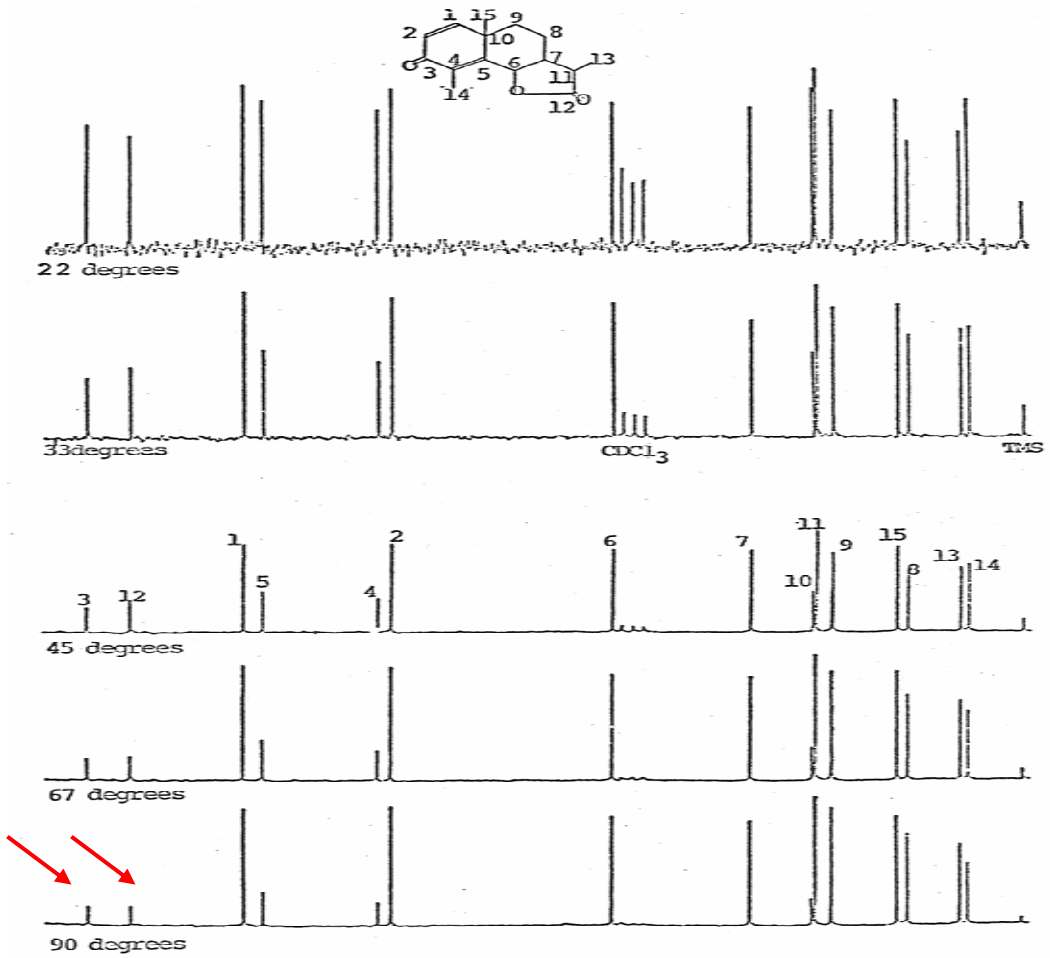
Relaxation

α-santonin

300 scans
2 second delay (τ or *tau*)

Shorter pulses – not as much M_0 tipped
So delay is long enough

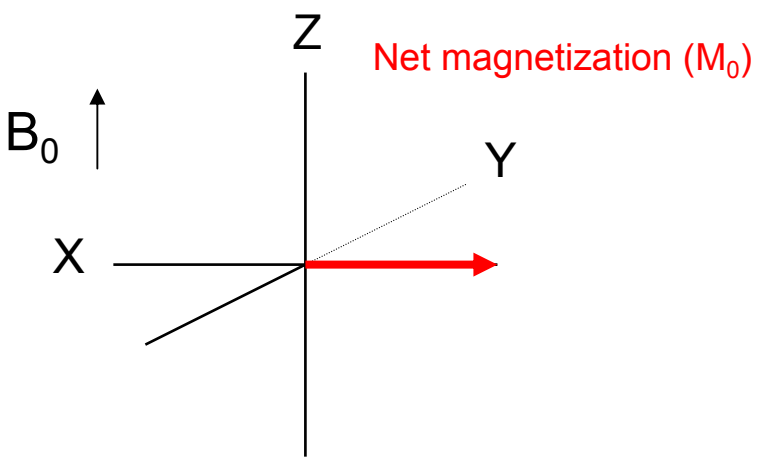
90^0 pulse – delay too short
Losing aromatic peaks

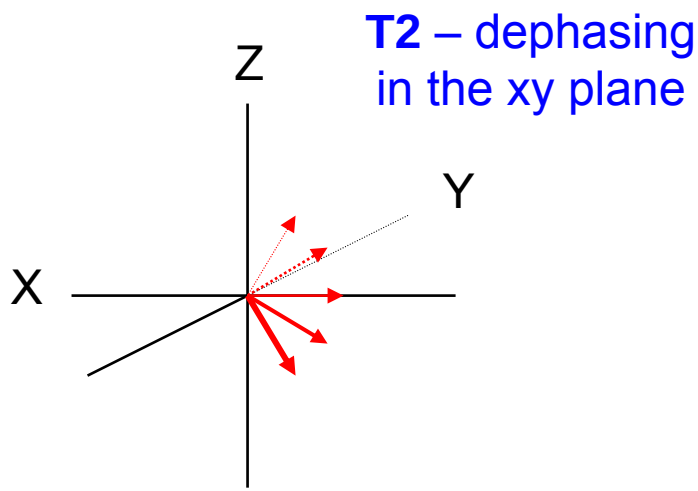


Relaxation

Spin-Spin or T2

- T2 relaxation – how long it takes for the spins to lose phase coherence, allowing the net magnetization (M_0) decay to 0 in the xy plane.
  - Spin flipping (+½ ↔ -½) due to fluctuating local dipolar magnetic fields (inhomogeneity of the field) causes some spins to ↑ (increase) in energy, some to ↓ (decrease), some to precess faster, others slower. Dephasing occurs.
  - If the acquisition time is shorter than T2, the end of the FID will be cut off (truncation!).
- Meanwhile, T1 relaxation is taking place.
  - T2 is always less than or equal to T1.





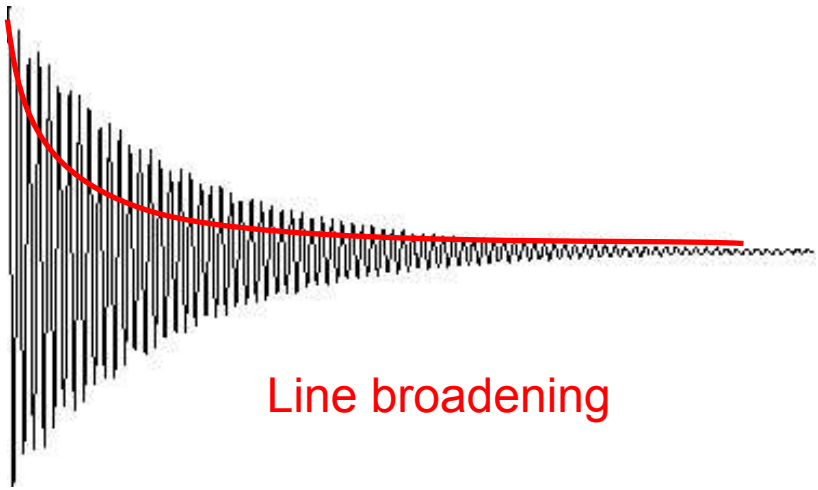
Apodization

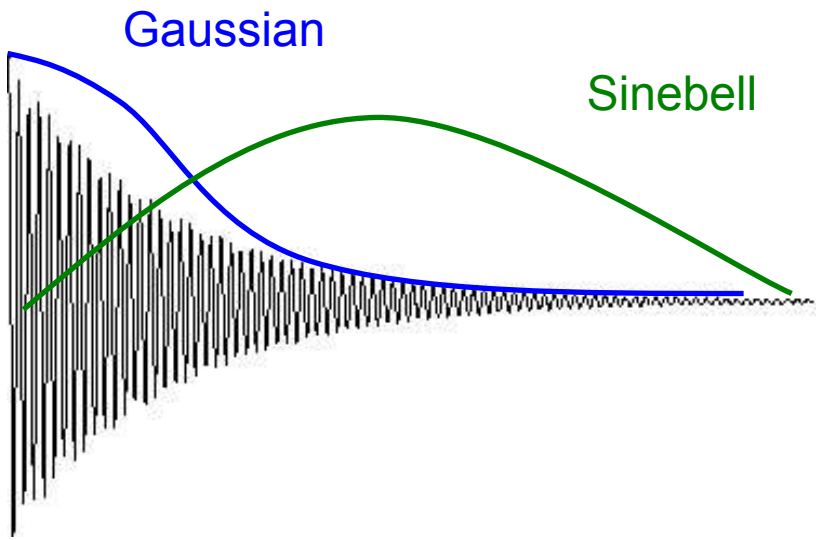
FID manipulation

- **Apodization** – when the FID is multiplied by some mathematical function to modify the spectrum.
  - Allows you to emphasize some quality of the spectrum at the expense of another.
  - This is done after the spectrum has been acquired.
  - Several different types of apodization.
    - **Sensitivity Enhancement** (line broadening) – enhances the first part of the FID, increasing sensitivity at the expense of resolution.
    - **Resolution Enhancement** (Gaussian, Sine Bell) – enhances the later part of the FID, increasing resolution at the expense of S/N.

Apodization

FID manipulation





Increased sensitivity
Decreased resolution

Decreased sensitivity
Increased resolution

Zero Filling

FID manipulation

- When an FID is Fourier transformed, the data (**np**, or number of points) consists of 2 parts.
  - **Real points** (np/2) from the cosine portion.
  - **Imaginary points** (np/2) from the sine portion, which are <u>not</u> displayed in the actual spectrum.
- Zero filling doubles number of real points used by adding an equal number of zeroes.
  - Increases the digital resolution.
  - FID needs to have decayed to 0 to be zero filled.

If np = 32,768, then 16,384 points would typically used to create the actual spectrum.
If it were zero filled, 16,384 zeros would be added to the end of the FID
so that 32,768 points would be used to create the spectrum.