Department of Chemistry
Instrumentation Facility

Massachusetts Institute of Technology

Introduction to NMR

Part 2

Revised 8/23/07

Setting Tube Depth

Varian

0.3 mL

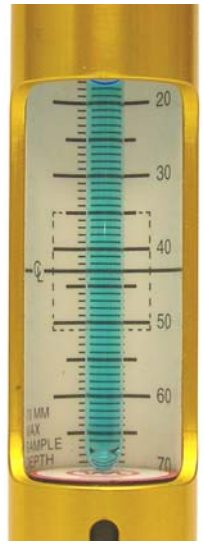
Positioned too low!

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!

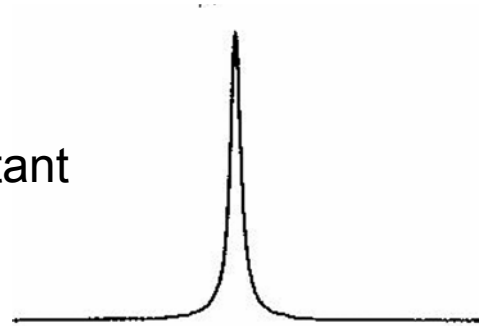
$$\frac{v}{B_0} = \gamma$$

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

Locking

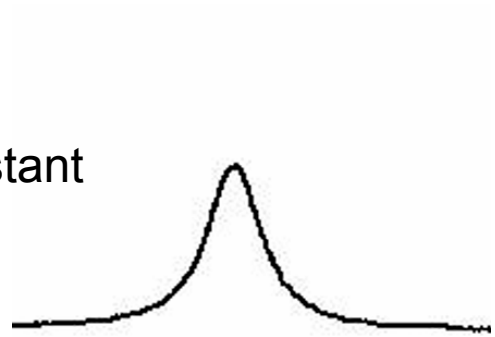
Accumulation over time

B_0 constant



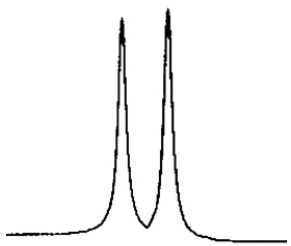
$\Delta\nu$ = small

B_0 not constant



$\Delta\nu$ = large

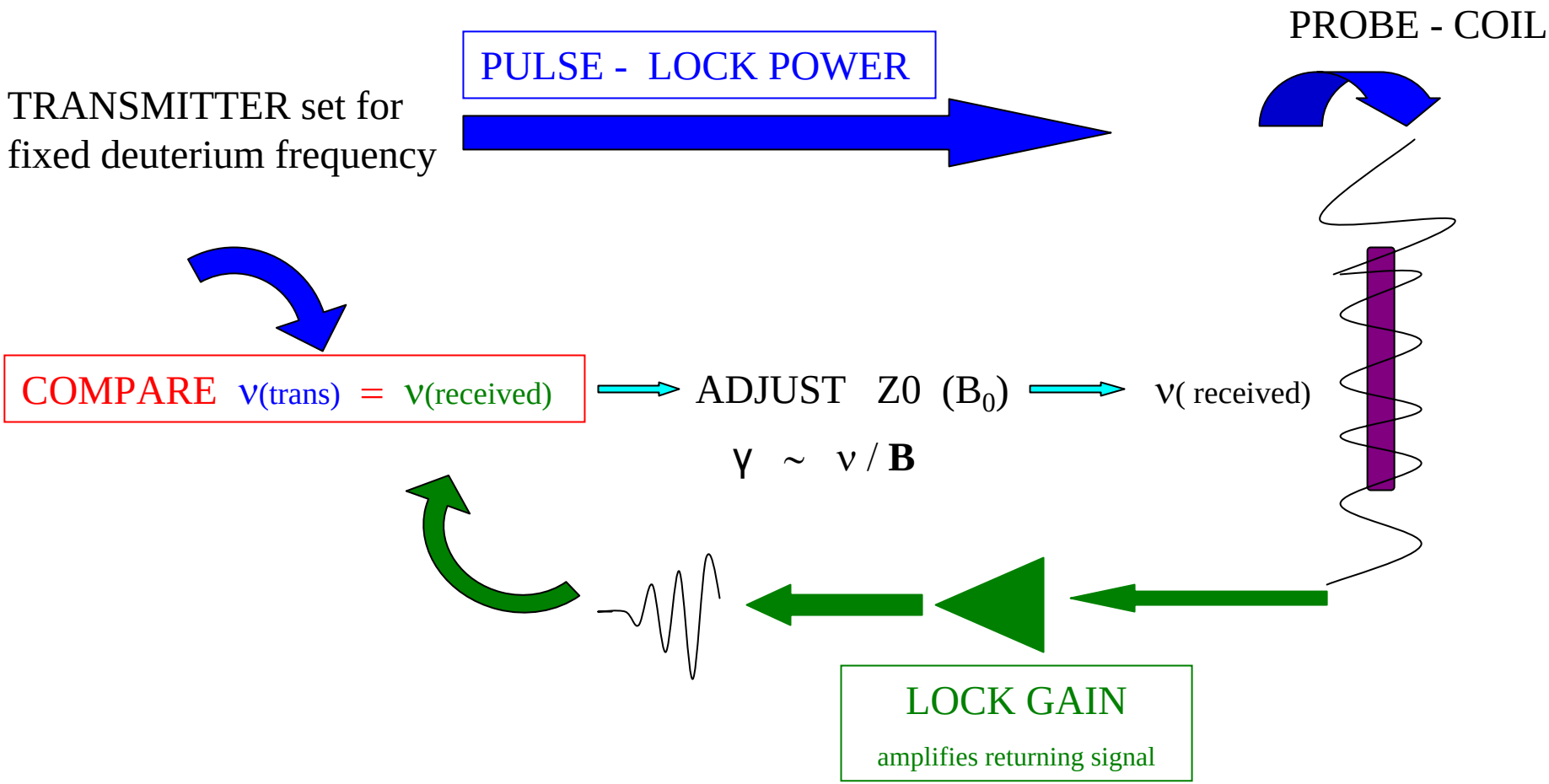
Can run unlocked, but run risk of getting broad peaks.



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

Locking

How does it work?

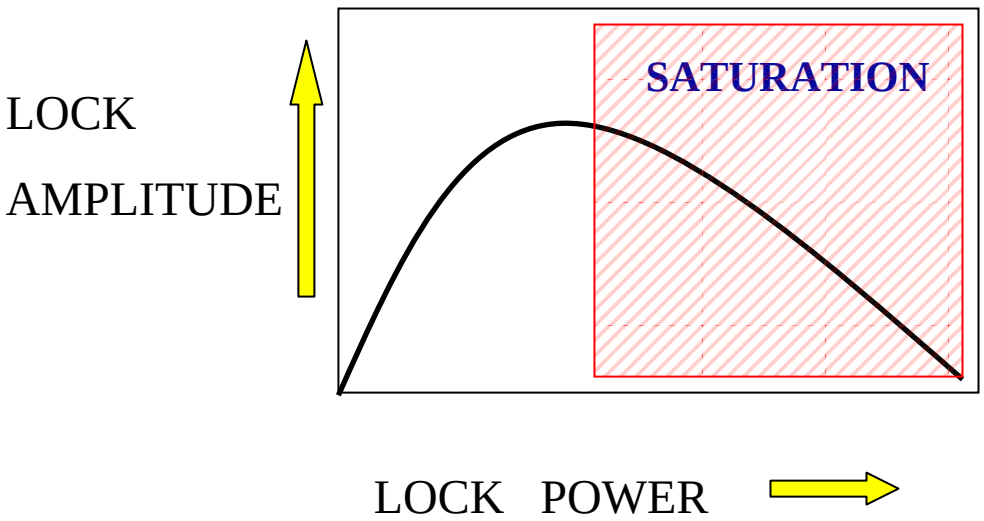


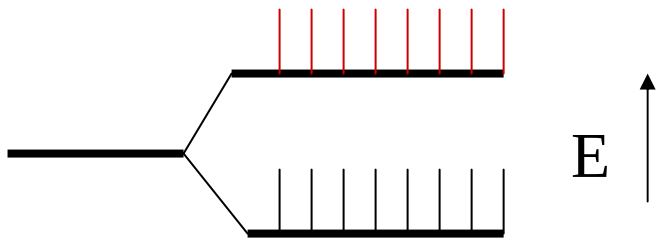
Goal of locking – match transmitted frequency (v trans) to the received frequency (v received). Once matched, said to be *on resonance*. Feedback loop is engaged (lock turned ON), and the instrument will "follow" any drift in the magnetic field.

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??)





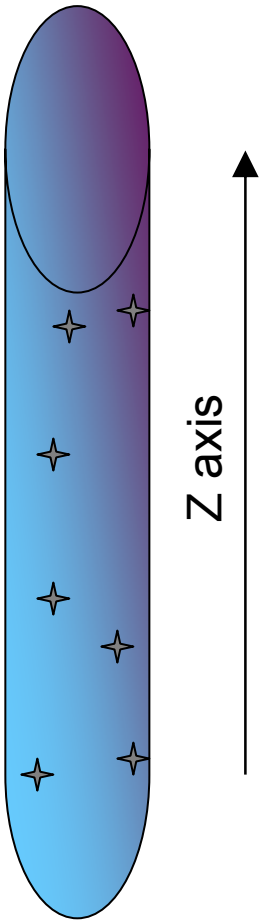
POPULATIONS EQUAL

SATURATION - NO SIGNAL

TOO MUCH POWER !!!

Shimming

ΔB_0 large



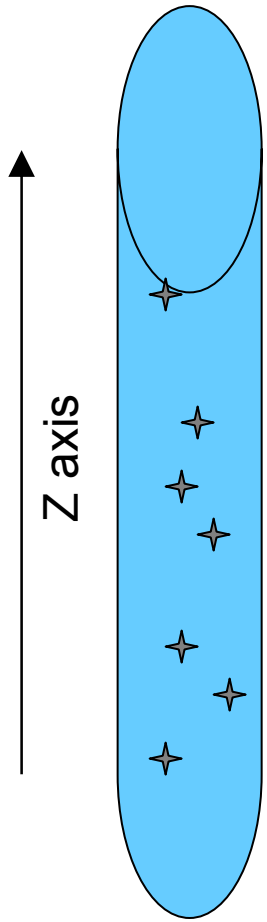
Z axis

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

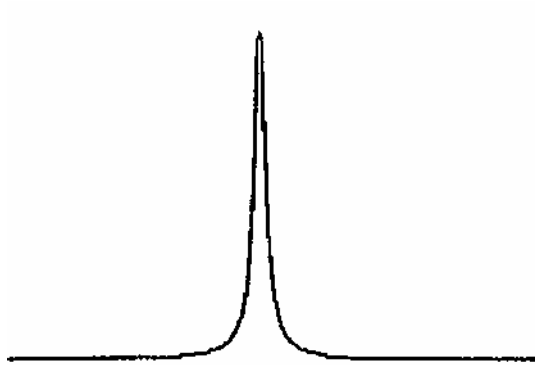
ΔB_0 large

Δv = large

Bad! ☹

NOT well shimmed

ΔB_0 small



Δv = small

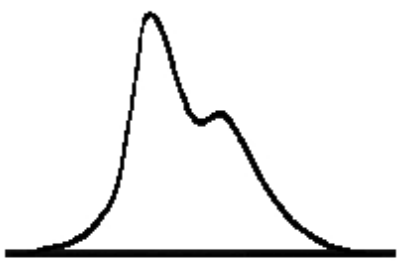
Good! ☺

Well shimmed

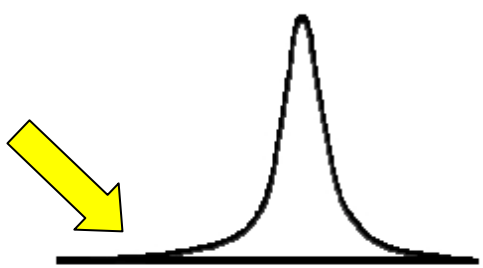
Bad Shimming

Wide, asymmetrical lines

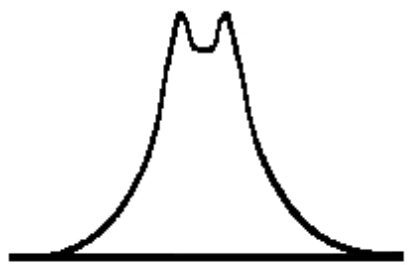
- How do we know when the shimming is good?
  - Amplitude of the lock
    - Have you adjusted the shims so that you have maximized your lock level?
  - Decay of the FID
    - Short, stubby FID = bad shimming
  - Examine actual peaks in finished spectrum
    - Are they all symmetrical? No tails or bumps?



Shoulder



Tail to the left



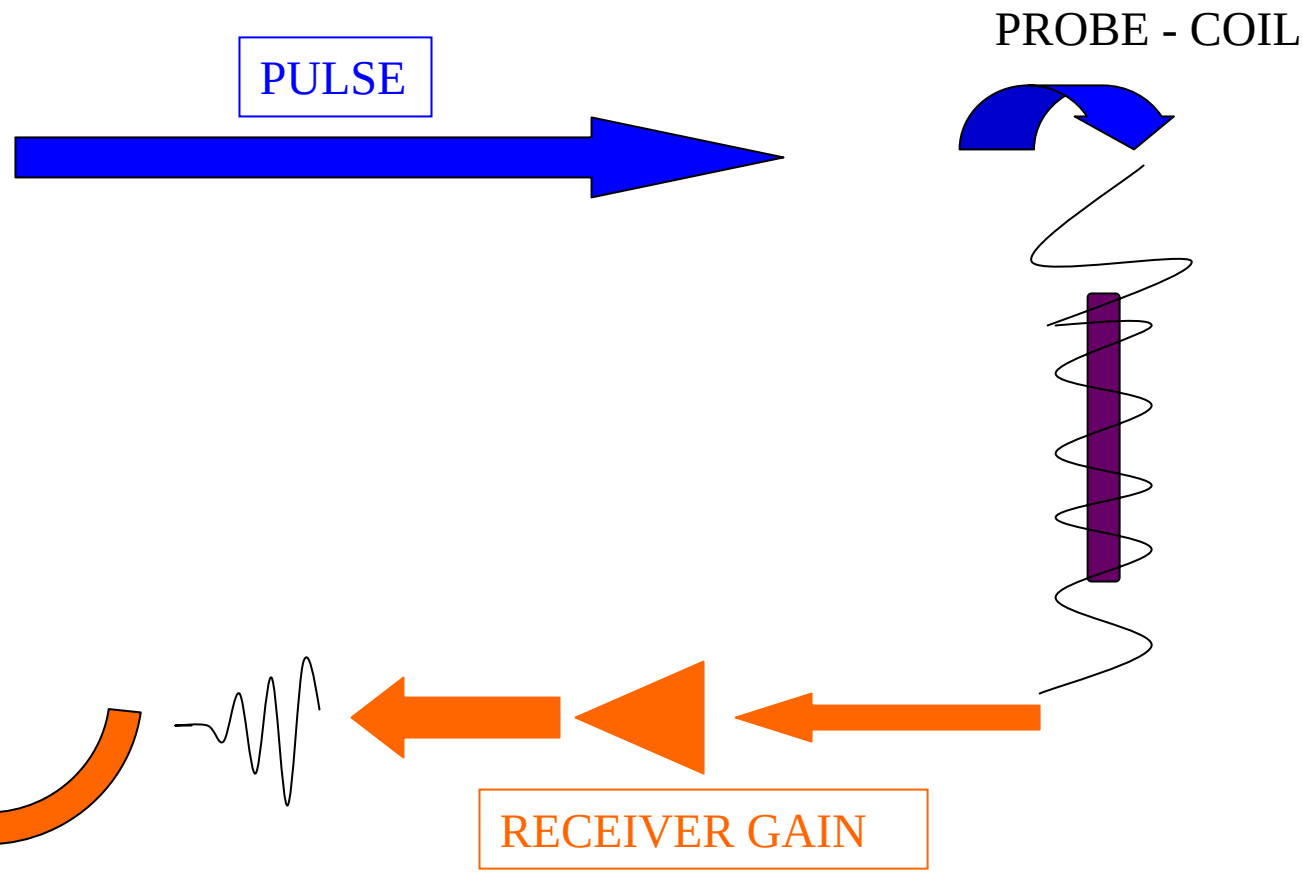
Splitting

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

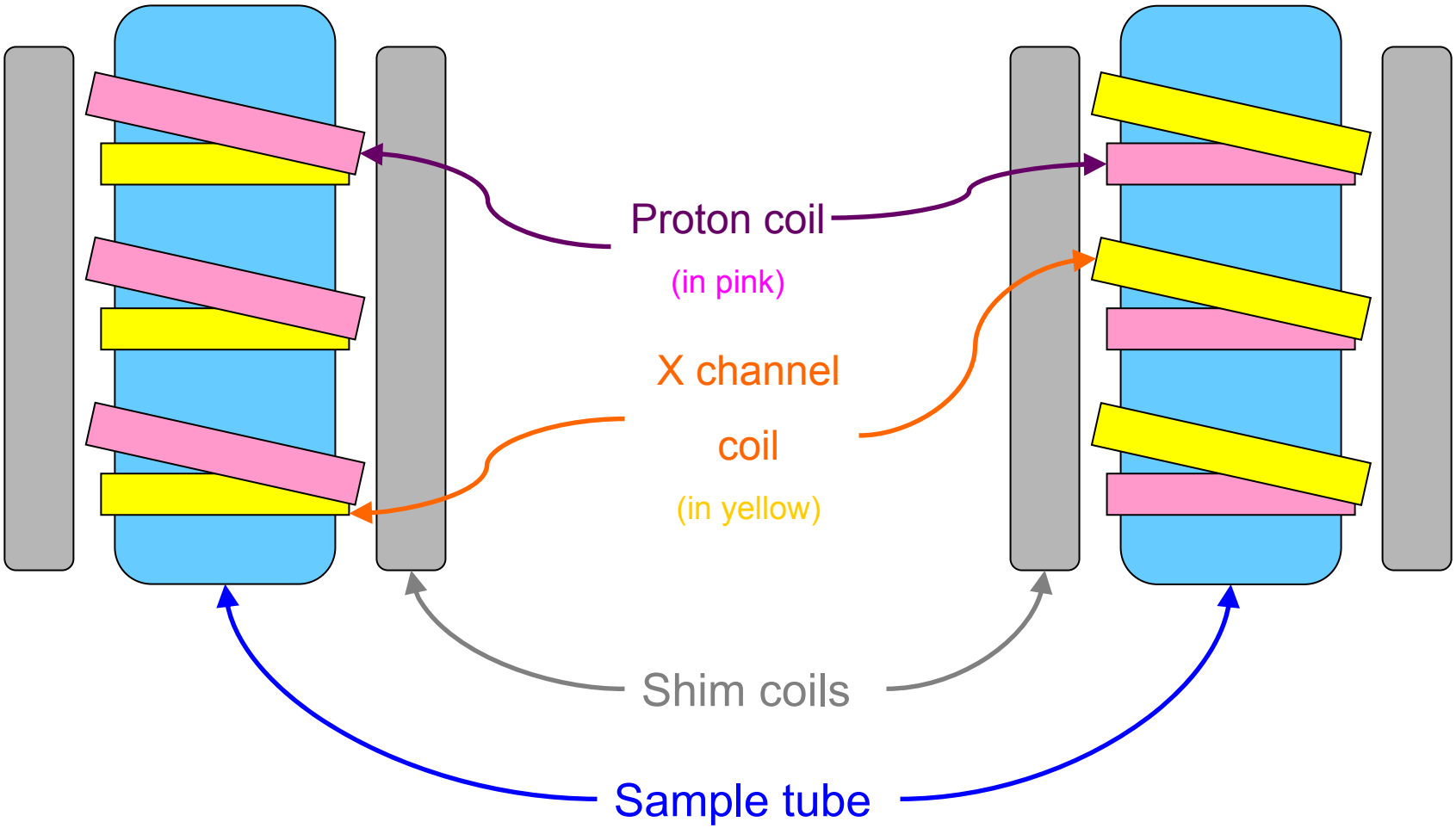
Which probe should I use?

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

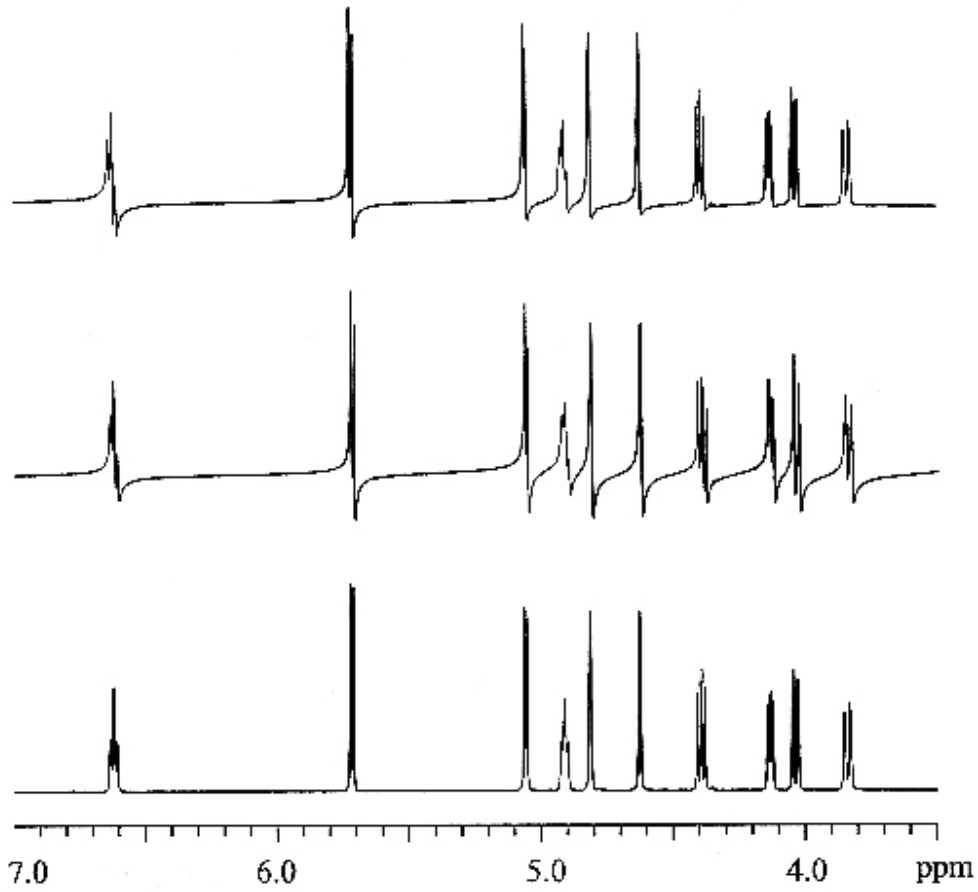
frequency dependent

BAD Zero Order Phasing

Not frequency dependent

Nicely phased!!!

Baseline is even on both sides of the peak.



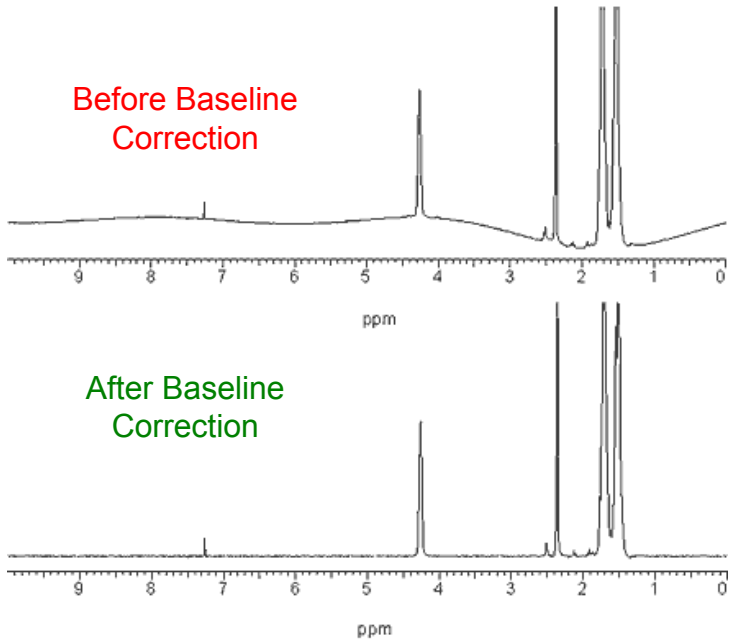
Baseline Correction

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.

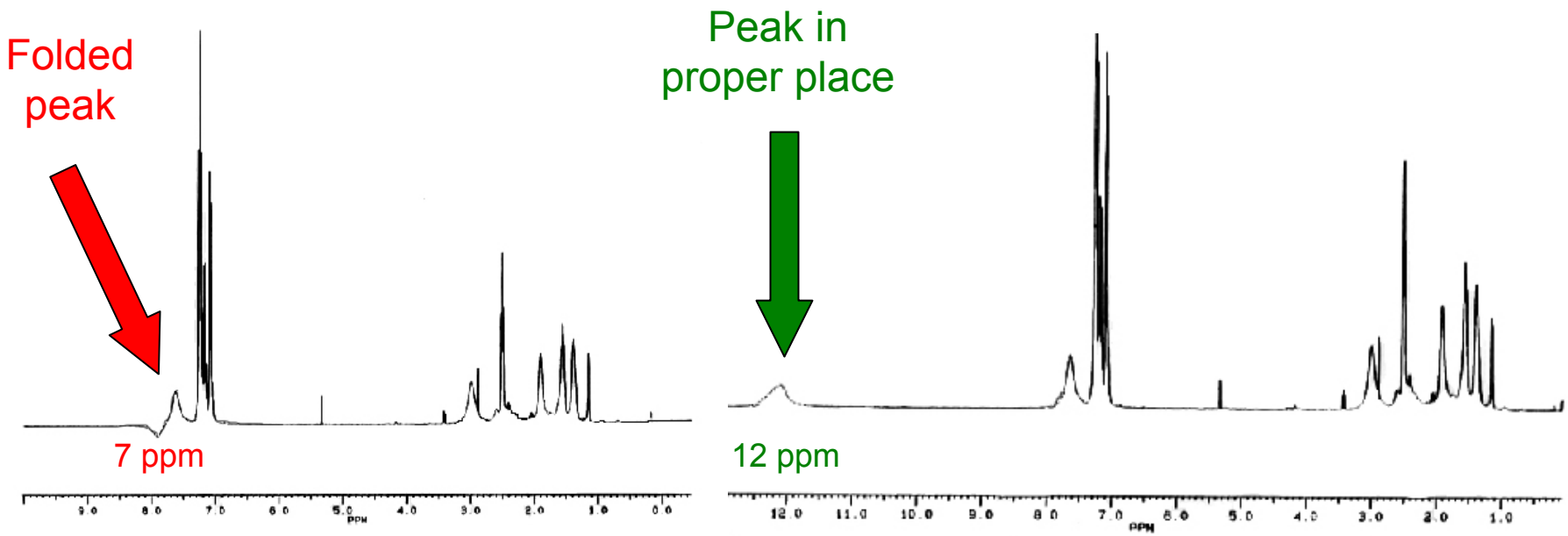
Reducing the pulse width and increasing the acquisition time can fix this.



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!

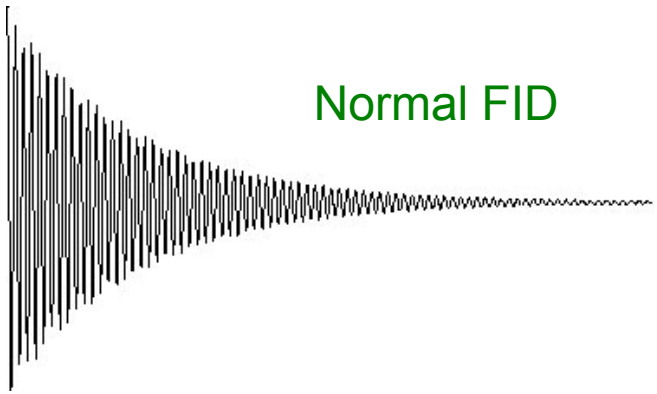


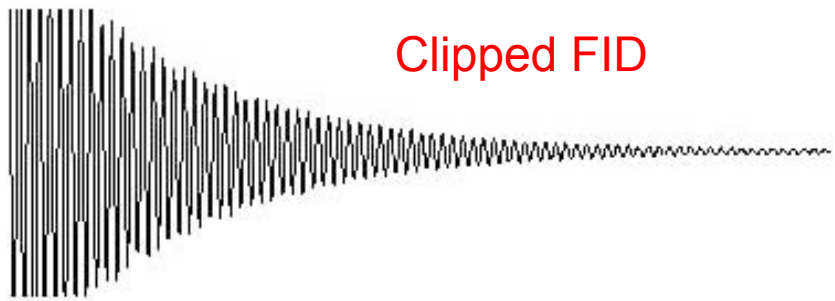
Bruker example

Clipping and Truncation

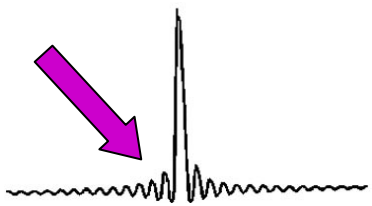
Cutting it short

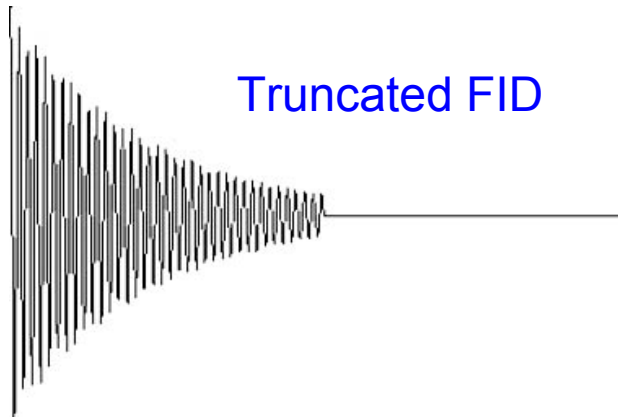
- Clipping – when the receiver gain is too high, and the top of the FID is clipped off. Produces broad peaks.





- 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.





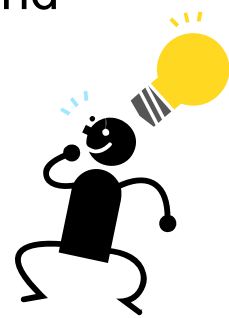
Signal to Noise

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

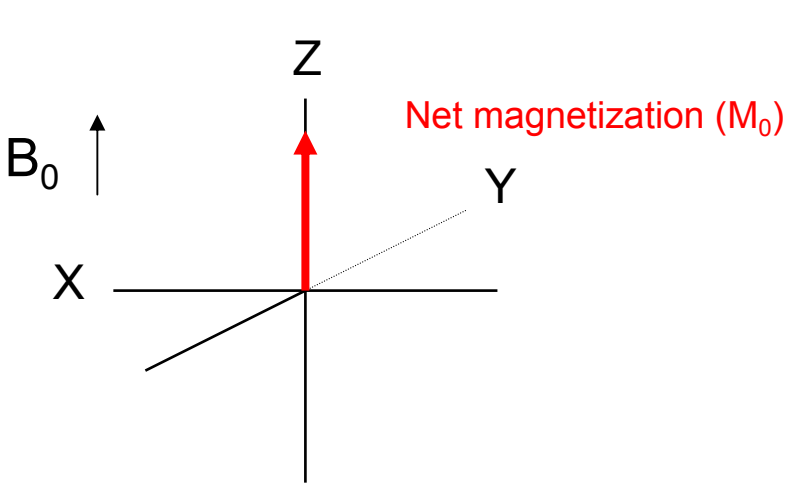
Notice that the longer you run, the less you gain over time. For example, there is not a huge gain in S/N between 3000 and 4000 scans.

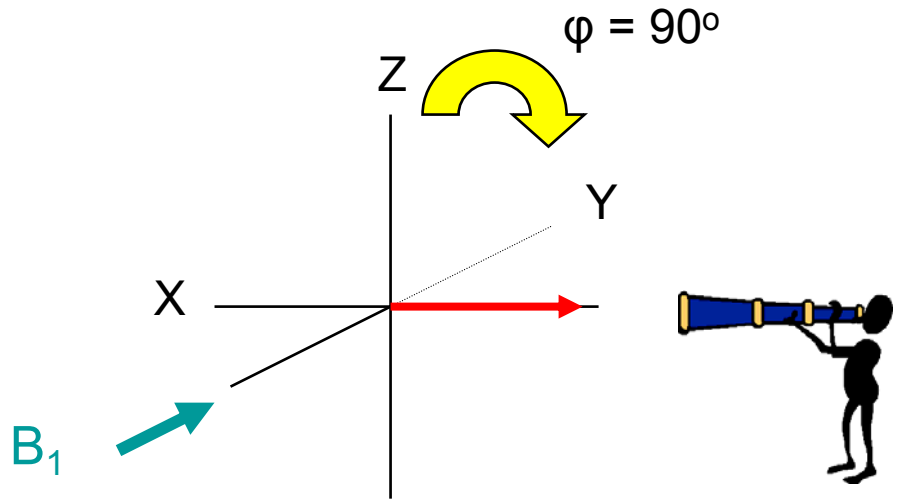


Relaxation

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.

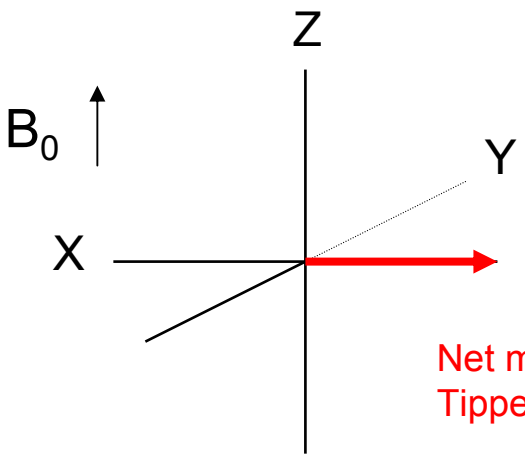




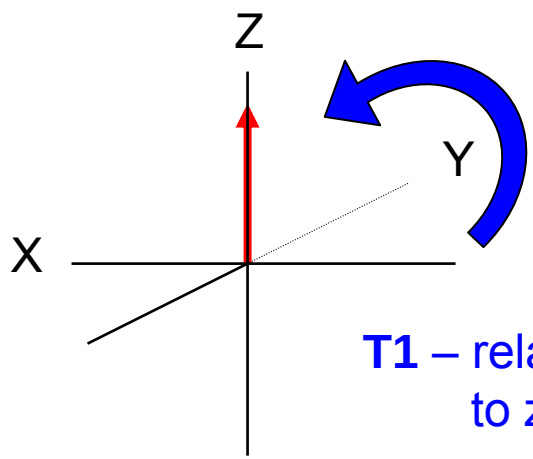
Relaxation

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

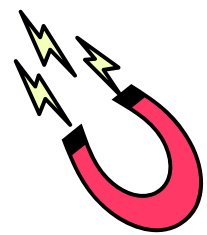


T1 – relaxing back to z axis

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.'



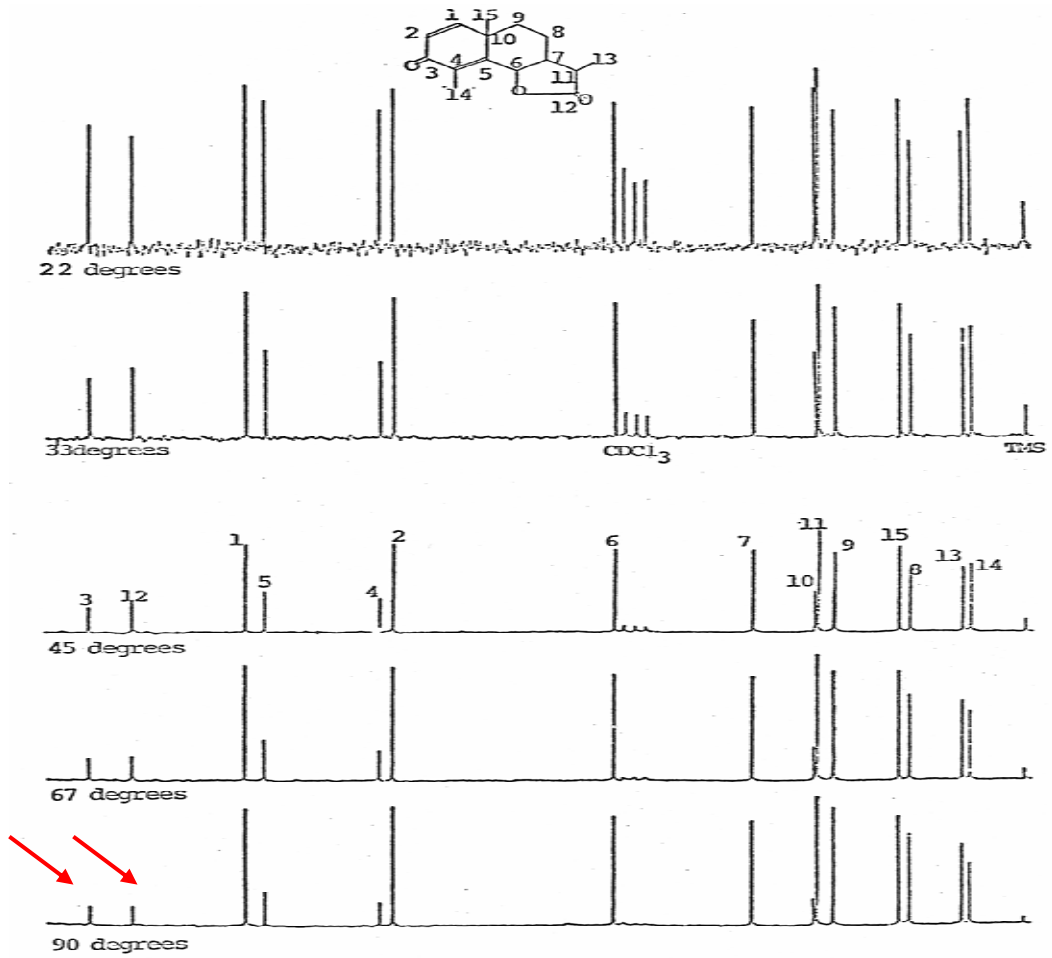
Relaxation

α-santonin

300 scans
2 second delay (τ or *tau*)

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

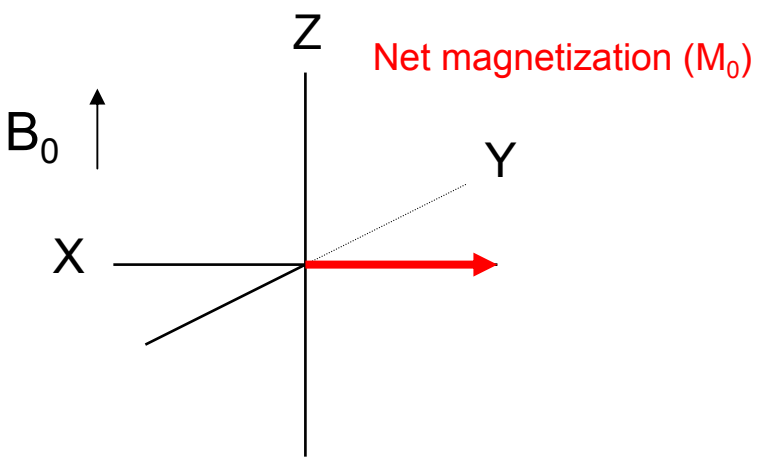
90^0 pulse – delay too short
Losing aromatic peaks

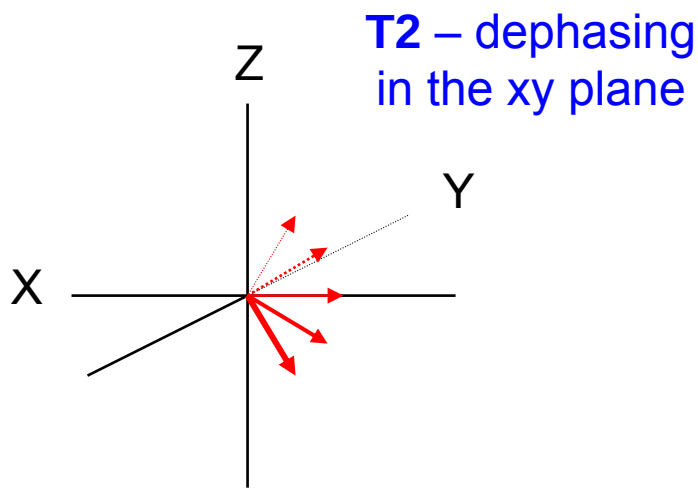


Relaxation

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.





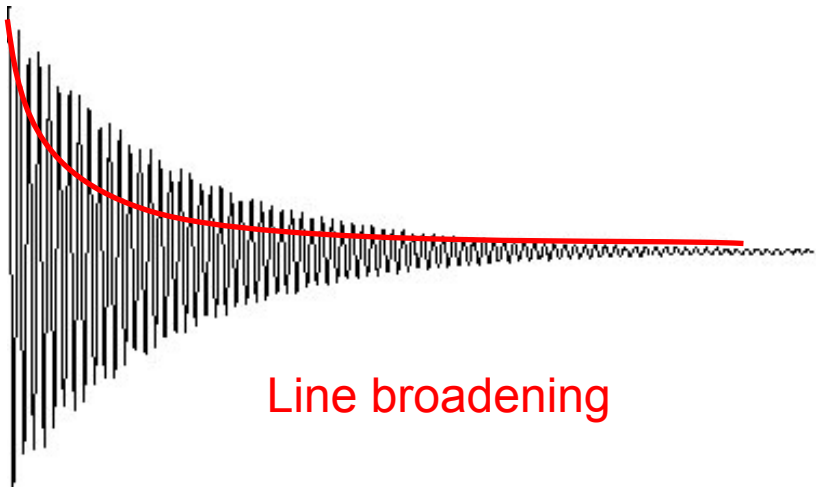
Apodization

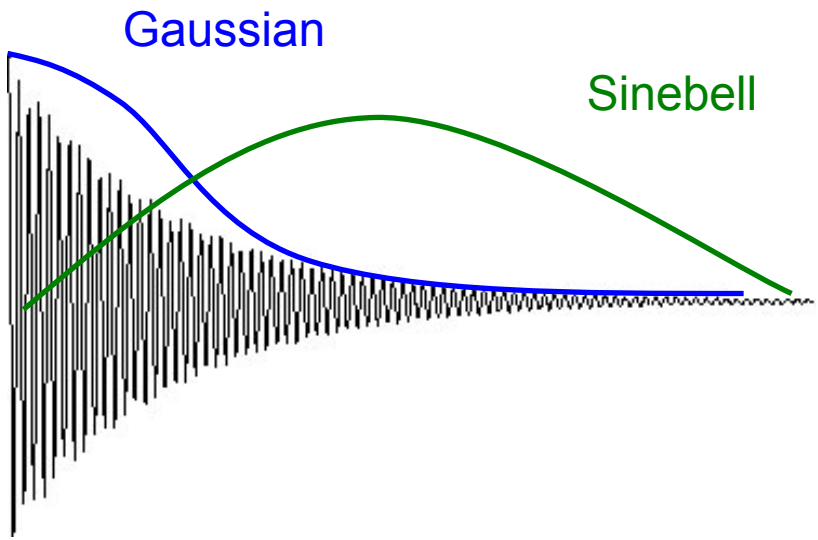
FID manipulation

- **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.

Credits

Special thanks to…..

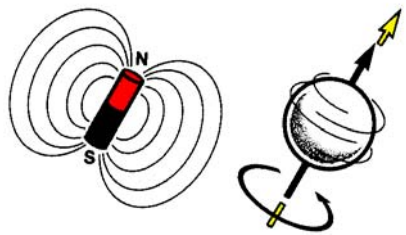
Kathleen Gallagher, University of New Hamsphire.

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



End of Part 2