

CHM 4380 / 8309B

Practical NMR Spectroscopy

Class Notes

Fall 2007

Instructor: Dr. Glenn A. Facey

History of NMR

1924 Pauli postulates that nuclei behave as spinning particles with quantized angular momentum.

1938 First NMR experiment using molecular beam methods.

1945 First NMR experiment on bulk materials conducted (Bloch (Stanford) and Purcell (Harvard)).

1949 Chemical shifts discovered.

1952 First commercial continuous wave NMR spectrometer (Varian).

1952 Bloch and Purcell receive Nobel Prize.

1957 First observation of ^{13}C NMR.

1958 Magic angle spinning introduced.

1962 First superconducting magnet used for NMR.

1965 ^1H decoupling implemented for ^{13}C .

1965 First Fourier transform NMR experiments carried out.

1969 First commercially available Fourier transform NMR spectrometer (Bruker).

1972 First high resolution NMR spectra of polycrystalline solids.

1974 Two dimensional NMR.

1980 First commercial MRI.

1985 n-dimensional triple resonance NMR.

1990 pulsed field gradients used routinely in pulse sequences.

1991 Richard Ernst wins Nobel prize for NMR.

1993 Commercially available cryogenically cooled NMR probes.

2002 Kurt Wuthrich wins Nobel prize for work on NMR of proteins.

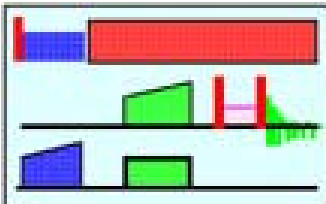
2003 Paul Lauterbur and Peter Mansfield win Nobel prize for work on MRI.

Recommended Reading

- Understanding NMR Spectroscopy***. James Keeler (2005).
Nuclear Magnetic Resonance. P.J. Hore (1995).
Experimental Pulse NMR. E. Fukushima and S.B.W. Roeder (1981).
Magnetic Resonance in Chemistry and Medicine Ray Freeman (2003).
200 and More Basic NMR Experiments. S. Berger and S. Braun (2004).
Multinuclear NMR. Ed. J. Mason (1987).
Nuclear Magnetic Resonance. R.K. Harris (1983).
Solid State NMR for Chemists. C.A. Fyfe (1983).
Modern NMR Techniques and their Applications in Chemistry. Edd. A.I. Popov and K. Hallenga (1991).
High Resolution NMR Techniques in Organic Chemistry. T.D.W. Claridge (1999).
Modern NMR Spectroscopy. J.K.M. Saunders and B.K. Hunter (1989).
Basic One- and Two- Dimensional NMR Spectroscopy. H. Friebolin (1991).
Handbook of High Resolution NMR. C. Brevard, P. Granger (1981).
Dynamic NMR Spectroscopy. J. Sandstrom (1982).

Online Textbook Recommended

Basics of NMR. Joseph P. Hornak <http://www.cis.rit.edu/htbooks/nmr/>



Elements Accessible by NMR

1 IA												18 VIIIA																							
Hydrogen 1 H 1.007 940 87(2) 1.008 105 08(2) 1.008 664 84(3)												Helium 2 He 4.002 603 254(15)																							
2 IIA												13 IIIA		14 IVA		15 VA		16 VIA		17 VIIA															
Lithium 3 Li 6.941 15(8) 7.016 004 55(8)		Beryllium 4 Be 9.012 116 87(9)												Boron 5 B 10.811 187(10) 11.009 305 5(10)		Carbon 6 C 12.010 738(10)		Nitrogen 7 N 14.006 438(4) 15.004 839 6(4)		Oxygen 8 O 15.999 431 6(3)		Fluorine 9 F 18.998 403 163(5)		Neon 10 Ne 19.992 479 868(5)											
Sodium 11 Na 22.989 769 28(2)		Magnesium 12 Mg 24.304 67(8)												Aluminum 13 Al 26.981 538 6(8)		Silicon 14 Si 28.085 579 9(9)		Phosphorus 15 P 30.973 761 99(8)		Sulfur 16 S 32.06 5(8)		Chlorine 17 Cl 35.45 3(8)													
3		4		5		6		7		8		9		10		11		12		13															
Potassium 19 K 39.098 309 1(4) 40.078 040 3(4)		Calcium 20 Ca 40.078 040 3(4)		Scandium 21 Sc 44.955 908 6(4)		Titanium 22 Ti 47.88 7(4) 48.930 029 0(4)		Vanadium 23 V 50.941 861 5(4) 51.940 509 1(4)		Chromium 24 Cr 51.996 163 5(4) 52.940 631 3(4)		Manganese 25 Mn 54.938 044 3(4)		Iron 26 Fe 55.845 859 5(4) 56.935 349 1(4)		Cobalt 27 Co 58.933 195 0(4) 59.933 041 3(4)		Nickel 28 Ni 58.693 4(4) 59.920 138 9(4)		Copper 29 Cu 63.546 800 5(4) 64.927 807 0(4)		Zinc 30 Zn 65.38 6(4) 66.926 969 9(4)		Gallium 31 Ga 69.723 17(4) 70.620 395 9(4)		Germanium 32 Ge 72.630 08(4) 73.921 149 0(4)		Arsenic 33 As 74.921 60(4) 75.923 175 0(4)		Selenium 34 Se 78.96 6(4) 79.916 364 0(4)		Bromine 35 Br 79.904 1(4) 80.911 94(4)		Krypton 36 Kr 83.6 3(4)	
Rubidium 37 Rb 85.467 8(4) 87.62 8(4)		Strontium 38 Sr 87.62 8(4)		Yttrium 39 Y 88.905 84(4)		Zirconium 40 Zr 91.224 4(4) 92.906 38(4)		Niobium 41 Nb 92.906 38(4)		Molybdenum 42 Mo 95.94 6(4) 97.905 44(4)		Technetium 43 Tc 98.906 250(4)		Ruthenium 44 Ru 98.906 250(4) 101.07 4(4)		Rhodium 45 Rh 101.07 4(4)		Palladium 46 Pd 106.363 5(4) 107.868 2(4)		Silver 47 Ag 107.868 2(4) 108.906 250(4)		Cadmium 48 Cd 112.411 8(4) 114.916 838 9(4)		Indium 49 In 114.916 838 9(4) 115.904 74(4)		Tin 50 Sn 118.710 7(4) 119.903 891 9(4)		Antimony 51 Sb 121.757 1(4) 123.742 088 9(4)		Tellurium 52 Te 127.603 3(4) 128.905 45(4)		Iodine 53 I 126.905 45(4) 127.929 111 9(4)		Xenon 54 Xe 131.29 4(4) 132.905 451 9(4)	
Cesium 55 Cs 132.905 451 9(4)		Barium 56 Ba 137.327 44(4)		Lanthanum 57 La 138.904 87(4) Ytterbium 70 Yb		Hafnium 72 Hf 178.49 6(4) 179.934 071 9(4)		Tantalum 73 Ta 180.947 88(4)		Tungsten 74 W 183.84 6(4) 186.207 1(4)		Rhenium 75 Re 186.207 1(4) 187.209 47(4)		Osmium 76 Os 190.23 6(4) 191.224 1(4)		Iridium 77 Ir 192.22 6(4) 193.224 1(4)		Platinum 78 Pt 195.084 9(4) 197.043 91(4)		Gold 79 Au 196.966 569 5(4) 200.938 271(4)		Mercury 80 Hg 200.59 6(4) 201.965 643 9(4)		Thallium 81 Tl 204.38 6(4) 208.980 401 9(4)		Lead 82 Pb 207.2 6(4) 208.980 401 9(4)		Bismuth 83 Bi 208.980 401 9(4)							
Lanthanum 57 La 138.904 87(4)		Thulium 69 Tm 168.930 4(4)		Ytterbium 70 Yb 173.054 688 9(4)																															

element: Hydrogen 1
 symbol: **H**
 isotope: 1.007 940 87(2), 1.008 105 08(2), 1.008 664 84(3)
 atomic weight: 1.007 940 87(2)
 spin number: 1/2
 frequency (MHz): 14.337 768 MHz

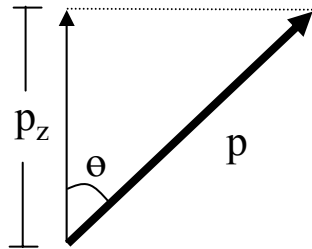
atomic number: 1
 frequency (MHz): 14.337 768 MHz

legend:
 white box: I=1/2 nuclei
 pink box: I=1/2 nuclei
 yellow box: I=1/2 and I=3/2 nuclei

From quantum mechanics we know that angular momentum, p , is quantized with quantum number I .

$$\mathbf{p} = \hbar (I (I + 1))^{1/2} \quad I = 0, 1/2, 1, 3/2, 2, 5/2 \dots$$

Angular momentum is a vector property, the direction of which is described by the quantum number, m , where,



$$\mathbf{P}_z = \hbar m \quad m = -I, (-I + 1), (-I + 2), \dots I$$

In classical terms, a nucleus can be thought of as a spinning charged particle. Any spinning charged particle generates a magnetic moment, μ , proportional to the angular momentum and since angular momentum is quantized with quantum number I so then must the magnetic moment be quantized.

$$\vec{\mu} = \gamma \vec{p} = \hbar (I (I + 1))^{1/2}$$

where γ is the gyromagnetic ratio for the nucleus being considered. It is an isotope dependant nuclear property. It can take on both positive and negative values.

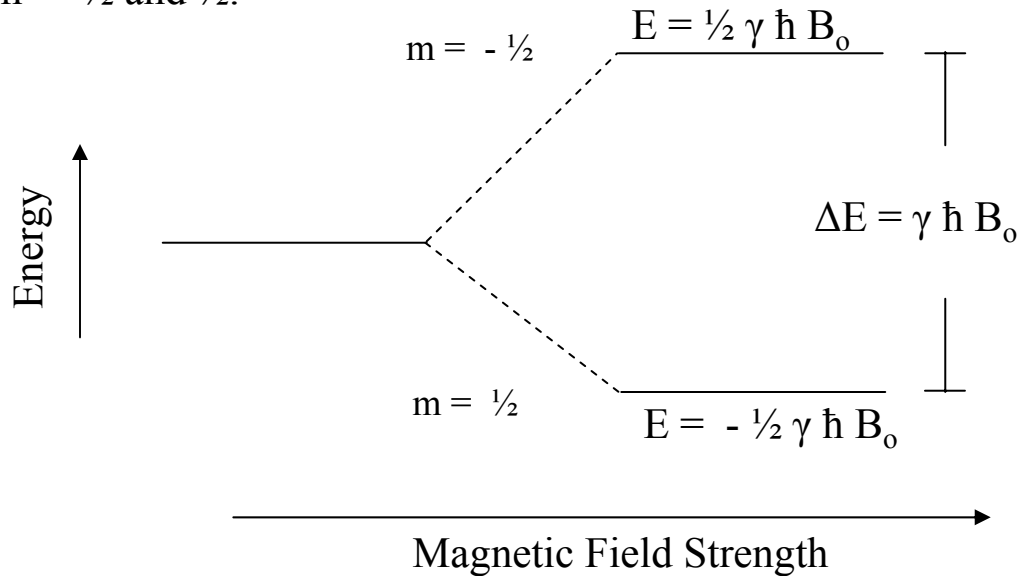
Nuclei with spin quantum number $I = \frac{1}{2}$ can be thought of as small bar magnets. If an external magnetic field, B_0 , is applied, the Energy, E , of the nucleus is given by:

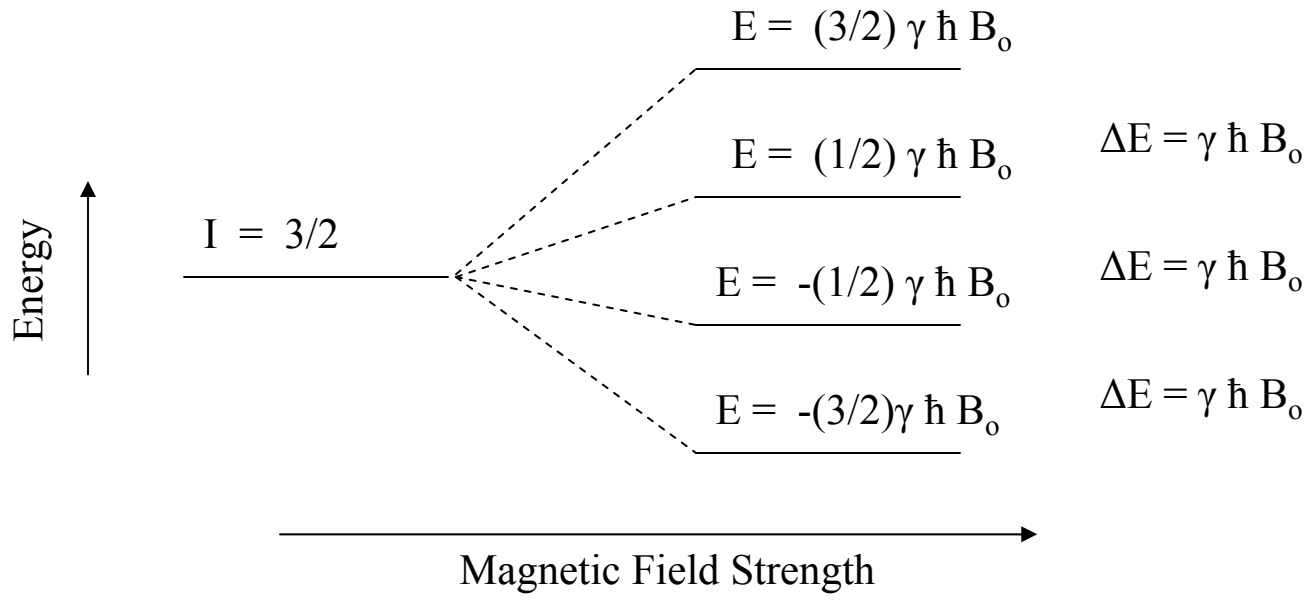
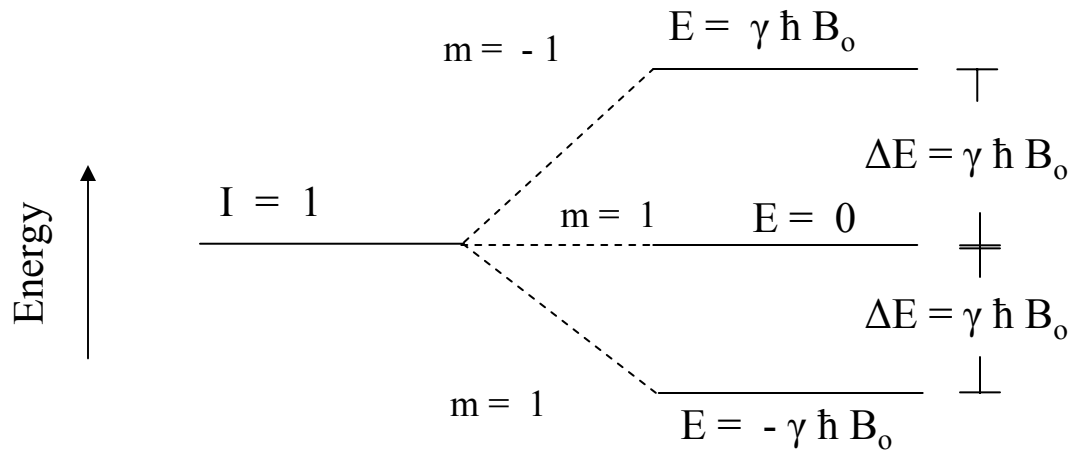
$$\mathbf{E} = -\vec{\mu} \cdot \mathbf{B}_0$$

If the direction of the applied external magnetic field is taken as the z direction (the axis of quantization) then,

$$\mathbf{E} = -\mu_z B_0 = -\gamma \hbar m B_0$$

For $I = \frac{1}{2}$, $m = -\frac{1}{2}$ and $\frac{1}{2}$.





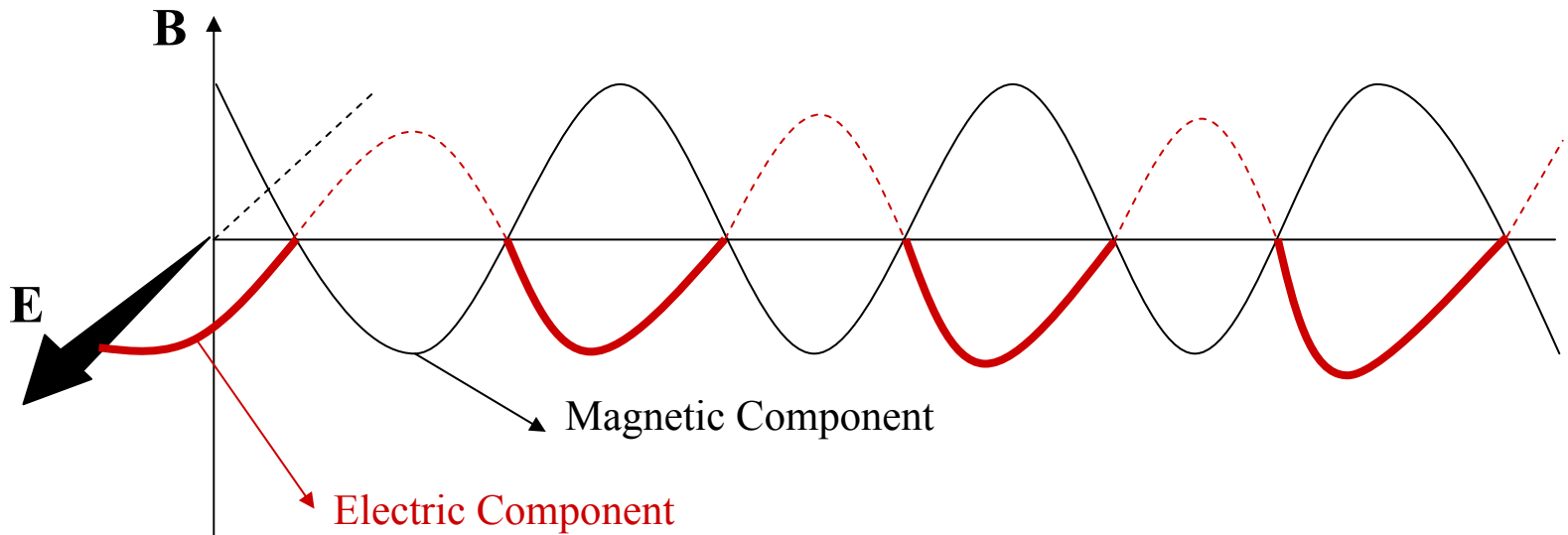
Since Energy and frequency are related by $\Delta E = h \nu$, we can express the energy as a frequency as follows:

$$\nu = (\gamma/2\pi) B_0 \quad \text{Larmor Equation}$$

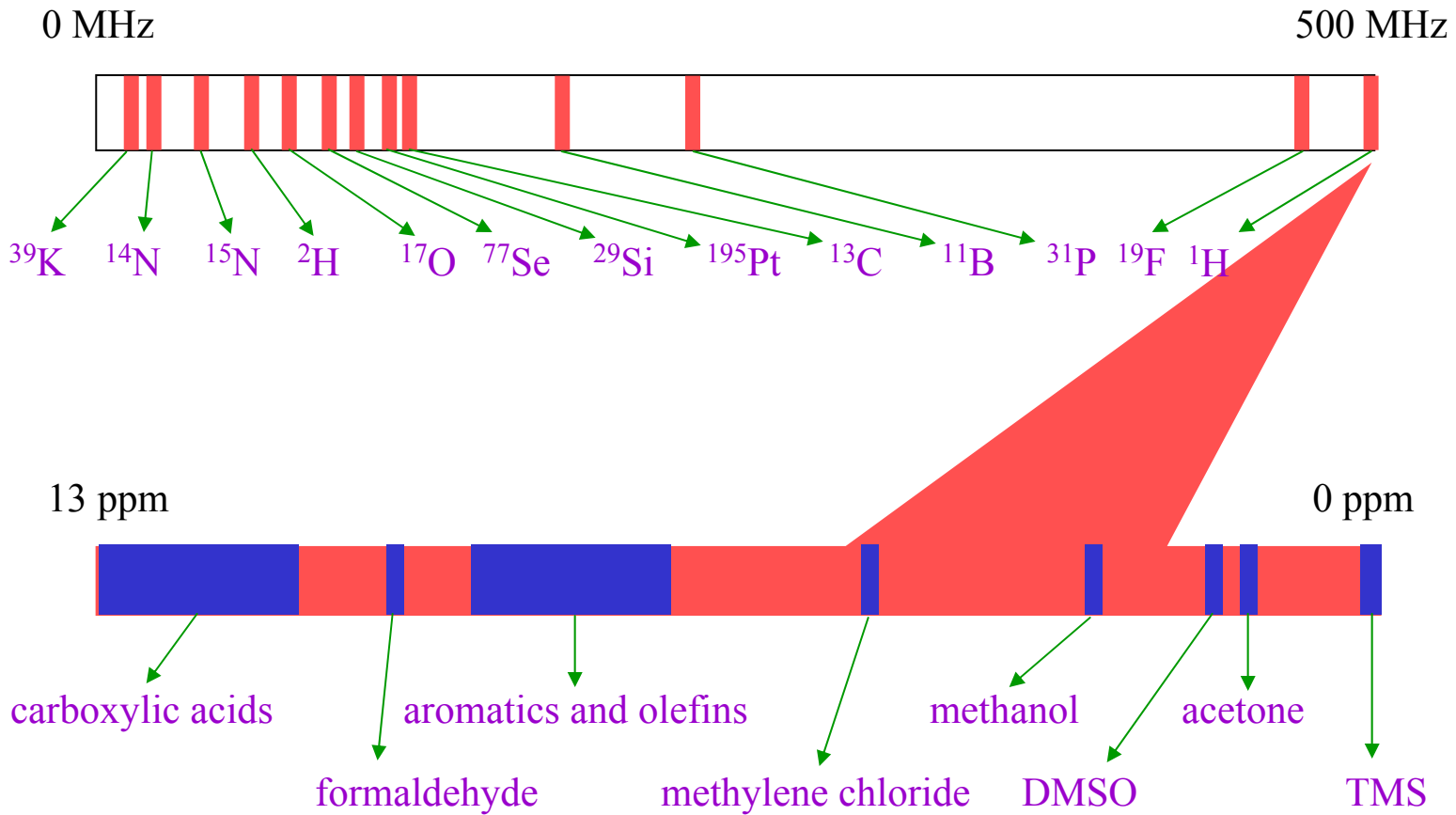
For readily available magnets, ν is in the radio frequency region of the electromagnetic spectrum. The Larmor frequency of a nucleus depends not only on the gyromagnetic ratio, γ , but also on the strength of the external applied magnetic field, B_0 .

In order to go from one energy state to another, electromagnetic radiation must be applied with an energy equal to the energy difference between the states. This is typically radio energy. Expressed differently, the frequency of the oscillating magnetic component of the electromagnetic wave must be equal to the Larmor frequency of the nucleus.

There is a quantum mechanical selection rule which states that $\Delta m = \pm 1$.



Resonance Frequencies of Various nuclei in an 11.7 Tesla Magnet



Excitation

One can satisfy the resonance condition (i.e. $\nu = (\gamma/2\pi) B_0$) in any one of 3 ways:

- o **A constant radio frequency can be applied to a sample while the magnetic field is continuously varied.**
- o **The radio frequency can be continuously varied while the sample is maintained in a constant magnetic field.**
- o **All relevant radio frequencies can be applied simultaneously to the sample while the sample is maintained in a constant magnetic field.**

The first of these methods is called “continuous wave”. It dominated NMR experimentation methods before the advent of Fourier transform methods.

The second method has also been used and is still used for certain specialized techniques.

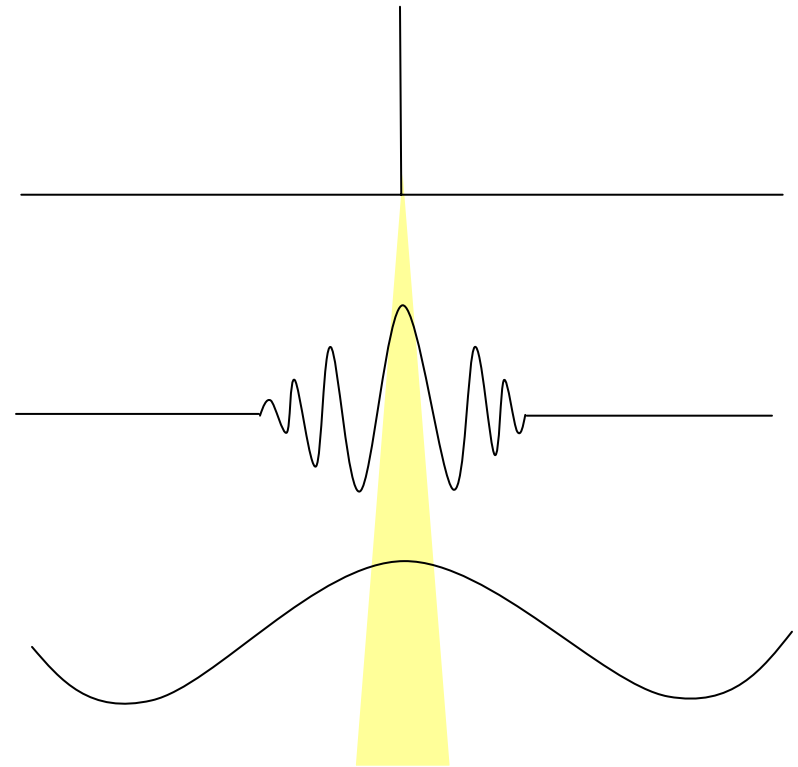
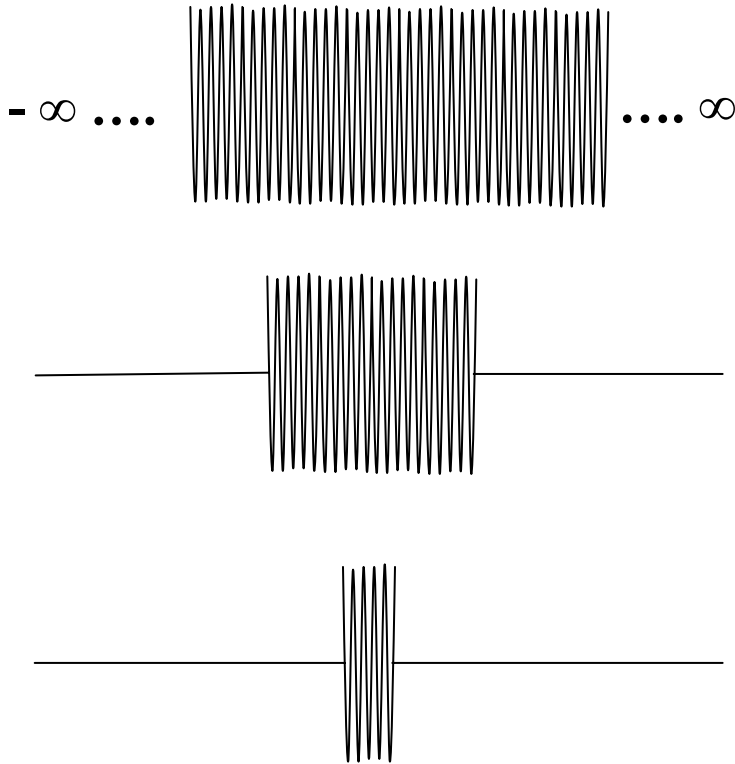
The third method is the basis for Fourier transform NMR and is used almost exclusively today.

In Fourier transform (FT) NMR spectrometers, a short (i.e. 1 – 100 μsec) pulse of radio frequency (rf) at the Larmor frequency, ν_0 , is applied to the sample. The excitation profile of the pulse is determined by Fourier analysis. Very long pulses (i.e. msec – sec) excite very narrow frequency ranges while very short pulses (μsec) excite very wide frequency ranges.

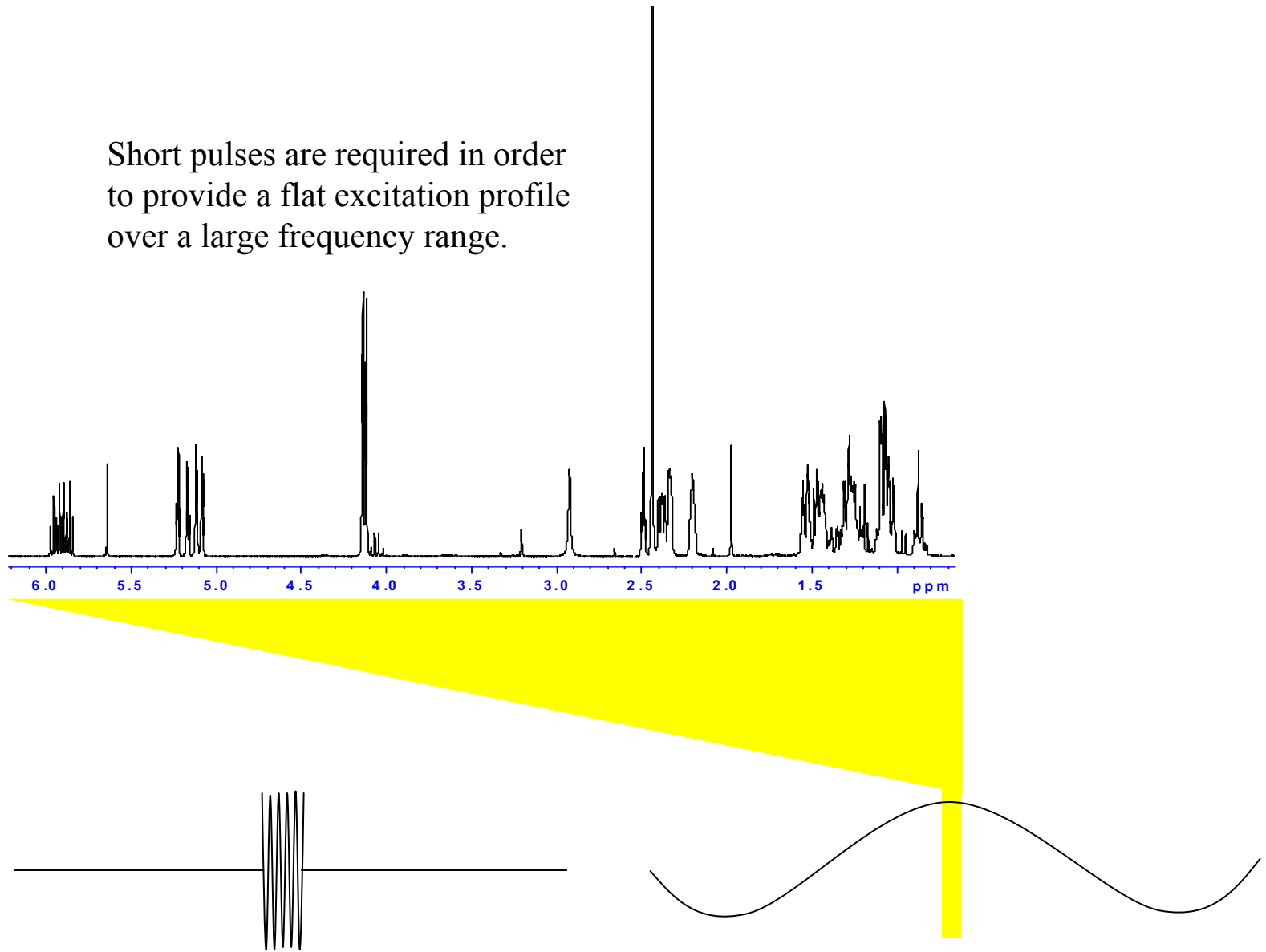
Excitation Profiles

Time

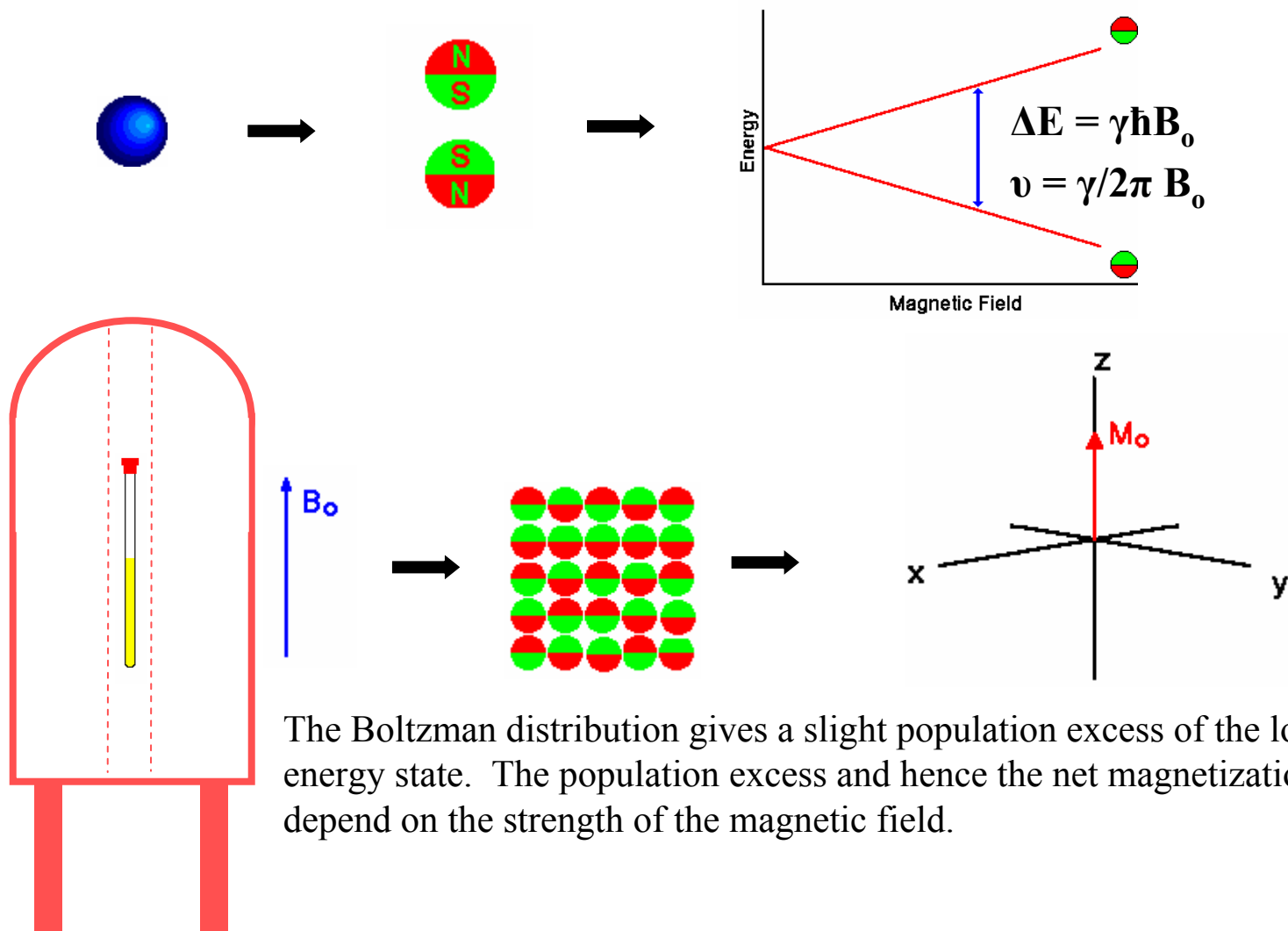
Frequency



Short pulses are required in order to provide a flat excitation profile over a large frequency range.

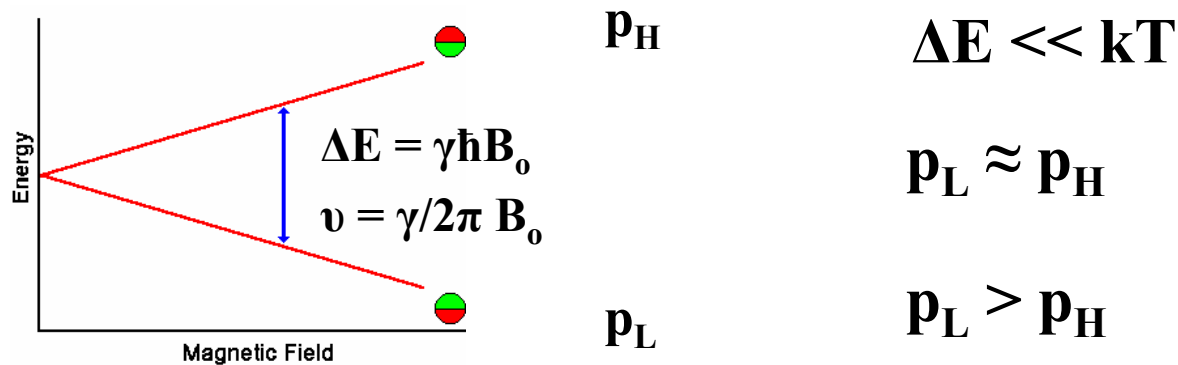


Spin $I = \frac{1}{2}$ Nuclei in a Magnetic Field



The Boltzman distribution gives a slight population excess of the lower energy state. The population excess and hence the net magnetization depend on the strength of the magnetic field.

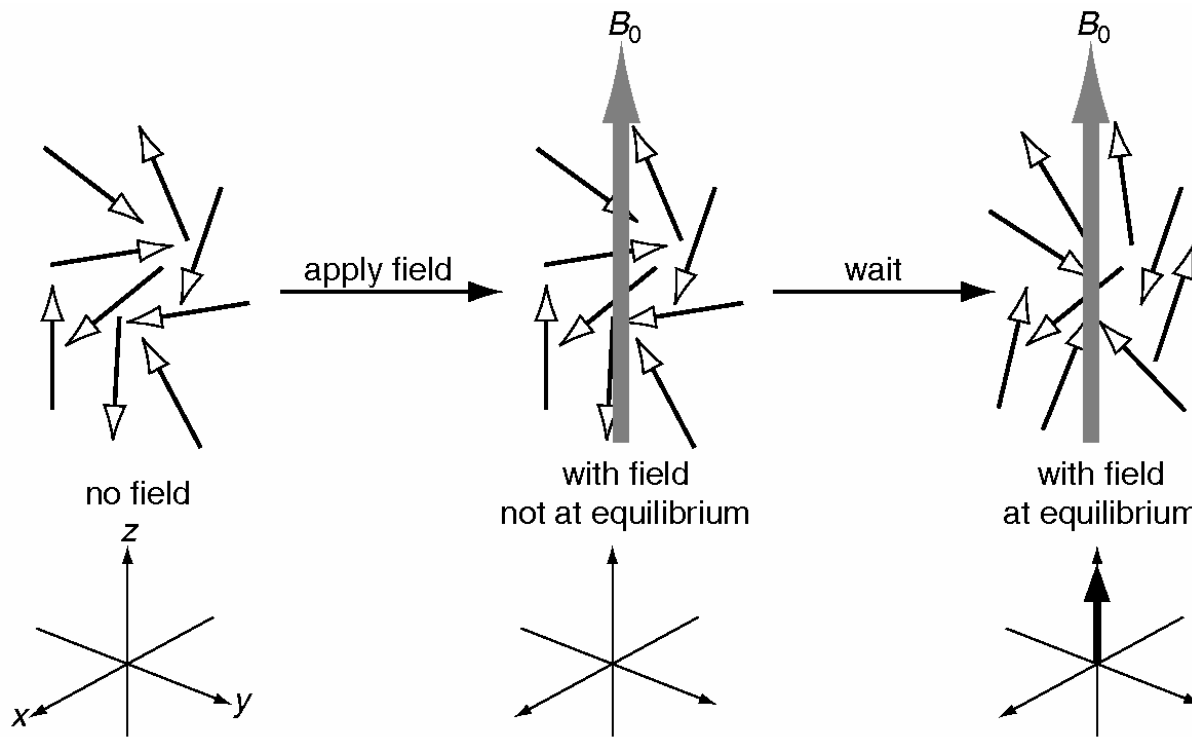
In a macroscopic sample containing spin $I = \frac{1}{2}$ nuclei, the Boltzmann distribution governs the population ratio between the low and high energy states. The energy difference between the states is much less than the available thermal energy so the ratio of populations is not much different than unity.



$$\frac{p_H}{p_L} = e \left(\frac{-(E_H - E_L)}{kT} \right) = e \left(\frac{-\gamma \hbar B_0}{kT} \right)$$

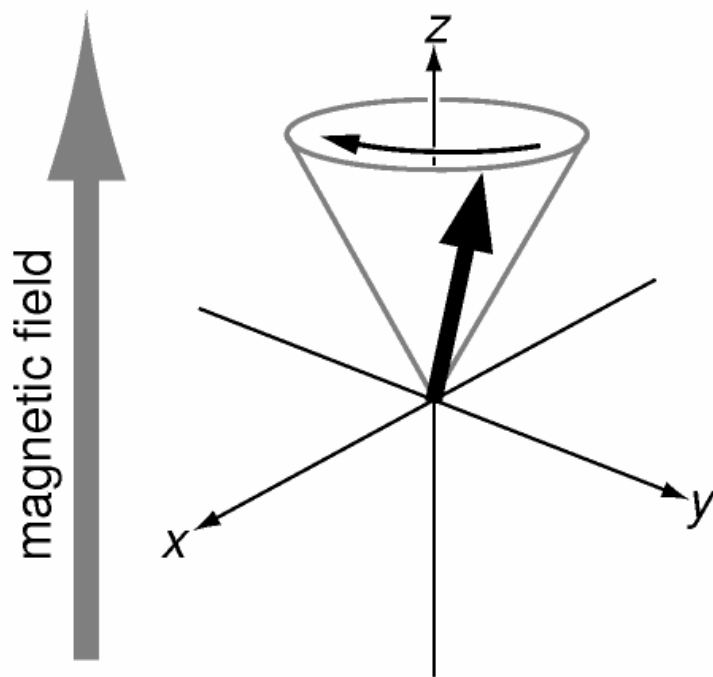
The sensitivity of an NMR measurement depends on the population ratio between the high and low states. Sensitivity therefore increases with increasing B_0 , γ and decreasing temperature.

There is no net magnetization in the absence of a magnetic field. The net bulk magnetization builds up when the sample is placed in the magnet.



Larmor Precession and the Vector Model

The equilibrium magnetization vector remains constant as a function of time. If, however for some reason, it gets diverted from the z axis, it rotates on the surface of a cone at the Larmor frequency ν_0 .



$$\nu_0 = \gamma/2\pi B_0 \quad \text{in Hz}$$

or

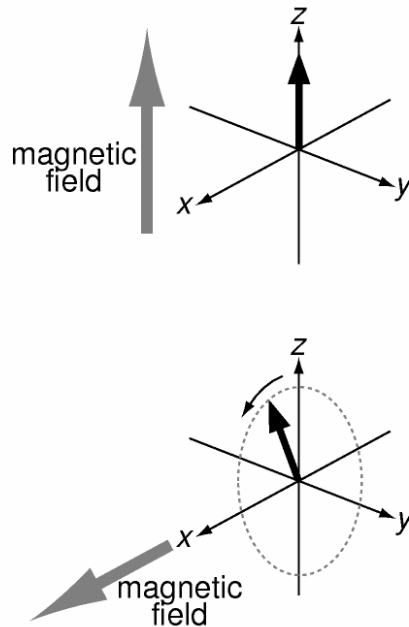
$$\omega_0 = \gamma B_0 \quad \text{in rad/sec}$$

The NMR spectrometer measures the time dependant magnetization in the x-y plane. This signal is processed to give the NMR spectrum.

Creating Transverse Magnetization

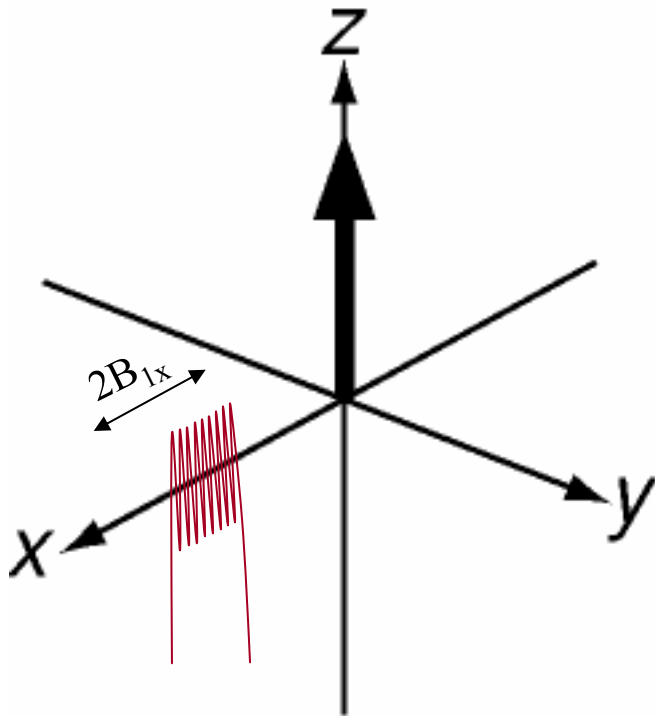
Since the NMR spectrometer measures transverse (i.e. x and y) magnetization we must have a way of creating it.

One way to do this would be to turn off the main magnetic field B_0 and quickly replace it with a magnetic field along one of the transverse axes.



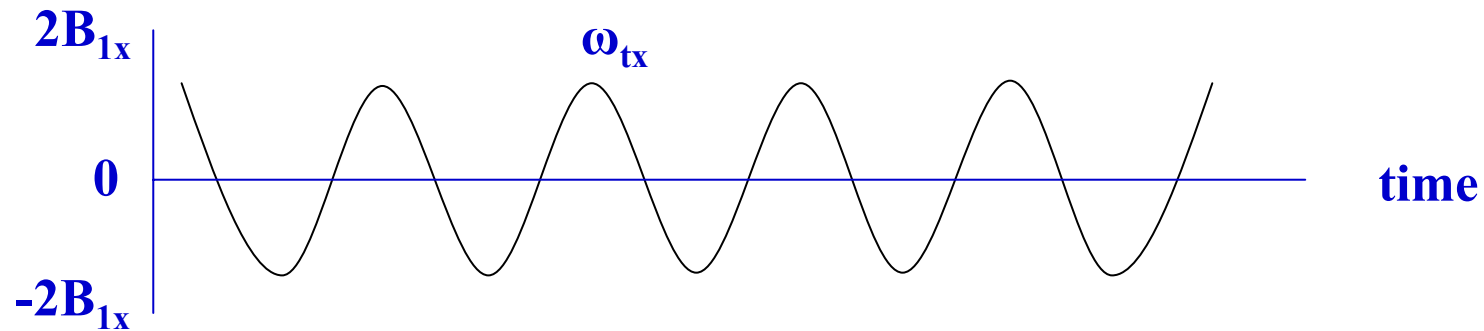
The equilibrium magnetization vector would then rotate according to the right hand screw rule to produce transverse magnetization which could be detected by the spectrometer.

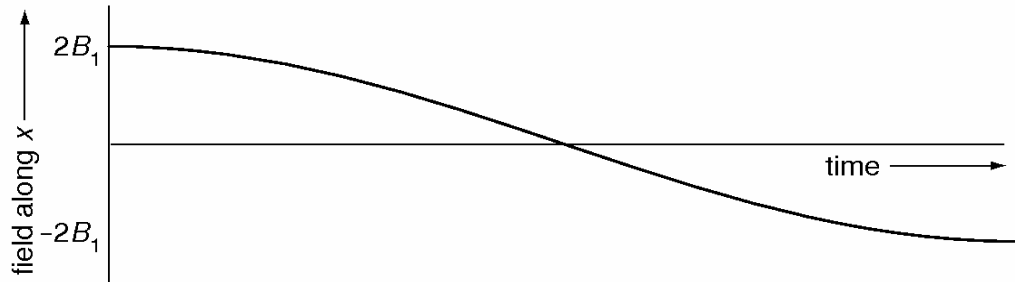
This is not practical as it is impossible to turn B_0 off and quickly replace it with another magnetic field.



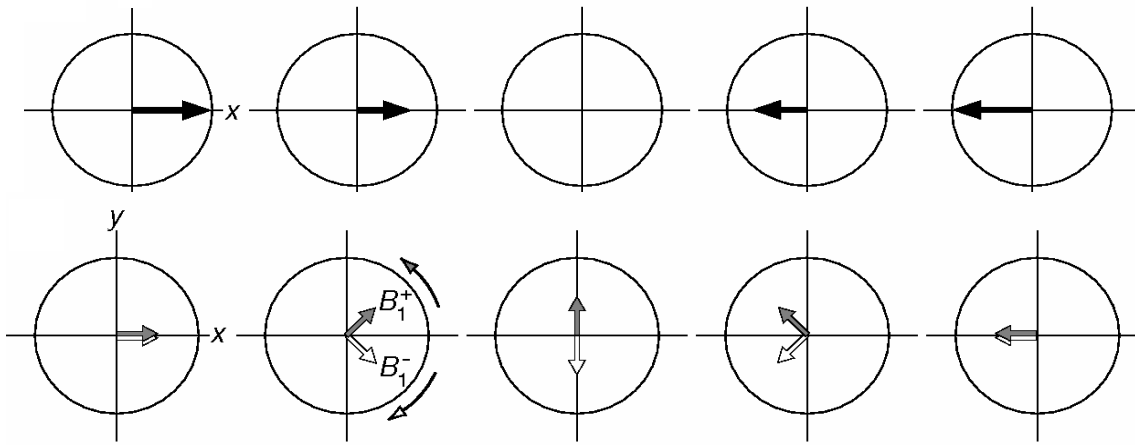
If we pass an alternating current at frequency ω_{tx} through a coil wound around the x axis, then we will generate a magnetic field, $2B_1$, about the x axis that oscillates at frequency ω_{tx} .

$$2B_{1x}(t) = 2B_{1x} \cos(\omega_{tx}t)$$





The oscillating field along the x axis can be decomposed into two fields rotating at ω_{tx} in opposite directions.

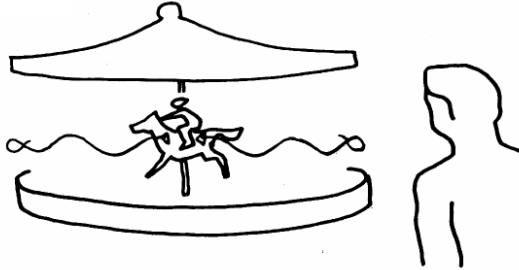


$$2B_{1x}(t) = 2B_{1x} \cos(\omega_{tx}t)$$

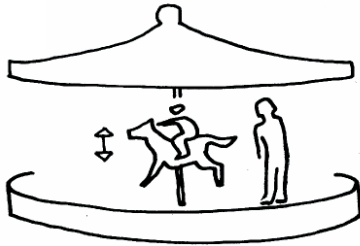
$$= B_{1x}(\cos(\omega_{tx}t) e_x + \sin(\omega_{tx}t) e_y) + B_{1x}(\cos(\omega_{tx}t) e_x - \sin(\omega_{tx}t) e_y)$$

$$= \mathbf{A} + \mathbf{B}$$

The Rotating Frame of Reference



Laboratory Frame



Rotating Frame

If we now assume that the x-y plane is rotating at ω_{tx} , then we have a stationary field of B_{1x} in the rotating frame and a field rotating at $2\omega_{tx}$

$$\begin{aligned} 2B_{1x}(t) &= \mathbf{B}_{1x} \mathbf{e}_x + \mathbf{B}_{1x}(\cos(2\omega_{tx}t) \mathbf{e}_x - \sin(2\omega_{tx}t) \mathbf{e}_y) \\ &= \mathbf{A} + \mathbf{B} \end{aligned}$$

In the Laboratory frame we know that:

$$\omega_0 = \gamma \mathbf{B}_0$$

In the rotating frame, the apparent precession frequency, Ω , is given by :

$$\Omega = (\omega_0 - \omega_{tx})$$

and the static magnetic field is reduced. The reduced magnetic field, \mathbf{B}_{red} , given by:

$$\mathbf{B}_{red} = \Omega/\gamma$$

If we choose the transmitter frequency, ω_{tx} to be equal to the Larmor frequency, ω_0 , such that Ω is zero then the static field experienced by the nuclei becomes zero. We can neglect the “B” term in the expression for the $2\mathbf{B}_{1x}(t)$ since $2\omega_{tx} \gg \omega_0$:

$$\begin{aligned} 2\mathbf{B}_{1x}(t) &= \mathbf{B}_{1x} \mathbf{e}_x + \cancel{\mathbf{B}_{1x}(\cos(2\omega_{tx}t) \mathbf{e}_x - \sin(2\omega_{tx}t) \mathbf{e}_y)} \\ &= \mathbf{A} + \cancel{\mathbf{B}} \\ &= \mathbf{B}_{1x} \end{aligned}$$

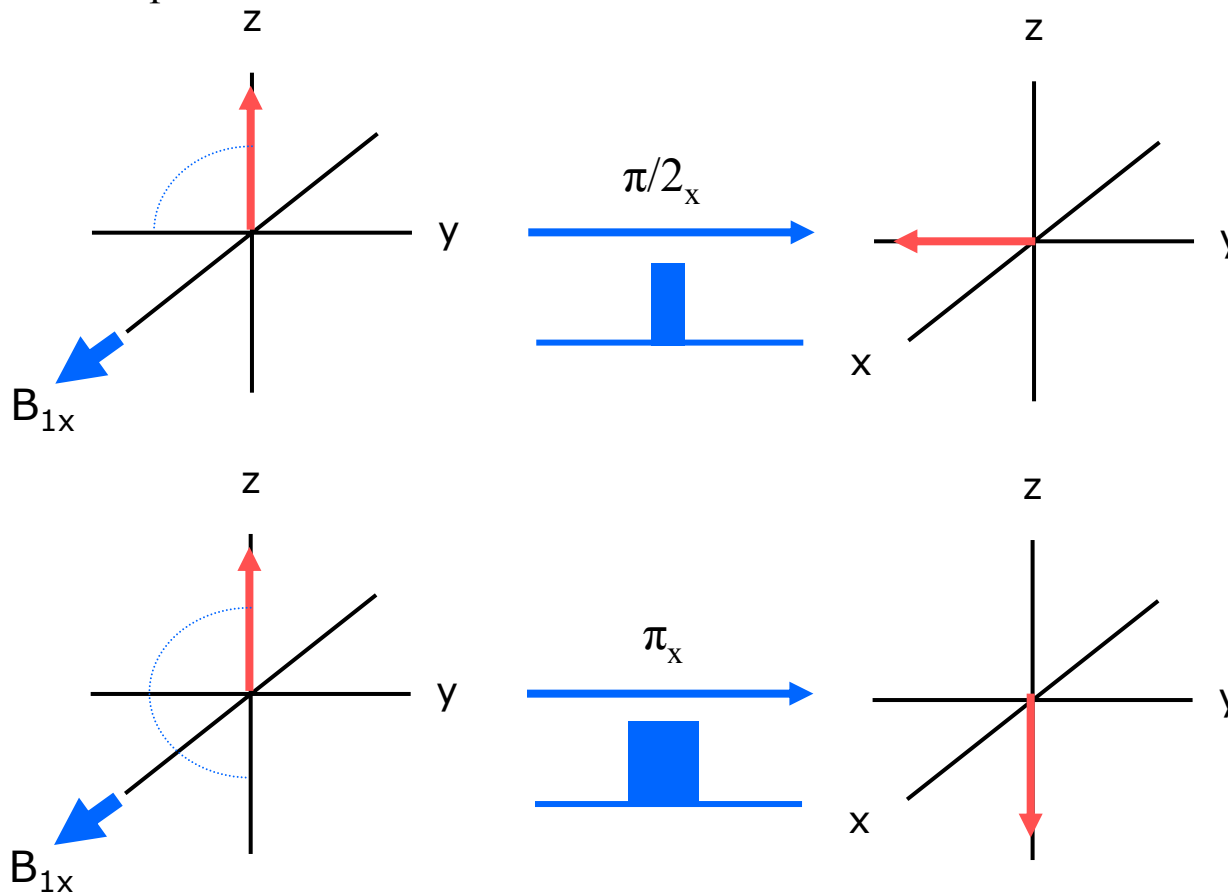
We have effectively turned \mathbf{B}_0 off and the nuclei therefore experience only the \mathbf{B}_{1x} magnetic field from the transmitter. **Transverse magnetization has been created.**

Effect of Radio Frequency Pulses

Pulses of radio waves at the Larmor frequency can be applied at any desired phase. During the pulse, the net magnetization vector rotates about the axis of the pulse phase. The rotation angle, α , is given by,

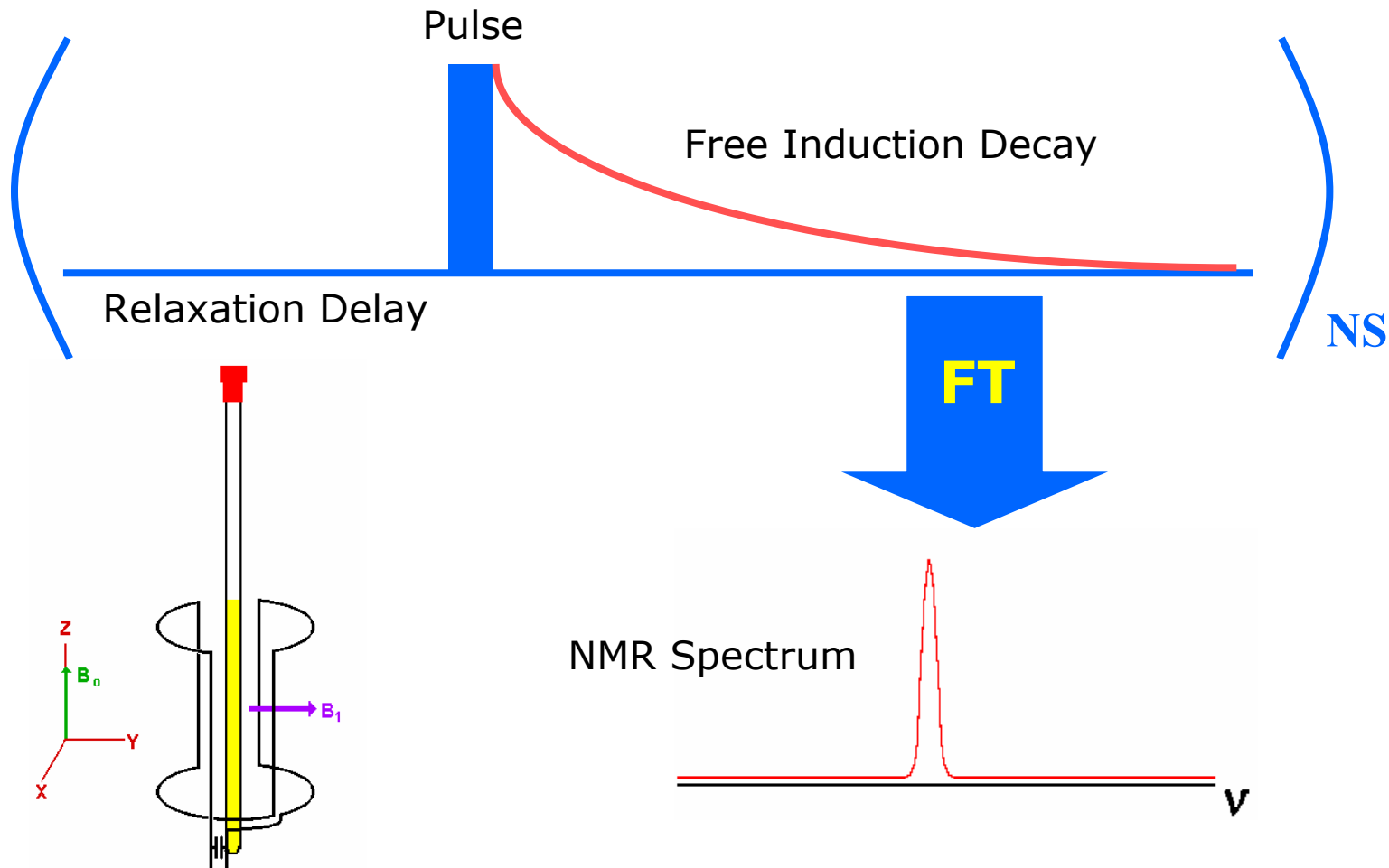
$$\alpha = \gamma B_1 t_p$$

Where γ is the gyromagnetic ratio for the nucleus, B_1 is the amplitude of the pulse and t_p is the duration of the pulse.

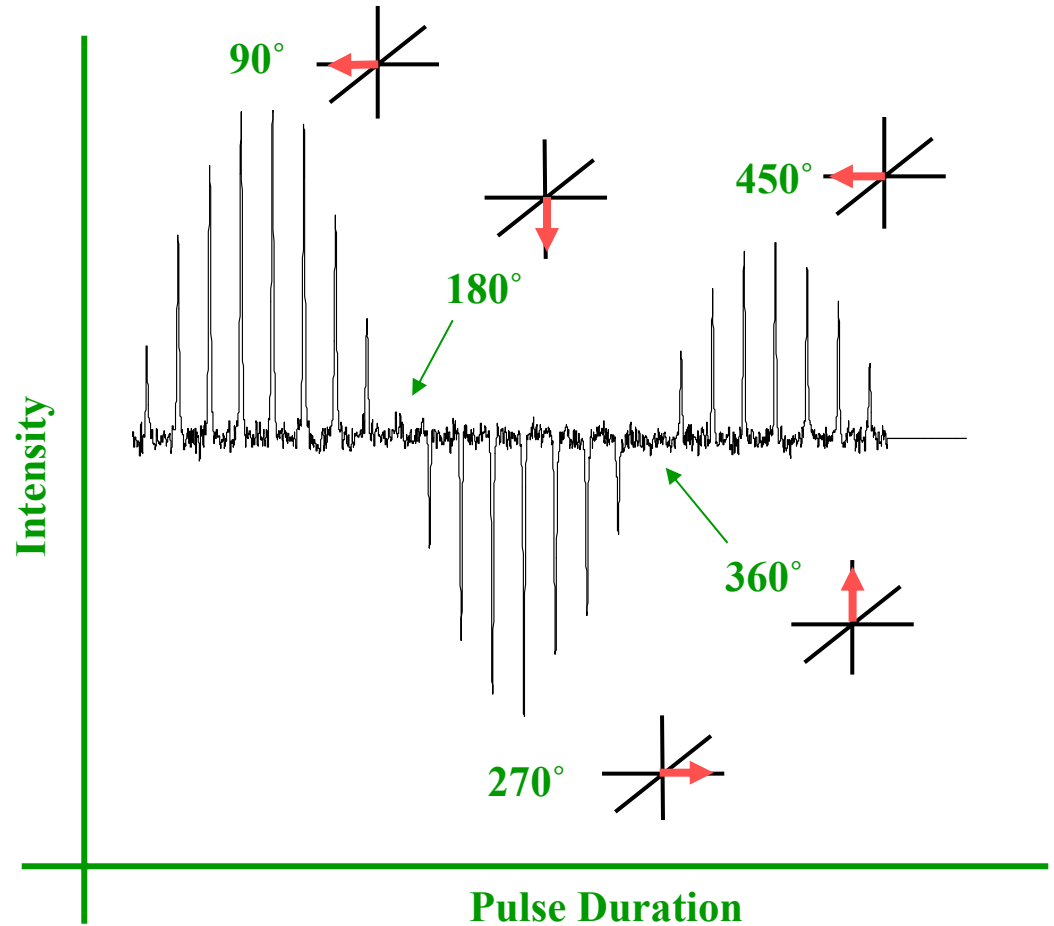
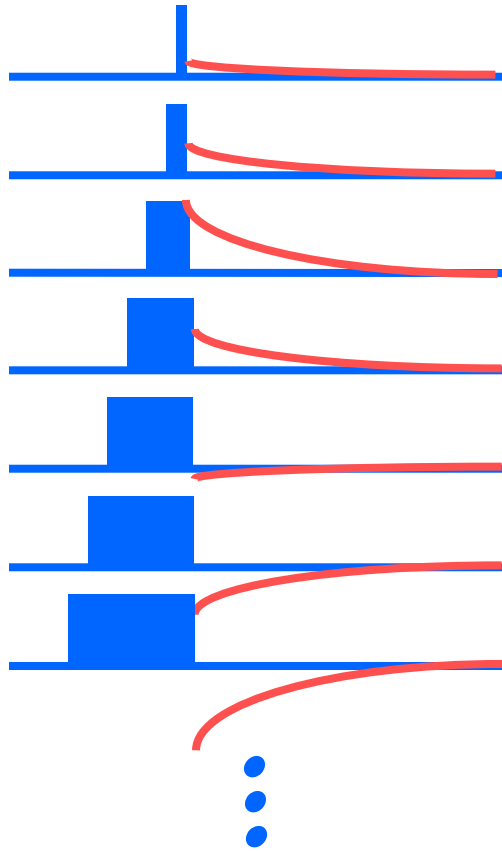


Delivery of Pulses and Detection of The NMR Signal

The change in magnetization in the xy plane after the pulse induces a time dependant voltage in a coil around the sample. This signal is called the Free Induction Decay (FID). The Fourier transform of this signal is the NMR spectrum.



Pulse Calibration

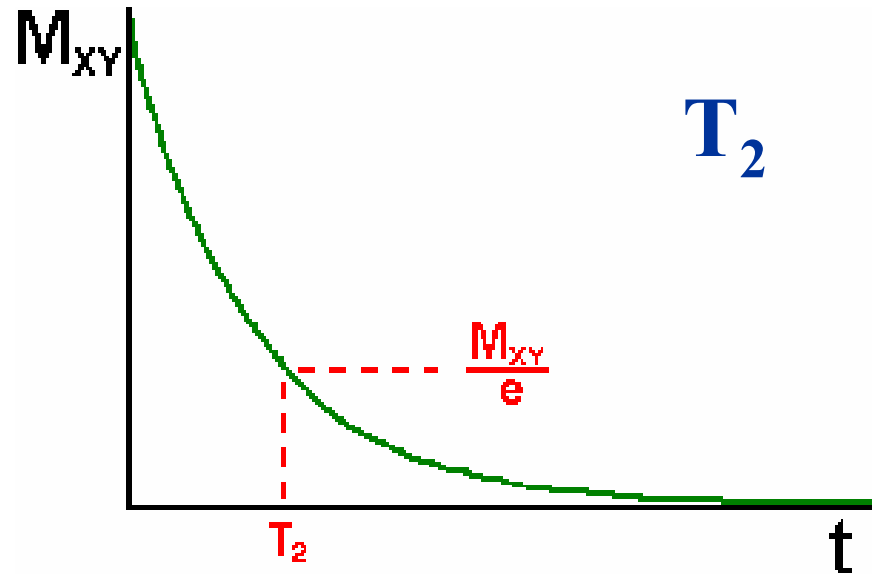


For NMR pulse sequences, it is very important to know what the 90 degree pulse duration must be set to at a particular power level.

Relaxation

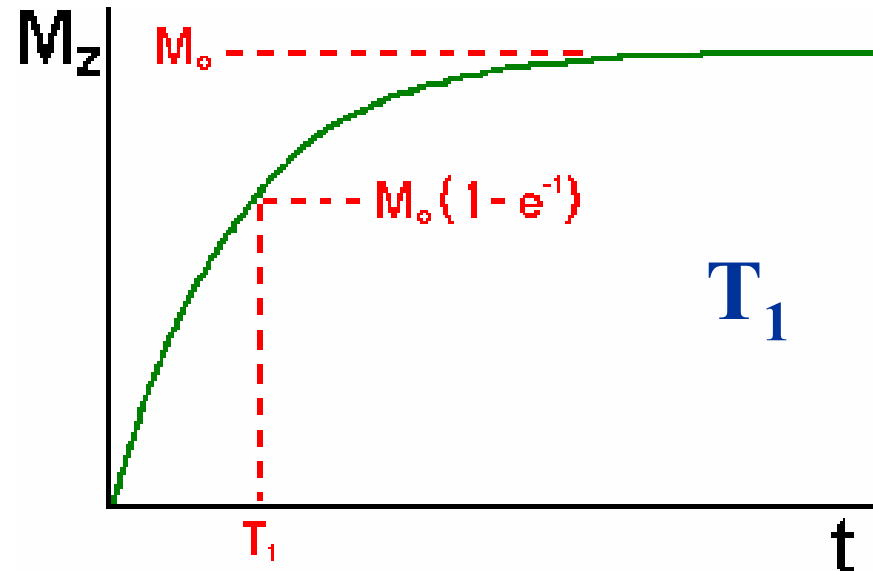
T_2 relaxation is the decay of transverse magnetization

Line Width

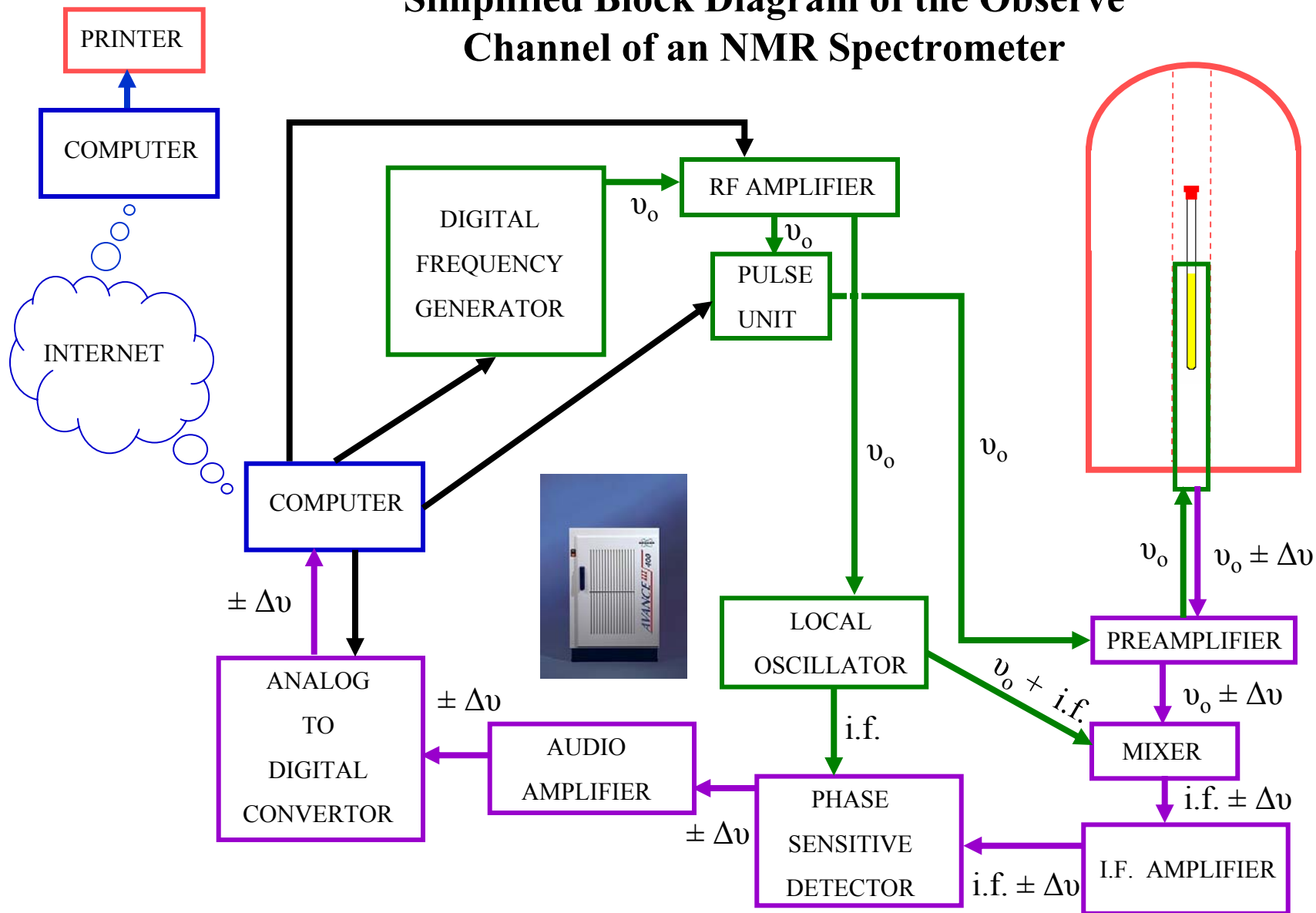


T_1 relaxation is the growth of magnetization along the z axis

Repetition Time



Simplified Block Diagram of the Observe Channel of an NMR Spectrometer



ν_0 is the frequency of the rf in the pulse. It is also called the **carrier frequency**. It is the frequency at which the rotating frame of reference rotates. The rf pulse is applied to the sample by way of a coil. The pulse provides a distribution of frequencies around ν_0 according to its excitation profile. The precession of the magnetic moments of the nuclei in the sample induces a voltage in the same coil used to transmit the rf to the sample. This rf voltage is represented as $\nu_0 \pm \Delta\nu$ where $\pm \Delta\nu$ is the distribution of frequencies in the NMR spectrum.

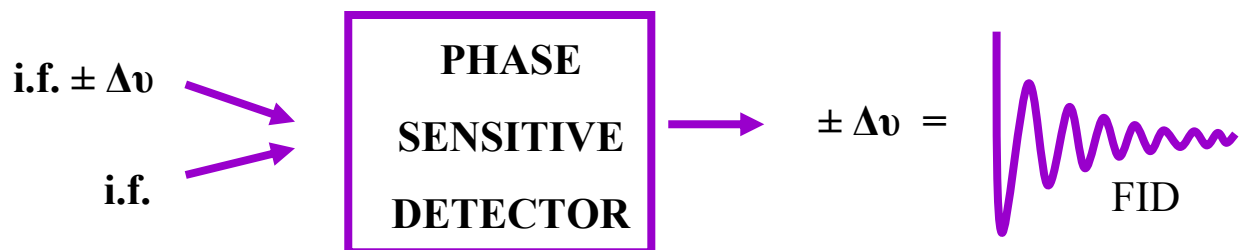


The raw NMR signal ($\nu_0 \pm \Delta\nu$) is mixed with a modulation signal composed of the carrier frequency and an intermediate frequency, i.f. The result is the intermediate frequency modulated by the frequency information of the NMR spectrum. The signal is thus mixed down to a convenient frequency to be handled by the spectrometer.

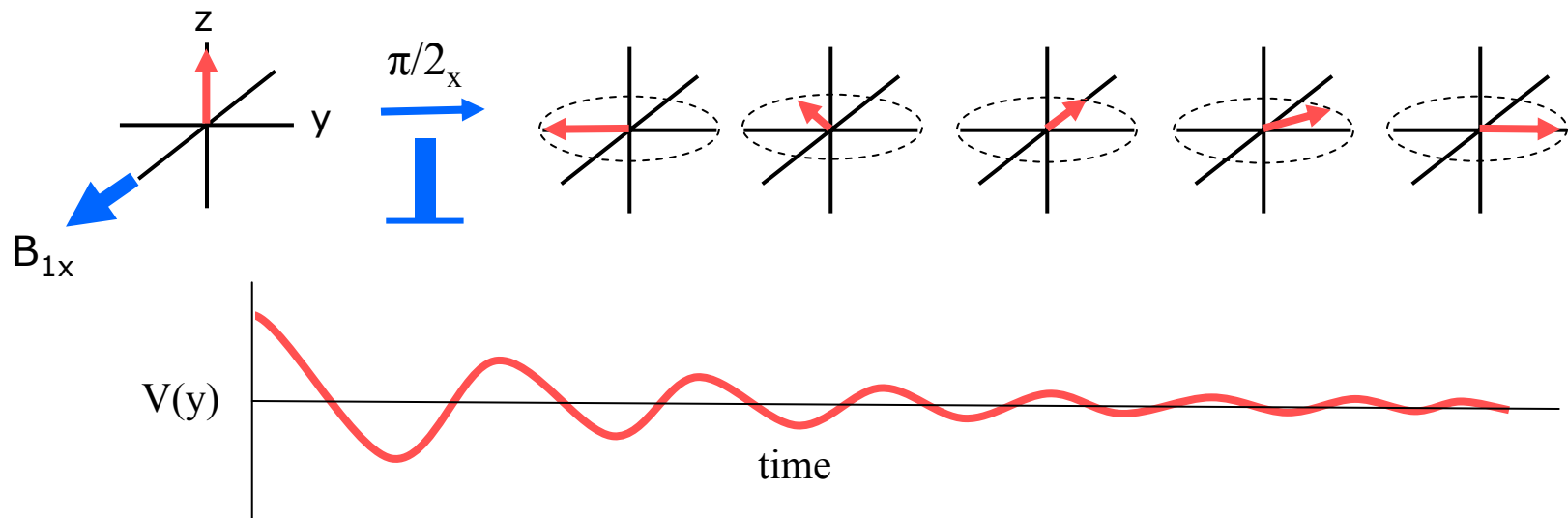


The intermediate frequency, i.f., is a convenient rf frequency chosen by the instrument manufacturer. It is chosen so as not to coincide with the Larmor frequency of any nucleus at the particular field strength of the spectrometer. Using such a frequency allows the spectrometer to treat all nuclei the same regardless of any frequency dependent electronic components.

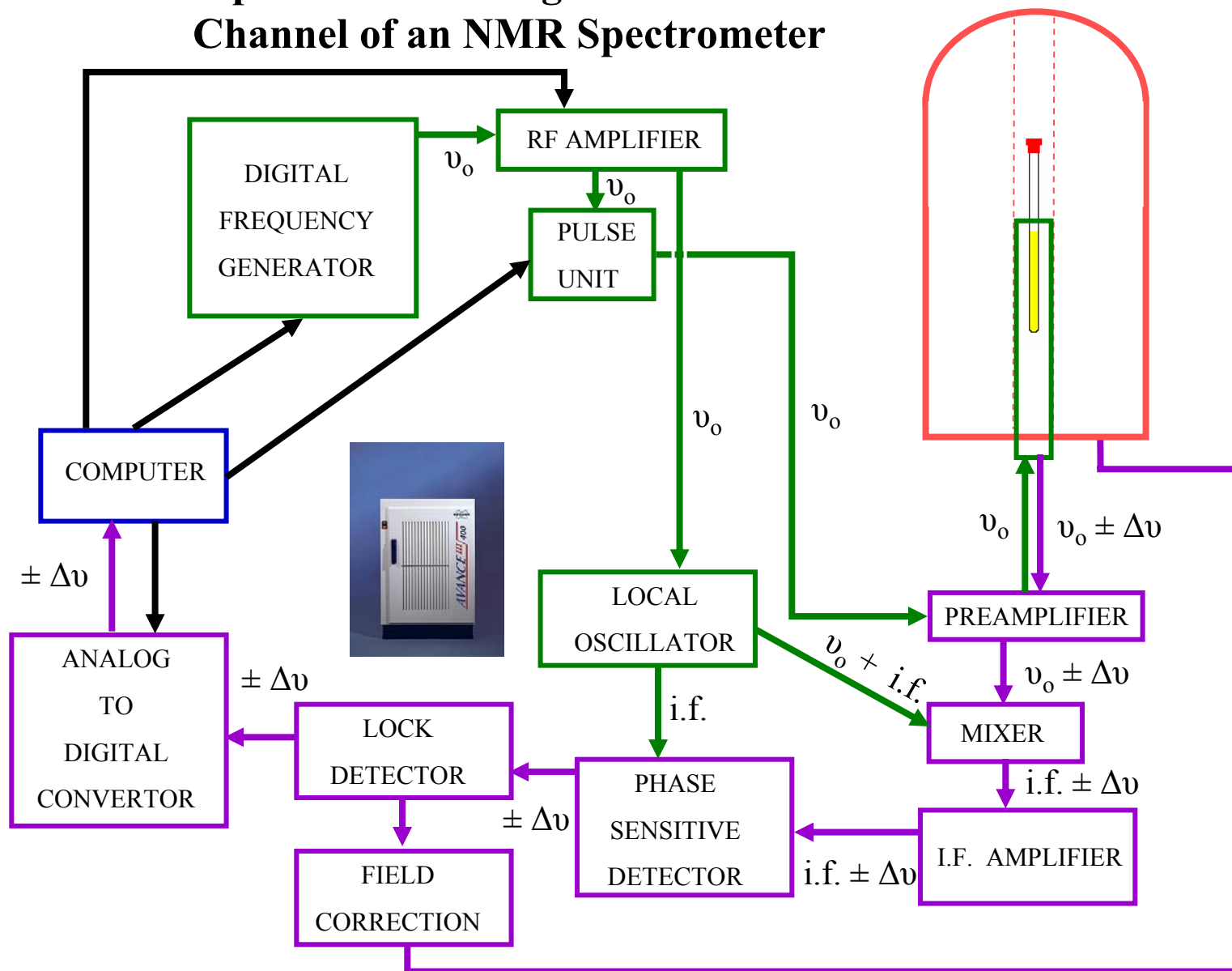
The pure intermediate frequency, i.f., and the i.f. modulated by, the information content of the NMR spectrum, $\pm\Delta\nu$, are fed into a phase sensitive detector. The output of the phase sensitive detector is exclusively the information content of the NMR spectrum, $\pm\Delta\nu$. This is what the user sees as the free induction decay, FID. It typically contains audio frequency signals as typical NMR spectral widths are of the order of tens to hundreds of kHz.



The FID is the voltage measured along a transverse axis in the rotating frame of reference. It contains time dependant signals with frequencies equal to the difference between the transmitter (carrier) frequency and the frequency of the NMR lines.

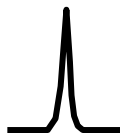


Simplified Block Diagram of the Lock Channel of an NMR Spectrometer



Field Stabilization Using a ^2H Lock

One uses the ^2H dispersion signal for the lock channel.

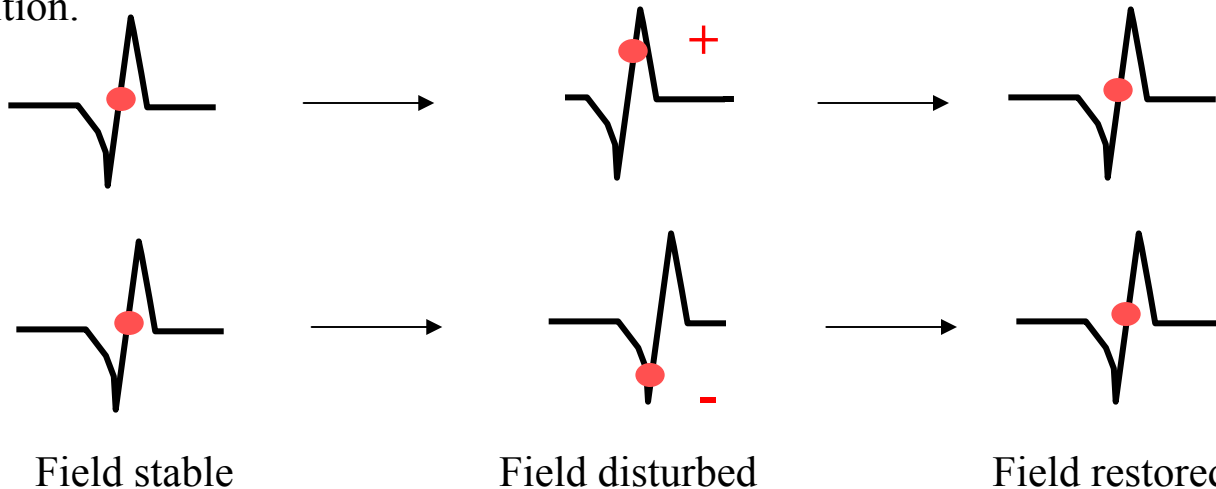


Absorption signal usually observed
in NMR spectra



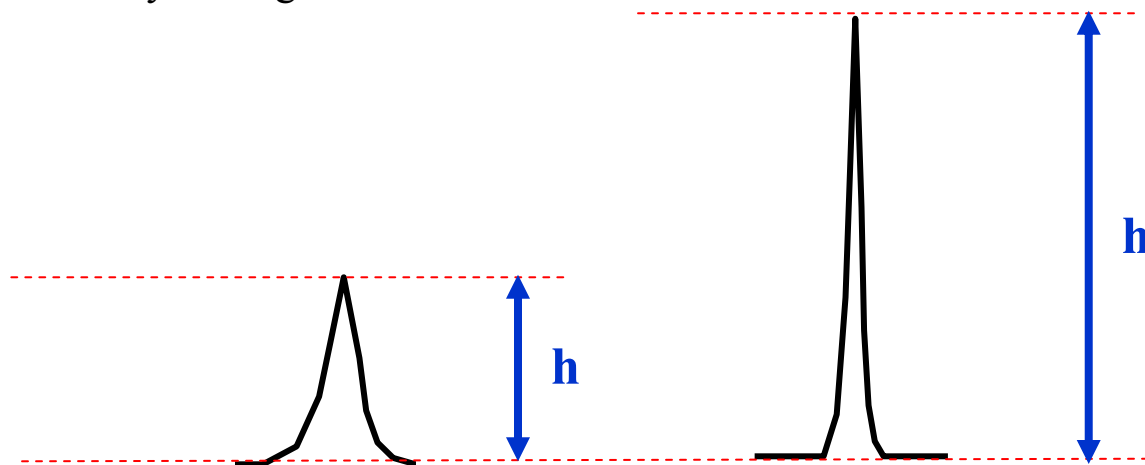
Dispersion signal not usually
observed in NMR spectra.

If the magnetic field is increased or decreased due to some unwanted external effect (e.g. metal object moved near the magnet), the lock dispersion signal will move since the frequency, ν , is proportional to the magnetic field, B_0 . The lock detector monitors the intensity of the center of the observation window. If the lock signal moves even by a small amount in either direction a large positive or negative signal will be detected. According to the sign of the measured intensity, a field correction device sends a current to the z^0 (i.e. B_0) shim coil to correct the field and return the lock signal to its original position.



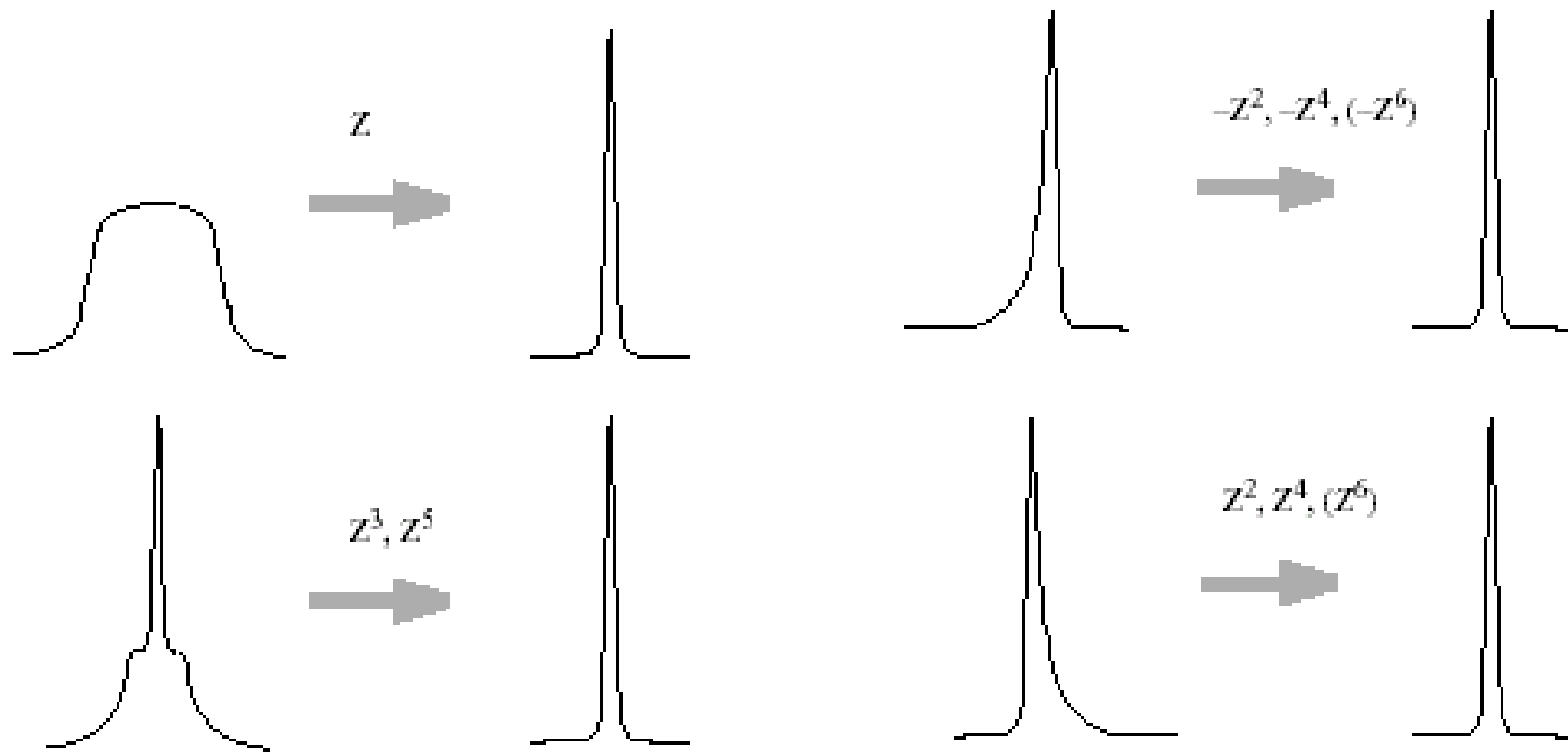
Shimming a Magnet Using the ^2H Lock Signal

The height of the absorption lock signal is a direct measure of how sharp or broad the ^2H signal of the solvent is, as the area of the signal is constant. Broad lines (or low lock levels) indicate that the magnet is inhomogeneous whereas sharp lines or high lock levels indicate that the magnet is very homogeneous



The height of the lock signal is monitored interactively while the properties of the magnetic field are adjusted by altering the currents in the shim coils (z , z^2 , z^3 , z^4 , z^5 , z^6 , x , y , xy , $(x^2 - y^2)$, xz , yz ... etc). The currents in the shim coils are adjusted to give a maximum lock signal. The higher the lock signal, the sharper the ^2H resonance of the solvent and therefore the more homogeneous is the magnet. The line shape and width, $\Delta\nu$, are directly related to the magnetic field homogeneity, ΔB_0 .

Effects of On-Axis Shims on Line Shapes



Shimming a Magnet Without a Lock

In spectrometers designed only for solids, there is rarely a lock channel as one does not use solvents. Instead, the magnetic field is shimmed interactively by looking at a free induction decay (FID) and adjusting the shims such that the FID extends out as far as possible in time and has an exponential shape. In general the homogeneity requirements are much less stringent for solids compared to liquids. For solids, one tends to shim a probe only once and not bother with the shims from sample to sample.



Bad Shims

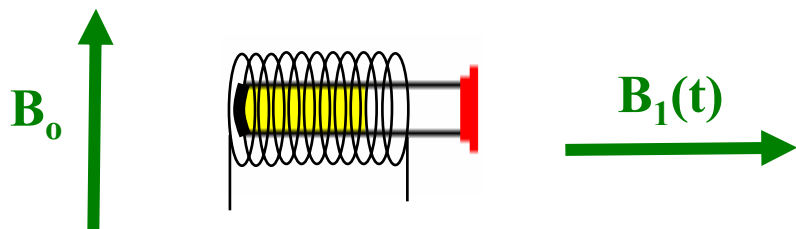


Good Shims



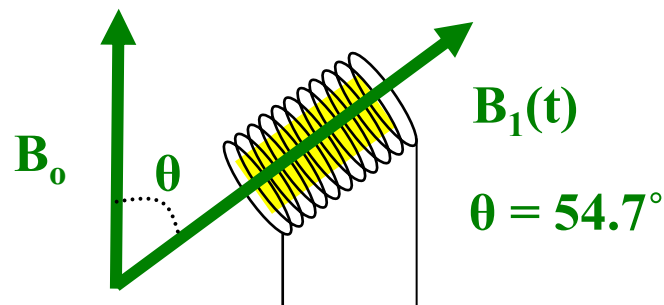
NMR Probes

The NMR probe must generate a fluctuating magnetic field, $B_1(t)$ perpendicular to the fixed magnetic field, B_0 at the Larmor frequency of the nucleus being observed. The most efficient way of achieving this is by transmitting the rf through a horizontal solenoid coil oriented perpendicular to the magnetic field./



This configuration is used for wide line solids applications. The major disadvantage is that the NMR probe must be removed from the magnet to change samples.

Magic Angle Spinning Probes for High Resolution Solid State NMR

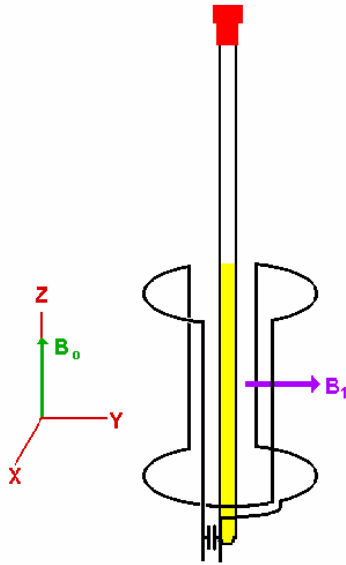


In “magic angle spinning” (MAS) probes for high resolution solids applications, the solenoid coil is oriented at 54.7° with respect to the magnetic field.



In such a configuration the usable power delivered to the sample is scaled down by a factor of $\sin(\theta) = 0.82$. A solenoid coil mounted parallel to the magnetic field would transmit no usable power to the sample

High Resolution NMR Probes for Liquids



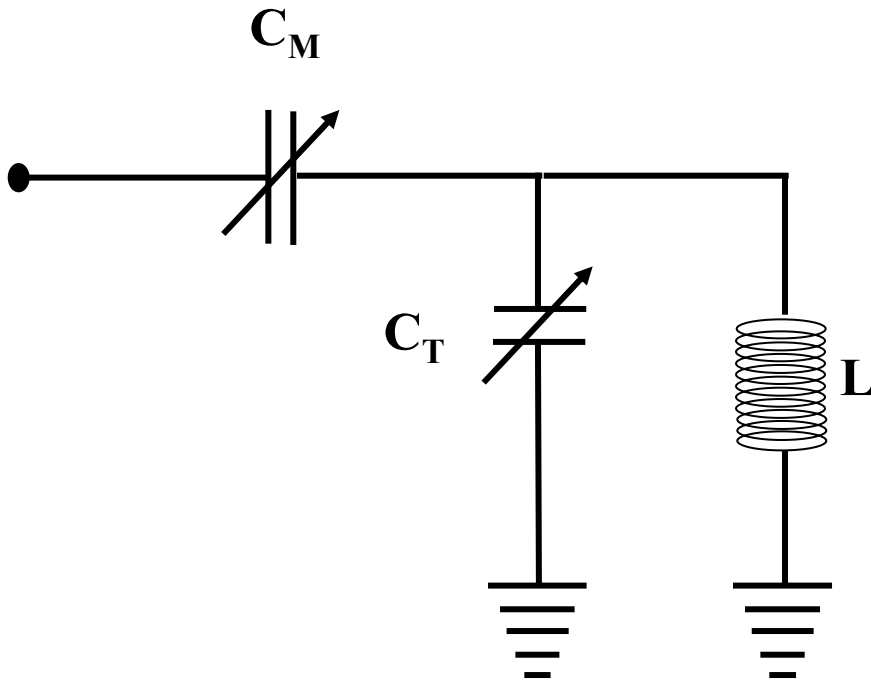
An alternative to the solenoid coil is the Helmholtz or saddle coil.

Like the solenoid coil oriented perpendicular to B_0 , the Helmholtz coil oriented parallel to B_0 will generate an oscillating rf field $B_1(t)$ perpendicular to B_0 . This configuration is used in high resolution NMR probes for liquids because it allows easy transport of samples in and out of the magnet without the need to remove the probe. It also allows one to spin the sample about B_0 which has the effect of averaging out transverse magnetic field inhomogeneity. Both sample transport and sample spinning are achieved with compressed air.



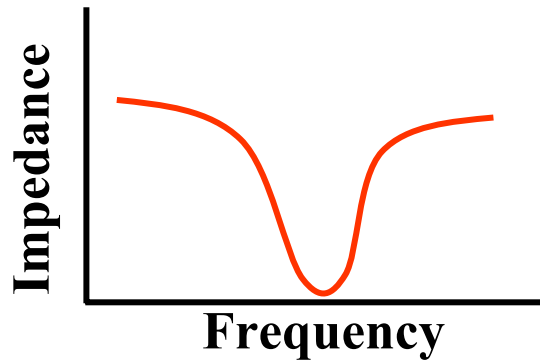
Tank Circuits

The electronics in NMR probes are very simple tank circuits. A single frequency probe can have the following circuit with only two capacitors and a coil.

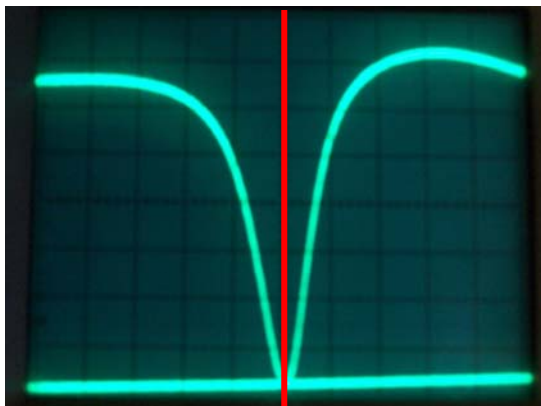
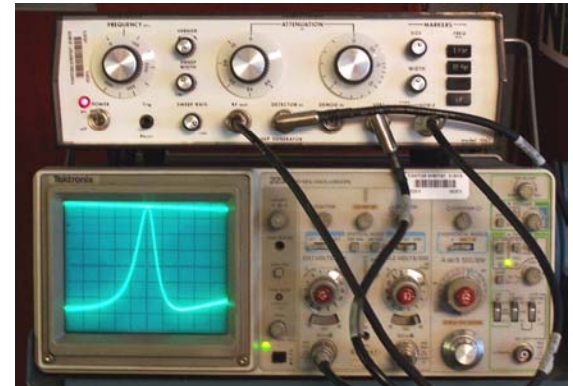


C_T is a tuning capacitor. It is used to tune the circuit to a specific frequency. C_M is a matching capacitor. It is used to alter the quality factor of the circuit. L is the coil in which the sample is placed. It may be a solenoid or Helmholtz coil. The inductance of the coil is an important property to consider in achieving the desired tuning range of the probe. The coil must be effective at both transmitting rf to the sample as well as detecting the small NMR signal from the sample.

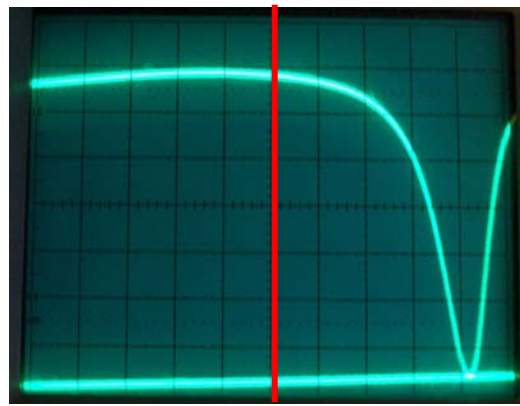
Probe Tuning and Matching



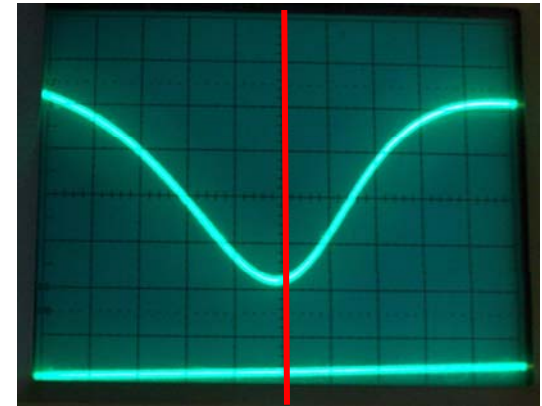
Tank circuits of this sort are band pass filters. They allow the passage of a narrow frequency range (perhaps one MHz) while rejecting other frequencies. The two variable capacitors, C_M and C_T are adjusted to make the circuit well tuned and well matched.



Tuned and matched



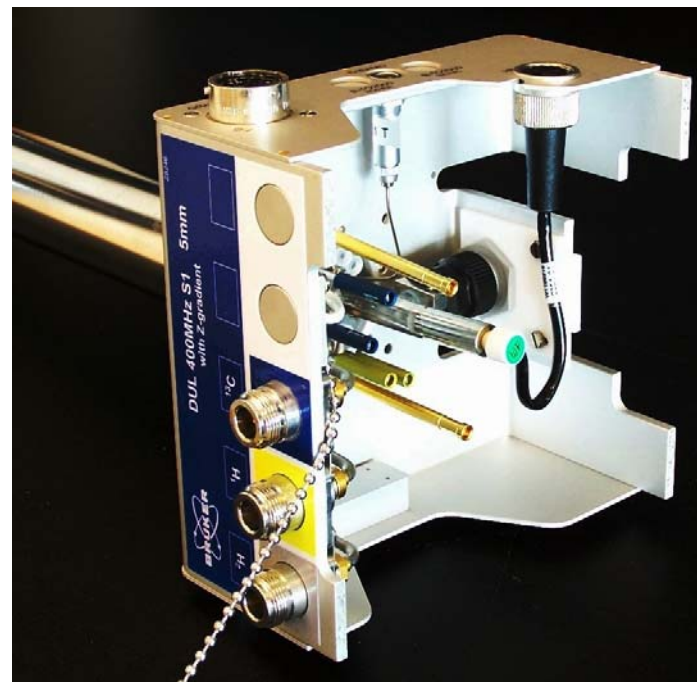
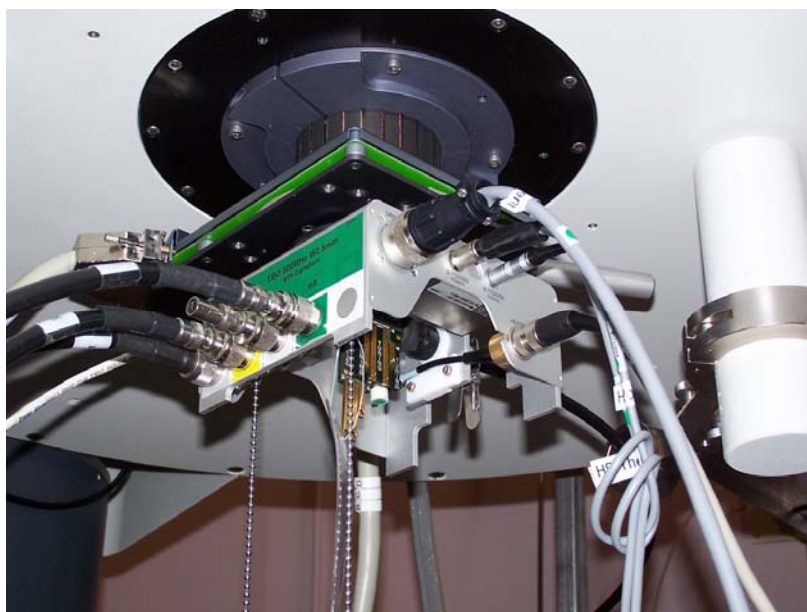
Poorly tuned, well matched



Well tuned, poorly matched

NMR Probes for Liquids

High resolution liquids NMR probes are often equipped with three separate rf channels: an X channel, tunable over a very wide frequency range (often $^{109}\text{Ag} - ^{31}\text{P}$); a ^1H channel (used for either observation or decoupling) and a ^2H channel (used for the lock). NMR probes are often equipped with heaters and thermocouples for the regulation of the sample temperature over a wide range. They are also equipped with pulsed field gradients required for more advanced experiments.



Types of Liquids NMR Probes

Single Frequency Probe – used to observe a single nucleus (usually protons) also equipped with a lock channel. These were usually delivered with very old systems and are quite rare now.

Dual Probe – designed to perform optimally for ^1H and a single X nucleus (usually ^{13}C) also equipped with a lock channel. The higher end models have variable temperature capability and pulsed field gradients.

Broadband Probe – has X, ^1H and ^2H channels. The X channel is tunable over a large frequency range (often ^{109}Ag – ^{31}P). The coil for the X channel is closest to the sample providing the highest possible sensitivity for the X nucleus. These probes also have variable temperature capability and pulsed field gradients.

Inverse Broadband Probe – exactly the same as the broadband probe except the ^1H rather than the X coil is closest to the sample providing the highest sensitivity for protons.

Triple Resonance Probe – has a tunable X channel, a fixed Y channel, a ^1H channel and a ^2H channel. These probes are typically configured for $^{13}\text{C}/^{15}\text{N}/^1\text{H}$ and used for the analysis of labeled proteins. They also have variable temperature capability and pulsed field gradients.

Four Nucleus Probe – the X channel has three tuning positions and is designed to observe 3 different isotopes. It also has a ^1H and ^2H channel and typically variable temperature capability and pulsed field gradients.

Microprobe – designed for reduced sample sizes with very small coils with high mass sensitivity. They are available in a wide variety of configurations.

Cryoprobe – a probe with cryogenically cooled electronics to improve the signal to noise ratio. These are typically triple resonance probes.

Types of Solids NMR Probes

Solenoid Probe – These are usually single frequency probes with exchangeable coils however they are available with proton decoupling capability as well. They are designed to handle high power efficiently and are used to observe broad lines.

Single Crystal Probe – A solenoid probe of either single frequency or X/¹H configuration used to observe the NMR spectra of single crystals. These probes are equipped with goniometer heads able to rotate the crystal about one or more axes.

Magic Angle Spinning (MAS) Probe – designed to spin a sample at an angle of 54.7° with respect to the magnetic field at rates of a few tens of Hz up to 70 kHz .(depending on sample size). They are available in single double and triple resonance configurations.

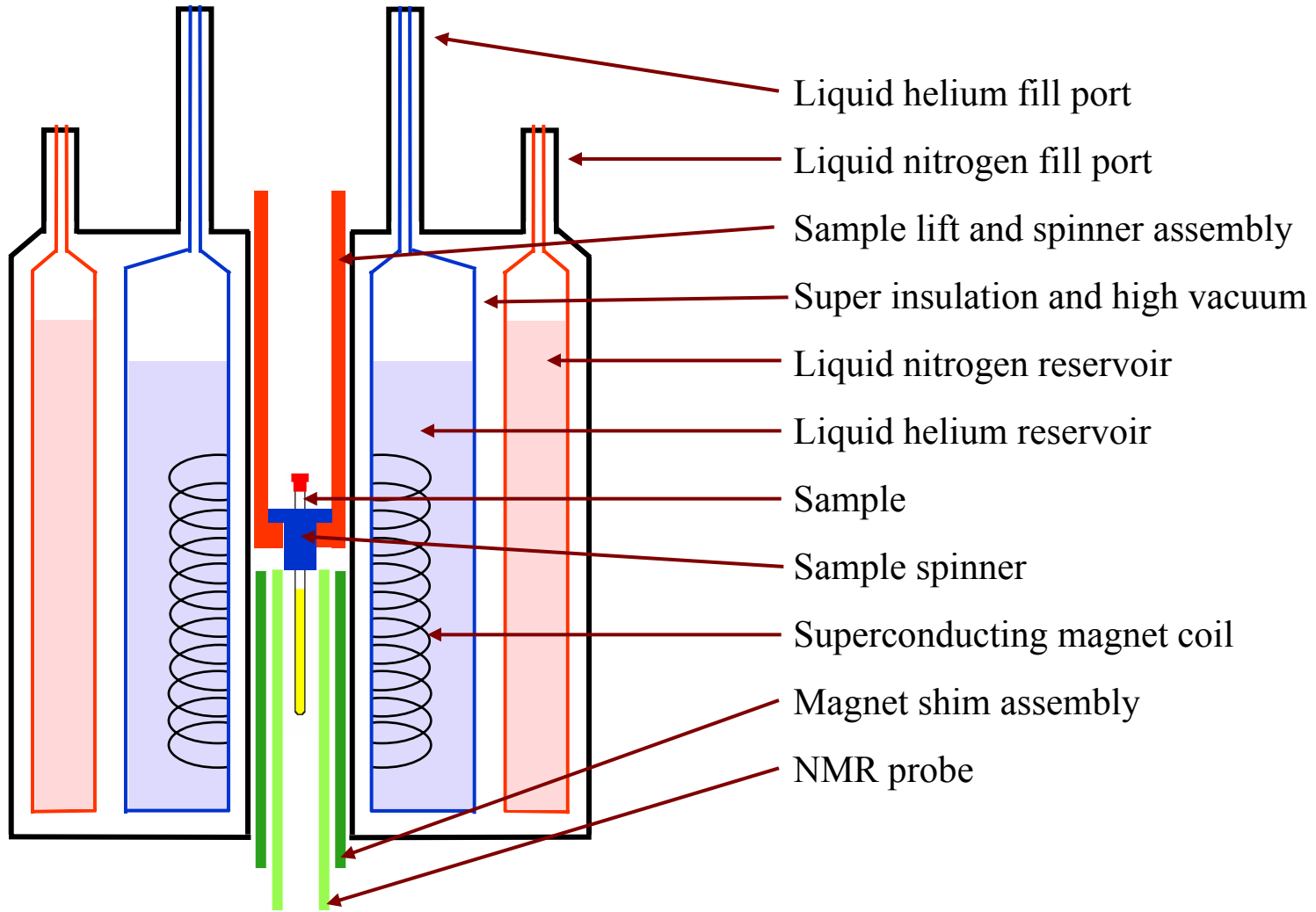
Variable Angle Spinning (VAS) Probe – like an MAS probe with the additional capability to being able to spin the sample at a wide range of angles with respect to the magnetic field.

Dynamic Angle Spinning (DAS) Probe – designed to spin a sample at one angle with respect to the magnetic field and then rapidly (eg.: tens of msec) switch to spinning at another angle.

Double Angle Rotation (DOR) Probe – designed to spin a sample about two different angles simultaneously.

Micro Imaging probe – usually tuned for protons. The probe is used with pulsed field gradients about 3 perpendicular axes to collect NMR images (magnetic resonance images, MRI).

The Magnet





The Magnet

- Single most expensive component of the NMR spectrometer (\$100,000 - \$10,000,000)
- Very good short and long range stability.
- Available at field strengths up to 21 T (^1H at 900 MHz).
- Field is very homogeneous.
- Cooled by liquid helium.
- Helium boil-off is reduced by super insulation, high vacuum and liquid nitrogen
- Very high field magnets require a pumping system based on the Joule-Thompson effect to reduce the temperature of the superconducting coil below that of the boiling point of liquid helium.
- Modern magnets are shielded to avoid large external stray fields.
- Once charged, the magnet requires no power.
- Available in narrow bore (54 mm), wide bore (89 mm) and super wide bore for liquid, solids and micro imaging, respectively.

Magnet Quench

If the temperature of the superconducting coil increases past the critical superconducting temperature (typically 7 K), the coil becomes resistive. All of the current (typically 100 Amps) is converted into heat which quickly boils off the liquid helium in a catastrophic “quench”.



Fourier Transforms

The Fourier transform is a mathematical conversion between time domain functions (FID's) and frequency domain functions (NMR spectra)

$$\mathbf{f(t)} \longrightarrow \mathbf{F(v)}$$

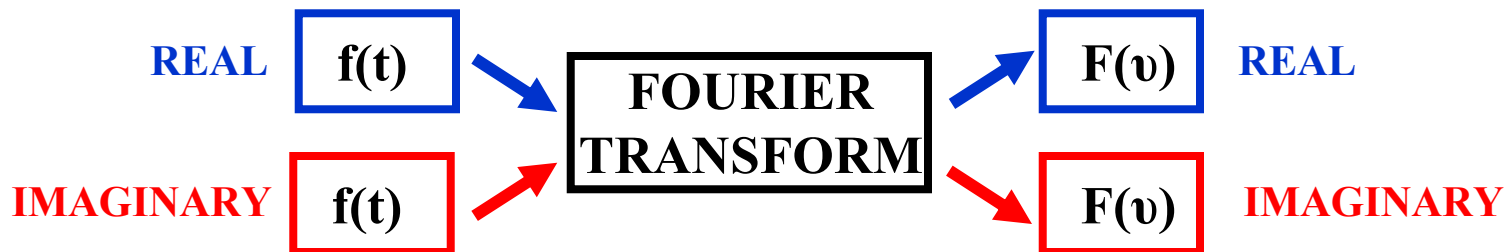
They are also used in FT-IR to convert Michaelson interferograms into infrared spectra, in ICR to convert cyclotron signals into mass spectra, in NQR and pulsed EPR spectroscopy. The mathematical definition is:

$$\mathbf{F(v)} = \int_0^{\infty} \mathbf{f(t)} e^{-2\pi i v t} dt$$

From the Euler identity, $e^{\pm i\phi} = \cos \phi \pm i \sin \phi$, we can rewrite the definition as follows:

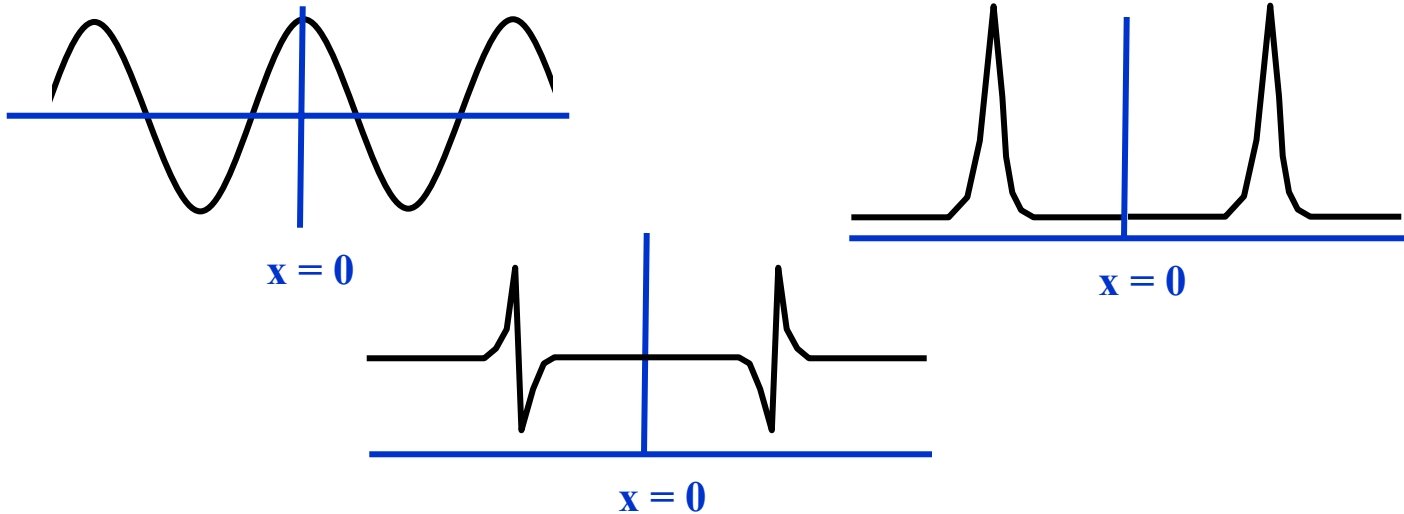
$$\mathbf{F(v)} = \int_0^{\infty} \mathbf{f(t)} [\mathbf{\cos (2\pi v t)} - \mathbf{i \sin (2\pi v t)}] dt$$

The Fourier transform is a complex operation requiring a real and an imaginary input. It produces both a real and an imaginary output.

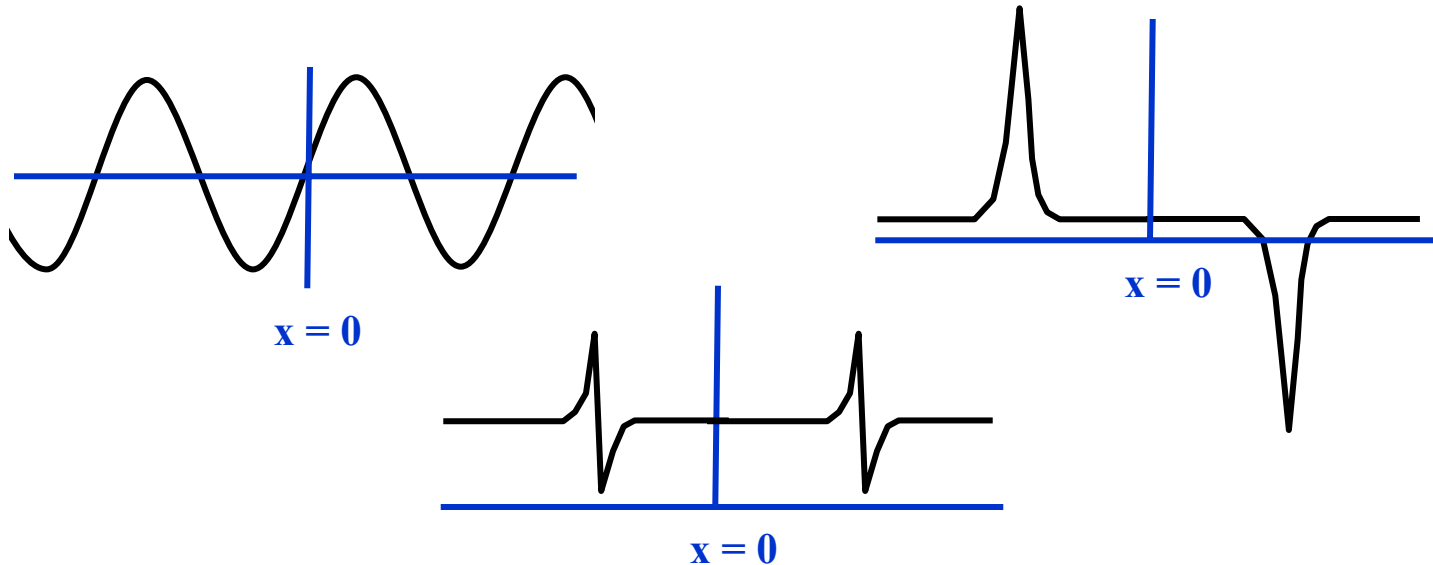


Useful Definitions

Even Function – A function $f(x)$ is even if $f(x) = f(-x)$. It is symmetric about $x = 0$.

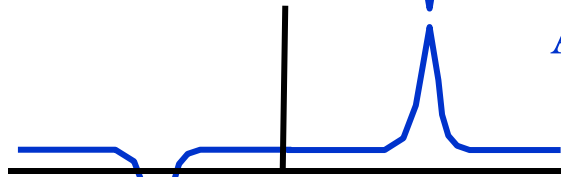
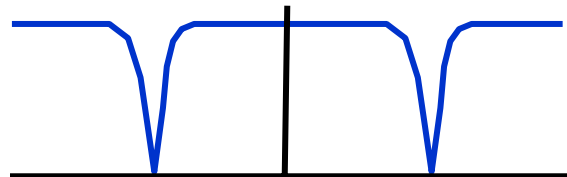
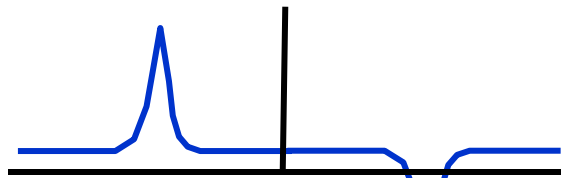
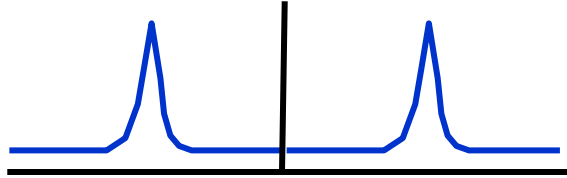


Odd Function – A function $f(x)$ is odd if $f(x) = -f(-x)$. It is not symmetric about $x = 0$.

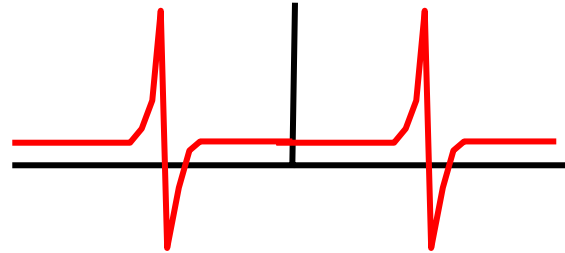
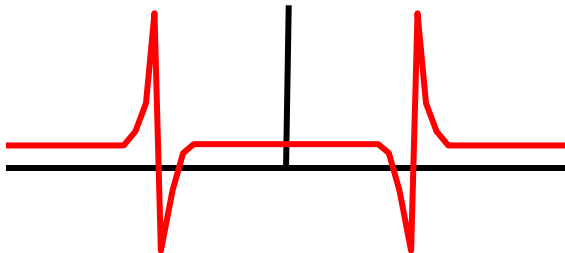


Properties of Fourier Transforms

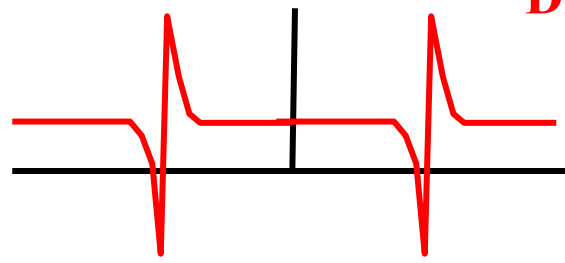
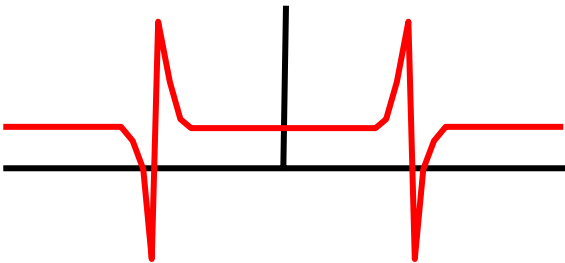
Property 1 – The output of a Fourier transform has both an absorption line shape and a dispersion line shape



Absorption Output

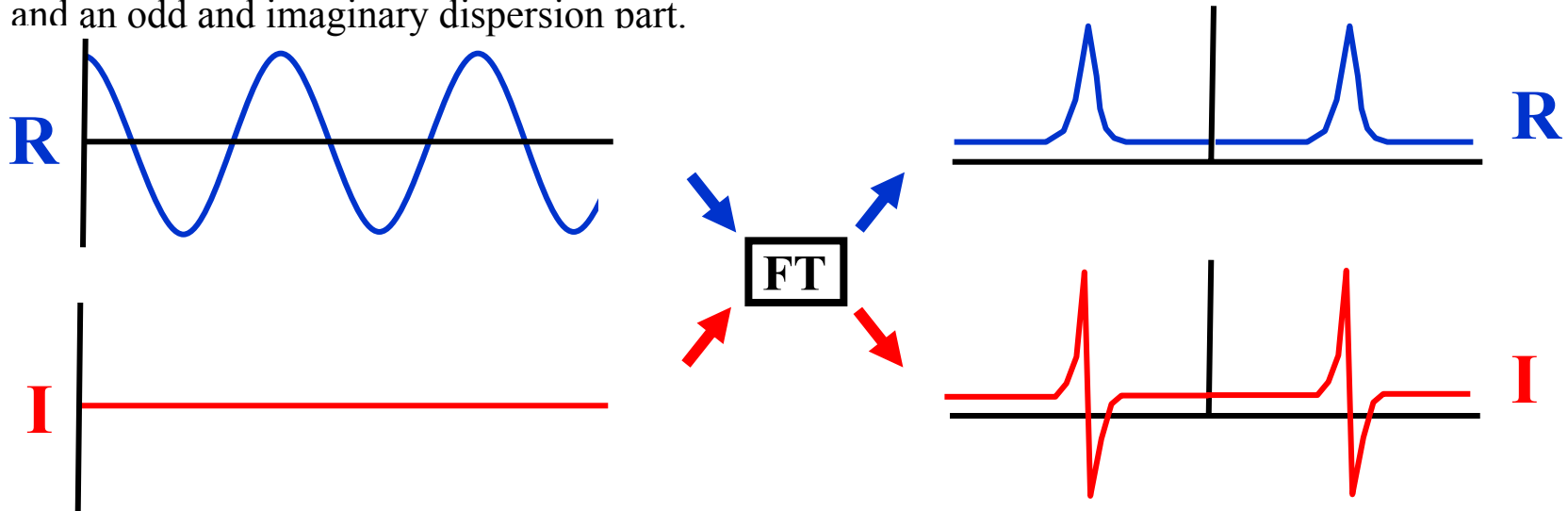


Dispersion Output

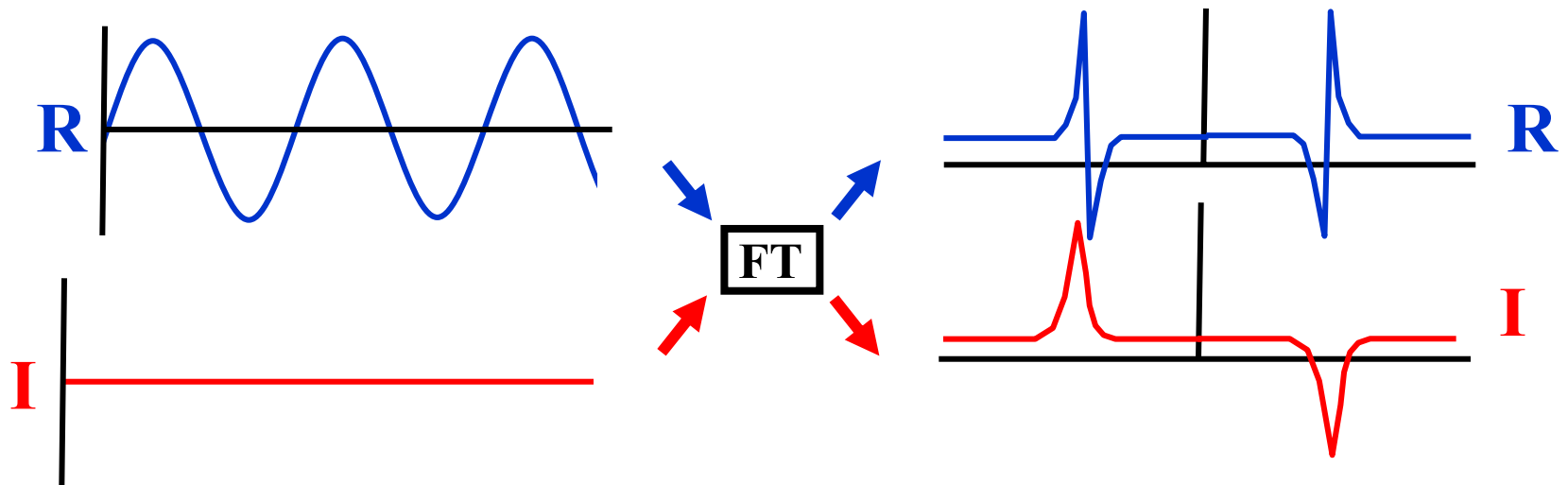


Properties of Fourier Transforms

Property 2 – The Fourier transform of a real even function has an even and real absorption part and an odd and imaginary dispersion part.

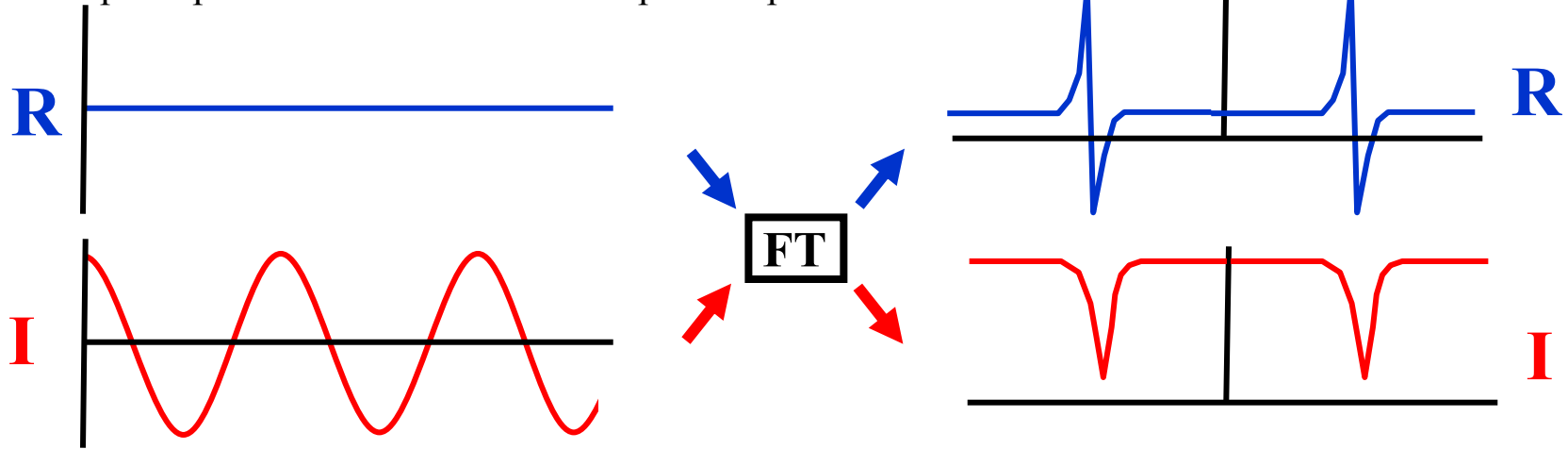


Property 3 – The Fourier transform of a real odd function has an odd and imaginary absorption part and an even and real dispersion part..

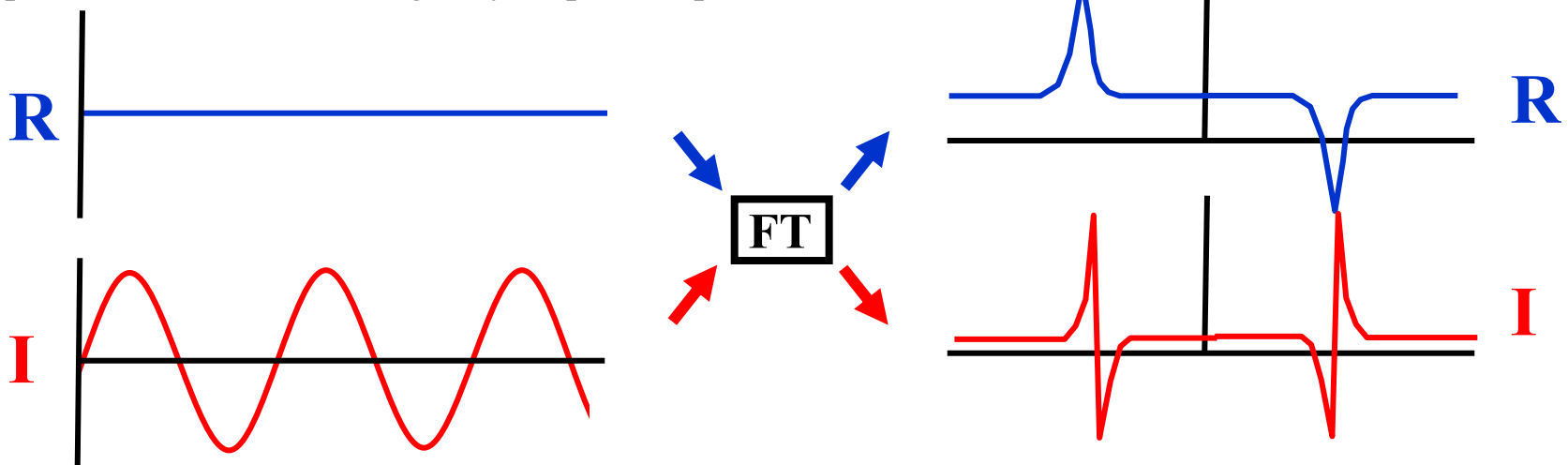


Properties of Fourier Transforms

Property 4 – The Fourier transform of an imaginary even function has even and imaginary absorption part and an odd and real dispersion part

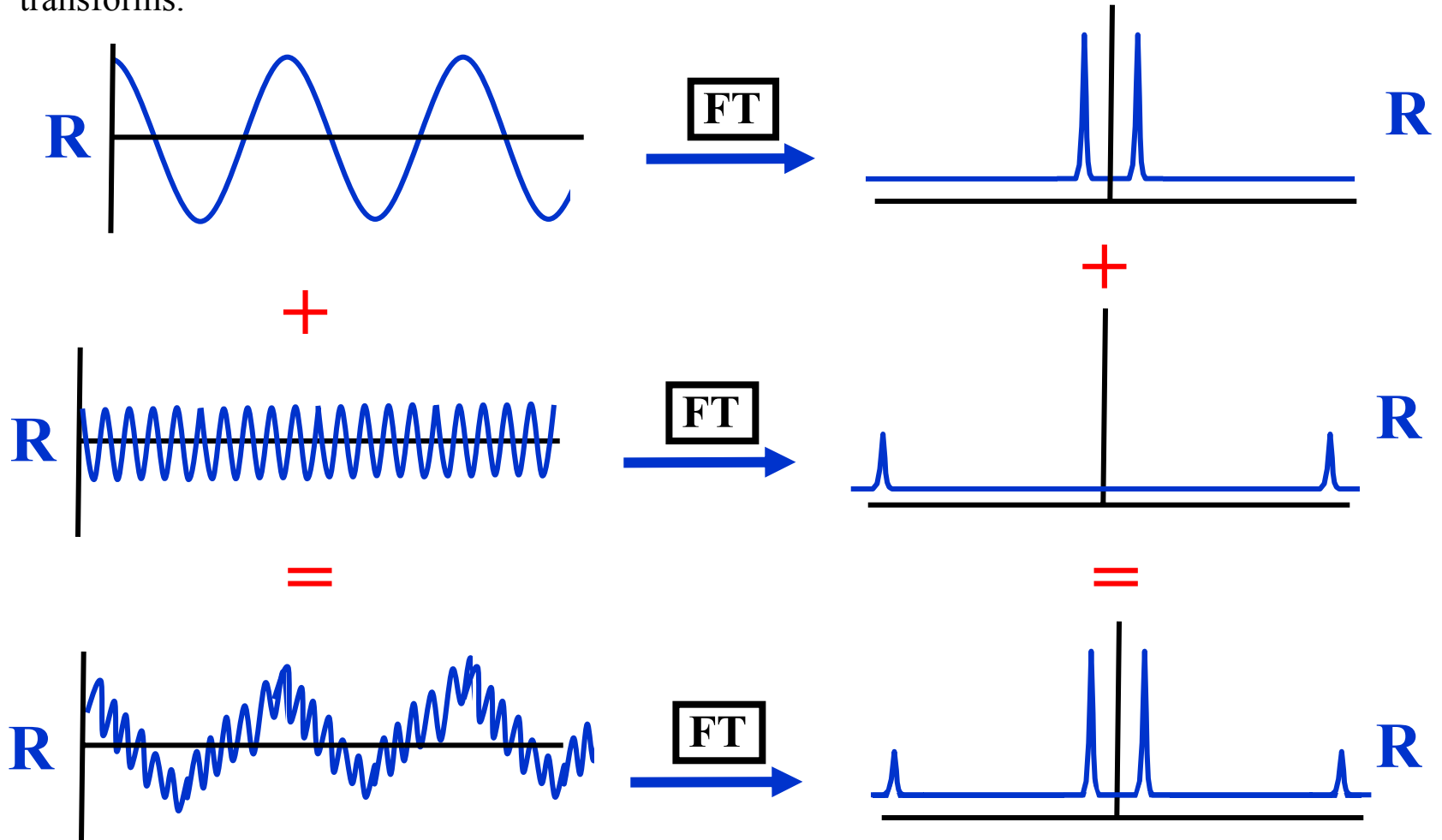


Property 5 – The Fourier transform of an imaginary odd function has an odd and real absorption part and an even and imaginary dispersion part..

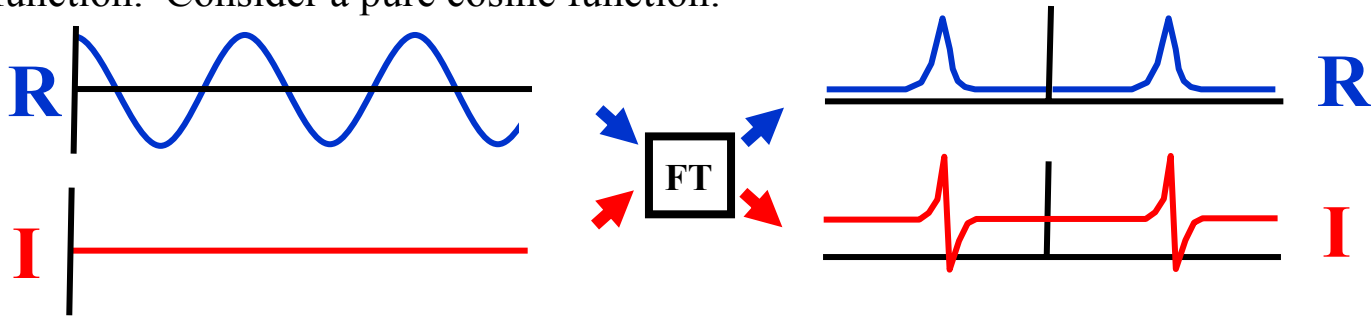


Properties of Fourier Transforms

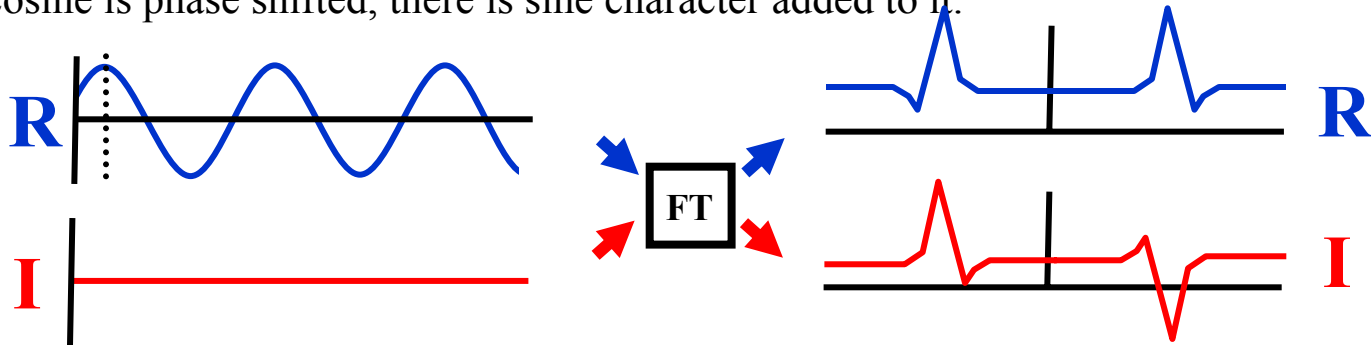
Property 6 – The Fourier transform of a sum of functions is the sum of individual Fourier transforms.



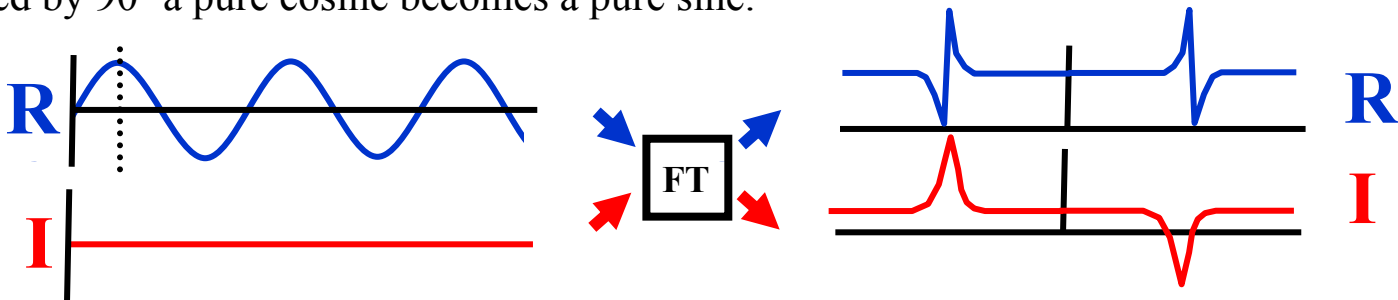
Property 7 – If $f(t)$ has Fourier transform $F(\nu)$, then $f(t+a)$ has Fourier transform $F(\nu) (\cos(a\nu) - i \sin(a\nu))$. Shifting the time origin of $f(t)$ changes the amount of even/odd character in the time domain function and hence the relative amounts of real and imaginary character in the frequency domain function. Consider a pure cosine function:



If the cosine is phase shifted, there is sine character added to it.

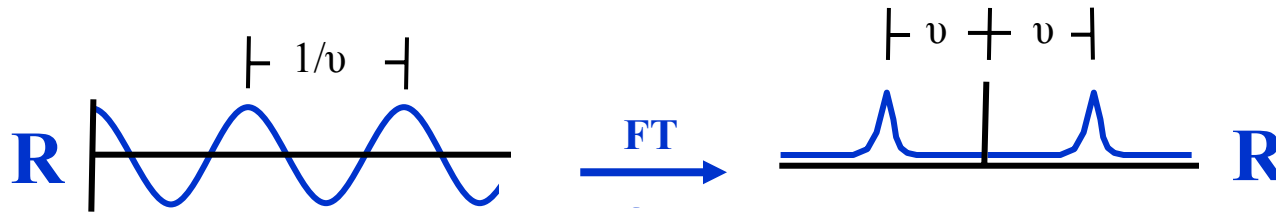


If shifted by 90° a pure cosine becomes a pure sine.

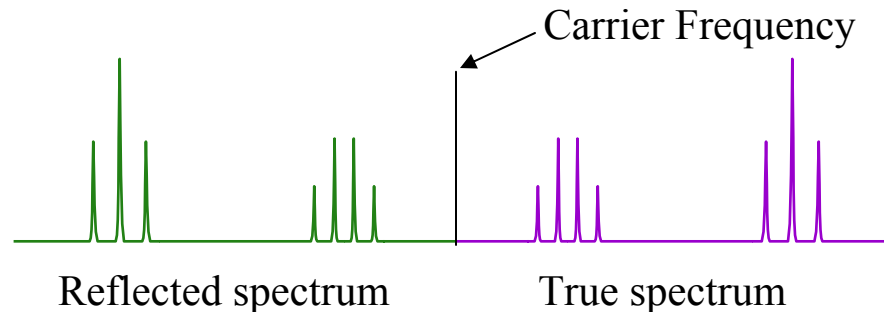


Single Channel Detection

If an FID is collected along the $-y$ axis and used as the “real” input for the Fourier transform, then one is unable to distinguish between positive and negative frequencies.

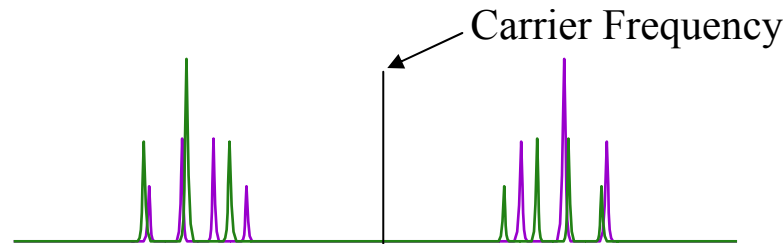


Instead of getting one line in the frequency spectrum representing the single frequency present in the time domain function, we get two. As a result, in single detection experiments, one must put the carrier frequency on one side of the spectrum.



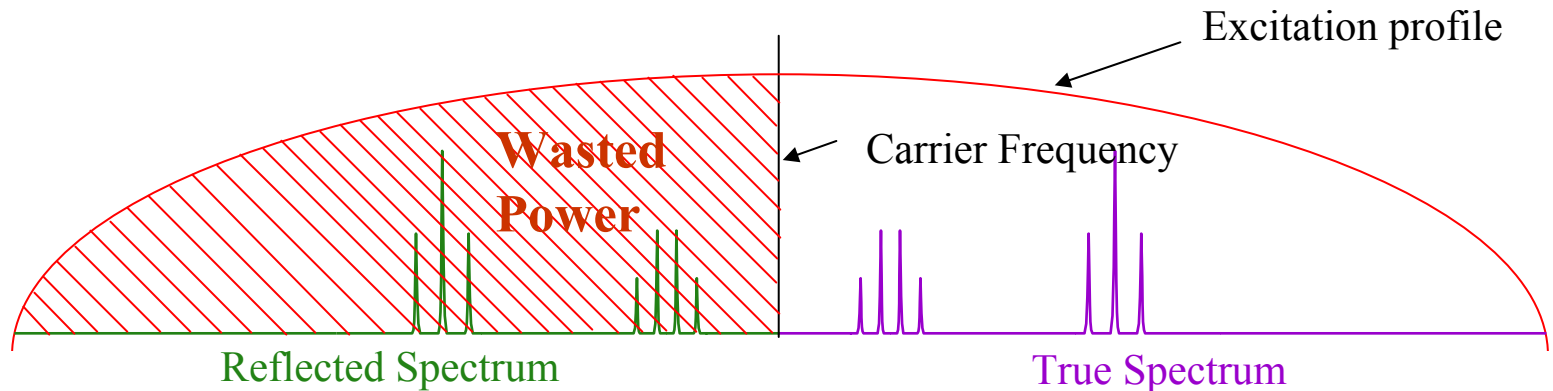
Disadvantages of Single Channel Detection

If the carrier is placed in the center of the true spectrum then there is overlap between the true spectrum and the reflected spectrum.



The disadvantages of single detection are:

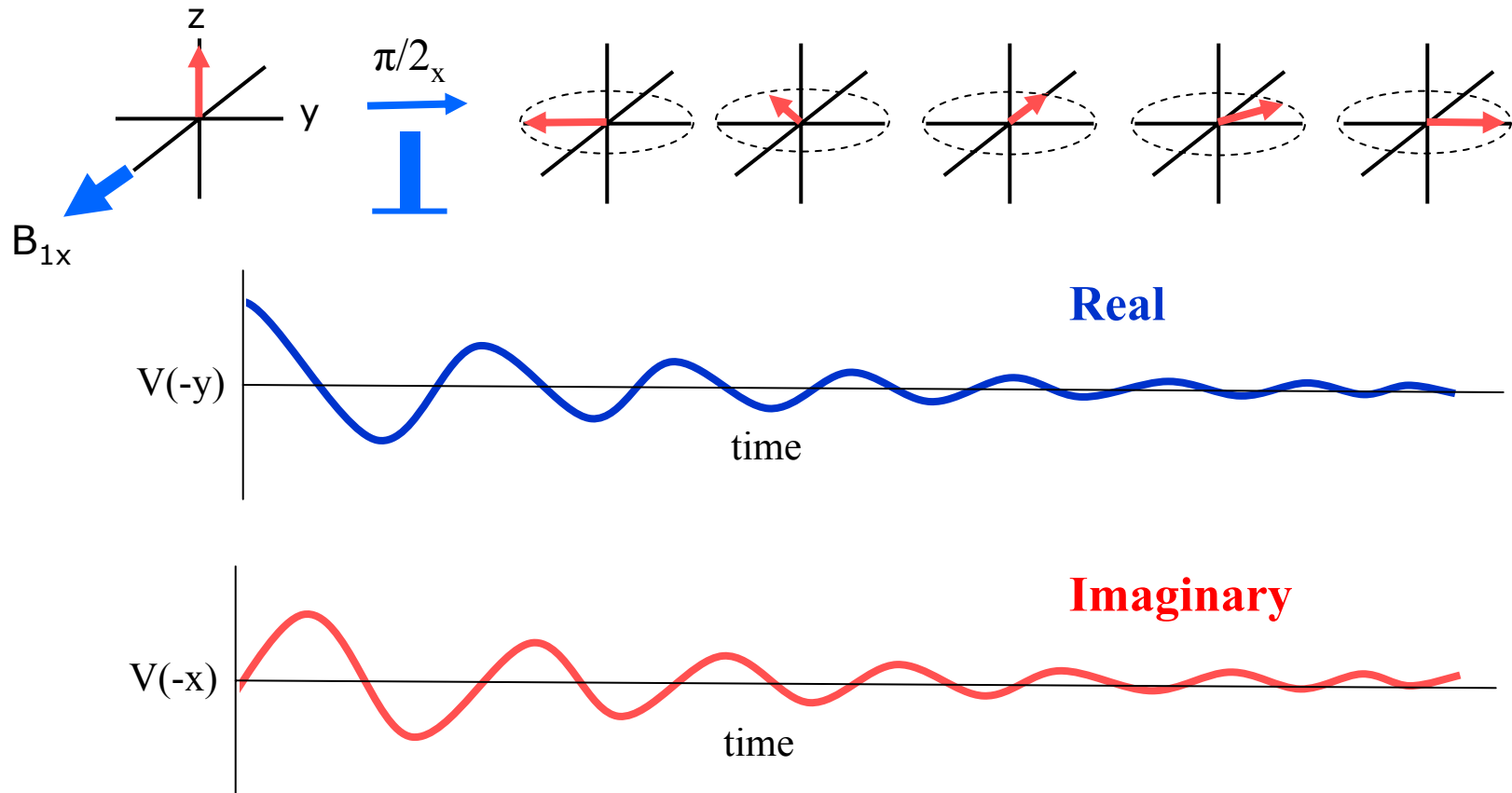
1. The excitation power is wasted as the carrier must be on one side of the spectrum.



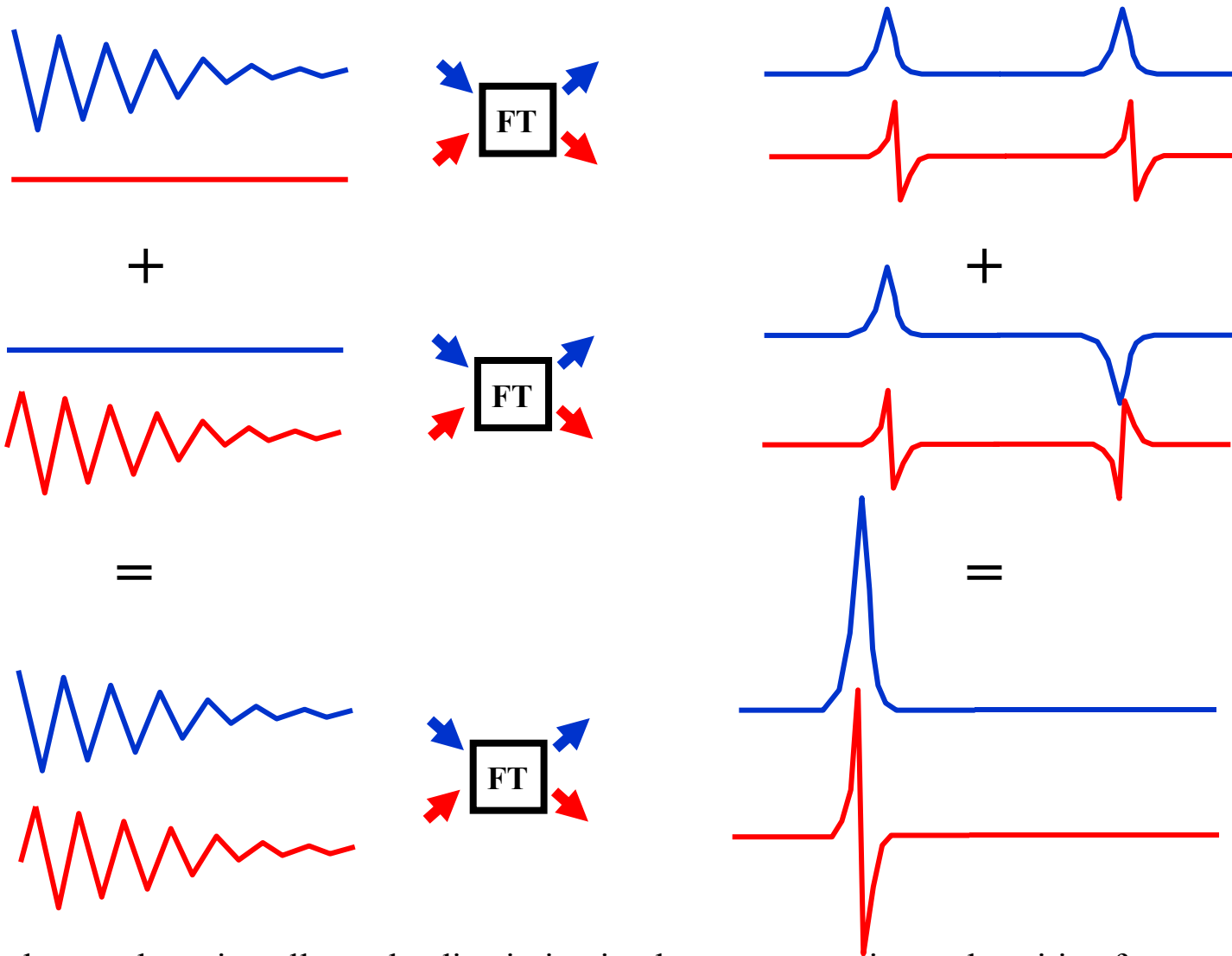
2. The signal to noise ratio is low as the intensity is split between the true spectrum and its reflection.

Quadrature Detection

One can avoid these two problems by using quadrature detection. Quadrature detection involves taking data on both the x and y axes of the rotating frame of reference. One of the signals is considered “real” and the other “imaginary”. Both are used as inputs to the Fourier transform.



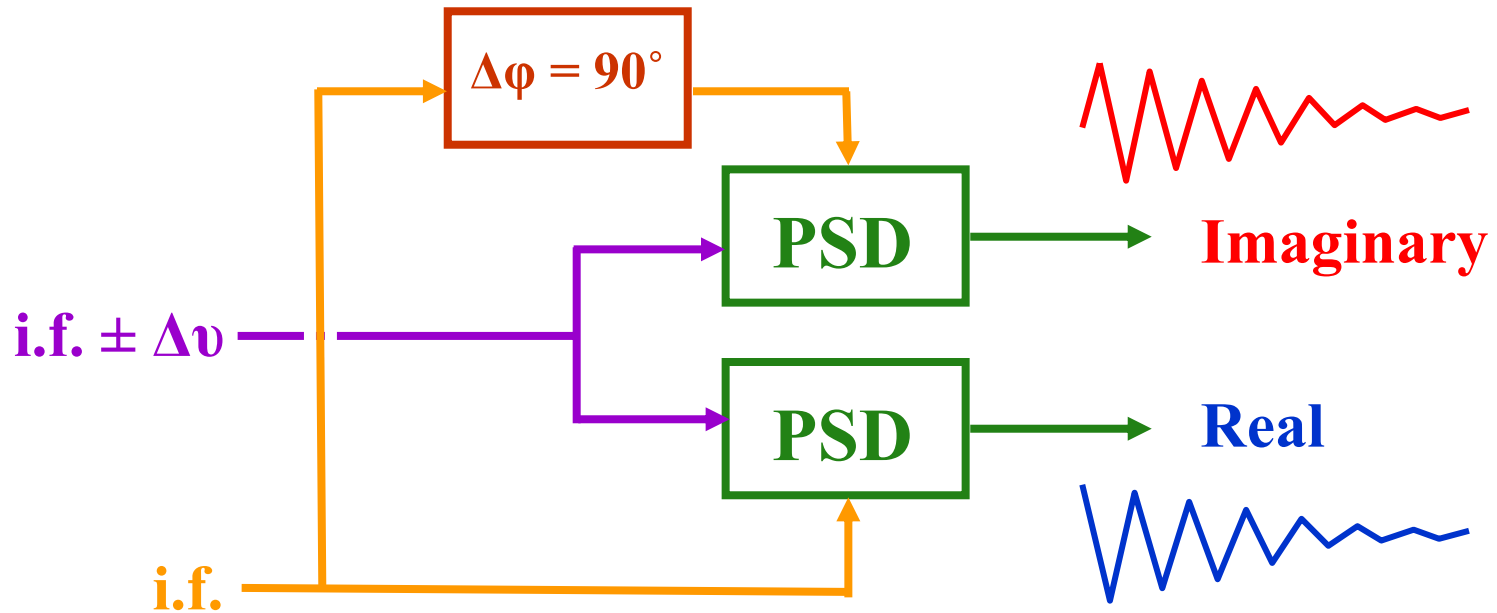
Quadrature Detection



Quadrature detection allows the discrimination between negative and positive frequencies and improves the signal to noise ratio by a factor of $\sqrt{2}$. Also, the carrier can be placed in the center of the spectrum without overlap problems.

Quadrature Detection

Since we do not have two perpendicular receiver coils, quadrature detection is generally achieved with two phase sensitive detectors and a 90° phase shifter.

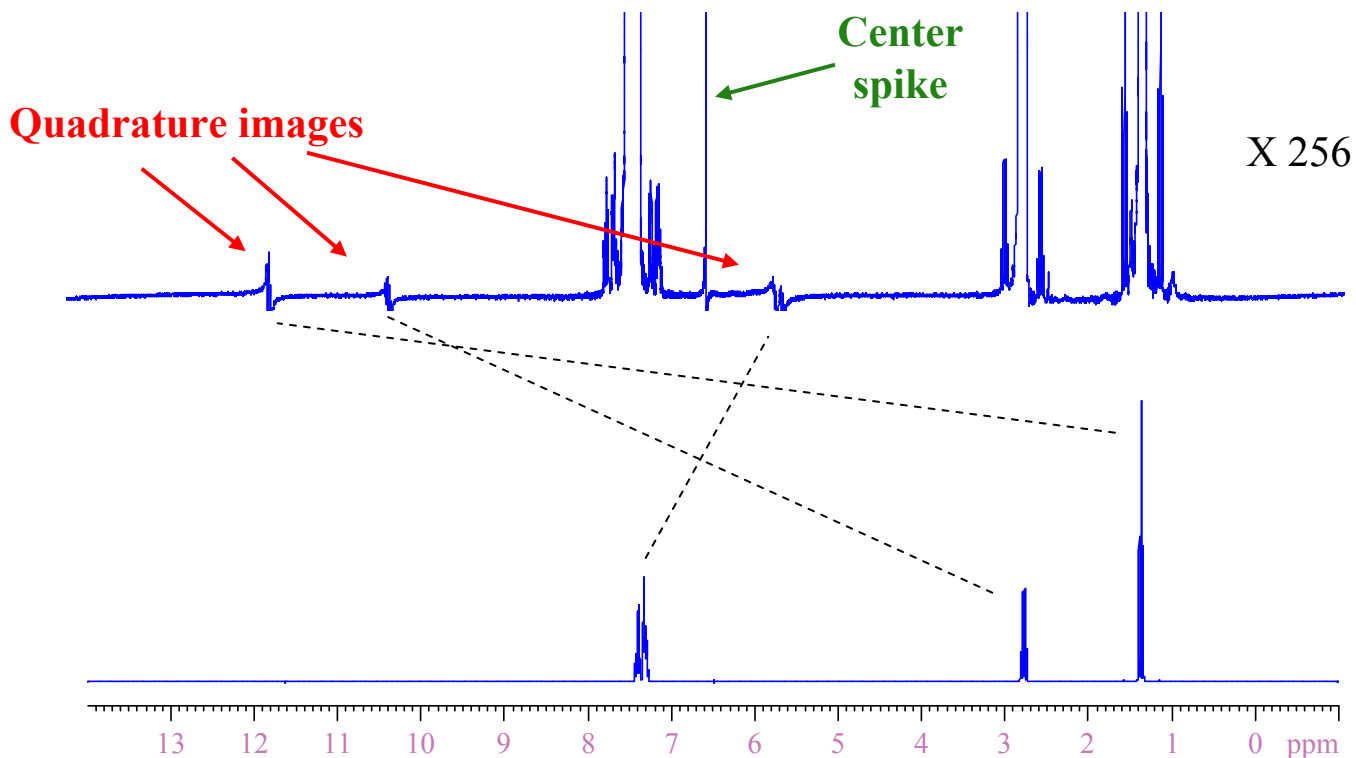


The real and imaginary data are collected simultaneously with the same receiver coil. The assignment of the real and imaginary channels is completely arbitrary. The important factor is that the relative phase difference between the two channels is always 90° .

Quadrature Artifacts

If the two phase sensitive detectors are not perfectly balanced, one will observe quadrature artifacts in the spectrum. These are small “ghost” images of large NMR signals symmetric about the carrier frequency. If there is an offset between the two phase sensitive detectors then there will be a spike in the exact center of the spectrum. These artifacts can be minimized by cycling the phases of both the pulse and the receiver.

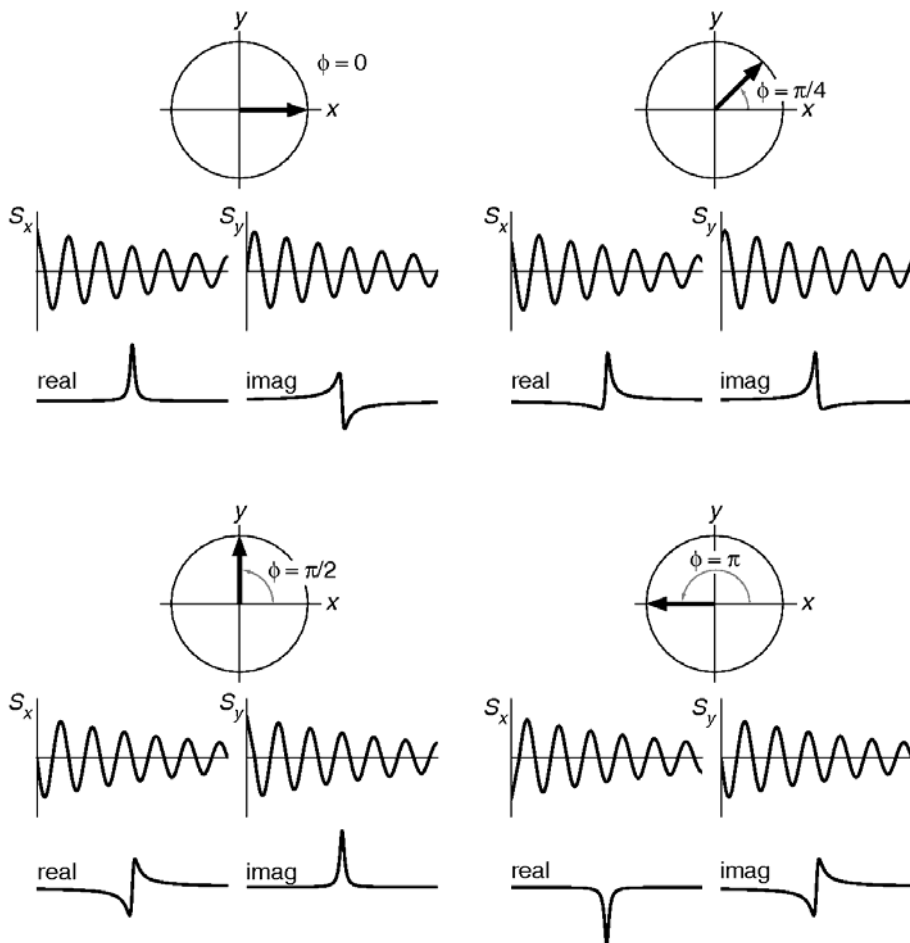
¹H NMR ethyl benzene



Phase

Despite the fact that we often talk about delivering pulses along the x axis and detecting signals on the (-y) axis of the rotating frame of reference, this is just a convention. The phases are completely arbitrary and are corrected after the data have been acquired. Here, the real FID is collected along the x axis.

The phase of the real FID affects the absorption / dispersion character of the spectrum. A spectrum that has pure absorption character is said to be “in phase”. The real FID’s collected are rarely purely even functions and therefore NMR spectra are rarely “in phase”. The phase is corrected after the data are collected.



Zero Order Phase Correction

The perfectly phased time domain signal can be represented as:

$$S(t) = S_0 \exp(i\Omega t) \exp(-t/T_2)$$

The spectrometer however collects data with an arbitrary phase, ϕ . This time domain signal can be represented as:

$$S(t) = S_0 \exp(i\Omega t) \exp(-t/T_2) \exp(i\phi)$$

which has Fourier transform:

$$S(\omega) = S_0 [A(\omega) + i D(\omega)] [\cos \phi + i \sin \phi]$$

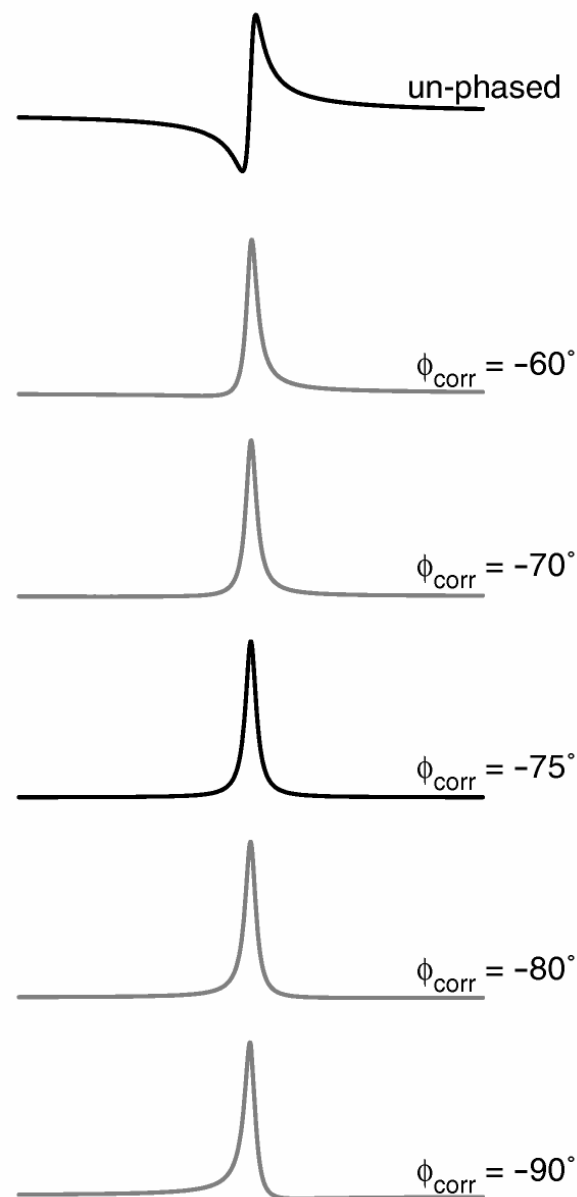
where $A(\omega)$ and $D(\omega)$ are the absorption and dispersion components, respectively. The real part is:

$$S_0 [(\cos \phi)A(\omega) - (\sin \phi)D(\omega)]$$

And the imaginary part is:

$$i S_0 [(\cos \phi)D(\omega) + (\sin \phi)A(\omega)]$$

The NMR software can therefore calculate an angle, ϕ (or it can be chosen interactively by the user) such that the real part of the spectrum is in absorption mode. This is called the zero order phase correction.

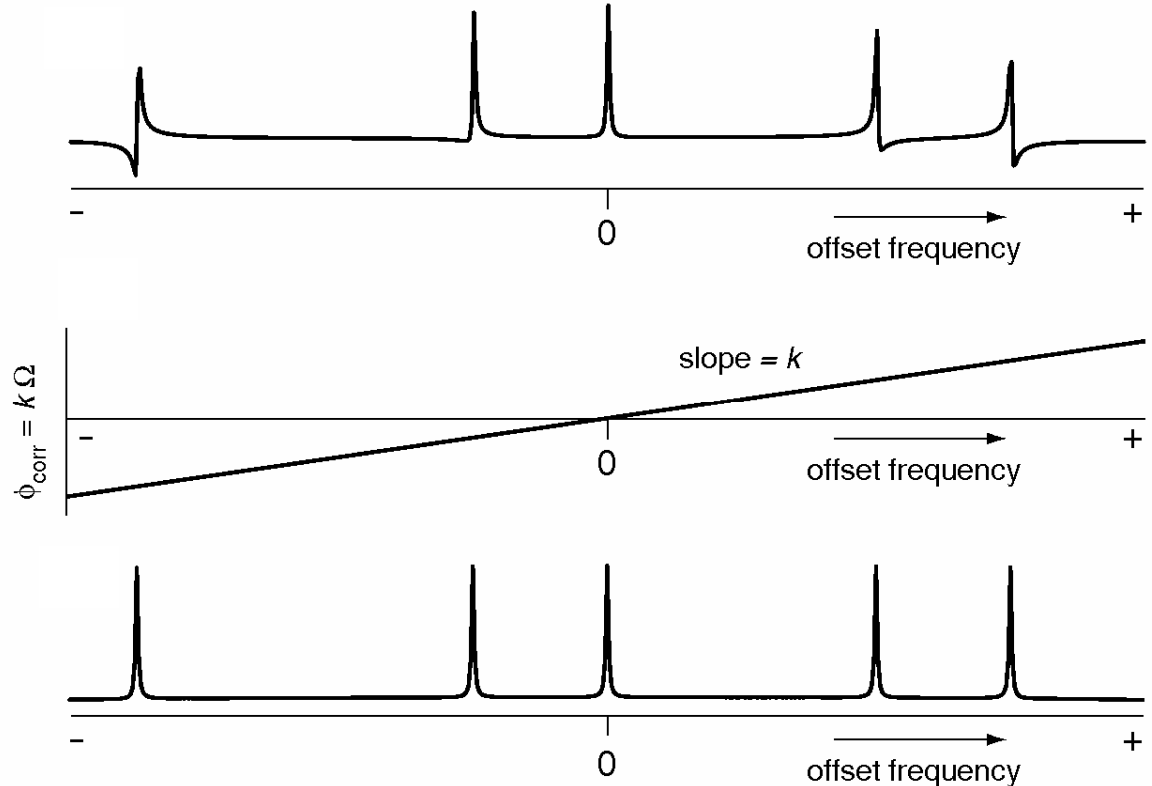


First Order Phase Correction

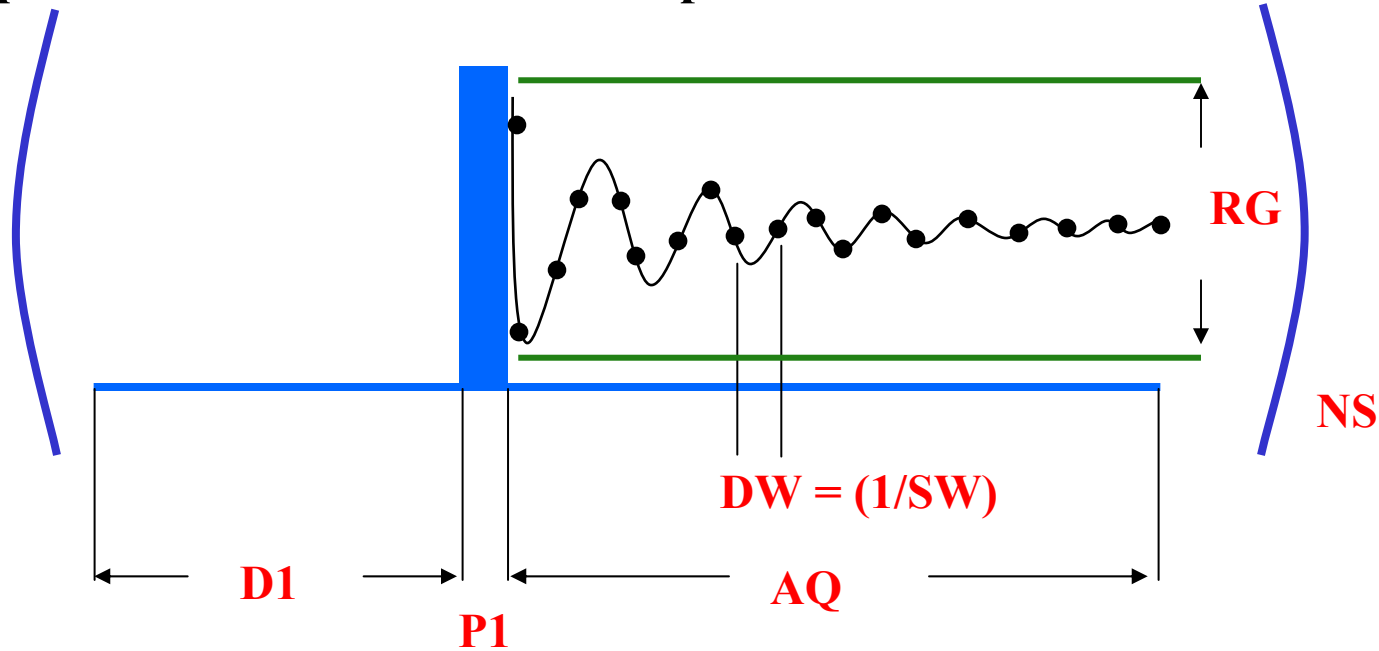
The 90° pulses we use are truly only 90° pulses for resonances at the carrier frequency (i.e. $\Omega = 0$). For peaks that are off-resonance (i.e. $\Omega \neq 0$), the pulses are not truly 90° and they produce both x and y magnetization. As a result the phase of an NMR signal is proportional to its offset from the carrier frequency.

$$\varphi = k \Omega$$

The spectrometer calculates the proportionality constant, k (or it is calculated interactively by the user) which will put all resonances in the spectrum in absorption mode regardless of their offset from the carrier. This is called first order phase correction.



Acquisition Parameters for a Simple One-Pulse NMR Measurement



D1 - Relaxation delay.

P1 - Pulse duration.

AQ - Acquisition time.

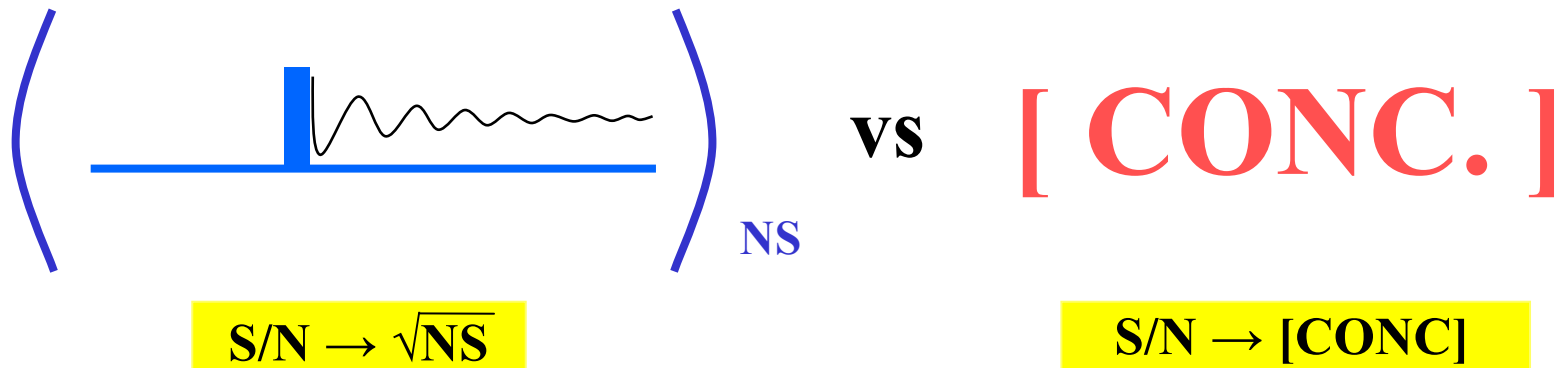
NS - Number of scans.

DW - Dwell time. The reciprocal of this time determines the spectral width, **SW**

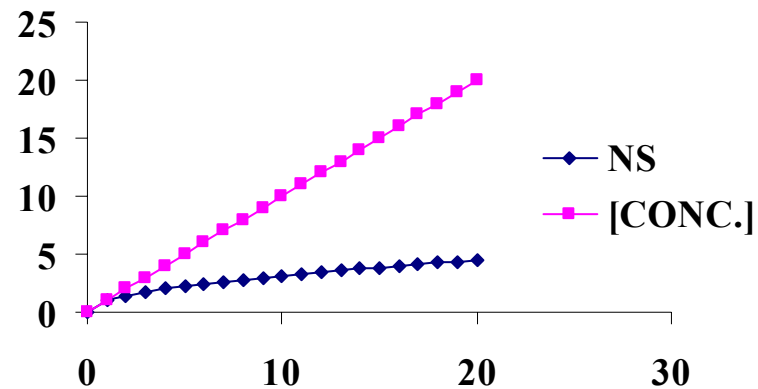
RG - Receiver gain.

Intelligent Choice of Acquisition Parameters – Number of Scans

The signal to noise ratio is proportional to the square root of the number of scans whereas it is directly proportional to the concentration.

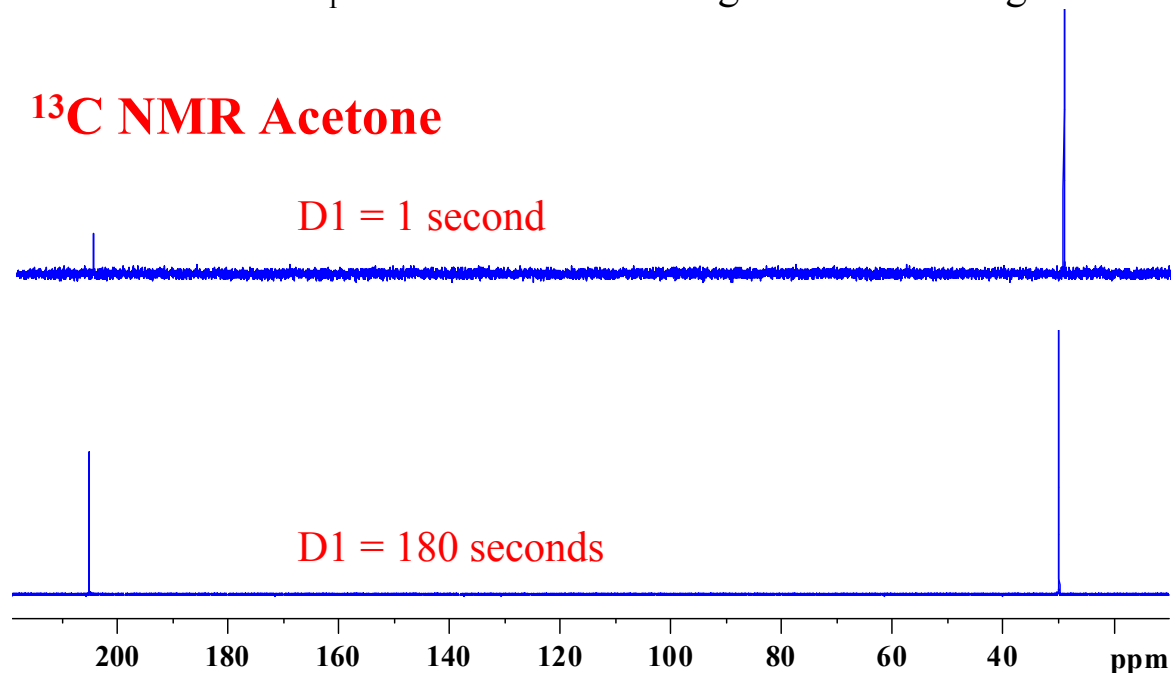


Signal to Noise Ratio vs. NS and [CONC.]



Intelligent Choice of Acquisition Parameters – Relaxation Delay

The relaxation delay, $D1$, should be chosen to allow sufficient time for equilibrium z magnetization to build up. If one requires quantitative results then the inter-pulse spacing (i.e. the sum of the relaxation delay, $D1$ and the acquisition time, AQ) should be greater than 5 times the T_1 of the slowest relaxing resonance being observed.



In the lower trace the signal to noise ratio is high and the ratio of carbonyl to methyl is 1:2 as expected. In the upper trace the signal to noise ratio is low because the magnetization was not allowed sufficient time to reach its equilibrium value after each pulse. Also, the ratio of carbonyl to methyl is much less than 1:2 since the carbonyl carbon has a longer T_1 than the methyl carbons.

Intelligent Choice of Acquisition Parameters – Acquisition Time

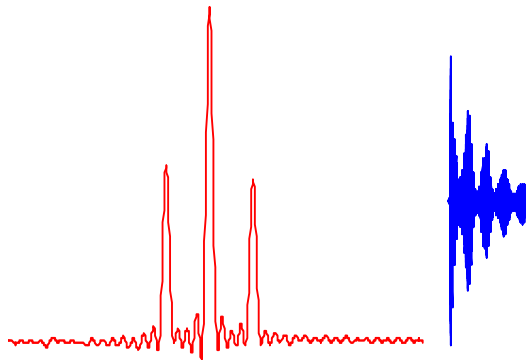
The acquisition time should be chosen such that the time domain signal has decayed into the noise. As a rule of thumb, one could use an estimate of the line width at half height, $\Delta\nu_{1/2}$, of the sharpest peak in the spectrum to estimate T_2^* , where:

$$T_2^* = 1 / (\pi \Delta\nu_{1/2})$$

The acquisition time should be at least 5 times T_2^* . Below are some examples of appropriate acquisition times in relation to line widths for typical solids and liquids.

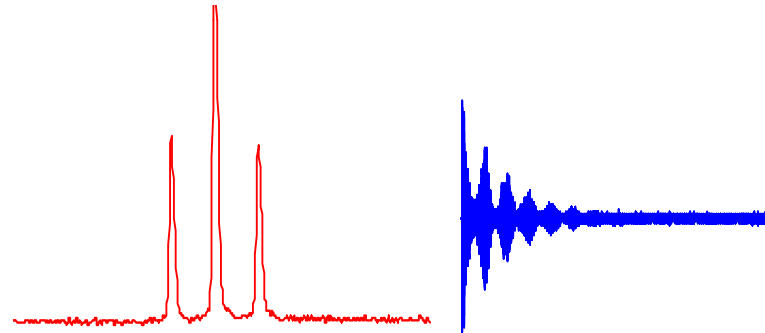
	$\Delta\nu_{1/2}$ (Hz)	AQ = 5 T_2^*
Solids	10,000	160 μ sec
	1,000	1.6 msec
	100	16 msec
Liquids	10	0.16 sec
	1	1.6 sec
	0.1	16 sec

Intelligent Choice of Acquisition Parameters – Acquisition Time

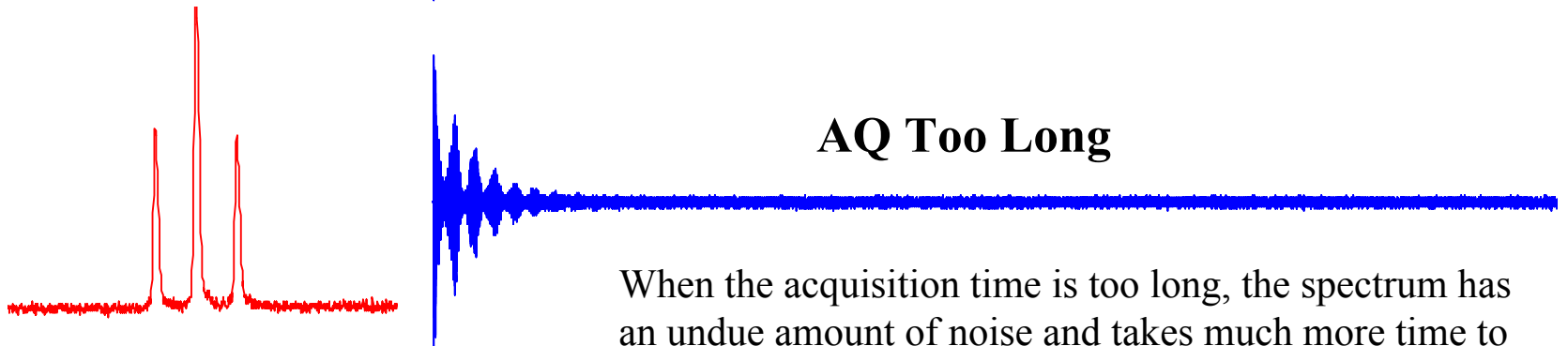


AQ Too Short

When the acquisition time is too short, the spectrum will have $(\sin x) / x$ distortions at the base of the peaks.



AQ Chosen Appropriately



AQ Too Long

When the acquisition time is too long, the spectrum has an undue amount of noise and takes much more time to collect.

Intelligent Choice of Acquisition Parameters – Pulse Duration

If the spin system is at equilibrium, one will always get the largest signal by using a 90° pulse in a single scan. This is not necessarily the most time efficient way to collect data when multiple scans are required. The optimum pulse flip angle, α_{opt} , is given by:

$$\alpha_{opt} = \arccos(\exp(-(AQ + D1)/T_1))$$

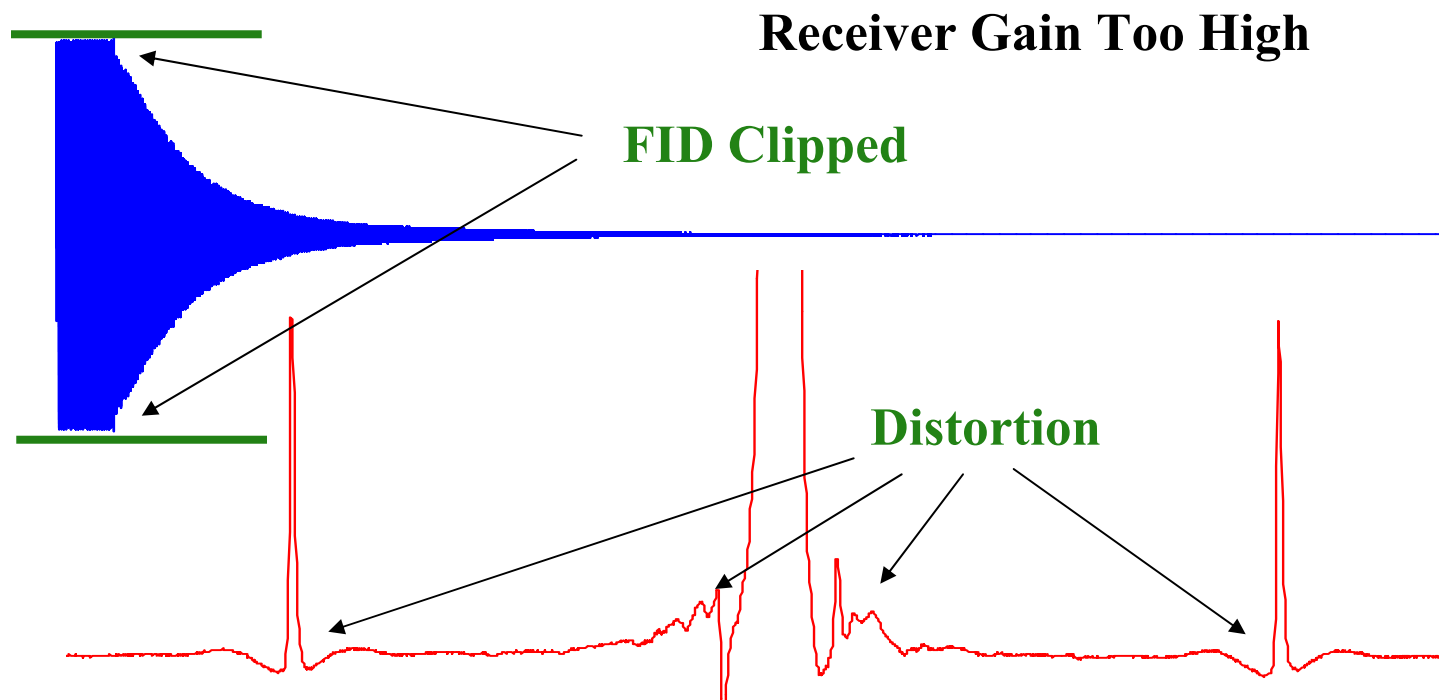
If we choose AQ such that $AQ = 5T_2^*$ and $D1 = 0$ so as to maximize the duty cycle of the receiver, we can set up the following table for a spectrum whose sharpest line is 0.5 Hz.

T_1	α_{opt}
1 sec	88°
5 sec	58°
20 sec	31°
60 sec	18°
1 hour	2°

Collecting data in this way will generate the largest possible signal per unit of data collection time when many scans have to be accumulated.

Intelligent Choice of Acquisition Parameters – Receiver Gain

The receiver gain of an NMR spectrometer is much like the volume control of a radio and it determines the maximum dynamic range of the NMR spectrometer. If the receiver gain is not set appropriately the NMR spectrum will either be distorted or will have a lower than necessary signal to noise ratio.



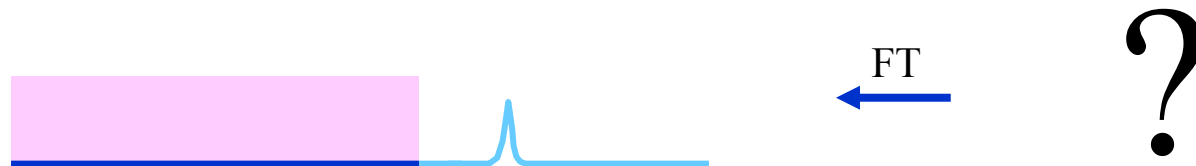
When the receiver gain is set too low, the spectrum is then free of distortion but the signal to noise ratio is lower than it can be.

Intelligent Choice of Acquisition Parameters – Spectral Width

The spectral width and carrier frequency should be set such that the entire spectrum of interest fits within the spectral width.



What happens when the spectral window does not include the spectrum?



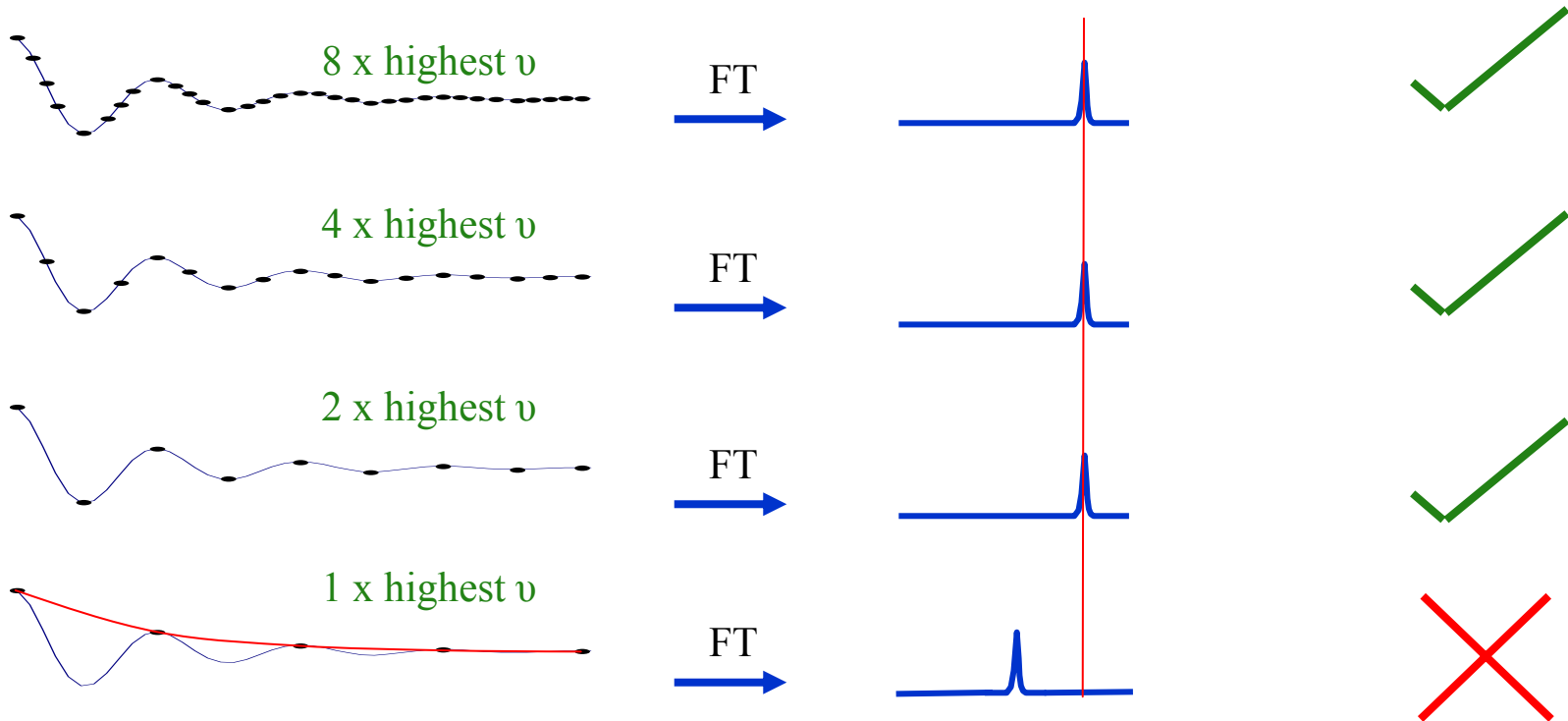
In order to answer this question one must consider the way the signals are filtered and digitized by the NMR spectrometer and be aware of the Nyquist Sampling Theorem.

The Nyquist Sampling Theorem

The Nyquist sampling theorem states that in order to represent time domain functions digitally, one must sample the signal at a rate of at least twice the highest frequency in the function. For an NMR spectrum acquired with quadrature detection, the highest frequency is $\frac{1}{2}$ the spectral width, SW. The theorem can therefore be stated as follows:

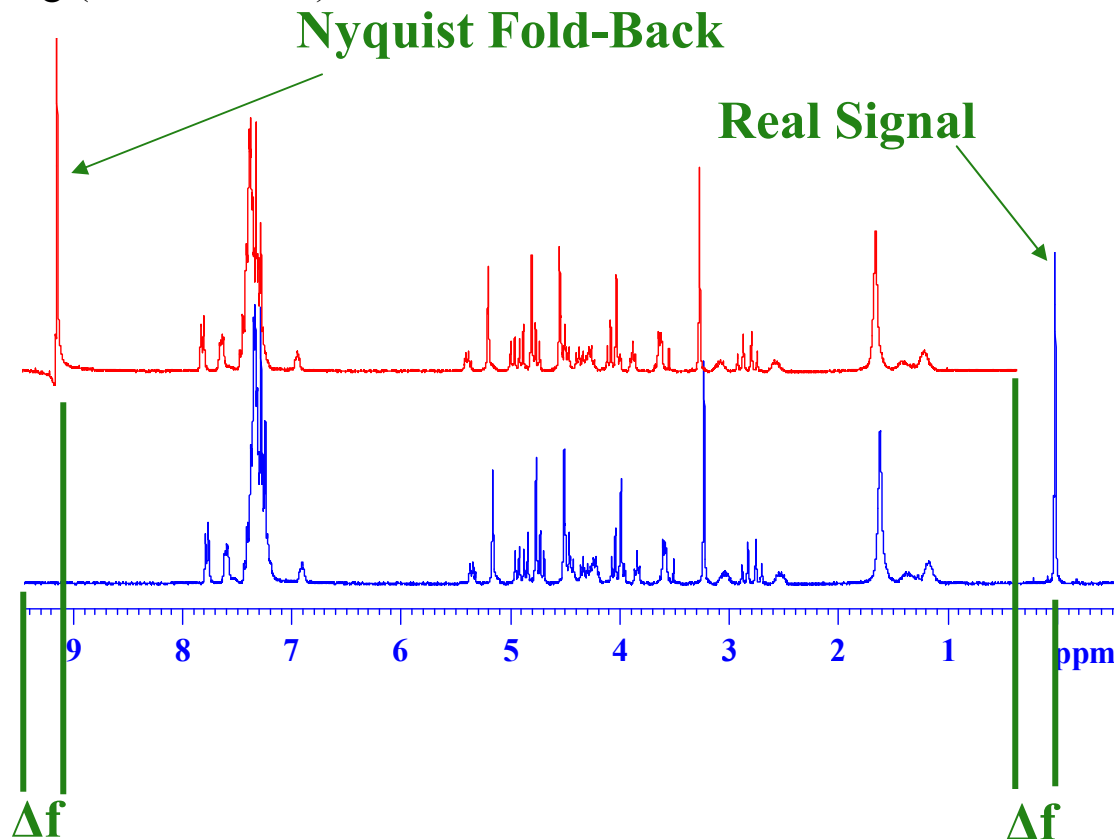
$$1 / DW \geq SW$$

Where DW is the dwell time – the time interval between points sampled in the FID.



Spectral Width and the Nyquist Sampling Theorem

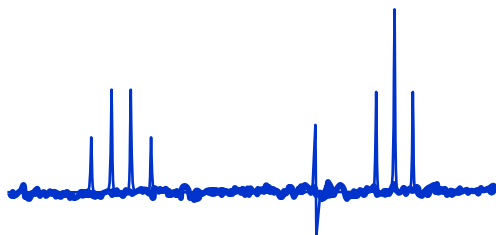
When one sets the spectral width on an NMR spectrometer, the dwell time is calculated by the spectrometer such that $DW = 1 / SW$. Also, the filter bandwidth is set at approximately $1.2 * SW$ to help filter out noise from outside of the spectral width which would be folded (or aliased) into the spectrum. If one uses a spectral width too small to contain all of the NMR signals, the sampling rate is not sufficiently fast to adequately digitize the FID and one sees aliasing (or fold-backs).



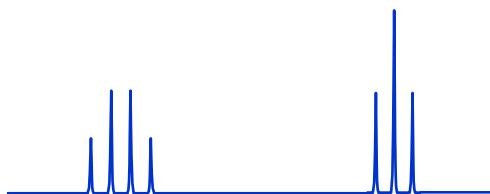
Any signals within the filter bandwidth outside of the range $\nu_0 \pm \frac{1}{2} SW$ will be folded into the spectrum. A resonance outside of the spectral width (but within the filter bandwidth) by Δf will be folded into the opposite side of the spectrum Δf away from the edge.

Digital Filtering

Modern NMR spectrometers have digital filters which supplement the analog filters to filter out unwanted signals (but more importantly unwanted noise) from outside of the desired spectral width. Digital filtering removes Nyquist fold-backs and improves signal to noise ratio.



Spectrum with Nyquist fold-back and folded in noise.

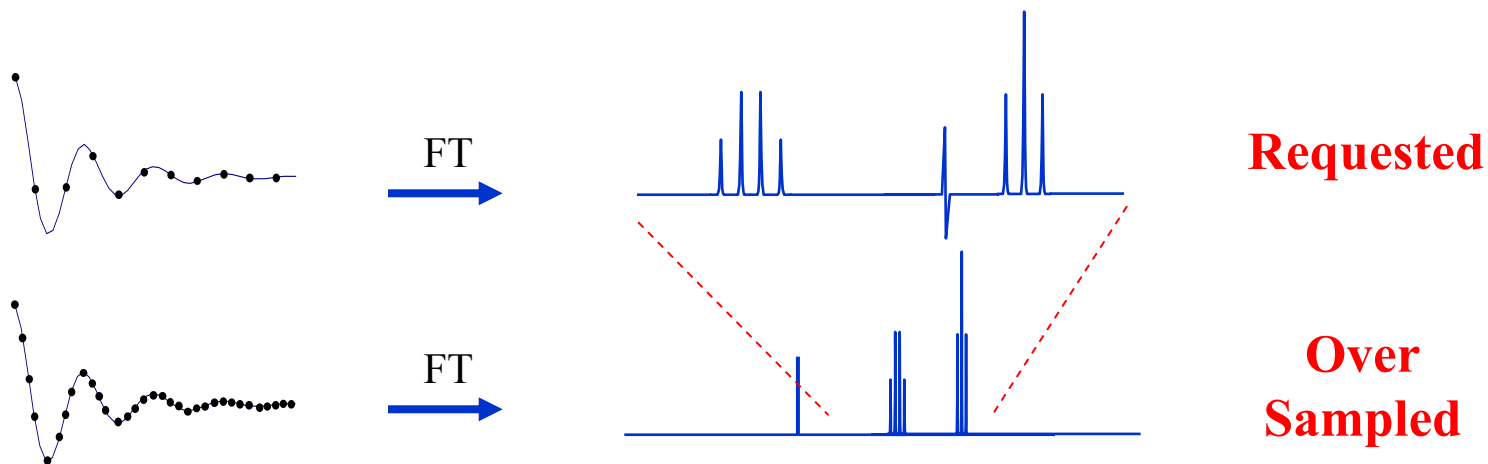


Digitally filtered spectrum

Digital filtering is now the standard on NMR spectrometers. It is carried out in three steps prior to Fourier transformation: over sampling, application of the digital filter and decimation.

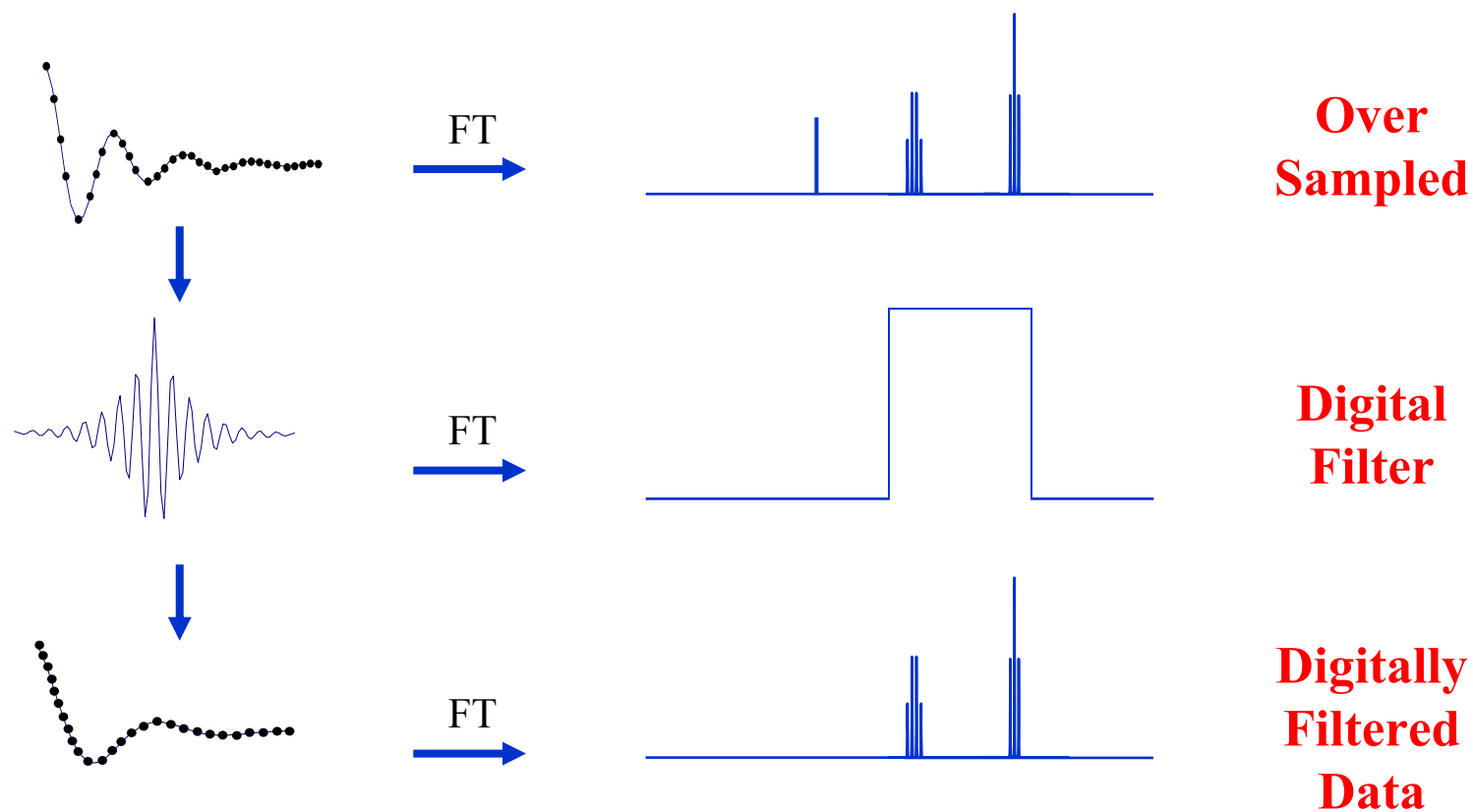
Digital Filtering – Over Sampling

Over Sampling – When a particular spectral width (SW), acquisition time (AQ) and number of acquisition points (NP) are requested by the user, the spectrometer will calculate the required dwell time (DW) to fulfill the Nyquist sampling theorem. When an FID is over sampled, the spectrometer minimizes the dwell time (or maximizes the spectral width) and increases the number of data points according to the hardware limit of the analog to digital converter.



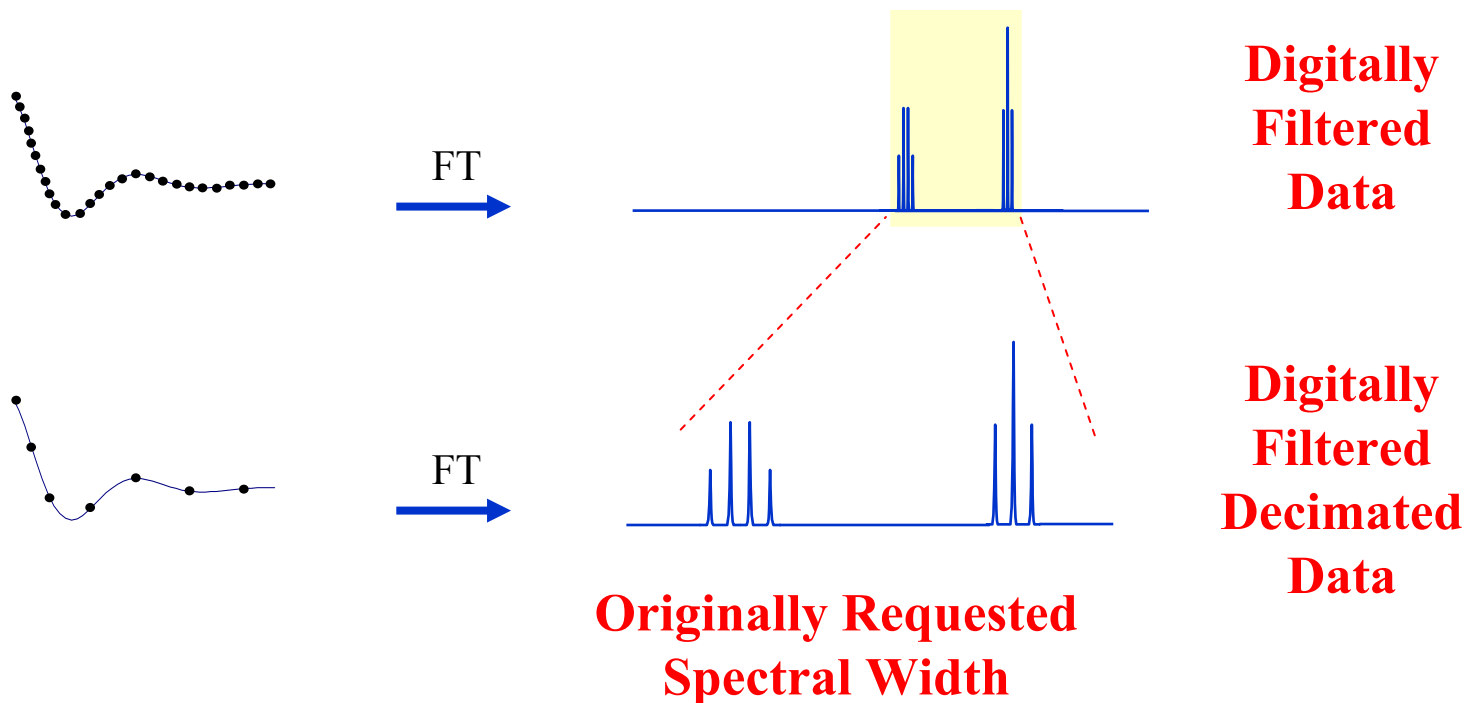
Digital Filtering – Applying the Digital Filter

Digital filters are applied mathematically to remove all frequencies greater than $\frac{1}{2}$ the requested spectral width from the FID. Although the mathematics is non-trivial, it involves treating the over-sampled FID with a function similar to a $(\sin(x))/x$ which will eliminate the high frequencies.



Digital Filtering – Decimation

Once the FID is digitally filtered, it is reduced in size such that the number of data points, the spectral width and the dwell time represent the values originally requested.



The digitally filtered decimated data provides the user with a spectrum of the requested spectral width free of Nyquist fold-backs and a high signal to noise ratio.

Resolution

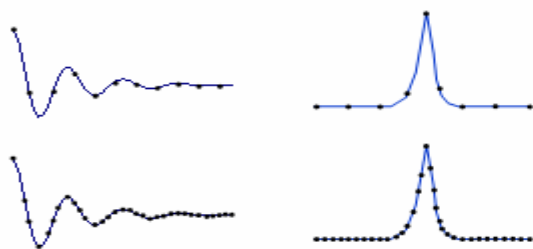
The true definition of resolution is the ability to distinguish or resolve two closely spaced peaks. Digital resolution on the other hand is defined as the number of points per unit frequency in the “real” Fourier transformed spectrum. Remember that the total number of points collected in the time domain, NP, is the sum of points in both the real and imaginary FIDs so the total number of points is split between the real and imaginary Fourier transformed spectra. The “real” spectrum contains NP/2 points. We can then define the digital resolution as:

$$\text{DRES} = \frac{1}{2} (\text{NP} / \text{SW})$$

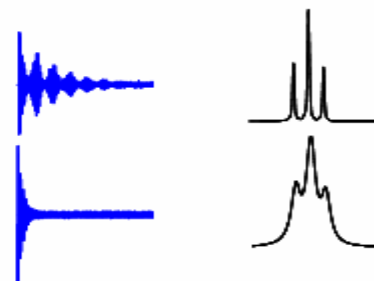
Since $\text{SW} = 1/\text{DW}$ and $\text{DW} = 2 \text{AQ} / \text{NP}$, we can rewrite the definition of digital resolution as:

$$\text{DRES} = \text{AQ}$$

The digital resolution of an NMR spectrum increases directly with acquisition time. The true resolution in an NMR spectrum depends, among other things, on the sample and the magnet homogeneity however, the longer lived the FID, the sharper the lines in a spectrum and the higher the probability of resolving two closely spaced signals.



Digital Resolution

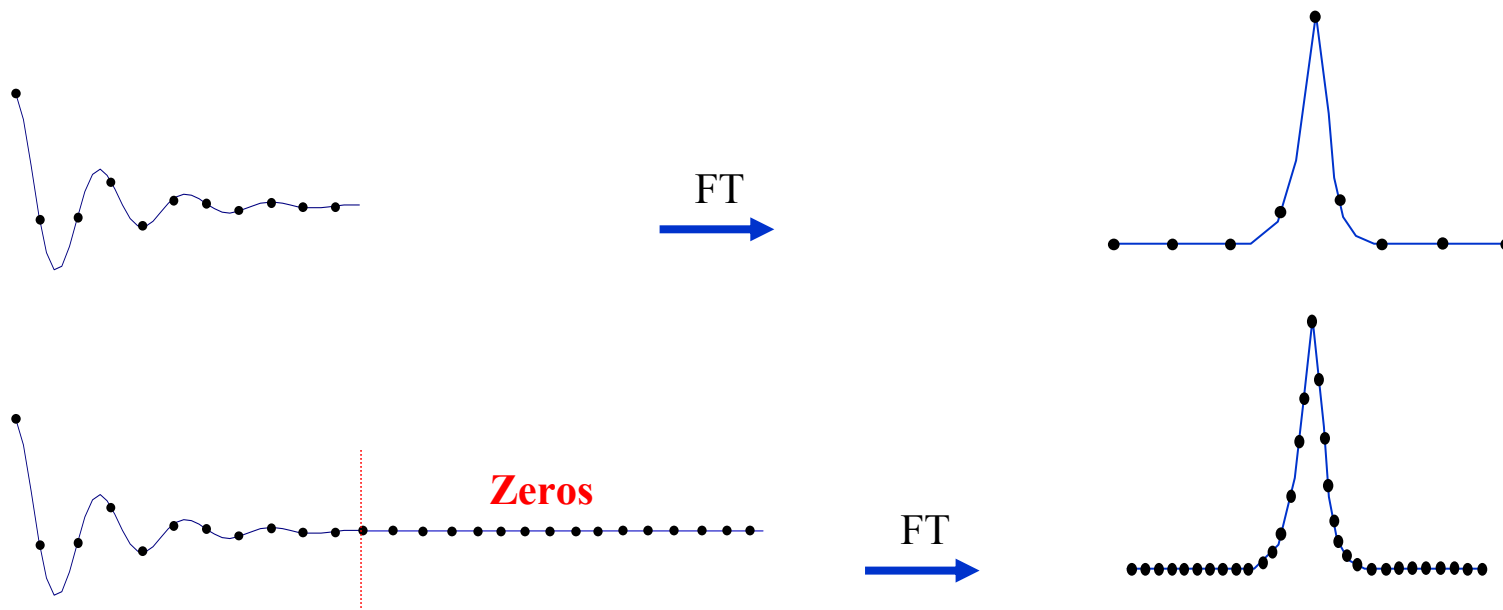


True Resolution

Data Processing – Zero Filling

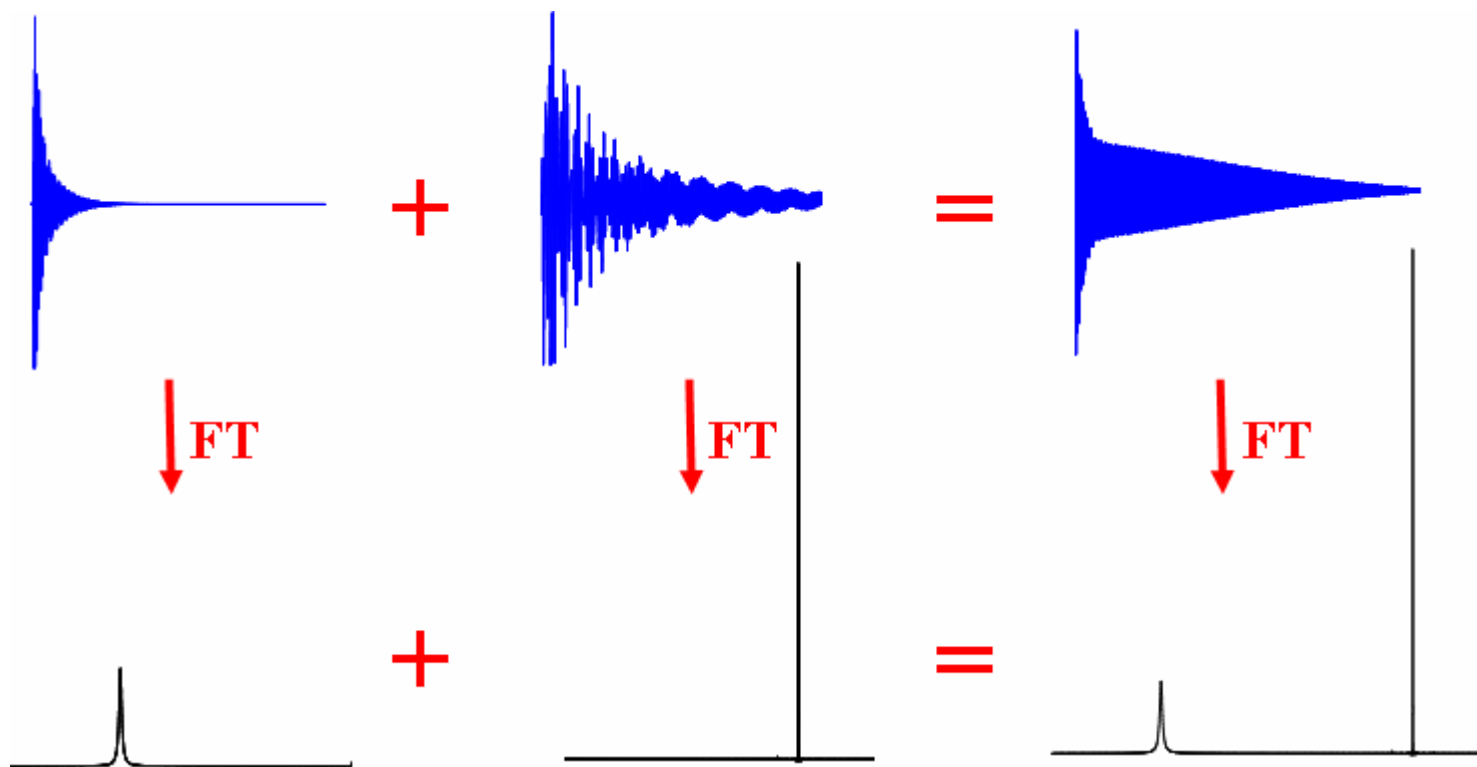
One can manipulate the time domain NMR data prior to Fourier transformation in order to enhance or diminish specific spectroscopic features.

Zero filling is the addition of zero intensity data points to the end of the FID. Zero filling artificially increases the acquisition time thereby improving the digital resolution in the Fourier transformed spectrum. It adds no new information to the spectrum. The apparent improvement in the data is cosmetic.



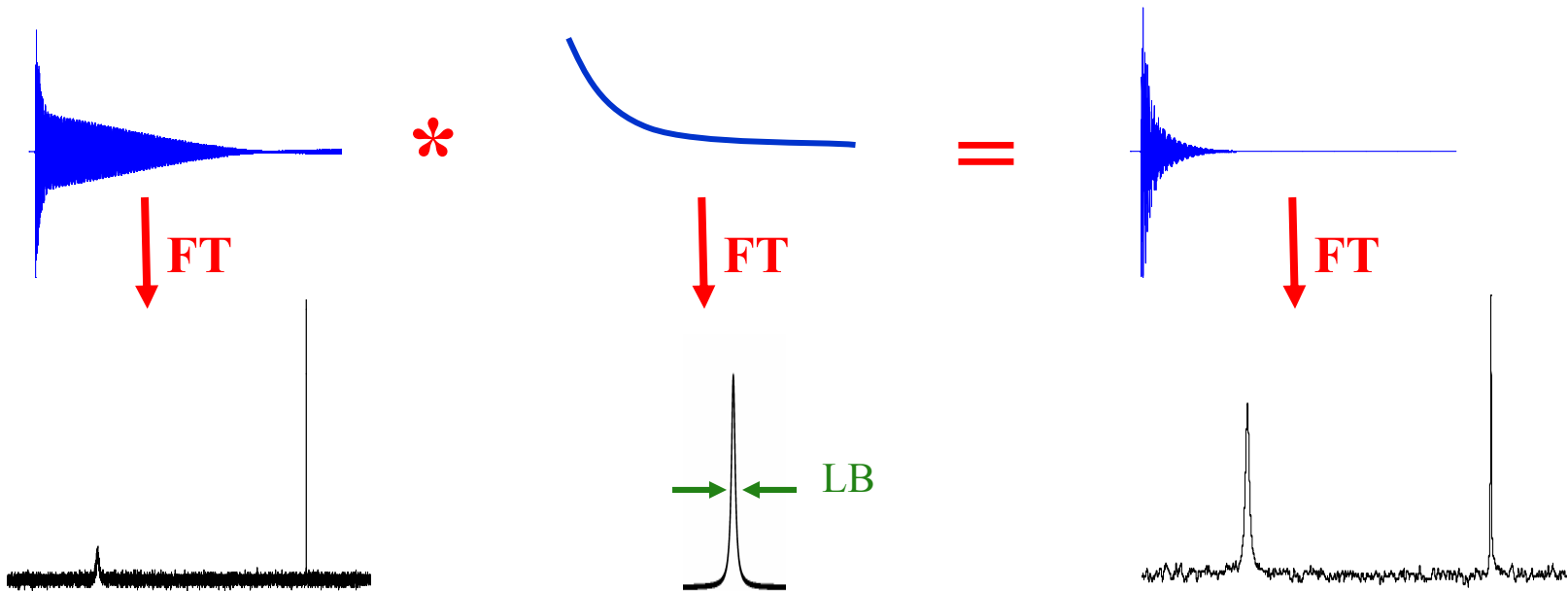
Data Processing – Information Content of the FID

Since time and frequency are reciprocals of one another, one expects that spectral features with small frequency distributions (i.e. sharp lines) require longer times in the FID to be defined. Conversely, spectral features with large frequency distributions (broad lines) require only short times in the FID to define them.



Data Processing – Line broadening

One can emphasize the broad features of an NMR spectrum by multiplying the FID by a window function which emphasizes the initial portion of the FID.



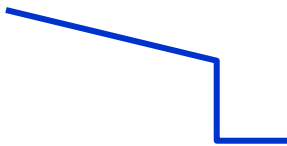
The exponential window function emphasizes broad features, broadens sharp features, increases the signal to noise ratio and maintains a Lorentzian line shape. Exponential line broadening is used routinely in FT NMR. One usually chooses a parameter, LB, (the width at half height of the Lorentzian related by FT to the exponential window function) to match the width at half height of the sharpest line of interest in the NMR spectrum. Choosing LB in this manner will improve the signal to noise ratio with a minimum loss in resolution.

Data Processing – Line broadening

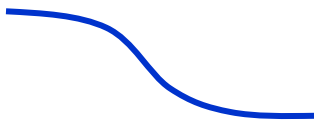
Other window functions can also be used for line broadening however these will distort the line shape from the natural Lorentzian shape.



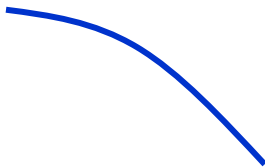
Box Function



Trapezoid



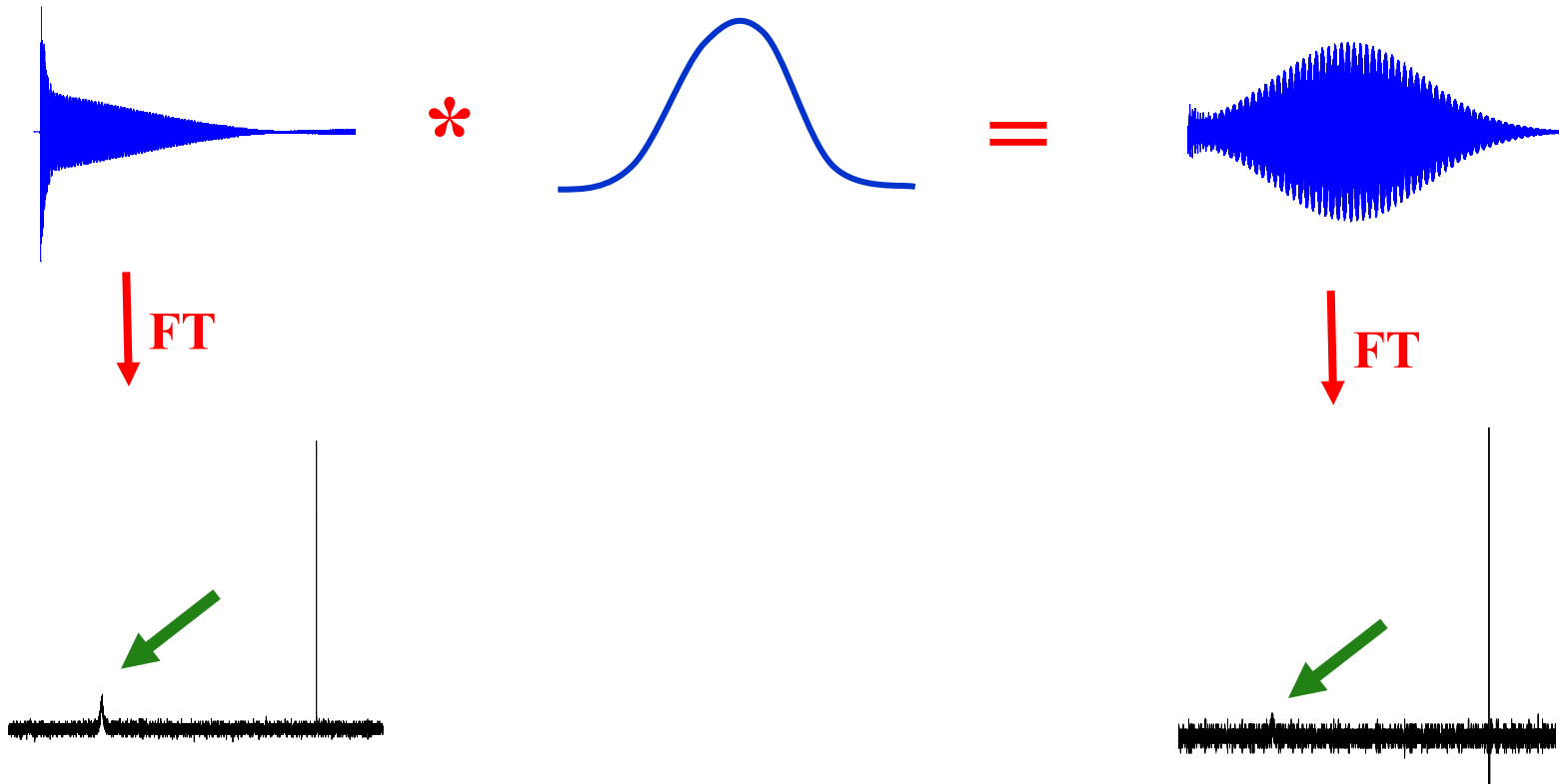
Half Gaussian



Sine Bell

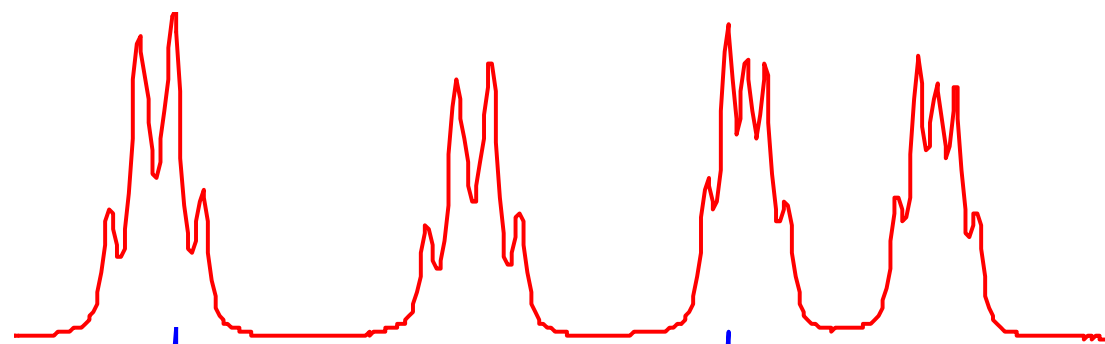
Data Processing – Resolution Enhancement

One can use a window function to emphasize later times in the FID. This will improve the resolution in the NMR spectrum.

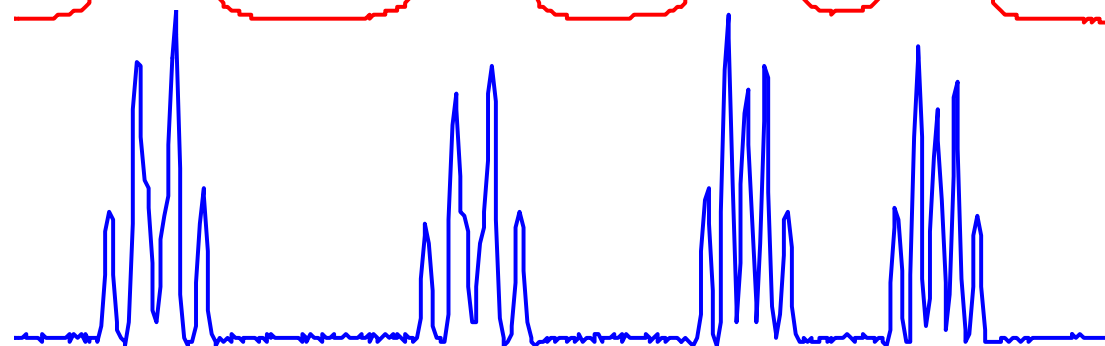


Resolution enhancement functions will increase the resolution, decrease the signal to noise ratio, diminish broad features and distort line shapes.

Example of Resolution Enhancement



Untreated Data

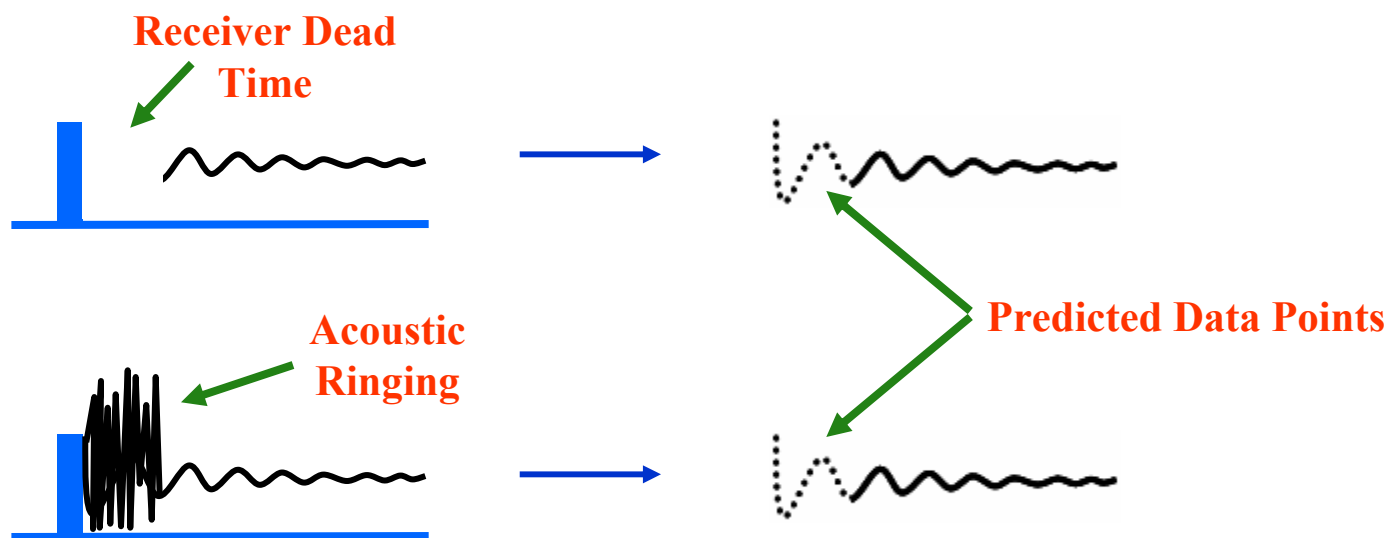


Data Treated With a
Gaussian Function

5.20 5.15 5.10 ppm

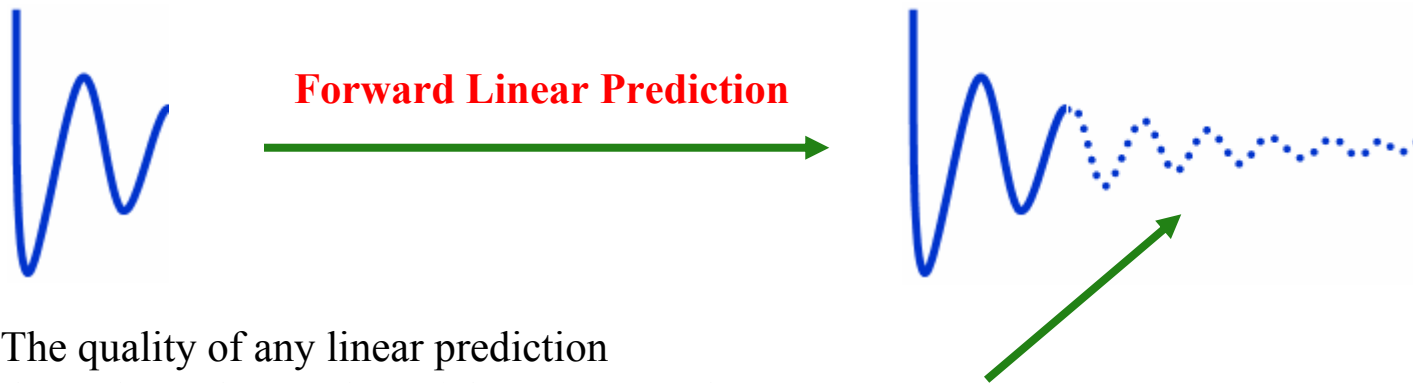
Backward Linear Prediction

There is often a problem with collecting NMR data (especially for solids) in that there is a dead time of 2 – 20 μsec associated with the receiver immediately following a pulse. If one is observing broad signals, then some of the data near the beginning of the FID is lost during this time and as a result the spectrum may suffer from severe phasing and baseline problems. There can also be problems with acoustic ringing in the NMR probe immediately following a pulse rendering the first few points of the FID meaningless. There are algorithms written into the NMR software to predict the behavior of an FID at times before the recorded acquisition in order to calculate the data that “should” exist at time zero (immediately following the pulse). These routines use the known observed behavior of the FID during the acquisition time to make the prediction.



Forward Linear Prediction

In forward linear prediction, the known observed data points of the FID are used to predict how the signal will behave after the receiver has been turned off. Forward linear prediction is used to improve resolution and decrease experiment times.



The quality of any linear prediction depends on the number of data points and on the number of data points used for the prediction. This technique must be used with caution and should always be noted in any published data.

**Predicted
Data
Points**

Chemical Interactions

Nuclei are exposed to magnetic fields other than B_0 and $B_1(t)$. The electrons and neighboring nuclei in molecules perturb the applied magnetic fields experienced by the nucleus. These perturbations contain chemical information and affect the NMR spectrum. It is these perturbations that make NMR such a useful, widespread technique.

Chemical Shielding

Circulating electrons in molecules behave much like currents in wires in that they generate magnetic fields. As a result, a nucleus will experience the main magnetic field, B_0 , modified by the effect of the local electronic environment in the molecule. The magnetic field, B , experienced by a nucleus can be written:

$$\mathbf{B} = \mathbf{B}_0 (1 - \sigma)$$

where σ is a shielding constant dependant on the local electronic environment around the nucleus. Both the magnitude and the sign of σ are determined by the molecular structure in which the nucleus resides. The Larmor equation must then be modified to give:

$$\nu = (\gamma/2\pi) \mathbf{B}_0 (1 - \sigma)$$

In practice, the shielding constant, σ , is inconvenient to measure, so the chemical shift, δ , is measured in terms of the difference in frequency between the nucleus of interest, ν , and the frequency of a reference compound, ν_{REF} .

$$\delta = 10^6 ((\nu - \nu_{\text{REF}}) / (\nu_{\text{REF}}))$$

δ is independent of B_0 and is reported in parts per million (ppm). δ and σ are related as follows:

$$\delta = 10^6 \left(\frac{\sigma_{\text{REF}} - \sigma}{1 - \sigma_{\text{REF}}} \right)$$

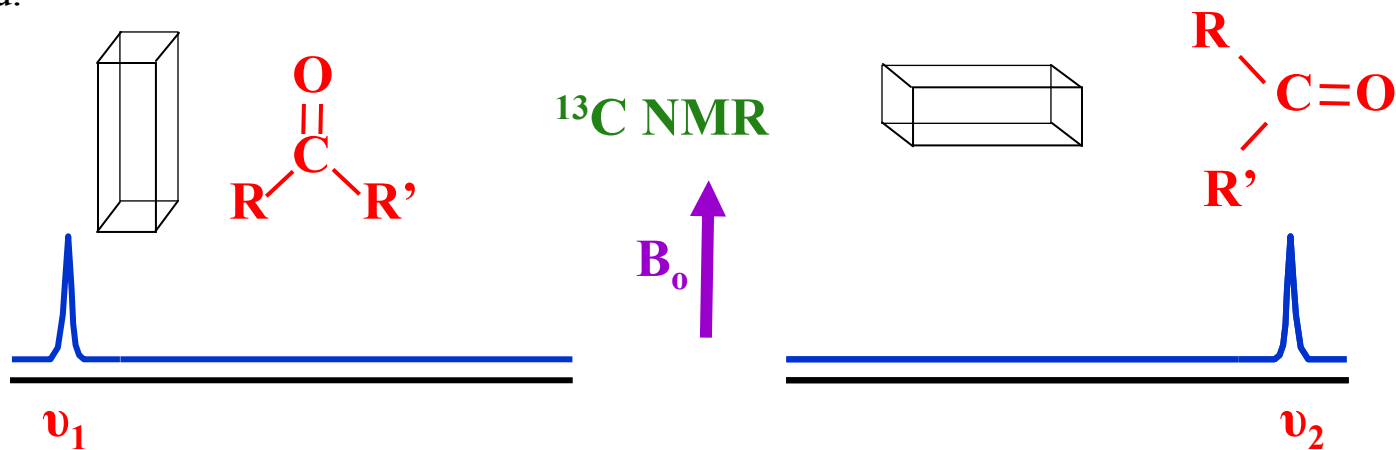
or

$$10^6 (\sigma_{\text{REF}} - \sigma)$$

since $\sigma_{\text{REF}} \ll 1$.

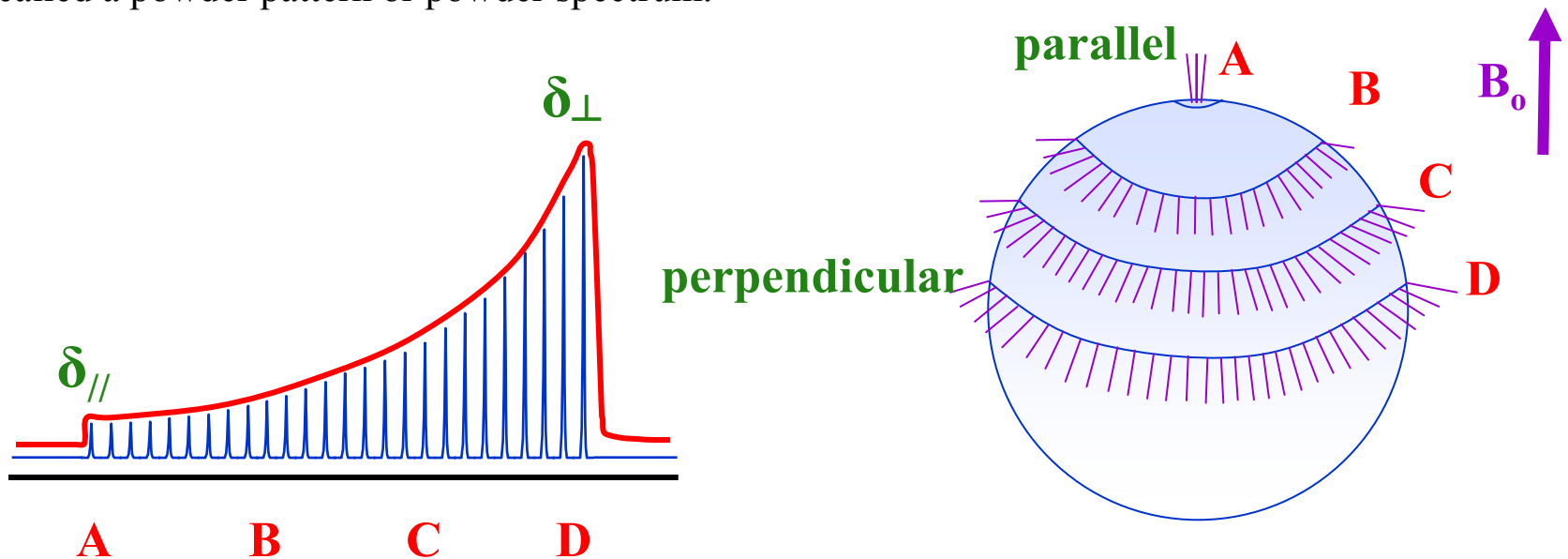
The above relationships are true for liquids or solutions where rapid isotropic molecular motion averages out any anisotropy.

If a molecule, fixed rigidly in a single crystal, is placed in a magnetic field, the frequency of the resonance depends on the orientation of the molecule with respect to the magnetic field.



Chemical Shielding

If a powder is placed in a magnetic field, all orientations of the crystallites (and molecules) are represented and one observes an envelope containing all possible frequencies. This is called a powder pattern or powder spectrum.



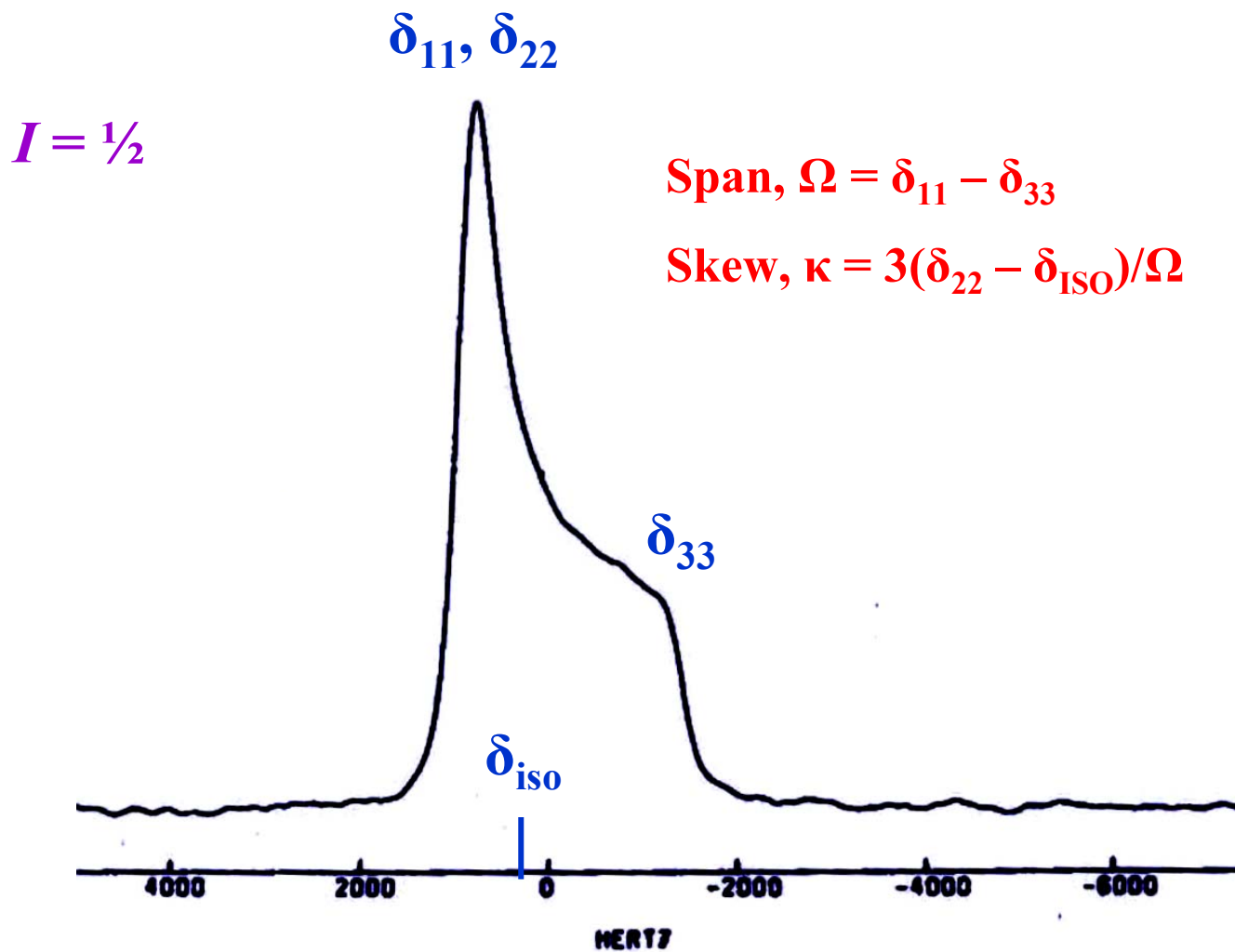
In general, the chemical shielding interaction must be described by a second rank tensor (i.e. a 3x3 matrix) to account for all of the symmetry.

$$\underline{\delta} = \begin{bmatrix} \delta_{11} & \delta_{12} & \delta_{13} \\ \delta_{21} & \delta_{22} & \delta_{23} \\ \delta_{31} & \delta_{32} & \delta_{33} \end{bmatrix} \quad \delta_{ij} = \delta_{ji}$$

In solution where rapid isotropic motion occurs, we see an average, δ_{iso} , where

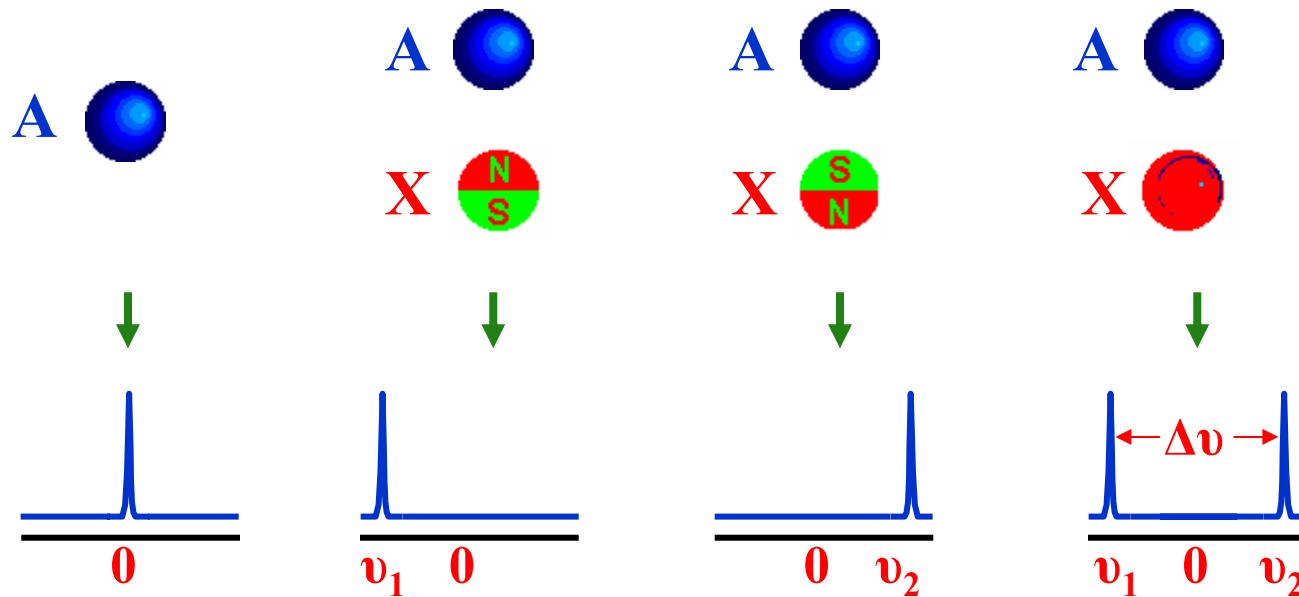
$$\delta_{\text{iso}} = \frac{1}{3} \text{Tr } \underline{\delta} = \frac{1}{3} (\delta_{11} + \delta_{22} + \delta_{33})$$

^{207}Pb spectrum of Solid Powdered PbNO_3



Dipolar Coupling

Every nucleus with $I > 0$ has a magnetic dipole and behaves like a small bar magnet generating local magnetic fields. These fields influence the resonance frequencies of the neighboring nuclei. Consider the NMR spectrum of a spin $I = \frac{1}{2}$ nucleus, A.



Dipolar Coupling

If A and X are hetero-nuclei, then the dipolar splitting, $\Delta\nu$, is:

$$\Delta\nu = (\hbar/2\pi) (\mu_0/4\pi) \gamma_A \gamma_X (1/r_{AX}^3) (3 \cos^2 \theta - 1) = R (3 \cos^2 \theta - 1)$$

If A and X are homo-nuclei, then the dipolar splitting, $\Delta\nu$, is:

$$\Delta\nu = (3\hbar/4\pi) (\mu_0/4\pi) \gamma^2 (1/r_{AX}^3) (3 \cos^2 \theta - 1) = (3/2) R (3 \cos^2 \theta - 1)$$

Where:

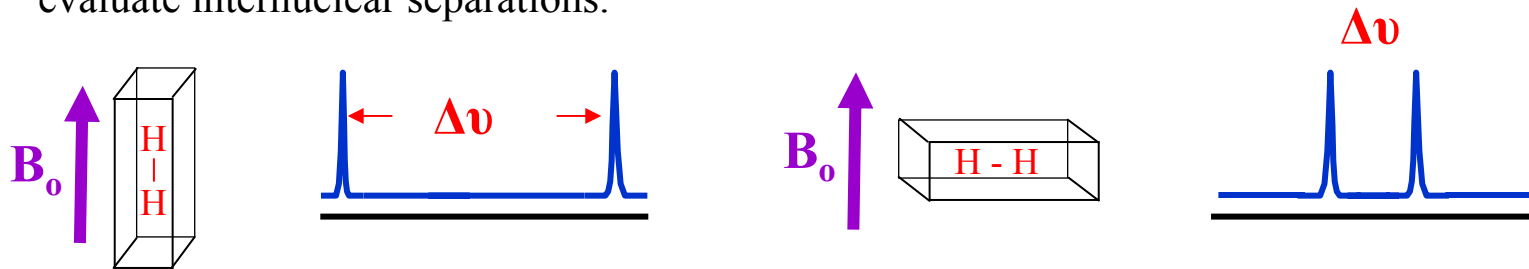
$$(\mu_0/4\pi) = 1 \cdot 10^{-7} \text{ kg m s}^{-2} \text{ A}^{-2}$$

r_{AX} = internuclear vector

θ = angle between the internuclear vector, r_{AX} and the magnetic field B_0 .

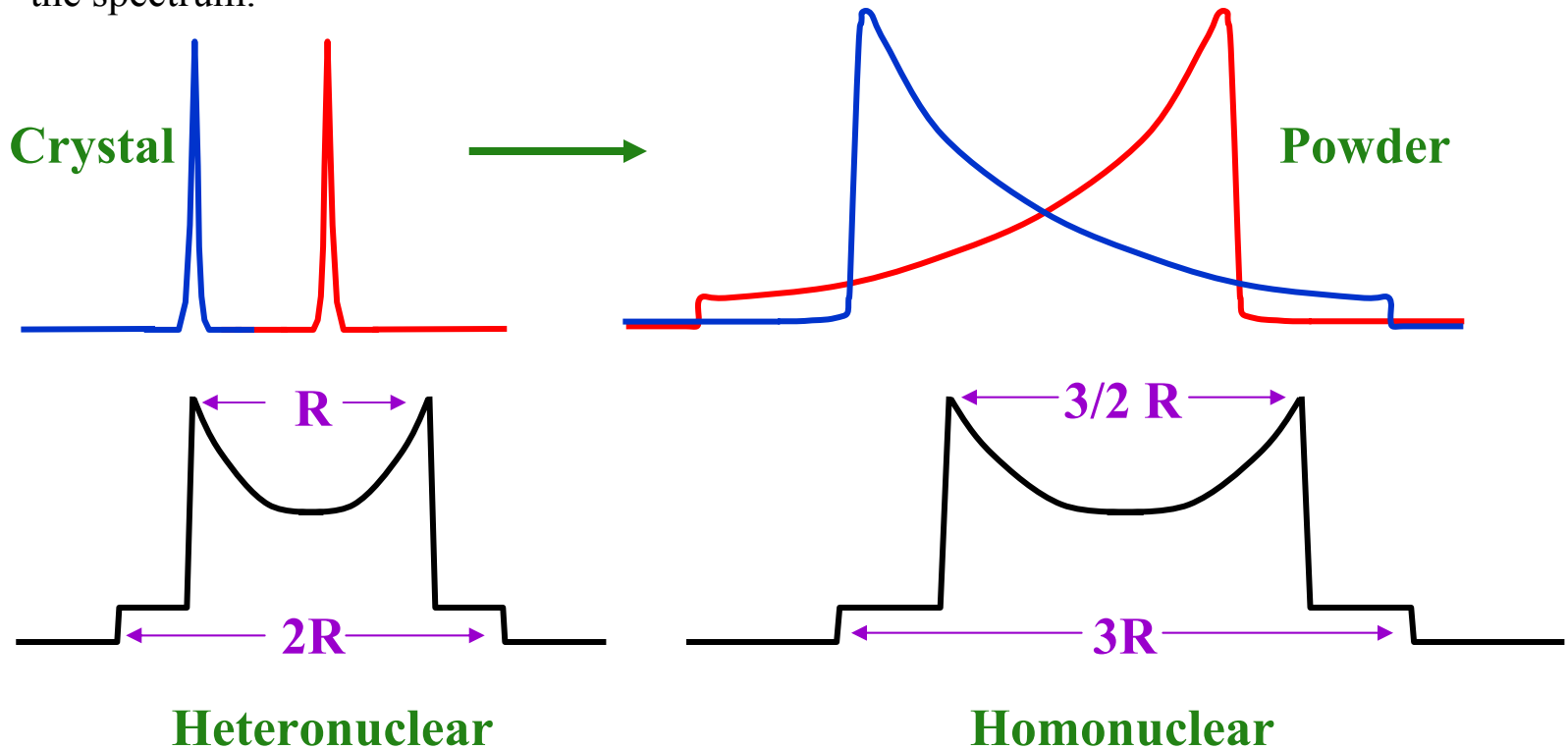
R = Dipolar coupling constant.

For single crystals, doublets are observed for the nuclei of a spin $I = 1/2$ pair. The frequency separation depends on the orientation of the crystal in the magnetic field and can be used to evaluate internuclear separations.



Dipolar Coupling

In powder samples, where all values of θ are represented, we get a powder line shape often called a “Pake Doublet”. The internuclear distance in the spin pair can be calculated from the spectrum.

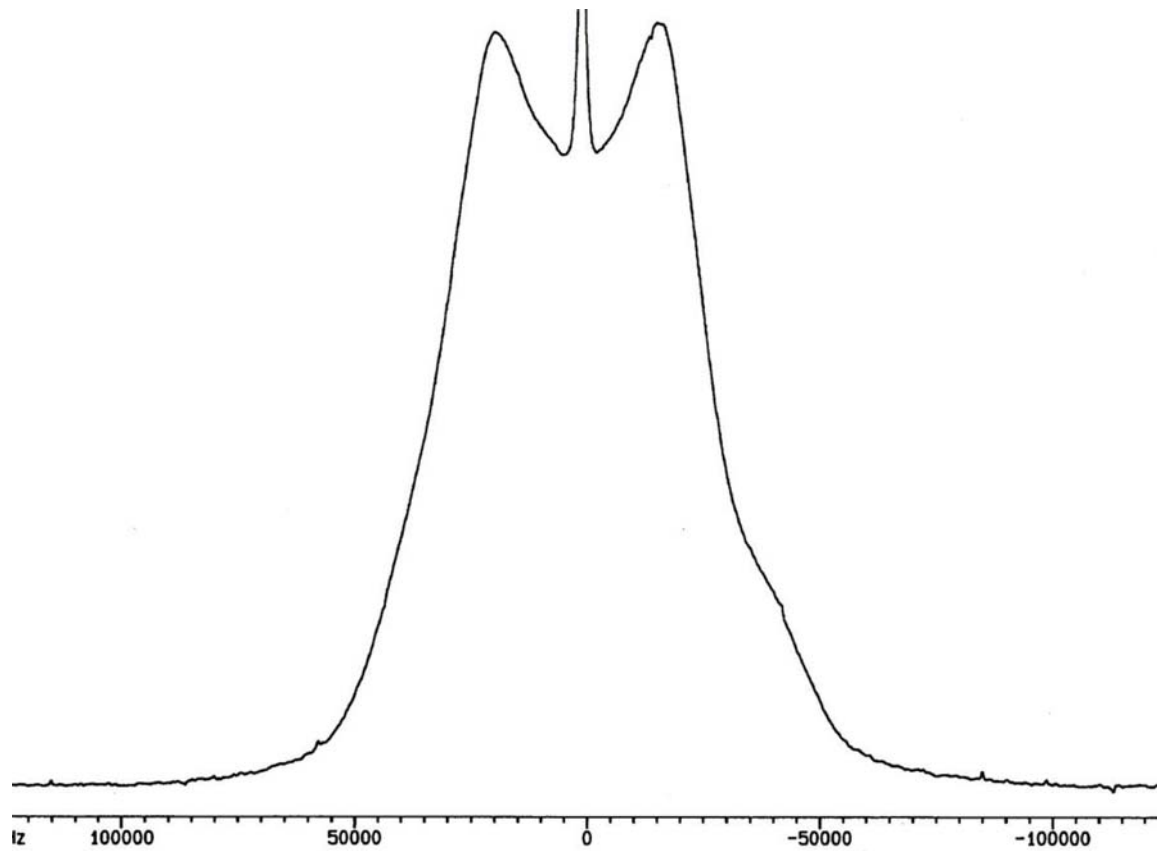


In solution where rapid isotropic motion occurs, the dipolar coupling is averaged to zero and is not directly observed in the spectrum. The dipolar coupling does however influence relaxation rates and NOE's. It can be exploited indirectly in the study of solutions to obtain geometric and structural information.

Dipolar Coupling

^1H NMR

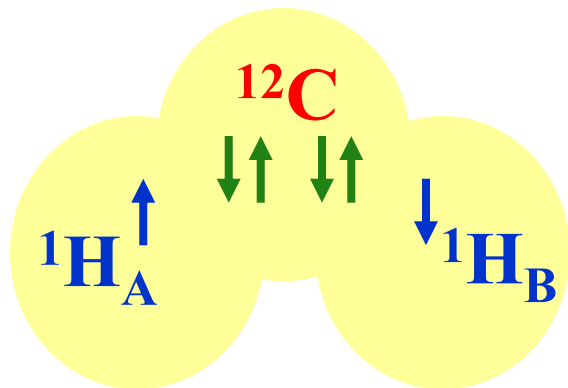
Powdered Calcium Sulfate Dihydrate (Gypsum)



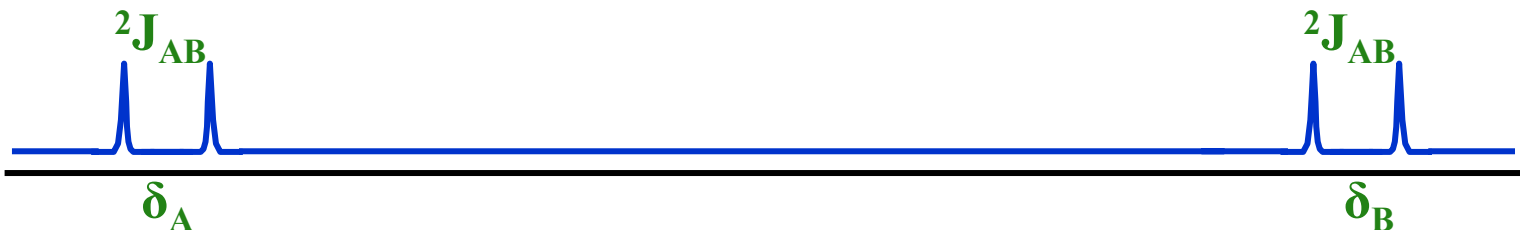
J Coupling

Unlike direct dipolar coupling which is mediated through space, J coupling is mediated through the electrons in chemical bonds. Most often it is isotropic in nature having no dependence on the orientation of the molecules with respect to the magnetic field. It can be thought of (albeit over simplistically) as follows:

Imagine the two protons of a methylene group with a delocalized “molecular orbital” containing 4 electrons.

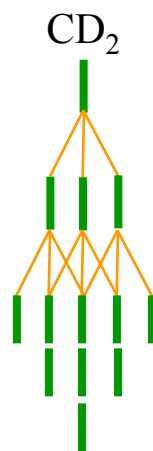
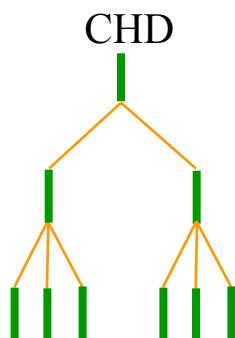
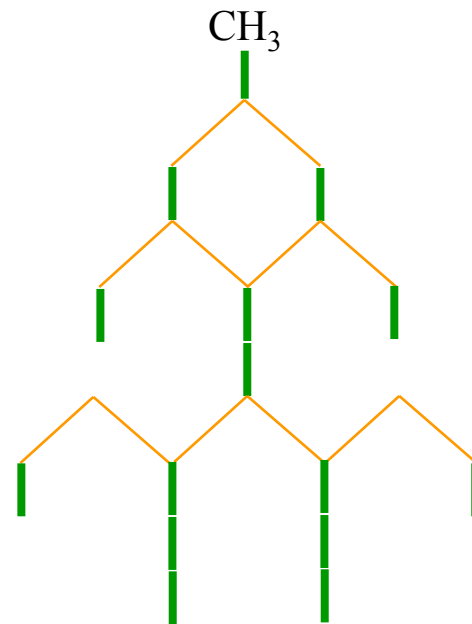
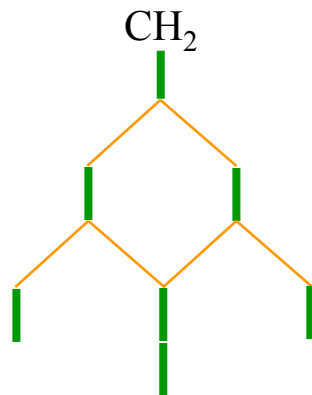
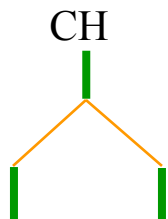


If proton A is “up” then the closest electron to it will favor being “down”. Since pairs of electrons are always of opposite spin, the next closest electron will favor being “up”. The next one will favor the spin “down” orientation and the fourth electron will favor being spin “up”. Since proton B is beside a spin “up” electron, it will favor being spin “down”. The energy differences are very small with respect to the thermal energy and hence proton A will “see” proton B in both the spin “up” and spin “down” orientations with almost equal probability. Proton A will therefore be split into a doublet, as will proton B.

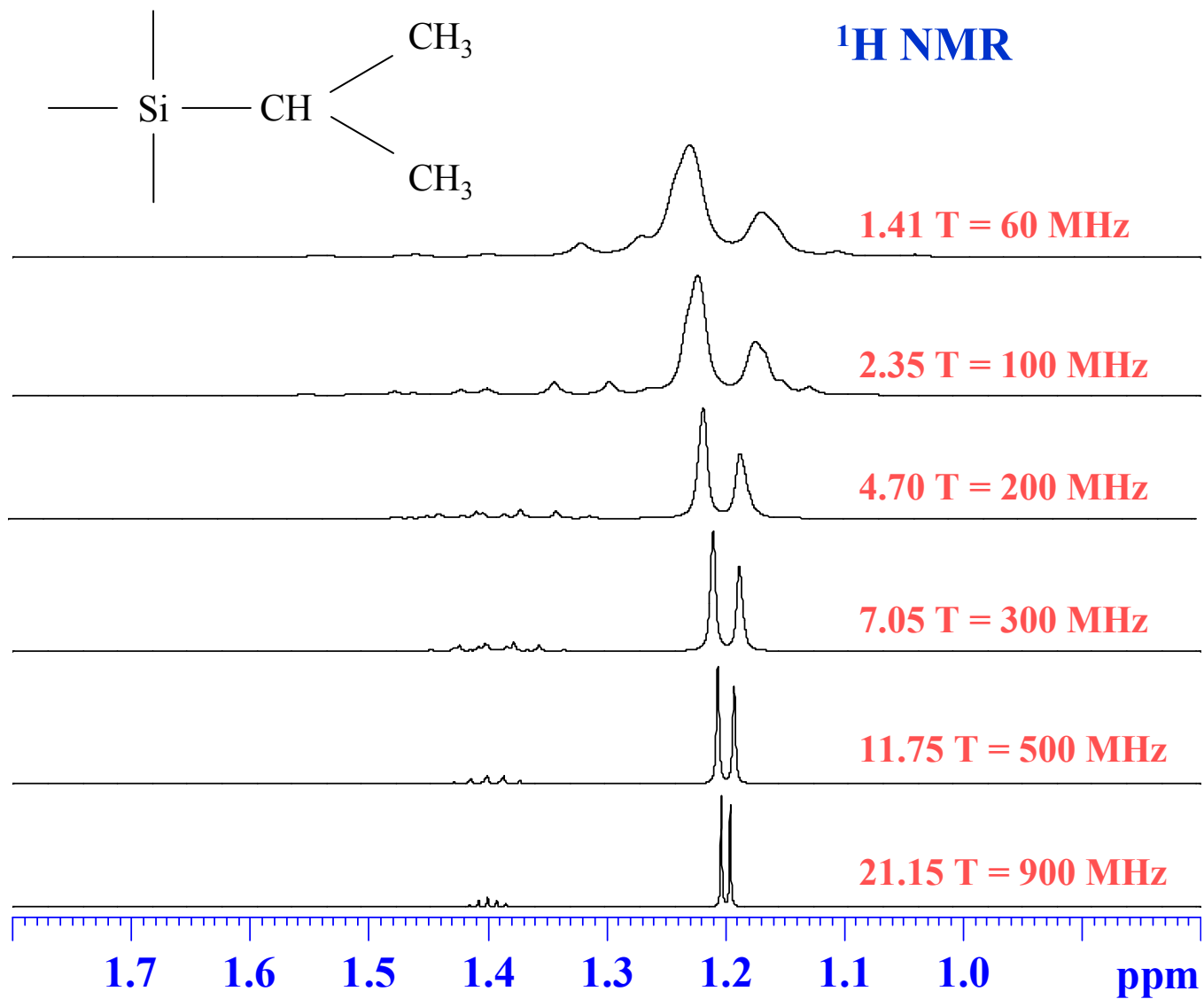


J Coupling

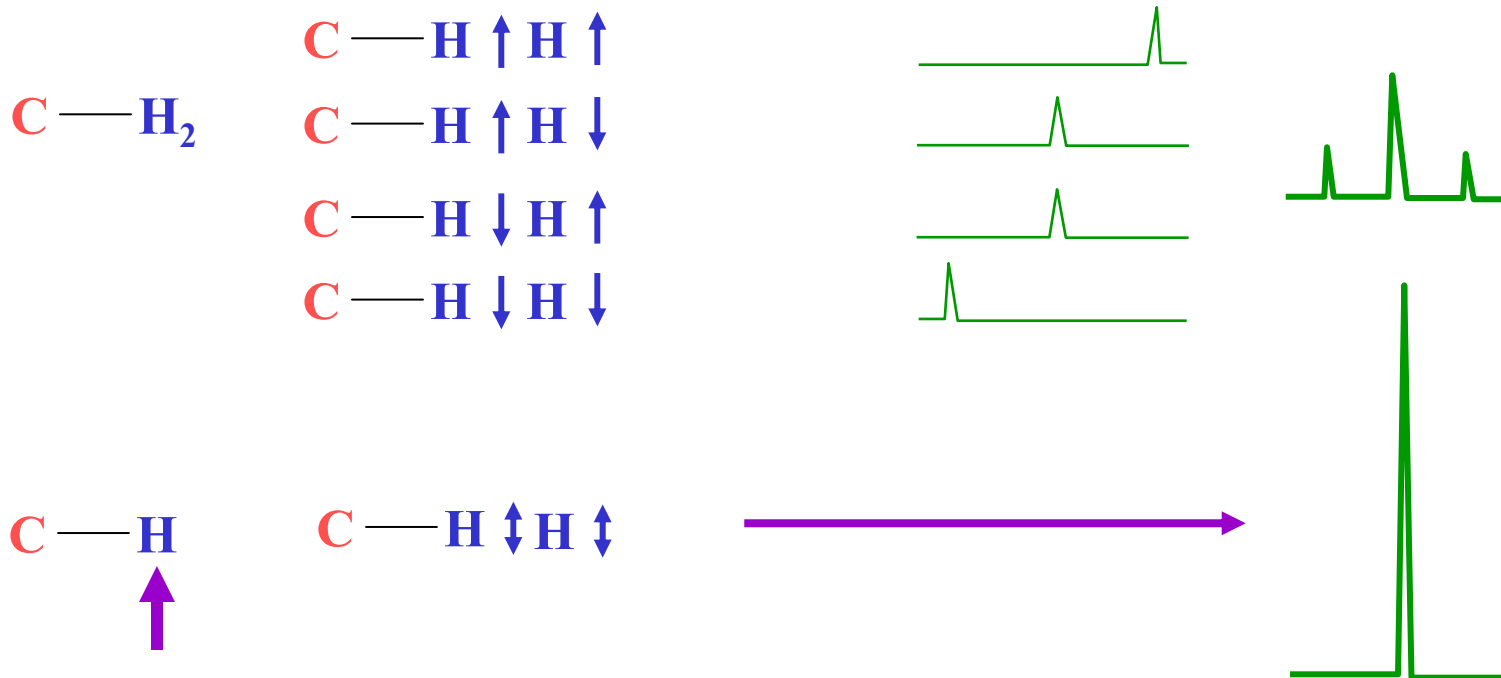
^{13}C NMR



Effect of the Magnetic Field Strength on a Spectrum



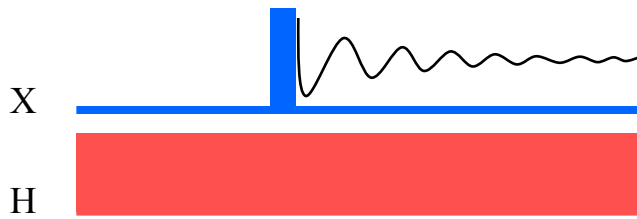
Coupling and Decoupling



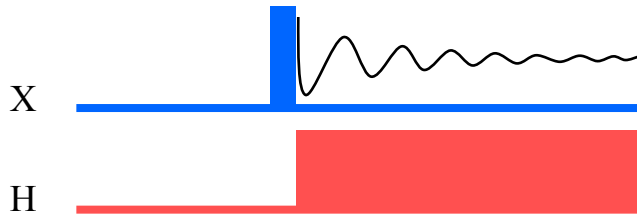
When protons are continuously irradiated they go from “spin up” to “spin down” on a very fast time scale such that the carbon “sees” an average state for each proton. Not only does this simplify the spectrum but it also leads to a very significant signal to noise ratio improvement.

Broadband Heteronuclear Decoupling

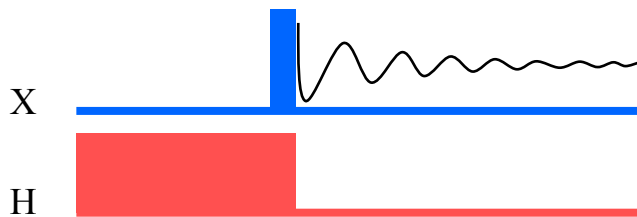
$X = {}^{13}\text{C}, {}^{31}\text{P}, {}^{15}\text{N}, \dots$



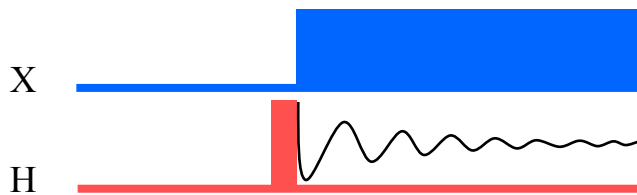
100 % Duty Cycle Proton Decoupling



Inverse Gated Proton Decoupling

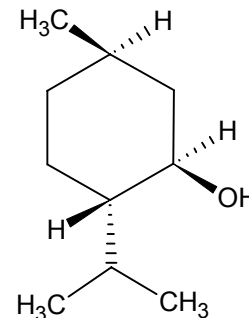


Gated Proton Decoupling



Inverse Gated X Decoupling

Examples of Proton Decoupling - Menthol



¹³C NMR

No Decoupling



Gated Decoupling



Inverse Gated Decoupling

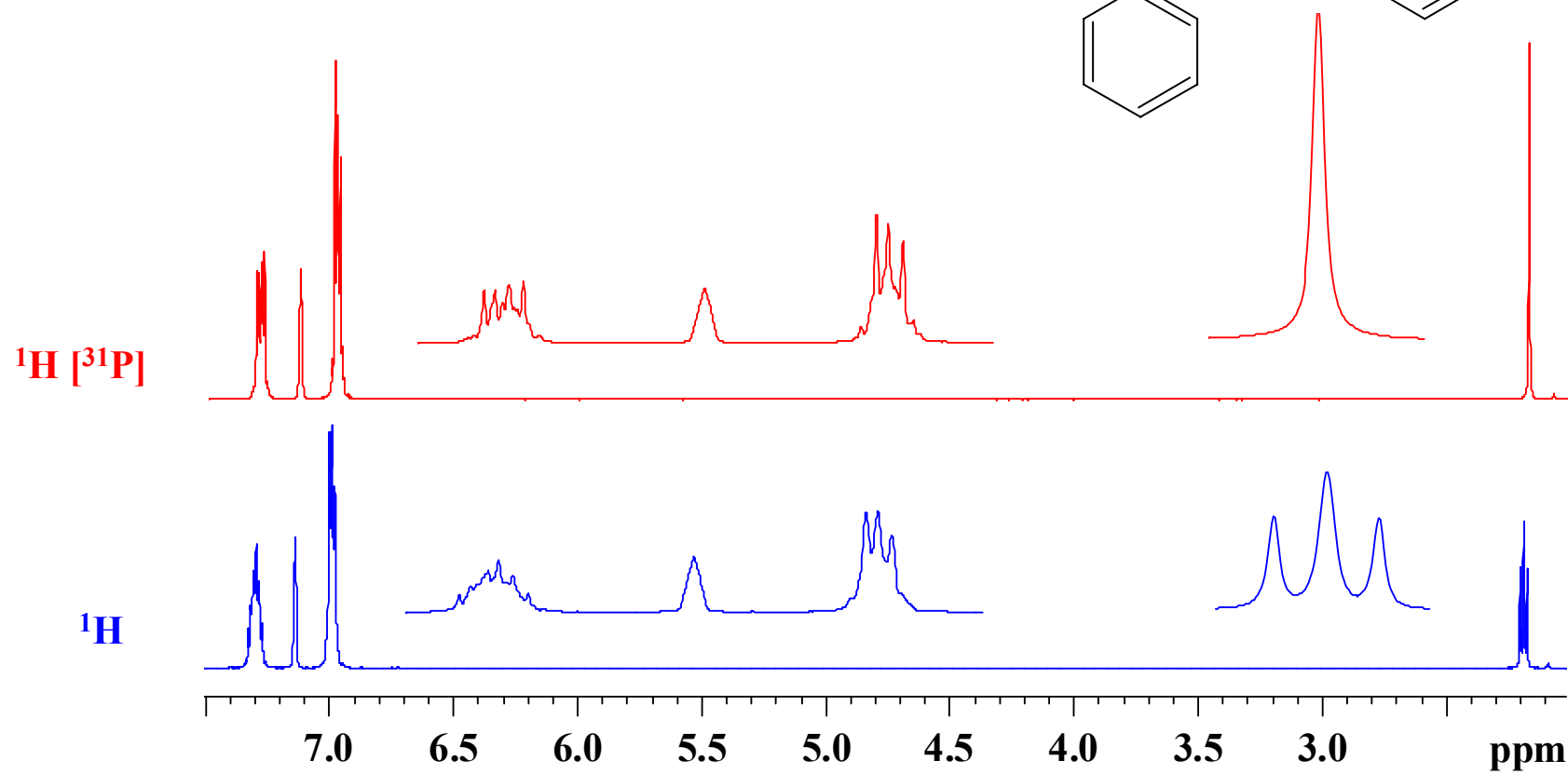
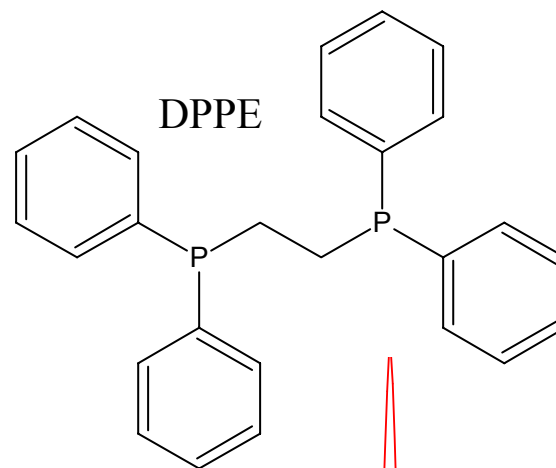
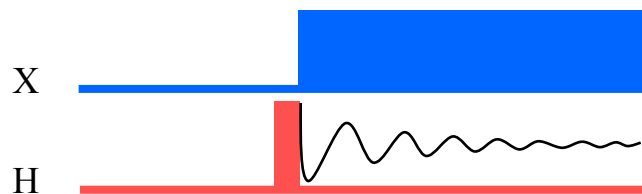


Decoupling
(100% Duty Cycle)

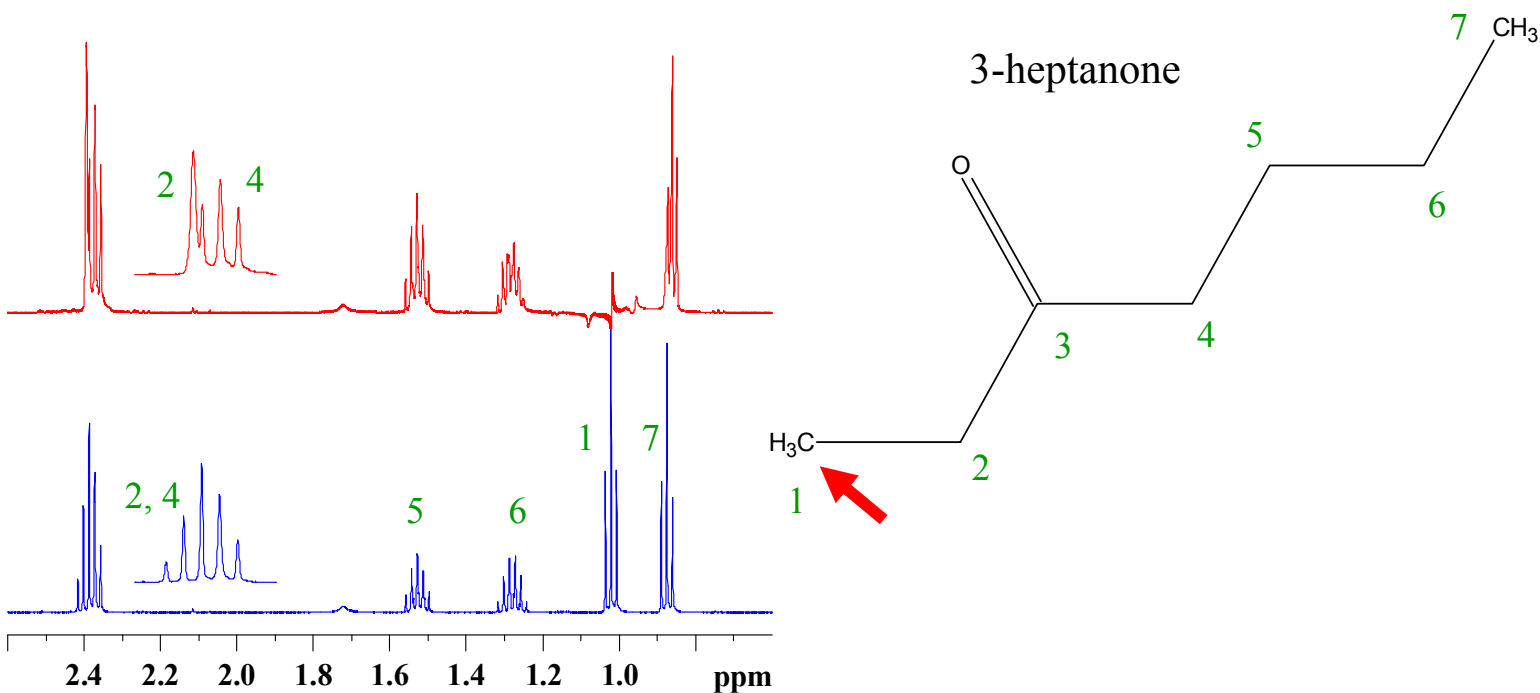
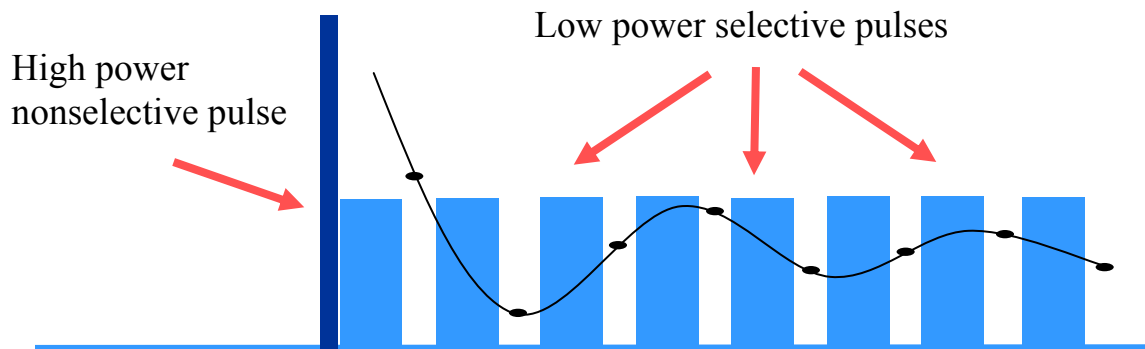


85 80 75 70 65 60 55 50 45 40 35 30 25 20 ppm

Inverse Gated X Decoupling



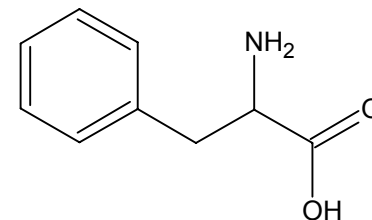
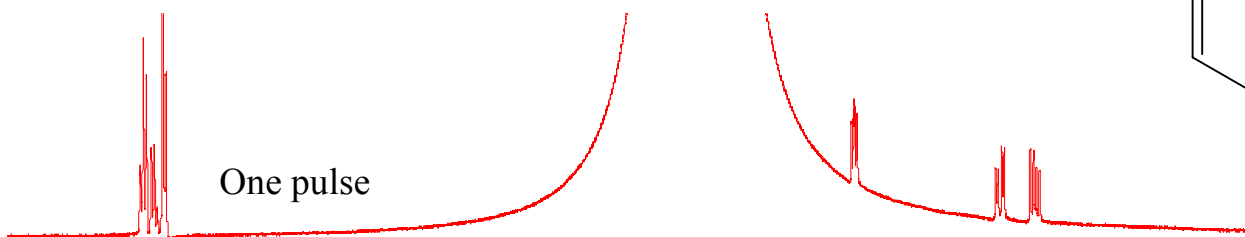
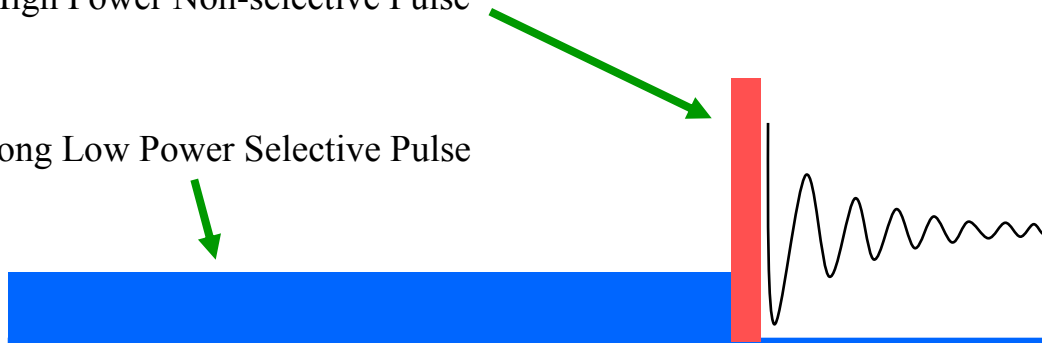
Homonuclear Decoupling



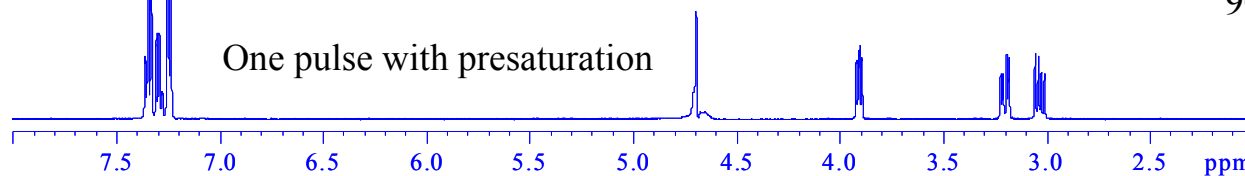
Solvent Suppression via Presaturation

Short High Power Non-selective Pulse

Long Low Power Selective Pulse



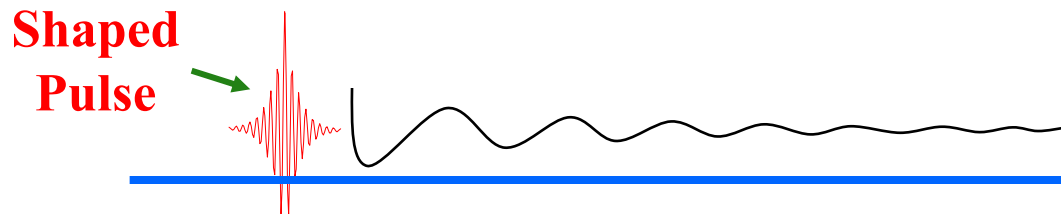
One pulse



One pulse with presaturation

90 % H₂O / 10% D₂O

Shaped Pulses for Selective Irradiation

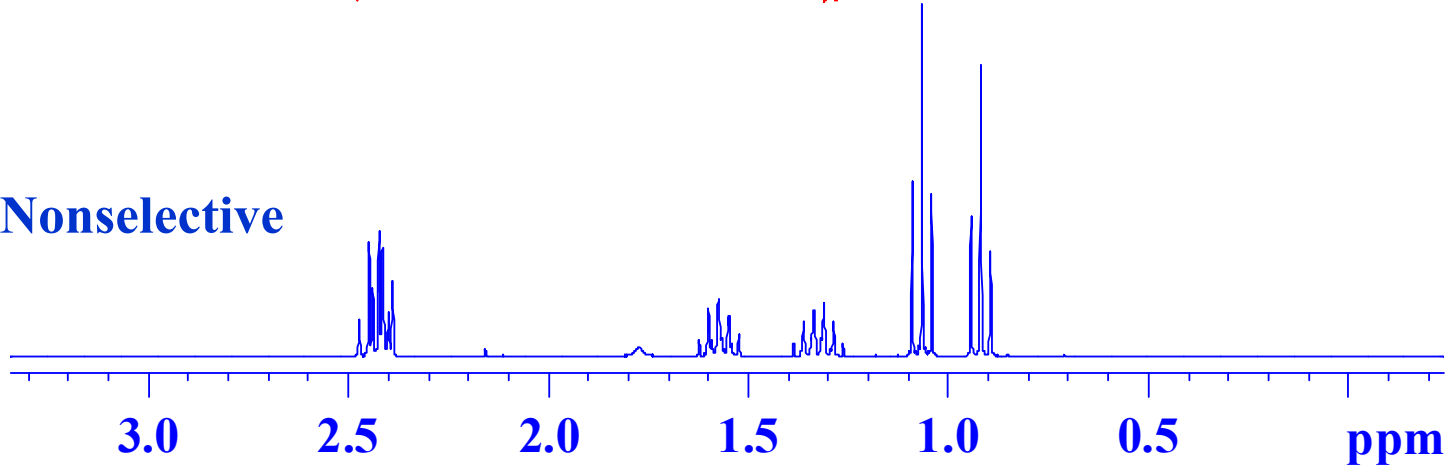


Shaped pulses are used to selectively irradiate a specific region of the spectrum

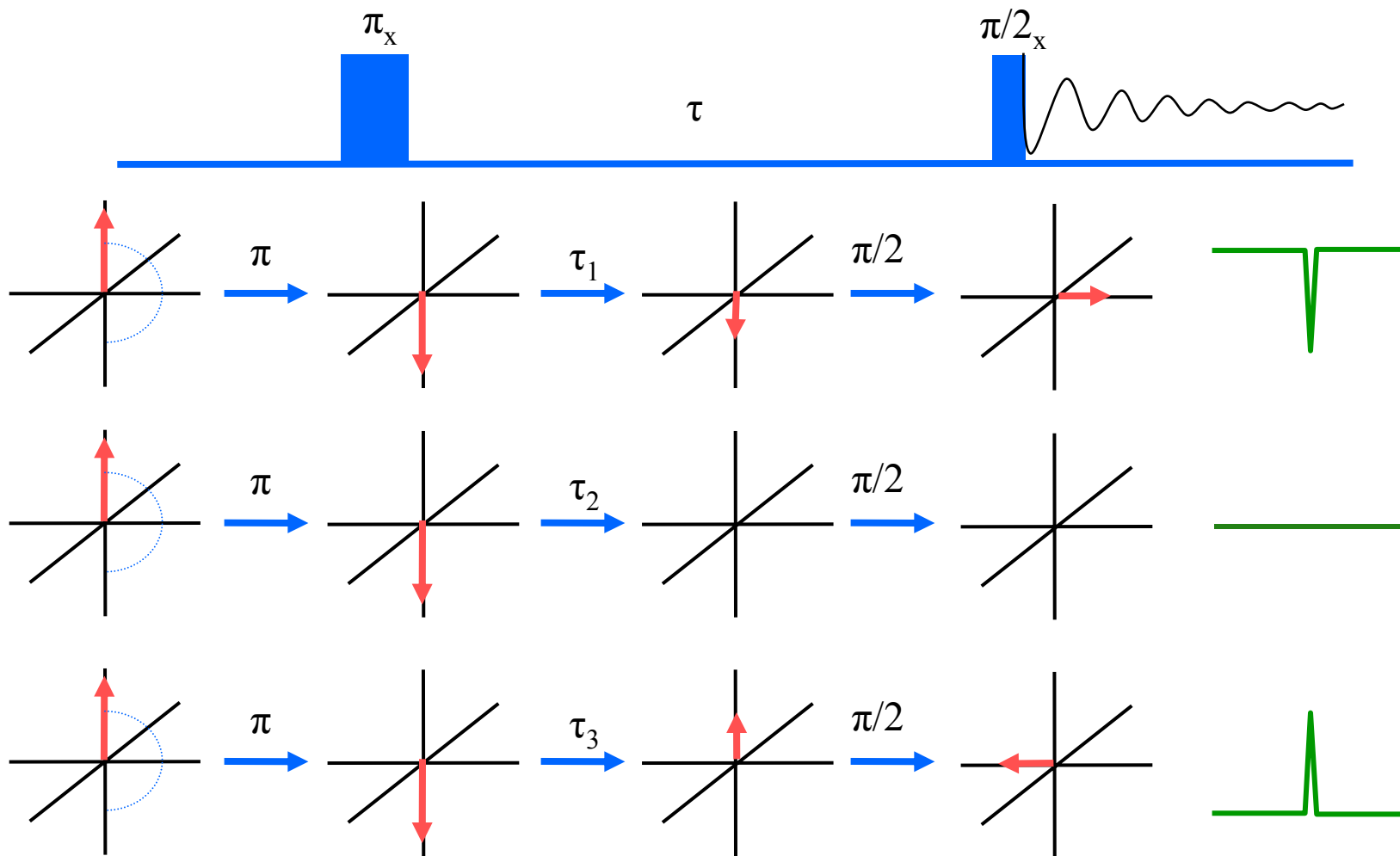
Selective



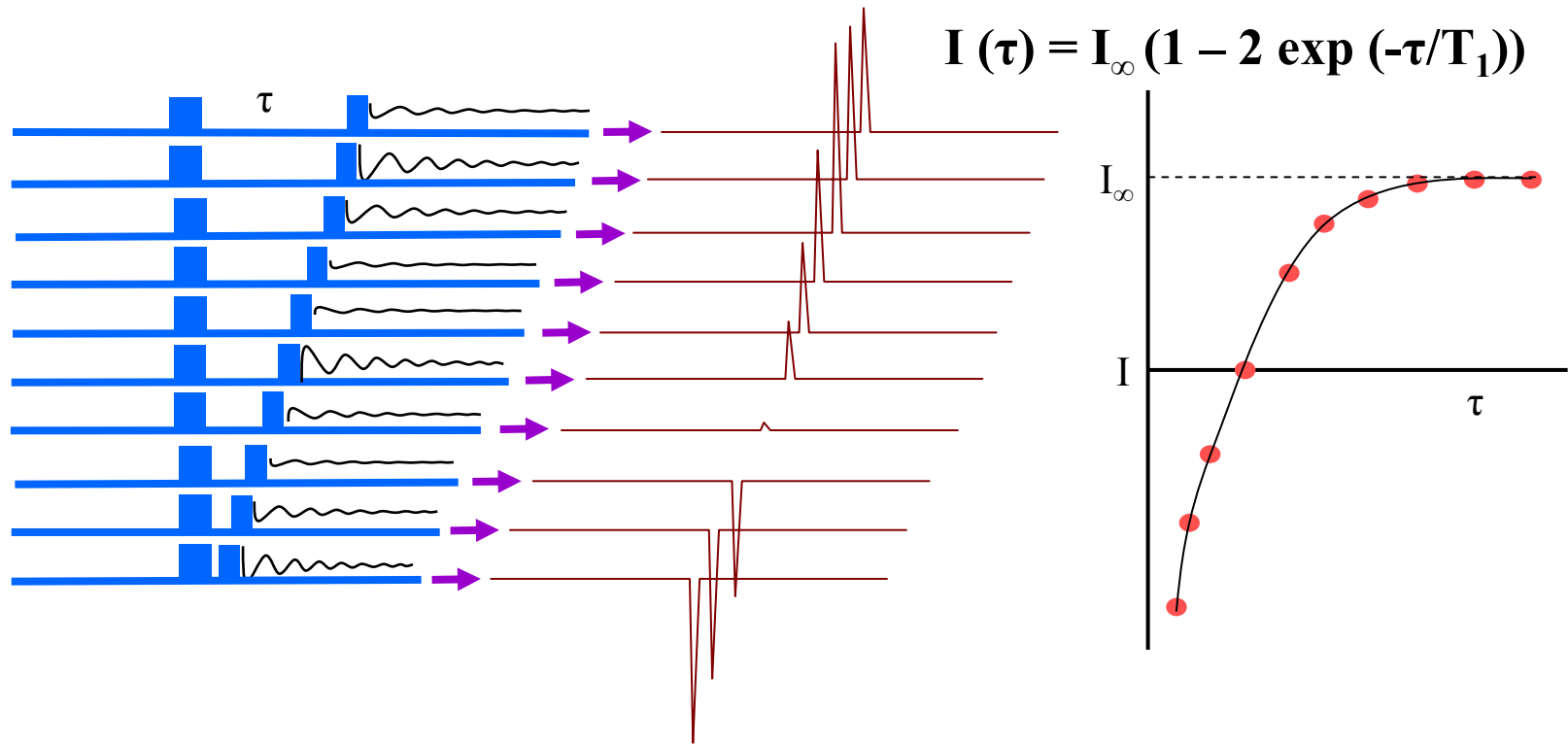
Nonselective



T_1 Relaxation Time Measurement

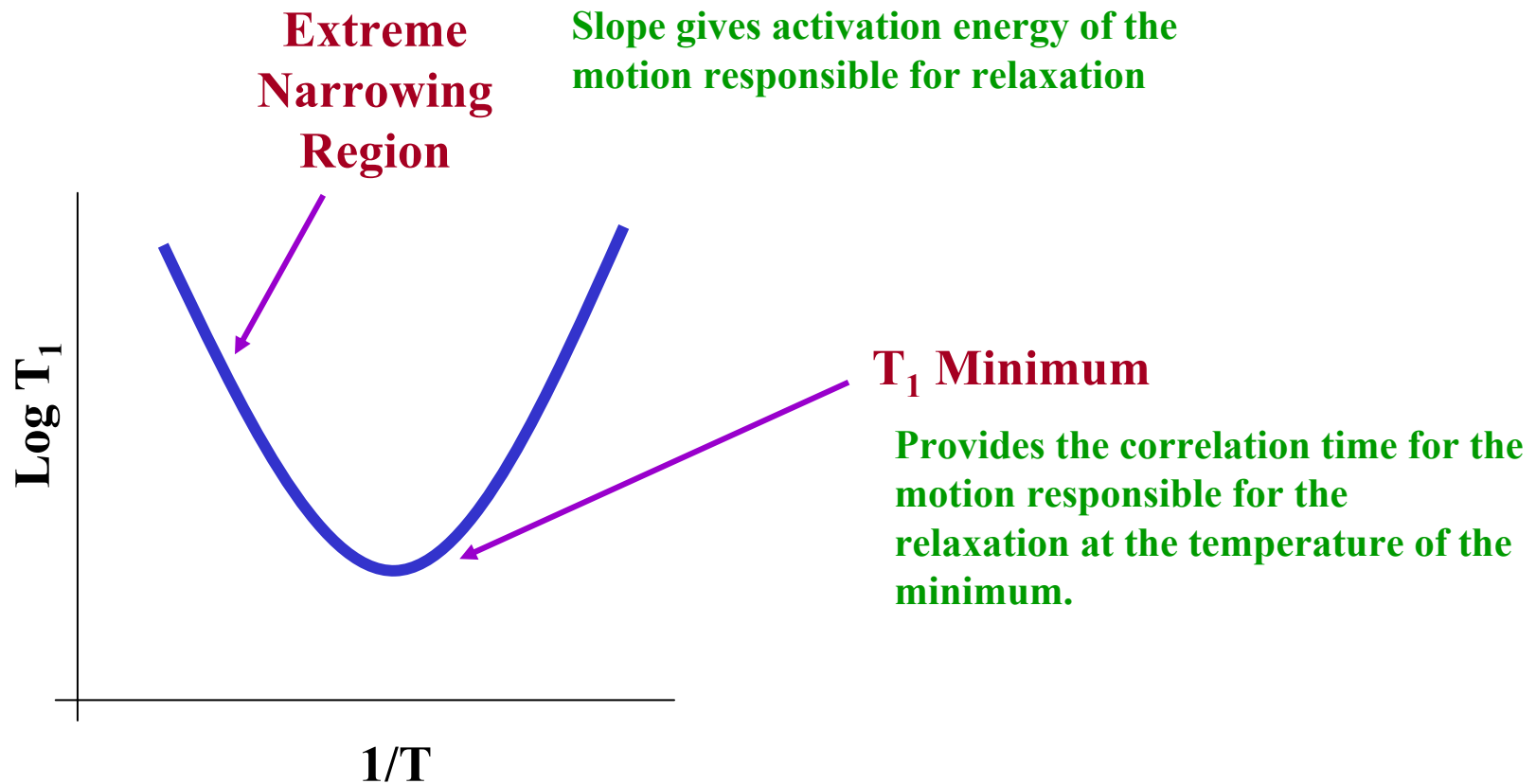


T_1 Relaxation Time Measurement



T_1 Relaxation as a Function of Temperature

Relaxation occurs by a number of mechanisms all of which depend on the degree of molecular motion. Relaxation is most efficient when the reciprocal of the motional correlation time is of the same order as the NMR resonance frequency.

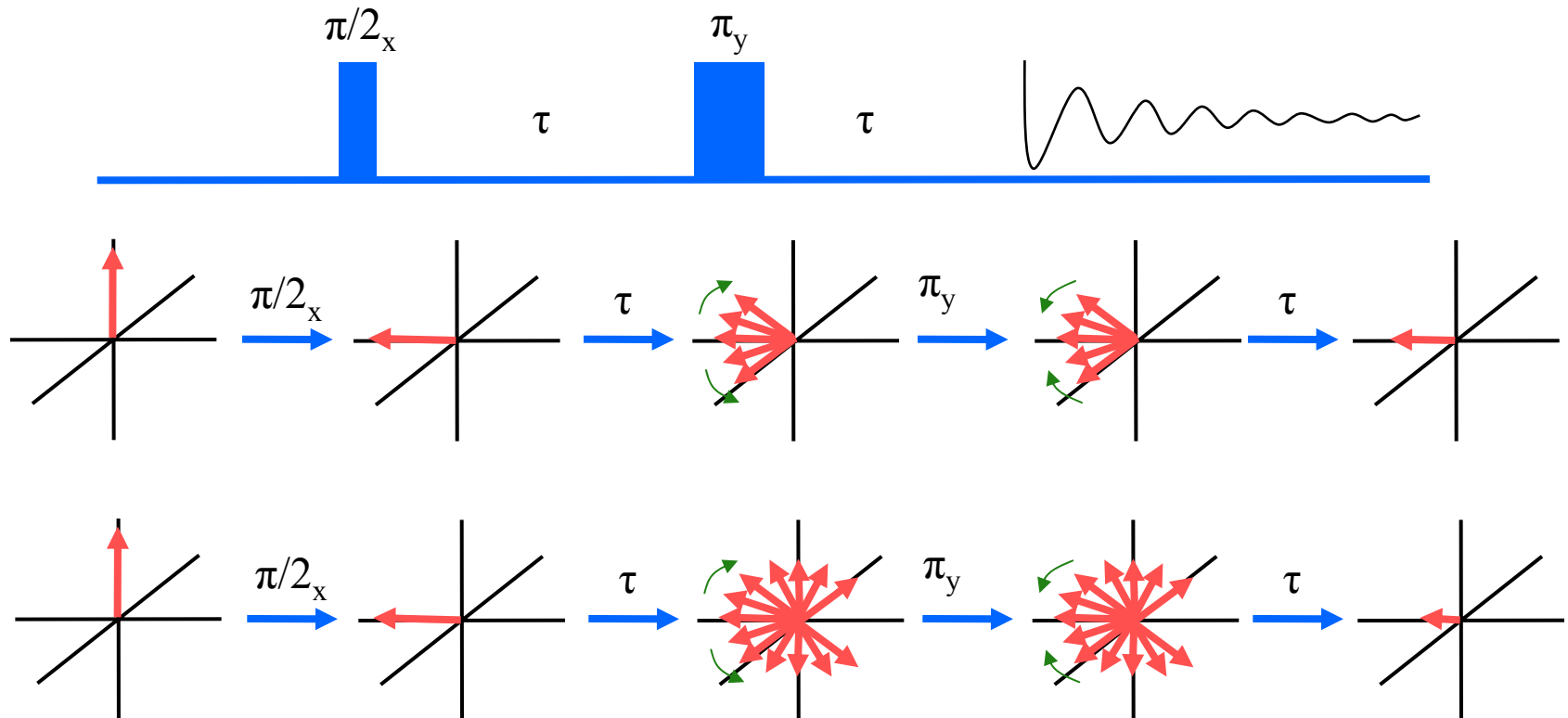


T_2 Relaxation Time Measurement

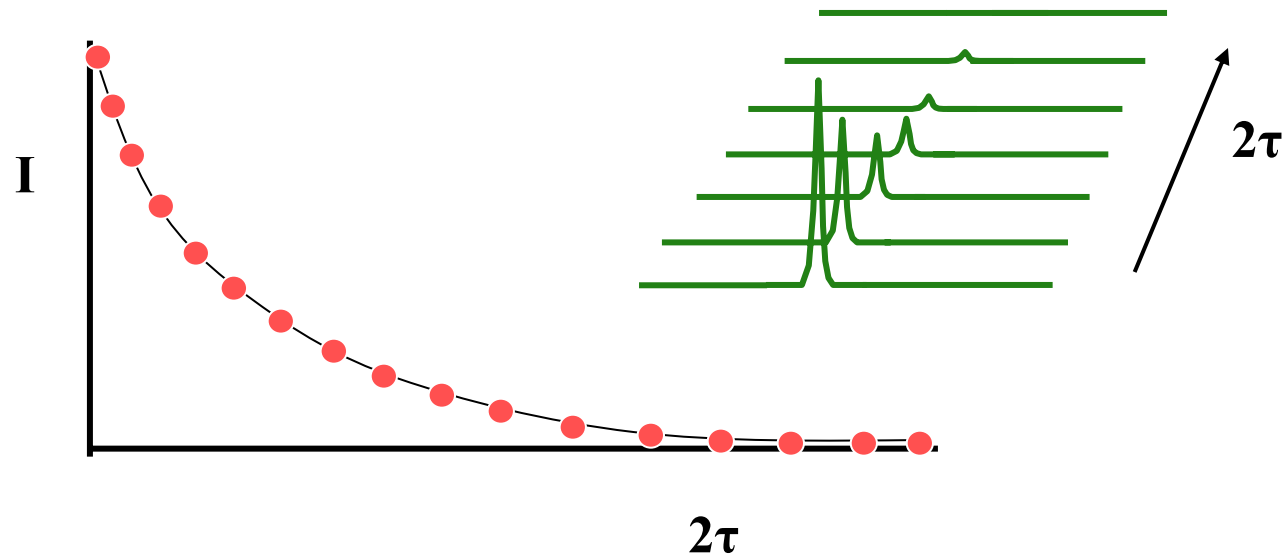
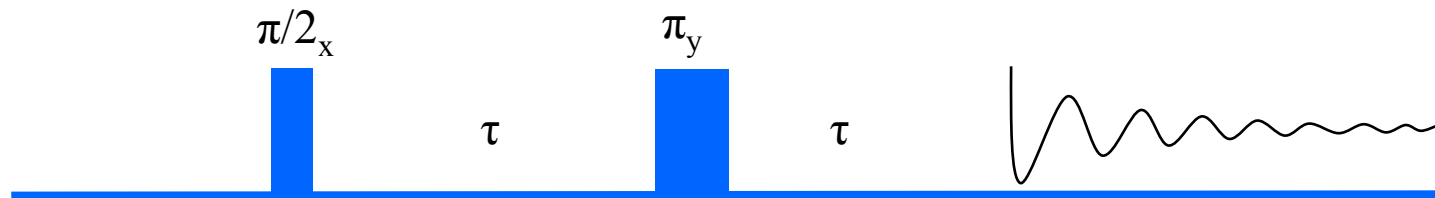
In principle, T_2 can be measured directly from the decay of the FID however, in practice, the FID will decay faster than expected due to magnetic field inhomogeneity.

$$(1/T_2^*) = (1/T_2) + (1/T_2^{\text{ih}})$$

where $(1/T_2^*)$ is the observed decay rate of the FID and $(1/T_2^{\text{ih}})$ is the decay rate due exclusively to magnetic field inhomogeneity. To measure T_2 , one must use a technique independent of magnetic field inhomogeneity.



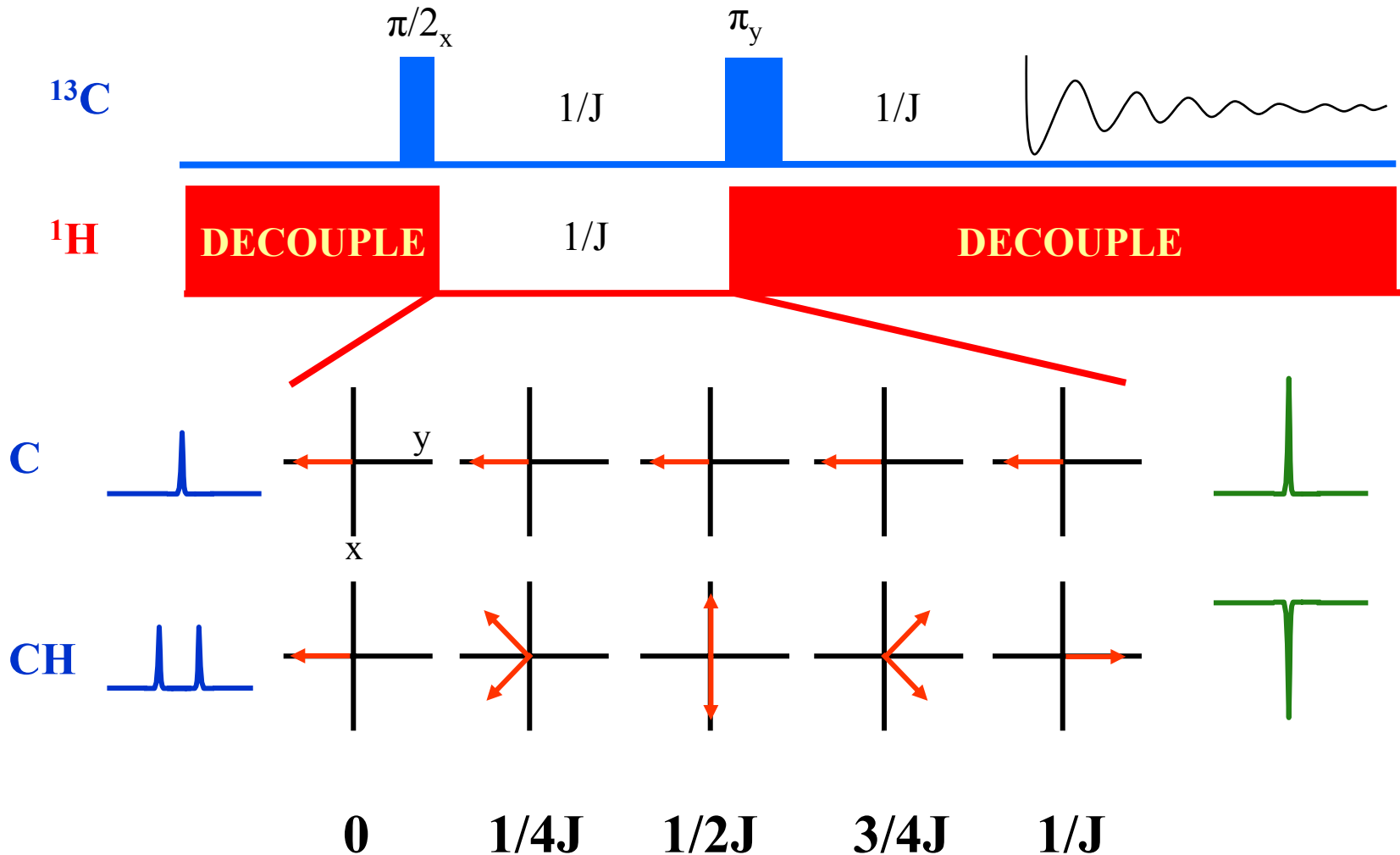
T_2 Relaxation Time Measurement



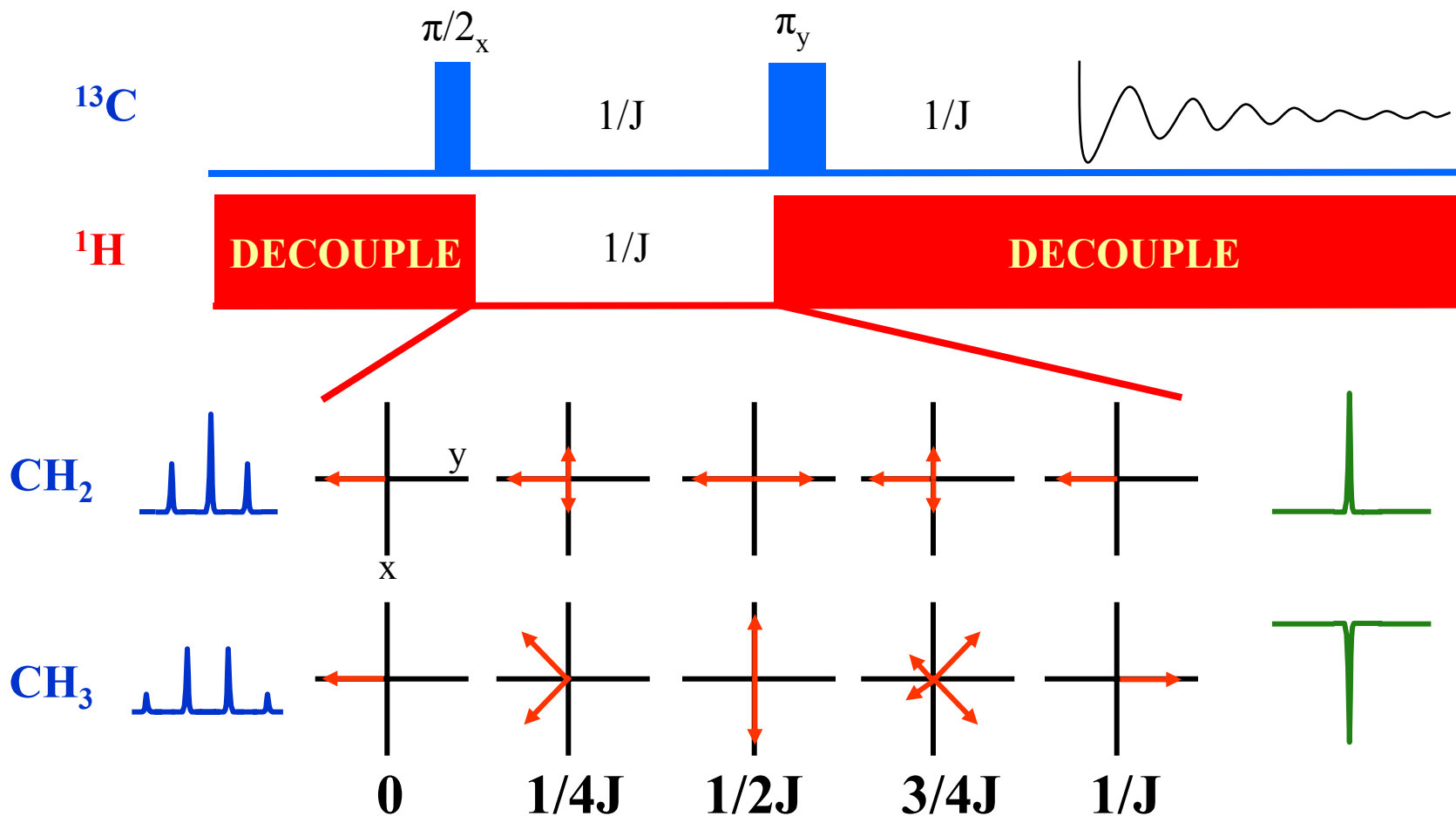
$$I(2\tau) = I_0 \exp(-2\tau/T_2)$$

J Modulated Spin Echo

The J modulated spin echo is used to assign the proton multiplicity of ^{13}C resonances. It is the main building block of the APT (attached proton test) experiment and the predecessor of the more modern DEPT experiments



J Modulated Spin Echo

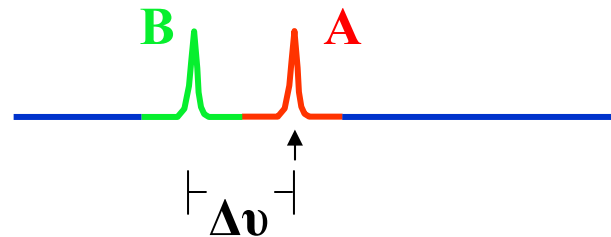


After the first $1/J$ delay, the phase of the ^{13}C signals is a function of the number of protons attached to the carbon.. If all signals were “on resonance” there would be no need for the π_y pulse or the second $1/J$ delay but since this is not the case the $-\pi_y - 1/J$ segment with the decoupler on serves to refocus chemical shift evolution during the first $1/J$ delay. This is completely analogous to how the T_2 sequence refocuses field inhomogeneity.

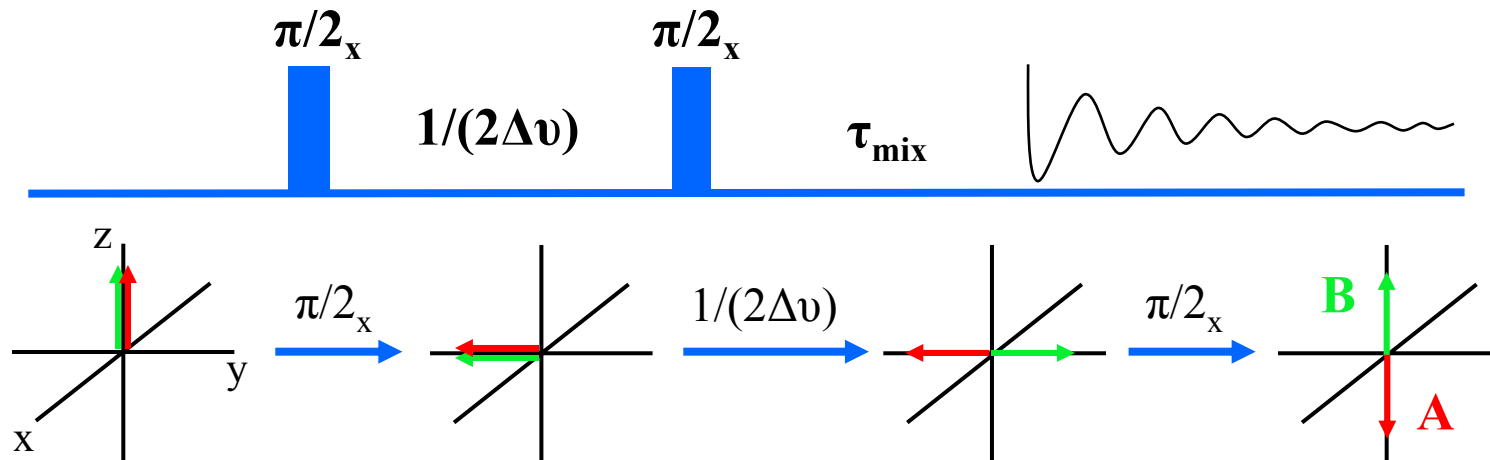
Inversion Transfer

Inversion transfer is used to detect chemical or spin exchange between two resonances. It is the one-dimensional analog to the 2D EXSY and 2D NOESY experiments.

Take as an example a two line spectrum with peaks A and B. We will assume that peak A is “on resonance”.

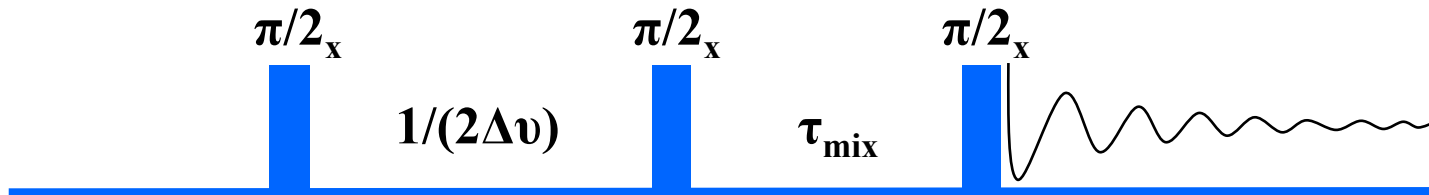


The principle of the sequence is to arrange each of the spin vectors on the $\pm z$ axis, allow exchange to occur and then observe the signals.

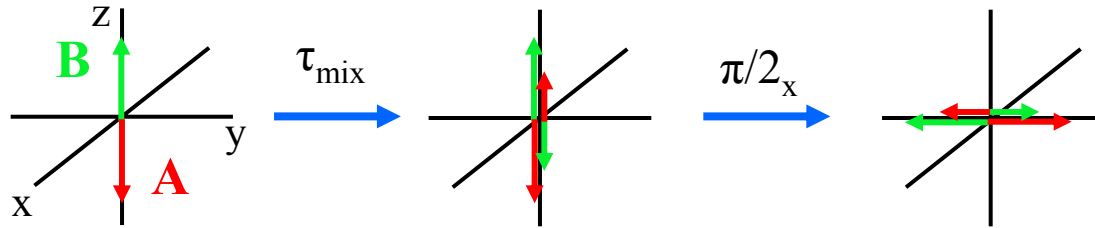


The $(\pi/2_x - 1/(2\Delta\nu) - \pi/2_x)$ segment of the pulse sequence selectively inverts the A resonance while leaving the B resonance unaffected.

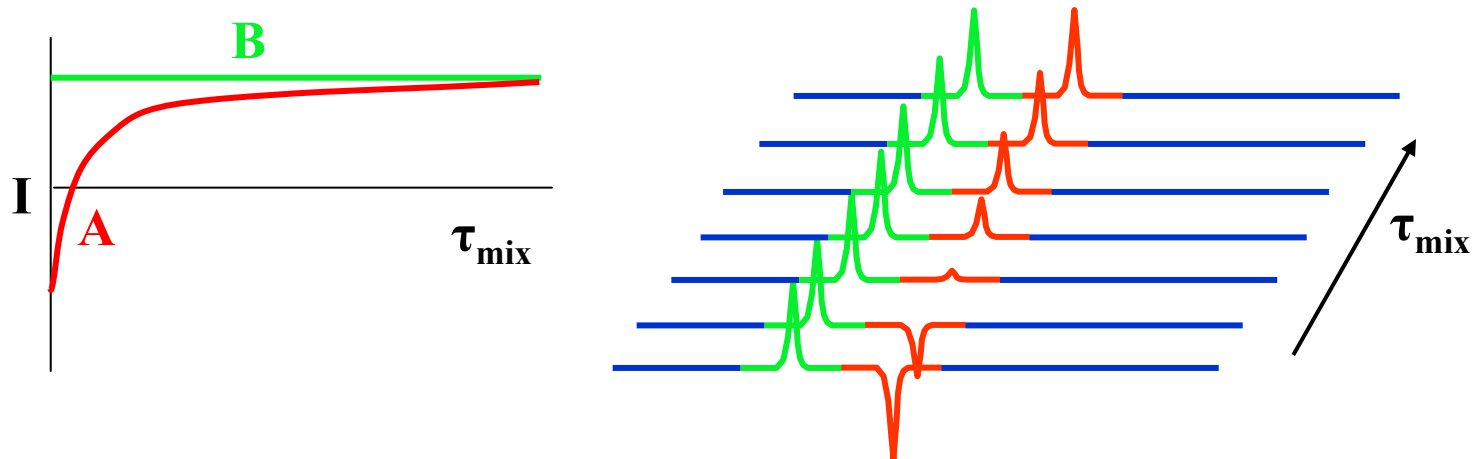
Inversion Transfer



During the mixing time, τ_{mix} , A will relax according to its T_1 and exchange between the two spins, A and B, may (or may not) occur. After the mixing period the second $\pi/2_x$ pulse is given and the receiver is turned on to observe the resonances.

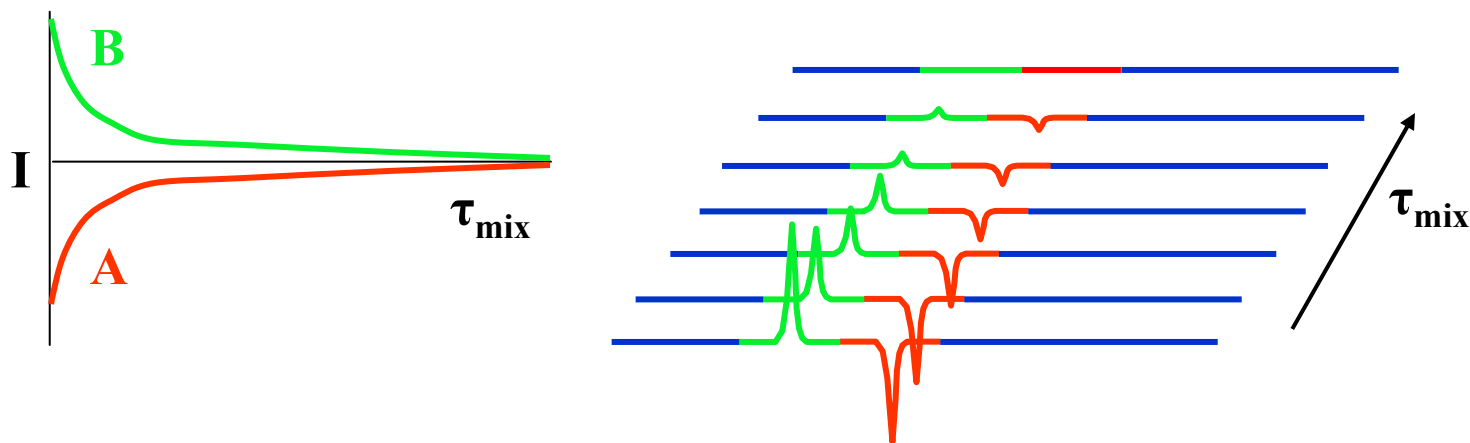


If no exchange occurs between A and B during the mixing time or if the exchange rate is much slower than the T_1 of A, then the only observable effect is that A will relax.

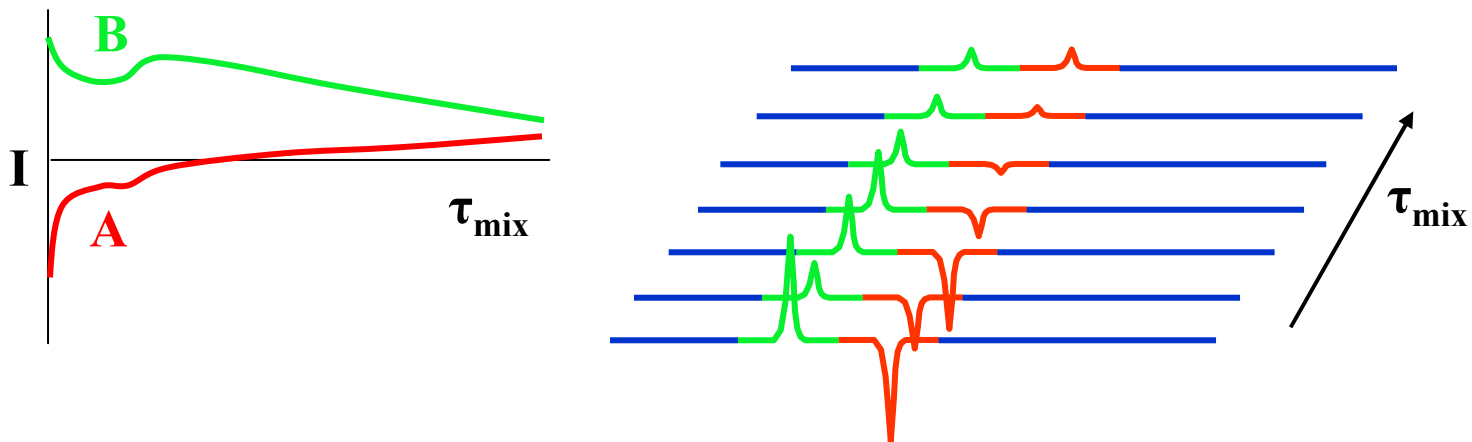


Inversion Transfer

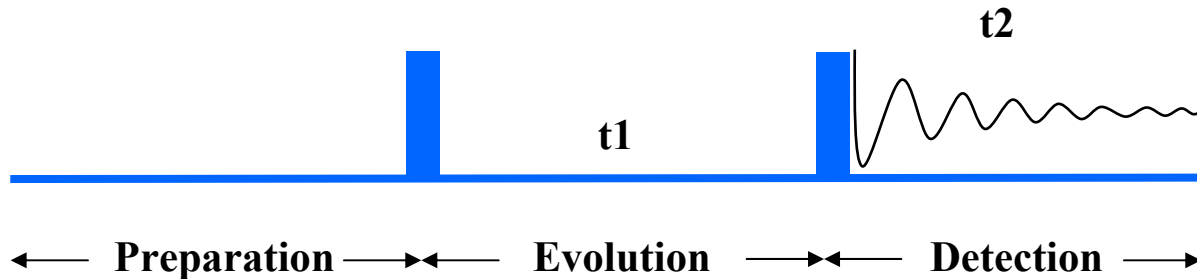
If exchange between A and B is fast with respect to the T_1 of A, then we have:



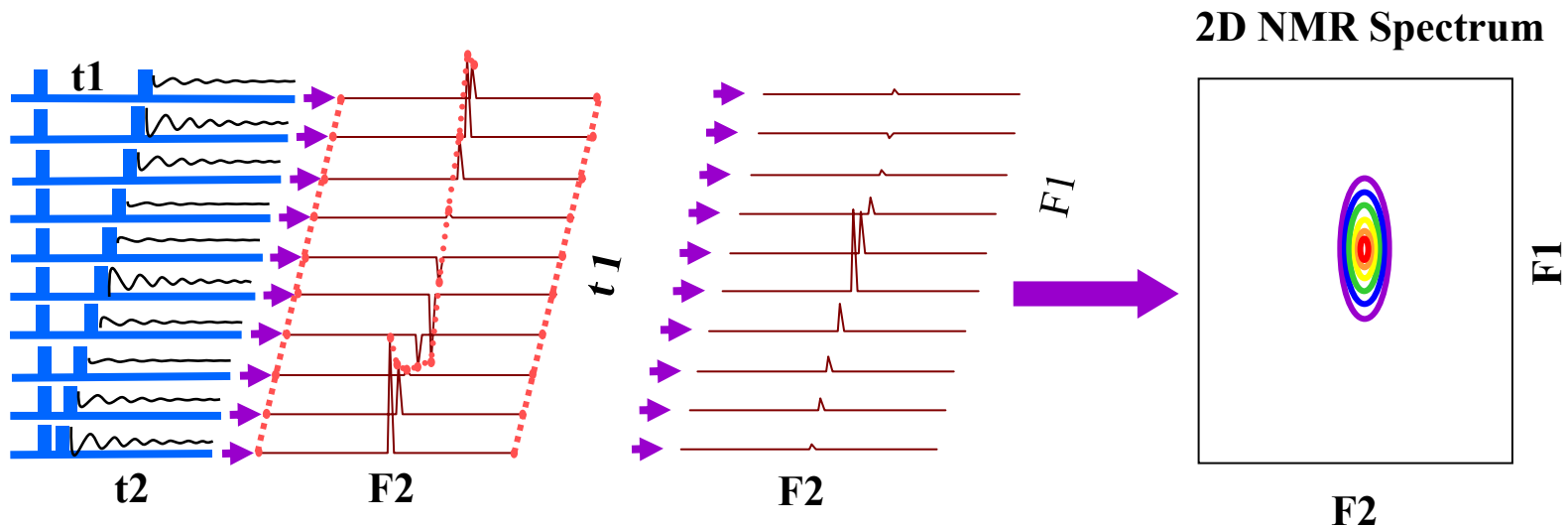
When the exchange rate is of the same order as the T_1 of A, we have:



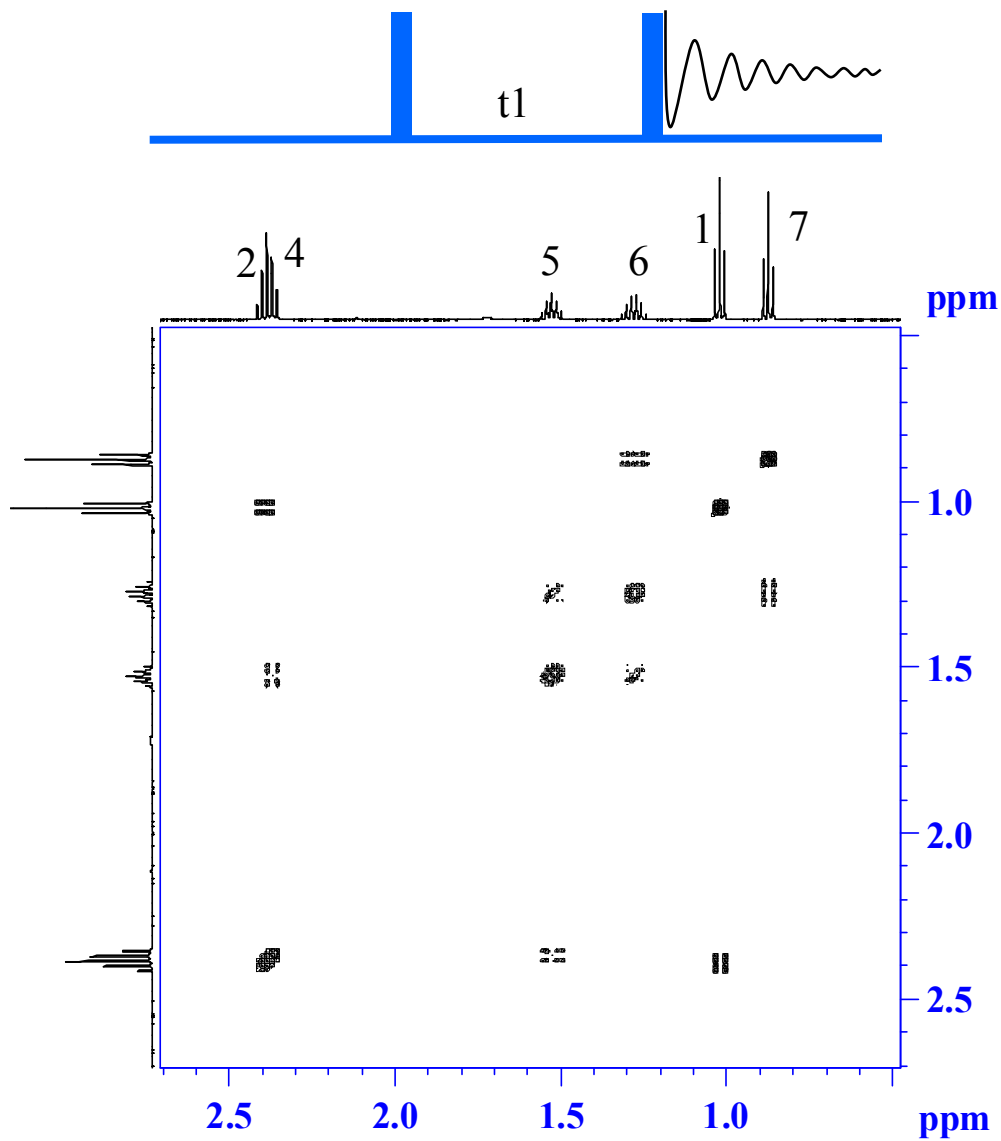
Two Dimensional NMR Spectroscopy



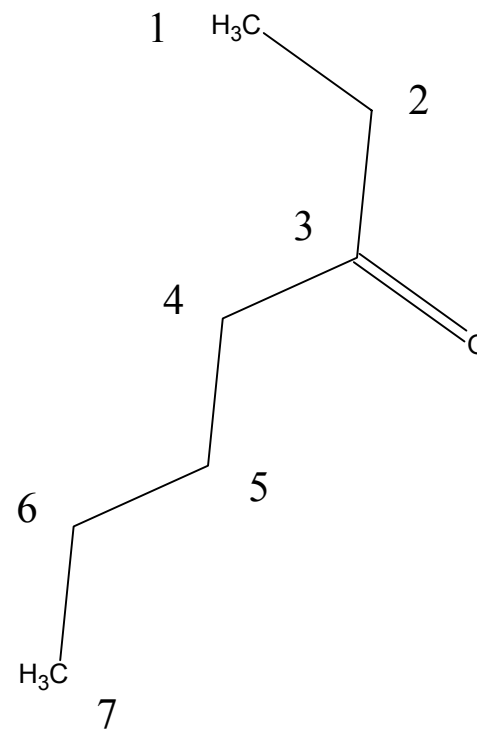
In 2D NMR, a series of FIDs are collected as a function of the evolution time, t_1 , to get signals, $S(t_1, t_2)$. Each of these FIDs is Fourier transformed with respect to t_2 to give a set of 1D NMR spectra, $S(t_1, F_2)$. This series of spectra is encoded with information about what happened during the evolution period. The information can be decoded by doing a second Fourier transform with respect to the evolution period, t_1 . Corresponding points in the NMR spectra, $S(t_1, F_2)$ are plotted against t_1 . The resulting interferograms are Fourier transformed to give a two dimensional contour map, $S(F_1, F_2)$. This is the 2D spectrum.



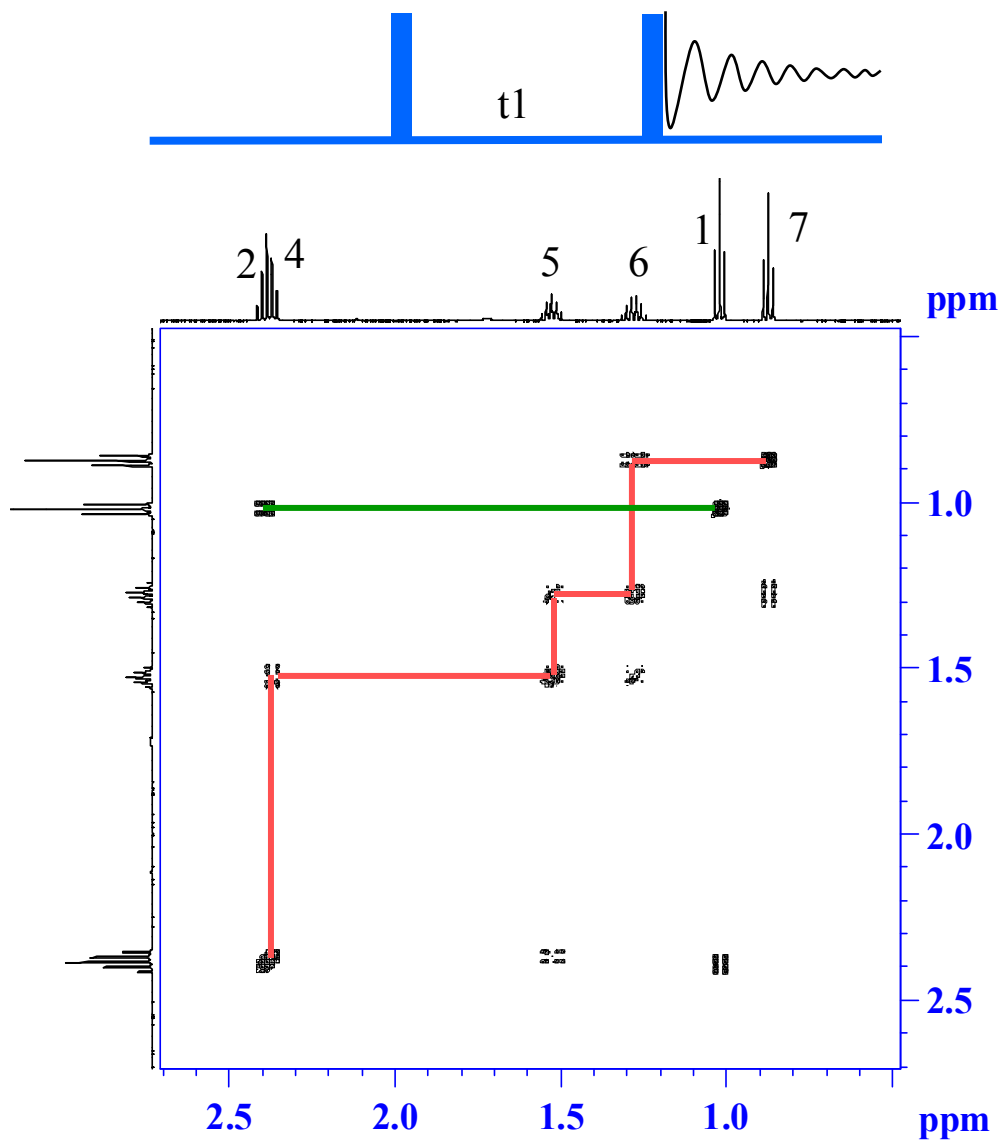
2D - COrrrelation SpectroscopY (COSY)



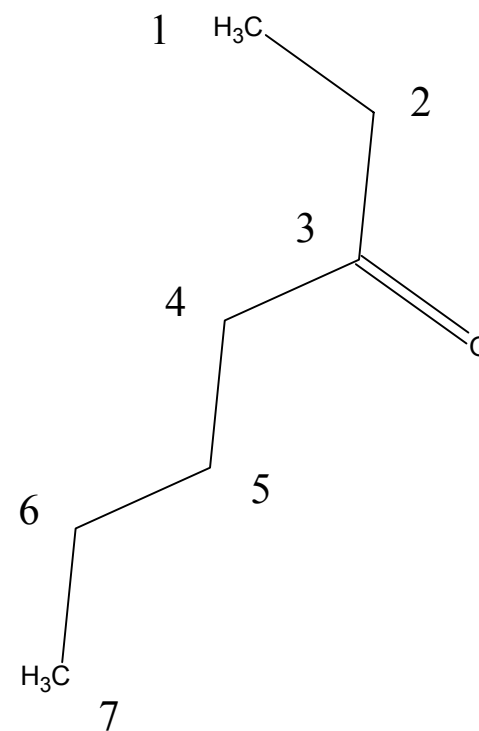
3-heptanone



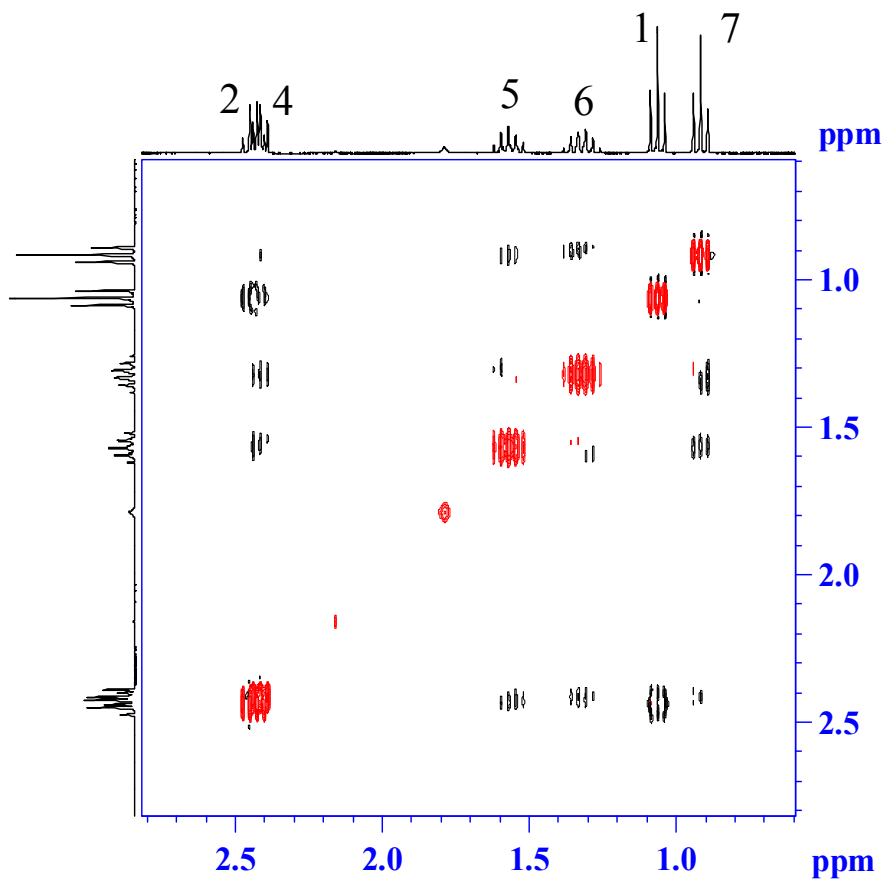
2D - COrrrelation SpectroscopY (COSY)



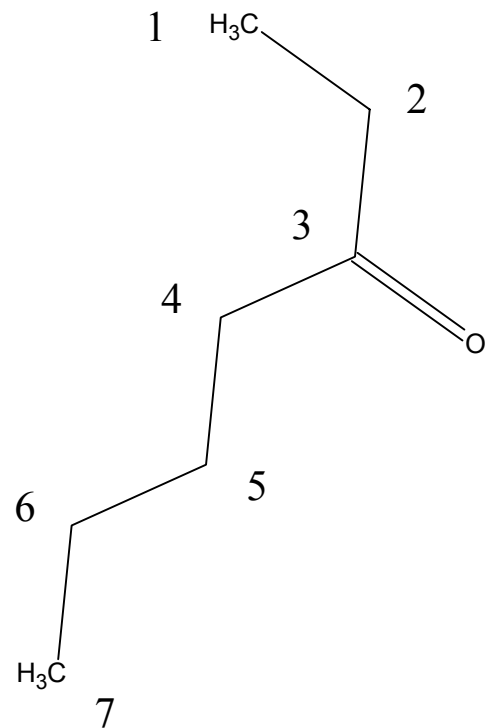
3-heptanone



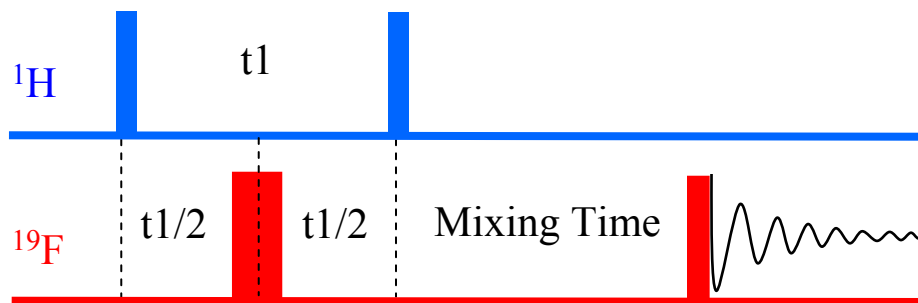
2D – Nuclear Overhauser Enhancement Spectroscopy (NOESY)



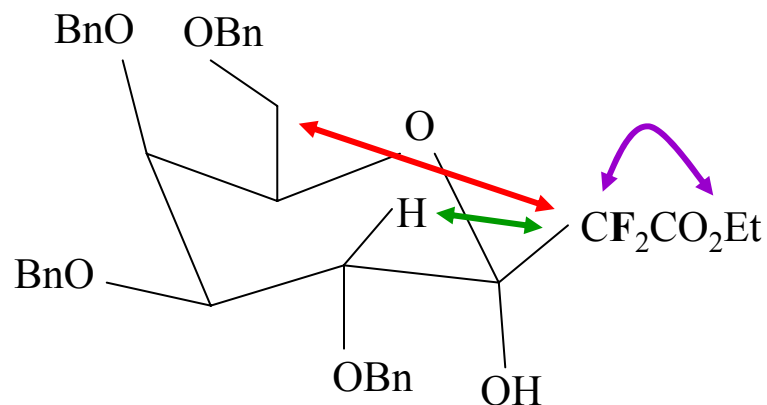
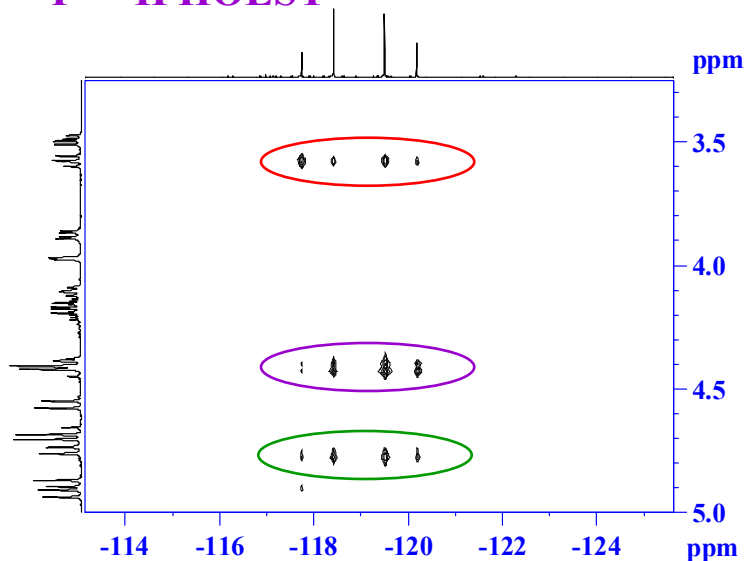
3-heptanone



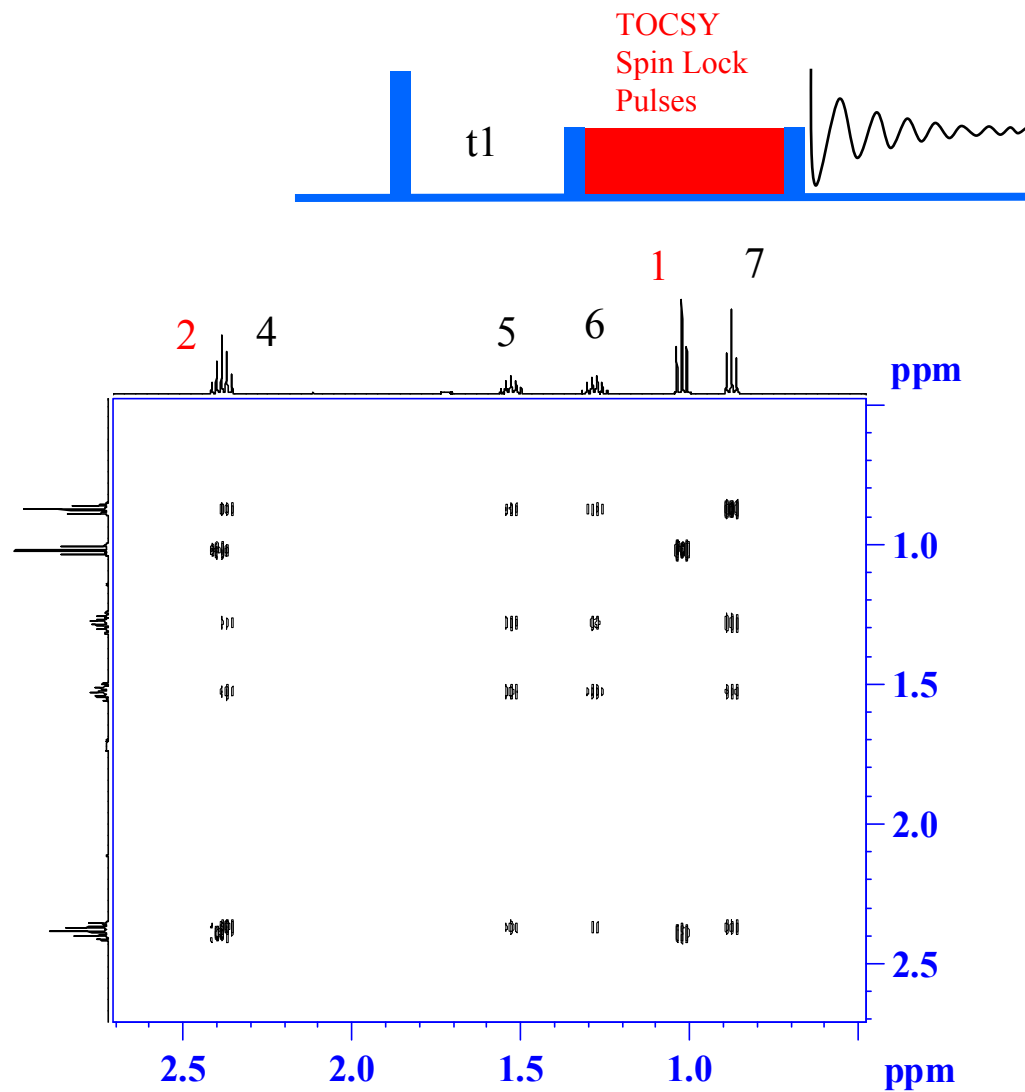
2D – Heteronuclear Overhauser Enhancement Spectroscopy (HOESY)



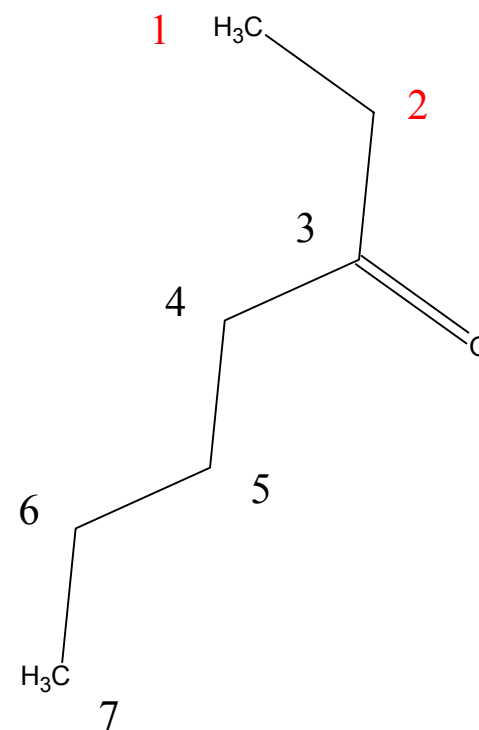
^{19}F – ^1H HOESY



2D – Total Correlation Spectroscopy (TOCSY)



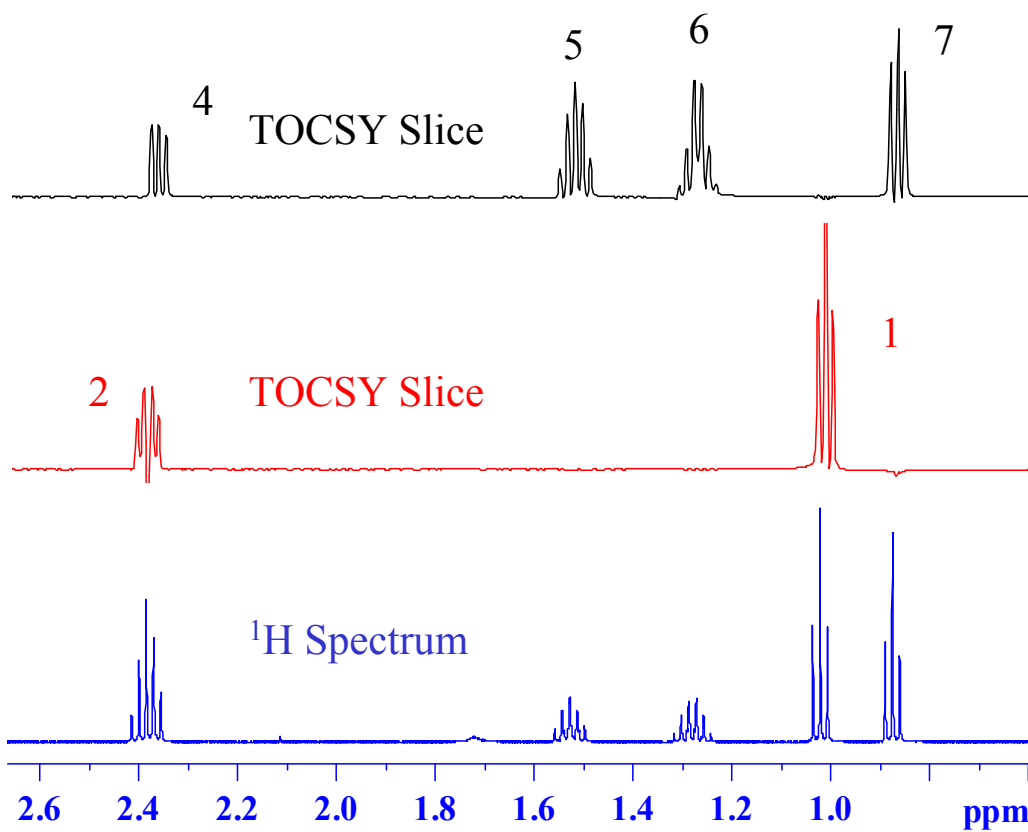
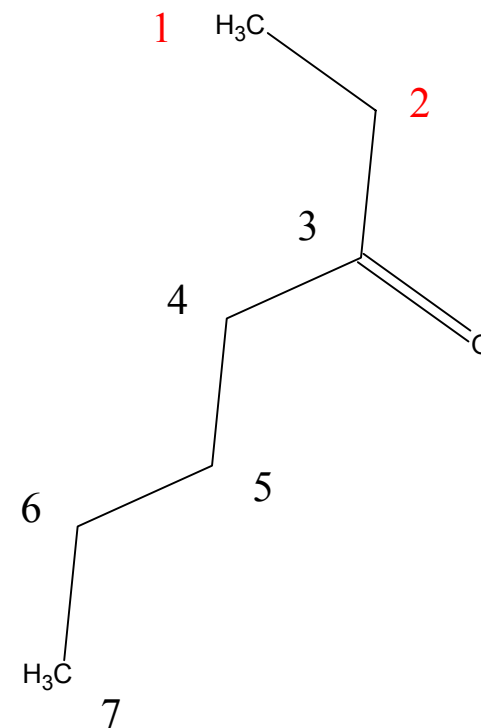
3-heptanone



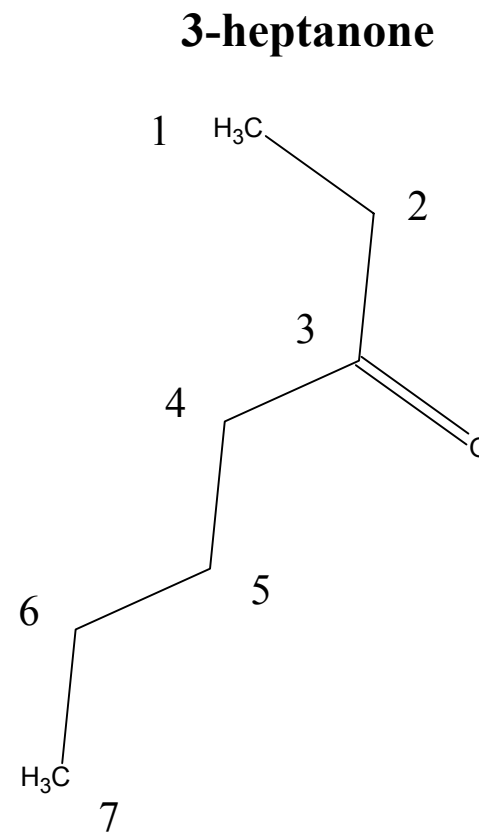
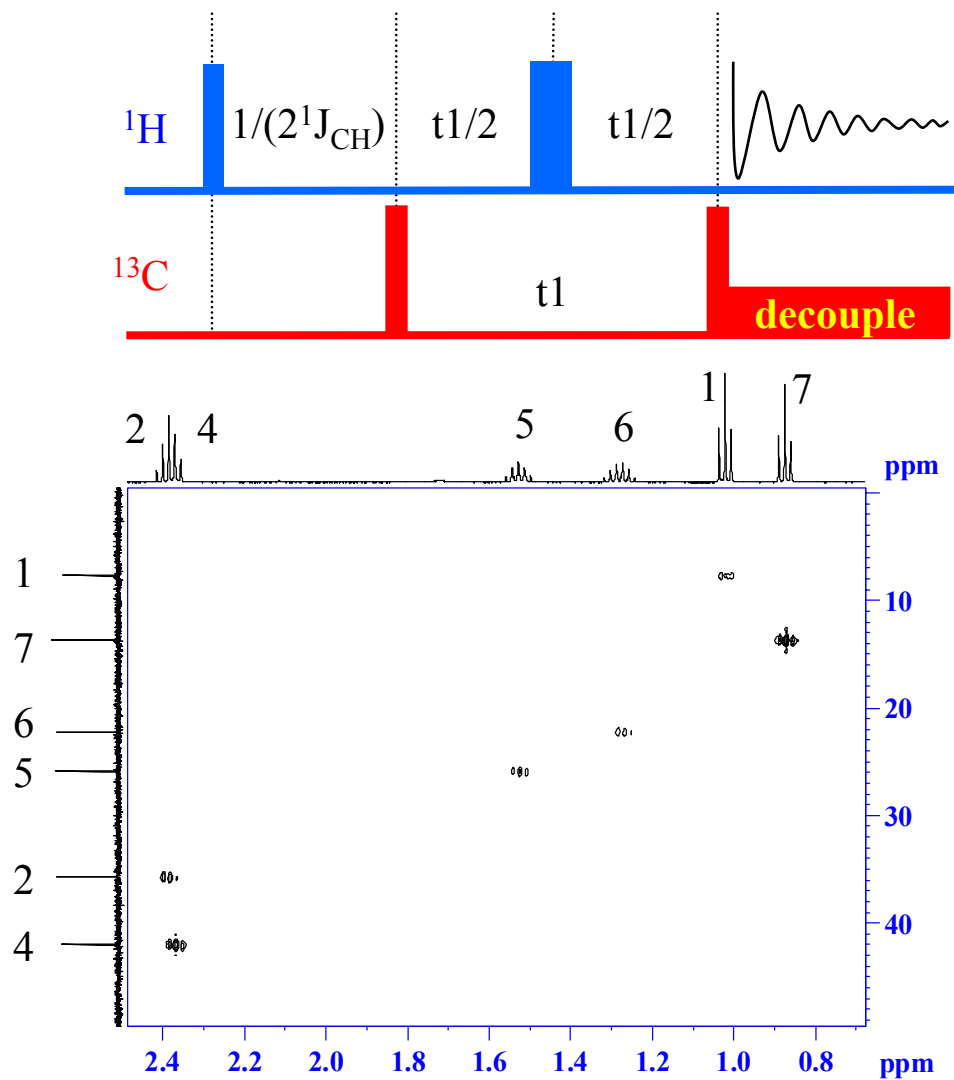
2D – Total Correlation Spectroscopy (TOCSY)



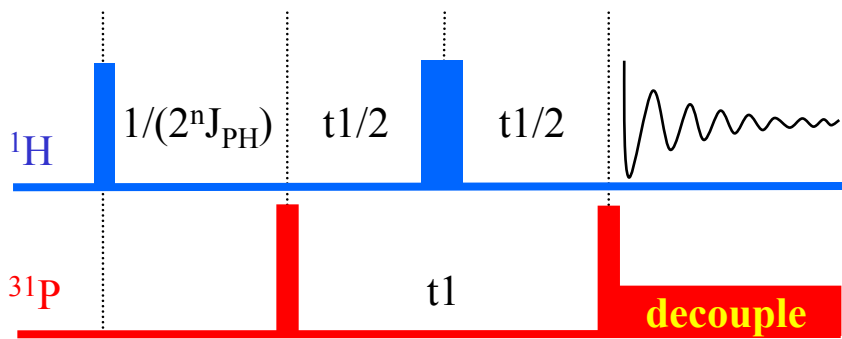
3-heptanone



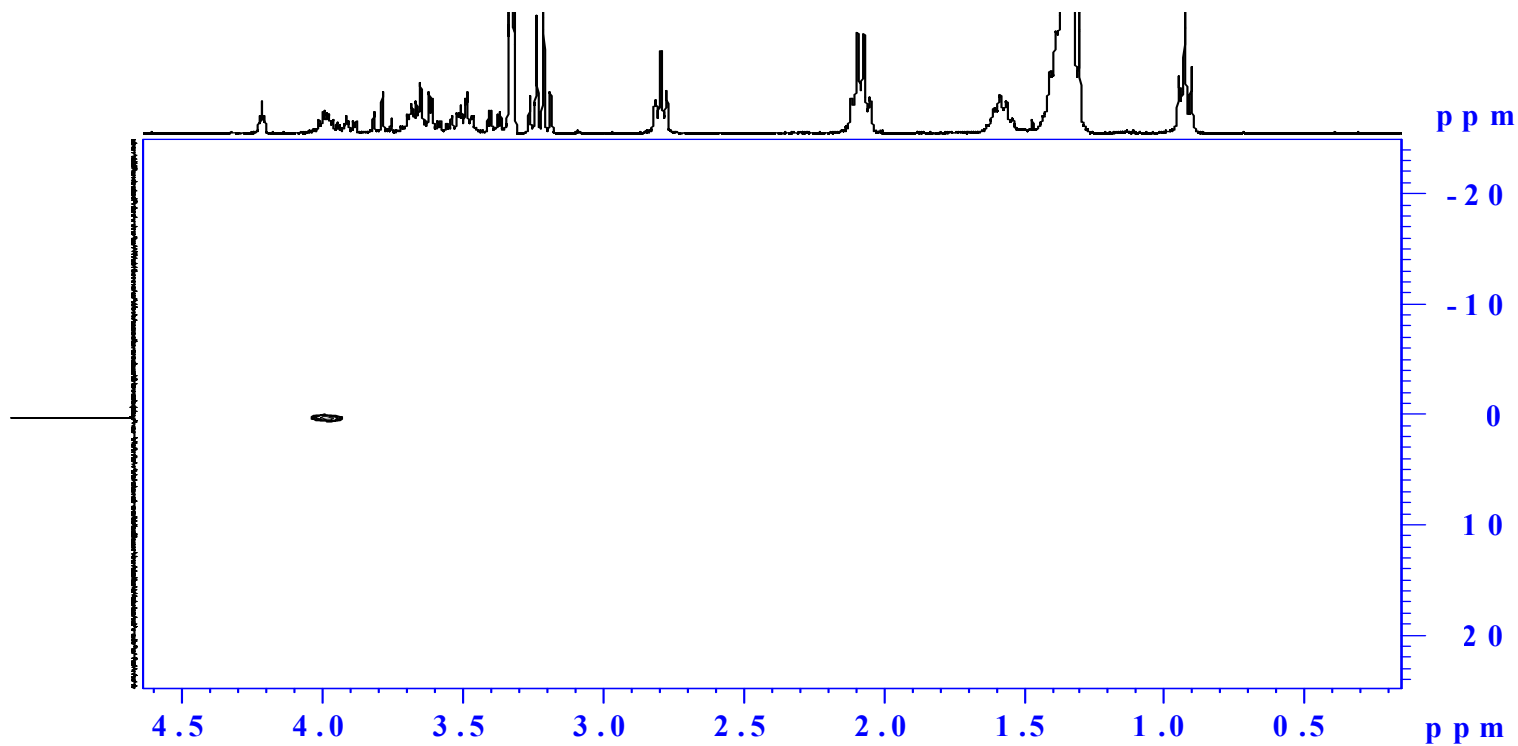
2D – Heteronuclear Multiple Quantum Coherence Spectroscopy (HMQC)



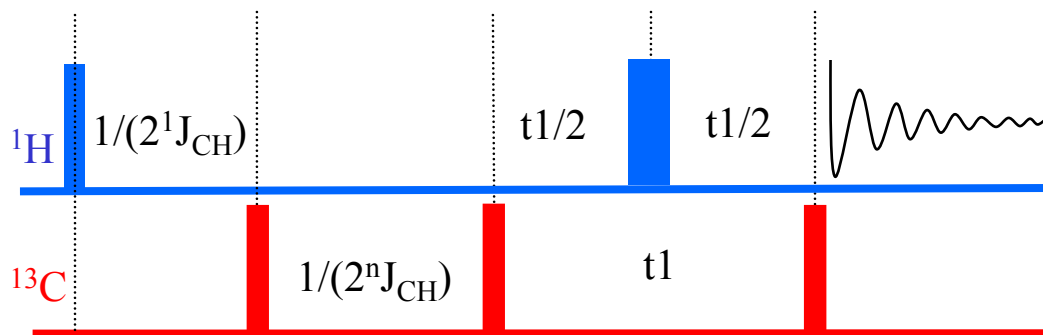
$^1\text{H} - ^{31}\text{P}$ 2D – Heteronuclear Multiple Quantum Coherence Spectroscopy (HMQC)



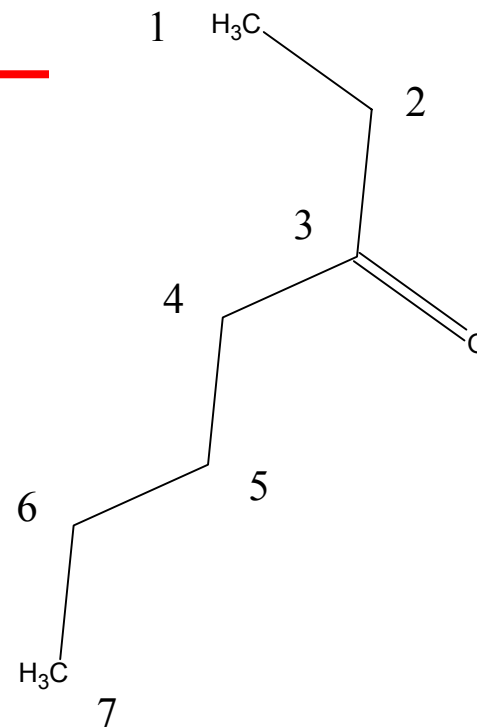
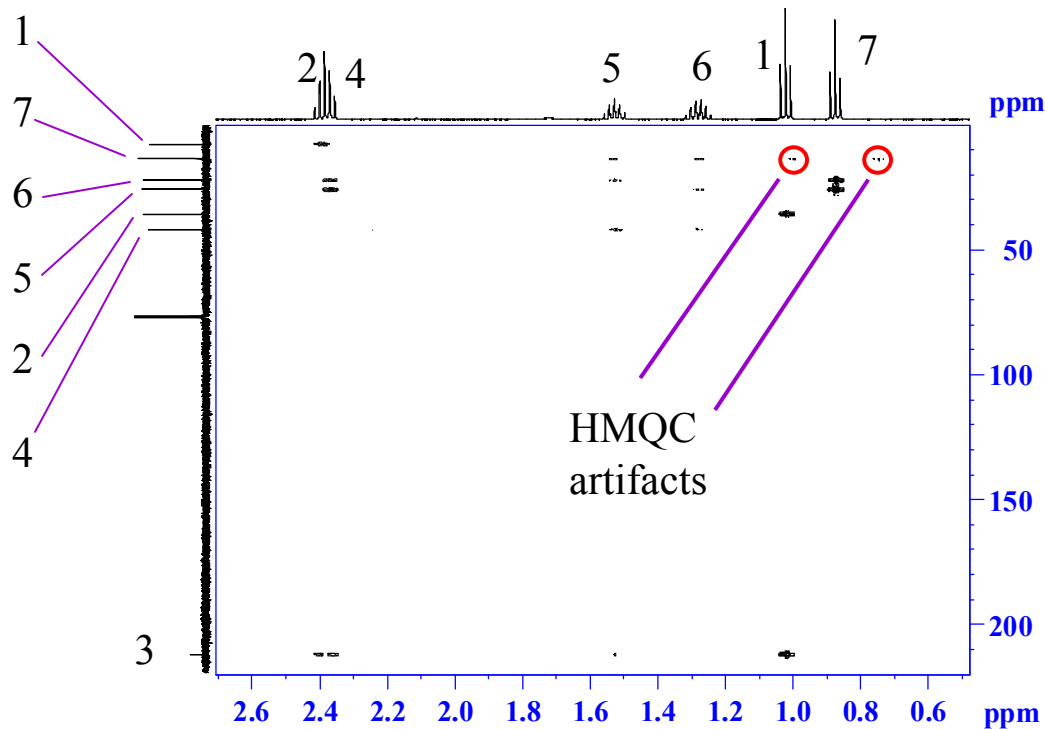
Complex Phospholipid



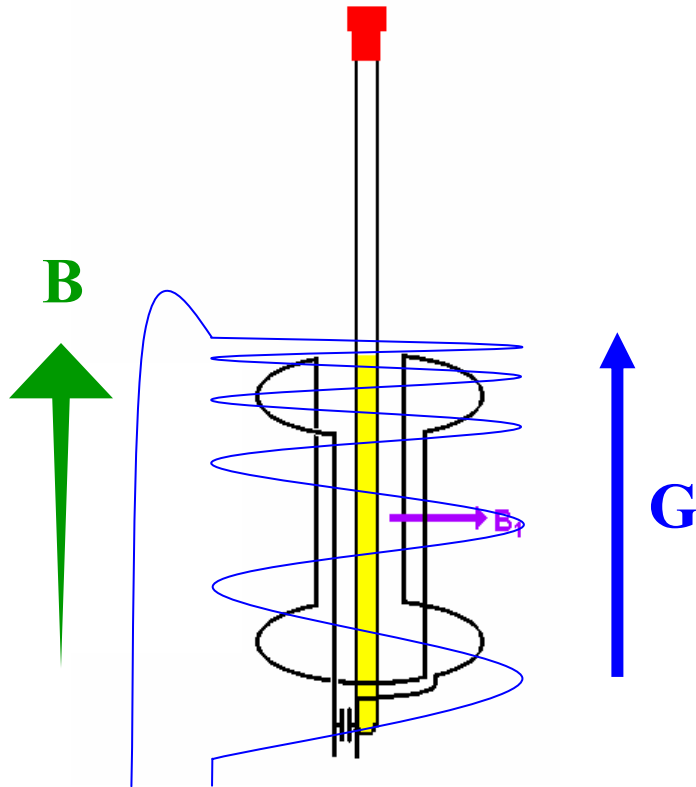
2D – Heteronuclear Multiple Bond Correlation Spectroscopy (HMBC)



3-heptanone



Pulsed Field Gradients



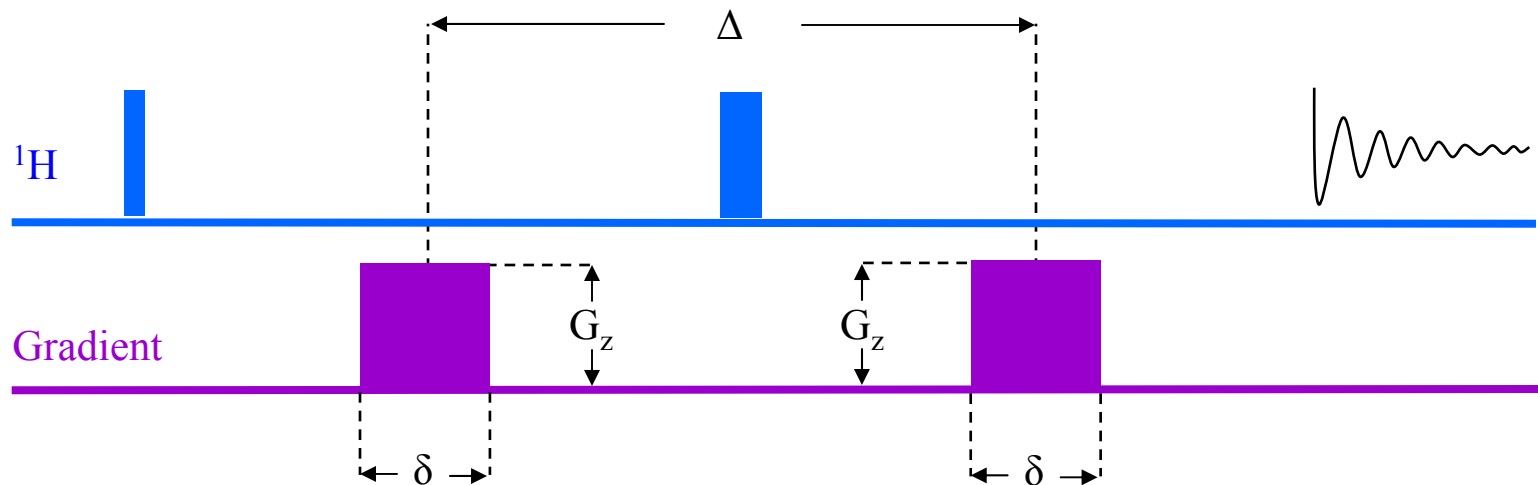
Uses for Pulsed Field Gradients

- Remove unwanted signals by destroying magnetic field homogeneity
- Eliminate or minimize the need to phase cycle pulses (gradient accelerated spectroscopy)
- Measure molecular diffusion
- Obtain magnetic resonance images

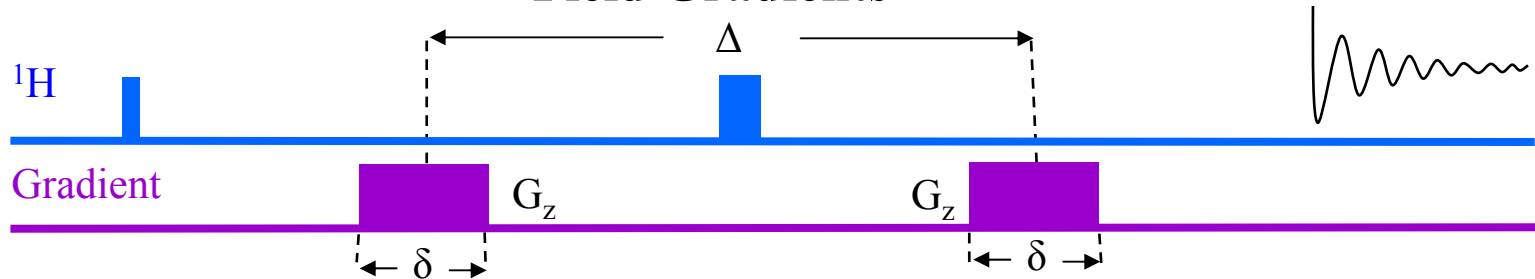
Measurement of Translational Diffusion Using NMR with Pulsed Field Gradients

One can use NMR with pulsed field gradients to measure translational diffusion constants of molecules. This may be useful in its own right but can also be used as a means of distinguishing monomers from dimers which may have identical NMR spectra. Monomers will diffuse faster than dimers because of their smaller size. This technique also has applications in the study of complexation, solvation and mixture analysis.

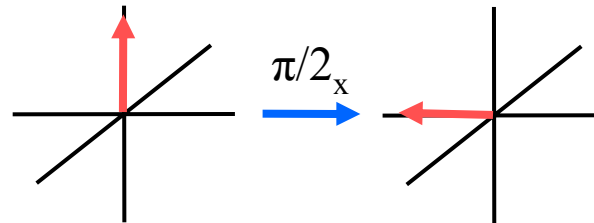
The technique uses a simple spin echo sequence with two pulsed field gradients of duration, δ , separated by time interval, Δ .



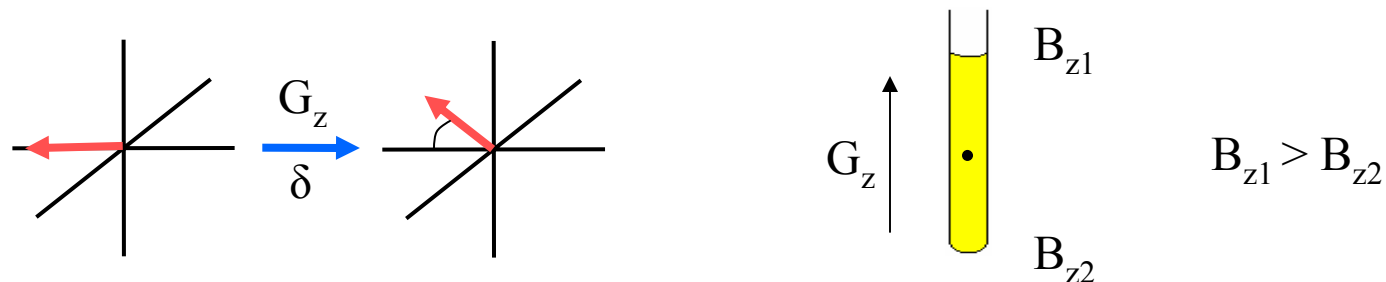
Measurement of Translational Diffusion Using NMR with Pulsed Field Gradients



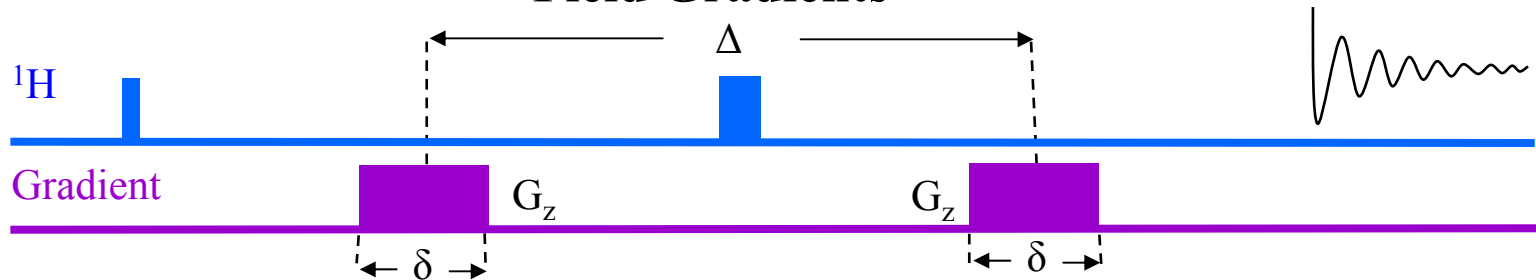
Let's consider how this sequence works for an "on-resonance" stationary spin:



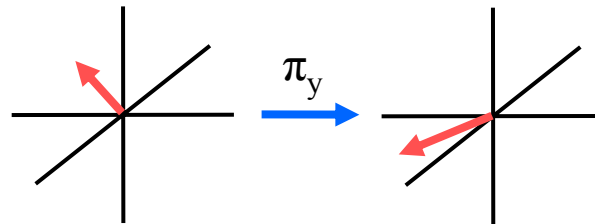
The gradient pulse is applied during period, δ . During that period of time the spin is no longer on resonance as it experiences a different magnetic field strength as a result of the gradient and therefore rotates in the rotating frame in accordance with both the duration of the gradient, δ and the strength of the gradient, G_z . After the gradient pulse, the spin vector is again stationary in the rotating frame as once again it experiences B_0 .



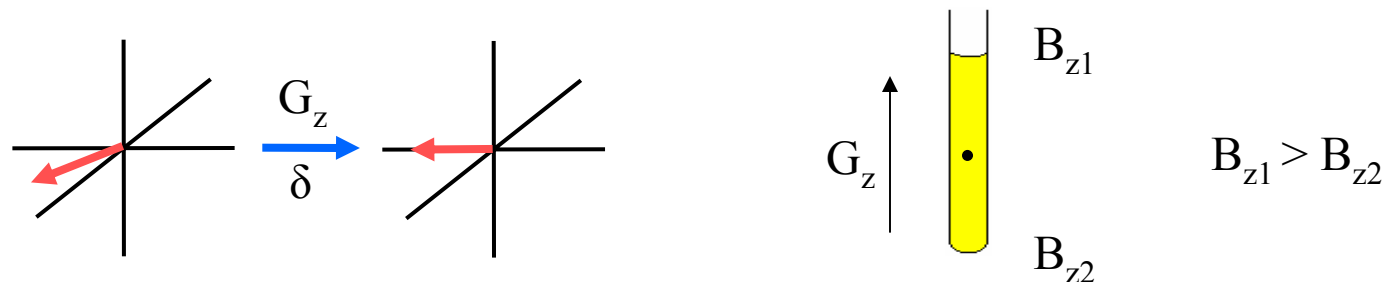
Measurement of Transnational Diffusion Using NMR with Pulsed Field Gradients



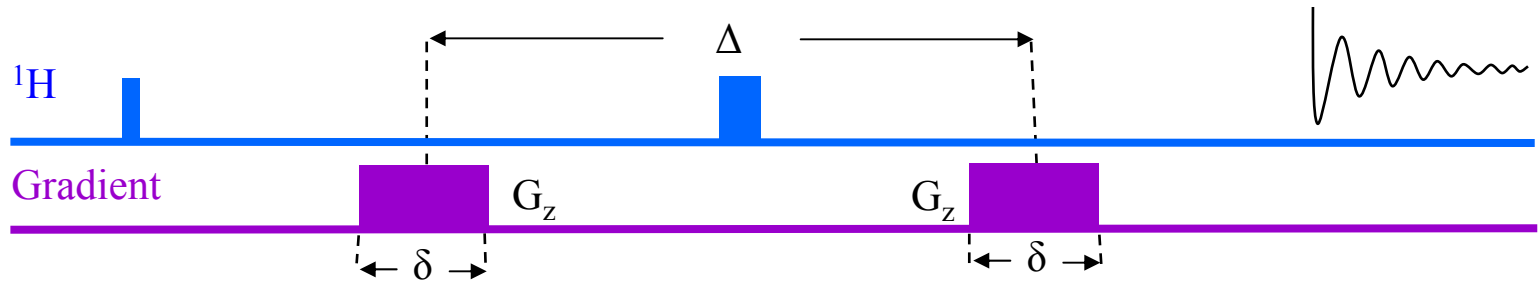
The π_y pulse flips the vector 180° about the y axis.



The second gradient pulse is applied. During the gradient pulse the spin vector again rotates in the rotating frame in accordance with both the duration of the gradient, δ and the strength of the gradient G_z . The second gradient pulse has the effect of canceling out the effect of the first gradient pulse and the spin vector is left on the $-y$ axis.

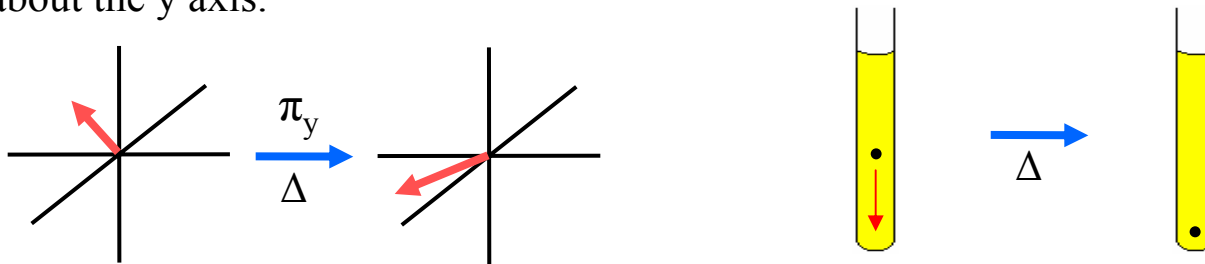


Measurement of Transnational Diffusion Using NMR with Pulsed Field Gradients

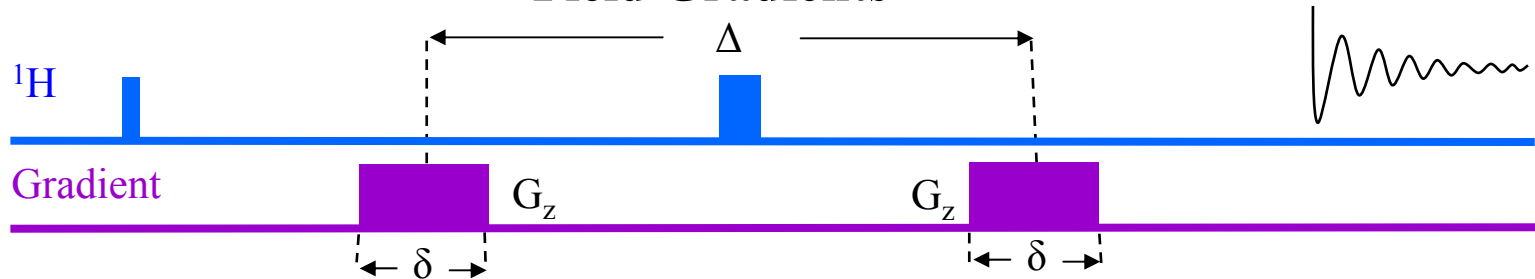


Since all peaks cannot be on-resonance, the π_y pulse is required to refocus their chemical shifts.

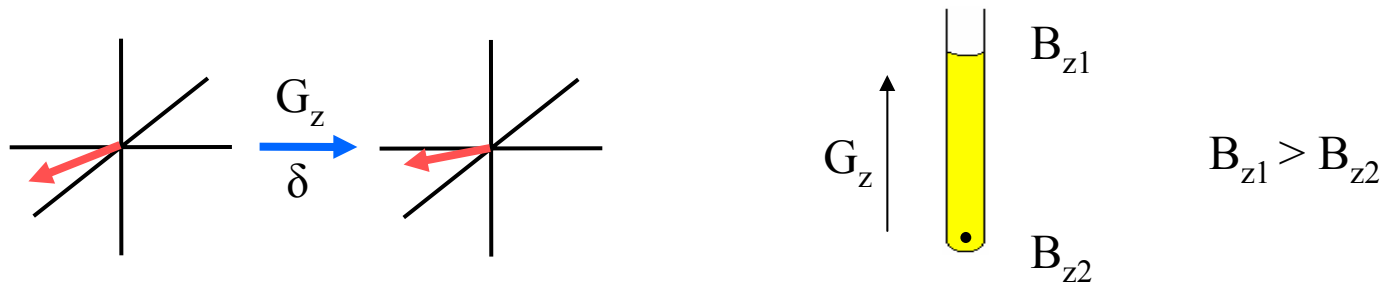
Now let's consider how this sequence works for an "on-resonance" spin which diffuses during the time period Δ . The spin vector behaves the same as that of the stationary spin up until the end of the first gradient pulse. During the time period, Δ the spin changes its position along the z axis due to molecular diffusion and the π_y pulse flips the vector 180° about the y axis.



Measurement of Transnational Diffusion Using NMR with Pulsed Field Gradients



The second gradient pulse is applied. During the gradient pulse the spin vector again rotates in the rotating frame in accordance with both the duration of the gradient, δ and the strength of the gradient G_z however, the speed of the rotation in the x-y plane is now different than it was before as the spin is in a different position in the NMR tube. In this case the second gradient pulse does not cancel out the effect of the first gradient pulse and the spin vector is left away from $-y$ axis, by an amount related to the extent of diffusion during time period, Δ . The result for the entire ensemble of spins, where some spins will have moved in one direction and others in the other direction, is that there will be less magnetization on the $-y$ axis and therefore a peak of lower intensity.



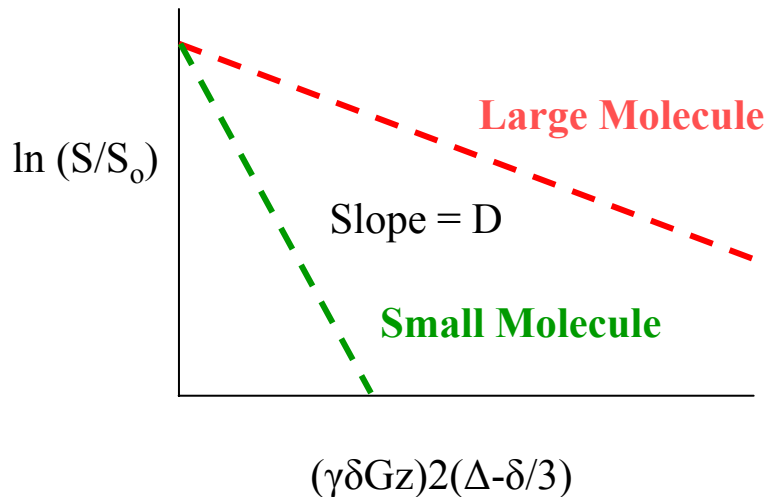
Measurement of Transnational Diffusion Using NMR with Pulsed Field Gradients

The intensity of the signal, S , for a diffusing spin can be written:

$$S = S_0 \exp (-(\gamma\delta G_z)^2 D (\Delta - \delta/3))$$

Where S_0 is the intensity of the signal when no gradients are applied and D is the diffusion constant.

The experiment is typically done by measuring spectra with a series of gradient strengths, G_z . The diffusion constant is evaluated by plotting $\ln (S/S_0)$ vs $(G_z \gamma \delta)^2 D (\Delta - \delta/3)$ for which the slope is $-D$.

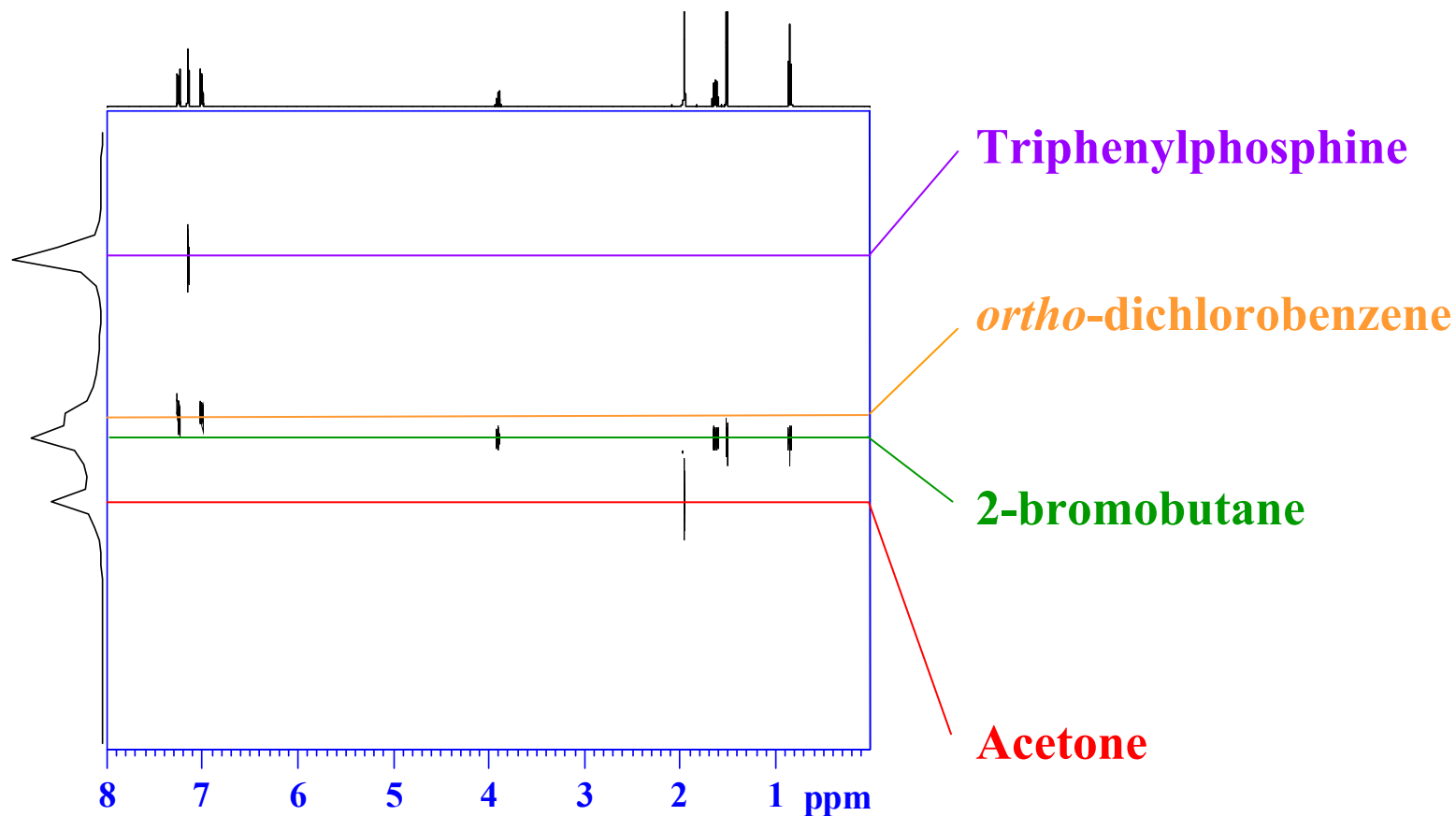


Measure signal intensity, S as a function of gradient strength, G_z .

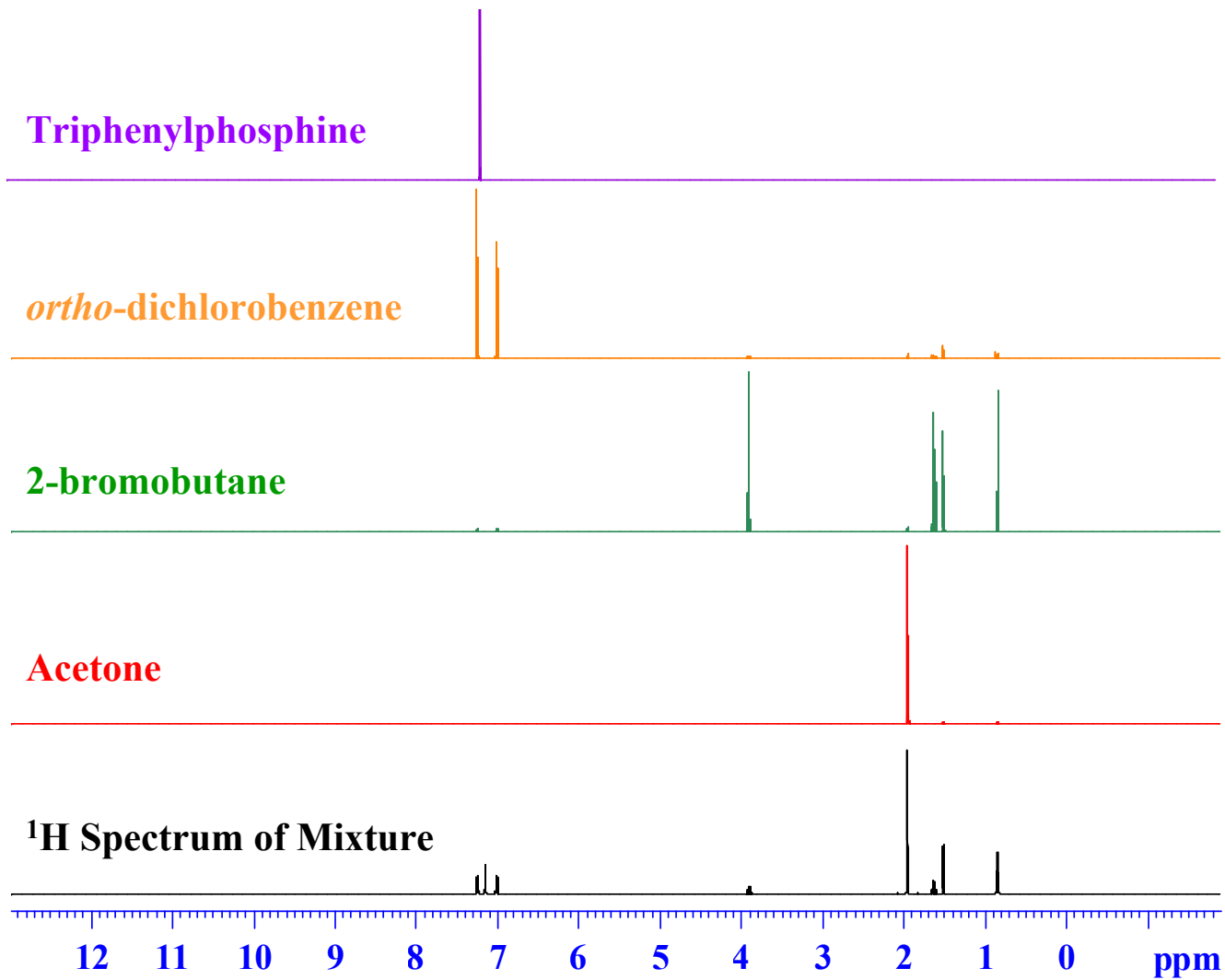
Diffusion Ordered Spectroscopy (DOSY) - Analysis of Mixtures

“NMR Chromatography”

The diffusion experiment can be performed in a pseudo-2D manner and each peak can be fit to the diffusion equation. The data are then displayed such that the diffusion constant is displayed along the vertical axis.

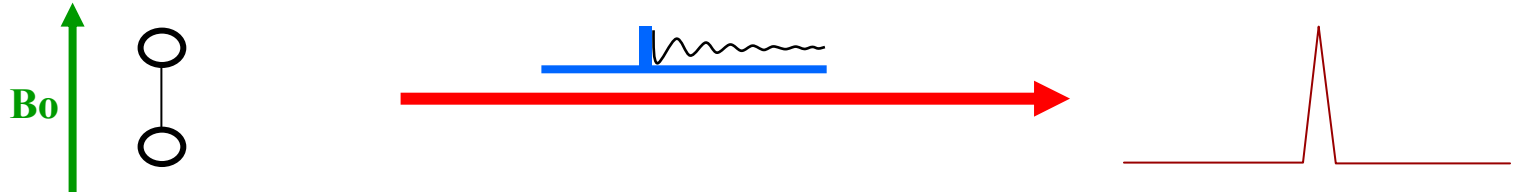


DOSY Mixture Analysis

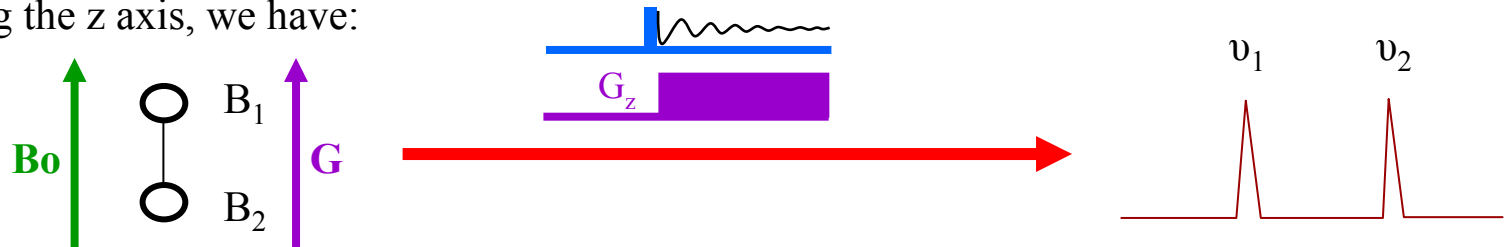


Magnetic Resonance Imaging (MRI)

NMR imaging is usually called magnetic resonance imaging (MRI). It uses NMR spectroscopy in a magnetic field with controlled inhomogeneity to give spatial information. If B_0 varies linearly with distance then the frequency of an NMR line will have the same dependence. Imagine a sample shaped like a dumbbell (two spherical vessels containing water) placed in a constant magnetic field.

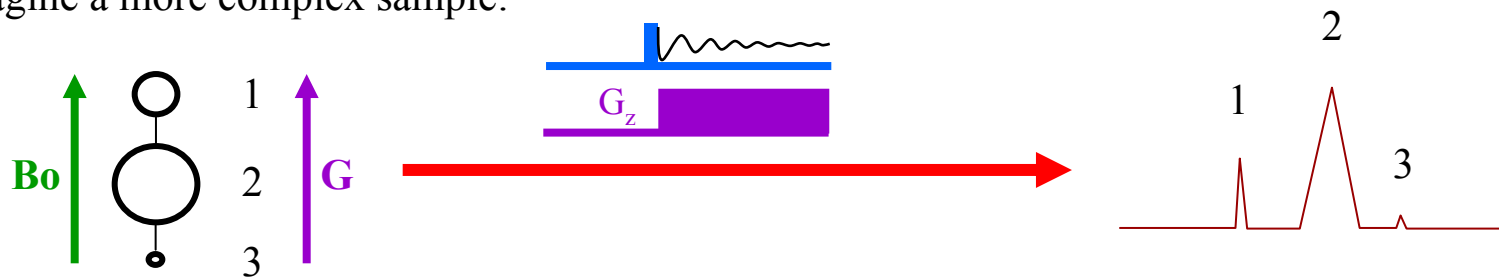


When the same sample is placed in a magnetic field with a linear magnetic field gradient along the z axis, we have:

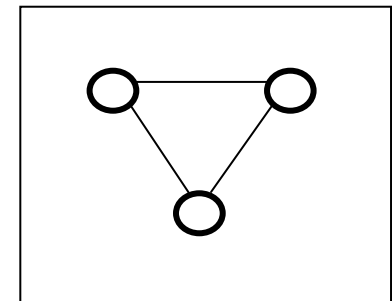
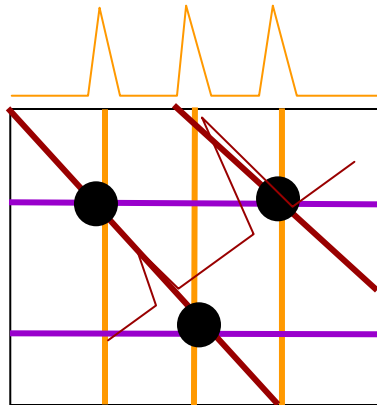
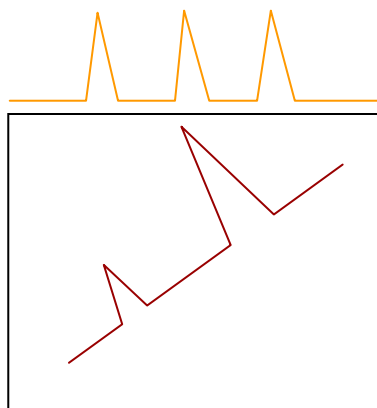
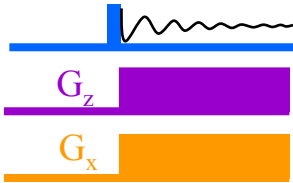
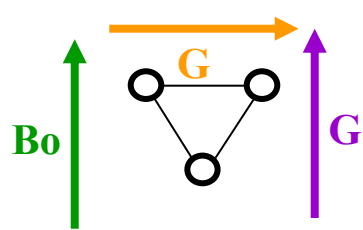
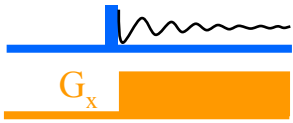
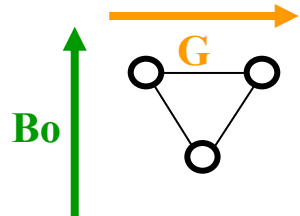
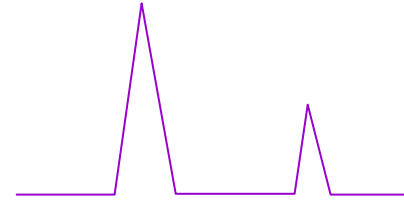
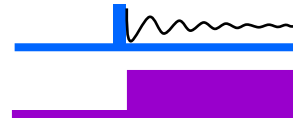
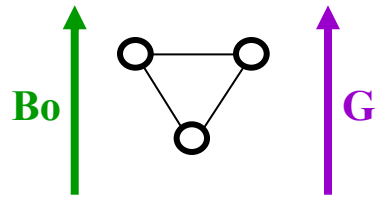


The spectrum is a one dimensional image of the sample where the frequency difference between the peaks is proportional to the distance between the water drops in the sample.

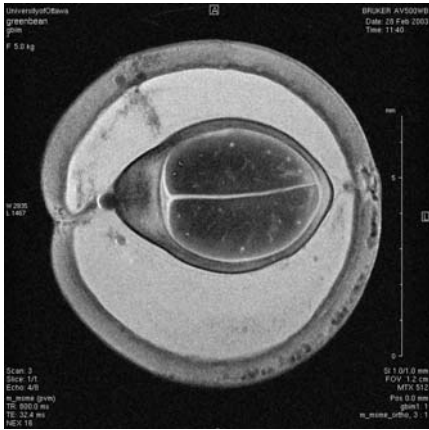
Imagine a more complex sample:



Two Dimensional MRI



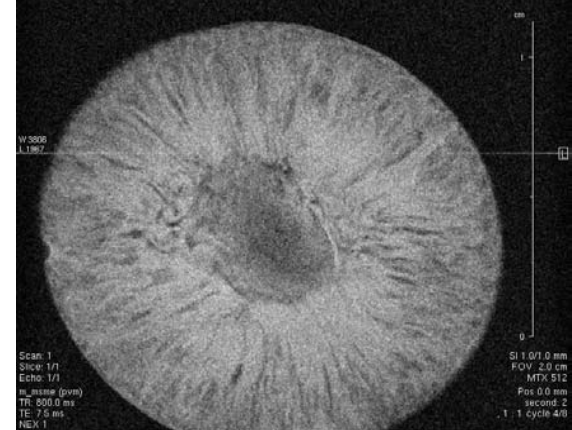
Magnetic Resonance Images



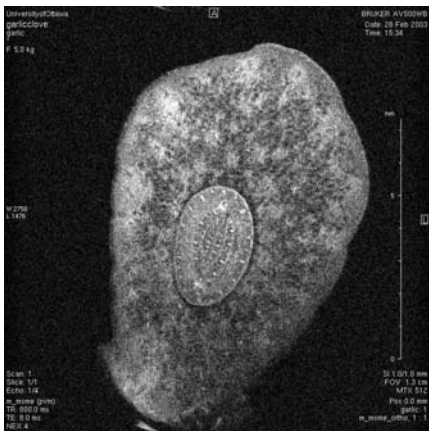
Green Bean



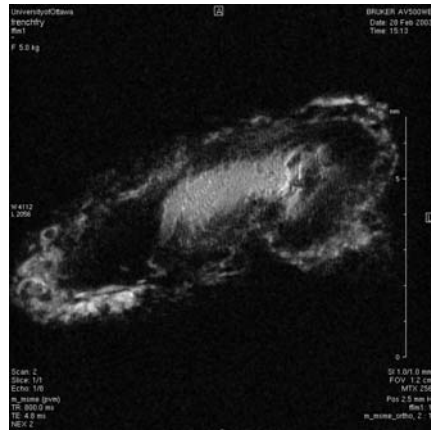
Apple Seed



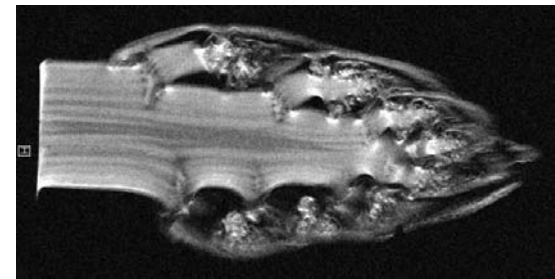
Seedless Grape



Garlic Clove



French Fry



Asparagus Tip