NMR spectroscopy

Prepared by Dr. Upali Siriwardane

For

CHEM 481 Lab

Objectives

1. Student should gain better understanding of NMR spectroscopy.
2. Student should gain experience in the acquisition, processing, and displaying NMR data.
3. Student should gain experience in interpreting NMR data in order to establish structure for unknown organic molecules.
4. Student should gain understanding in advanced 1Dimensional and 2Dimensional NMR techniques.

Information from ^{1}H-nmr spectra:

1. Number of signals: How many different types of hydrogens in the molecule.
2. Position of signals (chemical shift): What types of hydrogens.
3. Relative areas under signals (integration): How many hydrogens of each type.
4. Splitting pattern: How many neighboring hydrogens.

Introduction

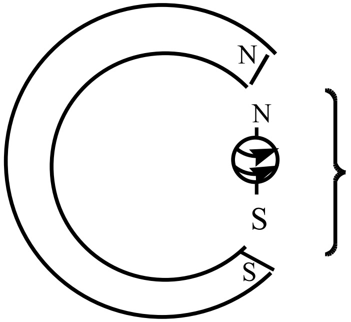
- The Nobel Prize has been awarded twice for work related to NMR. **F. Bloch** and **E.M. Purcell** received the Nobel Prize in Physics, in 1952, for the first experimental verifications of the phenomenon, and Prof. R.R. **Ernst** received the Nobel Prize in Chemistry, in 1991, for the development of the NMR techniques.
- Since its discovery 50 years ago, in 1945, it has spread from physics to chemistry, biosciences, material research and medical diagnosis.

Spin quantum number(I)

- Nuclear spin is characterized by a spin number, I, which can be zero or some positive integer multiple of 1/2 (e.g. 1/2, 1, 3/2, 2 etc.). Nuclei whose spin number, I= 0 have no magnetic moment(μ);eg. ^{12}C and ^{16}O show no NMR signal. Elements such as ^{1}H, ^{13}C, ^{19}F and ^{31}P have I=1/2, while others have even higher spin numbers:
- I=1 ^{14}N, ^{2}H
- I=3/2 ^{11}B, ^{35}Cl, ^{37}Cl, ^{79}Br, ^{81}Br.
- As the values for I increase, energy levels and shapes of the magnetic fields become progressively more and more complex.

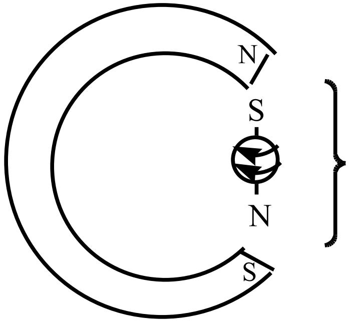
NMR = Nuclear Magnetic Resonance

Physical Principles:



α- spin state,
favorable,
lower energy

A spinning nucleus with it's magnetic field aligned **with** the magnetic field of a magnet



β- spin state,
unfavorable,
higher energy

A spinning nucleus with it's magnetic field aligned **against** the magnetic field of a magnet

Alignment with the magnetic field (called α) is lower energy than against the magnetic field (called β). How much lower it is depends on the strength of the magnetic field

NMR = Nuclear Magnetic Resonance

Physical Principles:

Boltzmann Distribution of Spin States

- In a given sample of a specific nucleus, the nuclei will be distributed throughout the various spin states available. Because the energy separation between these states is comparatively small, energy from thermal collisions is sufficient to place many nuclei into higher energy spin states. The numbers of nuclei in each spin state are described by the Boltzman distribution

Boltzman distribution

$$\frac{N_{upper}}{N_{lower}} = e^{-\gamma_N Hh/kT}$$

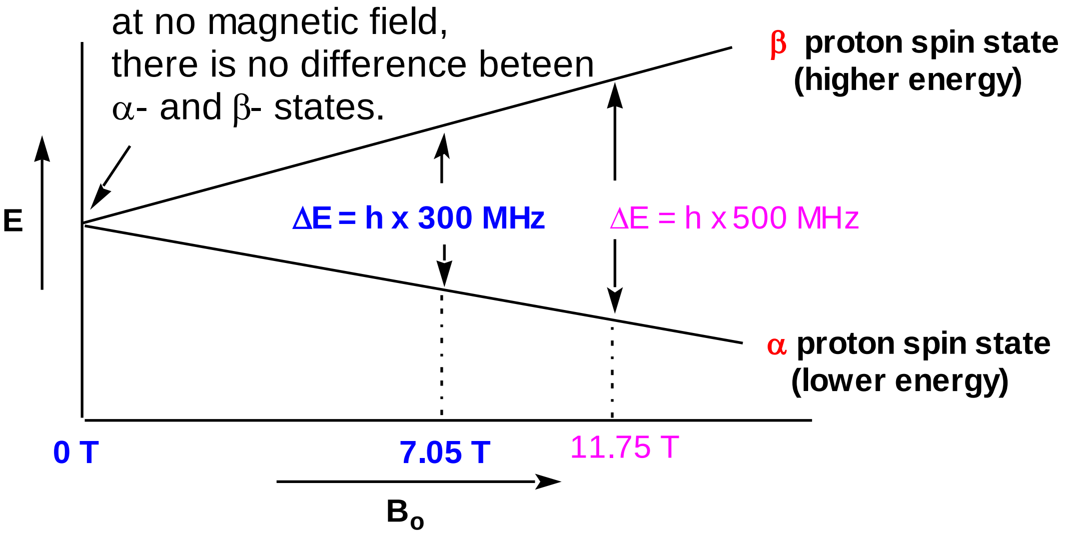
- where the N values are the numbers of nuclei in the respective spin states, is the magnetogyric ratio, **h** is Planck's constant, **H(B)** is the external magnetic field strength, k is the Boltzmann constant, and T is the temperature.
- In NMR, the energy separation of the spin states is comparatively very small and while NMR is very informative it is considered to be an insensitive technique .

Example: Boltzman distribution

- For example, given a sample of ^{1}H nuclei in an external magnetic field of 1.41 Tesla
- ratio of populations = $e^{((-2.67519\text{x}10\text{e}8 \text{ rad.s-1.T-1} * 1.41\text{T} * 6.626176\text{x}10\text{-}34 \text{ J.s}) / (1.380662\text{x}10\text{e-}23 \text{ J.K-1} * \text{K } 293))}$ = 0.9999382
- At room temperature, the ratio of the upper to lower energy populations is 0.9999382. In other words, the upper and lower energy spin states are almost equally populated with only a very small excess in the lower energy state.
- If N_0= 10^6 or 1,000,000 then N_j 999,938
- N_0- N_j =1,000,000 – 999,938 = 62
- 62 ppm excess in the ground state

NMR: Basic Experimental Principles

Graphical relationship between magnetic field (B_o) and frequency (ν) for ^{1}H NMR absorptions

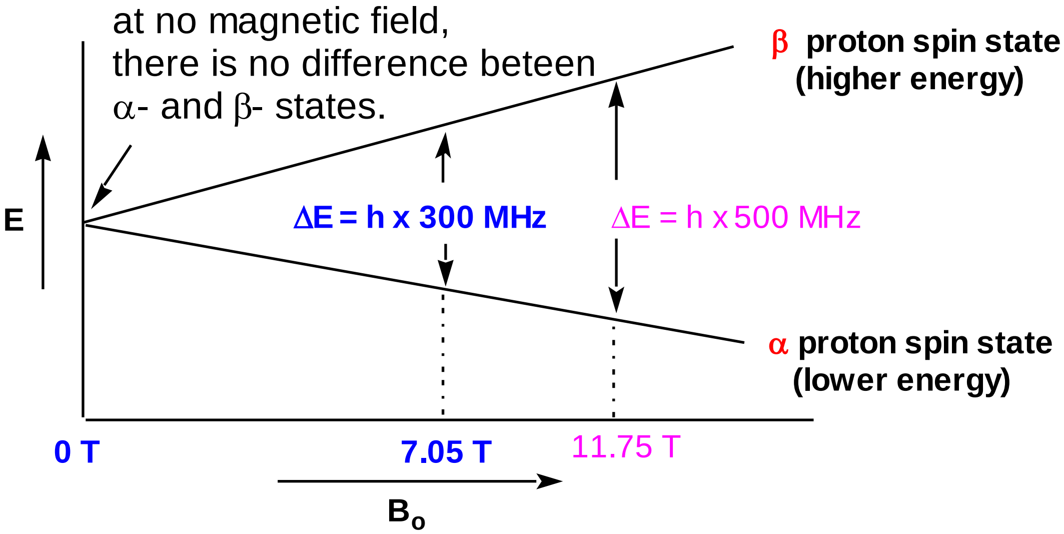

at no magnetic field, there is no difference beteen α- and β- states.

But there's a problem. If two researchers want to compare their data using magnets of different strengths, they have to adjust for that difference. That's a pain, so, data is instead reported using the "chemical shift" scale as described on the next slide.

NMR: Basic Experimental Principles

Imagine placing a molecule, for example, CH_4, in a magnetic field.
We can probe the energy difference of the α- and β- state of the protons by irradiating them with EM radiation of just the right energy.
In a magnet of 7.05 Tesla, it takes EM radiation of about 300 MHz (radio waves).
So, if we bombard the molecule with 300 MHz radio waves, the protons will absorb that energy and we can measure that absorbance.
In a magnet of 11.75 Tesla, it takes EM radiation of about 500 MHz (stronger magnet means greater energy difference between the α- and β- state of the protons)

Graphical relationship between magnetic field (B_o) and frequency (ν) for ^1H NMR absorptions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But there’s a problem. If two researchers want to compare their data using magnets of different strengths, they have to adjust for that difference. That’s a pain, so, data is instead reported using the “chemical shift” scale as described on the next slide.

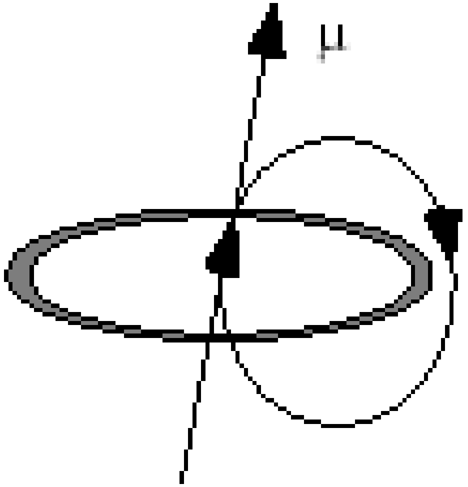
The Chemical Shift (Also Called δ) Scale

Here's how it works. We decide on a sample we'll use to standardize our instruments. We take an NMR of that standard and measure its absorbance frequency. We then measure the frequency of our sample and subtract its frequency from that of the standard. We then then divide by the frequency of the standard. This gives a number called the "chemical shift," also called δ, which does not depend on the magnetic field strength. Why not? Let's look at two examples.

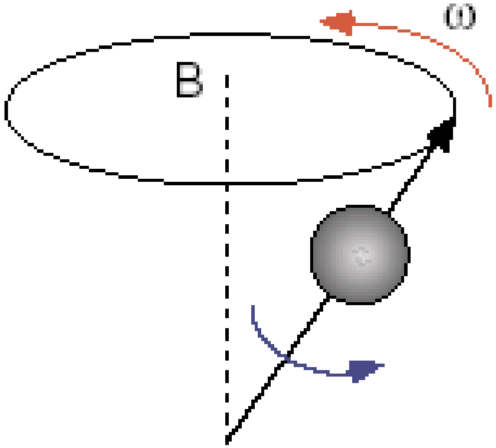
Imagine that we have a magnet where our standard absorbs at 300,000,000 Hz (300 megahertz), and our sample absorbs at 300,000,300 Hz. The difference is 300 Hz, so we take 300/300,000,000 = 1/1,000,000 and call that 1 part per million (or 1 PPM). Now lets examine the same sample in a stronger magnetic field where the reference comes at 500,000,000 Hz, or 500 megahertz. The frequency of our sample will increase proportionally, and will come at 500,000,500 Hz. The difference is now 500 Hz, but we divide by 500,000,000 (500/500,000,000 = 1/1,000,000, = 1 PPM).
It's brilliant.

Of course, we don't do any of this, it's all done automatically by the NMR machine.
Even more brilliant.

The Physical Basis of the NMR Experiment



- Imagine a charge travelling circularily about an axis builds up a magnetic moment
- It rotates (spins) about its own axis (the blue arrow) and **precesses** about the axis of the magnetic field B (the red arrow). The frequency of the precession (ω) is proportional to the strength of the magnetic field:



☐ $\omega = \gamma B_0$

γ = magnetogyro ratio

Magnetic field mrasured in Tesla

1 T = 10,000 gauss

Magnetogyric ratio(γ)

The larger the value of the magnetogyric ratio, the larger the

Magnetic moment (μ) of the nucleus and the easier it is to see by NMR spectroscopy.

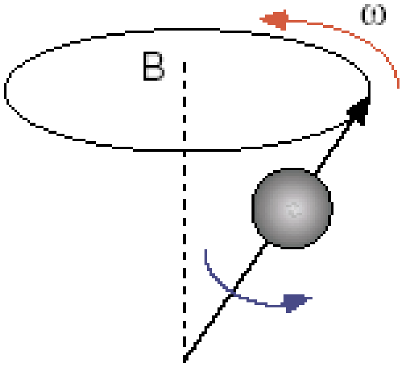
Energy difference (ΔE) between $I_z = +1/2$ and $I_z = -1/2$.

The Physical Basis of the NMR Experiment:

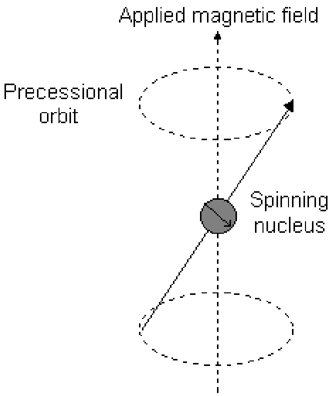
- Nuclear magnetic resonance, or NMR as it is abbreviated by scientists, is a phenomenon which occurs when the nuclei of certain atoms are immersed in a static strong magnetic field and exposed to a second oscillating magnetic field in the form of radiofrequency pulses, it is possible to transfer energy into the spin system and change the state of the system. After the pulse, the system relaxes back to its state of equilibrium, sending a weak signal that can be recorded.

Larmour frequency

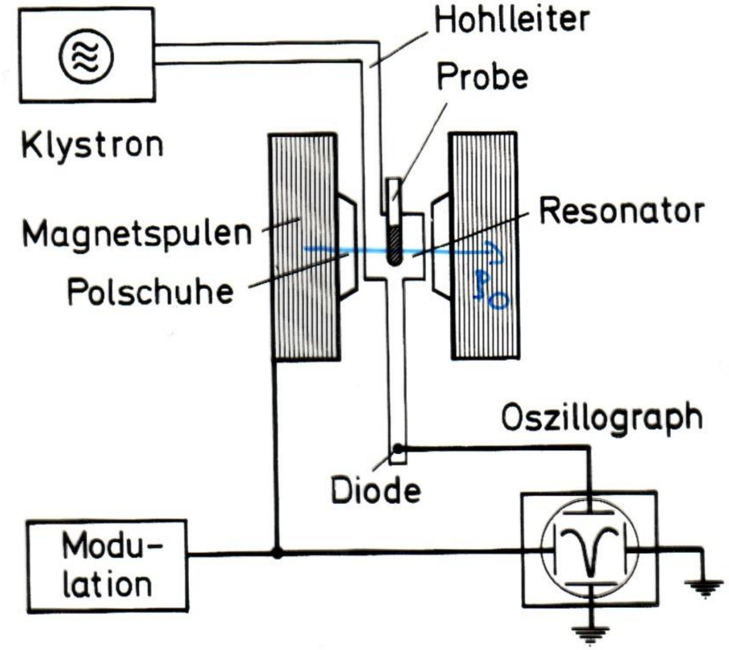
- **Precession**: The circular movement of the magnetic moment in the presence of the applied field.
- **Larmour frequency** : The angular frequency of the precessionis related to the external magnetic field strength B_0, by the gyromagnetic ratio γ :



$$\omega_0 = \gamma B_0$$



Classical NMR experiments



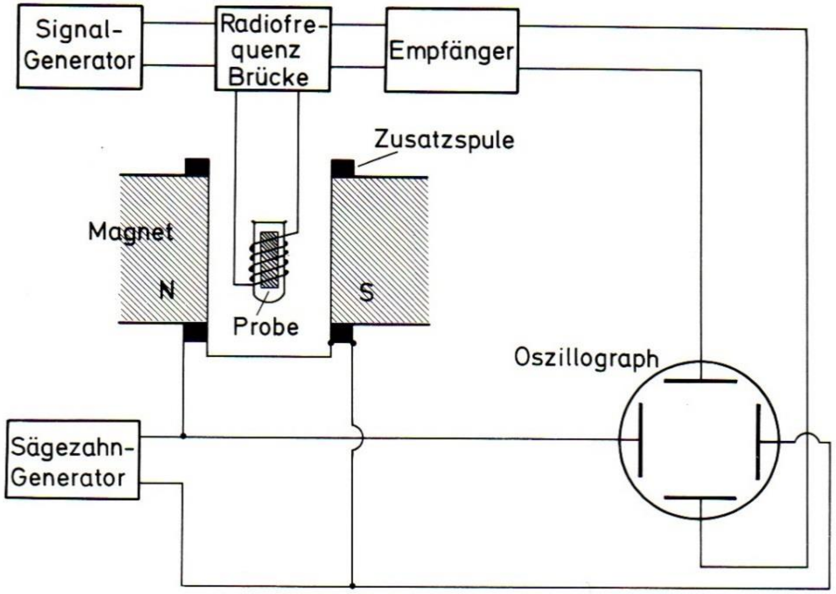
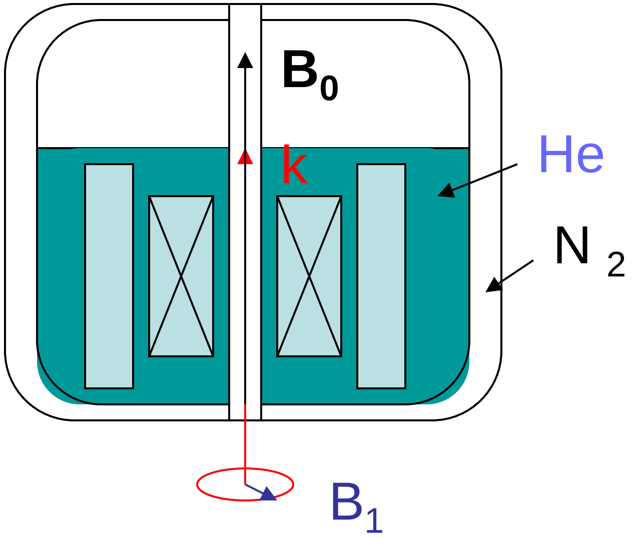


Abb. 20.18. Schema einer einfachen Kernspin-Resonanz-Apparatur. Die Probe befindet sich im Reagenzglas zwischen den Polschuhen eines homogenen Magneten. Das hochfrequente B_1-Feld wird über eine Brücke und eine Induktionsspule eingestrahlt. Zum besseren Nachweis der Resonanz kann das B_0-Feld durch eine Zusatzspule moduliert werden

Absorption signal

600 MHz Proton NMR Spectrometer

High frequency NMR spectrometers require very strong magnetic fields, which are produced using super-cooled coils (T = 4.2K, liquid He). The superconducting coils are surrounded by a giant vessel containing liquid N_2.



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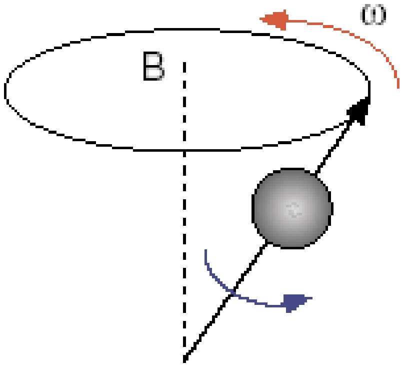
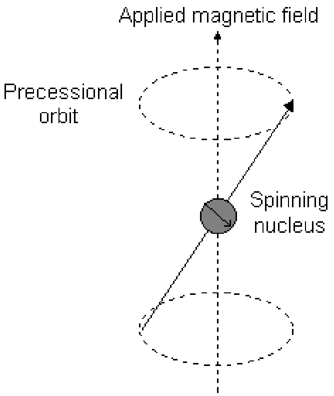


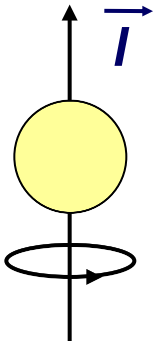
$$\omega_0 = \gamma B_0$$

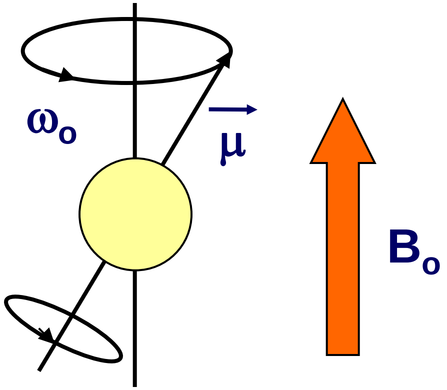


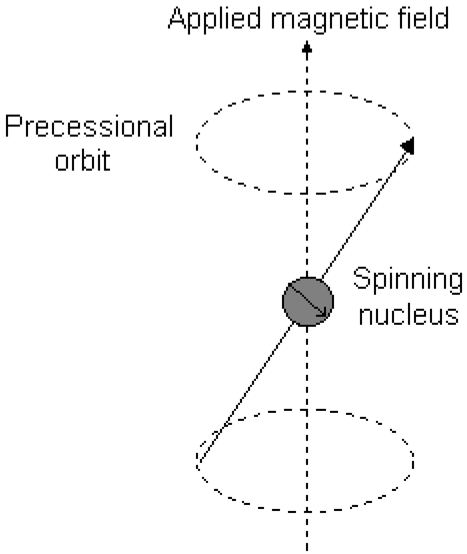
Classical View of NMR (*compared to Quantum view*)

Precession or ***Larmor*** frequency: $\omega = 2\pi\nu \Rightarrow \omega_o = \gamma B_o$ (radians)

angular momentum (I)







Simply, the nuclei spins about its axis creating a magnetic moment $\vec{\mu}$

Maxwell: Magnetic field ≡ Moving charge

Apply a large external field (B_o) and $\vec{\mu}$ will precess about B_o at its Larmor (ω) frequency.

Important: This is the same frequency obtained from the energy transition between quantum states

Quantum-mechanical treatment:

- The dipole moment μ of the nucleus is described in quantum-mechanical terms as

$$\mu = \gamma J$$

- Therein, J is the **spin angular momentum** and γ the magnetogyric ratio of the spin. When looking at single spins we have to use a quantum-mechanical treatment.
- Therein, the z-component of the angular momentum J is **quantitized** and can only take **discrete values**

$$\{J_z\} = m\frac{h}{2\pi}$$

- J is related to spin quantum number of the nuclei I

  -I,…,o,…,+I

Spin quantum number(I)

- Nuclear spin is characterized by a spin number, I, which can be zero or some positive integer multiple of 1/2 (e.g. 1/2, 1, 3/2, 2 etc.). Nuclei whose spin number, I= 0 have no magnetic moment(μ);eg. ^{12}C and ^{16}O show no NMR signal. Elements such as ^{1}H, ^{13}C, ^{19}F and ^{31}P have I=1/2, while others have even higher spin numbers:
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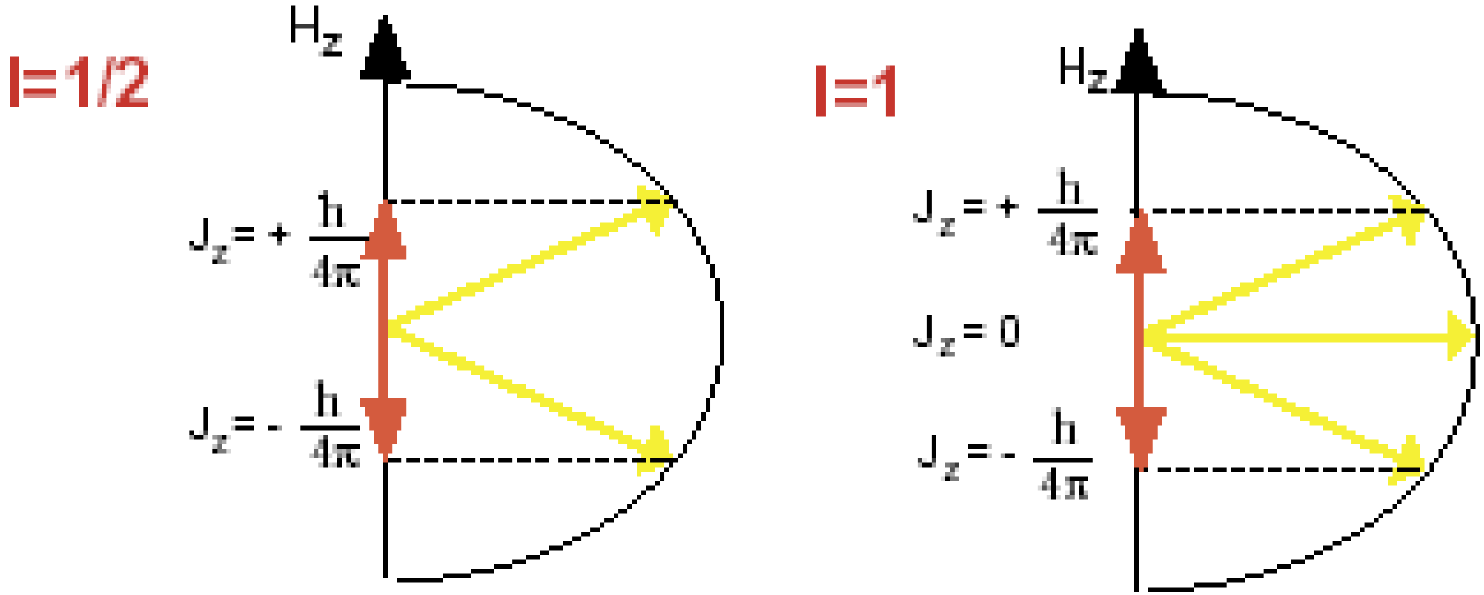
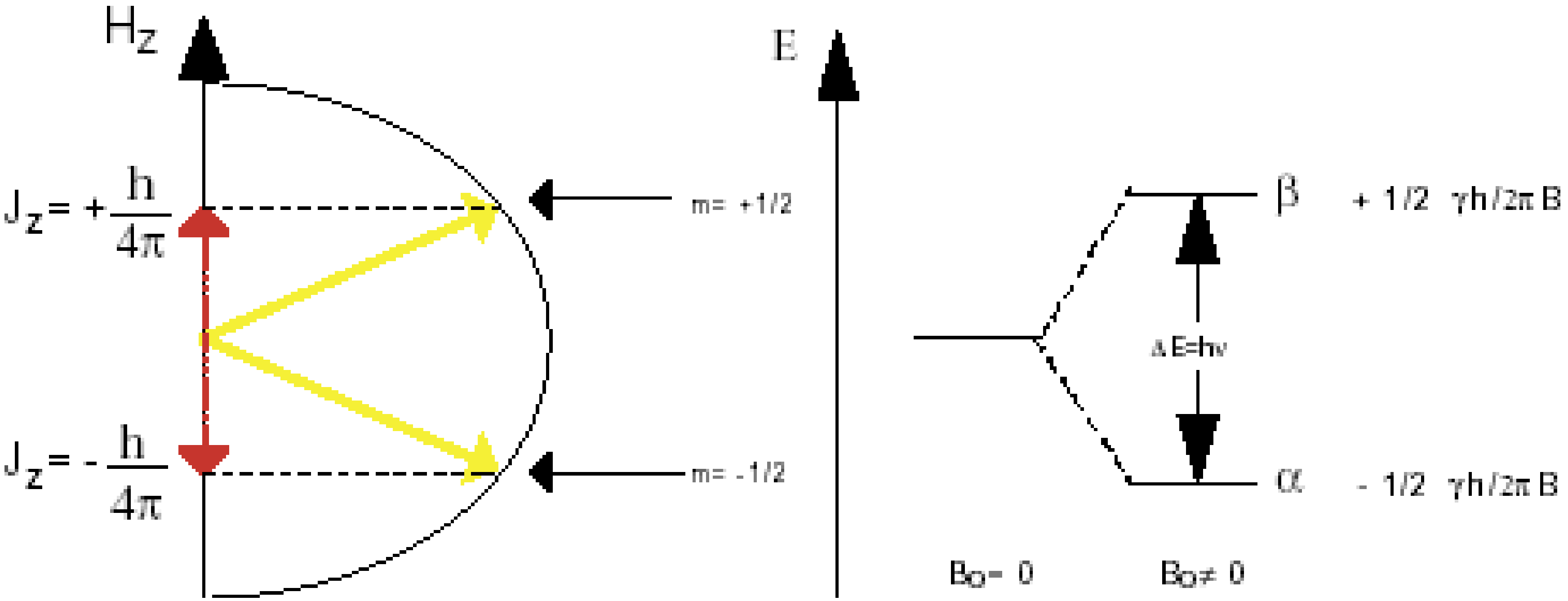
z-component of the angular momentum J



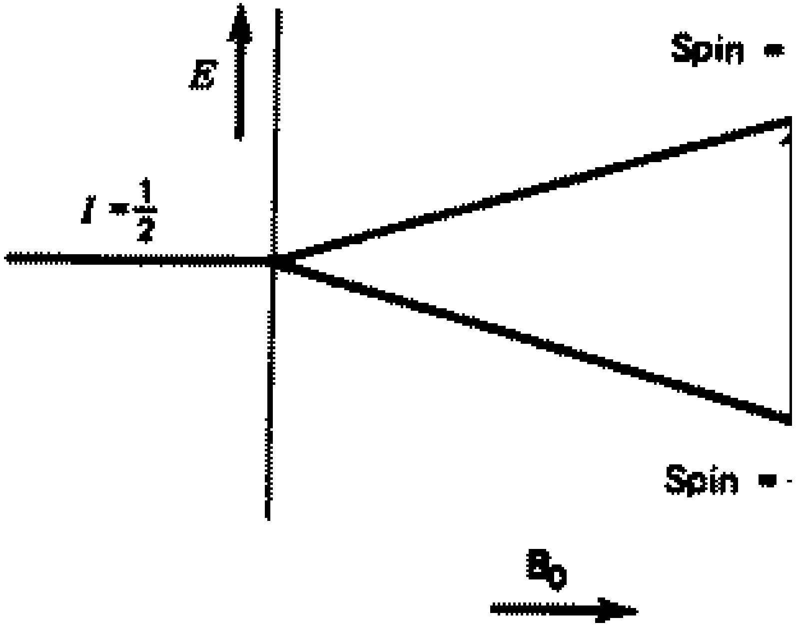
For I=1/2 nuclei, **m** can only be +1/2 or -1/2, giving rise to two distinct energy levels. For spins with I=1 nuclei three different values for Jz are allowed:

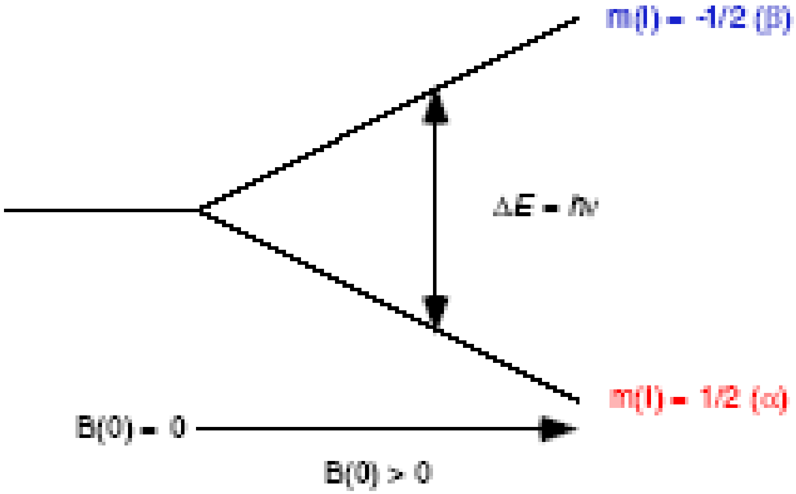
The energy difference ΔE,



- ***Zeeman effect***: splitting of energy levels in magnetic field
- The energy difference ΔE, which corresponds to the two states with m=±1/2, is then (the quantum-mechanical selection rule states, that only transitions with m= ±1 are allowed):

A Nuclei with I= 1/2 in a Magnetic Field





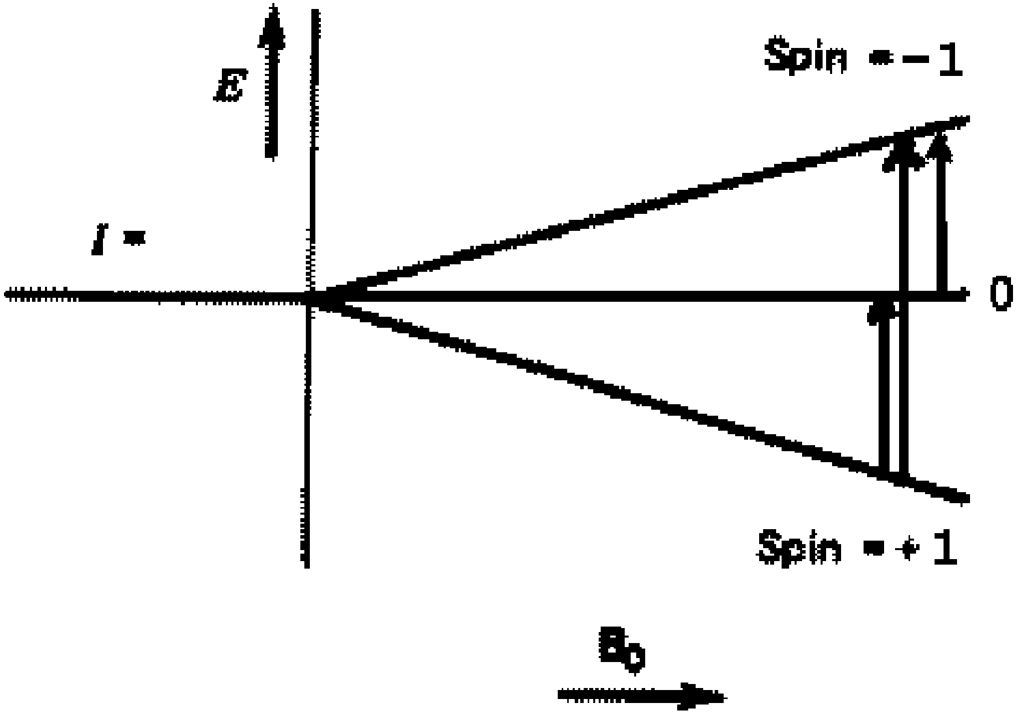
$\Delta \mathbf{E} = \mathbf{h}\,\nu$

$\nu = \gamma\, \mathbf{B_o} / 2\pi$

$\Delta \mathbf{E} = \gamma\, \mathbf{h}\, \mathbf{B_o} / 2\pi$

number of states = **2I+1**

A Nuclei with I= 1 in a Magnetic Field



number of states = **2I+1**

Boltzmann Distribution of Spin States

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Boltzman distribution

$$\frac{N_{\text{upper}}}{N_{\text{lower}}} = e^{-\gamma_N Hh/kT}$$

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Example: Boltzman distribution

- For example, given a sample of ^{1}H nuclei in an external magnetic field of 1.41 Tesla
- ratio of populations = $e^{\text{((-2.67519x10e8 rad.s-1.T-1 * 1.41T * 6.626176x10-34 J.s) / (1.380662x10e-23 J.K-1 *K 293))}}$ = 0.9999382
- At room temperature, the ratio of the upper to lower energy populations is 0.9999382. In other words, the upper and lower energy spin states are almost equally populated with only a very small excess in the lower energy state.
- If N_0= 10^6 or 1,000,000 then N_j 999,938
- N_0- N_j =1,000,000 – 999,938 = 62
- 62 ppm excess in the ground state

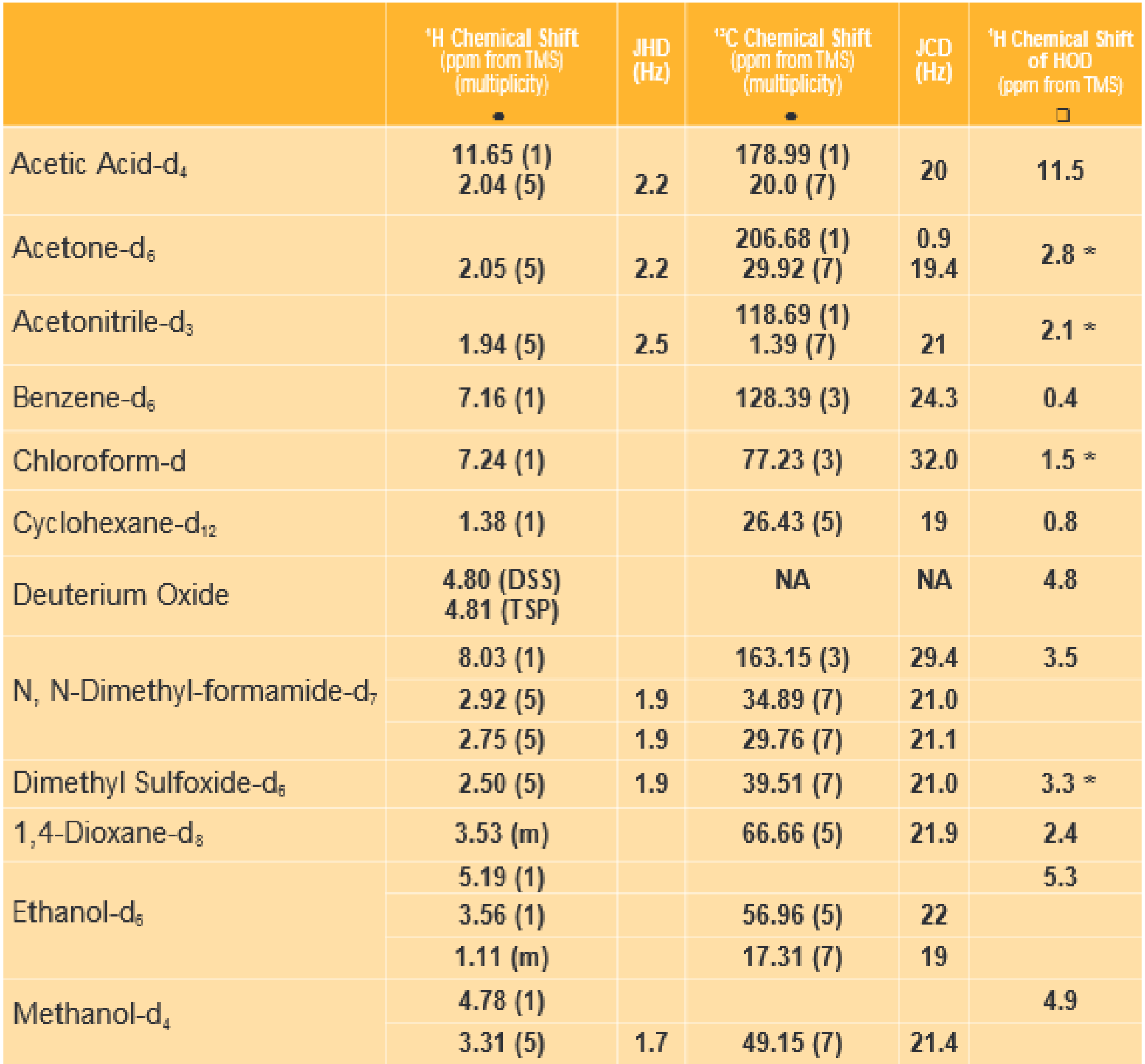
Saturation

- The condition that exists when the upper and lower energy states of nuclei are equal. (no observed signal by NMR)

δ values chemical shifts

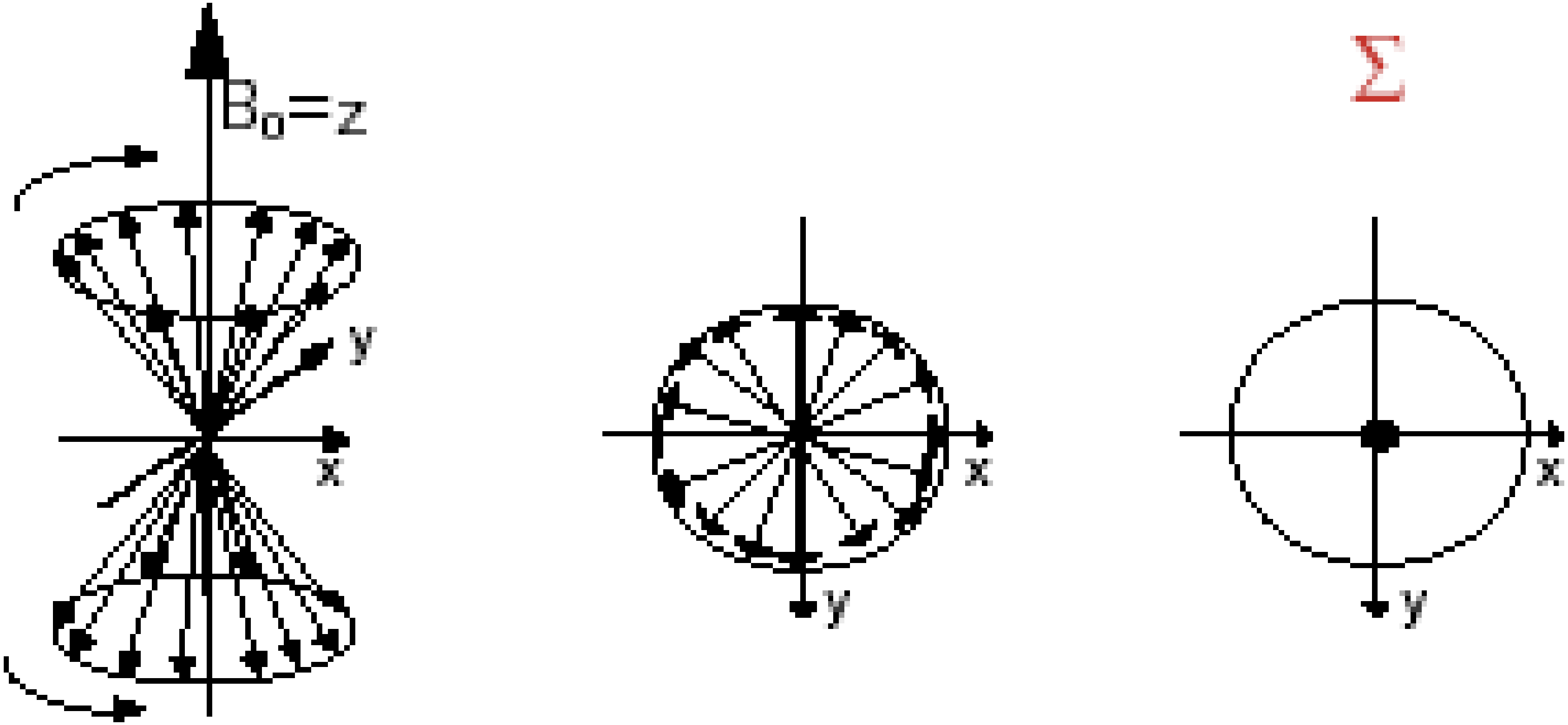
$$\delta\ (\text{ppm}) = \frac{\nu\ (\text{peak}) - \nu(\text{ref})}{\nu\ (\text{ref})} \times 10^6$$

Deuterated Solvents



	^{1}H Chemical Shift (ppm from TMS) (multiplicity) •	JHD (Hz)	^{13}C Chemical Shift (ppm from TMS) (multiplicity) •	JCD (Hz)	^{1}H Chemical Shift of HOD (ppm from TMS) ❑
Acetic Acid-d_4	11.65 (1) 2.04 (5)	2.2	178.99 (1) 20.0 (7)	20	11.5
Acetone-d_6	2.05 (5)	2.2	206.68 (1) 29.92 (7)	0.9 19.4	2.8 *
Acetonitrile-d_3	1.94 (5)	2.5	118.69 (1) 1.39 (7)	21	2.1 *
Benzene-d_6	7.16 (1)		128.39 (3)	24.3	0.4
Chloroform-d	7.24 (1)		77.23 (3)	32.0	1.5 *
Cyclohexane-d_{12}	1.38 (1)		26.43 (5)	19	0.8
Deuterium Oxide	4.80 (DSS) 4.81 (TSP)		NA	NA	4.8
N, N-Dimethyl-formamide-d_7	8.03 (1)		163.15 (3)	29.4	3.5
	2.92 (5)	1.9	34.89 (7)	21.0	
	2.75 (5)	1.9	29.76 (7)	21.1	
Dimethyl Sulfoxide-d_6	2.50 (5)	1.9	39.51 (7)	21.0	3.3 *
1,4-Dioxane-d_8	3.53 (m)		66.66 (5)	21.9	2.4
Ethanol-d_6	5.19 (1)				5.3
	3.56 (1)		56.96 (5)	22	
	1.11 (m)		17.31 (7)	19	
Methanol-d_4	4.78 (1)				4.9
	3.31 (5)	1.7	49.15 (7)	21.4	

Vector representation

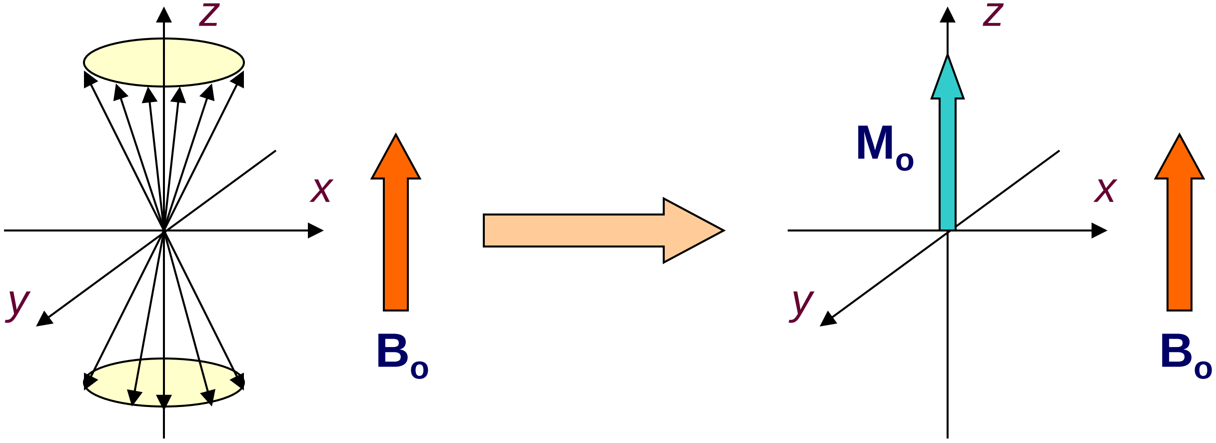


Bulk magnetization (M_o)

Now consider a real sample containing numerous nuclear spins:

M_o 🔔 **$(N\alpha - N\beta)$**

$$\vec{\mu} = \mu_x i + \mu_y j + \mu_z k$$

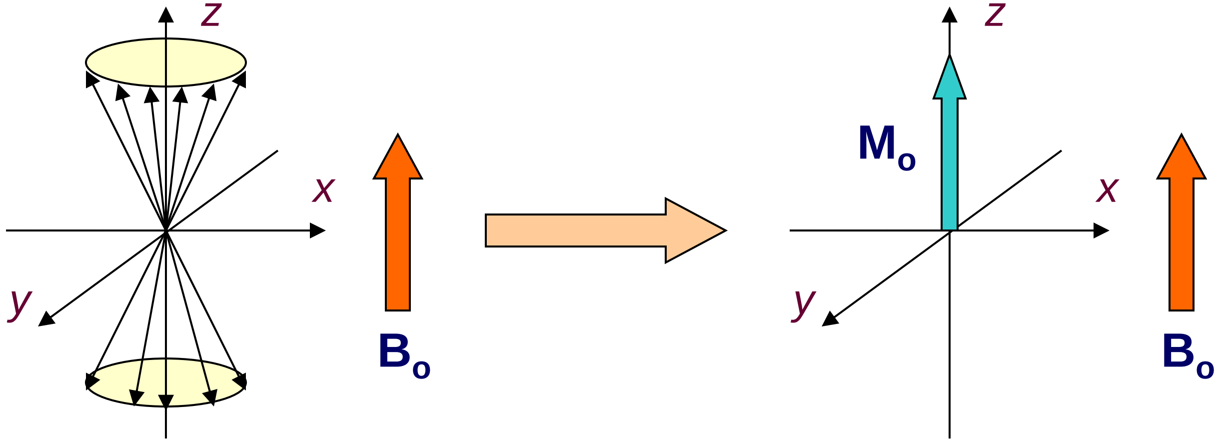


Since μ is precessing in the xy-plane, **M_o** $= \sum \mu_z k - \mu_{-z} k$

μ is ***quantized*** (α or β), **M_o** has a ***continuous*** number of states, bulk property.

An NMR Experiment

We have a net magnetization precessing about B_o at a frequency of ω_o with a net population difference between aligned and unaligned spins.

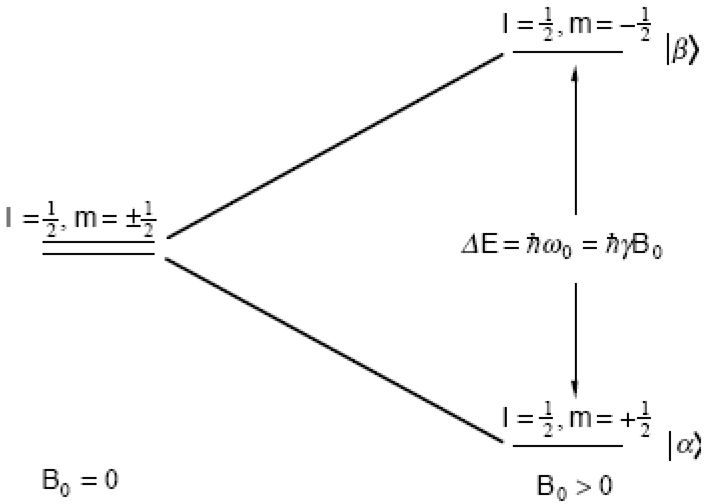


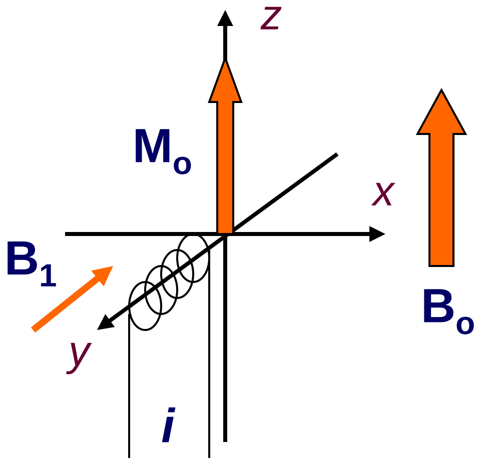
Now What?

Perturbed the spin population or perform spin gymnastics
Basic principal of NMR experiments

An NMR Experiment

To perturbed the spin population need the system to absorb energy.





Transmitter coil (y)

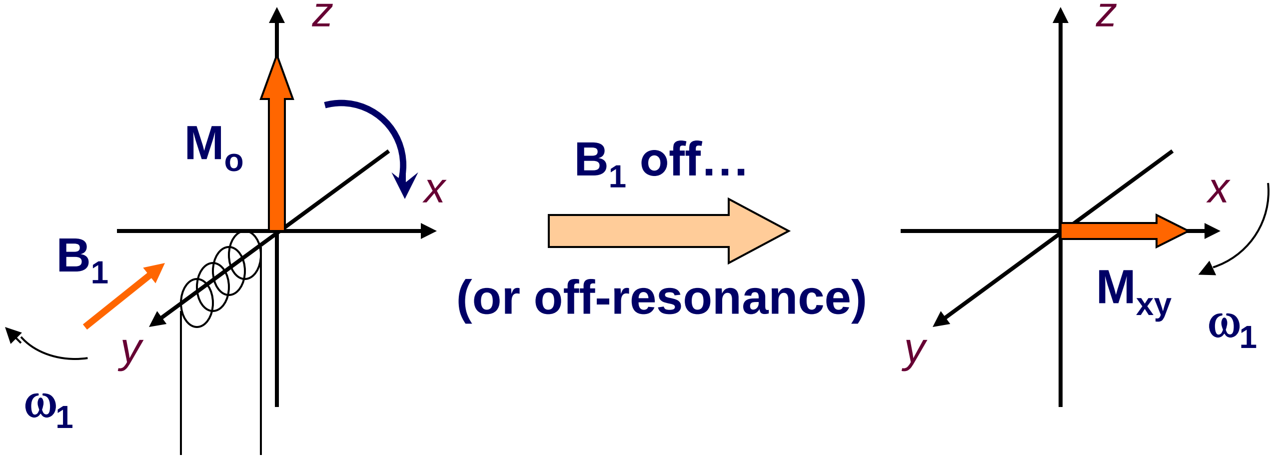
Two ways to look at the situation:

(1) quantum – absorb energy equal to difference in spin states

(2) classical - perturb M_o from an excited field B_1

An NMR Experiment

resonant condition: frequency (ω_1) of B_1 matches *Larmor* frequency (ω_o) energy is absorbed and population of ↓α and β↑ states are perturbed.

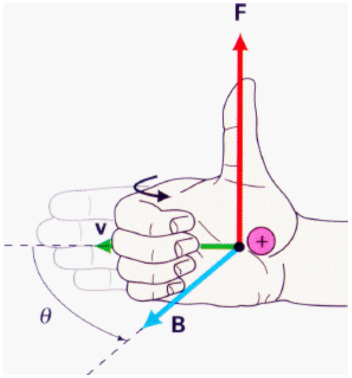


B_1 off…

(or off-resonance)

And/Or: *M_o now precesses about B_1 (similar to B_o) for as long as the B_1 field is applied.*

Again, keep in mind that individual spins flipped up or down (a single ***quanta***), but **M_o** can have a continuous variation.

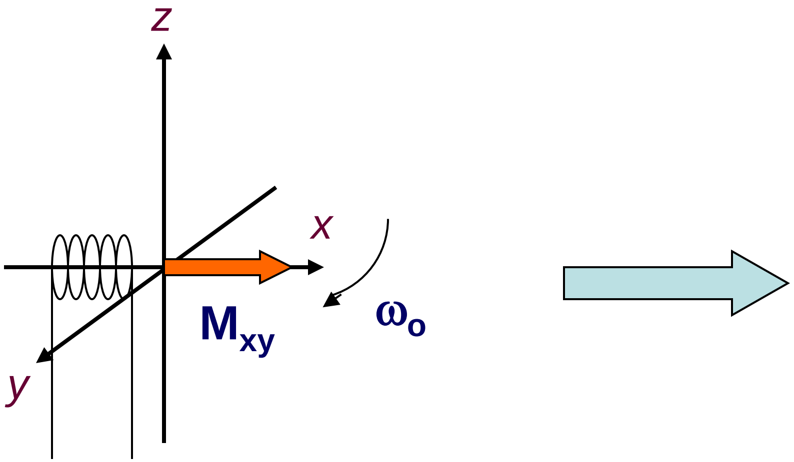


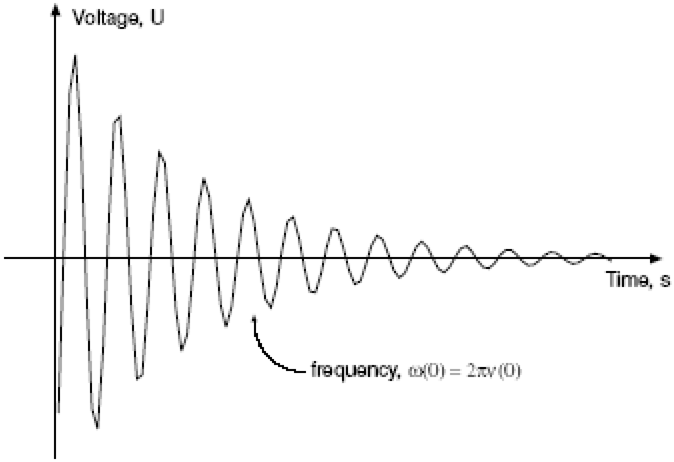
Right-hand rule

An NMR Experiment

What Happens Next?

The B_1 field is turned off and M_{xy} continues to precess about B_o at frequency ω_o.





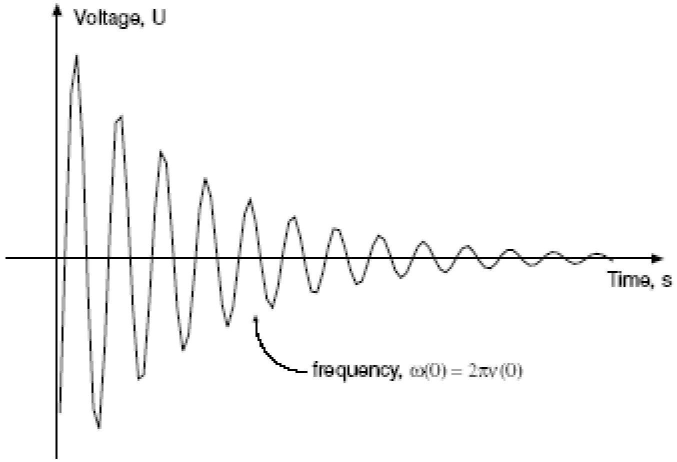
Receiver coil (x) ⇒ **NMR signal**

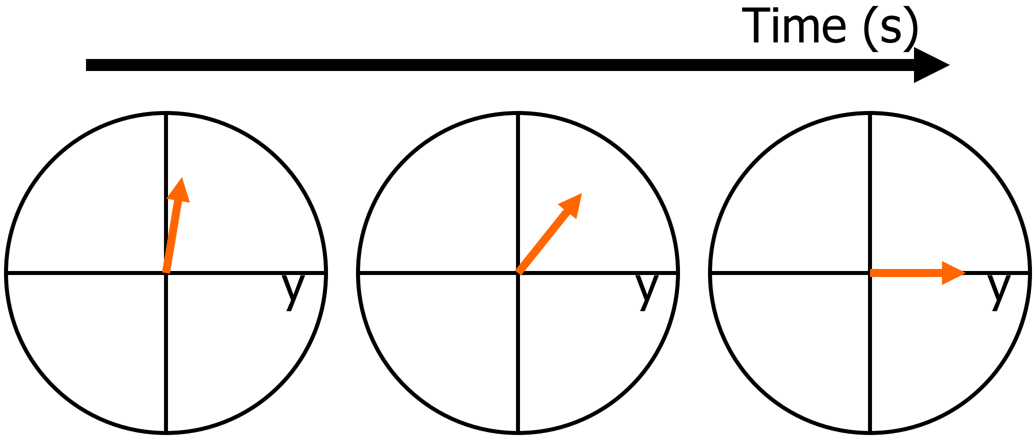
FID – Free Induction Decay

The oscillation of $\mathbf{M_{xy}}$ generates a fluctuating magnetic field which can be used to generate a current in a receiver coil to detect the NMR signal.

NMR Signal Detection - FID

Mxy is precessing about z-axis in the x-y plane

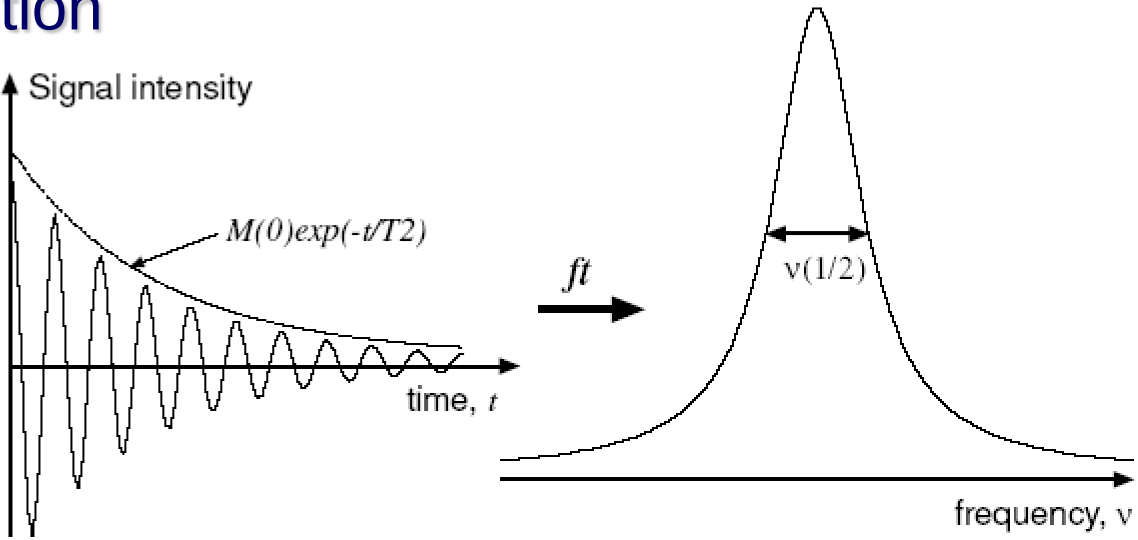




The FID reflects the change in the magnitude of Mxy as
the signal is changing relative to the receiver along the y-axis

Again, it is precessing at its Larmor Frequency (ω_o).

NMR Relaxation



$M_x = M_y = M_0 \exp(-t/T_2)$

Related to line-shape

$$\nu_{1/2} = \frac{1}{\pi T_2}$$

(derived from Hisenberg uncertainty principal)

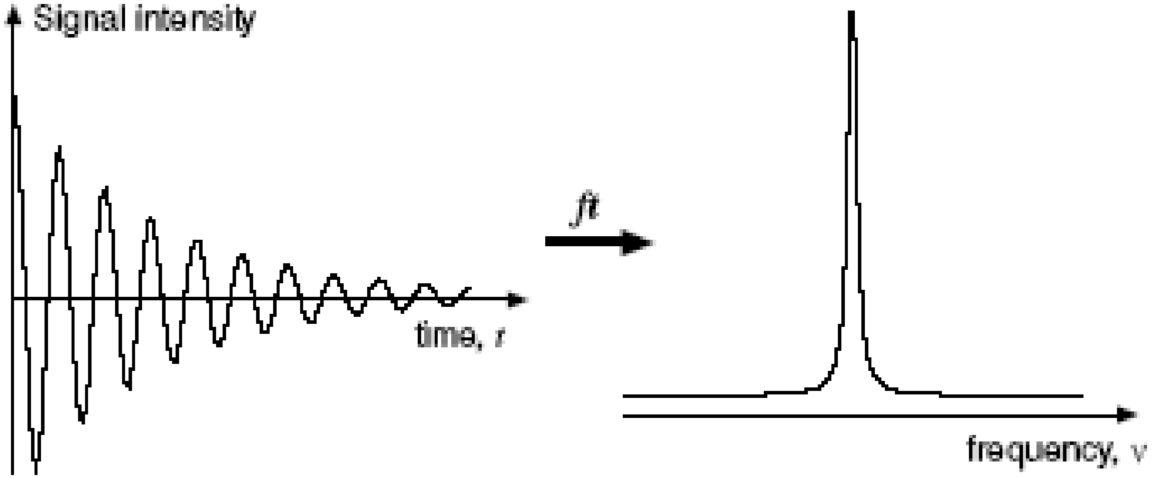
T_2 is the spin-spin (or transverse) relaxation time constant.
In general: T_1 T_2

Think of T_2 as the "randomization" of spins in the x,y-plane

Please Note: Line shape is also affected by the magnetic fields homogeneity

NMR Signal Detection - Fourier Transform

So, the NMR signal is collected in the Time - domain



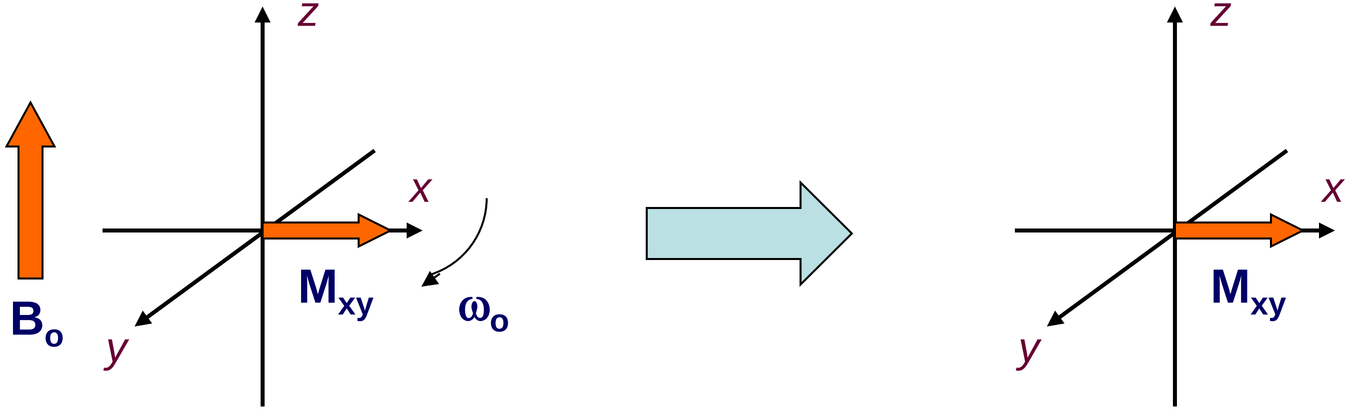
But, we prefer the frequency domain.

$$F(\nu) \propto \int_{-\infty}^{\infty} f(t) e^{-i2\pi\nu t}\, dt$$

Fourier Transform is a mathematical procedure that transforms time domain data into frequency domain

Laboratory Frame vs. Rotating Frame

To simplify analysis we convert to the rotating frame.



Laboratory Frame **Rotating Frame**

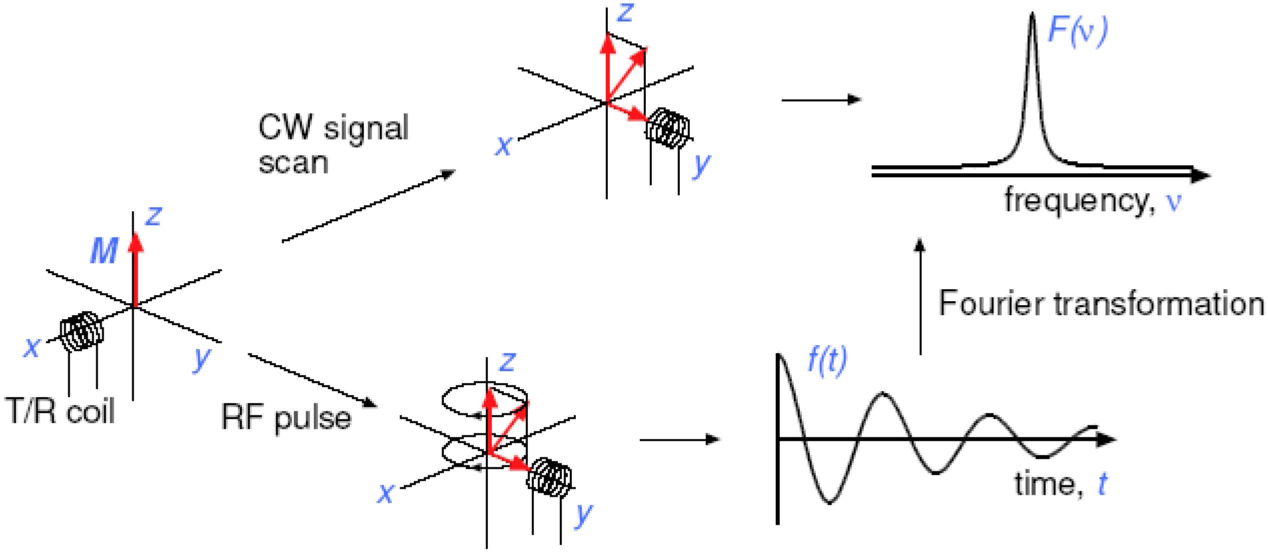
Simply, our axis now rotates at the Larmor Freguency (ω_o).
In the absent of any other factors, M_{xy} will stay on the x-axis

All further analysis will use the rotating frame.

Continuous Wave (CW) vs. Pulse/Fourier Transform

NMR Sensitivity Issue

A frequency sweep (CW) to identify resonance is very slow (1-10 min.)
Step through each individual frequency.



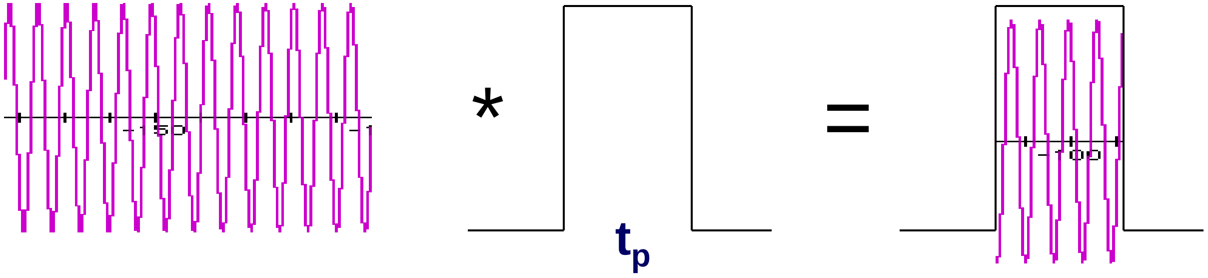
Pulsed/FT collect all frequencies at once in time domain, fast (N x 1-10 sec)

Increase signal-to-noise (S/N) by collecting multiple copies of FID
and averaging signal.

$$S/N \propto \sqrt{\text{number of scans}}$$

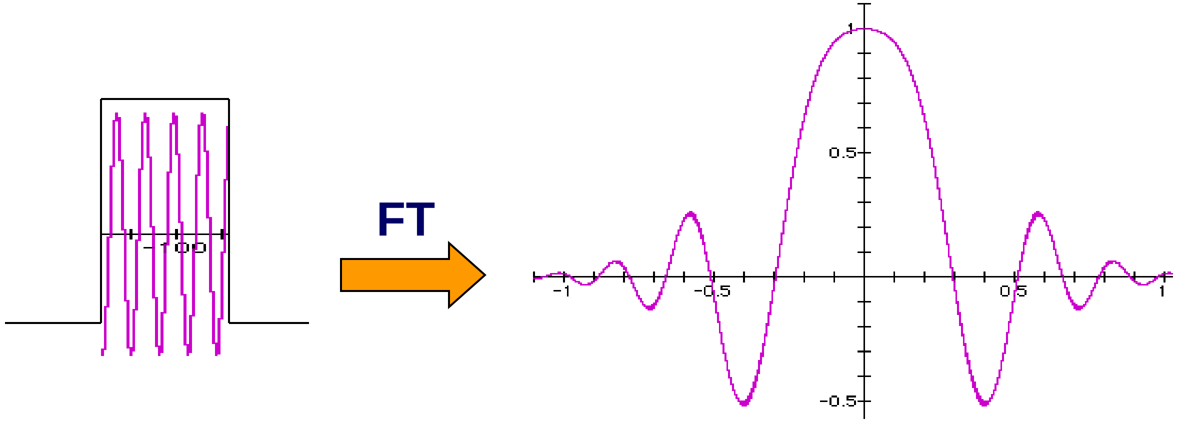
NMR Pulse

A radiofrequency pulse is a combination of a wave (cosine) of frequency ω_o and a step function



Pulse length (time, t_p)

The fourier transform indicates the pulse covers a range of frequencies

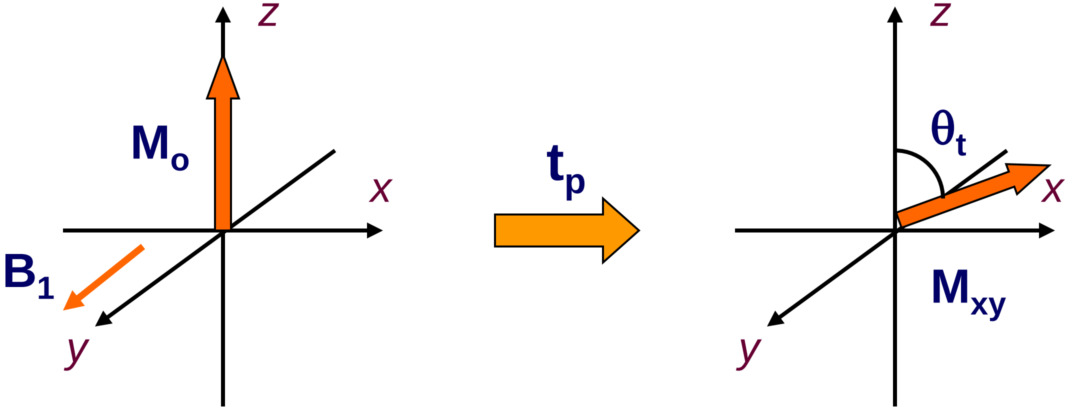


Hisenberg Uncertainty principal again: $\Delta\upsilon \cdot \Delta t \sim 1/2\pi$
Shorter pulse length – larger frequency envelope
Longer pulse length – selective/smaller frequency envelope

Sweep Width
$f \sim 1/t$

NMR Pulse

NMR pulse length or Tip angle (t_p)



$$\theta_t = \gamma * t_p * B_1$$

The length of time the B_1 field is on => torque on bulk magnetization (B_1)

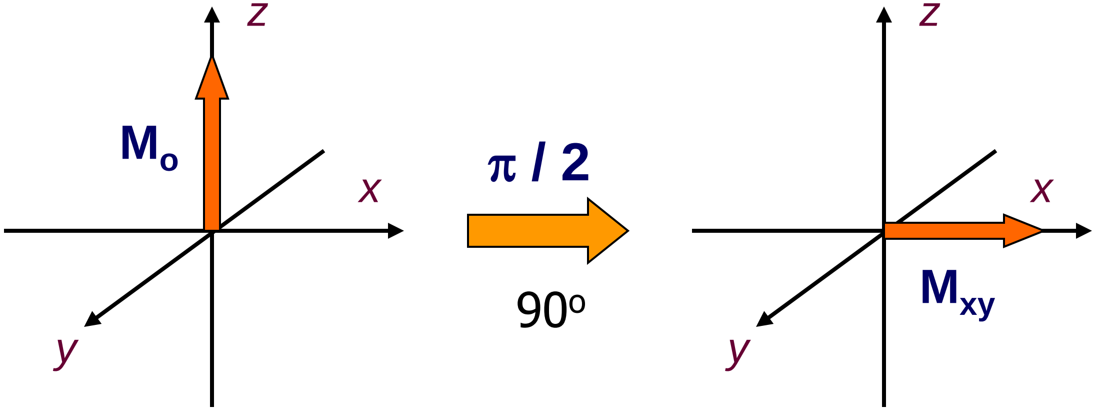
A measured quantity – instrument dependent.

NMR Pulse

Some useful common pulses

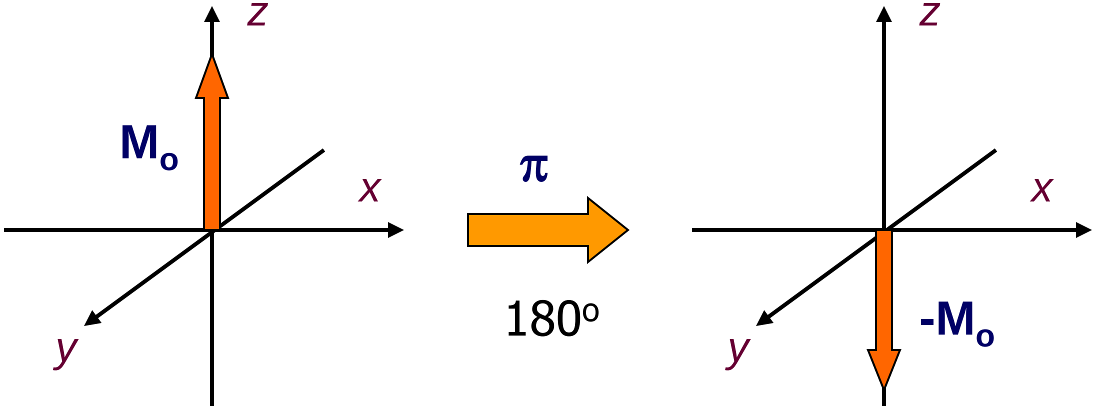
<u>90° pulse</u>

Maximizes signal in x,y-plane where NMR signal detected



<u>180° pulse</u>

Inverts the spin-population.
No NMR signal detected

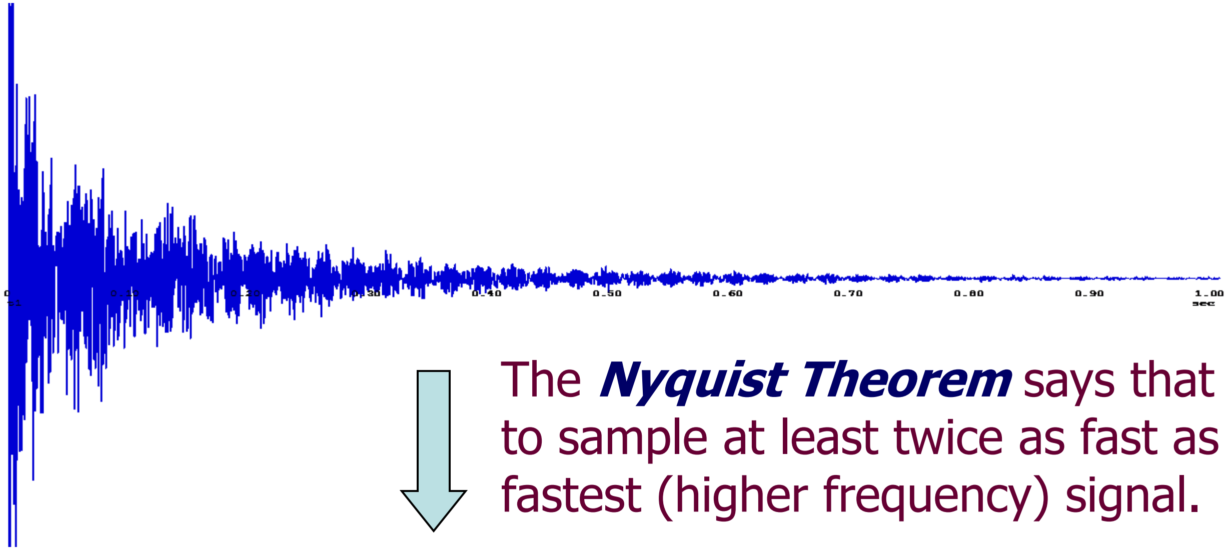


Can generate just about any pulse width desired.

NMR Data Acquisition

Collect *<u>Digital</u>* Data

ADC – analog to digital converter

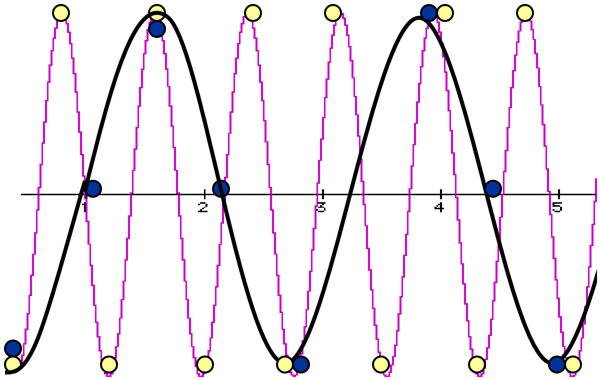


The ***Nyquist Theorem*** says that we have to sample at least twice as fast as the fastest (higher frequency) signal.

Sample Rate

○ - Correct rate, correct frequency

● -½ correct rate, ½ correct frequency
Folded peaks!
Wrong phase!



SR = 1 / (2 * SW)

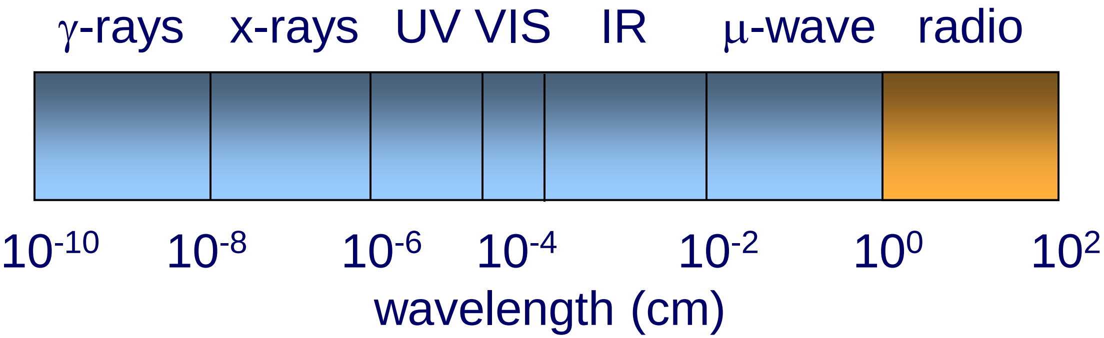
SR – sampling rate

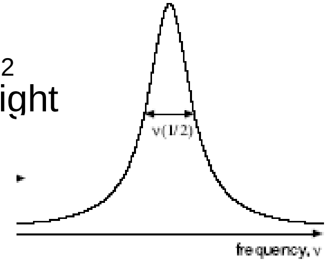
Information in a NMR Spectra

1) Energy $E = h\upsilon$

h is Planck constant

υ is NMR resonance frequency



Observable	Name	Quantitative	Information
Peak position	Chemical shifts (δ)	δ(ppm) = $\upsilon_{obs} - \upsilon_{ref}/\upsilon_{ref}$ (Hz)	chemical (electronic) environment of nucleus
Peak Splitting	Coupling Constant (J) Hz	peak separation (intensity ratios)	neighboring nuclei (torsion angles)
Peak Intensity	Integral	unitless (ratio) relative height of integral curve	nuclear count (ratio) T_1 dependent
Peak Shape	Line width	$\Delta\upsilon = 1/\pi T_2$ peak half-height 	molecular motion chemical exchange uncertainty principal uncertainty in energy

NMR Sensitivity

NMR signal depends on: signal (s) $\propto \gamma^4 B_o^2 N B_1 g(\upsilon)/T$

1) Number of Nuclei (N) (limited to field homogeneity and filling factor)
2) Gyromagnetic ratio (in practice γ^3)
3) Inversely to temperature (T)
4) External magnetic field ($B_o^{2/3}$, in practice, homogeneity)
5) B_1^2 exciting field strength

$$N_\alpha / N_\beta = e^{\Delta E / kT} \qquad \Delta E = \gamma h B_o / 2\pi$$

Increase energy gap -> Increase population difference -> Increase NMR signal

$$\uparrow \Delta E \equiv \uparrow B_o \equiv \uparrow \gamma$$

γ - Intrinsic property of nucleus can not be changed.

$(\gamma_H/\gamma_C)^3$ for ^{13}C is 64x $(\gamma_H/\gamma_N)^3$ for ^{15}N is 1000x

^1H is ~ 64x as sensitive as ^{13}C and 1000x as sensitive as ^{15}N !

Consider that the natural abundance of ^{13}C is 1.1% and ^{15}N is 0.37% relative sensitivity increases to ~6,400x and ~2.7×10^5x !!