



ESPECTROSCOPIA DE RESSONÂNCIA MAGNÉTICA NUCLEAR

Relações entre Comprimento de onda e Espectroscopia

Região Espectral	Energia do fóton	Mudanças na Energia Molecular
UV-Visível	~ 100 kcal/mol	Eletrônica
Infravermelho (IV)	~ 10 kcal/mol	Vibrações das ligações
Rádio	< 0,1 kcal/mol	Estados de spin nuclear em um campo magnético

Histórico da RMN

- 1937 **Rabi** prevê e observa o fenômeno da ressonância magnética nuclear
- 1946 **Bloch, Purcell** primeira ressonância magnética nuclear de uma amostra bruta
- 1953 **Overhauser** NOE (nuclear Overhauser effect)
- 1966 **Ernst, Anderson** RMN com transformada de Fourier
- 1975 **Jeener, Ernst** 2D NMR
- 1985 **Wüthrich** primeira elucidação estrutural de uma proteína pequena (BPTI) a partir das restrições de distâncias obtidas com NOE
- 1987 3D NMR + ^{13}C , ^{15}N marcação isotópica de proteínas recombinantes (resolução)
- 1990 gradientes de campo pulsado (supressão de artefatos)
- 1996/7 novos parâmetros estruturais de longo alcance:
- Residual acoplamentos dipolar de alinhamento parcial em meios líquidos cristalinos
- Projeção restrições ângulo de relaxamento com correlação cruzada TROSY (peso molecular >100 kDa)

Prêmios Nobel

- 1944 *Física* Rabi (Columbia)
- 1952 *Física* Bloch (Stanford), Purcell (Harvard)
- 1991 *Química* Ernst (ETH)
- 2002 *Química* Wüthrich (ETH)
- 2003 *Medicina* Lauterbur (University of Illinois in Urbana),
Mansfield (University of Nottingham)

História da RMN

Primeiro espectro de RMN na água

Espectro de ^1H RMN da Água

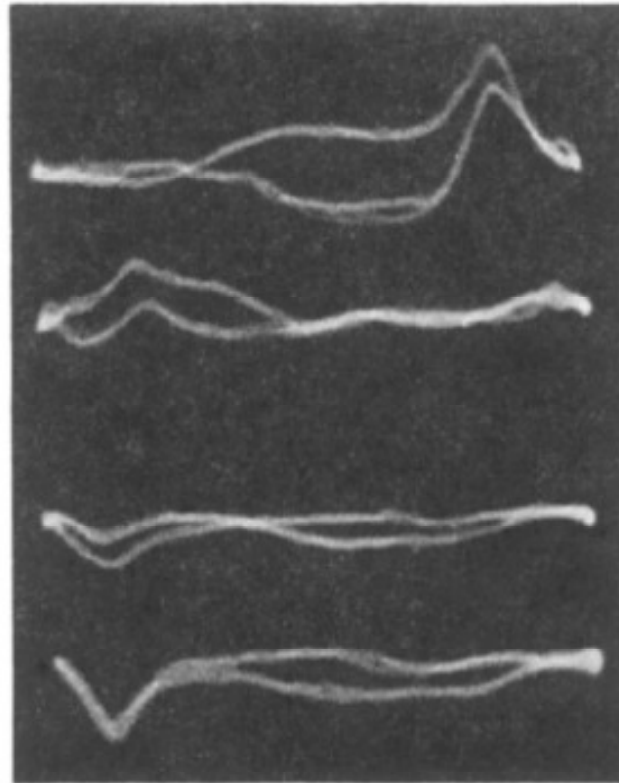
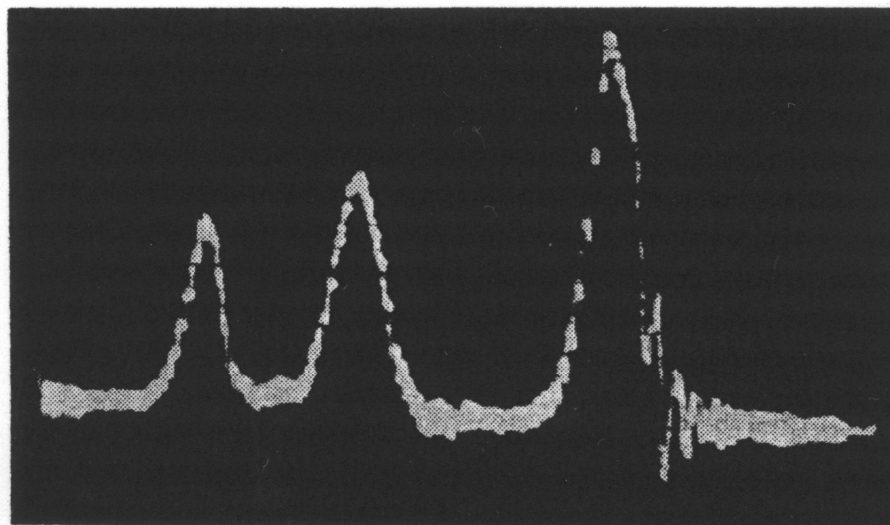


FIG. 10. Photographic record of the proton signal in water. The four traces from top to bottom correspond to the times t_1 , t_2 , t_3 , t_4 of Fig. 9. In the text they are referred to as a , b , c , d , respectively.

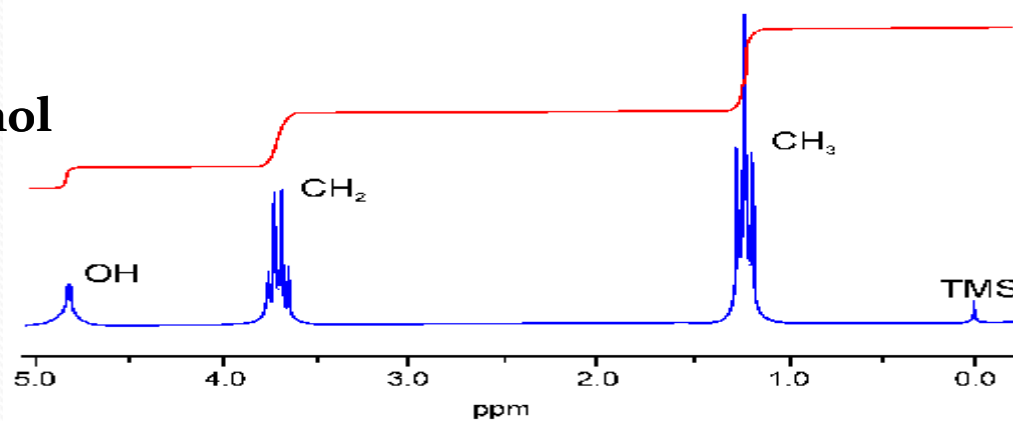
NMR History

Primeira observação do deslocamento químico

^1H NMR do etanol



Espectro Moderno do etanol



Arnold, J.T., S.S. Dharmatti, and M.E. Packard, *J. Chem. Phys.*, 1951. 19: p. 507.

Nuclear Magnetic Resonance

Introduction:

Nuclear Magnetic Resonance (NMR) measures the absorption of electromagnetic radiation in the radio-frequency region (~4-900 MHz)

- nuclei (instead of outer electrons) are involved in absorption process
- sample needs to be placed in magnetic field to cause different energy states

NMR was first experimentally observed by Bloch and Purcell in 1946 (received Nobel Prize in 1952) and quickly became commercially available and widely used.

Probe the Composition, Structure, Dynamics and Function of the Complete Range of Chemical Entities: from small organic molecules to large molecular weight polymers and proteins.

NMR is routinely and widely used as the preferred technique to rapidly elucidate the chemical structure of most organic compounds.

One of the *MOST* Routinely used Analytical Techniques

Typical Applications of NMR:

1.) Structural (chemical) elucidation

- ⇒ Natural product chemistry
- ⇒ Synthetic organic chemistry

- analytical tool of choice of synthetic chemists
- used in conjunction with MS and IR

2.) Study of dynamic processes

- ⇒ reaction kinetics
- ⇒ study of equilibrium (chemical or structural)

3.) Structural (three-dimensional) studies

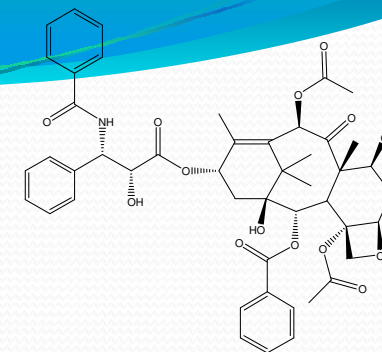
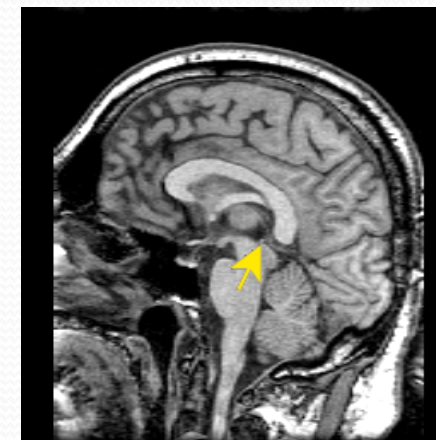
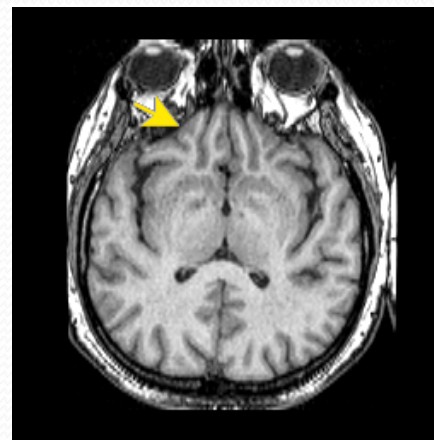
- ⇒ Proteins, Protein-ligand complexes
- ⇒ DNA, RNA, Protein/DNA complexes
- ⇒ Polysaccharides

4.) Drug Design

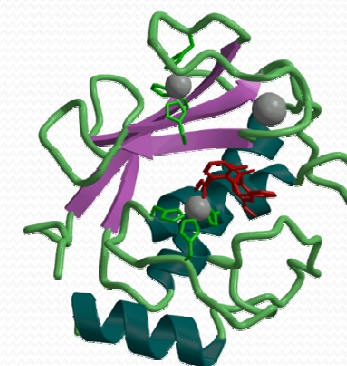
* Structure Activity Relationships by NMR

5.) Medicine -MRI

MRI images of the Human Brain



Taxol (natural product)



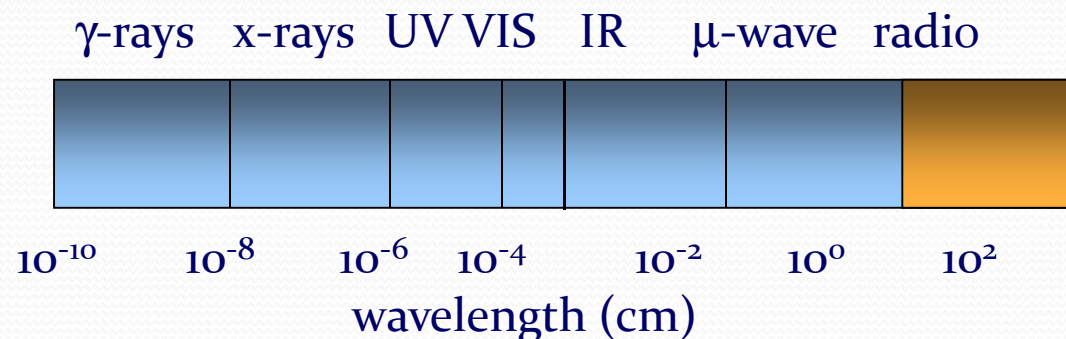
NMR Structure of MMP-13 complexed to a ligand

Informação em um Espectro de RMN

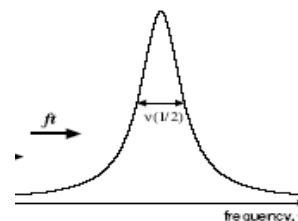
1) Energia $E = h\nu$

h é a constante de Planck

ν Frequência de RMN



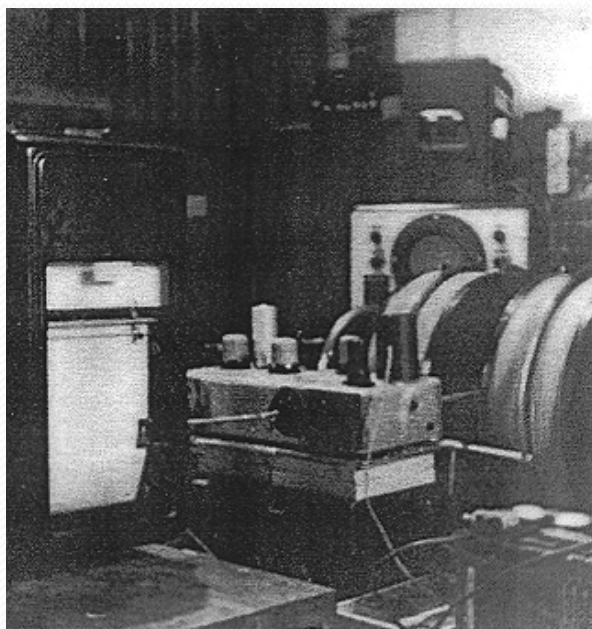
<u>Observações no sinal</u>	<u>Nome</u>	<u>Quantitativo</u>	<u>Informação</u>
Posição	Deslocamento Químico (d)	$d(\text{ppm}) = \frac{u_{\text{obs}} - u_{\text{ref}}}{u_{\text{ref}}}$ (Hz)	ambiente químico (eletrônico) do núcleo
Multiplicidade	Constante de Acoplamento (J) Hz	Separação dos picos (Graus de Intensidade)	Núcleos vizinhos (ângulos de torção)
Intensidade	Integral	sem unidade (razão) altura relativa da curva integral	Contagem nuclear (proporção) Dependente de T_1
Forma	Largura da Linha	$\Delta\nu = 1/\rho T_2$ Largura a $\frac{1}{2}$ altura	Movimento molecular permuta química princípio da incerteza incerteza na energia



NMR spectrometer



Bruker's
home
page

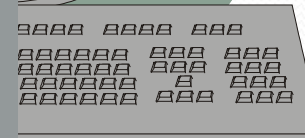
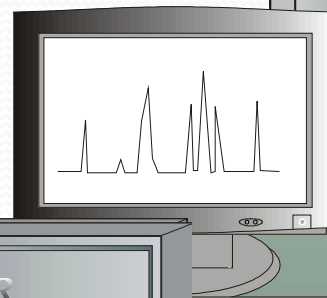


H. Pfeifer:
Pendulum
feedback receiver
Diplomarbeit,
Universität Leipzig,
1952

AVANCE 750
wide-bore in
Leipzig



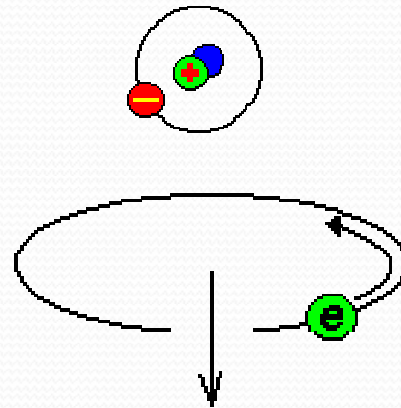
NMR spectrometer for liquids



A Basic Concept in ElectroMagnetic Theory

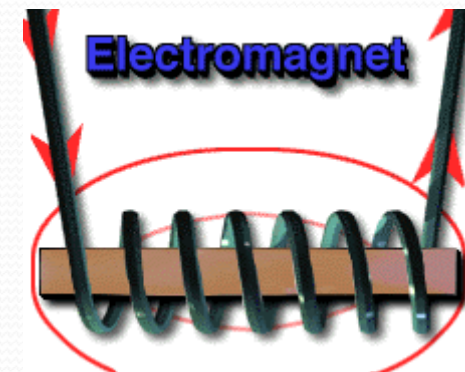
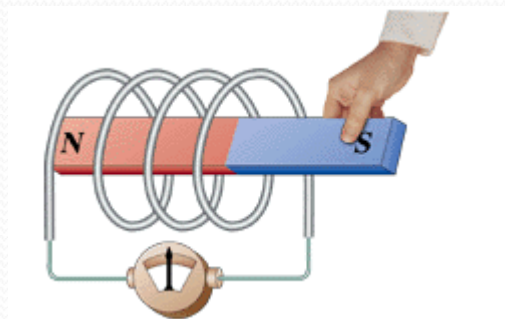
A Direct Application to NMR

A moving perpendicular external magnetic field will induce an electric current in a closed loop

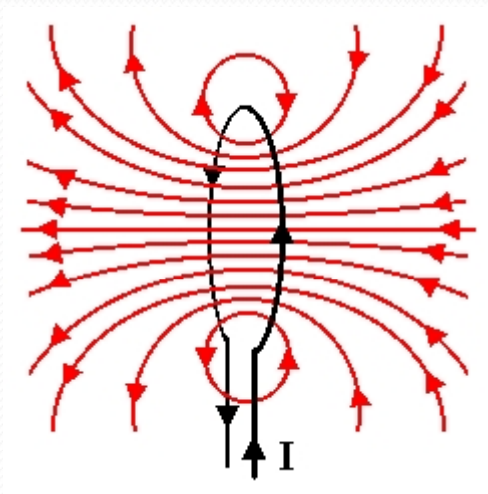


Magnetic field produced by circulating electron

An electric current in a closed loop will create a perpendicular magnetic field



A Basic Concept in ElectroMagnetic Theory



For a single loop of wire, the magnetic field, B through the center of the loop is:

$$B = \frac{\mu_0 I}{2R}$$

μ_0 – permeability of free space ($4\pi \times 10^{-7} \text{ T} \cdot \text{m} / \text{A}$)

R – radius of the wire loop

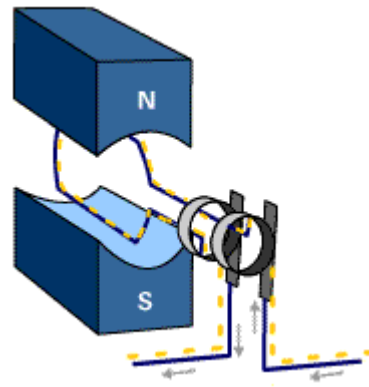
I – current

A Basic Concept in ElectroMagnetic Theory

Faraday's Law of Induction

- If the magnetic flux (Φ_B) through an area bounded by a closed conducting loop changes with time, a current and an emf are produced in the loop. This process is called induction.
- The induced emf is:

$$\xi = -\frac{d\Phi_B}{dt}$$

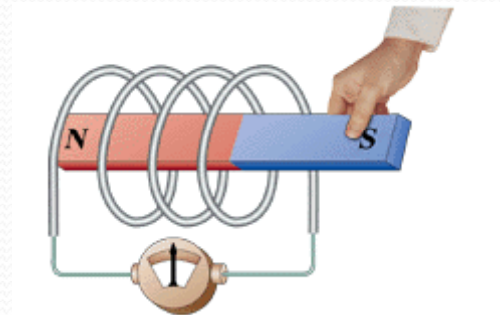


Simple AC generator

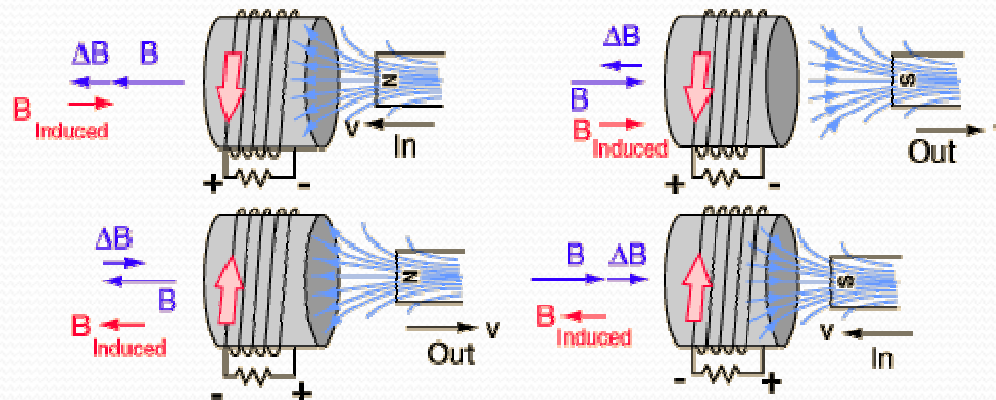
A Basic Concept in ElectroMagnetic Theory

Lenz's Law

- An induced current has a direction such that the magnetic field of the current opposes the change in the magnetic flux that produces the current.
- The induced emf has the same direction as the induced current



Direction of current follows motion of magnet



Theory of NMR

Quantum Description

Nuclear Spin (think electron spin)

- Nucleus rotates about its axis (spin)
- Nuclei with spin have angular momentum (p) or spin

1) total magnitude

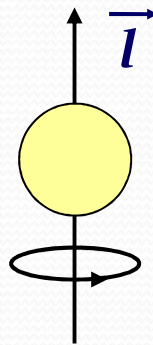
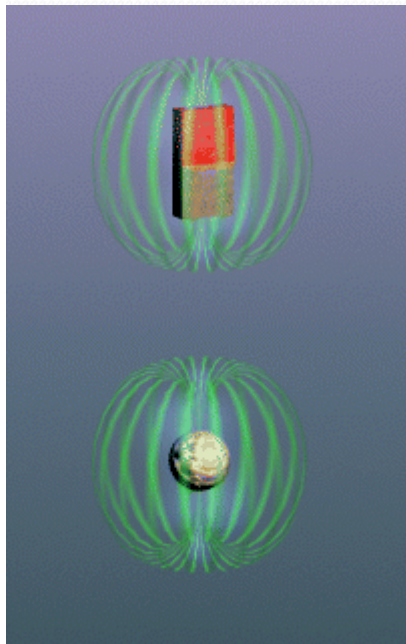
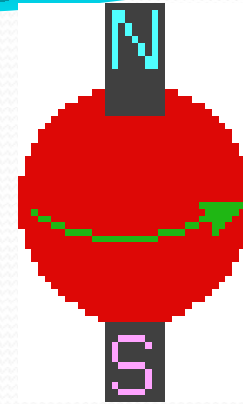
$$\hbar\sqrt{I(I+1)}$$

1) quantized, spin quantum number I

2) $2I + 1$ states: $I, I-1, I-2, \dots, -I$

$I=1/2$: $-1/2, 1/2$

3) identical energies in absence of external magnetic field



NMR Periodic Table

NMR “active” Nuclear Spin ($I = \frac{1}{2}$):

^1H , ^{13}C , ^{15}N , ^{19}F , ^{31}P

biological and chemical relevance

Odd atomic mass

$I = +\frac{1}{2}$ & $-\frac{1}{2}$

NMR “inactive” Nuclear Spin ($I = 0$):

^{12}C , ^{16}O

Even atomic mass & number

Quadrupole Nuclei Nuclear Spin ($I > \frac{1}{2}$):

^{14}N , ^2H , ^{10}B

Even atomic mass & odd number

$I = +1, 0$ & -1

Table 1.1 Nuclear properties of some of the elements

Element	Atomic mass	Spin I	Natural abundance (%)	Receptivity ($^{13}\text{C} = 1.00$)	Quadrupole moment (10^{30} m^2)	Resonant frequency (MHz) at 2.348 T
Hydrogen	1	1/2	99.985	5670	None	100.00
Deuterium	2	1	0.015	0.0082	0.287	15.35
Tritium	3	1/2	Radioactive	–	None	106.66
Helium	3	1/2	0.00014	0.0035	None	76.18
Lithium	6	1	7.42	3.58	-0.064	14.72
Lithium	7	3/2	92.58	1540	-3.7	38.87
Beryllium	9	3/2	100	78.8	5.3	15.06
Boron	10	3	19.58	22.1	7.4	10.75
Boron	11	3/2	80.42	754	4.1	32.08
Carbon	13	1/2	1.108	1.00	None	25.15
Nitrogen	14	1	99.63	3.70	1.67	7.23
Nitrogen	15	1/2	0.37	0.022	None	18.14
Oxygen	17	5/2	0.037	0.061	-2.6	13.56
Fluorine	19	1/2	100	4730	None	94.09
Neon	21	3/2	0.257	0.0036	9	7.90
Sodium	23	3/2	100	524	10	26.43
Magnesium	25	5/2	10.13	1.54	22	6.13
Aluminium	27	5/2	100	1170	14	26.08
Silicon	29	1/2	4.7	2.1	None	19.87
Phosphorus	31	1/2	100	377	None	40.48
Sulfur	33	3/2	0.76	0.098	-6.4	7.67
Chlorine	35(37)	3/2	75.53	20.2	-8.2	9.81
Potassium	39	3/2	93.1	2.69	5.5	4.67
Calcium	43	7/2	0.145	0.053	-5	6.74
Scandium	45	7/2	100	1720	-22	24.33
Titanium	49(47)	7/2	5.51	1.18	24	5.64
Vanadium	51(50)	7/2	99.76	2170	-5.2	26.35
Chromium	53	3/2	9.55	0.49	-15	5.64
Manganese	55	5/2	100	1014	40	24.84
Iron	57	1/2	2.19	0.00425	None	3.24
Cobalt	59	7/2	100	1560	42	23.73
Nickel	61	3/2	1.19	0.24	16	8.93
Copper	63(65)	3/2	69.09	368	-22	26.51
Zinc	67	5/2	4.11	0.67	15	6.25
Gallium	71(69)	3/2	39.6	322	11	30.58
Germanium	73	9/2	7.76	0.62	-17	3.48
Arsenic	75	3/2	100	144	29	17.18
Selenium	77	1/2	7.58	3.02	None	19.07
Bromine	81(79)	3/2	49.46	279	27	27.10
Krypton	83	9/2	11.55	1.24	27	3.86
Rubidium	87(85)	3/2	27.85	280	13	32.84
Strontium	87	9/2	7.02	1.08	16	4.35
Yttrium	89	1/2	100	0.676	None	4.92
Zirconium	91	5/2	11.23	6.05	-21	9.34
Niobium	93	9/2	100	2770	-32	24.55
Molybdenum	95(97)	5/2	15.72	2.92	-1.5	6.55
Technetium	99	9/2	Radioactive	–	-0.13	22.51
Ruthenium	99(101)	5/2	12.72	0.815	7.6	4.61
Rhodium	103	1/2	100	0.18	None	3.16
Palladium	105	5/2	22.23	1.43	65	4.58
Silver	109(107)	1/2	48.18	0.28	None	4.65
Cadmium	113(111)	1/2	12.26	7.69	None	22.18

Spin Quantum Numbers of Some Nuclei

The most abundant isotopes of C and O do not have spin.

Element ^1H	^2H	^{12}C	^{13}C	^{14}N	^{16}O	^{17}O	^{19}F	
Nuclear Spin Quantum No (I)	1/2	1	0	1/2	1	0	5/2	1/2
No. of Spin States	2	3	0	2	3	0	6	2

Elements with either odd mass or odd atomic number have the property of nuclear “spin”.

The number of spin states is $2\mathbf{I} + 1$,
where \mathbf{I} is the spin quantum number.

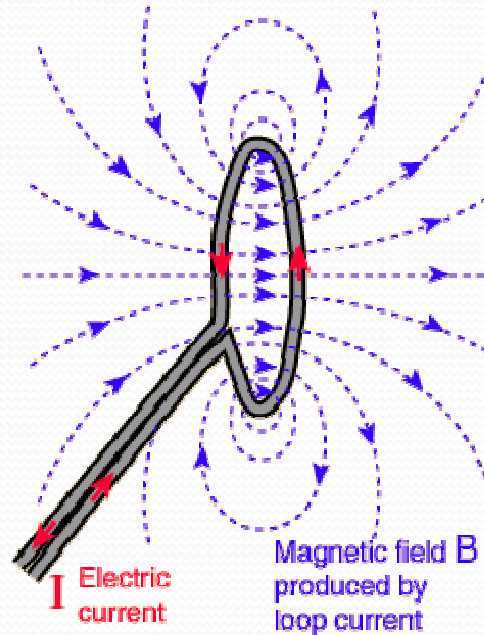
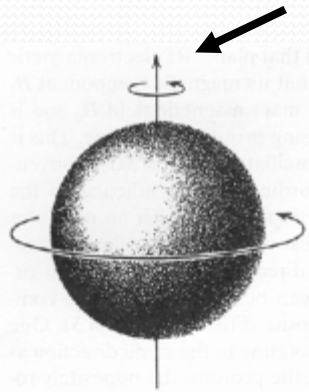
Spin dos Núcleos Atômicos

- Átomos de Spin $1/2$: número de massa é ímpar.
exemplos: ^1H e ^{13}C .
- Átomos de Spin 1 : número de massa é par.
exemplos: ^2H e ^{14}N .
- Átomos de Spin 0 : número de massa é par.
exemplos: ^{12}C e ^{16}O .

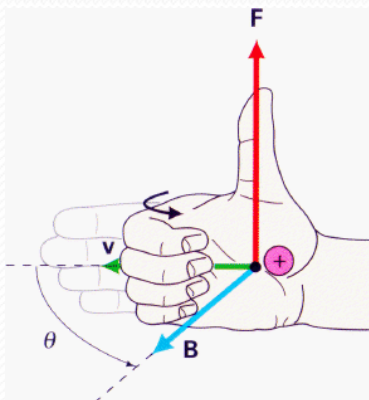
Magnetic Moment (μ)

- spinning charged nucleus creates a magnetic field

Magnetic moment



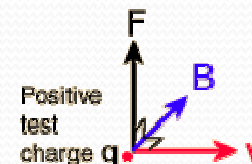
Similar to magnetic field created by electric current flowing in a coil



“Right Hand Rule”

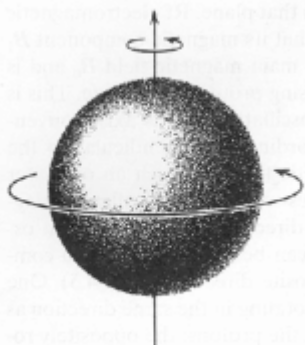
- determines the direction of the magnetic field around a current-carrying wire and vice-versa

$$\vec{F} = q\vec{v} \times \vec{B}$$



Gyromagnetic ratio (γ)

- related to the relative sensitivity of the NMR signal
- magnetic moment (μ) is created along axis of the nuclear spin



where:

$$\mu = \gamma p$$

$$\gamma = \frac{2\pi \mu}{h I} = \frac{\mu}{\hbar I}$$

p – angular momentum

γ – gyromagnetic ratio (different value for each type of nucleus)

- magnetic moment is quantized (m)
 $m = I, I-1, I-2, \dots, -I$

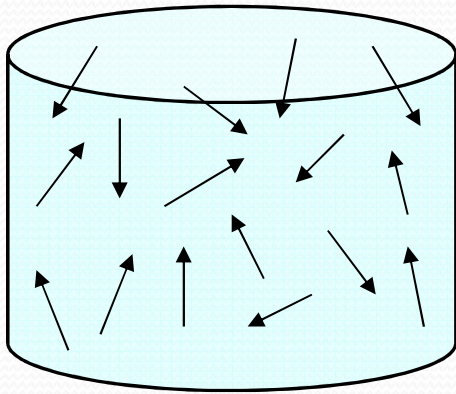
for common nuclei of interest:

$$m = +\frac{1}{2} \text{ \& \ } -\frac{1}{2}$$

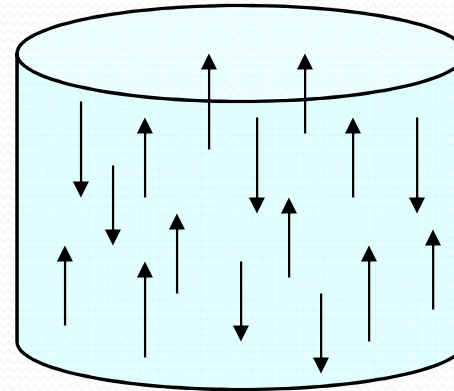
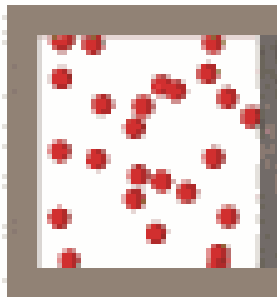
Isotope	Net Spin	γ / MHz T ⁻¹	Abundance / %
¹ H	1/2	42.58	99.98
² H	1	6.54	0.015
³ H	1/2	45.41	0.0
³¹ P	1/2	17.25	100.0
²³ Na	3/2	11.27	100.0
¹⁴ N	1	3.08	99.63
¹⁵ N	1/2	4.31	0.37
¹³ C	1/2	10.71	1.108
¹⁹ F	1/2	40.08	100.0

Magnetic alignment

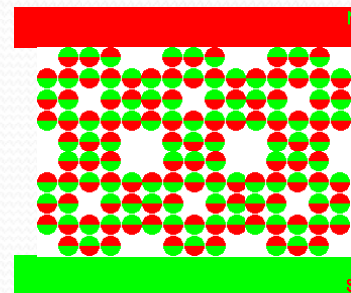
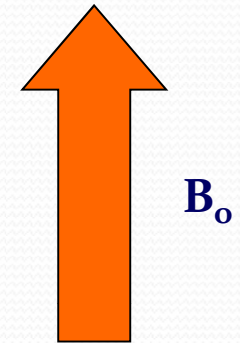
$$\uparrow = \gamma \mathbf{h} / 4\pi$$



In the absence of external field, each nuclei is energetically degenerate



Add a strong external field (B_0) and the nuclear magnetic moment: aligns with (low energy) against (high-energy)



Spins Orientation in a Magnetic Field (Energy Levels)

- Magnetic moment are no longer equivalent
- Magnetic moments are oriented in $2I + 1$ directions in magnetic field

♦ Vector length is:

$$\hbar\sqrt{I(I+1)}$$

♦ Angle (φ) given by:

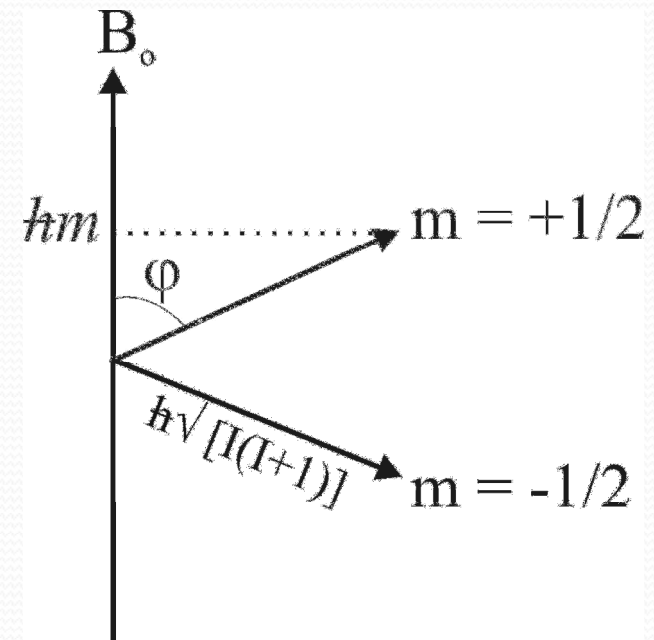
$$\cos \varphi = \sqrt{\frac{m}{I(I+1)}}$$

♦ Energy given by:

$$E = -\frac{m\mu}{I} B_0$$

where,

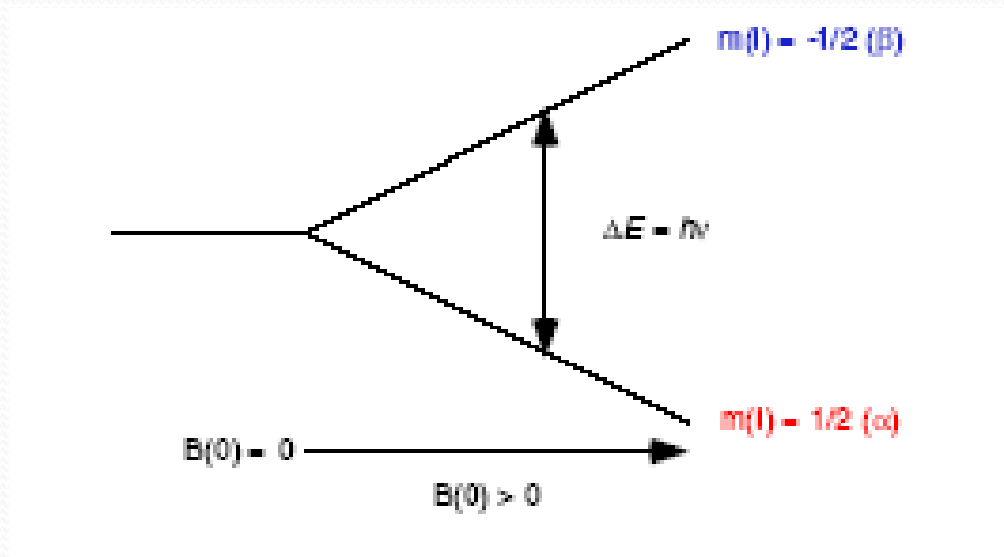
B_0 - magnetic field
 μ - magnetic moment
 h - Planck's constant



For $I = 1/2$

Spins Orientation in a Magnetic Field (Energy Levels)

- Magnetic moments are oriented in one of two directions in magnetic field (for $I = 1/2$)



- Difference in energy between the two states is given by:

$$\Delta E = \gamma h B_o / 2\pi$$

where:

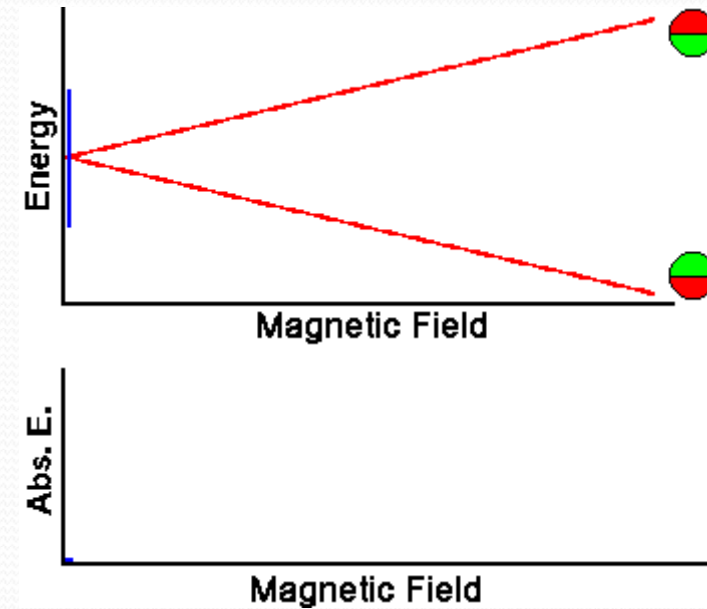
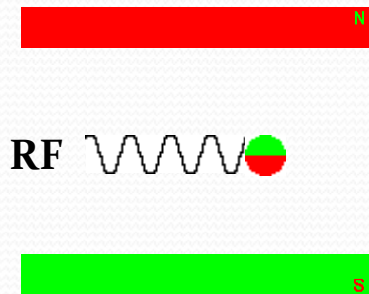
B_o – external magnetic field

h – Planck's constant

γ – gyromagnetic ratio

Spins Orientation in a Magnetic Field (Energy Levels)

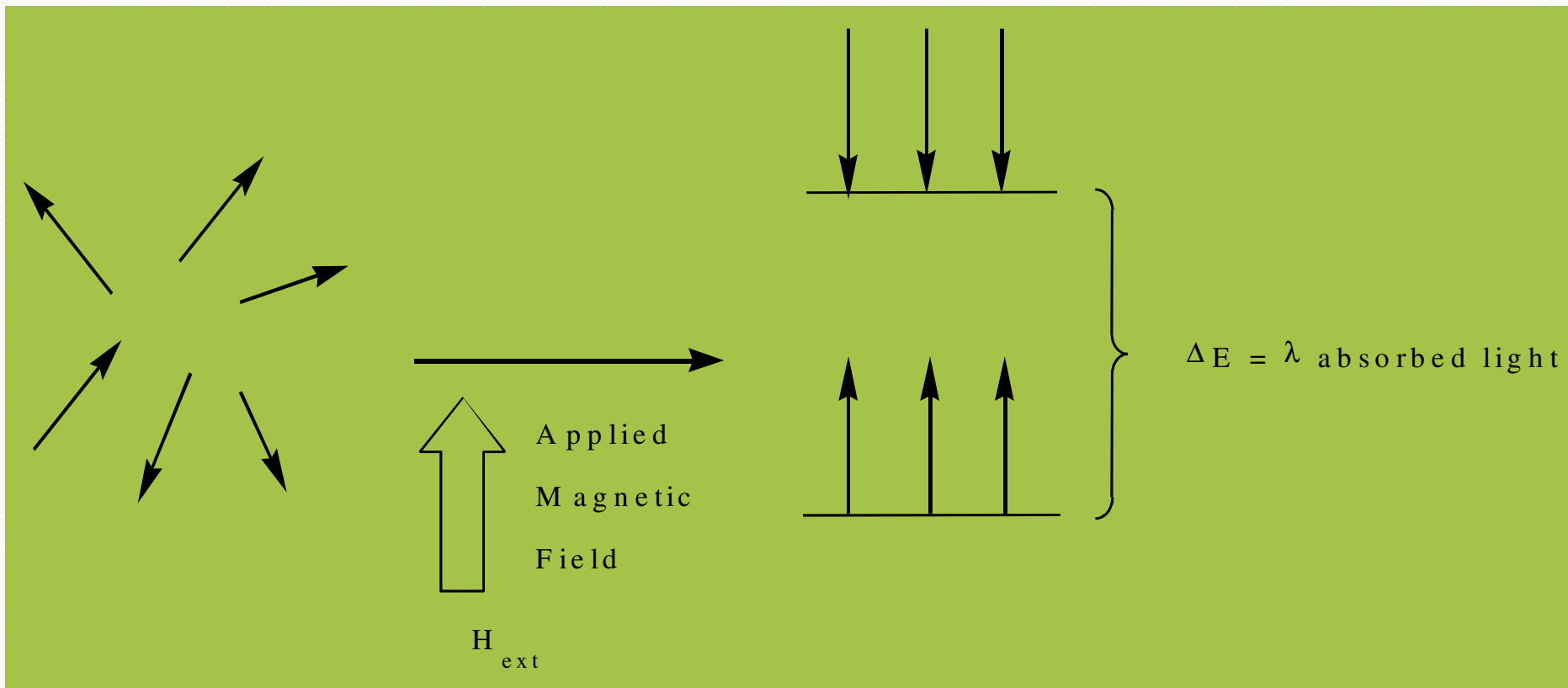
- Transition from the low energy to high energy spin state occurs through an absorption of a photon of radio-frequency (RF) energy



Frequency of absorption:

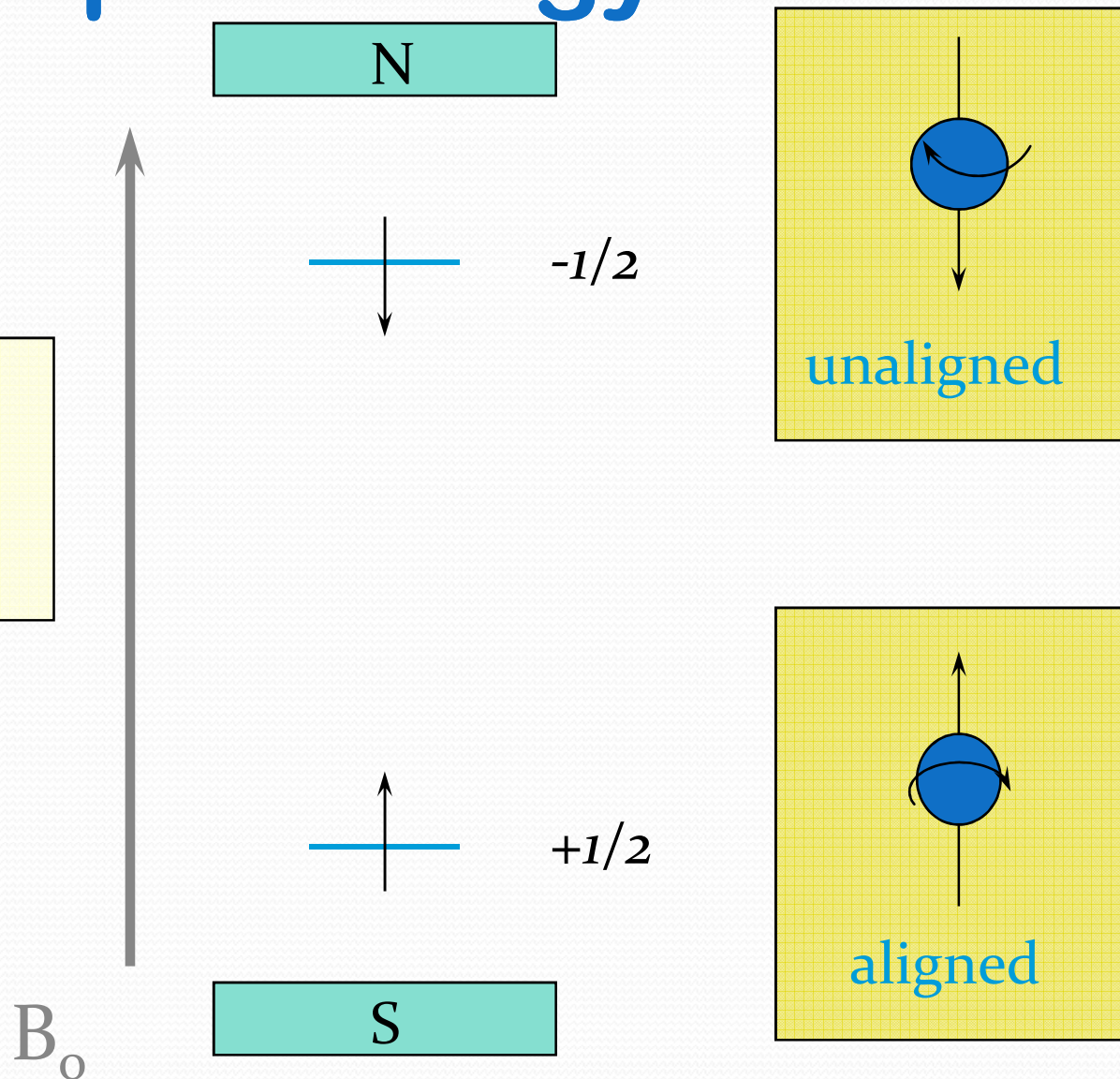
$$\nu = \gamma B_o / 2\pi$$

Estados de Energia de Prótons Submetidos a um Campo Magnético Externo



Nuclear Spin Energy Levels

In a strong magnetic field (B_0) the two spin states differ in energy.



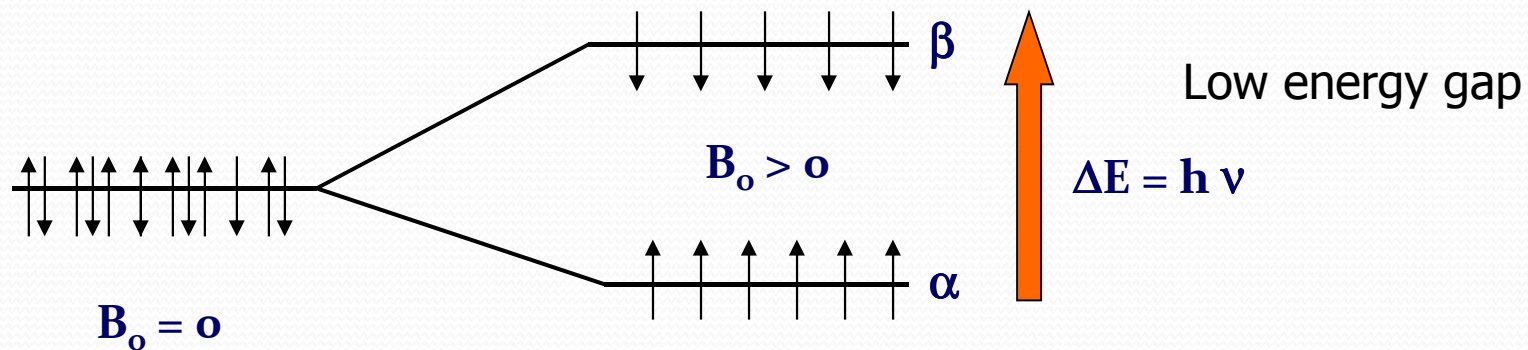
Ressonância Magnética Nuclear (RMN)

Nuclear Magnetic Resonance (NMR)

- **Ressonância** – fótons de ondas de rádio podem apresentar a diferença de energia exata entre os estados de spin $+ \frac{1}{2}$ and $- \frac{1}{2}$ resultando na absorção de fótons enquanto os prótons têm seus estados de spin alterados.
- **Magnética** – um campo magnético forte causa uma pequena diferença de energia entre os estados de spin $+ \frac{1}{2}$ e $- \frac{1}{2}$.
- **Nuclear** – núclídeos de spin $\frac{1}{2}$ (e.g. prótons) comportam-se como pequenas barras magnéticas.

NMR Signal (*sensitivity*)

- The applied magnetic field causes an energy difference between the aligned (α) and unaligned (β) nuclei
- NMR signal results from the transition of spins from the α to β state
- Strength of the signal depends on the population difference between the α and β spin states



- The population (N) difference can be determined from the Boltzmann distribution and the energy separation between the α and β spin states:

$$N_{\alpha} / N_{\beta} = e^{\Delta E / kT}$$

Resonance Frequencies of Selected Nuclei

Isotope Abundance B_0 (Tesla) Frequency(MHz) γ (radians/Tesla)

^1H	99.98%	1.00	42.6	267.53
		1.41	60.0	
		2.35	100.0	
		7.05	300.0	

^{13}C	1.108%	1.00	10.7	67.28
		2.35	25.0	
		7.05	75.0	

NMR Signal (sensitivity)

Since:

$$\Delta E = h\nu$$

and

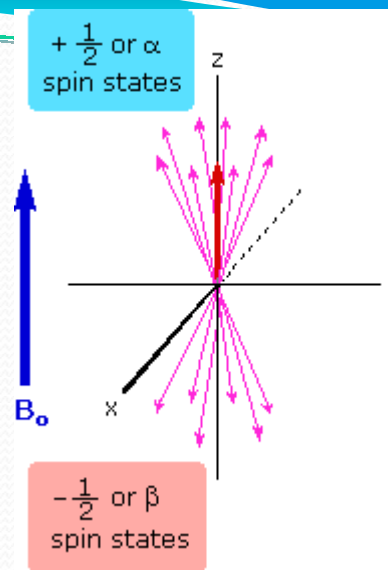
$$\nu = \gamma B_o / 2\pi$$

then:

$$N_\alpha / N_\beta = e^{\Delta E / kT}$$



$$N_\alpha / N_\beta = e^{(\gamma h B_o / 2\pi kT)}$$



The ΔE for ^1H at 400 MHz ($B_o = 9.39$ T) is 6×10^{-5} Kcal / mol

$$N_\alpha / N_\beta = 1.000060$$

Very Small !
~60 excess spins per million in lower state

NMR Sensitivity

NMR signal (**s**) depends on:

$$s \propto \gamma^4 B_0^2 N B_1 g(\nu) / T$$

- 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 ($B_0^{2/3}$, in practice, homogeneity)
- 5) B_1^2 exciting field strength (RF pulse)

$$N_\alpha / N_\beta = e^{\Delta E / kT}$$

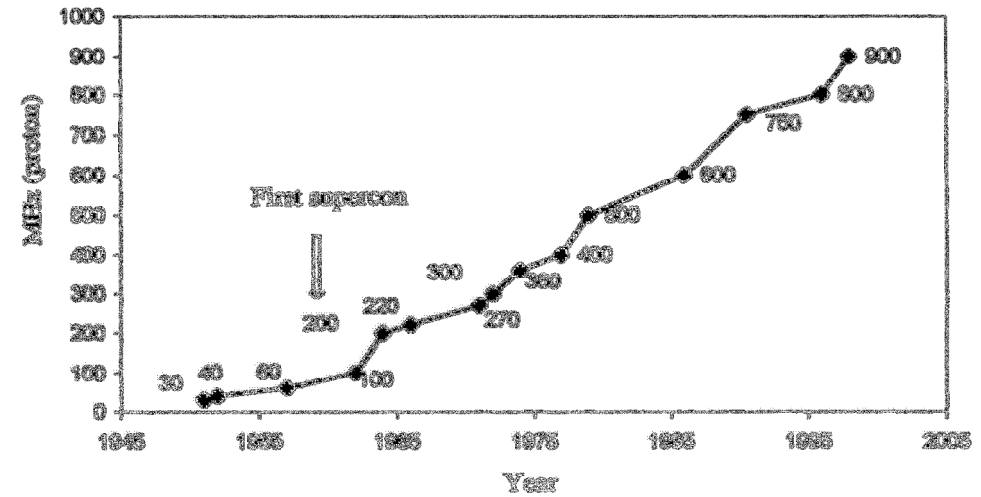
$$\Delta E = \gamma h B_0 / 2\pi$$

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

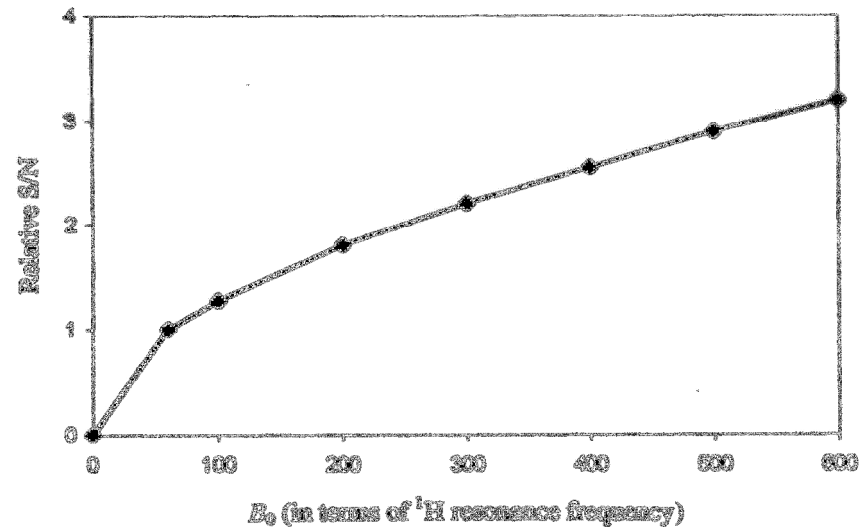
$$\uparrow \Delta E \equiv \uparrow B_0 \equiv \uparrow \gamma$$

NMR Sensitivity

Increase in Magnet Strength is a Major Means to Increase Sensitivity



Signal to noise improvement with magnetic field (sensitivity)



NMR Sensitivity

But at a significant cost!



~\$500,000



~\$1,500,000



~\$4,000,000

NMR Sensitivity

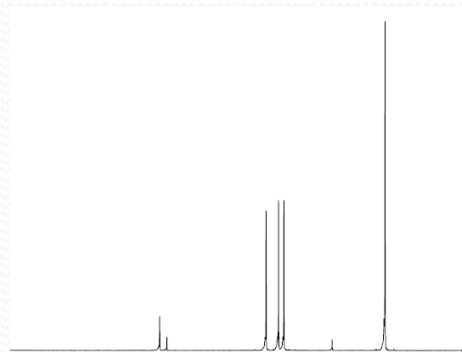
- Relative sensitivity of ^1H , ^{13}C , ^{15}N and other nuclei NMR spectra depend on
 - Gyromagnetic ratio (γ)
 - Natural abundance of the isotope

γ - Intrinsic property of nucleus can not be changed.

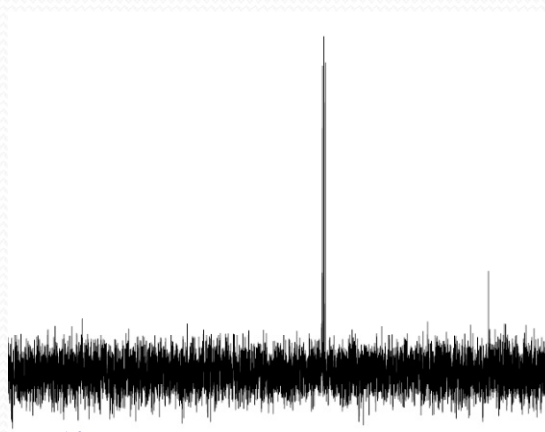
$$(\gamma_{\text{H}}/\gamma_{\text{C}})^3 \text{ for } ^{13}\text{C} \text{ is } 64\text{x} \quad (\gamma_{\text{H}}/\gamma_{\text{N}})^3 \text{ for } ^{15}\text{N} \text{ is } 1000\text{x}$$

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

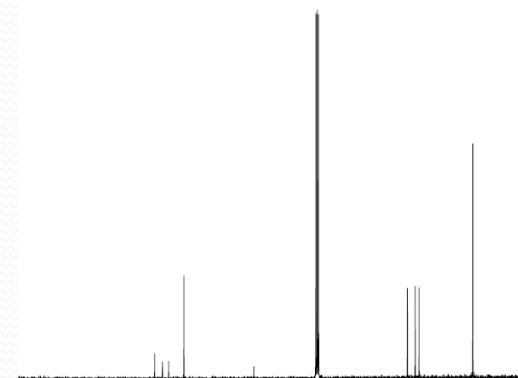
Consider that the natural abundance of ^{13}C is **1.1%** and ^{15}N is **0.37%**
relative sensitivity increases to ~**6,400x** and ~ **$2.7 \times 10^5\text{x}$** !!



^1H NMR spectra of caffeine
8 scans ~12 secs



^{13}C NMR spectra of caffeine
8 scans ~12 secs



^{13}C NMR spectra of caffeine
10,000 scans ~4.2 hours

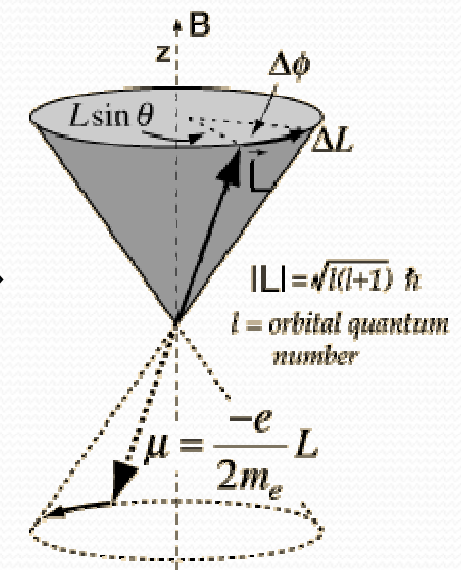
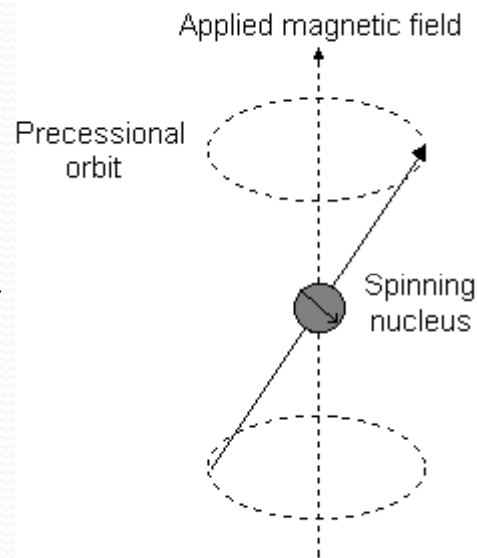
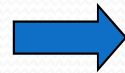
Theory of NMR

Classical Description

- *Spinning particle precesses around an applied magnetic field*



A Spinning Gyroscope
in a Gravity Field



$$\cos \varphi = \sqrt{\frac{m}{I(I+1)}}$$

Classical Description

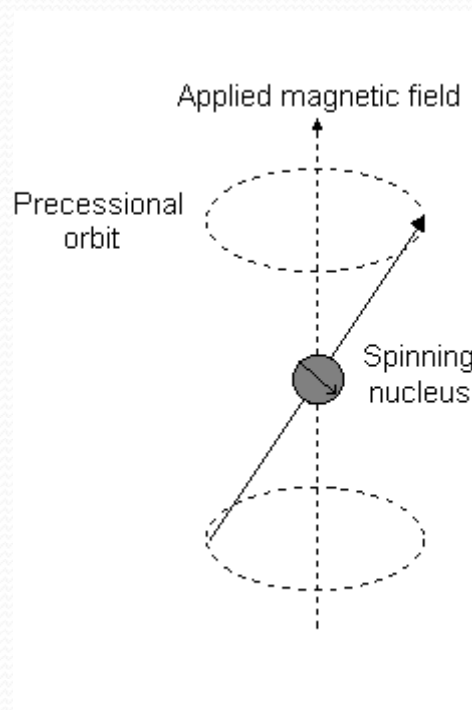
- Angular velocity of this motion is given by:

$$\omega_o = \gamma B_o$$

where the frequency of precession or **Larmor** frequency is:

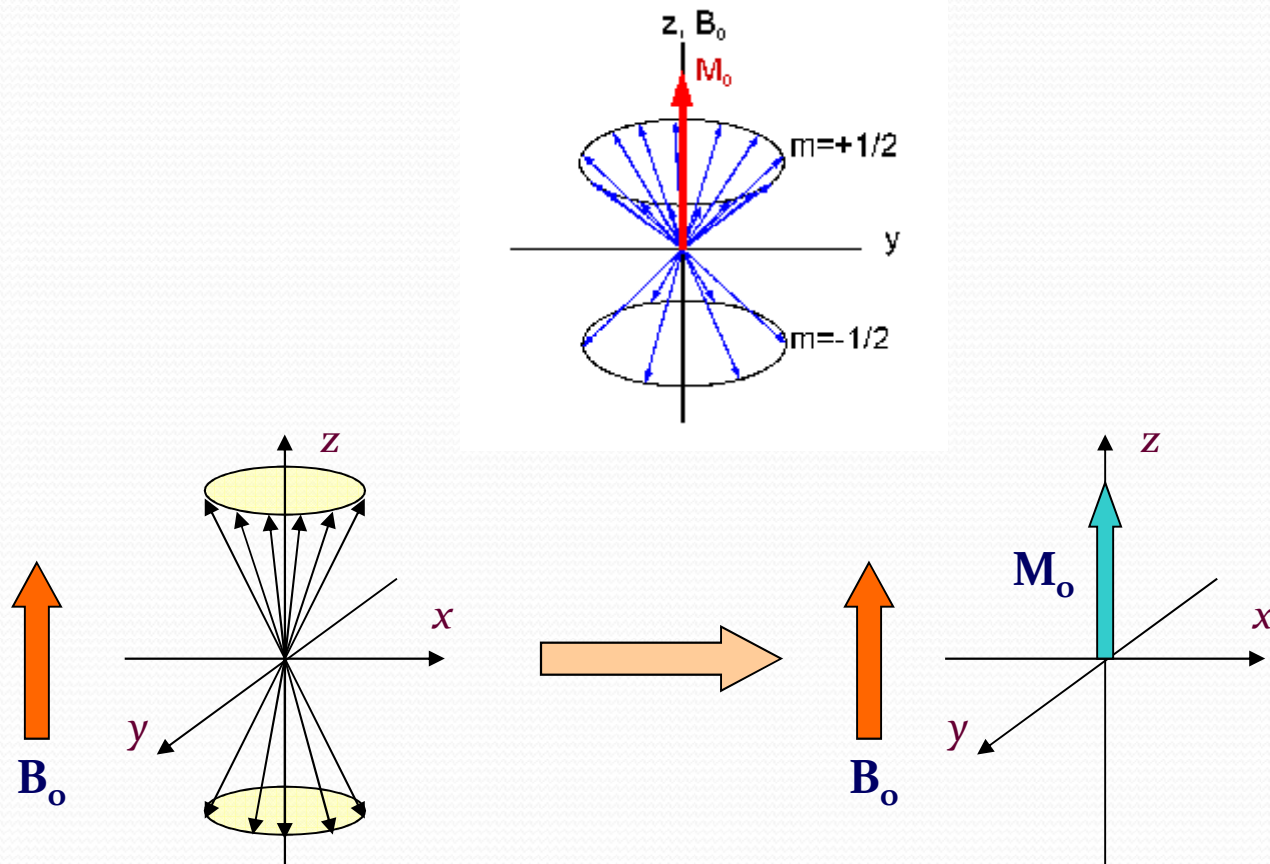
$$\nu = \gamma B_o / 2\pi$$

Same as quantum mechanical description



Classical Description


- **Net Magnetization**
 - Nuclei either align with or against external magnetic field along the z-axis.
 - Since more nuclei align with field, net magnetization (M_x, M_y) exists parallel to external magnetic field.
 - Net Magnetization along +Z, since higher population aligned with B_0 .
 - Magnetization in X,Y plane (M_x, M_y) averages to zero.



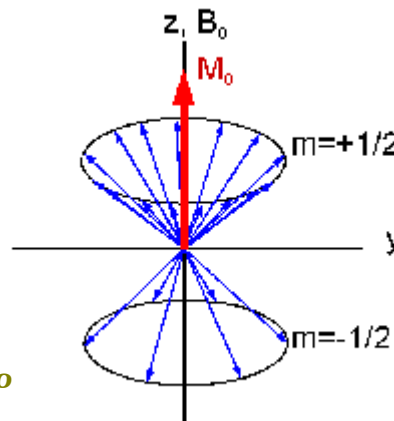
Classical Description

- *Observe NMR Signal*
 - Need to perturb system from equilibrium.
 - B_1 field (radio frequency pulse) with $\gamma B_0/2\pi$ frequency
 - Net magnetization (M_0) now precesses about B_0 and B_1
 - M_x and M_y are non-zero
 - M_x and M_y rotate at Larmor frequency
 - System absorbs energy with transitions between aligned and unaligned states
 - Precession about B_1 stops when B_1 is turned off

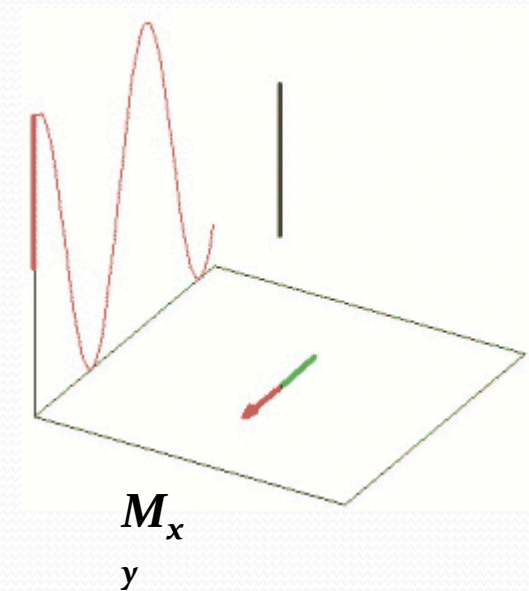
RF pulse



B_1 field perpendicular to B_0

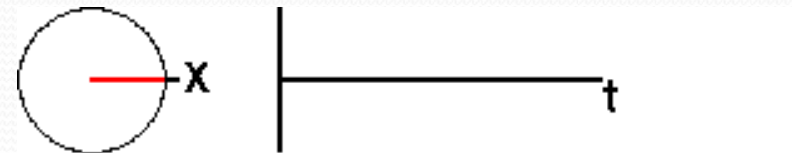
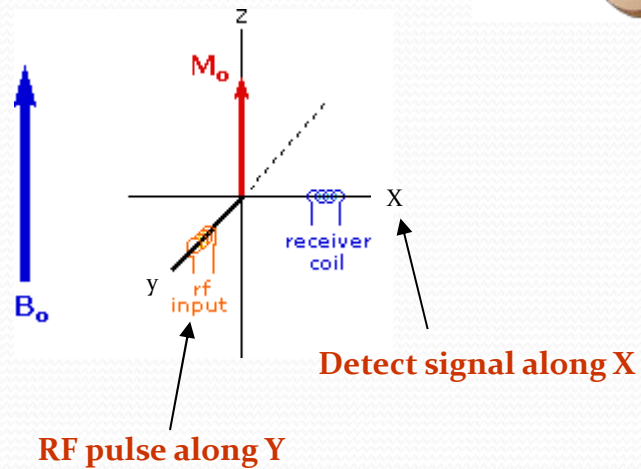
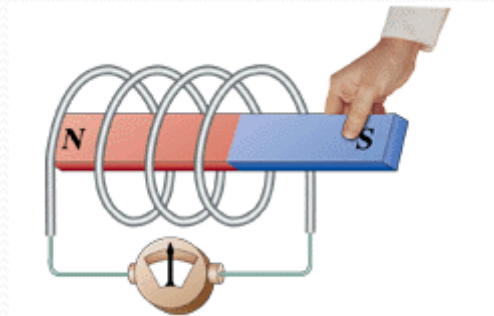


M_z



Classical Description

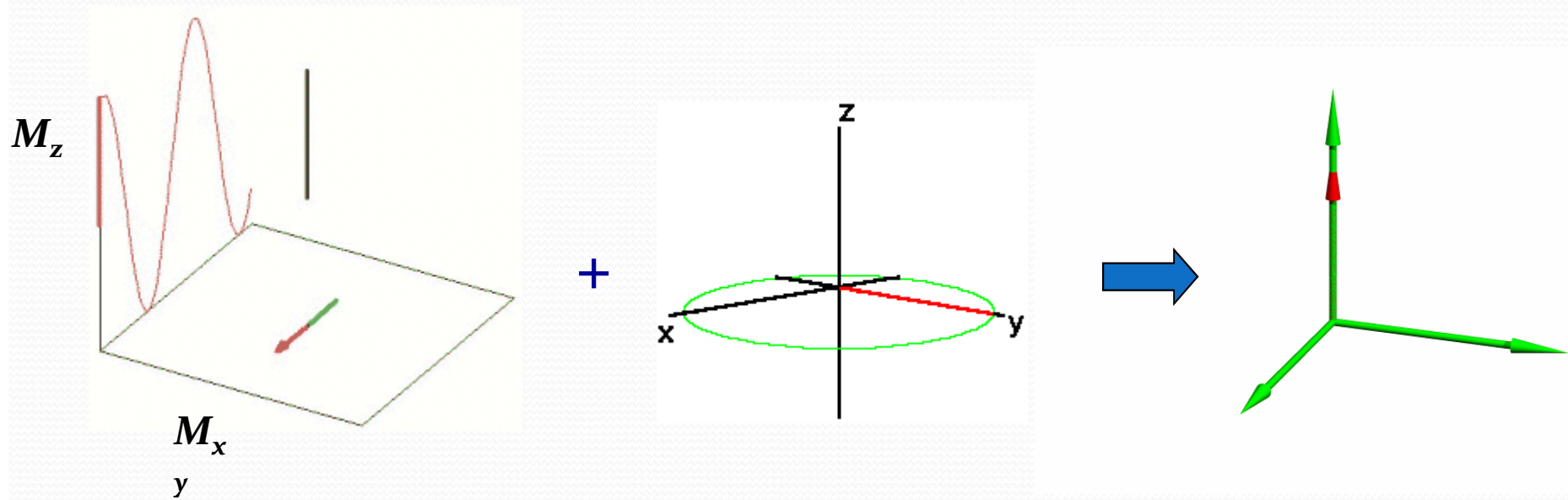
- *Observe NMR Signal*
 - **Remember:** a moving magnetic field perpendicular to a coil will induce a current in the coil.
 - The induced current monitors the nuclear precession in the X,Y plane



$$\nu = \gamma B_0 / 2\pi$$

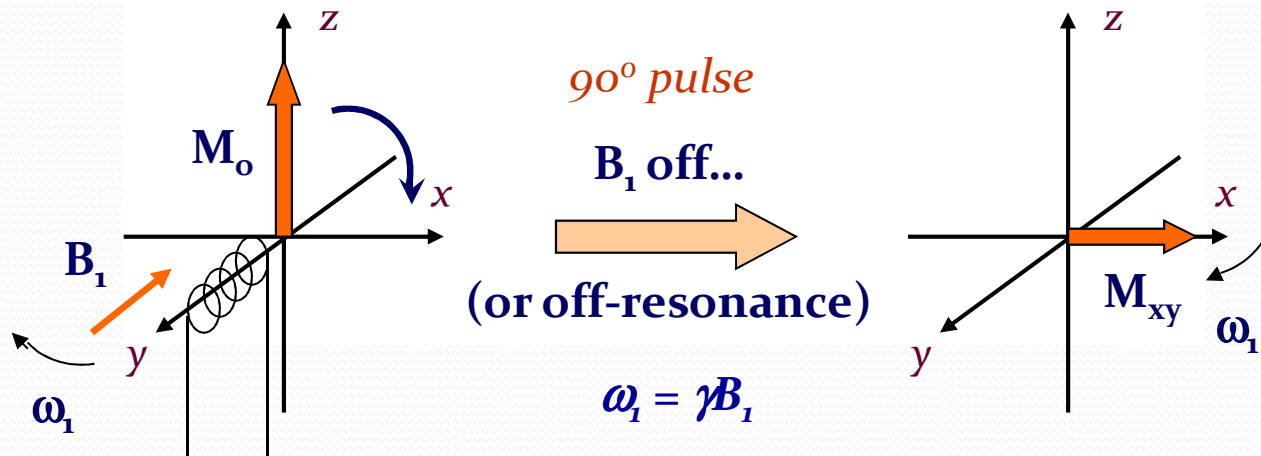
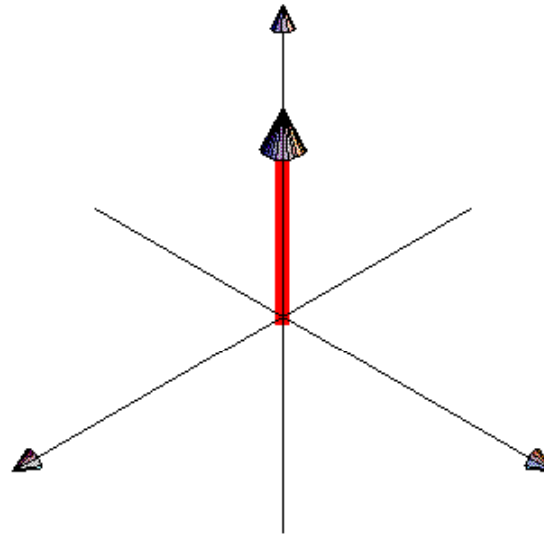
Classical Description

- *Rotating Frame*
 - To simplify the vector description, the X,Y axis rotates about the Z axis at the Larmor frequency (X',Y')
 - B_1 is stationary in the rotating frame



Classical Description

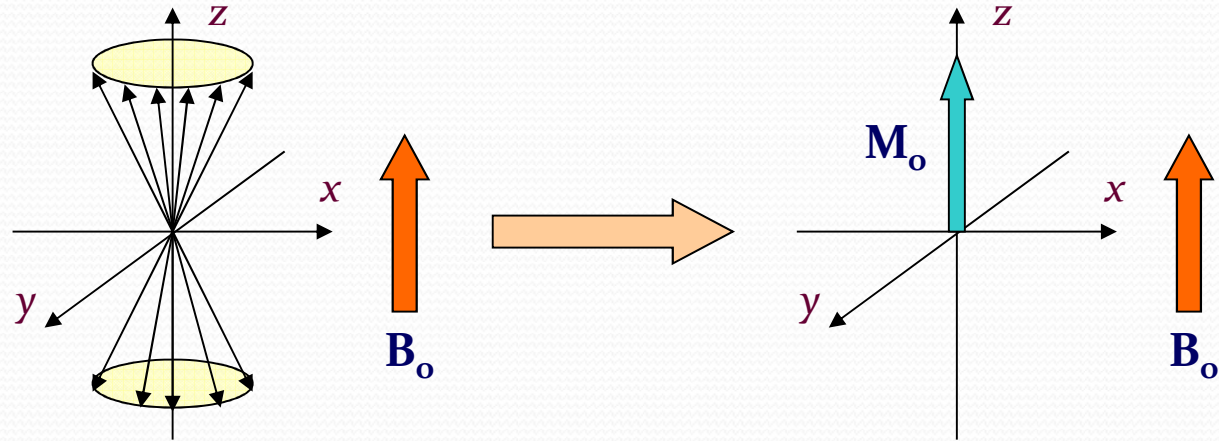
- *NMR Pulse*
 - Applying the B_1 field for a specified duration (Pulse length or width)
 - Net Magnetization precesses about B_1 a defined angle (90° , 180° , etc)



Net Magnetization

Classic View:

- Nuclei either align with or against external magnetic field along the z-axis.
- Since more nuclei align with field, net magnetization (M_o) exists parallel to external magnetic field



Quantum Description:

- Nuclei either populate low energy (α , aligned with field) or high energy (β , aligned against field)
- Net population in α energy level.
- Absorption of radio-frequency promotes nuclear spins from $\alpha \rightarrow \beta$.

