NMR spectroscopy

- Absorption (or emission) spectroscopy, as IR or UV. Detects the absorption of radiofrequencies (electromagnetic radiation) by certain nuclei in a molecule.

- Only nuclei with ***spin number*** (**I**) ≠ 0 can absorb/emit electromagnetic radiation.

    - Even atomic number & even mass ⇒ **I** = 0 (^{12}C, ^{16}O)
    - Even atomic mass & odd atomic number ⇒ **I** = whole integer (^{14}N, ^{2}H, ^{10}B)
    - Odd atomic mass ⇒ **I** = half integer (^{1}H, ^{13}C, ^{15}N, ^{31}P)

- The ***spin states*** of the nucleus (**m**) are ***quantized***:

$$\boxed{\mathbf{m = I, (I - 1), (I - 2), \ldots , -I}}$$

- Properly, **m** is called the ***magnetic quantum number***.

Background (continued)

- For ^{1}H, ^{13}C, ^{15}N, ^{31}P (biologically relevant nuclei) then:

$$\boxed{m = 1/2, -1/2}$$

- This means that only two states (energy levels) can be taken by these nuclei.

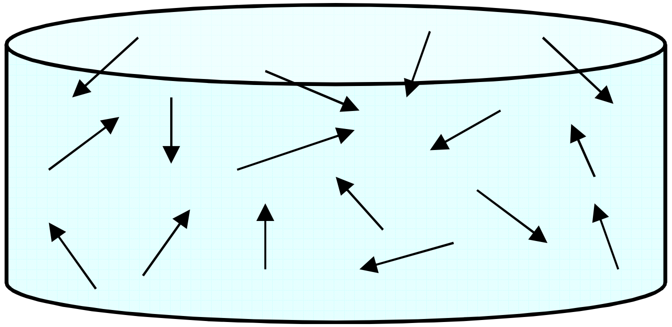
- Another important parameter of each particular nucleus is the ***magnetic moment*** (μ), which can be expressed as:

$$\boxed{\vec{\mu} = \gamma\, \mathbf{I}\, \mathbf{h} / 2\pi}$$

- It is a vector quantity that gives the direction and magnitude (or strength) of the 'nuclear magnet'
  - **h** is the Planck constant
  - γ is the gyromagnetic ratio, and it depends on the nature of each nuclei.
- Different nuclei have different magnetic moments.

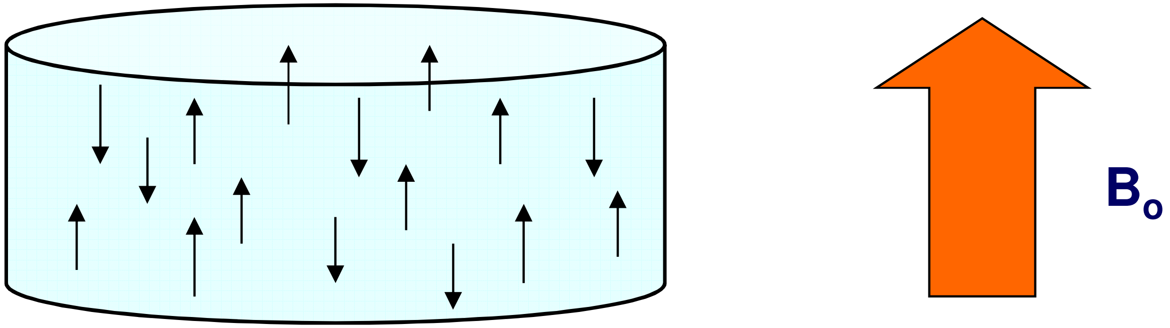
Effect of a magnetic field (for I = 1/2)

- In the ground state all nuclear spins are disordered, and there is no energy difference between them. They are ***degenerate***:



$\uparrow$ **= γ h / 4π**

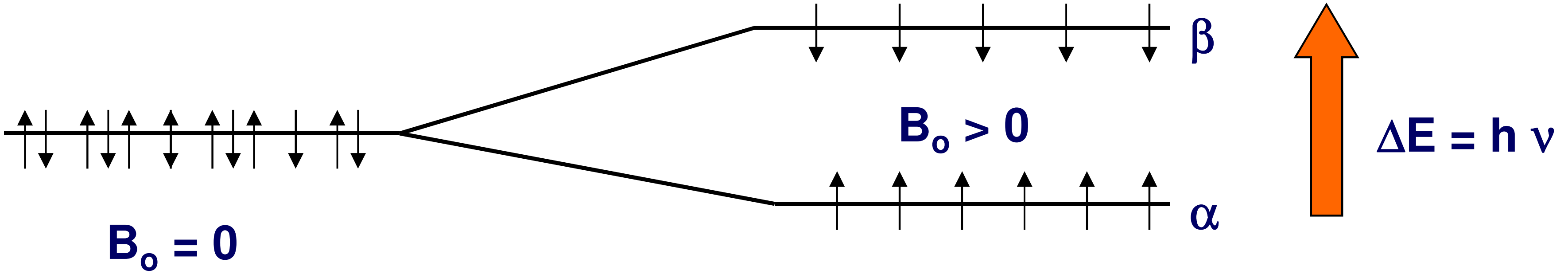
Since they have a magnetic moment, when we apply a strong external magnetic field (**B_o**), they orient either against or with it:



- There is always a small excess of nuclei (***population*** excess) aligned with the field than pointing against it.

Energy and populations

- Upon application of the external magnetic field we create an energy difference between nuclei aligned and against $\mathbf{B_o}$:



- Each level has a different ***population*** (***N***), and the difference between the two is related to the energy difference by the Boltzmann distribution:

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

- The ΔE for ^1H at 400 MHz ($\mathbf{B_o}$ = 9.5 T) is 3.8 x 10^{-5} Kcal / mol

$$\boxed{N_\alpha / N_\beta = 1.000064}$$

- The surplus population is small when compared to UV or IR.

Energy and sensitivity

- The energy (for a single spin) is proportional to the magnetic moment of the nuclei and the external magnetic field:

$$\mathbf{E = -\overrightarrow{\mu} \cdot \overrightarrow{B_o} \Rightarrow E_{(up)} = \gamma h B_o / 4\pi \text{ --- } E_{(down)} = -\gamma h B_o / 4\pi}$$

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

- This has implications on the energy (i.e., the intensity of the signal and sensitivity) that each nuclei can absorb:
  - Bigger magnets (bigger $\mathbf{B_o}$) make more sensitive NMR instruments.
  - Nuclei with larger γ absorb/emit more energy and are therefore more sensitive. Sensitivity is proportional to μ, to $\mathbf{N_\alpha - N_\beta}$, and to the 'coil magnetic flux', which are all dependent on γ. Therefore, it is proportional to γ^3.

$\gamma^{13}C$ = 6,728 rad / G

$\gamma^{1}H$ = 26,753 rad / G

^{1}H is ~ 64 times more sensitive than ^{13}C just because of the γ

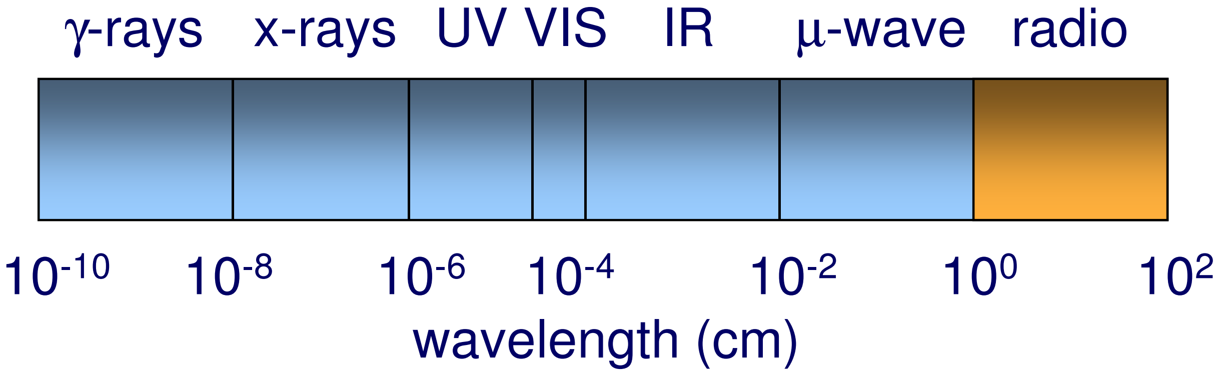
- If we consider natural abundance, ^{13}C (~1%) ends up being 6400 times less sensitive...

Energy and frequency

- Since energy is related to frequency, we can do some insightful math…

$$\left.\begin{array}{c}\Delta E = h\,\nu \\ \\ \Delta E = \gamma\, h\, B_o / 2\pi\end{array}\right\} \boxed{\nu = \gamma\, B_o / 2\pi}$$

- For ^1H in normal magnets (2.35 - 18.6 T), this frequency is in the 100-800 MHz range. For ^{13}C, 1/4 of that…

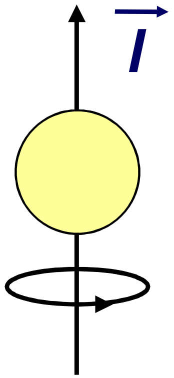


- To explain certain aspects of NMR, we need to refer to circular motion. Hz are not the best units to do so. We define the ***precession*** or ***Larmor*** frequency, ω:

$$\omega = 2\pi\nu \quad \Rightarrow \quad \boxed{\omega_o = \gamma\, B_o \text{ (radians)}}$$

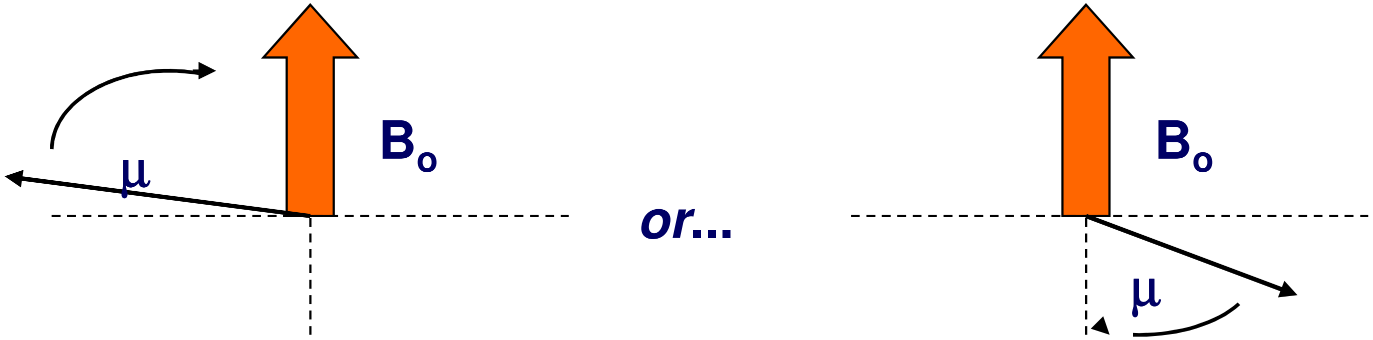
Precession and spinning tops

- What precession is ω_o associated with? One thing that we left out from the mix is the ***angular momentum***, ***I***, which is associated with all nuclei:



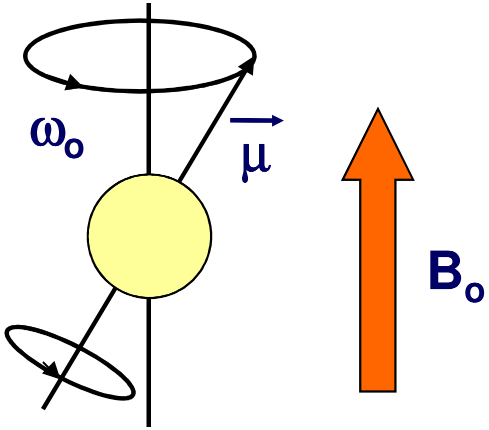
- Crudely, we can think of the nuclei as being spinning around its z axis. If we now consider those nuclei that have also a non zero μ, we have little spinning atomic magnets.

- Now, if we bring about a big $\mathbf{B_o}$, there will be an interaction between μ and $\mathbf{B_o}$ that generates a torque. No matter which is the original direction of μ, it will tend to align with $\mathbf{B_o}$:



Precession (continued)

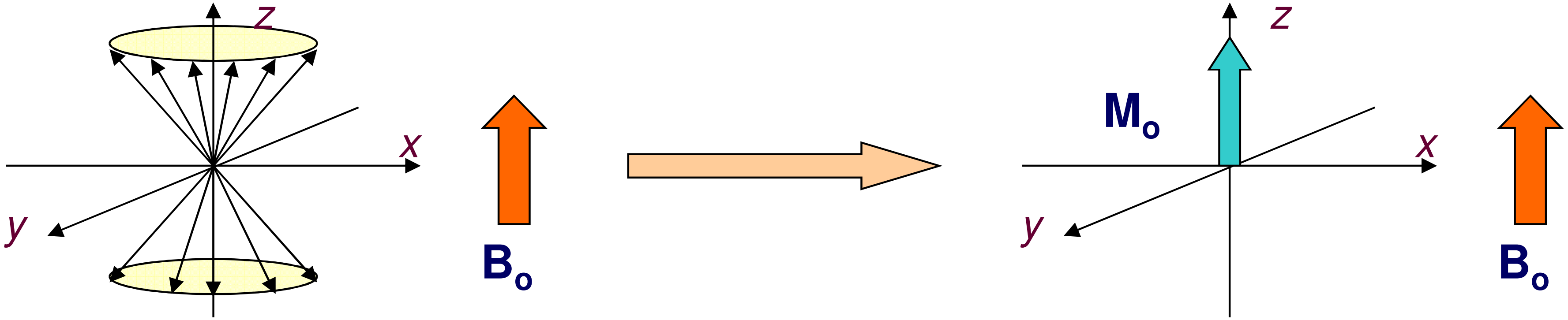
- Since the nuclei associated with μ is spinning due to ***I***, there are two forces acting on it. One that wants to bring it towards $\mathbf{B_o}$, and one that wants to keep it spinning. μ ends up precessing around $\mathbf{B_o}$:



- The best way to picture it is to imagine a spinning wooden top under the action of gravity.

Bulk magnetization

- We see the effects on macroscopic magnetization, **M_o**, which is directly proportional to the population difference (**$N_\alpha - N_\beta$**), in which contributions from different μs have been averaged:



- We can decompose each little μ in a **z** contribution and an **<xy>** plane contribution. The components in the **<xy>** plane are randomly distributed and cancel out. For the ones in **z**, we get a net magnetization proportional to **$N_\alpha - N_\beta$**.

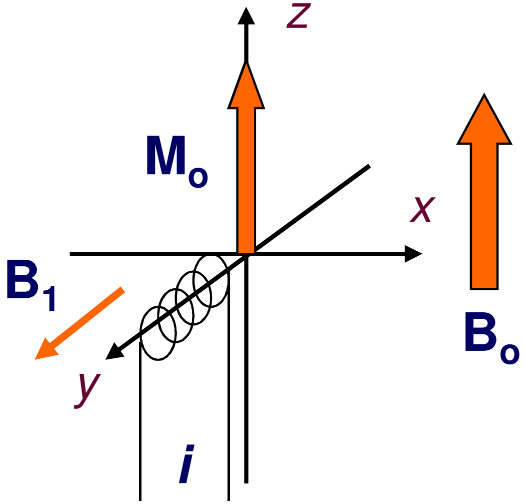
- Since this is (more or less) the situation in a real sample, we will from now on use **M_o** in all further descriptions/examples.

- There is an important difference between a μ and **M_o**. While the former is ***quantized*** and can be only in one of two states (α or β), the latter tells us on the whole spin population. It has a ***continuous*** number of states.

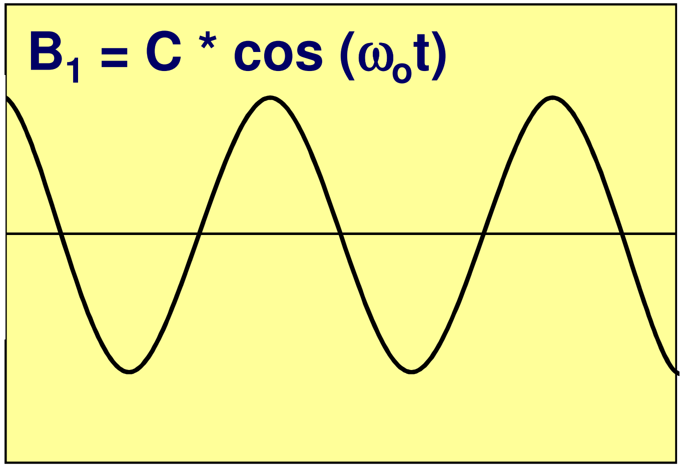
NMR excitation

So far, nothing happened. We need to do something to the system to observe any kind of signal. What we do is take it away from this condition and observe how it goes back to equilibrium. This means affecting the populations...

- We need the system to absorb energy. The energy source is an oscillating electromagnetic radiation generated by an alternating current:



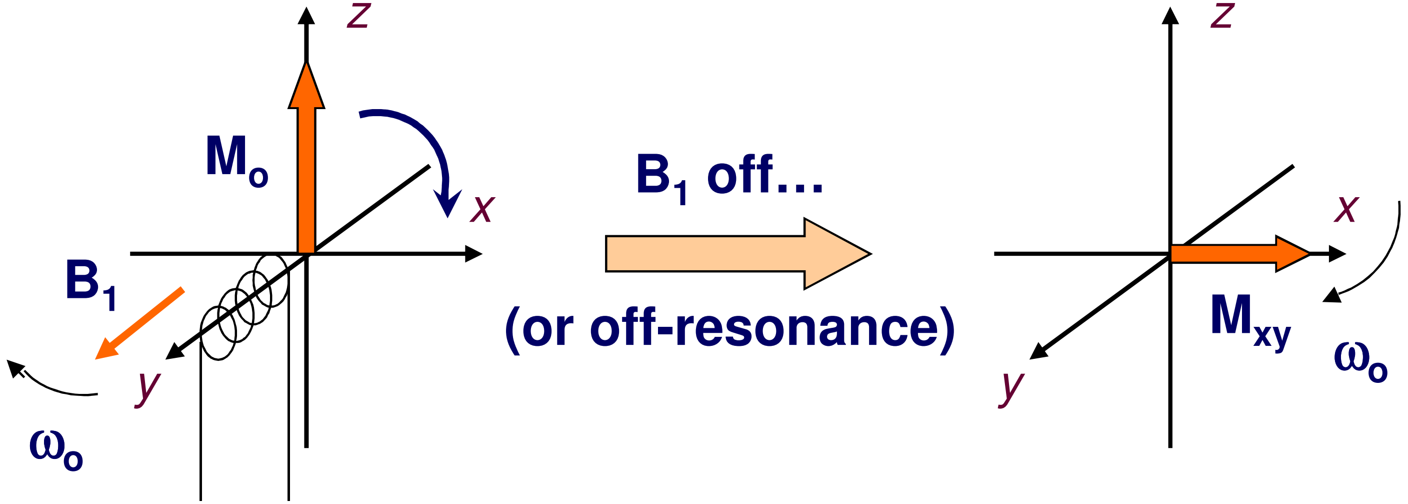
Transmitter coil (y)



$B_1 = C * \cos(\omega_o t)$

Resonance

- When the frequency of the alternating current is ω_o, we achieve a ***resonant condition***. The alternating magnetic field and **M_o** interact, there is a torque generated on **M_o**, and the system absorbs energy :



B_1 off…

(or off-resonance)

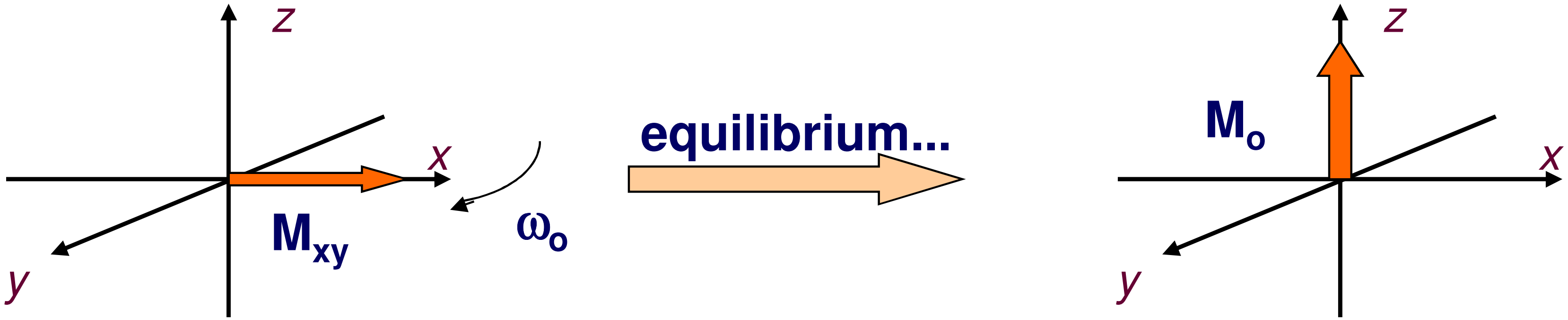
- Since the system absorbed energy, the equilibrium of the system was altered. We modified the populations of the **N_α** and **N_β** energy levels.

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

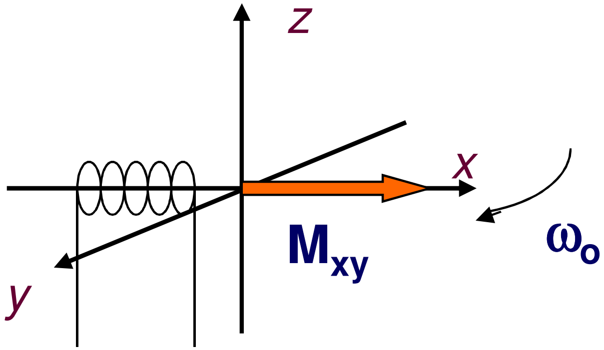
Return of M_o to equilibrium (and detection)

- In the absence of the external $\mathbf{B_1}$, $\mathbf{M_{xy}}$ will try to go back to $\mathbf{M_o}$ (equilibrium) by restoring the same $\mathbf{N_\alpha}$ / $\mathbf{N_\beta}$ distribution. This phenomenon is called ***relaxation***.

- $\mathbf{M_{xy}}$ returns to the **z** axis precessing on the **<xy>** plane:



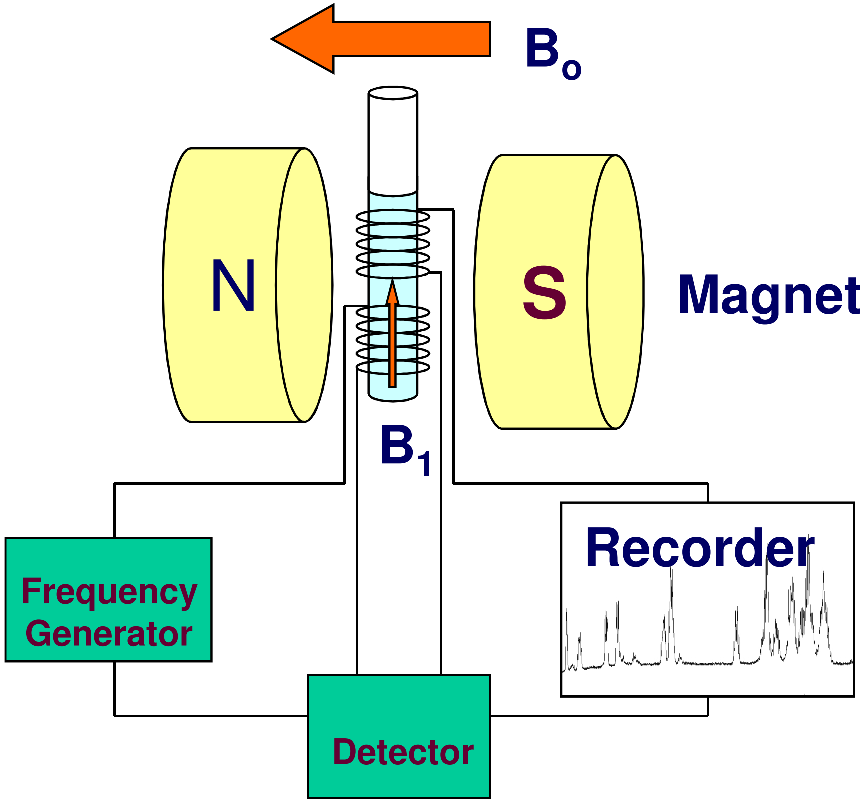
- The oscillation of $\mathbf{M_{xy}}$ generates a fluctuating magnetic field which can be used to generate a current in a coil:



Receiver coil (x) ⇒ **NMR signal**

NMR Instrumentation

- An NMR machine is basically a big and expensive FM radio.



- ***Magnet*** - Normally superconducting. Some electromagnets and permanent magnets (EM-360, EM-390) still around.

- ***Frequency generator*** - Creates the alternating current (at ω_o) that induces $\mathbf{B_1}$. Continuous wave or pulsed.

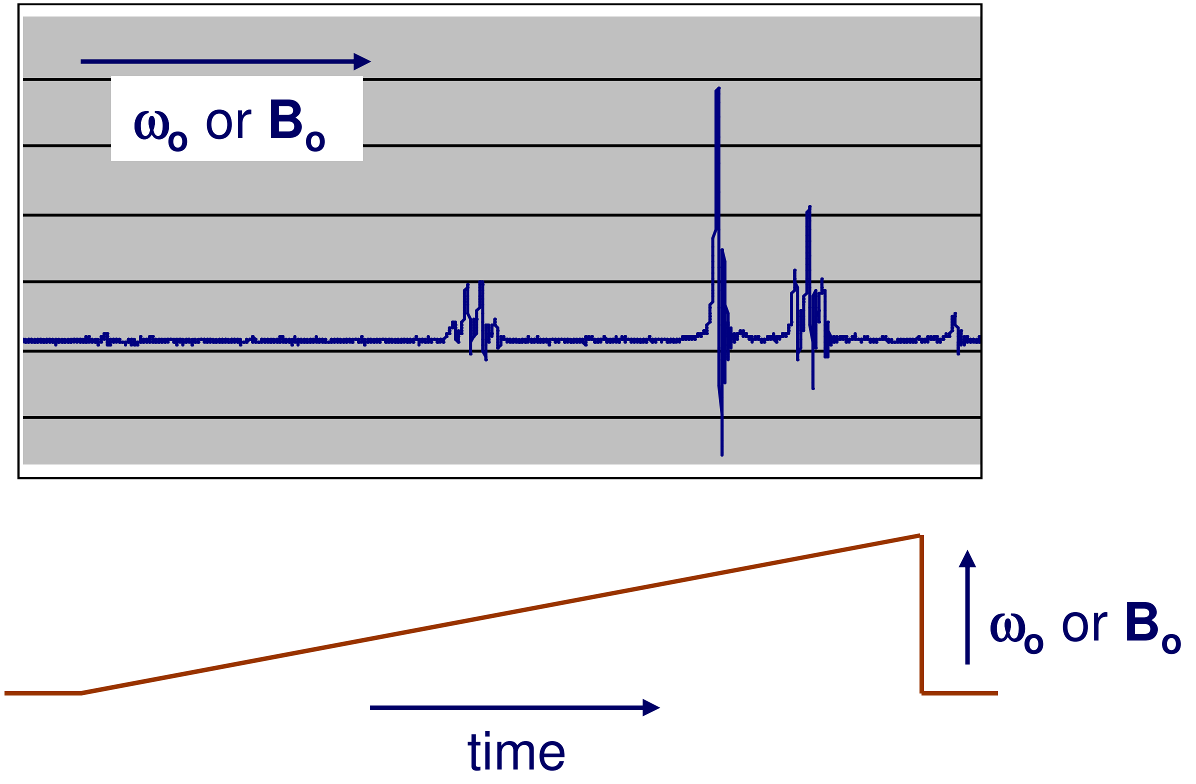
- ***Detector*** - Subtracts the base frequency (a constant frequency very close to ω_o) to the output frequency. It is lower frequency and much easier to deal with.

- ***Recorder*** - XY plotter, oscilloscope, computer, etc., etc.

Continuous Wave excitation

- It’s pretty *de mode*, and is only useful to obtain 1D spectra.

- The idea behind it is the same as in UV. We scan the frequencies continuously (or sweep the magnetic field, which has the same effect - **$\omega = \gamma$ B**), and record successively how the different components of **M_o** generate **M_{xy}** at different frequencies (or magnetic fields).



Fourier Transform - Pulsed excitation

- The way every NMR instrument works today.

- The idea behind it is pretty simple. We have two ways of tuning a piano. One involves going key by key on the keyboard and recording each sound (or frequency). The other, kind of brutal for the piano, is to hit it with a sledge hammer and record all sounds at once.

- We then need something that has all frequencies at once. A short ***pulse*** of radiofrequency has these characteristics.

- To explain it, we use another black box mathematical tool, the ***Fourier transformation***: It is a transformation of information in the time domain to the frequency domain (and *vice versa*).

$$S(\omega) = \int_{-\infty}^{\infty} s(t)\, e^{-i\omega t}\, dt$$

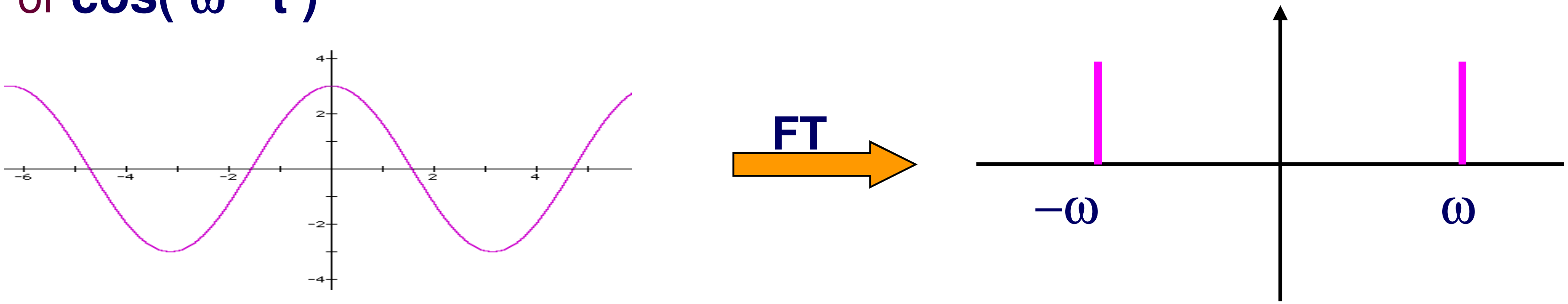
$$s(t) = 1/2\,\pi \int_{-\infty}^{\infty} S(\omega)\, e^{i\omega t}\, dt$$

- If our data in the time domain is periodical, it basically gives us its frequency components. Extremely useful in NMR, where all the signals are periodical.

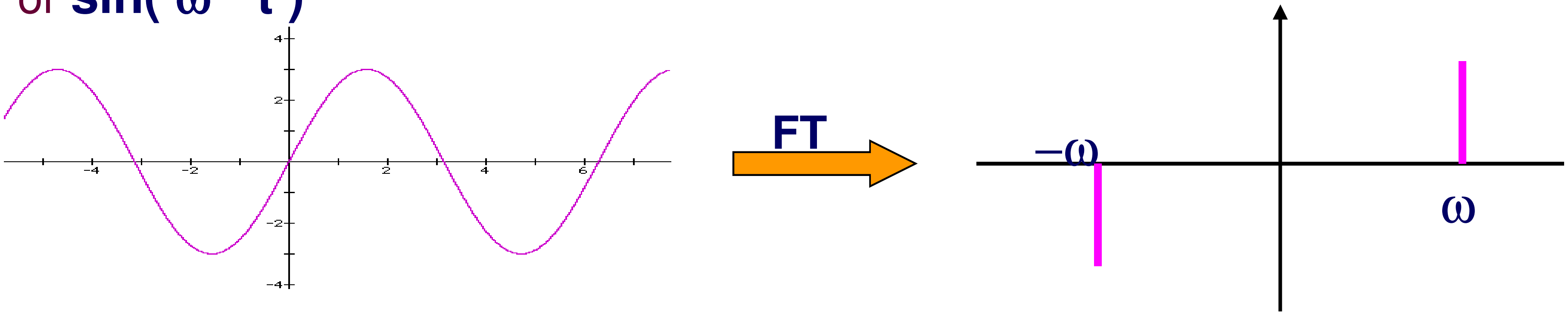
Fourier Transform of simple waves

- We can explain (or see) some properties of the FT with simple mathematical functions:

- For **cos(ω * t)**



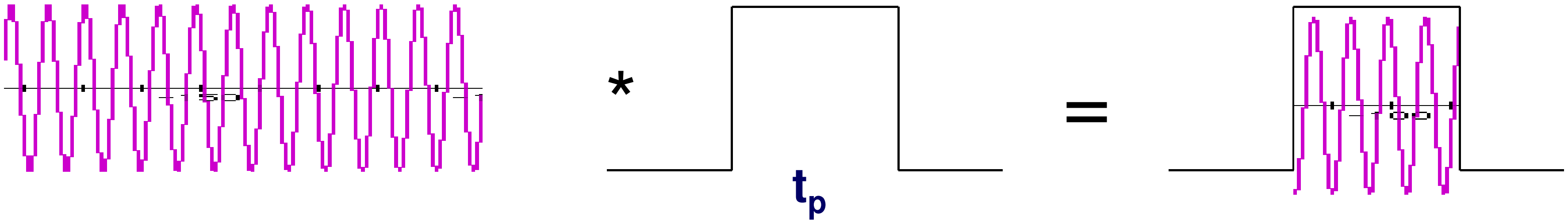
- For **sin(ω * t)**



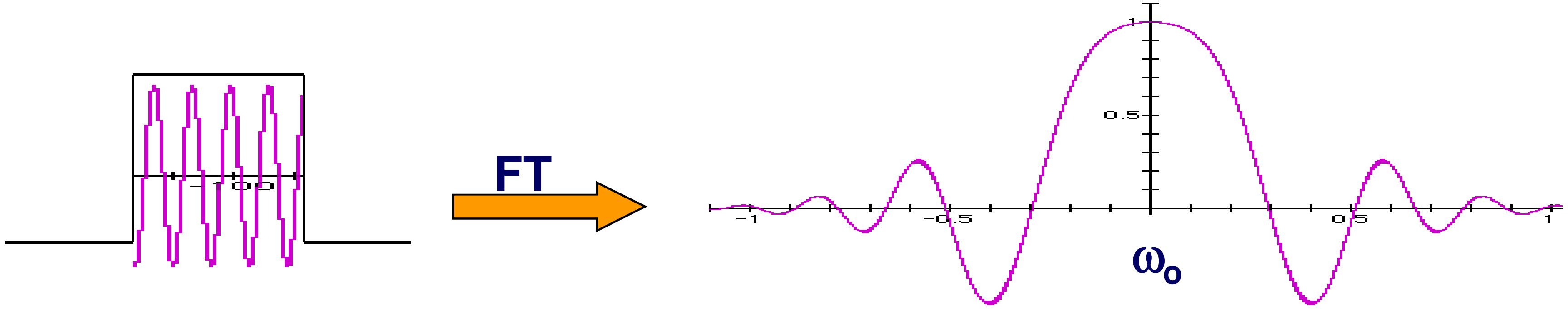
- The cosines are said to give absorptive lines, while sines give dispersive lines.

Back to pulses

- Now that we ‘master’ the FT, we can see how pulses work. A radiofrequency pulse is a combination of a wave (cosine) of frequency $\boldsymbol{\omega_o}$ and a step function:



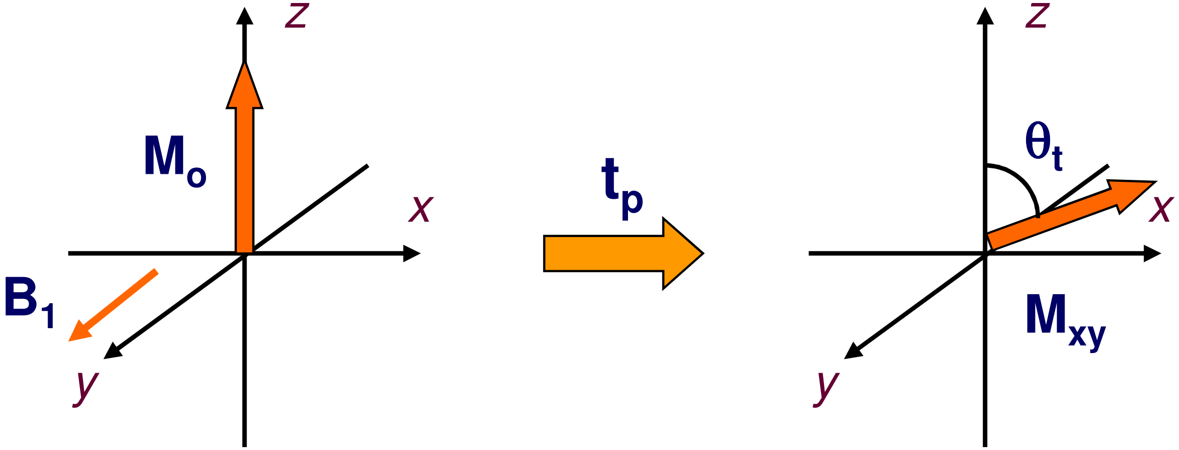
- This is the time domain shape of the pulse. To see the frequencies it really carry, we have to analyze it with FT:



- The result is a signal centered at $\boldsymbol{\omega_o}$ which covers a wide range of frequencies in both directions. Depending on the pulse width we have wider (shorter $\mathbf{t_p}$) or narrower (longer $\mathbf{t_p}$) ranges. Remember that $\mathbf{f \propto 1 / t}$.

Pulse widths and tip angles

- The pulse width is not only associated with the frequency range (or sweep width), but it also indicates for how long the excitation field $\mathbf{B_1}$ is on. Therefore, it is the time for which we will have a torque acting on the bulk magnetization $\mathbf{M_o}$:

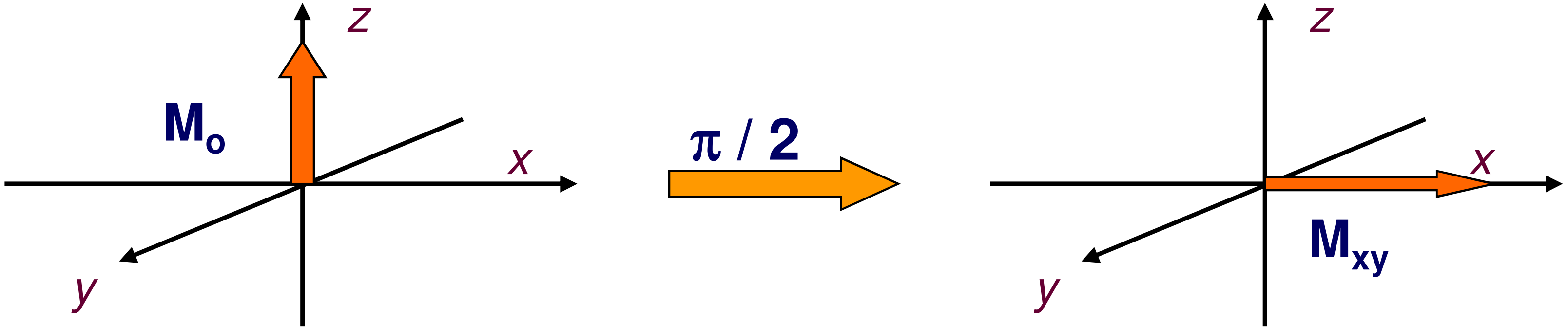


$$\boxed{\theta_t = \gamma * t_p * \mathbf{B_1}}$$

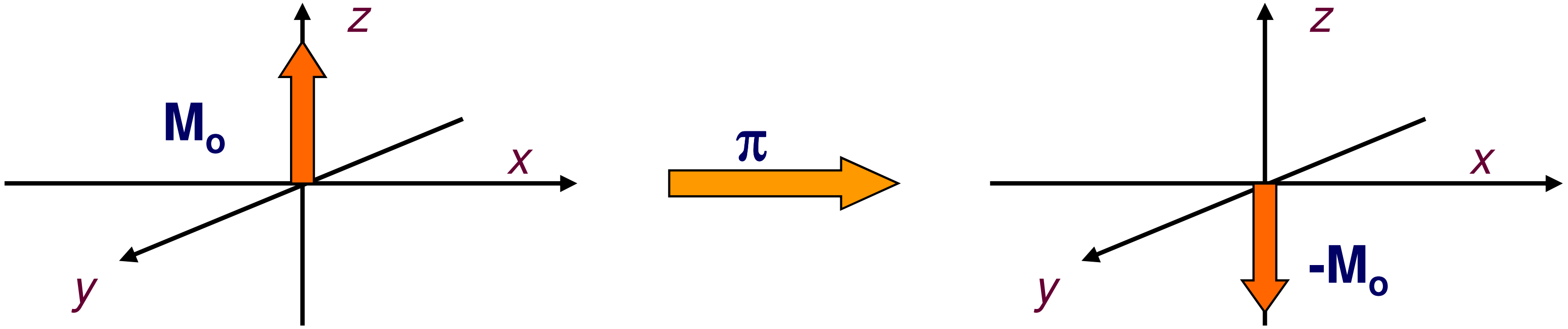
- As the pulse width for a certain flip angle will depend on the instrument ($\mathbf{B_1}$), we will therefore refer to them in terms of the rotation we want to obtain of the magnetization. Thus, we will have π / 4, π / **2**, and π pulses.

Some useful pulses

- The most commonly used pulse is the **π / 2**, because it puts as much magnetization as possible in the **<xy>** plane (more signal can be detected by the instrument):



- Also important is the **π** pulse, which has the effect of inverting the populations of the spin system...

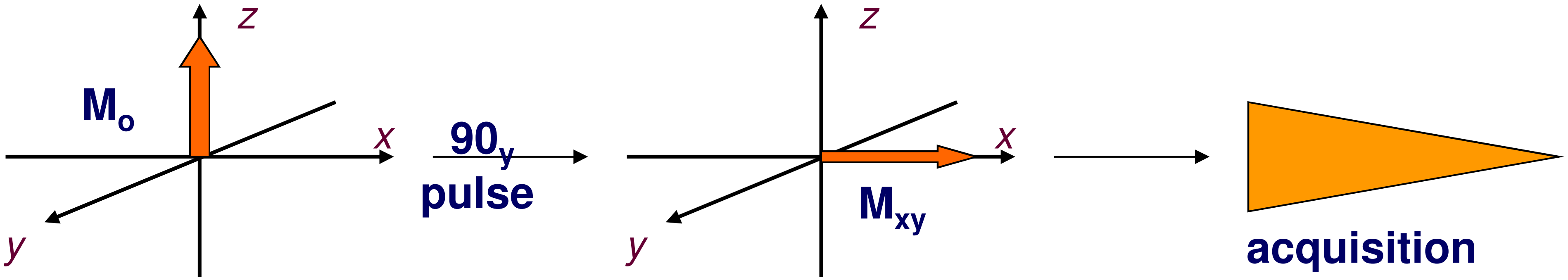


- With control of the spectrometer we can basically obtain any pulse width we want and flip angle we want.

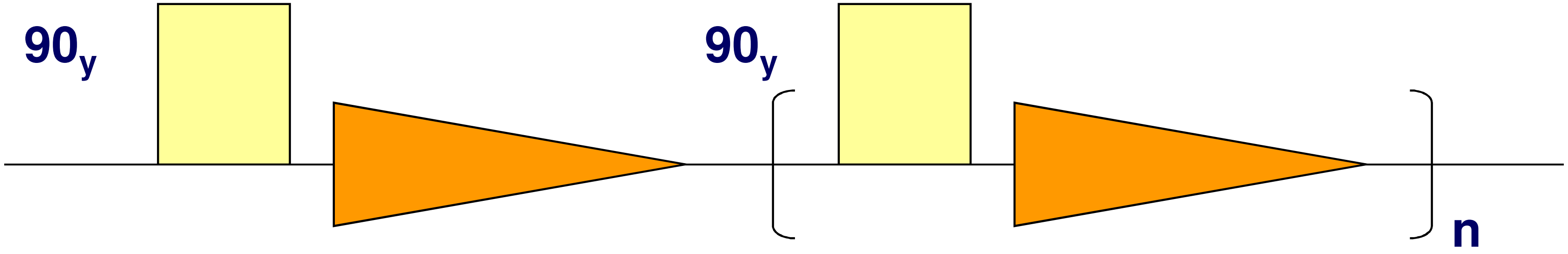
1D Pulse sequences

- We now have most of the tools to understand and start analyzing pulse sequences.

Vectors:



Shorthand:



- According to the direction of the pulse, we'll use $\mathbf{90_x}$ or $\mathbf{90_y}$ (or $\mathbf{90_\phi}$ if we use other phases) to indicate the relative direction of the $\mathbf{B_1}$ field in the rotating frame.

- The acquisition period will always be represented by an FID for the nucleus under observation (the triangle).

Relaxation phenomena

- So far we haven't said anything about the phenomena that brings the magnetization back to equilibrium. ***Relaxation*** is what takes care of this. There are two types of relaxation, and both are time-dependent exponential decay processes:

Longitudinal or Spin-Lattice relaxation (T_1):

- It works for the components of magnetization aligned with the **z** axis ($\mathbf{M_z}$).

 - Loss of energy in the system to the surroundings (lattice) as heat.
 - Dipolar coupling to other spins, interaction with paramagnetic particles, etc...

Transverse or Spin-Spin relaxation (T_2):

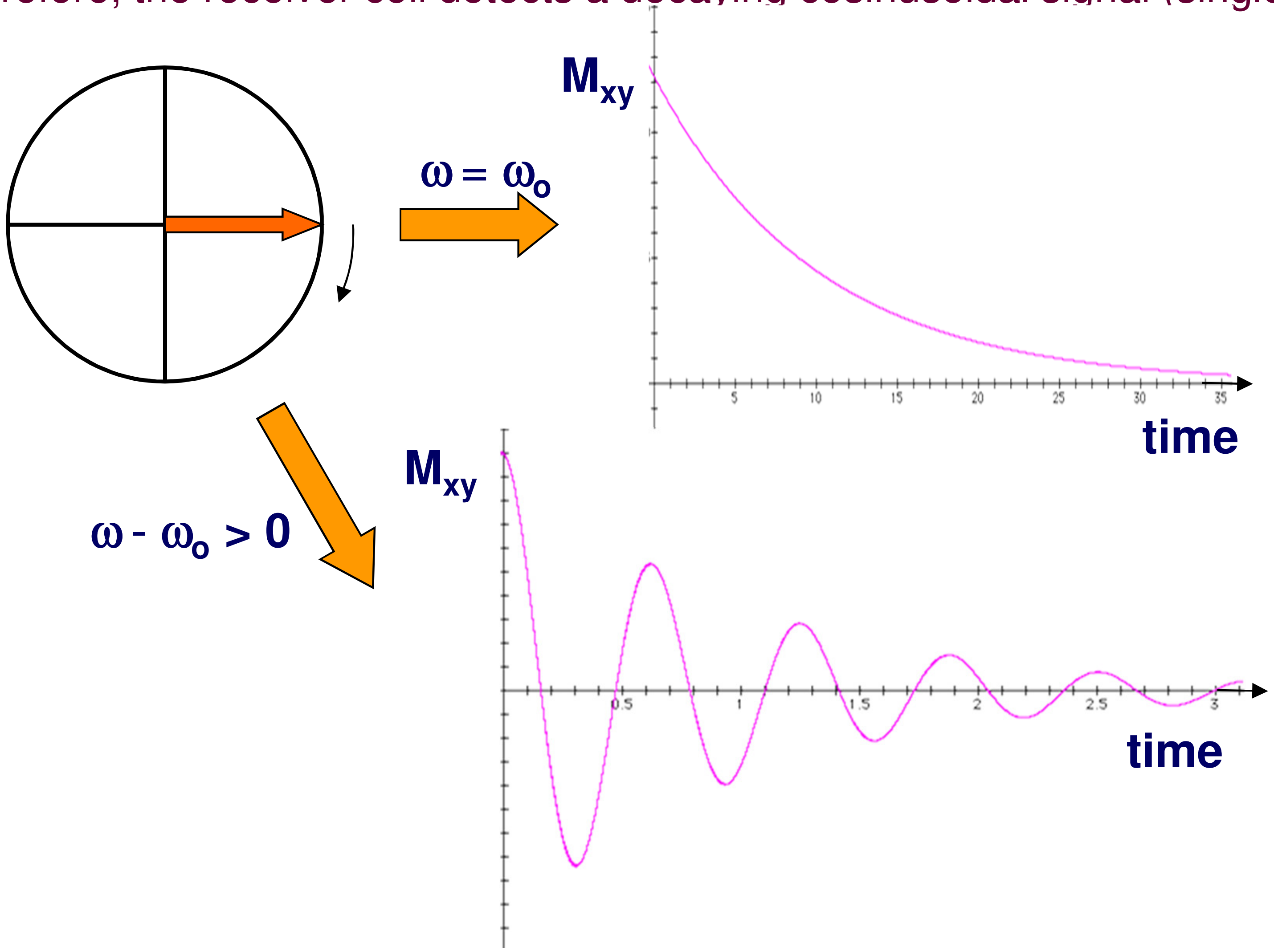
- It acts on the components of magnetization lying on the **<xy>** plane ($\mathbf{M_{xy}}$).

 - Spin-spin interactions dephase $\mathbf{M_{xy}}$
 - Also by imperfections in the magnet homogeneity (fanning out).
 - Cannot be bigger than T_1.

Free Induction Decay (FID)

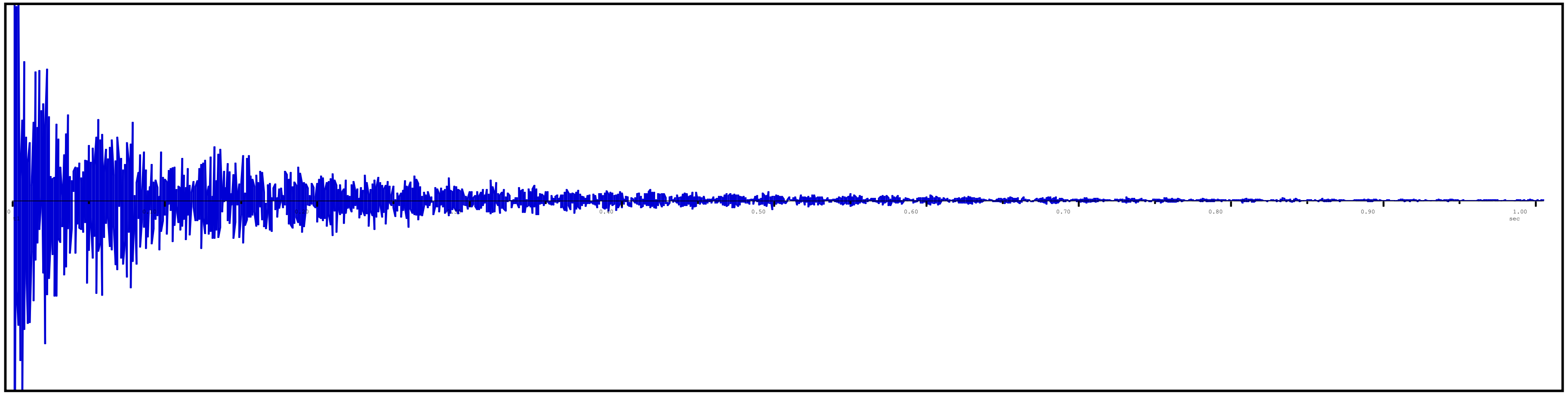
- Now, we are interested in analyzing the signal that appears in the receiver coil after putting the bulk magnetization in the **<xy>** plane (π / **2** pulse).

- We said earlier that the sample will go back to equilibrium (z) precessing. In the rotating frame, the frequency of this precession is $\omega - \omega_o$. The relaxation of $\mathbf{M_o}$ in the **<xy>** plane is exponential. Therefore, the receiver coil detects a decaying cosinusoidal signal (single spin type):

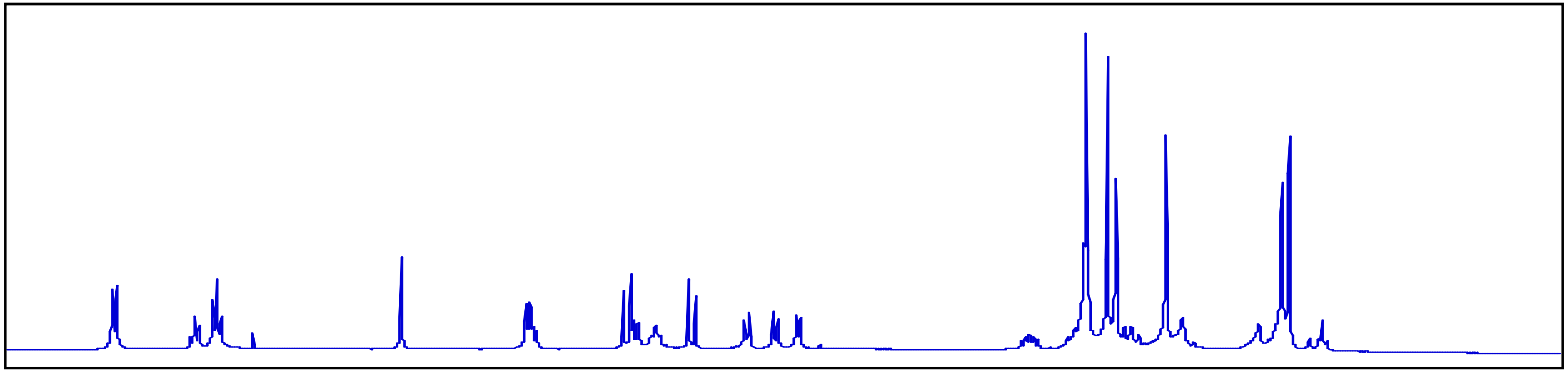


FID (continued)

- In a real sample we have hundreds of spin systems, which all have frequencies different to that of **B_1** (or ***carrier frequency***). Since we used a pulse and effectively excited all frequencies in our sample at once, we will se a combination of all of them in the receiver coil, called the ***Free Induction Decay*** (or ***FID***):

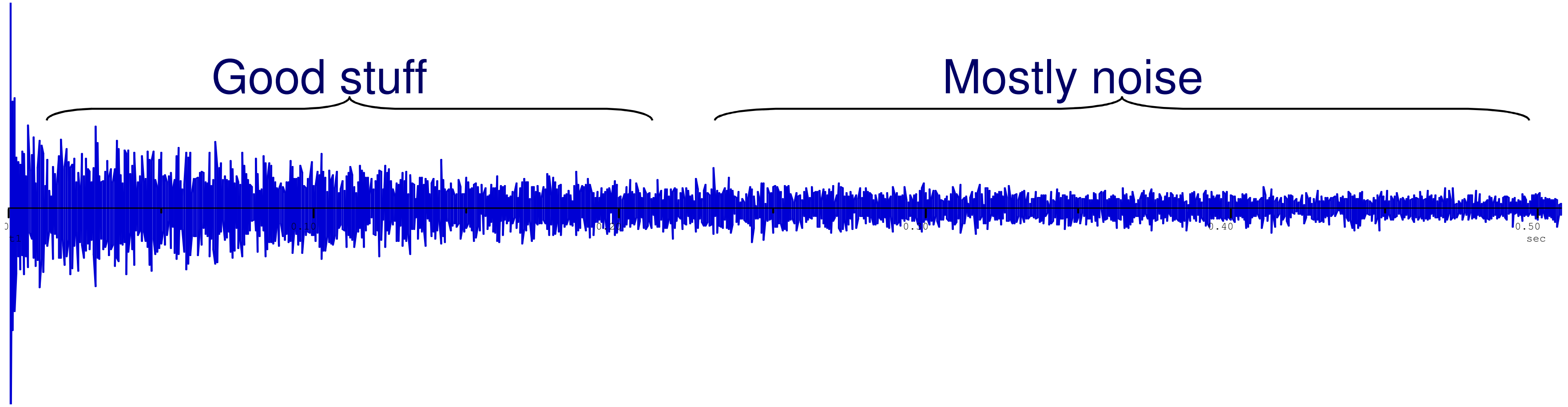


- The FT of this signal gives us the NMR spectrum:



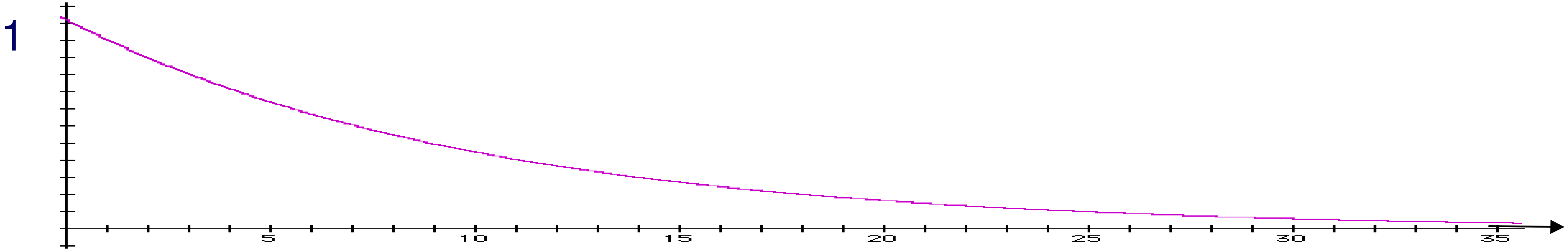
Data processing - Window functions

- Now we have the signal in the computer, properly sampled. There are some things we can do now a lot easier, and one of them is filtering. The real information in the FID is in the first section. As $\mathbf{M}_{\mathbf{xy}}$ decays, we have more and more noise:



- The noise is generally high frequency, and that is why NMR spectra have this jagged baseline. What if we could filter all the signals that were higher than a certain frequency?

- We use ***digital filtering***. Intuitively, it means multiplying the FID by a function that makes the noise at the end smaller:

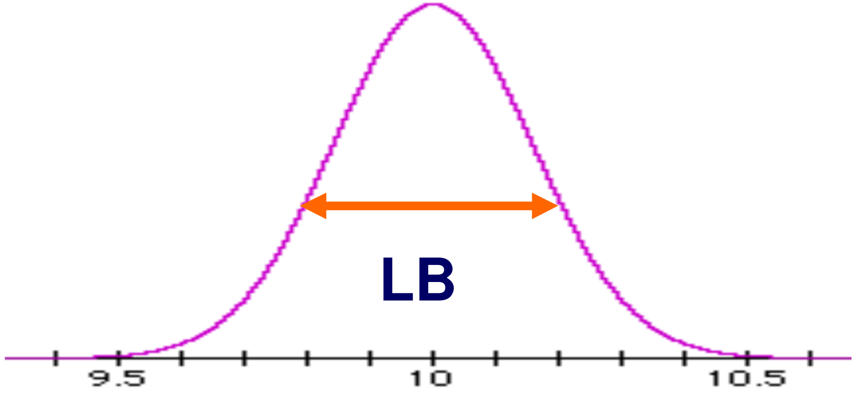


Window functions (continued)

- In this case, it is called ***exponential multiplication***, and has the form:

$$F(t) = 1 * e^{-(LB * t)} \quad \text{or} \quad F(t) = 1 * e^{-(t / \tau)}$$

- Why is that this removes high frequency noise? Actually, we are ***convoluting*** the frequency domain data with the FT of a decaying exponential. The FT of this function is a Lorentzian shaped peak with a width at half-height proportional to the rate of decay, or ***line broadening*** (***LB***), in Hz.

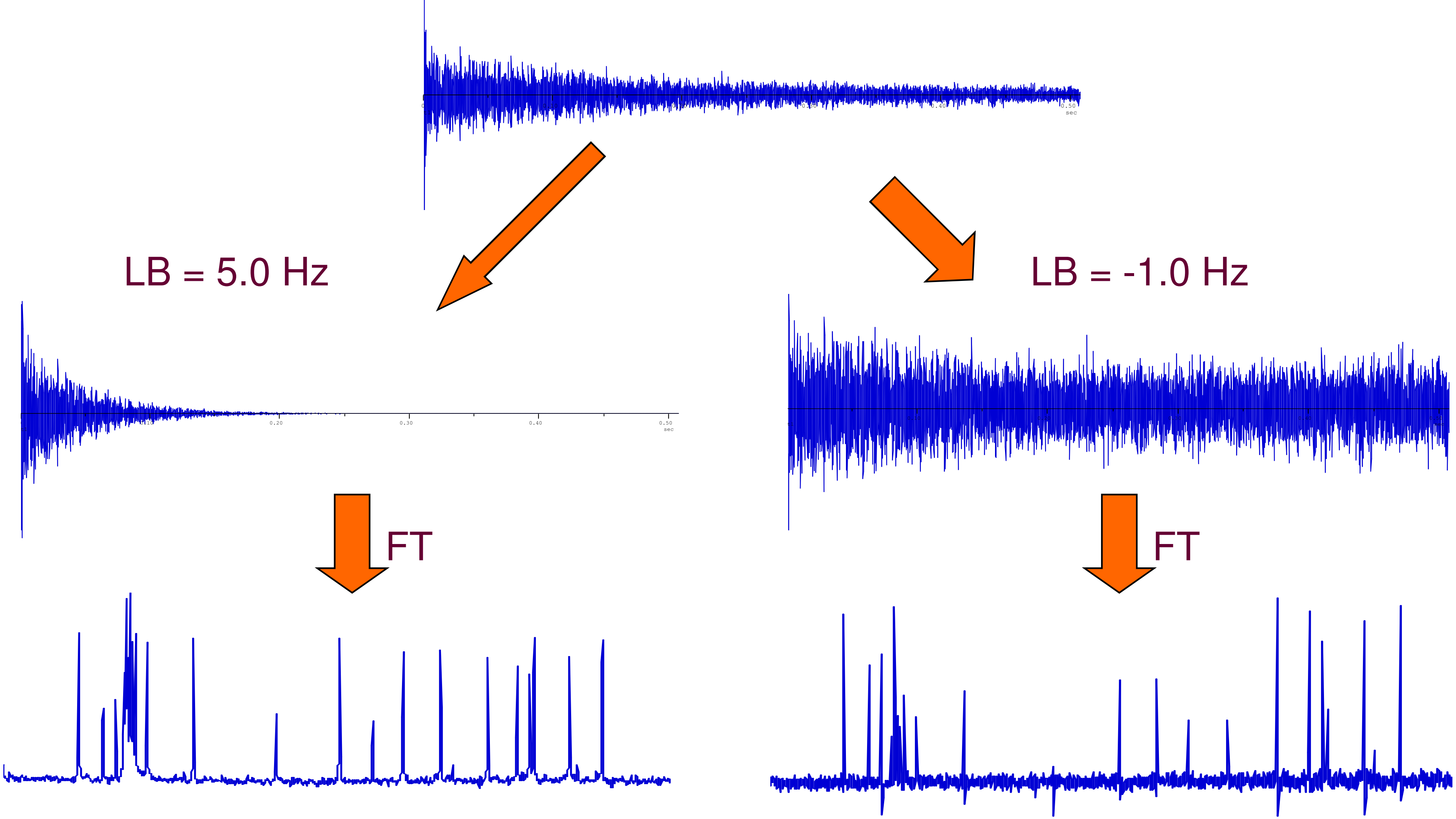


- Convolution makes the contribution of everything with a WAHH thinner than LB smaller in the spectrum (the scale here is bogus…).

- If we use an LB with the opposite sign, the exponential grows instead of decaying, letting signals with narrower widths to pass, improving resolution but lowering signal to noise ratio.

Sensitivity and resolution enhancement

- For the following raw FID, we can apply either a positive or negative LB factor and see the effect after FT:


LB = 5.0 Hz

LB = -1.0 Hz

FT

FT

Chemical shifts

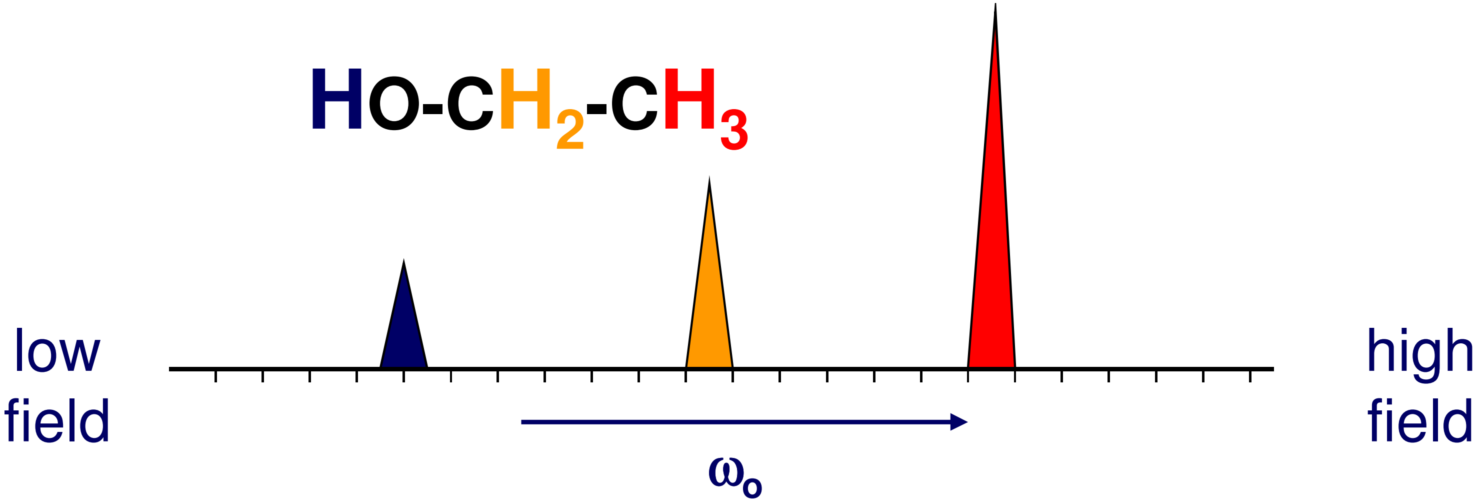
- If each type of nucleus has its characteristic ω_o at a certain magnetic field, why is NMR useful?

- Depending on the ***chemical environment*** we have variations on the magnetic field that the nuclei feels, even for the same type of nuclei. It affects the local magnetic field.

$$B_{eff} = B_o - B_{loc} \quad --- \quad B_{eff} = B_o(1 - \sigma)$$

- σ is the ***magnetic shielding*** of the nucleus. Factors that affect it include neighboring atoms, aromatic groups, etc., etc. The polarization of the bonds to the observed nuclei are also important.

- As a crude example, ethanol looks like this:



$HO\text{-}CH_2\text{-}CH_3$

The NMR scale (δ, ppm)

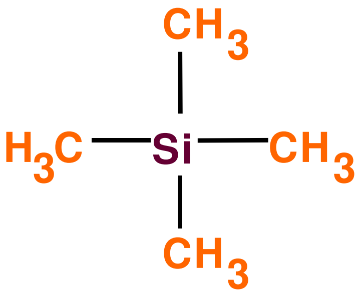
- We can use the frequency scale as it is. The problem is that since $\mathbf{B}_{loc}$ is a lot smaller than $\mathbf{B}_o$, the range is very small (hundreds of Hz) and the absolute value is very big (MHz).

- We use a relative scale, and refer all signals in the spectrum to the signal of a particular compound.

$$\boxed{\delta = \frac{\omega - \omega_{ref}}{\omega_{ref}}} \quad \text{ppm (parts per million)}$$

- The good thing is that since it is a relative scale, the δ in a 100 MHz magnet (2.35 T) is the same as that obtained for the same sample in a 600 MHz magnet (14.1 T).

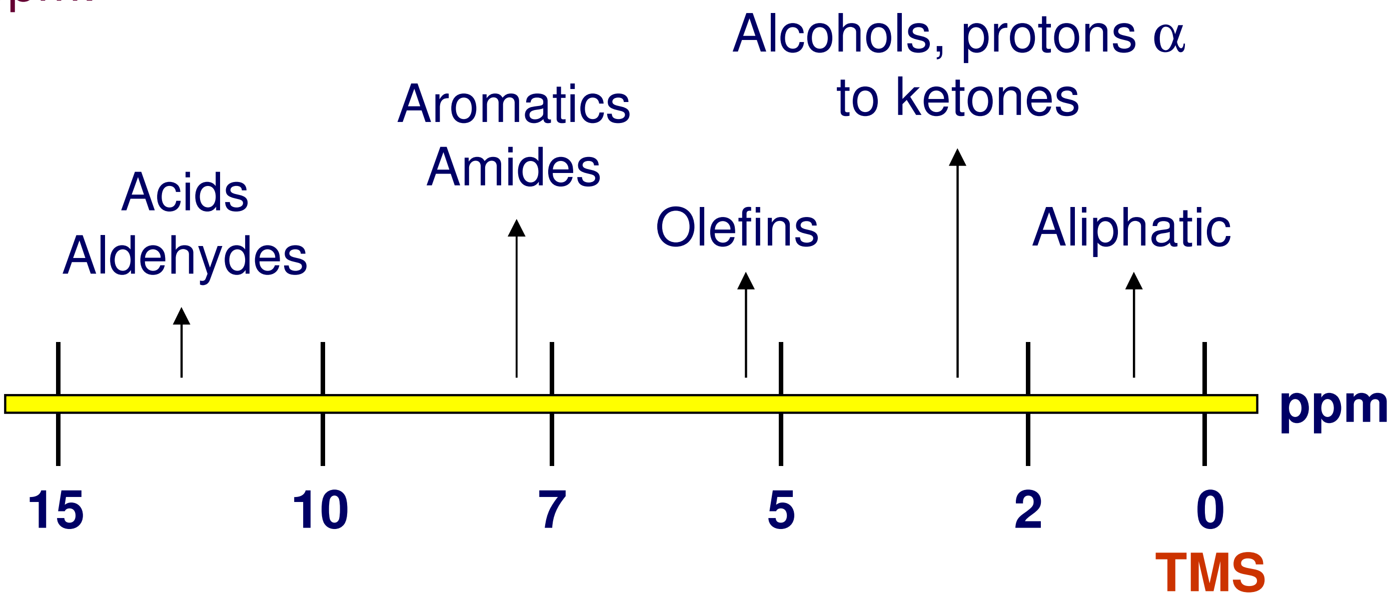
- ***Tetramethyl silane*** (**TMS**) is used as reference because it is soluble in most organic solvents, is inert, volatile, and has 12 equivalent ^{1}Hs and 4 equivalent ^{13}Cs:



- Other references can be used, such as the residual solvent peak, dioxane for ^{13}C, or **TSP** in aqueous samples for ^{1}H.

Scales for different nuclei

- For protons, ~ 15 ppm:



Acids
Aldehydes

Aromatics
Amides

Olefins

Alcohols, protons α
to ketones

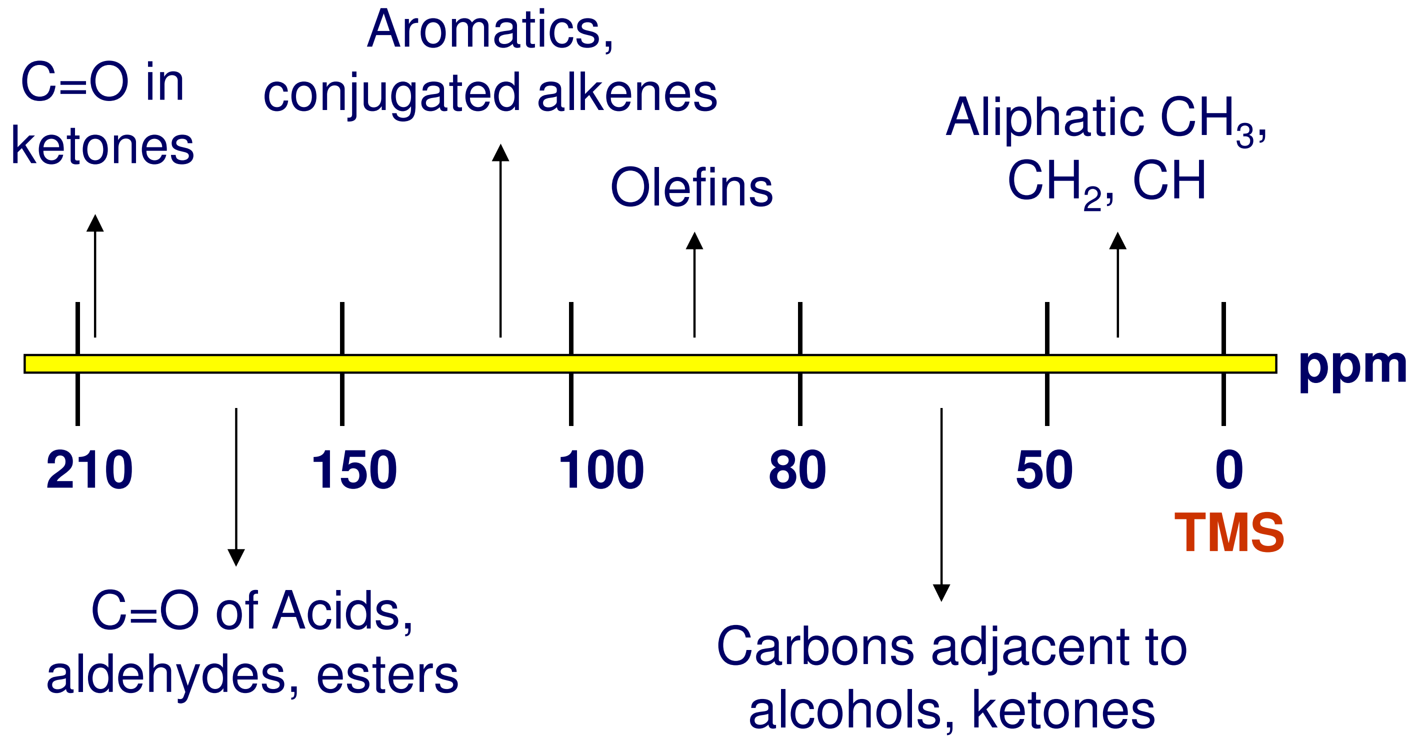
Aliphatic

ppm

15 10 7 5 2 0

TMS

- For carbon,
~ 220 ppm:



C=O in
ketones

Aromatics,
conjugated alkenes

Olefins

Aliphatic CH_3,
CH_2, CH

ppm

210 150 100 80 50 0

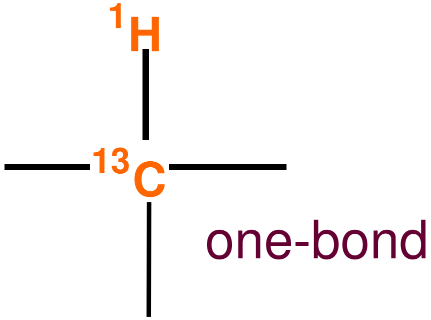
TMS

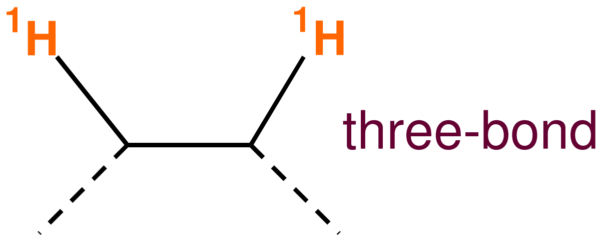
C=O of Acids,
aldehydes, esters

Carbons adjacent to
alcohols, ketones

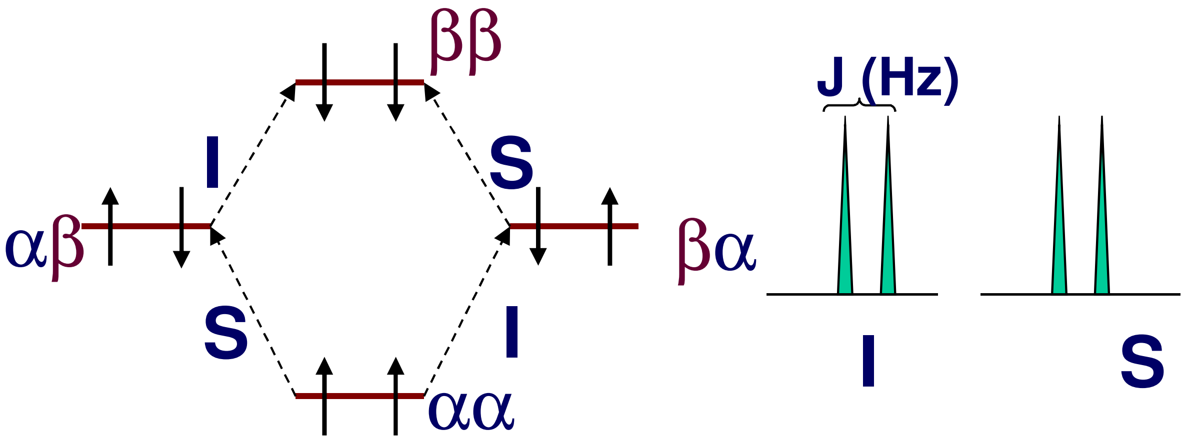
Coupling Constants

- The energy levels of a nucleus will be affected by the spin state of nuclei nearby. The two nuclei that show this are said to be ***coupled*** to each other. This manifests in particular in cases were we have through bond connectivity:





- Energy diagrams. Each spin now has two energy 'sub-levels' depending on the state of the spin it is coupled to:



- The magnitude of the separation is called ***coupling constant*** (**J**, Hz).
- Coupling patterns are crucial to identify spin systems in a molecule and to the determination of its chemical structure.