# Data acquisition

- There are certain things that we have to take into account before and after we take an FID (or the spectrum, the FID is not that useful after all).

- Some deal with the detection system. Since a computer will be acquiring the data, we can only take certain number of samples from the signal (***sampling rate***). How many will depend on the frequencies that we have in the FID.

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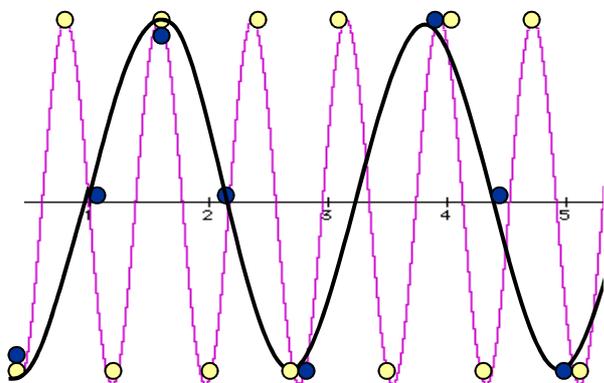

$$SR = 1 / (2 * SW)$$

- If we sample twice as fast as the frequency, where the dots are ○ we, are in the clear.

- On the other hand, if we go too slow and sample at half the speed at ● we end up with a digitized signal in the computer at 1 / 2 of the real frequency. These peaks will ***fold over*** with the wrong phase in our spectrum. This is called ***aliasing***.

Quadrature detection

- In the old days the frequency of **B₁** (carrier) was somewhere higher than all other frequencies. This was done to avoid having frequencies faster (or slower) than the carrier, so the computer always knew the sign of the frequencies in the FID.
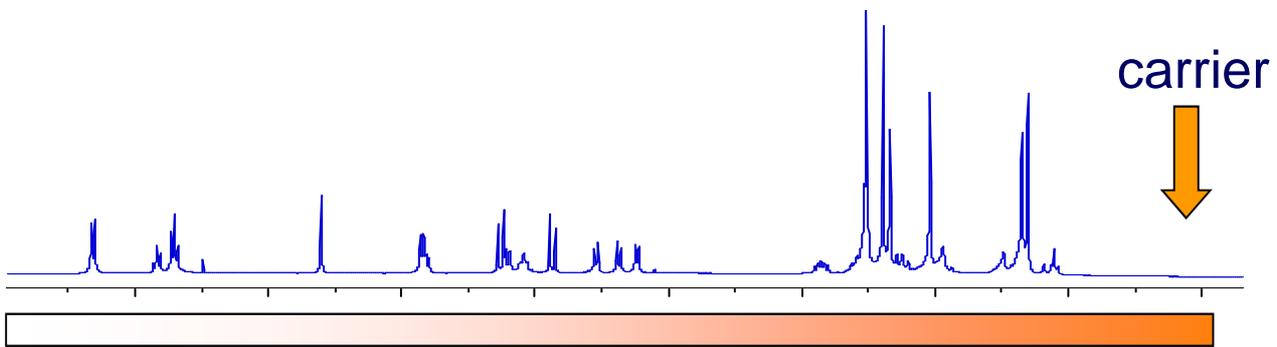
carrier

- There are two problems. One, noise, which is always there, is not sampled properly and its aliased into our spectrum. Also, in order to excite lines far from the carrier, we need very good pulses, which is never the case.

- Considering this, the best place to put the carrier is the center of the frequency spectrum:
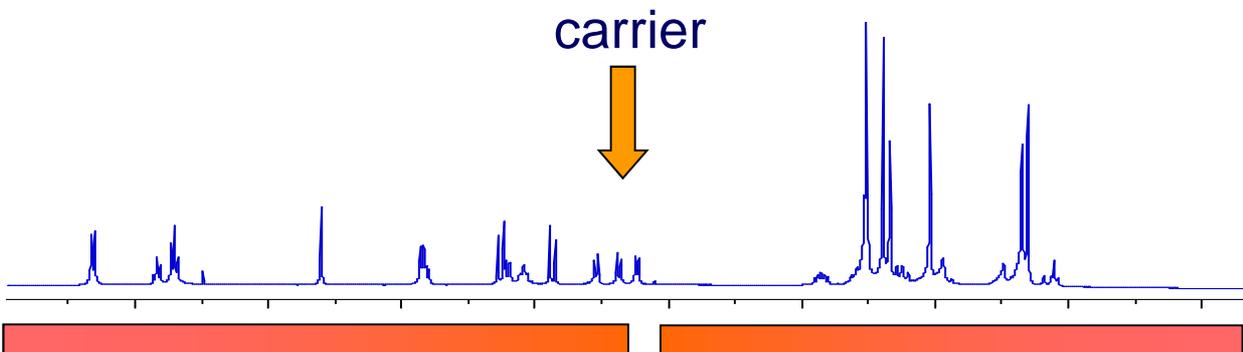
carrier

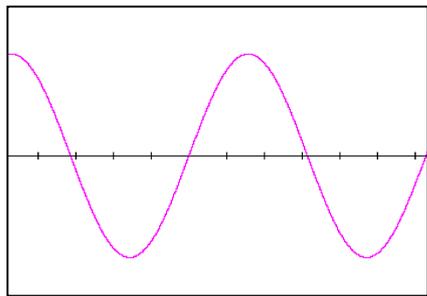- There are several things we have to consider before doing it.

Quadrature detection (continued)

• How can we tell which frequency is going faster or slower relative to the carrier? The trick is to put 2 receiver coils at 90 degrees (with a phase shift of 90 degrees) of each other:
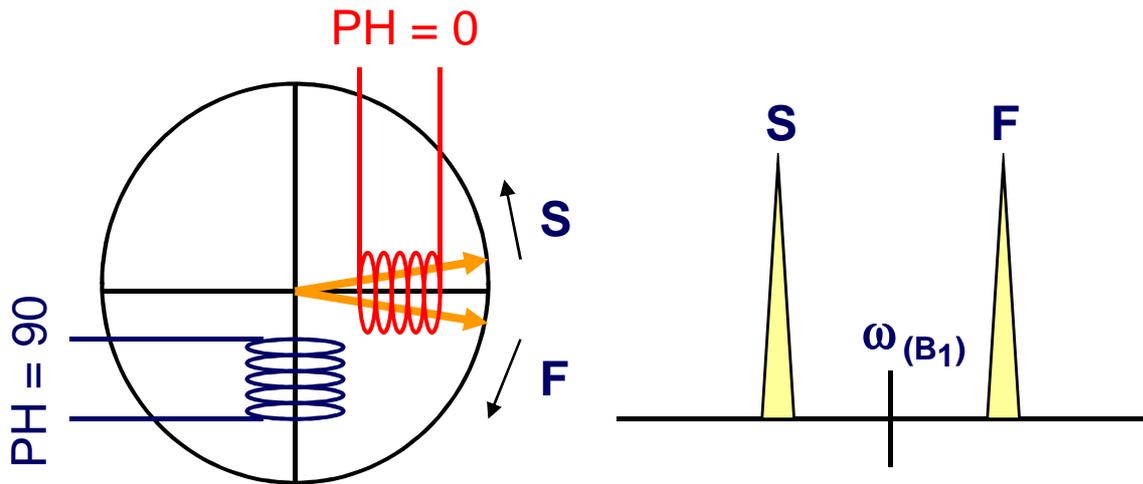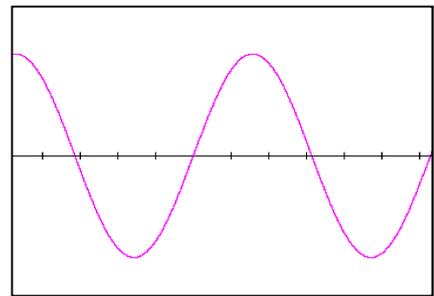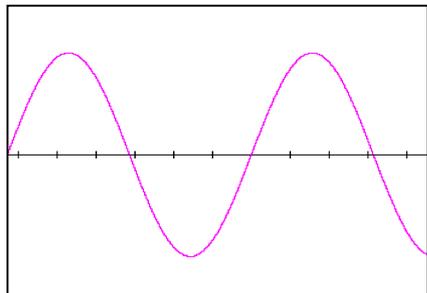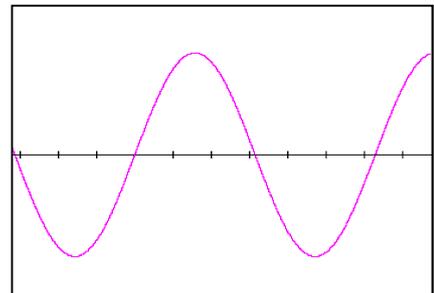

• The phase of the **F**aster signals is opposite to that of the **S**lower signals, and the computer is then able to sort this out.

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. Most information in the FID is in the first section. As M_{xy} decays, we have more and more noise:
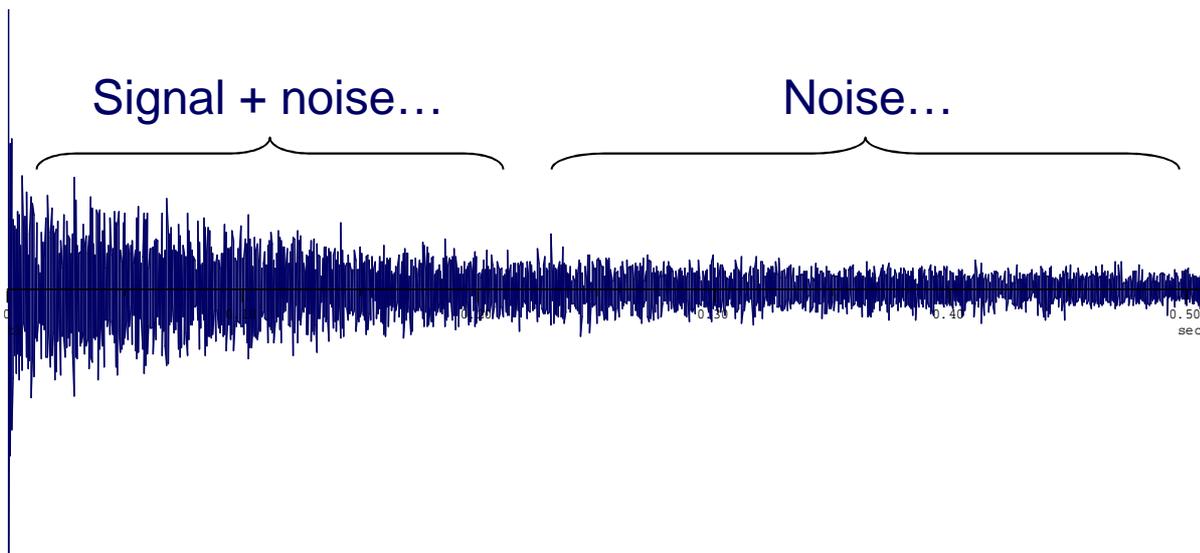
- The noise is generally high frequency, and this is why NMR spectra have this jagged baseline. What if we could filter all the signals that were higher than a certain frequency?

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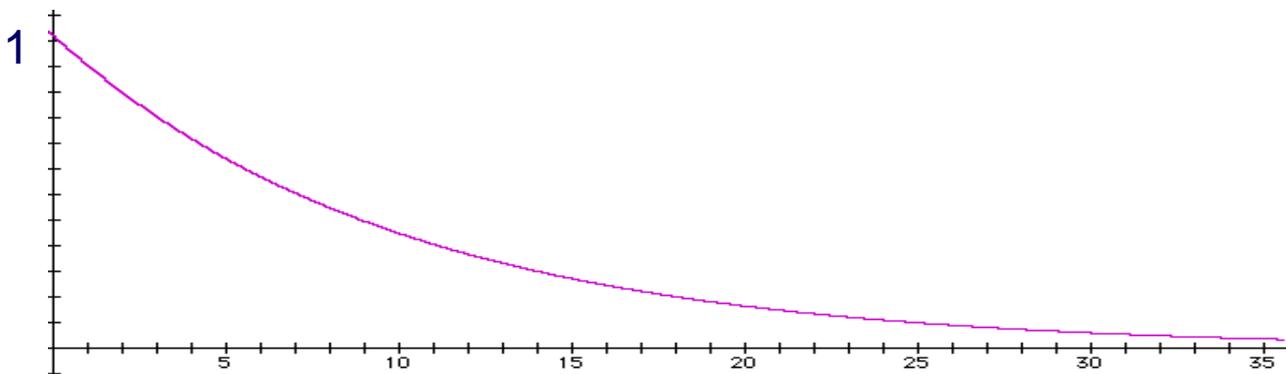
Window functions (continued)

- In this case, it is called **exponential multiplication**, and has the form:
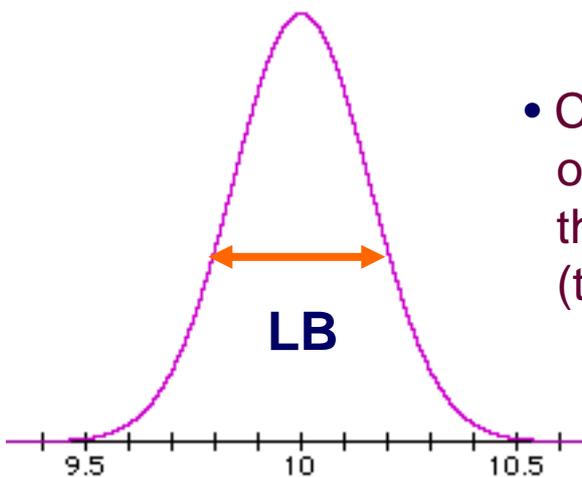
$$F(t) = 1 * e^{-(LB * t)} \quad - or - \quad F(t) = 1 * e^{-(t/\tau)}$$

- Why is it 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 (WAHH) proportional the decay rate, or **line broadening** (**LB**), in Hz.

LB

- 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
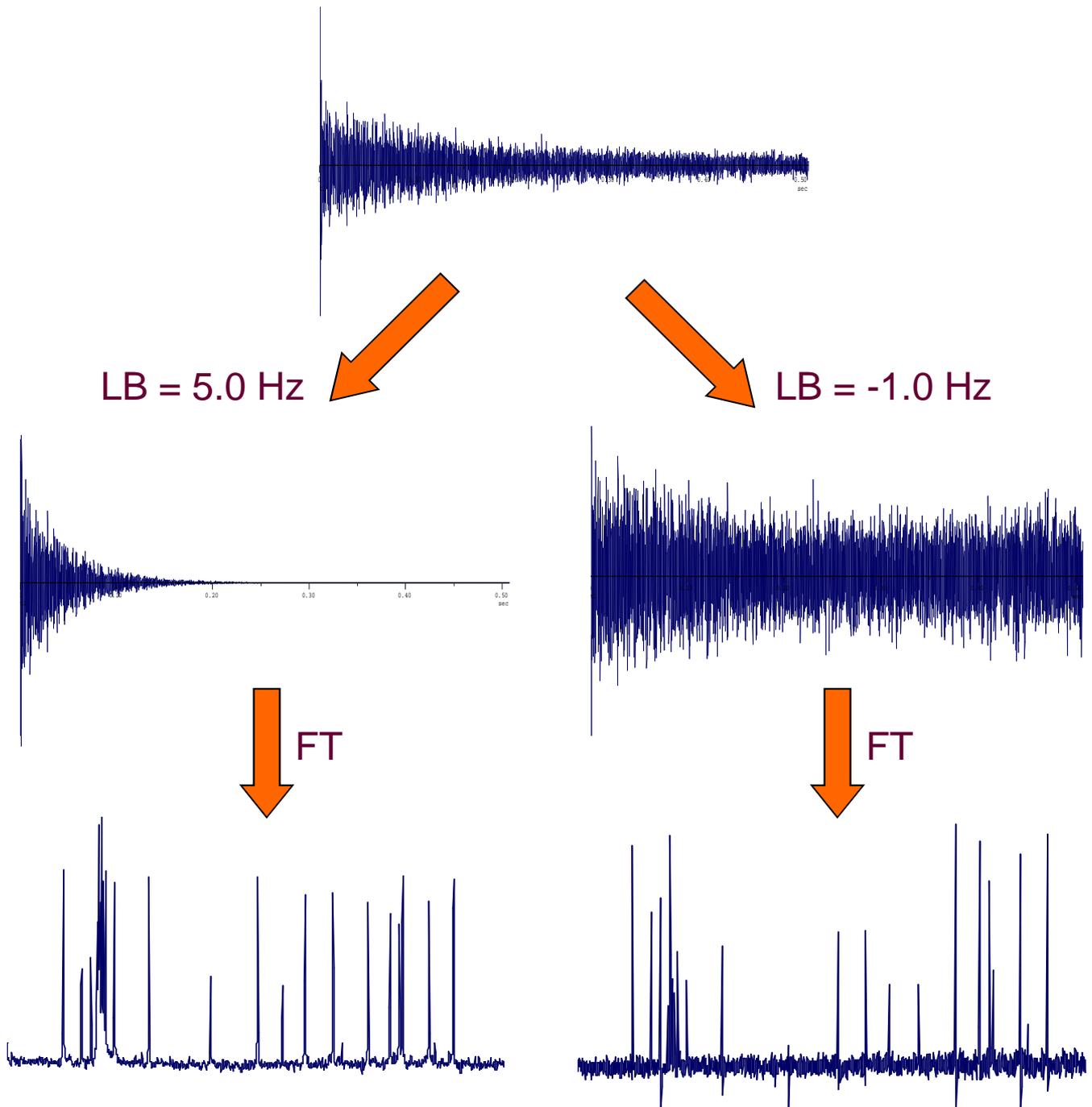
Other useful window functions

- Gaussian/Lorentzian: Improves resolution and does not screw up sensitivity as bad as resolution enhancement alone.
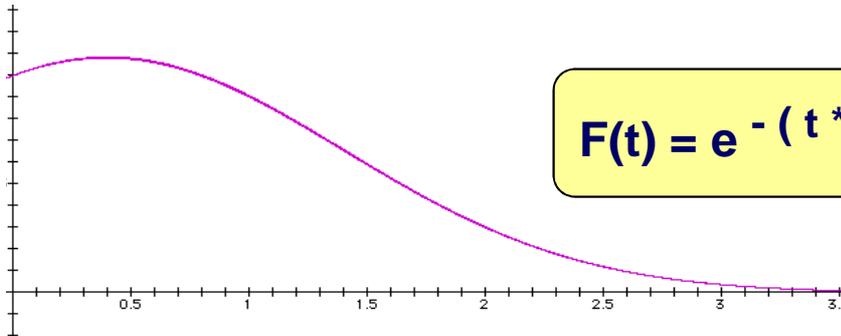
$$F(t) = e^{-(t * LB + \sigma^2 t^2 / 2)}$$

- Hanning: Another resolution/sensitivity enhancement combo.
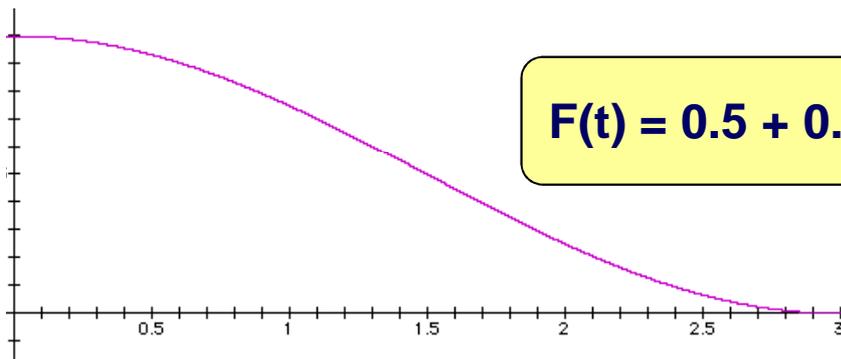
$$F(t) = 0.5 + 0.5 * \cos(\pi t / t_{max})$$

- Cosine/Sine: Employed mostly for 2D spectra.

$$F(t) = \cos(\pi t / t_{max})$$
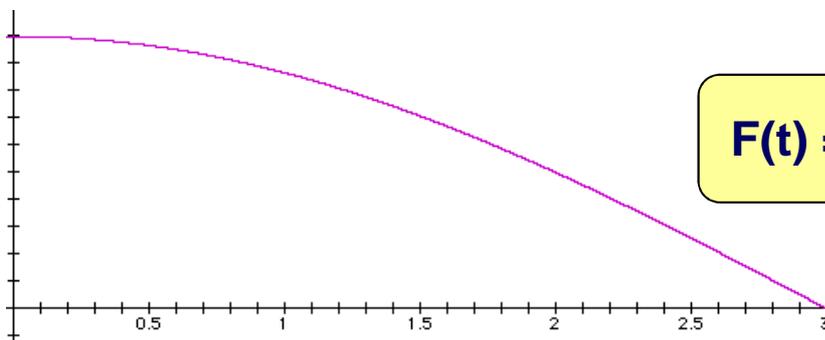
- The right window function depends on the experiment, and, as usual, there is a lot of fooling around involved...

Data size and zero-filling

- Another important consideration is the **data size** (**SI**, in bytes). Remember that it was related to the spectral width (sampling rate). It is also related to the time we will sample the FID. Longer sampling times means more data.

- In the good old days, memory, and thus the size of the data, was awfully scarce. Most machines would only allow 16K (16384) points to be taken, which meant that if we wanted good resolution, we could only sample for short periods.

- Even if we have plenty memory, more acquisition time limits the number of repetitions we can do in a certain period.

- We now define the **digital resolution** (**DR**) as the number of Hz per point in the FID for a given spectral width:

$$DR = SW / SI$$

DR - digital resolution (Hz/point)
SW - spectral width (Hz)
SI - data size (points)

- So, for a **SW** of 5 KHz and an FID of 16K, we have a digital resolution of 0.305 Hz/point.

- One obvious problem from this is that if we have a large **SW** and a small **SI**, our resolution may not be able to pick some of the line separations in our spectra.

Zero-filling (continued)

- Is there any way we can increase our digital resolution (I.e., the number of points) without having to acquire for longer times? The trick is called *zero-filling*.

- What we do is increase the number of data points prior to the FT by adding zeroes at the end of the FID. We usually add a power of 2 number of zeroes (8K, 16K, etc.).

8K data   8K zero-fill

8K FID   16K FID
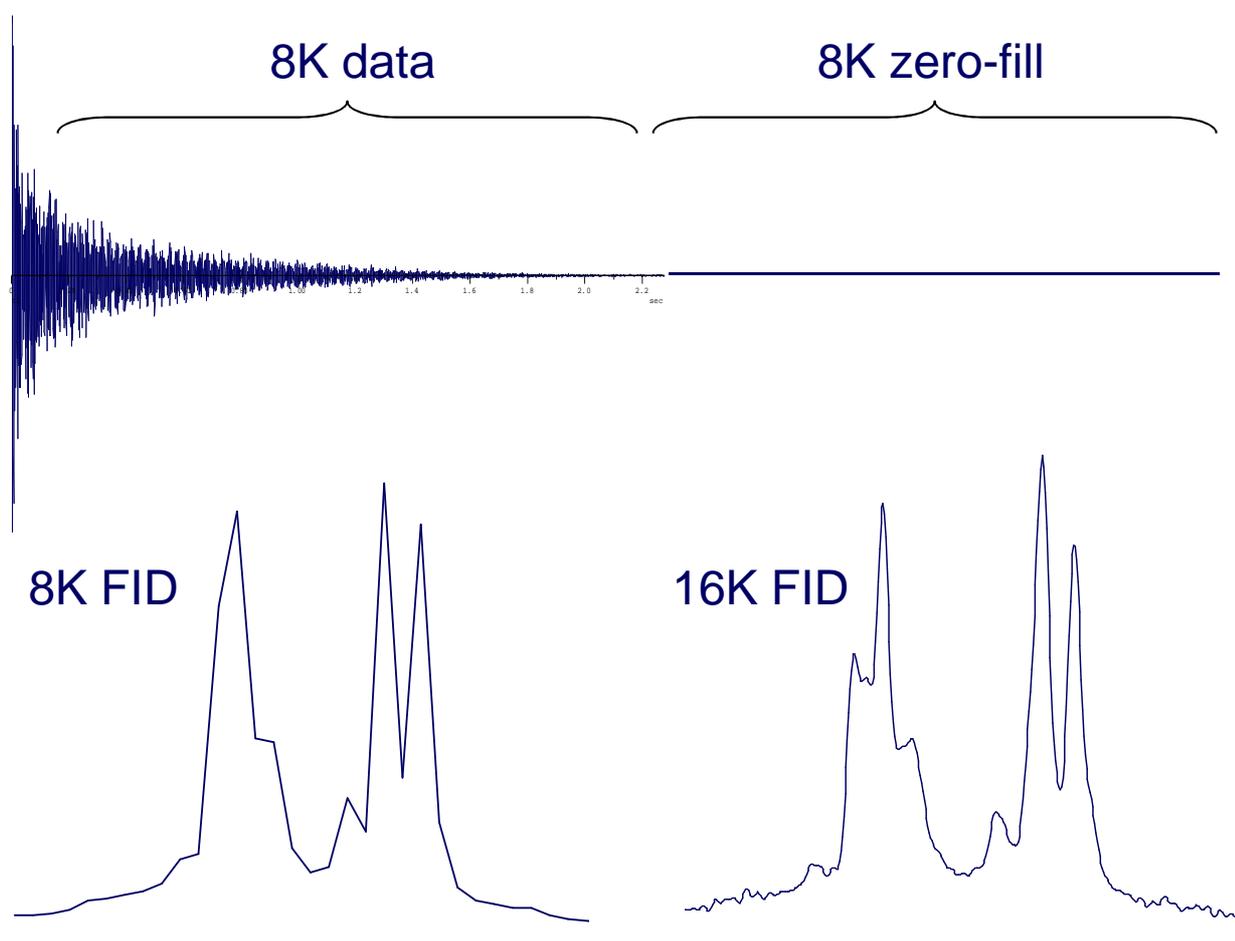
- In this way, we increase the points per Hz ratio, and can in many cases improve the spectrum. However, it does not help if we have really crappy data from the start (we don't get good resolution if we did not sample enough…).

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 (M_z).

  - Loss of energy in the system to the surroundings (lattice) as heat.
  - Dipolar coupling to other spins, interaction with paramagnetic particles, etc...
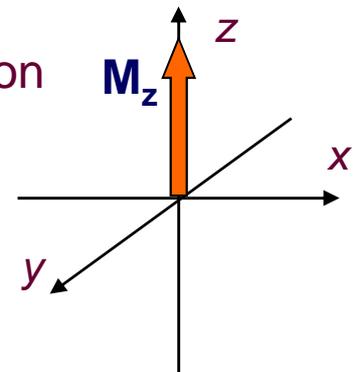
Transverse or Spin-Spin relaxation (T_2):

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

  - Spin-spin interactions (**J**) dephase M_{xy}
  - Also by imperfections in the magnet homogeneity (***fanning out***).
  - Cannot be bigger than T_1.
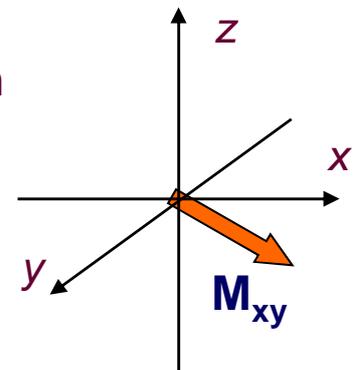
- In order to understand relaxation from a phenomenological point of view, we have to introduce the ***Bloch equations***, which describe the evolution of the spin system with time under the effects of magnetic fields as well as relaxation.

Bloch equations

• We know that the magnetic field interacts with magnetization (or the angular momentum) generating a torque that tips it. We usually deal with B_1 in the **<xy>** plane and M_o in the **z** axis. However, the Bloch equations are for any case, and describe variations of **M** with time:

$$dM_x(t) / dt = \gamma \left[M_y(t) * B_z - M_z(t) * B_y \right] - M_x(t) / T_2$$

$$dM_y(t) / dt = \gamma \left[M_z(t) * B_x - M_x(t) * B_z \right] - M_y(t) / T_2$$

$$dM_z(t) / dt = \gamma \left[M_x(t) * B_y - M_y(t) * B_x \right] - (M_z(t) - M_o) / T_1$$

• The γ appears because it's L (average angular momentum) which generates the torque. Without trying to understand very well were they come from, we can se that the variation of **M** in one axis depends on the other two.

• We'll analyze the solution for the differential equations for an ideal case in which we have magnetization on the **<xy>** plane exclusively (after a $\pi / 2$ pulse and a certain ω…):

$$M_x(t) = M_o * \cos(\omega_{eff} t) * e^{- t / T_2}$$

$$M_y(t) = M_o * \sin(\omega_{eff} t) * e^{- t / T_2} \quad - \quad \omega_{eff} = \omega_o - \omega$$

$$M_z(t) = M_o * (1 - e^{- t / T_1})$$

Bloch equations (continued)

- Graphically, we have the following:
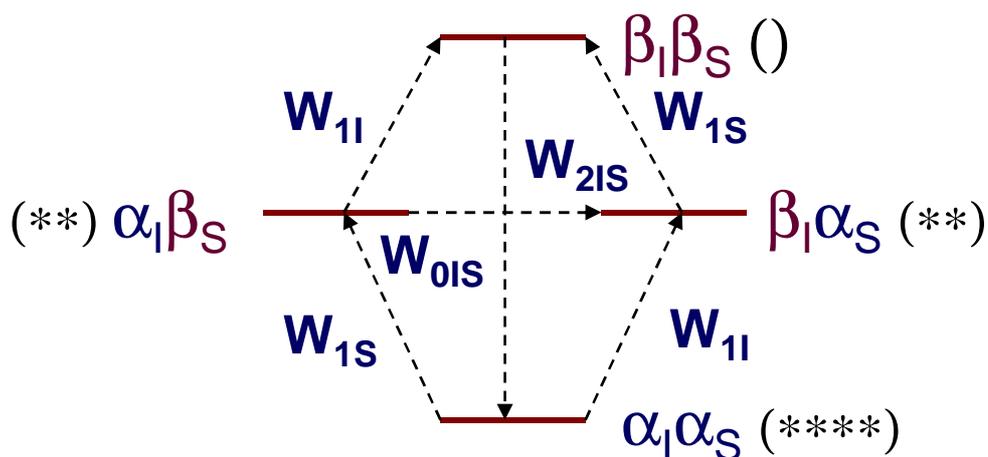
$$M_z(t) = M_o * \cos(\omega_{eff}t) * e^{-t/T_2}$$

$$M_y(t) = M_o * \sin(\omega_{eff}t) * e^{-t/T_2}$$

$$M_z(t) = M_o * (1 - e^{-t/T_1})$$
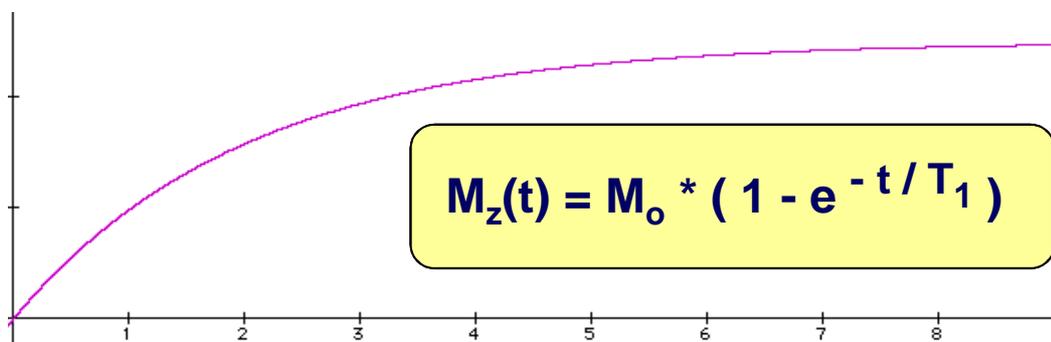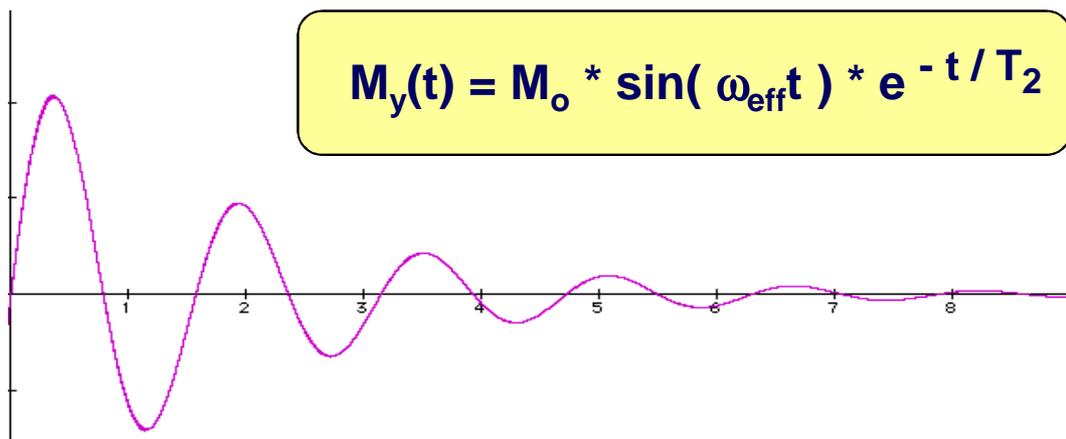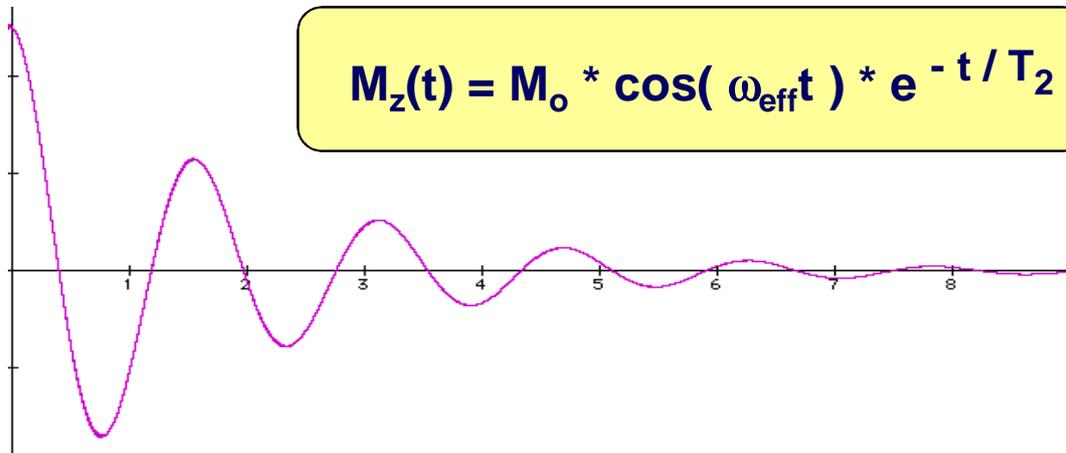
- From these equations, we can deduce that the best LB factor to use is $1/T_2$...

Nuclear Overhauser Effect (NOE)

- The **NOE** is one of the ways in which the system (a certain spin) can release energy. Therefore, it is profoundly related to relaxation processes. In particular, the NOE is related to exchange of energy between two spins that are not scalarly coupled ($J_{IS} = 0$), but have **dipolar coupling**.

- The NOE is evidenced by enhancement of certain signals in the spectrum when the equilibrium (or populations) of others nearby are altered. We use a two spin system energy diagram to explain it:

$$\beta_I \beta_S \ ()$$

$$W_{1I} \qquad W_{1S}$$

$$W_{2IS}$$

$$(**) \ \alpha_I \beta_S \qquad\qquad W_{0IS} \qquad\qquad \beta_I \alpha_S \ (**)$$

$$W_{1S} \qquad\qquad W_{1I}$$

$$\alpha_I \alpha_S \ (****)$$

- **W** represents a **transition probability**, or the rate at which a certain transition can take place. For the system in equilibrium we can have W_{1I} and W_{1S} transitions, which represents **single quantum** transitions.

- W_{0IS} and W_{2IS} are **zero** and **double quantum** transitions, are forbidden and have a much lower probability.

Nuclear Overhauser Effect (continued)

- The W_{1I} and W_{1S} transitions, are related to spin-lattice or longitudinal relaxation.

- Here we see that relaxation due to dipolar coupling takes place when the spins give away energy by processes that occur at frequencies close to $\omega = \gamma * B_o$, which include the movement (translation, rotation) and collision of spins.

- We now *saturate* the **S** transition, which means that we make both its energy levels equal. The populations of the **S** transitions are now the same:
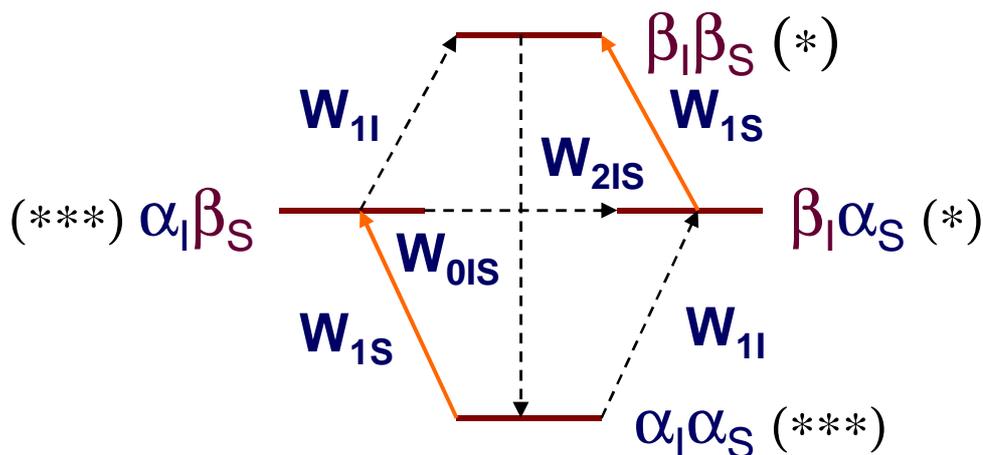
- The W_{1S} transitions are not possible (we have the same populations in these levels), and the W_{1I} is not happening (we have not affected the equilibrium for this spin). The W_{0IS} and W_{2IS} become the only way **S** can relax.

- These relaxation pathways for **S** also involve transitions of **I**, so thus the enhancement of this signal. W_{2IS} will give positive enhancement of **I**, and W_{0IS} will give negative enhancements.

Nuclear Overhauser Effect (even more…)

- We cannot detect W_{2IS} or W_{0IS}, but they affect the way the spin system relaxes. One has a rate close to twice ω, while the other one is almost zero. So one will be related to very slow motions, and the other one to fast tumbling...

- If we now put all this in a big equation (the ***Solomon equations***) we get something that will help us see several things. We have:

$$\eta = \gamma_I \, / \, \gamma_S * \frac{W_{2IS} - W_{0IS}}{2 * W_{1S} + W_{2IS} + W_{0IS}}$$

- First, if the molecule tumbles rapidly (all small organic gunk) we have that under saturation of the **I** transitions W_{2IS} will dominate, so the maximum enhancement for **S** is $\gamma_I \, / \, \gamma_S$. If we are looking at the ^{13}C signal while decoupling (saturating) ^1H, we get a theoretical enhancement of ~ 4.

- If the molecule tumbles slowly, as a protein, W_{0IS} dominates, and we have a maximum NOE of - $\gamma_I \, / \, \gamma_S$. Since here we are interested in ^1H - ^1H NOE, the theoretical enhancement will be ~ -1 (wishfull thinking…).

Nuclear Overhauser Effect (ugh…)

- This is all theory. There are other, competing, relaxation processes ocurring simultaneously.

- Also, the 'in the middle's' are not so clear cut, and we will not deal with them for the moment.

- It is useful to compare the frequency of the spin system to the molecular **tumbling rate** or **correlation time**, τ_c.

- $\omega * \tau_c << 1$ - This means that the molecule tumbles fast, and we have positive enhancements. It is called the **extreme narrowing condition** (small molecules, non-viscous solvents).

- $\omega * \tau_c >> 1$ - This means that the molecule tumbles slowly, and we have negative enhancements. It is called the **diffusion limit** (proteins, viscous solvents).

- $\omega * \tau_c \approx 1$ - These are the 'in the middle's,' and we can have situations in which the NOE goes to zero. It will happen for certain medium sized molecules and it depends on the base frequency of the NMR

- There is one things that we left out from our treatment, which is the dependence of the NOE with the distance between **I** and **S**. We will see this later in more detail.