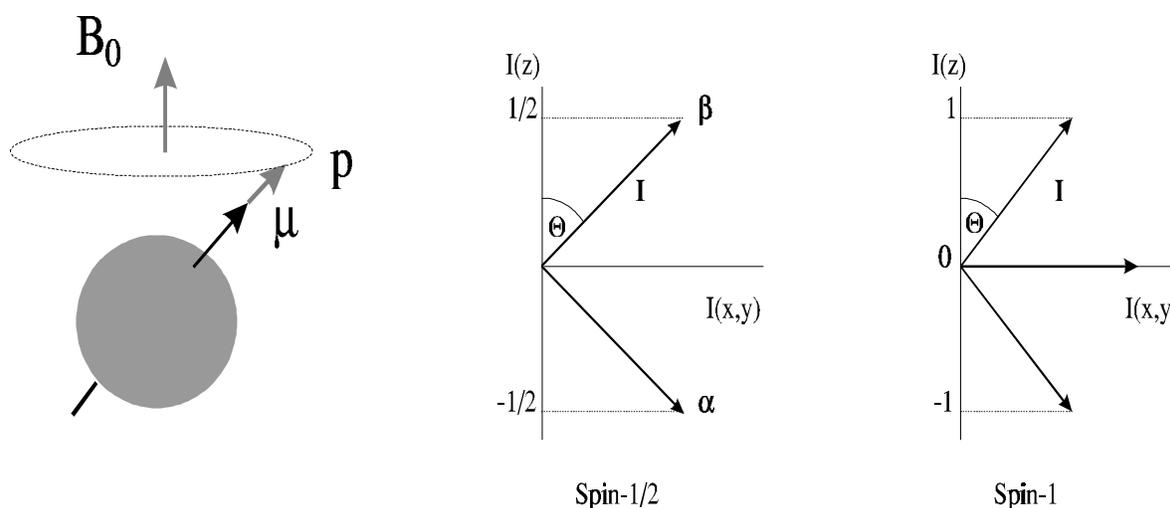


2 Basis Principles of FT NMR

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Nuclei in magnetic fields

Atomic nuclei are composed of nucleons, i.e., protons and neutrons. Each of these particles shows a property named "spin" (behaving like an angular momentum) that adds up to the total spin of the nucleus (which might be zero, due to pairwise cancellation). This spin interacts with an external magnetic field, comparable to a compass-needle in the Earth's magnetic field (for spin- $1/2$ nuclei).



left: gyroscope model of nuclear spin. Right: possible orientations for spin- $1/2$ and spin-1 nuclei in a homogeneous magnetic field, with an absolute value of $|I| = \sqrt{I(I+1)}$. Quantization of the z component I_z results in an angle Θ of 54.73° (spin- $1/2$) or 45° (spin-1) with respect to the z axis.

- in a magnetic field, both I and I_z are quantized
- therefore the nuclear spin can only be orientated in $(2I + 1)$ possible ways, with quantum number m_I ranging from $-I$ to I ($-I, -I+1, -I+2, \dots, I$)
- the most important nuclei in organic chemistry are the spin- $1/2$ isotopes ^1H , ^{13}C , ^{15}N , ^{19}F , and ^{31}P (with different isotopic abundance)
- as spin- $1/2$ nuclei they can assume two states in a magnetic field, α ($m_I = -1/2$) and β ($m_I = +1/2$)

Usually the direction of the static magnetic field is chosen as z axis, and the magnetic quantum number m_l often called m_z , since it describes the size of the spin's z component in units of $h/2\pi$:

$$I_z = m_z h/2\pi \quad [2-1],$$

resulting in a magnetic moment μ :

$$\mu = \gamma I \quad [2-2]$$

$$\mu_z = m_z \gamma h/2\pi \quad [2-3]$$

γ being the isotope-specific gyromagnetic / magnetogyric constant (ratio).

The interaction energy of a spin state described by m_z with a static magnetic field B_0 in z direction can then be described as:

$$E = -\mu B_0 = \mu B_0 \cos \Theta \quad [2-4]$$

$$E = \mu_z \gamma B_0 h/2\pi \quad [2-5]$$

For the two possible spin states of a spin- $1/2$ nucleus ($m_z = \pm 1/2$) the energies are

$$E_{1/2} = \frac{0.5ghB_0}{2p} \quad [2-6a]$$

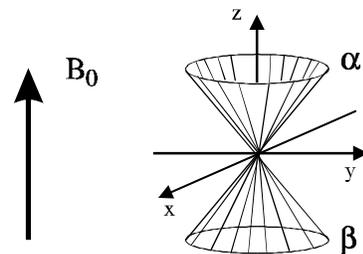
$$E_{-1/2} = \frac{-0.5ghB_0}{2p} \quad [2-6b]$$

The *energy difference* $\Delta E = E_{1/2} - E_{-1/2} = h\nu = \omega h/2\pi$ corresponds to the energy that can be absorbed or emitted by the system, described by the Larmor frequency ω :

$$\Delta E = \gamma B_0 h/2\pi \quad [2-7]$$

$$\boxed{\omega_0 = \gamma B_0} \quad [2-8]$$

The Larmor frequency can be understood as the precession frequency of the spins about the axis of the magnetic field B_0 , caused by the magnetic force acting on them and *trying* (I_z is quantized!) to turn them completely into the field's direction (like a toy gyroscope "feeling" the pull of gravity).

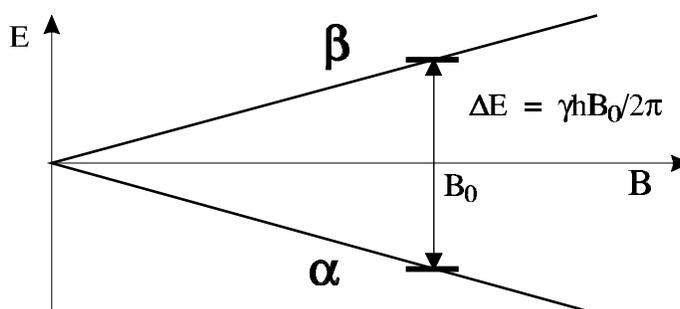


According to eq. 2-8, this frequency depends only on the magnetic field strength B_0 and the spin's gyromagnetic ratio γ . For a field strength of 11.7 T one finds the following resonance frequencies for the most important isotopes:

Isotope	γ (relative)	resonance frequency at 11.7 T	natural abundance	relative sensitivity*
^1H	100	500 MHz	99.98 %	1
^{13}C	25	125 MHz	1.1 %	10^{-5}
^{15}N	-10	50 MHz	0.37 %	10^{-7}
^{19}F	94	455 MHz	100 %	0.8
^{29}Si	-20	99 MHz	4.7 %	10^{-3}
^{31}P	40	203 MHz	100 %	0.07

- also taking into account typical linewidths and relaxation rates

The energy difference is proportional to the B_0 field strength:



How much energy can be absorbed by a large ensemble of spins (like our NMR sample) depends on the population difference between the α and β state (with equal population, rf irradiation causes the same number of spins to absorb and emit energy: no net effect observable!).

According to the BOLTZMANN equation

$$\frac{N(\mathbf{a})}{N(\mathbf{b})} = \exp \frac{\Delta E}{kT} = \exp \frac{g\hbar B_0}{2pkT} \quad [2-9]$$

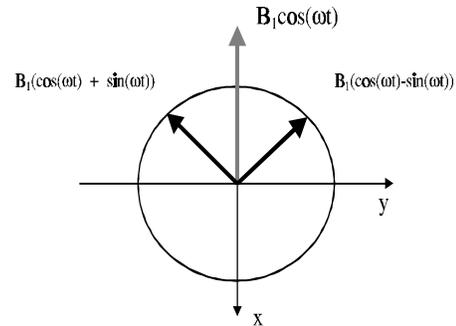
For 2.35 T (= 100 MHz) and 300 K one gets for ^1H a population difference $N(\alpha)-N(\beta)$ of ca. $8 \cdot 10^{-6}$, i.e., less than $1/1000$ % of the total number of spins in the sample!

Irradiation of an oscillating electromagnetic field

Absorption

Resonance condition: rf frequency has to match Larmor frequency
 = rf energy has to match energy difference between α and β level.

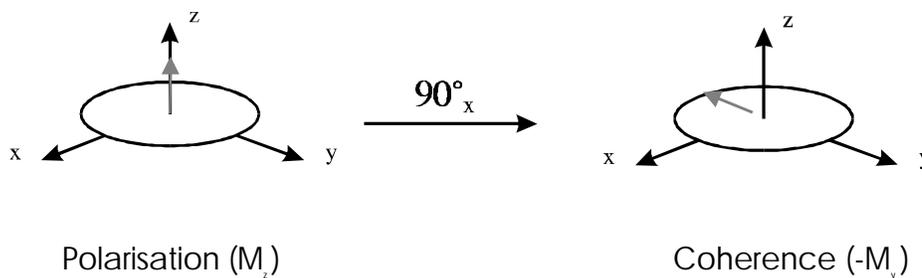
a linear oscillating field $B_1 \cos(\omega t)$ is identical to the sum of two counter-rotating components, one being exactly in resonance with the precessing spins.



Rotating coordinate system

Switching from the lab coordinate system to one rotating "on resonance" with the spins (and B_1) about the z axis results in both being static. Generally all vector descriptions, rf pulses etc. are using this *rotating coordinate system*!

Now the effect of an rf irradiation (a pulse) on the macroscopic (!) magnetization can be easily described (keeping in mind the gyroscopic nature of spins):



The flip angle β of the rf pulse depends on its field strength B_1 and duration τ :

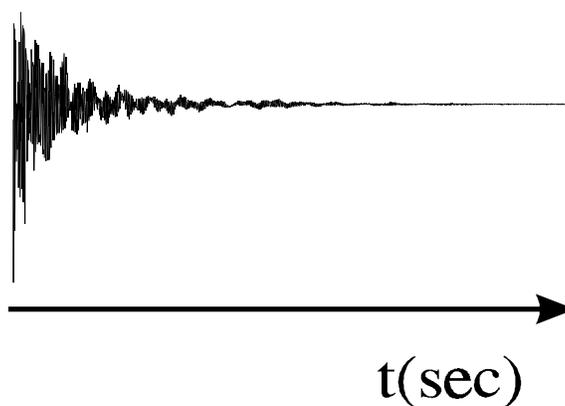
$$\beta = \gamma B_1 \tau_p \quad [2-11]$$

Being composed of individual nuclear *spins*, a *transverse* (in the x,y plane) macroscopic magnetization $M_{x,y}$ (*coherence*) starts precessing about the z axis with the Larmor frequency (in the lab coordinate system) under the influence of the static B_0 field, e.g., after a 90°_x pulse:

$$M_{-y}(t) = M_{-y} \cos(\omega t) + M_x \sin(\omega t) \quad [2-12]$$

thus inducing a voltage / current in the receiver coil (which is of course fixed in the probehead in a transverse orientation): the FID (free induction decay)

typical ^1H FID of a complex compound (cyclic hexapeptide)



According to eq. [2-12], the FID can be described as sine or cosine function, depending of its phase.

Relaxation

The excited state of coherence is driven back to BOLTZMANN equilibrium by two mechanisms:

1) spin-spin relaxation (transverse relaxation)

$$dM_{x,y} / dt = -M_{x,y} / T_2 \quad [2-13]$$

corresponds to a loss of phase coherence \Rightarrow magnetization is spread uniformly across the x,y plane: decay of net transverse magnetization / FID (entropic effect)

due to the B_0 field being not perfectly uniform for all spins (disturbance by the presence of other spins); this inherent T_2 relaxation is increased by experimental inhomogeneities (bad shim!): T_2^*

2) spin-lattice relaxation (longitudinal relaxation)

$$dM_z / dt = - \frac{M_z - M_0}{T_1} \quad [2-14]$$

due to the excited state's dissipation of *energy* into the "lattice", i.e., other degrees of freedom (molecular vibrations, rotations etc.), until the BOLTZMANN equilibrium is reached again (M_z).

In the BOLTZMANN equilibrium, all transverse magnetization must have also disappeared: $T_2 \leq T_1$; $T_1 = T_2$ for "small" molecules; however, T_1 can also be much longer than T_2 (important for "relaxation delay" between scans) !

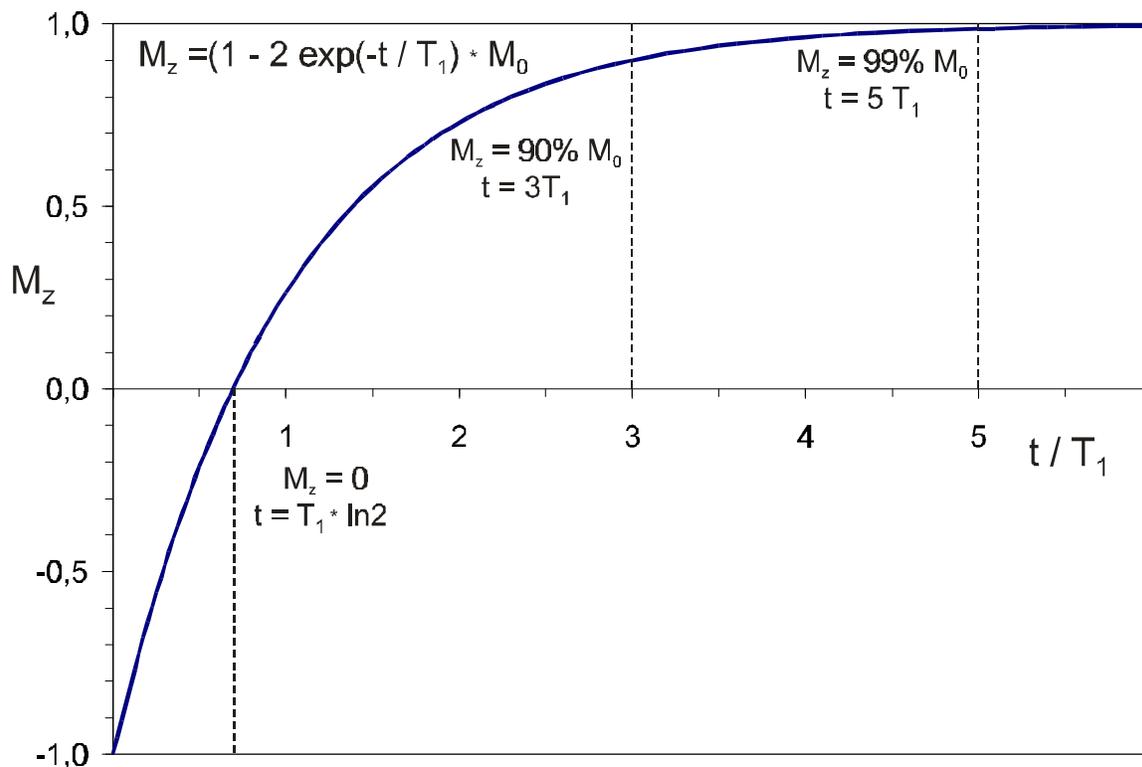
Measuring T_1 :

To avoid T_2 relaxation, the system must be brought out of BOLTZMANN equilibrium without creating $M_{x,y}$ magnetization: a 180° pulse converts M_z into M_{-z} , then T_1 relaxation can occur during a defined period τ . For detection of the signal, the remaining M_z / M_{-z} component is turned into the x,y plane by a 90° pulse and the signal intensity measured:

180° - τ - 90° -acquisition

inversion-recovery experiment

From integration of eq, [2-14], one gets zero signal intensity at time $\tau_0 = T_1 \ln 2 \approx 0.7 T_1$



T₂ and linewidth

Due to the characteristics of FT, the linewidth depends on the decay rate of the FID:

$$lw_{1/2} = \frac{1}{\pi T_2} \quad [2-15]$$

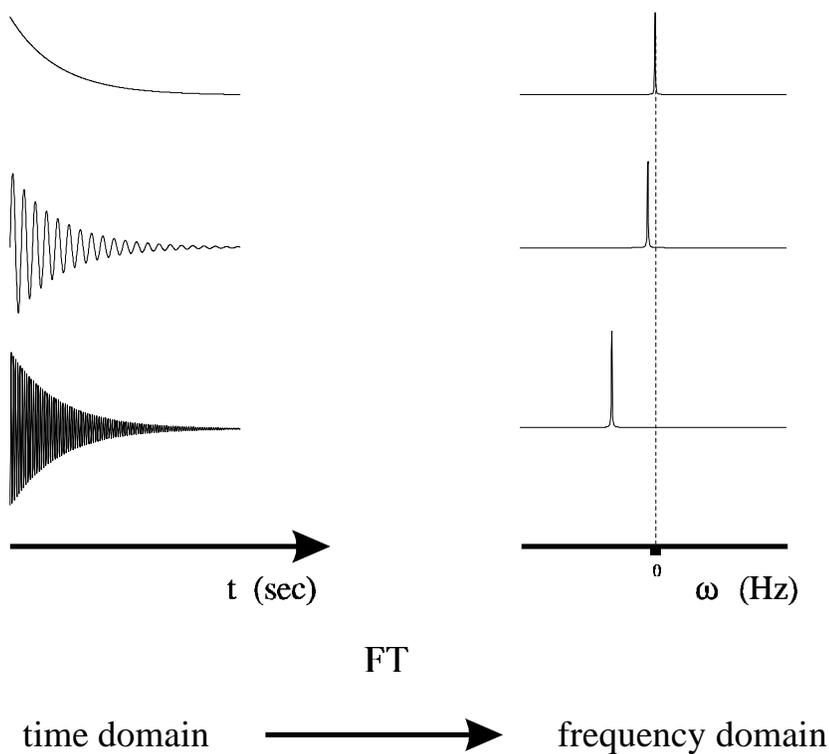
(for the linewidth at half-height)

The FID being a composed of exponentially decaying sine and cosine signals, eq. [2-12] should read

$$M_y(t) = \{M_y \cos(\omega t) + M_x \sin(\omega t)\} \exp(-t/T_2) \quad [2-16]$$

Chemical Shift

Resonance frequencies of the same isotopes in different molecular surroundings differ by several ppm (parts per million). For resonance frequencies in the 100 MHz range these differences can be up to a few 1000 Hz. After creating a $M_{x,y}$ coherence, each spin rotates with its own specific resonance frequency ω , slightly different from the B_1 transmitter (and receiver) frequency ω_0 . In the rotating coordinate system, this corresponds to a rotation with an offset frequency $\Omega = \omega - \omega_0$.



Sensitivity

The signal induced in the receiver coil depends

1. on the size of the polarisation M_z to be converted into $M_{x,y}$ coherence by a 90° pulse, which is

(from BOLTZMANN equation) $\propto \frac{g_{exc} B_0}{T}$

2. *and* on the signal induced in the receiver coil at detection, depending on the magnetic moment of the nucleus detected γ_{det} and its precession frequency $\omega = \gamma_{det} B_0$, *in summa*

$$S \propto \gamma_{det}^2 B_0$$

unfortunately the noise also grows with the frequency, i.e., $\sqrt{\gamma B_0}$.

The complete equation for sensitivity is thus

$$\boxed{S/N = n \gamma_{exc} \gamma_{det}^{3/2} B_0^{3/2} (NS)^{1/2} T^{-1} T_2} \quad [2-17]$$

n = number of nuclei in the sample, NS = number of scans acquired

Conclusions:

- importance of detecting the nucleus with the highest γ (i.e., ^1H), important in heteronuclear H,X correlation experiments: "inverse detection"
- double sample concentration gives double sensitivity, but to get the same result from longer measuring time, one needs *four times* the number of scans!
- sensitivity should increase at lower temperatures (larger polarisation), but lowering the temperature usually also reduces T_2 , leading to a loss of S/N due to larger linewidths.

Basic Fourier-Transform NMR Spectroscopy

In FT NMR (also called pulse-FT NMR) the signal is generated by a (90°) rf pulse and then picked up by the receiver coil as a decaying oscillation with the spins resonance frequency ω .

Generating an audio frequency signal

The rf signal (ω) from the receiver coil is "mixed" with an rf reference frequency ω_0 (usually the same used to drive the transmitter), resulting in an "audio signal" with frequencies $\Omega = \omega - \omega_0$. The "phase" of the receiver (x or y) is set electronically by using the appropriate phase for the reference frequency ω_0 (usually, a complex signal –i.e., the x and y component – is detected simultaneously by splitting the primary rf signal into two mixing stages with 90° phase shifted reference frequencies).

The Analog-Digital Converter (ADC)

For storage and processing the audio frequency signal has to be digitized first. There are two critical parameters involved:

1. dynamic range

describes how fine the amplitude resolution is that can be achieved; usually 12 bit or 16 bit. 16 bit corresponds to a resolution of $1:2^{15}$ (since the FID amplitudes will go from -2^{15} up to 2^{15}), meaning that features of the FID smaller than $1/32768$ of the maximum amplitude will be lost!

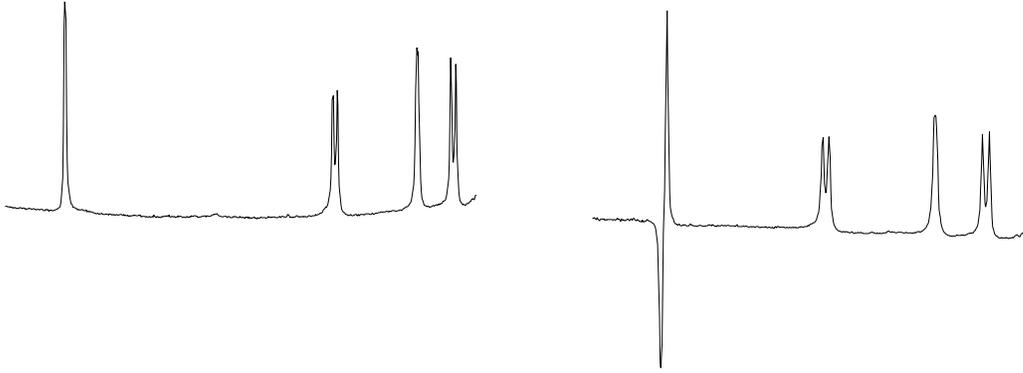
2. time resolution

corresponds to the minimum *dwell time* that is needed to digitize a single data point (by loading the voltage into a capacitor and comparing it to voltages within the chosen dynamic range). This needs longer for higher dynamic range, limiting the range of offset frequencies that can be properly detected (the sweep width SW).

High resolution spectrometers: dynamic range 16 bit, time resolution ca. $6 \mu\text{s}$ (= 133,333 Hz SW)

Solid state spectrometers: dynamic range 9 bit, time resolution ca. $1 \mu\text{s}$ (= 1,000,000 Hz SW)

NYQUIST frequency: the highest frequency that can be correctly detected from digitized data, corresponding to two (complex) data points per period. After FT, the spectral width will go from -NYQUIST freq. to +NYQUIST freq.. Signals with absolute offset frequencies ω larger than the NYQUIST freq. will appear at wrong places in the spectrum (folding):



Usually electronic band pass filters are set automatically to suppress signals (and noise!) from *far outside* the chosen spectral range. Really sharp edges are only possible with digital signal processing.

Characteristic for folded signals:

- out of phase (but: phase error varies!)
- due to the band pass filter, signal intensity decreases with offset (of the unfolded signal) beyond the spectral width
-

Fourier Transformation

All periodic functions (e.g., of time t) can be described as a sum of sine and cosine functions:

$$f(t) = a_0/2 + a_1\cos(t) + a_2\cos(2t) + a_3\cos(3t) + \dots \\ + b_1\sin(t) + b_2\sin(2t) + b_3\sin(3t) + \dots$$

The coefficients a_n and b_n can be calculated by FOURIER transformation:

$$F(\omega) = \int_{-\infty}^{+\infty} f(t)\exp\{i\omega t\}dt$$

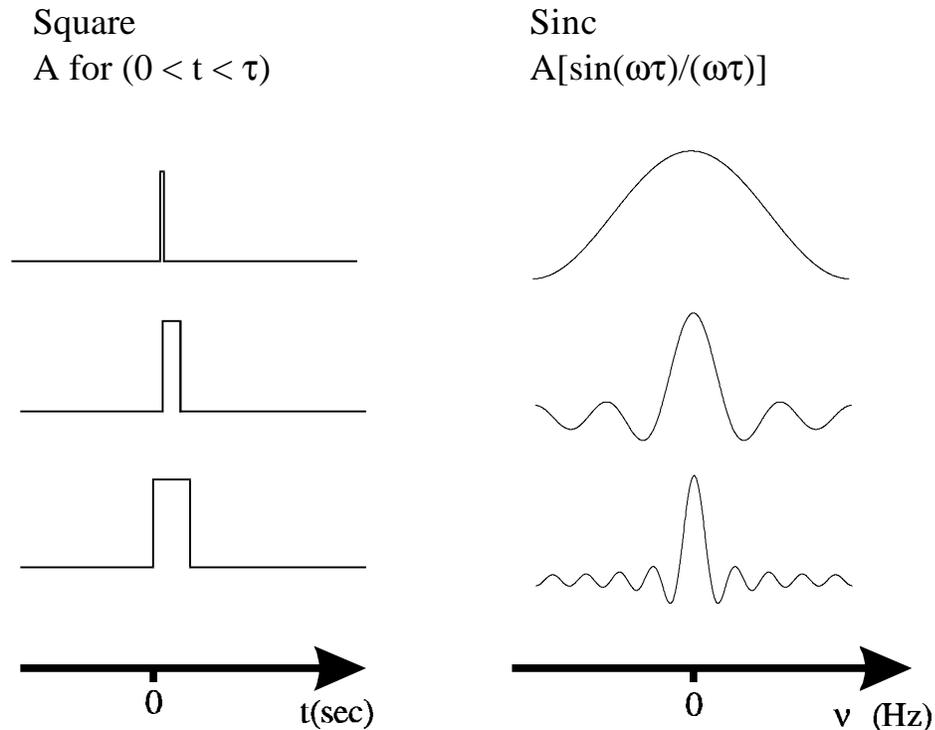
with $\exp\{i\omega t\} = \cos(\omega t) + i \sin(\omega t)$

$F(\omega)$ – the FOURIER transform of $f(t)$ – is a complex function that can be divided into a real and an imaginary part:

$$\operatorname{Re}(F(\omega)) = \int_{-\infty}^{+\infty} f(t)\cos(\omega t)dt \qquad \operatorname{Im}(F(\omega)) = \int_{-\infty}^{+\infty} f(t)\sin(\omega t)dt$$

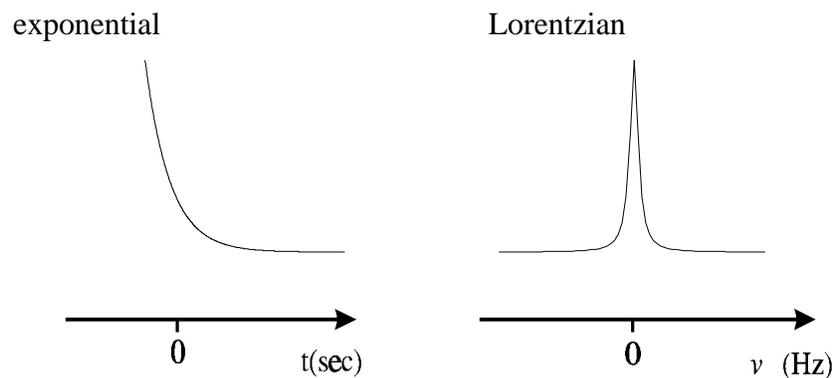
Let's look at some important FOURIER pairs, i.e., $f(t)$ and $F(\omega)$:

1) square function — sinc function



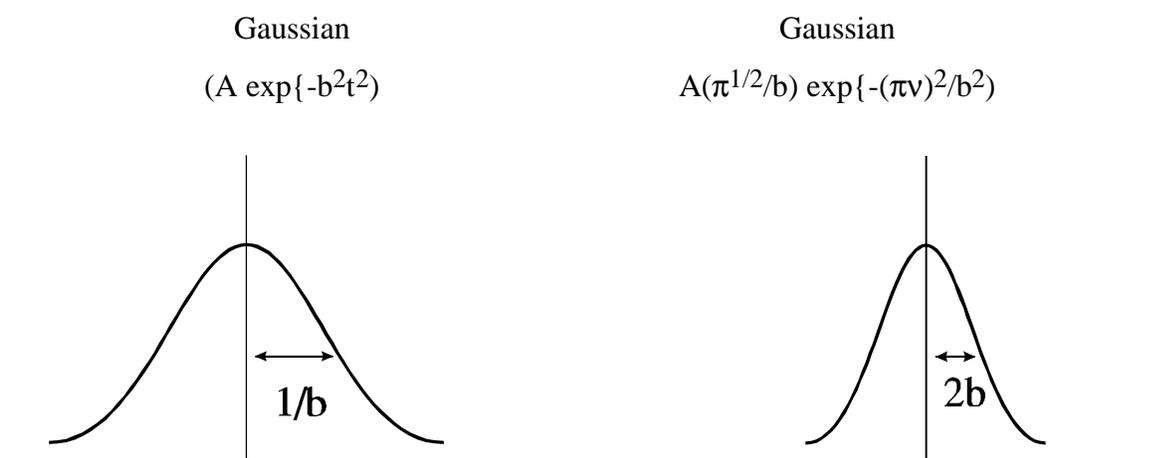
Since normal rf pulses are square shaped (in the time domain), their excitation profile (in the frequency domain) is given by its FOURIER transform, the sinc function (approximation for $\beta \ll 180^\circ$). The excitation band width is proportional to the *reciprocal* of the pulse duration, pulses must be short enough to keep the "wiggles" outside the range of interest.

2) exponential function — Lorentzian function



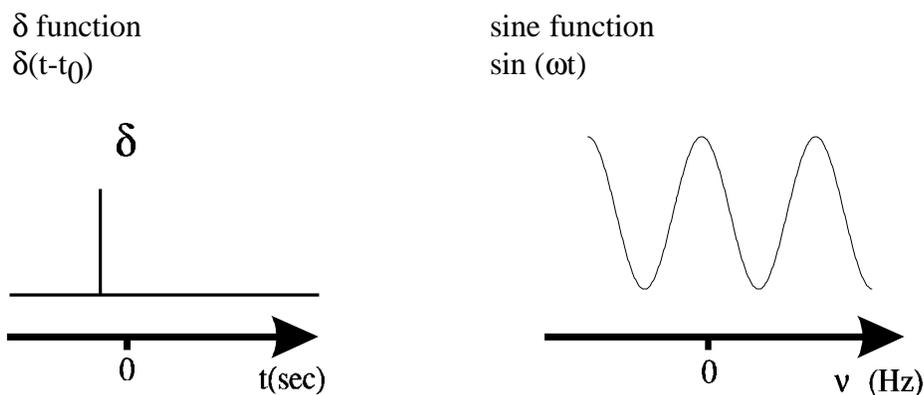
Since all signals are supposed to decay exponentially in time, the Lorentzian is the "natural" line shape in the (frequency) spectrum; the faster the exponential decay, the broader the Lorentzian.

3) Gaussian function — Gaussian function



FOURIER transform of a Gaussian is another Gaussian, the widths of both again show a reciprocal relationship

4) δ function — sine (or cosine) function / $\exp\{-i\omega t\}$



the extreme case of an infinitely narrow (and intense) square function; also: a sine function as FID (no relaxation) corresponds to an infinitely sharp line in the spectrum.

Important properties of FOURIER transformation

- the two functions of a FOURIER transform pair can be converted into each other by FT oder inverse FT (same algorithm, just sign flip from $i\omega t$ to $-i\omega t$),
- FT and iFT are linear operations, i.e.:

$$A f(t) \xrightarrow{FT} A F(\omega) \quad (A = \text{complex constant})$$

$$f(t) + g(t) \xrightarrow{FT} F(\omega) + G(\omega)$$

- broadening in one dimension leads to narrowing in the FT dimension:

$$f(A t) \xrightarrow{FT} \frac{1}{A} F(\omega/A)$$

- a time shift of the FID leads to a phase twist ϕ of the spectrum:

$$f(t - \tau) \xrightarrow{FT} F(\omega) \exp(-i\phi t)$$

- a convolution (= changing all lineshapes) in the frequency domain can be easily done by multiplying the time domain signal with the FOURIER transform of the desired lineshape (= the apodization function $g(t)$) prior to FT:

$$f(t) g(t) \xrightarrow{FT} \int_{-\infty}^{+\infty} F(\omega) G(\omega - \omega_k) d\omega_k$$

- the signal integral in the spectrum corresponds to the amplitude of the FID at $t=0$, meaning that apodization of the FID prior to FT affects all signal integrals in the same way (by a factor of $g(t=0)$), so that relative signal integrals (not signal heights!!!) do not change.
- also a non-zero integral in the FID (i.e., a DC offset caused by the electronic amplifiers) results in a non-zero "signal" at $\Omega=0$ Hz, i.e., a spike at the transmitter / receiver reference frequency ω_0 .

In NMR we are further limited by

- a) not knowing $f(t)$ from $-\infty$ to ∞ , but only from 0 to AQ (= length of acquisition time)
- b) not acquiring a continuous, but a digitized FID signal, with values known only for $t = n \Delta t$

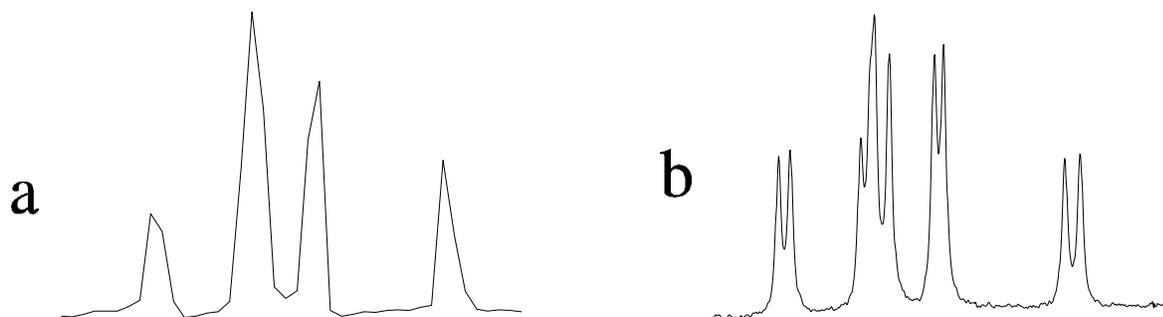
As a result, we have to perform a discrete FT, with a sum instead of an integral (cf. p. 16), and we also get a discrete spectrum as the result, with data points in small frequency steps (the digital resolution), AND we only get a spectrum with a limited spectral width (SW).

$$SW = 1/DW \qquad \text{digital spectral resolution} = 1/AQ$$

With the (very time-consuming!) original algorithm, a FT can be calculated on any number of data points in the FID. Data processing usually relies on the much faster Fast FOURIER Transform (FFT) = COOLEY-TUKEY algorithm, which can only convert 2^n data points in the FID into 2^n spectral data points. Missing points in the FID are usually filled up to the next power of 2 with "zero" points.

Zerofilling

Since *zerofilling* increases the apparent length of the FID (= "AQ"), this results in a higher digital resolution in the spectrum! This does not increase the information content of the FID, only the way the spectral information is distributed between the real and imaginary parts of the spectrum. Therefore a real gain in resolution is limited to zerofilling up to 2 AQ (in theory) or ca. 4 AQ (*in praxi*). In this case one really gets more information in the spectrum, e.g., multiplett patterns that were not visible without zerofilling.



a: without zerofilling; b: with zerofilling up to 4 AQ.

Apodization

The exponential decay of the FID results in a Lorentzian as the natural lineshape. By multiplying the FID with a window function the spectral lineshapes can be changed (=convoluted) to wide variety of other shapes, affecting *spectral* resolution as well as signal-to-noise (usually in opposite directions).

Linewidth is related to the speed of decay (T_2^*). Multiplication of the FID with a function increasing with time mimicks a slower relaxation = narrower lines. HOWEVER, since now the weight of the last parts of the FID (with relatively low S/N) is increased relative to the first part of the FID (with

high S/N), the S/N of the resulting spectrum will deteriorate! Getting rid of the noisy part of the FID (by multiplying it with a decaying window function) results in better S/N, but also in broader lines due to "faster relaxation".

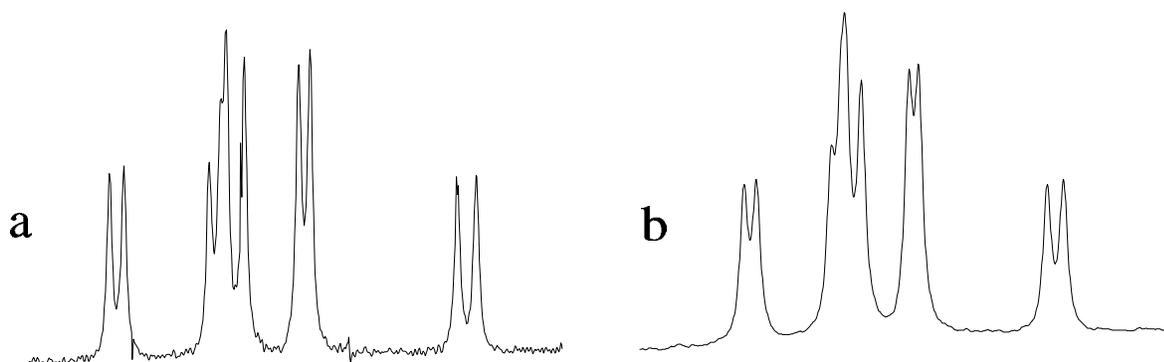
shape of the FID:

$$S = A \cdot \exp\{-t/T_2\}$$

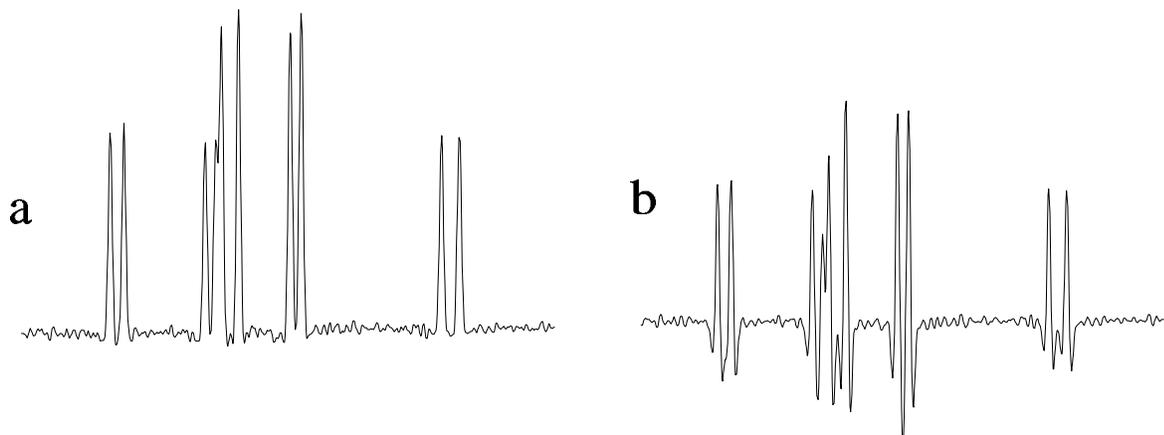
after exponential multiplication:

$$\begin{aligned} S &= A \exp\{-t/T_2\} \exp\{-at\} \\ &= A \exp\{-t(a + 1/T_2)\} \end{aligned}$$

matched filter : $a = 1/T_2$; doubles the linewidth and is supposed to be a good compromise, without excessive line broadening (often used in ^{13}C NMR).



Application of the matched filters; a: without window function, b: with matched filter.



Lorentzian-to-Gaussian transformation for resolution enhancement; a: perfect parameter setting results in baseline separation of the signals; b: oops - that was too much!

A widely used method for resolution enhancement (with concomitant loss of S/N!) is the Lorentzian-to-Gaussian transformation:

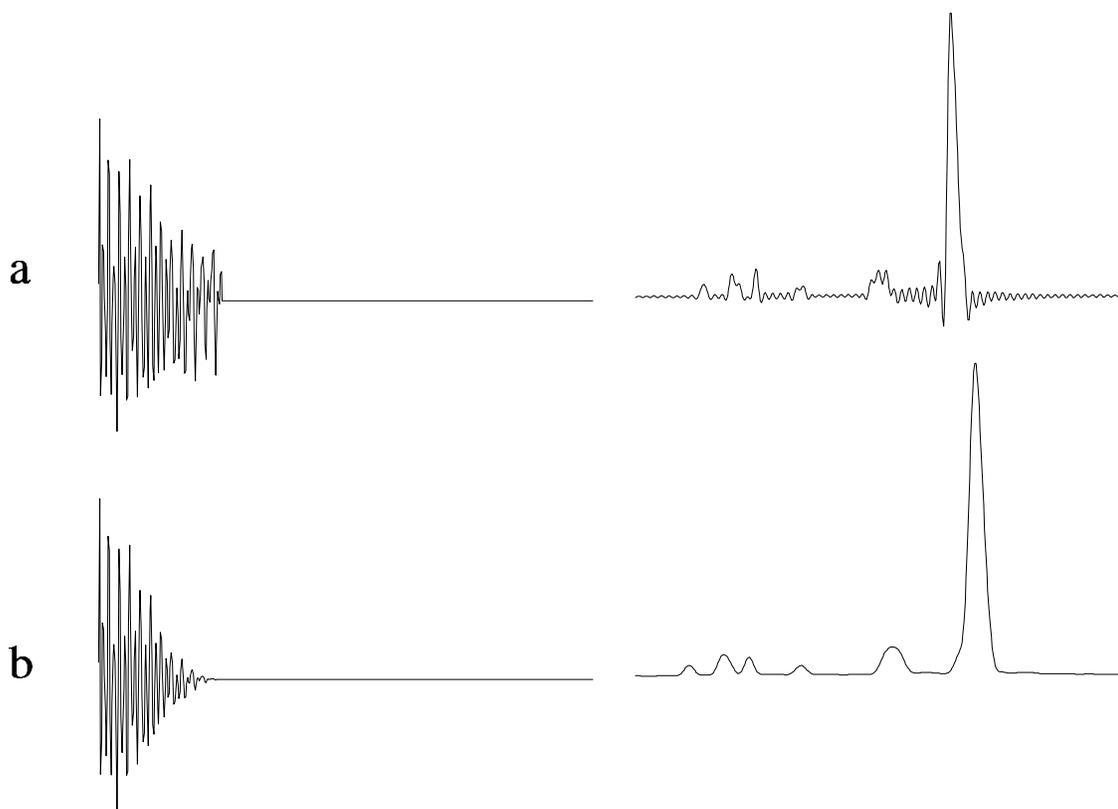
$$S = A \cdot \exp\{-t/T_2\} \exp\{-at\} \exp\{-bt^2\} \quad [2-19]$$

With $a = -1/T_2$ and $b > 0$ the exponential decay is replaced by one leading to a Gaussian lineshape: the narrower basis yields better line separation. Usually the maximum of the Gaussian window function is also shifted from $t=0$ towards a later point in time, thus reducing the apparent decay rate and leading to narrower lines (and reduced S/N).

Truncation

If zerofilling is applied to a *truncated* FID that has not decayed to zero at the end, the result will be the multiplication of a perfect FID with a step function. After FT, all signals in the spectrum will be convoluted with the step function's FOURIER transform, i.e., all lineshapes will contain *sinc* wiggles.

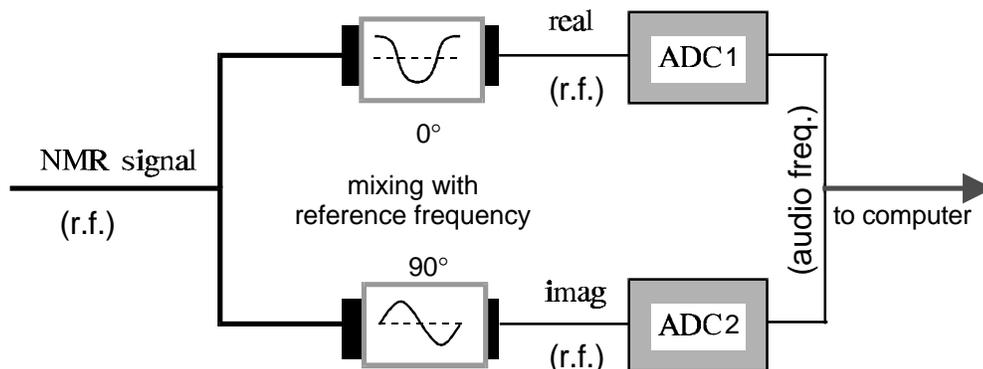
To avoid wiggles, the FID has to be brought to zero before zerofilling, usually by applying an appropriate window function (alternative: linear prediction).



a: truncated FID and resulting spectrum, each signal line is "convoluted" with a sinc function, resulting in very annoying "wiggles", esp. from the more intense signals. b: properly apodized FID (with an exponential function) and its FOURIER transform, linewidths are somewhat larger than in (a) due to the choice of window function.

Quadrature detection

The spectral range is limited by \pm NYQUIST frequency. After mixing with ω_0 to get the audio frequency signal, only the frequency offset Ω relative to ω_0 is retained. With complex data, the sign of Ω is readily obtained from the complex FT (STATES-HABERKORN-RUBEN or *echo-antiecho* in 2D).



However, if the spectrometer's receiver cannot digitize the two (x and y) components of the FID simultaneously, then only a real FID is obtained. It still contains the absolute values of Ω , but not their sign, so that the resulting spectrum is symmetric with respect to ω_0 .

How to get a normal spectrum without mirror peaks from a real FID / the REDFIELD trick

1. Set the transmitter frequency to the center of desired spectral range (for best excitation).
2. Acquire data points at twice the rate required by the desired spectral range, i.e., at

$$\Delta t = \frac{DW}{2} = \frac{1}{2SW}$$

This gives you – after FT – twice the desired spectral width.

3. Don't acquire the data points with a constant phase, but with a 90° phase shift between subsequent points: (i.e., $x, y, -x, -y, x, \dots$).

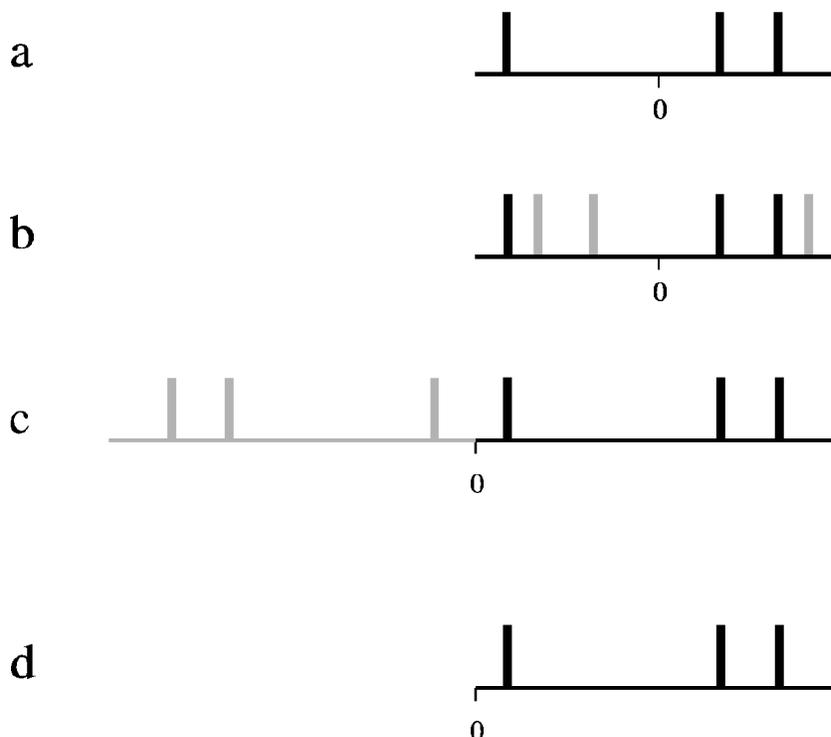
A 90° shift every $\frac{1}{2SW}$, i.e., 360° in $\frac{2}{SW}$, corresponds to an apparent rotation of the receiver coil with a frequency of $\frac{SW}{2}$ (in addition to ω_0). This is identical to shifting the receiver reference frequency by $\frac{SW}{2}$, to the edge of the desired SW.

4. Due to the real FT, the spectrum appears symmetric to the apparent receiver frequency, but the half outside the chosen spectral range is just discarded.

The same principle occurs in 2D (3D, ...) NMR as time proportional phase incrementation, TPPI.

Implications:

- Artifacts occurring at ω_0 (axial peaks, DC offset) appear at the edge of the spectrum.
- Folded signals fold into the *full* ($2*SW$) spectrum and appear as mirror images in the "half spectrum" kept after the real FT, apparently they "fold in from the edge closer to them".



(a) the "correct" spectrum; (b) spectrum obtained from a real FID, containing mirror images of all peaks; (c) again mirror images from a real FID, but with twice the sampling rate (=double SW) and REDFIELD trick to shift apparent receiver reference frequency; (d) after discarding the left half of *c*.

Signal phases and phase correction

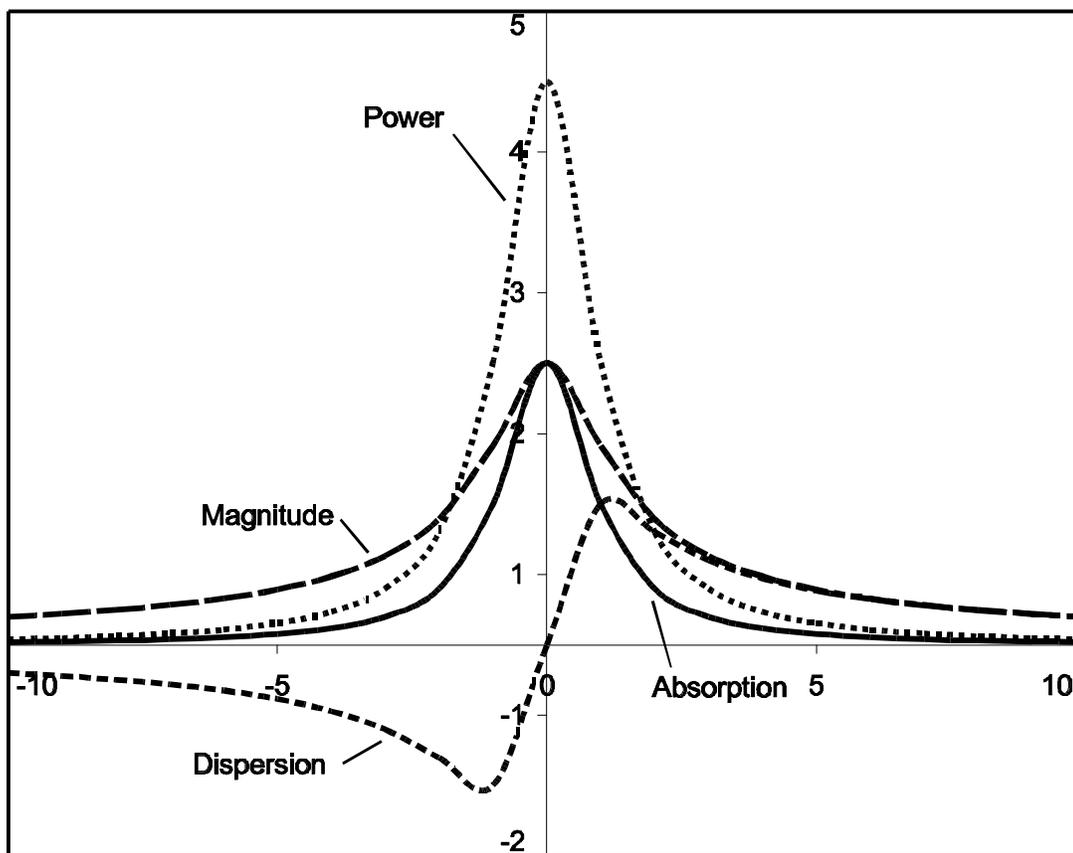
NMR lines consist of an absorptive (A) and a dispersive (D) component. The real part *Re* of the spectrum (displayed on screen / plot) and the imaginary part *Im* (hidden on the hard drive) are usually linear combinations of both, so that another linear combination (phase correction) is necessary to make the real part purely absorptive:

$$\text{Re} = A \cos(\phi) + D \sin(\phi)$$

$$\phi = \text{const.} \quad \text{for zero order correction}$$

$$\text{Im} = A \sin(\phi) - D \cos(\phi)$$

$$\phi = k \Omega t \quad \text{for linear (first order) correction}$$



Comparison of absorption, dispersion, magnitude and power mode lineshape (for a Lorentzian line)

$$\text{Magnitude / absolute value mode} \quad \sqrt{(\text{Re}^2 + \text{Im}^2)}$$

$$\text{Power mode} \quad (\text{Re}^2 + \text{Im}^2)$$

Absolute value mode spectra must not be integrated! Due to the very slow drop-off of signal amplitude, the integrals do *not* converge (i.e., infinite integral contribution from a signal's tails) and are completely meaningless.

Power spectra may be integrated; however, the signal integrals correspond to the *square* of the number of protons involved.

Absolute value (and also power spectra) show a very unpleasant "natural" lineshape, derived from the broad dispersive component. The best way to improve resolution (at the cost of S/N) is probably a "pseudo-echo" window function, i.e., the first half period of a sine or \sin^2 function (starting and ending with zero).