Lecture notes Chem 781 - PART 2:

Experimental details of excitation and acquisition:

- show schematics of NMR spectrometer

Major components:

- **Magnet:** generates static B_0 field (see scheme). Also contains sample transport, spin assembly and shim coils.
- **Probe:** transmits radio frequency field and receives NMR signal. Typically solution probe contains two coils, one for 1H/19F and 2H (lock), and one broadband tunable from 31P downwards. Also standard on modern probes are gradient coils to apply a linear field gradient for controlled periods of time. Also specialty probes like solid state probes available.
- **Console:** RF-electronics, generate and control frequencies, pulse control, amplification and digitization of signal, acquisition control
- **Computer (workstation):** user interface, post acquisition processing

Signal detection:

The x,y magnitization osscilating at ν_0 will induce a voltage in the receiver coil. Note that ν_0 is in the laboratory frame, hence ossciliation is in MHz range.

Preamplifier: The signal is amplified such that ADC is fully utilized, but not overloaded (see below).

Mixer: Transmitter frequency ω_{RF} is subtracted from signal. That is equivalent to observing the signal in the rotating frame: Only the offset oscillation with respect to the carrier (Ω) is digitized and analyzed, which is only in the order of Hz or kHz rather the initial MHz signal. The principle is identical to a radio where subtraction of the MHz carrier signal leaves the audio signal only.

Analog-digital converter (ADC:)

The analog audio signal (voltage) is converted into discrete data points, each representing a number according to its intensity. One obtains TD points (TD = time domain data points).

- **Vertical resolution (dynamic range):** only a limited number of bits will be available: maximum and minimum value which can be digitized. For example, a 16 bit ADC can take values from -32767 to 32767 ($\pm[2^{15}-1]$). Any signal larger than the maximum number will be cut off at the top. If the signal is too small weak signals in the presence of a strong one will not be digitized at all since any number smaller than "1" will be represented as "0".

  Example: 1mM sample in H_2O $M_z/M_z(H_2O) = c/(2 \cdot c_{H2O}) = (1 \cdot 10^{-3}\ mol\ l^{-1})/(2 \cdot 55\ mol\ l^{-1})$

  $\approx 1 \cdot 10^{-5} = 1{:}100{,}000$

  Deuterated solvents or solvent suppression techniques are required to properly detect signal in dilute samples.

  On modern spectrometers, the optimization of the signal intensity can be performed automatically, on the Bruker DPX300 the command is **rga**

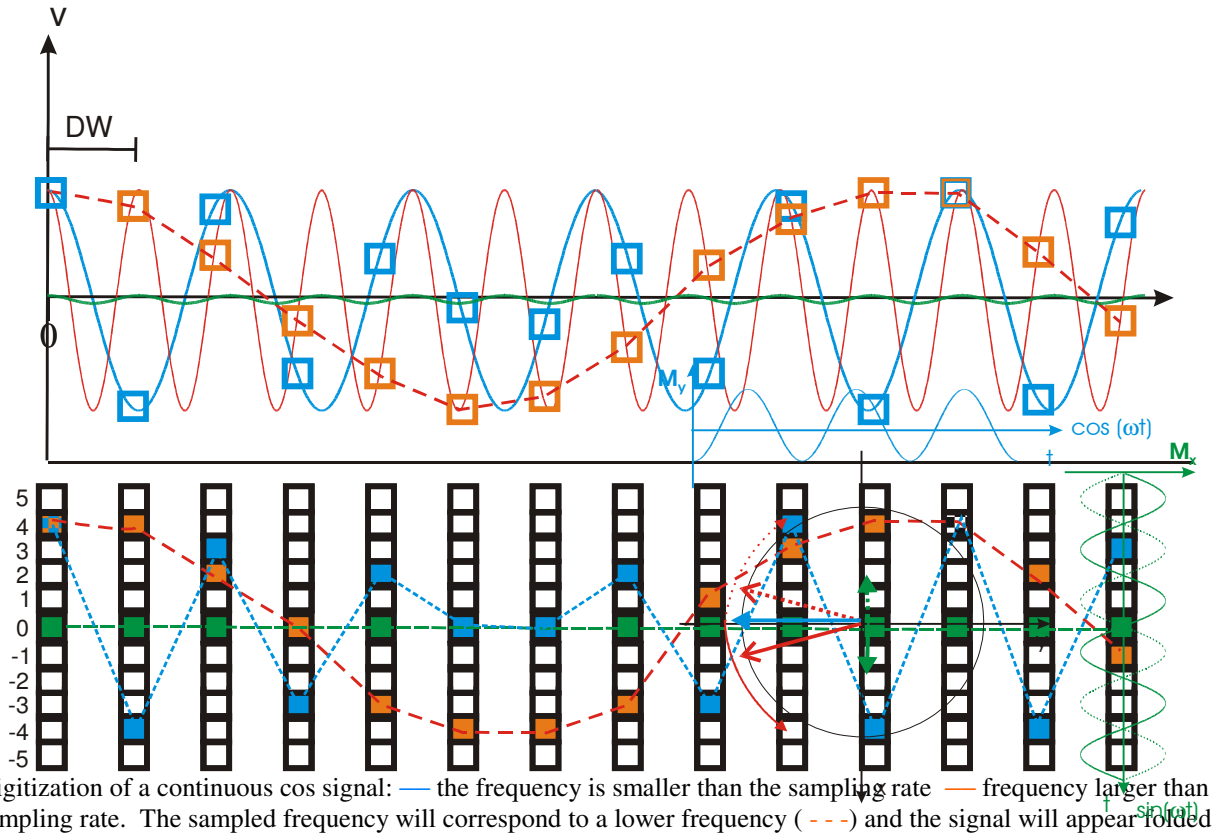
- **Sampling rate:** The sampling rate is determined by maximum frequency to be digitized. In general, a sine function needs least 2 data points per period. That results in a *dwell time* of the receiver of

$$DW = 2\pi/(2\cdot\Omega_{max}) = 1/(2\cdot SWH) \qquad \textbf{(2.1)}$$

  SWH is the spectral width measured in Hz set on the spectrometer. Normally the operator sets this value with the parameter *SW* which is measured in ppm rather Hz. The time required to accumulate *TD* data points is then

$$AQ = TD\cdot DW = TD/(2\cdot SWH) \qquad \textbf{(2.2)}$$

  For a typical 1D ^1H NMR spectrum (2^{15} = 32,768 points with a spectral width of 20 ppm = 6000 Hz at 300 MHz) one obtains AQ = 2.7 s.



Digitization of a continuous cos signal: — the frequency is smaller than the sampling rate — frequency larger than sampling rate. The sampled frequency will correspond to a lower frequency (- - -) and the signal will appear folded into the window. Limited word length of the receiver will also prevent small signals to be acquired in the presence of large ones

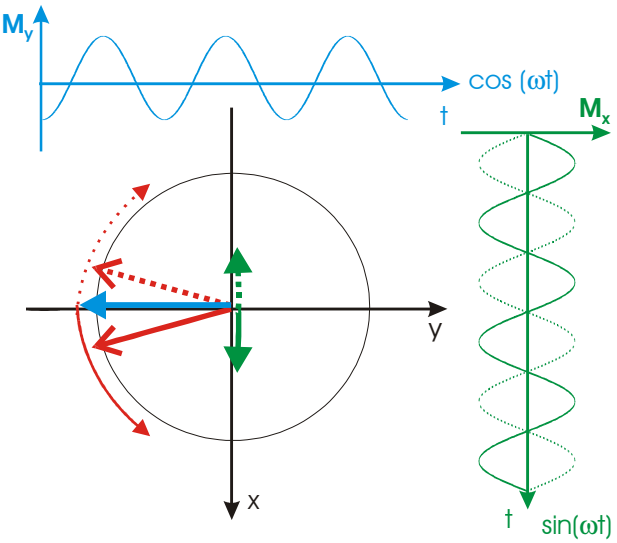
If the frequency becomes larger than the required sampling rate (i.e. if there are signals outside the selected window) the signal will still be detected, but digitized with the wrong frequency and would appear as a "folded" peak inside the window. On newer spectrometers, digital filters allow the removal of these peaks during digitization, however the problem persists in the indirect dimension of two dimensional spectra.

Quadrature detection:

After subtraction of carrier frequency ν_{RF} the sign of rotation Ω can be positive (faster than ν_{RF}) or negative (slower than ν_{RF}). One detector measures only the projection of the signal on *one* axis (x OR y in the rotating frame). The **direction** of rotation (clockwise or counter clockwise rotation, positive or negative frequency) can not be distinguished.

Possible solution: Set the offset frequency on edge of spectrum: Impractical for general use, and one would waste range by acquiring half of the window without signal.

Better: Use two detectors which differ in phase by 90° and collect both cosine and sine components of signal separately:



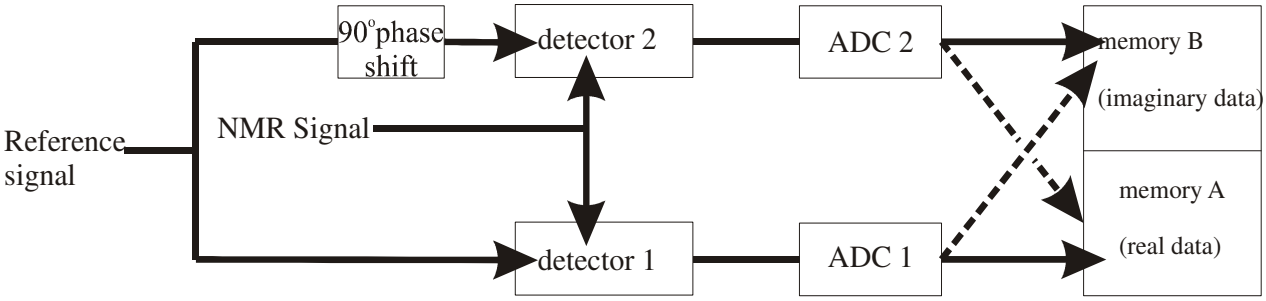
receiver 1 (cosine component) receiver 2sine component)

$$M_{x,y} = - M_0 \mathbf{I}_y \cos(\Omega t) + M_0 \mathbf{I}_x \sin(\Omega t)$$

In practice, simultaneous recording of x- and y- component with two detectors is realized by splitting the NMR signal and subtracting one time the carrier frequency, the other time the carrier signal phase shifted by 90°.

Advantage: The carrier frequency can be set in center of spectrum, and the AD converter can run with lower frequency since $\nu_{max} = 2\pi\ \Omega_{max} = ½ \cdot$ SWH

Problem: two pieces of hardware are used, so subtraction artefacts arising from the combination of the two data can occur (Quadratur images)



Fourier transform and lineshape:

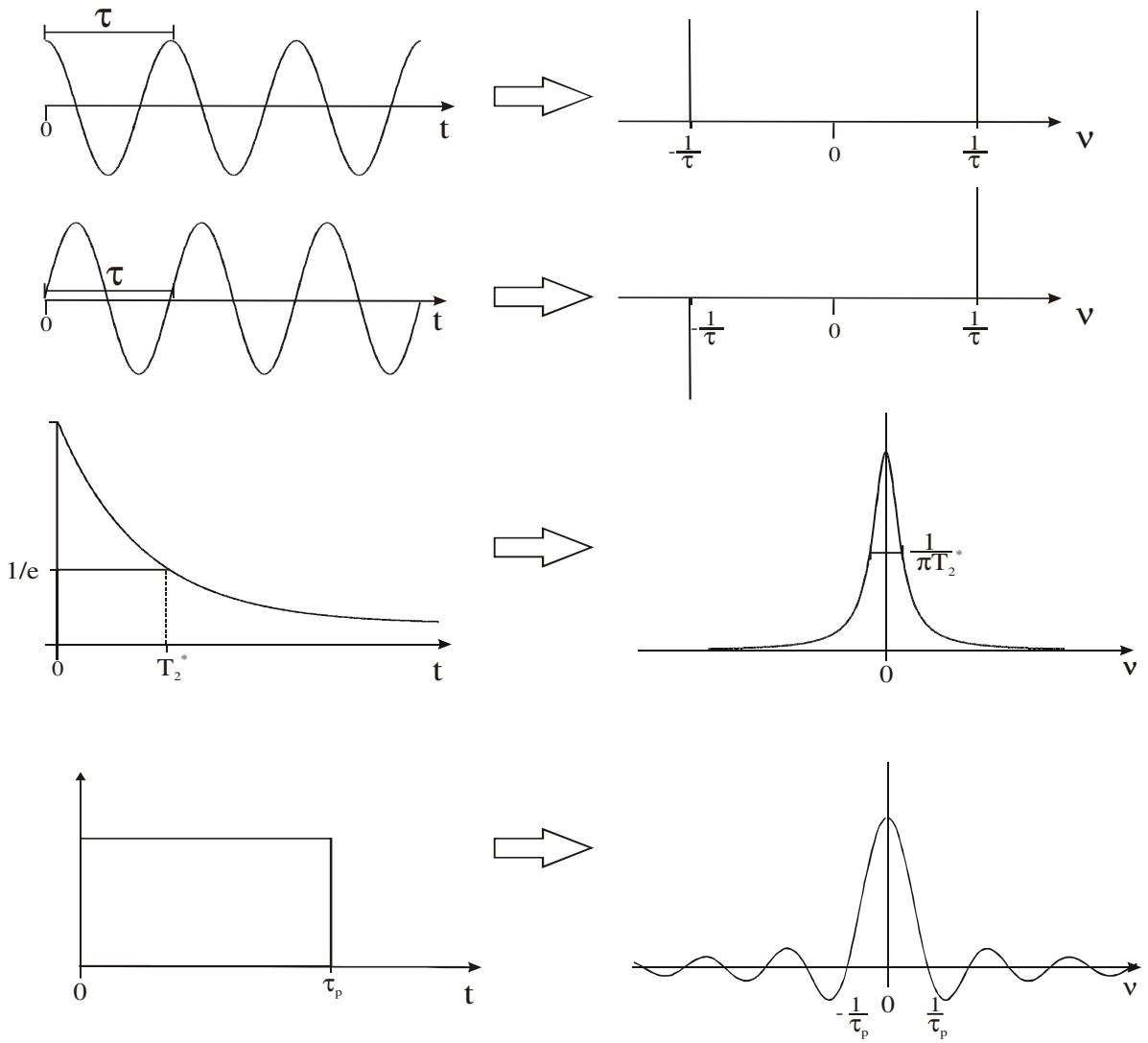
The *time* dependent signal has to be converted into a function of *frequency*. This can be achieved by a process called **fourier transformation** ("extraction of frequencies contained in oscillation").

The process works both ways, and the time dependent function S(ω) and the frequency dependent function S(t) form a Fourier pair.

$$S(\omega) = \int_{-\infty}^{+\infty} S(t)[\cos(\omega t) - i\sin(\omega t)]dt$$

and

$$S(t) = \int_{-\infty}^{+\infty} S(\omega)[\cos(\omega t) + i\sin(\omega t)]d\omega \qquad \textbf{(2.3)}$$



Examples of fourier pairs: from top cosine/spike, sine/spike, exponential decay / lorentzian, rectangular/sinc

Quadrature detection yields *two* time dependent signals (cosine, detector 1 and sine, detector 2) as input, which can be considered mathematically as real and imaginary components of a complex function. For a single NMR signal at frequency Ω and relaxation time T_2^* that results in

$$S(t) = R(t) + I(t) = e^{-\frac{t}{T_2^*}}[\cos(\Omega t) + i\sin(\Omega t)] \quad \textbf{(2.4)}$$

Consequently Fourier transform yields a complex frequency signal $S(\omega) = R(\omega) + i\, I(\omega)$:

$$f(t) = \int_0^\infty [R(\omega) + iI(\omega)][\cos(\omega t) + i\sin(\omega t)]d\omega \quad \textbf{(2.5)}$$

If the real and imaginary components in the time domain spectrum are pure cosine and sine functions (no phase error), then the real component will give a pure absorption spectrum and the imaginary component will give rise to a pure dispersion spectrum:

$$R(\omega) = A(\omega) \text{ and } I(\omega) = D(\omega) \qquad \textbf{(2.6)}$$

with

$$A(\omega) = \frac{\frac{1}{T_2^*}}{(\frac{1}{T_2^*})^2 + (\omega - \Omega)^2} \qquad \textbf{(2.7)}$$

and

$$D(\omega) = \frac{\omega - \Omega}{(\frac{1}{T_2^*})^2 + (\omega - \Omega)^2} \quad (2.8)$$

Normally one is only interested in the absorption signal (real signal, cosine component), and the dispersion part (imaginary, sine component) can be discarded (but see phase correction below).

The absorption signal is a Lorentz function. The maximum of the signal is at $\omega = \Omega$, and the width at half height is

$$\Delta\omega_{1/2} = \frac{2}{T_2^*} \quad or \quad \Delta\nu_{1/2} = \frac{1}{\pi T_2^*} \quad \textbf{(2.9)}$$

Consequence of complex fourier trasform:

The number of points in final spectrum (***SI***) is *half* of that in original time domain signal. ***TD*** points means ***TD***/2 cosine ("real") and ***TD***/2 sine ("imaginary") points, therefore ***SI*** = TD/2.

In practice, one normally sets SI = TD, equivalent to doubling the number of data points by adding zeros at the end of the signal.

For computational reasons, ***SI*** always needs to be a power of 2.

Window functions:

Exponential : Multiplying the FID with an exponentially decaying function will enhance the first high signal to noise part of the signal compared to the end of signal resulting in improvement of S/N at cost of resolution

decay constant of exponential has to be matched to decay of signal (parameter LB)

default: for ^{1}H LB = 0.3, ^{13}C LB = 3

LB < 0 gives growing exponential: results in improved resolution, but tremendous cost of S/N better:

Gaussian **:** resolution enhancement with less cost of S/N (function goes towards 0 at end)

LB < 0, 1> GB > 0 gives shift of maximum of gaussian

Sine, squared sine**:** Brings a not fully decayed signal smoothly to zero at the end. Important for short sampling times (2D NMR)

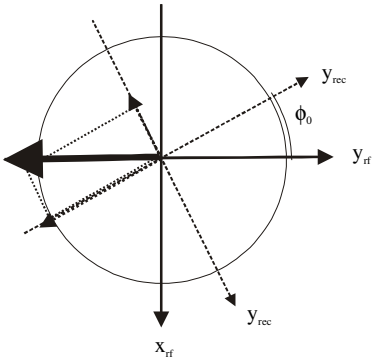
Examples will be provided in lecture and lab.

Phase Errors:

Up to this point it was assumed that at the beginning of data acquisition all magnetization was aligned parallel to the -y axis and are detected as pure sine- and cosine functions. That would result in the pure absorption and dispersion line shapes after Fourier transform . However in reality the signal most likely is not pure absorption, but contains dispersion contribution, because the initial magnetization is not perfectly aligned with the -y axis.

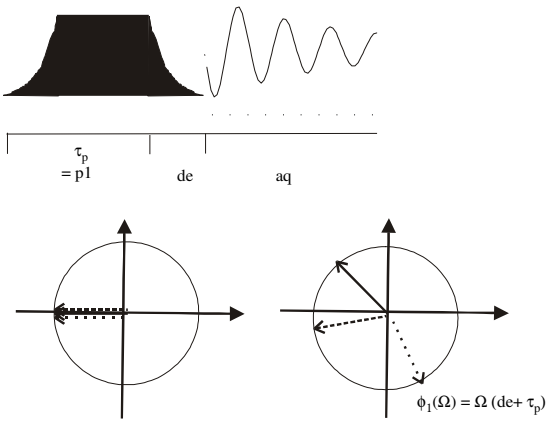
The error normally can be expressed by two terms, one constant for all signals (zero order) and one dependent on the position of the signal in the spectrum (first order):

- **Zero Order (constant):** The reference frequency in the phase sensitive detector is not exactly in phase with the transmitter, which is equivalent to say that x- and y-axes of the receiver in the rotating reference frame are not perfectly aligned with the x- and y axes of the transmitter. The reason is that the signals for the transmitter pulse travels a different pathway in the spectrometer as the reference signal.



  In practice it is more convenient to correct for that error by manipulating the x and y (sine and cos) components of the spectrum afterwards than trying to perfect the spectrometer design.

- **First order (linear):** proportional to frequency offset of signal



  The excitation pulse is of finite length, and for and for a nucleus with $\nu_0 \neq \nu_{RF}$ ($\Omega \neq 0$) some precession will take place during τ_P. In addition, the pulse will not fall off to zero power instantaneously but will require a dead time after the pulse (pre acquisition delay). The begin of data acquisition will therefore not at t = 0, but at t = τ_P + DE, and nuclei with

different ν_0 will no longer be aligned, but dephased proportional to their offset frequency..

If one combines zero and first order effects the total phase error can be described as

$$\phi = \phi_0 + \phi_1 (\Omega) = \phi_0 + ({}^{n}/_{SI})\, \phi_1 \text{ (n = specific data point, SI total number of points)}$$

Phase error means that the two components detected during quadrature detection are not pure sine and cosine, but mixed, and therefore the final real and imaginary spectra will be not pure absorption and dispersion, but also mixed phase.
However, pure absorption and dispersion spectra can be obtained by a linear combination of real and imaginary data:

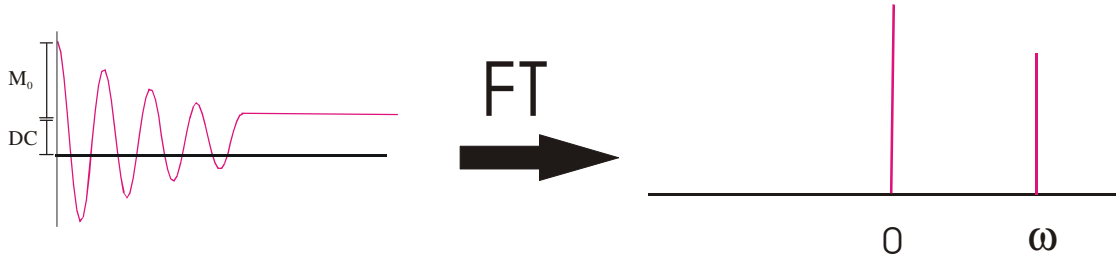
$$A(\omega) = R(\omega) \cos \phi + I(\omega) \sin \phi \text{ and } \quad D(\omega) = I(\omega) \cos \phi - R(\omega) \sin \phi \quad \textbf{(2.10)}$$

This correction can be obtained interactively or automatically by the command **apk** on the Bruker spectrometer, as long both real and imaginary data are available.

Artefact suppression by phase cycling

1) **Constant DC-offset** (imperfect baseline, will cause artefact at zero freq.):

<u>solution:</u> subtract by turning phase of pulse by 180° and subtracting spectra:



1st scan: pulse $(\pi/2)_x$	$-M_0\mathbf{I}_y\cos(\Omega t) + M_0\,\mathbf{I}_x \sin(\Omega t) + DC;$	add to memory
2nd scan: pulse $(\pi/2)_{-x}$	$+M_0\,\mathbf{I}_y \cos(\Omega t) - M_0\,\mathbf{I}_x \sin(\Omega t) + DC;$	subtract from memory
total	$-2\,M_0[\,\mathbf{I}_y \cos(\Omega t) + \mathbf{I}_x \sin(\Omega t)\,]$	

Phase cycling adds desired signal and subtracts unwanted signal.

2) **Imbalance of the two receivers** used for quadrature detection will cause image peak symmetric to the center of spectrum (since two different pieces of hardware are used)

Solution: Cycle phase of pulse x/y and interchange detector 1 and 2:

scan	P1	rec. 1 (I_y)	rec. 2 (I_x)	R(t)	I(t)	receiver phase
1	x	-cos	+sin	+ rec.1	+ rec.2	x
2	y	+sin	+cos	- rec. 2	+ rec. 1	y

This cycle is normally combined with the DC-suppression cycle to give a 4-step phase cycle (CYCLOPS).

scan	P1	rec. 1 (I_y)	rec. 2 (I_x)	R(t)	I(t)	receiver phase
1	x	-cos	+sin	+ 1	+2	x
2	y	+sin	+cos	- 2	+ 1	y
3	-x	+cos	-sin	- 1	- 2	-x
4	-y	-sin	-cos	+ 2	- 1	-y

The different combinations of adding/substracting the receiver outputs to real and imaginary data are equivalent to having the receiver along different axes.

Short notation:

P1	x y -x -y	or in Bruker pulse programs:	PH1 0 1 2 3
receiver	x y -x -y		PH31 0 1 2 3

Signal accumulation:

Phase cycle subtracts artefacts, adds up signal:

NS scans: signal will grow by factor NS, artifacts will subtract for NS = n·4 (for CYCLOPS cycle)

however: random noise will not cancel out (opposed to popular belief) but grow by $\sqrt{NS}$

Signal to noise ratio will therefore grow only as $NS / \sqrt{NS} = \sqrt{NS}$ i.e. to achieve **twice** the signal / noise one needs **four times** as many scans.

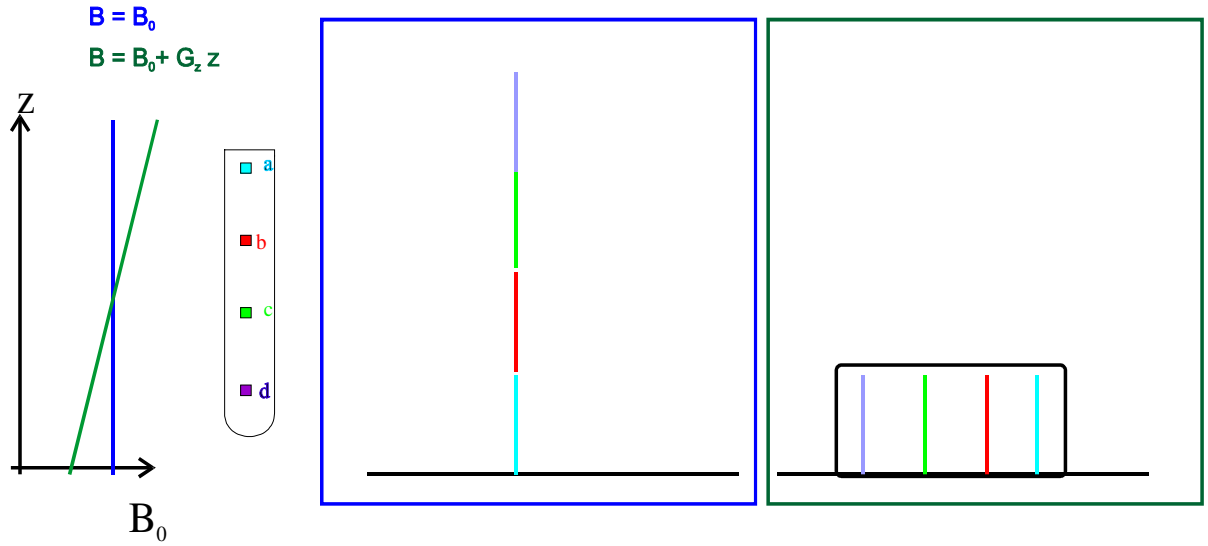
Pulsed Field Gradients

One disadvantage of phase cycling is that several scans are required to remove undesired signals. Also in cases where the undesired signal is larger than the desired signal the receiver gain (***rg***) has

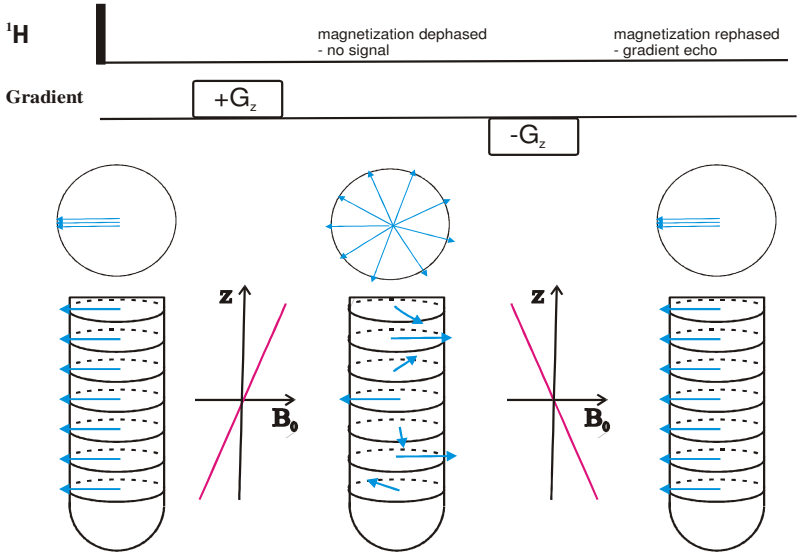
still to be adjusted using the undesired signal rather the desired one.

Modern spectrometers allow removal of artefacts/unwanted signals within **one** scan using pulsed field gradients:

- gradients coils are t into probe in addition to shim coils
- The effect is linear z-gradient similar to a bad z-shim, but usually much stronger. Also pulsed field gradients can be turned on for only a few ms at a time during the experiment
- They can be used to selectively dephase x,y magnetization while not effecting z-magnetization.
- A signal is also observed for signals where subsequent gradients cancel out, while every other signal is dephased (short T_2^*).
- The advantage over phase cycling is that it is all done in one scan, no subtraction artifacts occur and in case of good signal to noise experiment time is kept to minimum
- if gradient on during acquisition, can be used for imaging (MRI) or gradient shimming

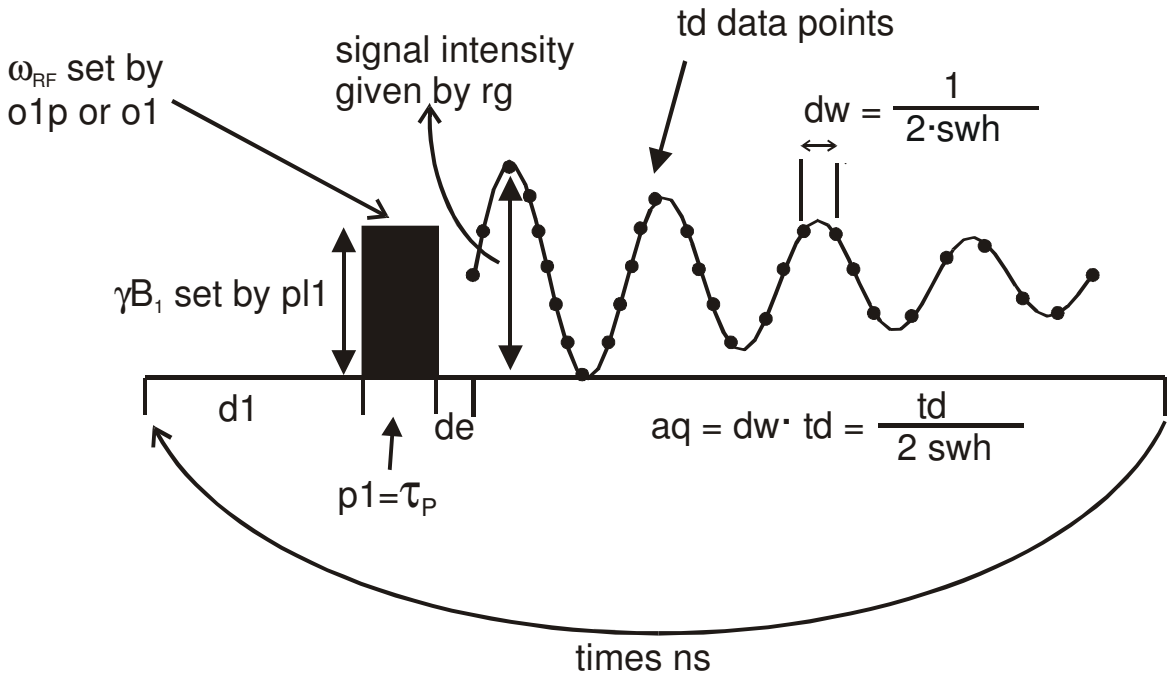


Effect of field gradiend applied during acquisition (imaging): In a homogeneous field, all parts of the sample resonate at the same frequency. With a gradient applied, the frequency will depend on the z-position in the sample



Gradient Echo:
The effect of two consecutive gradiends on x,y magnetization will cancel out.

Summary of the one pulse experiment:



Experiment time = ns (d1+aq+p1+de)

On Bruker spectrometers, the following pulse program is used (; denotes comments):

```
;zg
1 ze              ;Clear Memory
2 d1              ;relaxation delay, wait for z magnetization to build up
  p1 ph1          ;apply rf pulse with relative phase according to phase program ph1
  go=2 ph31       ;Wait delay de, turn on receiver, monitor signal according to phase program ph31
                  jump back to “2" for ns times to repeat more scans
  wr #0           ;write spectrum to disk
exit
ph1=0 2 2 0 1 3 3 1               ;CYCLOPS phase cycling of p1
ph31=0 2 2 0 1 3 3 1              ;and the receiver
;pl1 : f1 channel - power level for pulse (default)
;p1 : f1 channel -  high power pulse
;d1 : relaxation delay; 1-5 * T1
```