NMR Spectroscopy Basics

by

Radovan Fiala, Karel Kubicek, and Pavel Kaderavek

CEITEC, Masaryk University

Nuclear spin

Atomic nuclei consist of protons and neutrons (nucleons)

Protons and neutrons have spin ½

Spins tend to compensate each other but often not completely
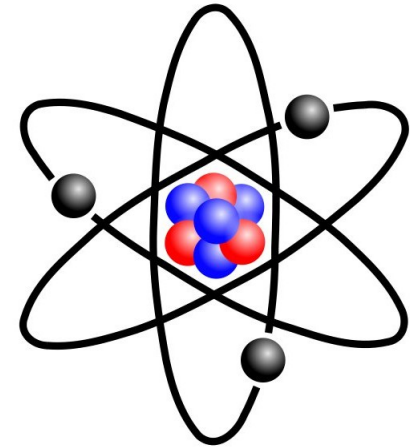
Resulting spin quantum number of the nucleus is

$$I = k * ½ \qquad k \text{ is integer } 0, 1, 2 ....$$

Magnetic spin quantum number – number of possible spin states

$$m = -I, -I+1, -I+2 ......I-2, I-1, I$$

Examples

I = ½, m = -½, +½   2 spin states   example: ^{1}H
I = 1, m = - 1, 0, 1   3 spin states   example: ^{2}H

Proton
Neutron

Magnetic moment

Only nuclei with non-zero spins have magnetic moments and are active in NMR

Magnetic moment

$$|\mu| = \gamma \hbar \sqrt{I(I+1)}$$
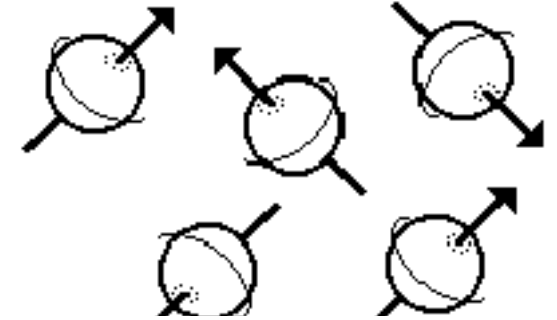
I = quantum number

$$\mu_z = \gamma \hbar m$$

$m = I, I-1, I-2\ldots -I$ = allowed states
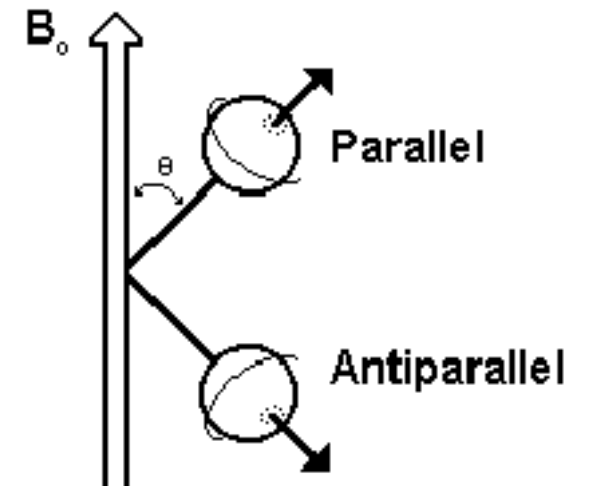
$\hbar = h/2\pi$ h Planck's constant γ magnetogyric ratio, specific to isotopes

Isotopes differ by the number of neutrons and have generally different spins.

In NMR, we refer to isotopes rather than elements, e.g. ^1H (proton) or ^{13}C instead of hydrogen and carbon
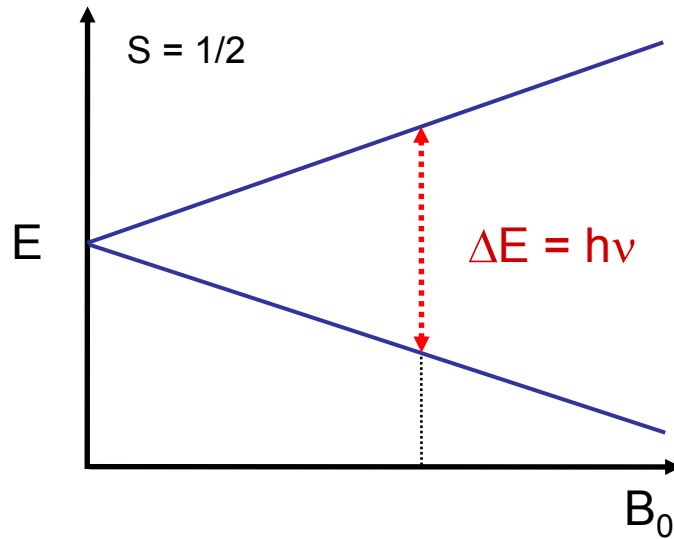
No magnetic field

B_o

Parallel

θ

Antiparallel

In magnetic field B_0

Spins in magnetic field

S = 1/2

$\Delta E = h\nu$

E

B_0

$\Delta E = h\gamma B_0/2\pi$ $\quad$ B_0 external magnetic field

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

$h = 6{,}626.10^{-34}$ J.s $\quad$ h is Planck's constant

γ Magnetogyric ratio, specific for each isotope

For ^1H $\quad$ $\gamma = 42\ 494\ 369$ s^{-1}.T^{-1}

For 100 MHz resonance frequency you need magnet

$B_0 = 100$ s^{-1} /42.494 s^{-1}.T^{-1} $\cong$ 2.35 T

Research grade NMR spectrometers are produced usually with ^1H resonance frequencies in multiples of 100 MHz, currently from 400 MHz up to 1,200 MHz (1.2 GHz), corresponding to magnets from 9.4 T to 28.2 T. For protein studies, high-field spectrometers with resonance frequencies 600 MHz or higher are commonly used.

On Earth, spins are always subject to a magnetic field, but the magnetic field of Earth is only about 50 µT.

NMR and electromagnetic spectrum

$$10^7$$

NMR

**Noninvasive
Low sensitivity**

← Increasing Frequency (ν)

| 10^{24} | 10^{22} | 10^{20} | 10^{18} | 10^{16} | 10^{14} | 10^{12} | 10^{10} | 10^{8} | 10^{6} | 10^{4} | 10^{2} | 10^{0} | ν (Hz) |

| γ rays | | X rays | UV | | IR | | Microwave | FM | | AM | | Long radio waves |

Radio waves

| 10^{-16} | 10^{-14} | 10^{-12} | 10^{-10} | 10^{-8} | 10^{-6} | 10^{-4} | 10^{-2} | 10^{0} | 10^{2} | 10^{4} | 10^{6} | 10^{8} | λ (m) |

Increasing Wavelength (λ) →

Visible spectrum

| 380 | V | 450 | B | 495 | G | 570 | Y | 590 | O | 620 | R | 750 |
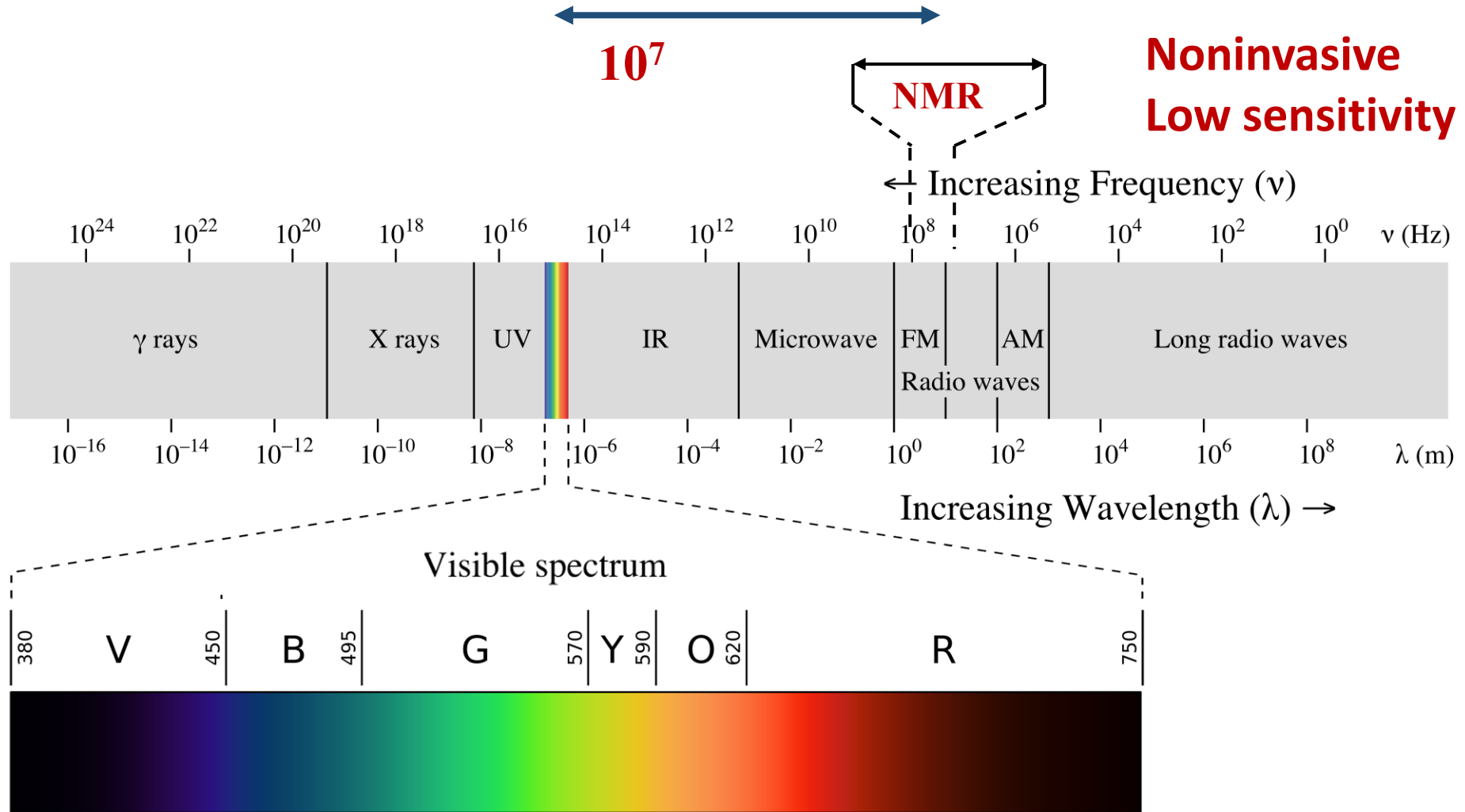
Chemical shift

Nuclei do not experience the external magnetic field B_0 only,
But also the fields of other particles, especially electrons.
Net magnetic field at a nucleus

$$B = B_0 (1 - \sigma) \qquad \sigma \quad \text{nuclear shielding}$$

Resonance frequencies differ slightly depending on the location of the nucleus in the molecule.
Resonance frequencies are field dependent, which is impractical – values are not comparable between different spectrometers.
Chemical shift – frequency difference relative to a suitable standard, expressed in ppm.

$$\delta = 10^6 (\upsilon - \upsilon_{ref}) / \upsilon_{ref}$$

For 1H, TMS (tetramethylsilane) is a common reference compound
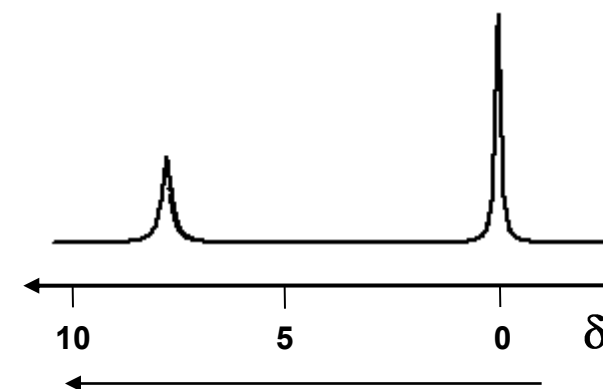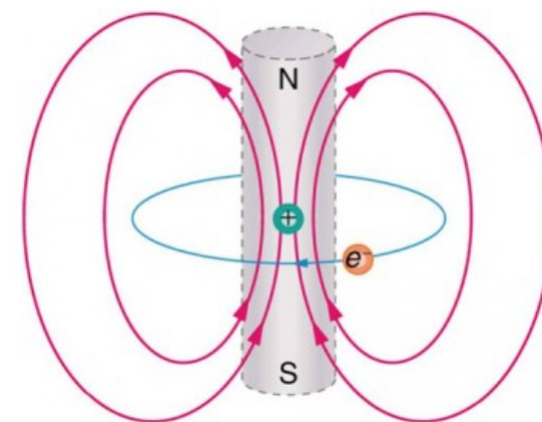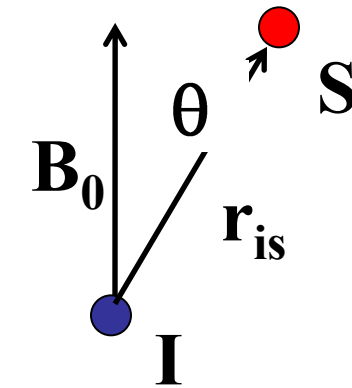
Spin-spin interactions

Dipolar coupling

Direct interaction between nuclear spins

Depends on the orientation of the internuclear vector with respect

to external magnetic field

Important in solid-state spektra

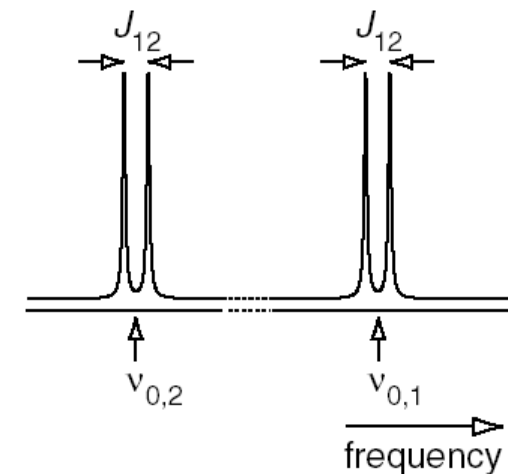In liquids manifests itself only through relaxation phenomena

Scalar / J-coupling

Interaction of nuclei through the cloud of electrons

Does not depend on the external magnetic field

Causes splitting of signals (doublets for spins ½)

Scalar interaction constant J (in Hz, no dependence on B_0)

Reaching the resonance - CW

Continuous wave (CW)

Irradiation frequency is changed (swept) over the range. When resonance occurs, the irradiation energy is absorbed which is recorded as a signal. The resulting record of intensities vs. frequency is the spectrum.

To get undistorted spectrum the process must be SLOW (minutes)

frequency sweep

f_1 Hz f_2

ppm

spectrum
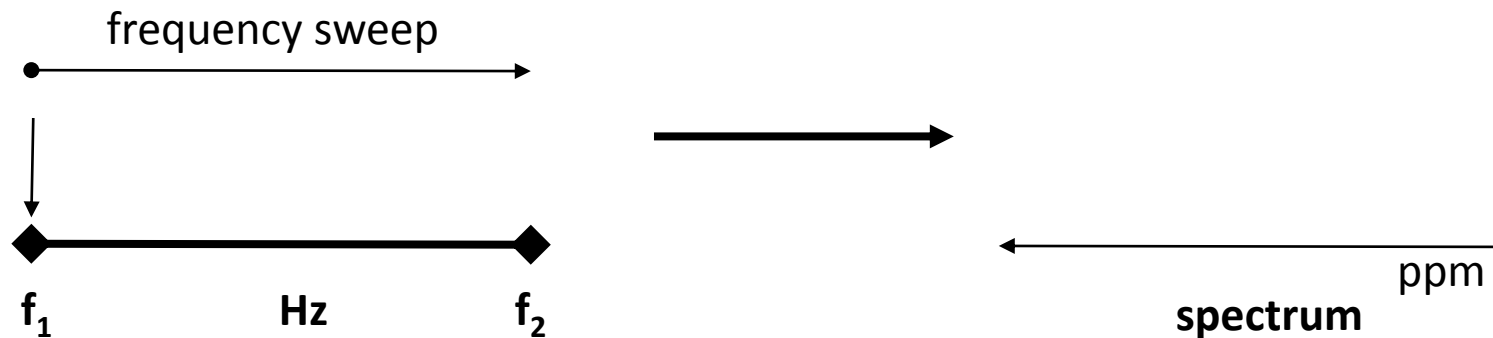
# Reaching the resonance - PFT

Pulsed with Fourier Transform

The spin system is irradiated by a single short high intensity pulse. This is equivalent to irradiating a range of frequencies. The shorter and more intense the pulse is, the broader range of frequencies is affected. After the pulse, the response of the spin system is recorded as a function of time. The record is FID (Free Induction Decay). The spectrum is produced by Fourier Transform of an FID.

RF pulse 90°

FT

t

FID – time domain

Spectrum - frequency domain

ppm
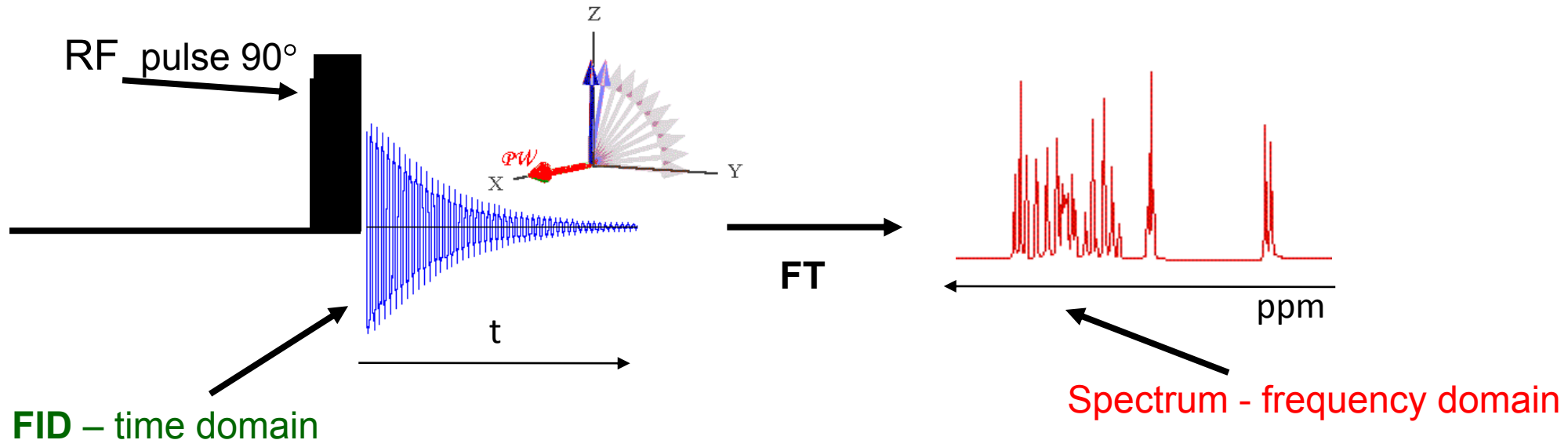
Relaxation

Spin-lattice relaxation - T$_1$

Transferring the energy into surroundings (solvent/'lattice'). The spin system returns into equilibrium.
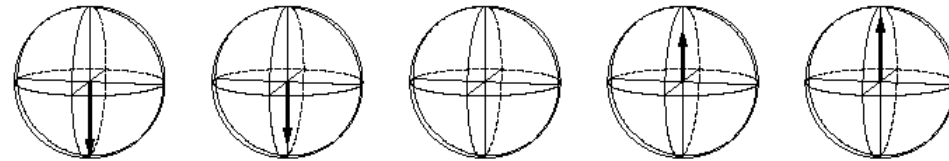
Spin-spin relaxation - T$_2$

The intensity of the signal in FID is dropping with time (Free Induction Decay) due to loss of coherence. The energy is still in the spin system but randomly oriented nuclear magnetic moment average to zero.

Exponential decay
$\exp(-t/T_2)$
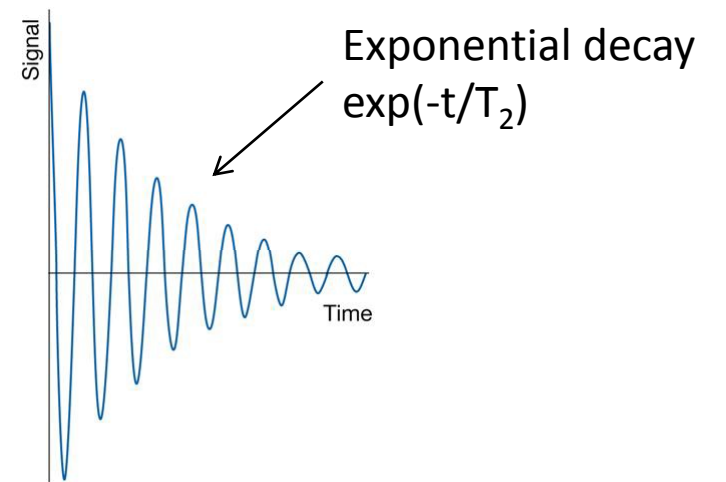
Resolution

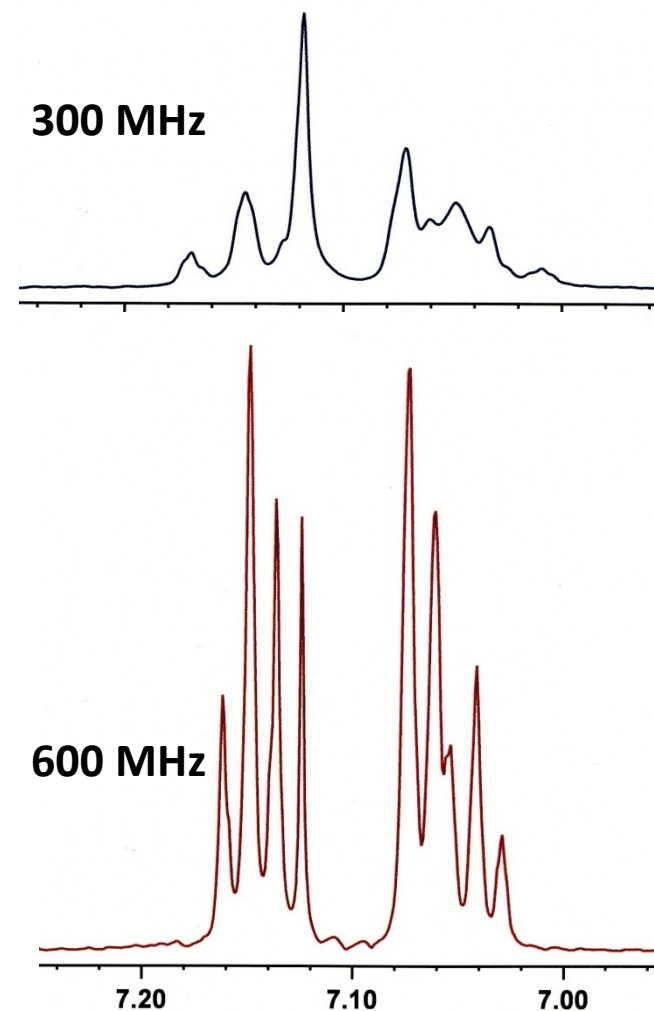Position of signals is given by the chemical shift (relative number)

The with of 1 ppm in Hz (absolute scale) depends on magnetic

field:

at 500 MHz, 1 ppm = 500 Hz,

at 1000 MHz, 1 ppm = 1,000 Hz

If the linewidth is the same, signal appear farther from each

other at higher field.

Resolution and sensitivity increase linearly with magnetic field

300 MHz

600 MHz

7.20   7.10   7.00

Aromatic part of ethylbenzene spectrum

Sensitivity

Energy difference between spin levels are very small.

Boltzmann distribution $N_\alpha = N_\beta \cdot \exp(-\Delta E/k_B T)$

$\Delta E = h\nu$, h Planck's constant, k_B Boltzmann constant

N_α, N_β – polulations of the spin states

For protons at 500 MHz and 303 K, the population difference

between the ernergy levels is less than 0.01%!

We work with **a small fraction od nuclei – sensitivity** of NMR is inherently **low**

For proteins, you need about **0.5 ml of sample with 0.5 mM** concentration.

Improving sensitivity by signal accumulation – more scans

Signal-to-noise ratio increases with square root of number of scans: $S/N \sim \sqrt{ns}$
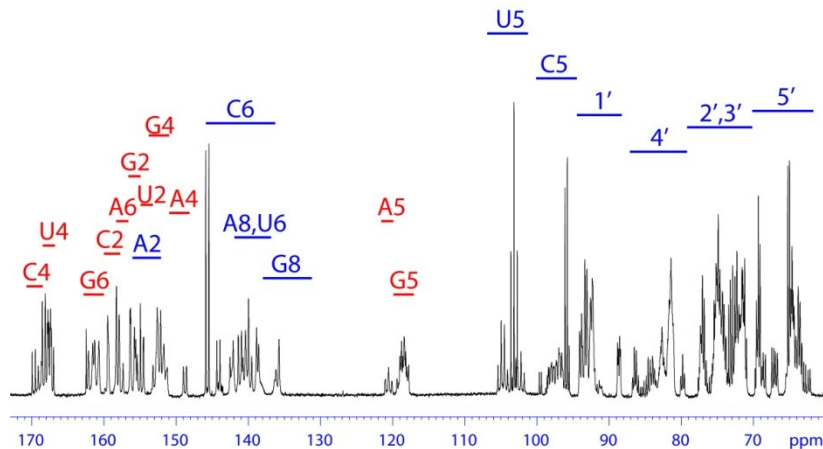
Increasing Resolution

Biopolymers: repetition of identical units (nucleotides, amino acids)
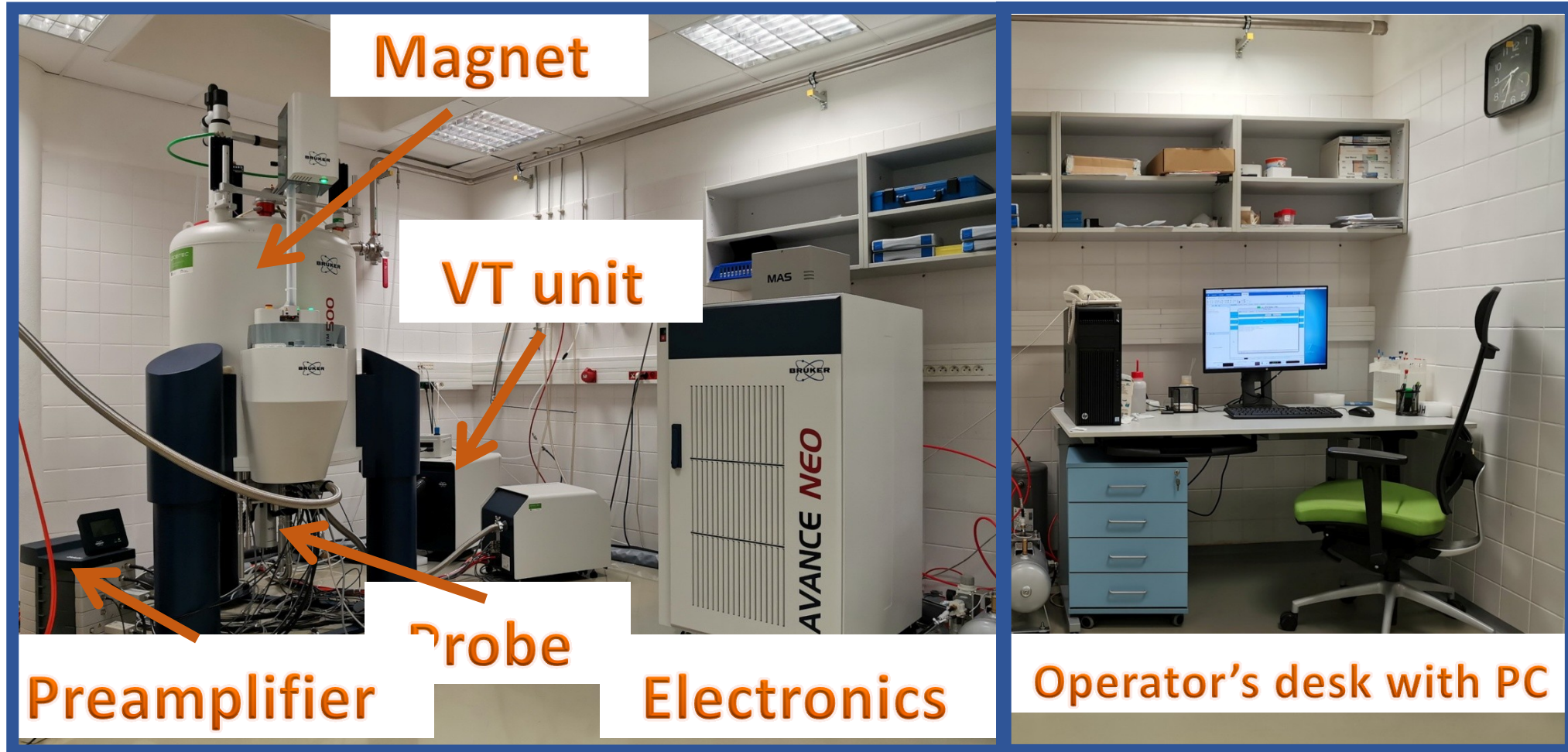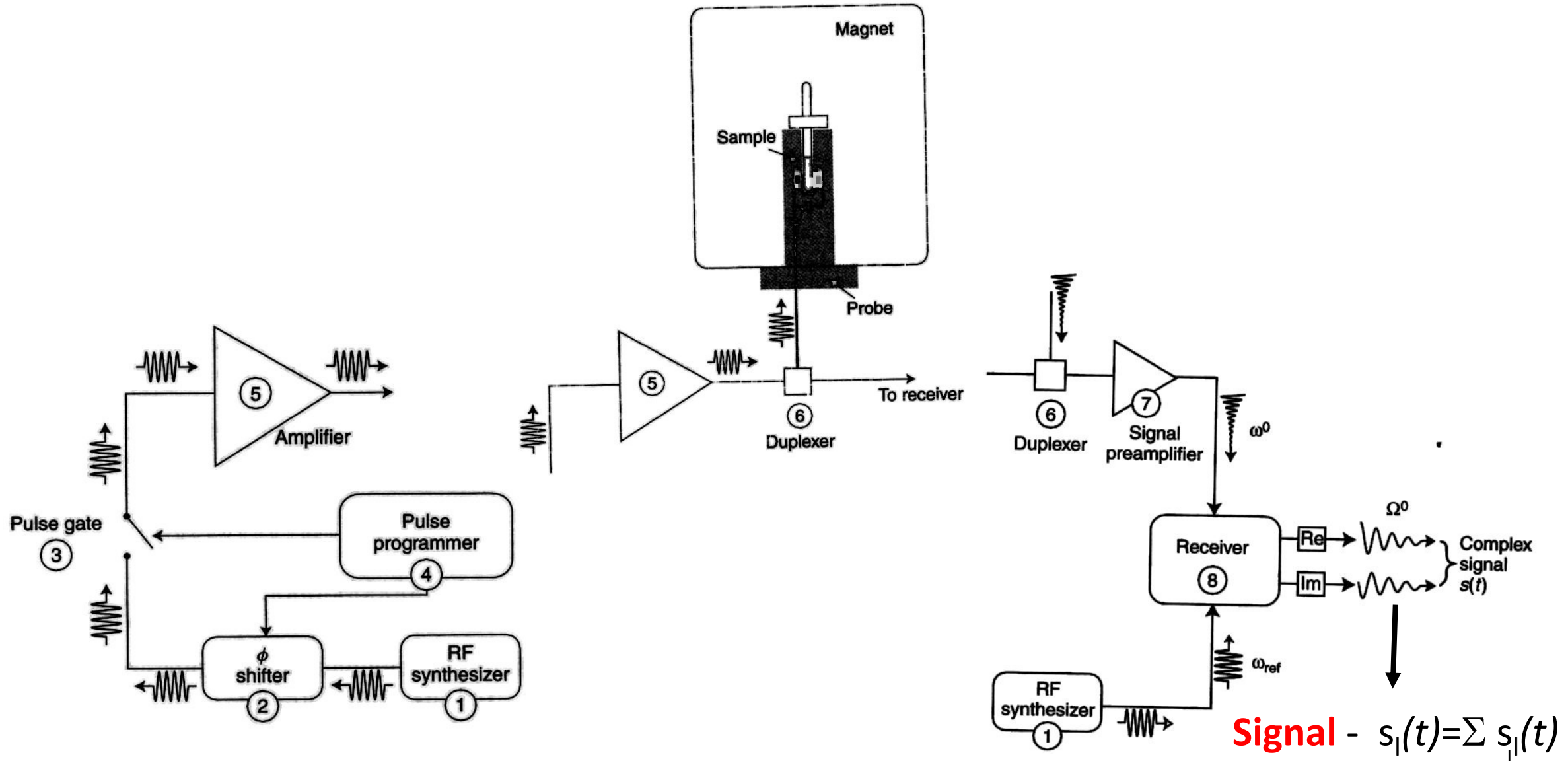
High resolution is needed

High magnetic field
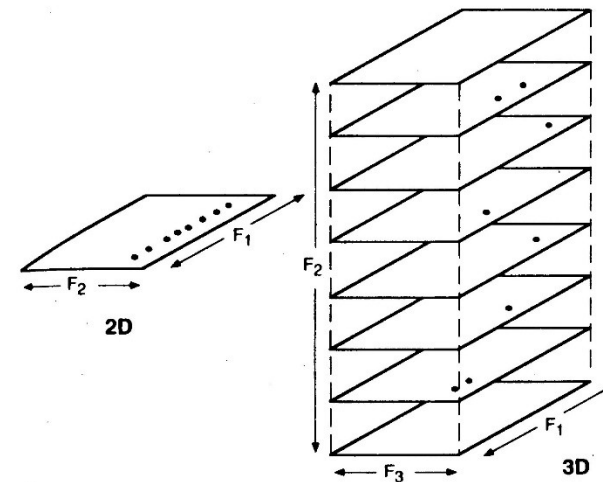
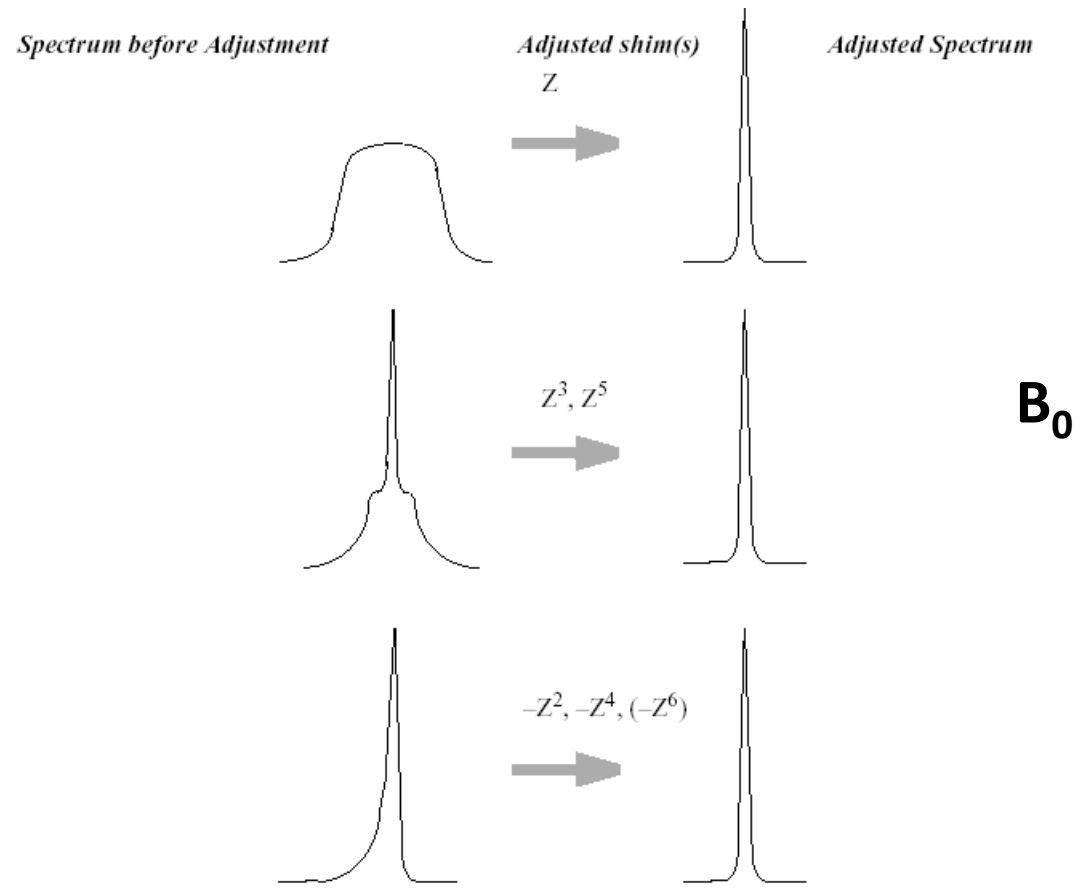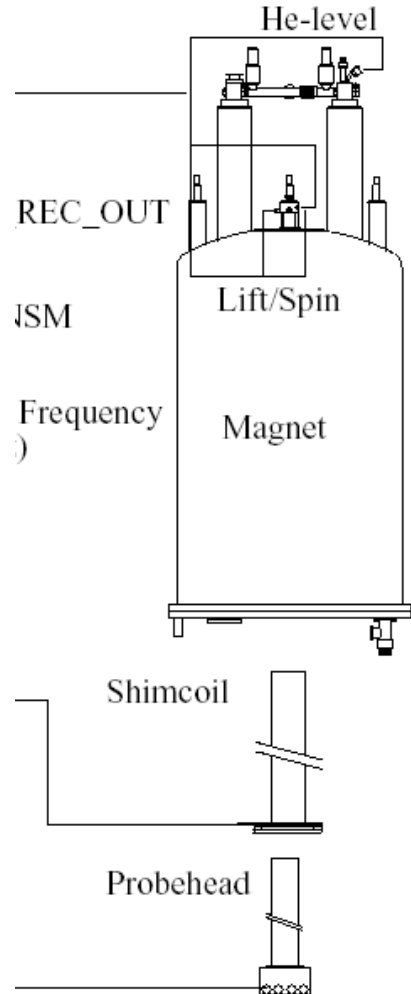Increasing number of dimensions

NMR Spectrometer
Major parts

Magnet

VT unit

Preamplifier

Probe

Electronics

Operator's desk with PC

NMR Spectrometer - Scheme

Signal - $s_I(t) = \sum s_{II}(t)$

The Magnet

He-level

REC_OUT

NSM

Frequency

Lift/Spin

Magnet

Shimcoil

Probehead

Spectrum before Adjustment

Adjusted shim(s)

Adjusted Spectrum

Z

Z^3, Z^5

$-Z^2, -Z^4, (-Z^6)$

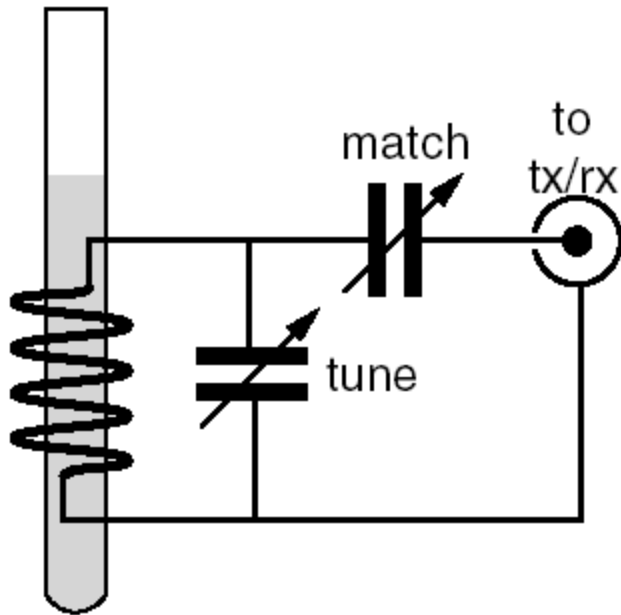B_0

Adjusting the magnetic field homogeneity

The Probe

Houses the sample
Changes electric current into magnetic field and back.
Must be tuned for best sensitivity
Inverse probes, X-nuclei detection probes
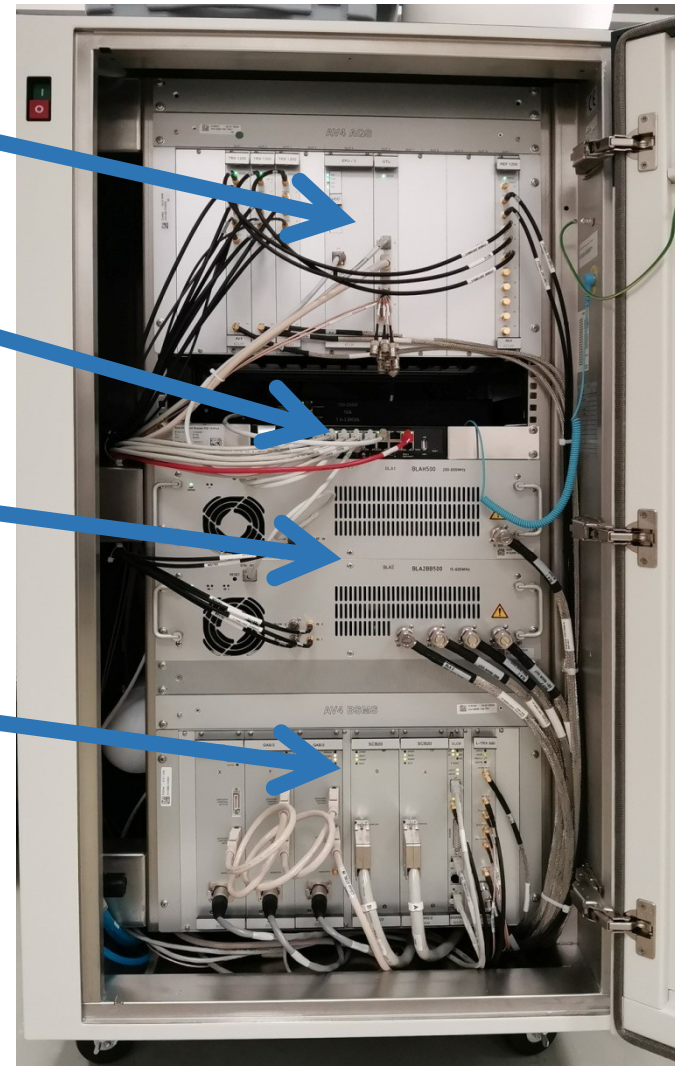Room temperature probes, cryoprobes

Electronics

Frequency synthesis & signal processing
Computer (real-time control)

Power amplifiers

Magnet control
sample insert & eject
shimming
temperature control

How the NMR Spectrometer Works

Transmitter – power *vs.* rf field induction

$$L_p = 10 \times \log_{10} \frac{P_{out}}{P_{in}}; \quad [dB]$$

$$10 \times \log_{10} \frac{P_{out}}{P_{in}} = 10 \times \log_{10} \frac{1}{2} = -3.0$$

Relative power ratio expressed in decibel
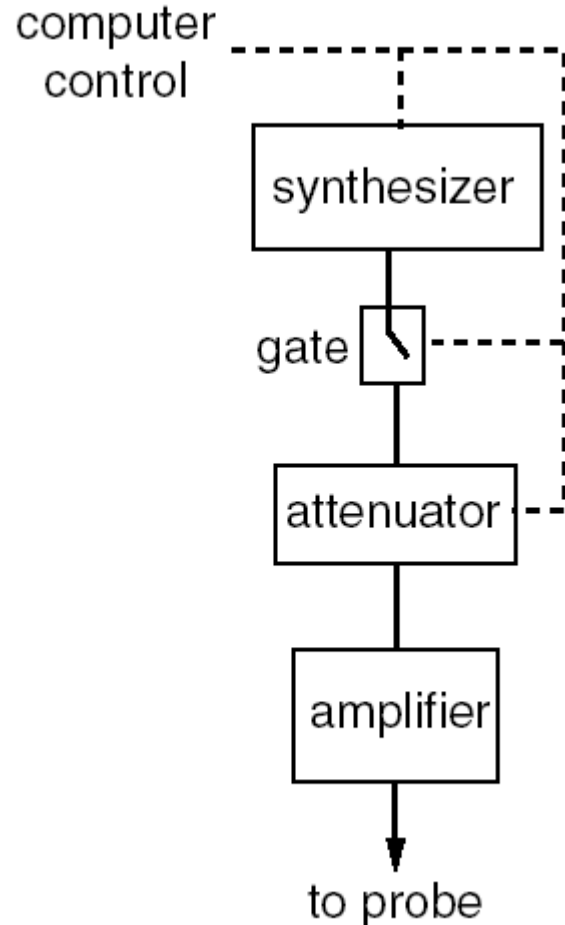**1 dB: P1/P2 = 1.2589254**

computer
control

synthesizer

gate

attenuator

amplifier

to probe

$$!!!!!!!!B_1 \sim I \sim P^{1/2} \ (P=RI^2)!!!!!!!!!$$

$$\text{power ratio} = \left(\frac{\omega_1^{new}/2\pi}{\omega_1^{init}/2\pi} \right)^2 .$$

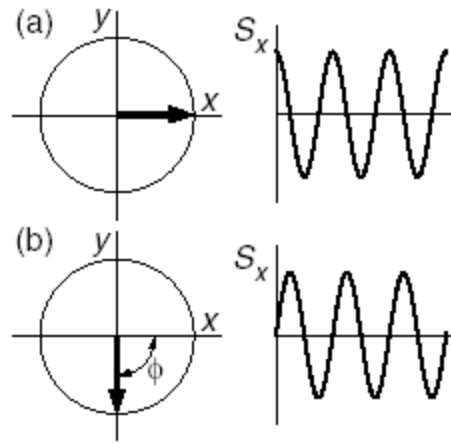$$\text{power ratio in dB} = 10 \log_{10} \left(\frac{\omega_1^{new}/2\pi}{\omega_1^{init}/2\pi} \right)^2$$

$$= 20 \log_{10} \left(\frac{\omega_1^{new}/2\pi}{\omega_1^{init}/2\pi} \right) .$$

Relative rf field ratio ex-pressed in decibel
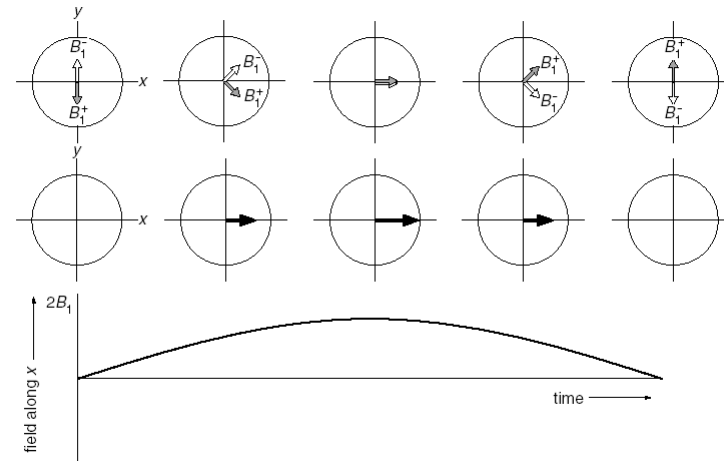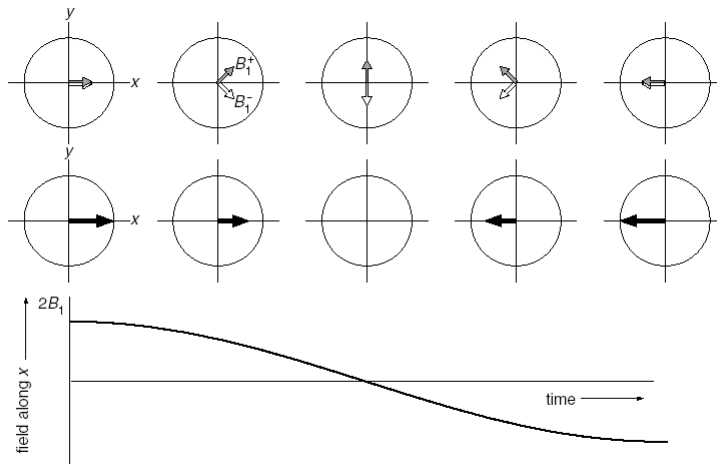**1 dB: ω1/ω2 = 1.120185**

How the NMR Spectrometer Works

Transmitter – phase shifted pulses
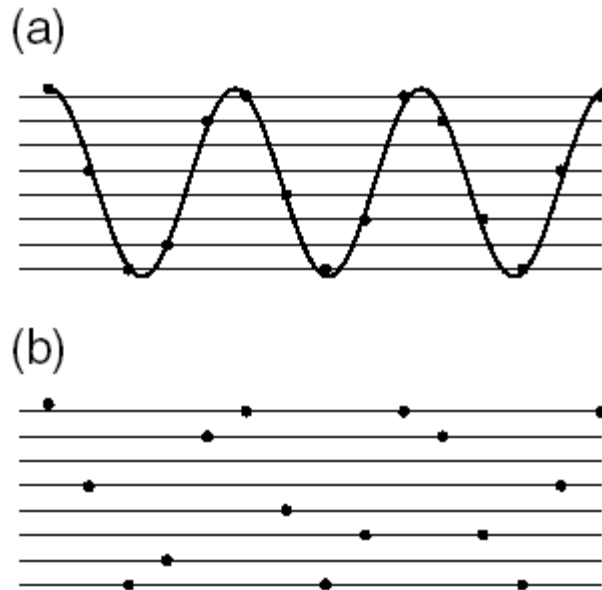

$$2B_1 \cos \omega_{RF} t.$$

$$2B_1 \sin \omega_{RF} t.$$

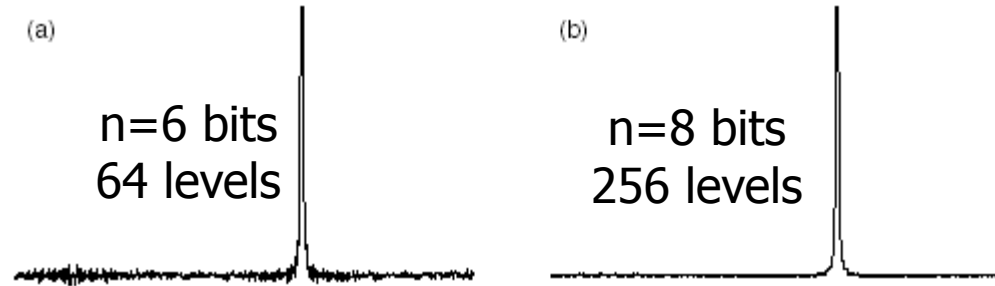# How the NMR Spectrometer Works

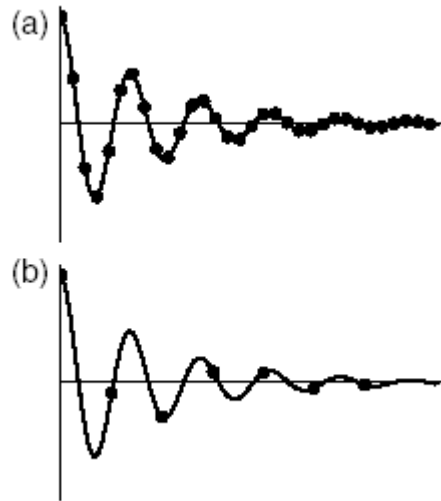3bit A/D converter (8 levels)
$2^n = 8$

A/D converter

(a)

(b)

n=6 bits
64 levels

n=8 bits
256 levels

A/D converter typically 16 bits
i.e. 65 536 levels
32 bits
i.e. 4 294 967 296 levels

How the NMR Spectrometer Works

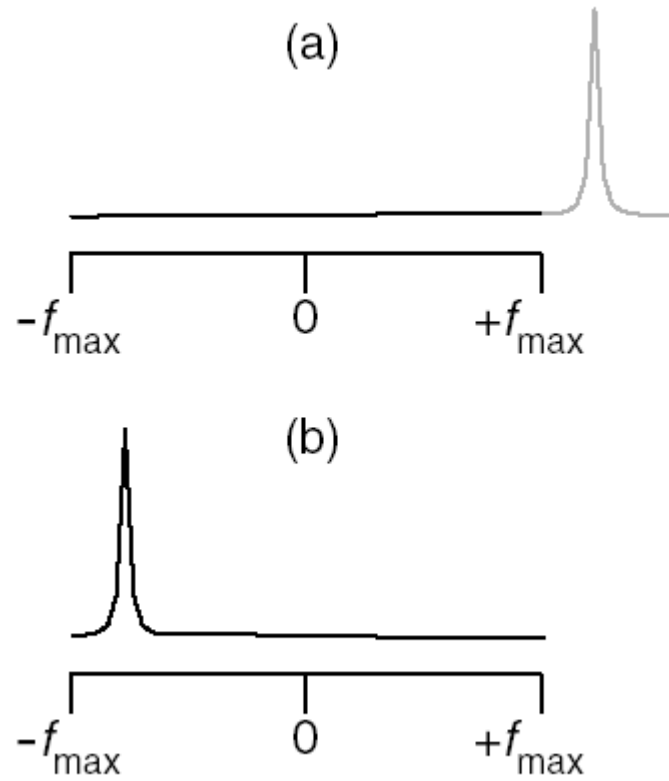## A/D converter – sampling frequency

Nyquist frequency

$$f_{max} = \frac{1}{2\Delta};$$

$$\Delta = \frac{1}{2f_{max}}.$$

f_{max} = 1 kHz => Δ = 500 μs

How the NMR Spectrometer Works
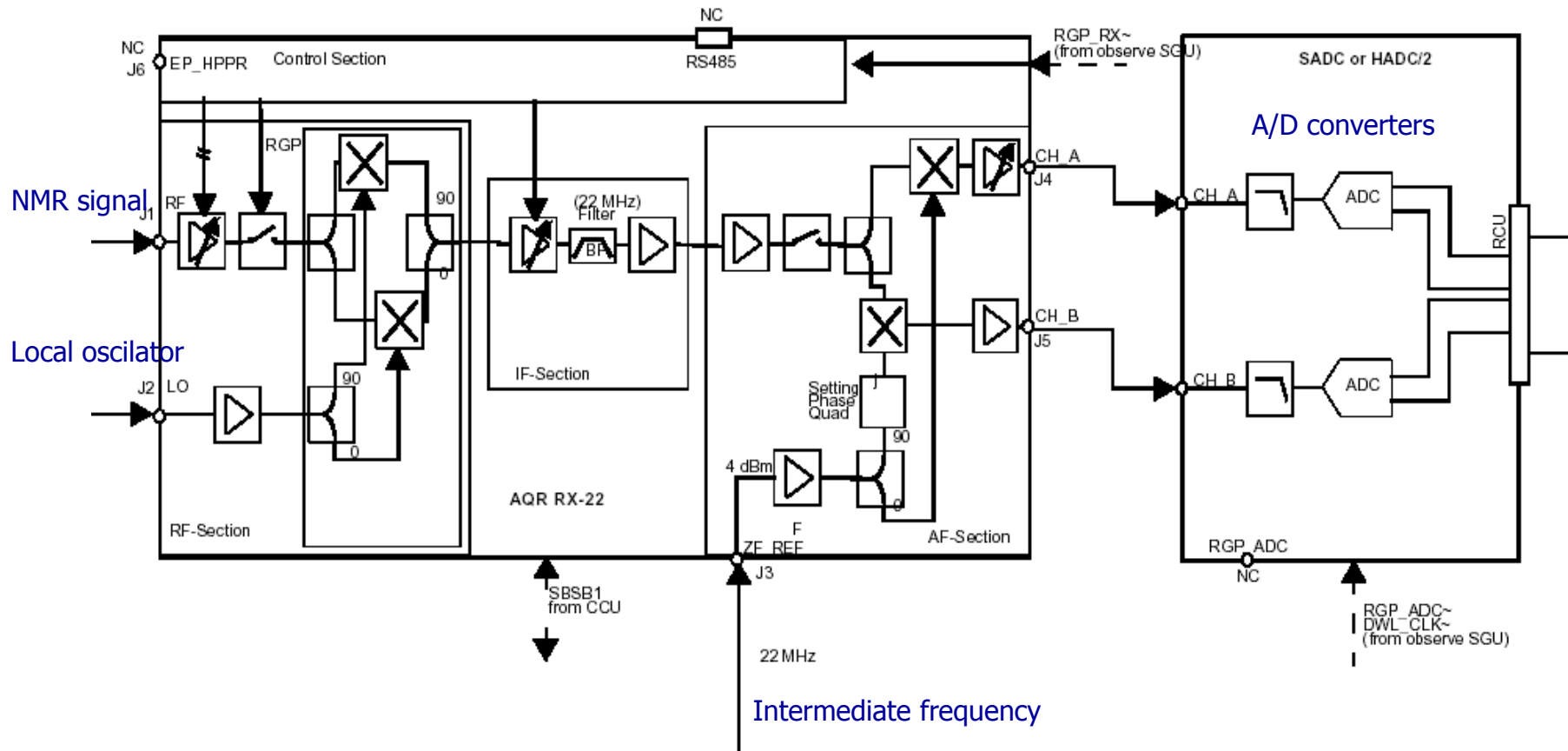
A/D-converter – signal folding

(a)

$-f_{max}$ 0 $+f_{max}$

(b)

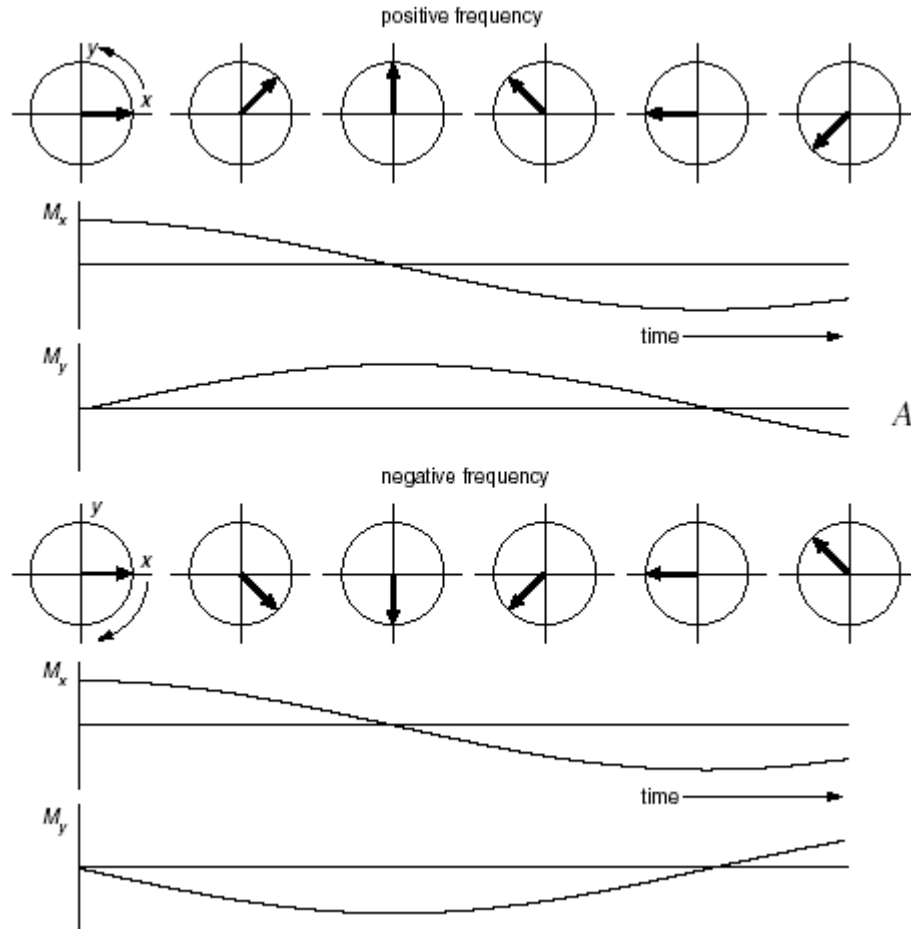$-f_{max}$ 0 $+f_{max}$

How the NMR Spectrometer Works

Receiver

How the NMR Spectrometer Works

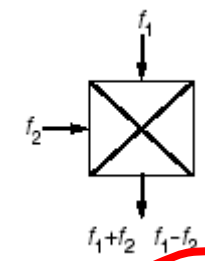## Quadrature detection

mixer

$$A \cos \omega_0 t \times \cos \omega_{rx} t = \tfrac{1}{2} A \left[\cos(\omega_0 + \omega_{rx}) t + \cos(\omega_0 - \omega_{rx}) t \right]$$

$$A \cos \omega_0 t \times (-\sin \omega_{rx} t) = \tfrac{1}{2} A \left[-\sin(\omega_0 + \omega_{rx}) t + \sin(\omega_0 - \omega_{rx}) t \right]$$

cosA.cosB=1/2(cos(A+B) + cos(A-B))

cosA.sinB=1/2(sin(A+B) - sin(A-B))

How the NMR Spectrometer Works

Quadrature detection

$$+ \cos(\omega_0 - \omega_{\mathrm{rx}})t\,]$$

$$+ \sin(\omega_0 - \omega_{\mathrm{rx}})t\,]$$
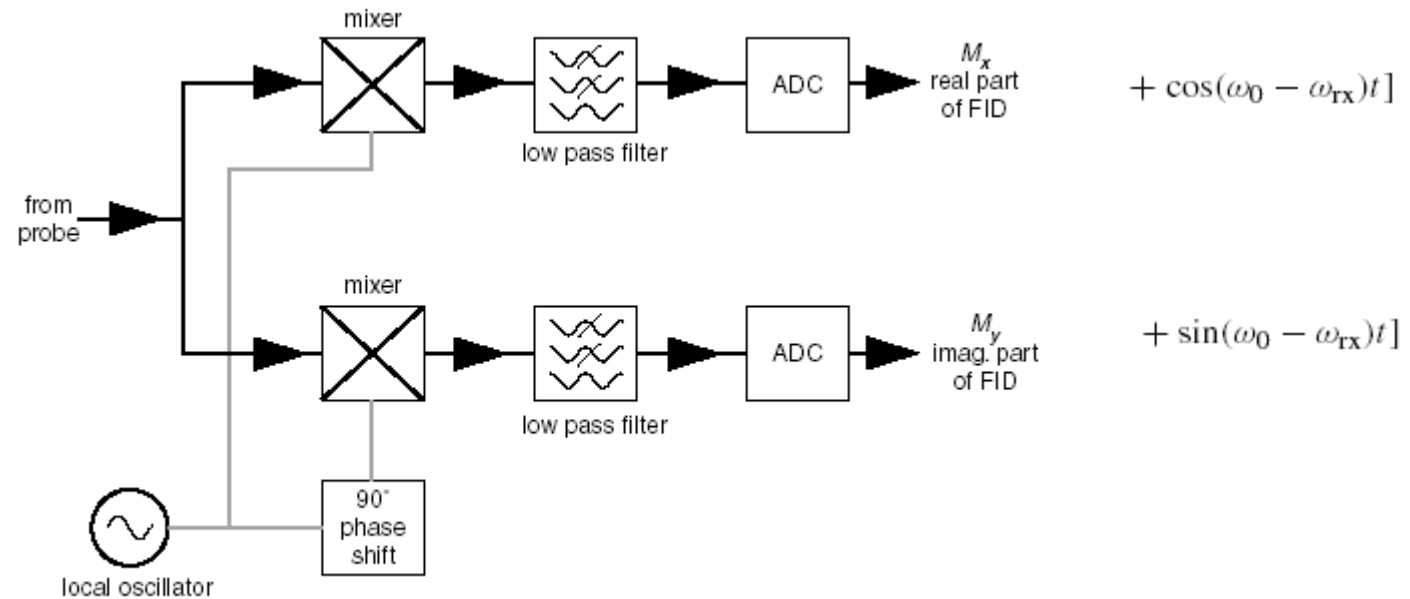
Intermediate frequence

Frequency $\omega_{\mathrm{o}} + \omega_{\mathrm{rx}}$ removed by filter

How the NMR Spectrometer Works

Quadrature detection – time *vs.* frequency

Spectral width f_{sw} Acqusition time t_{acq}

N – number of acquisition points
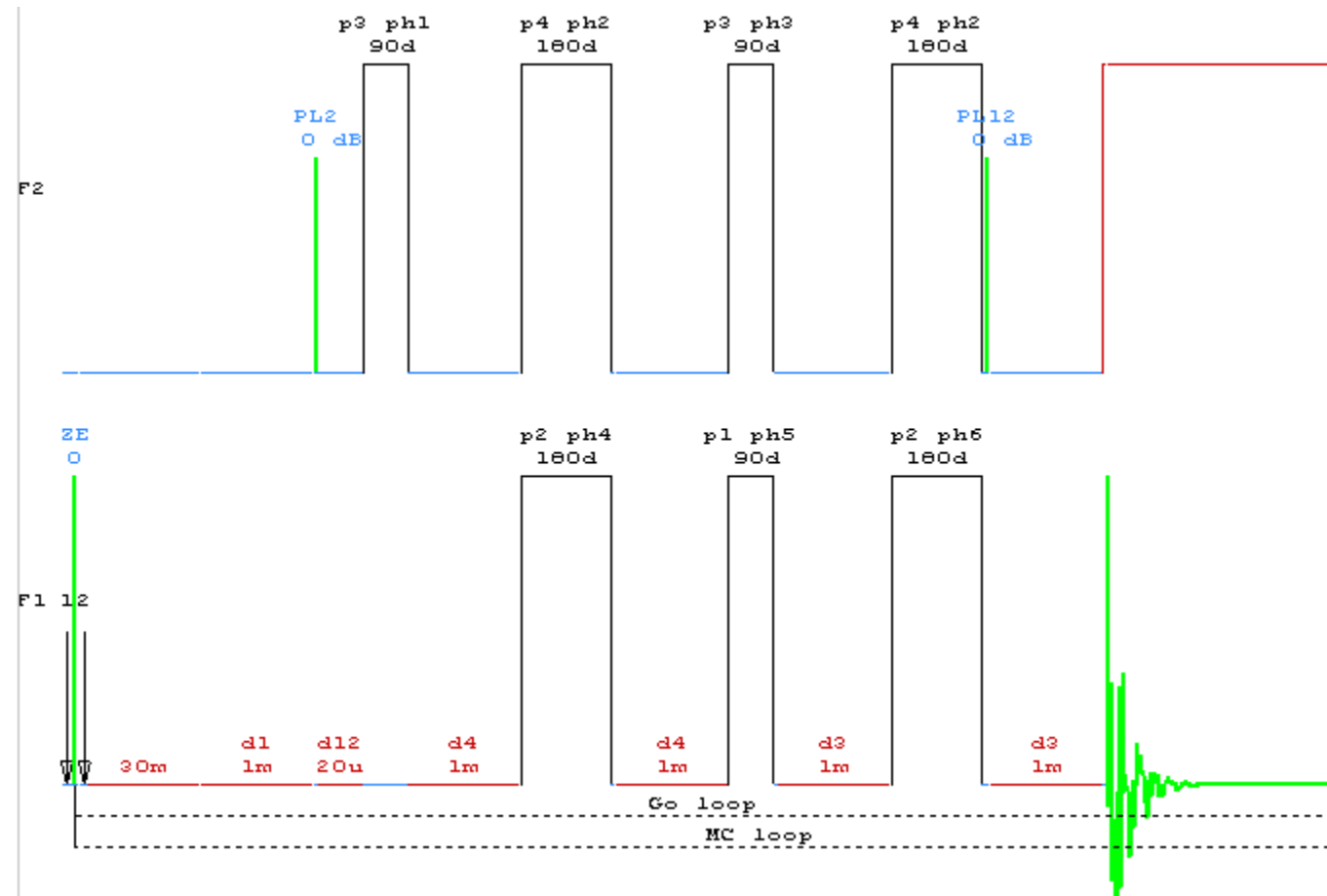
$$\Delta = \frac{1}{f_{SW}} \qquad\qquad N = \frac{t_{acq}}{\Delta}.$$

Δ – sampling interval

Pulse programmer

INEPT with refocusing

How the NMR Spectrometer Works
Pulse programmer

```
;ineptrd
;avance-version (02/05/31)
;INEPT for non-selective polarization transfer
;with decoupling during acquisition

#include <Avance.incl>

"p2=p1*2"
"p4=p3*2"
"d3=1s/(cnst2*cnst11)"
"d4=1s/(cnst2*4)"
"d12=20u"


1 ze
2 30m do:f2
  d1
  d12 pl2:f2
  (p3 ph1):f2
  d4
  (center (p4 ph2):f2 (p2 ph4) )
  d4
  (p3 ph3):f2 (p1 ph5)
  d3
  (center (p4 ph2):f2 (p2 ph6) )
  d3 pl12:f2
  go=2 ph31 cpd2:f2
  30m do:f2 mc #0 to 2 F0(zd)
exit
```

```
ph1=0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
ph2=0 2
ph3=1 1 3 3
ph4=0 2
ph5=0 0 0 0 1 1 1 1 2 2 2 2 3 3 3 3
ph6=0 2 0 2 1 3 1 3
ph31=0 0 2 2 1 1 3 3
```
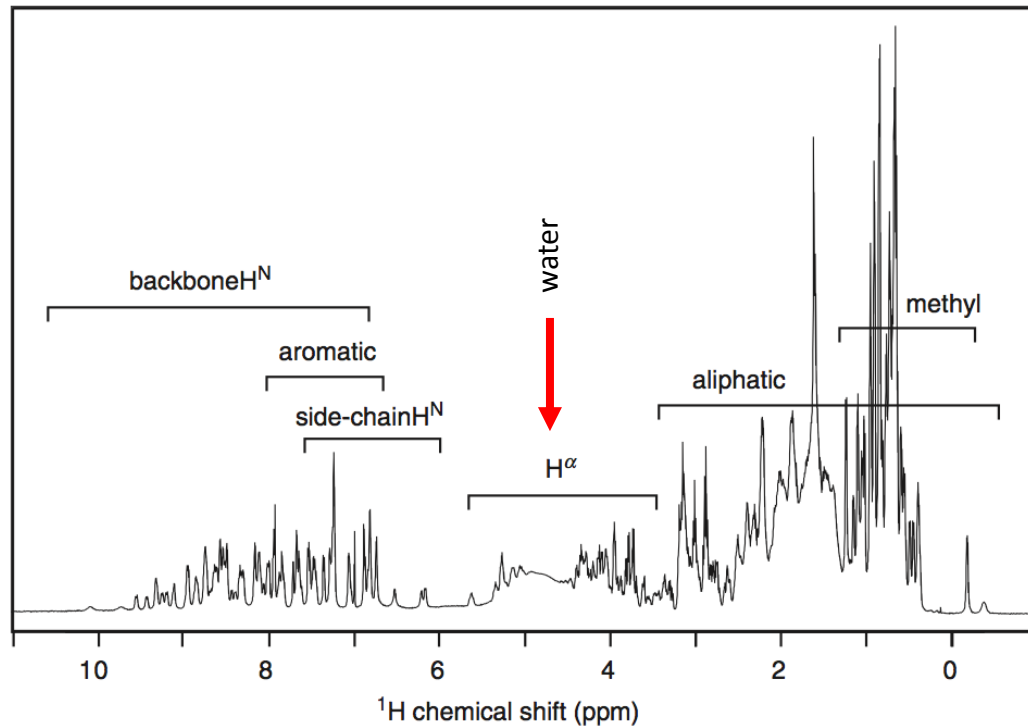
```
;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;d1 : relaxation delay; 1-5 * T1
;d3 : 1/(6J(XH))  XH, XH2, XH3 positive
;     1/(4J(XH))  XH only
;     1/(3J(XH))  XH, XH3 positive, XH2 negative
;d4 : 1/(4J(XH))
;d12: delay for power switching                [20 usec]
;cnst2: = J(XH)
;cnst11: 6  XH, XH2, XH3 positive
;       4  XH only
;       3  XH, XH3 positive, XH2 negative
;NS: 4 * n, total number of scans: NS * TD0
;DS: 16
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence

;$Id: ineptrd,v 1.8 2002/06/12 09:05:00 ber Exp $
```
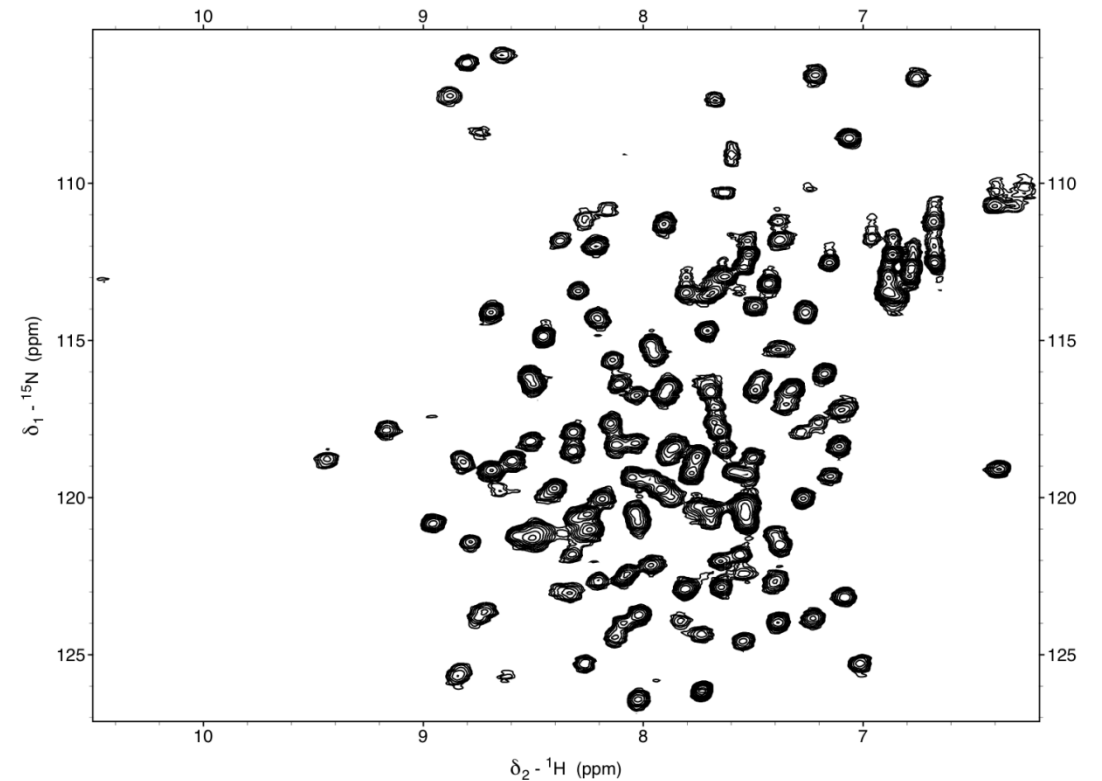
23.10.2012

Examples

1D proton spectrum of a protein

¹H-¹⁵N HSQC of a well folded protein at 293 K
with approximately 155 amino acids, 600MHz

¹H 1D, Cavanagh et al., Protein NMR Spectroscopy, 2007

Additional reading

[Keeler-2002-Understanding_NMR_Spectroscopy.pdf (cam.ac.uk)](Keeler-2002-Understanding_NMR_Spectroscopy.pdf)

YouTube videos

NMR Spectroscopy Visualized

Introduction to NMR spectroscopy Part 1, Part 2

The End

Thank you for your attention

Questions, comments?
radovan.fiala@ceitec.muni.cz