Basic methods of biomolecular structural studies

Basics of MRI

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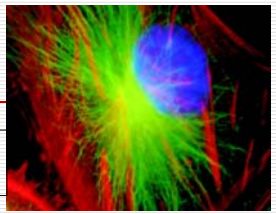
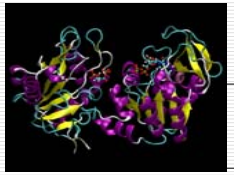
Biomolecular structure:



Actin filaments
Microtubules
nucleus

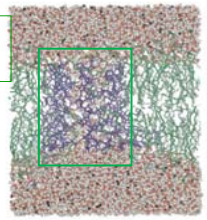
- cells and cellular compartments

- structure of macromolecules and functional complexes



Polypeptide chain and secondary structural units in a two-domain protein

Ion-transport protein embedded in a bilayer membrane



Methods for structural studies

1. Cellular compartments and associated molecules

Size range – resolution of available techniques ?

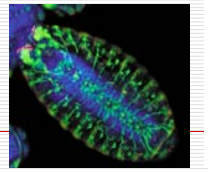
~μm

Resolution of **light microscopy**

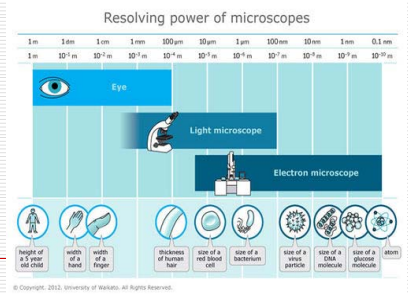
Abbe's principle: $\delta = 0.61\frac{\lambda}{n\sin\alpha} \geq \frac{\lambda}{2} \geq 200nm$

Confocal techniques
Multiphoton ecxitation
Fluorescence labelling

Drosophila melanogaster embrio



Central nerve system – blue
Microtubular structures - green



2. Macromolecular complexes

AFM, TIRFM, superresolution microscopy (+fluorescence labelling)

Electron microscopy

Optical absorption and fluorescence spectroscopy

IR and CD spectroscopy

3. Macromolecular structure with atomic resolution

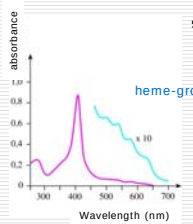
X-ray diffraction: atomic resolution (in crystalline state!)

NMR spectroscopy: atomic resolution in smaller units (high conc.!)

(Mass spectrometry)

What is „spectroscopy" ?

Absorption or emission
„spectrum"



Probability of absorption
Probability of emission

Energy – type quantity : photonenergy, particle energy
frequency
1/f, λ , 1/λ

Basics of NMR spectroscopy and MRI

Figures: Kastler-Patay: MRI orvosoknak, Folia Neuroradiologica, 1993

(Nuclear) Magnetic Resonance (Imaging)

Nuclear Magnetic Resonance Imaging
- history

NMR - spectroscopy
Bloch, Purcell, 1946
Nobel price in physics, 1952


Felix Bloch
1912-1977


Edward Mills Purcell
1906-1983

MRI - *first diagnostic image: 1973*
- *first layer-image 1977*
- *first human brain diagnostics 1980*

EPR Electron Paramagnetic Resonance – spectroscopy
based on electron – magnetic dipoles

I. Phenomenon of Nuclear Magnetic Resonance

Source of nuclear magnetic moment μ

1. *Constituents of atomic nuclei:*

protons and neutrons

P **N**

- Show quantized behavior.

- Have self angular momentum: **spin**

quantum number: $S_N=S_P=1/2$

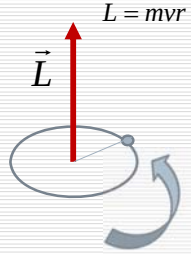
like the electron!

Source of nuclear magnetic moment μ

2. *Quantized behavior of the angular momentum* $\vec{L}$

Classical description of a rotating body with mass m, velocity v and radius r

$L = mvr$



- ***Magnitude*** is quantized

Planck's constant

$$|\vec{L}| = L = \frac{h}{2\pi}\sqrt{l(l+1)}$$

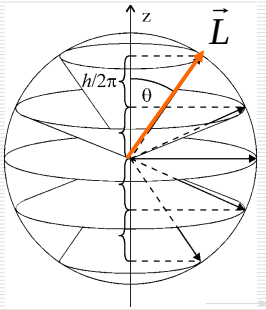
l= 0,1,2,..n-1

Orbital quantum number: *l*

Example: electron „rotating" on one orbital

2. Quantized behavior of the angular momentum

- ***direction*** is also quantized**:** *only those are allowed for which the projection onto* ***one*** *axis (z) obeys the formula:*



$$L\cos\Theta = L_z = \frac{h}{2\pi}m_l$$

(m_l=0,+/-1,…+/-l)

„m„ magnetic quantum number

2*l* +1 –kind of orinetations

H (z) magnetic field : defines a direction

If l= 2 5 orientations

L_x and L_y are not determined

3. Quantized behavior of the ***spin***-angular momentum

- ***Magnitude*** is quantized, but s=1/2

$$|\vec{S}| = \frac{h}{2\pi}\sqrt{s(s+1)}$$

- ***direction*** is also quantized**:** *only those are allowed for which the projection onto* ***one*** *axis (z) obeys the formula:*

$$S\cos\theta = S_z = \frac{h}{2\pi}m_s \qquad m_s=+1/2,\ -1/2 \qquad (2xS+1)=2 \quad \text{-kinds}$$

S_x and S_y are not defined

4. The angular momentum of a charge leads to

magnetic dipole moment μ

Classic view: orbiting electron → moving charge → magnetic dipole

Proportionality: $\left|\vec{\mu}_l\right| = \frac{e}{2m_e c}\left|\vec{L}\right|$

-e, m_e charge and mass of electron, c speed of light

Projection onto the z axis $\vec{\mu}_{l,z} = -\frac{e}{2m_e c}\frac{h}{2\pi}m_l = -\frac{eh}{4\pi m_e c}m_l$

μ_B Bohr magneton

Spin angular momentum of the electron also generates magnetic dipole

$\left|\vec{\mu}_{s,z}\right| = -\frac{eh}{4\pi m_e c}2m_s = \mu_B$

m_s= +/- ½

Two orientations

Bohr magneton

$$\mu_B = \frac{eh}{4\pi m_e c}$$

Magnitude of the projection of the magnetic dipole moment onto the z axis (Magnetic field vector) that belongs to the spin angular momentum of the electron

*5. **Spin**-angular momentum of **nucleons** also generates magnetic moments of quantized nature*

Orientation is opposite,

$\mu_N < \mu_P$

electron $\left|\overrightarrow{\mu_{e,z}}\right| = -2 * m_s * \mu_B = -\mu_B$

neutron $\left|\overrightarrow{\mu_{N,z}}\right| = -2 * m_s * (1.91) * \mu_g$

proton $\left|\overrightarrow{\mu_{P,z}}\right| = 2 * m_s * (2.79) * \mu_g$

m_P~1840m_e!!

$\mu_g << \mu_B$

$\mu_g = \frac{eh}{4\pi m_n c}$

Giromagnetic constant

The electron has much larger spin-magnetic moment

Magnitude of nuclear magnetic moments

Pairs of equi-energetic nucleons with opposite spin states cancel each others magnetic moments

Momentum of an atomic nucleus ≠ 0, in case of odd no. of protons or neutrons

Example: Tritium

$^3_1H \rightarrow two.\dot{}neutrons \rightarrow \sum\vec{\mu}_N = 0$

$\rightarrow\rightarrow one.\dot{}proton \rightarrow \mu_{nucleus} = \mu_P = 2.79\mu_g \approx 3\mu_g$

Simplified relation

$$\vec{\mu}_N \approx -\frac{2}{3}\vec{\mu}_P$$

Magnetic moments of live tissues

^{1}H ^{13}C ^{19}F ^{23}Na ^{31}P stable isotopes in human tissues with non-compensated moments

Atom	I h-ban	μ μ_K-ben	Atom	I h-ban	μ μ_K-ben
n	1/2	-1,91	$^{12}_{6}C$	0	0
p	1/2	+2,79	$^{13}_{6}C$	1/2	+0,7
$^{2}_{1}H$	1	-0,86	$^{14}_{7}N$	1	+0,4
$^{3}_{1}H$	1/2	+3	$^{15}_{7}N$	1/2	-0,28
$^{3}_{2}He$	1/2	-2,1	$^{16}_{8}O$	0	0
$^{4}_{2}He$	0	0	$^{17}_{8}O$	5/2	-1,9
$^{6}_{3}Li$	1	+0,8	$^{36}_{17}Cl$	2	+1,3
$^{7}_{3}Li$	3/2	-3,2	$^{115}_{49}In$	9/2	+5,5
$^{9}_{4}Be$	3/2	-1,2	$^{208}_{82}Pb$	0	0
$^{10}_{5}B$	3	-1,8	$^{209}_{83}Bi$	9/2	+4

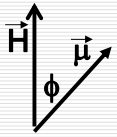
$\vec{h} = \frac{h}{2\pi}$

2/3-d of atoms is H!

It has outstandingly large momentum

Proton-MRI

Energy of classical magnetic dipoles in magnetic field



$$E = E_0 - \left|\vec{H}\right| * \left|\vec{\mu}\right| * \cos\phi$$

Energy without magnetic field

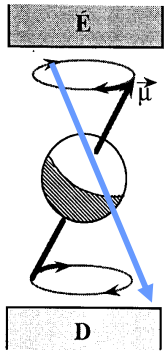
Projection of magnetic moment onto the magnetic field vector

E decreases if $\phi \longrightarrow 0$ →

→ **the dipoles become oriented *parallel* to the magnetic field**

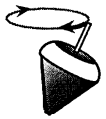
Proton-moments in magnetic field

Two orientations, and precession



"parallel" orientation

Energetically favourable E_1 state



$E_1 < E_2$

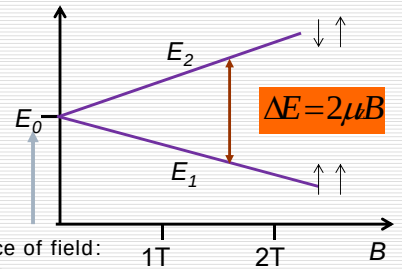
"antiparallel" orientation

E_2 state

Zeeman effect - Zeeman splitting

In magnetic field, the two orientations of spin-moments mean two energetic states

$$\Delta E = E_2 - E_1 = (E_o - E_{magn.2}) - (E_0 - E_{magn.1}) =$$
$$= \mu B \cos\phi + \mu B \cos\phi = 2\mu_z B$$



The energy difference (Zeeman splitting) linearly increases with the strength of the magnetic field

In absence of field: E_0 energy

T:Tesla

1 Tesla= 10 000 Gauss

Larmor frequency: the frequency of precession – depends on B

$$\Delta E = 2\mu B = hf$$

Proton-momentum

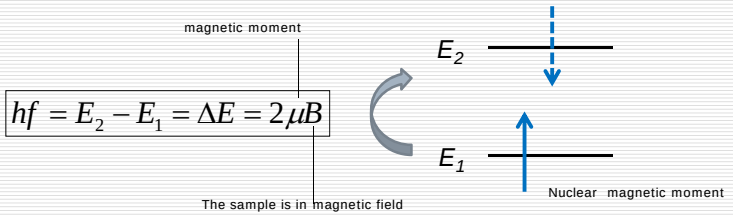
The two frequencies are identical!

What frequency is able to induce the excitation of the E1 to E2 transition ?

$$hf = \Delta E = 2\mu B$$

Absorption of a photon of Larmor frequency induces the transition from E1 to E2

Phenomenon of Nuclear Magnetic Resonance

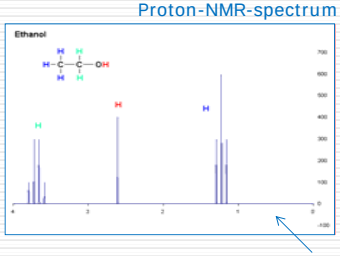


Irradiation of the sample with electromagnetic photons of exactly that energy which is able to excite the transition of the magnetic moments from state E1 to state E2

The transition is „in resonance" with the photonenergy

II. Nuclear Magnetic Resonance spectroscopy

Proton-NMR-spectrum

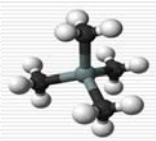


Local environment of a selected nucleus (e.g. H) change the external magnetic field (B_0)→ →change the exciting photon energy → → ***„chemical shift"***

$$hf' = 2\mu B_0(1 \pm \sigma)$$

$$\frac{hf' - hf_0}{hf_0}(ppm)$$

Photonenergies that are absorbed identify the chemical bonding → →***chemical structure***



Reference compound: Tetramethylsilane→ hf_0

^{1}H, ^{13}C, ^{15}N, ^{31}P are used

In vivo applications

Role of the strength of B

*Spin (1/2)-related dipole moment vectors of opposite orientation in a sample **cancel each other!***

What will be the resultant vector?

Two energy levels with populations N1 and N2
***Boltzmann distribution**: N1>N2*

$$\frac{N_2}{N_1} = e^{-\frac{\Delta E}{kT}}$$

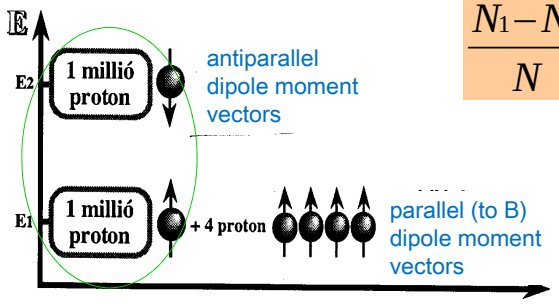
Proton, B=0.5T
$\Delta E = 2\mu B \cong 10^{-7}$ eV
kT (310 K) = 0.027eV

Very small ~ 0! → $N_2 \sim N_1$

larger B →larger ΔE → larger population difference → larger resultant

The effect must be based on the small number of uncancelled vectors (parallel to B).

Even if B is as strong as possible, small signal is expected in any measurements based on nuclear magnetic moments



$$\frac{N_1 - N_2}{N} \approx 10^{-8}$$

$$\sum \vec{\mu_i} = \vec{M}$$

Symbol M: sum of Proton moments Parallel to B

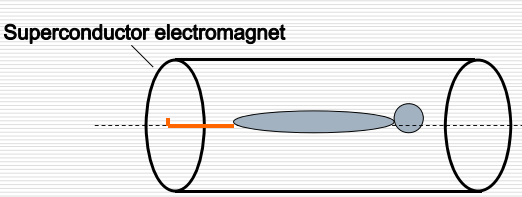
III. Application for Diagnostic Imaging - MRI

the image is produced from data obtained

- after ceasing (terminating) the excitation

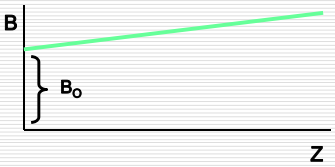
- while the dipoles return to their original orientation, parallel to the magnetic field.

Parameters of the diagnostic measurement



B is large → ΔE is larger
no of uncomppensated momenta is larger

EXPENSES!



Direction of B is II to Z
B increases linearly along Z
B_o+B(Z)

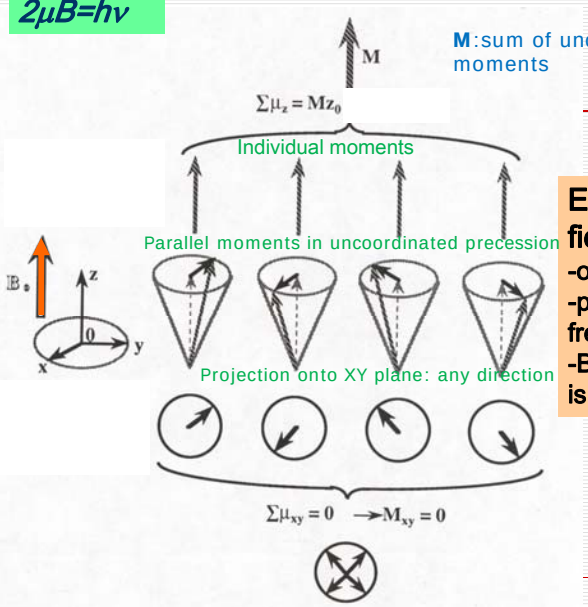
10 000 Gauss ↔ 0.5 G on surface of Earth

Special caution!

Metals, implants, pacemaker……

Protons inside the patient in presence of B

$2\mu B = h\nu$



M:sum of uncompensated parallel moments

Effect of magnetic field
-orientation
-precession with the same frequency
-BUT: phase of precession is arbitrary

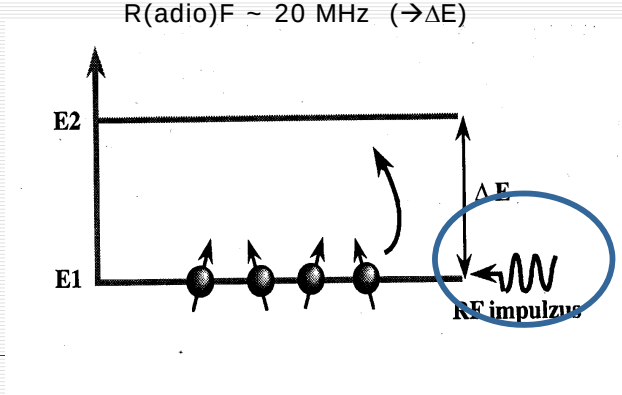
$M_{xy}=0$

Excitation by hf electromagnetic radiation: resonance at a given Z ⟶ protons in one layer

$\Delta E = 2\mu B = hf$

Electromagnetic radiation is by the AC field of an induction coil.

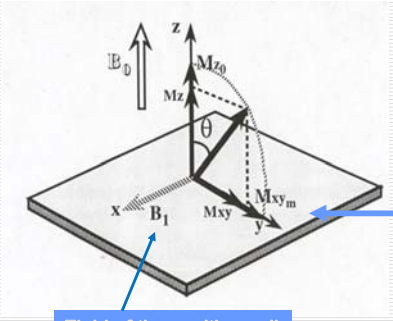
R(adio)F ~ 20 MHz (→ΔE)



Effect of radiation - in one slice at a given Z

- 1. Energy-transition E1 ⟶ E2
- 2. Change of orientation parallel ⟶ antiparallel
- 3. The phase of external radiation is enforced onto the precession of moments
  ⟶ moments turn around *in phase*

The change of orientation in the presence of radiation takes time



Field of the exciting coil precession also around B1
$h\nu_1 = 2\mu B_1$

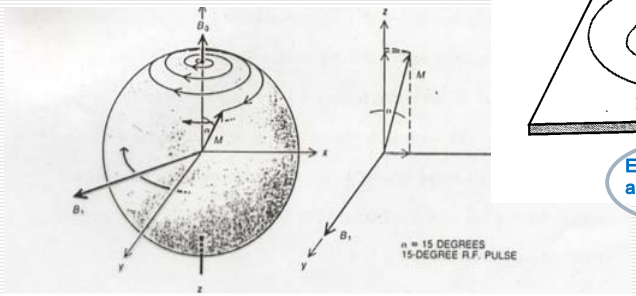
Protocols depending on the duration time

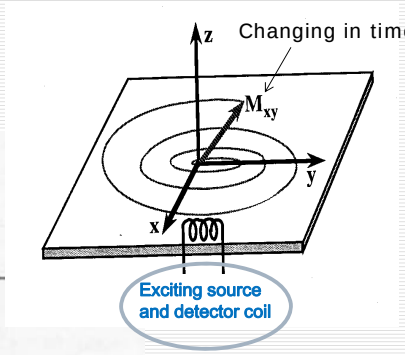
90 degrees pulse technique
Pulse lasts until turning into the XY plane

180 degrees pulse
Total conversion

Change of orientation is by precession → changing magnetic field in the XY plane induces voltage in the detector coil.

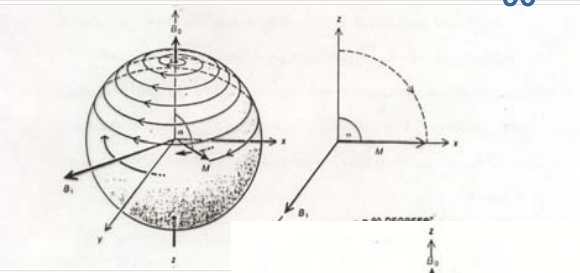
e.g. at 15°



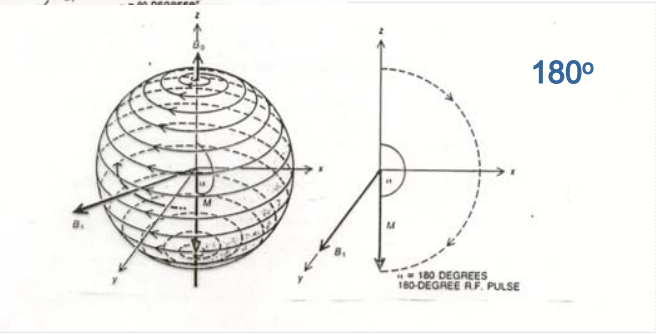


Some phases in excitation

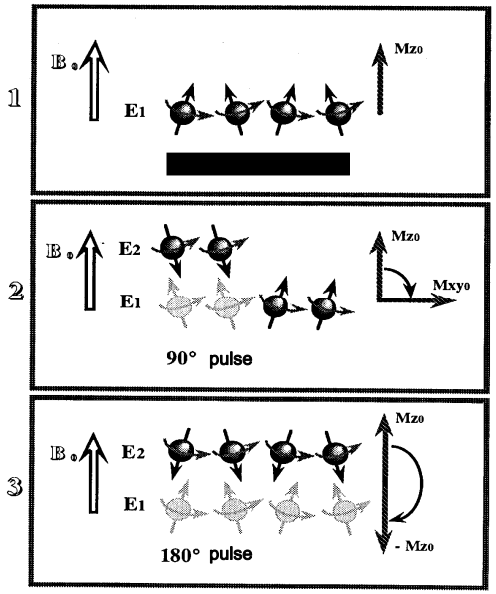
90°



180°



Change of orientation in energy-level concept



90° -pulse

50% change In orientation

M_z=0

We discuss the case of using 90° pulse

Data for the MR-image are obtained from the relaxation of orientation after switching the exciting pulse off

Example: relaxation after 90° pulse

M_z changes from 0 to max (relaxation time T1)

M_{xy} changes from max to 0 because of :

-change in orientation (back to parallel)

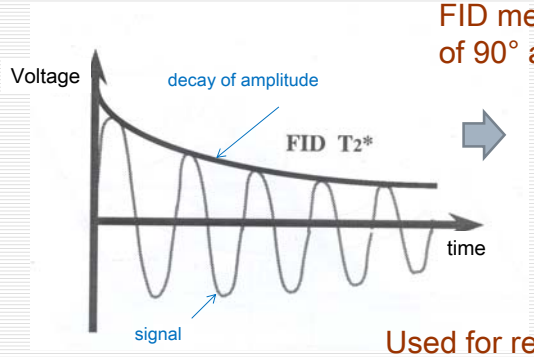
-coordiantion of precession is degrading (relaxation time T2)

ρ density of protons ~ magnitude of the signal

(In real measurement: combination of 90° and 180° pulses)

Measured signal: induced voltage in the coil placed in the XY plane:

Free Induction Decay



FID measured after combination of 90° and 180° pulses

τ_1, τ_2, ρ determined

Used for regulating pixel brightness: *Imaging*

Diagnostic significance of the parameters

T1 spin-lattice relaxation time

Surrounding molecules

T1: 500 – 1000 ms

Max signal is proportional to the ρ proton density

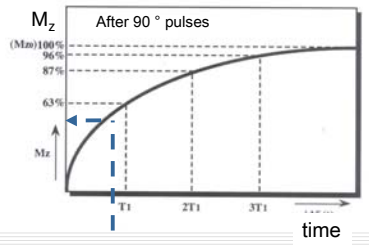
T1

$$M_Z = M_{Zo}\left(1 - e^{-\frac{t}{\tau}}\right)$$

Relaxation is fast (T1 small) if molecular vibrations can absorb the ΔE photonenergy

ΔE is small→ collective motions of large molecules can be excited
e.g. Hydrogens of large proteins, or of lipid compartments

$$f_{water} >> f_{Larmor}$$



e.g.T1 small--- Mz(t) large --- bright pixel --- lipids are bright

Spin-lattice relaxation times (T1) of various tissues at f_{Larmor}=100 MHz

Szövet	Tissue	T_1 (s)
bör	skin	0.62 ± 0.02
vállizom	muscle	1.02 ± 0.03
nyelőcső	gullet	0.80 ± 0.11
gyomor	stomach	0.77 ± 0.07
máj	liver	0.57 ± 0.03
lép	spleen	0.70 ± 0.05
tüdő	lung	0.79 ± 0.06
csont	bones	0.55 ± 0.03
prosztata	prostate	0.80 ± 0.01
vese	kidney	0.86 ± 0.03
agy	brain	1.00 ± 0.02

Diagnostic significance of the parameters

T2 spin-spin relaxation time

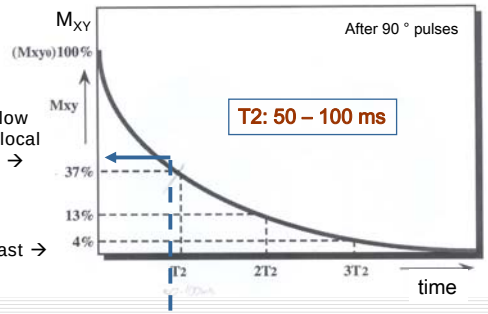
T2

$$M_{XY} = M_{XY,0}e^{-\frac{t}{\tau}}$$

Surrounding local nuclear magnetic fields – inhomogeneity helps in loss of coordination in precession

Large molecules→ proteins, lipids → slow vibration → maintained differences in local B during relaxation → rapid relaxation → T2 is small

Aqueous environment: motions are fast → differences are quickly averaged → slow loss of coordinated precession



Lipids ->T2 small-> Mxy small -> pixel is dark

Aqueous -> T2 large-> bright pixel

How to define the pixels within the XY plane of a measred slice?

1. The excitation frequency selects a certain cross sectional slice

   hf= 2μB(Z) f → B → z all protons at z will respond

2. *B(X) gradient field* applied during relaxation *changing linearly with the X position →*

   hf= 2μ (B(Z) + B(X)) → precession f changes

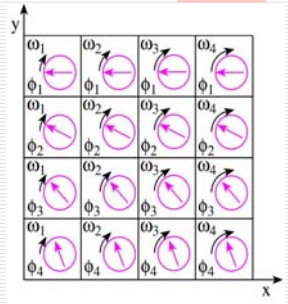
3. *B(Y) gradient field* applied for a short time at the beginning of relaxation *changes the phase* of the signal linearly in function of Y

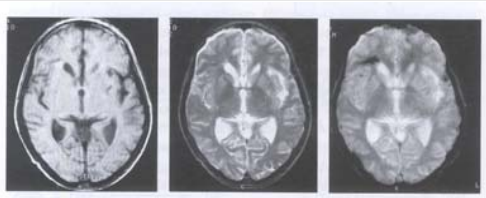
*The **signal** in the induction coil is **the sum of AC signals of distinct frequency and phase***

$\omega = 2\pi f$

Fourier analysis →
resolving the contribution of each f and ϕ pixels in XY →

$\rho_{i,j}, \tau_{1,i,j}, \tau_{2,i,j}$





T1 coding
White matter is bright

T2 coding
Grey matter is bright

Combined coding involving ρ

MRI as diagnostic method

-**non-invasive** – but contrast materials are used

-Metabolic/chemical **fine details of soft tissues** are differentiated

-primarily brain diagnostics (lipids), but very broad field:

neck, chest, abdomain (liver, spleen, pancreas, kidney….) joints, muscles of the sceleton

-Bone does not cause shadow: **spinal cord can be seen** ⟷ US, CT

-**Good resolution**: ~5 mm slices, 1.5x1.5 mm pixels– very good

-**3D reconstruction**

But: - very expensive method (device: large B, superconducting coil..)
- multiple slices require long data acquisition time
- B leads to problems – pace makers, implants, etc.

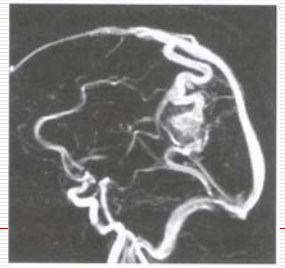
END of lecture in 2015

Additional reading: Special MRI techniques – progress

1. Angiography

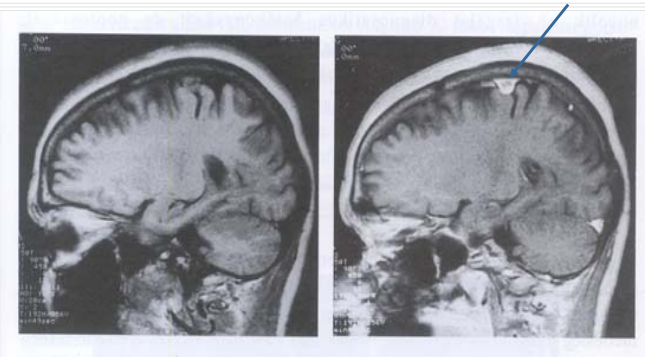
Blood flow in or out of the slice changes the signal in the relaxation -> analysis yields the pixels of blood vessels

Malformation in the region of artéria cerebri média in
arterio-venosus flow
-based on phase analysis



2. Use of contrast enhancement: T1 és T2-contrast

T1 weighted image for diagnostics of meningeoma
Gadolinium contrast makes the tumor bright



Paramagnetic atoms : T1 becomes shorter in unhealthy tissues of brain -> bright Gd, Mn, Ba – farmacons

T2 contract atoms are ferromagnetic: shorten T2 in healthy tissues → dark

3. Functional MRI- fMRI

BOLD : Blood Oxygen Level Dependent signal Ogawa, 1990

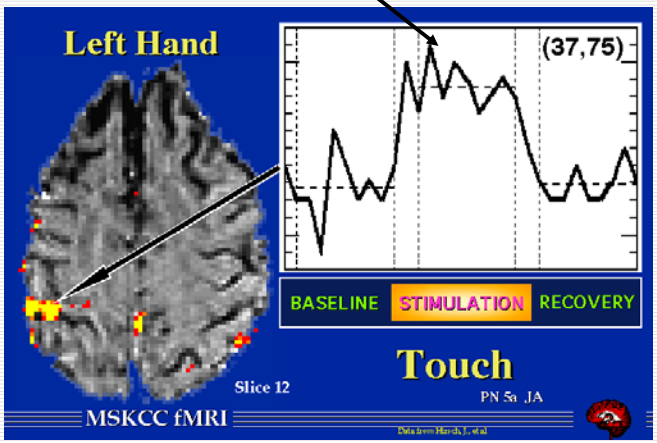
Main idea:
oxy hemoglobin : diamagnetic – no magnetic moment
deoxy hemoglobin: paramagnetic – significant momentum

=> ***Hb oxydation state acts as endogen contrast agent***

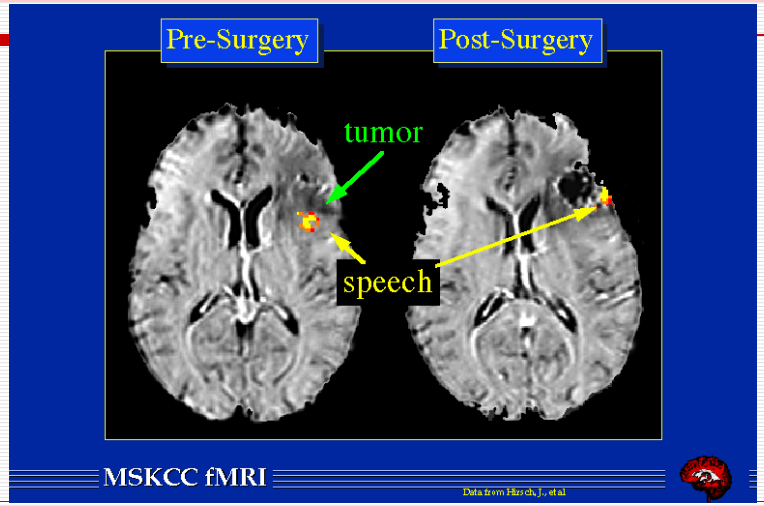
Used in diagnostics of functional regions in brain:
visual cortex, motor cortex, speech center

Neuron activity ↑ blood flow ↑ oxyHb ↑ T2 ↑ signal intensity ↑

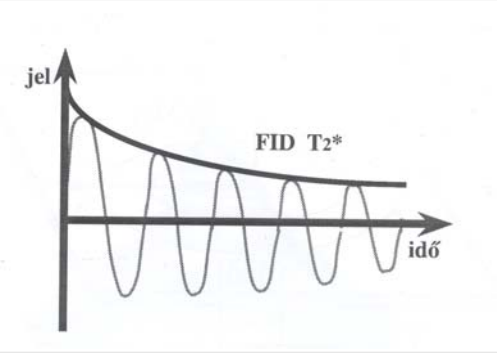
Haemodynamic response functions – short detection time: 1-2 minutes



fMRI – surgical applications: functionally important regions are visualized before surgical intervention



Az indukált feszültség valódi jelalakja : Free Induction Decay FID



T1 és T2 paraméterek megjelenítése

⟷ Mz(t) és Mxy(t) mérése ?

Gyakorlati megvalósítás: impulzus-gerjesztések kombinációjával