BIOMOLECULAR STRUCTURAL DYNAMICS

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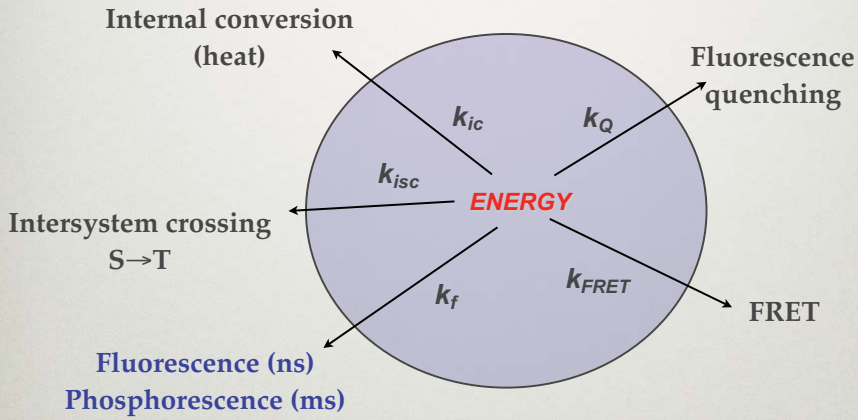
OBJECTIVES OF INVESTIGATION

- Understanding biomolecular **structure**
- and **function** (processes, states, transitions, interactions, movement, etc.)

FATE OF ABSORBED ENERGY



Radiative *and* non-radiative transitions!

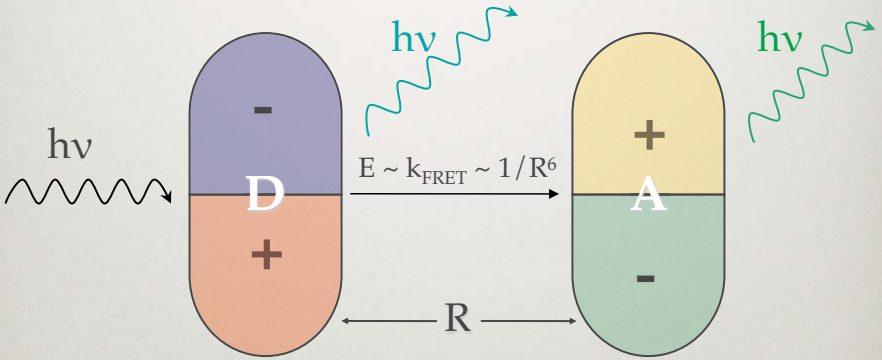
1. FLUORESCENCE RESONANCE ENERGY TRANSFER

In general:

- Occurs by non-radiative dipole-dipole interaction between an excited ***donor*** and an proper ***acceptor*** molecule under certain conditions (spectral overlap and close distance).
- ***Fluorescence Resonance Energy Transfer (FRET):*** if the participants of the transfer are fluorophores.

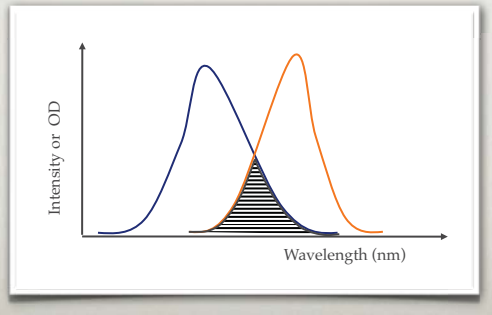
FRET

- Acceptor (A) emission contributes to the relaxation of the excited donor (D) molecule.



Conditions of FRET

- **Fluorescent** donor and acceptor molecules.
- The distance **(R)** between donor and acceptor molecules is 2-10 nm!
- **Overlap** between the emission spectrum of the donor and the absorption spectrum of the acceptor.

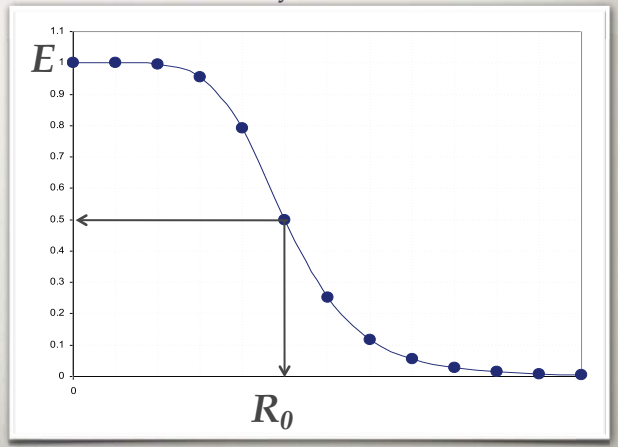


Distance dependence of FRET

$$E = \frac{R_0^6}{R_0^6 + R^6}$$

Förster-distance
(Distance at which transfer efficiency (E) is 0.5)

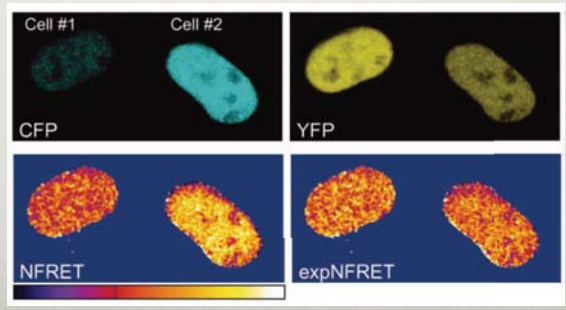
Actual distance between fluorophores



Applications of FRET

- **Molecular ruler:** distance measurement on the nm (10^{-9}m) scale.
- High sensitivity!
- **Applications:**
  - Measurement of ***interactions*** between molecules.
  - Measurement of ***structural*** changes on molecules.

FRET microscopy

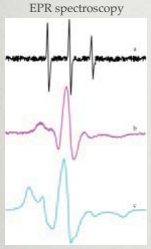


2. "Radiospectroscopies":

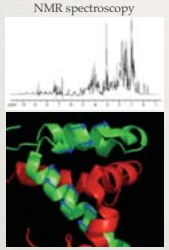
revolutionized physics, chemistry, biology and medicine

- Electronspin resonance (***ESR***, electron paramagnetic resonance - ***EPR***)
- Nuclear magnetic resonance (***NMR***, ***MRI***)

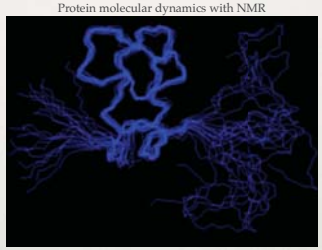
EPR spectroscopy



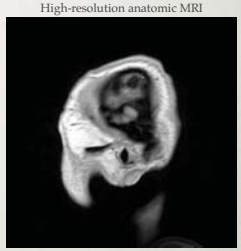
NMR spectroscopy



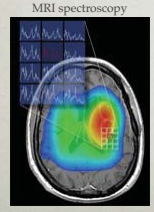
Protein molecular dynamics with NMR



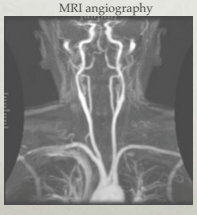
High-resolution anatomic MRI



MRI spectroscopy

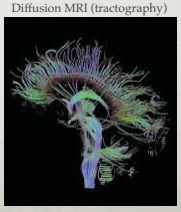


MRI angiography

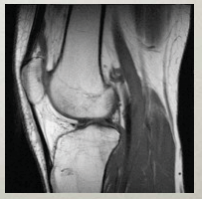


Functional MRI (fMRI)

Diffusion MRI (tractography)



Musculoskeletal MRI

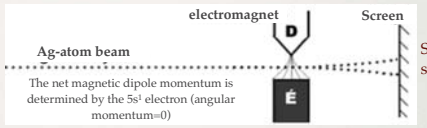


Atomic, molecular systems may behave as elementary magnets

Stern-Gerlach experiment (1922)

Otto Stern (1888-1969)

Walther Gerlach (1889-1979)



Silver beam splits in two

In an inhomogenous magnetic field, in addition to torque, net force arises on the magnetic dipoles:

The spin magnetic moment may take on two values (+1/2, -1/2)

Nuclear magnetic resonance, NMR
Nobel-prize, 1952

Isidor Rabi (1898-1988)

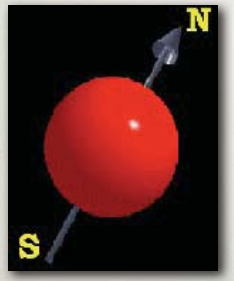
Felix Bloch (1905-1983)

Edward Mills Purcell (1912-1997)

Magnetic resonance: resonance-type absorption of electromagnetic energy by a sample placed in magnetic field.

Systems with net spin: elementary magnets

- Elementary particles (p, n, e) have ***spin*** (due to angular momentum).
- Based on the number of elementary particles and certain organizational principles within the system (e.g., Pauli exclusion principle), ***net spin*** may arise.
- Nucleus: odd mass number - half nuclear spin (^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P); even mass number, odd atomic number - nuclear spin is whole number; even mass and atomic numbers - nuclear spin is zero.
- Electron: net electron spin only in molecules containing stable unpaired electrons (e.g., free radicals).
- Because of spin and charge, the system possesses ***magnetic momentum***.



Top

Nuclear magnetic momentum:

$$M_N = \gamma_N L$$

γ_N = gyromagnetic ratio (ratio of magnetic momentum and angular momentum)

L = nuclear spin ($L = \sqrt{l(l+1)}\hbar$), l=net spin quantum number.

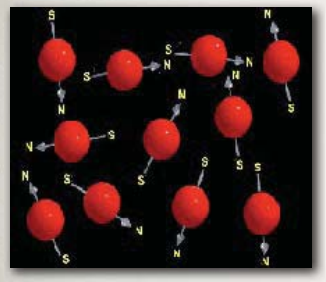
Electron magnetic momentum:

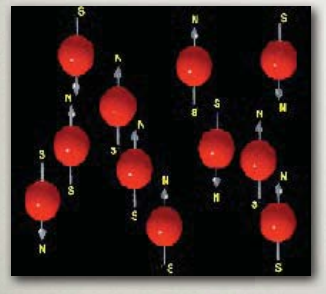
$$M_e = -g\mu_\beta \sqrt{S(S+1)}$$

g = electron's g-factor (dimensionless ratio that describes the relationship between magnetic momemtum and gyromagnetic ratio)

μ_β = Bohr magneton (unit of the electron's magnetic dipole momentum)

S = spin quantum number





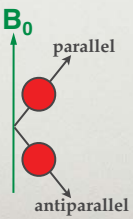
In the absence of magnetic field:

orientation of elementary magnets is random

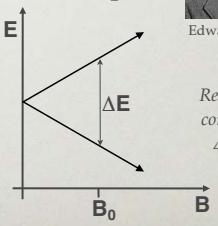
Paramagnetism: magnetism arises upon placing the sample in external magnetic field (magnetic dipoles become oriented).

In magnetic field:

elementary magnets become oriented



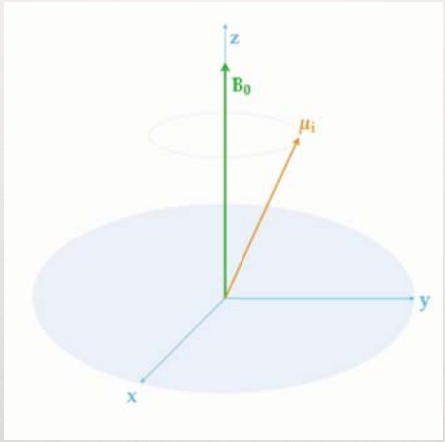
energy levels split



Edward Purcell, 1946

Resonance condition: ΔE=hf

Nuclear Magnetic Resonance: Spin Precession



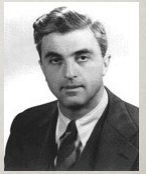
Precession or Larmor frequency:

$$\omega_0 = \gamma B_0$$

$$f_{Larmor} = \frac{\gamma}{2\pi} B_0$$

Resonance condition:

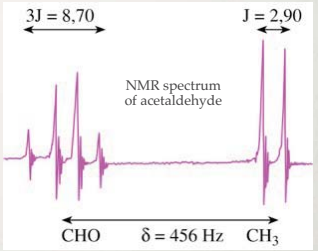
$$\Delta E = \frac{h\omega_0}{2\pi}$$



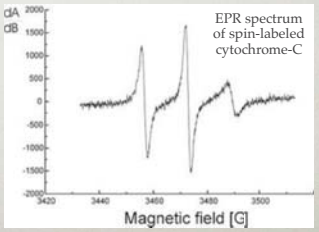
Felix Bloch, 1946

NMR and EPR spectroscopy

- NMR spectrum: intensity of absorbed electromagnetic energy as a function of frequency.
- Area under the "NMR-line" is proportional to the number of nuclei.
- Presence (and structure) of local electron cloud affects the local magnetic field: frequency condition is shifted ("chemical shift"). It opens the possiblity of chemical structure determination.



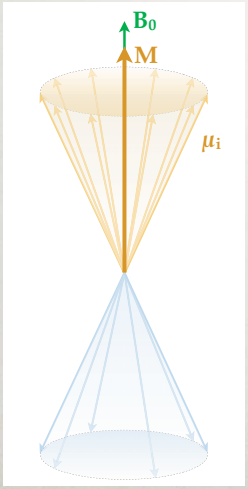
- EPR spectrum: intensity of absorbed electromagnetic energy as a function of magnetic field.
- Magnetic field is smaller, and frequency of EM radiation is greater (microwave) than in NMR.
- Spin-labeling: attachment of a molecule containing stable unpaired electron (radical).
- Rotational dynamics in the time range of 10^{-4} - 10^{-2} s may be measured.



Net magnetization

Due to spin access in different energy states

Low energy state
parallel in case of proton



High energy state
antiparallel in case of proton

B_0 = magnetic field
M = net magnetization

Ratio of magnetic spins in high- (antiparallel) and low-energy (parallel) states:

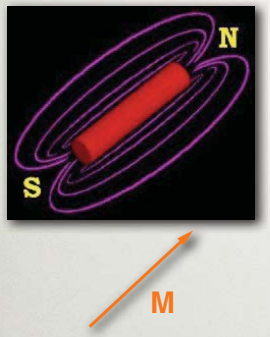
$$\frac{N_{antiparallel}}{N_{parallel}} = e^{-\frac{\Delta E}{k_B T}}$$

Boltzmann distribution

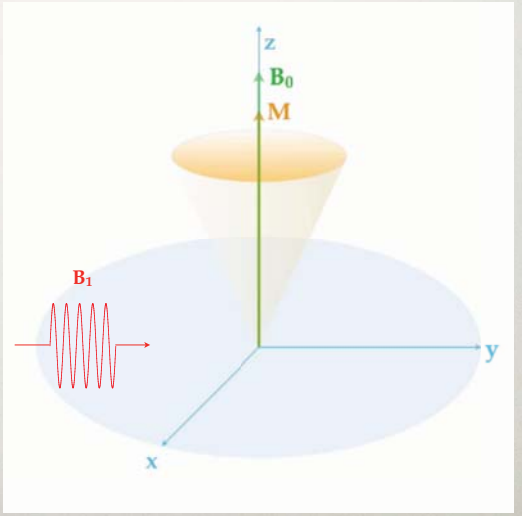
Excitation

Using radio frequency radiation

Resonance condition: Larmor frequency

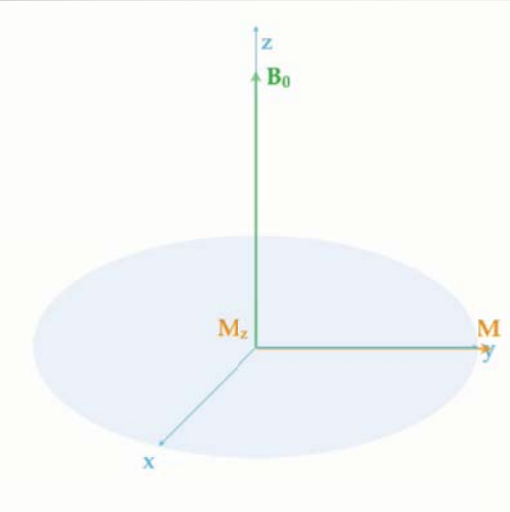


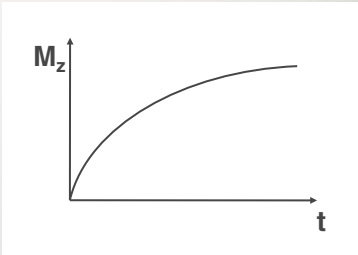
B_0 = magnetic field
M = net magnetization
B_1 = irradiated radio frequency wave



Spin-lattice relaxation

T1 or longitudinal relaxation

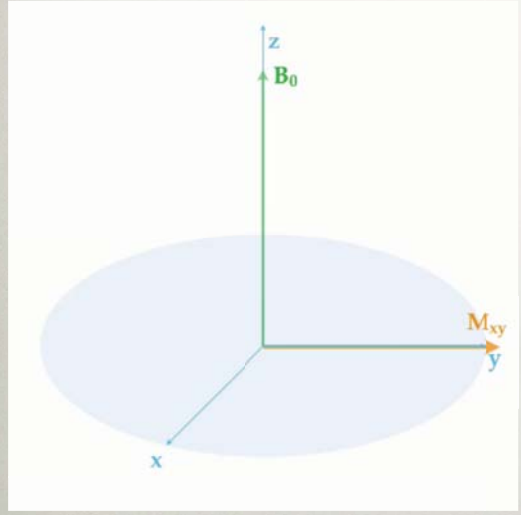


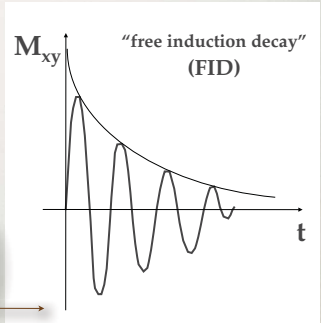


T1 relaxation time:
depends on interaction
between elementary magnet (proton)
and its environment

Spin-spin relaxation

T2 or transverse relaxation



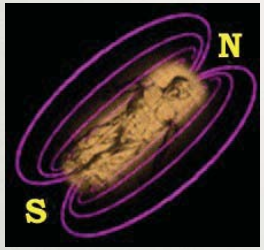


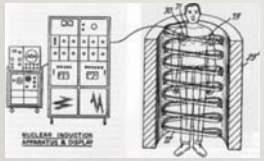
T2 relaxation time:
depends on interaction between
elementary magnets (protons)

MRI is a non-invasive "tomographic" method

MRI:

Net magnetization of the human body is generated





Damadian's patent figure

Raymond V. Damadian
(1936-)

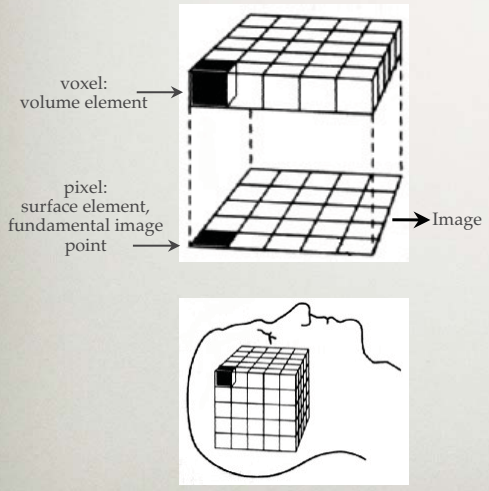
"Indomitable"

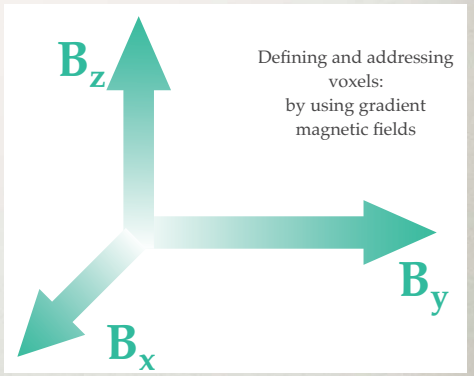
Nobel-prize (2003)

Paul C. Lauterbur
(1929-)

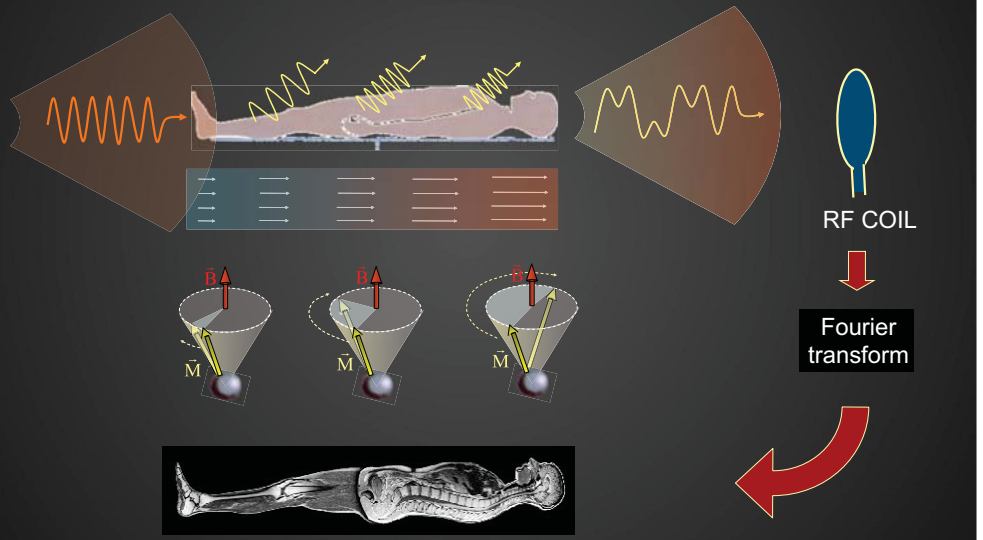
Peter Mansfield
(1933-)

MRI Imaging I:
Spatial resolution

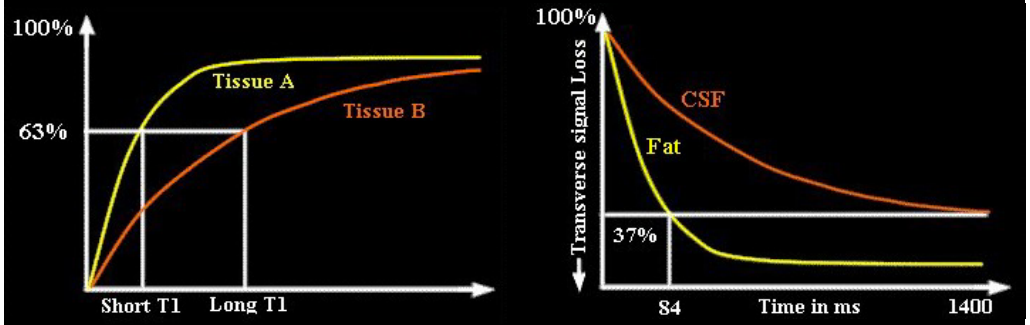


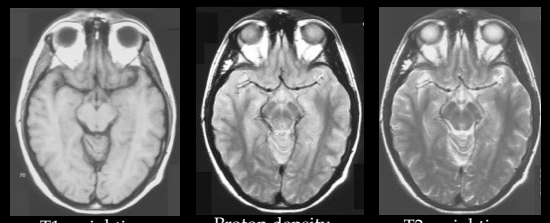


Spatial encoding of the NMR signal is based on frequency changes in the precession

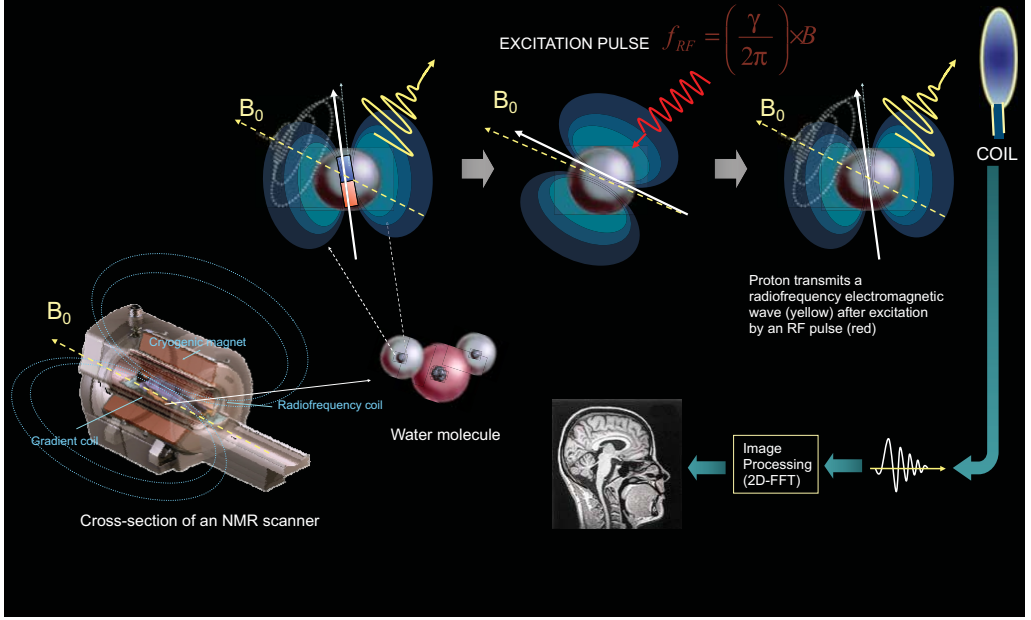


MRI Imaging II:
Color depth based on relaxation times



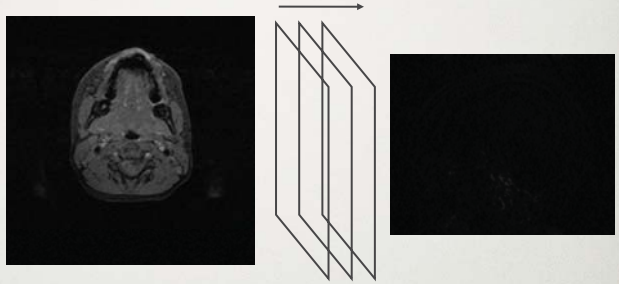


Nuclear Magnetic Resonance Imaging: Summary

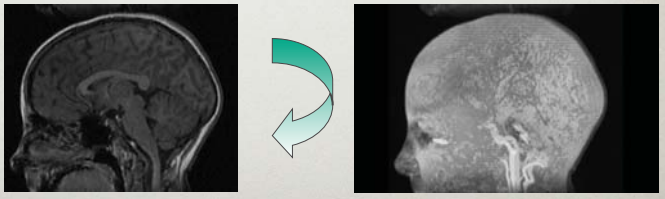


MRI:
Image manipulation

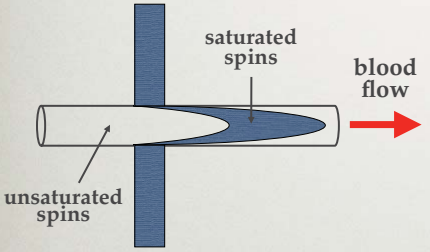
Reslicing in perpendicular plane



Spatial projection („volume rendering")



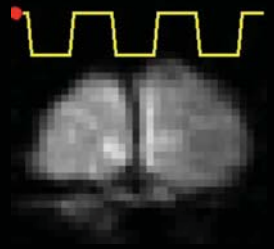
MRI:
Non-invasive angiography



MRI movie
Based on high time resolution images

Opening and closing of aorta valve

Functional MRI (fMRI)
High time resolution images recorded synchronously with physiological processes



Effect of light pulses on visual cortex

Superposed MRI and PET sequence

PET activity: during eye movement
Volume rendering