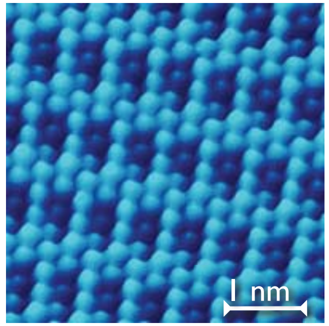
Structure and dynamics of Biomolecular systems

Fluorescence, Single-molecule biophysics, radio spectroscopies (EPR, NMR, MRI)

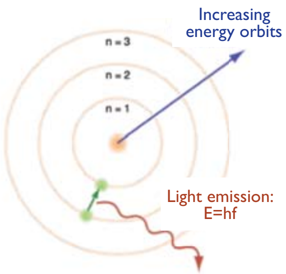
John Dalton (1766-1844)



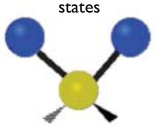
Oxygen atoms on a rhodium single crystal

Fluorescence and its special applications

Energy transitions in an atom

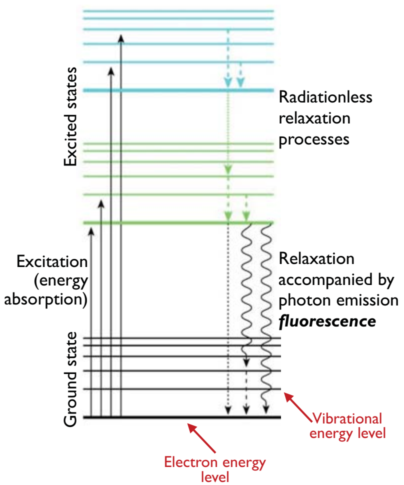


The energy of a molecule is influenced by its motional states



Vibration

Energy transitions in a molecule

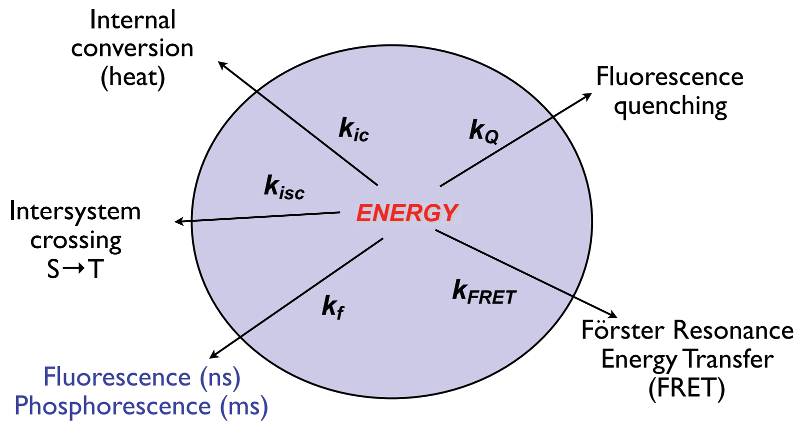


Fluorescence in a genetically modified species:

GFP: ~27 kDa, 238 aa; 11-strand β-barrel; chromophore formed from the Ser65-Tyr66-Gly67 residues of the central helix

"Green mouse"

Fate of absorbed energy

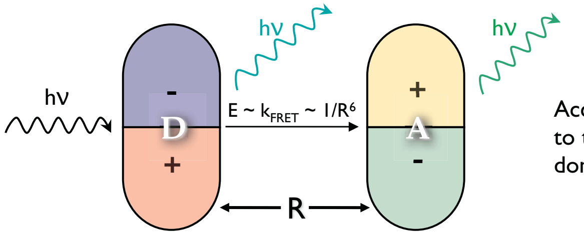


Radiative and non-radiative transitions!

Förster Resonance Energy Transfer (FRET)

- Occurs by non-radiative dipole-dipole interaction between an excited ***donor*** and an proper ***acceptor*** molecule under certain conditions (spectral overlap and close distance).
- ***F**luorescence **R**esonance **E**nergy **T**ransfer (FRET):* if the participants of the transfer are fluorophores.

Theodor Förster
(1910-1974)



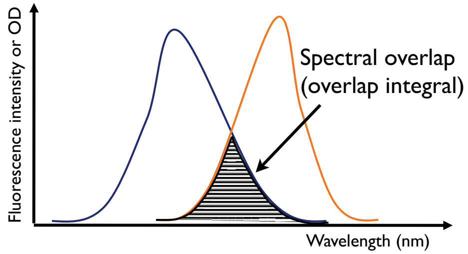
Acceptor (A) emission contributes to the relaxation of the excited donor (D) molecule!

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.

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

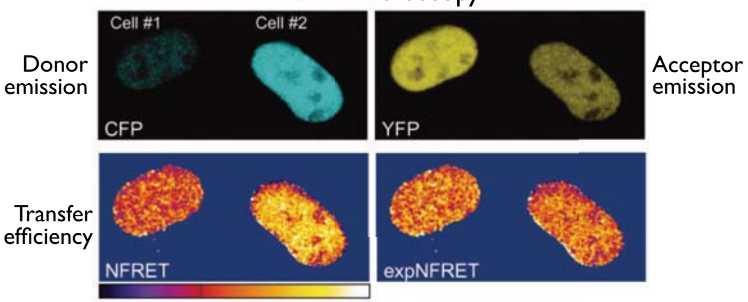
R_0: **Förster-distance**
(Distance at which transfer efficiency (E) is 0.5)
R: Actual distance between fluorophores (D-A)



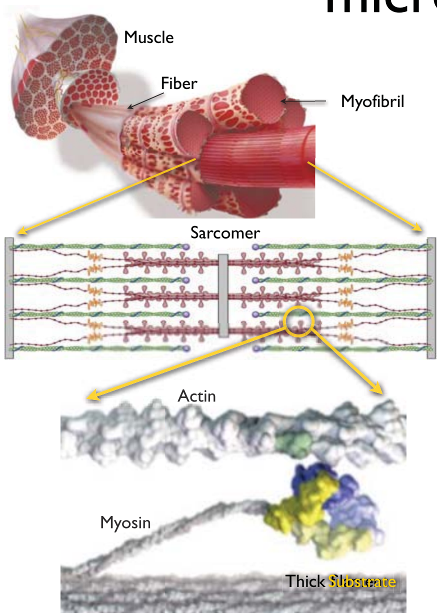
Application of FRET

- **Molecular ruler:** distance measurement in the nm (10^{-9}m) range.
- Highly sensitive (see power function)!
- **Applications:**
  - Measurement of **intermolecular interactions**.
  - Measurement of **intramolecular structural changes**.

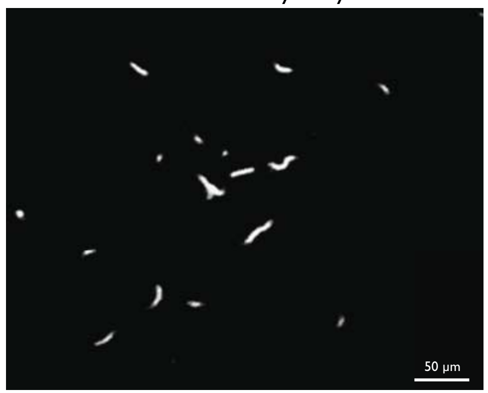
FRET mmicroscopy



Sensitized fluorescence video microscopy



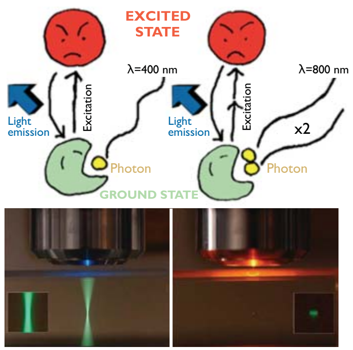
In vitro motility assay



Motility of rhodamine-labeled single actin filaments on a myosin-coated surface

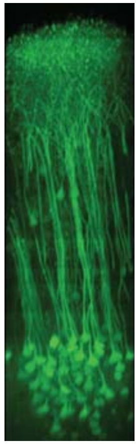
Multiphoton fluorescence microscopy

- •Energy of two or more photons is summed during excitation
- •Excitation, hence emmission, is only in the focal point (limited photodamage)
- •Excitation with long-wavelength (IR), short (fs) light pulses
- •Large (upt to 2 mm) penetration distance due to long wavelength

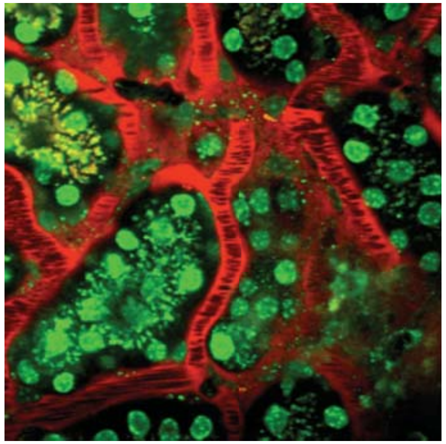


Single photon fluorescence

Multiphoton fluorescence



Cortical pyramidal cells



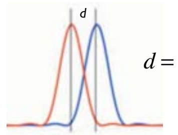
Green: proximal kidney tubules; Red: albumin (plasma)

Super-resolution microscopy

Nobel-prize in chemistry, 2014

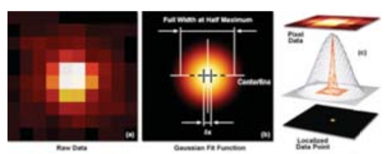
Resolution problem is converted into position-determination problem

Resolution problem (Abbé)

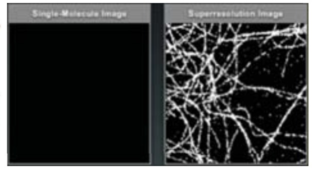


$$d = \frac{0.61\lambda}{n \sin\alpha}$$

Position determination problem
(precision depends on photon count)

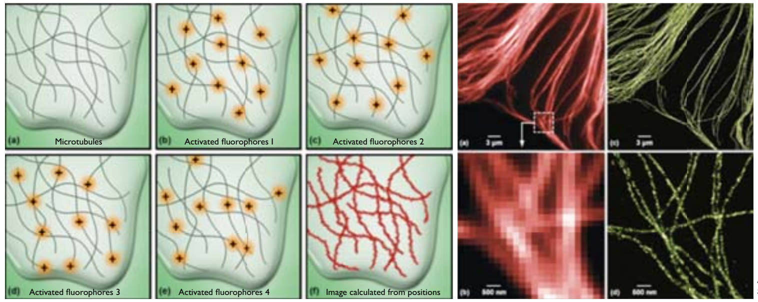


"Stochastic" data collection, single fluorophores



STORM ("stochastic optical reconstruction microscopy"); PALM ("photoactivated localization microscopy")

Data collection process



Microtubular system

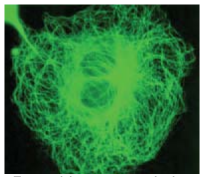
Resolved distance:

$$! = \frac{!}{\sqrt{"}} \frac{\lambda}{"\#\#\$\% \alpha}$$

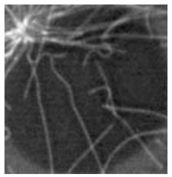
N: photon number

Single-molecule biophysics

1. Individuals (spatial and temporal trajectories) can be idenditfied in an ensemble



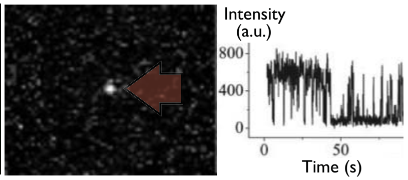
Ensemble - microtubular system



Single microtubules - treadmilling

2. Stochastic events may be discovered

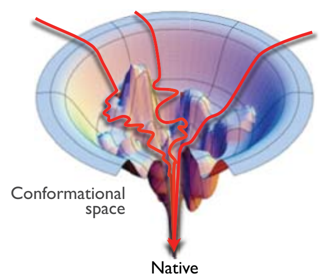
Ensemble - intensity



Single quantum dot - blinking

3. Parallel-pathway processes may be described

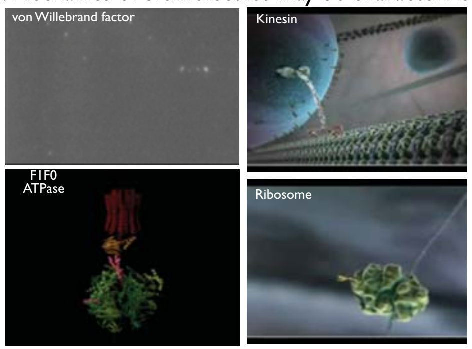
Unfolded state



Conformational space

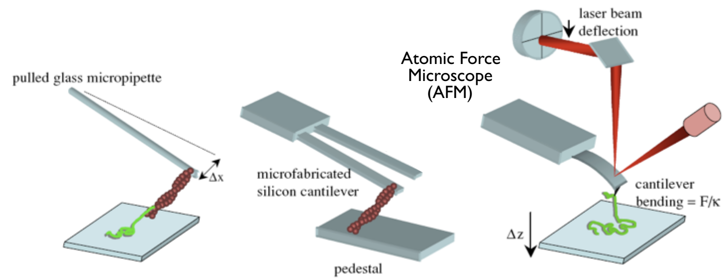
Native state

4. Mechanics of biomolecules may be characterized

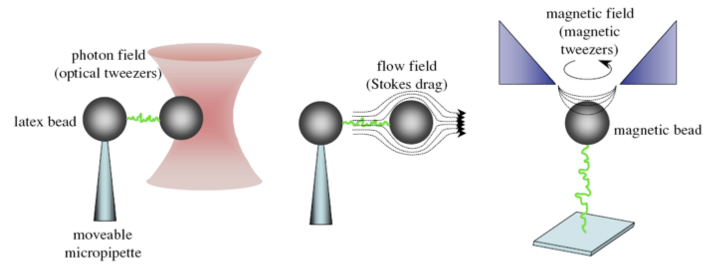


Manipulation of single molecules

Cantilever methods

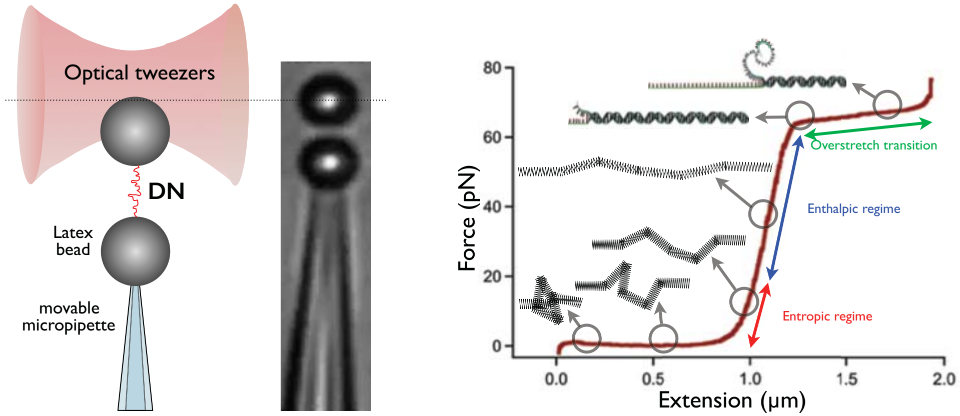


Field-based methods



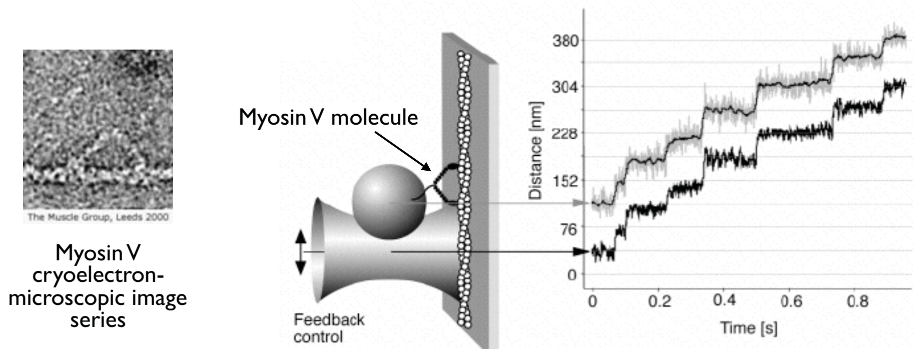
Measurable parameters I. Force

How much force develops during the stretch of a single dsDNA molecule?



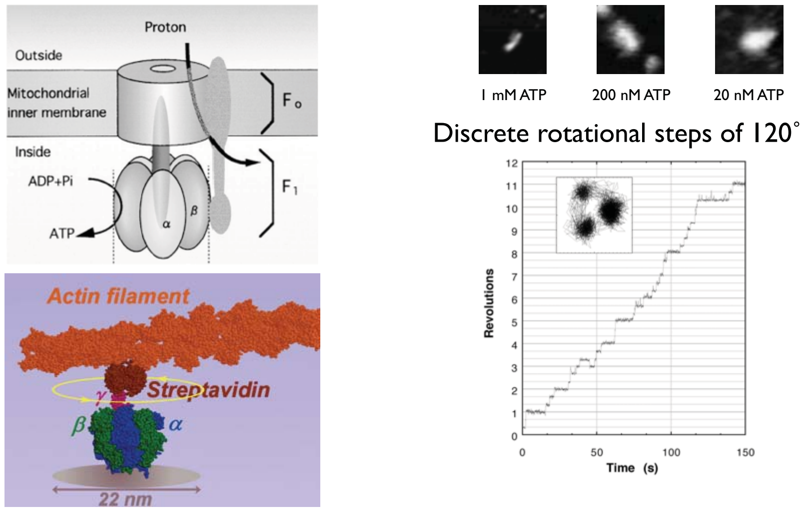
Measurable parameters II. Distance

What is the step size of a motor protein?



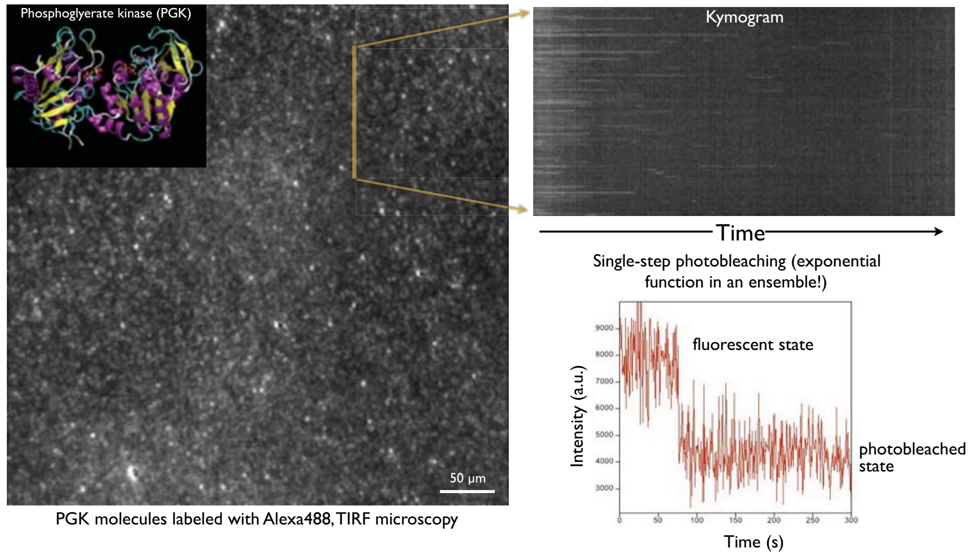
Measurable parameters III. Rotational angle

How does the ATP synthase work?



Measurable parameters IV. Fluorescence

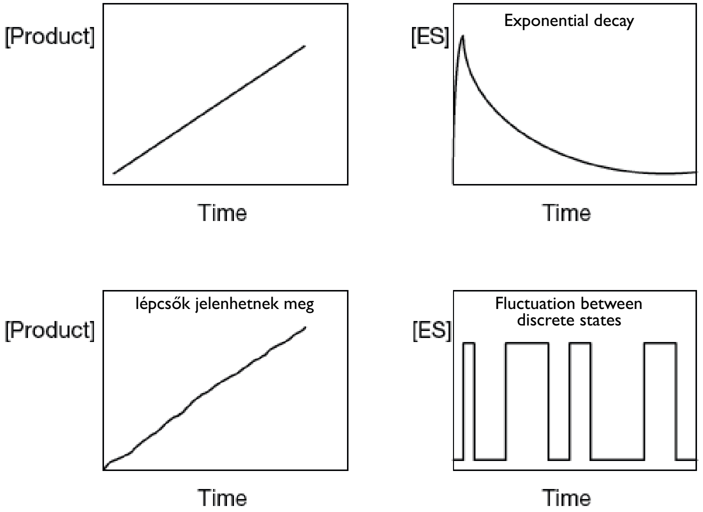
What are the conformational states of a molecule?



Ensemble *versus* single molecule behavior

Ensemble:

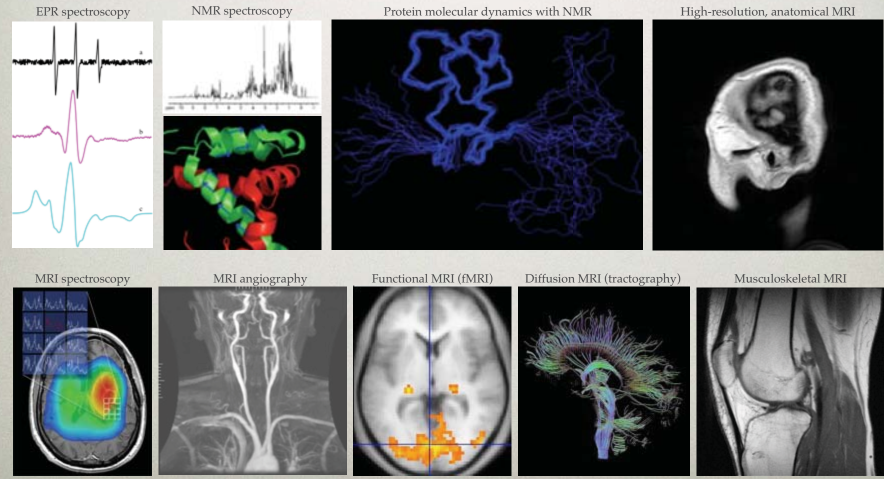
Single molecule:



"Radio spectroscopies":

Revolutionized physics, chemistry, biology and medicine

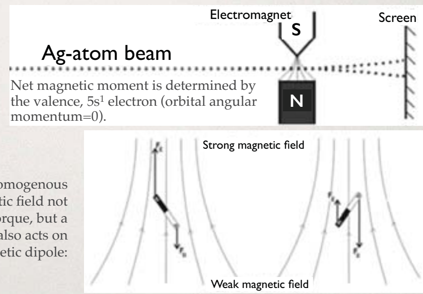
- Electronspin resonance (*ESR*, electron paramagnetic resonance - *EPR*)
- Nuclear Magnetic Resonance (*NMR, MRI*)



Atomic, molecular systems may behave as elementary magnets

Stern-Gerlach experiment (1922)

Otto Stern (1888-1969) Walther Gerlach (1889-1979)



The beam splits in two.

In an inhomogenous magnetic field not only torque, but a net force also acts on the magnetic dipole:

The spin magnetic moment may attain two discrete values.

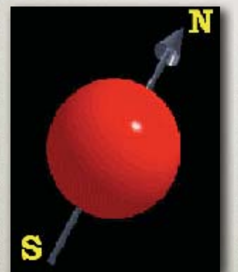
Nuclear magnetic resonance, NMR) Nobel-prize, 1952

Isidor Rabi (1898-1988) Felix Bloch (1905-1983) Edward Mills Purcell (1912-1997)

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

Systems with net spin: elementary magnets

- Elementary particles (p, n, e) have their own ***spin***.
- Depending on the number of elementary particles and organizational principles (e.g., Pauli principle), ***net spin*** emerge within the system.
- Atomic nucleus: odd mass number - half nuclear spin (^1H, ^{13}C, ^{15}N, ^{19}F, ^{31}P); even mass number, odd atomic number - whole nuclear spin; even mass and atomic number - zero nuclear spin.
- Electron: net electron spin within a mmolecular system containing a stable unpaired electron (e.g., free radicals).
- Because of charge and net spin ***magnetic moment*** emerges.



Nuclear magnetic moment:

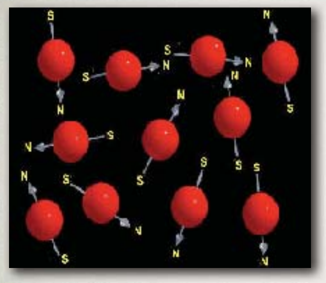
$$M_N = \gamma_N L$$

γ_N = gyromagnetic ratio (ratio of magnetic moment and angular momentum.)
L = nuclear spin ($L = \sqrt{l(l+1)}\hbar$), l= spin quantum number.

Magnetic moment of the electron:

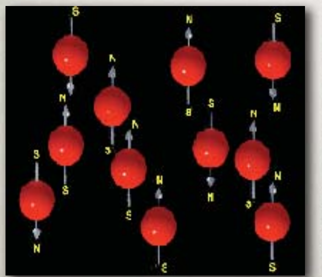
$$M_e = -g\mu_\beta\sqrt{S(S+1)}$$

g = electron's g-factor (dimensionless number that describes the relationship between magnetic moment and gyromagnetic ratio)
μ_β = Bohr's magneton (unit of the electron's magnetic moment)
S = spin quantum number

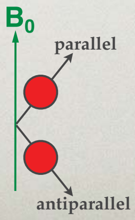


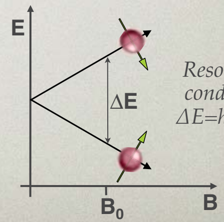
In absence of magnetic field:
random orientation of elementary magnets

Paramagnetism: magnetism emerging in external magnetic field (caused by the orientation of magnetic dipoles).



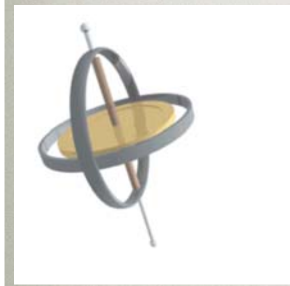
In magnetic field:



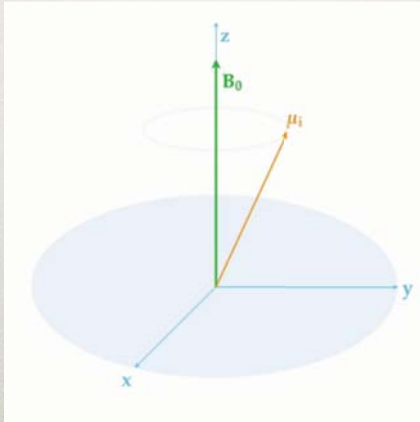


Resonance condition:
$\Delta E = hf = \mu B_0$

Precession



Top, gyroscope



Precession or Larmor frequency:

$$\omega_0 = \gamma B_0$$

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

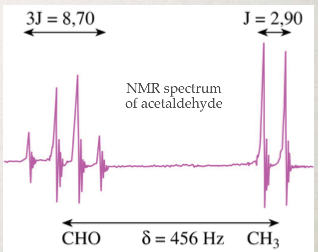
Resonance condition:

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

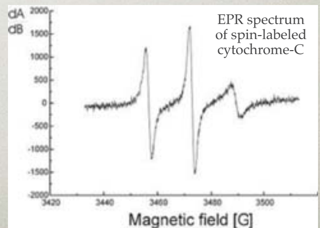
Felix Bloch, 1946

NMR and EPR spectroscopy

- NMR spectrum: intensity of absorbed electromagnetic radtion as a function of frequency.
- The area under the "NMR-line" is proportional to the number of absorbind atomic nuclei.
- The electron cloud distorts the local magnetic field, therefore the frequency condition is shifted: "chemical shift". Chemical structure determination is possible.

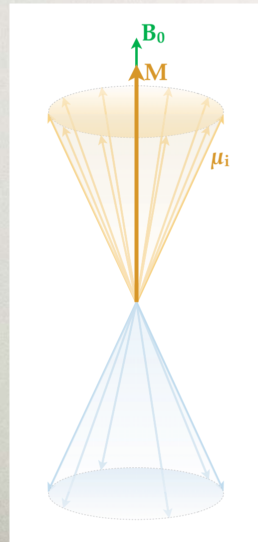


- EPR spectrum: intensity of electromagnetic radiation as a function of magnetic field.
- Magnetic field is lower, but radiation frequencies are greater (microwave) than in NMR.
- Spin-labeling: attachment of a chemical containing a stable unpaired electron.
- Dynamics of rotational motion can be measured ub the 10^{-4} - 10^{-2} s time range.



Net magnetization

Due to spin access in different energy states



B_0 = magnetic field
M = net magnetization

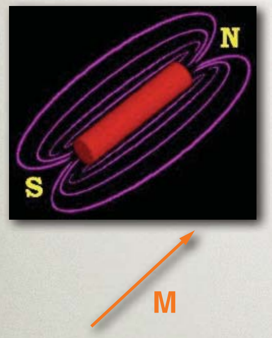
Low-energy state
parallel in case of the proton

High-energy state
anti-parallel in case of the proton

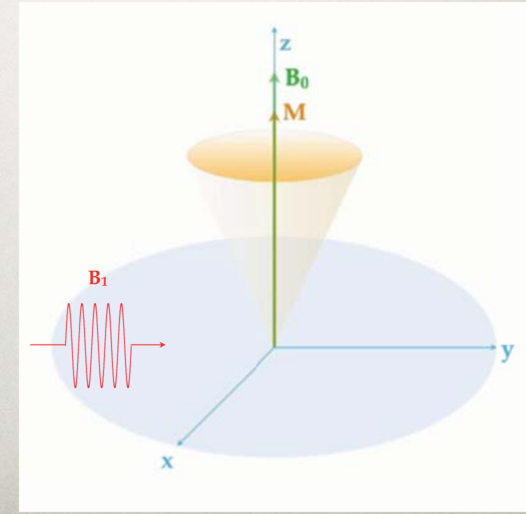
Excitation

with radio frequency electromagnetic radiation

Resonance condition: Larmor frequency

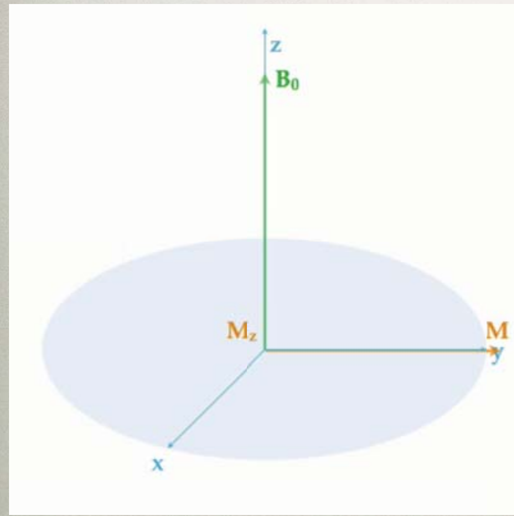


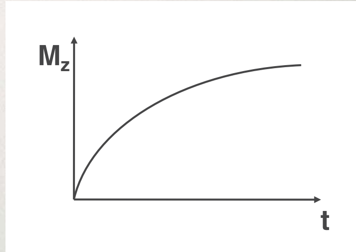
B_0 = magnetic field
M = net magnetization
B_1 = irradiated radio frequency wave



Spin-lattice relaxation

T1 or longitudinal relaxation

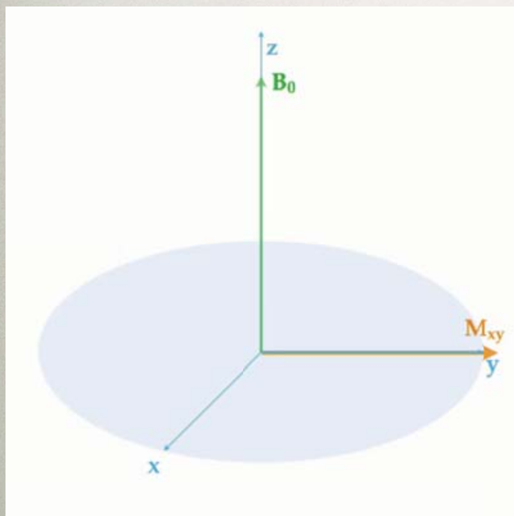


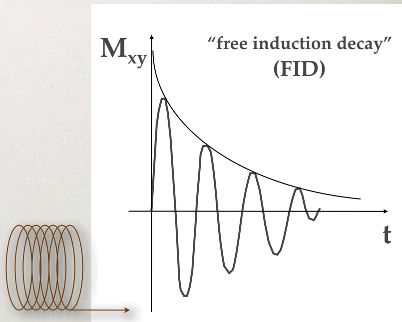


T1 relaxation time:
depends on interaction
between elementary magnet (proton)
and its environment

Spin-spin relaxation

T2 or transverse relaxation

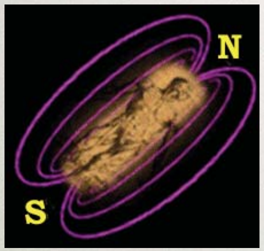




T2 relaxation time:
depends on interaction between
elementary magnets (protons)

MRI:

Net magnetization of the human body is generated



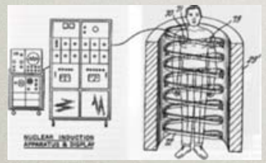
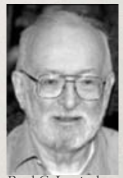


Figure from Damadian's patent file.

Raymond V. Damadian
(1936-)

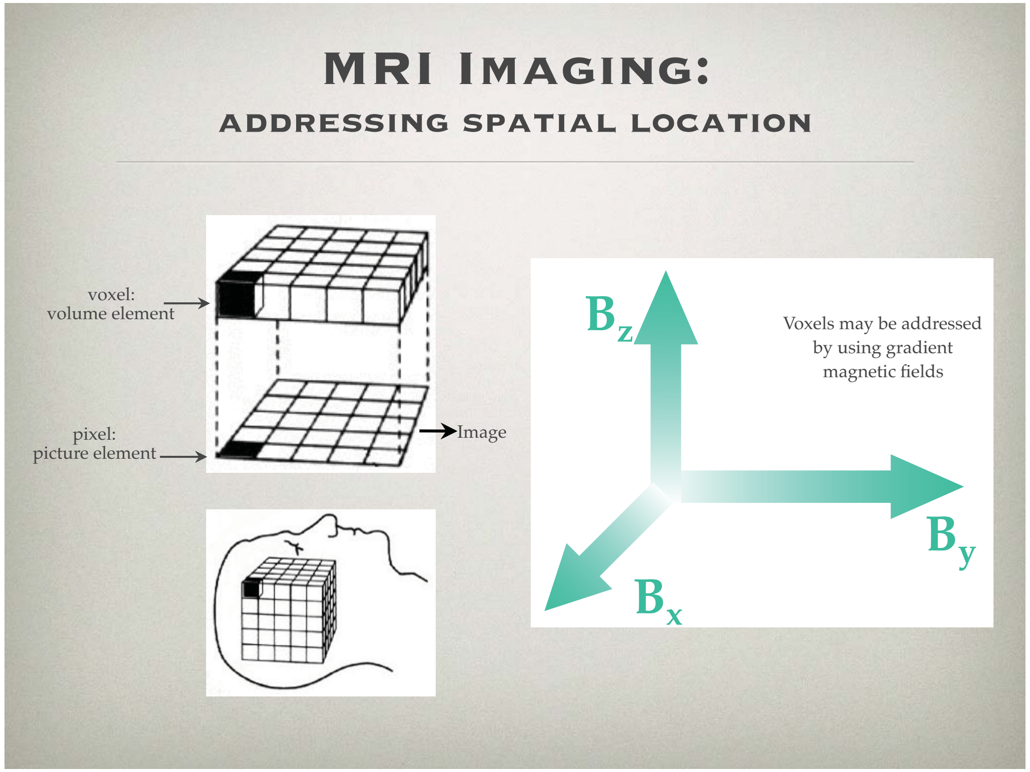
The "Indomitable"

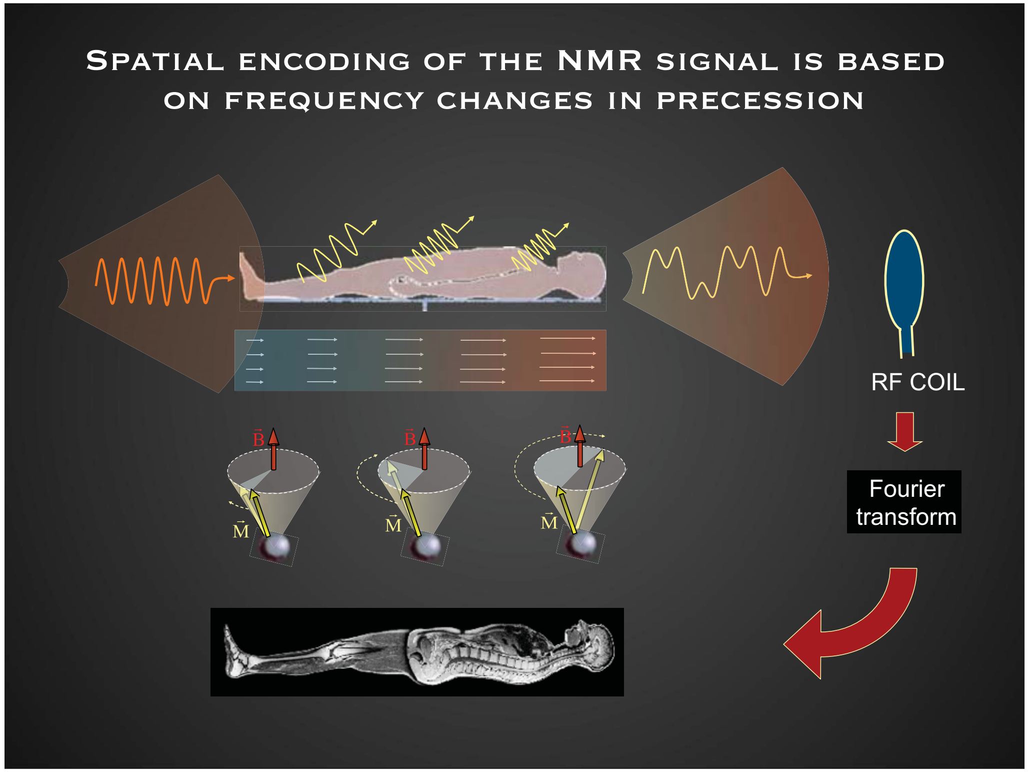
Nobel-prize (2003)

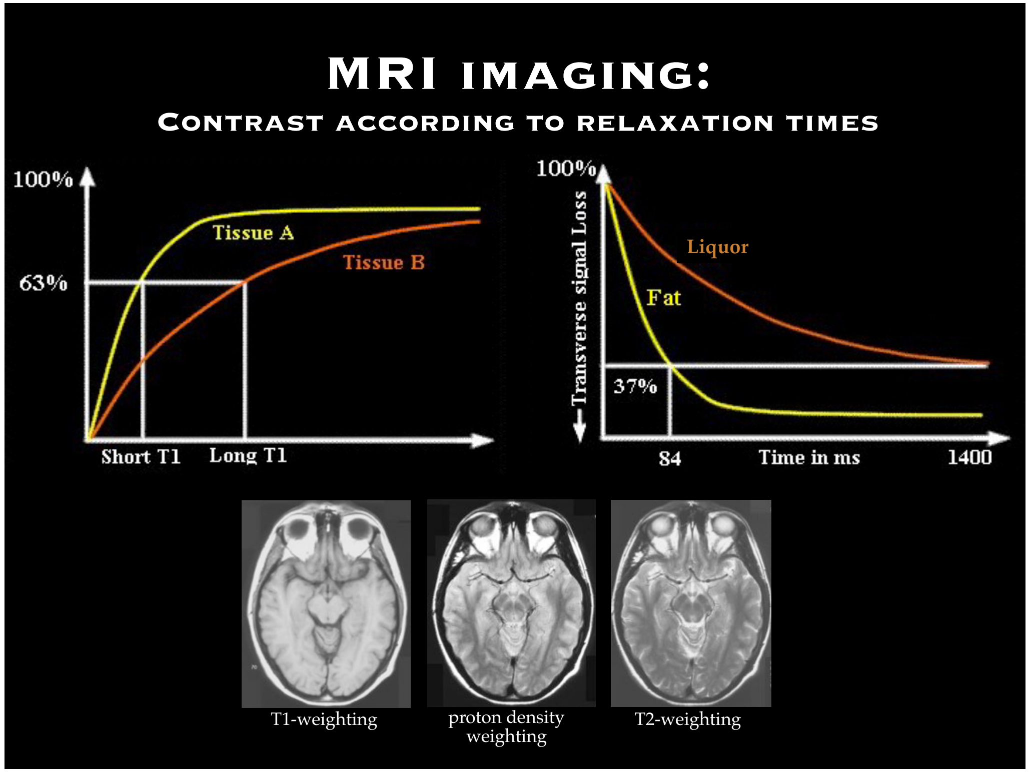


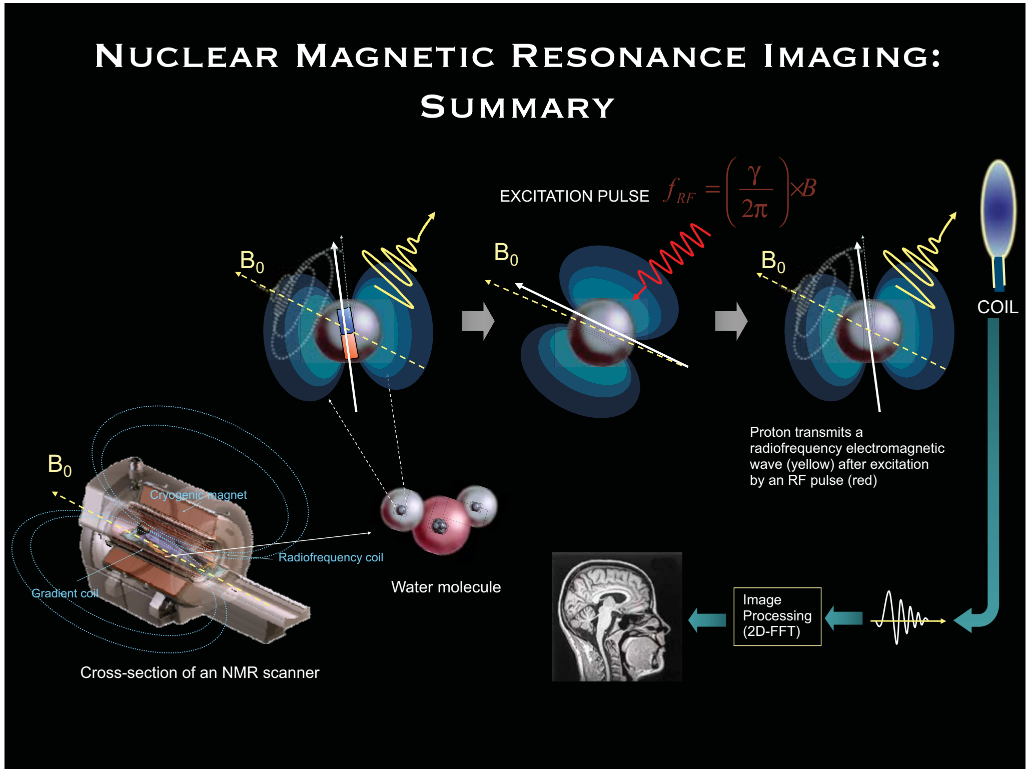
Paul C. Lauterbur
(1929-)

Peter Mansfield
(1933-)


MRI Imaging:
addressing spatial location
voxel:
volume element
pixel:
picture element
Image
Bz
By
Bx
Voxels may be addressed
by using gradient
magnetic fields

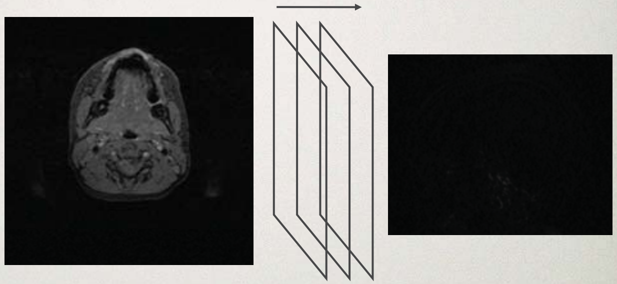

Spatial encoding of the NMR signal is based
on frequency changes in precession
RF COIL
Fourier
transform
B
M


MRI imaging:
Contrast according to relaxation times
100%
63%
Tissue A
Tissue B
Short T1
Long T1
100%
Transverse signal Loss
Liquor
Fat
37%
84
Time in ms
1400
T1-weighting
proton density
weighting
T2-weighting

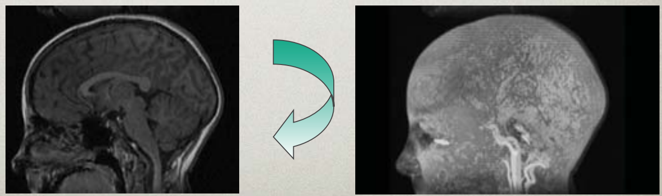

Nuclear Magnetic Resonance Imaging:
Summary
EXCITATION PULSE
$f_{RF} = \left(\frac{\gamma}{2\pi}\right) \times B$
B0
B0
B0
COIL
Proton transmits a
radiofrequency electromagnetic
wave (yellow) after excitation
by an RF pulse (red)
B0
Cryogenic magnet
Radiofrequency coil
Gradient coil
Water molecule
Cross-section of an NMR scanner
Image
Processing
(2D-FFT)

MRI:
Image manipulation

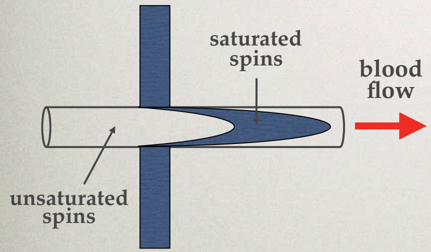
Reslicing in the transverse plane



Spatial projection („volume rendering”)



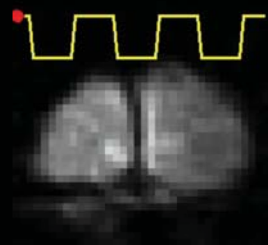
MRI:
Non-invasive angiography



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

Cary and Michael Huang (http://htwins.net)