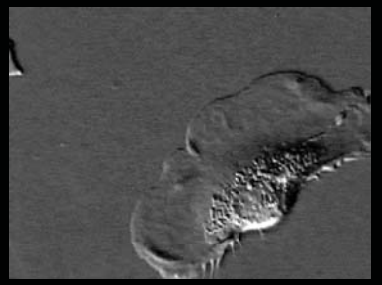
Investigation of biomolecular systems

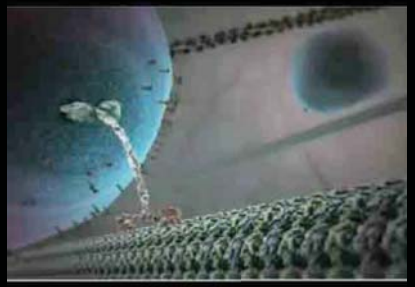
Spectroscopies and microscopies

Living cell: ensembles of molecular machines



Crawling keratinocyte

Microtubule dynamic instability



Vesicle transport with kinesin

Protein synthesis on the ribosome

http://multimedia.mcb.harvard.edu

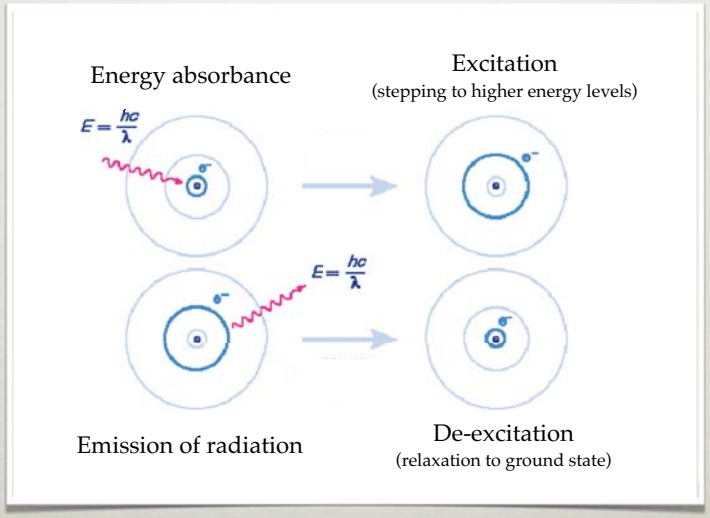
Methods

- Spectroscopy:
  -interaction between electomagnetic radiation and matter
  -absorption; fluorescence (FRET); NMR
- Microscopy:
  -image formation about objects invisible to the eye
  -getting around the Abbe criterion

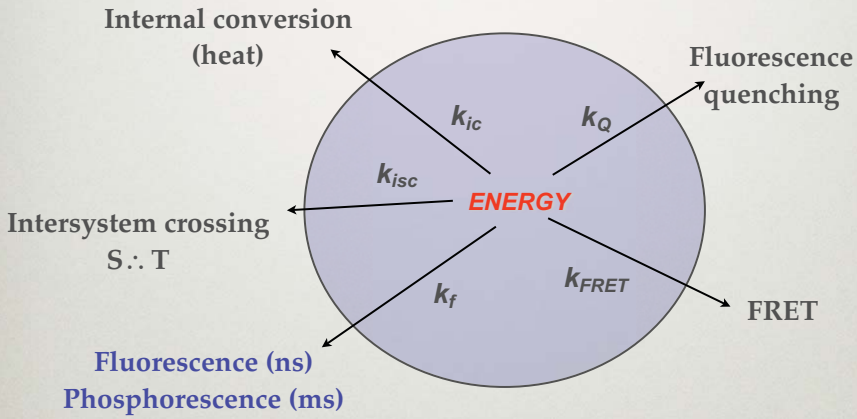
LUMINESCENCE

- Relaxation from excited state followed by light emission
- Radiation emitted by matter in excess of thermal emission
- "Cold light"
- Processes of fluorescence and phosphorescence

Simplified steps of luminescence



Fate of absorbed energy



Radiative *or* non-radiative transitions!

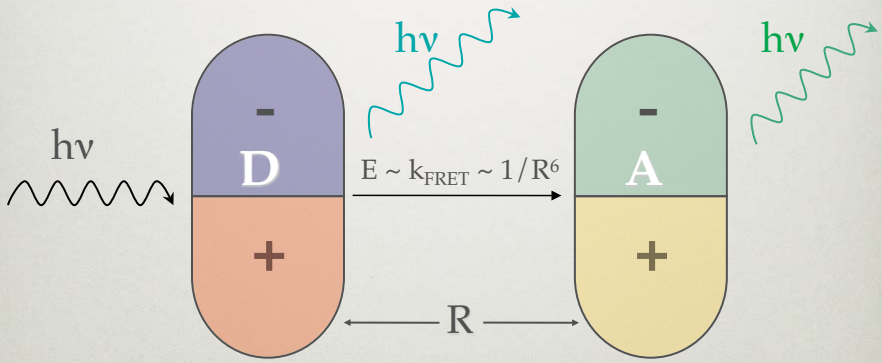
Fluorescence Resonance Energy Transfer

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 florophores.

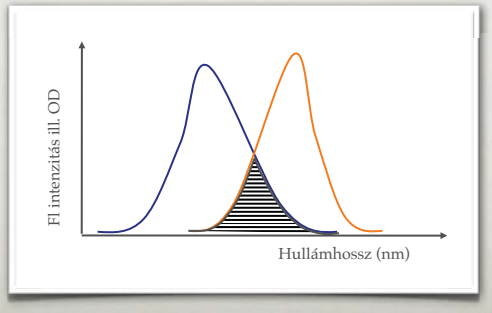
FRET

- Acceptor emission contributes to the relaxation of the excited donor 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**.

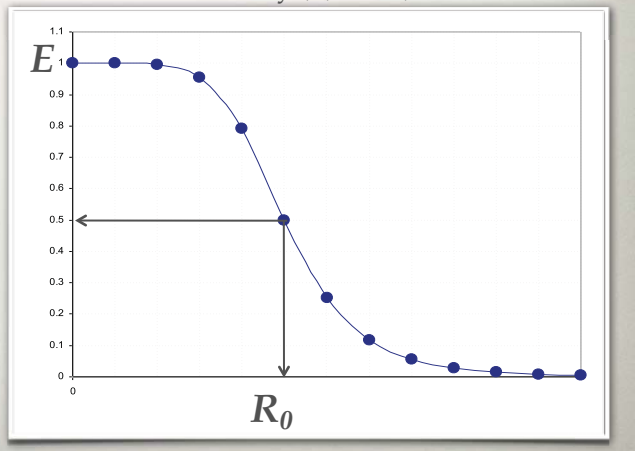


Distance dependence of FRET

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

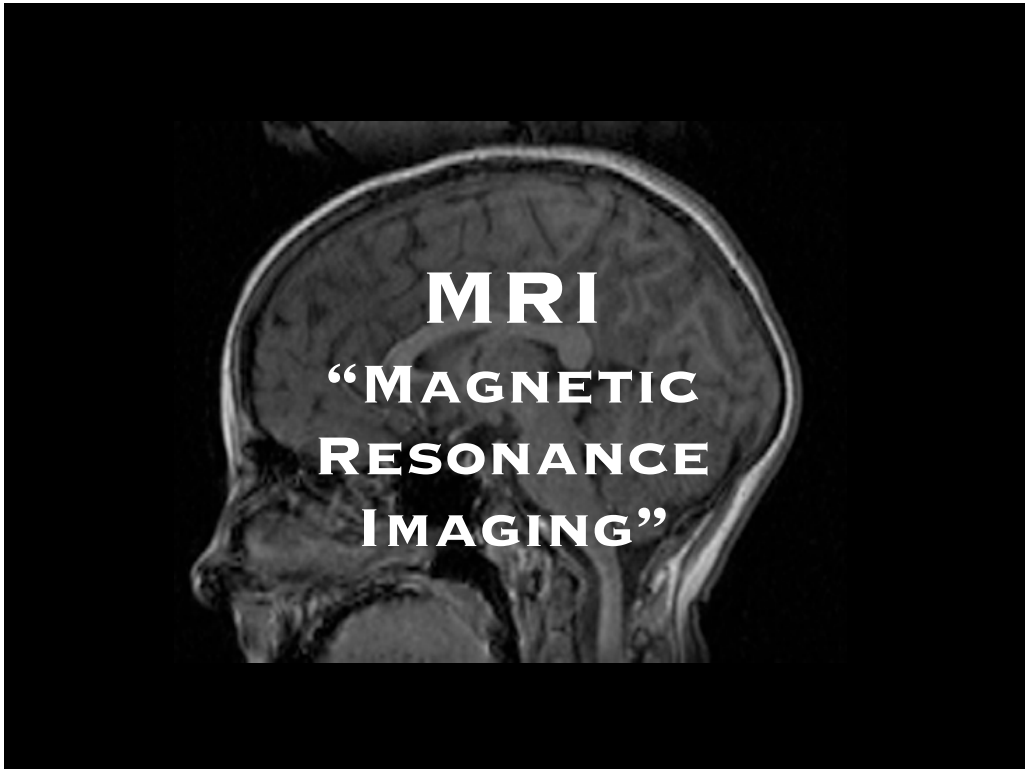
Förster-distance
(Distance at which transfer efficiency (E) is 0.5)

Actual distance between fluorophores

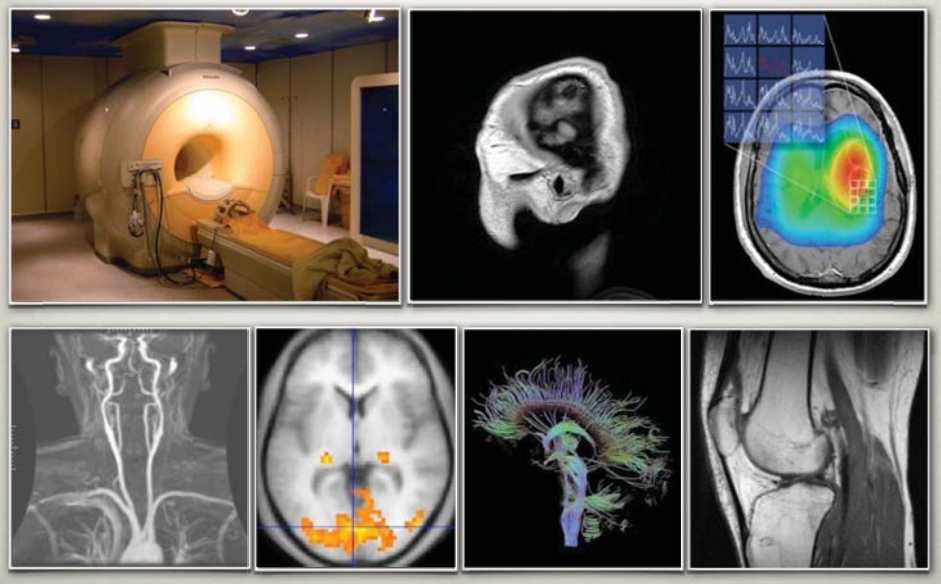


Applications of FRET

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

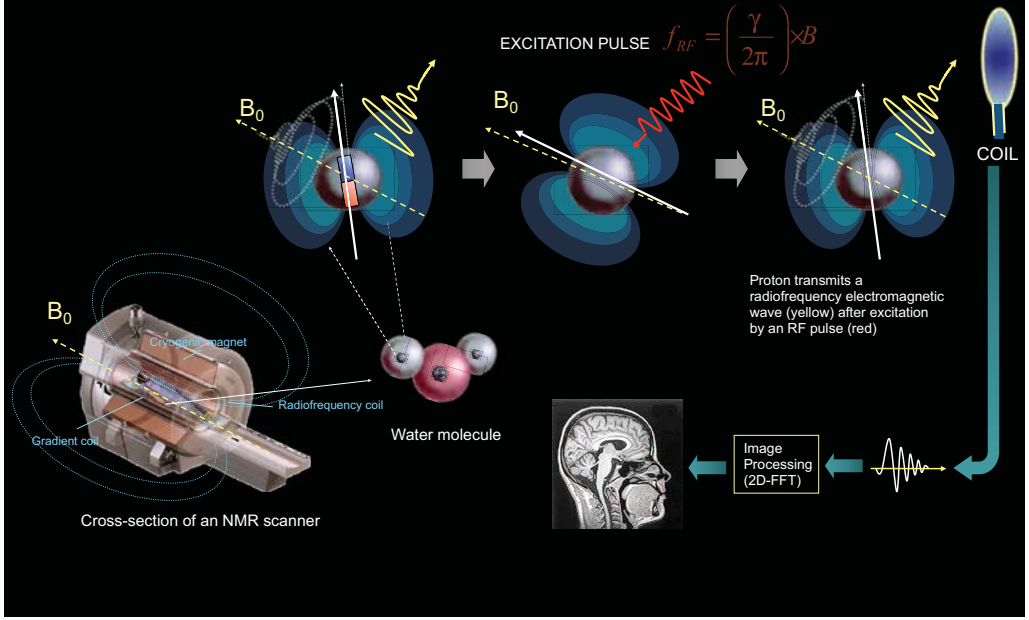


MRI is a revolutionary device



MRI is a non-invasive tomographic method

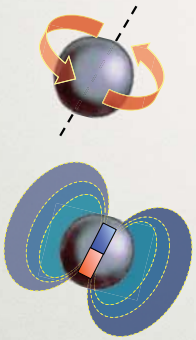
Nuclear Magnetic Resonance Imaging: Basic principle

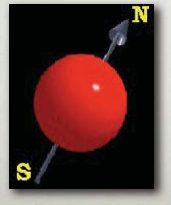


Atomic nuclei with nuclear spin: elementary magnets

Otto Stern

W. Gerlach





Atomic nuclei have mass: $m_{proton} = 1{,}67.10^{-24}$ g

Atomic nuclei carry angular momentum: $L = \sqrt{l(l+1)}\hbar$

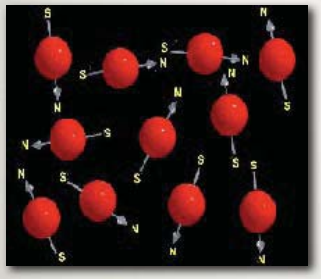
l = spin quantum number

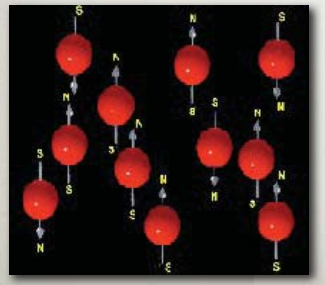
Atomic nuclei carry charge: $q_{proton} = 1{,}6.10^{-19}$ C

Atomic nuclei possess magnetic moment: $\mu_i = \gamma L$

γ = gyromagnetic ratio
L = angular momentum

Nuclear Magnetic Resonance (NMR)

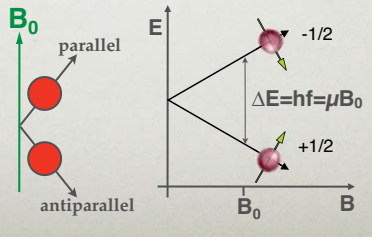




In absence of magnetic field:
random orientation of elementary magnets

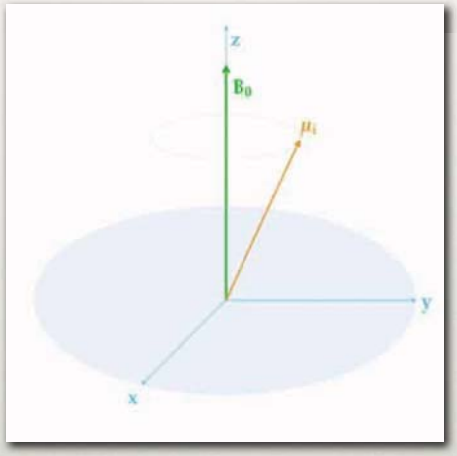
In magnetic field:

elementary magnets orient | energy levels split



Edward Purcell, 1946

Nuclear Magnetic Resonance: Spin Precession



Precession or Larmor frequency:

$$\omega_0 = \gamma B_0$$

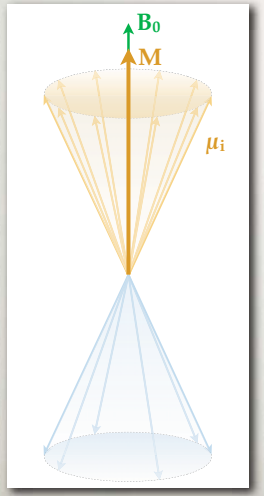
$$f_{Larmor} = \frac{\gamma}{2\pi} B_0$$

Felix Bloch, 1946

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:

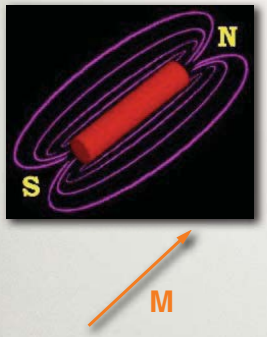
$$\frac{N_{antiparallel}}{N_{parallel}} = e^{-\frac{\Delta E}{k_B T}}$$

Boltzmann distribution

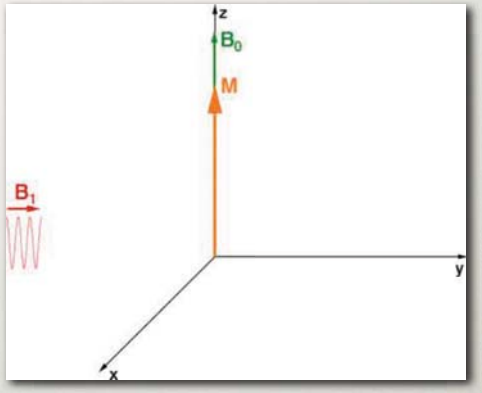
Excitation

Using radio frequency radiation

Resonance condition: Larmor frequency

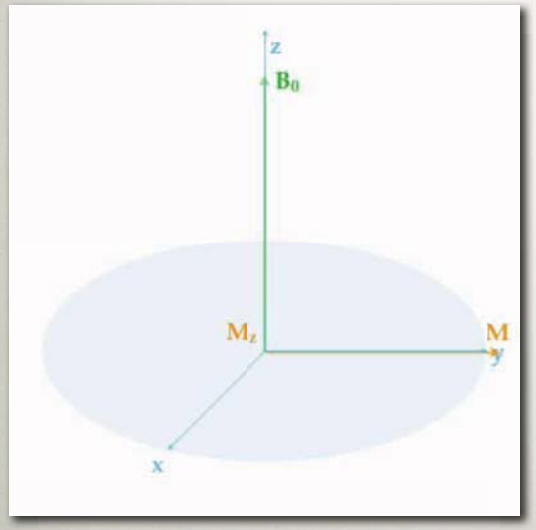


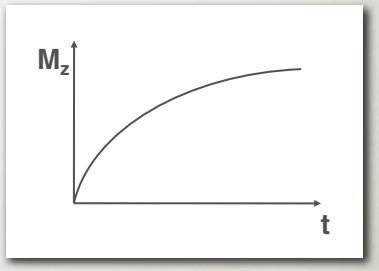
B_0 = magnetic field
M = net magnetization
B_1 = irradiated radio frequency wave



Spin-lattice relaxation

T1 or longitudinal relaxation

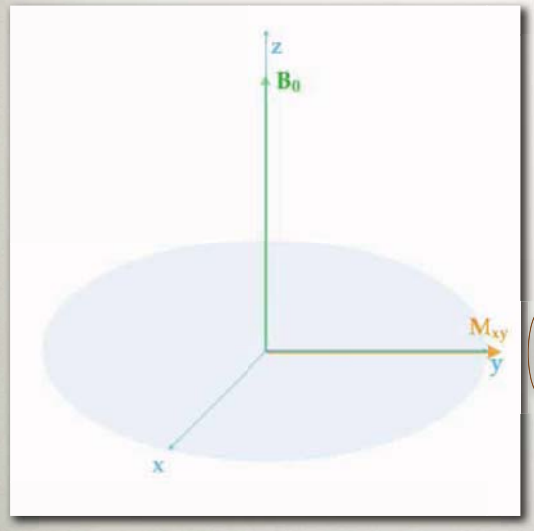


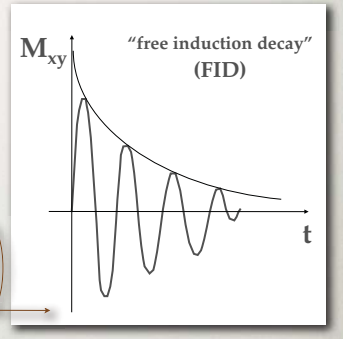


T1 relaxation time:
depends on interaction
between elementary magnet (proton)
and its environment

Spin-spin relaxation

T2 or transverse relaxation



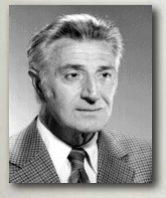


T2 relaxation time:
depends on interaction between
elementary magnets (protons)

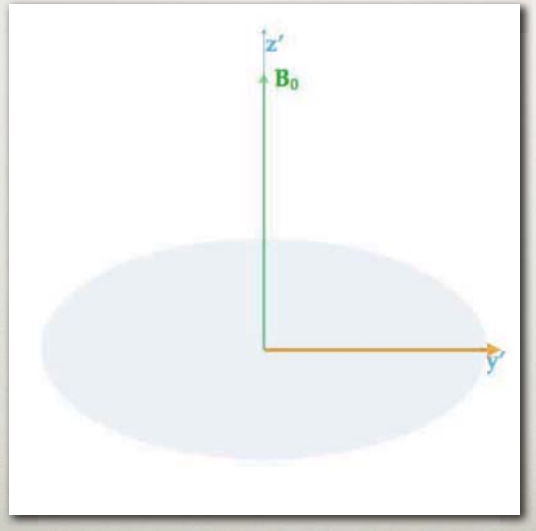
Spin-spin relaxation

T2 or transverse relaxation

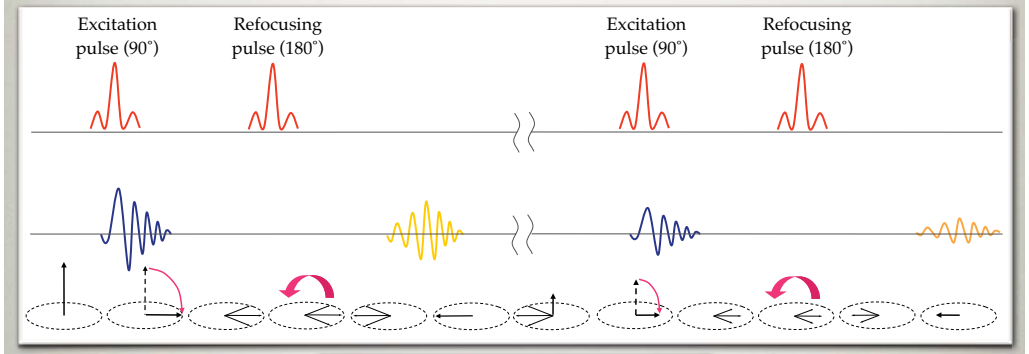
Repetitive pulses of excitation and subsequent relaxation: spin-echo sequence

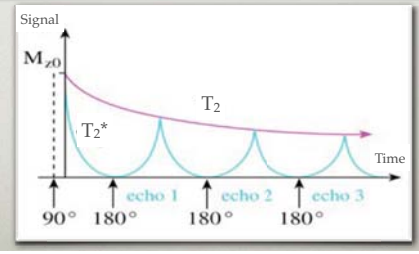


Erwin Hahn, 1949

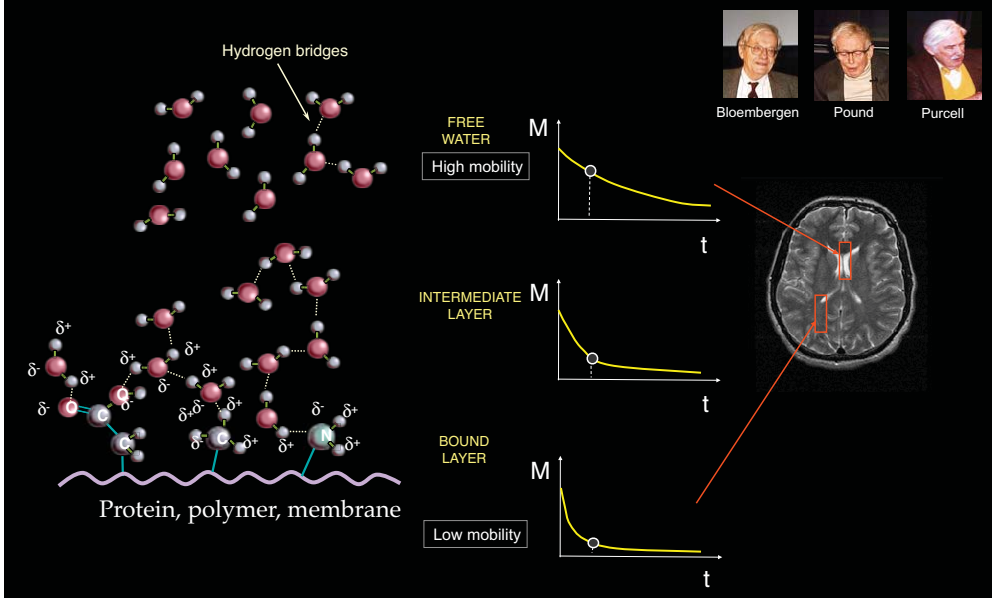


The spin-echo experiment

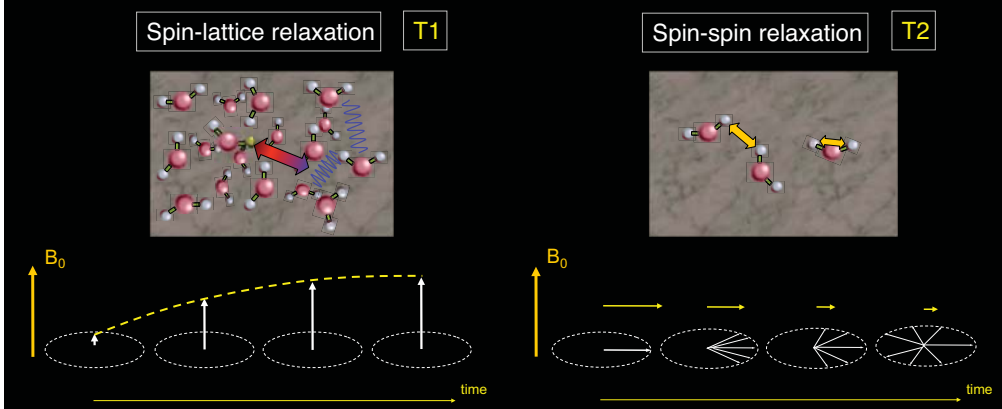




Contrast in MR images is determined by the interaction of spin systems

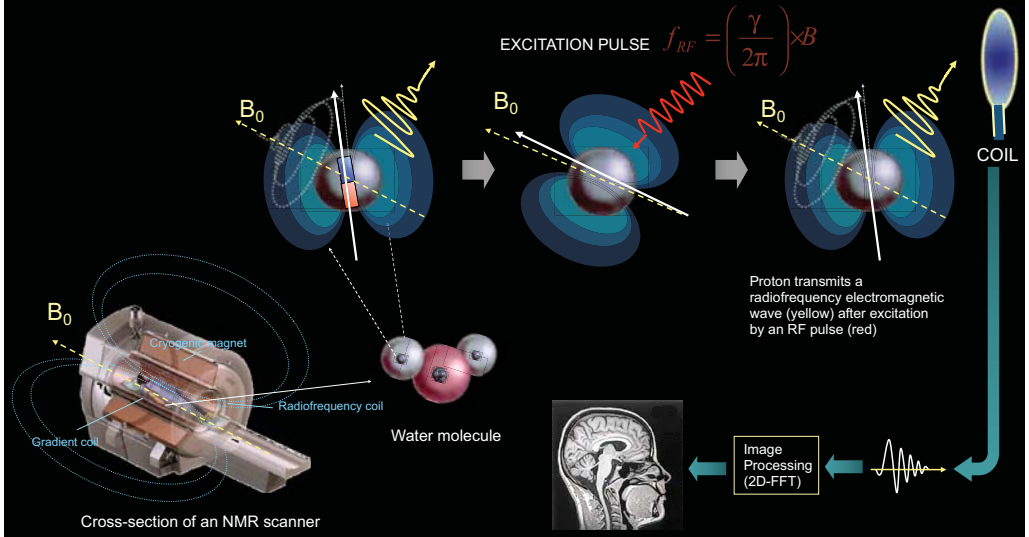


Nuclear Magnetic Resonance Imaging: Two important relaxation mechanisms

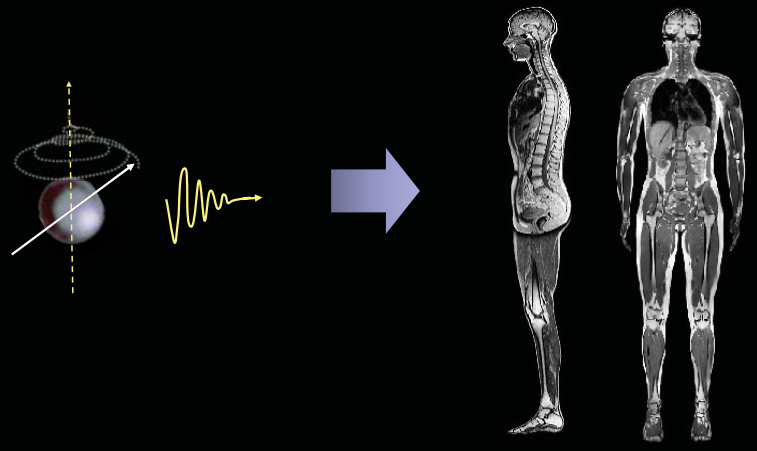


Spin-lattice relaxation (T1)	Spin-spin relaxation (T2)
Restoration of longitudinal magnetization	Dephasing of transverse magnetization
Energy transfered to lattice (phonons)	Energy transferred between spins
Entropy increases	No entropy change of total spin system
Repopulation of spins between spin energy levels	No repopulation of spins between spin energy levels
Interactions with magnetic field fluctuations at Larmor frequency	Interactions with magnetic field fluctuations at low frequency

Nuclear Magnetic Resonance Imaging: Basic principle

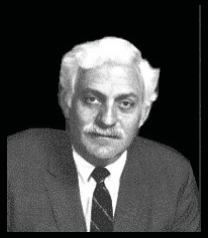


From nuclear magnetic resonance signal to Magnetic Resonance Imaging

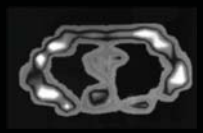


First NMR experiments in vivo

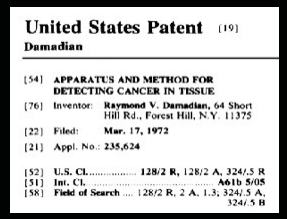
Downstate Medical Center - Brooklyn, 1972



Raymond V. Damadian

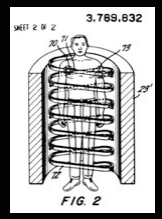


First MRI scan

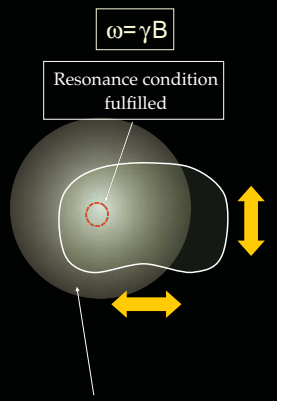


United States Patent [19]
Damadian

[54] APPARATUS AND METHOD FOR DETECTING CANCER IN TISSUE
[76] Inventor: Raymond V. Damadian, 64 Short Hill Rd., Forest Hill, N.Y. 11375
[22] Filed: Mar. 17, 1972
[21] Appl. No.: 235,624
[52] U.S. Cl. ..... 128/2 R, 128/2 A, 324/.5 R
[51] Int. Cl. A61b 5/05
[58] Field of Search .... 128/2 R, 2 A, 1.3; 324/.5 A, 324/.5 B

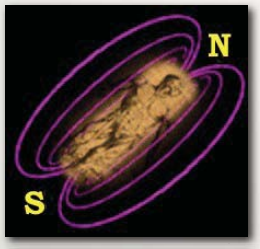


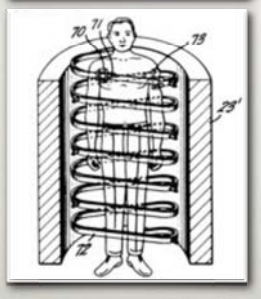
1970: detection of lengthened relaxation times in cancerous tissues
1972: theoretical development of human in vivo 3D NMR
1977: first human MRI image



MRI:

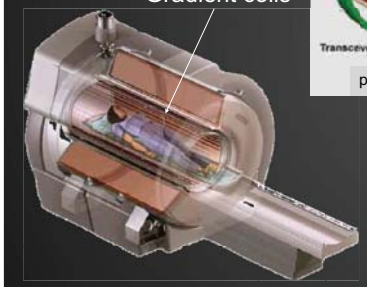
Net magnetization of the human body is generated

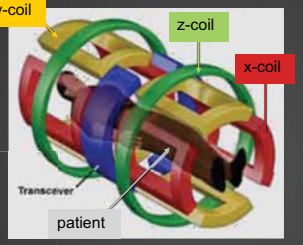


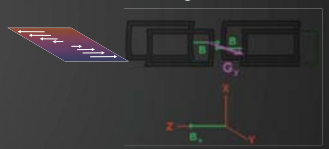


"Indomitable"

Spatial encoding of the NMR signal: Imaging gradients

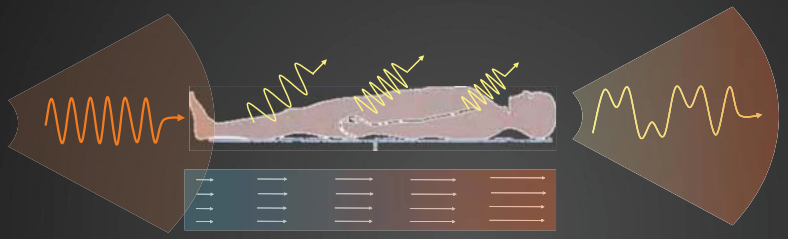




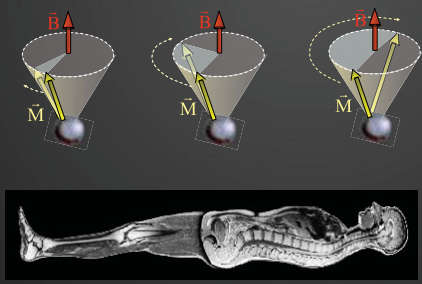


IMPORTANT NOTE:
The magnetic field is always in the Z-direction

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



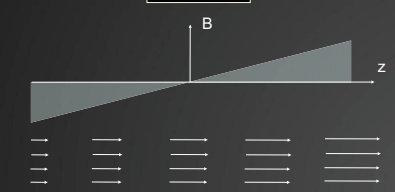
RF COIL

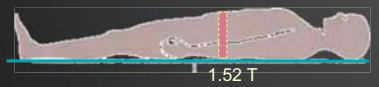


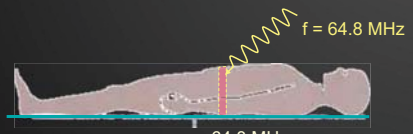
Fourier transform

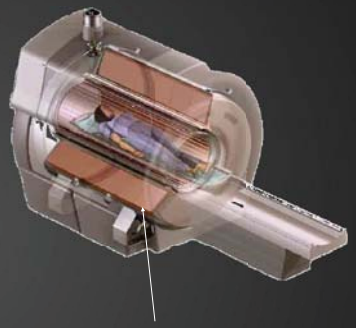
Spatial encoding: Slice selection

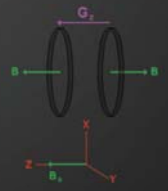
$\omega = \gamma B$







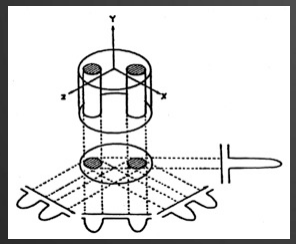


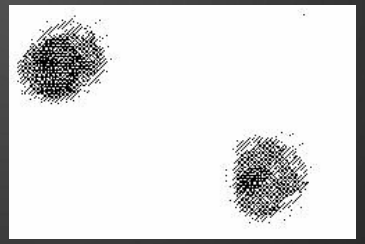


NMR scanner with backprojection

Paul Lauterbur, 1973
Illinois

Peter Mansfield, 1973
Nottingham

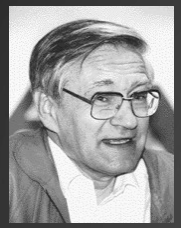




Nature 242, (1973), 190-191

Nobel prize for physiology and medicine (Lauterbur & Mansfield) in 2003

NMR scanner with 2D Fourier transformation



Richard Ernst, 1974
Zürich

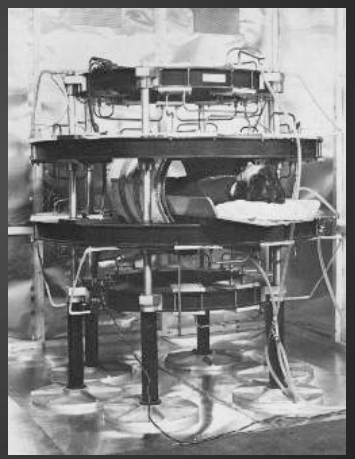
NMR Fourier Zeugmatography

ANIL KUMAR, DIETER WELTI, AND RICHARD R. ERNST

Laboratorium für Physikalische Chemie, Eidgenössische Technische Hochschule, 8006 Zürich, Switzerland

Received August 2, 1974

A new technique of forming two- or three-dimensional images of a macroscopic sample by means of NMR is described. It is based on the application of a sequence of pulsed magnetic field gradients during a series of free induction decays. The image formation can be achieved by a straightforward two- or three-dimensional Fourier transformation. The method has the advantage of high sensitivity combined with experimental and computational simplicity.



Nobel price for chemistry in 1991

The first MRI scanners ...

Interventional MRI unit

Open MRI unit

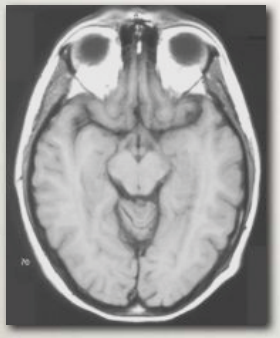
Mobile MRI unit

… and recent ones

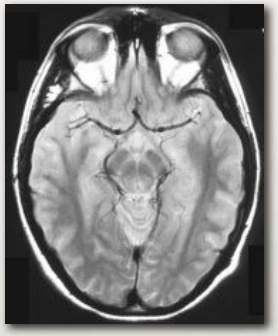
MRI Imaging:

Color resolution (contrast)

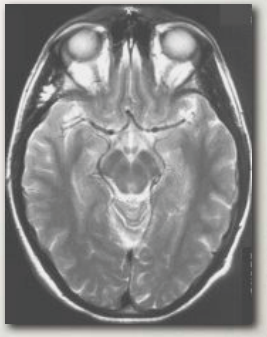
based on spin density and relaxation times



T1-weighing



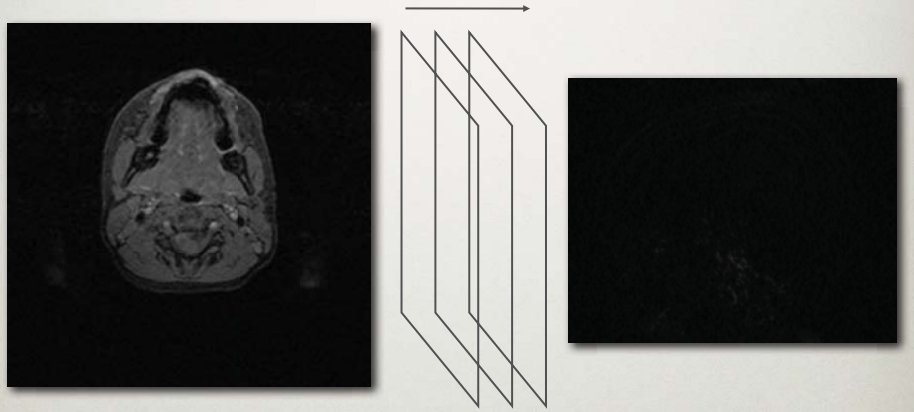
Proton density-weighing



T2-weighing

MRI:

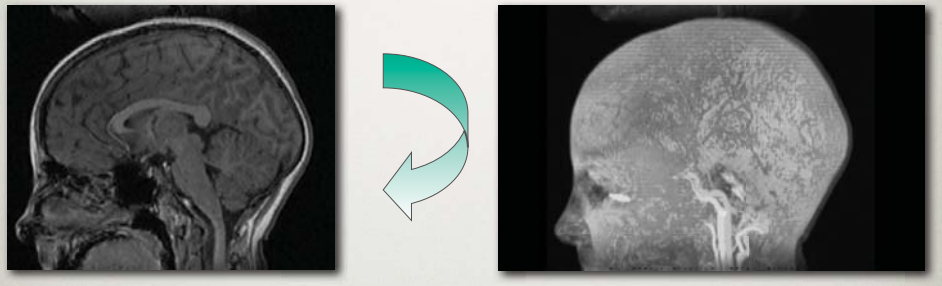
Image manipulation I



Reslicing in perpendicular plane

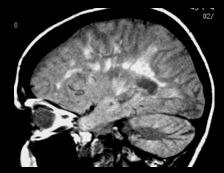
MRI:

Image manipulation II

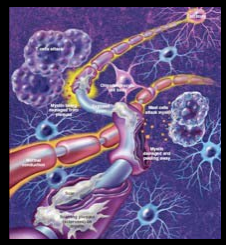


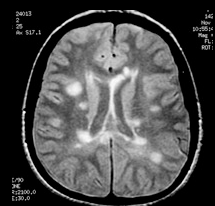
Spatial projection
(„volume rendering")

Anatomical imaging:
Multiple sclerosis

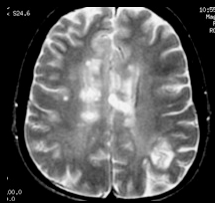


Proton density
(sagital)

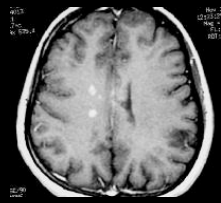




Proton density
(transverse)

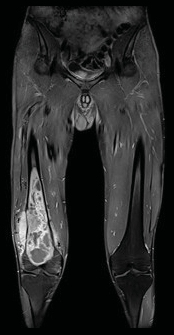


T2 weighted
(transverse)

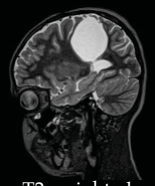


T1 weighted
With contrast agent

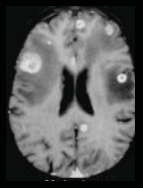
Anatomical imaging: Oncology



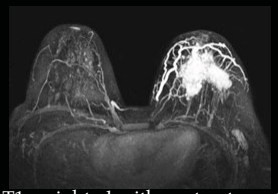
T2 weighted
(chondrosarcoma)



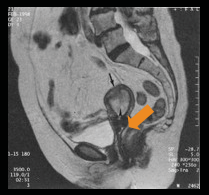
T2 weighted
(cyst)



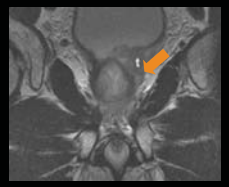
Protondensity
(Brain metastasis)



T1 weighted with contrastagent
(Breast carcinoma)

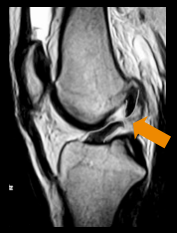


T2 weighted
(cervix carcinoma)

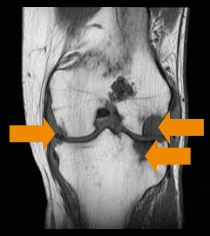


T2 weighted
(prostate tumor)

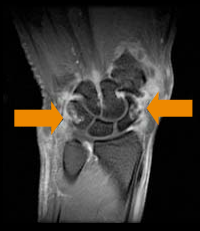
Anatomical imaging Bone and soft tissue



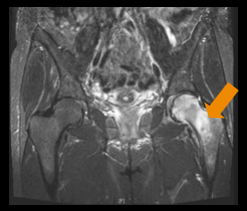
T2 weighted
(torn ligaments)



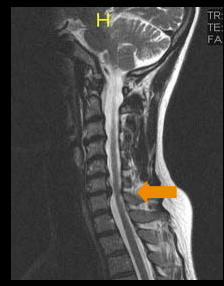
Rheumatoid arthritis
knee



Rheumatoid arthritis
whrist

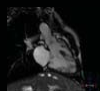


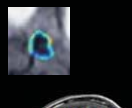
Osteoporosis (femur)

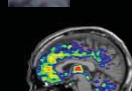


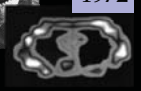
T2 weighted
(hernia)

There is more to MRI than anatomical imaging ...









1972

2008

First NMR images

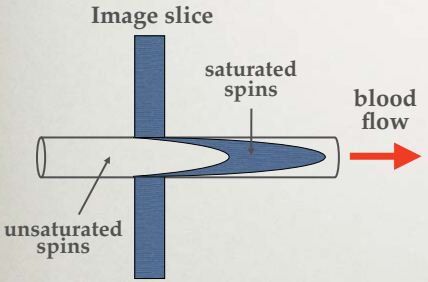
'State of the art'

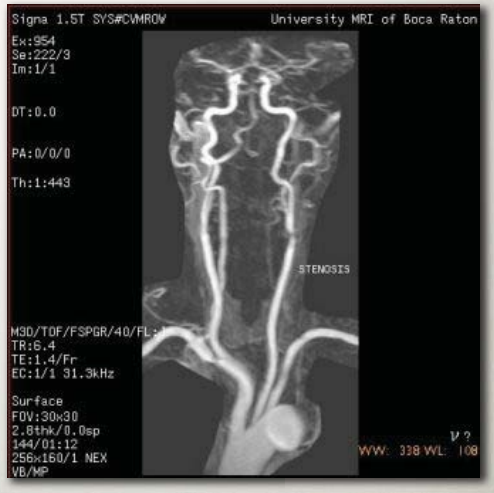
- 3D images
- dynamic images
- sharp image resolution

In research phase

- quantitative imaging
- cell-specific contrast agents
- hyperpolarized MRI
- in vivo spectroscopy
- functional imaging
- 'multimodality' imaging

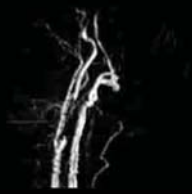
MRI:
Non-invasive angiography



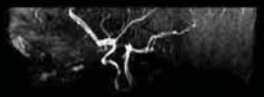


MRI:

Non-invasive angiography



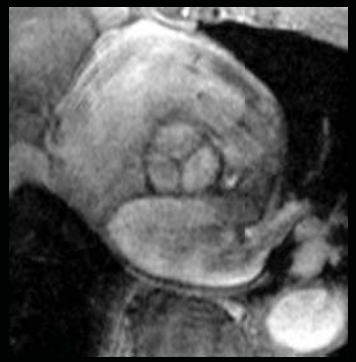
arteria carotis



Circulus arteriosus Willisii

MRI movie

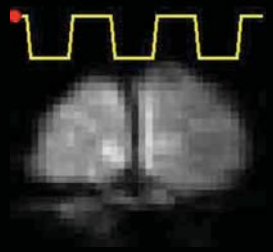
Based on high time resolution images



Opening and closing of aorta valve

Functional MRI (fMRI)

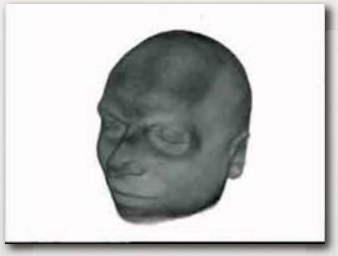
High time resolution images recorded synchronously with physiological processes



Effect of light pulses on visual cortex

Superposition of MRI on other information (PET)

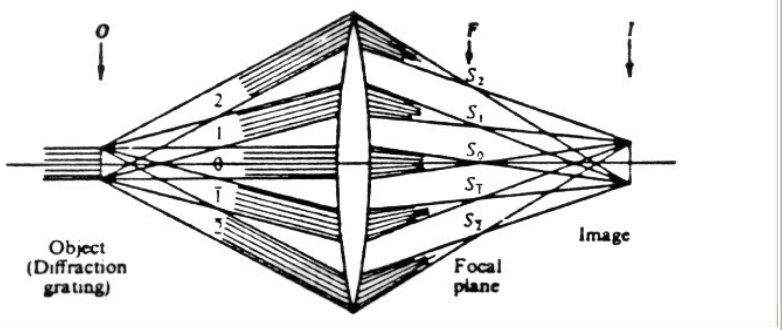
Superposed MRI and PET sequence



PET activity: during eye movement
Volume rendering

Microscopy

Image formation in the light microscope



Due to diffraction: image of a point object is an Airy disk

Smallest resolved distance (Abbé):

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

Possibilities of improving microscope resolution

1. Improving the parameters of the Abbe formula
2. Conversion of the resolution problem into a positioning problem
3. Non-diffraction-limited imaging

1. Reduction of wavelength: Electron microscopy

1. Electron as wave,
De Broglie (1924):

$$\lambda = \frac{h}{mv}$$

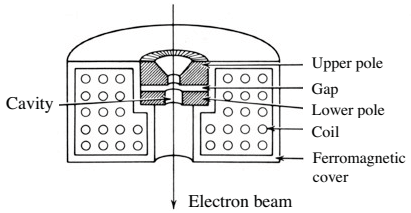
λ=wavelength
h=Planck's constant
m=electron mass
v=electron travel velocity

2. Deflecting the electron beam: with magnetic lens

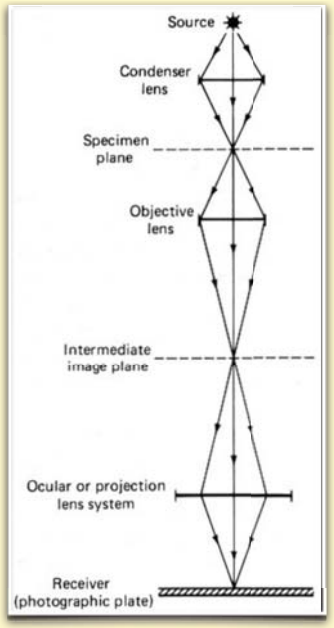
$$F = eBV_e \sin\alpha$$

F=force on electron
e=electron charge
B=magnetic induction
V_e=electon velocity
α=angle between electron path and magnetic field

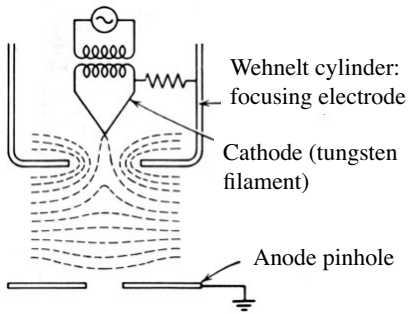
Magnetic lens:
electron beam spirals through it.



The electron microscope



Source: elektron gun



Large vacuum: 10^{-2} Pa

Resolution and contrast in the electron microscope

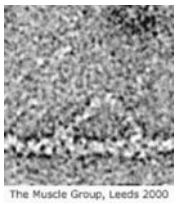
A. Theoretical resolution:
Modified Abbe-formule (for small α angles)
Based on electon velocity (100000 km/s), d=0.005 nm

$$d = \frac{\lambda}{\alpha}$$

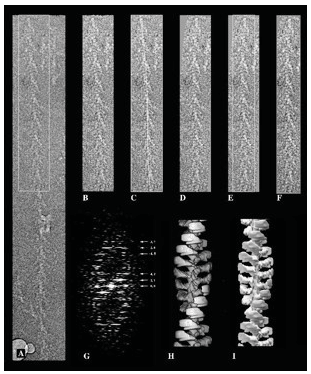
B. Real resolution: limited by small NA, ~0.1 nm.
Because of small NA, depth of focus is large (several μm).

C. Practical resolution in biological samples:
1/10 of section thickness.

D. Contrast mechanism: electron diffraction
Contrast enhancement: by electron dense dyes

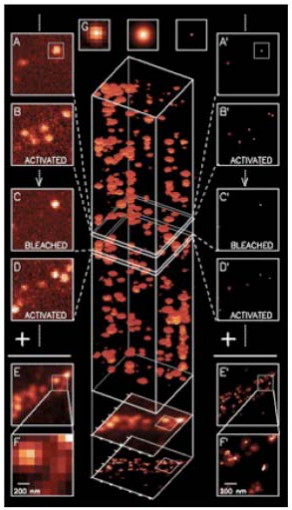


Cryo-electron microscopy, particle-analysis image reconstruction

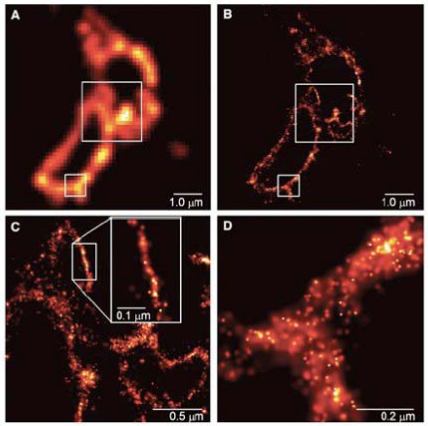


3. Conversion of resolution problem into positioning problem

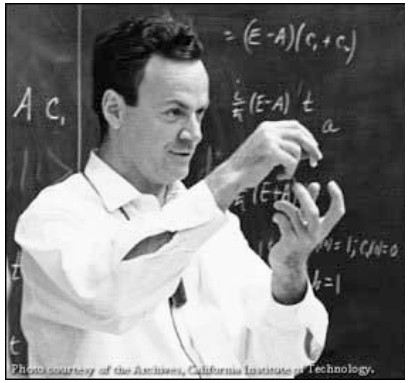
Photo-Activated Localization Microscopy (PALM)



CD63, lysosome transmembrane protein

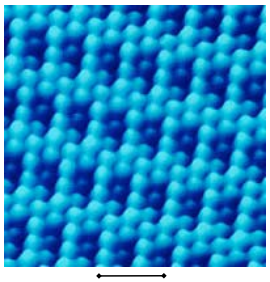


3. Non-diffraction-limited microscopy
Atomic Force Microscopy, AFM



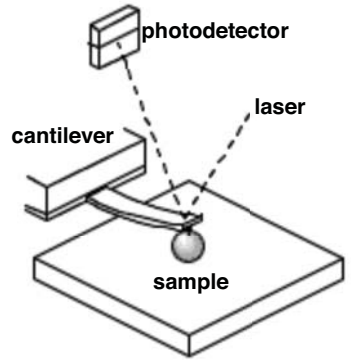
Richard P. Feynman:
"There is plenty of room at the bottom"
December 29, 1959

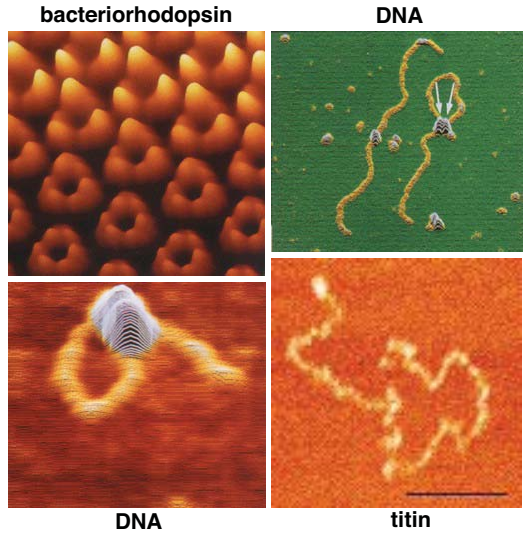
Oxygen atoms on the surface of rhodium crystal



Unit of nanoworld:
1 nanometer

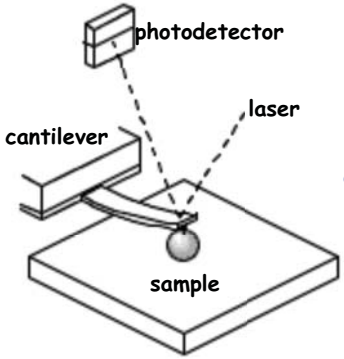
Atomic Force Microscope: AFM

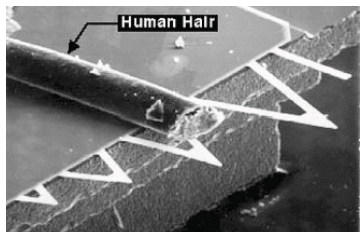


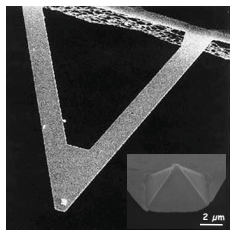


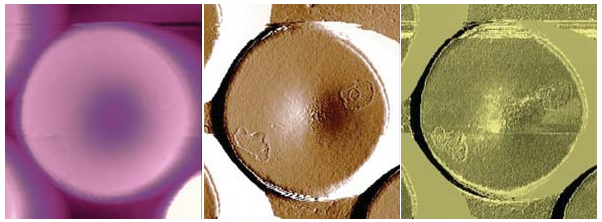
AFM Foundations

Image formation by scanning

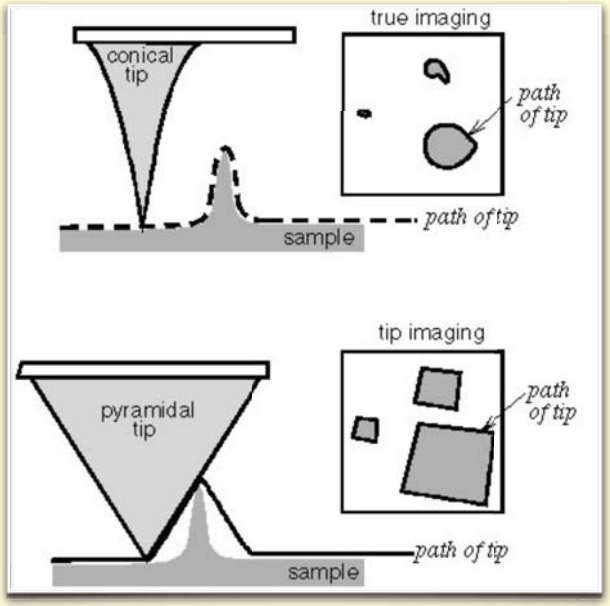




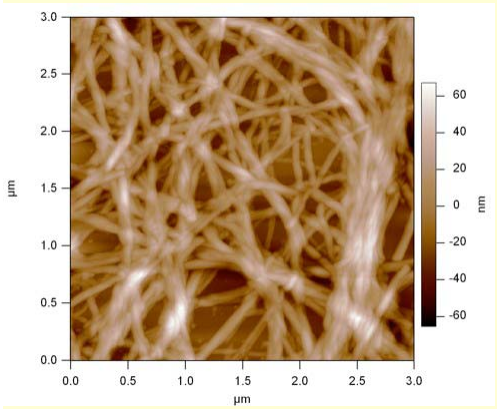




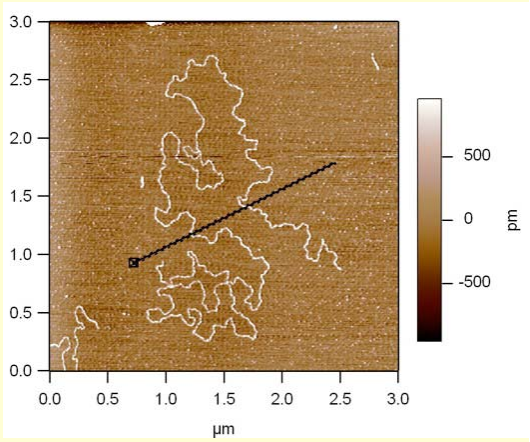
Imaging errors

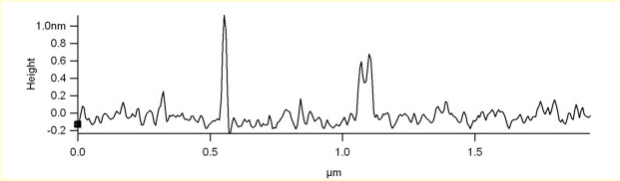


Beta-amyloid fibrils

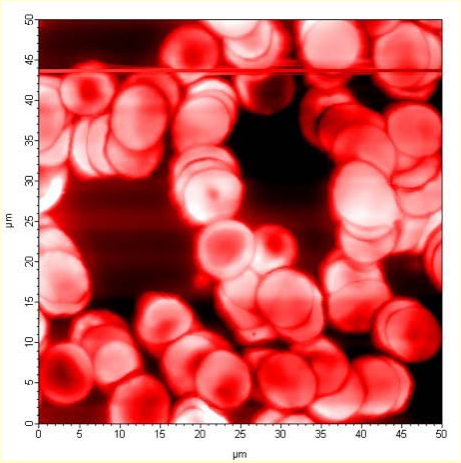


DNA

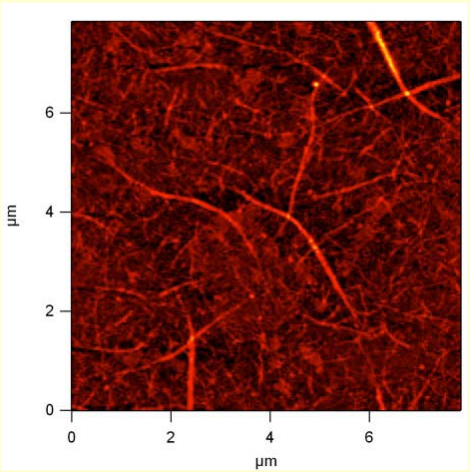




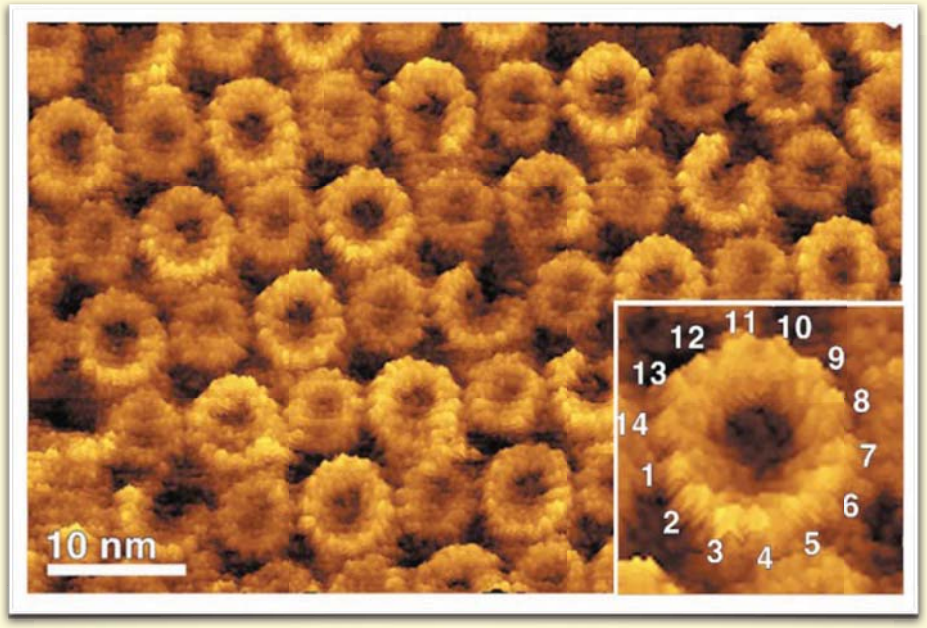
Red blood cells (smear)



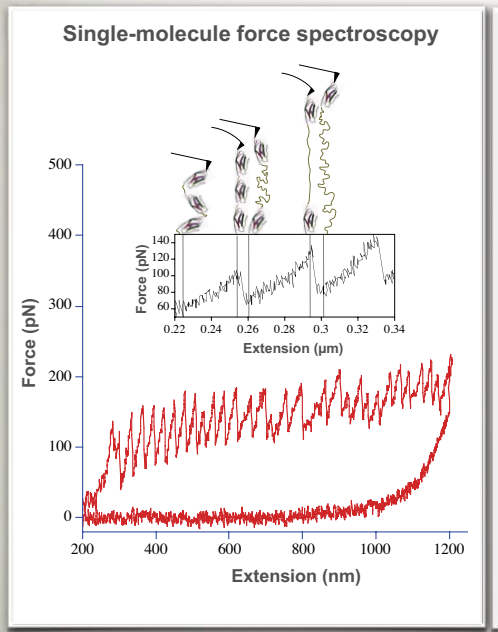
Fibrin network

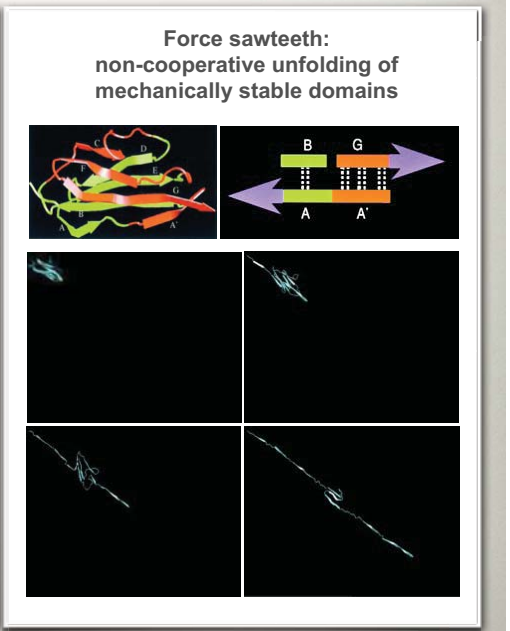


ATP-SYNTHASE



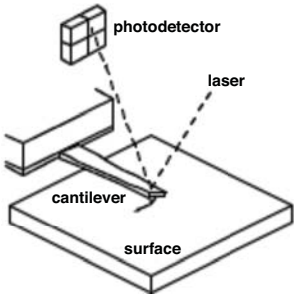
Manipulation of molecules with AFM



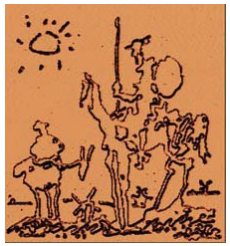


Nanoindentation, nanolithography

Lithos: stone, graphein: to draw



Pablo Picasso: "Don Quixote" drawn in polycarbonate surface



1 μm