

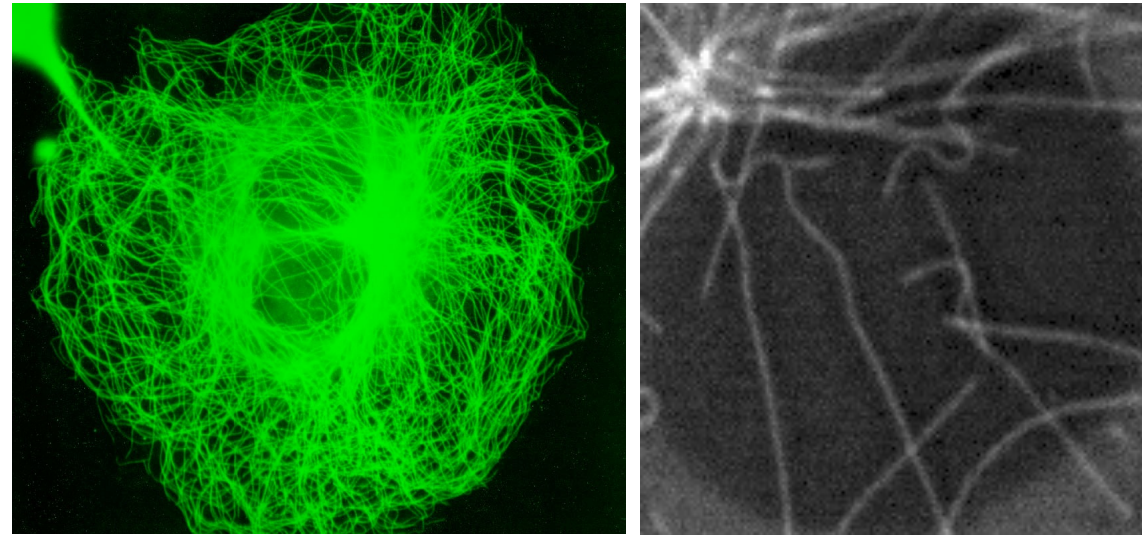
# SINGLE-MOLECULE BIOPHYSICS RADIO SPECTROSCOPIES (NMR, ESR, MRI)

MIKLÓS KELLERMAYER



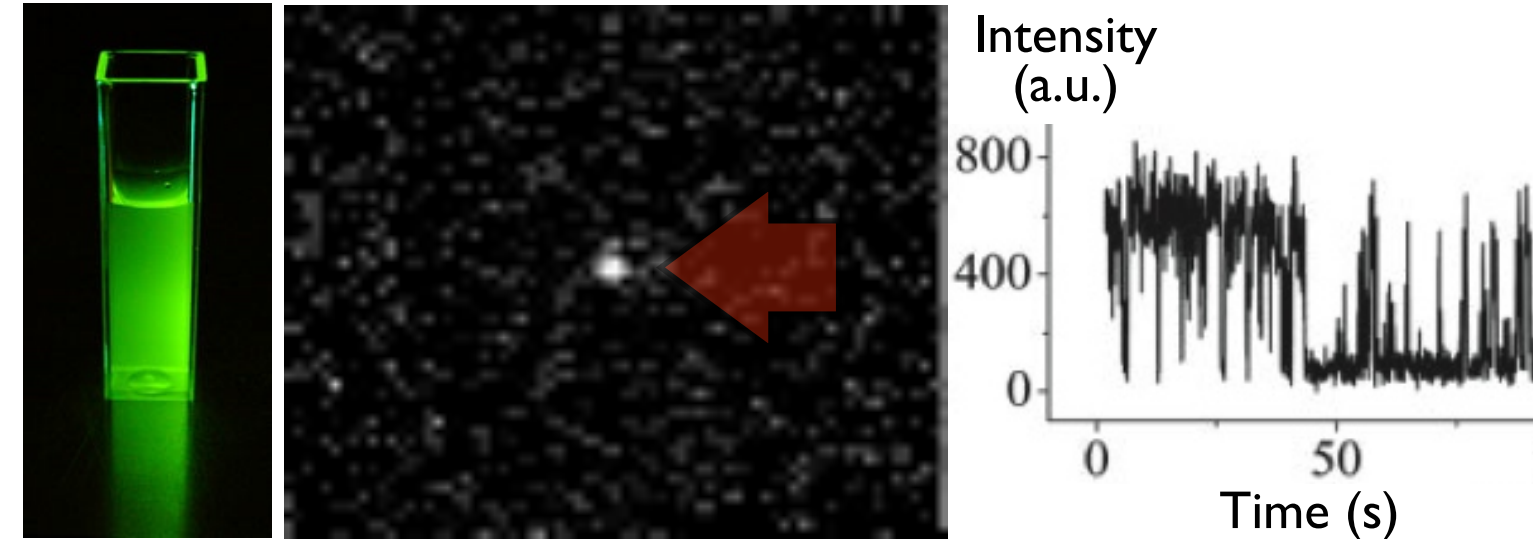
# Single-molecule biophysics

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



Ensemble - microtubular system      Single microtubules - treadmilling

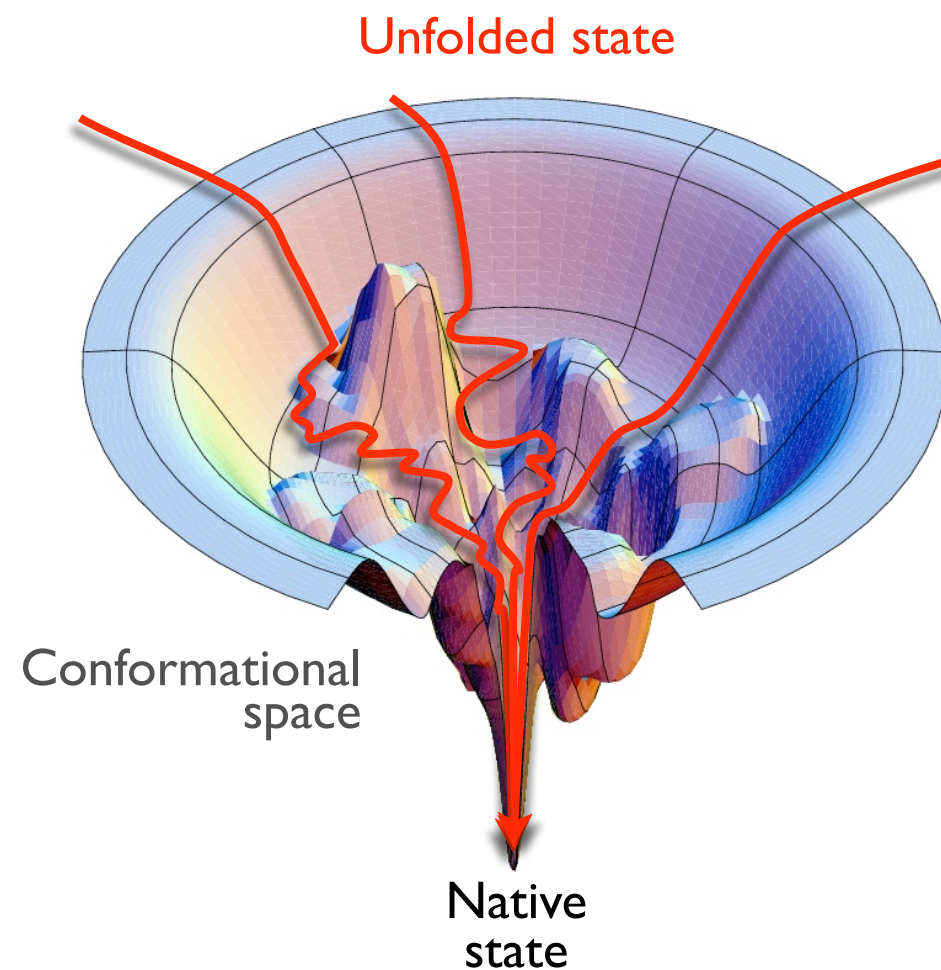
2. Stochastic events may be discovered



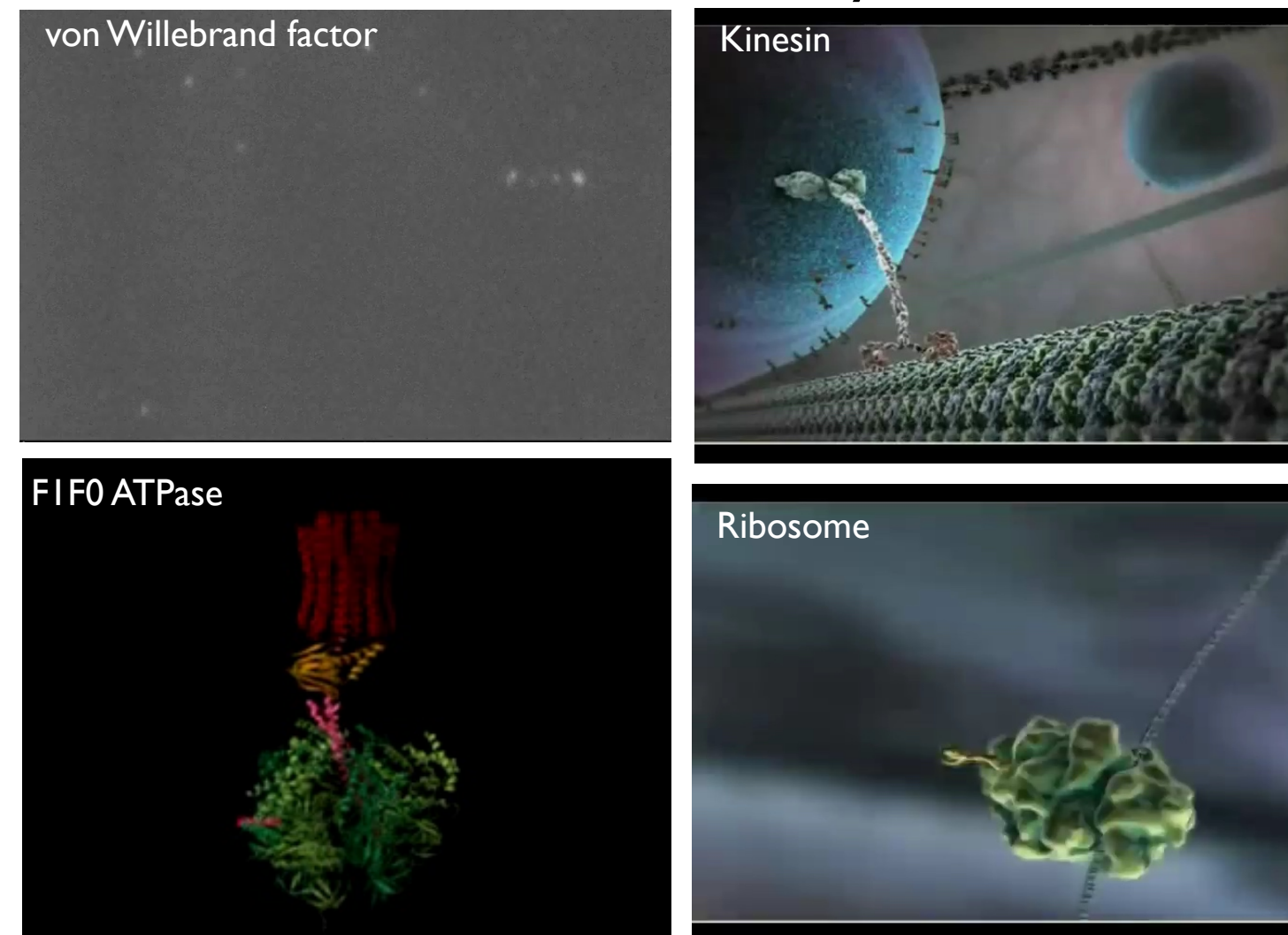
Ensemble - intensity

Single quantum dot - blinking

3. Parallel-pathway processes may be described

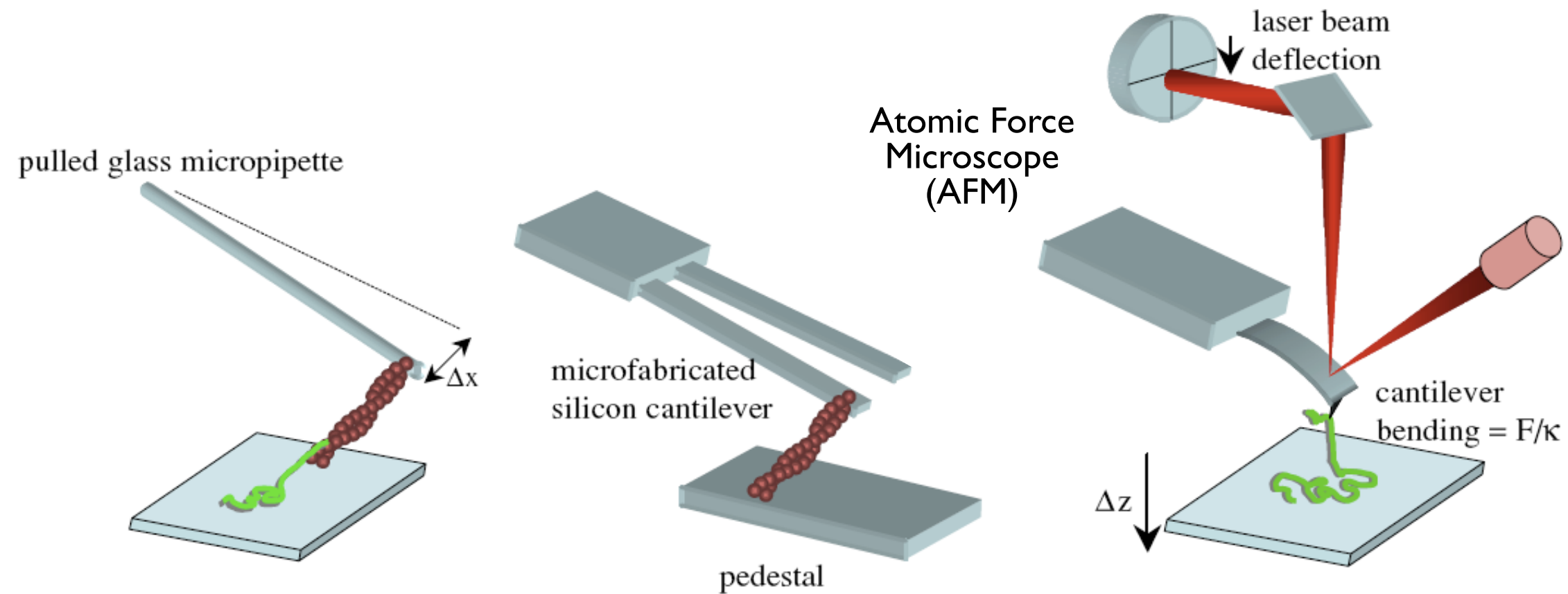


4. Mechanics of biomolecules may be characterized

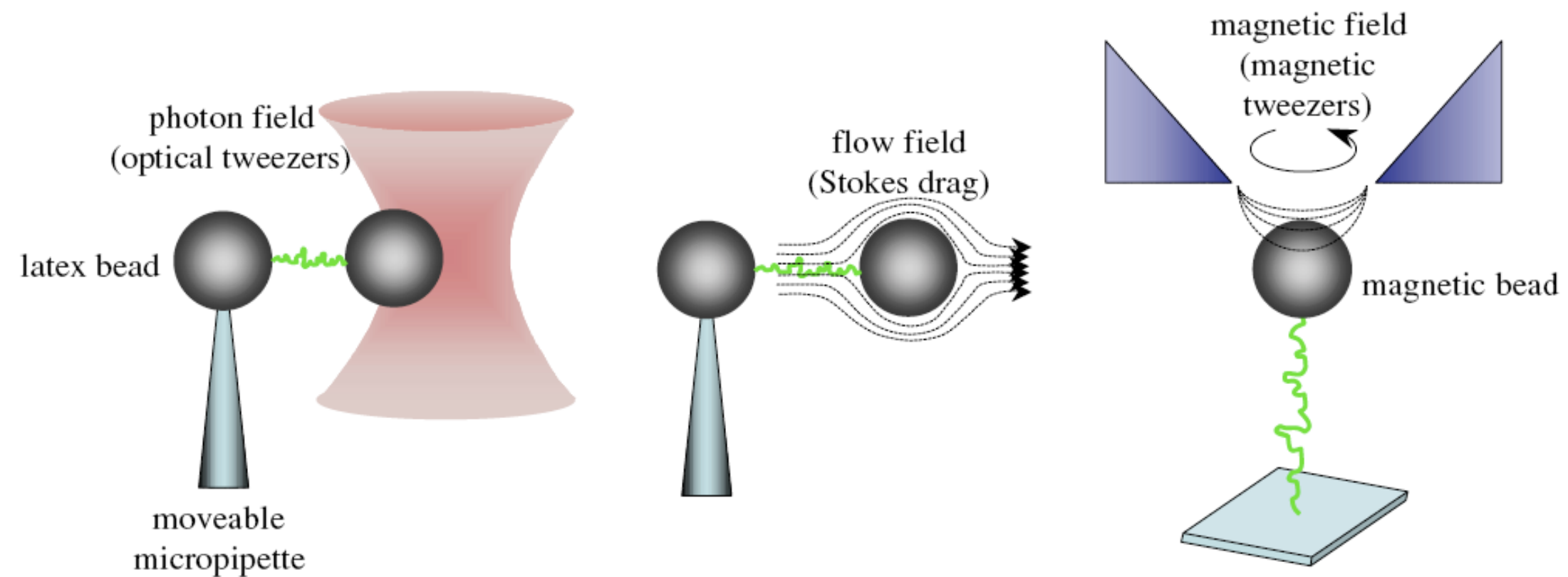


# Manipulation of single molecules

## Cantilever methods



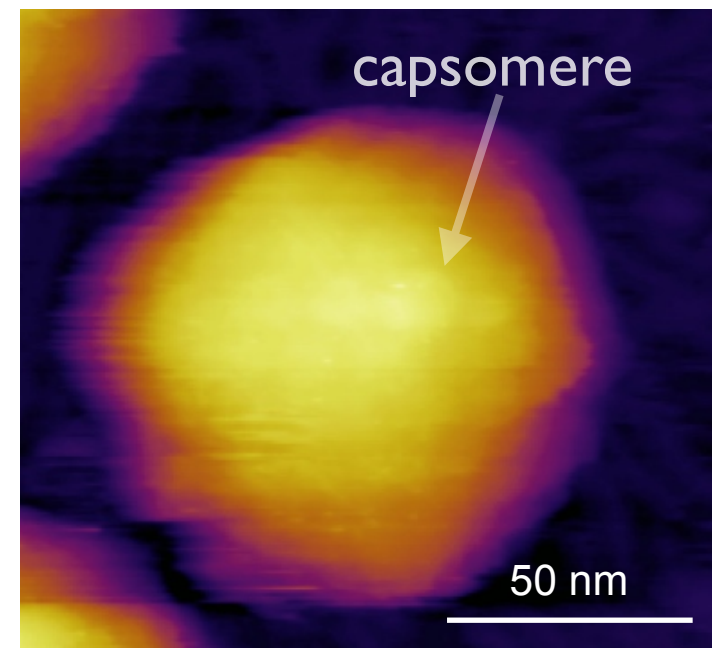
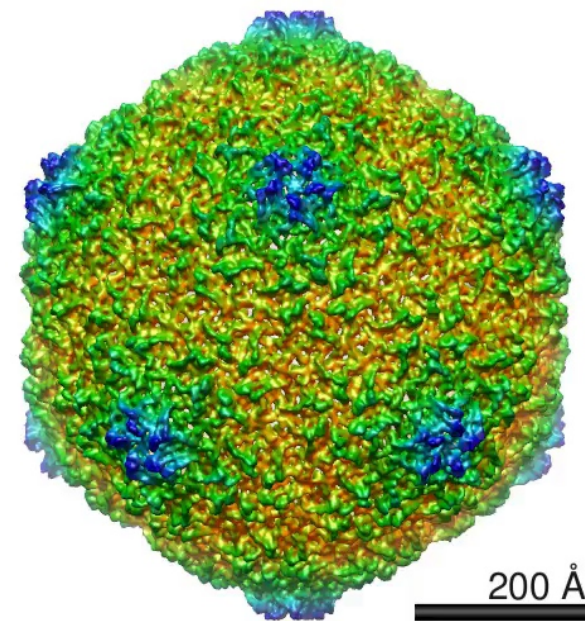
## Field-based methods





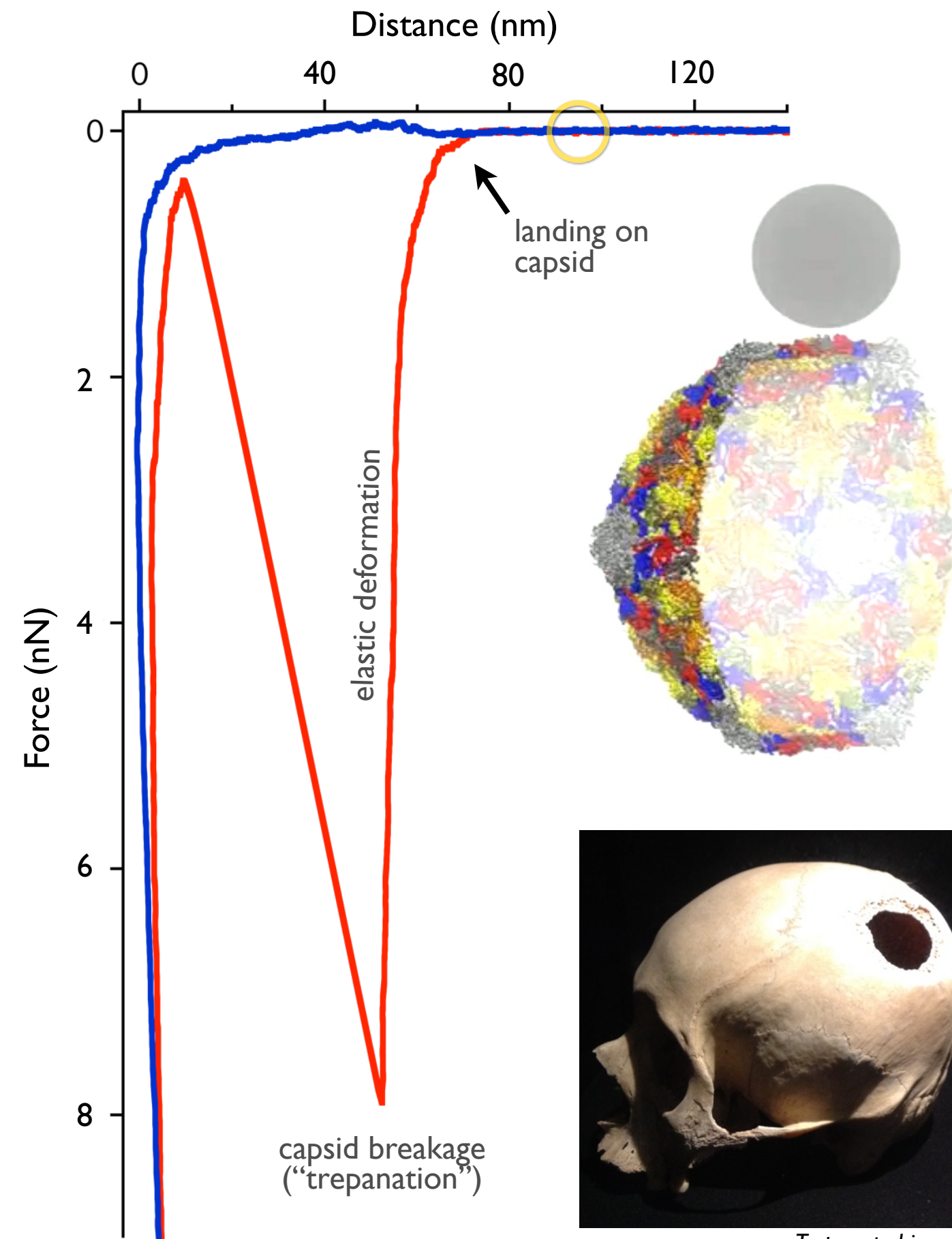
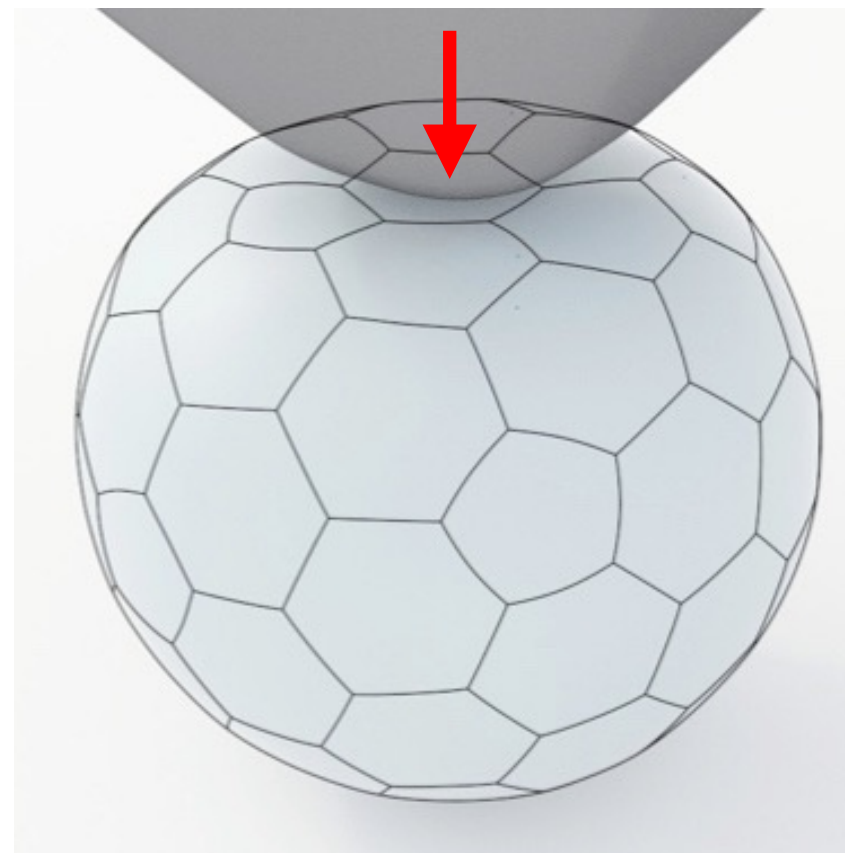
# Measurable parameters I. Force

T7 bacteriophage



T7 phage particles immobilized on mica surface

Nanoindentation  
of viral capsid

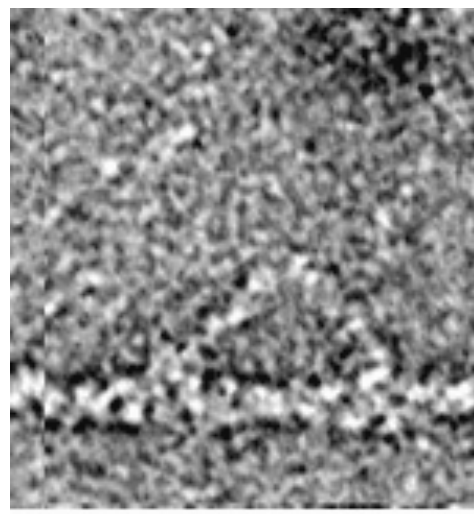


Trepanated inca skull,  
Museo Rafael Larco Herrera, Lima



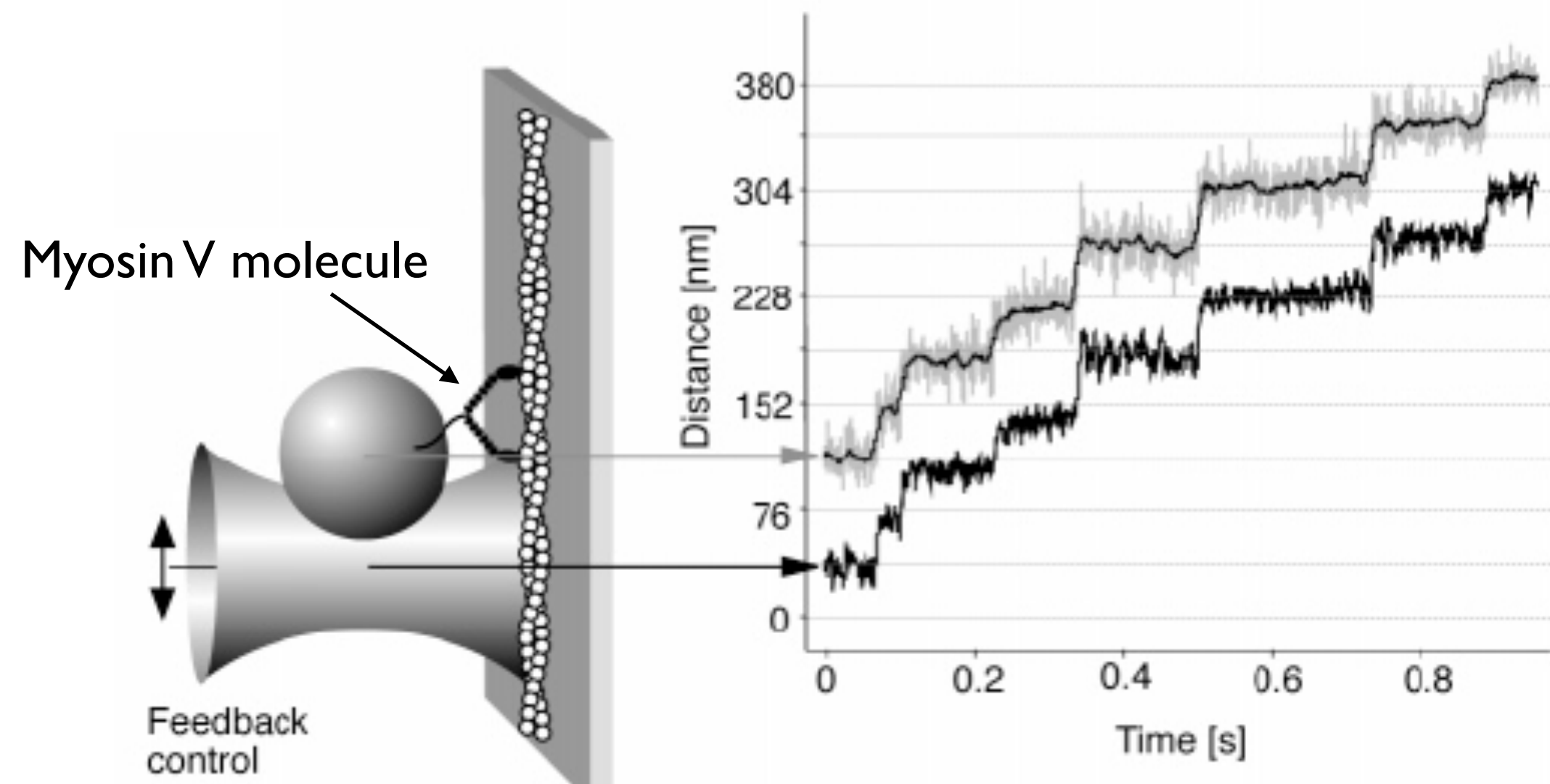
# Measurable parameters II. Distance

What is the step size of a motor protein?



The Muscle Group, Leeds 2000

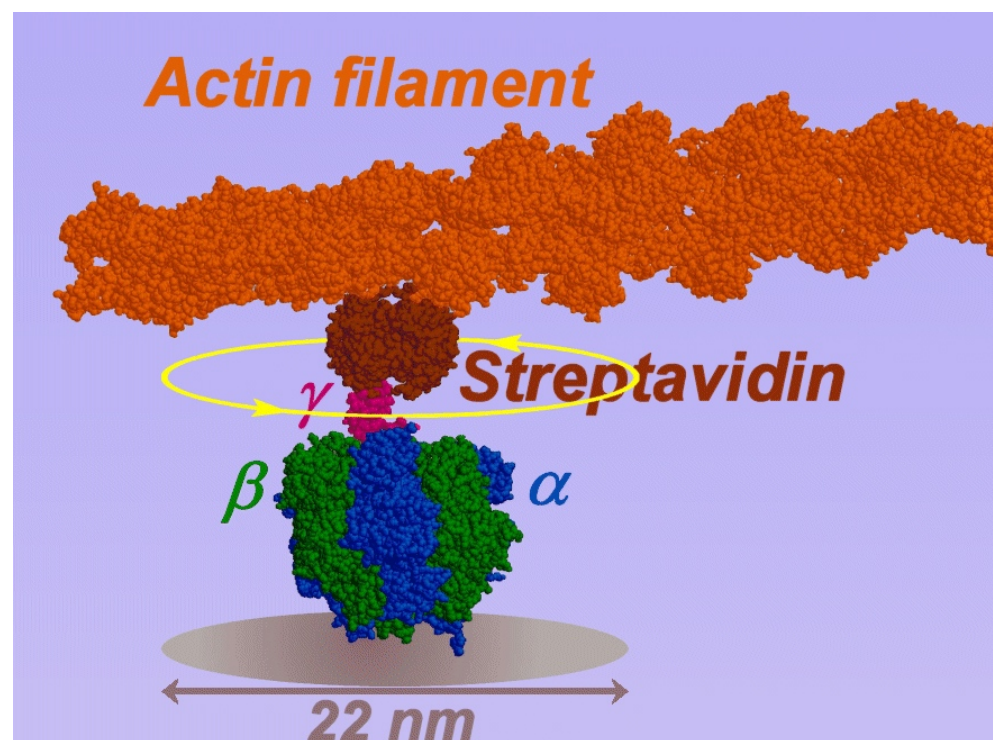
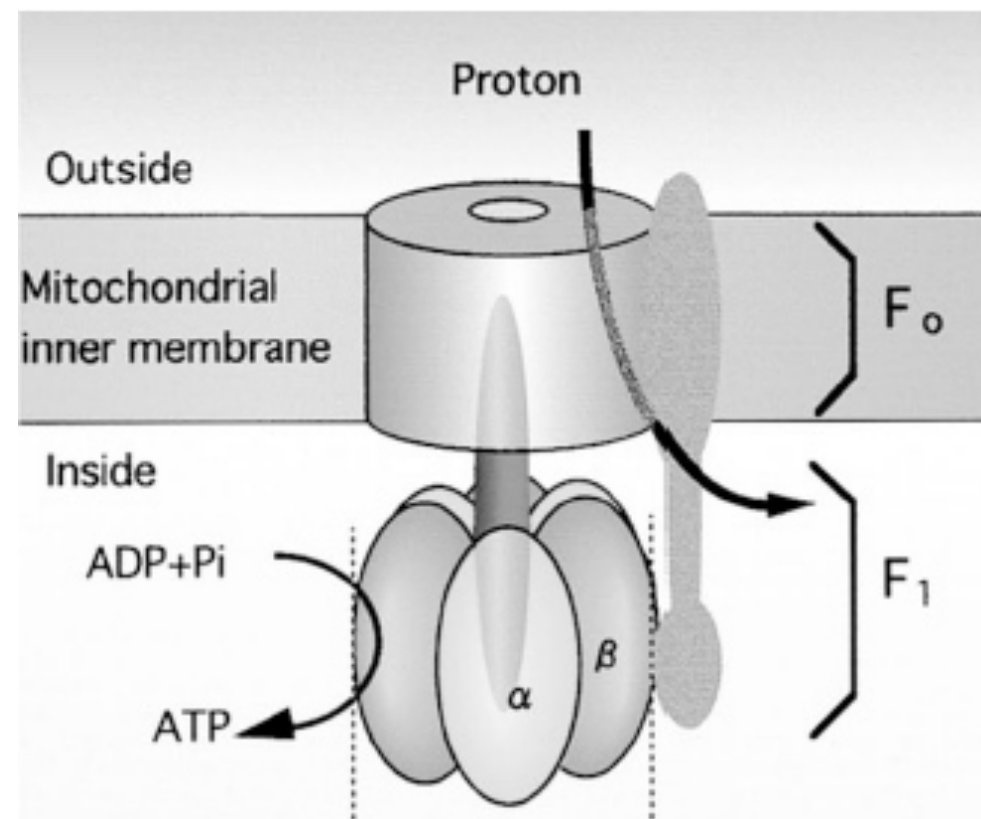
Myosin V  
cryoelectron-  
microscopic image  
series



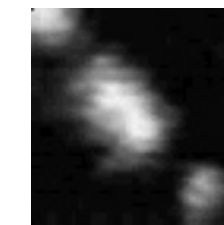


# Measurable parameters III. Rotational angle

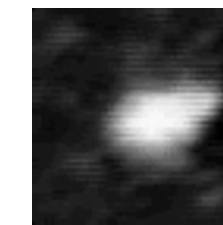
How does the ATP synthase work?



1 mM ATP

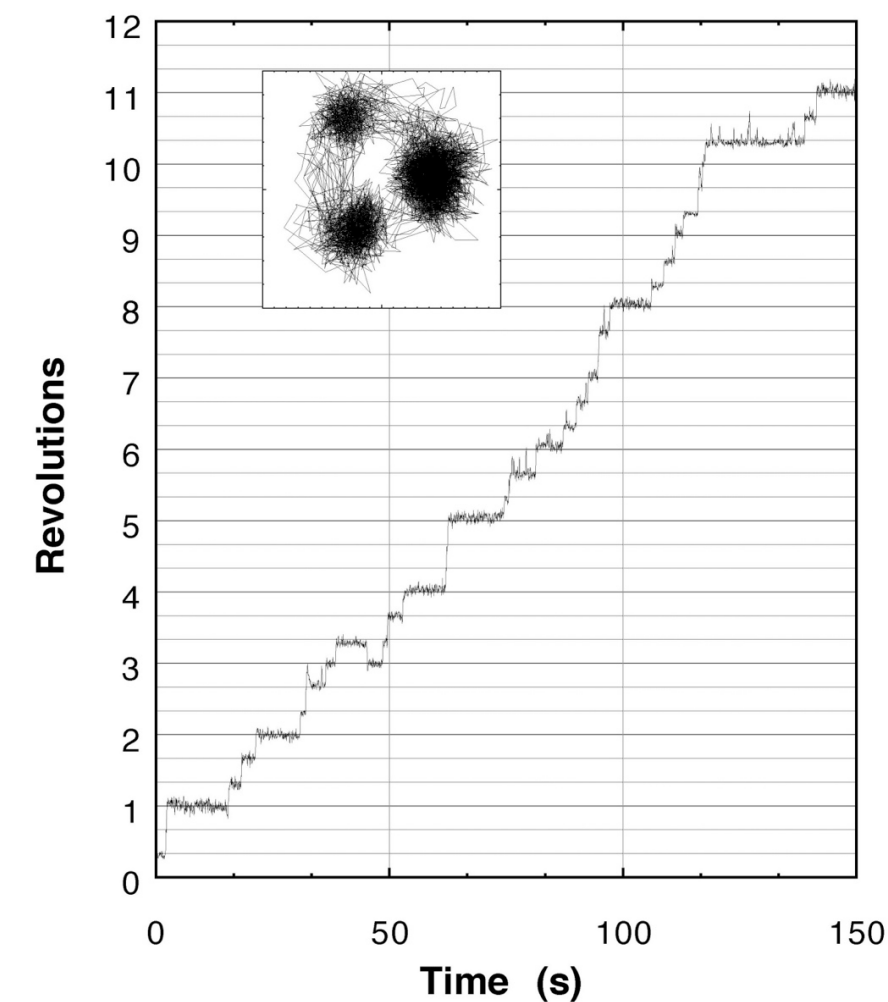


200 nM ATP



20 nM ATP

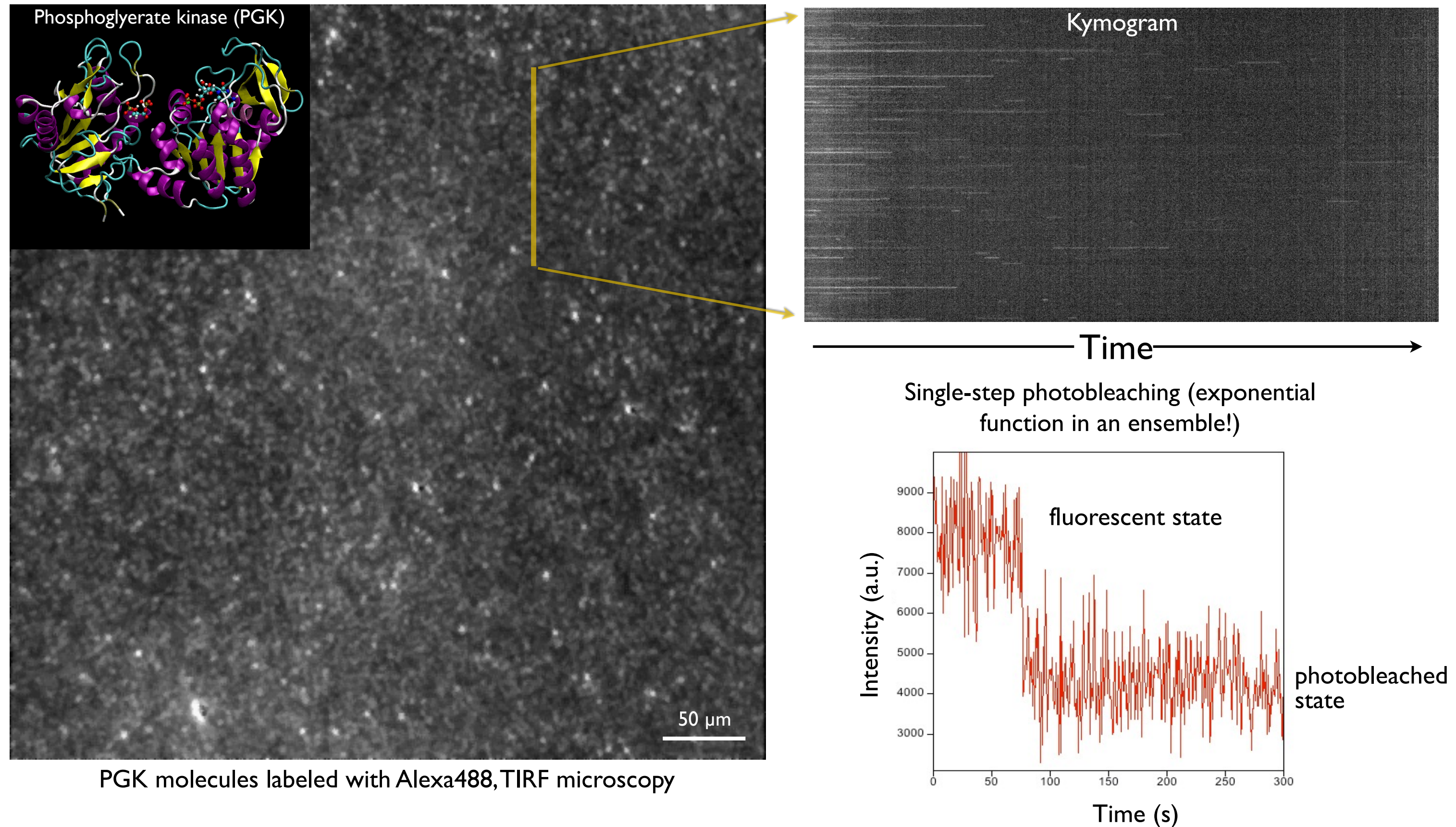
Discrete rotational steps of  $120^\circ$





# Measurable parameters IV. Fluorescence

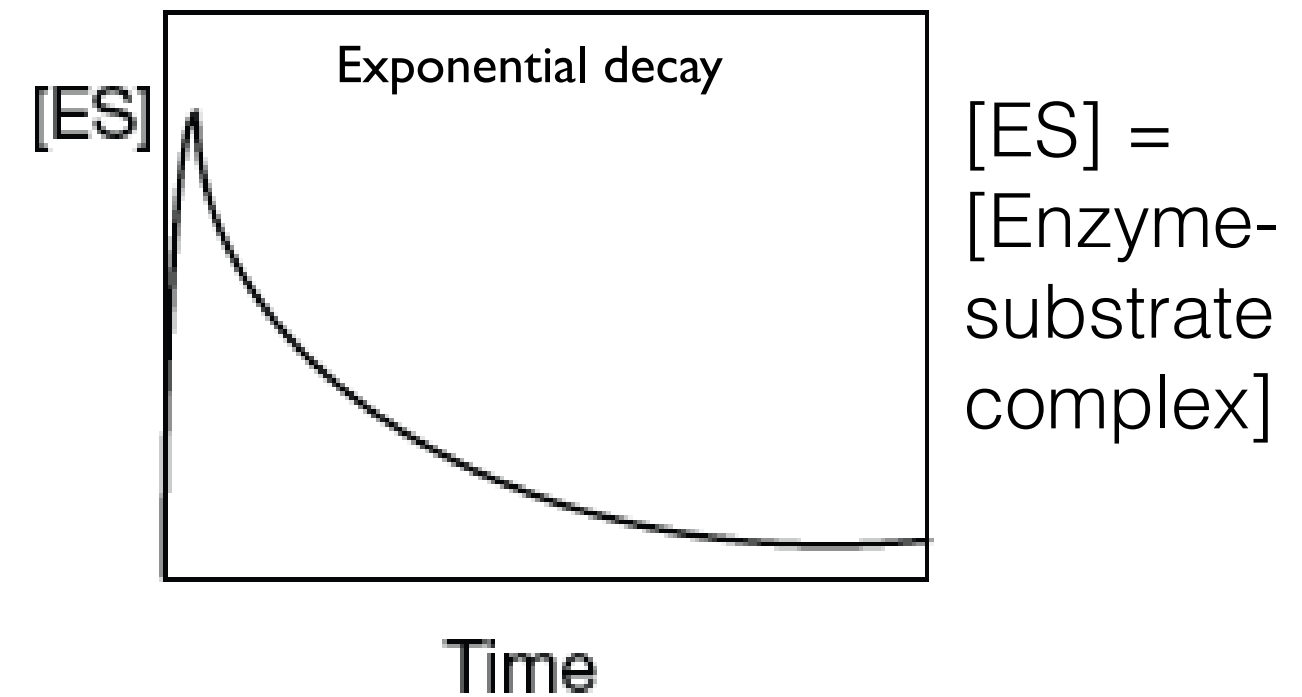
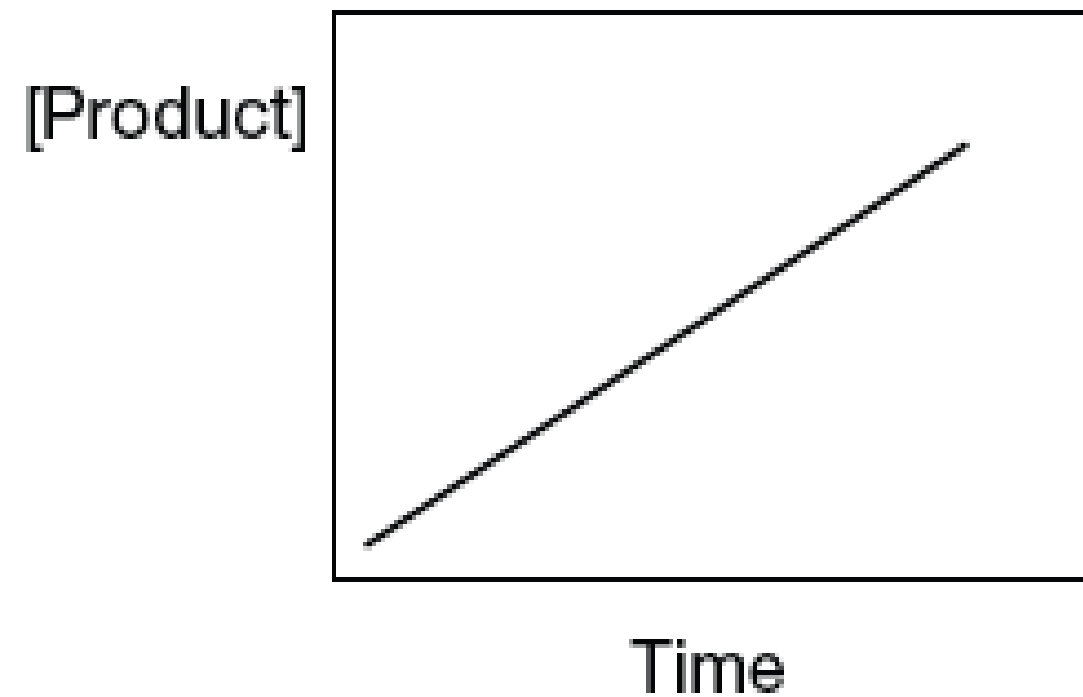
What are the conformational states of a molecule?



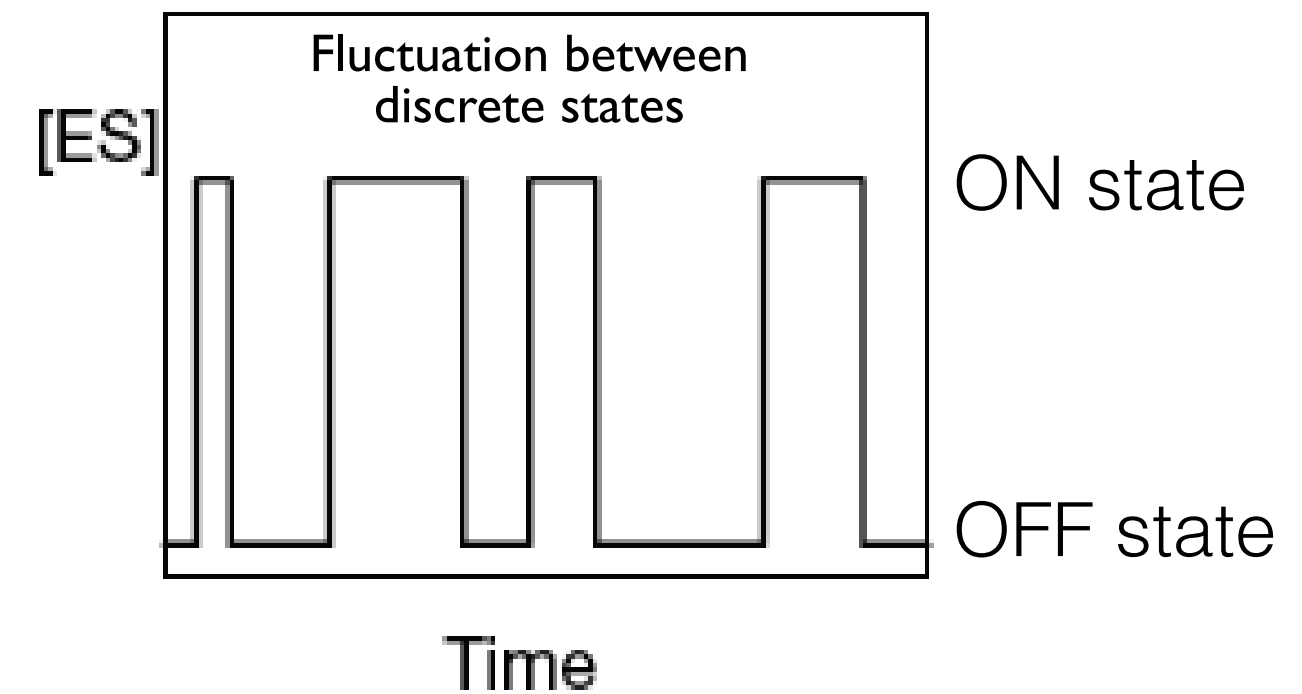
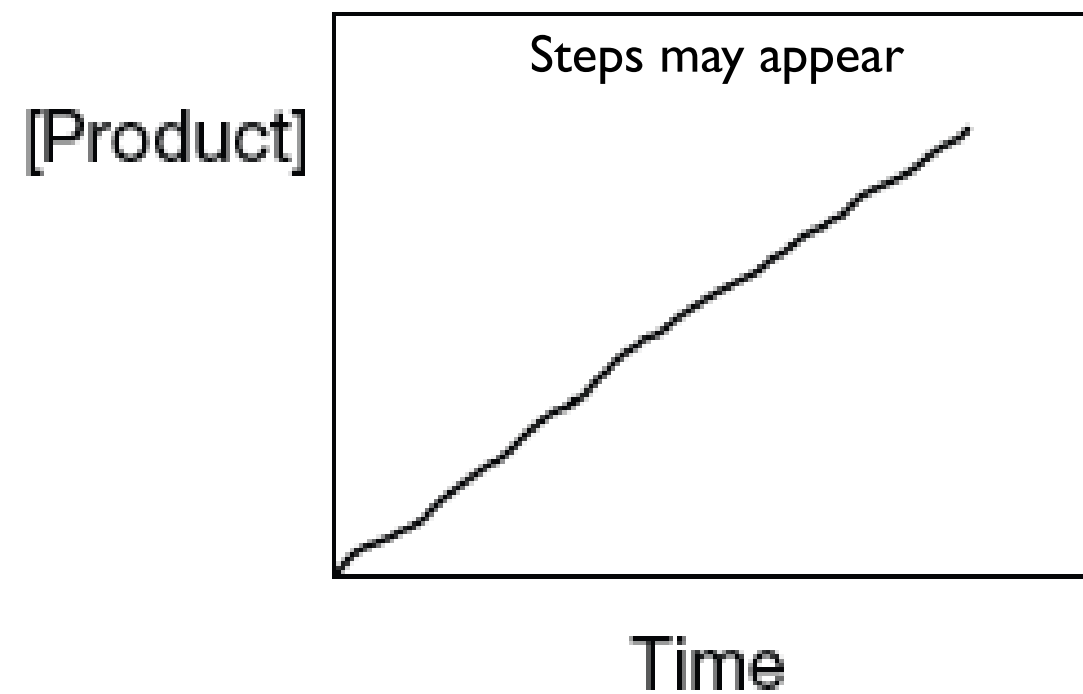


# Ensemble *versus* single molecule behavior

Ensemble:



Single molecule:



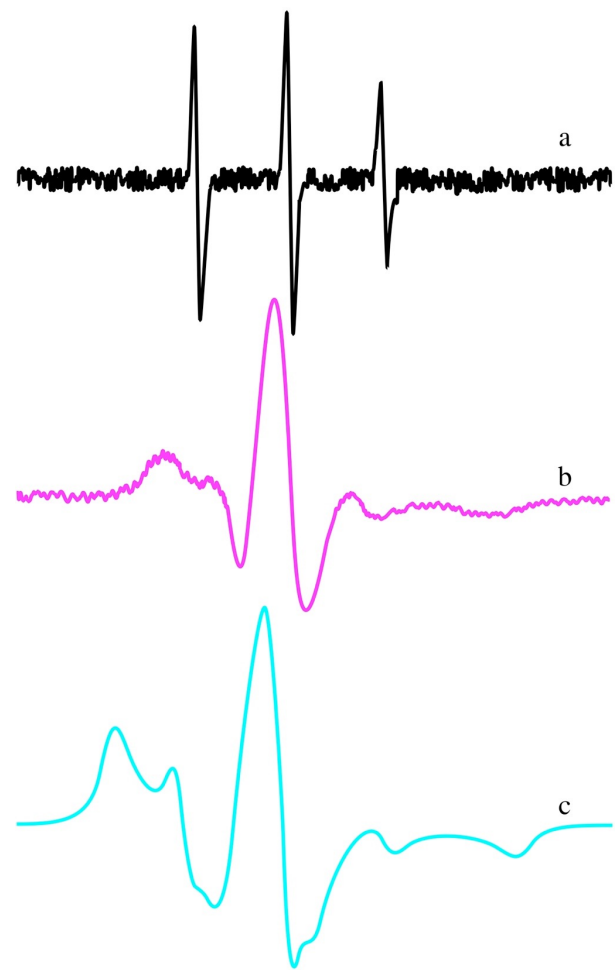


# Radio spectroscopies

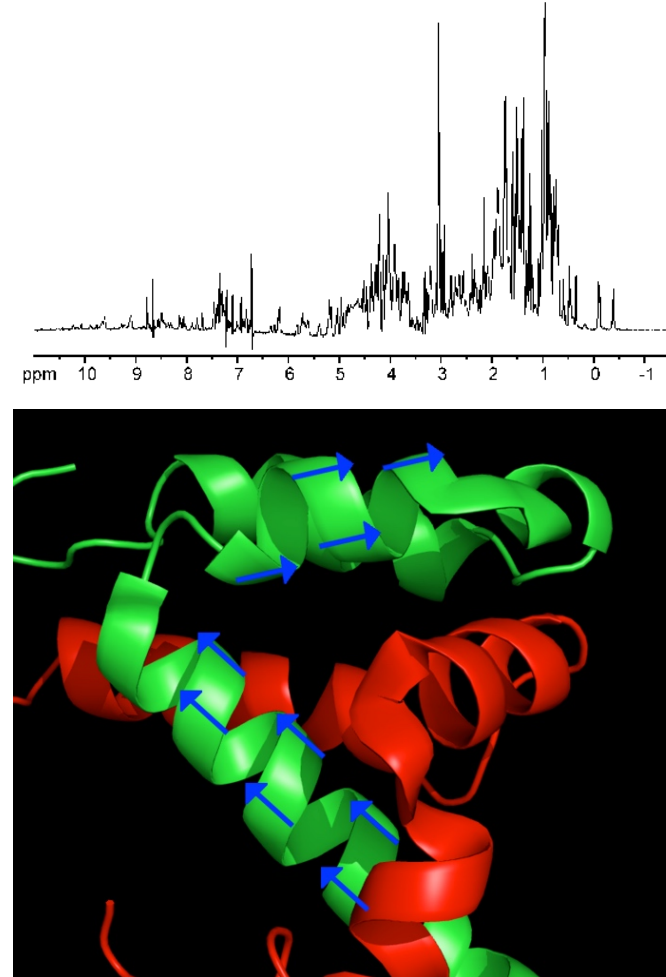
Revolutionized physics, chemistry, biology and medicine

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

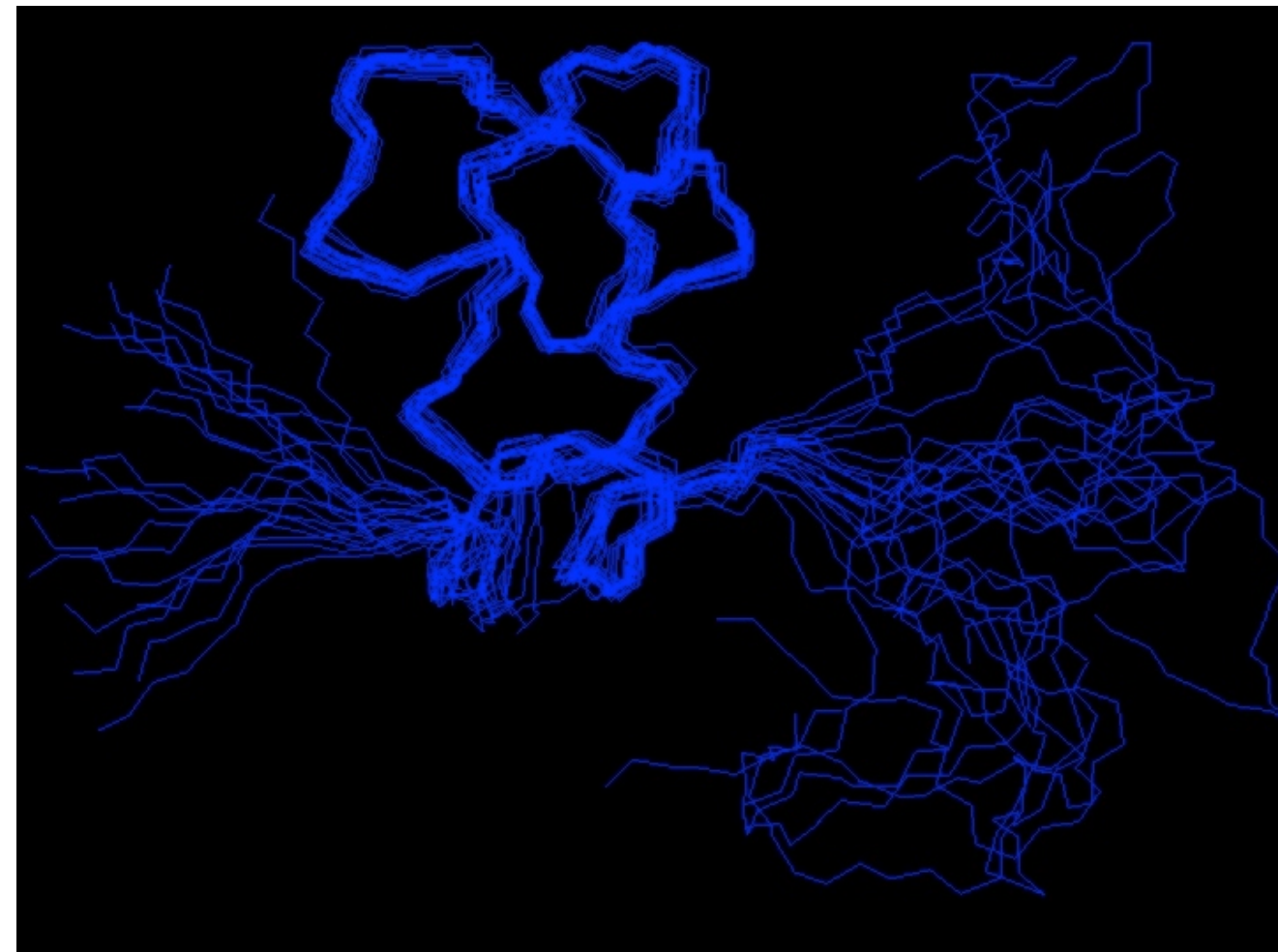
EPR spectroscopy



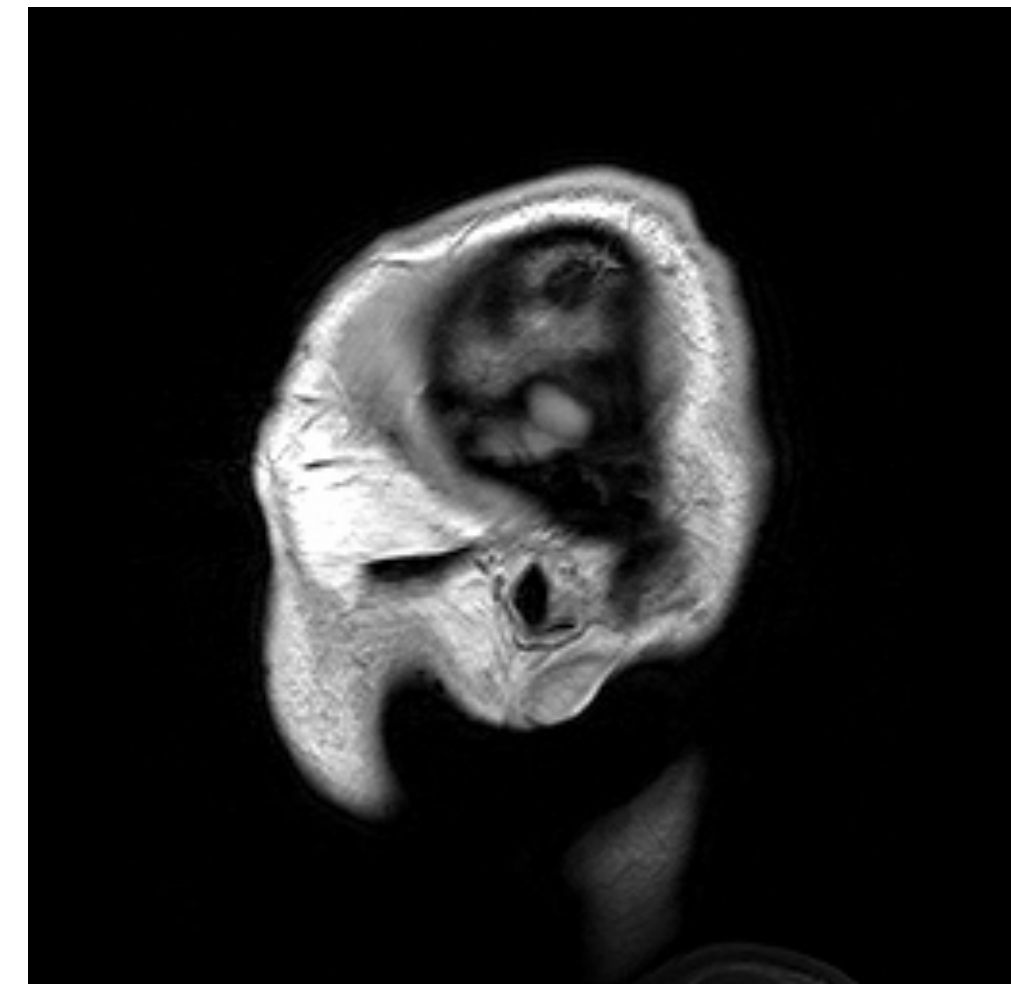
NMR spectroscopy



Protein molecular dynamics with NMR



High-resolution, anatomical MRI





# Atomic, molecular systems may behave as elementary magnets

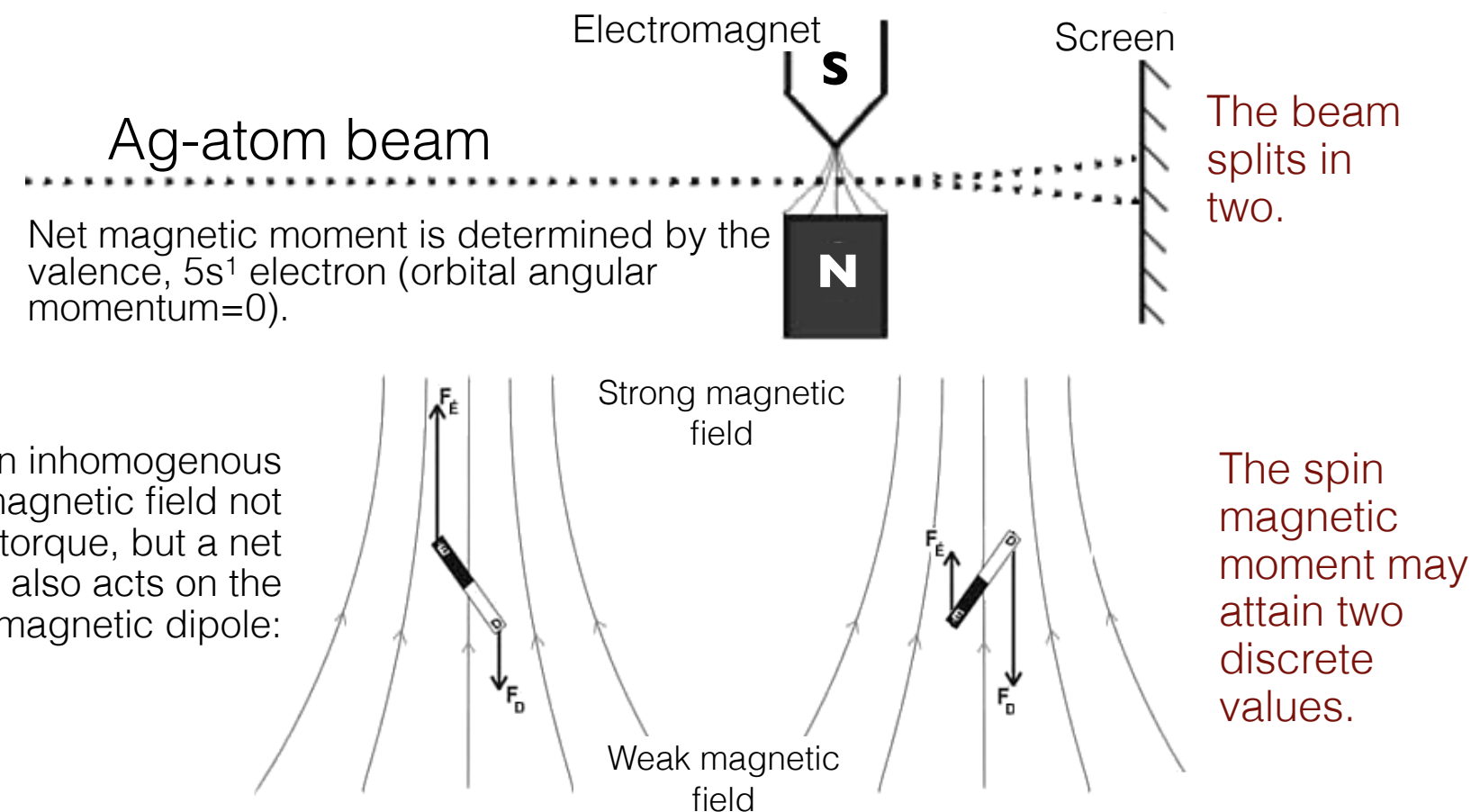
## Stern-Gerlach experiment (1922)



Otto Stern  
(1888-1969)



Walther Gerlach  
(1889-1979)



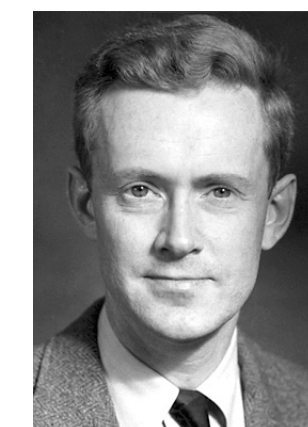
## Nuclear magnetic resonance, (NMR) Nobel-prize, 1952



Isidor Rabi  
(1898-1988)



Felix Bloch  
(1905-1983)



Edward Mills Purcell  
(1912-1997)

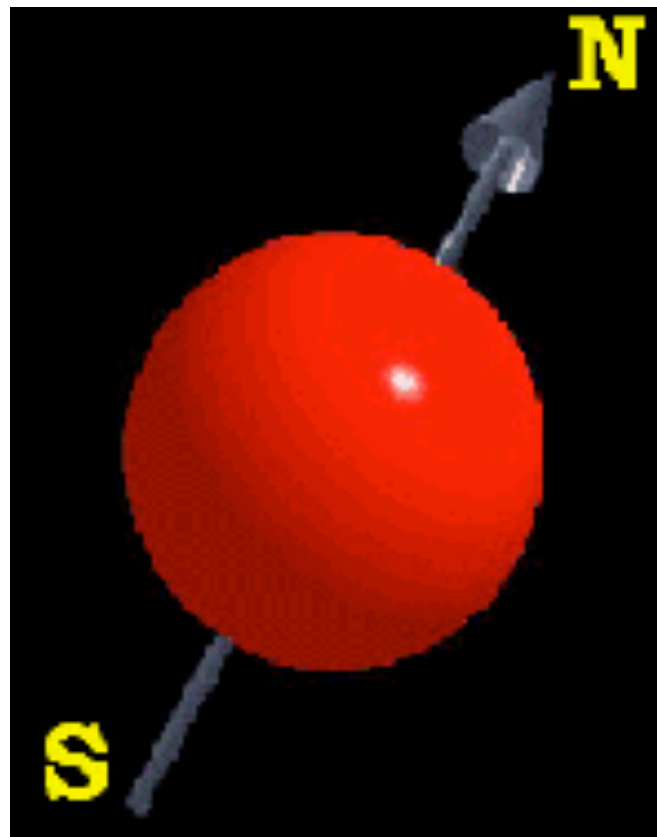
**Magnetic resonance:** resonance-absorption of electromagnetic energy by a material placed in magnetic field.



# Systems with net spin: elementary magnets

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- 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* emerges within the system.
- Atomic nucleus: odd mass number - half nuclear spin ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{19}\text{F}$ ,  $^{31}\text{P}$ ); even mass number, odd atomic number - whole nuclear spin; even mass and atomic number - zero nuclear spin.
- Electron: net electron spin within a molecular system containing a stable unpaired electron (e.g., free radicals).
- Because of *charge* and *net spin*, *magnetic moment* emerges.



Nuclear magnetic moment:

$$M_N = \gamma_N L$$

$\gamma_N$  = 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:

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

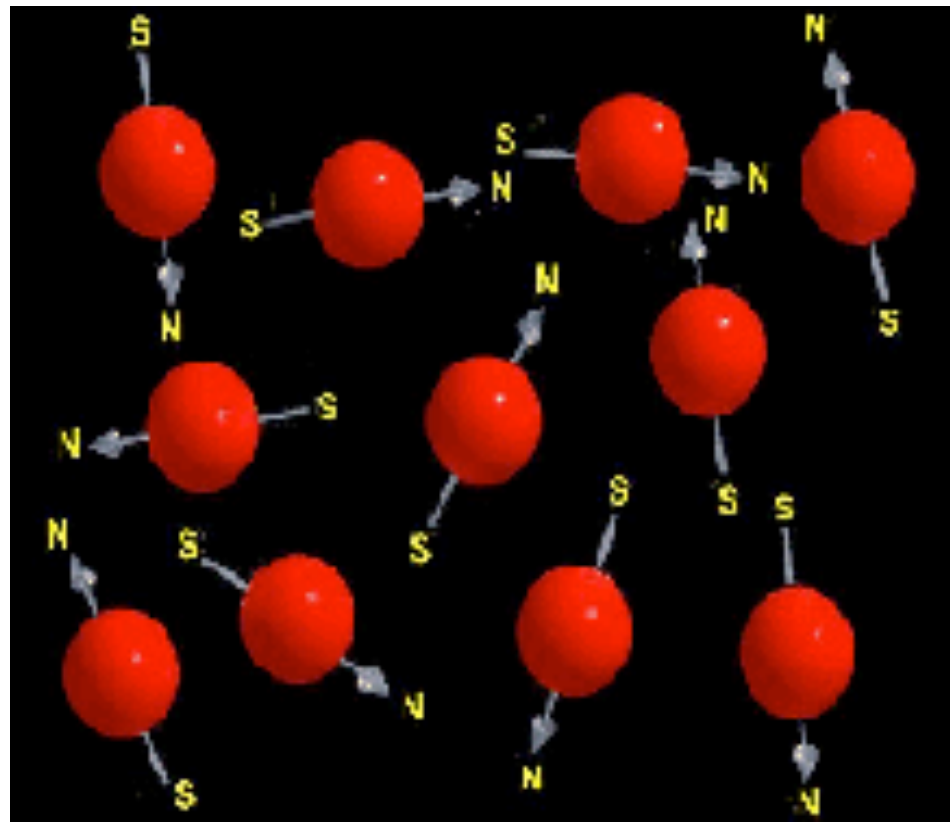
$g$  = electron's g-factor (dimensionless number that describes the relationship between magnetic moment and gyromagnetic ratio)

$\mu_\beta$  = Bohr's magneton (unit of the electron's magnetic moment)

$S$  = spin quantum number

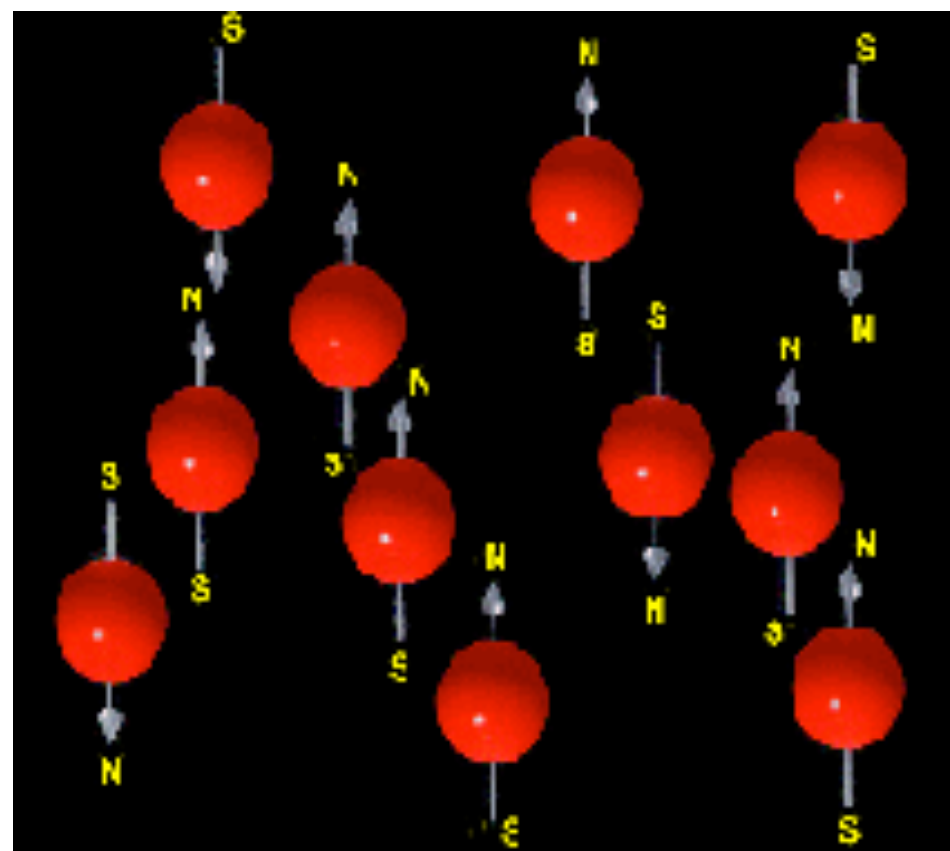


# In external magnetic field the elementary magnets orient



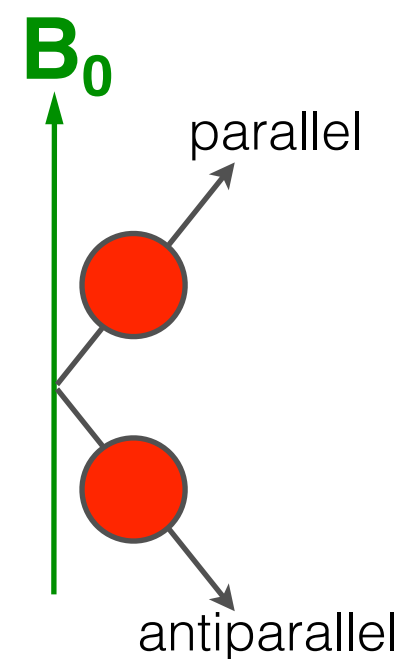
In absence of magnetic field:  
random orientation of elementary magnets

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

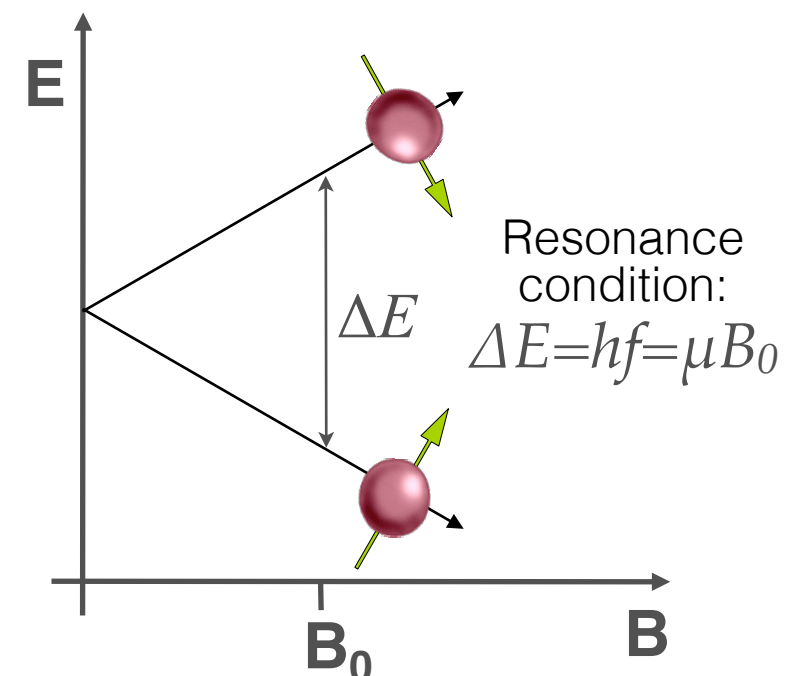


In magnetic field:

elementary  
magnets orient



energy  
levels split

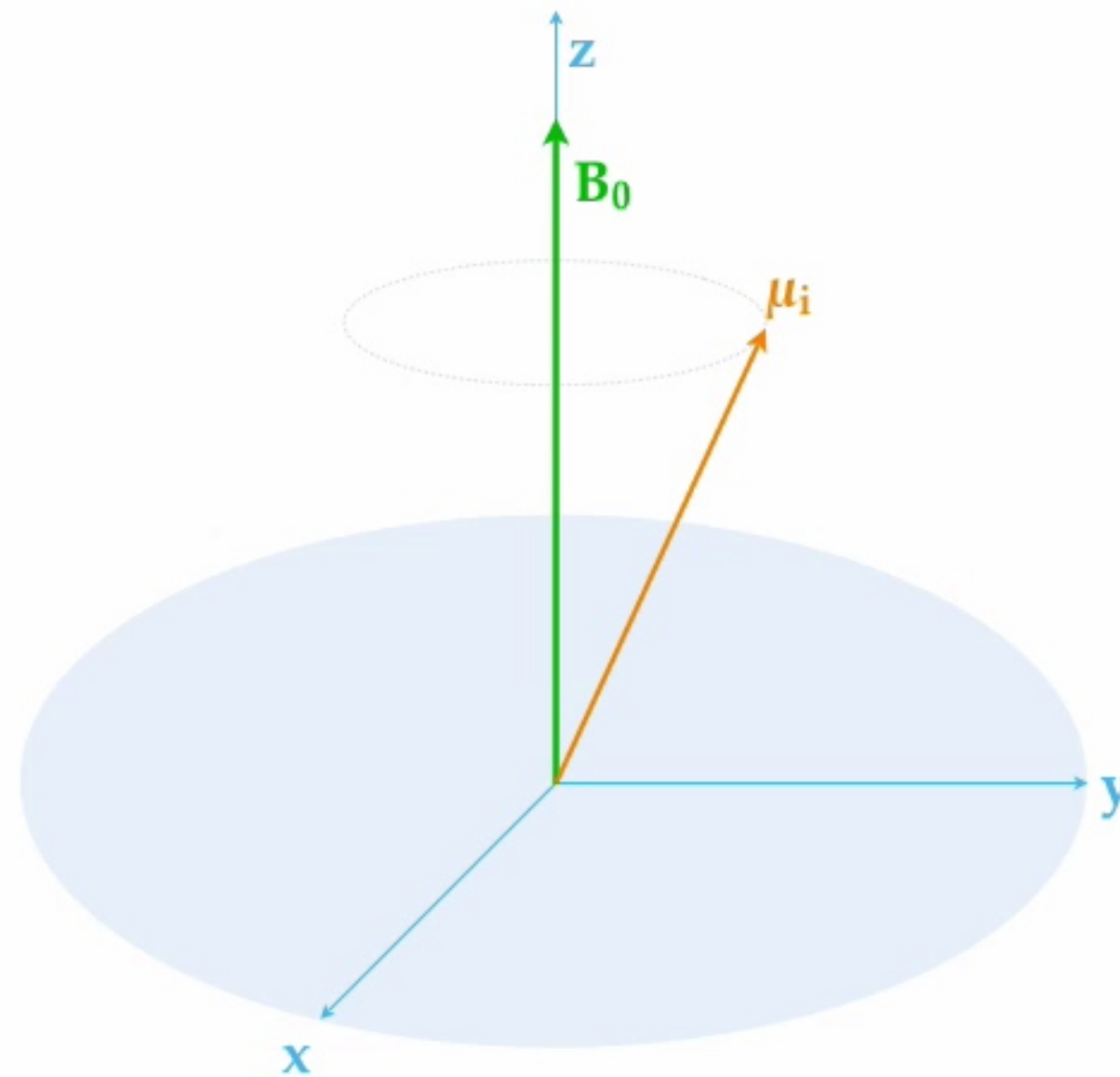


Edward Purcell,  
1946

# Oriented elementary magnets do precessional motion



Precession of a top



Precession of an elementary magnetic moment ( $\mu_i$ ) in magnetic field ( $B_0$ ) within a reference  $xyz$  space

Precession or Larmor frequency:

$$\omega_0 = \gamma B_0$$
$$f_{Larmor} = \frac{\gamma}{2\pi} B_0$$

Resonance condition:

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

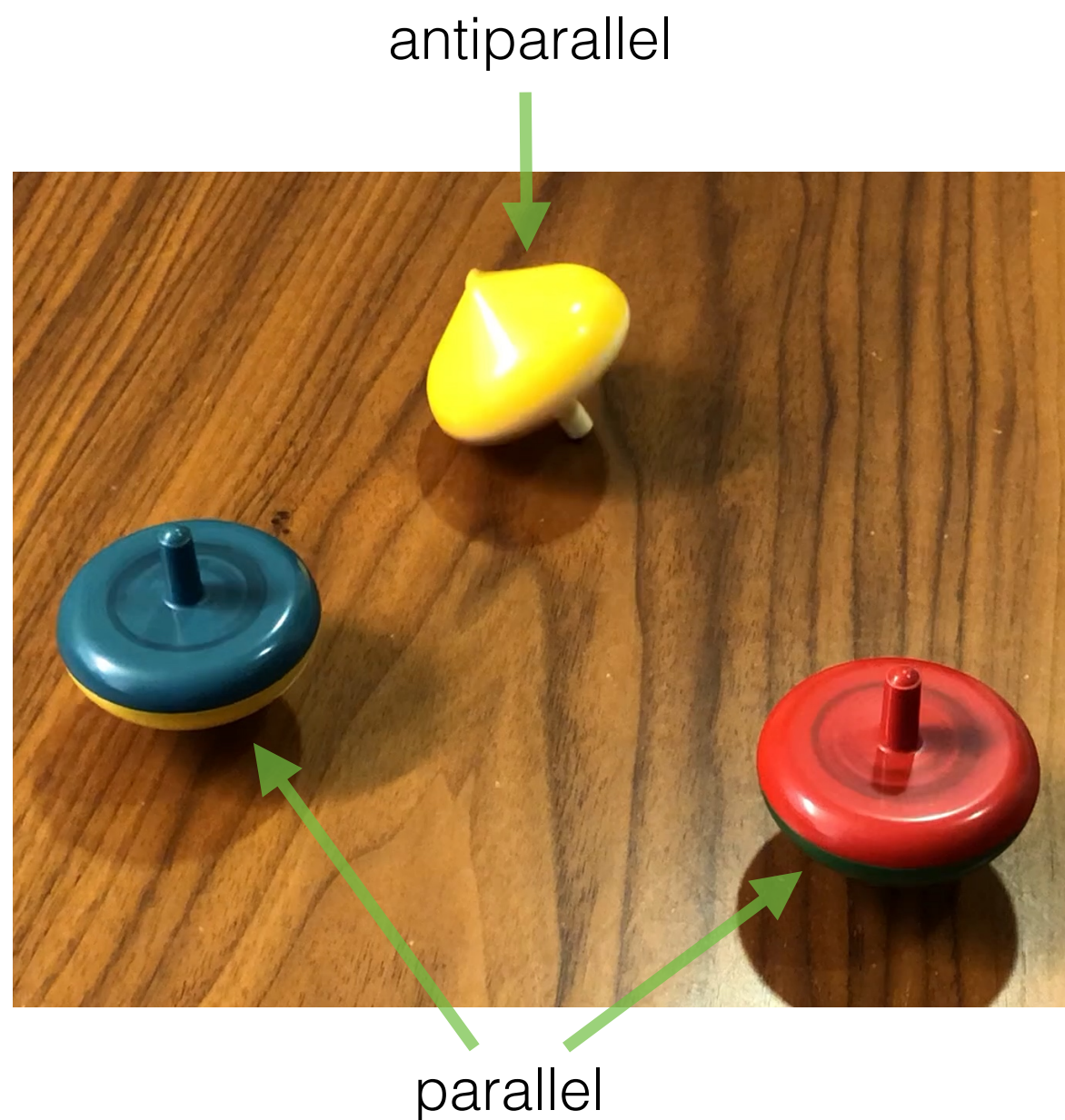


Felix Bloch, 1946

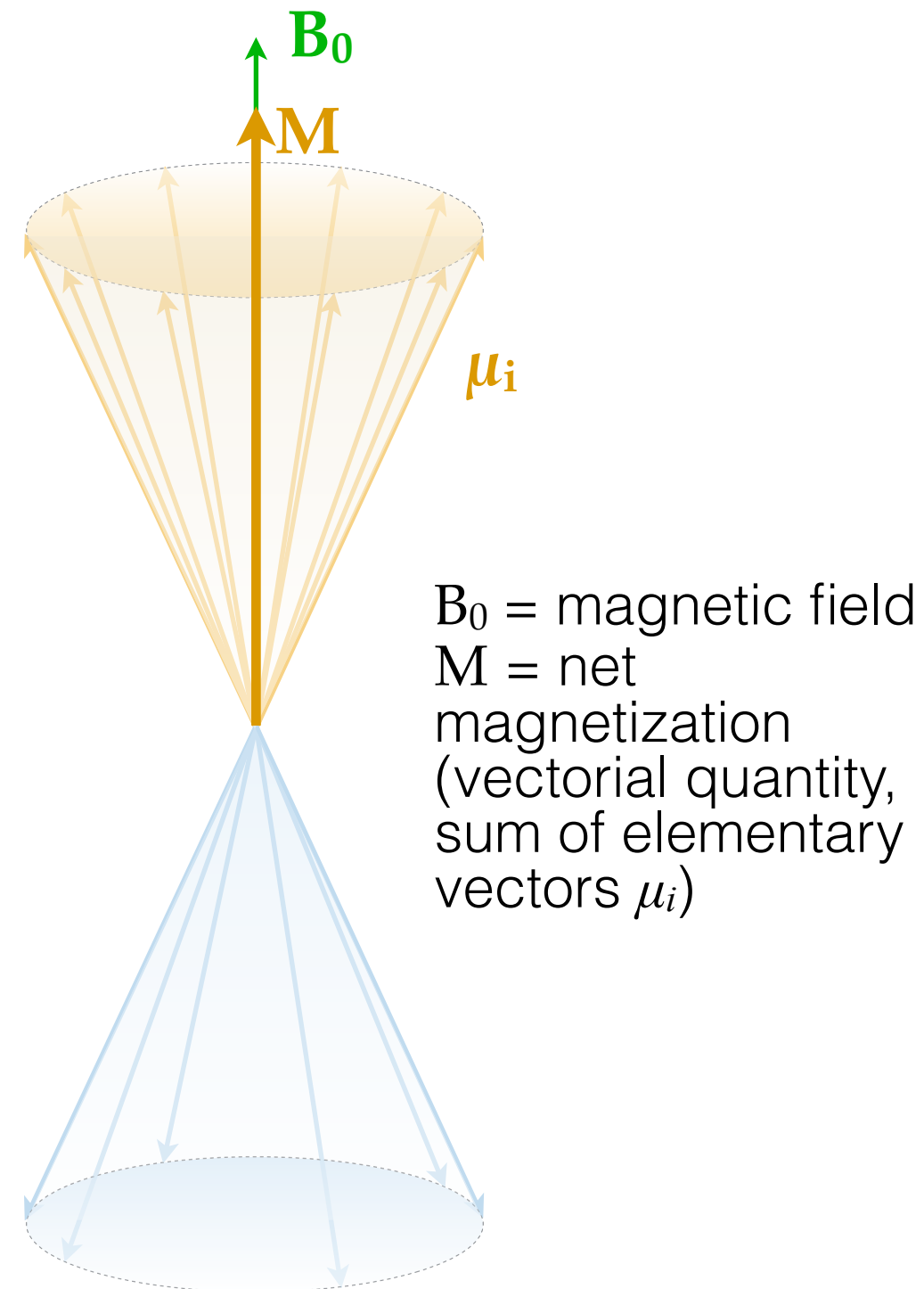


# Net (macroscopic) magnetization

Due to spin access in different energy states



Low-energy state  
parallel in case of proton ( $^1\text{H}$ )



High-energy state  
antiparallel in case of  $^1\text{H}$

Ratio of low- and high-energy spin populations is determined by the Boltzmann distribution:

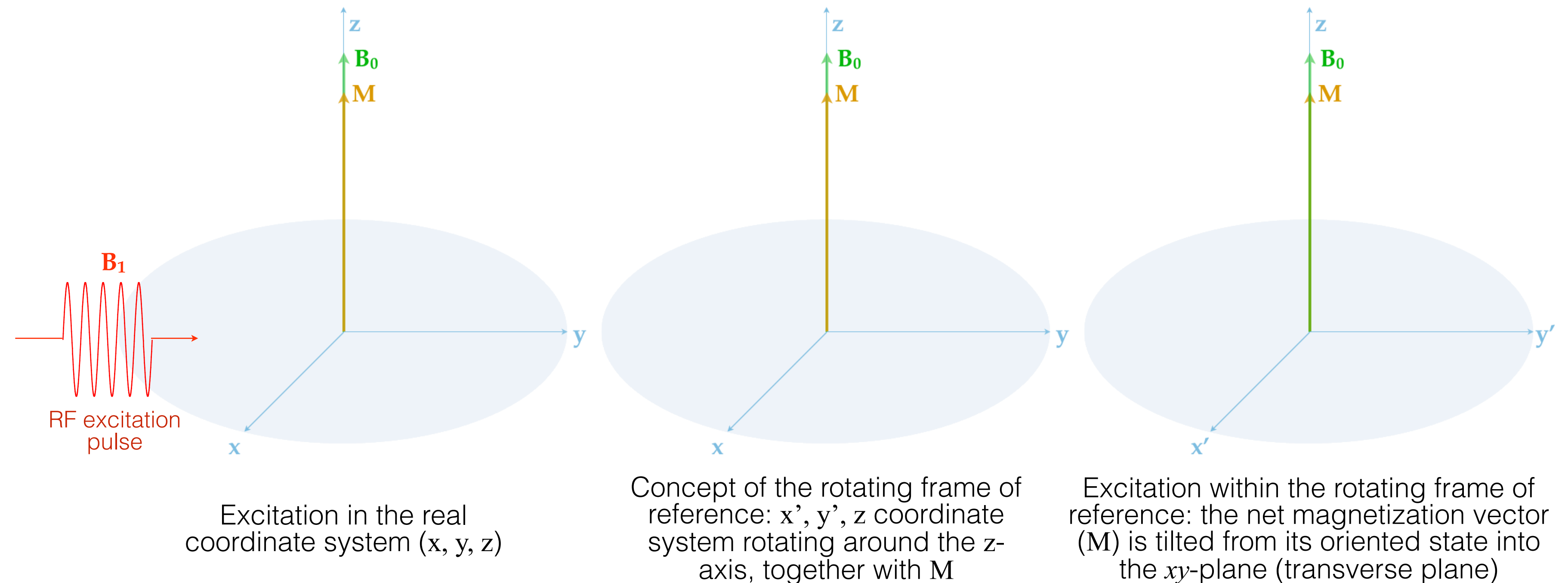
$$\frac{N_{\text{antiparallel}}}{N_{\text{parallel}}} = e^{-\frac{\Delta E}{k_B T}}$$

N.B.: magnetic field in MRI is 20-50-thousand times as strong as the earth's magnetic field.

# Excitation

## Resonance condition, Larmor frequency

Employed electromagnetic radiation: radiowaves (NMR, MRI), microwaves (ESR)



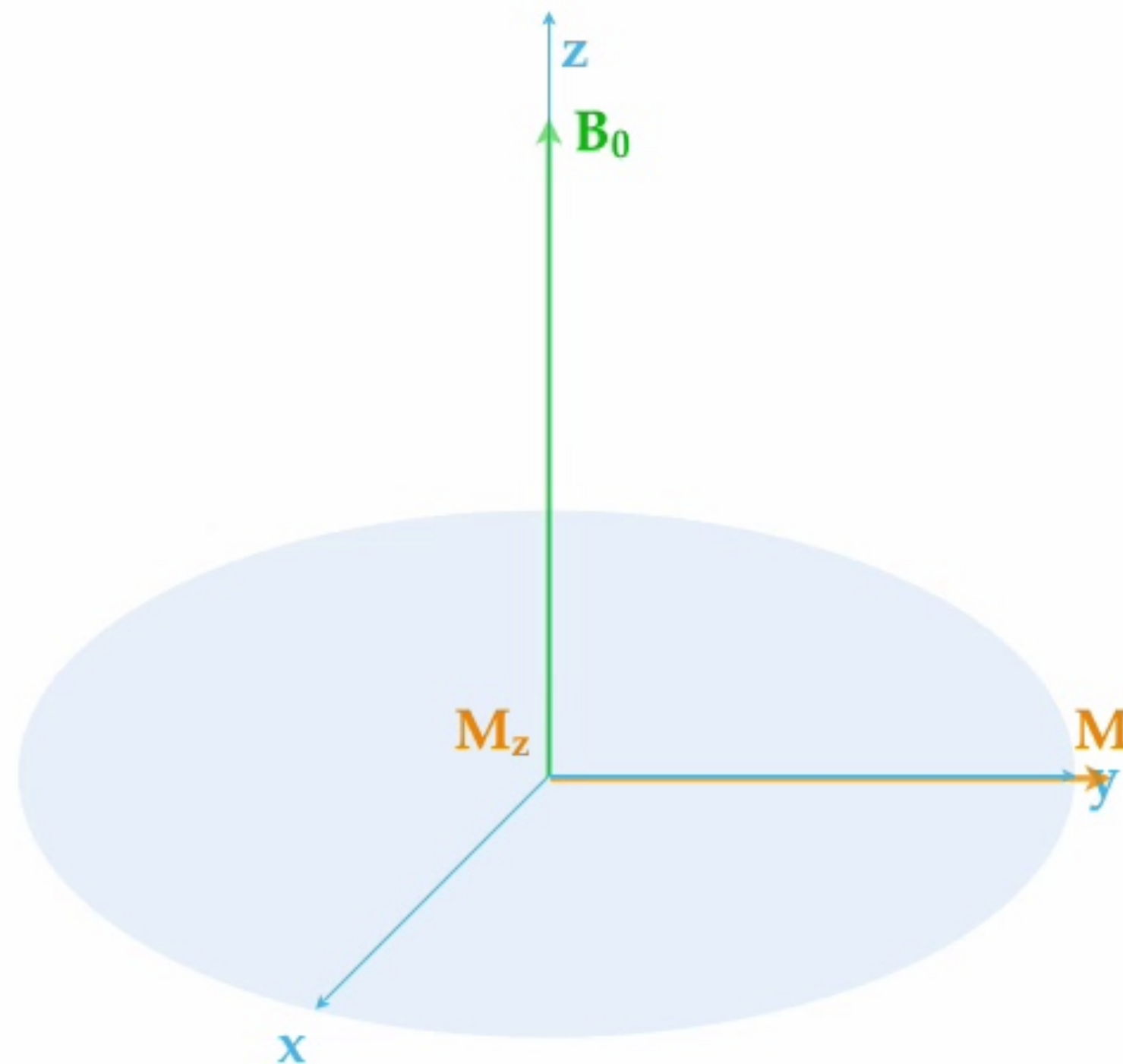
$B_0$  = magnetic field  
 $M$  = net magnetization  
 $B_1$  = irradiated radio frequency wave



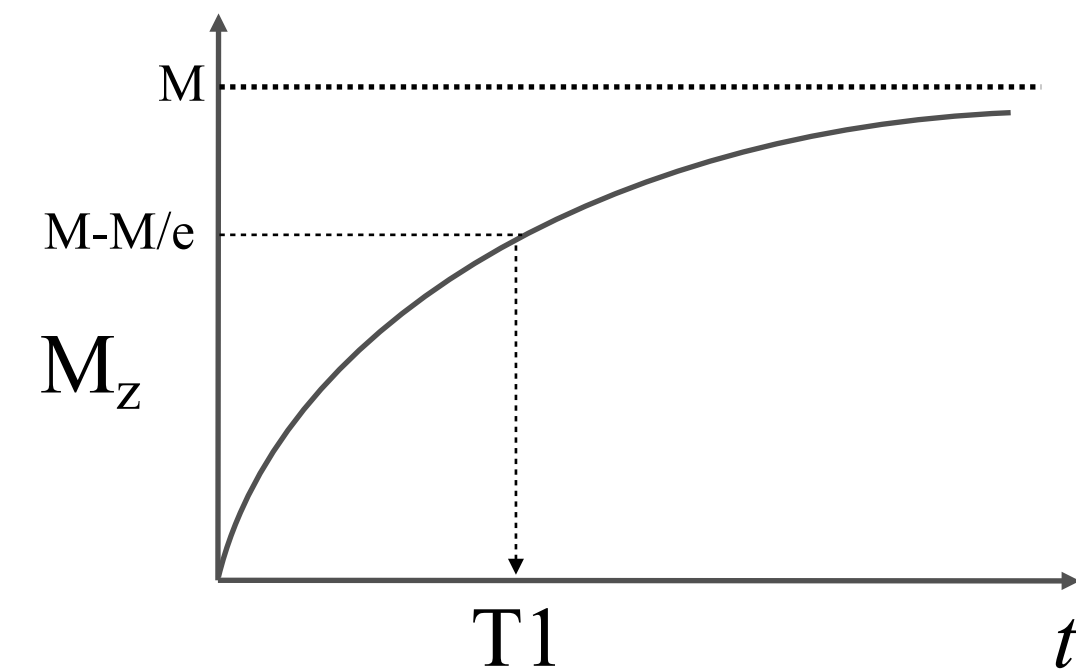
# Spin-lattice relaxation

## T1 or longitudinal relaxation

**T1 relaxation process:** return (relaxation) of the  $z$ -axis vectorial component of  $M$  ( $M_z$ ) towards the direction of the external magnetic field



$M_z$ :  $z$ -axis vectorial component of  $M$

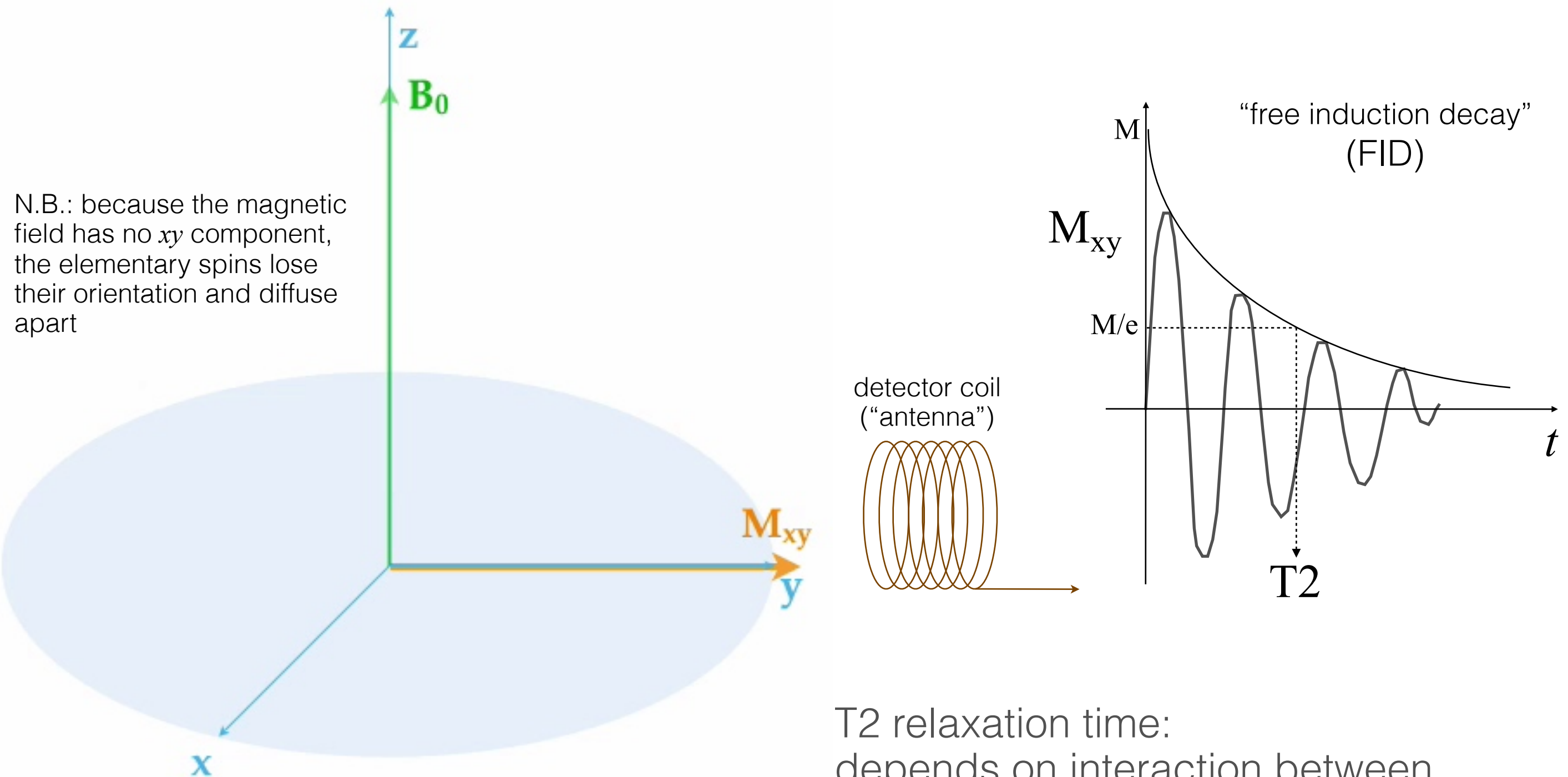


T1 relaxation time:  
depends on interaction  
between elementary magnet (proton)  
and its environment (lattice)

# Spin-spin relaxation

## T2 or transverse relaxation

**T2 relaxation process:** diffusion (spreading) of the elementary magnetic moments ( $\mu_i$ ) resulting in the decay of the transverse( $xy$ )-plane vectorial component of  $M$  ( $M_{xy}$ )



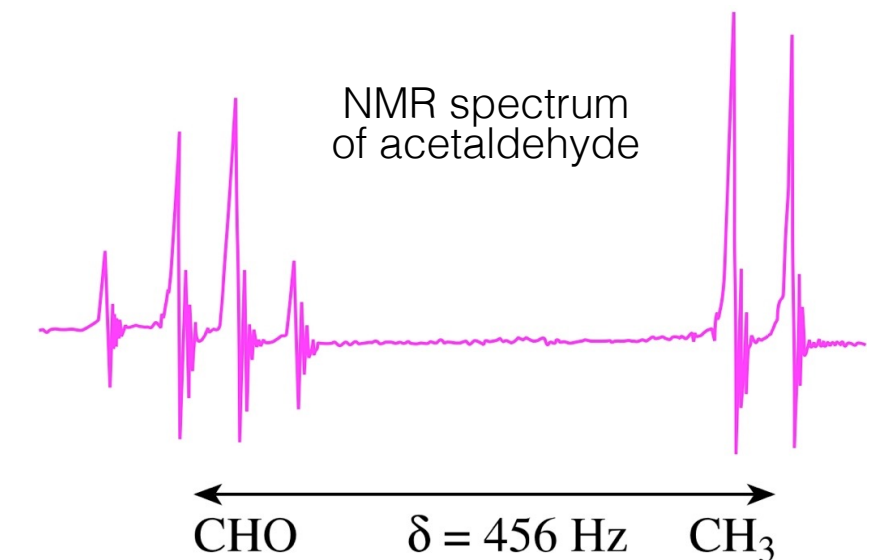
$M_{xy}$ :  $xy$ -plane vectorial component of  $M$

T2 relaxation time:  
depends on interaction between  
elementary magnets (spins, protons);  
process occurs in the transverse ( $xy$ ) plane

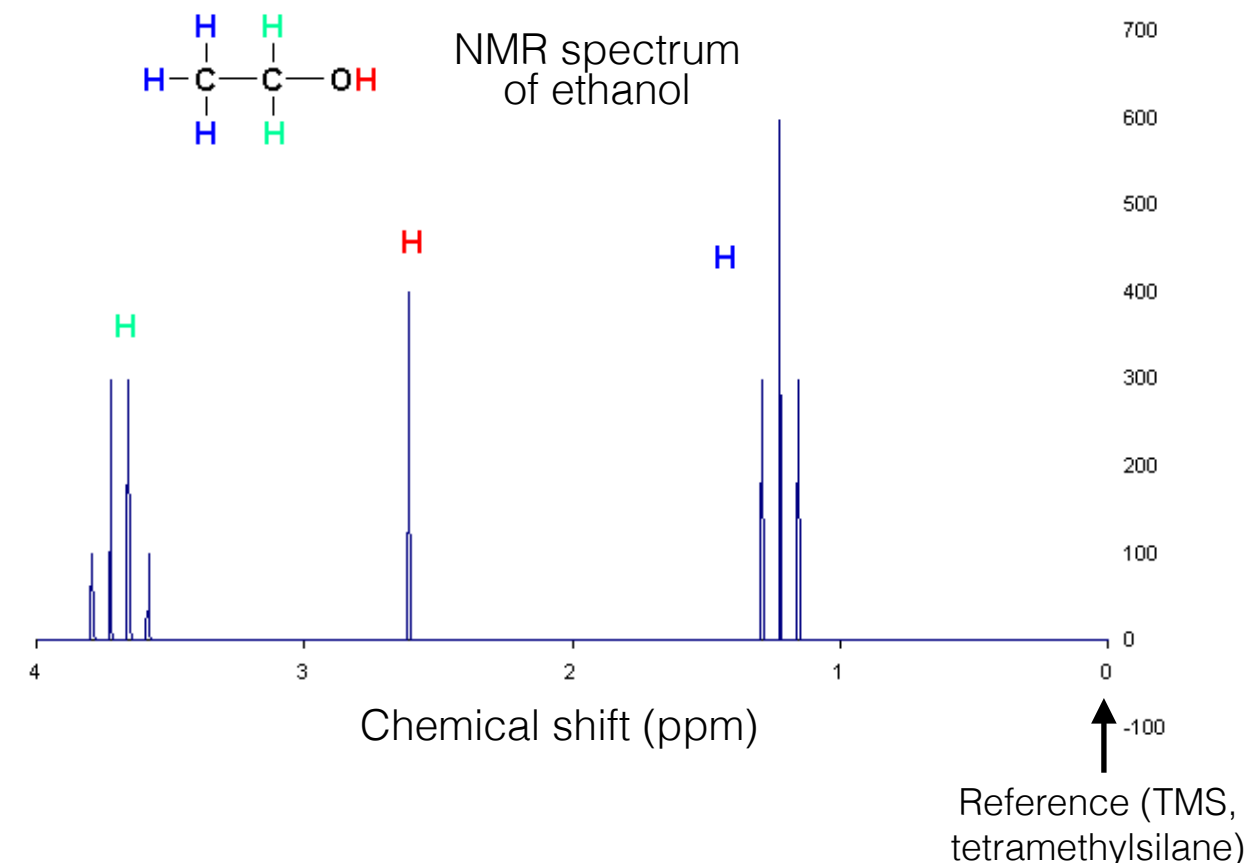


# NMR spectroscopy

- NMR spectroscopy or Magnetic Resonance Spectroscopy (MRS)
- Spectroscopic method for measuring the local magnetic field around atomic nuclei. We measure the resonance frequencies of the nuclei positioned in the magnetic field.
- NMR spectrometer: superconducting magnet (cooled with liquid He), large magnetic field (spectral resolution is proportional to field strength).
- NMR spectrum: intensity of absorbed electromagnetic radiation as a function of frequency.
- The area under the “NMR-line” is proportional to the number of absorbing atomic nuclei.
- The electron cloud distorts the local magnetic field, therefore the frequency condition is shifted: “chemical shift”. Chemical structure determination is possible.
- Protein NMR: possibility of measuring dynamics and the detection of disordered protein elements



900 MHz NMR, 21.1 T magnet



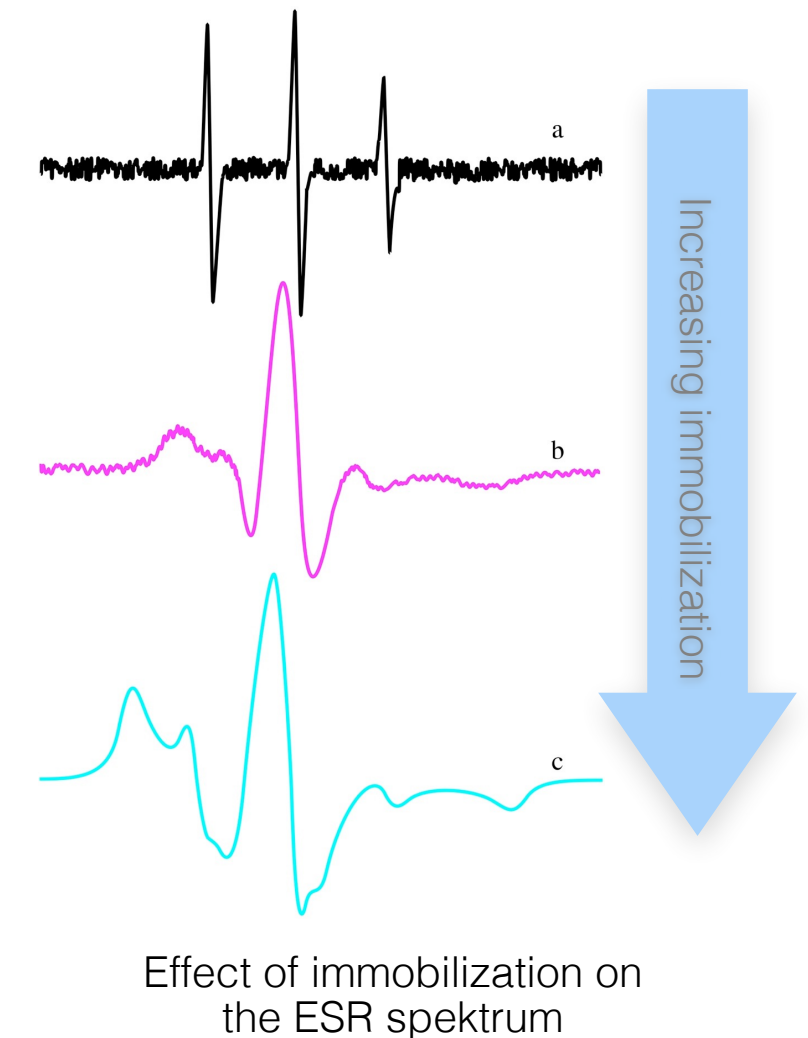
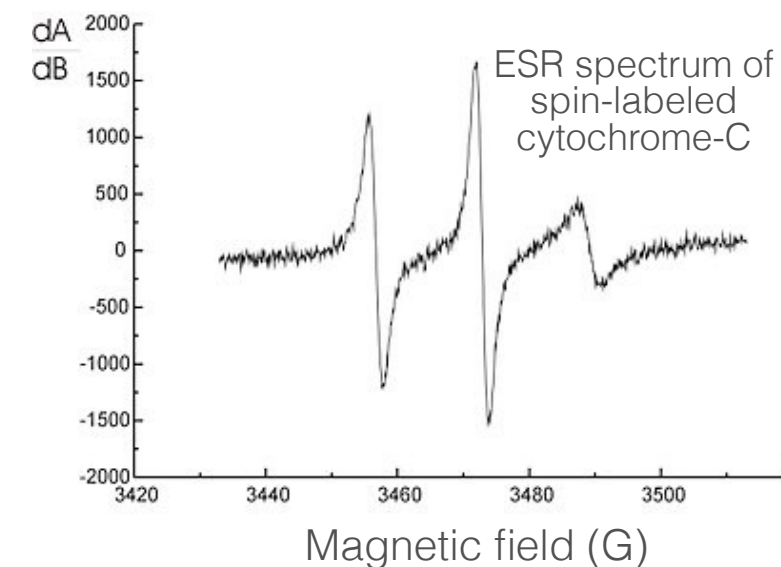
Somatomedin B domain  
(superimposed structures)

# ESR spectroscopy

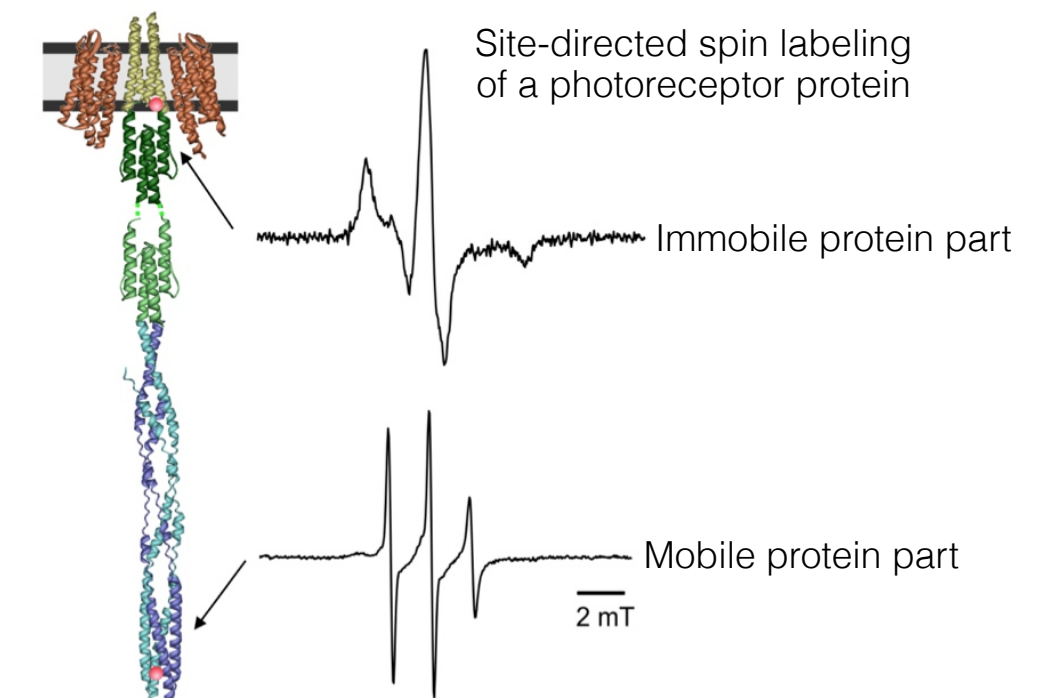
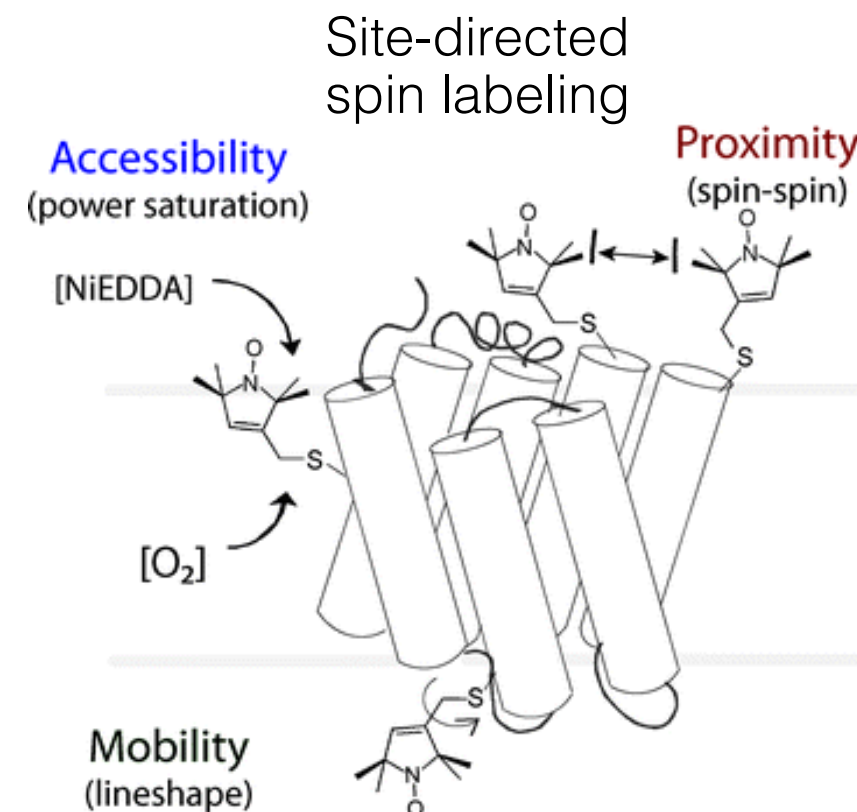
- Electron spin resonance (ESR) or electron paramagnetic resonance (EPR) spectroscopy.
- Spectroscopic method for investigating materials containing unpaired electron.
- EPR spectrum: intensity of electromagnetic radiation as a function of magnetic field.
- Magnetic field is lower, but radiation frequencies are greater (microwave) than in NMR.
- Spin-labeling: attachment of a chemical containing a stable unpaired electron. Site-directed spin labeling: spin labeling of reactive (mostly -SH) residues introduced into the targeted protein by molecular biological tools (point mutation).
- Dynamics of rotational motion can be measured up to the  $10^{-4}$  -  $10^{-2}$  s time range.



Jevgenij Zavoisky, 1944

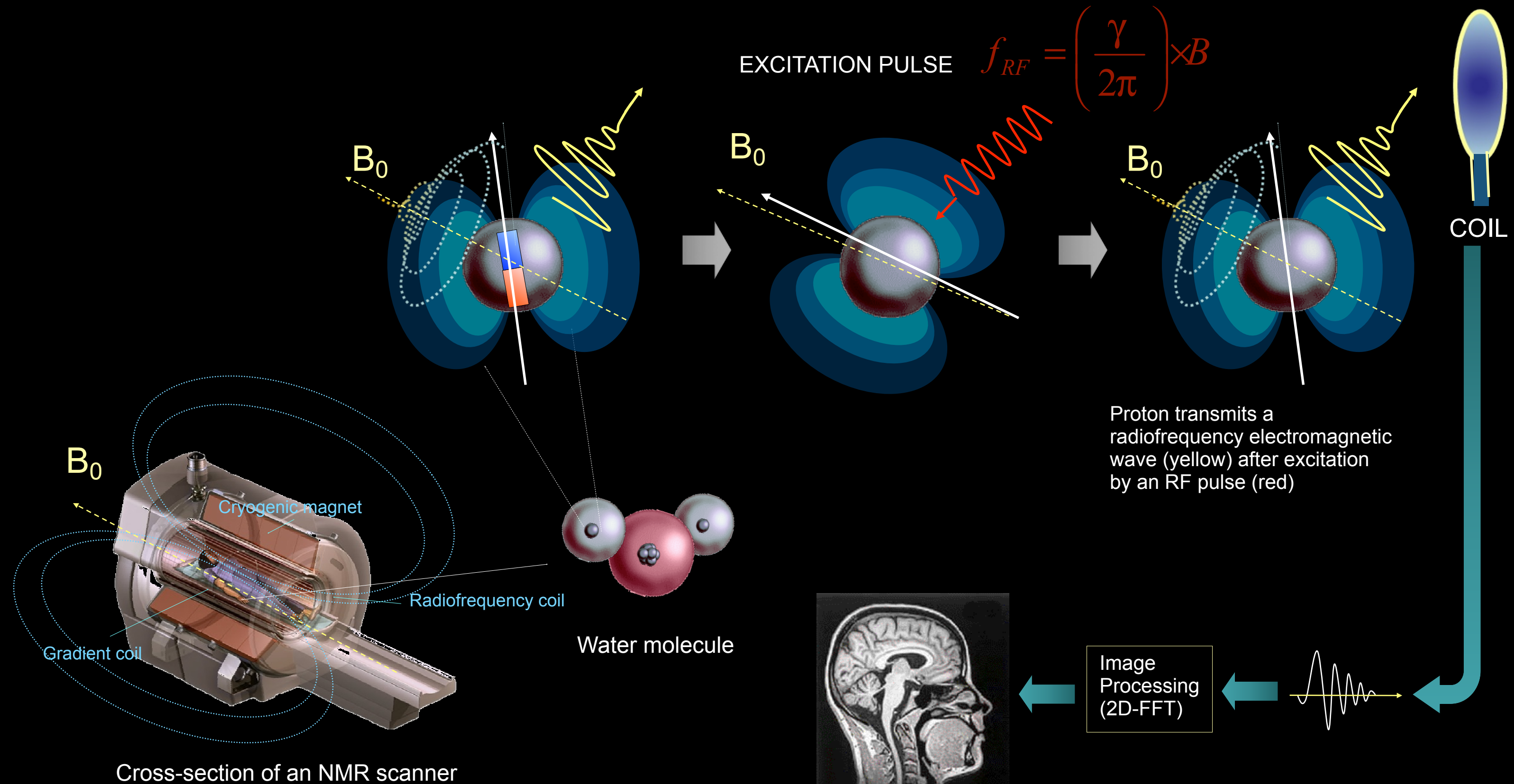


ESR spectroscopy workstation



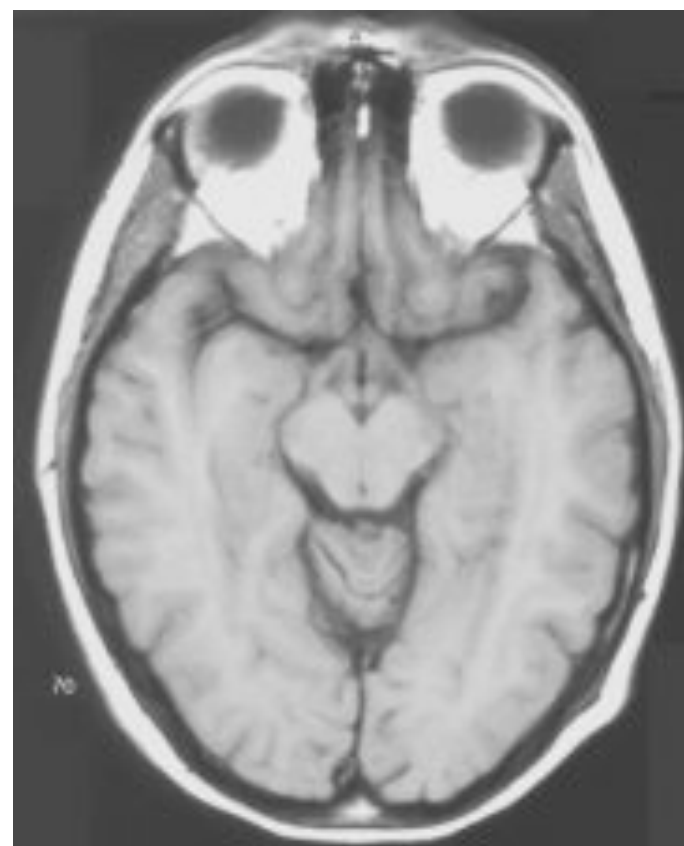
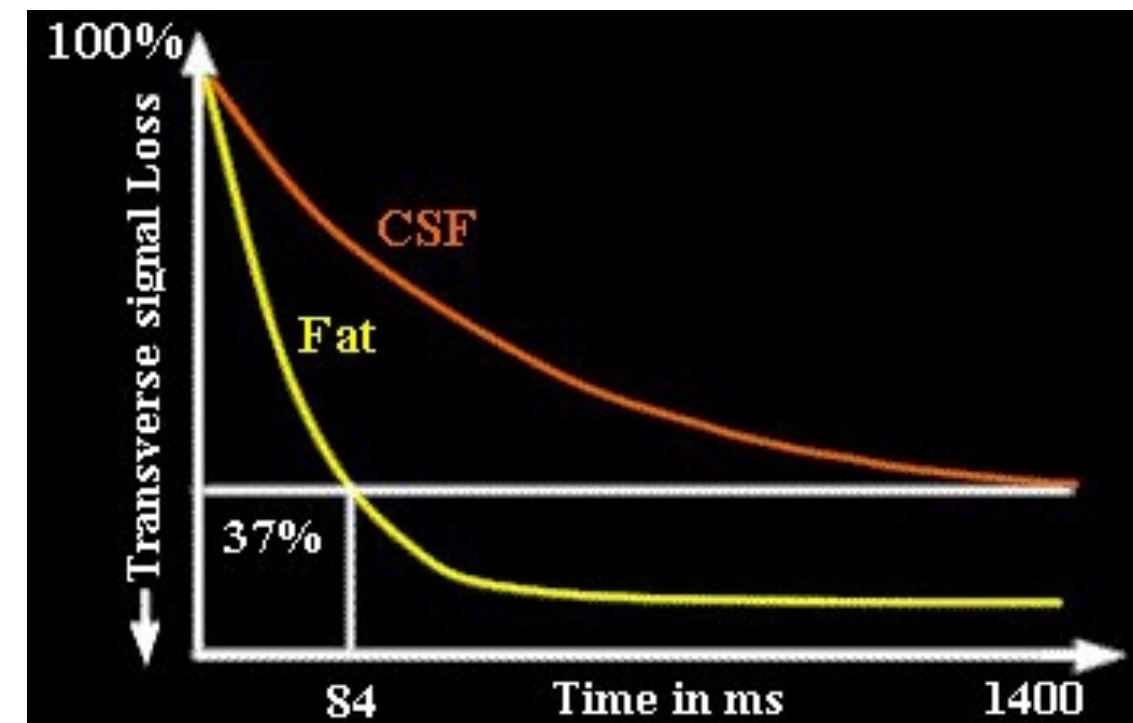
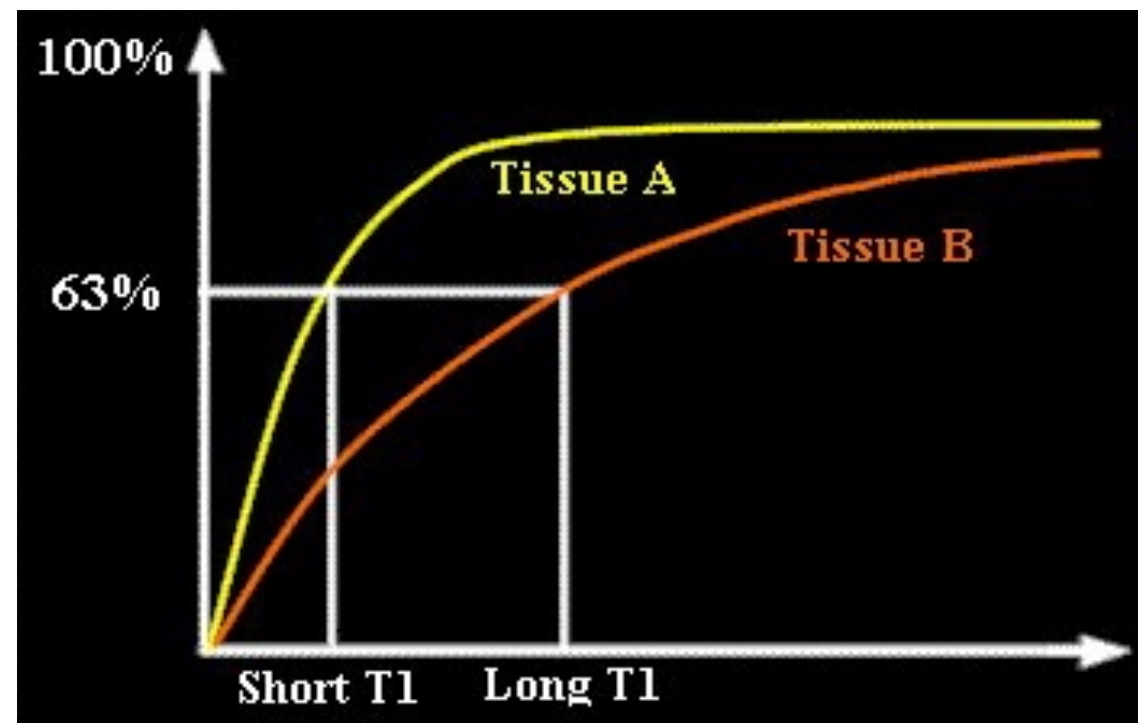


# MRI: the human body is macroscopically magnetized

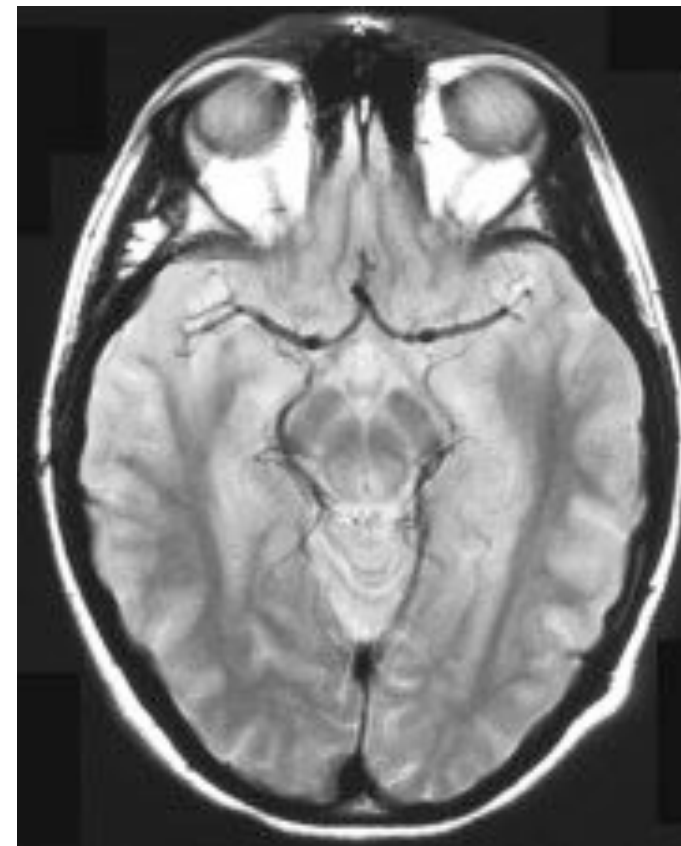


# MRI 1: contrast

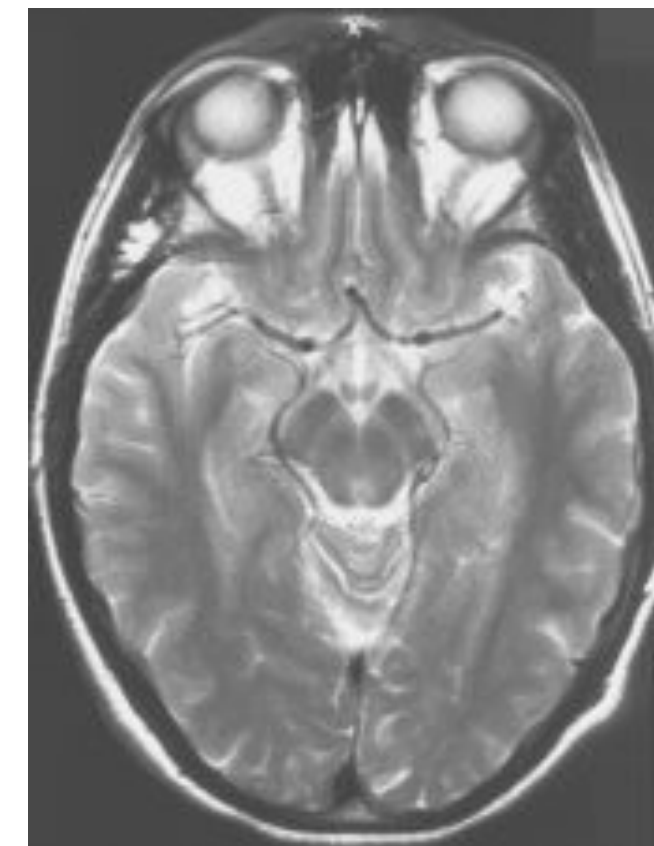
Color contrast based on spin density (proton density, PD) and relaxation times (T1, T2)



T1-weighting



proton density-weighting

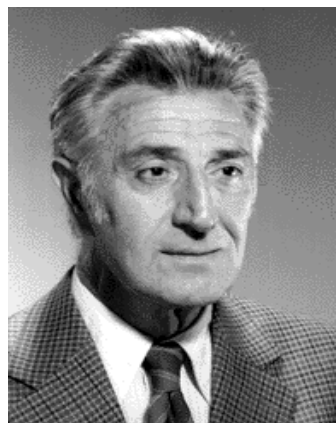


T2-weighting

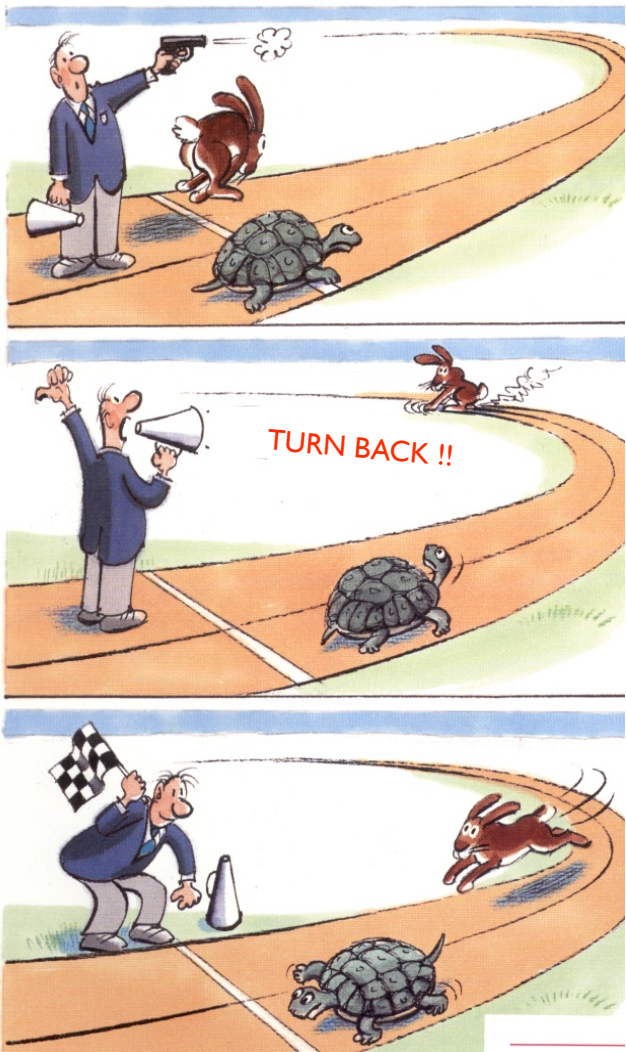
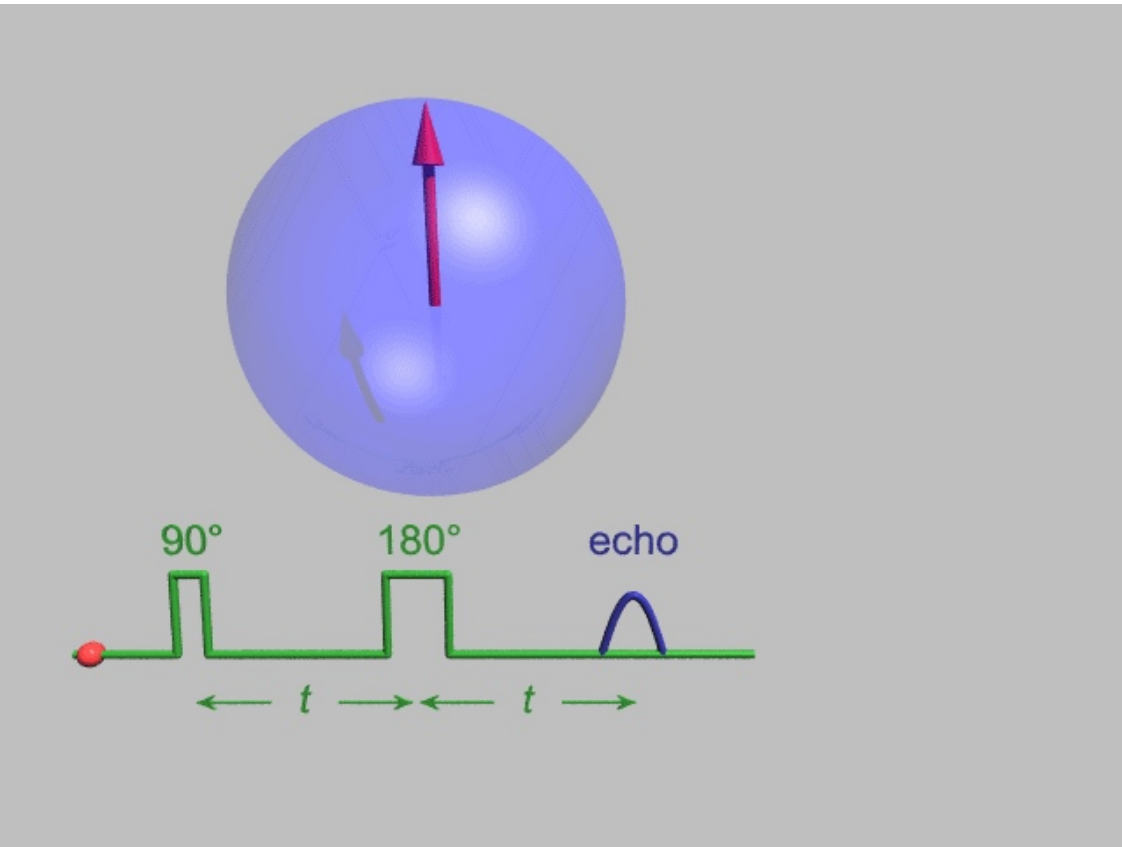
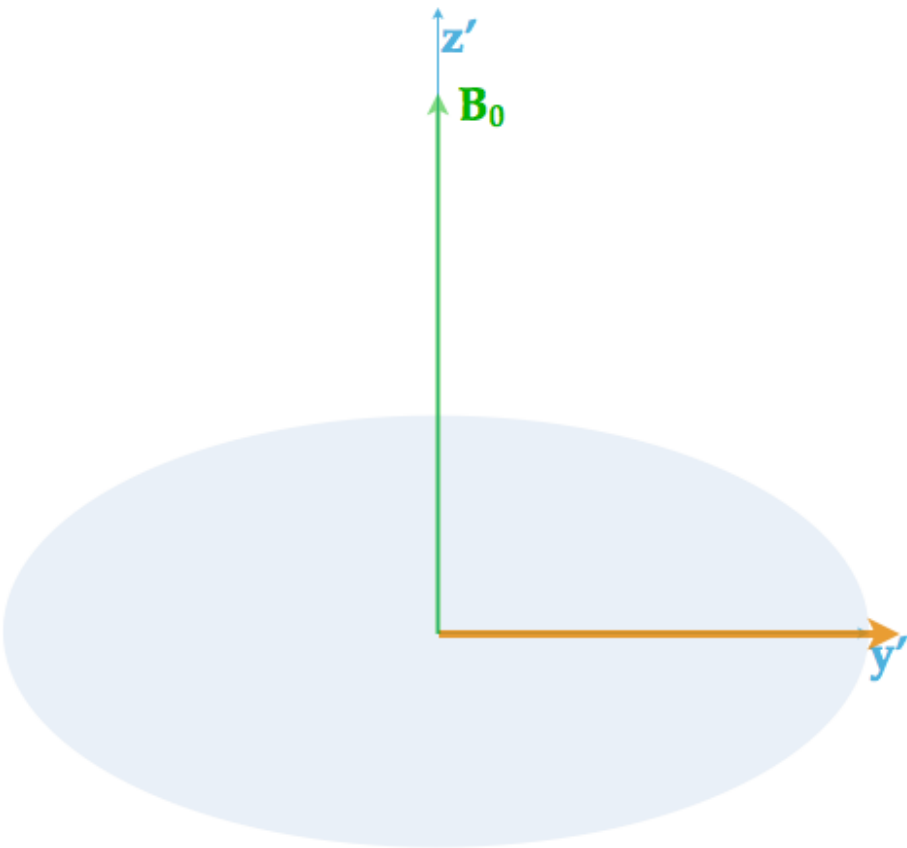


# Measuring relaxation time: the spin-echo experiment

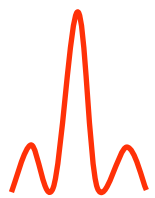
Repeating excitation, refocusing and “echo” pulses: spin-echo sequence



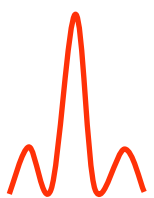
Erwin Hahn, 1949



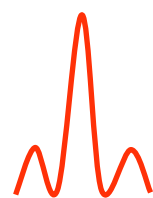
Excitation pulse (90°)



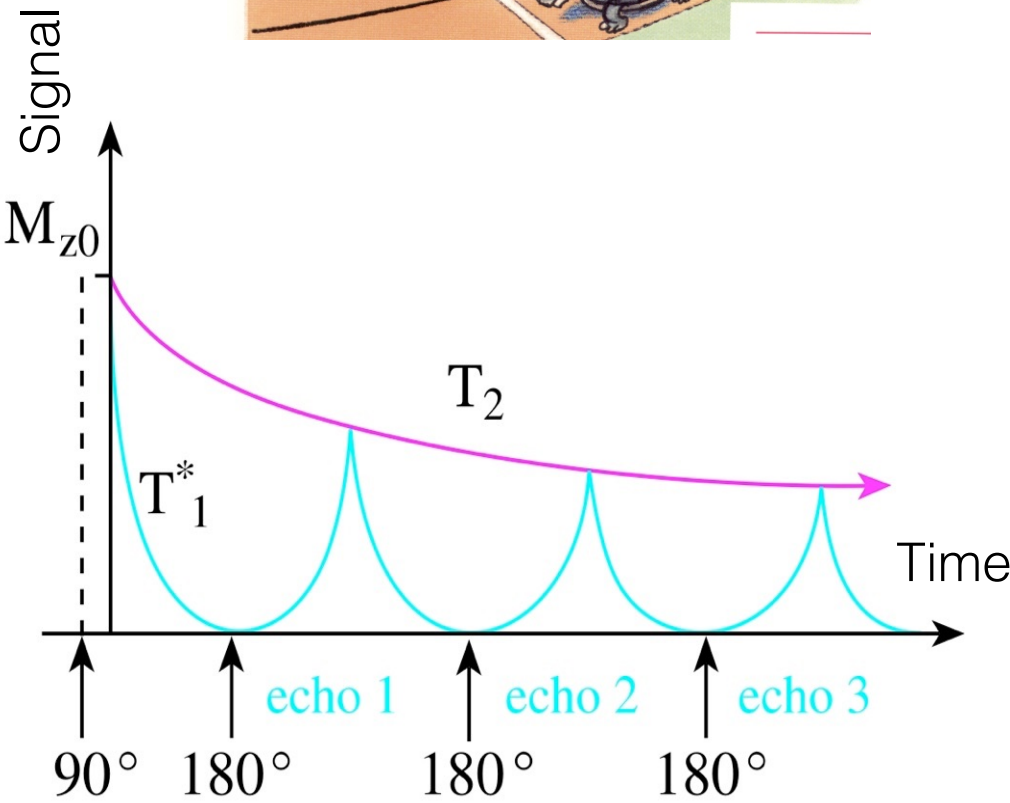
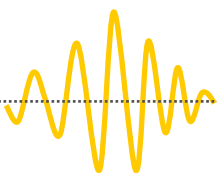
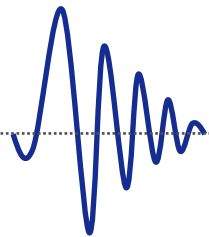
Refocusing pulse (180°)



Refocusing pulse (180°)



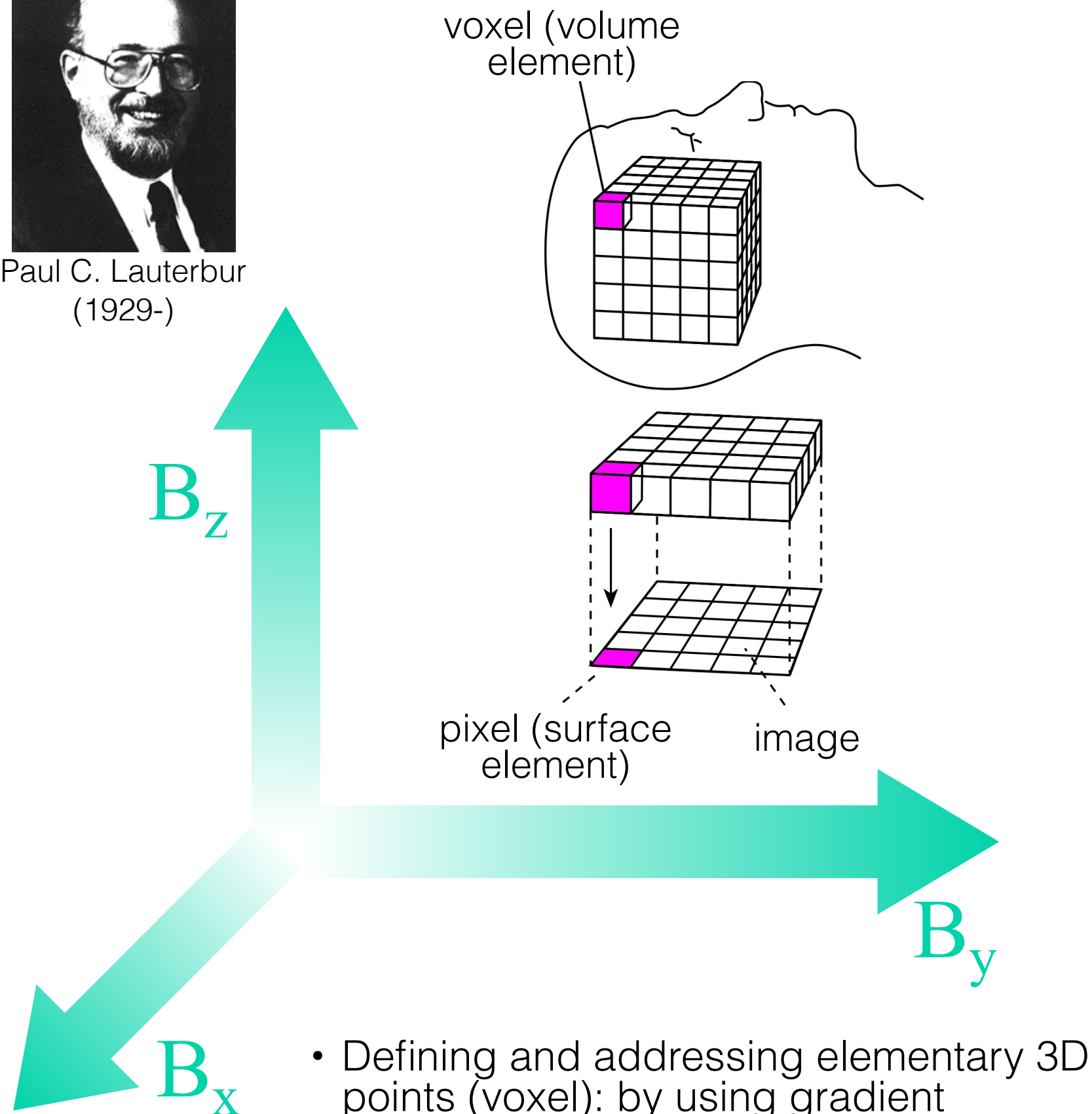
Knocking sounds in MRI:  
pulse generation



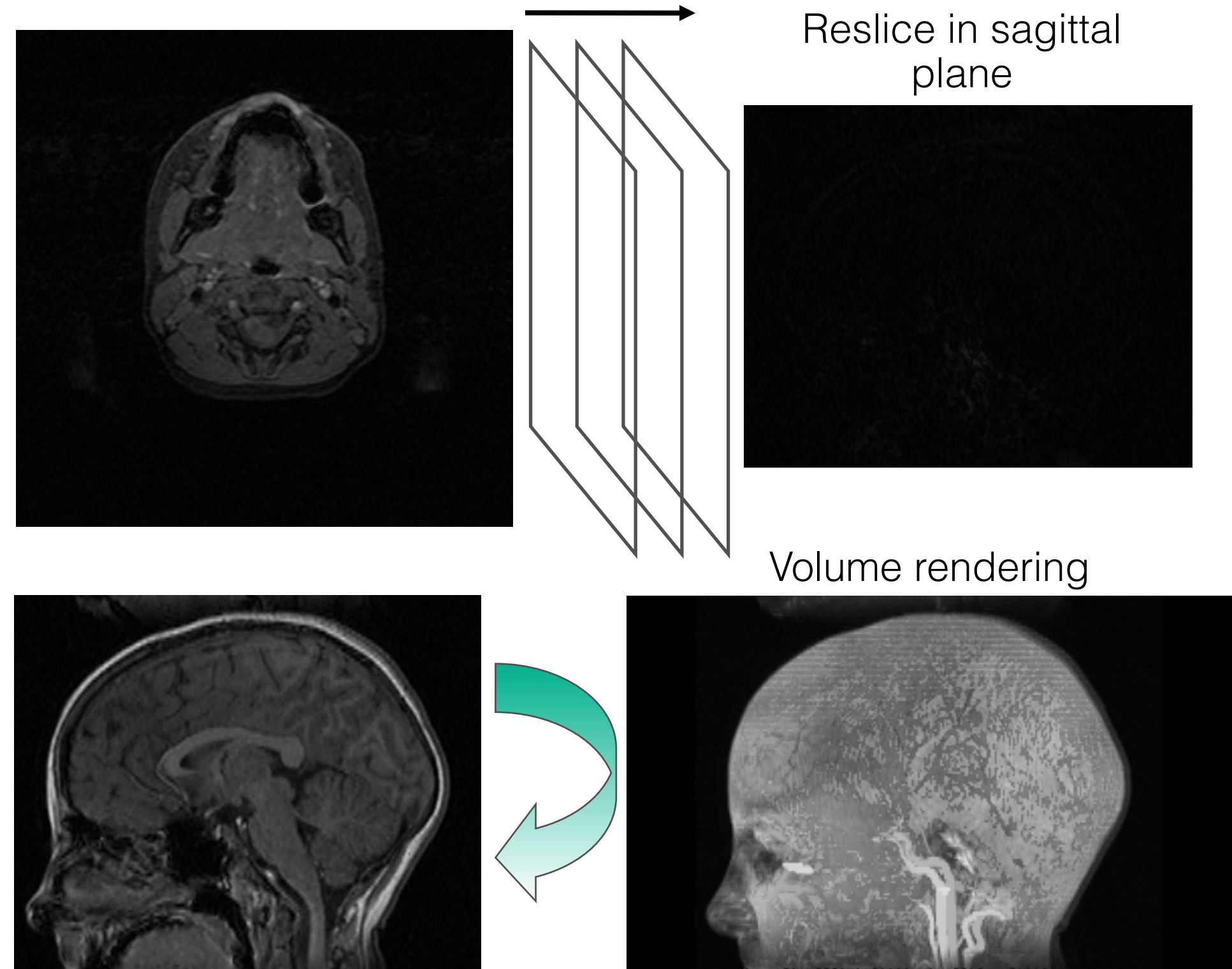
# MRI 2: spatial encoding



Paul C. Lauterbur  
(1929-)

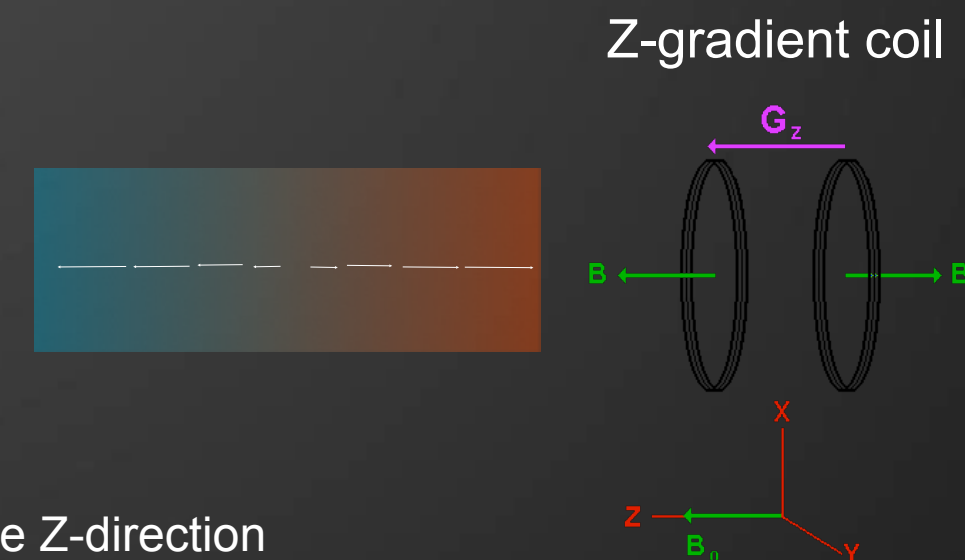
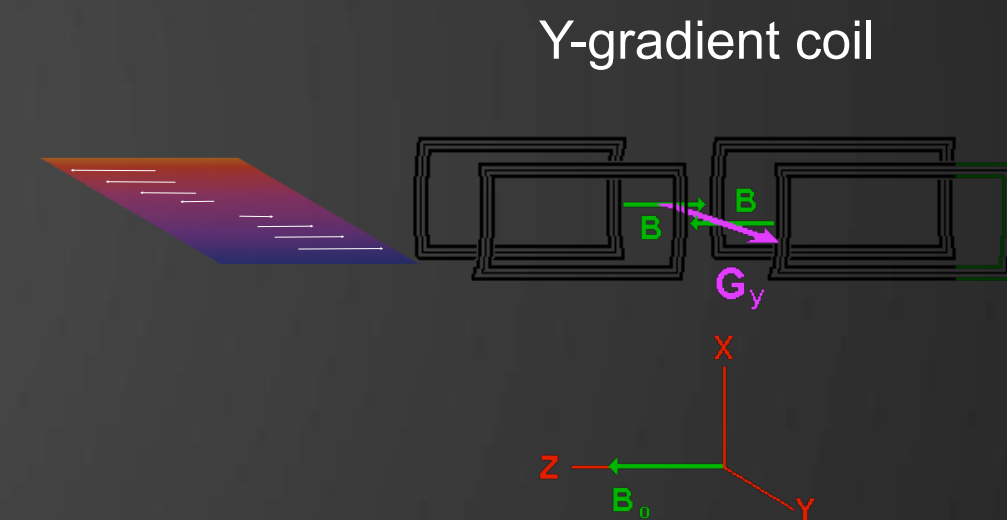
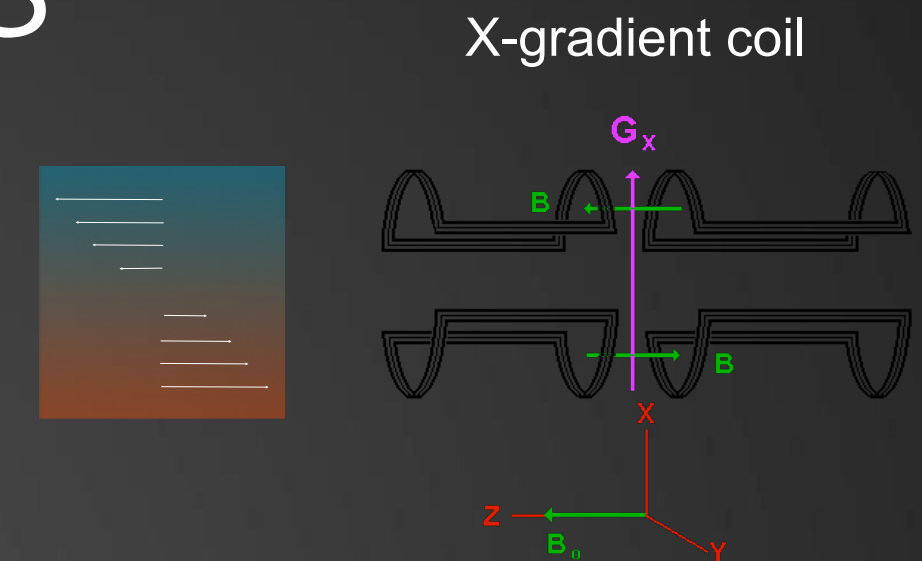
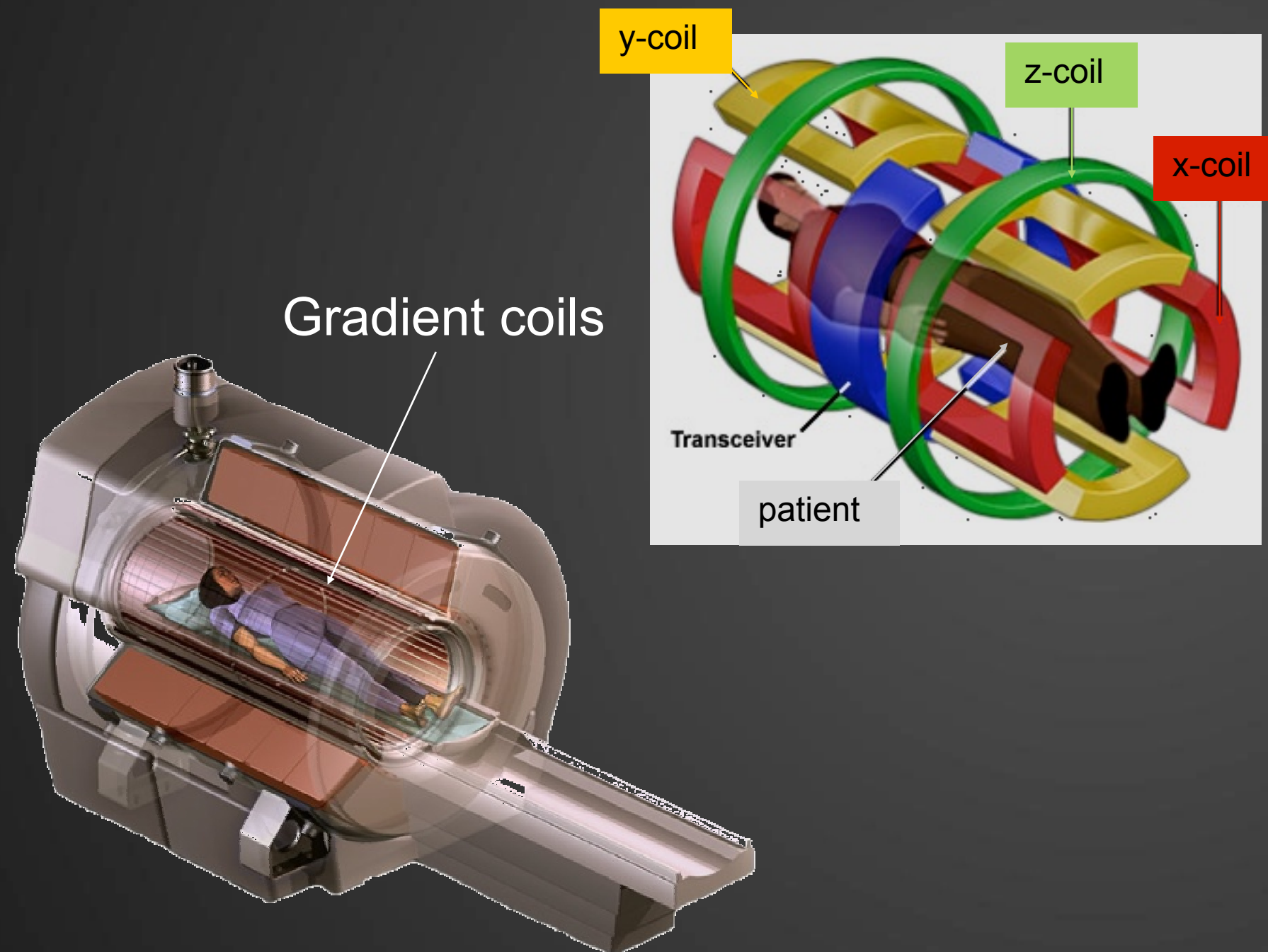


- Defining and addressing elementary 3D points (voxel): by using gradient magnetic field
- Foundations: resonance condition





# Generation of gradient fields: with gradient coils



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

# MRI 3: image reconstruction

## 1. Back projection

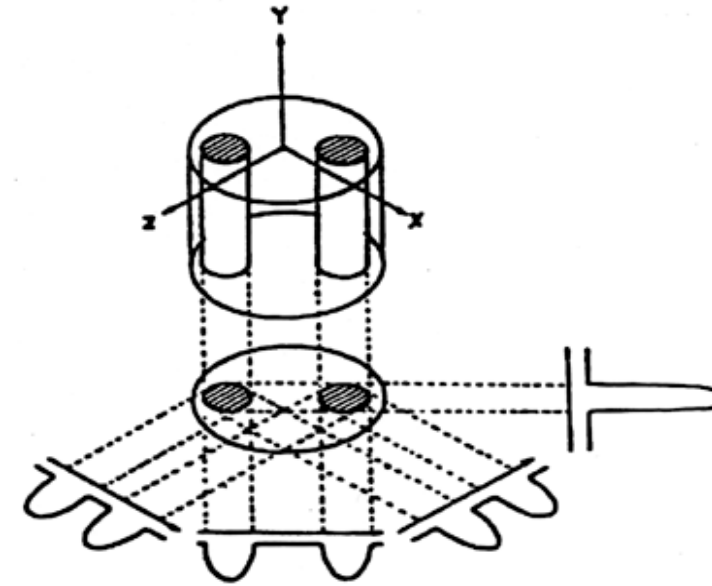
- as in CT scanning



Paul Lauterbur,  
1973, Illinois



Peter Mansfield,  
1973, Nottingham



Principle of back  
projection



Reconstructed image (cross  
section of two test tubes)

## 2. 2D Fourier transformation

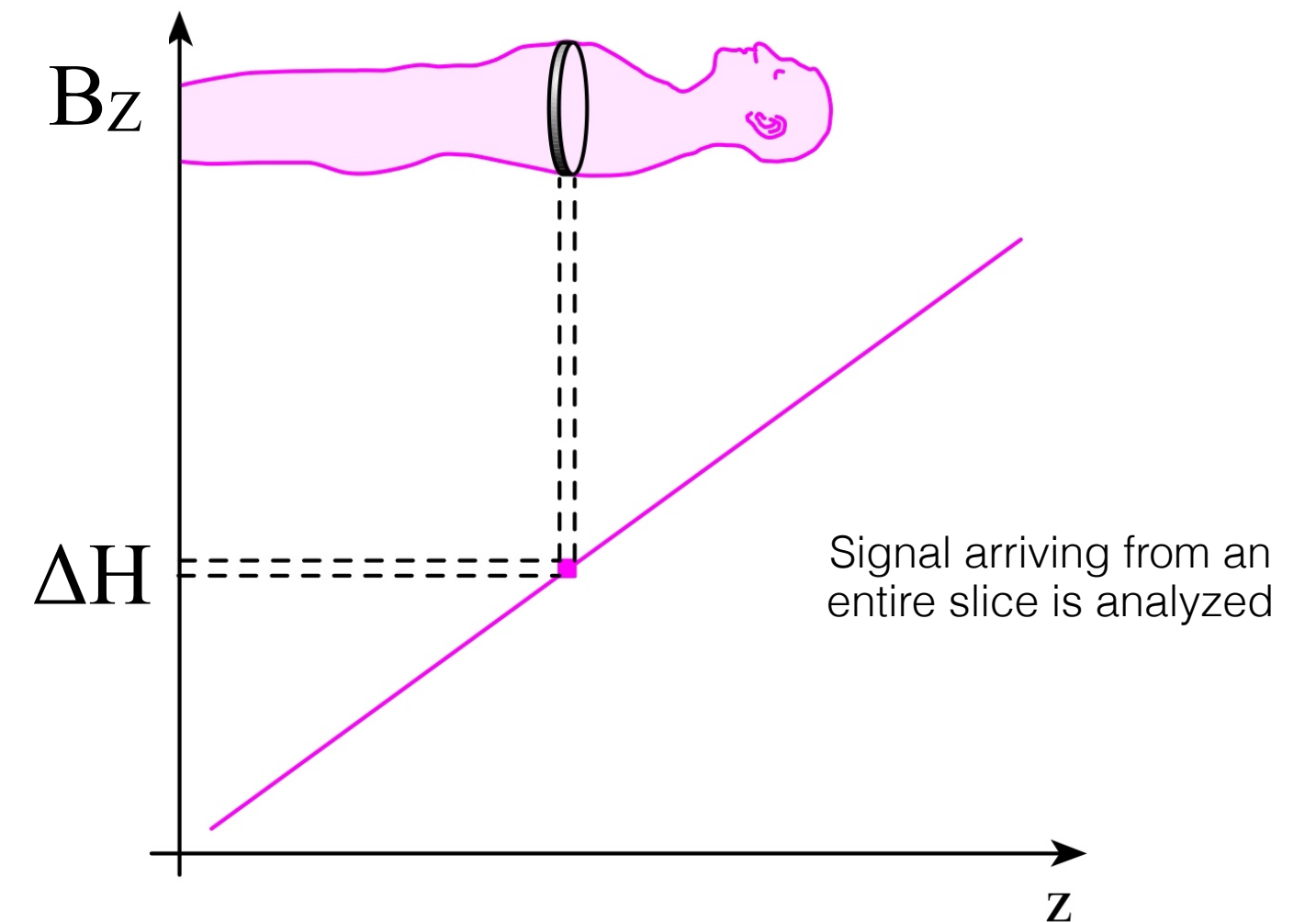
- currently used method
- „NMR Fourier  
Zeugmatography“



Richard Ernst,  
1974, Zürich

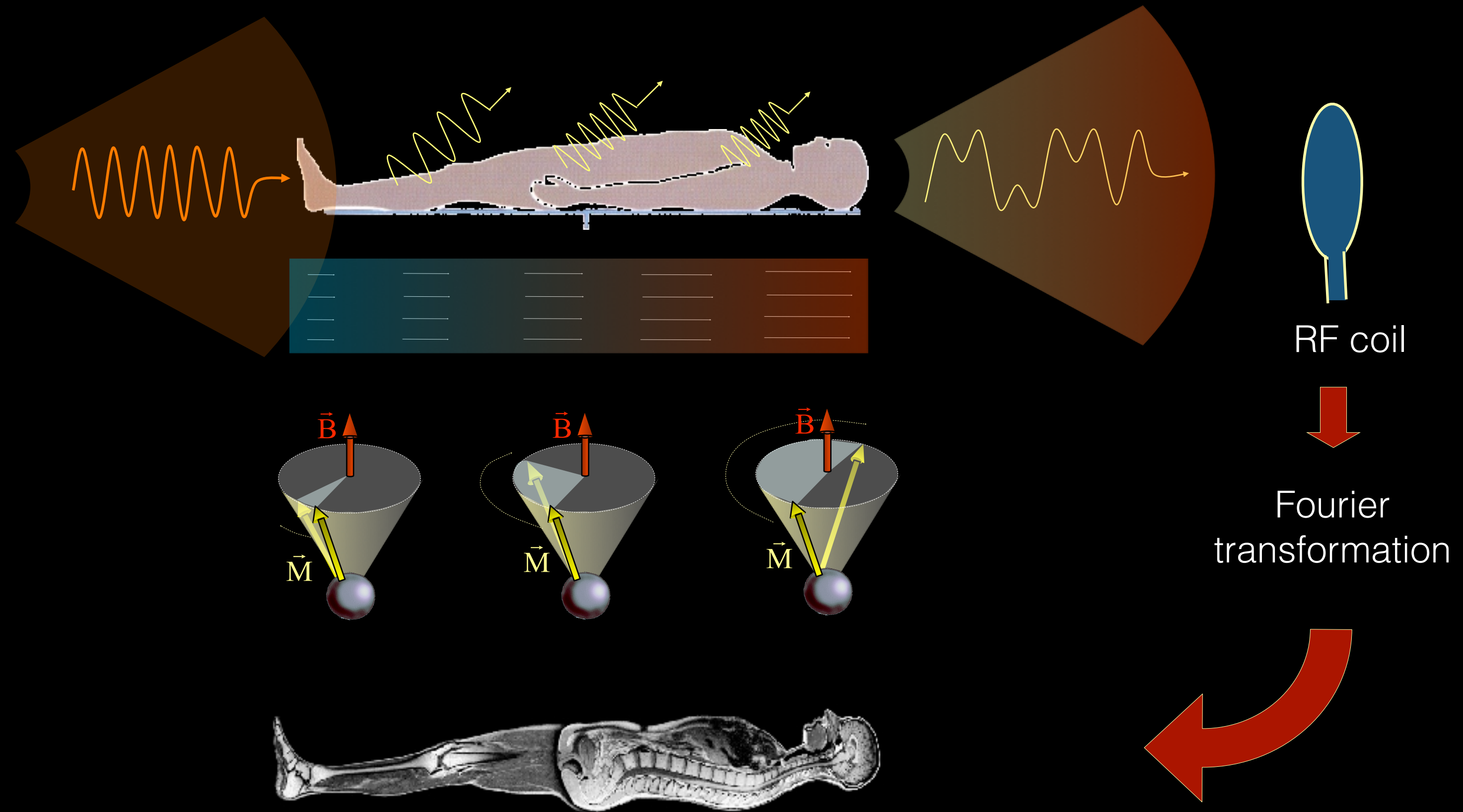


“MRI Scanner Mark One”,  
Aberdeen, Scotland





# MRI: spatial coding and image reconstruction based on the resonance condition ( $B_0$ -dependent $\omega$ )



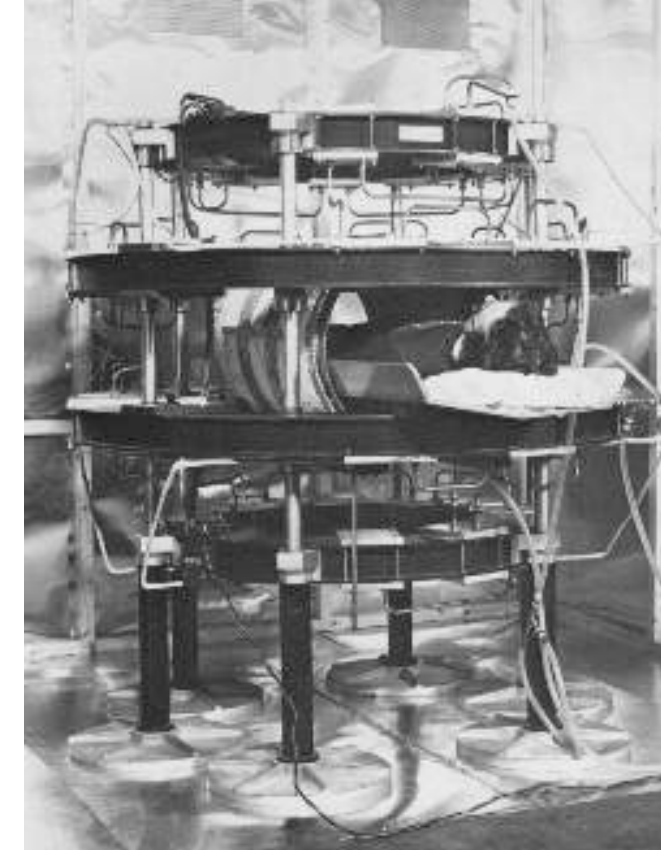


# MRI 4: scanners

Early times



Indomitable (Damadian)



MRI Scanner Mark One (Ernst)

Present



3T MRI



Open MRI unit



Interventional MRI unit



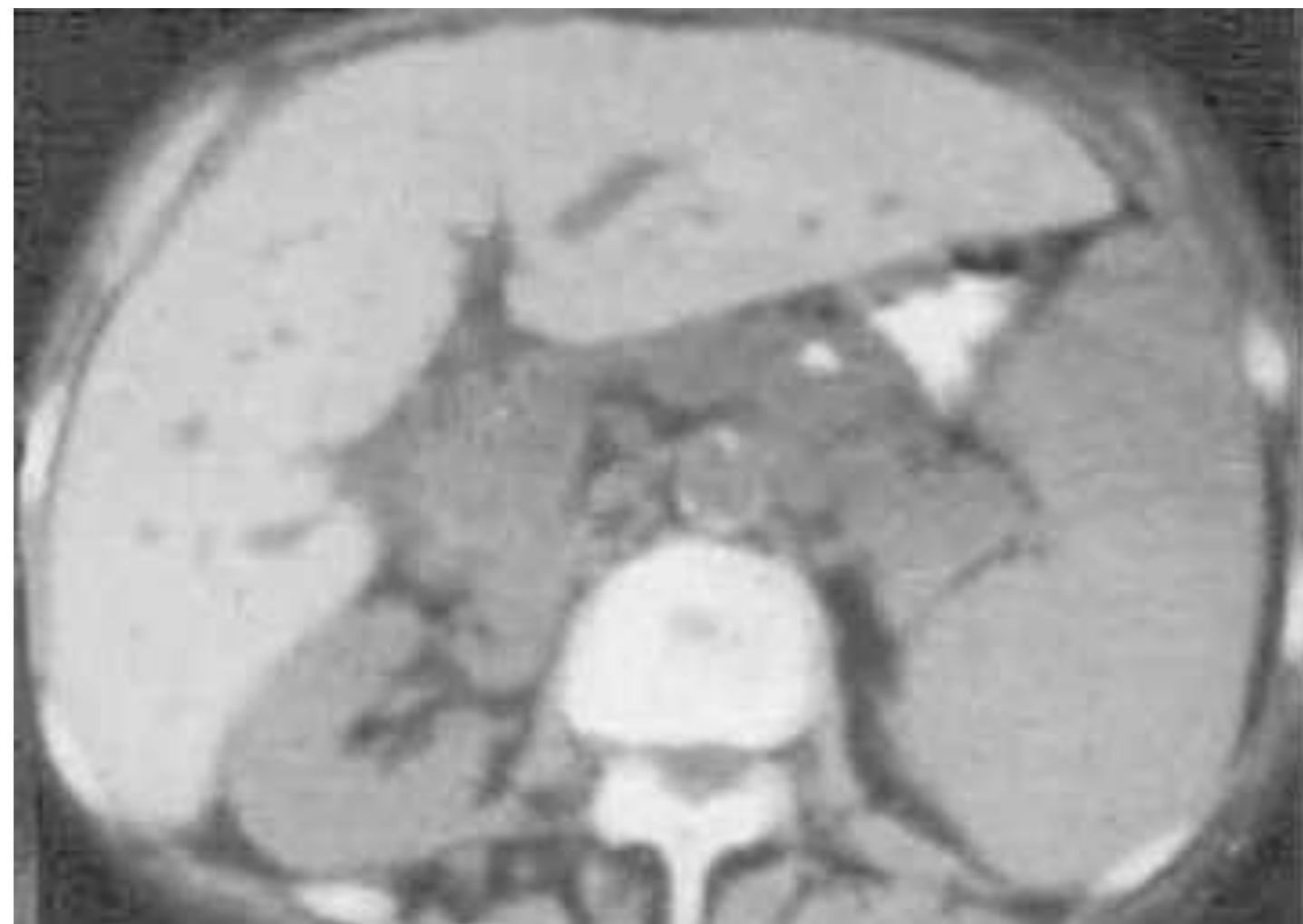
Mobile MRI



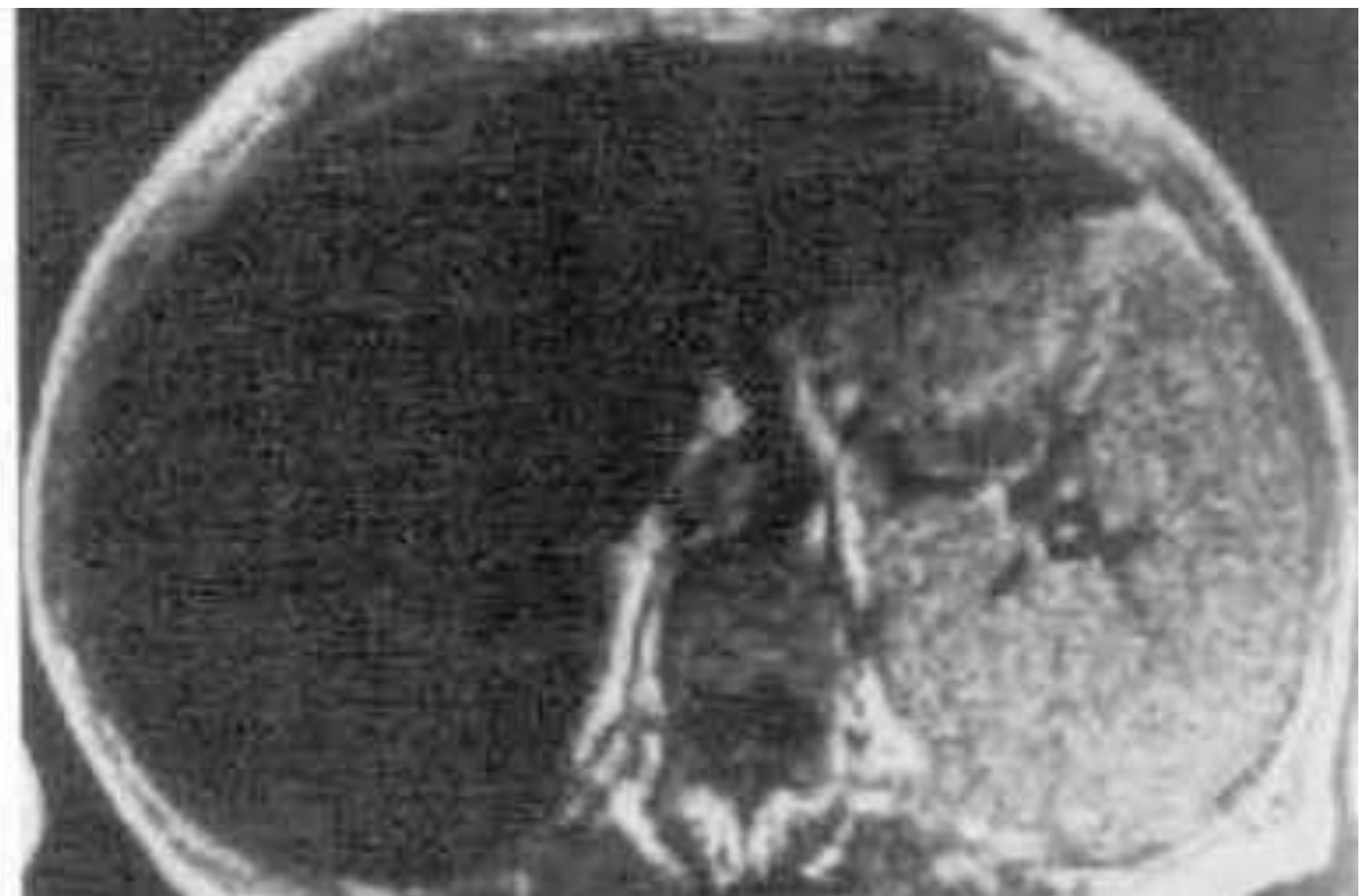
# MRI 5: contrast agents

**Positive:** paramagnetic elements (T1 contrast): Gd, Mn

**Negative:** superparamagnetic, ferromagnetic (T2 contrast): FeIII, MnII



CT



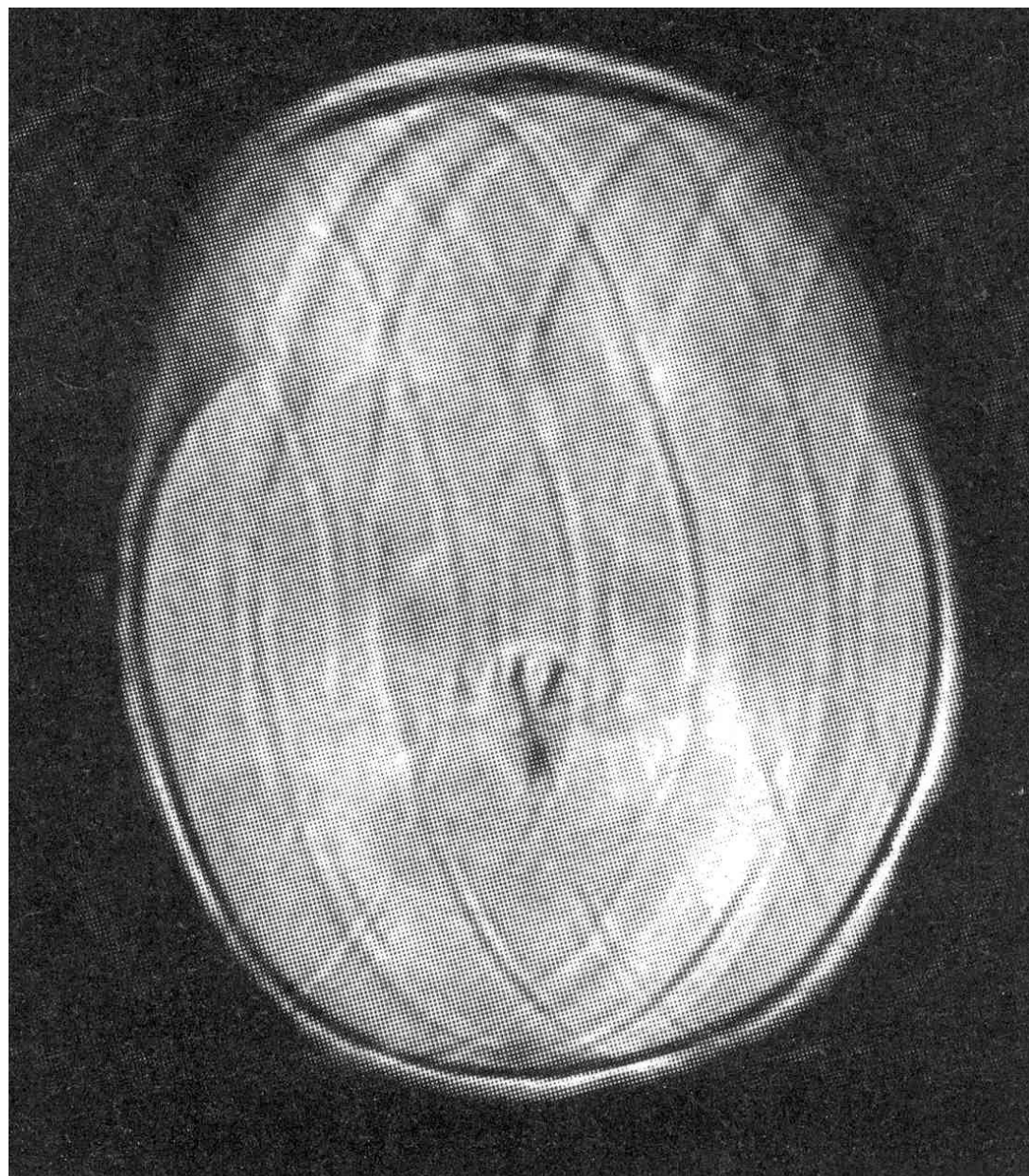
MR T2

Haemochromatosis hepatis (iron accumulation in liver)

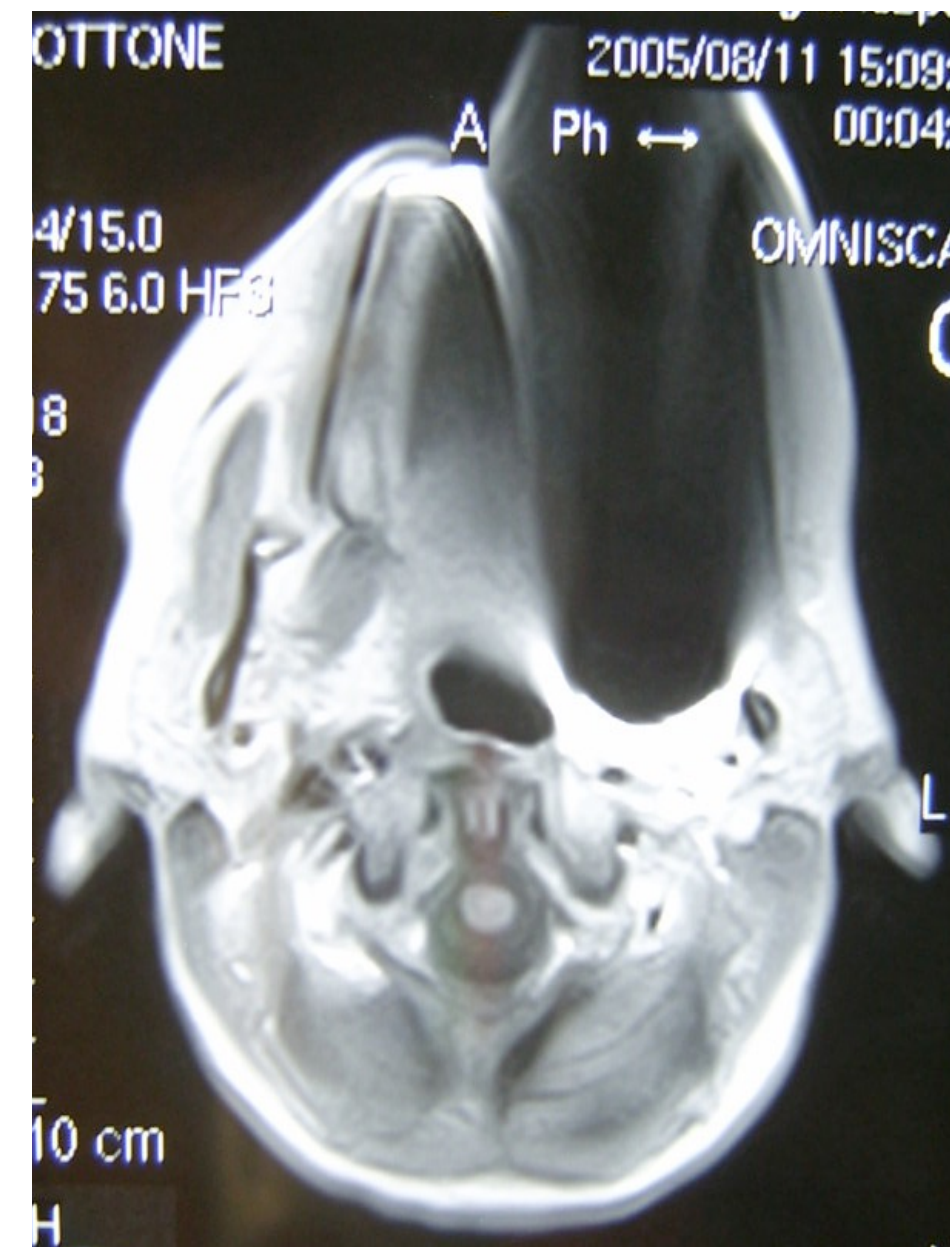


# MRI 6: artefacts

- Motion
- Metals (implants, injury)



Motion artefact

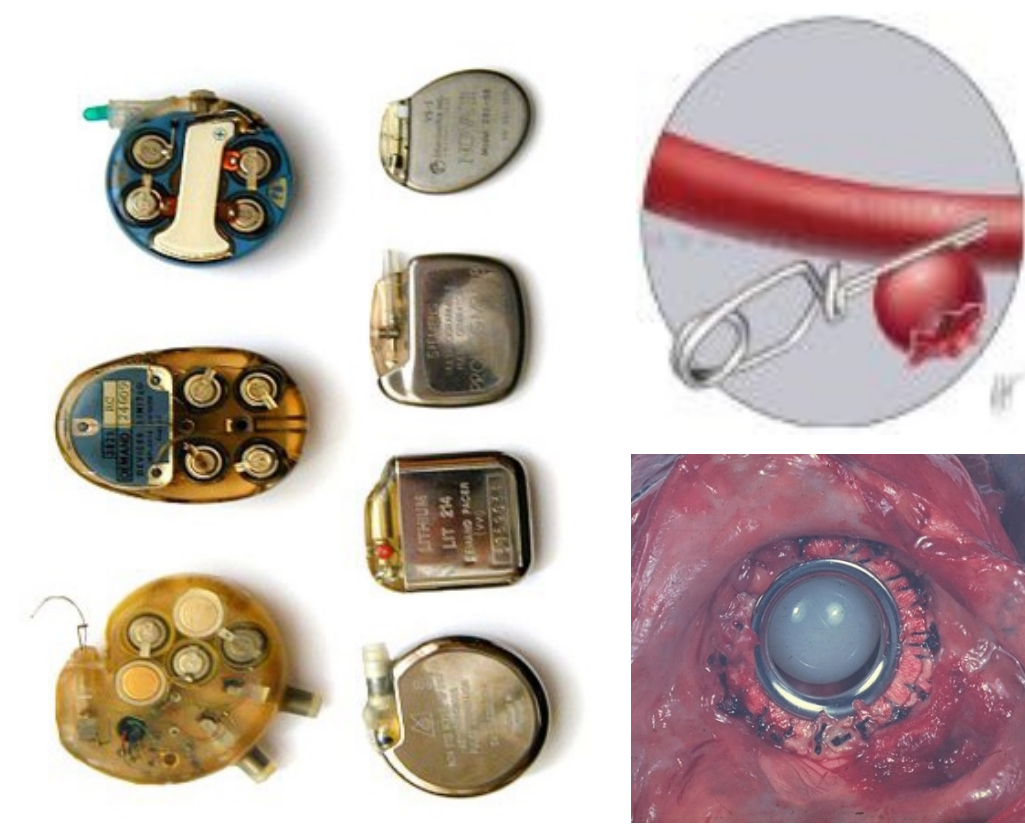
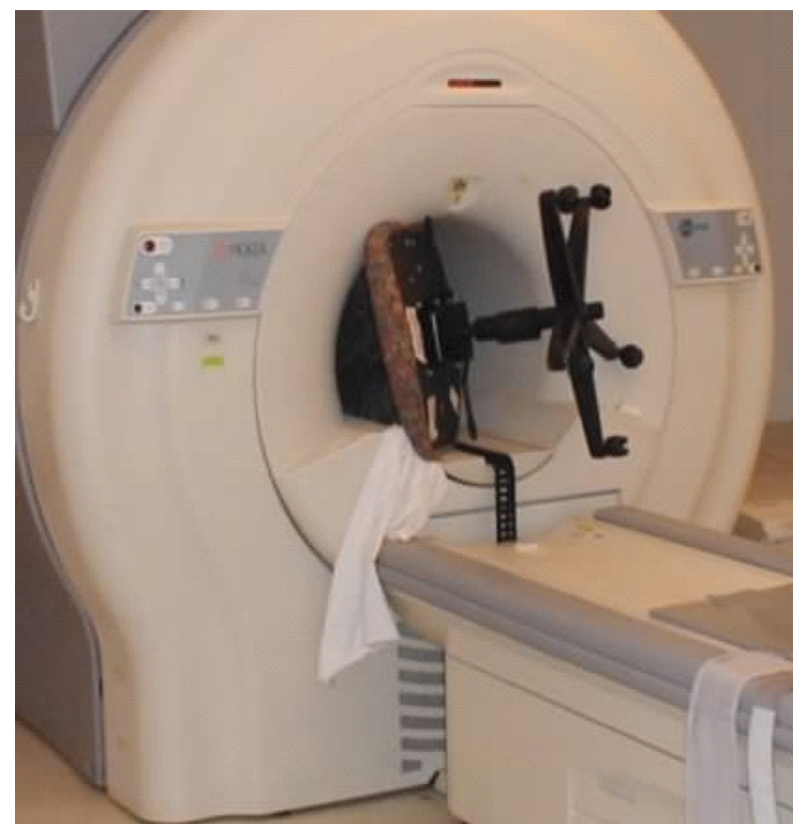
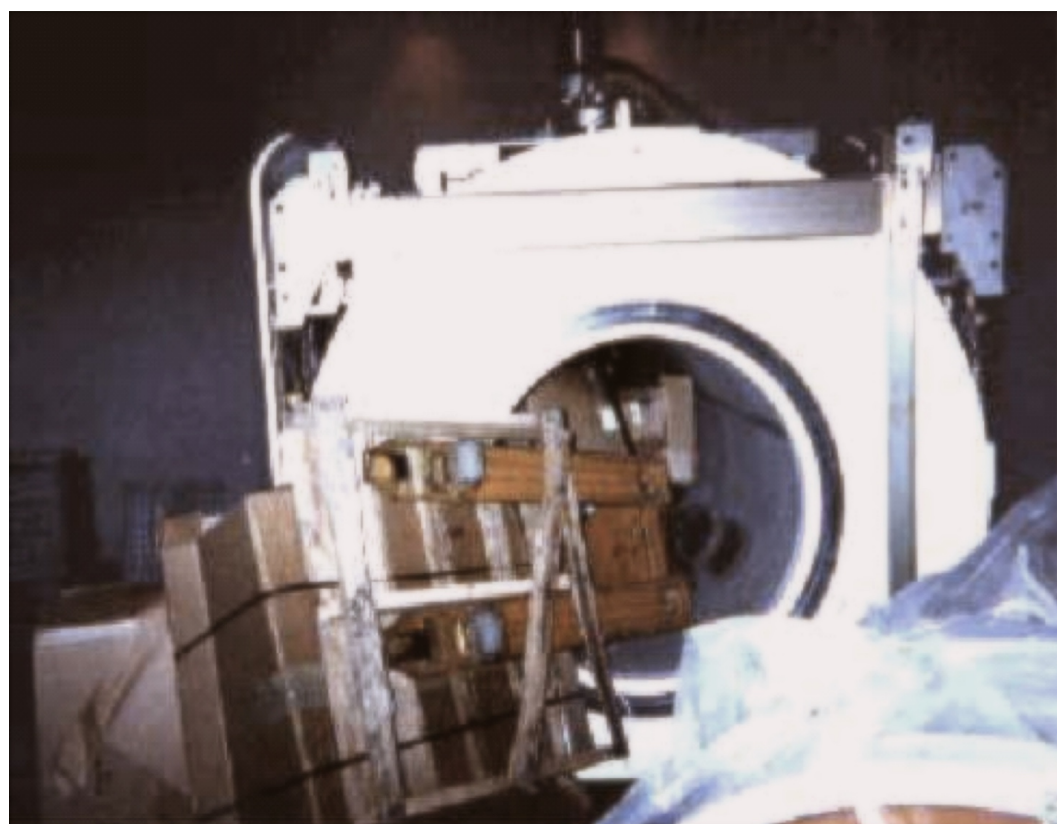


Metal in the orbit of the eye



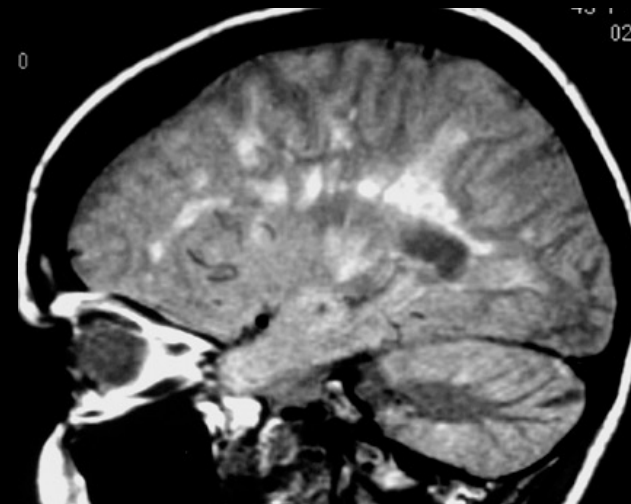
# MRI 7: dangers, contraindications

- Static magnetic field - metal objects  
Contraindications: implanted devices (pacemaker, defibrillator, hearing aids, drug delivery devices), neurostimulators, brain aneurysm clamps, early cardiac valve implants
- Gradient field - induced current
- Radio frequency field - thermal effects (lens, testis)

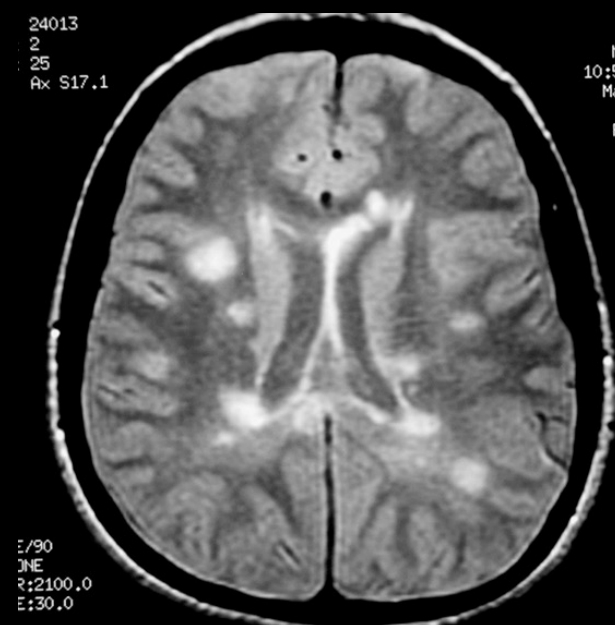
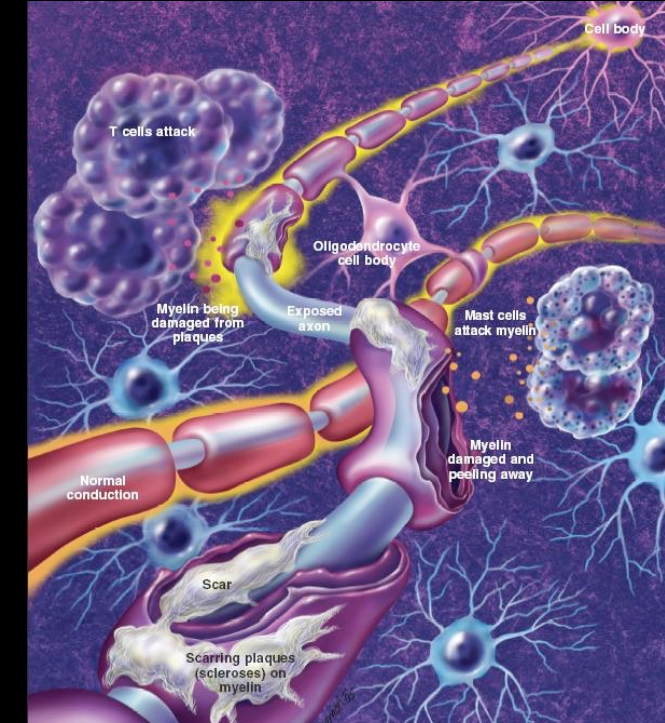




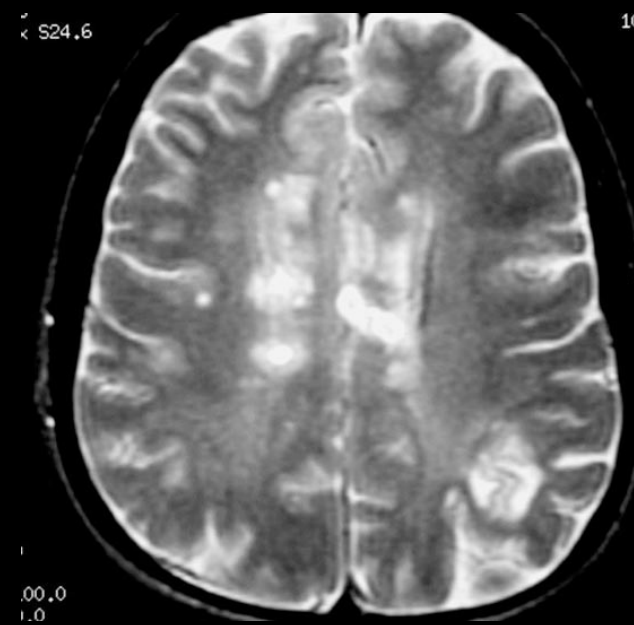
# Applications: Anatomical imaging - Multiple sclerosis



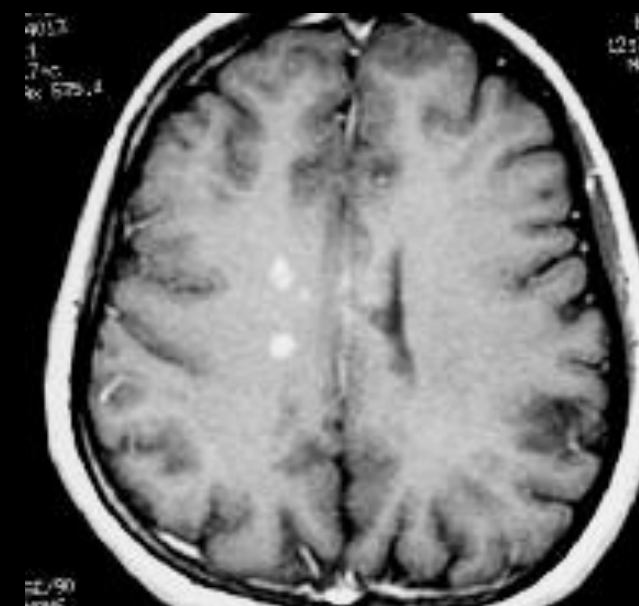
Proton density  
(sagittal)



Proton density  
(transverse)



T2 weighted  
(transverse)



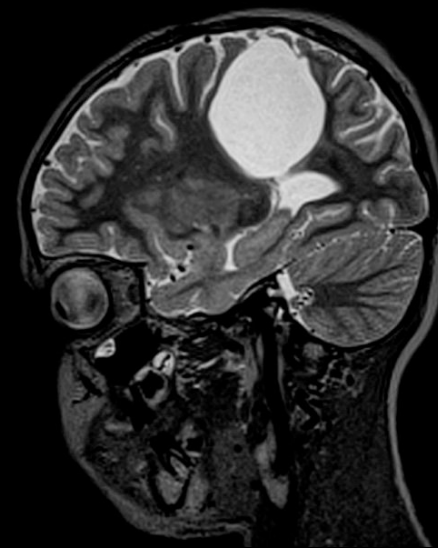
T1 weighted  
With contrast agent



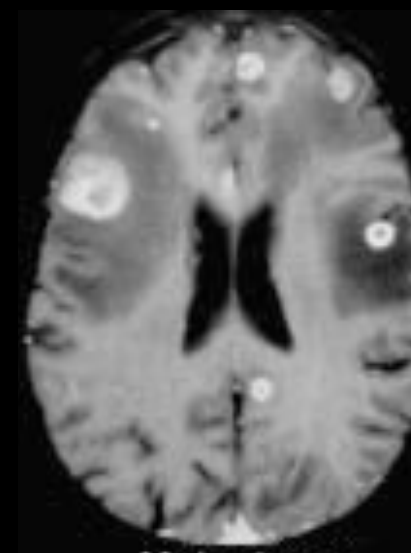
# Anatomical imaging: Oncology



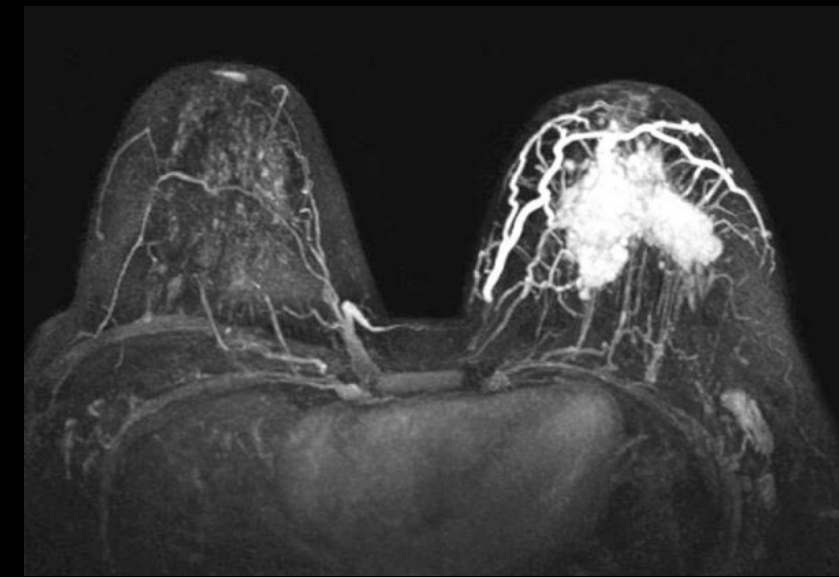
T2 weighted  
(chondrosarcoma)



T2 weighted  
(cyst)



Proton density  
(Brain metastasis)



T1 weighted with contrastagent  
(Breast carcinoma)



T2 weighted  
(cervix carcinoma)



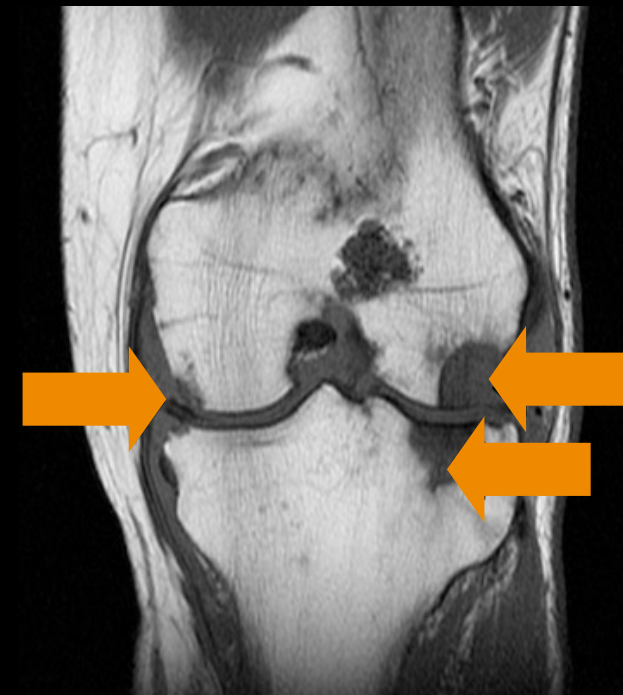
T2 weighted  
(prostate tumor)

# Anatomical imaging

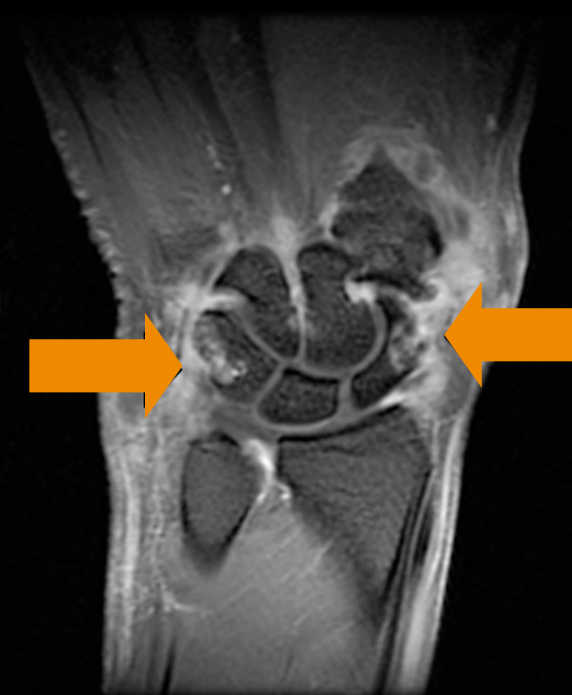
## Bone and soft tissue



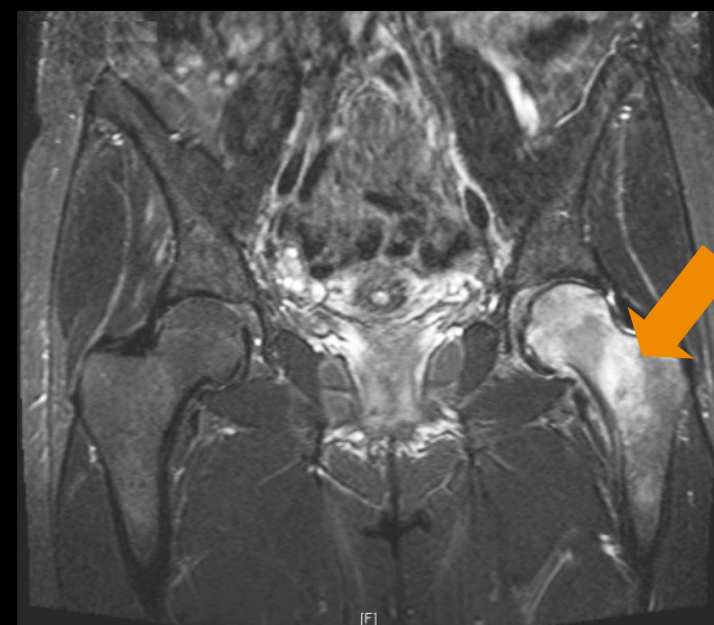
T2 weighted  
(torn ligaments)



Rheumatoid arthritis  
knee



Rheumatoid arthritis  
wrist



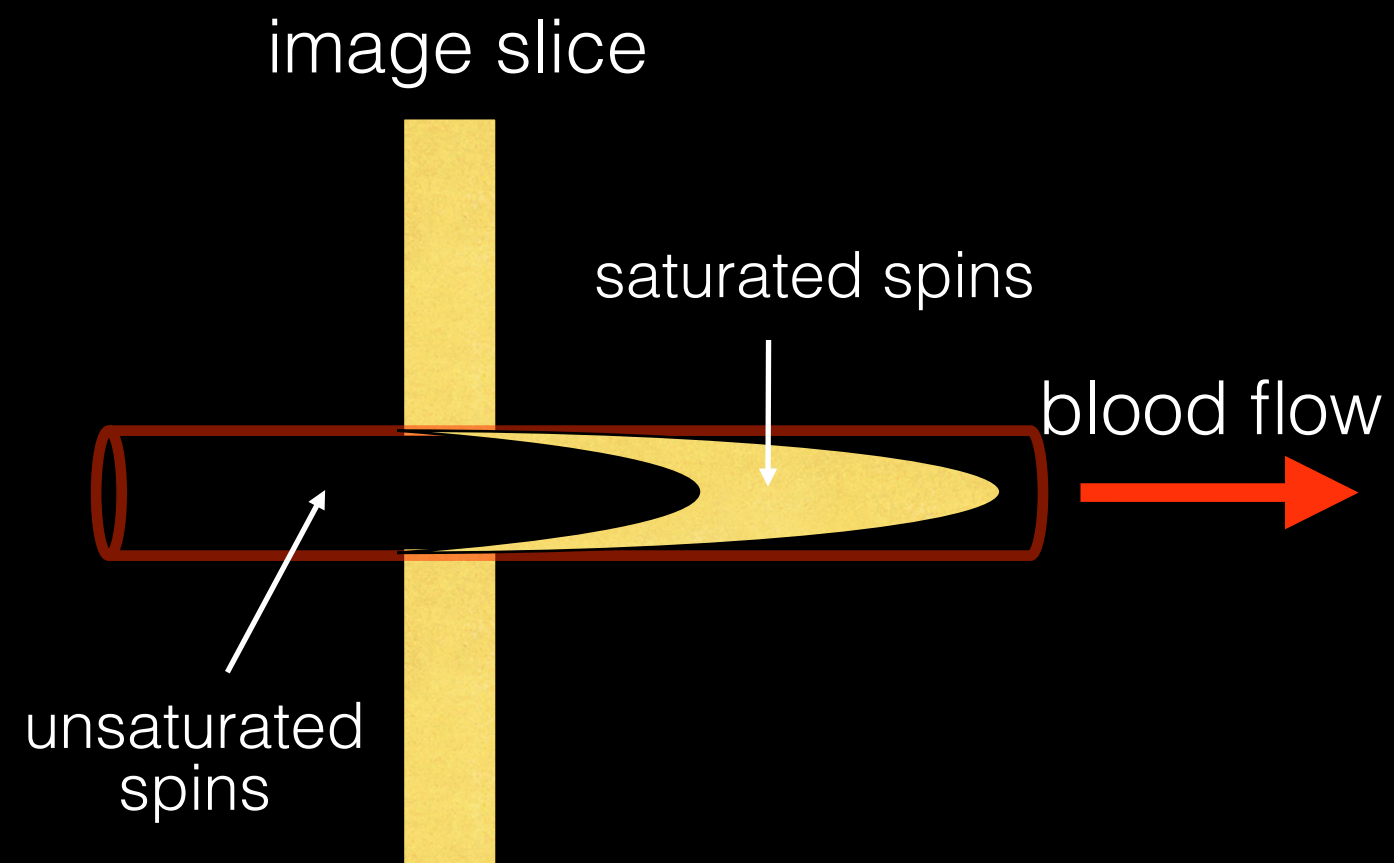
Osteoporosis (femur)



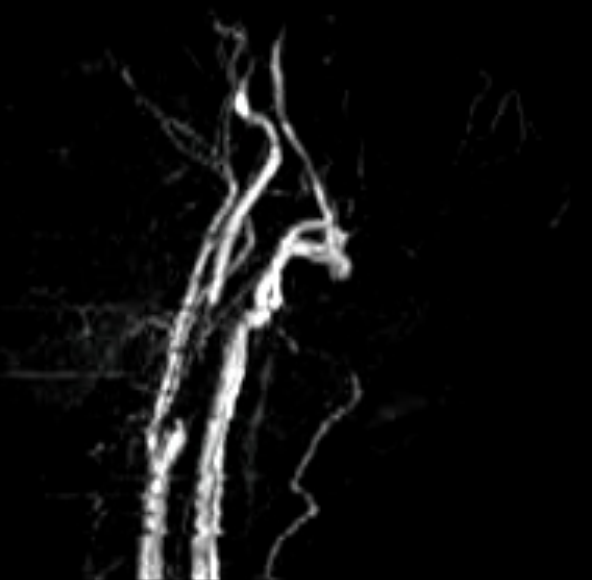
T2 weighted  
(hernia)



# MRI — Non-invasive angiography



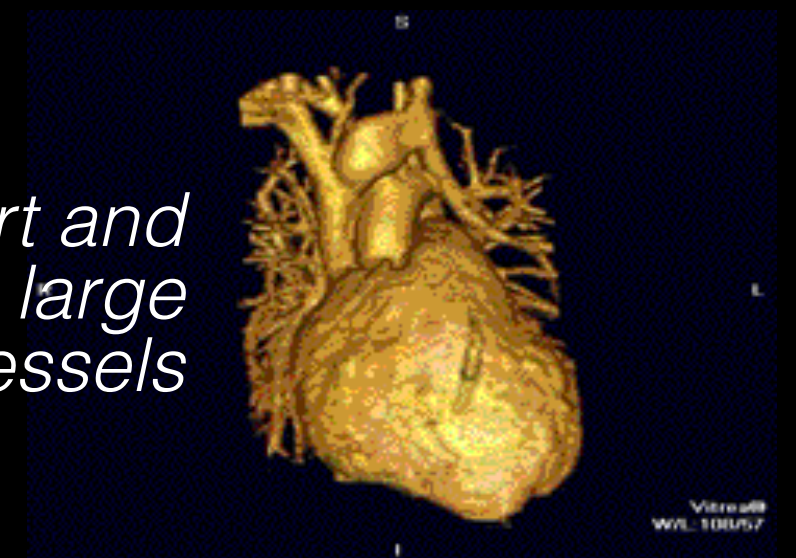
*Arteria  
carotis*



*Circulus  
arteriosus  
Willisii*



*Heart and  
large  
vessels*



# Time-resolved MRI (ECG-gating required)



Blood flow across the cardiac chambers

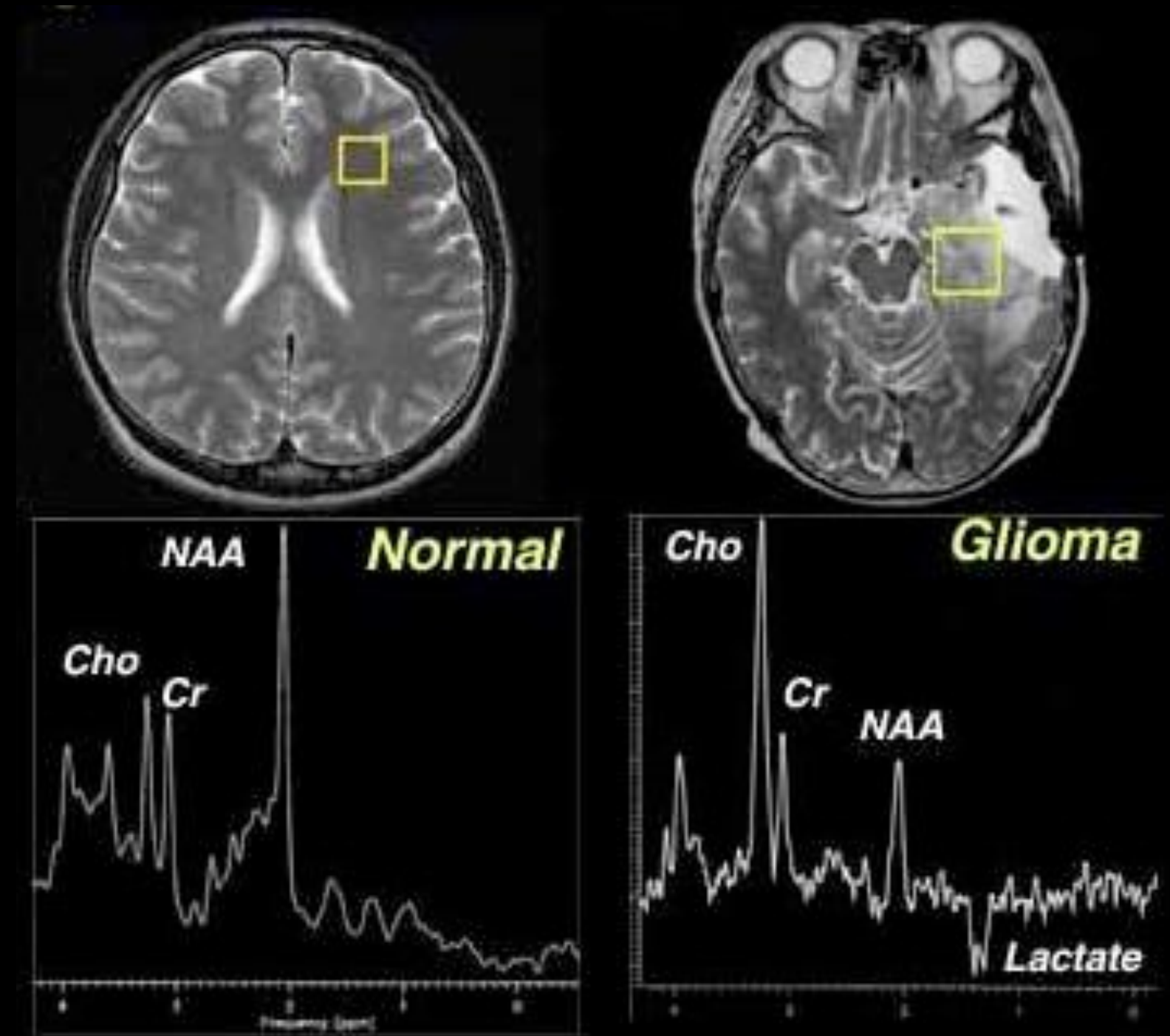


Opening and closing of aorta valve

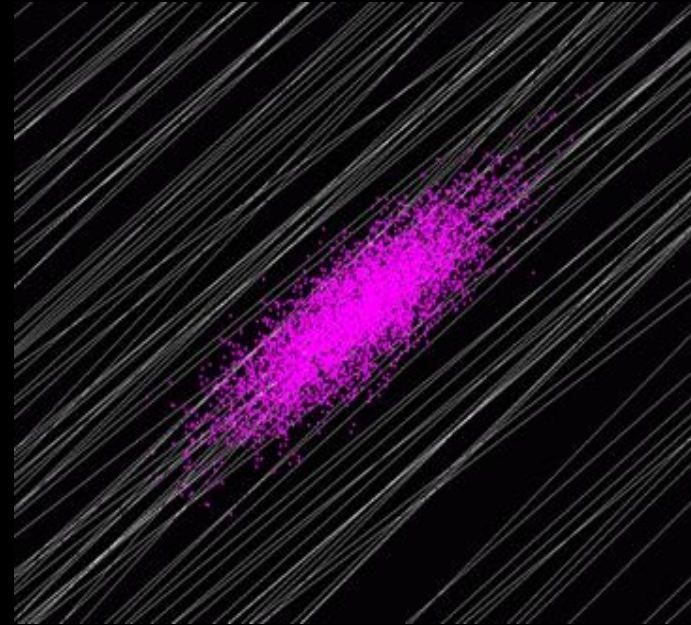
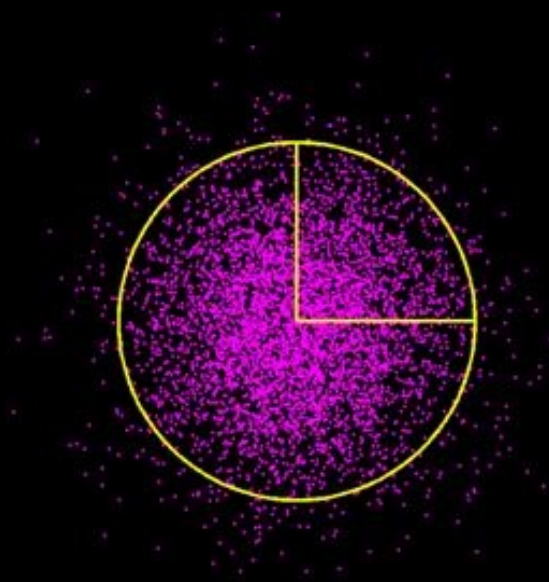


# MR Spectroscopy (MRS)

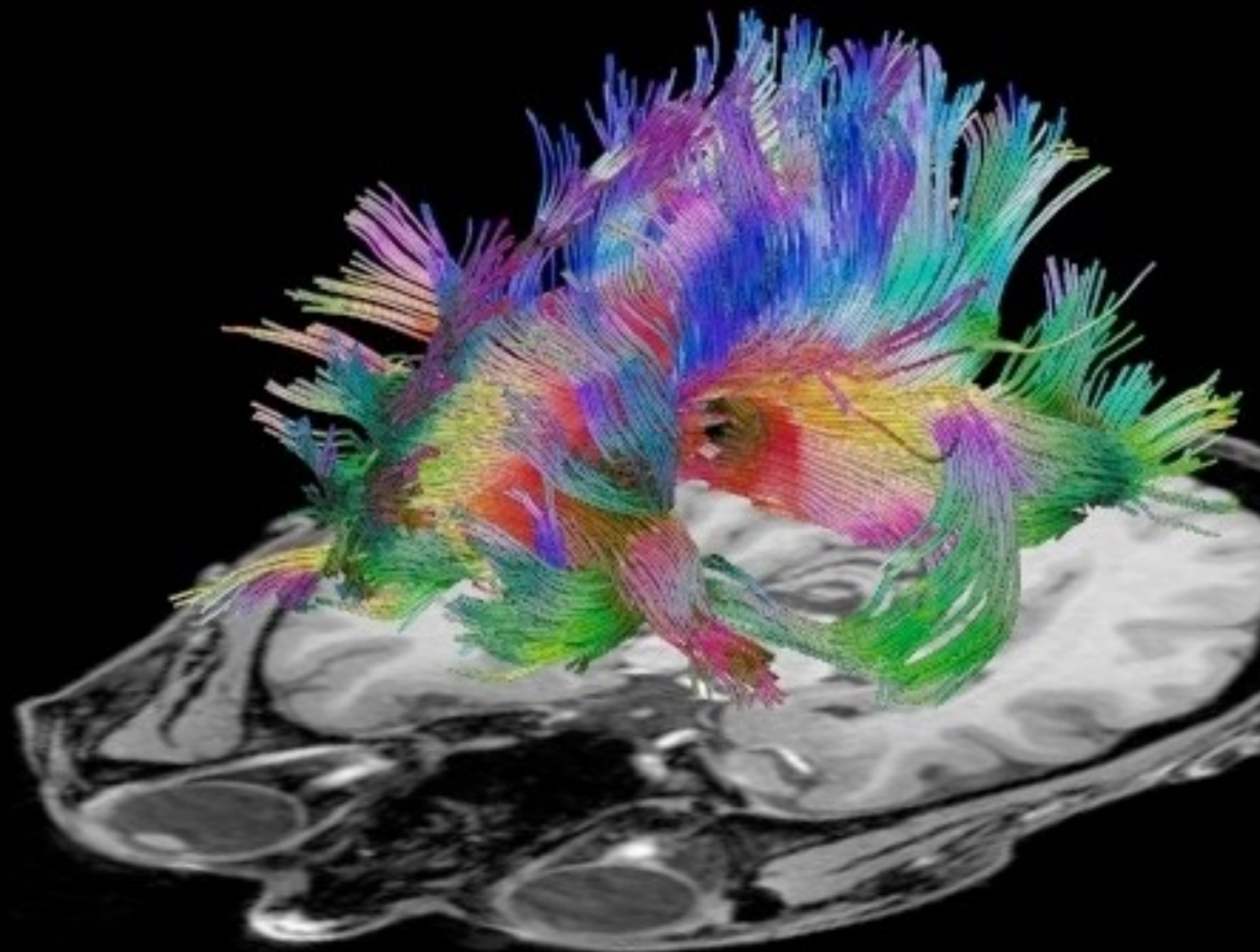
- Chemical shift
- Identification of metabolites
- Tumor diagnostics



# Diffusion imaging



Anisotropic water diffusion:  
contrast generation



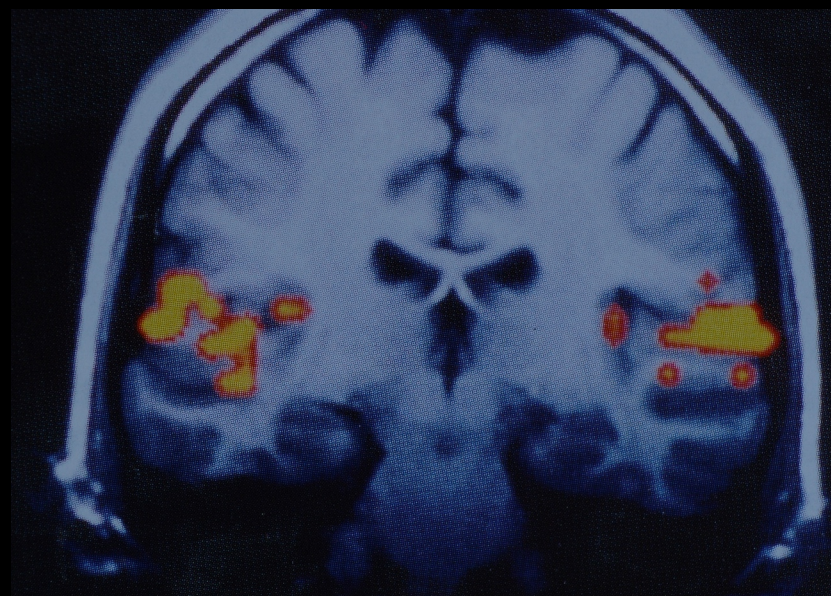
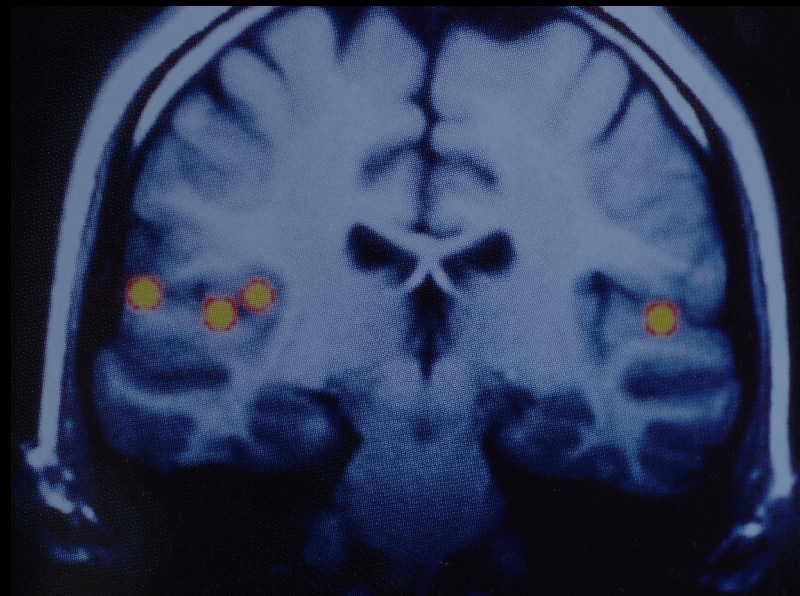
Imaging neural tracts:  
tractography

*Corpus callosum*

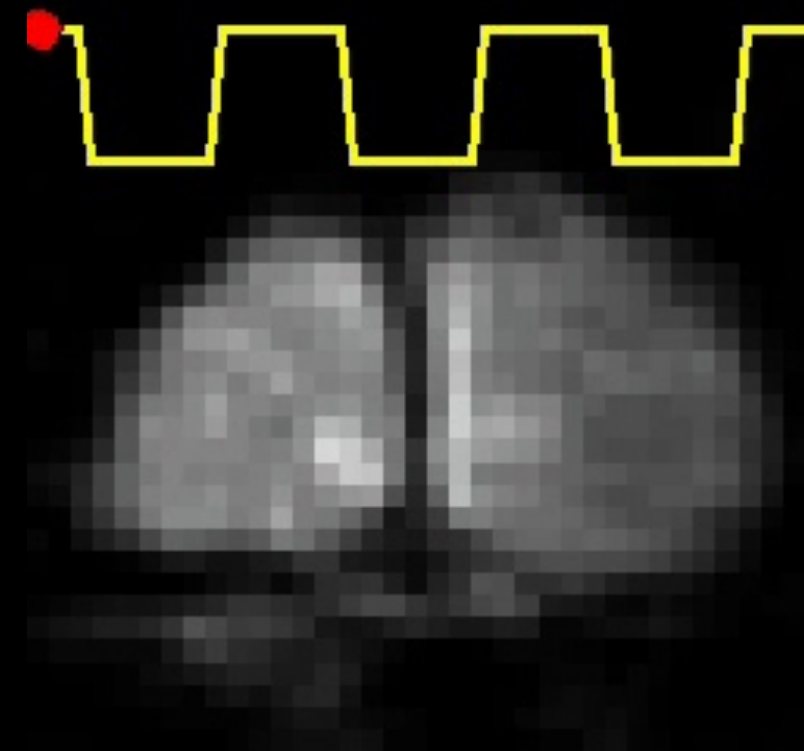


# Functional MRI (fMRI)

High time resolution images recorded synchronously with physiological processes



Activation in the  
auditory cortex



Effect of light pulses on  
the visual cortex

# Superposition of MRI and PET



Intracranial tumor



PET signal: cortical areas activated  
during eye movement  
Volume rendering



# Feedback



<http://report.semmelweis.hu/linkreport.php?qr=J6XCRWMCMR5DF9FR>