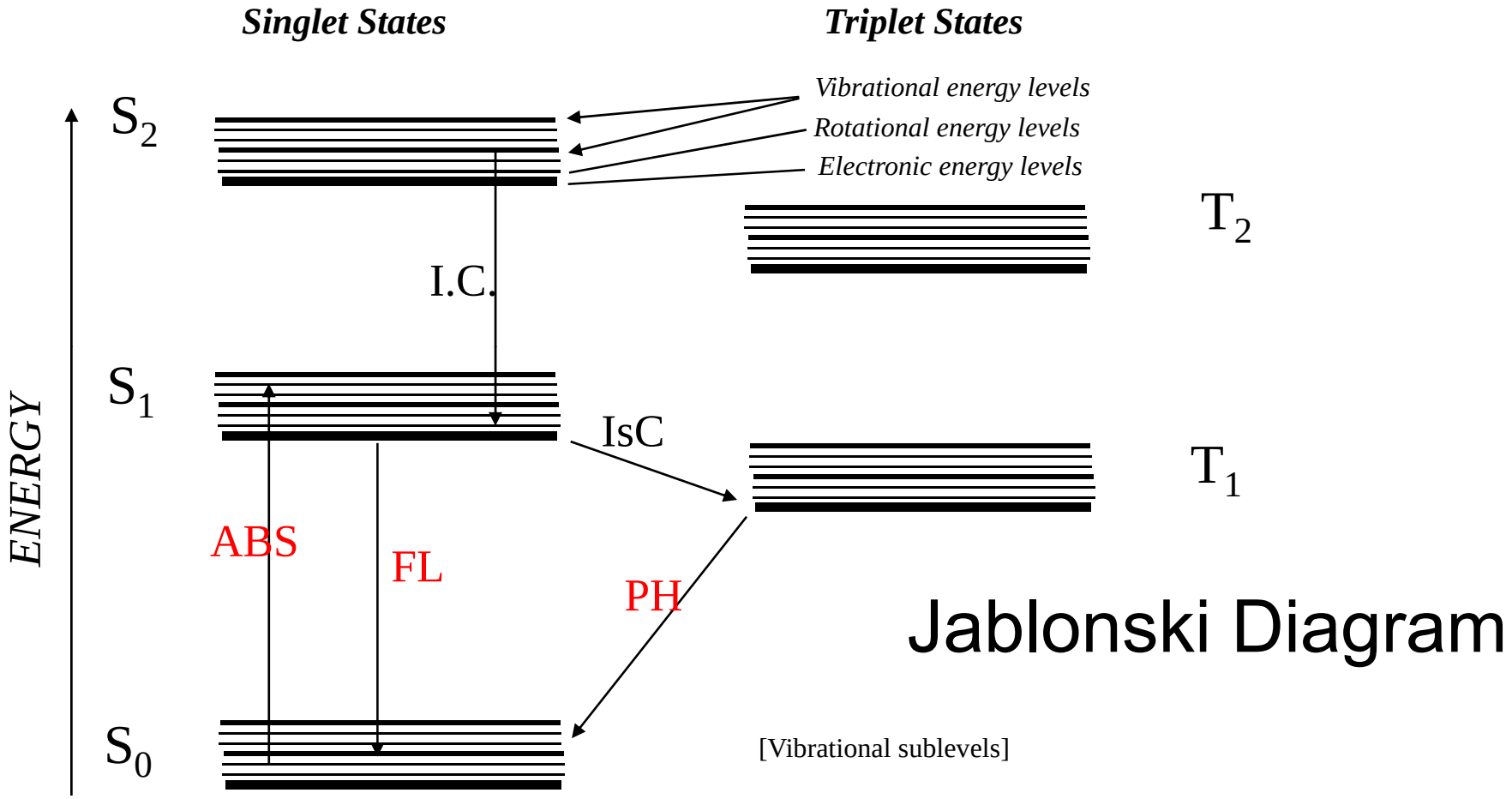
Summary on Methods

- Absorption spectroscopy
- Fluorescence spectroscopy
- Raman Scattering
- Circular Dichroism
- Confocal microscopy
- Quantum Dot imaging
- Optical Tweezers
- Dynamic light scattering
- Surface Plasmon Resonance
- Scanning probe microscopy
- Langmuir-Blodgett Technique
- Quartz Crystal Microbalance
- Differential Scanning Calorimetry
- Ultrasound imaging
- X-ray imaging
- Isotope labeling
- Chromatography
- Electrophoresis
- Photodynamic therapy,
- Mass spectrometry,
- Scanning electron microscopy
- X-ray diffraction
- Neutron diffraction
- NMR spectroscopy
- Magnetic Resonance Imaging
- Positron EmissionTomography
- Transmission Electron microscopy
- Patch Clamp technique
- Radiotherapy
- Electroencephalography (EEG)
- Photonic crystals

Methods based on interaction of light with the sample (molecule, object, cell)

- Absorption spectroscopy
- Fluorescence spectroscopy
- Raman Scattering
- Circular Dichroism
- Optical and fluorescence microscopy
- Confocal microscopy
- Quantum Dot imaging
- Optical Tweezers
- Dynamic light scattering

Absorption and fluorescence



ABS - Absorbance *S 0.1.2 - Singlet Electronic Energy Levels*

FL - Fluorescence *T 1,2 - Corresponding Triplet States*

I.C.- Nonradiative Internal Conversion *IsC - Intersystem Crossing* *PH - Phosphorescence*

Absorption and fluorescence Spectroscopy

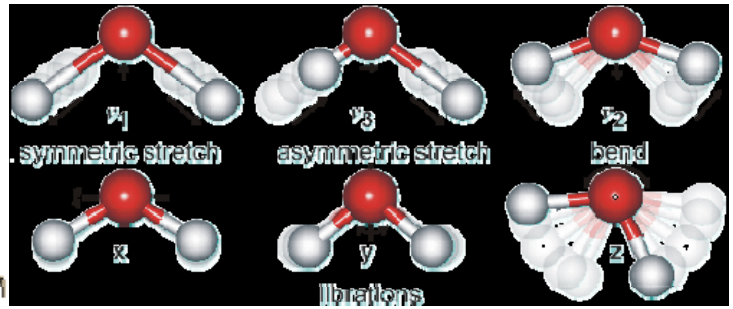
Absorption of a visible light leads to electronic transitions – (spectrophotometry, UV-vis spectroscopy),

Absorption of IR – transitions between different vibrational energy levels (IR spectroscopy)

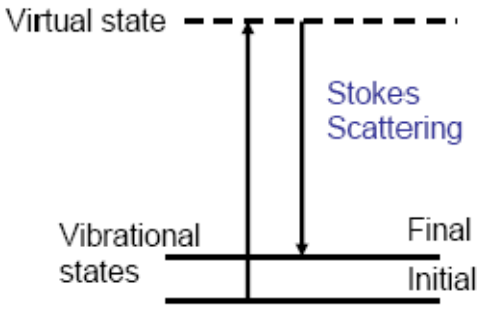
- Luminescence is the emission of light by a substance. It occurs when an electron returns to the electronic ground state from an excited state and loses it's excess energy as a photon.
- Luminescence is a collective name given to three related emission types: Fluorescence, Phosphorescence, Chemiluminescence

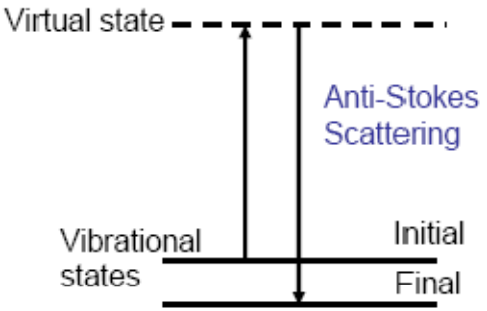
- Samples are substances analyzed in solution,
- These methods provide a chemical signature of a substance, detection, help to follow chemical reactions
- IR – signature of secondary structure of molecules

Raman Scattering



- Rayleigh scattering is elastic scattering – the photon energies of the scattered photons is not changed.
- Scattering in which the scattered photons have either a higher or lower photon energy is called Raman scattering.
- This kind of scattering involves exciting some vibrational mode of the molecules, giving a lower scattered photon energy (Stockes shift), or scattering off an excited vibrational state of a molecule which adds its vibrational energy to the incident photon (Anti-Stokes shift).





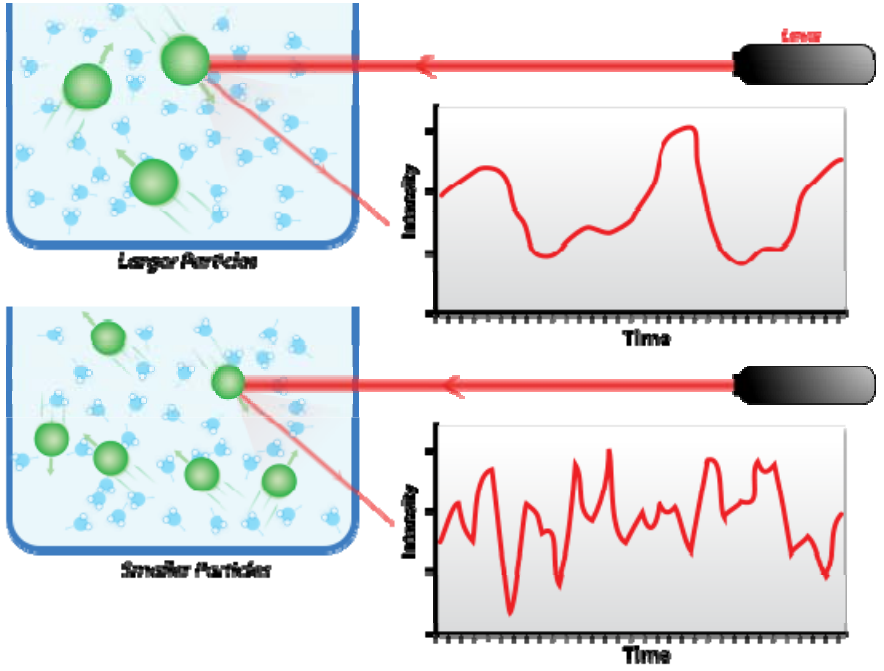
Raman Spectroscopy:

- a spectroscopic technique used to study vibrational, rotational, and other low-frequency modes in a molecule,
- relies on inelastic scattering of light by molecules,
- gives vibrational information for the chemical bonds in molecules.
- provides a fingerprint by which the molecule can be identified, especially useful for secondary structures
- molecules and substances in solutions are studied

- It can be used also for imaging – by collecting Raman spectra at each point of the sample – micron resolution
- but specific elements can be recognized

Dynamic light scattering (DLS):

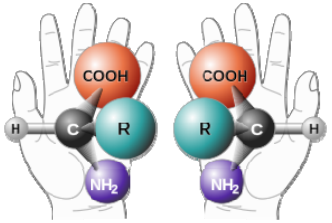
- technique used to determine the size and motion (diffusion coefficient) of small particles in solution
- based on scattering of light from the particles
- change in light Intensity with time when light interacts with moving particles
- Biomolecules and particles size and aggregation can be studied
- Estimation of molecular weight
- Shape of protein

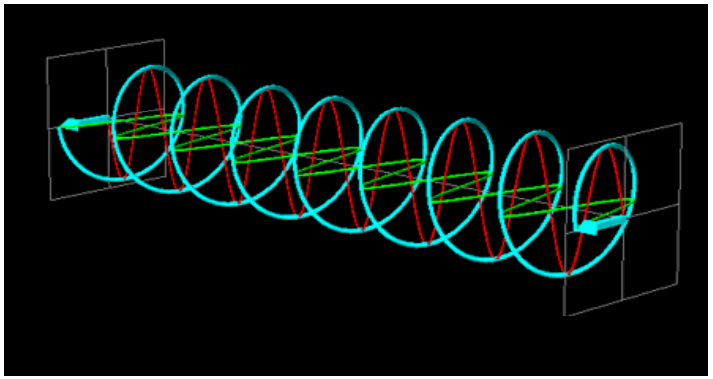


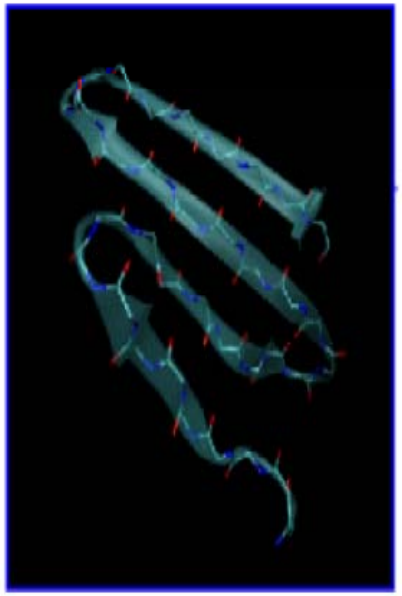
Samples – optically clear solutions of pure substances

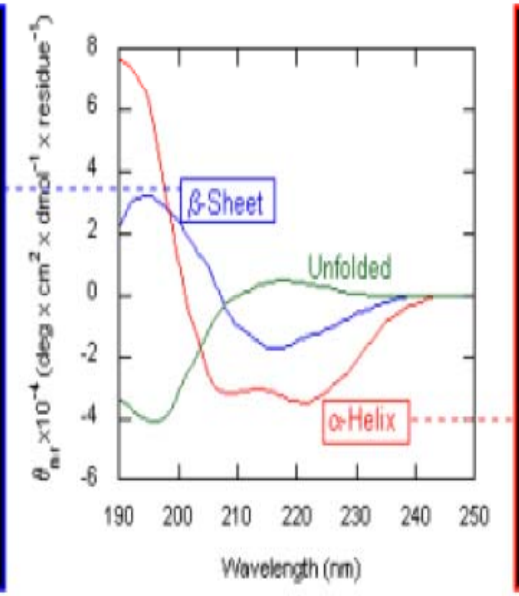
Circular Dichroism (CD)

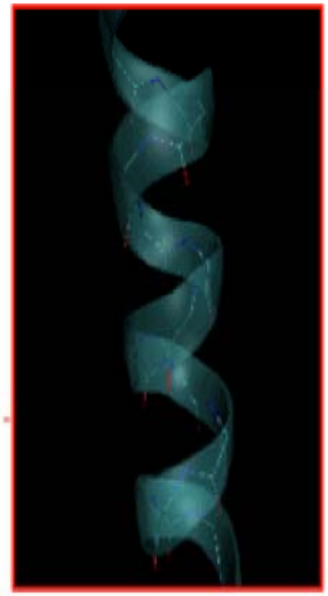
- A form of spectroscopy which analyzes the different amounts absorption of left- and right-handed circularly polarized light on chiral molecules and stereo-isomers





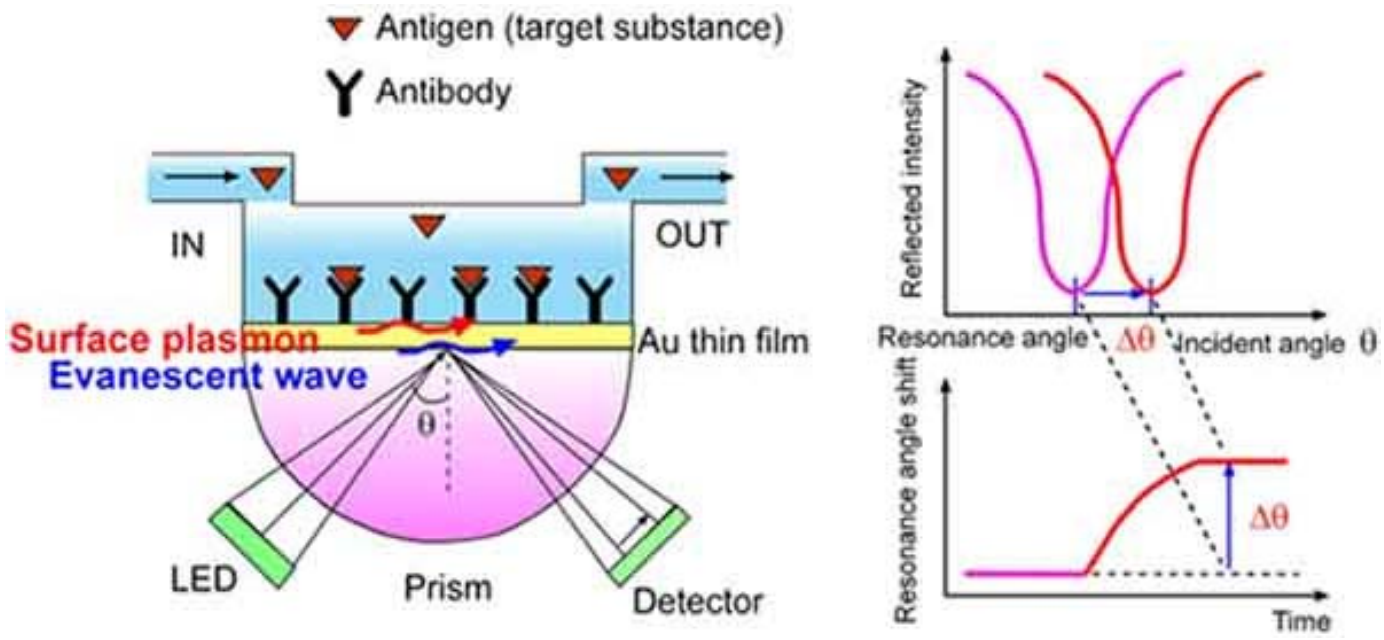






Used to determine the secondary structure of macromolecules specifically - stereo-isomers
Molecules (pure substances) in solution are analyzed

Surface plasmon resonance (SPR)



- Surface sensitive spectroscopic technique, based on excitation of electron oscillations (plasmons) in thin metal surfaces upon interaction with light. Plasmons resonance is sensitive to adsorption of molecules to the gold surface.
- Used to detect the binding of biological molecules onto arrays of probe biomolecules covalently attached to chemically-modified gold surfaces, used in biosensing

Confocal microscopy

- Optical microscopy – transmission or reflection of light from the sample
- Fluorescence microscopy – fluorescence label
- Confocal microscopy – fluorescence microscopy which collects images from planes (slices) and allows for reconstitution of 3D images
- Application – imaging life cells, cell organels, tissues labelled with fluorescent probes . Allows to study distribution/aggregation of biomolecules in cells

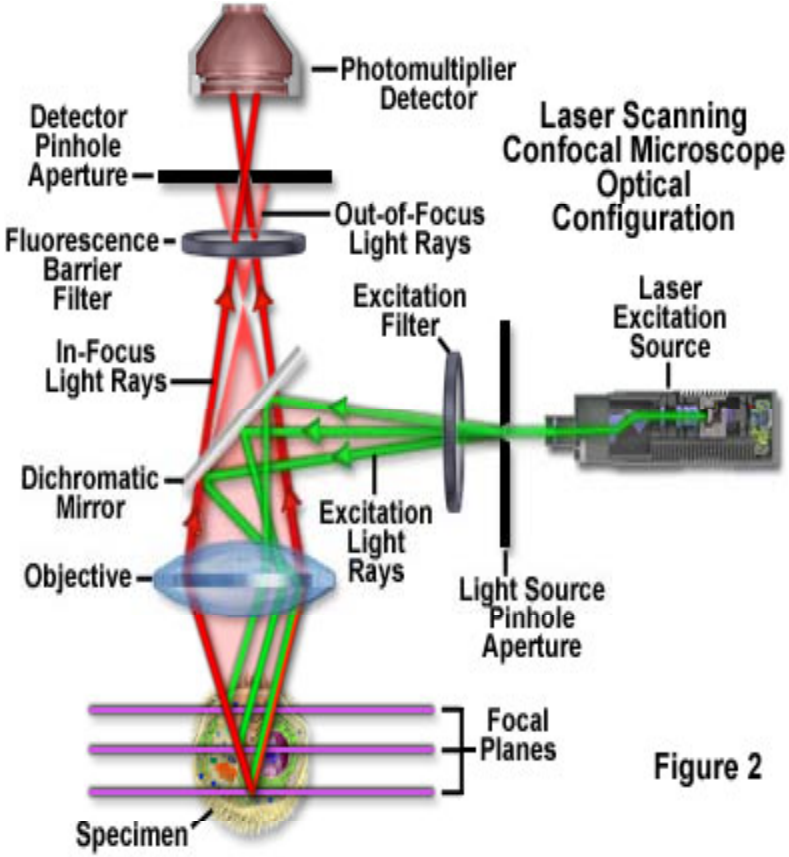


Figure 2

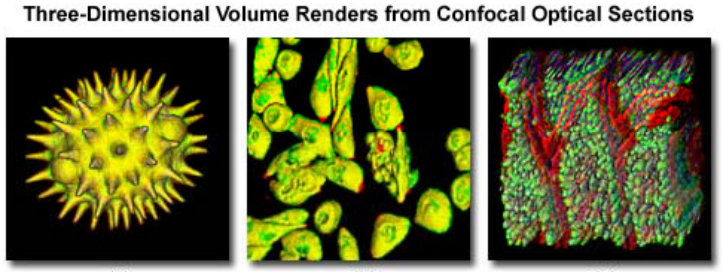
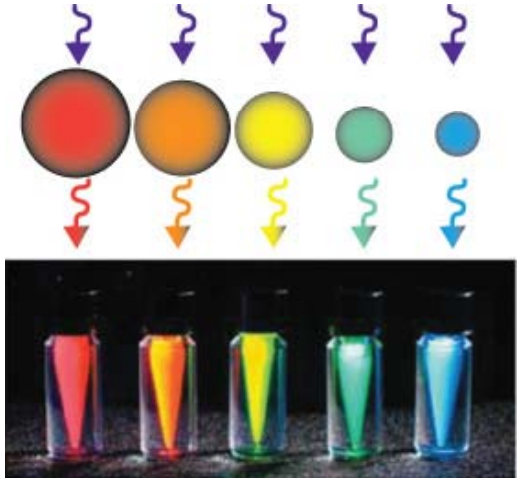
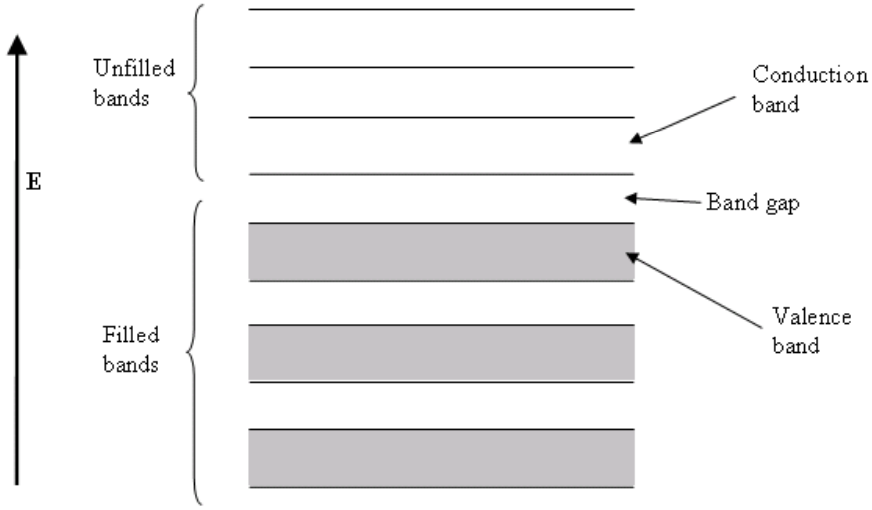
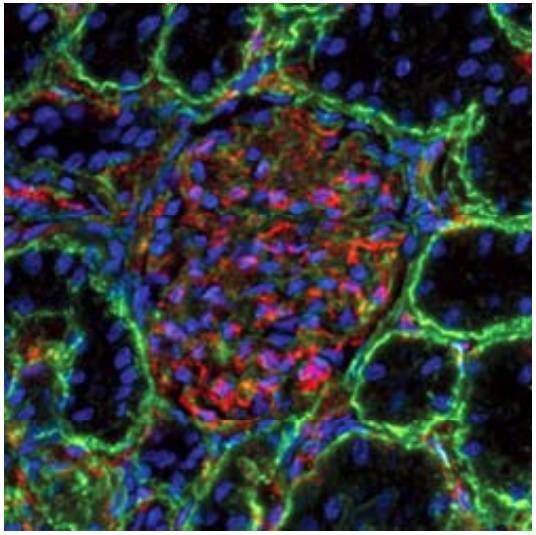


Figure 7

Quantum dot imaging





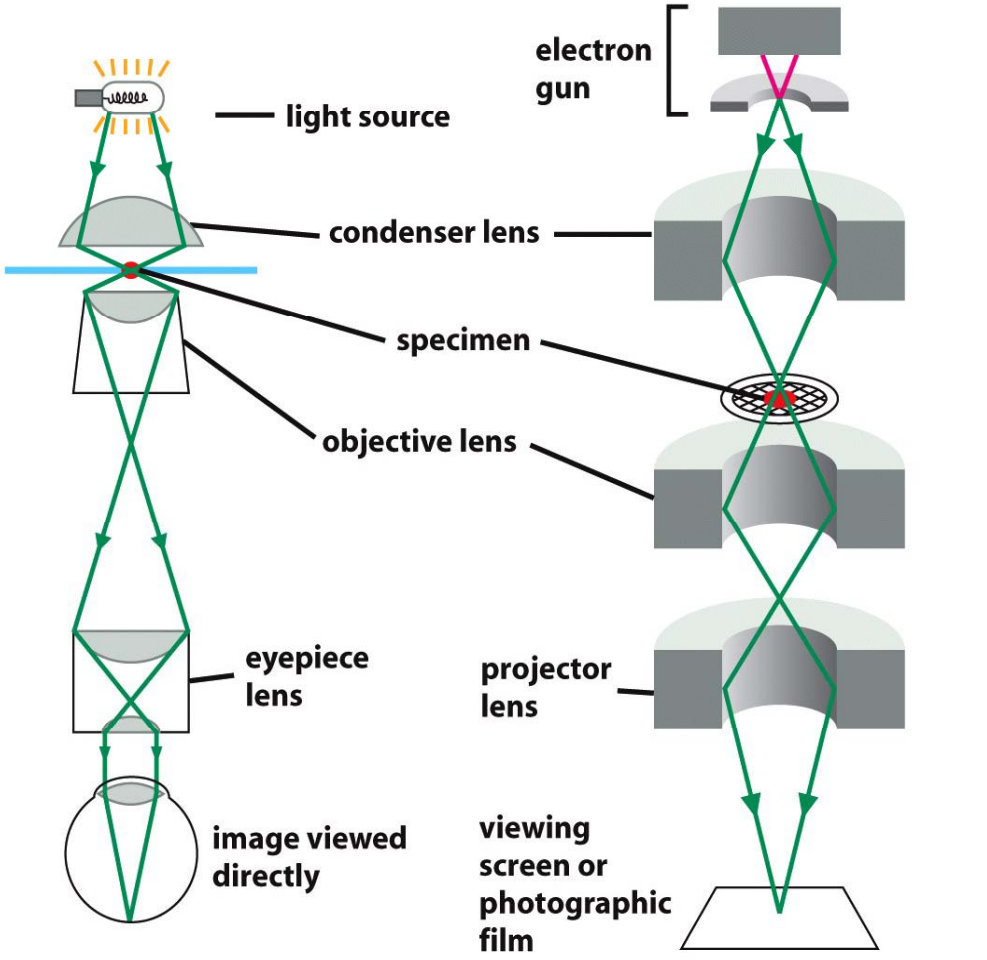


Mouse kidney section

- A quantum dots are nm size semiconductor nano particles (1-20 nm) which fluoresce upon interacting with light.
- Fluorescence wavelength of quantum dots depends on their size and have a broad spectrum from red to blue.
- Quantum dots are widely used as fluorescence labels for cell imaging in biology research and tissue imaging in medical imaging & diagnostics.
- Quantum dots can be coated with lipid films and other functional molecules for the purposes of biocompatibility and specific binding.

The Electron Microscopes

- The electron microscope is used to resolve fine structure with high resolution.
- Rather than using light, high speed electrons beams are used. As with light, resolution is a function of wavelength (diffraction limited). The wavelength associated with accelerated electron beam is very small thus theoretical resolution of electron microscopy is 0.002 nm (100,000 X that of the light microscope).
- When electrons are passed through specimen it's called **Transmission Electron Microscopy (TEM).**
- When electrons are reflected/scattered – **Scanning Electron Microscopy (SEM)**
-



Light Microscope

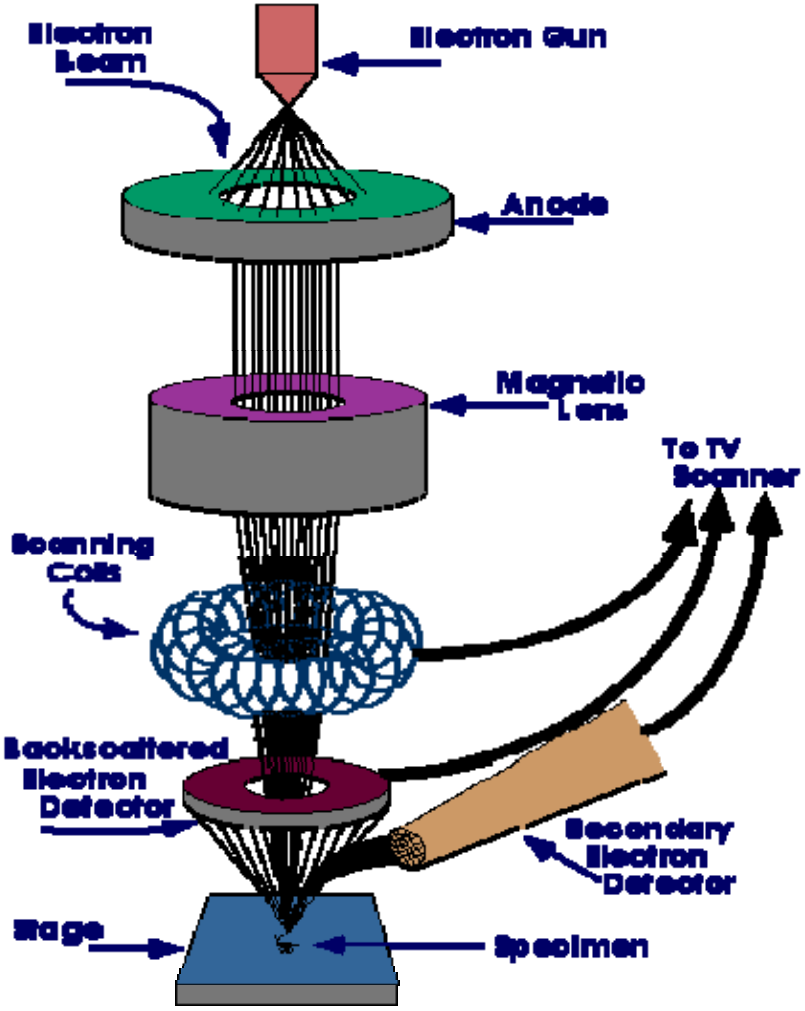
T Electron Microscope

Scanning electron microscopy

- Interaction of scanning electron beam with the sample surface
- Back-scattered electrons are analyzed by the detector to produce the image
- Sample has to be fixed on the substrate and coated with thin metallic film (gold)

Applications:

imaging various surfaces, biological samples as well (dried and coated with gold),
high resolution 3D images, 10 nm.



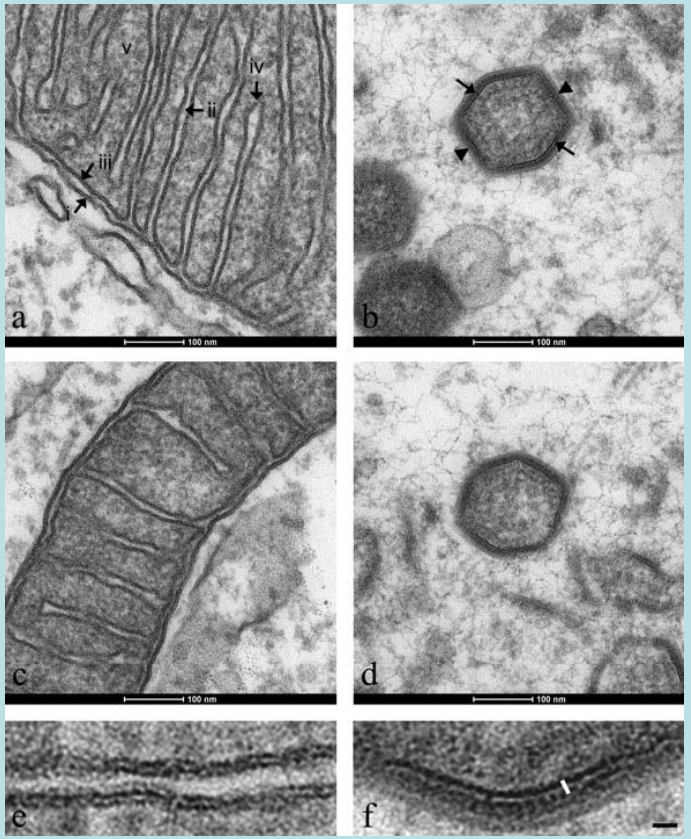
Transmission electron microscopy

Transmission Electron Microscopy (TEM) is a microscopy technique that uses beams of energetic electrons to provide morphologic, compositional and crystallographic information on various samples.

Samples require preparation :
sliced thin enough to allow passage of electrons - electron transparency:

dehydration, freezing, chemical fixation,

High resolution, A, nm,



Scanning probe microscopy methods

- STM
- tunneling of electrons between 2 electrodes under electric field
- requires conductive surface
- works in air or vacuum

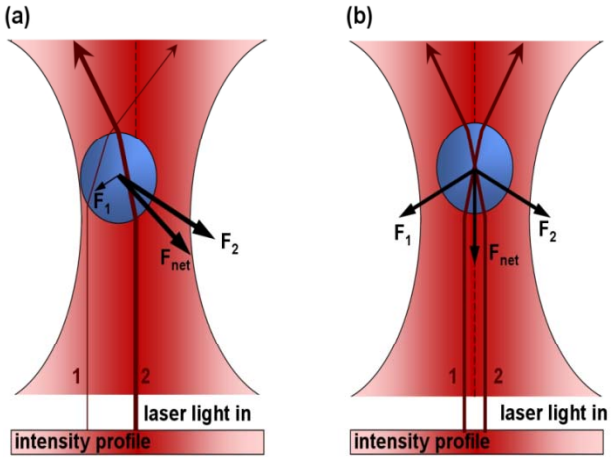
- AFM
- van der Waals interaction between tip and sample
- can image non conductive surfaces
- works in air and in liquid media

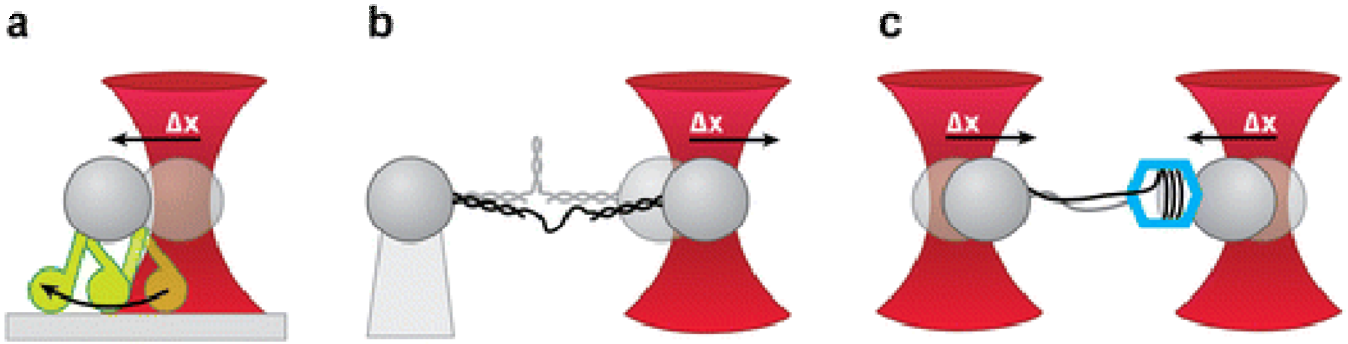
- SNOM
- interaction of light with the sample at the near-field
- requires fluorescence label
- works in air and in liquid media

The principle of scanning probe microscopy methods is interaction of sharp scanning probe (tip) with the sample surface. The interaction can be – tunnelling current, forces, and interaction of light coming through the probe with the sample. Resolution is not limited by diffraction limit, but is limited by the sharpness of the probe – few nm to atomic resolution.

Optical Tweezers

- a focused laser beam provides a force which can physically hold and move microscopic transparent dielectric objects
- Allows for the investigation of week forces and dynamics at the single molecule level, attached to these beads

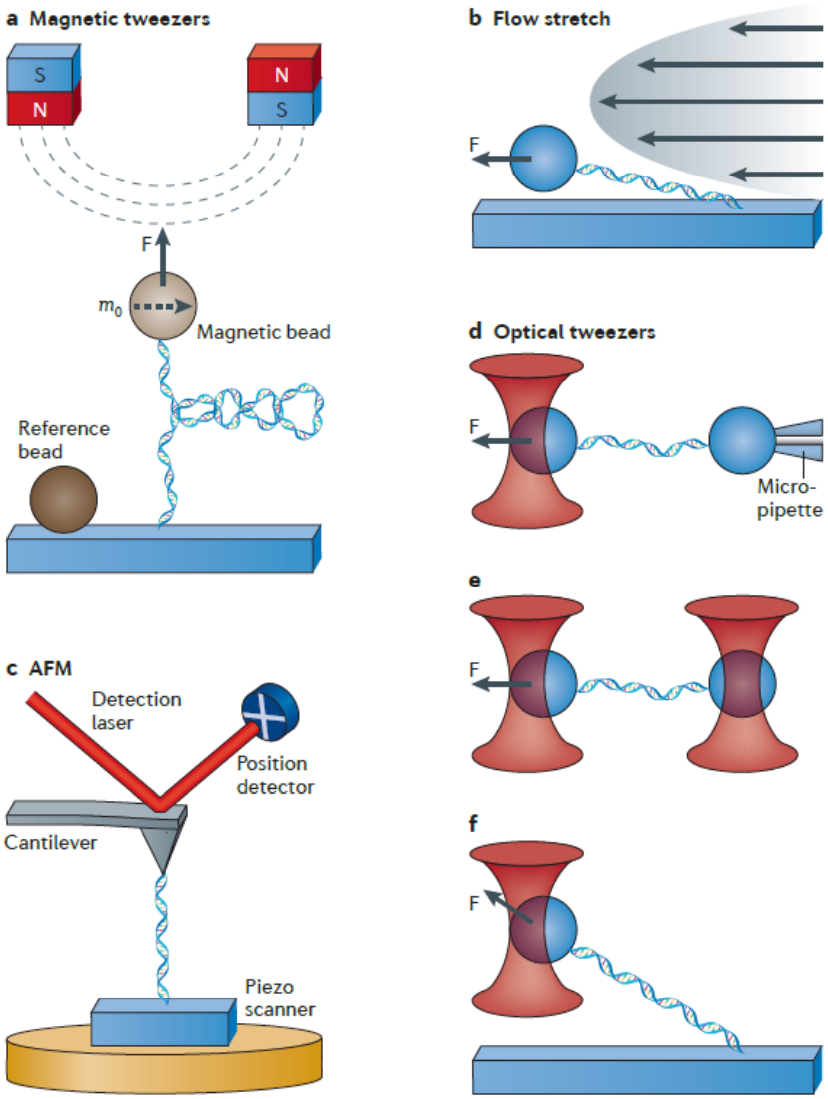


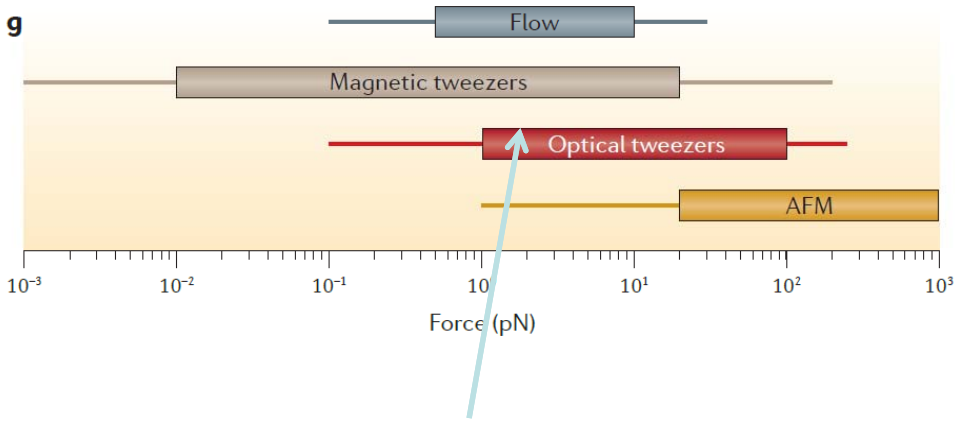


Moffitt JR, et al. 2008.
Annu. Rev. Biochem. 77:205–28.

Applications:
Nanomanipulations
Binding forces between single molecules can me measured

Single-molecule force manipulation techniques



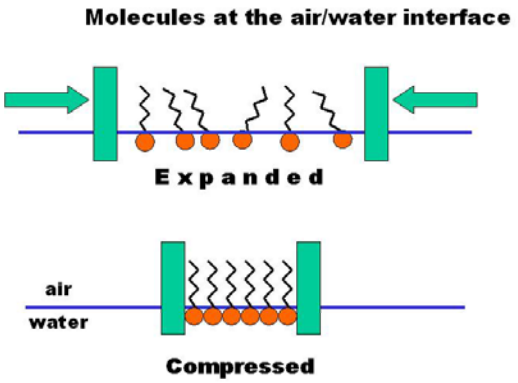


Optimal range for studying most biological processes

doi:10.1038/nrg3316

Langmuir-Blodgett Technique

used to study the properties of monolayers of amphiphilic molecules.



1. Make monolayers of amphiphilic molecules at the air-water interface
2. Study surface tension of the monolayer films (compression)
3. Deposit monolayers or multilayers on solid support
4. The structure of the supported films can be studied through other methods (such as microscopy)

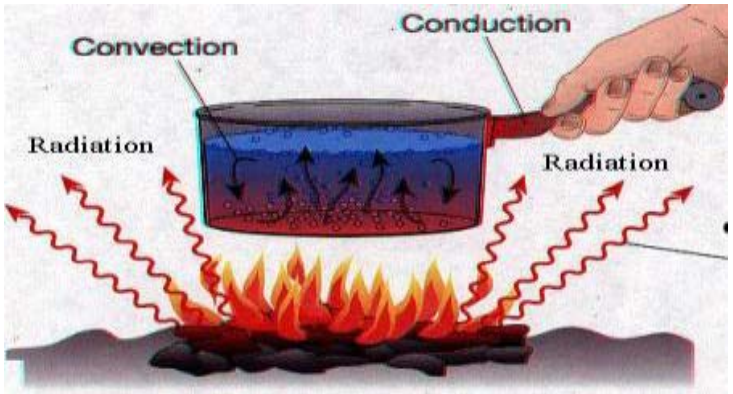
Differential Scanning Calorimetry

- Measurement of heat flow in one sample with comparison to the amount of heat flow in control sample while heated at the constant rate in both samples
- Analysis of phase transitions which are associated with heat transfer
- Applications: Material properties characterizations: glass, metals, polymers
- Phase transitions in biomolecules and assembiles: proteins, lipids,
- Denaturation in proteins and other biomolecules.

The heat transfer is measured
C is the heat capacity:

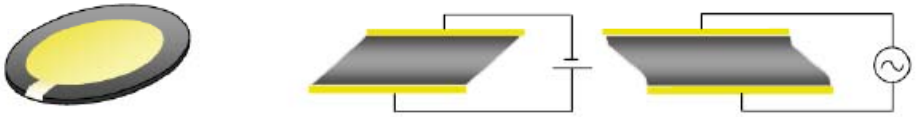
$$Q = C\Delta T$$

Heat transfer is subject to different properties based on which type of heat transfer, for this reason a measure of heat flow is used to characterize the samples
Samples – solutions of molecules or solid states – polymers, glass



Quartz Crystal Microbalances

- Is a very precise method of measuring mass and the kinetics of absorption of biomolecules to a surface, Mass sensitivity of 1 ng/cm2
- Quartz crystal is excited to oscillate at its resonant frequency f,



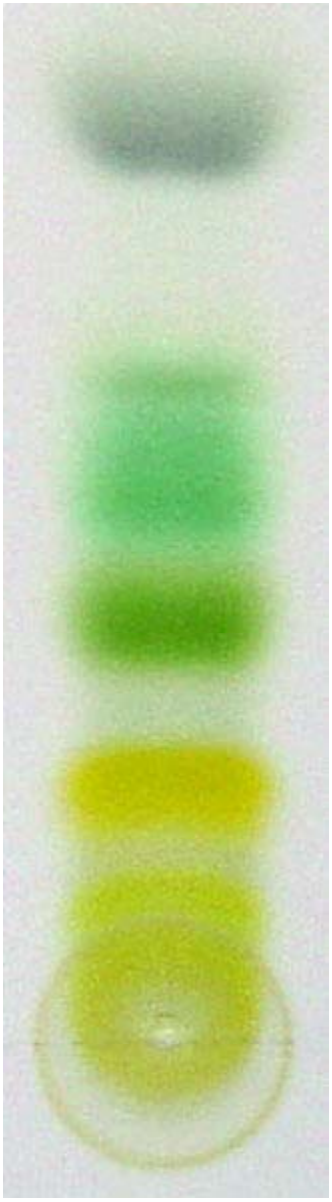
$\Delta f \sim \Delta m/C$

C – constant for the crystal

- Adding mass to crystal changes the resonant frequency
- Allows to obtain information about shape and weight of molecule, as well as to measure the kinetics of the adsorption
- Applications: Interaction of molecules with surfaces, Chemical reaction monitoring, Biomedical Sensors, Metal deposition monitoring, Immunosensors for allergenic foods

Chromatography

- Allows separation of molecules in complex solutions
- Based on different interaction of molecules with immobile state (beads in the column or paper) – due to this some molecules will move faster through the column than others under the gravitation force or applied pressure and thus can be separated.
- Applications: analysis of water, food contaminations, analysis of hydrocarbon mixtures in gasolines, analysis of biomolecules in mixtures - protein separation
- Samples – solution/mixtures of molecules



Electrophoresis

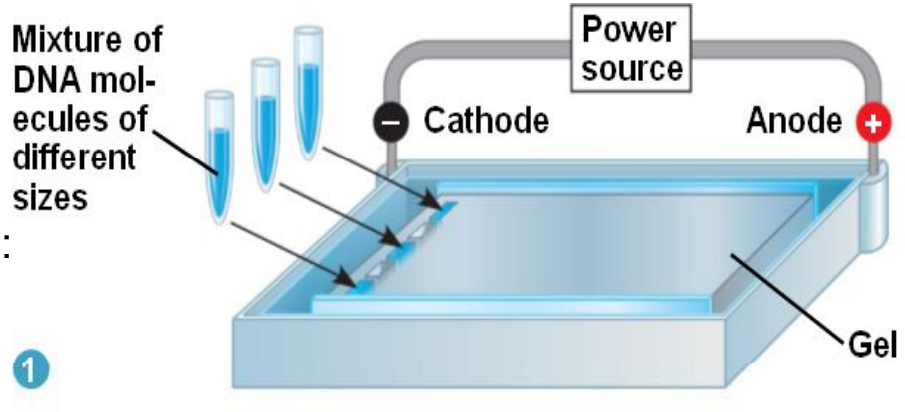
separation technique where an electric field causes charged particles to move through a separation matrix (gel or paper).

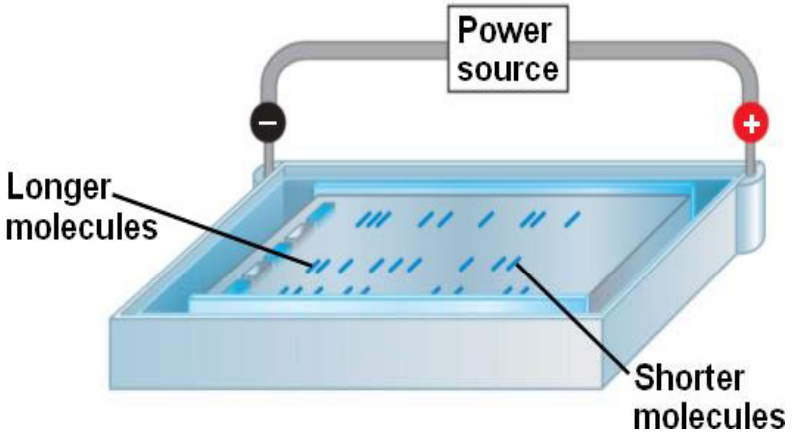
An electric field exerts an electrostatic force on a charged object moving through the field: F=qE

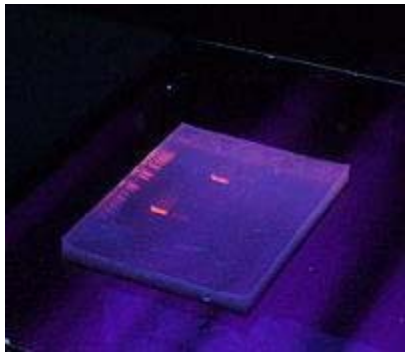
samples - solutions of substances, mixtures

applications:

- used to separate charged particles (molecules, ions) according to mass, charge and size.
- used to analyze DNA and proteins in basic research and forensic science

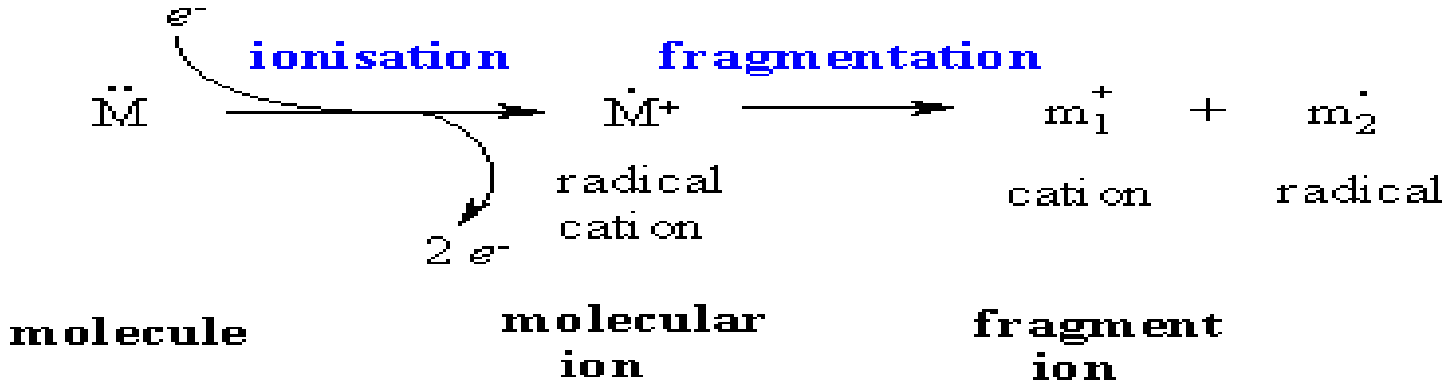






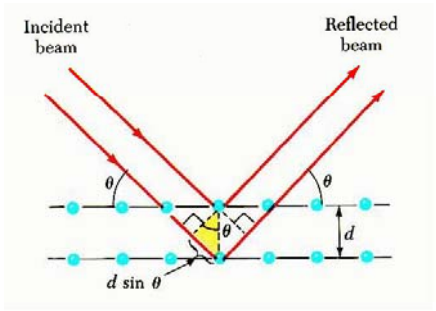
Need a method to observe the results - e.g. ethidium bromide with UV, Coomassie Blue

Mass spectrometry



- The identification of compounds and/or elements contained in an unknown material, method of determining chemical composition.
- The substance is broken into ions by ionizing radiation and the ions are accelerated in the tube under electromagnetic field, when the ions pass the secondary magnetic field their path deviates from the straight line and they lend on the detector at different positions, defined by their mass to charge ratio (M/z).
- Applications: investigation of structure of the complex substances by analyzing fragment ions; widely used to analyze protein structures. The substance needs to be broken into smaller pieces.

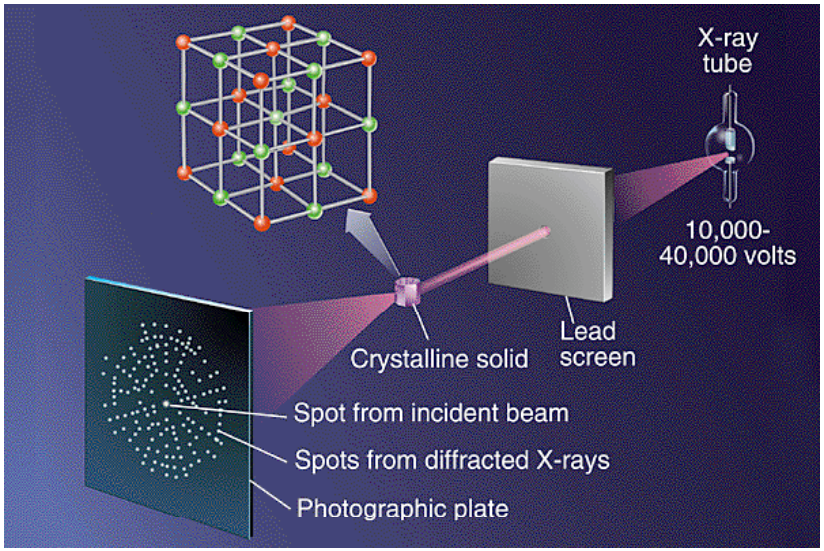
X-Ray diffraction



X-ray diffraction technique is based on the elastic scattering of x-rays from structures that have long range order (crystalline or ordered structures).

- The diffracted x-rays interact with an crystalline thin film and form a pattern which can be interpreted into a structure. Diffraction pattern is defined by periodicity of crystalline structure, dimensions of the lattice.

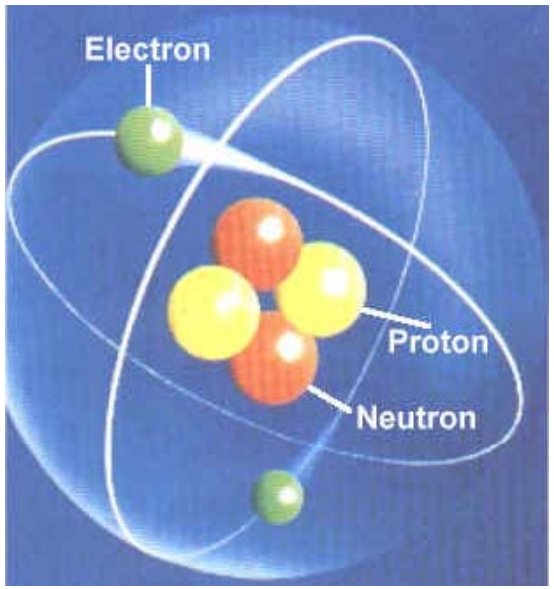
- Applications:
- Determining structure of crystallized substances (distances/angles between atoms):
- In solid state and material research,
- Protein crystallography
- Structure of periodic stacks of lipid membrane



Neutron Diffraction

Neutron diffraction is also known as elastic neutron scattering

- Neutrons are used to determine the structure and motion (vibrational, etc.) of atoms within a structure
- Neutrons have wavelengths that correspond well to inter atomic distances
- (2 A), and energy similar to elementary excitations (20meV).

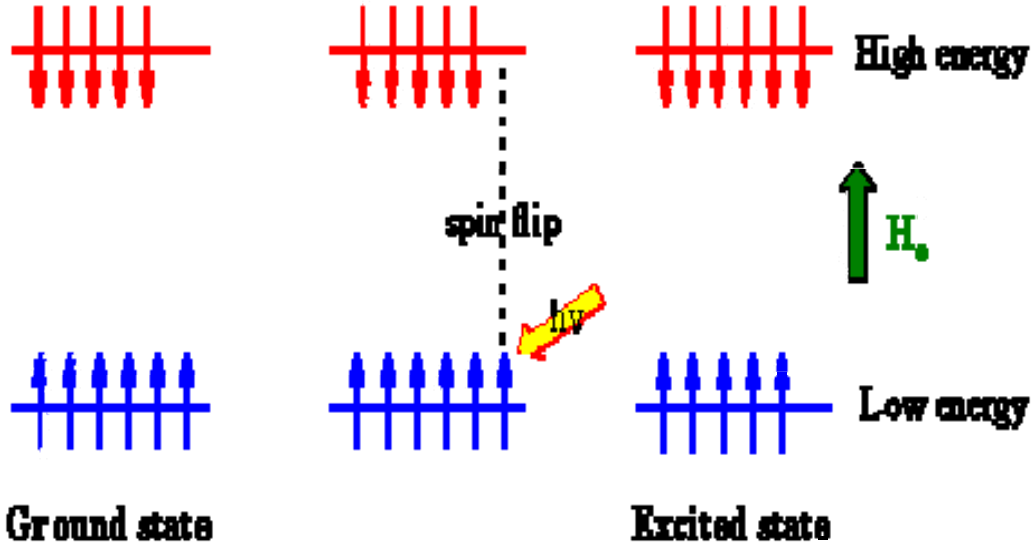


Neutrons are less likely to interact with the electron clouds of biological molecules because their cross sections varies between elements, which allows us to distinguish between isotopes, especially for hydrogen which is largely invisible in X-ray diffraction.

Nuclear Magnetic Resonance (NMR) spectroscopy

Is a form of spectroscopy, relies upon inherent magnetic moments in the nucleus of atoms. Is useful in determining the structure and nature of complex molecules. Samples - pure substances in solution with atoms of nuclear spin 1/2 , 3/2 pr 5/2 are NMR active.; 1 and 0 are not active

based on alignments of nuclear spins in the magnetic field, and transitions between energy levels created due to alignment of these spins in magnetic filed (parallel and anti-parallel alignments) as well as spin-spin coupling



NMR spectroscopy

Different atoms have different energy differences/frequencies

An NMR spectrum is a plot of the ***radio frequency applied*** against ***absorption***.

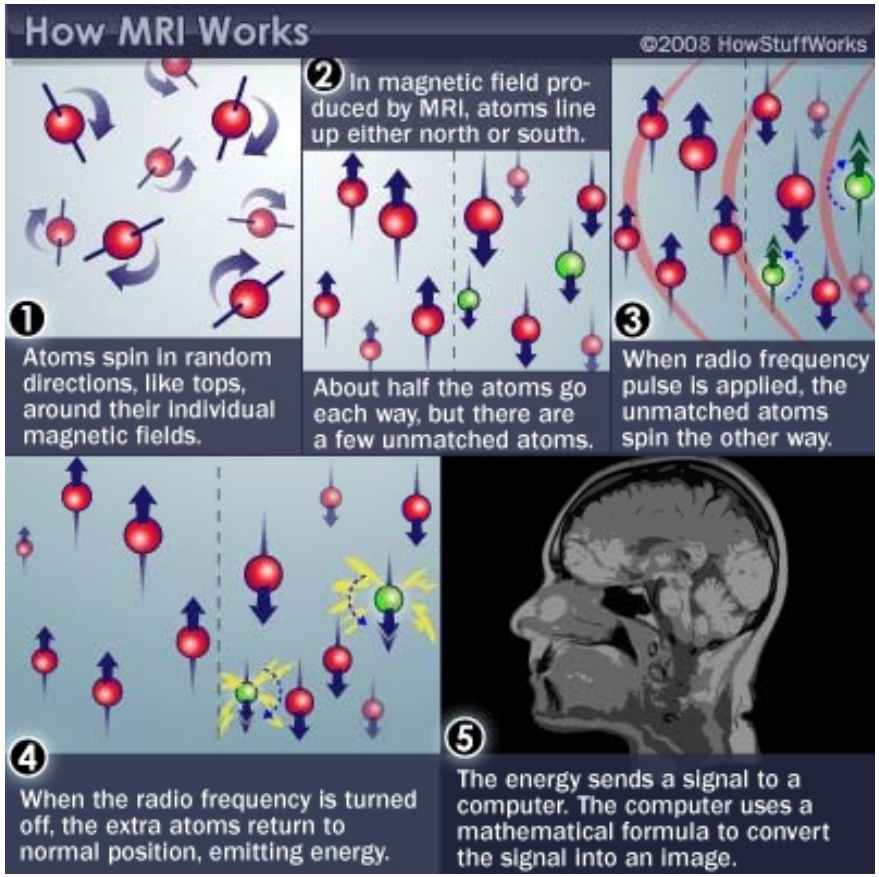
A signal in the spectrum is referred to as a ***resonance***.

The frequency of a signal is known as its ***chemical shift***.

Absorption spectra are obtained and compared to standard, chemical shift is characterization of atom and its environment- neighbors

Magnetic Resonance Imaging

- External Radio wave pulses are sent to alter the alignment of nuclear spins in magnetic field, usually protons.
- As the atoms return back to their relaxed state, they produce radio waves captured by the detector
- Different atoms from different tissues have differing speeds at which they return to the relaxed state.
- The frequency and intensity of relaxing atoms are used distinguish tissues and to produce the images of slices of the person's body.
- These images are composed together to form a 3D image of the part of interest.

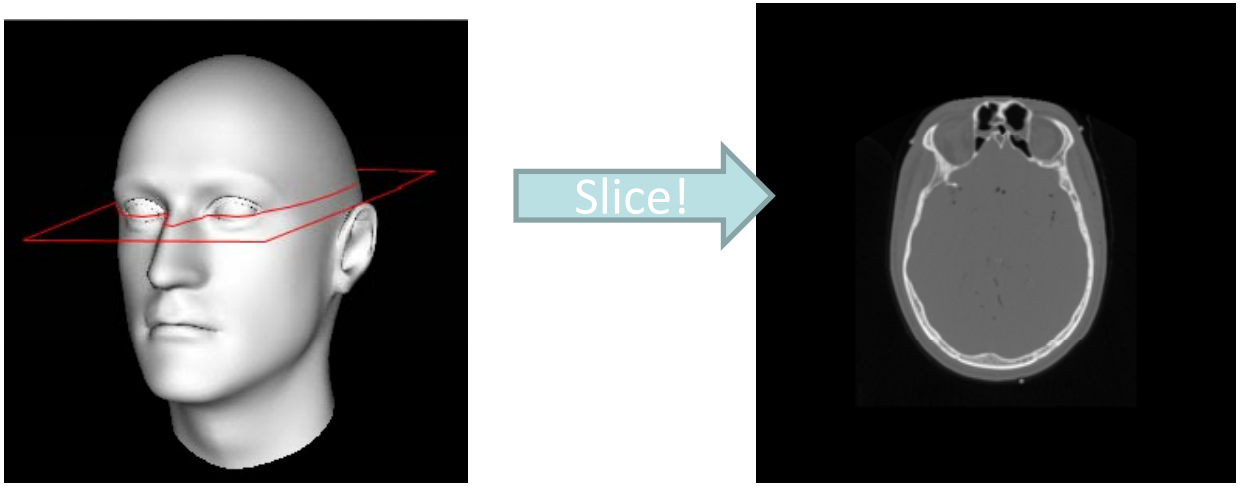


Applications in medicine for non-invasive tissue imaging and diagnostics with mm and micrometer resolution

Tomography

- Definition of Tomography:
  - Non invasive Imaging of body/organism – imaging sections(slice) using some kind of wave energy
  - Many types of wave energy
    - X-Ray (X-Ray Computed Tomography – CT/CAT)
    - Optical (near IR light) (Optical Coherence Tomography)
    - Positron Emission (Position Emission Tomography –PET)
    - … tens of others

Applications in medicine for non-invasive tissue imaging and diagnostics with mm and micrometer resolution

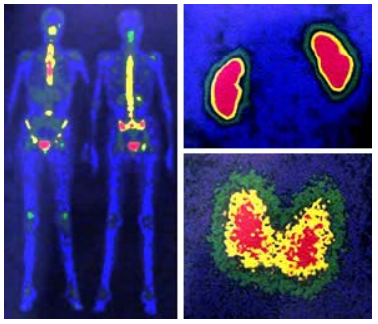


Isotope labeling

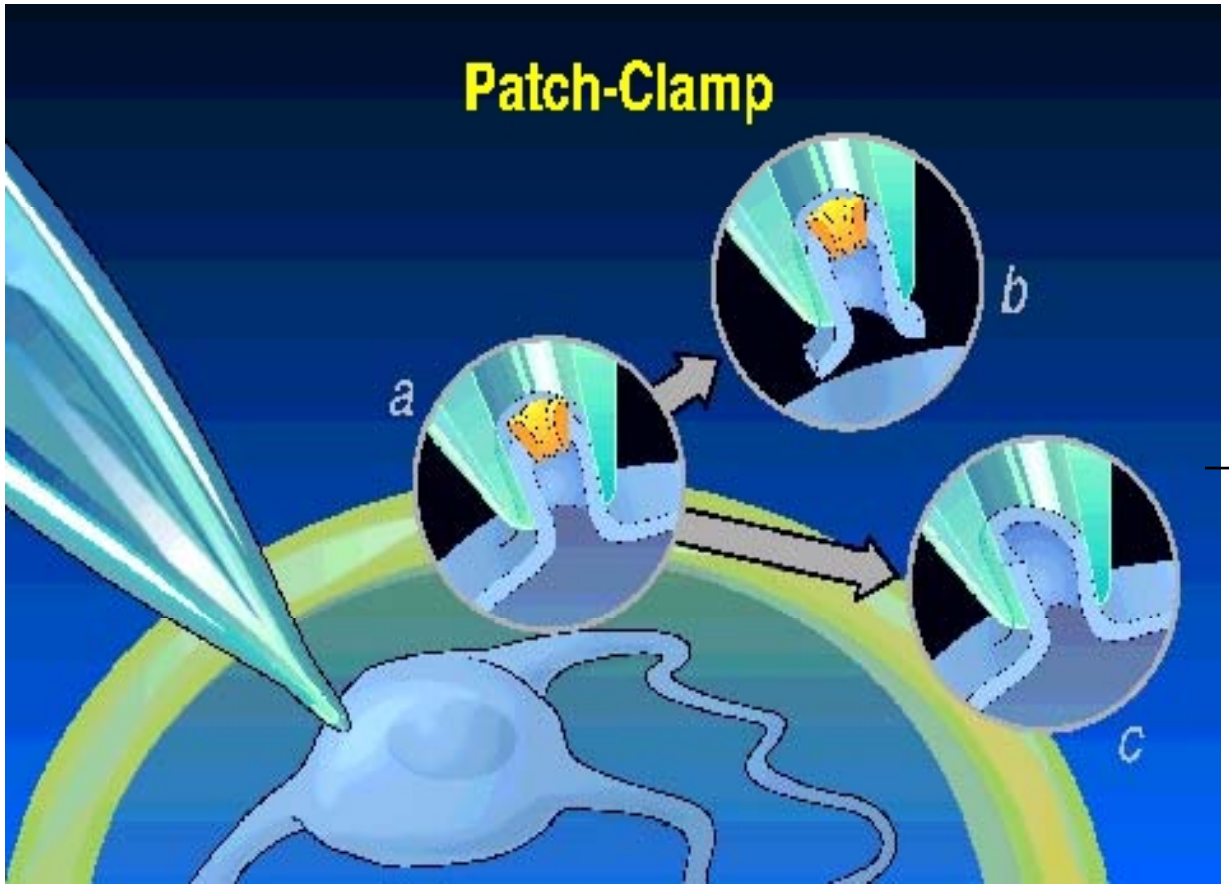
- Isotopes are different atomic forms of the same element. They differ by the amount of neutrons located within the nucleus.
- These different masses contribute to slightly different physical properties, but all atoms of the same element maintain their chemical properties.
- The different masses of atoms in the molecules can be detected by the different vibrational frequencies with Infrared spectroscopy or Mass/charge ratio by mass spectrometry.

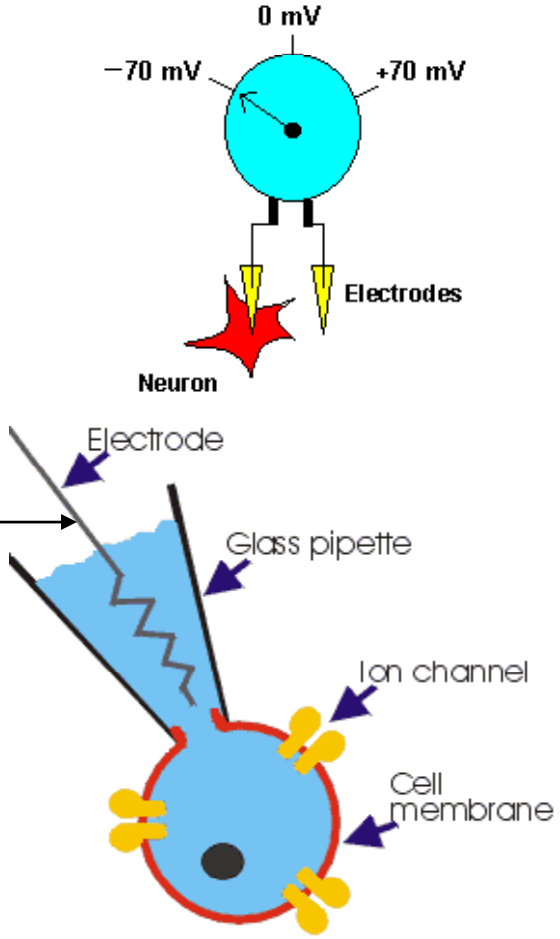
- Applications:

  To track substances as they move through different types of systems, biological organisms as well,
- Chemical reactions can be followed when substances are 'tagged' by integrating an isotope into their chemical structure.
- Carbon-dating (Carbon-14)
- Molecular Biology and Biotechnology: DNA and protein labeling,
- Medical Applications:
- Mapping the inside of the body using a
- short lived radioisotope as an imaging agent .
- Isotope labelling of biological molecules can
- be tracked through the body to look for diseases - cancer



Patch Clamp

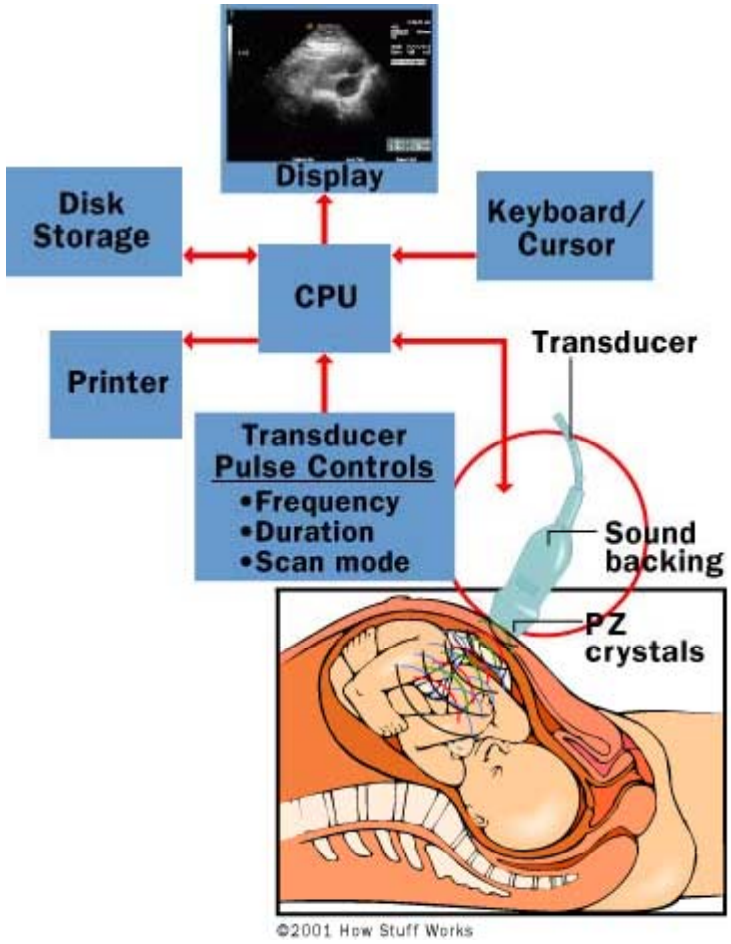




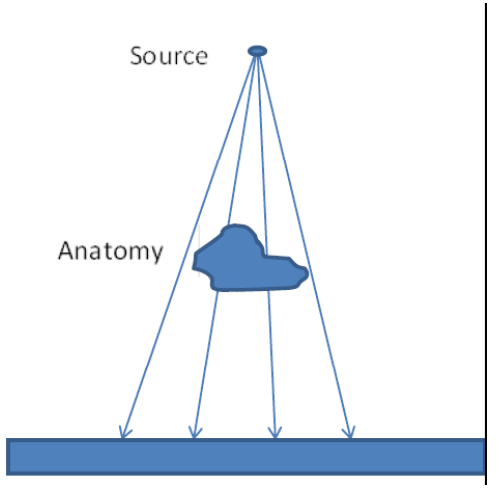
- Patch clamp is an electrophysiology technique, which allows the study of single or multiple ion channels, **allows to measure membrane potential, conductance or ion current of single ion channels**
- Particularly useful for the study of excitable tissues such as neuron cells

Ultrasound imaging

- Imaging tool (mm resolution) with a wide range of applications primarily in medical diagnostics
- Measures reflectivity of tissue to sound waves(1)
  - Ultrasound waves & echo signals
- Signals are processed to create an image
- Non-invasive, quick, portable and free of radiation risk
- Real-time & cross-sectional
- Frequencies at 1 to 18 MHz



X-ray imaging



- X-rays are high energy electromagnetic radiation
- Use of x-rays based on their ability to pass through matter
- Higher frequency rays and lower density materials allow furthest infiltration of x-rays
- Images are based on unequal diffusion of x-rays through materials
- Used in medicine for diagnostics
- Can be damaging at high exposure

Photodynamic therapy

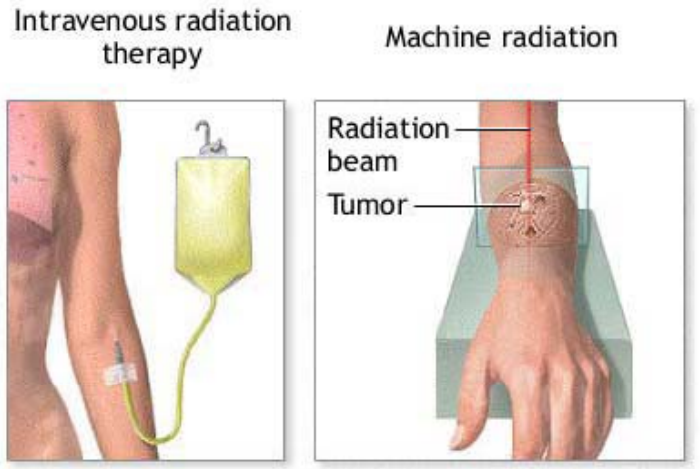
- Photodynamic Therapy, or PDT, is a medical treatment involving activation of a photosensitizer drug by light of a specific wavelength

- How it works: light is absorbed by photosensitizer drug, photosensitizer molecule gets excited, transfers the energy to oxygen. Singlet oxygen (or Reactive Oxygen Species, OH radical) are created – this is a toxic form of oxygen which destroys surrounding cells where it is injected

- Applications: treatment of cancer and skin diseases, eye retina, requires light exposure of the area saturated with photosensitizer drug

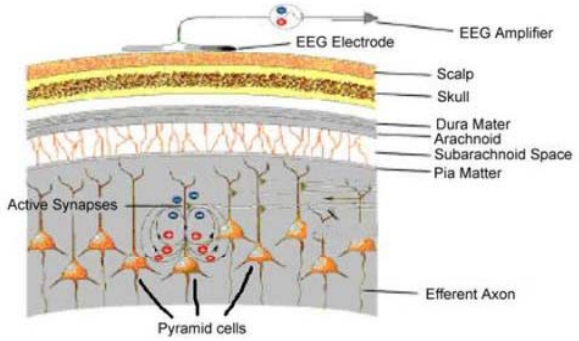
Radiotherapy

- The use of beams of ionizing radiation or energetic charged particles to control or kill cells - treatment.
- Ionizing radiation is electromagnetic waves that have sufficient energy to ionize (liberate an electron) an atom when they interact with it.
- Direct damage is breaking molecules,
- Indirect damage – ROS produced by breaking water, which later damage the biomolecules
- Applications: treatment of
  - Cancer
  - Damaged or defective nerve cells
  - Inflammatory Diseases (eye disease)
  - Skin disease



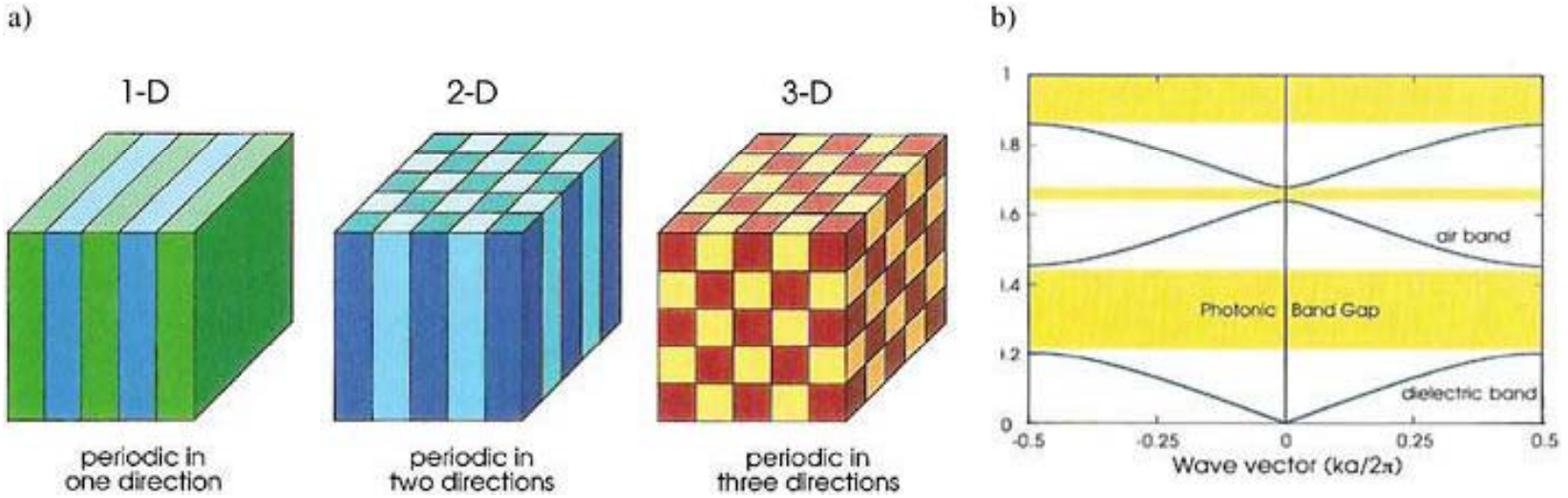
Electroencephalography (EEG)

- Electrical activity recorded using Small metal discs called electrodes placed on the scalp in special positions.
- Measure of voltage potential resulting from ionic current of action potentials of large amount of neurons
- oscillations of potentials represent different states of brain functioning
- Brain activity and associated diseases can be monitored: epileptic and sleep disorders,
- attention
- study



http://faculty.vassar.edu/abbaird/resources/brain_science/images/eeg_machine.jpg

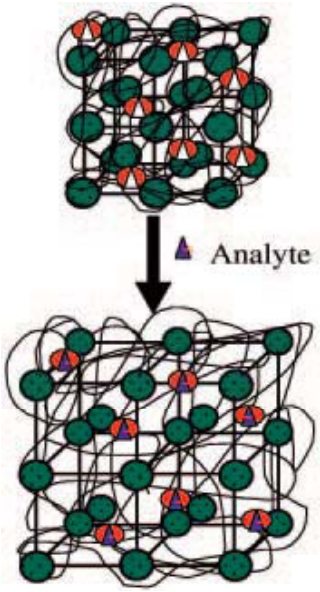
Photonic crystals

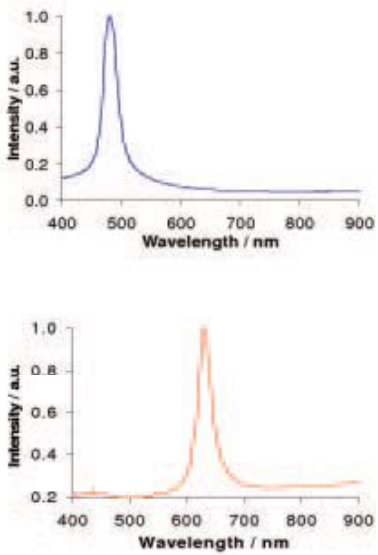


- Photonic crystals are periodic dielectric nanostructures that affect the propagation of electromagnetic waves by forbidding a certain frequency range of light. They trap or guide photons, in a similar way as semiconductors for electron transport.
- Priodicity of photonic crystals can be tuned to allow or forbid propagation of certain wavelengthes.
- Used as optical traps and waiveguides and in imaging as light sources
- Used in biosensing as well - next slide

Photonic Crystal Glucose-Sensing Material for Noninvasive Monitoring of Glucose in Tear Fluid

- *Alexeev et al, Clinical Chemistry 50:12,* 2353–2360 (2004)





Photonic crystal was made of cubic array of colloidal particles embedded in polymer hydrogel, its periodicity defines the wavelength of diffracted light. When Glucose binds inside the crystal, hydrogel swells, the lattice periodicity changes and the crystal diffracts light of a different wavelength.
The crystal is embedded in the contact lens and changes color upon glucose binding

Questions on methods

- Out of the list of methods pick as many answers as possible,
- Each correct answer will count as +1,
- each incorrect answer will count as -1,
- at the end the sum will be calculated

Example Questions on methods

- What methods are used in medicine for diagnostics and non-invasive imaging of live organisms
- What methods are analytical techniques to separate mixtures of substances?
- What methods are used to image cells and their content
- What method is used to separated DNA by length
- What methods are used in biosensing
- What methods are used to study neuron cell activity
- What methods can give information on 3-D structure of protein?
- What methods are used to follow changes in structure during chemical reaction?
- What methods are used in medicine as therapeutic approaches to treat diseases?

- **What physical methods can give information on 3-D structure of protein? Pick as many as you want, correct answer +1, incorrect answer -1**

- Langmuir-Blodgett technique
- MRI
- SPR
- Patch Clamp technique
- X-ray diffraction
- X-ray imaging

imaging

- Name high resolution imaging techniques
-
- Which are diffraction limited?
- Which can image in liquid
- Which method(s) need metallic coating of the sample?