Introduction to Biomedical Engineering

Biomedical optics II

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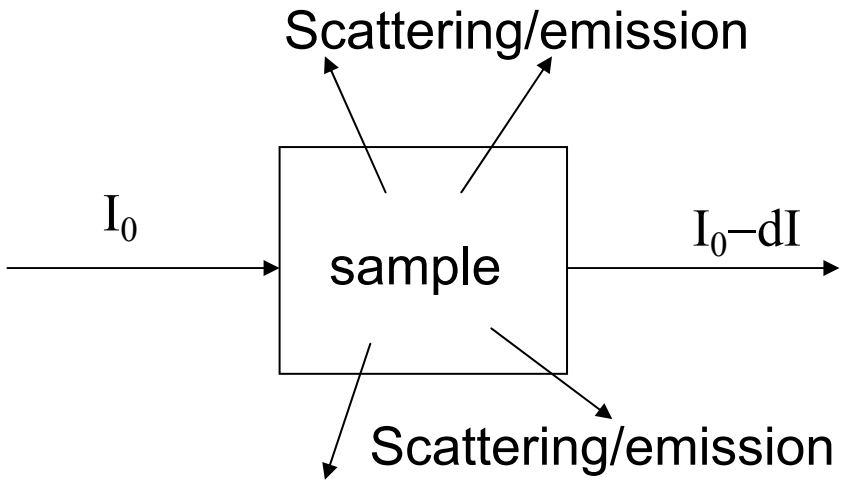
Outline

- Chapter 17: Biomedical optics and lasers
- Fundamentals of light
- Light-matter interaction
- Optical imaging
- Optical sensing: spectroscopy
- Using light for manipulation
  - Laser tweezers, laser scissors
- Using light for therapy

Optical detection – overview

- Why use optical detection?
  - Non-invasive, minimally disturbing
  - Fast $\Rightarrow$ real-time monitoring
  - Sensitive
  - High spatial resolution
- Limitations?
  - Penetration depth
  - Source of signal/contrast

Light-matter interaction



I_0: intensity of incident light
Total loss of incident light
dI = absorption + scattering

Attenuation of incident light in biological samples

$$I(x) = I_0 \cdot e^{-\mu_t x} = I_0 \cdot e^{-(\mu_a + \mu_s)x}$$

μ_t: attenuation coefficient
μ_a: absorption coefficient
μ_s: scattering coefficient

x: light path length

Spectroscopy

- Measure wavelength dependent light intensity <u>after</u> interaction of light with sample
- Wavelength is related to energy levels of molecules ($E=h\nu \Rightarrow \nu$ is frequency of light, h is Planck's constant 6.63×10^{-34} J·s)
- Can be used to identify types of molecules and quantitatively measure amount (concentration)
- Many different techniques: e.x. absorption, fluorescence, Raman scattering, diffuse scattering

Absorption spectroscopy

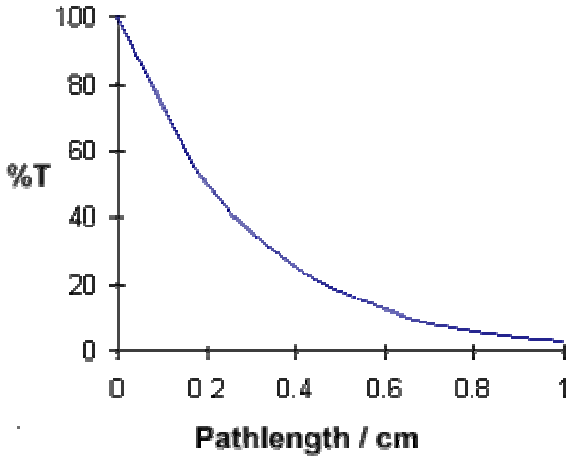
If $\mu_a >> \mu_s$, attenuation is due to absorption only (ex. in a solution)

Beer-Lambert's law

$$I(\lambda) = I_0(\lambda) \cdot e^{-\mu_a(\lambda)L} = I_0(\lambda) \cdot e^{-\varepsilon'(\lambda)CL}$$

where C is the concentration of the absorbing compound in the solution;
L is the path length of incident light in sample (cm)

Light intensity decays exponentially due to absorption



Note: in biological tissues, the absorption coefficient μ_a is used

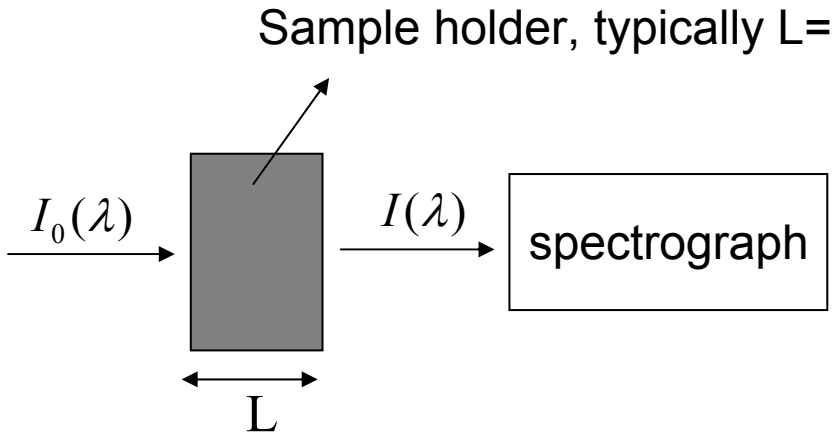
$$I(\lambda) = I_0(\lambda) \cdot e^{-\mu_a(\lambda)L}$$

Absorption spectroscopy

For molecules dissolved in solution

$$I(\lambda) = I_0(\lambda) \cdot e^{-\varepsilon'(\lambda)CL}$$

The wavelength dependent absorption of the molecule, $\varepsilon(\lambda)$, can be measured



The absorbance is defined as (also called optical density)

$$A(\lambda) = \log\left(\frac{I_0(\lambda)}{I(\lambda)}\right) = \varepsilon(\lambda)CL$$

where $\varepsilon(\lambda)$ is the molar extinction coefficient ($M^{-1}cm^{-1}$) of the compound

Absorption spectroscopy

In an atom or a molecule, electrons exist in certain “orbitals”, which have distinct energy levels

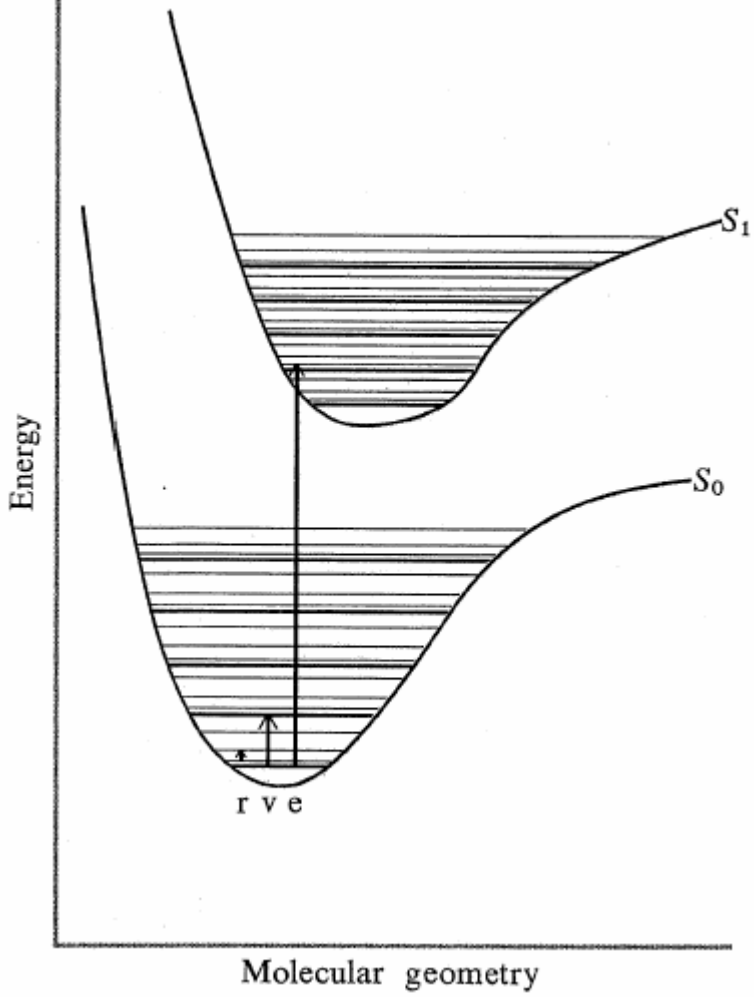
Chemical bonds in a molecule can also vibrate or rotate in certain preferred modes

S: Electronic energy levels
v: vibrational energy levels
r: rotational energy levels

Energy difference corresponds to wavelength of light

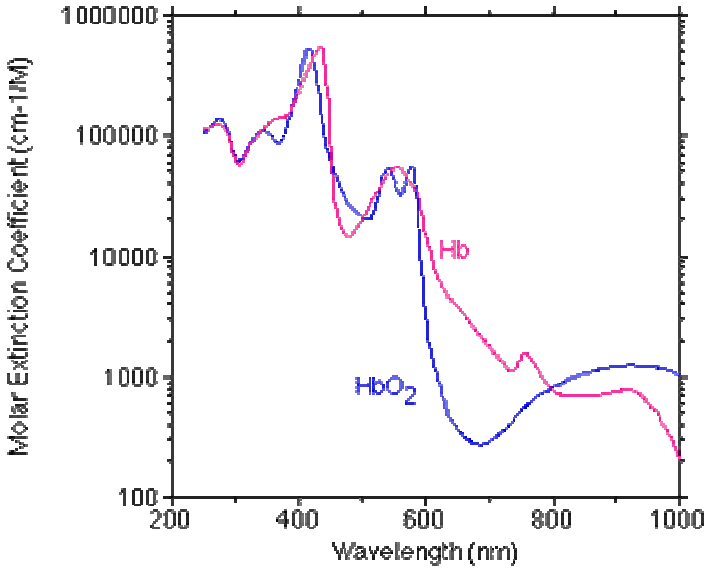
$$E_f - E_i = h\nu = hc/\lambda$$

Molecular energy levels



Absorption spectroscopy – example

Hemoglobin absorption spectra



Note the difference between oxygen carrying and non-oxygen carrying hemoglobin

Fluorescence

Absorption of incident light

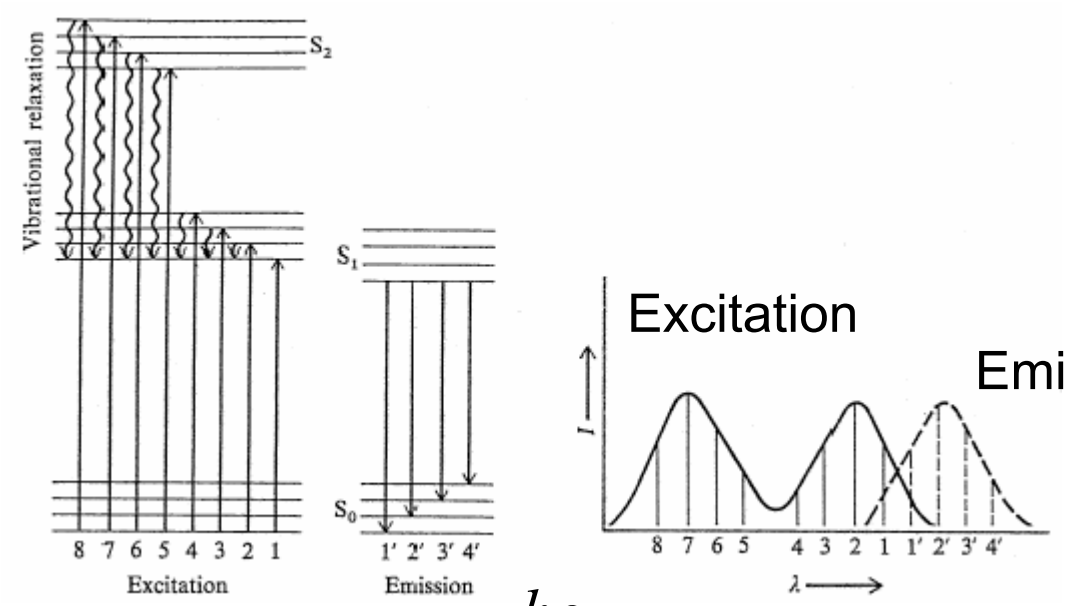
- Electrons are excited to higher energy level

Emission of fluorescence

- Some of the electrons returning to the ground energy level emit light (the probability of emission is called quantum yield)

First excited electronic energy level

Ground (unexcited) electronic energy level



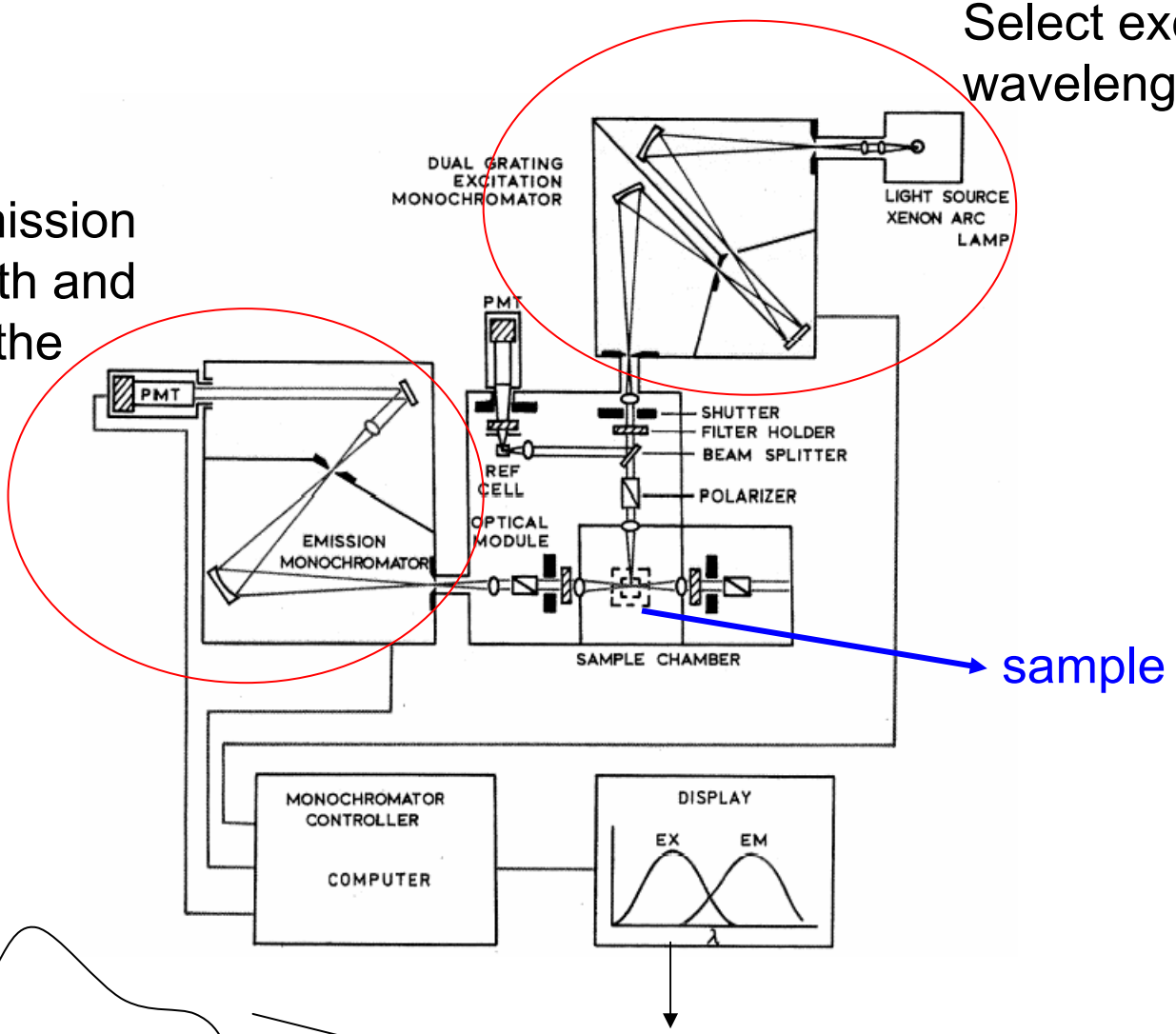
$$E_{high} - E_{low} = h\nu = \frac{hc}{\lambda}$$

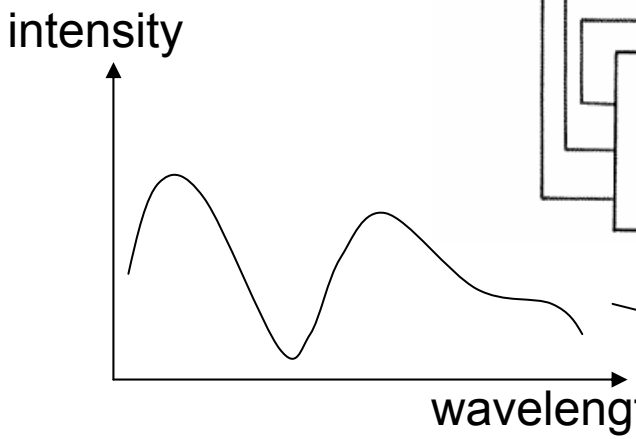
$\lambda_{Em} > \lambda_{Ex}$

Fluorescence spectroscopy instrumentation

Select excitation wavelength

Select emission wavelength and measure the intensity

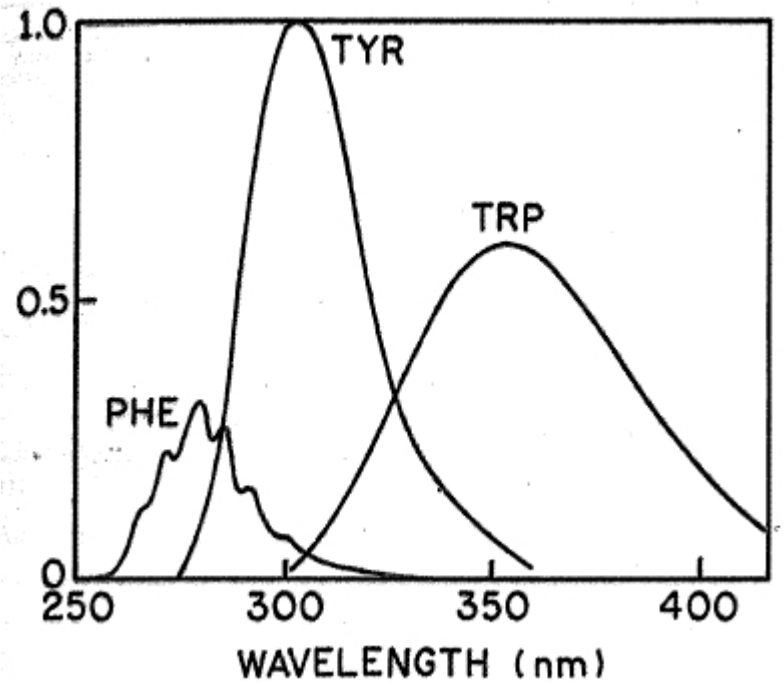




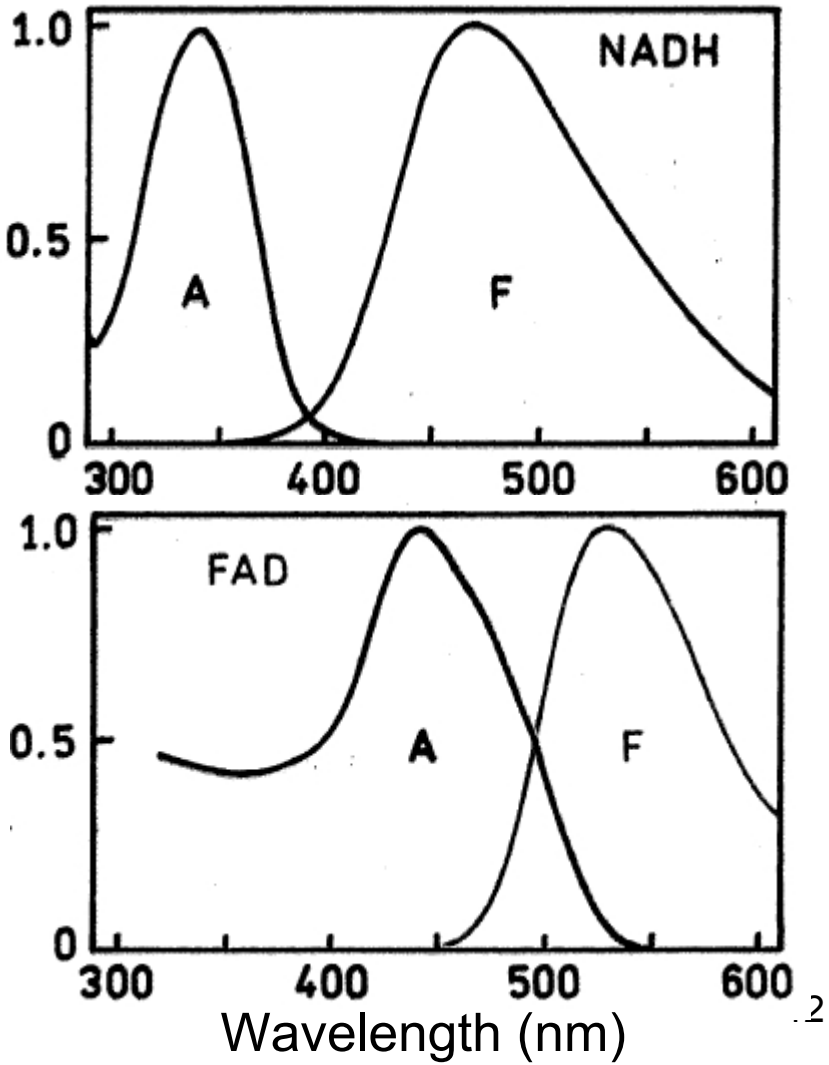
Plot measured intensity vs. wavelength

Fluorescence spectra of biomolecules

Emission spectra of 3 amino acids



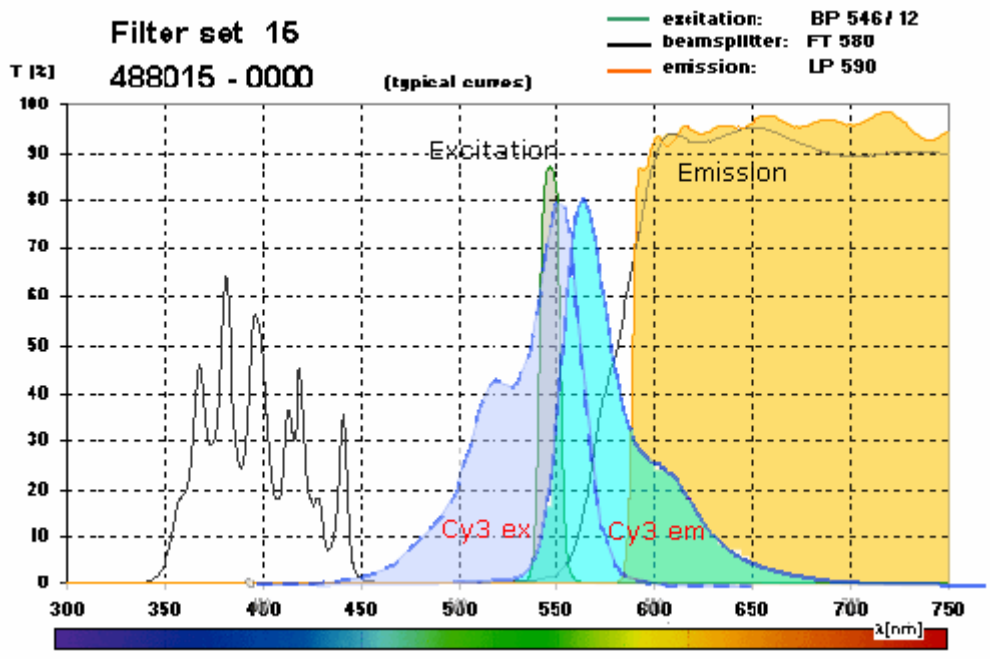
Fluorescence spectra of enzyme cofactors



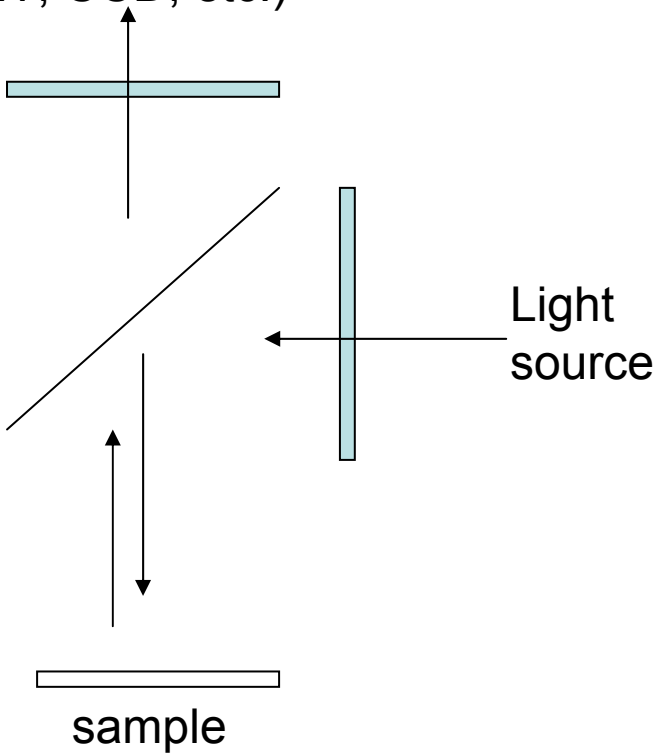
Fluorescence detection

If the absorption and emission characteristics (such as peak wavelengths) are known, optical filters can be used to select proper excitation wavelength and detect intensity of fluorescence emission

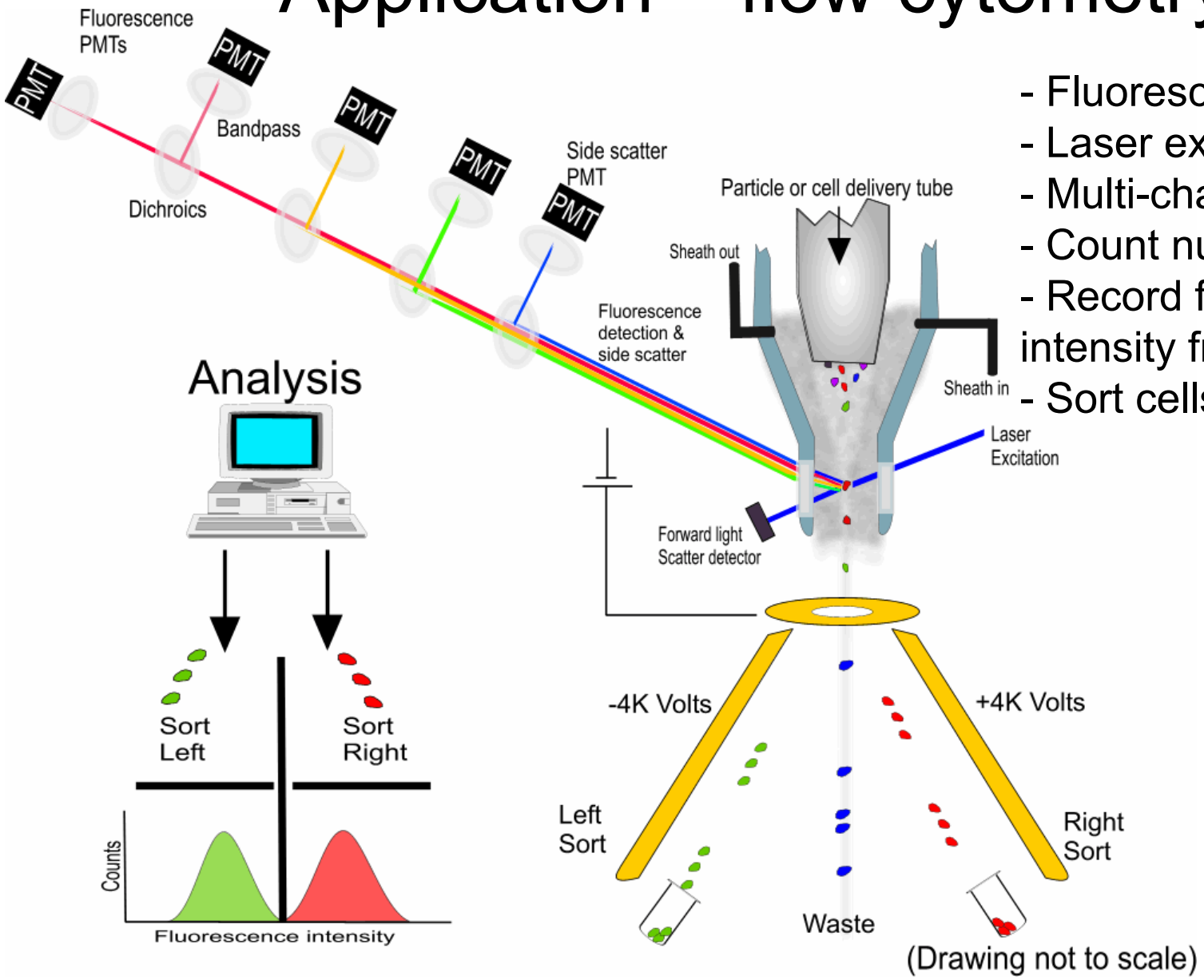
Example: Cy3



Detector (photodiode, PMT, CCD, etc.)



Application – flow cytometry

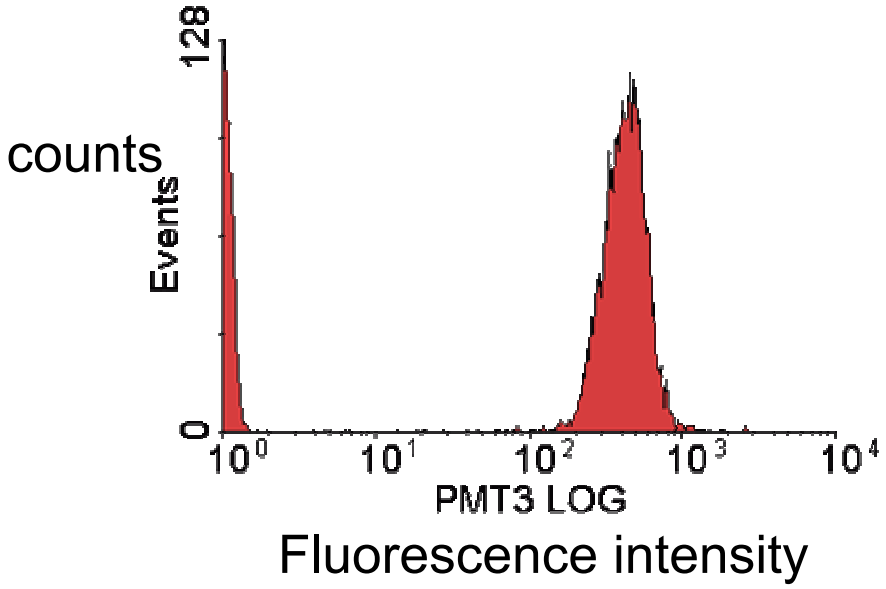


- Fluorescence labeling
- Laser excitation
- Multi-channel detection
- Count number of cells
- Record fluorescence intensity from each cell
- Sort cells

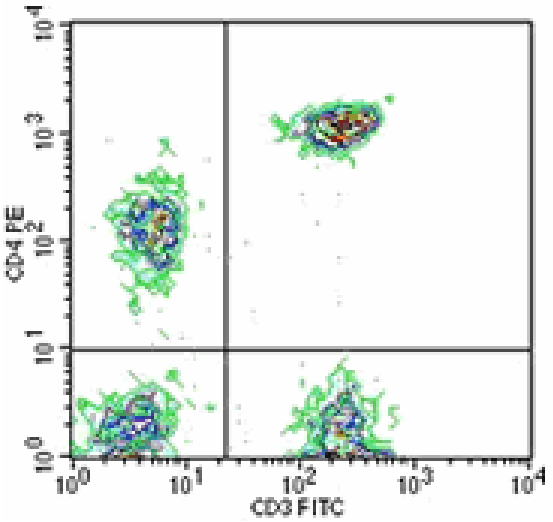
Dr. J. Paul Robinson, Purdue University Cytometry Laboratories

Flow cytometry data

Histogram of single fluorescence intensity from individual cells



Scatter plot of fluorescence intensities of 2 labels (each binding to a specific target)

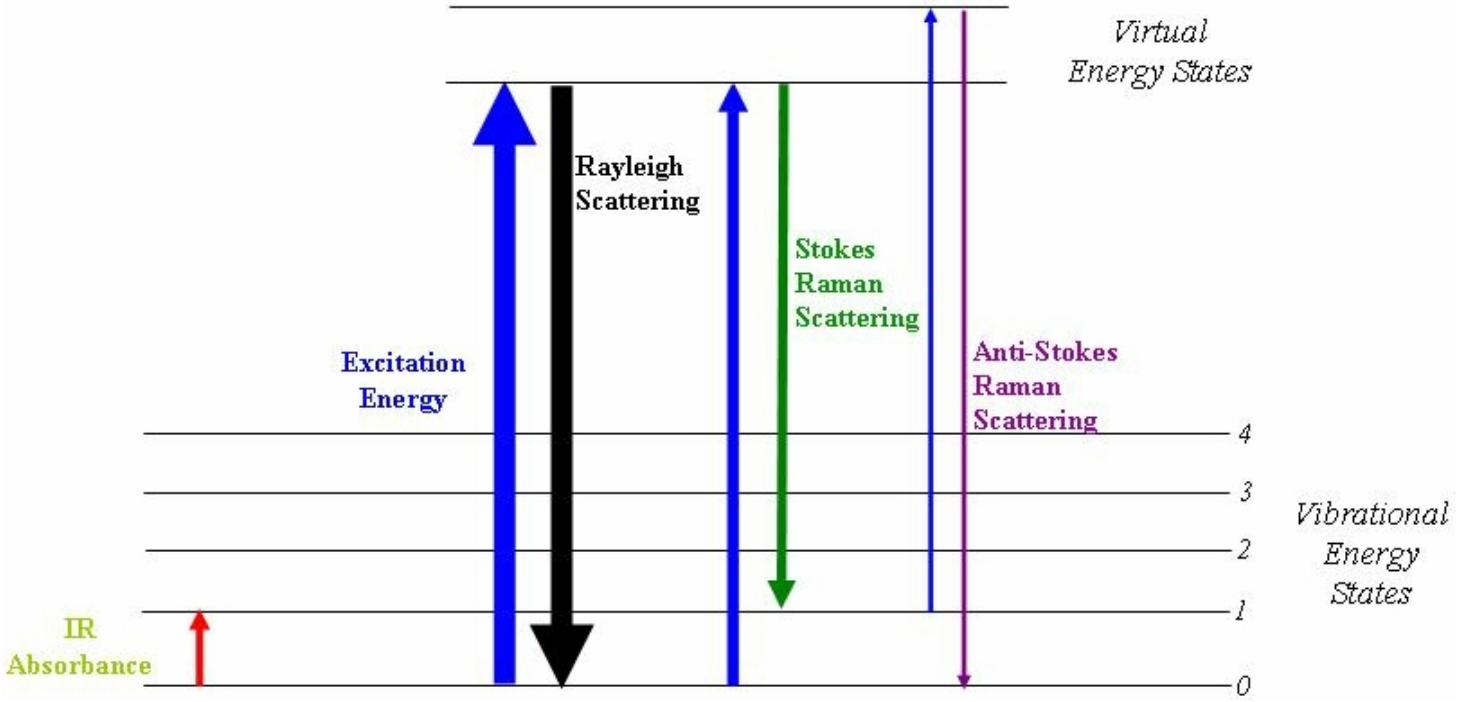


Raman scattering

Scattering is redirection of light due to interaction between light and matter

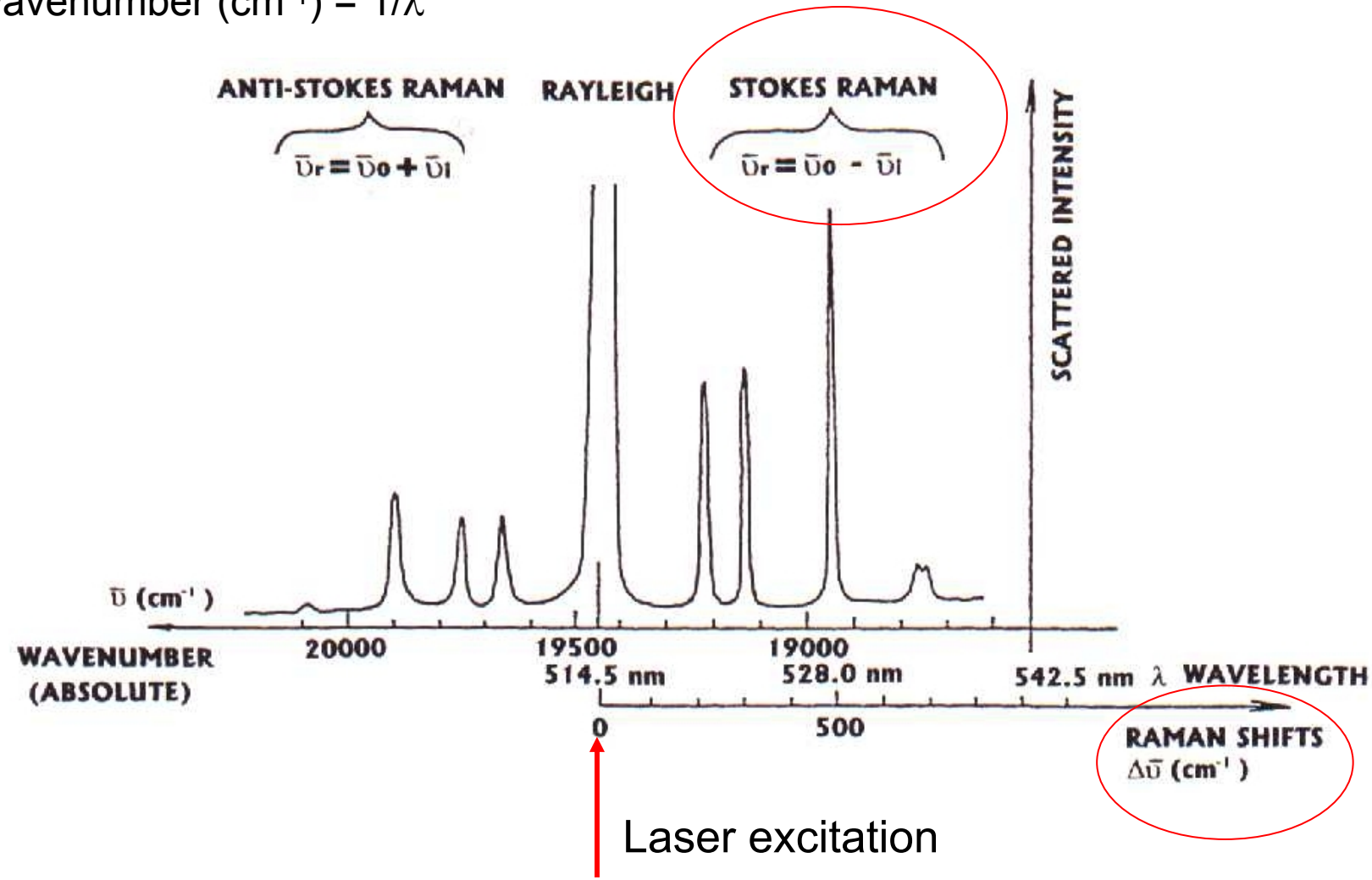
In Raman scattering (inelastic scattering), energy transfer occurs and the difference in photon energy between the scattered and incident light is related to the structure of the molecule

Stokes Raman scattering is much more likely than anti-Stokes since at room temperature most electrons are in the lowest vibrational state



Raman spectroscopy

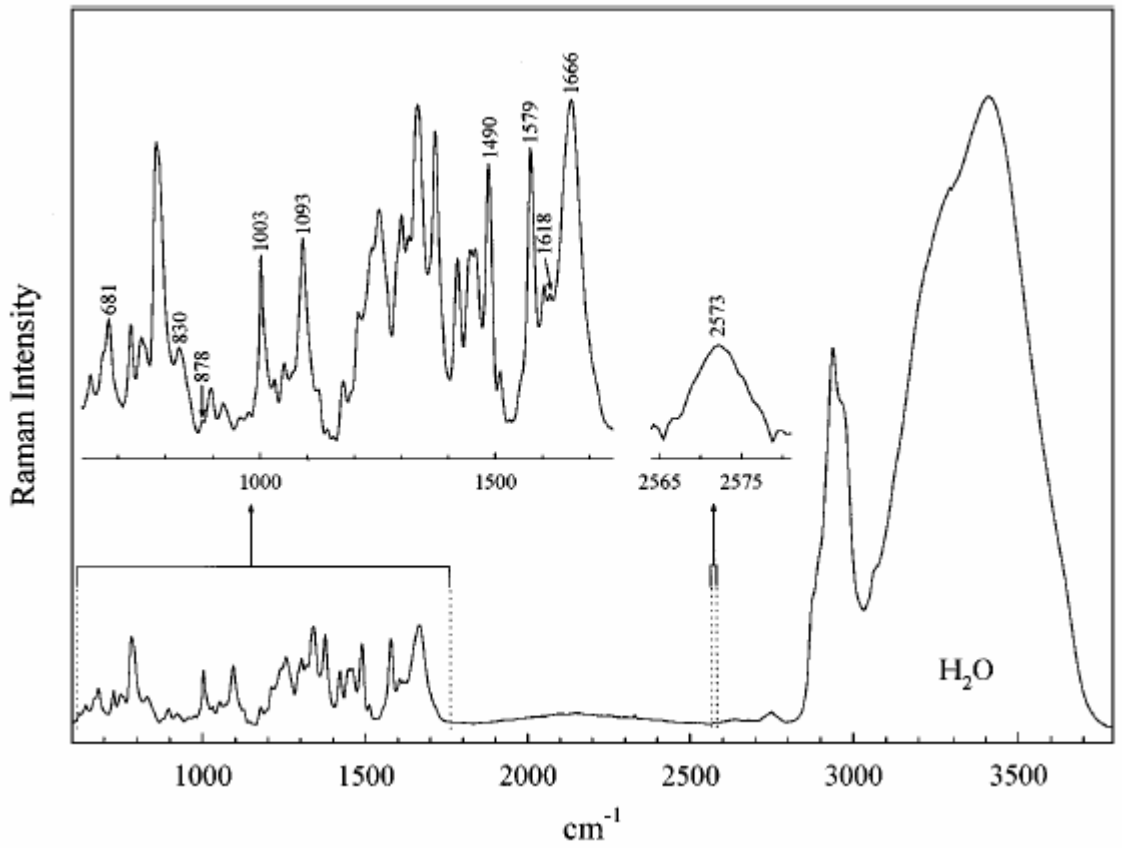
The <u>difference in energy of light</u> (called Raman shift) is expressed in wavenumber (cm^{-1}) = $1/\lambda$



Raman spectroscopy

Since there are many possible vibrational modes in a molecule, Raman scattering often has many spectral peaks, which enable identification of molecules via matching with spectral databases

Example: Raman spectrum of P22 virus

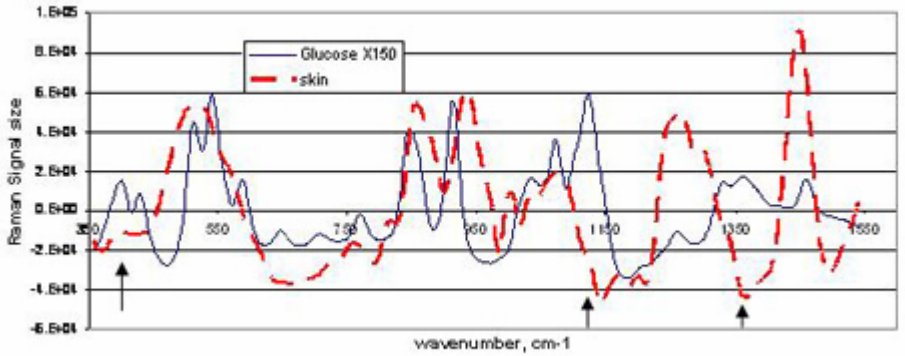


Thomas, *Annual Rev. Biophys. Biomol. Struct.* **28**, 1-27 (1999)

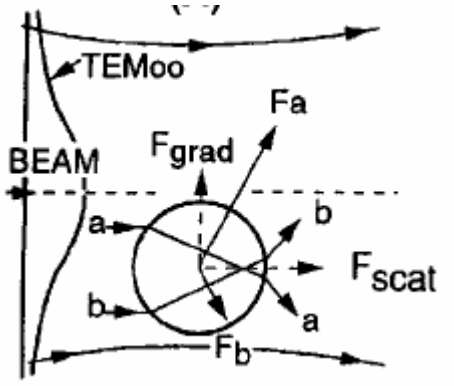
Raman spectroscopy

Handheld Raman systems

Potential application with huge market: non-invasive detection of blood glucose for patients with diabetes

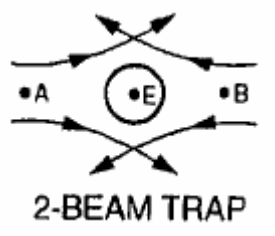


Optical trap – introduction



A small spherical particle under laser radiation

1. Refraction of light at interfaces
2. Conservation of momentum results in Fa, Fb
3. Fa > Fb due to the intensity profile of the laser beam
4. The net force is pushing the sphere forward and toward the center of the beam

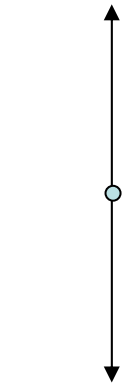


The sphere can be trapped using two laser beams propagation in opposite directions

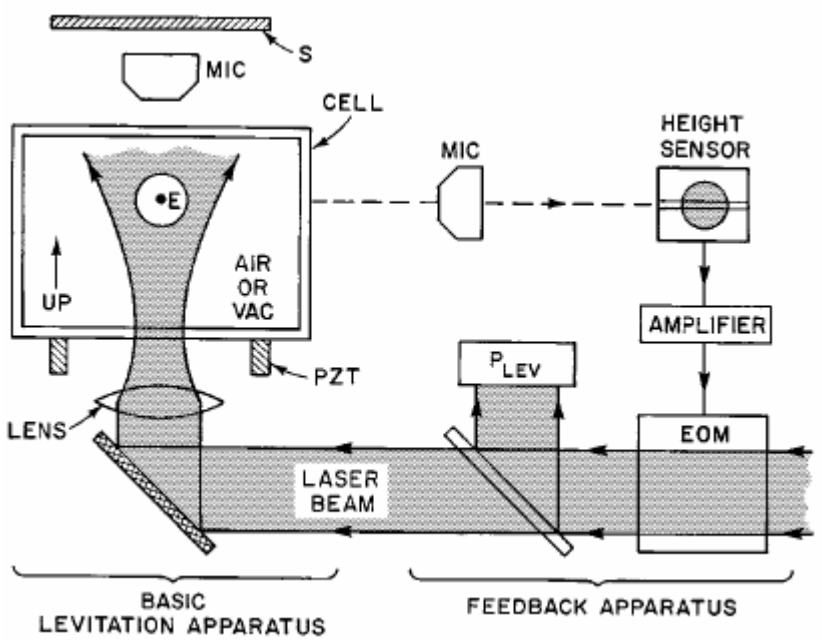
IEEE J Selected topics in quantum electronics **6**, 841-856 (2000)

Optical levitation trap

scattering force



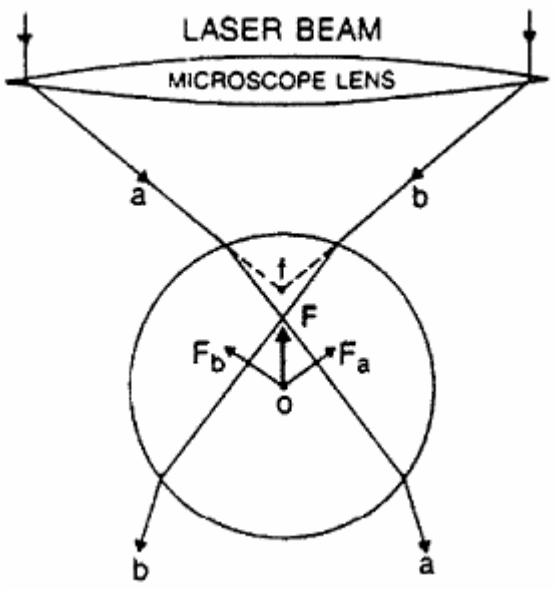
gravity

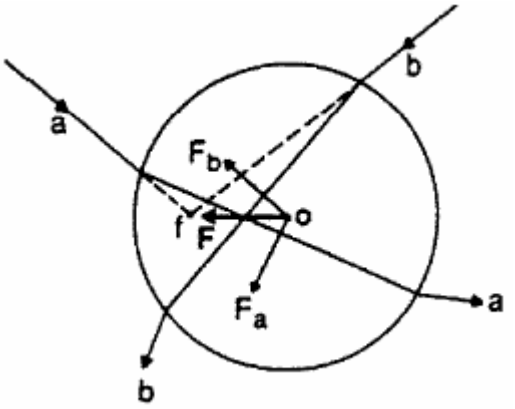


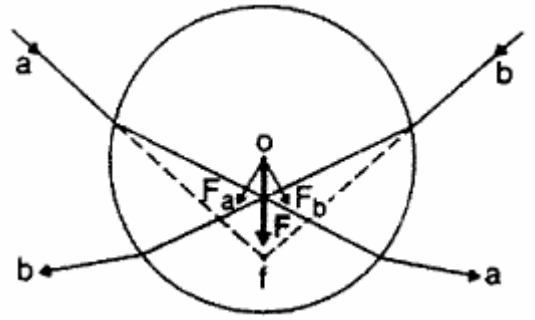
Micrometer particles can be “lifted” and moved around

Optical tweezers

Ray-optics illustrating the stability of optical tweezers





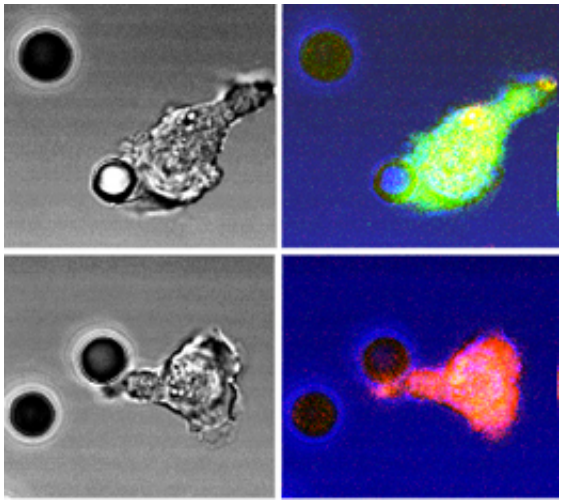


Any displacement of the particle generates force that pulls the particle back to the central position

Typically near IR lasers such as Nd:YAG 1064nm are used for less photon energy and less damage to biological samples

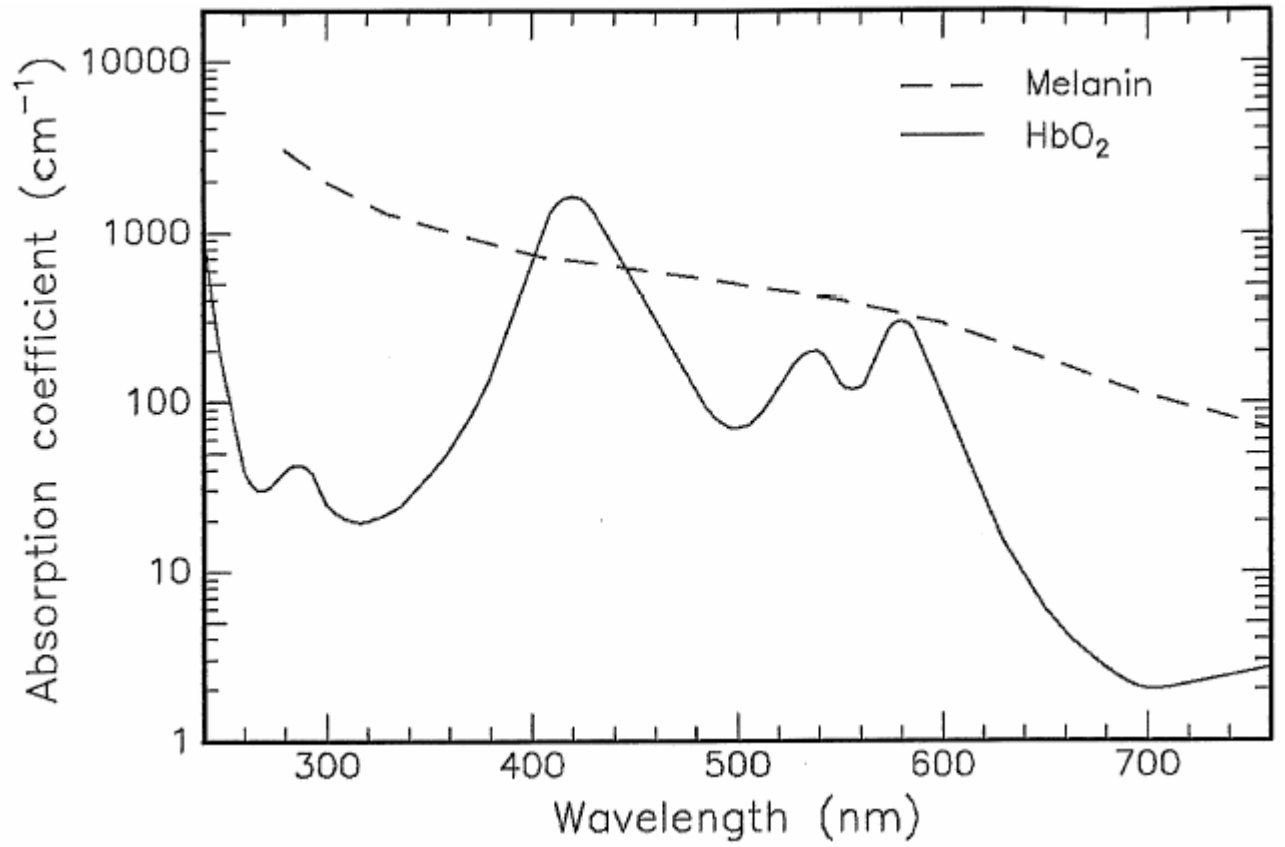
Optical tweezers applications

- Moving cells and organelles
- Study of molecular motors such as interactions of kinesins and dyneins with microtubules and actin filaments
- Study of mechanical properties of microtubules, actin filaments, and DNA bio-polymers
- Isolation and separation of bacteria and other biopolymers *in vivo*



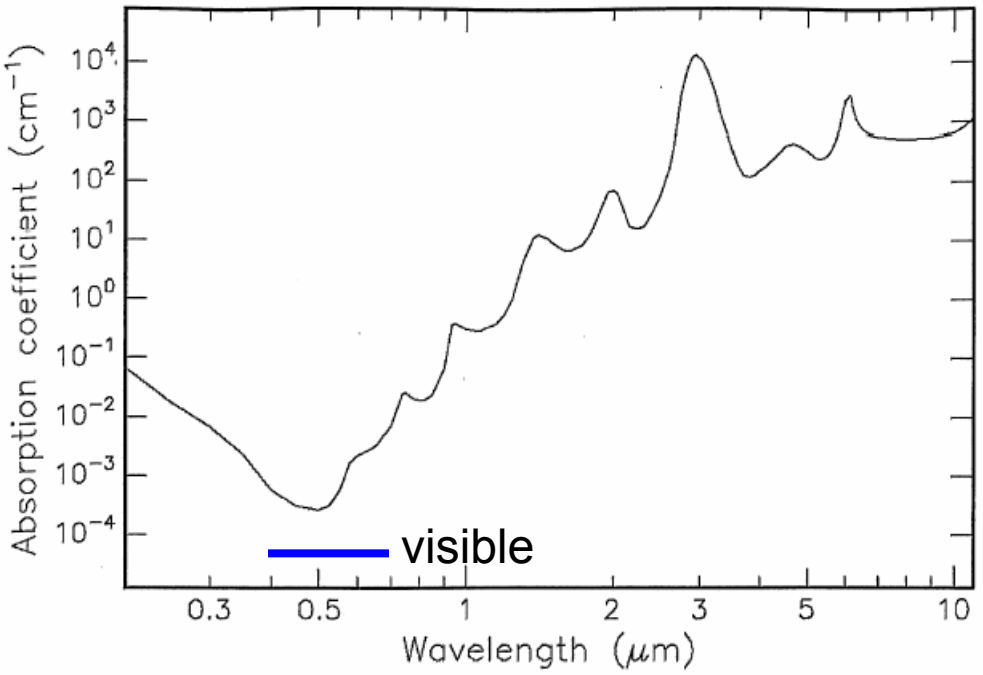
Use of Laser for treating diseases

Interaction between laser and tissue: Absorption spectra of main absorbers: melanin in skin and hemoglobin (HbO_2) in blood



Laser-tissue interaction (cont.)

Absorption of water



The broad absorption band in the IR of water is used to transfer optical energy into kinetic energy (heat) in tissue

In the visible range, light absorption by water is low, which can be used for *in vivo* interrogation of tissue

In UV, light scattering in tissue increases significantly ($\propto 1/\lambda^4$) $\Rightarrow$ less penetration depth in tissue

Laser-tissue interaction (cont.)

Thermal effects of laser radiation (raised temperature in tissue)

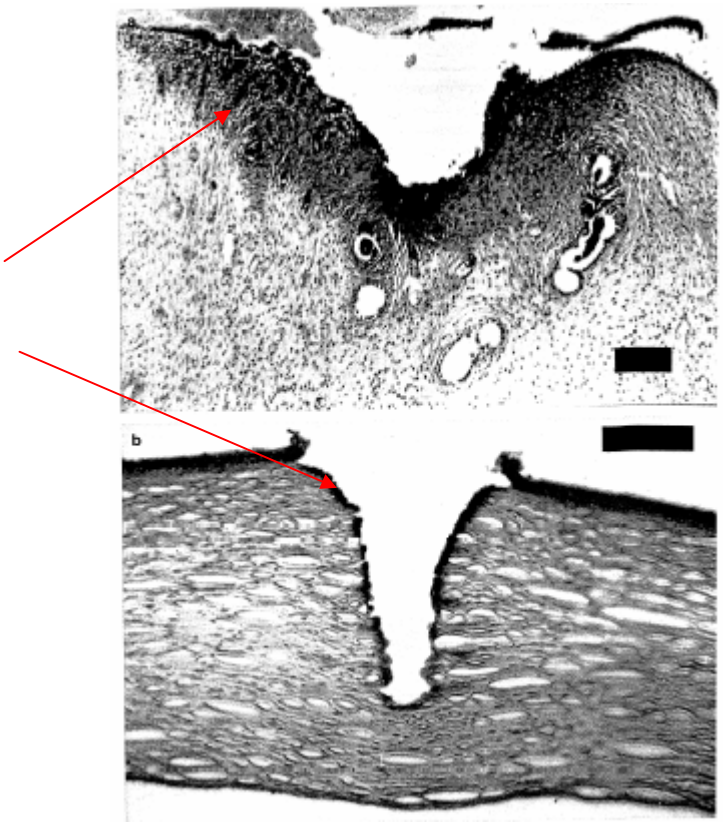
Temperature	Biological effect
37°C	Normal
45°C	Hyperthermia
50°C	Reduction in enzyme activity, cell immobility
60°C	Denaturation of proteins and collagen, coagulation
80°C	Permeabilization of membranes
100°C	Vaporization, thermal decomposition (ablation)
> 100°C	Carbonization
> 300°C	Melting

Note that protein and collagen denature at around 60°C

Heating tissue at 60°C can lead to cell necrosis $\Rightarrow$ can be used to kill cancer cells

Tissue coagulation

Uterine tissue of rat (top) and human cornea (bottom) coagulated with lasers (>60°C); H&E stained histologic slides



Laser ablation

With enough photon energy, laser can be used as a scalpel. Even better, tissue can be removed very precisely without any appearance of thermal damage

Dissociation energy of some chemical bonds present in biological tissues

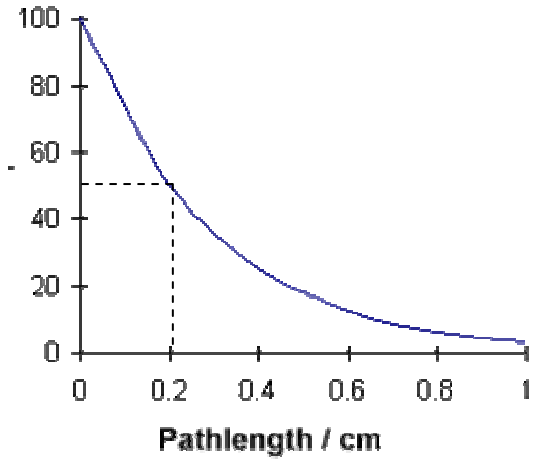
Type of bond	Dissociation energy (eV)
C=O	7.1
C=C	6.4
O–H	4.8
N–H	4.1
C–O	3.6
C–C	3.6
S–H	3.5
C–N	3.0
C–S	2.7

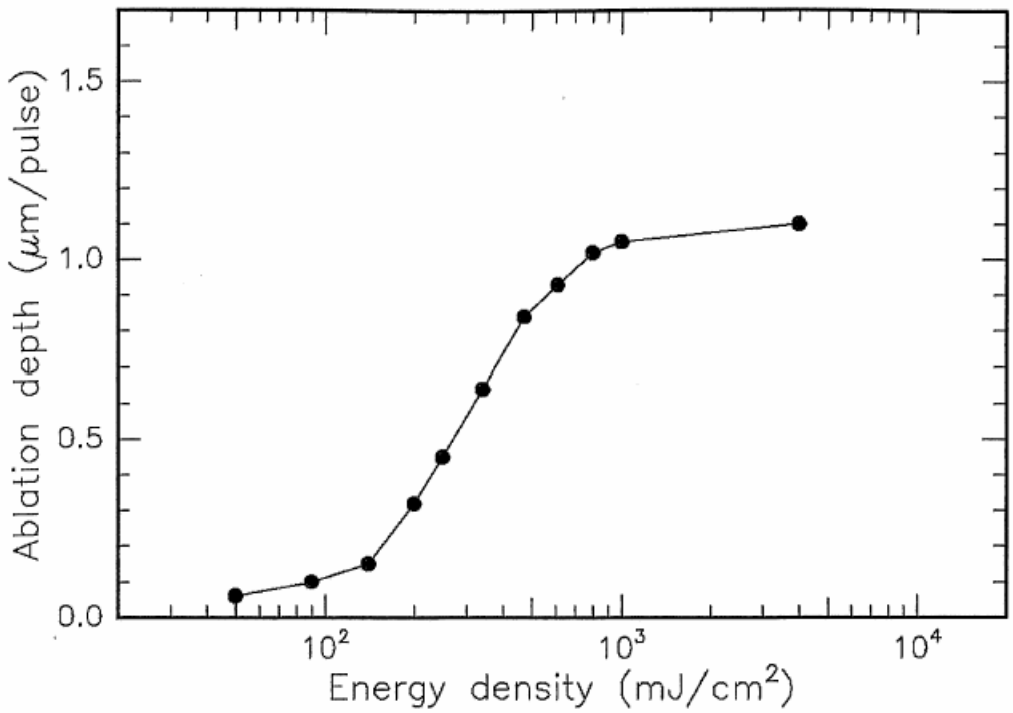
1eV = 1.6×10^{-19} J

The required energy corresponds to UV radiation and can be supplied by excimer lasers such as ArF @193nm and KrF @248nm, or 4th harmonic of a Nd:YLF @263nm

Laser ablation (cont.)

The absorption of laser energy in tissue follows Beer-Lambert's law
There exists a threshold at which the absorbed energy is high enough to cause decomposition of tissue





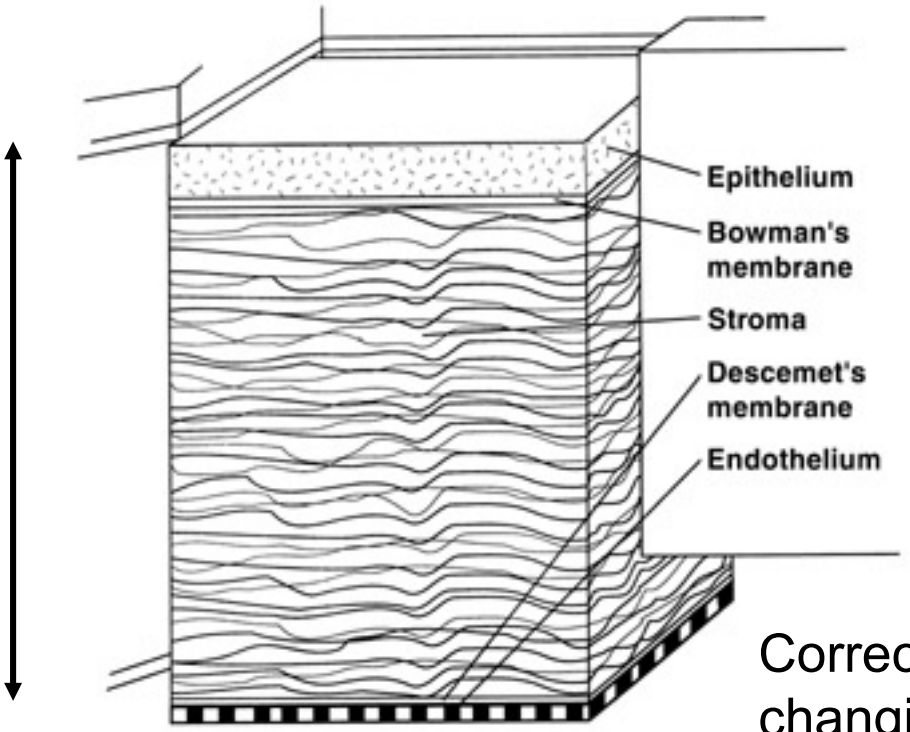
Ablation of rabbit cornea obtained with an ArF laser (14ns pulse duration)

Typical pulse duration: 10-100 ns

Typical power density: 10^7-10^{10} W/cm^2

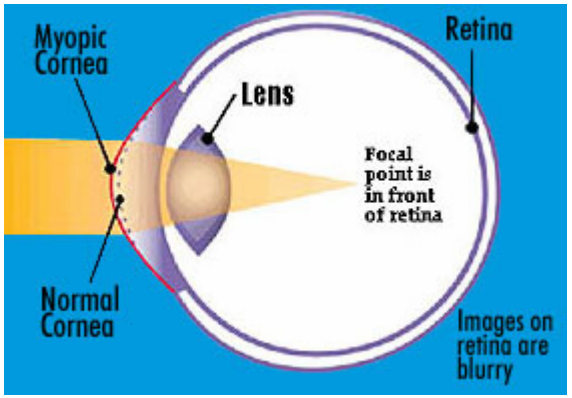
Application – Laser surgery for myopia

Structure of cornea



500-700 μm

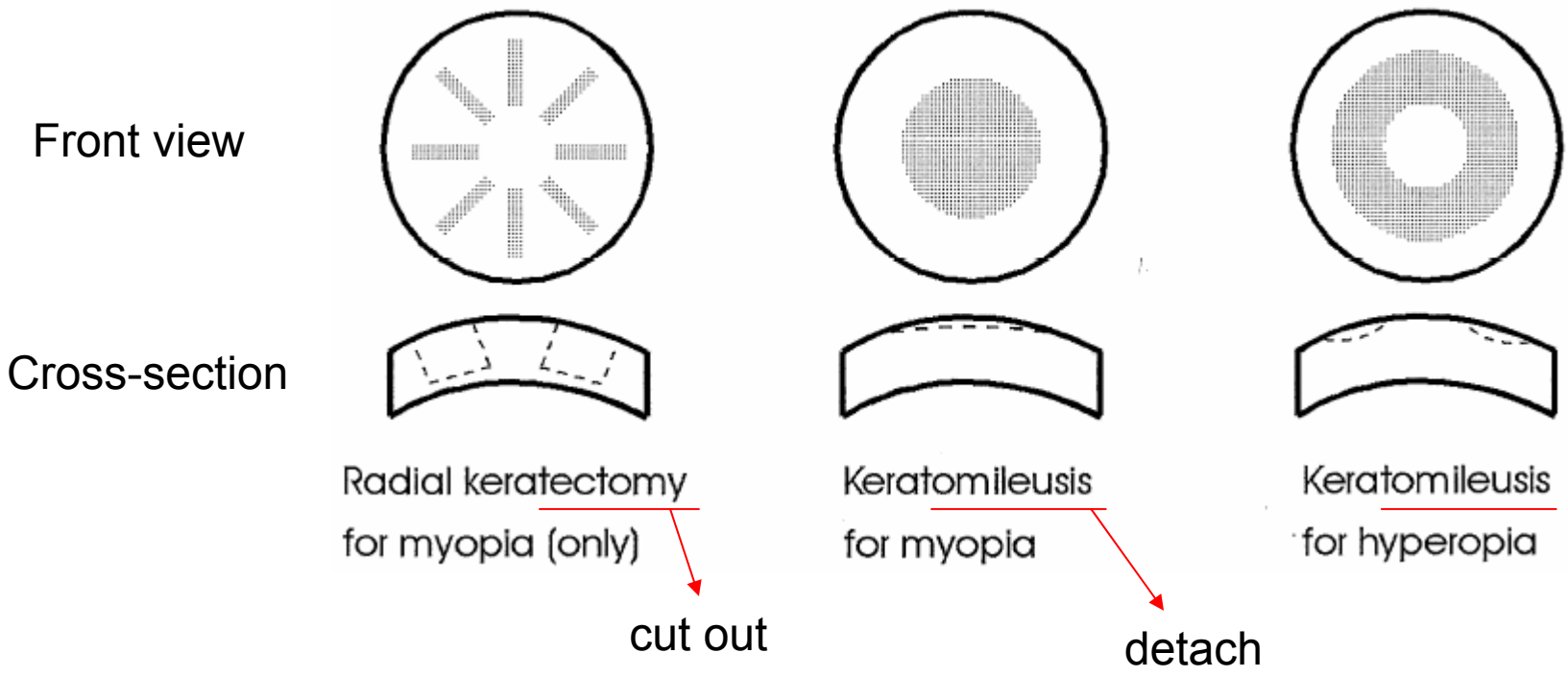
Focus of light is in front of the retina in nearsightedness (myopia)



Correction of the focus is achieved by changing the curvature of the cornea

Eye laser surgery (cont.)

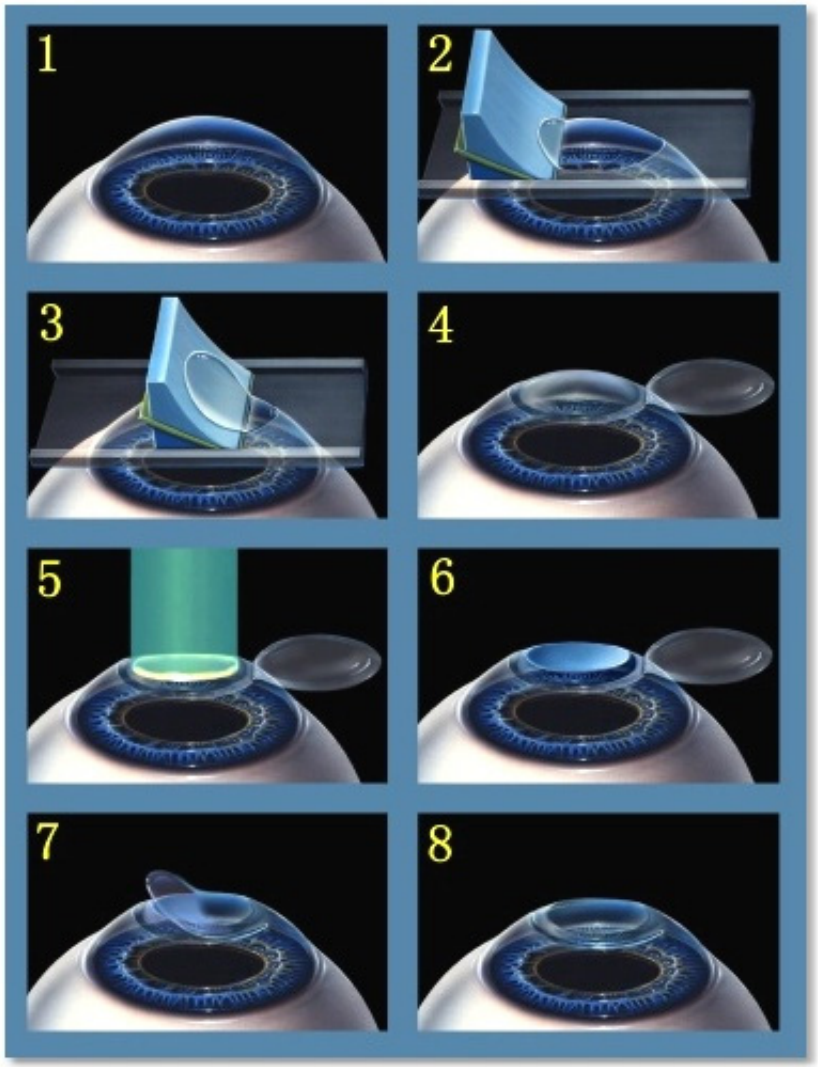
Schemes for correction of myopia and hyperopia



Tensile force leads to flattening of the surface

Directly “shape” the cornea

LASIK



Laser Assisted In-Situ Keratomileusis

A “flap” of cornea is formed

Change in curvature of cornea is achieved by removing stroma instead of the epithelium $\Rightarrow$ faster recovery and less chance of side effects such as opacification of the cornea

Photodynamic therapy (PDT)

Photochemical interaction: light can induce chemical effects within macromolecules or tissues

1. A chromophore compound that absorbs light efficiently is called a photosensitizer
2. Photosensitizer is excited by laser irradiation
3. Decay of this excited photosensitizer (to its original state) results in various reactions that eventually cause oxidation of essential cell structures

Photodynamic therapy (PDT)

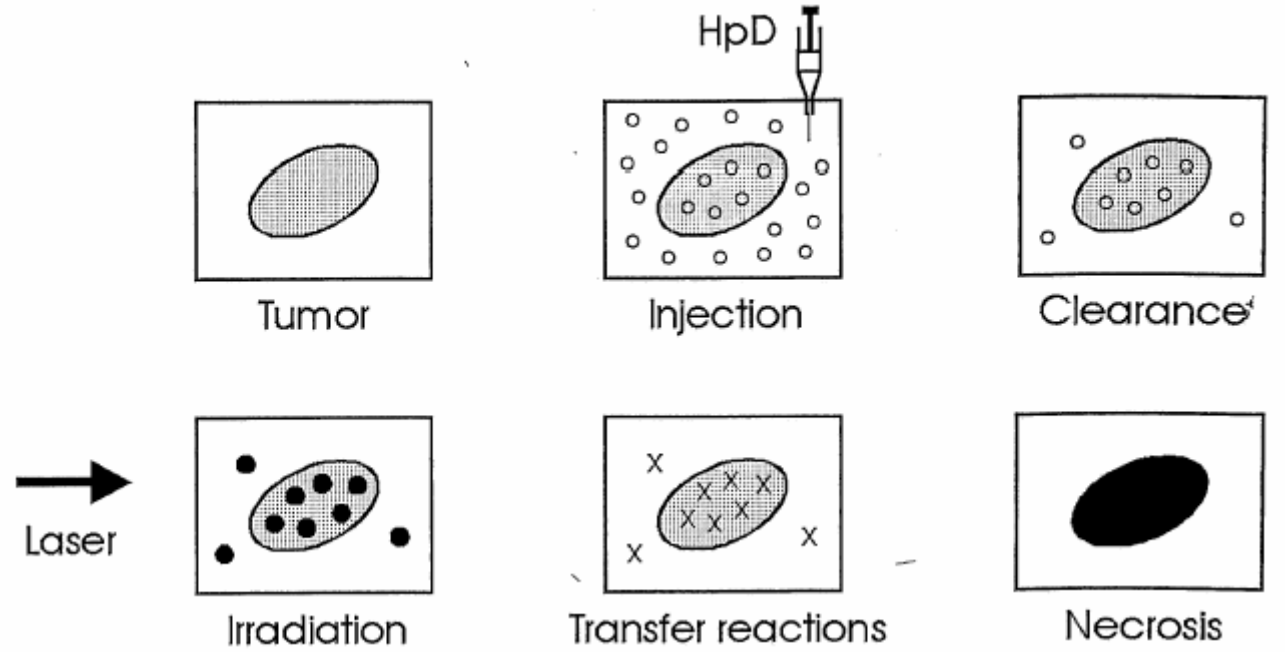
In order to have therapeutic effects for cancer, the photosensitizer molecules need to be at higher concentrations in tumor cells than in normal cells

Example: hematoporphyrin (HpD)

- In healthy tissue: most HpD is cleared from tissue after 48-72 hours
- In tumor cells: concentration does not decrease much in 3 days

PDT example

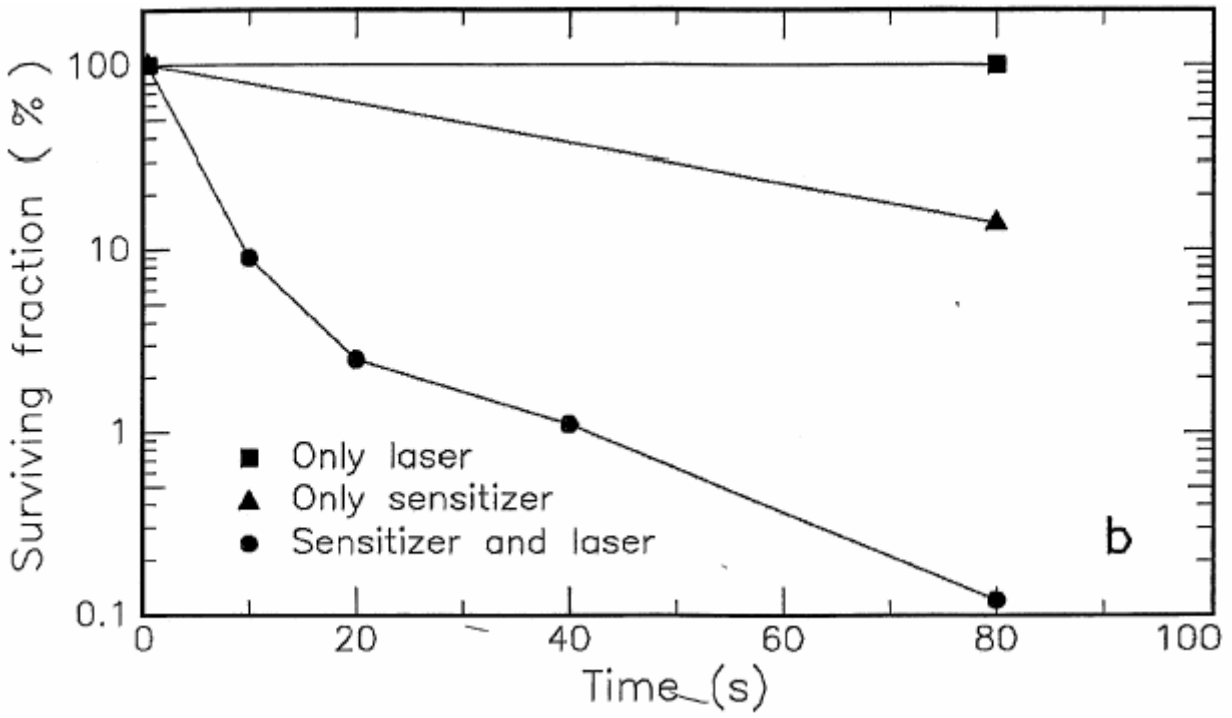
2.5-5mg per kg body weight
IV injection



Laser irradiation:
3-7 days after injection of HpD
He-Ne laser 633nm

Photodynamic therapy (PDT)

Example: cell viability in PDT using HpD



Note: photosensitizers are usually organic dyes that also emit fluorescence, which can be used to measure the concentration of the photosensitizer and diagnose cancer $\Rightarrow$ simultaneous diagnosis and therapy of tumors!

References

- Laser-Tissue Interactions: Fundamentals and Applications, by Markolf H. Niemz
  - Ch3: interaction mechanisms
  - Ch4: Medical Applications of Lasers, 4.1 Laers in Ophthalmology