Contemporary optical spectral methods for diagnostics in medicine

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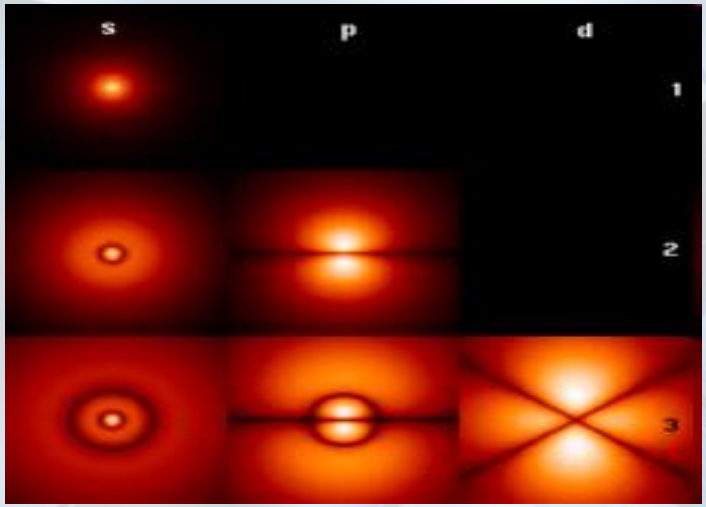
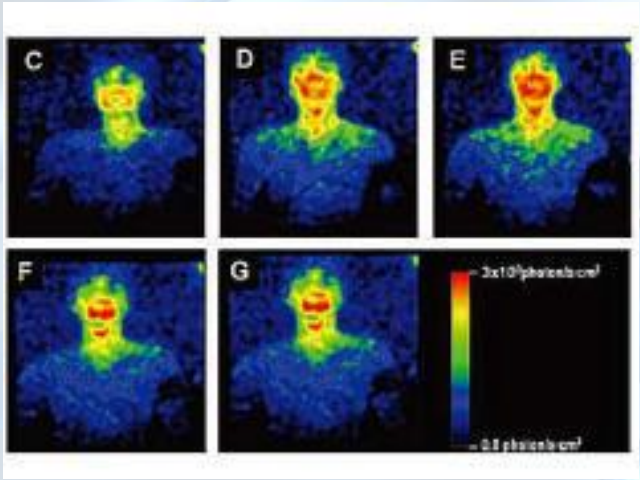
Ocean of EM waves



Atoms and molecules emit

All space object emit

All living creatures emit



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Spectroscopic observation

- **Spectroscopy** – a science exploring the interaction of electromagnetic radiation with the matter.
- This interaction involves processes of radiation, absorption, refraction, scattering, and polarization of electromagnetic radiation.
- Spectral measurements detect, separate and register energy changes in nuclei, atoms, ions, molecules producing resonant peaks, arranged in spectra.
- **Optical spectroscopy** – measurements are in the region of optical range of the electromagnetic irradiation, UV+visible+IR spectral regions, i.e. from ~100 nm up to 100 mocrons.

Electromagnetic radiation :

Gamma-raysи:

10^{-12} – 3.10^{-15} m

X-rays:

10^{-9} – 10^{-12} m

Ultraviolet rays:

4.10^{-7} – 10^{-9} m (1nm)

Visible light:

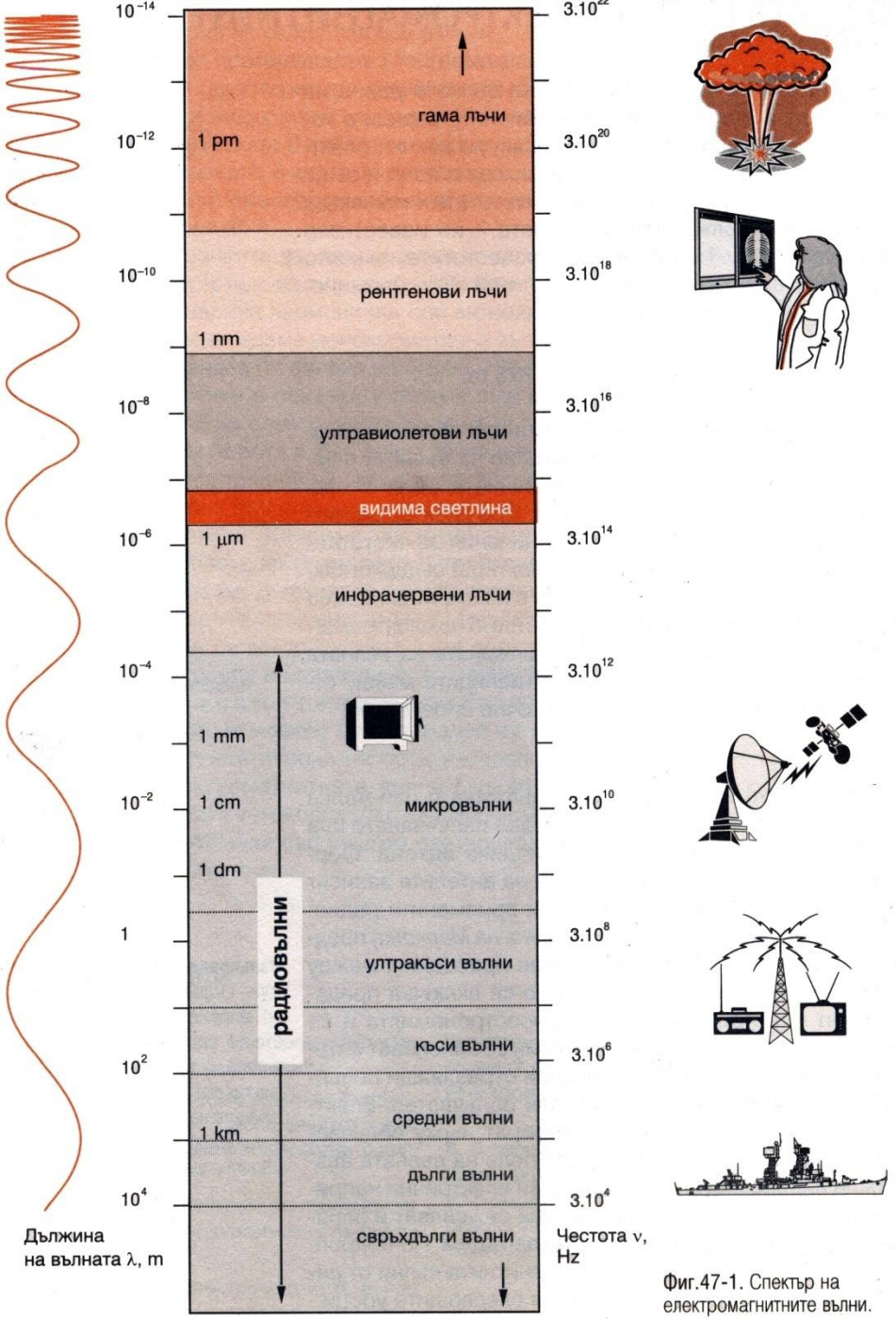
8.10^{-7} – 4.10^{-7}m

Infrared rays:

3.10^{-3} – 8.10^{-7} m

Radiowaves:

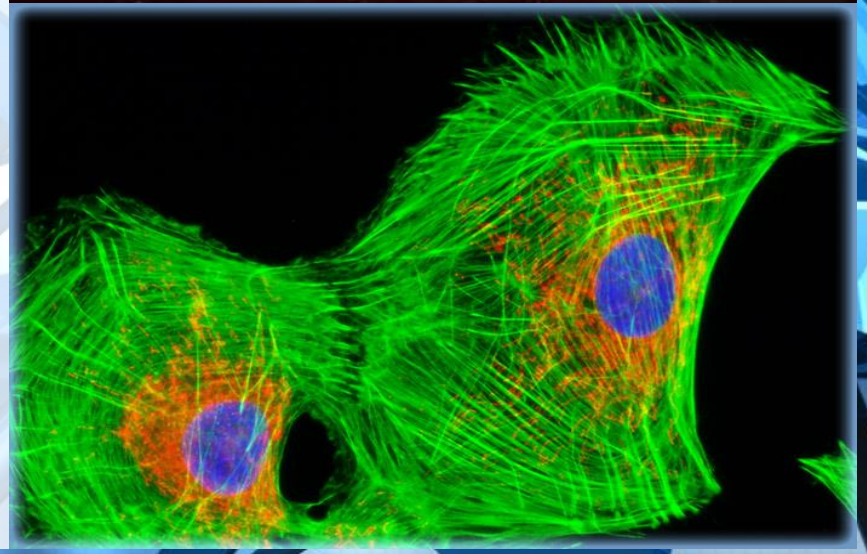
3.10^{5} – 3.10^{-3}m



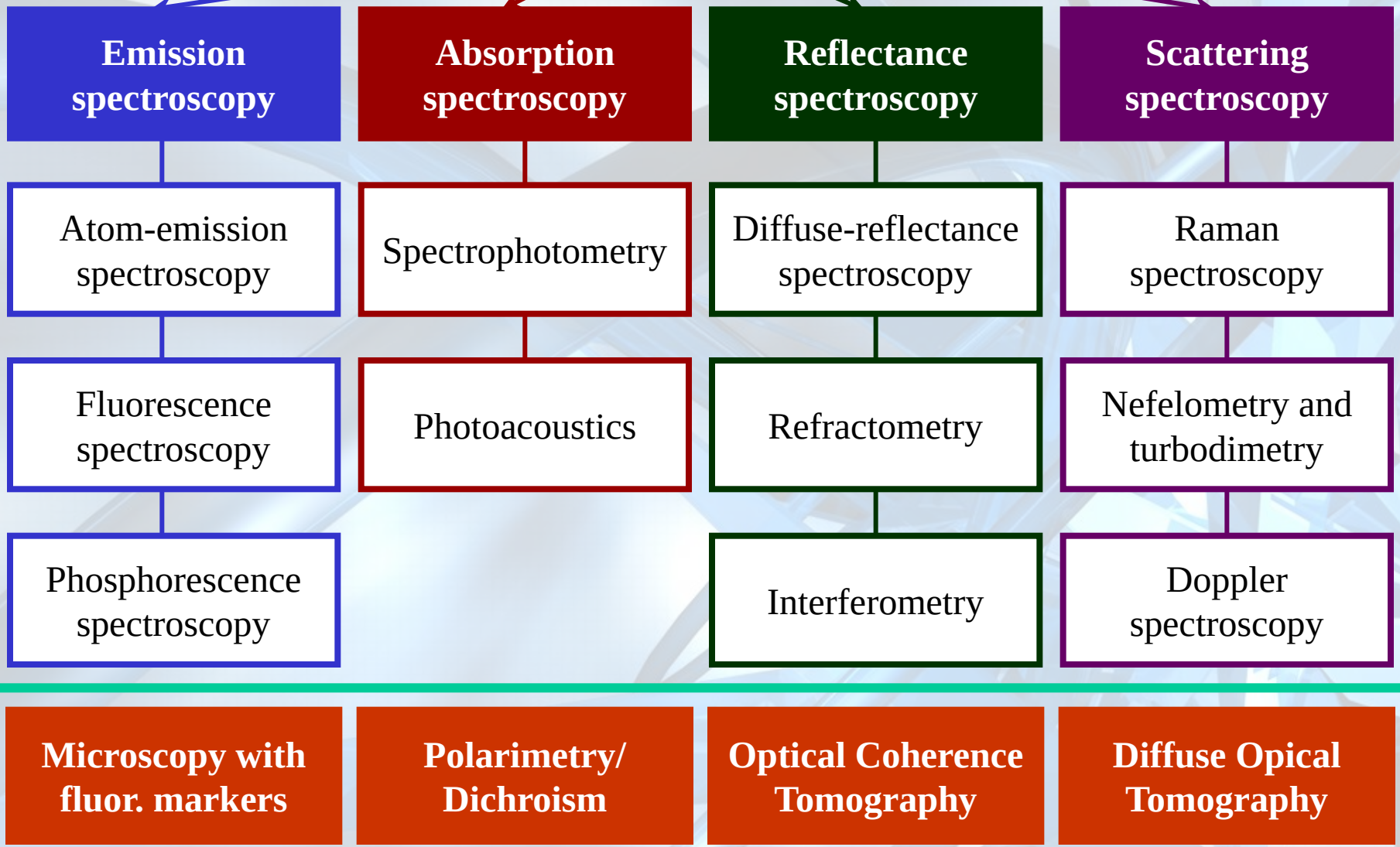
Фиг.47-1. Спектър на електромагнитните вълни.

Advantages of spectroscopic measurements

- Data can be obtained remotely from the object of investigation - spectral studies can determine the composition, temperature and even the movement of distant and inaccessible objects;
- Qualitative and quantitative analysis - it is possible to determine the quantity of the element by the intensity of the emitted spectral lines;
- Sensitivity - Spectral analysis can detect the presence of very small amounts (10^{-11} - 10^{-12} g) of a substance, even single molecules and atoms;
- Spectroscopy is widely used in astronomy, metallurgy, chemical analysis, geology (for analysis of ores and minerals), forensic science, control of the composition of harmful substances in the natural environment (eg emissions to air, soil and water), in medicine and others.



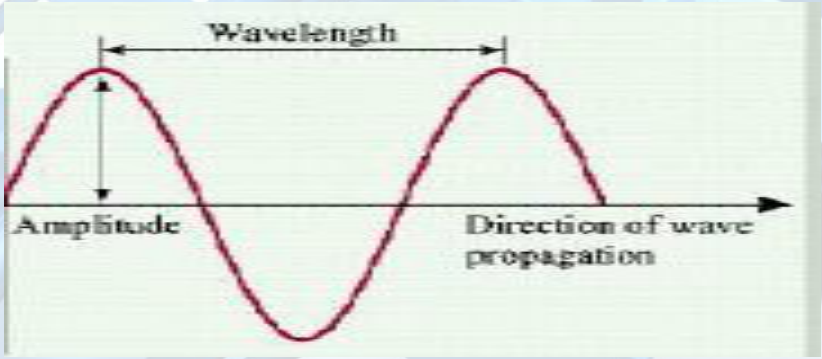
Spectral methods in medicine



Spectroscopic quantities

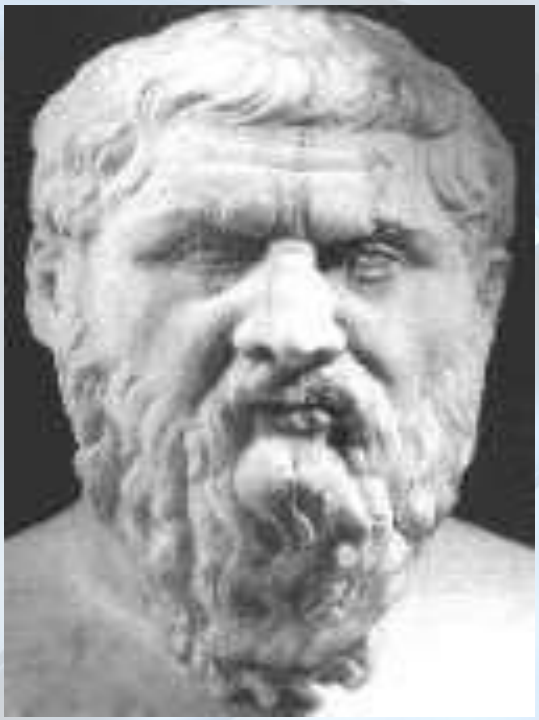
- **Period T [s] -** the smallest interval of time after which all properties characterizing the wave are repeated
- **Wavelength λ [*m*] -** the distance between two equivalent points of the harmonic wave.
- **Frequency ν [*Hz*] -** the number of waves that pass through a given point for one second.
- **Wave number ν [cm^{-1}] -** number of whole wavelengths contained in 1 cm in the direction of the distribution of EMW
- **Energy E[*J*]** – in spectroscopy very often is used unit eV . 1 eV = $1{,}60201.10^{-19}$ J
- **Intensity *I*[*cd*]** – light flow crossing per unit time through unit area

$$E = h.\nu = \frac{h.c}{\lambda} = \frac{h.(c_0/n)}{\lambda}$$

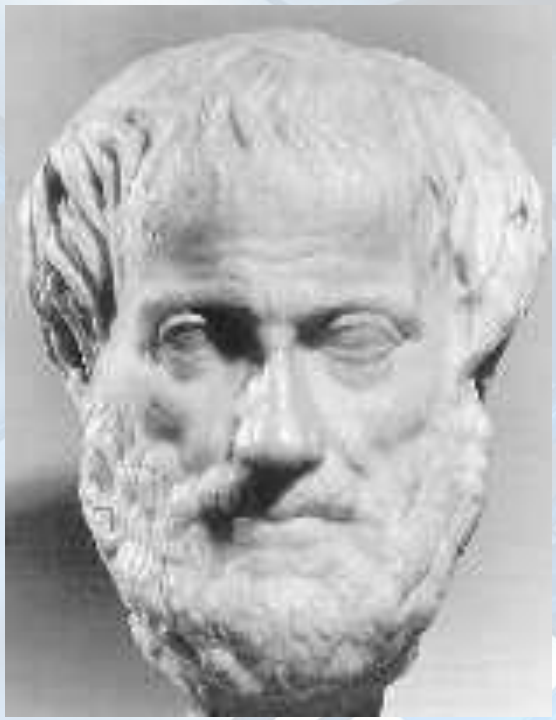


A bit of history...

- All starting from ancient Greeks...

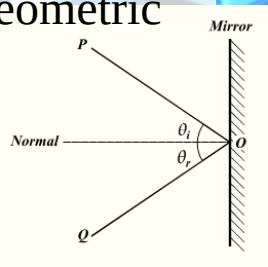


Plato (427-347 BC)
He believed that the eyes emitted a "fire" that people used to see the objects



Aristotle (384-322 BC)
The light emits out of a source, and one sees the reflection of it from the object

Euclid (~ 325-265 BC)
Founder of the geometric optics



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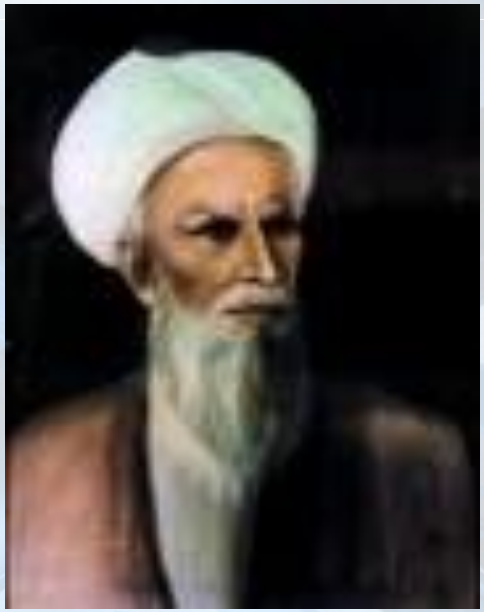
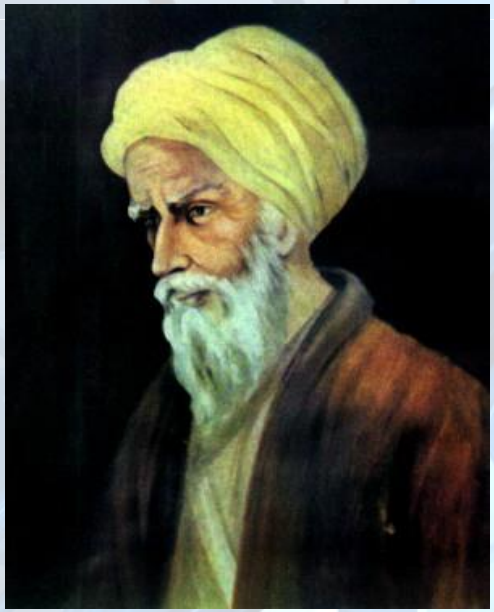
A bit more history...

Galen of Pergamas (Claudius Gelenus: 130-201)
Describes anatomical elements of the eye
Identifies the lens as the main tool of the eye
But ... he believes in the radiation theory



Abu Bakr Muhammad ibn Zekaria al-Razi
Mohammad ibn Zakariya al-Razi (864-930), Persia
First describes the contraction of the pupil in response to light irritation
Supports the Democritus theory of atoms



Abu Ali al-Hassan ibn al-Haysam Abu Ali al-Hasan ibn al-Haytham (965-1040), Kair (Katib-al-Manazir - 7 volumes, translation at 1270)
Describes the anatomy of the eye
Creates vision theory – used up to 17 century for teaching in Europe universities
Describes primary and secondary light. sources, geom. distribute. of light, colors, describes laws of refraction and reflection of light

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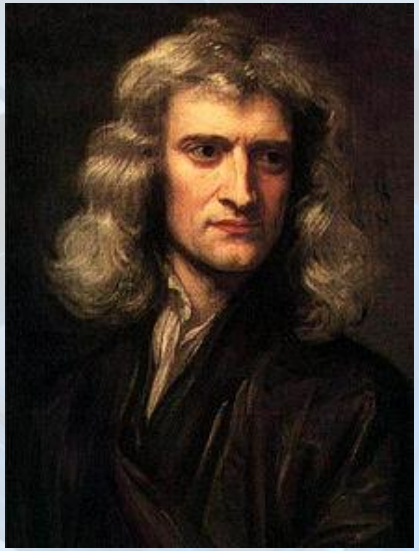
Formation of the contemporary view for light nature

Johan Kepler (1571-1630)
Creates a theory of image formation on the retina (eye eyes)
Law of Refraction at Small Angles of Fall

Francesco Maria Grimaldi (1618-1663)
Observes and describes diffraction –in 1660 year
Before him - the corpuscular light theory was used (from Aristotel is in effect - then - the light is a wave



Sir Isaac Newton (1642-1727)
Independent colors
Different colors are excited with characteristic vibrations - the "feeling" of red is longer than the others

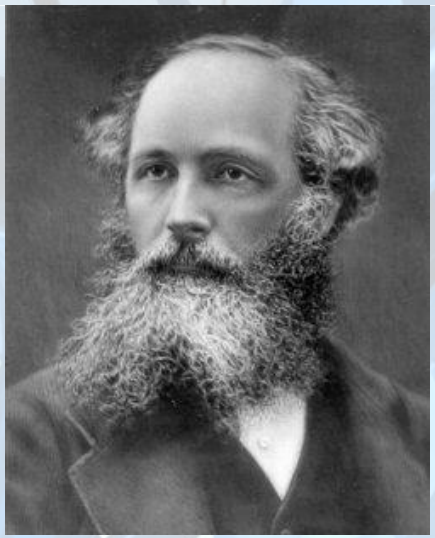
Christian Huygens (1629-1695)
Each point on the primary wave front is a source of secondary spherical waves

Formation of contemporary vision about the light nature – from 17 up to the beginning of 20 century

Thomas Young (1773-1829)
1801-1803 an experiment was carried out with the two slits - light interference was found

Augustine Fresnel (1788-1827)
1818: Creates an mathematical model for wave theory for light, and for its diffraction

Michael Faraday (1791-1865)
1845: Demonstrated the electromagnetic nature of light by altering its polarization in a strong magnetic field



James Maxwell (1831-1879)
1873: The Theory of Electromagnetic Wave Propagation.
Light - EM disturbance in the form of a wave propagating through matter

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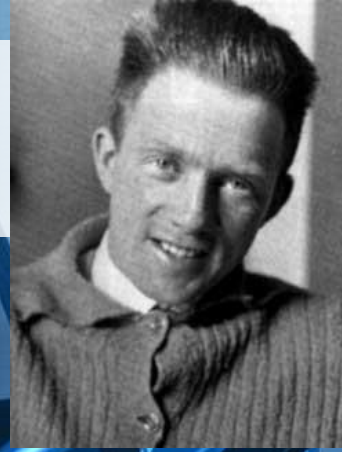
Quantum mechanics

Max Planck

Niels Bohr

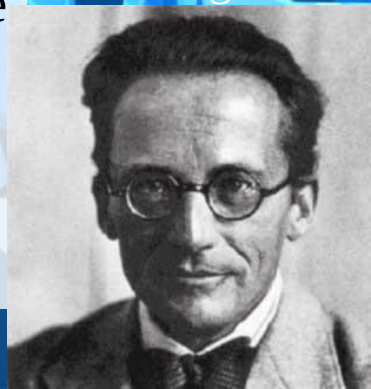
Louis de Broglie

- 1900: Planck postulates that an oscillating electric system transmits its energy to an EM field of quanta
- 1905: Einstein - photoelectric effect - light consists of quanta- photons that interact with electrons as particles
- 1900-1930 - photons, protons, electrons, neutrons - also appear and have behavior as waves and particles
- The particlewith impulse p is associated with a wavelength $p \sim h/\lambda$
- Quantum mechanics describes the way , how the light is absorbed and radiated by atoms



Werner Heisenberg

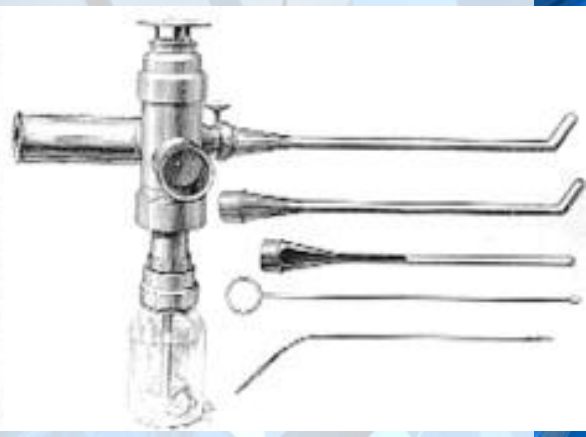
Erwin Schrödinger



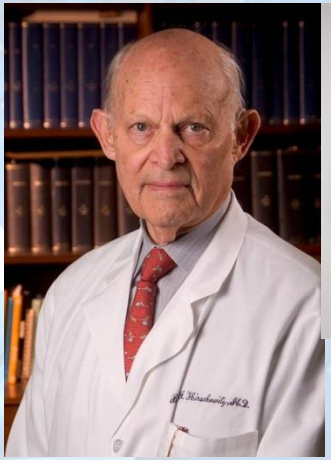
Optical methods in diagnostics - history...

Gulio Cezare Aranzio (1529-1589)
For the first time he used a light source for an endoscopic procedure - focusing sunlight through a bottle of water in the nasal cavity

Philip Bozzini (1773-1809) - "Lichtleiter“



Antoine Jean Desormeaux (1815–1894)



1957 r. – first fiberoptic endoscope

Basil Isaac Hirschowitz (29.05.25-19.01.13)

Biomedical optics – why?

- Non-invasive
- High resolution
- Opportunity for repeat meas. and / or continuous monitoring
- In vivo research (artifact reduction)
- High sensitivity / accuracy
- Low price
- No side effects
- Ability to obtain functional information
- Portable systems
- Real-time measurements
- Monitoring of therapeutic procedures
  - Precise dosimetry
  - Physiological influence

Biomedical optics – why not?

- Insufficient data on the spectral characteristics of the normal tissues and pathology
- False - positive results (due to high sensitivity)
- Low specificity
- Tissues are strongly scattering media - optical scattering - complex algorithms for data analysis
- Overlapping of fluorescence spectra of endogenous fluorophores
- Weak signals (fluorescence, Raman spectroscopy)

Basic optical tissue characteristics

- Scattering coefficient- μs [cm-1]
- Absorption coefficient- μa [cm-1]
- Anisotropy factor g [-]
- Henney-Greenstein function

$$g = \int_0^{\pi} \int_0^{2\pi} P(\cos\theta) \sin\varphi \cos\theta d\theta d\varphi$$

- Light in the tissues is propagated strongly anisotropic;
- g varies between 0,7-0,95 for biotissues (~30^{o})
- μs’ = μs (1-g)

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Light absorption in the tissues

- $dI = -\mu a\, I\, dx$
- $I = I_0\, e^{-\mu a\, x}$, x – tissues tickness
- $\mu_a = \rho\sigma_a$, σ is the absorption section, and ρ is the density of the absorbing particles, giving the law of Beer-Lambert
- $I = I_0\, exp(-\rho\sigma\, x)$
- Reciprocal value of the absorption coefficient $1/\mu a$ [mm] represents the average free way that passes a photon before being absorbed

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Henney-Greenstein function

$$p(\theta) = \frac{1}{4\pi} \frac{1 - g^2}{(1 + g^2 - 2g\cos\theta)^{3/2}}$$, such that $\int_0^{\pi} p(\theta)\, 2\pi \sin\theta\, d\theta = 1$

and $\int_0^{\pi} p(\theta) \cos\theta\, 2\pi \sin\theta\, d\theta = g$

- More standard is presented as:

$$p(\cos\theta) = \frac{1}{2} \frac{1 - g^2}{(1 + g^2 - 2g\cos\theta)^{3/2}}$$, such that $\int_{-1}^{1} p(\cos\theta)\, d(\cos\theta) = 1$

and $\int_{-1}^{1} p(\cos\theta) \cos\theta\, d(\cos\theta) = g$

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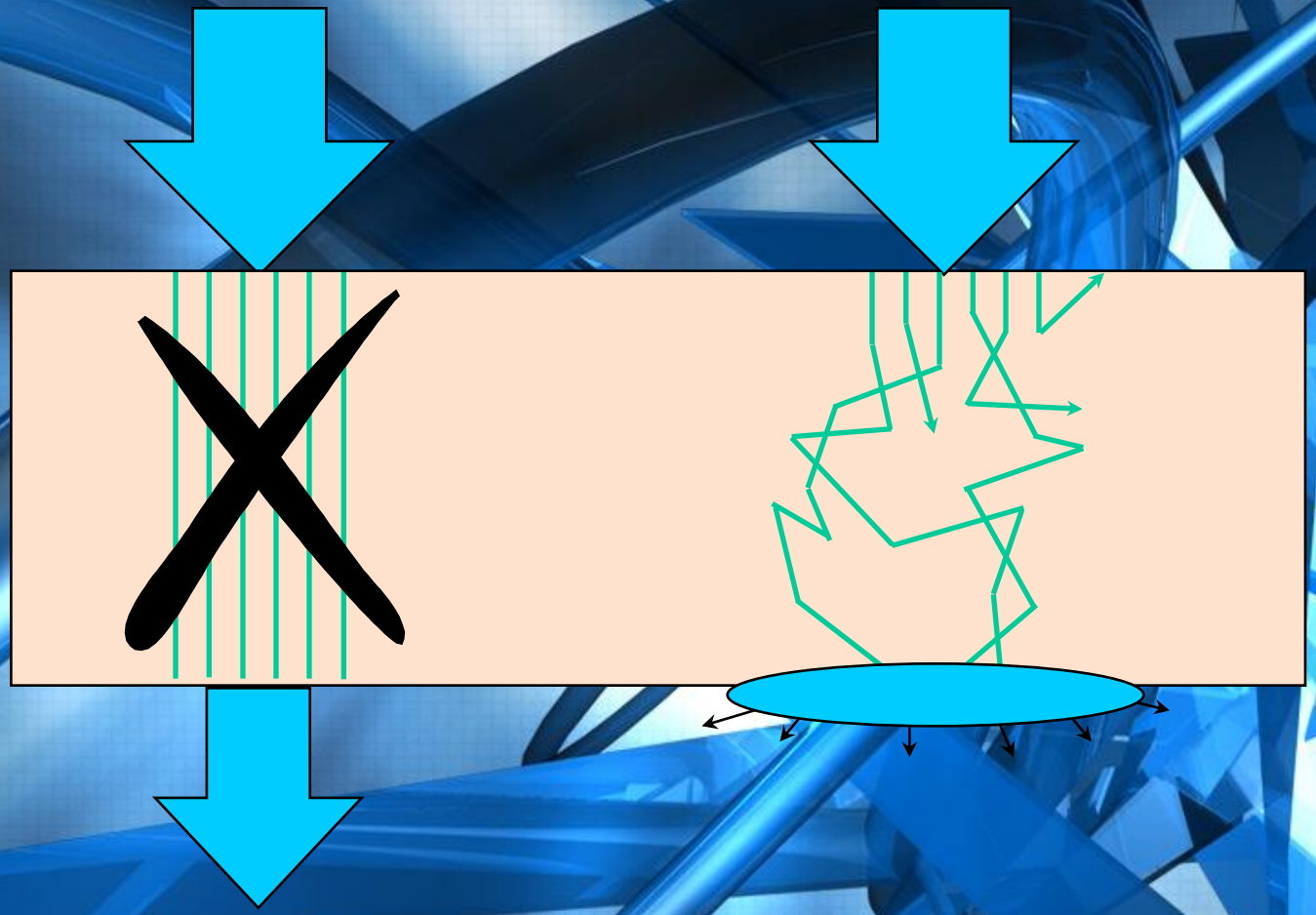
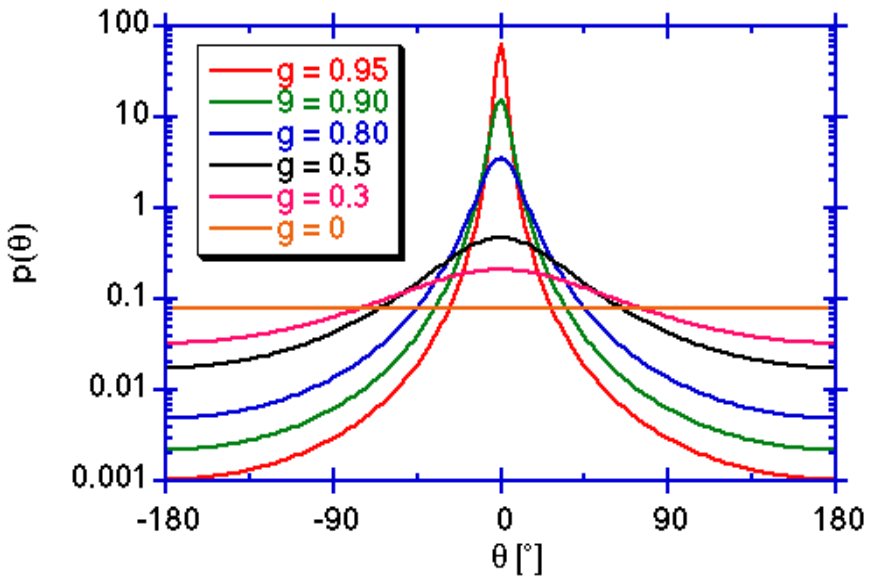
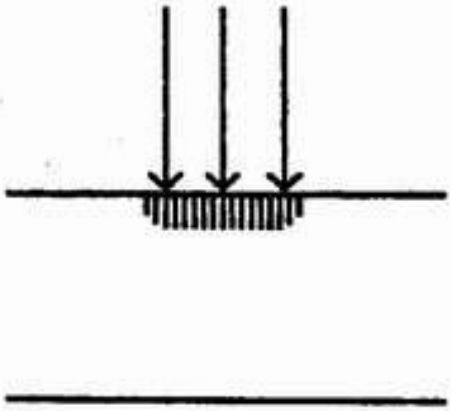
Light propagation into the tissues

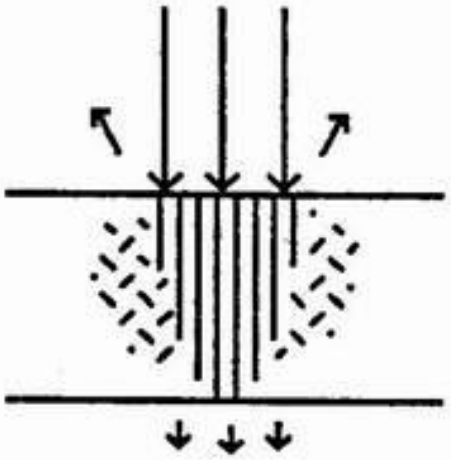


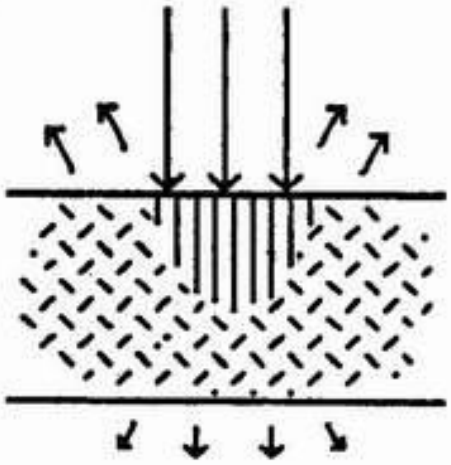
Light distribution into the tissues

- 1-predominant absorption;
- 2 - near values for absorption and scattering coefficients;
- 3-predominant scattering

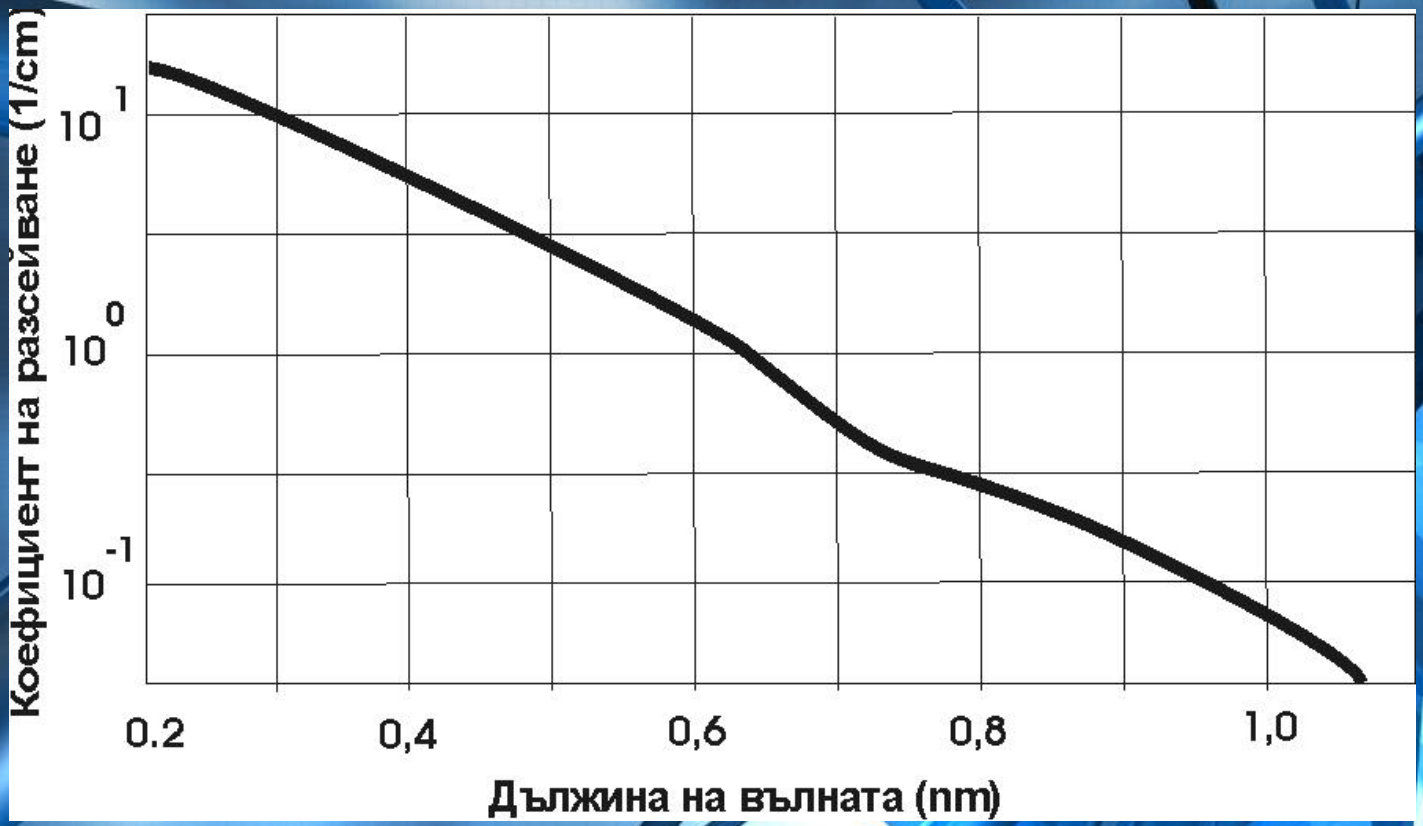






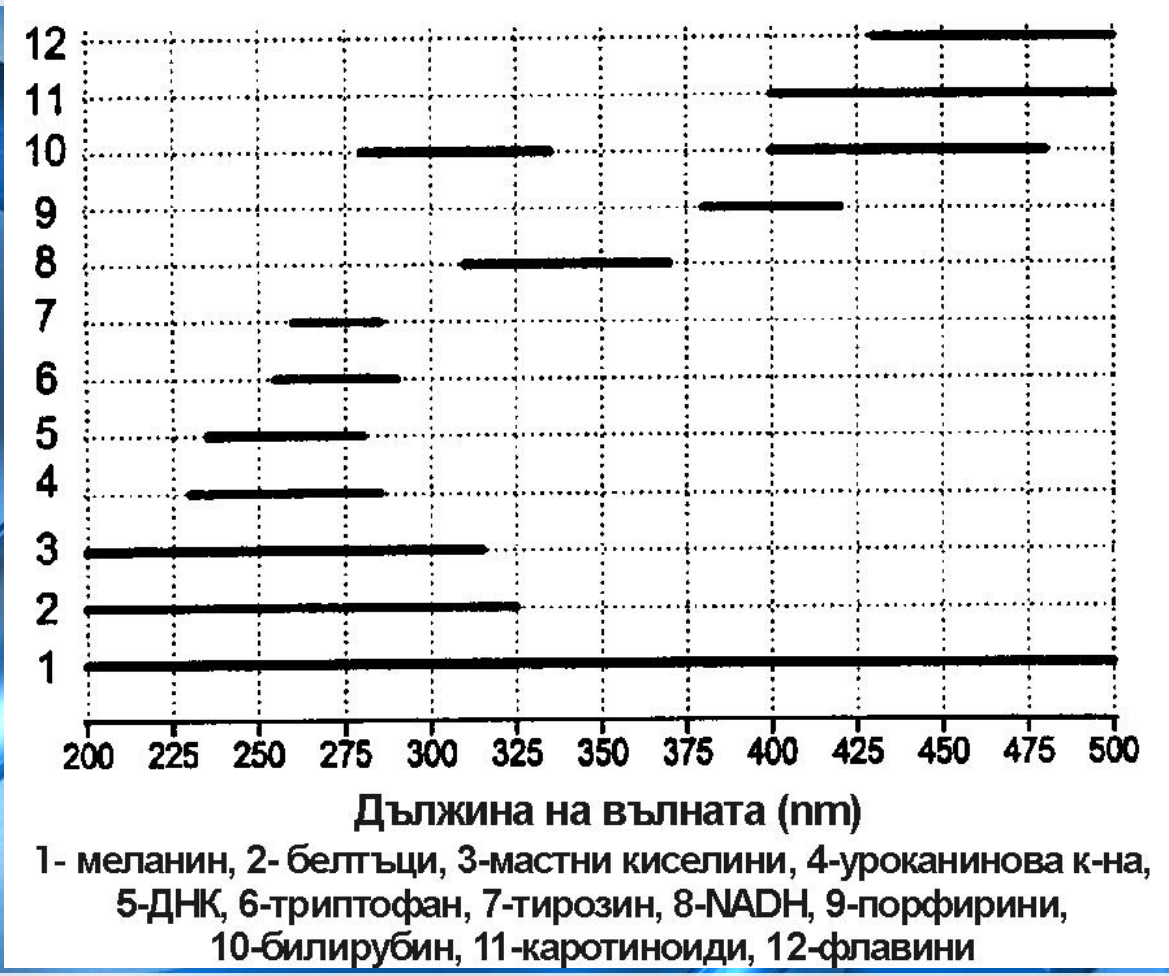


Properties, associated with the structure



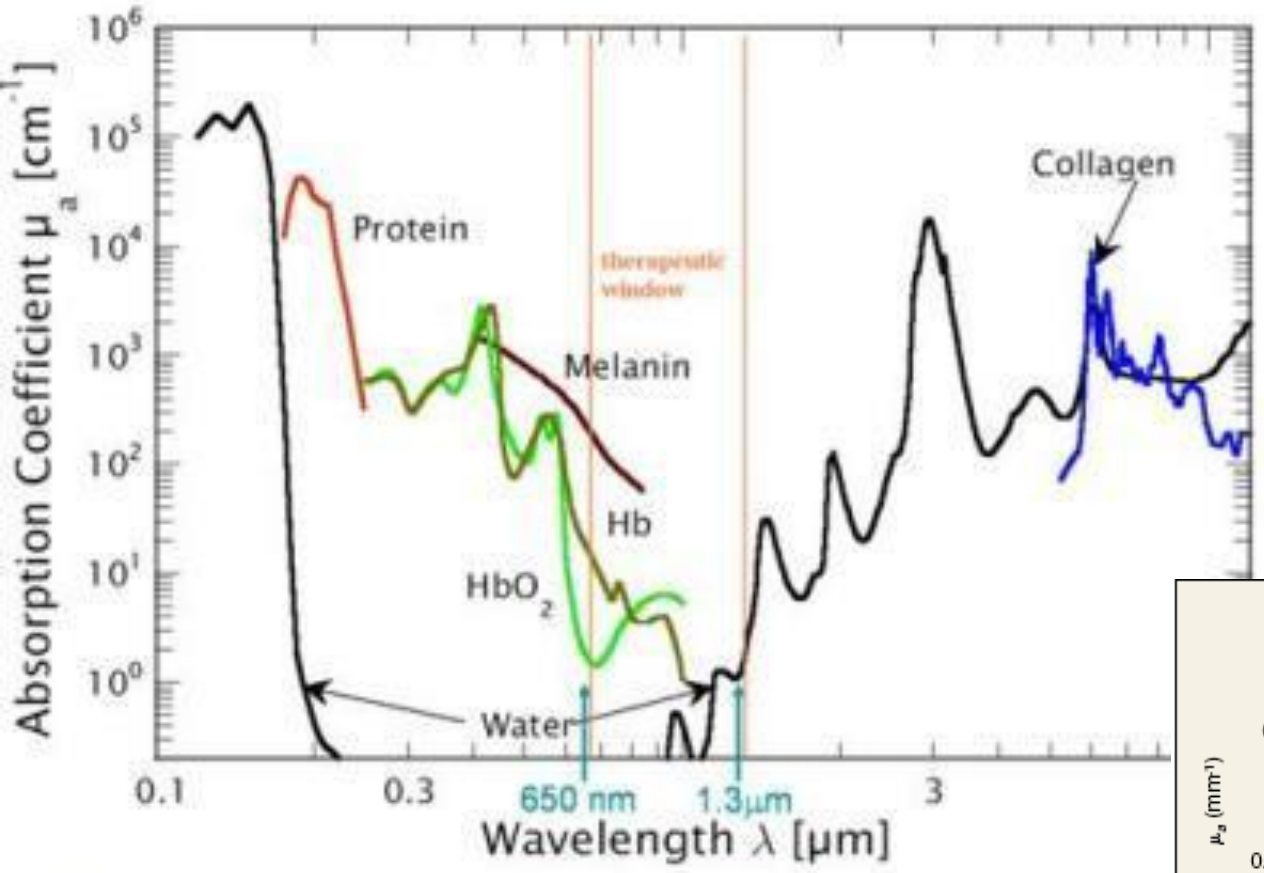
Dependence of the scattering coefficient on the wavelength for soft tissues

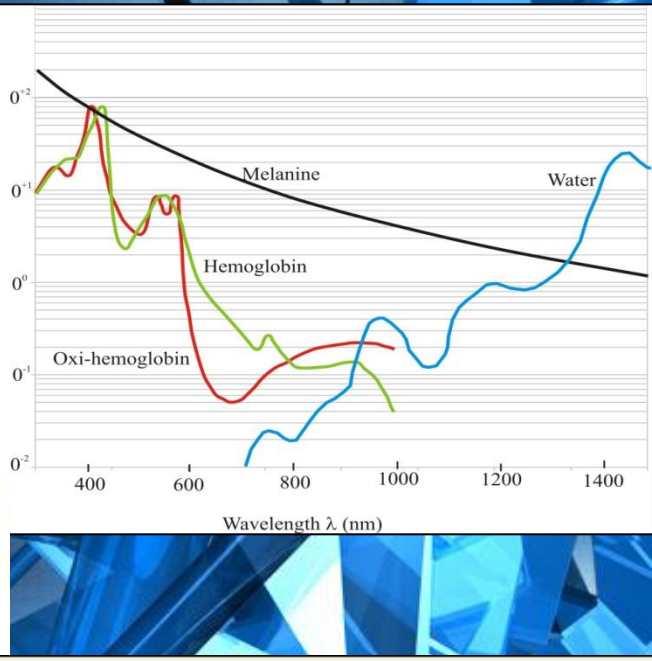
Properties, associated with the content

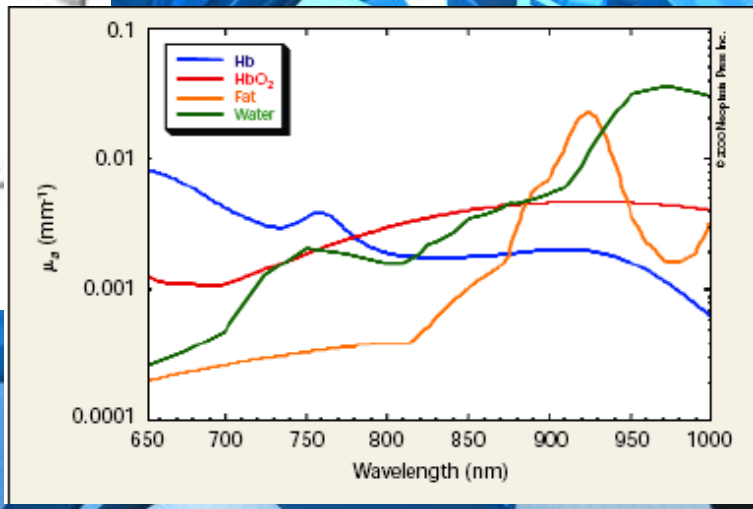


Spectral regions of absorption for typical skin chromophores

“Therapeutic window”







Therapeutic window- example

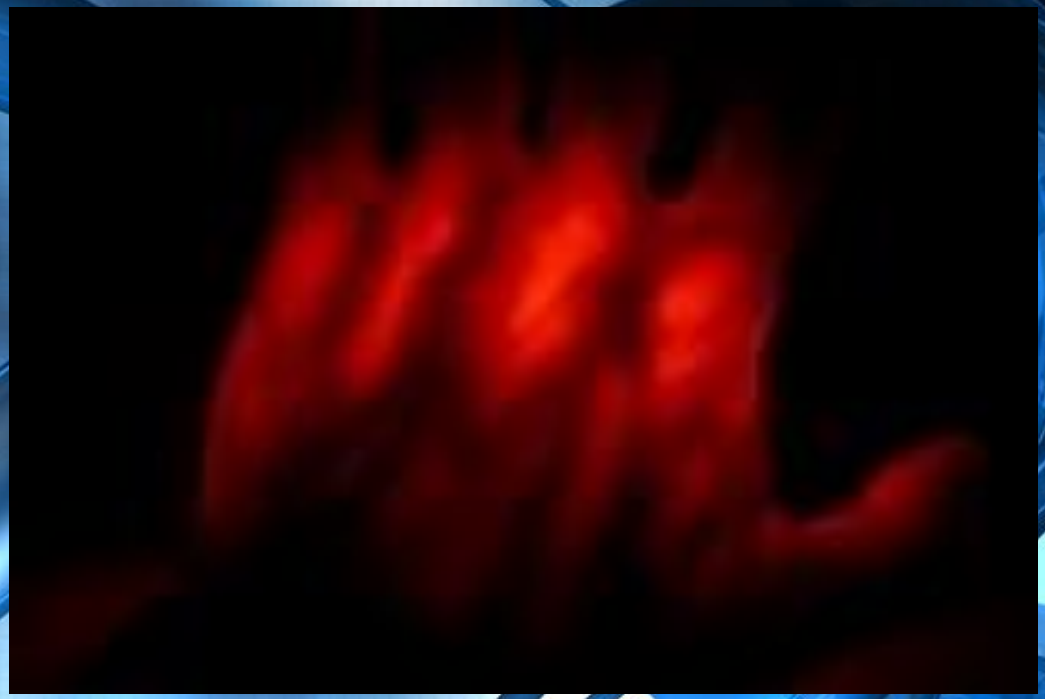
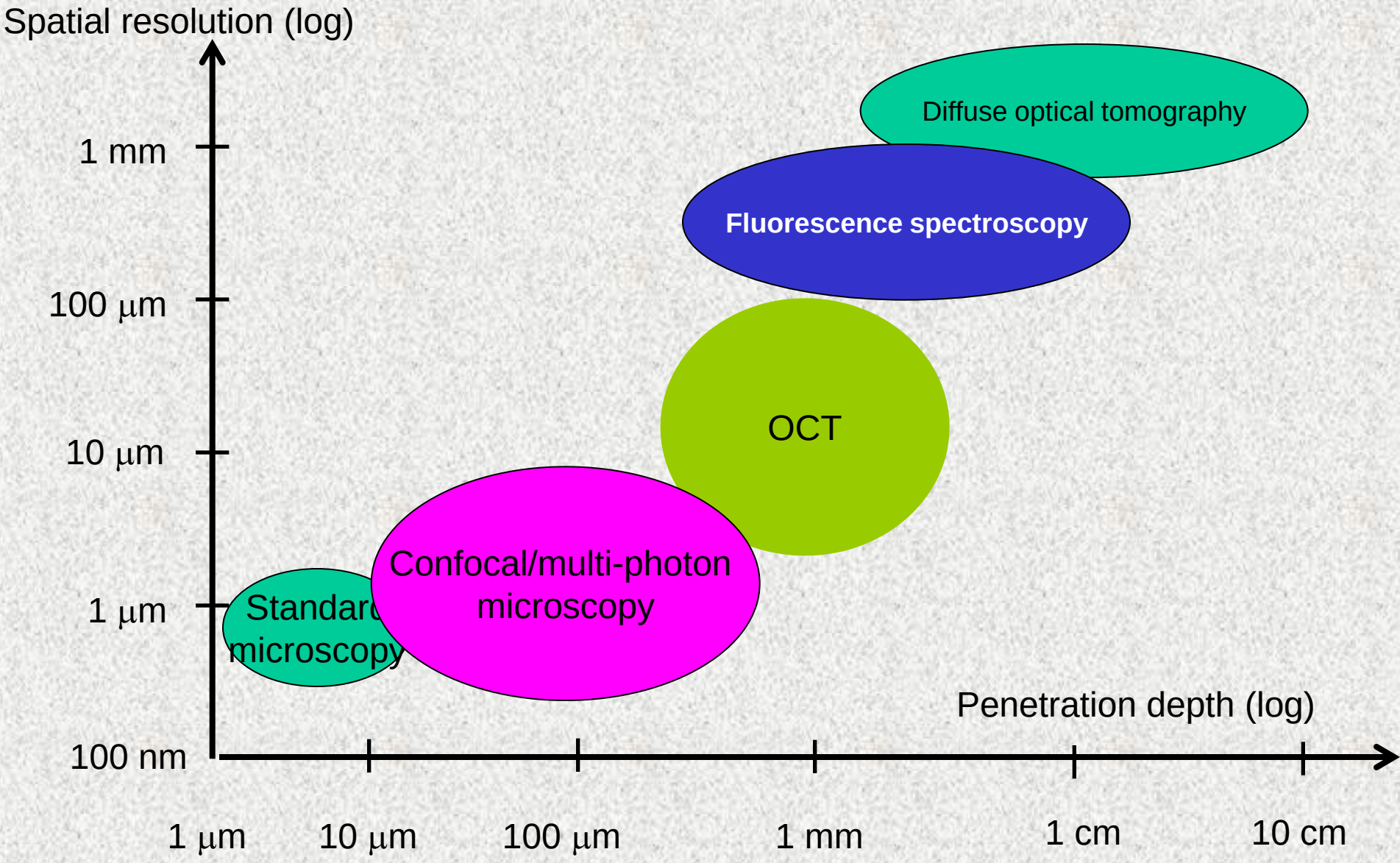


Photo of a hand - only red light is visible due to low absorption and high scattering in the 600-900 nm area, the blood absorbs more strongly in the range 700-800 nm

Which method to choose?



Optical biopsy- advantages

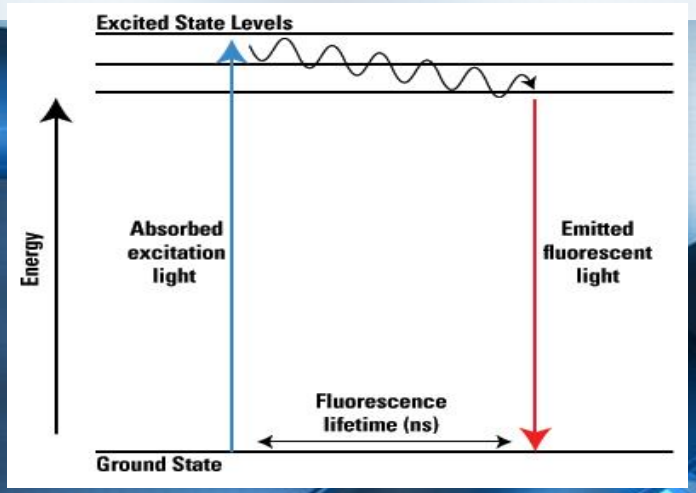
Spectroscopy methods: functional information

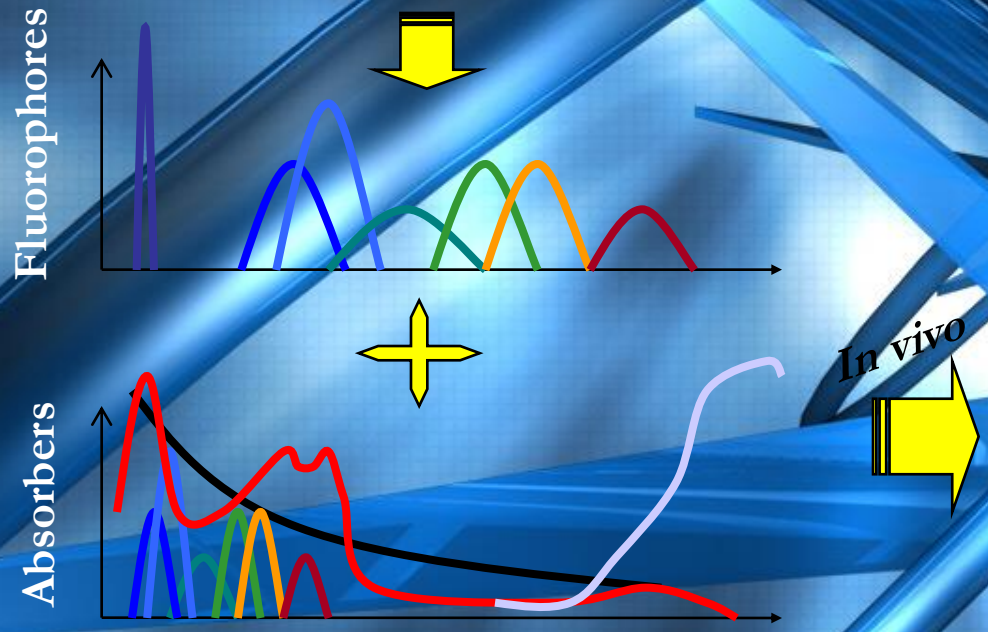
- **Diffuse reflectance**
  - Depth of penetration - from x1 microns to x1 cm, depending on λ, detection geometry, source-detector distance
  - Absorption
    - Oxygenation of tissues, saturation with oxygen
    - Oxygen consumption
    - Hemodynamics
  - Scattering
    - Structural changes of the intercellular matrix
    - Changes in cell nucleiклетките
- **Light scattering**
  - Depth of penetration - from x1 to x100 microns, depending on specimen scattering properties
  - inelastic (Raman) scattering
    - Biochemical content
  - Elastic scattering
    - Provides information about size distribution of nuclei, mitochondria, etc., tissue structure
    - Spatial resolution - may potentially detect changes in the order of 100 nm
- **Fluorescence**
  - Depth of penetration - from x1 microns to x1 cm, depending on the optical properties of the object, the applied λ, the source-detector geometry
  - Endogenous fluorescence - cellular and tissue biochemistry - NADH / FAD, porphyrins, oxidized lipids and for tissue structure - collagen, elastin, cross-links
  - Exogenous fluorescence - lesion boundaries, type of formations
  - Fluorescence markers - antigen expression and molecular beacons (enzyme activity)

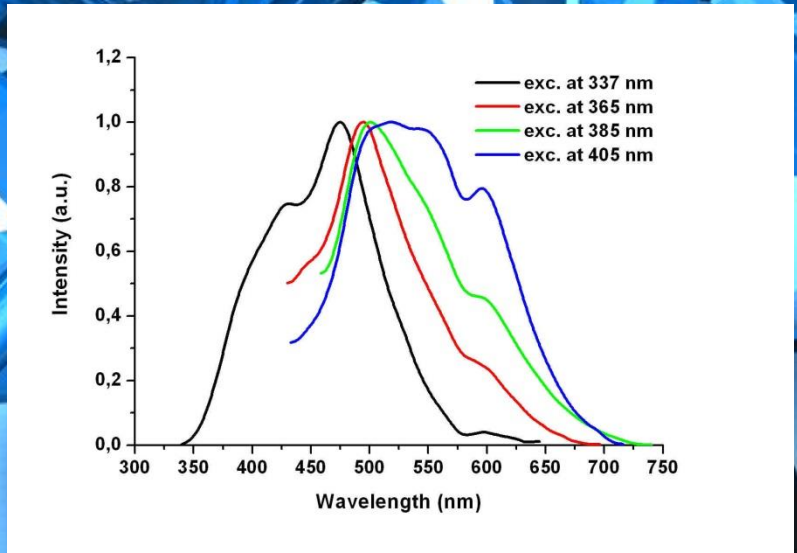
Tissue fluorescence

- Detecting tumors
- Detecting pre-cancerous conditions
- Biopsy monitoring
- Monitoring of patients
- Monitoring of therapeutic procedures
- Detection of atherosclerotic plaques
- Detecting bacterial infections

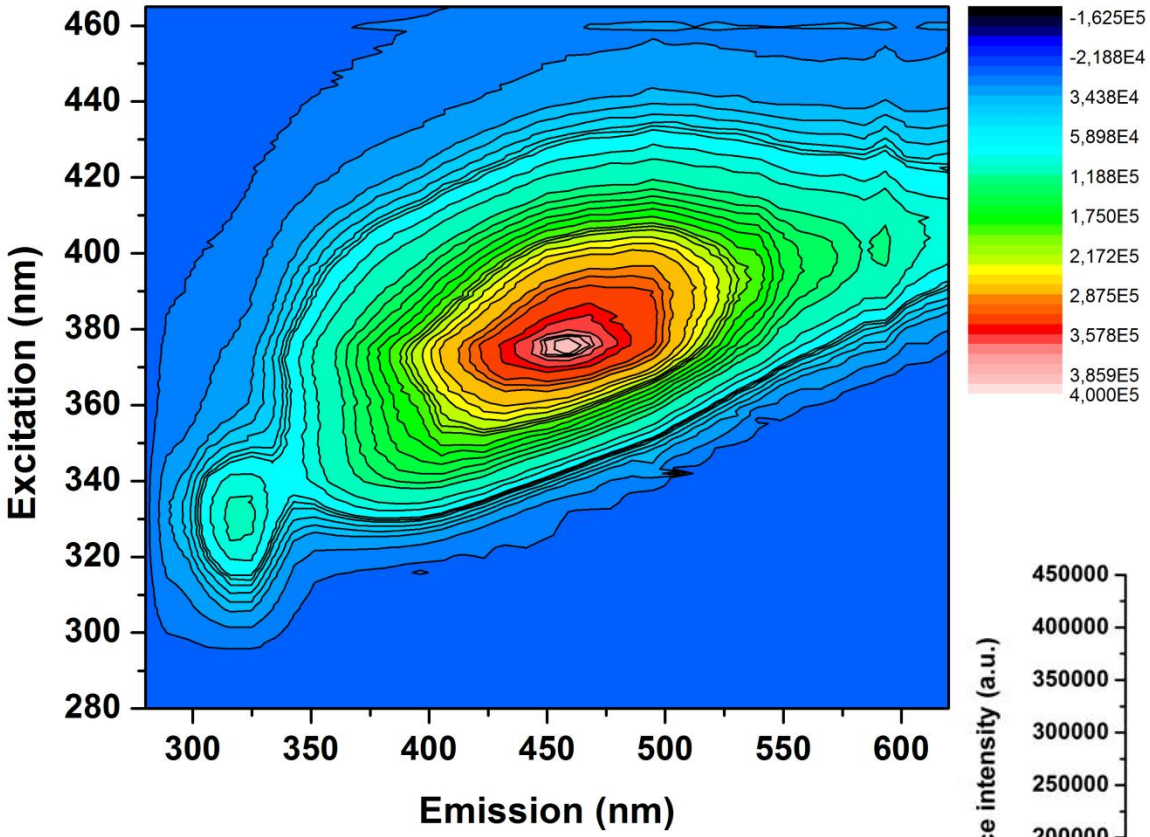
Tissue fluorescence



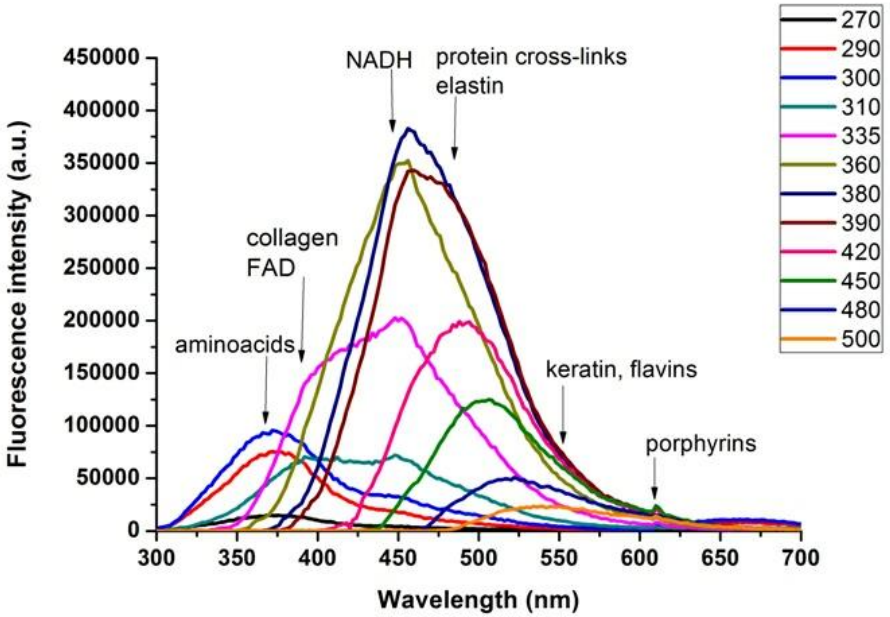




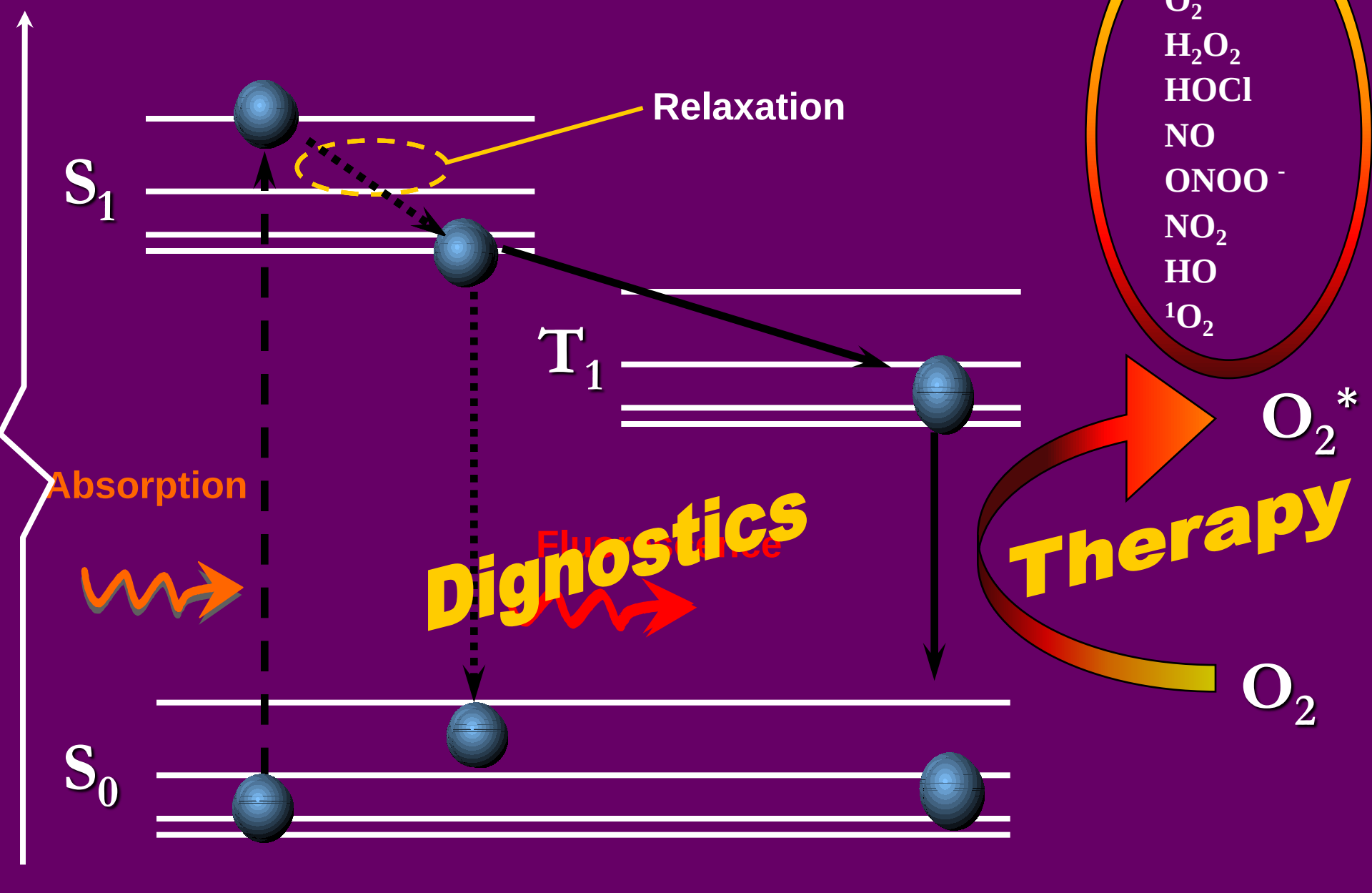
Skin EEM data



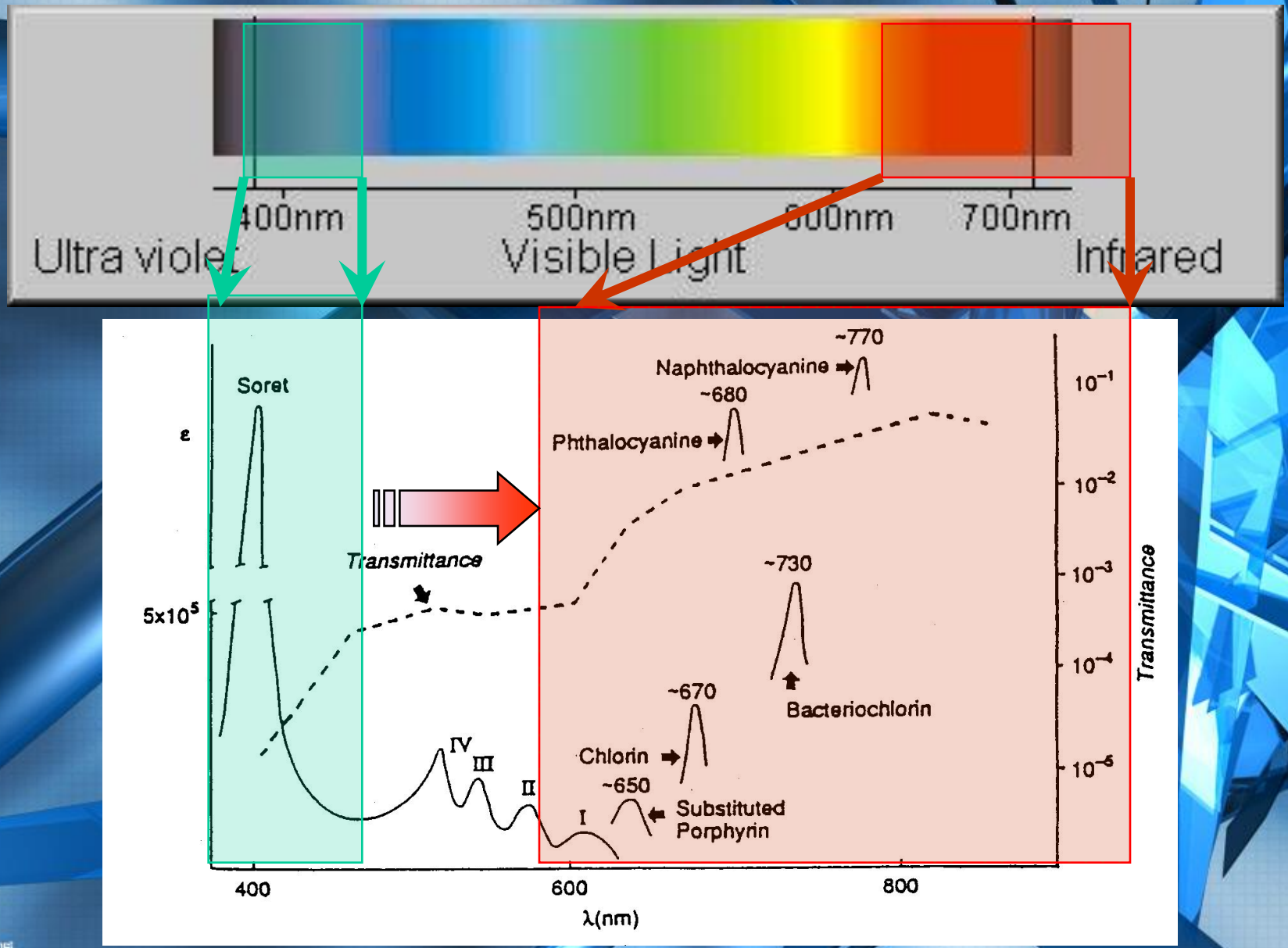
Autofluorescent EEM matrix of normal skin, phototype 2



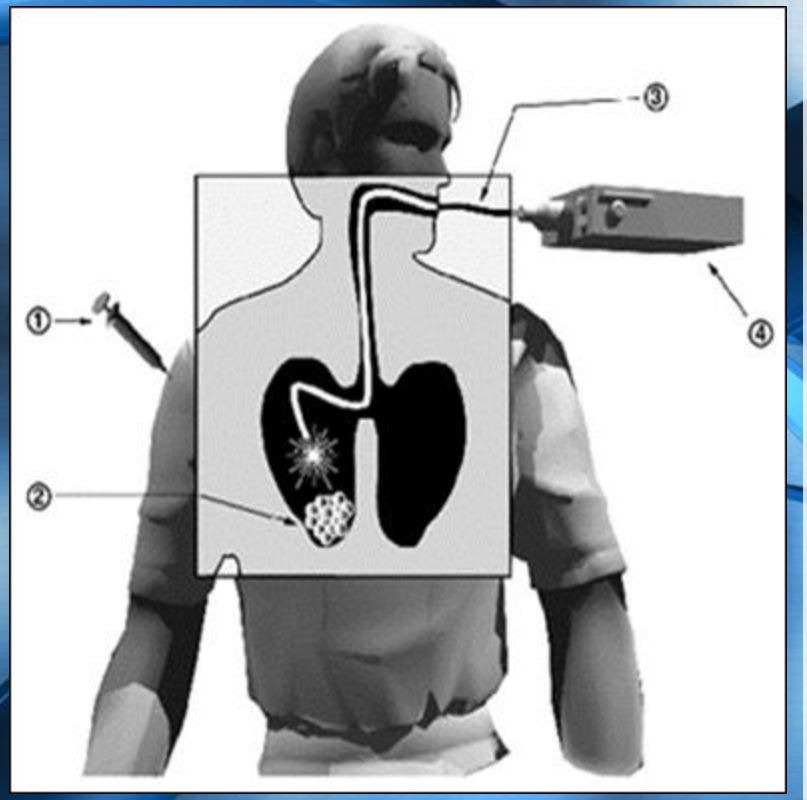
Principles of PD and PDT



Spectral position of PD and PDT



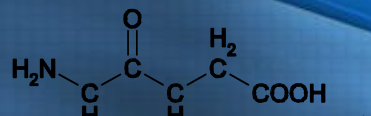
PDT of tumors – major steps

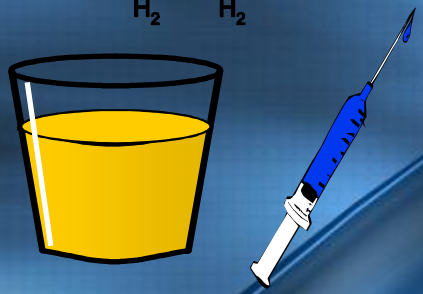


1. In the body is introduced light-sensitive chemical compound (photosensitizer)
2. The drug selectively accumulates in the tumor tissue where it is irradiated with a laser of appropriate wavelength
3. The photosensitizer transmits the laser energy and creates a toxic form of oxygen and free radicals
4. Tumor cells are destroyed with a minimal damage to surrounding healthy cells

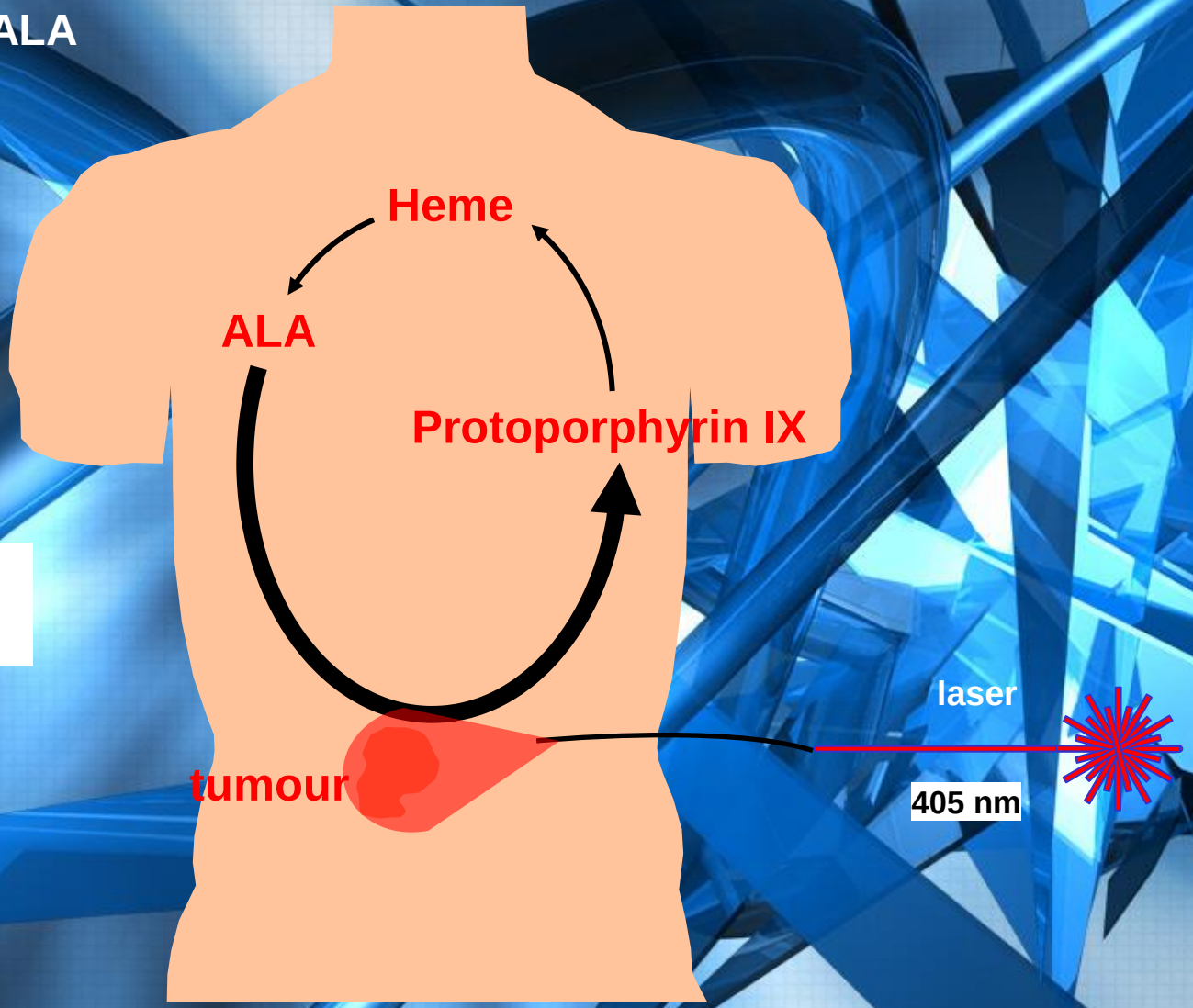
ALA-PDT

Administration of ALA



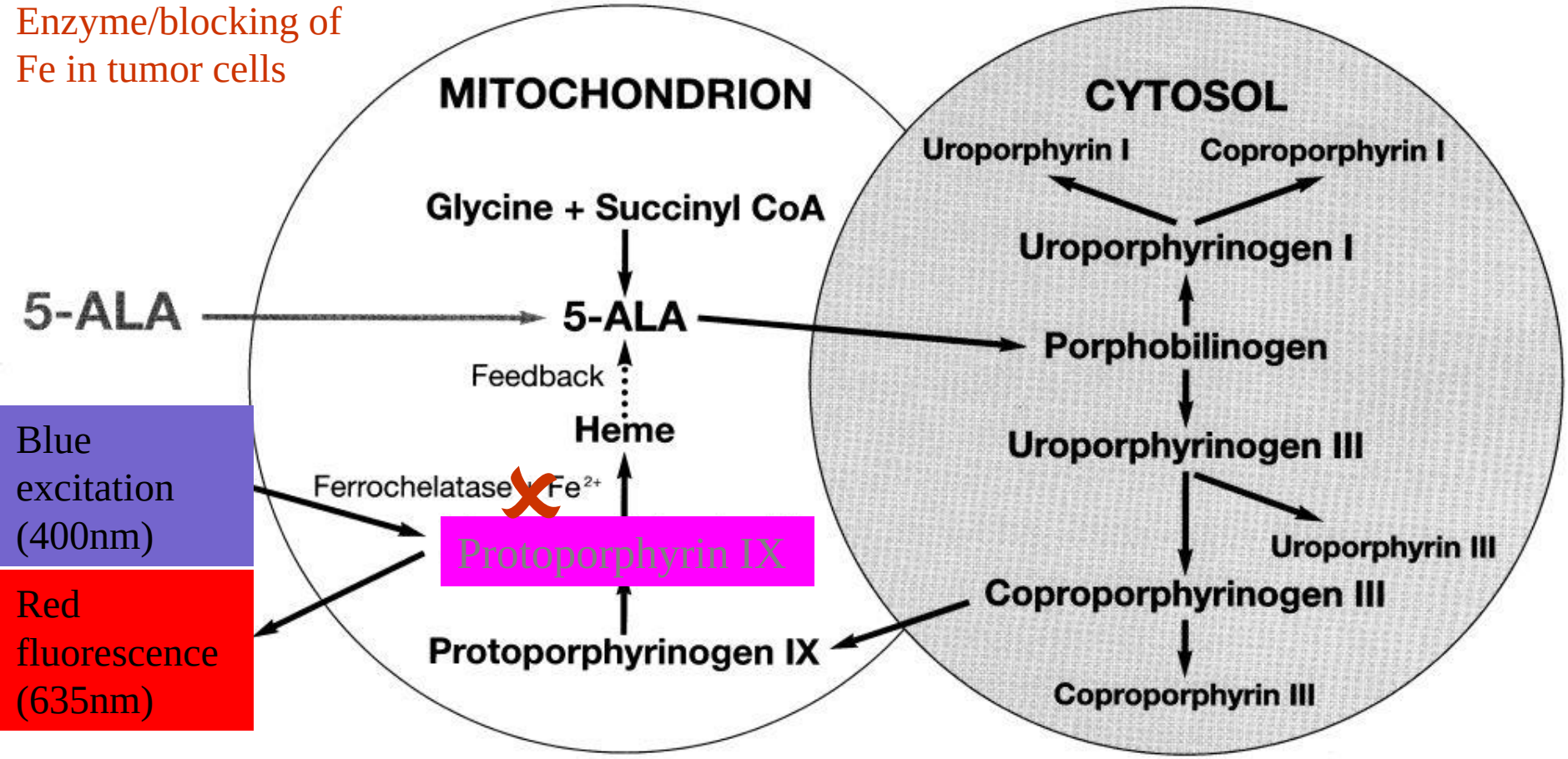


Synthesis Protoporphyrin IX



Metabolism of δ-ALA in the cells

Enzyme/blocking of
Fe in tumor cells

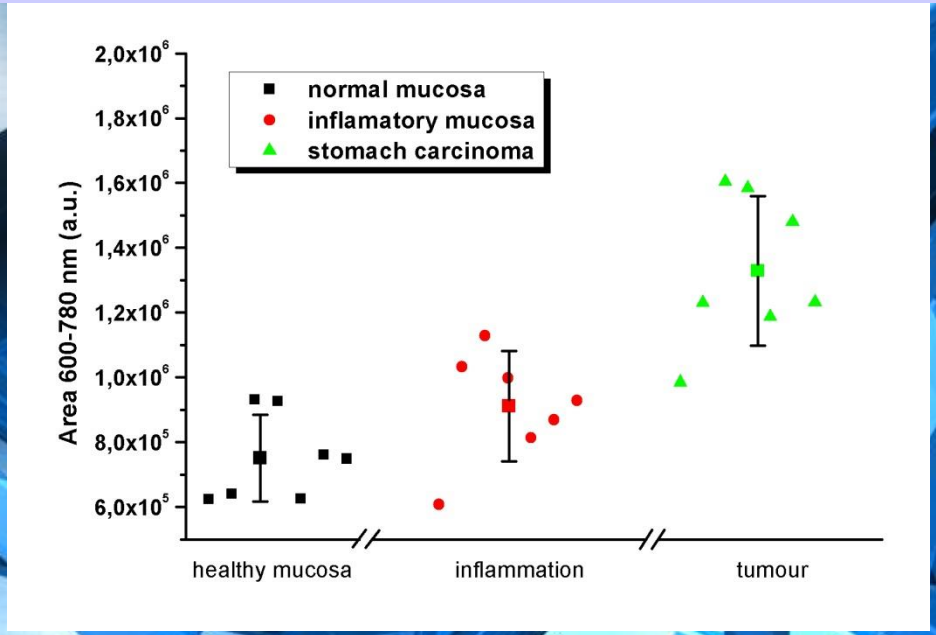


Blue excitation (400nm)

Red fluorescence (635nm)

2-D fluorescence visualization of oesophageal carcinoma using 5-ALA/PpIX

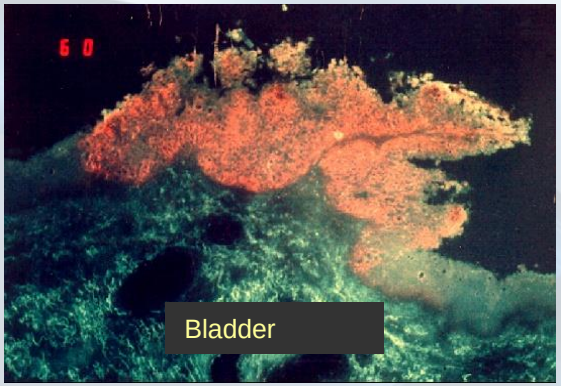
Image from photosensitized mucosa of esophagus using standard video-endoscopic CCD camera

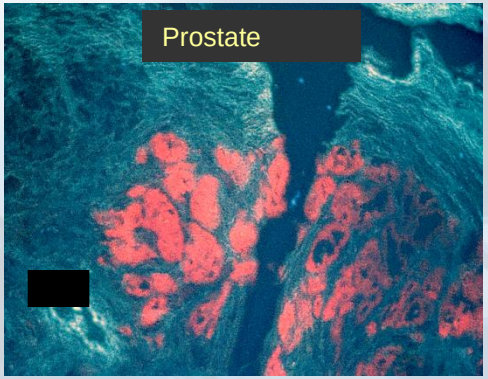


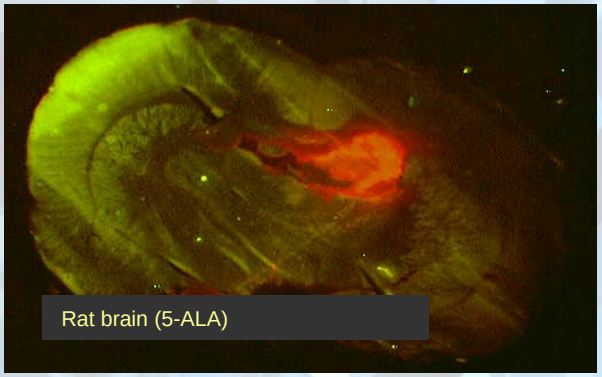
Comparison of the integrated fluorescence signal for the region 600-780 nm, calculated for all cases detected from stomach normal mucosa, inflammation, and carcinoma. Lines represent the mean values of the areas calculated.

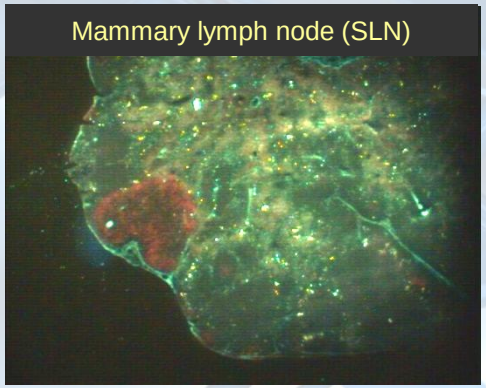
M. Ortner et al., Endoscopy 35: 663-668 (2003)

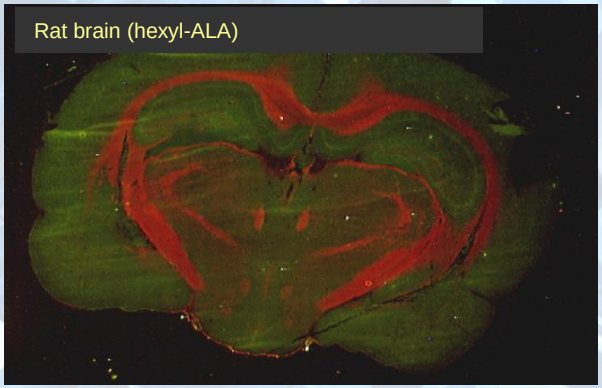
Examples – exogenous fluorescence

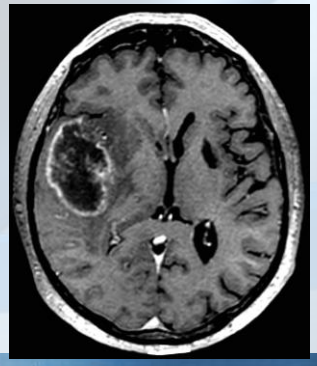


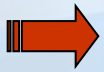


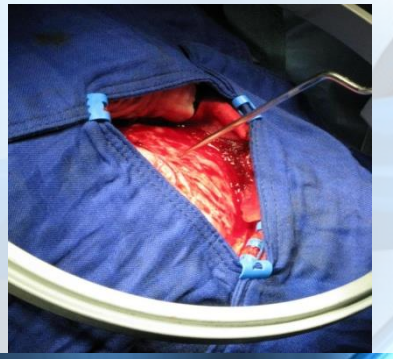


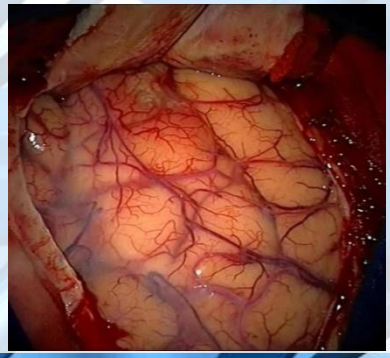


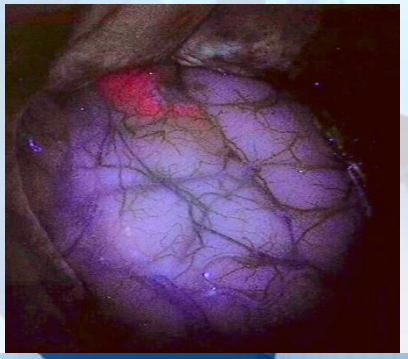












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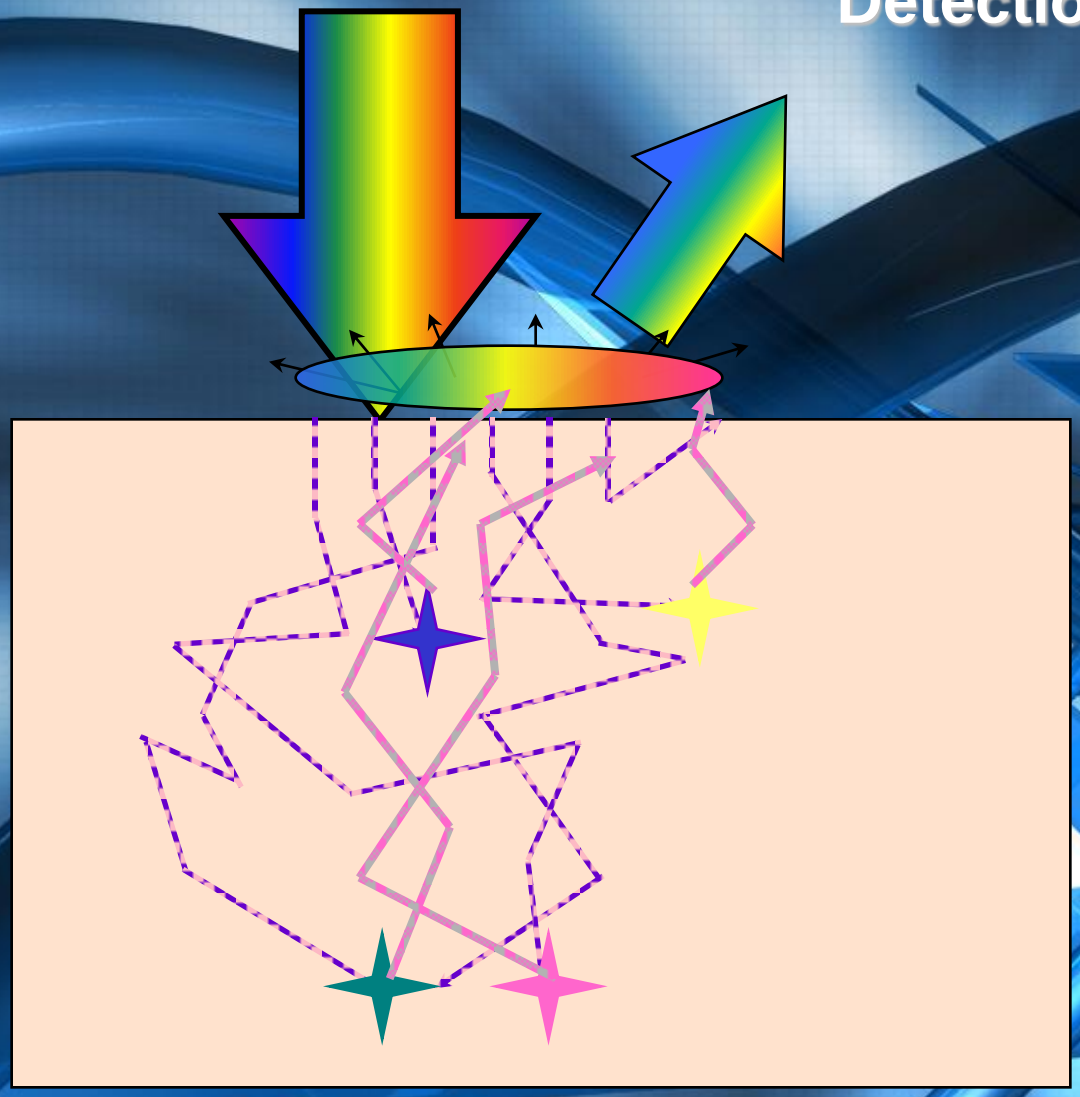
Light scattering spectroscopy

- Detecting tumors
- Detection of pre-cancerous changesБаретов хранопровод
  - Cervix
  - Tumors of the oral cavity
- Management/control of biopsies
- Non-invasive patient monitoring

Tissue reflectance

Detection of reflected signal



1) Light in a broad spectral range penetrates to the tissue

2) In the volume there are substances that absorb at different wavelengths - part of the light is absorbed by them

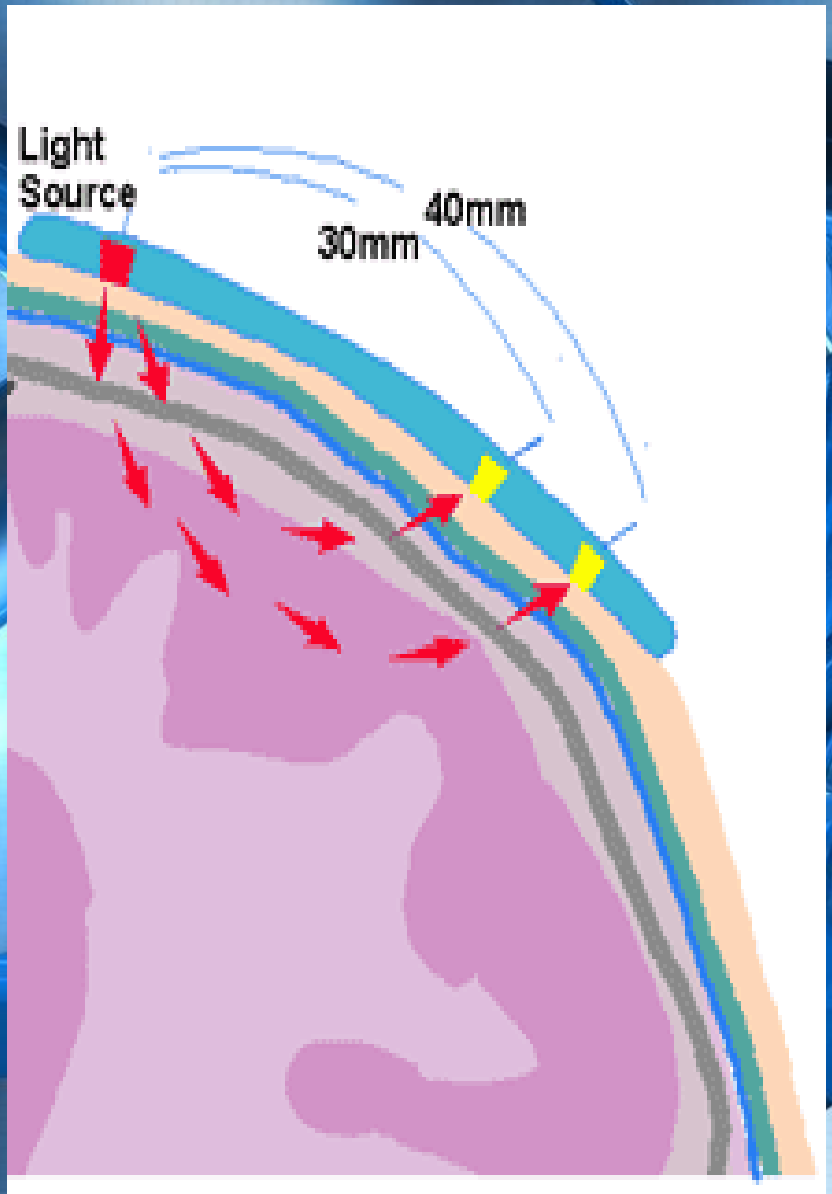
3) The light is scattered in the volume of the tissue and part is coming back and goes to the surface

4) The reflected signal, consisting of the specular reflection and of the signal, scattered into the volume of the tissue, is detected by a spectrometer

Diffuse optical tomography and spectroscopy

- Analysis of brain functions
- Pulse oximetry
- Consumption of oxygen in muscle tissues
- Bilirubin test for newborns
- Detecting breast cancer
- Arthritis
- Atherosclerotic plaques

(NIRS) Diffuse optical tomography of the brain in the near infrared region



Pulse oximetry

The pulse oximeter can provide information on:

1. Saturation of arterial blood hemoglobin with oxygen (associated with each molecule)
2. Pulse
3. Photoplasmogram - change of blood volume in the examined area

The pulse oximeter can NOT provide information on:

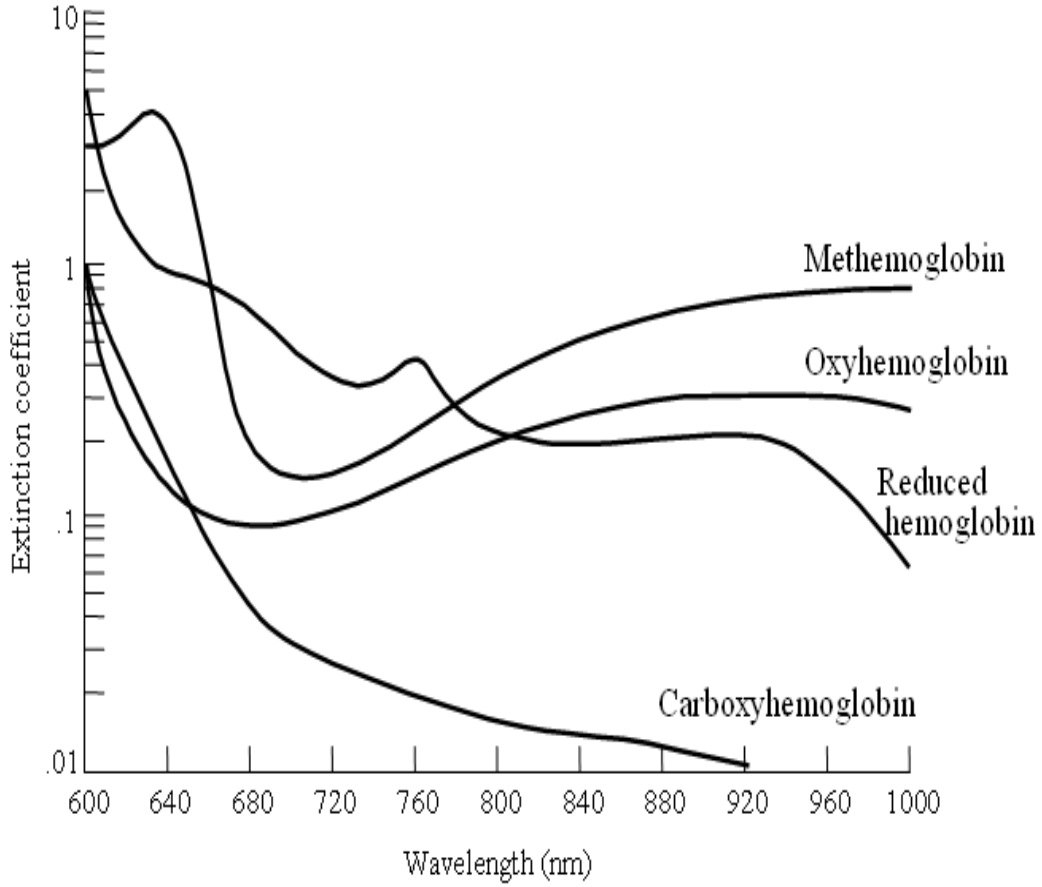
1. Oxygen content in the blood
2. The amount of dissolved oxygen in the blood
3. Respiratory volume or breathing rate
4. Arterial pressure

Pulse oximetry is used for:

- Continuous monitoring of patients
- Monitoring in operating and intensive wards
- Ambulatory monitoring
- Control of oxygen therapies
- Diagnosis of night apnea
- Respiratory support for the patients
- When transporting patients with emergency care
- At an advanced stage of "heavy" pregnancies and births

photooxyhemometry

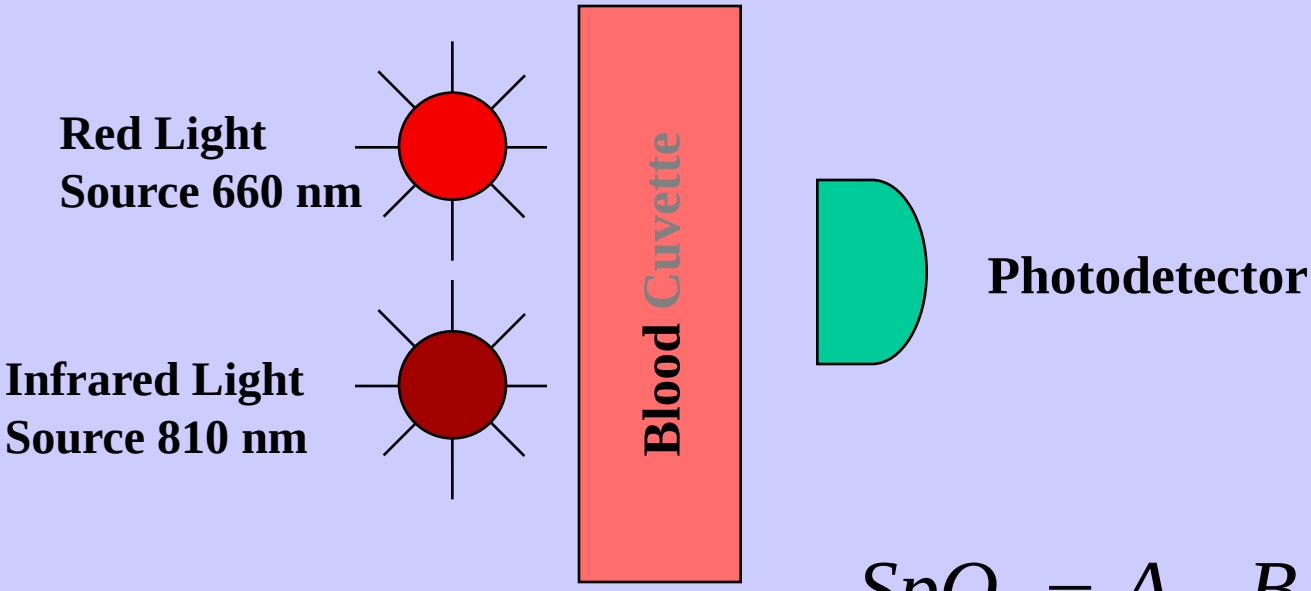
Определение сатурации
и кислородом



$$SpO_2 = \frac{[HbO_2]}{[HbR]+[HbO_2]} * 100\%$$

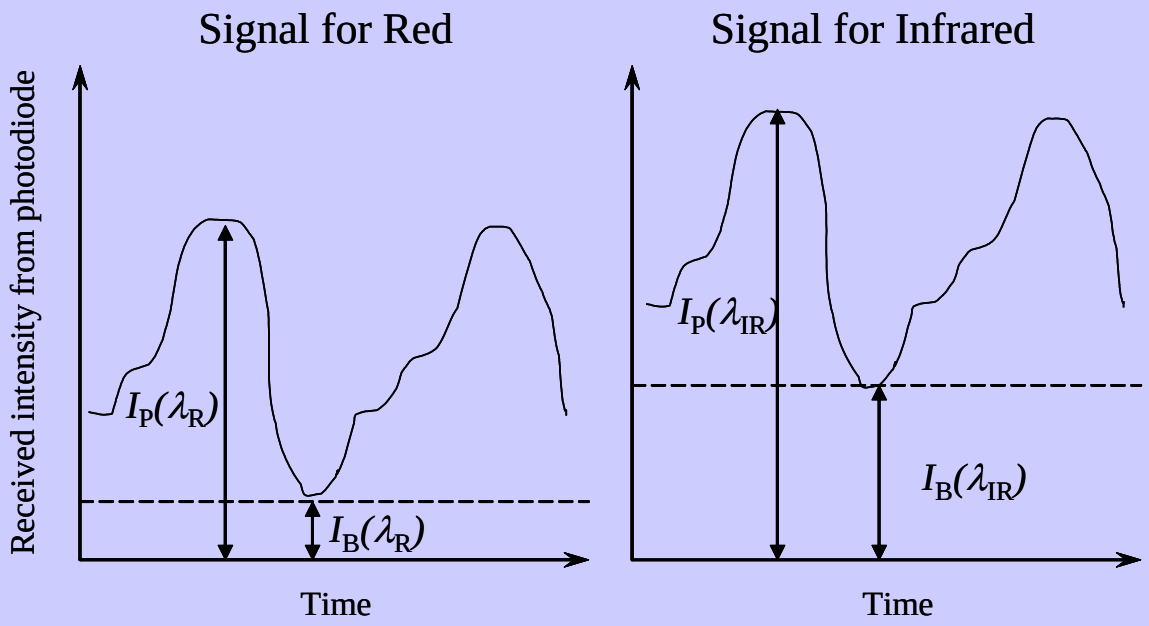
$$SpO_2 = 1 - \frac{[HbR]}{[HbR]-[HbO_2]}$$

Practical determination of SpO_2 in photooxyhemometry



$$SpO_2 = A - B \cdot \frac{D(660нм)}{D(810нм)}$$

D(660), D(810) – optical density of the blood at λ = 660 и 810 нм
A and **B** - constants



Indicator of blood oxygenation

$$R_{OS} = \frac{\Delta A(\lambda_R)}{\Delta A(\lambda_{IR})} = \frac{\ln\left(\frac{I_P(\lambda_R)}{I_B(\lambda_R)}\right)}{\ln\left(\frac{I_P(\lambda_{IR})}{I_B(\lambda_{IR})}\right)}$$

$I_p(\lambda r)$, $I_p(\lambda ir)$ и $I_b(\lambda r)$, $I_b(\lambda ir)$ – pulsed and permanent compounds of the absorption in the red and infrared detection channels

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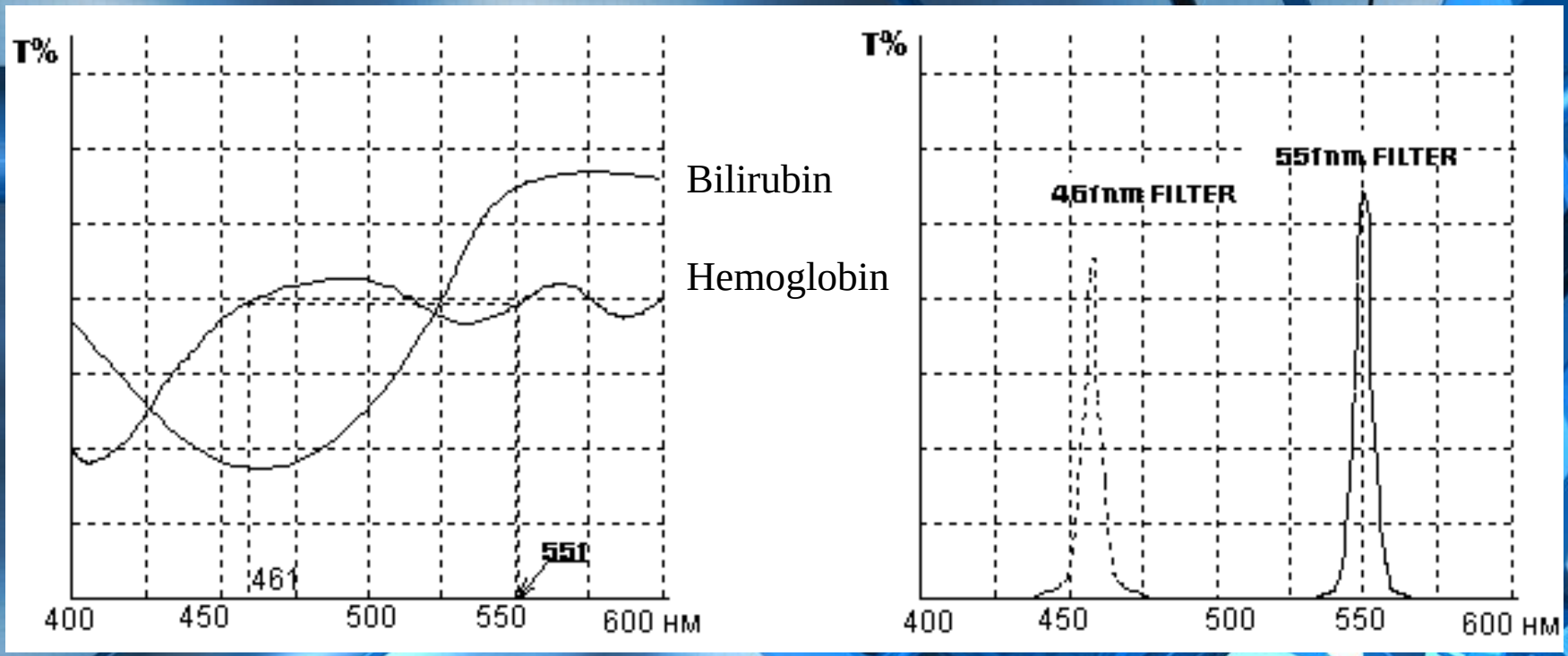
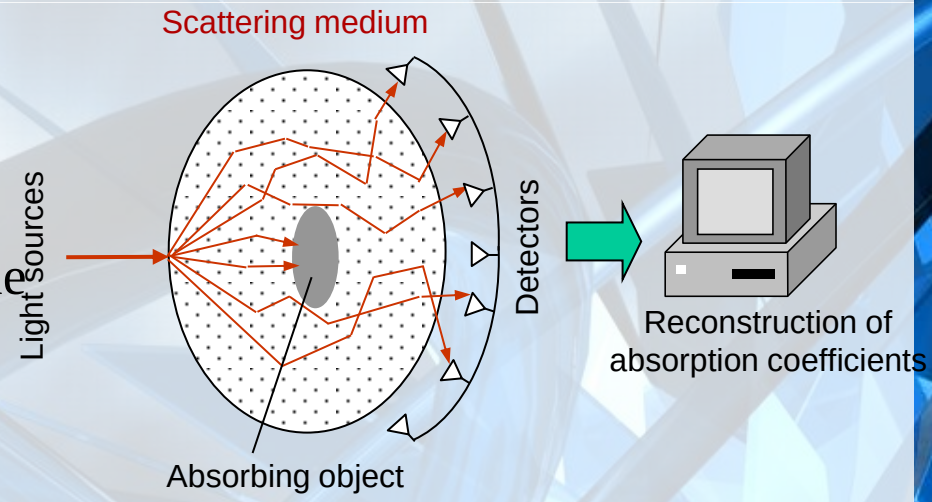
Bilirubinometry



Bilirubinometers are designed to directly measure the total amount of bilirubin. Two wavelengths of 461 and 551 nm are used. Maximum bilirubin uptake is observed at 460 nm and the second filter is used to separate the effect of hemoglobin uptake. Hematocrit capillaries in a very wide range - 0-513 μmol / l, minimal uncertainty (5%), 2 drops of blood (~ 5 μl), reagents are not added.

Diffuse optical tomography

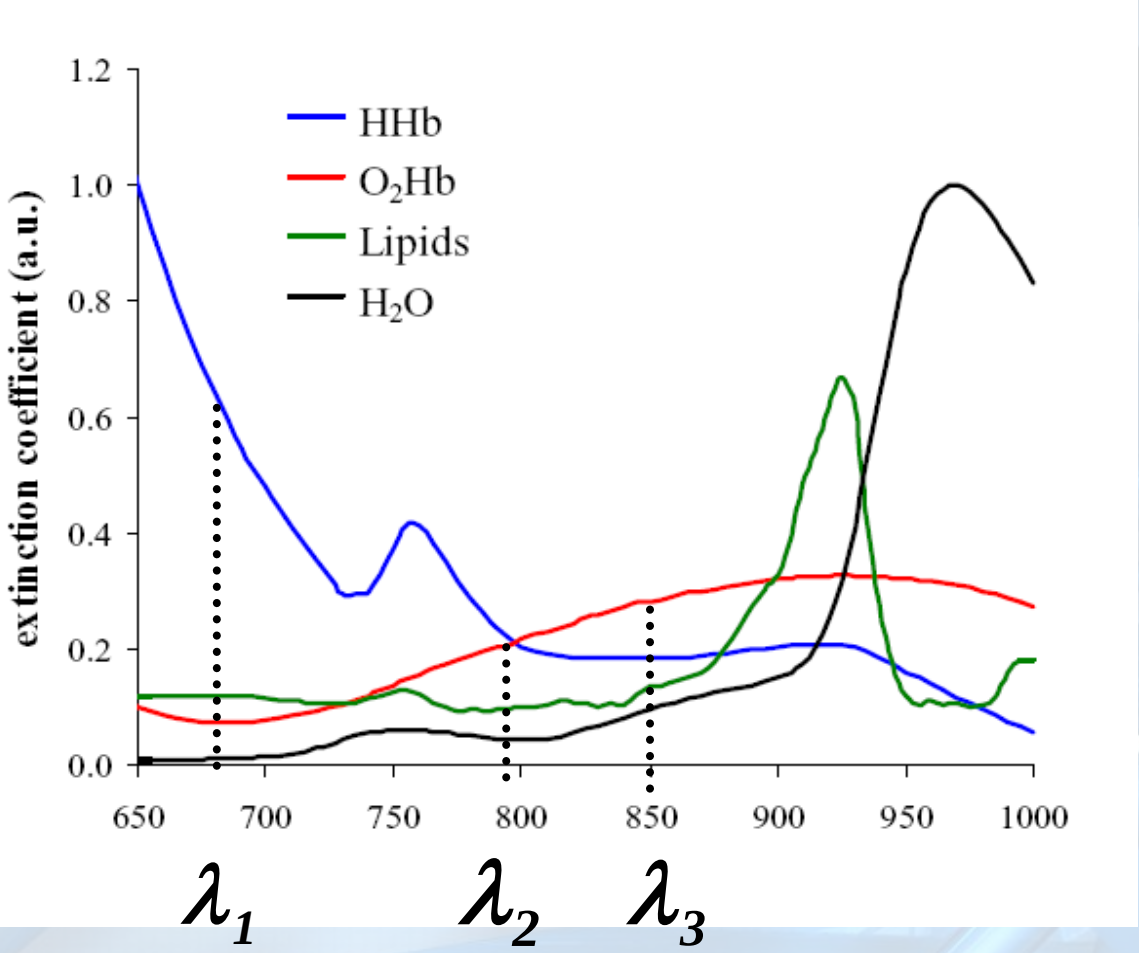
- Non-invasive
- High contrast
- Opportunity for 3-D reconstruction
- Functional information about the tissue



- Mammography - Not suitable for screening due to the radiation dose
- Ultrasound - insufficient specificity for patients over 40 years of age

- Disadvantages - resolution - 2-3 mm
- The data is obtained after solving the backward task, which is not well defined (ill-posed)

Measurement principle



684 nm ~ (HHb)
790 nm ~ (HHb+ O2Hb)
850 nm ~ (O2Hb)

Characterization of the tissue

Different dependence of the absorption coefficient on the wavelength

↓

Evaluation of tissue composition

↓

Assessment of the functional state of the tissue:

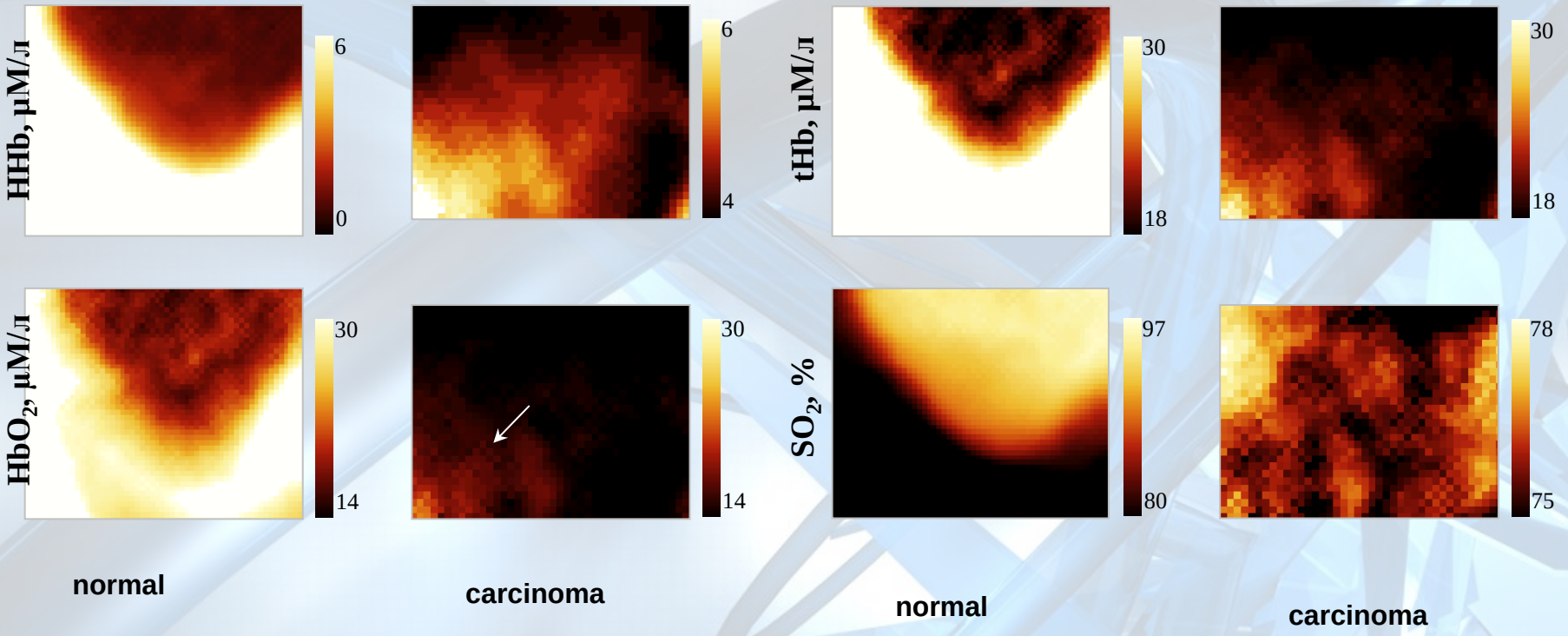
-Lesion type - benign-malignant;

- an angiogenesis study of the tumor;

- Determining the degree of hypoxia of the tumor;

- monitoring of treatment

Differences between healthy and diseased tissue

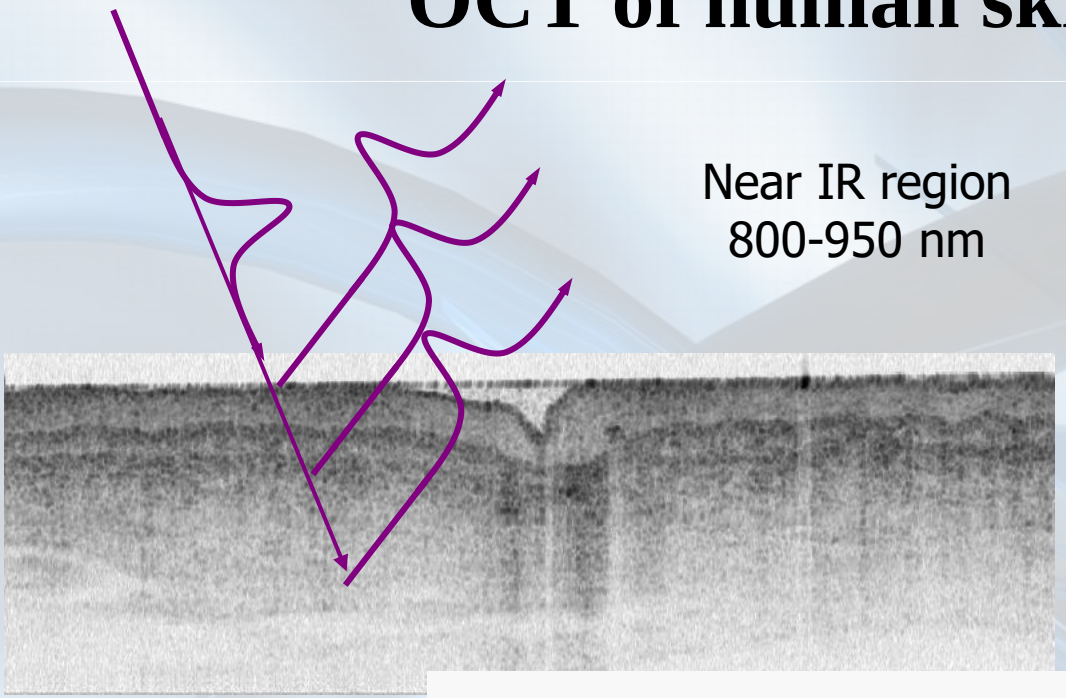


Distribution of the concentrations of deoxy-, oxy- and total hemoglobin, saturation of the blood with oxygen

Optical coherent tomography

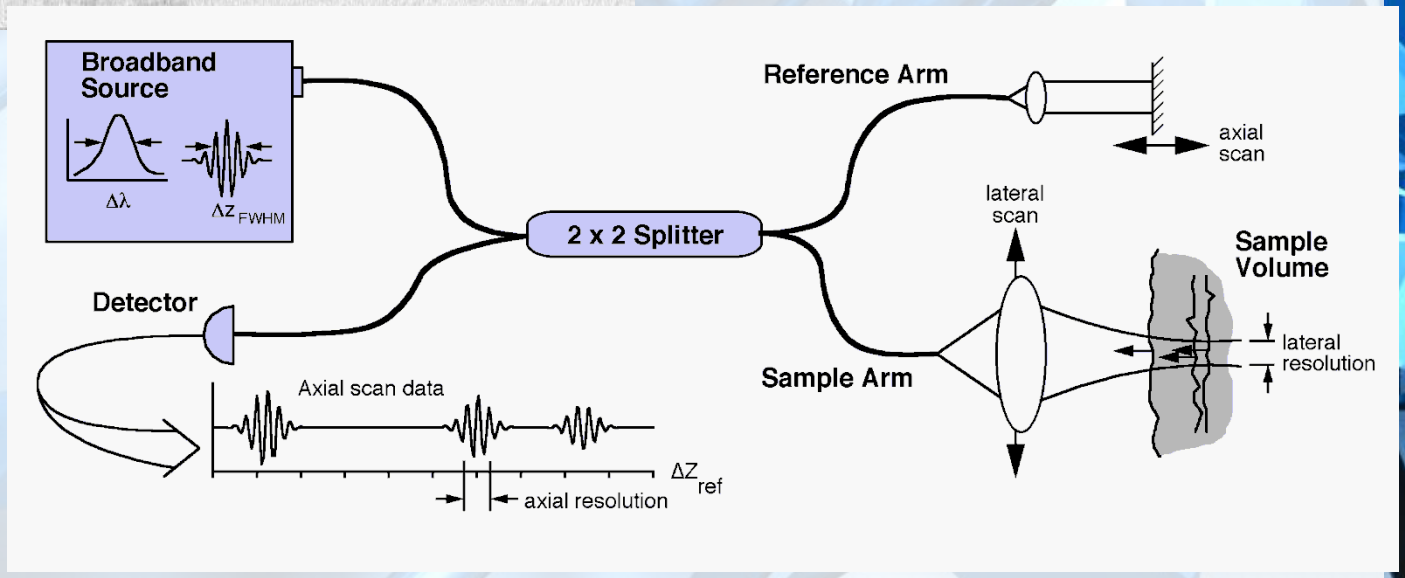
- Non-invasive detection of morphological changes
- Applications
  - Detecting cancerous formations
  - Diagnosis of the eyes and eye diseases
  - Detection of atherosclerotic plaques
  - Analysis of laboratory animals (developmental biology)

OCT of human skin- principles



Near IR region
800-950 nm

Michelson interferometer
To measure delays of scattered photons



OCT system for clinical applications

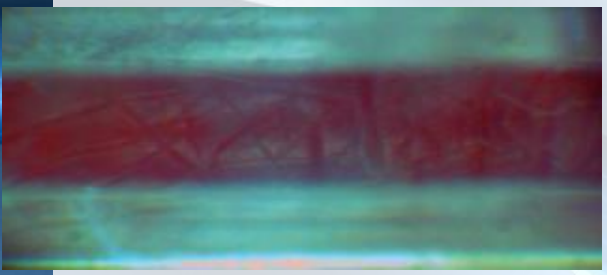
Thorlabs OCP930SR Spectral Radar OCT imaging system

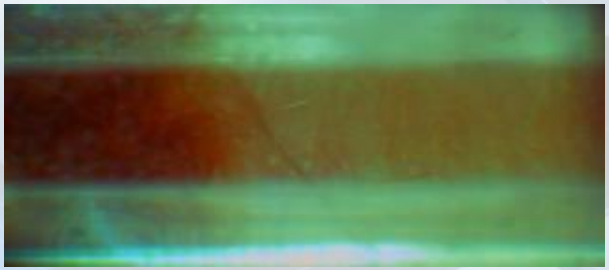
100 nm spectral bandwidth, imaging depth of ~1.6mm, lateral resolution - 20 μm, axial resolution - 6.2 μm.

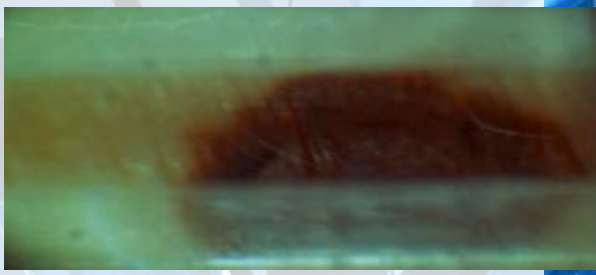
930 nm light source, optical power - 2 mW.

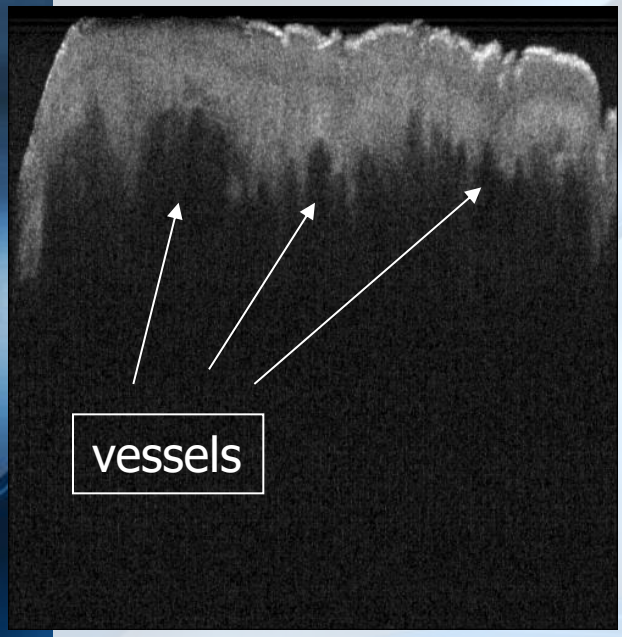
Images have been acquired at 8 frames per second, image width being set to a maximum of 6 mm and image size to 512 rows.

OCT of skin benign lesions

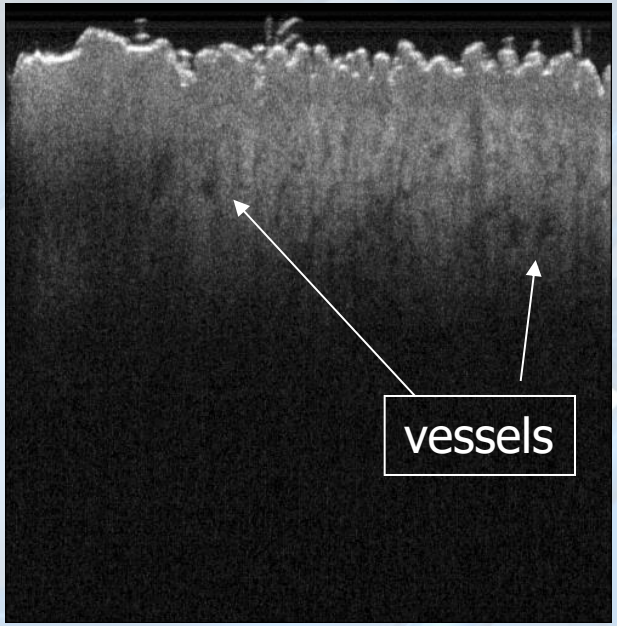




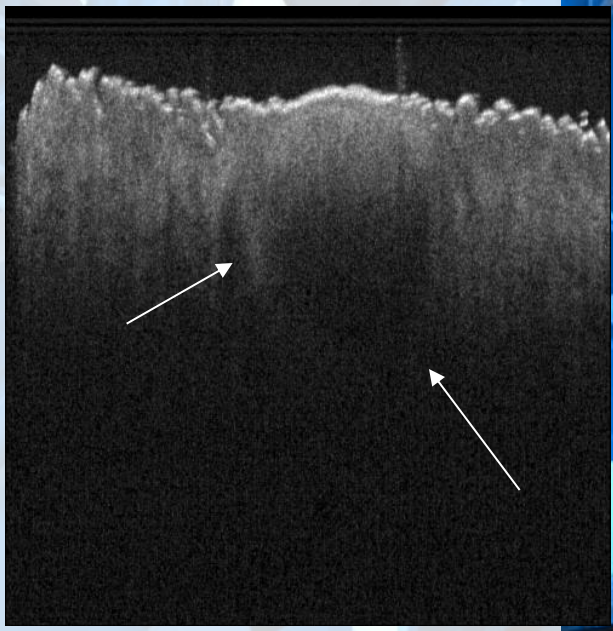




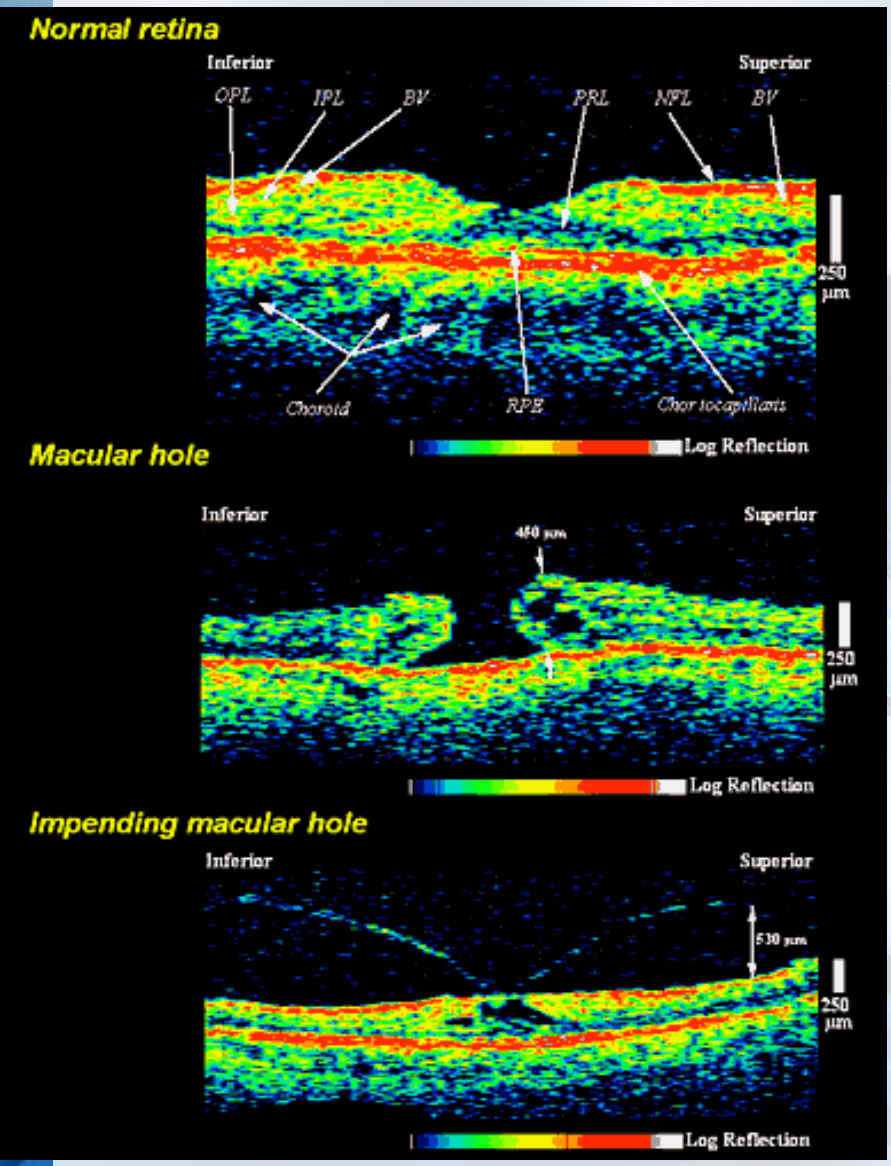
hemangioma



Actinica keratosis



Basocellular papiloma



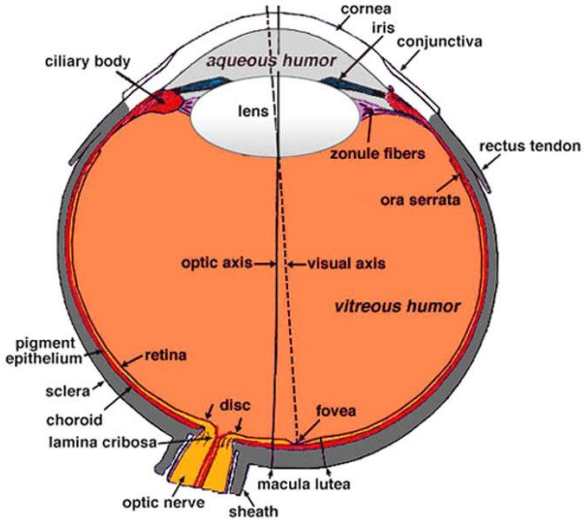


Fig. 2. Sagittal horizontal section of the adult human eye.

Normal eye

Patient with visual impairment (20/80): Macula disruption

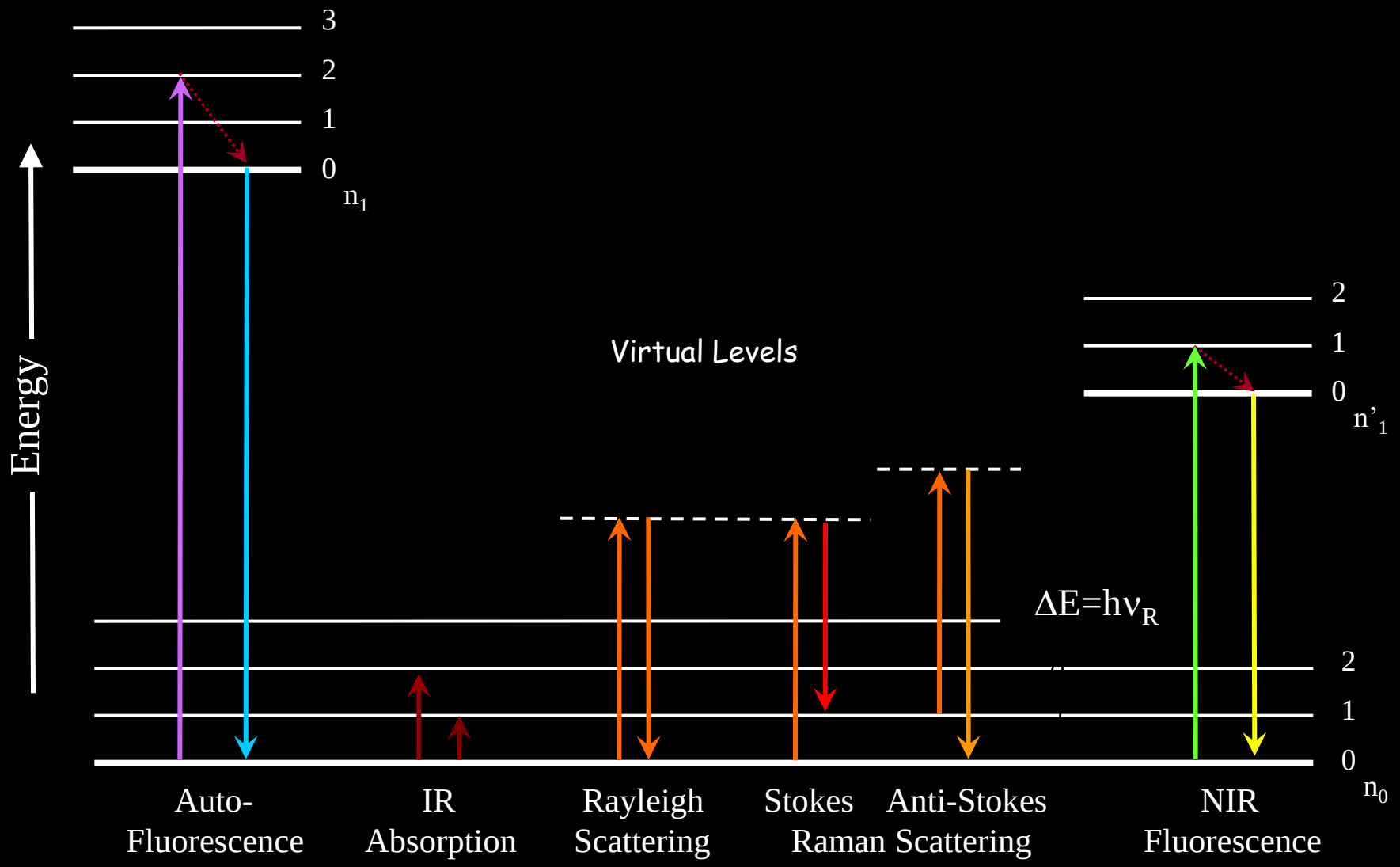
Initial macular disruption that can be recovered

http://rleweb.mit.edu/Publications/currents/cur11-2/11-2oct.htm

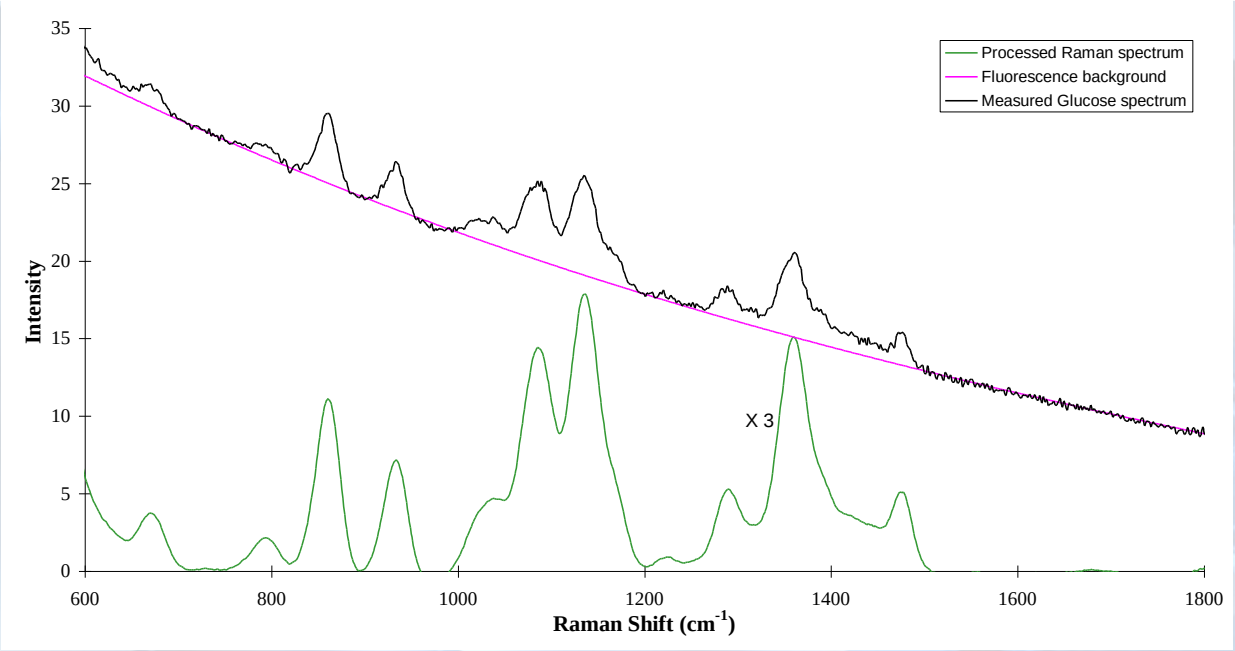
Raman scattering

- Detection of atherosclerosis
- Detecting tumors
- Determination of blood composition
- Detecting bacterial infections

Photo-Molecular Interactions

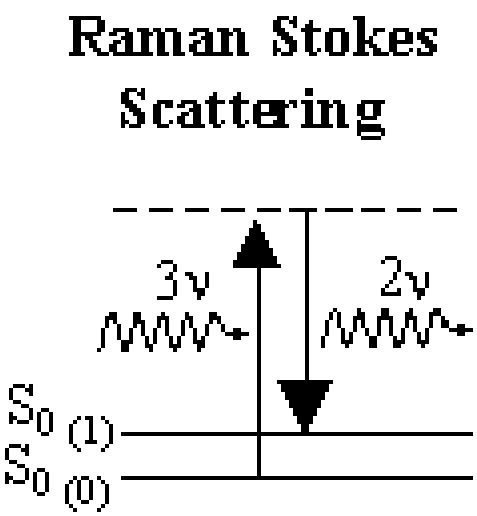


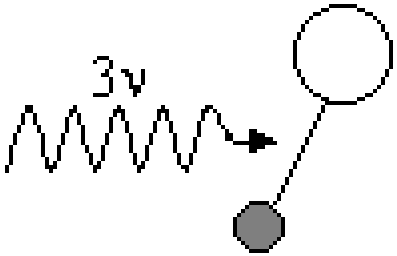
Raman scattering

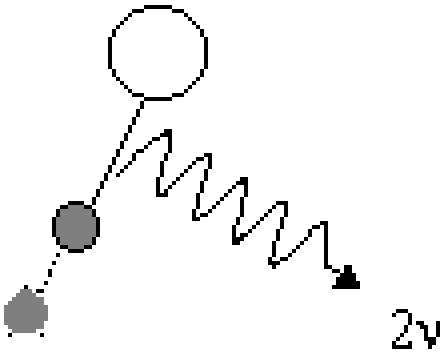


The inelastic scattering of the photons , as they excite to virtual vibrational states and emit a photon with a lower frequency (Stocks) or higher (anti-Stocks)

The signal is significantly weaker than the fluorescence signal
Peaks are specific for a particular type of molecular bonds

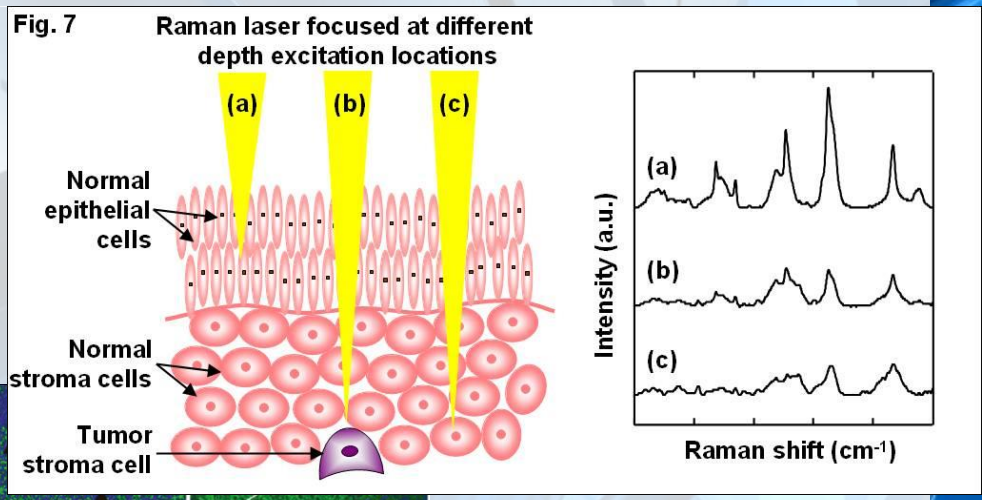


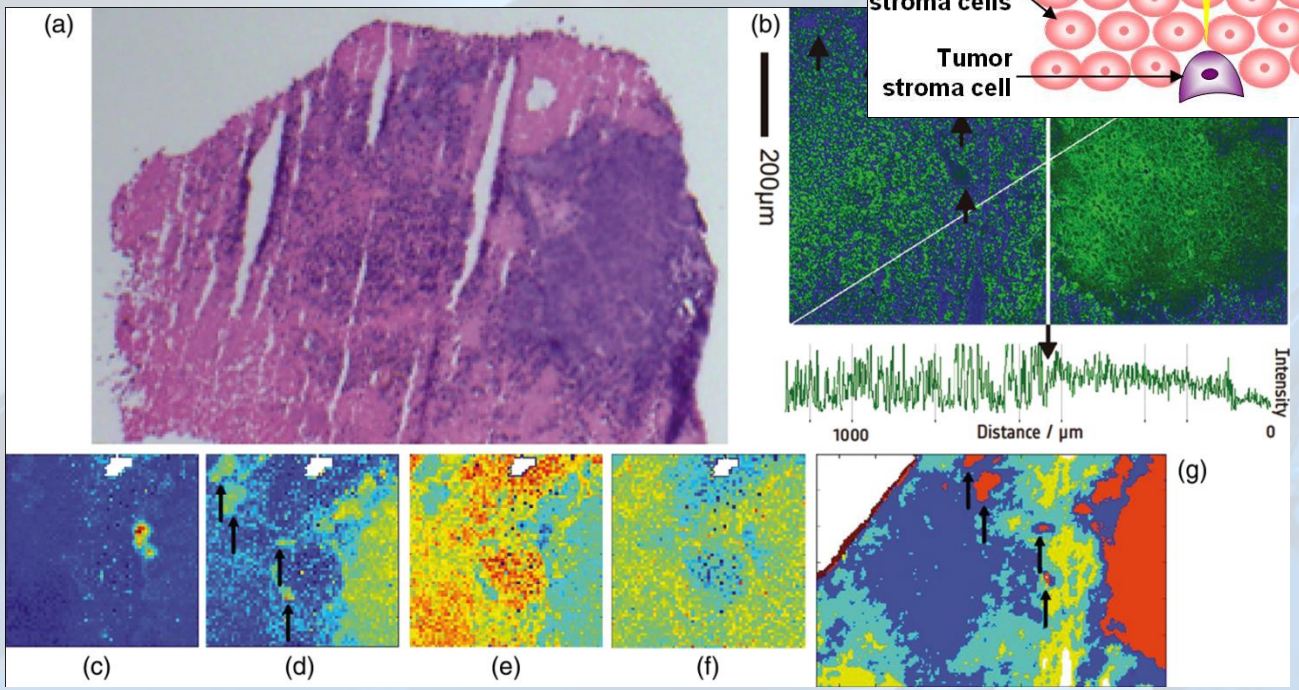




Raman scattering to detect tumor cells

Brain tumour- GBM





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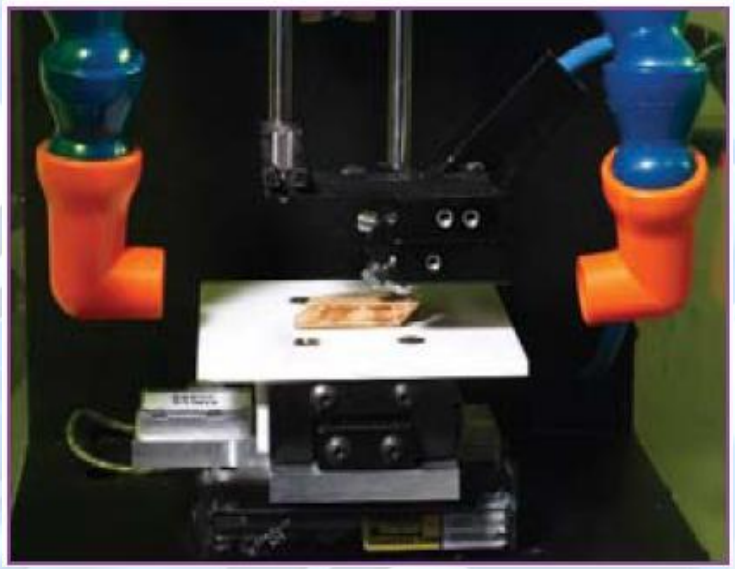
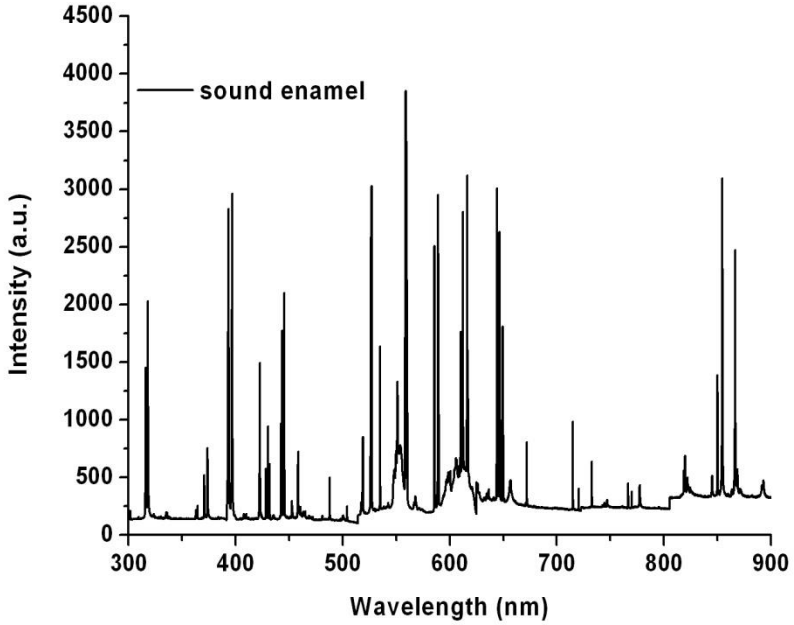
Atomic-emission spectroscopy

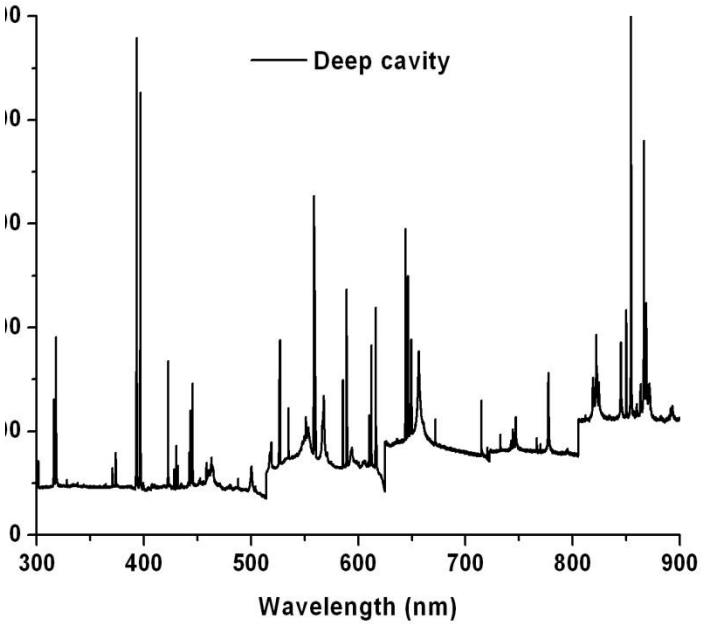
LIBS 2500Plus (Ocean Optics Inc.):
- 7 spectrometric channels – 200 – 980 nm
- Q-switched Nd:YAG laser, 200 mJ
- sample chamber
- fiber bundle for 7-channels LIBS system

- highly sensitive
- in real time
- traces of heavy metals
- forensic science



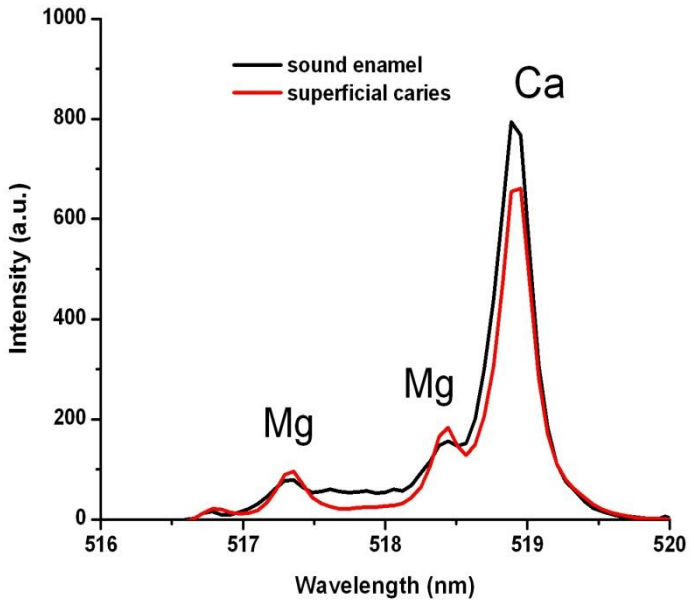
LIBS - results



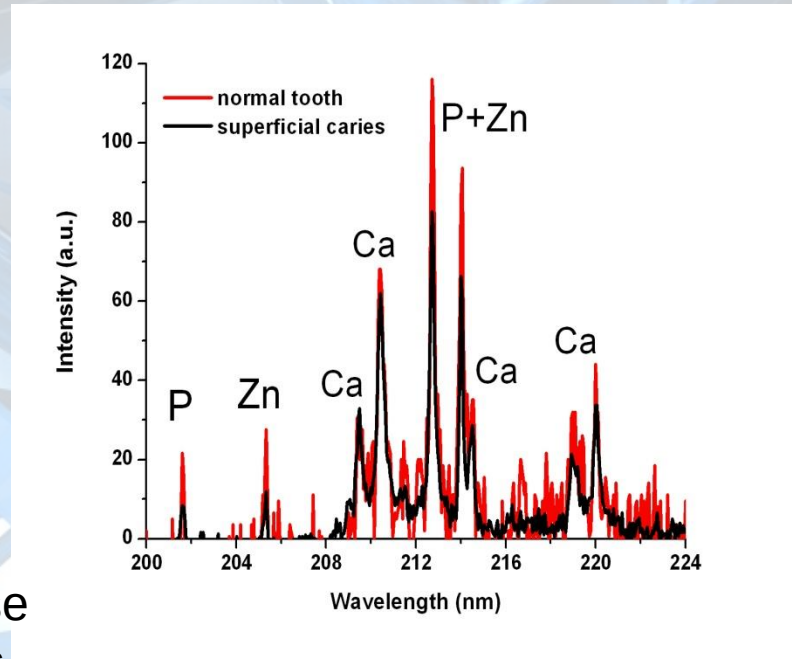


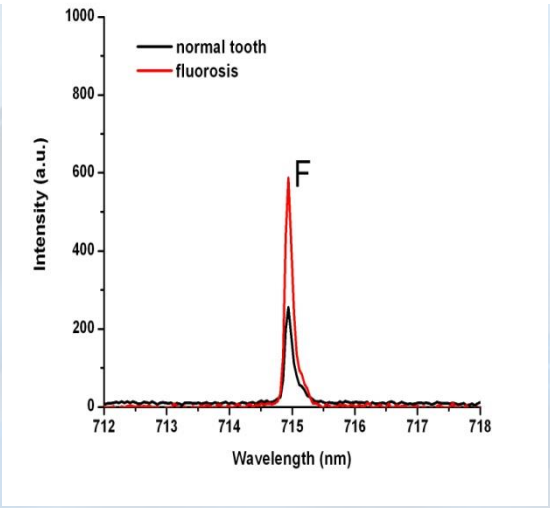
Atomic lines of Ca, P - hydroxyapatite
C, Zn, Cu - bacterial traces, porphyrins, organic matter
Mn, Cu, Fe - in the toothpaste;
Sn, Ti, Ag - traces associated with toothpastes;
Microelements - Na, Al, K, Mg, Si, H - related to the ion exchange of Ca and P in the demineralisation process of the solid tissues

LIBS results



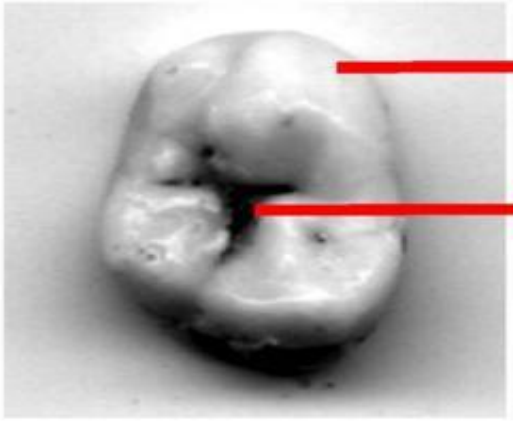
Ca decreases in excess of Mg - the ion exchange process in the carious lesions





A sharp increase in F on fluorosis - Ca ion exchange

Zn and P increase associated with organic matter and bacterial metabolism products

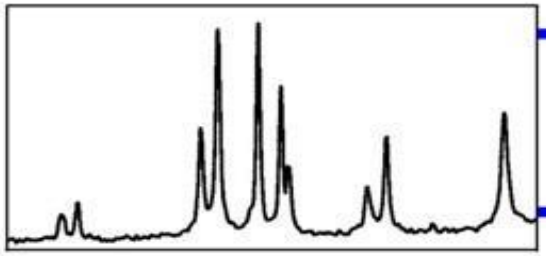


Reference spectra
"healthy tissue"

Reference spectra
"carious tissue"

Generation of discriminant models:
"healthy" and "carious"
and reference library

unknown sample



Discriminant model
Healthy / Carious

YES

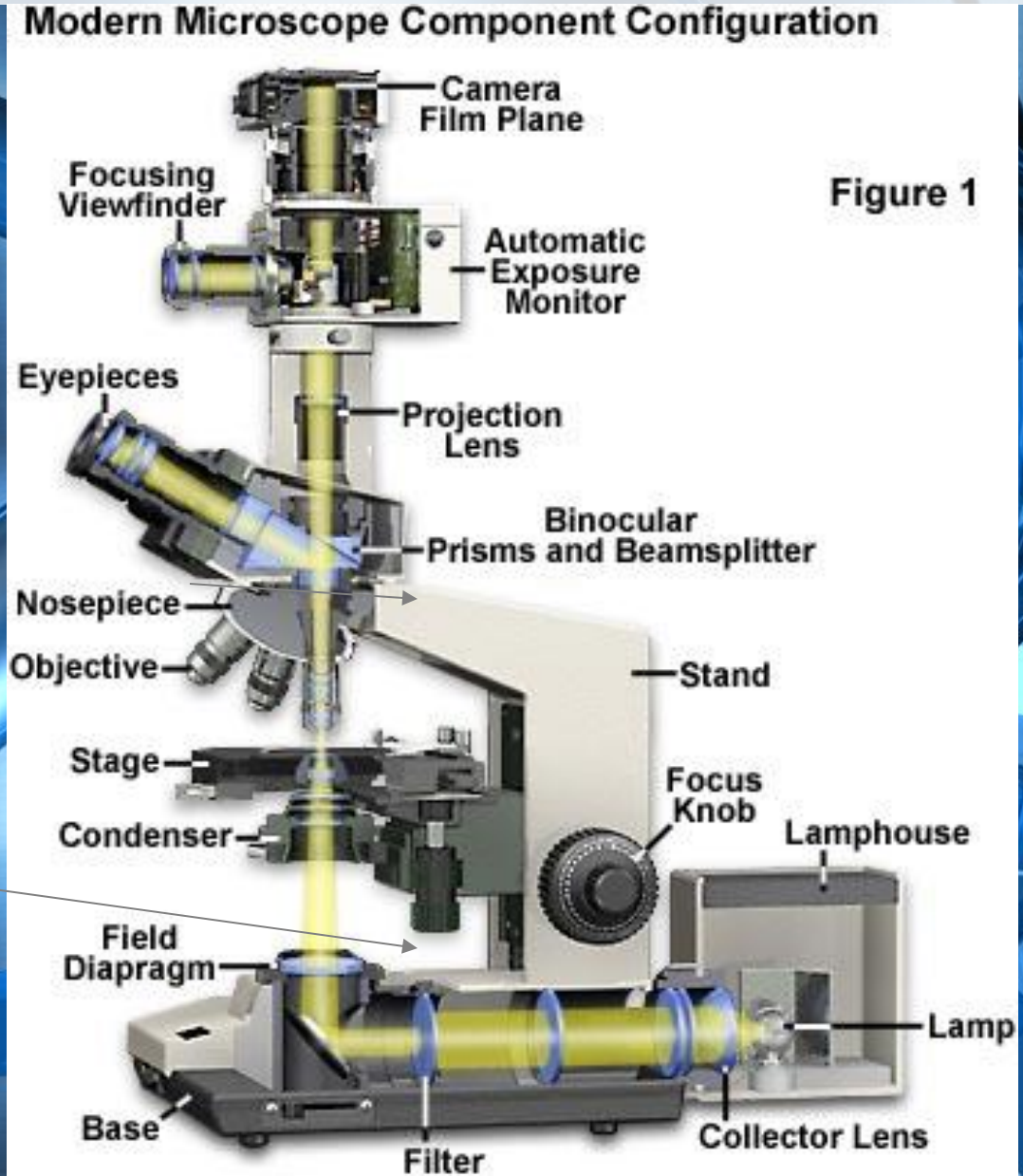
NO

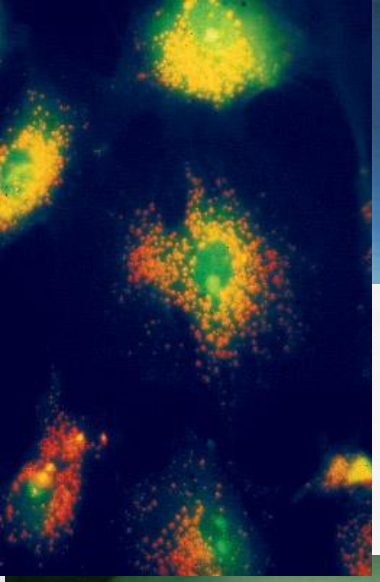
Estimated SE ~ 95%, SP ~ 92%

Optical microscopy

- Cell microscopy
  - Analysis of basic cell functions of healthy and diseased cells
  - Analysis of the role of specific proteins and cellular components and their interaction
- Tissue (intravital) microscopy
  - Analysis of interactions in the cell matrix that lead to the induction, development and / or regression of disease states
- Drug / therapeutic interactions and optimization
- Early detection

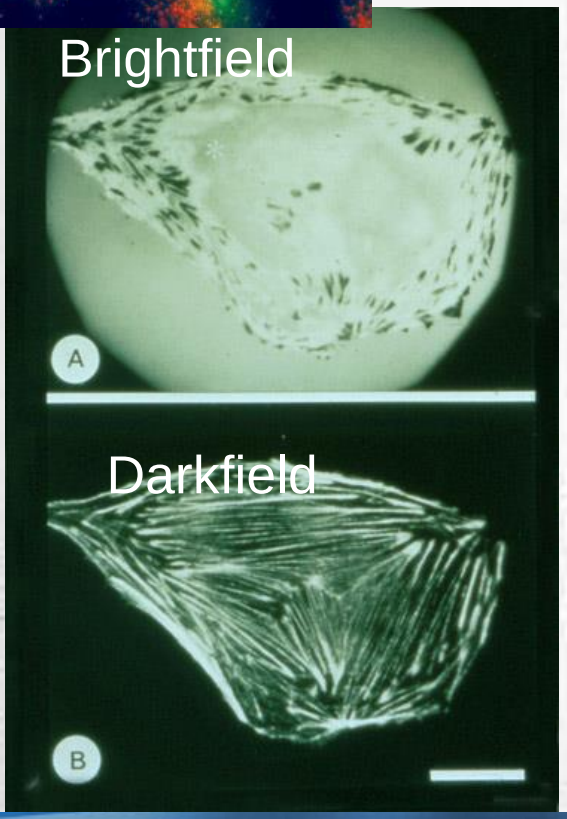
Contemporary microscope

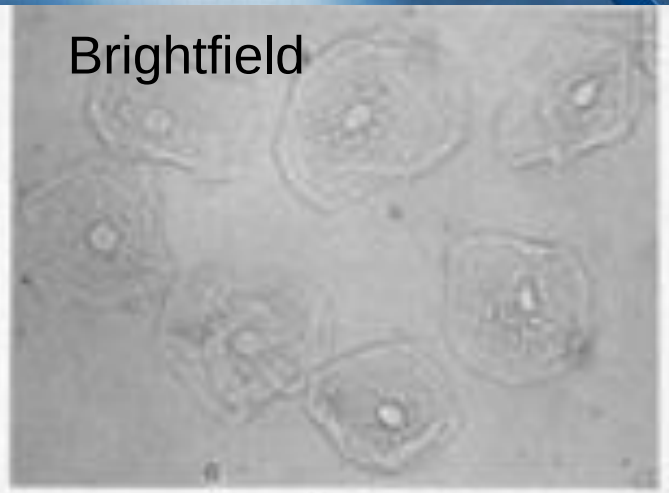


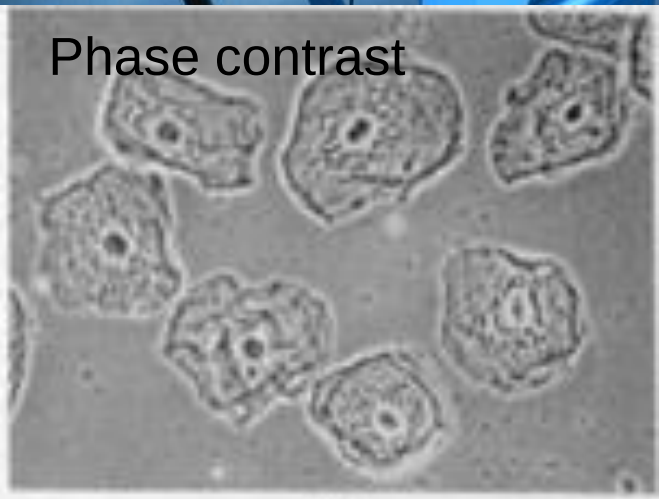


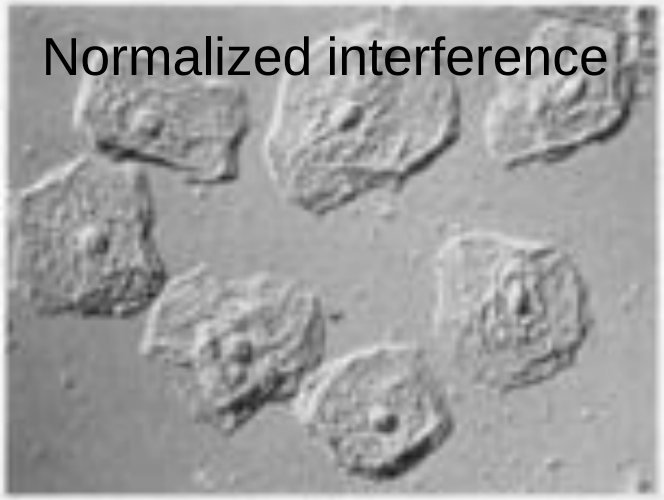
Fluorescence

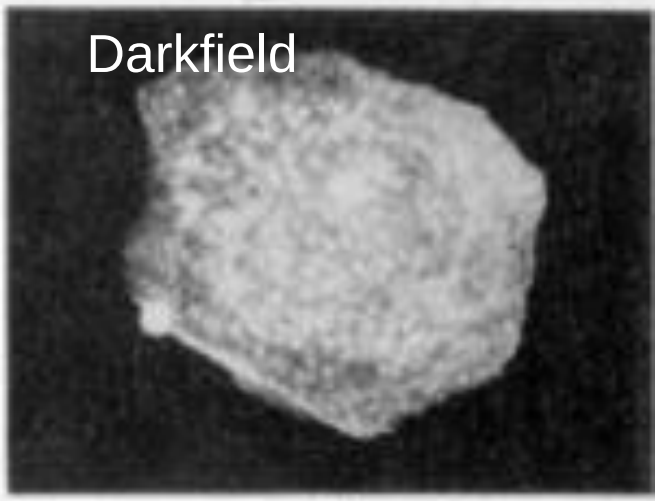
Index of refraction











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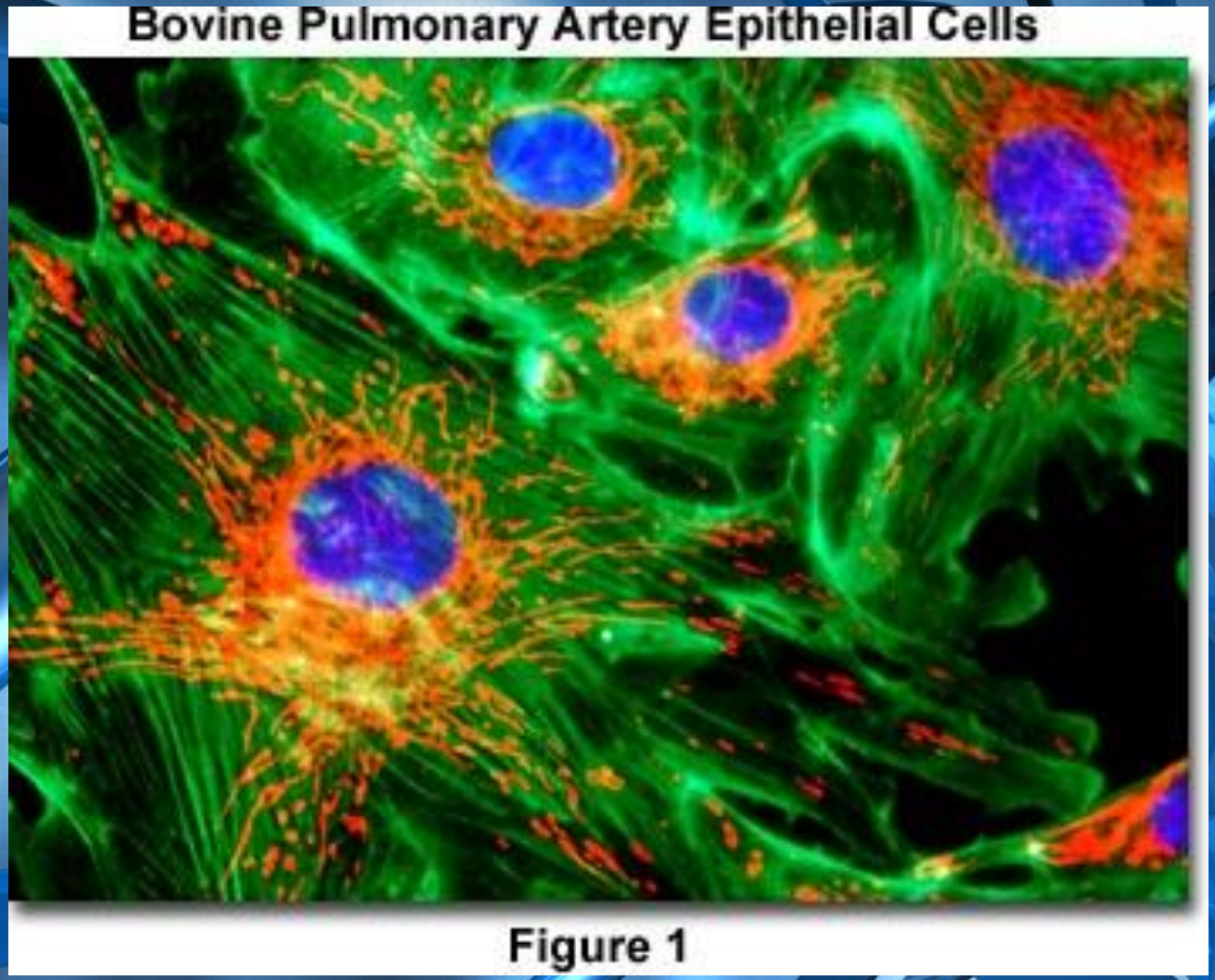
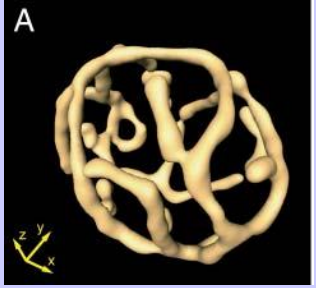
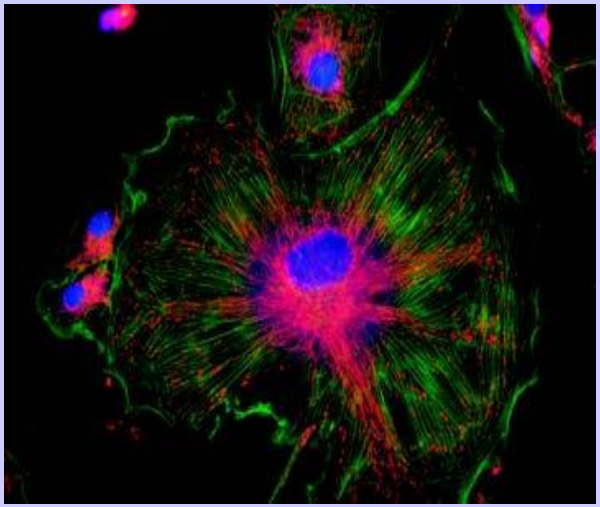
Multi-Wavelength Immunofluorescence Microscopy





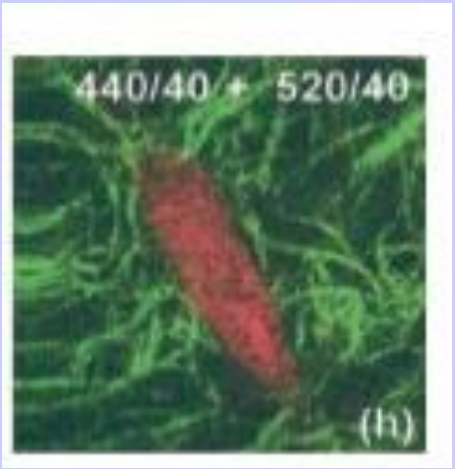
4-Pi microscopy
Mitochondrial network
of live bacterial cell
80 nm res

From organelles to cells



Triple stained endothelial
Cell of pulmonary artery

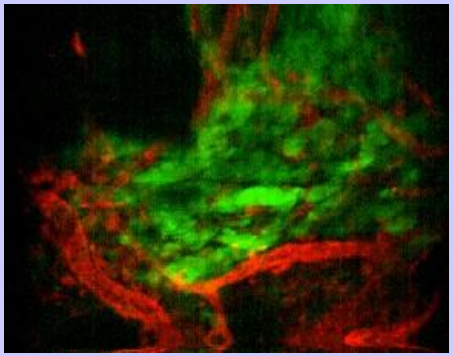
From cells to tissues

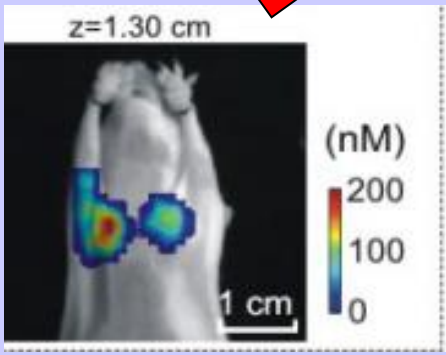


From tissues to animals

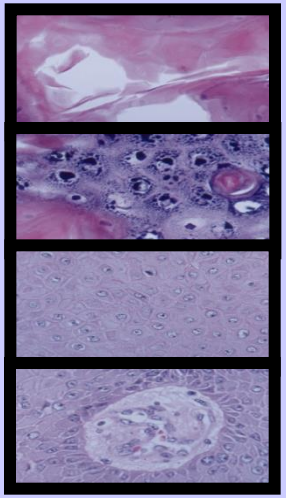
Engineered tissue:
Fibroblast (red) in
collagen matrix (green)
Endogenous signal

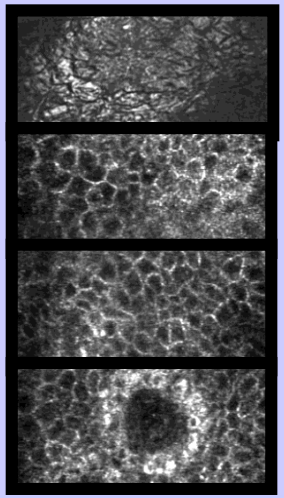
Tumors and
blood vessels
imaged in vivo





From animals to humans



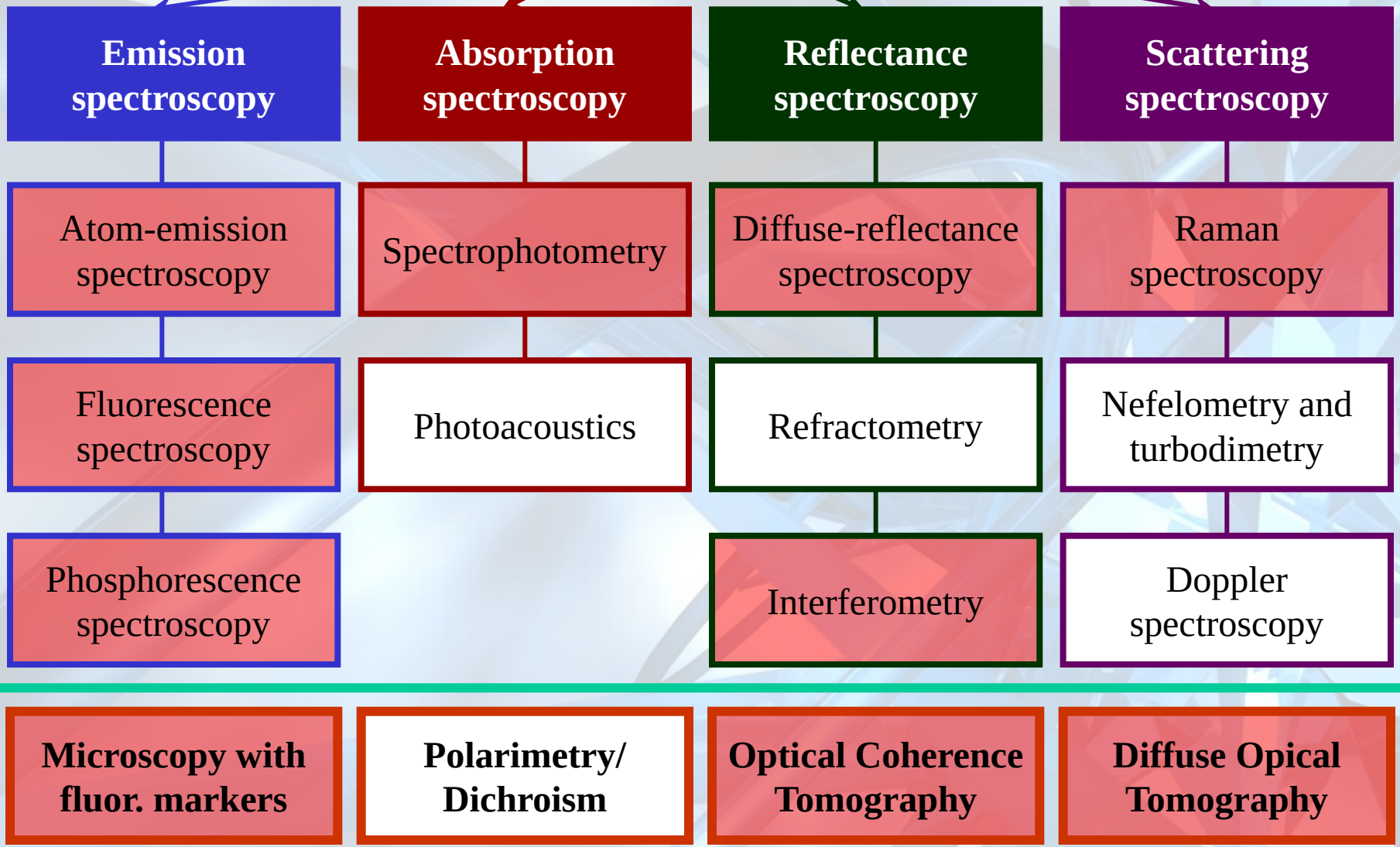


"*En face*" SECTION of human skin

Multimodal optical detection methods

- Objective: Biochemical and morphological information is collected to obtain a more accurate (more sensitive, more specific) detection
- Combinations:
- Fluorescence + diffuse reflection + scattering
- Fluorescence + Raman
- OCT + fluorescence
- Reflection and fluorescence 2-D images
- Reflection and colorimetry

Spectral methods in medicine



Thank you very much for your attention!