

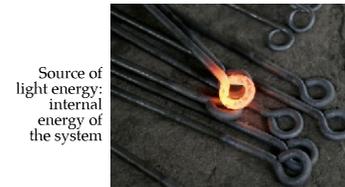
LIGHT EMISSION

MIKLÓS KELLERMAYER

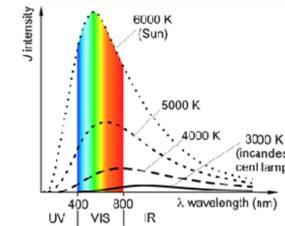
SOURCE OF EMISSION

1. Thermal (black body) radiation

Mechanism: thermal motion of atoms, molecules

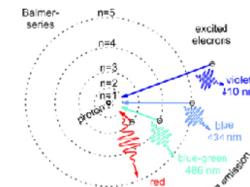


Source of light energy: internal energy of the system

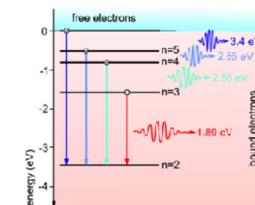


2. Luminescence

Mechanism: emission of excited-state energy



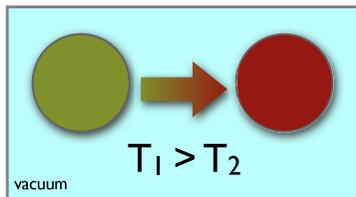
Source of light energy: energy of excited state



“Black-body” (Thermal) radiation

One way of generating of light (besides *luminescence*)

Electromagnetic radiation emitted from all matter due to its possessing thermal energy



Heat exchange, temperature equilibration



- High-temperature objects emit light.
- The greater the temperature of the body, the smaller the wavelengths that appear in its emission spectrum.

... what is a “black body”...?

A black body absorbs all the light falling on it

Objects not only emit radiation but absorb it as well.

Ratio of spectral emissive power (M) and absorptivity (α) is constant (Kirchoff's law):



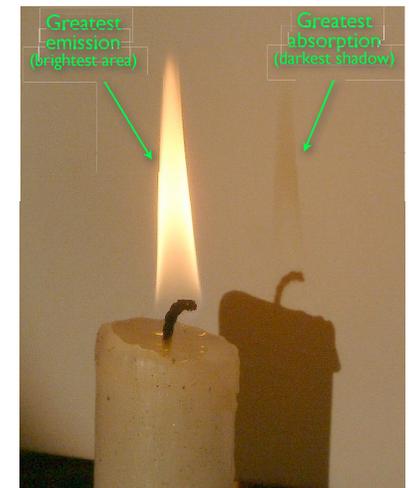
Gustav Robert Kirchoff (1824-1887)

$$\frac{M_{\lambda i}}{\alpha_{\lambda i}} = \frac{M_{\lambda j}}{\alpha_{\lambda j}}$$

For a black body (BB):

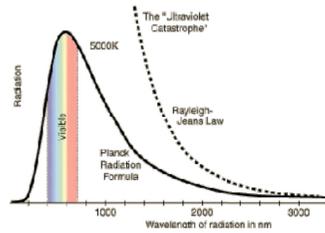
$$\alpha_{\lambda BB} = 1$$

- That is, the black body absorbs all light that it is exposed to (nothing is reflected).
- The black body is an ideal object for investigating temperature-dependent emission.



Black-body radiation

Properties and inferences



Stefan-Boltzmann law:

$$M_{BB}(T) = \sigma T^4$$

M_{BB} = emissive power, area under emission spectrum.

Wien's displacement law:

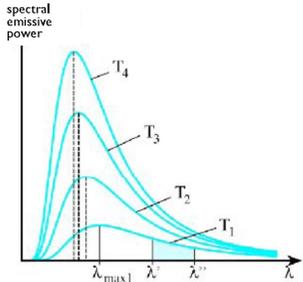
$$\lambda_{max} T = const$$

Planck's law of radiation:

$$E = hf$$

h = Planck's constant (6.626×10^{-34} Js).

Meaning: energy is absorbed and emitted in discrete packets (*quanta*).



Jozef Stefan (1835-1893)



Ludwig Eduard Boltzmann (1844-1906)



Wilhelm Wien (1864-1928)

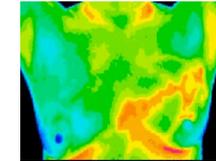


Max Karl Ernst Ludwig Planck (1858-1947)

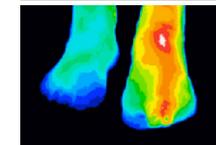
APPLICATIONS OF THERMAL RADIATION

Thermography, infradiagnostics

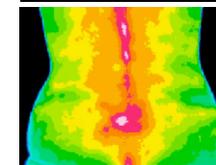
"Seeing through" a non-absorbing layer



Breast carcinoma



Inflammation



Chronic musculoskeletal stress (pain)

Airport thermography

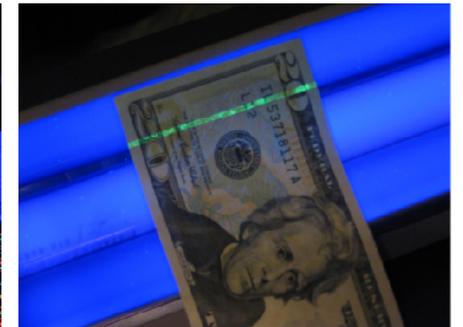


Detection of febrile condition, prevention of epidemics

Definitions of luminescence

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

Emission by luminescence: everywhere



Photoluminescence

Luminescence everywhere

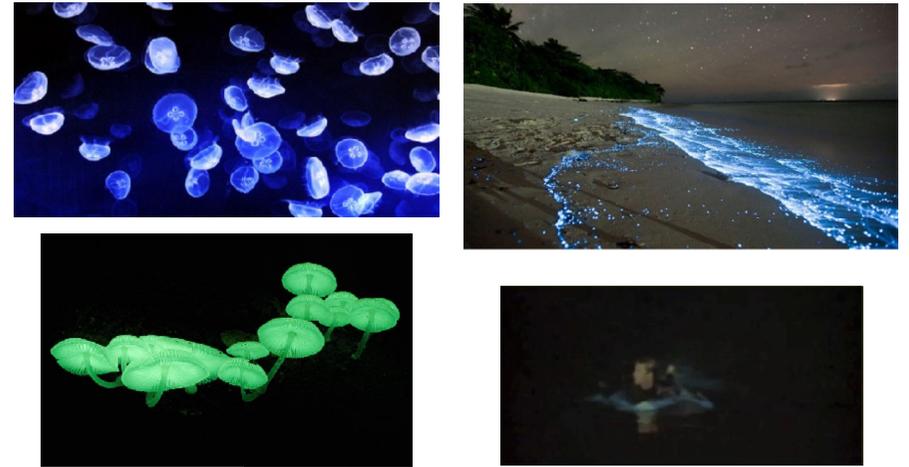


Display lights

Radioluminescence

Aurora borealis

Luminescence everywhere



Bioluminescence

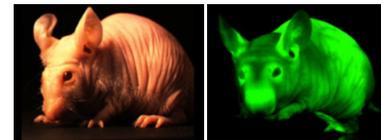
Luminescence everywhere



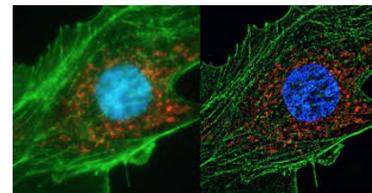
Bioluminescence
Firefly



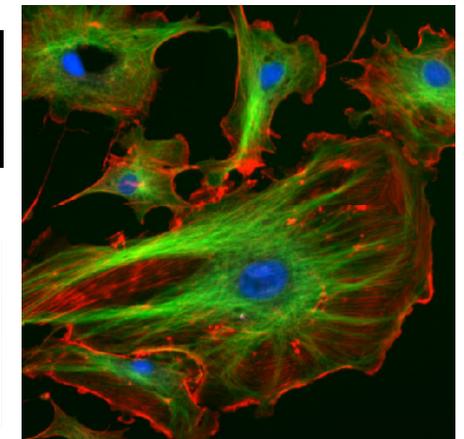
Luminescence everywhere



GFP-mouse (green nude mouse)



Superresolution microscopy (Nobel-prize 2014)



Epifluorescence microscopy (cytoskeletal system)

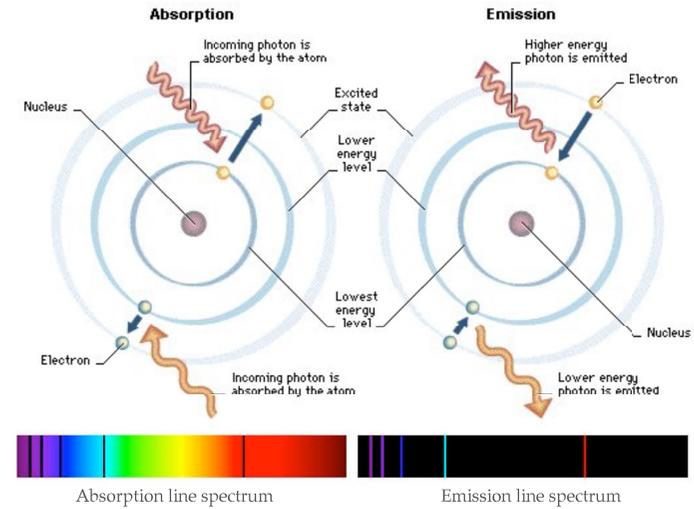
Fluorescence

Types of luminescence

(a) Excitation Mode	Luminescence Type
absorption of radiation (UV/VIS)	photoluminescence
chemical reaction	chemiluminescence, bioluminescence
thermally activated ion recombination	thermoluminescence
injection of charge	electroluminescence
high energy particles or radiation	radioluminescence
friction	triboluminescence
sound waves	sonoluminescence

(b) Excited State (Assuming Singlet State)	Luminescence Type
first excited singlet state	fluorescence, delayed fluorescence
lowest triplet state	phosphorescence

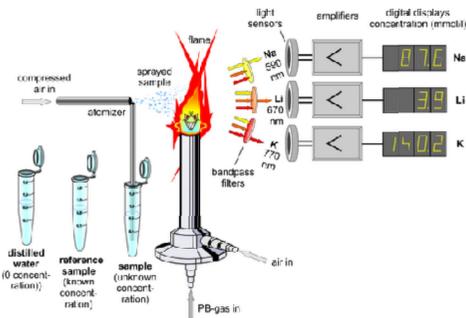
ABSORPTION AND EMISSION BY AN ATOM



APPLICATION OF EMISSION SPECTROSCOPY

Flame photometry

Qualitative and quantitative analysis of alkali metals

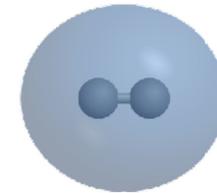


Clinical practice: determination of serum ions (Na⁺, K⁺)



STATE OF A MOLECULE IS AFFECTED BY ITS MOTIONAL MODES

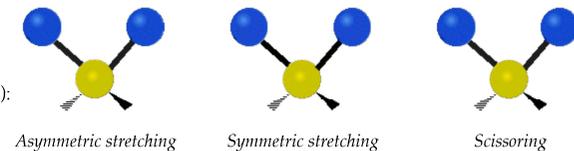
Molecule: atoms connected by chemical bonds
Simplest case: diatomic molecule (e.g., hydrogen molecule)



Molecules *vibrate* and *rotate*!

Vibration: periodic motion *along* the axis of the covalent bond
Rotation: periodic motion *around* the axis of the covalent bond

Examples of vibrational motion in the triatomic methylene group (-CH₂-):



ENERGY OF A MOLECULE



Max Born
(1882-1970)



J. Robert Oppenheimer
(1904-1967)

Born-Oppenheimer approximation:

$$E_{total} = E_e + E_v + E_r$$

Important notions:

- Types of energy states are independent (not coupled)
- Energy states are non-continuous, but discrete
- Transition between states involves packets (quanta) of energy
- Scales of transition energies between different states are different:

$$E_e \sim 100\times E_v \sim 100\times E_r$$

$$\sim 3 \times 10^{-19} \text{ J } (\sim 2 \text{ eV}) > \sim 3 \times 10^{-21} \text{ J} > \sim 3 \times 10^{-23} \text{ J}$$

("Rule of thumb": *ultraviolet* > *visible* > *infrared*)

SPIN STATES

Wolfgang Pauli
(1900-1958)



Pauli's exclusion principle:

- Each quantum state can be occupied by a single electron.
- Within an atom there cannot be two electrons for which all four quantum numbers are identical.

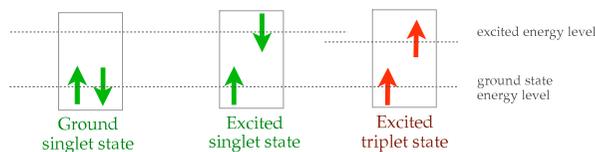
fully occupied subshell: spin pairing (opposite-spin electrons pair)

Singlet and triplet states:

number of orientations of magnetic moment associated with net spin state (in magnetic field) = $2S+1 = 1$ (*singlet*) or 3 (*triplet*). (S = net spin, e.g., in fully occupied subshell $(+1/2)+(-1/2) = 0$)

S: singlet state: paired electrons with opposite spins, net spin (S) = 0, number of orientations ($2S+1$) = 1.

T: triplet state: there are identical spin-state electrons in the molecule, net spin = 1 (e.g., $(+1/2)+(+1/2) = 1$), number of orientations ($2S+1 = 2+1$) = 3.

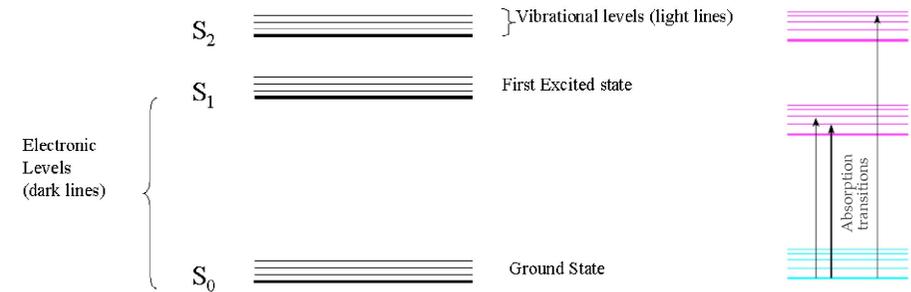


REPRESENTATION OF ENERGY STATES

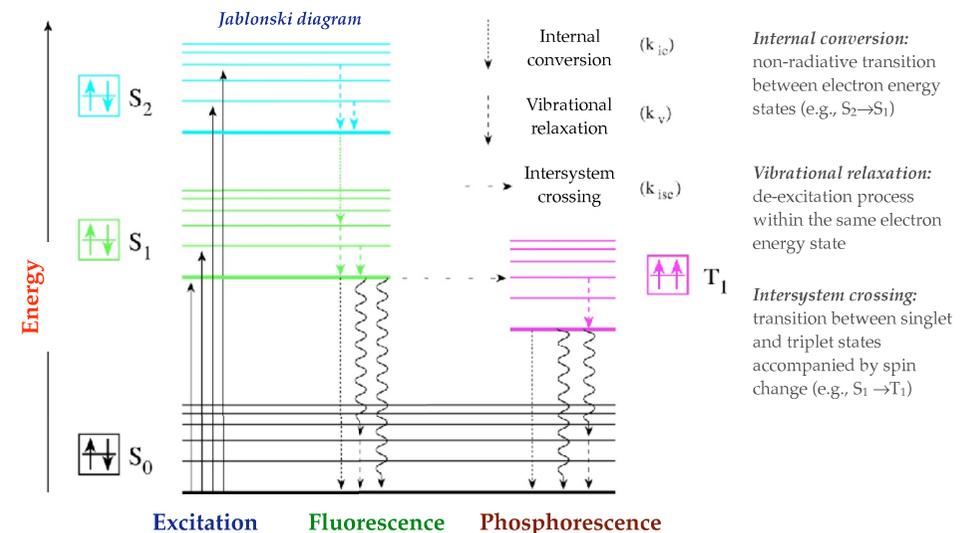
Jabłoński diagram:
illustrates the electronic states of a molecule and the transitions between them (with arrows)



Alexander Jabłoński
(1898-1980)



PROCESSES OF LUMINESCENCE



Internal conversion: non-radiative transition between electron energy states (e.g., $S_2 \rightarrow S_1$)

Vibrational relaxation: de-excitation process within the same electron energy state

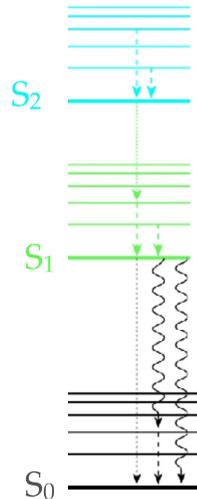
Intersystem crossing: transition between singlet and triplet states accompanied by spin change (e.g., $S_1 \rightarrow T_1$)

KASHA'S RULE

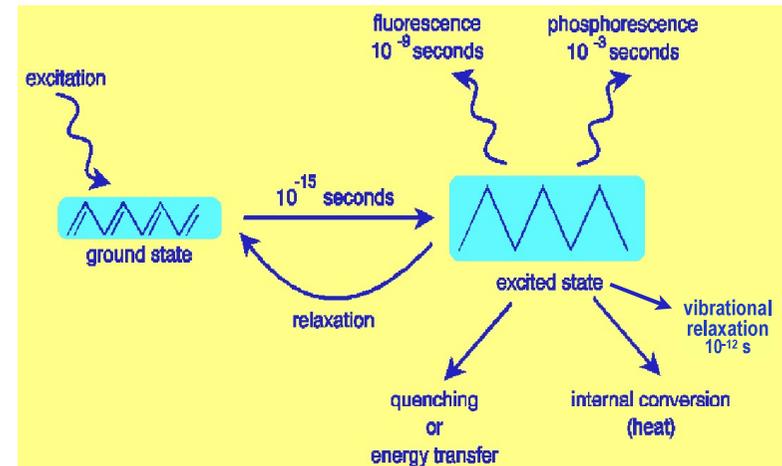
Photon emission (fluorescence or phosphorescence) occurs only from the lowest-energy excited electronic state (i.e., S_1 or T_1) of a molecule.



Michael Kasha (1920-) American physicist

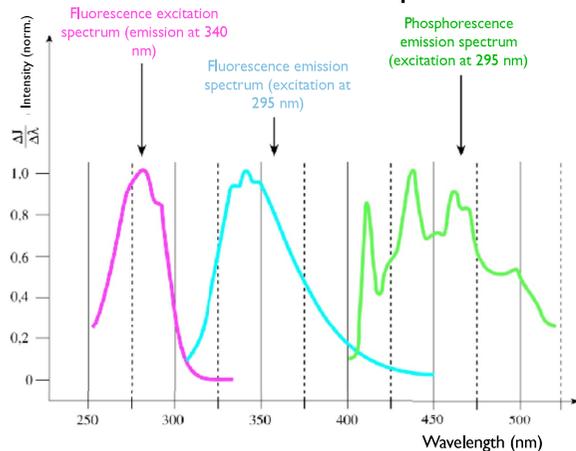


TIME SCALE OF TRANSITIONS



Characterization of luminescence

Luminescence spectra



- Band spectra
- Fluorescence excitation and emission spectra are symmetric
- Stokes shift



George Stokes (1819-1903)

Fluorescent dyes: "fluorophores"
By the specific attachment fluorophores, non-fluorescent molecules may also be studied (fluorescent labeling)

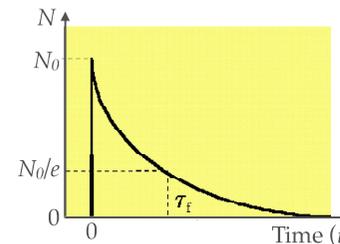
CHARACTERIZATION OF LUMINESCENCE II.

Quantum yield

$$\Phi = \frac{\text{number of emitted photons}}{\text{number of absorbed photons}} \leq 1 \quad \Phi = \frac{k_f}{k_f + k_{nr} + k_{isc} + k_Q}$$

k_{nr} = non-radiative transition rate constants

Excited-state lifetime (τ)



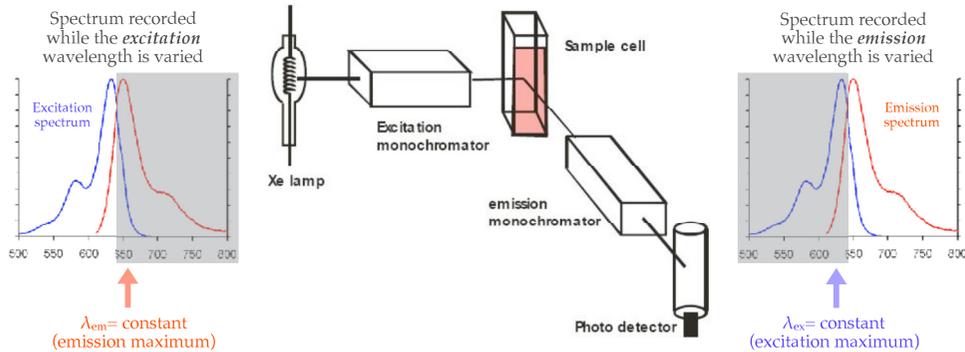
$$N = N_0 e^{-(k_f + k_{nr})t}$$

N = molecules in excited state
 t = time
 k_f = fluorescence rate constant
 k_{nr} = non-radiative transition rate constant
 τ = fluorescence lifetime

$$\tau = \frac{1}{k_f + k_{nr}}$$

MEASUREMENT OF FLUORESCENCE

Fluorescence spectrometer
("Steady-state" spectrofluorometer)

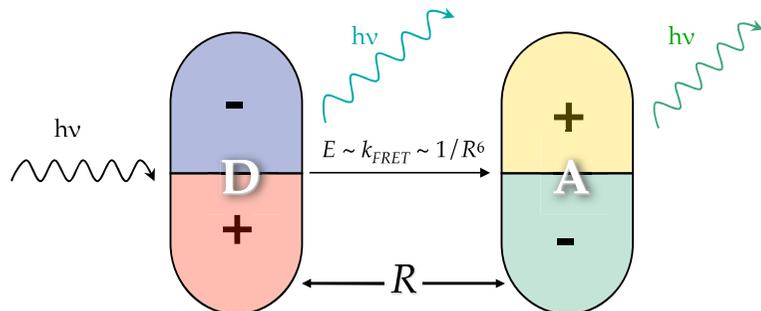


Förster Resonance Energy Transfer (FRET)

- Occurs by non-radiative dipole-dipole interaction between an excited **donor (D)** and an proper **acceptor (A)** molecule under certain conditions (spectral overlap and close distance).
- Fluorescence Resonance Energy Transfer (FRET)**: if the participants of the transfer are fluorophores.



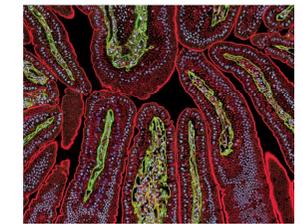
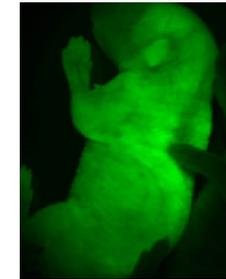
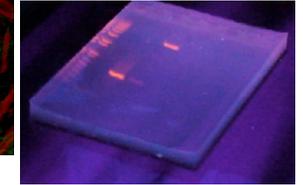
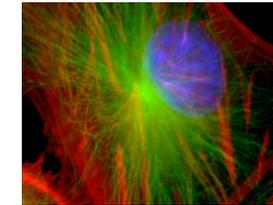
Theodor Förster
(1910-1974)



Biomedical applications of fluorescence

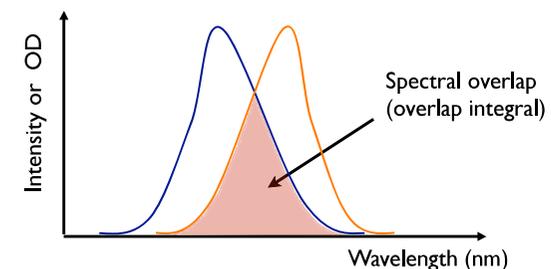
A few examples:

- Fluorescence microscopy
- DNA sequencing (chain termination method)
- DNA detection (EtBr)
- DNA microarray
- Immunofluorescence
- Fluorescence-activated cell sorting (FACS)
- Förster resonance energy transfer (FRET)
- Fluorescence recovery after photobleaching (FRAP)
- Fluorescent protein conjugation technologies
- Quantum dots
- etc...



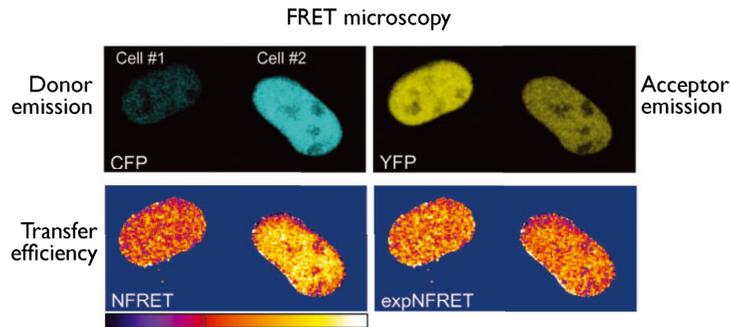
Conditions of FRET

- Fluorescent** donor and acceptor molecules.
- The distance (**R**) between donor and acceptor molecules is 2-10 nm!
- Overlap** between the emission spectrum of the **donor** and the absorption spectrum of the **acceptor**.



Applications of FRET

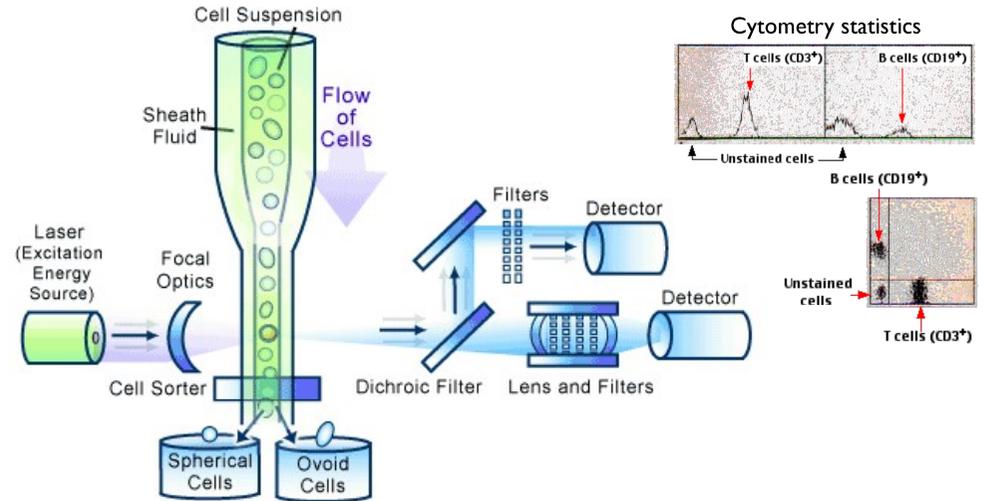
- **Molecular ruler:** distance measurement on the nm (10^{-9} m) scale.
- High sensitivity (see sixth-power dependency)!
- **Applications:**
 - Measurement of **interactions** between molecules.
 - Measurement of **structural** changes on molecules.



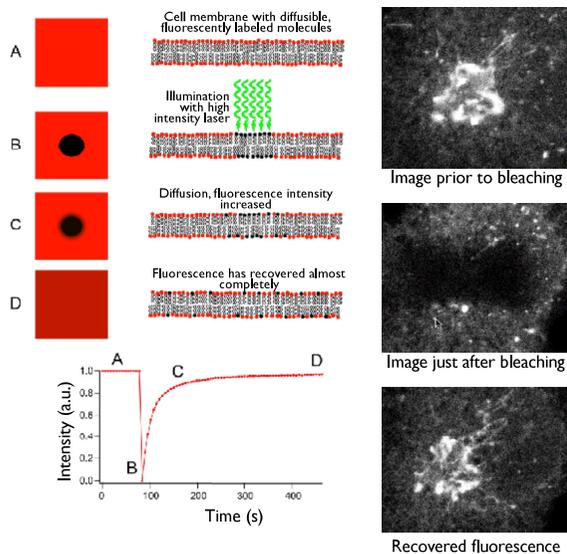
Fluorescence activated cell sorter (FACS)

Flow cytometry

- A cell suspension, fluorescently labeled by using specific antibodies, is analyzed cell by cell
- Numerous parameters are measured simultaneously (fluorescence intensity at several wavelengths, small- and large-angle scatter)
- Statistical analysis
- If needed, cells can be separated according to their fluorescence



Fluorescence Recovery After Photobleaching (FRAP)

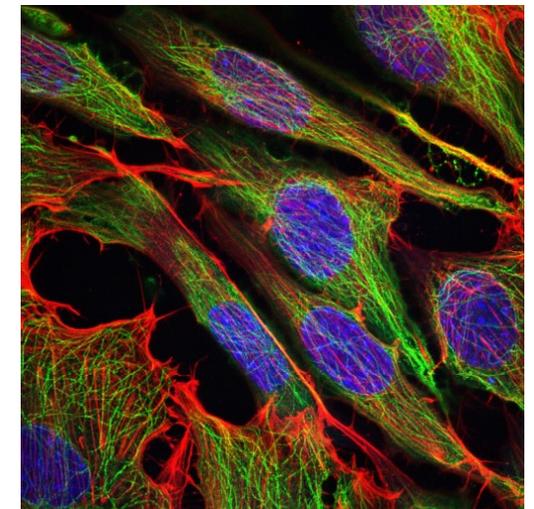
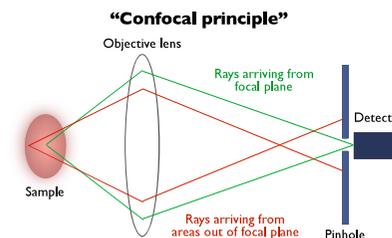
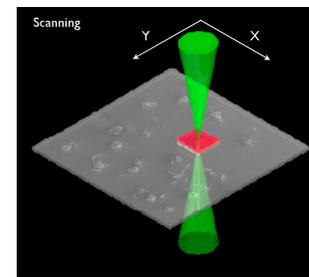


The diffusion coefficient can be determined from the time-dependent recovery of fluorescence as:

$$D = \frac{w^2}{4t_D}$$

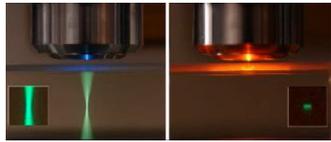
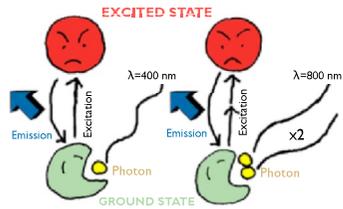
D = diffusion coefficient
w = width of bleached area
t_D = time constant

Laser scanning confocal microscopy

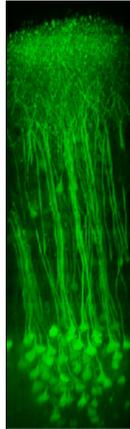


Multiphoton microscopy

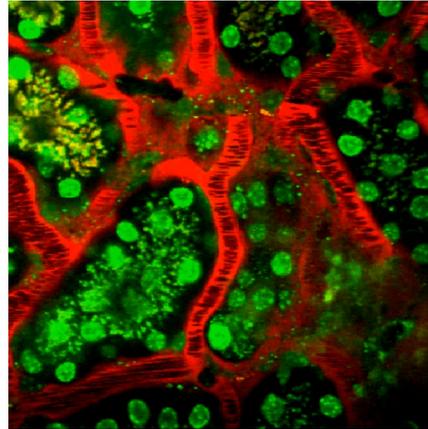
- Energy of two (or more) photons are added during excitation
- Excitation (hence emission) only in the focal point (limited photodamage)
- Excitation with long wavelength (near-IR), short (fs) light pulses
- Large (up to 2 mm) penetration due to long wavelength



Single-photon fluorescence Two-photon fluorescence



Cortical pyramidal cells

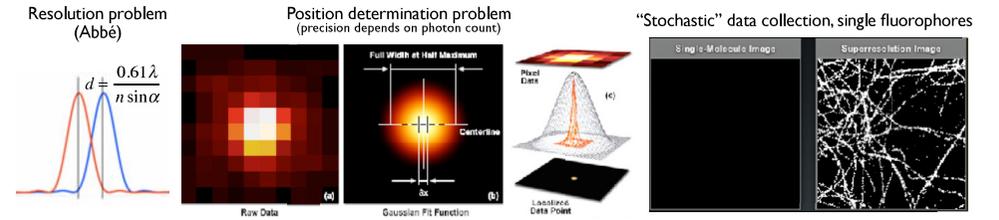


Green: proximal kidney tubules; Red: albumin (plasma)

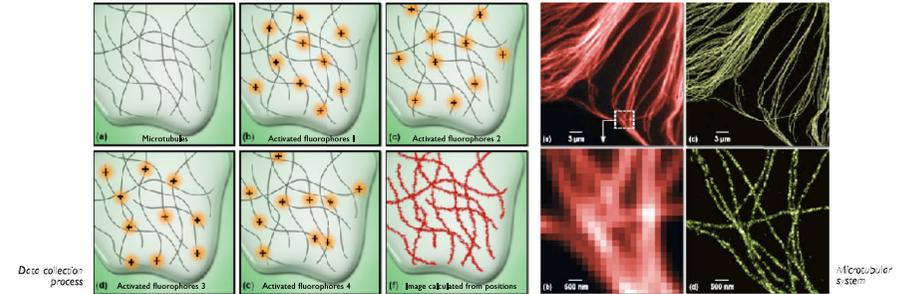
Super-resolution microscopy

Chemistry Nobel-prize, 2014

Resolution problem is converted into position-determination problem

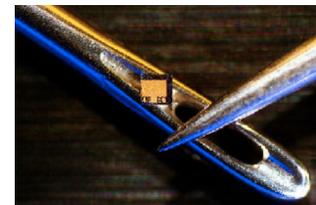


STORM ("stochastic optical reconstruction microscopy"); PALM ("photoactivated localization microscopy")

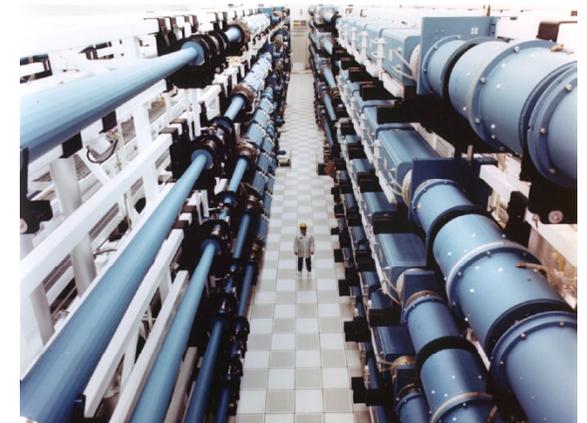


LASER

Lasers are everywhere



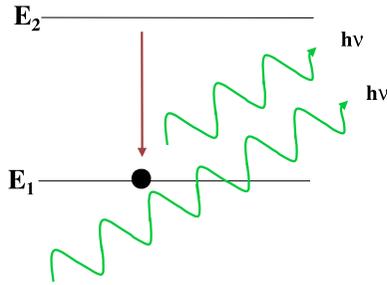
5 mW diode laser
few mms



Terawatt NOVA laser - Lawrence Livermore Laboratories
Size of a football field

Laser:

“Light Amplification by Stimulated Emission of Radiation”



Luminescent light source based on light amplification.
MASER: Microwave Amplification by Stimulated Emission of Radiation

LASER HISTORY IN A NUTSHELL



- **1917** - *Albert Einstein*: theoretical prediction of stimulated emission.
- **1946** - *G. Meyer-Schwickerather*: first eye surgery with light.
- **1950** - *Arthur Schawlow and Charles Townes*: emitted photons may be in the visible range.
- **1954** - *N.G. Basov, A.M. Prochorow, and C. Townes*: ammonia maser
- **1960** - *Theodore Maiman*: first laser (ruby laser)
- **1964** - *Basov, Prochorow, Townes (Nobel-prize)*: quantum electronics
- **1970** - *Arthur Ashkin*: laser tweezers
- **1971** - *Dénes Gábor (Nobel-prize)*: holography
- **1997** - *S. Chu, W.D. Phillips and C. Cohen-Tanoudji (Nobel-prize)*: atom cooling with laser.
- 2013, october 8: *NIF (National Ignition Facility, USA)*: launching nuclear fusion with 192 laser beams, positive energy balance.

Principles of laser I. stimulated emission

<p>1. Absorption</p> <p>Frequency of transition: $n_{12} = N_1 B_{12} \rho(\nu)$</p> <p>$\Delta E = E_2 - E_1 = h\nu$ energy quantum is absorbed.</p>	<p>2. Spontaneous emission</p> <p>Frequency of transition: $n_{21} = N_2 A_{21}$</p> <p>$E_2 - E_1$ photons travel independently in all directions.</p>	<p>3. Stimulated emission</p> <p>Frequency of transition: $n_{21} = N_2 B_{21} \rho(\nu)$</p> <p>In the presence of external field. Field energy is increased. Phase, direction and frequency of emitted and external photons are identical.</p>
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Explanation: two-state atomic or molecular system.
 E_1, E_2 : energy levels, $E_2 > E_1$
 $\rho(\nu)$: spectral energy density of external field.
 N_1, N_2 : number of atoms or molecules on the given energy level.
 B_{12}, A_{21}, B_{21} : transition probabilities (Einstein coefficients), $B_{12} = B_{21}$

Principles of laser II. Population inversion

Light amplification depends on the relative population of energy levels.

J (pink wavy arrow) enters an "Active medium" box of length Δx . K (pink wavy arrow) exits. The output intensity is $J + \Delta J$.

$\Delta J = JK(N_2 - N_1)\Delta x$

J = intensity
 K = constant
 x = distance travelled within medium
 N_1, N_2 = number of atoms on the E level

N.B.: If dJ is positive, more light exits the medium, than has entered!

E_2 level: 2 smiley faces

E_1 level: 6 sad smiley faces

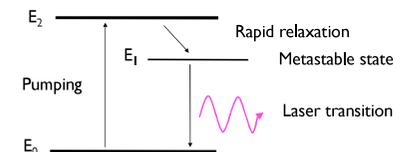
Thermal equilibrium

E_2 level: 6 smiley faces

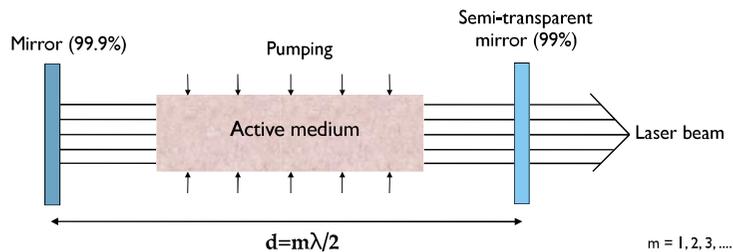
E_1 level: 2 sad smiley faces

Population inversion

- Population inversion only in multiple-state systems!
- Pumping: electrical, optical, chemical energy



Principles of laser III. Optical resonance



Resonator:

- two parallel (or concave) mirrors
- part of the exiting light is coupled back into the medium
- positive feedback -> self-excitation -> resonance

• Optical switch in the resonator: Q-switch, pulsed mode

Properties of laser light I.

1. Small divergence

Parallel (collimated) beam

2. Large power

In continuous (CW) mode, tens, hundreds of W (e.g., CO₂ laser)

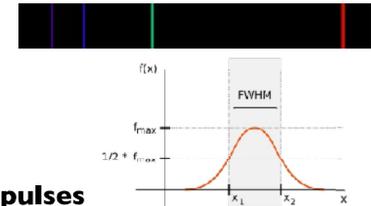
In Q-switched mode, momentary power is enormous (GW)

Because of small divergence, large spatial power density.

3. Small spectral bandwidth

"Monochromaticity"

Large spectral energy density



4. Often polarized

5. Possibility of extremely short pulses

ps, fs

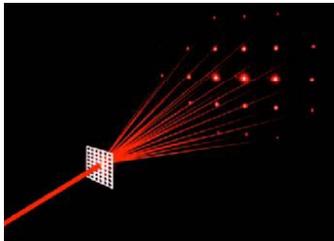
Properties of laser light II.

6. Coherence

phase identity, interference tendency

temporal coherence (phase identity of photons emitted at different times)

spatial coherence (phase identity across beam diameter)



Application: holography, optical coherence tomography

Types of lasers

Based on active medium:

1. Solid state lasers

Metal doping in crystals or glasses; Ruby, Nd-YAG, Ti-sapphire

Red-infrared spectral range; CW, Q-switched mode, large power

2. Gas lasers

Best known: He-Ne laser (10 He/Ne). Small energy, wide use

CO₂ laser: CO₂-N₂-He mixture; λ~10 μm; Huge power (100 W)

3. Dye lasers

Dilute solution of organic dyes (e.g., rhodamine, coumarine); Pumped by another laser.

Large power (Q-switched mode); Tunable

4. Semiconductor lasers

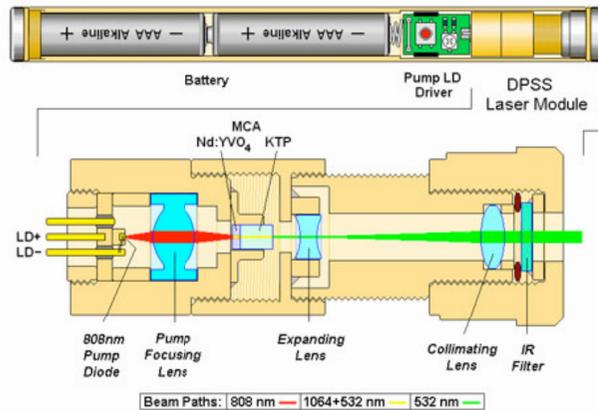
On the boundary of p- and n-type doped semiconductors.

No need for resonator mirrors (total internal reflection)

Red, IR spectral range. Huge CW power (up to 100W)

Beam characteristics are not very good. Wide use because of small size.

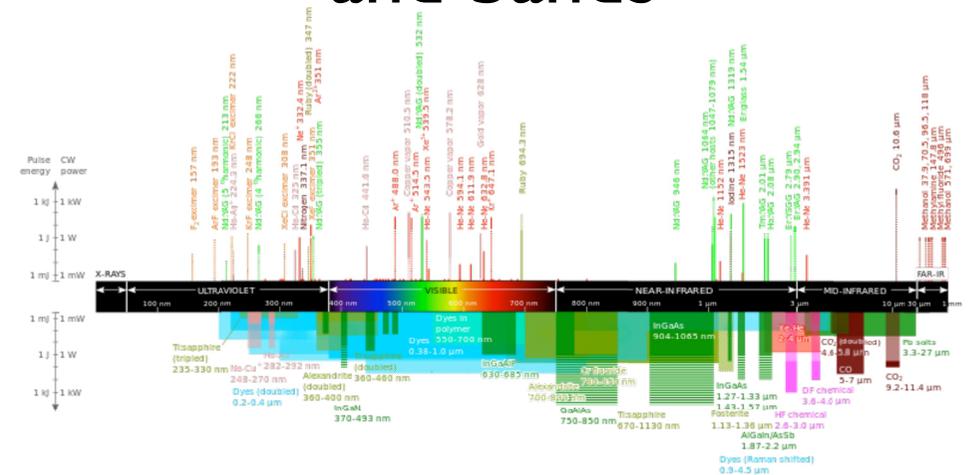
The green laser pointer



- Steps:
1. Diode laser (808 nm) pumps
 2. Solid state laser (neodimium-yttrium-vanadate) generates 1064 nm light
 3. KTP (potassium titanyl phosphate) crystal doubles frequency (halves wavelength): 532 nm (green)

*Notes:
 DPSS: diode-pumped solid state
 MCA: multiple crystal assembly
 LD: laser diode

Lasers, spectral lines and bands



Laser lines are available from X-rays to infrared light.

Application of lasers based on power

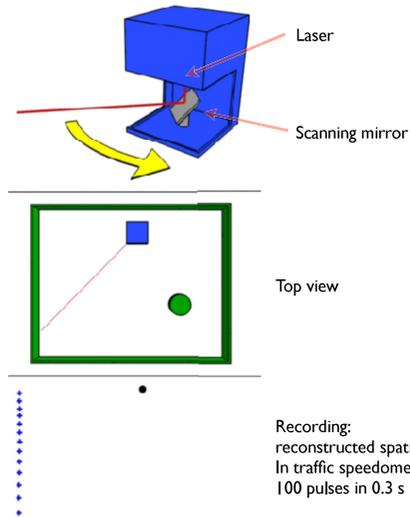
- 5 mW – CD-ROM drive
- 5–10 mW – DVD player or DVD-ROM drive
- 100 mW – high-speed CD-RW writer
- 250 mW – DVD-R writer
- 1–20 W – solid-state laser for micromachining
- 30–100 W – surgical CO₂ laser
- 100–3000 W – industrial CO₂ laser (laser cutter)
- 1 kW – 1 cm diode laser bar

Factors to be considered in laser applications

- Steerability (small divergence)
- Power
- Monochromaticity
- Coherence

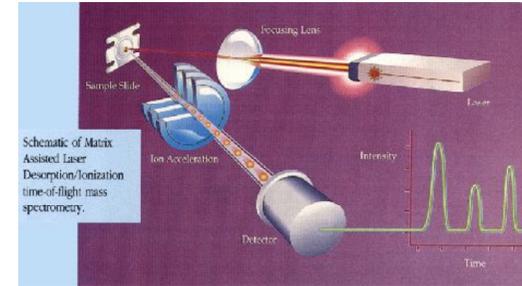
Speed measurement with laser

LIDAR: "Light Detection and Ranging"

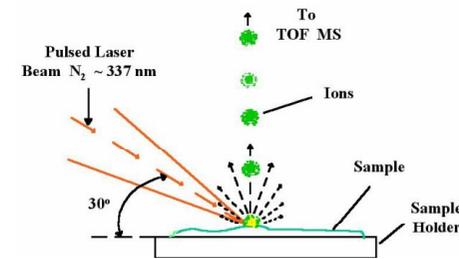


Steerability

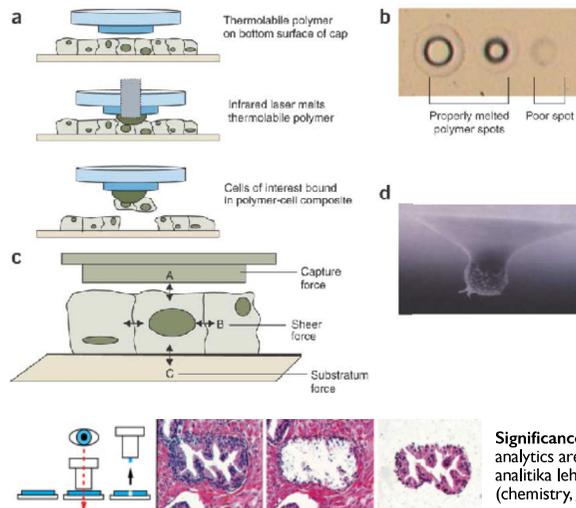
MALDI-TOF: matrix-assisted laser desorption/ionization time of flight mass spectrometry



Power density



"Laser capture microdissection"

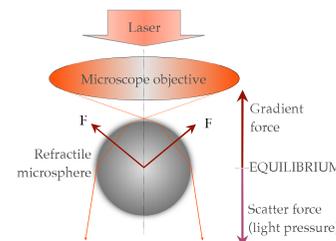


Power density

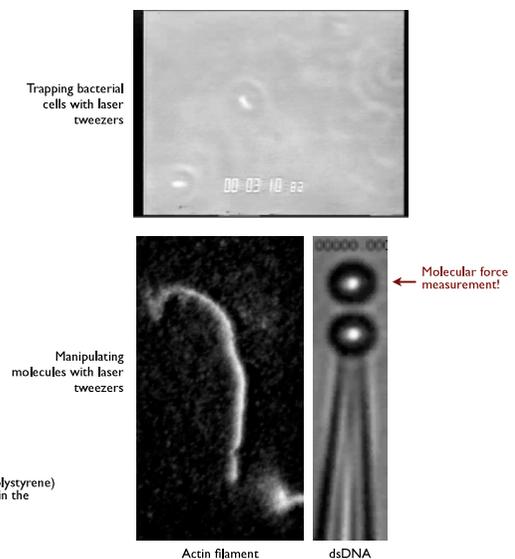
Significance: local analytics are possible
analitika lehetősége (chemistry, genetics)

Laser tweezers

Power density



In the laser tweezer momentum change occurs between the photons and the trapped particle:

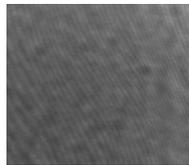
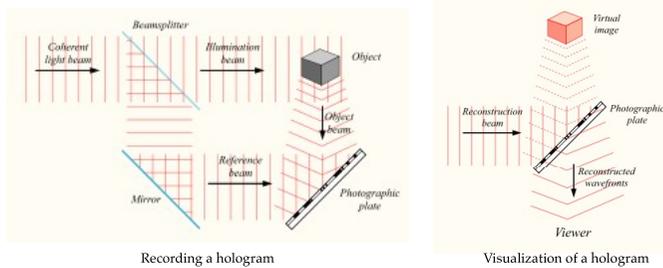


Holography

Coherence



Dénes Gábor
(1900-1979)

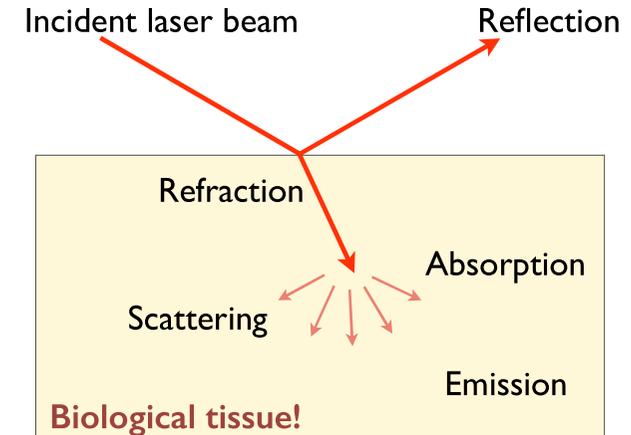


Surface of a hologram recording



Holograms

Biomedical applications of lasers I.



The effects depend not only on the properties of the laser, but also on those of the biological tissue: absorbance, transmittivity, light-induced reactions.

Biomedical applications of lasers II.

Surgical disciplines: “laser knife”, coagulation, blood-less surgery. Tumor removal, tattoo removal. CO₂ and Nd:YAG lasers, holmium laser lithotripsy (urology).

Dermatology: wide-spread uses (tattoo removal, naevus removal, etc.)

Dentistry: caries absorbs preferentially.

Photodynamic tumor therapy: laser activation of photosensitive chemicals preferentially taken up by the tumor.

Ophthalmology: Retina lesions, photocoagulation, glaucoma, photorefractive keratectomy (PRK).

Dermatological applications: I. Considerations

1. Employed wavelengths:

- Argon: 488 or 514.5 nm
- Ruby: 694 nm
- Alexandrite: 755 nm
- Pulsed diode array: 810 nm
- Nd:YAG: 1064 nm

2. Pulse width

3. Size of illuminated area (8-10 mm diameter)

4. Energy flux (J/cm²)

5. Repetition rate (accumulation effects)

6. Epidermal cooling (gels, liquids, sprays, air)

Dermatological applications:

2. Hair removal

Phototricholysis, photoepilation

Basis: selective photothermolysis

selective absorption by chromophores

Employed chromophores:

1. Carbon (exogenous, carbon or graphite-containing creams)
2. Hemoglobin (endogenous)
3. Melanin (endogenous)



Before treatment

After treatment

Dermatological applications:

4. Naevus removal



Before treatment

After treatment

Dermatological applications:

3. Tattoo removal

Q-switched Nd:YAG laser (1064 nm)



Before treatment

After treatment

Dermatological applications:

5. Removal of superficial blood vessels, veins



Before treatment

After treatment



Before treatment

2 years after treatment

Dermatological applications:

6. Resurfacing

1993. Adrian
CO₂, Erbium:YAG laser



Wrinkle removal



Sun damage



Rhinophyma (sebaceous gland hypertrophy, fibrosis)

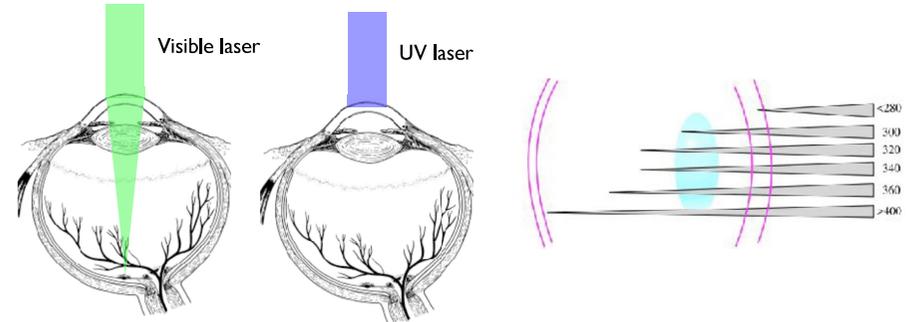


Systemic epidermal naevus

Ophthalmologic applications:

I. Principles

Transmittivity of optical media is wavelength-dependent



Ophthalmologic applications:

2. LASIK

“Laser-assisted In Situ Keratomileusis”
One type of refractive laser eye surgery

History:

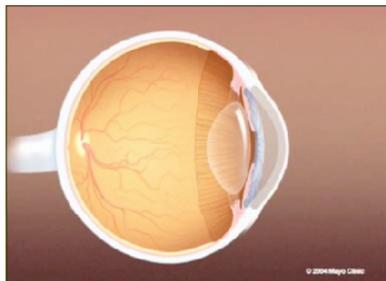
Jose Barraquer, 1970: construction of a microkeratome, with which he was able to cut lines and lobes in the cornea with laser (keratomileusis).

Lucio Buratto (Italian) and Ioannis Pallikaris (Greek), 1990: combination of keratomileusis photorefractive keratectomy.

Thomas and Tobias Neuhann (Germany), 1991: automated microkeratome.

Steps:

1. Removal of contact lens (7-10 days prior to treatment)
2. Scanning the topography of the cornea with low-power laser.
3. Cutting and lifting a layer of the cornea with femtosecond laser.
4. Removal of material from the corneal stroma (few tens of microns). Excimer laser (193 nm).

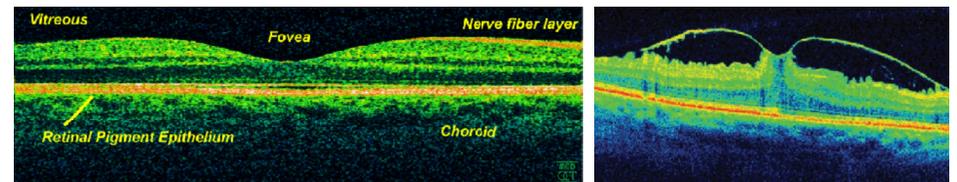
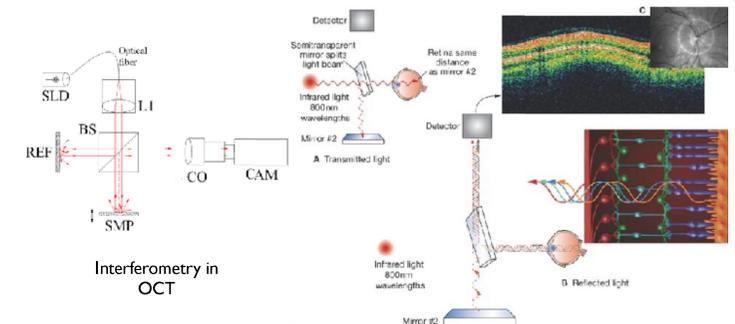


OCT

Optical Coherence Tomography

- non-invasive
- contrast-agent free
- near microscopic resolution

Principles: light rays reflected in deeper tissue layers can be separated from scatter by using interferometry. The spatial position of the reflecting layers can be determined. The structure of the illuminated sample can be resolved within 1-2 mm depth.



Normal retina

Macula degeneration

Photodynamic therapy

Photodynamic therapy (PDT):

Roswell Park Cancer Institute 1970's.

Three-component tumor therapeutic method:

1. Photosensitizing agent, 2. Light, 3. Oxygen.

Steps:

1. Administration of photosensitizing agent (aminolevulinic acid, ALA).
2. Incubation for few hours. ALA is transformed into protoporphyrin IX.
3. Illumination of target area with diode laser (few minutes).
4. Protoporphyrin absorbs \rightarrow excited singlet state \rightarrow triplet state \rightarrow energy transfer with triplet oxygen \rightarrow excited, reactive oxygen \rightarrow tissue reaction
5. The illuminated area necrotizes in a few days.

