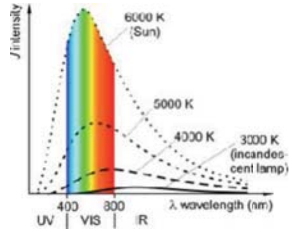
Light emission

Miklós Kellermayer

Source of emission

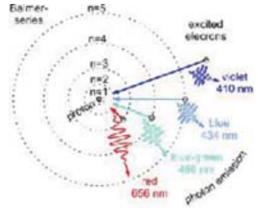
1. Thermal (black body) radiation

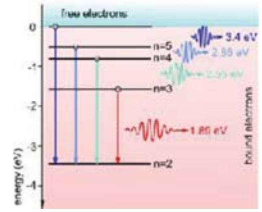
Mechanism: thermal motion of atoms, molecules



2. Luminescence

Mechanism: emission of excited-state energy

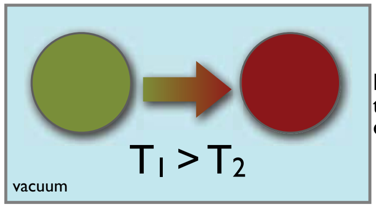




"Black-body" (Thermal) radiation

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

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



Heat exchange, temperature equilibration

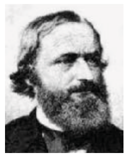
- High-temperture objects emit light.
- The greater the temperature of the body, the smaller the wavelengths that appear in its emission spectrum.

... 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):



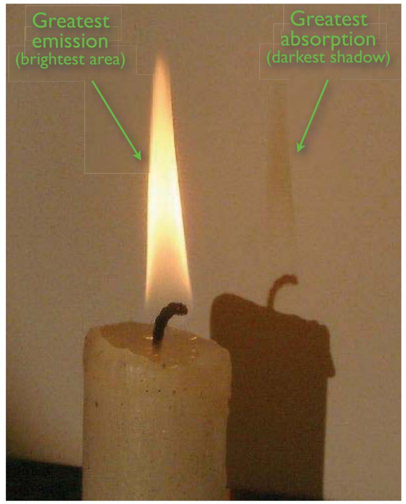
Gustav Robert **Kirchoff**
(1824-1887)

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

For a black body (BB):

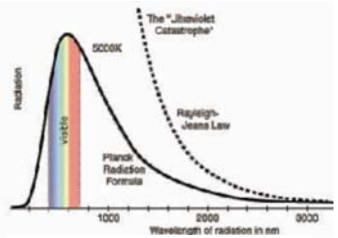
$$\alpha_{\lambda BB} = 1$$

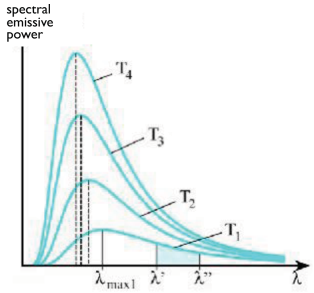
- That is, the black body absorbs all light that it is exposed to (nothing is reflected).
- The black body is an ideal object for investigating temperature-dependent emission.



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:

$$E = hf$$

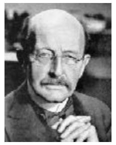
h = Planck's constant (6.626 x 10^{-34} Js).

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)

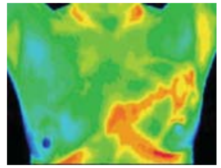
Applications of thermal radiation

Thermography, infradiagnostics

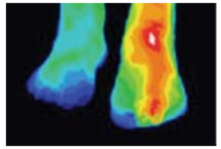
"Seeing through" a non-absorbing layer

Airport thermography

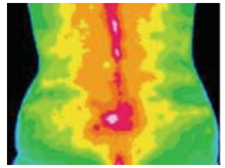
Detection of febrile condition, prevention of epidemics



Breast carcinoma



Inflammation



Chronic musculoskeletal stress (pain)

Definitions of luminescence

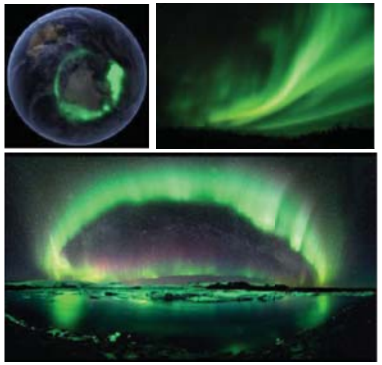
- Relaxation from excited state followed by light emission
- Radiation emitted by matter in excess of thermal emission
- "Cold light"
- Processes of fluorescence and phosphorescence

Emission by luminescence: everywhere

Photoluminescence

Luminescence everywhere

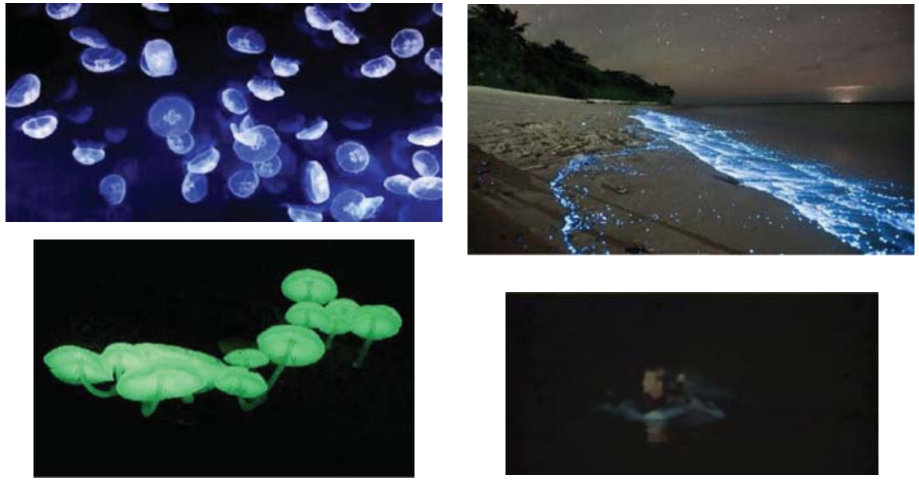
Display lights



Aurora borrealis

Radioluminescence

Luminescence everywhere

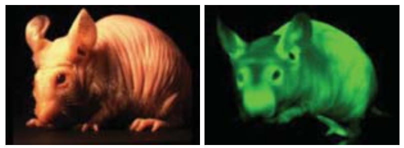


Bioluminescence

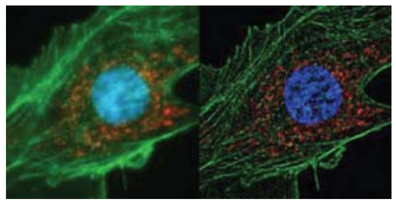
Luminescence everywhere

Bioluminescence
Firefly

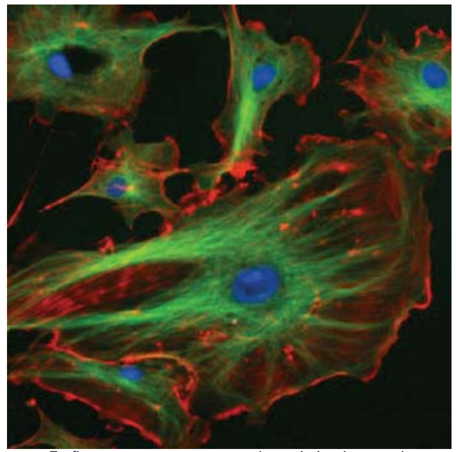
Luminescence everywhere



GFP-mouse (green nude mouse)



Superresolution microscopy (Nobel-prize 2014)



Epifluorescence microscopy (cytoskeletal system)

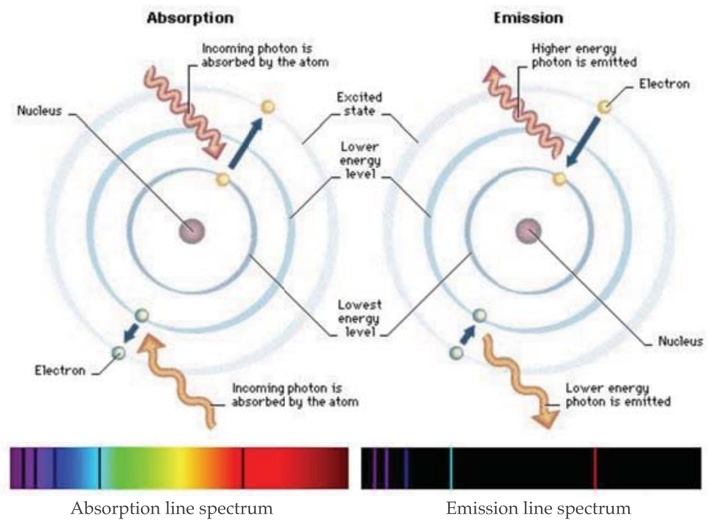
Fluorescence

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

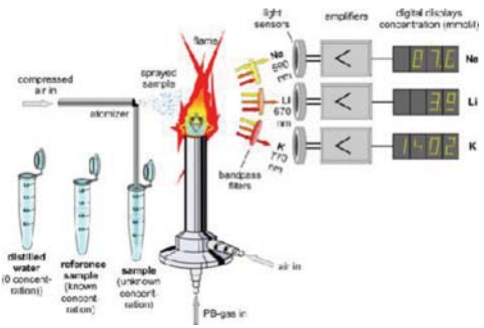
Absorption and emission by an atom



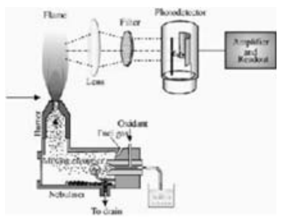
Application of emission sectroscopy

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 ($-CH_2-$):

Asymmetric stretching *Symmetric stretching* *Scissoring*

Energy of a molecule


Max Born
(1882-1970)


J. Robert Oppenheimer
(1904-1967)

Born-Oppenheimer approximation:

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

Iportant 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:

$$E_e \overset{\sim 100x}{>} E_v \overset{\sim 100x}{>} E_r$$

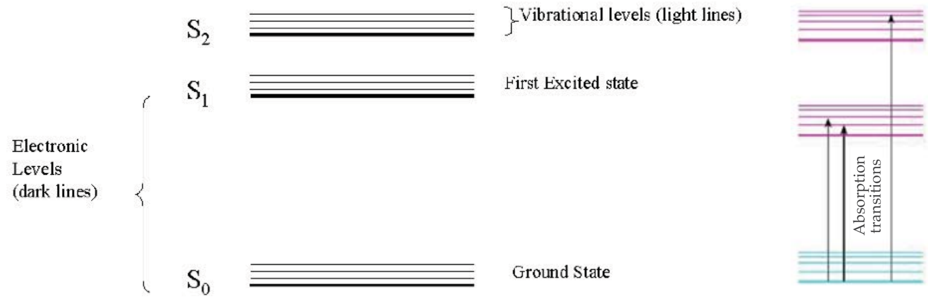
~3x10^{-19} J (~2 eV) > ~3x10^{-21} J > ~3x10^{-23} J

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

Representation of energy states

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


Alexander Jabłoński
(1898-1980)



Spin states


Wolfgang Pauli
(1900-1958)

Pauli's exclusion principle:

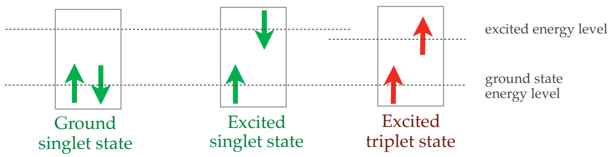
- Each quantum state can be occupied by a single electron.
- Within an atom there cannot be two electrons for which all four quantum numbers are identical.

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

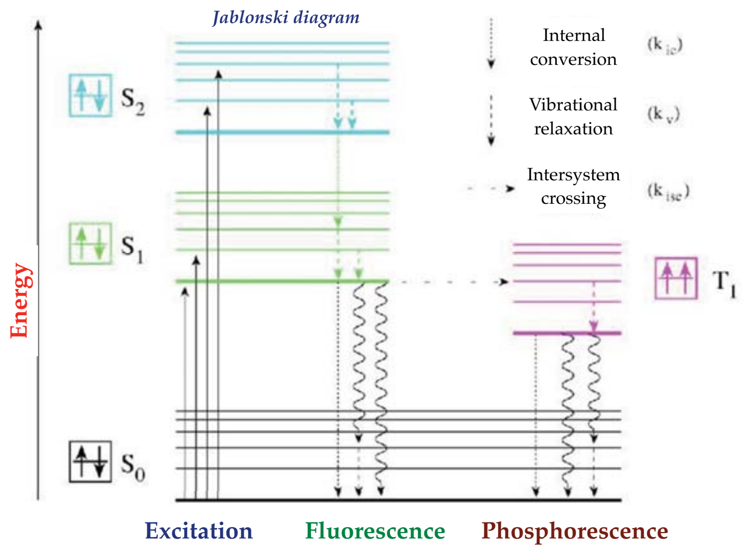
Singlet and ***triplet*** states:
number of orientations of magnetic moment associated with net spin state (in magnetic field) = 2S+1 = 1 (*singlet*) or 3 (*triplet*). (S = net spin, e.g., in fully occupied subshell (+1/2)+(-1/2) = 0)

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.



Processes of luminescence



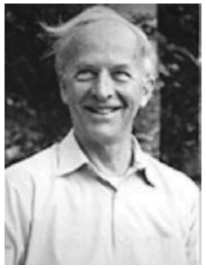
Internal conversion: non-radiative transition between electron energy states (e.g., $S_2 \rightarrow S_1$)

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

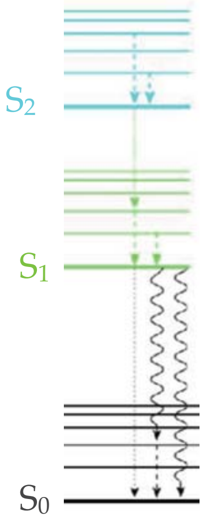
Intersystem crossing: transition between singlet and triplet states accompanied by spin change (e.g., $S_1 \rightarrow T_1$)

Kasha's rule

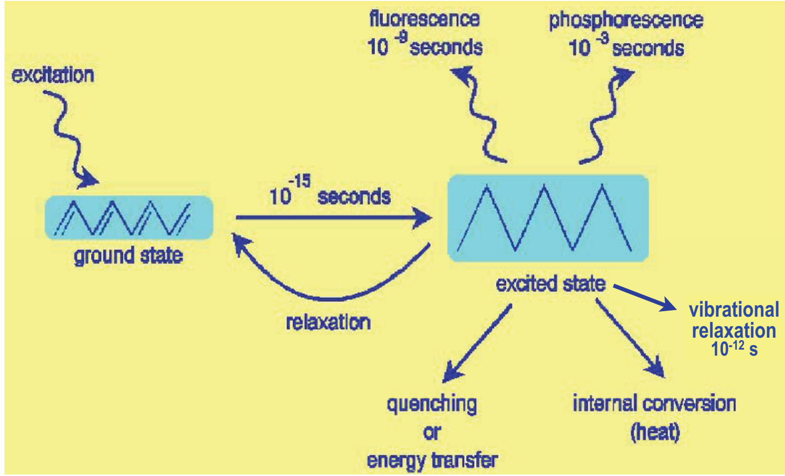
Photon emission (fluorescence or phosphorescence) occurs only from the lowest-energy excited electronic state (i.e., S_1 or T_1) of a molecule.



Michael Kasha (1920-)
American physicist

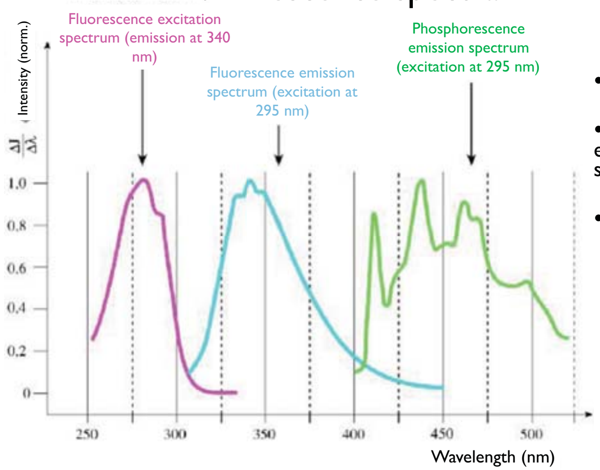


Time scale of transitions



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)

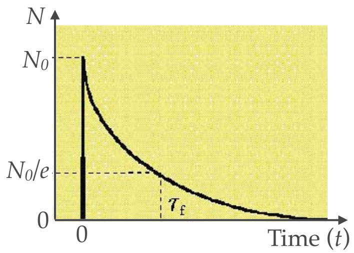
Chracterization of luminescence II.

Quantum yield

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

k_{nr}=non-radiative transition rate constants

Excited-state lifetime (τ)



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

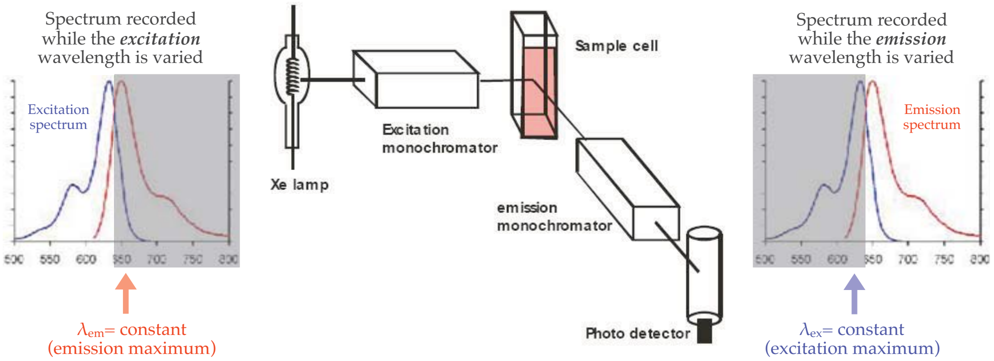
$$\tau = \frac{1}{k_f + k_{nr}}$$

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

Measurement of fluorescence

Fluorescence spectrometer

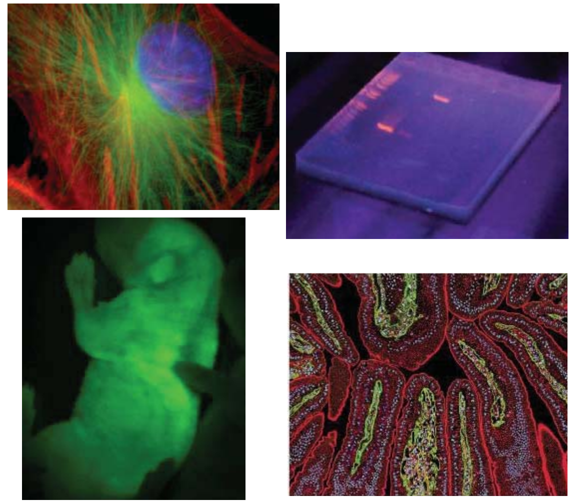
("Steady-state" spectrofluorometer)



Biomedical applications of fluorescence

A few examples:

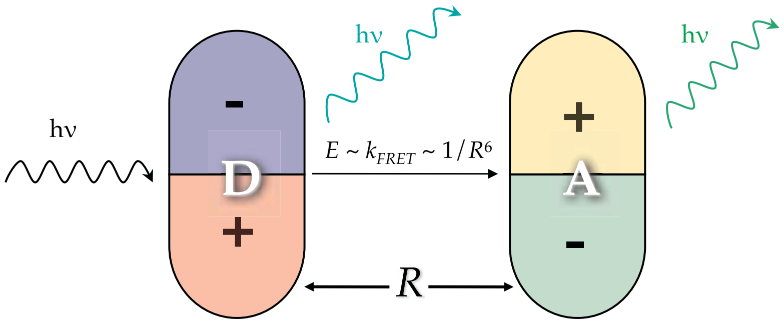
- Fluorescence microscopy
- DNA sequencing (chain termination method)
- DNA detection (EtBr)
- DNA microarray
- Immunofluorescence
- Fluorescence-activated cell sorting (FACS)
- Förster resonance energy transfer (FRET)
- Fluorescence recovery after photoleaching (FRAP)
- Fluorescent protein conjugation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



Förster Resonance Energy Transfer (FRET)

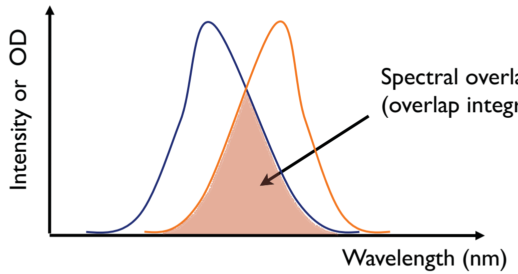
- Occurs by non-radiative dipole-dipole interaction between an excited ***donor (D)*** and an proper ***acceptor (A)*** molecule under certain conditions (spectral overlap and close distance).
- ***<u>F</u>luorescence <u>R</u>esonance <u>E</u>nergy <u>T</u>ransfer (FRET):*** if the participants of the transfer are fluorophores.

Theodor Förster (1910-1974)



Conditions of FRET

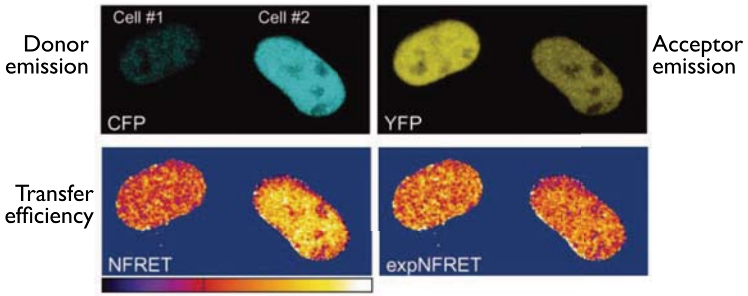
- **Fluorescent** donor and acceptor molecules.
- The distance **(R)** between donor and acceptor molecules is 2-10 nm!
- **Overlap** between the emission spectrum of the **<u>donor</u>** and the absorption spectrum of the **<u>acceptor</u>**.



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.

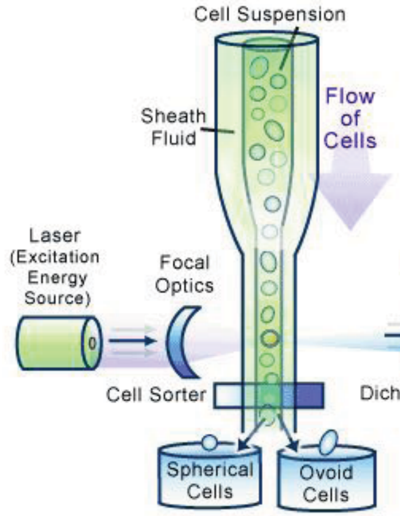
FRET microscopy



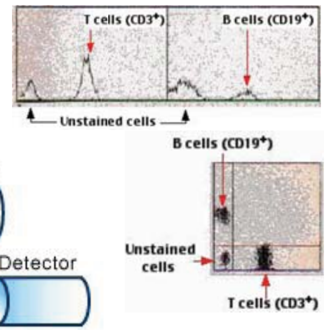
Fluorescence activated cell sorter (FACS)

Flow cytometry

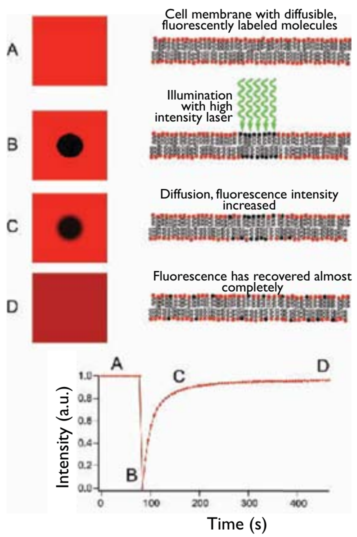
- A cell suspension, fluorescently labeled by using specific antibodies, is analyzed cell by cell
- Numerous parameters are measured simultaneously (fluorescence intensity at several wavelengths, small- and large-angle scatter)
- Statistical analysis
- If needed, cells can be separated according to their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



Cytometry statistics



Fluorescence Recovery After Photobleaching (FRAP)



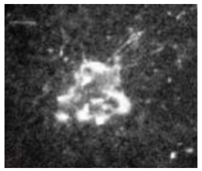


Image prior to bleaching

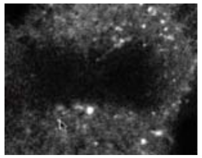
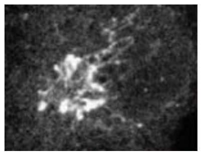


Image just after bleaching



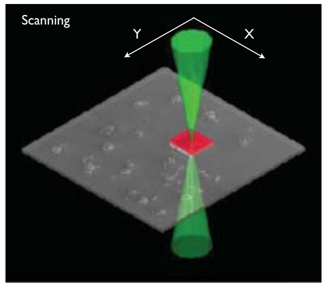
Recovered fluorescence

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

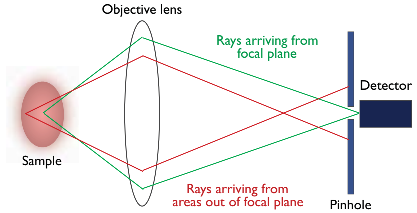
$$D = \frac{w^2}{4t_D}$$

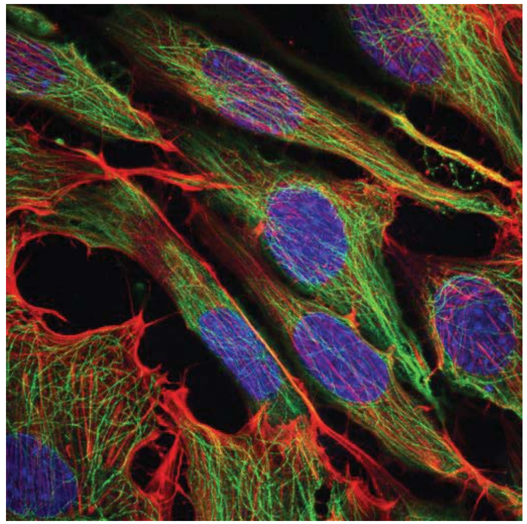
D = diffusion coefficient
w = width of bleached area
t_D = time constant

Laser scanning confocal microscopy



"Confocal principle"

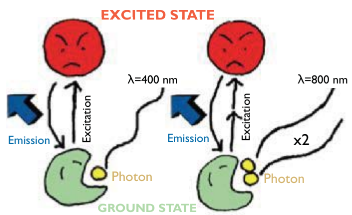


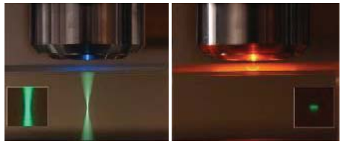


Green: microtubules; Red: actin; Blue: nuclei

Multiphoton microscopy

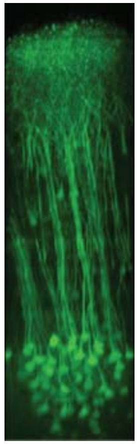
- •Energy of two (or more) photons are added during excitation
- •Excitation (hence emission) only in the focal point (limited photodamage)
- •Excitation with long wavelength (near-IR), short (fs) light pulses
- •Large (up to 2 mm) penetration due to long wavelength



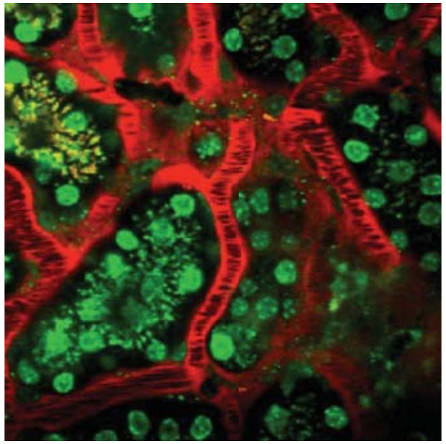


Single-photon fluorescence

Two-photon fluorescence



Cortical pyramidal cells



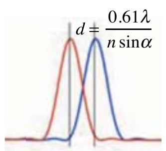
Green: proximal kidney tubules; Red: albumin (plasma)

Super-resolution microscopy

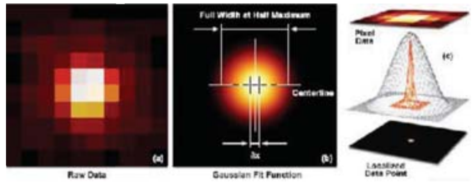
Chemistry Nobel-prize, 2014

Resolution problem is converted into position-determination problem

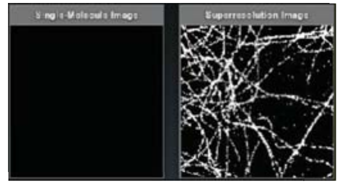
Resolution problem (Abbé)



Position determination problem (precision depends on photon count)

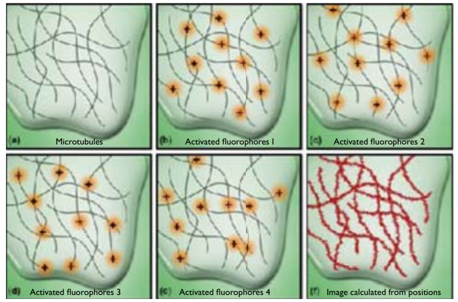


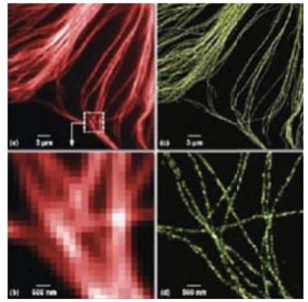
"Stochastic" data collection, single fluorophores



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

Data collection process





Microtubular system

Laser

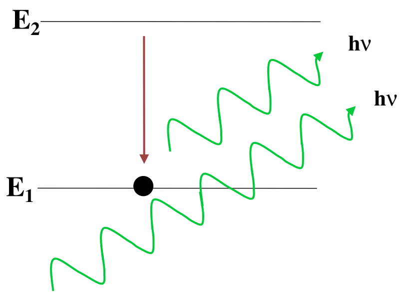
Lasers are everywhere

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"



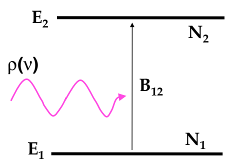
Luminescent light source based on light amplification.
MASER: Microwave Amplification by Stimulated Emission of Radiation

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. Basow, A.M. Prochorow, and C. Townes:* ammonia maser
- **1960** - *Theodore Maiman:* first laser (ruby laser)
- **1964** - *Basow, Prochorow, Townes (Nobel-prize):* quantum electronics
- **1970** - *Arthur Ashkin:* laser tweezers
- **1971** - *Dénes Gábor (Nobel-proze):* 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

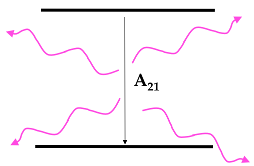
1. Absorption



Frequency of transition:
$n_{12}=N_1B_{12}\rho(\nu)$

$\Delta E= E_2-E_1=h\nu$
energy quantum is absorbed.

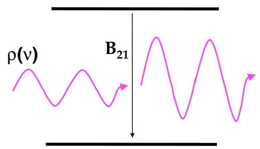
2. Spontaneous emission



Frequency of transition:
$n_{21}=N_2A_{21}$

E_2-E_1 photons travel independently in all directions.

3. Stimulated emission



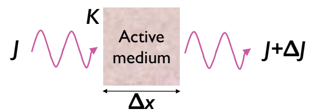
Frequency of transition:
$n_{21}=N_2B_{21}\rho(\nu)$

In the presence of exernal field. Field energy is increased. Phase, direction and frequency of emitted and external photons are identical.

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

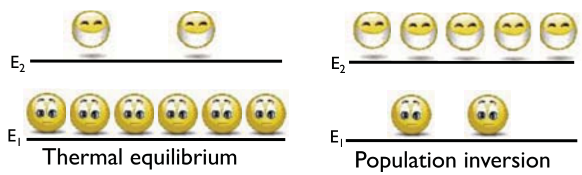
Light amplification depends on the relative population of energy levels.



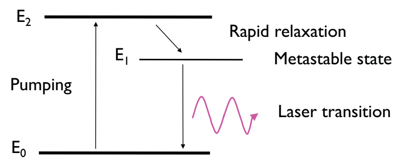
$\Delta J=JK(N_2-N_1)\Delta x$

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

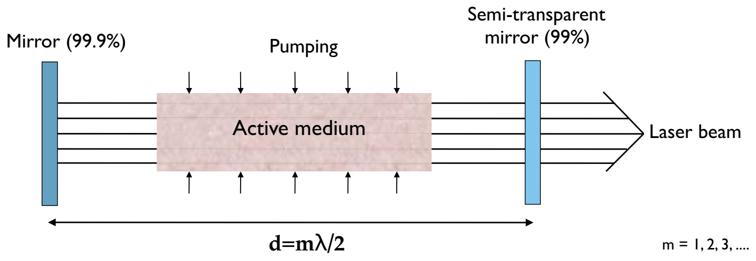
N.B.: If *dJ* is positive, more light exits the medium, than has entered!



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



Principles of laser III. Optical resonance



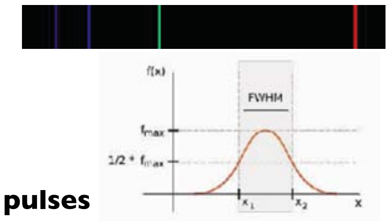
Resonator:

- 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

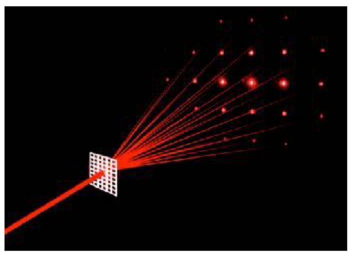
Properties of laser light I.

1. **Small divergence**
   Parallel (collimated) beam
2. **Large power**
   In continuous (CW) mode, tens, hundreds of W (e.g., CO_2 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:

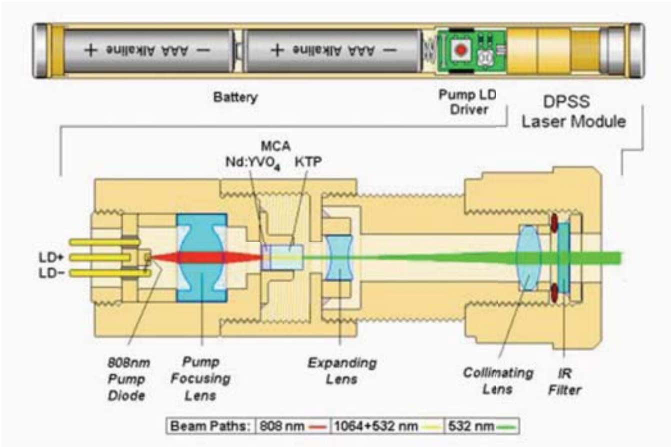
1. Solid state lasers
Metal doping in crystals or glasses; Ruby, Nd-YAG, Ti-sapphire
Red-infrared spectral range; CW, Q-switched mode, large power

2. Gas lasers
Best known: *He-Ne laser* (10 He/Ne). Small energy, wide use
CO_2 laser: CO_2-N_2-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)

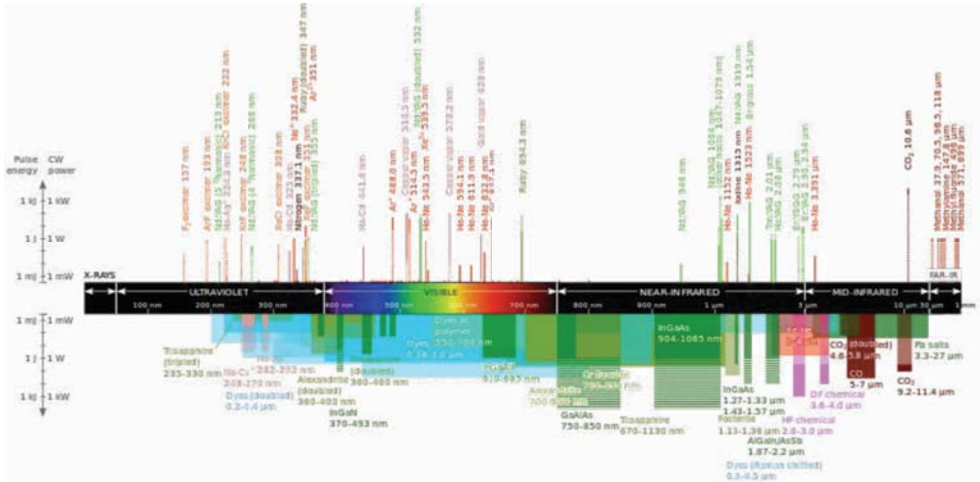
*Notes:

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

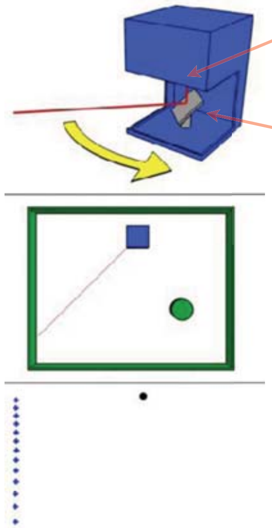
- 5 mW – CD-ROM drive
- 5–10 mW – DVD player or DVD-ROM drive
- 100 mW – high-speed CD-RW writer
- 250 mW – DVD-R writer
- 1–20 W – solid-state laser for micromachining
- 30–100 W – surgical CO_2 laser
- 100–3000 W – industrial CO_2 laser (laser cutter)
- 1 kW – 1 cm diode laser bar

Factors to be considered in laser applications

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

Speed measurement with laser

LIDAR: "Light Detection and Ranging"

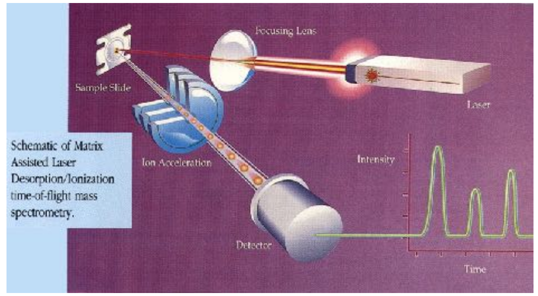


Steerability

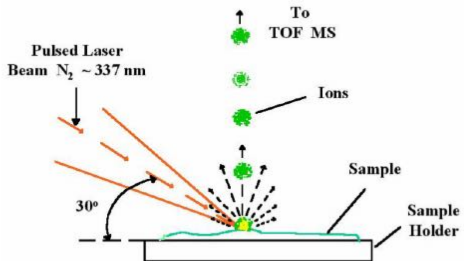
Recording:
reconstructed spatial arrangement.
In traffic speedometer:
100 pulses in 0.3 s

MALDI-TOF:

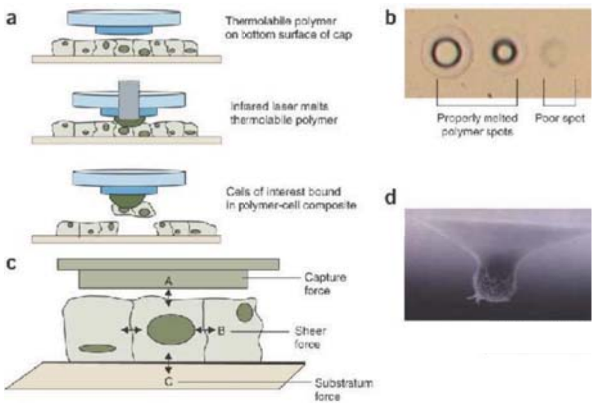
matrix-assisted laser desorption/ionization time of flight mass spectrometry



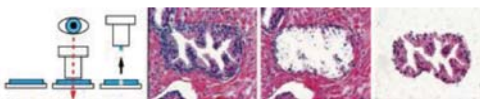
Power density



"Laser capture microdissection"



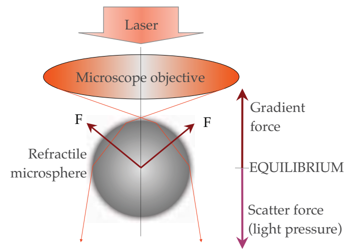
Power density



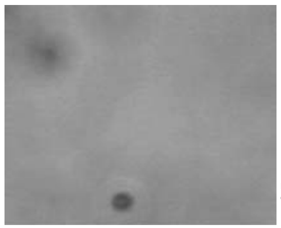
Significance: local analytics are possible
analitika lehetősége
(chemistry, genetics)

Laser tweezers

Power density

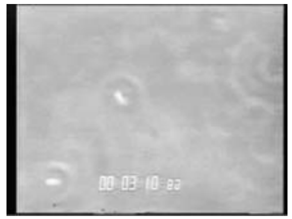


In the laser twweezer momentum change occurs between the photons and the trapped particle:



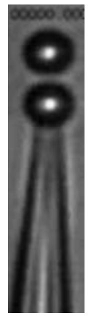
3 μm latex (polystyrene) microspheres in the optical trap

Trapping bacterial cells with laser tweezers



Manipulating molecules with laser tweezers

Actin filament



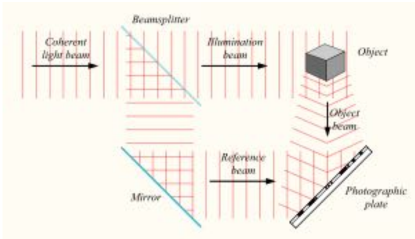
Molecular force measurement!

dsDNA

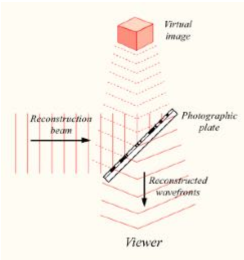
Holography

Coherence

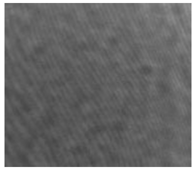
Dénes Gábor
(1900-1979)



Recording a hologram



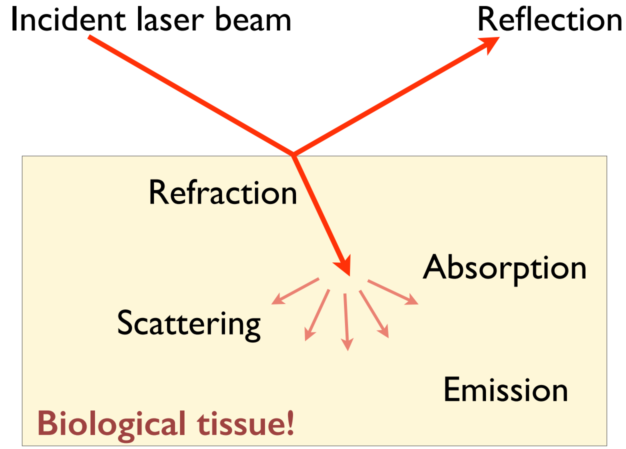
Visualization of a hologram



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. CO_2 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 acivation of photosensitive chemicals
preferentially taken up by the tumor.

Opthalmology: Retina lesions, photocoagulation, glaucoma, photorefractive keratektomy (PRK).

Dermatological applications:

1. 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/cm^2)
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)

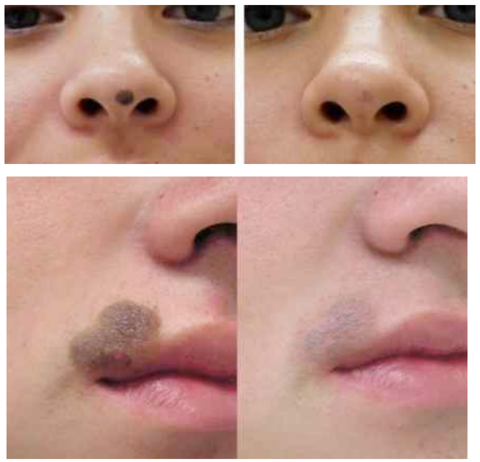
Before treatment After treatment

Dermatological applications:
3. Tattoo removal

Q-switched Nd:YAG laser (1064 nm)

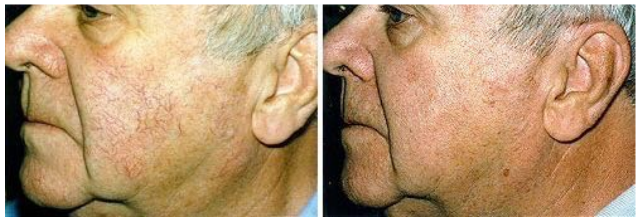
Before treatment After treatment

Dermatological applications:
4. Naevus removal



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
CO_2, Erbium:YAG laser


Wrinkle removal

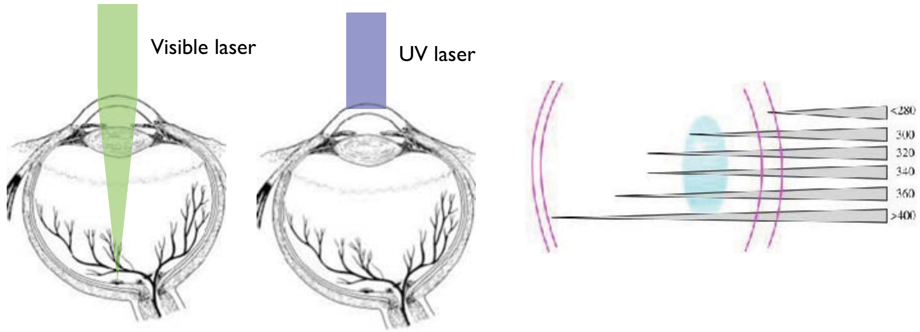

Sun damage


Rhinophyma (sebaceous gland hypertrophy, fibrosis)


Systemic epidermal naevus

Ophthalmologic applications:
1. Principles

Transmittivity of optical media is wavelength-dependent

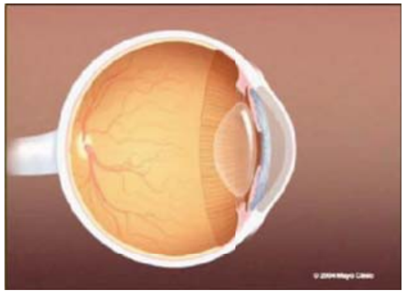


Ophthalmologic applications:
2. LASIK

"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.

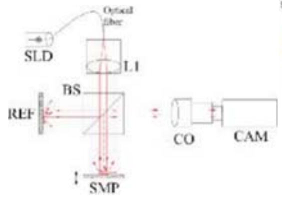
Steps:
1. Removal of contact lens (7-10 days prior to treatment)
2. Scanning the topography of the cornea with low-power laser.
3. Cutting and lifting a layer of the cornea with femtosecond laser.
4. Removal of material from the corneal stroma (few tens of microns). Excimer laser (193 nm).



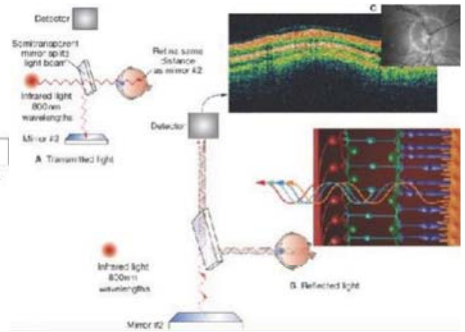
OCT

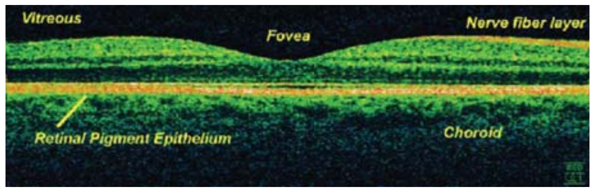
Optical Coherence Tomography
- non-invasive
- contrast-agent free
- near microscopic resolution

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 sampe can be resolved within 1-2 mm depth.

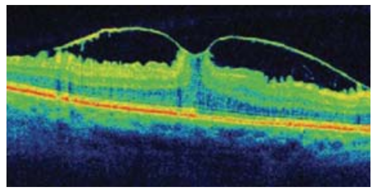


Interferometry in OCT





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