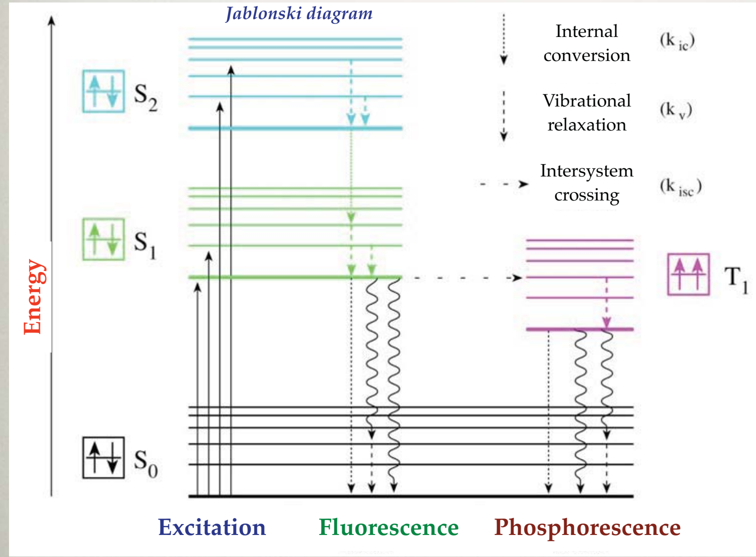
Luminescence cont'd

Miklós Kellermayer

Processes of luminescence



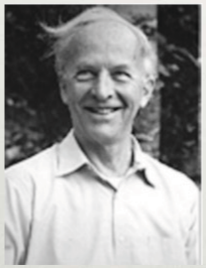
Internal conversion: non-radiative transition between electron energy states (e.g., $S_2 \rightarrow S_1$)

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

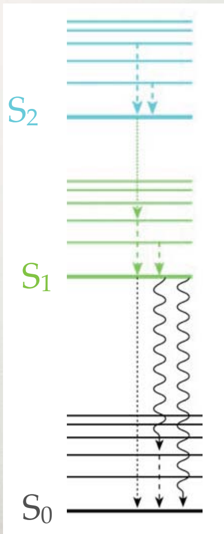
Intersystem crossing: transition between singlet and triplet states accompanied by spin change (e.g., $S_1 \rightarrow T_1$)

Kasha's rule

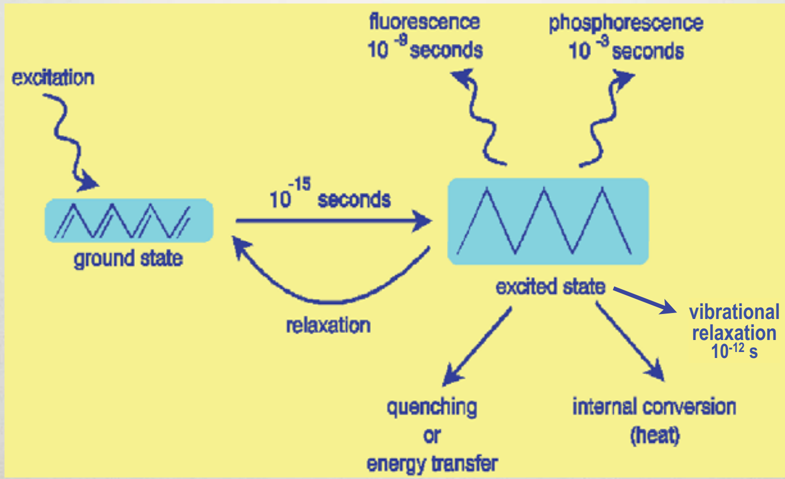
Photon emission (fluorescence or phosphorescence) occurs only from the lowest-energy excited electronic state (i.e., S_1 or T_1) of a molecule.



Michael Kasha (1920-)
American physicist

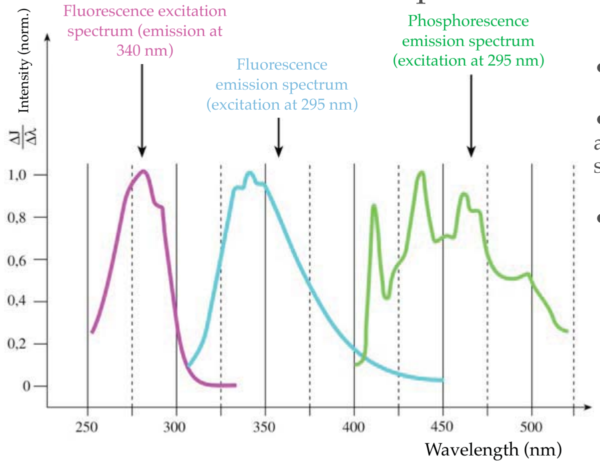


Time scale of transitions



Characterization of luminescence I.

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)

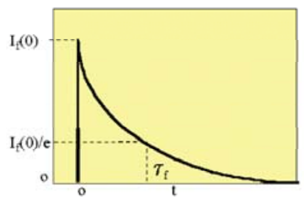
Chracterization of luminescence II.

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



$$\frac{dN}{dt} = -\left(k_f + k_{nr}\right) \cdot N$$

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

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

N=molecules in excited state

t=time

k_f=fluorescence rate constant

k_{nr}=non-radiative transition rate constant

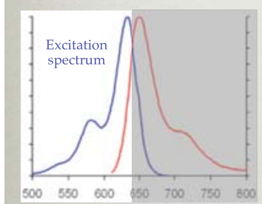
τ=fluorescence lifetime

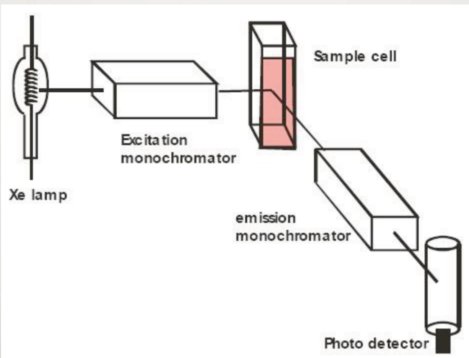
Measurement of fluorescence

Fluorescence spectrometer

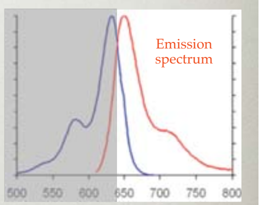
("Steady-state" spectrofluorometer)

Spectrum recorded while the ***excitation*** wavelength is varied



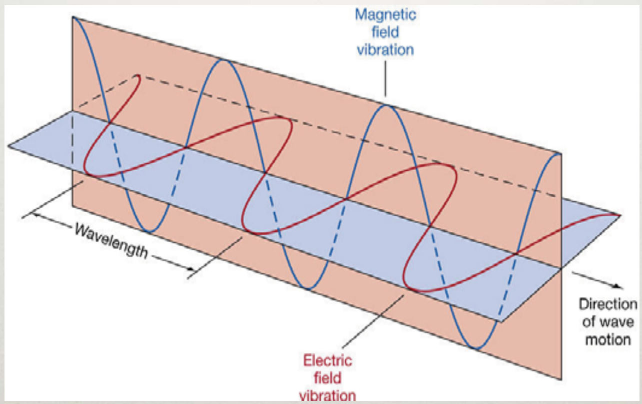


Spectrum recorded while the ***emission*** wavelength is varied

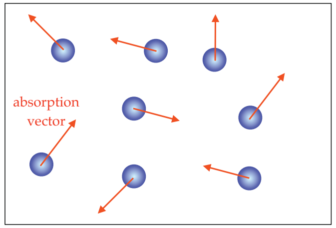


Light is electromagnetic radiation

- Propagating electromagnetic disturbance.
- Transverse wave.
- Therefore, it can be polarized.



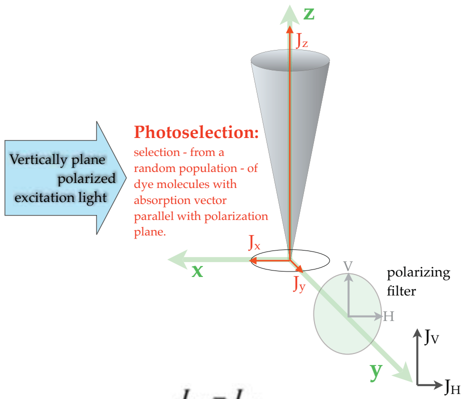
Excitation with polarized light



Absorption and emission vectors of fluorophores: determine the probabilities of photon absorption and emission.

Absorption is maximal if absorption vector and electrical polarization axis of light are parallel.

Absorption depends on $\cos^2\alpha$ (α is the angle between absorption axis and electrival polarization vector).



Polarization: $p = \dfrac{J_{VV} - J_{VH}}{J_{VV} + J_{VH}}$

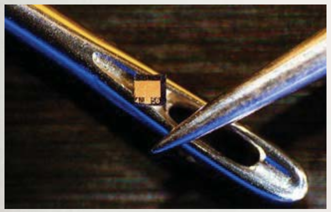
Anisotropy: $r = \dfrac{J_{VV} - J_{VH}}{J_{VV} + 2J_{VH}}$

- additive quantity
- In the denominator: total excitation intensity ($J_{VHx}=J_{VHy}$)

Laser

Miklós Kellermayer

Lasers are everywhere



5 mW diode laser
few mms

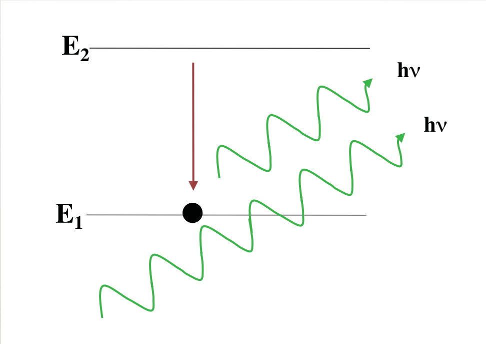
Terawatt NOVA laser - Lawrence Livermore Laboratories
Size of a football field

Laser

1. What is the laser?
2. Short laser history
3. Foundations of laser function
4. Properties of laser light
5. Types of lasers
6. Biomedical applications of lasers

Laser:

"Light Amplification by Stimulated Emission of Radiation"



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

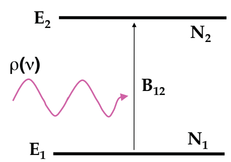
Laser history in a nutshell

- **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

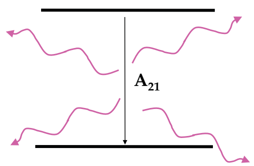
1. Absorption



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

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

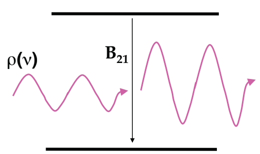
2. Spontaneous emission



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

E_2-E_1 photons
travel independently in all directions.

3. Stimulated emission



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

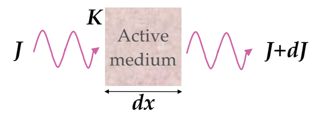
In the presence of exernal field.
Field energy is increased.
Phase, direction and frequency of emitted and external photons are identical.

Explanation: two-state atomic or molecular system.
E_1, E_2 : energy levels, $E_2>E_1$
$\varrho(\nu)$: 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

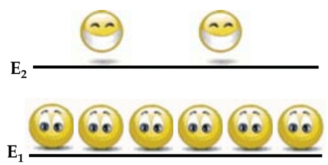
Light amplification depends on the relative population of energy levels.



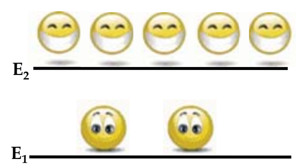
$$dJ=JK(N_2-N_1)dx$$

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!

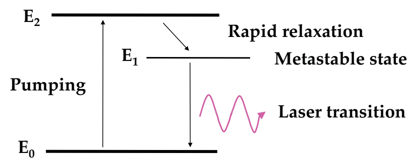


Thermal equilibrium

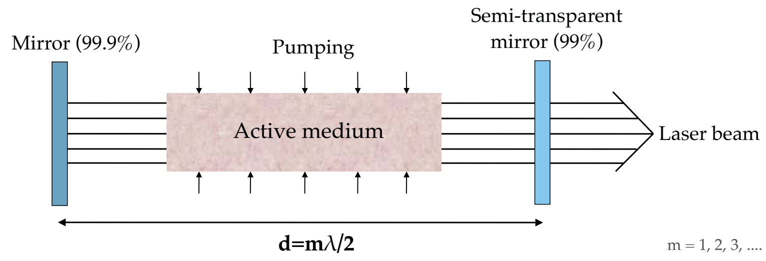


Population inversion

- 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

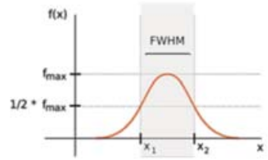
Optical switch in the resonator: Q-switch, pulsed mode

Properties of laser light I.

1. **Small divergence**
   Parallel (collimated) beam

2. **Large power**
   In continuous (CW) mode, tens, hundreds of W (e.g., 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

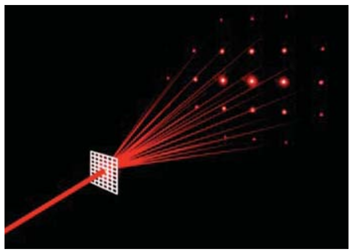


4. **Often polarized**

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:

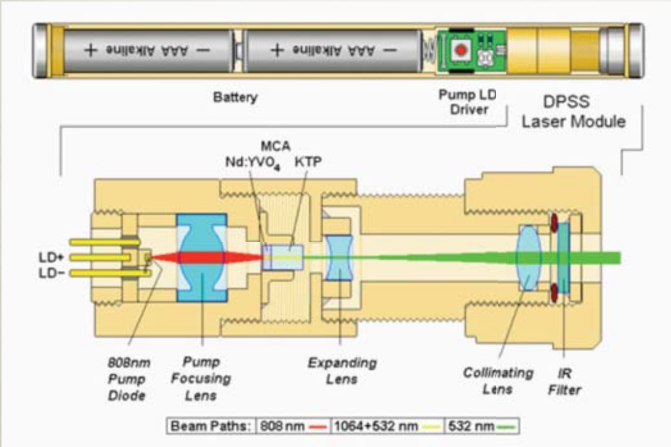
1. Solid state lasers
Metal doping in crystals or glasses; Ruby, Nd-YAG, Ti-sapphire
Red-infrared spectral range; CW, Q-switched mode, large power

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)

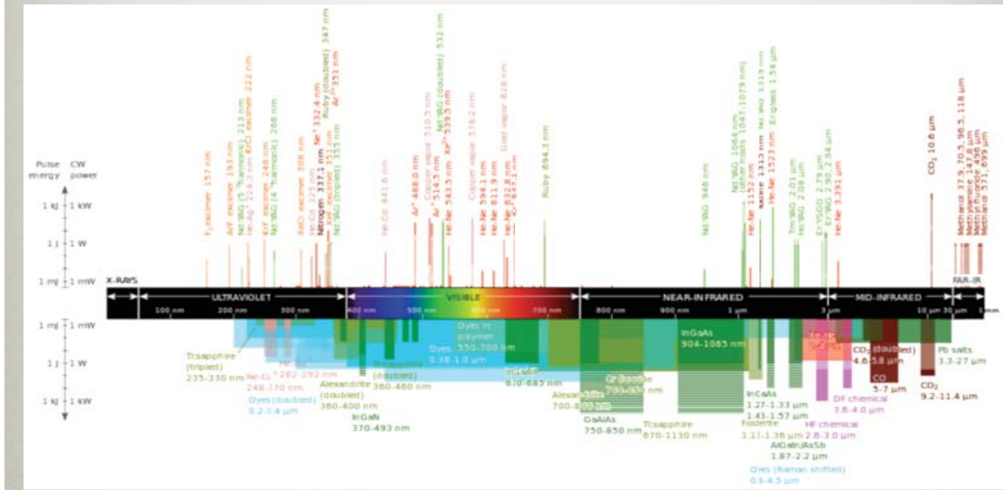
*Notes:

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

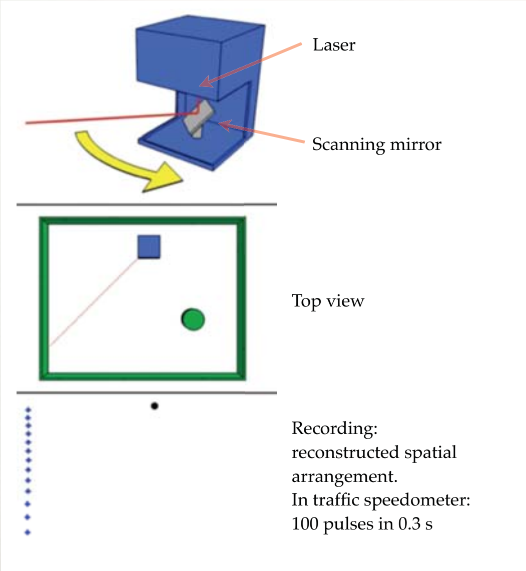
- 5 mW – CD-ROM drive
- 5–10 mW – DVD player or DVD-ROM drive
- 100 mW – high-speed CD-RW writer
- 250 mW – DVD-R writer
- 1–20 W – solid-state laser for micromachining
- 30–100 W – surgical CO_2 laser
- 100–3000 W – industrial CO_2 laser (laser cutter)
- 1 kW – 1 cm diode laser bar

Factors to be considered in laser applications

- Steerability
- Power
- Monochromaticity
  (see fluorescence applications in next lecture!)
- Coherence

Speed measurement with laser

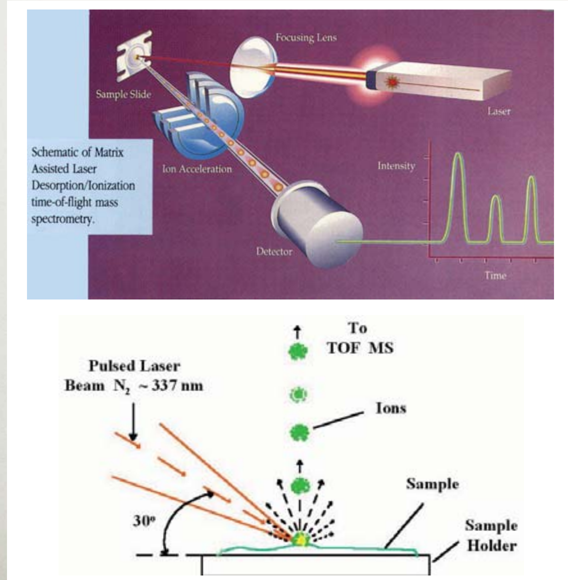
LIDAR: “Light Detection and Ranging”



Steerability

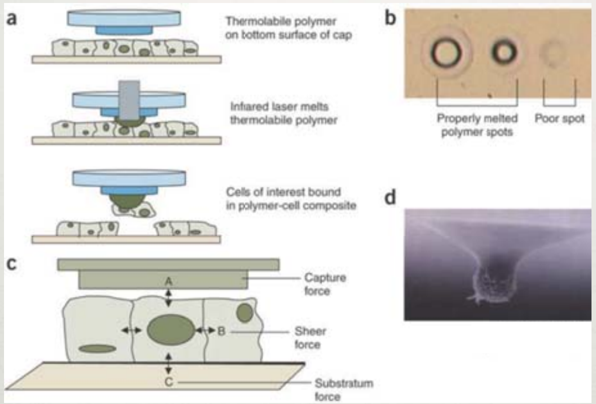
MALDI-TOF:

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

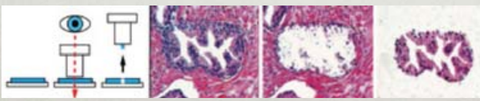


Power density

“Laser capture microdissection”



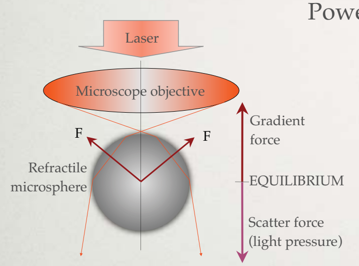
Power density



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

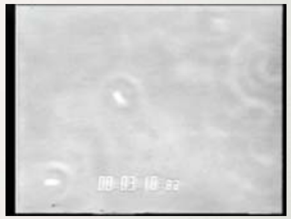
Laser tweezers

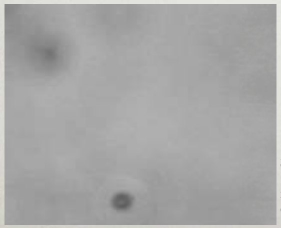
Power density



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

Trapping bacterial cells with laser tweezers

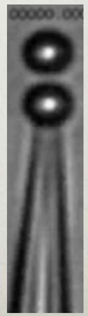




3 μm latex (polystyrene) microspheres in the optical trap

Manipulating molecules with laser tweezers

Actin filament



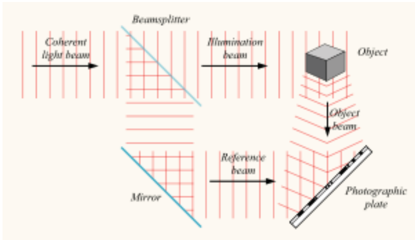
dsDNA

Molecular force measurement!

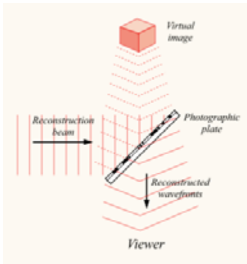
Holography

Coherence

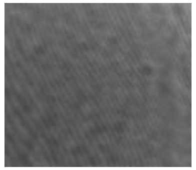
Dénes Gábor
(1900-1979)



Recording a hologram



Visualization of a hologram



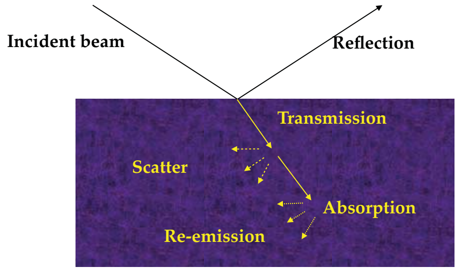
Surface of a hologram recording

Holograms

Biomedical applications of lasers I.

Principles:

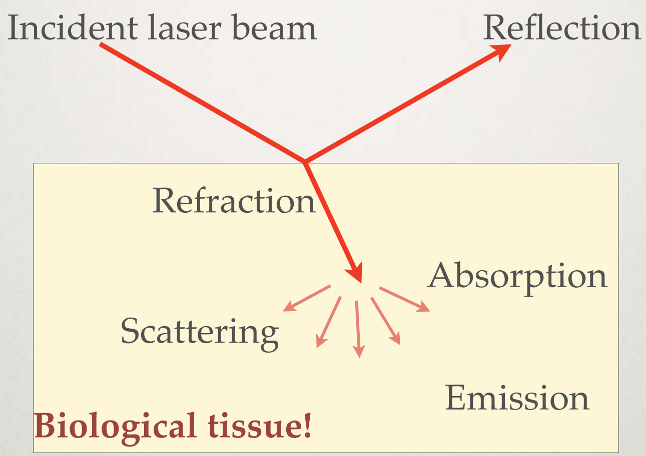
1. Interaction of light with matter



2. Properties of laser beam:
Focusing, wavelength, power

3. Properties of biological tissue:
Transmittivity, absorbance, light-induced reactions

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)**

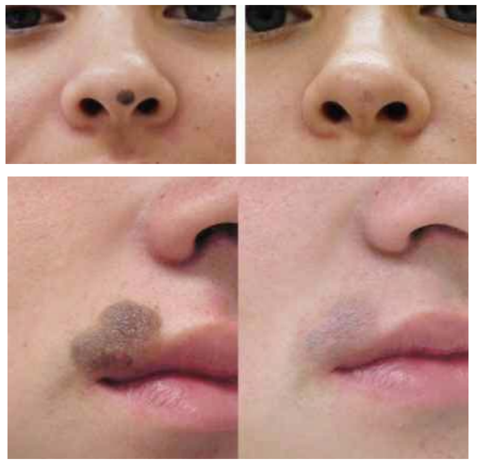
Before treatment — After treatment

Dermatological applications:
3. Tattoo removal

Q-switched Nd:YAG laser (1064 nm)

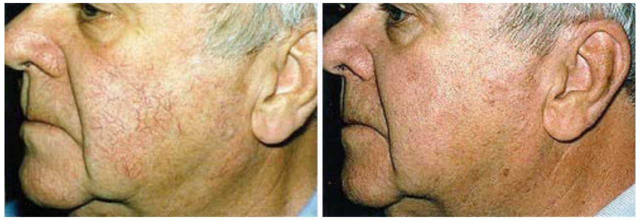
Before treatment — After treatment

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. Bőr felületi módosítása ("resurfacing")

1993. Adrian

CO_2, Erbium:YAG laser

Wrinkle removal

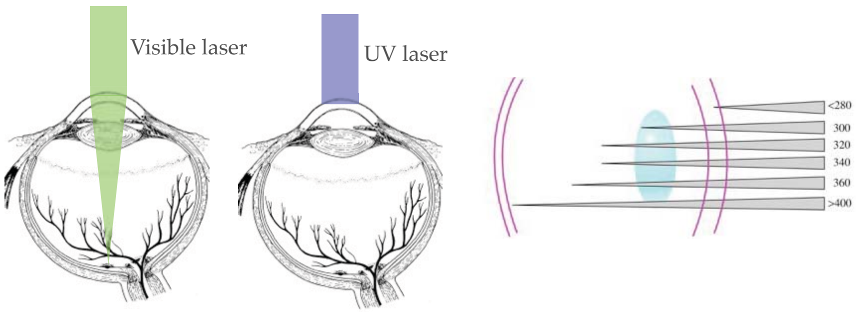
Sun damage

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.

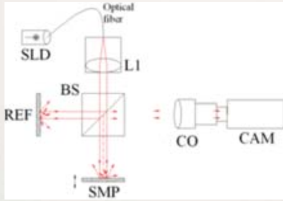
Steps:

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).

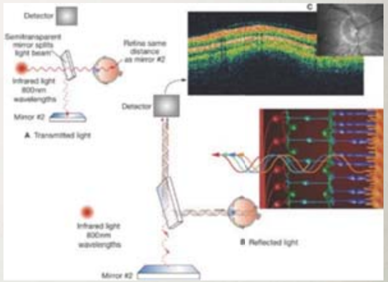
OCT

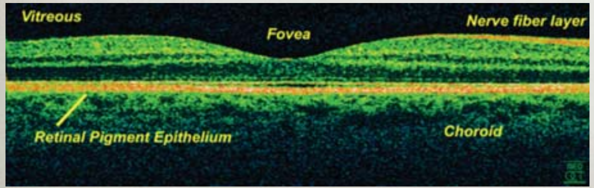
Optical Coherence Tomography
- non-invasive
- contrast-agent free
- near microscopic resolution

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.

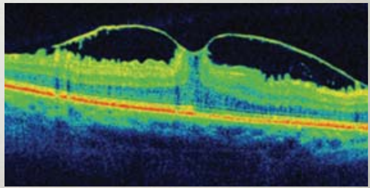


Interferometry in OCT





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.