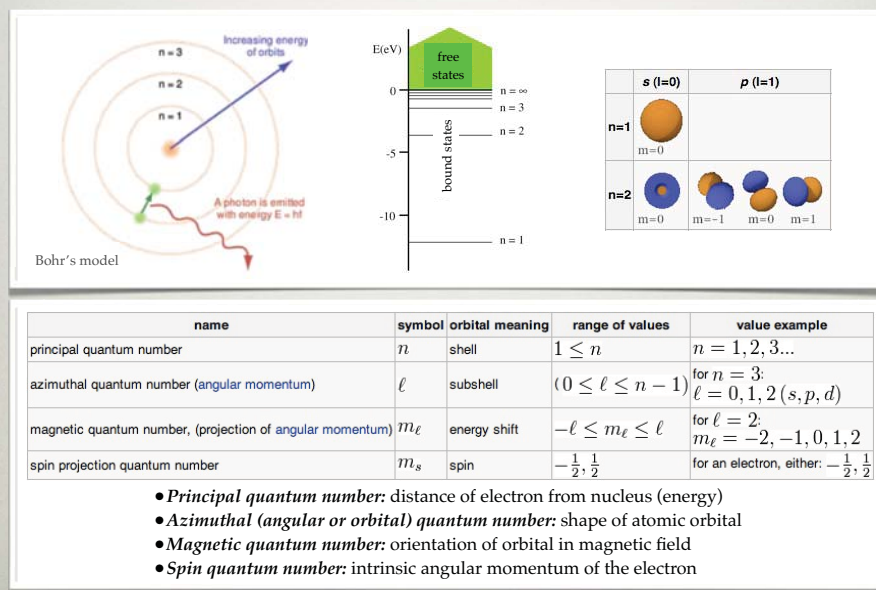


LUMINESCENCE

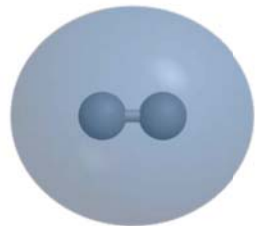
MIKLÓS KELLERMAYER

ATOMIC STRUCTURE



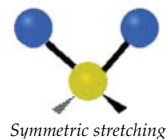
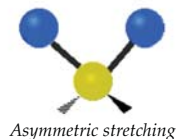
MOLECULAR STRUCTURE

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



Molecules *vibrate* and *rotate*!

Examples of vibrational motion in triatomic group ($-\text{CH}_2-$):



ENERGY OF A MOLECULE

Born-Oppenheimer approximation:

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

Important notions:

Types of energy states are independent (not coupled)

Energy states are non-continuous, but discrete

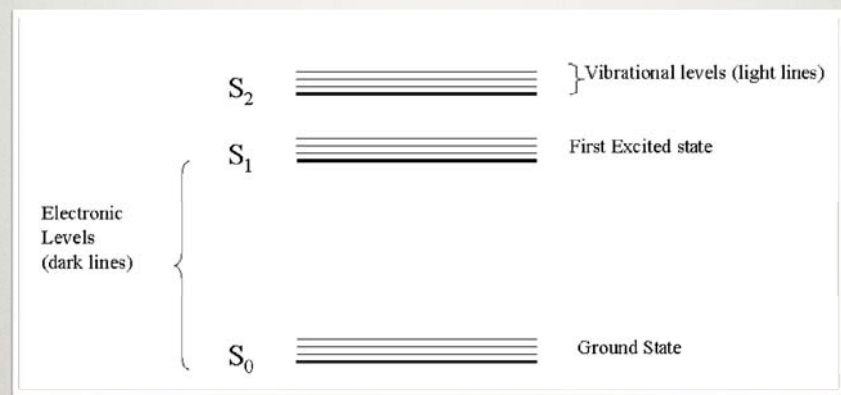
Transition between states involves packets (quanta) of energy

Scales of transition energies between different states are different:

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

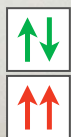
$$\sim 3 \times 10^{-19} \text{ J } (\sim 2 \text{ eV}) > \sim 3 \times 10^{-21} \text{ J} > \sim 3 \times 10^{-23} \text{ J}$$

REPRESENTATION OF ENERGY STATES



S: singlet state; paired electrons with opposite spins
(N.B.: Pauli's principle)

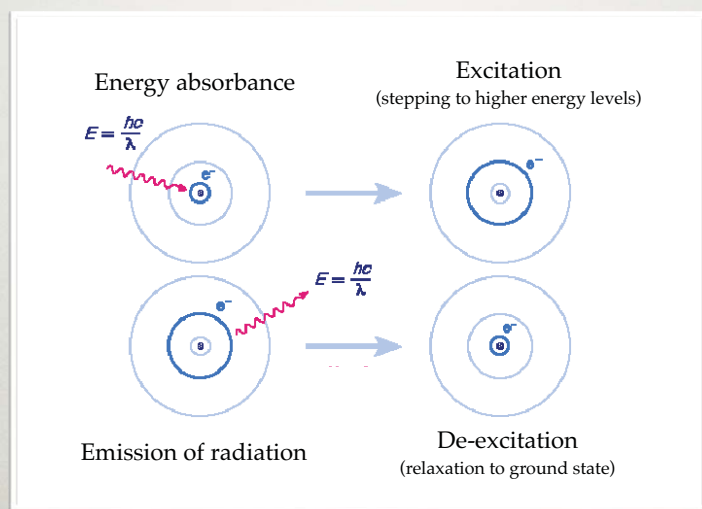
T: triplet state; paired electrons with like spins



DEFINITION 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

SIMPLIFIED STEPS OF LUMINESCENCE



TYPES OF LUMINESCENCE

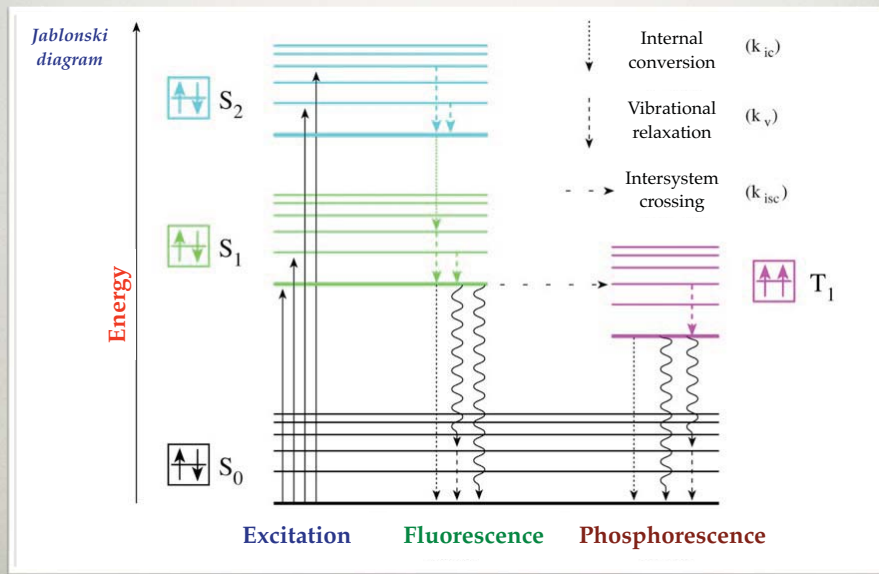
(a) Excitation Mode	Luminescence Type
absorption of radiation (UV/VIS)	photoluminescence
chemical reaction	chemiluminescence, bioluminescence
thermally activated ion recombination	thermoluminescence
injection of charge	electroluminescence
high energy particles or radiation	radioluminescence
friction	triboluminescence
sound waves	sonoluminescence
(b) Excited State (Assuming Singlet State)	Luminescence Type
first excited singlet state	fluorescence, delayed fluorescence
lowest triplet state	phosphorescence

Bioluminescence



Firefly

PROCESSES OF LUMINESCENCE

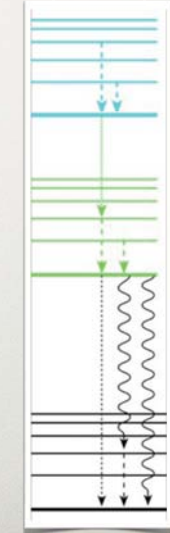


KASHA'S RULE

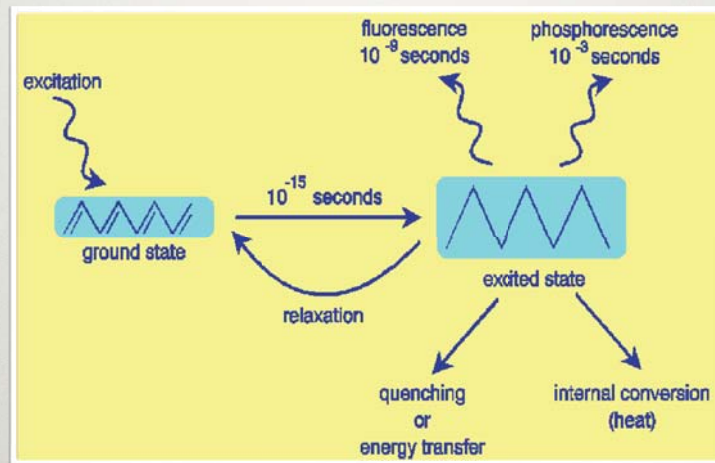
Photon emission (fluorescence or phosphorescence) occurs only from the lowest-energy excited electronic state of a molecule.



Michael Kasha (1920-) American physicist

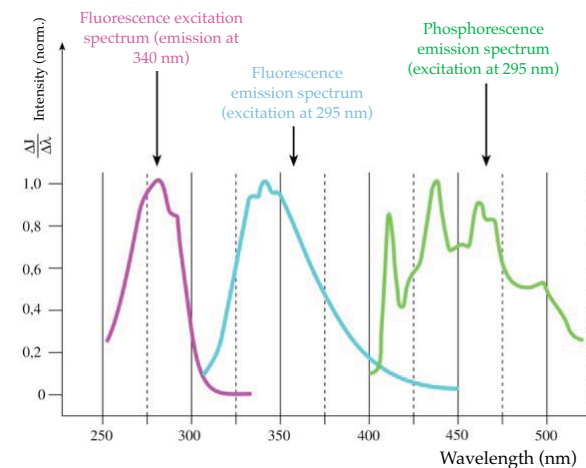


TIME SCALE OF TRANSITIONS



CHARACTERIZATION OF LUMINESCENCE I.

Luminescence spectra



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

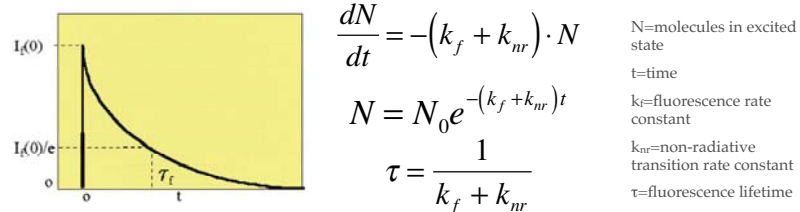
CHARACTERIZATION OF LUMINESCENCE II.

Quantum yield

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

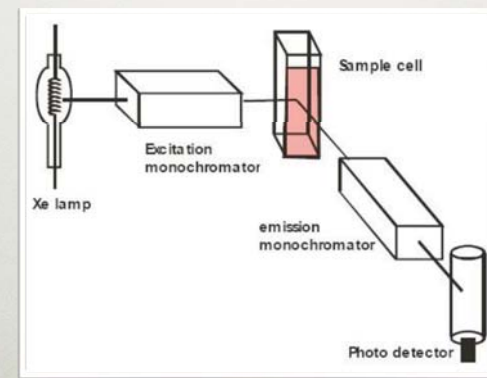
k_{nr} =non-radiative transition rate constants

Excited-state lifetime



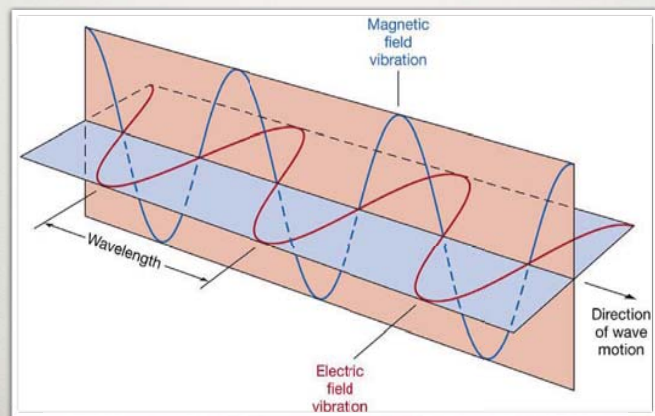
MEASUREMENT OF FLUORESCENCE

Fluorescence spectrometer ("Steady-state" spectrofluorometer)

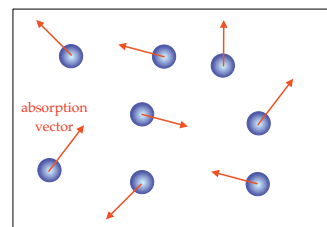


LIGHT IS ELECTROMAGNETIC RADIATION

- Propagating electromagnetic disturbance.
- Transverse wave.
- Can be polarized.



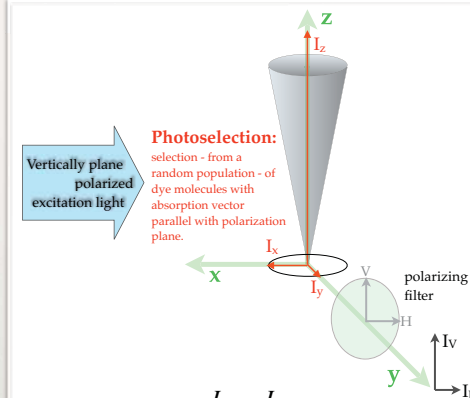
POLARIZATION, ANISOTROPY



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 electrical polarization vector).

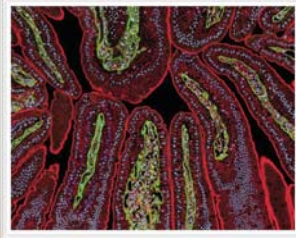
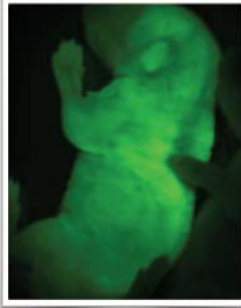
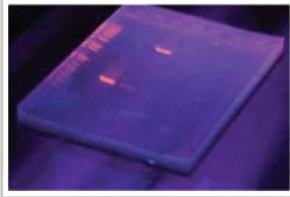
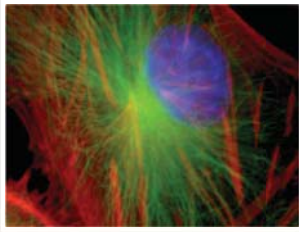


Polarization: $p = \frac{I_{VV} - I_{VH}}{I_{VV} + I_{VH}}$

Anisotropy: $r = \frac{I_{VV} - I_{VH}}{I_{VV} + 2I_{VH}}$

BIOMEDICAL APPLICATIONS OF FLUORESCENCE

- ✦ 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



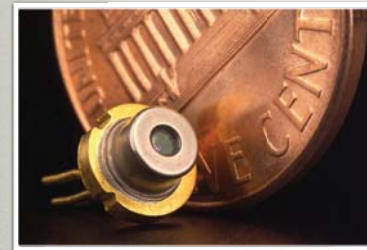
SUMMARY

- Molecular structure and energy states are important in luminescence.
- Luminescence is molecular de-excitation followed by radiation.
- Fluorescence spectra are characterized by Stokes shift.
- Quantum yield and lifetime are important luminescence parameters.

LASER

BASIC PRINCIPLES, CHARACTERISTICS, APPLICATIONS

LASERS ARE EVERYWHERE



5 mW diode laser
few mm



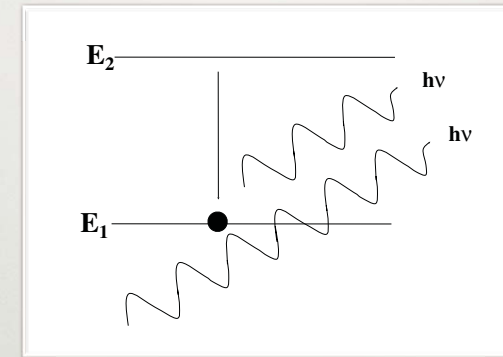
Terawatt NOVA laser
Lawrence Livermore
Laboratories
Size of a football field

LASER

1. What is 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 AMPHIFICATION BY STIMULATED EMISSION OF RADIATION”



MASER: Microwave Amplification by Stimulated Emission of Radiation

LASER HISTORY IN A NUTSHELL

1917 - *Albert Einstein*:

theoretical prediction of stimulated emission.

1946 - *G. Meyer-Schwickerath*: 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)

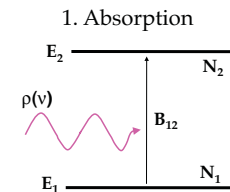
1964 - *Basow, Prochorow, Townes (Nobel-prize)*: quantum electronics

1970 - *Arthur Ashkin*: laser tweezers

1971 - *Dénes Gábor (Nobel-prize)*: holography

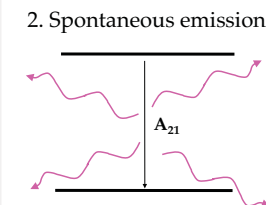
1997 - *S. Chu, W.D. Phillips and C. Cohen-Tannoudji (Nobel-prize)*:
atom cooling with laser.

PRINCIPLES OF LASER I. STIMULATED EMISSION



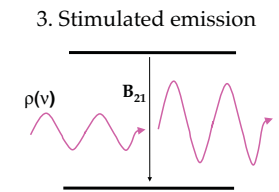
Frequency of transition:
 $n_{12} = N_1 B_{12} \rho(\nu)$

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



Frequency of transition:
 $n_{21} = N_2 A_{21}$

$E_2 - E_1$ photons
travel independently in all
directions.



Frequency of transition:
 $n_{21} = N_2 B_{21} \rho(\nu)$

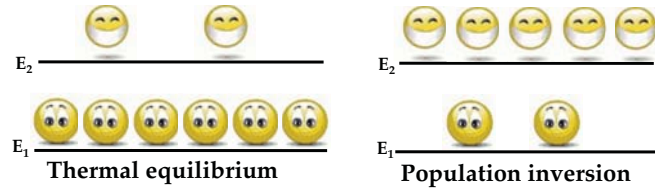
In the presence of external field.
Field energy is increased.
Phase, direction and frequency of
emitted and external photons are
identical.

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), $B_{12} = B_{21}$

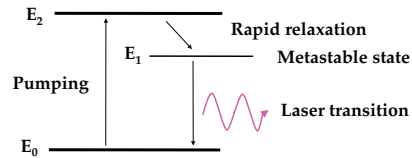
PRINCIPLES OF LASER II. POPULATION INVERSION

Light amplification depends on the relative population of energy levels.

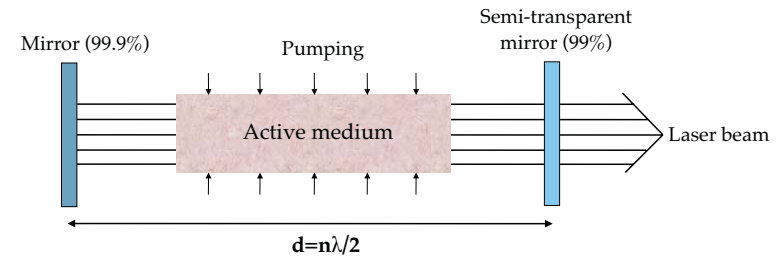
$$F \xrightarrow[\text{dz}]{\text{Active medium}} F+dF \quad dF=FA(N_2-N_1)dz$$



- 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

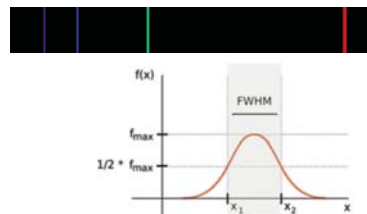
Parallel (collimated) beam

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

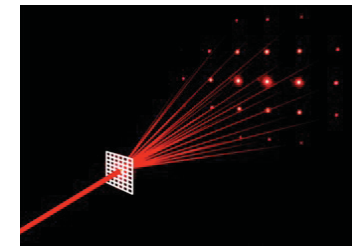
PROPERTIES OF LASER LIGHT II.

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

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; $\lambda \sim 10 \mu\text{m}$; 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.

APPLICATION OF LASERS

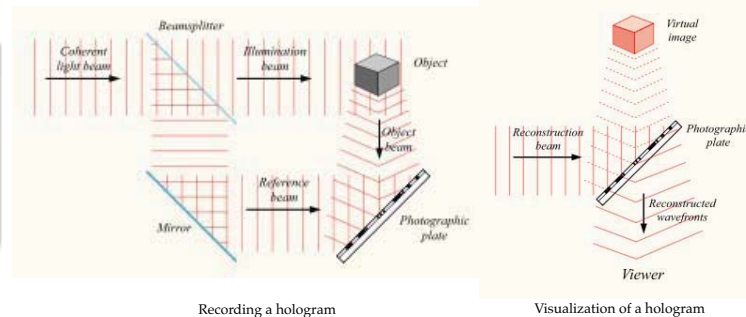
BASED ON POWER

- 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

HOLOGRAPHY



Dénes Gábor



Surface of a hologram recording

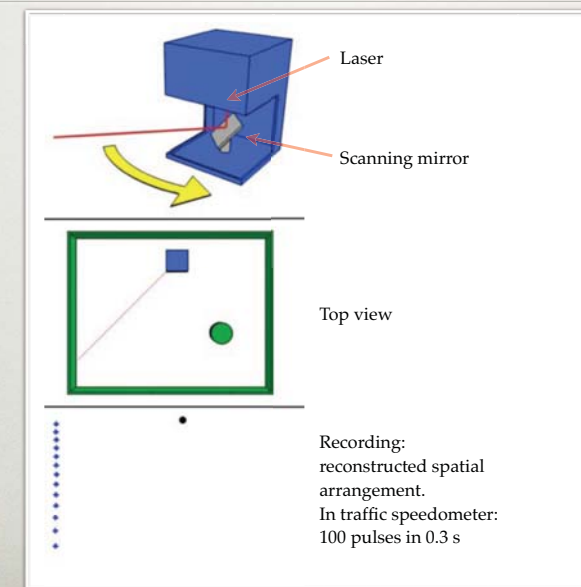


Holograms



SPEED MEASUREMENT WITH LASER

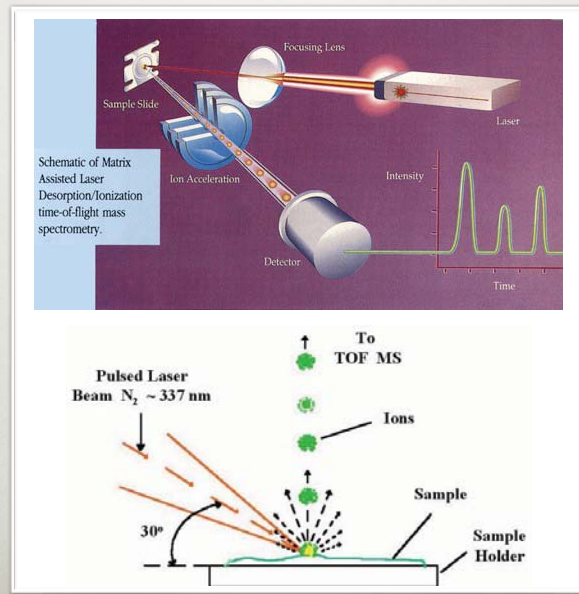
LIDAR: "LIGHT DETECTION AND RANGING"



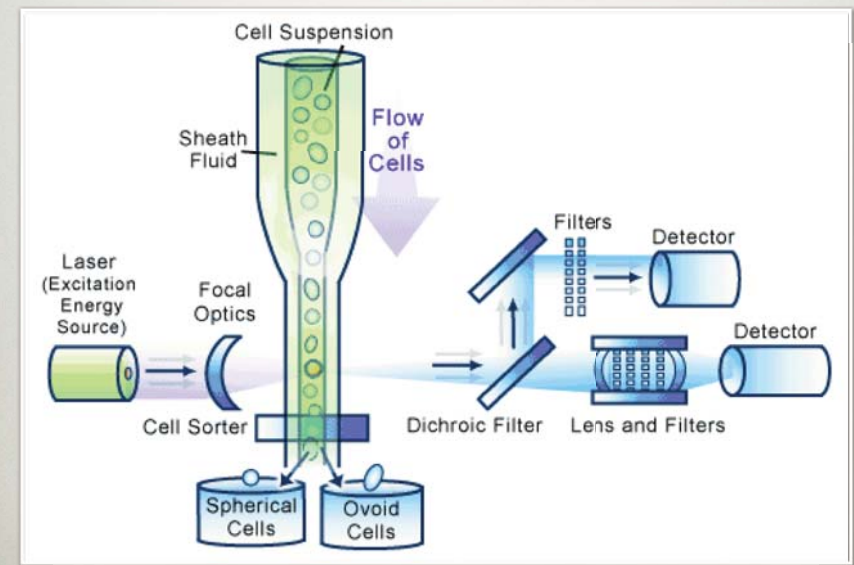
Recording:
reconstructed spatial
arrangement.
In traffic speedometer:
100 pulses in 0.3 s

MALDI-TOF:

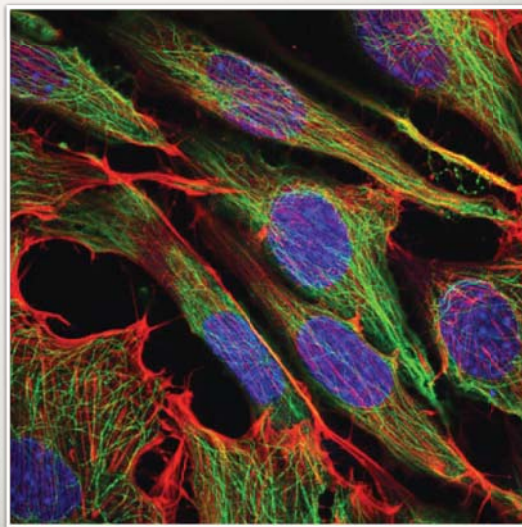
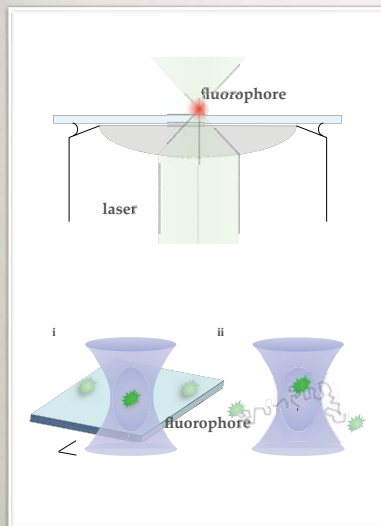
MATRIX-ASSISTED LASER DESORPTION/IONIZATION
TIME OF FLIGHT MASS SPECTROMETRY



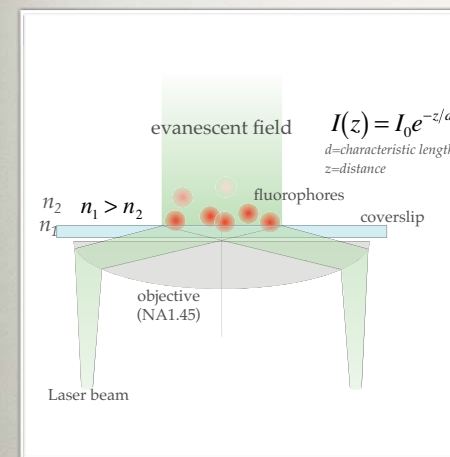
FLUORESCENCE ACTIVATED CELL SORTER (FACS)



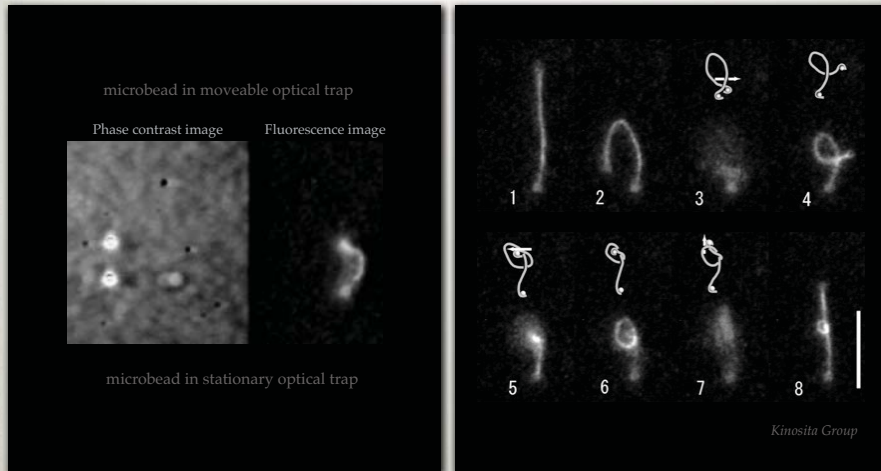
LASER SCANNING CONFOCAL MICROSCOPE



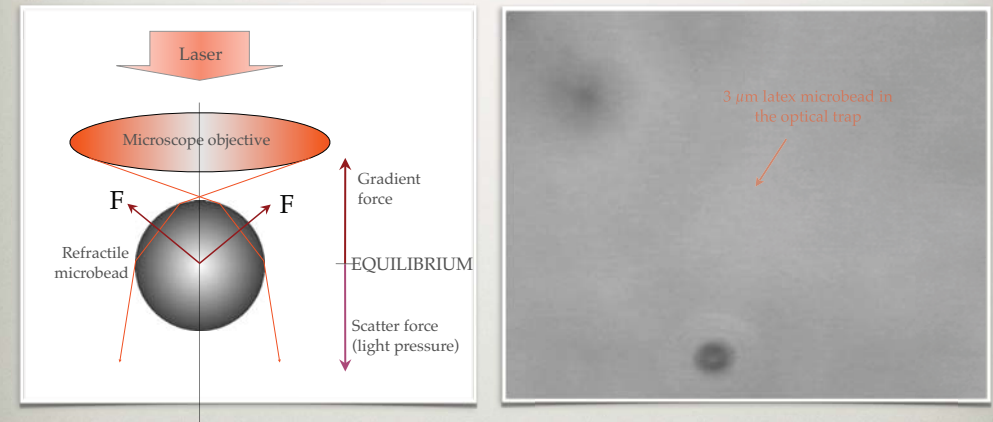
TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY (TIRFM)



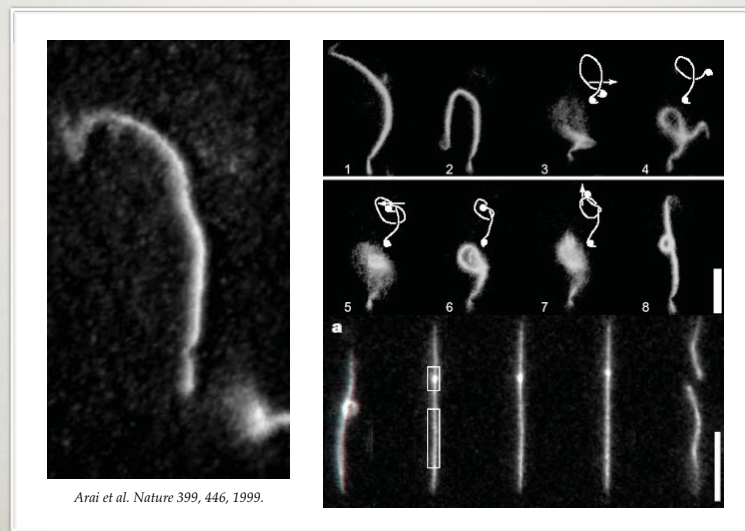
TYING A KNOT ON A SINGLE DNA MOLECULE



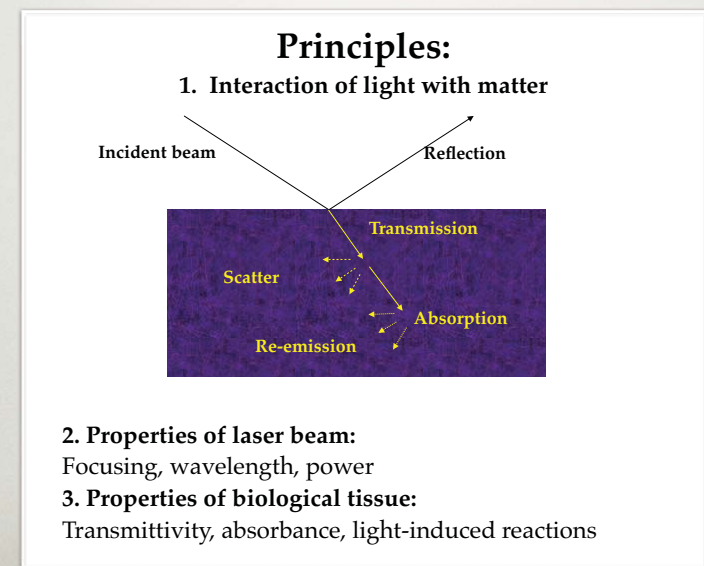
OPTICAL TWEEZERS



TYING A KNOT ON A SINGLE ACTIN FILAMENT WITH OPTICAL TWEEZERS



BIOMEDICAL APPLICATIONS OF LASERS I.



BIOMEDICAL APPLICATIONS OF LASERS II.

Surgical disciplines: "laser knife", coagulation, blood-less surgery. Tumor removal, tattoo removal. CO₂ and Nd:YAG lasers.

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: 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/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: 3. TATTOO REMOVAL

Q-switched Nd:YAG laser (1064 nm)

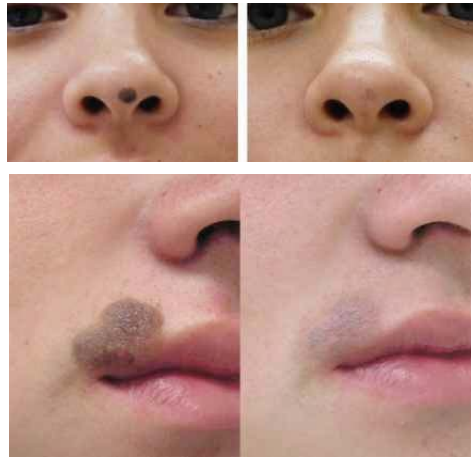


Before treatment

After treatment

DERMATOLOGICAL APPLICATIONS:

4. NAEVUS REMOVAL

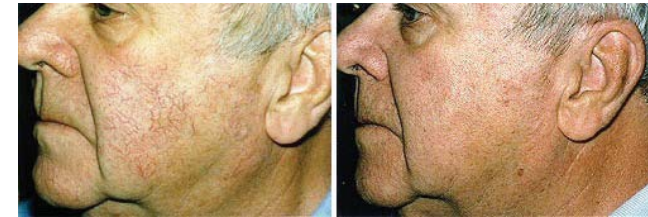


Before treatment

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
CO₂, Erbium:YAG laser



Wrinkle removal



Sun damage



Rhinophyma

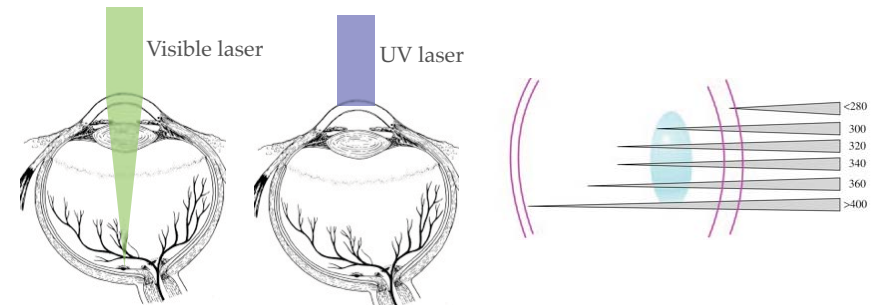


Systemic epidermal naevus

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).

Photorefractive keratectomy (PRK)

Another type of refractive laser eye surgery.

But, there is no layer removal. The surface restructuring is smaller. However, it is more painful, and regeneration is slower.

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 -> excited singlet state -> triplet state -> energy transfer with triplet oxygen -> excited, reactive oxygen -> tissue reaction
5. The illuminated area necrotizes in a few days.

LASER: KEY WORDS

What is needed for laser function?

- Stimulated emission
- Population inversion
- Pumping
- Optical resonance

What are the properties of laser light?

- Monochromatic
- Coherent
- Large power