Thermal radiation
Luminescence

Medical Biophysics I. 30 October, 2024

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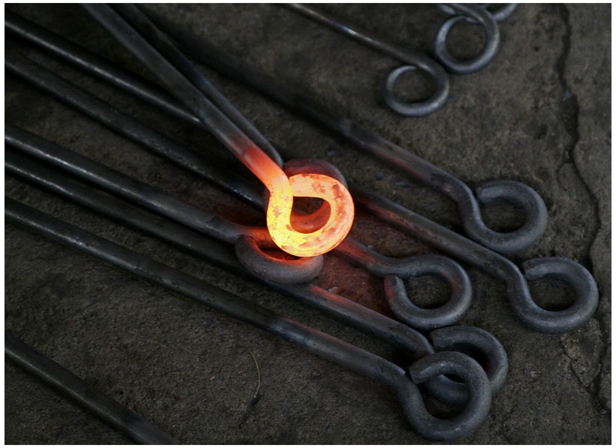
SEMMELWEIS EGYETEM 1769

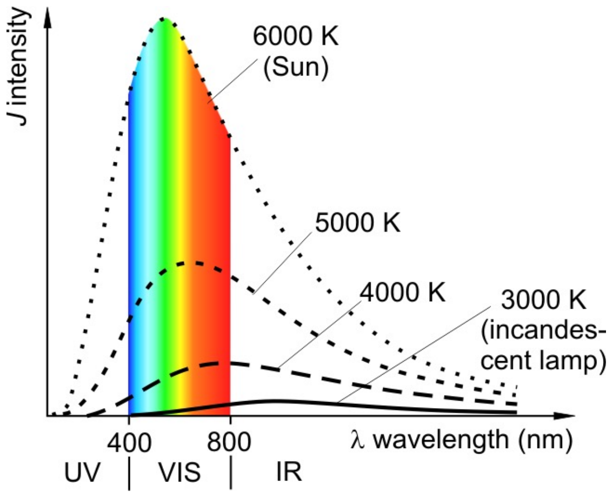
Sources of light emission

1. Thermal (black body) radiation

Mechanism: thermal motion of atoms, molecules

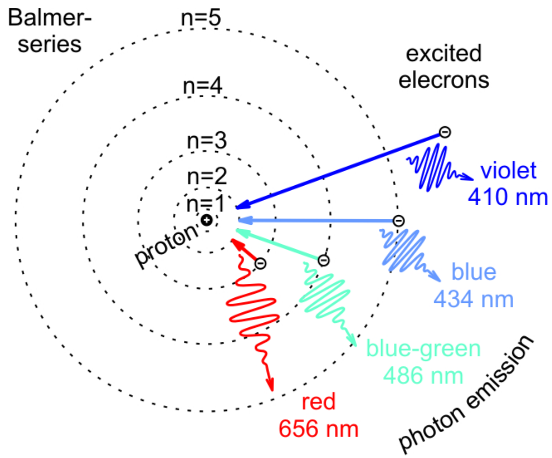
Source of light energy: internal energy of the system



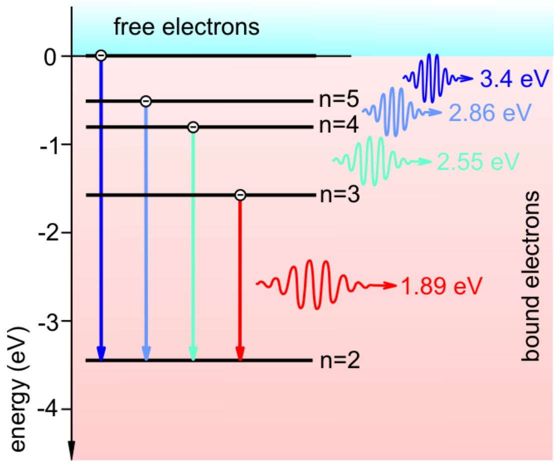


2. Luminescence

Mechanism: emission of excited-state energy

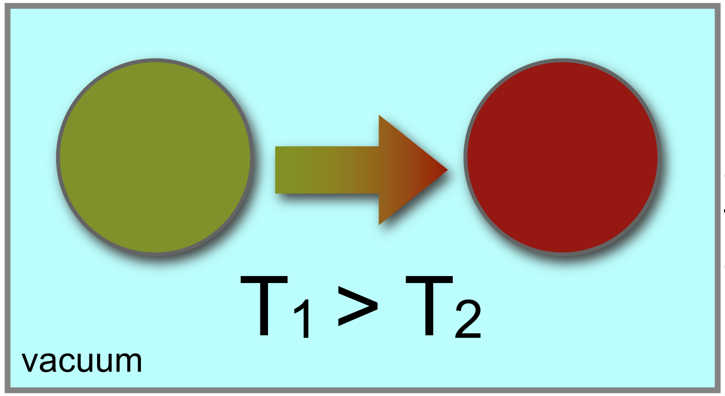


Source of light energy: energy of excited state

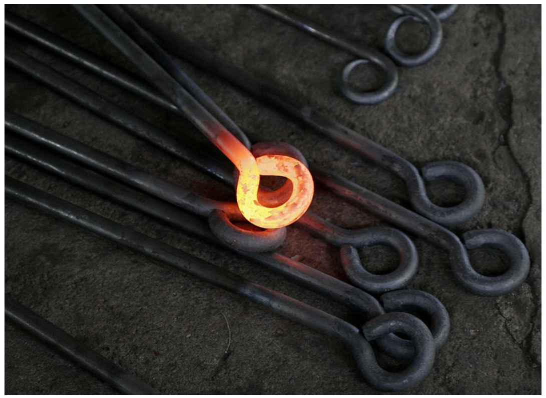


“Black-body” (Thermal) radiation

- 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-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”...?

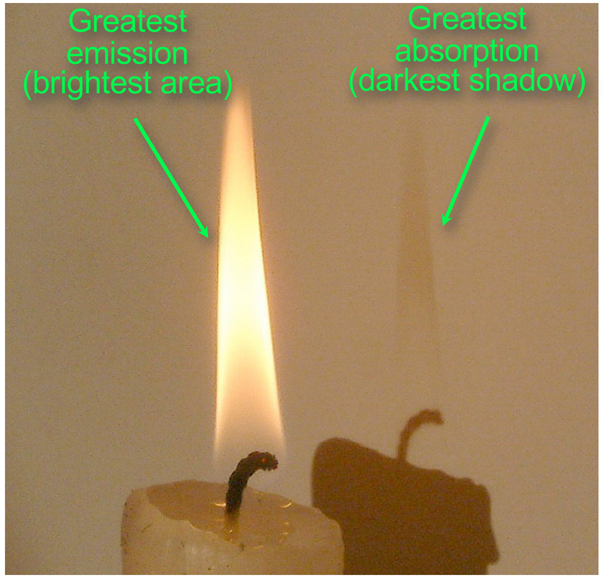
Black body absorbs all light falling on it

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)

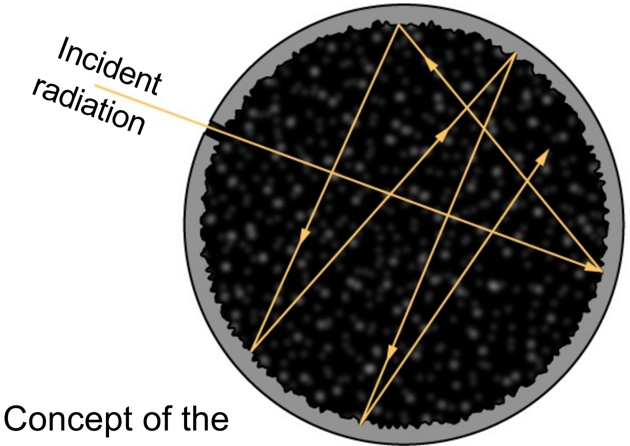
$$\frac{M_{\lambda i}}{\alpha_{\lambda i}} = \frac{M_{\lambda j}}{\alpha_{\lambda j}}$$



For a black body (BB):

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

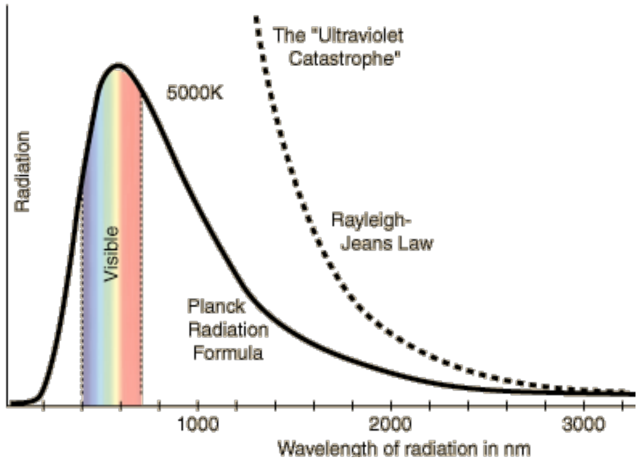
- That is, the black body absorbs all light that it is exposed to (nothing is reflected).
- The black body is an ideal object for investigating temperature-dependent emission.

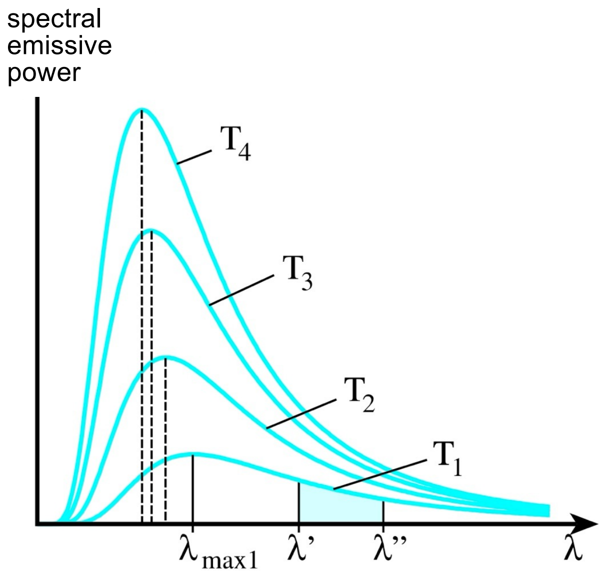


Concept of the absolute black body

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:

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

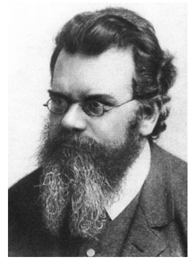
Planck's law of radiation:

$$E = hf$$

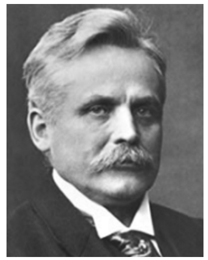
h = Planck's constant (6.626 x 10^{-34} Js).

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

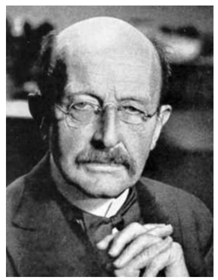
Jozef Stefan (1835-1893)



Ludwig Eduard Boltzmann (1844-1906)



Wilhelm Wien (1864-1928)



Max Karl Ernst Ludwig Planck (1858-1947)

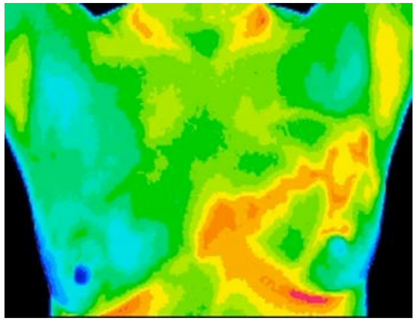
Applications of thermal radiation

Thermography, infradiagnostics

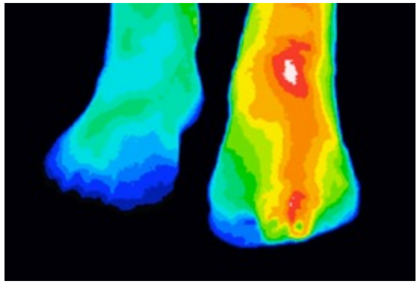
“Seeing through” a non-obsorbing layer

Airport thermography

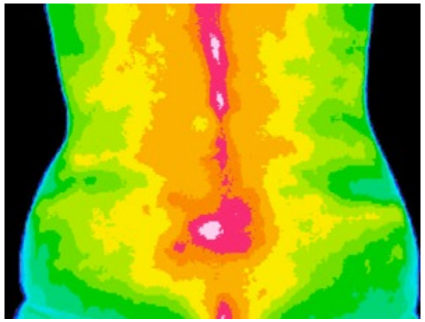
Detection of febrile condition, prevention of epidemics



Breast carcinoma



Inflammation



Chronic musculoskeletal stress (pain)

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

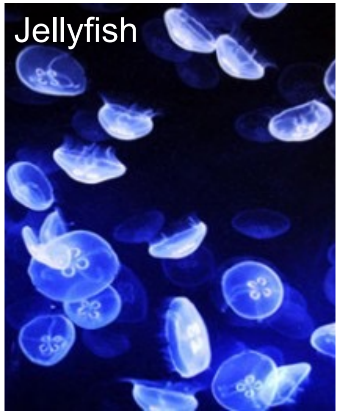
Luminescence is everywhere

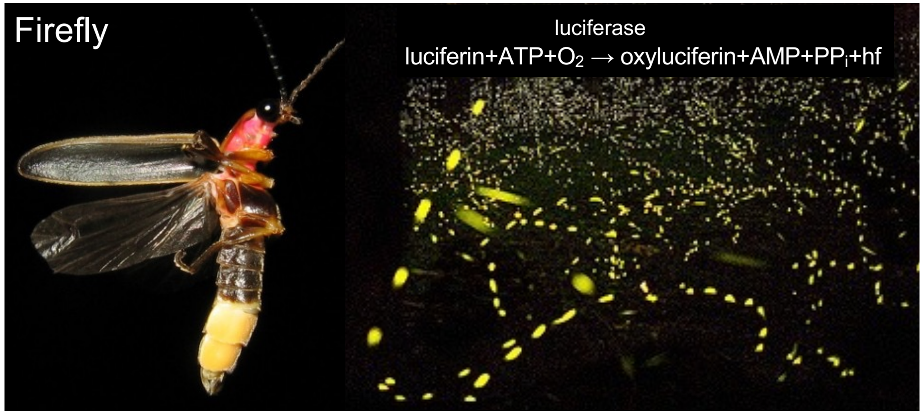
Photoluminescence

Luminescence is everywhere

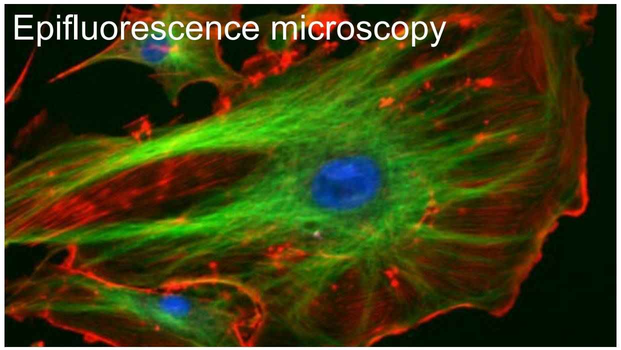
Radio-
luminescence

Bio-/chemi-
luminescence





Fluorescence
applications

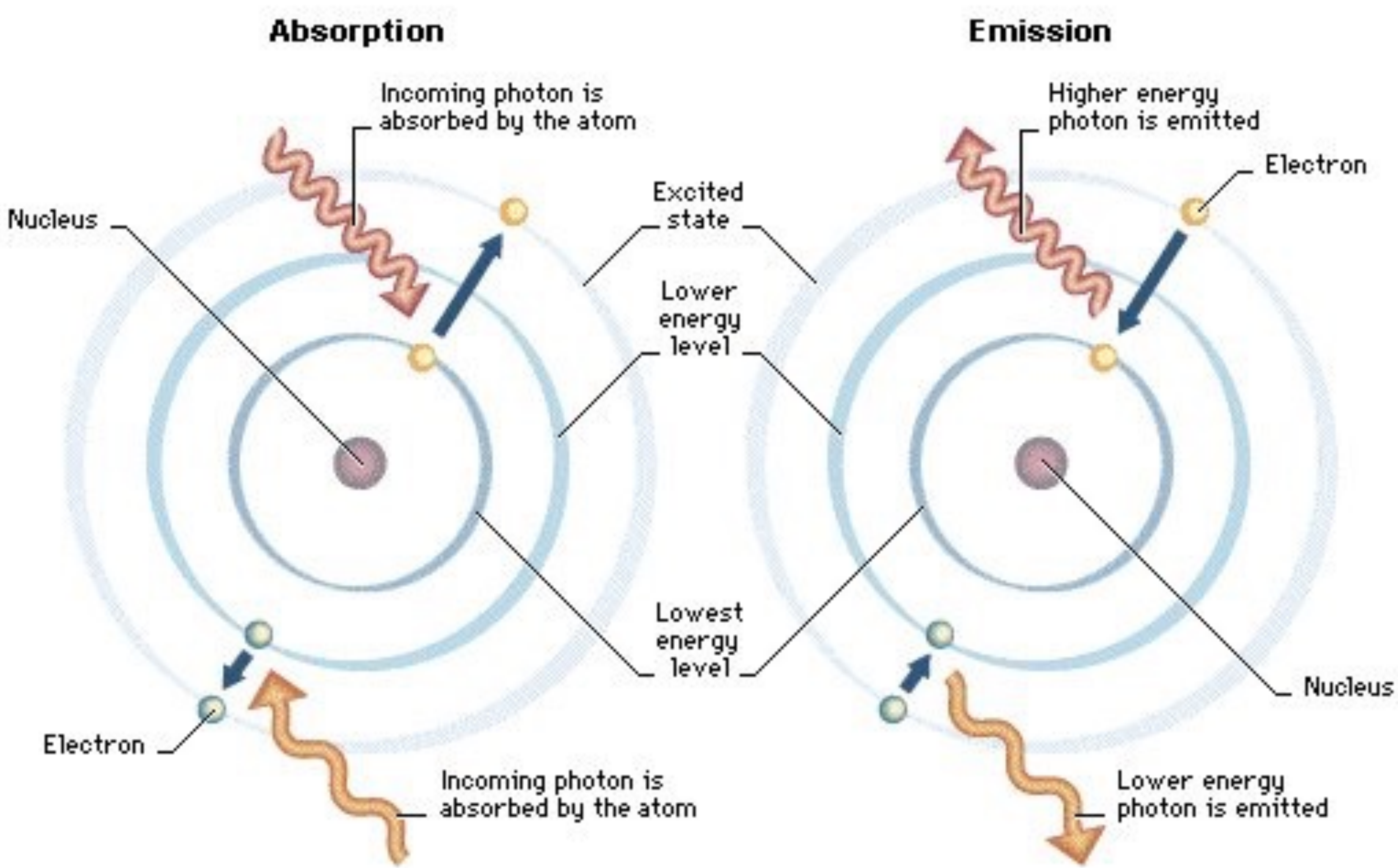


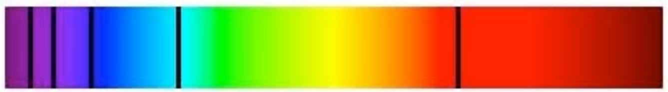
Types of luminescence

(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





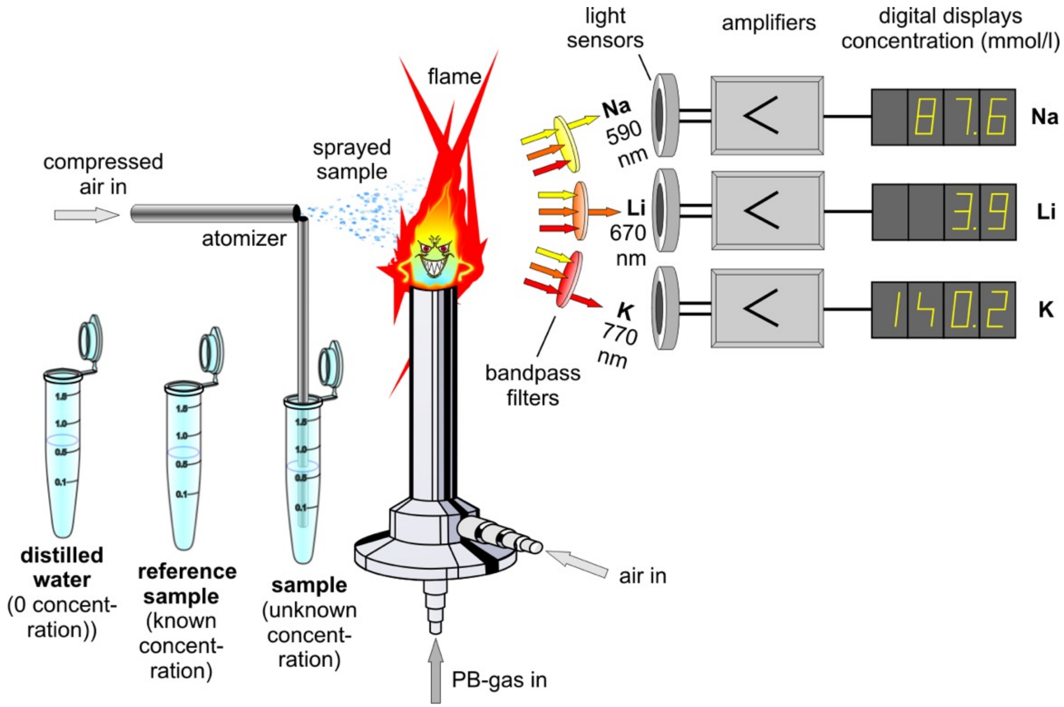
Absorption line spectrum

Emission line spectrum

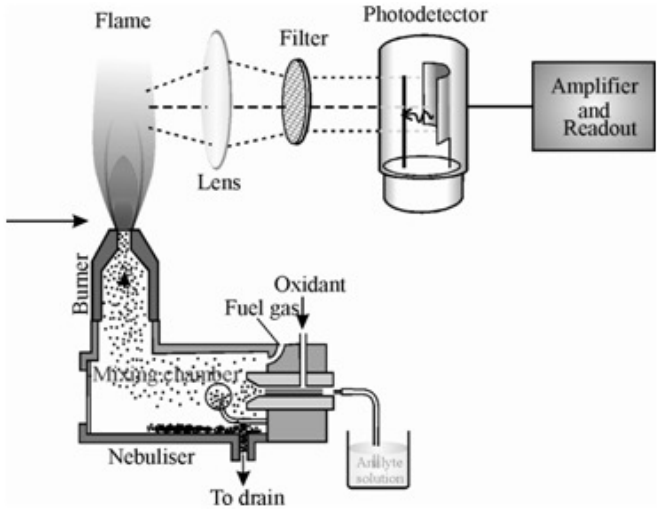
Application of emission sectroscopy

Flame photometry

Qualitative and quantitative analysis of alkali metals

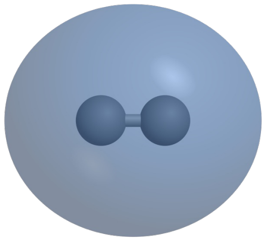


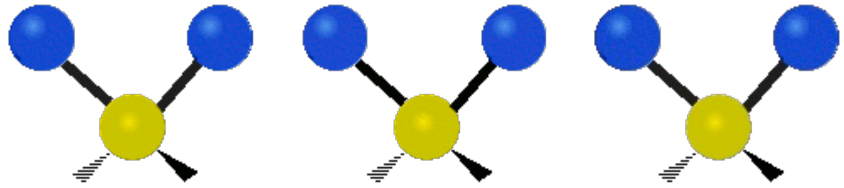
Clinical practice: determination of serum ions (Na^+, K^+)



The emission by an excited molecule is non-linear, because its energy levels are complex

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

Energy of a molecule: Born-Oppenheimer - approximation:

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

- 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.

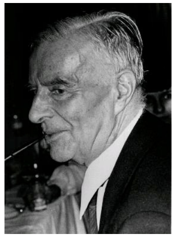
Scaling of transition energies:

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

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

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

Representation of energy states

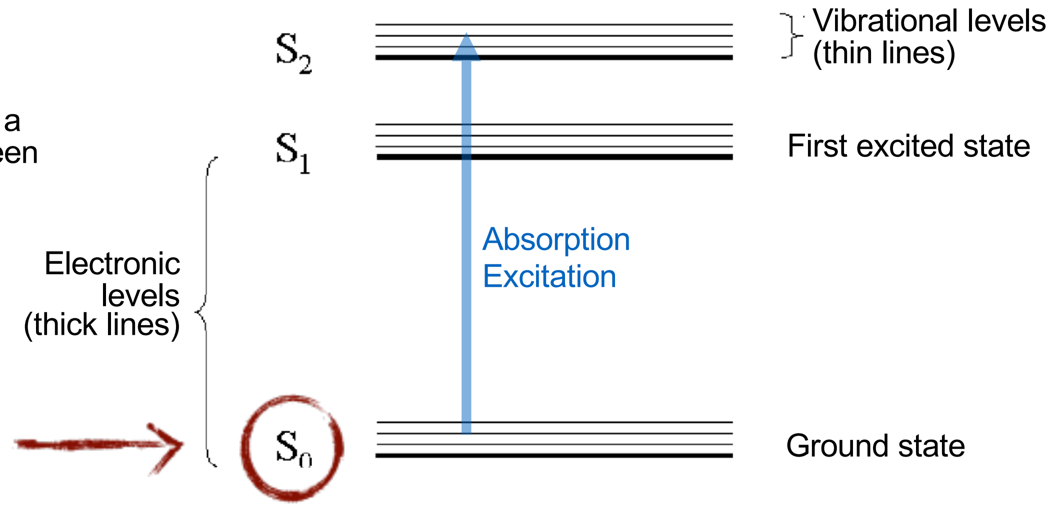


Alexander Jabłoński
(1898-1980)

Jabłoński diagram:

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

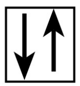
What is this "S" (singlet) state?



Spin states – Pauli's exclusion principle

Wolfgang Pauli
(1900-1958)

- 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.

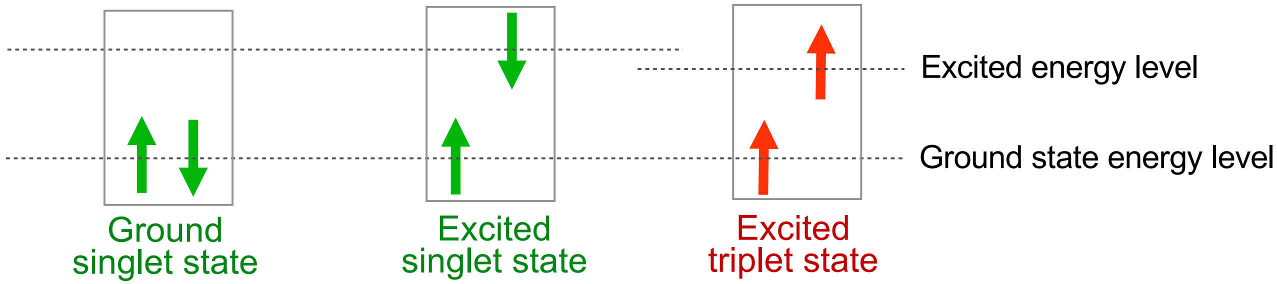


fully occupied subshell:
spin pairing
(opposite-spin electrons pair)

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)

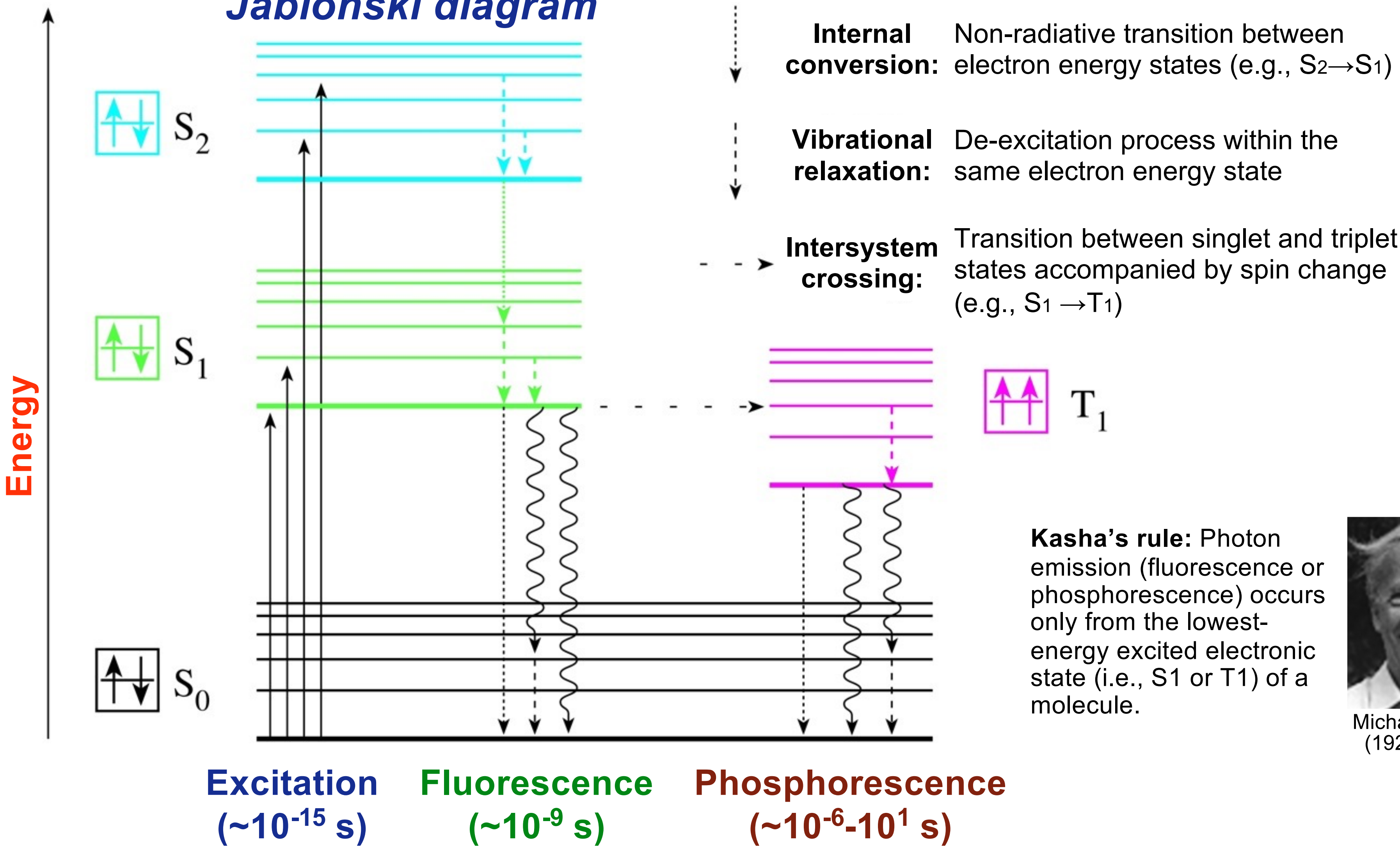
S: singlet state: paired electrons with opposite spins, net spin (S) = 0, number of orientations (2S+1) = 1.

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.

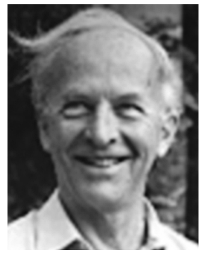


Processes of luminescence

Jablonski diagram



Kasha's rule: Photon emission (fluorescence or phosphorescence) occurs only from the lowest-energy excited electronic state (i.e., S1 or T1) of a molecule.

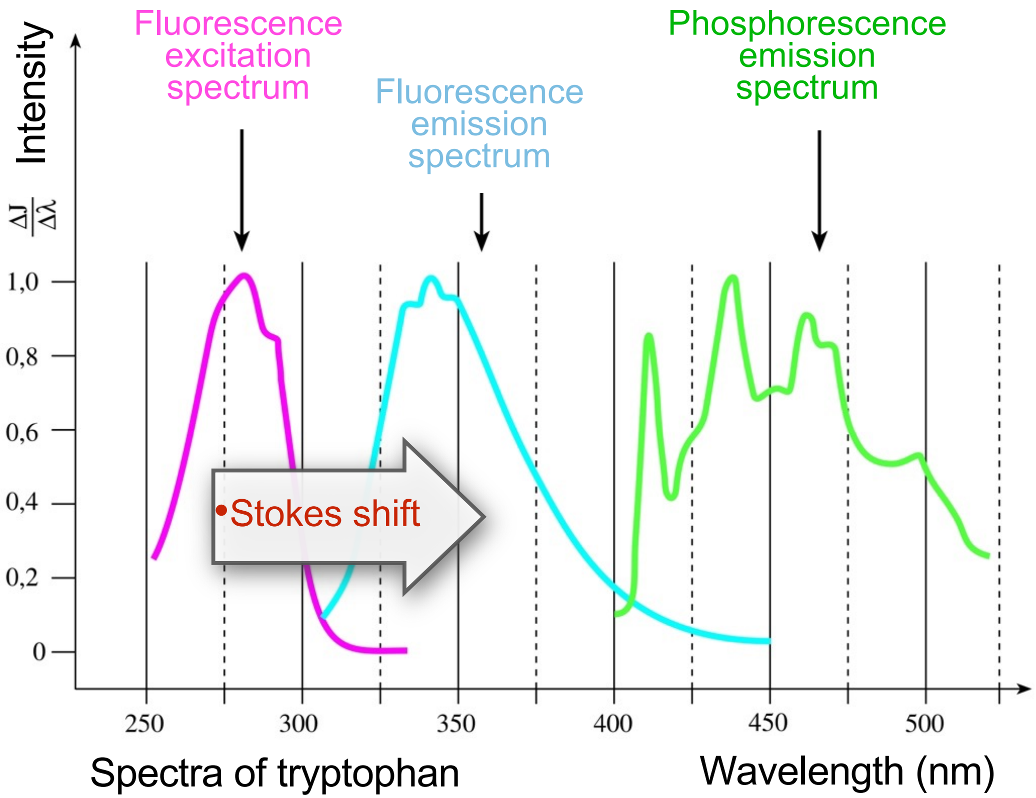


Michael Kasha (1920-2013)

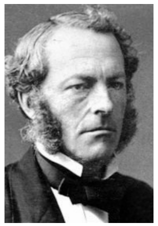
Characterization of luminescence

1. Spectra

- Band spectra
- Excitation-emission are mirror-symmetric

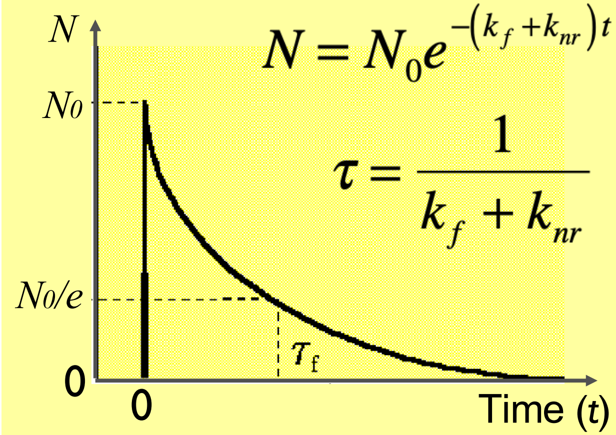


Spectra of tryptophan



George Stokes
(1819-1903)

2. Excited-state lifetime (τ)



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

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

k_f = fluorescence rate constant

k_{nr} = rate constant of non-radiative transitions

3. Quantum yield (Φ)

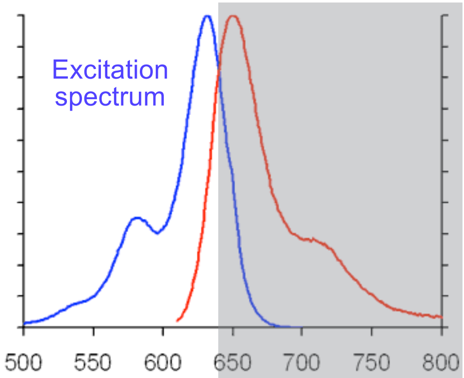
$$\Phi = \frac{k_f}{k_f + k_{nr}} = \frac{N_{emitted\ photons}}{N_{absorbed\ photons}} \leq 1$$

Measurement of luminescence

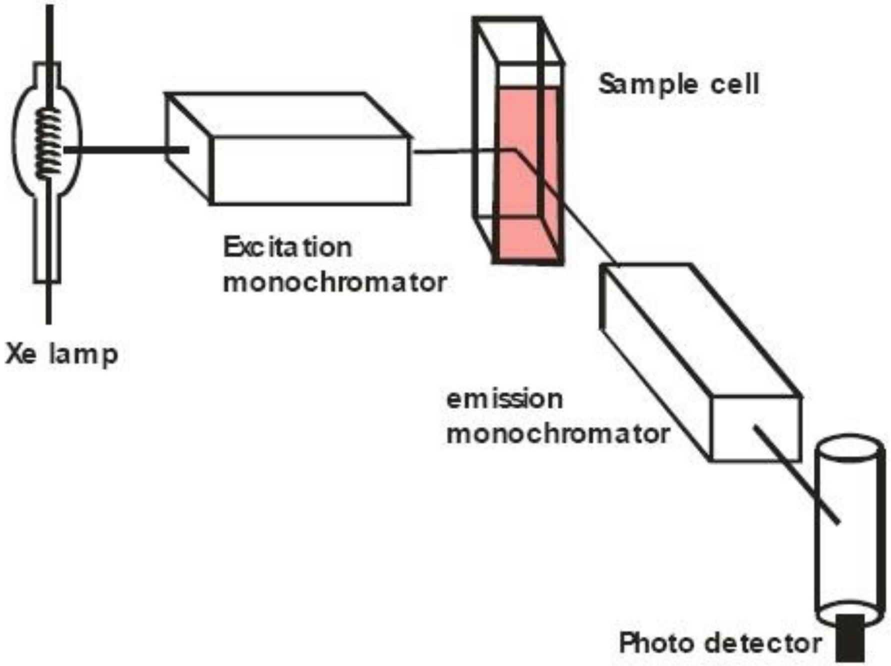
Fluorescence spectrometer

("Steady-state" spectrofluorometer)

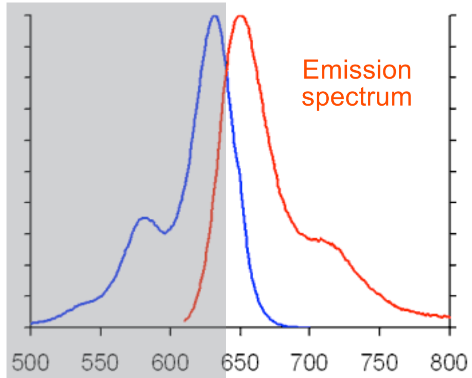
Spectrum recorded while the ***excitation*** wavelength is varied



λ_{em}= constant
(emission maximum)



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

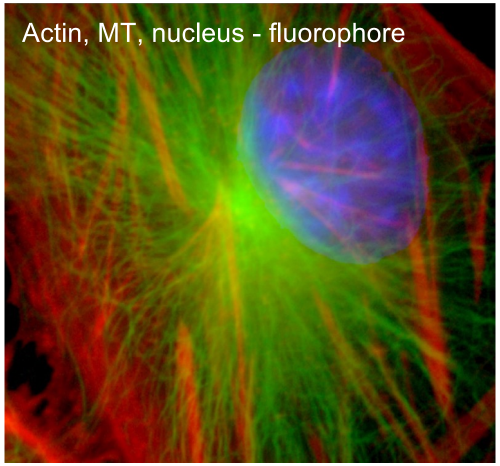


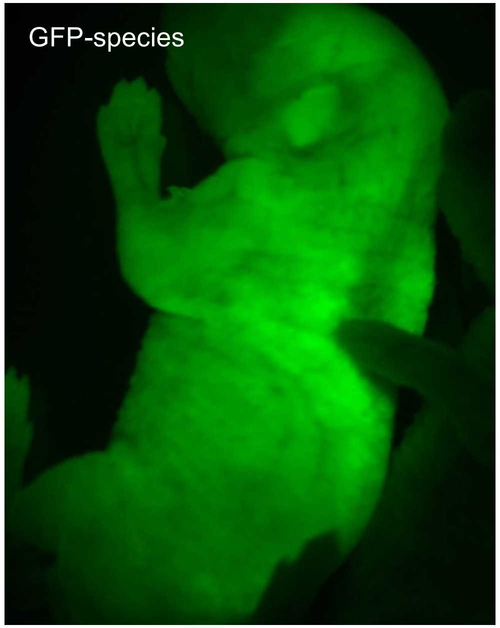
λ_{ex}= constant
(excitation maximum)

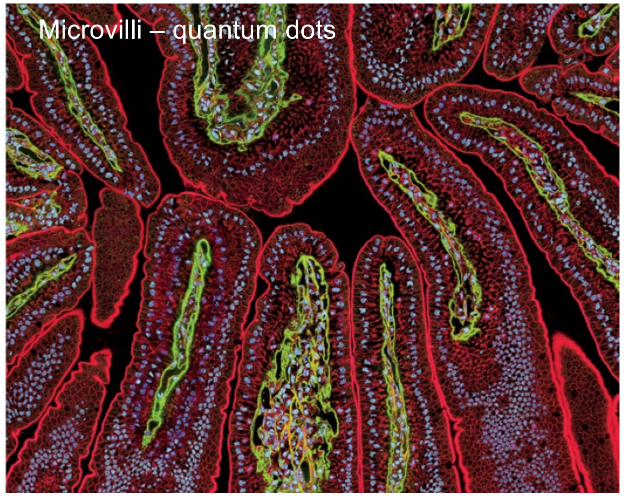
Biomedical applications of fluorescence

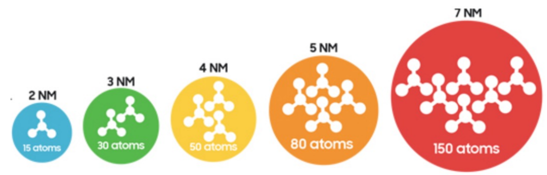
- Fluoresent labeling with fluorophores
- DNA sequencing (chain termination), DNA staining (EtBr), DNA microarray technology
- Immunofluorescence
- Fluorescent protein conjugation techniques (GFP)
- FRET (Fluorescence Resonance Energy Transfer)
- FRAP (Fluorescence Recovery After Photobleaching)
- FACS (Fluorescence Activated Cell Sorting)
- Quantum-dot labeling
- Etc., etc.

Moungi G. Bawendi, Louis E. Brus, Alexei I. Ekimov
kémiai Nobel-díj 2023



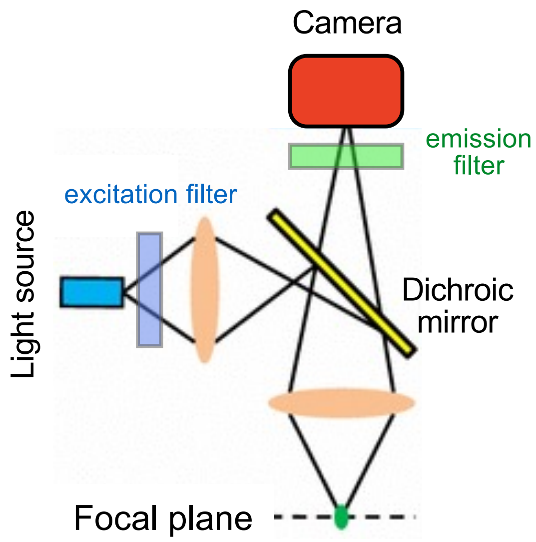






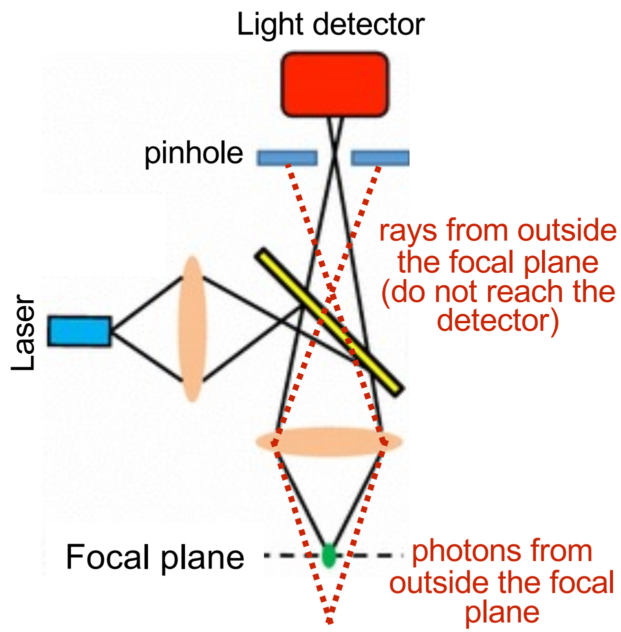
Epifluorescence microscope and Laser scanning confocal microsope

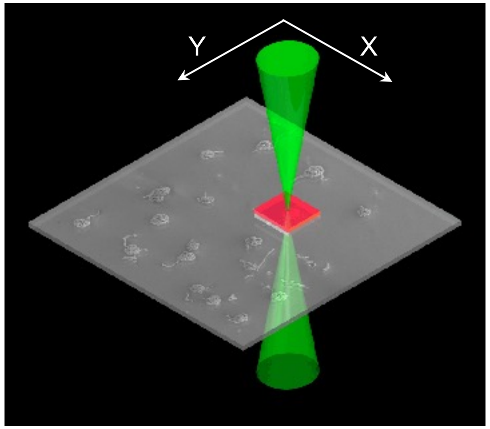
„**Epi**”:
from above



Confocality:
focal points of the excitation- and emission-side geometric optics are identical.

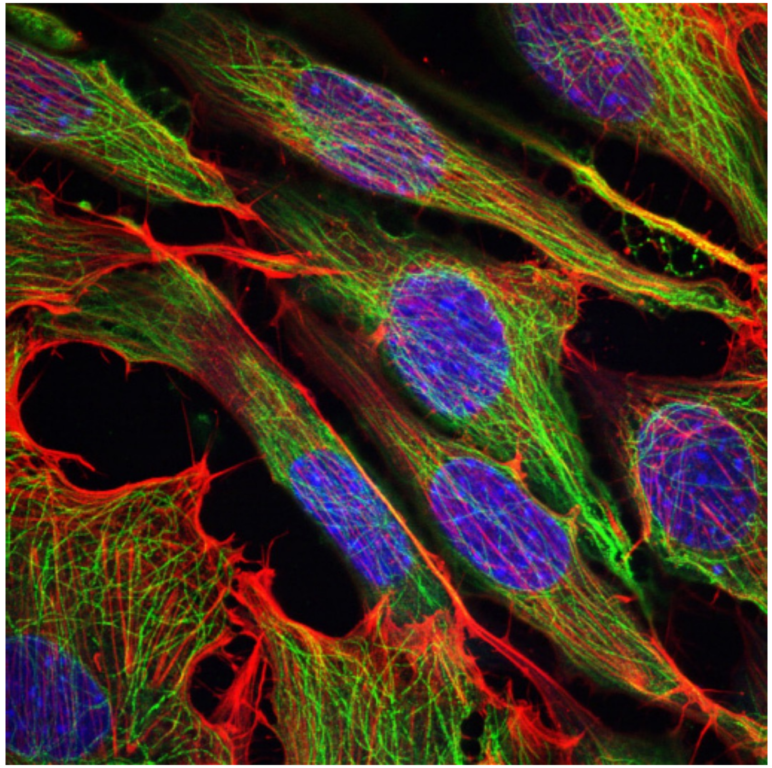
Confocal principle:
photons that are not from the focal point can be filtered by using a „pinhole”.





Scaning, point-by-point data collection

Green: microtubules;
Red: actin;
Blue: nucleus



Super-resolution microscopy

Chemistry Nobel-prize, 2014

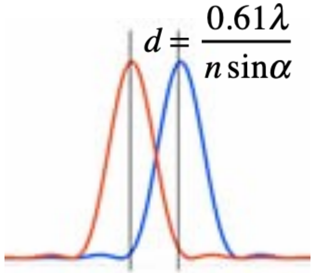

Eric Betzig


Stefan Hell


William E. Moerner

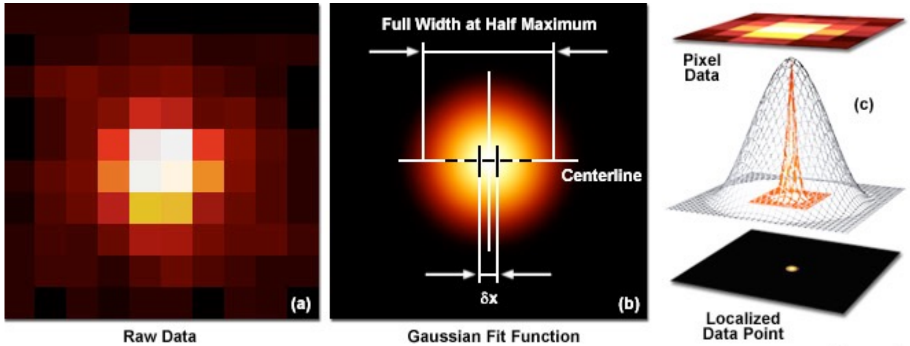
Resolution problem is converted into position-determination problem

Resolution problem (Abbé)

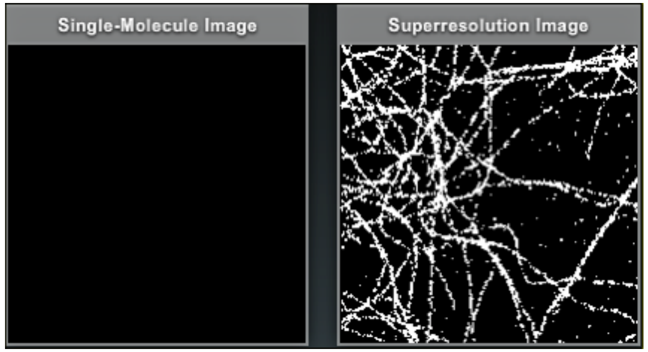


$$d = \frac{0.61\lambda}{n \sin\alpha}$$

Position determination problem
(precision depends on photon count)

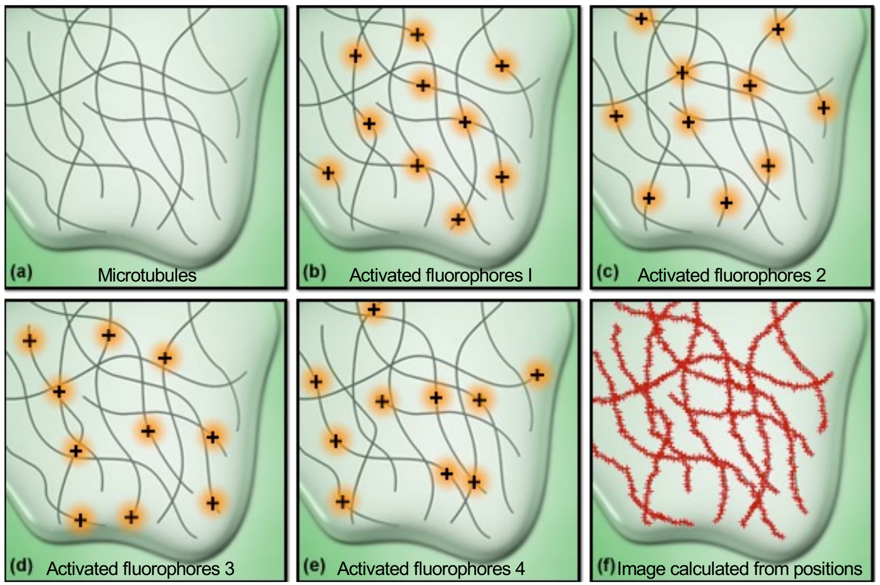


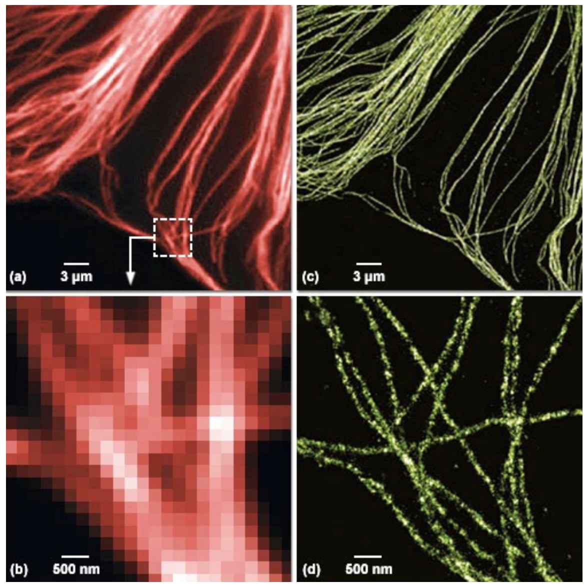
“Stochastic” data collection, single fluorophores



STORM (“stochastic optical reconstruction microscopy”); PALM (“photoactivated localization microscopy”)

Data collection process





Microtubular system

Feedback

https://feedback.semmelweis.hu/feedback/pre-show-qr.php?type=feedback&qr=DCQ4DIJQDTA4FUQH