Biophysics I

7. Luminescence

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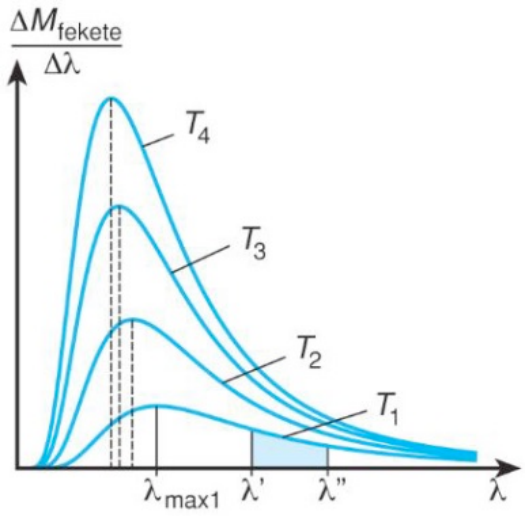
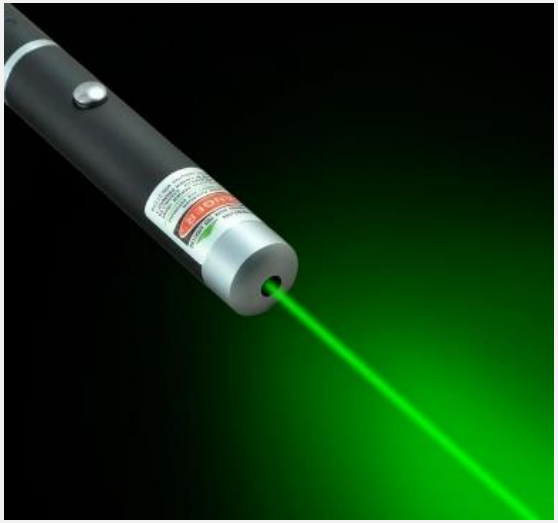
Generation of light

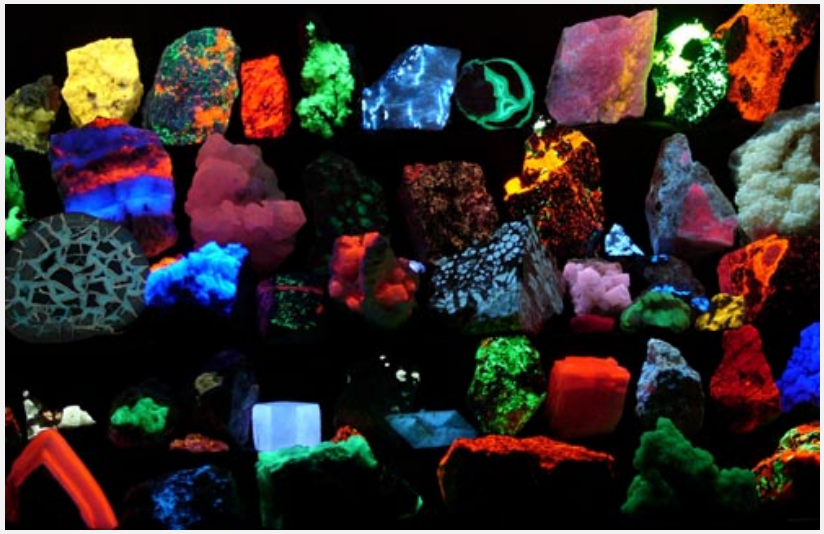
- Thermal (black-body) radiation
- Luminescence
- Laser

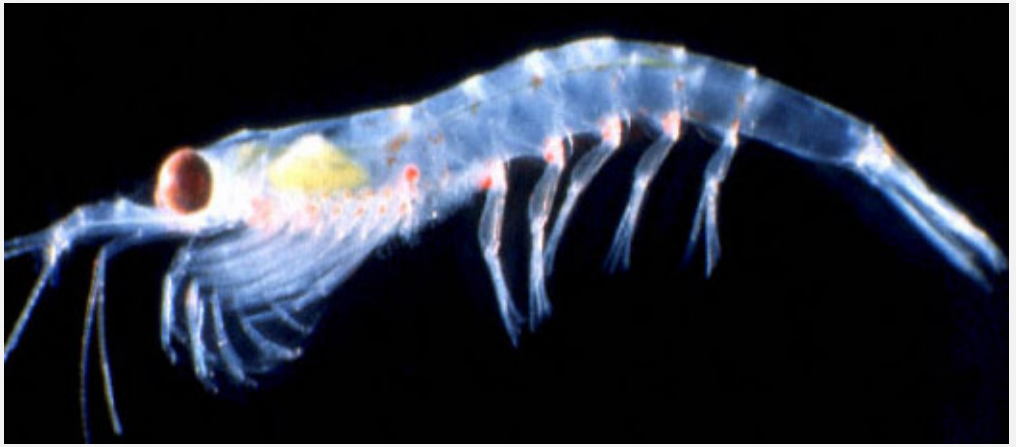




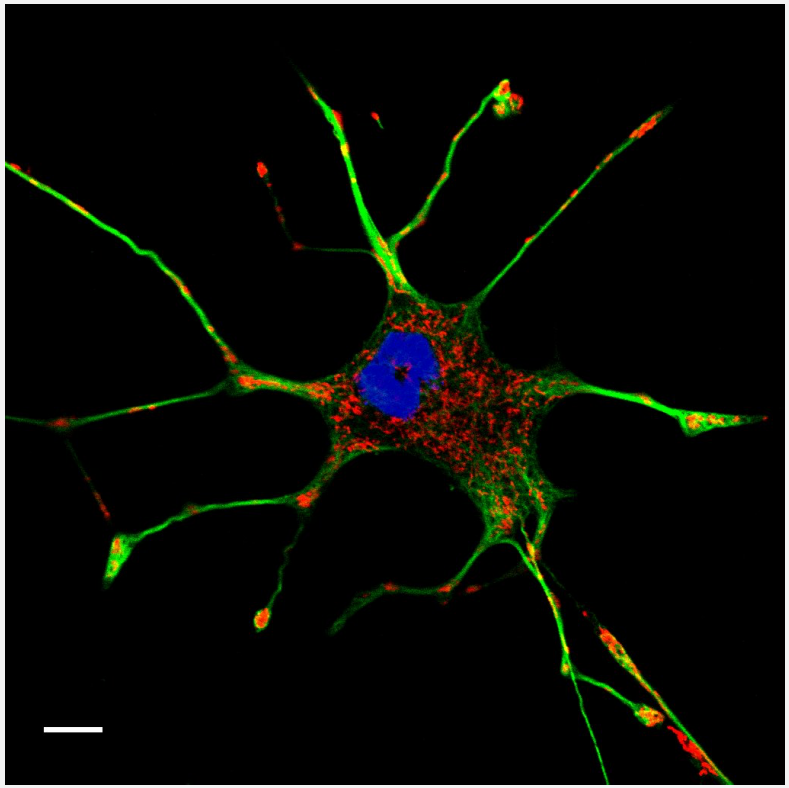
Luminescence: radiations released above the thermal radiation of a body (cold light).

Luminescence in Nature



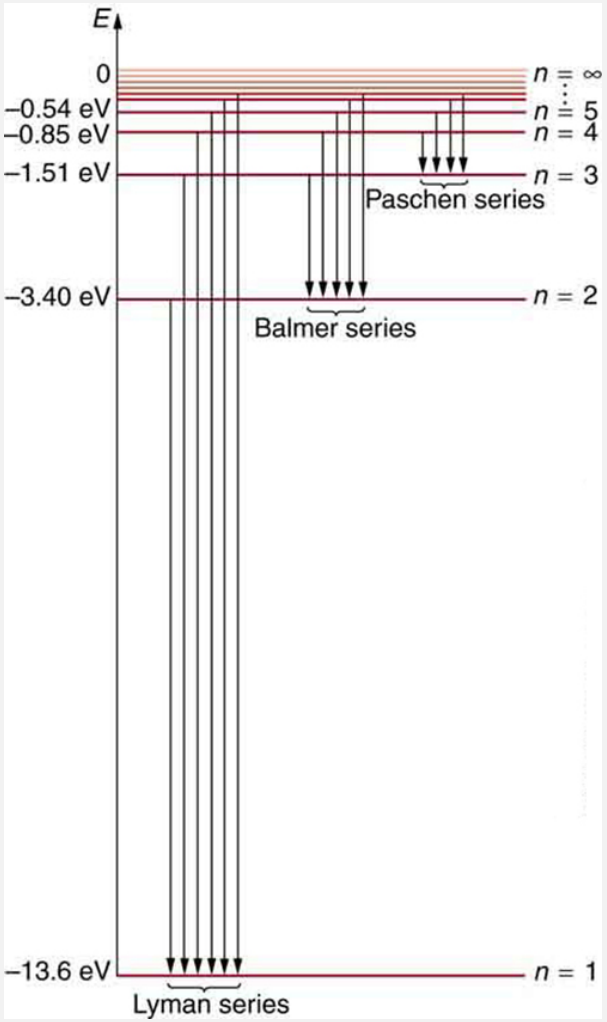


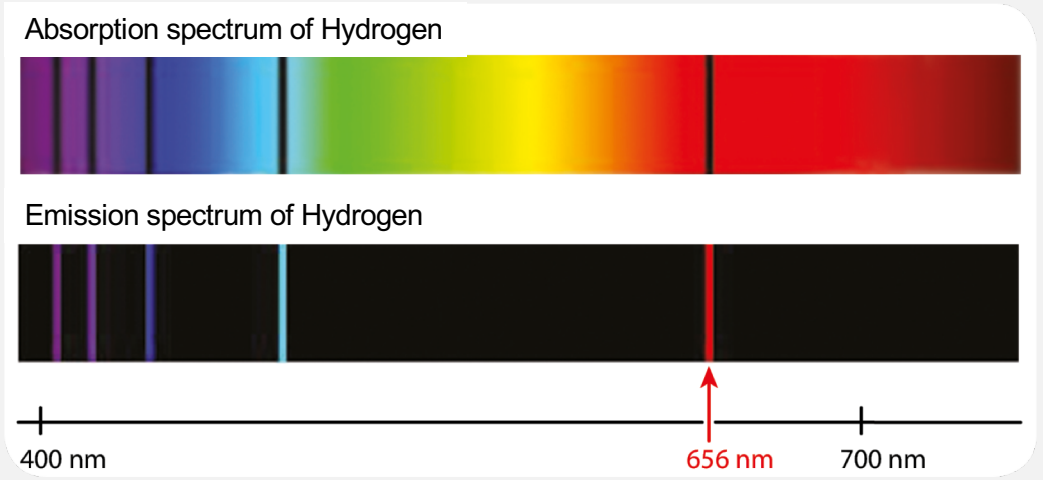
Applied Luminescence

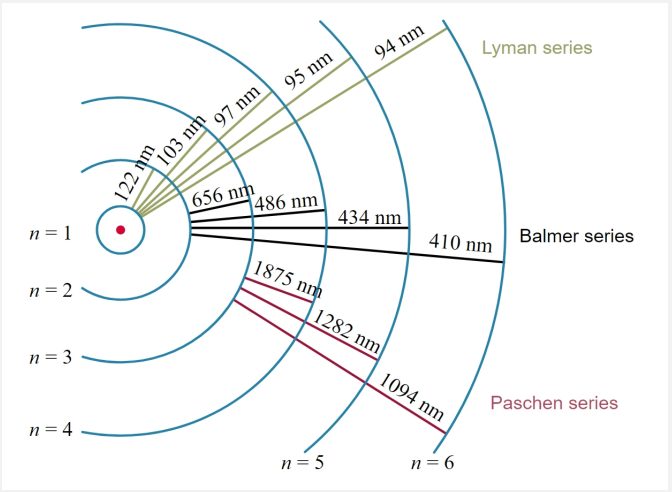


Atomic energy levels

Jablonski diagram

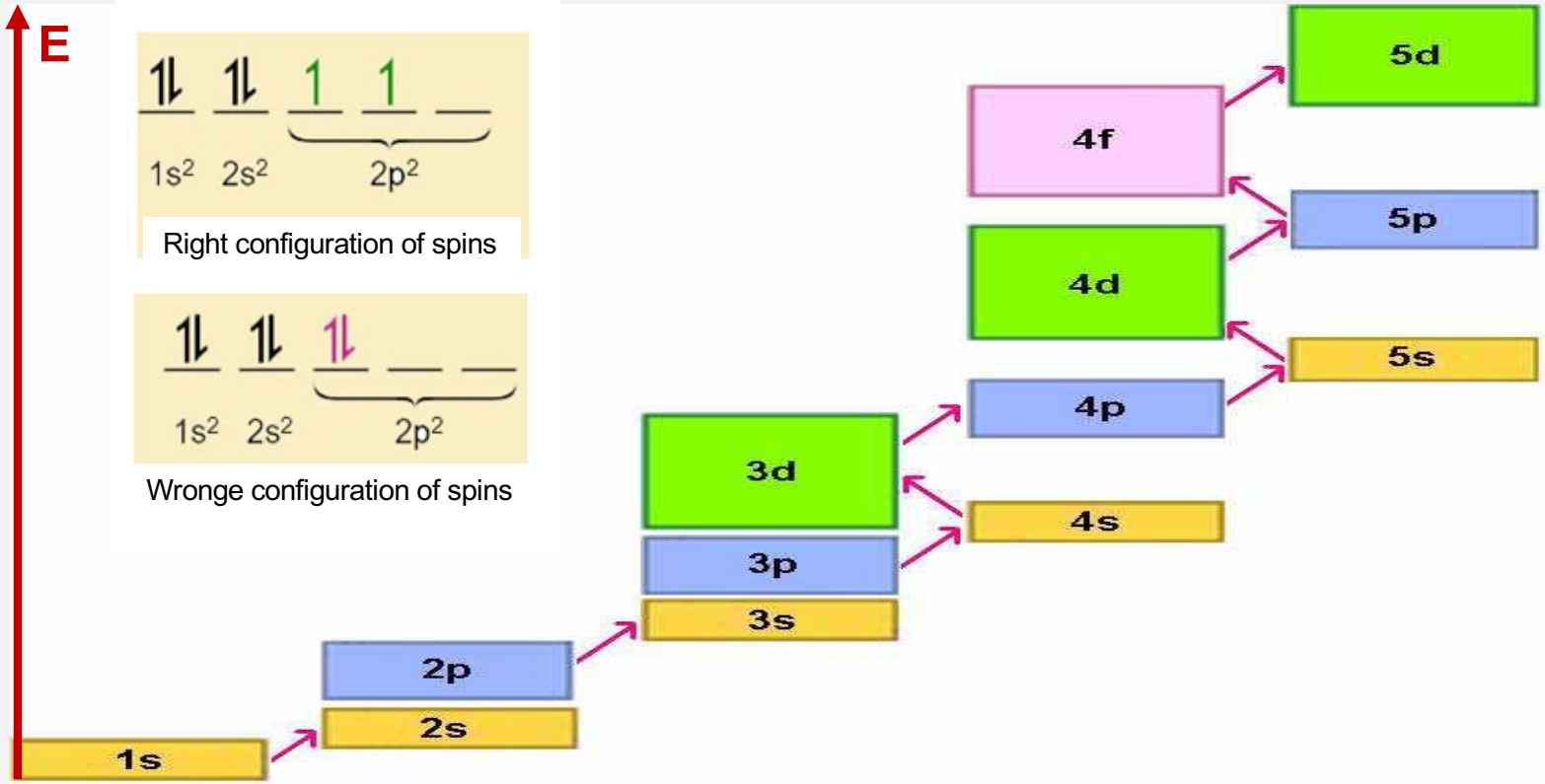






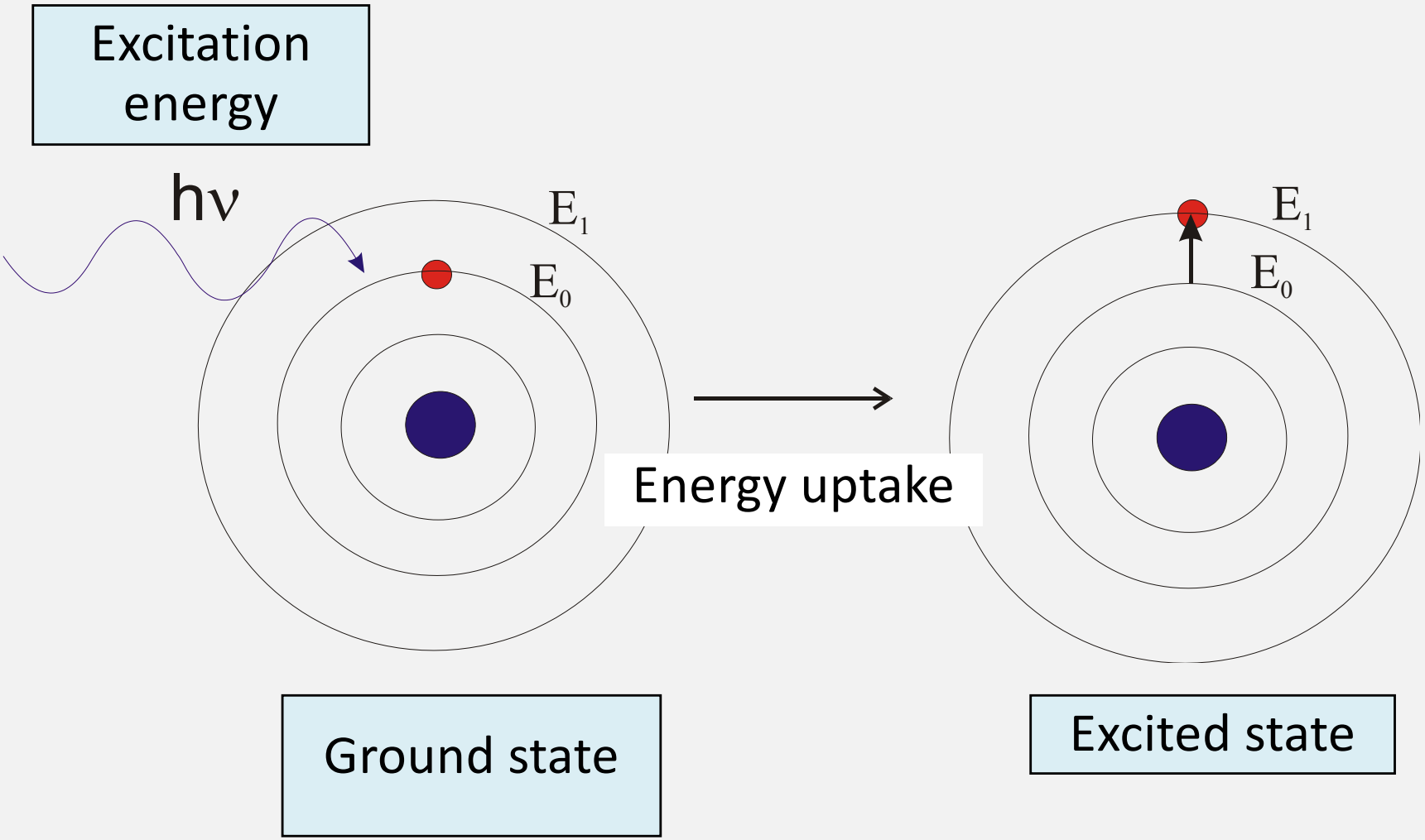
Niels Bohr (1913): Electrons are situated in non-radiating stationary orbitals with discrete amount of energies. Transition between stationary orbitals is possible only if the electron gets, or releases, the exact energy difference between the orbitals.

Atomic energy levels



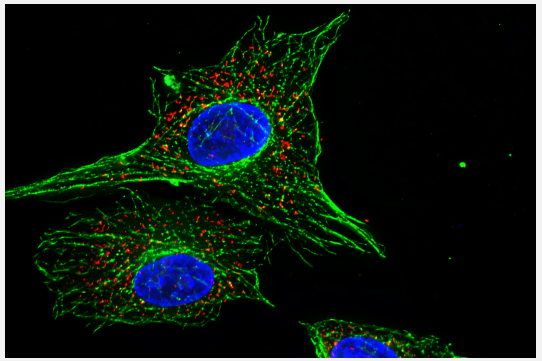
- Electrons occupy the lowest possible energy state (ground state)
- Pauli's exclusion principle: no two identical electrons, having the same quantum numbers, may occupy the same quantum state simultaneously
- Hund's rule: the lowest energy state is the one with the maximum net spin value.

Let’s consider a single atom



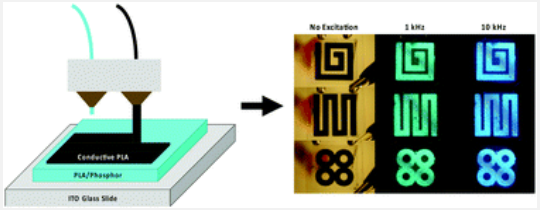
Typical excitation modes

- absorbtion of photons: ***photoluminescence***



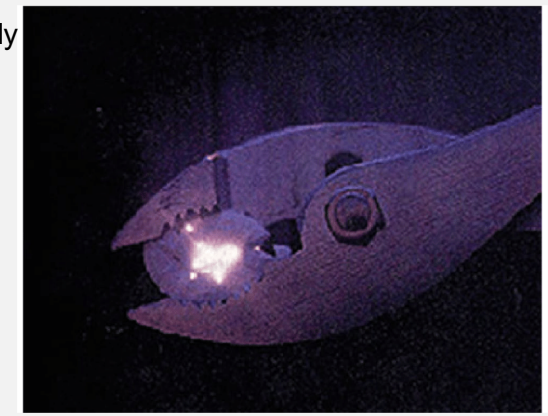
- energy of a chemical reaction: ***chemo/bioluminescence***

- high electric field or current: ***electroluminescence***



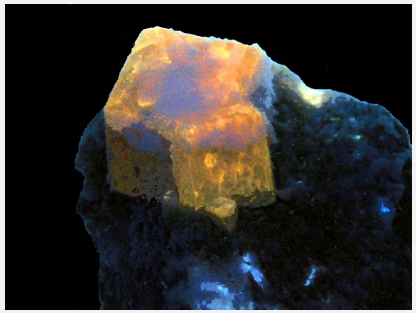
- mechanical deformation: ***triboluminescence***

mint candy

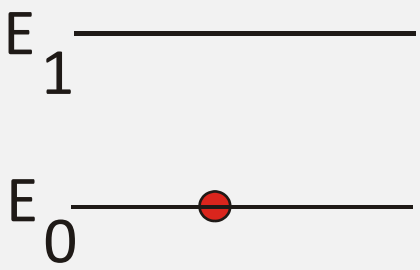


- heat: ***thermoluminescence***

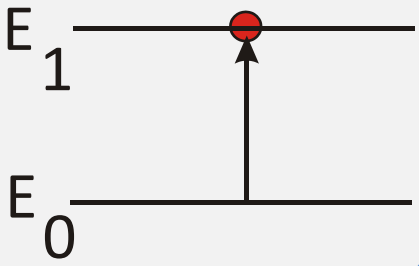
Wulfenit



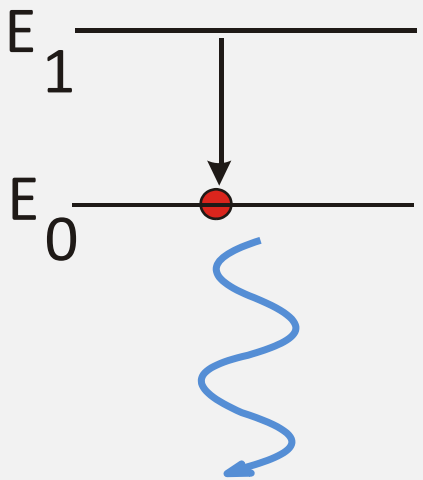
Mechanism of relaxation



excitation of an external electron

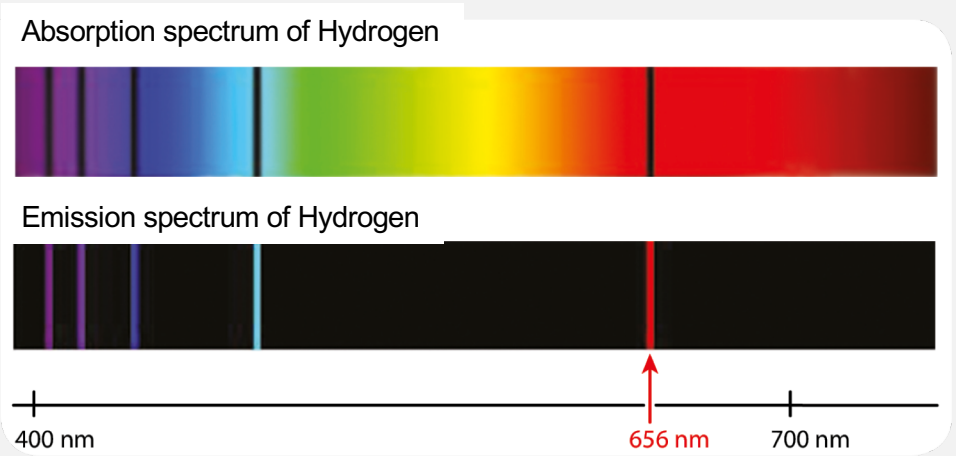


spontaneous relaxation of the electron back to ground state

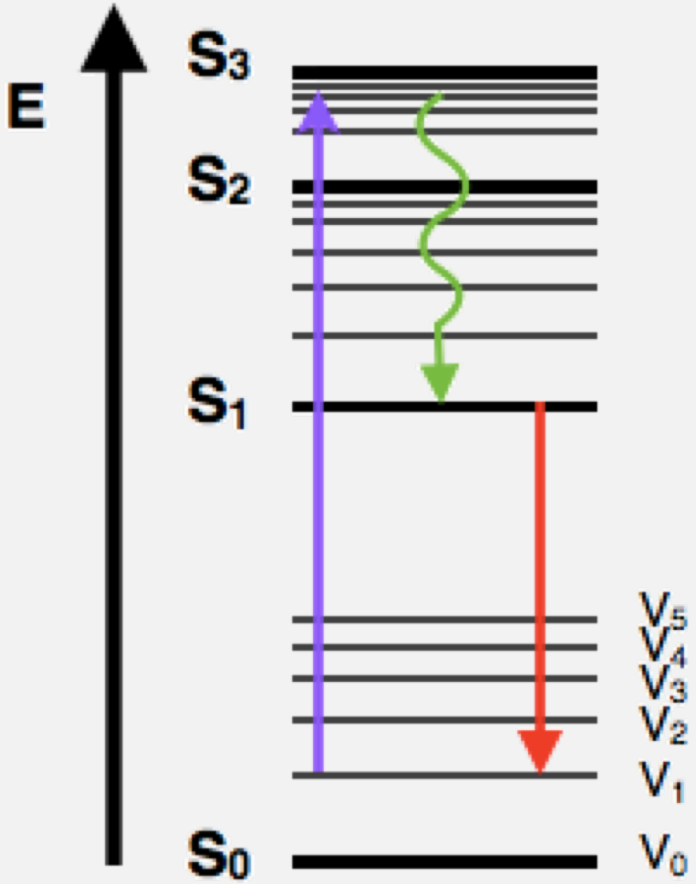


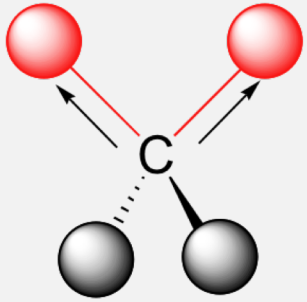
light emission

$hf=E_1-E_0$

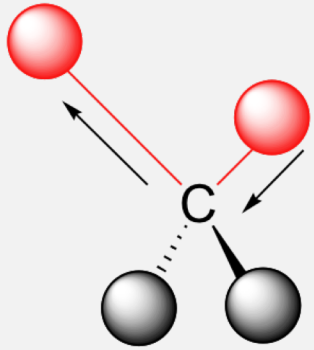


Energy levels in Molecules

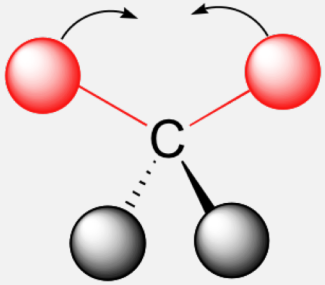




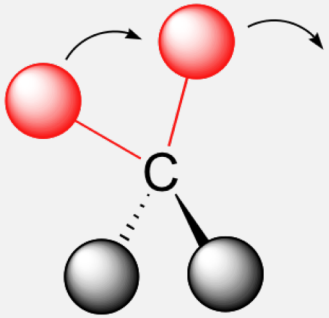
symmetric stretching



asymmetric stretching

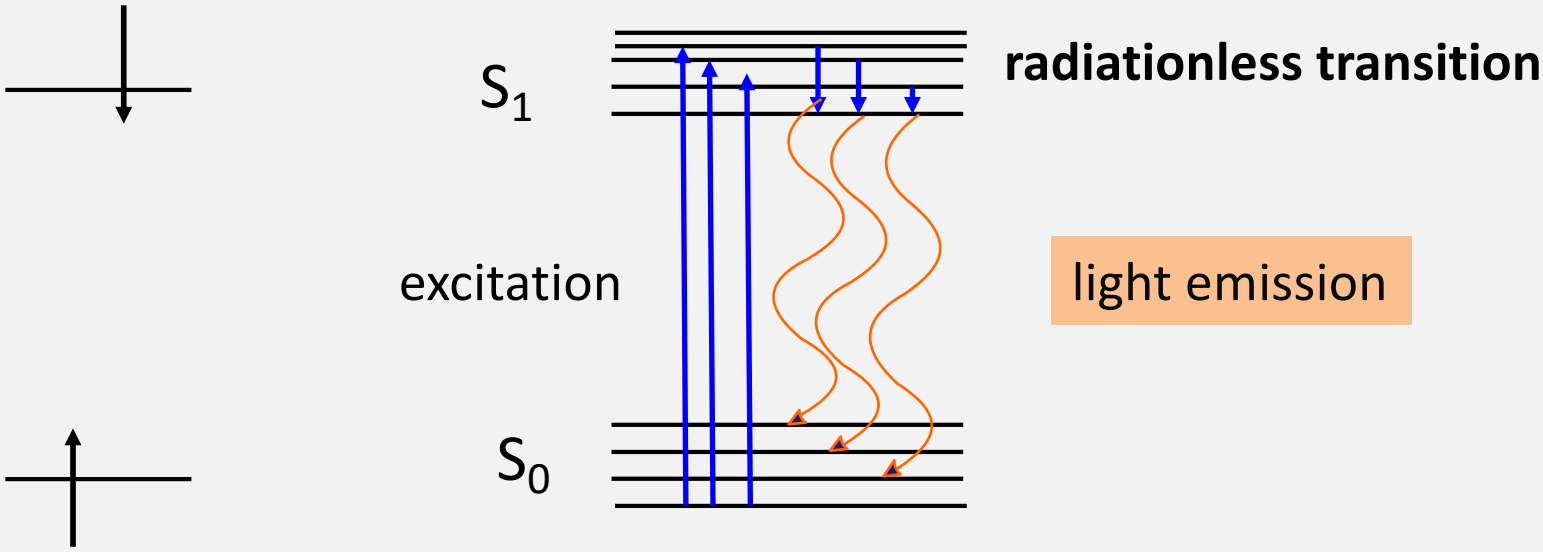


scissoring



rocking

Mechanism of Fluorescence

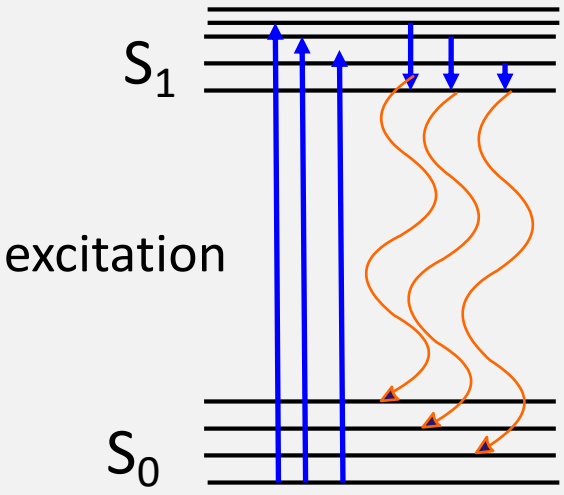


Singlet state:

paired electrons with net spin of 0 (multiplicity of 1)

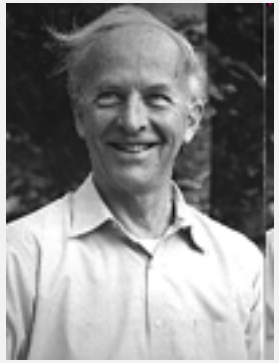
Fluorescence

Excitation and relaxation (light emission) in singlet states



Kasha's rule:

light emission emanates from the relaxation of the lowest vibrational level of the first excited state to ground state



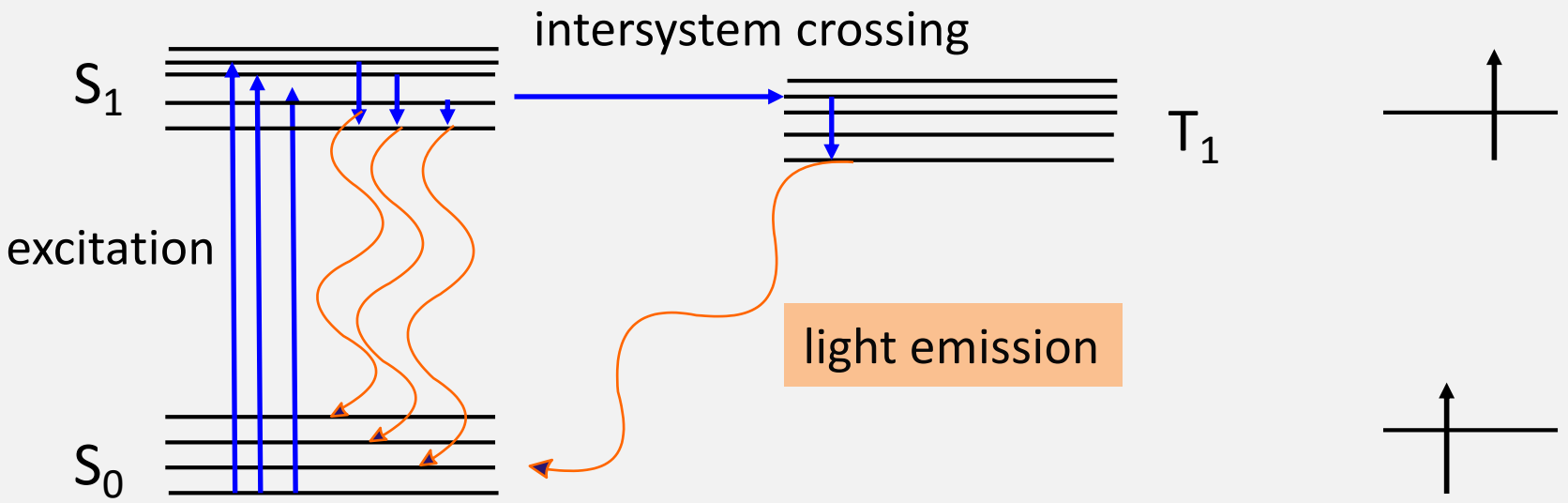
$$E_{excitation} \geq E_{fluorescence}$$

$$\lambda_{excitation} \leq \lambda_{fluorescence}$$

Stokes's shift

$$E = h*c/\lambda$$

Mechanism of Phophorescence



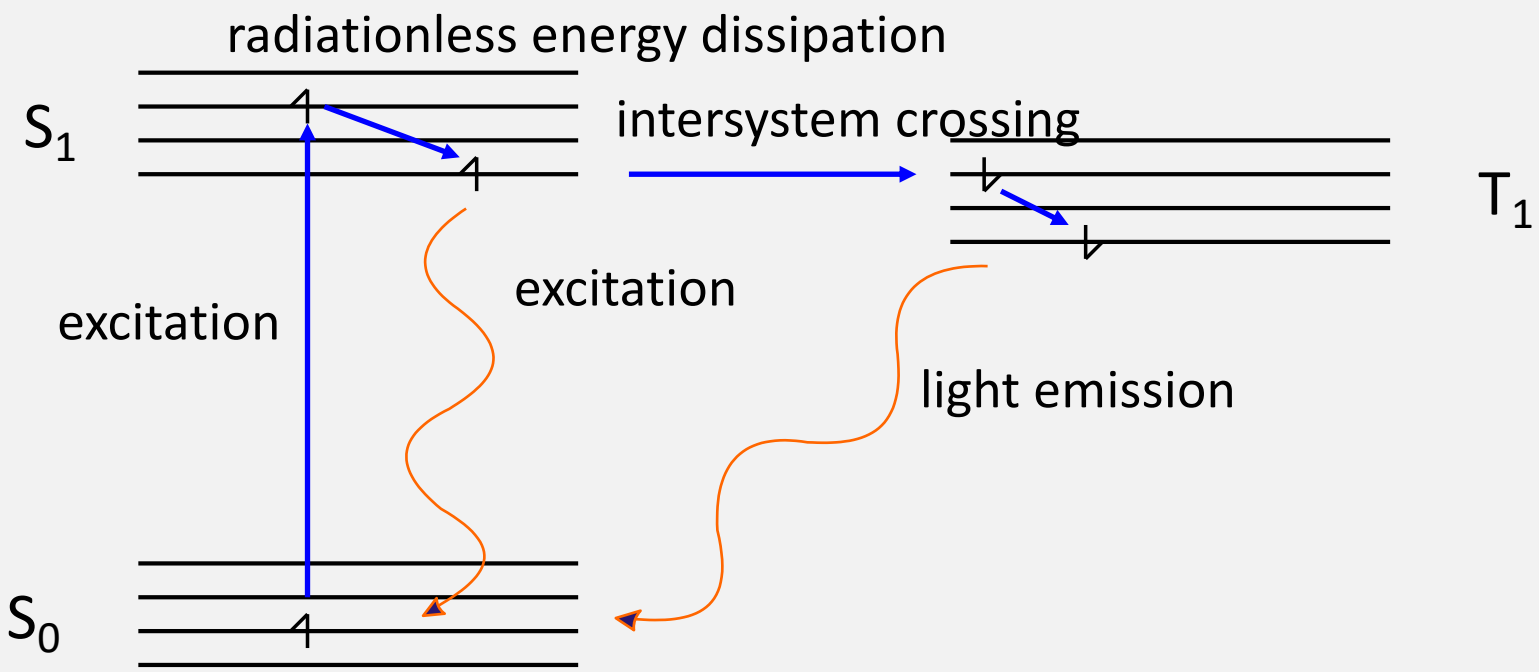
Phosphorescence

Relaxation after spin transition

Triplet state:

electrons with unpaired spins (multiplicity of 3)
Metastable state

Energy-relationship of luminescence modes



Stokes's shift

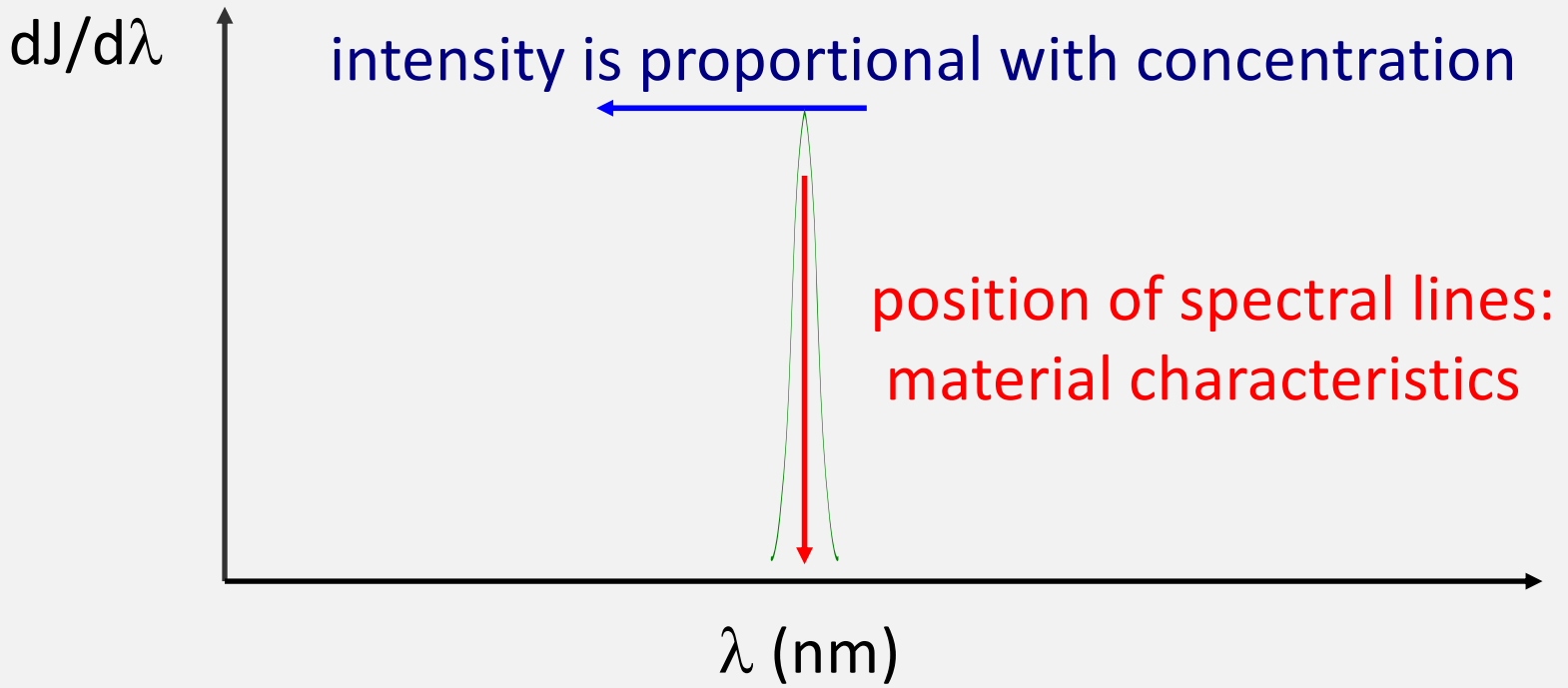
$$E_{excitation} \geq E_{fluorescence} > E_{phosphorescence}$$

$$\lambda_{excitation} \leq \lambda_{fluorescence} < \lambda_{phosphorescence}$$

Characteristics of emitted light

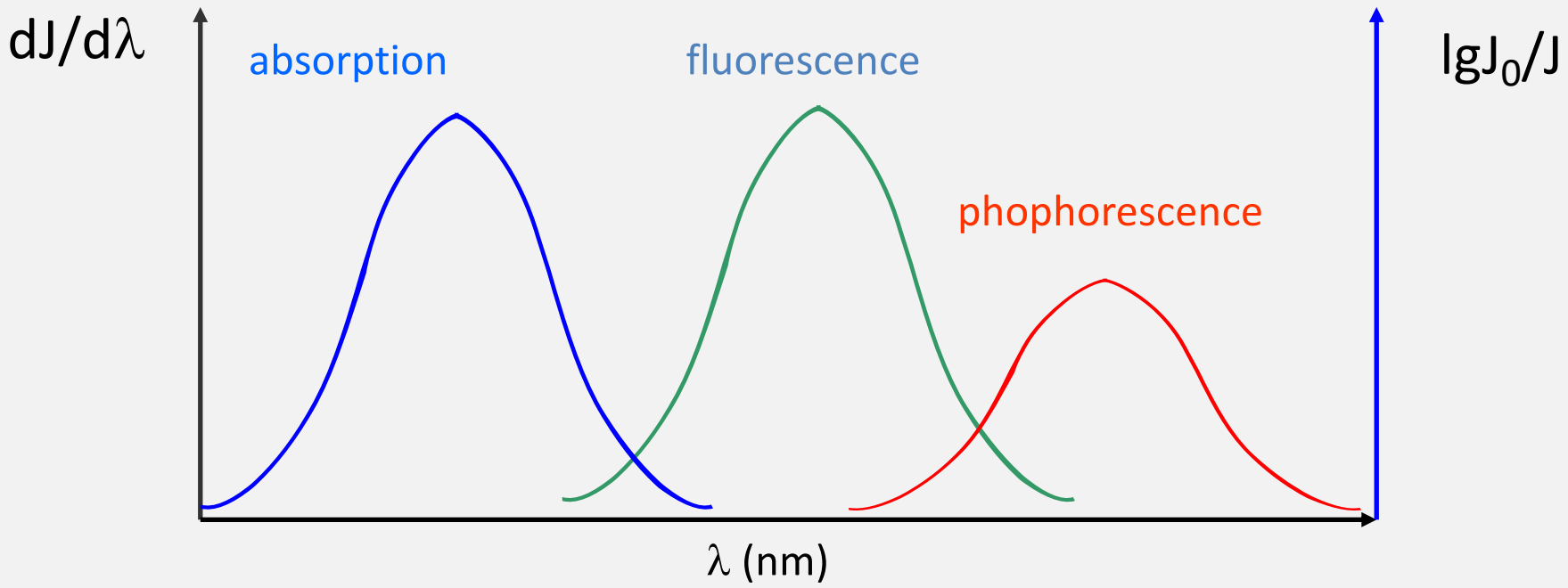
Spectrum – distribution of intensities along wavelength

Line-spectrum in the case of atoms:



Characteristics of emitted light

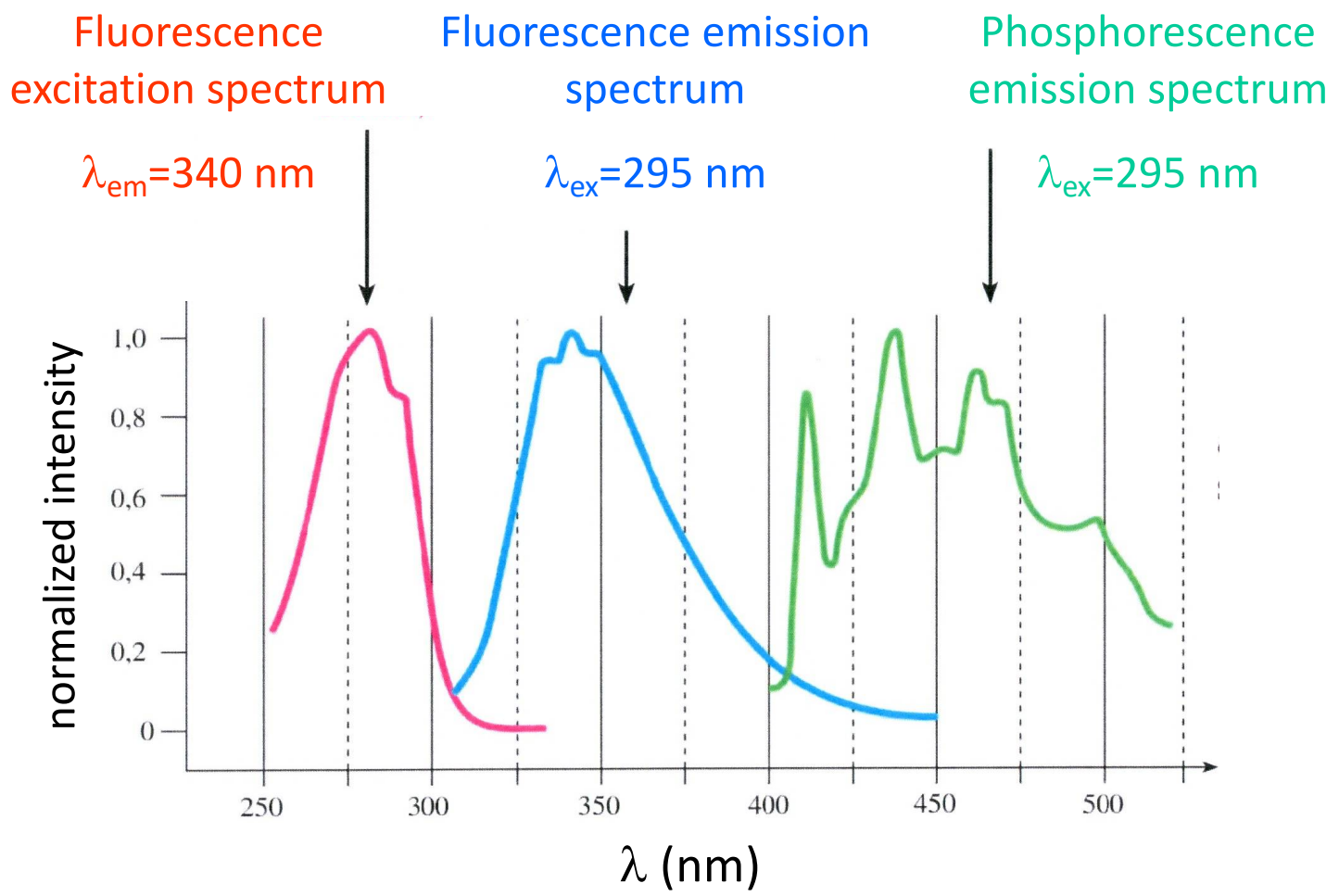
Band-spectrum in case of molecules



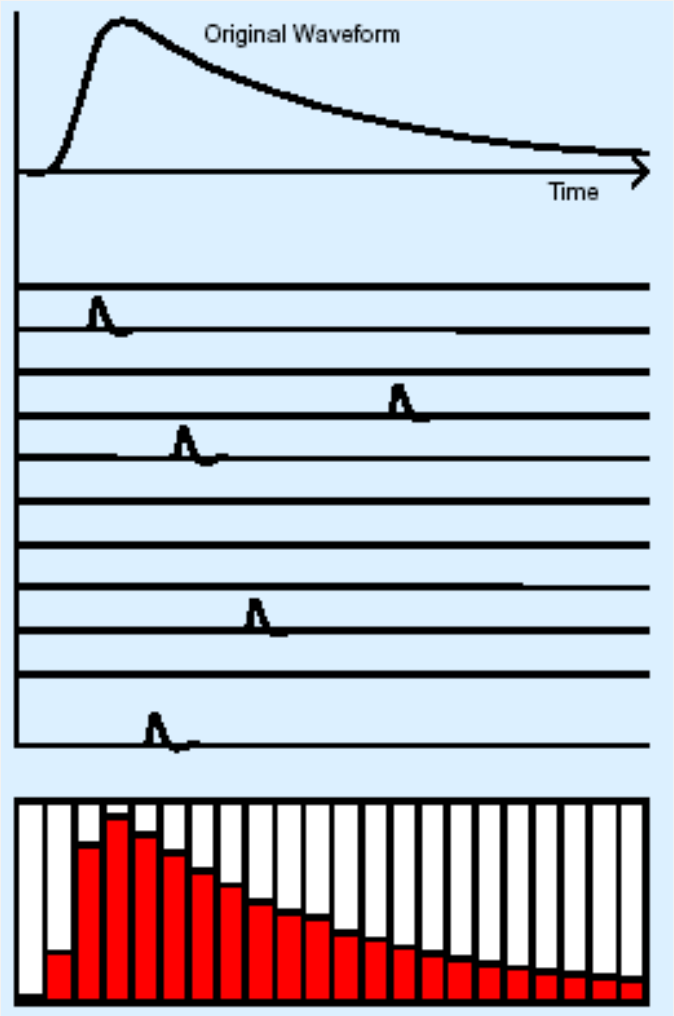
$$\lambda_{excitation} \leq \lambda_{fluorescence} < \lambda_{phosphorescence}$$

Stokes's shift

Spectra of tryptophan



Excited-state lifetime



Single photon counting

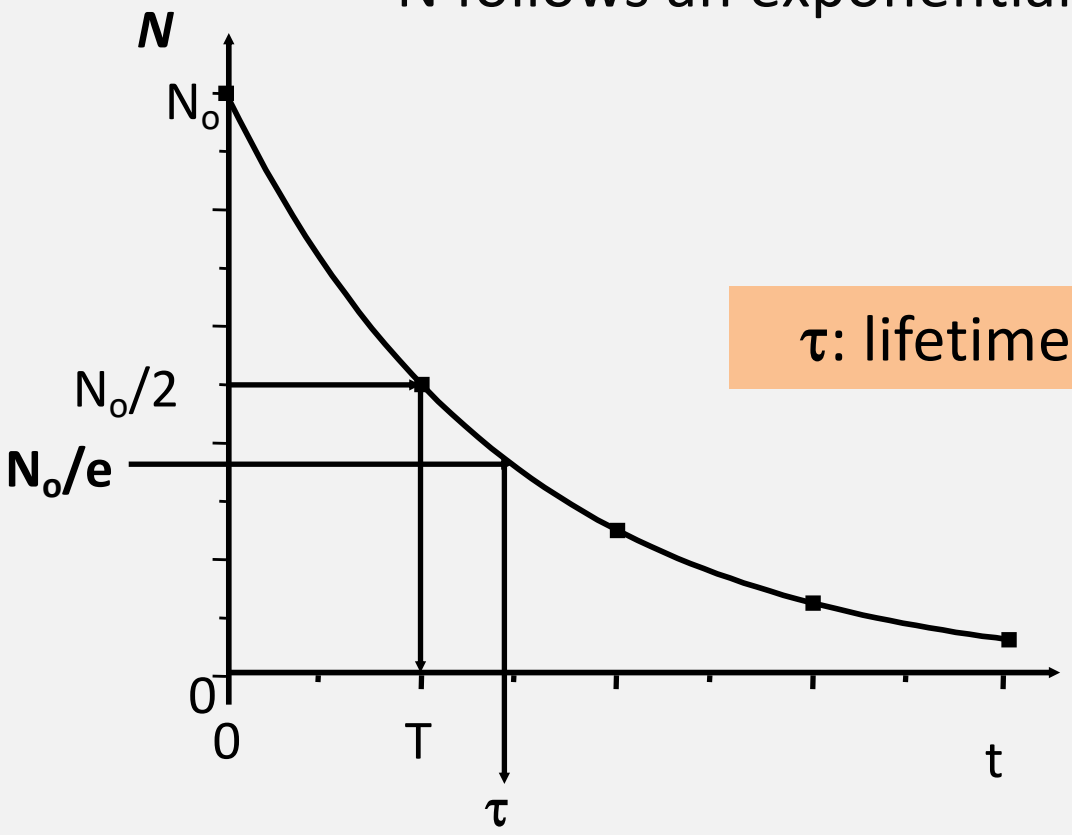
Measuring of time-delay between excitation and photon emission.

Statistical analysis of large number of measurements.

time after excitation

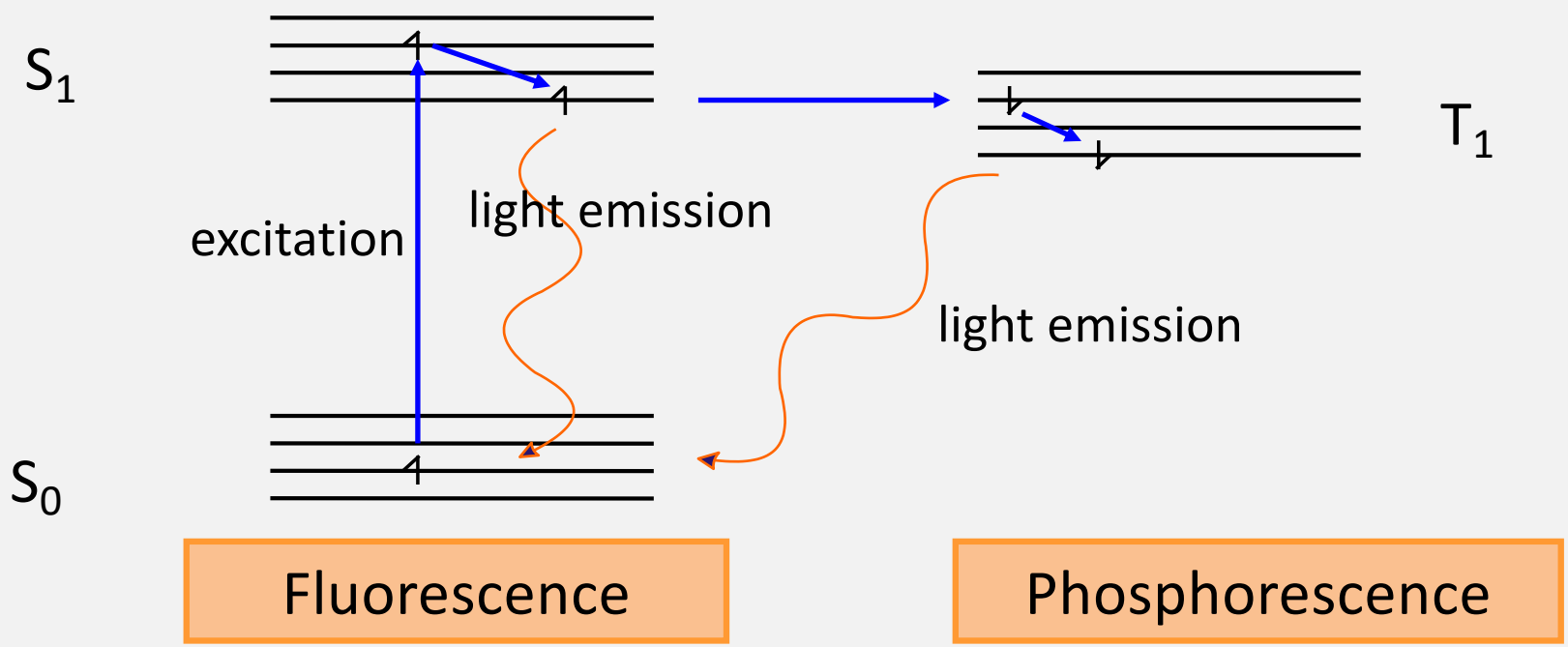
Number of excites electrones $\longrightarrow$ $N = N_0 e^{-\frac{t}{\tau}}$

N follows an exponential decay function.



τ: lifetime, T: half-life

Typical excited-state lifetimes



Fluorescence	Phosphorescence
fast relaxation	slow relaxation
$10^{-9} – 10^{-7}$ s	$10^{-3} – 10^{2}$ s

Is excitation always followed by photon emission?

- Excited state decay can be caused by mechanisms other than photon emission and are therefore often called "non-radiative transitions„.
- These can include: chemical reaction, dynamic collisional quenching, near-field dipole-dipole interactions, internal conversion and intersystem crossing.

Is excitation always followed by photon emission?

Fluorescence quantum yield (Q_F)

Q_F = number of emitted photons / number of absorbed photons

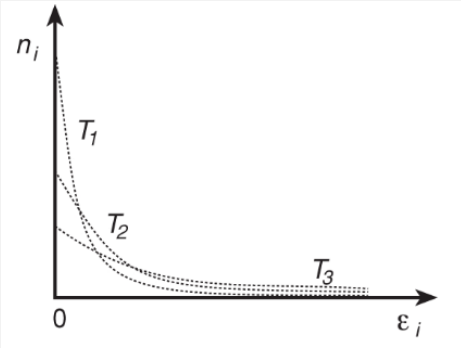
$$Q_F \leq 1$$

Boltzmann-distribution

The distribution of energy among the possible states (degrees of freedom).

When characterizing a macro-state of the system, only the number of particles (n_0, n_1, n_2,...) is given, having the energies of e_0, e_1, e_2,...

$$n_i = n_0 e^{-\frac{\varepsilon_i - \varepsilon_0}{kT}}$$



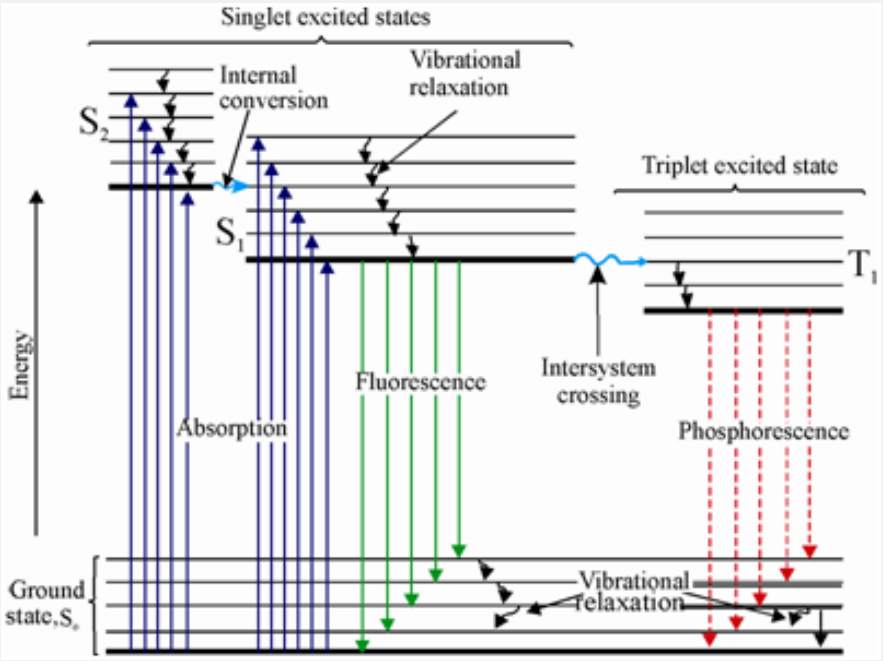
Number of particles in excited to ground states: $n_1/n_0 = e^{-hf/kT}$

IIf λ = 550 nm, than at room temperature $n_1/n_0 \sim 10^{-40}$!!!

Luminescence summary

Types of luminescence:

- fluorescence
- phosphorescence



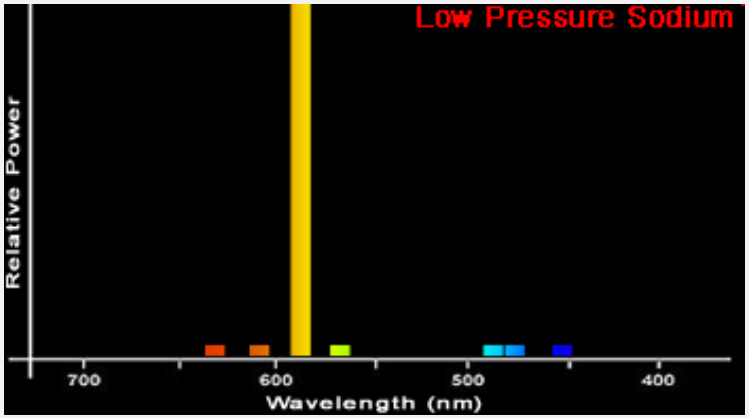
Characteristics:

- emission spectrum:
  - type
  - maximum λ position
  - amplitude
- lifetime
- quantum yield

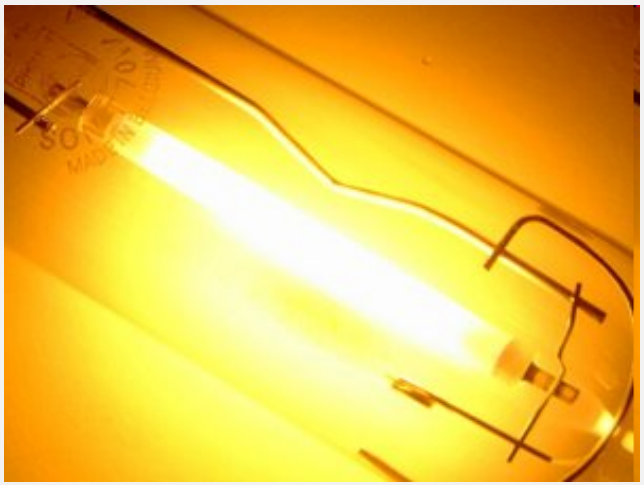
Application fields of luminescence

- Light sources
- concentration determination
- fluorescence spectroscopy
- fluorescence microscopy
- dosimetry
- structure determination
- cell/tissue labeling
- safety control … many more

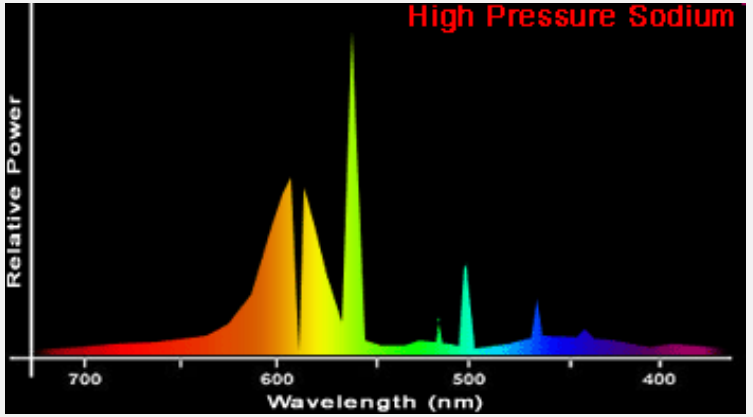
Luminescent light sources



Low-pressure Na-vapor lamp

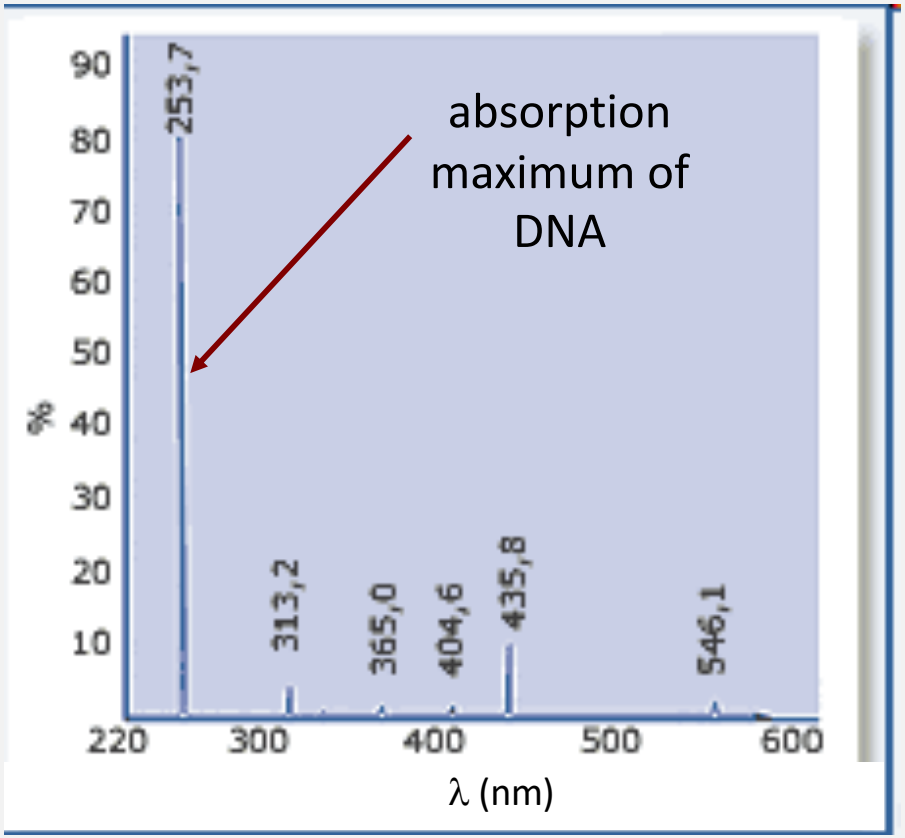


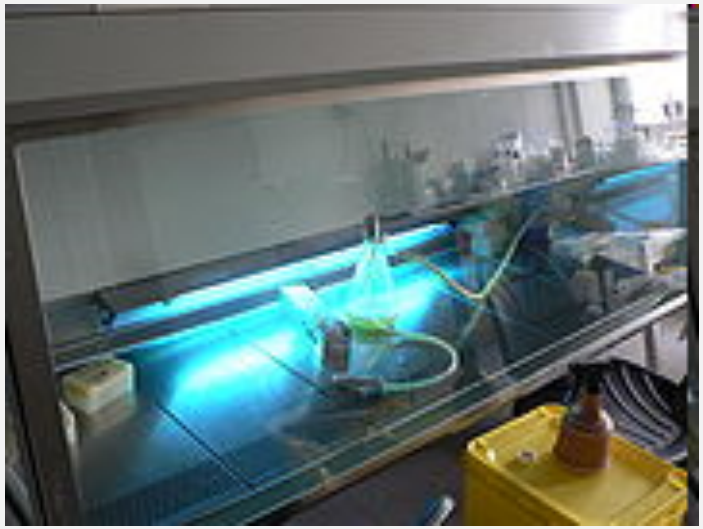
Metal-vapor lamps



High-pressure Na-vapor lamp

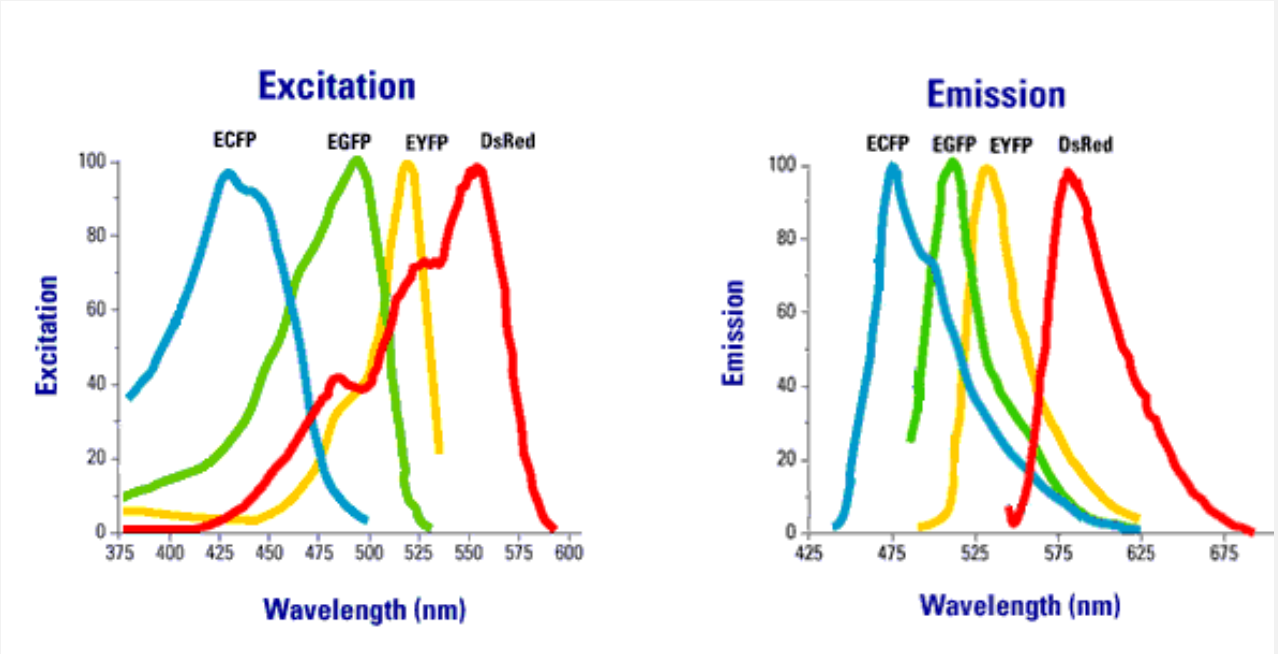
Low-pressure Hg-vapor lamp

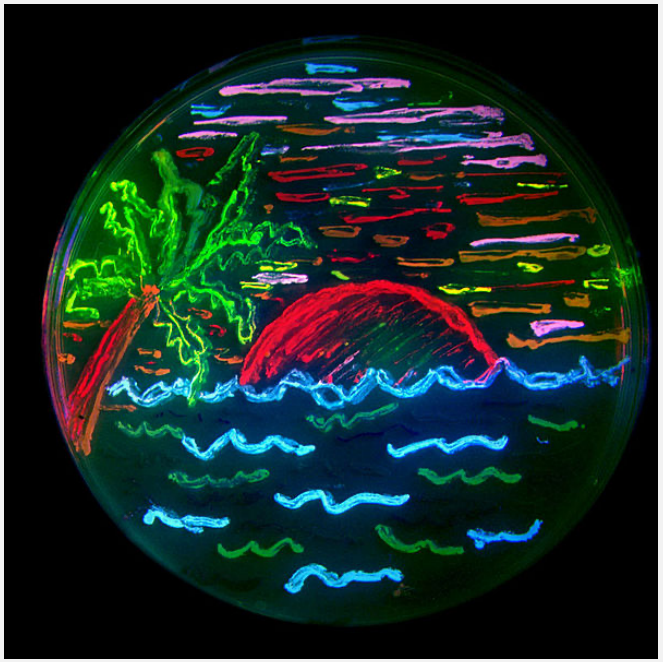


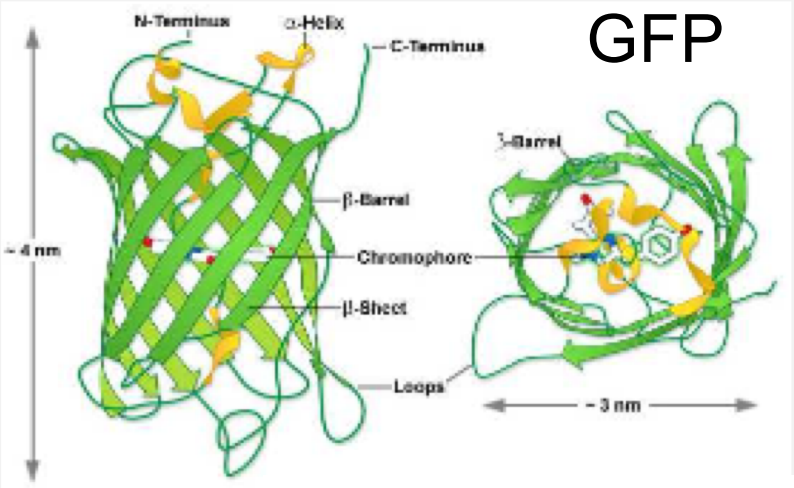


application: germicid lamp

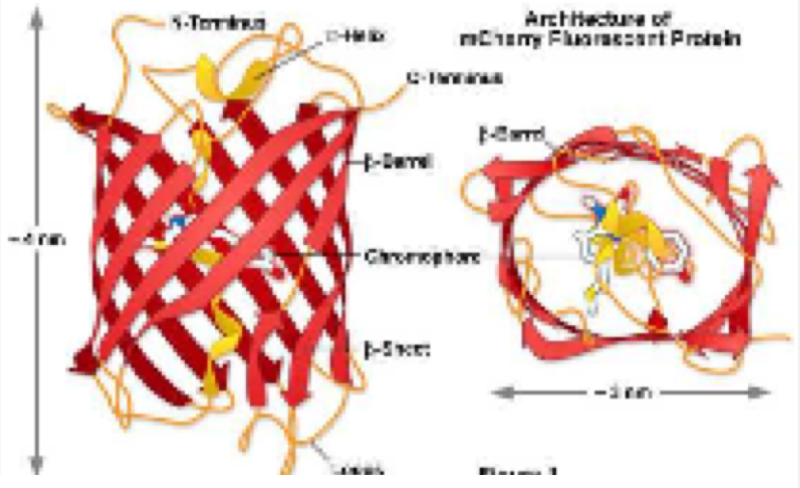
Fluorescence-based methods are wide-spread in medical research and diagnostics





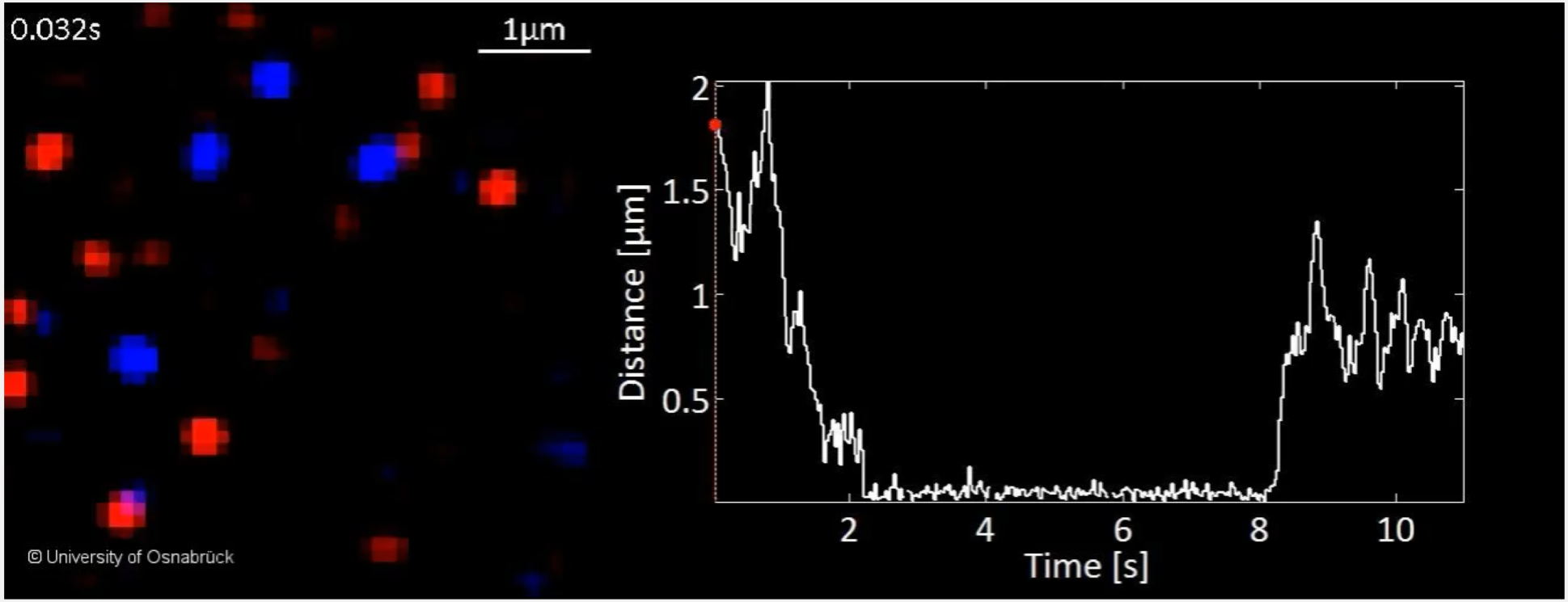


GFP (jellyfish)

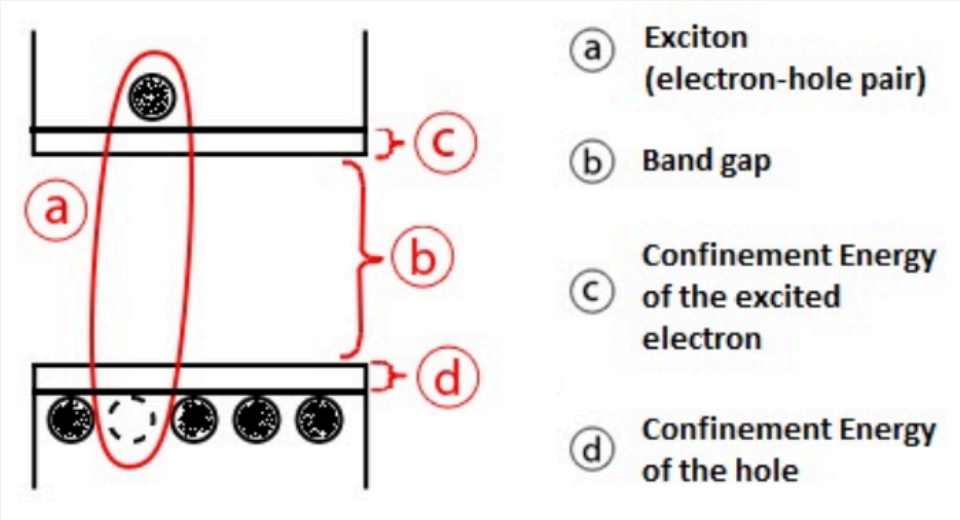


DsRed (red coral)

Fluorescent dye-labeling to follow interaction of proteins in real-time



Fluorescent quantum dots (QD)

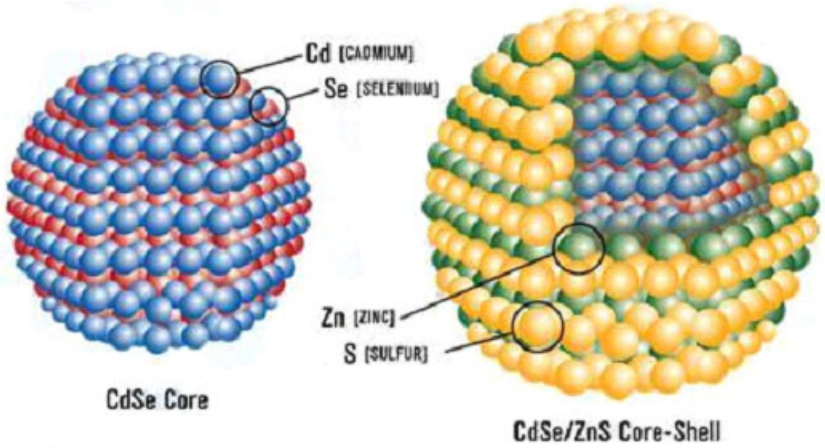


$$E_{\text{confinement}} = \frac{\hbar^2\pi^2}{2a^2}\left(\frac{1}{m_e}+\frac{1}{m_h}\right) = \frac{\hbar^2\pi^2}{2\mu a^2}$$

$$E_{\text{exciton}} = -\frac{1}{\epsilon_r^2}\frac{\mu}{m_e}R_y = -R_y^*$$

$$E = E_{\text{bandgap}} + E_{\text{confinement}} + E_{\text{exciton}}$$

$$= E_{\text{bandgap}} + \frac{\hbar^2\pi^2}{2\mu a^2} - R_y^*$$



The energy of confined electron-hole pair (exciton) depends on the diameter of the semiconductor nanoparticle.

Fluorescent quantum dots (QD)

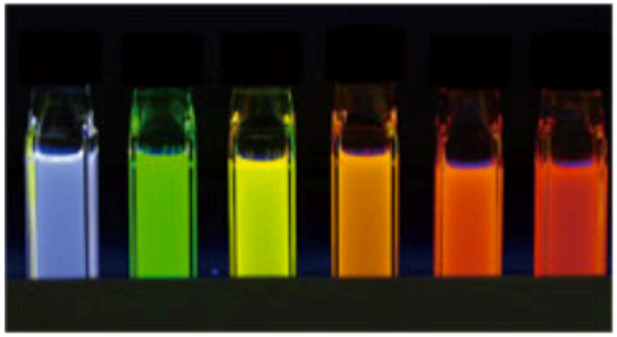
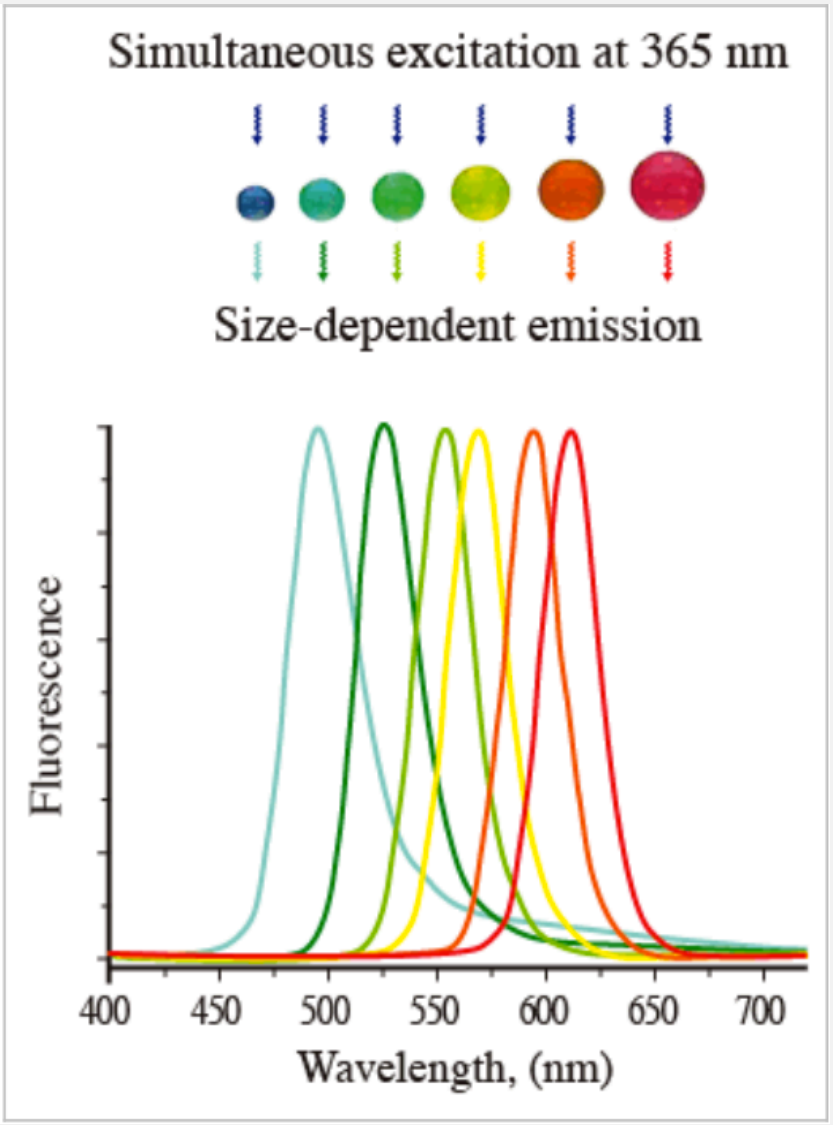
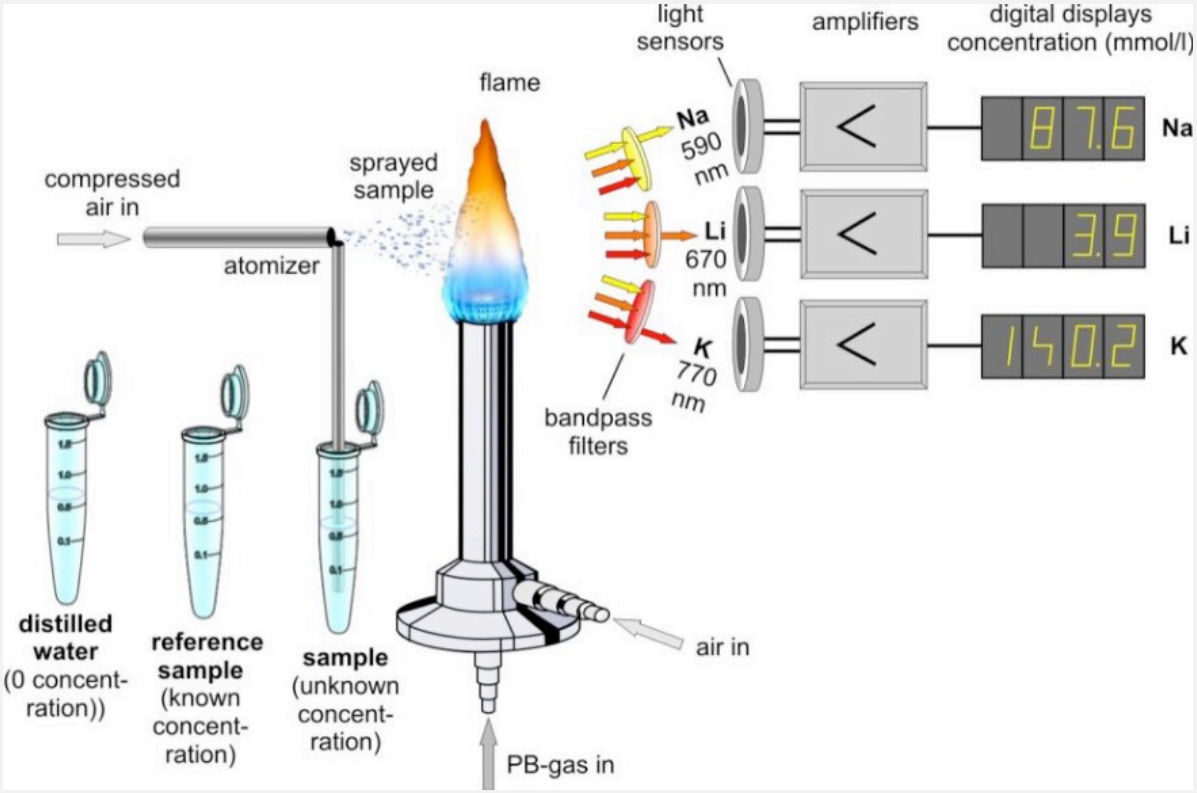


Figure 1: Fluorescence emitted from quantum dots. Blue fluorescence can be emitted from small particles of approximately 2 nm in diameter, green from ~3 nm particles, yellow from ~4 nm particles, and red from large particles of ~5 nm.
The wavelength of the excitation light is 365 nm.

Excitation spectra overlap to ~400 nm, so only one excitation wavelength is proper to a set of QDs.

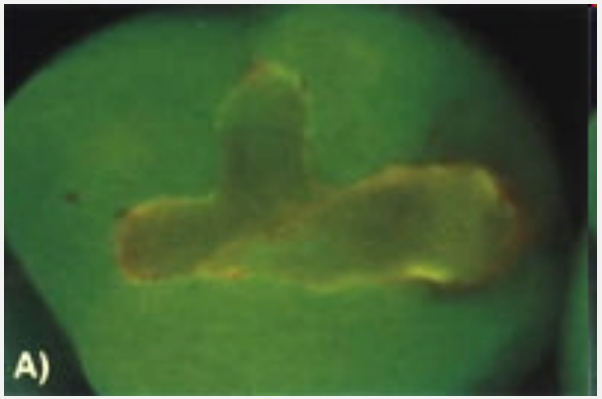


Flame photometer

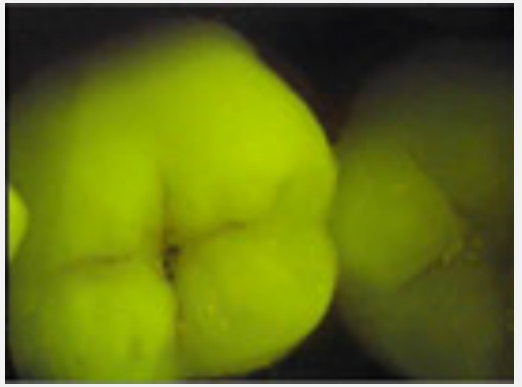


Quantitative determination of K^+, Li^+ és Na^+ ions

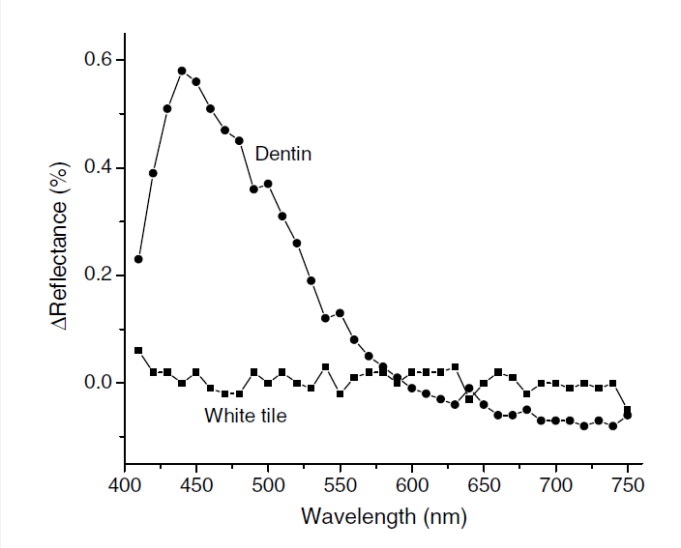
Applications in dental medicine



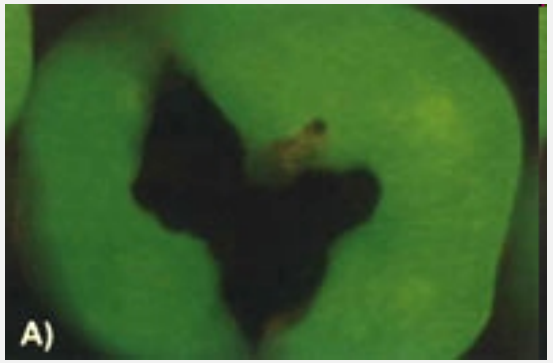
Red fluorescence indicates the activity of cariogenic bacteria



Auto-fluorescence of teeth. When teeth are illuminated with high intensity blue light they will start to emit light in green.



Lee, Journal of Biomedical Optics 20(4), 040901 (April 2015)



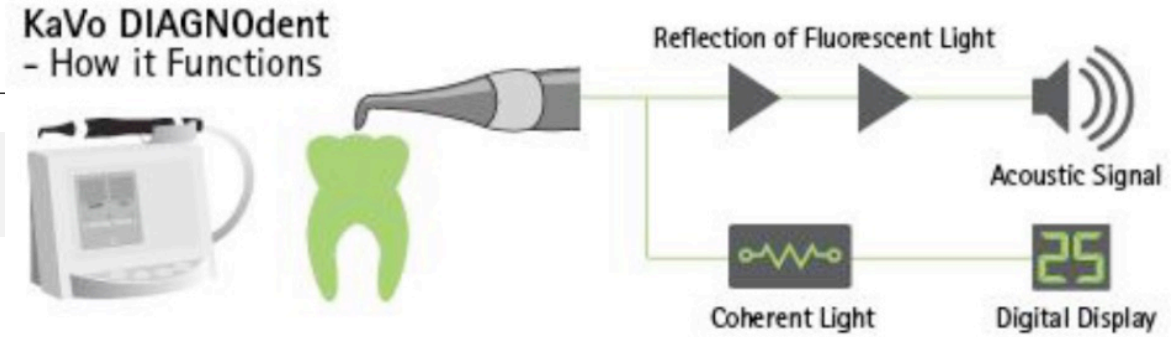
amalgam restoration

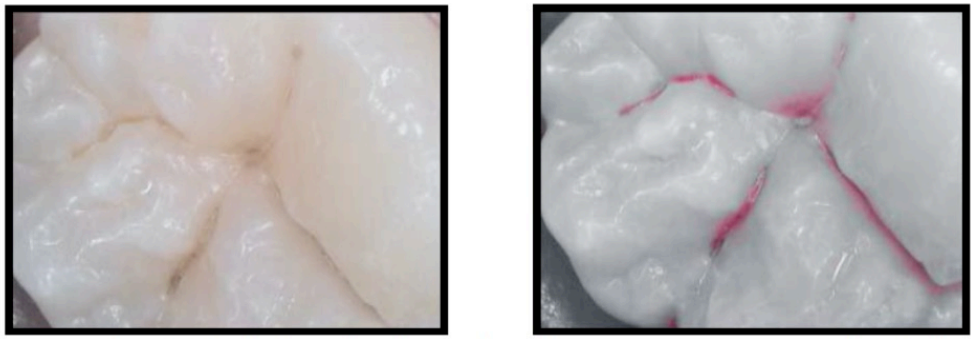
0 – 14	No special measures.
15 – 20	Usual prophylactic measures.
21 – 30	More intensive prophylaxis or restoration: indication is dependent on: *Caries activity. *Caries risk. * Recall interval, etc.
from 30	Restoration and more intensive prophylaxis.

Figure (5) Spectra camera with spacer on (Kurtzman, 2010).

Table 2: Interpretation of Spectra data (Kurtzman, 2010).

Displayed Color	GREEN → BLUE → RED → ORANGE → YELLOW				
Displayed Number	1 → 5				
Depth of Involvement	Sound Enamel	Initial Enamel Caries	Deep Enamel Caries	Initial Dentin Caries	Deep Dentin Caries





SOPROCARE. (A) Carious lesion invisible in DAYLIGHT mode. (B) Carious lesion visible in CARIO mode

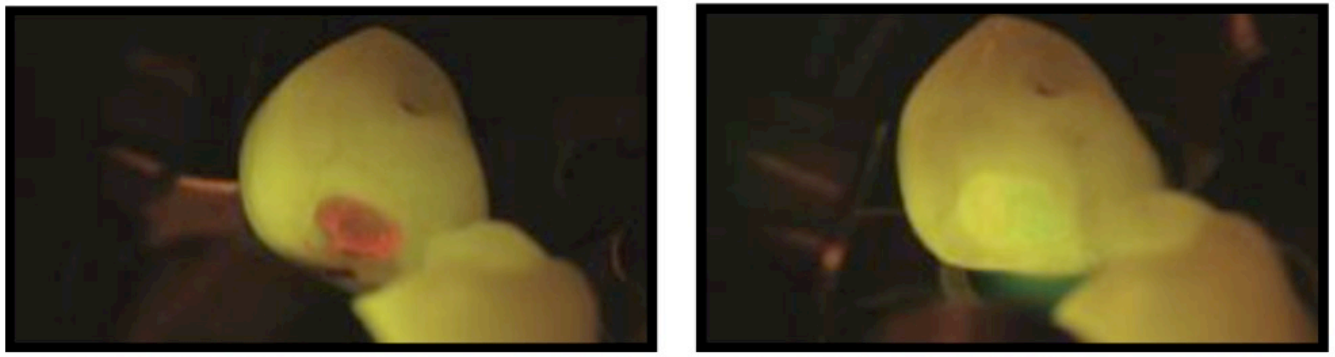
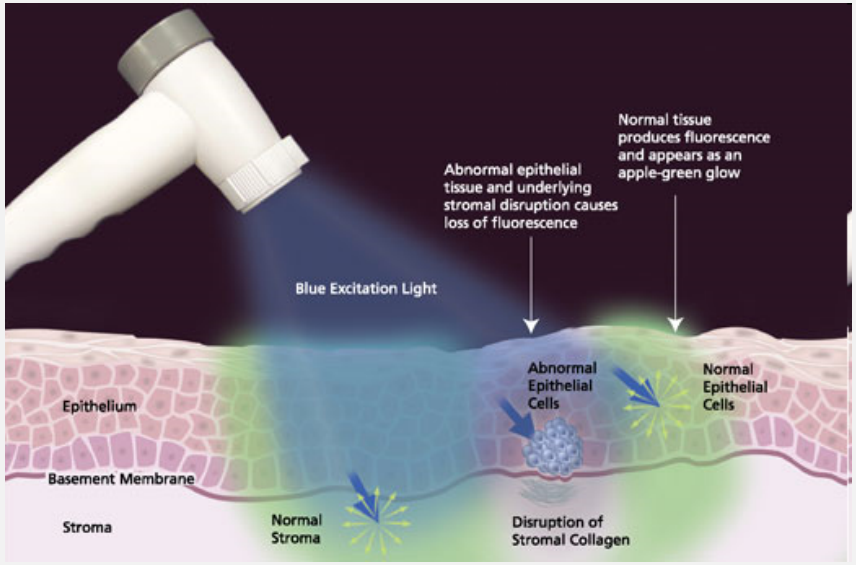


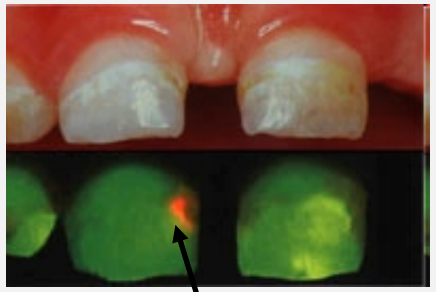
Figure (8) Photos showed cavity illumination with Facelight before and after caries excavation (21).

Mualla, Journal of Dental and Medical Sciences. 15:3, 2016, PP 65-75

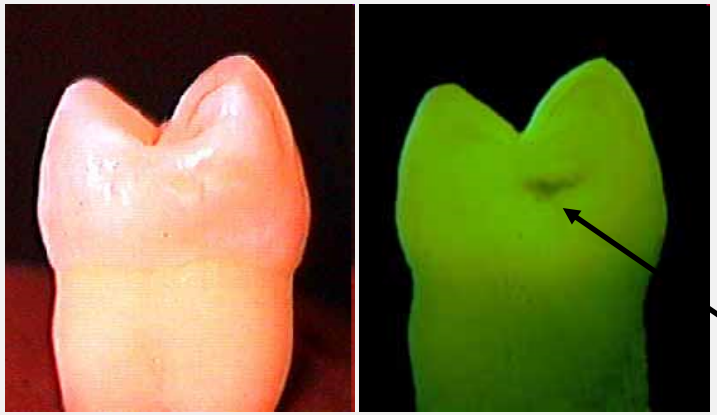


Healthy and malignant tissues
different fluorescent properties

native and fluroescent
images



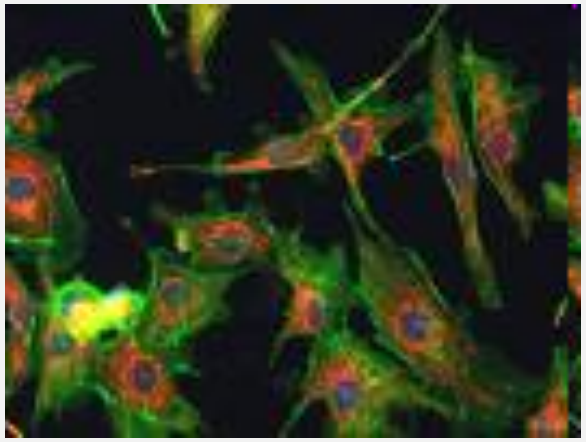
Active caries

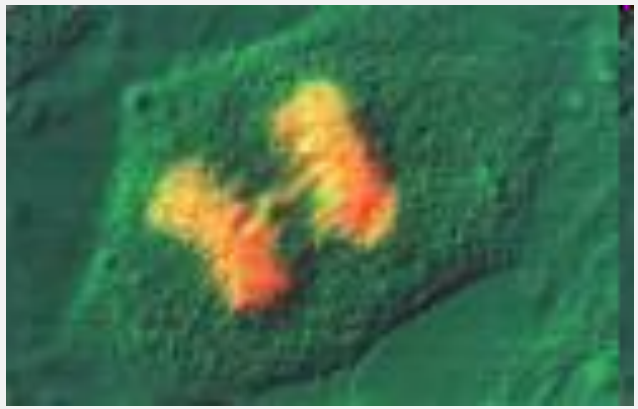


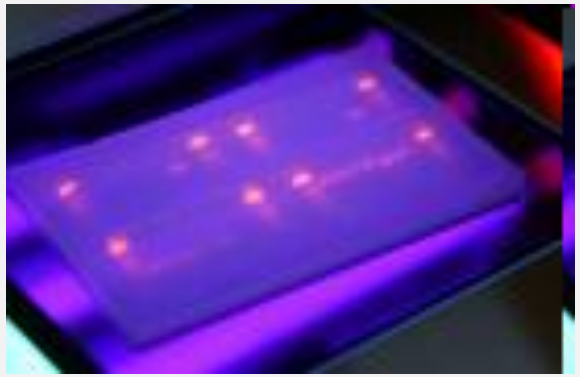
native and fluroescent
images

caries

Many applications in the lab







Checklist:

Luminescence

ground- and excited states

modes of excitation

Jablonski diagram

Fluorescence

Phosphorescence

Kasha’s rule

Stokes’s shift

lifetime

quantum yield

applications

(Boltzmann diistribution)

Damjanovich, Fidy, Szöllősi: Medical Biophysics

II. 2.2

2.2.4

2.2.6

VI.3.3

3.3.1

3.3.2

3.3.3