

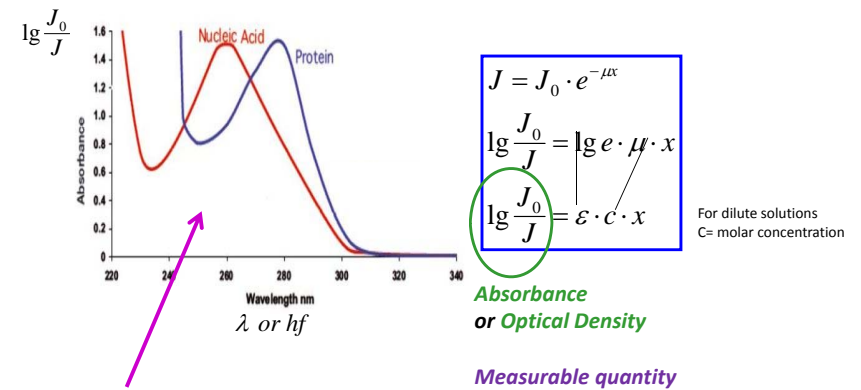
Light absorption affecting the human body Phenomena that produce light

Prof. Judit Fidy
2015, October 28

Reminder.....

Absorption spectroscopy - spectrophotometry

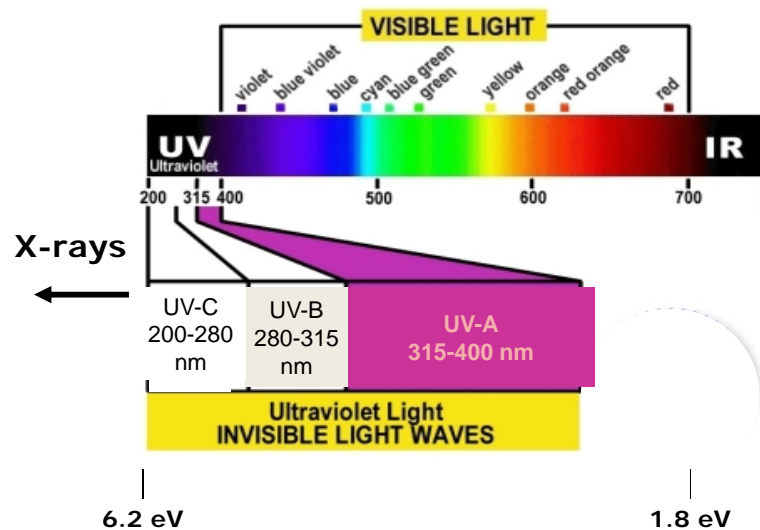
Measuring what photonenergies (λ -s) are absorbed - depends on μ



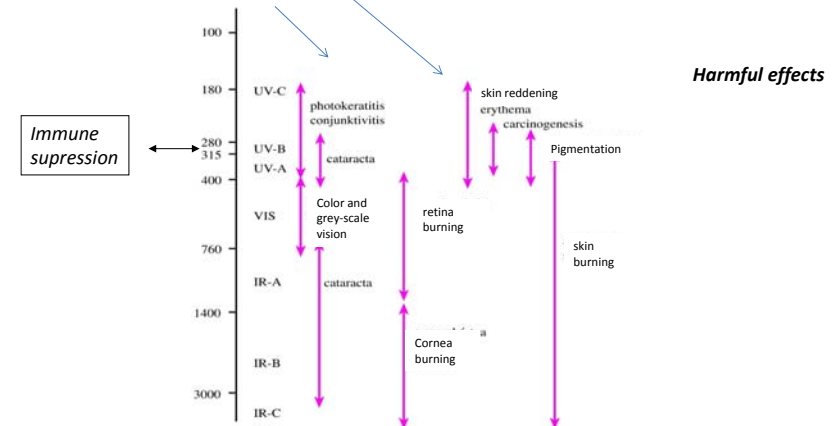
Absorption spectrum – characteristic for the electronic states of the molecule

Targets of light absorption in the human body

Because of distinct effects, the UV range is divided into three regions

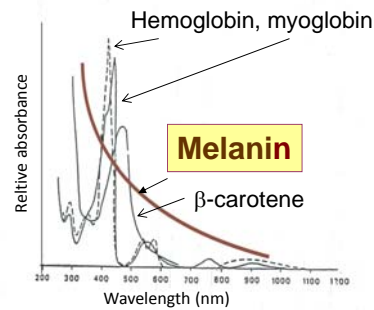


Targets of light absorption in the human body primary targets: eye, skin



Positive effects: Synthesis of vitamin D (UVA); stimulation of hormones, immune system, metabolism (VIS); etc. ---- exact details in many cases are not known

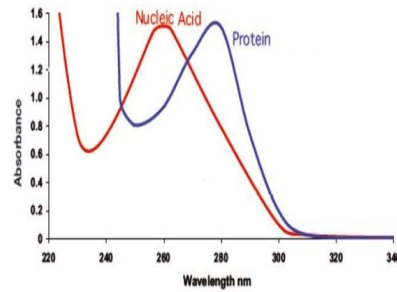
What are the molecules that absorb light?



+Exogen chromophores

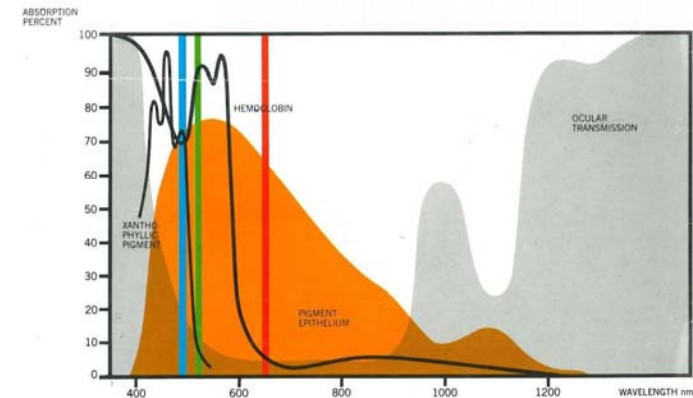
- colored foods
- medications
- cosmetics
-

Endogen chromophores

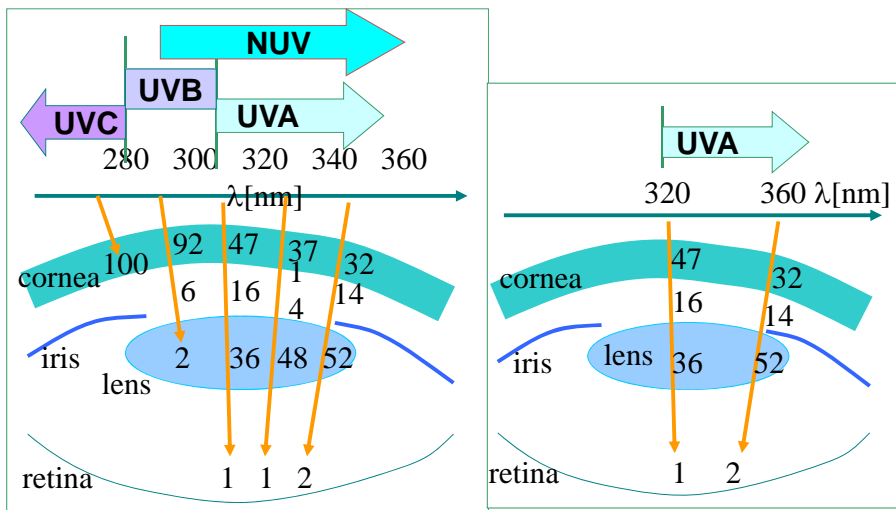


What are the molecules that absorb light – in the eye?

Spectral characteristics of the eye

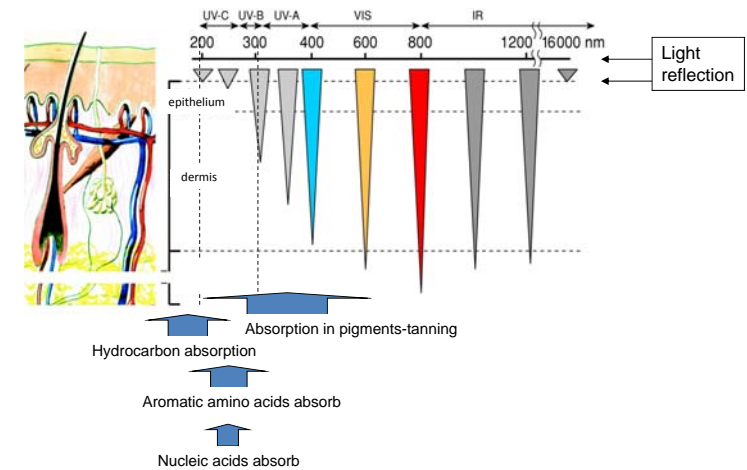


Depth of penetration - in eye



Depth of penetration - in skin

Longer wavelength → deeper penetration



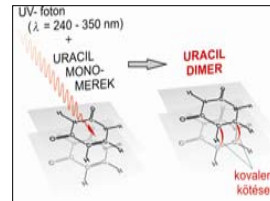
Possible consequences of light absorption: photochemical reactions

Direct photochemical effects of UV light → DNA, RNA defects

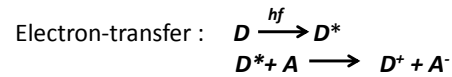
dimerization of pyrimidine bases : cytosine, uracil, thymine

crosslinking with proteins

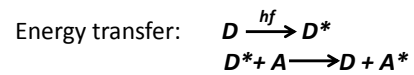
photohydration



Indirect effects



reactive free radical



A=oxygen A^* =**reactive singlet oxygen**

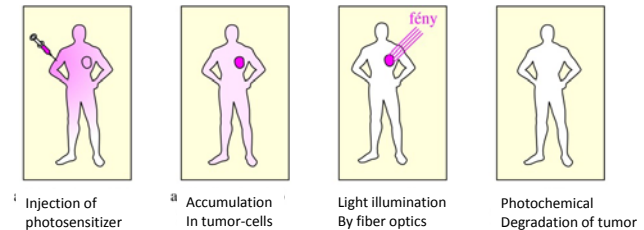
Therapeutic use of photochemical effects by photosensitizers

Photosensitizer molecule:

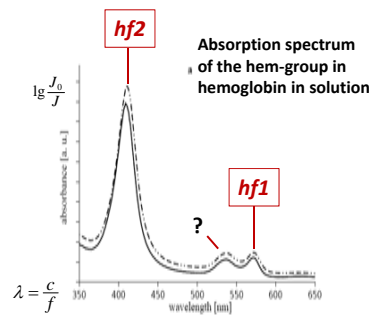
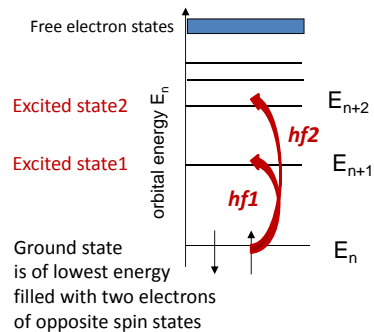
high efficiency of light absorption

high efficiency of generating free radicals or singlet oxygen

high affinity to target cells



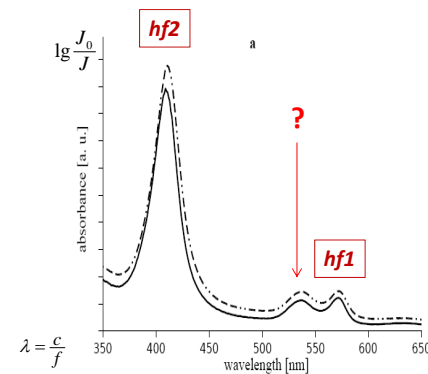
Schematics used to interpret the absorption spectrum by electronic transitions between atomic/molecular orbitals



Simplified view: - out of the filled orbitals, only that of the highest energy is shown: E_n

- the highest occupied energy level is filled by two electrons of opposite spins (most of light absorbing/emitting molecules)

The schematics is too simple!



$\Delta E = hf$ fulfilled, still:

1. great differences in the absorbances of the various transitions

2. broad bands instead of sharp maxima

Answer 1: the probability of electronic excitation depends on the relation of the quantum numbers of the two states → **selection rules apply**

Before Answer 2 - terminology

Singlet state: all electrons in the molecule are spin-paired $\sum_j s_j = 0$

Triplet state: one pair of electrons is spin-unpaired $\sum_j s_j = 1$

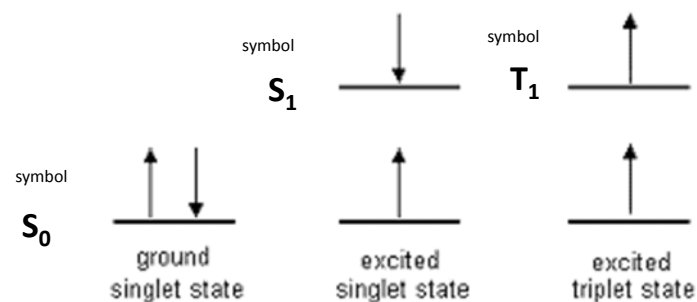
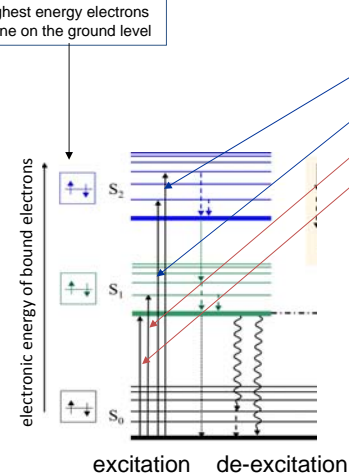


Photo-excitation: $S_0 \rightarrow S_1$ or $S_0 \rightarrow S_2$ etc.

$S_0 \rightarrow T$
is not allowed

Completed schematics for aromatic hydrocarbons
e.g. heme in hemoglobin

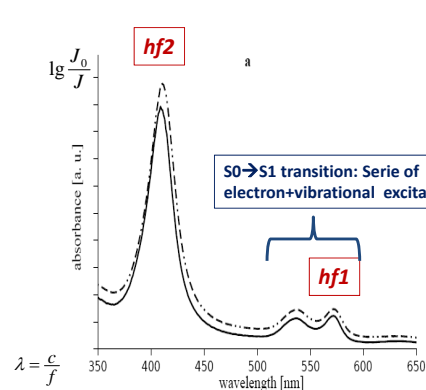
Spin states of the two
highest energy electrons
- one on the ground level



Absorption:

Broad variety of possibilities
for electronic excitation due
to vibrational modes of the
molecule

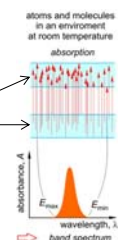
The schematics was too simple!



$\Delta E = hf$ fulfilled, still:

2. **broad bands instead of sharp maxima for molecules not in vacuum**

Broadened discrete levels due to variety of environment



Answer 2: two reasons: 1. vibronic transitions in molecules

2. Boltzmann distribution on energy levels in the environment
→ variety in electric fields perturb the electron-energies

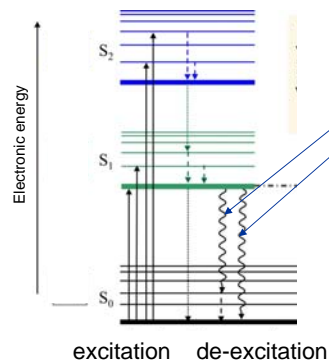
Phenomena that produce light - outline

- Spontaneous light emission from excited electronic state : **Luminescence**
- Light produced by induced emission: LASER (next week)
- **Thermal radiation**
- LED (last week): light emission in the recombination of electrons and holes in semiconductor diodes, induced by electric voltage

Spontaneous light emission from excited electronic state : Luminescence

Two kinds: $S_1 \rightarrow S_0$ transition **Fluorescence**
 $T_1 \rightarrow S_0$ transition **Phosphorescence**

Fluorescence

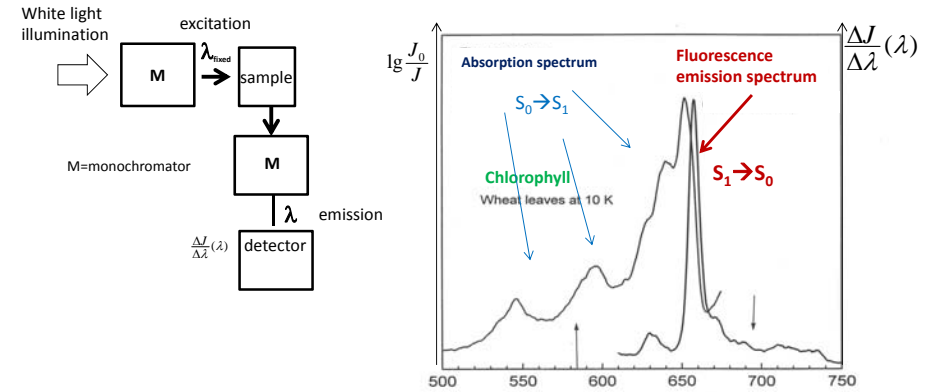


De-excitation by photon emission between singlet states

1. Kasha's rule

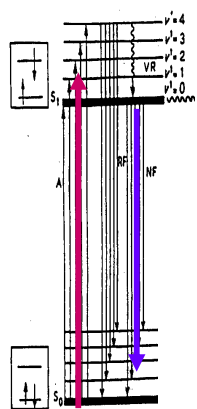
In a sample of molecules in interaction with the environment, fluorescence occurs only from the vibrational state of **lowest energy** within the **lowest electronic excited state**.

2. Fluorescence spectroscopy

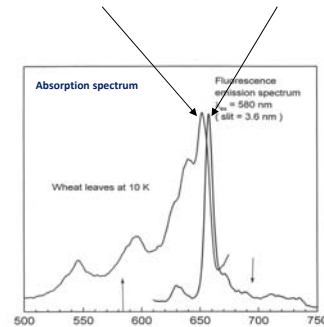


3. Stokes-shift in fluorescence spectroscopy

Average of absorbed and emitted photon energies of the electronic transitions between the same orbitals do not coincide



Absorption takes place from the lowest vibronic state in S_0

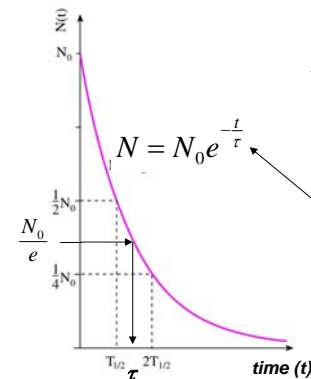


$$hf_{abs} > hf_{flu}$$

$$\lambda_{abs} < \lambda_{flu}$$

For maximum positions

4. Lifetime of excited states – fluorescence decay



After instantaneous excitation of N_0 number of molecules, they return to the ground state

Exponential decay with time

τ is the excited state lifetime

N_0 molecules were excited at $t=0$. The number of excited molecules, N decreases with the time after excitation

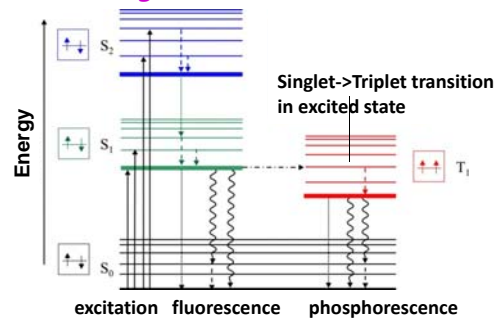
The lifetime of singlet excited states (S_1) is short $\sim 10^{-9}$ s

Phosphorescence

Selection rules forbid the change of spin state upon excitation and spontaneous emission:

$S_0 \rightarrow T_1$ and $T_1 \rightarrow S_0$ are of very low probability

Jablonski diagram



1. The lifetime of triplet excited states

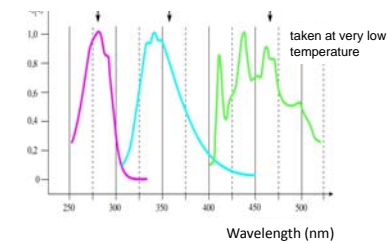
The $T_1 \rightarrow S_0$ transition is of low probability \rightarrow the lifetime is long : $\mu s - s$
metastable state

2. Relation of absorption-, fluorescence-, and phosphorescence spectra

Spectra of the *Tryptophan* amino acid

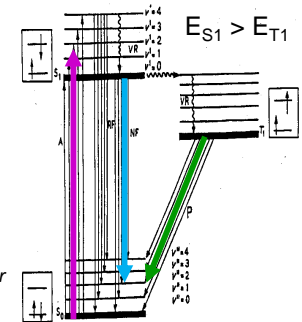
absorption fluorescence phosphorescence (spectra are normalized for comparison)

$$\lambda_{\max} < \lambda_{\max} < \lambda_{\max}$$



Phosphorescence emission is typically of **very low intensity**

other de-excitation phenomena compete with light emission
long lifetime \rightarrow increased probability of de-excitation by other phenomena (heat generation)



Applications of luminescence

Various ways for electronic excitation and spontaneous light emission

- Light photon absorption: **photoluminescence**
- Chemical activation: **chemoluminescence** - **bioluminescence**
- Collision with charged particles accelerated by electric field: **electroluminescence**
- Excited state after mechanical deformation: **triboluminescence**
- Thermal population of excited state : **thermoluminescence** (see lab.practice)

Still: luminescence – even fluorescence - is not abundant in nature!

$$\Phi_F = \frac{N_{fluor}}{N_{abs}} = \frac{k_{fluor}}{k_{fluor} + k_{heat} + k_{ext}} \approx \int F(f) df = J_{fluor, band}$$

Quantum efficiency of fluorescence

k : speed of de-excitation (light emission, heat generation, external interaction)

$$\tau = \frac{\Phi}{k_{fluor}} = \frac{1}{k_{fluor} + k_{heat} + k_{ext}}$$

lifetime

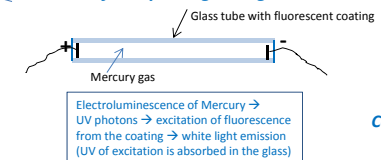
Most of the molecules do not relax from excited electronic state by light emission!

1. Light sources in everyday life



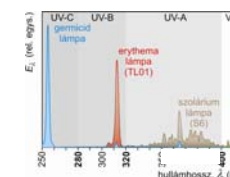
Examples for electroluminescence, combined electro- and photo-luminescence in lab. practice

F-tubes for replacing sunlight



Compact form of F-tube

2. Light sources for special (Medical) purposes - based on the emission wavelength

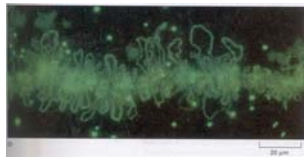


Electroluminescence of Mercury gas at various pressures

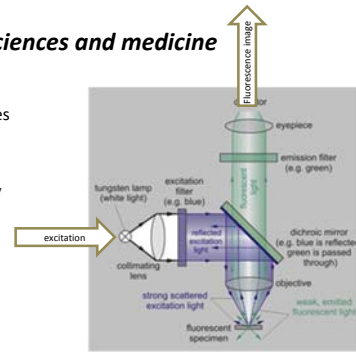
- low pressure: Germicidal lamp - $\lambda = 254$ nm absorbed in DNA of bacteria \rightarrow sterilization of laboratories
- medium pressure: Erythema lamp - $\lambda = 280 - 320$ nm those with $\lambda \sim 310$ nm treat psoriasis, vitiligo
- high pressure: Solarium lamps for tanning, light source for excitation of photosensitizers in dermatology

3. Fluorescence microscopy – applied in life sciences and medicine

Basis: the tissues naturally have very little fluorescence →
→ imaging will show the site of attached fluorescent dyes by photoluminescence

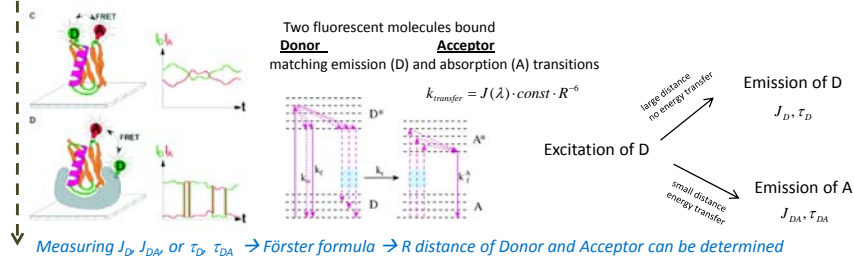


A step in gene expression:
proteins processing RNA
are colored green by an antibody

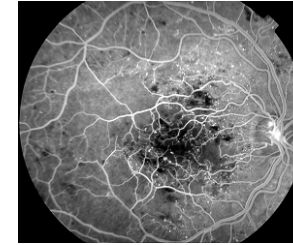


For reading.....not discussed in the lecture

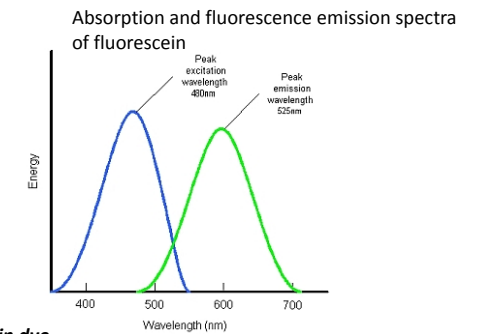
FRET: Closeness – distance R – of molecules studied by Förster-type Resonance Energy Transfer



Angiography using fluorescence dyes



Blood vessels of the retina visualized by **fluorescein dye**
Image taken by reflection mode fluorescence microscopy



4. Fluorescence spectroscopy used for qualitative and quantitative analysis

- lab. Practice: spectrum is characteristic for the molecule → identification
- medical laboratory: flame photometry → intensity of selected emission lines → quantitative analysis

Thermal (black body) radiation - reminder (previous lecture)

All material objects that are at non-zero absolute temperature emit electromagnetic radiation that originates from internal vibrations of the composing atoms leading to charge acceleration and dipole oscillation.

Kirchhoff's law: objects that have intense thermal radiation emission, are also efficient absorbers of the same radiation

Definitions:

$$\alpha = \frac{E_{absorbed}}{E_{total incident}}$$

Efficiency of absorption

$$M_{\lambda} = \frac{\Delta P}{\Delta A} = (J_{emitted})$$

Total emitted power in all directions at λ wavelength



Thermal (black body) radiation - reminder (previous lecture)

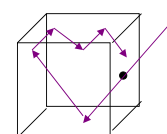
Kirchhoff's law:

$$\frac{M_{\lambda,i}}{\alpha_{\lambda,i}} = \frac{M_{\lambda,j}}{\alpha_{\lambda,j}} = const.$$

The ratio of emission and absorption efficiencies at all λ -s are the same for all objects (i and j)

Absolute Black body: fully absorbs all incident energies :

$$\alpha = 1 \Rightarrow M \text{ is also the highest}$$



Black body: metal box with a small hole. A beam that gets in will never find its way out.

The human body is 95% black body

Thermal (black body) radiation - reminder (previous lecture)

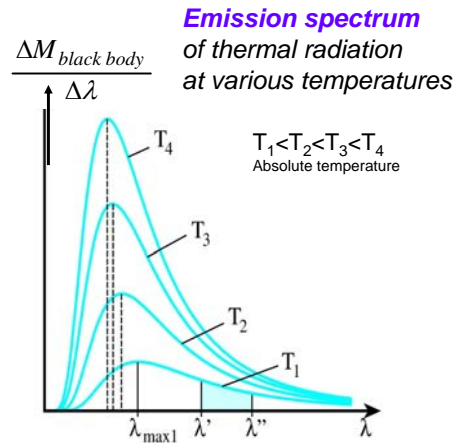
Stefan –Boltzmann law:

$$M_{total} = \sigma \cdot T^4$$

Emitted intensity at all λ :
„area below the curve” of the
emission spectrum

Stefan–Boltzmann constant

$$\sigma = 5.7 \cdot 10^{-8} \frac{W}{m^2 \cdot K^4}$$



The emission spectrum is continuous, it covers a
broad range in λ (photonenergy) with a maximum

Thermal (black body) radiation - reminder (previous lecture)

Stefan –Boltzmann law:

$$M_{total} = \sigma \cdot T^4$$

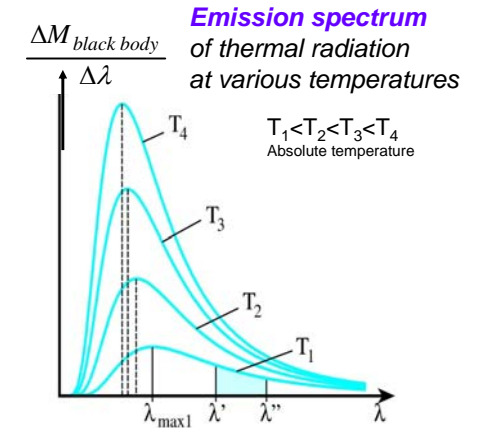
Body temperature strongly influences
the emitted intensity.

Wien's displacement law

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

2.898x10⁶ nmK

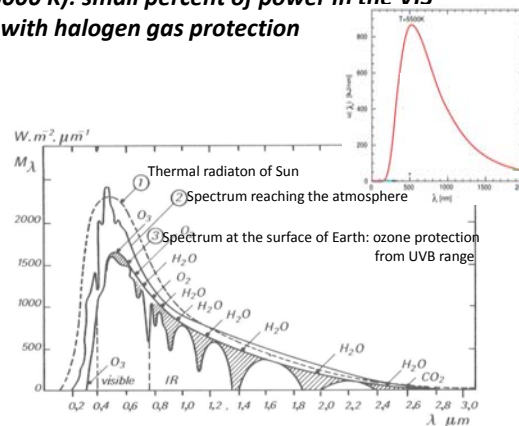
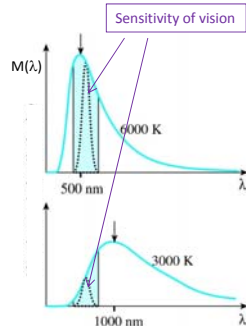
The wavelength of maximum intensity shifts to
shorter wavelengths when T is increased



Practical importance of thermal radiation

1. Light sources of everyday life

- Sun as black-body radiator at 6000 K + ozone protection (UVB -!)
- incandescent lamps (~3000 K): small percent of power in the VIS nowadays only with halogen gas protection

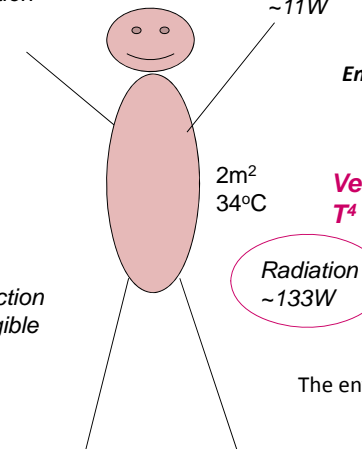


2. Thermal equilibrium of human body

Perspiration
~17W

Convection
~11W

Conduction
- negligible



Energy exchange with the environment

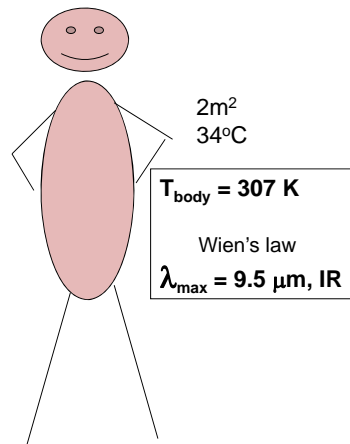
$$\Delta M = \sigma \cdot (T_{body}^4 - T_{envir.}^4)$$

**Very significant – depends on
 T^4 of the environment!**

The environment is also a thermal radiator!

3. Thermal radiation of human body used in diagnostics

Below 700 K (430 C) the radiation is mostly IR, not visible



Telethermography

Mapping the intensity of IR radiation emitted by the human body over a given surface by IR camera

inflammations, changes in blood circulation, metabolic changes in tumors..... lead to temperature changes i.e. changes in the intensity of IR radiation



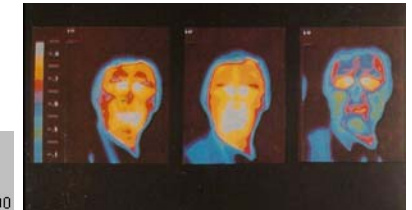
Color coded temperature maps

Capabilities of human IR imaging

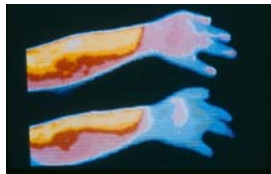


A trailer driver is getting tired during a day of driving

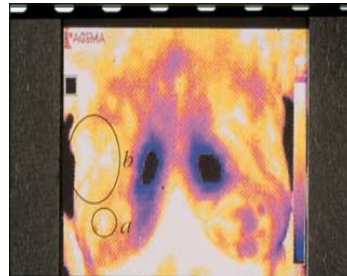
morning noon night



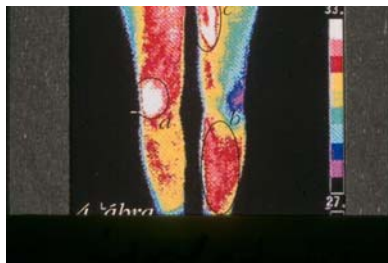
Examples for diagnostic applications – telethermography



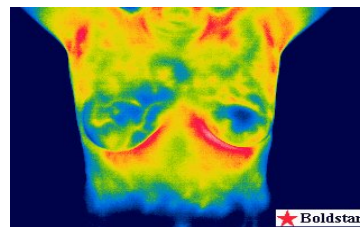
Effect of smoking



Breast tumor



Inflammation and thrombosis



A healthy dog....

Thank you for your attention!