Medical Biophysics I.

Interactions of light with matter.

Light scattering, light absorption. Basic principles of absorption spectrometry.

(Help to understand and learn exam question 12. and to measurement „Light absorption”.)

8th Lecture, 25th October 2016

12th exam question

How the light can interact with atoms and molecules?

Basic concepts: Light scattering, Rayleigh-scattering, Mie-scattering, light absorption.

Phenomena: Blue sky, white and grey clouds, transparency.

Physical quantities: Light intensity (J), electric dipole momentum (p), absorbance (A).

Laws, relations and the way leading to them: Attenuation of light intensity passing through medium. Dependence of scattered light intensity on the wavelength. Lambert–Beer-law.

Applications: Measurement of static light scattering and absorption, absorption spectrometry, determination of concentration. Dark field microscope.

Repetition I. - Light

What is light? – visible electromagnetic wave

Wave: quantity(ies) changing periodically in SPACE and TIME

Electromagnetic wave:

electric field (E) and magnetic flux density („field” (B)) -||-

$$\vec{F} = q \cdot \vec{E} \qquad \vec{F_L} = q \cdot \vec{v} \times \vec{B}$$

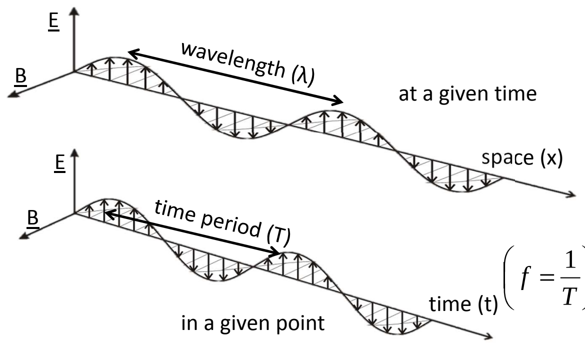
Electric field is defined as the electric force per unit charge

Magnetic fields are produced by electric currents (moving charges), which can be macroscopic currents in wires, or microscopic currents associated with electrons in atomic orbits

Repetition I. - Light

$$\underline{E} = A_E \cdot \sin\left(\frac{2\cdot\pi}{\lambda}\cdot x + \varphi_0\right)$$
$$\underline{B} = A_B \cdot \sin\left(\frac{2\cdot\pi}{\lambda}\cdot x + \varphi_0\right)$$

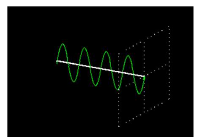
$$\underline{E} = A_E \cdot \sin\left(\frac{2\cdot\pi}{T}\cdot t + \varphi_0\right)$$
$$\underline{B} = A_B \cdot \sin\left(\frac{2\cdot\pi}{T}\cdot t + \varphi_0\right)$$



$$\left(f = \frac{1}{T}\right)$$

TIME and SPACE: $c = \lambda \cdot f$

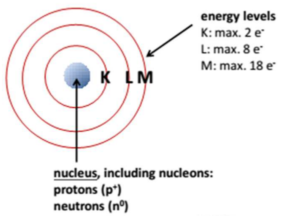
$$\varepsilon = h \cdot f \qquad E = \sum N \cdot \varepsilon_\lambda \qquad J = \frac{E}{A \cdot t}$$

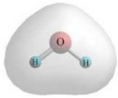


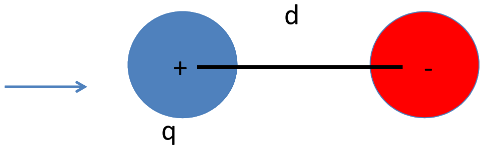
Visible:

400 nm 800 nm

Repetition II.
Matter: Atoms and Molecules







Dipole moment vector extent:

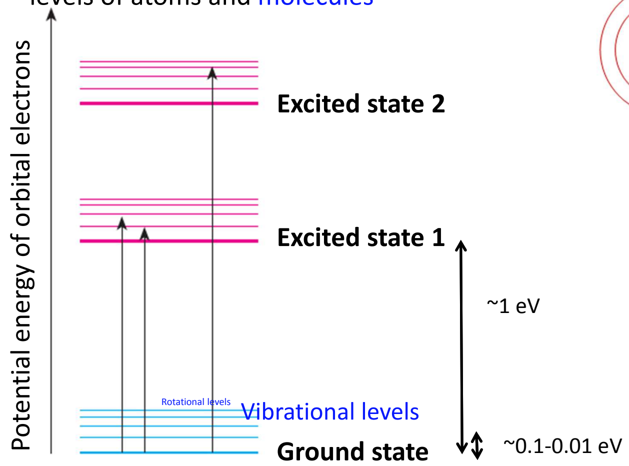
$$\left|\vec{d}\right| = d \cdot q$$

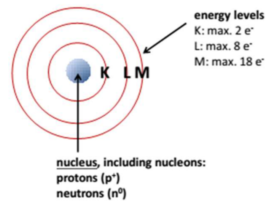
Polarizability (α): extent of a dipole that is generated by a unit of electric force-field

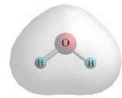
$$\vec{d} = \alpha \cdot \vec{E}$$

Repetition II.
Atoms and Molecules

Electronic and vibrational energy levels of atoms and molecules



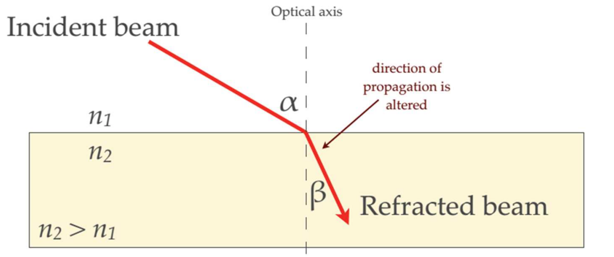


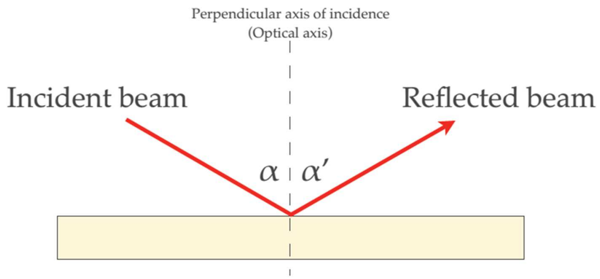


Molecules: + „vibration” and „rotation”

~0.01-0.001 eV

InteractionS – repetition



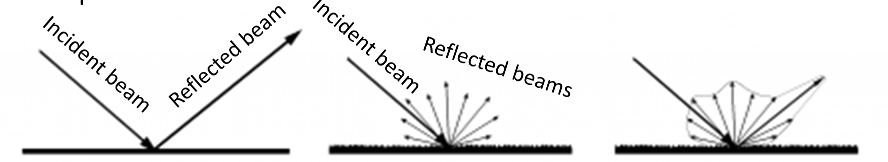


InteractionS – repetition

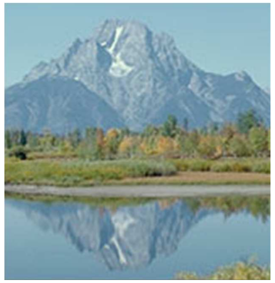
Experiment 1.

InteractionS – Reflection

Specular reflection | Diffuse reflection | Mixed reflection



Without (<λ) irregularity



With irregularity

InteractionS – Reflection

Coefficient of reflection

$$\rho_{\lambda} = \frac{J_{reflected(\lambda)}}{J_{incident(\lambda)}}$$

depends on:
wavelength
incident angle
material (refractive indices ...)

$$\rho_{\lambda} = \frac{J_{reflected(\lambda)}}{J_{incident(\lambda)}} = \left(\frac{n_2 - n_1}{n_2 + n_1}\right)^2$$

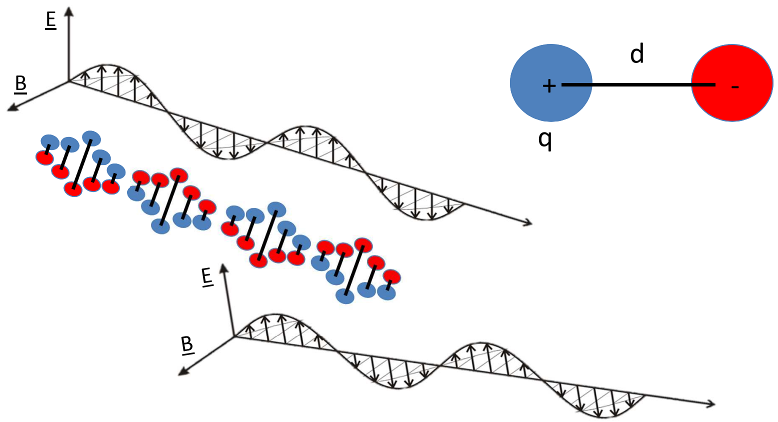
equation is true **IF** incident angle is 0°, material is transparent (see later)

InteractionS

Experiment 2.

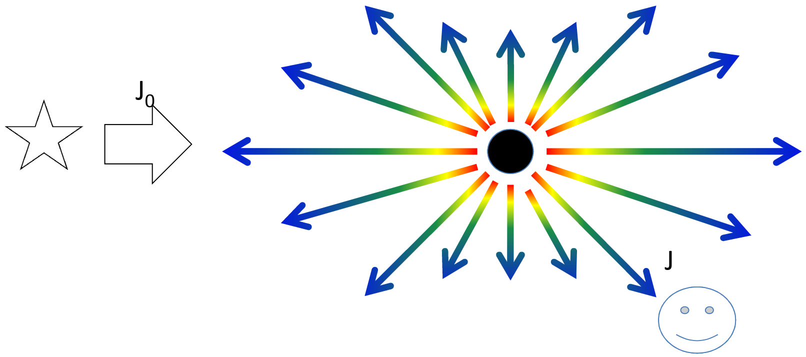
InteractionS – Scattering

1. Elastic scattering (*f* does NOT change)



Interaction**S** – Rayleigh Scattering

Small, individual particles – individual oscillations without interference

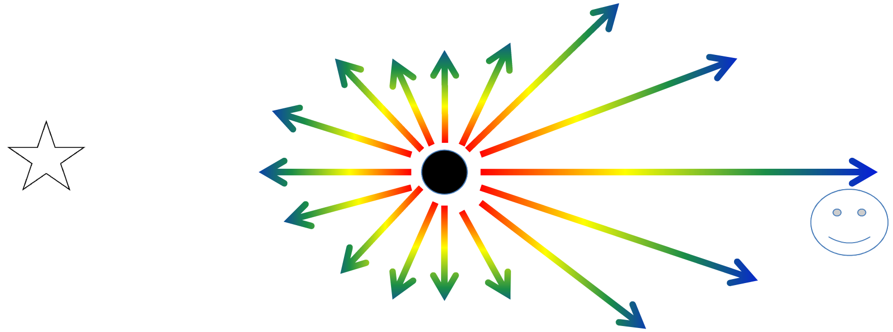


$$J_\lambda = J_0 \cdot \frac{8 \cdot \pi^4 \cdot N \cdot \alpha^2}{\lambda^4 \, R^2} \cdot \left(1 + \cos^2(\beta)\right)$$

N: number of scatterers
α: polarizability
λ: wavelength
R: distance from scatterer
β: observing angle

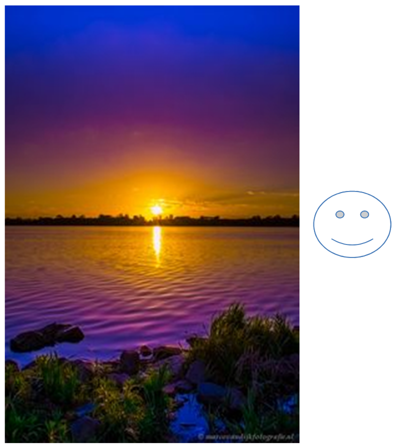
Interaction**S** – Mie Scattering

Large particles, group of atoms, molecules (size > λ/10)
– constructive interference



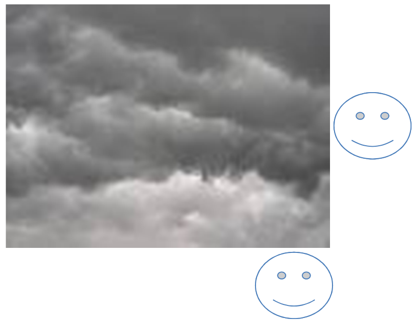
wavelength independent
large forward scattering
Size dependent:
- Size increasing: intensity increasing
- Size increasing further (> λ*10) : intensity decreasing

Interaction**S** – Rayleigh Scattering



Blue sky
Red sunset

Interaction**S** - Mie scattering



Glare, white cloud

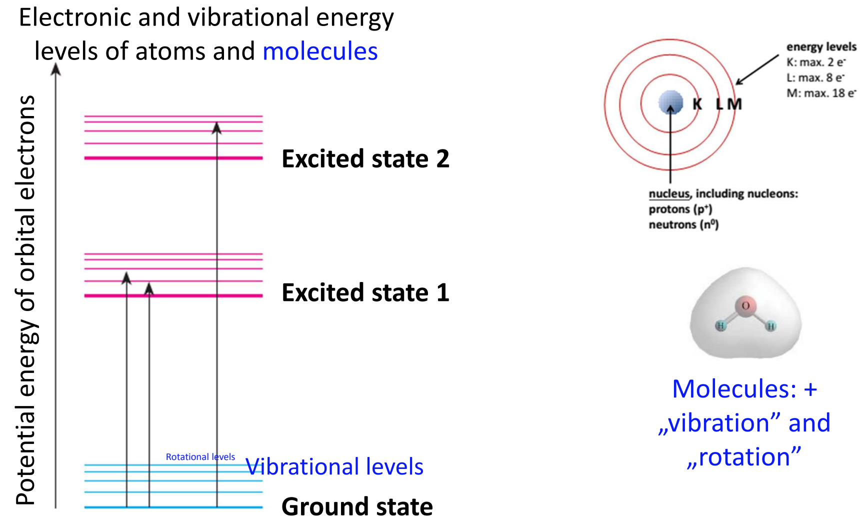
Gray cloud

InteractionS

Experiment 3.

InteractionS - Absorption

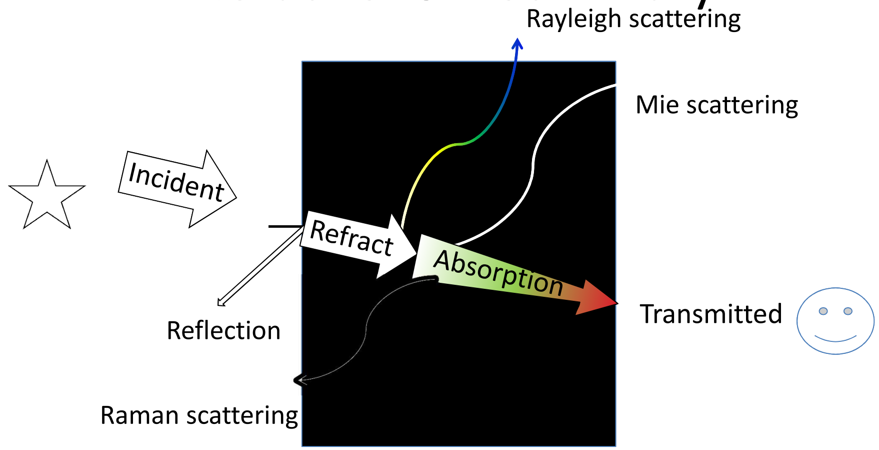
Resonance: induced oscillation – energy levels



InteractionS - Scattering

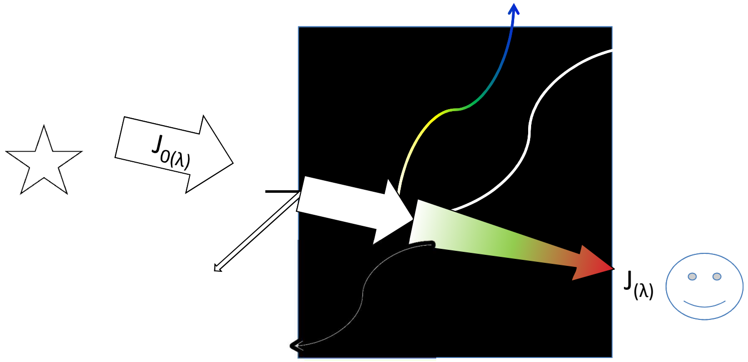
2. Inelastic scattering (*f* changes)
- energy absorption between vibrational levels + re-emission
  - short life time in the excited state (~fs)
- Raman scattering
  - Stokes: scattered ε reduced
  - Anti-Stokes: scattered ε increased

InteractionS - Summary



Which one? – Probabilities!
depends on: wavelength
incident angle
material

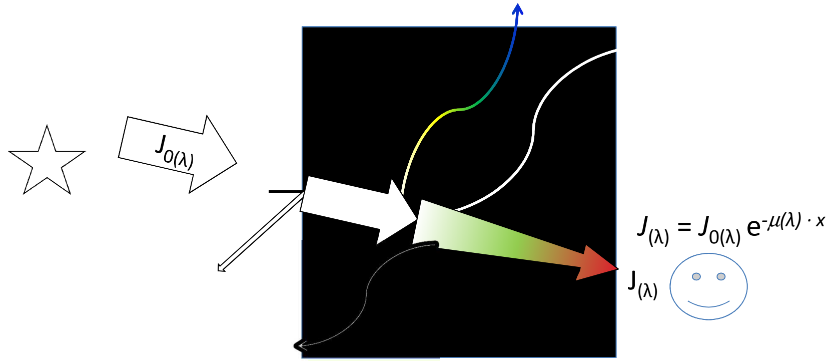
Intensity attenuation - Decay law



$J_{(\lambda)} = J_{0(\lambda)}\, e^{-\mu(\lambda) \cdot x}$

J: intensity
μ: attenuation coefficient
x: layer thickness

Transmittance, Optical density

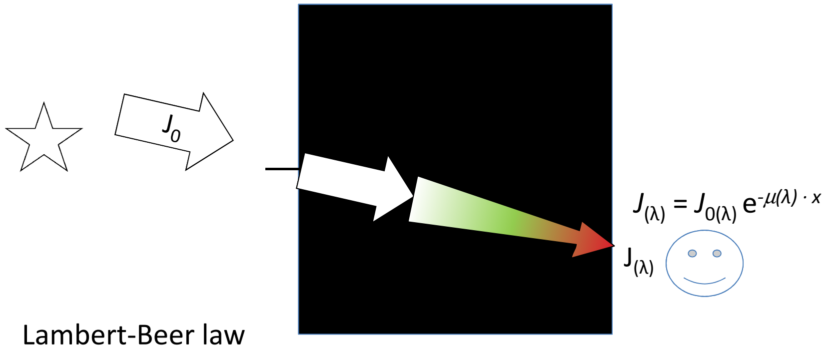


$$T = \frac{J_{(\lambda)}}{J_{0(\lambda)}} \quad (\%)$$

$$OD = \log \frac{J_{0(\lambda)}}{J_{(\lambda)}} = \log e \cdot \mu_{(\lambda)} \cdot x$$

T: transmittance
OD: optical density

Absorption



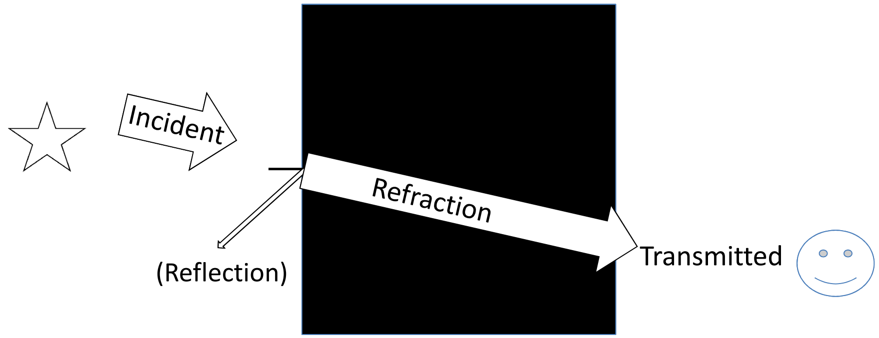
Lambert-Beer law

$$A = \log \frac{J_{0(\lambda)}}{J_{(\lambda)}} = \log e \cdot \mu_{(\lambda)} \cdot x = \varepsilon_{(\lambda)} \cdot c \cdot x$$

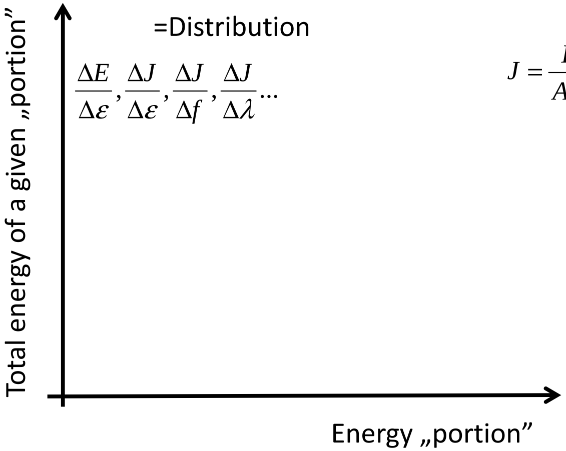
Conditions!!!

μ: absorption coefficient
ε: (decadic) molar extinction coefficient
c: concentration

Transparency



Spectrum



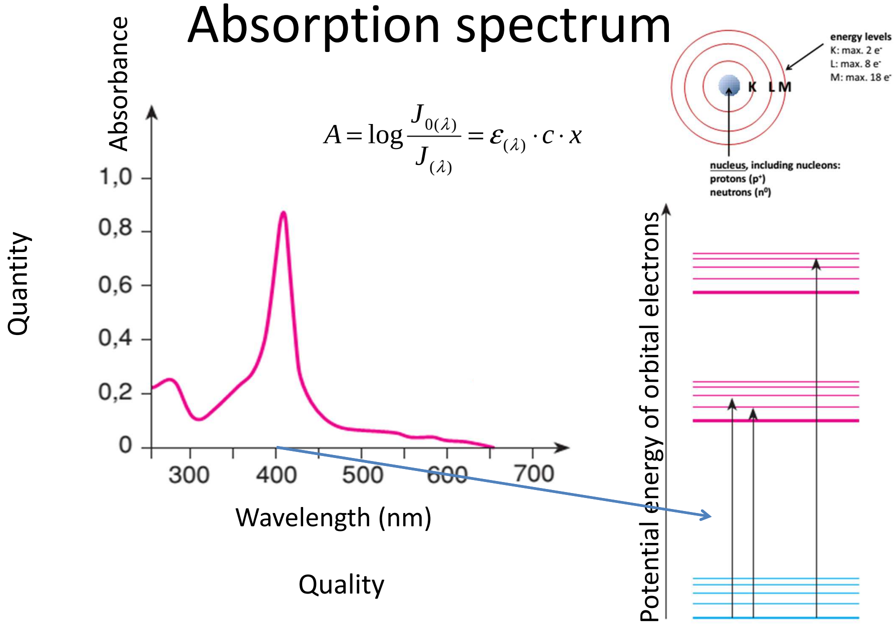
$$\frac{\Delta E}{\Delta \varepsilon}, \frac{\Delta J}{\Delta \varepsilon}, \frac{\Delta J}{\Delta f}, \frac{\Delta J}{\Delta \lambda} \ldots$$

$$J = \frac{E}{A \cdot t} = \frac{\sum N \cdot \varepsilon_\lambda}{A \cdot t}$$

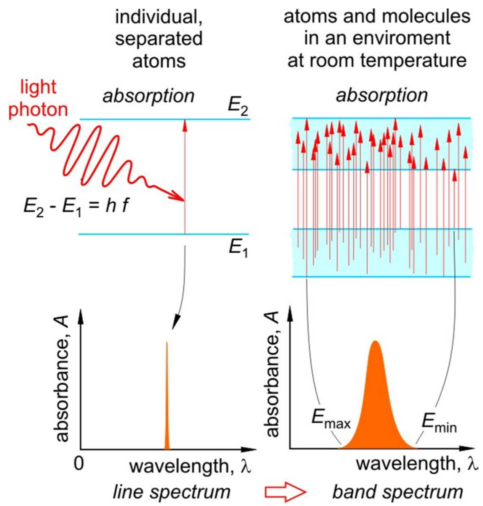
Eg.: ε,f,λ,1/f….

Absorption spectrum

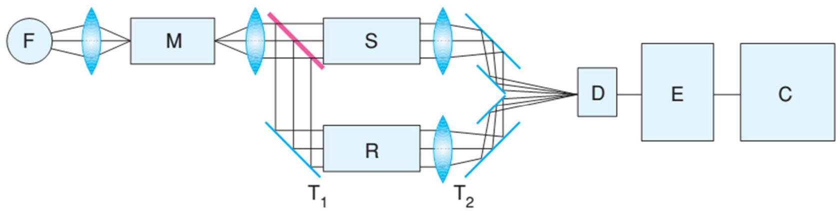
$$A = \log \frac{J_{0(\lambda)}}{J_{(\lambda)}} = \varepsilon_{(\lambda)} \cdot c \cdot x$$



Spectrum broadening



Absorption spectrum measurement



Monocromators:

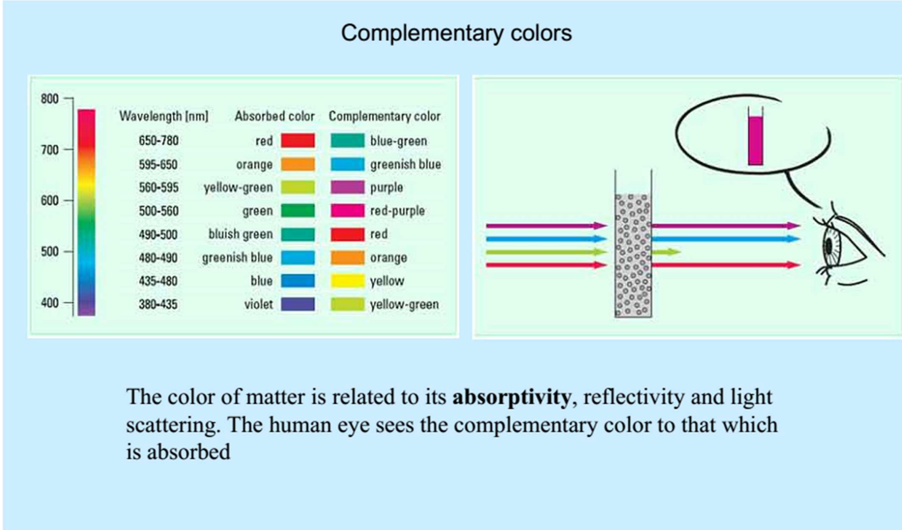
- Prism: refraction – advantage: total intensity
- Optical grating: diffraction (interference image) – advantage: linear („ghosts”: harmonics)

Scattering measurements

Concentration measurements:
Turbidimetry: (high intensity of scattering)
measuring OD (condition: no absorption)
Nephelometry: (low intensity of scattering)
collecting scattered light in a given angle

Particle size and geometry measurement:
Dynamic light scattering: (size)
collecting scattered light in a given angle
scattered intensity changes in time
Static light scattering: (size, geometry)
collecting scattered light in a given angle range (multiple angle)

Color - absorption



Colors

?