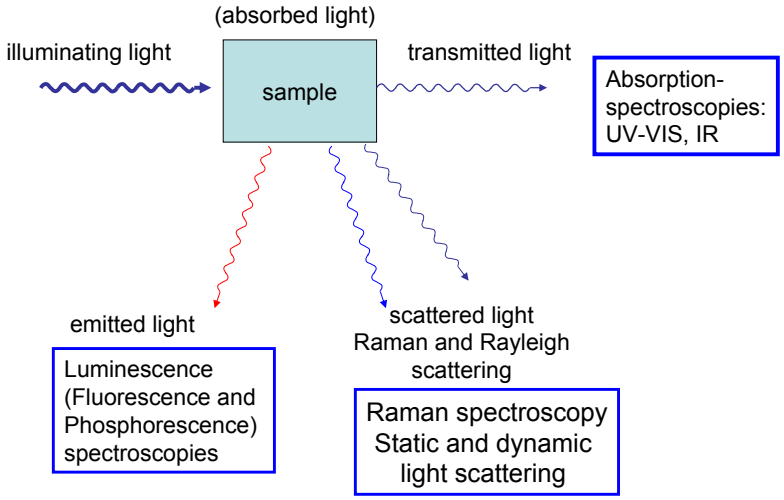
Optical spectroscopic techniques

László Smeller

What happens if a sample is illuminated by light?

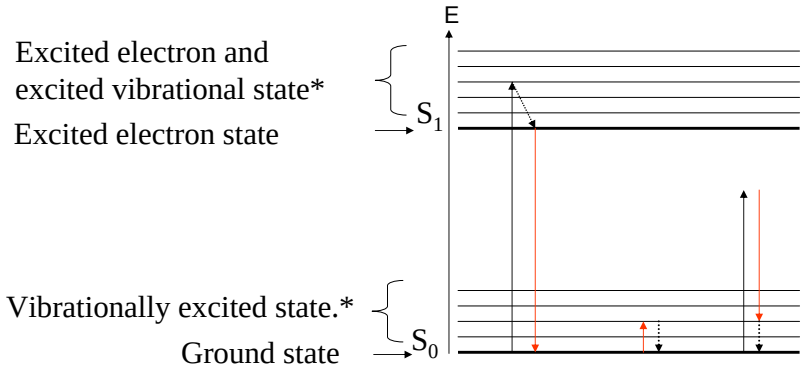


Spectroscopy
(Absorption and emission spectroscopy)

- Analysis of the wavelength dependence of the transmitted or emitted light.
- Information:
  - identification of atoms and molecules,
  - detection of changes in the molecular structure (conformation)
  - determination of the concentration

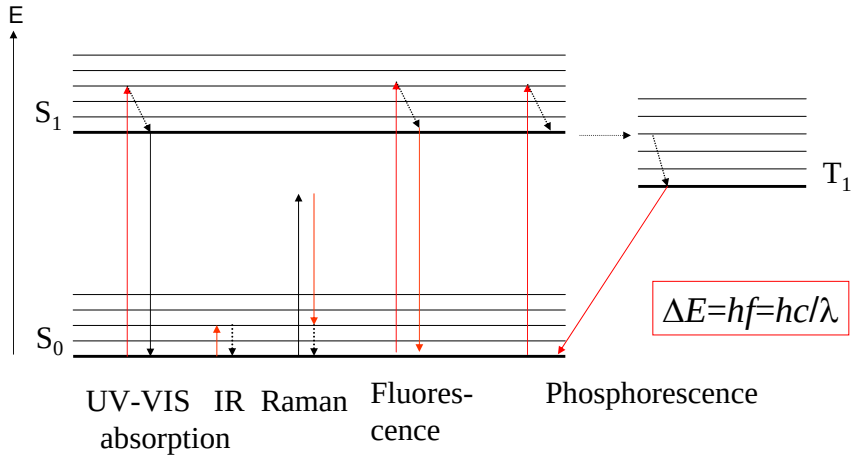
Why is light absorbed or emitted?

Jablonski diagram



*only for molecules! (not for atoms)

Why is light absorbed or emitted?



Absorption spectroscopy (UV-VIS)

As a reminder:

- law of absorption: $J=J_0 \cdot e^{-\mu x}$ where μ(material,c,λ)
- Lambert-Beer law:

$$A = \lg \frac{J_0}{J} = \varepsilon(\lambda) c x$$

- spectrum: $A(\lambda)$
- measurement: spectrophotometer
  (details: see pract. exc.)
  reference solution (J_0)
- information: identification (λ_{max}), concentration (A)

Infrared spectroscopy

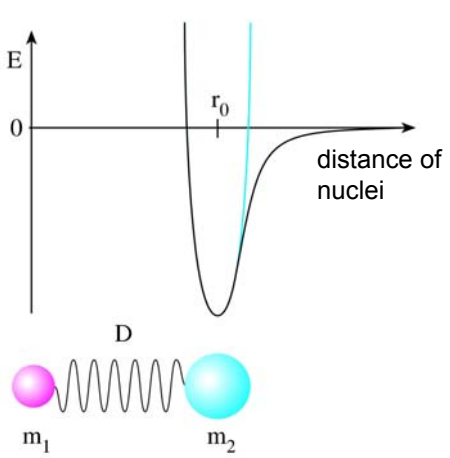
- Infrared light: λ=800 nm - 1 mm
  MIR (mid-infrared) : 2,5-50 μm
- absorption spectroscopy
- the absorbed infrared radiation excite molecular vibrations
- very specific for the structure of the molecule
- special method for detection:
  FT spectrometer

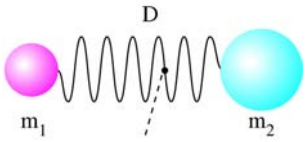
Molecular vibrations

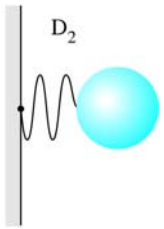
The electrons are light ($m_e << m_{nucleus}$), they can follow the movements of the nuclei easily, therefore the movements of the nuclei are independent of the movements of the electrons.

Classical physical description: the chemical bond is represented by a spring

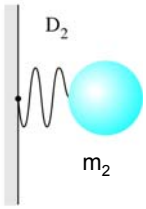
Molecular vibrations:





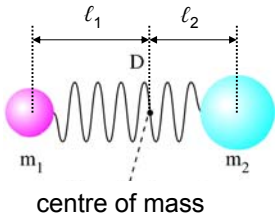


known from elementary mechanics:



$$f = \frac{1}{2\pi}\sqrt{\frac{D_2}{m_2}}$$

$$\frac{m_2}{m_1} = \frac{\ell_1}{\ell_2}$$



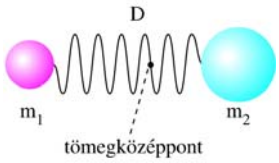
$$\frac{D_2}{D} = \frac{F/D}{F/D_2} = \frac{\Delta\ell}{\Delta\ell_2} = \frac{\ell}{\ell_2} =$$

$$= \frac{\ell_1 + \ell_2}{\ell_2} = \frac{\ell_1}{\ell_2} + 1 = \frac{m_2}{m_1} + 1 = \frac{m_1 + m_2}{m_1}$$

$$F = D\Delta\ell$$

it follows: $\frac{m_1 + m_2}{m_1} = \frac{D_2}{D}$, substituting in

$$f = \frac{1}{2\pi}\sqrt{\frac{D_2}{m_2}}$$



frequency of the vibration:

$$f = \frac{1}{2\pi}\sqrt{\frac{D(m_1 + m_2)}{m_1 m_2}}$$

$$m_{red} = \frac{m_1 m_2}{m_1 + m_2}$$ is called as reduced mass

The frequency: $f = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}}$

The wavelength: $\lambda = \frac{c}{f} = 2\pi c\sqrt{\frac{m_{redukált}}{D}}$

In the IR spectroscopy the wavenumber (ν) is used, which is the reciprocal of λ:

$$\nu = \frac{1}{\lambda} = \frac{1}{2\pi c}\sqrt{\frac{D}{m_{redukált}}}$$

ν: number of waves in a unit length [cm^{-1}]

Example: CO

The measured wavenumber: ν= 2143 cm^{-1}

⇨ λ=4,67μm ⇨ f=6,43 10^{13} Hz

m_C=2·10^{-26} kg, m_O=2,7·10^{-26} kg

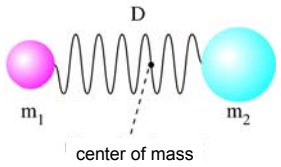
} ⇨ D=1875 N/m

if ν is known, D can be calculated

if D is known, ν can be calculated

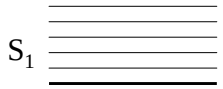
Classical vs. quantum physics

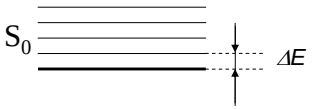
Classical physical picture



$$f = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}}$$

Quantum mechanical picture





$\Delta E = hf$

resonance with the light with frequency f

=

Vibrations of the large molecules

Molecule consisting of N atoms:

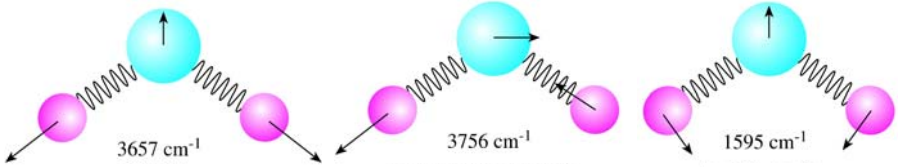
- 3N degree of freedom,
  - 3-3 are the rotations and translations of the whole molecule
- 3N-6 vibrational degree of freedom (3N-5 for the linear molecules)
- 3N-6 independent normal vibrations

Normal vibrations

All the atoms vibrate

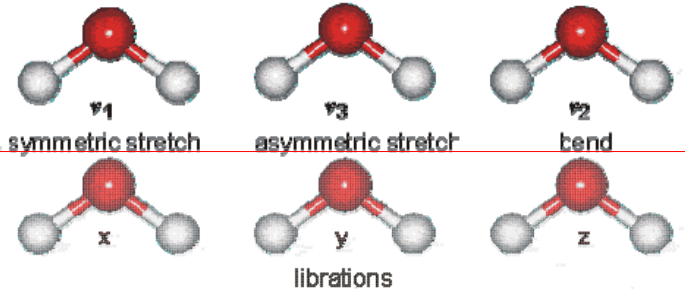
- with the same frequency but
- with different amplitude and
- in different direction.

Example: water



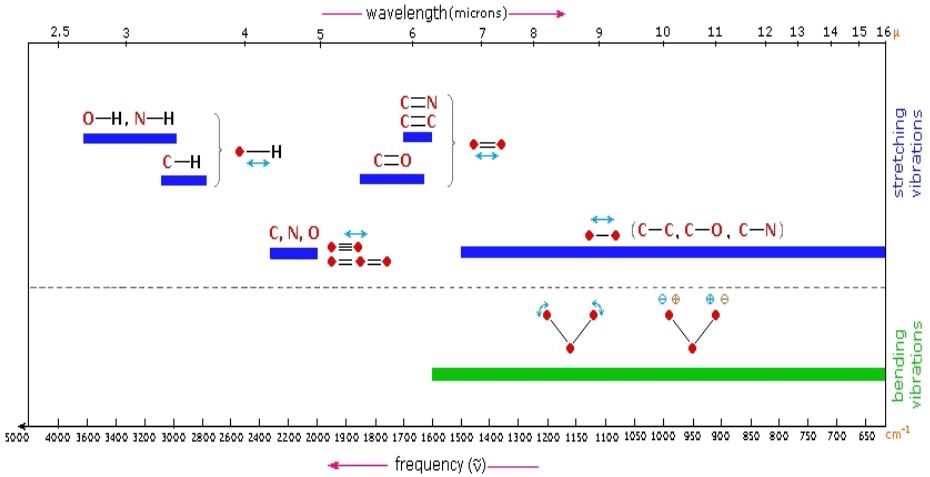
symmetric stretching asymmetric stretching bending

Normal vibrations of water

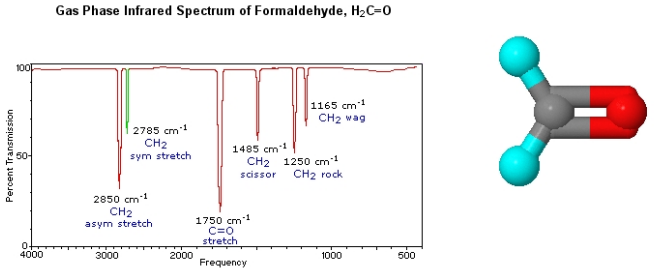


These are no vibrations! These are rotations!

Typical vibrational frequencies (wavenumbers)



Example: Formaldehyde



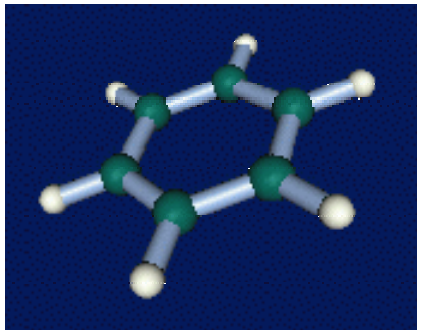
- View CH_2 Asymmetric Stretch
- View CH_2 Symmetric Stretch
- View C=O Stretch
- View CH_2 Scissoring
- View CH_2 Rocking
- View CH_2 Wagging

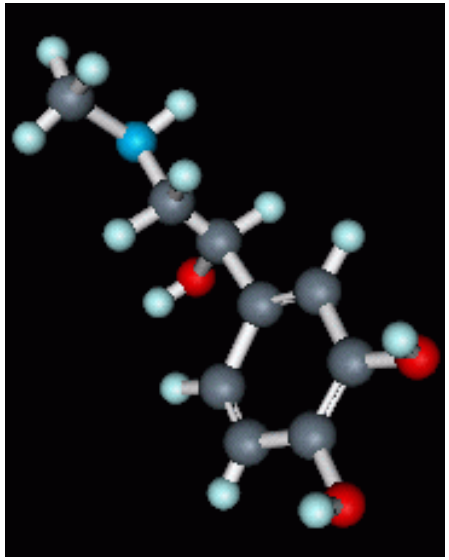
- Ball&Stick Model
- Spacefill Model
- Stick Model
- Motion Off

http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm

Flavin

Benzol



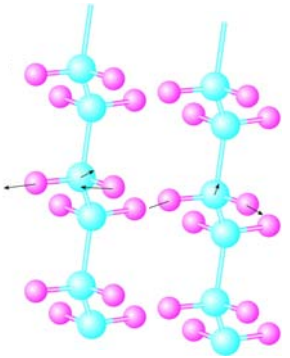


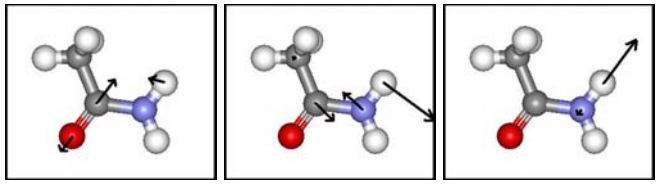
Vibrations of the macromoleclues

Complex global vibrations

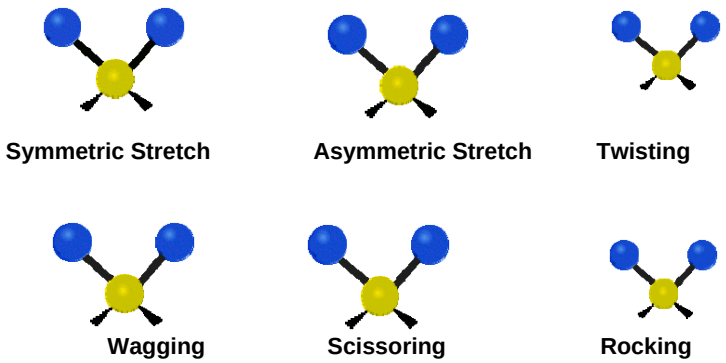
Localised vibrations, e.g.:

- CH_2 vibrations of the lipids
- amid vibrations of proteins (acetamide)





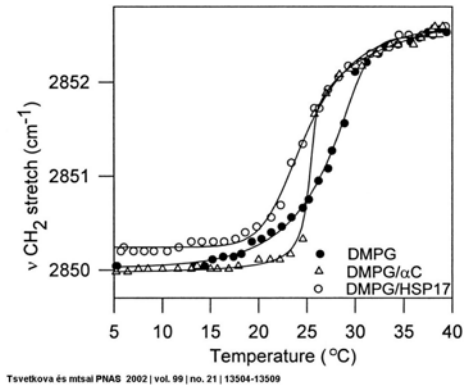
Lipids



Types of Vibrational Modes. Figure from Wikipedia

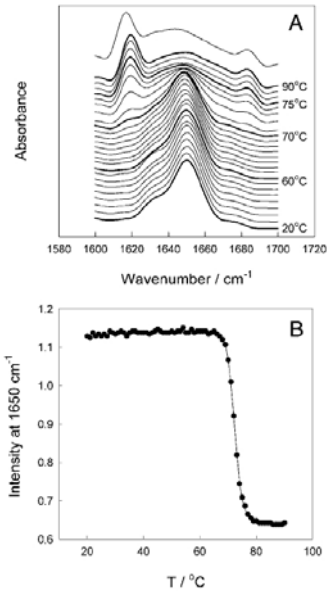
Applications

phase transitions in lipids



Tsvetkova és mtsai PNAS 2002 | vol. 99 | no. 21 | 13504-13509

protein denaturation



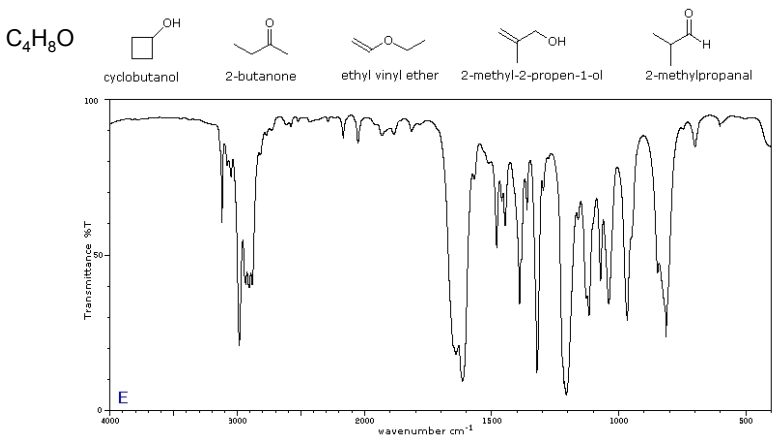
Meersman et al. Biophys J.

Pharmaceutical applications

- synthesis: identification of the intermedate and the end product
- determination and justification of the molecular structure
- detection of the metabolites
- quality control (purity)

- Remark.: Lambert-Beer law is valid, determination of concentration is possible

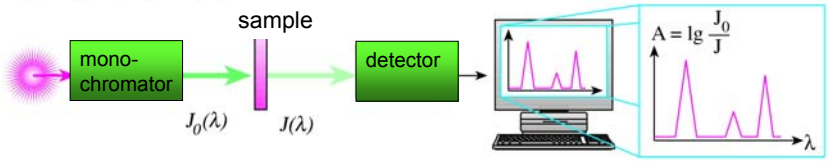
Example: Identification of molecules



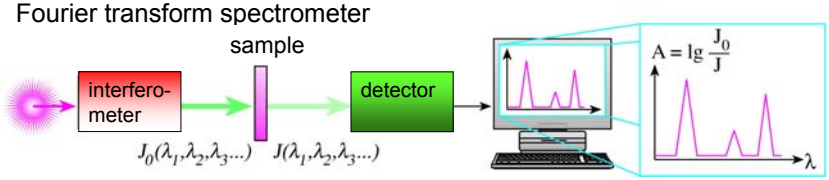
http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm

The technique of the measurement : Fourier transform spectrometer (FTIR)

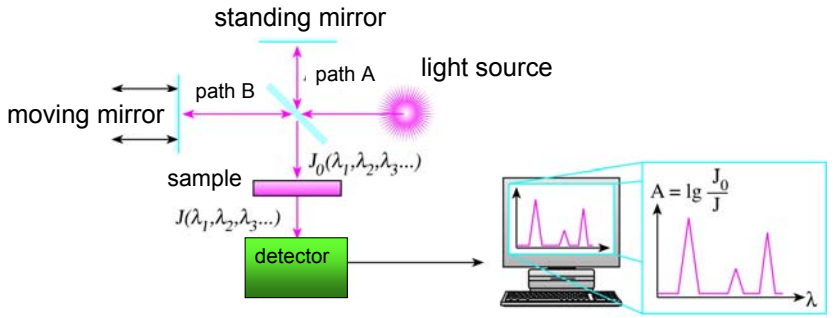
conventional (dispersion) spectrometer



Fourier transform spectrometer



book 6.17



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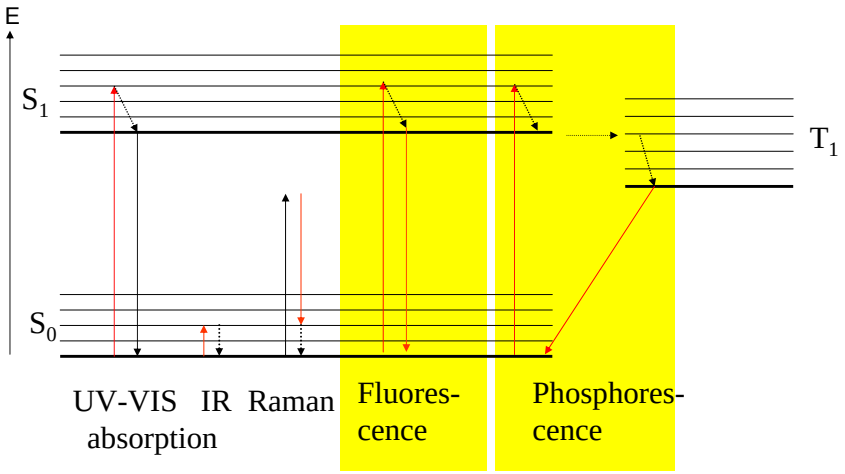
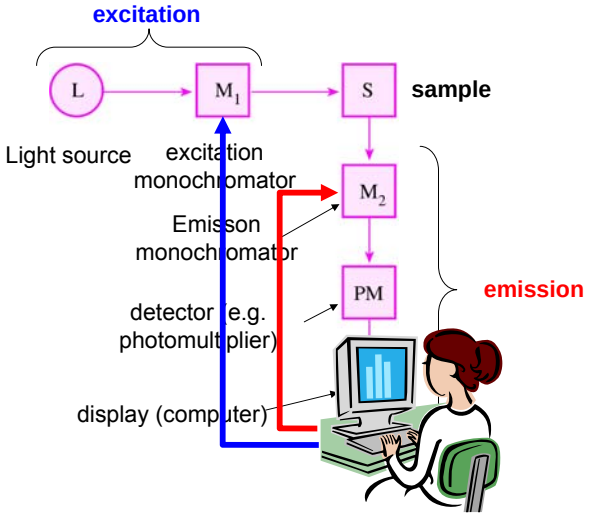
Luminescence spectroscopy



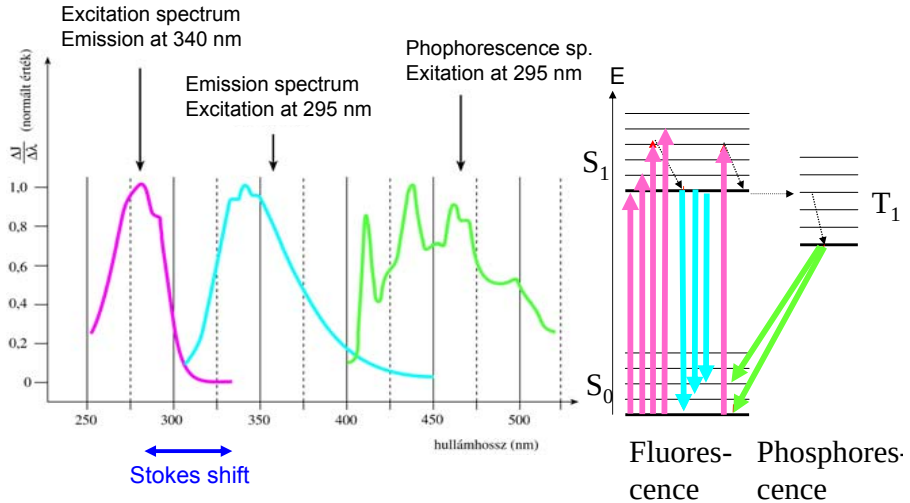
Measurable quantities in Fluorescence Spectroscopy

- Wavelength of the exciting light
- Wavelength of the emitted light (fluor., phosph.)
- Time dependence of the emitted light
- Polarisation of the emitted light
- Intensity of the emitted light

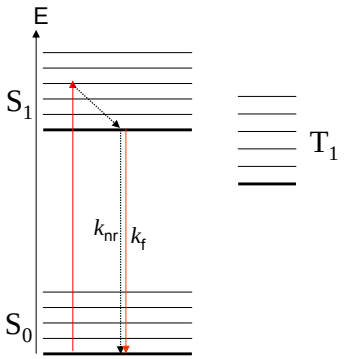
Scheme of the fluorescence spectrometer



Excitation and emission spectra



Fluorescence quantum yield(Q)



Quantum yield: Q =

$$= \frac{\text{number of emitted photons}}{\text{number of absorbed photons}}$$

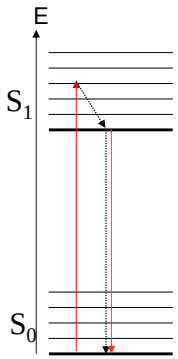
$$Q_f = \frac{k_f}{k_f + k_{nr}}$$

k_f probability of the transition with light emission (fluoresc.)

k_{nr} probability of nonradiating transition

dyes, fl. markers Q≈1

The lifetime of the excited state



From N excited molecules during Δt time

$-\Delta N=(k_f+k_{nr})N\Delta t$ will go back to ground state.

Differential equation:

$$\frac{dN}{dt} = -(k_f + k_{nr})N$$

Solution: $N = N_0 e^{-(k_f + k_{nr})t} = N_0 e^{-\frac{t}{\tau}}$

where $\tau = \frac{1}{k_f + k_{nr}}$ is the lifetime of the excited state

Decay of the fluorescence intensity

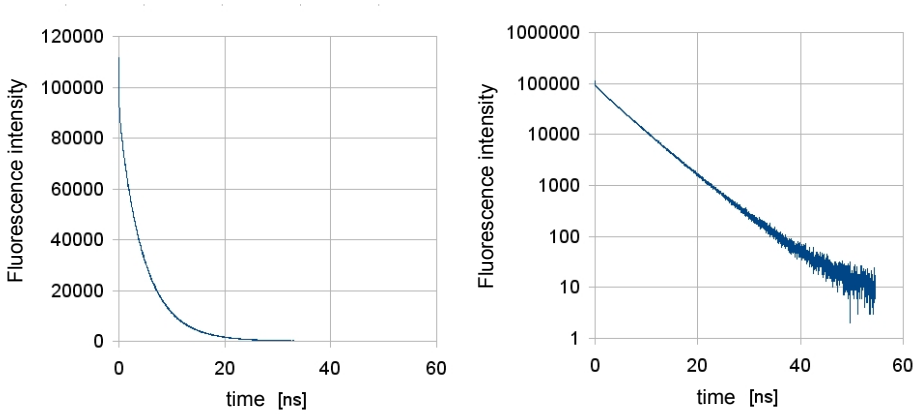
The number of emitted photons is proportional with ΔN-el, i.e. it is proportional also with N,-which means it is decays exponentially with the decay constant of τ.

How to measure?
-Pulsed illumination (flashlamp, or pulse laser)
-Photon counting as function of time
Quantum yield and life time can be also defined for phosphorescence, using similar definitions.

Typical lifetimes:

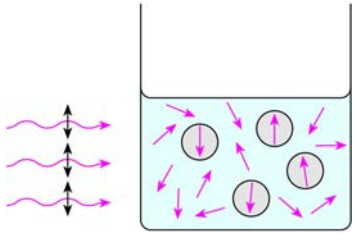
τ_{fluor} ns $\tau_{phosphor}$ μs ...s

Example: tryptophan



Fluorescence polarisation

illumination with polarized light



polarization degree of the emitted light is measured

The fluorescent molecule can rotate between the absorption and the emission ⇨ dinamic information

rotational correlation time (how fast the rotational diffusion is?)

Ligth scattering

Rayleigh $\lambda_{scatt}=\lambda_{illum}$

Raman $\lambda_{scatt} \neq \lambda_{illum}$

Raman scattering:

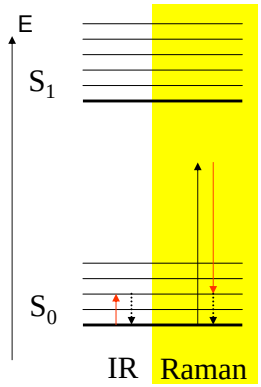
$\lambda_{scatt} \neq \lambda_{illum} \Rightarrow f_{scatt} \neq f_{illum}$

$\Rightarrow E_{photon,scatt} \neq E_{photon,illum}$

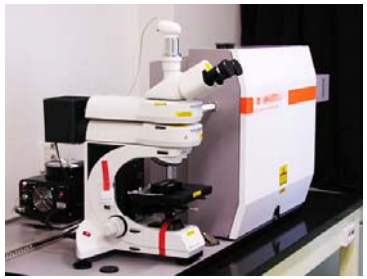
Where is the energy?

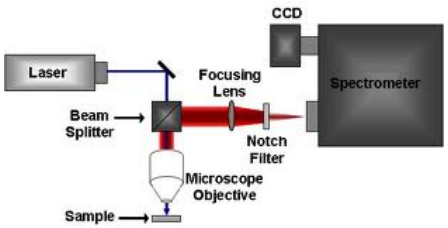
Excites vibrations of the molecule (cfr. IR)

very weak (~10^{-8})

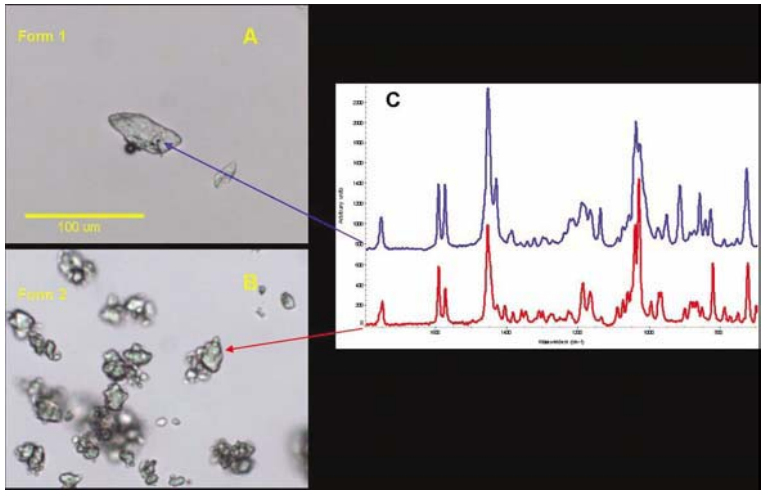


Equipment





Pharmaceutical application



Rayleigh scattering

Size of the particle: $a << \lambda$

The scattered intensity:

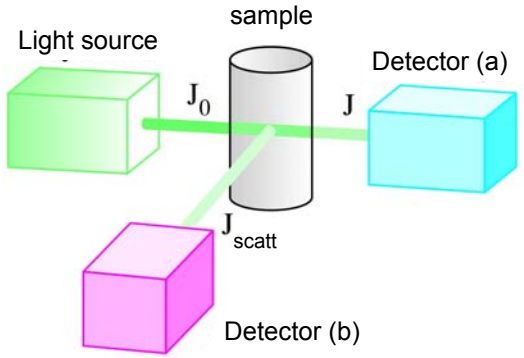
$$J_{scatt} \sim J_0 N \frac{a^6}{\lambda^4}$$

Information: size, contentration (quantity)
(e.g. colloids)

Meaurement of the Rayleigh scattering

if $J_{scatt} << J_0$

J_{scatt} is measured (Nephelometry)



If $J_{scatt} \approx J_0$

J is measured (turbidimetry)

The same technique as for the absorption specctrosopy but now J is reduced due to the scattering (and not due to absorption).