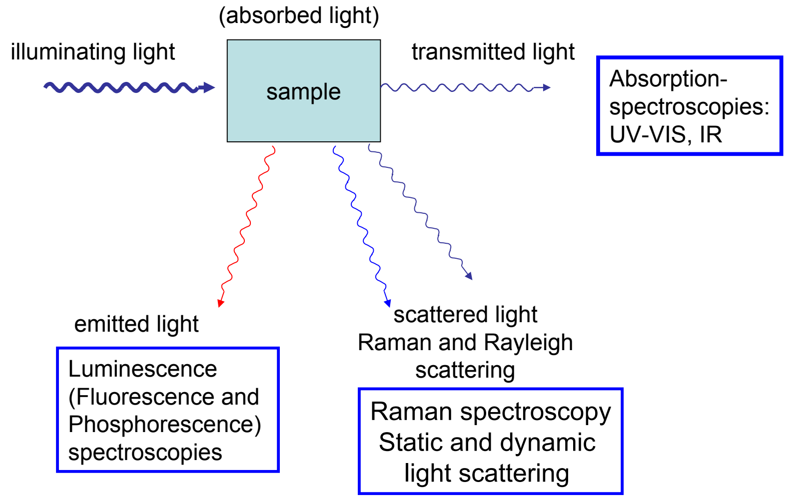
Optical spectroscopic techniques

László Smeller

What happens if a sample is illuminated by light?

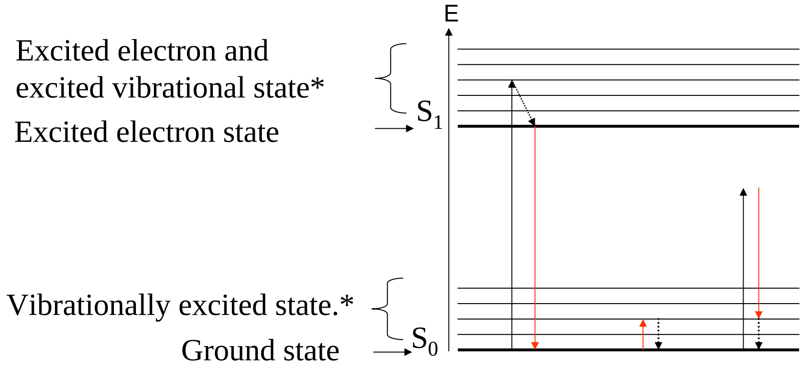


Spectroscopy
(Absorption and emission spectroscopy)

- 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

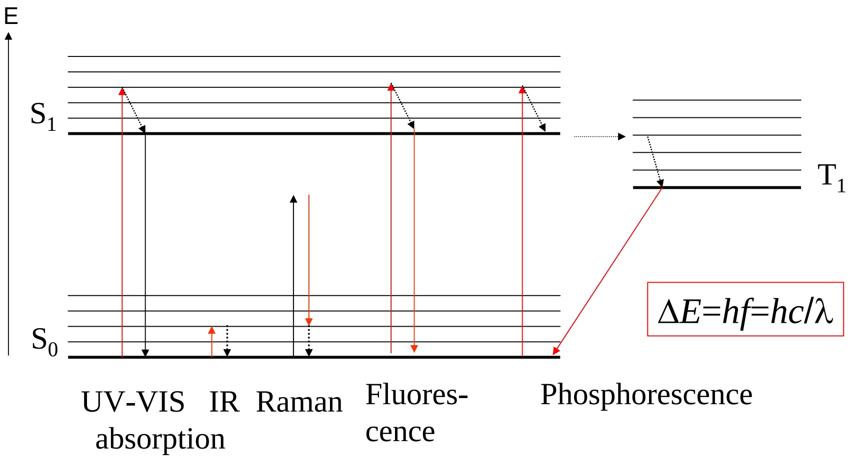
Why is light absorbed or emitted?

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

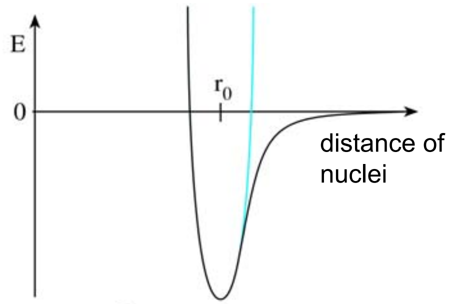
- Infrared light: λ=800 nm - 1 mm
  MIR (mid-infrared) : 2,5-50 μm
- absorption spectroscopy
- the absorbed infrared radiation excite molecular vibrations
- very specific for the structure of the molecule
- special method for detection:
  FT spectrometer

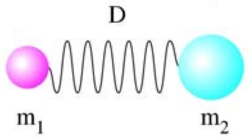
Molecular vibrations

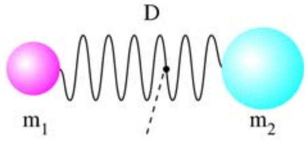
The electrons are light ($m_e << m_{nucleus}$), they can follow the movements of the nuclei easily, therefore the movements of the nuclei are independent of the movements of the electrons.

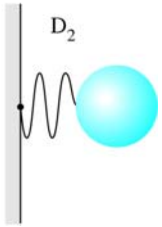
Classical physical description: the chemical bond is represented by a spring

Molecular vibrations:

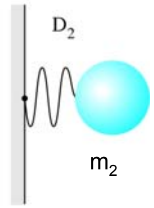






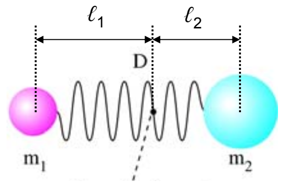


known from elementary mechanics:



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

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

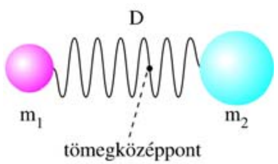


$$\frac{D_2}{D} = \frac{F/D}{F/D_2} = \frac{\Delta\ell}{\Delta\ell_2} = \frac{\ell}{\ell_2} =$$

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

$$F = D\Delta\ell$$

substituting $\frac{m_1 + m_2}{m_1} = \frac{D_2}{D}$ into $f = \frac{1}{2\pi}\sqrt{\frac{D_2}{m_2}}$



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

Frequency with the reduced mass: $f = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}}$

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=$2\cdot10^{-26}$ kg, m_O=$2{,}7\cdot10^{-26}$ kg

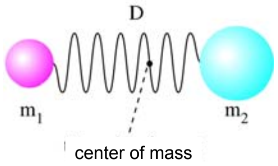
⇨ D=1875 N/m

if ν is known, D can be calculated

if D is known, ν can be calculated

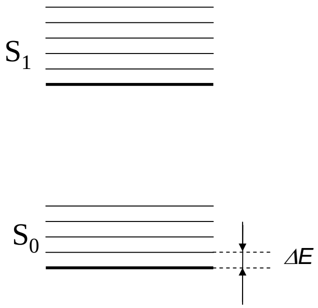
Classical vs. quantum physics

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 = $\Delta E = hf$

Vibrations of the large molecules

Molecule consisting of N atoms:

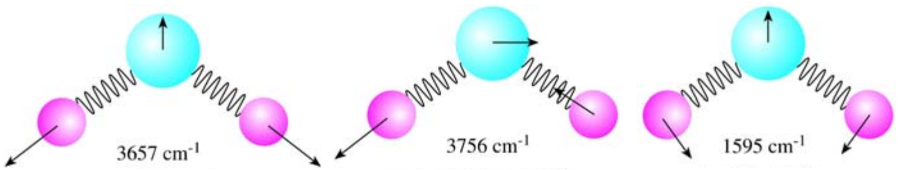
- 3N degree of freedom,
  3-3 are the rotations and translations of the whole molecule
- 3N-6 vibrational degree of freedom
  (3N-5 for the linear molecules)
- 3N-6 independent normal vibrations

Normal vibrations

All the atoms vibrate

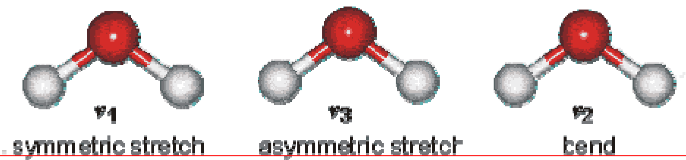
- with the same frequency but
- with different amplitude and
- in different direction.

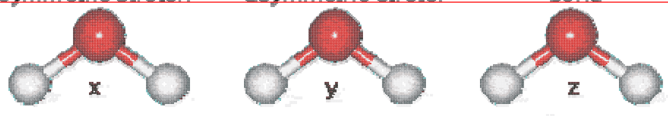
Example: water



symmetric stretching | asymmetric stretching | bending

Normal vibrations of water

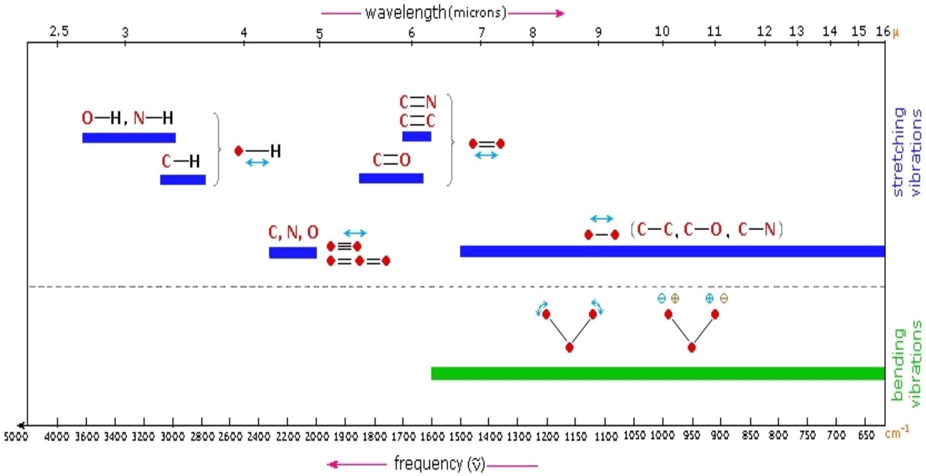




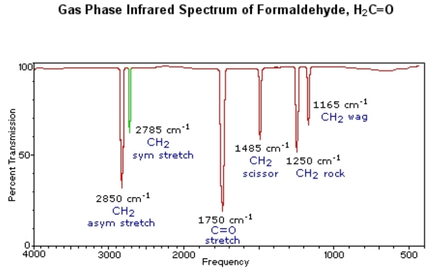
librations

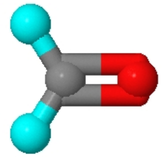
These are no vibrations! These are rotations!

Typical vibrational frequencies (wavenumbers)



Example: Formaldehyde



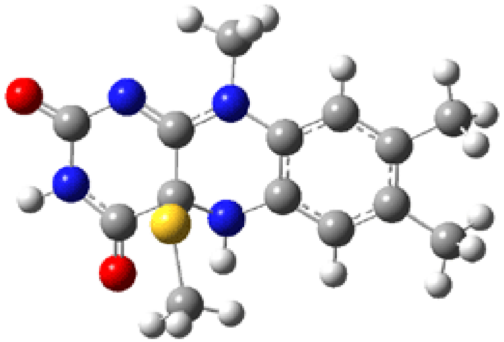


View CH_2 Asymmetric Stretch
View CH_2 Symmetric Stretch
View C=O Stretch
View CH_2 Scissoring
View CH_2 Rocking
View CH_2 Wagging

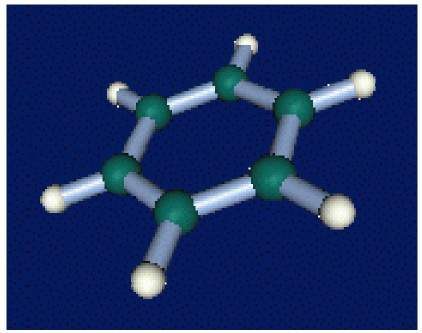
Ball&Stick Model
Spacefill Model
Stick Model
Motion Off

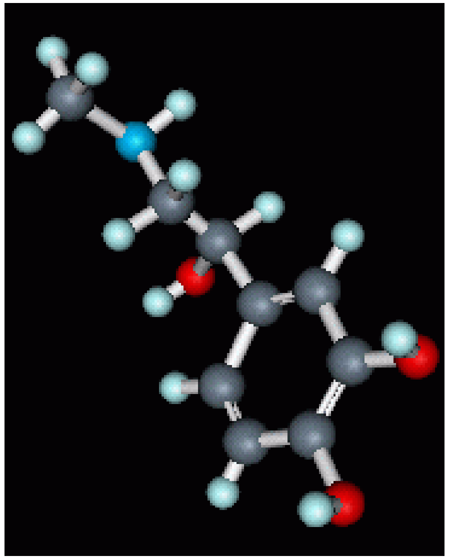
http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm

Flavin



Benzol



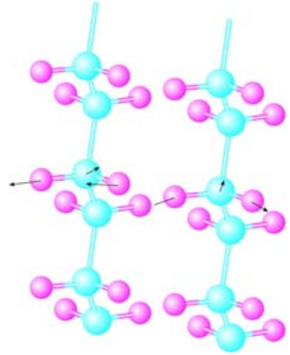


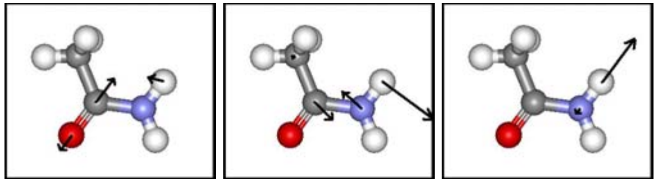
Vibrations of the macromoleclues

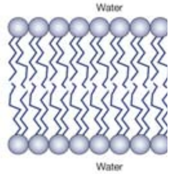
Complex global vibrations

Localised vibrations, e.g.:

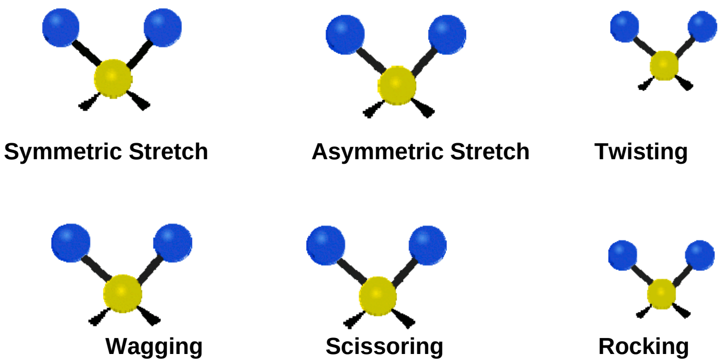
- CH_2 vibrations of the lipids
- amid vibrations of proteins (acetamide)







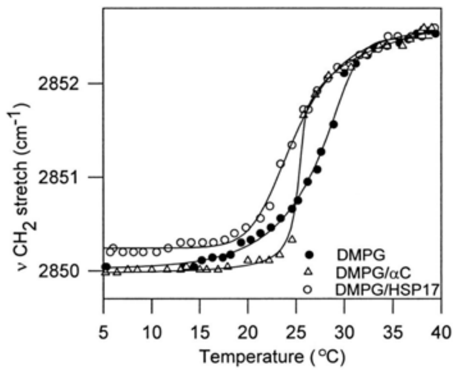
Lipids



Types of Vibrational Modes. Figure from Wikipedia

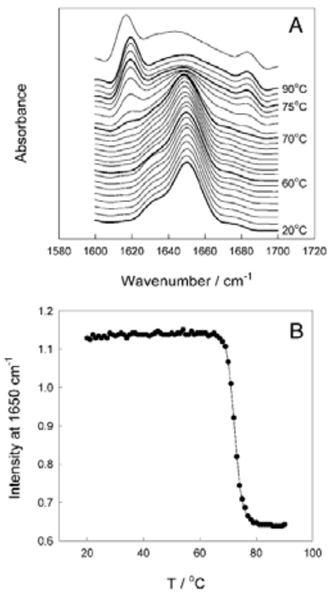
Applications

phase transitions in lipids



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

protein denaturation



Meersman et al. Biophys J.

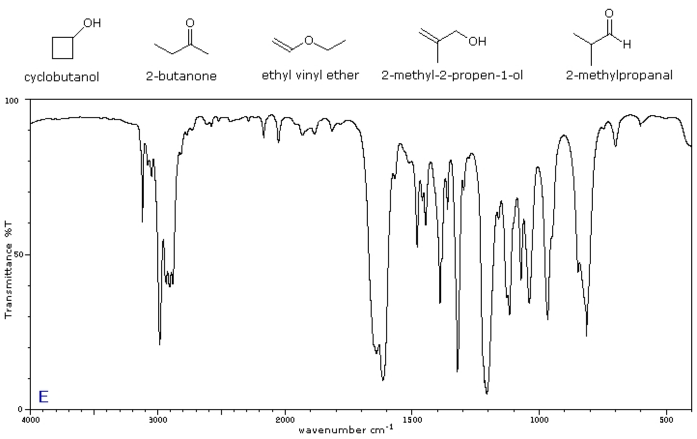
Pharmaceutical applications

- synthesis: identification of the intermediate 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

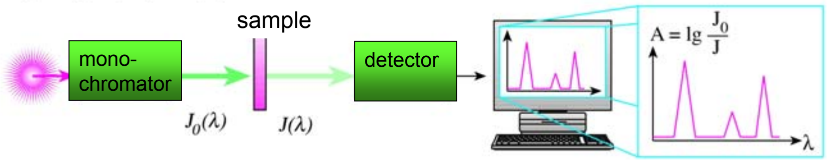
C_4H_8O



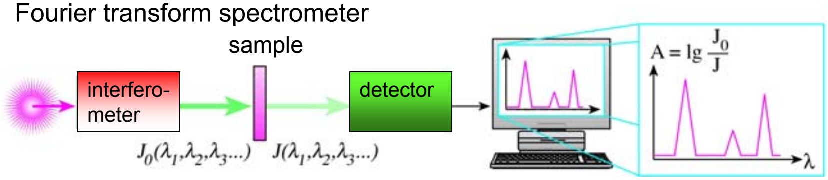
http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm

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

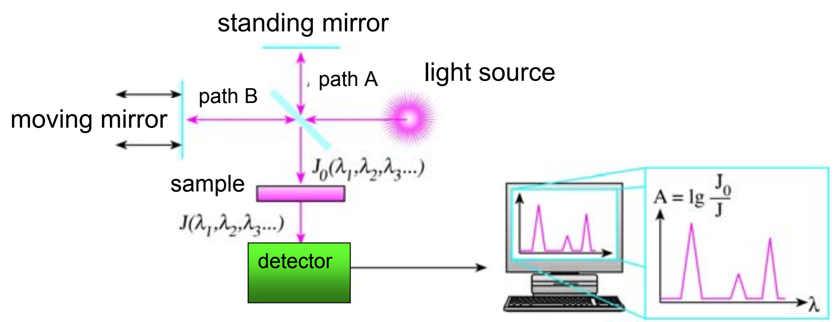
conventional (dispersion) spectrometer



Fourier transform spectrometer



book 6.17



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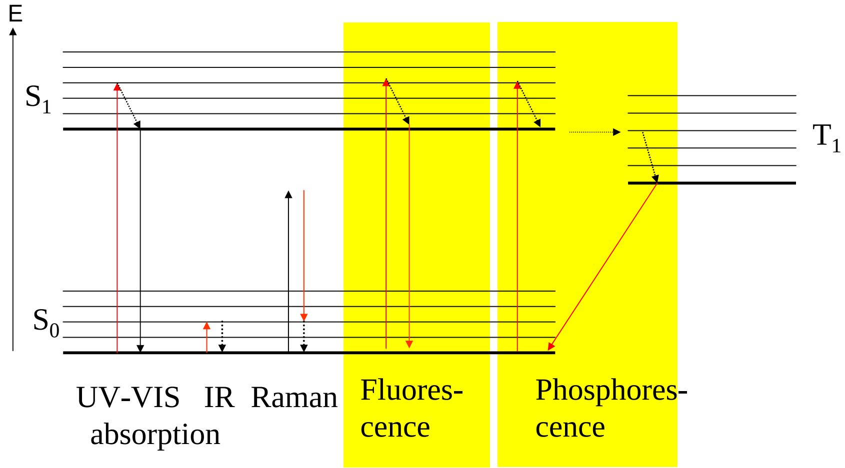
Luminescence spectroscopy



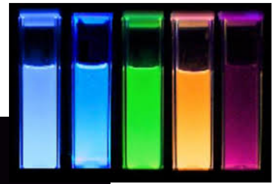
Which molecues are fluorescent?

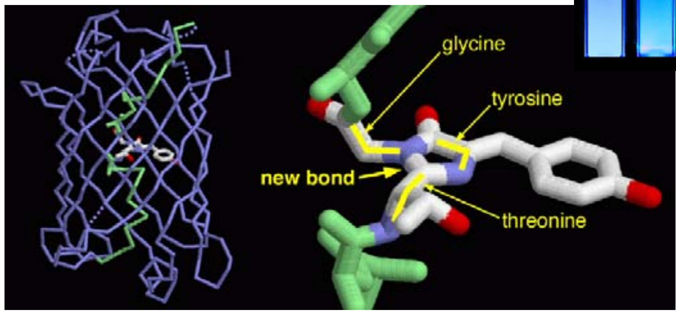
Amino acids (tryptophan, tyrosine, phenylalanine)

Fluorescent dyes

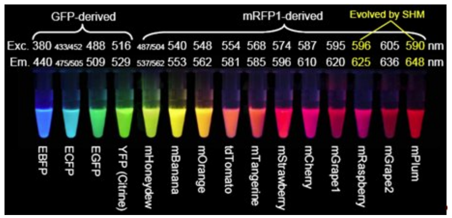
GFP

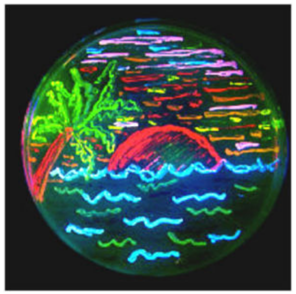
…





Aequorea victoria

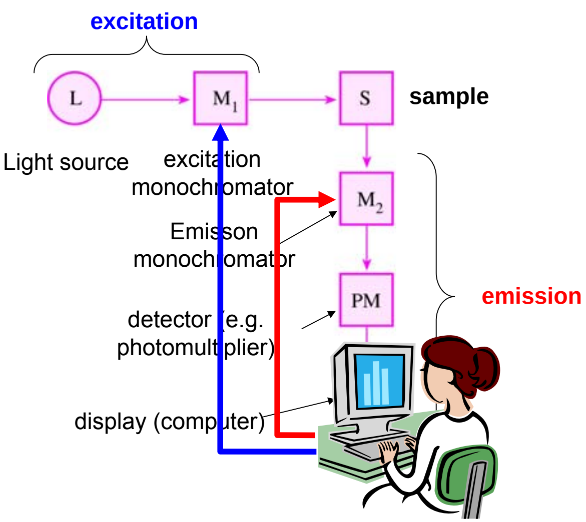




Measurable quantities in Fluorescence Spectroscopy

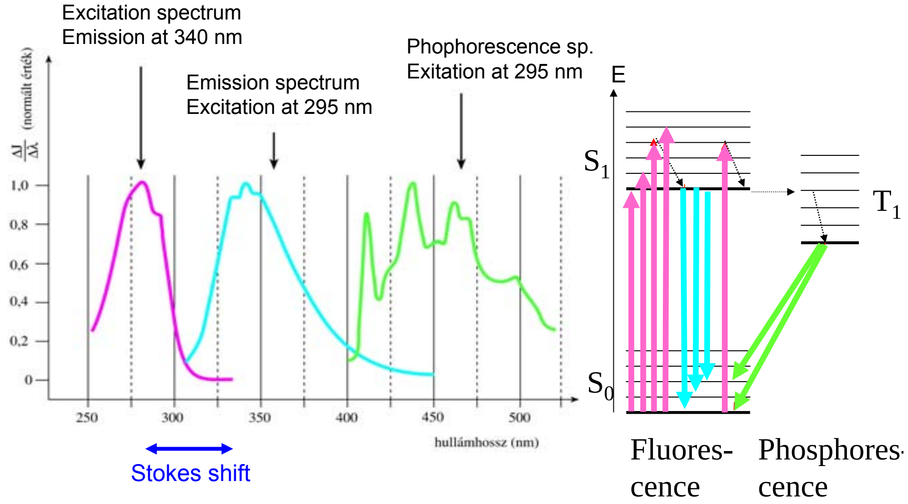
- Wavelength of the exciting light
- Wavelength of the emitted light (fluor., phosph.)
- Time dependence of the emitted light
- Polarisation of the emitted light
- Intensity of the emitted light

Scheme of the fluorescence spectrometer



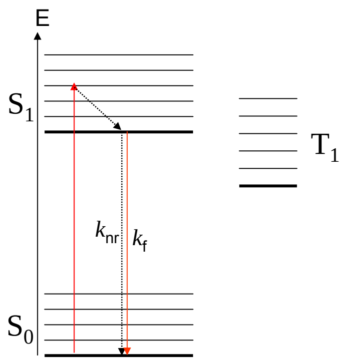
6.26

Excitation and emission spectra



6.25.

Fluorescence quantum yield(Q)



Quantum yield: Q =

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

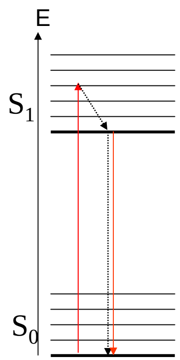
$$Q_f = \frac{k_f}{k_f + k_{nr}}$$

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

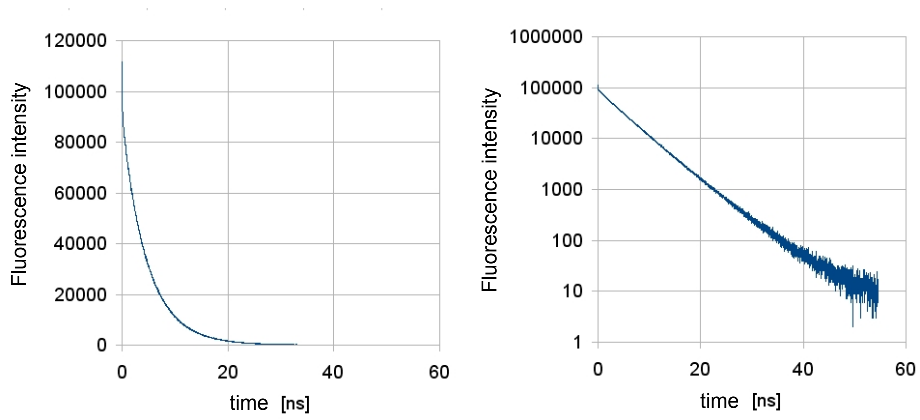
The number of emitted photons is proportional with ΔN, i.e. it is proportional also with N, which means it is decays exponentially with the decay constant of τ.

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:

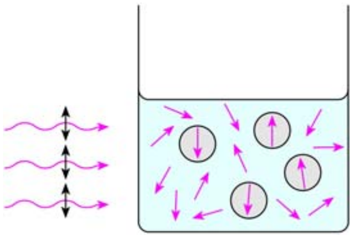
τ_{fluor} ns $\tau_{phosphor}$ μs ...s

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?)

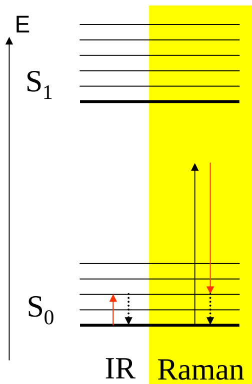
Ligth scattering

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

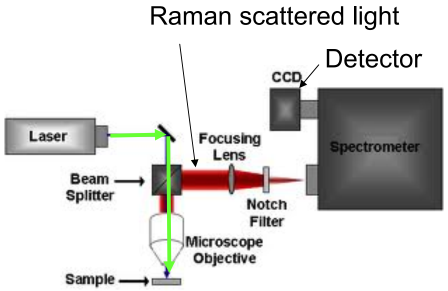
Raman
$\lambda_{scatt} \neq \lambda_{illum}$

Raman scattering:
$\lambda_{scatt} \neq \lambda_{illum}$ ⇨ $f_{scatt} \neq f_{illum}$
⇨ $E_{photon,scatt} \neq E_{photon,illum}$

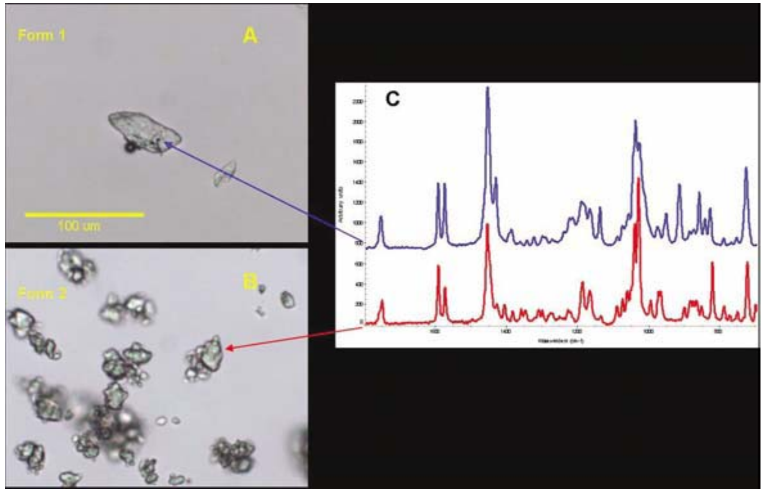
Where is the energy?
Excites vibrations of the molecule (cfr. IR)
very weak (~10^{-8})



Equipment



Pharmaceutical application



Rayleigh scattering

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

The scattered intensity: $$J_{scatt} \sim J_0 N \frac{a^6}{\lambda^4}$$

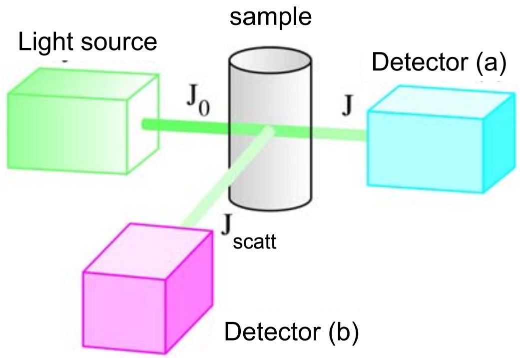
Information: size, contentration (quantity)

(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).