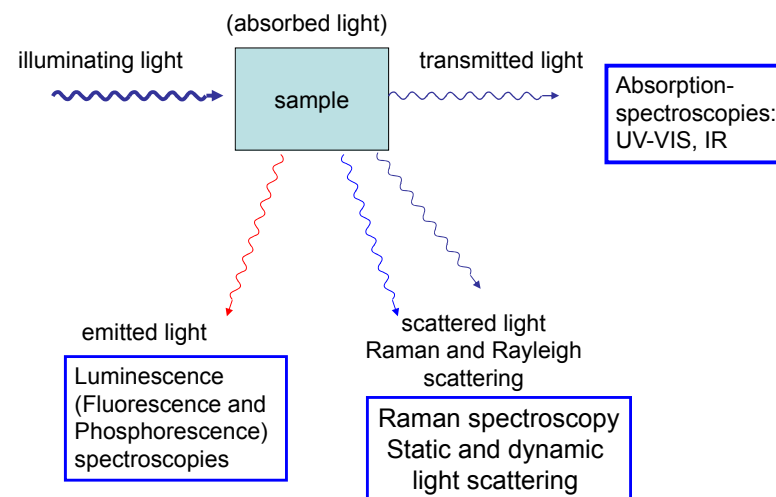


Optical spectroscopic techniques

László Smeller

What happens if a sample is illuminated by light?

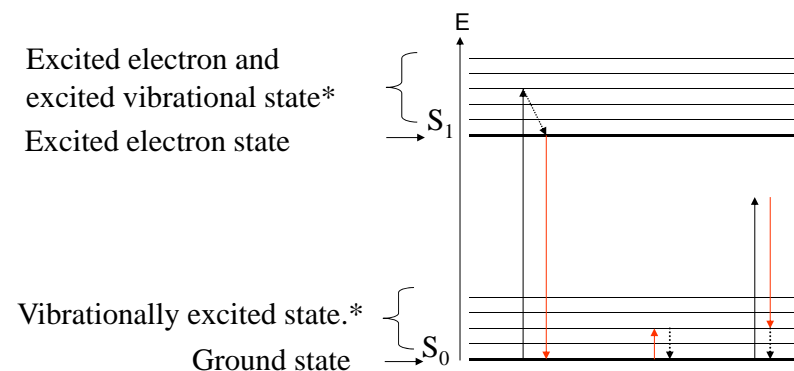


Spectroscopy (Absorption and emission spectroscopy)

- 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

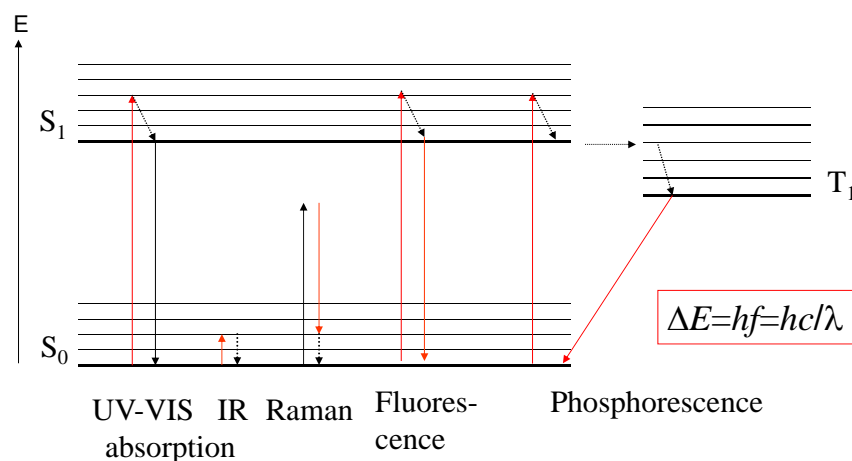
Why is light absorbed or emitted?

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 $\mu(\text{material}, c, \lambda)$

- Lambert-Beer law:

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

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

Infrared spectroscopy

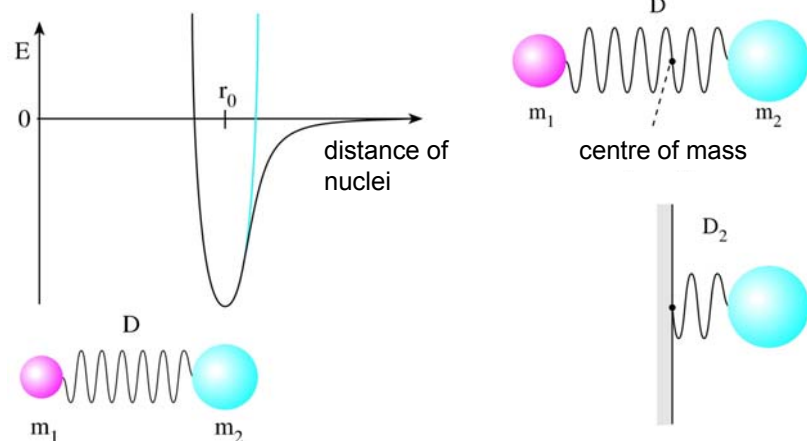
- Infrared light: $\lambda = 800 \text{ nm} - 1 \text{ mm}$
MIR (mid-infrared) : $2,5 - 50 \mu\text{m}$
- absorption spectroscopy
- the absorbed infrared radiation excite molecular vibrations
- very specific for the structure of the molecule
- special method for detection:
FT spectrometer

Molecular vibrations

The electrons are light ($m_e \ll m_{\text{nucleus}}$), 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:

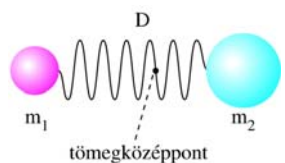
$$f = \frac{1}{2\pi} \sqrt{\frac{D_2}{m_2}}$$

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

$$\begin{aligned} \frac{D_2}{D} &= \frac{F/D}{F/D_2} = \frac{\Delta\ell}{\Delta\ell_2} = \frac{\ell}{\ell_2} = \\ &= \frac{\ell_1 + \ell_2}{\ell_2} = \frac{\ell_1}{\ell_2} + 1 = \frac{m_2}{m_1} + 1 = \frac{m_1 + m_2}{m_1} \end{aligned}$$

$F = D\Delta\ell$

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

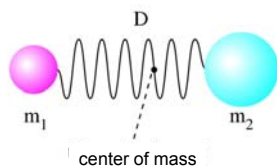
The measured wavenumber: $\nu = 2143 \text{ cm}^{-1}$

$$\left. \begin{aligned} \Rightarrow \lambda &= 4,67 \mu\text{m} \Rightarrow f = 6,43 \cdot 10^{13} \text{ Hz} \\ m_C &= 2 \cdot 10^{-26} \text{ kg}, m_O = 2,7 \cdot 10^{-26} \text{ kg} \end{aligned} \right\} \Rightarrow D = 1875 \text{ N/m}$$

if ν is known, D can be calculated
if D is known, ν can be calculated

Classical vs. quantum physics

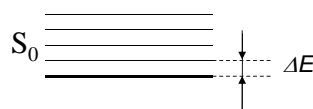
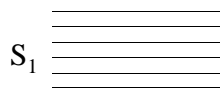
Classical physical picture



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

resonance with the light with frequency f

Quantum mechanical picture



$$\Delta E = hf$$

=

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

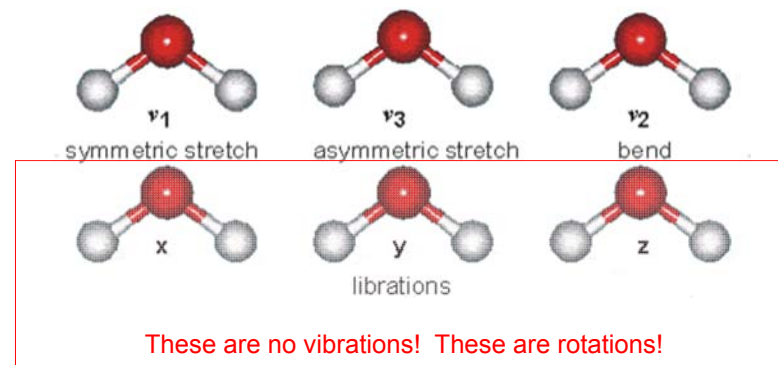
All the atoms vibrate

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

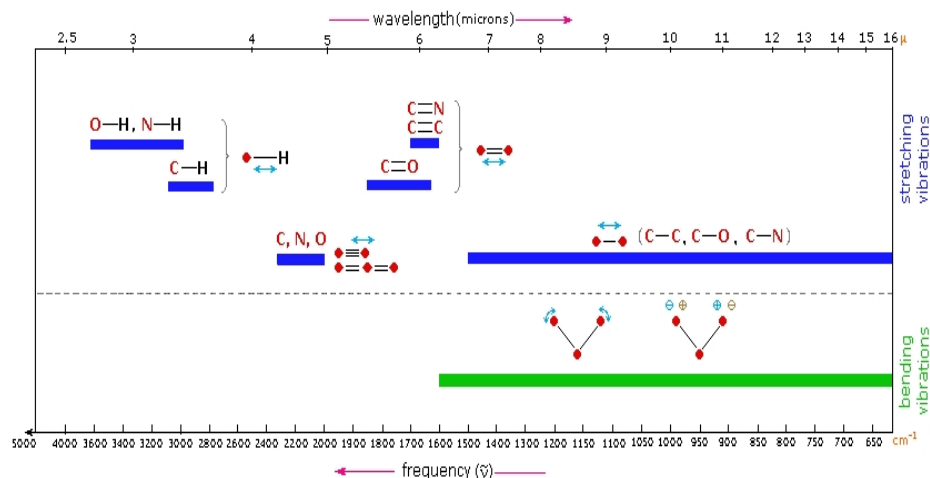
Example: water



Normal vibrations of water

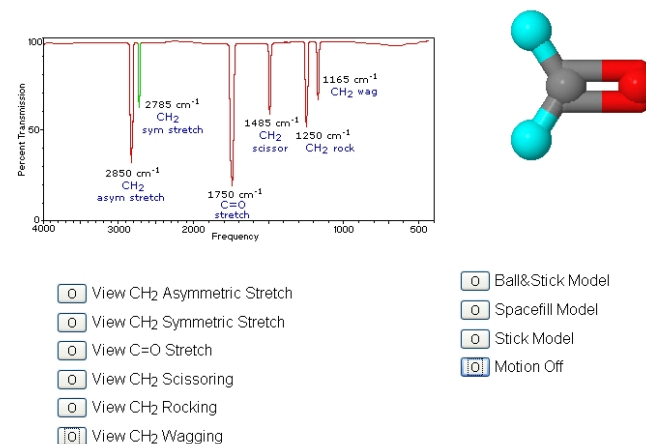


Typical vibrational frequencies (wavenumbers)



Example: Formaldehyde

Gas Phase Infrared Spectrum of Formaldehyde, $\text{H}_2\text{C=O}$

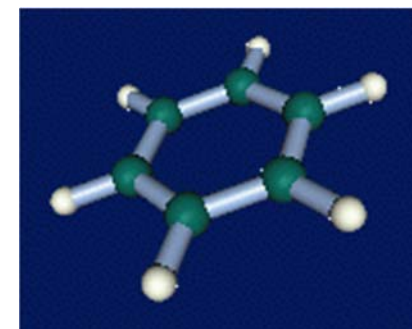


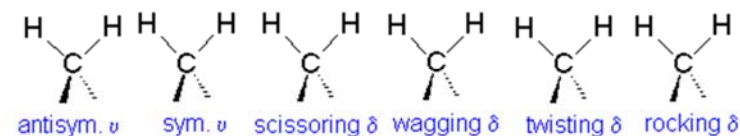
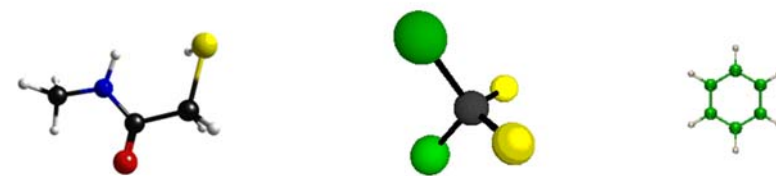
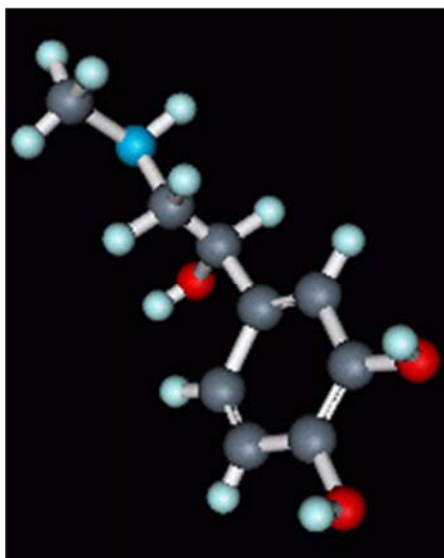
<http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm>

Flavin



Benzol



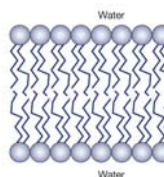
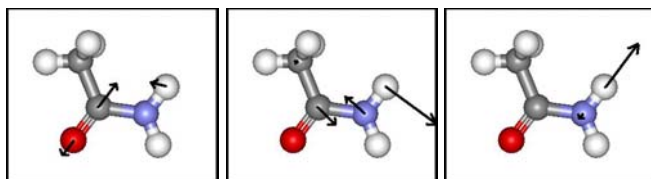
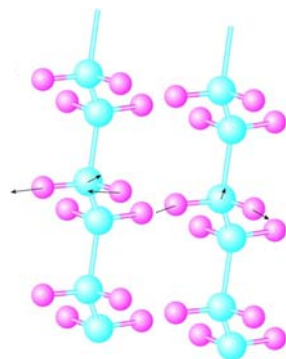


Vibrations of the macromolecules

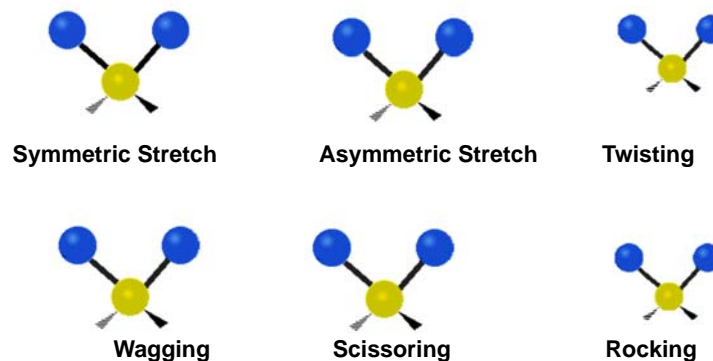
Complex global vibrations

Localised vibrations, e.g.:

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

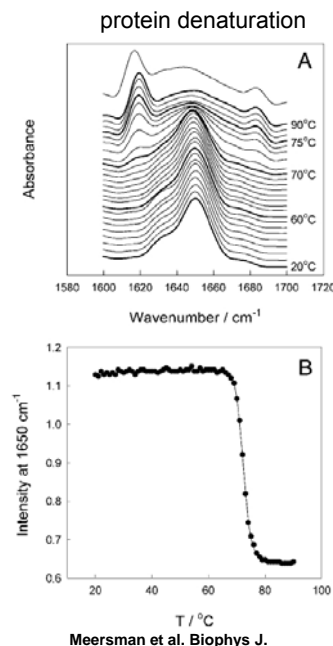
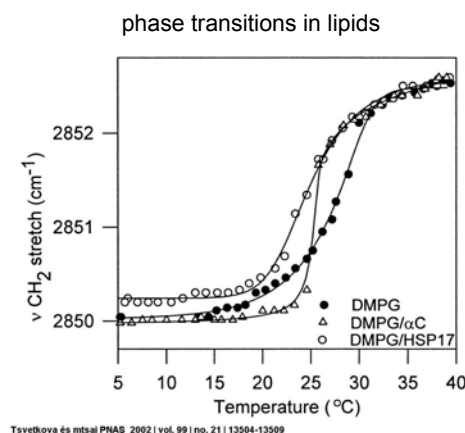


Lipids



Types of Vibrational Modes. Figure from Wikipedia

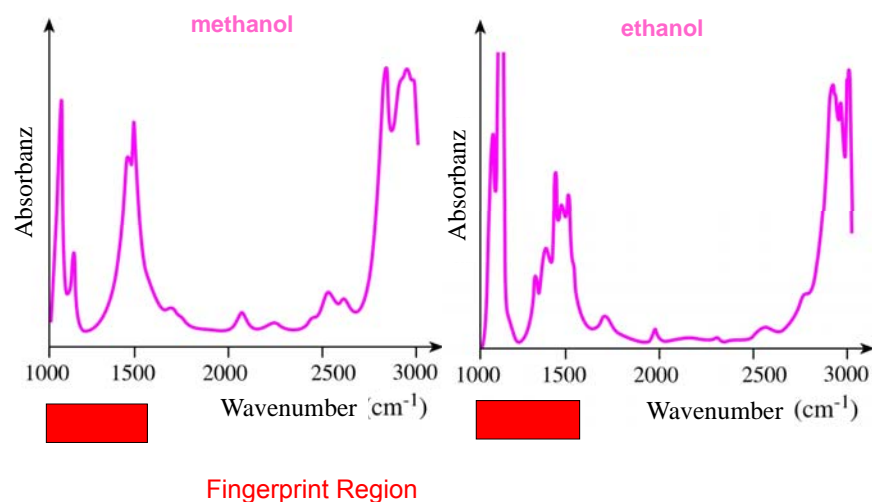
Applications



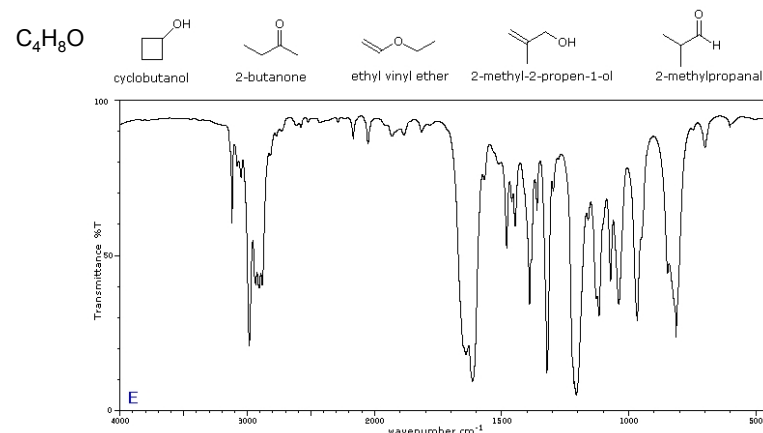
Pharmaceutical applications

- 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

Identification of the Molecules

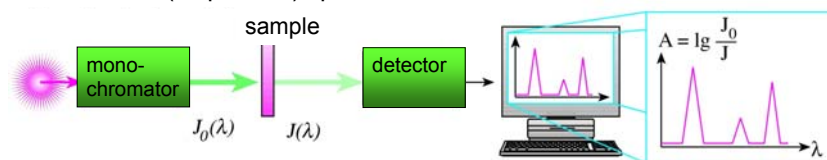


Example: Identification of molecules

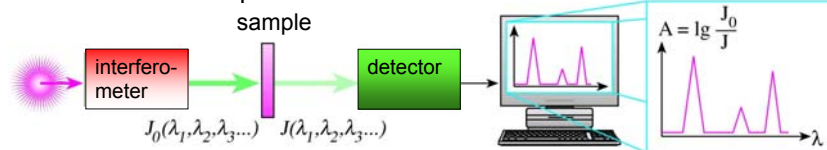


The technique of the measurement : Fourier transform spectrometer (FTIR)

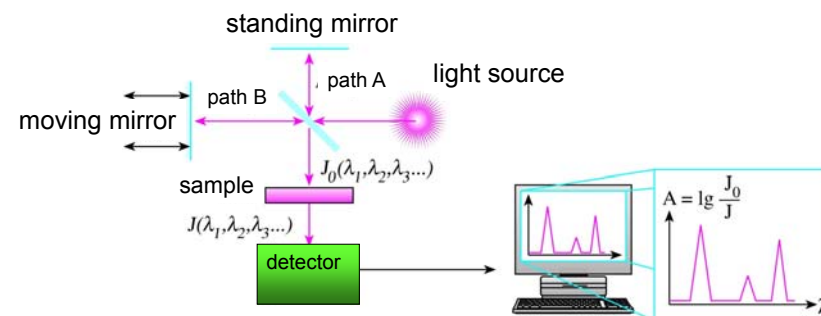
conventional (dispersion) spectrometer



Fourier transform spectrometer

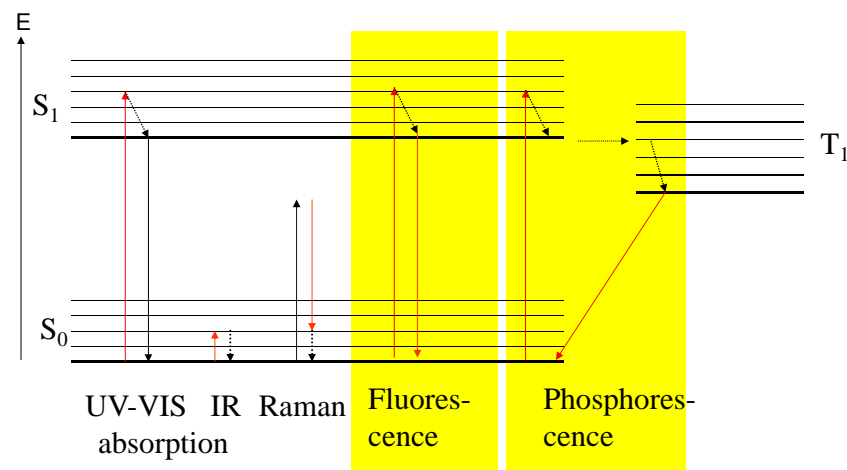


book 6.17



book 6.18

Luminescence spectroscopy



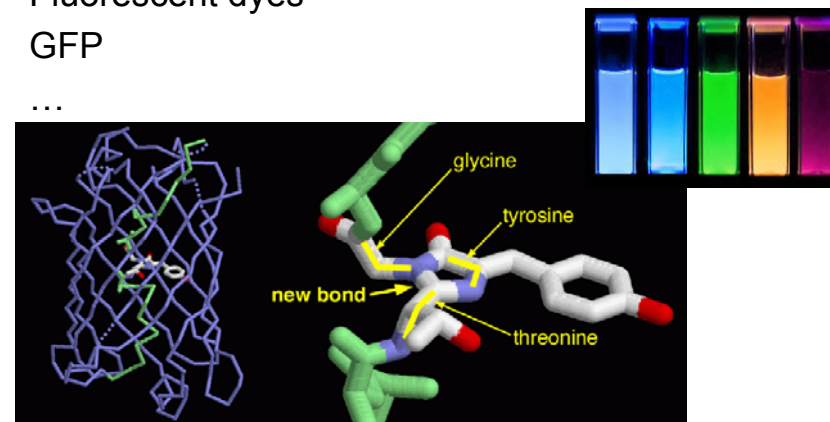
Which molecules are fluorescent?

Amino acids (tryptophan, tyrosine, phenylalanine)

Fluorescent dyes

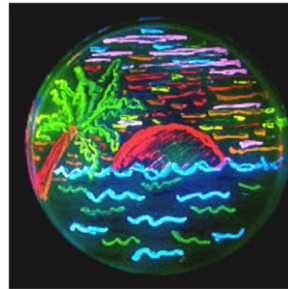
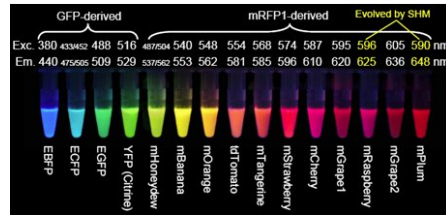
GFP

...





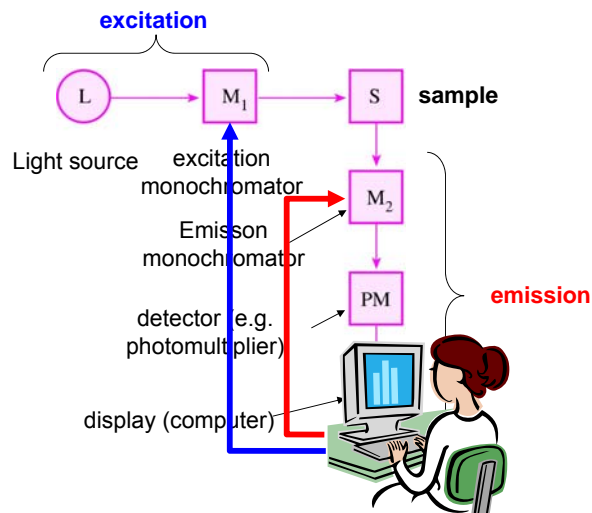
Aequorea victoria



Measurable quantities in Fluorescence Spectroscopy

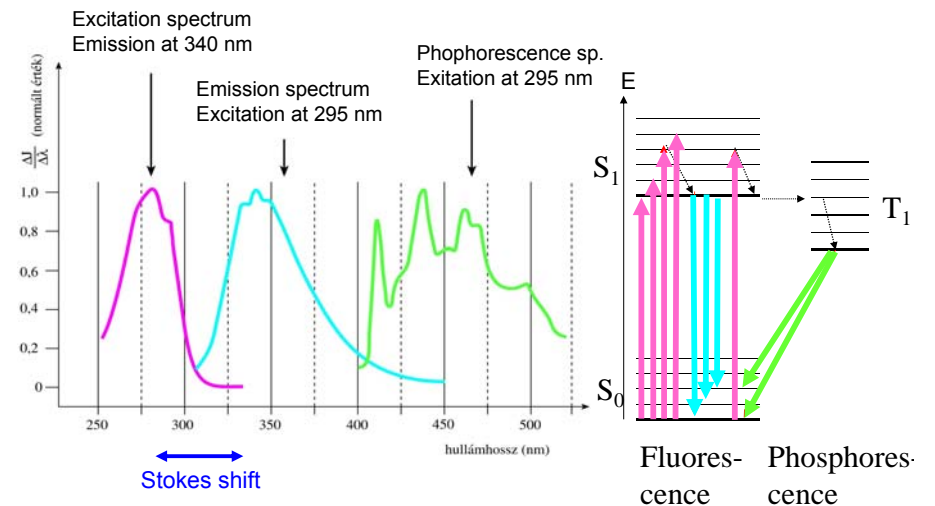
- Wavelength of the exciting light
- Wavelength of the emitted light (fluor., phosph.)
- Time dependence of the emitted light
- Polarisation of the emitted light
- Intensity of the emitted light

Scheme of the fluorescence spectrometer



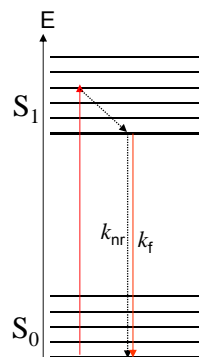
6.26

Excitation and emission spectra



6.25.

Fluorescence quantum yield(Q)



Quantum yield: $Q =$
 $= \frac{\text{number of emitted photons}}{\text{number of absorbed photons}}$

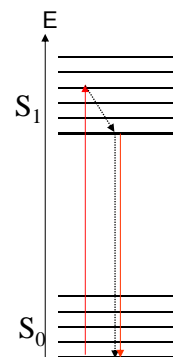
$$Q_f = \frac{k_f}{k_f + k_{nr}}$$

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

k_{nr} probability of nonradiating
 transition

dyes, fl. markers $Q \approx 1$

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 decays exponentially with the decay
 constant of τ .

How to measure?

-Pulsed illumination (flashlamp, or pulse laser)

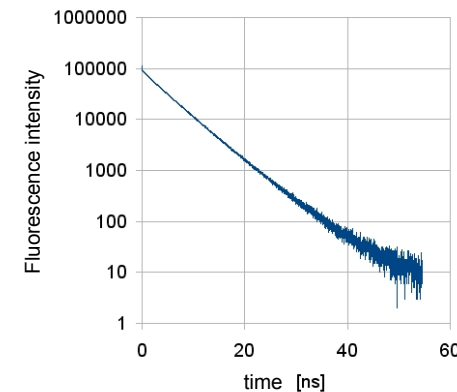
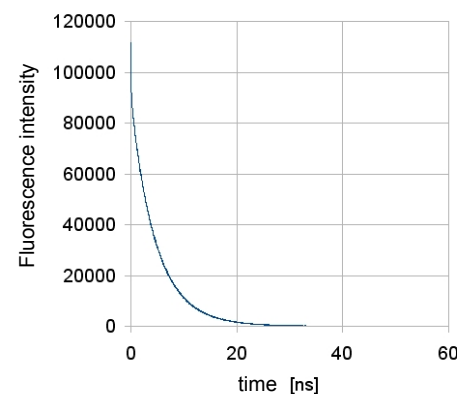
-Photon counting as function of time

Quantum yield and life time can be also defined
 for phosphorescence, using similar definitions.

Typical lifetimes:

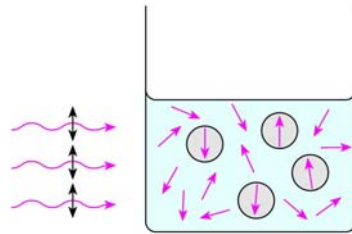
τ_{fluor} ns τ_{phosphor} $\mu\text{s} \dots \text{s}$

Example: tryptophan



Fluorescence polarization

illumination with
polarized light



polarization degree of the emitted light is measured
The fluorescent molecule can rotate between the
absorption and the emission \Rightarrow
dynamic information: rotational correlation time
(how fast is the rotational diffusion?)

6.28

Ligh scattering

Rayleigh

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

Raman

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



Chandrasekhra
Venkata Raman

Raman scattering:

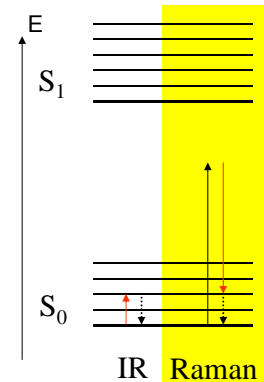
$$\lambda_{\text{scatt}} \neq \lambda_{\text{illum}} \Rightarrow f_{\text{scatt}} \neq f_{\text{illum}}$$

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

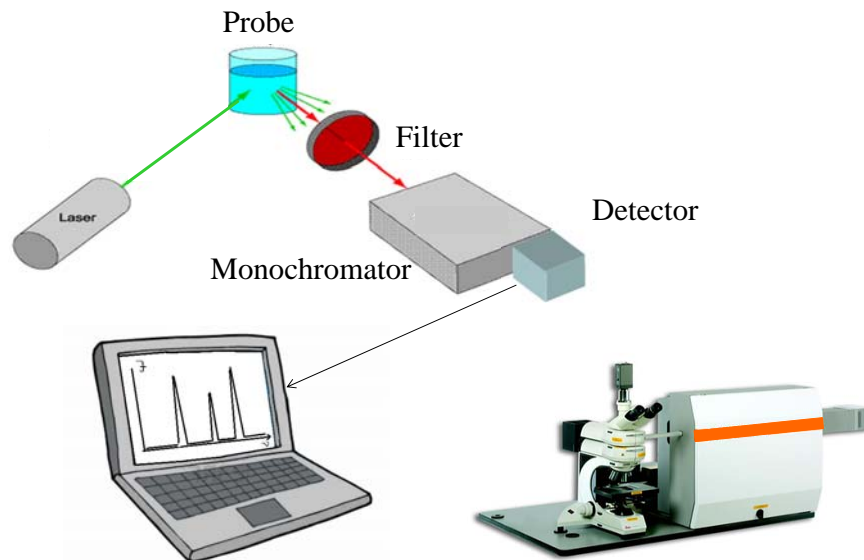
Where is the energy?

Excites vibrations of the
molecule (cfr. IR)

very weak ($\sim 10^{-8}$)



Raman Spectrometer



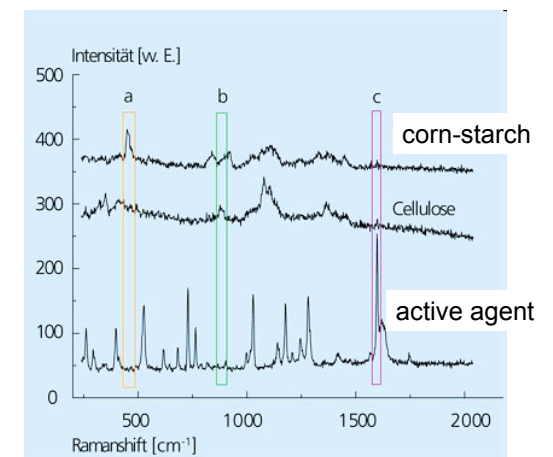
Raman-Scattering

Vibrations are
specific for the
molecules

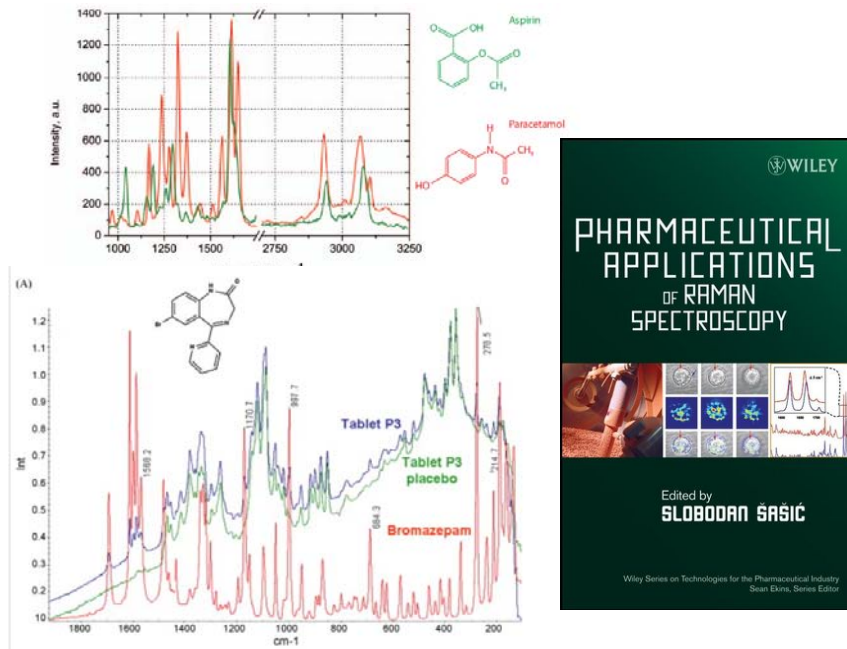


Raman
Spectroscopy

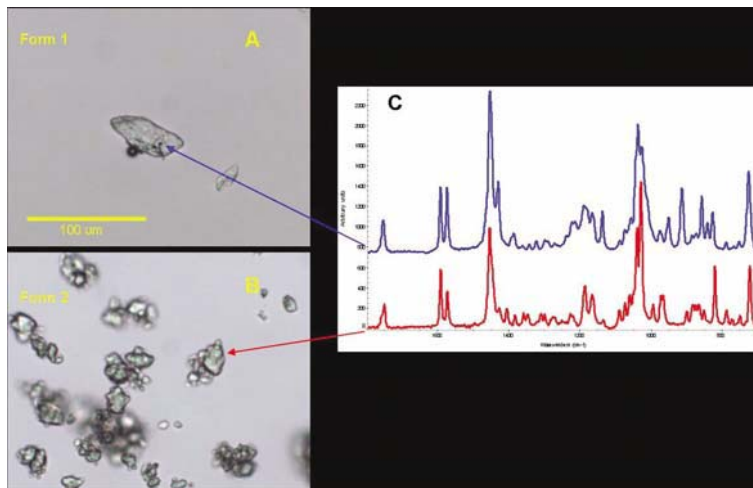
E.g.: active agent
content of a tablet



Handheld Raman spectrometer for identification of materi



Pharmaceutical application



Rayleigh scattering

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

The scattered intensity:

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

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

Measurement of the Rayleigh scattering

if $J_{\text{scatt}} \ll J_0$

J_{scatt} is measured
(Nephelometry)

If $J_{\text{scatt}} \approx J_0$

J is measured
(turbidimetry)

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

