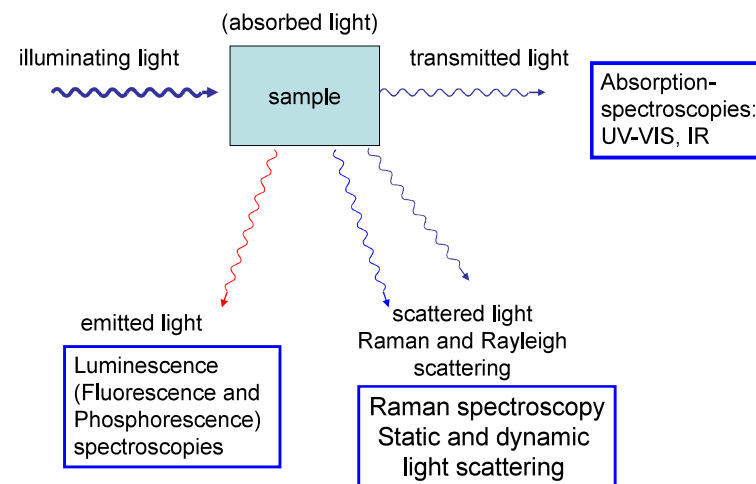


Optical spectroscopic techniques

László Smeller

What happens if a sample is illuminated by light?



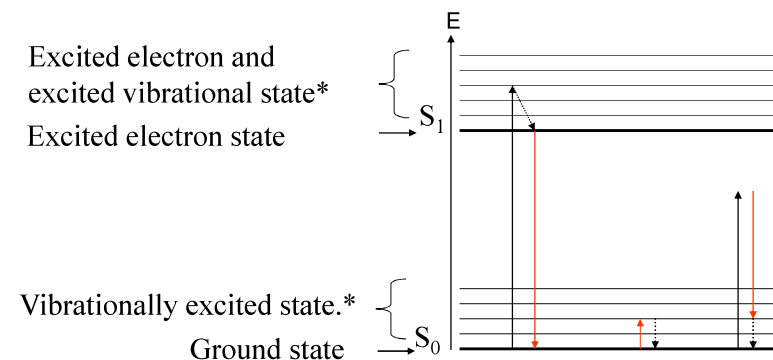
Spectroscopy

(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

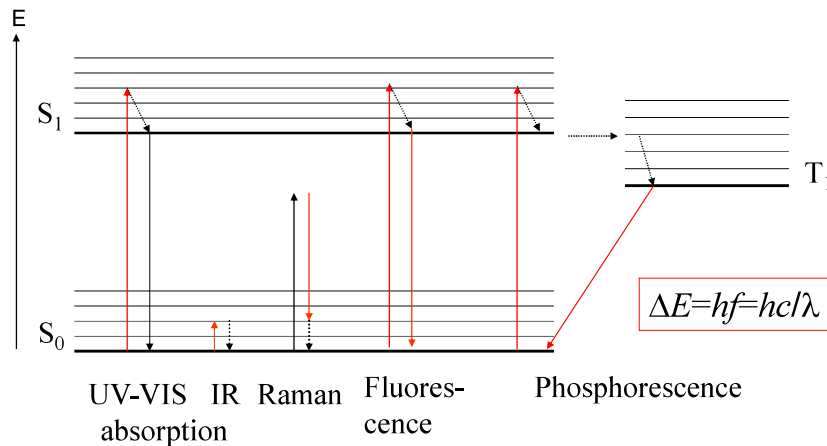
Why is light absorbed or emitted?

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

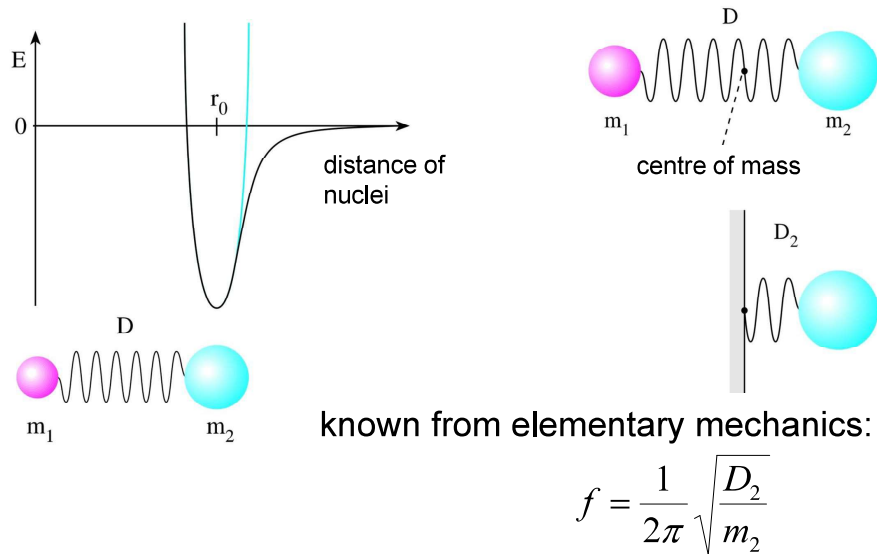
- Infrared light: $\lambda = 800 \text{ nm} - 1 \text{ mm}$
MIR (mid-infrared) : $2,5 - 50 \mu\text{m}$
- absorption spectroscopy
- the absorbed infrared radiation excites molecular vibrations
- very specific for the structure of the molecule
- special method for detection:
FT spectrometer

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:



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

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

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

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

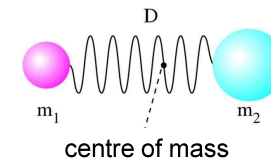
Example: CO

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

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

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

Reduced mass: $m_{red} = \frac{m_1 m_2}{m_1 + m_2}$



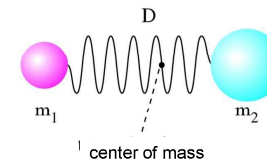
Frequency of the vibration with the reduced mass:

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

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

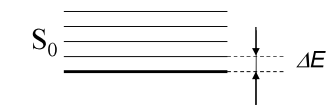
Classical vs. quantum physics

Classical physical picture



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

Quantum mechanical picture



resonance with the light with frequency f

$\Delta E = hf$

Vibrations of the large molecules

Molecule consisting of N atoms:

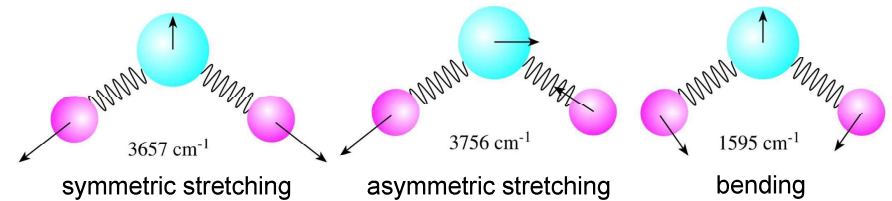
- $3N$ degree of freedom,
3-3 are the rotations and translations of the whole molecule
- $3N-6$ vibrational degree of freedom ($3N-5$ for the linear molecules)
- $3N-6$ independent normal vibrations

Normal vibrations

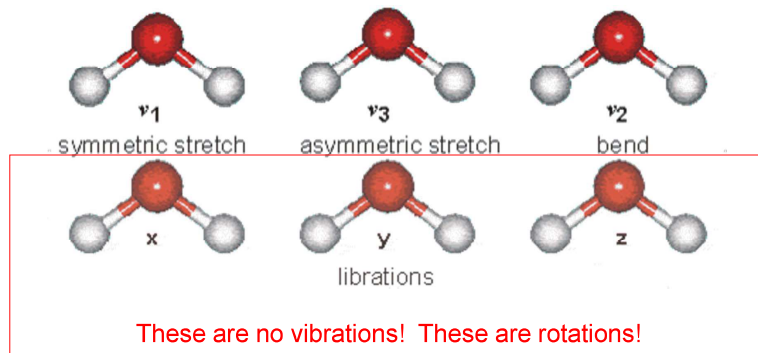
All the atoms vibrate

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

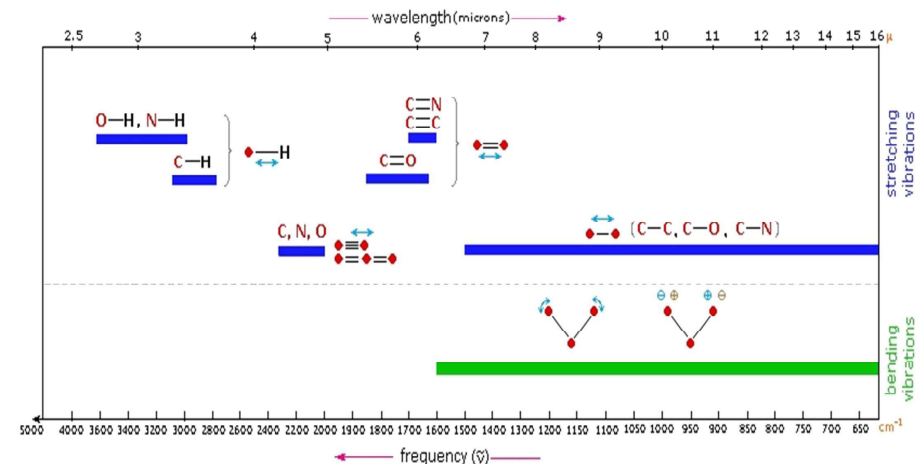
Example: water



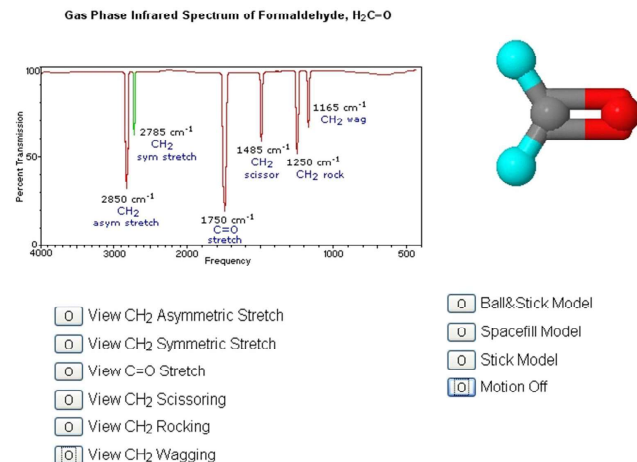
Normal vibrations of water



Typical vibrational frequencies (wavenumbers)



Example: Formaldehyde



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

See also: <https://www.youtube.com/watch?v=TmYXML3PenM>

For further examples of molecular vibrations see:

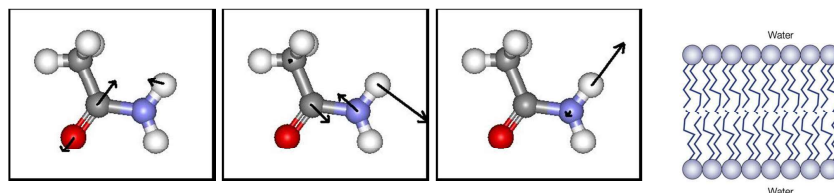
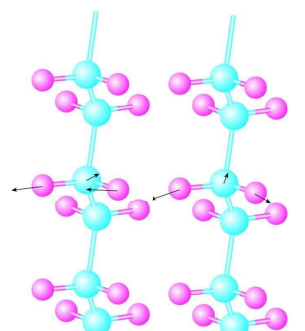
- Water:
<https://www.youtube.com/watch?v=1uE2lvkKW0>
- CO_2 :
<https://www.youtube.com/watch?v=W5gimZIFY6I>
- Ammonia:
<https://www.youtube.com/watch?v=aSiJ2bt1jwQ>
- Ethanol:
<https://www.youtube.com/watch?v=O5dulWd-OnQ>
- Benzene:
<https://www.youtube.com/watch?v=NA9etutSt7A>
- ...

Vibrations of the macromolecules

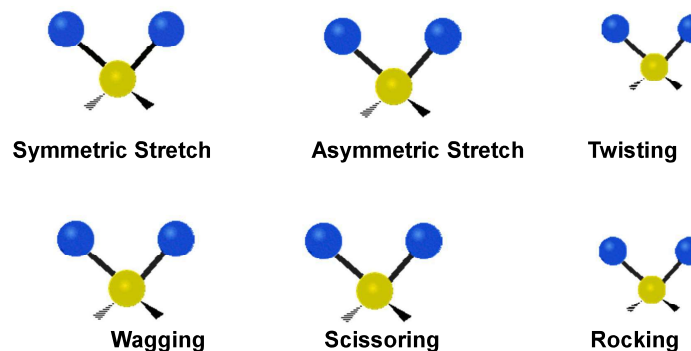
Complex global vibrations

Localised vibrations, e.g.:

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

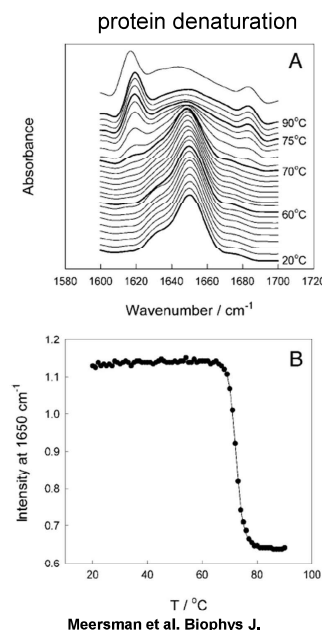
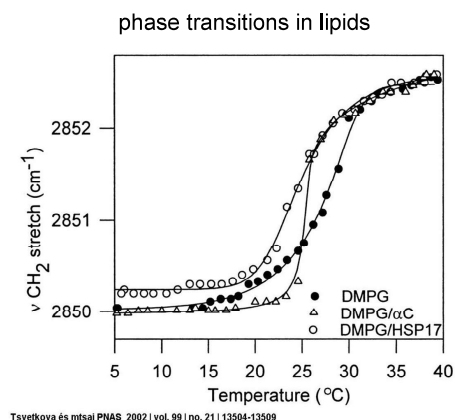


Lipids



Types of Vibrational Modes. Figure from Wikipedia

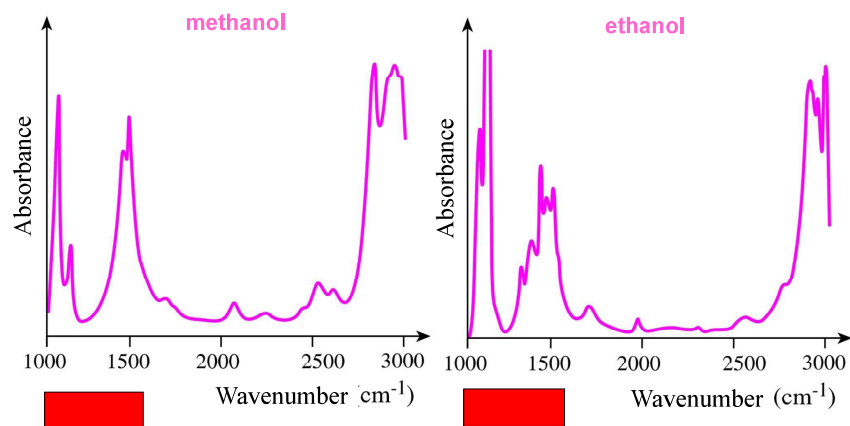
Applications



Pharmaceutical applications

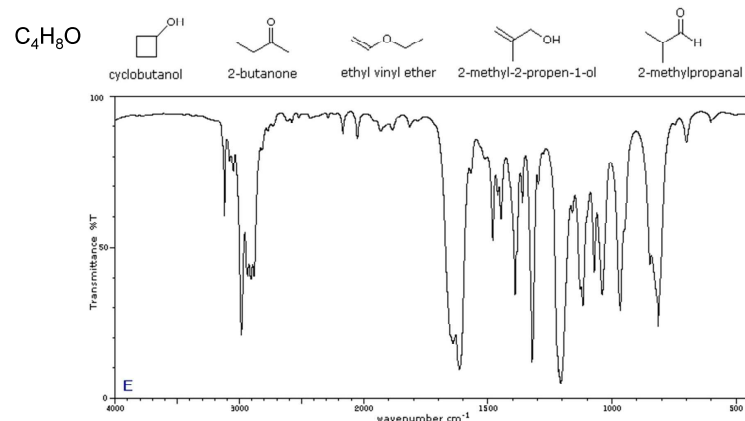
- synthesis: identification of the intermediate and the end product
 - determination and justification of the molecular structure
 - detection of the metabolites
 - quality control (purity)
- Remark.: Lambert-Beer law is valid, determination of concentration is possible

Identification of the Molecules



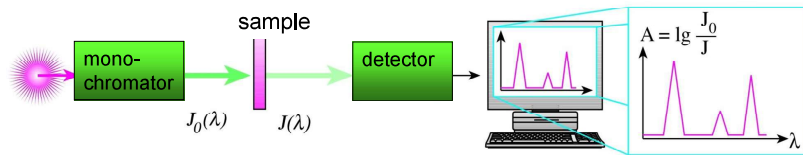
Fingerprint Region

Example: Identification of molecules

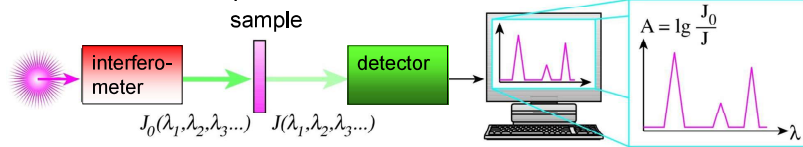


The technique of the measurement : Fourier transform spectrometer (FTIR)

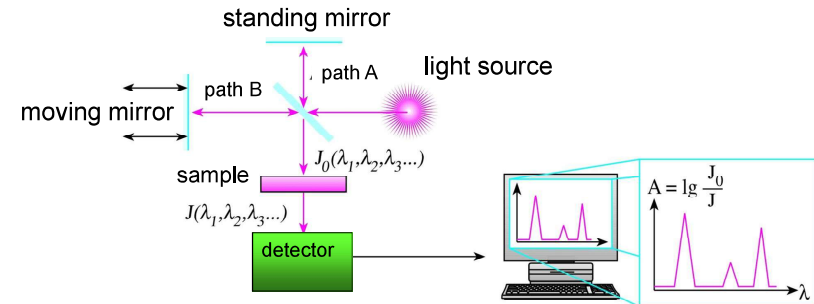
conventional (dispersion) spectrometer



Fourier transform spectrometer

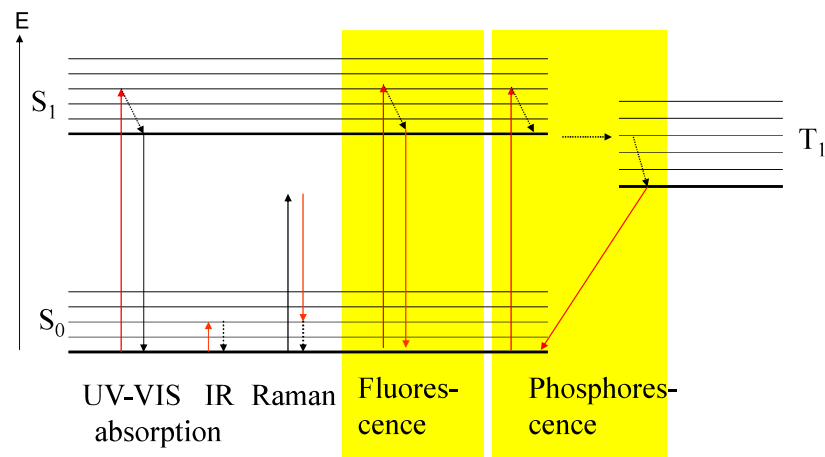


book 6.17



book 6.18

Luminescence spectroscopy

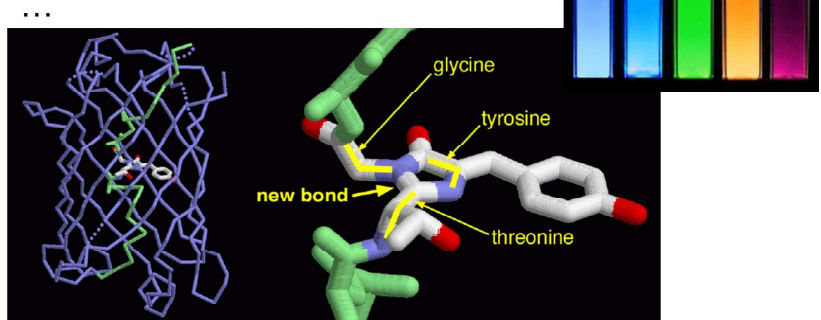


Which molecules are fluorescent?

Amino acids (tryptophan, tyrosine, phenylalanine)

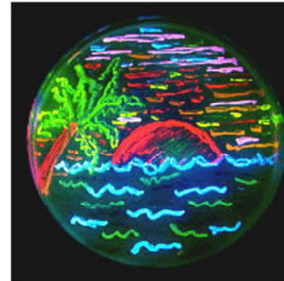
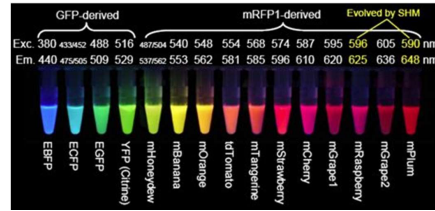
Fluorescent dyes

GFP





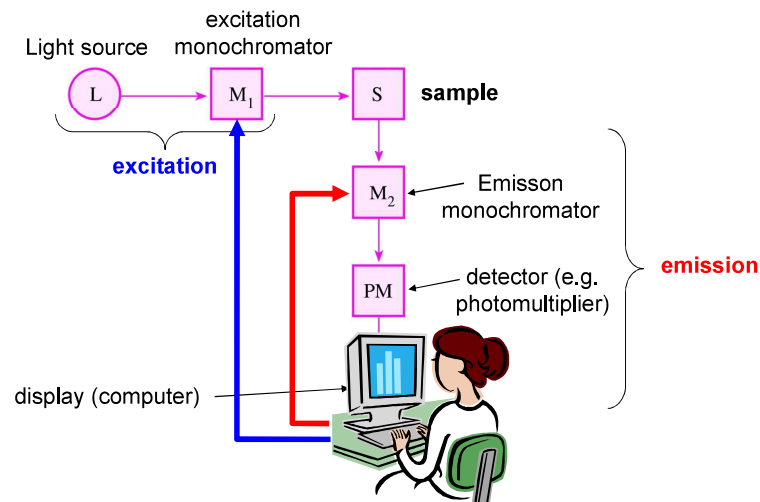
Aequorea victoria



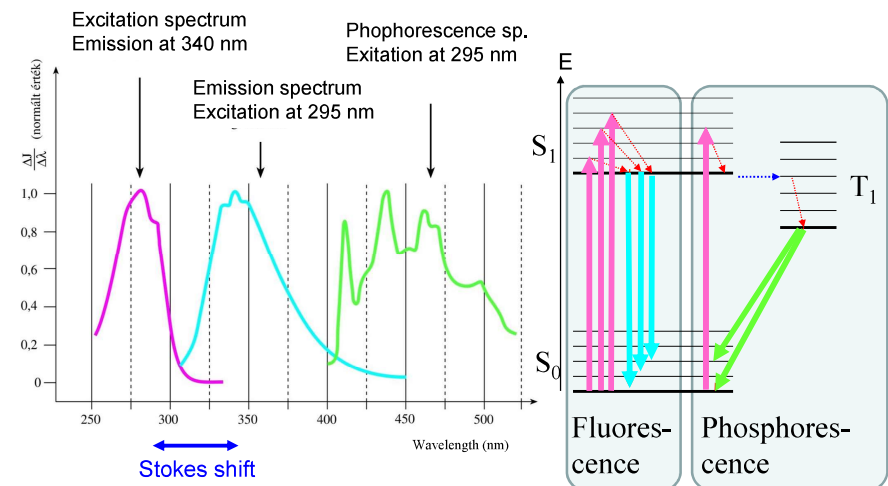
Measurable quantities in Fluorescence Spectroscopy

- 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





Excitation and emission spectra

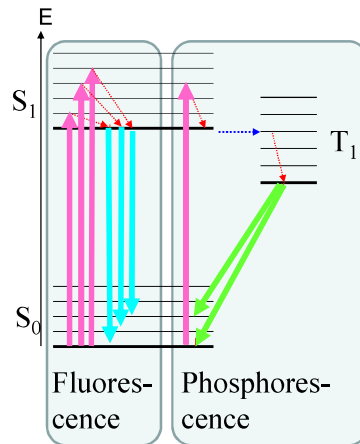


Kasha's rule

Kasha's rule:
the excited molecule first reaches the lowest vibrational level and photon emission occurs always from this state to any vibrational level of the ground (S_0) state.

 Vibrational relaxation (c.a. 10^{-12} s)

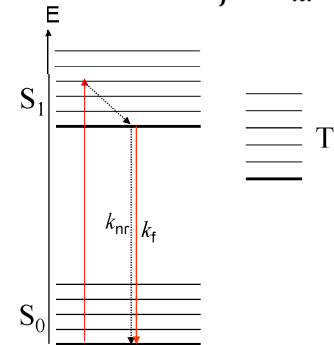
 Intersystem crossing



Fluorescence quantum yield(Q)

Quantum yield (Q_F):

$$Q_F = \frac{k_f}{k_f + k_{nr}} = \frac{\text{number of photons emitted}}{\text{number of photons absorbed}}$$



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

k_{nr} probability of nonradiating transition

For dyes, fl. markers $Q \approx 1$

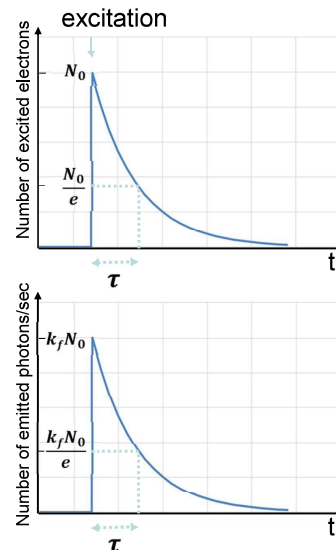
Luminescence lifetime

$$N = N_0 e^{-\frac{t}{\tau}}$$

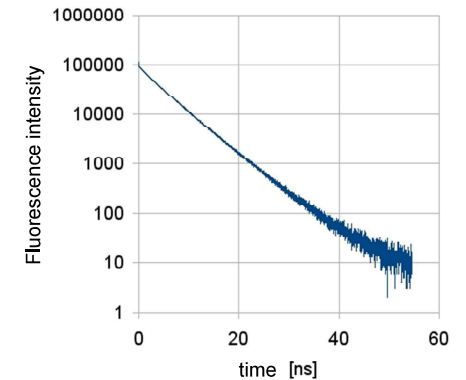
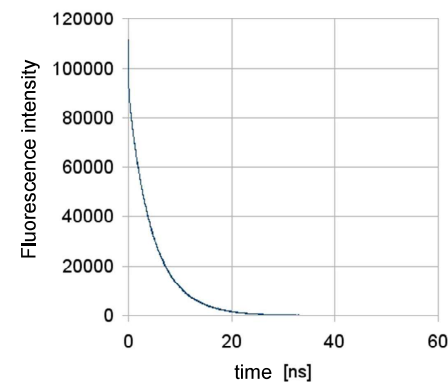
$$\tau = \frac{1}{k_f + k_{nr}}$$

k_f : Rate of photon producing transitions

k_{nr} : Rate of non-radiative transitions

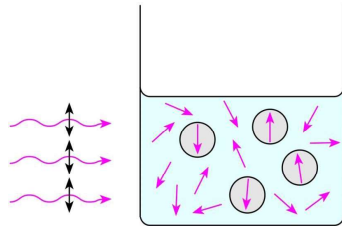


Example: tryptophan



Fluorescence polarization

illumination with
polarized light



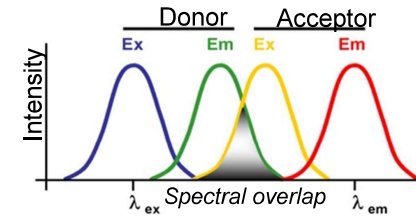
polarization degree of the emitted light is measured
The fluorescent molecule can rotate between the
absorption and the emission \Rightarrow
dynamic information: rotational correlation time
(how fast is the rotational diffusion?)

6.28

FRET

Förster Resonance Energy Transfer

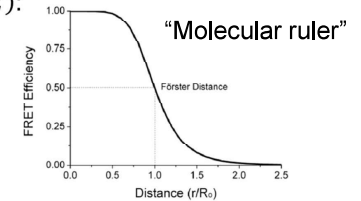
Energy transfers from donor without emission to acceptor in dipole-dipole interactions. Requires spectral overlap between donor emission and acceptor absorption.



FRET efficiency or quantum yield (E):

$$E = \frac{1}{1 + (r/r_0)^6}$$

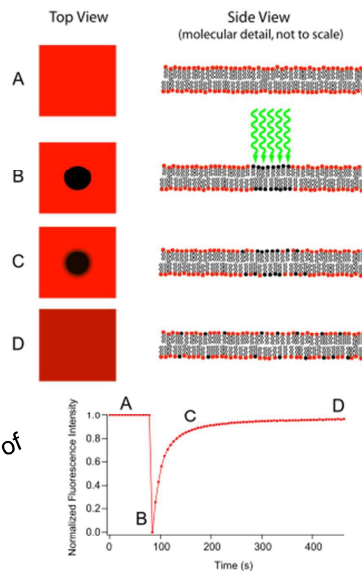
r_0 : Förster distance



FRAP

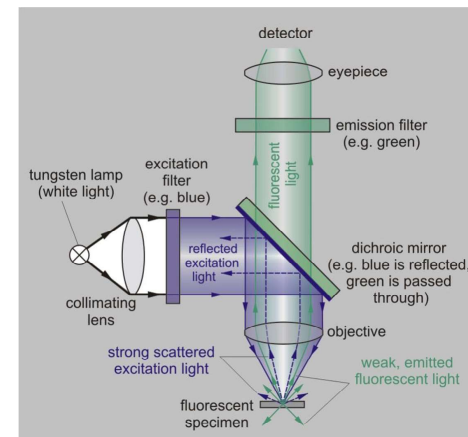
Fluorescence Recovery After
Photobleaching

Photobleaching:
The permanent loss of
fluorescence due to photochemical
reactions.



Lateral diffusion of
lipid molecules

Fluorescence microscope



Ligth scattering

Rayleigh

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

Raman

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



Raman scattering:

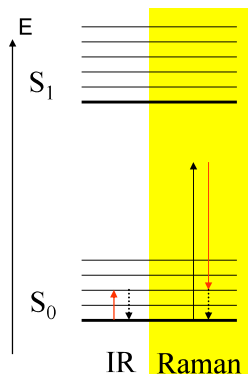
$$\lambda_{\text{scatt}} \neq \lambda_{\text{illum}} \Rightarrow f_{\text{scatt}} \neq f_{\text{illum}}$$

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

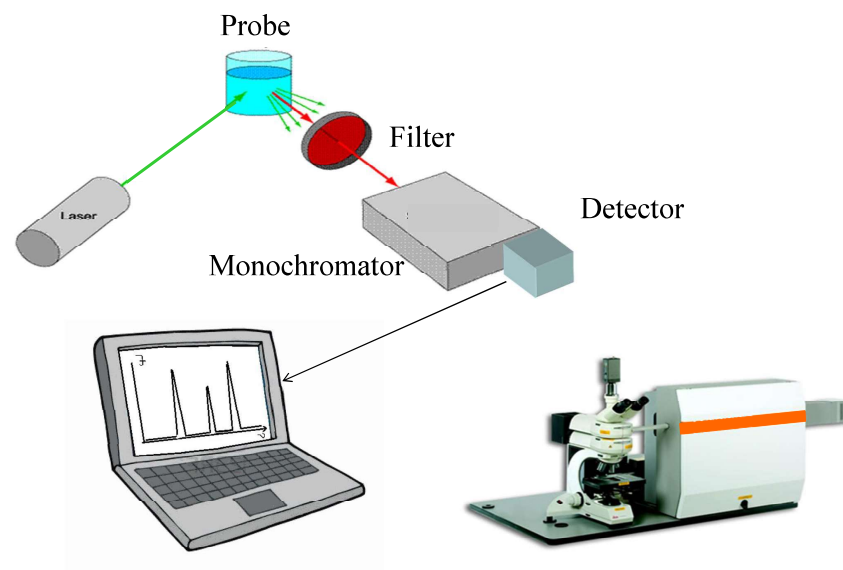
Where is the energy?

Excites vibrations of the molecule (cfr. IR)

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



Raman Spectrometer



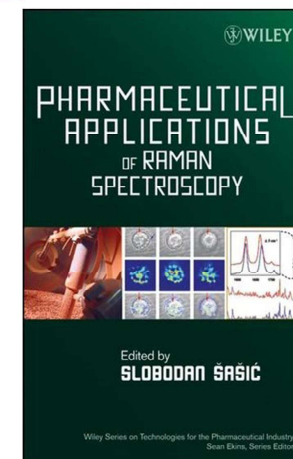
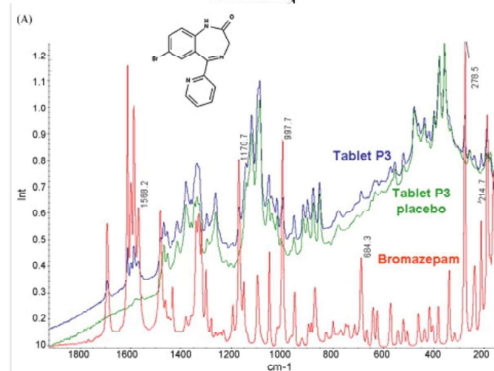
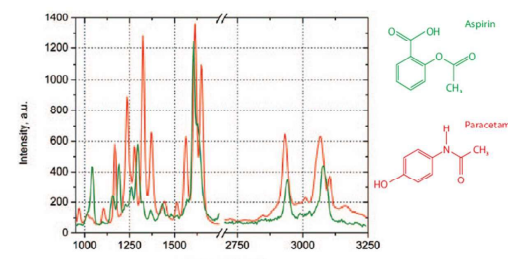
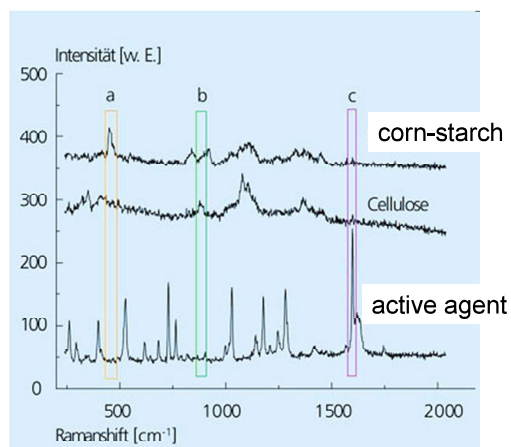
Raman-Scattering

Vibrations are specific for the molecules



Raman Spectroscopy

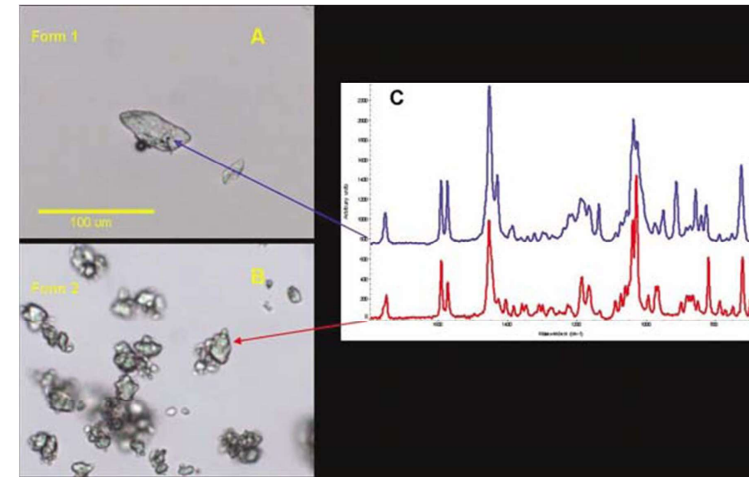
E.g.: active agent content of a tablet



Handheld Raman spectrometer for identification of materials



Pharmaceutical application



Rayleigh scattering

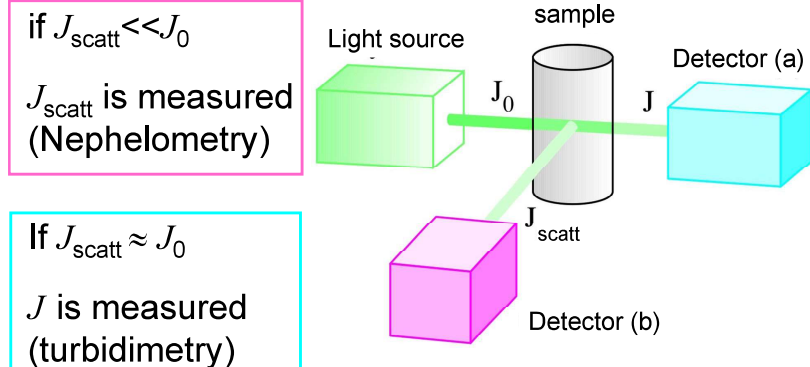
Size of the particle: $a \ll \lambda$

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



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