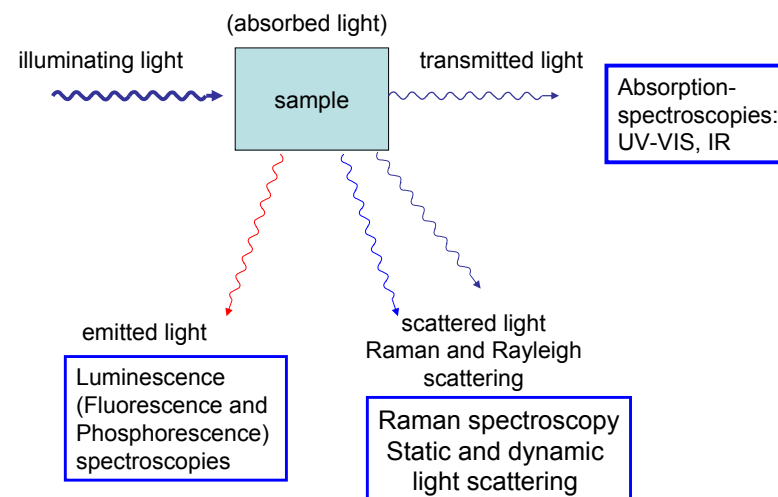


# Optical spectroscopic techniques

László Smeller

## What happens if a sample is illuminated by light?

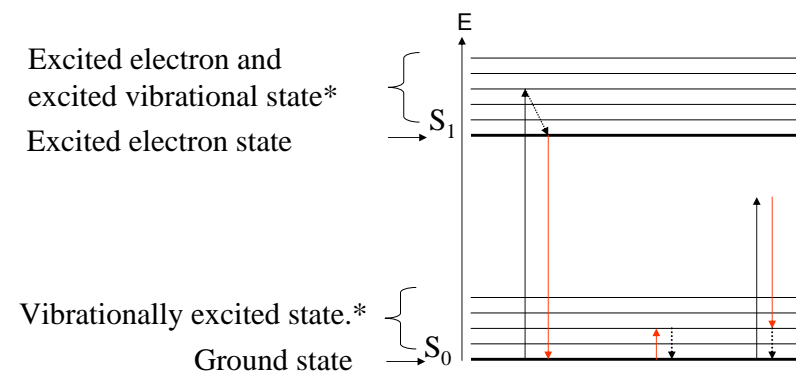


## Spectroscopy (Absorption and emission spectroscopy)

- 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

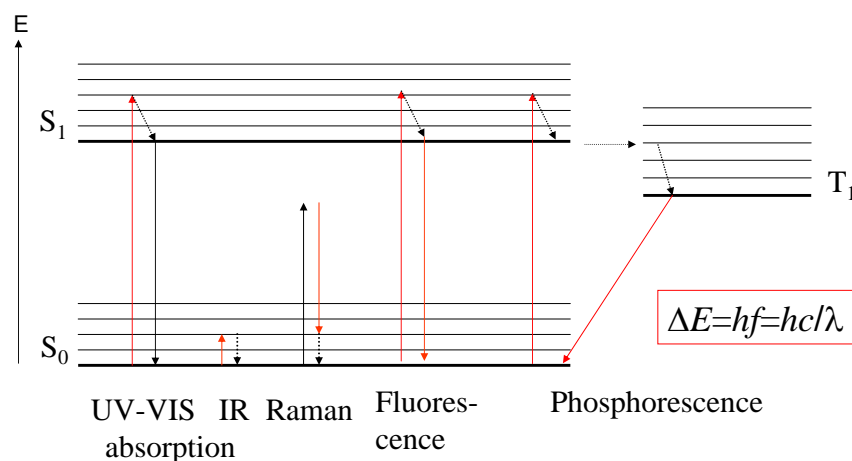
## Why is light absorbed or emitted?

### 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 ( $\lambda_{\max}$ ), concentration (A)

## Infrared spectroscopy

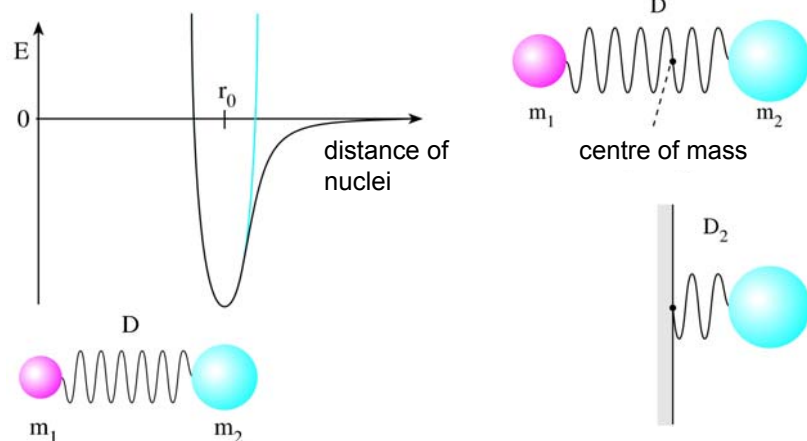
- Infrared light:  $\lambda = 800 \text{ nm} - 1 \text{ mm}$   
MIR (mid-infrared) :  $2,5 - 50 \text{ } \mu\text{m}$
- absorption spectroscopy
- the absorbed infrared radiation excite molecular vibrations
- very specific for the structure of the molecule
- special method for detection:  
FT spectrometer

## 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

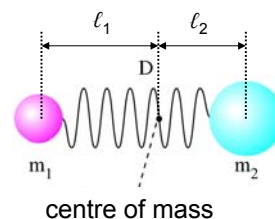
## Molecular vibrations:



known from elementary mechanics:

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

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

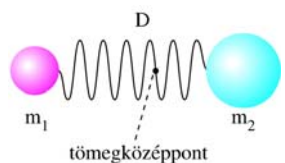


$$\frac{D_2}{D} = \frac{F/D}{F/D_2} = \frac{\Delta\ell}{\Delta\ell_2} = \frac{\ell}{\ell_2} =$$

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

$$F = D\Delta\ell$$

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 ( $\nu$ ) is used, which is the reciprocal of  $\lambda$ :

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

$\nu$ : number of waves in a unit length [ $\text{cm}^{-1}$ ]

Example: CO

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

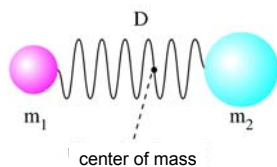
$$\Rightarrow \lambda = 4,67 \mu\text{m} \Rightarrow f = 6,43 \cdot 10^{13} \text{ Hz} \quad \left. \begin{array}{l} \\ m_C = 2 \cdot 10^{-26} \text{ kg}, m_O = 2,7 \cdot 10^{-26} \text{ kg} \end{array} \right\} \Rightarrow D = 1875 \text{ N/m}$$

if  $\nu$  is known,  $D$  can be calculated

if  $D$  is known,  $\nu$  can be calculated

## Classical vs. quantum physics

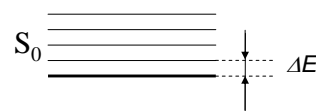
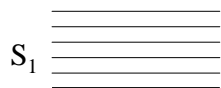
Classical physical picture



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

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

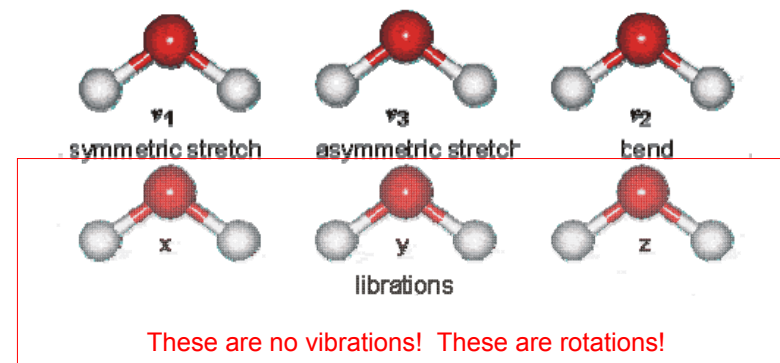
All the atoms vibrate

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

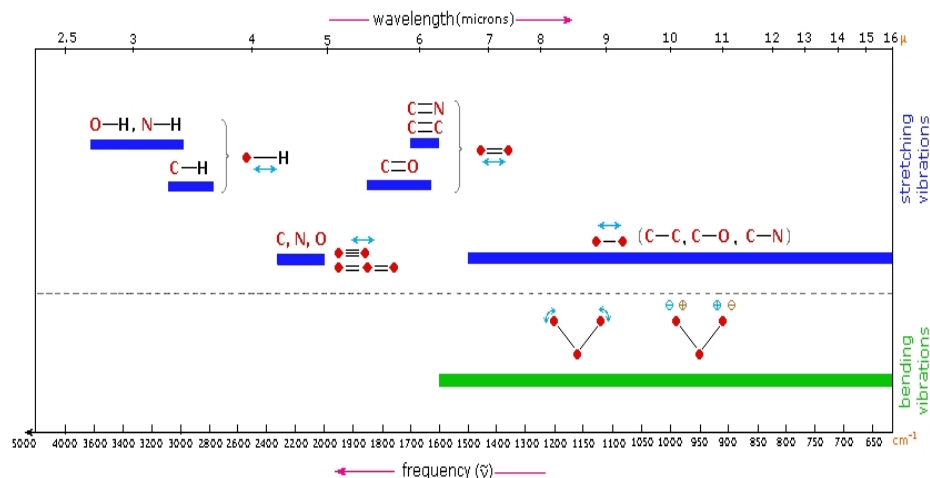
Example: water



## Normal vibrations of water

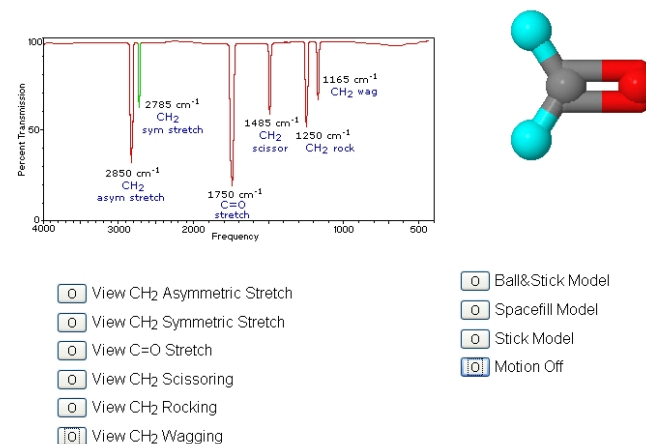


## Typical vibrational frequencies (wavenumbers)



## Example: Formaldehyde

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

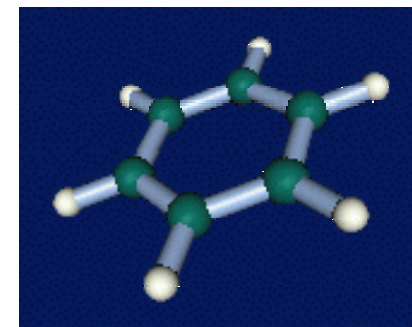


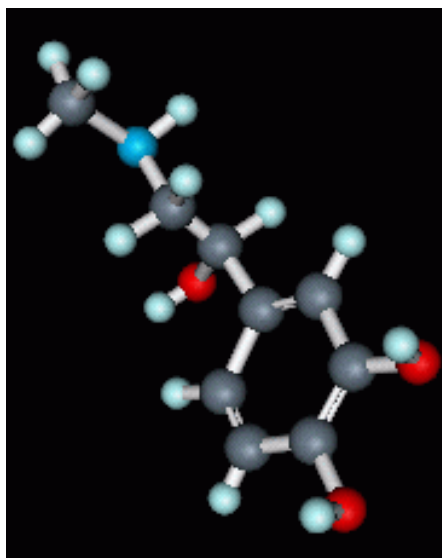
<http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm>

## Flavin



## Benzol



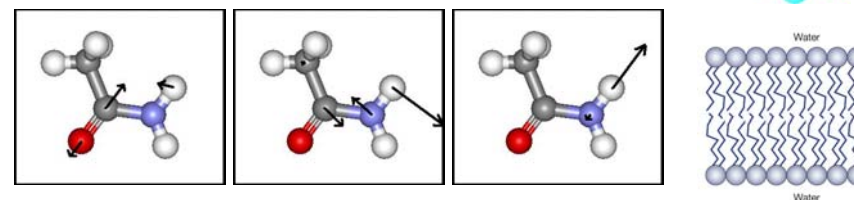
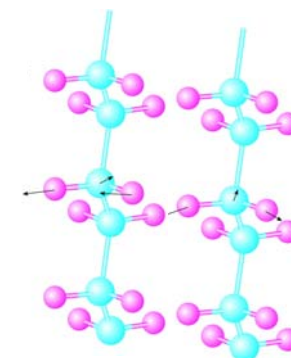


## Vibrations of the macromolecules

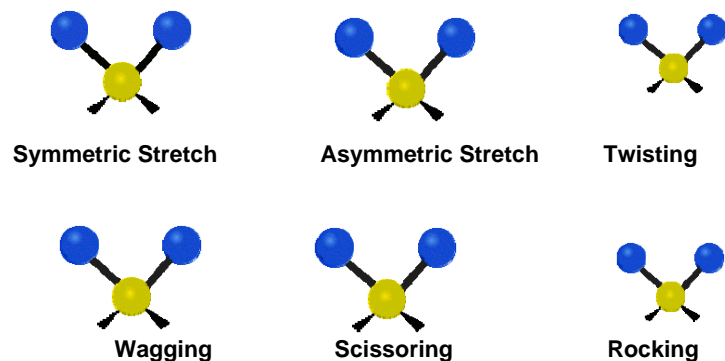
Complex global vibrations

Localised vibrations, e.g.:

- $\text{CH}_2$  vibrations of the lipids
- amid vibrations of proteins (acetamide)

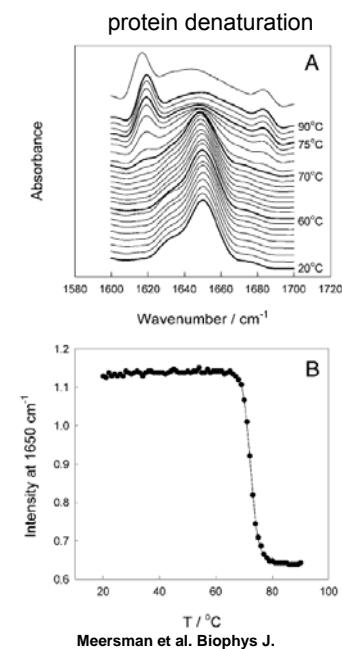
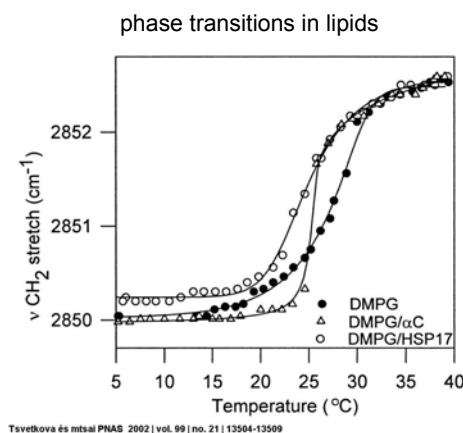


## Lipids



Types of Vibrational Modes. Figure from Wikipedia

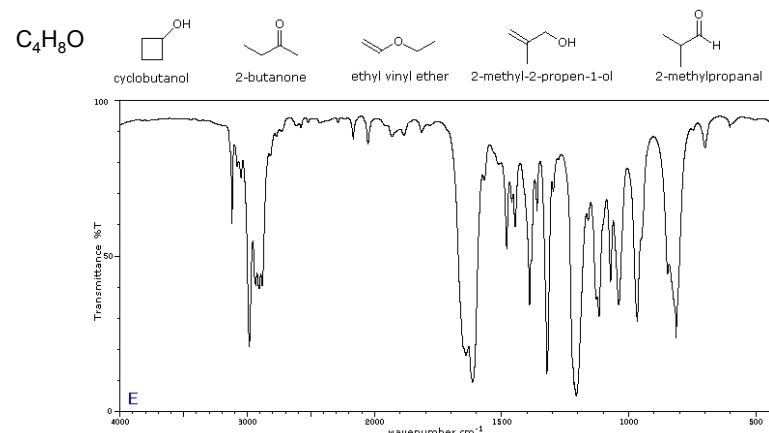
## Applications



## 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

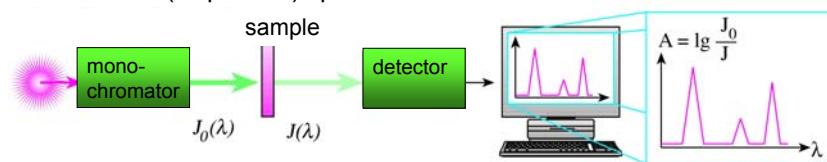
## Example: Identification of molecules



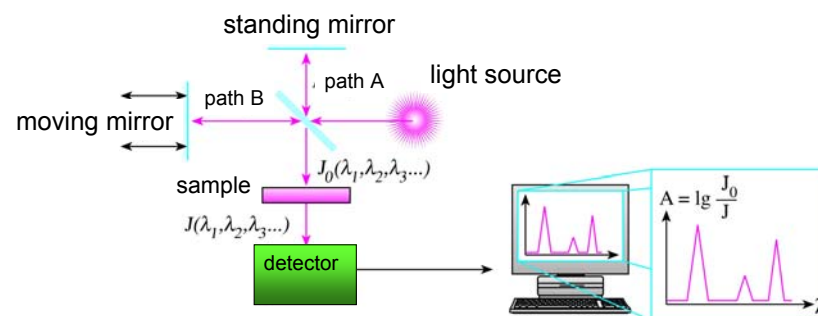
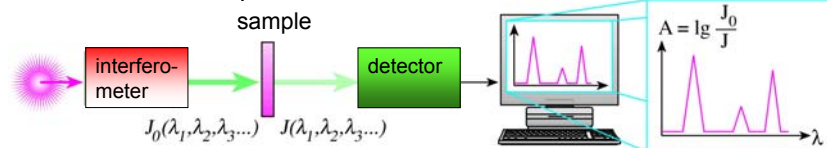
<http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm>

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

conventional (dispersion) spectrometer

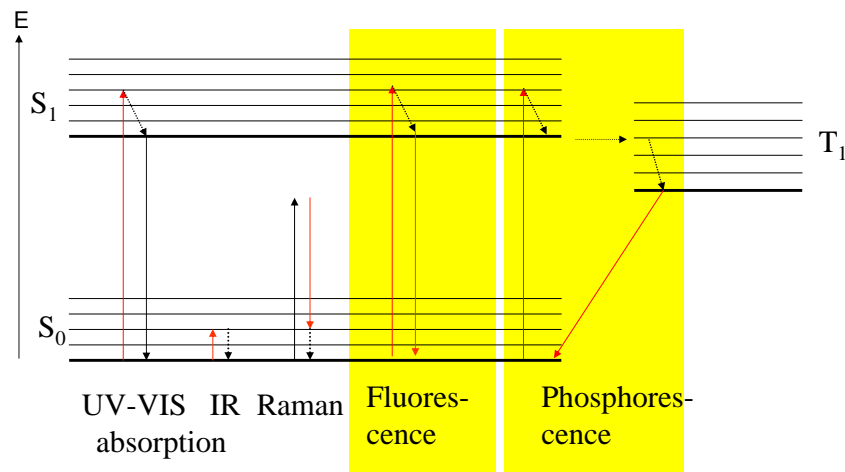


Fourier transform spectrometer





# Luminescence spectroscopy

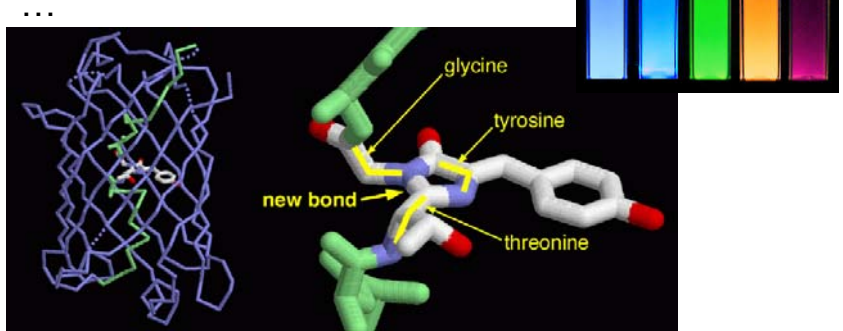


## Which molecules are fluorescent?

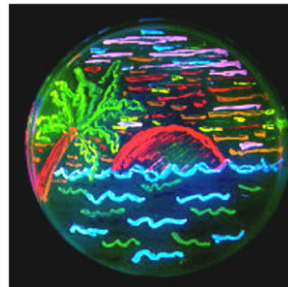
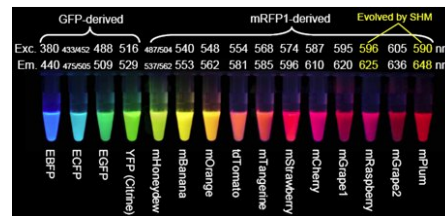
Amino acids (tryptophan, tyrosine, phenylalanine)

Fluorescent dyes

GFP



Aequorea victoria

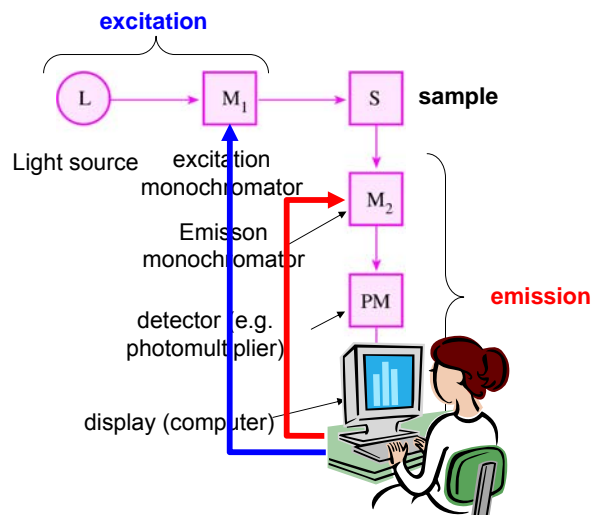


## Measurable quantities in Fluorescence Spectroscopy

- 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

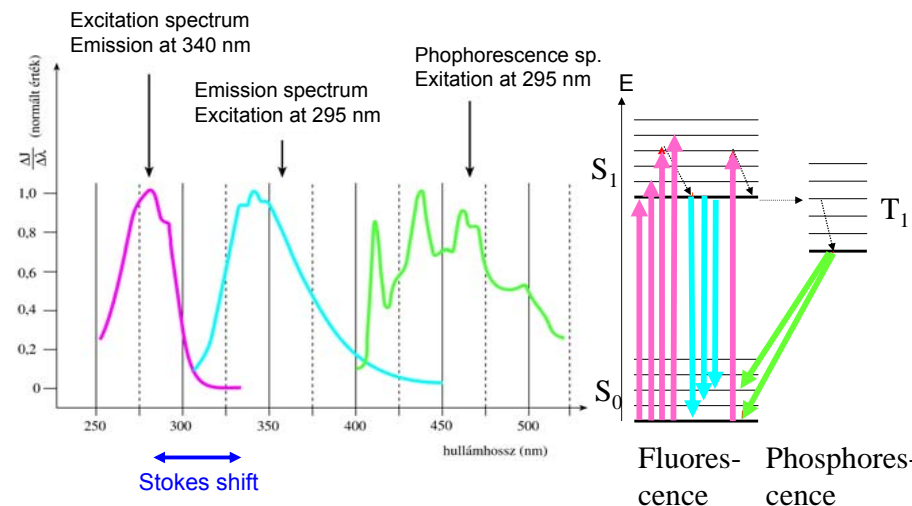


## Scheme of the fluorescence spectrometer



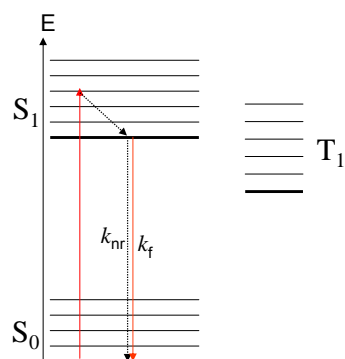
6.26

## Excitation and emission spectra



6.25.

## Fluorescence quantum yield(Q)



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

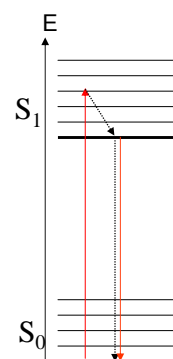
$$Q_f = \frac{k_f}{k_f + k_{nr}}$$

$k_f$  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  $\Delta t$  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  $\Delta N$ , i.e. it is proportional also with  $N$ , which means it decays exponentially with the decay constant of  $\tau$ .

How to measure?

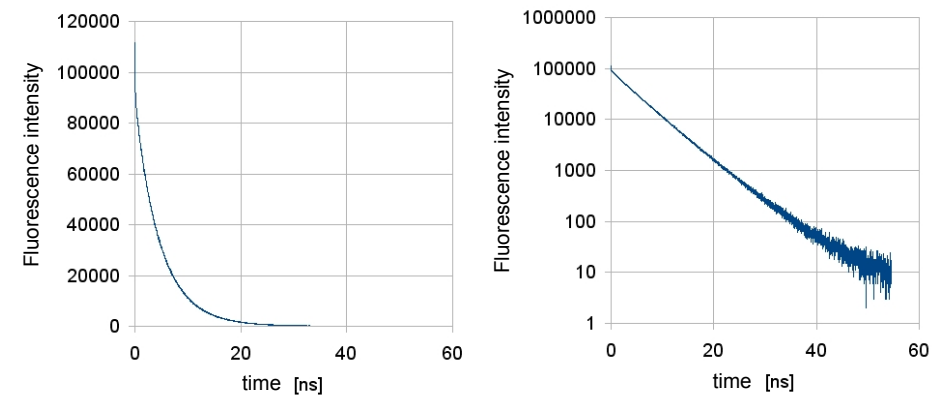
- Pulsed illumination (flashlamp, or pulse laser)
- Photon counting as function of time

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

Typical lifetimes:

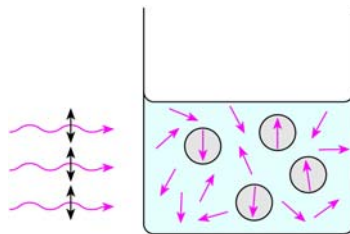
$\tau_{\text{fluor}}$  ns       $\tau_{\text{phosphor}}$   $\mu\text{s}$  ... s

## 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 the rotational diffusion is?)

## Light scattering

Rayleigh

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

Raman

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

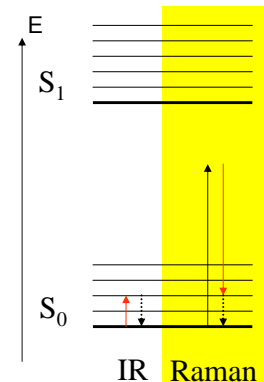


Raman scattering:

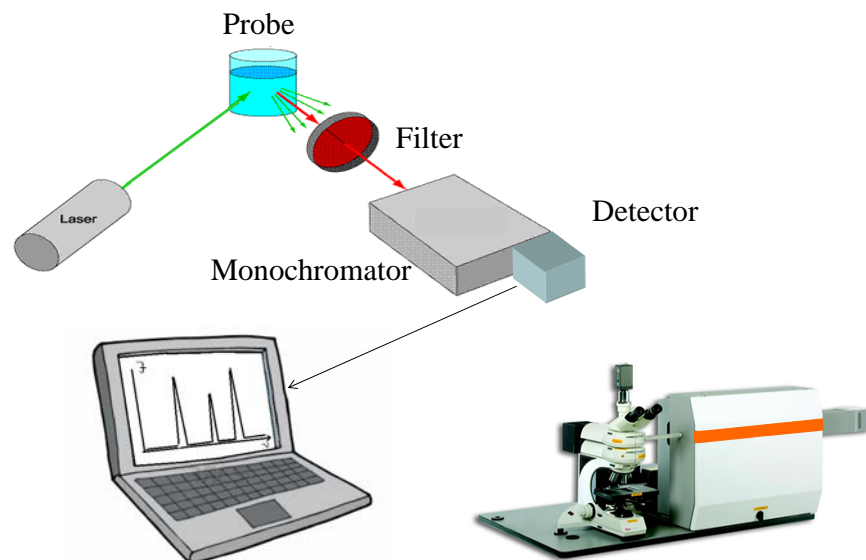
$$\lambda_{\text{scatt}} \neq \lambda_{\text{illum}} \Rightarrow f_{\text{scatt}} \neq f_{\text{illum}}$$

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

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



## Raman Spectrometer



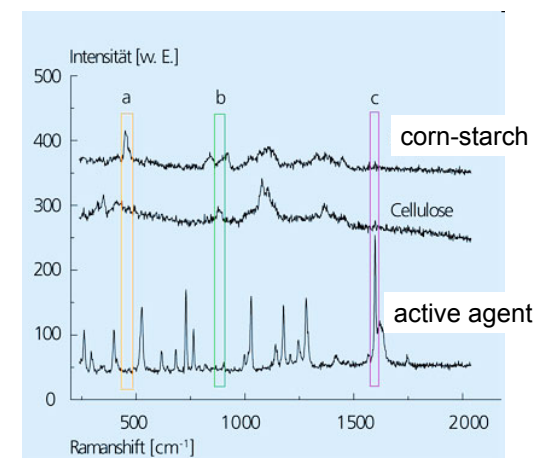
## Raman-Scattering

Vibrations are specific for the molecules

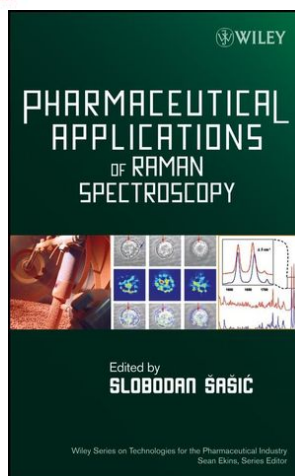
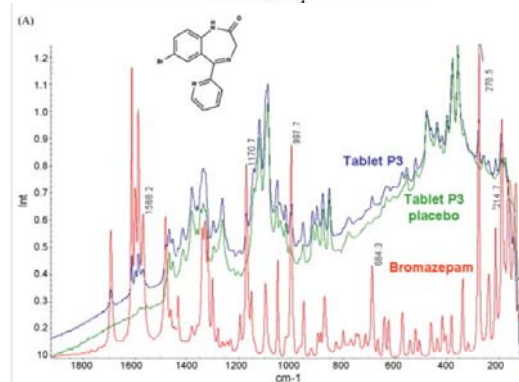
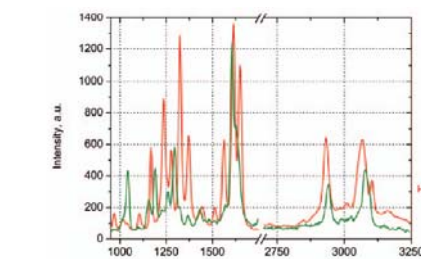


Raman Spectroscopy

E.g.: active agent content of a tablet



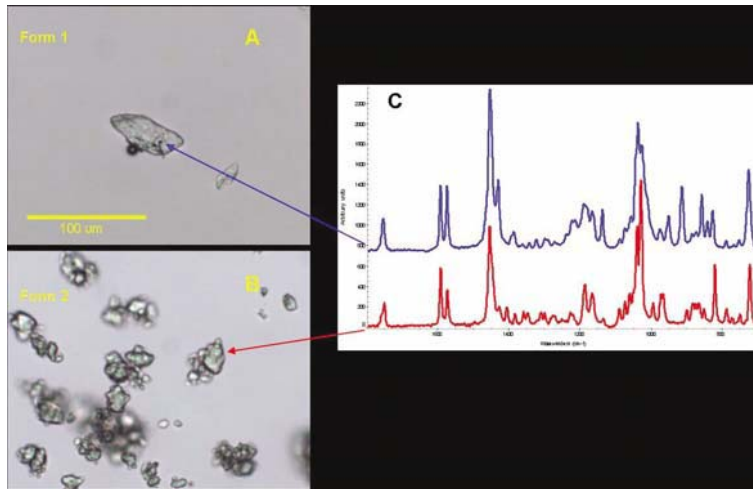
<http://www.igb.fraunhofer.de/de/kompetenzen/grenzflaechentechnik/oberflaechenanalytik/ausstattung/konfokale-mikroskopie-spektroskopie/raman-spektroskopie-tablette.html>



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

$J_{\text{scatt}}$  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).

