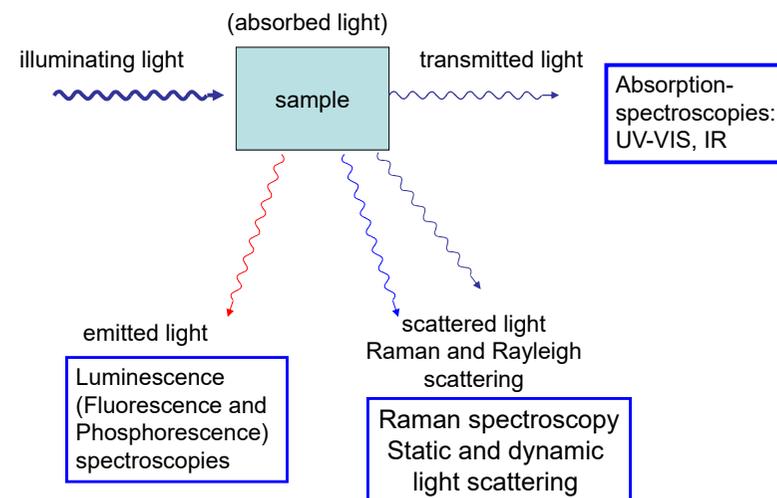


# Optical spectroscopic techniques

László Smeller

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

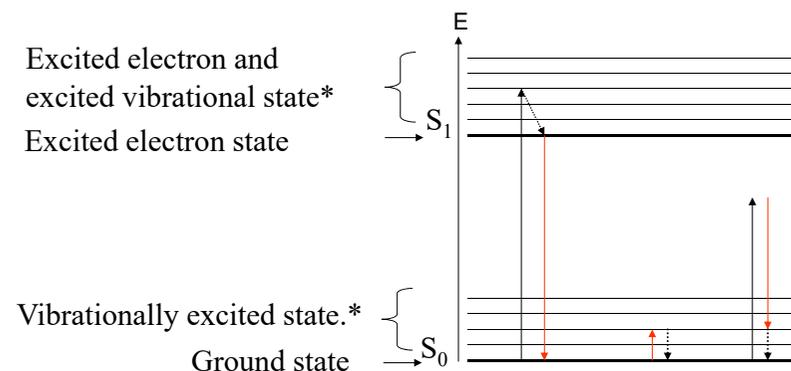


## Spectroscopy (Absorption and emission spectroscopy)

- 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

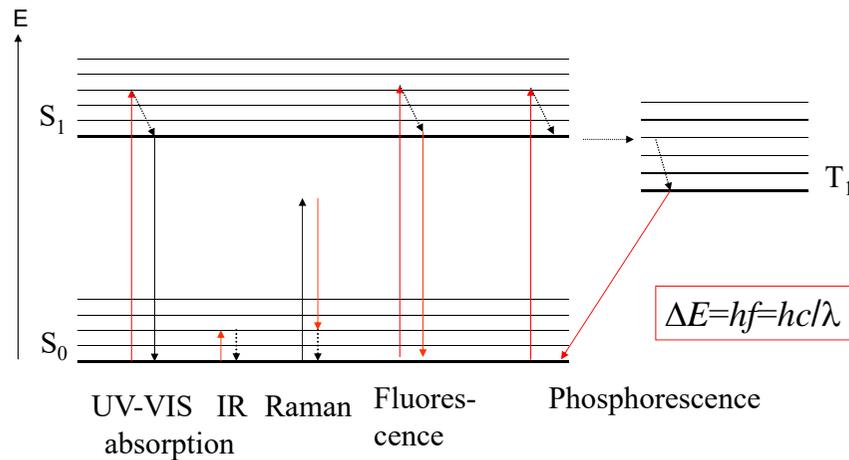
## Why is light absorbed or emitted?

### 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

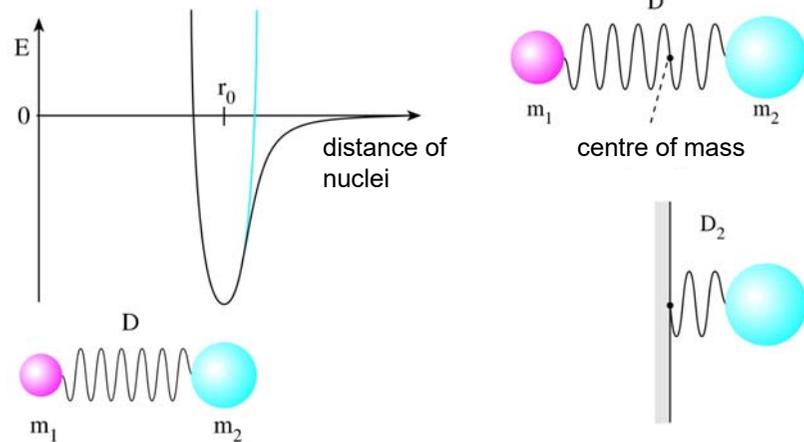
- Infrared light:  $\lambda = 800 \text{ nm} - 1 \text{ mm}$   
MIR (mid-infrared) :  $2,5 - 50 \mu\text{m}$
- absorption spectroscopy
- the absorbed infrared radiation excite molecular vibrations
- very specific for the structure of the molecule
- special method for detection:  
FT spectrometer

## Molecular vibrations

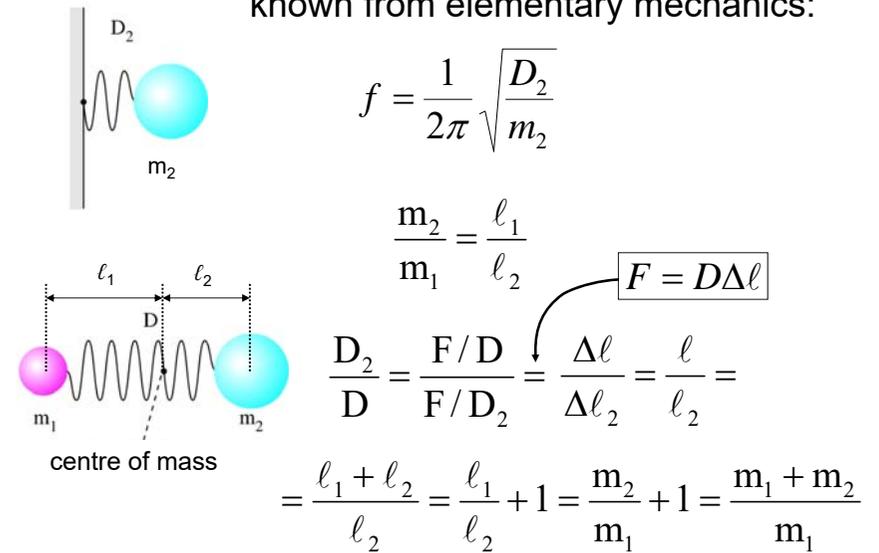
The electrons are light ( $m_e \ll m_{\text{nucleus}}$ ), they can follow the movements of the nuclei easily, therefore the movements of the nuclei are independent of the movements of the electrons.

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

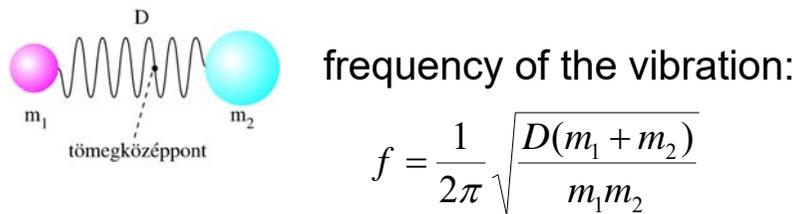
# Molecular vibrations:



known from elementary mechanics:



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



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

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

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

In the IR spectroscopy the wavenumber ( $\nu$ ) is used, which is the reciprocal of  $\lambda$ :

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

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

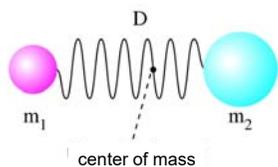
Example: CO

The measured wavenumber:  $\nu = 2143 \text{ cm}^{-1}$   
 $\Rightarrow \lambda = 4,67 \mu\text{m} \Rightarrow f = 6,43 \cdot 10^{13} \text{ Hz}$   
 $m_C = 2 \cdot 10^{-26} \text{ kg}, m_O = 2,7 \cdot 10^{-26} \text{ kg}$  }  $\Rightarrow D = 1875 \text{ N/m}$

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

# Classical vs. quantum physics

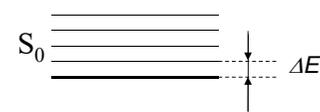
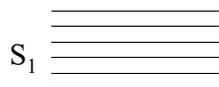
Classical physical picture



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

resonance with the light with frequency  $f$

Quantum mechanical picture



$$\Delta E = hf$$

=

# Vibrations of the large molecules

Molecule consisting of N atoms:

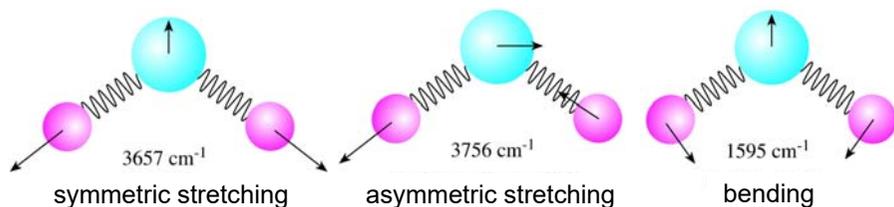
- 3N degree of freedom,
  - 3-3 are the rotations and translations of the whole molecule
- 3N-6 vibrational degree of freedom (3N-5 for the linear molecules)
- 3N-6 independent normal vibrations

# Normal vibrations

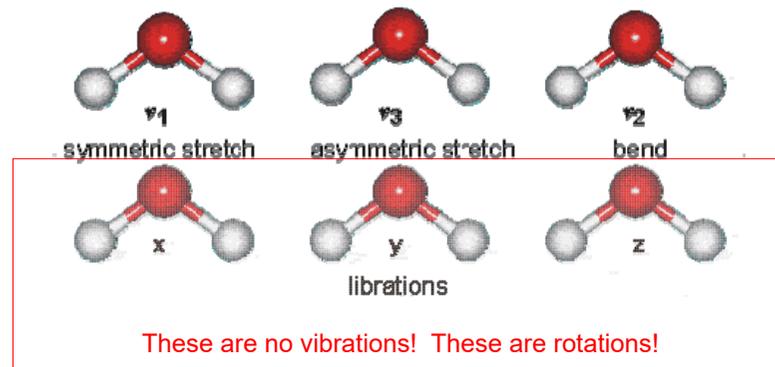
All the atoms vibrate

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

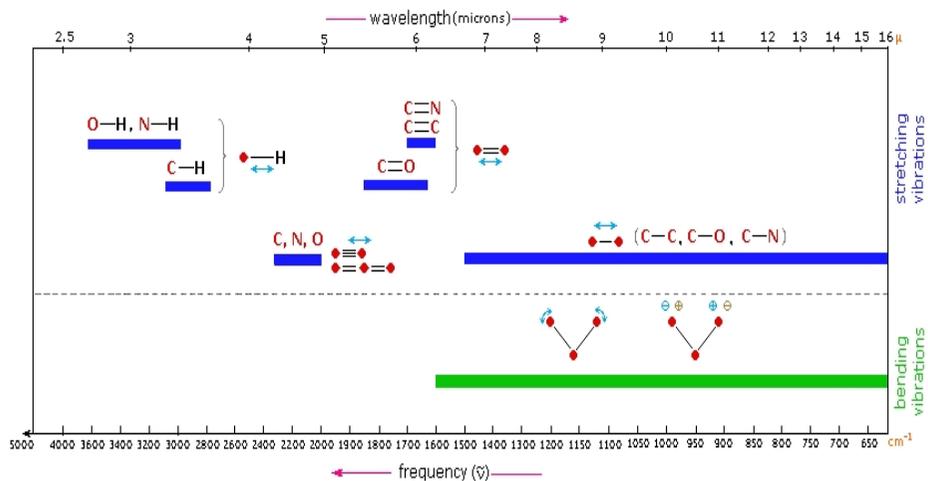
Example: water



# Normal vibrations of water

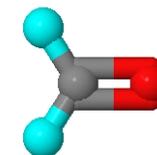
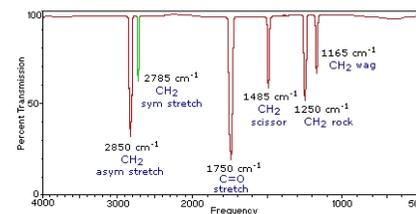


## Typical vibrational frequencies (wavenumbers)



## Example: Formaldehyde

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

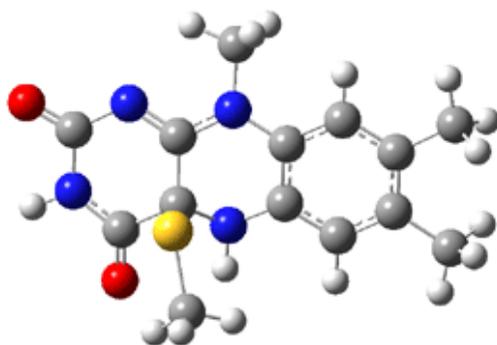


- View CH<sub>2</sub> Asymmetric Stretch
- View CH<sub>2</sub> Symmetric Stretch
- View C=O Stretch
- View CH<sub>2</sub> Scissoring
- View CH<sub>2</sub> Rocking
- View CH<sub>2</sub> Wagging

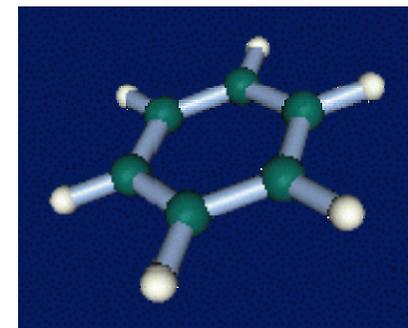
- Ball&Stick Model
- Spacefill Model
- Stick Model
- Motion Off

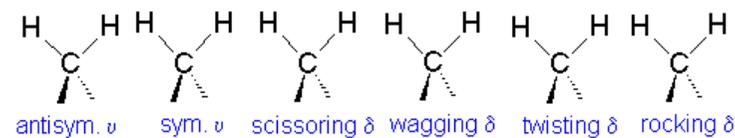
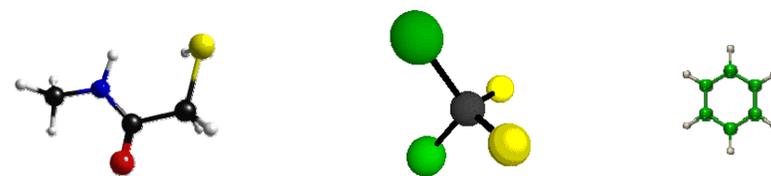
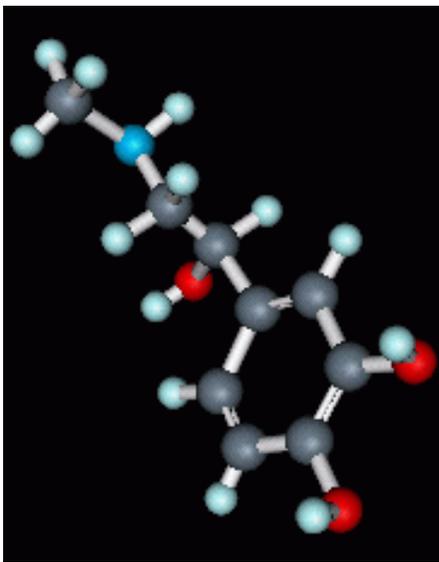
<http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm>

## Flavin



## Benzol



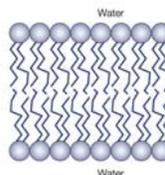
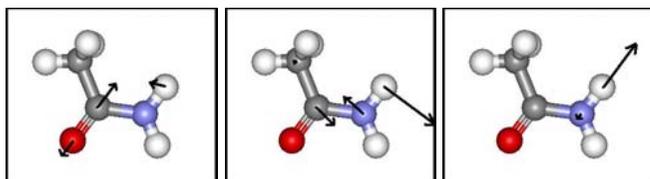
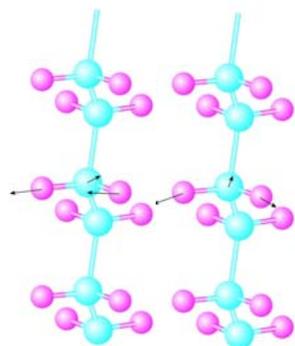


## Vibrations of the macromolecules

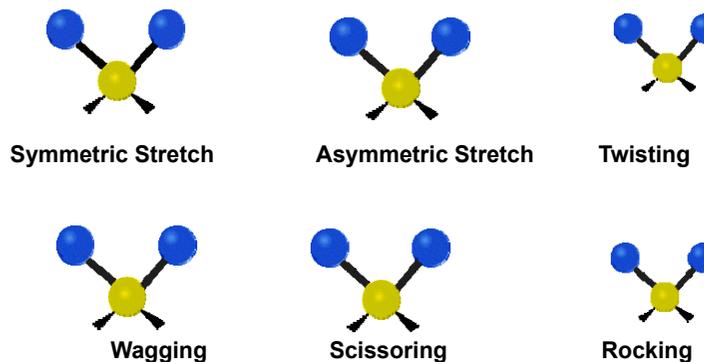
Complex global vibrations

Localised vibrations, e.g.:

- CH<sub>2</sub> vibrations of the lipids
- amid vibrations of proteins (acetamide)



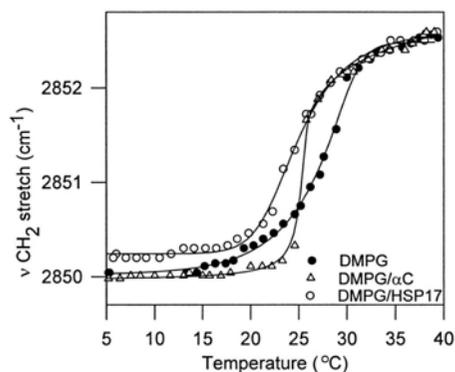
## Lipids



Types of Vibrational Modes. Figure from Wikipedia

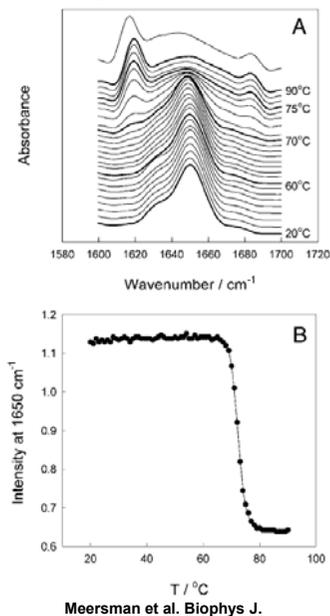
# Applications

phase transitions in lipids



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

protein denaturation

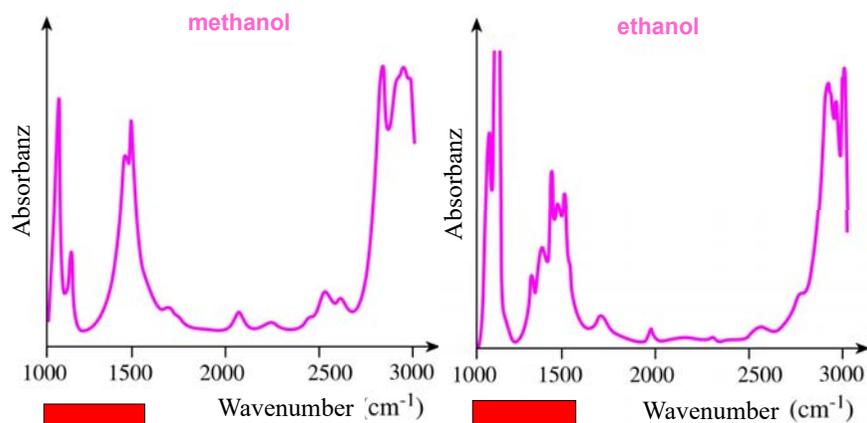


Meersman et al. Biophys J.

# Pharmaceutical applications

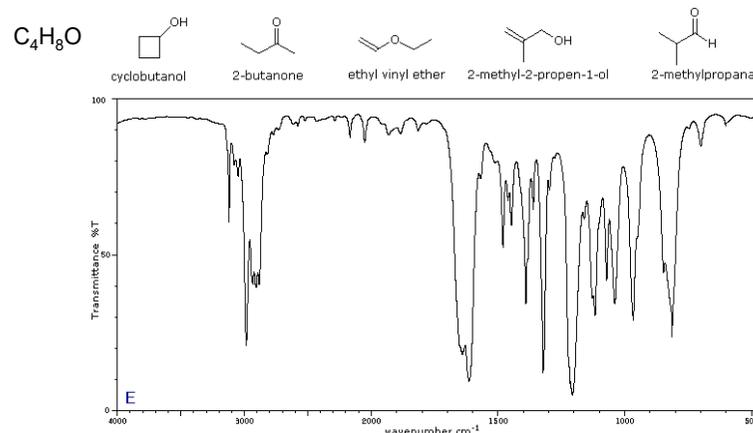
- synthesis: identification of the intermediate and the end product
- determination and justification of the molecular structure
- detection of the metabolites
- quality control (purity)
- Remark.: Lambert-Beer law is valid, determination of concentration is possible

# Identification of the Molecules



Fingerprint Region

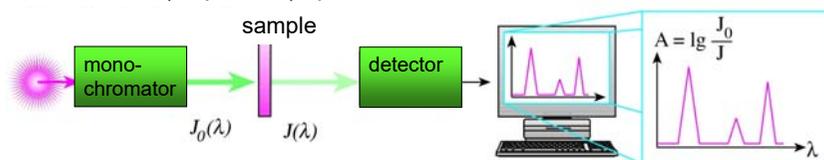
# Example: Identification of molecules



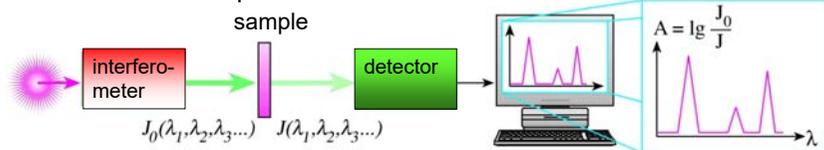
<http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm>

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

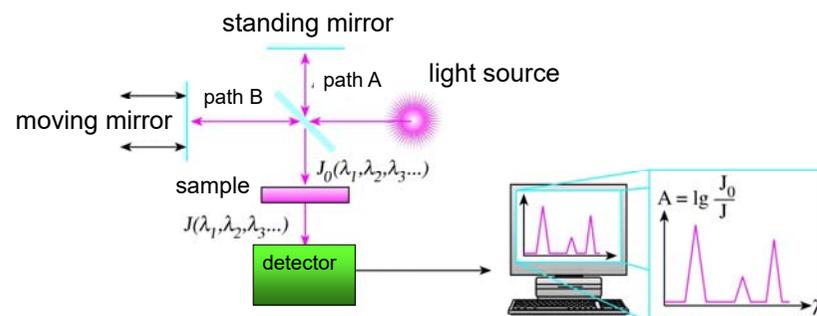
conventional (dispersion) spectrometer



Fourier transform spectrometer

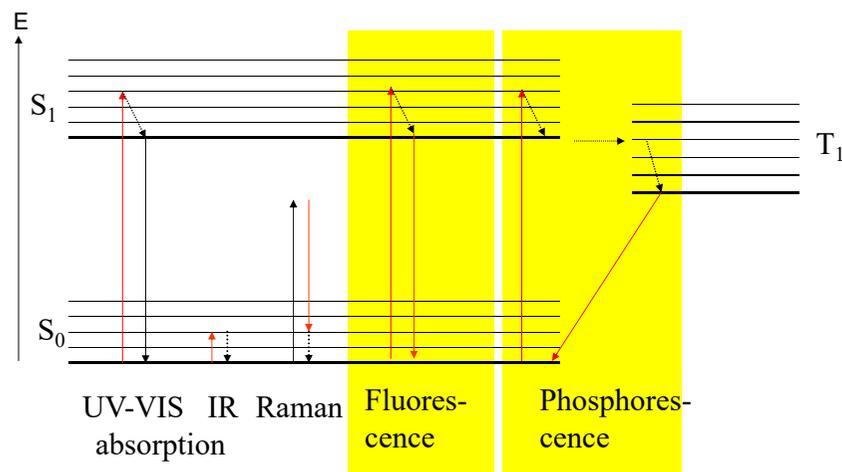


book 6.17



book 6.18

# Luminescence spectroscopy

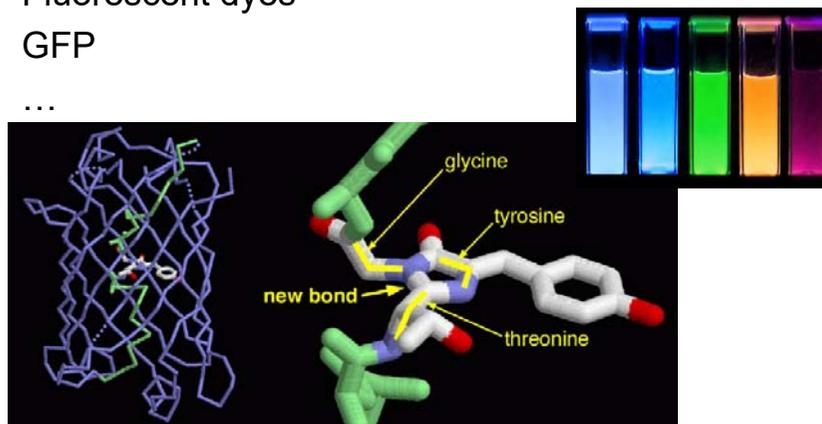


# Which molecules are fluorescent?

Amino acids (tryptophan, tyrosine, phenylalanine)

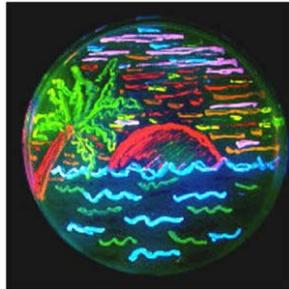
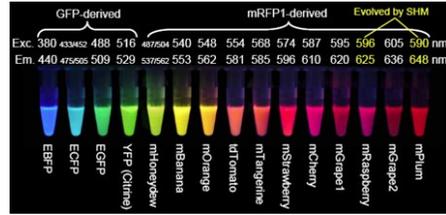
Fluorescent dyes

GFP





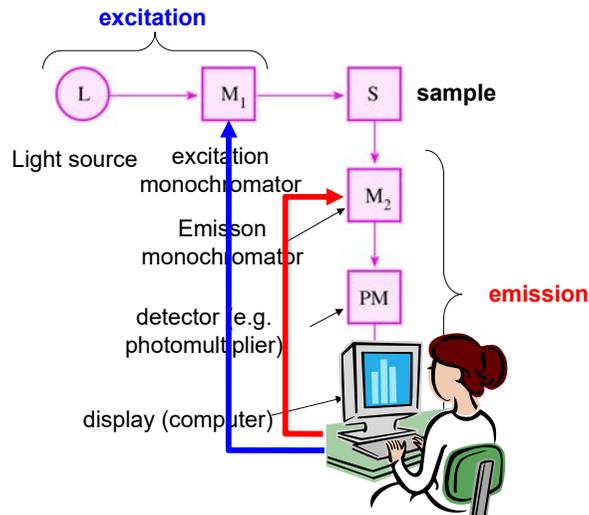
Aequorea victoria



## Measurable quantities in Fluorescence Spectroscopy

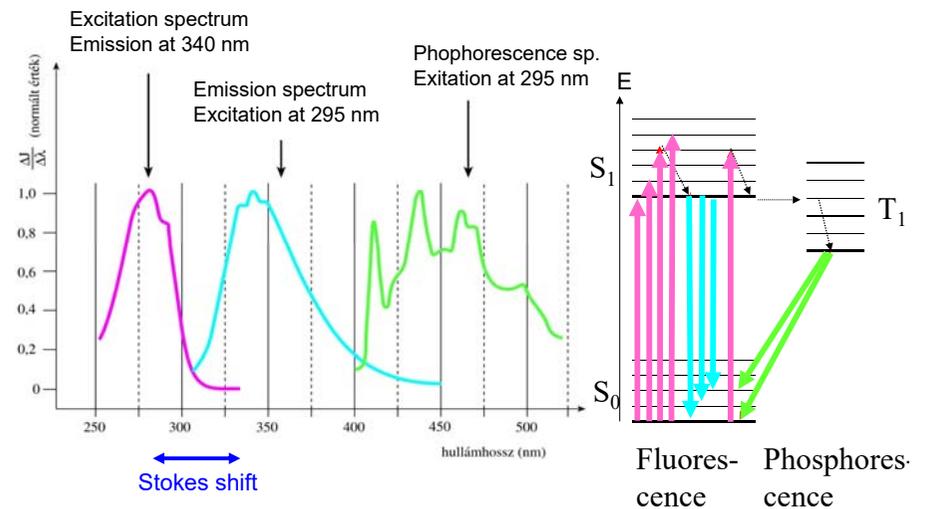
- Wavelength of the exciting light
- Wavelength of the emitted light (fluor., phosph.)
- Time dependence of the emitted light
- Polarisation of the emitted light
- Intensity of the emitted light

## Scheme of the fluorescence spectrometer



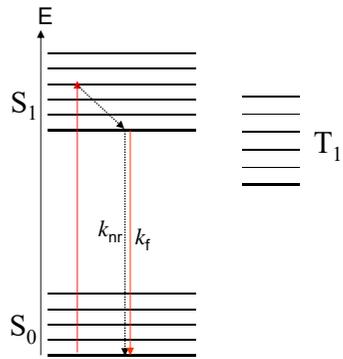
6.26

## Excitation and emission spectra



6.25.

## Fluorescence quantum yield(Q)

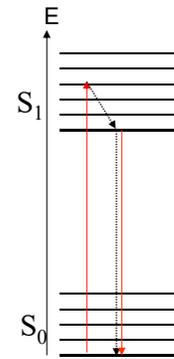


Quantum yield:  $Q =$   
 $= \frac{\text{number of emitted photons}}{\text{number of absorbed photons}}$

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

$k_f$  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  $\Delta t$  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?

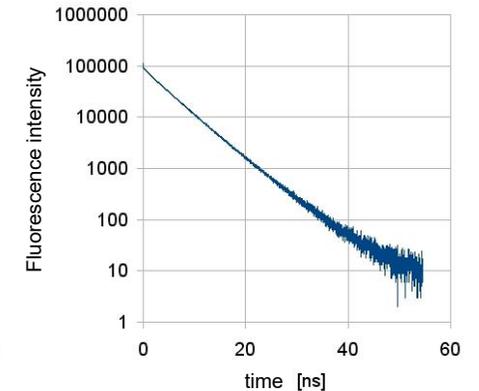
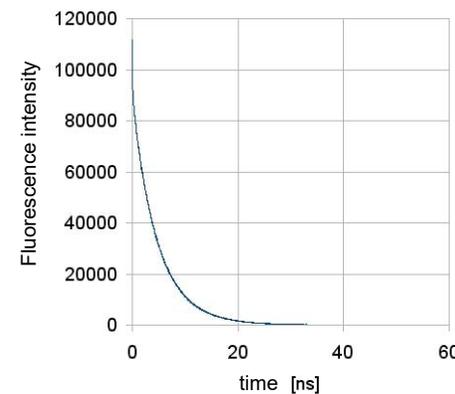
- Pulsed illumination (flashlamp, or pulse laser)
- Photon counting as function of time

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

Typical lifetimes:

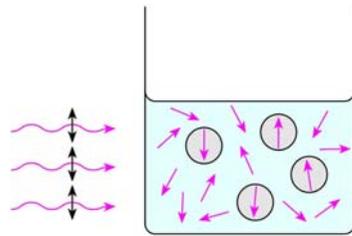
$\tau_{\text{fluor}}$  ns       $\tau_{\text{phosphor}}$   $\mu\text{s} \dots \text{s}$

## 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

# Ligth scattering

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

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



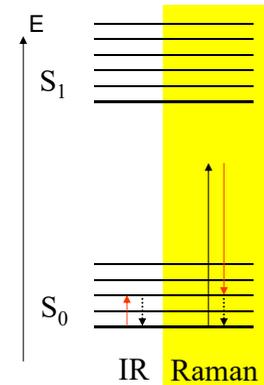
Chandrasekhra Venkata Raman

Raman scattering:

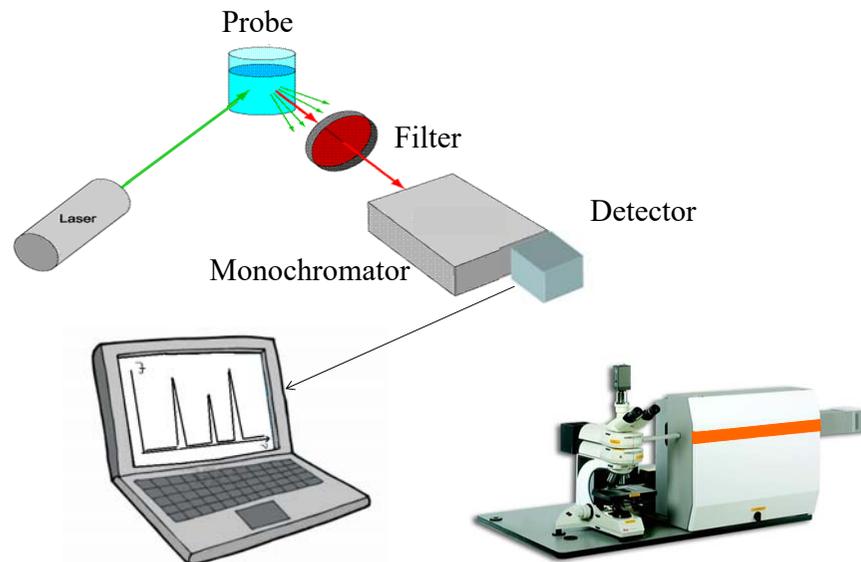
$$\lambda_{scatt} \neq \lambda_{illum} \Rightarrow f_{scatt} \neq f_{illum}$$

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

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



# Raman Spectrometer



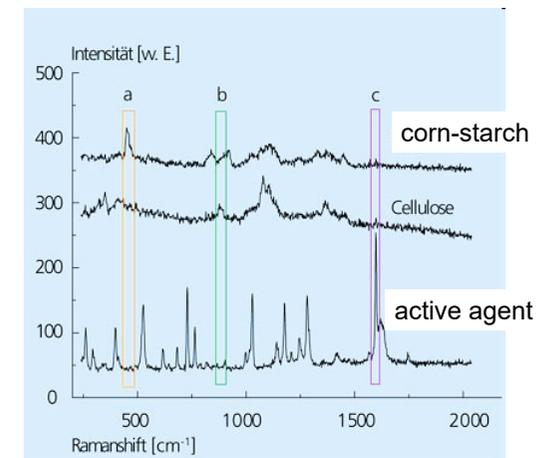
# Raman-Scattering

Vibrations are specific for the molecules



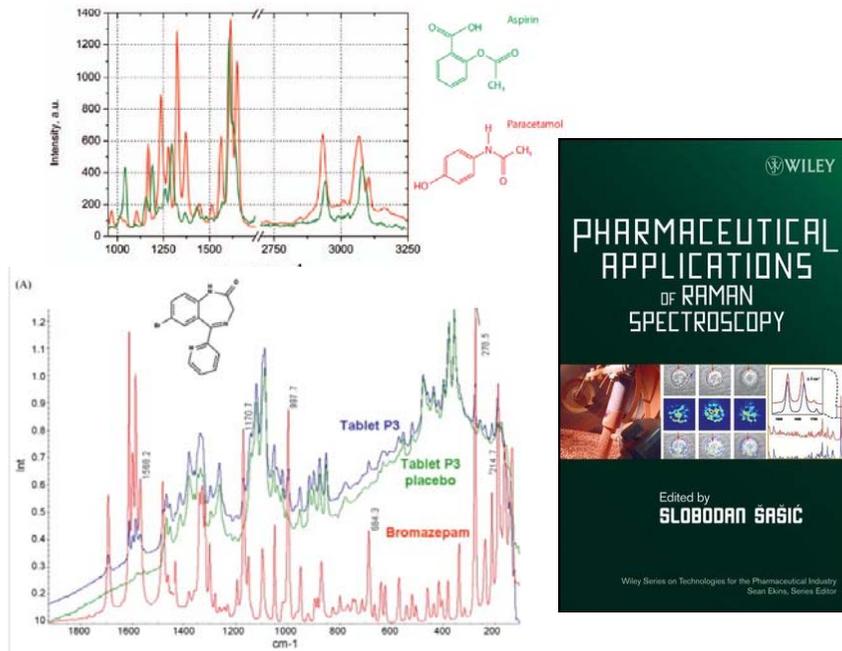
Raman Spectroscopy

E.g.: active agent content of a tablet

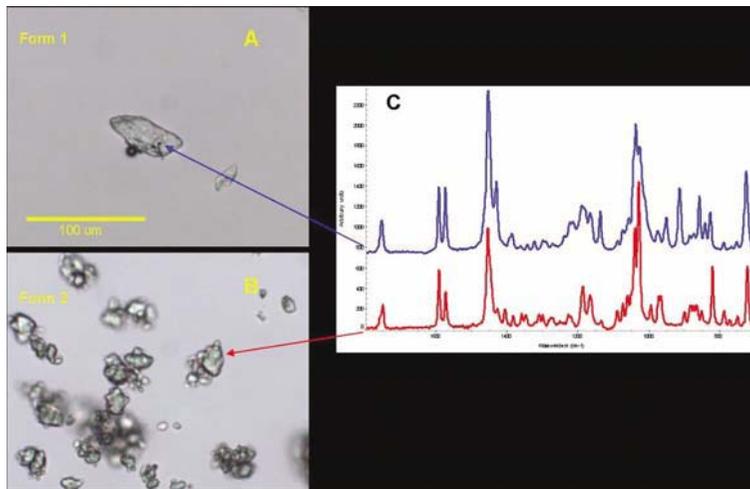


<http://www.igb.fraunhofer.de/de/kompetenzen/grenzflaechentechnik/oberflaechenanalytik/ausstattung/konfokale-mikroskopie-spektroskopie/raman-spektroskopie-tablette.html>

## 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).

