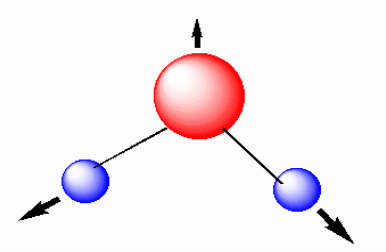
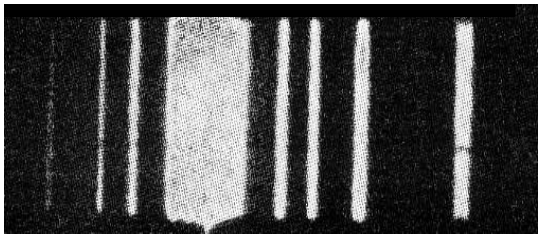
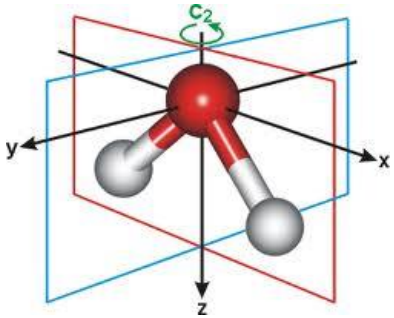
The Principle of Raman Spectroscopy and its Application in Microscopy







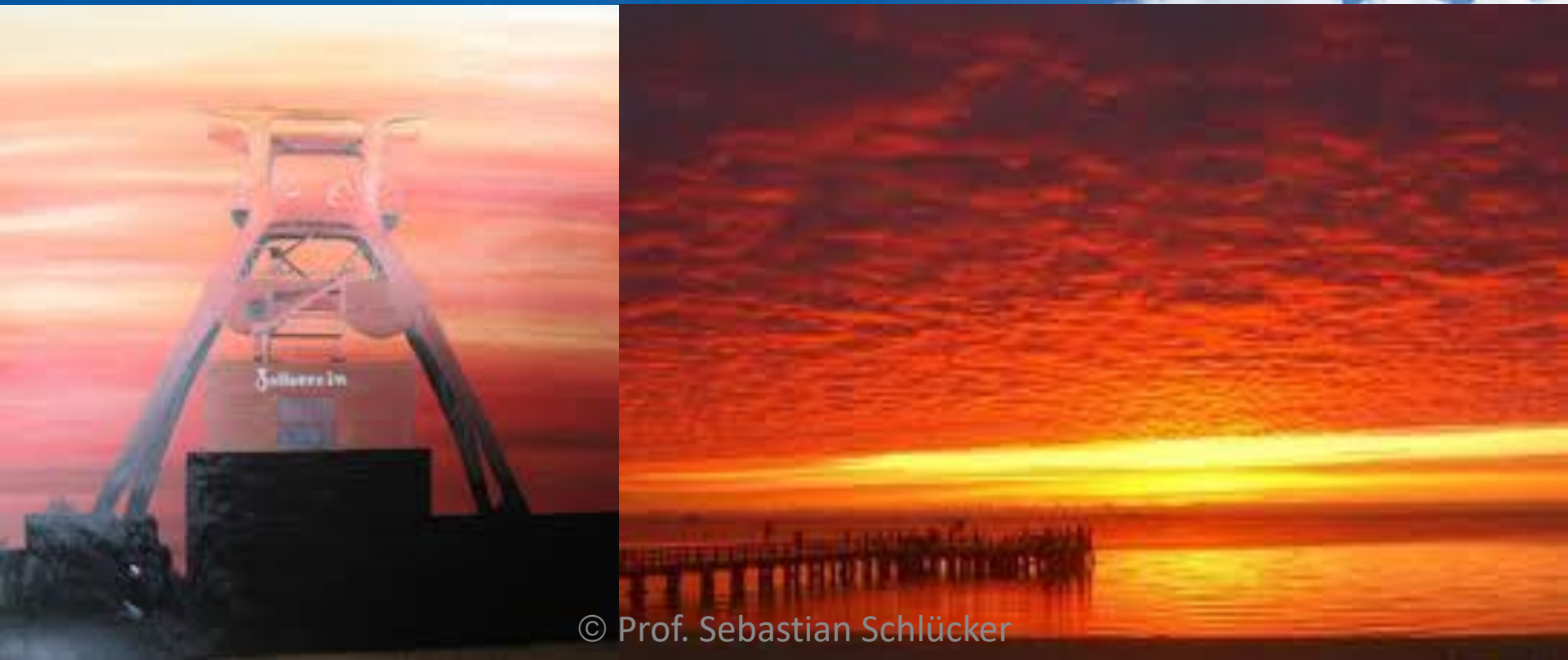
Sebastian Schlücker
Chair of Physical Chemistry I
Department of Chemistry
University Duisburg-Essen (UDE)

http://www.uni-due.de/schluecker-lab/

eMail: sebastian.schluecker@uni-due.de

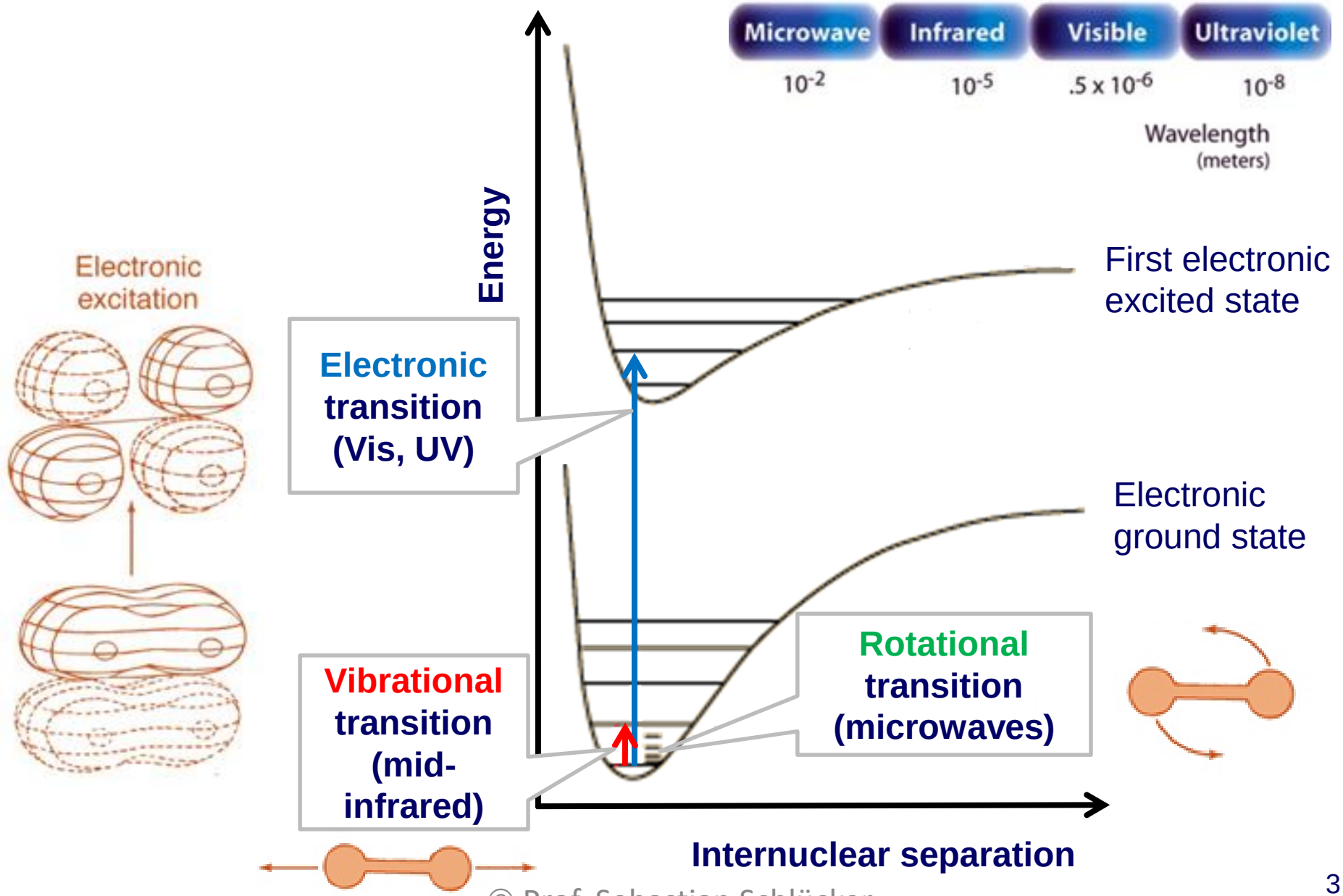
© Prof. Sebastian Schlücker

Spectroscopy
= Light – Matter Interaction



© Prof. Sebastian Schlücker

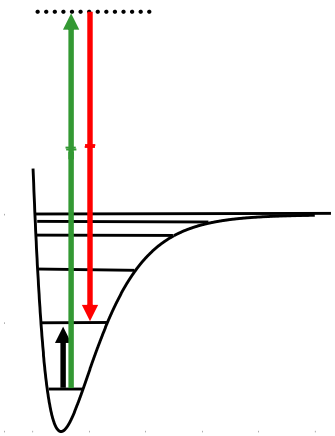
Spectroscopy Probes Differences in Energy



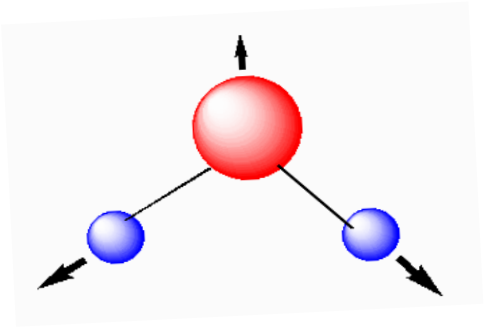
© Prof. Sebastian Schlücker

Overview 1: Topics for Today

MIR and **Vibrational Raman Spectroscopy**

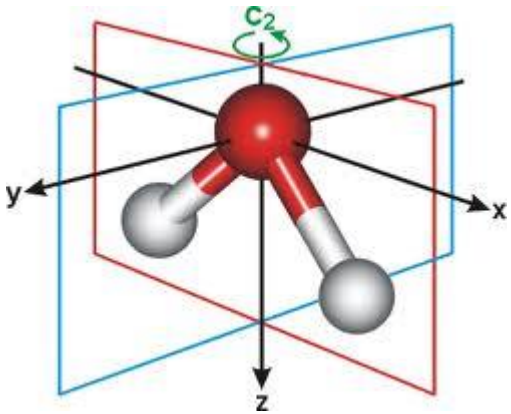


Molecular Vibrations, Energy Levels, Quantum Chemistry



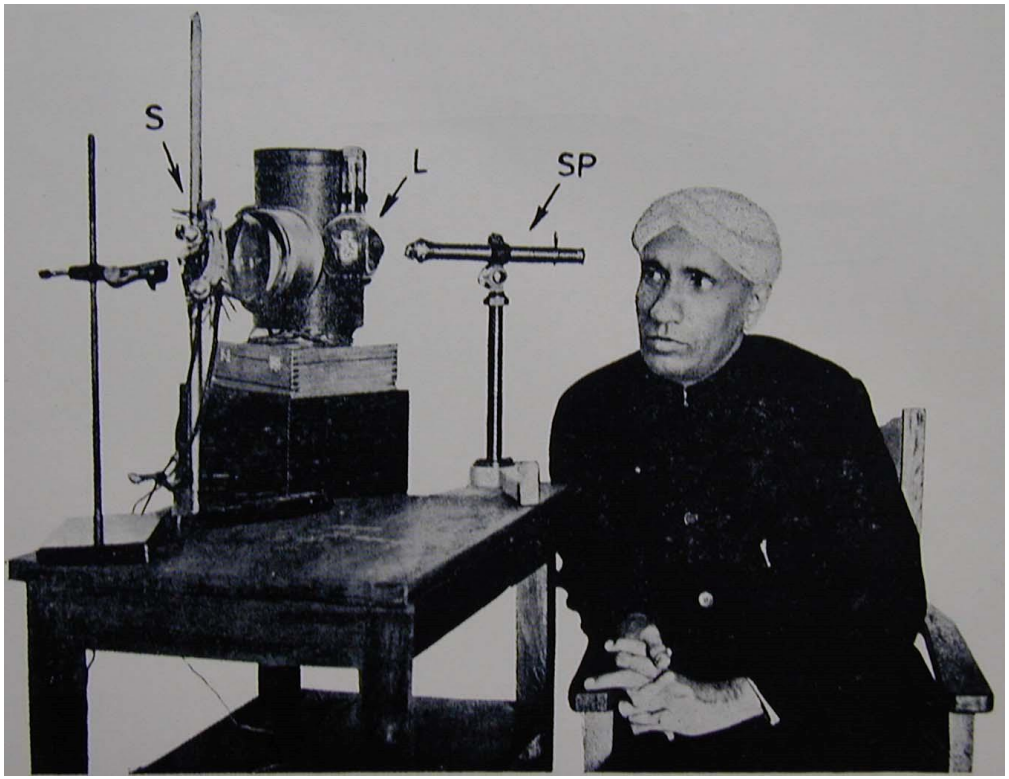
Case Study: Water

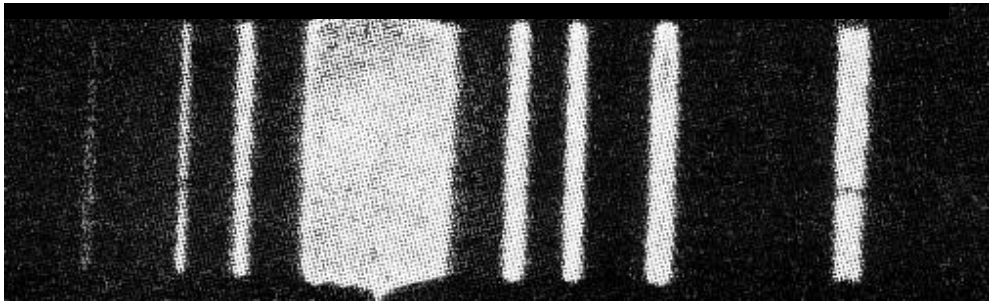
Molecular Symmetry and Group Theory



© Prof. Sebastian Schlücker

Discovery of the Raman Effect (1928; NPP 1930)

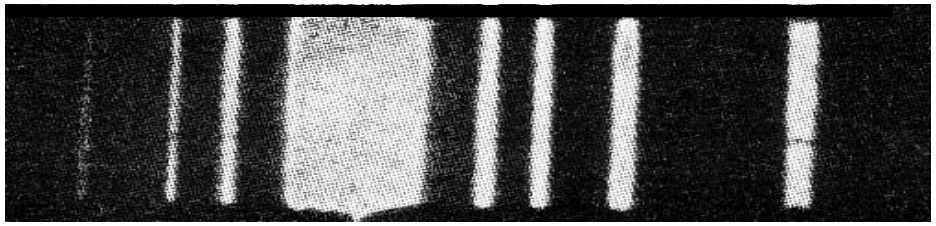




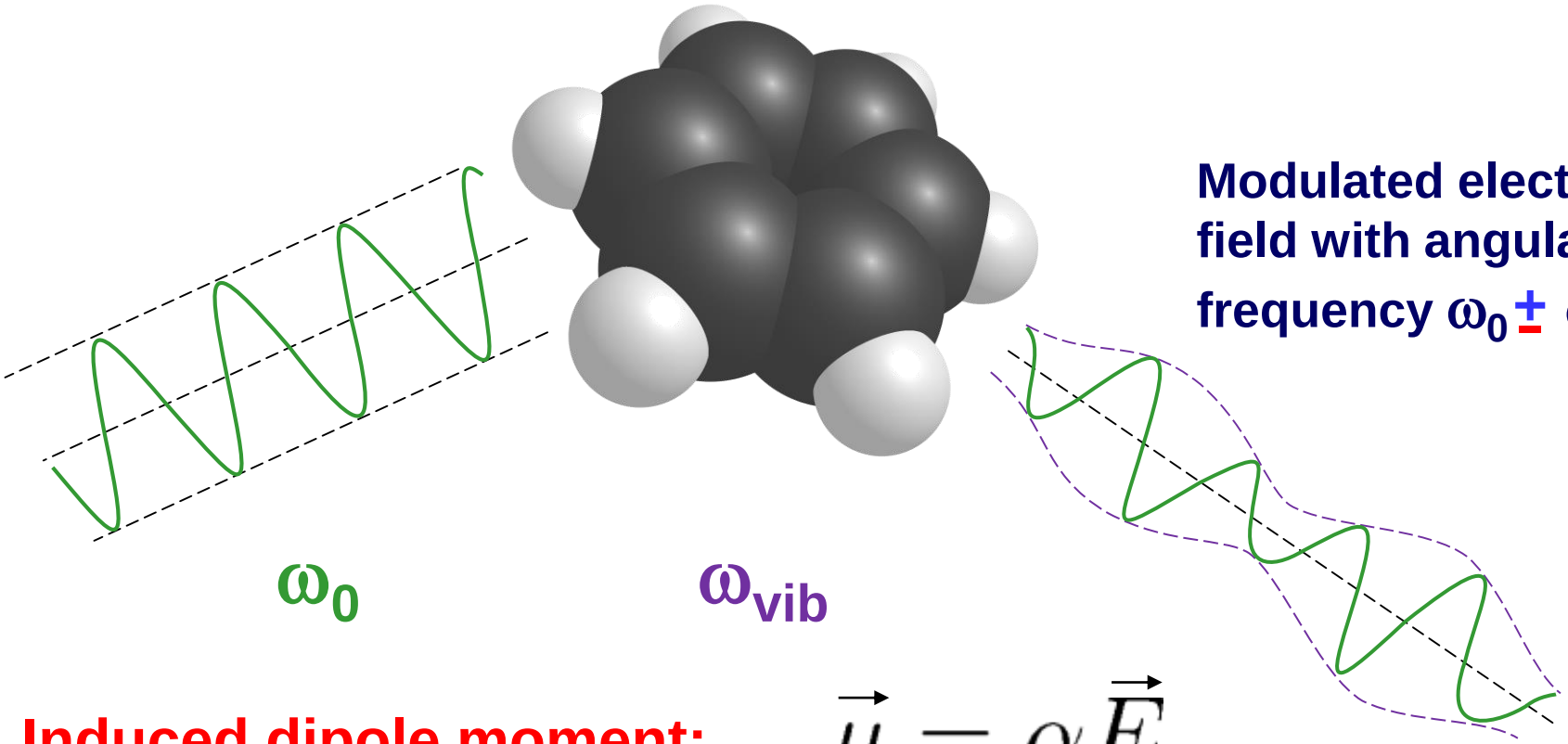
aS R S

© Prof. Sebastian Schlücker

Modulation of the incident electric field by vibrating molecule



$\omega_0 + \omega_{vib}$ ω_0 $\omega_0 - \omega_{vib}$



Modulated electric field with angular frequency $\omega_0 \pm \omega_{vib}$

<u>Induced</u> dipole moment: $\vec{\mu} = \alpha \vec{E}$

© Prof. Sebastian Schlücker

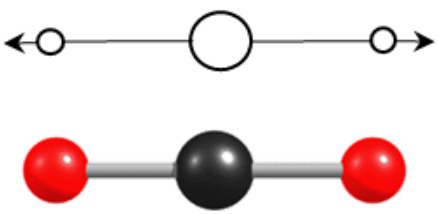
Modulation of the incident electric field by vibrating molecule

Induced dipole moment: $\vec{\mu} = \alpha \vec{E}$

$$\mu = \underbrace{\alpha_0 E_0 \cos \omega_0 t}_{\text{Rayleigh scattering}} + \underbrace{\frac{1}{2}\boxed{\left(\frac{\partial \alpha}{\partial q}\right)_0} q_0 E_0 \cos\left(\omega_0 - \omega_R\right) t}_{\text{Stokes Raman scattering}}$$

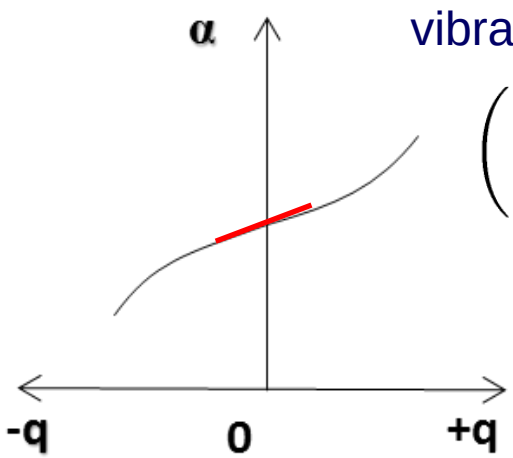
$$+\underbrace{\frac{1}{2}\left(\frac{\partial \alpha}{\partial q}\right)_0 q_0 E_0 \cos\left(\omega_0 + \omega_R\right) t}_{\text{Anti-Stokes Raman scattering}}$$

Example:
symm. stretch CO_2



Raman-active vibration since

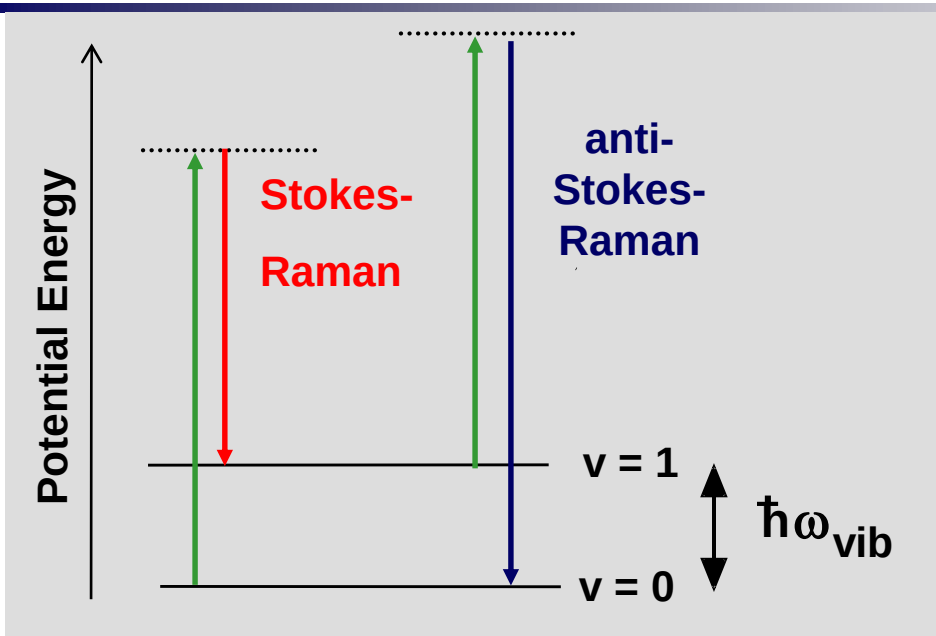
$$\left(\frac{\partial \alpha}{\partial q}\right)_0 \neq 0$$

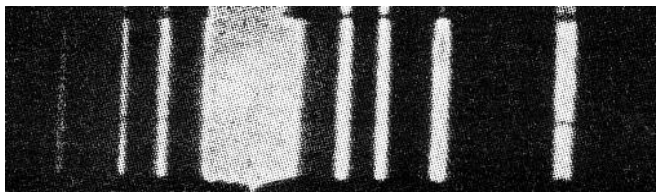


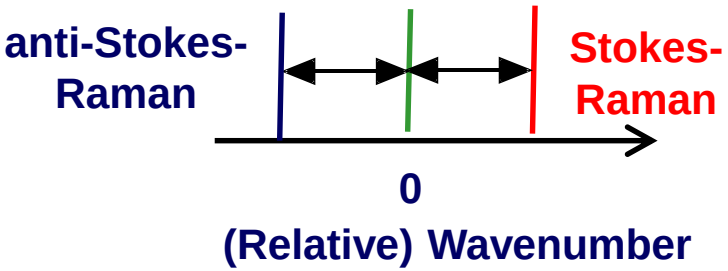
Selection Rule of Raman Scattering:
change of polarizability when passing through the equilibrium position (q=0)

© Prof. Sebastian Schlücker

Raman is Routinely Performed at the Stokes Side







Relative Intensities: **Boltzmann Factor**

$$\frac{N(v=1)}{N(v=0)} = e^{-\Delta E/k_B T} = e^{-\hbar\omega_{vib}/k_B T}$$

At room temperature:

$$k_B T \approx 200\ \text{cm}^{-1} \text{ or } 1\ \text{eV}/40 = 25\ \text{meV}$$

Helpful: 500 nm = 20 000 cm^{-1} ≈ 2.5 eV

Examples:

S-S stretch

$$\approx 500\ \text{cm}^{-1}$$

$$e^{-500/200}$$

$$= e^{-5/2}$$

$$\approx 8\%$$

C-H stretch (aromatic)

$$\approx 3000\ \text{cm}^{-1}$$

$$e^{-3000/200}$$

$$= e^{-15}$$

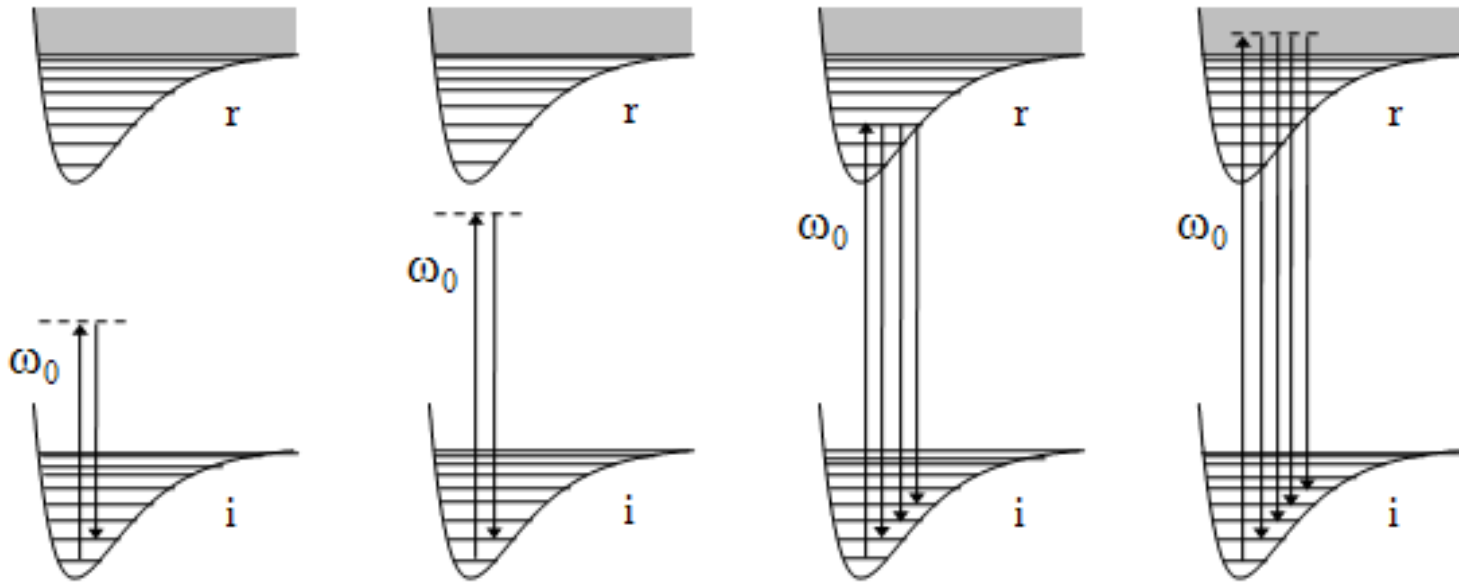
$$\approx 3 \cdot 10^{-5}\ \%$$

© Prof. Sebastian Schlücker

Quantum-mechanical Description of Raman Scattering

$$I_{fi} = C I_o \omega_{sc}^4 \sum_{\rho,\sigma} \left| (\alpha_{\rho\sigma})_{fi} \right|^2$$

$$(\alpha_{\rho\sigma})_{fi} = \sum_r \left[\frac{\langle f|\mu_\rho|r\rangle\langle r|\mu_\sigma|i\rangle}{\hbar\omega_{ri} - \hbar\omega_0 - i\Gamma_r} + \frac{\langle f|\mu_\sigma|r\rangle\langle r|\mu_\rho|i\rangle}{\hbar\omega_{rf} + \hbar\omega_0 - i\Gamma_r} \right]$$



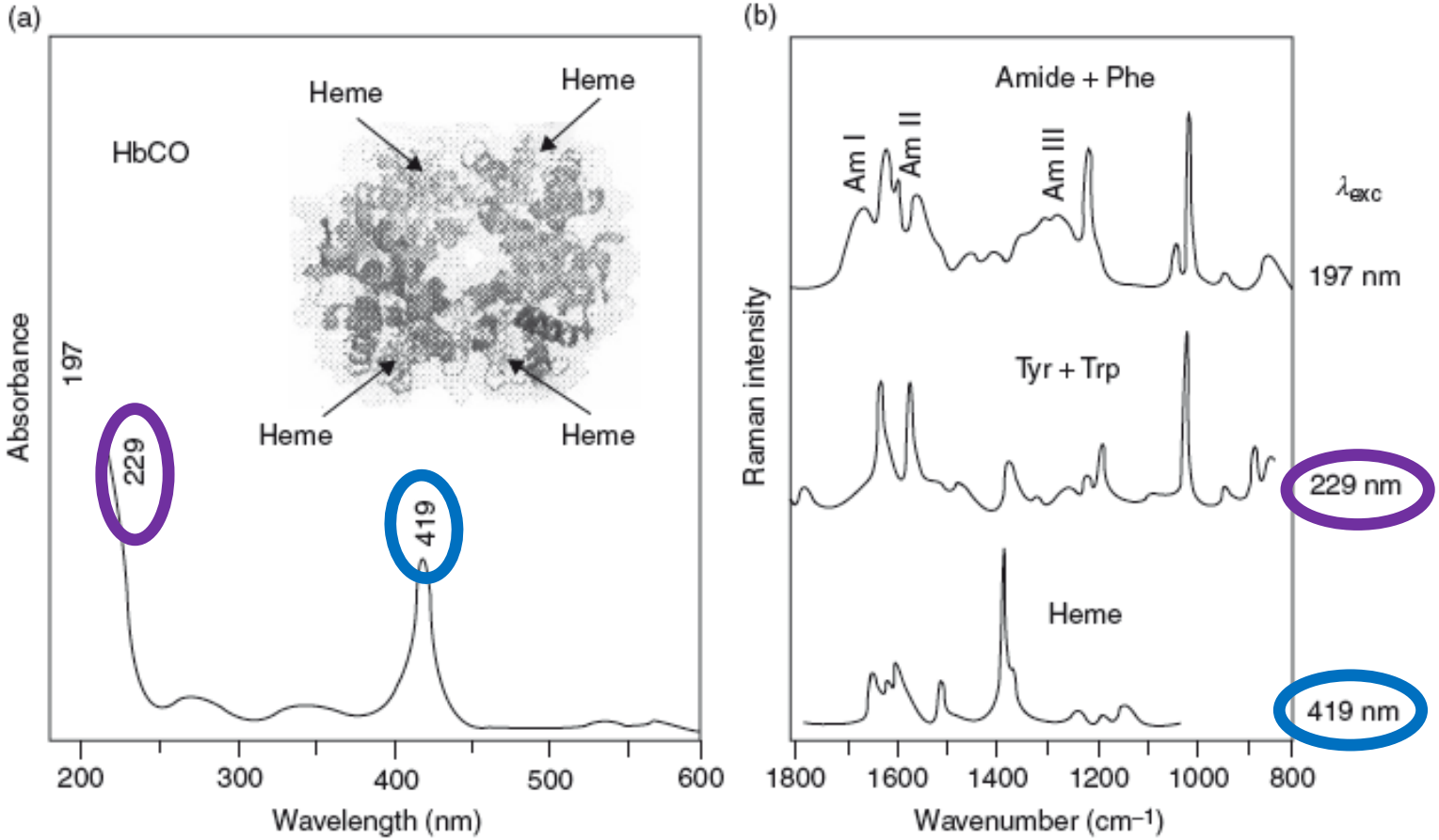
a) Normal Raman (b) Pre-resonance Raman (c) Discrete resonance Raman (d) Continuum resonance Raman

© Prof. Sebastian Schlücker

Case Study Hemoglobin: Resonance Raman (RR) Scattering

Sensitivity:
Enhanced Raman signal: dilute samples

Selectivity:
Probe only the chromophoric subunit

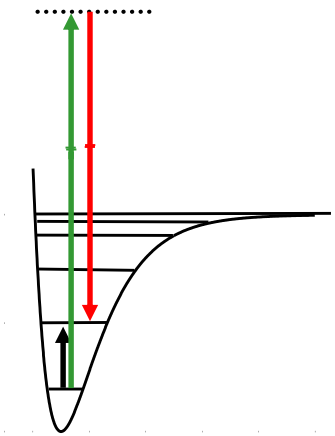


Schlücker S and Srivastava SK. Resonance Raman Applications. In: John Lindon, George Tranter and David Koppenaal, editors. Encyclopedia of Spectroscopy and Spectrometry, 2nd edition, Vol 3. Oxford: Elsevier; 2010. pp. 2426–2434.

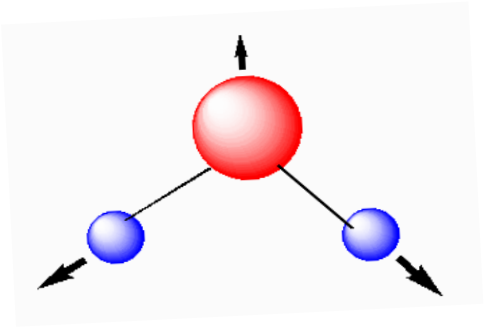
© Prof. Sebastian Schlücker

Molecular Vibrations – Vibrational Spectroscopy – Symmetry

MIR and Vibrational Raman Spectroscopy

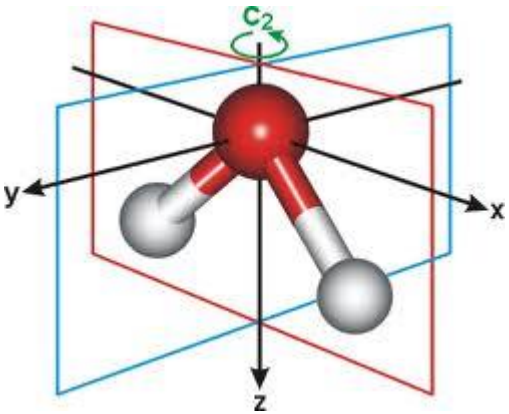


Molecular Vibrations, Energy Levels, Quantum Chemistry



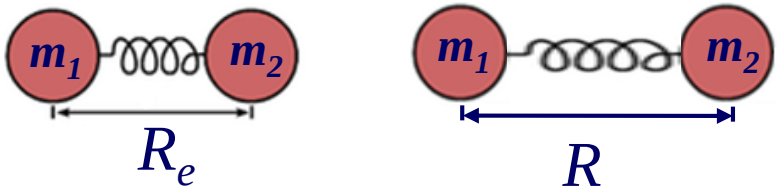
Case Study: Water

Molecular Symmetry and Group Theory

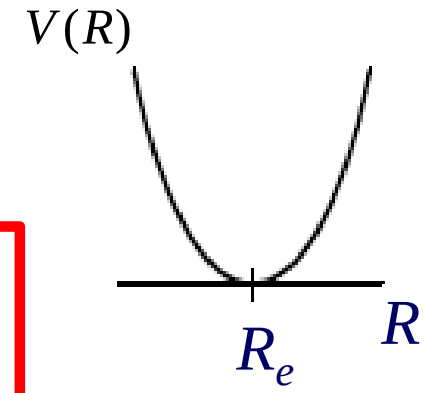


© Prof. Sebastian Schlücker

The Harmonic Oscillator: Eigenfrequency



$$\mu = \frac{m_1 m_2}{m_1 + m_2}$$



$$\boxed{\omega = 2\pi\nu = \sqrt{\frac{k}{\mu}}}$$

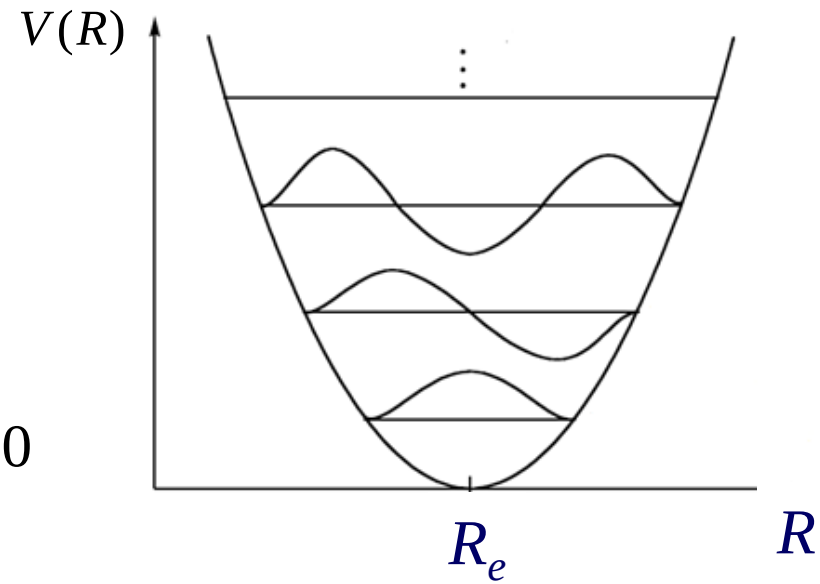
Classical

QM $\quad H\psi_{vib} = E_{vib}\psi_{vib}$

$$\{V(R) + T(R)\}\psi_{vib} = E_{vib}\psi_{vib}$$

$$V(R) = \frac{1}{2}k(R - R_e)^2$$

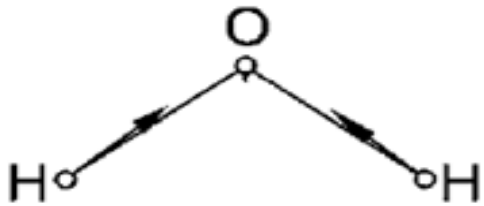
$$\frac{\partial^2 \psi_{vib}}{\partial R^2} + \frac{2\mu}{\hbar^2}\left[E_{vib} - \frac{1}{2}k(R - R_e)^2\right]\psi_{vib} = 0$$



© Prof. Sebastian Schlücker

Normal Modes of Vibration

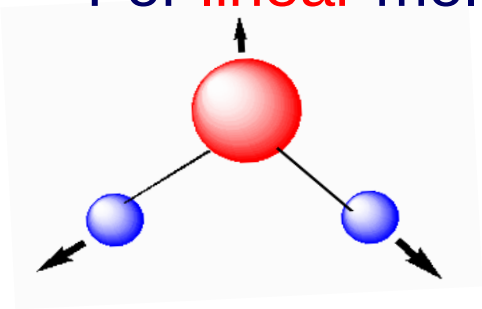
A mode of vibration is called normal mode of vibration when all atoms in the molecule oscillate with *same frequency* and *in phase*.
The *center of mass must not change*.



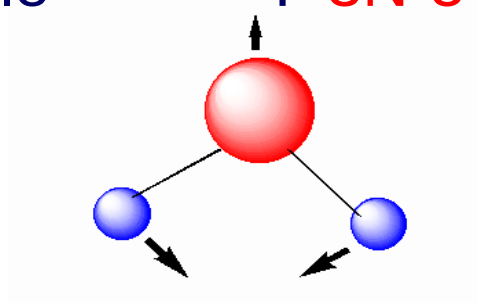
Number of normal modes of vibration for a polyatomic molecule with N atoms

For non-linear molecule : 3N-6 (All – 3 Trans – 3 Rot)

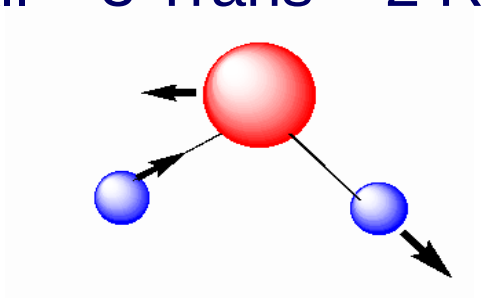
For linear molecule : 3N-5 (All – 3 Trans – 2 Rot)



Symmetric stretching



Bending

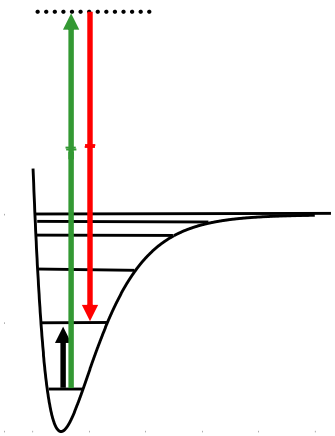


Anti-symm. stretching

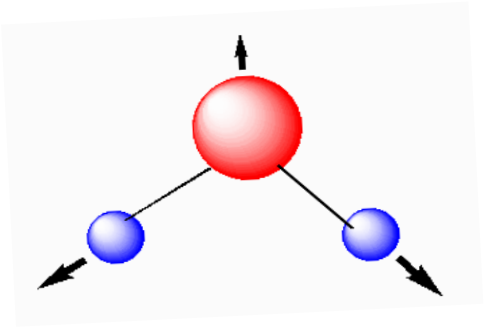
© Prof. Sebastian Schlücker

Molecular Vibrations – Vibrational Spectroscopy – Symmetry

MIR and Vibrational Raman Spectroscopy

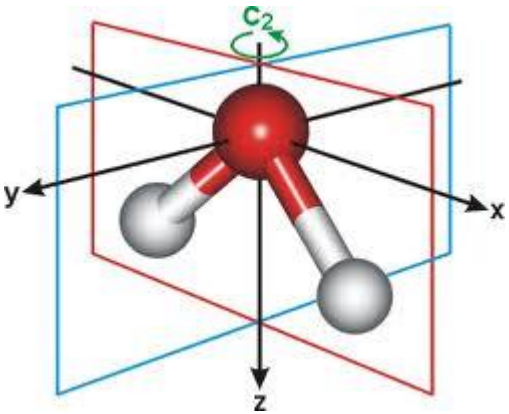


Molecular Vibrations, Energy Levels, Quantum Chemistry



Case Study: Water

Molecular Symmetry and Group Theory

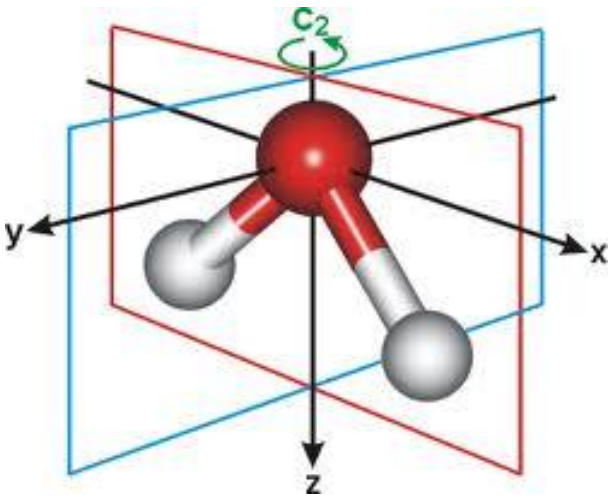


© Prof. Sebastian Schlücker

Case Study Water: Symmetry Elements

Symmetry Elements

Identity	Axis of Rotation	Mirror Planes	
E	C_2	$\sigma_v(xz)$	$\sigma'_v(yz)$



Character Table

C_{2v}	E	C_2	$\sigma_v(xz)$	$\sigma'_v(yz)$		
A_1	1	1	1	1	z	x^2, y^2, z^2
A_2	1	1	−1	−1	R_z	xy
B_1	1	−1	1	−1	x, R_y	xz
B_2	1	−1	−1	1	y, R_x	yz

Irreducible Represen-tations

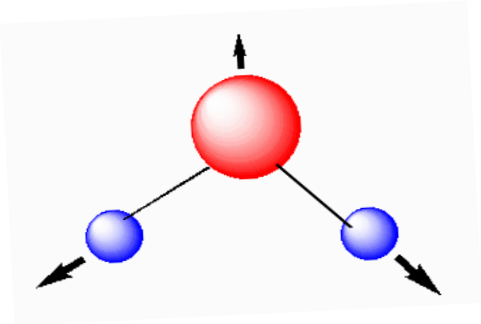
IR + Rotation

Raman

(Cotton: Chemical Applications of Group Theory)

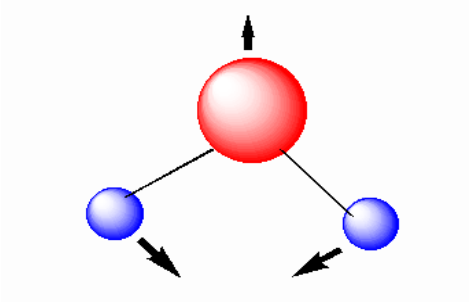
© Prof. Sebastian Schlücker

Case Study Water: Symmetry of Normal Modes

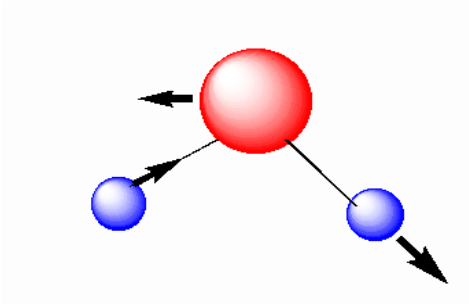


Symmetric stretching

$\nu(a_1)$

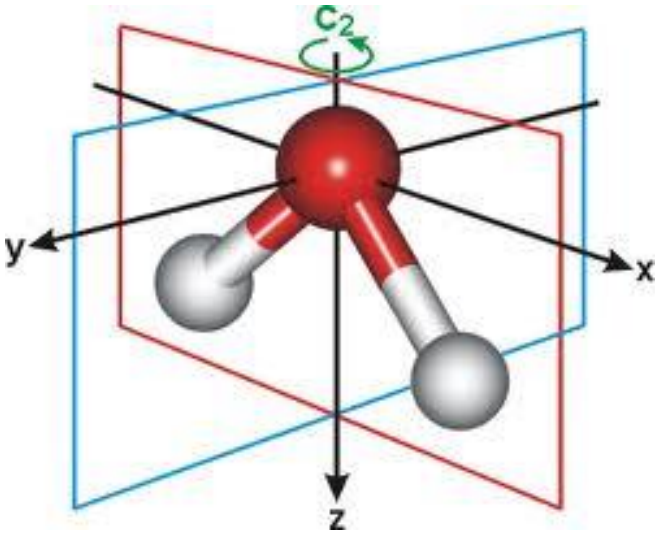


Bending



Anti-symm. stretching

C_{2v}	E	C_2	$\sigma_v(xz)$	$\sigma'_v(yz)$
A_1	1	1	1	1
A_2	1	1	−1	−1
B_1	1	−1	1	−1
B_2	1	−1	−1	1



© Prof. Sebastian Schlücker

Overview 2: Raman Microscopies

RAMAN MICROPROBE AND MICROSCOPE WITH LASER EXCITATION

M. DELHAYE and P. DHAMELINCOURT
Service de Spectrochimie infra-rouge et Raman C.N.R.S., Université de Lille, B.P. 36, 59650 Villeneuve d'Ascq, France

(Received 13 November, 1974)

Linear techniques (cw lasers):	Nonlinear techniques (pulsed lasers):
Resonance Raman	CARS
SERS, iSERS	SRS
TERS	SE(CARS/SRS)

© Prof. Sebastian Schlücker

Overview 2: Raman Microscopies

RAMAN MICROPROBE AND MICROSCOPE WITH LASER EXCITATION

M. DELHAYE and P. DHAMELINCOURT

Service de Spectrochimie infra-rouge et Raman C.N.R.S., Université de Lille, B.P. 36, 59650 Villeneuve d'Ascq, France

(Received 13 November, 1974)

Linear techniques (cw lasers):	Nonlinear techniques (pulsed lasers):
Resonance Raman	CARS
SERS, iSERS	SRS
TERS	SE(CARS/SRS)

© Prof. Sebastian Schlücker

The first "Raman Microprobe" (1975)

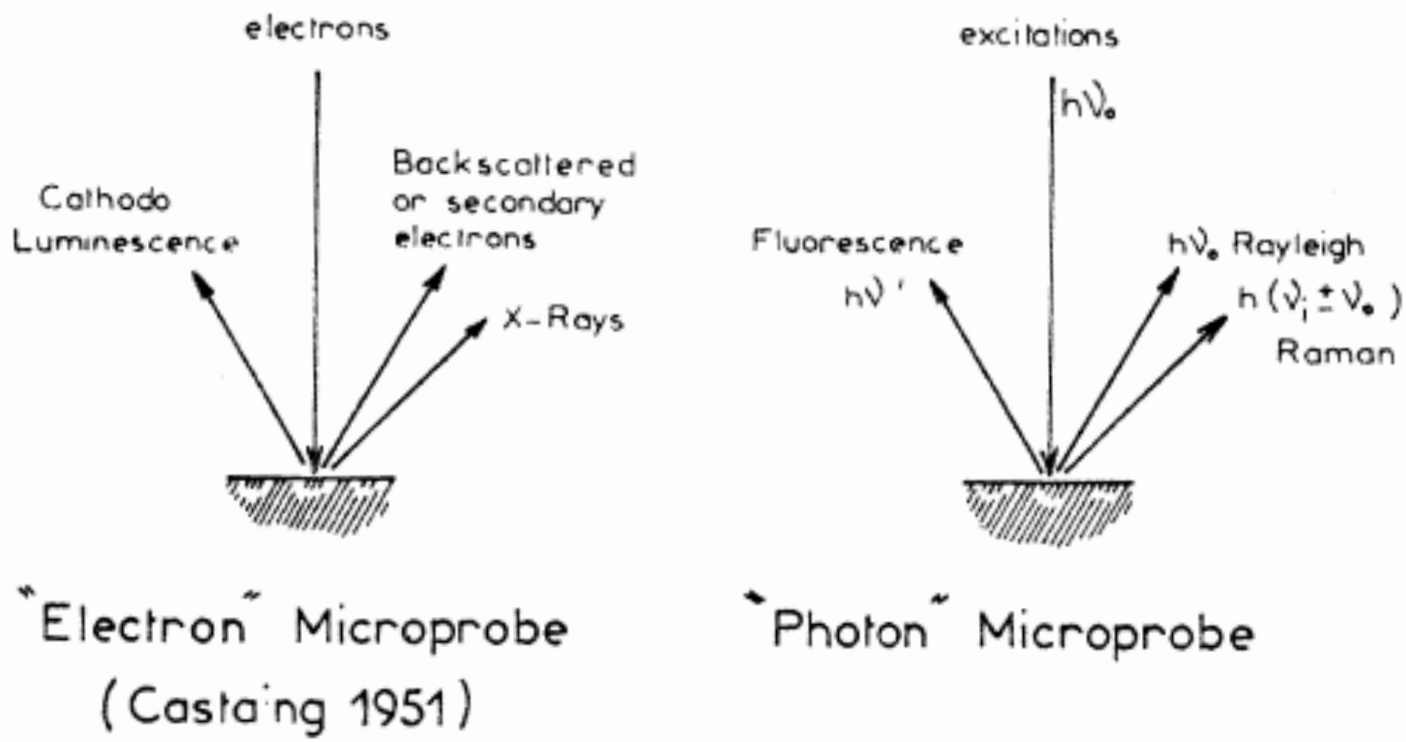


Fig. 1. Basic principles of the Electron Microprobe and the Raman Microprobe.

© Prof. Sebastian Schlücker

The first "Raman Microprobe" (1975)

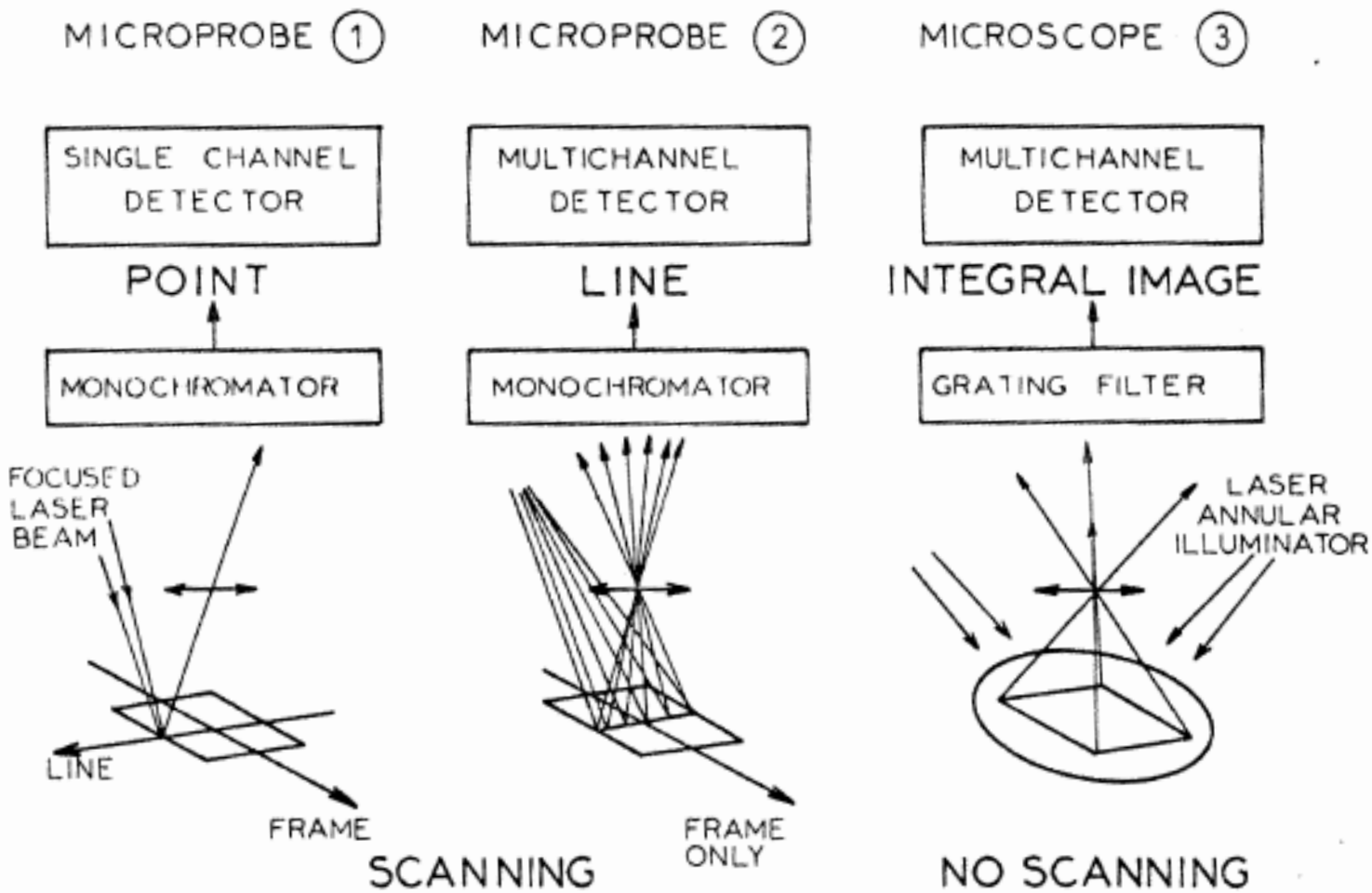


Fig. 2. Schematic representation of the methods for obtaining images of samples with characteristic Raman frequencies.

© Prof. Sebastian Schlücker

The first "Raman Microprobe" (1975)

Rayleigh

30μ

Raman

850 cm^{-1}

2056 cm^{-1}

Fig. 11. The Raman Microscope working as a probe: The sample is a mixture of chromate and ferrocyanide microcrystals with dust particles. *Upper*: Micrograph obtained with the exciting line. *Lower left*: Micrograph of the same area obtained in a characteristic Raman frequency of the chromate ion. *Lower right*: Micrograph of the same area obtained in a characteristic frequency of the $-C \equiv N$ group.

© Prof. Sebastian Schlücker

Overview 2: Raman Microscopies

RAMAN MICROPROBE AND MICROSCOPE WITH LASER EXCITATION

M. DELHAYE and P. DHAMELINCOURT
Service de Spectrochimie infra-rouge et Raman C.N.R.S., Université de Lille, B.P. 36, 59650 Villeneuve d'Ascq, France

(Received 13 November, 1974)

Linear techniques (cw lasers):

Resonance Raman

SERS, iSERS

TERS

Nonlinear techniques (pulsed lasers):

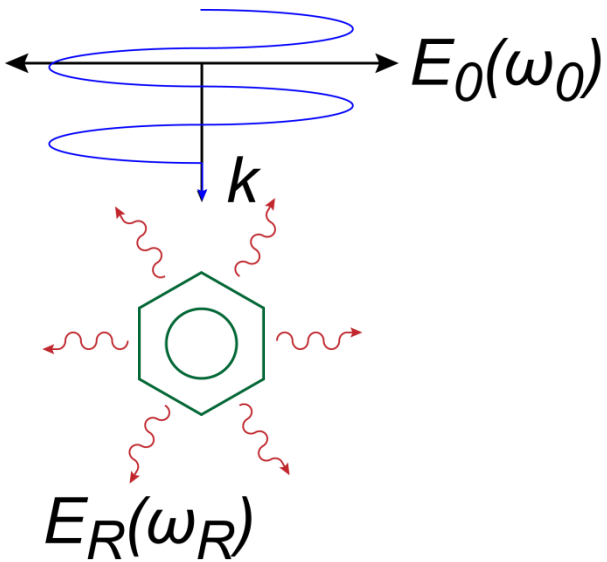
CARS

SRS

SE(CARS/SRS)

© Prof. Sebastian Schlücker

Normal versus Surface-Enhanced Raman Scattering (SERS)



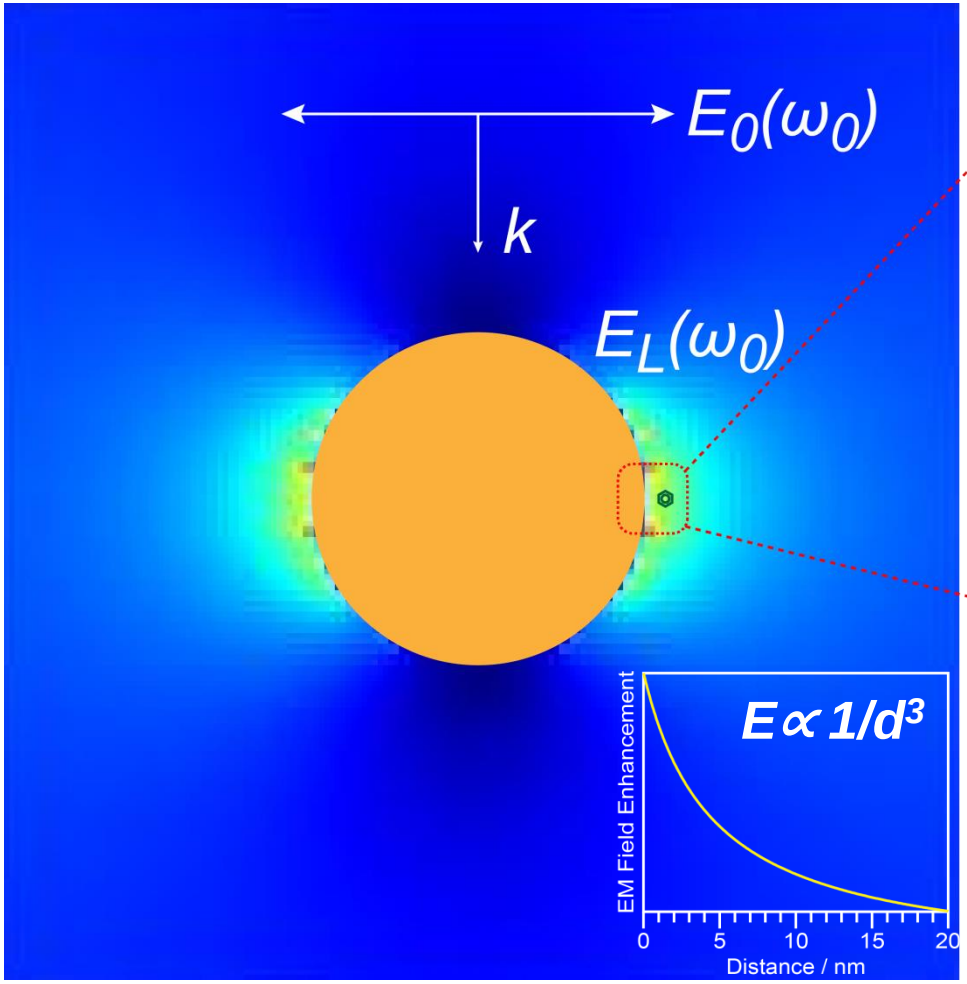
Normal Raman:

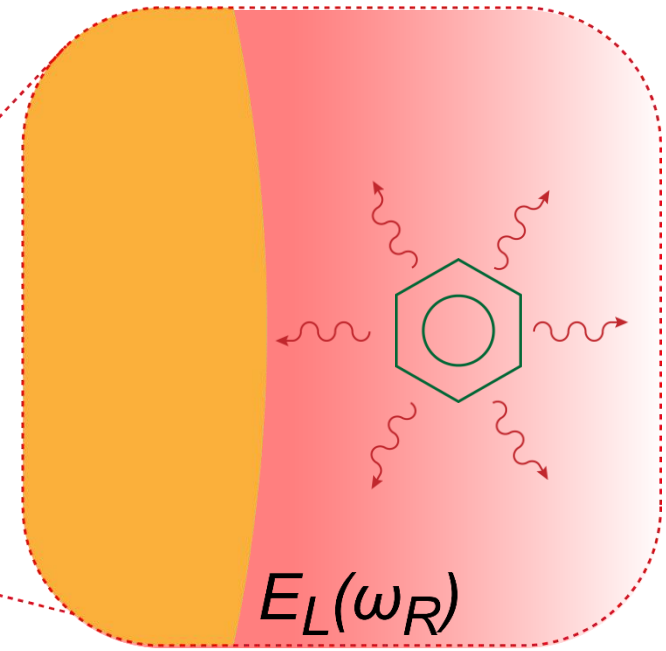
$$I_R \propto E^2$$

© Prof. Sebastian Schlücker

Normal versus Surface-Enhanced Raman Scattering (SERS)

The Electromagnetic Enhancement (EM) Contribution





Surface-Enhanced Raman Spectroscopy: Concepts and Chemical Applications
S. Schlücker, *Angew. Chem. Int. Ed.*, **2014**, 53, 4756-4795.

SERS:

$$I_{SERS} \propto |E_{loc}(\omega_0)|^2 \cdot |E_{loc}(\omega_0 + \omega_R)|^2$$

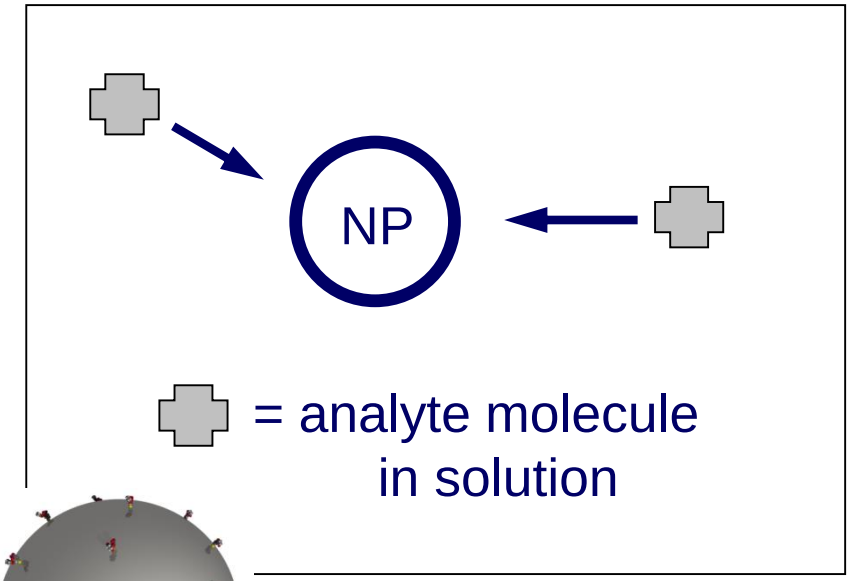
$$\approx |E_{loc}|^4$$

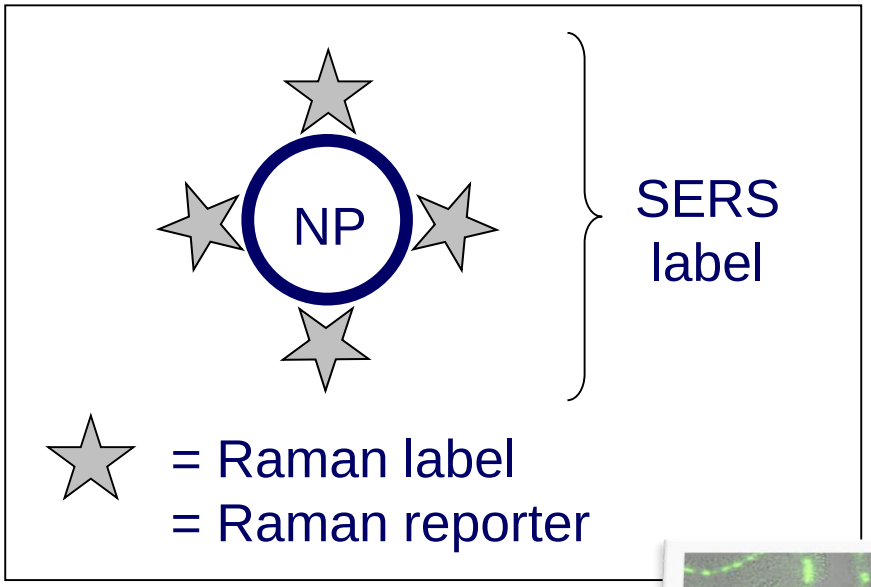
Surface selectivity $I_{SERS} \propto 1/d^{12}$ **Sensitivity**

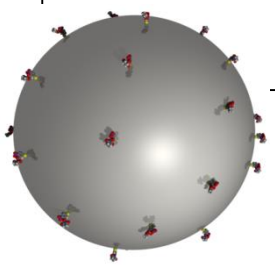
© Prof. Sebastian Schlücker

SERS Applications with Noble Metal Colloids

Label-free vs. SERS labels (nanotags)





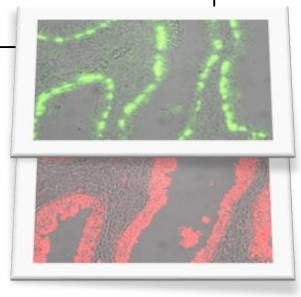


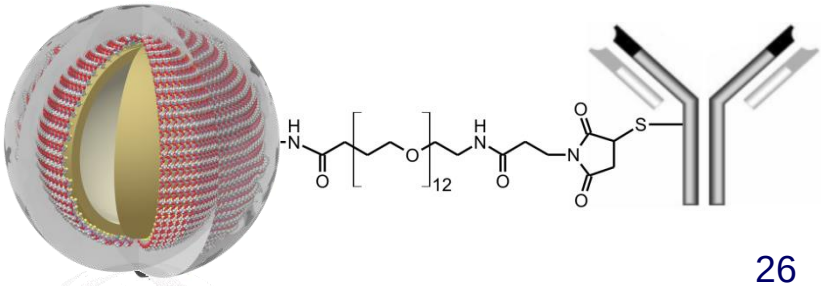
- **Detection of Analytes**

  biomolecules, drugs, explosives, food components, pharmaceuticals, pigments (e.g., in paintings)

- **Chemical Reaction Monitoring**

- **Immunoassays**
- **iSERS Microscopy**





© Prof. Sebastian Schlücker

Overview 2: Raman Microscopies

RAMAN MICROPROBE AND MICROSCOPE WITH LASER EXCITATION

M. DELHAYE and P. DHAMELINCOURT
Service de Spectrochimie infra-rouge et Raman C.N.R.S., Université de Lille, B.P. 36, 59650 Villeneuve d'Ascq, France

(Received 13 November, 1974)

Linear techniques (cw lasers):	Nonlinear techniques (pulsed lasers):
Resonance Raman	CARS
SERS, iSERS	SRS
TERS	SE/TE & CARS/SRS

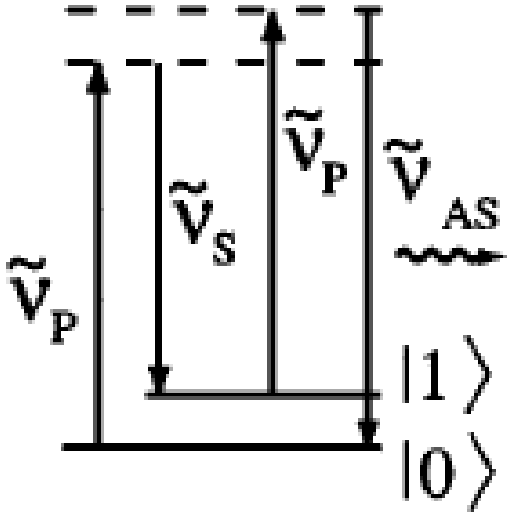
© Prof. Sebastian Schlücker

Coherent Anti-Stokes Raman (CARS) Microscopy

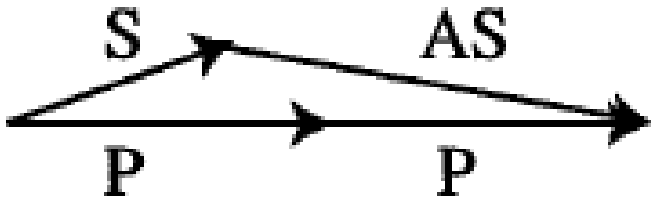
VOLUME 82, NUMBER 20 PHYSICAL REVIEW LETTERS 17 MAY 1999

Three-Dimensional Vibrational Imaging by Coherent Anti-Stokes Raman Scattering

Andreas Zumbusch,* Gary R. Holtom, and X. Sunney Xie†

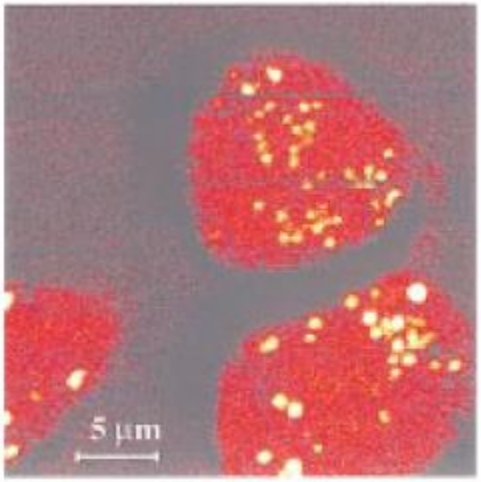


energy conservation



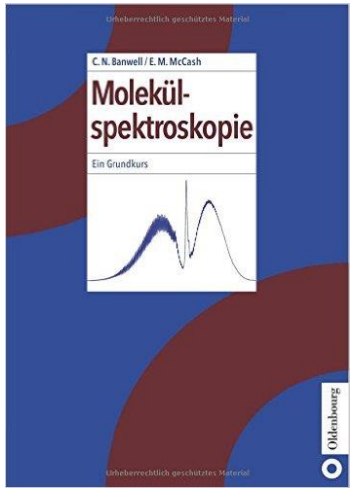
momentum conservation

lipid distribution in cells

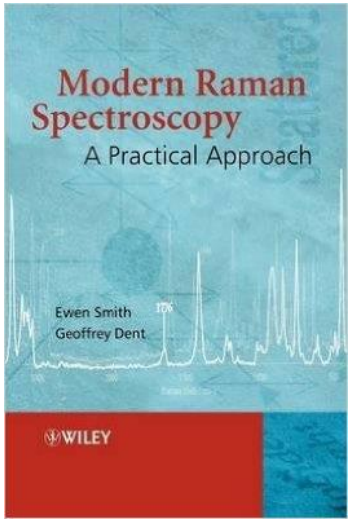


© Prof. Sebastian Schlücker

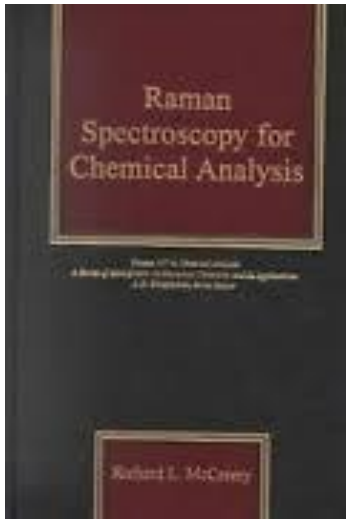
Literature: Molecular/Raman Spectroscopy & Microscopy



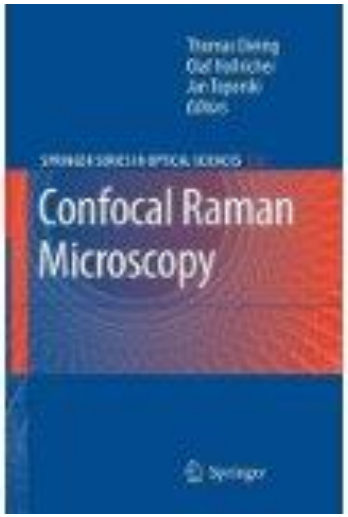
Molecular Spectroscopy, Banwell McCash



Modern Raman Spectroscopy Smith & Dent



Raman Spectroscopy for Chemical Analysis, McCreery



Confocal Raman Microscopy, Dieing, Hollricher, Toporski (Eds.)

© Prof. Sebastian Schlücker

Thank you
for your attention !

PDF of slides@
https://mint-lernen.de/raman/

© Prof. Sebastian Schlücker