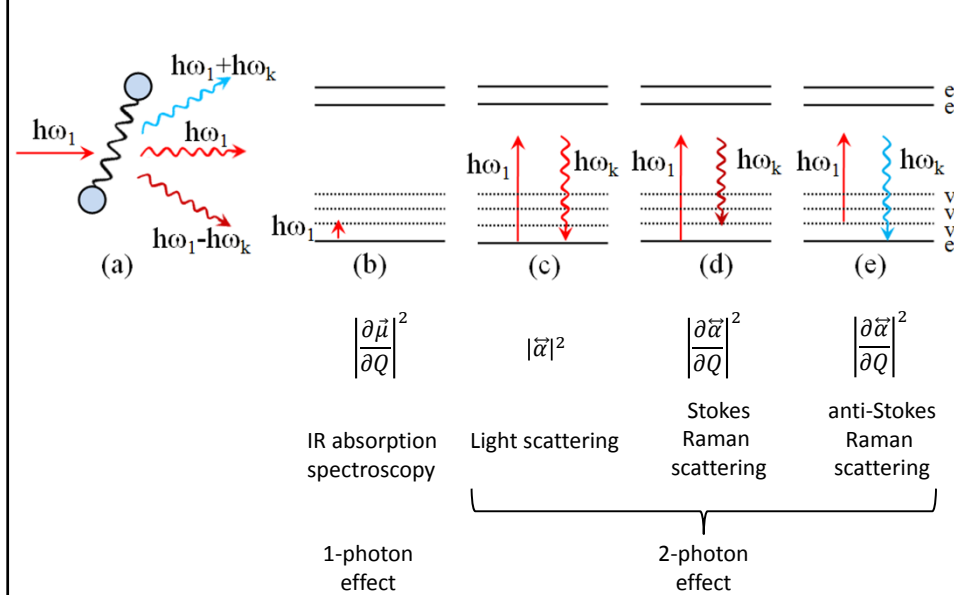


# Fundamentals of Raman spectroscopy

Part1  
Cees Otto

## Vibrational Spectroscopy



## Nanometers, wavenumbers and relative wavenumbers

Absolute wavenumbers:

$$\sigma [\text{in cm}^{-1}] = \frac{10^7}{\lambda [\text{in nm}]}$$

Example: 500 nm corresponds to 20000 cm<sup>-1</sup>

Relative wavenumbers:

$$\sigma_{\text{Raman}} [\text{cm}^{-1}] = \frac{10^7}{\lambda_{\text{exc}} [\text{in nm}]} - \frac{10^7}{\lambda_{\text{sc}} [\text{in nm}]}$$

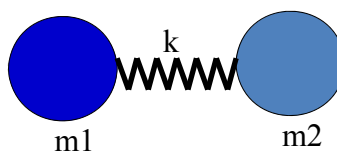
Example: A Raman band at 1020 cm<sup>-1</sup> and excited with a laser wavelength of 500 nm scatters light at a wavelength of 527 nm.

A vibration that absorbs light at 1020 cm<sup>-1</sup> absorbs light in the infrared at a wavelength of 9.8 μm (Check everything!)

## The harmonic oscillator

The Schrödinger equation for the harmonic oscillator describes atomic vibrations in molecules, which are also called **molecular vibrations**:

For a di-atomic molecule:



$$\nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}}$$

The **frequency** of the vibration depends on the “spring” Constant, **k**, and the reduced mass, **μ**, of the atoms involved in the motion.

$$\frac{1}{\mu} = \frac{1}{m_1} + \frac{1}{m_2}$$

The frequency is expressed in cm<sup>-1</sup>

$$1 \text{ cm}^{-1} \sim 30 \text{ GHz} \quad \nu [\text{Hz}] = \tilde{\nu} [\text{cm}^{-1}] c [\text{cm/s}]$$

Motions of light atoms (C-H, N-H, O-H) have high frequencies: ~ 2700-4000 cm<sup>-1</sup>

Motions of heavy atoms (S-S- bridges) have low frequencies: < 600 cm<sup>-1</sup>

Motions involving C, N, O can be anywhere between 600 - 2700 cm<sup>-1</sup>

Tables relate chemical groups with vibration frequencies are available in the literature

## Raman spectroscopy

- 1: Band positions
- 2: Band width
- 3: Amplitude of a band  
(related to Raman cross section)

## A comparison between cross sections

Electronic (UV-Vis) Absorption spectroscopy:	$10^{-20} \text{ m}^2$
Fluorescence spectroscopy:	$Q \times 10^{-20} \text{ m}^2$
Vibrational (IR) absorption spectroscopy:	$10^{-23} \text{ m}^2$
Resonance Raman spectroscopy:	$10^{-29} \text{ m}^2$
Non-resonant Raman spectroscopy:	$10^{-33} \text{ m}^2$
Surface Enhanced Raman Scattering:	$10^{-7} \text{ m}^2$

Surface enhanced Raman scattering cross sections vary widely in literature reports. There seems to be consensus developing that estimates the SERS cross sections between 6 to 8 orders of magnitude larger than the “normal” non-resonant and resonant Raman cross sections.

So:  
Surface Enhanced Raman Scattering:  $10^{-21}$  to  $10^{-27} \text{ m}^2$

Reported literature values are even as high as  $10^{-16} \text{ m}^2$ . This requires further research.

## Fluorescence “cross section”

$$n_{fl}^{det} = \sigma_{abs} N_{mol} \frac{P}{A \hbar \omega} Q_{fl} E^{det}$$

$$\sigma_{abs} \approx 10^{-20} m^2$$

### Fluorescence imaging

$\lambda_{exc} = 457$  nm.  
128 x 128 pixels  
image time : 8 seconds  
power: 5 microwatt

**A(rea):  $2.4 \cdot 10^{-13} m^2$**

**E<sup>det</sup> : 6 - 13 %**

**Intensity:  $\sim 10^7 W/m^2$**   
**Flux density:  $\sim 10^{26} 1/(m^2s)$**

$$n_{fl}^{det} = 10^5 N_{mol}$$

## Raman cross section

$$n_R^{det} = \sigma_R (3m - 6) N_{mol} \frac{P}{A \hbar \omega} E^{det}$$

$$\sigma_R \approx 10^{-33} m^2 \text{ (non-res.)}$$

**A(rea):  $2.4 \cdot 10^{-13} m^2$**

**E<sup>det</sup> : 6 - 13 %**

### Raman imaging

$\lambda_{exc} = 647$  nm.  
64 x 64 pixels  
image time : 1800 seconds  
power: 60 miliwatt

Status:2003

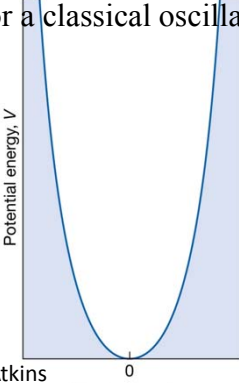
**Intensity:  $\sim 10^{11} W/m^2$**   
**Flux density:  $\sim 10^{30} 1/(m^2s)$**

$$n_R^{det} = (3m - 6) 10^{-4} N_{mol}$$

$\sim 10$  photons/day.mol.vib

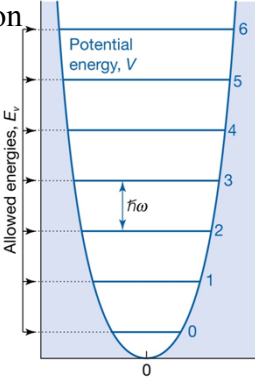
### The harmonic oscillator

The parabolic potential for a classical oscillation



From Atkins

The energy states of a QM harmonic oscillator form a "ladder"



Allowed energies,  $E_v$

$E_v = (v + \frac{1}{2})h\nu_0$

Transitions can only occur between consecutive states:  $\Delta v = \pm 1$

$\Delta E = E_{v+1} - E_v = h\nu_0$

In non-linear molecules with "n" atoms  $3n-6$  vibrations exist. Each vibration has a distinct force constant and reduced mass and, therefore, frequency. Raman spectra contain information on the vibration frequencies. Chemical analysis is possible because the frequencies are unique for molecular groups.

### Band positions

Hooke's law:  $F = -kx$  with "k", the force constant. "x" is the displacement from the equilibrium position

The relation between "force" and "potential energy" is:  $F = -\frac{\partial V}{\partial x}$

Follows:  $V = \frac{1}{2}kx^2$  a parabolic potential

The S. eq. for the harmonic oscillator becomes:

$$-\frac{\hbar^2}{2\mu} \frac{\partial^2 \psi(x)}{\partial x^2} + \frac{1}{2}kx^2 \psi(x) = E\psi(x)$$

The wave functions:  $\psi_v(x) = N_v H_v\left(\frac{x}{\alpha}\right) e^{-\frac{x^2}{2\alpha^2}}$  ( $v=0,1,2,3,\dots$ )

The eigenvalues:  $E_v = (v + \frac{1}{2})h\nu_0$   $\nu_0 = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}}$   $\alpha = \left(\frac{\hbar^2}{mk}\right)^{1/4}$

The energy difference between adjacent levels is:  $\Delta E = E_{v+1} - E_v = h\nu_0$

## The wavefunctions of the harmonic oscillator

$$\psi_v(x) = N_v H_v\left(\frac{x}{\alpha}\right) e^{-\frac{x^2}{2\alpha^2}} \quad (v=0,1,2,3,\dots)$$

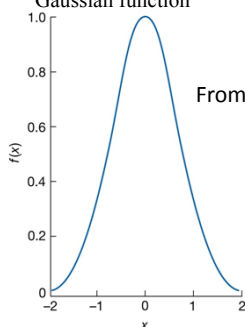
with  $H_v$  the Hermite polynomials (See page 340, table 12.1).

$\alpha$  is proportional to the steepness of the parabolic function, meaning large  $\alpha$  shallow potential well, small  $\alpha$  steep potential well.

$$\alpha = \left(\frac{\hbar^2}{mk}\right)^{1/4}$$

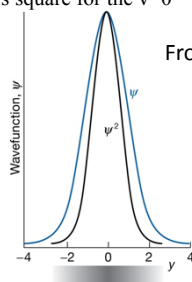
The normalization constant  $N_v$  is:  $N_v = \left(\frac{1}{\alpha \pi^{1/2} 2^v v!}\right)^{1/2}$

Fig. 8.19 The graph of the Gaussian function



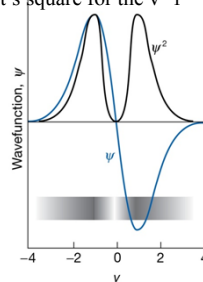
From Atkins

Fig. 8.20 the wavefunction and its square for the  $v=0$



From Atkins

Fig. 8.21 the wavefunction and its square for the  $v=1$



## Hermite polynomials

The wave functions for the harmonic oscillator look as follows:

$$\psi_v(x) = N_v H_v\left(\frac{x}{\alpha}\right) e^{-\frac{x^2}{2\alpha^2}} \quad (v=0,1,2,3,\dots)$$

The polynomial functions  $H_v\left(\frac{x}{\alpha}\right)$  are a power series of the argument and take the following form (with  $y=x/\alpha$ ):

$$H_v(y) = 1 \quad (v=0)$$

$$H_v(y) = 2y \quad (v=1)$$

$$H_v(y) = 4y^2 - 2 \quad (v=2)$$

$$H_v(y) = 8y^3 - 12y \quad (v=3)$$

$$H_v(y) = 16y^4 - 48y^2 + 12 \quad (v=4)$$

$$H_v(y) = 32y^5 - 160y^3 + 120y \quad (v=5)$$

See page 302-306 for more details.

## Some properties of the harmonic oscillator

$x=0$  is the equilibrium position of two nuclei connected with a “spring”.  
The expectation value for  $x$  and  $x^2$ , respectively  $\langle x \rangle$  and  $\langle x^2 \rangle$  is:

$$\langle x \rangle_v = 0$$

$$\langle x^2 \rangle = \left(v + \frac{1}{2}\right) \frac{\hbar}{\sqrt{mk}}$$

Interpretation:

The average value of the position is equal to the equilibrium constant, so the oscillator is equally likely to be found on either side of the equilibrium position.

The mean square displacement is proportional to the vibrational quantum number.

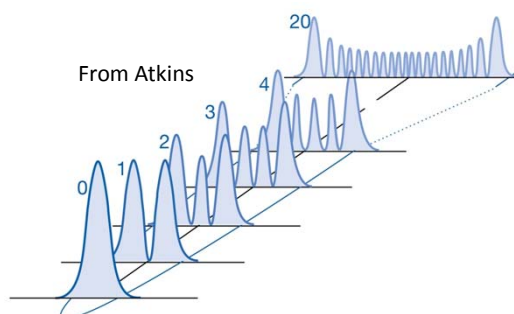


Fig. 8.23 p. 304

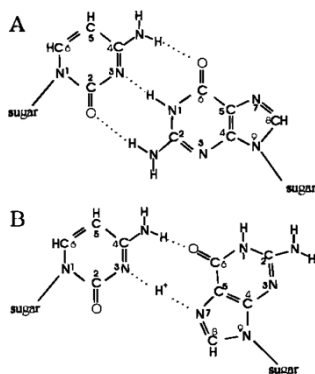
The probability distribution for the first 5 states of a harmonic oscillator and the state  $v=20$ . Note how the regions of highest probability move towards the turning points of the classical motion as “ $v$ ” increases.

## Molecular vibrations in DNA measured with Raman spectroscopy

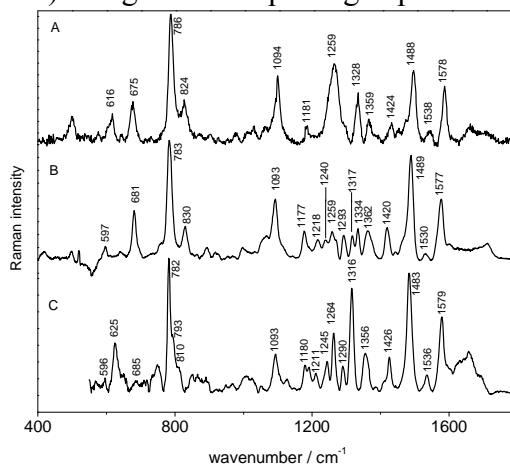
Poly(dG-dC).poly(dG-dC): C) Z-form: Watson Crick LH-helix

B) B-form: Watson-Crick RH-helix

A) Hoogsteen basepairing at pH=4.3



Changes in physico-chemical Parameters (pH, T, etc.) gives rise to profound changes in the Raman spectrum.



G.M.J. Segers-Nolten, N.M. Sijtsma, C. Otto, *Biochemistry* 36(43), 13241-13247 (1997)

## Band positions

Hooke's law

$$F = -\frac{\partial V}{\partial x} = -k x$$

For  $3n-6$  vibrations

$$\nu_i = \frac{1}{2\pi} \sqrt{\frac{k_i}{\mu_i}}$$

The force constant,  $k_i$ , are obtained from :

$$k = \frac{\partial^2 V}{\partial x^2}$$

Substituting Hooke's law in the Newton equation:

$$\mu \frac{\partial^2 x}{\partial t^2} = -k x$$

And solving the equation of motion results in:

$$x(t) = A \sin(2\pi \nu_i t)$$

with

$$\nu_i = \frac{1}{2\pi} \sqrt{\frac{k_i}{\mu_i}}$$

How does this work out for  $N$  atoms?

## Band Positions

The dynamic model for molecular vibrations may start from  $N$  individual atoms.

Each of these atoms has a  $(x,y,z)$  Cartesian coordinate system attached.

Displacement of each atom along any of its coordinates may affect other atoms

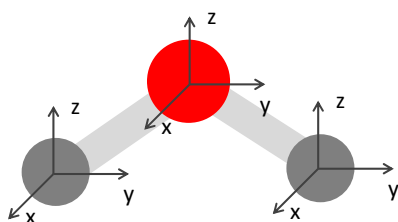
along their  $(x,y,z)$  coordinates through the spring constant. For a three-atomic molecule nine (9) degrees of freedom exist. Three degrees of freedom are connected to translations of the center of mass of the molecule (translations of the molecule),

Three degrees of freedom are connected to the rotation of the molecule around the center of mass (rotations of the molecule).

$3N-6$  degrees of freedom are connected to internal motion of atoms with respect to the center of mass. These are the molecular vibrations.

In a linear molecule  $3N-5$  vibrations exist.

The description of vibrations in cartesian coordinates of the atoms is not chemically intuitive. A coordinate systems based on internal coordinates is more suited.





## Band positions

Let us first look at a description in Cartesian coordinates. First substitute the solution to the Newton equation back into it.

We obtain: 
$$-4\pi^2 v^2 \mu x = -k x$$

N atoms have 3N degrees of freedom and 3N equations of motion result. The motion of each atom in the x-, y- and z-direction is driven back to equilibrium by a spring constant and by coupling, via spring constants, to each of the other degrees of freedom. For a three-atomic molecule we obtain 9 coupled equations of motion:

$$\begin{aligned} -4\pi^2 v^2 \mu_1 x_1 &= -k_{xx}^{11} x_1 - k_{xy}^{11} y_1 - k_{xz}^{11} z_1 - k_{xx}^{12} x_2 - k_{xy}^{12} y_2 - k_{xz}^{12} z_2 - k_{xx}^{13} x_3 - k_{xy}^{13} y_3 - k_{xz}^{13} z_3 \\ -4\pi^2 v^2 \mu_1 y_1 &= -k_{yx}^{11} x_1 - k_{yy}^{11} y_1 - k_{yz}^{11} z_1 - k_{yx}^{12} x_2 - k_{yy}^{12} y_2 - k_{yz}^{12} z_2 - k_{yx}^{13} x_3 - k_{yy}^{13} y_3 - k_{yz}^{13} z_3 \\ -4\pi^2 v^2 \mu_1 z_1 &= -k_{zx}^{11} x_1 - k_{zy}^{11} y_1 - k_{zz}^{11} z_1 - k_{zx}^{12} x_2 - k_{zy}^{12} y_2 - k_{zz}^{12} z_2 - k_{zx}^{13} x_3 - k_{zy}^{13} y_3 - k_{zz}^{13} z_3 \\ &\cdot \\ &\cdot \\ -4\pi^2 v^2 \mu_3 z_3 &= -k_{zx}^{31} x_1 - k_{zy}^{31} y_1 - k_{zz}^{31} z_1 - k_{zx}^{32} x_2 - k_{zy}^{32} y_2 - k_{zz}^{32} z_2 - k_{zx}^{33} x_3 - k_{zy}^{33} y_3 - k_{zz}^{33} z_3 \end{aligned}$$

## Band positions

The notation is much more transparent in matrix form using mass-weighted coordinates and force constants:

$$\tilde{x}_i = \sqrt{m_i} x_i$$

$$\tilde{k}_{xy}^{12} = \frac{k_{xy}^{12}}{\sqrt{m_1} \sqrt{m_2}}$$

The equations become:

$$-4\pi^2 v^2 \begin{pmatrix} \tilde{x}_1 \\ \tilde{y}_1 \\ \tilde{z}_1 \\ \tilde{x}_2 \\ \tilde{y}_2 \\ \tilde{z}_2 \\ \tilde{x}_3 \\ \tilde{y}_3 \\ \tilde{z}_3 \end{pmatrix} = - \begin{pmatrix} \tilde{k}_{xx}^{11} & \tilde{k}_{xy}^{11} & \tilde{k}_{xz}^{11} & \tilde{k}_{xx}^{12} & \tilde{k}_{xy}^{12} & \tilde{k}_{xz}^{12} & \tilde{k}_{xx}^{13} & \tilde{k}_{xy}^{13} & \tilde{k}_{xz}^{13} \\ \tilde{k}_{yx}^{11} & \tilde{k}_{yy}^{11} & \tilde{k}_{yz}^{11} & \tilde{k}_{yx}^{12} & \tilde{k}_{yy}^{12} & \tilde{k}_{yz}^{12} & \tilde{k}_{yx}^{13} & \tilde{k}_{yy}^{13} & \tilde{k}_{yz}^{13} \\ \tilde{k}_{zx}^{11} & \tilde{k}_{zy}^{11} & \tilde{k}_{zz}^{11} & \tilde{k}_{zx}^{12} & \tilde{k}_{zy}^{12} & \tilde{k}_{zz}^{12} & \tilde{k}_{zx}^{13} & \tilde{k}_{zy}^{13} & \tilde{k}_{zz}^{13} \\ \tilde{k}_{xx}^{21} & \tilde{k}_{xy}^{21} & \tilde{k}_{xz}^{21} & \tilde{k}_{xx}^{22} & \tilde{k}_{xy}^{22} & \tilde{k}_{xz}^{22} & \tilde{k}_{xx}^{23} & \tilde{k}_{xy}^{23} & \tilde{k}_{xz}^{23} \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \tilde{k}_{xx}^{31} & \tilde{k}_{xy}^{31} & \tilde{k}_{xz}^{31} & \tilde{k}_{xx}^{32} & \tilde{k}_{xy}^{32} & \tilde{k}_{xz}^{32} & \tilde{k}_{xx}^{33} & \tilde{k}_{xy}^{33} & \tilde{k}_{xz}^{33} \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \end{pmatrix} \begin{pmatrix} \tilde{x}_1 \\ \tilde{y}_1 \\ \tilde{z}_1 \\ \tilde{x}_2 \\ \tilde{y}_2 \\ \tilde{z}_2 \\ \tilde{x}_3 \\ \tilde{y}_3 \\ \tilde{z}_3 \end{pmatrix}$$

## Band positions

Introducing the following shorthand notation for the Cartesian coordinates of the atoms:

$$\xi = \begin{pmatrix} \tilde{x}_1 \\ \tilde{y}_1 \\ \tilde{z}_1 \\ \tilde{x}_2 \\ \tilde{y}_2 \\ \tilde{z}_2 \\ \tilde{x}_3 \\ \tilde{y}_3 \\ \tilde{z}_3 \end{pmatrix}$$

the equation becomes:  $-4\pi^2 \nu^2 \xi = -\vec{k} \xi$

This is an eigenvalue equation and can be solved by standard means once one knows the matrix of force constants.

The solution contains the **3N frequencies** associated with the 3N degrees of freedom. The 6 degrees of freedom associated with translations and rotations will have a frequency close to "zero". The frequencies of the 3N-6 (or 3N-5 for a linear molecule) will be different from "zero" and can be associated with the frequencies of internal modes of freedom, the molecular vibrations, of a molecule.

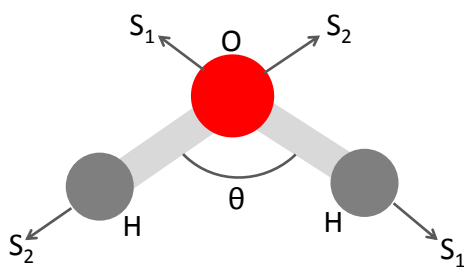
The **amplitude** of each of the atoms in each of the vibrations can also be obtained.

This is a classical model for the nuclei.

Real nuclei are not "point-like" but need to be described by a vibrational wave function, which reflects the probability to find an atom at a certain position. The classical approach gives very reasonable results for the frequencies in spite of this approximation.

## Internal Coordinates

Internal coordinates are defined according to "common sense" or chemical intuition. Several types of internal coordinates can be defined. In the three-atomic molecule below, two types occur, namely **bond stretch motions** and a **bending motion**.



Two stretch motions can be distinguished, one for each bond between the atoms. The angle between the two OH-groups varies around the equilibrium value  $\theta$ .

This angle defines a bending coordinate.

For more complicated molecules other internal coordinates can be conveniently defined as well. The most common ones are listed below:

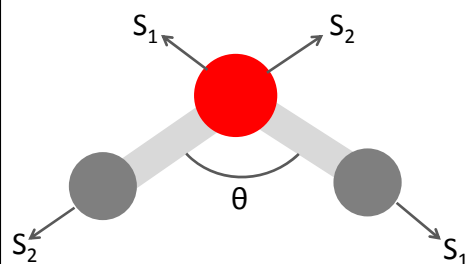
- Bond stretch coordinates
- In plane Bending coordinates
- Angle between a bond and a plane defined by two bonds
- Torsion coordinate

Other coordinates may be defined whenever a molecule "suggests" this.

## Normal coordinates

The internal coordinates do not form a symmetry optimized set of motions for the nuclei. For instance in the example below for "water", it is hard to imagine that the oxygen atom will simultaneously move to the left and to the right. The symmetry optimized motions of the

of the nuclei are the normal coordinates. One normal coordinate is associated with each normal mode of motion.



The normal modes form an orthogonal system of motions. They are linear combinations of the internal coordinates (and also of the Cartesian coordinates).

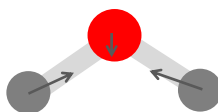
### Properties of Normal Modes are:

- Each normal normal mode behaves like a harmonic oscillator
- A normal mode is a concerted motion of many atoms
- A normal mode does not translate or rotate the molecule
- All atoms involved in a normal mode pass simultaneously through the equilibrium position
- Normal modes are independent, that is they form an orthonormal set, that is they do not interact.

## Example of Normal Modes

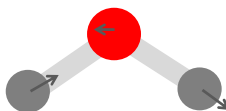
Normal modes can be constructed from the internal coordinates by forming symmetry-adapted linear combinations. For a three-atomic system this is rather straight forward, as follows:

$$Q_{sym} = S_1 + S_2$$



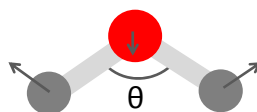
The length of the arrows represent the amplitude of the motion of the atom in the normal mode.

$$Q_{anti-sym} = S_1 - S_2$$

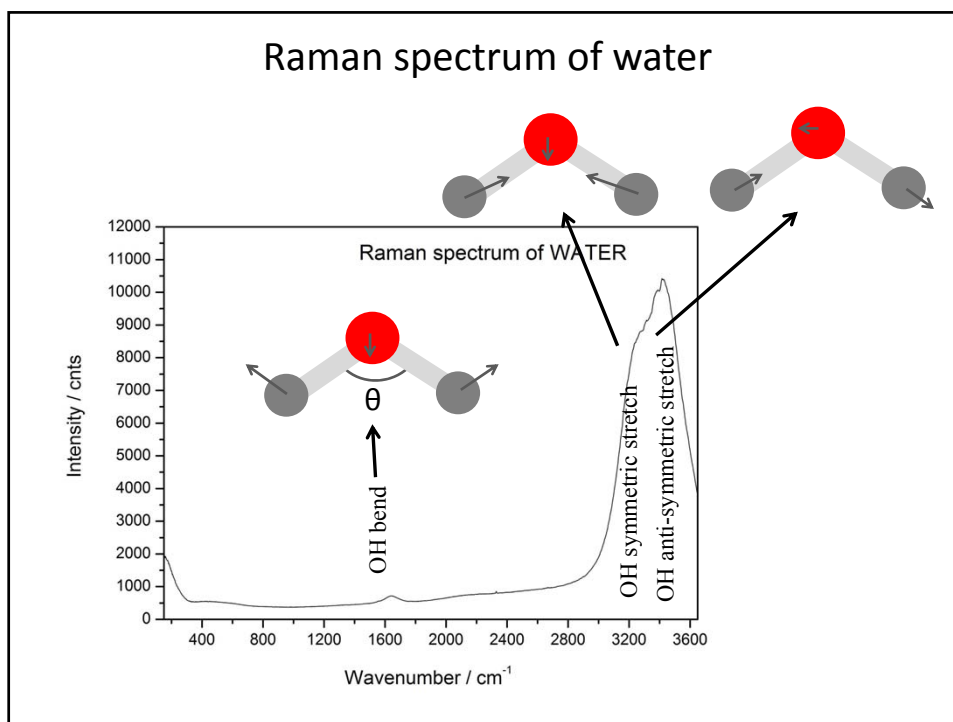


In the drawings the amplitude is greatly exaggerated. The amplitude of atomic vibrations in molecules is typically of the order of 1% of the inter-atomic distance. The inter-atomic distance is typically 0.1 nm.

$$Q_{bend} = \theta$$

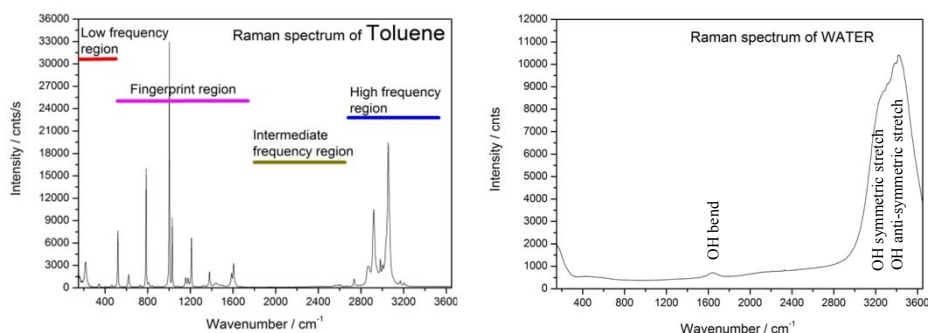


The amplitude of the vibrations is therefore typically 1 pm (1 picometer). Light atoms, like H, have somewhat larger amplitudes.



## Raman spectra of different compounds

Raman spectra of different compounds are different. A compound can be determined from the measured Raman spectrum. Raman spectroscopy is an **analytical chemical** technique!! In mixtures of compounds the Raman spectra are partially overlapping. Complicated Raman spectra result. The spectrum of the mixture can be viewed as a "pattern". Raman pattern recognition is an important approach for medical applications concerning cell and tissue Raman spectroscopy. **Multivariate analysis methods** provide powerful tools to perform pattern recognition in Raman spectra.



## Raman cross section

The previous “classical” introduction explains qualitatively all aspects but does not explain quantitative aspects of Raman scattering.

A quantum-mechanical derivation of the molecular polarizability gives the right treatment.

We will not do the derivation, but the result of the treatment provides the expression for the Raman polarizability for each normal mode (and for the polarizability in general).

The formula explain also intensities of light scattering for normal modes and is helpful to understand the difference in non-electronic resonance Raman scattering and electronic resonance Raman scattering.

## The Raman scattering cross section

We had:  $n_R^{\text{det}} = \sigma_R (3m - 6) N_{\text{mol}} \frac{P}{A \hbar \omega} E^{\text{det}}$  For (3m-6)vibrations

The (dipolar) scattering depends on direction and this becomes:

$$n_{\text{Raman}} = N \frac{P_L}{A \hbar \omega_L} \int \frac{d\sigma_R}{d\Omega} d\Omega \quad \text{For one (1) vibration}$$

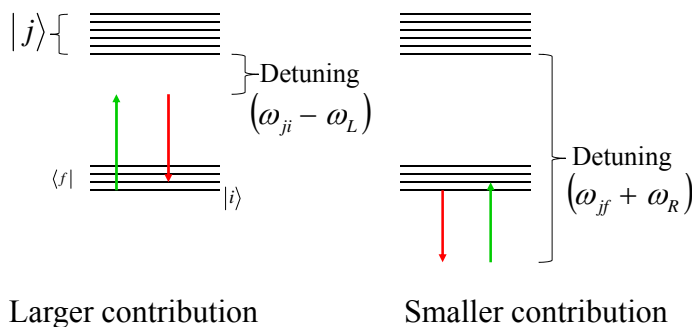
This formula recognizes through the integral over the angular dependence that the scattering is not equal in all directions.

The angle-dependent scattering cross section is:

$$\frac{d\sigma_R}{d\Omega} = \frac{(\omega_L \mp \omega_{\text{vib}})^4}{(4\pi \epsilon_0)^2} \frac{\eta_R}{\eta_L c^4} |\tilde{\alpha}_{\text{Raman}}|^2$$

### The Raman polarizability

$$\tilde{\alpha}_{Raman} = \sum_j \left\{ \frac{\langle f | \vec{\mu} \cdot \vec{\epsilon}_R | j \rangle \langle j | \vec{\mu} \cdot \vec{\epsilon}_L | i \rangle}{\hbar(\omega_{ji} - \omega_L) + i\Gamma_{ji}} + \frac{\langle f | \vec{\mu} \cdot \vec{\epsilon}_L | j \rangle \langle j | \vec{\mu} \cdot \vec{\epsilon}_R | i \rangle}{\hbar(\omega_{jf} + \omega_R) + i\Gamma_{jf}} \right\}$$

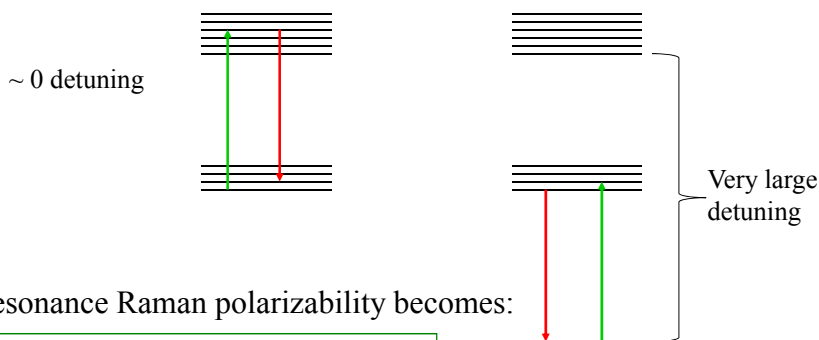


Understand / Explain the formula!

27

### The resonance Raman polarizability

$$\tilde{\alpha}_{Raman} = \sum_j \left\{ \frac{\langle f | \vec{\mu} \cdot \vec{\epsilon}_R | j \rangle \langle j | \vec{\mu} \cdot \vec{\epsilon}_L | i \rangle}{\hbar(\omega_{ji} - \omega_L) - i\Gamma_{ji}} + \frac{\langle f | \vec{\mu} \cdot \vec{\epsilon}_L | j \rangle \langle j | \vec{\mu} \cdot \vec{\epsilon}_R | i \rangle}{\hbar(\omega_{jf} + \omega_R) + i\Gamma_{jf}} \right\}$$



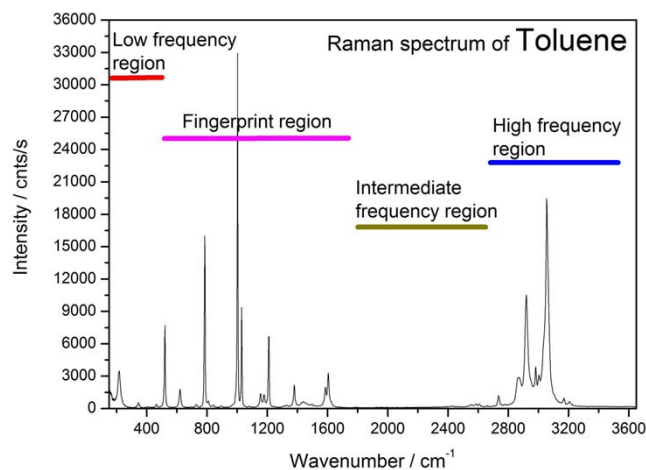
The resonance Raman polarizability becomes:

$$\tilde{\alpha}_{Raman} = \sum_j \left\{ \frac{\langle f | \vec{\mu} \cdot \vec{\epsilon}_R | j \rangle \langle j | \vec{\mu} \cdot \vec{\epsilon}_L | i \rangle}{\hbar(\omega_{ji} - \omega_L) - i\Gamma_{ji}} \right\}$$

28

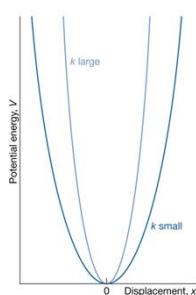
## The Raman spectrum of a poly-atomic molecule: toluene

Harmonic Oscillator selection rule:  $\Delta v = \pm 1$



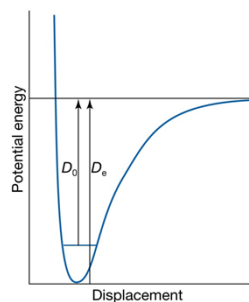
39 fundamental modes are Raman active. They are all observed.

## The potential energy is not parabolic



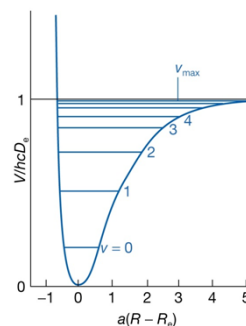
$$V = \frac{1}{2} k x^2$$

The harmonic potential is an approximation to the actual potential energy



A bond that can be dissociated must be described by a different potential energy.

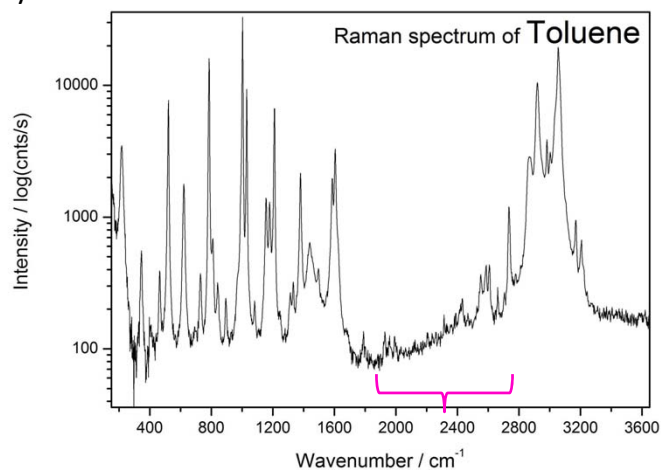
The Morse potential energy is a useful description



The vibrational energy levels are progressively closer when the dissociation is approached

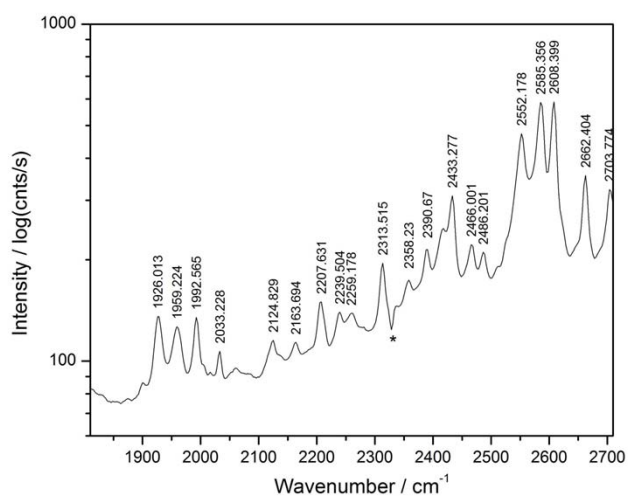
## Overtone and combination bands in a polyatomic molecule: toluene

Many more modes than 39 fundamental modes are observed.



Actually, approx. 112 modes have been observed so far.

## The intermediate region improved



All these modes are overtones or combination bands.



## Raman spectroscopy

Raman spectroscopy to understand matter by probing electrons and vibrations

Material Science

Raman spectroscopy as a contrast method in microscopy

Label-free microscopy

Raman spectroscopy to determine molecular number densities

Label-free sensing

## Raman Chemical Analysis

$$n_R [\# / s] = \sigma_R (3m-6) C L^2 \frac{I}{\hbar\omega} l\Omega$$

Raman cross section

**Sensitivity**

Concentration molecules of interest

**Detectivity**

Local field enhancement

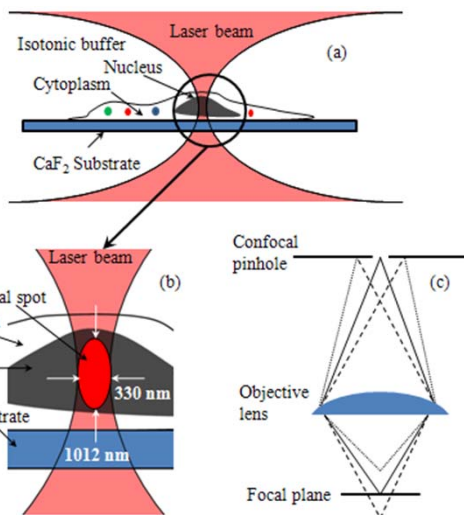
**Sensitivity**

Molecular vibrations for chemical identification

**Specificity**

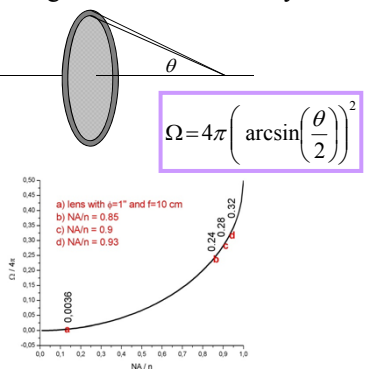
Optical engineering of the optimal illumination/detection strategy

## Hyperspectral Confocal Raman Microscopy



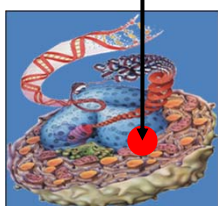
**Confocal effect:**  
Reduction of out-of-focus light  
Sharper imaging

**High NA:**  $\theta = \arcsin\left(\frac{NA}{n}\right)$   
Higher resolution  
Large collection efficiency



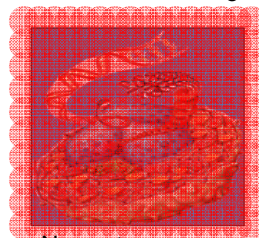
## Measurement Modes

Single point measurement



Single spectrum  
Not representative for the whole cell

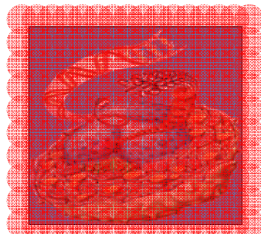
Confocal Raman imaging



N spectra  
N=1024, 4096, ....

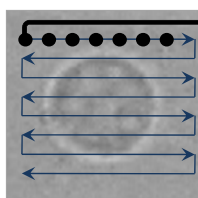
Progresses to less time consuming

Rapid Scanning mode



Single spectrum  
Representative and fast

## Confocal Raman measurement procedure



Schematic view of the raster scanning for imaging of the cells

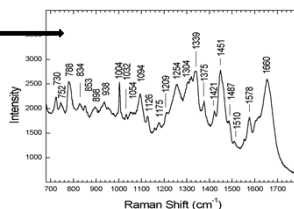
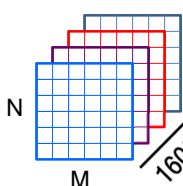


Table : Raman peak assignments

S.No	Peak position (cm) <sup>-1</sup>	Peak Assignment
1	788	Nucleus: O-P-O symmetric stretch
2	853	Tyrosine
3	938	Protein: $\alpha$ - helix
4	1004	Phenylalanine
5	1032	Phenylalanine
6	1094	Nucleus: O-P-O symmetric stretch
7	1126	Protein: C-N stretch
8	1254	Adenine
9	1304	Adenine
10	1339	Adenine
11	1451	C-H deformation
12	1660	Protein: Amide 1
13	1045	Collagen (Proline)
14	2335	Nitrogen (atmosphere)
**	590	Phosphate PO <sub>4</sub> <sup>3-</sup>
*	960	Phosphate PO <sub>4</sub> <sup>3-</sup>
+	1070	Carbonate CO <sub>3</sub> <sup>2-</sup>

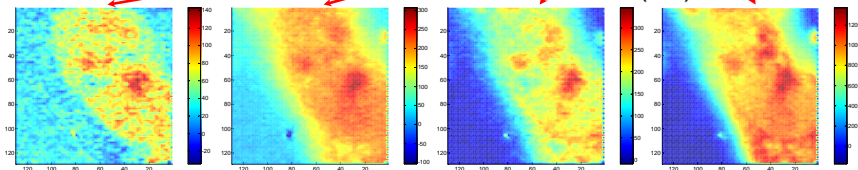
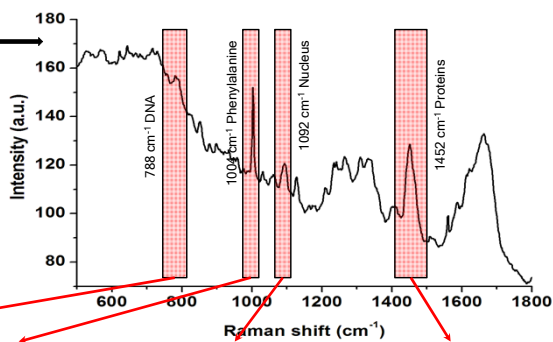
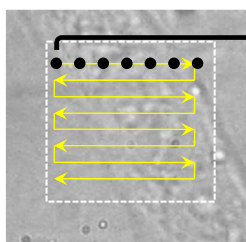


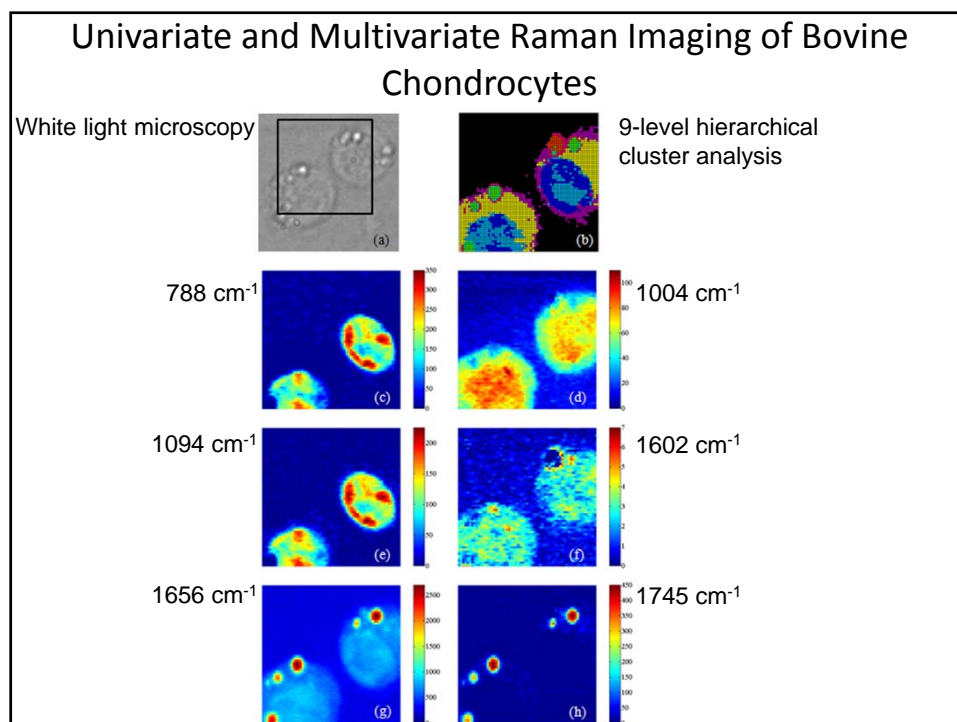
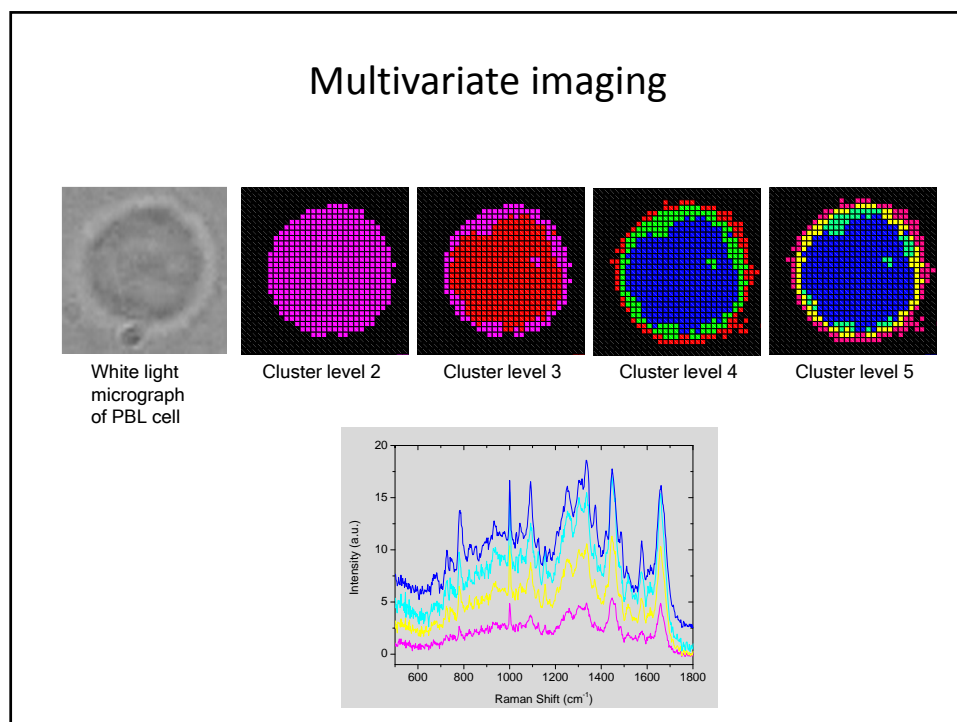
Data obtained :  
 Each frame = 32 x 32 pix  
 Each pix = 1600 data points  
 => 1.64 million data points

Hyper spectral data cube

Rich chemical information from cell with light

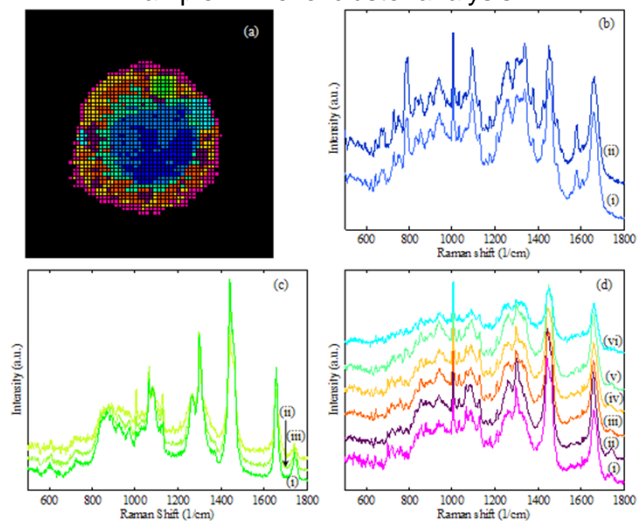
## Univariate imaging





## Datamining Molecular Information

Example: 12-level cluster analysis



Improving chemical resolution

END