Methods for the examination of biomolecules

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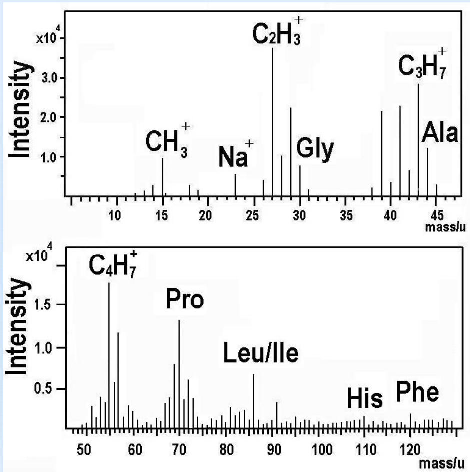
Which kind of information are important?

- **Composition** – chemical elements, molecules
- **Structure –** crystal structure **-** 3D structure
- **Distribution**
- **Types of chemical bonds, conformation**
- **Dynamics**
- **Magnetic and electrical properties, …**

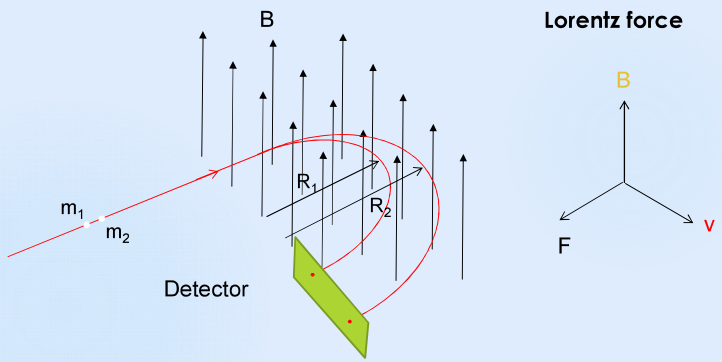
Mass spectrometry

Mass spectrometry

- - information on chemical elements and molecules in the sample

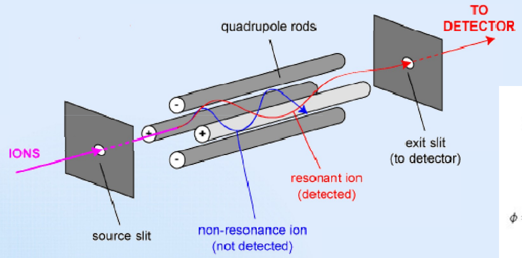


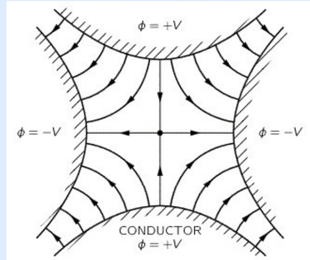
Mass spectrometry – physical principle (older type)



$$F = B.Q.v = \frac{m.v^2}{R} \Rightarrow R = \frac{m.v}{Q.B}$$

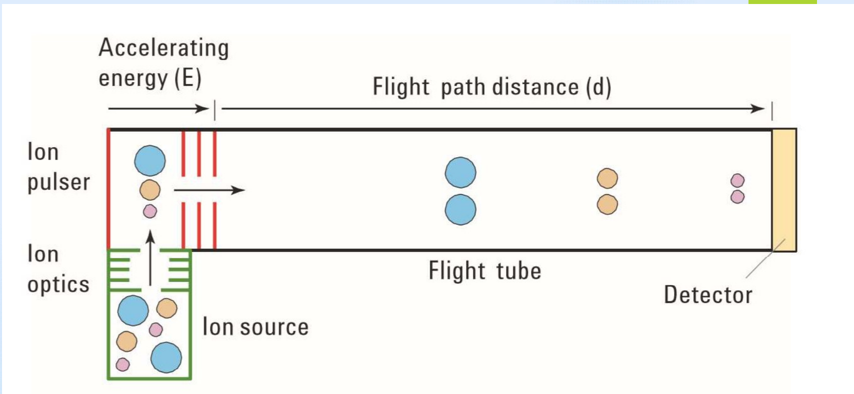
Mass spectrometry – physical principle (new type)





A narrow m/z range is selected.
Tunable with the voltages applied to the rods.

Principle of the *Time of Flight (TOF)*



$E = \frac{1}{2} m.v^2 \Rightarrow v^2 = 2.E/m$

$v = d/t$

$\Big\} \; t \sim \sqrt{m}$

What is important?

Sample must be in ionized state

How to accomplish that?

- Electrospray ionization
- Electron ionization
- Matrix-assisted laser desorption/ionization

Electrospray ionization

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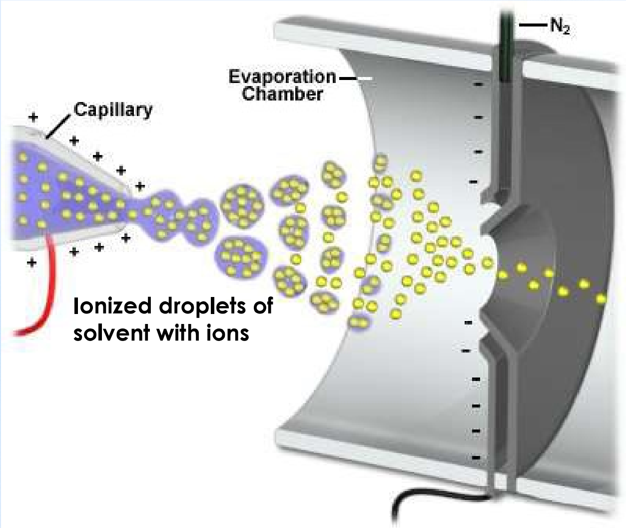

Electron ionization

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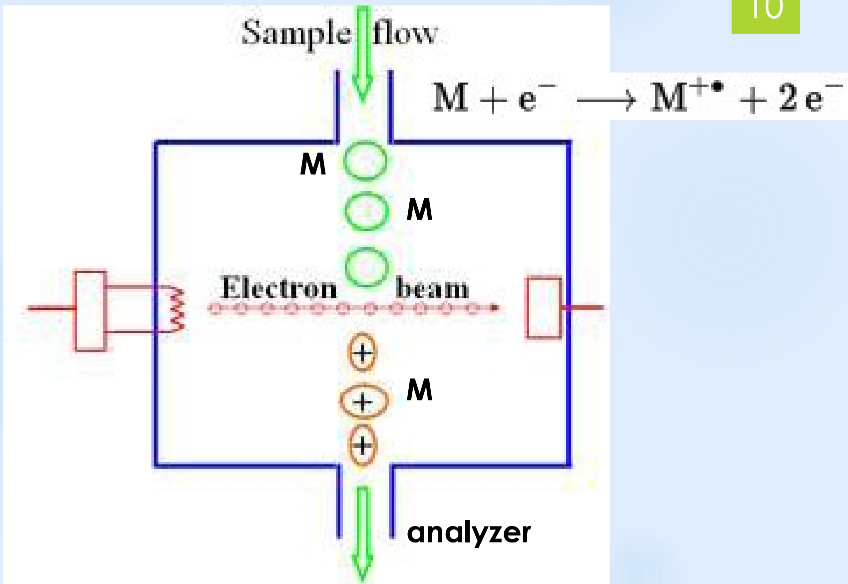

Matrix-assisted laser desorption/ionization

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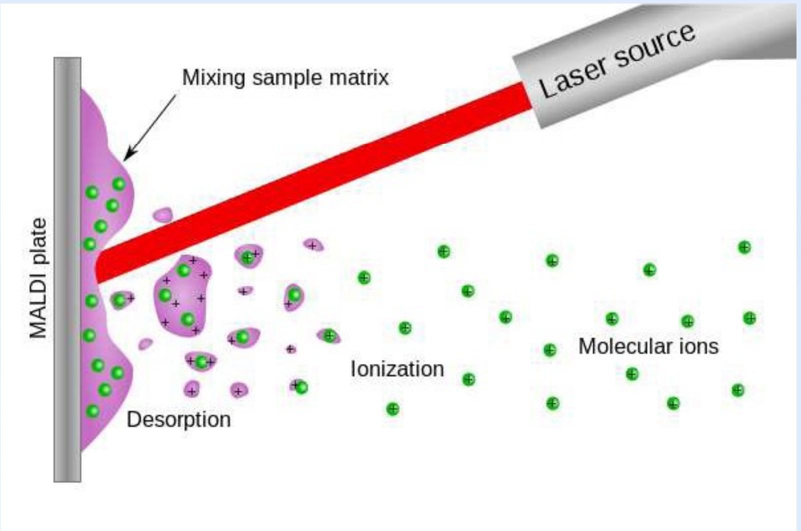

Disadvantage: sample destruction

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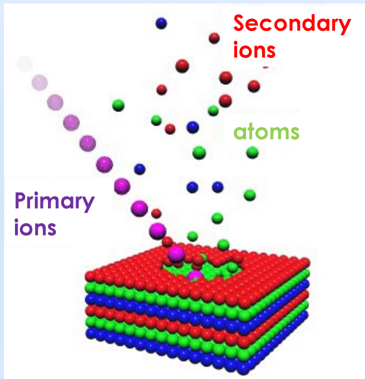
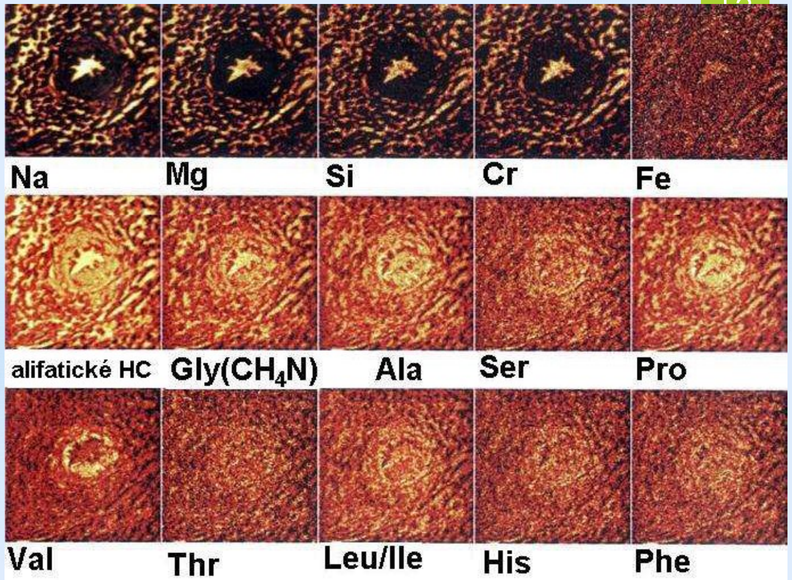
Secondary ion mass spectroscopy



Advantage: space resolution

Map of chemical elements and compounds



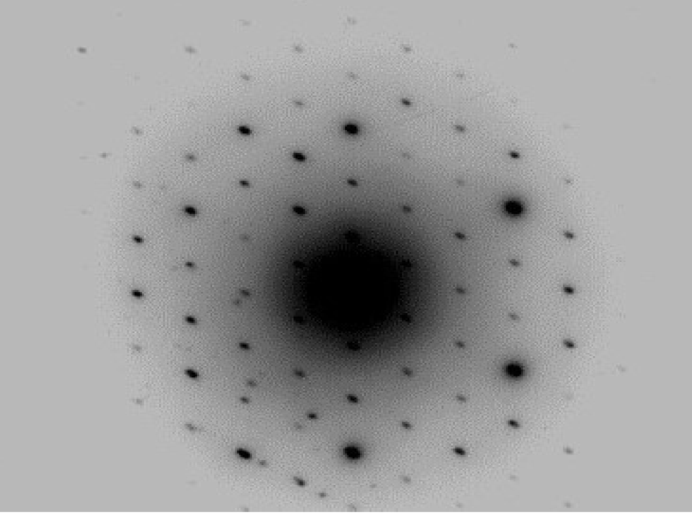
Advantages:

- High sensitivity
- Discrimination between isotopes (^{12}C, ^{13}C , ...)
- information on chemical bonds
- detection of light chem. elements and compounds

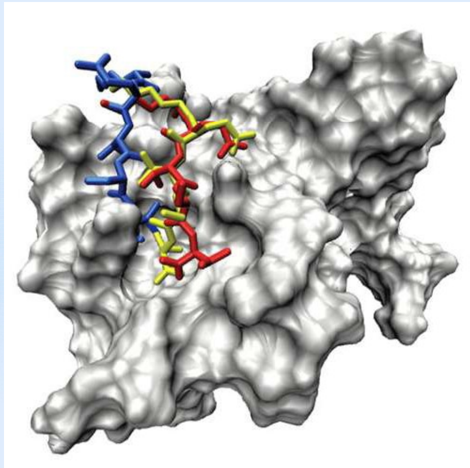
Drawbacks:

- Different sensitivity of chem. elements in different environments (copper in spleen vs copper in liver)
- Different sensitivity of chem. Elements in the same environment (copper vs iron in liver)

Diffraction
X-ray, electron, neutron



What is the relevance of the structural information?

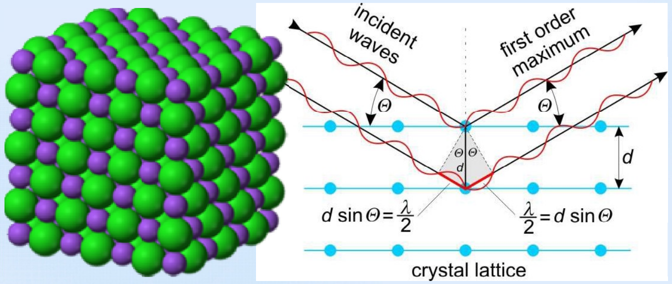


Understanding the enzymatic, protein, (poly)saccharides function, docking of small molecules …

Different structure = different function

From:http://www.cipsm.de

Diffraction – physical principle

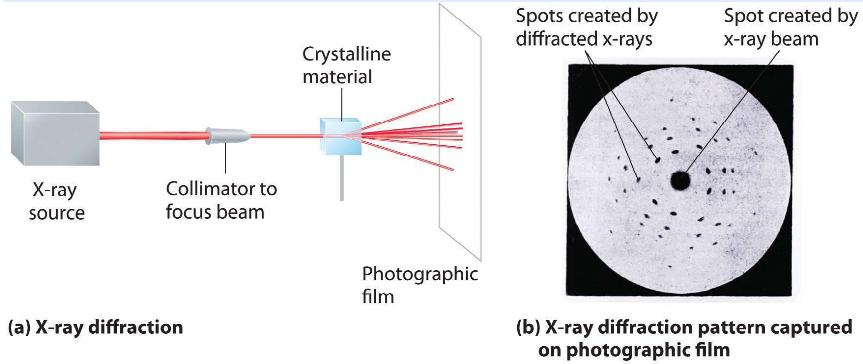


$$\sin\Theta_{max} = \frac{k\lambda}{2d}$$

$$d = \frac{k\lambda}{2\sin\Theta_{max}}$$

λ – wavelength of X-ray (electrons, neutrons), Θ_{max} – the angle of incidence at which the maximum intensity occurs, k – integer, d – distance of diffracting planes

Diffraction – physical principle



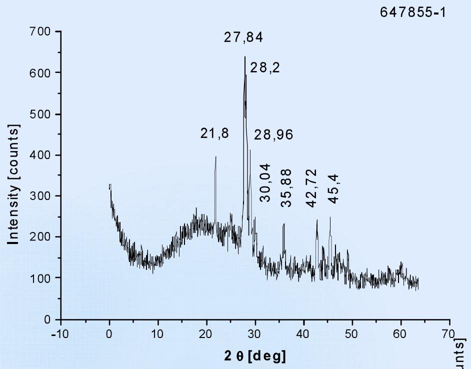
(a) X-ray diffraction

(b) X-ray diffraction pattern captured on photographic film

Diffraction

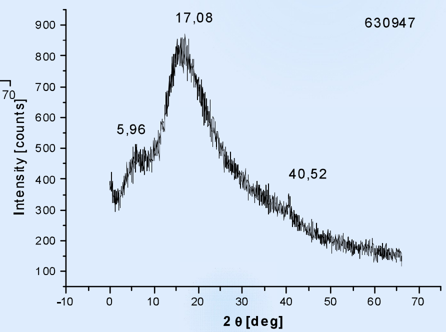
- **Powder diffraction**
- **Laue diffraction**
- **Small angle diffraction**

Powder diffraction – human spleen



Sample with crystals

Amorphous sample



Powder diffraction – human spleen

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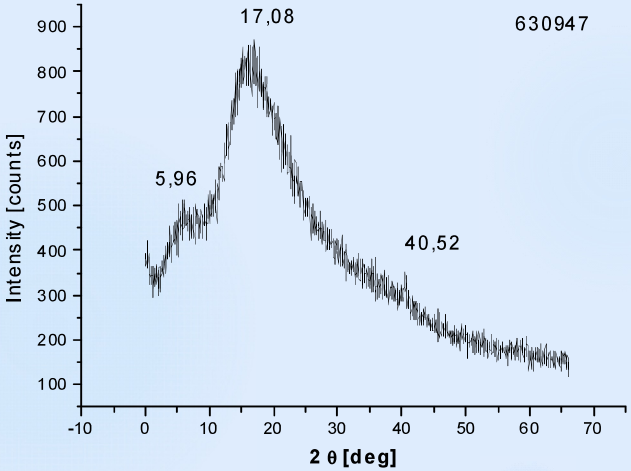
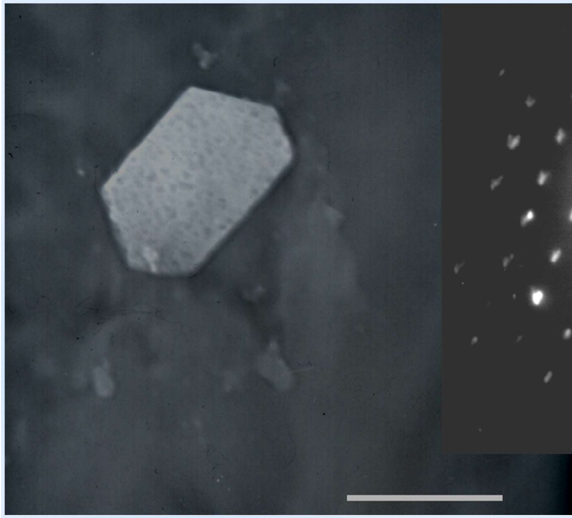
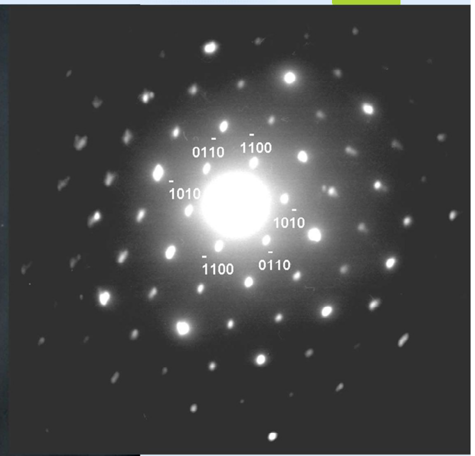


Results – transmission electron microscopy





Fe, O, Cu – high level

Fig. 2 Human brain, globus pallidus. Iron-rich particle of regular shape. Scale bar = 1 µm. TEM. Diffraction pattern of hematite α-Fe_2O_3 particle.

Investigation of 3D structure of macromolecules by x-ray diffraction

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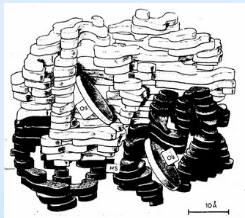
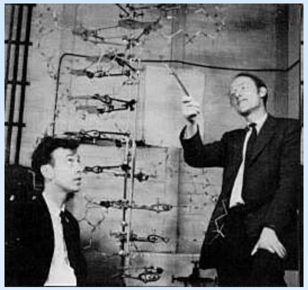
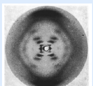


Nobel prize 1962
Globular protein
M. F. Perutz,
J. C. Kendrew

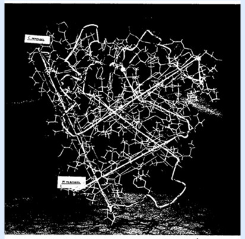
mioglobin:~1200 atoms



Nobel prize 1962
Structure of DNA
Francis Crick
James Watson
Maurice Wilkins



Rosalind Franklin



Investigation of 3D structure of macromolecules by x-ray diffraction

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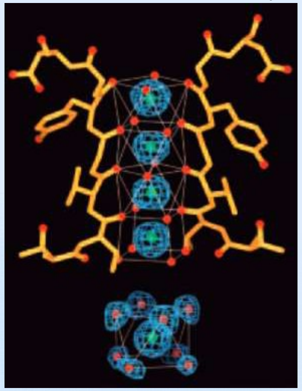
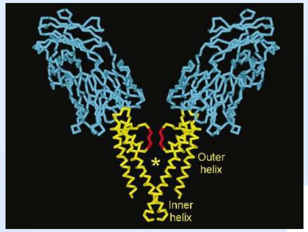
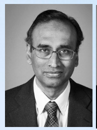
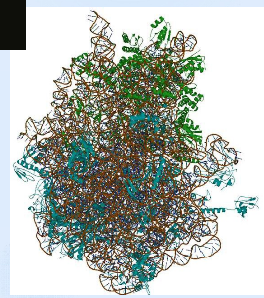


Nobel prize 2003
Memebrane channel
Roderick MacKinnon





Nobel prize 2009
Ribosome
V. Ramakrishnan, T. A. Steitz, A. E. Yonath
30S subunit: ~35000 atoms,
50S subunit:~64000 atoms



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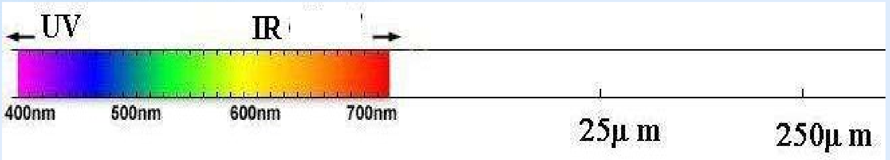
IR spectroscopy



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IR spectroscopy

- information on molecular structure, chemical bonds, conformation, configuration of molecules

IR spectroscopy – physical principle

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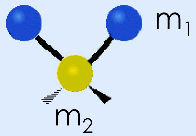
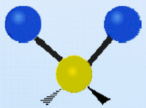


Symmetric stretching

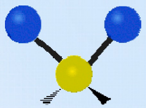
$$f = \frac{1}{2\pi}\sqrt{\frac{D(m_1 + m_2)}{m_1 m_2}}$$

D – constant

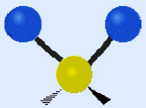
m_1, m_2 – *mass of atoms*



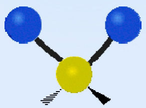
Asymmetric stretching



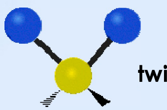
Scissoring



rocking



wagging



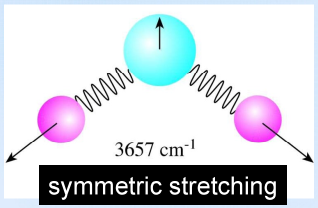
twisting

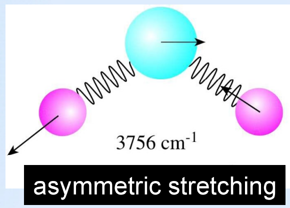
IR spectroscopy – vibrations

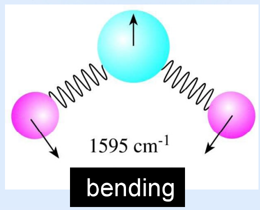
All the atoms vibrate

- with different amplitude and
- in different direction

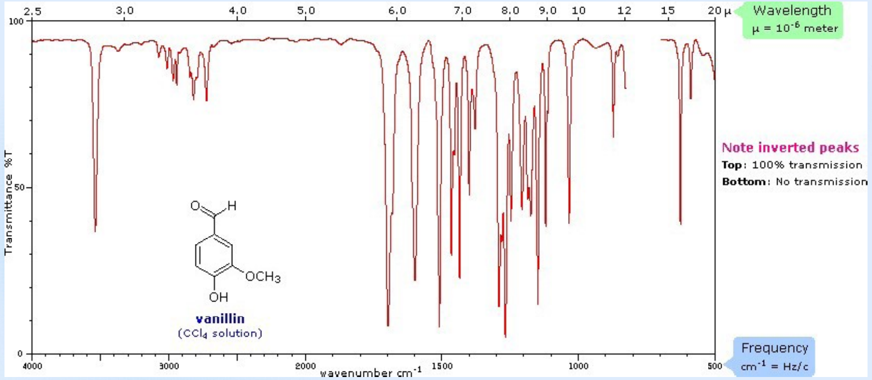
Example: water





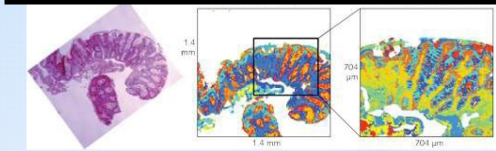


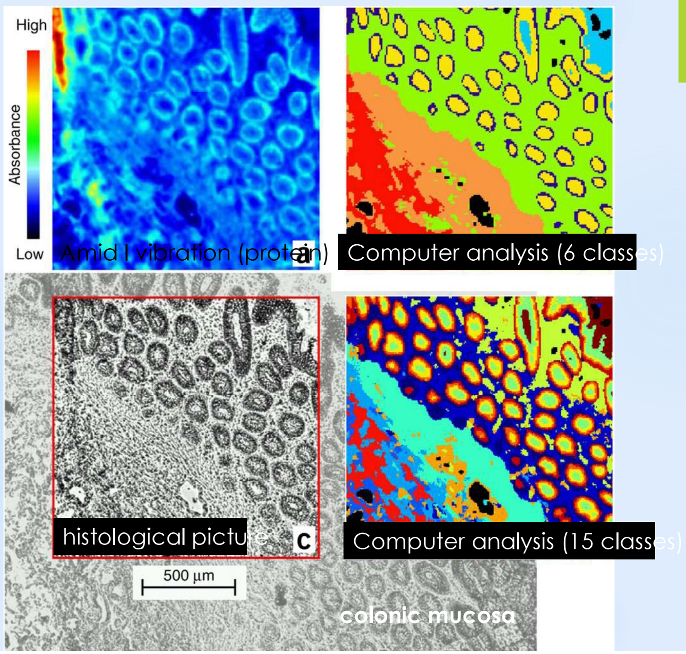
IR spectroscopy – spectrum



Infrared microscopy

[redacted]





Conclusion: mass spectrometry
diffraction
IR spectrometry

Ex vivo methods

in vivo methods
(MRI, EPR, PET, CT, ...)

Thank you very much for your attention