# Solid-state analysis in pharmaceutical industry

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# Topics Overview

- Generics
- Drug Discovery and Development
- Legal Aspects
- Solid forms
- Electromagnetic Spectrum
- Microscopy
- Particles size distribution
- Infrared spectroscopy
- Raman spectroscopy
- Mapping
- Thermal analysis

# Differences between Original and Generics Analysis

None

Generics contain the same type of compounds like the originals

Generics are also the drug product

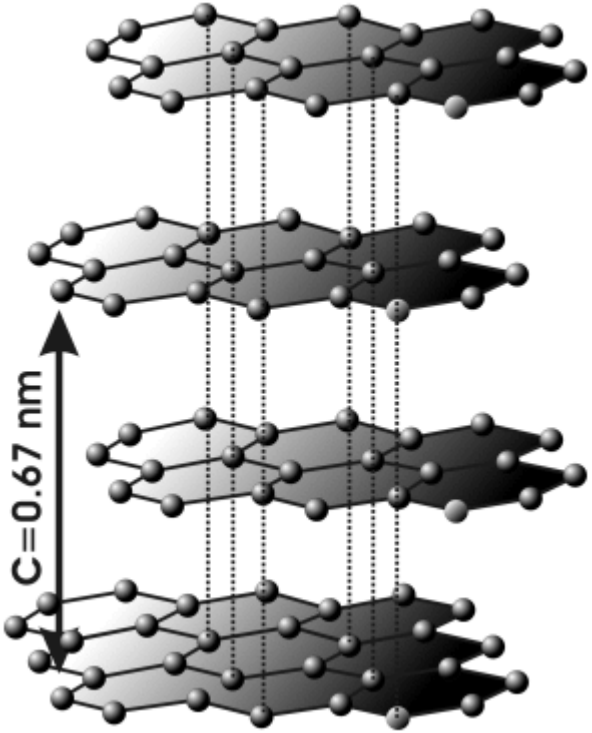
The goal is the same for generic and innovative drugs – safety and efficacy

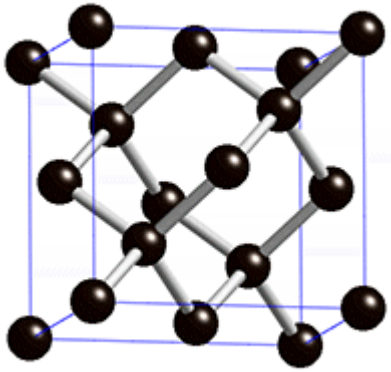
Reverse engineering – as much information on the original drug product as possible

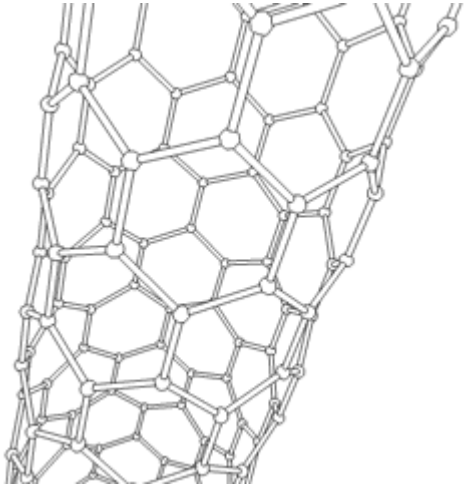
# Allotropy

The property of chemical elements to exist in more than one crystalline lattice

Examples: graphite vs. Diamond vs. Other forms



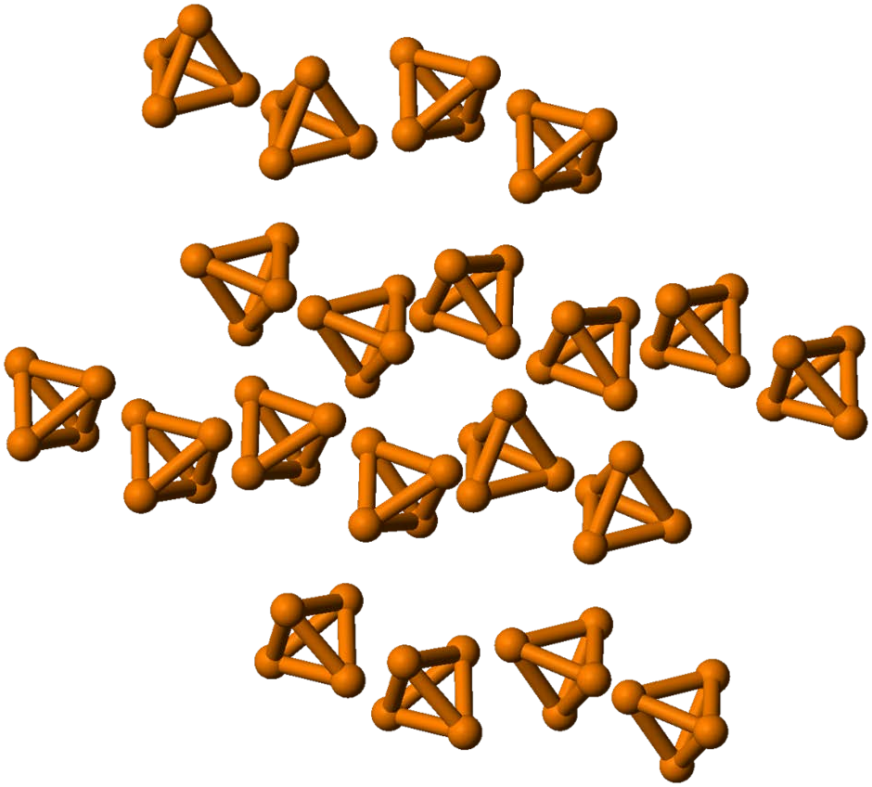


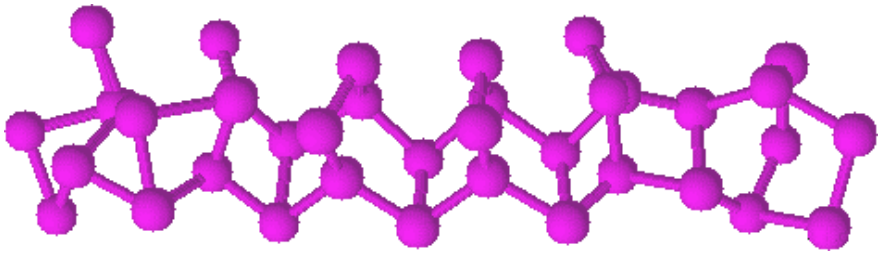
Autor: Anton – Na Commons přeneseno z de.wikipedia.; original upload 7. Feb 2004 by Anton, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=350220

https://cs.wikipedia.org/wiki/Uhl%C3%ADk

# Allotropy

## Phosphorus white and red (and others)





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https://commons.wikimedia.org/w/index.php?curid=1311089

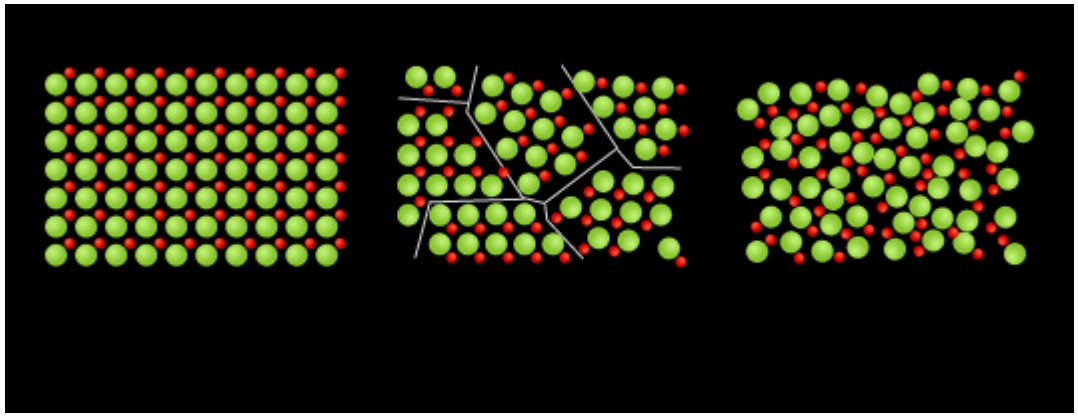
Autor: Ondřej Mangl – ChemSketch, Volné dílo,
https://commons.wikimedia.org/w/index.php?curid=2135934

# Polymorphism

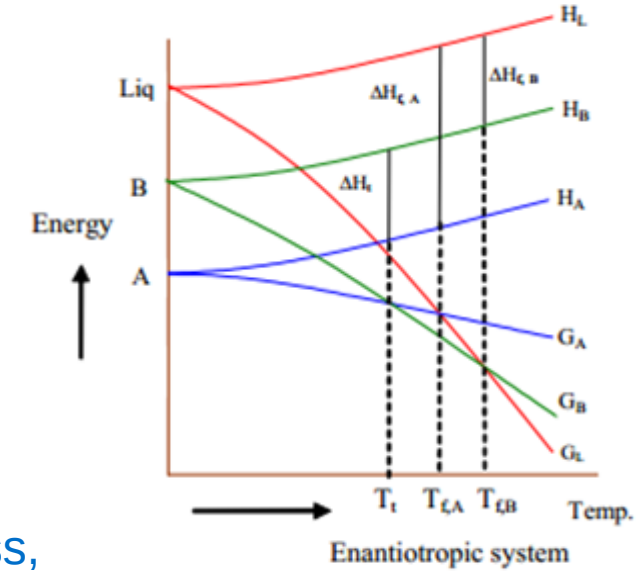
Polymorphism – The property of chemical compounds to exist in more than one crystalline lattice.

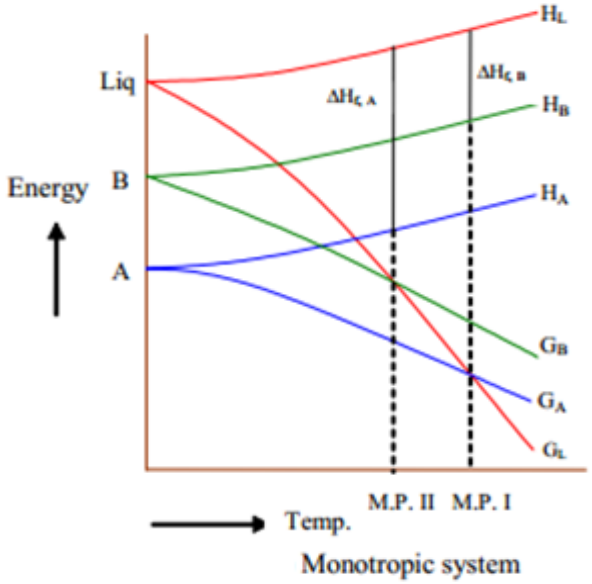
Different polymorphs – Different properties similarly to allotropes: density, hardness, reactivity, dissolution rate

Amorfy – bez pravidelné struktury



http://www.physics-in-a-nutshell.com/article/1
Crystalline - amorphous




http://chemistry.tutorvista.com/inorganic-chemistry/polymorphism.html

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# Solid State Analytical Techniques – the most commonly used methods in pharmaceutical practise

Microscopy and particle size Analysis

- Optical
- Electron
- Image Analysis
- …

X-ray diffraction

- Single- crystal
- Powder diffraction

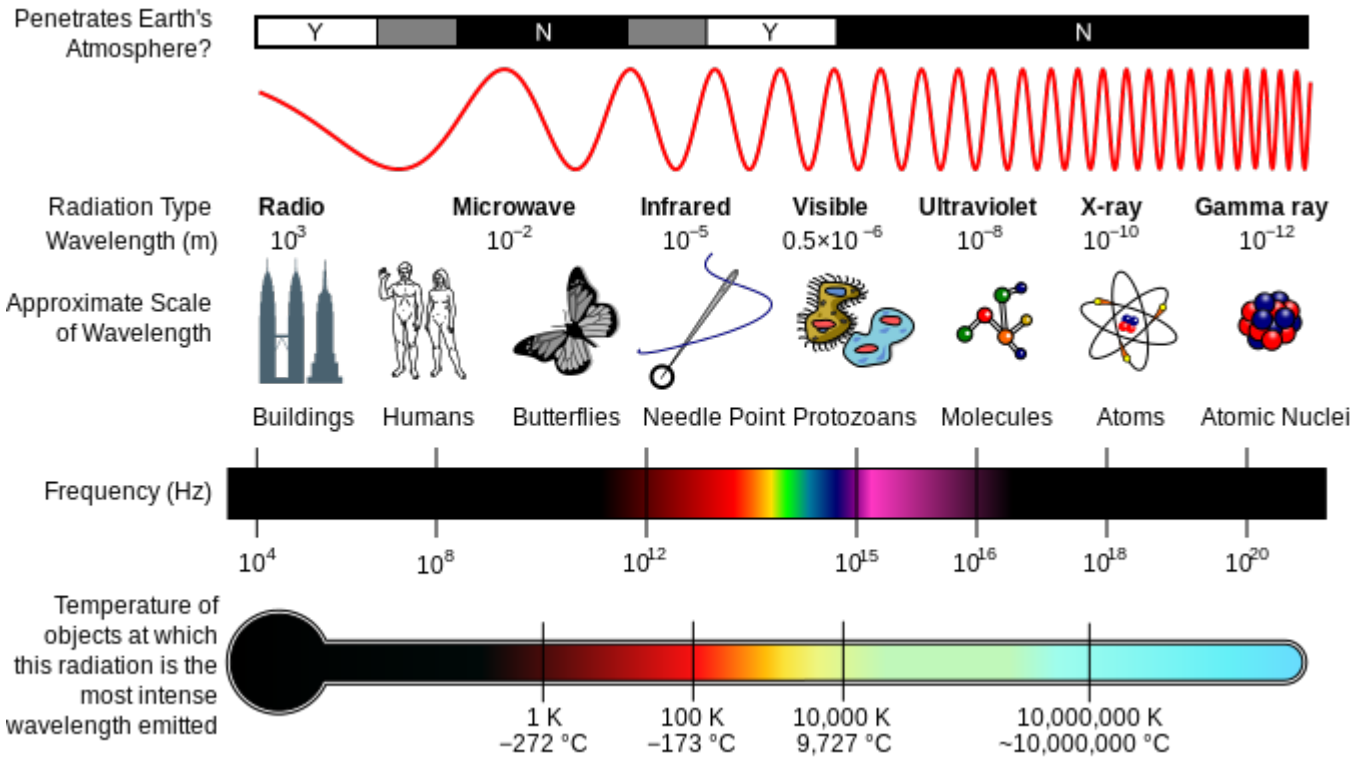
Thermal Analysis

- Differential Scanning Calorimetry (DSC)
- Thermogravimetric Analysis (TGA)
- Dynamic Vapour Sorption (DVS)
- …

Molecular spectroscopy

- Infrared
- Raman
- Nuclear Magnetic Resonance (NMR)
- Mass Spectroscopy
- …

# Electromagnetic Spectrum




By Inductiveload, NASA - self-made, information by NASABased off of File:EM Spectrum3-new.jpg by NASAThe butterfly icon is from the P icon set, File:P biology.svgThe humans are from the Pioneer plaque, File:Human.svgThe buildings are the Petronas towers and the Empire State Buildings, both from File:Skyscrapercompare.svg, Public Domain, https://commons.wikimedia.org/w/index.php?curid=2974242

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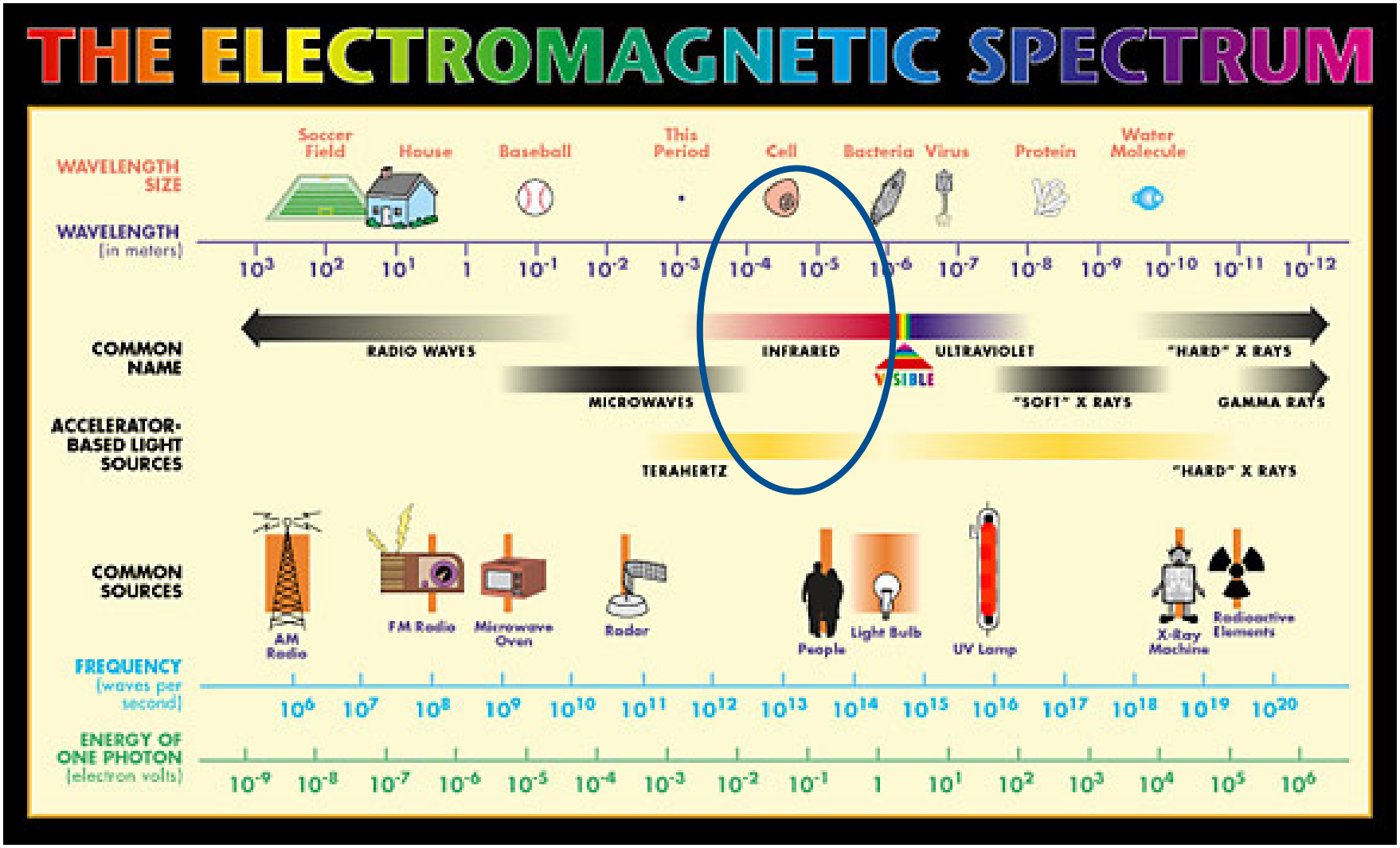
# Infrared Spectroscopy




http://www2.lbl.gov/MicroWorlds/ALSTool/EMSpec/EMSpec2.html

# Electromagnetic Spectrum

| Wavelength μm | Wavenumber $cm^{-1}$ | Region |
|---|---|---|
| 0.78-2.5 | 12800-400 | Near infrared (NIR) |
| 2.5-40 | 4000-200 | Mid infrared (MIR) |
| 40-1000 | 200-5 | Far infrared (FIR) |

# Infrared Spectroscopy

- Onephotonic transition between two vibrational levels
- Interaction with the incidental photon
- Mid IR Region (MIR) – the most universal method in development and QC
- Near IR Region (NIR) – mainly in QC
- Far IR (FIR) – mainly in primary research

# Infrared Spectroscopy

3N-6 (3N-5 for linear molecules) degrees of freedom (vibrational modes):

3 degrees of freedom for one atom (independent translation – 3 coordinates)

For N-atomic molecule 3 x N = 3N degrees of freedom (translation, rotation, vibration)

For IR vibration 3 rotations (2 for linear molecule) and 3 translation doesn‘t count, thus:

3N-6 degrees of freedom for non-linear molecule vibrations

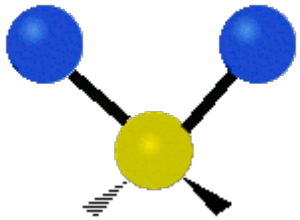
# Infrared Spectroscopy

Dipole moment **change** condition

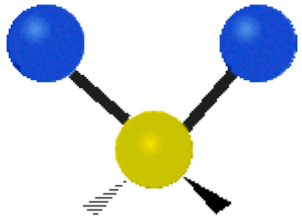
$$\frac{\partial p}{\partial q} \neq 0$$

, where *q* is a normal coordinate

Stretching vibrations – the bond length changes



Symetric

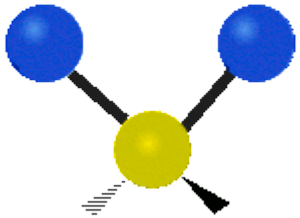


Antisymetric

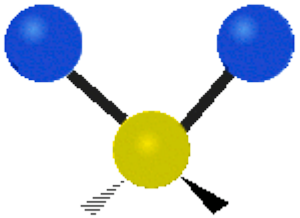
www.wikipedia.org

# Infrared Spectroscopy

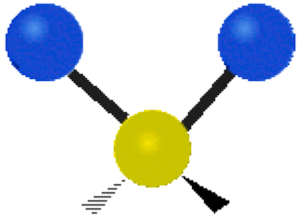
## Deformation and bending modes – bonding angle changes



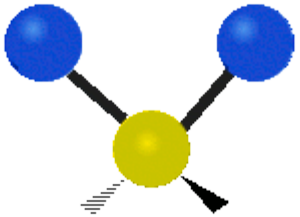
Twisting



Wagging



Rocking



Scissoring

www.wikipedia.org

# Infrared Spectroscopy

Because of dipole change condition, homoatomic molecules do not absorb IR radiation ($O_2$, $N_2$ , $H_2$ , …)

Molecules exhibiting dipole moment absorb usually strongly, because the dipole change is high (C=O, S=O, N=O, O-H, …)

(Dis)advantage for pharmaceutical analysis, as formulations often contain saccharides containing many hydroxyl groups

# Infrared Spectroscopy – Instrumentation

Source:

MIR: ceramic rod T 1000-1200 °C

NIR: halogen nebo tungsten bulb

FIR: mercury lamp

Detector:

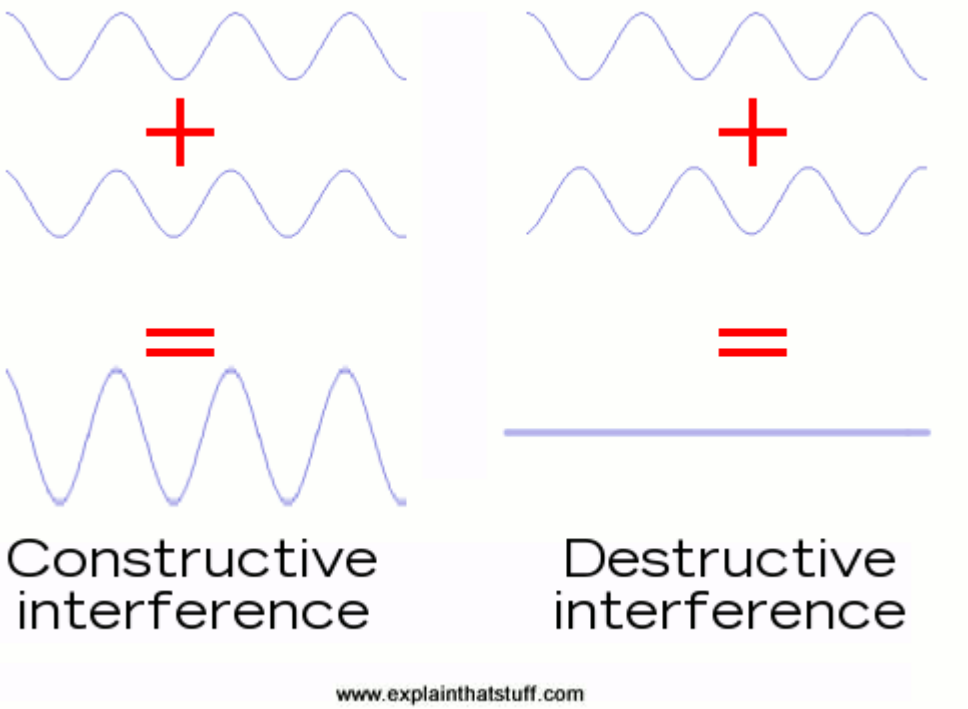
MIR: deuterated triglycin sulphate (DTGS), mercury-cadmium tellurid (MCT)

NIR: PbSe, PbS, Ge, MCT

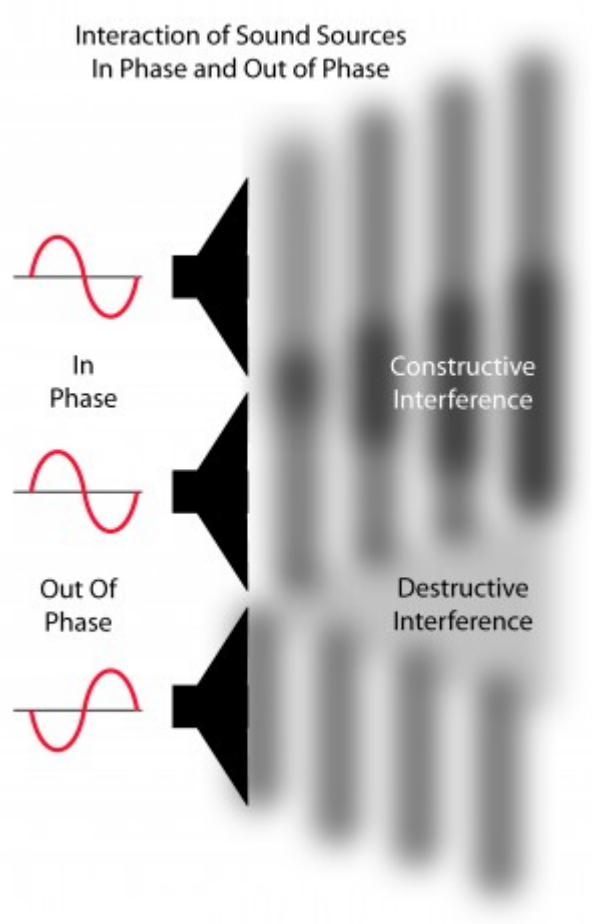
FIR: DTGS

Optical elements must be IR stransparent: KBr, ZnSe, $CaF_2$, CsI, Si, diamond, …

# Interference

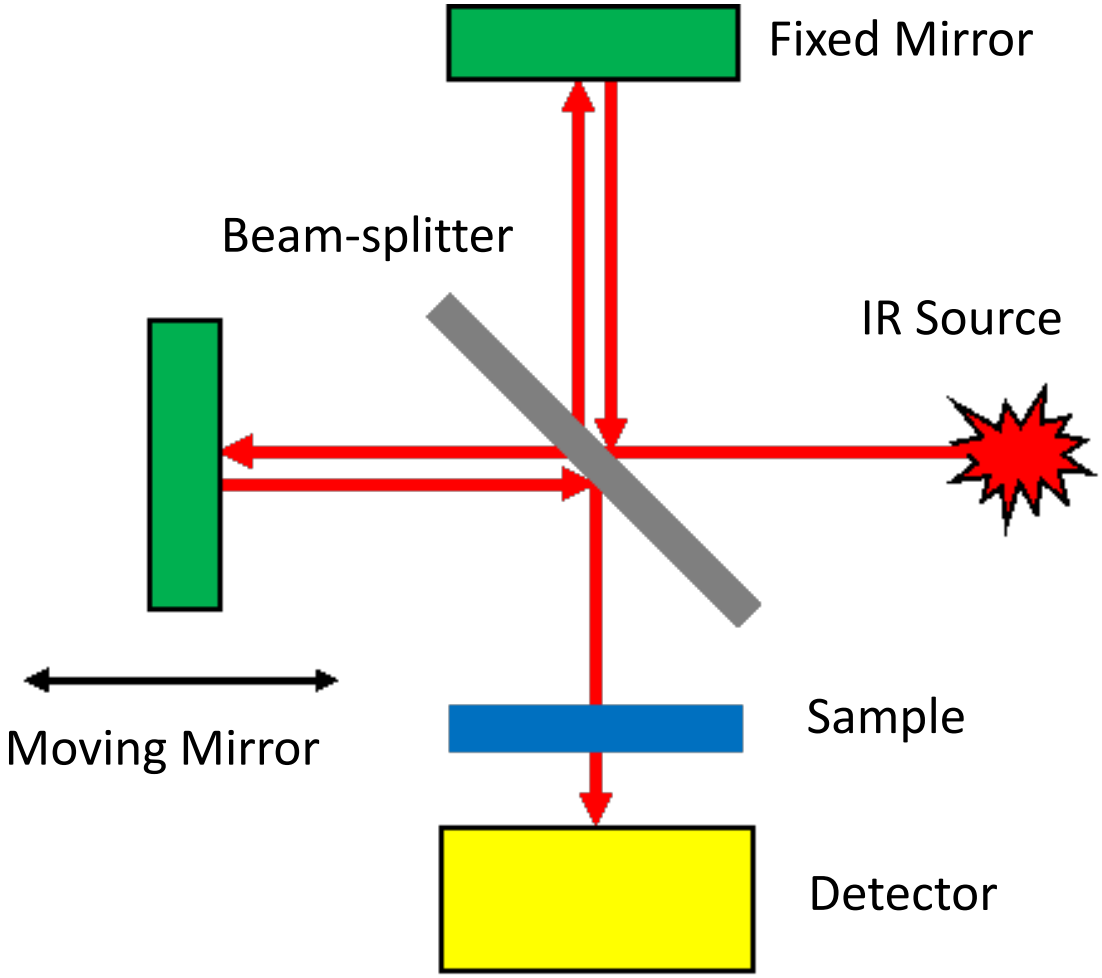



https://www.ligo.caltech.edu/LA/page/what-is-interferometer




http://www.ctgclean.com/tech-blog/2011/10/ultrasonics-sound-interactions-of-sound-waves/

# Infrared Spectroscopy - Interferometer



# Infrared spectroscopy – measurement techniques

## Transmission

- Solids, liquids nad gasses incl. emulsions and suspensions

## Reflective

- Liquids, solids, emulsions and suspensions

## Fiber Techniques

## Microscopic techniques

# Infrared spectroscopy – Transmission

Transmission:

Cuvettes – for liquids and gasses and suspensions of solids (Nujol, Fluorolube)

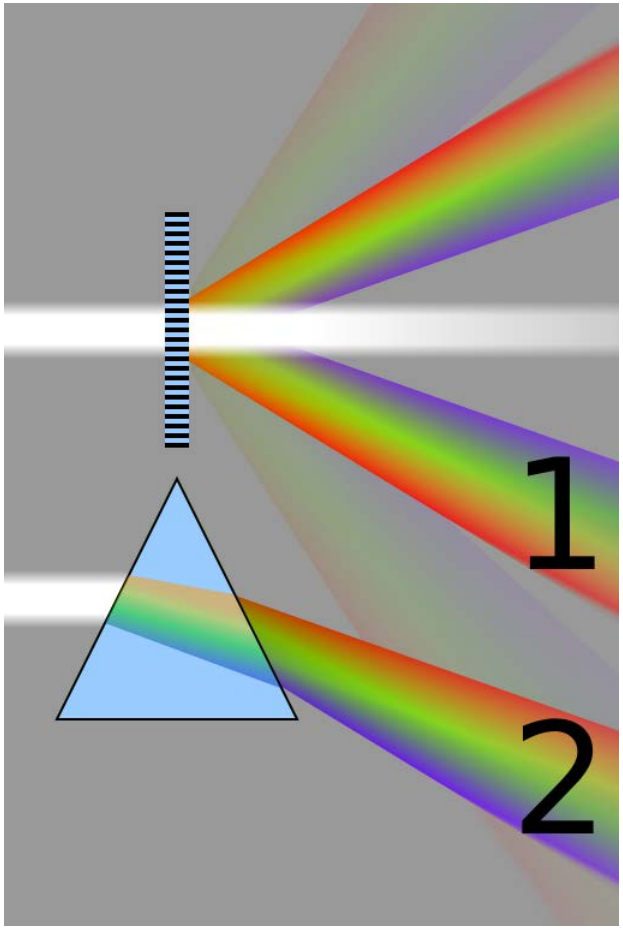
- Transparent cuvettes – KBr, $CaF_2$, $BaF_2$, KRS-5 (TlBr-TlI)
- Resistance to the analyzed material

Discs – with potassium bromide

- Possible polymorph changes
- Possible interaction with groups such as: –NHx, -COOH, …

# Electromagnetic Spectrum – Effects

Diffraction and refraction:

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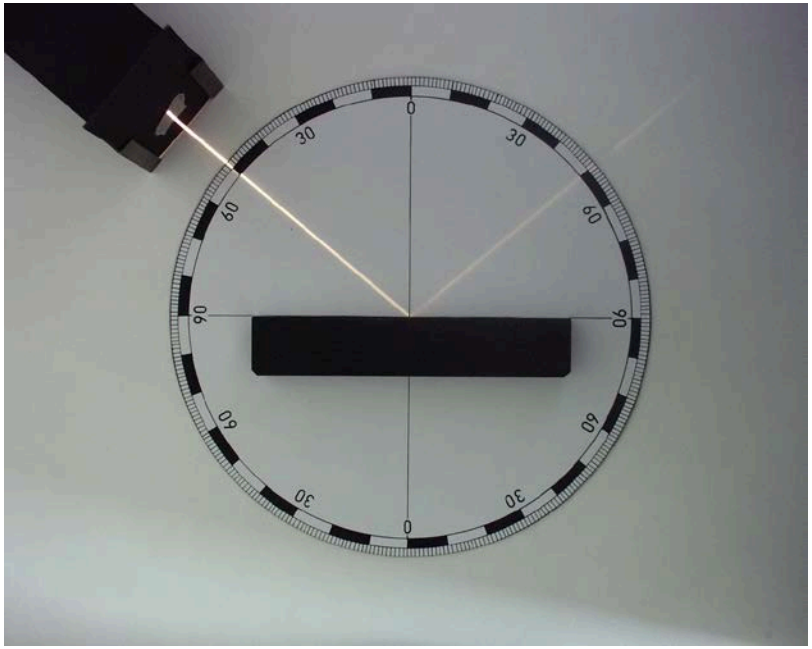
Reflection: Same medium => Same speed, Wavelength

Refraction: Different medium => Different Wavelengths

Diffraction: Same medium => Wavelengths differentiation

Reflection:

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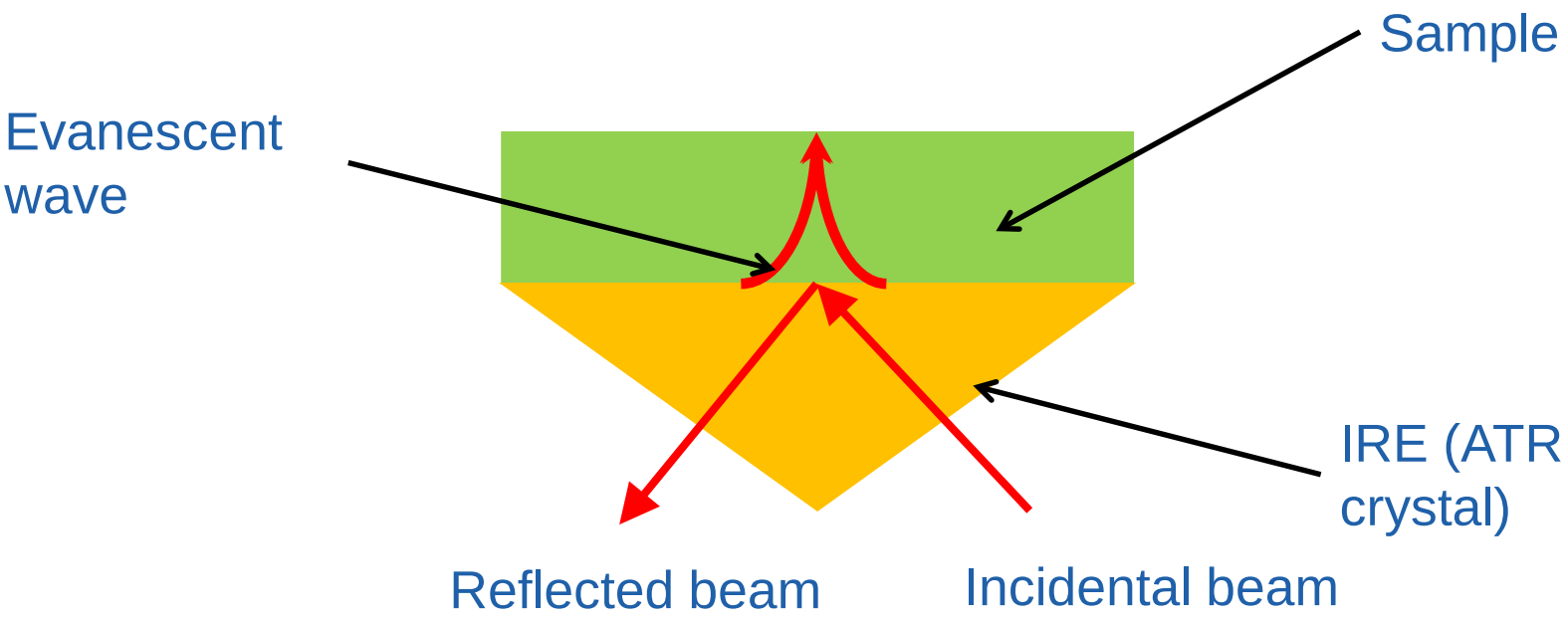
# Infrared spectroscopy – Reflection

Attenuated Total Reflection (ATR)



# Infrared spectroscopy – Reflection

Attenuated Total Reflection (ATR):

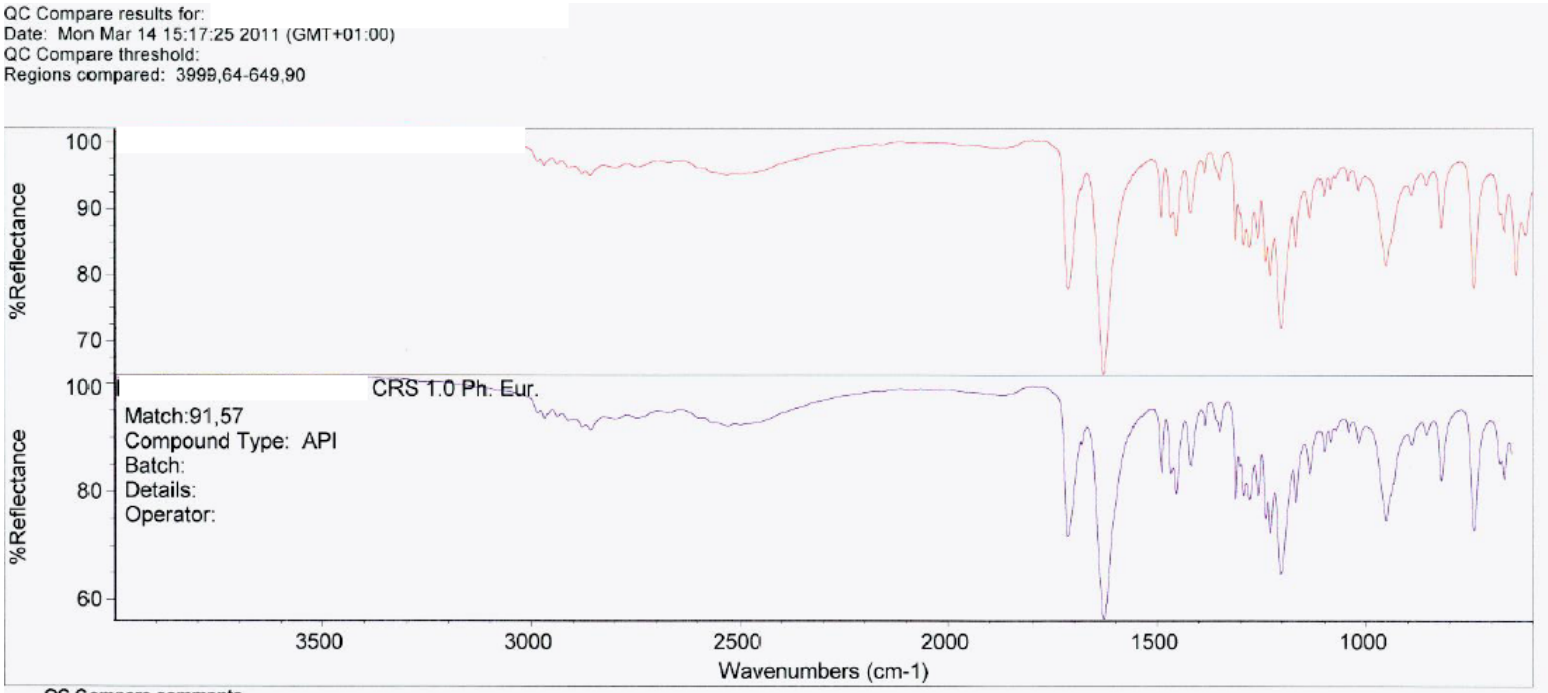
Tight contact of the IRE and the sample is necessary

Critical Angle (reflection only, not the refraction): $$\theta_c = \sin^{-1}\frac{(n_2)}{(n_1)}$$

Depth of penetration: $$d_p = \frac{\lambda}{2\pi n_1\sqrt{\left(\sin^2\theta - \left(\frac{n_2}{n_1}\right)^2\right)}}$$

θ angle of incidental beam, $n_2$ refractive index of the sample, $n_1$ refractive index of IRE

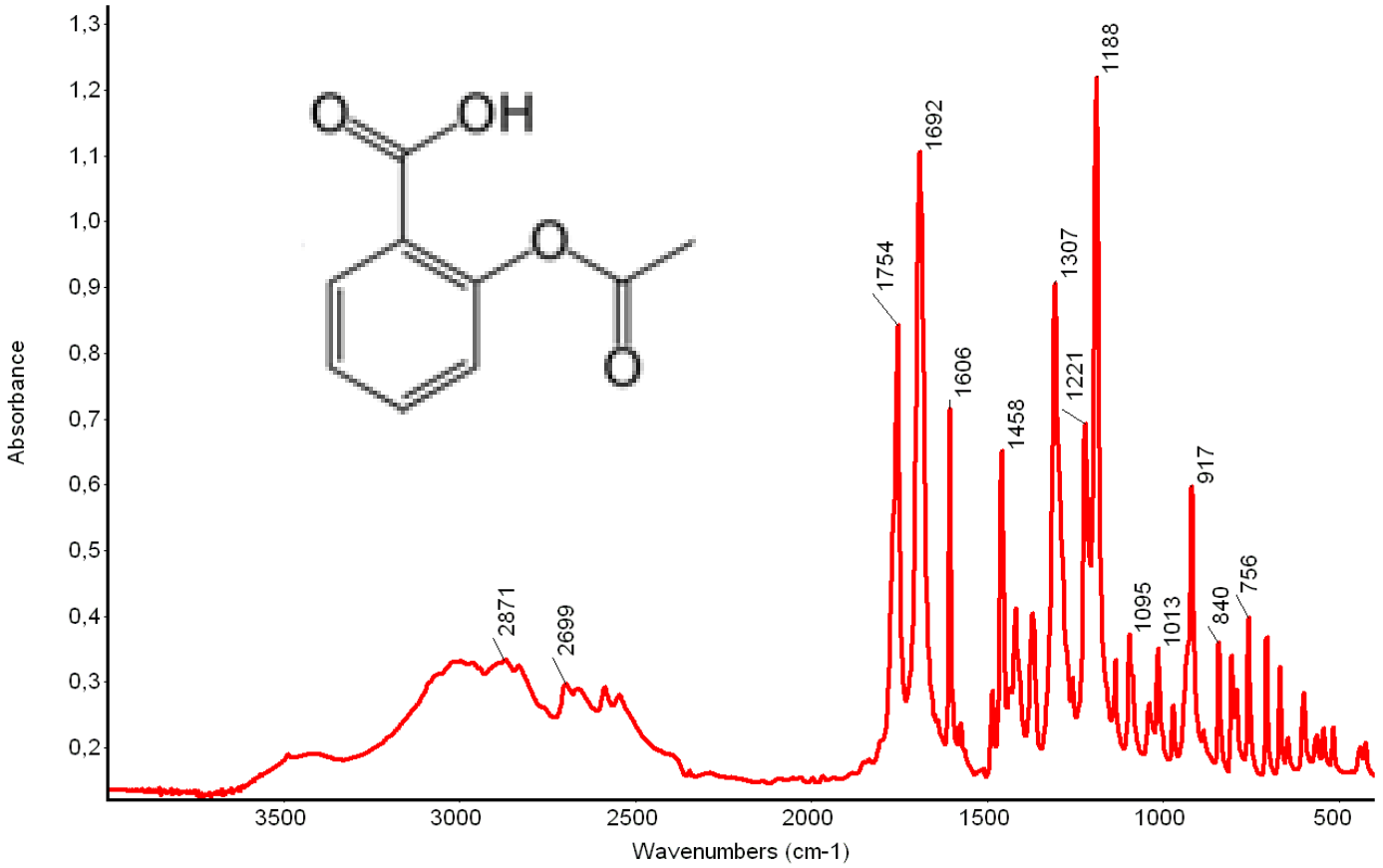
# Infrared spectroscopy – Identity confirmation




**The spectrum matches API.**

**The best match value is 91,57 and the critical match value is 80,00.**

# Infrared spectroscopy – Bands assignment




3000-2600 $cm^{-1}$ broad band ν(O-H), 3100 – 3000 $cm^{-1}$ ν(C-H) aromatic, 3000-2800 $cm^{-1}$ ν(C-H) aliphatic, 1754 $cm^{-1}$ ν(C=O) esther, 1692 $cm^{-1}$ ν(C=O) acid, 1606 $cm^{-1}$ ν(C=C) aromatic, 1458 δ(CH3), 1221 $cm^{-1}$ $\nu_{as}$(C-O), 1188 $cm^{-1}$ $\nu_{sym}$(C-O).

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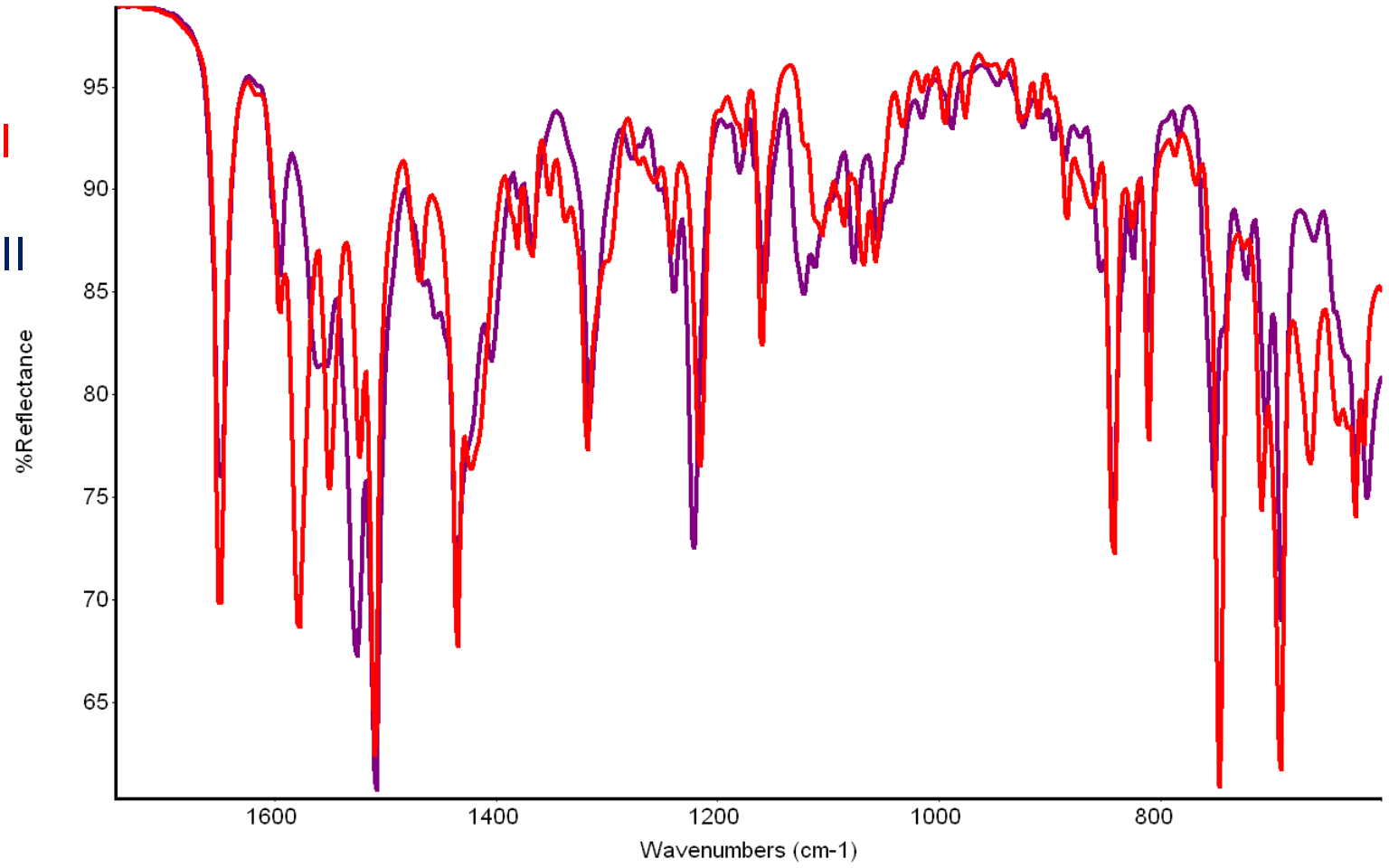
# Infrared spectroscopy – Polymorph differentiation

Form I

Form II

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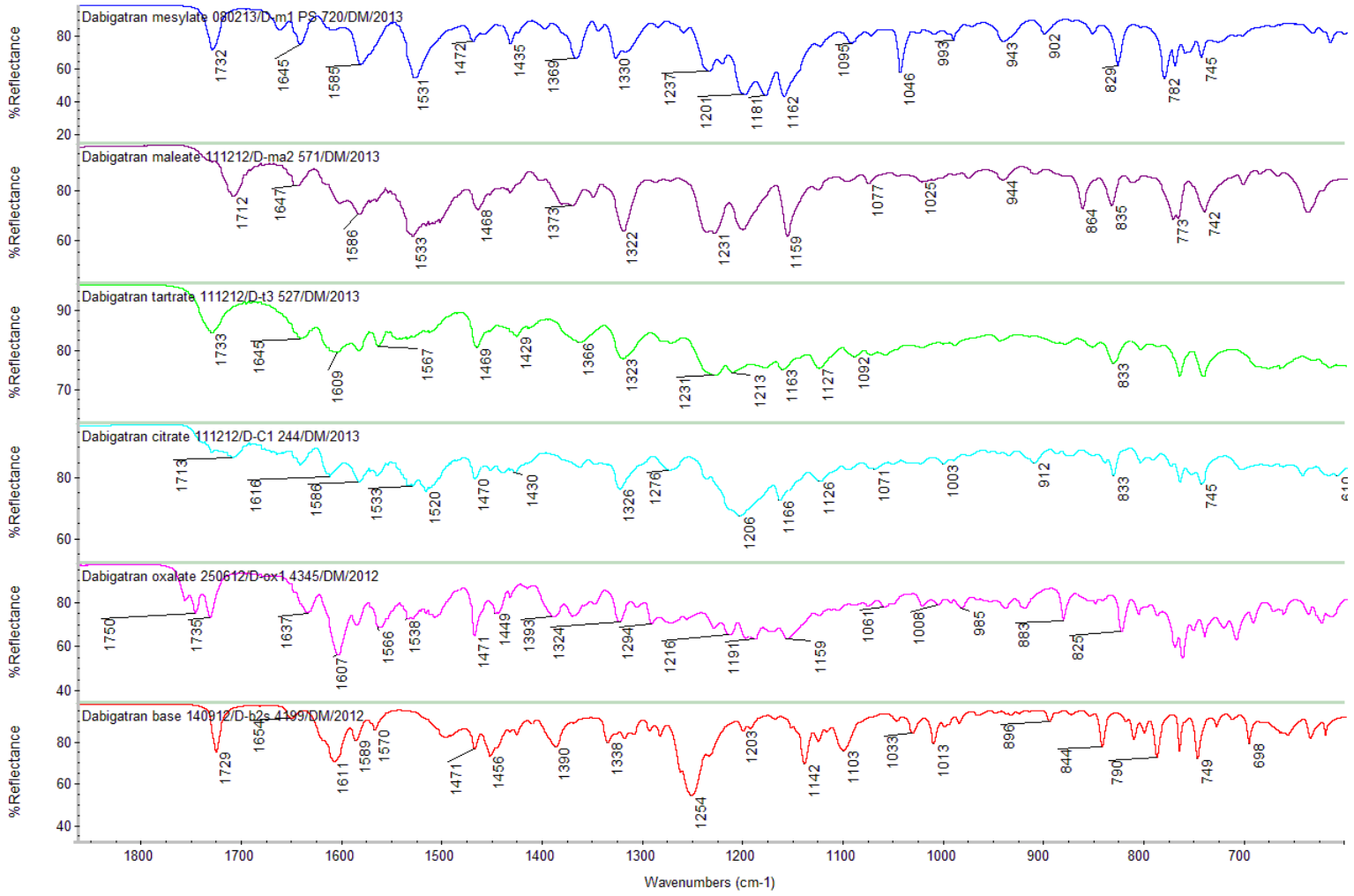
# Infrared spectroscopy – Salt differentiation



# Raman Spectroscopy

Sir Chandrasekhara Venkata Raman (1888-1970)

- Nobel Prize 1930

Raman Effect (together with Sir Kariamanickam Srinivasa Krishnan) - 28 February 1928

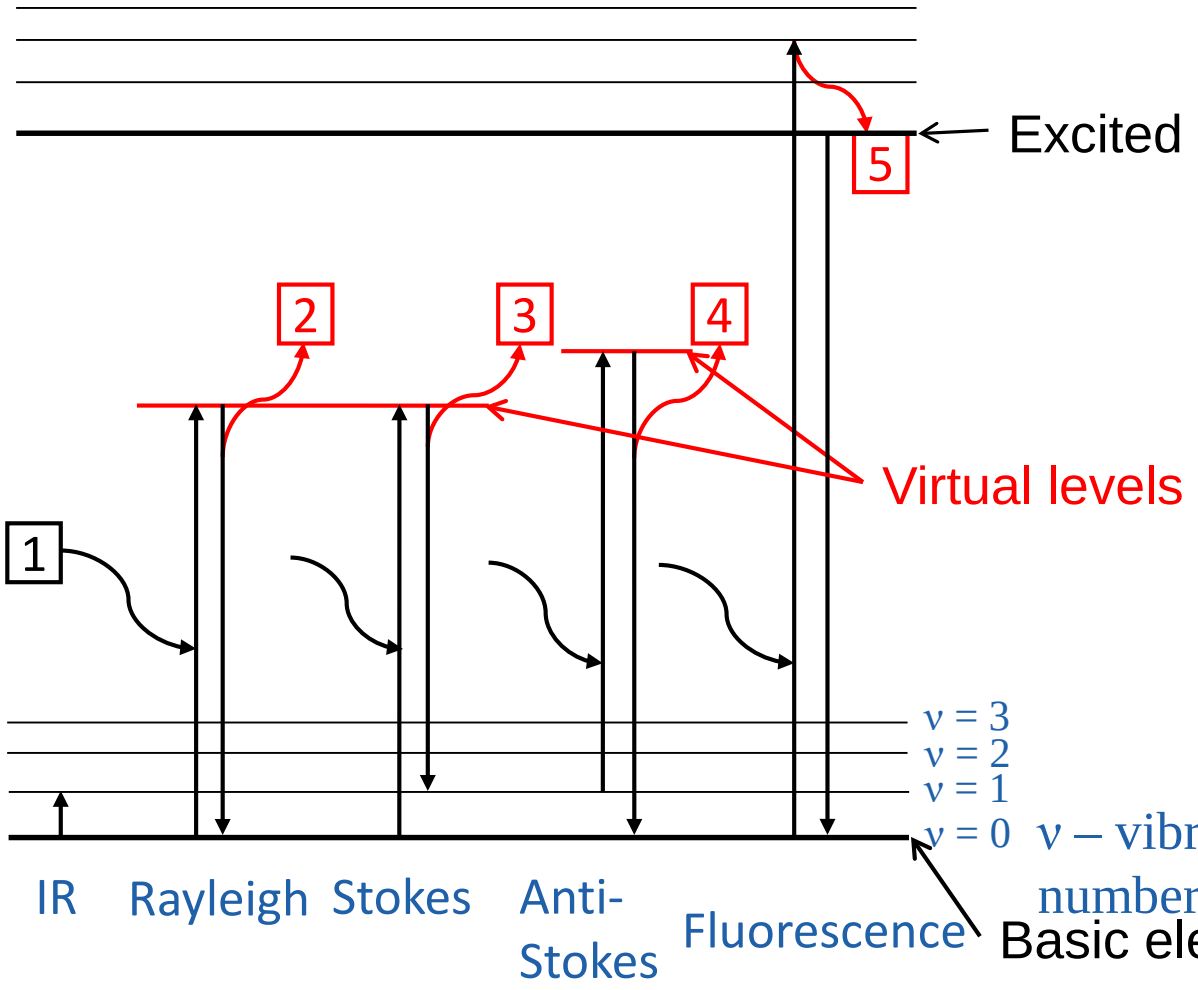
- Non-elastic scattering in liquids
- Published sooner than

Grigory Samuilovich Landsberg and Leonid Isaakovich Mandelstam - 21 February 1928

- Soviet Union
- In crystals

Predicted by Austrian Adolf Gustav Stephan Smekal (1923)

# Raman Spectroscopy




1 – absorbed photon ($\nu_0$)
2 – scatered photon ($\nu_0$)
3 – scatered photon ($\nu_0 - \nu_{vib}$)
4 – scatered photon ($\nu_0 + \nu_{vib}$)
5 – fluorescence

# Raman Spectroscopy

Comparison with IR spectroscopy:

Infrared: one-photonic effect (absorption)

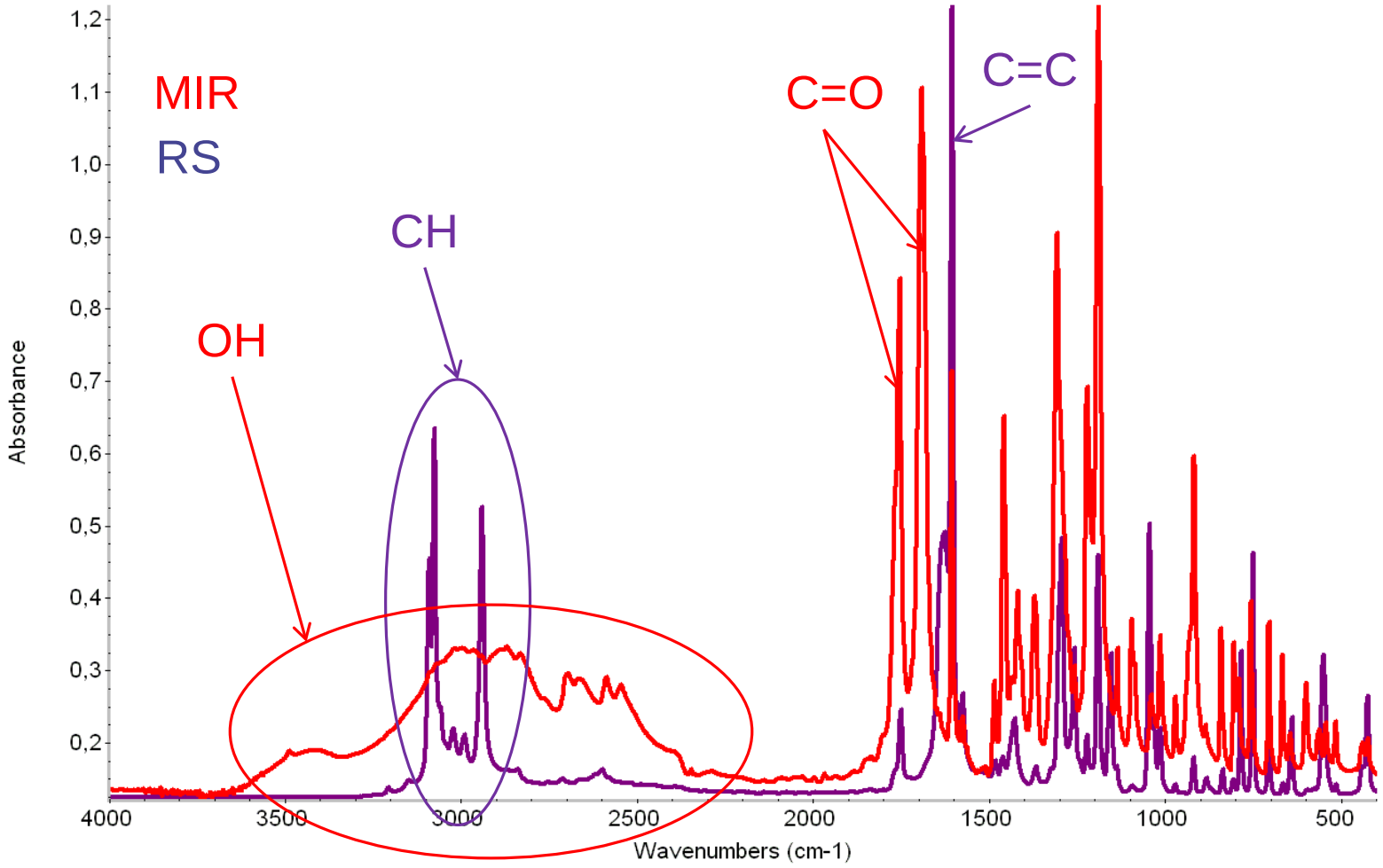
Principle: $\frac{\partial p}{\partial q} \neq 0$ Intensity: $\left(\frac{\partial p}{\partial q}\right)^2$

Raman: Two-photonic effect (non-elastic scattering)

Principle: $\frac{\partial \alpha}{\partial q} \neq 0$ Intensity: $\left(\frac{\partial \alpha}{\partial q}\right)^2$

Identical vibrations – different intensity

# IR vs. Raman Spectroscopy



# Raman Spectroscopy

Instrumentation:

Source:

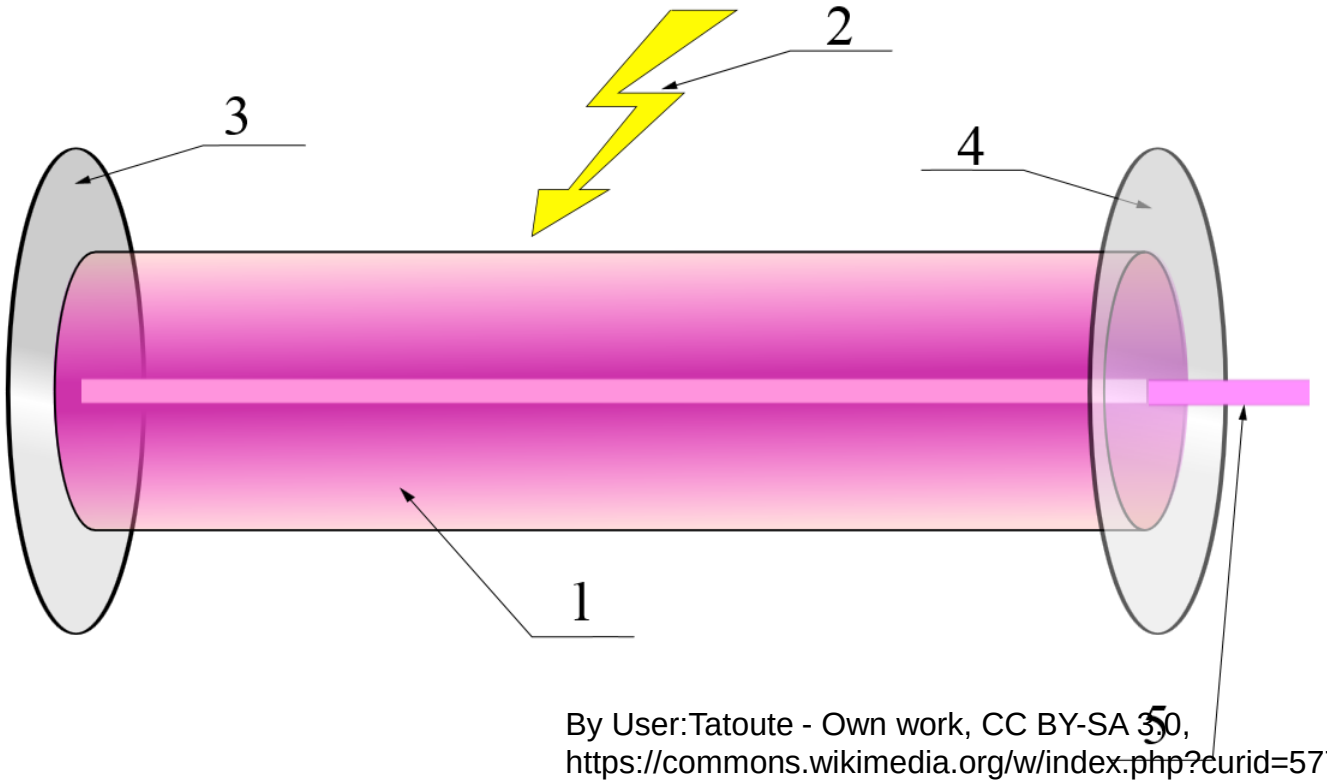
- Lasers (monochromatic, coherent – identical frequency, direction, phase of photons
  - NIR Nd:YAG (1064 nm), diode (785 nm), …
  - VIS Nd:YAG (532 nm), He-Ne (633), Ar (514, 488, 458), …
  - UV Ar (244, 257), HeCd (325), …
- Mercury lamp

Detectors

- Ge, CCD, photomultipliers, ..

# Electromagnetic Spectrum – Laser

## Light Amplification by Stimulated Emission of Radiation




By User:Tatoute - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=577575

1 – medium, 2 – energy pump, 3 – high reflection mirror, 4 – partially transparent mirror, 5 – laser beam

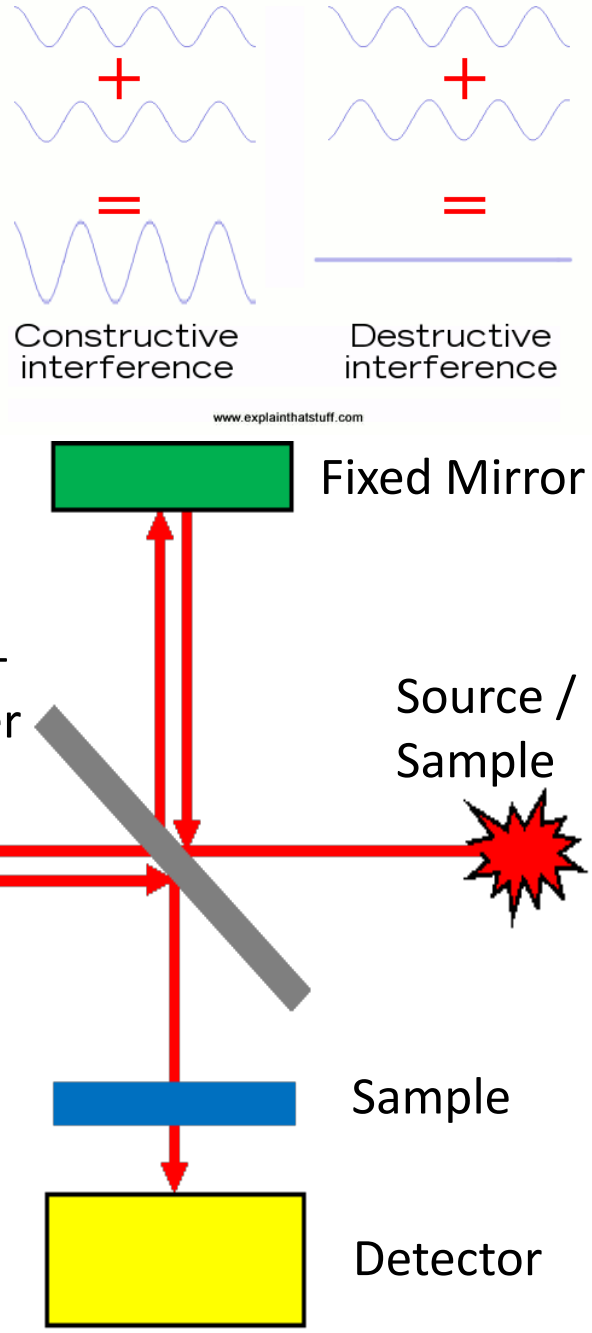
# Raman Spectroscopy

Dispersive elements

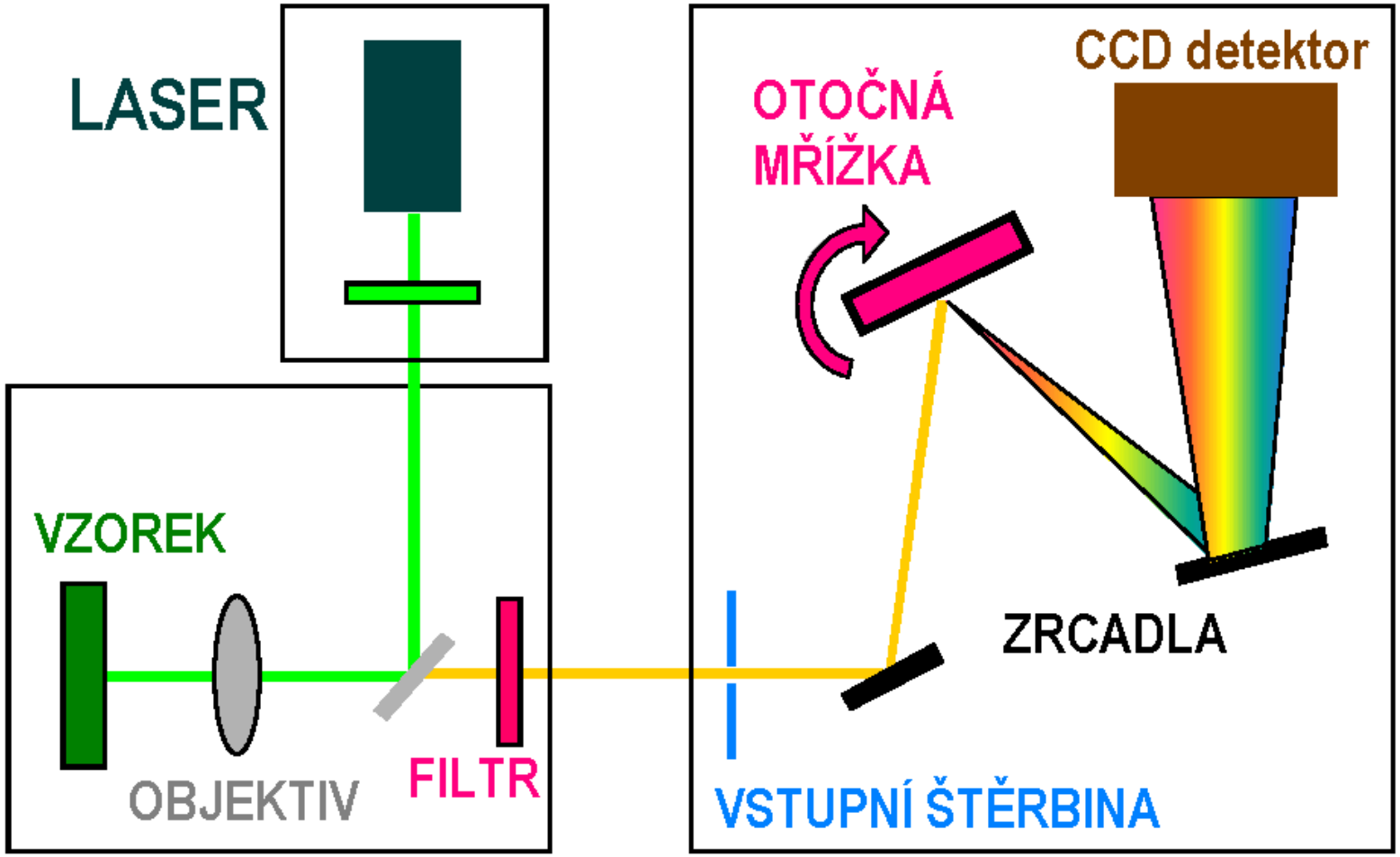
- Gratings
- Lower accumulation time
- Better signal to noise
- Higher risk of sample burning
- UV and VIS lasers

Interferometric systems

- Lower risk of fluorescence
- Higher acqusition time
- Lower spectra intensity
- NIR lasers

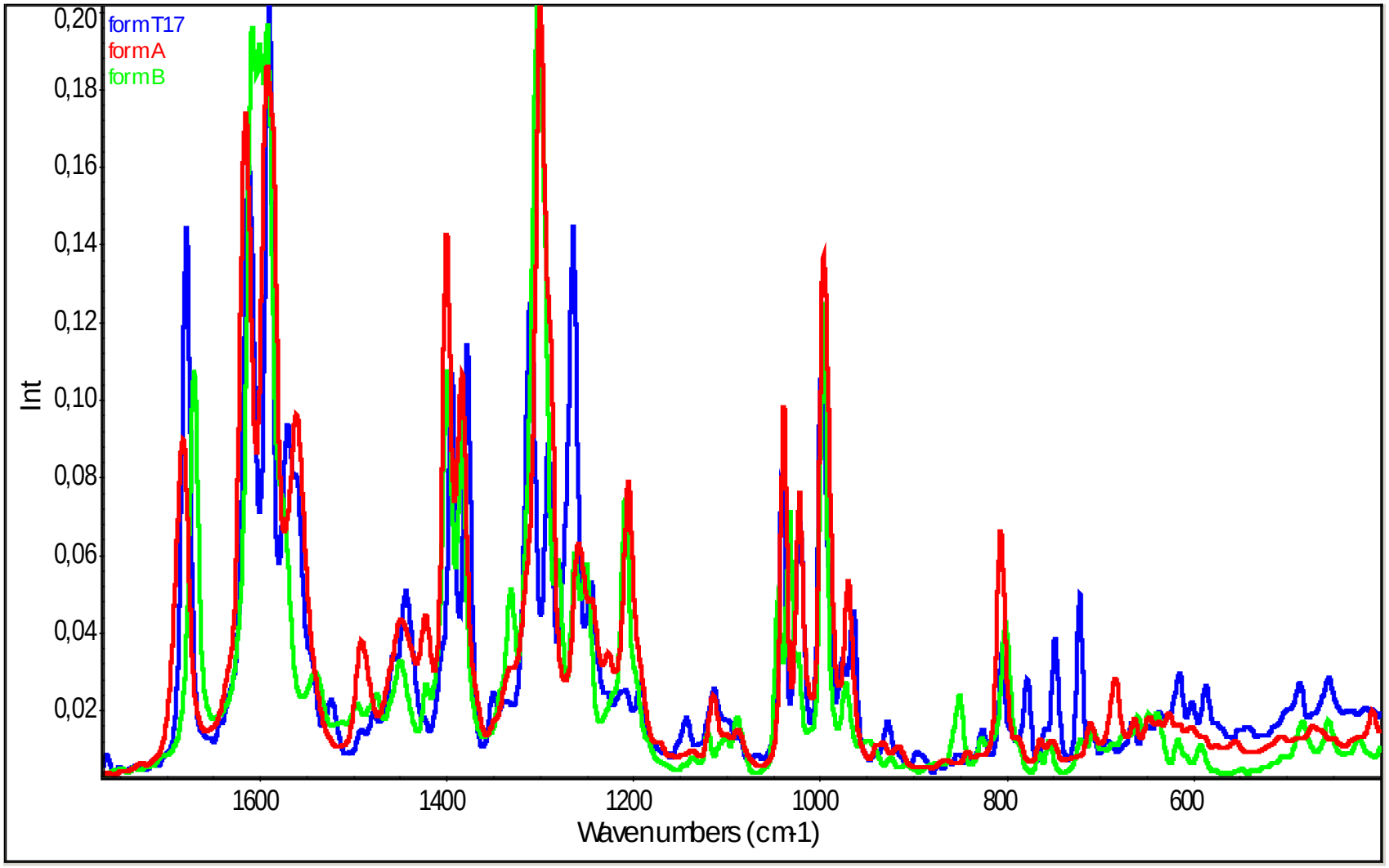


# Raman Spectroscopy




P. Matějka, přednášky VŠCHT Praha

# Raman Spectroscopy



# Raman Spectroscopy – Application in pharmaceutical industry

Same as IR plus:

Possible to measure through glass and plastic packages (no contamination risk)

Possible to measure aqueous samples – low OH band intensity

No or minimal sample pretreatment

Intensive symetric vibrations (C=C, N=N, …) – advantage for API identification in the mixtures and/or dosage form

Possible to calculate the temperature of the sample

# Microscopy – Sample preparation

Many approaches

Consider what you want to achieve

Directly on a glassy carrier, Petri dish, …

Sectioning is carried out by a knive, blades, abrasive paper, …

For thin sections of hard, soft or small materials: embedding into a matrix and consequent sectioning or abrasion

Embedding is appropriate among others for mineralogy, restoration, histology, tissue engineering and pharmaceutics

# Microscopy – Sample preparation

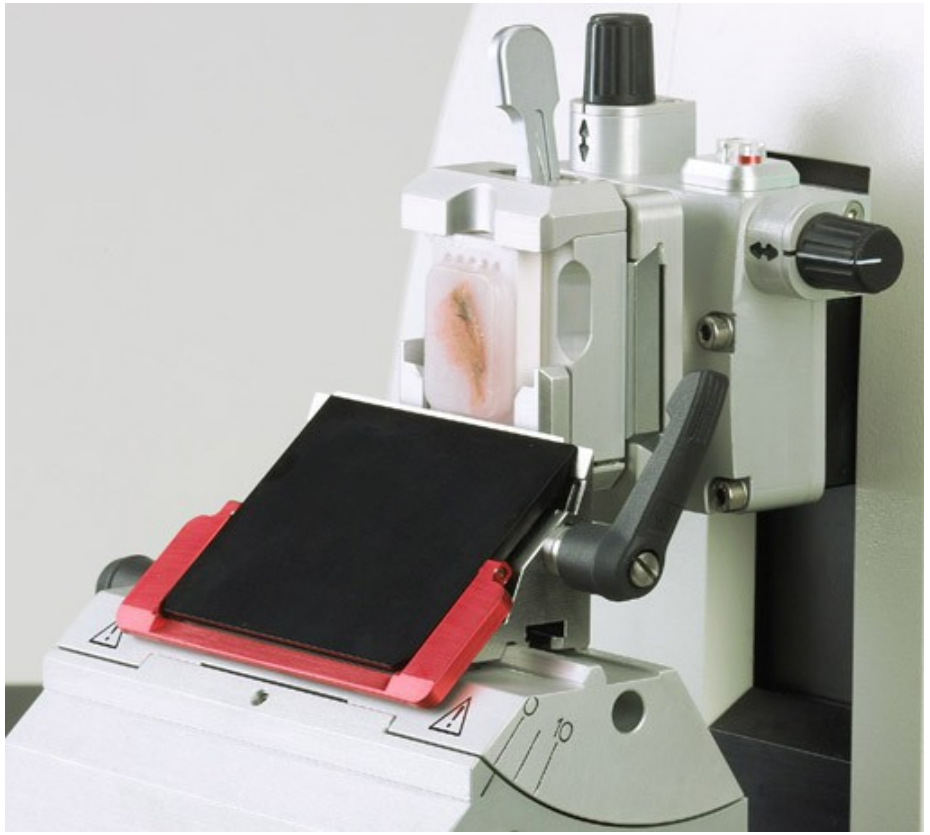
Sample is embedded into an appropriate medium blocks (parraffin, resins, polyurethans, …)

https://www.leicabiosystems.com/

http://sites.gsu.edu/neuroscience-core/training/histology/

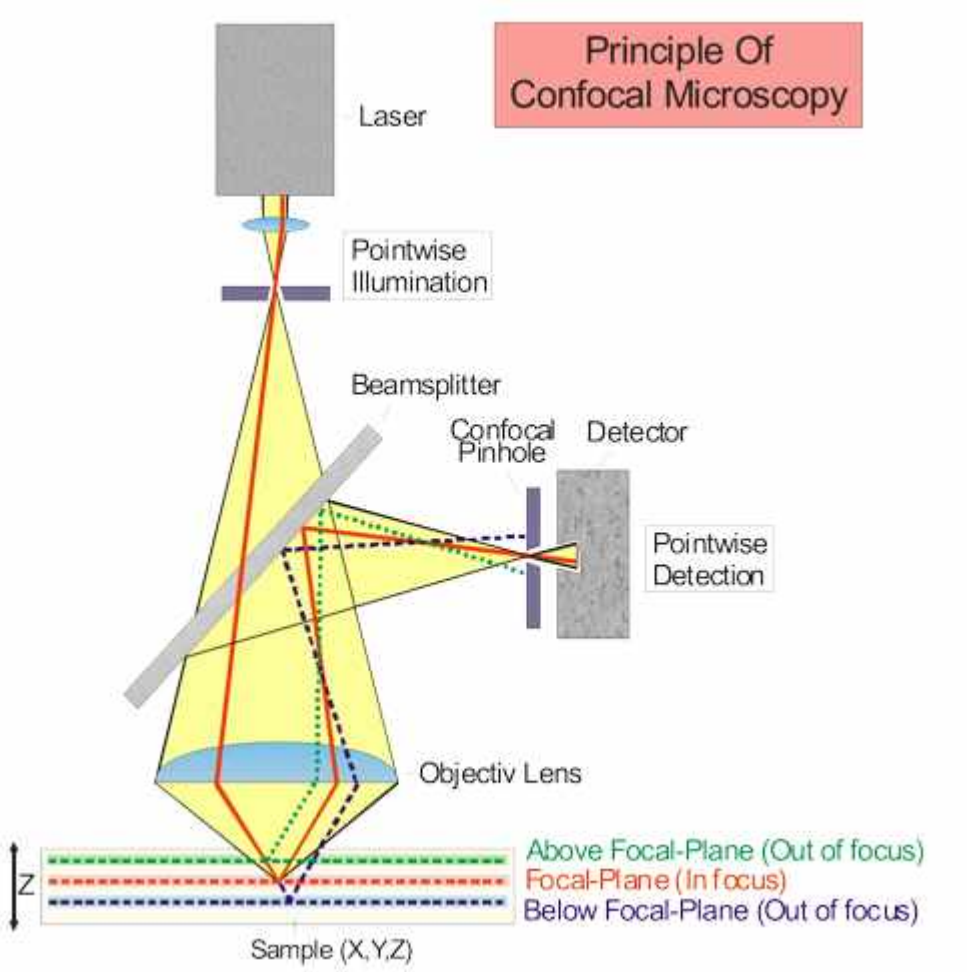
# Microscopy – Sample preparation

Blocks are consequently abraded or sectioned by microtome

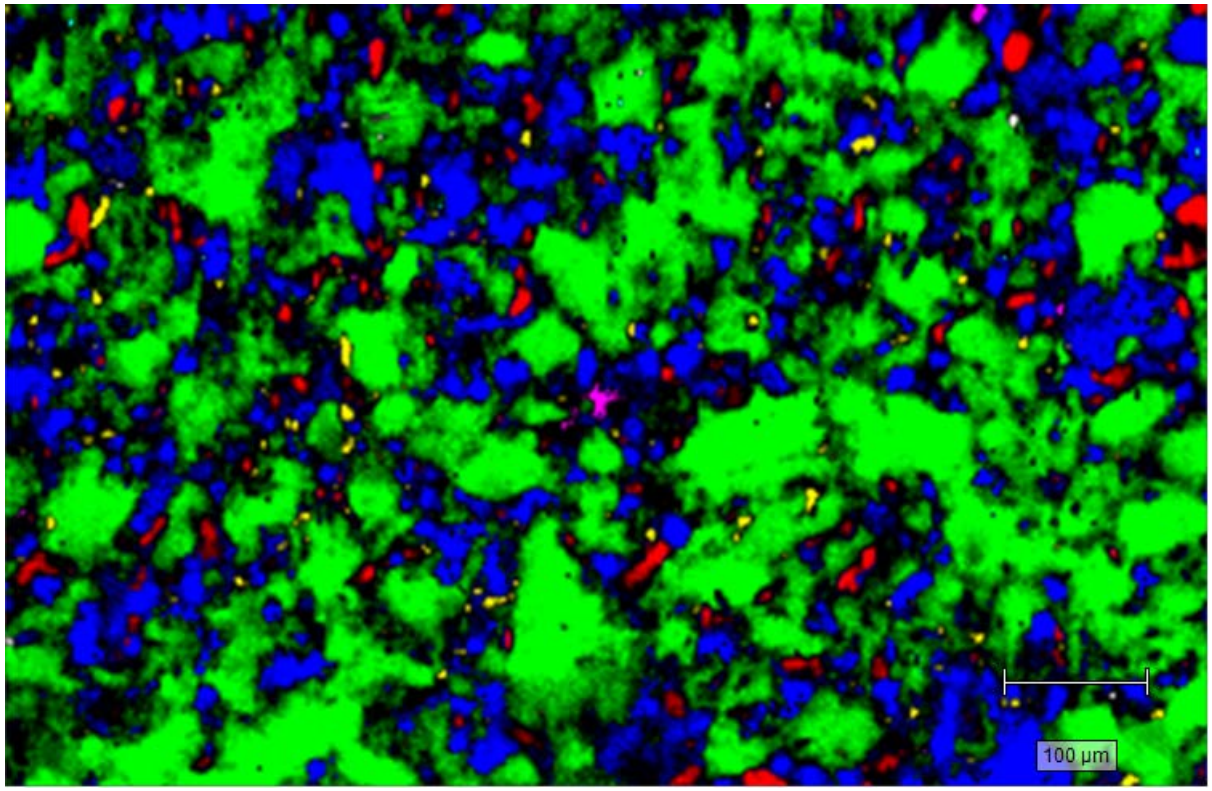
https://www.leicabiosystems.com/

# Raman Spectroscopy – microscopy, confocality




http://fb6www.uni-paderborn.de/.../microscope_e.htm

# Raman spectroscopy – mapping



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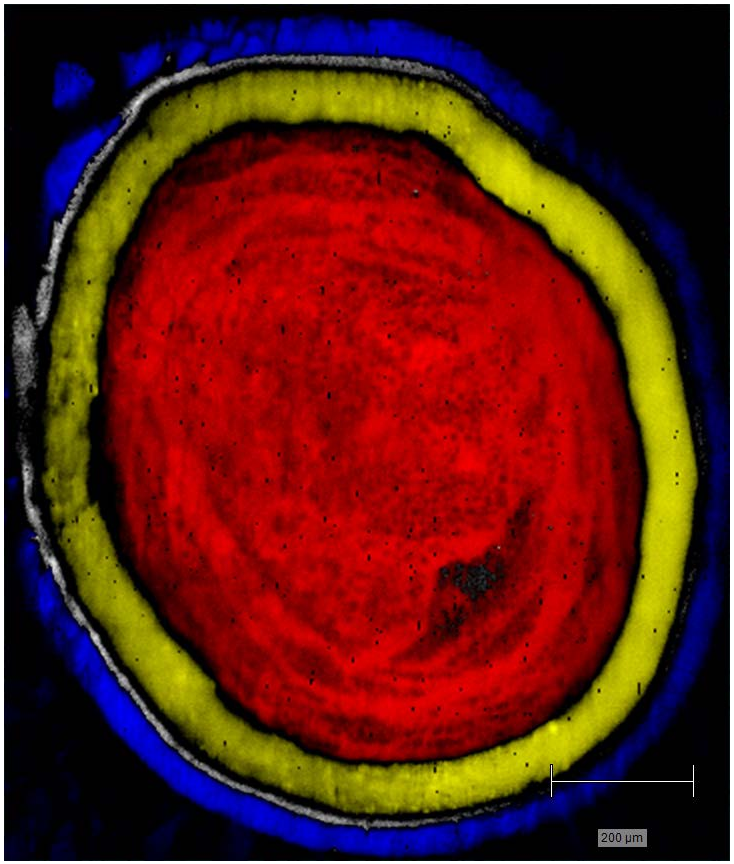
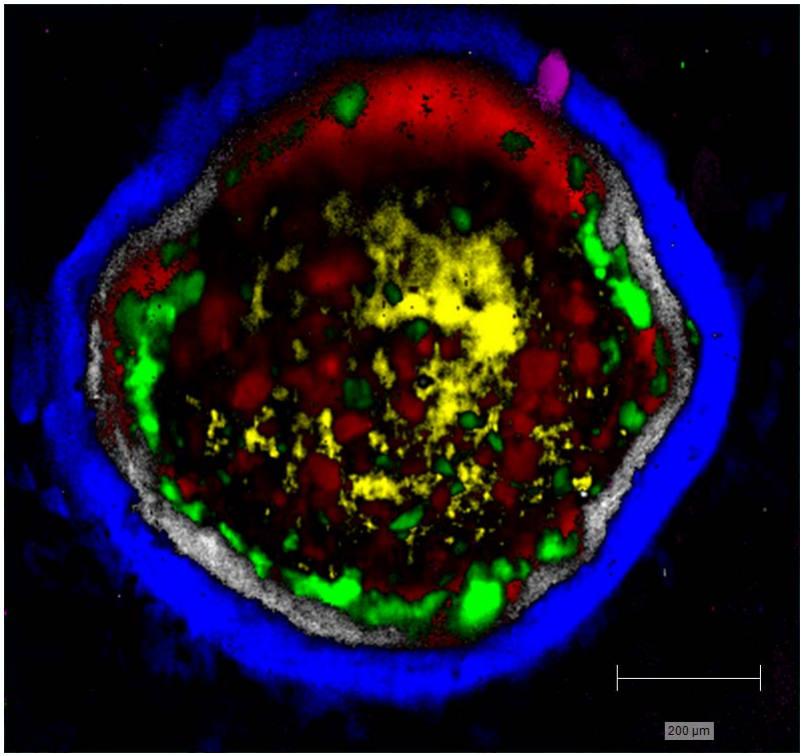
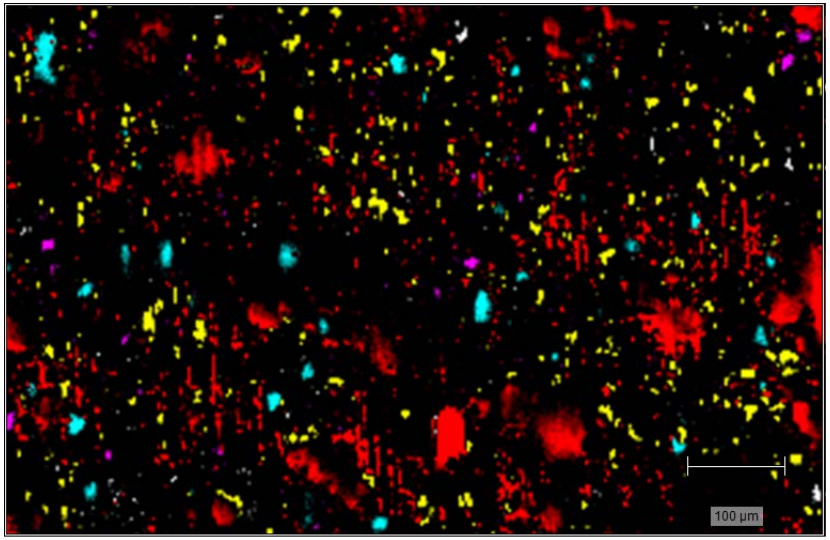
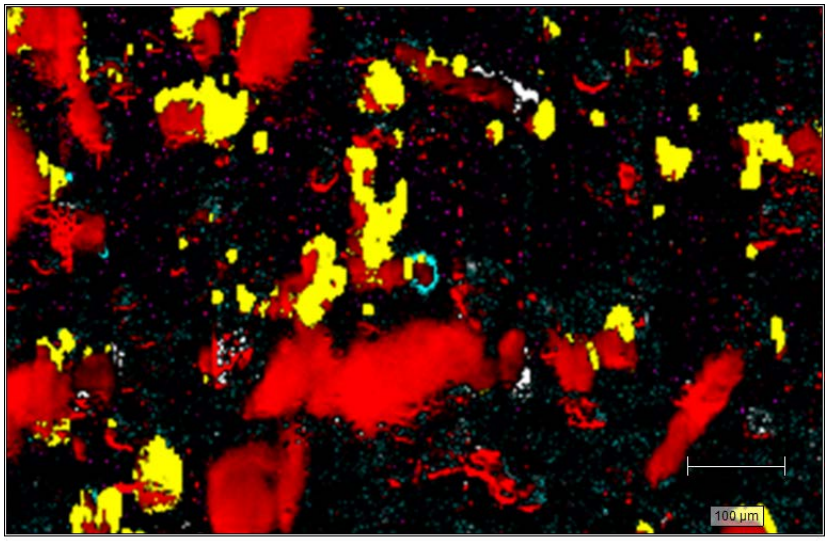
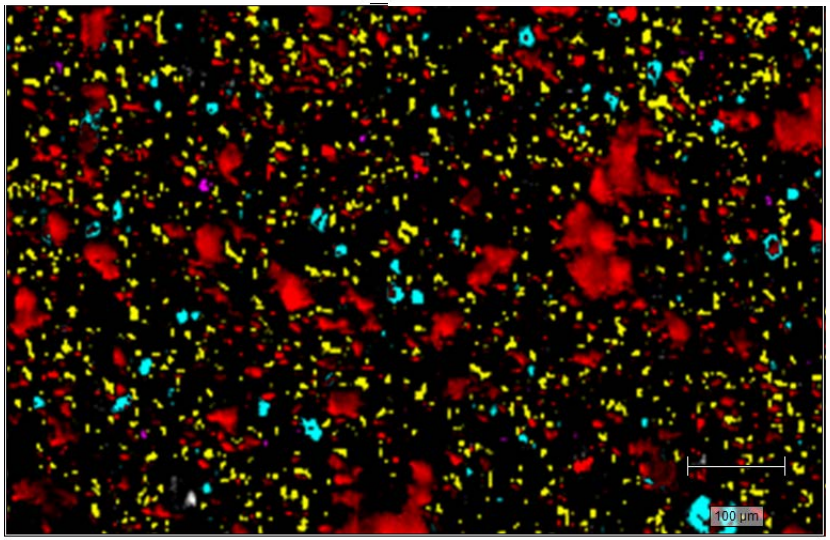


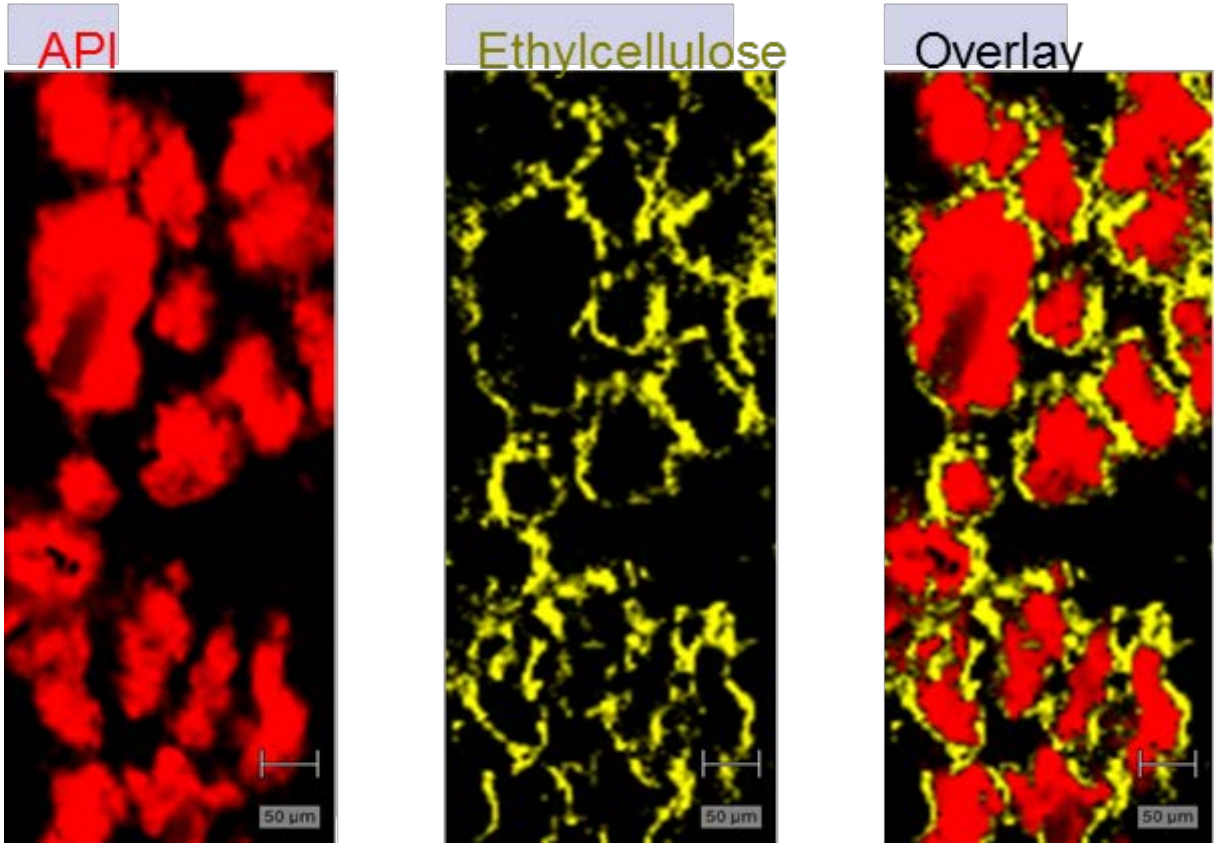
# Raman spectroscopy – mapping



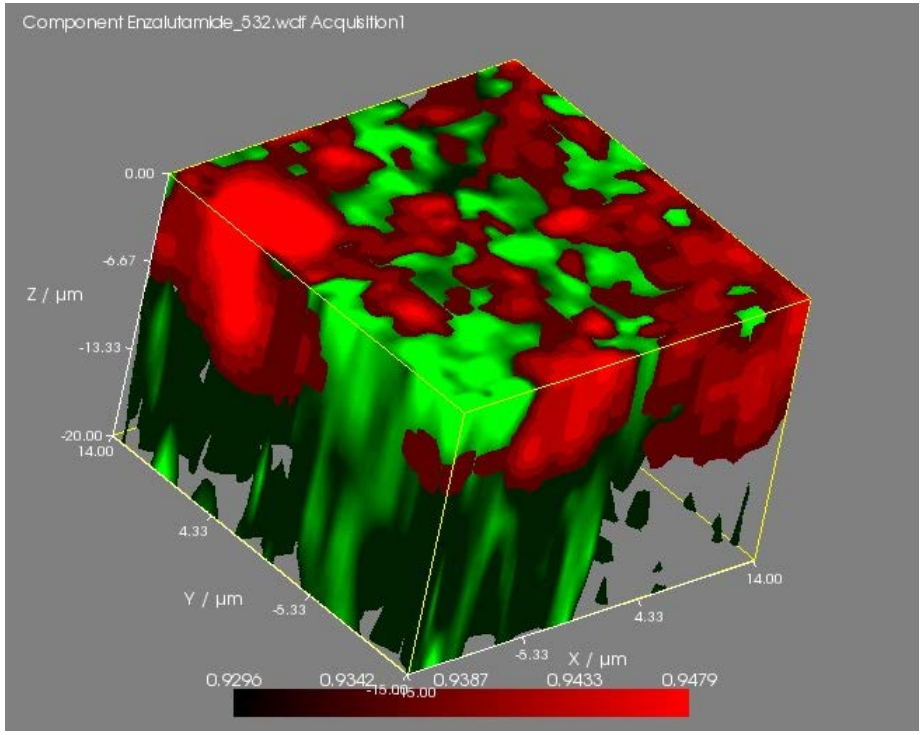



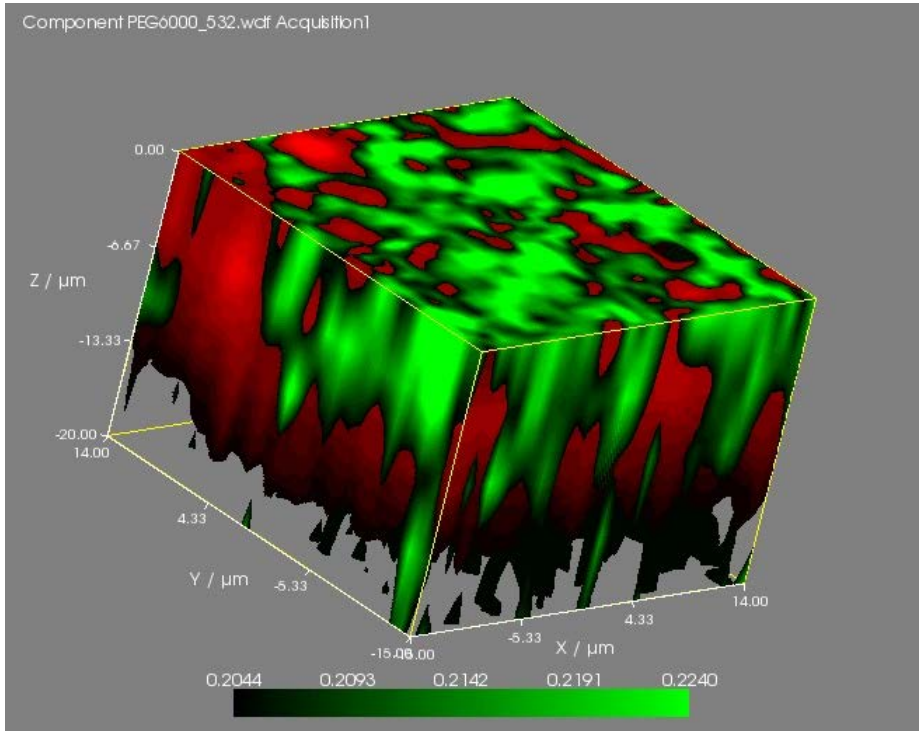



# Raman spectroscopy – mapping



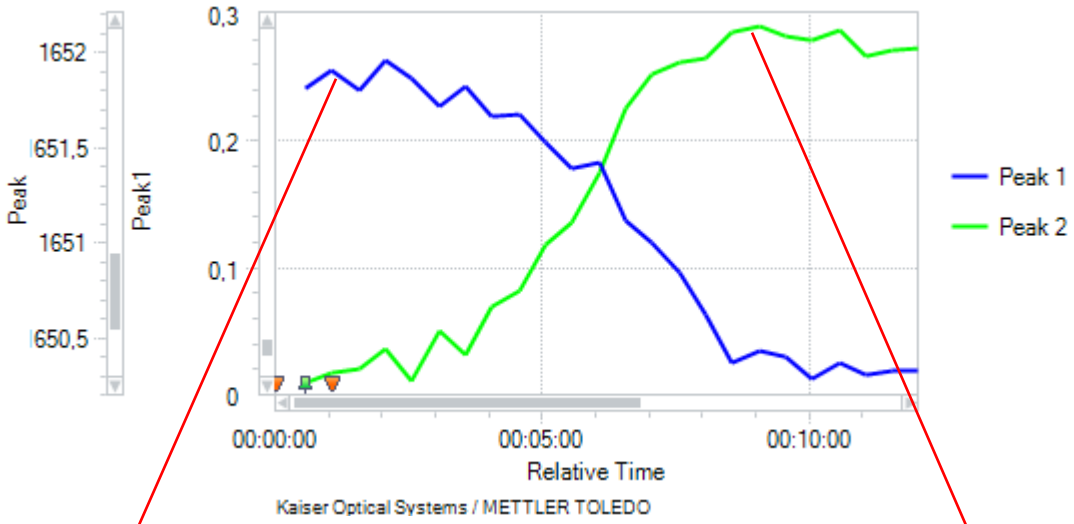
# Raman spectroscopy – mapping

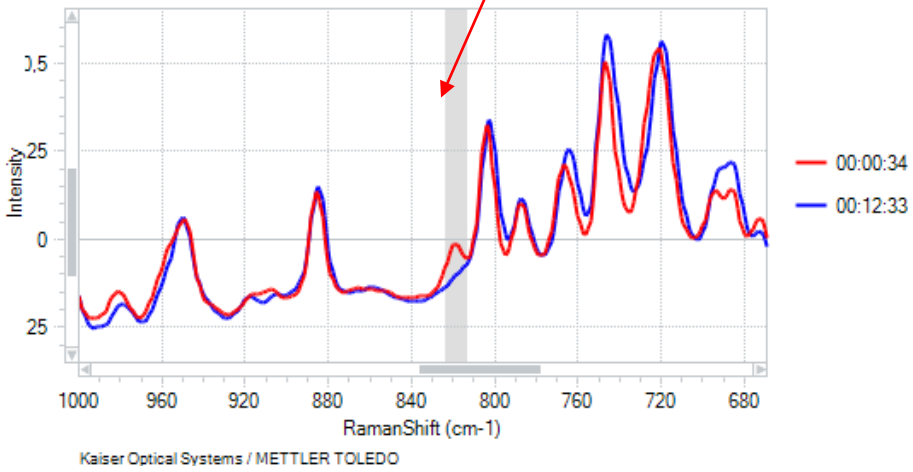


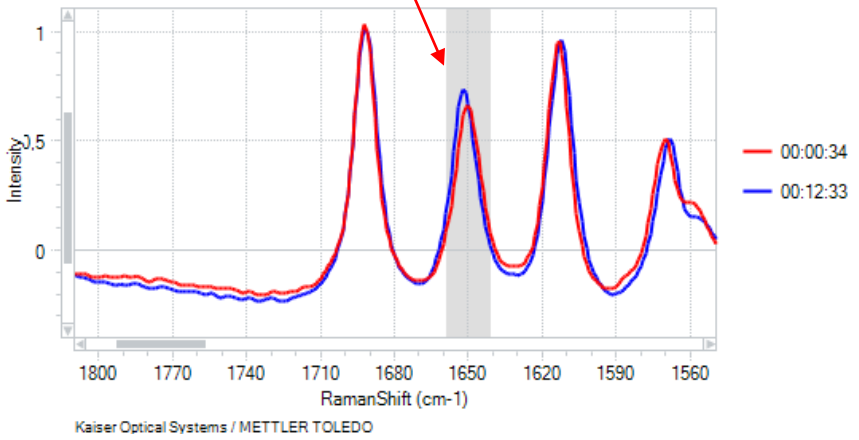



# Raman Spectroscopy – Probes

Solid form transformation

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# **Raman Spectroscopy:** Other Applications

- All research and development fields, e.g.:
- Medicine – tissues diagnostics
- Geology – mineral analysis
- Electrotechnics – semiconductor applications, microchips
- Chemical industry
- Food-Processing industry
- Pharmaceutical industry
- Metallurgy
- Rescue Systems
- Armed Forces & criminalistics & Forensic Sciences

# Thermal Analysis

Physico-chemical properties are observed

- Changes of mass, energy, conductivity, etc. during heating
- Different principles

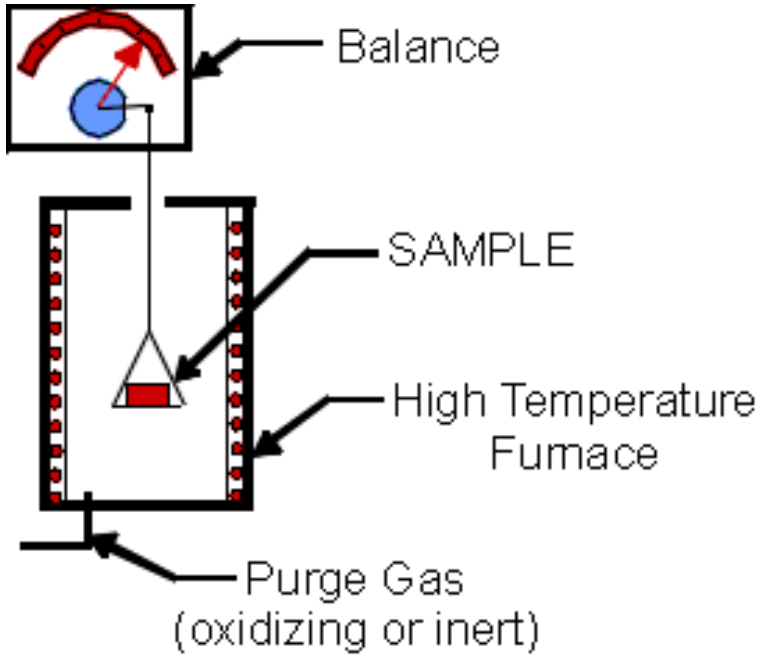
# Thermogravimetry (TGA)

Accurate balances

Weight change during heating

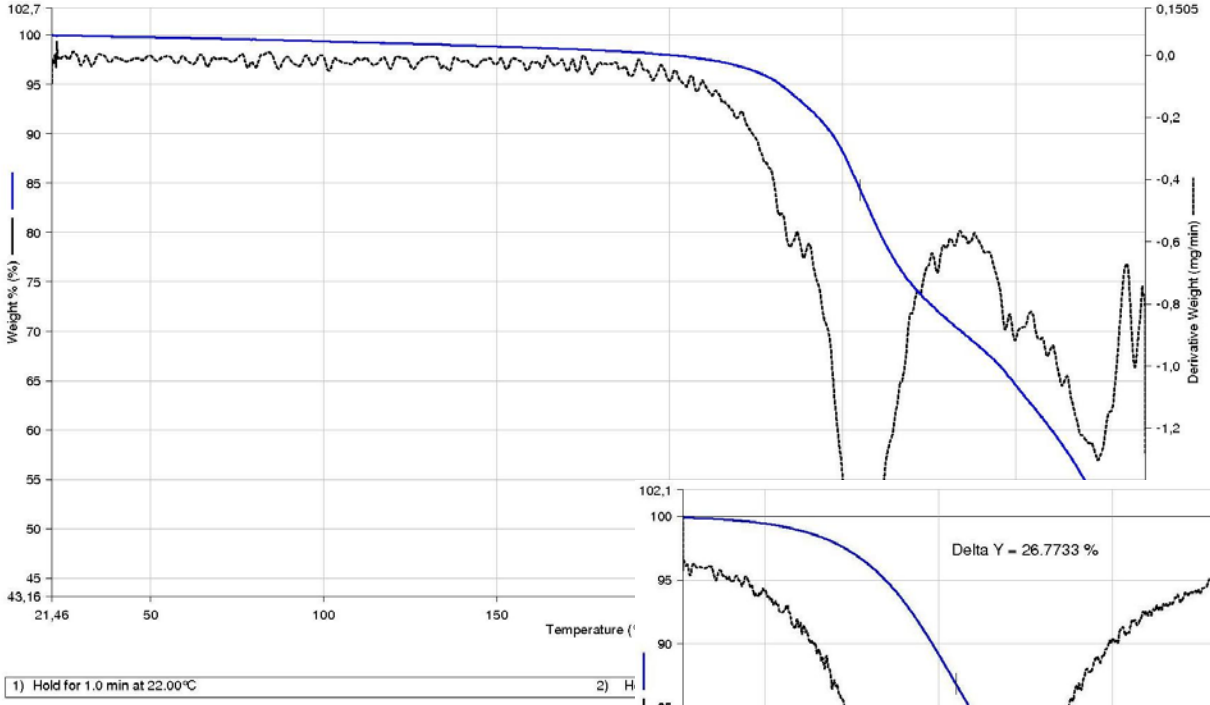
Application:

- Water / moisture content
- Thermal stability
- Thermal decomposition
- Weight changes

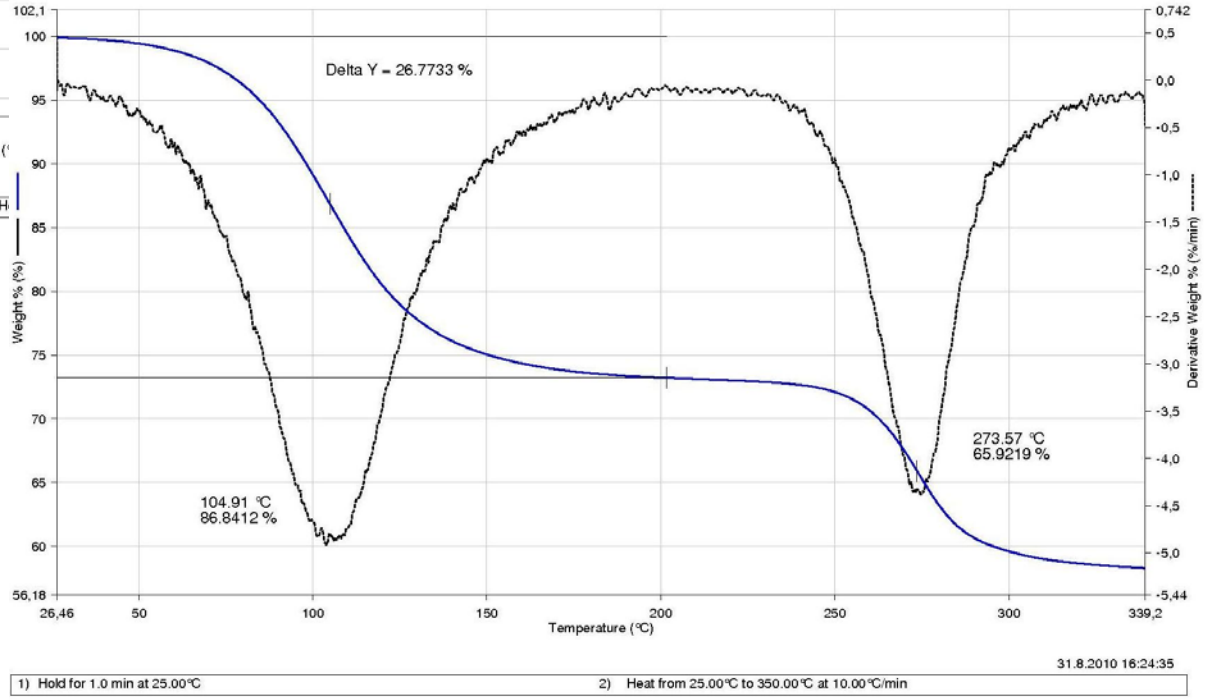



http://radchem.nevada.edu/classes/chem455/lecture_22__thermal_methods.htm

# Thermogravimetry (TGA)




Water-free vs. 7 mol water

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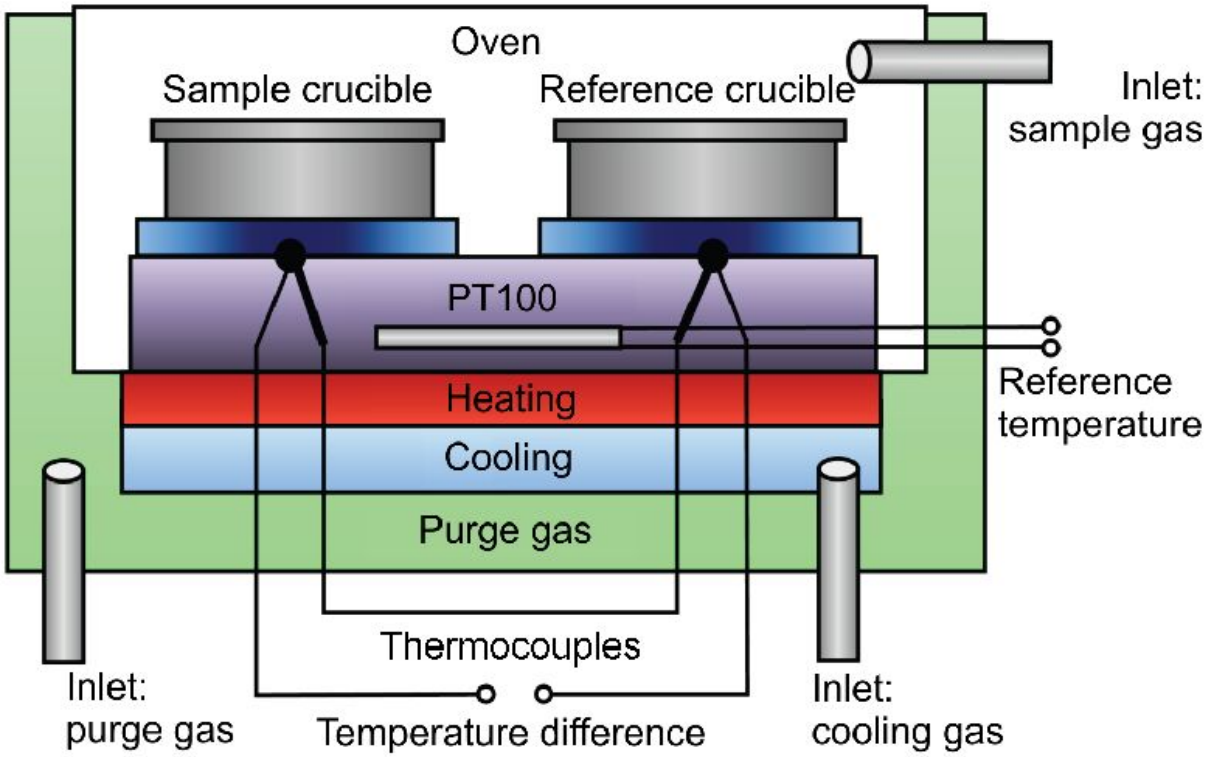
# Differential Scanning Calorimetry (DSC)

Method

- Amount of supplied energy in order to keep isothermal conditions (heated sample and reference)

Application:

- Melting point
- Glassy transition temperature
- Phase transitions
- Polymorphism
- Thermal decomposition
- Dehydratation

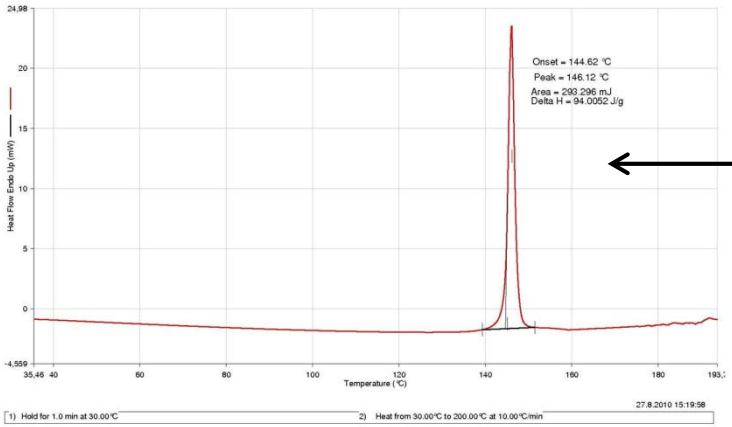



References:

- DSC – empty cup
- MicroDSC – reference: according to specific thermal capacity: corundum, water and undekan (90 % of measurement)

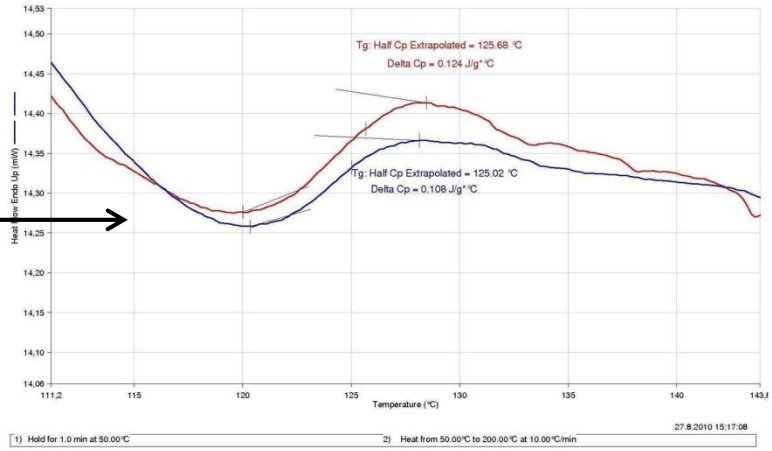
http://www.intechopen.com/books/applications-of-calorimetry-in-a-wide-context-differential-scanning-calorimetry-isothermal-titration-calorimetry-and-microcalorimetry/thermal-analysis-of-phase-transitions-and-crystallization-in-polymeric-fibers

# Differential Scanning Calorimetry (DSC)




Crystalline

Amorphous



# Dynamic Vapour Sorption

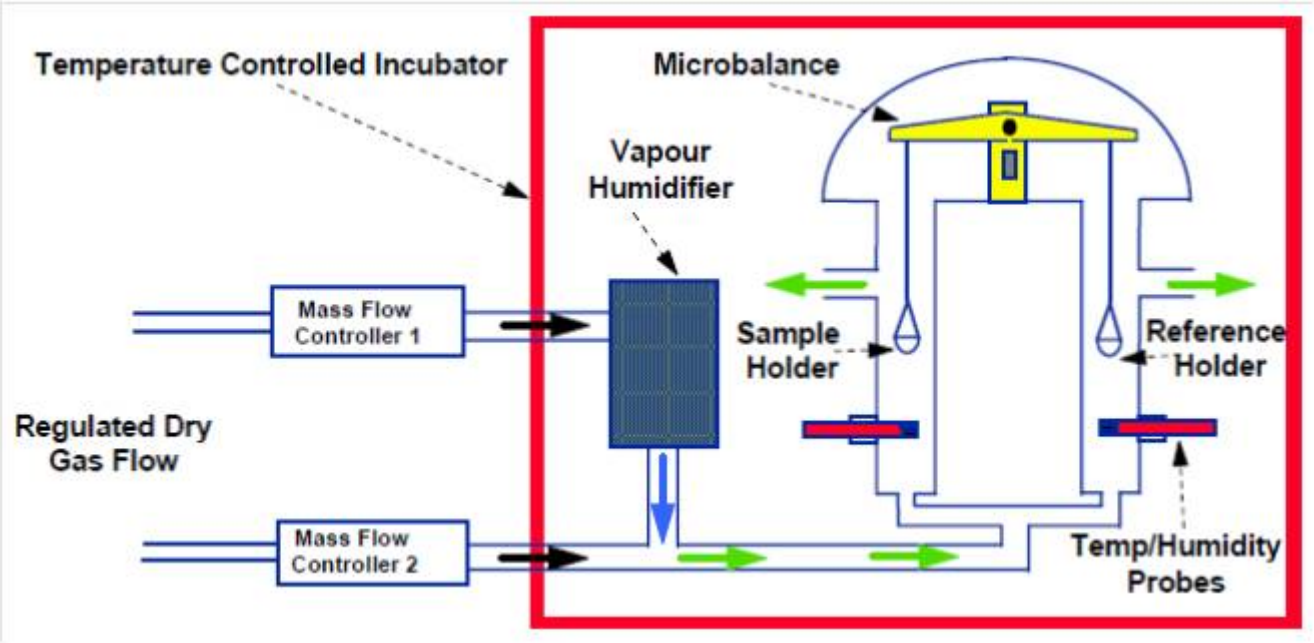
Hygroscopicity

- A potential of substances to sorb and retain water
- Affects physico-chemical properties and material stability
- Registration of weight changes depending on the controlled relative humidity, time and temperature

# Dynamic Vapour Sorption

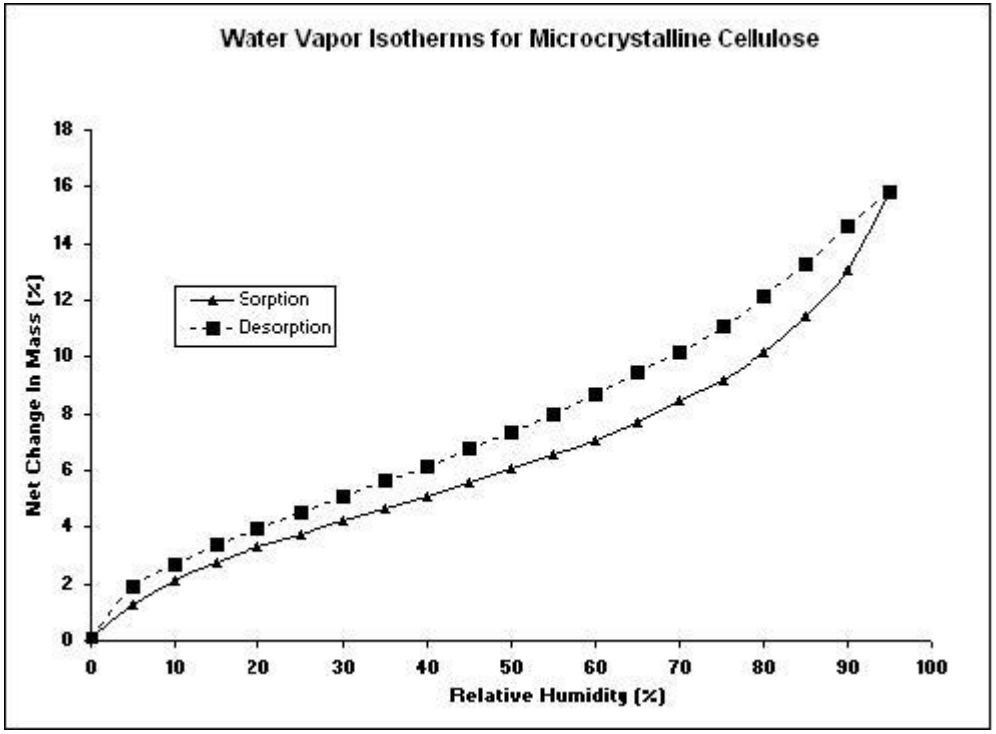
Adsorption – surface interaction, no penetration into the bulk

Absorption – penetration of water into the bulk




http://dunia-wahyu.blogspot.cz/2012/06/dynamic-vapour-sorption-dvs-analysis.html

# Dynamic Vapour Sorption




By Dvstechnique - Own work, Public Domain, https://commons.wikimedia.org/w/index.php?curid=7321903

# Surface Area Analysis

Adsorption of gas (helium or nitrogen) or mercury on the material surface

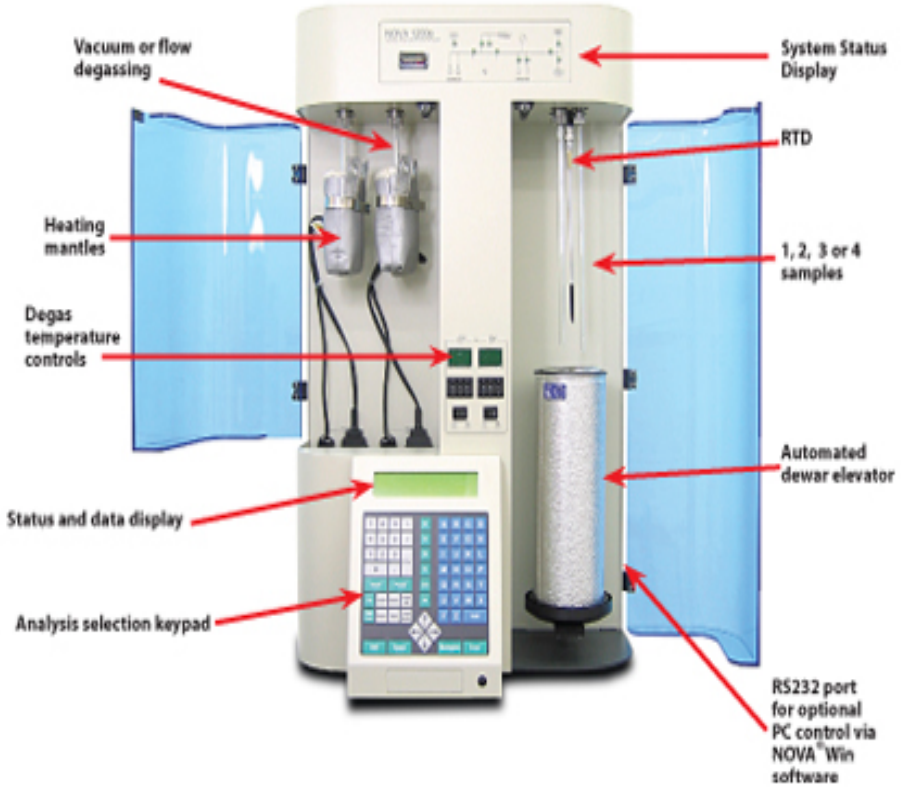
Environment: T = -196 °C (liquid nitrogen boiling point)

Non-covalent interactions (van deer Waals), between gas and surface

Sample preparation – degas (vacuum)

Higher surface = larger interaction area

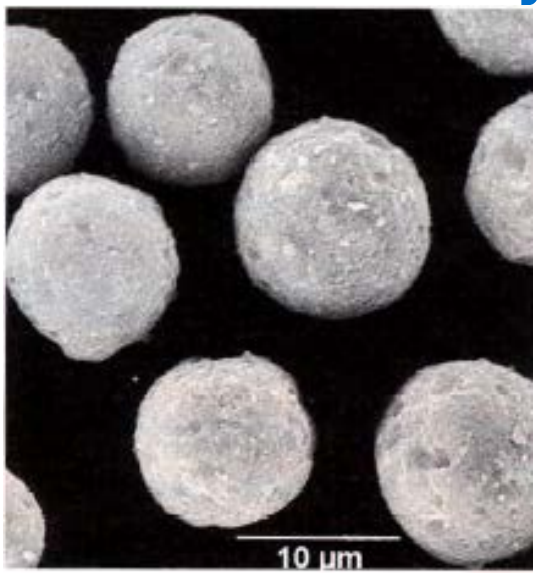
# Surface Area Analysis




Rear of Instrument: RS 232 Port for PC Control via NOVAWin 2.0 Printer Port

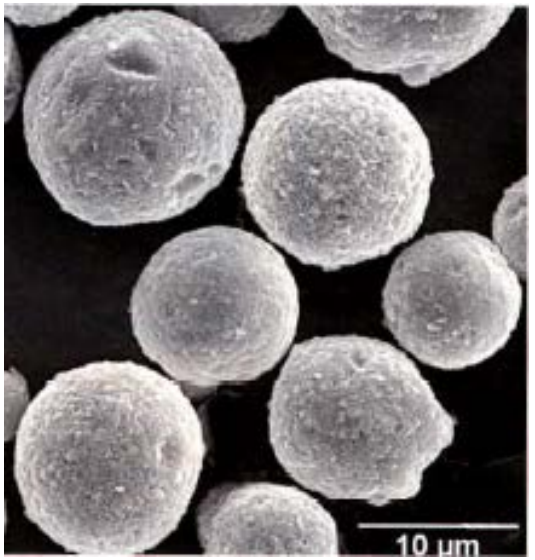
http://www.quantachrome.com/gassorption/nova_series.html

# Surface Area Analysis




$5m^2/g$


$150m^2/g$




$10m^2/g$

# Thank you

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