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Raman spectroscopy

Leena Pitkänen
28.4.2025

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Raman spectroscopy?

Spectroscopic technique to determine vibrational modes of molecules

Structural information

- **Chemical structure, functional groups**
- **Crystallinity**
- **Molecular interactions**

C. V. Raman

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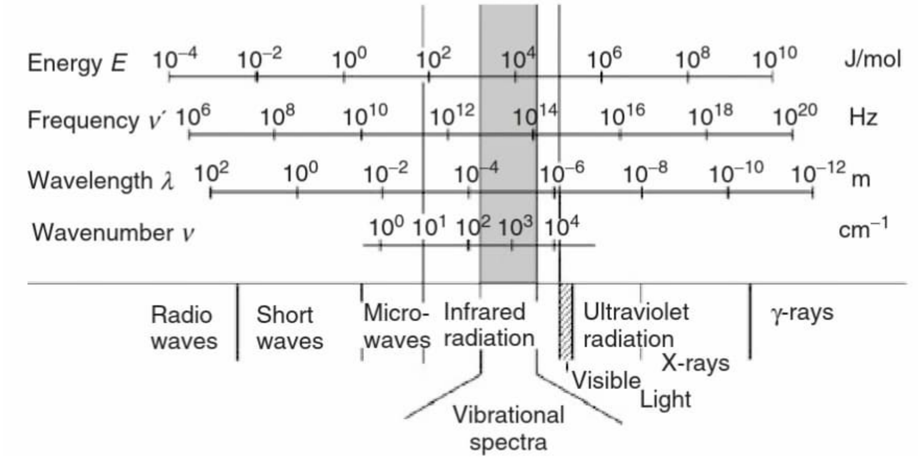
Electromagnetic radiation



- **Electromagnetic radiation in the near visible light changes the status of molecular vibrations**
  - **Vibrational spectra of molecules**

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Leng, Y., Materials characterization: Introduction to Microscopic and Spectroscopic methods, 2009, Wiley-VCH

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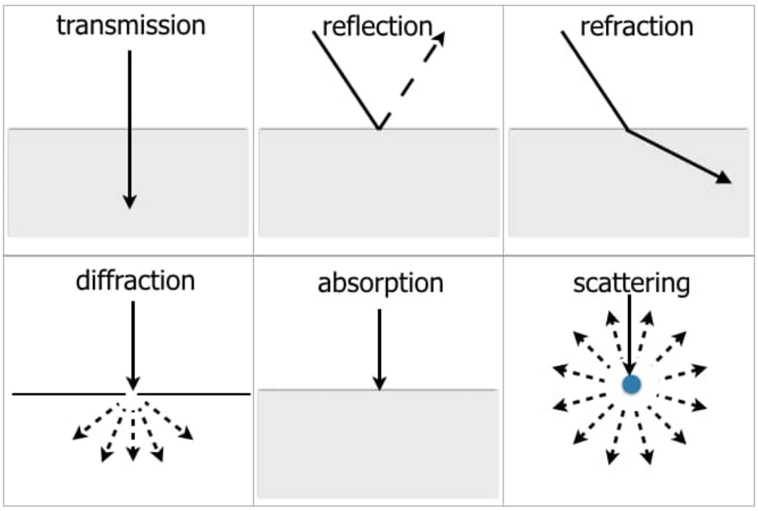
Behavior of light



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https://www.mrwaynesclass.com/lightOptics/reading/index02.html

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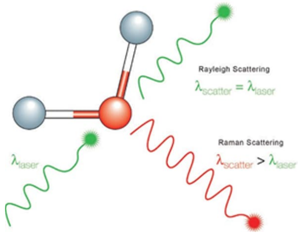
Light scattering



Light scattering can be either elastic or inelastic:

1. Elastic scattering: scattered light has the **same** frequency than incident radiation (Rayleigh).
2. Inelastic scattering: scattered light has **higher** frequency than the incident radiation (anti-Stokes).
3. Inelastic scattering: scattered light has **lower** frequency than the incident radiation (Stokes →Raman).

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https://www.horiba.com/en_en/raman-imaging-and-spectroscopy/

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Rayleigh scattering

- **Most of the scattered light is at the same wavelength as the incident light**

→ **Rayleigh scattering**

Useful when measuring polymer
molar masses in solution!

$$\mathrm{LS} \sim \boldsymbol{M} \cdot c \cdot (\partial n/\partial c)^2$$

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M = molar mass
c = concentration
$\partial n/\partial c$ = refractive index increment

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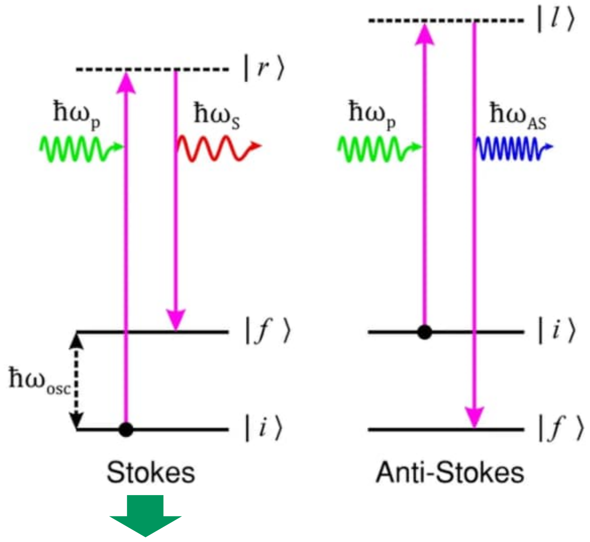
Inelastic scattering

The photon frequency decreases → the final energy level of molecular vibration is higher than the initial energy



The photon frequency increases → the final energy level of molecular vibration is lower than the initial energy

Stronger intensity

Jones et al. Nanoscale Research Letters (2019) 14:231

Raman (Stokes) scattering

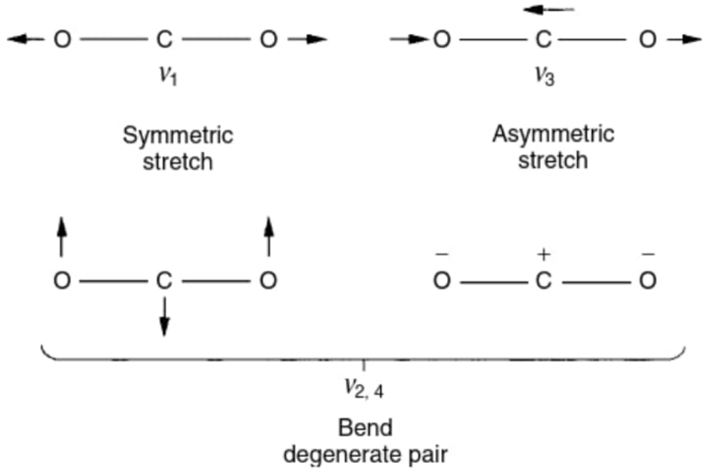
- **Small amount of light (typically 0.0000001%) is scattered at different wavelength than the incident light**
  - This scattering depends on the chemical structure of the analyte
  - Raman spectrum commonly shows a number of peaks showing the intensity and wavelength position of the Raman scattered light
  - Each peak corresponds to a specific molecular bond vibration

C – C **C – H** **C = C** **N – O**

https://www.horiba.com/en_en/raman-imaging-and-spectroscopy/

Molecular vibrations

Normal modes of vibrations in a CO_2 molecule



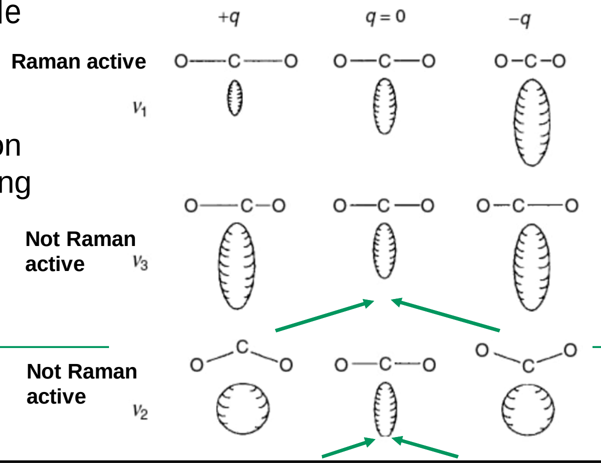
Can be detected with Raman

Leng, Y., Materials characterization: Introduction to Microscopic and Spectroscopic methods, 2009, Wiley-VCH

Raman active molecules

- **In Raman active molecules the vibration mode must cause polarizability changes**
  - Change in size, shape or orientation of the electron cloud that surrounds the molecule

The change in ellipsoid electron cloud should be changing during vibration (not return back)



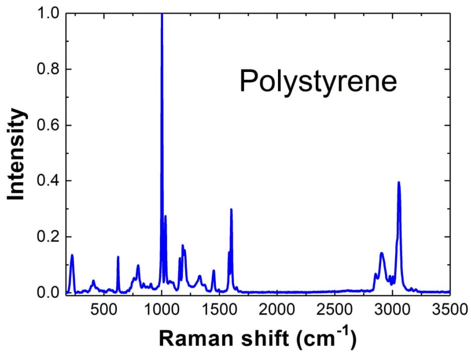
Vibration modes of CO_2

Wave number ($\overline{V}$)

- **Reciprocal of the wavelength in the units of cm-1**

$$\overline{v} = \frac{1}{\lambda}$$

Absolute wavenumber



Polystyrene raman spectrum | Raman for life (ramanlife.com)

Relative wavenumber

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

Example: The 532 nm laser is used and the analyte scatters light at 554 nm. What is the Raman wavenumber?

746 cm-1

Common Raman vibrations

Functional Group/ Vibration	Region	Raman	InfraRed
Lattice vibrations in crystals, LA modes	10 - 200 cm^{-1}	strong	strong
δ(CC) aliphatic chains	250 - 400 cm^{-1}	strong	weak
υ(Se-Se)	290 -330 cm^{-1}	strong	weak
υ(S-S)	430 -550 cm^{-1}	strong	weak
υ(Si-O-Si)	450 -550 cm^{-1}	strong	weak
υ(Xmetal-O)	150-450 cm^{-1}	strong	med-weak
υ(C-I)	480 - 660 cm^{-1}	strong	strong
υ(C-Br)	500 - 700 cm^{-1}	strong	strong
υ(C-Cl)	550 - 800 cm^{-1}	strong	strong
υ(C-S) aliphatic	630 - 790 cm^{-1}	strong	medium
υ(C-S) aromatic	1080 - 1100 cm^{-1}	strong	medium
υ(O-O)	845 -900 cm^{-1}	strong	weak
υ(C-O-C)	800 -970 cm^{-1}	medium	weak
υ(C-O-C) asym	1060 - 1150 cm^{-1}	weak	strong

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Raman Bands - HORIBAhttps://static.horiba.com › Horiba › Raman_Tutorial

Common Raman vibrations

υ(CC) alicyclic, aliphatic chain vibrations	600 - 1300 cm^{-1}	medium	Medium
υ(C=S)	1000 - 1250 cm^{-1}	strong	weak
υ(CC) aromatic ring chain vibrations	*1580, 1600 cm^{-1}	strong	medium
	*1450, 1500 cm^{-1}	medium	medium
	*1000 cm^{-1}	strong/medium	weak
δ(CH3)	1380 cm^{-1}	medium	strong
δ(CH2) δ(CH3) asym	1400 - 1470 cm^{-1}	medium	medium
υ(C-(NO2))	1340 - 1380 cm^{-1}	strong	medium
υ(C-(NO2)) asym	1530 - 1590 cm^{-1}	medium	strong
υ(N=N) aromatic	1410 - 1440 cm^{-1}	medium	-
υ(N=N) aliphatic	1550 - 1580 cm^{-1}	medium	-
δ(H2O)	~1640 cm^{-1}	weak broad	strong
υ(C=N)	1610 - 1680 cm^{-1}	strong	medium
υ(C=C)	1500 - 1900 cm^{-1}	strong	weak
υ(C=O)	1680 - 1820 cm^{-1}	medium	strong
υ(C≡C)	2100 - 2250 cm^{-1}	strong	weak
υ(C≡N)	2220 - 2255 cm^{-1}	medium	strong
υ(-S-H)	2550 - 2600 cm^{-1}	strong	weak
υ(C–H)	2800 - 3000 cm^{-1}	strong	strong
υ(=(C-H))	3000 - 3100 cm^{-1}	strong	medium
υ(≡(C-H))	3300 cm^{-1}	weak	strong
υ(N-H)	3300 - 3500 cm^{-1}	medium	medium
υ(O-H)	3100 - 3650 cm^{-1}	weak	strong

Raman **IR**

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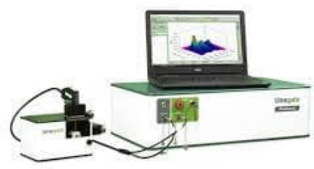
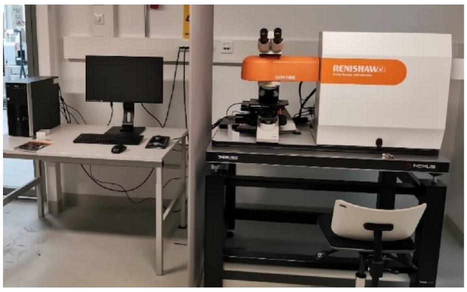
Raman instruments

- **Raman microscopy**
- **Raman with probe options**
- **Portable Raman devices**

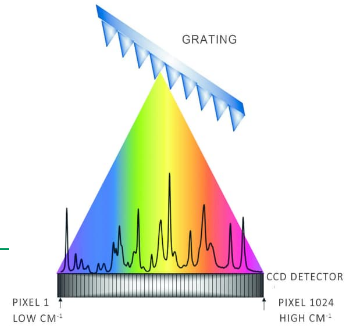



Diffraction gratings

- **Disperses Raman-scattered light according to its wavenumbers (before detector)**
  - Diffracts the different wavelengths in discrete angles
- **Gratings affect the spectral resolution (*i.e.* how small wavenumber differences can be detected)**
- **Grating surface contains parallel lines**
  - Each grating has line density l/mm (lines/mm)
  - The higher the line density, the better the spectral resolution
  - With a lower l/mm grating more spectral range will be acquired

CCD detector

- **CCD (charged-coupled device) detectors most commonly used**
  - Converts photon signals to electric signals
  - Can detect and record light intensity of discrete wavelengths separated by the diffraction grating



https://www.horiba.com/fra/ramanspectrometerpresentation/

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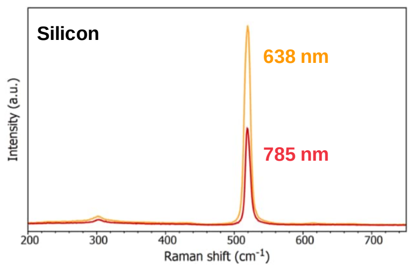
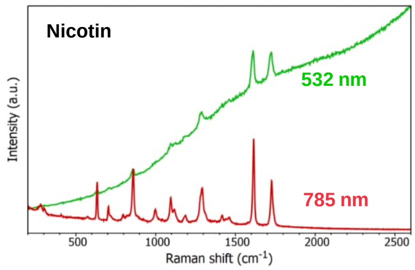
Laser wavelengths in Raman

- **Raman scattering intensity is proportional to $1/\lambda^4$**
  $\rightarrow$ Raman wavelength ↑
  Raman scattering intensity ↓
- **Common wavelengths: 244 nm, 488 nm, 532 nm, 785 nm, 1064 nm**





Fewer molecules absorb in the near-infrared region

https://www.edinst.com/blog/lasers-for-raman-spectroscopy/

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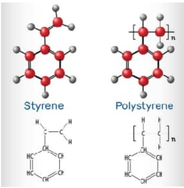
What kind of samples can be analyzed with Raman?

- **Basically anything!**



Pixabay

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Challenging in respect of Raman…

- **Metallic materials**
  - High reflection of electromagnetic waves
- **Fluorescent samples**
  - *E.g.* lignin
  - Try different wavelengths, time-gated Raman
- **Very dark samples which absorb light**
  - Weak scattering
- **Very polar molecules (H_2O)**
  - Benefit: analytes dispersed in water can be analyzed

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Comparison of Raman and IR

- Often complementary techniques, both are fast
- Raman works for aqueous samples (IR suffers water absorption effects)
- IR might work better for samples with fluorescence or if the concentration of the analyte is low
- Raman works better in the lower frequencies → crystal lattice vibrations for distinguishing different crystalline forms

Sample preparation

- Basically, no sample preparation is need
- The smoother the sample surface, the better spectrum
- Sometimes powder samples or very “fluffy” samples might be challenging

→ compressing the samples

Raman is non-destructive method

- **In principle, Raman is non-destructive**
- **Control the laser power in order to avoid any structural changes caused by the heat**
  - Samples might burn!
  - Rotation of the sample

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Is Raman surface active method?

- **With confocal Raman it is possible to do depth analysis**
  - Depth (Z) resolution
  - Possible to analyze different layers of the sample

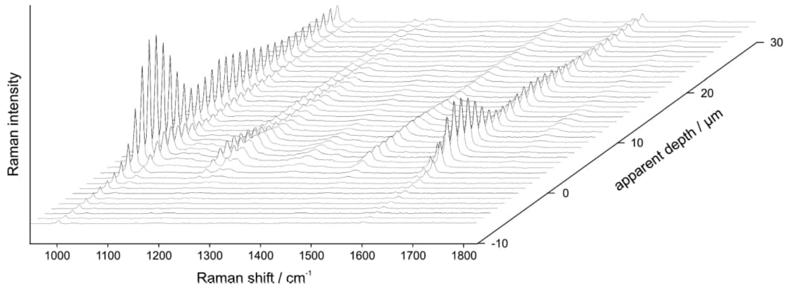

FIG. 5. Raman spectra for the PS/PMMA laminate using a dry objective, plotted as a function of apparent depth below the surface.

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Applied Spectroscopy (2003) 57:1468

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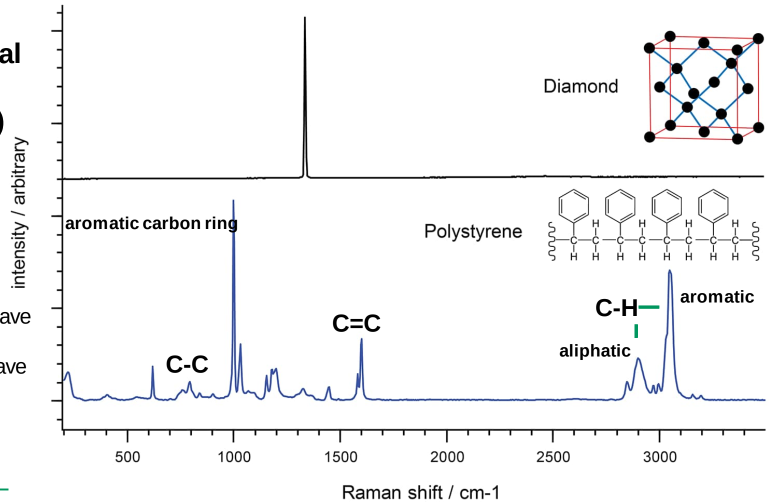
Examples
Raman spectra for diamond and polystyrene

Regular array of identical atoms → one peak (one molecular environment)



- Heavy atoms and weak bonds have low Raman shifts
- Light atoms and strong bonds have high Raman shifts

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http://www.renishaw.com/en/raman-spectra-explained--25807

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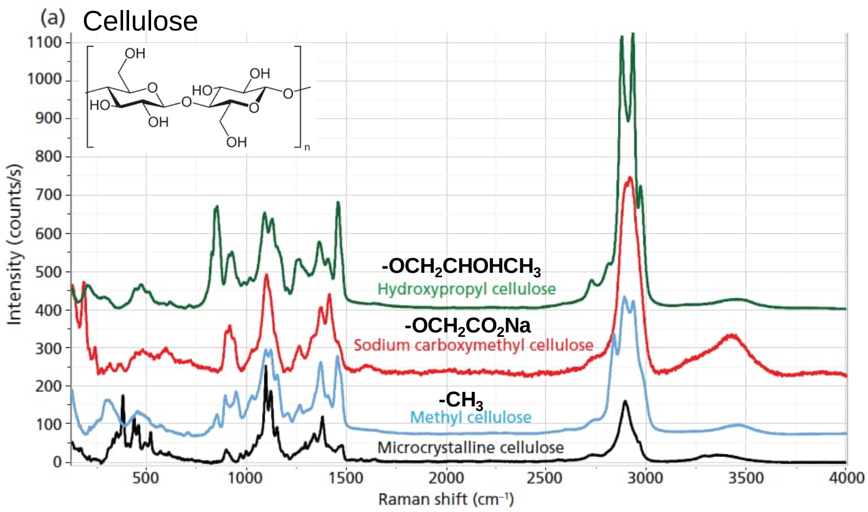
Examples
Cellulose and modified celluloses

Whole spectrum



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Adar F. Characterizing Modified Celluloses Using Raman Spectroscopy. Spectroscopy (2016) 31:22-27.

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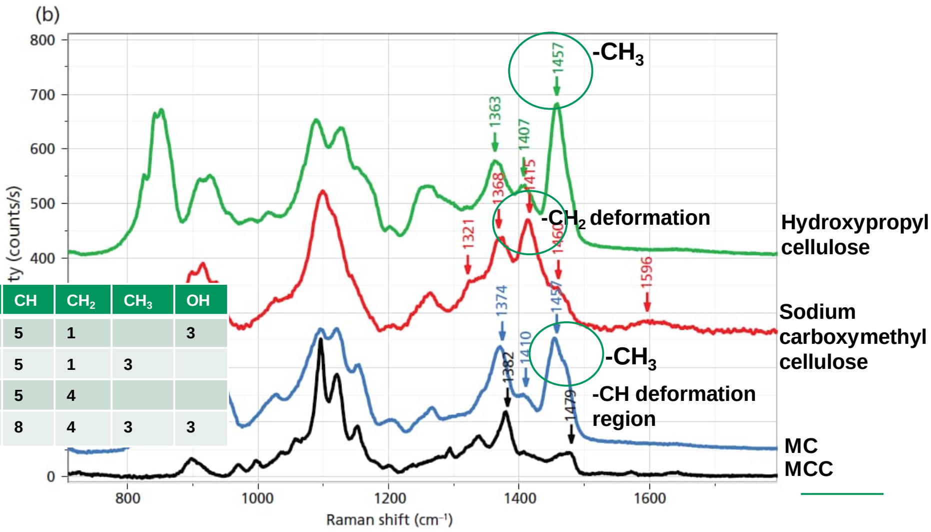
Examples
Cellulose and modified celluloses

Fingerprint region



	CH	CH_2	CH_3	OH
MCC	5	1		3
Methyl cellulose	5	1	3	
Sodium carboxymethyl cellulose	5	4		
Hydroxypropyl cellulose	8	4	3	3

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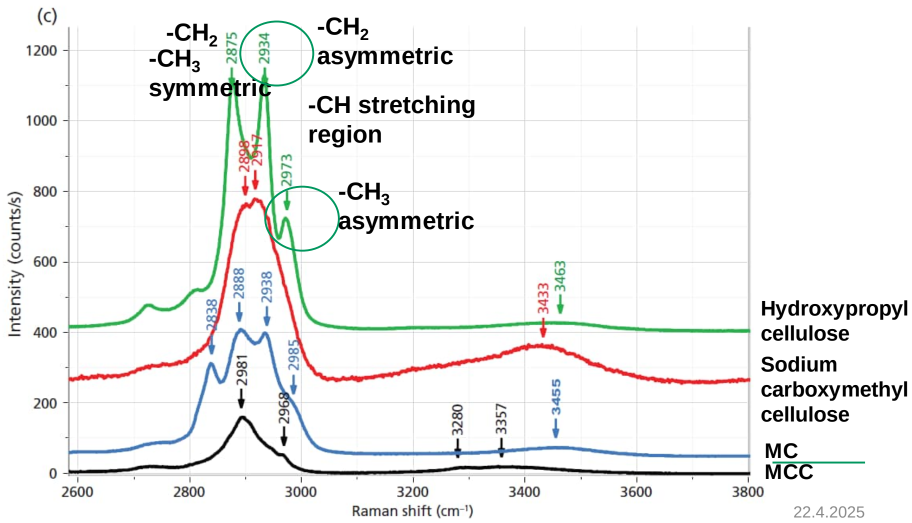
Examples
Cellulose and modified celluloses

CH-OH region



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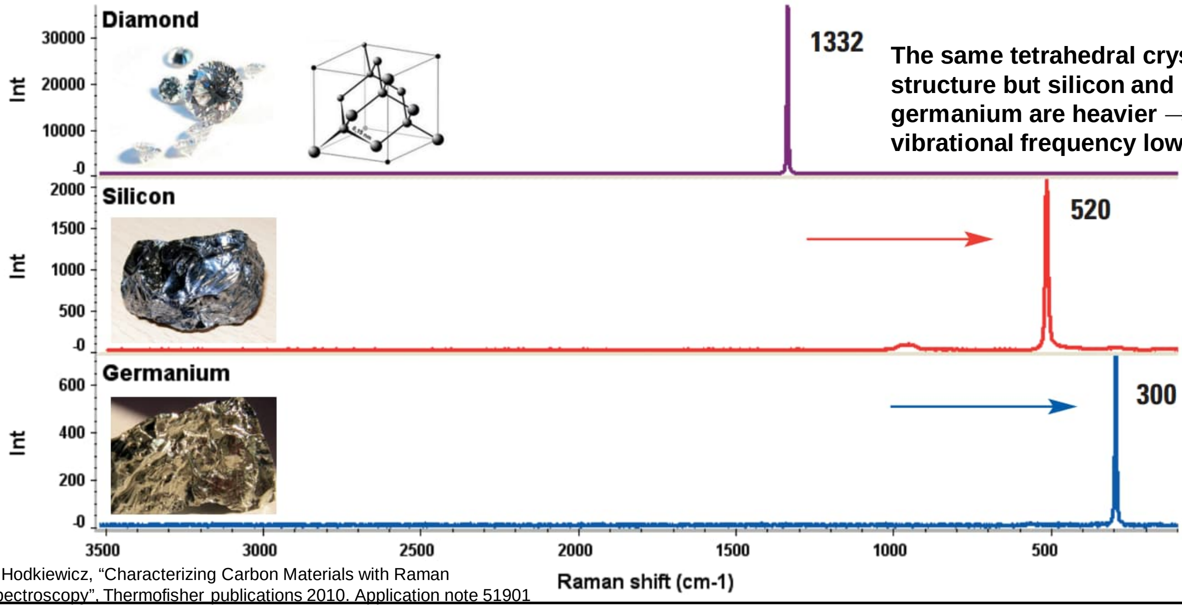
Examples
Carbon materials



The same tetrahedral crystal structure but silicon and germanium are heavier → vibrational frequency lower

J. Hodkiewicz, "Characterizing Carbon Materials with Raman Spectroscopy", Thermofisher publications 2010. Application note 51901

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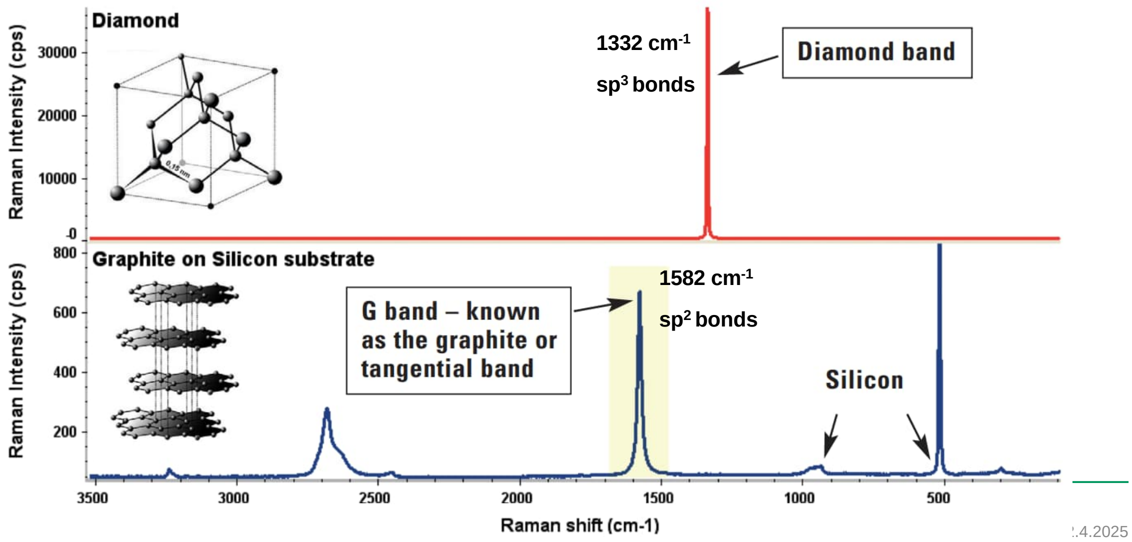
Examples
Carbon materials

Raman is sensitive to the orientation of the bonds



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sp^2 bonds have higher energy and frequency than sp^3 bonds

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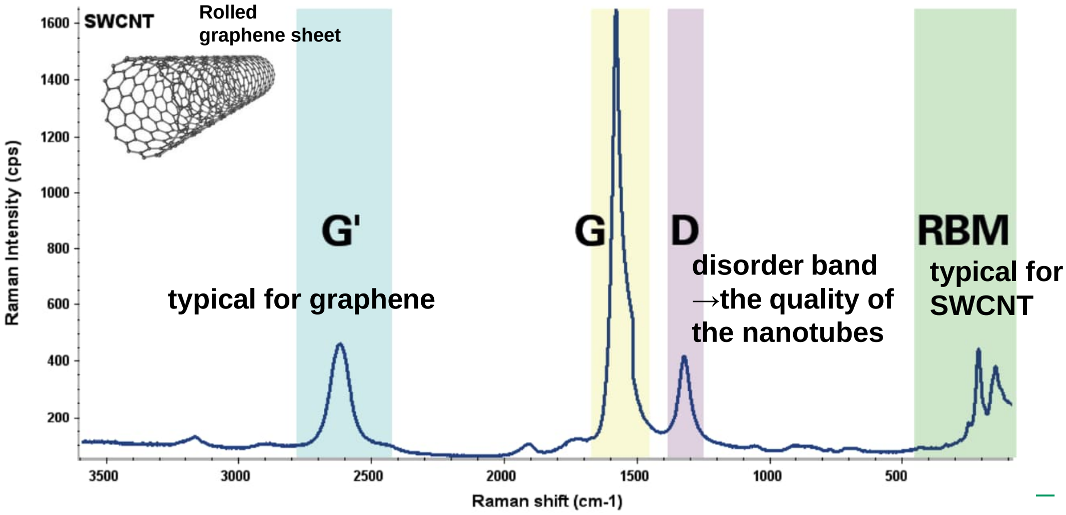
Examples
Carbon materials –carbon nanotubes



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RBM, radial breathing mode bands → tube expansion and contraction

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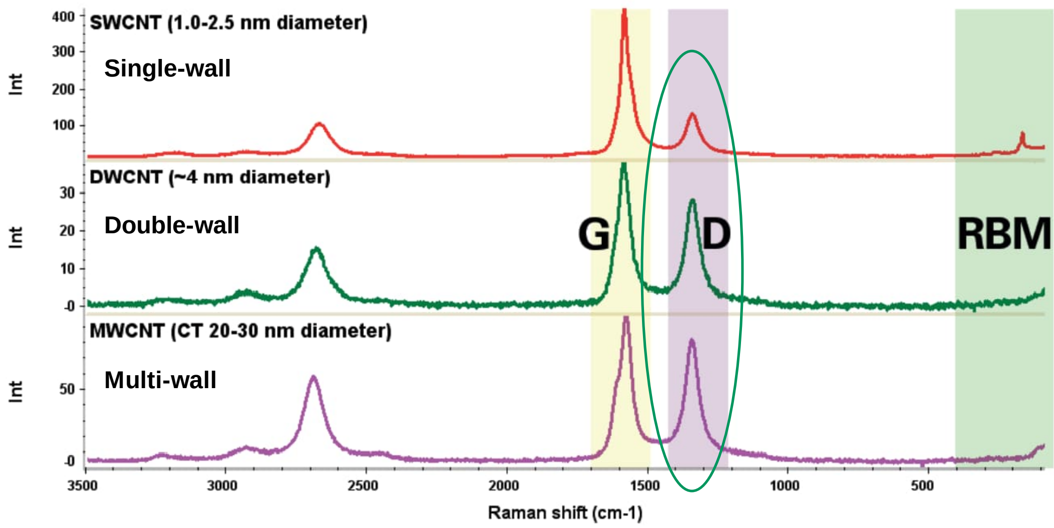
Examples
Carbon materials –carbon nanotubes



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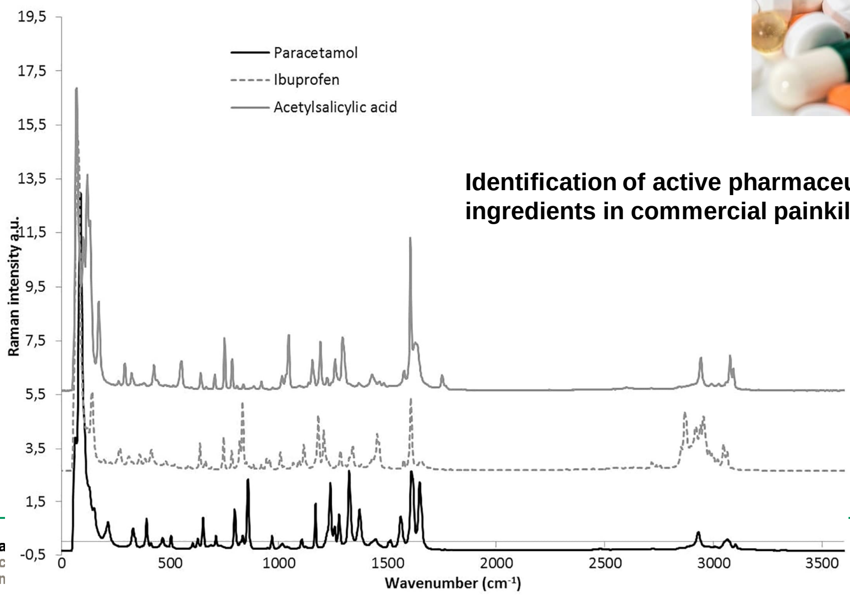
Raman for quality control

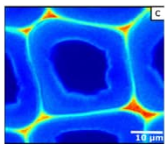


Identification of active pharmaceutical ingredients in commercial painkillers

Vibrational Spectroscopy (2020) 111,103147

Raman imaging

- **Combines Raman and microscopy to produce “chemical image”**
- **Raman spectrum from different locations of the sample is collected to build a map in which every pixel consist of an individual spectrum**
- **The amount of spectral data defines the resolution (commonly less than 1 µm resolution can be achieved)**



Belt et al., 2017

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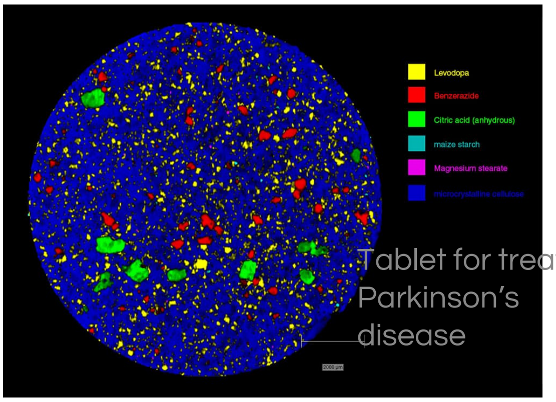
Raman imaging

https://youtu.be/1AhK_3RGAq4



Tablet for treating Parkinson's disease

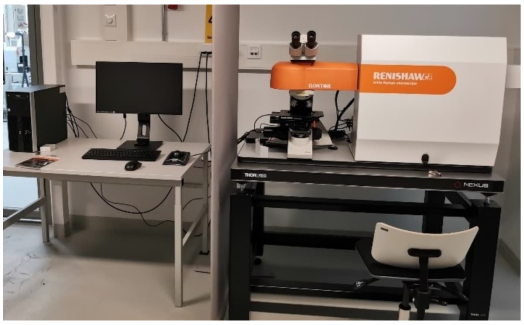
https://blue-scientific.com/large-area-raman-mapping-fast-high-resolution/

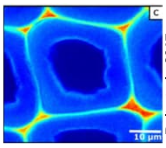
Raman spectroscopy at CHEM

- **Renishaw inVia confocal Raman**
- **Timegate Raman**
- **Renishaw UV-Raman**

Renishaw inVia confocal Raman microscope

- Two lasers: 532 nm (green) and 785 nm (NIR)
- Single spectrum acquisition, depth profiles and area mapping with lateral resolution of < 1µm
- Spectral resolution of 1 cm^{-1} with high resolution gratings
- Objectives: 20x, 100x (air), 64x (water-immersion)
- Gratings: 2400/1800 l/mm (“high-resolution” = 1 cm^{-1} res.) and 830 (possibility to measure broader wavenumber range)

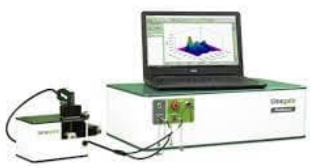


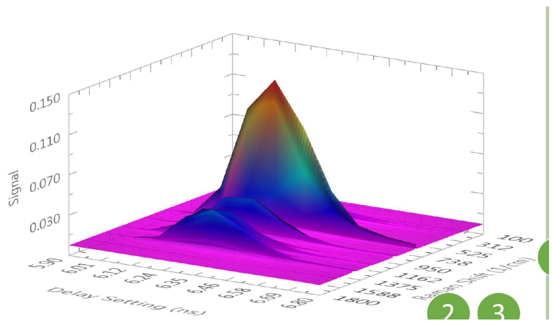


Belt et al., 2017

Timegate Raman

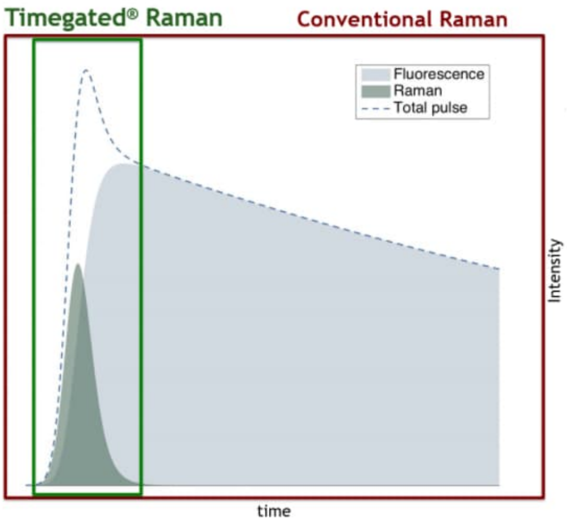
- Allows time resolved measurements
- 532 nm (green) pulsed laser
- Possibility to use probes (BWTek standard probe and touch Raman immersion probe or microscope





Supression of fluorencence

- **Picosecond range laser excitation source and time-gated single photon counting array detector**
  - *Capturing of instantaneous Raman scattering signal while rejecting the longer average delay fluorescence interference*

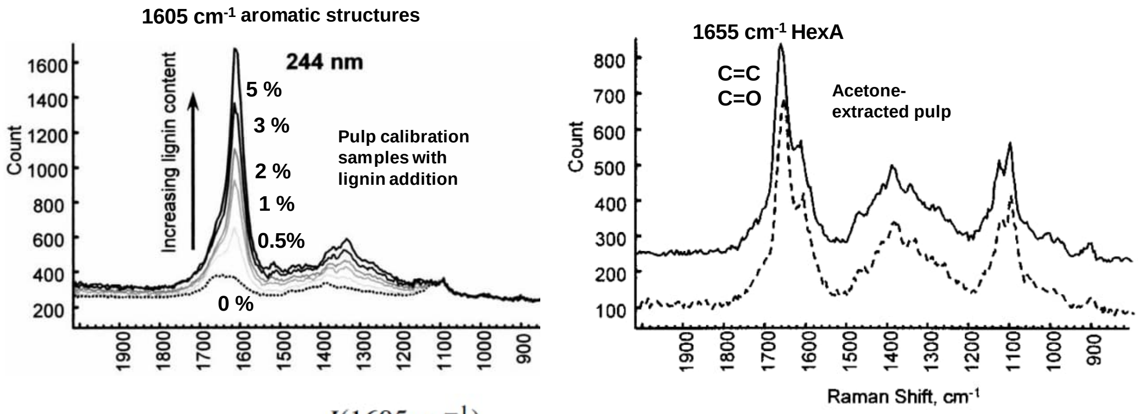


https://www.timegate.com/timegated_technology

Renishaw UV-Raman

- **Wavelengths of 244 nm or 257 nm**
- **High excitation with low wavelength laser (efficiency of Raman scattering $1/\lambda^4$)**
- **Less issues with fluorescence (fluorescence typically occurs at wavelengths longer than 300 nm)**

Quantification of lignin and hexenuronic acid using UV-Raman



$$Lignin(\%) = 0.32 \cdot \frac{I(1605cm^{-1})}{I(1094cm^{-1})}$$

$$HexA(mmol/kg) = 4.39 \cdot \frac{I(1655cm^{-1})}{I(1094cm^{-1})} - 1.14$$

cellulose

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Jääskeläinen et al., Journal of Wood Chemistry and Technology, 25: 51–65, 2005

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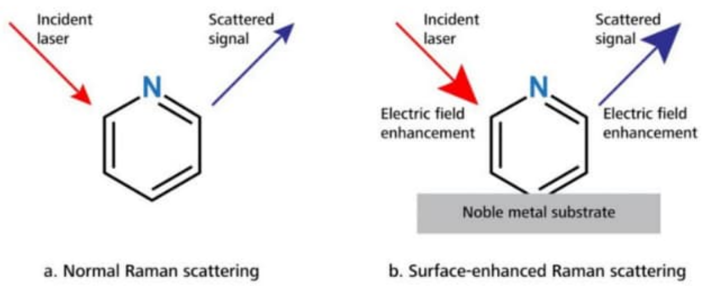
Surface-enhanced Raman spectroscopy (SERS)



- **Valence electrons of metal → localized plasmons which enhance incident light as well as scattered signal**

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Raman spectroscopy

Laboratory demos

PUU1 building (Vuorimiehentie 1)
Abio hall, lab 260, 2nd floor

Meet at the lobby!