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Explore the future

HORIBA Instruments Inc.

HORIBA Scientific

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Optical Spectroscopy

2019 May 21

The goal

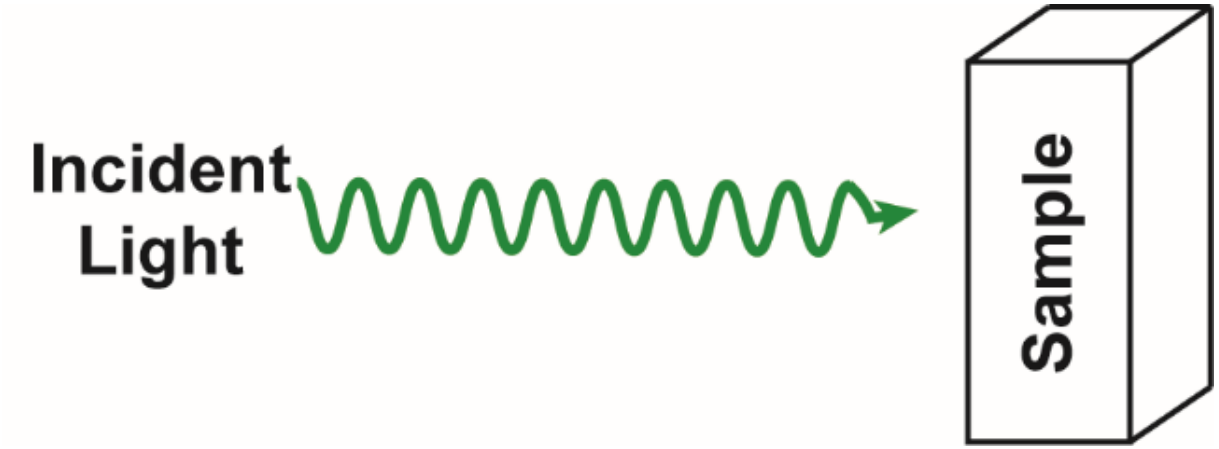
The Tutorial will address Fundamentals of the techniques (UV-VIS, Fluorescence, IR and Raman), advantages and limitations of these techniques. Instrumentation and typical applications will be discussed during tutorial and the examples of applications will be shown.

Why use optical spectroscopy?

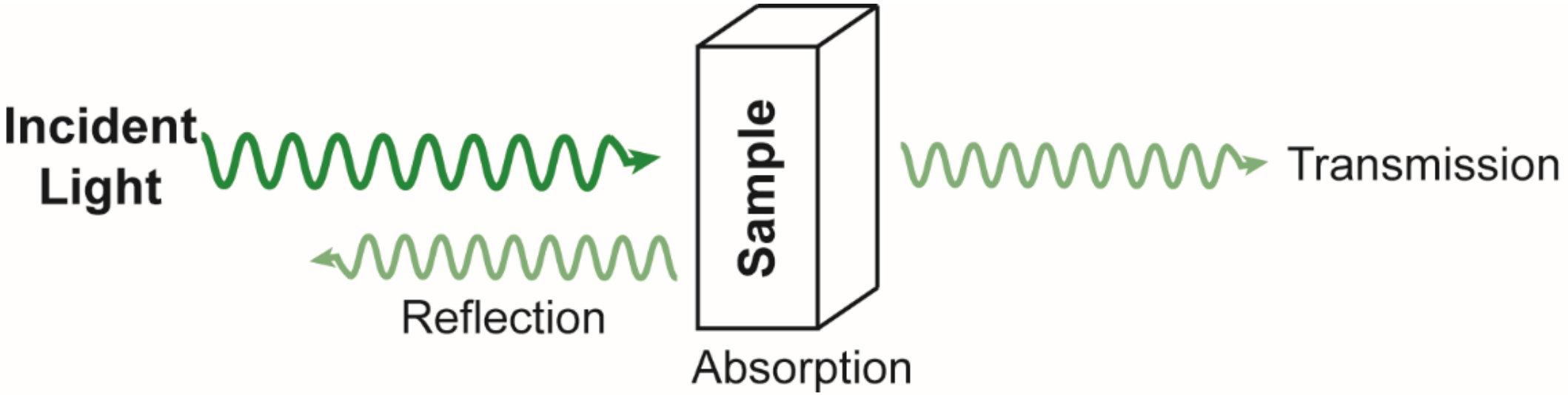
- **Chemical information**
- **Sensitive**
- **Typically nondestructive**
- **Detect analytes or monitor chemical changes *in situ*, *in operando*, or *in vivo***
- **Can have spatial and temporal resolution**

What is optical spectroscopy?

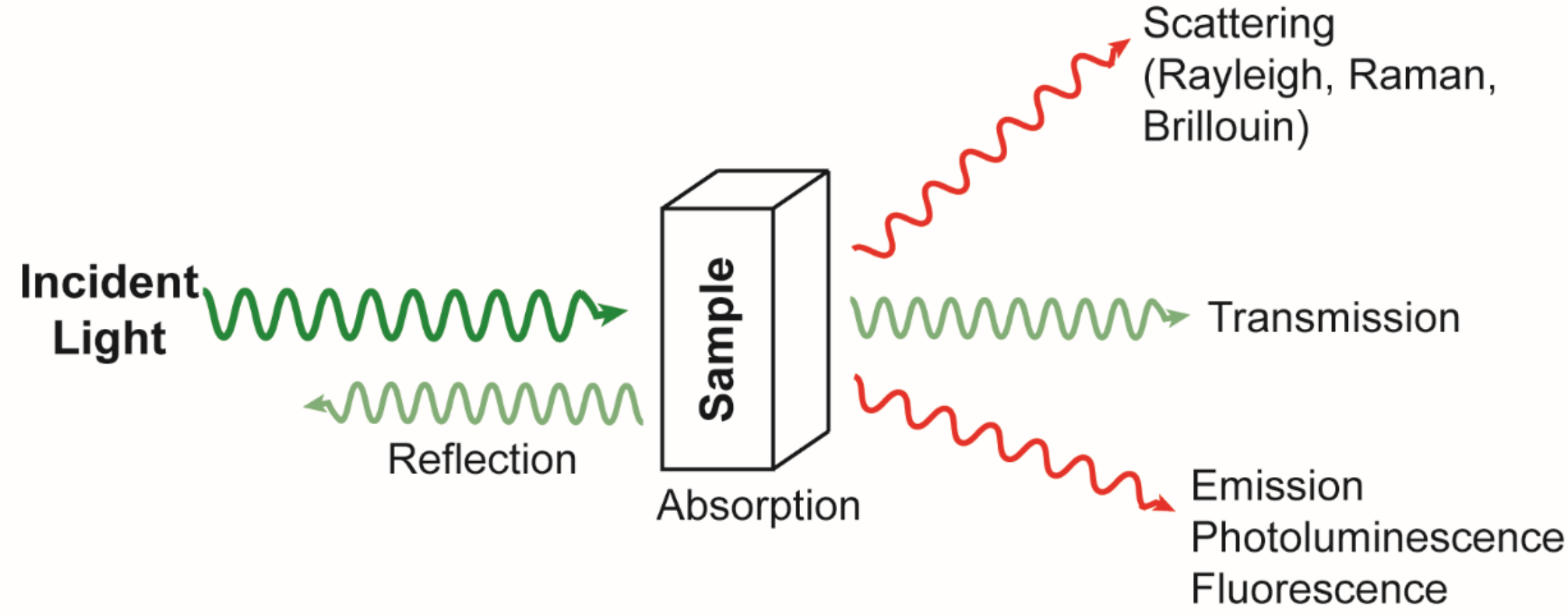
Interaction of (UV, visible, IR) light with matter



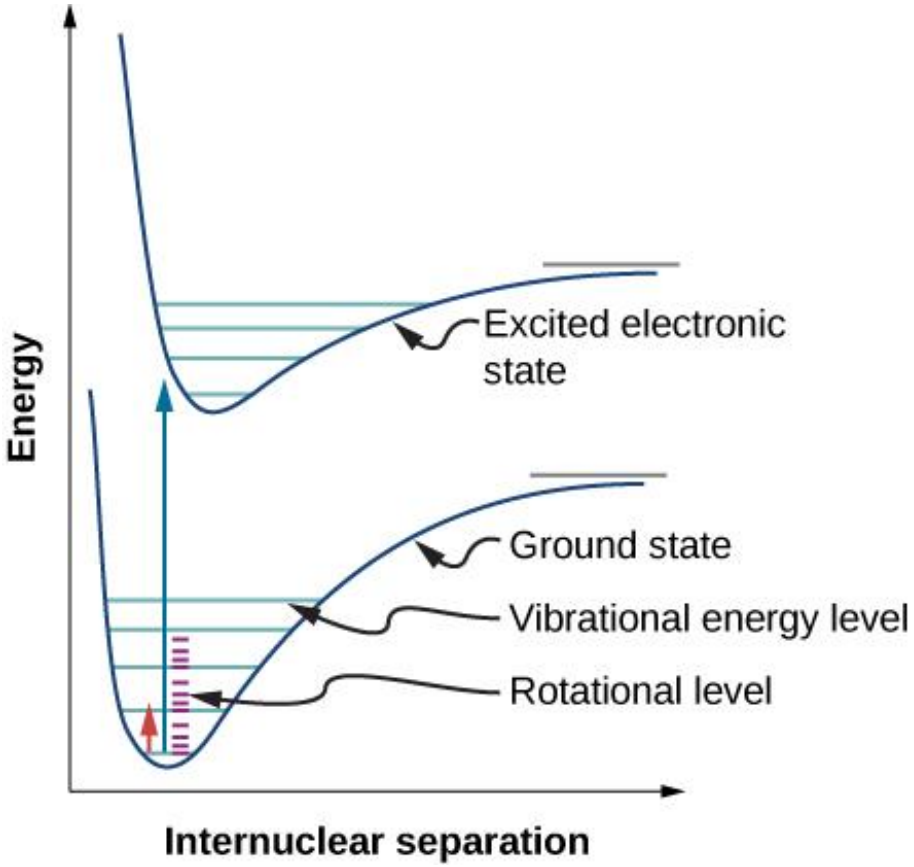
Interaction of light with matter

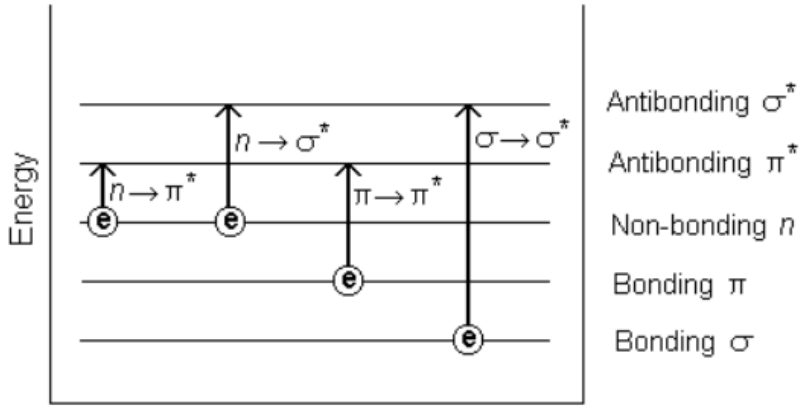


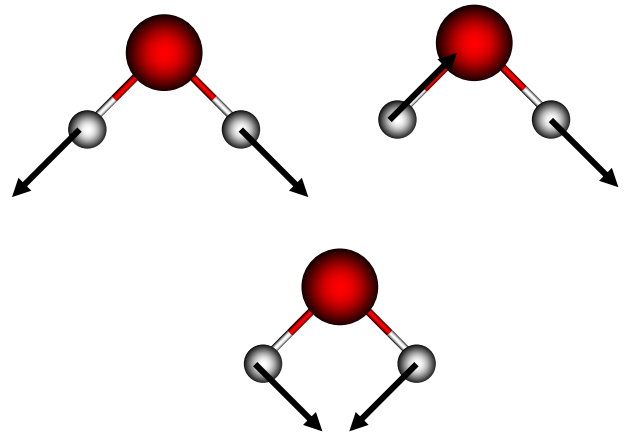
Interaction of light with matter



What chemical info can we get?

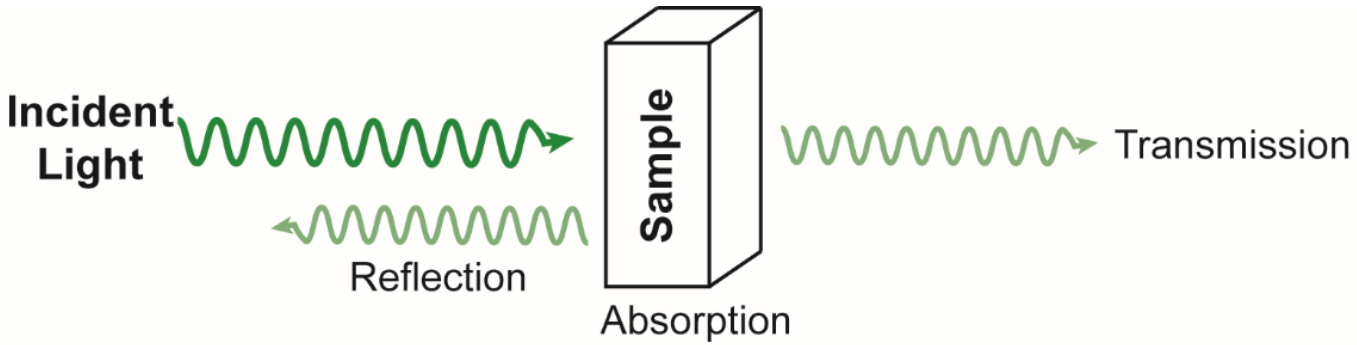


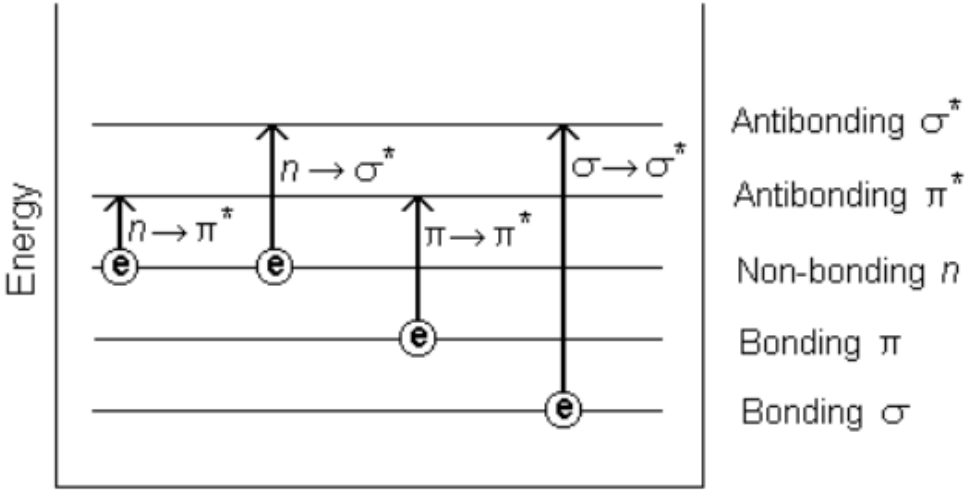




- **Electronic states**
  - **Some chemical identification capability**
  - **Electron transfer**
  - **Photochemistry**
- **Vibrational states**
  - **“Fingerprints”**
  - **Molecular bonds**

UV-VIS Optical Spectroscopy



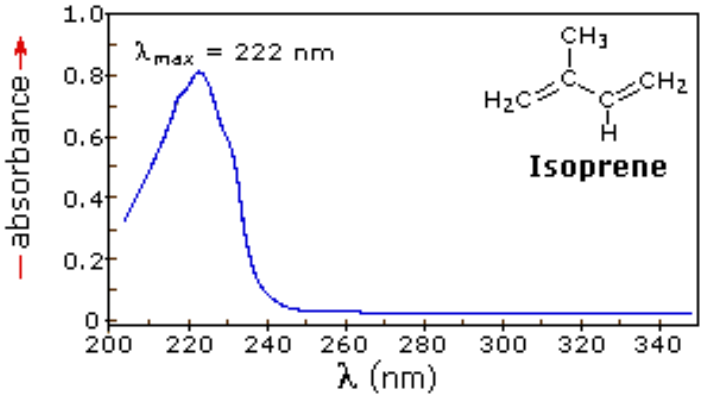


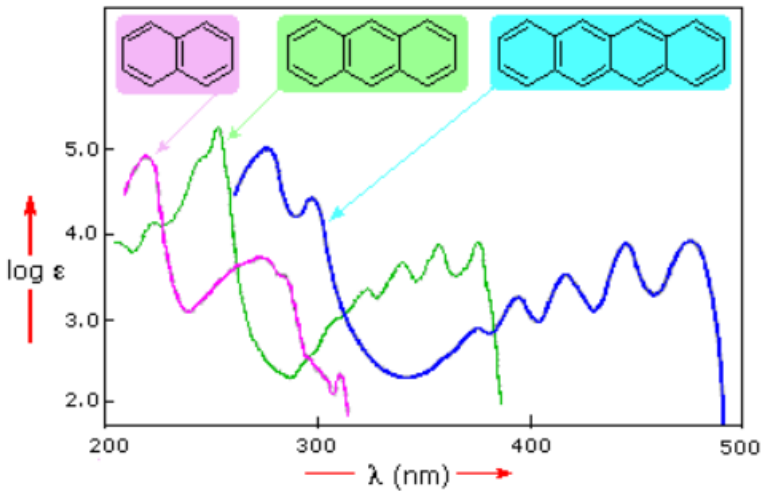
- **Colorimetry**
- **Transition of electron from lower to higher level**

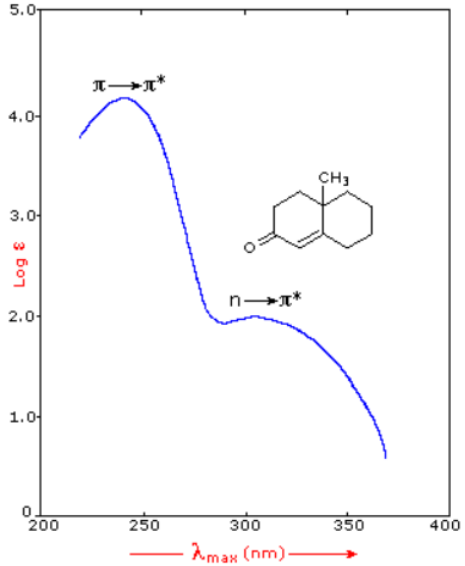
UV-VIS Applications

Chromophore	Example	Excitation	λ_{max}, nm	ε	Solvent
C=C	Ethene	π –> π*	171	15,000	hexane
C≡C	1-Hexyne	π –> π*	180	10,000	hexane
C=O	Ethanal	n –> π* π –> π*	290 180	15 10,000	hexane hexane
N=O	Nitromethane	n –> π* π –> π*	275 200	17 5,000	ethanol ethanol
C-X X=Br X=I	Methyl bromide Methyl Iodide	n –> σ* n –> σ*	205 255	200 360	hexane hexane

- **Electronic transitions**
- **Quantification**
- **Reaction monitoring (for some cases)**







Quantification with UV-Vis

The *Beer-Lambert law (or Beer's law)* is the linear relationship between absorbance and concentration of an absorbing species. The general *Beer-Lambert law* is usually written as:

$$A = a(\alpha) \times b \times c$$

where *A* is the measured absorbance, $a(\alpha)$ is a wavelength-dependent absorptivity coefficient, *b* is the path length, and *c* is the analyte concentration. When working in concentration units of molarity, the *Beer-Lambert law* is written as:

$$A = \varepsilon \times b \times c$$

where ε is the wavelength-dependent molar absorptivity coefficient with units of M^{-1} cm^{-1}.

Experimental measurements are usually made in terms of transmittance (T), which is defined as:

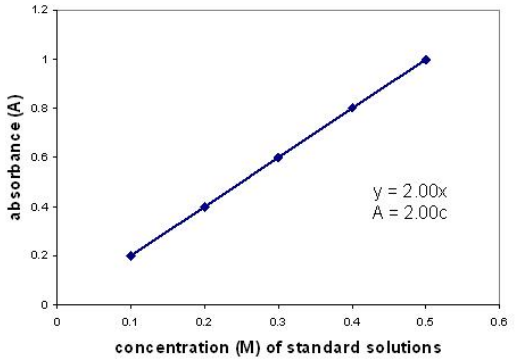
$$T = I/I_0$$

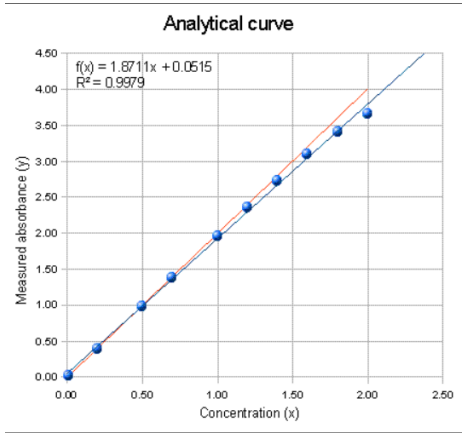
where I is the light intensity after it passes through the sample and I_o is the initial light intensity. The relation between *A* and *T* is:

$$A = -Log(T) = -Log(\frac{I}{I_0})$$

Beer's Law Limitations

The linearity of the *Beer-Lambert law* is limited by chemical and instrumental factors. Causes of nonlinearity include:





1) deviations in absorption coefficients at *high concentrations (>0.01M)* due to electrostatic interactions between molecules in close proximity

2) scattering of light due to particulates in the sample

3) fluorescence or phosphorescence of the sample

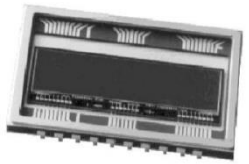
4) changes in refractive index at high analyte concentration

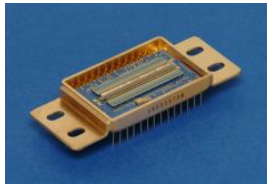
5) shifts in chemical equilibria as a function of concentration

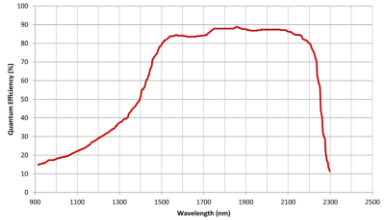
6) non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band

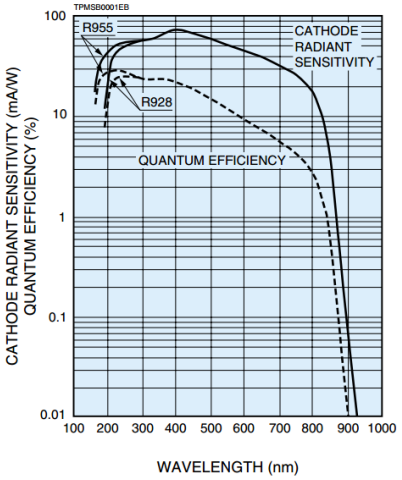
7) stray light

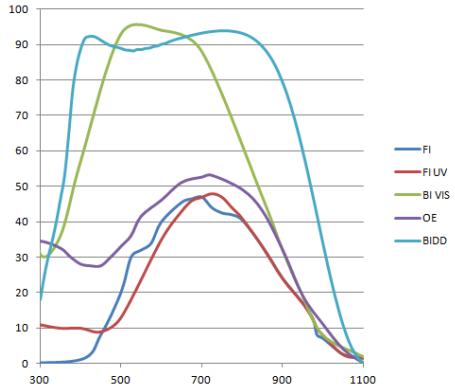
UV-VIS Instrumentation

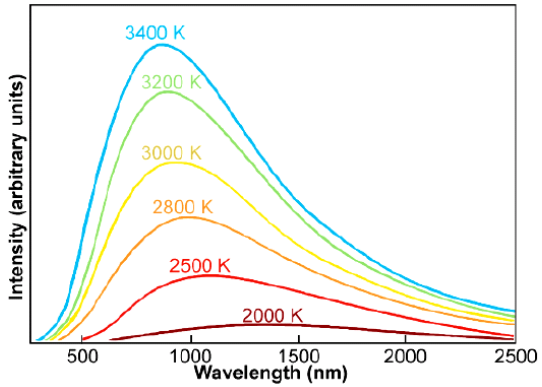


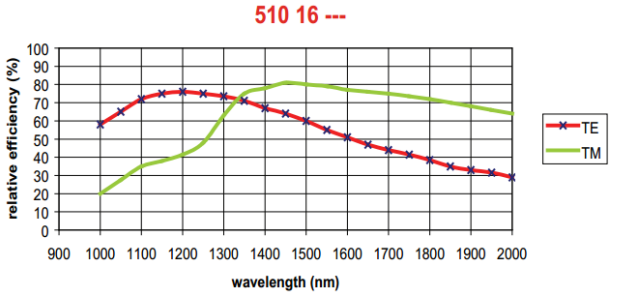












What is fluorescence?

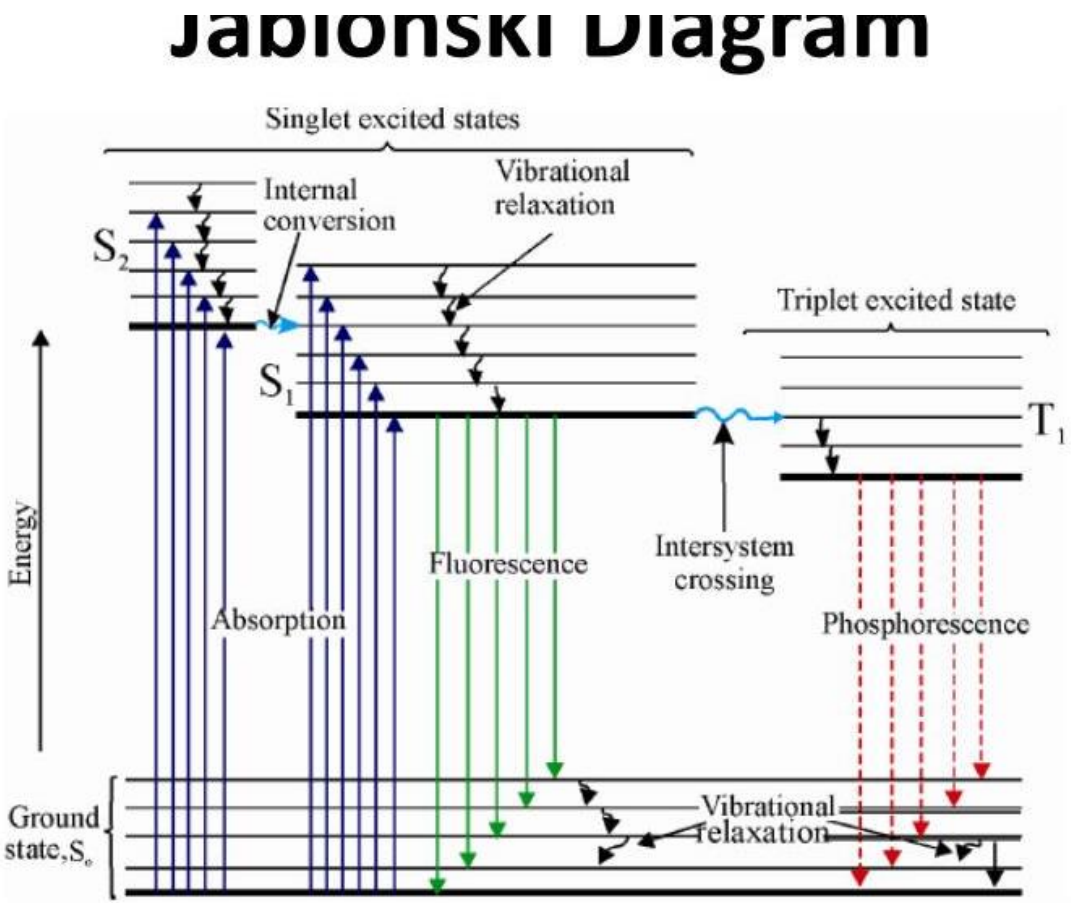
The term fluorescence is actually one type of luminescence. Luminescence, broadly defined, is light emission from a molecule. There are several types of luminescence.

Luminescence is light emission from a molecule

- **Photoluminescence** (Light energy stimulates emission)
- **Chemiluminescence** (Chemical energy stimulates emission)
- **Electroluminescence** (Electrical energy stimulates emission)

What is fluorescence?

Jablonski Diagram



Fluorescence is a type of photoluminescence where:

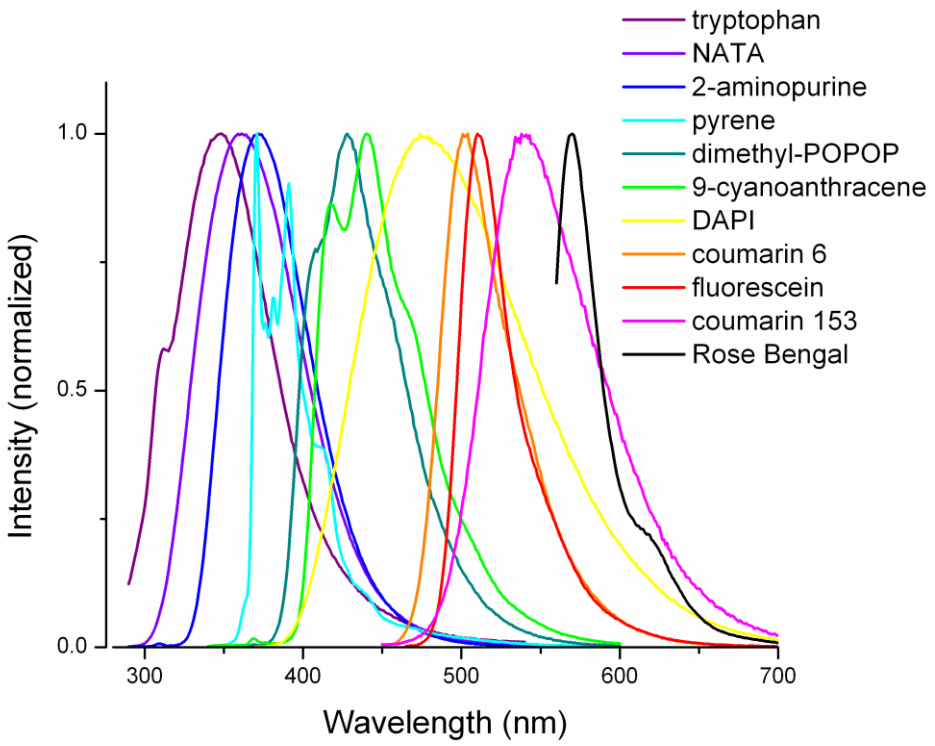
- Light energy excites molecule to excited state
- Excited state rapidly loses thermal energy to environment through vibrations
- Photon is emitted at terminal low-lying singlet excited state
- Fluorescence competes for other nonradiative processes including energy transfer and heat loss

When we measure a fluorescence spectrum, we are typically looking at the intensity at which a molecule emits , the wavelength or energy at which it emits, and also the time which the molecule spends in the excited state. This is the fluorescence lifetime.

Fluorescence benefits

- Sensitivity
- Selectivity
- Many molecules fluoresce naturally
- Others can be made to fluoresce with tags or dyes
- No significant sample prep – samples can be measured as is

A Spectrum of Fluorescence Dyes



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- Amino acids (Trp, Phe, Tyr)
- Base pair derivatives (2-AP)
- Chlorophylls
- FP's (fluorescent proteins)
- Organic dyes (fluoresceins, rhodamines)
- Rare earth elements
- Semiconductors
- Quantum dots
- SWCNT's
- Solar cells
- Pigments, brighteners
- Phosphors
- Many more…

Typical Fluorescence Techniques

- Excitation and Emission Scanning and Mapping
- Anisotropy and Polarization
- Microtiter Plate Based Assays
- Macro and Microscopic Spatial Resolution and Imaging
- Solid Sample and Solution Phase Sampling
- Remote Fiber Optic Probing
- Quantum Yield: Integrating Sphere - Thin Films, powders and Solutions
- Temperature scanning
- Fluorescence Resonance Energy Transfer (FRET)
- Time-resolved fluorescence (TRF) using time-correlated single photon counting (TCSPC)

Fluorescence Spectra

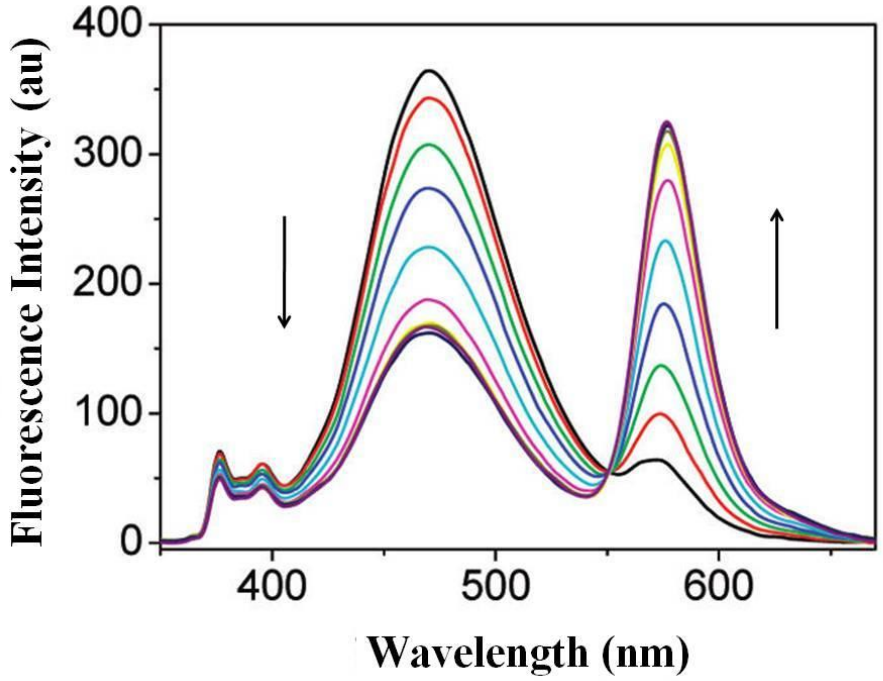
Fluorescence Emission spectrum: Fix the excitation wavelength and scan the emission wavelength

Excitation spectrum: Fix the emission wavelength and scan the excitation to excite the sample over a range of wavelengths (Analogous to Absorbance Spectrum)

Emission and Excitation spectra are typically used to see how a sample is changing

Spectral peak wavelength and/or intensity may change with

- Temperature
- Concentration
- Interactions with other molecules (quenchers, energy transfer,etc.)
- Solvent environment (pH, polarity, ion concentration, etc.)

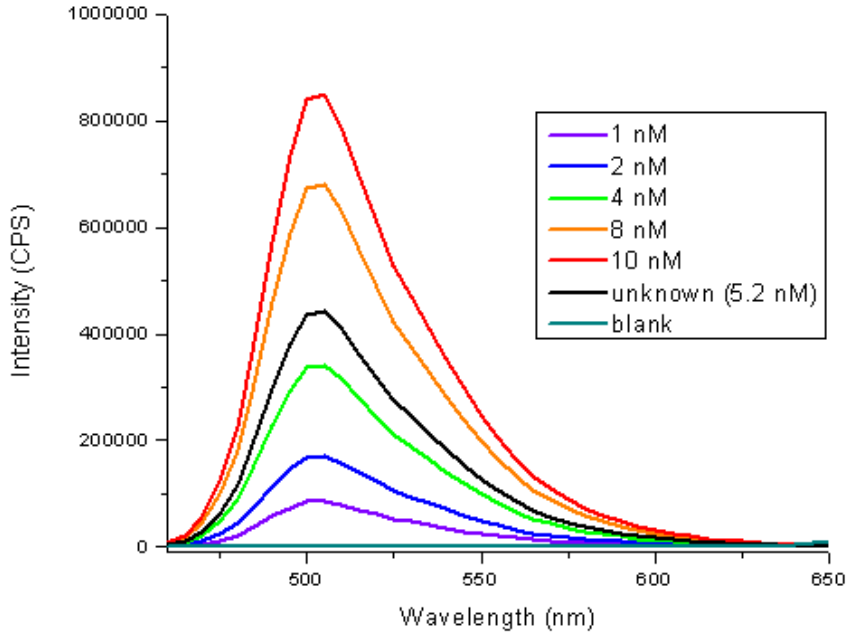


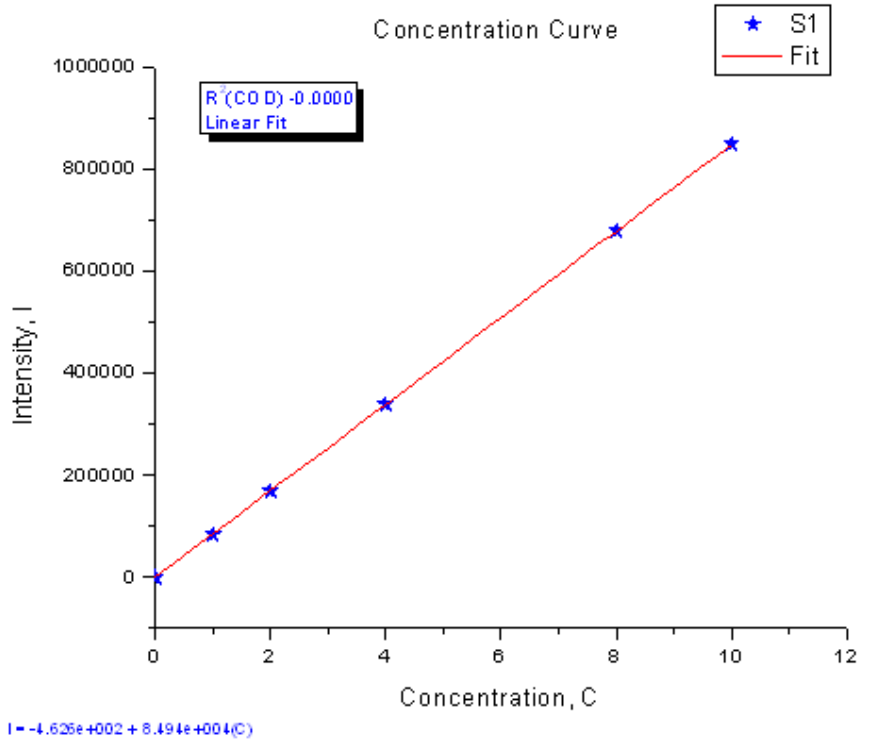
Concentration Curves

Fluorescence intensity is linear with concentration of the fluorophore

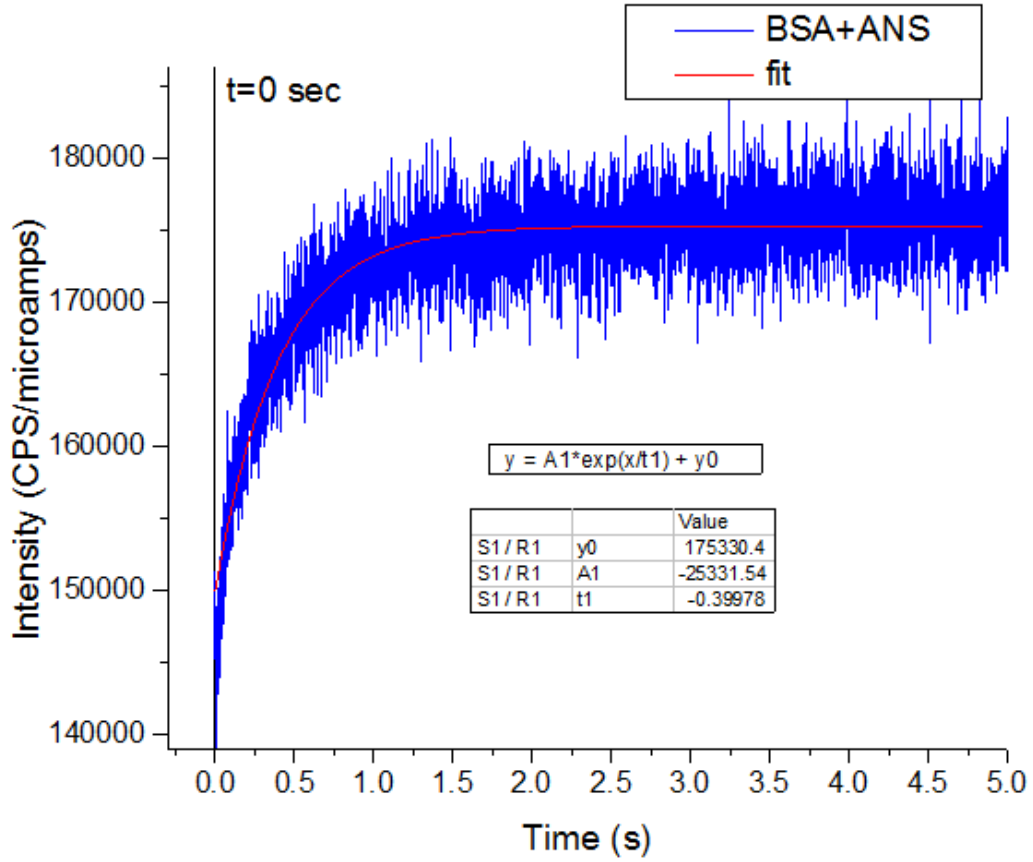
microspheres (01)

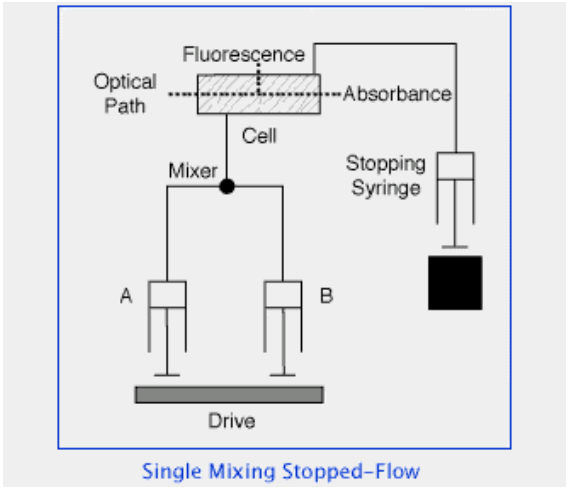
	Sample(L)	Sample Type(L)	Concentration(L)	Group Name(L)	S1	Trial(S1)	StdErr(S1)	S1c
					CPS		(%)	CPS
1	Sample 1	Blank	N/A	Group 1	747	10	0.82	1102
2	Sample 2	Standard	1	Group 1	84975	8	1.1	85330
3	Sample 3	Standard	2	Group 1	169950	5	0.45	170305
4	Sample 4	Standard	4	Group 1	339901	7	0.32	340256
5	Sample 5	Standard	8	Group 1	679803	3	0.05	680158
6	Sample 6	Standard	10	Group 1	849753	4	0.04	850108
7	**Sample 7**	**Unknown**	**5.2**	Group 1	441872	3	0.02	442227
8								





Fluorescence - Binding kinetics



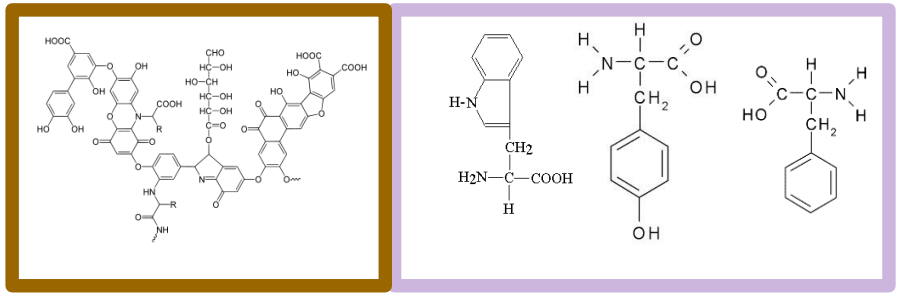


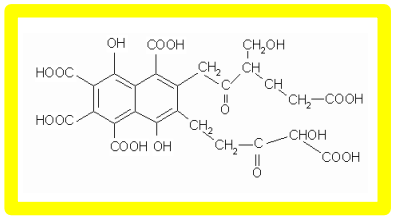
Stopped flow mixing: 1 uM BSA + 0.5 uM ANS
Ex: 280 nm, Em: 350 nm; 1.0 ms integration time, 1.0 ms increment

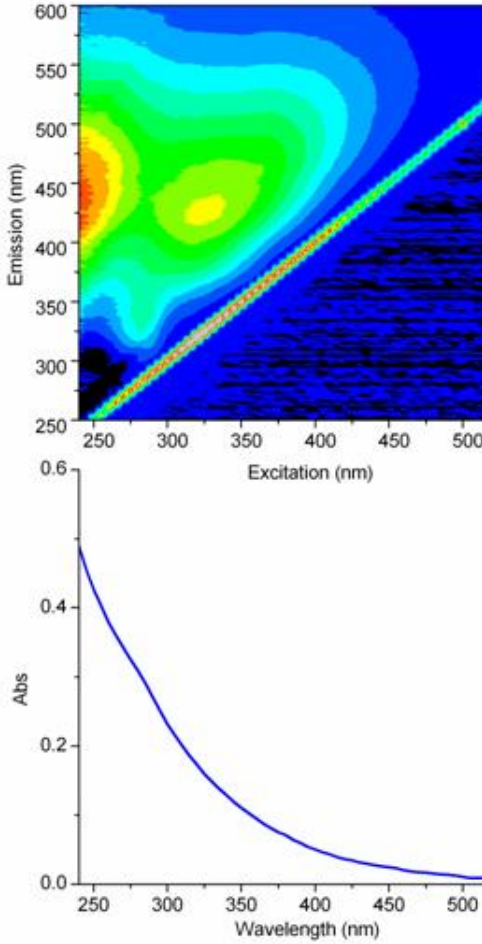
Fluorescence – EE Matrix

- Measure emission spectrum over range of excitation wavelengths – gives a contour plot (λ_{ex} vs λ_{em} vs FL intensity)

- EEMs measure absorbance and emission of all *fluorescent* Components
- Dissolved organic matter
- Food science
  - Wine
  - Olive oils
  - Dairy
- Petroleum/oil/PAHs



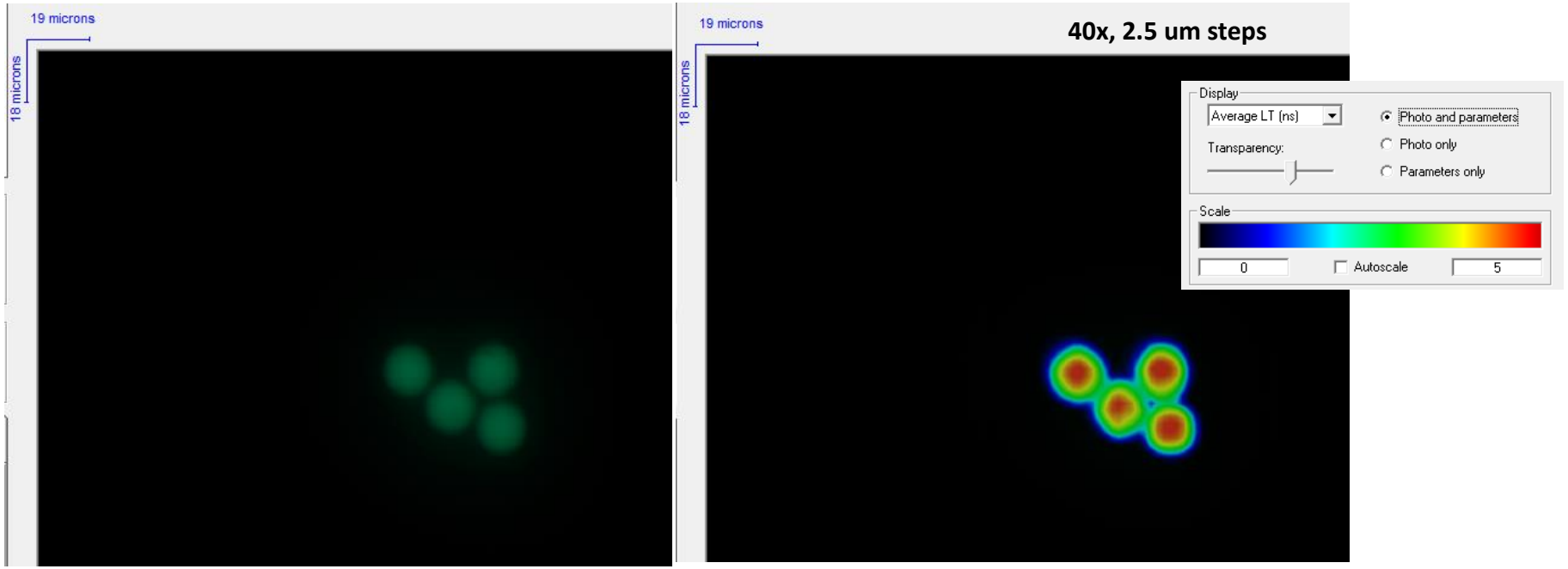




Fluorescence – Imaging

- Measure lifetime decays across spatial area
- Detect defects in PV materials
- FRET for intracellular protein dynamics

FRET = Fluorescence Resonance Energy Transfer

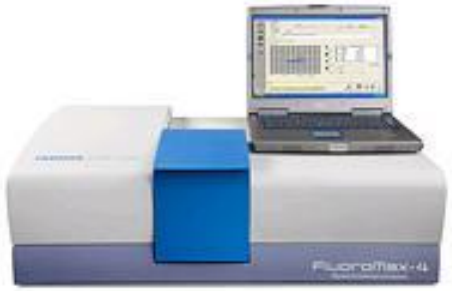


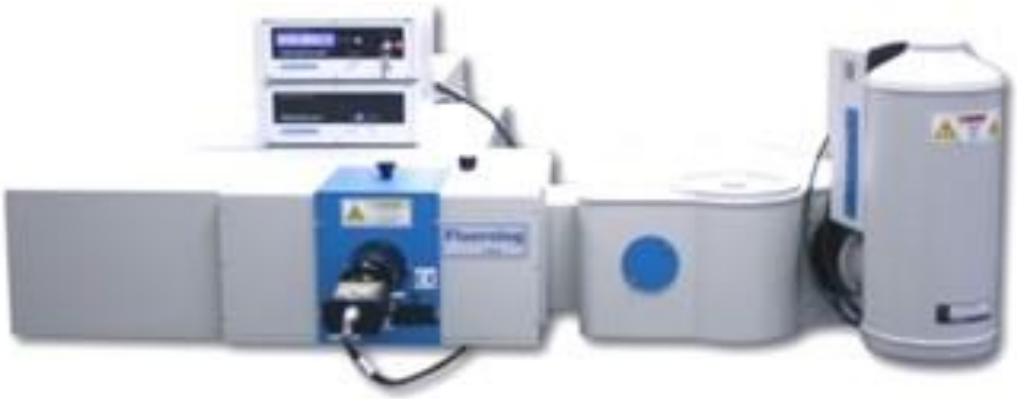
Fluorescence image

FLIM map >1000 decays in 1 minute

FLIM = Fluorescence-lifetime imaging microscopy

Fluorometers





Vibrational Spectroscopy

- IR and Raman spectroscopy are the most common and complimentary to each other.
- Applications of vibrational spectroscopy
  - Molecular spectroscopy
    - Vibrations of atoms within molecules
    - Gas, liquid and solid
  - Solid state and material science
    - Vibrations of atoms within molecules (e.g. molecular crystals, amorphous materials)
    - Vibrations between atoms and molecules (e.g. amorphous materials)
    - Vibrations of crystalline lattice
    - From liquid to solid (e.g. gel, crystal)

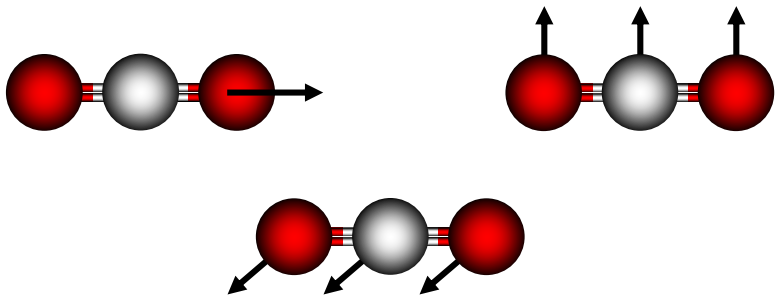
Movements in a Molecule

There are 3N possible movements in a molecule made of N atoms, each of which moving in one of three directions, x, y and z. There are three transitional movements: all atoms in the molecule moving in x, y or z direction at the same time. There are three rotational movements around x, y or z-axisLinear molecules are exceptions because two axes that are perpendicular to the molecular axis are identical. The rest of movements are vibrational movements:

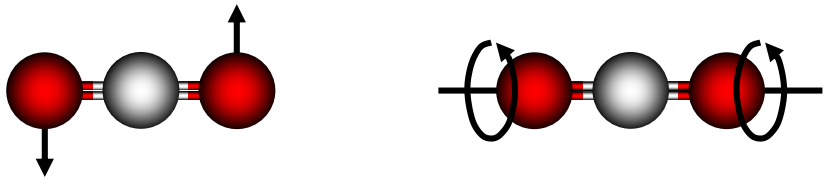
for linear molecules, 3N – 5 movements and for non-linear molecules, 3N – 6 movements

Carbon Dioxide (linear)

Translational movements



Rotational movements



There are three atoms in each molecule, 3 × 3 = 9 total possible movements.

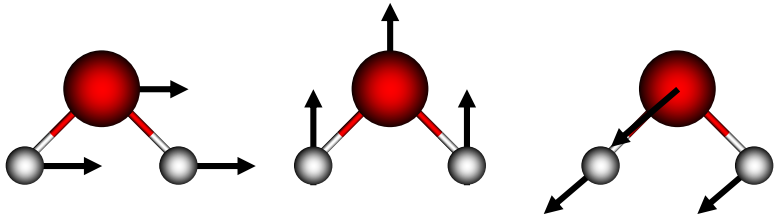
There are three transitional movements for each molecule.

There are two rotational movements for CO_2 (linear) and three for H_2O (non-linear)

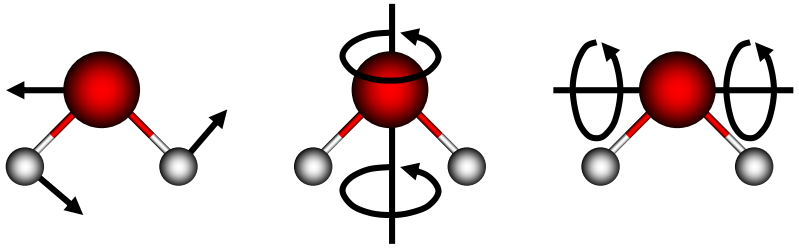
Therefore, there are four vibrational movements for CO_2 (linear) and three for H_2O (non-linear).

Water (non-linear)

Translational movements



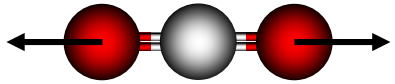
Rotational movements



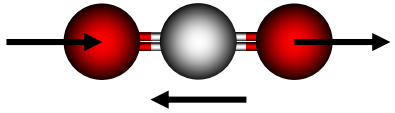
Vibrational Normal Modes

Carbon Dioxide (linear)

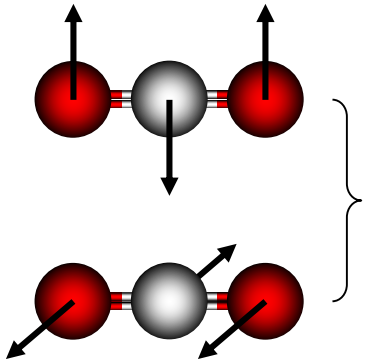
Symmetric stretching



Asymmetric stretching



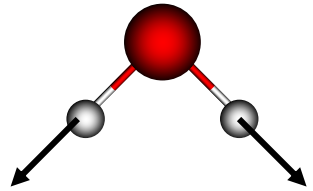
Bending



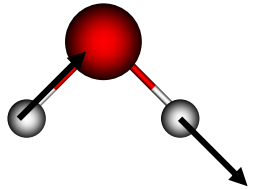
Degenerated modes
Different motions, the same frequency

Water (non-linear)

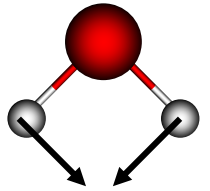
Symmetric stretching



Asymmetric stretching



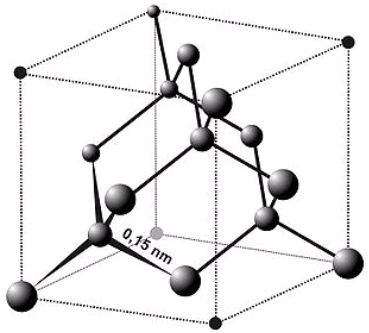
Bending

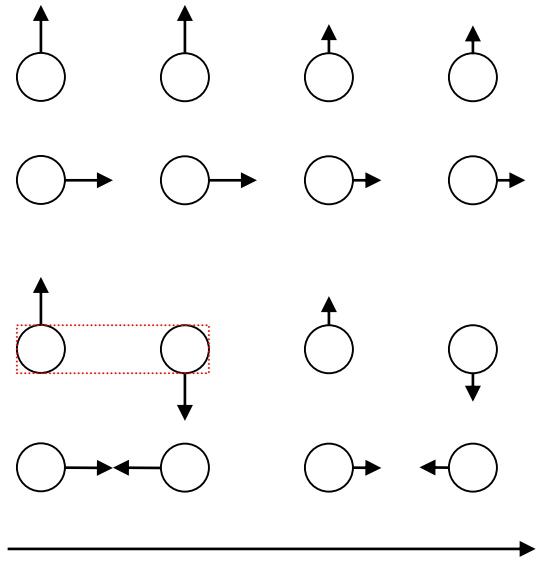


Movements in Crystals

- **3 acoustic phonons**: all atoms (molecules or ions) in a unit cell move to the same direction, and each unit cell moves by slightly different amplitudes. Brillouin Scattering.
- **3N-3 optical phonons**: atoms (molecules or ions) in a unit cell move against each other, and each unit cell moves by slightly different amplitudes.

Diamond (Cubic)





Transverse Acoustic (TA) Mode

Longitudinal Acoustic Mode (LA)

Transverse Optical (TO) Mode

Longitudinal Optical (LO) Mode

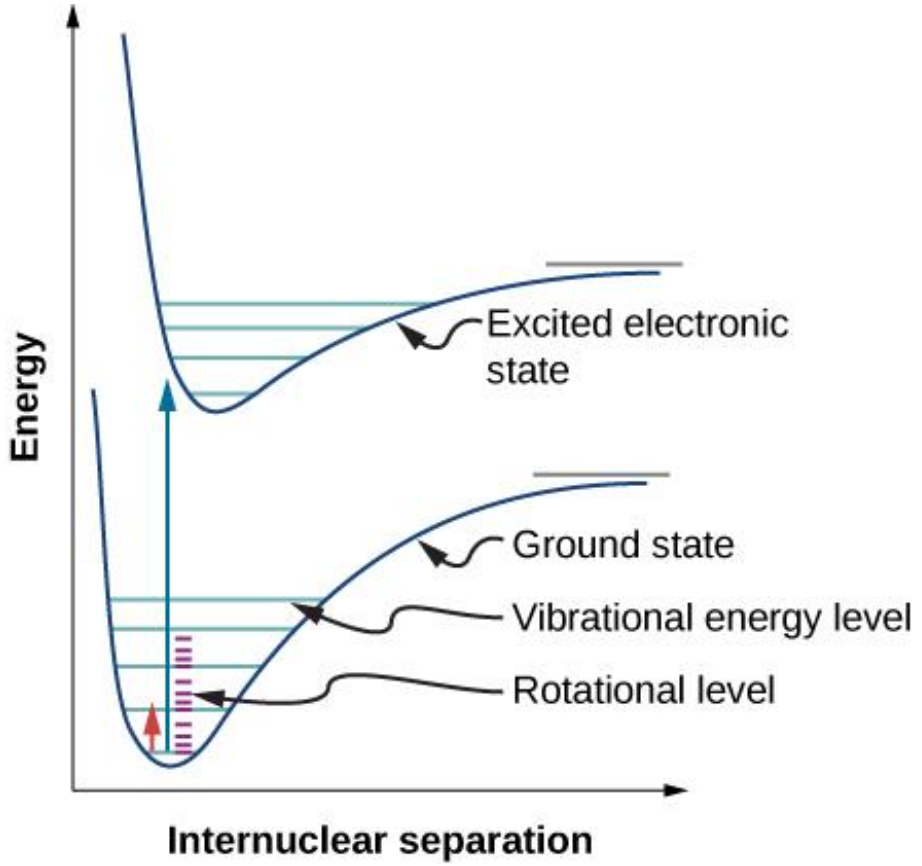
Direction of Propagation

What can Vibrational Spectra tell us?

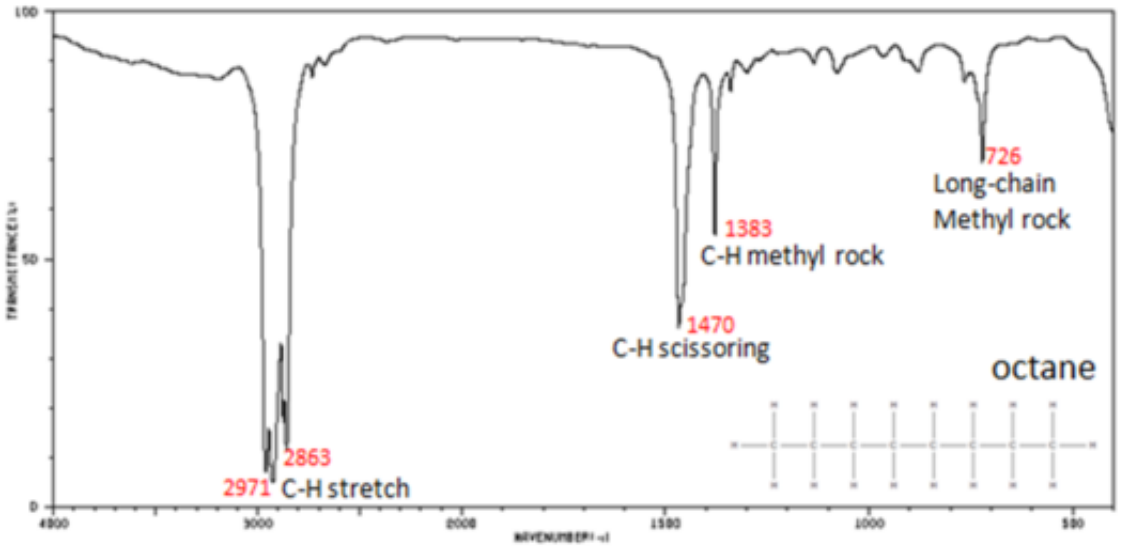
- Vibrational frequencies are characteristic of chemical bonds or groups of bonds in a specific molecule: normal modes
- The spectra provide fingerprints representing the set of bonds present in the material
- Vibrational frequencies are sensitive to details of the structure and local environment of a molecule, such as crystal phase, local strain, and degree of crystallinity
- Relative intensities within a spectrum can quantify the orientation of the bond w.r.t. the incoming laser polarization

Infrared Spectroscopy

While polychromatic light (typically 25,000 – 2,500 nm or 400 – 4000 cm^{-1}) passes through or reflects from the sample, photons whose frequencies match those of vibrational normal modes are absorbed.



Infrared Spectra



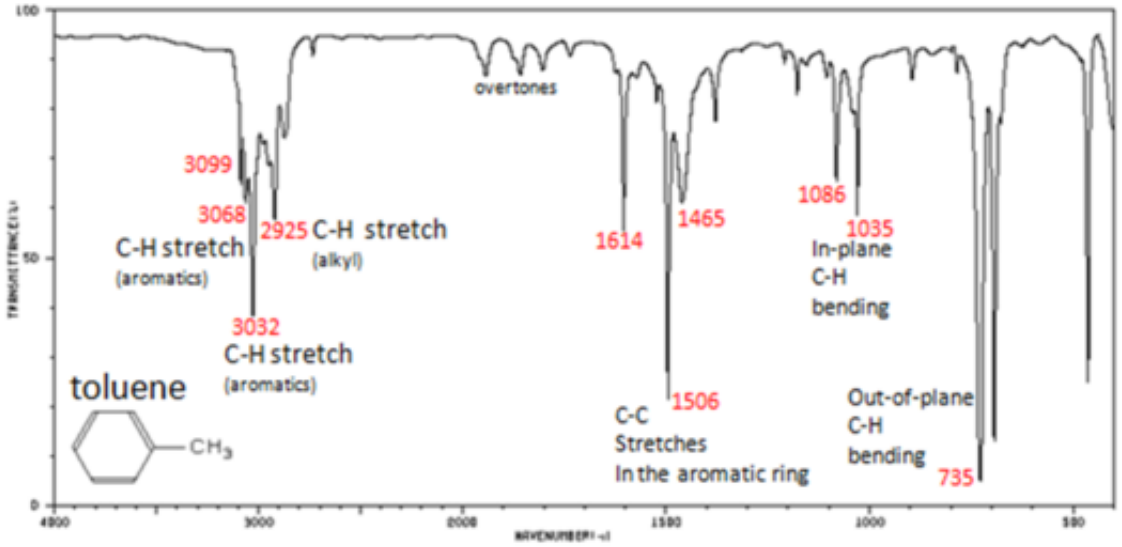
octane

C–H stretch from 3000–2850 cm^{-1}

C–H bend or scissoring from 1470-1450 cm^{-1}

C–H rock, methyl from 1370-1350 cm^{-1}

C–H rock, methyl, seen only in long chain alkanes, from 725-720 cm^{-1}



toluene

C–H stretch from 3100-3000 cm^{-1}

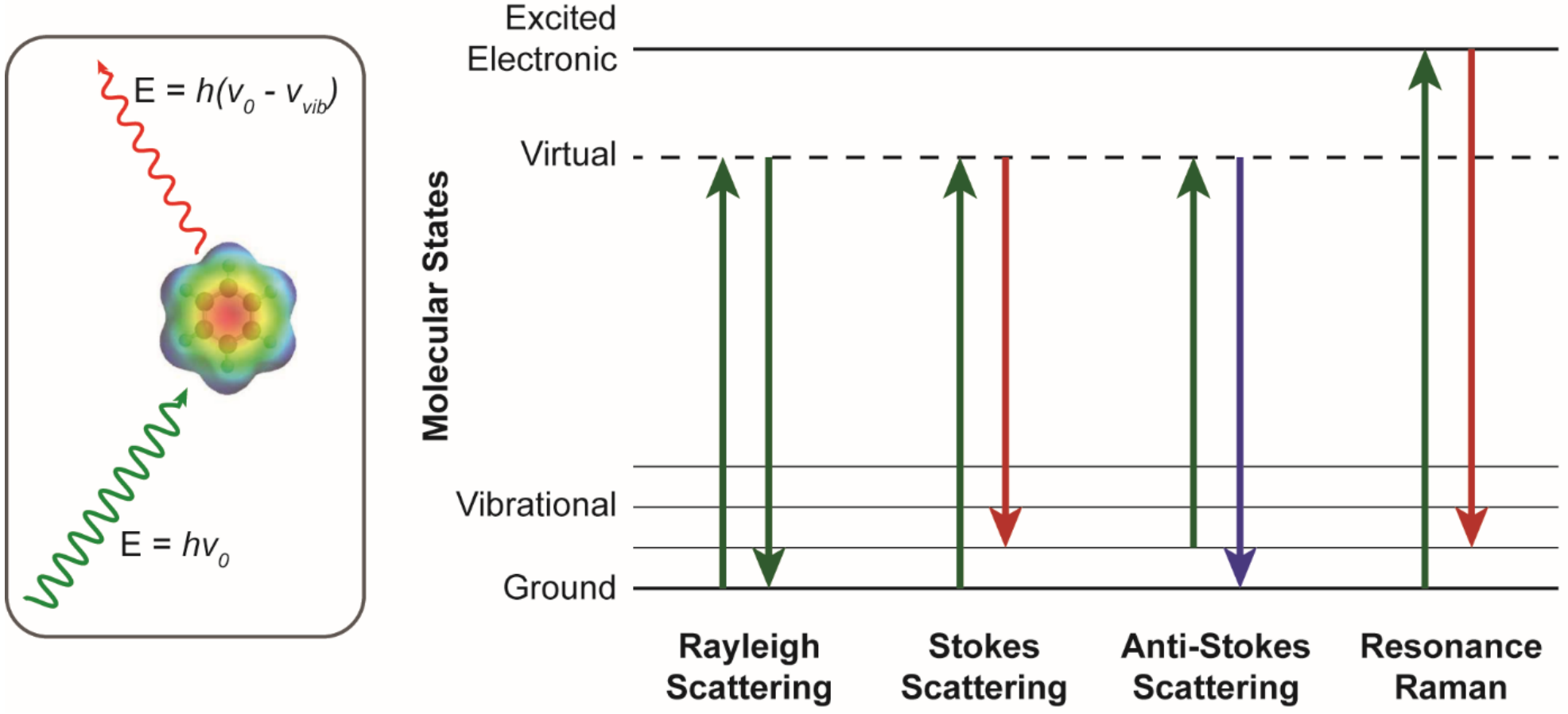
overtones, weak, from 2000-1665 cm^{-1}

C–C stretch (in-ring) from 1600-1585 cm^{-1}

C–C stretch (in-ring) from 1500-1400 cm^{-1}

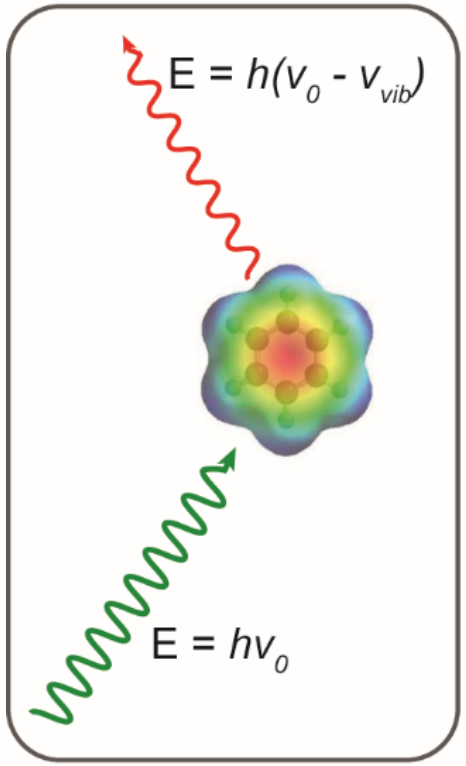
C–H "oop" from 900-675 cm^{-1}

Raman Spectroscopy

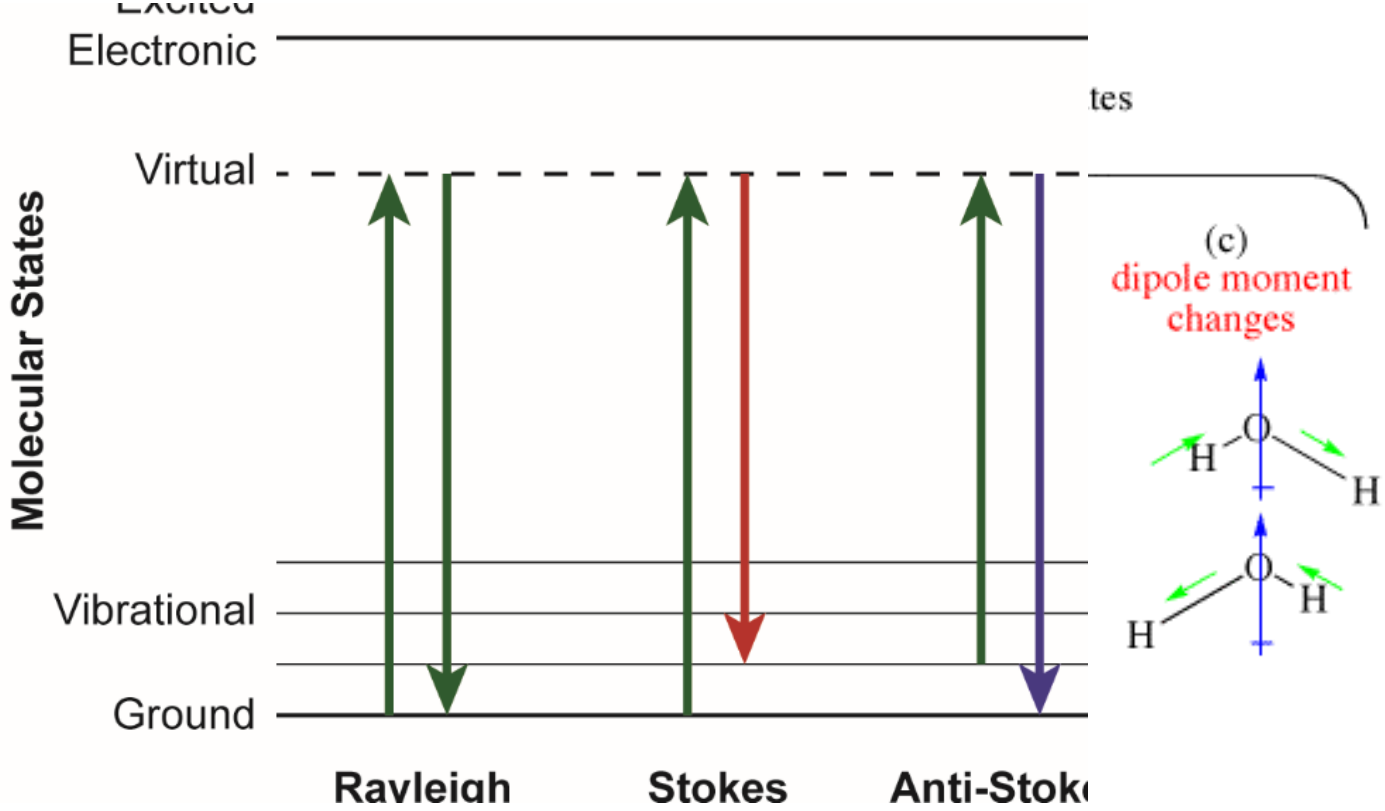


Raman vs IR Spectroscopy

Raman



IR



Normal modes can be IR active, Raman active, or both.

Raman vs IR Spectroscopy

Raman

- Scattering
- Better spatial resolution
- No water background
- Little sample preparation
- Can excite electronic transitions simultaneously
- Stokes/anti-Stokes

- 1 in 10^{10} photons
- Fluorescence: enemy

IR

- Absorption
- Stronger signal
- No issues with fluorescence

- Difficulties with sample prep
- Studies in water difficult

Raman Spectroscopy

Raman observes laser energy changes as it excites a molecular vibrations.

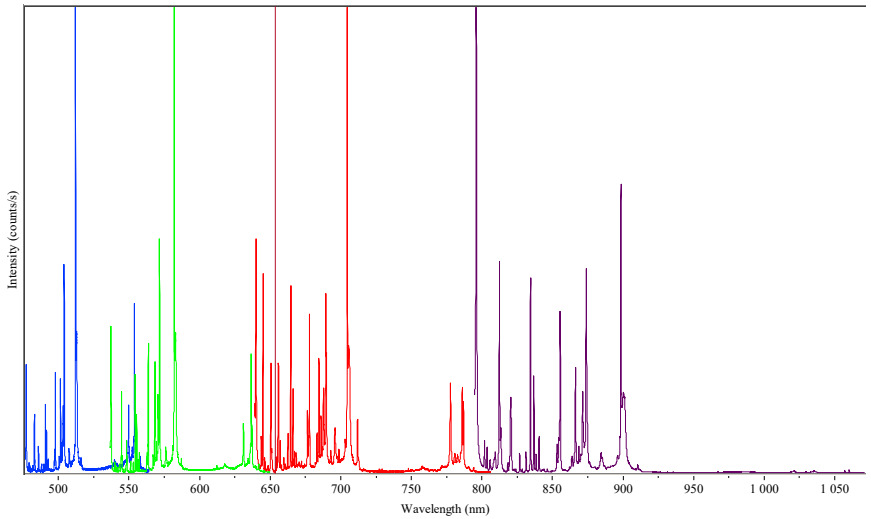
The unit used is relative to the energy ν

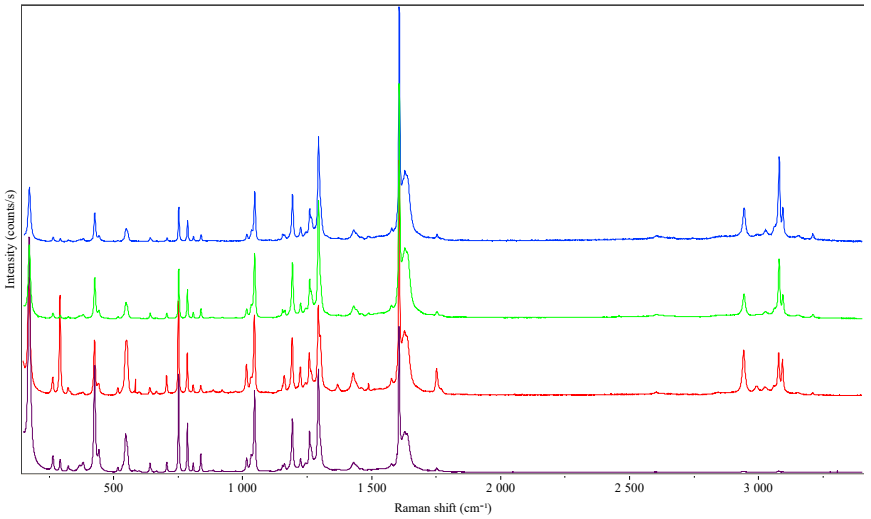
$\nu\ (cm^{-1}) = 1/\lambda\ (cm)$

Laser – 532 nm = 18797.0 cm^{-1}
Si peak – 547.15 nm = 18276.4 cm^{-1}

$\nu_{raman} = [\ 1/\lambda_{laser} \pm\ 1/\lambda_{peak}]$

18797.0 cm^{-1} – 18276.4 cm^{-1} = 520.6 cm^{-1}





Raman Spectroscopy

How big the Raman Shift?

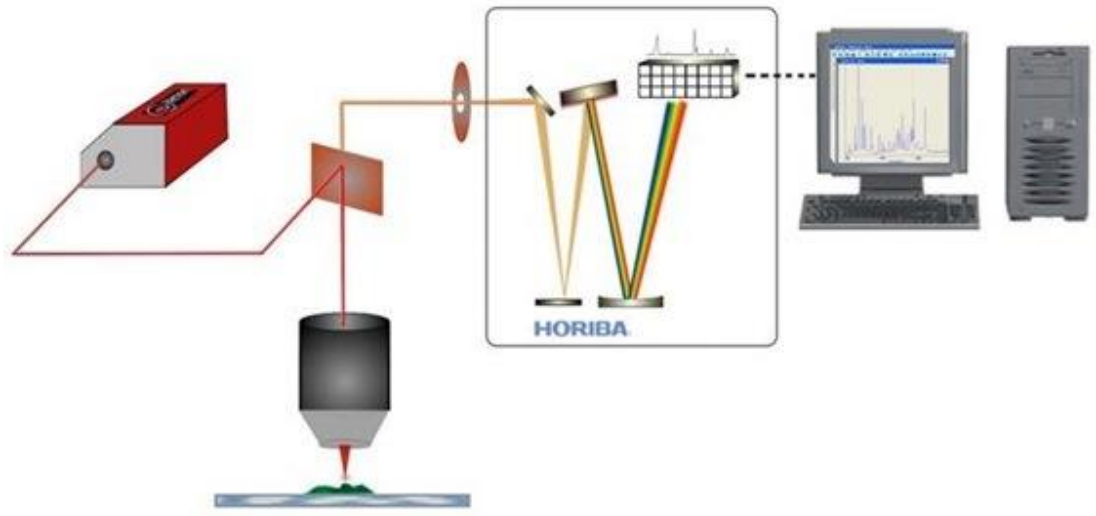
Si, 520.6 cm^{-1}

Laser, nm	Stokes, nm	Difference, nm
325	330.63	5.59
473	484.94	11.94
532	547.15	15.15
632.8	654.36	21.56
785	818.45	33.45
1064	1126.39	62.39

Raman Spectroscopy

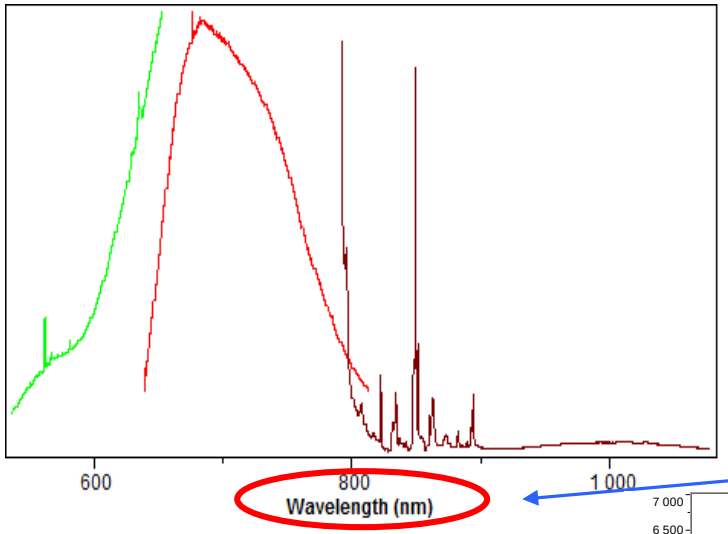
A Confocal Raman Microscope is made from essentially 4 components

- Laser source - to provide monochromatic light
- Confocal Microscope and Sampling optics - to deliver the laser to the sample and collect the Raman signal
- Rejection filter- to remove the Rayleigh scattered light and transmit only the Raman signal
- Spectrometer and detector - to disperse the Raman scattered light into its different wavelengths and detect its intensity (producing the spectrum)



Raman Spectroscopy

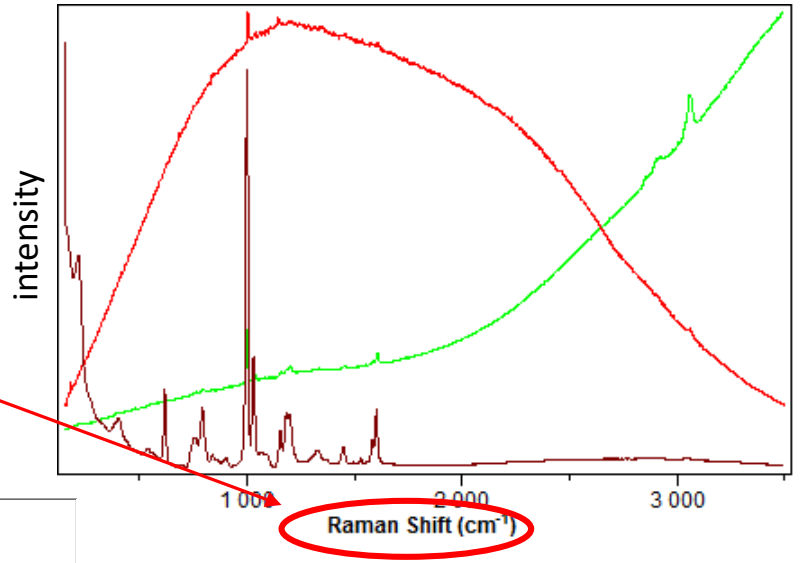
Laser source - to provide monochromatic light – from UV to NIR

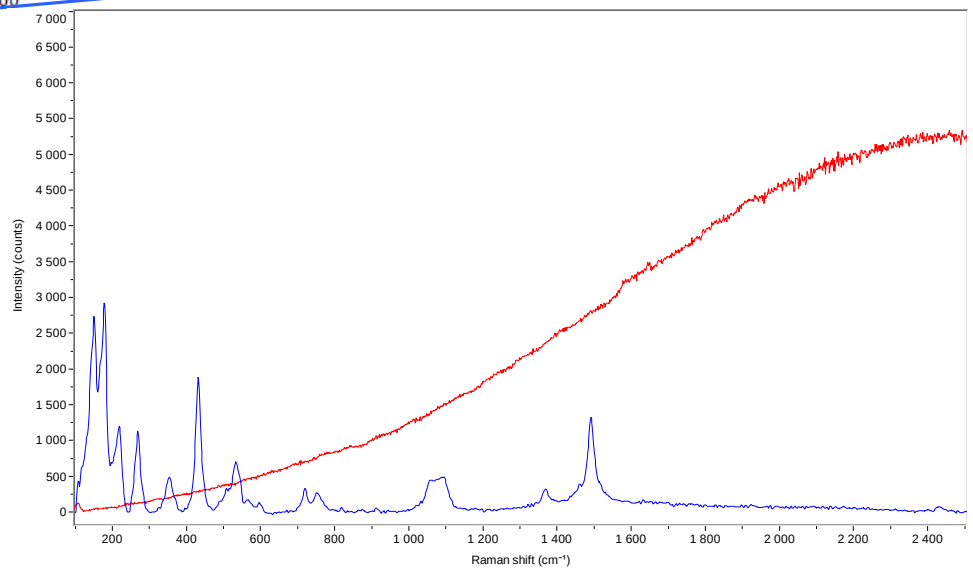


Green spectrum: 532 nm laser
Red spectrum: 633 nm laser
Dark red spectrum: 785 nm laser

Raman is wavelength **independent**

Fluorescence is wavelength **dependent**



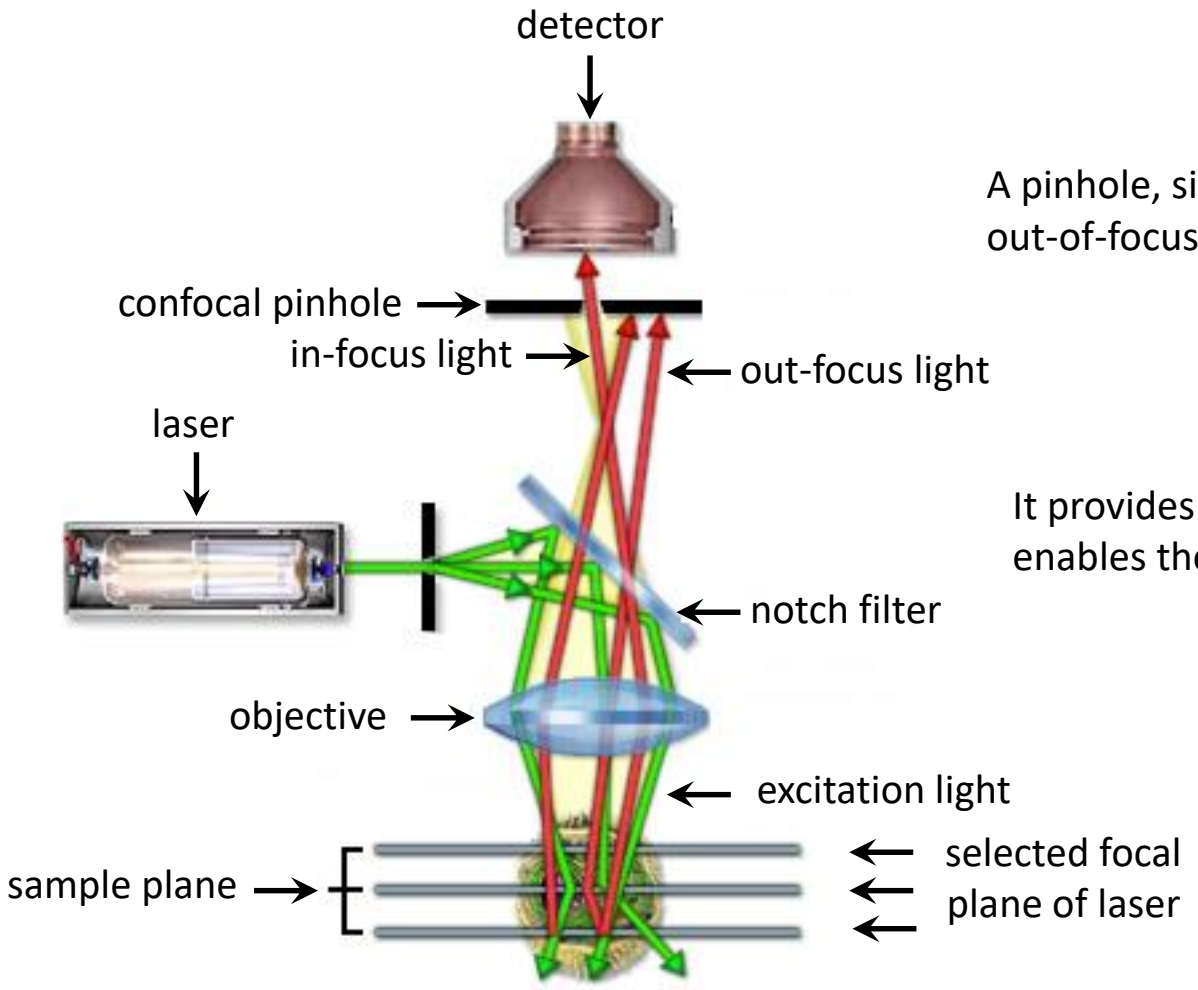


Blue spectrum: 473 nm laser

Red spectrum: 785 nm laser

Raman Spectroscopy

Confocal microscope and sampling optics



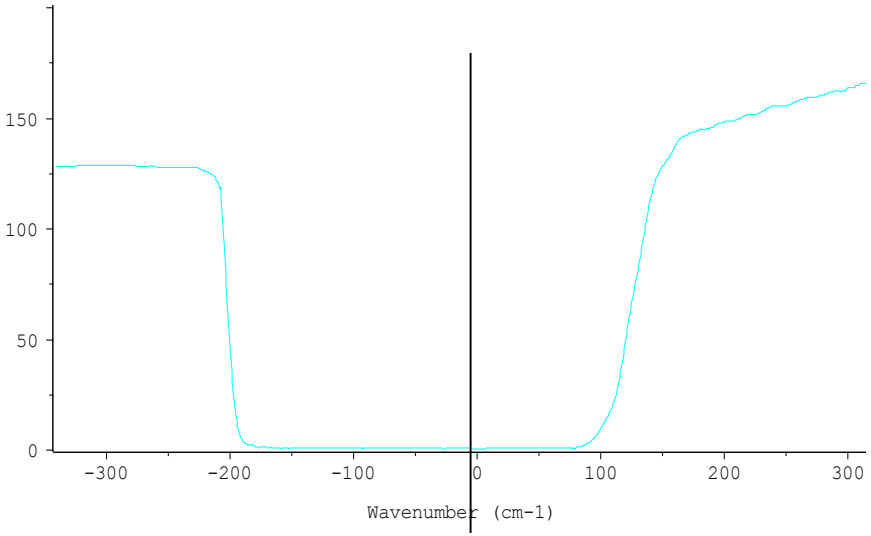
A pinhole, situated in a conjugated focal plane, prevents out-of-focus light from being detected.

It provides the axial discrimination of the signal and enables the depth profiling.

Raman Spectroscopy

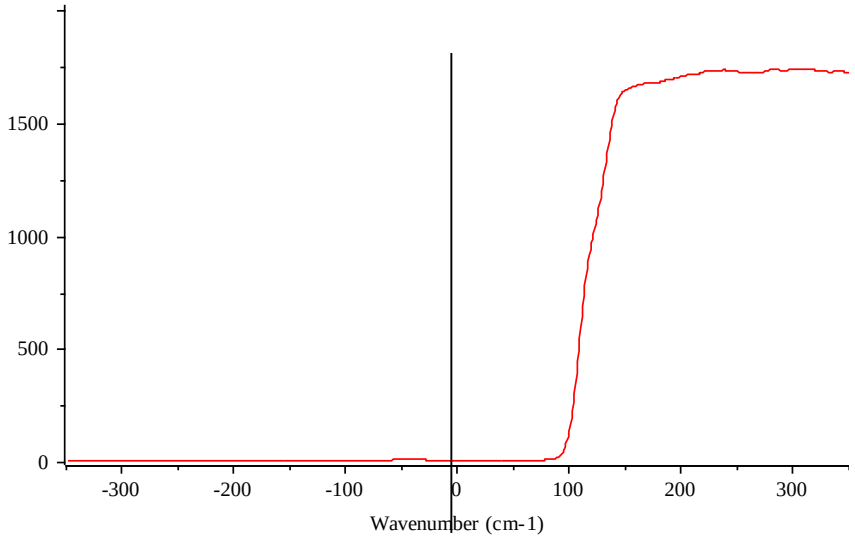
Rejection filter

White light spectrum with a notch filter



Stokes and Anti-Stokes Raman

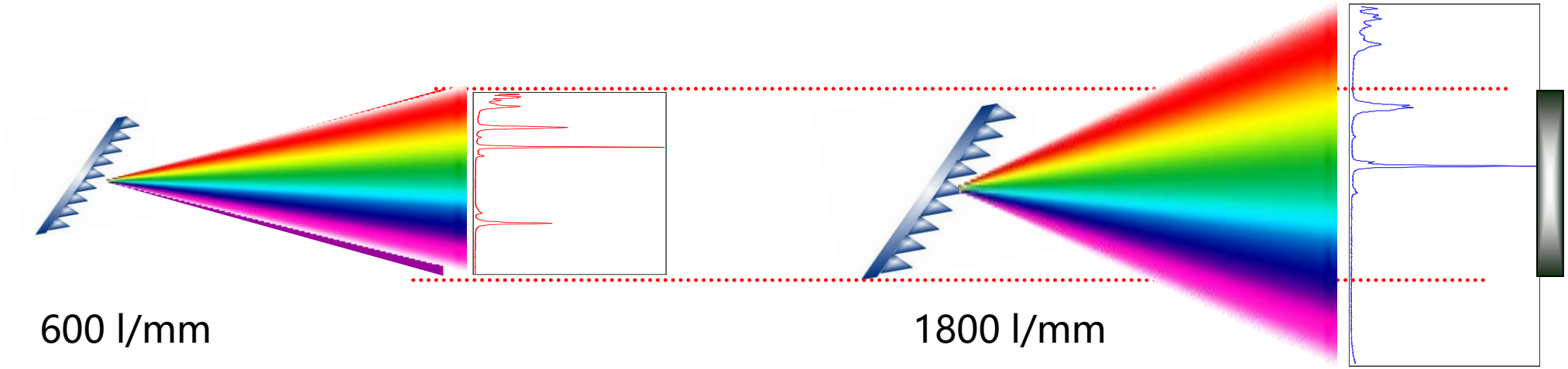
White light spectrum with an edge filter

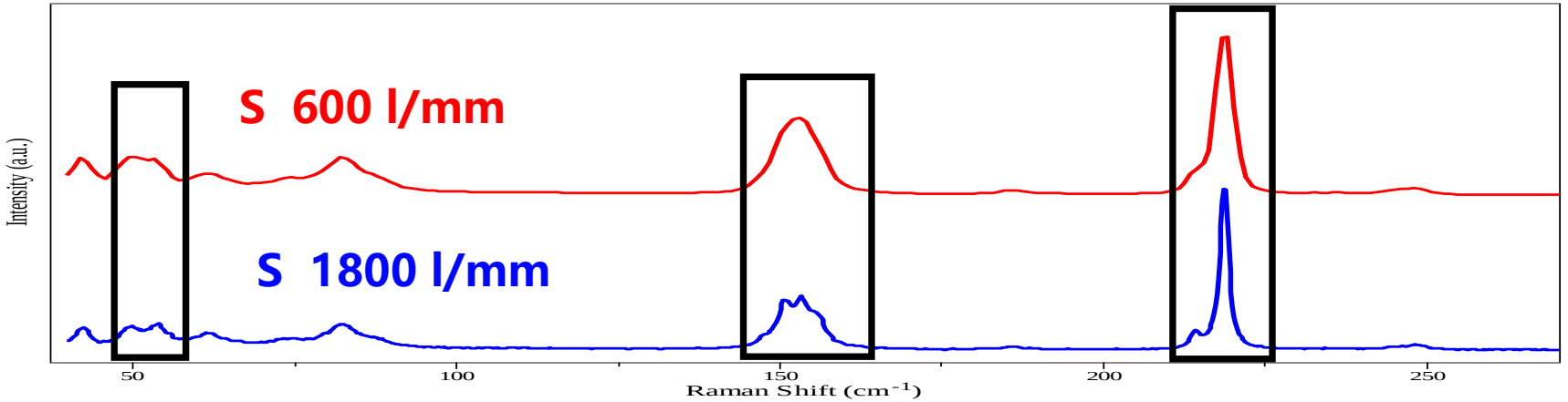


Stokes Raman only

Raman Spectroscopy

Spectrometer: gratings and spectral resolution

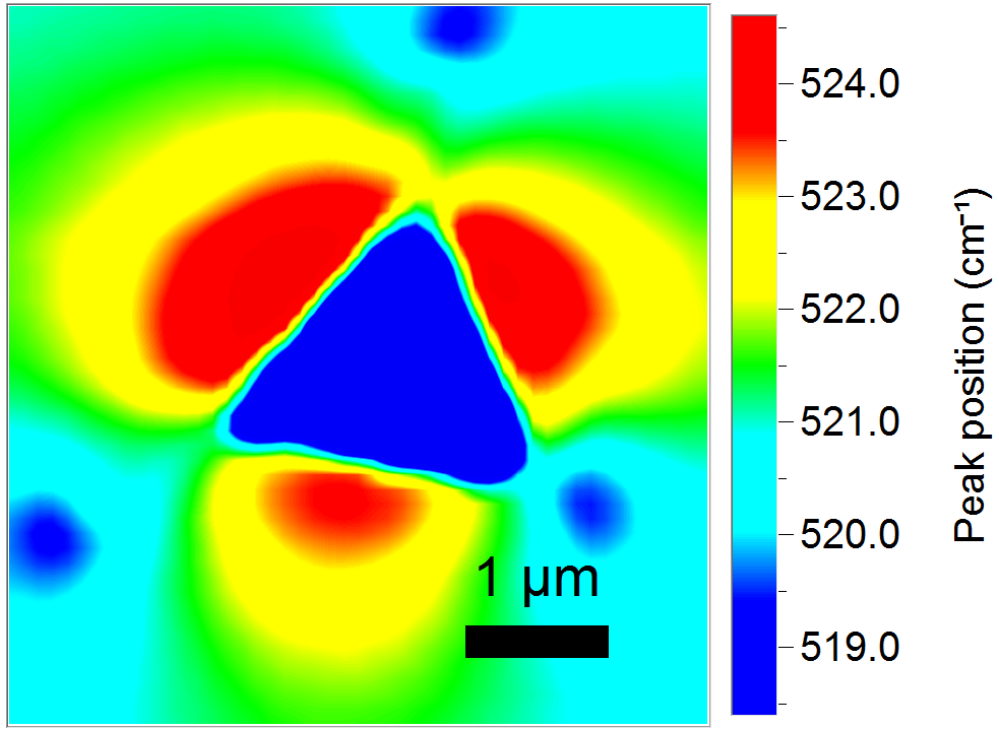




Raman Spectroscopy

Nano-indented Silicon

Peak position map

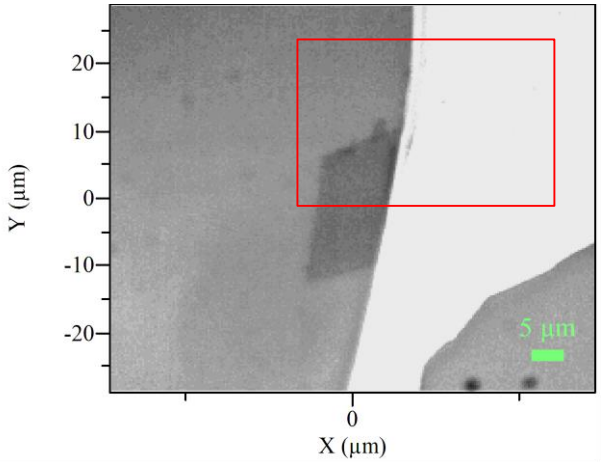


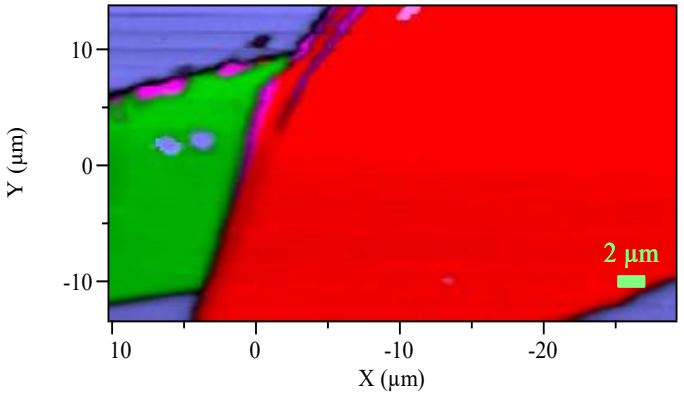
Map of Berkovich tip nano-indented Silicon, showing stress-induced peak shifts.

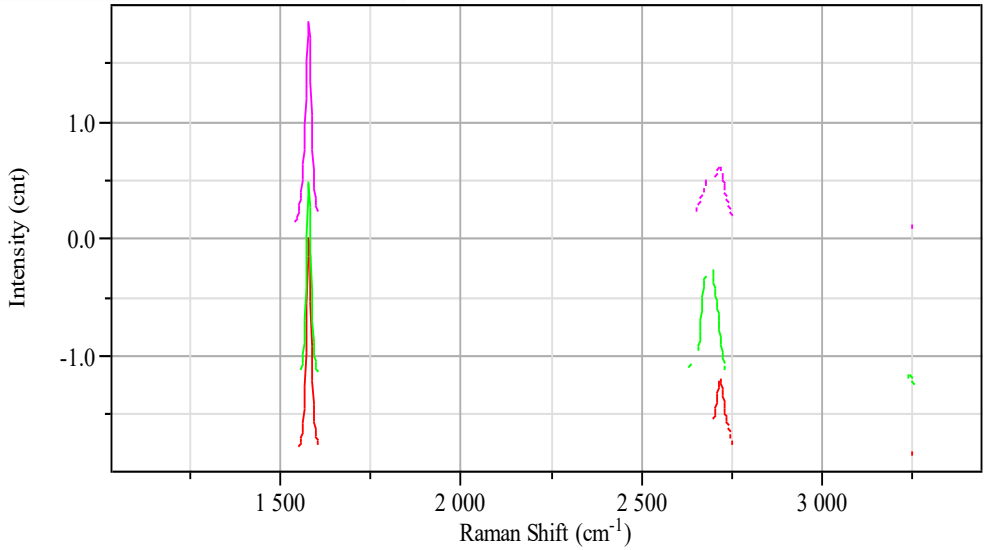
System:	LabRAM HR Evo
Laser:	532 nm
Grating:	1800 gr/mm
XY stage:	DuoScan
Objective:	x100
Acq. Time:	0.2 s x 2
Step X:	0.1 µm
Step Y:	0.1 µm
No. pixels:	2,450 (49X x 50Y)

Raman Spectroscopy

Graphene



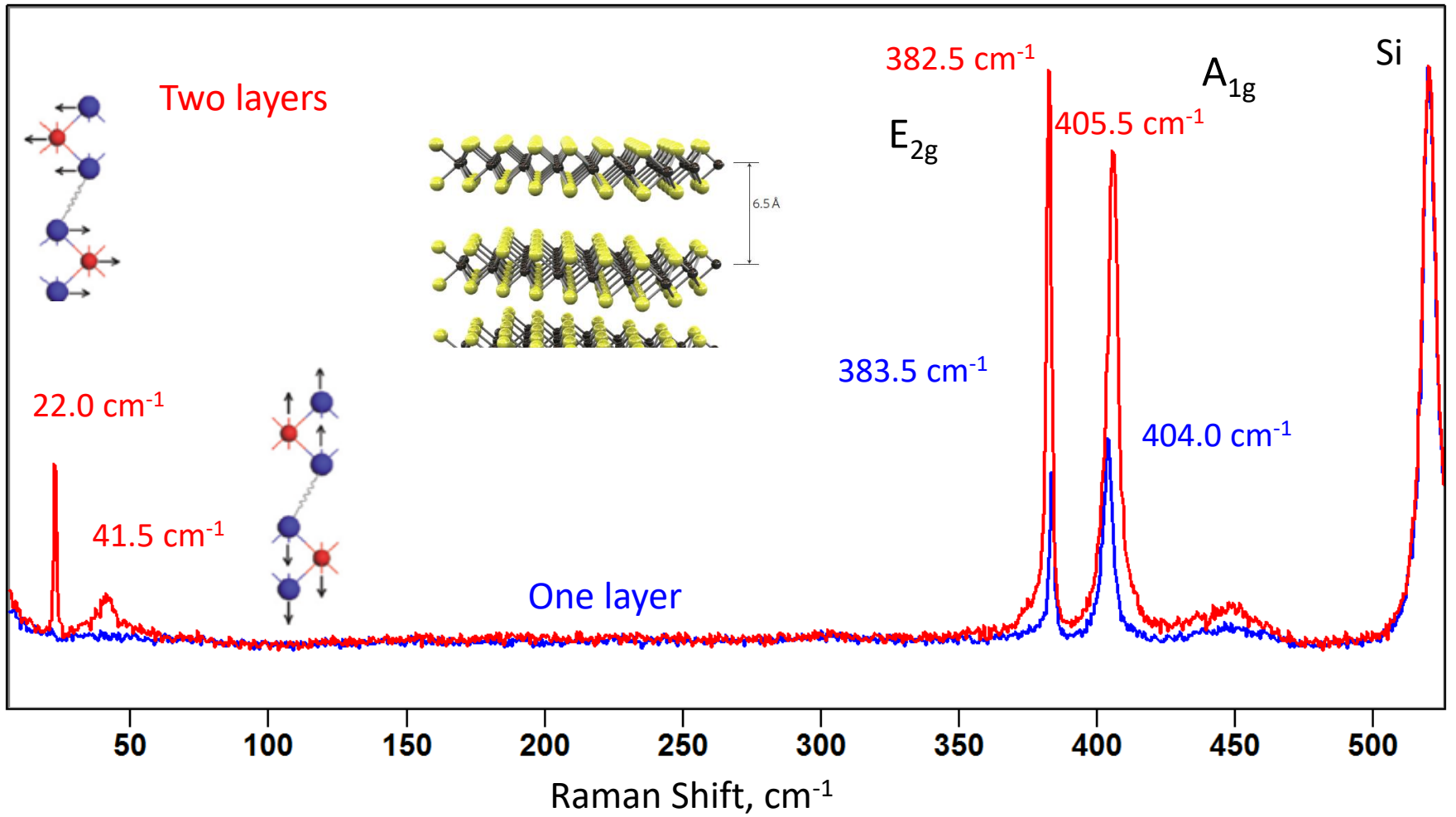




graphene bilayer
graphene multilayer
graphene edges
SiO_2/Si substrate

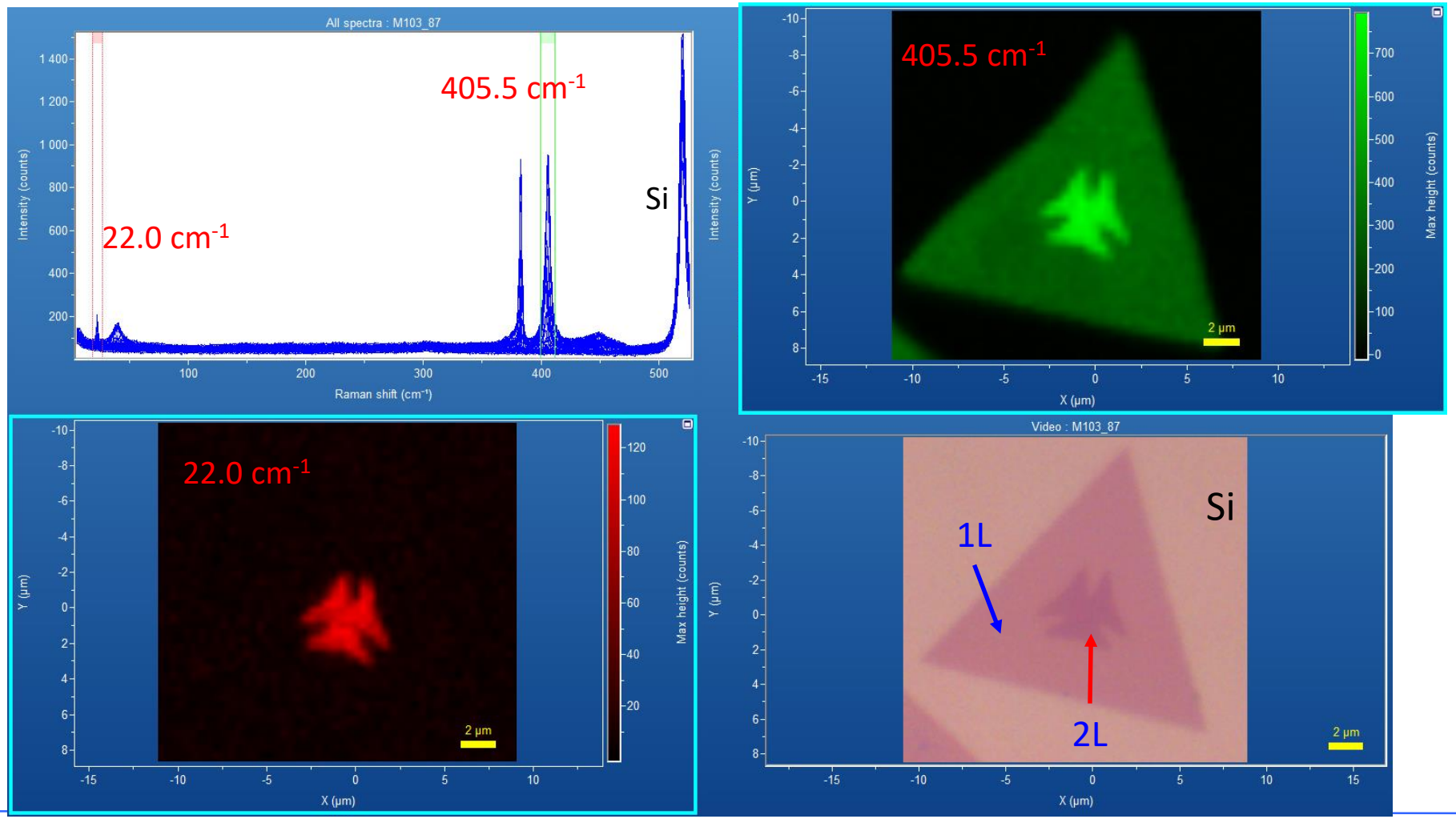
Raman Spectroscopy

Raman Spectra from one and two layers of MoS_2



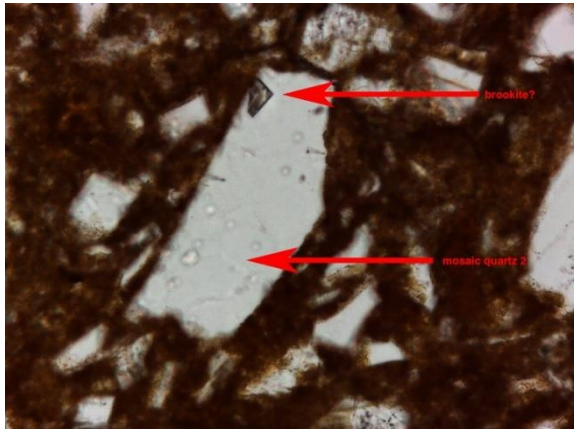
Raman Spectroscopy

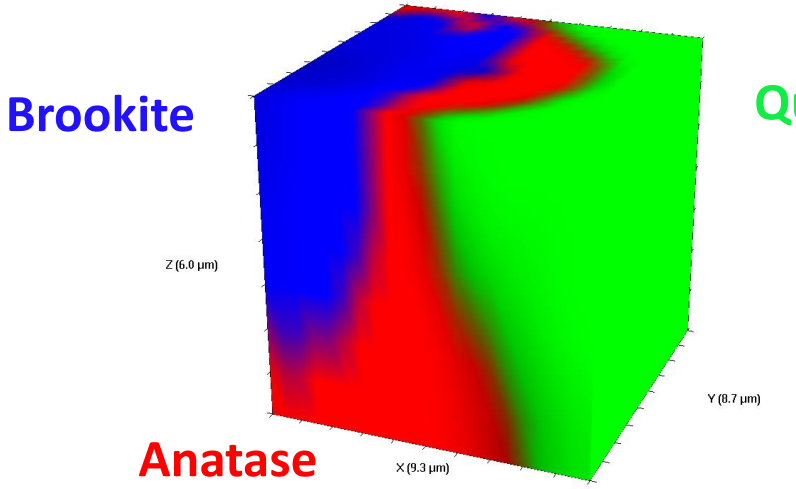
Raman Imaging of one and two layers of MoS_2

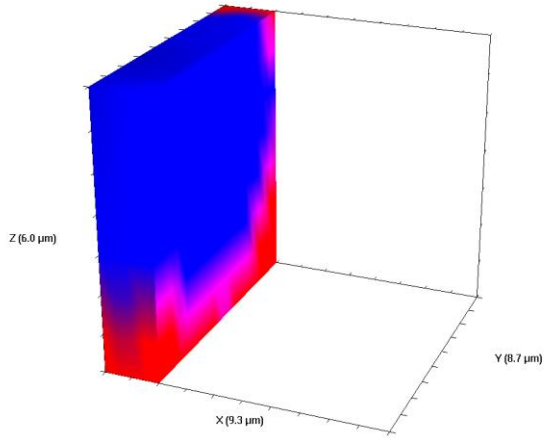


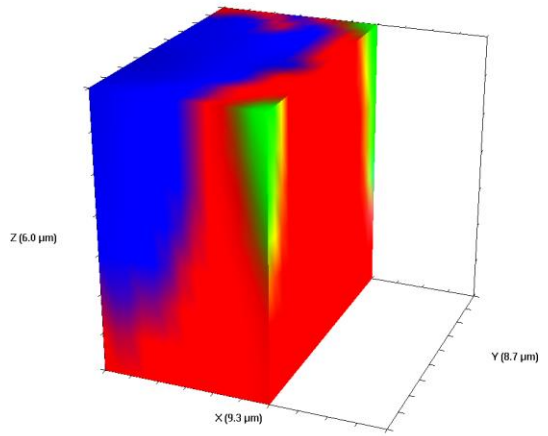
Raman Spectroscopy

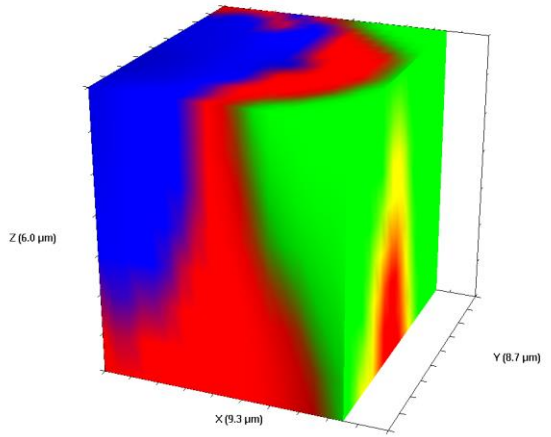
Solid inclusion of TiO_2 (anatase and brookite) in quartz





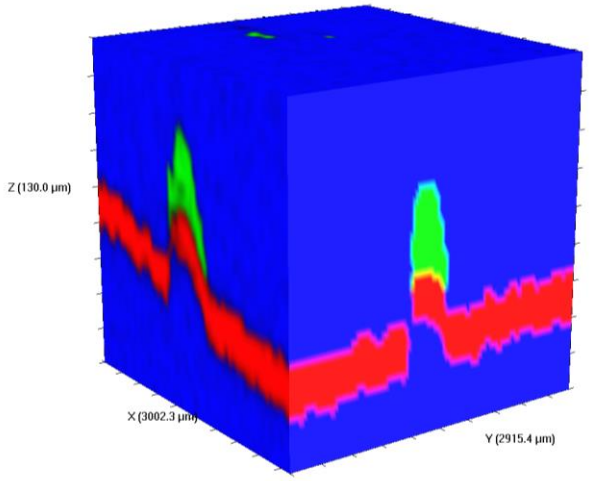


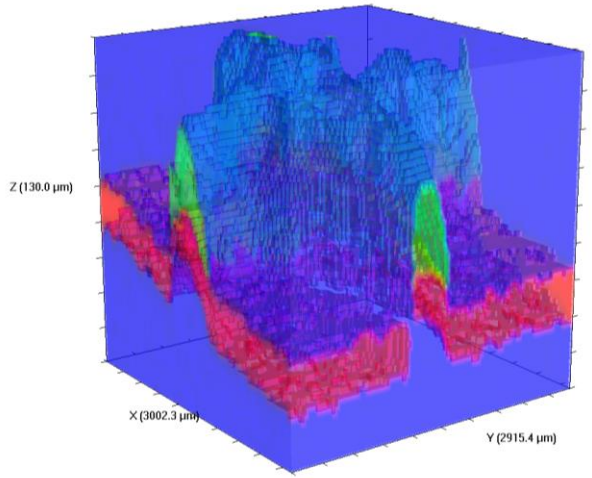


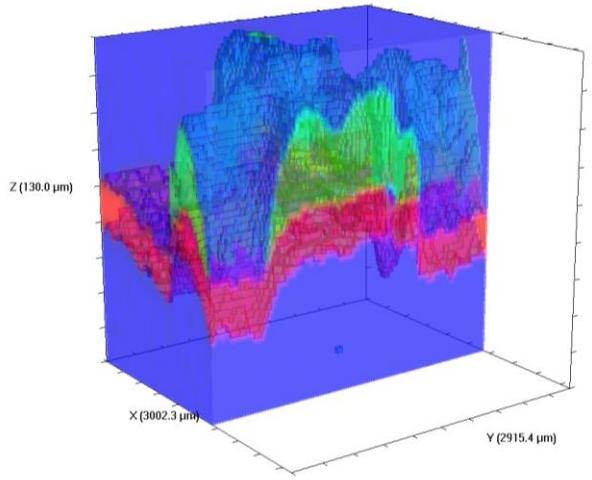


Raman Spectroscopy

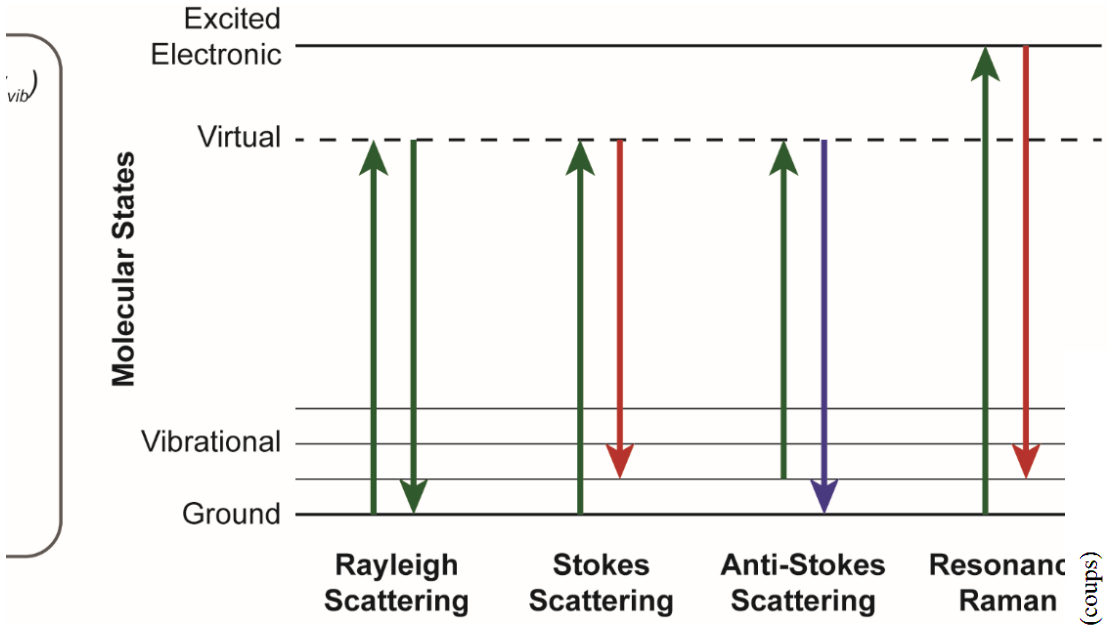
Adhesive polymer



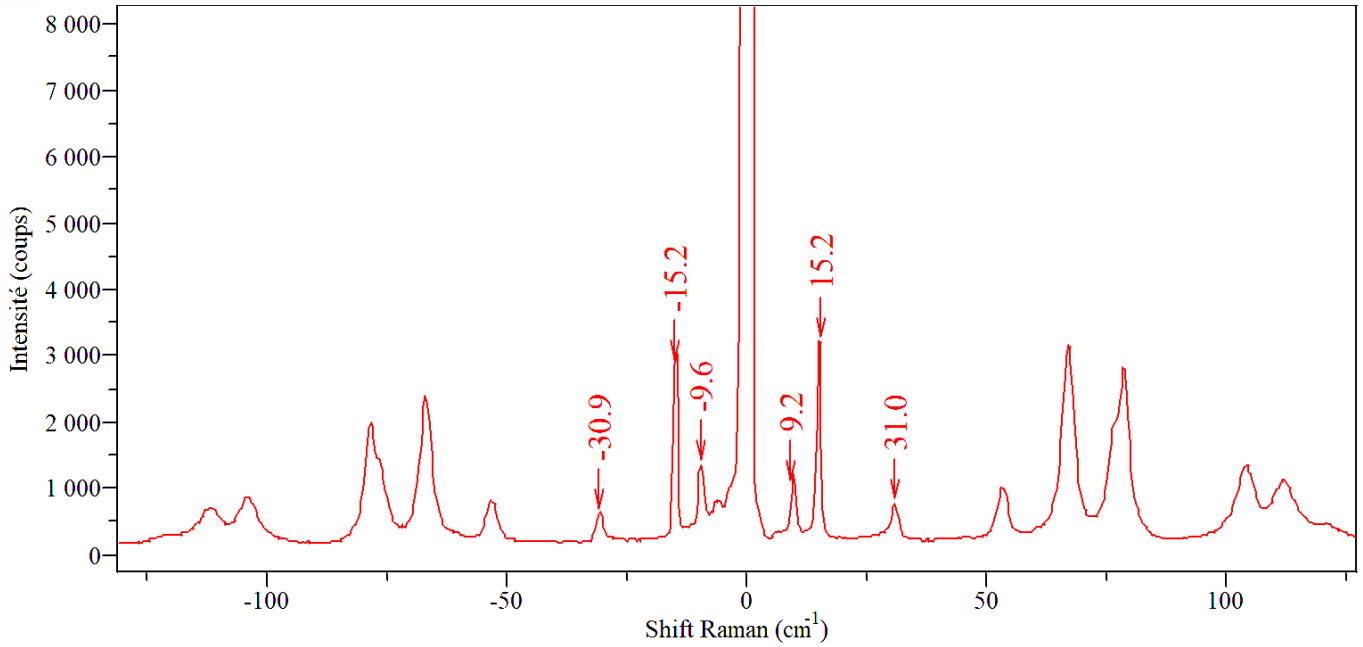




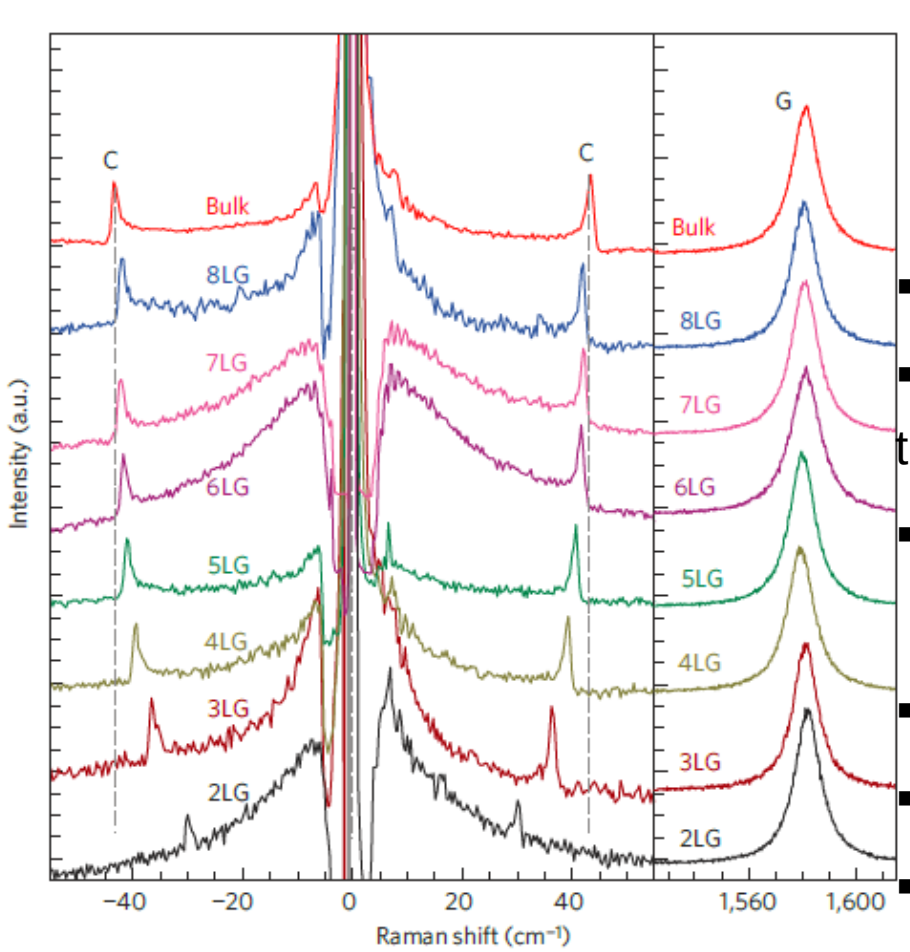
Raman Thermometry

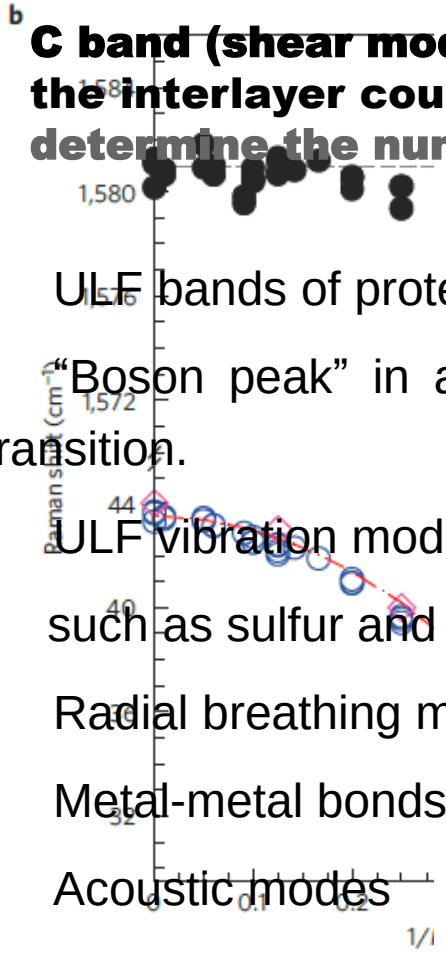


Cysteine spectrum @ 633 nm, 1800gr/mm grating



Low Wavenumber Raman





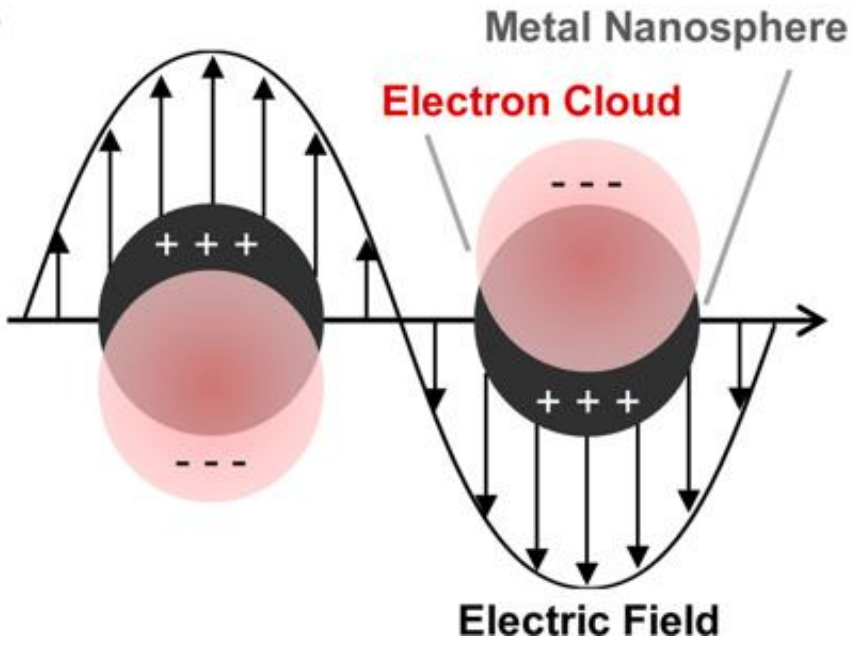
C band (shear mode): allows direct measurement of the interlayer coupling, its peak position allows to determine the number of layers

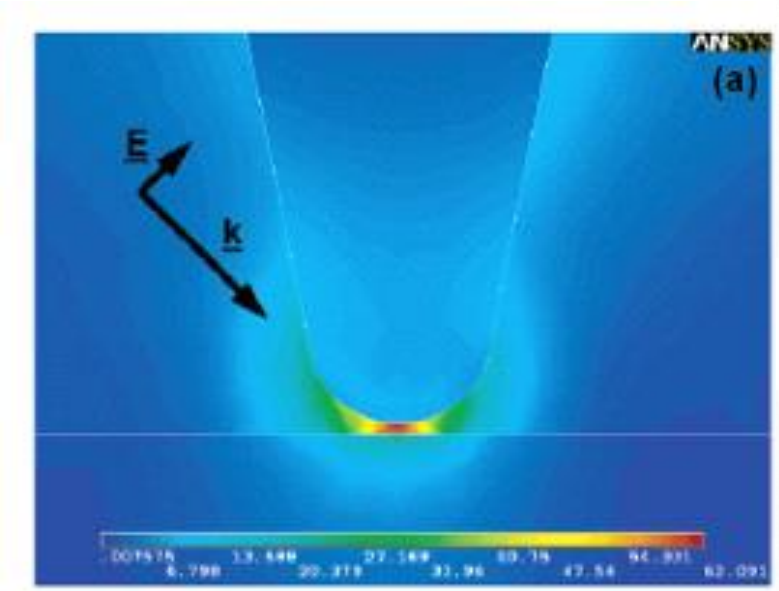
- ULF bands of protein molecule.
- "Boson peak" in amorphous glasses to study the liquid-glass transition.
- ULF vibration modes in minerals and organic materials, such as sulfur and L-Cystine.
- Radial breathing mode of carbon nanotubes and quantum dots.
- Metal-metal bonds
- Acoustic modes

The shear mode of multilayer graphene, P.H. Tan, Nature Materials, 2012

Tip-Enhanced Raman Spectroscopy

Localized Surface Plasmon Resonance (LSPR)





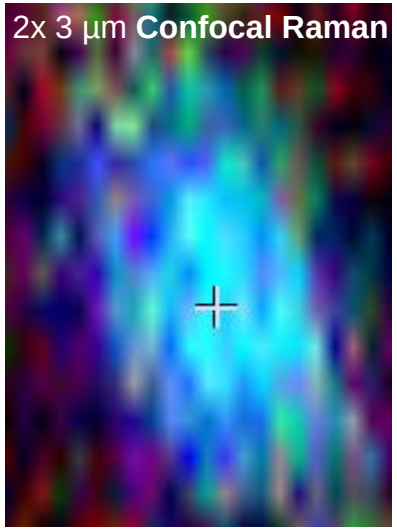
- As high as 10^8 enhancement for SERS, 10^6 for TERS
- Plasmonic “hot spots” provide additional electromagnetic enhancement

Camden, J. P.; Dieringer, J. A.; Wang, Y.; Masiello, D. J.; Marks, L. D.; Schatz, G. C.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2008**, *130* (38), 12616–12617.

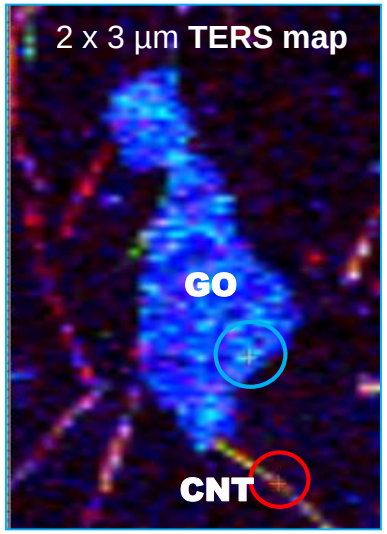
J. Phys. Chem. B 110, 6692, 2006
Optics Express 21, 25271, 2013

TERS: Better Spatial Resolution

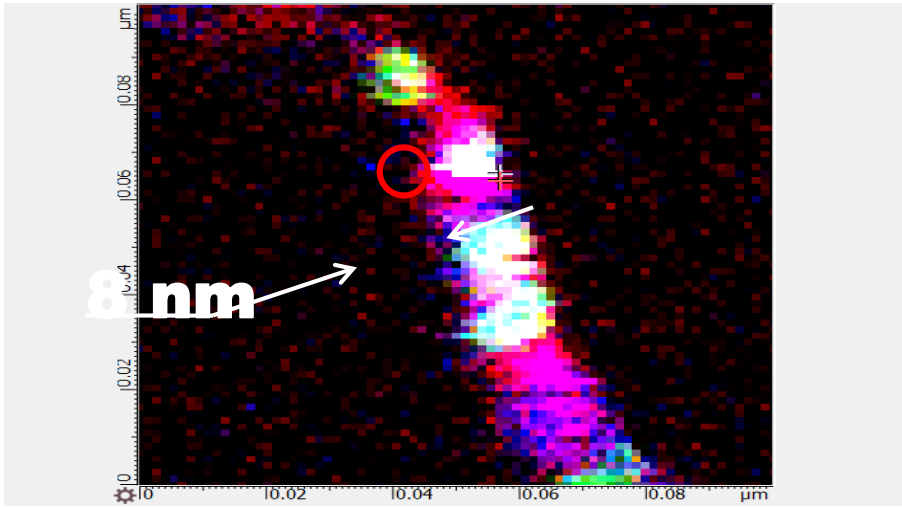
Confocal Raman and TERS of the same area, graphene oxide and CNTs on Au



Confocal Raman
13 mW; integration 1 s/pixel



TERS
130 µW; integration 0.2 s/pixel



100 nm x 100 nm (75 x 75 pixels), 50 ms per pixel

Optical resolution capability: 8 nm

The goal

The Tutorial will address Fundamentals of the techniques (UV-VIS, Fluorescence, IR and Raman), advantages and limitations of these techniques. Instrumentation and typical applications will be discussed during tutorial and the examples of applications will be shown.

Thank you

Thank you

감사합니다

Cảm ơn

ありがとうございました

Dziękuję

धन्यवाद

Grazie

Merci

谢谢

நன்ற

ขอบคุณครับ

Obrigado

Σας ευχαριστούμε

شُكْرا

Tack ska ni ha

Большое спасибо

Danke

Gracias