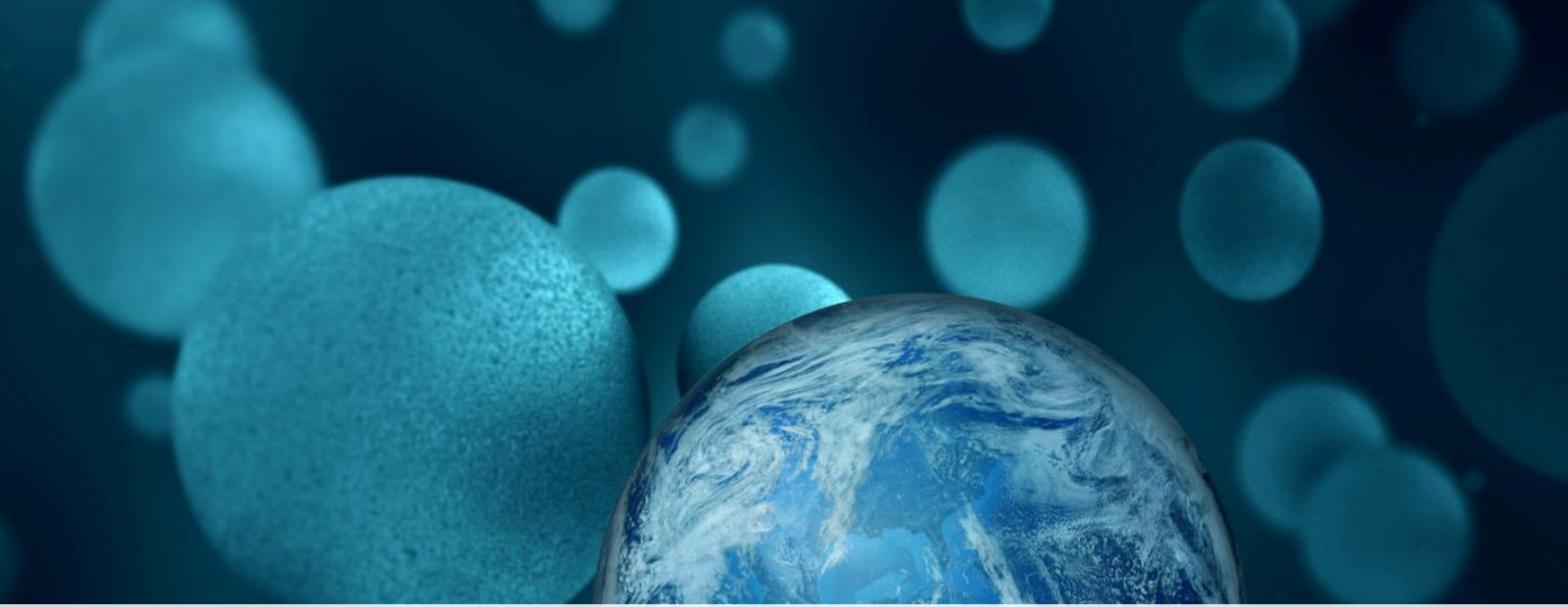

ThermoFisher SCIENTIFIC

Raman Theory and Applications

송신헌
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sinheon.song@thermofisher.com

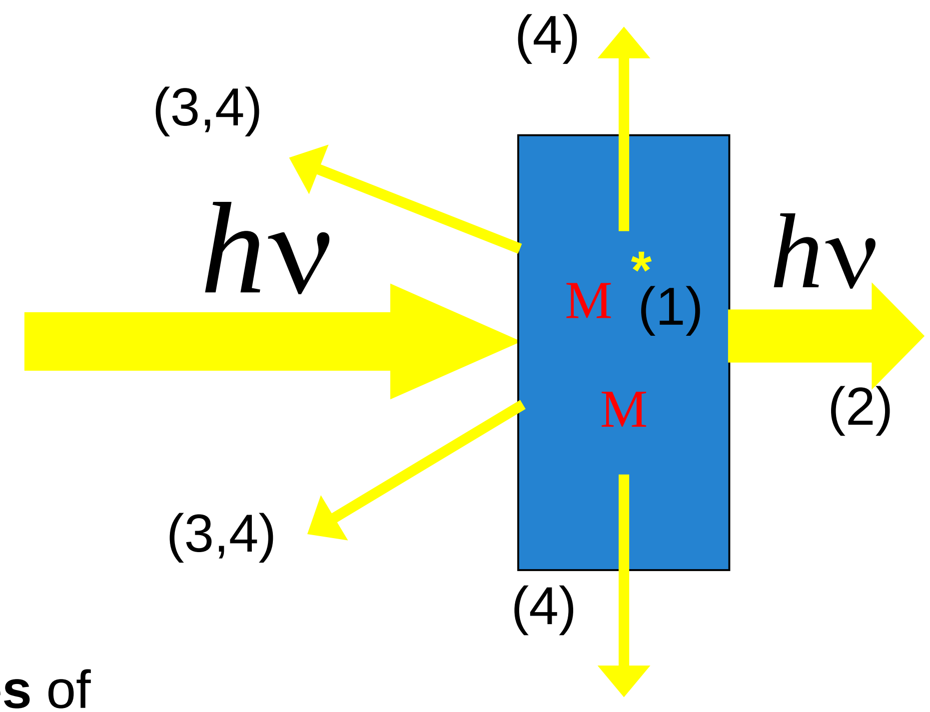
Basics of Raman Spectroscopy

First Raman instrument: History

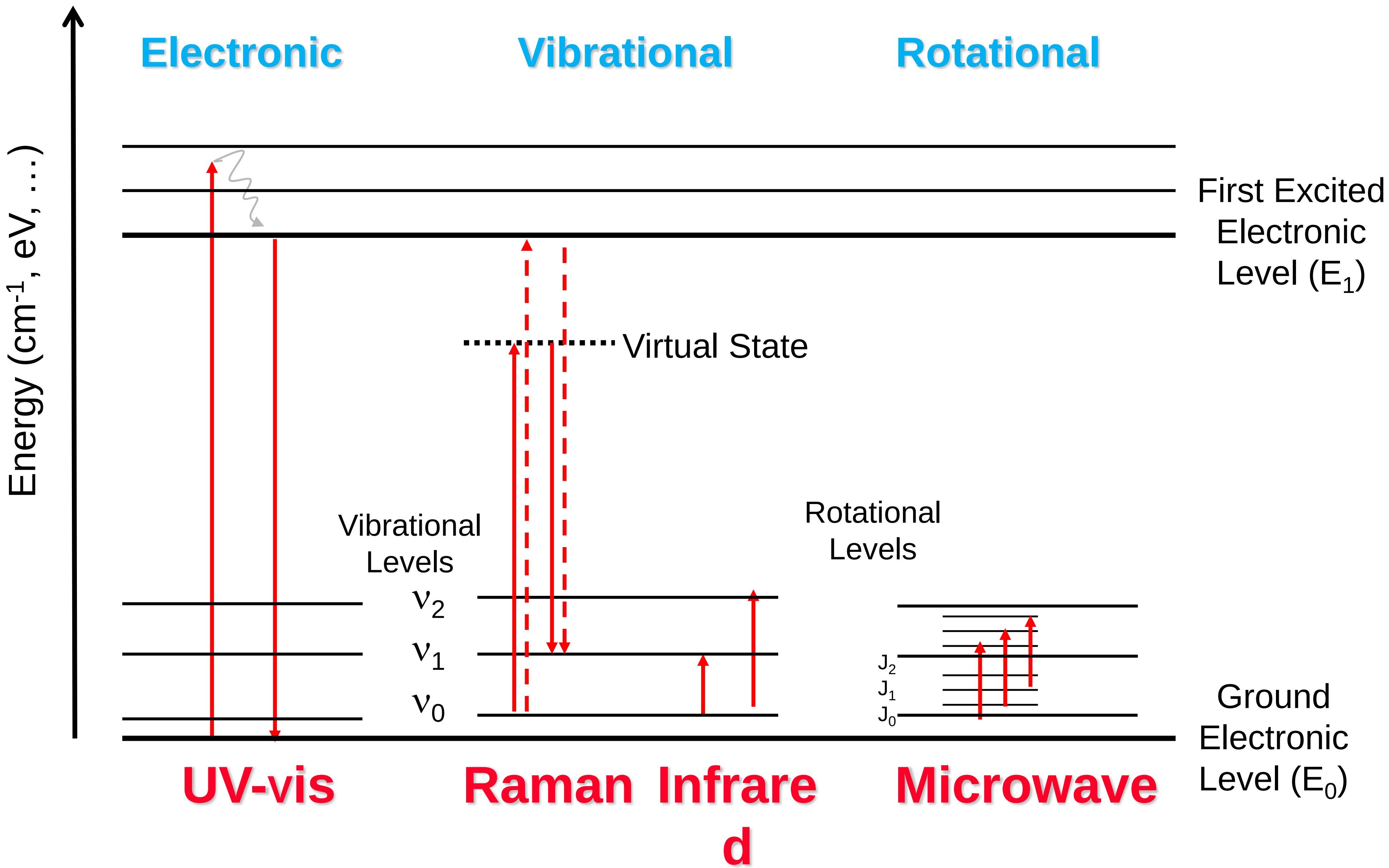
- Sunlight as a source and a narrow band photographic filter to create monochromatic light and a "crossed" filter to block this monochromatic light

Interaction Between Electromagnetic Radiation and Substances

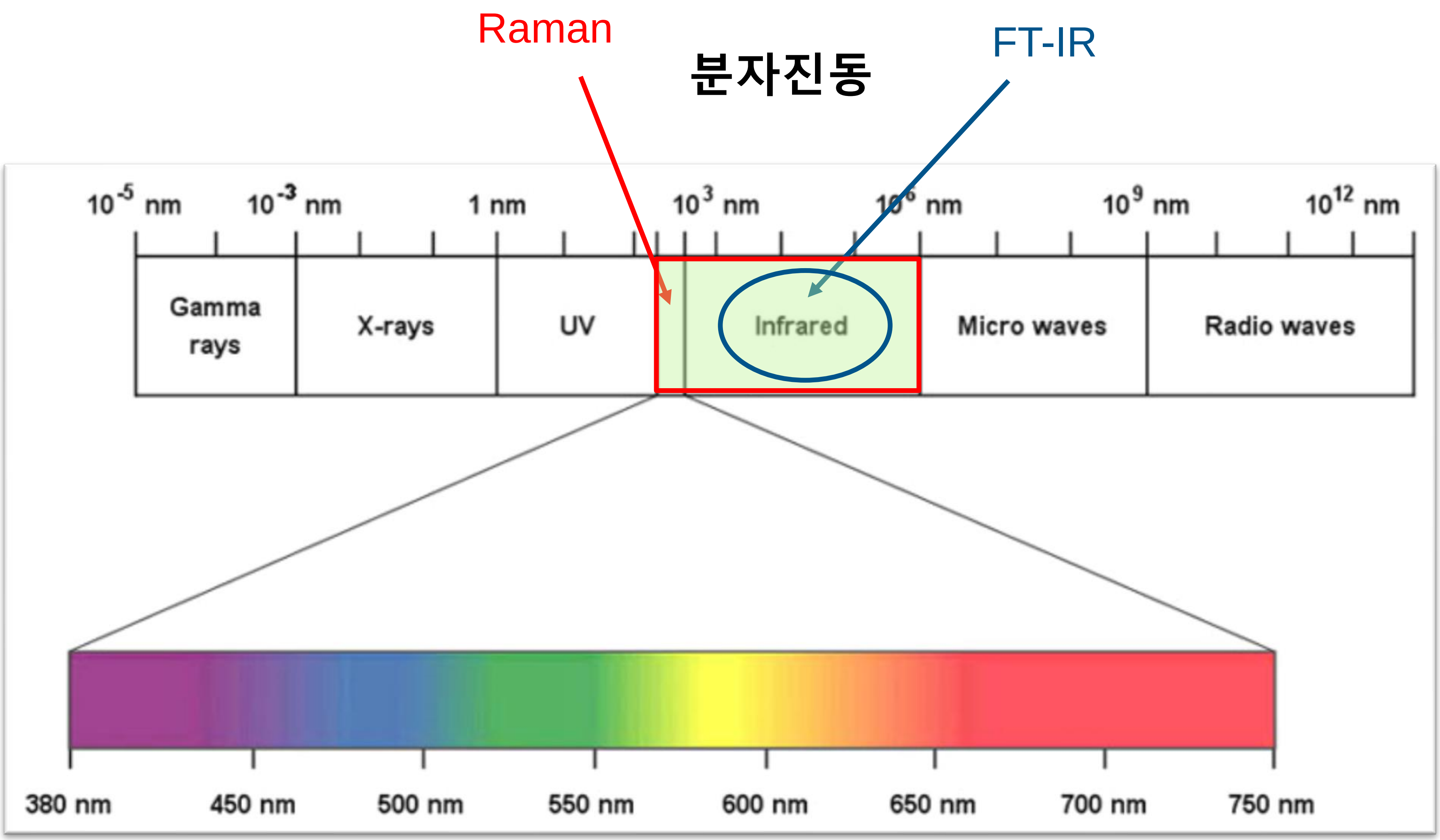
- When a beam of electromagnetic radiation is passed through a substance, it interacts with the substance and can be:
  - absorbed (1)
  - transmitted (2)
  - reflected (3)
  - scattered (4)
- depending upon:
  - its **frequency**
  - the **structure of molecules** of the substance it encounters.



Energy Transitions Studied by Molecular Spectroscopy



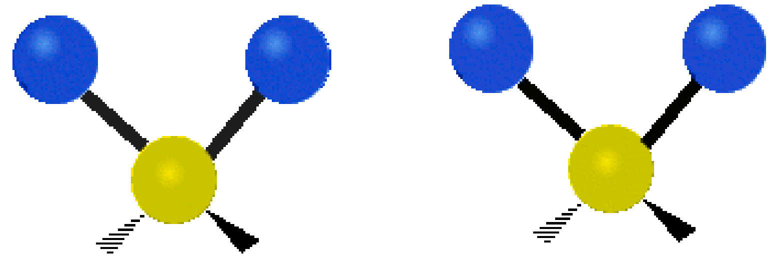
What is Raman Spectroscopy?

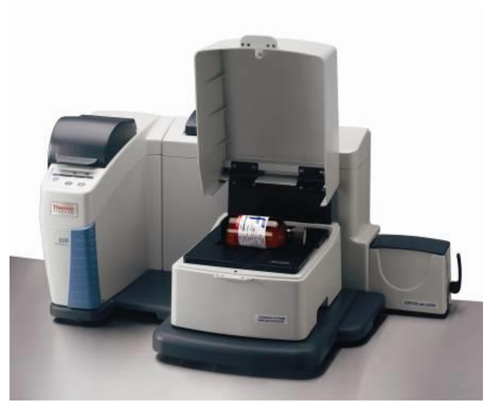


Electromagnetic spectrum

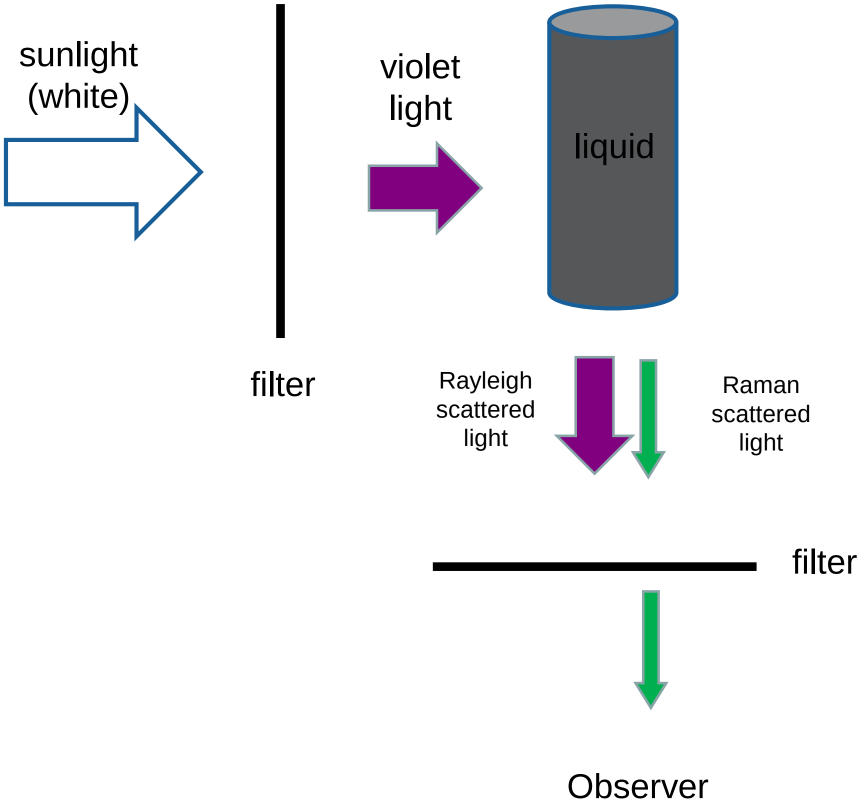
What is Raman Spectroscopy?

- Complementary technique to infrared (IR) spectroscopy
- Uses light to probe covalent chemical bonds by looking at vibrations
- Provides detailed molecular information: sensitive to even slight changes in bond angle or strength
- Useful for identifying unknown solids and liquids, including both inorganic and organic materials
- Sensitive to changes in structure, morphology, and even temperature





A Brief History of Raman Spectroscopy



1928 C.V. Raman discovered the Raman effect during light scattering experiments

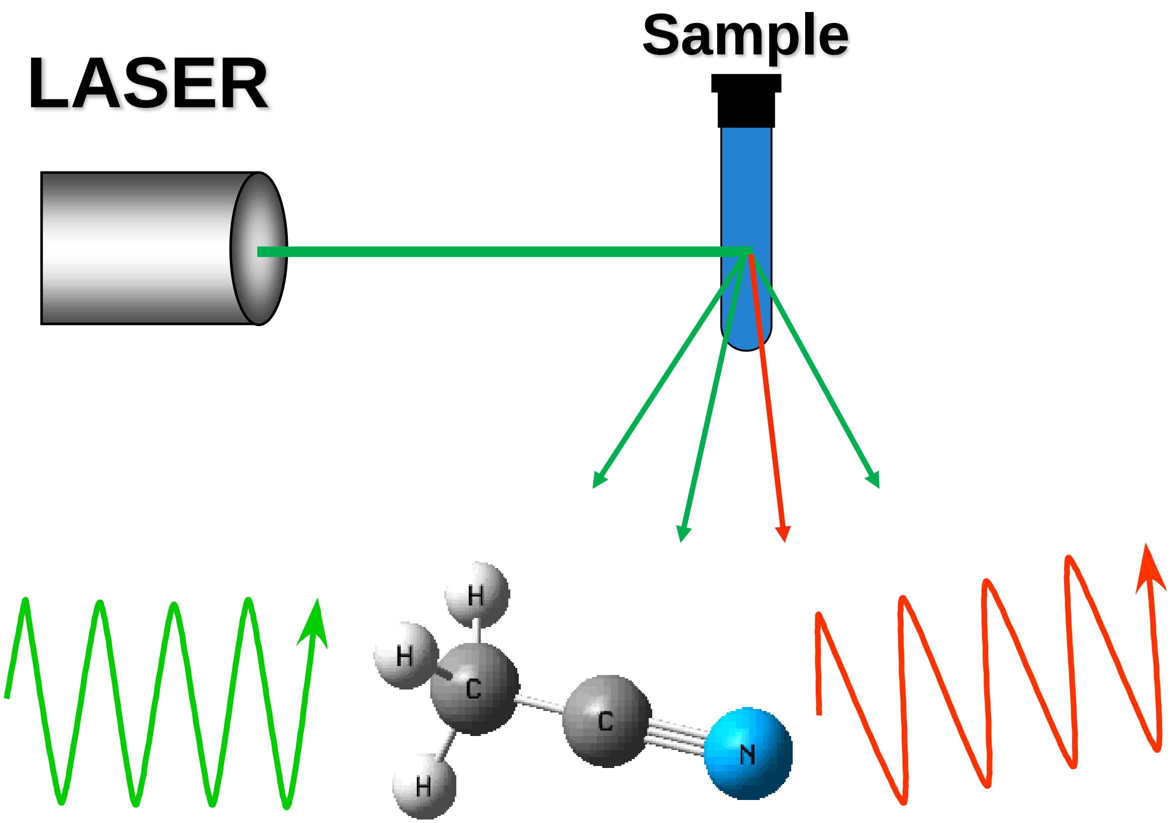
1930 Raman received the Nobel Prize in Physics

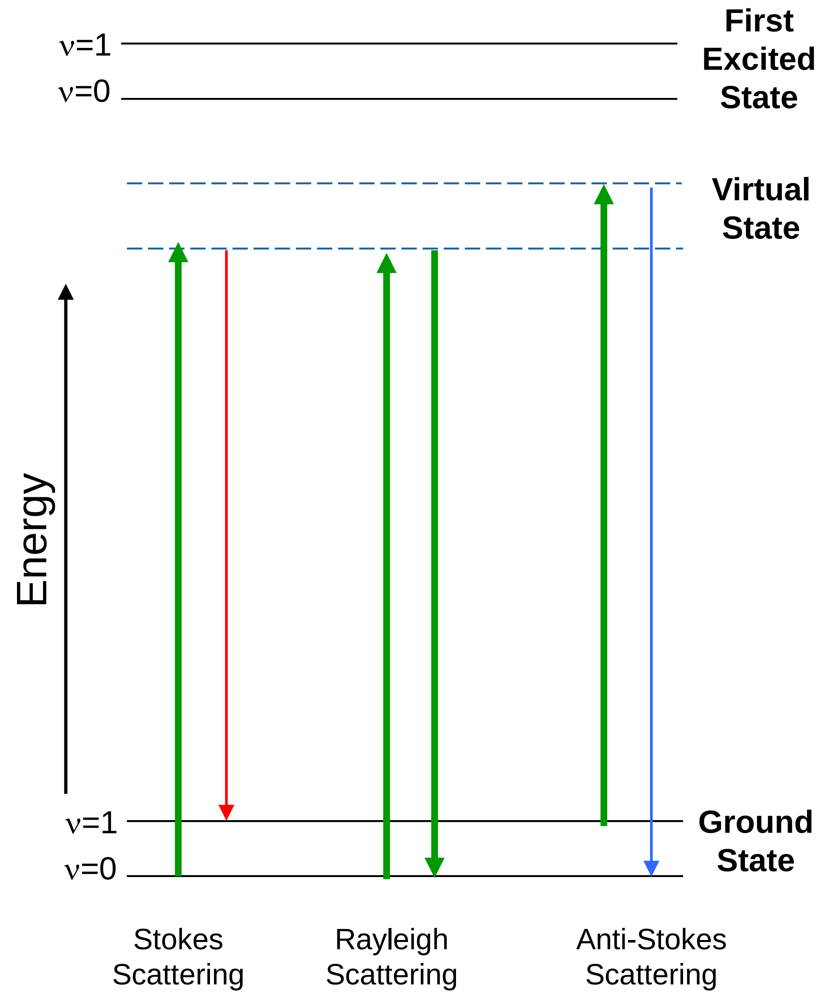
*1928- Owen Richardson, thermionic emission

*1929- Louis de Broglie, wave-like properties of electrons

This experiment was repeated for different liquids and each liquid scattered the light differently

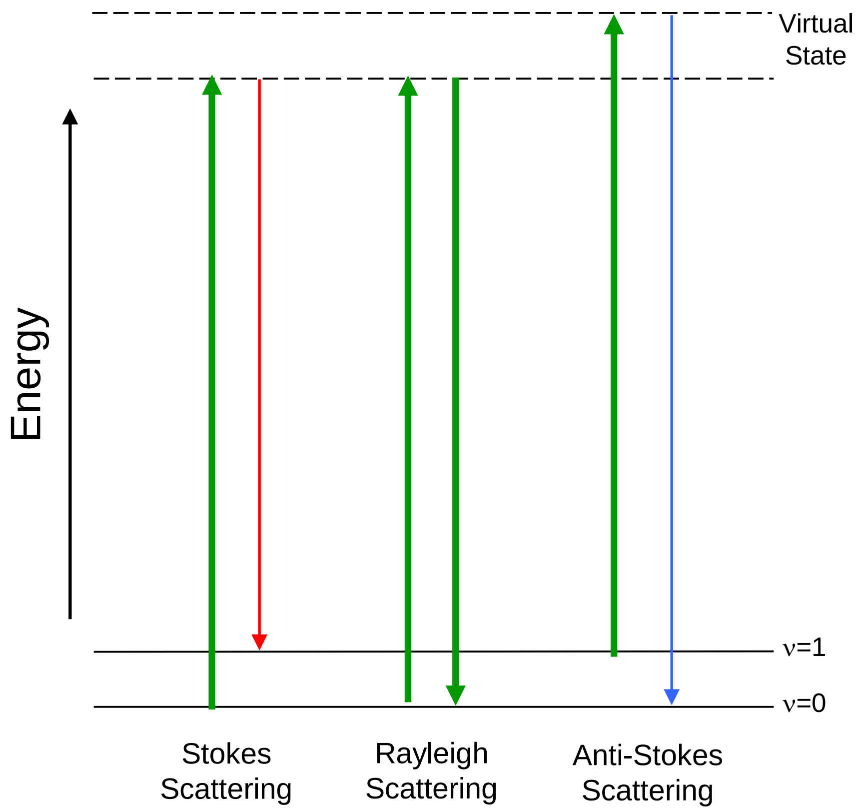
Raman Spectroscopy

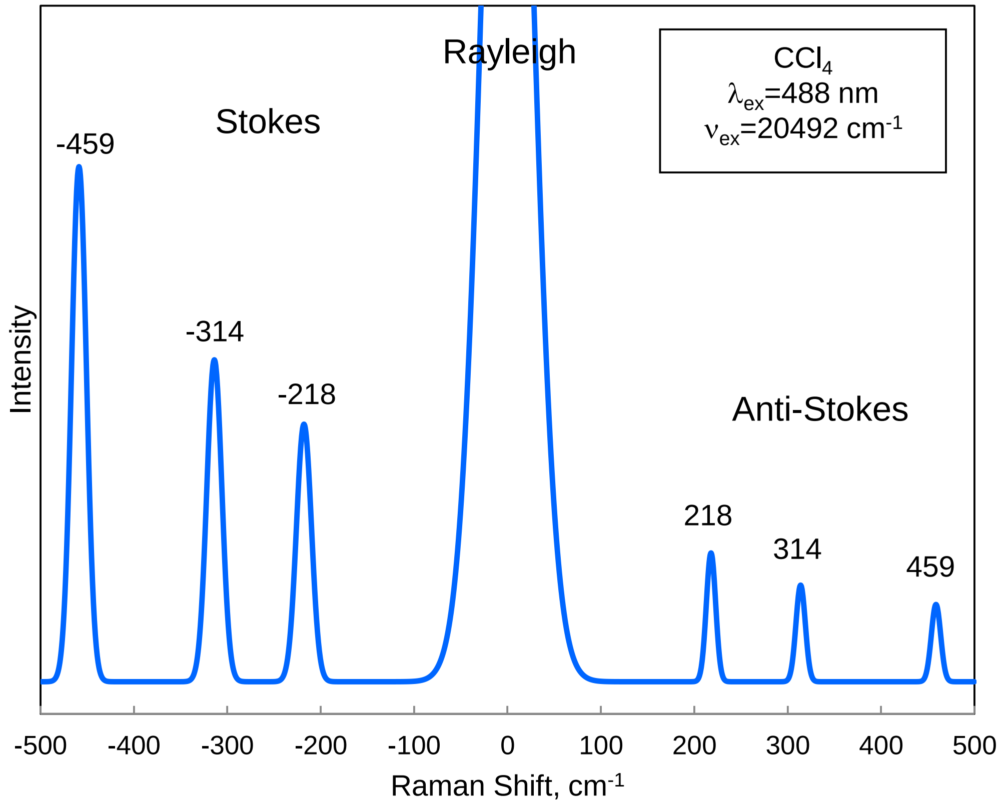




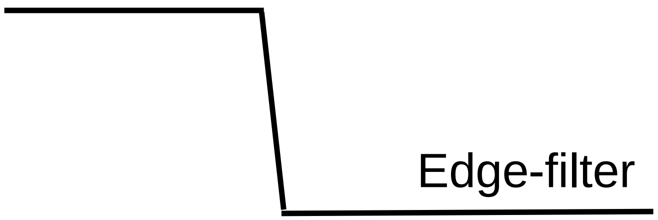
- Light scattering technique – Raman scatter
- Difference between incident and scattered light
- The difference corresponds to differences in vibrational states

Stokes and Anti-Stokes – Filter Off Rayleigh Scatter

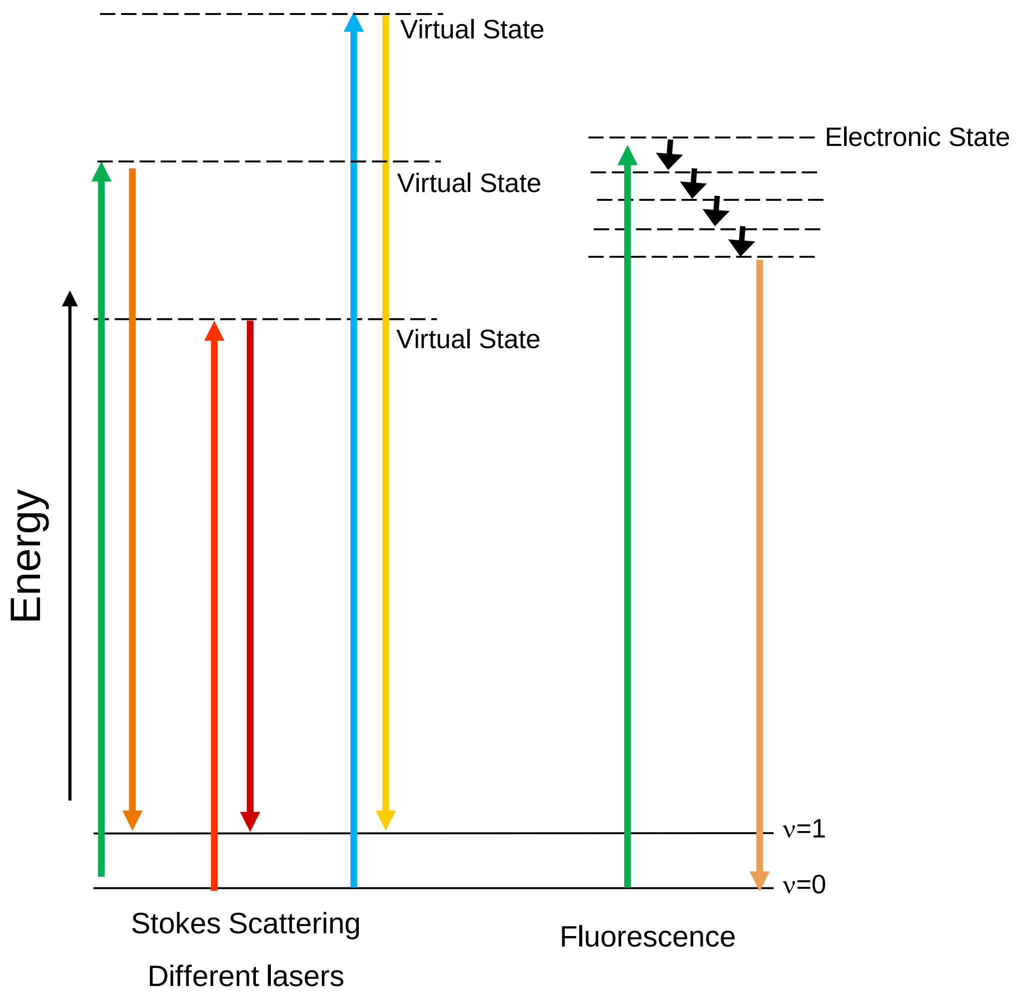




Rejection Filter



Balance most efficient laser with avoiding severe fluorescence

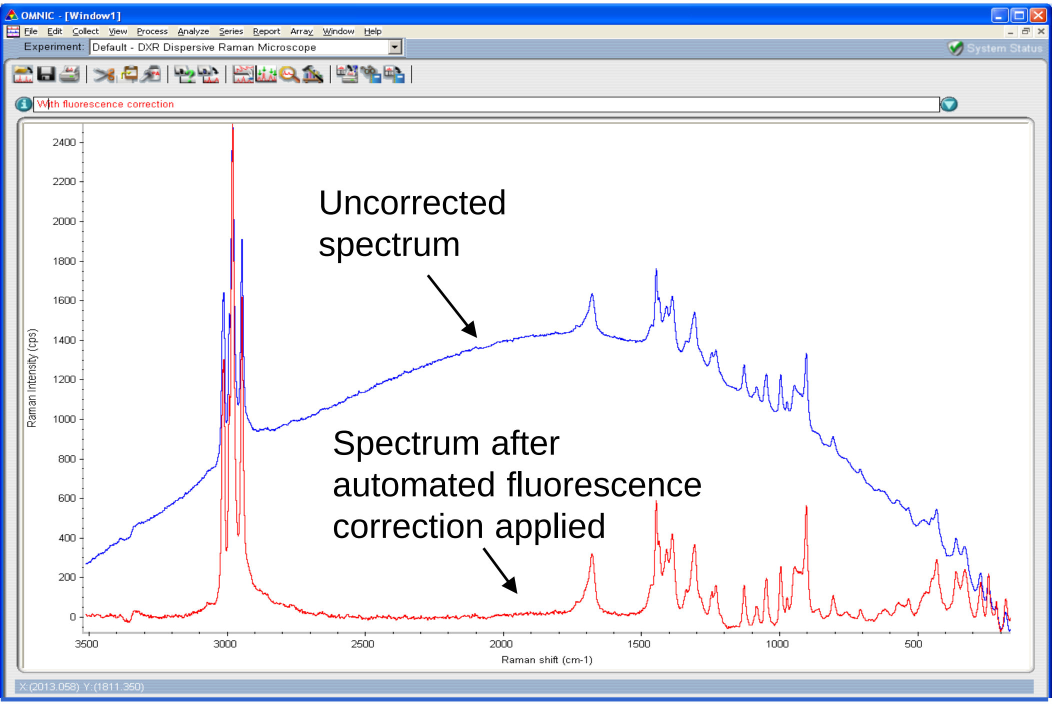


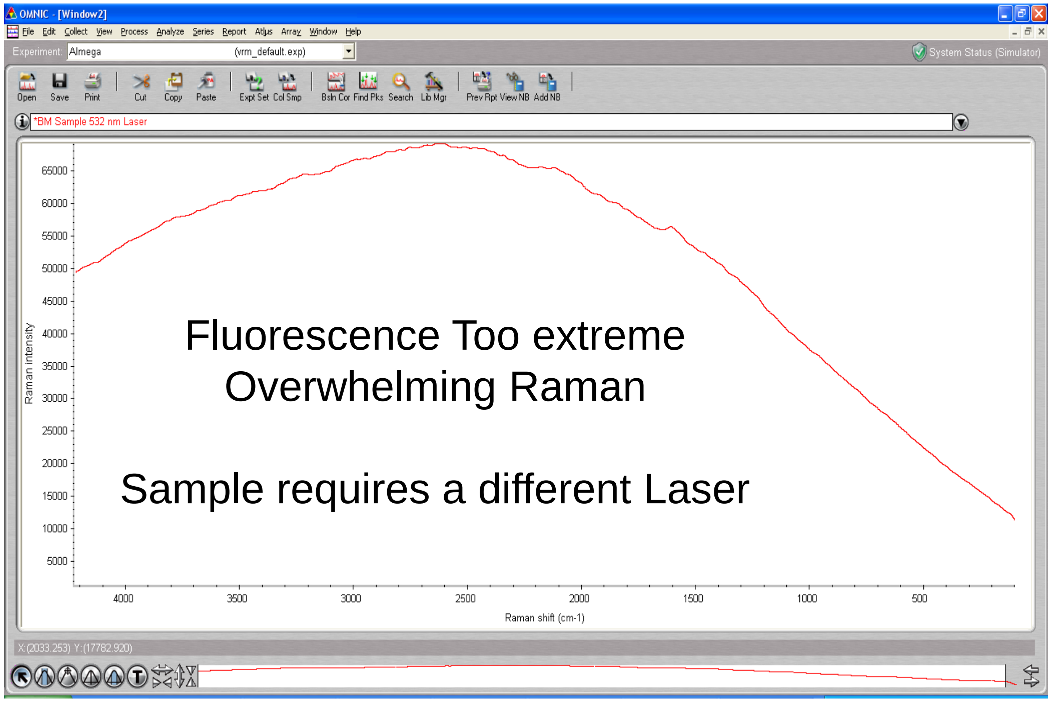
Laser Choices: 455 nm, 532 nm, 633 nm, 785 nm

$$I_{scatter} \propto \frac{1}{\lambda_{ex}^4}$$

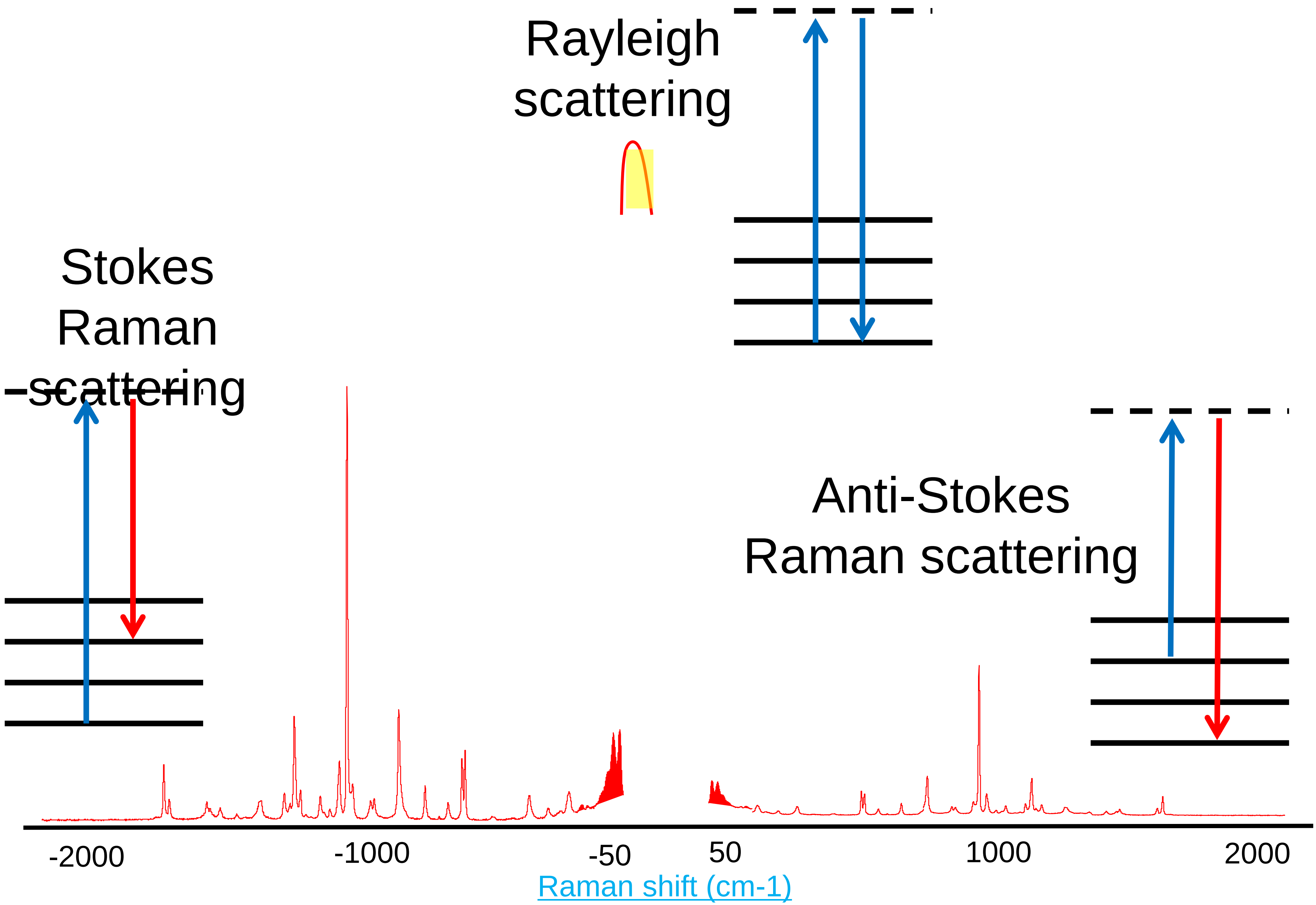
Longer Wavelength Lasers (785 nm)	Shorter Wavelength Laser (532 nm)
	More Efficient Raman Scatter (lower laser power)
Typically Less Fluorescence (sample dependent)	
	Better Spatial Resolution

Avoiding Fluorescence – Change Excitation Wavelength



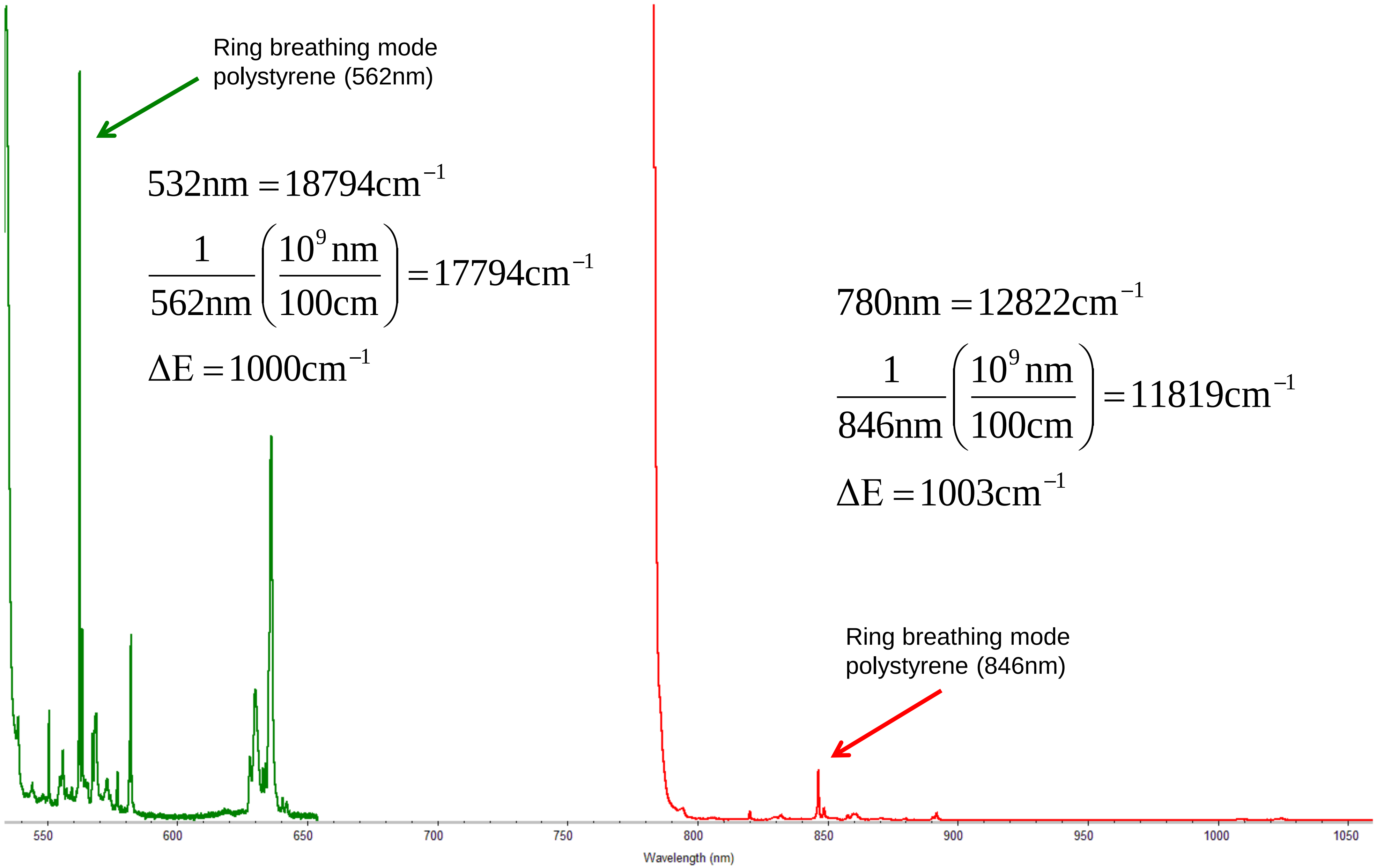


If the fluorescence is not too extreme it can be compensated for using a software correction otherwise changing the excitation wavelength (laser) might be the best solution.



Rayleigh scattering
Stokes Raman scattering
Anti-Stokes Raman scattering
-2000
-1000
-50
50
1000
2000
Raman shift (cm-1)

Why use Raman shift?



$$532\text{nm} = 18794\text{cm}^{-1}$$

$$\frac{1}{562\text{nm}}\left(\frac{10^{9}\,\text{nm}}{100\text{cm}}\right) = 17794\text{cm}^{-1}$$

$$\Delta E = 1000\text{cm}^{-1}$$

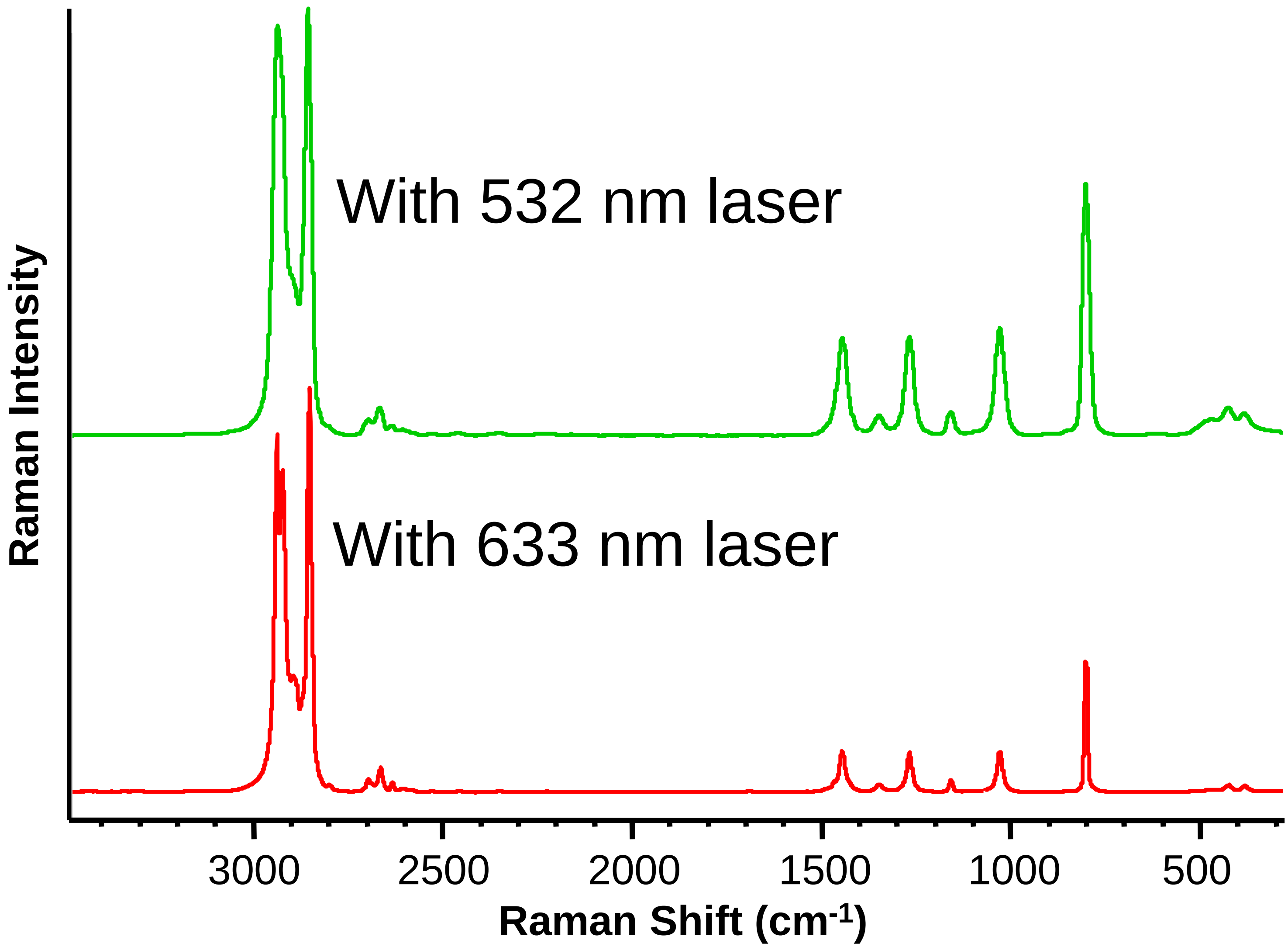
$$780\text{nm} = 12822\text{cm}^{-1}$$

$$\frac{1}{846\text{nm}}\left(\frac{10^{9}\,\text{nm}}{100\text{cm}}\right) = 11819\text{cm}^{-1}$$

$$\Delta E = 1003\text{cm}^{-1}$$

What's with all the lasers?

The vibrational shifts are independent of laser frequency

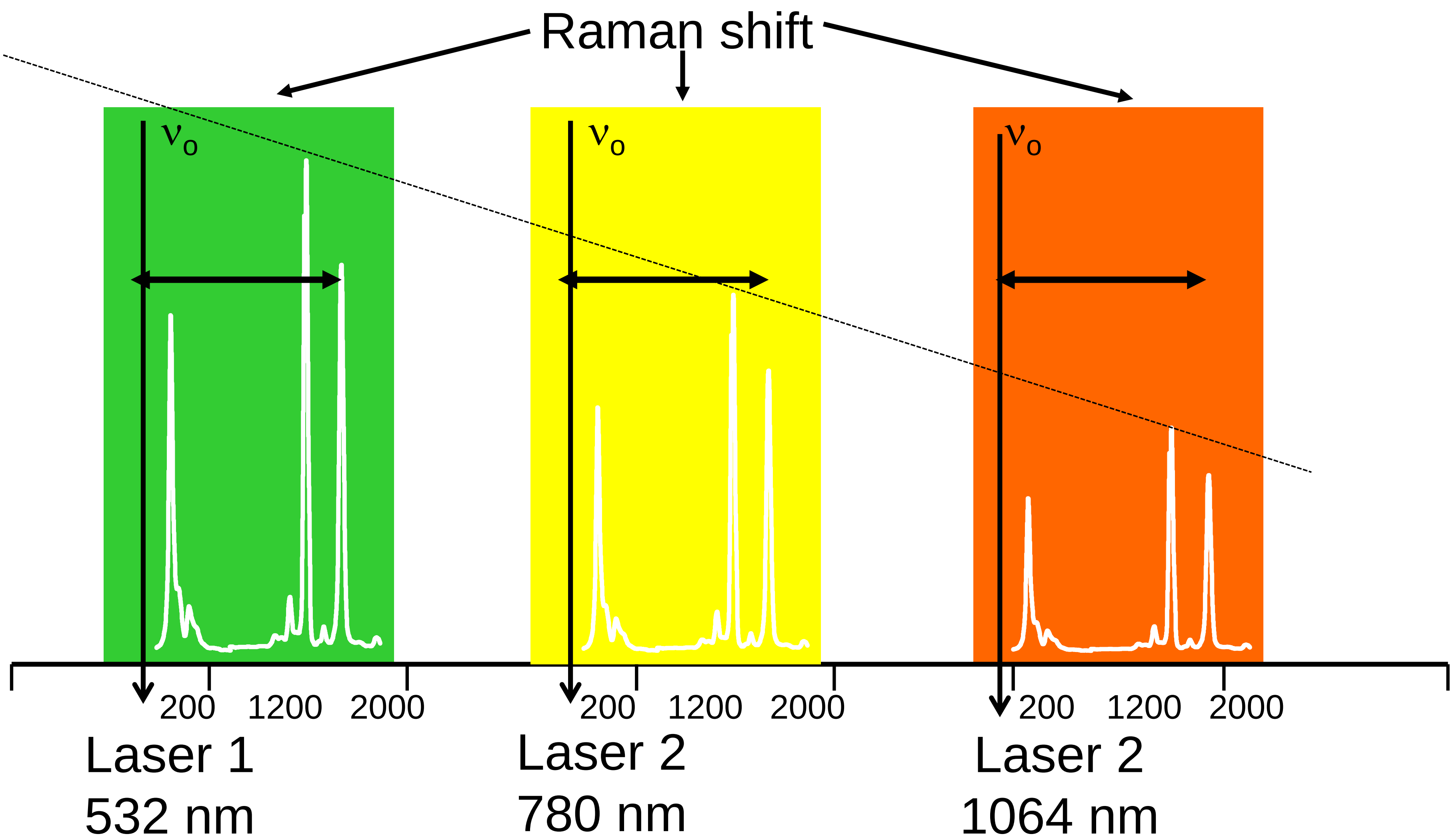


$$I_{scatter} \propto \frac{1}{\lambda_{ex}^4}$$

455 nm
532 nm
633 nm
780 nm

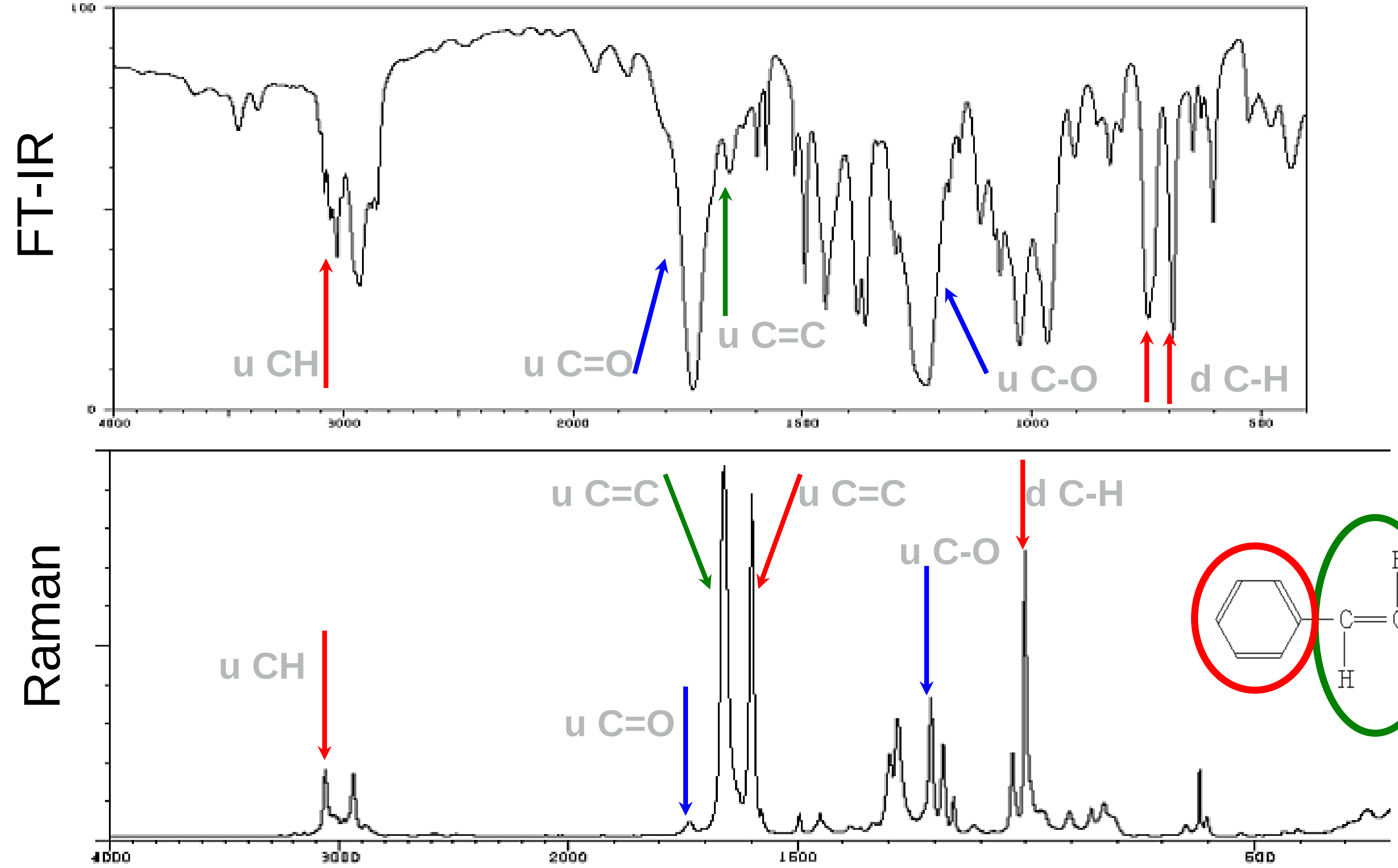
Influence of laser excitation on Raman spectrum

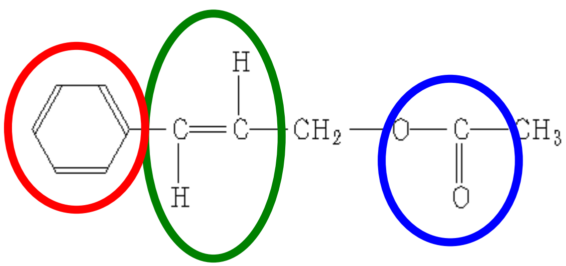
- Different laser source give rise to the same Raman signal at different wavelength but the "Raman Shift" is egual, νo-ΔEvib



Raman & FTIR Spectroscopy Provide Complementary Information

- Raman and FT-IR give similar structural information
  - Example:Trans-cinnamyl acetate





 Proprietary & Confidential

Rules of thumb for Raman scattering

- The frequency of the laser is independent of the frequency of the vibration

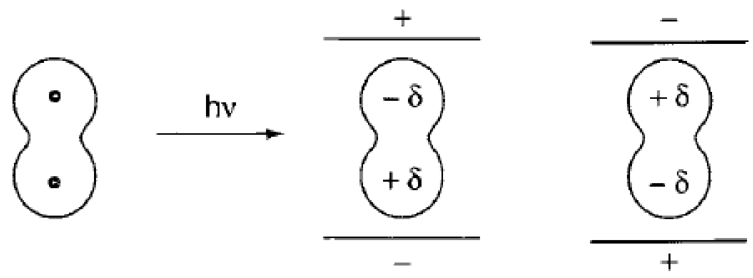
- The polarizability of the bond must change during the vibration

$$\frac{dP}{dq} \neq 0$$

Rules of thumb for Raman scattering

$P = \alpha E.$

분자가 전기장(laser beam)내에 놓이면 핵은 –로 전자는 +극으로 이동하며 전자구름의 변화가 생김



$$P_x = \alpha_{xx}E_x + \alpha_{xy}E_y + \alpha_{xz}E_z,$$
$$P_y = \alpha_{yx}E_x + \alpha_{yy}E_y + \alpha_{yz}E_z,$$
$$P_z = \alpha_{zx}E_x + \alpha_{zy}E_y + \alpha_{zz}E_z.$$

실제 분자내에서는 매질이 anisotropic하여, E가 x,y,z방향으로 구성되어 있음

$$\begin{bmatrix} P_x \\ P_y \\ P_z \end{bmatrix} = \begin{bmatrix} \alpha_{xx} & \alpha_{xy} & \alpha_{xz} \\ \alpha_{yx} & \alpha_{yy} & \alpha_{yz} \\ \alpha_{zx} & \alpha_{zy} & \alpha_{zz} \end{bmatrix} \begin{bmatrix} E_x \\ E_y \\ E_z \end{bmatrix}$$

Polarizability tensor

Tensor 구성 성분중 하나가 진동중에 바뀌면 Raman active

Rules of thumb for Raman scattering

$$E = E_0 \cos 2\pi \nu_0 t,$$

$$P = \alpha E = \alpha E_0 \cos 2\pi \nu_0 t.$$

분자가 진동운동을 하면, 전하의 분포 즉 편극율이 변함
핵과 결합에 참여하는 전자사이의 거리가 커지면 결합은 쉽게 외부전기장에 의하여 변함(편극율은 핵간의 거리 q에 의존)

$$q = q_0 \cos 2\pi \nu_m t,$$

$$\alpha = \alpha_0 + \left(\frac{\partial \alpha}{\partial q}\right)_0 q_0 + \dots$$

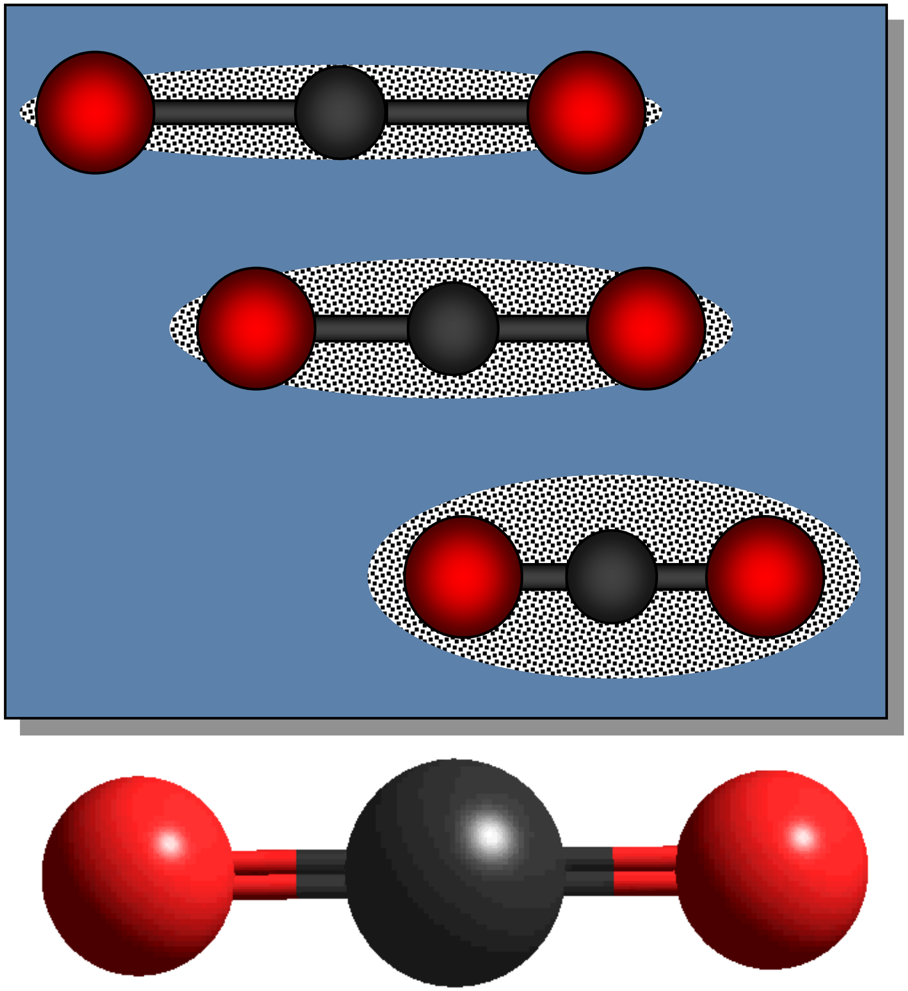
주파수 v_m으로 진동할때 어느 순간의 편극율

$$\begin{aligned} P &= \alpha E_0 \cos 2\pi \nu_0 t \\ &= \alpha_0 E_0 \cos 2\pi \nu_0 t + \left(\frac{\partial \alpha}{\partial q}\right)_0 q E_0 \cos 2\pi \nu_0 t \\ &= \alpha_0 E_0 \cos 2\pi \nu_0 t + \left(\frac{\partial \alpha}{\partial q}\right)_0 q_0 E_0 \cos 2\pi \nu_0 t \cos 2\pi \nu_m t \\ &= \alpha_0 E_0 \cos 2\pi \nu_0 t \\ &\quad + \frac{1}{2}\left(\frac{\partial \alpha}{\partial q}\right)_0 q_0 E_0 [\cos\{2\pi(\nu_0 + \nu_m)t\} + \cos\{2\pi(\nu_0 - \nu_m)t\}] \end{aligned}$$

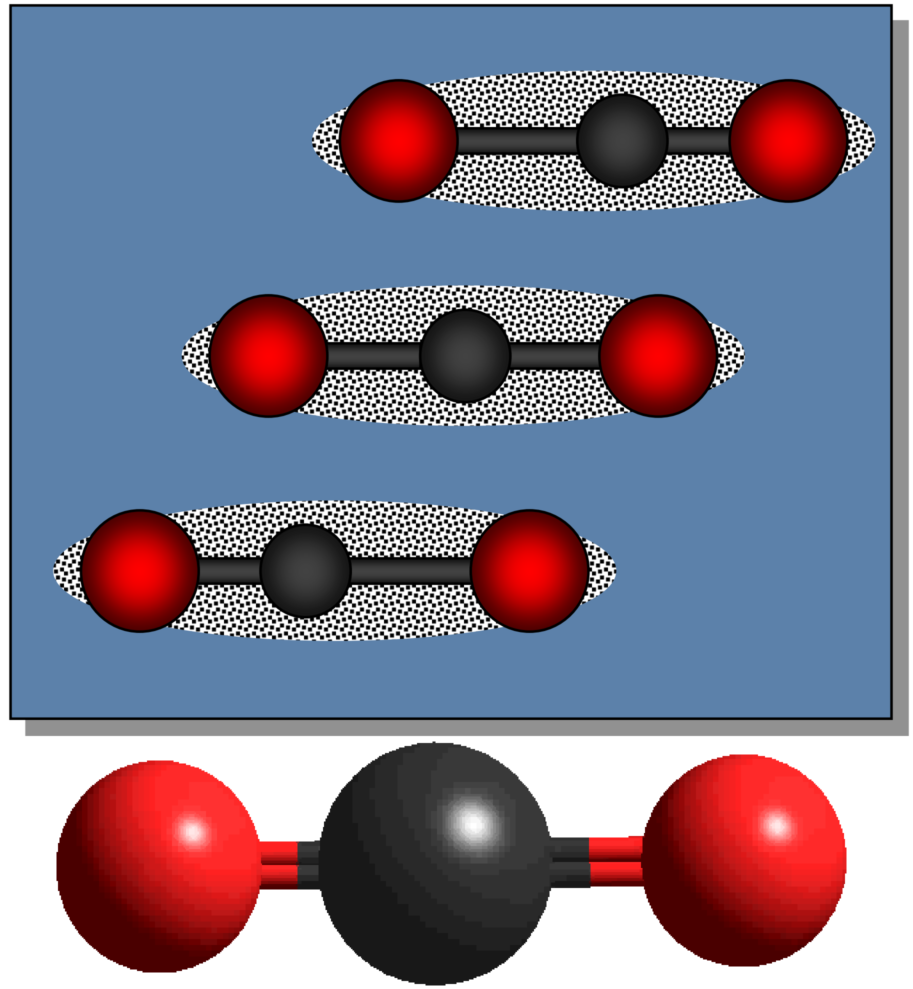
Polarizability of CO_2

Polarizability: the tendency of the electron cloud of an atom to be distorted from its normal shape

Symmetric Stretch: Raman Active

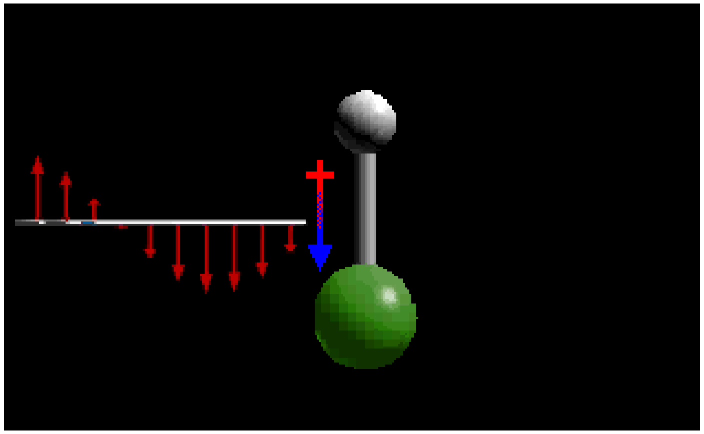


Antisymmetric Stretch: Not Raman Active



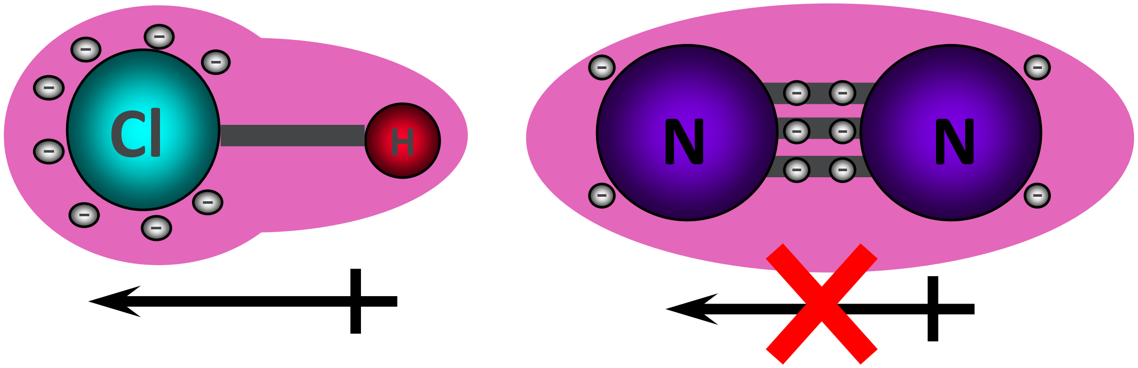
Selection rules for infrared activity

- The frequency of the light must be identical to the frequency of the vibration (resonance)

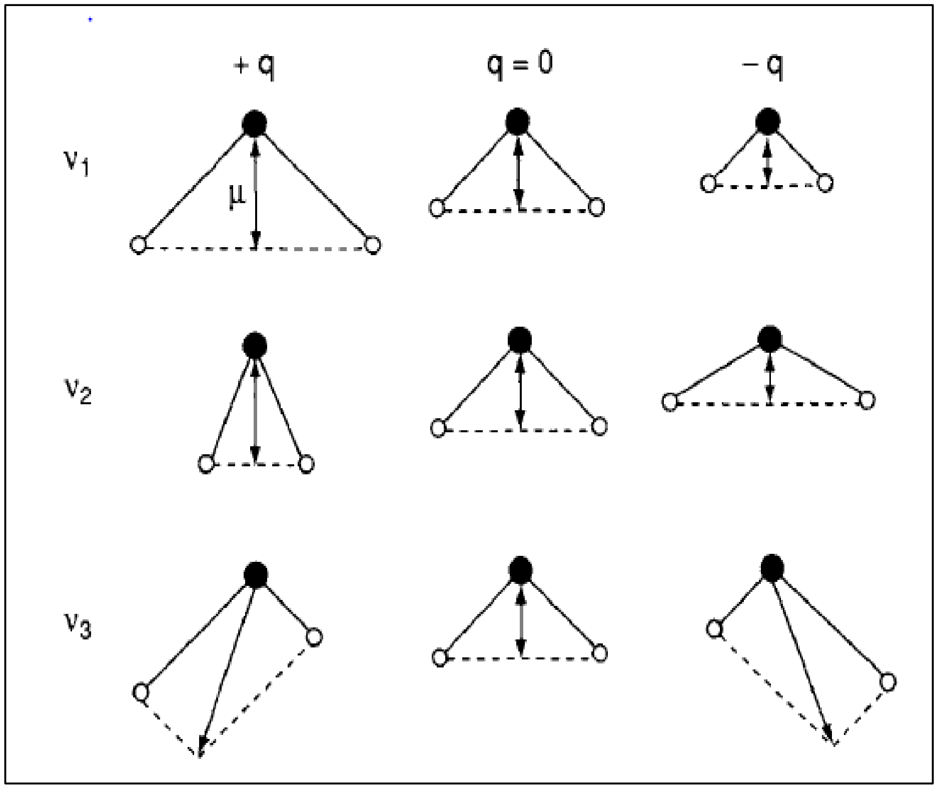


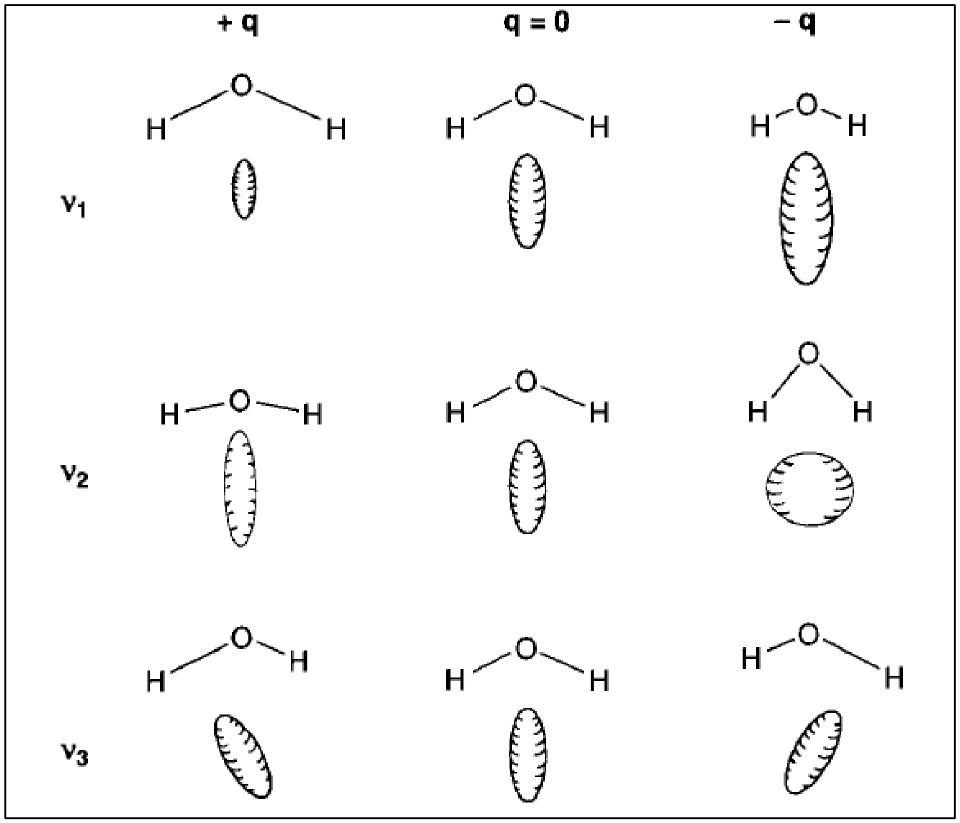
- The dipole moment (μ) of the molecule must change during the vibration

$$\frac{d\mu}{dq} \neq 0$$

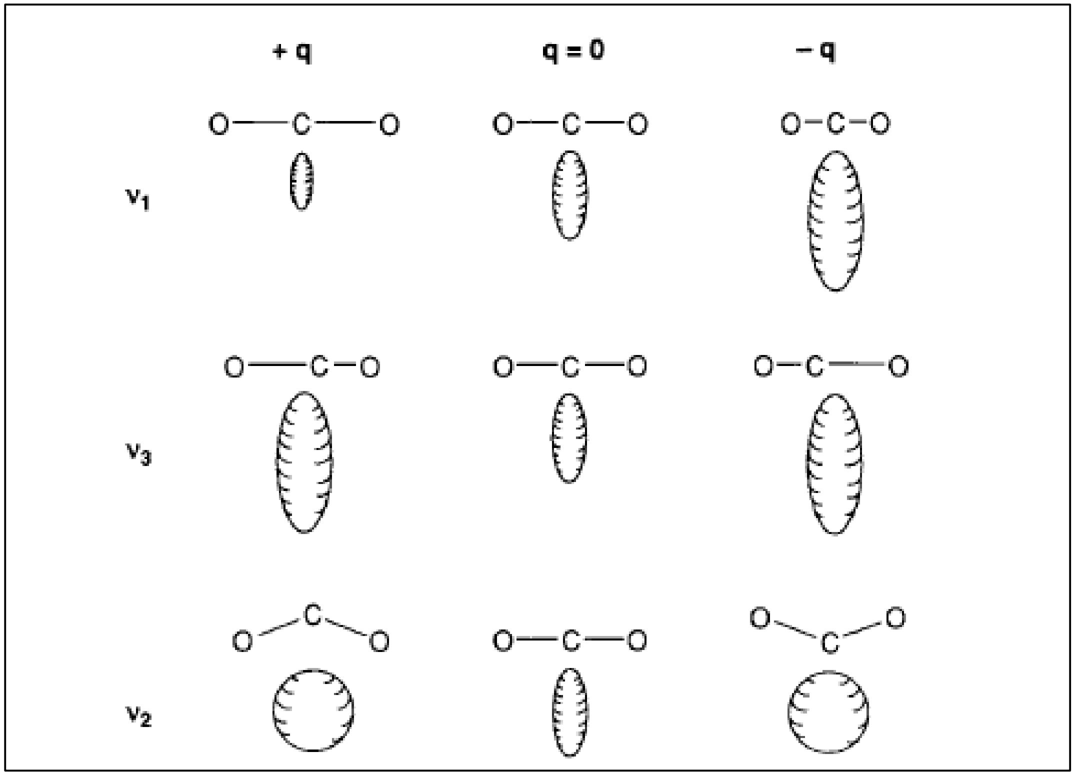


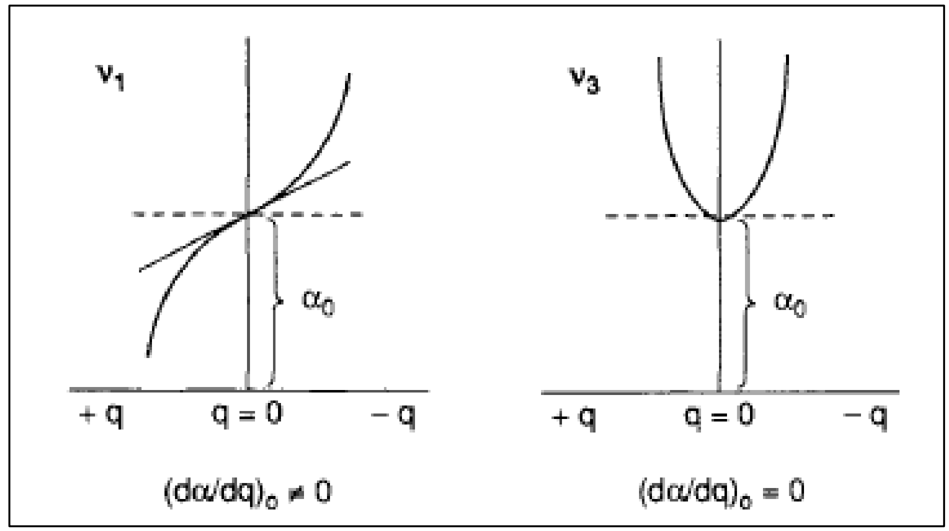
Dipole moment & Polarizability of H_2O





Dipole moment & Polarizability of CO_2





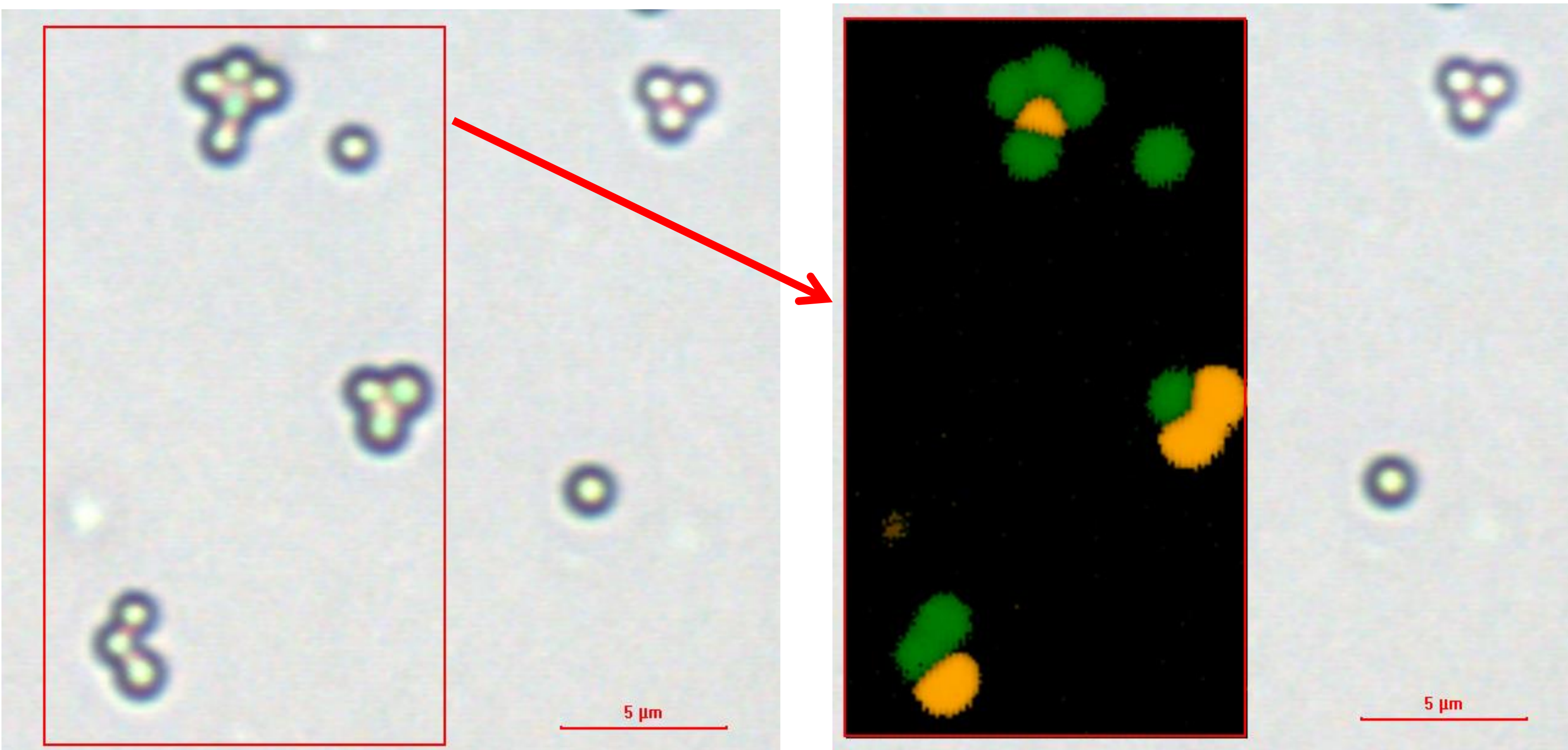
Comparison of Infrared & Raman spectroscopy

	Infrared	Raman
Measurement	Absorption	Emission
Activation of vibration	Dipole moment	Polarizability
Major functional groups	O-H, N-H, C=O	C=C, Aromatics
Sample preparation	Flat, transparent, destructive	No preparation Non-destructive
Water sample	X	O
Quartz/glass	X	O
Particle size	최소 10 um 이상	1 um 이하 가능

Sampling Accessories

- Sampling Accessories
  - Versatility, Interchangeability, Functionality

High Res Discrimination between similar 1µm particles



532 nm laser, 6.0 mW, 100X objective

25000 spectra, 0.1 µm pixel size

Acquisition parameters: 100 Hz (10 ms/spectrum), 20 scans

Polystyrene   Polymethyl methacrylate

Spatial resolution

- Laser spot size

$$D = \frac{1{,}22\lambda}{NA}$$

- Spectrograph Aperture

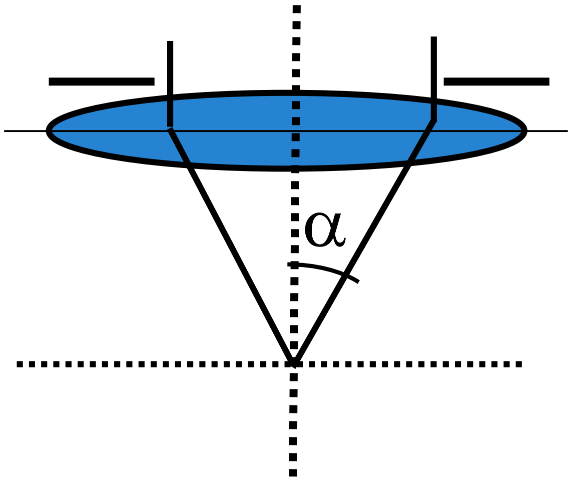
For smaller aperture, spatial resolution will be better

Spatial resolution of X,Y axes is ~ 0.5 ㎛

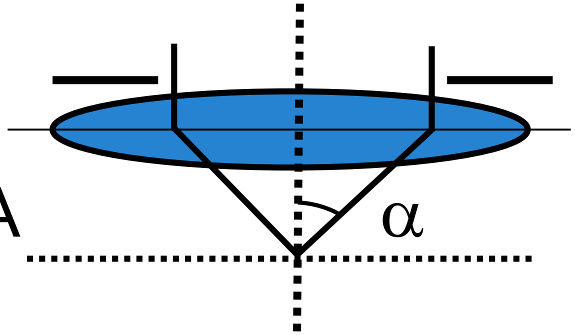
Numerical Aperture (NA)

- Collection Optics → Objectives

Magnification	NA
4X	0.1
10X	0.25
20X	0.40
50X	0.75
100X	0.90
NA = Numerical Aperture	



N.A. = nsinα



Laser spot size

$$D_{x,y} = \frac{1.22 \times \lambda}{N.A.}$$

Magnification	NA
4X	0.1
10X	0.25
20X	0.40
50X	0.75
100X	0.90
NA = Numerical Aperture	

100x objective with N.A. of 0.9

$$D_{x,y}(633\ nm) = \frac{1.22 \times 0.633\ \mu m}{0.90} = 0.86\ \mu m$$

$$D_{x,y}(532\ nm) = \frac{1.22 \times 0.532\ \mu m}{0.90} = 0.72\ \mu m$$

Spatial resolution

dependent on laser and objective

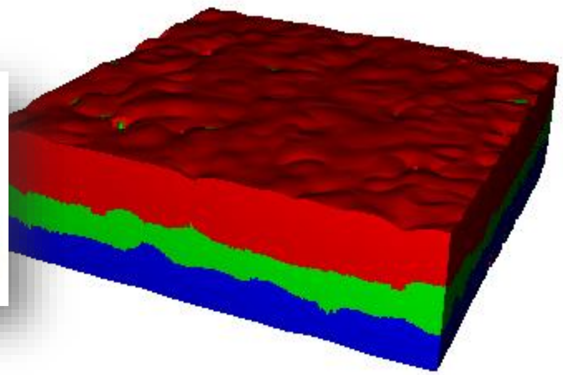
Objective	785 nm laser	633 nm laser	532 nm laser	455 nm laser
10x	3.1 µm	2.5 µm	2.1 µm	1.8 µm
20x	1.9 µm	1.6 µm	1.3 µm	1.1 µm
50x	1.0 µm	0.8 µm	0.7 µm	0.6 µm
100x	0.9 µm	0.7 µm	0.6 µm	0.5 µm

Applications

Representative Applications of Raman Spectroscopy

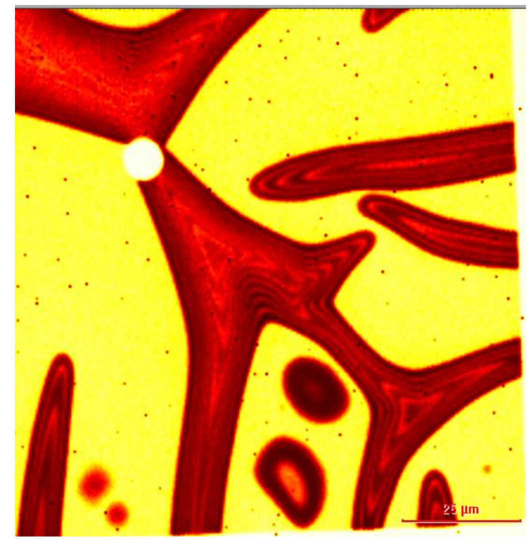
Polymers and Packaging

Subsurface analysis to **identify inclusions** and **verify layers** without sample preparation



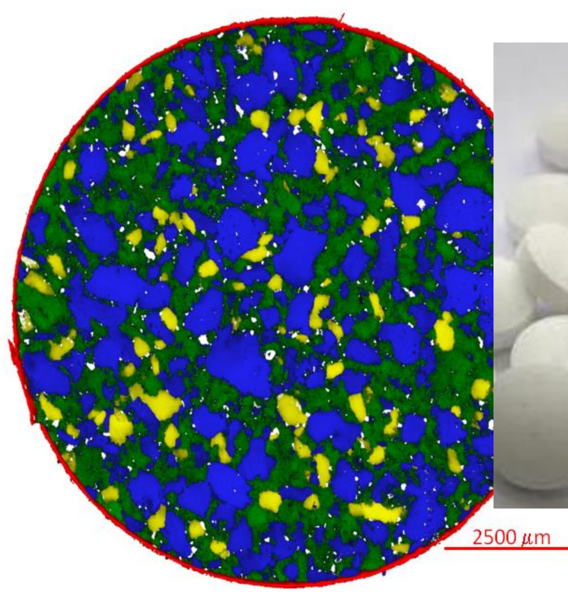
Semiconductors and Thin Films

Variation in **stress distributions** and **crystallinity** across an entire wafer; **Identify contaminants and defects**



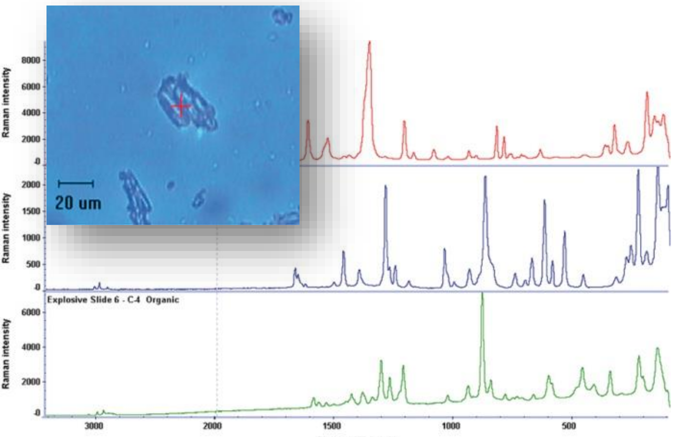
Pharmaceuticals

Full tablet imaging for **content uniformity** and **formulation analysis**



Today Raman is Being Used in Many Different Areas

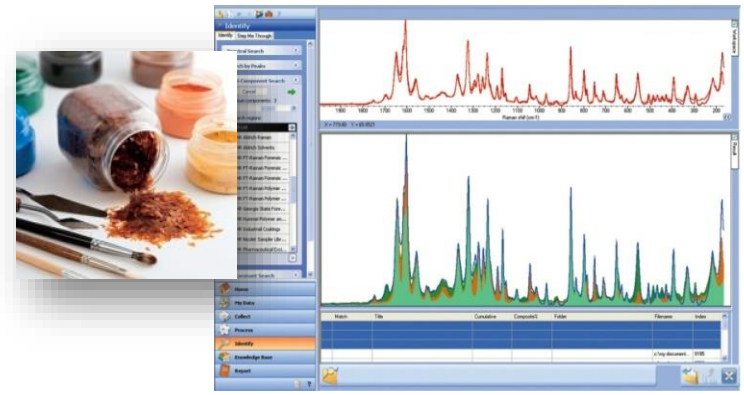
Forensic Science



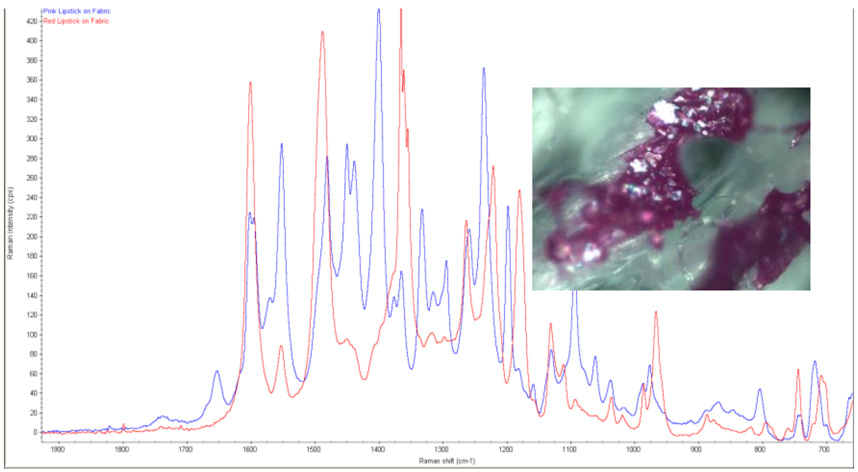
Identification of components in explosives residue

Art Conservation and Archeology

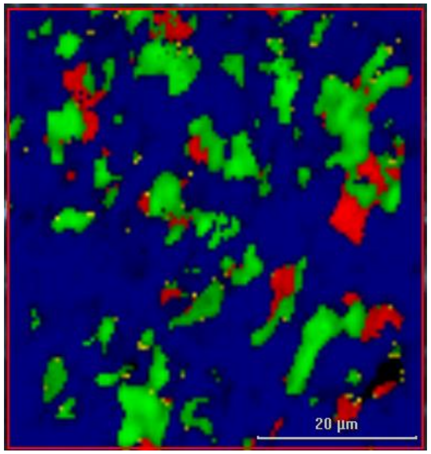
Identification and discrimination of paint pigments using DXR microscopy and fiber optic analysis



Analysis of lipstick



Energy Storage - Lithium Ion Batteries



Analysis of cathode components

- Carbon Black
- Li Mn Ni Oxide #1
- Li Mn Ni Oxide #2

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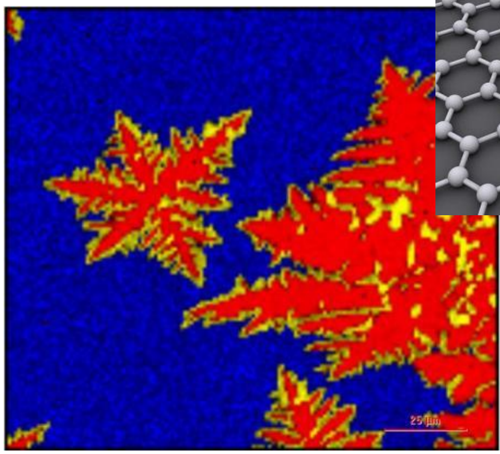
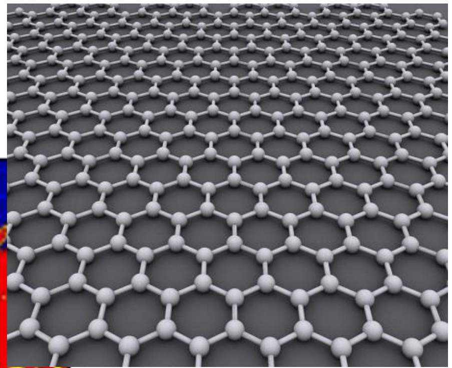
Raman Solves Many Problems





Carbon Nanomaterials

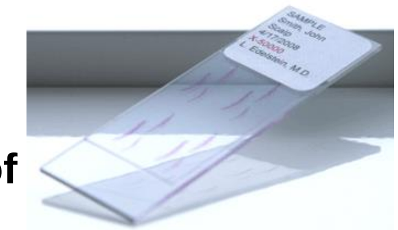
Analysis of graphene **growth mechanisms** and **distribution of defects**

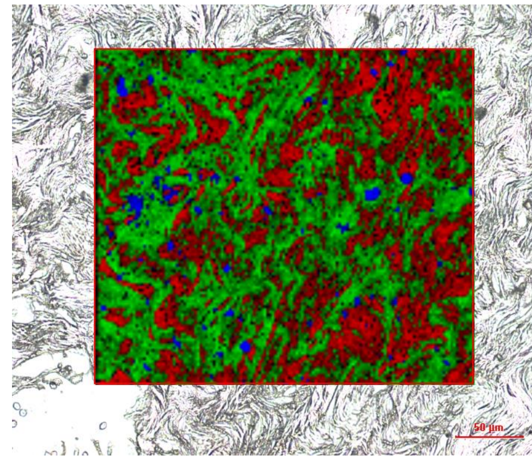
Life Science

Spectroscopic evaluation of cancerous tissue and bacterial biofilms to **expand understanding**

Cell Nuclei   Collagen

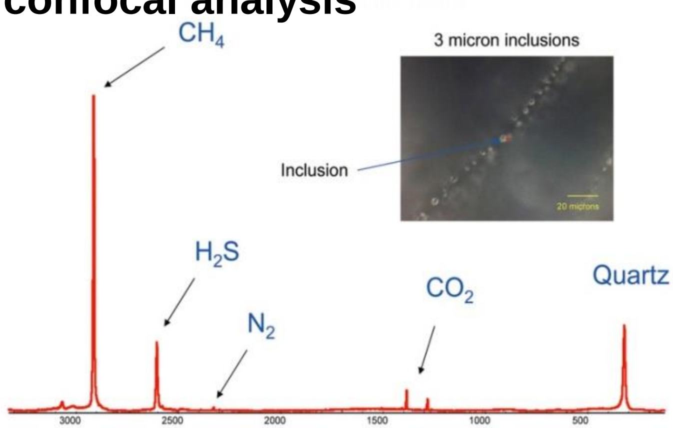
Glass Slide



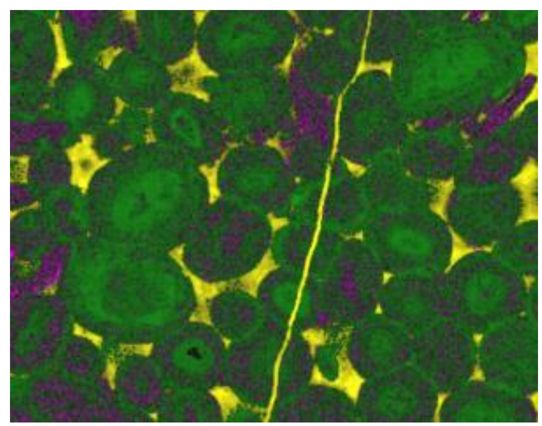


Geology / Mineralogy

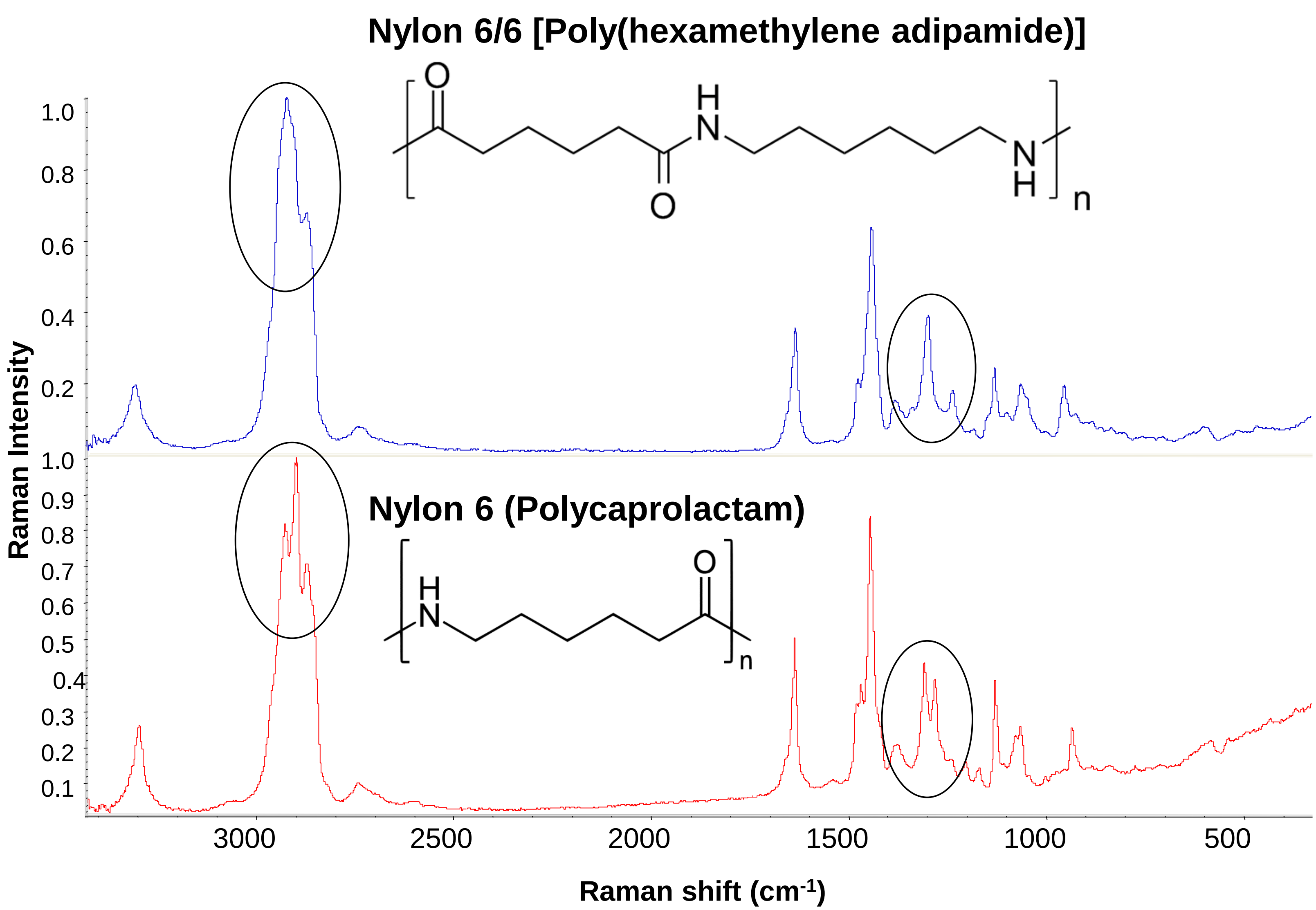
Identification of **inclusions** in minerals using DXR **confocal analysis**



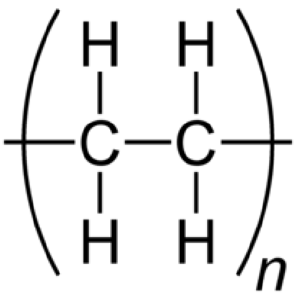
Rapid non-destructive **identification of minerals**

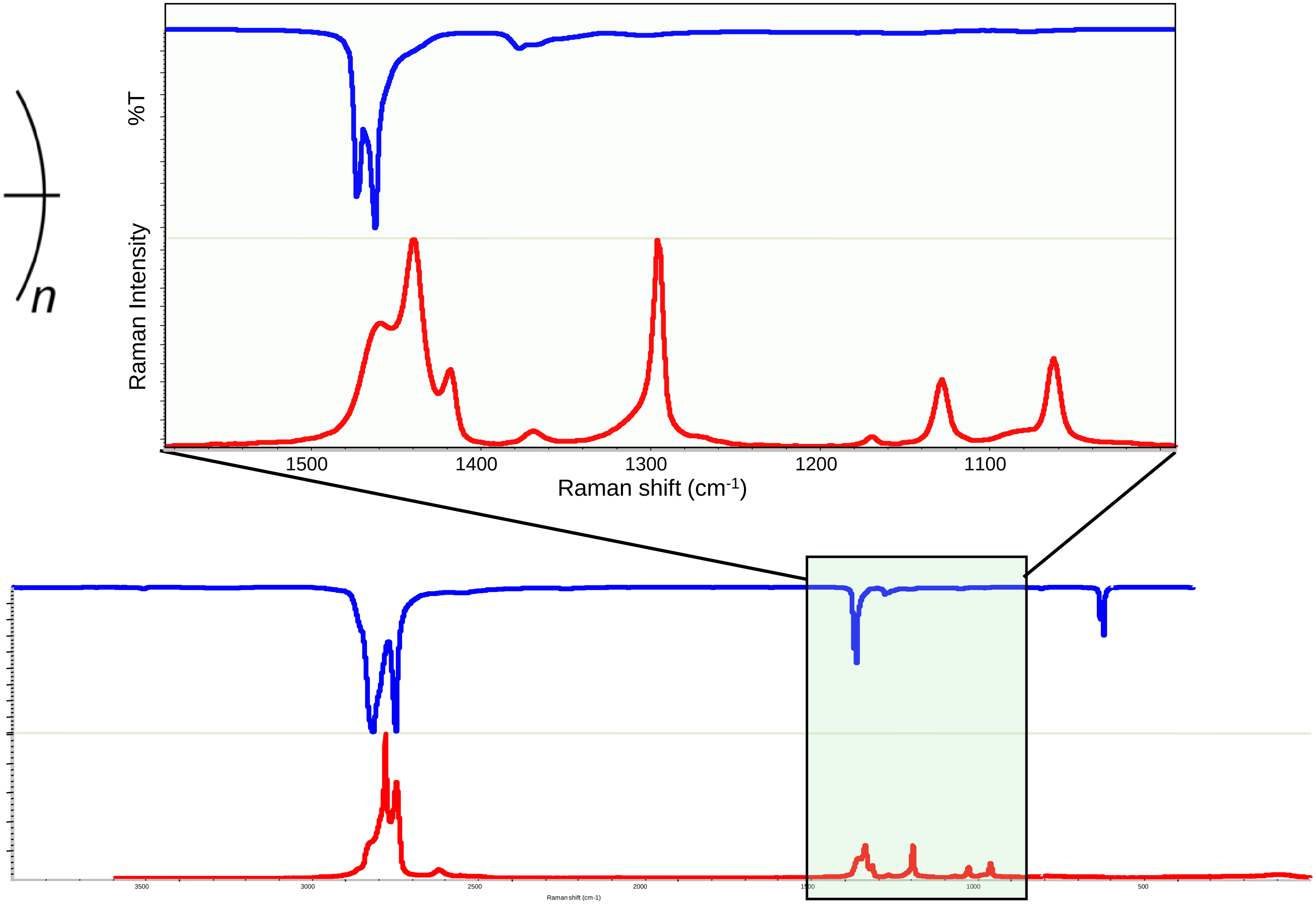


Identify – Advantage - Raman Can Be More Specific



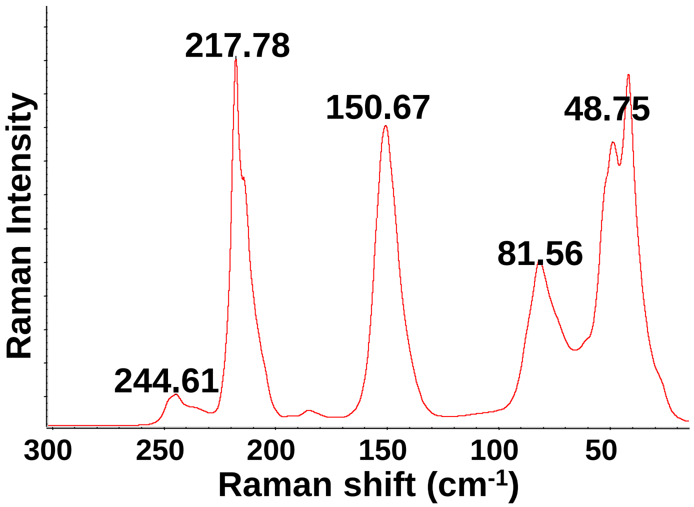
- Polyethylene Spectra



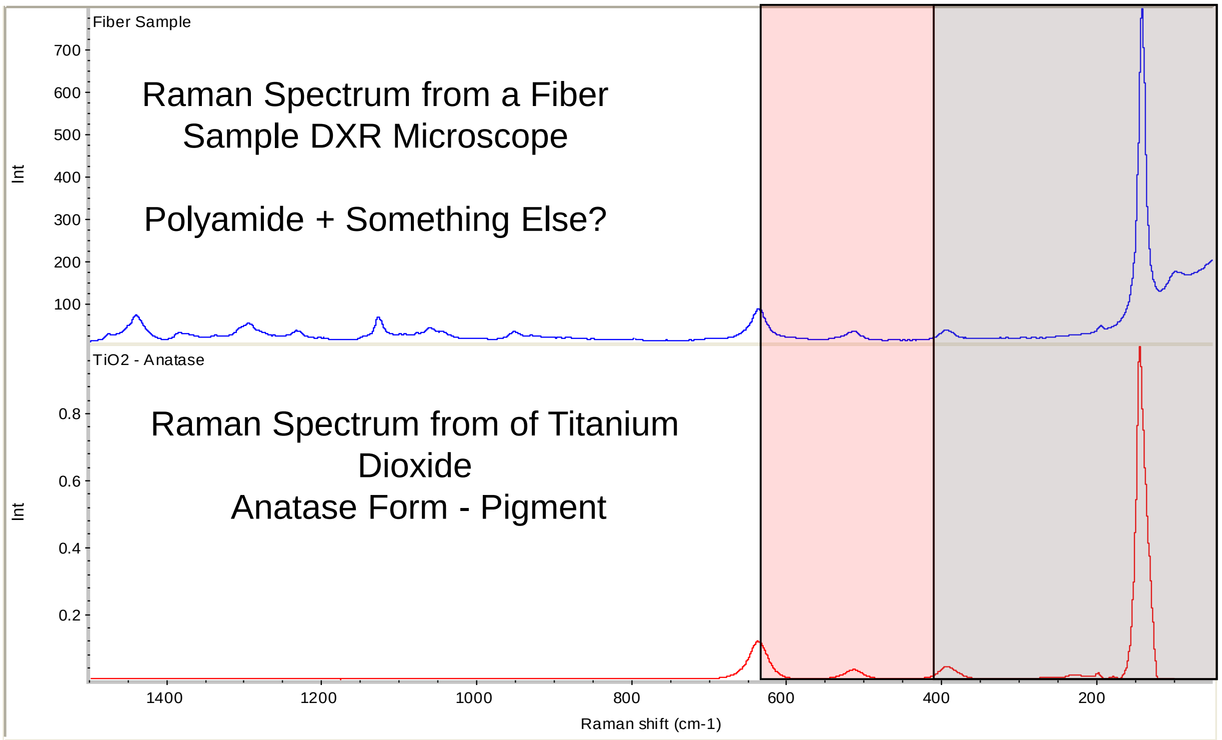


Identify – Advantage – Access to More Information

- Raman provides easy access to far-IR vibrations
  - Typical spectrum extends to 50 cm^{-1}
  - Great for analysis of many inorganics
  - Atmospheric water vapor not a concern



Sulfur

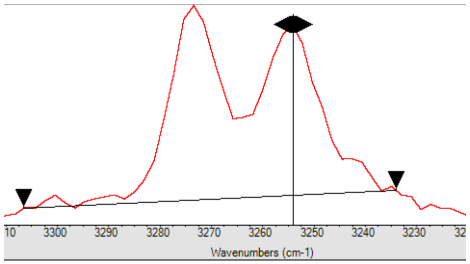


Pigments in Polymers

Differentiating – Distinguish Between Similar Materials

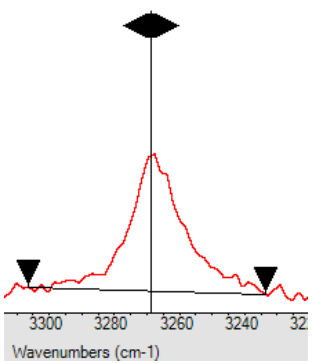
Pharmaceutical Polymorphs

3273, 3254 cm^{-1}



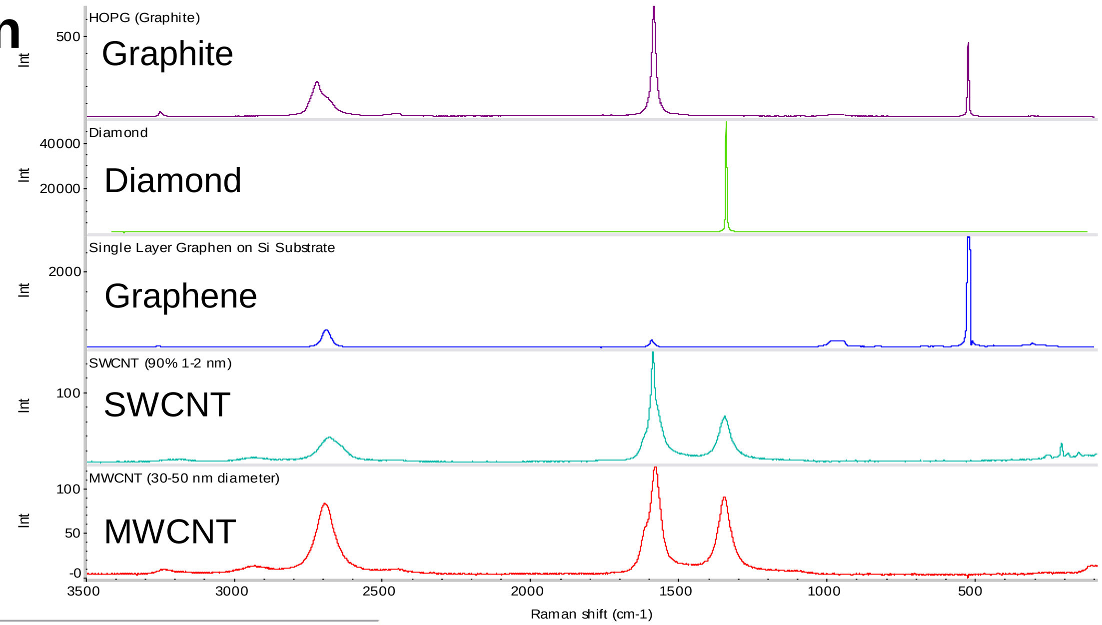
(monoclinic form)

3267cm^{-1}

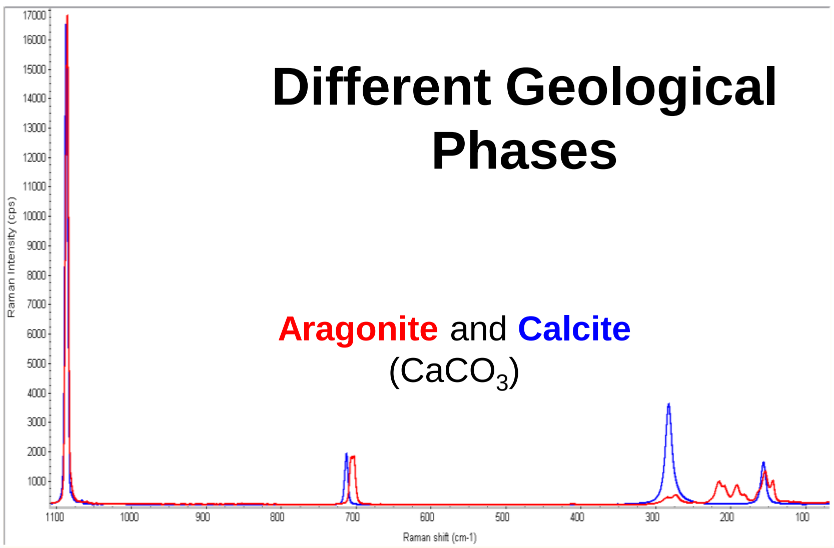


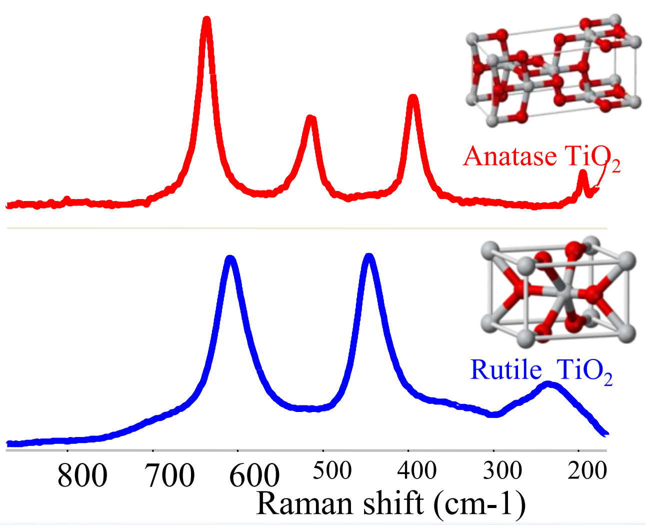
(triclinic form)

Different forms of Carbon



Different Geological Phases





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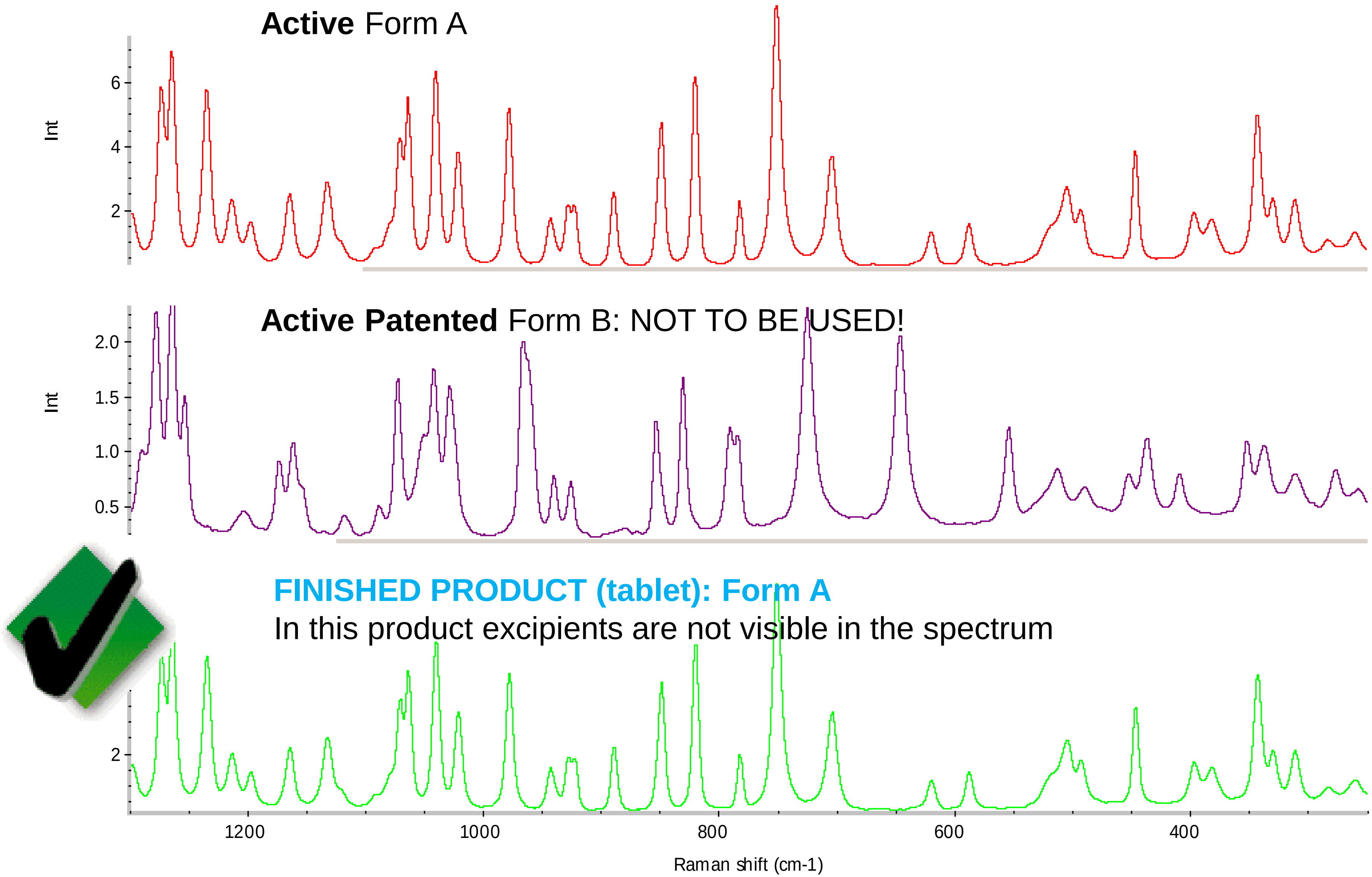
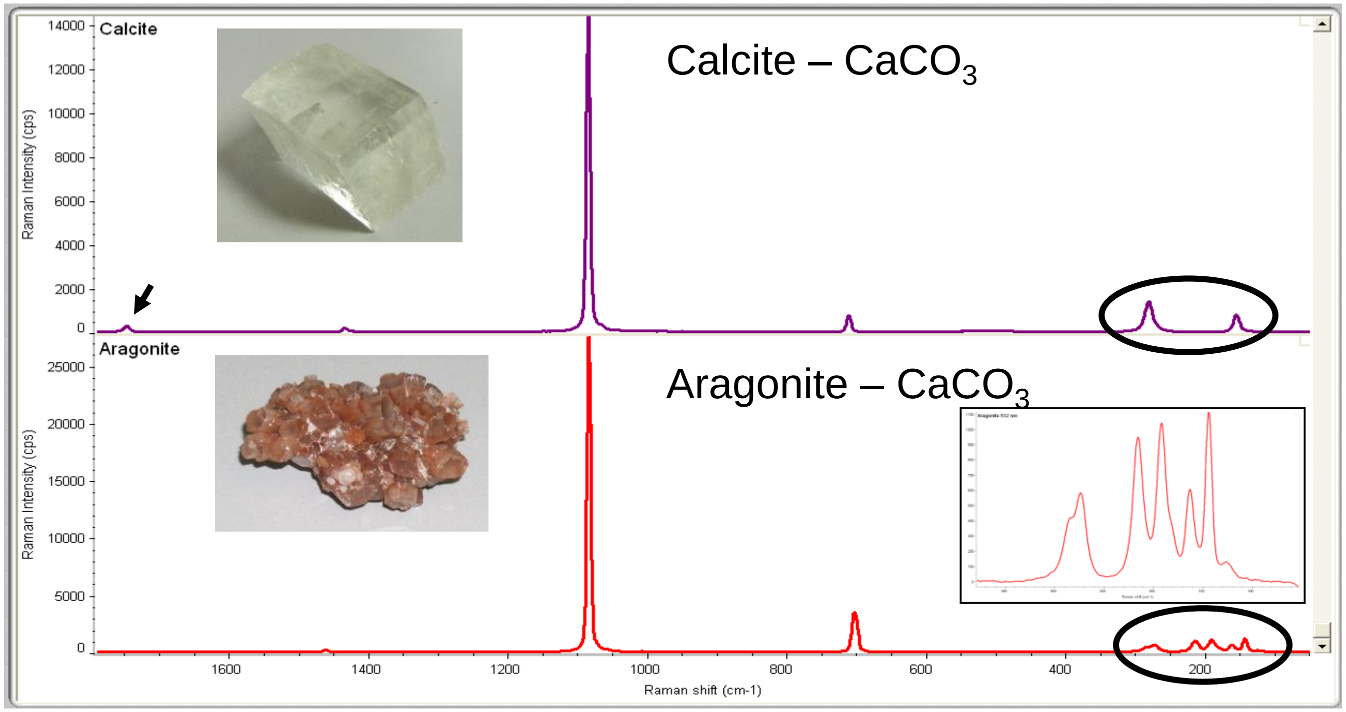
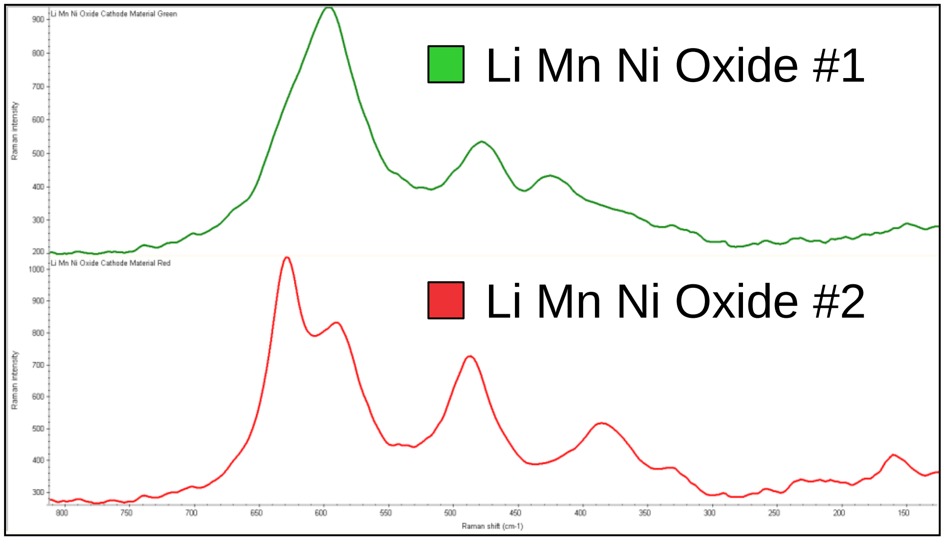
Differentiating Polymorphic Forms with Raman Spectroscopy



Polymorphs are not only Encountered in Pharmaceuticals





Li Mn_x Ni_y O_z Spinels used as cathode materials in Li ion batteries

 Proprietary & Confidential

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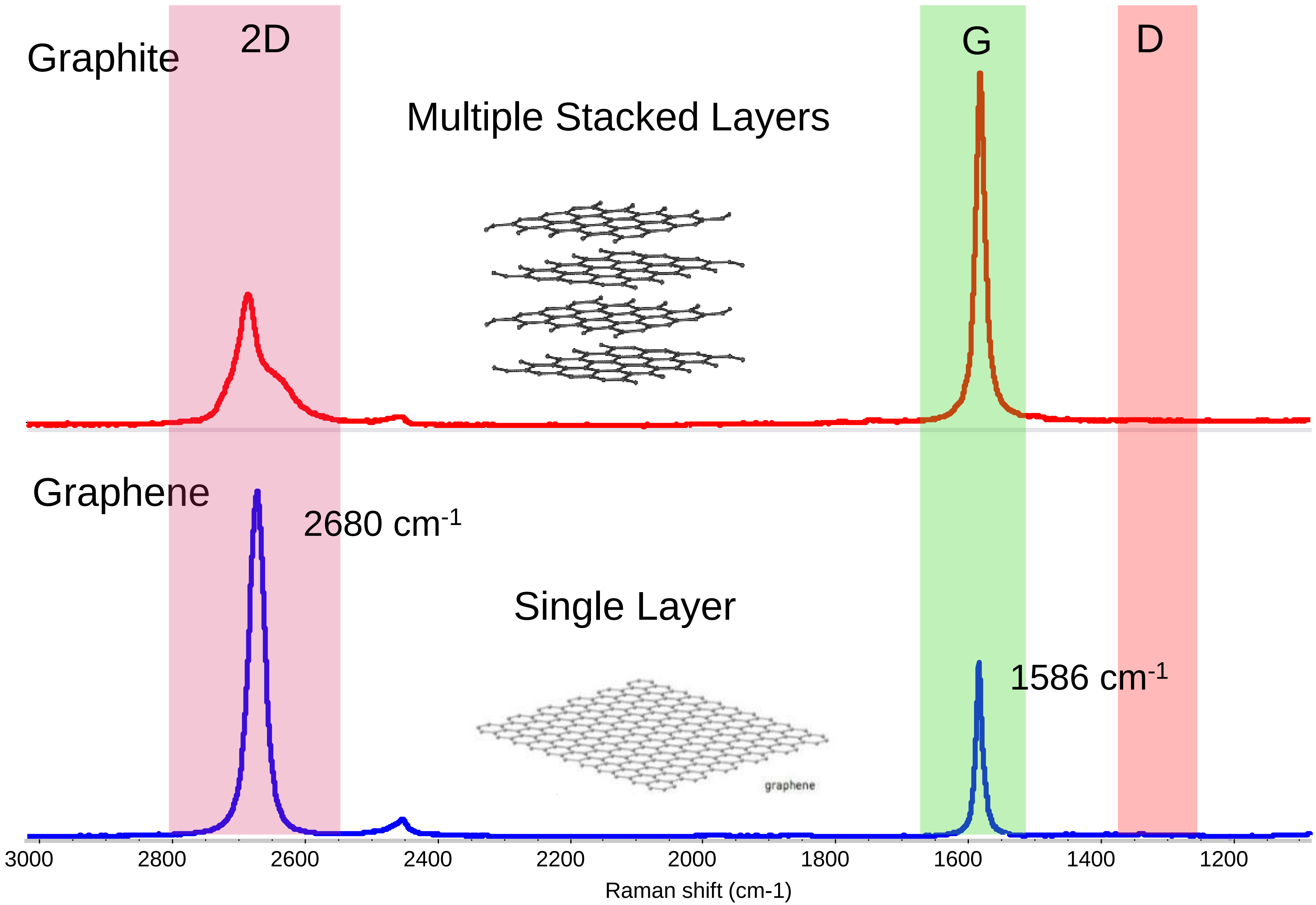
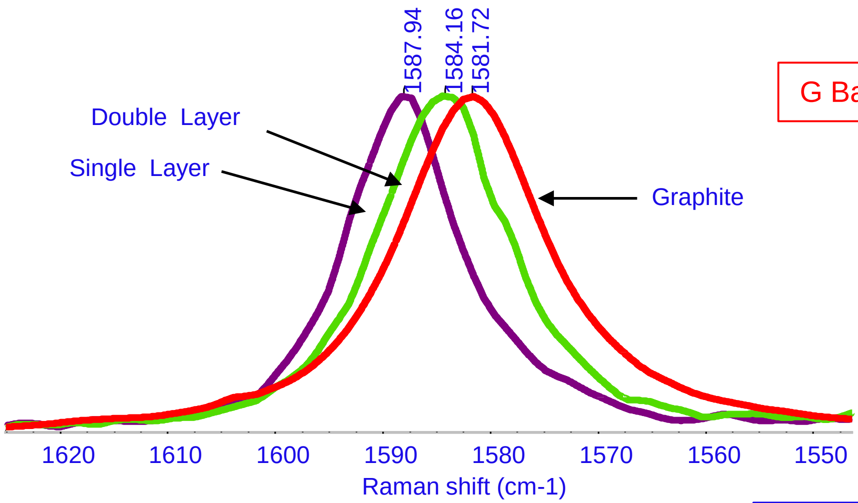
Raman Can Provide Additional Structural Information



Raman Spectroscopy can be Used to Evaluate the Number of Layers

$\omega_G = 1581.6 + 11/(1 + n^{1.6})$

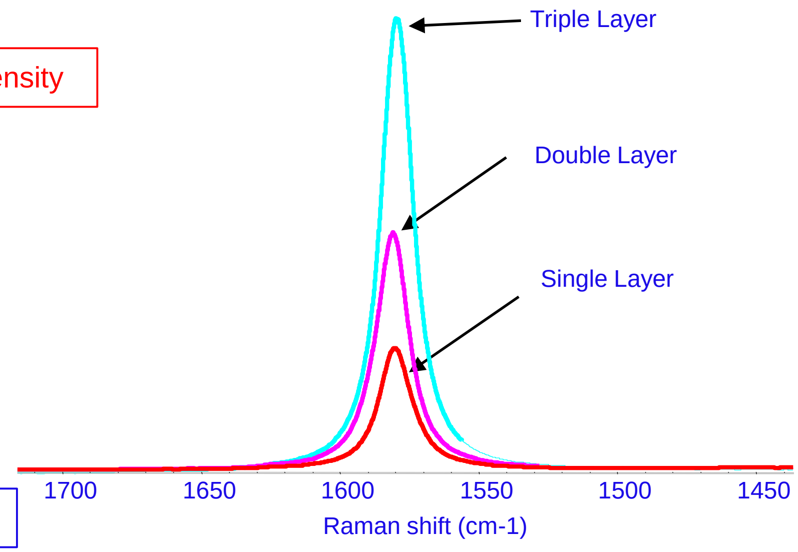


Wang,Hui; Cao,Xuewei; Feng,Min; and Lan,Guoxian

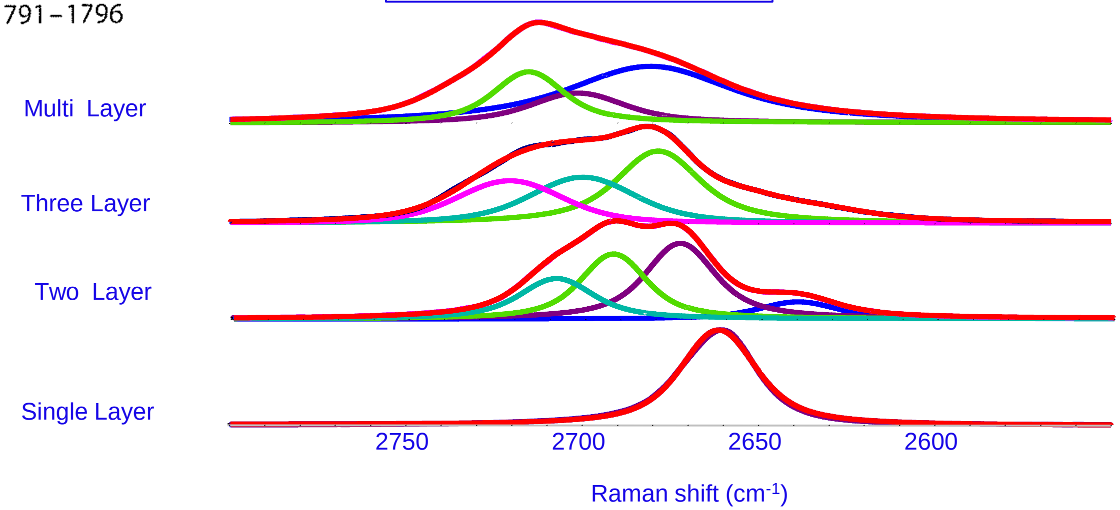
J. Raman Spectrosc. **2009**, *40*, 1791–1796

G Band Position and Intensity

Linear increase in G band intensity with # of layers present



2D Band Shape Analysis



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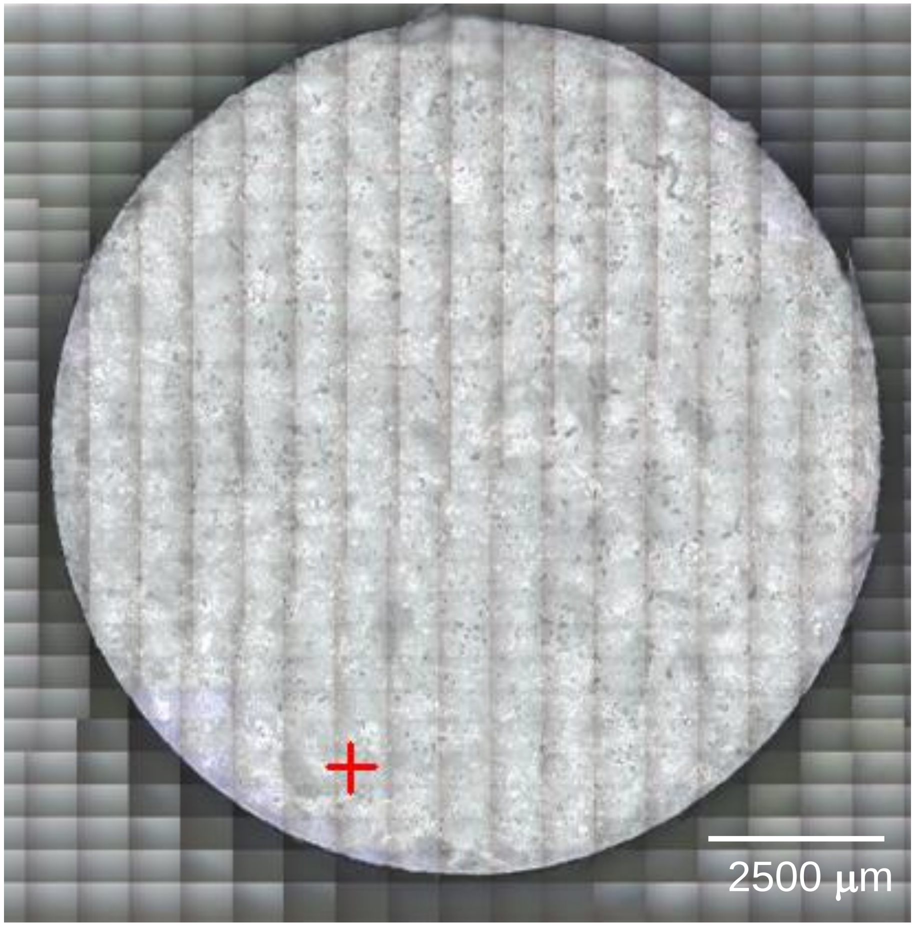
Tablet Imaging Example



Video Mosaic Image
(10X objective, 100X total magnification)

<u>Migraine Relief Tablet</u>
11 mm diameter, 676 mg

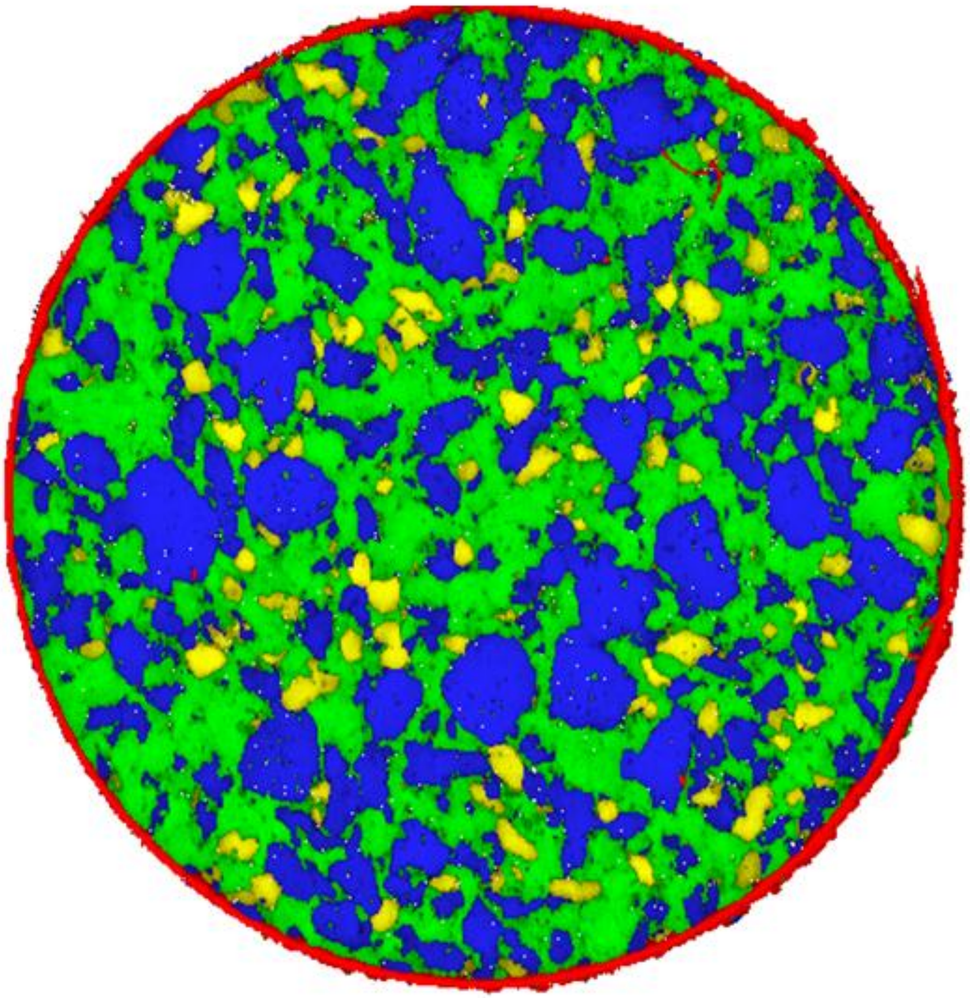
APIs

Acetaminophen	250 mg (37%)
Aspirin	250 mg (37%)
Caffeine	65 mg (9.6 %)

Inactive
corn starch, microcrystalline cellulose, sodium lauryl sulfate, sodium starch, glycolate, crospovidone, polyethylene glycol, polyvinyl alcohol, povidone, stearic acid, talc, titanium dioxide

Imaging the Whole Tablet

Raman MCR Image



Aspirin Acetaminophen Caffeine Titanium Dioxide

Area Imaged - 11 x 11 mm^2
10X objective
Image Pixel Size - 25 μm
226,000 spectra
Exposure Time 1.8 ms (550 spectra per
532 nm laser,

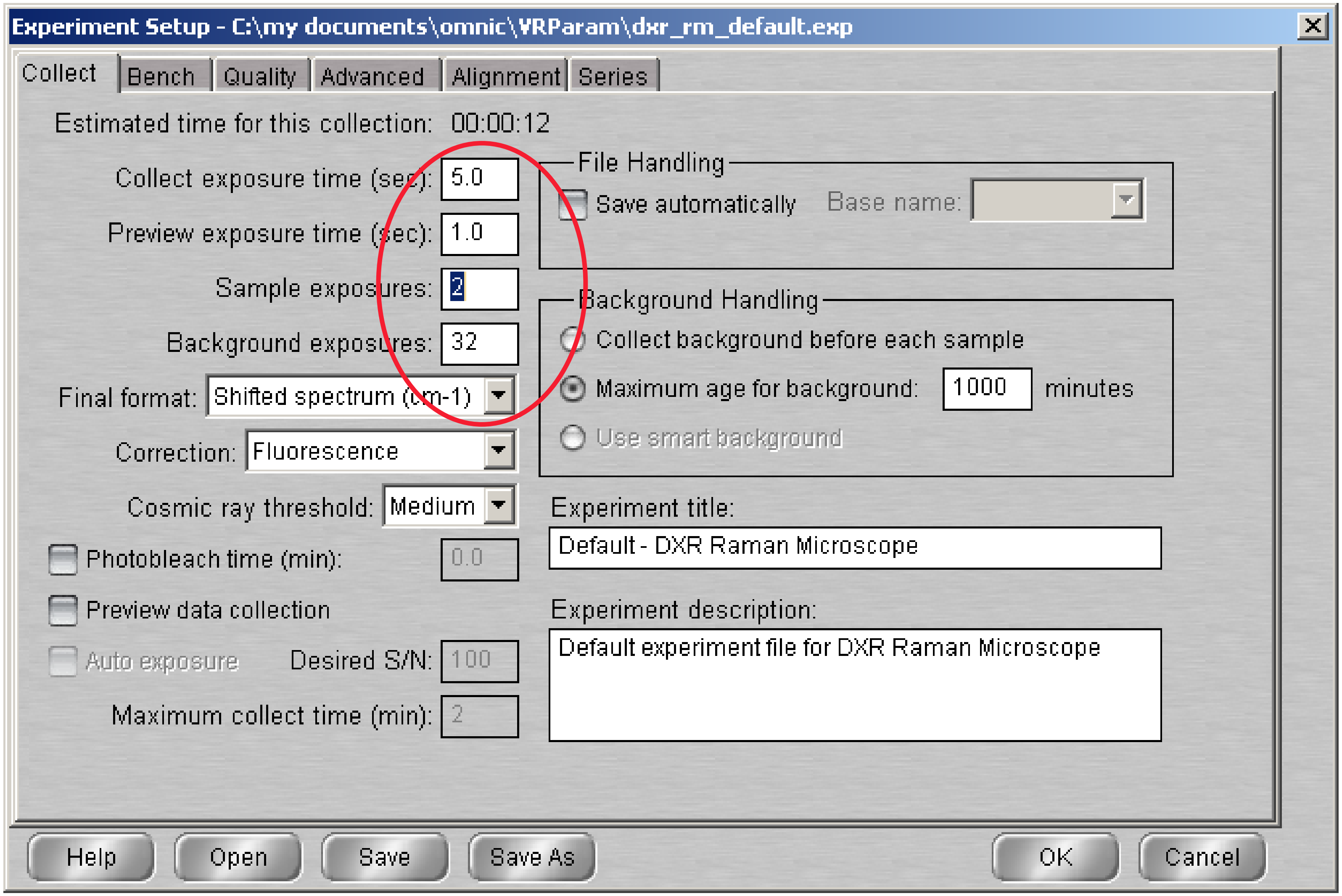
8 minute collect time!!

Experiment Setup

Objectives

- *Collect Tab*
  - *Setting Number of Scans and Resolution*
  - *Auto Exposure*
  - *Background Handling Options*
  - *Experiment Descriptions and Titles*
  - *File Handling Options*
  - *Final Format*
  - *Fluorescence Correction*
- *Bench Tab*
- *Advanced Tab*

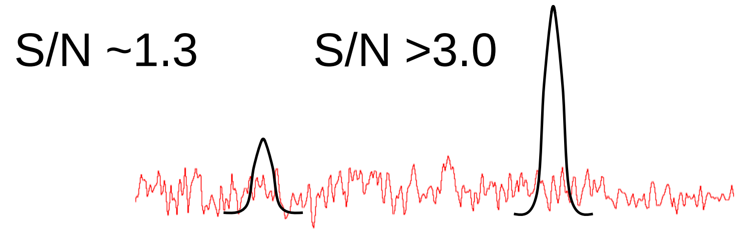
Experiment setup—collect tab



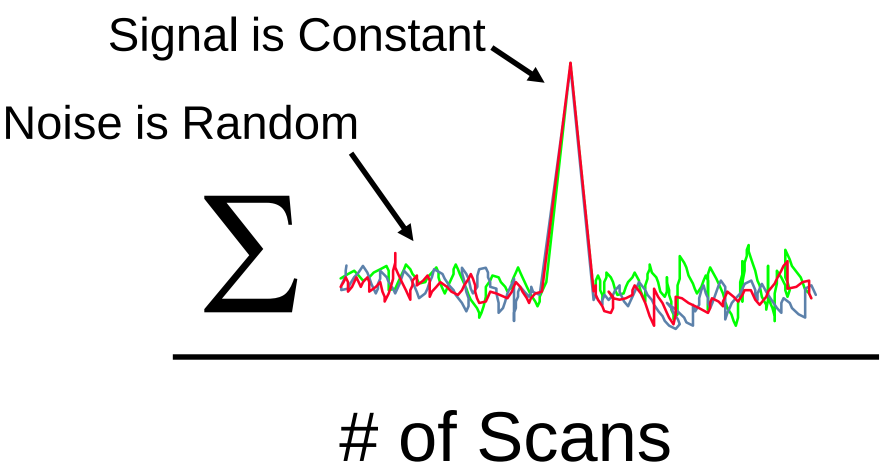
Setting number of exposures

S/N = Signal to Noise Ratio

Instrumental Noise: extraneous and random fluctuation in measured intensity.



Signal Averaging

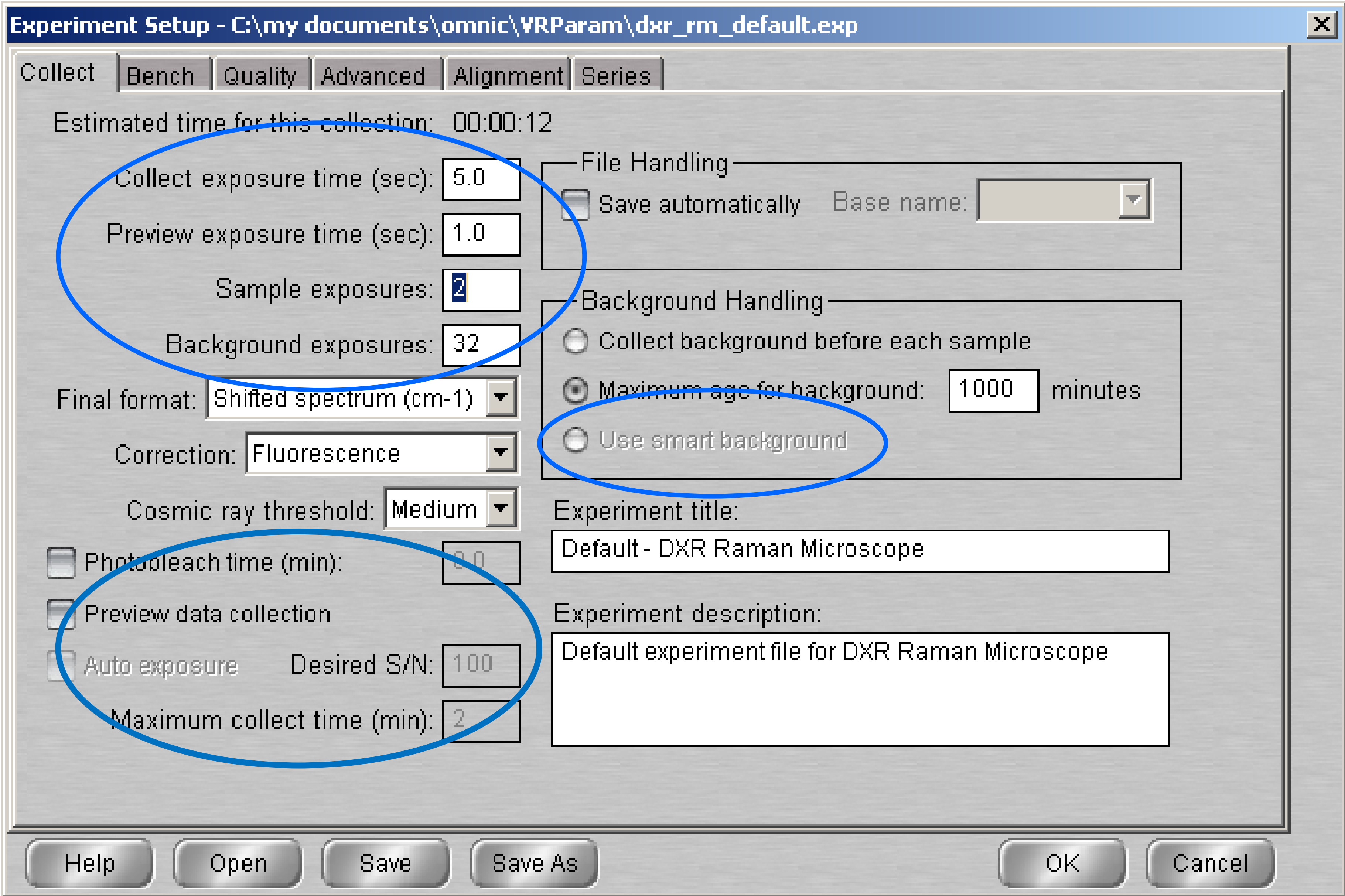


$$\uparrow \frac{S}{N} = \frac{nS_n}{\sqrt{n}N_n} = \sqrt{\uparrow n}\,\frac{S_n}{N_n}$$

S: Signal, N: Noise, n: number of scans

D. A. Skoog and J. J. Leary, *Principles of Instrumental Analysis 4th ed.* Fort Worth: Saunders College Publishing, 1992.

Auto exposure



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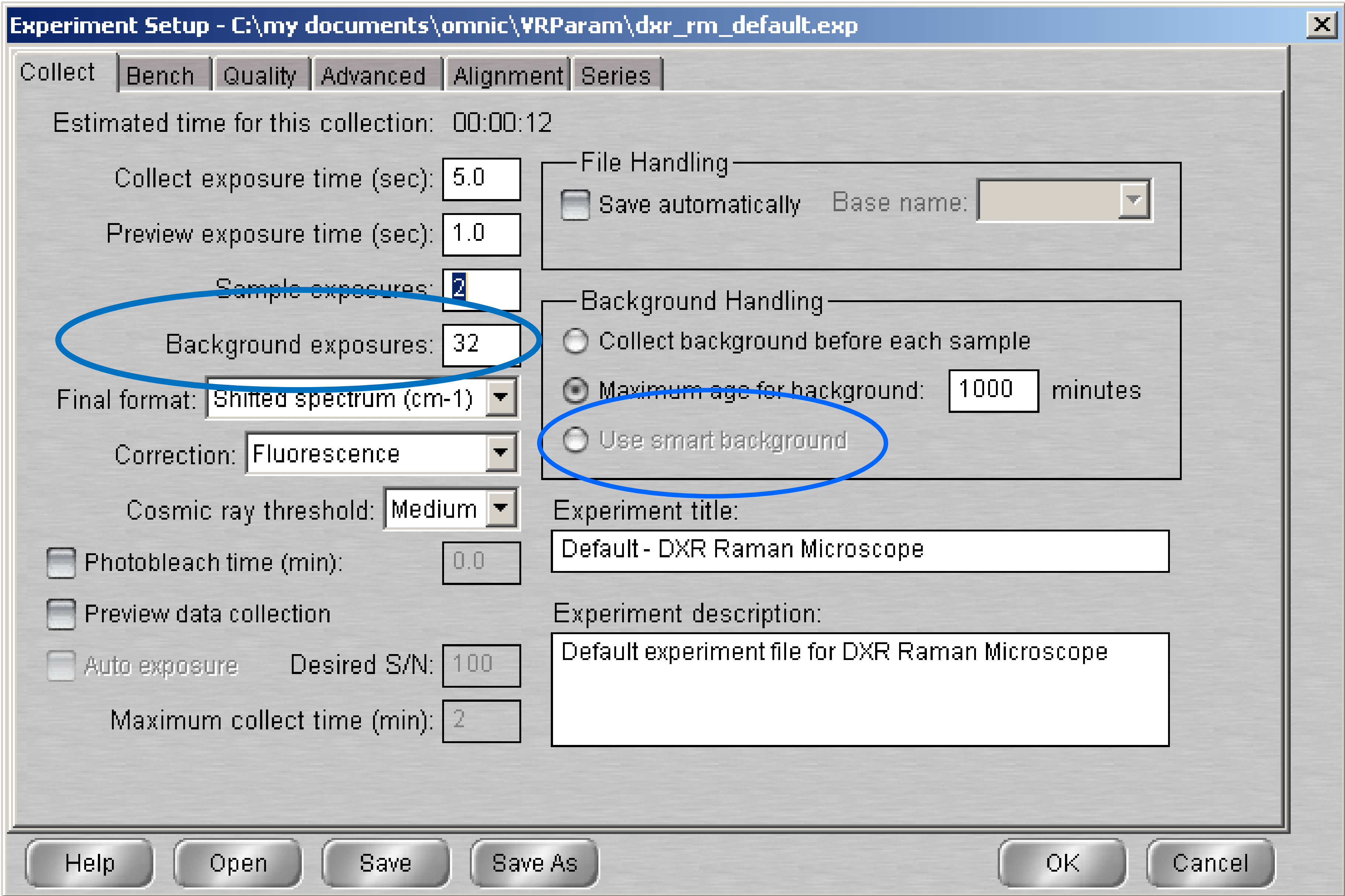
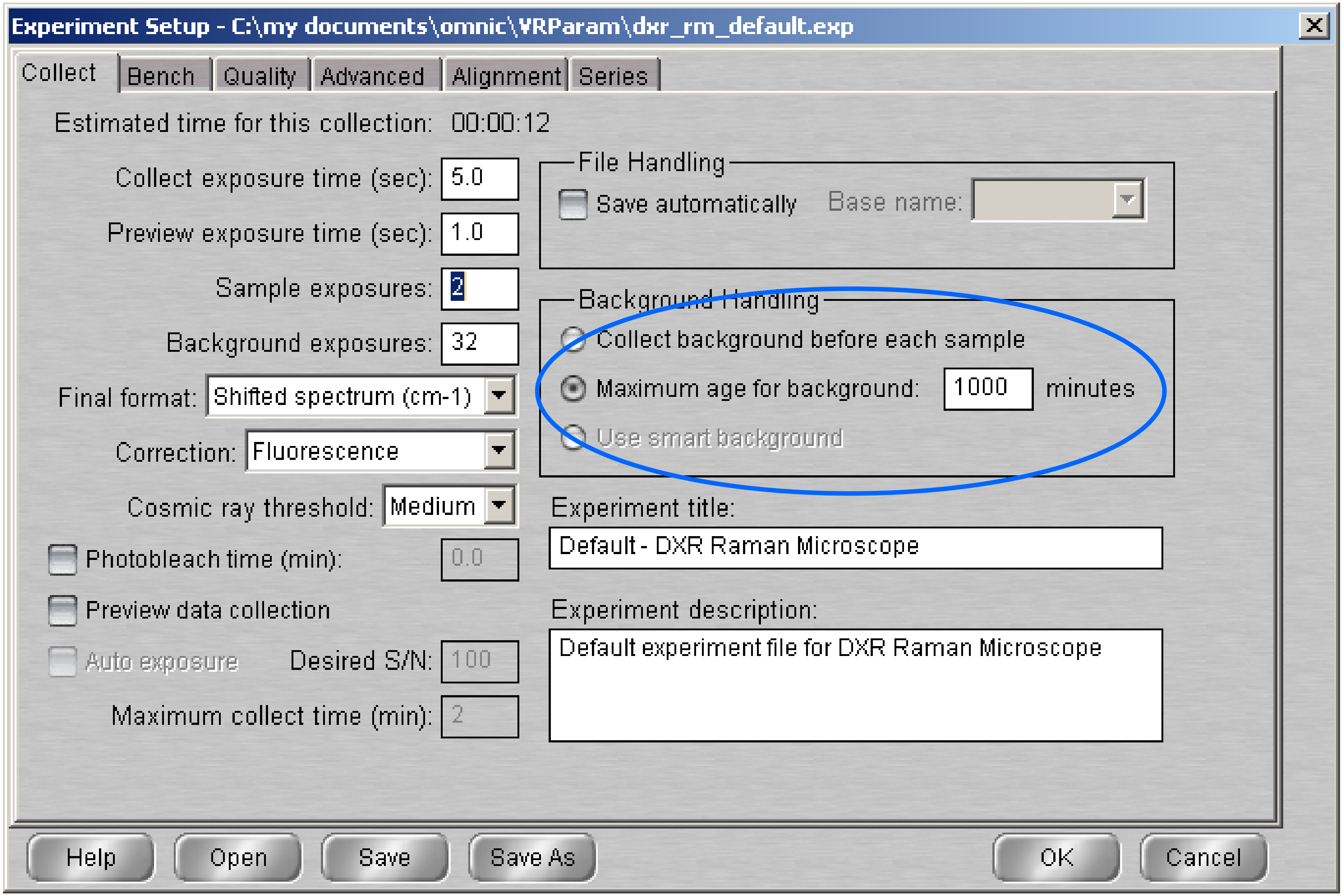
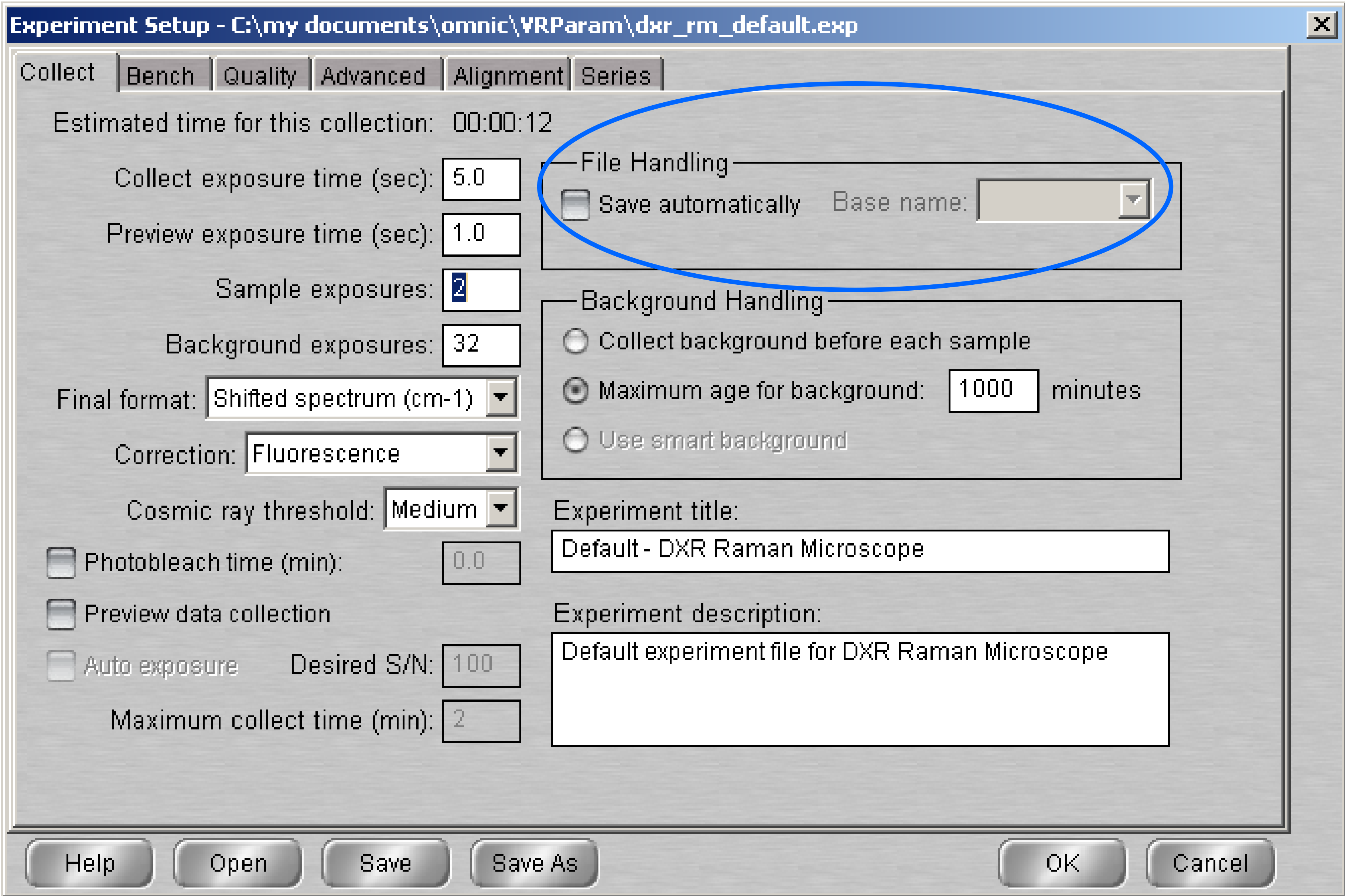
Smart background



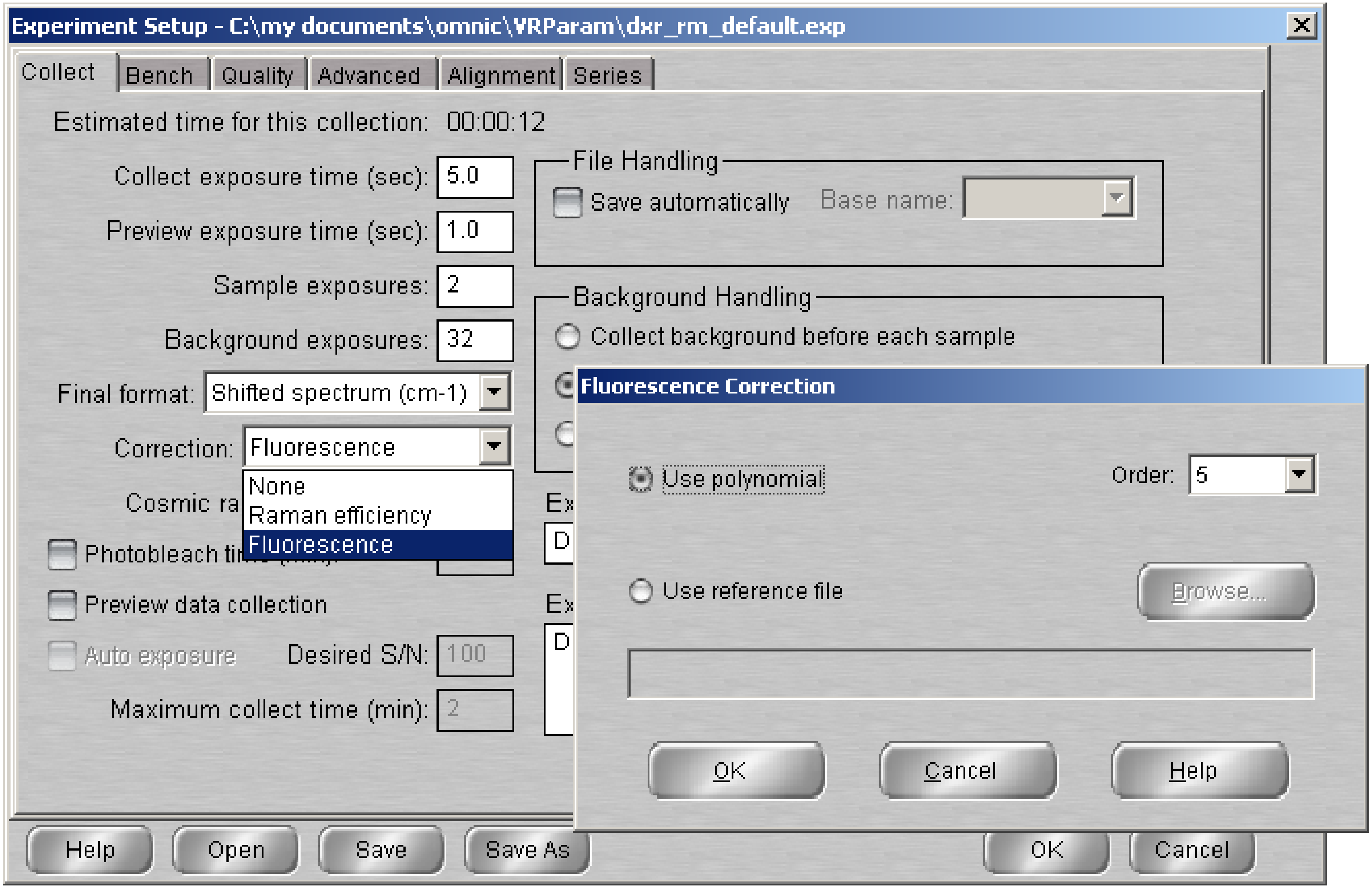
Other background options



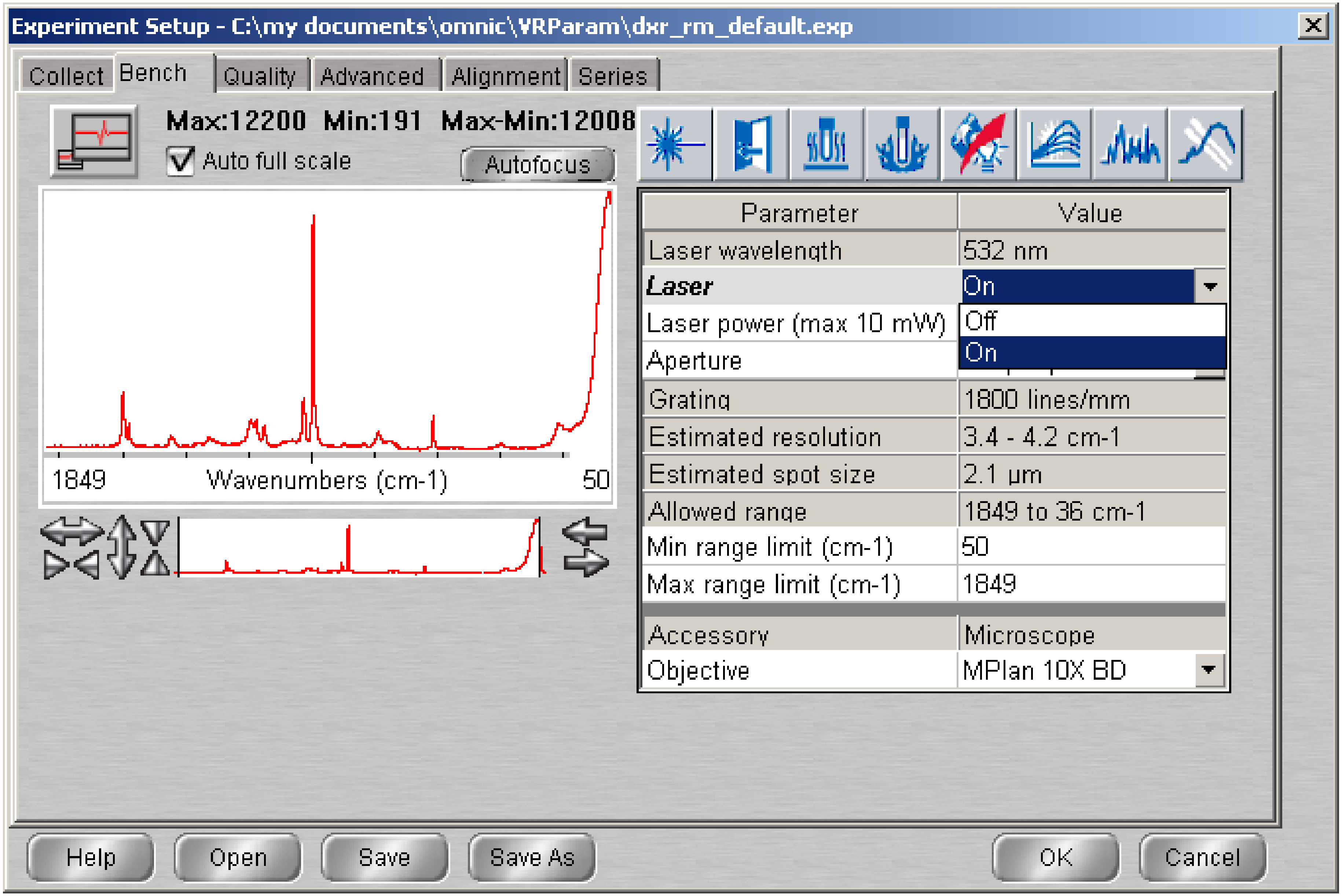
File handling options



Fluorescence correction

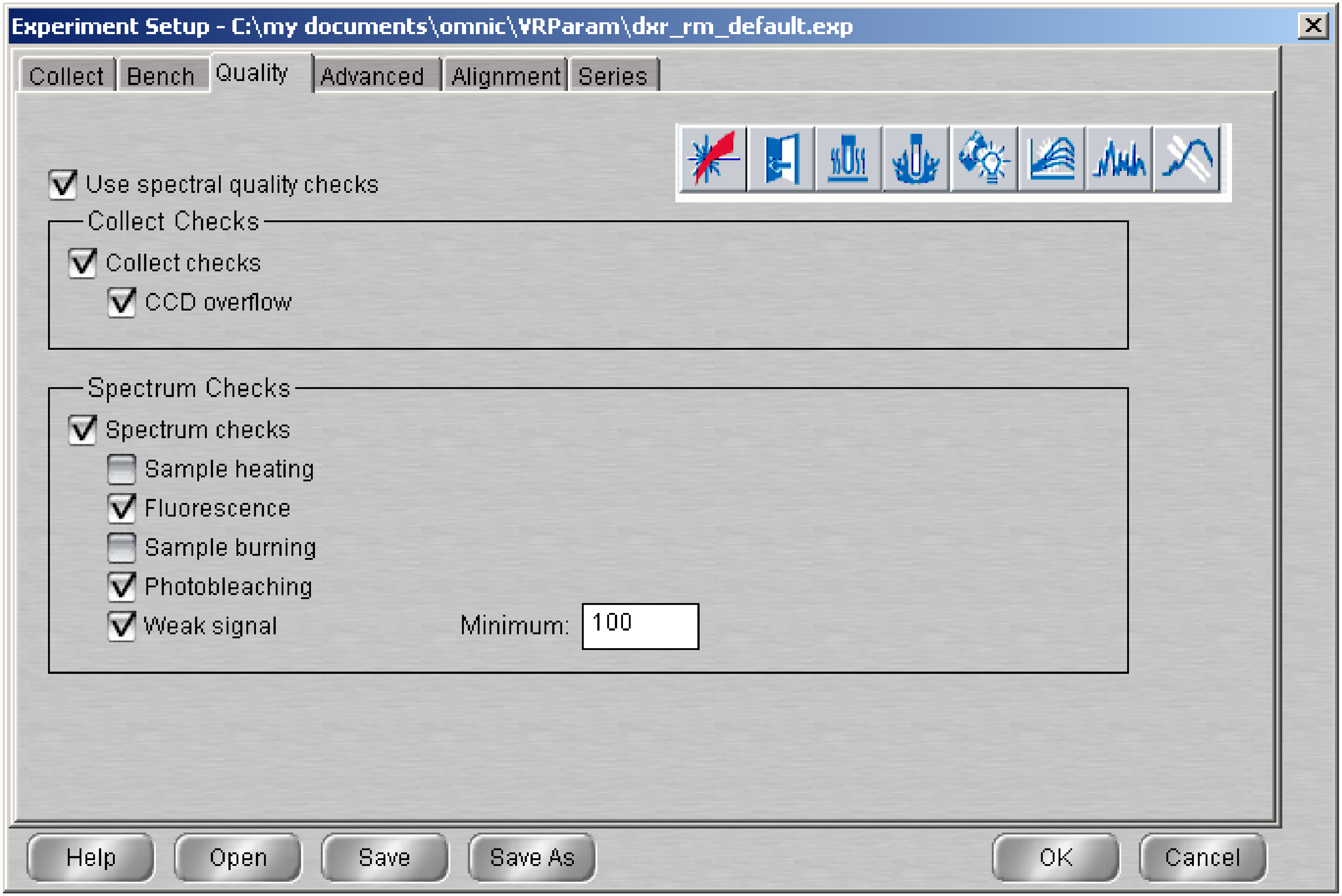


Bench tab

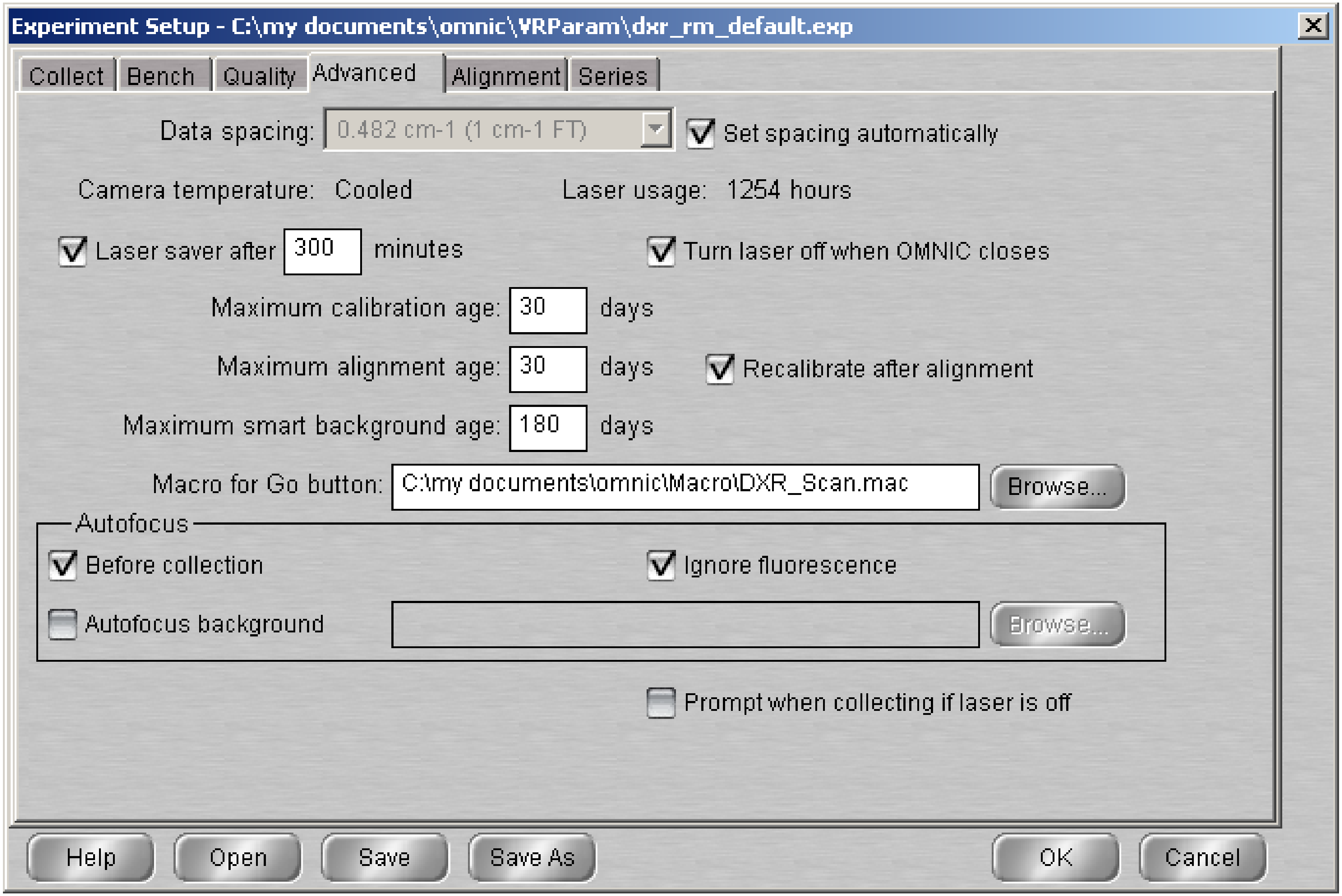


Experiment Setup - C:\my documents\omnic\VRParam\dxr_rm_default.exp
Collect
Bench
Quality
Advanced
Alignment
Series
Max:12200 Min:191 Max-Min:12008
Auto full scale
Autofocus
1849
Wavenumbers (cm-1)
50
Parameter
Value
Laser wavelength
532 nm
Laser
On
Laser power (max 10 mW)
Off
On
Aperture
Grating
1800 lines/mm
Estimated resolution
3.4 - 4.2 cm-1
Estimated spot size
2.1 µm
Allowed range
1849 to 36 cm-1
Min range limit (cm-1)
50
Max range limit (cm-1)
1849
Accessory
Microscope
Objective
MPlan 10X BD
Help
Open
Save
Save As
OK
Cancel

Bench tab icons and quality checks



Advanced options



Summary

- *Collect Tab*
  - *Setting Number of Scans and Resolution*
  - *Auto Exposure*
  - *Background Handling Options*
  - *Experiment Descriptions and Titles*
  - *File Handling Options*
  - *Final Format*
  - *Fluorescence Correction*
- *Bench Tab*
  - *Beam Path/Accessory*
  - *Ions and Quality Checks*
- *Advanced Tab*