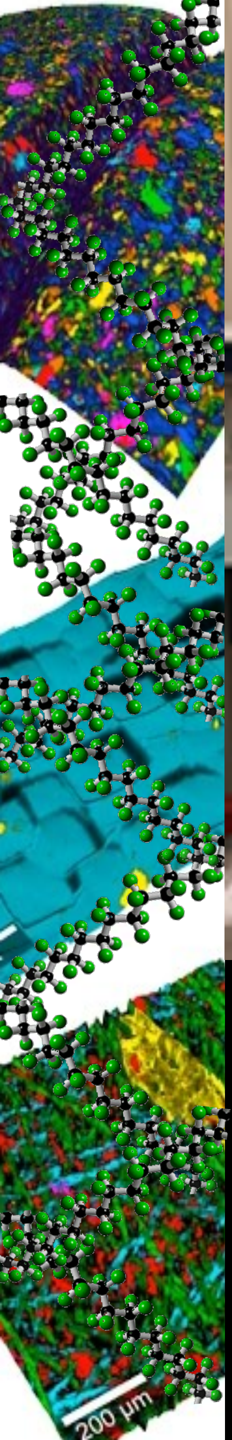
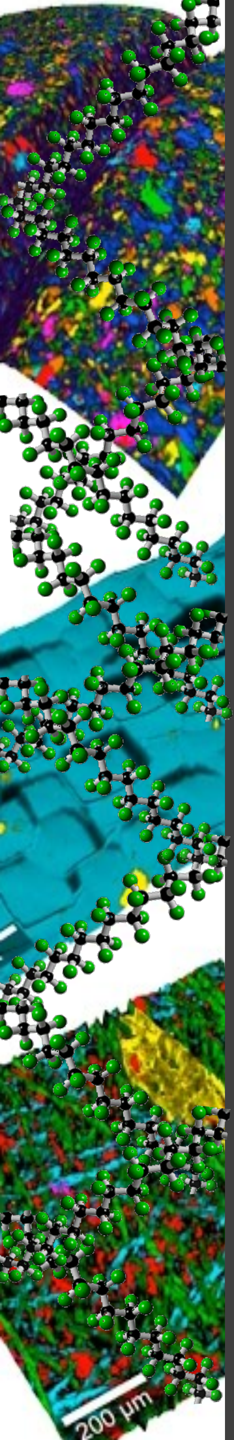


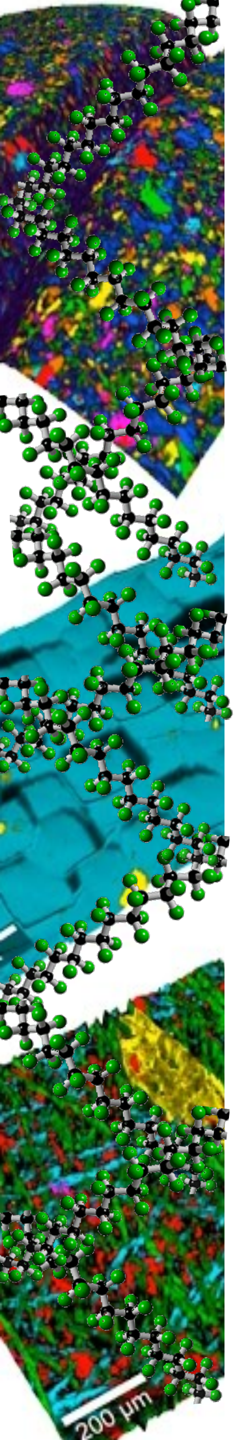
Espectroscopía y microscopía avanzada: Raman

Dra. Viviana Jehová González Velázquez

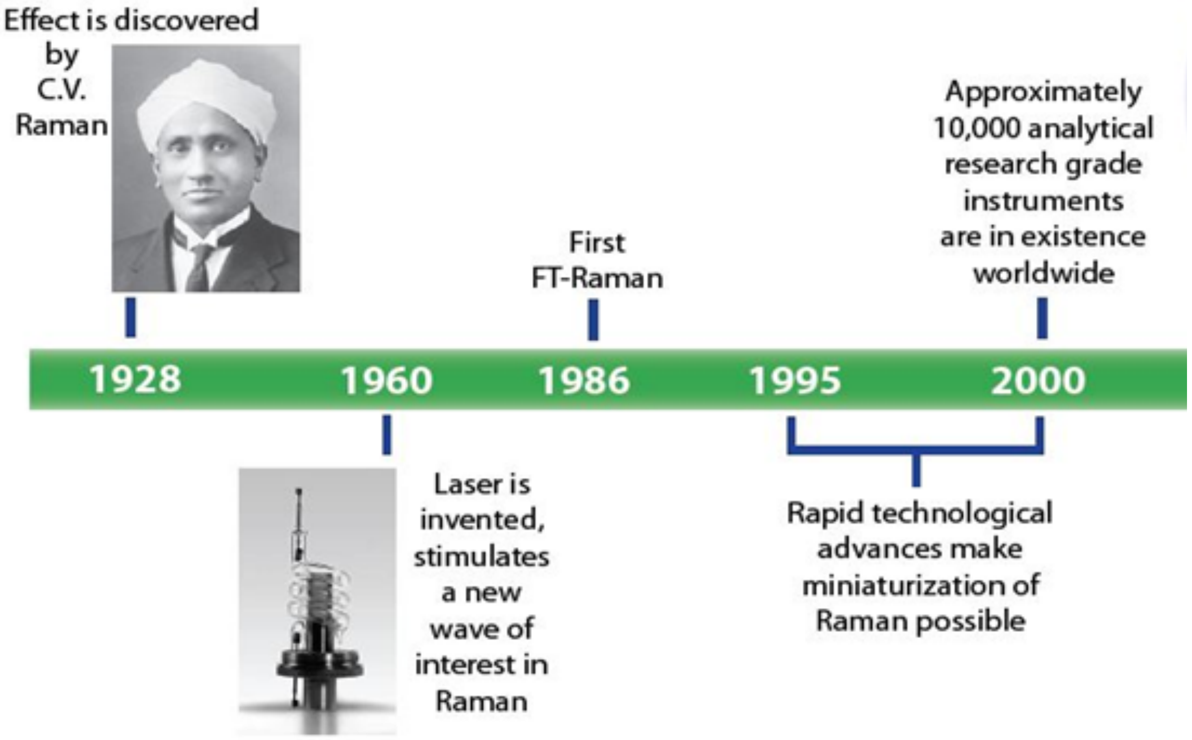


Index

- Introduction.
- Theoretical principles of the technique.
- Instrumentation.
- Applications of Raman Spectroscopy.
- Conclusion.



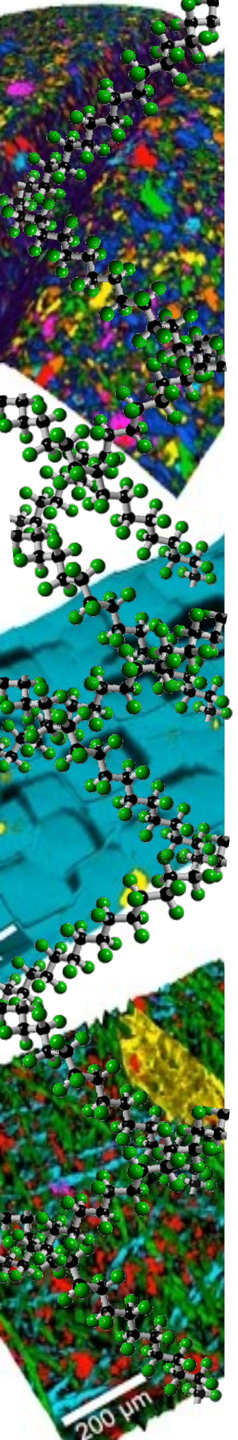
Introduction



Descripción del efecto Raman. Por Chandrasekhara Venkata Raman.

Un alumno de un físico indio observó un cambio de color en un rayo y su equipo no podía eliminar este efecto. Sospecharon que esto se debía a una propiedad de la sustancia.

Publicación en *Nature* de Raman y Krishnan sobre radiación secundaria.

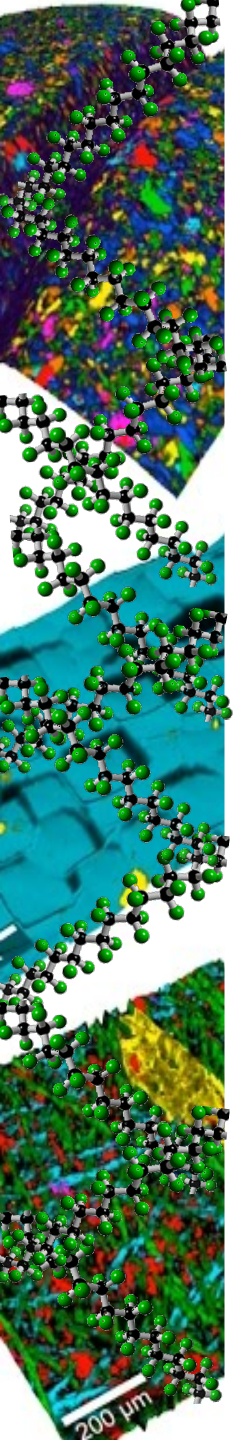


What is Raman Spectroscopy ?

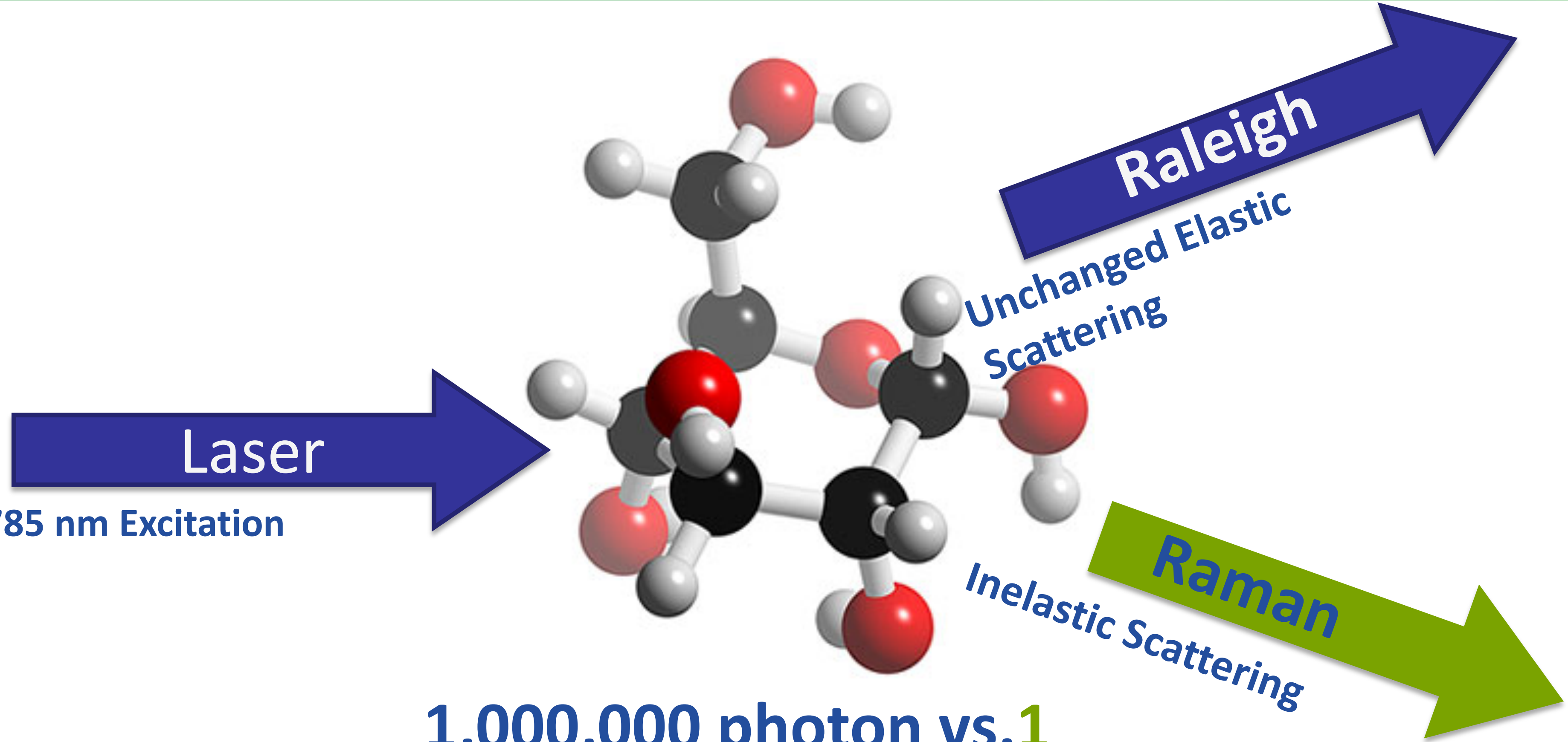
- Raman spectroscopy is a form of molecular spectroscopy – the scattering of electromagnetic radiation by atoms or molecules. The Raman signal is an invaluable tool for molecular fingerprinting.

  - Advantages of Raman Spectroscopy
    - Little to no sample preparation required
    - Perform analysis directly
    - Enables both qualitative and quantitative analysis
    - Highly selective
    - Fast analysis times
    - Insensitive to aqueous absorption bands

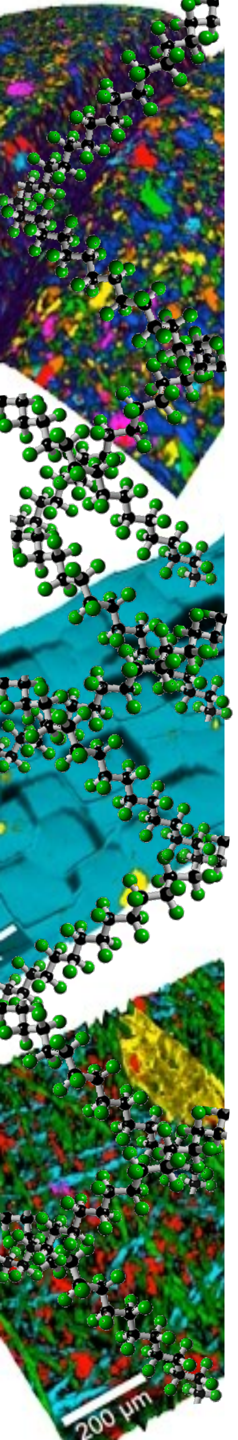
KEY: Sensitivity, S/N, performance/cost, reproducibility, qualitative/quantitative



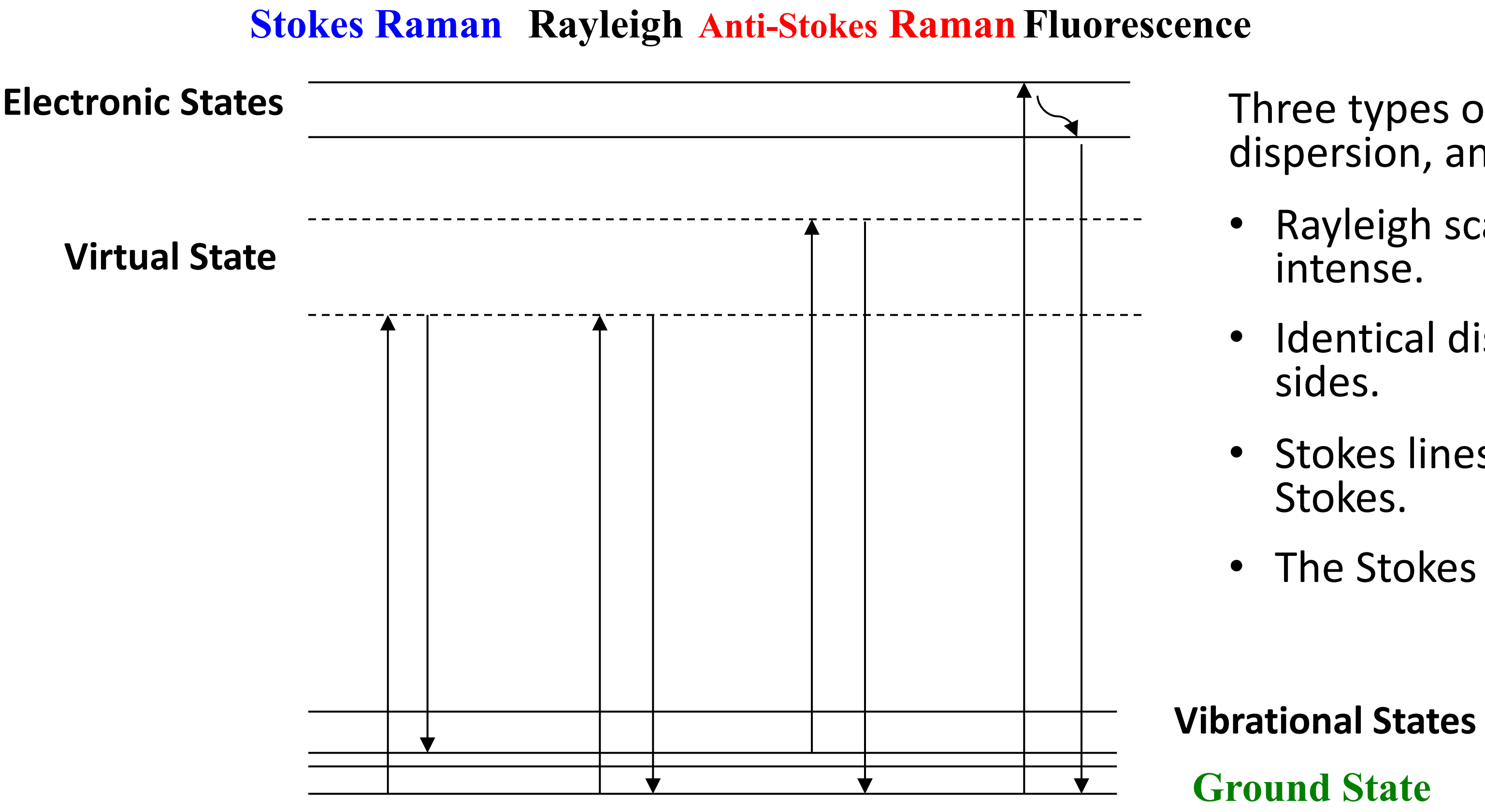
Raman Scattering



1,000,000 photon vs.1

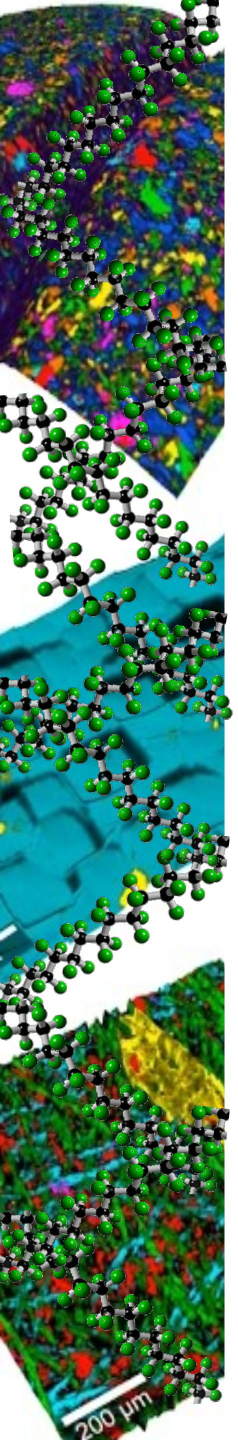


Energy Diagram for Raman Scattering

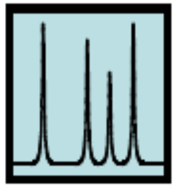
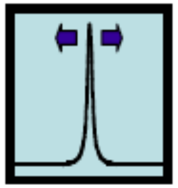
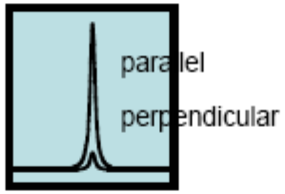
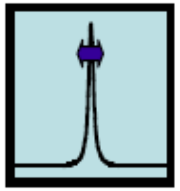


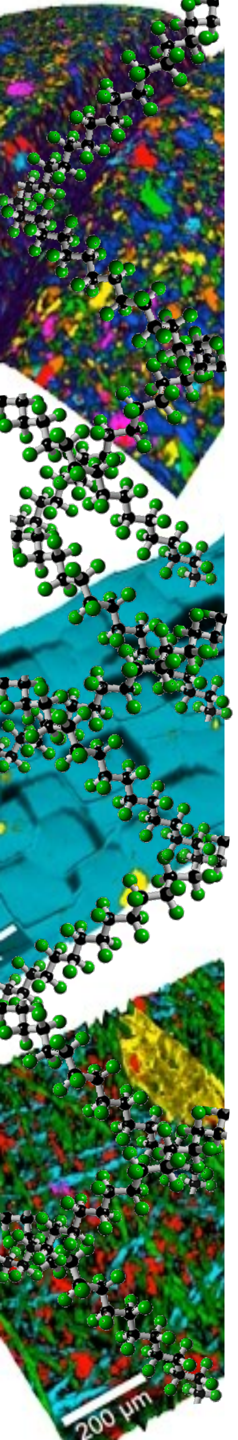
Three types of radiation emitted. Stokes dispersion, anti-Stokes and Rayleigh.

- Rayleigh scattering is significantly more intense.
- Identical displacement models on both sides.
- Stokes lines more intense than anti-Stokes.
- The Stokes part of the spectrum is used.



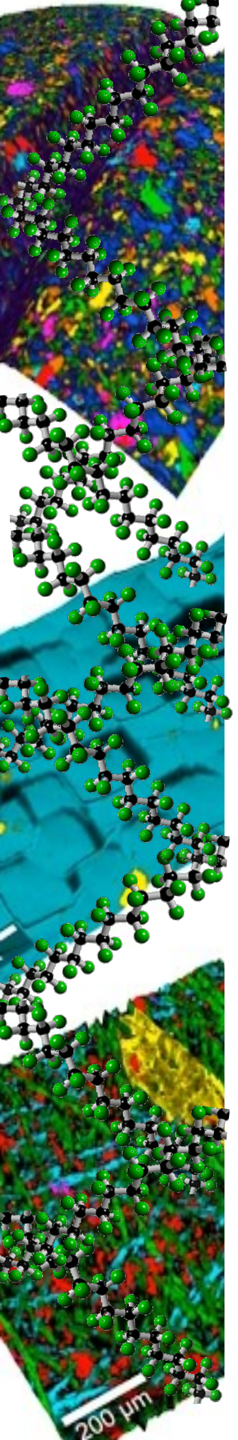
Information From Raman Spectroscopy

 characteristic Raman frequencies	→	composition of material	e.g. MoS_2, MoO_3
 changes in frequency of Raman peak	→	stress/strain state	e.g. Si 10 cm^{-1} shift per % strain
 polarisation of Raman peak	→	crystal symmetry and orientation	e.g. orientation of CVD diamond grains
 width of Raman peak	→	quality of crystal	e.g. amount of plastic deformation



Raman Strengths

- Compatible with fiber optics and glass cells
- High information content, including non-composition info such as crystallinity, orientation, particle size,…
- Can be used to analyze aqueous solutions without interference of water (as can be case in FTIR or NIR)
- Little sample preparation
- Applicable to very tiny samples or locations on samples (micro- work)



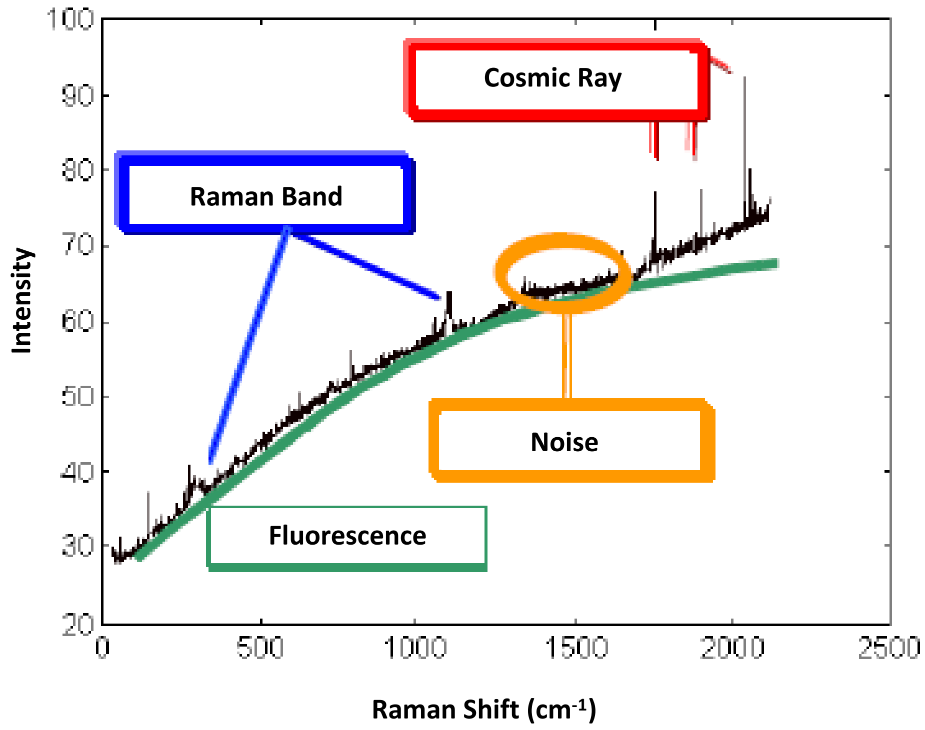
Raman Limitations & problems

Limitations:

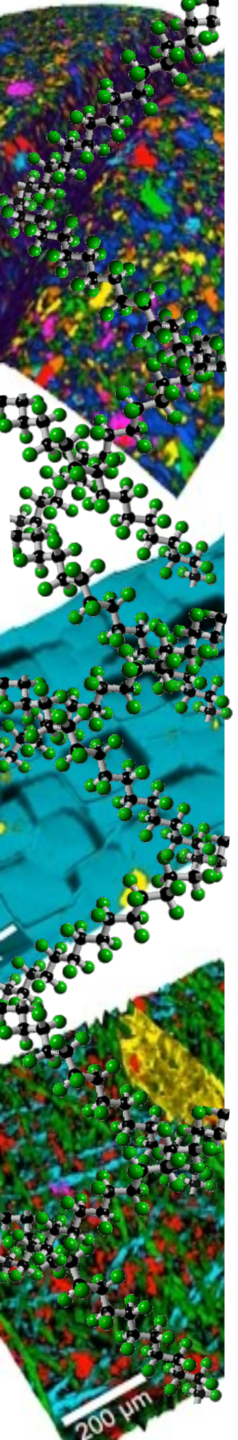
- Weak signal (efficiency ~ 10^{-8}): typical LOD ~0.1%
- Fluorescence interference
- Sample heating or photobleaching can interfere; can't examine black or deeply colored materials
- High information content (interferences)
- Laser source may have fluctuations
- Need an internal standard or standardization for quantitative work

Problems:

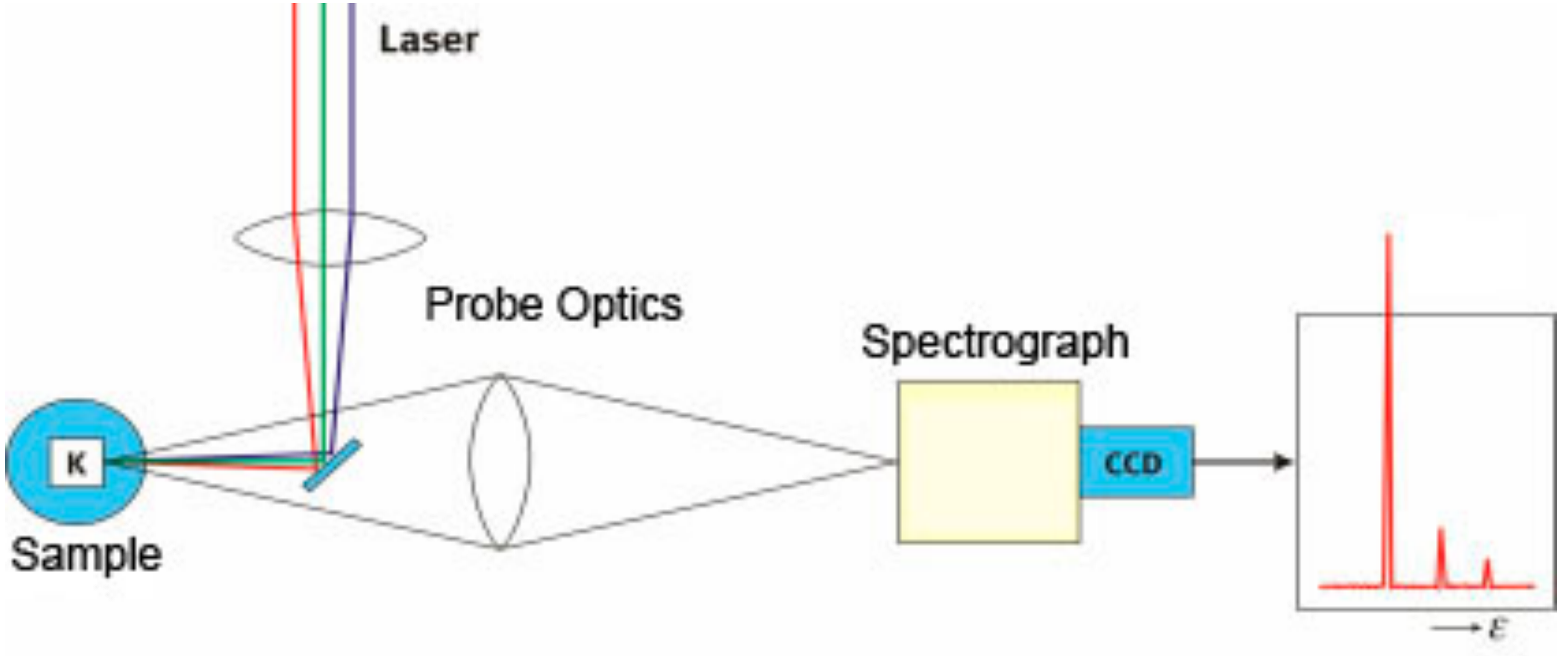
- The noise.
- Fluorescence.
- Calibration errors.



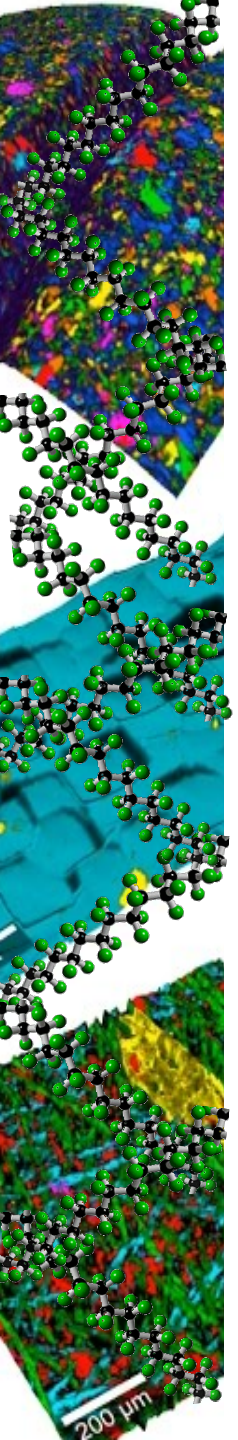
Raman Diagram



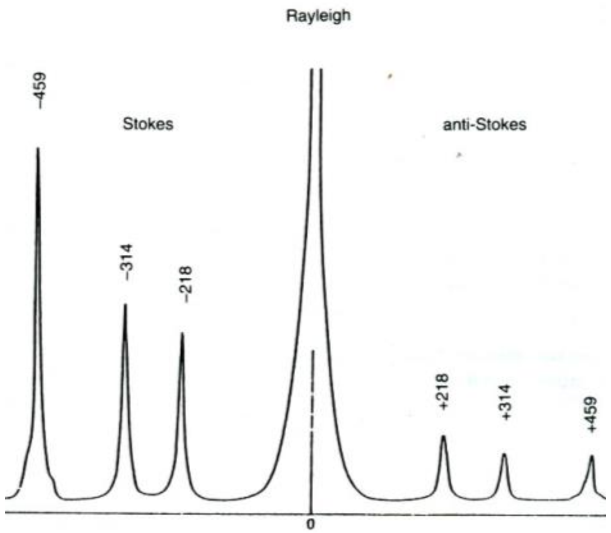
A Raman Instrument consists of a laser, sampling optics (probe), and an optical spectrometer. Because of very weak Raman scattering signals lasers are used as intense excitation sources.



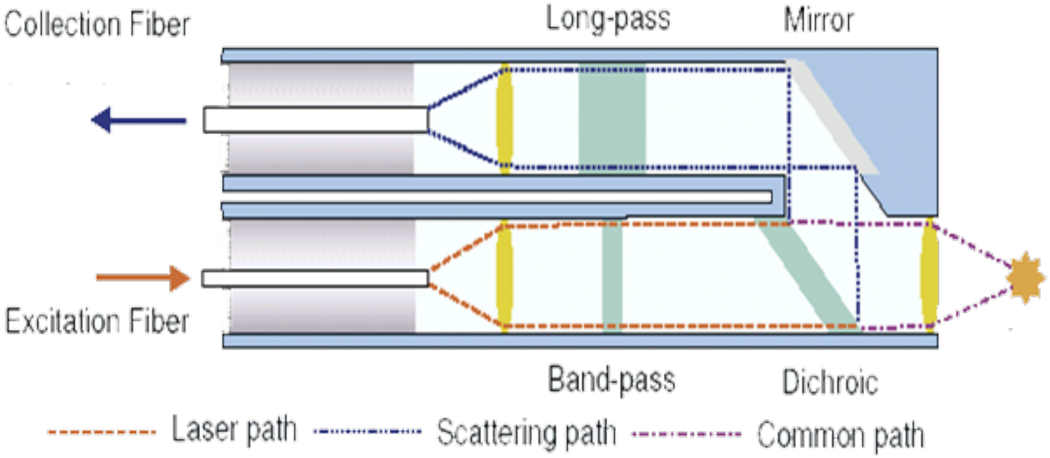
Importance of Rayleigh Rejection



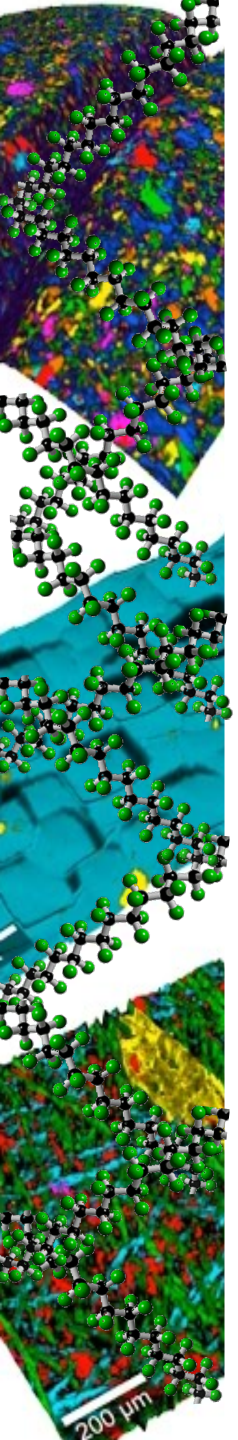
Since the Rayleigh scattering can be up to 10,000,000 times stronger than the Raman scattering, it is imperative that it be filtered out.

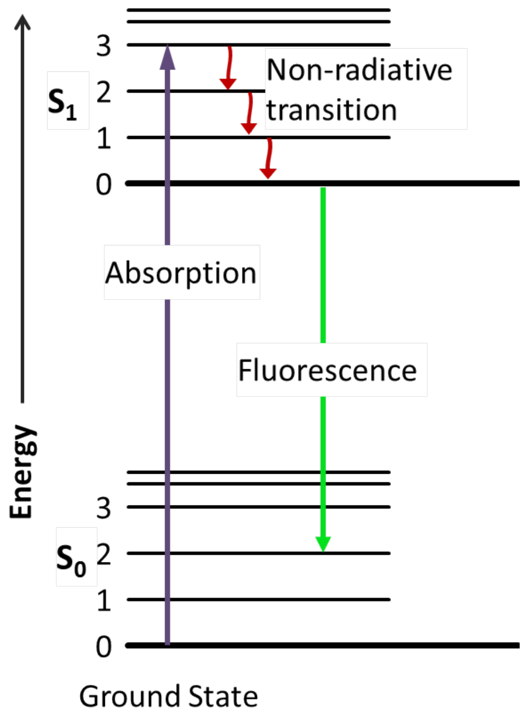


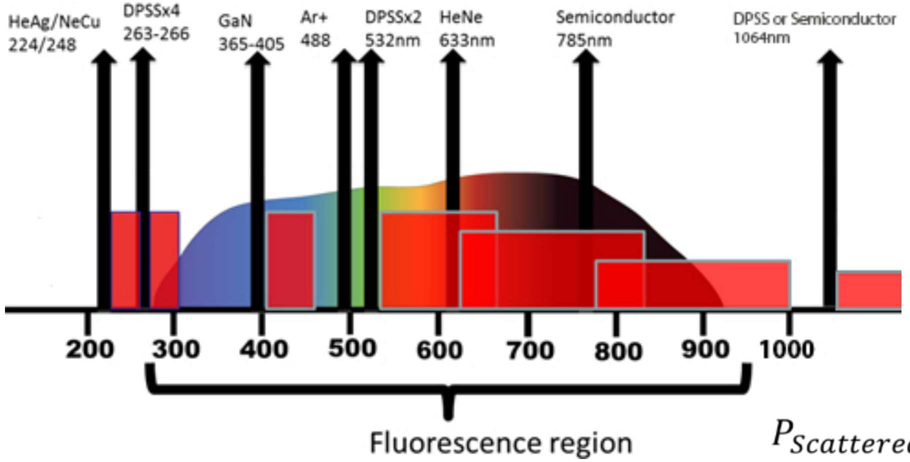
- The quality of the Long-pass filter will determine the wavenumber cut-on of the spectrometer (e.g. 175cm^{-1}or 65cm^{-1})
- The anti-Stokes scattering does not provide any additional information (except in thermal studies) so it is also filtered out.



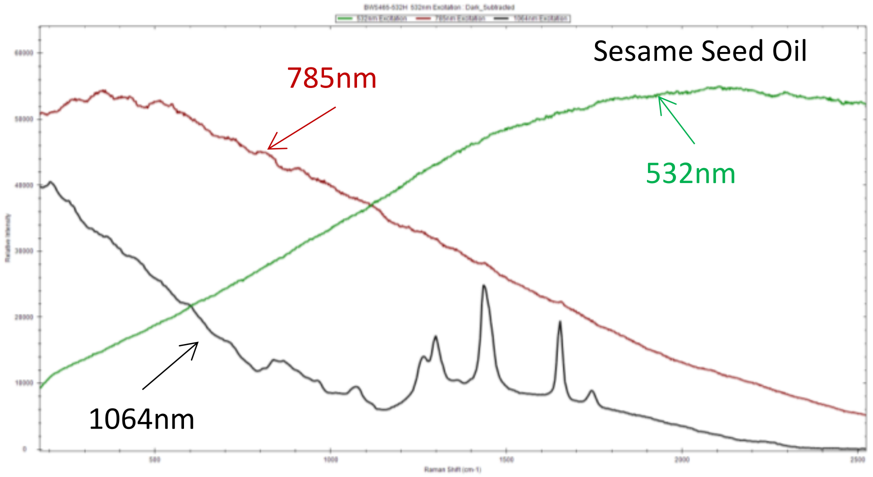
Excitation Wavelengths

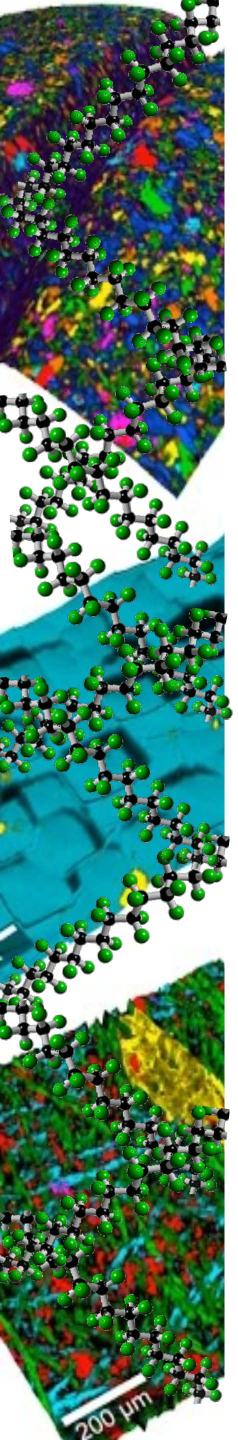






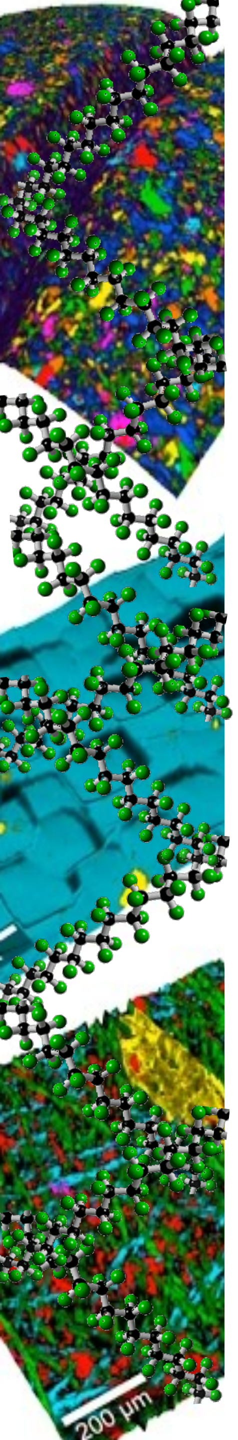
$$P_{Scattered} \propto \frac{I_o}{\lambda^4}$$





Mid-IR	NIR reflectance	Raman
Mechanism: absorption by fundamental molecular vibrational modes	**Mechanism**: absorption by harmonics of fundamental vibrational modes of X-H bonds (*e.g.*, N-H, O-H)	**Mechanism**: inelastic scattering by vibrational, rotational, low frequency molecular modes.
Molecular Specificity: high	**Molecular Specificity:** low	**Molecular Specificity:** high
Signal Strength: strong	**Signal Strength:** weak	**Signal Strength:** weak
Energy: 600-4000 cm^{-1}	**Energy**: 4000-12,500 cm^{-1}	**Energy**: 65-4000 cm^{-1} shift
Sampling: direct material contact required, glass and water appear opaque; can rarely see through container materials	**Sampling**: non-contact, but close proximity to material required (<5 mm)	**Sampling**: container interference is mitigated by accessories
Hardware: probes expensive and fragile; typically short in length, and inflexible	**Hardware**: longer fiber optics possible (meters); quartz optics; dispersive, interferometry common	**Hardware**: CCD detection; quartz optics; long fiber optics common (kilometers in some cases)

Raman spectroscopy is best at symmetric vibrations of non-polar groups while IR spectroscopy is best at the asymmetric vibrations of polar groups.



Key Instrumentation for Renishaw Raman

- Stabilized laser: 532nm, 633nm, 785nm
- Running measurements such as:
- line scans
- area mapping
- volume scans
- Software: Wire 4.4

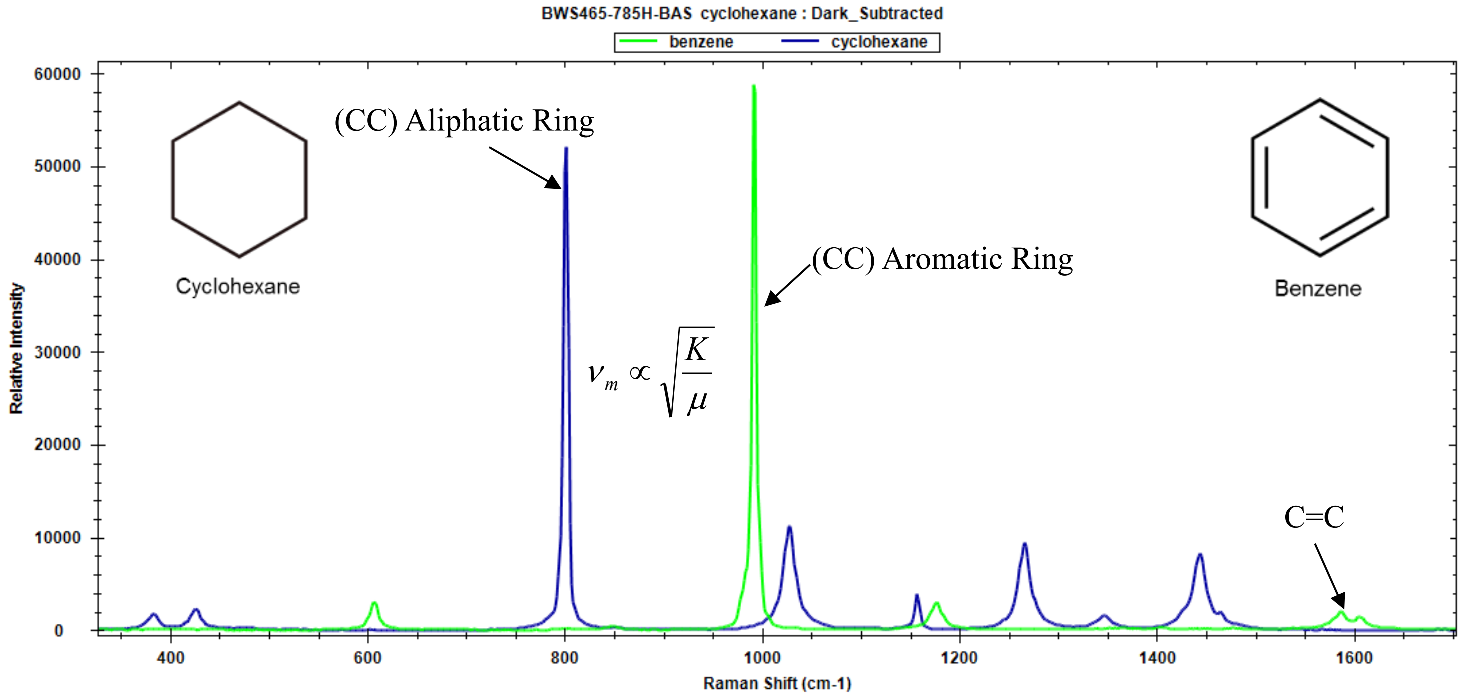
Láser de He-Ne, cuya longitud de onda es λ=632.8nm.

Láser de Ar, cuya longitud de onda es λ=514.4 nm.

Láser semiconductor IR, cuya longitud de onda es λ=785nm.

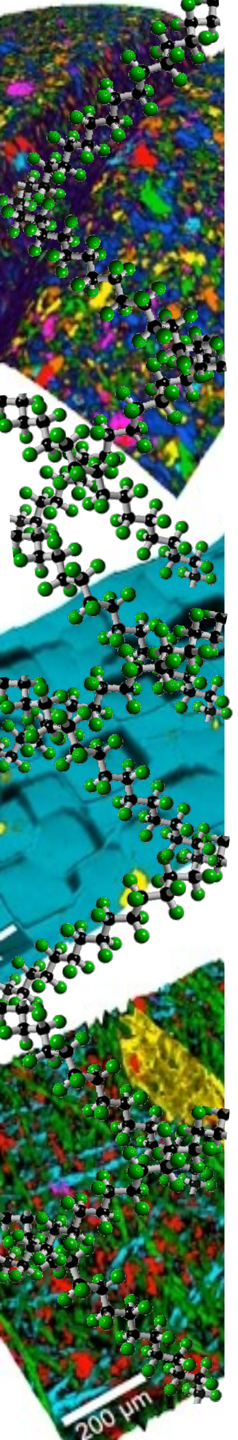
Raman Spectrum

A Raman spectrum is a plot of the intensity of Raman scattered radiation as a function of its frequency difference from the incident radiation (usually in units of wavenumbers, cm^{-1}). This difference is called the *Raman shift*.

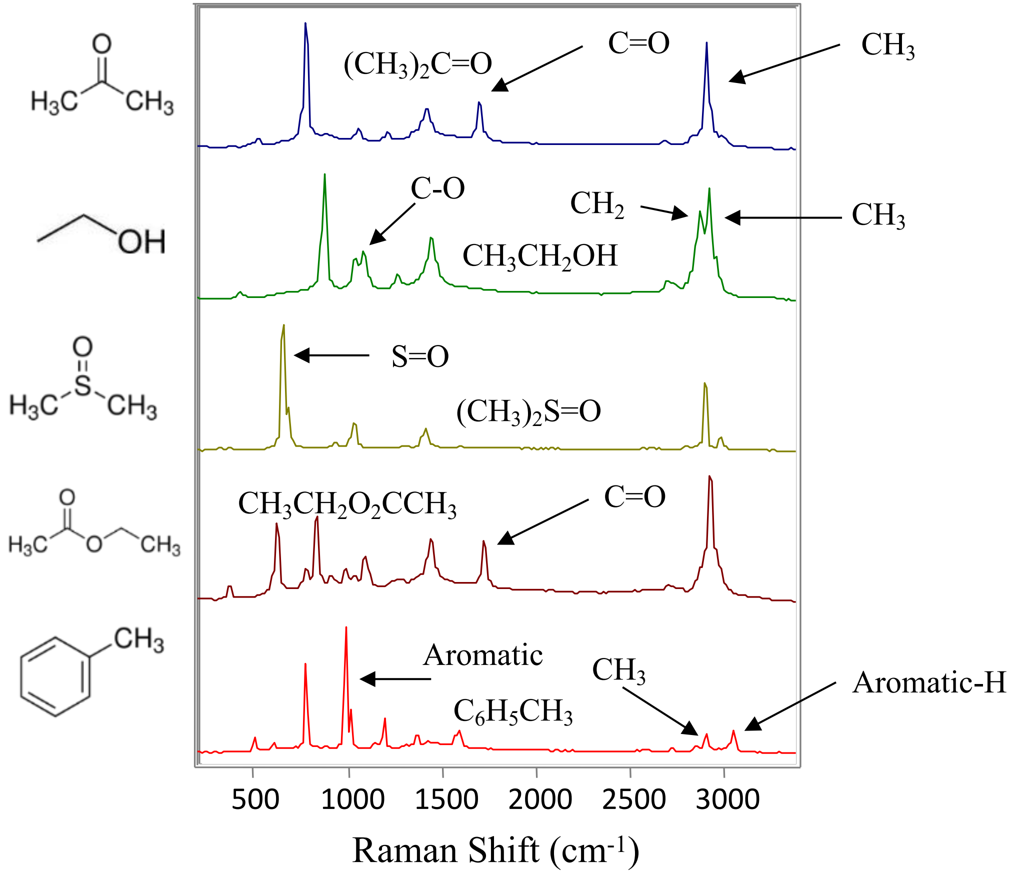


The intensity of the Raman peaks depends on:

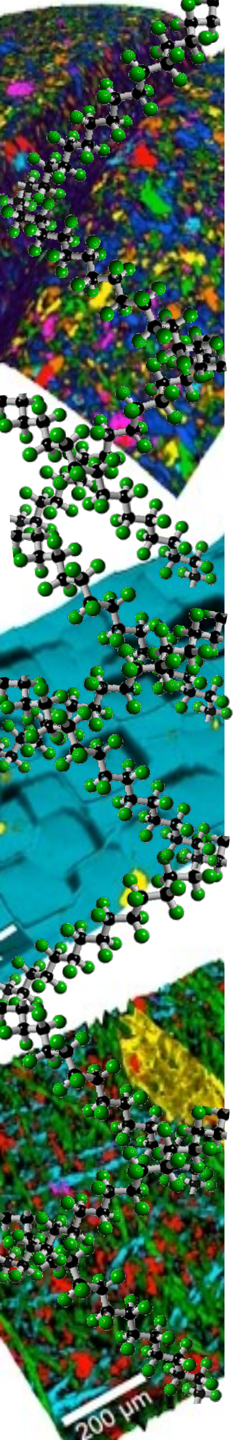
- Polarity of the molecule.
- Intensity of the source.
- Concentration of the active group.



Raman Spectral Information

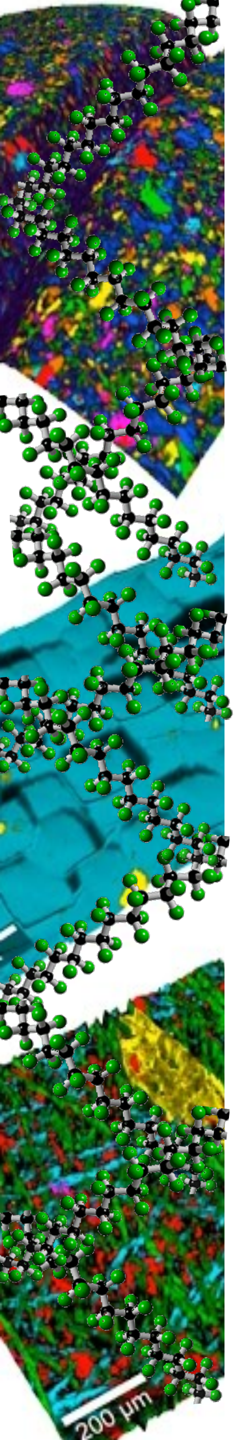


Functional Group/ Vibration	Region
Lattice vibrations in crystals, LA modes	10 - 200 cm^{-1}
δ(CC) aliphatic chains	250 - 400 cm^{-1}
υ(Se-Se)	290 -330 cm^{-1}
υ(S-S)	430 -550 cm^{-1}
υ(Si-O-Si)	450 -550 cm^{-1}
υ(Xmetal-O)	150-450 cm^{-1}
υ(C-I)	480 - 660 cm^{-1}
υ(C-Br)	500 - 700 cm^{-1}
υ(C-Cl)	550 - 800 cm^{-1}
υ(C-S) aliphatic	630 - 790 cm^{-1}
υ(C-S) aromatic	1080 - 1100 cm^{-1}
υ(O-O)	845 -900 cm^{-1}
υ(C-O-C)	800 -970 cm^{-1}
υ(C-O-C) asym	1060 - 1150 cm^{-1}
υ(CC) alicyclic, aliphatic chain vibrations	600 - 1300 cm^{-1}
υ(C=S)	1000 - 1250 cm^{-1}
υ(CC) aromatic ring chain vibrations	*1580, 1600 cm^{-1} *1450, 1500 cm^{-1} *1000 cm^{-1}
δ(CH3) δ(CH2)	1380 cm^{-1}
δ(CH3) asym δ(CH2)	1400 - 1470 cm^{-1}
δ(CH3) asym	1400 - 1470 cm^{-1}
υ(C-(NO2))	1340 - 1380 cm^{-1}
υ(C-(NO2)) asym	1530 - 1590 cm^{-1}
υ(N=N) aromatic	1410 - 1440 cm^{-1}
υ(N=N) aliphatic	1550 - 1580 cm^{-1}
δ(H2O)	~1640 cm^{-1}
υ(C=N)	1610 - 1680 cm^{-1}
υ(C=C)	1500 - 1900 cm^{-1}
υ(C=O)	1680 - 1820 cm^{-1}
υ(C≅C)	2100 - 2250 cm^{-1}
υ(C≅N)	2220 - 2255 cm^{-1}
υ(-S-H)	2550 - 2600 cm^{-1}
υ(C–H)	2800 - 3000 cm^{-1}
υ(=(C-H))	3000 - 3100 cm^{-1}
υ(≅(C-H))	3300 cm^{-1}
υ(N-H)	3300 - 3500 cm^{-1}
υ(O-H)	3100 - 3650 cm^{-1}



Raman can measure through a broad class of packaging

Bottles	Thickness
Amber Glass	< 2 mm
Clear Glass	< 3 mm
High Density Polyethylene (HDPE)	< 1 mm
Teflon FEP	< 1 mm
Polystyrene	< 1 mm
Vials	
Amber and Clear Glass	< 1 mm
Bags	
Polypropylene (PP)	< 0.1mm
Polyethylene (PE), Low-Density Polyethylene (LDPE)	< 0.1mm



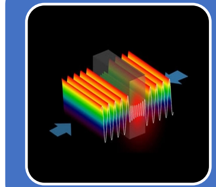
Applications of Raman Spectroscopy

Pharmaceutical

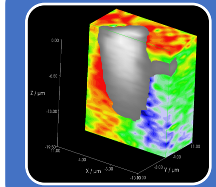
Biomedicine

Forensic

Polymers



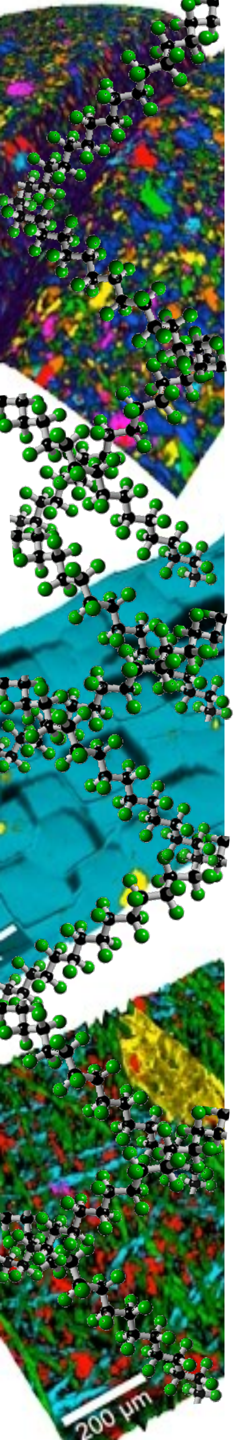
Nanotechnology



Aplicación en semiconductores

Aplicaciones en arte y arqueología

Oil Industry



What can Raman do for you?

Strong Raman Signal

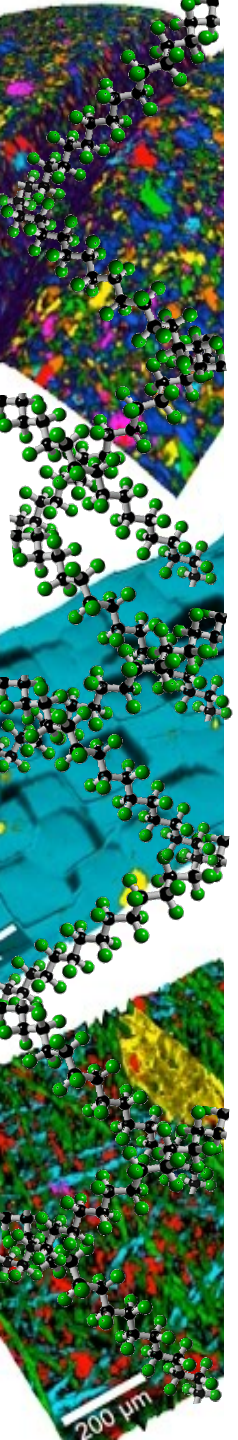
- Active Pharmaceutical Ingredients
- Alcohols
- Antibiotics
- Antioxidants
- Buffers
- Coatings
- Diluents
- Emulsifiers
- Excipients
- Flavors
- Fragrances
- Lubricants
- Monomers and polymers
- Polyatomic inorganics
- Preservatives
- Solvents
- Vitamins

Weak Raman Signal

- Materials that are dark in color
- Highly fluorescing molecules
- Fillers/binding agents
- Glass
- Thin-walled plastics
- Water

No Raman Signal

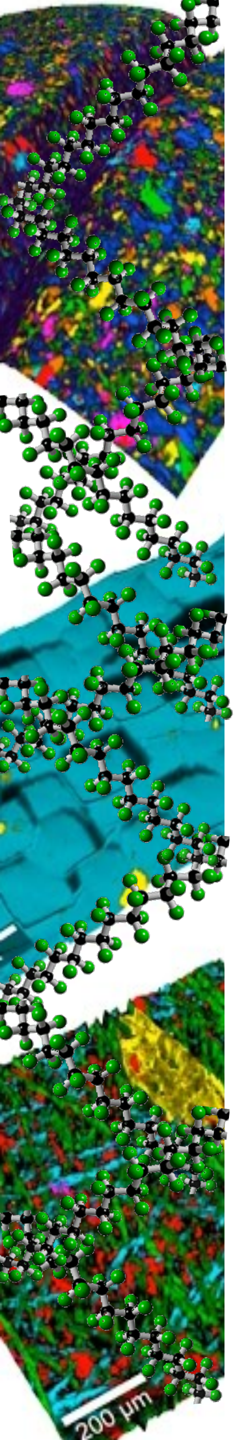
- Black materials
- Metals
- Mono-atomic ions

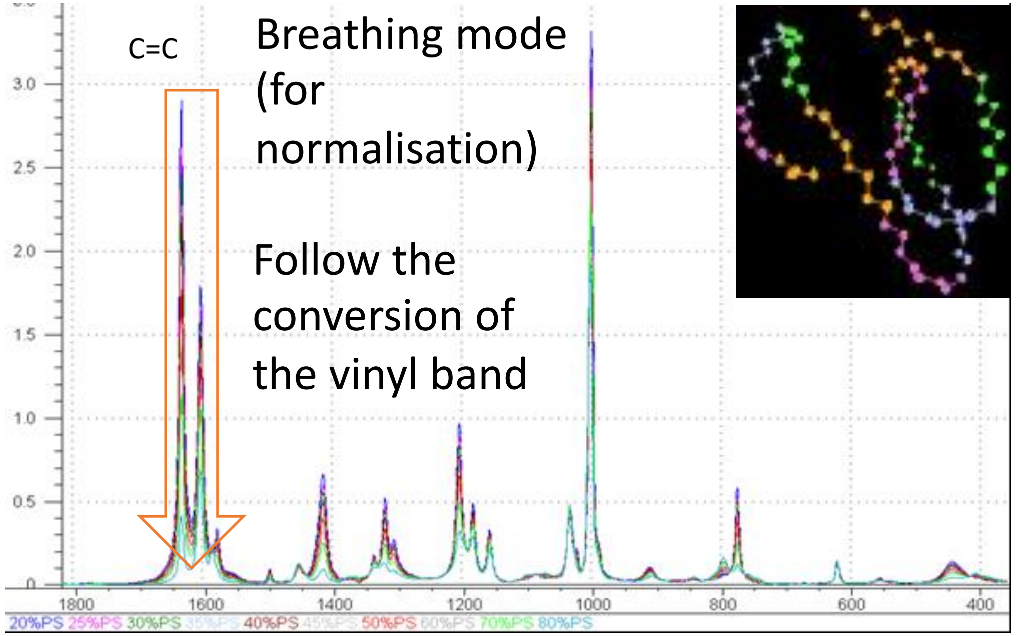


Applications in Polymer Science

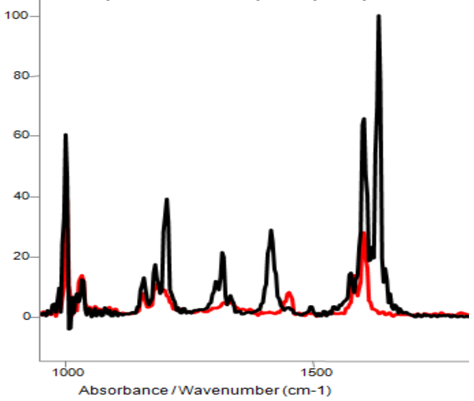
- Raman spectroscopy helps to meet needs of the polymer, additive, compounding and masterbatch industries, by allowing for an audit trail to certify the link between the quality of raw materials and finished product.
- Raman has many applications in both QA and QC including incoming material inspections, measurement of polymer grade, blend ratios, additives, and ageing.
- It can aid in the optimisation of formulations for desired properties and performance by predicting:
    - Processing properties – MFI, liquid viscosity
    - Thermal properties – glass transition, melting point
    - Physical properties – density, mechanical modulus and strength, impact strength
    - Fire properties – UL scores, LOI, flame retardants

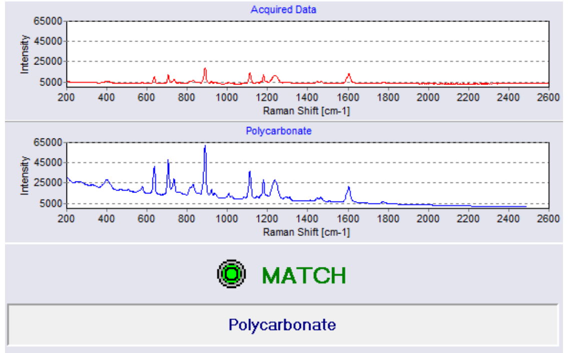
Polymer Reaction Monitoring





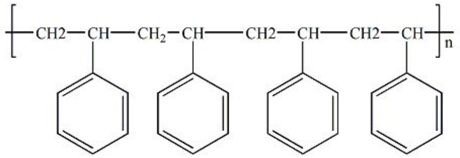
styrene -> polystyrene



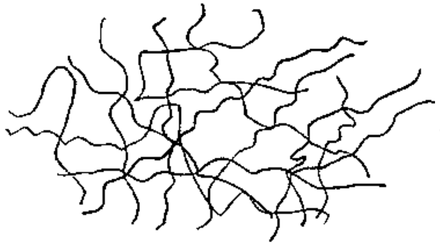


Chemical composition

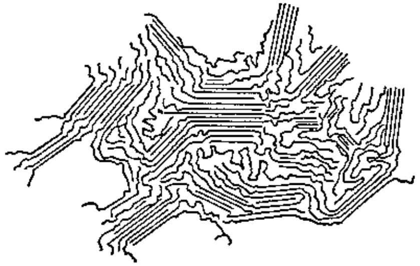
Polymerization degree



Crystallinity

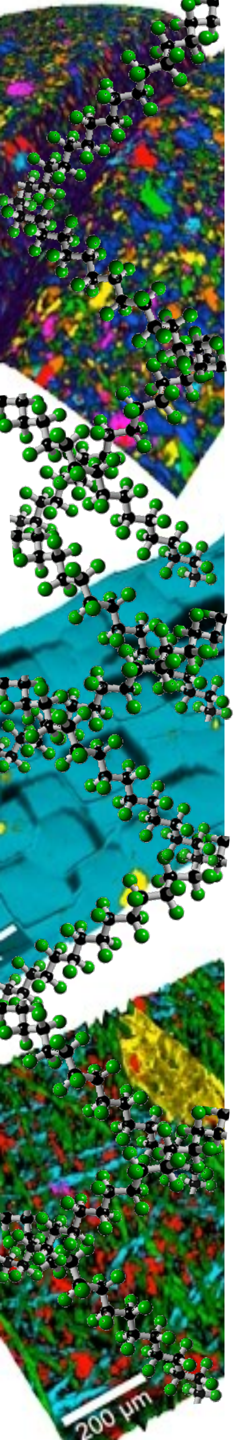


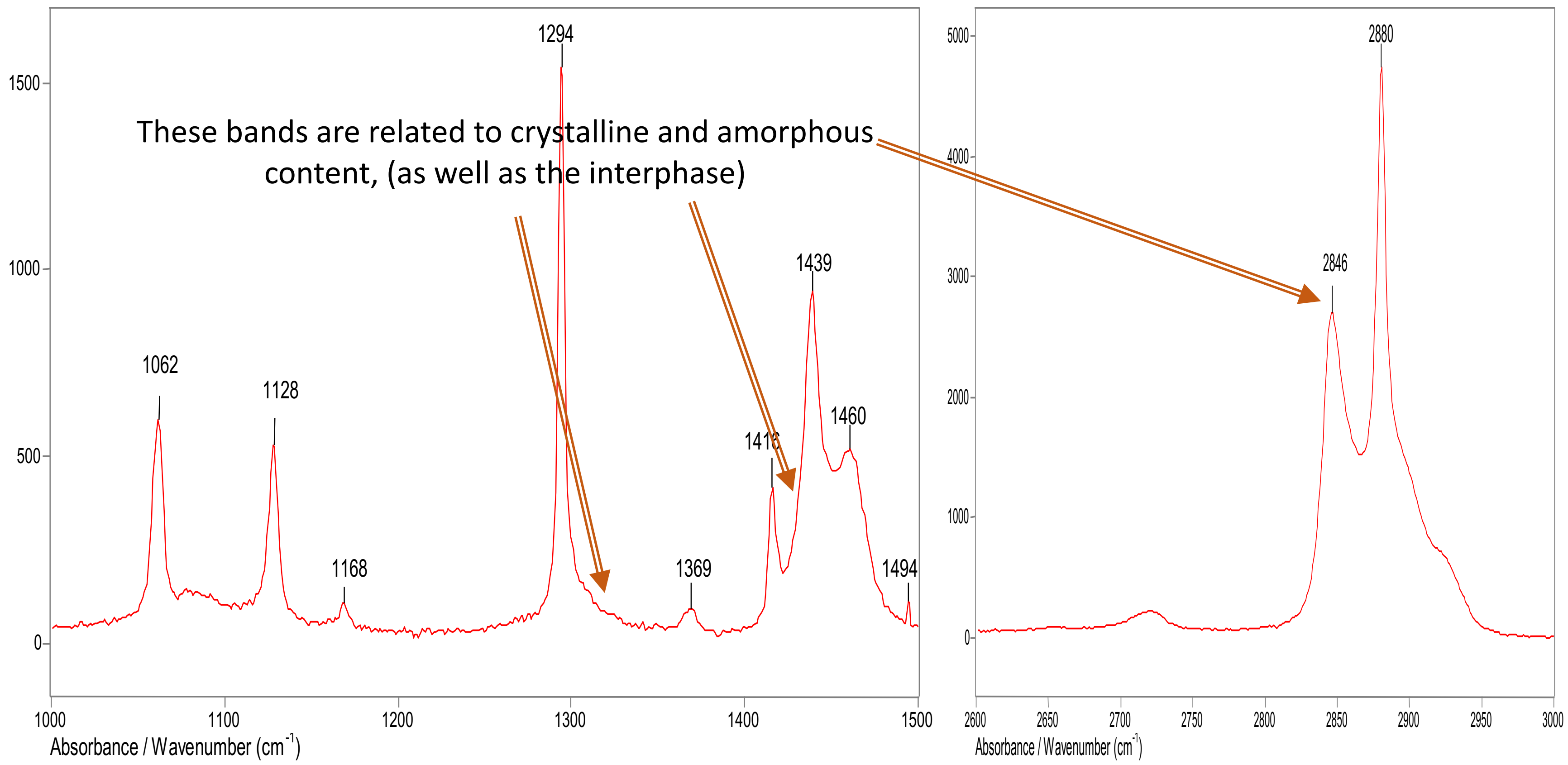
Polímero de estructura amorfa

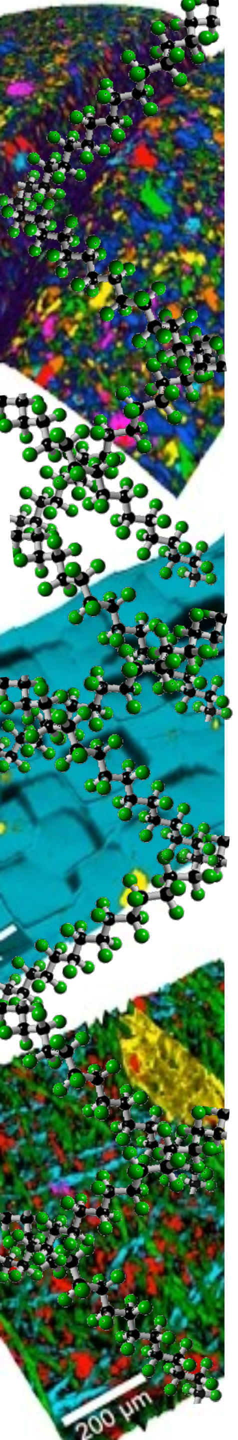


Polímero de estructura cristalina

Polymer Crystallinity





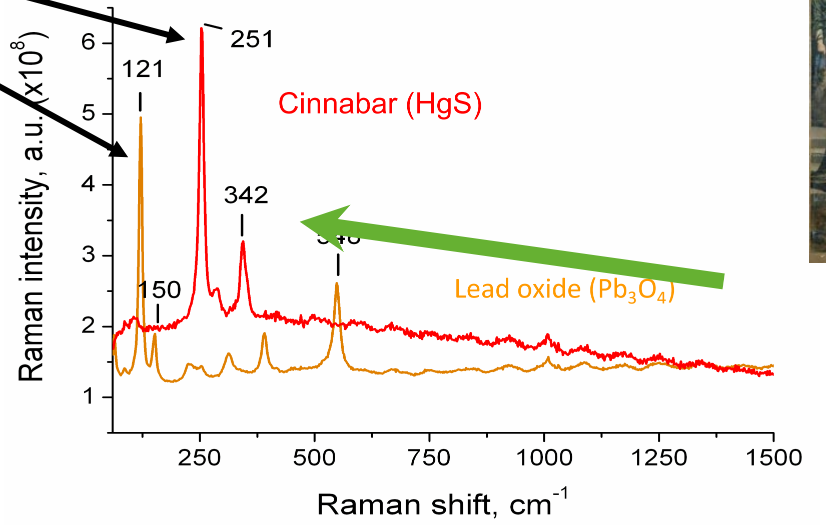


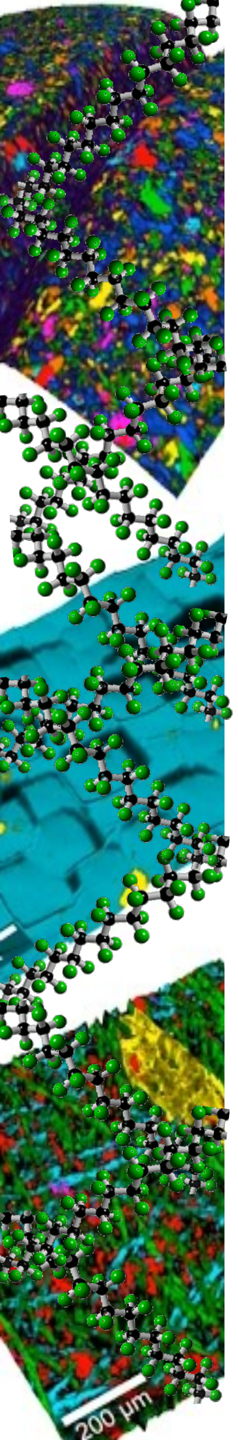
Raman Microscopy of Ancient Pigments

Analysis of pigments on the ceiling of a cathedral in Spain using a Raman spectrometer connected to a tripod-mounted video microscope for precision alignment.

- Assist in the restoration of minimal damage
- Prevents counterfeiting

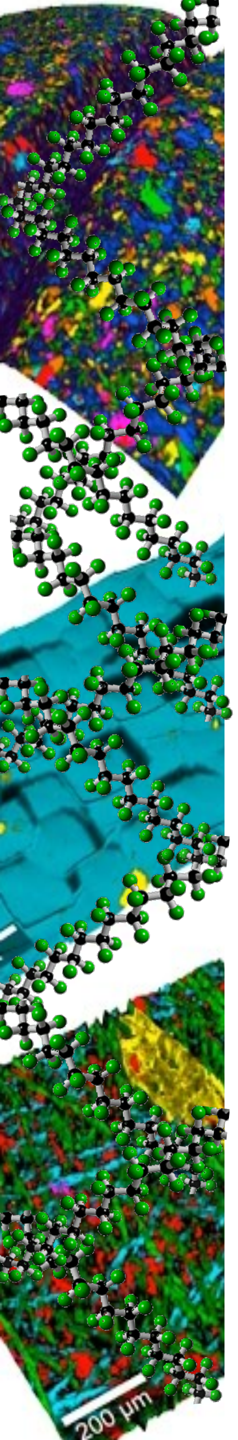
Courtesy of *M.J. Ayora Cañada y A. Dominguez*
Universidad de Jaén





Applications in the Semiconductor Industry

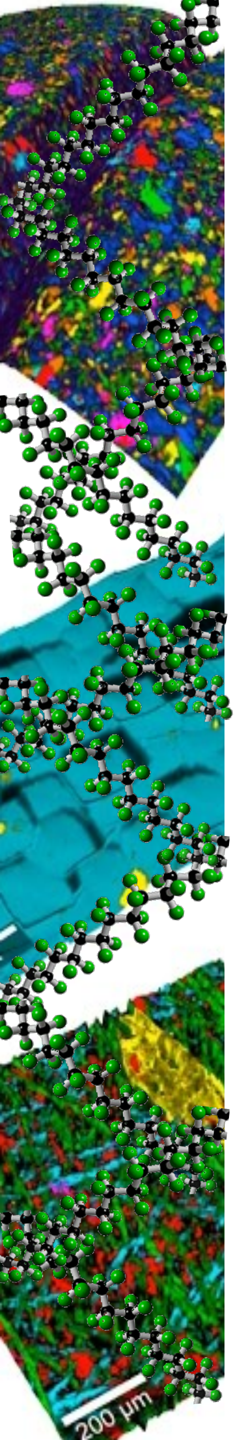
- Allows a structural study and industrial monitoring



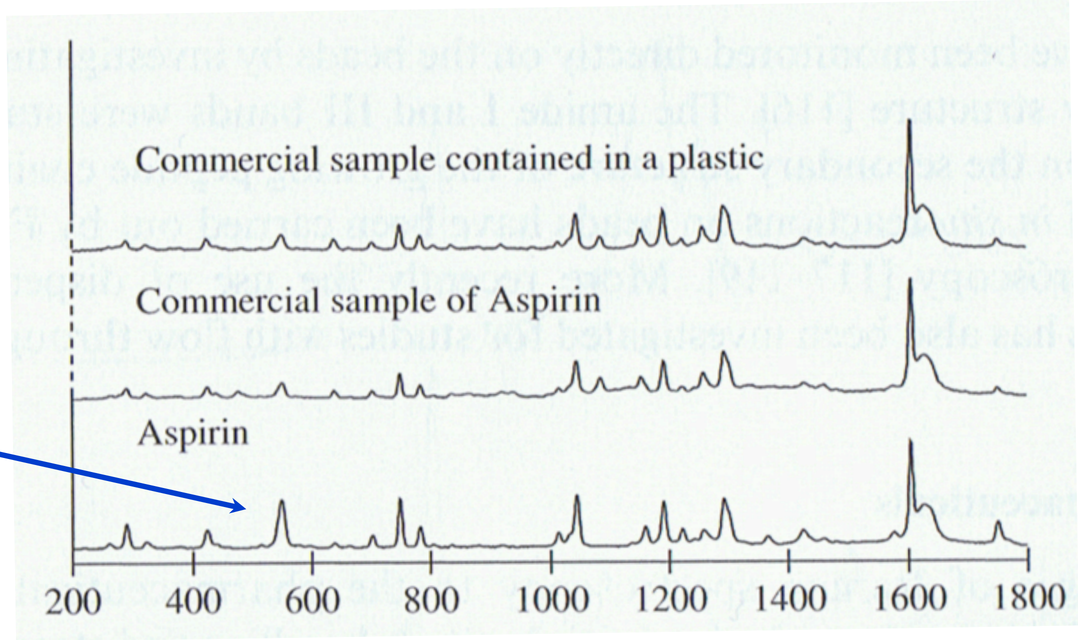
Applications in the Pharmaceutical Industry

- Identification
  - Verification of the identity of incoming raw materials.
  - Identification and analysis of counterfeit drug products.
- Process Control
  - Real time quantitative analysis for process analytical control (PAT) such as blending, titration, and polymorphic transition monitoring.
  - Water does not interfere with Raman
  - In situ analysis of aqueous cultures
  - Fast and safe quality control

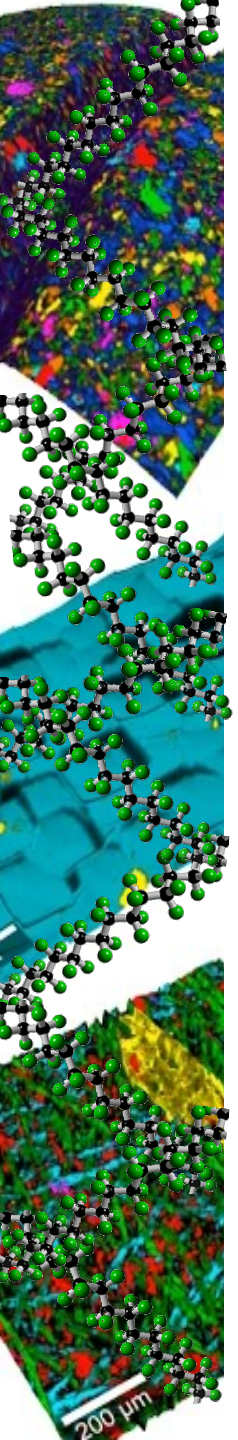
Applications in the Pharmaceutical Industry



It is possible to test certain substances in the same container



Certain differences for reasons of purity

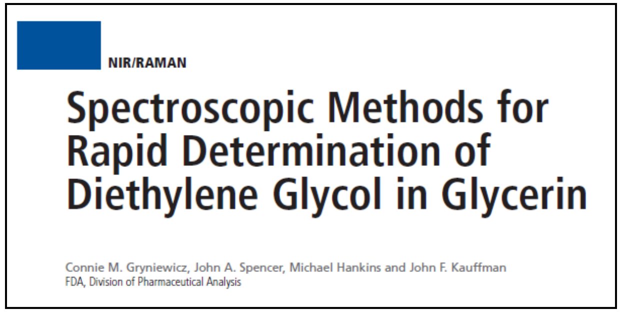


Counterfeit Drug Identification

- According to the World Health Organization's estimates, ~10-15% of the world's drug supply (and about 1% in the US) is counterfeit - at a value of about $200B in 2010

- Raman Spectroscopy is currently being used for not only identification of counterfeit drug products but also to analyze the quality and purity

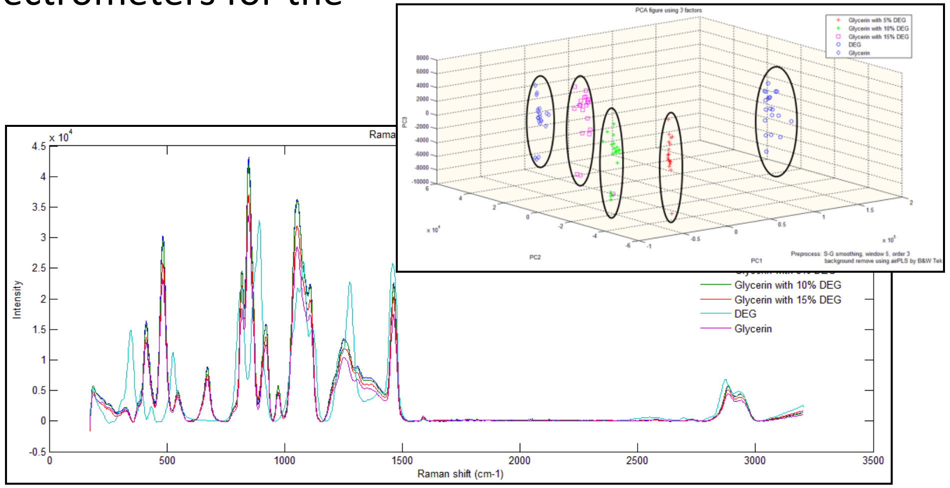
- For example, the FDA is currently using Raman spectrometers for the identification of glycerin contaminated with DEG.

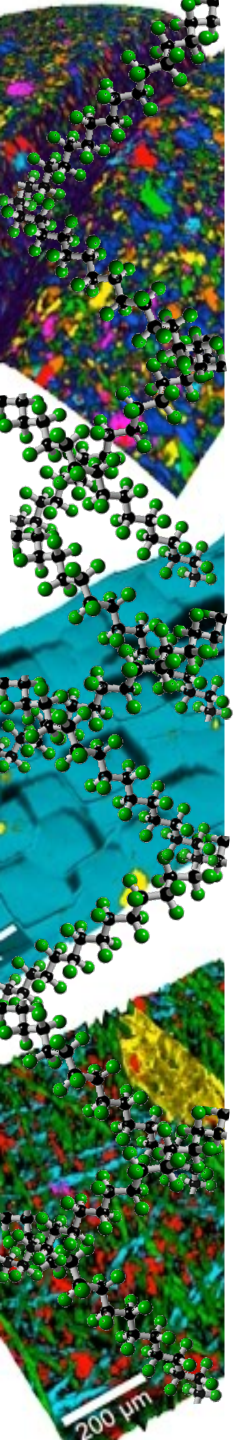


NIR/RAMAN

Spectroscopic Methods for Rapid Determination of Diethylene Glycol in Glycerin

Connie M. Gryniewicz, John A. Spencer, Michael Hankins and John F. Kauffman
FDA, Division of Pharmaceutical Analysis





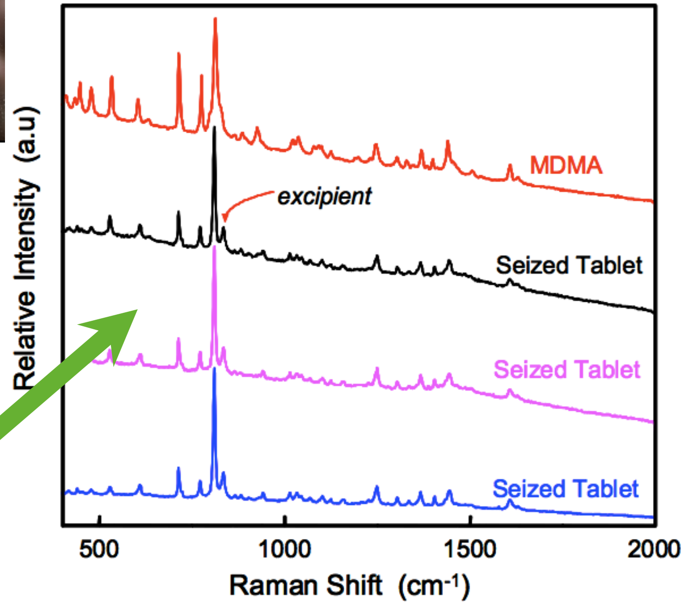
Applications in Forensic Analysis

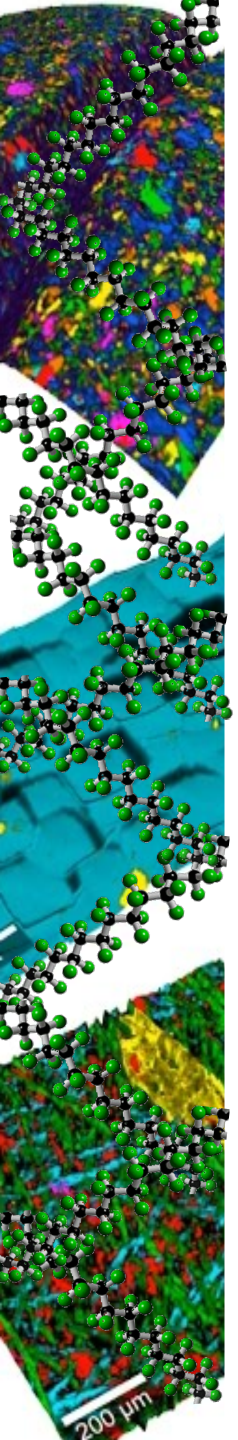
- Nondestructive Narcotic Drug Identification

- Explosives Identification:
  - Exact Chemical Compositions of Material (i.e. PETN, RDX)
  - Binding Agents Within Explosive Materials

- Identification and Analysis of Toxic Solvents and Bio-warfare Agents

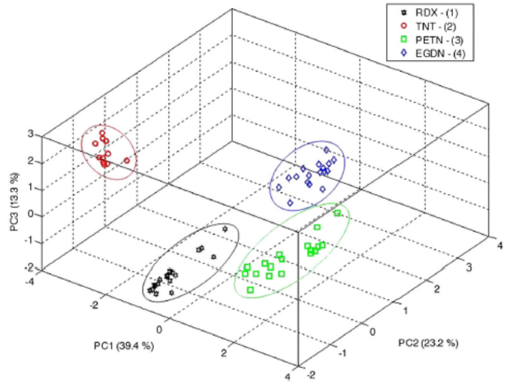
- Trace Forensic Evidence Analysis:
  - Including Fibers, Fabrics, Pigments, Inks, etc.

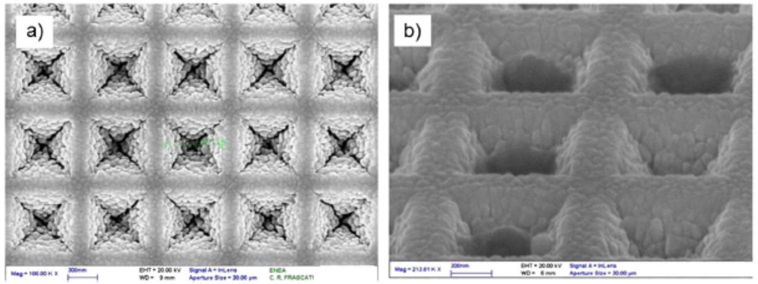




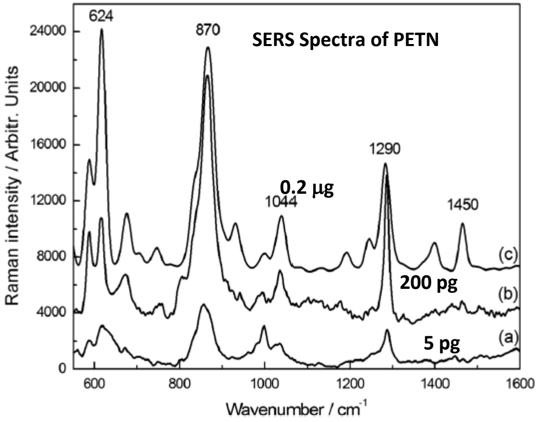
Identification of Explosives

- Raman spectrometers are also well suited for the identification of explosives and hazardous materials.
- Typically surface enhanced Raman spectroscopy (SERS) is utilized for this application because it allows for the detection of trace levels of explosives.
- For example, a in a recent publication it was shown that PETN could be detected at concentrations as low as 5 pg.





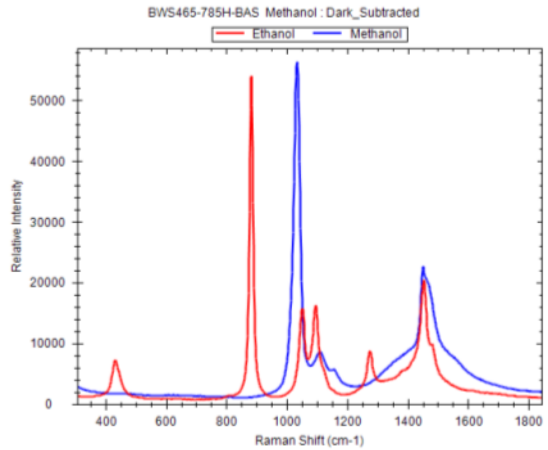
SEM image of SERS substrate used in the measurement.



S. Botti et al., J. Raman Spectrosc. 2013, 44, 463–468

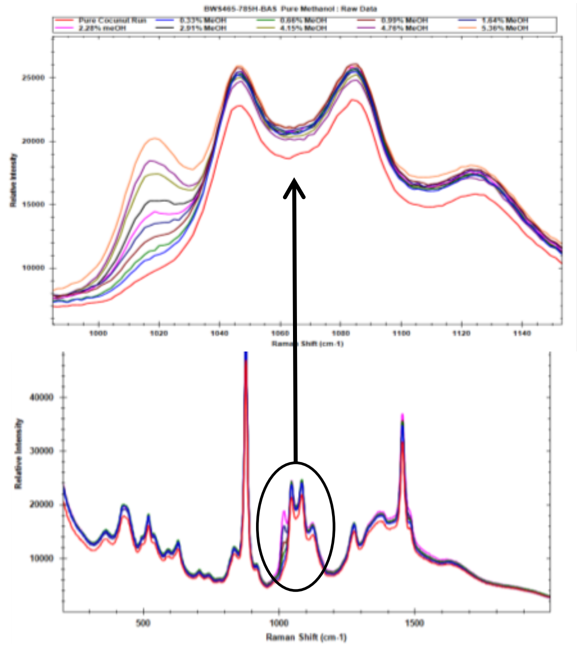
Product Contamination – Methanol-Laced Spirits

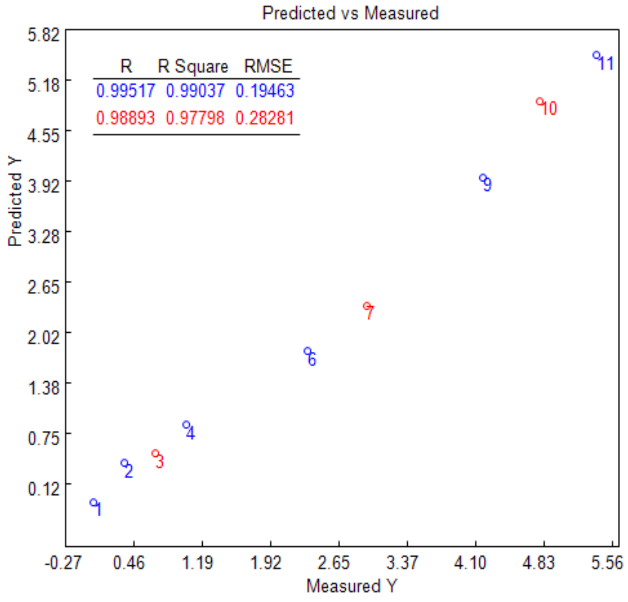
- Over the past several years an alarming trend has become evident that there are serious issues with contaminated alcohol within the EU, and in particular Eastern Europe.
- Studies have shown that the maximum tolerable concentration of methanol in alcoholic beverages with about 40% alcohol is about 2% (v/v) by volume.
- In September of 2012 when the Czech Republic banned the sale of hard liquor after 20 people died from the consumption of methanol-laced spirits.
- After an exhaustive study of different screening tools the Czech Republic turned to the use of Raman spectroscopy as the screening tool of choice for the identification and quantification of methanol in contaminated spirits.

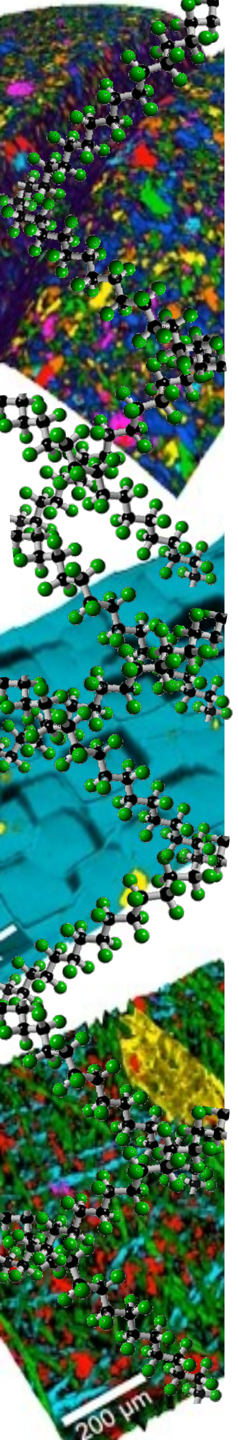


Example using Methanol-Laced Coconut Rum

- CH_3 bending vibration at 1013 cm^{-1} increases with methanol concentration. A PLS regression method can be developed to readily measure concentration of MeOH in alcoholic beverage by Raman spectroscopy

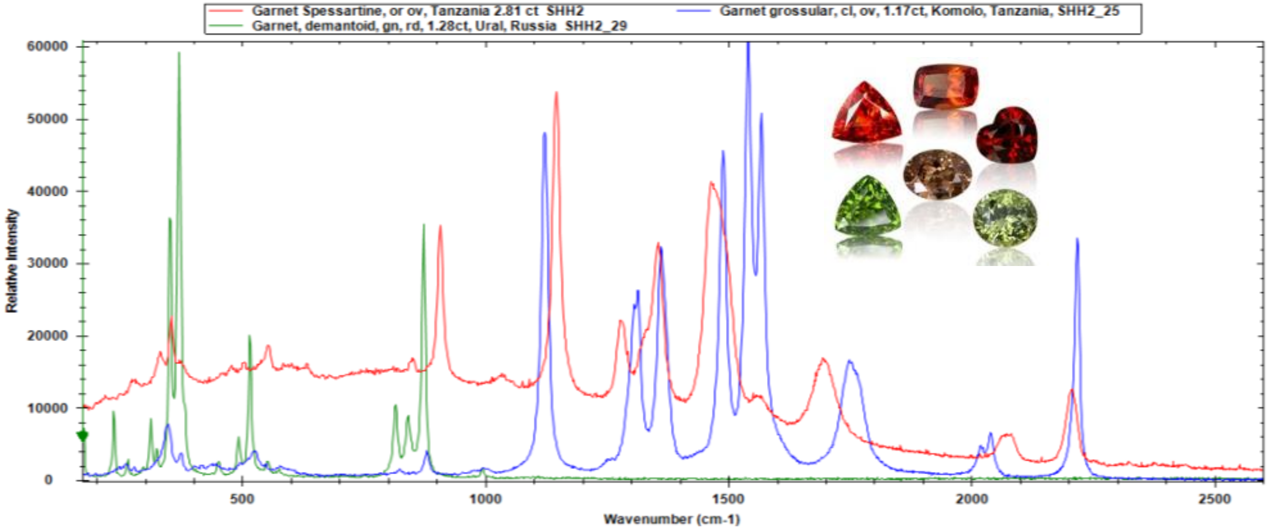




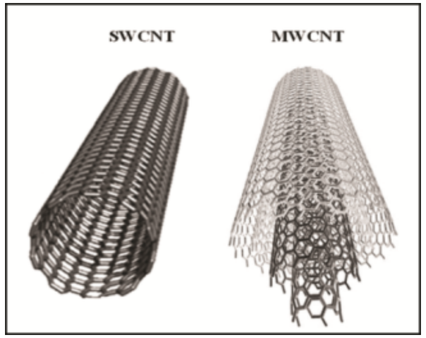


Analysis of Garnet Gemstones

- Garnets are a class of silicate minerals which include a number of varieties with the general form $X_3Y_2(SiO_4)_3$.
- Raman spectroscopy's high selectivity allows for the differentiation of the different garnet varieties. Andradite and grossular fall into the ugrandite group of garnets (calcium in X site), while spessartine falls into the pyralspite group (aluminum in Y site).



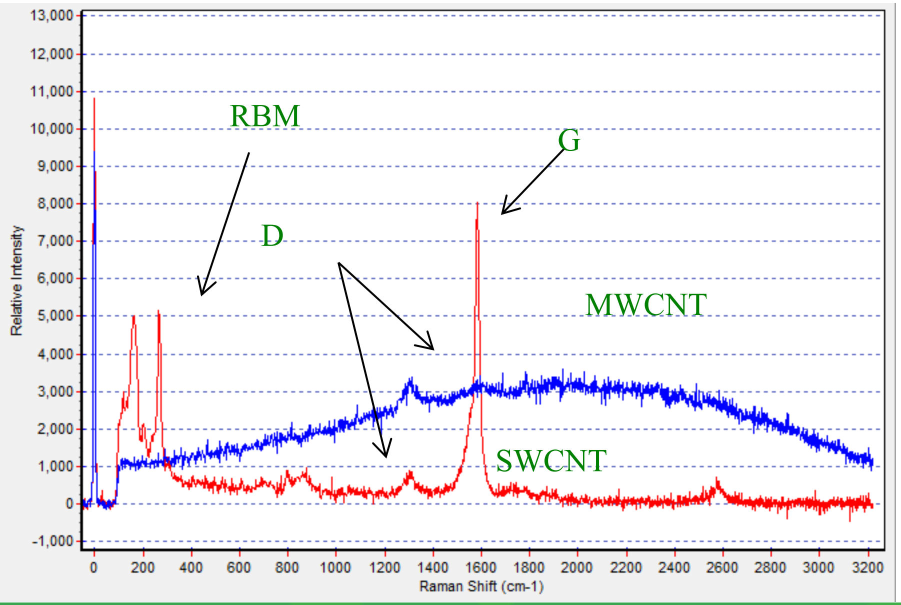
Raman Analysis of Carbon Nanotubes



RBM – Radial breathing modes, which probe the lattice structure of the CNT allowing for the calculation of tube diameter.

D Band – Disorder band, measures the degree of amorphism of the CNT.

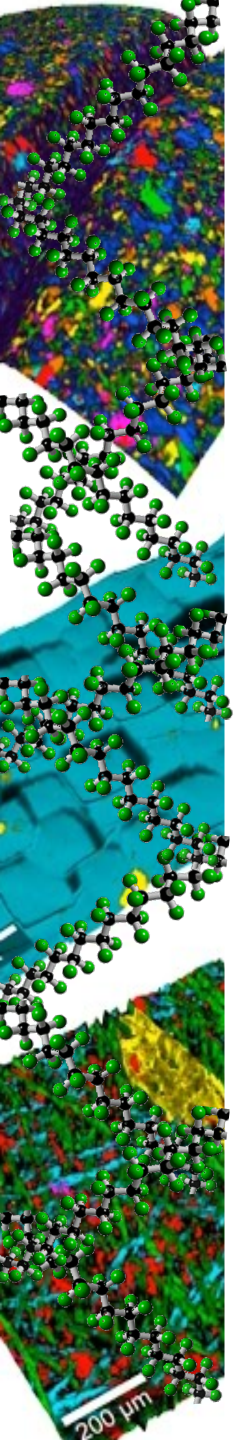
G Band – Tangential Mode, measures the degree of crystallinity (diamond like structure) of the CNT.



Applications in Geology and Mineralogy

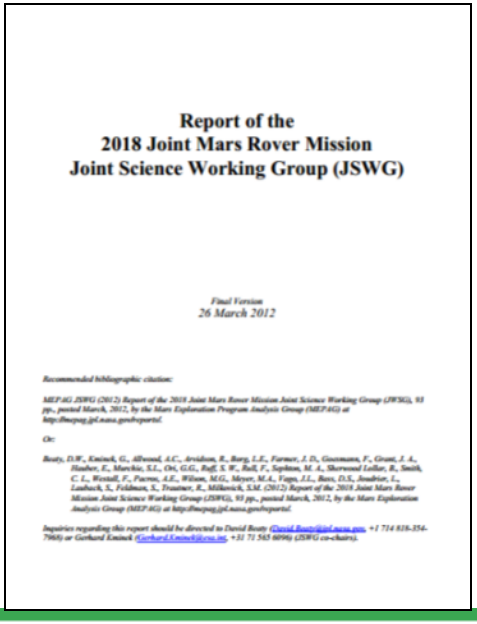
- Raman spectrometers are ideal for the identification of gemstones and minerals, including polymorphs and isomorphs.
- Non-contact, non-destructive sampling allows for analysis of precious or scarce samples, unlike other techniques such as LIBS.
- Anti-counterfeiting of precious, such as identification of diamond from zircon

Images Courtesy of Prof. Rull University of Valladolid



Raman evaluated for Geological survey on Mars

Before testing for life on other planets, feasibility studies are done on barren areas of the Earth. One such place is Rio Tinto in Spain, where conditions are analogous to Mars, where Raman spectrometers were evaluated for the joint NASA/EAS 2018 Mars rover mission.



Report of the
2018 Joint Mars Rover Mission
Joint Science Working Group (JSWG)

Final Version
26 March 2012

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Or:

Beaty, D.W., Kminek, G., Allwood, A.C., Arvidson, R., Borg, L.E., Farmer, J. D., Goesmann, F., Grant, J. A., Hauber, E., Marchis, S.L., Ori, G.G., Ruff, S. W., Rull, F., Sephton, M. A., Sherwood Lollar, B., Smith, C. L., Westall, F., Pacros, A.E., Wilson, M.G., Meyer, M.A., Vago, J.L., Bass, D.S., Joudrier, L., Laubach, S., Feldman, S., Trautner, R., Milkovich, S.M. (2012) Report of the 2018 Joint Mars Rover Mission Joint Science Working Group (JSWG), 93 pp., posted March, 2012, by the Mars Exploration Analysis Group (MEPAG) at http://mepag.jpl.nasa.gov/reports/.

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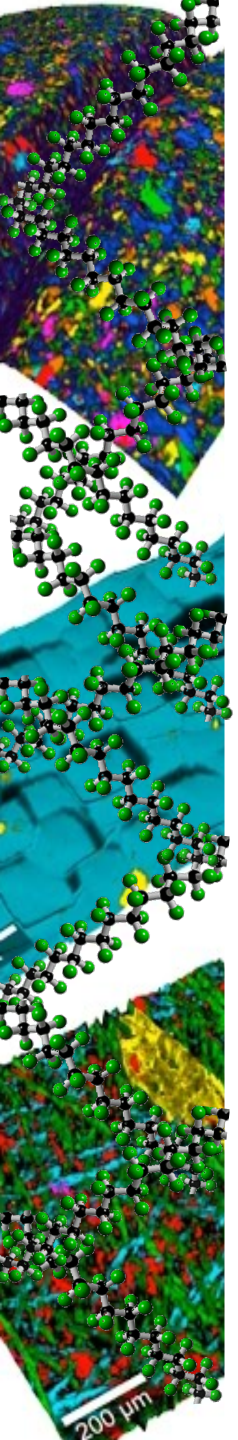
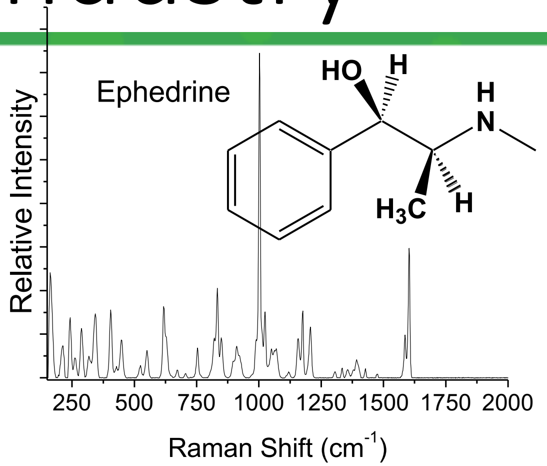
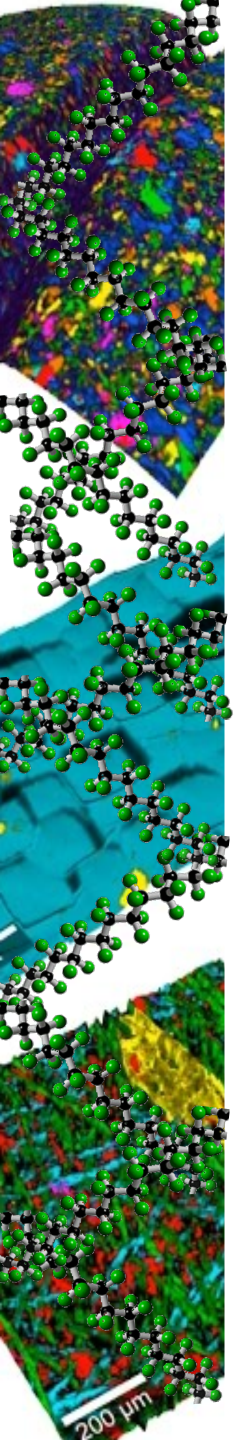
Images Courtesy of Prof. Rull University of Valladolid



Food & Agriculture Industry

- Measuring chain length and extent of saturation of fatty acids in edible oils
- Meat product quality analysis
- Product contamination
- SERS analysis of food contaminants including bacteria, antibiotics, dyes, etc.
- Analysis of components in grain kernels
- Raw material identification/verification for the food and beverage industries

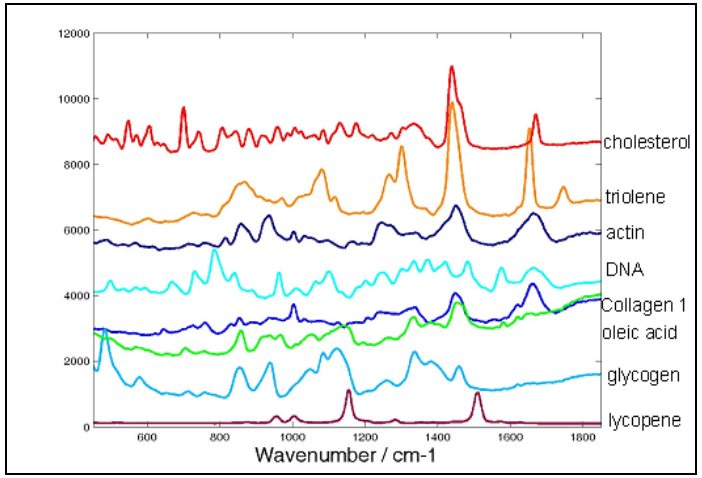


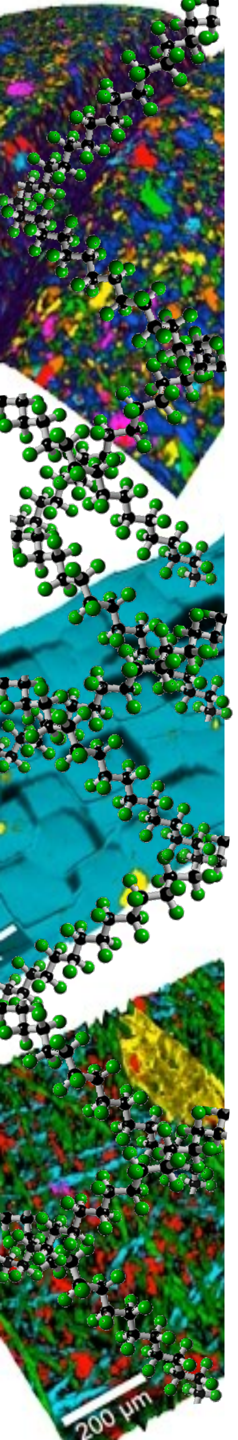


Applications in the Biomedical Diagnostics

- Raman spectroscopy is becoming more pervasive in biomedical diagnostics because of the demand for near real time and minimally invasive analysis. Applications include: biopsies, cytology, drug efficacy studies, histopathology, surgical targets and treatment monitoring.
- Some of the most active research areas are the analysis of abnormalities in tissue samples such as brain, arteries, breast, bone, cervix, embryonic media; and the identification of biomarkers for early stage detection of various diseases.

- Raman has also been used to investigate blood disorders such as anemia, leukemia and thalassemias (inherited blood disorder), as well as understanding cell growth in bacteria, phytoplankton, viruses and other micro-organisms.





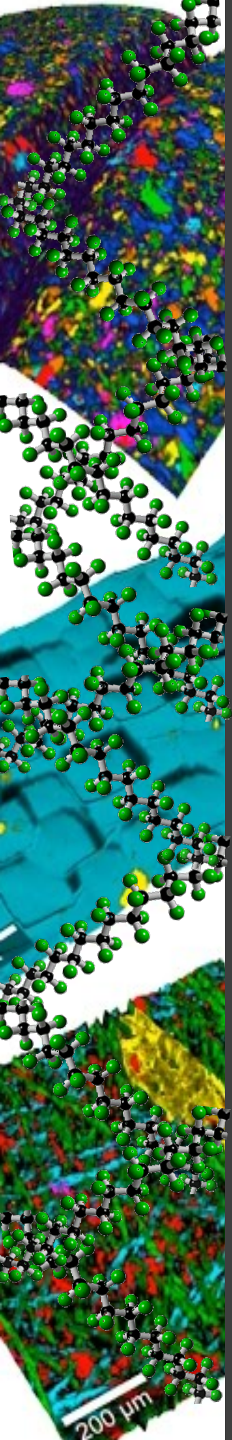
Conclusions

Advantages:

- All types of aggregation states (solid, liquid and gas)
- No sample preparation required.
- Non-destructive technique.
- Obtaining the Raman spectrum is fast.
- Glass containers can be used.

Disadvantages:

- Cannot be applied to metals or alloys.
- The Raman effect is very weak.
- Interference with materials showing fluorescence.



Thank you