Chemical and Biological Applications of Raman Spectroscopy

Amanda Prance [a], Janet Peachey [a], Rheannon Harvey [b], Maria Hepel [a], Magdalena Stobiecka [a], Robert Ewy[b]

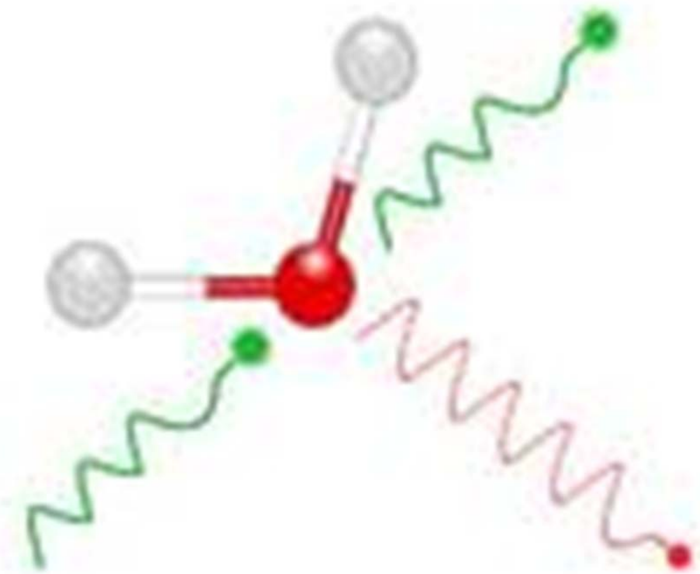
[a] Department of Chemistry, [b] Department of Biology, State University of New York at Potsdam, 13676 Potsdam, NY, U.S.A

Raman spectroscopy

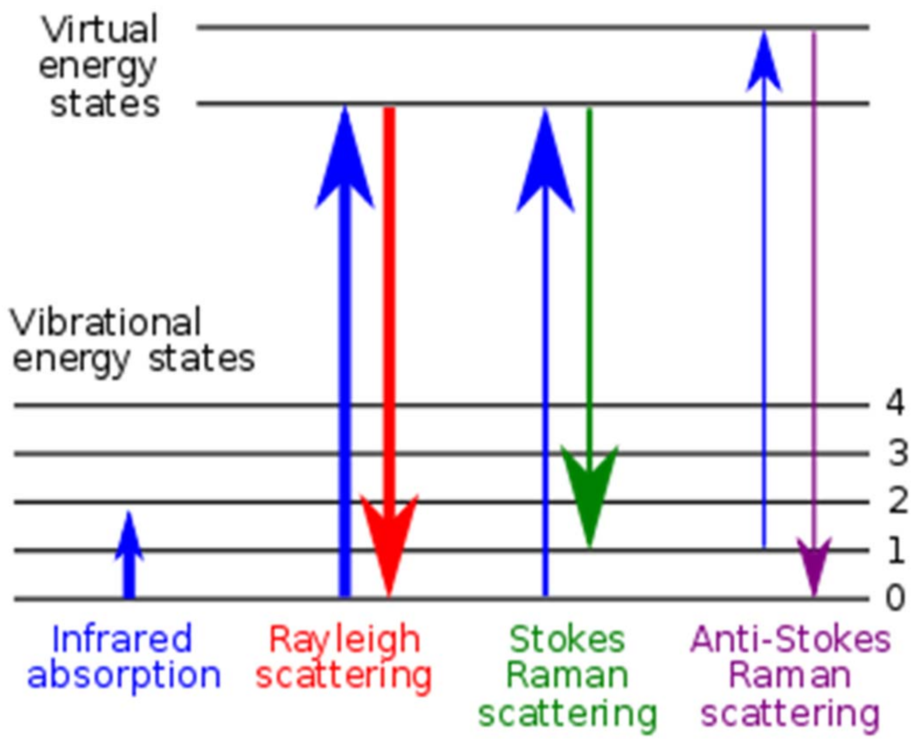
Raman spectroscopy is an invaluable technique for investigations of pharmaceutically and medically relevant molecules. It is used in many fields where non-destructive microscopic chemical analysis and imaging is required. Both Biologists and Chemists use Raman Spectroscopy to identify chemical compounds, their functional groups, and to determine the conformation of complex biomolecules, such as proteins and DNA. It can be used to rapidly characterise the chemical composition and structure of a sample, whether solid, liquid, gas, gel, slurry or powder. It is particularly amenable to *in vivo* measurements.

Raman spectroscopy and the infrared absorption spectroscopy, are the most widely used techniques which give information about the structure and properties of molecules by providing detailed characteristics of their vibrational transitions. Raman analysis can provide key information easily and quickly.

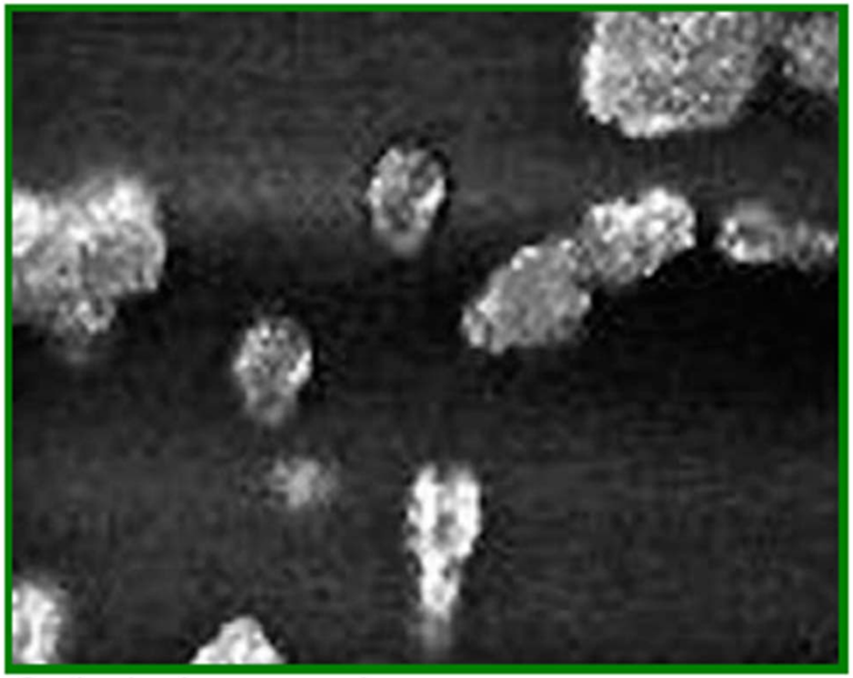
The Raman spectroscopy is based on an inelastic light scattering by molecules (the Raman effect). In the Raman scattering process, a photon interacts momentarily with a molecule and is then scattered into surroundings in all directions. During the brief interaction with molecule, photon loses or gains energy which is then detected and analyzed.



Light scattered from a molecule



Virtual
energy
states
Vibrational
energy states
4
3
2
1
0
Infrared
absorption
Rayleigh
scattering
Stokes
Raman
scattering
Anti-Stokes
Raman
scattering

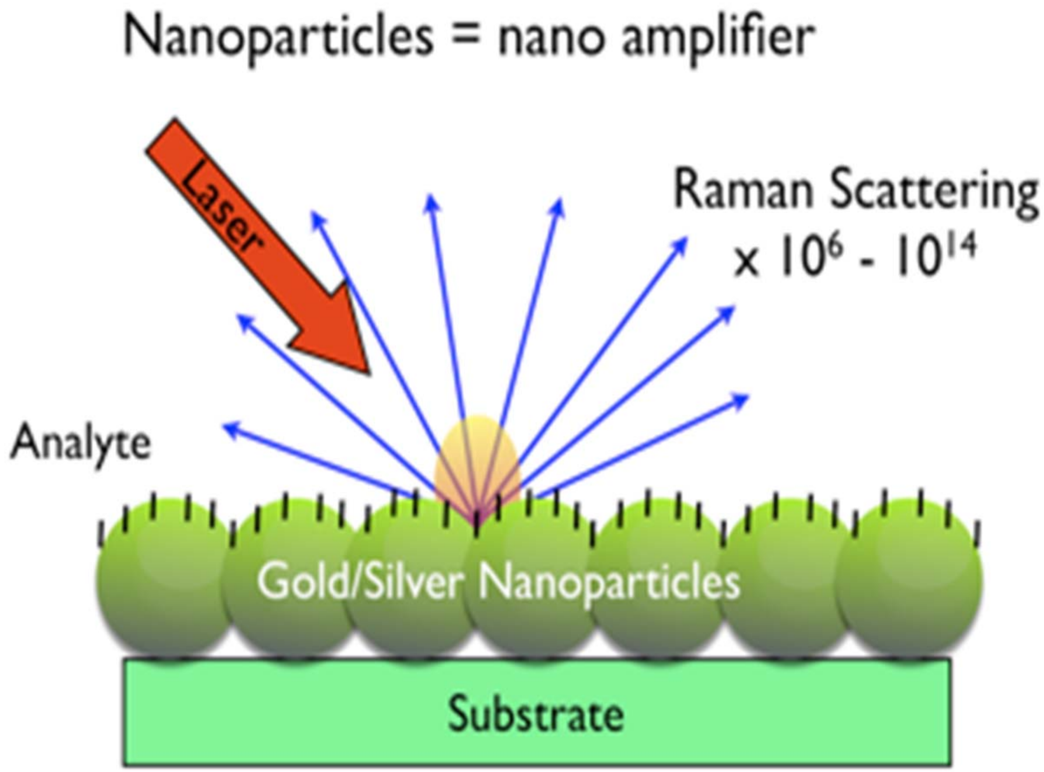


Optical microscopy images

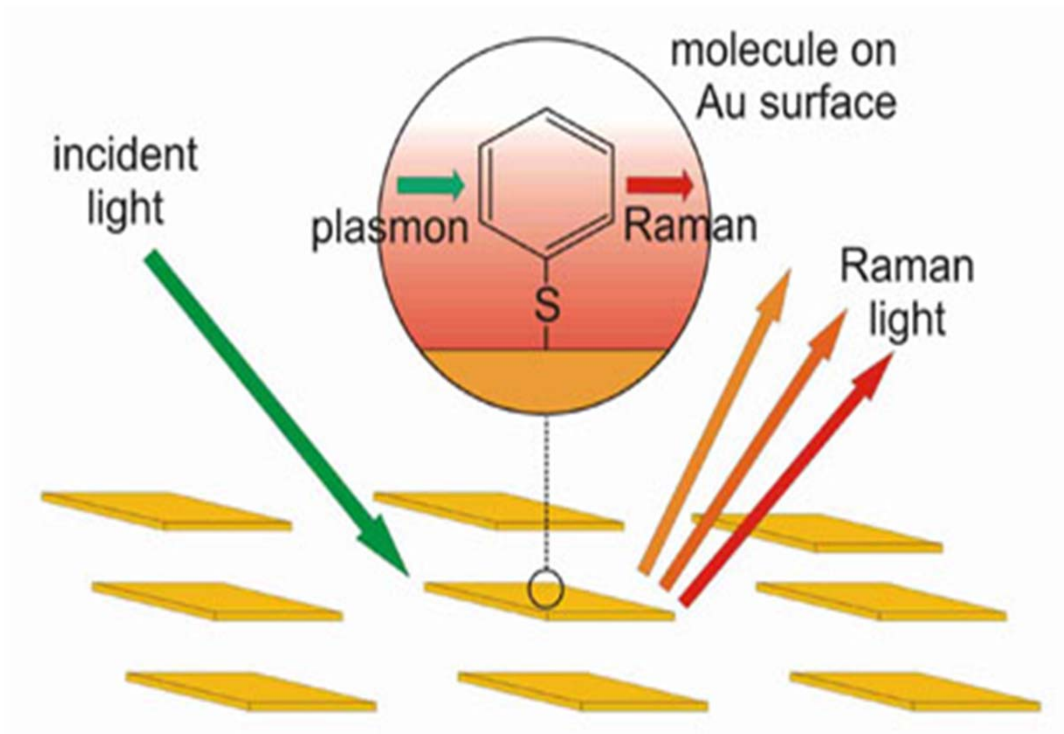
http://www.horiba.com *Raman Application Note*, Pharmaceutics 01

Surface enhanced Raman spectroscopy

The enhancement of the Raman signal has been obtained in surface-enhanced Raman spectroscopy (SERS) by molecules adsorbed on rough metal surfaces (silver, metal nanoparticles). SERS can be used to identify the specific single nucleotide polymorphisms and to identify and classify microorganisms at the species and subspecies level. This technique has been also applied for rapid identification of microorganisms in clinical diagnosis.


Nanoparticles = nano amplifier
Laser
Raman Scattering
x 10^6 - 10^{14}
Analyte
Gold/Silver Nanoparticles
Substrate

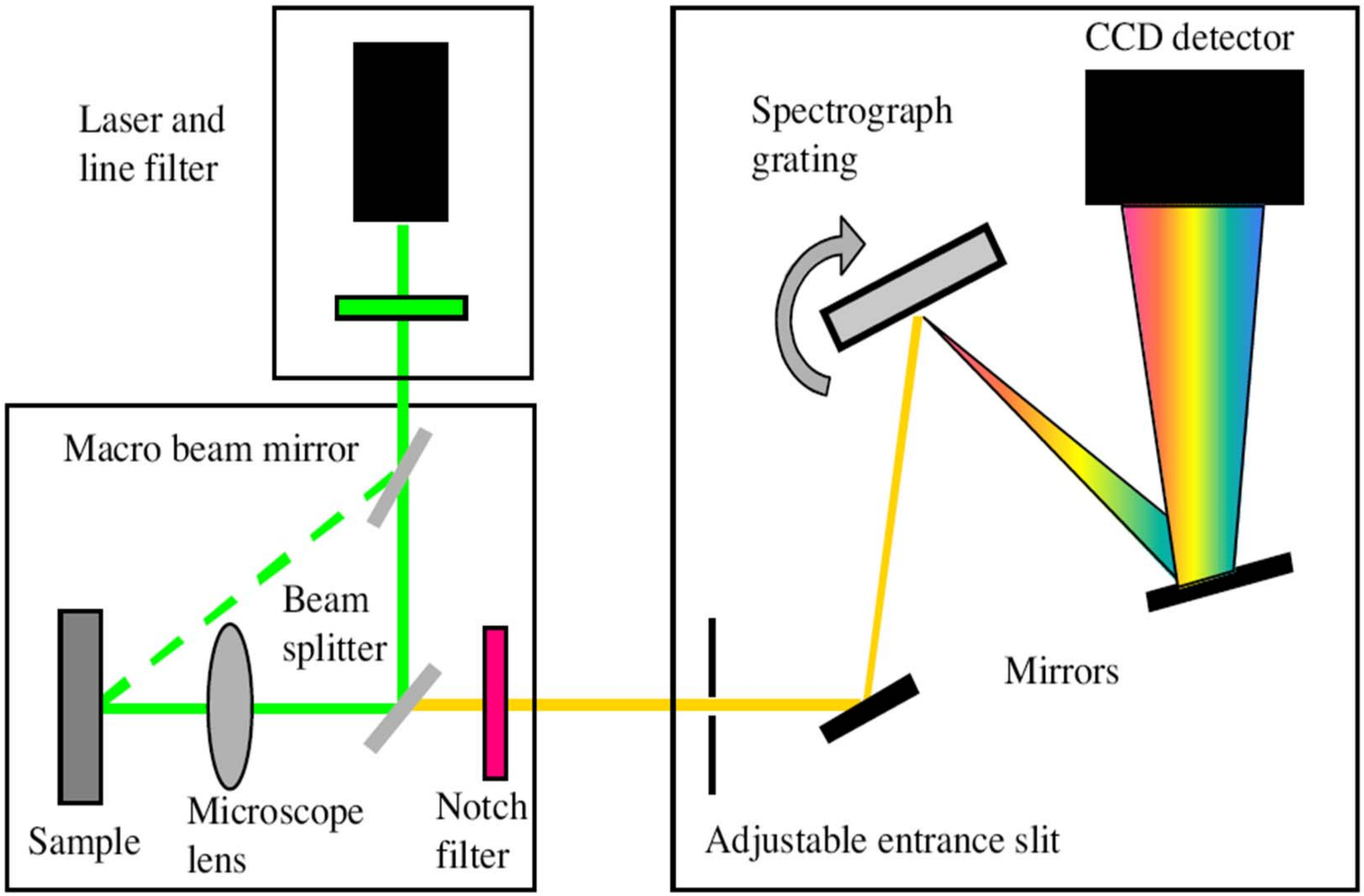
Au or AgNPs



Rough Au surface

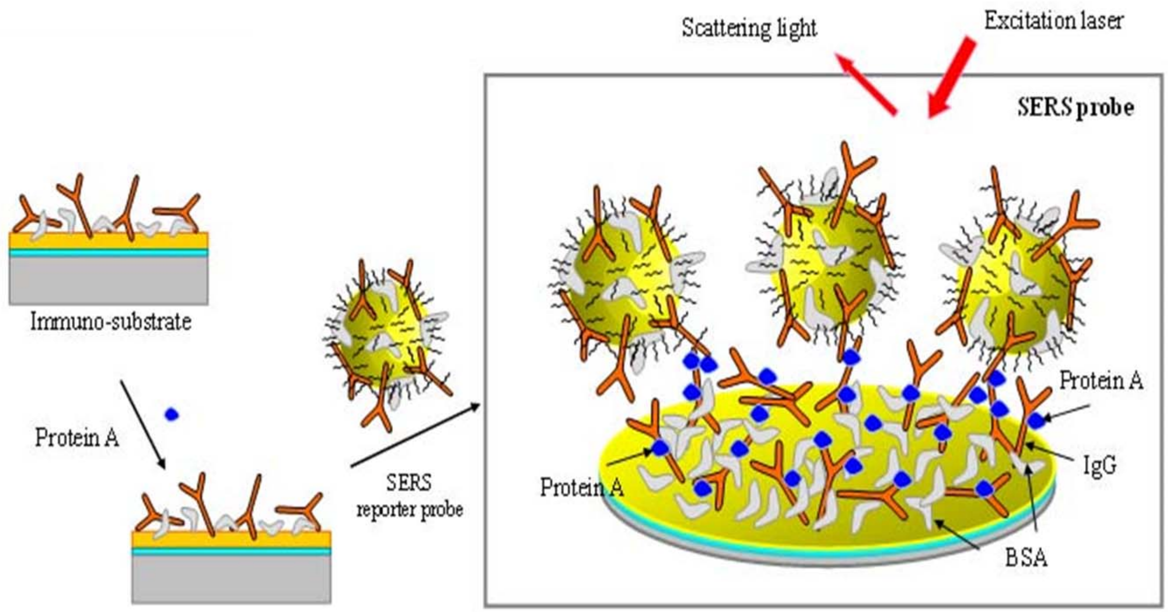
http://www.thermoscientific.com

Simplified Raman spectrometer layout



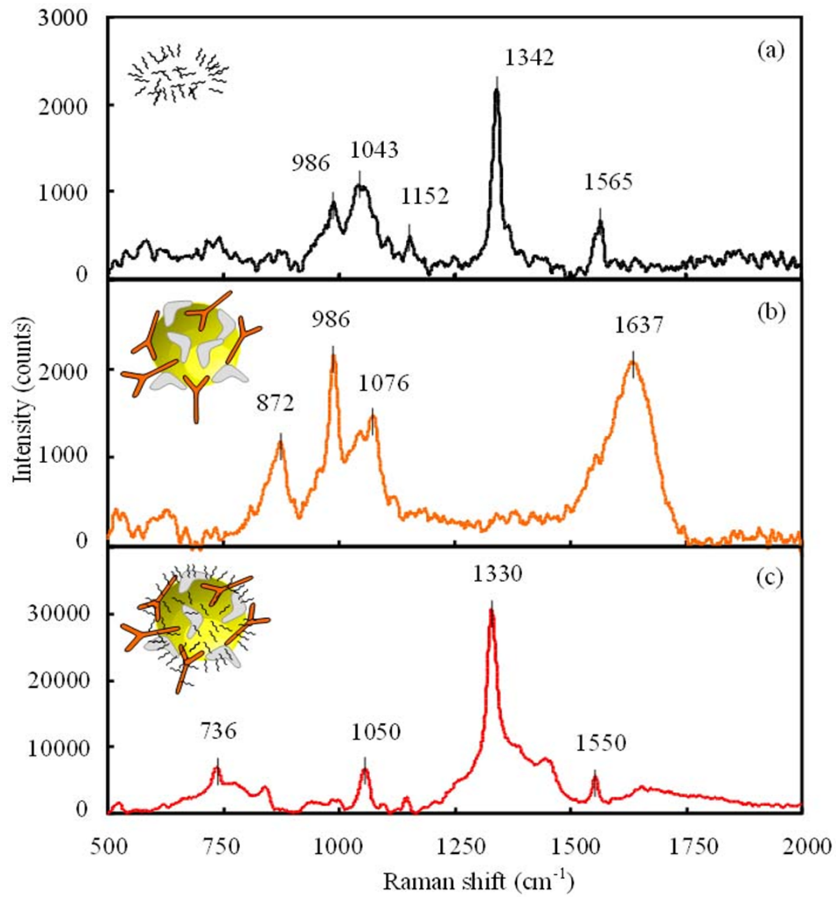
Biology

Characterisation of Biomolecules



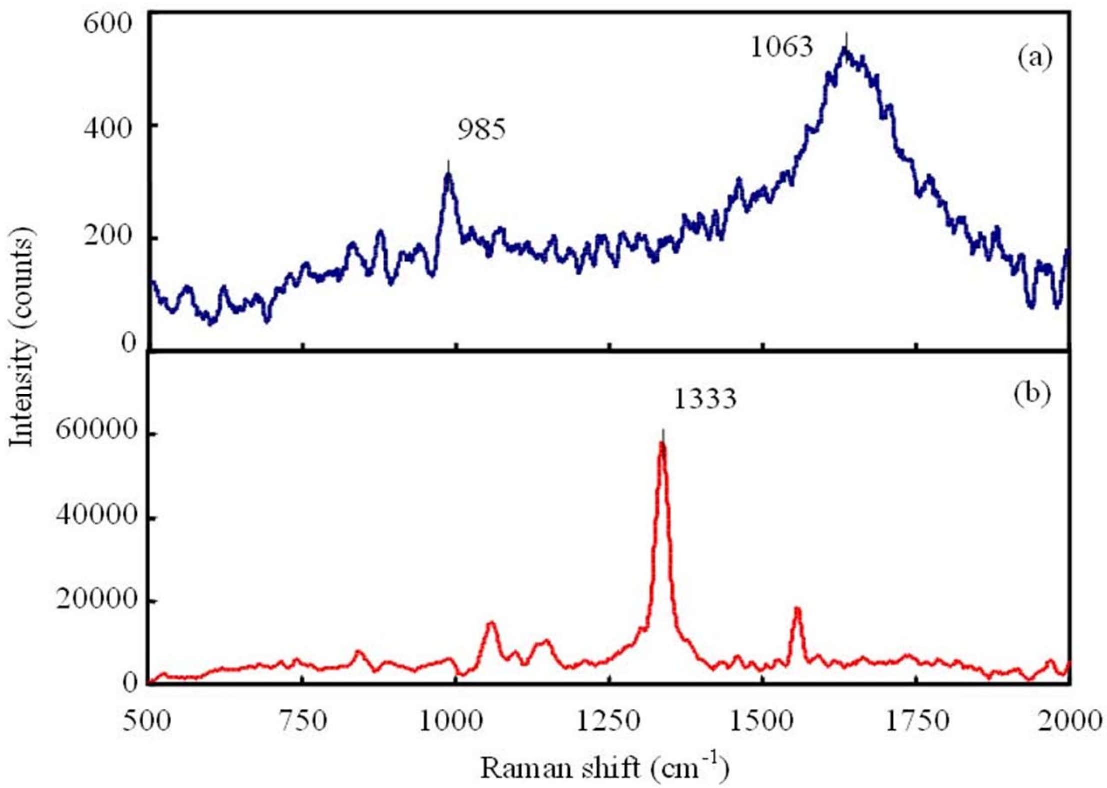
SERS-based immunoassay: The illustration of the sandwich interaction among the Raman reporter labeled immunoAuNP, the antigen, and the immunosubstrate.

C-C Lin, Y-M Yang, Y-F Chen, T-S Yang, H-C Chang, *Biosensors and Bioelectronics*, 2008, 24, 178–183



The Raman and SERS spectra of DTNB and IgG. (a) the Raman spectrum of DTNB (1 mM DTNB in d_2H_2O); (b) SERS spectrum of BSA-IgG-AuNP; (c) SERS spectrum of TNB-BSA-IgG-AuNP. Radiation of 633 nm was used for excitation.

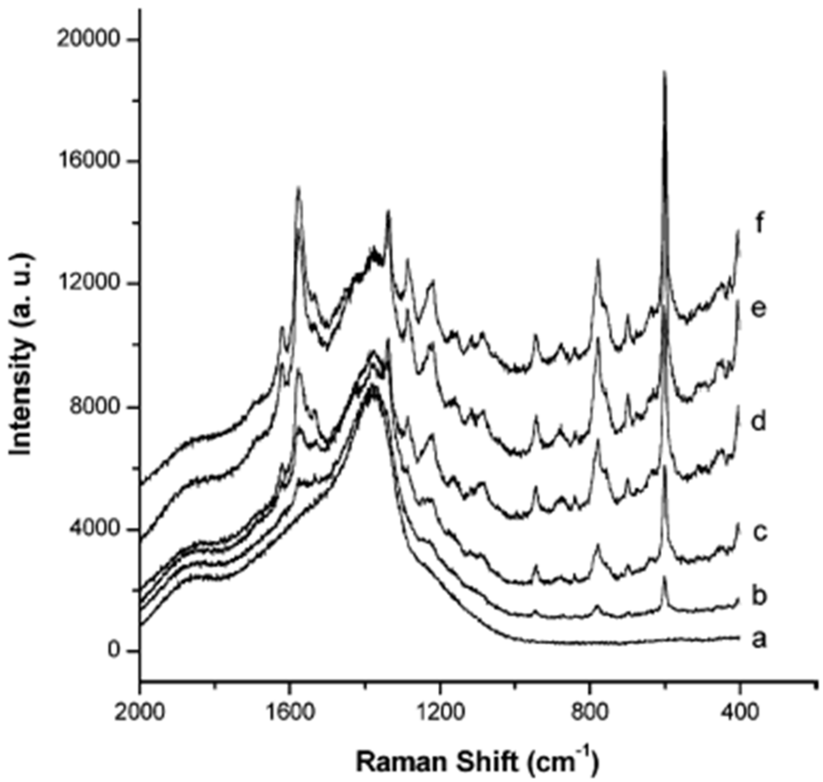
C-C Lin, Y-M Yang, Y-F Chen, T-S Yang, H-C Chang, *Biosensors and Bioelectronics*, 2008, 24, 178–183

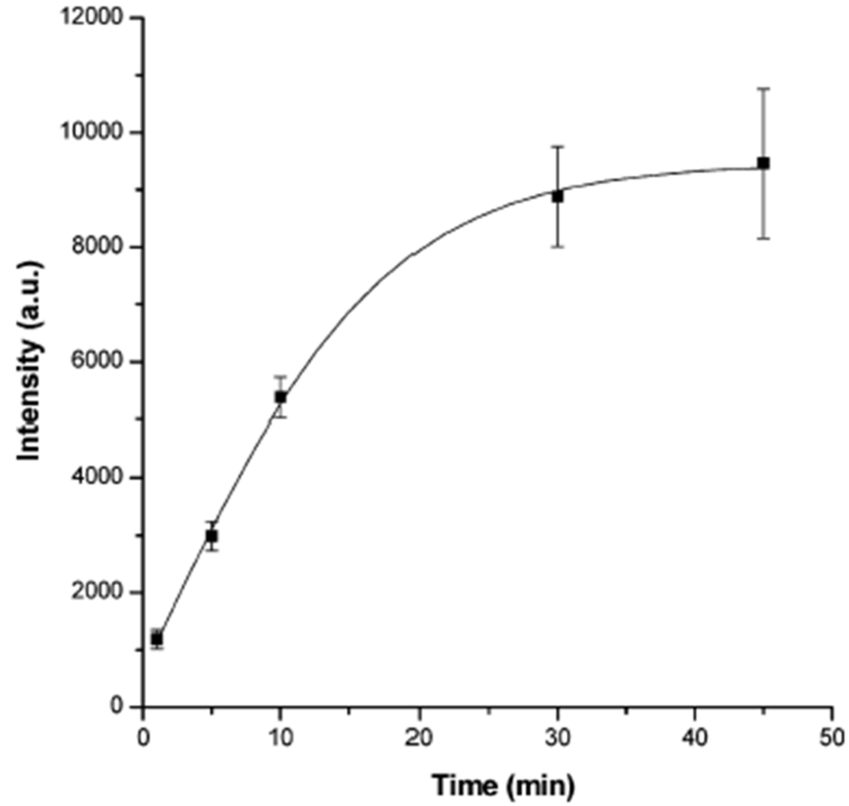


SERS spectra for protein A detection in immunoassay. (a) SERS spectrum of protein A (1 μg/mL). (b) SERS spectrum of the immunostrate/protein A/TNB-labeled immuno-AuNP. The spectra were obtained using 633 nm excitation.

C-C Lin, Y-M Yang, Y-F Chen, T-S Yang, H-C Chang, *Biosensors and Bioelectronics*, 2008, 24, 178–183

Alkaline Phosphatase

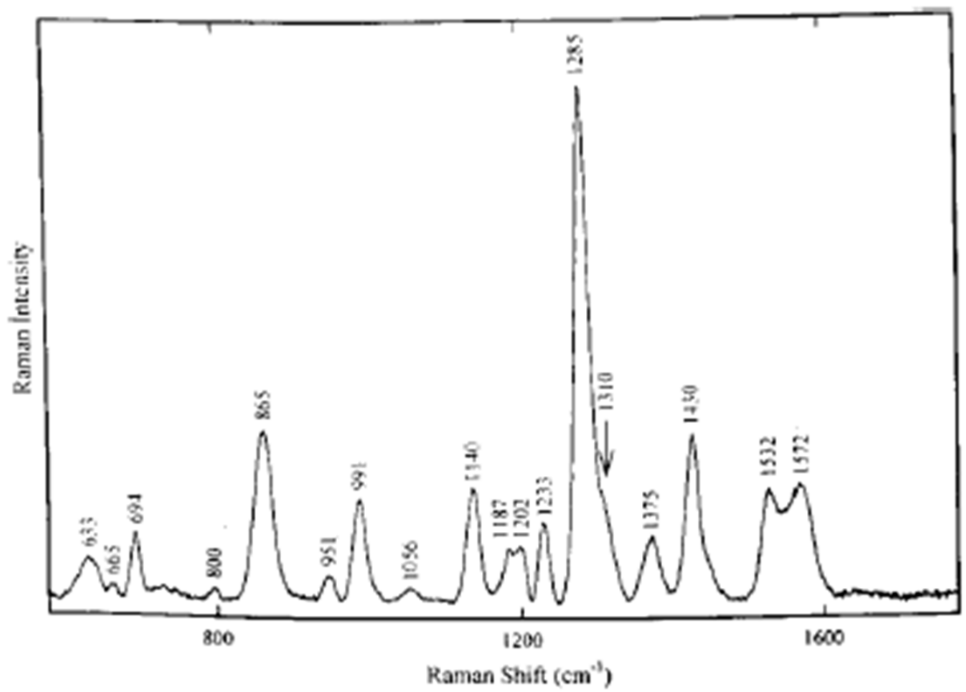




Time-dependent SERS spectra of alkaline phosphatase catalyzed hydrolysis of BCIP, C_{BCIP} = 0.4 mg/mL, C_{ALP} = 4.1 x 10 $^{-10}$ M; a) background without ALP, time: 1, 5, 10, 30, 45 min

C. Ruan, W. Wang, B. Gu, *Anal.Chem.* 2006, 78, 3379-3384

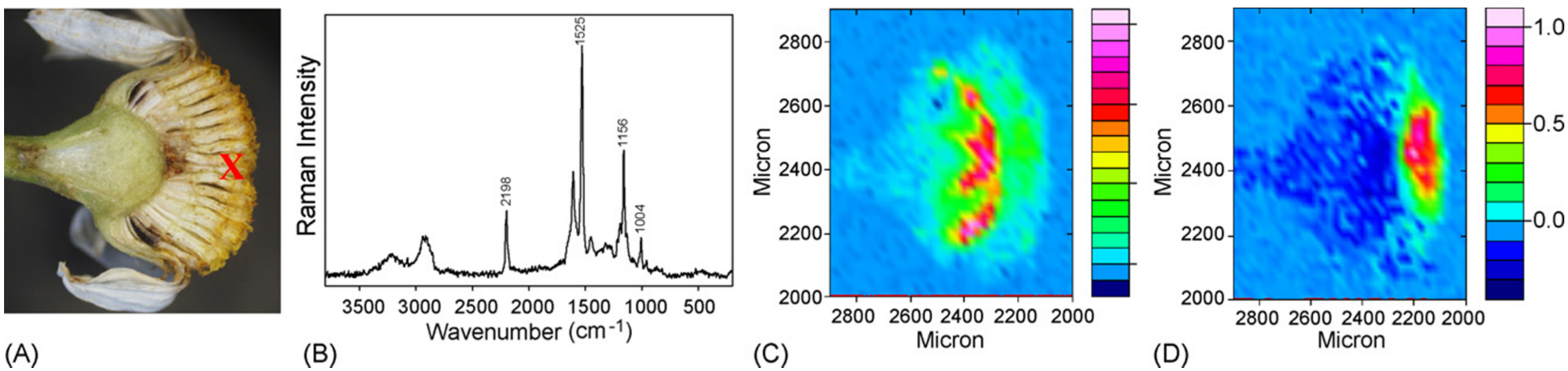
6-Mercaptopurine (6-MP)



SERS spectrum of 10^{-3} M 6MP with 488-nm laser excitation

A. Vivoni, S-P. Chen, D. Ejeh, C. M. Hosten, *Langmuir*, 16, 2000, 3310-3316

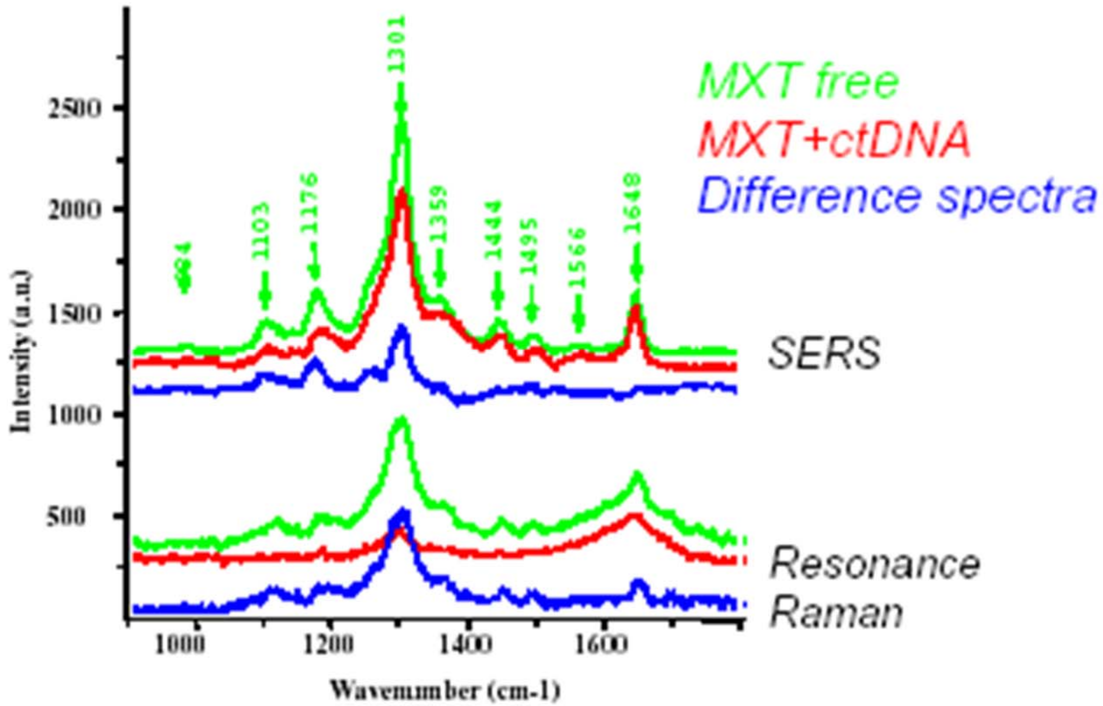
chamomile (*Chamomilla recutita) inflorescence*



Microscopic image of chamomile (*Chamomilla recutita) inflorescence (A), FT-Raman spectrum (B)* Raman mapping showing the distribution of polyacetylenes (C) and carotenoids (D).

A. Kudelski, *Talanta*, 2008, 76,1-8

Drug/DNA Interactions

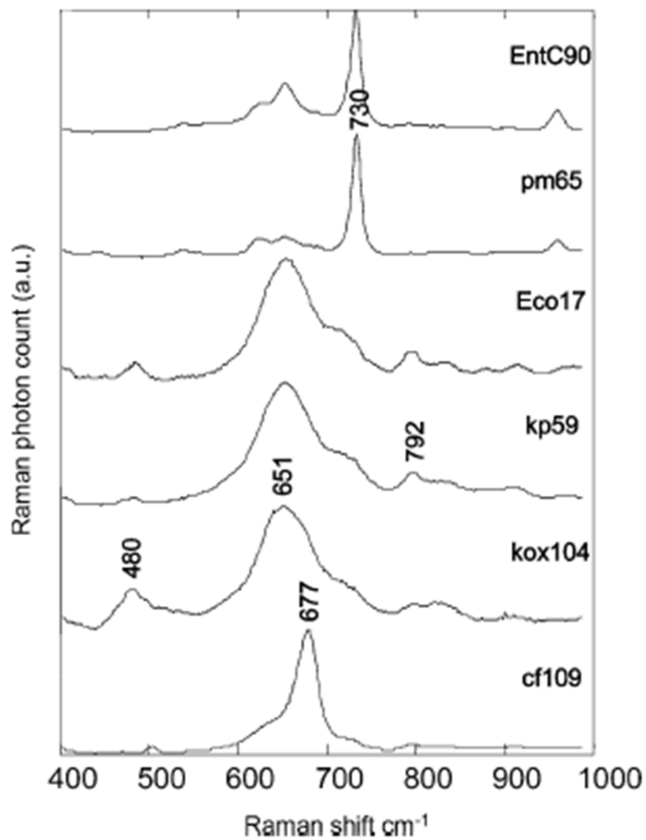


Comparative SERS and resonance Raman spectra

http://www.horiba.com *Raman Application Note*, Bio 05

Single Cell and Viral Analysis

Pathogens

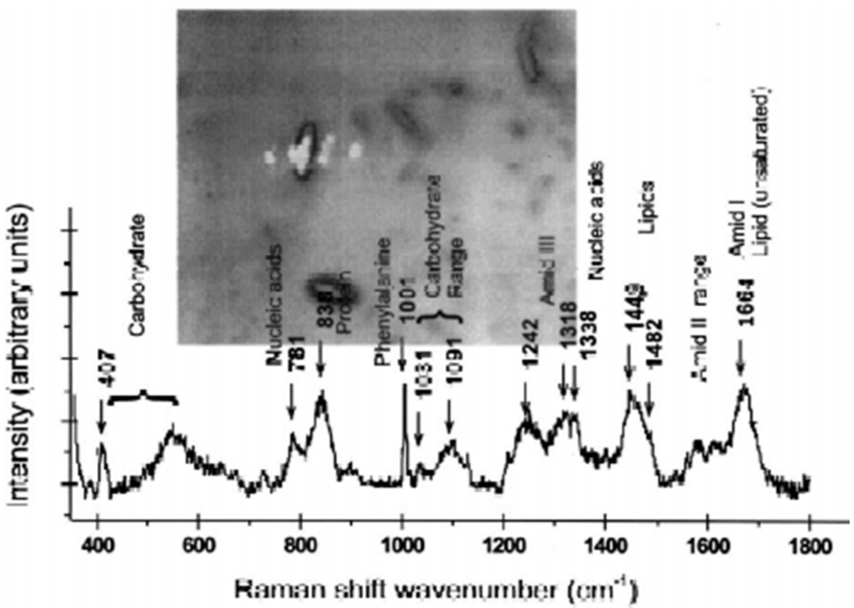


SERS signals for urinary tract bacteria: *Enterococcus* spp., *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Citrobacter freundii*

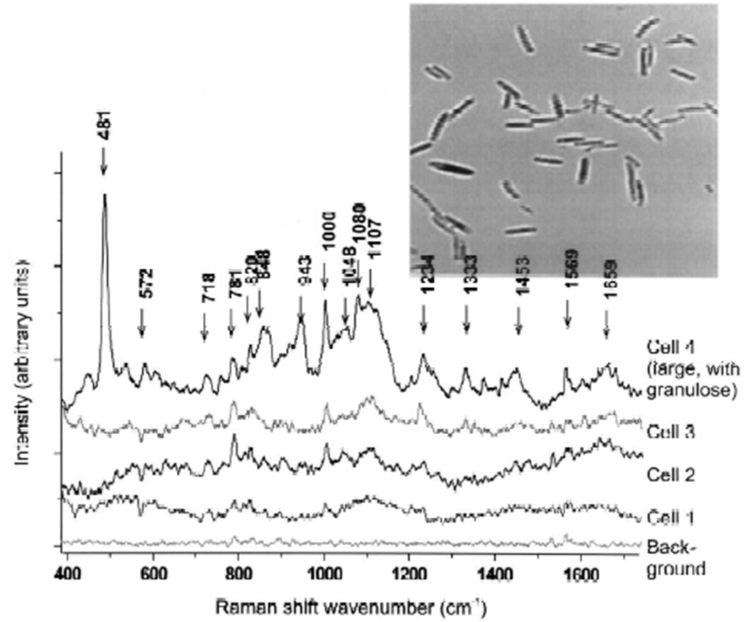
Intrinsic SERS signals for adenovirus (Ad), rhinovirus (rhino), and HIV

P.J. Vikesland, K.R. Wigginton, *Environ.Sci.Technol.,* 2010, 44, 3656-3669

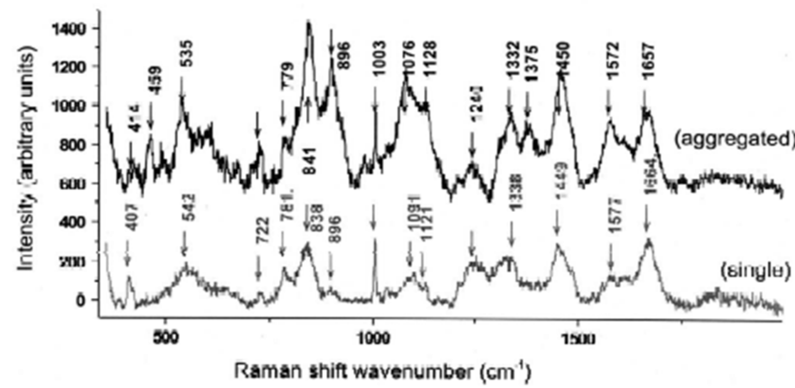
Bacteria Cells



Raman spectrum of a single cell of *Clostridium acetobutylicum*, of a size of about 2 x 4 μM



Different cells from the ‘early’sample

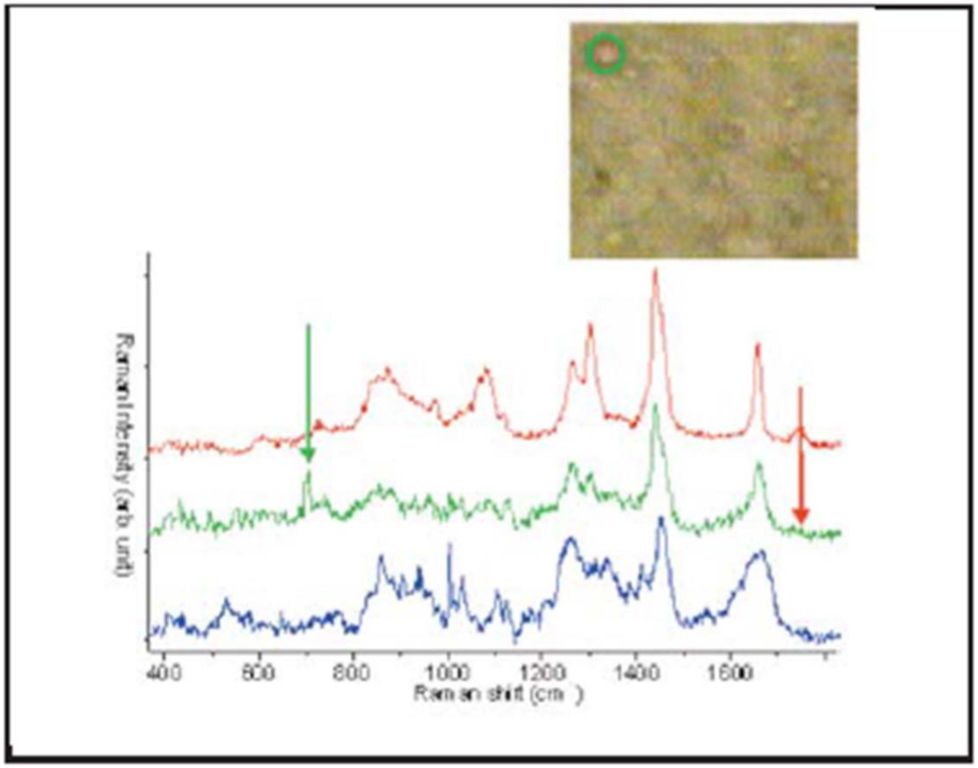


Spectra of the middle sample: Single and aggregates cells

K.C. Schuster, E. Urlaub, J.R. Gapes, *Journal of Microbiological Methods*, 2000, 42, 29-38

Medicine

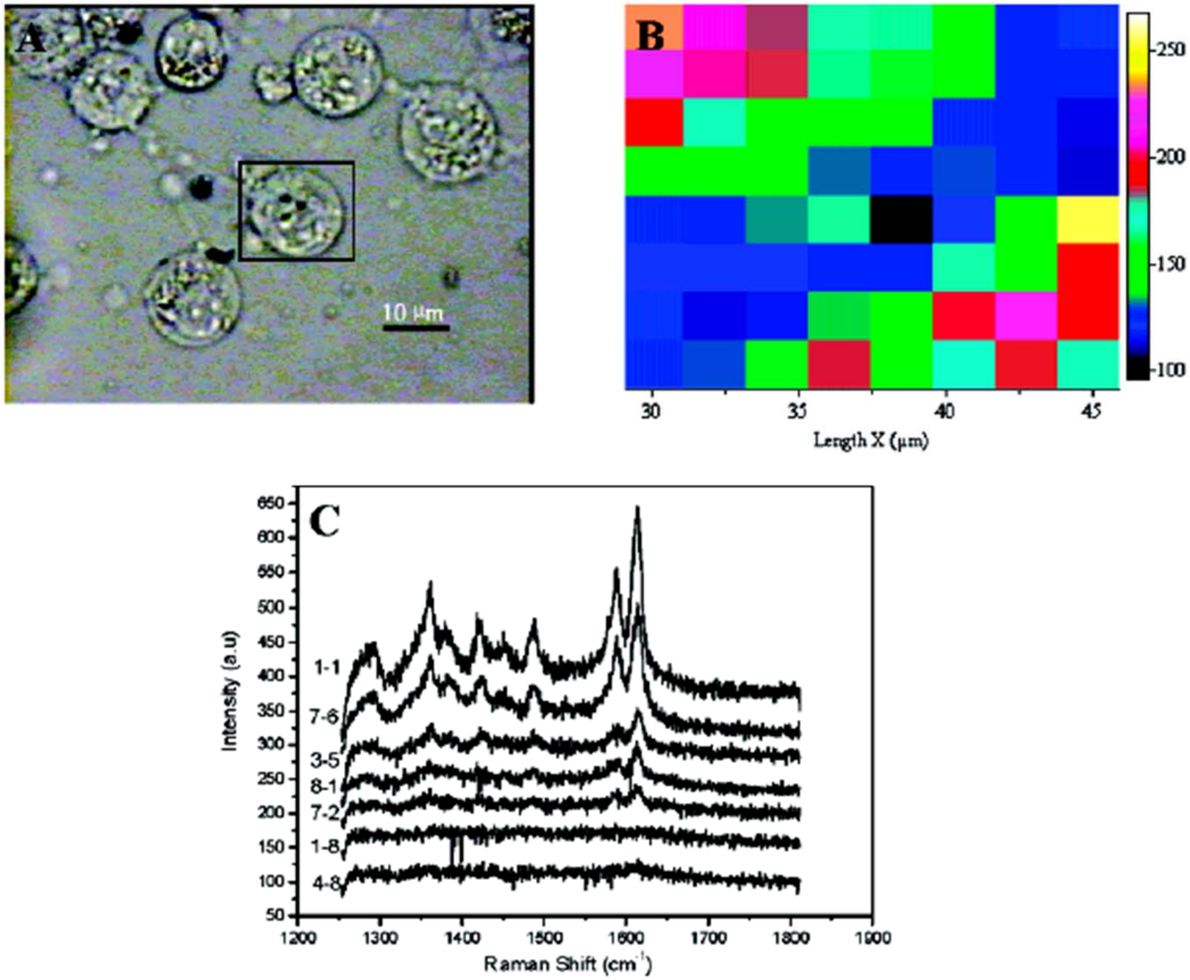
ApoE-knok-out Mouse



Optical micrograph of the lumen side of an aorta from an ApoE knock-out mouse. A globule is marked with a green circle. Raman spectra of globule (green), a fatty region (red), and a proteinaceous-rich region (blue)

http://www.horiba.com *Raman Application Note*, Bio 08

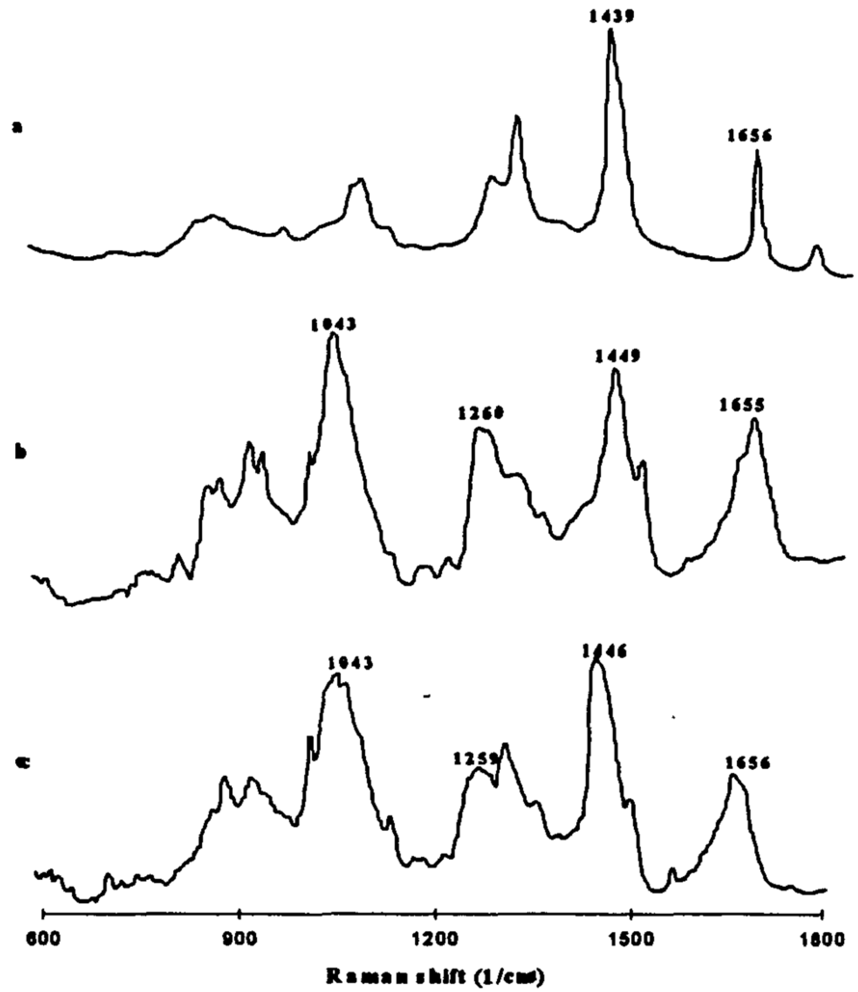
Optical Probes in Living Cells



A) The photomicrograph of the cells, (B) SERS spectra mapping of the BG-AuNPs in the living cell indicated in the rectanglar box, (C)SERS spectra of BG in the living cell

Y. Wang, D. Li, P. Li, W. Wang, W. Ren, S. Dong, E. Wang, *J Phys Chem C*, 2007, 111, 16833-16839

Cancer Detection

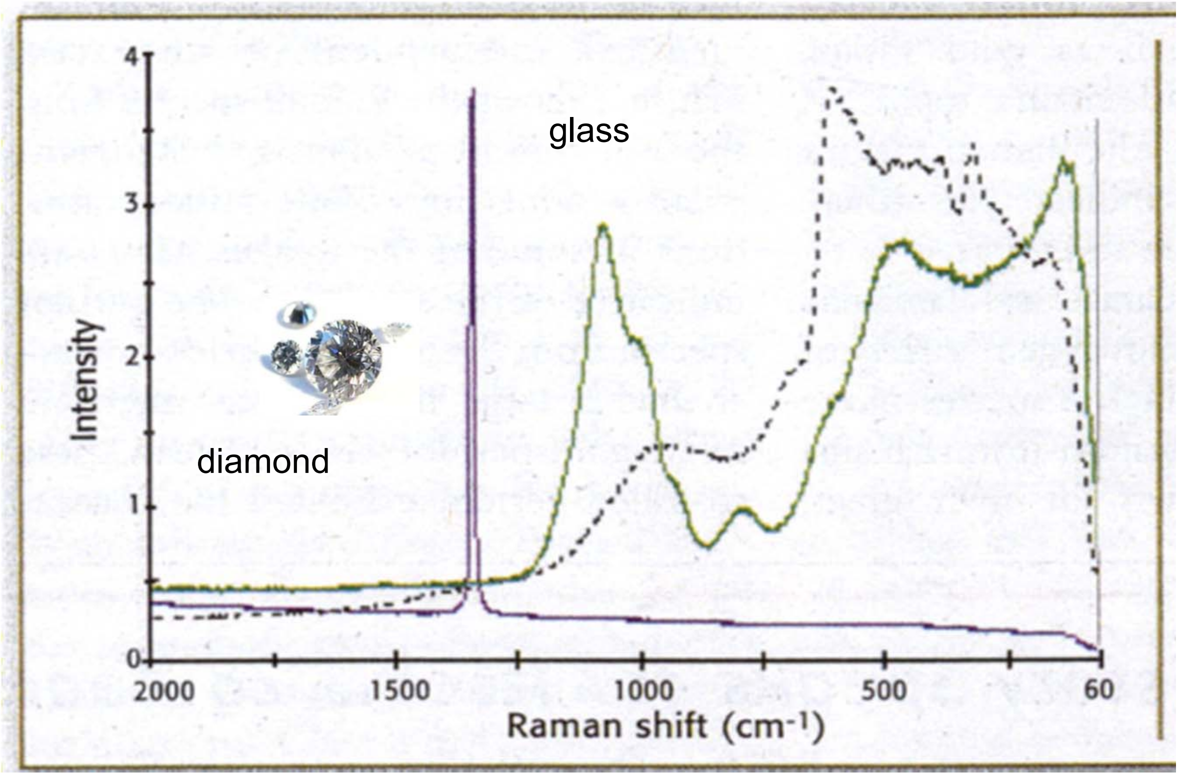


Raman spectra at 784 nm excitation pf (a) normal breast, (b) malignant, (c) benign tissue

A. Mahadevan-Jansen, R. Richards-Kortun, *Proceedings -19th International Conference IEEE/EMBS*, 1997, Chicago

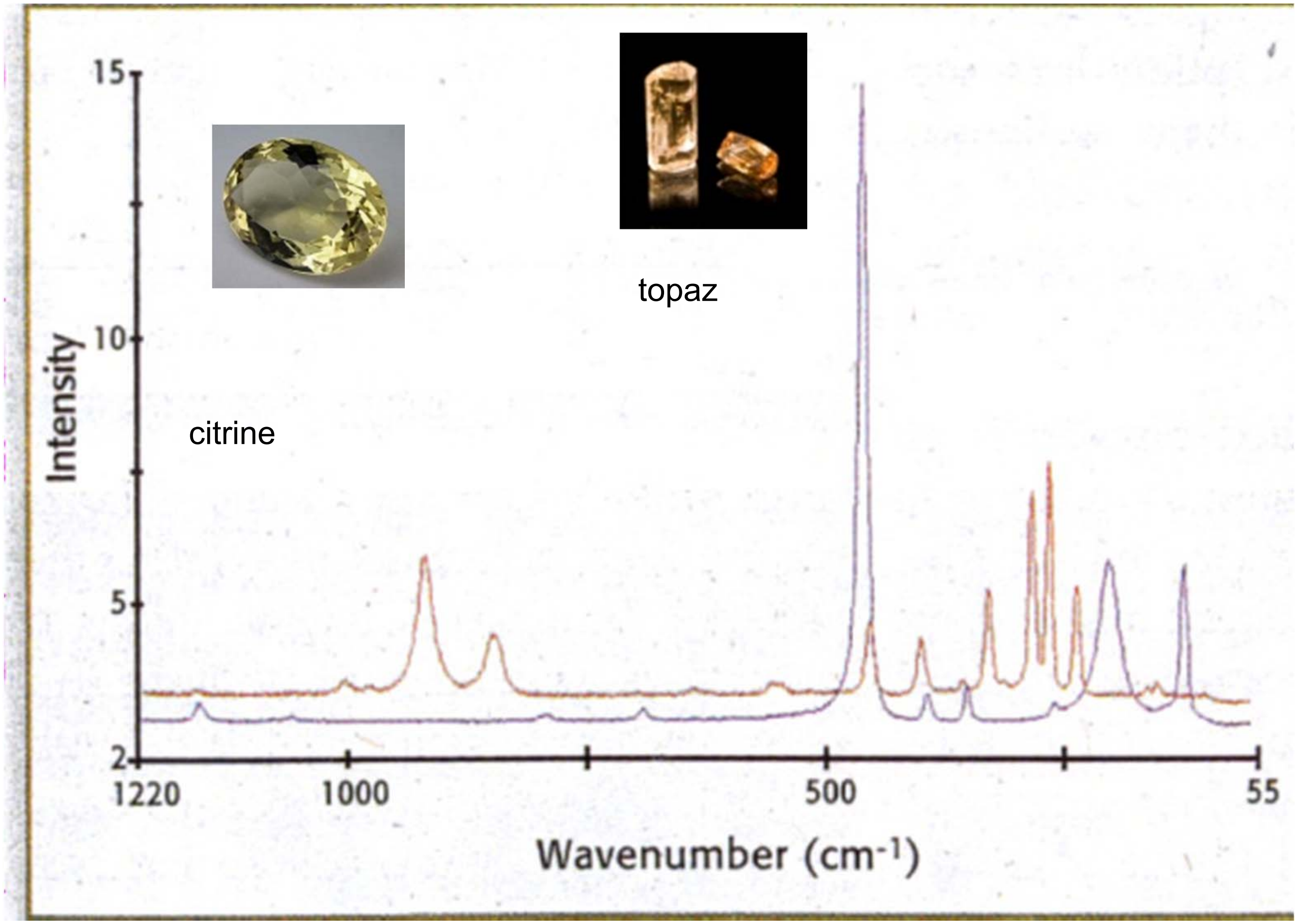
Geology and Mineralogy

Gemstone Identification



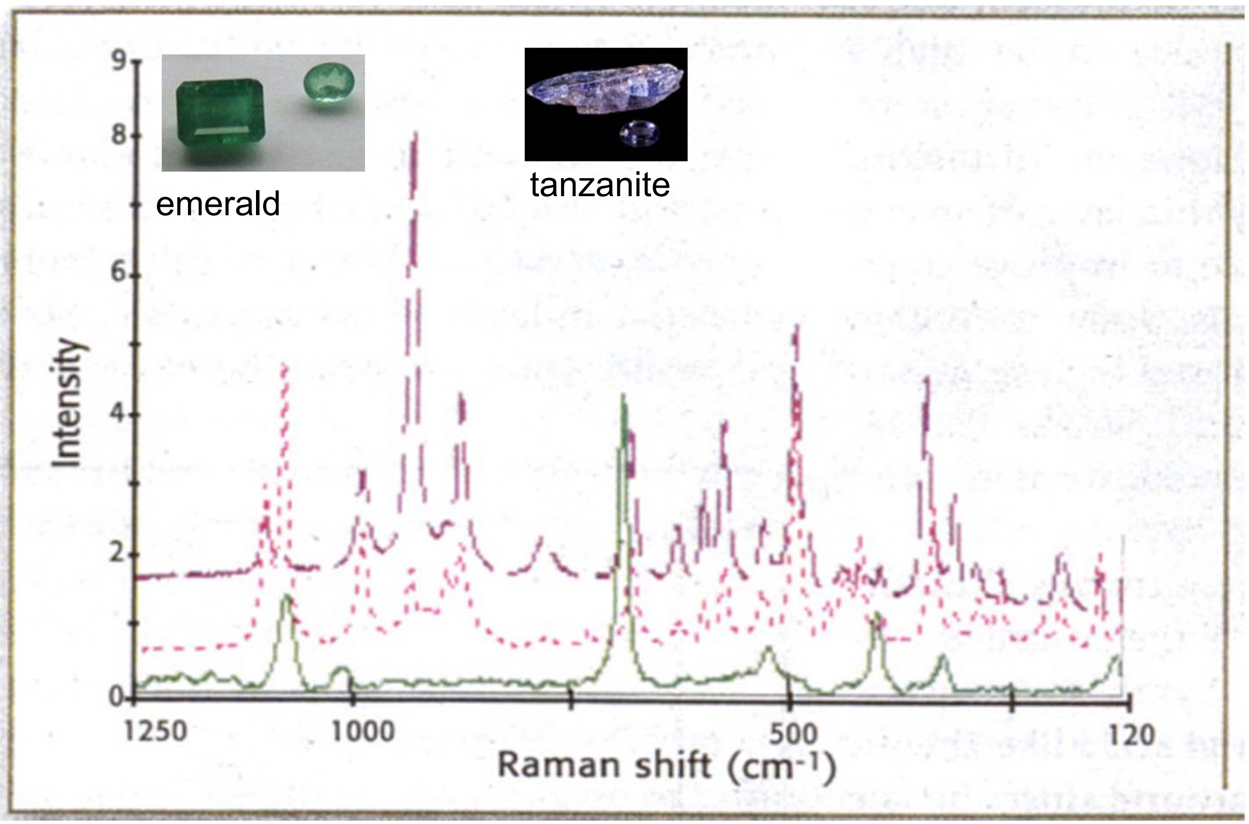
Differences between glass and diamond

A. L. Jenkins and R. A. Larsen, Spectroscopy , 2004, 19 (4)



Differences between citrine and topaz

A. L. Jenkins and R. A. Larsen, Spectroscopy , 2004, 19 (4)



Differences between emerald and two tanzanites

A. L. Jenkins and R. A. Larsen, Spectroscopy , 2004, 19 (4)

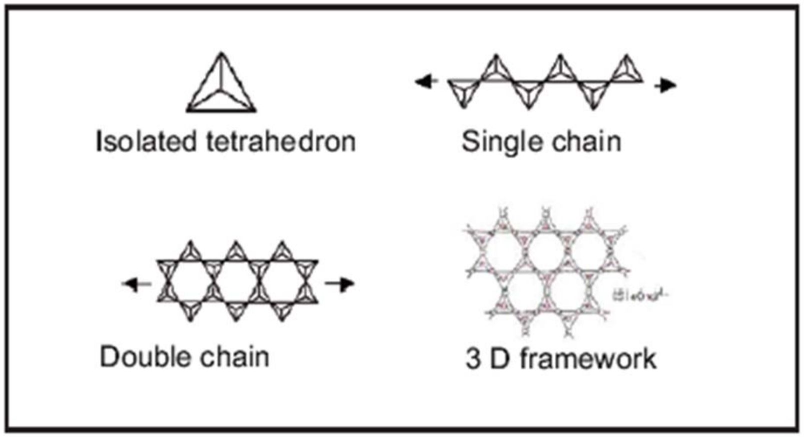
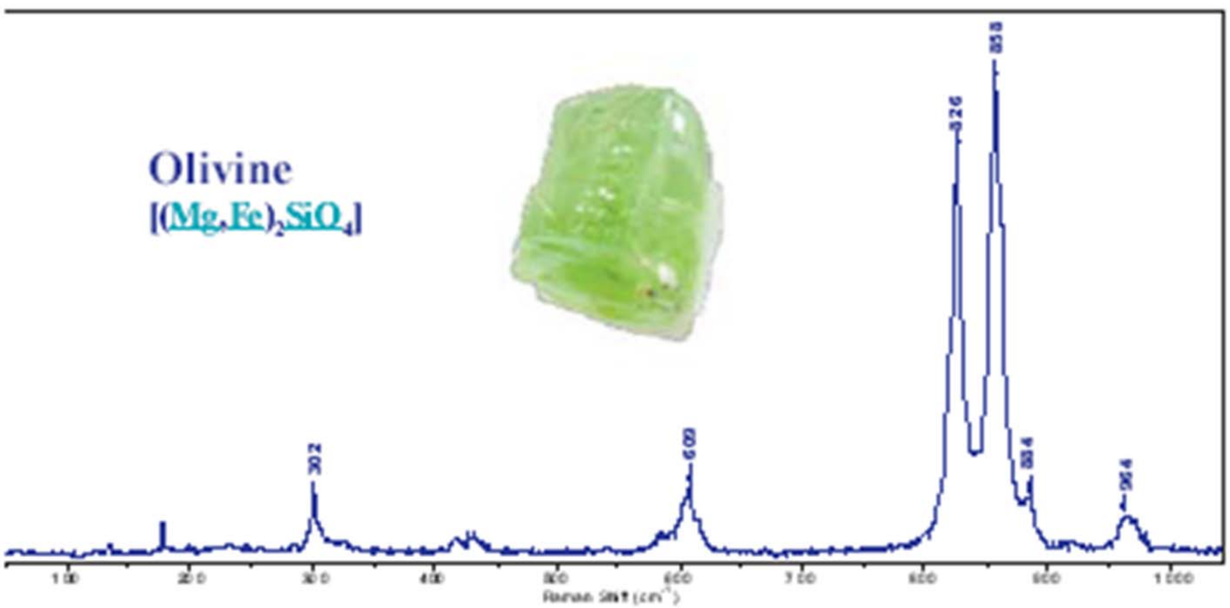
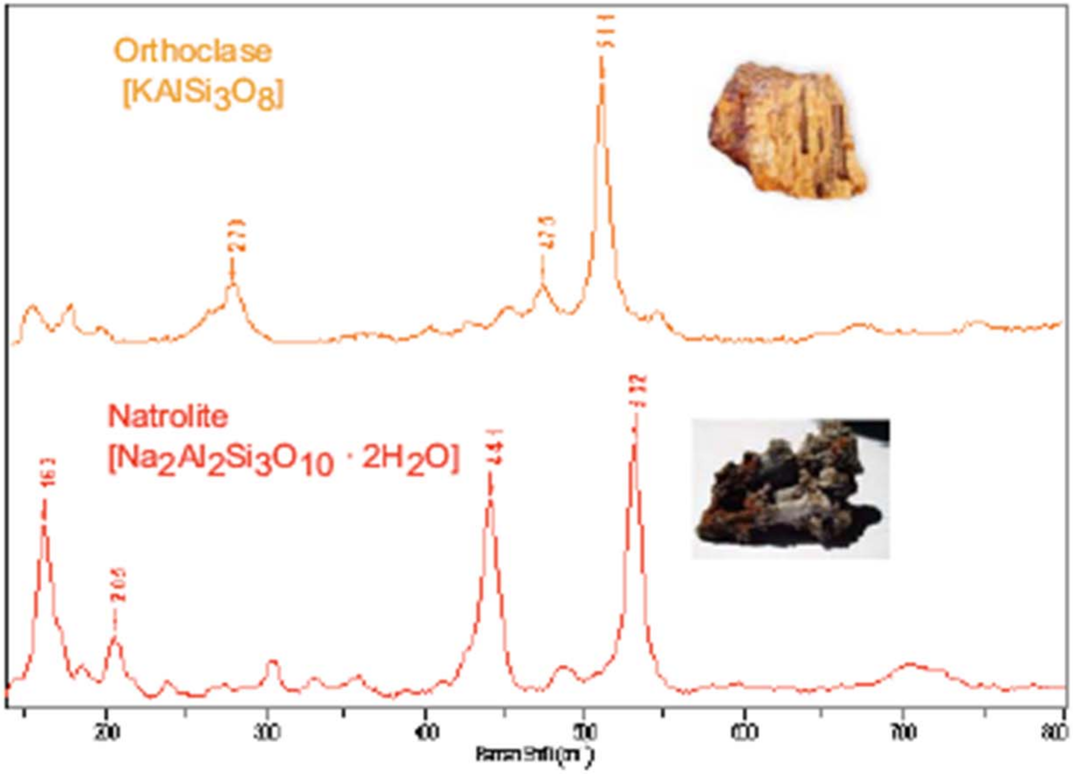


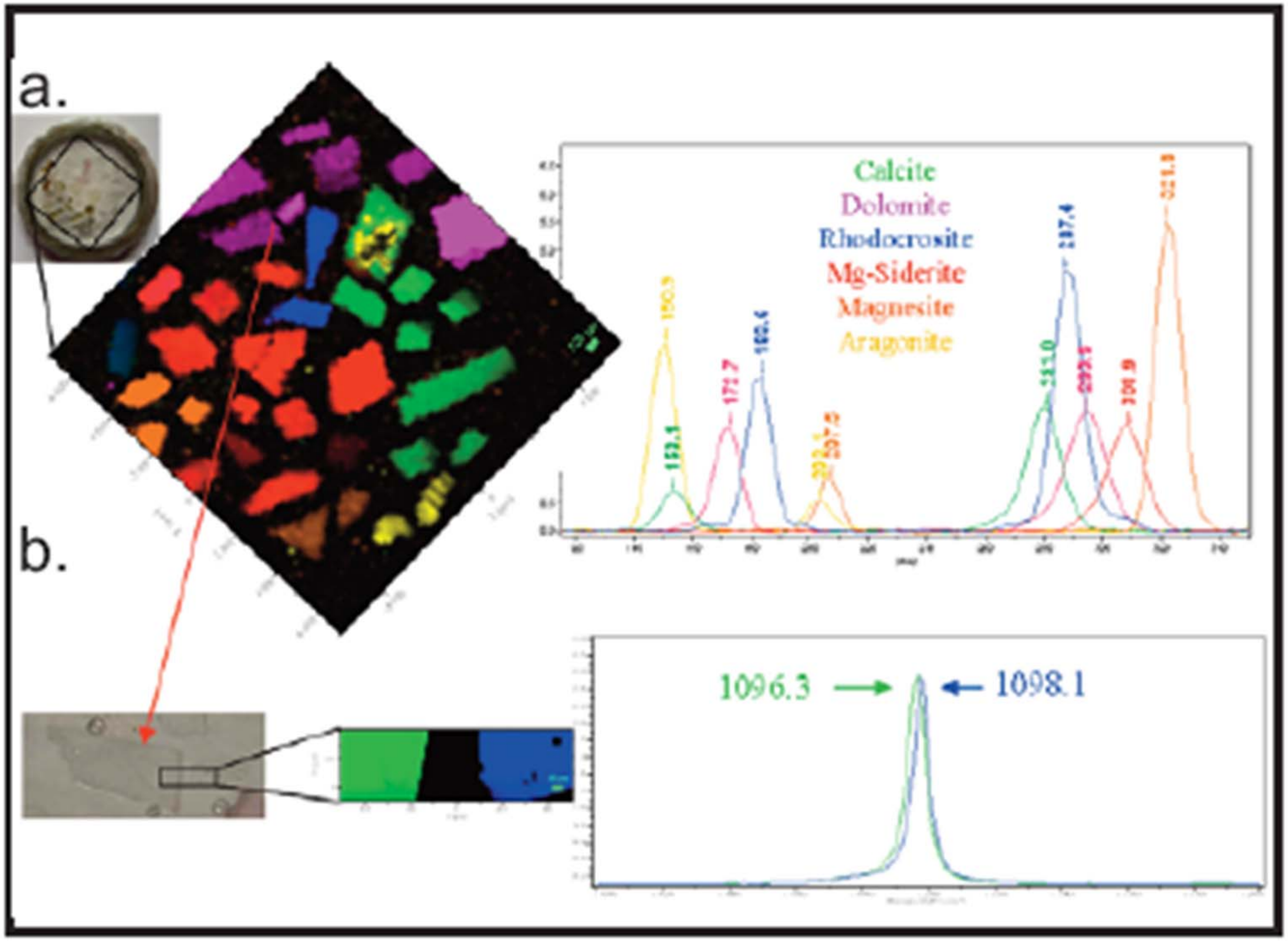
Figure 1: Different tetrahedral arrangements

Mineral Identification





Natrolite and orthoclase Raman spectra

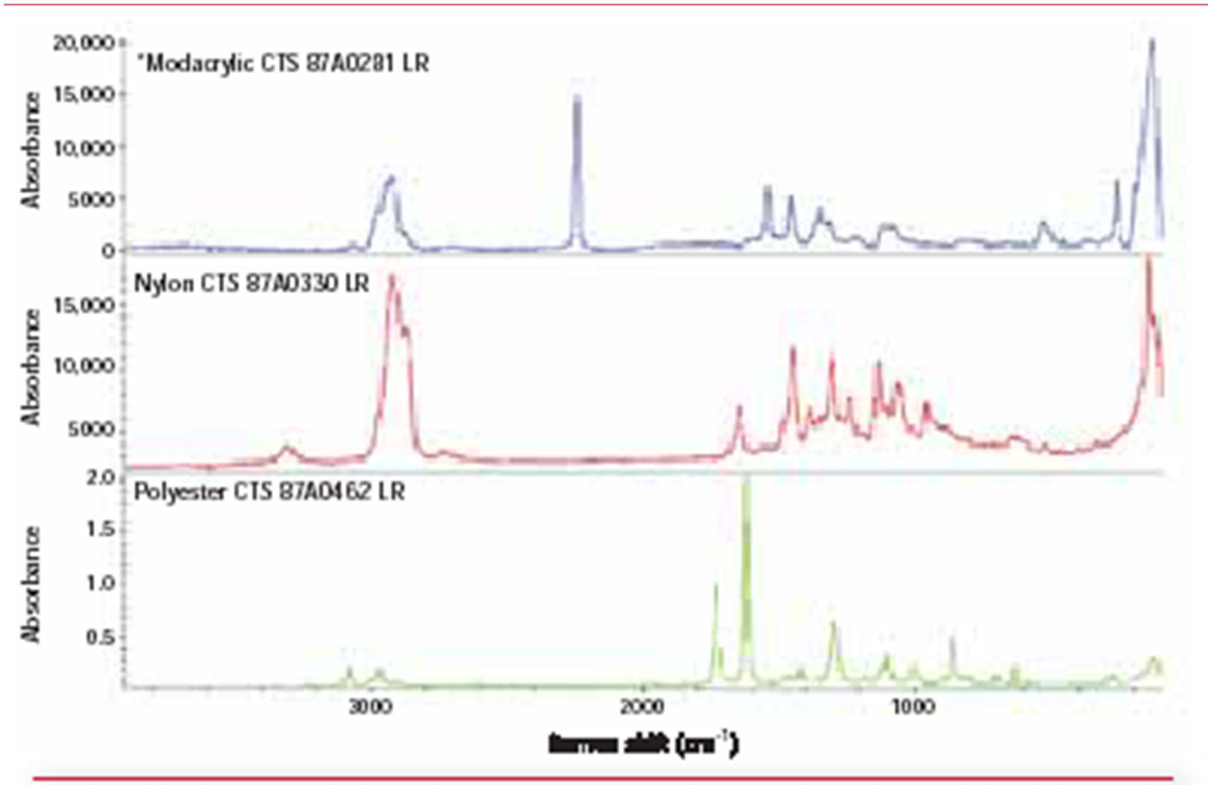


a) Raman imaging of different types of carbonates crystals, b) High spectral resolution map of two dolomite crystals based on the small spectral shift existing between the two crystals spectra

http://www.horiba.com *Raman Application Note*, Geology 01

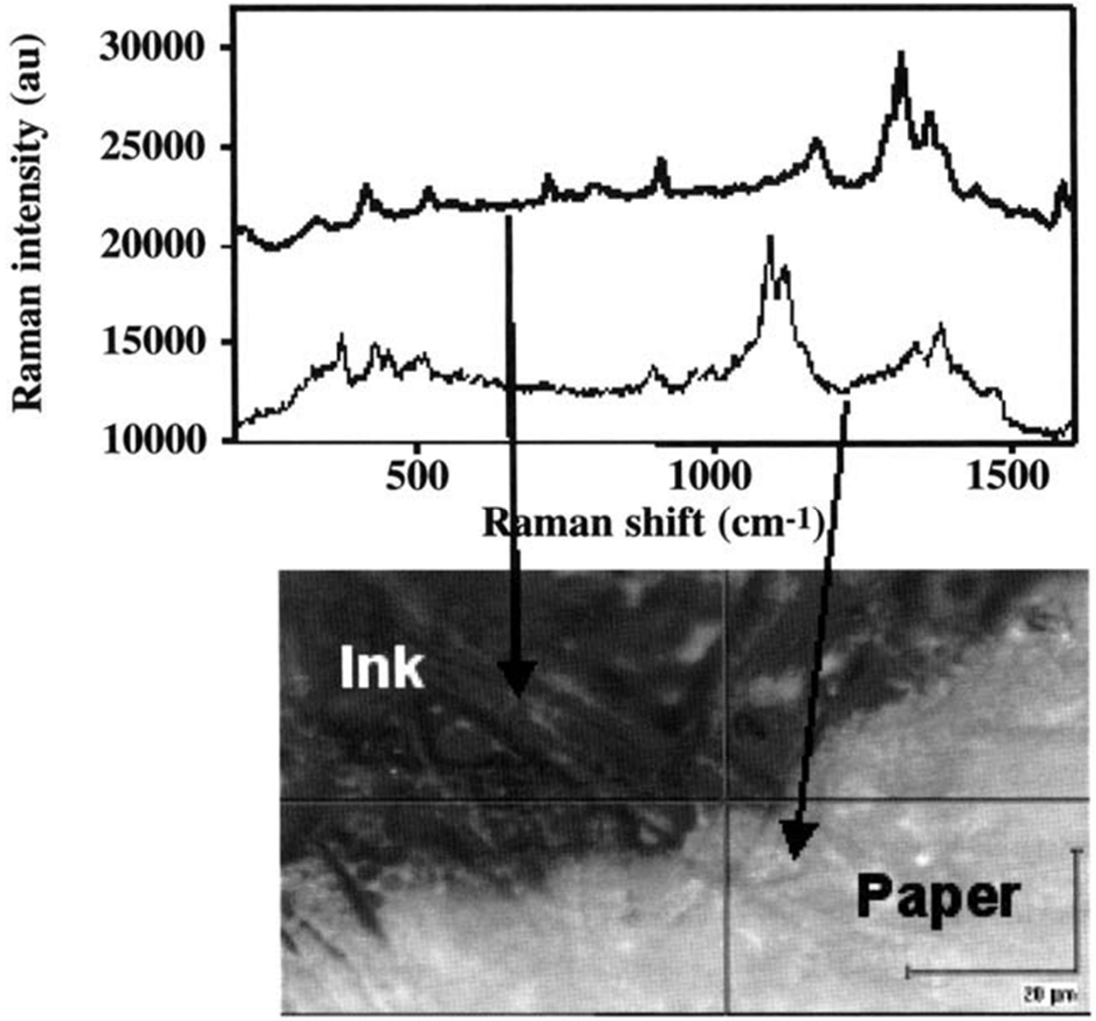
Forensic science

Analysis of Fibers



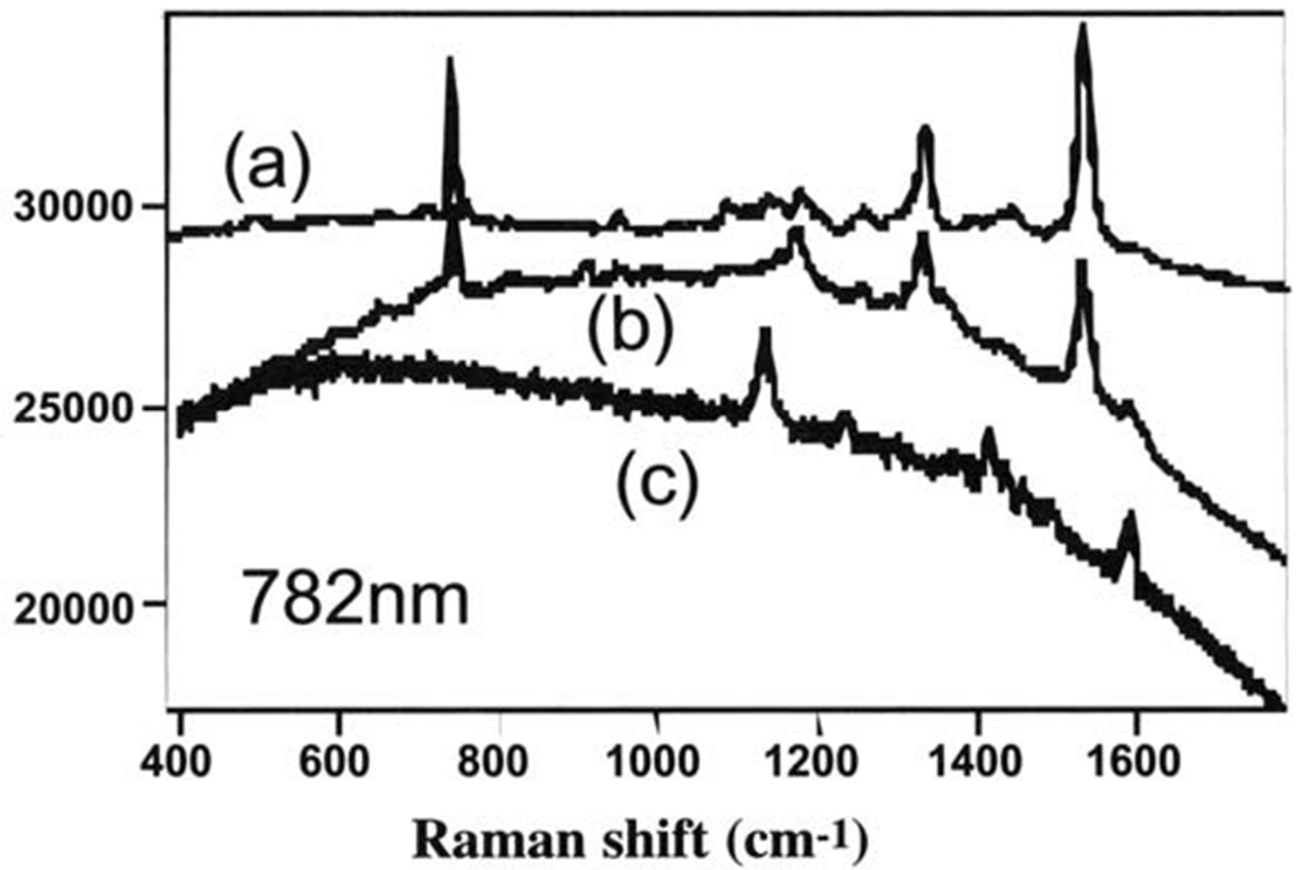
FT-Raman spectra of three fibers: Modacrylic, Nylon and Polyester

M.H. Wall, L. De Noble, R. Hartman, *Spectroscopy*, 2005



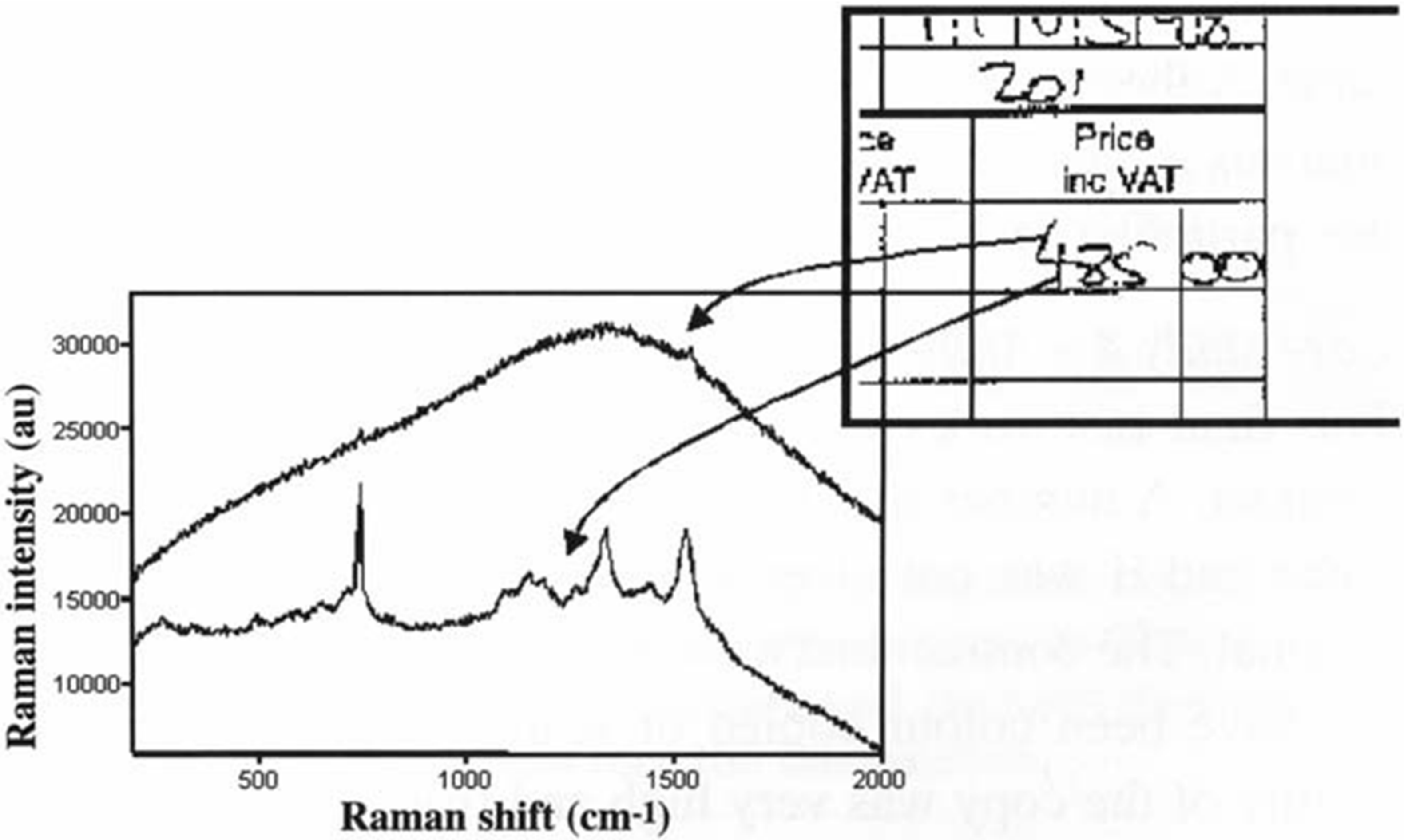
Raman spectra from an 'inked' and 'uninked' paper fiber

M. Claybourn, M. Ansell, *Science & Justice*, 2000, 40, 261-271



Raman spectra taken for different pens: (a) green Bic®, (b) blue Parker ball pen®, (c) red Berol®

M. Claybourn, M. Ansell, *Science & Justice*, 2000, 40, 261-271

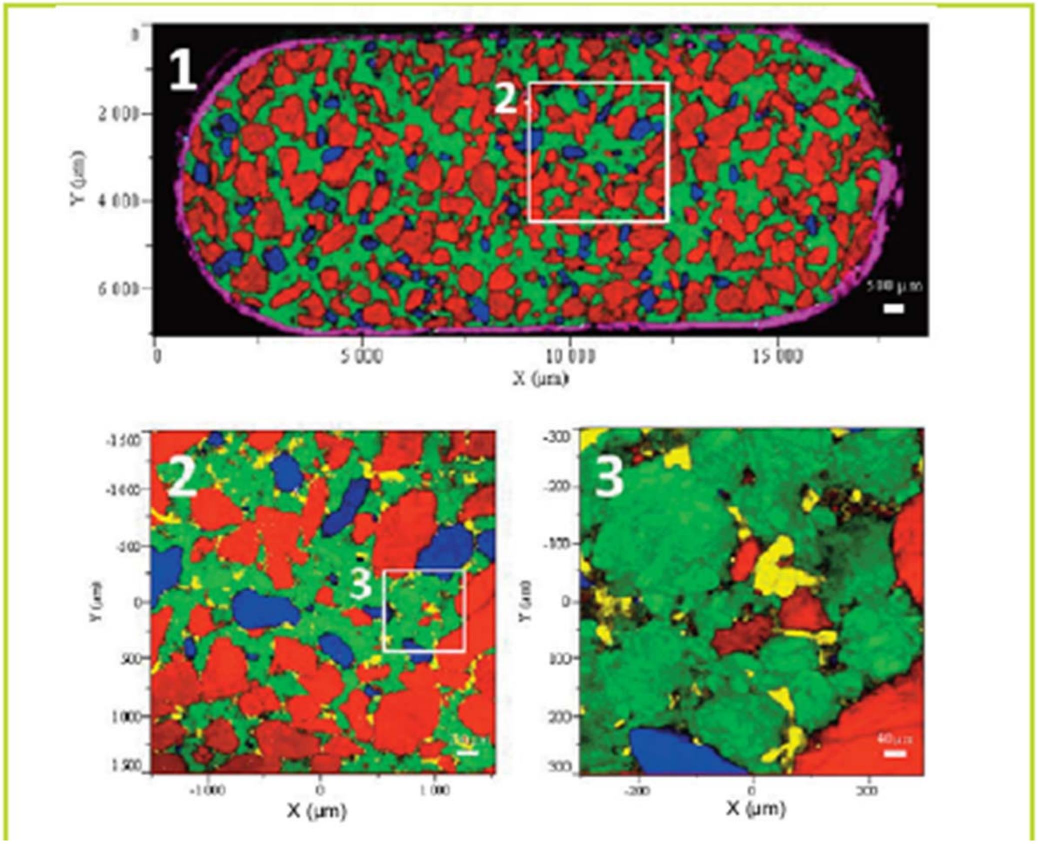


Raman spectra taken from a suspect on an insurance claim document

M. Claybourn, M. Ansell, *Science & Justice*, 2000, 40, 261-271

Chemistry, Pharmaceuticals and Cosmetics

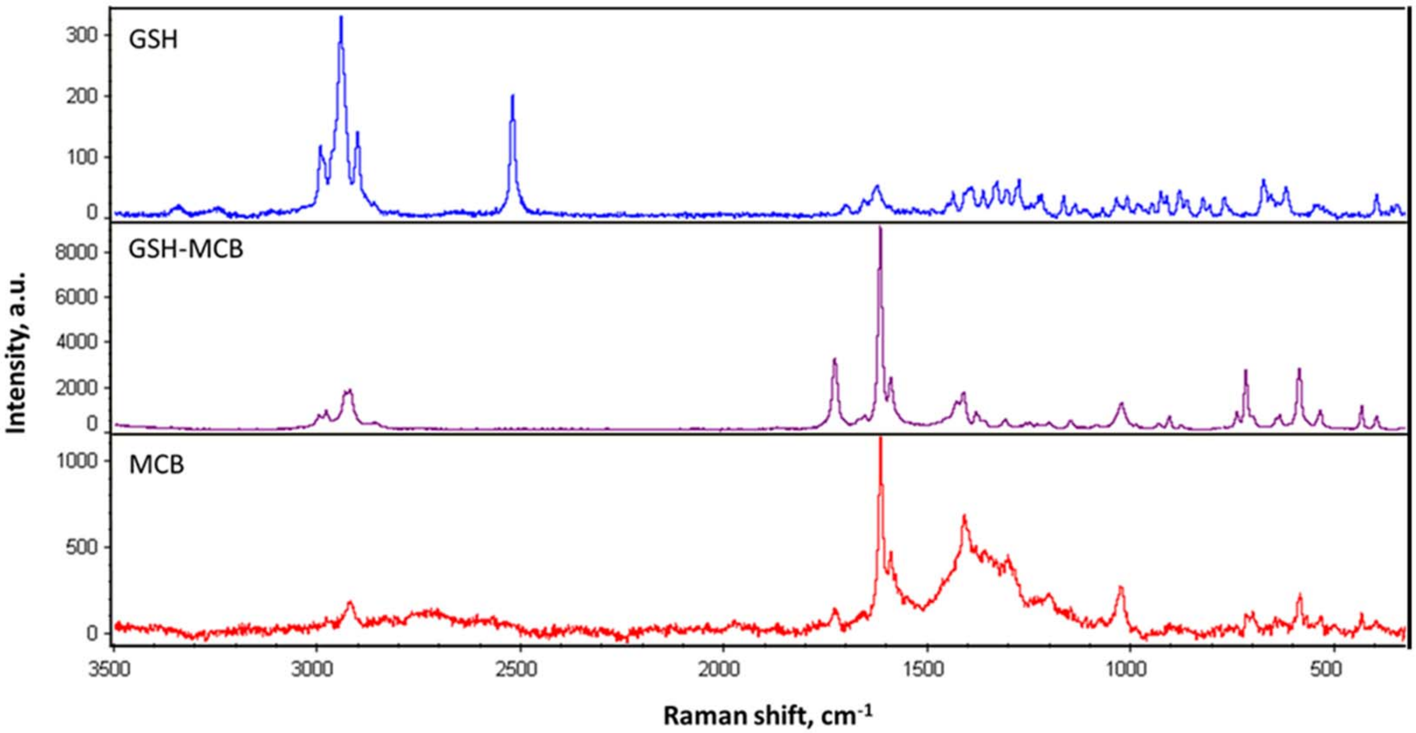
Compound Distribution in Tablets



Colour-coded Raman images of a pharmaceutical tablet the spatial distribution of the various components

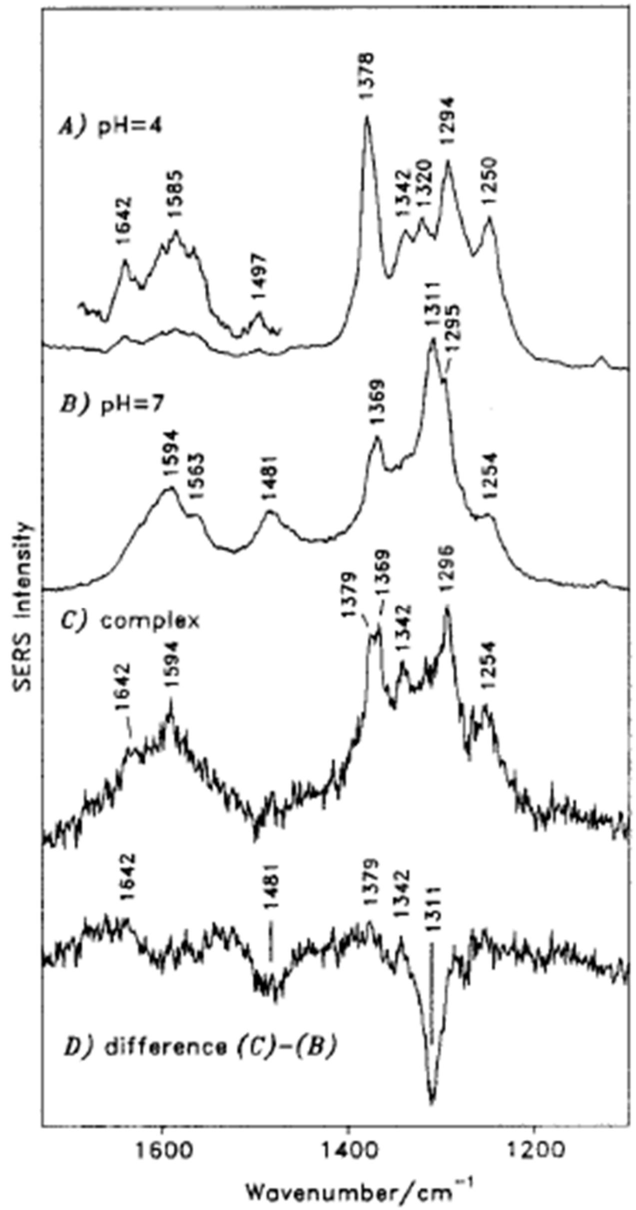
http://www.horiba.com *Raman Application Note*, Pharmaceutical

Molecular Interactions



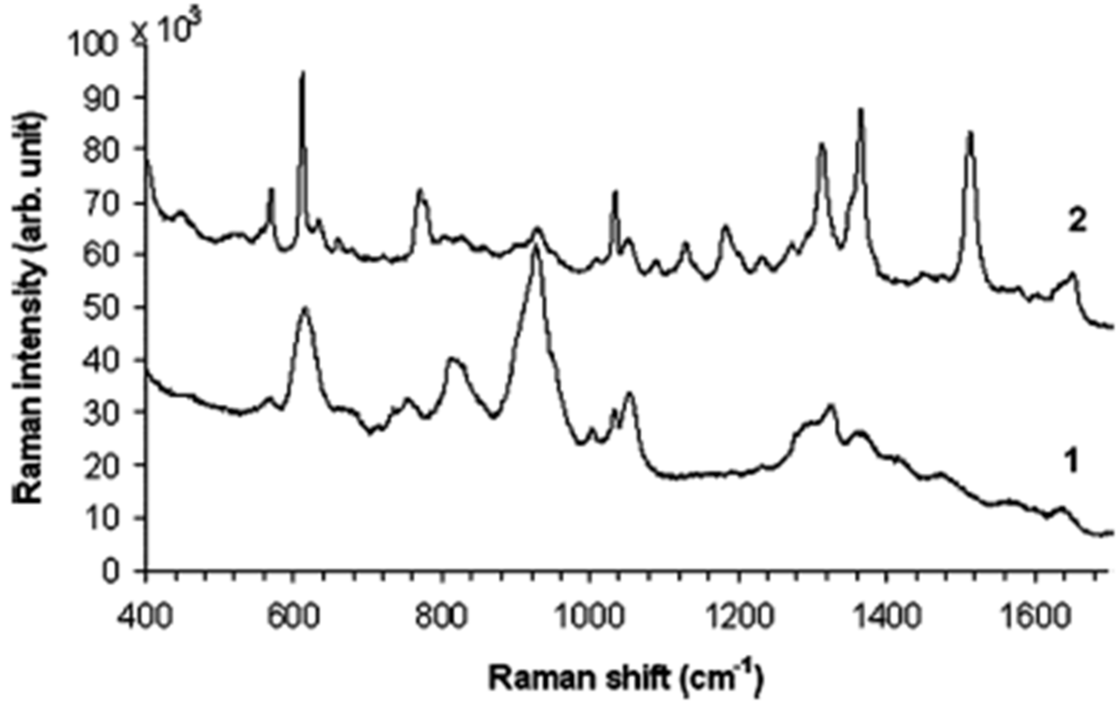
Raman spectra of glutathione (GSH), monochlorobimane (MCB), and their complex.

M. Hepel, M. Stobiecka, *Journal of Photochemistry and Photobiology A*, 2011, 225, 72-80.



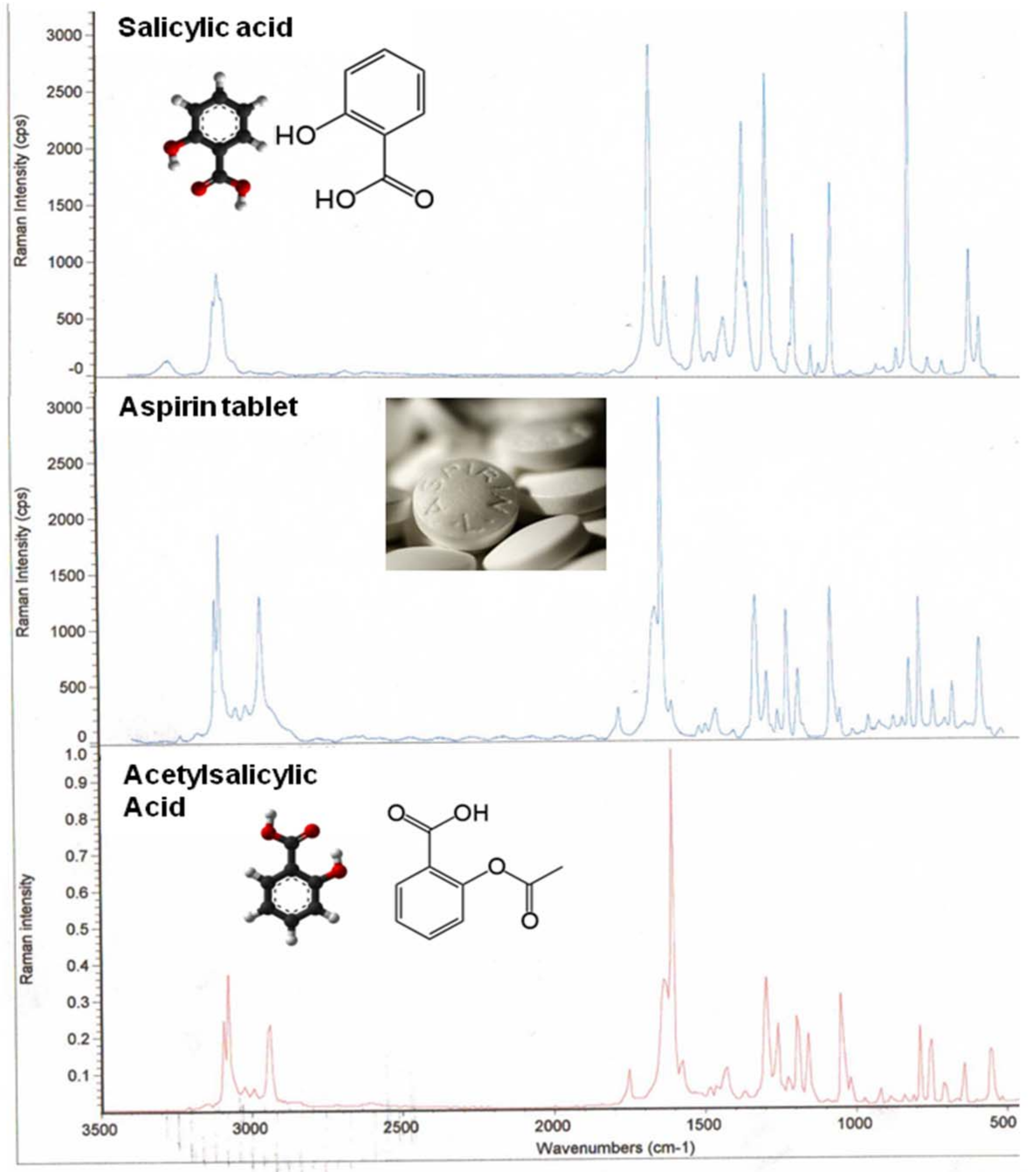
SERS spectra of hypericin (3x10-6 M) at (A) pH 4 and (B) pH 7; (C) SERS spectrum of hypericin – HSA complex and (D) difference spectrum between the SERS of the complex and SERS of hypericin at neutral pH

P.Miškovský, D. Jancura, S.Sánchez-Cortés, E.Kočišová, L.Chinsky, JACS, 1998, 120, 6374-6379

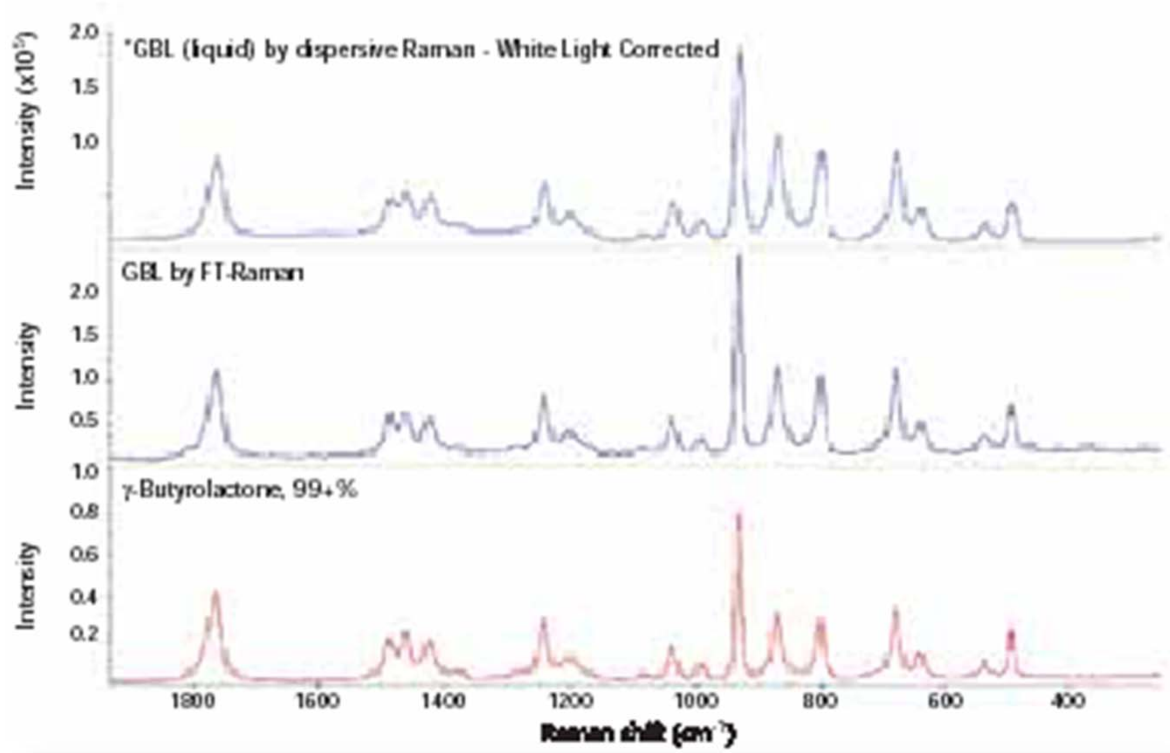


SERS spectra of Rhodamine 6G adsorbed on AgNPs (1) and their complexes with Pluronic L121 (2). C_{Rh6G} = 250 nM

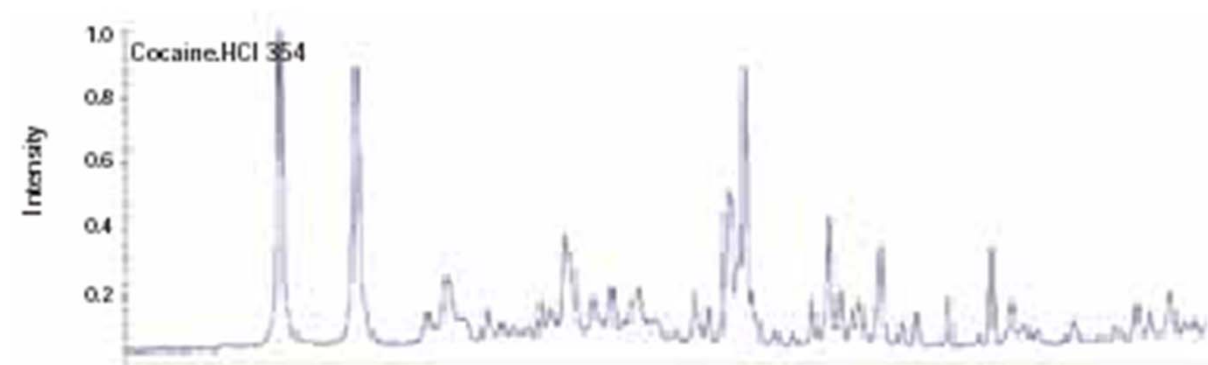
T.I. Abdullin, O.V. Bondar, Y.G.Shtyrlin, M. Kahraman, M. Culha, *Langmuir*, 2010, 26, 5153-5159



Salicylic acid, Aspirin tablet and Acetylsalicylic acid Raman spectra



γ-butyrolactone (GBL), a precursor to GBH (Liquid Ecstasy)



Cocaine Raman spectra

M.H. Wall, L. De Noble, R. Hartman, *Spectroscopy*, 2005

References

1. Lin C-C, Yang Y-M, Chen Y-F, Yang T-S, Chang H-C, *Biosensors and Bioelectronics*, 2008, 24, 178–183
2. Ruan C., Wang W., Gu B., *Anal. Chem.,* 78, 2006, 3379
3. Vivoni A, Chen S, Ejeh D, Hosten C., *Langmuir*, 16, 2000, 3310-3316
4. Kudelski A., *Talanta*, 76, 2008, 1-8
5. Vikesland P., Wigginton K., Environ. *Sci.Technol.,* 44, 2010, 3656
6. Schuster K.C., Urlaub E., Gapesa J.R. , *Journal of Microbiological Methods* 42, 2000, 29–38
7. Wang Y., Li D., Li P., Wang W., Ren W., Dong S., Wang E., *J Phys Chem C*, 111, 2007, 16833
8. Mahadevan-Jansen A., Richards-Kortun R., *Proceedings -19th International Conference IEEE/EMBS*, 1997, Chicago
9. Jenkins A. L. and Larsen R. A. , Spectroscopy , 2004, 19 (4)
10. Wall M.H., De Noble L., Hartman R., *Spectroscopy*, 2005
11. Claybourn M., Ansell M., Science & Justice, 2000, 40, 261-271
12. P.Miškovský, D. Jancura, S.Sánchez-Cortés, E.Kočišová, L.Chinsky, JACS, 1998, 120, 6374-6379
13. Abdullin T., Bondar O., Shtyrlin Y., Kahraman M., Culha M., *Langmuir*, 26, 2010, 5153
14. Raman Application Note http://www.horiba.com

Acknowledgements:

This project was supported by the **National Science Foundation CCLI grant**