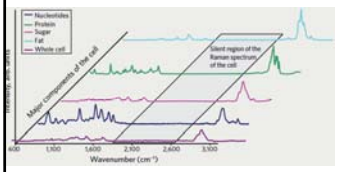
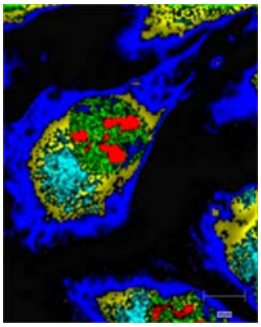
Babeș-Bolyai University Cluj-Napoca
Biomolecular Physics Department - BPD

Raman and non-linear Raman methods

Why IR and Raman spectroscopy?

Molecular specific information





Raman image of human osteosarcoma (bone cancer) cells, showing the nuclei (green), nucleoli (red), membrane-bound organelles (cyan) and the cell body (yellow thick region, blue membranous area).

Medical imaging

Publications and search engines

- Elsevier
  http://www.sciencedirect.com/Springer
- Spinger
  http://link.springer.com/
- RSC
  http://www.rsc.org/
- IOP
  http://www.rsc.org/
- ACS
  http://pubs.acs.org/
- Wiley
  http://onlinelibrary.wiley.com/

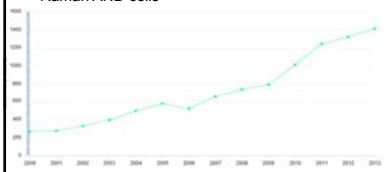
SCOPUS https://www.scopus.com/
WEB OF SCIENCE http://webofknowledge.com/

European Conference on Molecular Spectroscopy – EUCMOS Cluj-Napoca 2012

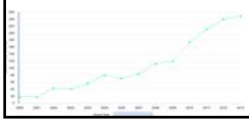
Bio-medical interest for Raman spectroscopy

www.scopus.com

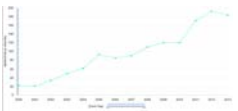
Raman AND cells



Raman AND cancer



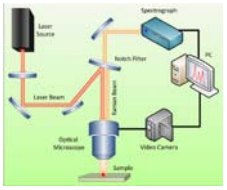
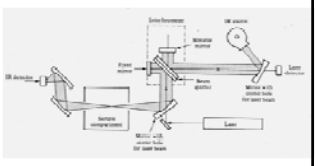
Raman AND diagnosis



Raman and FTIR spectrometers in Cluj

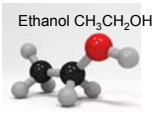
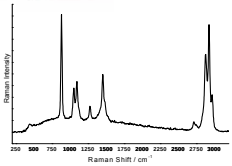
- UBB
  - Physics Faculty
    - FT-Raman, FTIR (Bruker), multilaser (UV-VIS-NIR 6 lines) Raman -AFM microscope (Renishaw), DeltaNu Raman (532, 633 nm)
  - ICEI
    - Multilaser Raman-AFM (Witec), Ocean Optics Raman (785 nm)
- USAMV
  - FT-Raman, FTIR (Bruker, Shimadzu), Dispersive Raman microscope (633, 785 nm)
- UAD
  - FTIR, Dispersive Raman microscope (Bruker)
- ITIM
  - Multilaser Raman-AFM microscope (Solaris)

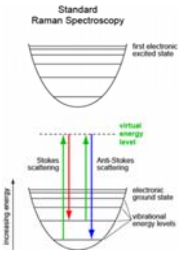
Raman and FTIR spectrometers

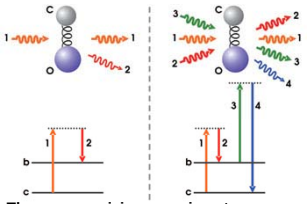
Raman spectroscopy

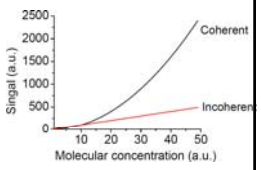
Ethanol CH_3CH_2OH



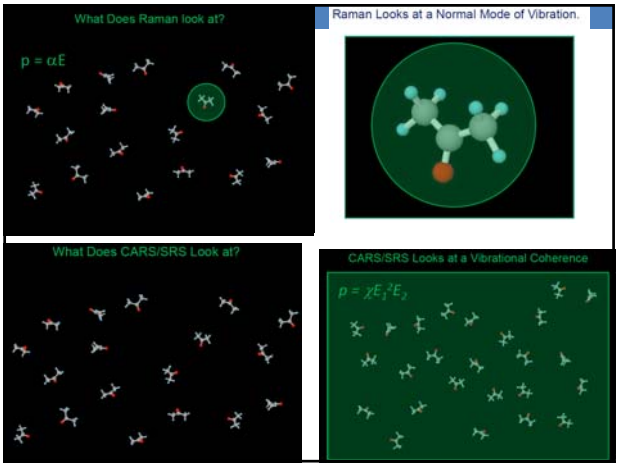
Coherent anti-Stokes Raman Spectroscopy (CARS)



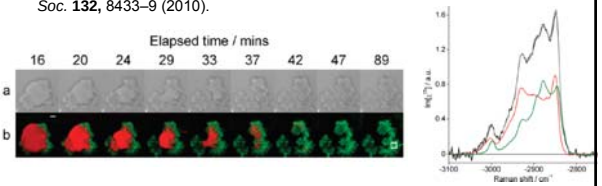


Three wave mixing experiments

The first recordings of Coherent Anti-Stokes Raman Scattering go back to the 6os of the last century, when two researchers of the **Scientific Laboratory at the Ford Motor Company**, P. D. Maker and R. W. Terhune, published an article about their experiments. They simply called their work "three wave mixing experiments". Almost ten years later, in the middle of the 70s, R.F. Begley, A.B. Harvey and R. L. Byer of Stanford University showed advantages of CARS over Raman spectroscopy and first applications on biological samples.



Day, J. P. R., Rago, G., Domke, K. F., Velikov, K. P. & Bonn, M. Label-free imaging of lipophilic bioactive molecules during lipid digestion by multiplex coherent anti-Stokes Raman scattering microspectroscopy. *J. Am. Chem. Soc.* **132,** 8433–9 (2010).

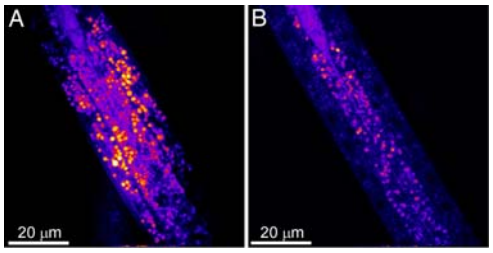


lipophilic drugs progesterone, Vitamin D3

the oil is hydrolyzed by pancreatic lipase, first at margins at water/oil interface due to water solubility of enzyme

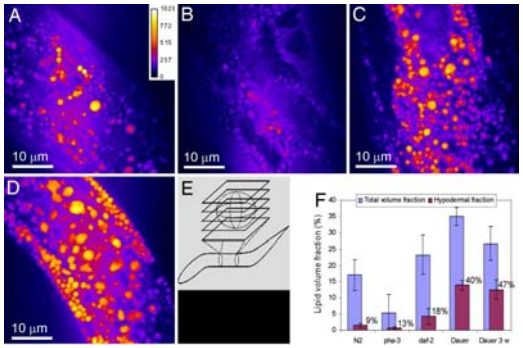
CARS images display enhanced contrast compared to the bright-field images. Furthermore, one can distinguish regions of the image in which both phases coexist.

Better understanding of the fundamental mechanisms behind **metabolic diseases** requires methods to monitor lipid stores on single-cell level *in vivo*. *We have used Caenorhabditis elegans (*worm at, about 1 mm in length*) as a* model organism to demonstrate the limitations of fluorescence microscopy for imaging of lipids compared with coherent anti-Stokes Raman scattering (CARS) microscopy, the latter allowing chemically specific and label-free imaging in living organisms.



Comparison of CARS and fluorescence microscopy (Nile red-stained)

Lipids storage, different sections



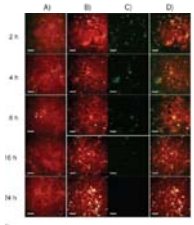
Interactions of pathogenic organisms with cellular membrane

viruses and bacteria necessarily involve interactions at **cellular membranes** for, at the minimum, the entry of the pathogen into its host cell to begin infection.

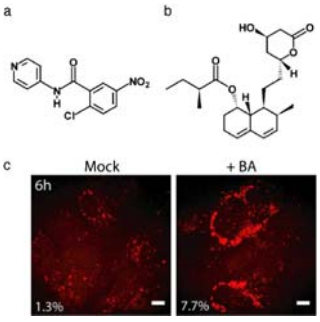
Indeed, nearly all viruses of concern to human health have some specific **interactions with lipids**, including human immunodeficiency virus (HIV), hepatitis C virus (HCV) and the influenza virus.

there is great potential for applying CARS microscopy to study host-virus interactions in HCV and other viral infections.

HCV **induces the accumulation of lipid droplets** (LDs) on which the HCV core protein is known to reside



Interactions of drugs with cells



Significant lipid accumulation was observed after a 6-h treatment with BA and was mainly localized around the perinuclear regions of the cell appearing as larger LD aggregates

Malignant/non-malignant breast cancer cells
-identification of tumour margins

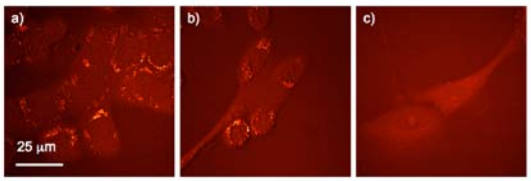
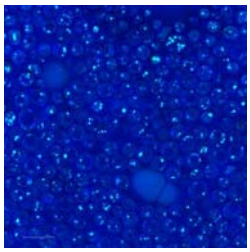


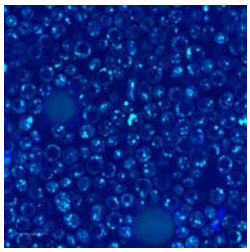
Figure 12: CARS images of a) live nonmalignant (MCF-12A); b) mildly malignant (MCF-7) and c), malignant (MBA-MB-231) breast cancer cells. The bright spots are the lipid droplets. Note that the droplet concentration in malignant cells is lower than in normal cells.

CARS Microscopy Leica Microsystems

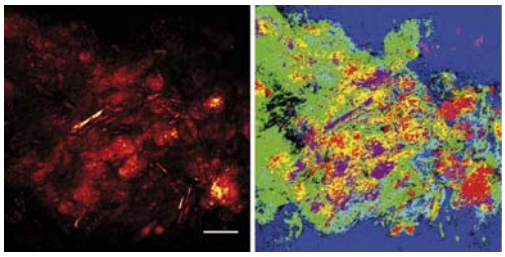
Staining standard fluorescence samples is often time-consuming and expensive and may influence typical properties of living cells as they act in a chemical way. Furthermore, dyes lose intensity and alter the sample. They often cause phototoxicity, harm the specimen and consequently may influence the result of the experiment.
CARS overcomes these drawbacks by the intrinsic characteristics of the method. CARS does not require labeling because it is highly specific to molecular compounds which are based on vibrational contrast and chemical selectivity. The crucial advantage of this method is that the sample remains almost unaffected



In vivo imaging of yeast cells. Yeast cells in one week old medium. The lipid droplets are small due to the lack of sugar. The bright blue parts are lipid droplets inside the yeast cells (at 2,850 cm^{-1}).

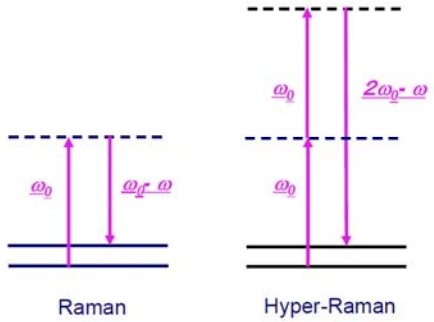


Yeast cell cultured over three hours in fresh medium. The result are bigger lipid droplets which indicate that there is enough sugar available for the cells. The dark blue parts are generated by water at a wave number of 3,250 cm^{-1}

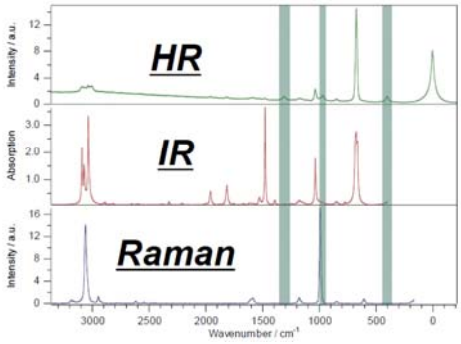


This is a CARS image of an atherosclerotic lesion in a mouse aorta. The left panel shows a regular CARS image of the lesion at 2,845 cm^{-1}, highlighting the lipophilic components. The right panel shows a spectral decomposition (principal component analysis) of the CARS signal in the 2,750–3,050 cm^{-1} range, showing that different areas in the image correspond to different lipophilic compounds. Each color in the image corresponds to a different CARS spectrum. Scale bar is 50 microns

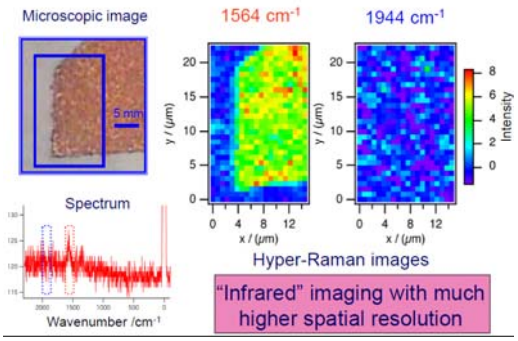
Raman Scattering and Hyper-Raman Scattering



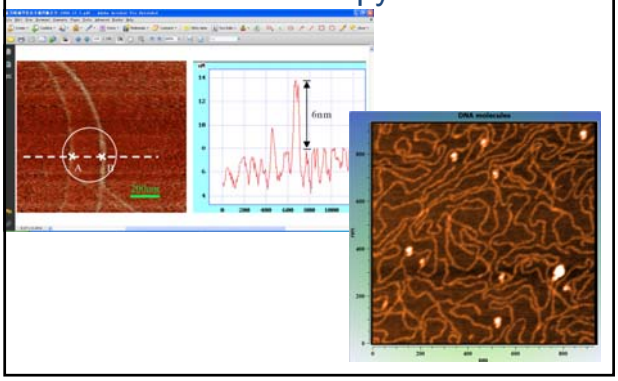
Complete Vibrational Spectra of Benzene

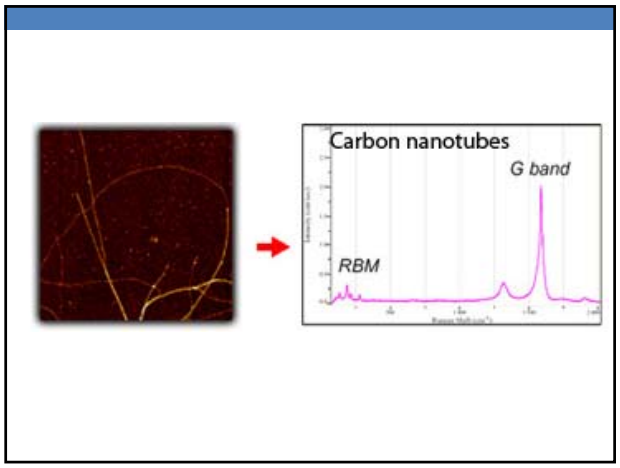


Hyper-Raman Imaging with an Infrared-active Mode

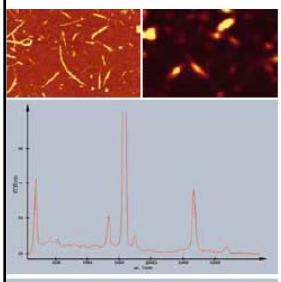


Atomic force microscopy AFM

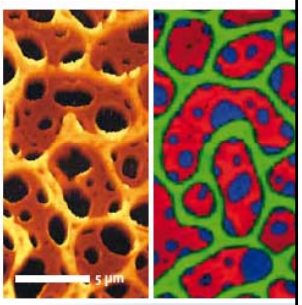




Renishaw Raman/AFM system

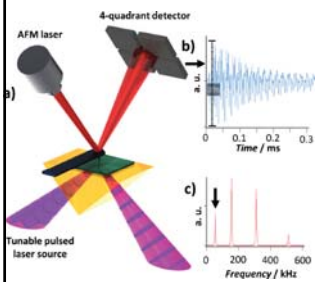


AFM and Raman images of carbon nanotubes deposited on a Si-substrate along with typical Raman spectrum of a carbon nanotube



Confocal Raman and AFM topography image of a polymer blend on glass

Chemical imaging beyond the diffraction limit

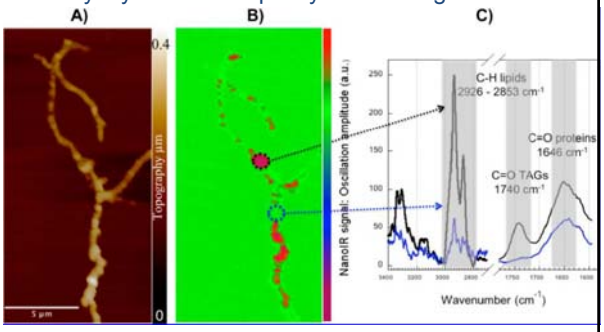


Schematic of the ***Photothermal induced resonance*** (PTIR) set up: if the IR laser pulses (purple) are absorbed by the sample (green) it rapidly expands and deflect the AFM cantilever; the amplitude of the deflection, which is proportional to the energy absorbed, is measured by the AFM four quadrant detector. The PTIR signal analysis is carried out either by (b) determining the maximum of peak to peak deflection during the cantilever ring down (blue signal) or by (c) Fourier transformation determining the amplitude of one of the cantilever normal modesof vibration (red signal).

Lahiri, B., Holland, G. & Centrone, A. Chemical Imaging: Chemical Imaging Beyond the Diffraction Limit: Experimental Validation of the PTIR Technique (Small 3/2013). *Small* **9,** 488–488 (2013).

AFM-IR

TriAcylGlycerols in Streptomyces microorganisms



Homework

Leica-Microsysthems
http://www.leica-microsystems.com/science-lab/step-by-step-guide-to-the-molecular-basics-of-cars-microscopy/

EUCMOS 2012 Cluj-Napoca, Book of Abstracts
http://www.phys.ubbcluj.ro/eucmos2012/