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Raman spectroscopy of biological samples

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Explore the future

Automotive Test Systems | Process & Environmental | Medical | Semiconductor | Scientific

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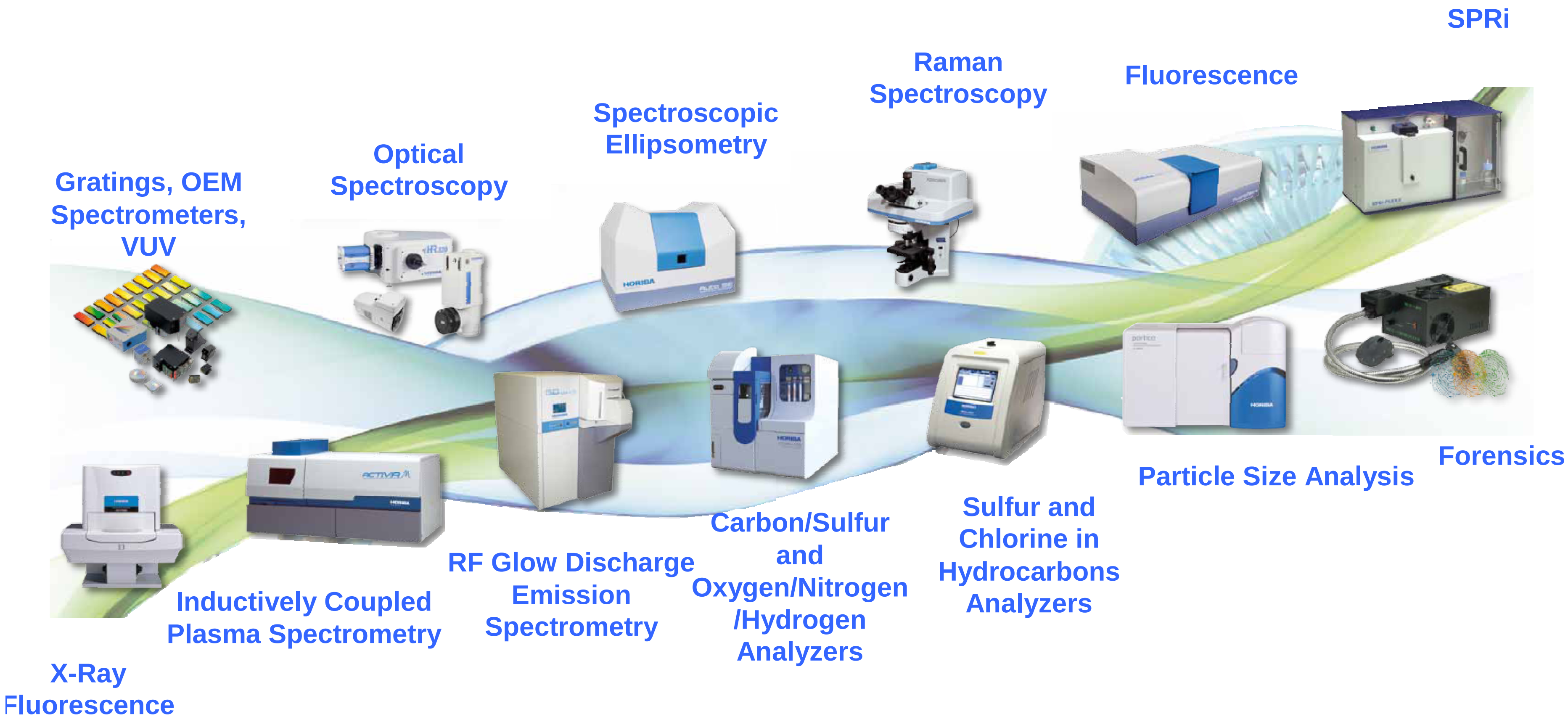

Explore the future

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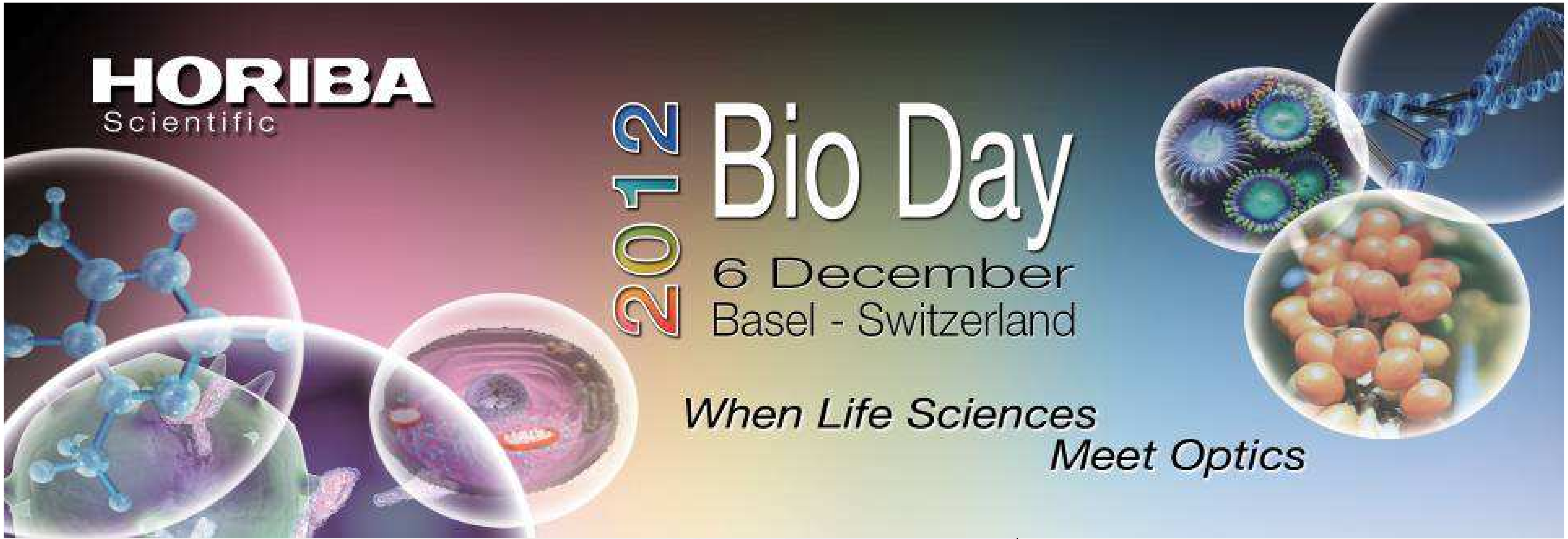


Session 1 - Molecular Characterization

Use of fluorescence to study aggregation of biopharmaceuticals — *Tudor Arvinte, University of Geneva and Therapeomic Basel (Switzerland)*

Size and charge characterization of nanoparticules used in bio-applications — *Christelle Mégier, HORIBA Scientific (France)*

Title to be confirmed — *Marité Cárdenas Gómez, University of Copenhagen (Denmark)*

Session 2 - Clinical Applications

SPRi-MS coupling for the identification and characterization of clinical biomarkers — *Wilfrid Boireau, FEMTO-ST Institute Besançon (France)*

Analysis of sperm DNA quality by Raman microspectroscopy — *Victoria Sanchez, University of Münster (Germany)*

Demonstration of the Instruments

Particle Characterization
Raman
SPRi
Fluorescence
Spectroscopic Ellipsometry
Electrochemistry, micro volume measurement

Opticsvalley

Raman spectroscopy

High selectivity to chemical species, molecular bondings
From micron spot analysis to big areas fast mapping
From Research to Analytical applications

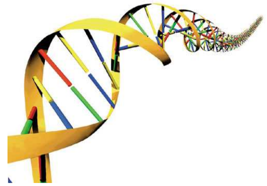
Within the last 10 years,

12 European Research Projects

23'000 Scientific Publications

6 new Raman spectrometers

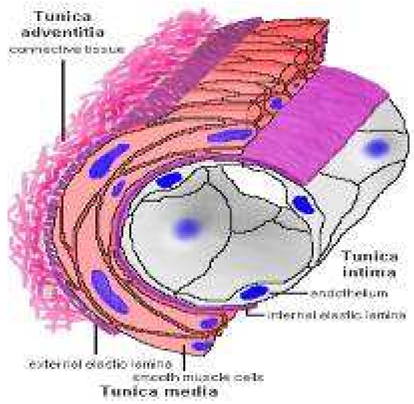
Applications in Biology

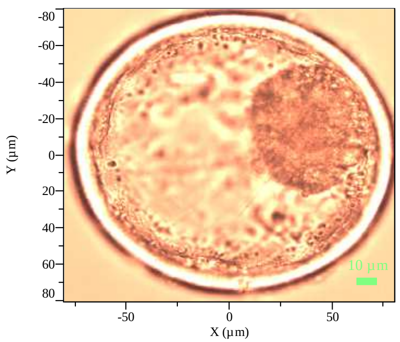


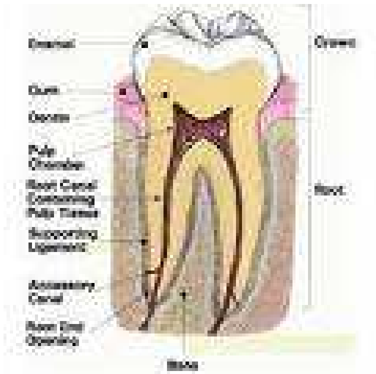
Biology is a natural science concerned with the study of life and living organisms, including their structure, function, growth, origin, evolution, distribution.

In biology, Raman Spectroscopy is mainly used to study:

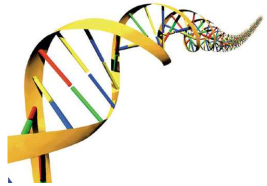
- DNA Analysis
- Lipids, proteins & amino acids
- Bacteria classification and recognition
- Drug / Cell interaction
- Diagnosis & prognostic for cancer
- Dental prostheses







Advantages of Raman spectroscopy in Biology



- **Sensitivity to many different functional groups:** access to C=C, S-S, C-S bonds (that are weak in IR)

- **Highly selective fingerprint:** similar compounds can be discriminate

- **Non-invasive and non-destructive method, no sample preparation**

- **Compatibility with aqueous solutions**

- **High spatial resolution:** Single cell level analysis, intracellular imaging are achievable

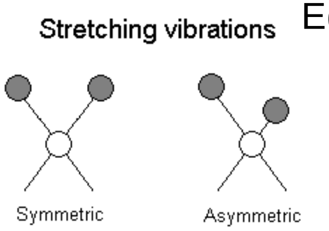
- **Sensitivity to Molecular orientation**: polarization measurements

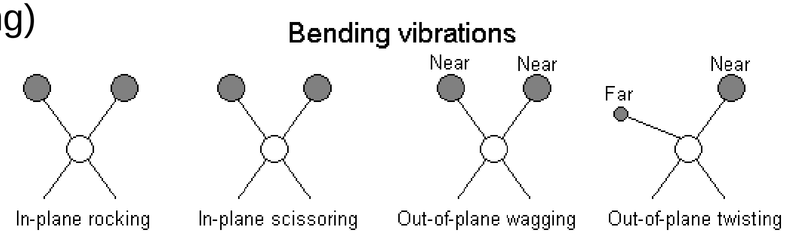
- **Measurements can be done in vivo or in vitro**

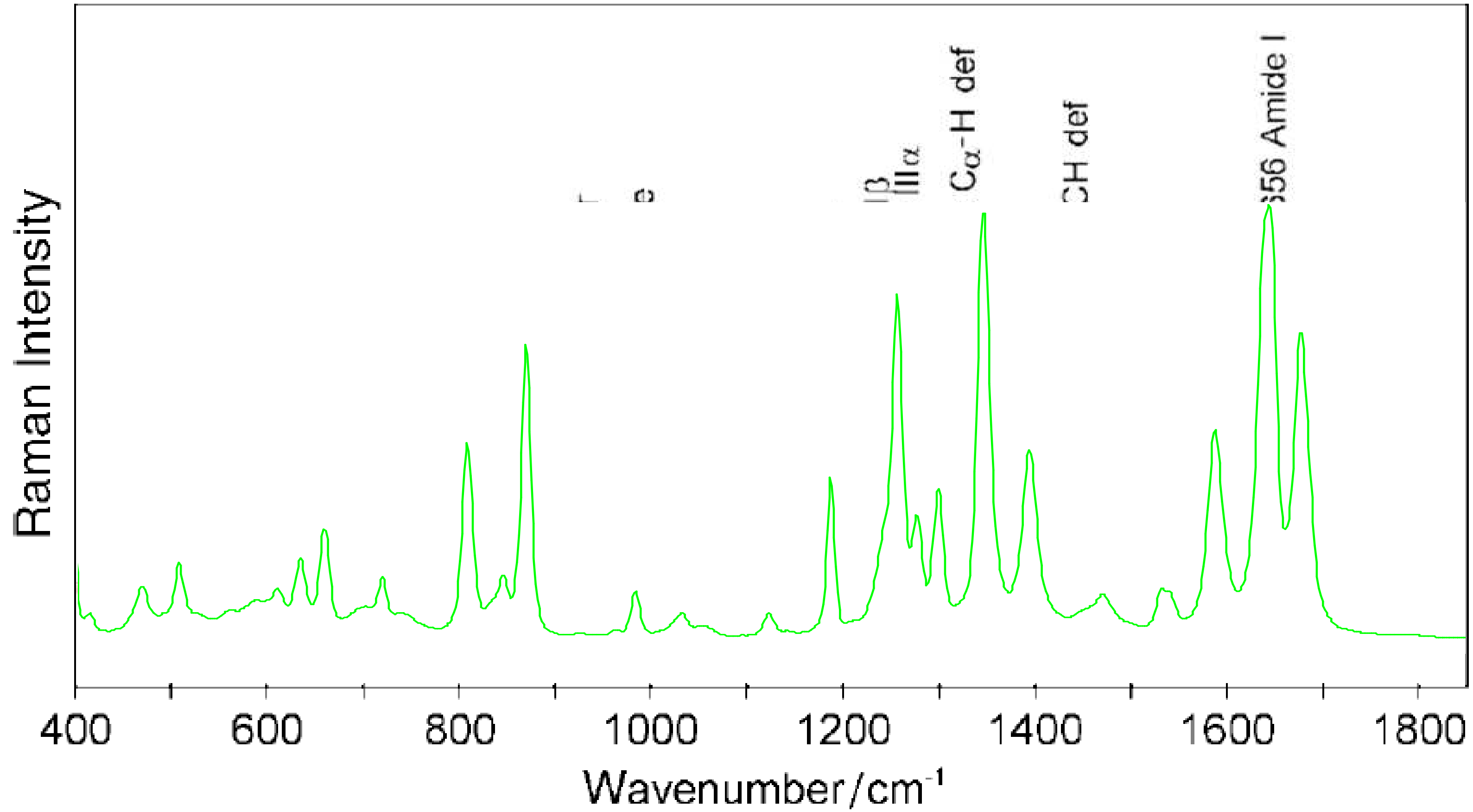
- **SERS, TERS and Resonance effects:** can highly increase sensitivity

Raman analysis of proteins

E(bending) < E(stretching)







Raman analysis of proteins

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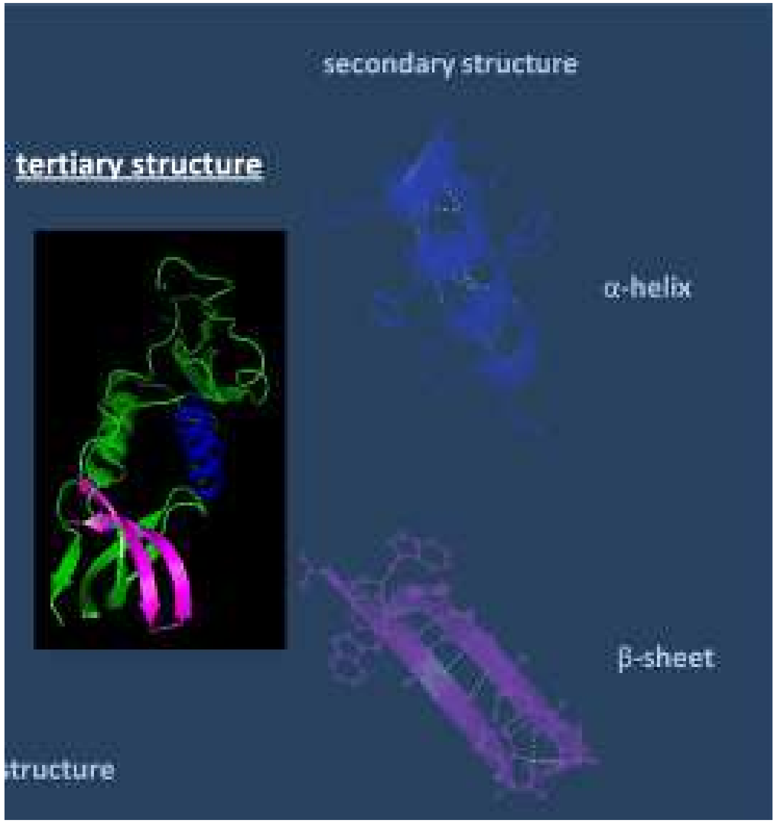
- Size of proteins makes spectrum complex
  - Polypeptide backbone
    - Secondary structure
  - Amino acids in side chains
    - H-bonding
    - Environment
    - Intermolecular interactions
- Raman spectroscopy provides unique information about :
  - Chemical composition
  - Conformational structure
- Reactions can be monitored by Raman to study the mechanisms of the reaction and derive bio-physical properties



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Raman analysis of proteins

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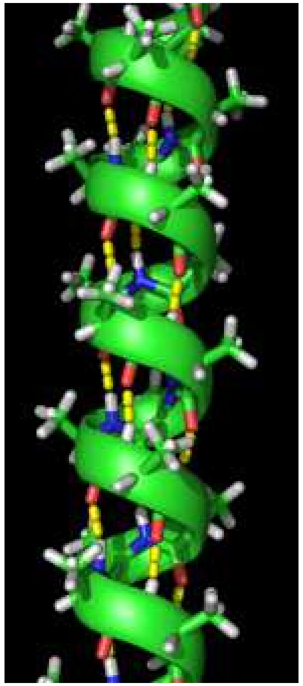
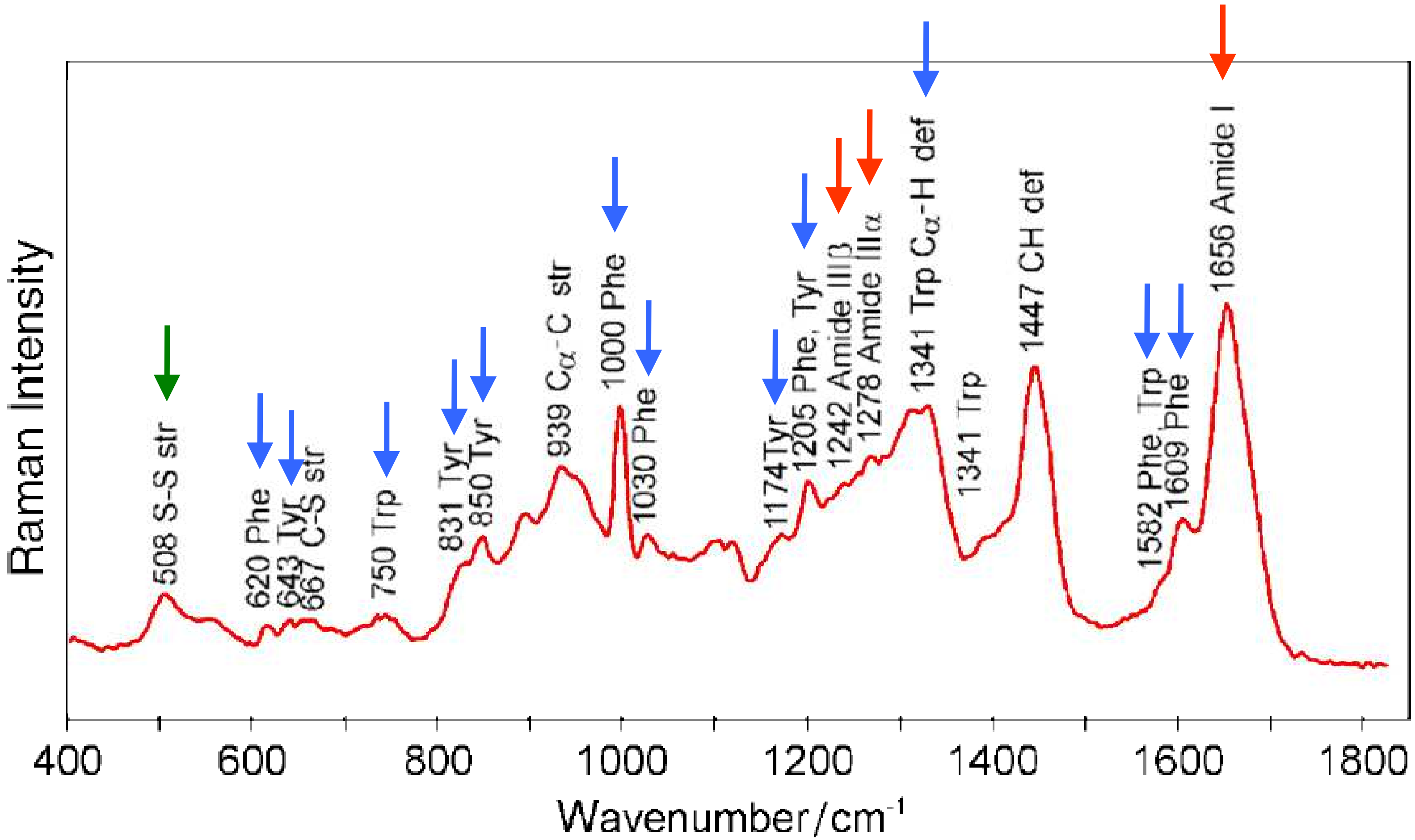
- Amide bonds involved in H-bonding
  - Strongly dependent on 2º / 3º structure

Wavenumber Range	Band	Strength	Secondary Structure
1665-1672	Amide I	strong	beta sheet
1660-1670	Amide I	strong	random coil
1645-1655	Amide I	strong	alpha helix
1270-1300	Amide III	weak	alpha helix
1243-1253	Amide III	moderate	random coil
1229-1235	Amide III	strong	beta sheet



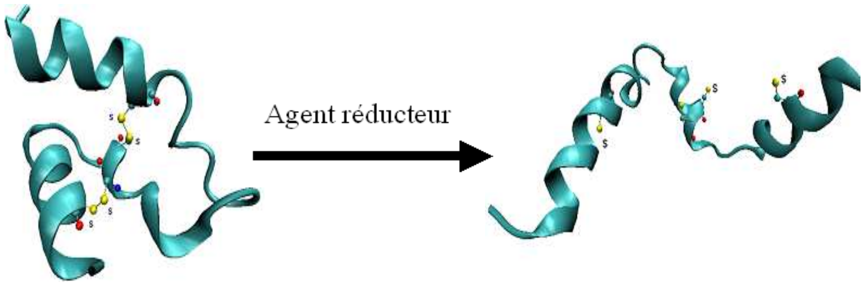
Raman analysis of proteins



backbone **amino acids** **S-S**

Biomolecules analyses: proteins

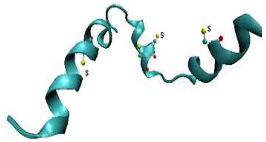
- Example of the disulfide bridge breaking in proteins



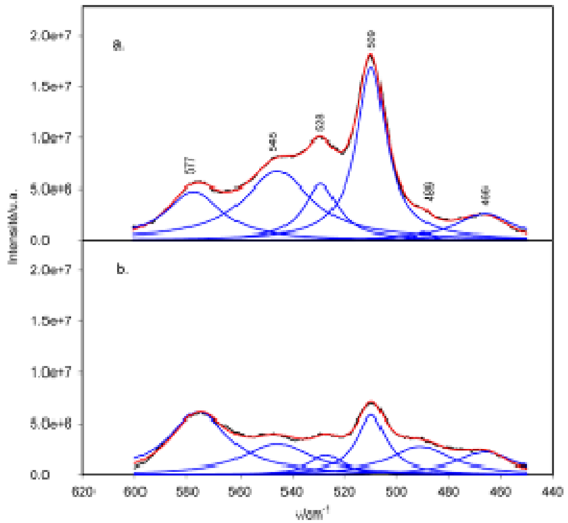
- Disulfide bridge:
  - stabilization of the protein structure
  - denaturation of the protein when broken

- Protein used: albumine (BSA)
  - BSA reacts with reducing agents (DTT, phosphine) to break the S-S bonds.
  - Depending on the conditions, the reaction is equilibrated or total
  - The kinetic of the reaction is monitored by measuring Raman spectra over time

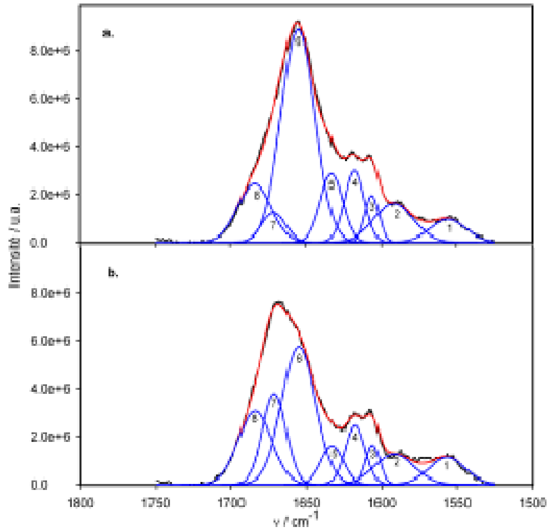
Biomolecules analyses: proteins



- Example of the disulfide bridge breaking in proteins



S-S bond before/after reaction



Amide I bond before/after reaction

- Contributions of the different peaks are calculated after deconvolution
  - calculation of physical parameters, such as reaction rate, free enthalpy, activation energy
  - information about the structure of the denatured protein

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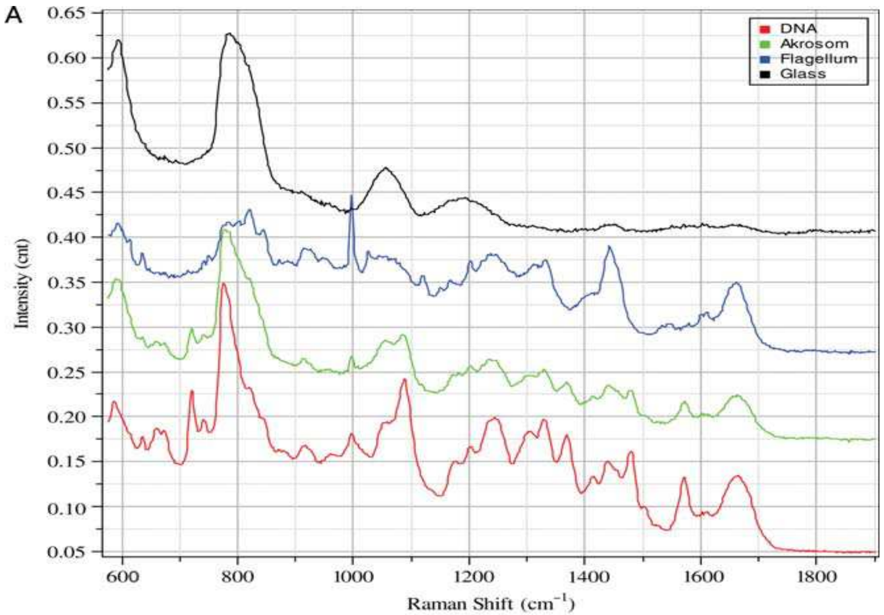
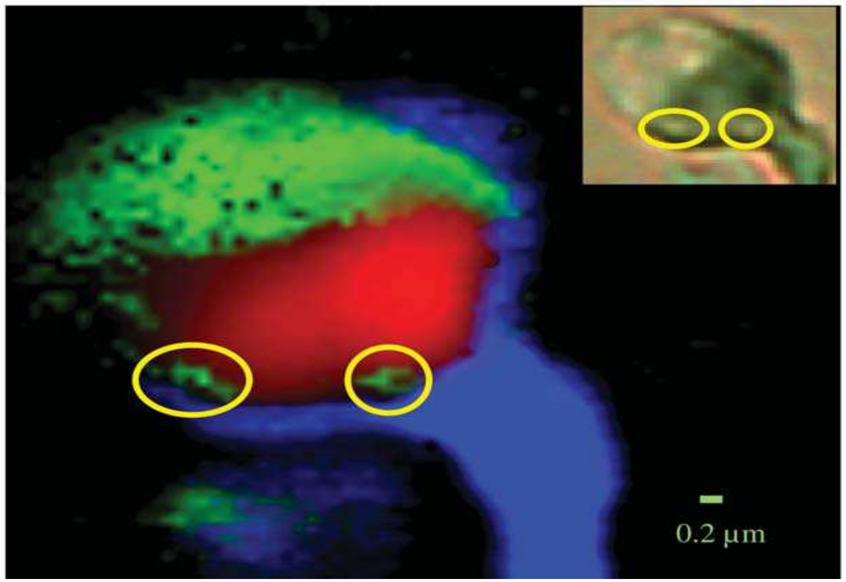
Biomolecules analyses: DNA

- Example of DNA in semen
  - Male infertility is often not well diagnosed, thus therapeutic options are limited
  - Nuclear DNA damage of semen is one of most crucial cause, but it currently lacks of reliable method to assess the status of the sperm's DNA

A



B



  - Mapping of a sperm head with spectra obtained every 50 nm. The colours represent the different parts of the head, based on spectral information.

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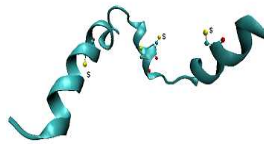
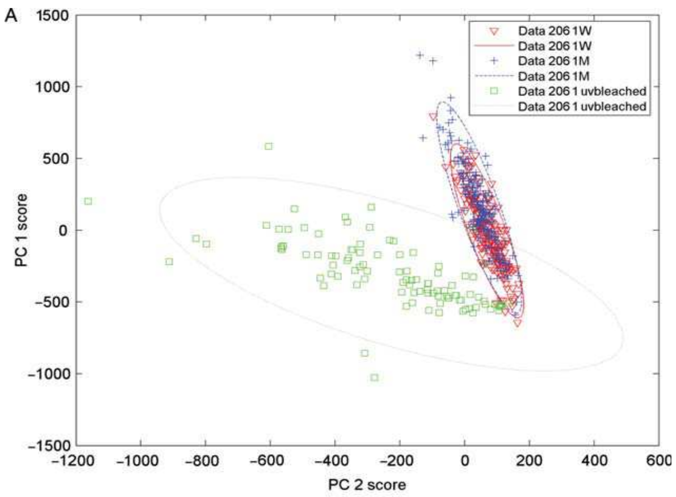
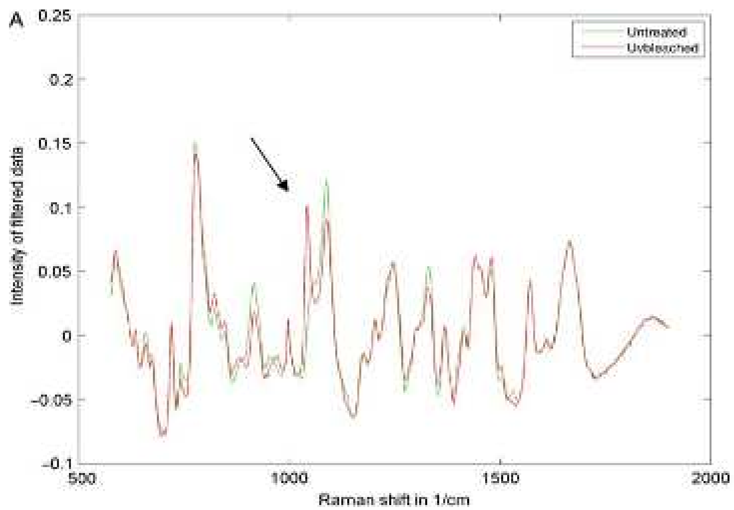
Biomolecules analyses: DNA



- Example of DNA in semen
  - Semen collected from 8 donors. 200 sperm/sample/treatment were analyzed by Raman
  - Half of the population is irradiated with UV-B; the other half remain untreated
  - UV-B causes damages on nuclear DNA

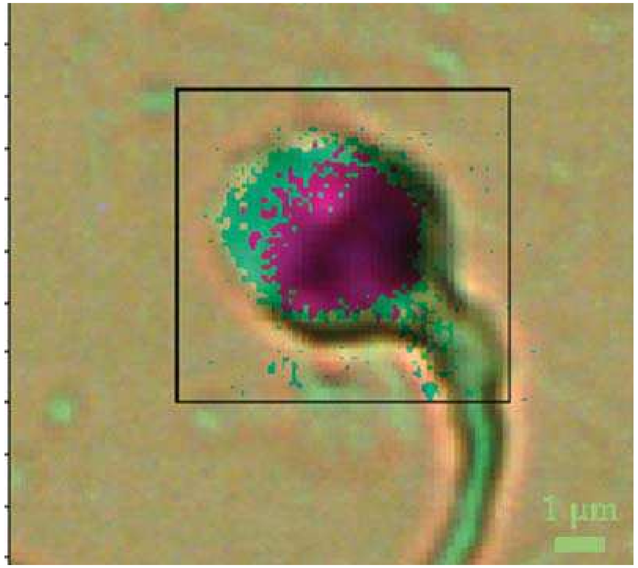




  - 2 populations are clearly identified in the PCA plot: the untreated one vs the UV-bleached one
  - Averaged spectra of both population effectively show significant differences, assigned to modifications of nucleotide bases (phosphate band) due to UV-B irradiation

Biomolecules analyses: DNA

- Example of DNA in semen
  - Distibution of damaged and undamaged DNA within the sperm nucleus



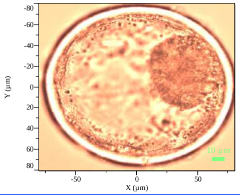
Damaged

Undamaged

  - Using the scores of the PCA (principal component analysis), the distribution of the damaged DNA can be visualized
  - It is mainly located in the periphery (under the acrosomal cap)

Cells analysis : sorting and imaging

Cells analyses



- Raman: interesting tool for cells analysis
  - Spatial resolution
  - Confocality
  - Complementary to fluorescence

- Used to analyse single cells for identification purposes

- Used to analyse cells content
  - Drug location and interaction in cells
  - SERS to enhance the signal of low concentrated compounds

European Research Projects - Life sciences

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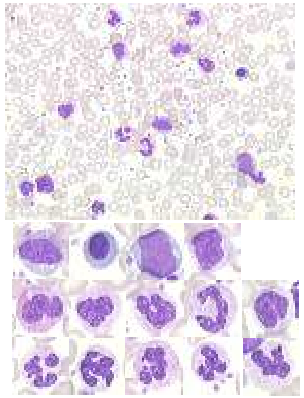
Disease diagnostic and prognostic

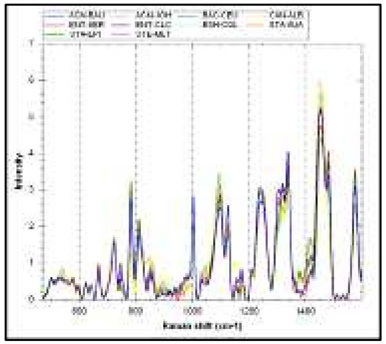
- **IHMO 2008-2010**

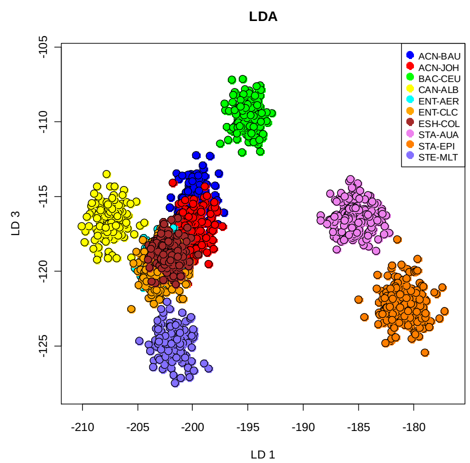
  Hybrid **I**maging **M**icroscopy for **O**ncology
  - Diagnostic and prediction of tumors
  - Blood smear analysis



- **DIAGRAM 2009-2010**

  Detection and **I**dentification of bacteria by **Ram**an spectroscopy and SERS on nanostructured surfaces





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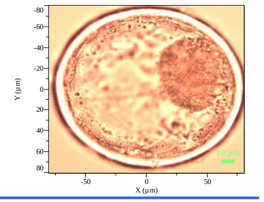
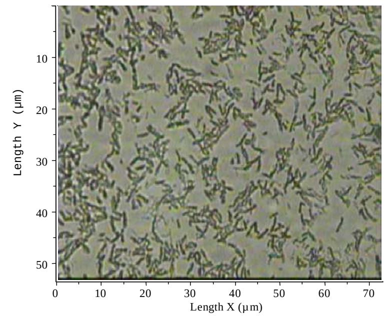
Cells analyses: bacteria



- Bacteria identification and classification
  - More than 3800 spectra of different species / strains
  - Classification according to the species and strain



	Total number of spectra	Number of wrongly classified strain spectra	Recognition rate for strains (%)	Number of wrongly classified species spectra	Recognition rate for species (%)
B. pumilus DSM 27	57	11	80.7	7	87.7
B. pumilus DSM 361	69	10	85.5	5	92.8
B. sphaericus DSM 28	53	8	84.9	3	94.3
B. sphaericus DSM 396	42	6	85.7	6	85.7
B. subtilis DSM 10	306	7	97.7	5	98.4
B. subtilis DSM 347	42	3	92.9	3	92.9
E. coli DSM 423	94	19	79.8	0	100.0
E. coli DSM 429	90	29	67.8	0	100.0
E. coli DSM 498	134	25	81.3	4	97.0
E. coli DSM 499	83	42	49.4	1	98.8
E. coli DSM 613	86	23	73.3	0	100.0
E. coli DSM 1058	71	15	78.9	0	100.0
E. coli DSM 2769	108	30	72.2	0	100.0
M. luteus DSM 348	619	3	99.5	3	99.5
M. luteus DSM 20030	48	4	91.7	3	93.8
M. lylae DSM 20315	45	4	91.1	4	91.1
M. lylae DSM 20318	20	1	95.0	1	95.0
S. cohnii DSM 6669	67	1	98.5	1	98.5
S. cohnii DSM 6718	65	11	83.1	9	86.2
S. cohnii DSM 6719	63	10	84.1	5	92.1
S. cohnii DSM 20260	65	4	93.9	1	98.5
S. epidermidis 195	74	3	96.0	3	96.0
S. epidermidis 2682	141	5	96.5	0	100.0
S. epidermidis DSM 1798	112	46	58.9	1	99.1
S. epidermidis DSM 3269	93	33	64.5	0	100.0
S. epidermidis DSM 3270	110	48	56.4	0	100.0
S. epidermidis DSM 20042	106	42	60.4	0	100.0
S. epidermidis ATCC 35984	805	4	99.5	4	99.5
S. warneri DSM 20036	71	9	87.3	4	94.4
S. warneri DSM 20316	67	4	94.0	2	97.0
average recognition rate	**3806**		**82.7**		**96.3**

- 96% identification @ specie level
- 83 % identification @ strain level
- Use of chemometrics to build up classification models

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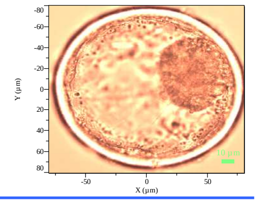
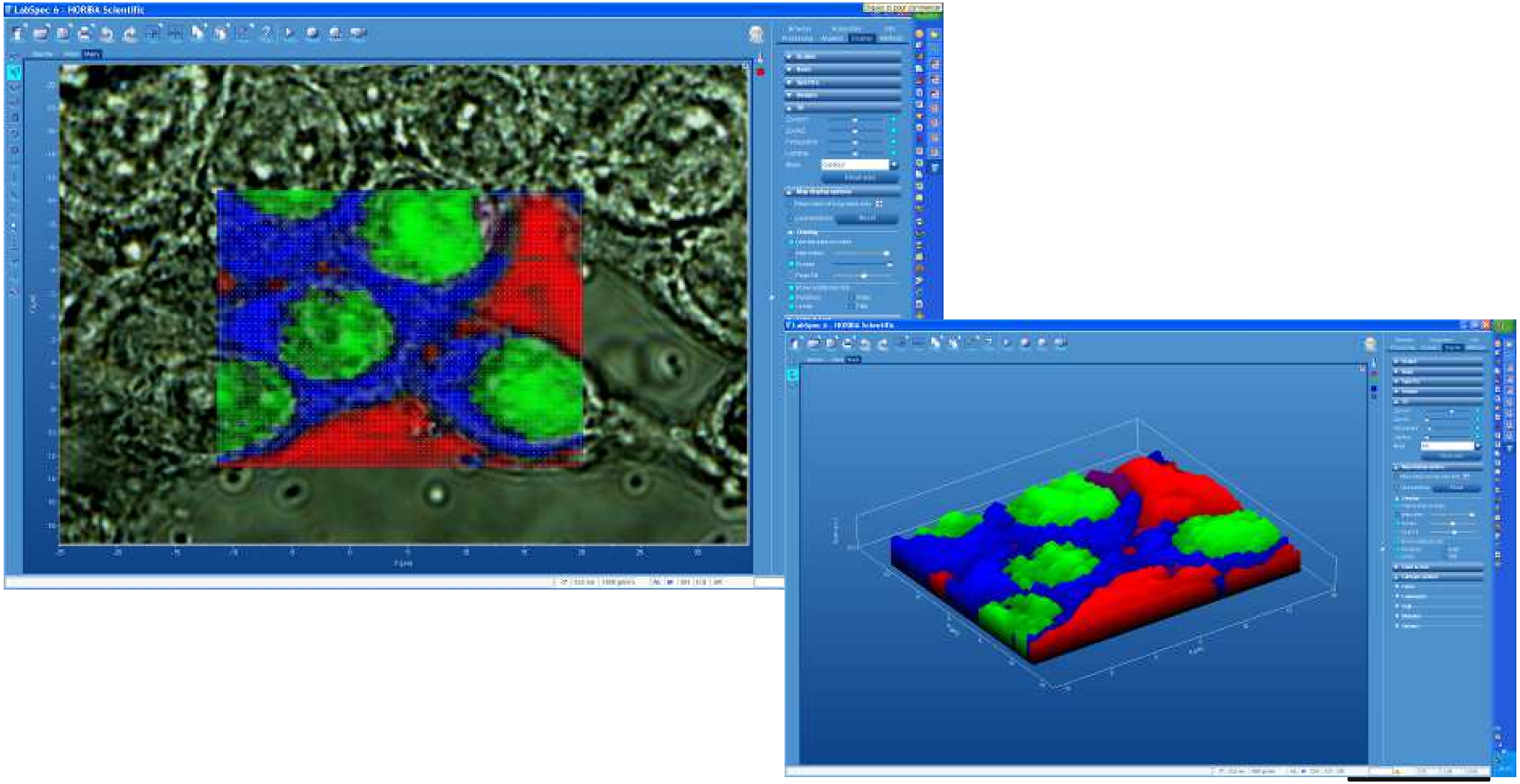
Cells analysis: lymphocytes

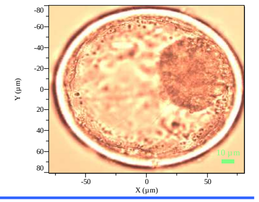


Lymphocytes classification

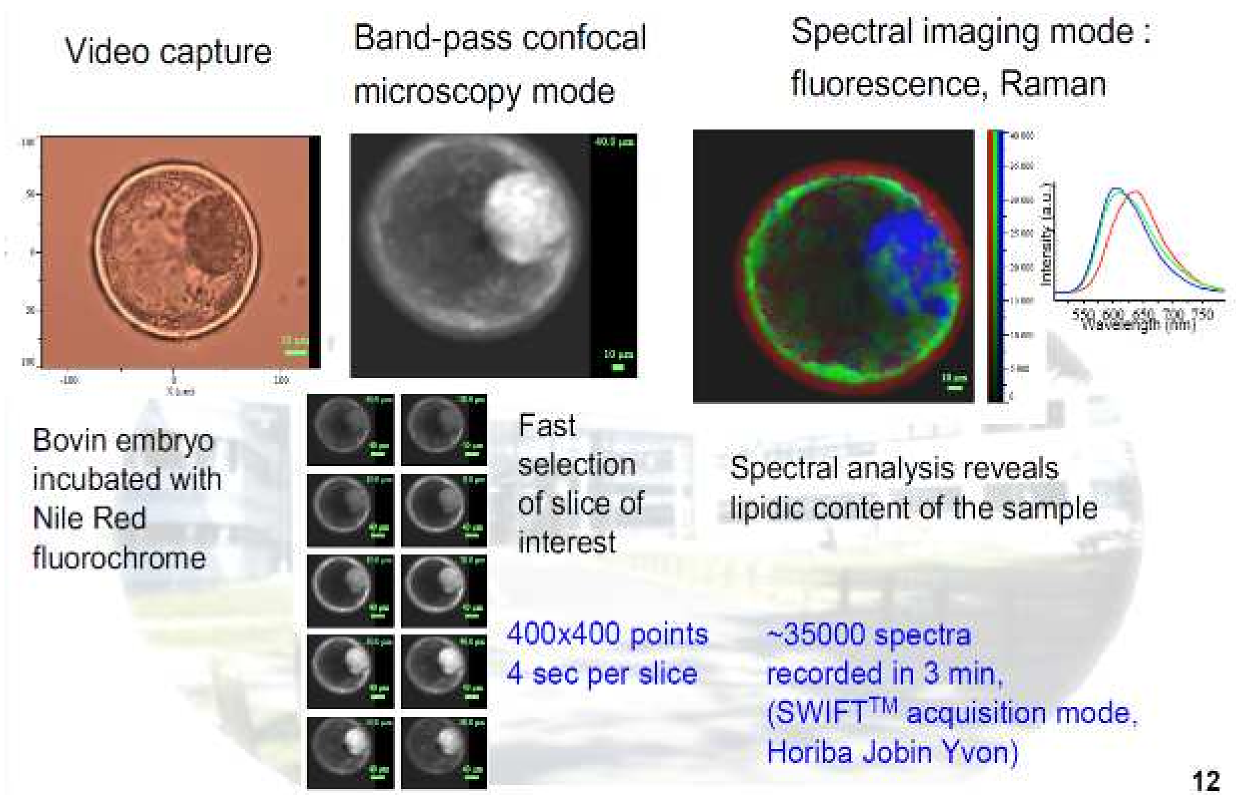
- Raman signature is used to lymphocytes classification

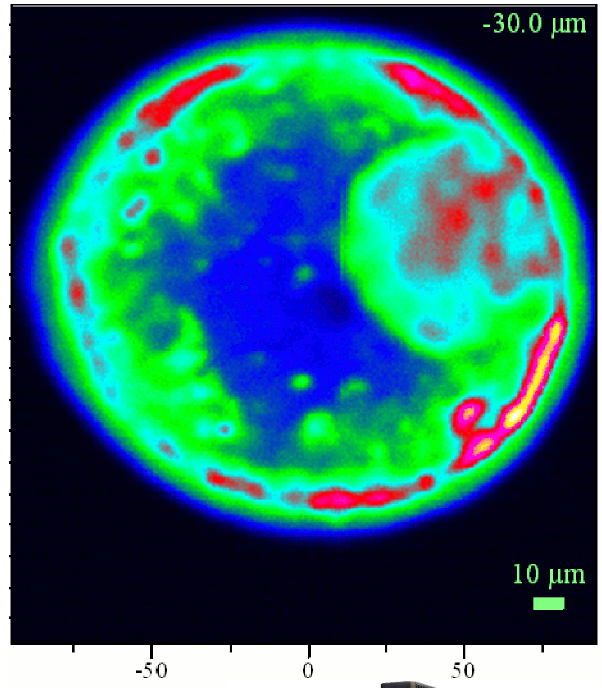


Cells analyses

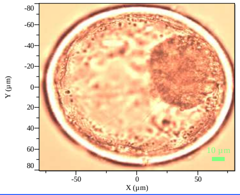


20 slices, with 400 x 400 data points
(over a 203um x 185um area)





Cells analyses: chemotherapy optimization



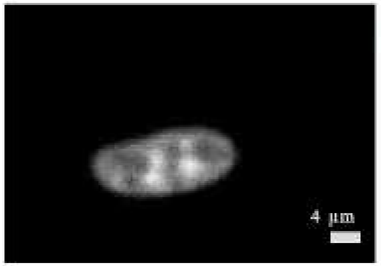
XploRA INV confocal fluorescence spectral imaging

Drug (doxorubicine) delivery in breast cancer cell

Video capture

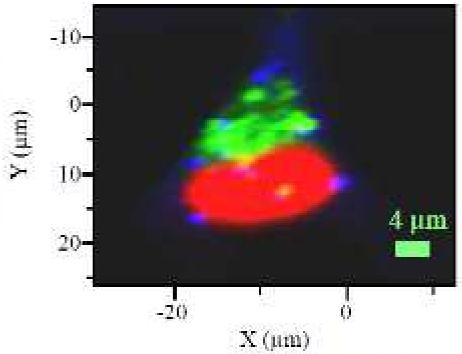
MCF-7 cancer cell
Treated with DOX

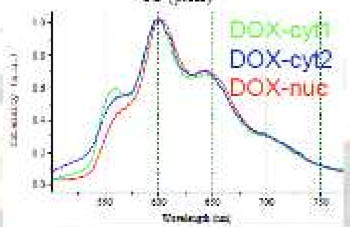
Band-pass confocal microscopy mode



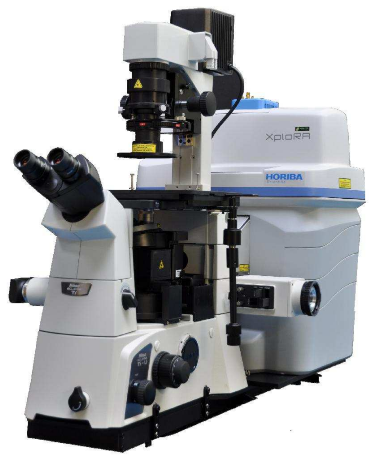
Most intense DOX fluorescence is in the nucleus

Spectral imaging mode : fluorescence, Raman





Spectral analysis reveals drug molecular interactions



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Tissues imaging
Diagnosis, pronostic for cancer

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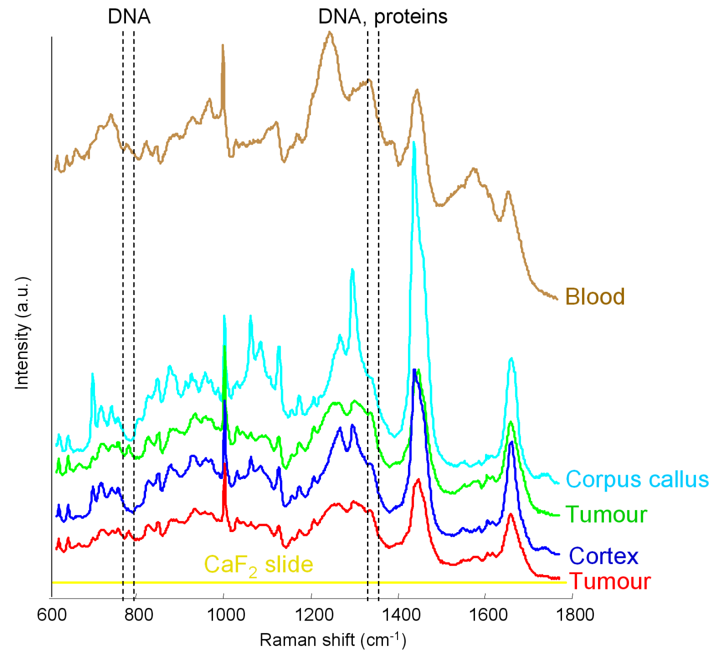
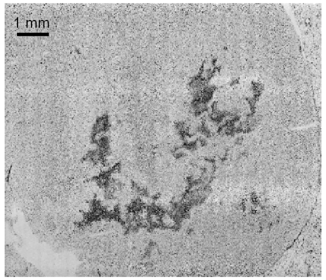
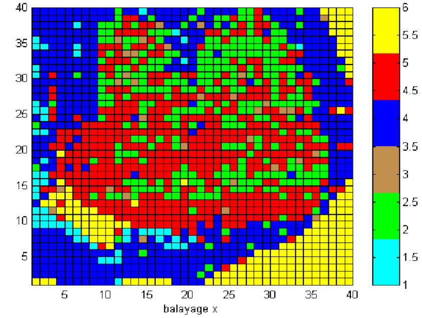
Diagnosis and tissue analysis

- Raman provides detailed information on cell/tissue biochemistry
- Probes DNA, RNA, lipids, proteins, carbohydrates etc
- Clustering of spectra identifies tissue type
- Two classifications of Brain cancer tumour
- **Red** tumour areas match identification by pathologist
- **Green** areas are likely to be 'early' stage tumours





Photomicrograph



Raman mapped image

Diagnosis and tissue analysis

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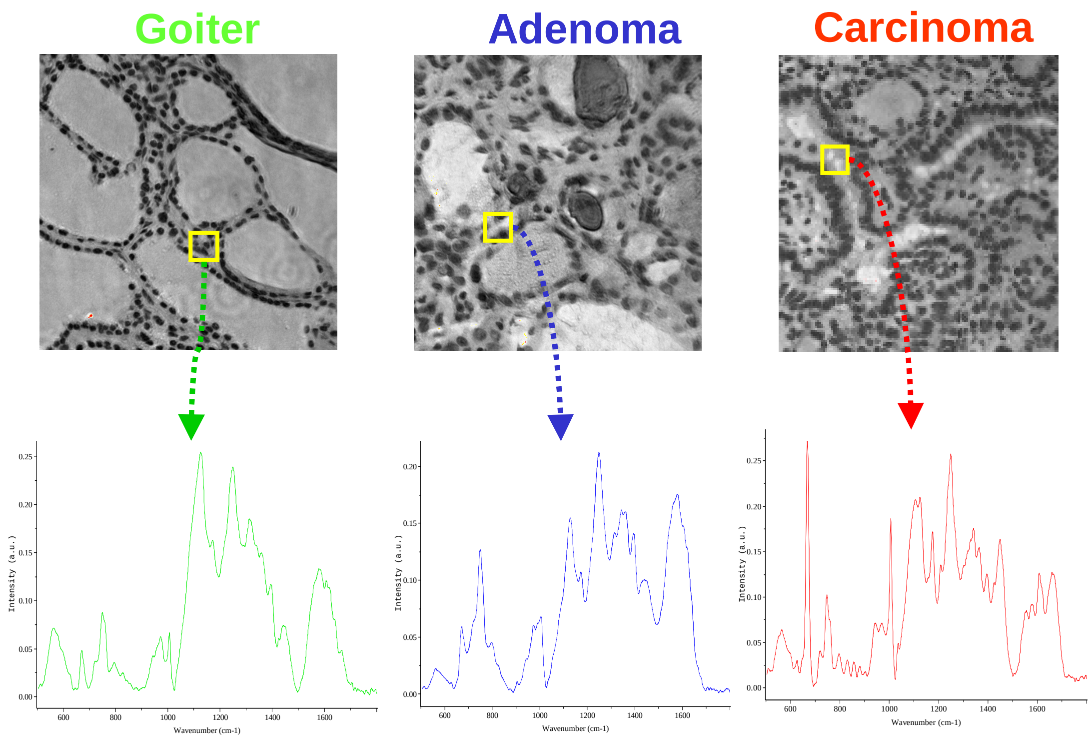
Characterization of human thyroid tumor tissue

- Raman spectroscopy allows to distinguish between tissue corresponding to non-tumorous **goiter**, benign **adenoma** and malign **carcinoma.**

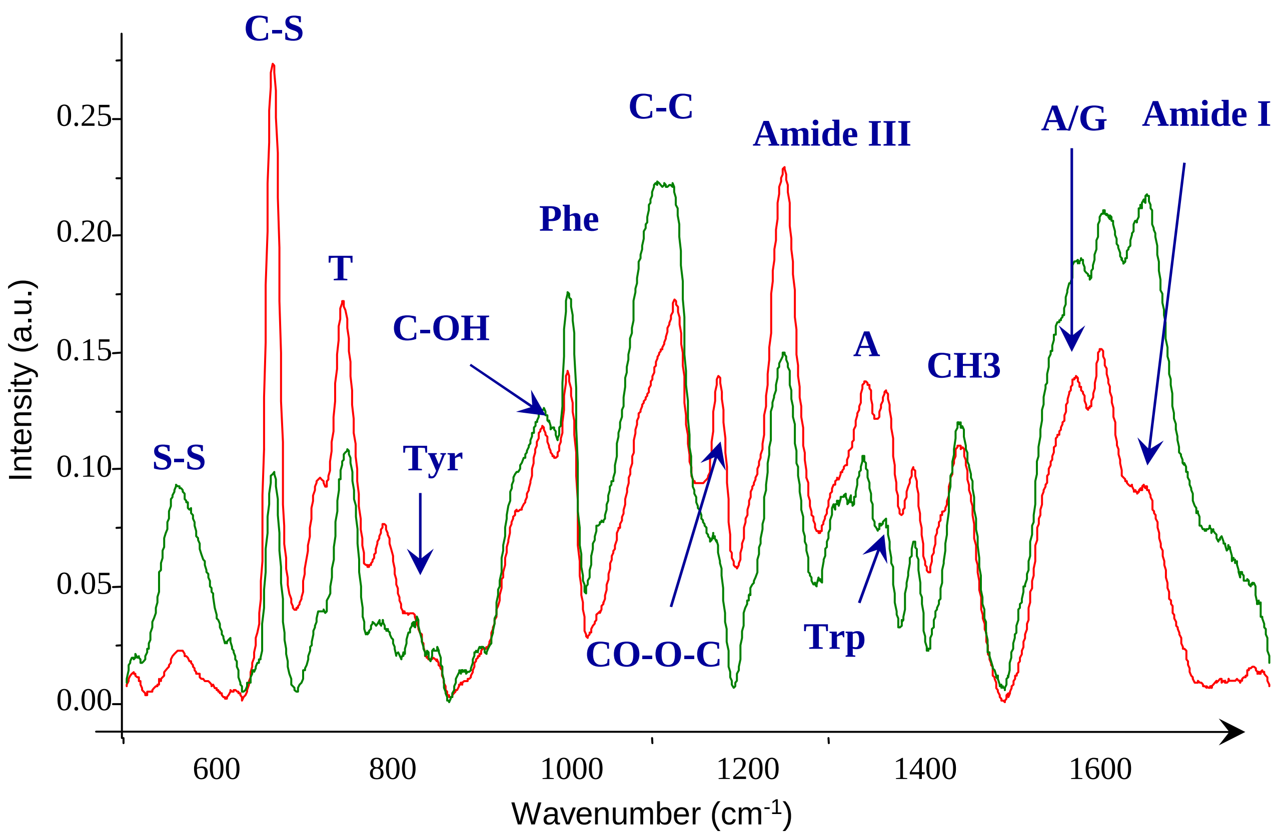


Diagnosis and tissue analysis

HORIBA Scientific

Characterization of human thyroid tumor tissue

- Peak identification comparison: **goiter** and **carcinoma.**



T: thymine
Tyr: tyrosine
Phe: phenylalanine
Trp: tryptophan
A: adenine
G: guanine

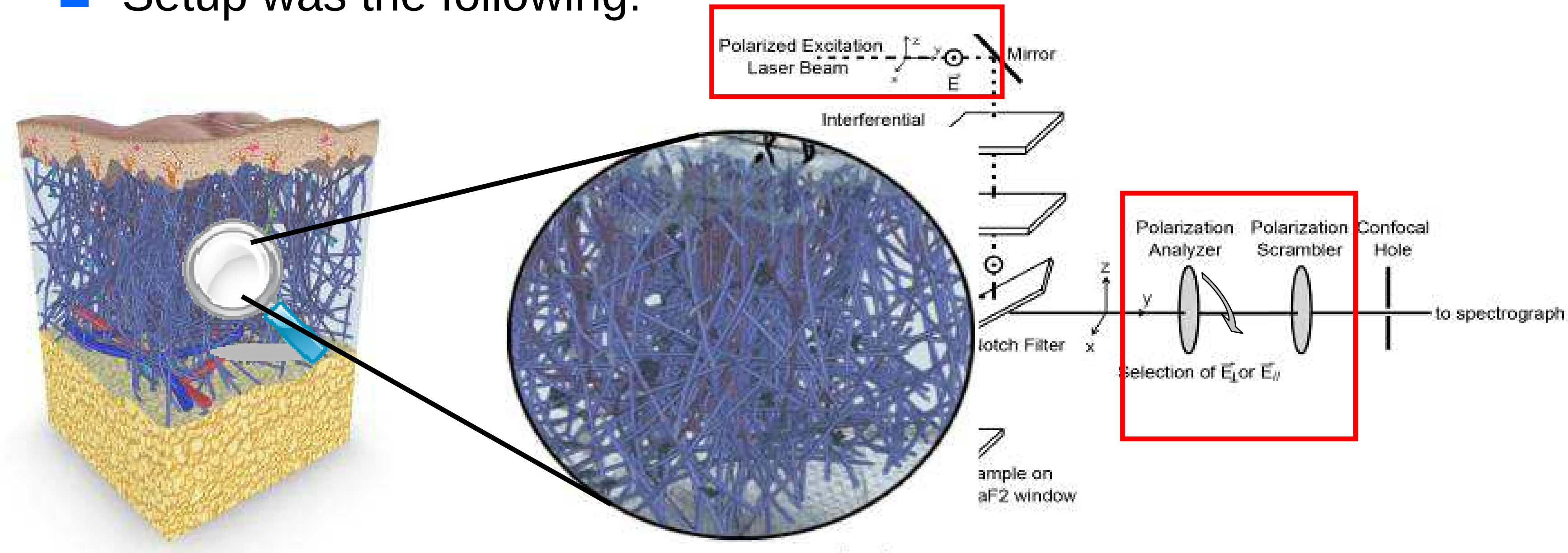
Probing tumour tissues in skin basal cells carcinoma using polarized Raman microspectroscopy

HORIBA Scientific

Key Reference Olivier Piot, Elodie Ly, Michel Manfait

Probing tumour and peritumoral tissues in superficial and nodular skin basal cells carcinoma using polarized Raman microspectroscopy to be submitted at ICORS 2010 conference - UMR6237 MEDyC, University of Reims Champagne Ardennes, Reims, France. Appl. Spectrosc. 2008 ; 62 (10) : 1088-1094

- Polarized Raman has been used mostly for oriented macromolecules such as fibers (silk, hair…), DNA, viruses, bones and isolated cells
- Applied to skin tissues, it was evidenced that polarized Raman can bring complementary information
- Setup was the following:



www.skinscience.fr

Probing tumour tissues in skin basal cells carcinoma using polarized Raman microspectroscopy

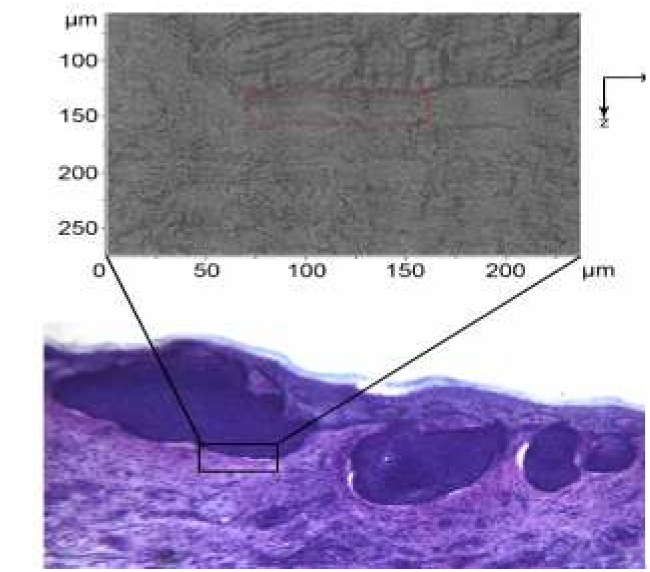
Basal cell carcinoma (N=5)

4 areas were studied

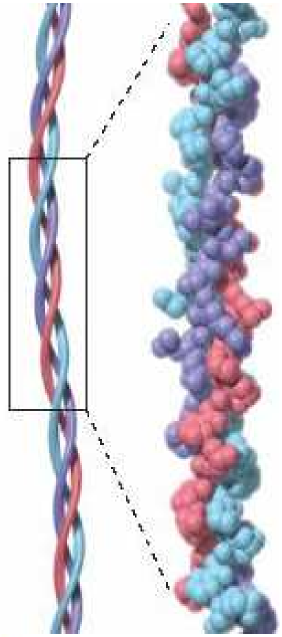
- Peritumoral dermis (=close to the tumour)
- Healthy dermis (=far from the tumour)
- Healthy epidermis
- Tumour

3 spectra were acquired for each area

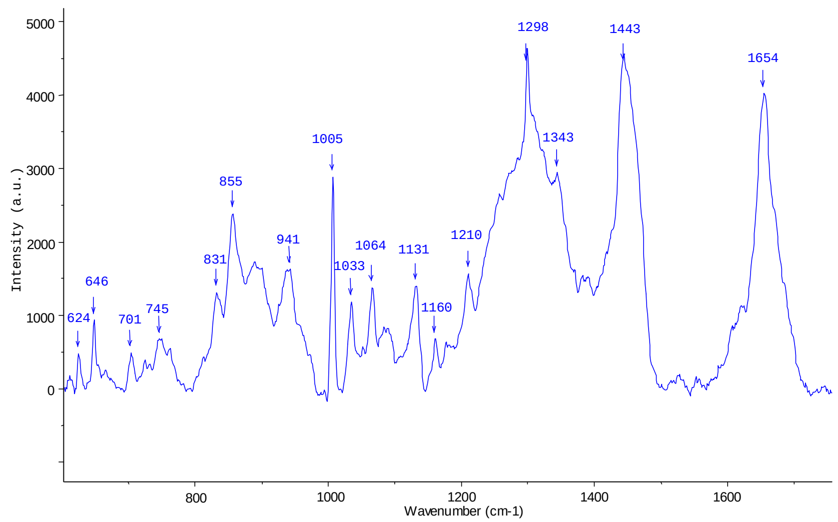
- Standard Raman
- Polarized Raman I//
- Polarized Raman I⊥



Tumour invasion analysis



Collagen triple helix



Skin typical Raman spectrum

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Probing tumour tissues in skin basal cells carcinoma using polarized Raman microspectroscopy

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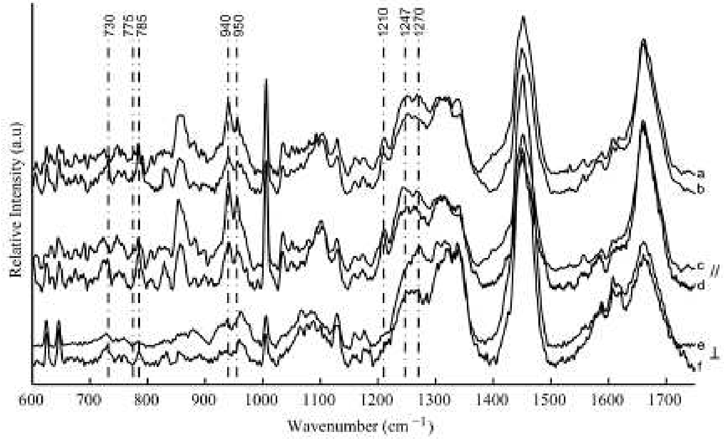
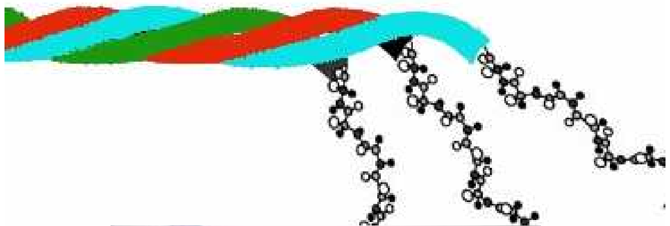


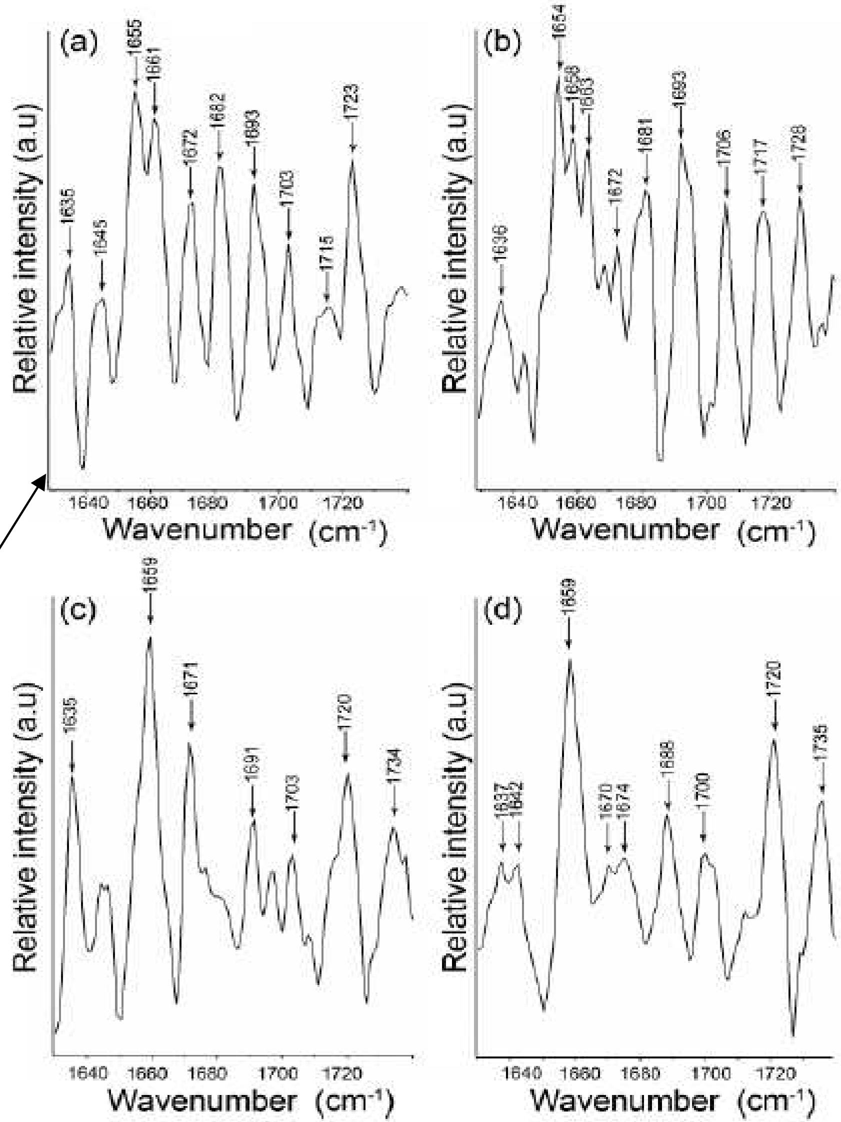
➔ degradation of collagen in 3 alpha chains

➔ transient state of collagen degradation?

(middle peak might be related to double alpha chain)



Standard Raman | **Raman Polarized //**



Peritumoral dermis

Healthy dermis

Tips and tricks to optimize your Raman measurements of biological samples

- Get a dedicated fully automated system with
    - multiple lasers & fast mapping (fluorescence, photosensitivity issues…)
    - immersion objectives
- Use appropriate microscope slides in CaF_2 or fused quartz
- Hold your sample with optical tweezers
- Enhance your signals through plasmonic resonance effects (SERS, TERS)

NEW in 2010 Innovation & Performance

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Product release

- XploRA Inverted:
  - Inverted Nikon Ti-U microscope for applications in biology, lifesciences, and nanomaterials
  - Mid-range spectral resolution (<2cm^{-1})
  - Fully automated system
  - 3 lasers in Vis-NIR (473 to 785nm), 4 gratings
  - High thoughput microscope without compromise on microscopy visualisation
  - Compatible with Ultra fast imaging

NEW in 2012 Innovation & Performance

HORIBA Scientific

■ LabRAM HR Evolution:

- Fully achromatic system from 200nm to 2.1µm
- Multiple microscopes available, including double microscope configuration (upright, inverted)
- Highest Spectral resolution for single stage spectrograph
  - ✓ down to 0.5cm^{-1} FWHM in visible with 2400g/mm grating
- Fully automated system
  - ✓ Up to 6 lasers from 229nm to 1064nm
  - ✓ Up to 3 simultaneous detectors
- Improved spatial resolution
  - ✓ Down to <400nm FWHM in XY
  - ✓ Down to <1µm FWHM in Z

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NEW in 2012 Innovation & Ease of use

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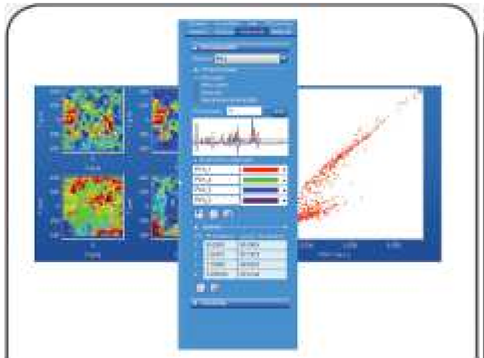
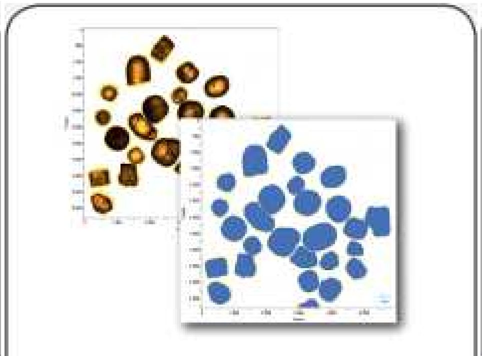
LabSpec 6
Spectroscopy Suite

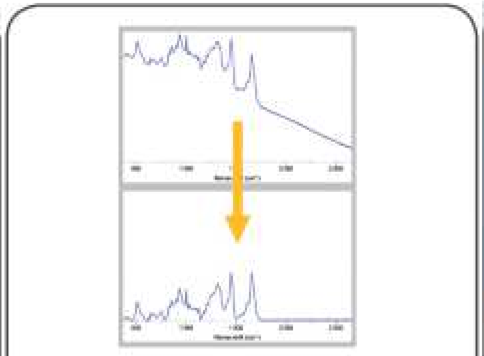
- ✓ Advanced automation and customization
- ✓ Fully Integrated Multivariate Analysis module
- ✓ Particle Finder module
- ✓ On-the-fly automated fluorescence removal “FLAT”
- ✓ Real 3D imaging, XYZ and chemical information



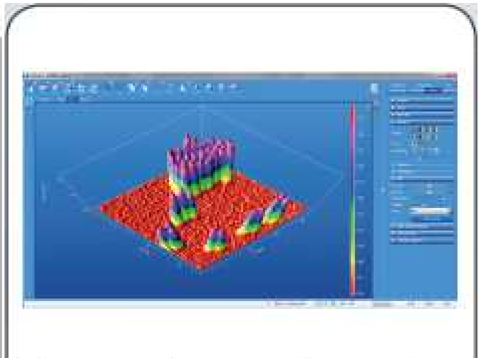
Fully Integrated Multivariate Analysis module*



ParticleFinder module* for automated particle location and analysis



FLAT correction
On-the-fly automated Fluorescence Removal



3D image rendering with variable smoothing, lighting and palettes

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Select appropriate microscope slides

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- Ban plastic or glass microscope slides which give a strong fluorescence signal when excited in visible and/or NIR

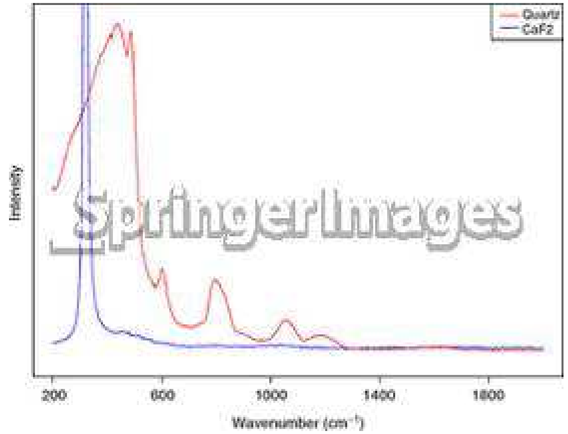

Fig 2
Comparison of background Raman spectra derived from quartz versus CaF2 slides.
Note the increased background of quartz slides below 1,200 cm−1 wavenumbers.

- Go for fused quartz (10$) or best Calcium fluoride slides (35$)
  - Crystran (UK)
  - GM Associates (USA)

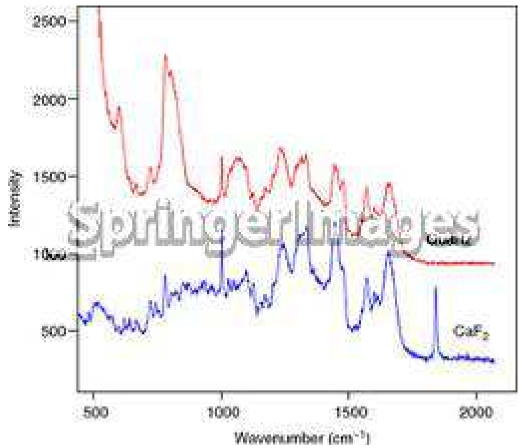

Fig 3
Raman spectra derived from E. coli cells on either Quartz or CaF2 slides for single cells with 20 s integration.
Note the higher baseline in Quartz derived spectra due to inherent Raman background for Quartz (Fig.) and the diversity of spectral peaks obtained from a single cell.

Raman combined with Laser tweezers for bacteria analysis

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- Photons exert a force on any material to which they are incident
- A focussed laser beam exerts sufficient force to be able to hold and move small objects (typically in the size range 0.1-10 µm)
- Combination of Raman and laser tweezers allows individual bacteria to be:
  - Held in place
  - Interrogated using Raman
  - Classified according to species / age / ^{13}C labelling
  - Moved to a storage point
  - Dropped

Manipulation of small particles in fluids

785nm laser, 300mW, on LabRAM HR

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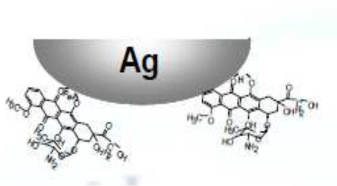
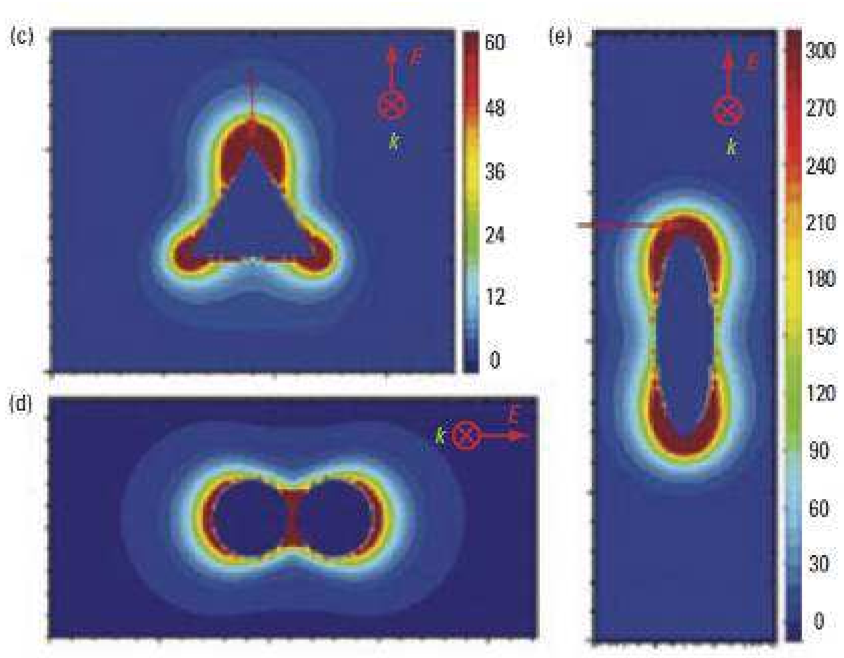
SERS: improve weak Raman signals



- Surface Enhanced Raman Scattering (SERS) has long been used to **enhance weak Raman signals** by means of surface plasmon resonance, allowing detection of chemical species at **very low concentration**
- SERS exploits the **generation of highly localized fields in the near field of adapted metallic nanostructures** for enhancing spontaneous Raman scattering
- Increases in sensitivity can be by **many orders of magnitude**, improving from 10^{-3}M for spontaneous (normal) Raman, to 10^{-5}M for resonance Raman, and **up to 10^{-12}M for SERS**
- Common metals used for SERS include **gold and silver**, and these can either be used in the form of a **nano-scale roughened surface** onto which the sample is adsorbed, or as a **colloid suspension**



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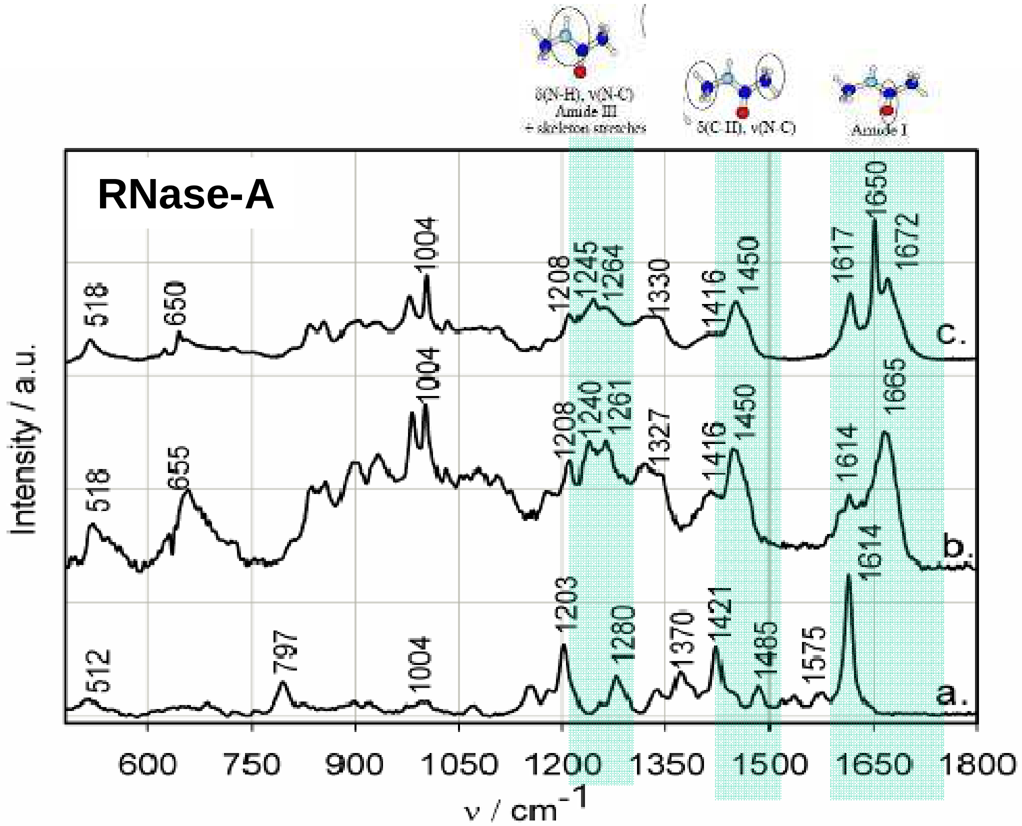
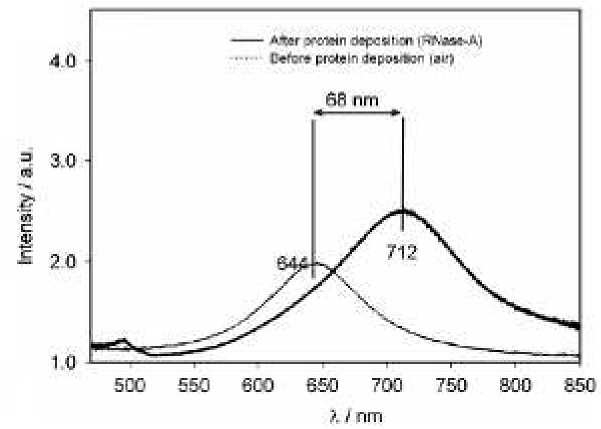
SERS for proteins – Developing a reproducible nanobiosensor

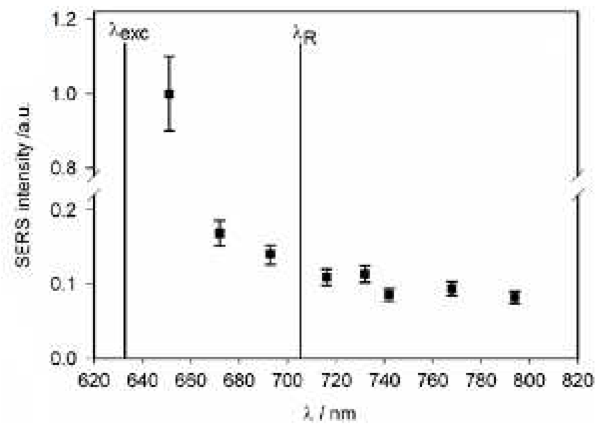
SERS substrates : gold nanocylinders arrays



(a) SERS spectrum measured for RNase-A at 1mM concentration
(b) Raman spectrum of RNase-A in aqueous solution at 1mM
(c) Raman spectrum of RNase-A in powder state

LSPR characterisation





SERS intensity as a function of the position of the plasmon resonance calculated for the RNase-A Raman bands located at 1614 cm^{-1}. The excitation wavelength, (λ_{exc}) is 632.8 nm and the considered Raman band (λ_R) for RNase-A is located at 705 nm.

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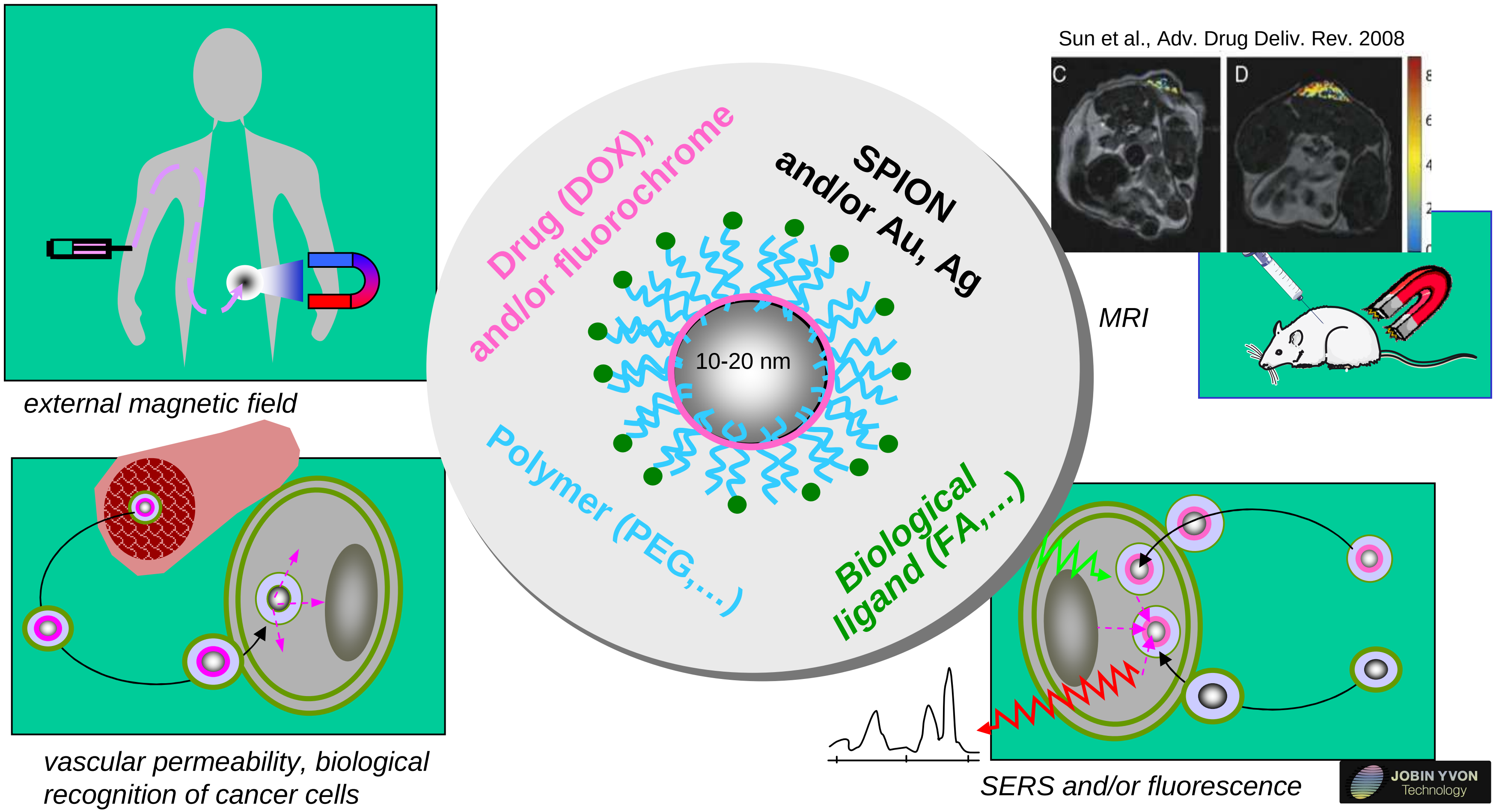
(C. David et al. Nanotechnoloy (2010))

Nanoparticles for drug targeting and imaging

Courtesy of Pr I. Chourpa, group « Magnetic nanovectors for chemotherapy », EA 4244

Anticancer drug targeting | **Imaging of anticancer drugs/cancer cells**



external magnetic field

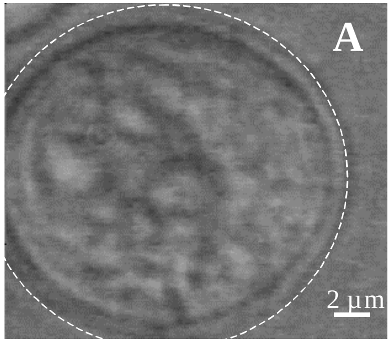
vascular permeability, biological recognition of cancer cells

SERS and/or fluorescence

Methodological coupling: Simultaneous co-detection SERRS-fluorescence on the same spectral image

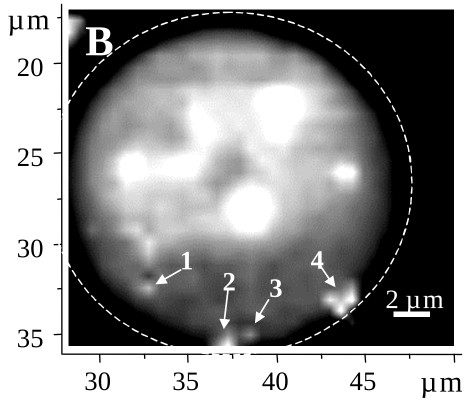
Courtesy of Pr I. Chourpa, group « Magnetic nanovectors for chemotherapy », EA 4244

Video capture

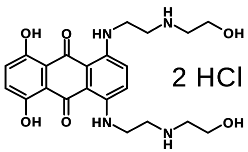


MCF-7 cancer cell treated with 1 µM MTX and incubated with NP Ag-citrate

Spectral intensity

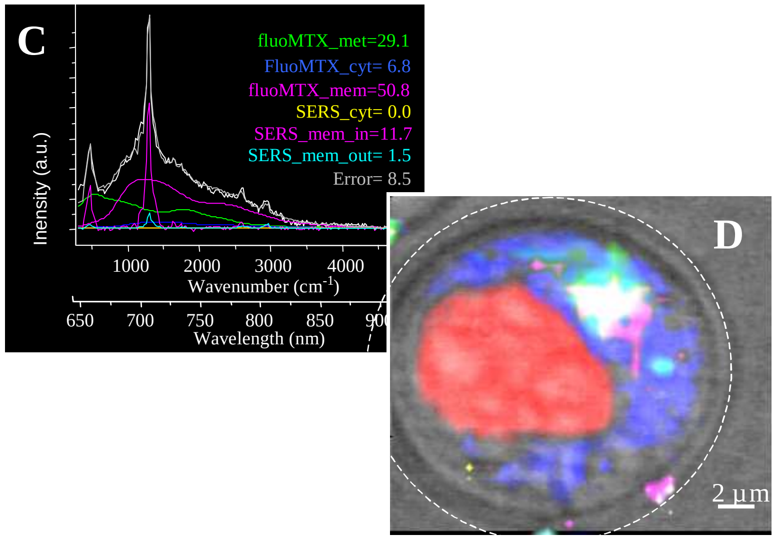


MTX :



Spectral analysis :

SERRS and fluorescence



Rem 1:

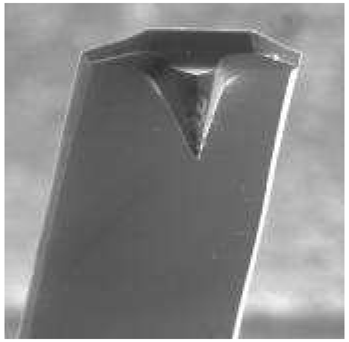
Aggregates ≤ 1 µm are detectable

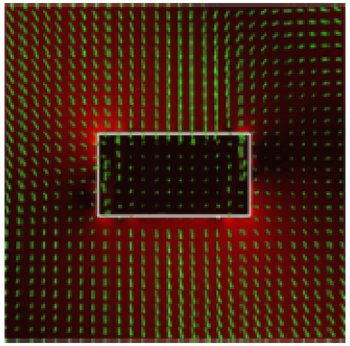
Rem 2 :

SERRS and fluorescence spectral informations are complementary

From colocalized AFM-Raman…
…to Tip Enhanced Raman Spectroscopy

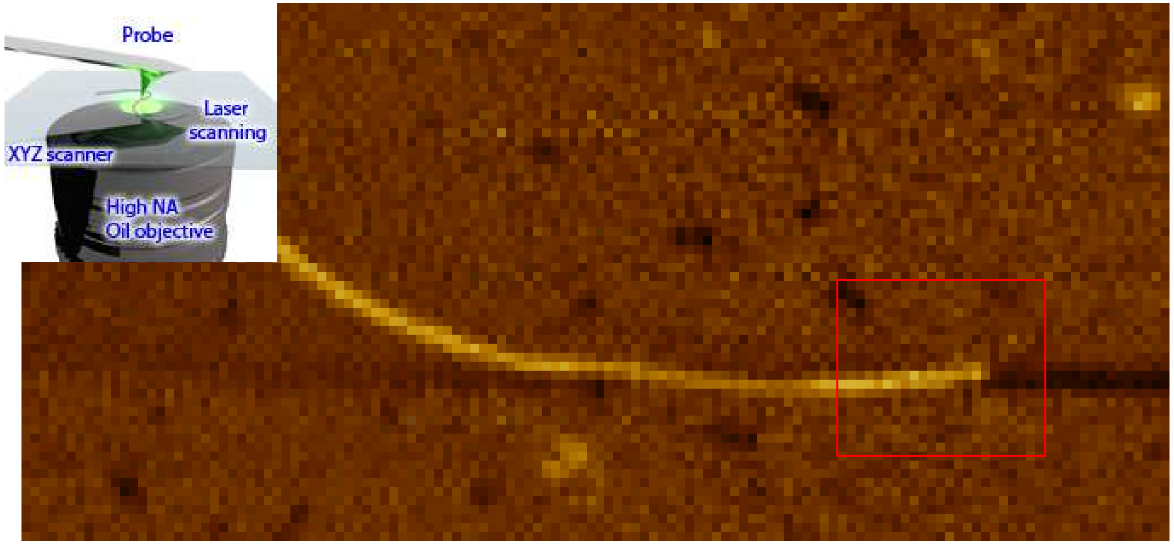
- A combination of various techniques and effects:
  - Scanning Probe microscopy
    - spatial resolution (near-field, imaging)
  - Surface plasmon resonance
    - signal enhancement necessary because Raman is a weak scattering
  - Optical spectroscopy
    - Raman 'sensor' (excitation collection detection)

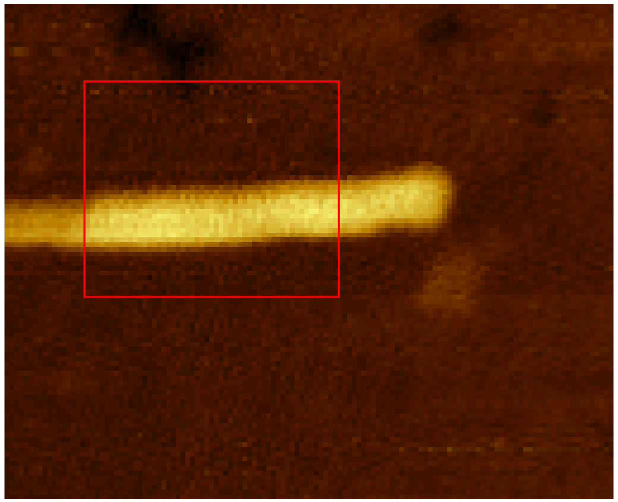


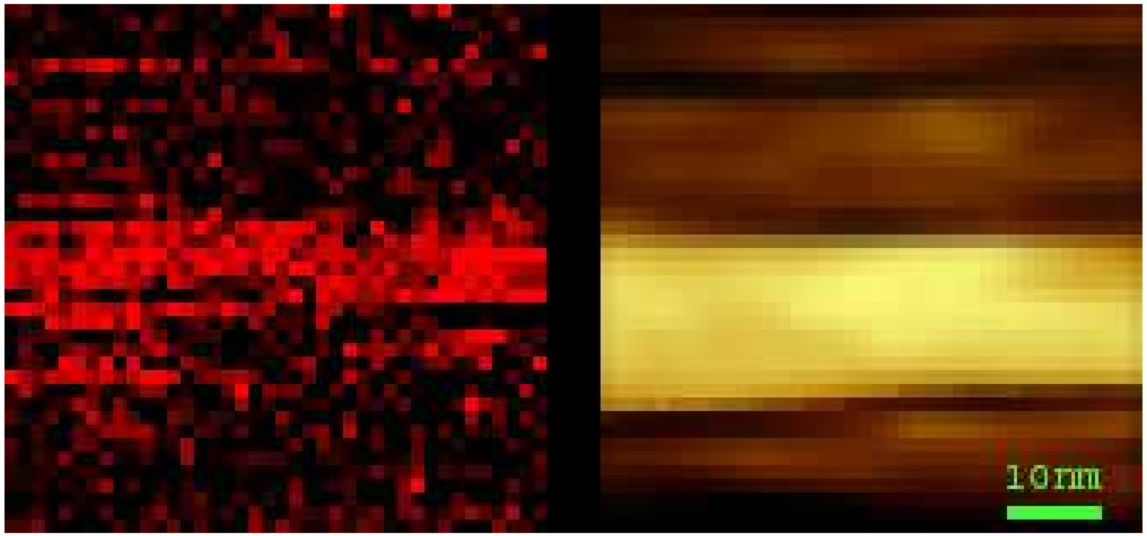


TERS Imaging results

- DNA:
  Raman resolution
  <15nm
- Inverted
  microscope
- Oil immersion







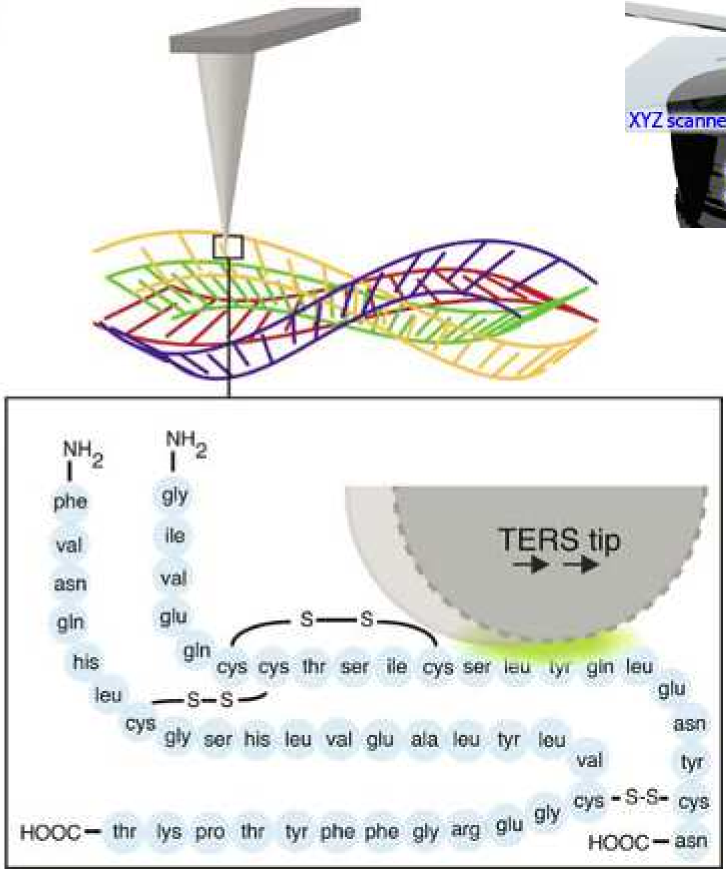
Tip Enhanced Raman Spectroscopy

HORIBA Scientific

- TERS profile across a single amyloid fibril

a)

b)



Deckert et al. J. Biophotonics 1–5 (2012)
DOI 10.1002/jbio.201100142

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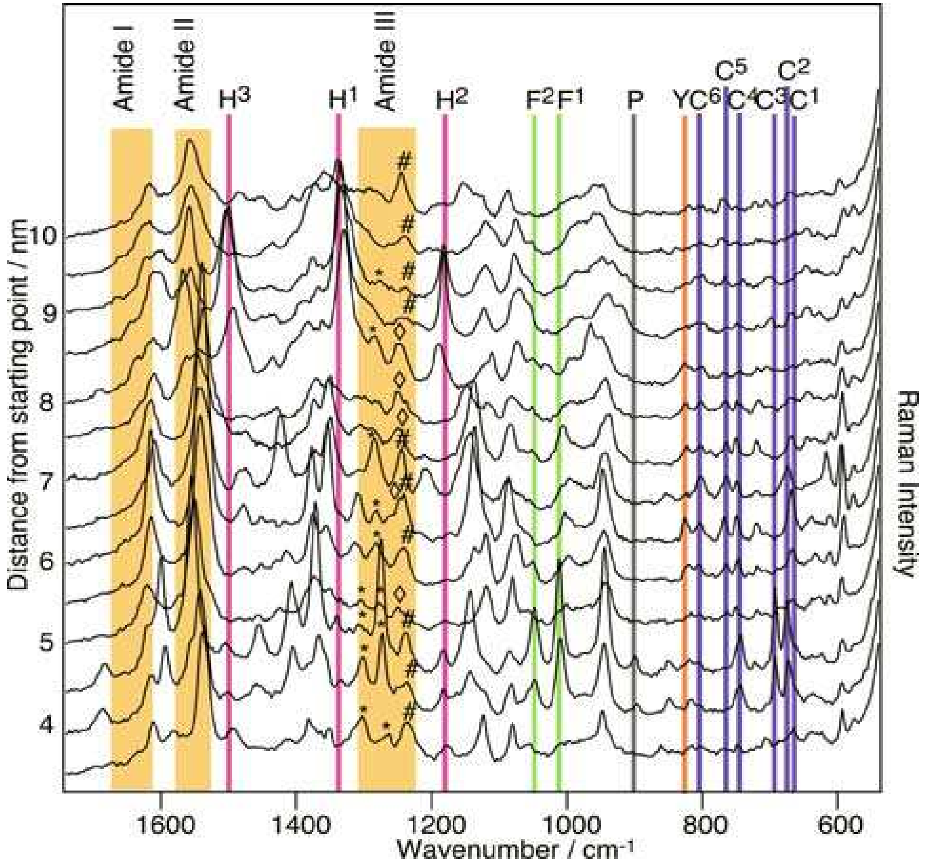
Tip Enhanced Raman Spectroscopy

Courtesy of Deckert et al. J. Biophotonics 1–5 (2012) DOI 10.1002/jbio.201100142

TERS profile across a single amyloid fibril.

- The study of amyloid structure and growth has been motivated by their implication in many human diseases. There are ~20 diseases associated with excessive deposits of amyloid plaques in the affected tissue or organ including Alzheimers disease (AD), Parkinsons disease (PD), type II diabetes, and spongiform encephalopathies.

- Raman bands of the different constituents of amino acids are distinguished



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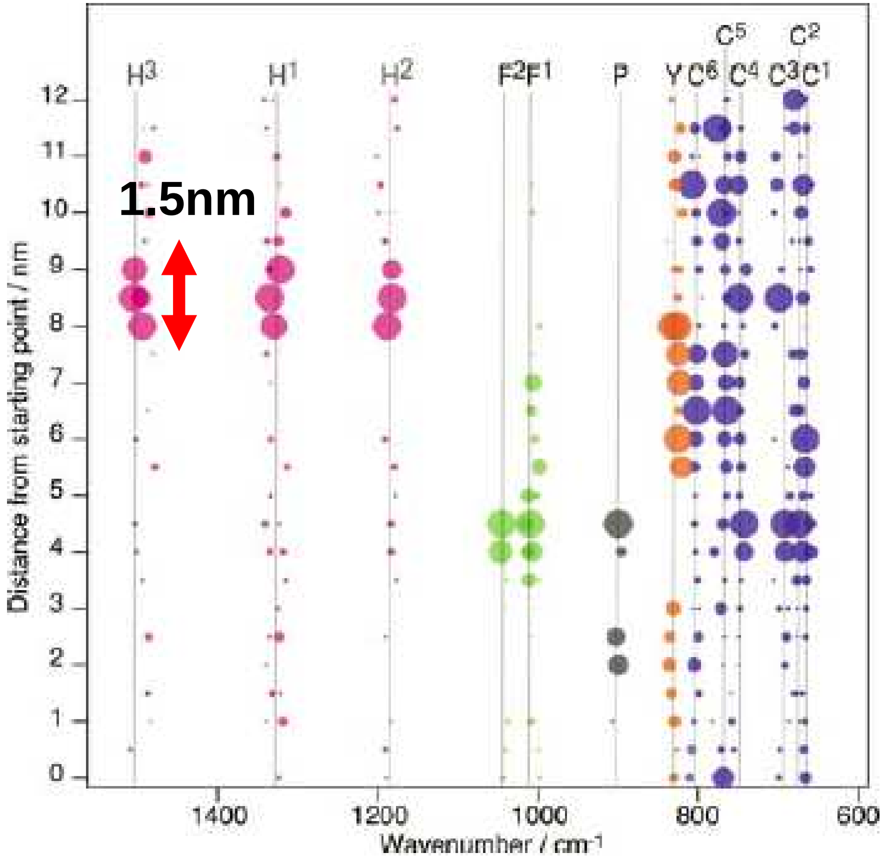
Tip Enhanced Raman Spectroscopy

HORIBA Scientific

- TERS profile across a single amyloid fibril

- TERS signal intensity tracking of amino acid signals of the fibril, blue: cystine (C), orange: tyrosine (Y), grey: proline (P), green: phenylalanine (F), purple: histidine (H).
- The sequence is clearly visible and shows a **resolution better than 2nm**



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Conclusion

- Raman as tool for bio-medical investigations
  - at biomolecules, cells and tissue levels
  - used for medical investigation and diagnosis, pharmacokinetics, biophysics, …
- Complementary technique to traditional ones:
  - Brings chemical specific information on the sample
  - Micron-scale spatial resolution (imaging)
  - Confocality
  - Coupling to confocal fluorescence
  - Non destructive
- Tips and Tricks
  - Use of appropriate substrates/matrix (CaF_2 or quartz slides, SERS nanocolloids)
  - Use of optical tweezers combined to Raman to hold the biomolecule during spectra acquisitions
  - SERS Increases highly the sensitivity for low concentrated samples
  - TERS increases both sensitivity and spatial resolution

Happy Researching,

Thank you!

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