RAMAN SPECTROSCOPY FOR BIOMEDICAL APPLICATIONS

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Outline

Introduction

Raman spectroscopy

Surface enhanced Raman spectroscopy

- Biological objects
- Analysis of the exhaled breath
- SERS substrate development

Coherent Raman techniques

- Stimulated Raman scattering

Research areas

Carbon based materials

- Amorphous carbon
- Nanodiamond
- Polymers
- Silicon carbide

Glasses

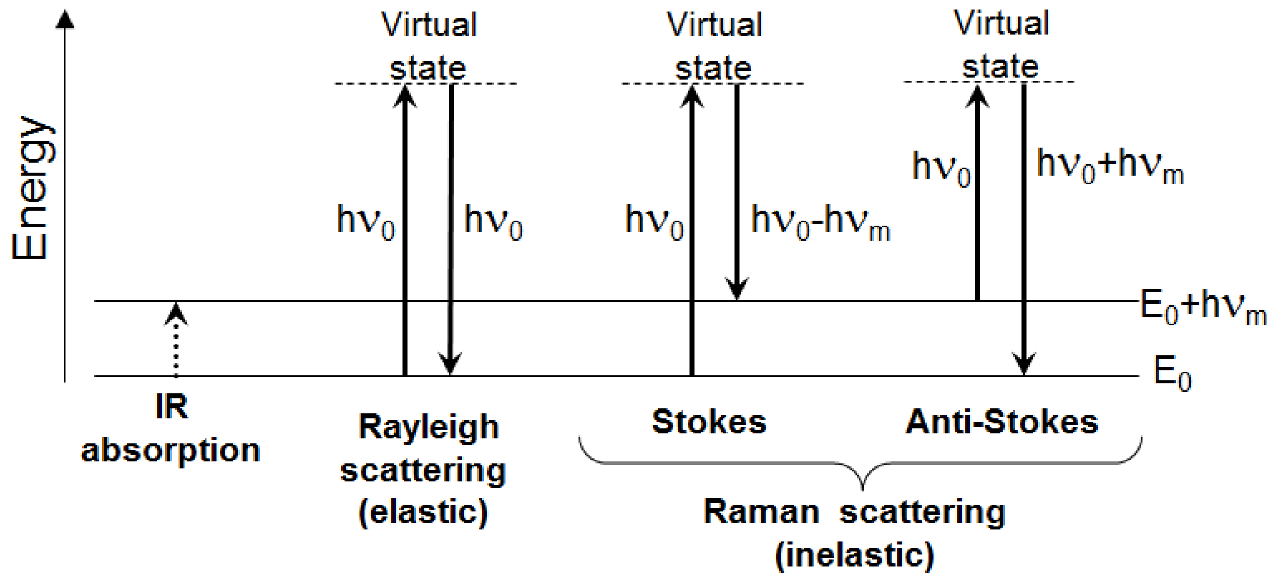
- Chalcogenides
- Photoinduced volume changes and mass transport

Raman spectroscopy

- Material characterization
- Development of optical systems for Raman measurements
- Surface Enhanced Raman spectroscopy
- Biological applications

Raman scattering

Inelastic light scattering due to the interaction of the incident light with matter.



$$\mu = \alpha_0 \, E_0 \cos(2\pi\nu_0 \cdot t) + \frac{1}{2}\left(\frac{\partial \alpha}{\partial q}\right)_{q=0} q_0 E_0 \left[\cos\left(2\pi\{\nu_0 - \nu_m\}\cdot t\right) + \cos\left(2\pi\{\nu_0 + \nu_m\}\cdot t\right)\right]$$

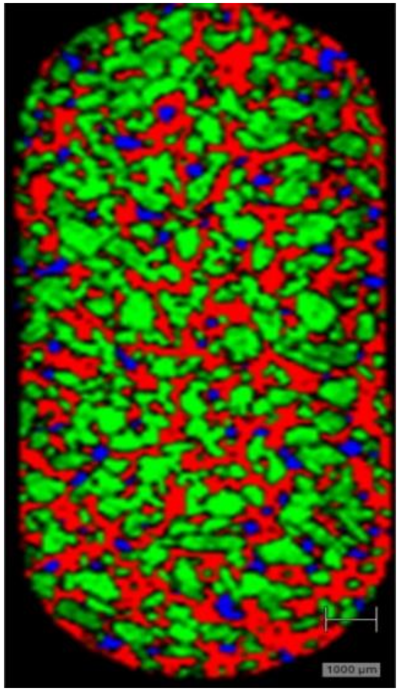
Selection rule: A vibrational transition is Raman active if the polarizability changes during the normal vibration.

For IR: A vibrational transition is infrared active if the dipole momentum changes during the normal vibration.

Applications

Material properties affecting the characteristic vibrations

- Composition
- Structural parameters
- Conformation
- Presence of isotopes
- Chemical reactions
- Temperature
- Internal stress



Increasing the sensitivity

- **Use if higher excitation energies**

  Raman intensity I ~ ν^4

  ~$\mathbf{10^3}$ enhancement

$$I_{RS} = I_{exc}(\nu_{exc}-\nu_m)^4\Sigma|(\alpha_{ij})_m|^2$$

I_{RS} –Raman scattering intensity
I_{exc} – intensity of the excitation
$h\nu_{exc}$ – excitation energy
$h\nu_m$ – energy of the scattered phot.
α_{ij}- – polarizability

Drawbacks

- Damage of the sample
- Strong photoluminescence could overlap the Raman bands
- Requires special instrumentation

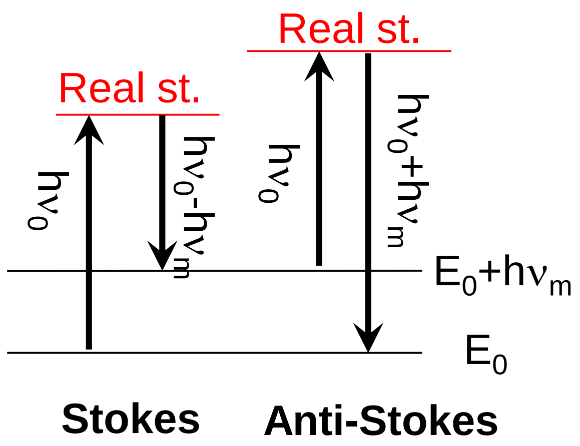
Increasing the sensitivity

$$(\alpha_{ij})_{mn} \propto (\nu_{et}-\nu_{exc})^{-1}$$

α_{ij}- – polarizability
$h\nu_{et}$ – energy of a real el. transition
$h\nu_{exc}$ – excitation energy

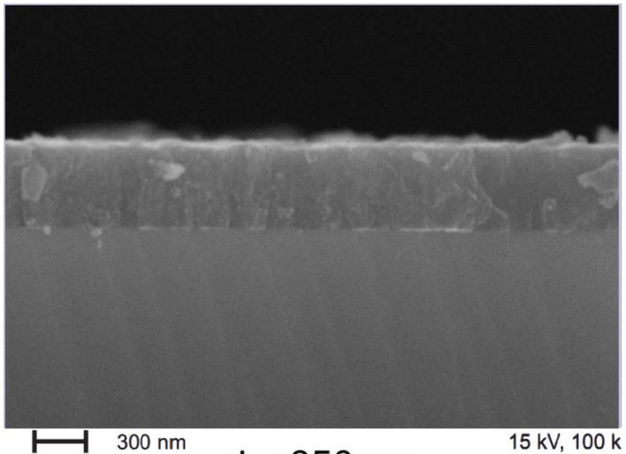
- **Use if higher excitation energies**
  Raman intensity I ~ ν^4
  ~**10³** enhancement

- **Resonant Raman scattering**
  When the energy of the incident photons is equal or close to the energy of an existing electronic transition of the sample.
  ~10³ – 10⁵ enhancement



Raman spectrum of nanodiamond

Excitation with different energies



d = 350 nm

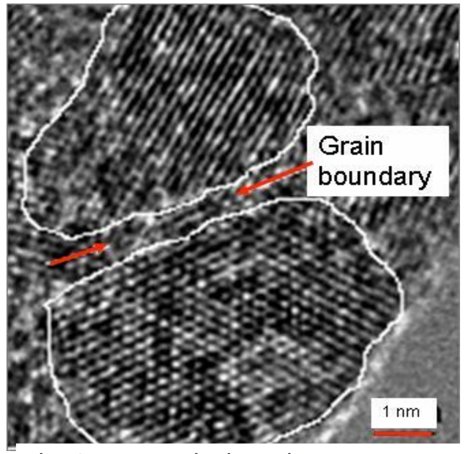
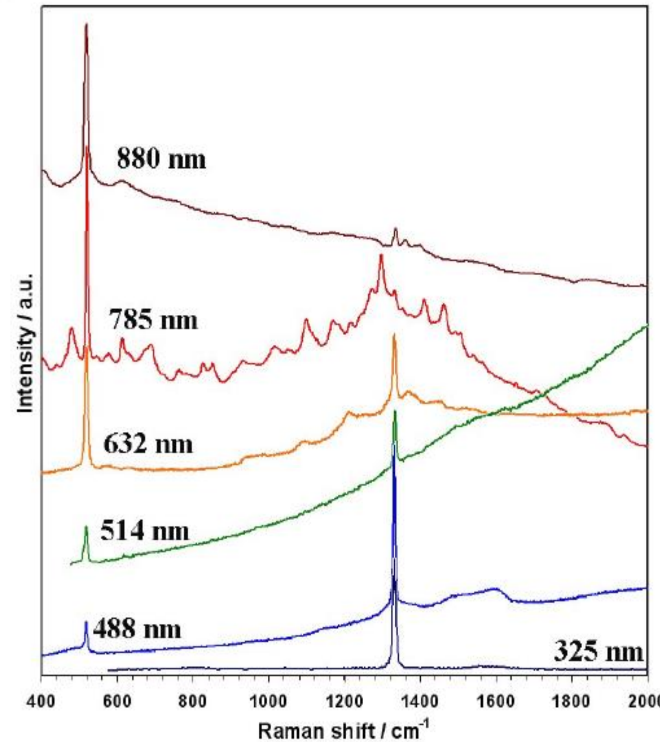
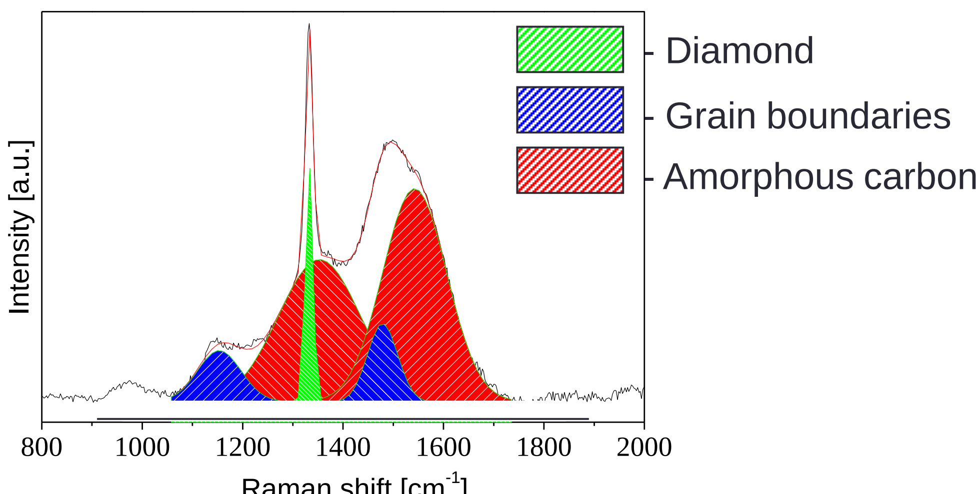


*Fig. 2. Transmission Electron Microscope (TEM) image showing the grain sizes in ultrananocrystalline diamond***





Selective enhancement allows to focus on different constituents of the nanodiamond film

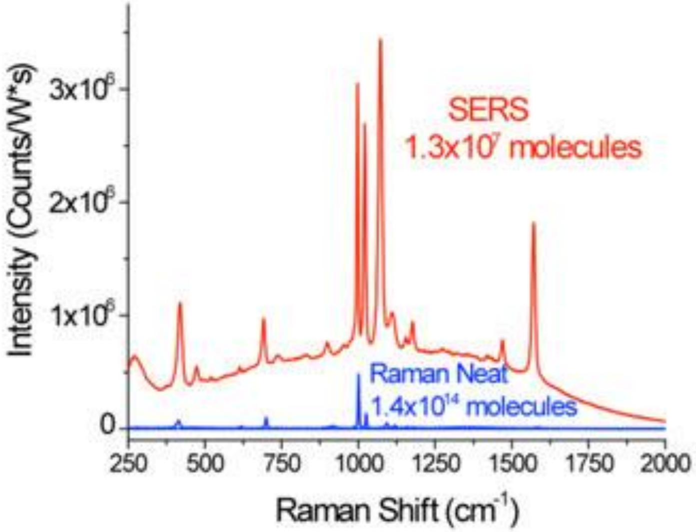
P.W. May et al. MRS Proceedings 1039 (2007)

Increasing the sensitivity

- **Use if higher excitation energies**
  Raman intensity I ~ $1/\lambda^4$
  ~**10^3** enhancement

- **Resonant Raman scattering**
  When the energy of the incident photons is equal or close to the energy of an existing electronic transition of the sample.
  ~10^3 – 10^5 enhancement

- **Surface Enhanced Raman Scattering (SERS)**
  Enhancement of the incident and/or scattered intensity by interaction with localized surface plasmons of metallic nanoparticles or surfaces with nanoscale roughness.
  ~10^7 – 10^{10} enhancement



Types of SERS

Electromagnetic enhancement

- Interaction of the electromagnetic field of light with surface plasmons
- 10^7-10^8 enhancement
- The enhancement strongly depends on the distance from the metallic surface:

$$G = \left[\frac{r}{r+d}\right]^{10}$$

r – curvature of the SERS surface

d – distance of the molecule from the surface

Chemical enhancement

- Charge transfer between the metallic surface and the adsorbed molecule
- Enhancement of one monolayer

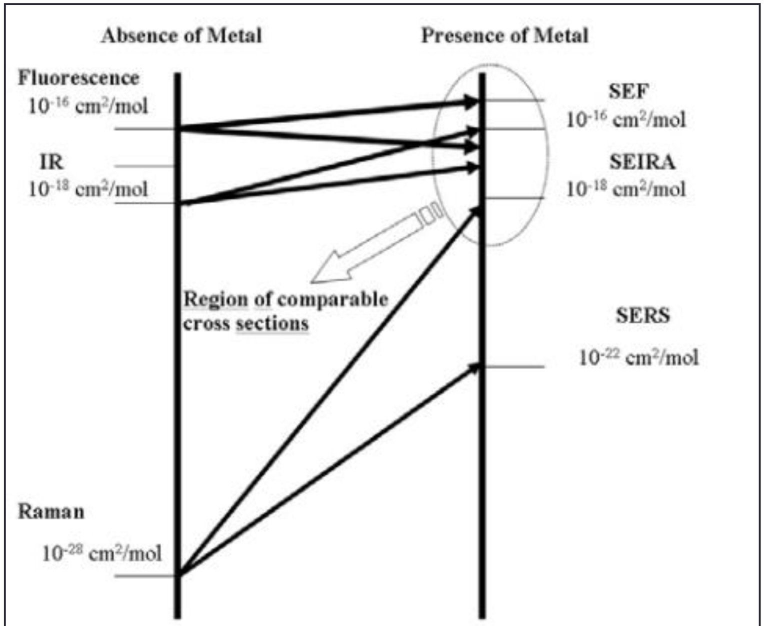
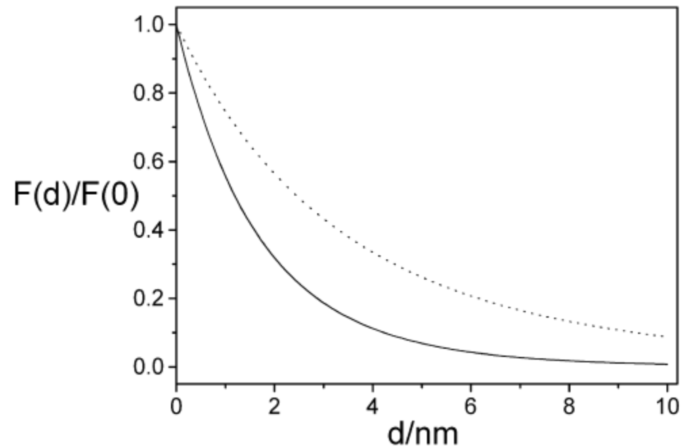


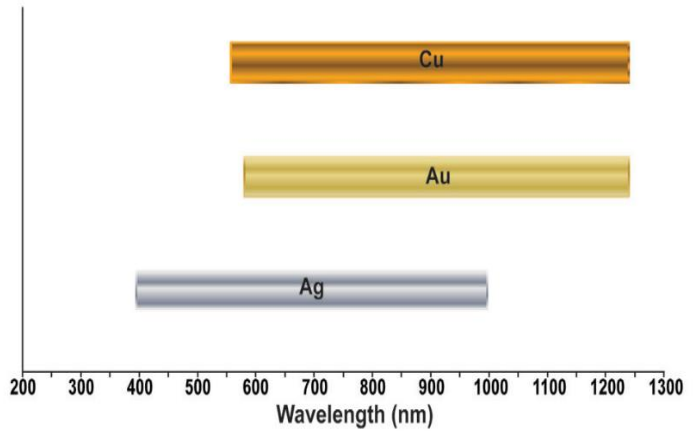
Fig. 1. Spectroscopic signal gain (expressed in terms of cross-section) observed in absence and presence of metal nanoparticles in fluorescence, IR absorption and Raman scattering.

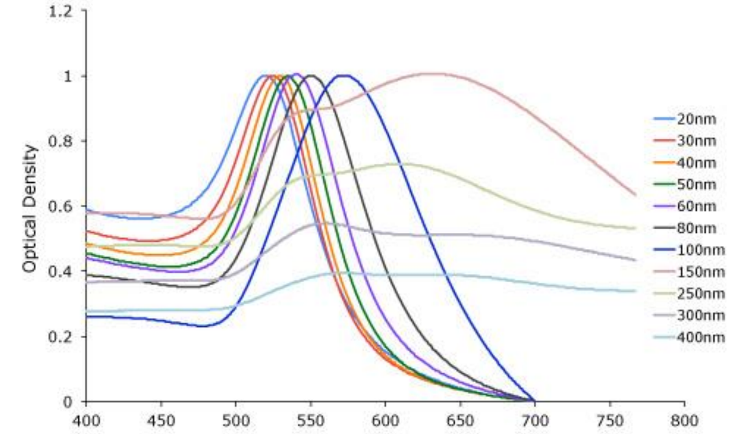


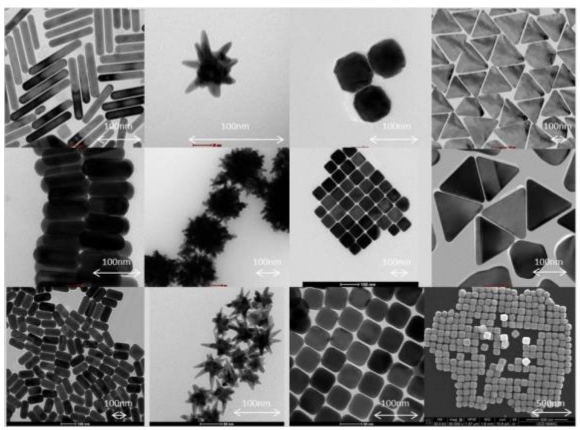
F. Siebert, Vibrational Spectroscopy for Life Sciences, Wiley, 3008

SERS active materials

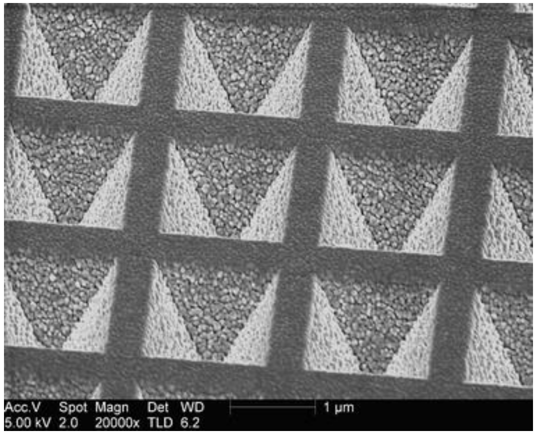
- Gold, silver and copper
- Nanoparticles and surfaces with nanoscale roughness
- Most solutions utilize localized plasmons







Gold nanoparticles



Gold coated patterned surface

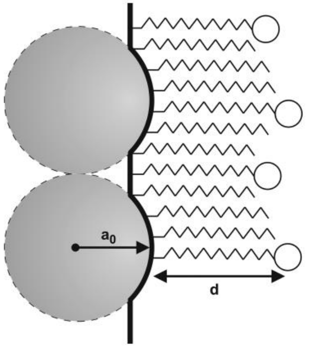


Fig. 2.11 Simplified view of a sub-microscopically rough metal surface (thick solid line) approximating the surface roughness for semi-spheres of radius a_0. Molecules contributing to the SER scattering (hollow spheres) are separated from the surface via spacers of length d.

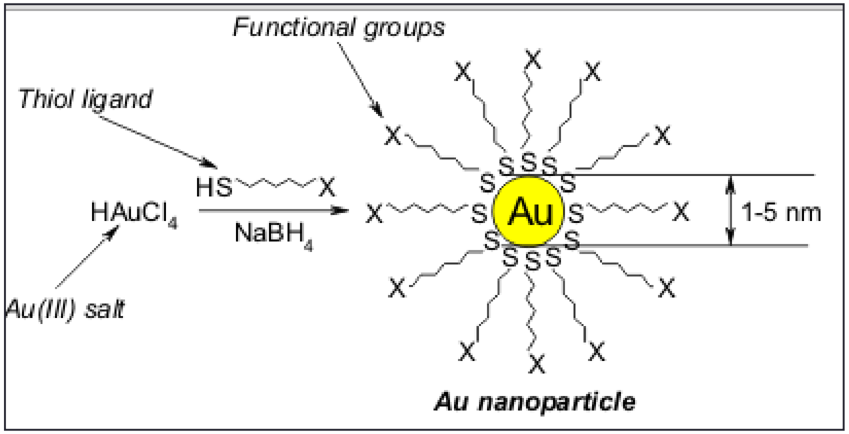
Surface functionalization

Gold is easy to functionalize via thiol chemistry.

Surface functionalization makes SERS active surfaces sensitive to specific molecules.

This approach can be used for selective SERS enhancement and detection of molecules, proteins, viruses, etc.

Multiplex SERS detection requires differentiation between the SERS agents with different functionalization.



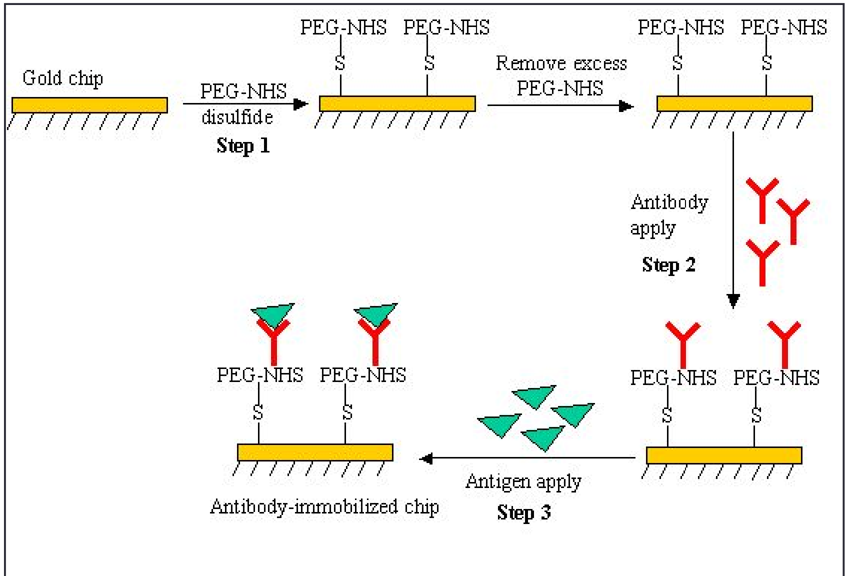


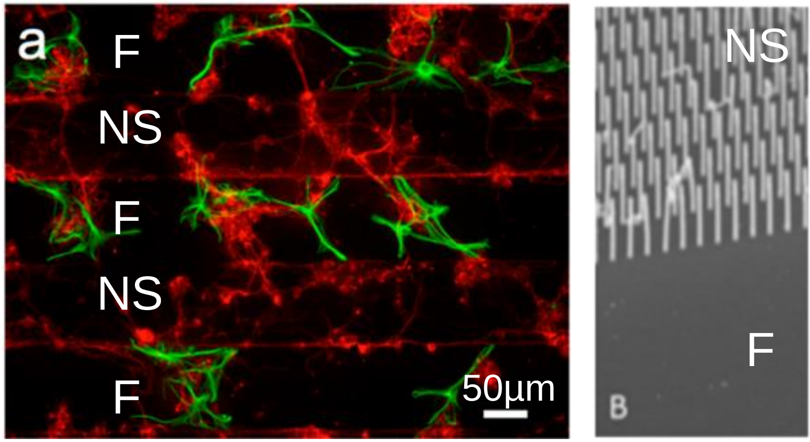
Fig.7.1. Immobilization of gold surface with antibody via PEG as a linker

www.als-japan.com, www.york.ac.uk

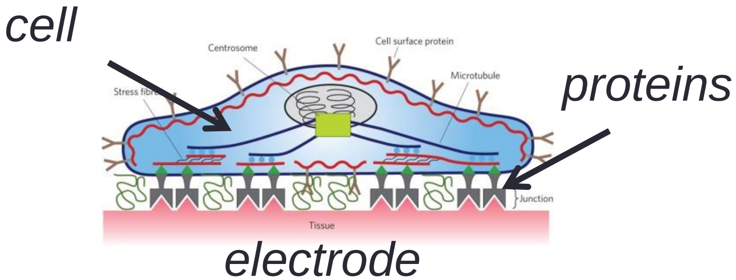
SERS on biological samples

Brain electrode implants

- Brain's self-healing causes the implanted brain electrodes to be surrounded with so called glial cells that make difficult to access the neurons.
- Glial overgrowth can be avoided by using nanostructured electrode surfaces.
- Brain cells are connected to the electrode surface with proteins.
- **Aim:** determine the type of interconnecting proteins and analyze their behavior using Raman spectroscopy.
- .

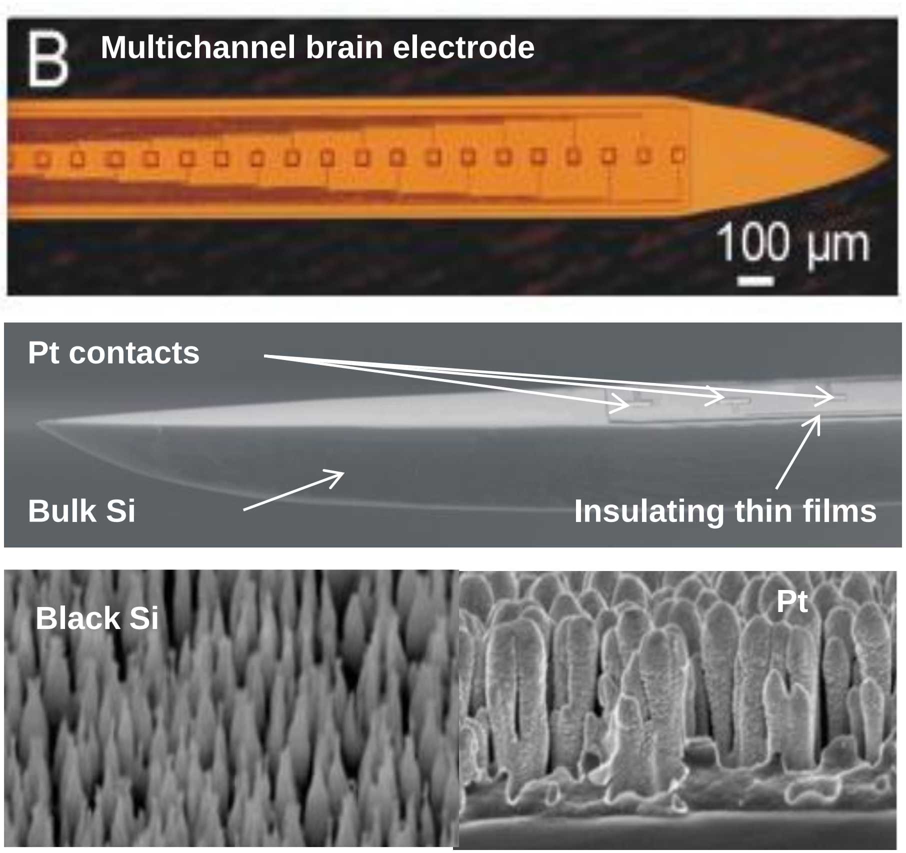


Elecrode surface with nanostructu-red (NS) and flat (F) regions
Red – neurons
Green – glial cells



G. Piret et al., ACS Appl. Mater. Interfaces 2015, 7, 18944−18948

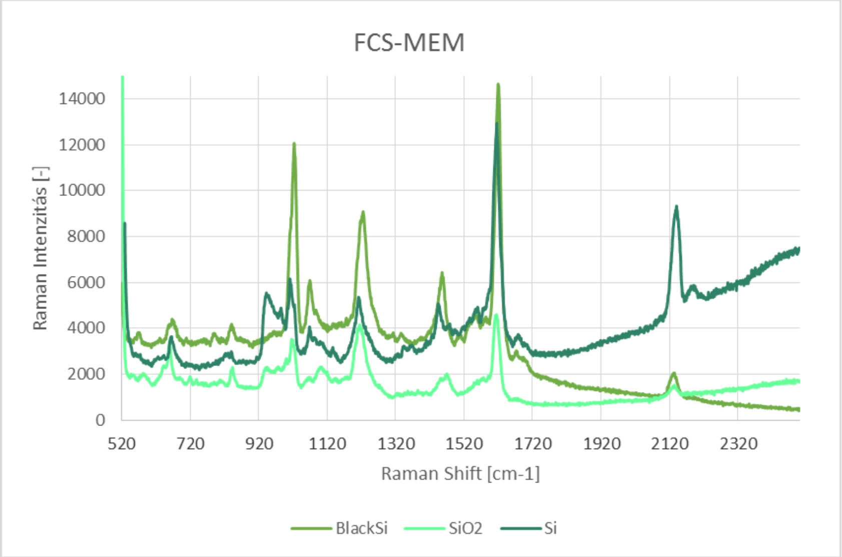
Electrodes and proteins

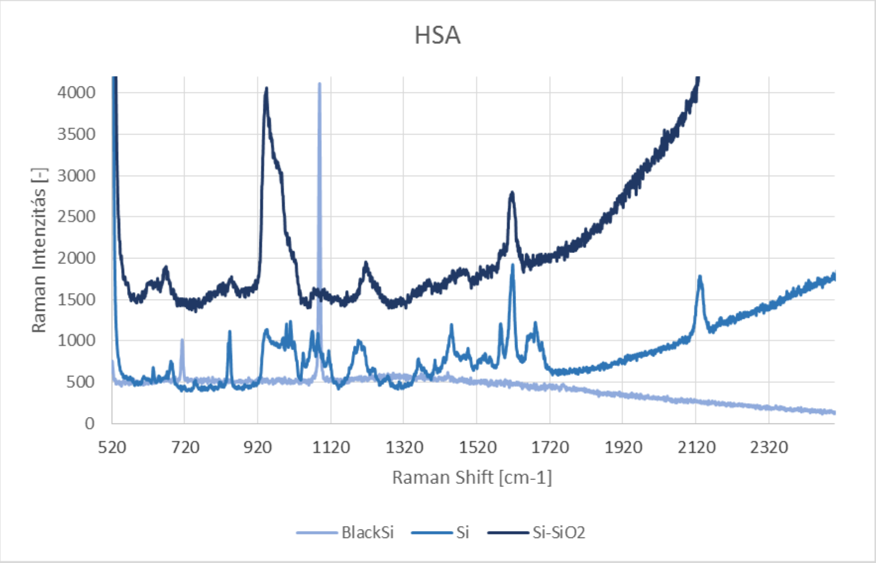


Nanostructured Si and Pt surfaces

- SERS on nanostructured electrode surfaces.
- No enhancement was observed after coating the nanostructured surface with Au.
- Alternative: SERS with gold nanoparticles on different surfaces (Si, SiO_2, Black Si)
- Tested proteins: HSA, FCS, PLL
- Mixing of proteins with Au colloid, drying droplets on the surface.

SERS of proteins on different surfaces





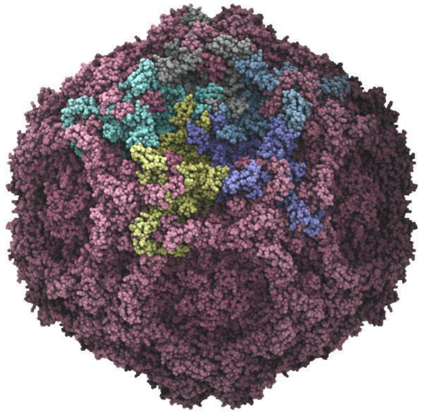
- SERS spectrum depends on the substrate.
- Possible reasons:
  - wetting properties of the surface
  - conformation of the proteins
  - specific hotspots for Au attachment

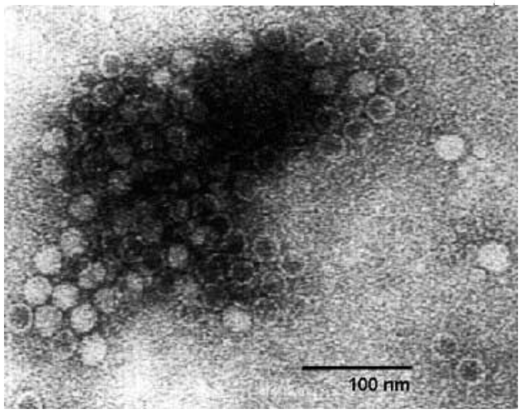
SERS fingerprint of proteins

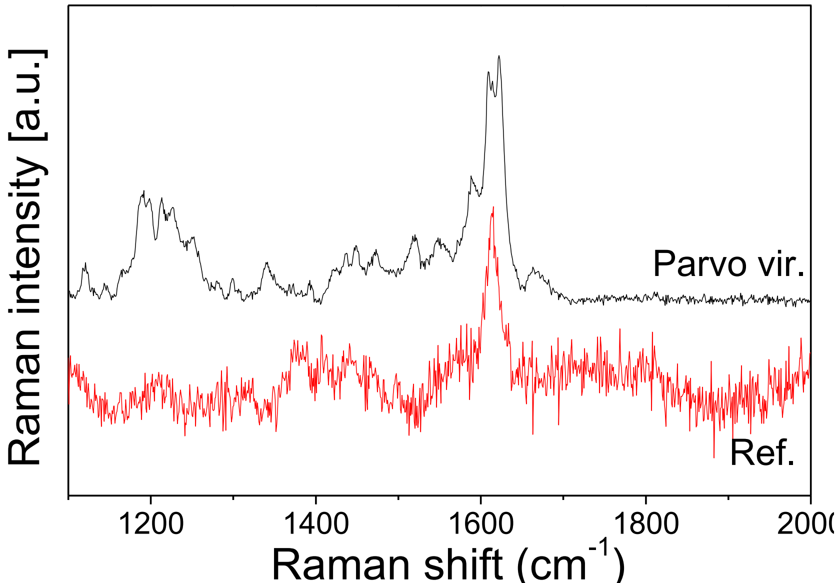
HSA	FCS	PLL
v[cm^{-1}]	v[cm^{-1}]	v[cm^{-1}]
630		
670	670	670
850	850	
	1020	
	1070	
	1140	
1100		
1220	1220	1220
1400		
	1455	
		1525
	1570	
1620	1620	1620
	1690	
1830		
	2140	
		2145-2200

SERS of viruses

- SERS was used to study porcine parvo virus
- The uncleaned virus in the residues of the cell growth culture and the uninfected growth culture were mixed with 20 nm colloidal gold and dried on Si surface
- Clear differences were observed in the spectra







Next step – attach porcine parvo antibodies to the gold surface

Analysis of the exhaled breath

It was shown that the exhaled breath contains volatile organic compounds that can be used as disease markers.

Diseases
Lung cancer, asthma, COPD, tuberculosis, diabetes, helicobacter pyroli, cystic fibrosis, etc.

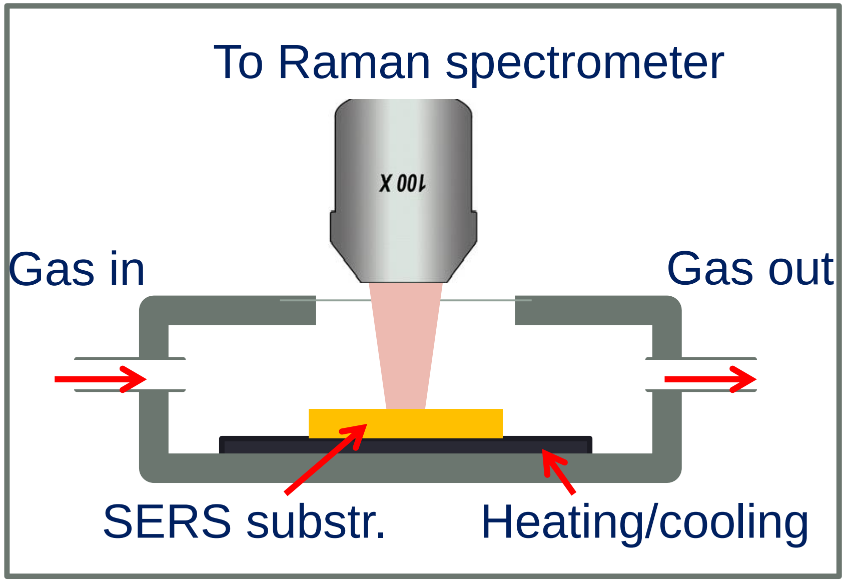
Typical VOCs
Methanol, ethanol, acetone, n-pentane, isoprene, benzene, etc.

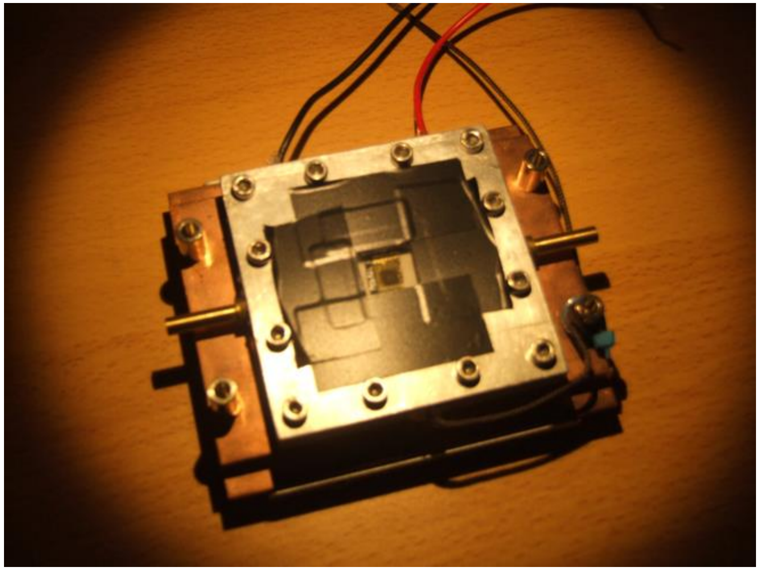
Methods
Mass spectrometry, gas chromatography, ion mobility spectroscopy, electronic nose, dogs, etc.

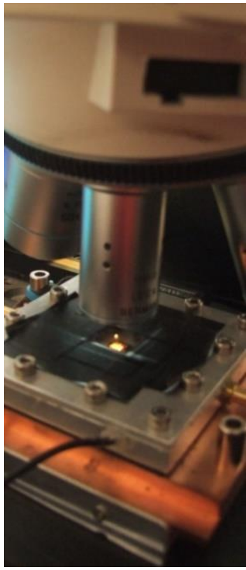
Aim
Use Raman spectroscopy to detect VOCs in exhaled breath.

Pathogen and disease	Source	Analysis method	VOC biomarker (no. of VOCs)[a]
Mycobacterium tuberculosis			
TB	Breath	GC-MS	Methyl nicotinate
TB	Breath	GC-MS	Profile (14)
TB (active)	Breath	GC-MS	Profile (10)
TB	Breath	GC-MS	Profile (8)
TB	Breath	GC-TOF-MS	Profile (7)
TB	Urine	GC-MS	Profile (5)
Pseudomonas aeruginosa			
Lung infection (CF patients)	Breath	SIFT-MS	Hydrogen cyanide
	Breath	SIFT-MS	Methylthiocyanate
	Breath	GC-MS	2-Aminoacetophenone
	Breath	IMS	Profile (21)
	Breath	GC-TOF-MS	Profile (22)
	Sputum	SPME-GC-MS	2-Nonanone and 2,4-dimethyl-1-heptene
Aspergillus fumigatus			
Lung infection	Breath	GC-MS	2-Pentylfuran
Vibrio cholerae			
Cholera	Feces	GC-MS	*p*-Menth-1-en-8-ol
Helicobacter pylori infections	Breath	GC-MS	Isobutane/2-butanone and ethyl acetate
	Breath	PTR-MS	Hydrogen cyanide/hydrogen nitrate

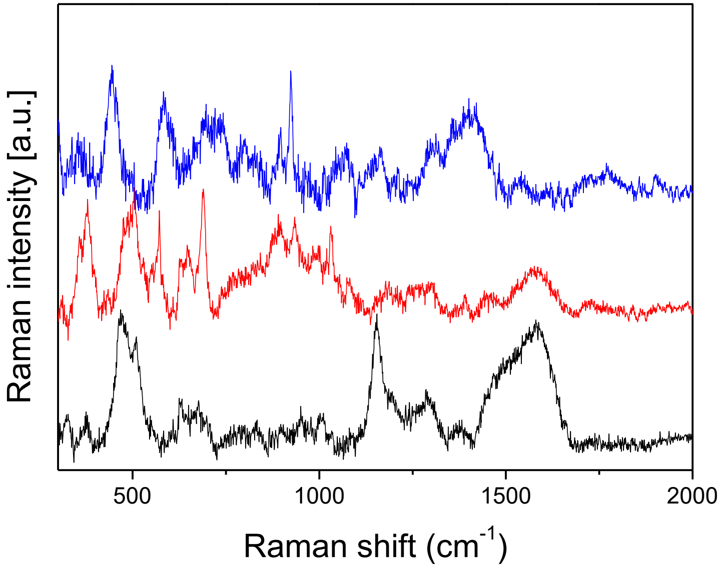
Flow cell for SERS measurements







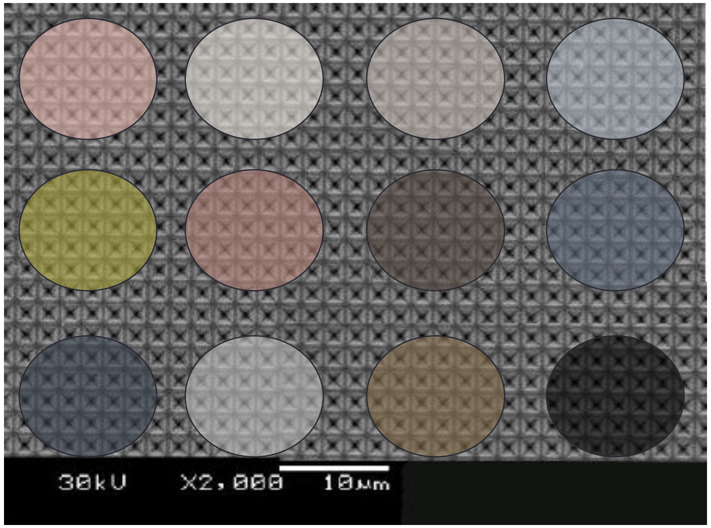
- Gas flow cell with top optical window for SERS measurements
- The substrate was cooled with a Peltier element in order to facilitate the condensation of the molecules from the exhaled breath.
- Nitrogen was used as carrier gas

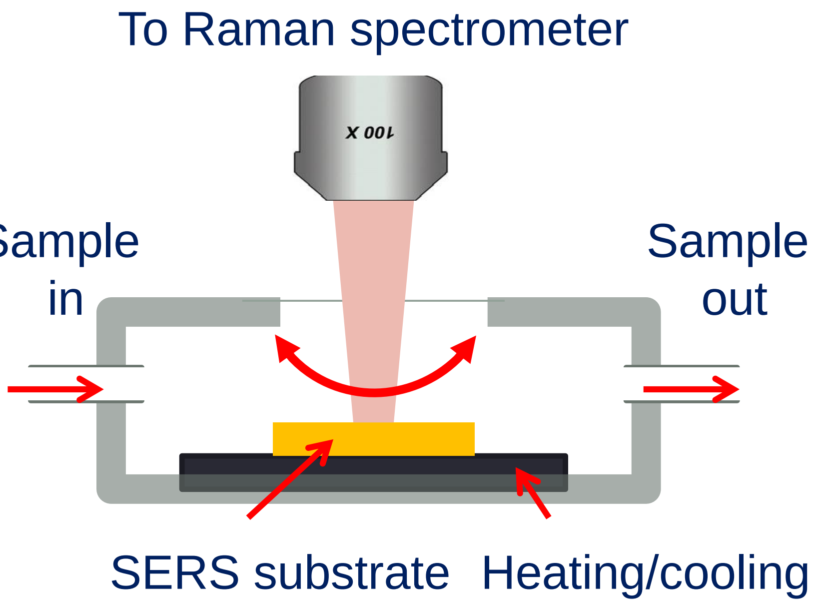


SERS spectra of exhaled breath

SERS platform

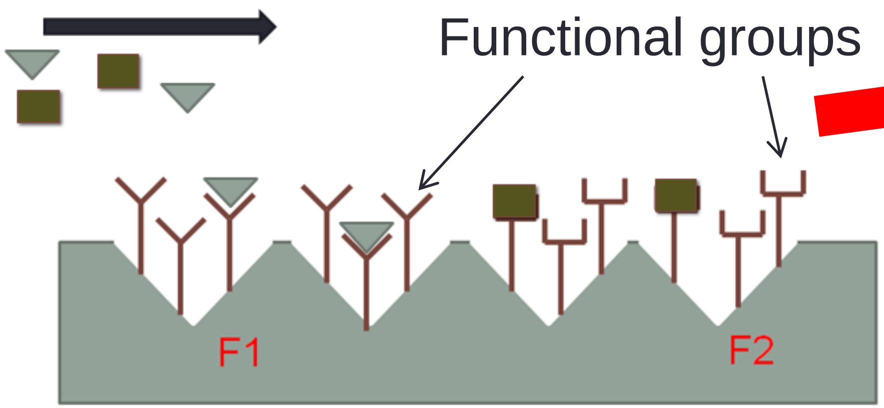
- Functionalization of the different areas of the SERS substrate with different functional groups
- Use scanning Raman measurements
- The spectrum of a given area changes when the appropriate molecule is attached to the functional group
- Possibility to fabricate arrays to detect several analytes
- *Not only for exhaled breath*





Functionalized SERS array

SERS of exhaled breath, fluids, etc.

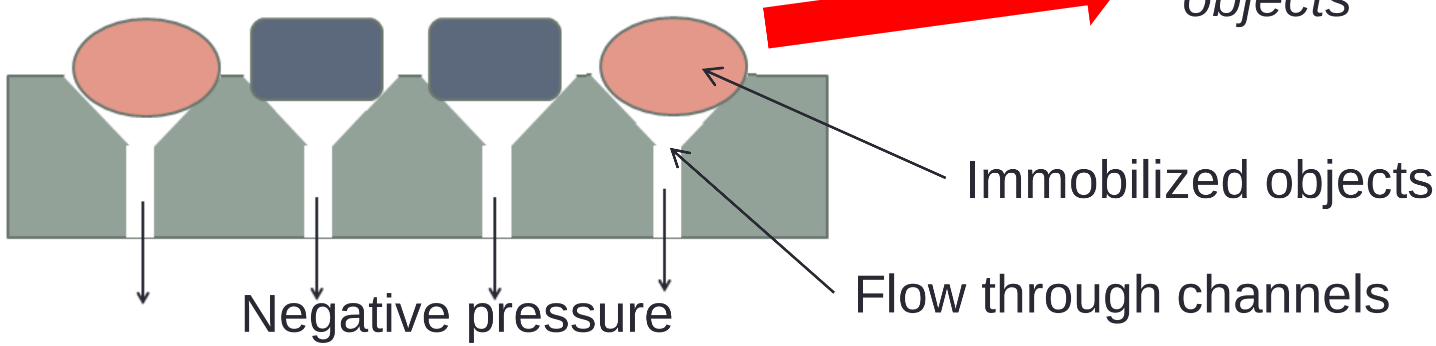


Changes in the SERS spectrum of the functionalized region

SERS study of single cells, etc.

Matching the diameter of the flow through channels < cell size

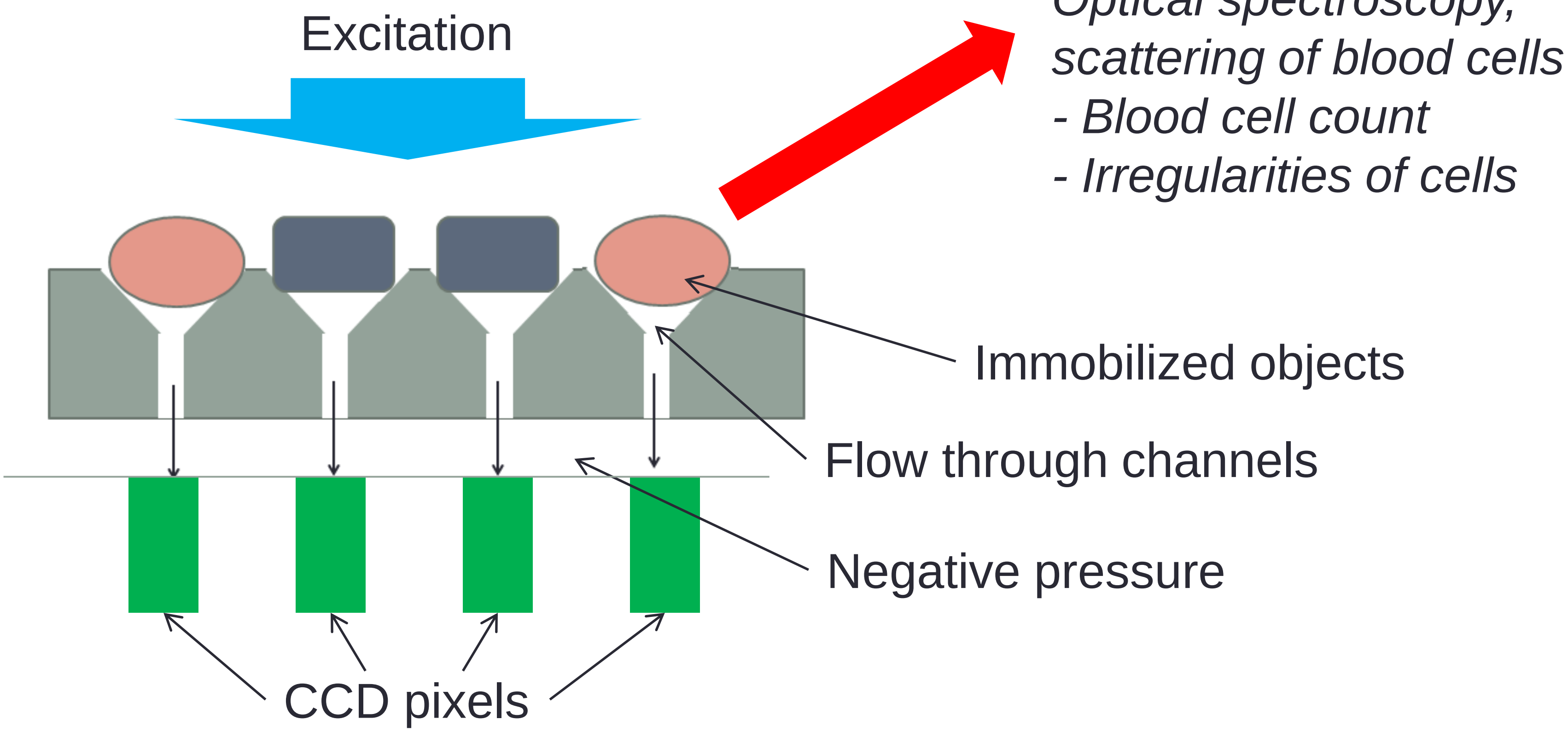
SERS spectrum of individual immobilized objects



Functionalized SERS array

Analysis of blood cells

Matching the period of the flow through channels with pixels of a CCD camera

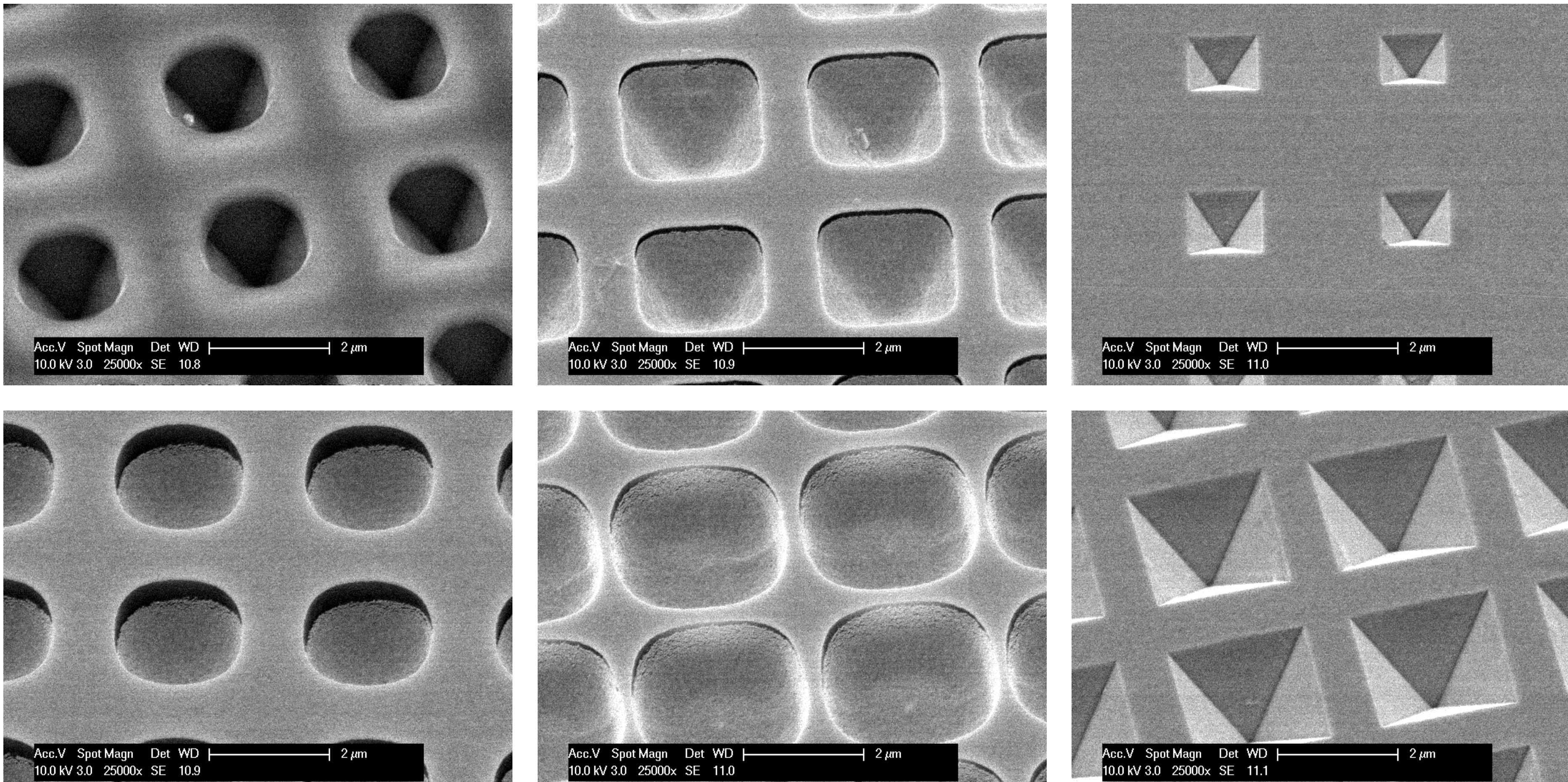


One channel for each cell

Development of SERS substrates

Periodical structures prepared in silicon by isotropic and anisotropic etching.

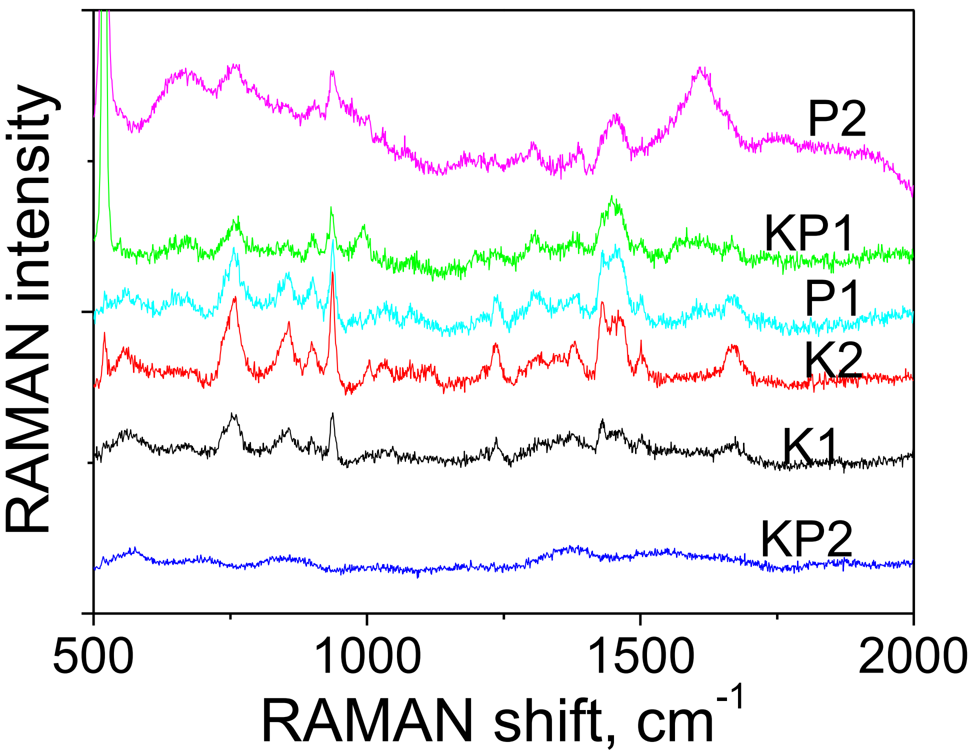
1 and 2 micron periods.



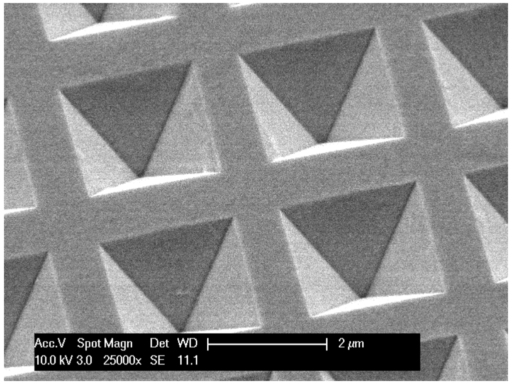
Hemispheres　　Rounded pyramids　　Pyramids

Development of SERS substrates

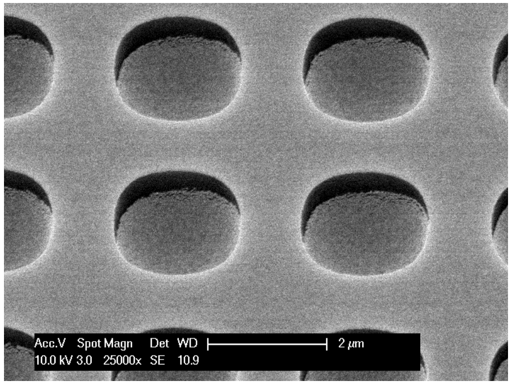
Enhancement on different substrates.



K2



P2



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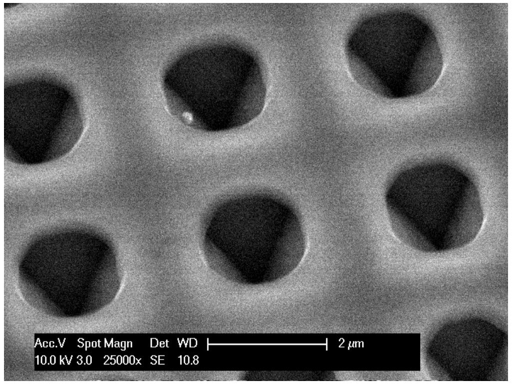
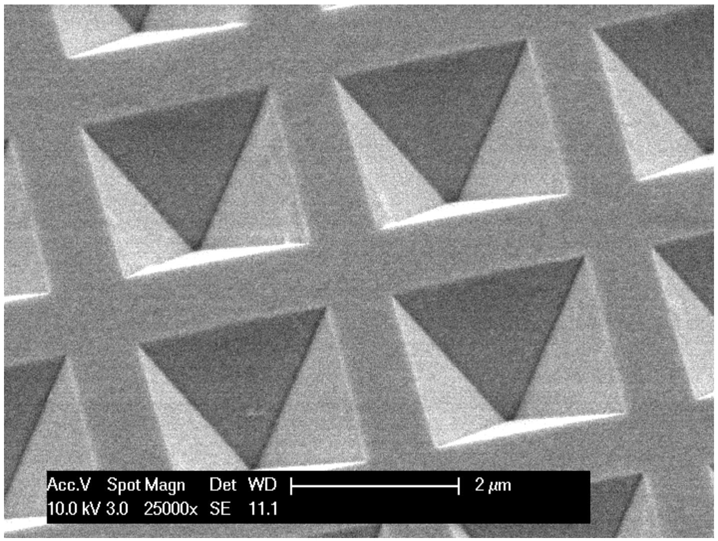
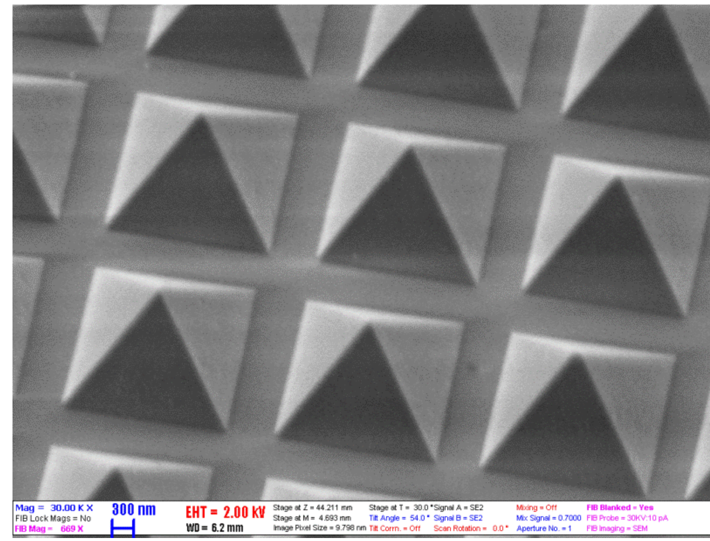


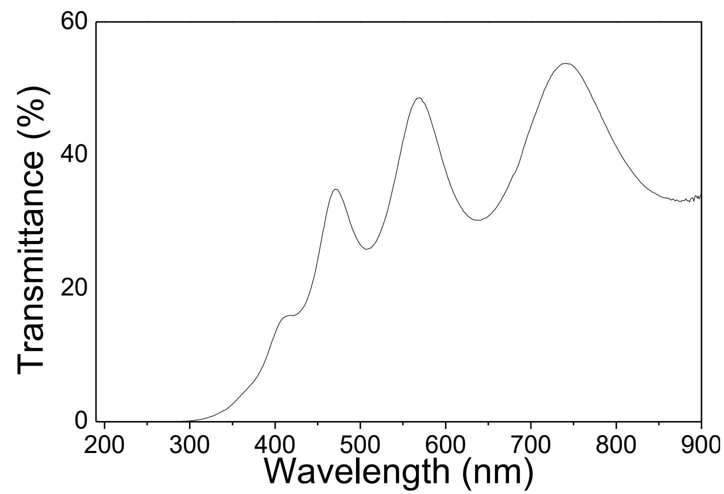
Optically transparent substrate



Carbon nitride
+ Si etching



Fabrication of optically transparent substrate for backside excitation by coating the Si substrate with carbon nitride and subsequent etching of silicon.

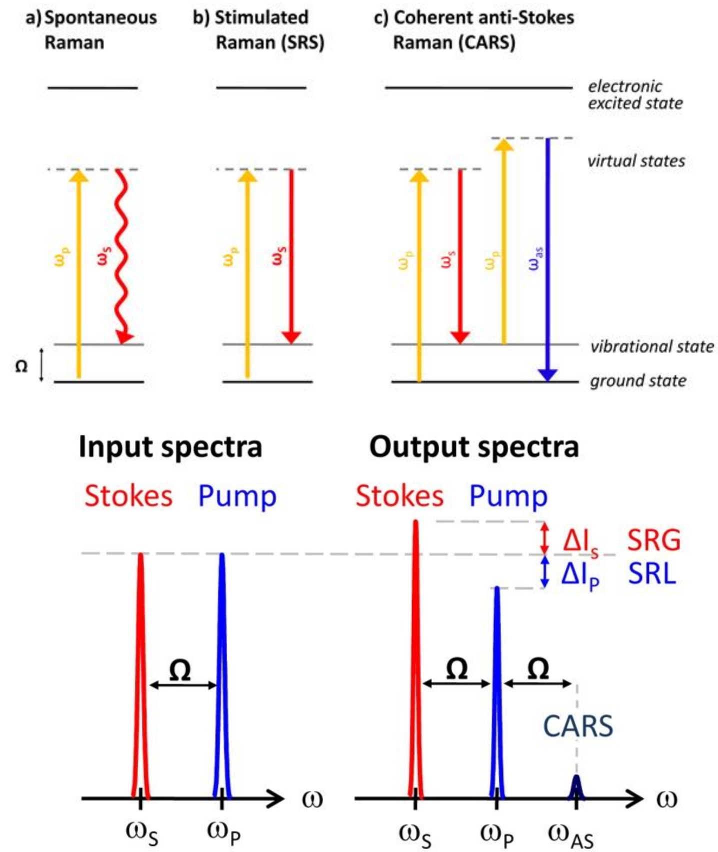


Coherent Raman techniques

Non-linear effects

- *Coherent anti-Stokes Raman scattering (CARS)*
- *Stimulated Raman scattering (SRS)*

- Strong detection signal, much sensitive than normal Raman
- Processes occur only at the focus, allowing 3D imaging
- These techniques can be used with strong PL background
- Distinct Raman fingerprints of different molecules allow label-free imaging of biological samples
- Require moderate laser powers tolerable by living biological samples
- Deep penetration in biological tissues when using near-infrared photon energies

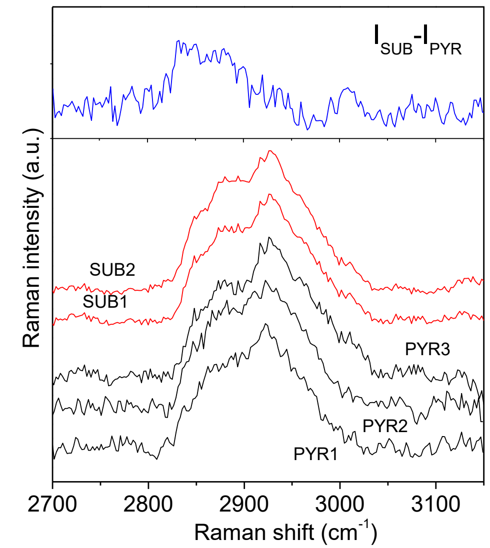


http://bernstein.harvard.edu/

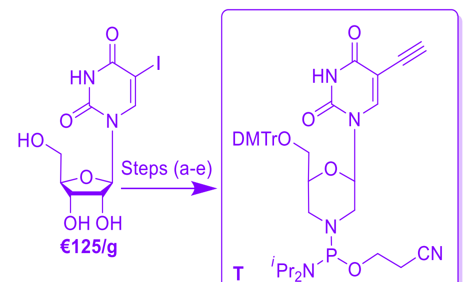
In vivo visualization of neuronal nuclear functions

NEURAM – Visual Genetics
H2020 Fet-Open project
Starts in October, 2016

- *Develop a label-free SRS imaging system for in vivo monitoring of neuron activity*
- *Preparation of alkyne (C≡CH) tags for labelling of single genes*
- *Develop tools for loading alkyne-tagged nucleosides and nucleotide analogues into neurons*
- *Visualize and track endogenous transcription at single gene level using SRS*



532 nm excited Raman spectra of subicular and pyramidal brain cells



(a) ≡—TMS, $Pd(PPh_3)_4$ (10 mol%), CuI (20 mol%), TEA (2.0 equiv), DMF, rt; (b) TBAF, THF, rt. *Or* K_2CO_3, Water, MeOH; (c) DMTr-Cl, NEt_3, Py, rt, Ar; (d) $NaIO_4$, $(NH_4)_2B_4O_7$, CH_3OH, rt; then $NaCNBH_3$, CH_3OH, rt; (e) NC⁀O-$P(N^iPr_2)_2$, DCI, CH_2Cl_2, rt, Ar.

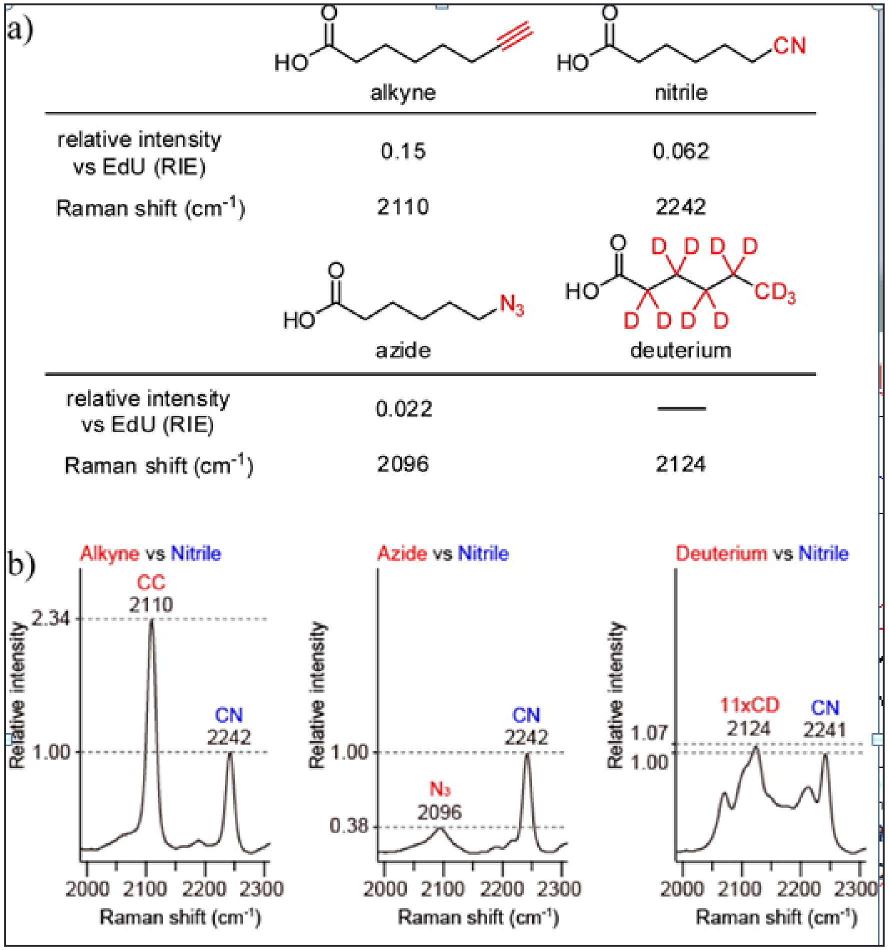
Synthesis of morpholino-T alkyne tag

Alkyne tagging of biological objects

The high sensitivity of SRS allows to use functional tags for labelling biological objects.

Alkyne, nitrile, etc. tags rarely present in biological objects. They have strong Raman signal in region far from peaks of other vibrations of biological objects.

Functional tags can be attached to proteins, genes, etc. without affecting their function and allow to study their mechanisms



H. Yamakoshi, J. Am. Chem. Soc., 2012, 134 (51), pp 20681–20689

Nanostructures and Applied Spectroscopy Group

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