Sensitive Raman Spectroscopy

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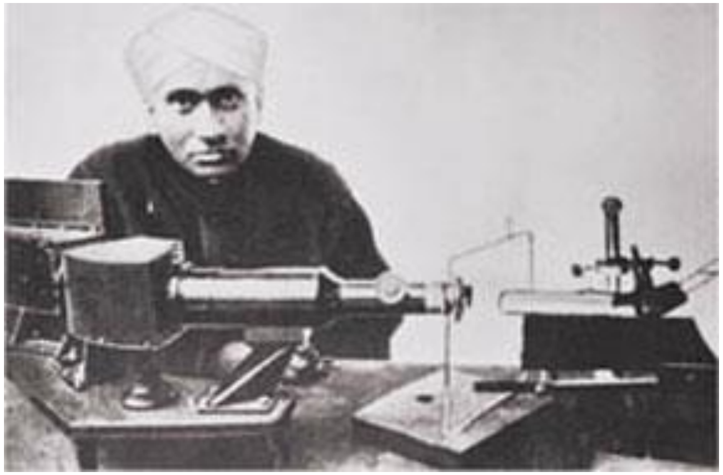
Raman scattering

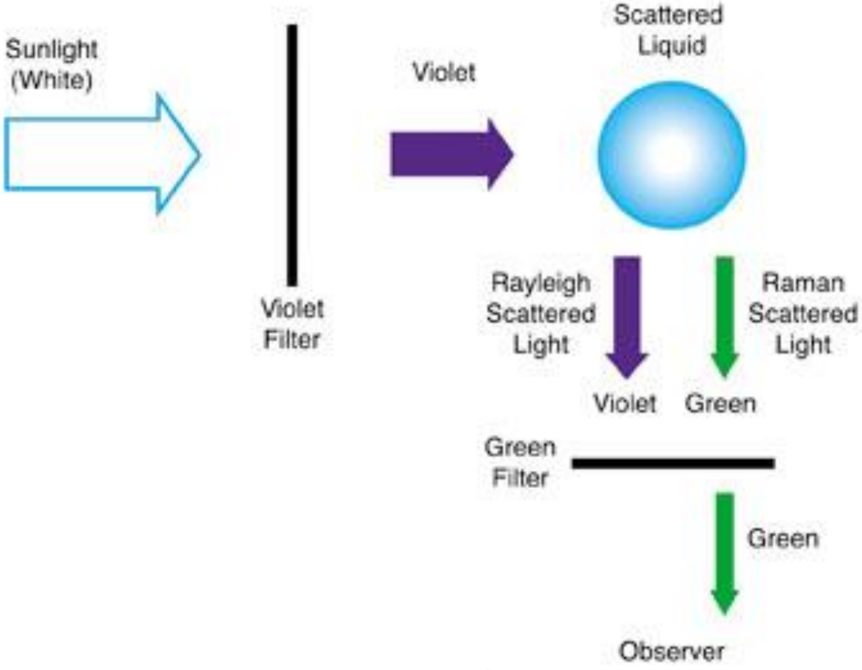
C. V. Raman in 1928
Nobel Prize, 1930

Investigation of light scattering by water droplets with focused sunlight and filters

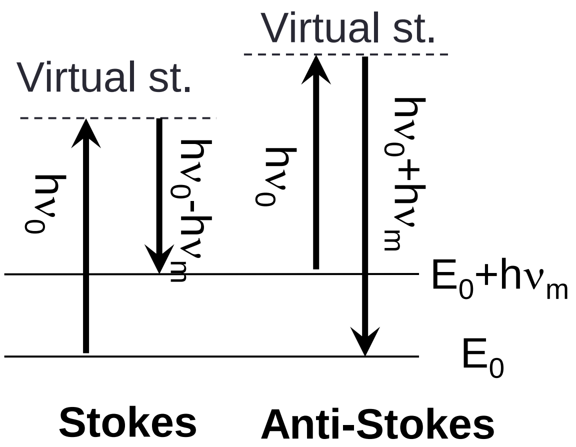
Appearance of a different (green) color in the scattered (violet) light, which had 10^{-7} times lower intensity

G. Landsberg, L. Mandelstam
Combinatorial scattering in crystals





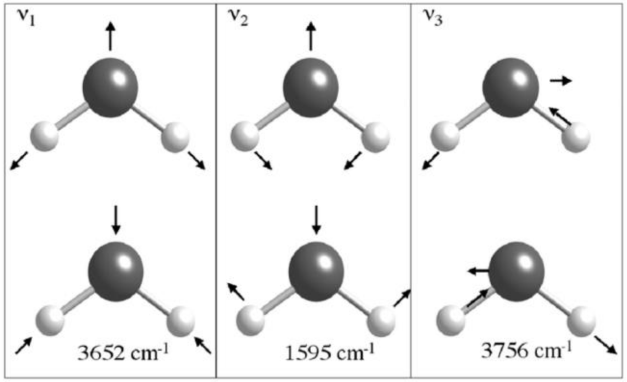
Raman scattering

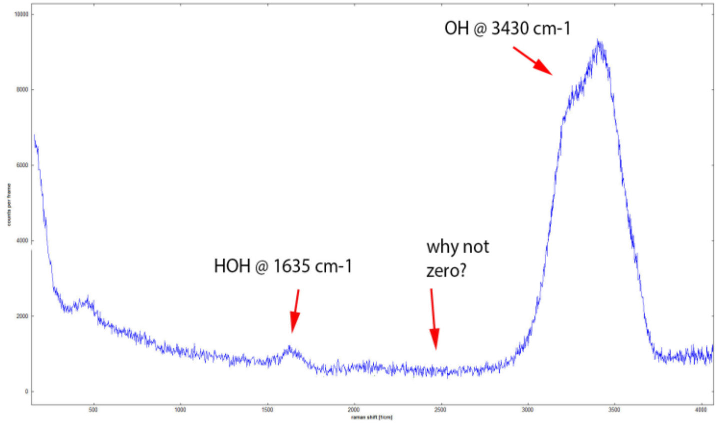


Inelastic light scattering due to the interaction of the incident light with matter.

The energy difference of the excitation and Raman scattered light equals to the energy of a normal vibration of the medium (molecule, crystal).

In general the Raman shift is independent of the excitation energy.





Applications

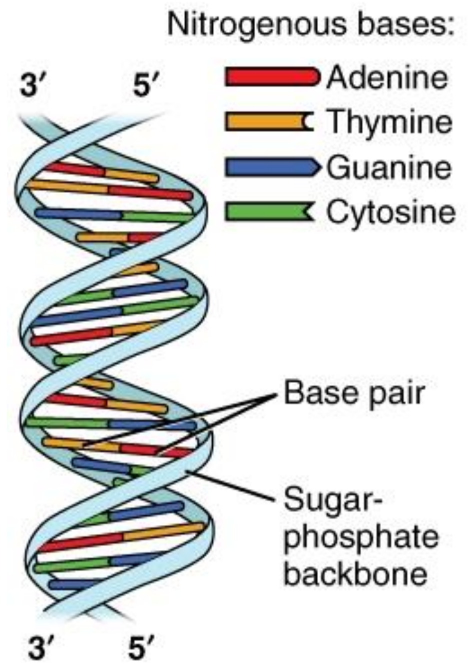
Information related to the vibrations of the medium

- Composition
- Conformation of the molecule
- Crystal lattice parameters
- Bond formation
- Isotope detection
- Trace elements and defects
- Temperature
- Internal stress

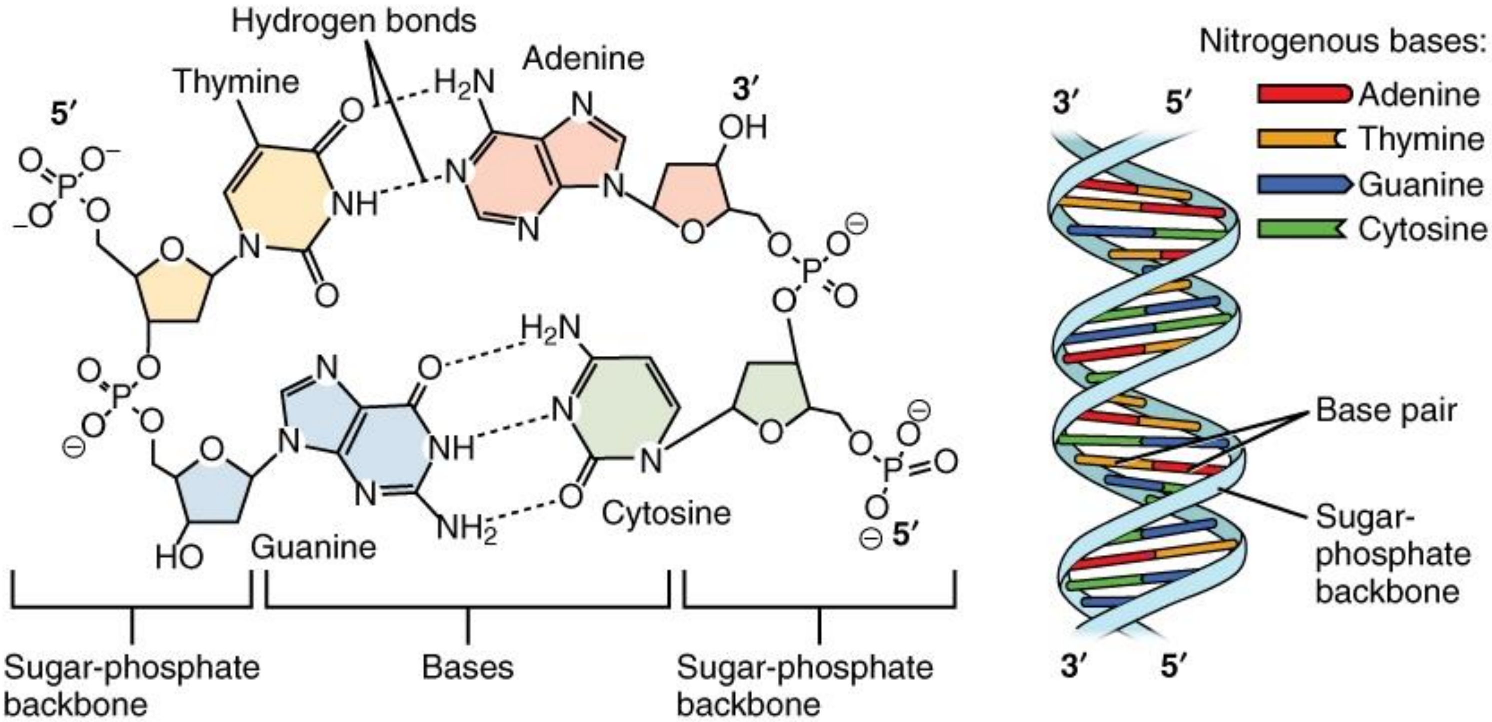
Journal of Raman Spectroscopy **keyword cloud**

DNA hybridization

- Duplex formation by two polynucleotide chains.
- A method to identify specific DNA sequences through the detection of the pairing of their single-stranded form with a known complementary single stranded DNA.
- The basis of PCR and other genotyping techniques.



Duplex formation



Four nucleotides – two base pair types:

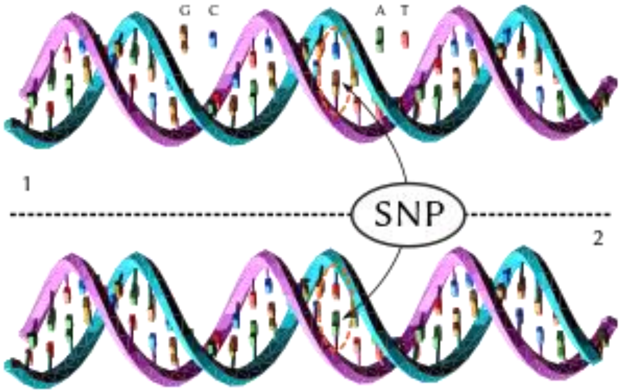
- Cytosine – Guanine (3 hydrogen bonds)
- Adenine – Thymine (2 hydrogen bods)
- (In RNA: Adenine – Uracil)

The nucleotides in the chains are connected by sugar and phosphate groups

Single nucleotide polymorphism (SNP)

A substitution of a single nucleotide at a specific position in the genome.

- 600 million SNPs in the human genome
- A person has 4-5 million SNPs
- Majority is harmless/unknown
- Influence the response to pathogens, chemicals, drugs, vaccines
- Cause genetic and other diseases
- Potential biomarkers



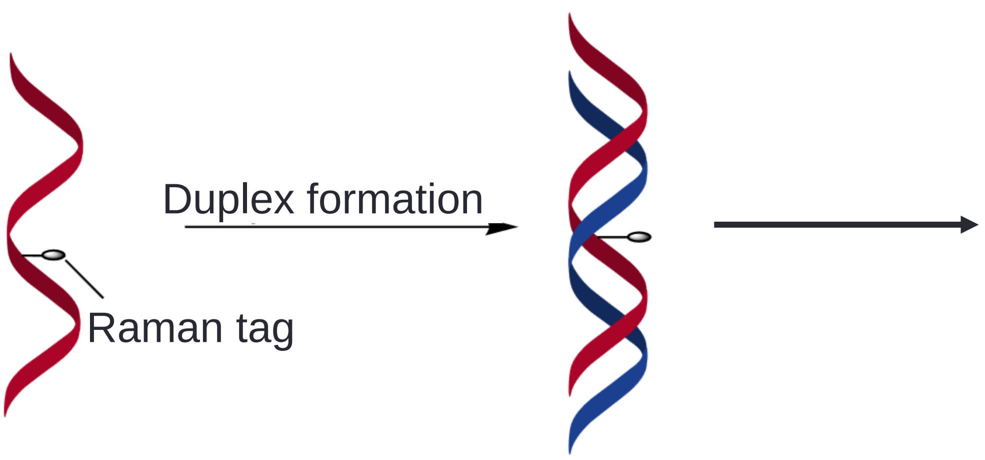
TGGACTCTCTCAATG - test
CATTGAGA**G**AGTCCA - sample

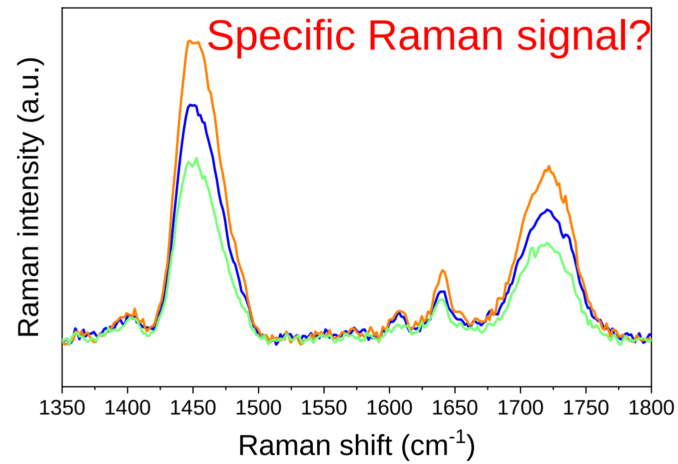
TGGACTCTCTCAATG - test
CATTGAGAAAGTCCA - sample

SNP detection – non-perfect or mis-match hybrdization

Detection of DNA hybridization

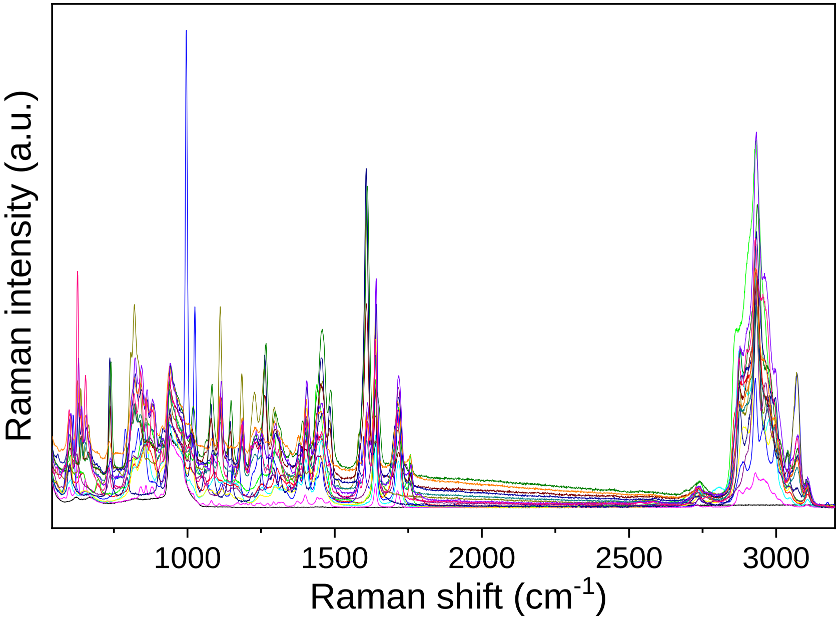
Marking specific sites of the test DNA sequence with a Raman tag.





- The Raman signal does not overlap with other Raman modes of the system
- No/minimal effect on the properties of the tagged molecule;

Tags for vibrational spectroscopy

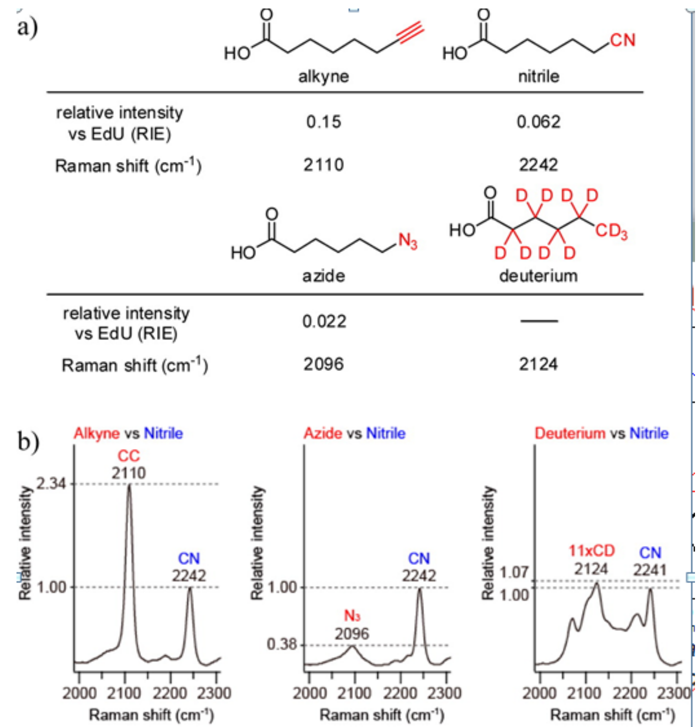


Typical Raman spectra of biological samples

- 1800 – 2600 cm^{-1} – no Raman signal
- This region can be used to detect the Raman response of tags

Tags

Alkyne	**–C≡C–H**
Nitrile	**–C≡N**
Azide	**–N=N=N**
Deuterium	**–C–D**



Alkyne has the highest Raman cross-section.

(H. Yamakoshi, J. Am. Chem. Soc., 2012)

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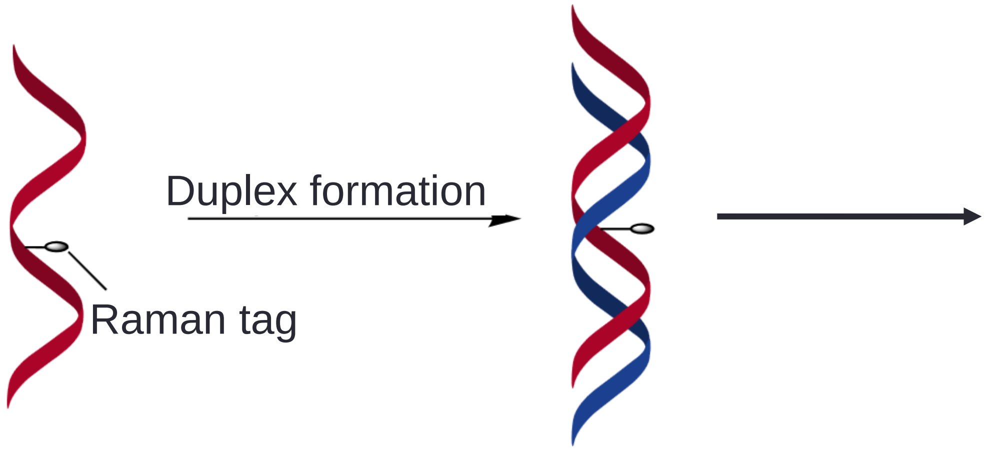
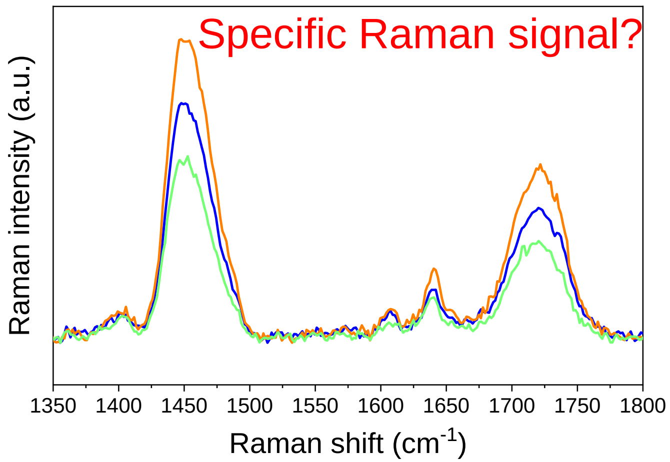
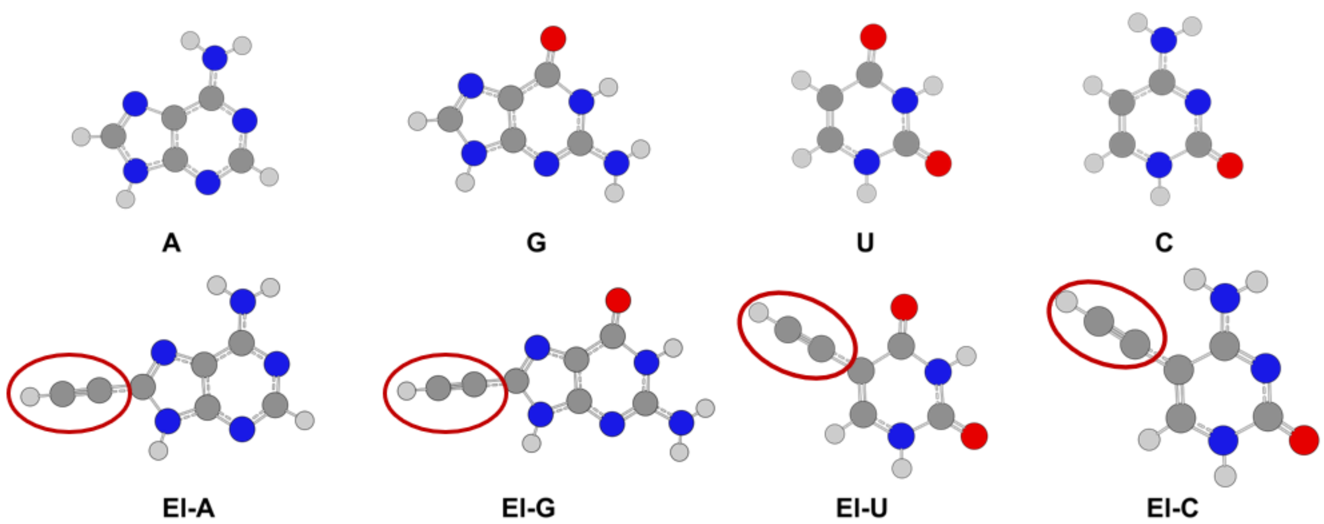






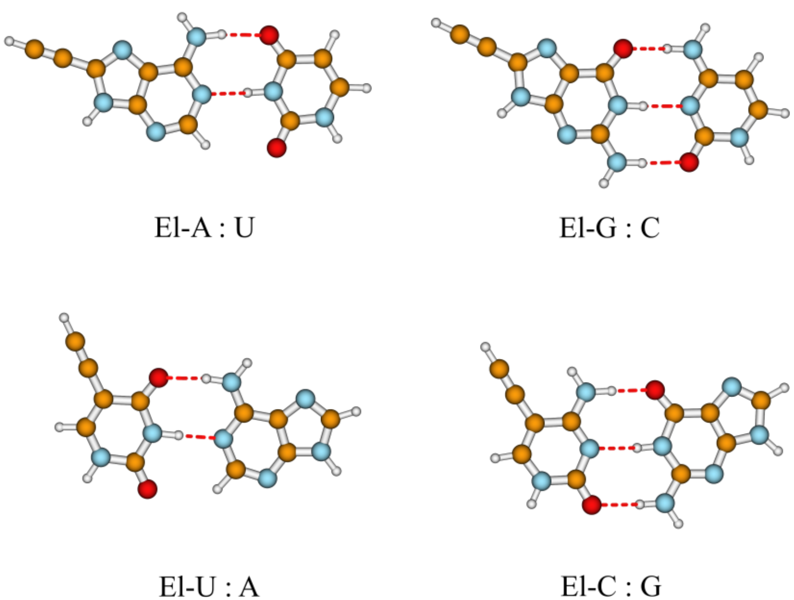
Nucleobase

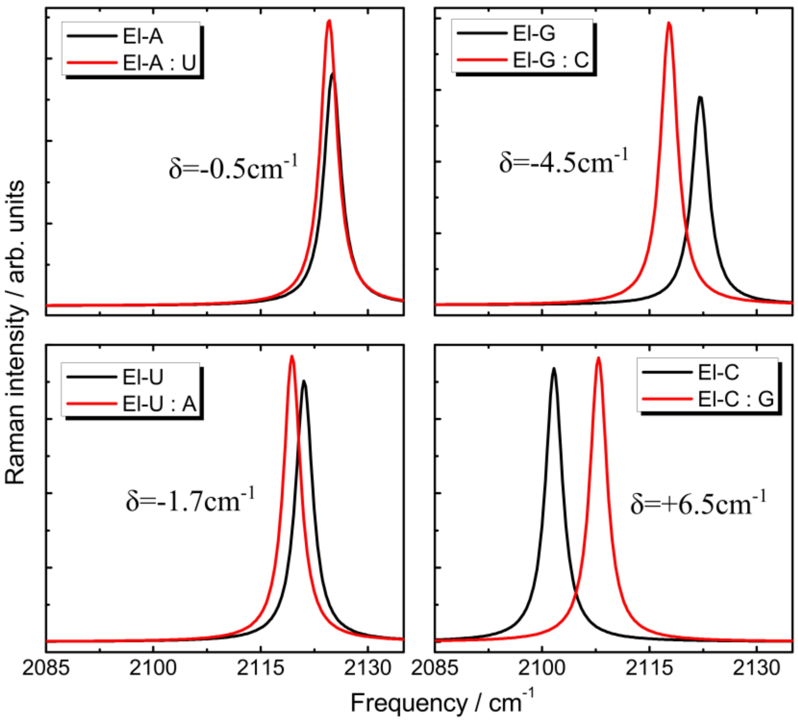
Alkyne tagged nucleobase

Modeling I

Nucleobase complex formation

Shift of the alkyne Raman peak position upon nucleobase complex formation by alkyne tagged nuclebase (El-A, El-G, El-U, El-C) and their complementers (U, C, A, G).





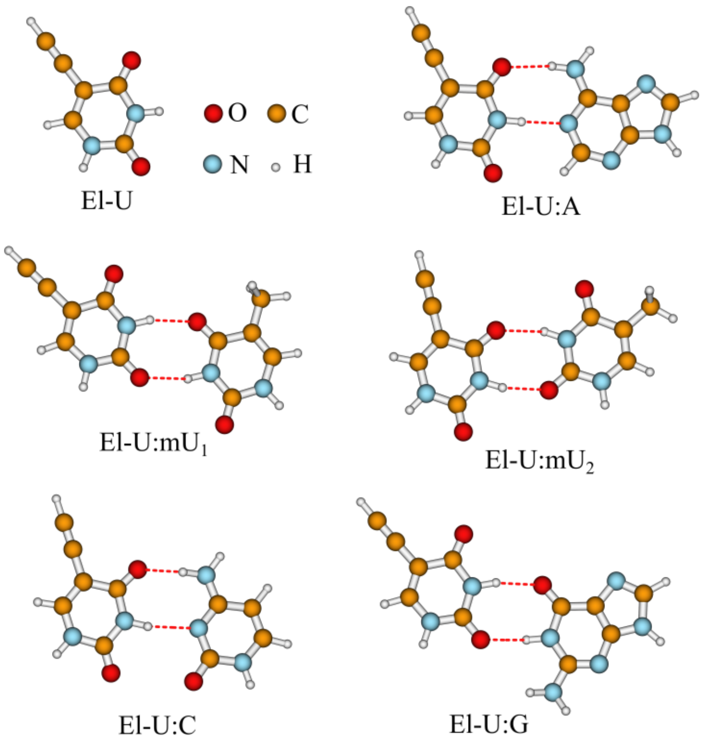
DFT: B3LYP/6-311++G(df,pd)

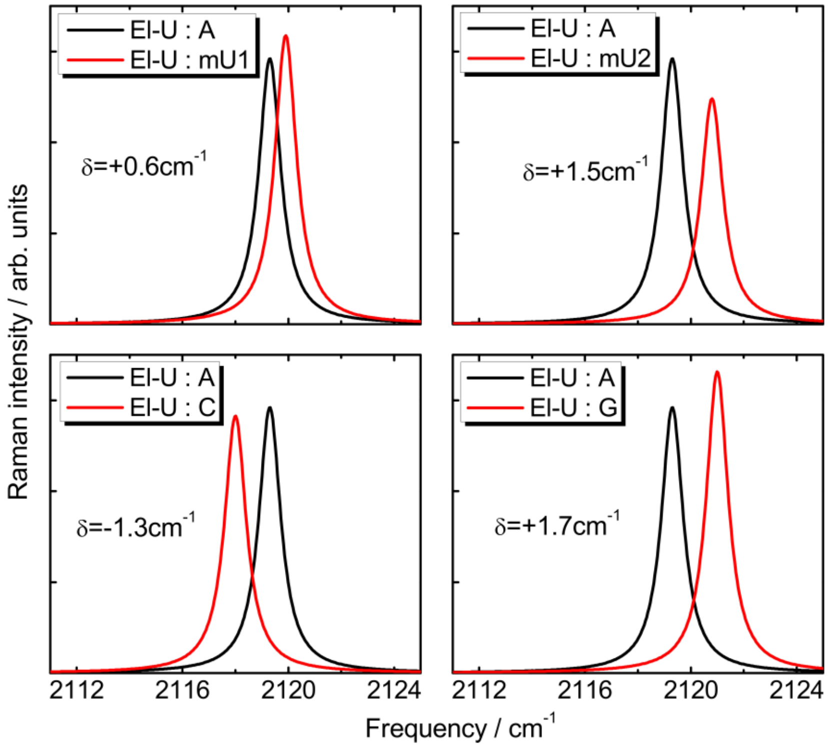
The Raman peaks were simulated by Lorenzians of 10 cm^{-1} FWHM having intensities obtained from the simulations.

Modeling II

Matched and mis-matched hybridization

Shift of the alkyne Raman peak position upon nucleobase complex formation by the alkyne tagged base (El-U) with matching (A) and mis-matching (U, C, G) bases.

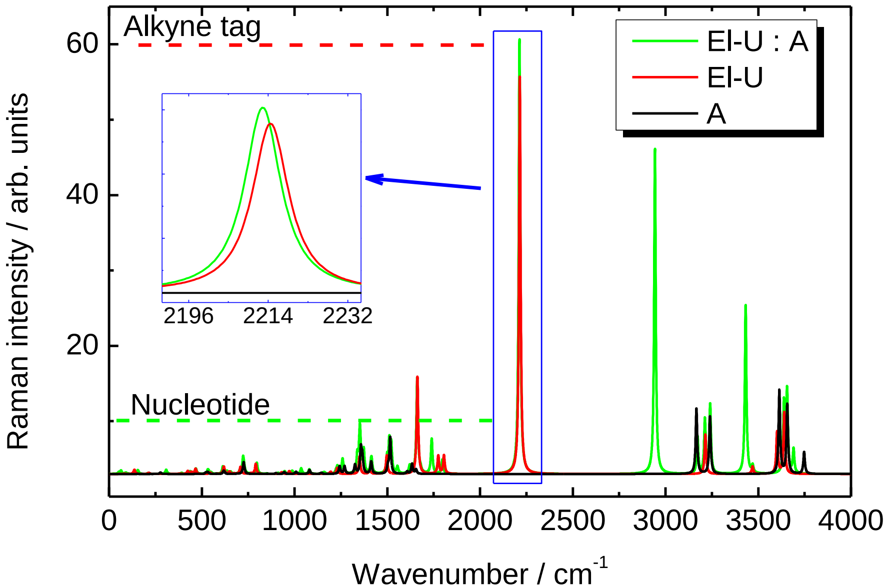




DFT: B3LYP/6-311++G(df,pd)

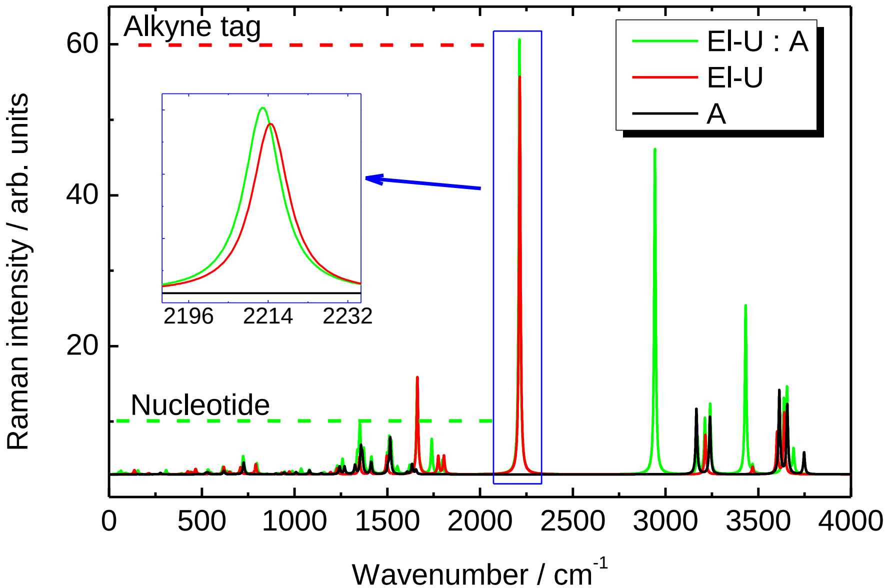
The Raman peaks were simulated by Lorenzians of 10 cm^{-1} FWHM having intensities obtained from the simulations.

Raman experiments



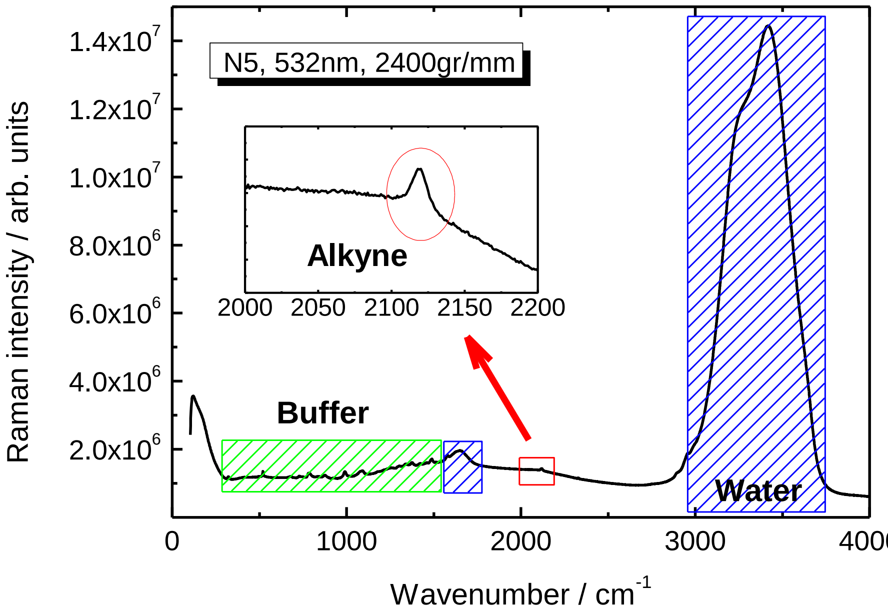
Simulated Raman spectra of ethynyl-labeled nitrogenous base molecules (El-U and A) and their complex (El-U : A). (B3LYP/6-311++G(df,pd), FWHM:10 cm^{-1})

Raman experiments



Simulated Raman spectra of ethynyl-labeled nitrogenous base molecules (El-U and A) and their complex (El-U : A).
(B3LYP/6-311++G(df,pd), FWHM:10 cm^{-1})

Alkyne Raman mode (EdU in water)



Typical conditions

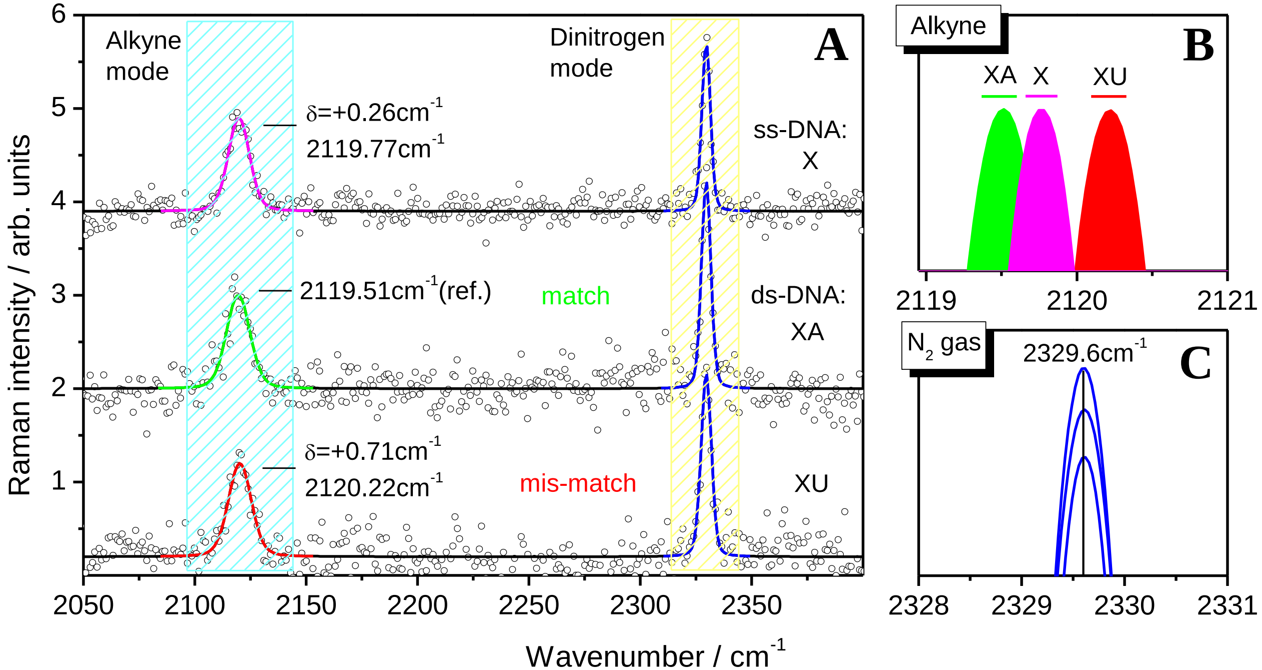
Alkyne tagged DNA probe (one tag)
0,5 mM concentration
532 nm excitation
15 min. measurement time

Raman experiments

Match and mis-match differentiation

X – alkyne tagged uracil

S1 - TTTTGGTGTAXTCAAGGCTCC
S2 - GGAGCCTTGAATACACCAAAA
S3 - GGAGCCTTGAUTACACCAAAA

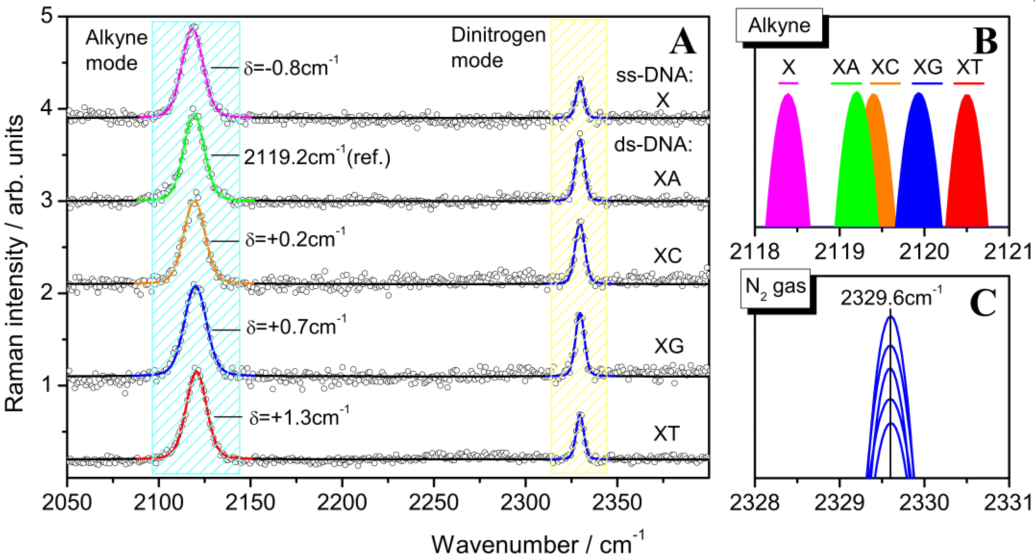


Sample	Alk. freq cm^{-1}	FWHM cm^{-1}	N_2 freq cm^{-1}	FWHM cm^{-1}
S1 (ss DNA probe)	2119.77	11.35	2329.60	4.59
D12 (S1+S2) - XA match	2119.51	12.50	2329.60	4.98
D13 (S1+S3) - XU mis-match	2120.22	12.19	2329.60	5.44

Raman experiments

X – alkyne tagged uracil

Mis-match differentiation



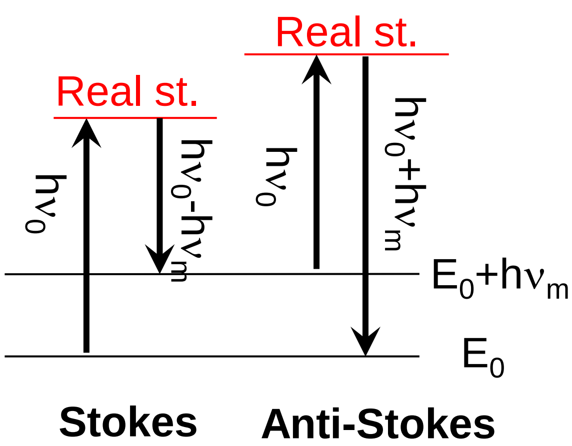
Structure	ν (cm^{-1})	Δν (cm^{-1})
Sample SM1 ss-DNA TGGACTCXCTCAATG	2118.4	-0.8
Sample 1 ds-DNA (match) TGGACTCXCTCAATG CATTGAGAGAGTCCA	2119.2	0.0 (ref.)
Sample 4 ds-DNA (mis-match) TGGACTCXCTCAATG CATTGAGTGAGTCCA	2120.5	+1.3
Sample 8 ds-DNA (mis-match) TGGACTCXCTCAATG CATTGAGCGAGTCCA	2119.4	+0.2
Sample 9 ds-DNA (mis-match) TGGACTCXCTCAATG CATTGAGGGAGTCCA	2119.9	+0.7

- The technique can distinguish between the single-stranded, matching and mis-matching double-stranded DNA sequences based on the position of the Raman band of the alkyne tag attached to the probe sequence.
- It can detect single nucleotide polymorphism and determine the type of the substituent nucleobase.

Increasing the Raman sensitivity

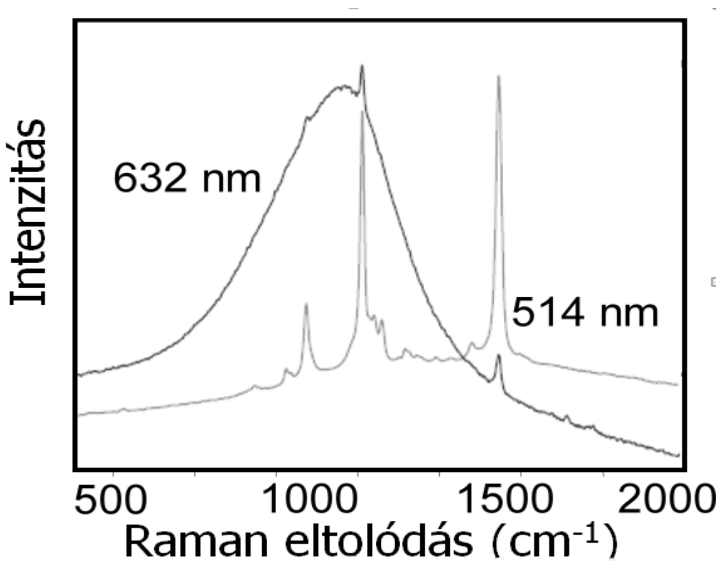
- **Increase the excitation energy**
  Raman intensity I ~ $1/\lambda^4$
  ~$\mathbf{10^3}$ enhancement

  Possible damage to the sample
  Strong photoluminescence backgound



- **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

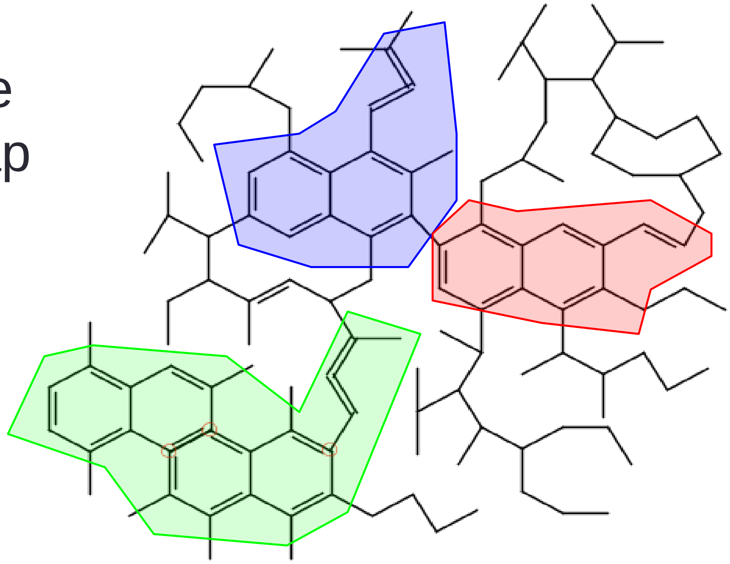
  Excitation of concurrent photolminescence



Selective enhancement

Can be used for the characterization of composite materials consisting of units with specific band gap

- Nanotube bundles
- Nanodiamonds
- Amorphous carbon
- Carbon fibres
- Polymers
- Etc.

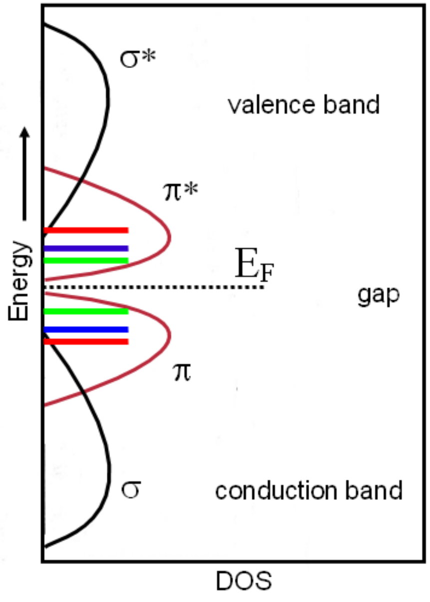


Amorphous carbon

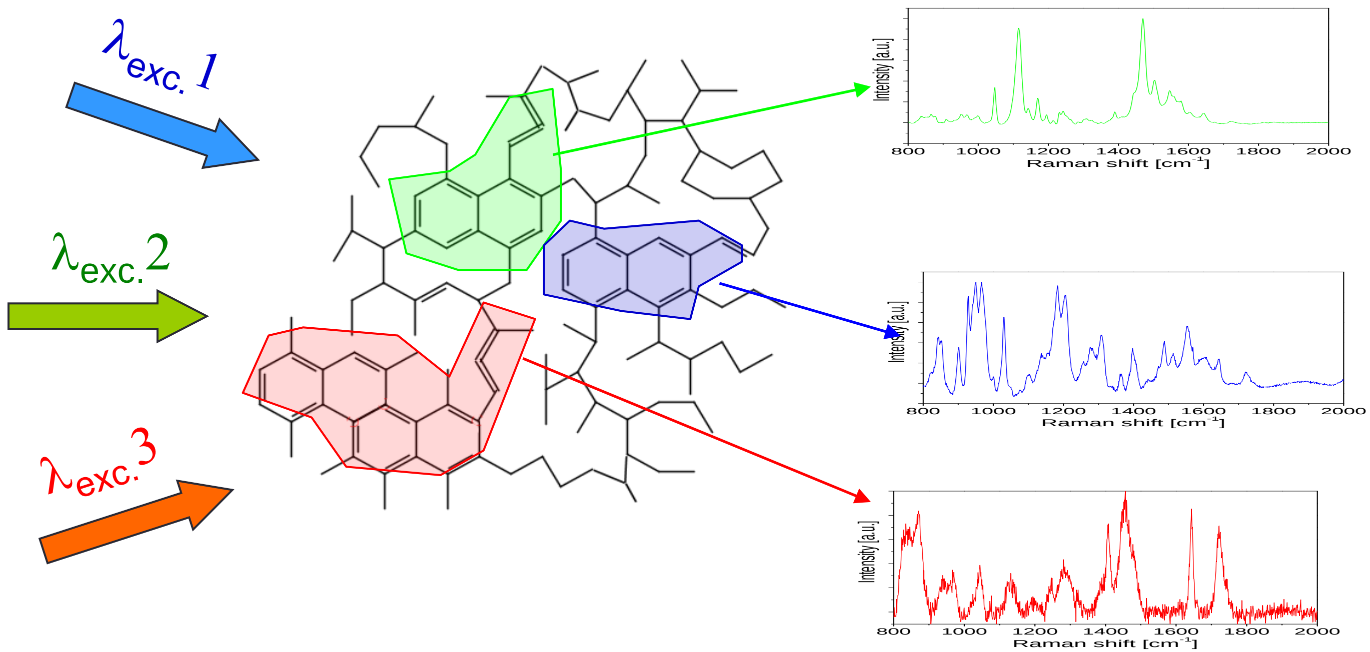
sp^2 and sp^3 hybridized carbon (+ hydrogen)
sp^2 carbon atoms form clusters embedded into the matrix of sp^3 hybridized carbon network.

The sp^3 structure with σ bonds has large band gap.

The sp^2 clusters with π bonds have small band gap, the value of which depends on the cluster size and topology.

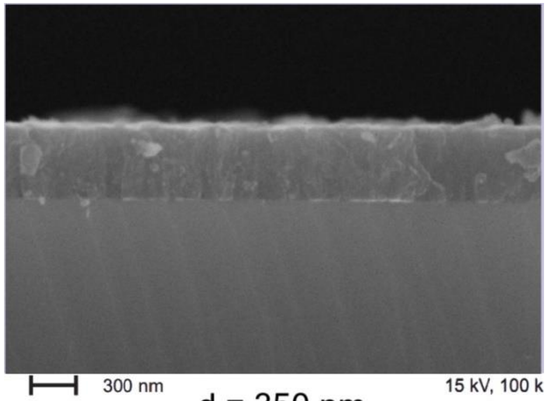


Selective enhancement



Large band gap – small cluster
Small cluster – higher frequency vibrations
High excitation energy – resonant excitation
of small clusters

Nanocrystalline diamond (NCD)



d = 350 nm

Diamond crystallites

- sp^3 hybridized carbon atoms
- σ bonds – wide band gap

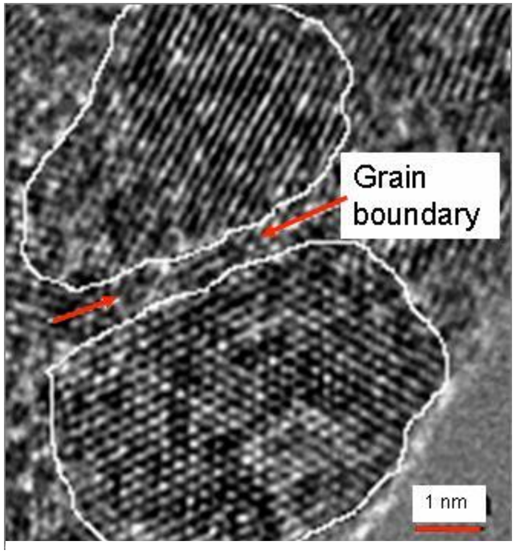
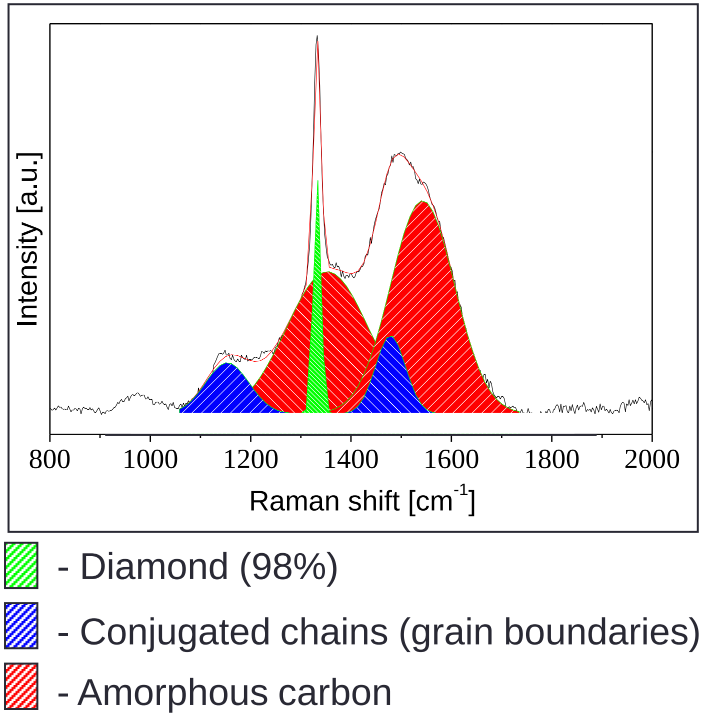


*Fig. 2. Transmission Electron Microscope (TEM) image showing the grain sizes in ultrananocrystalline diamond***

Intergrain region + interface

- amorphous carbon
- sp^2 + sp^3 hybridized carbon atoms
- σ and π bonds – narrow band gap
- some level of ordering on the interface

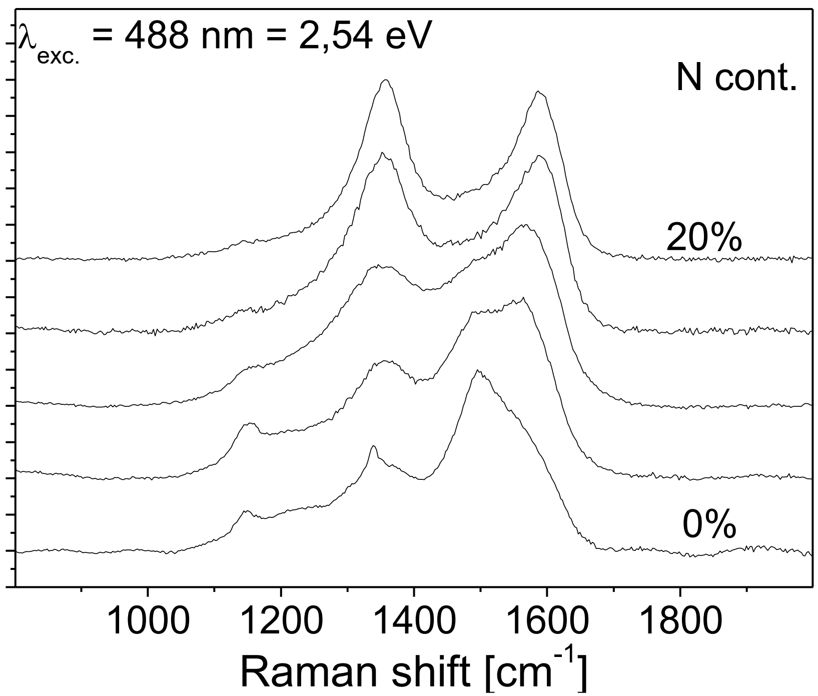
Typical Raman spectrum of nanodiamond (488 nm excitation).



F. Cleri et al., Europhys. Lett., 46 (5), pp. 671-677 (1999)
http://www.thindiamond.com
http://theory.materials.uoc.gr

Addition of nitrogen to NCD

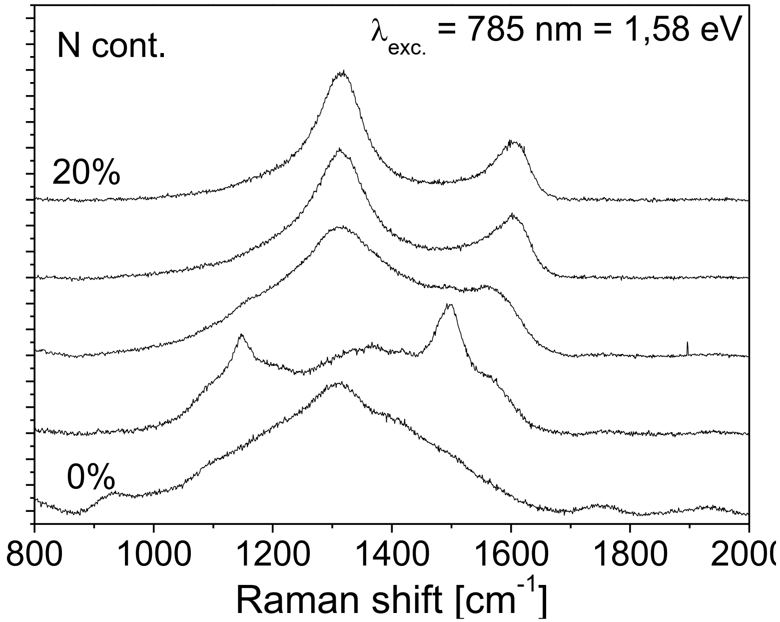
Nitrogen containing NCD films were grown using microwave-enhanced chemical vapor deposition from $CH_4 + H_2 + N_2$ mixture.



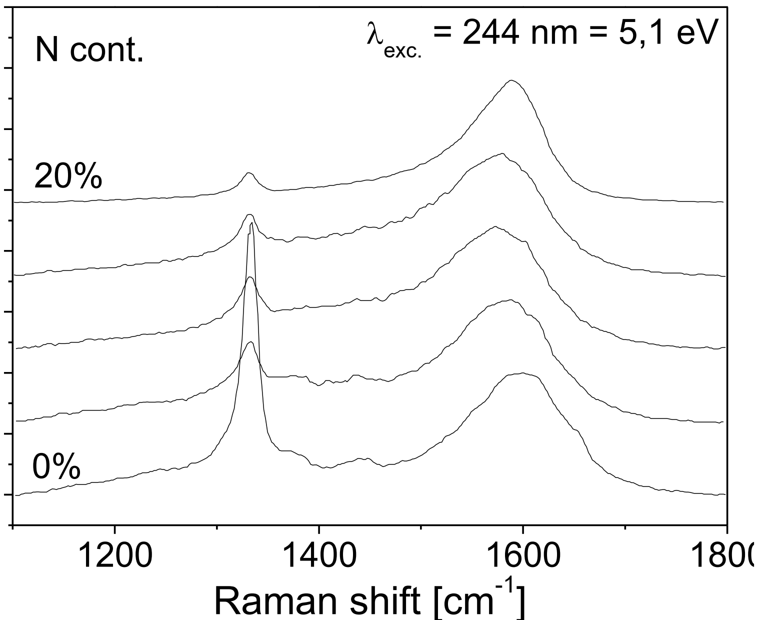
- Typical NCD spectrum at 0% nitrogen.
- The diamond peak decreases with nitrogen content.
- G peak shifts to higher wavenumbers.
- The 20% nitrogen sample is dominated by the amorphous carbon D and G peaks.

Addition of nitrogen causes gradual transformation to an amorphous carbon structure.

Addition of nitrogen to NCD

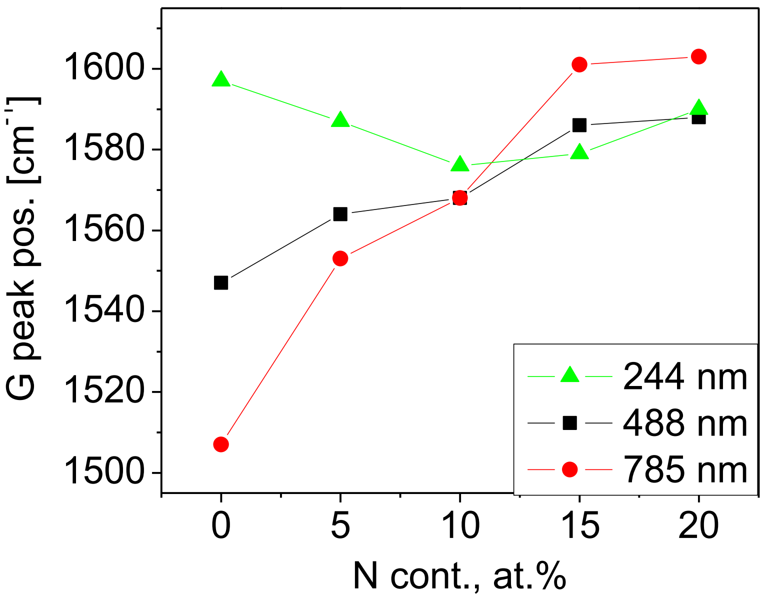


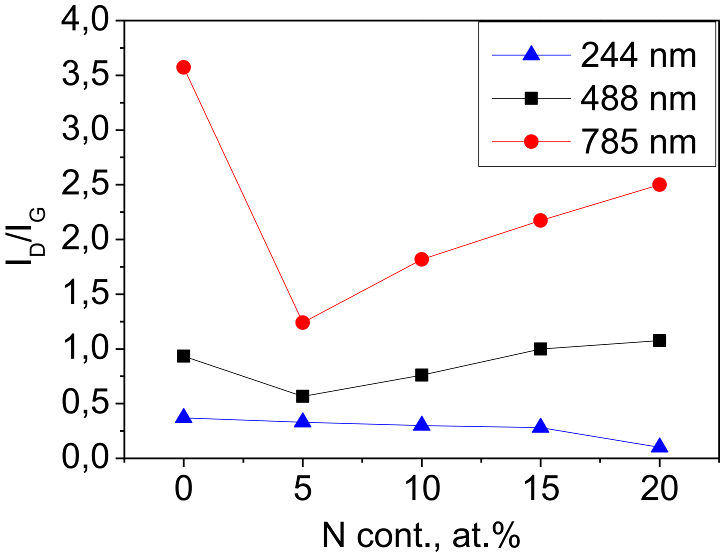
- No diamond peak in the 0% sample.
- Strong Raman contribution in the D band region.
- G band increases and shifts to higher wavenumbers with nitrogen content.
- Transformation into amorphous carbon.



- Detectable diamond peak in the spectrum of the 20% sample.
- Weak D and strong G band.
- G peak first shifts to lower, then to higher wavenumbers with nitrogen content.

Addition of nitrogen to NCD





488 and 785 nm excitation:

- The G peaks shifts to the region characteristic for graphite and condensed six-fold rings with nitrogen content.
- The I_D/I_G ratio increases.

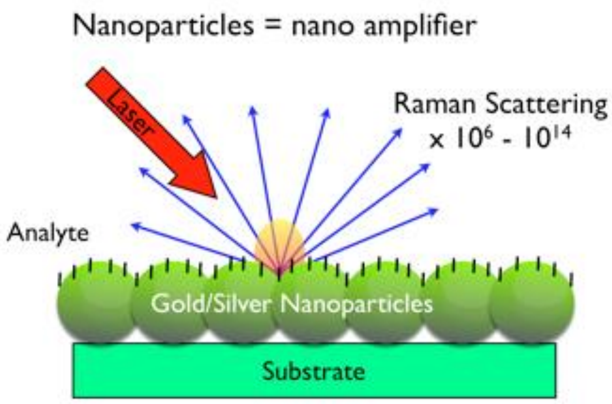
The Raman spectra indicate graphitization.

244 nm excitation:

- G peak position has a U-shaped behavior
- The I_D/I_G ratio decreases.

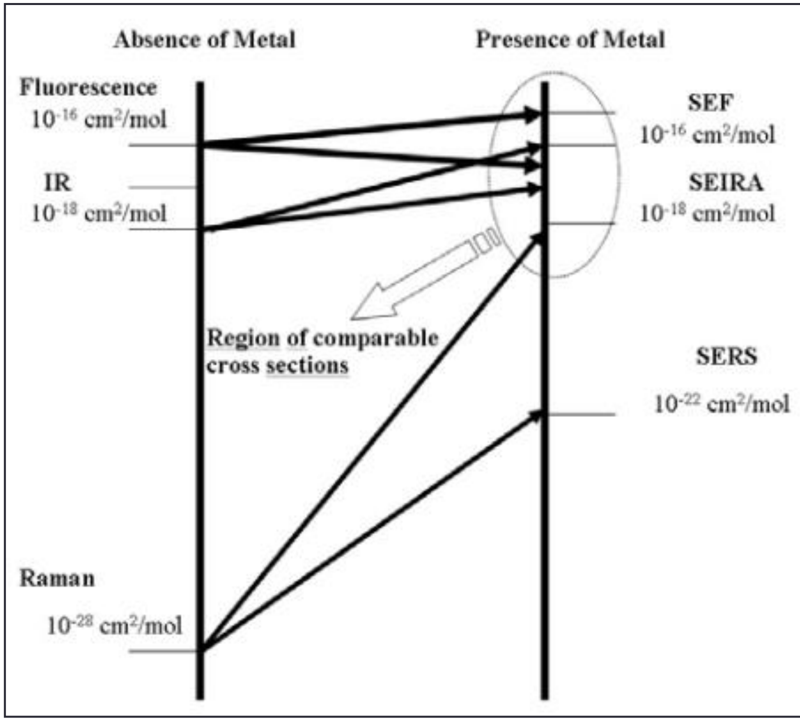
The Raman spectra indicate that the way of incorporation of nitrogen into small clusters depends on the nitrogen content.

Surface-enhanced Raman scattering (SERS)



Resonant amplification of the electromagnetic field of excitation and/or Raman scattered photons by plasmons of metallic nanoparticle nanoantennas (or nanostructured surfaces).

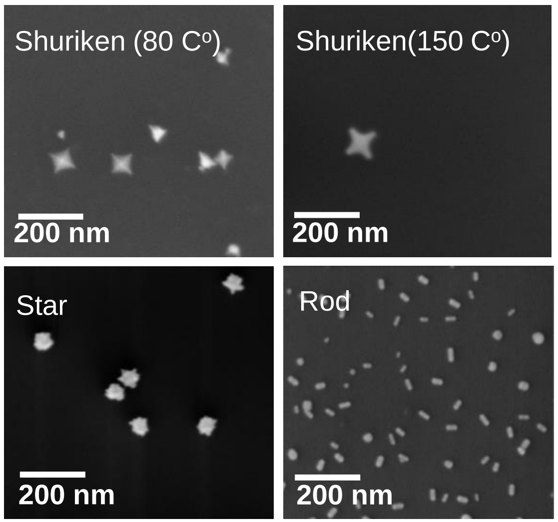
- 10^6–10^{10} Raman enhancement
- Proven single-molecule sensitivity
- Works with other light-matter interaction processes as well

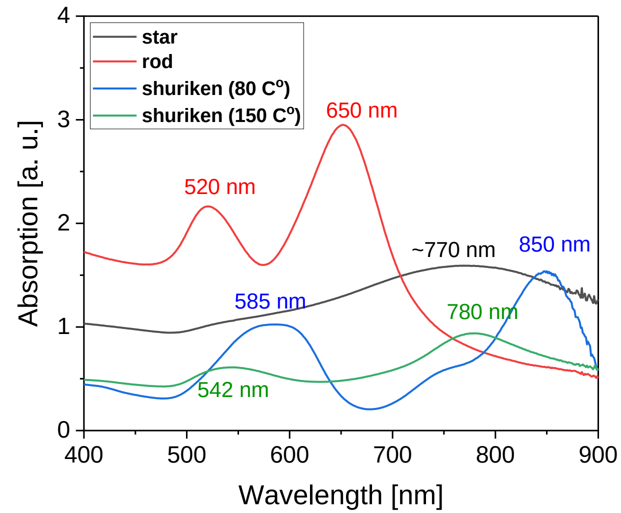


K. Kneipp et al., Surface Enhanced Raman Scattering, Springer, 2006

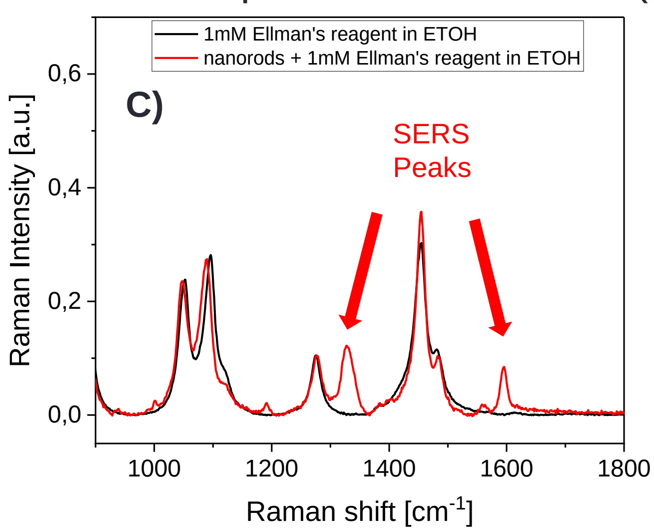
Synthesis of SERS substrates

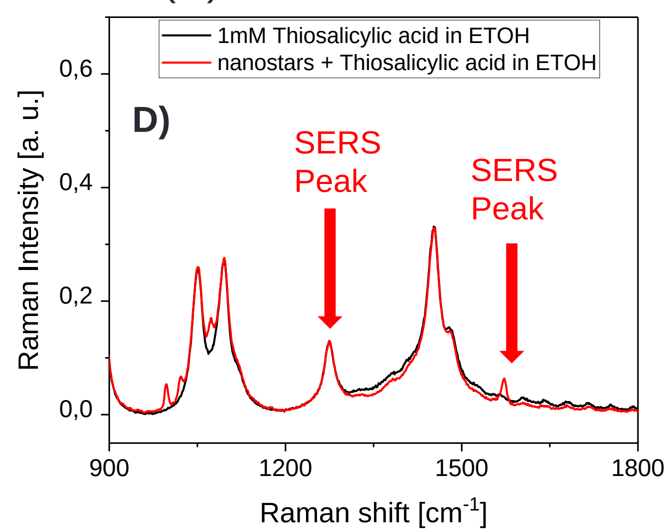
Synthesis of gold nanoparticles for SERS measurements, the plasmon resonance absorption band of which can be tuned over the visible range of the optical spectrum.





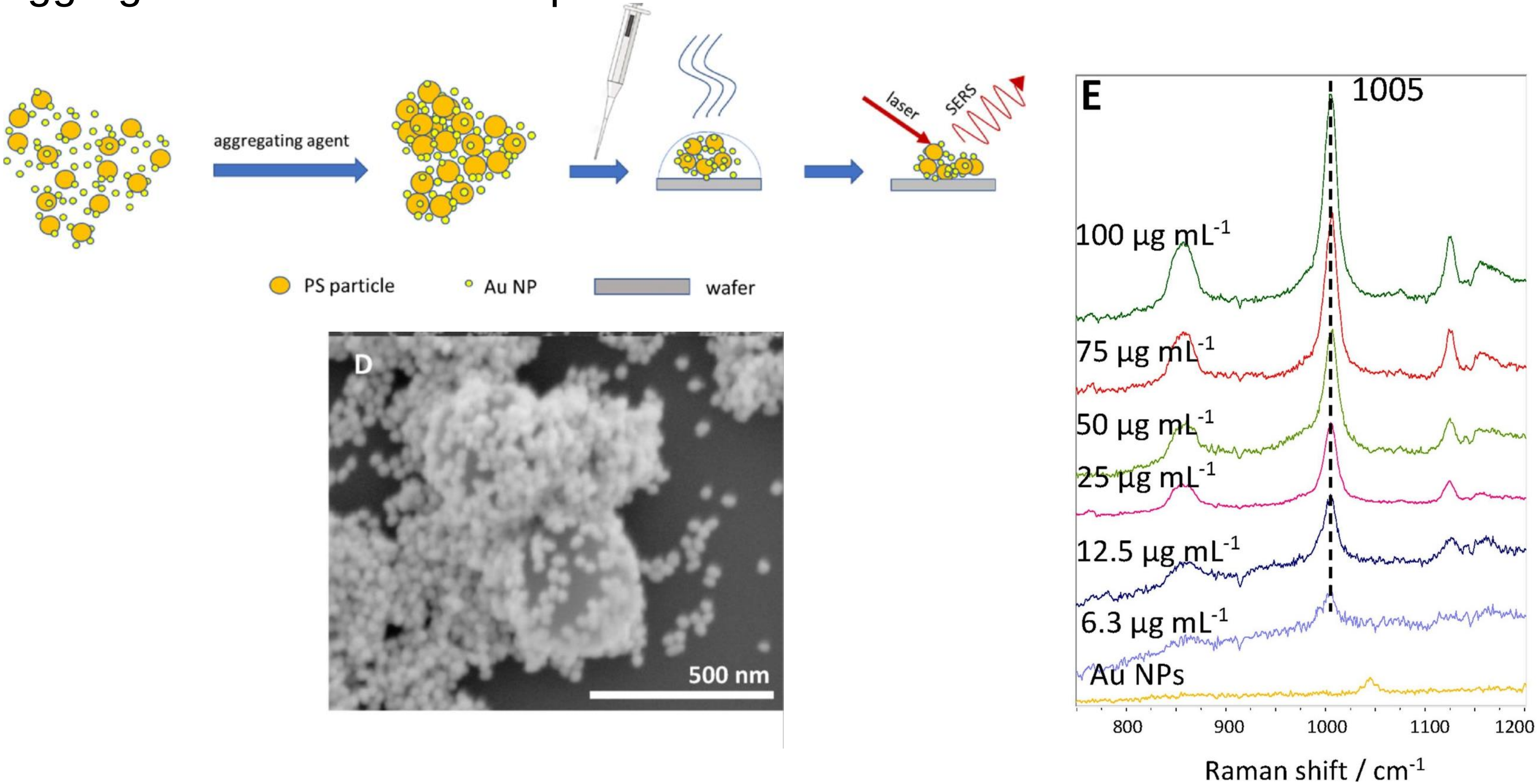
Detection of two compounds with 633 nm (C) and 785 nm (D) excitation in low concentrations.





SERS detection of microplastics

Detection of polystyrene and polyethylene with SERS of gold nanoparticles aggregated onto the microspalstics.

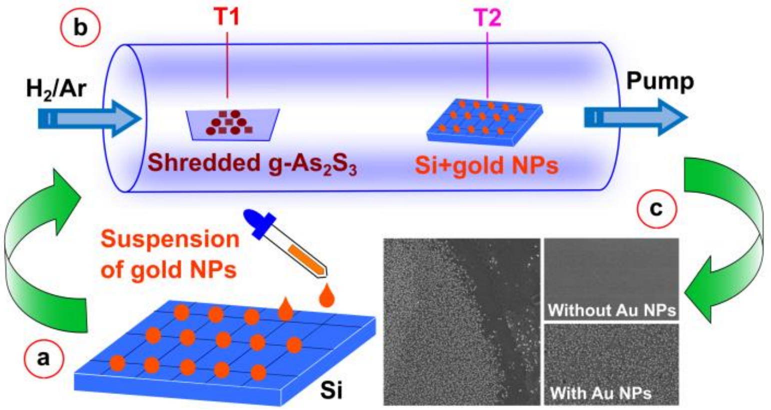


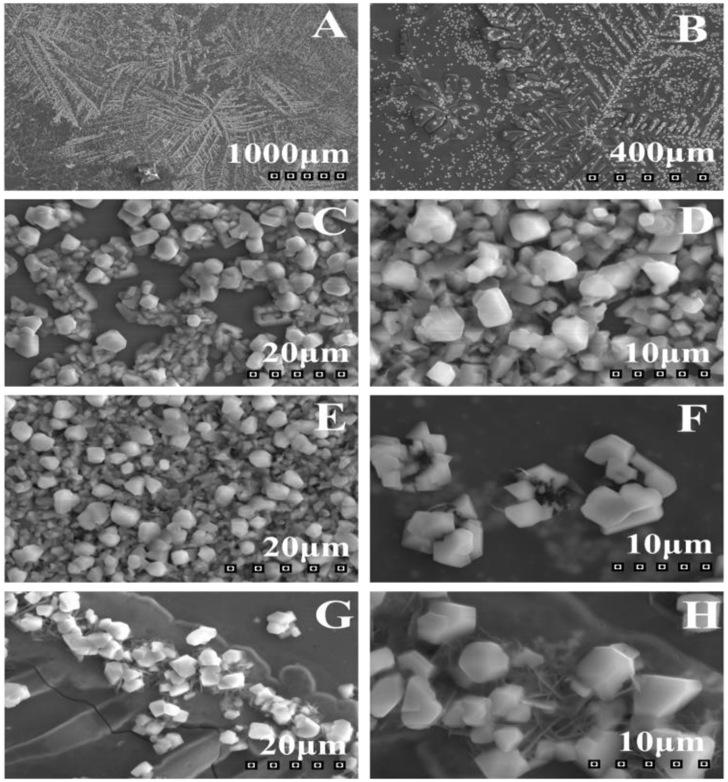
- The technique was showing a 6.3 µg/mL detection limit.

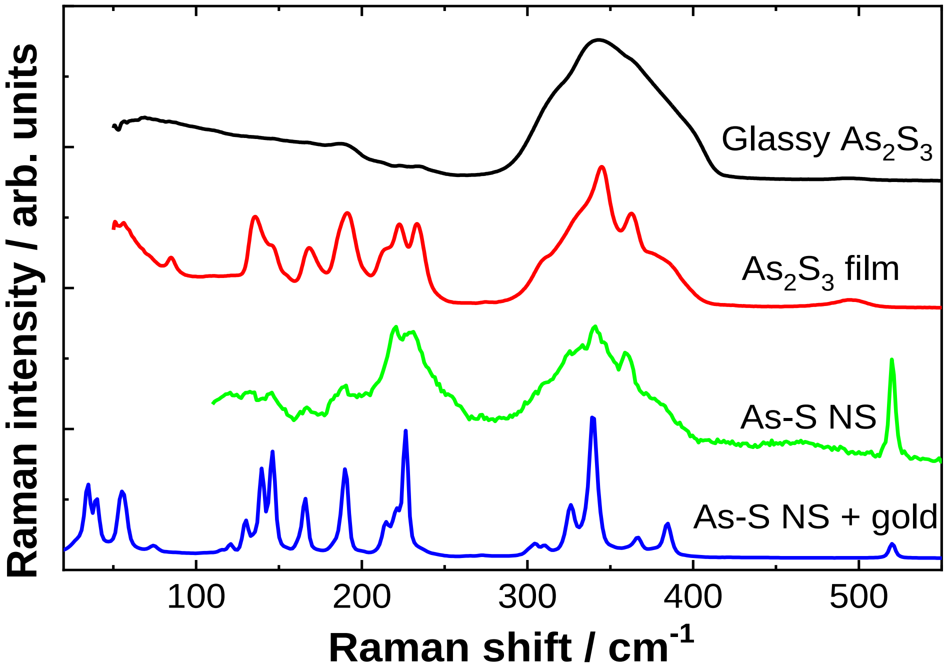
Mikac, L. et al., Appl. Surf. Sci. 608, 155239 (2023)

Gold NP catalyzed thermal CVD

Growth of arsenic-sulphur structures onto a silicon substrate covered with gold nanoparticles.







R. Holomb, O. Kondrat, V. Mitsa, A. Mitsa *et al.*, *J. Alloys Compd.* **894** (2022) 162467.

Acknowledgements

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