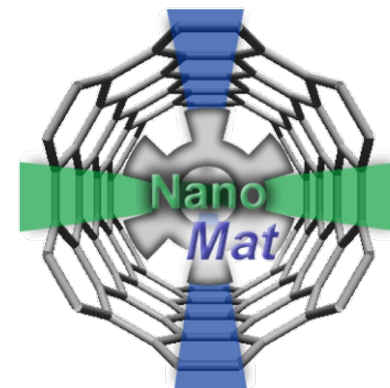


Microscopía Raman Confocal & Microscopia de Fuerza Atómica

Dr. Ricardo Faccio

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Facultad de Química
Universidad de la República
rfaccio@fq.edu.uy

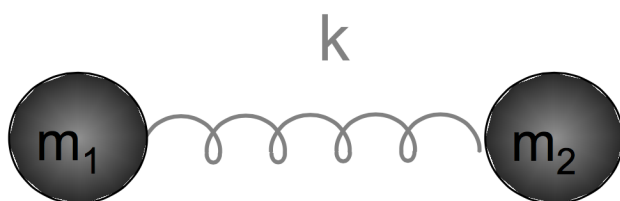


Punteo

- ▣ Modos Vibracionales
- ▣ Reglas de Selección: IR vs Raman
- ▣ Microscopia Óptica y Confocal
- ▣ Microscopia Raman Confocal
- ▣ Aplicaciones de MicroRaman
- ▣ Microscopia de Fuerza Atómica
- ▣ Aplicaciones de MicroRamanAFM

http://cryssmat.fq.edu.uy/ricardo/litiasis_rf.zip

1. Modos Vibracionales



$$V = \frac{1}{2} kx^2$$

$$\nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}}; \quad \frac{1}{\mu} = \frac{1}{m_1} + \frac{1}{m_2}$$

Bond	Bond Energy [kcal (kJ)]	Stretching Frequency (cm ⁻¹)
<i>Frequency dependence on bond energies</i>		
C—C	83 (350)	1200
C=C	146 (611)	1660
C≡C	200 (840)	2200

stronger
bond

$\bar{\nu}$ increases

Bond	Bond Energy [kcal (kJ)]	Stretching Frequency (cm ⁻¹)
<i>Frequency dependence on atomic masses</i>		
C—H	100 (420)	3000
C—D	100 (420)	2100
C—C	83 (350)	1200

heavier
atoms

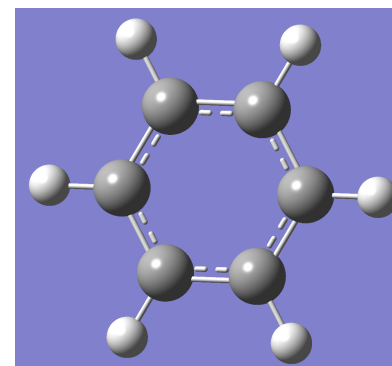
$\bar{\nu}$ decreases

1. Modos Vibracionales

□ Número de modos depende de los grados de libertad

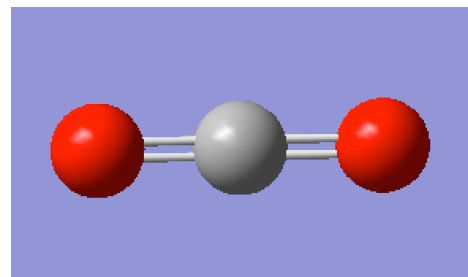
■ Moléculas no Lineales

- $N^{\circ} \text{ modos} = 3N - 6$
- Ejemplo: Benceno
 - 30 modos



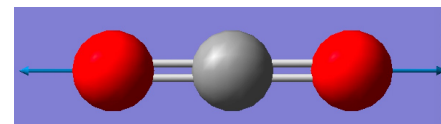
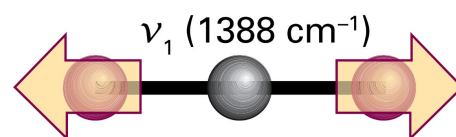
■ Moléculas Lineales

- $N^{\circ} \text{ modos} = 3N - 5$
- Ejemplo: CO_2
 - 4 modos

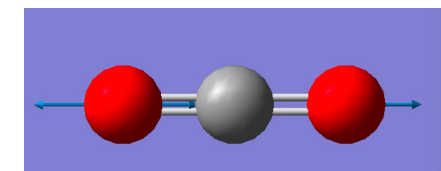
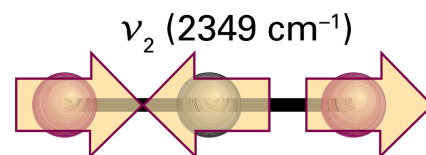


Modos Vibracionales del CO₂

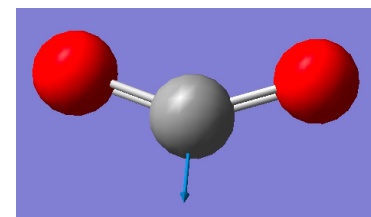
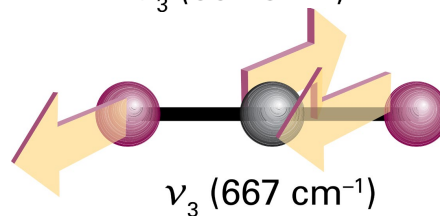
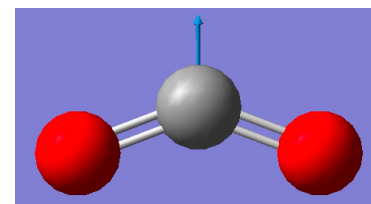
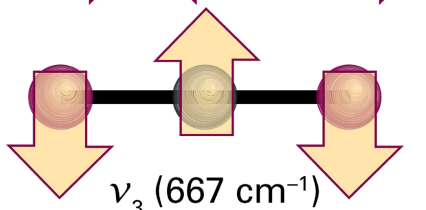
Estiramiento
simétrico



Estiramiento
antisimétrico

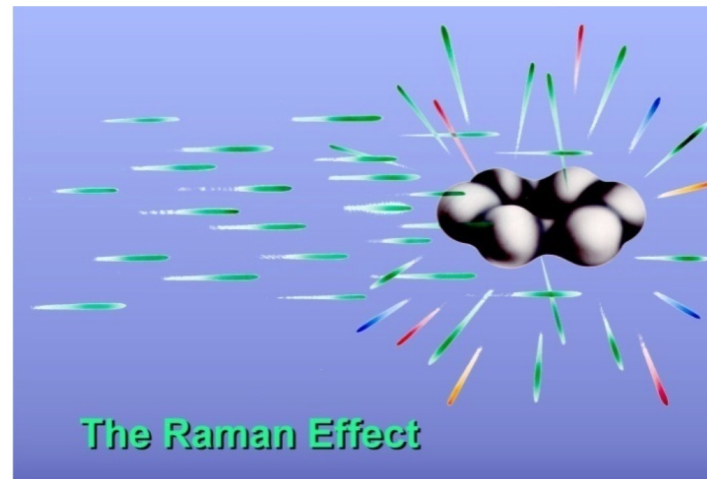
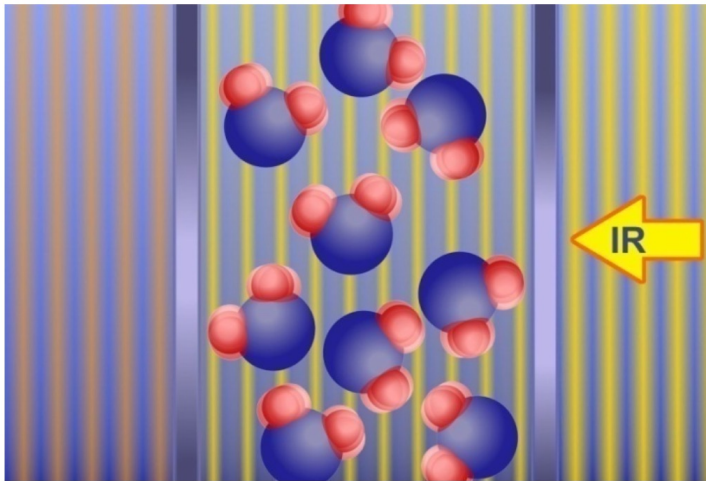


Deformación
degenerada

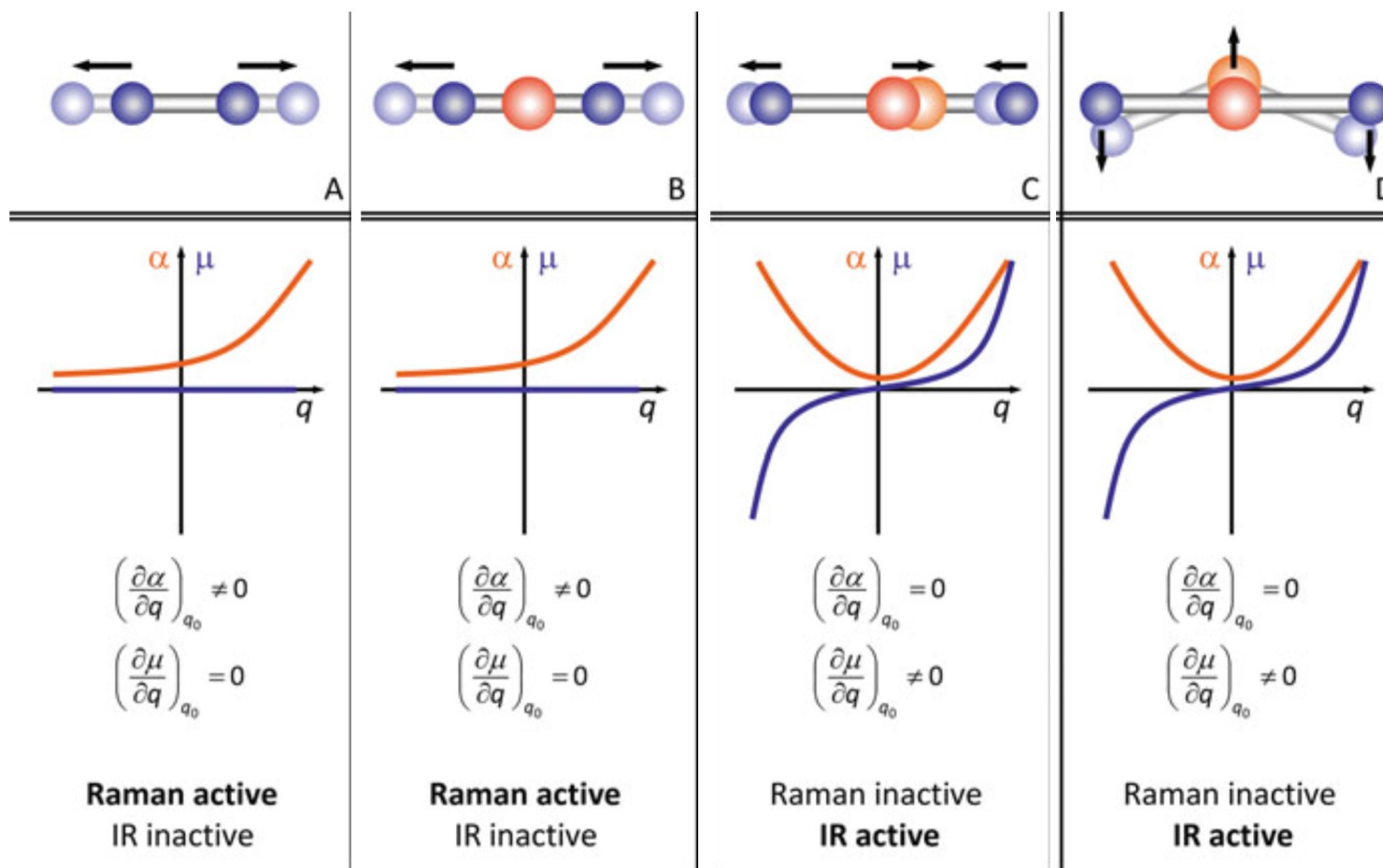


Reglas de Selección

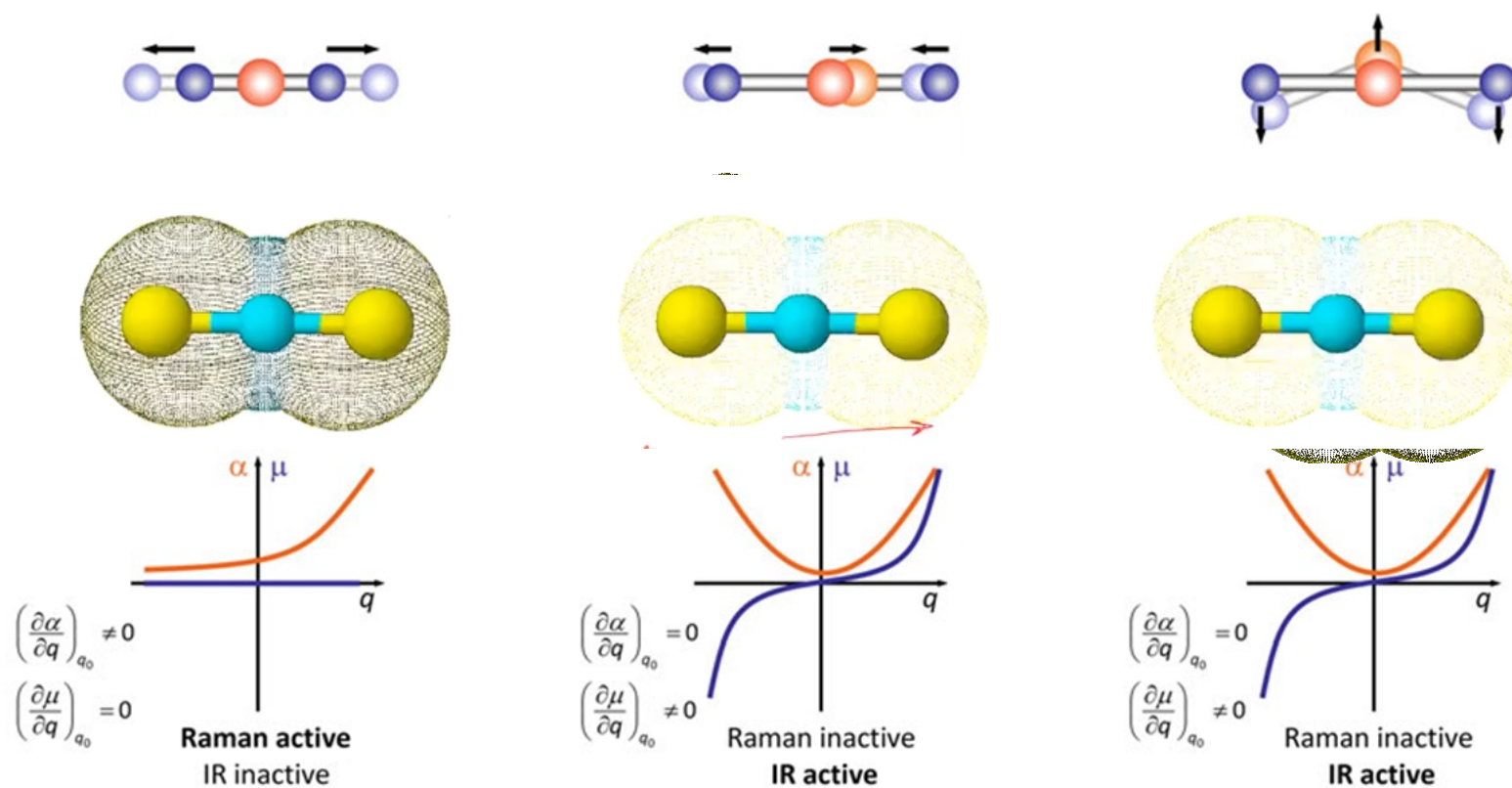
- IR: Absorción de fotón por dipolo (μ) del modo normal de vibración.
- Raman: Dispersión inelástica mediada por momento dipolar inducido (polarizabilidad α) generado por la OEM.



Reglas de Selección: IR vs Raman

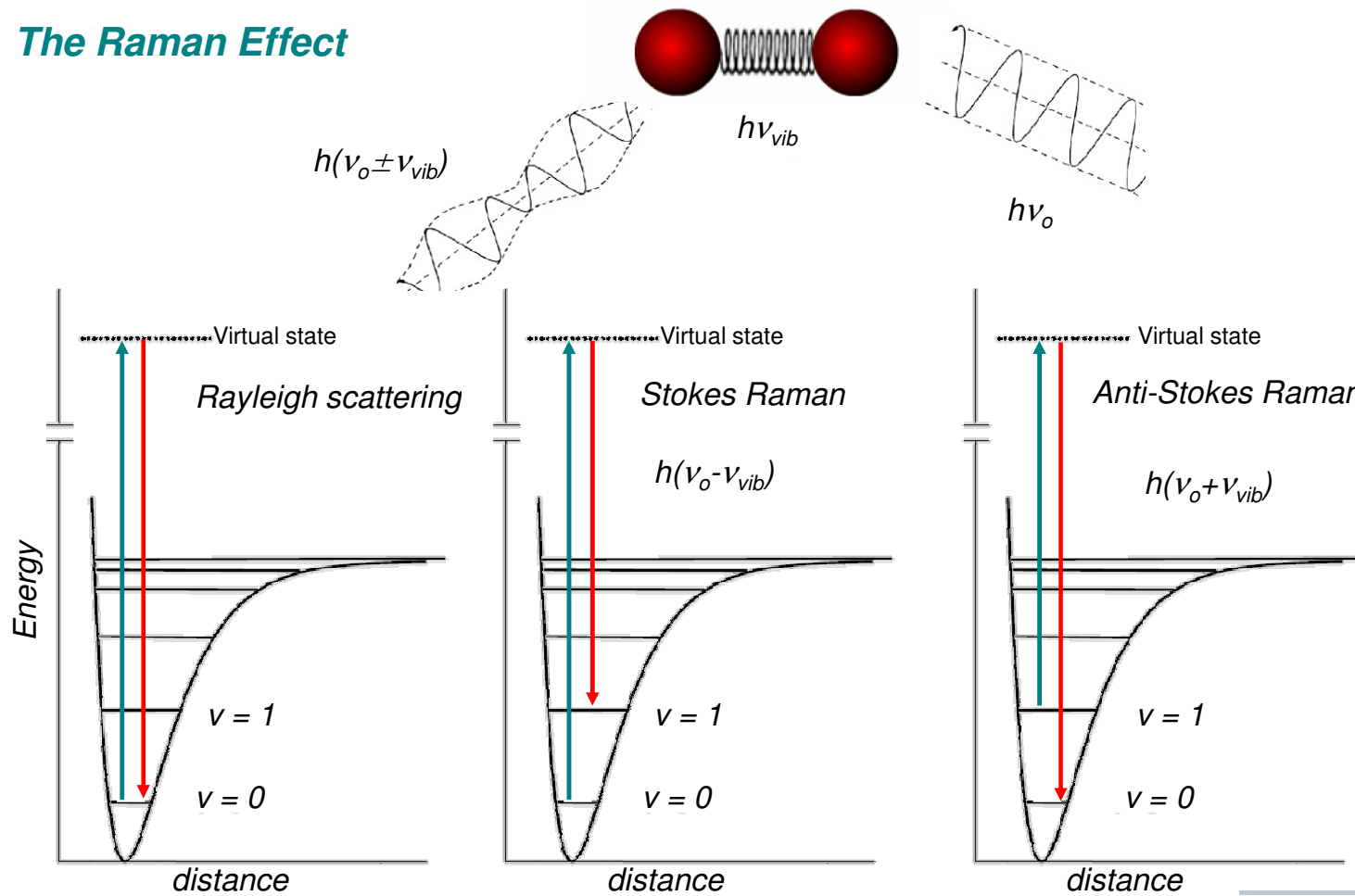


Reglas de Selección: IR vs Raman



El efecto Raman

The Raman Effect

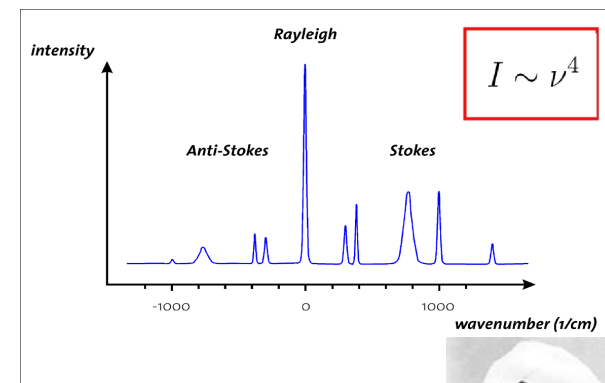
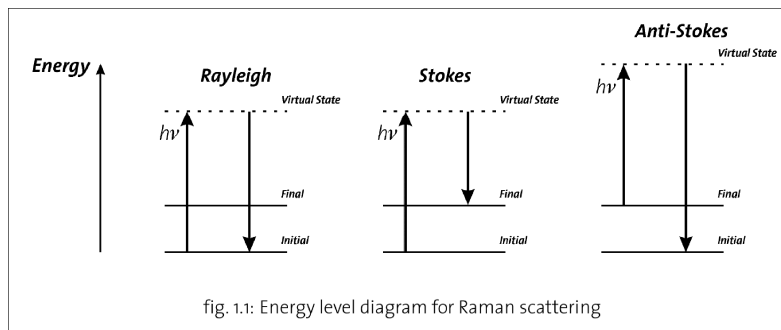
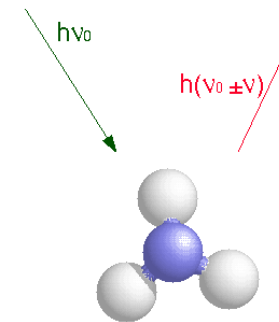


Octubre de 2023

El efecto Raman

The Raman Effect

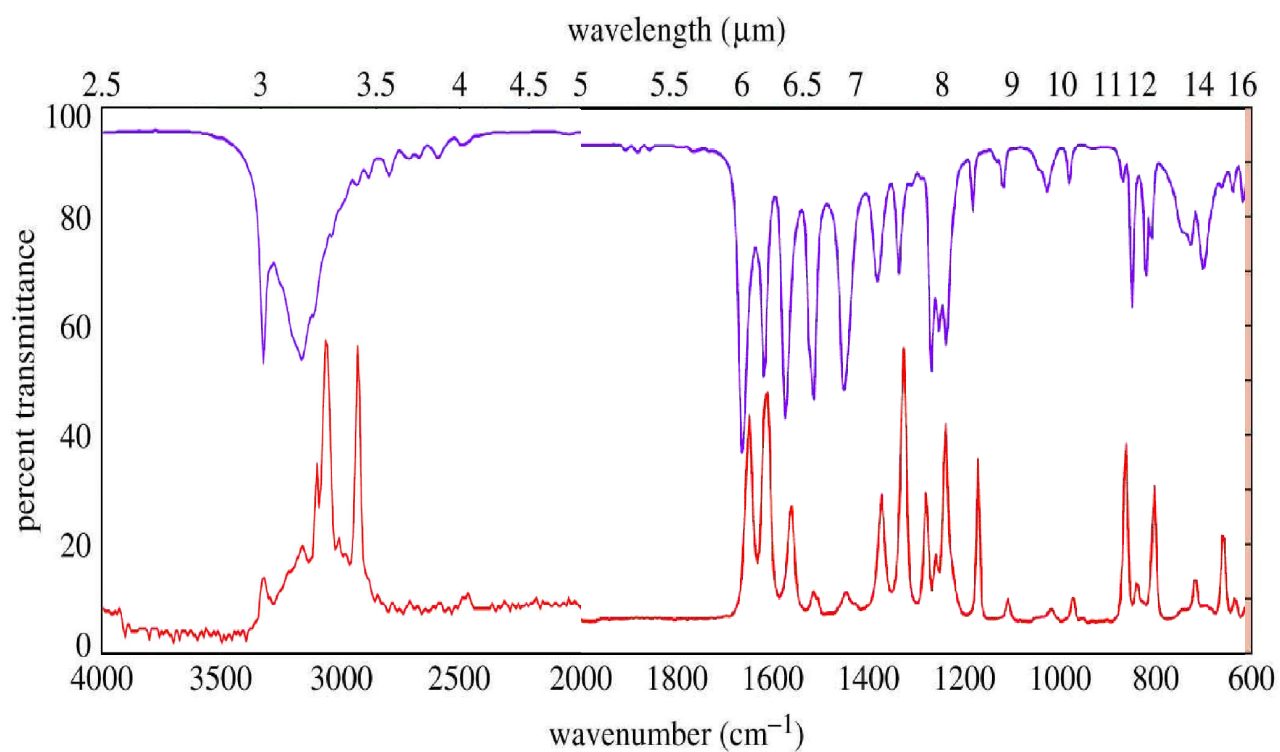
- non-resonant excitation (Stokes) or annihilation (Anti-Stokes) of a vibrational quantum
- energy shift between the exciting and scattered photon is characteristic for the molecules involved in the scattering process



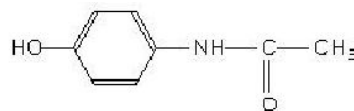
- theoretical prediction 1923 by A. Smekal
- experimentally discovered 1928 by Sir Chandrasekhara Raman, Nobel Prize 1930



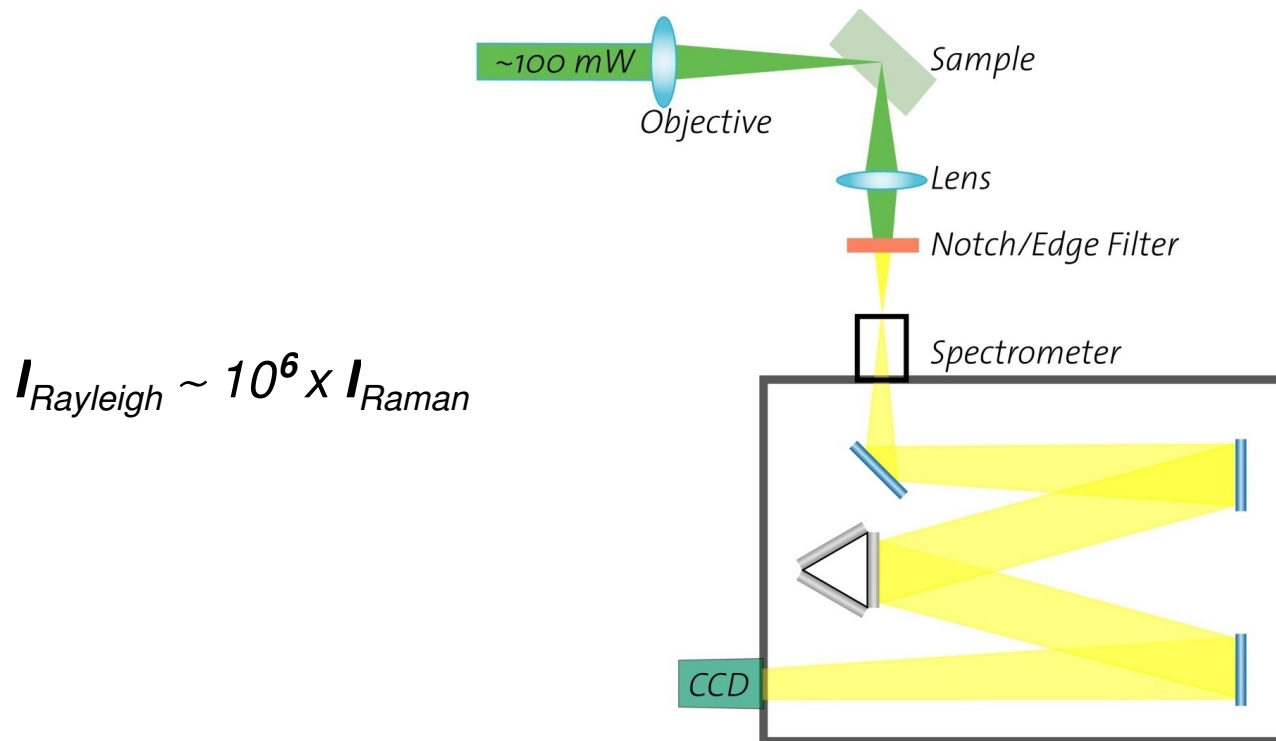
Infrarrojo vs. Raman



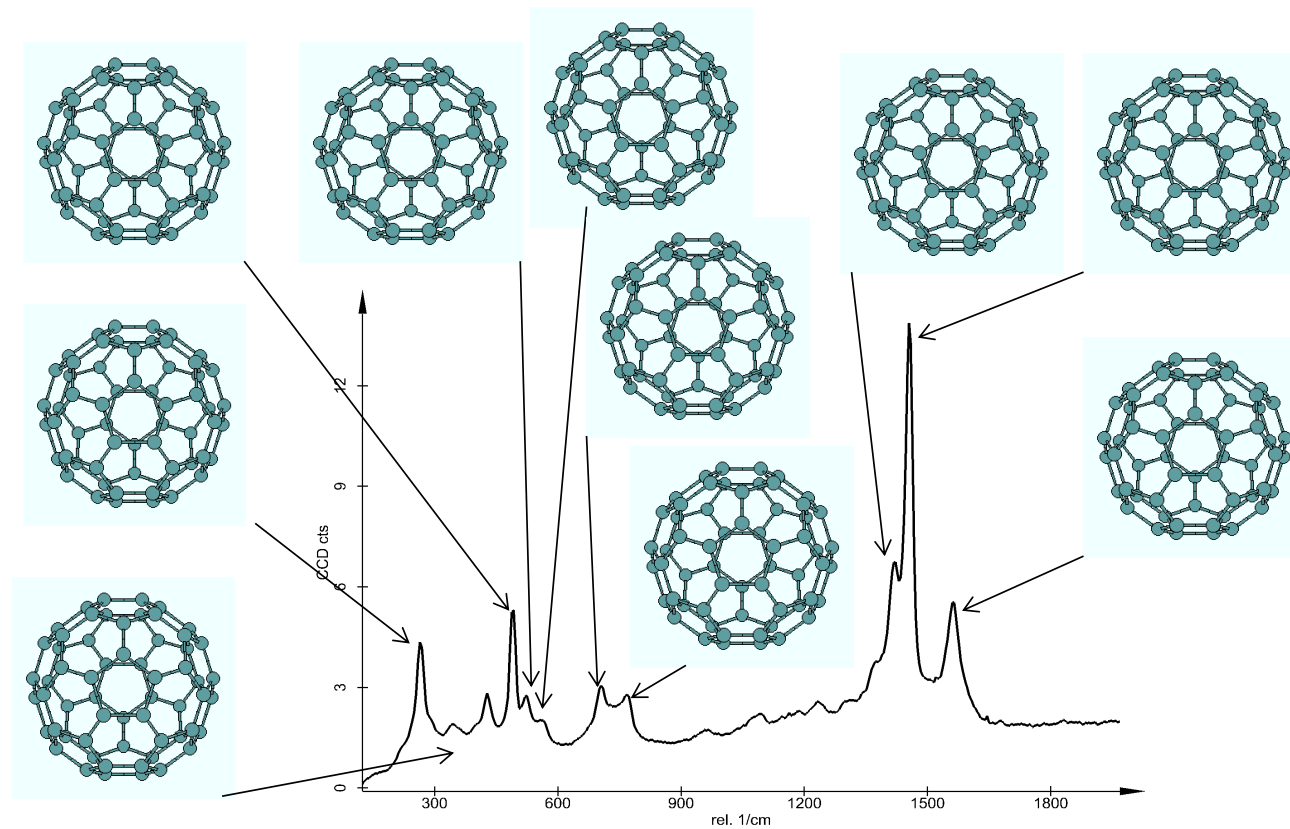
Paracetamol



Esquema Típico de Experimento



Espectroscopia Raman

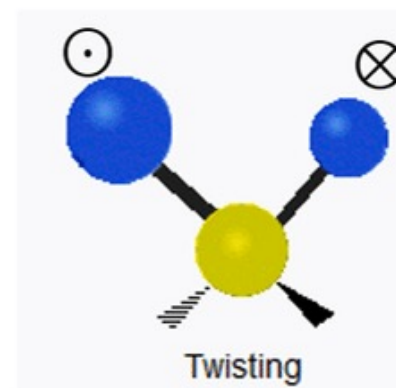
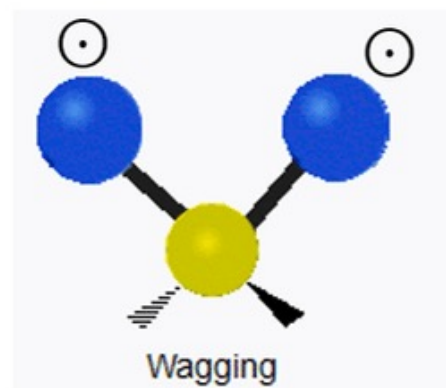
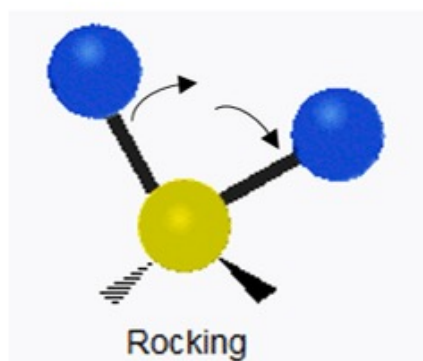
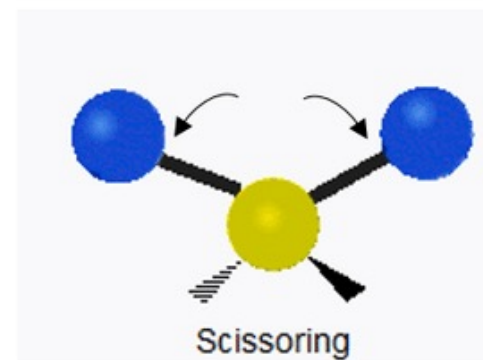
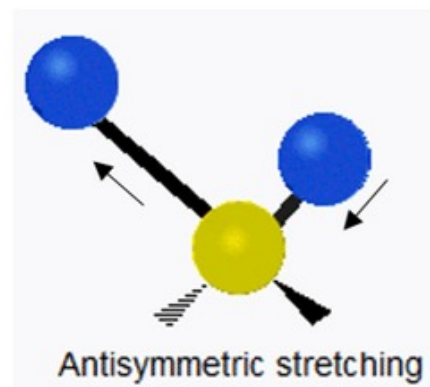
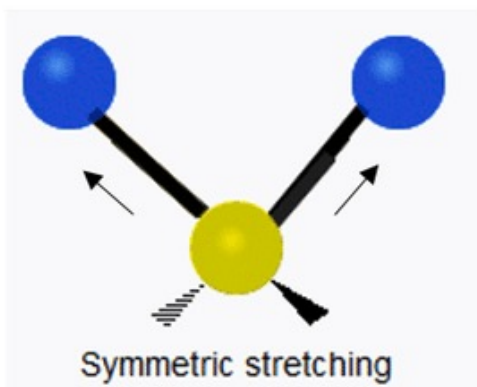


Identificación de Bandas

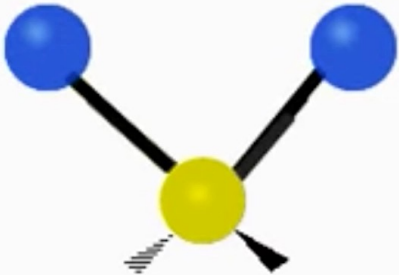
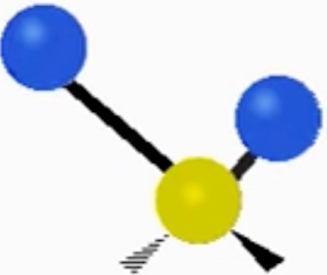
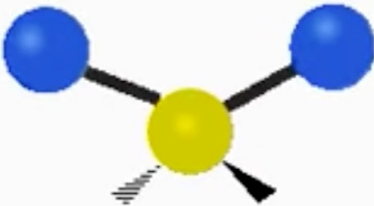
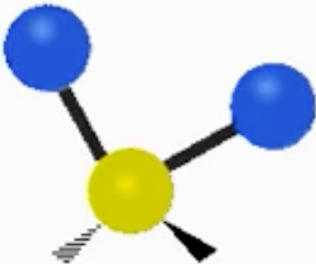
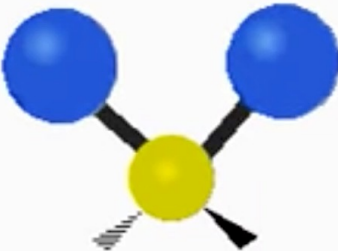
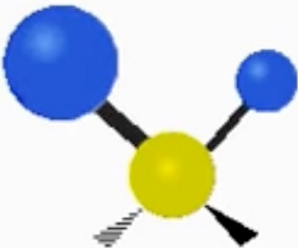
Functional Group/ Vibration	Region	Raman	InfraRed
Lattice vibrations in crystals, LA modes	10 - 200 cm^{-1}	strong	strong
$\delta(\text{CC})$ aliphatic chains	250 - 400 cm^{-1}	strong	weak
$\nu(\text{Se-Se})$	290 -330 cm^{-1}	strong	weak
$\nu(\text{S-S})$	430 -550 cm^{-1}	strong	weak
$\nu(\text{Si-O-Si})$	450 -550 cm^{-1}	strong	weak
$\nu(\text{Xmetal-O})$	150-450 cm^{-1}	strong	med-weak
$\nu(\text{C-I})$	480 - 660 cm^{-1}	strong	strong
$\nu(\text{C-Br})$	500 - 700 cm^{-1}	strong	strong
$\nu(\text{C-Cl})$	550 - 800 cm^{-1}	strong	strong
$\nu(\text{C-S})$ aliphatic	630 - 790 cm^{-1}	strong	medium
$\nu(\text{C-S})$ aromatic	1080 - 1100 cm^{-1}	strong	medium
$\nu(\text{O-O})$	845 -900 cm^{-1}	strong	weak
$\nu(\text{C-O-C})$	800 -970 cm^{-1}	medium	weak
$\nu(\text{C-O-C})$ asym	1060 - 1150 cm^{-1}	weak	strong

$\nu(\text{CC})$ alicyclic, aliphatic chain vibrations	600 - 1300 cm^{-1}	medium	Medium
$\nu(\text{C}=\text{S})$	1000 - 1250 cm^{-1}	strong	weak
$\nu(\text{CC})$ aromatic ring chain vibrations	*1580, 1600 cm^{-1}	strong	medium
	*1450, 1500 cm^{-1}	medium	medium
	*1000 cm^{-1}	strong/medium	weak
$\delta(\text{CH}_3)$	1380 cm^{-1}	medium	strong
$\delta(\text{CH}_2)$ $\delta(\text{CH}_3)$ asym	1400 - 1470 cm^{-1}	medium	medium
$\delta(\text{CH}_2)$ $\delta(\text{CH}_3)$ asym	1400 - 1470 cm^{-1}	medium	medium
$\nu(\text{C}-(\text{NO}_2))$	1340 - 1380 cm^{-1}	strong	medium
$\nu(\text{C}-(\text{NO}_2))$ asym	1530 - 1590 cm^{-1}	medium	strong
$\nu(\text{N}=\text{N})$ aromatic	1410 - 1440 cm^{-1}	medium	-
$\nu(\text{N}=\text{N})$ aliphatic	1550 - 1580 cm^{-1}	medium	-
$\delta(\text{H}_2\text{O})$	~1640 cm^{-1}	weak broad	strong
$\nu(\text{C}=\text{N})$	1610 - 1680 cm^{-1}	strong	medium
$\nu(\text{C}=\text{C})$	1500 - 1900 cm^{-1}	strong	weak
$\nu(\text{C}=\text{O})$	1680 - 1820 cm^{-1}	medium	strong
$\nu(\text{C}\equiv\text{C})$	2100 - 2250 cm^{-1}	strong	weak
$\nu(\text{C}\equiv\text{N})$	2220 - 2255 cm^{-1}	medium	strong
$\nu(-\text{S}-\text{H})$	2550 - 2600 cm^{-1}	strong	weak
$\nu(\text{C}-\text{H})$	2800 - 3000 cm^{-1}	strong	strong
$\nu(=\text{C}-\text{H})$	3000 - 3100 cm^{-1}	strong	medium
$\nu(\equiv\text{C}-\text{H})$	3300 cm^{-1}	weak	strong
$\nu(\text{N}-\text{H})$	3300 - 3500 cm^{-1}	medium	medium
$\nu(\text{O}-\text{H})$	3100 - 3650 cm^{-1}	weak	strong

Coordenadas Internas



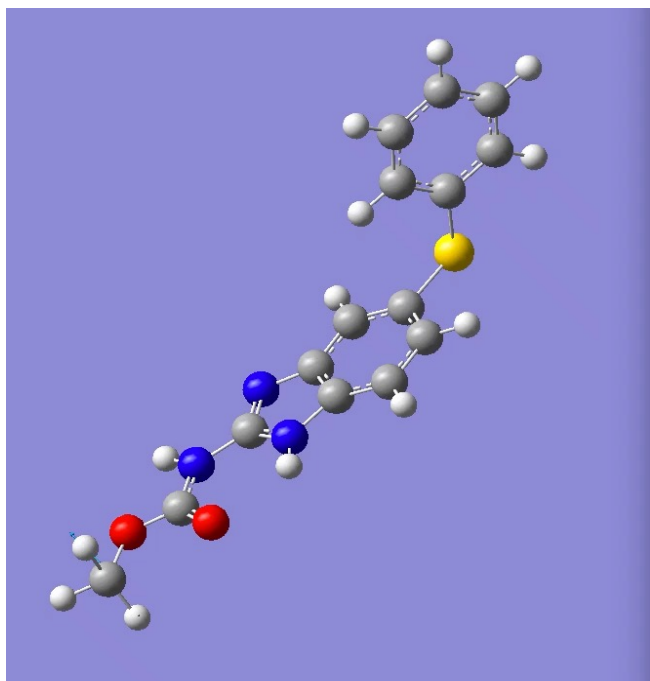
Coordenadas Internas

Symmetrical stretching	Antisymmetrical stretching	Scissoring
		
Rocking	Wagging	Twisting
		

Métodos Teóricos

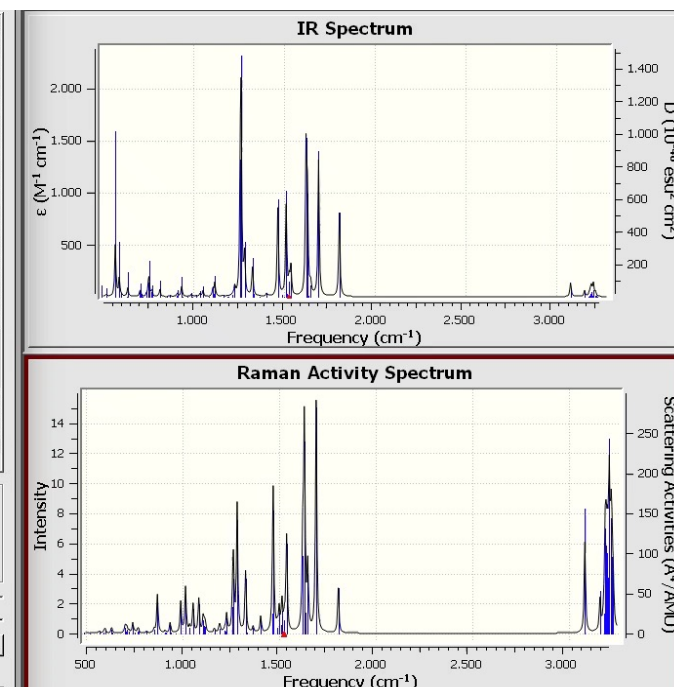
■ Cálculos *ab initio* o *Primeros Principios*

■ *DFT*

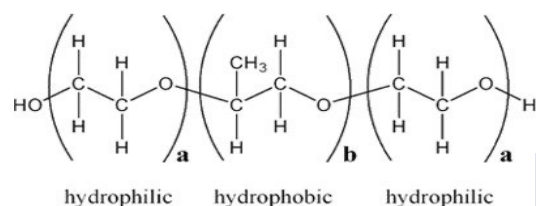


Mode #	Frequency	Infrared	Raman Activity	Depolar-1
71	1488.33	4.0951	7.4331	0.4913
72	1498.41	1.6350	26.2724	0.4348
73	1510.74	245.9288	10.9452	0.6723
74	1513.40	6.9058	27.9007	0.7498
75	1525.75	12.7557	16.4005	0.5506
76	1528.29	36.7918	1.3782	0.6505
77	1536.90	78.5299	112.3579	0.3368
78	1620.40	397.0939	96.7108	0.2349
79	1628.26	257.9796	239.6510	0.3693
80	1634.30	4.0029	25.7055	0.5202
81	1646.36	30.5415	75.0445	0.5860
82	1689.67	377.9050	281.7096	0.3373
83	1804.45	233.3730	56.6451	0.2683
84	3081.42	39.4745	155.3709	0.0212
85	3159.30	18.0586	53.2119	0.7493

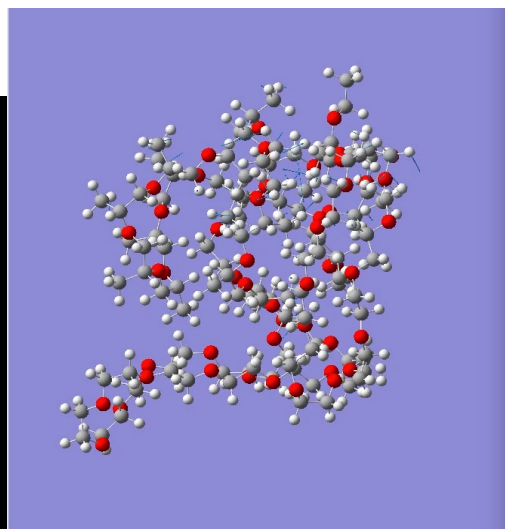
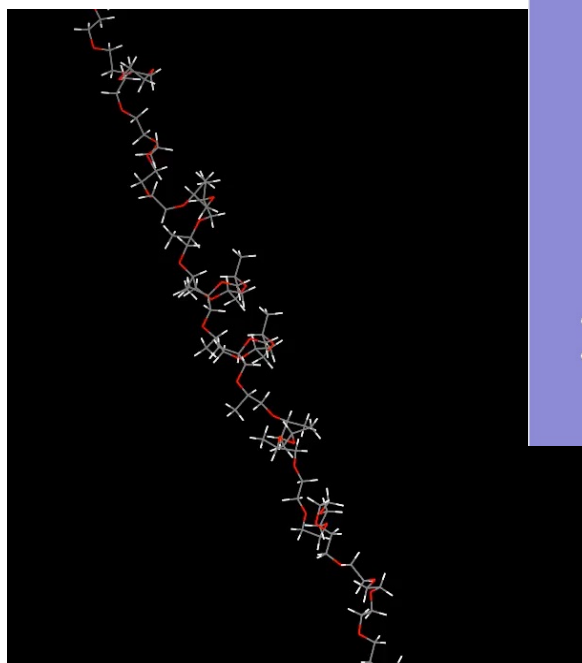
Animate Vibration:
☐ Stop Animation
Repeats: Frames per Cycle: Frame Delay (msec):
Displacement Amplitude:
☒ Show Displacement Vectors Scale:
☐ Show Dipole Derivative Unit Vector Scale:
☐ Manual Displacement:
Scale frequencies?



Poloxamer P184

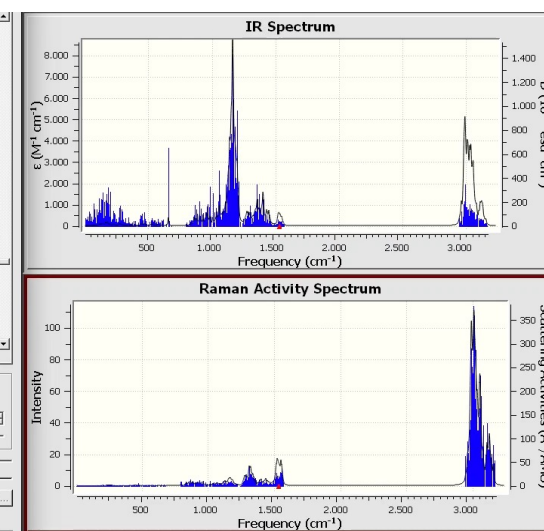


Classical MD → Structural Optimization ab initio Calculations
→ Phonons and polarizabilities determination



Mode #	Frequency	Infrared	Raman Activity	Dipole
910	1531.73	2.5095	21.6991	0.731
911	1533.41	4.4399	14.3750	0.746
912	1533.54	15.6748	18.5910	0.739
913	1534.14	4.0442	10.9234	0.743
914	1534.85	8.9271	18.0199	0.722
915	1535.13	8.2443	3.7176	0.700
916	1535.28	2.3846	9.3043	0.699
917	1536.93	11.6751	7.7106	0.756
918	1537.16	6.4488	14.3888	0.743
919	1537.67	3.8143	18.7628	0.725
920	1537.80	14.7864	6.7754	0.641
921	1537.93	0.2661	2.1380	0.705
922	1538.18	3.1835	8.7477	0.712
923	1538.40	9.8712	6.1394	0.756
924	1538.88	13.3642	9.3387	0.743

☐ Animate Vibration
☒ Stop Animation
 Repeats: Frames per Cycle: Frame Delay (msec):
 Displacement Amplitude:
☒ Show Displacement Vectors Scale:
☐ Show Dipole Derivative Unit Vector Scale:
☐ Manual Displacement:
 Scale frequencies?



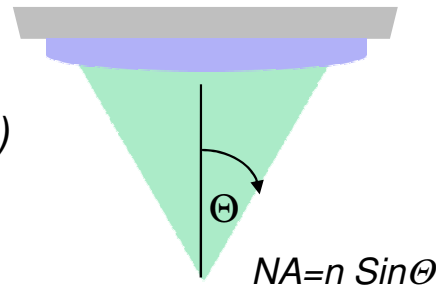
poly(ethylene oxide)/poly(propylene
oxide)/poly(ethylene oxide)
Molecular Dynamics Simulation

Microscopia Óptica Clásica

What is the resolution limit of a classical optical microscope?

Ernst Abbé (1840-1905): diffraction theory

$$\Delta x \geq 0,61 \times (\lambda / NA)$$

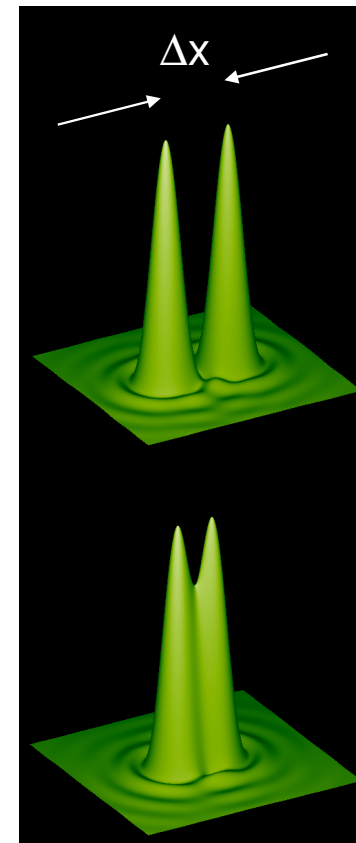


air: $NA \leq 0,95$ (340nm @ 532nm exc.)

water: $NA \leq 1,2$ (270nm @ 532nm exc.)

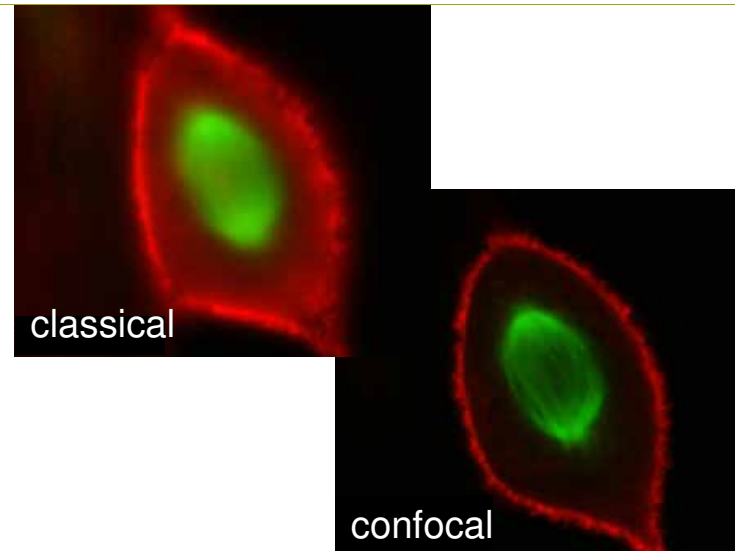
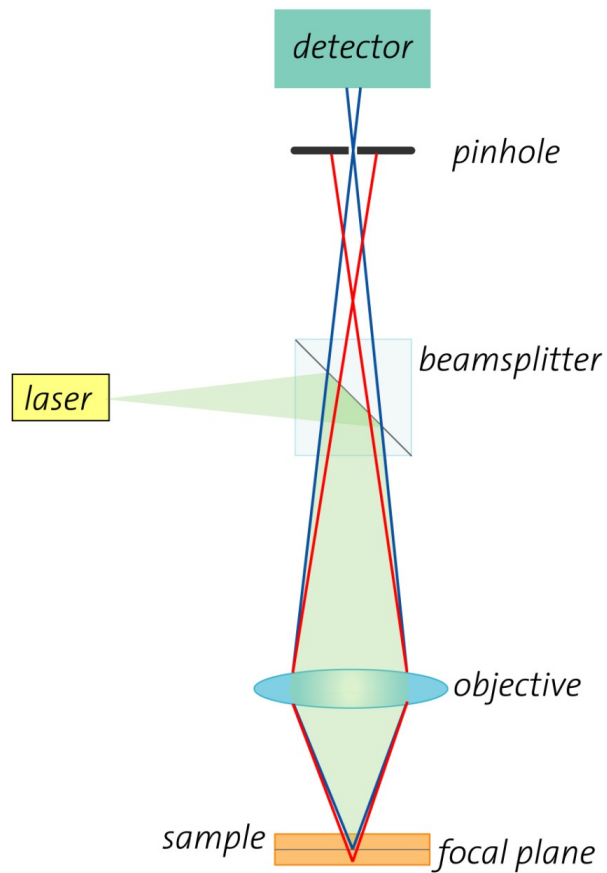
oil: $NA \leq 1,4$ (230nm @ 532nm exc.)

resolution limit $\approx \lambda / 2$



Microscopia Confocal

confocal microscopy

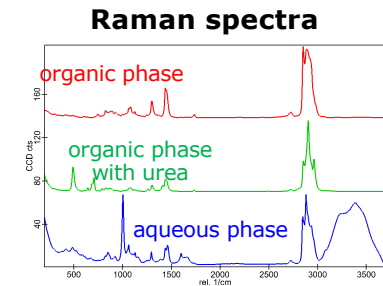
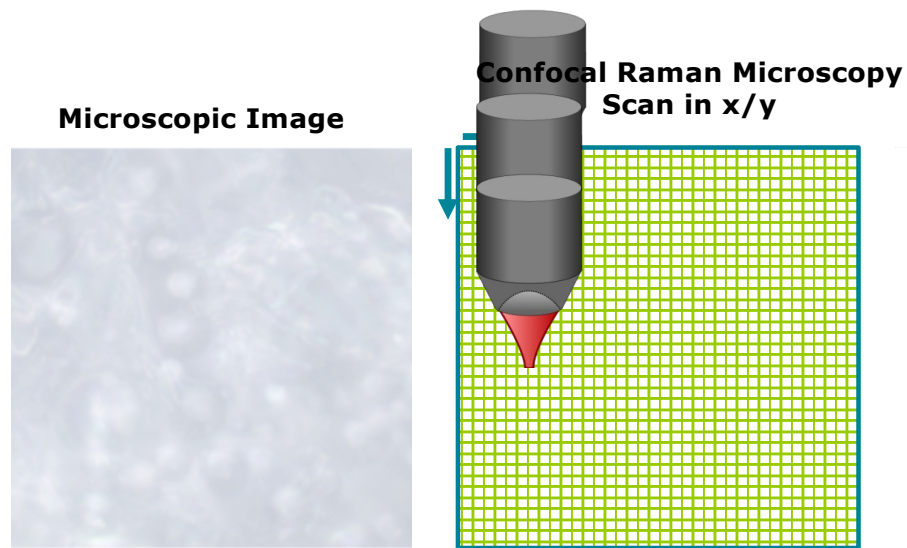


- much smaller background
- 3-D information
- slightly higher resolution possible

spatial, but NO chemical information

Confocal Raman imaging (2D)

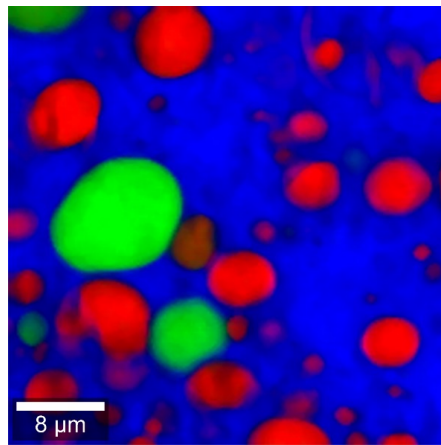
- Example: Emulsion (hand cream with urea)



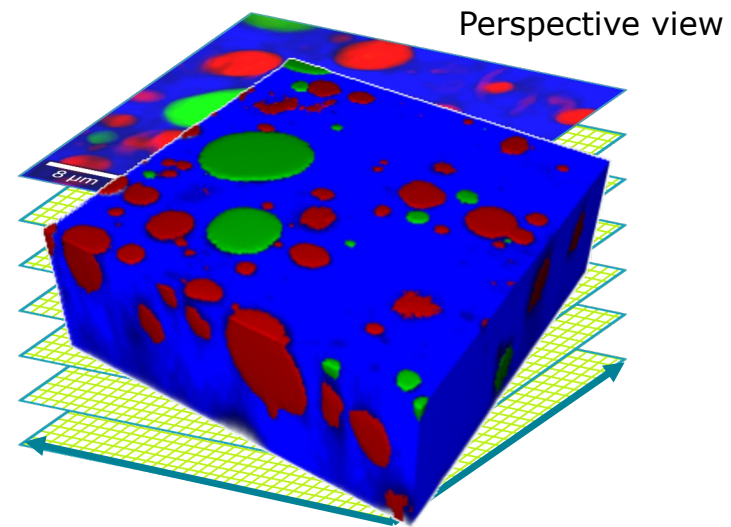
Chemical
information

Chemical
information
& spatial
resolution

Raman imaging – 3D



2D Raman image

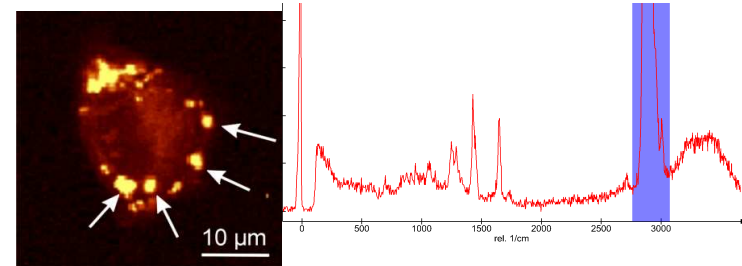


3D Raman image

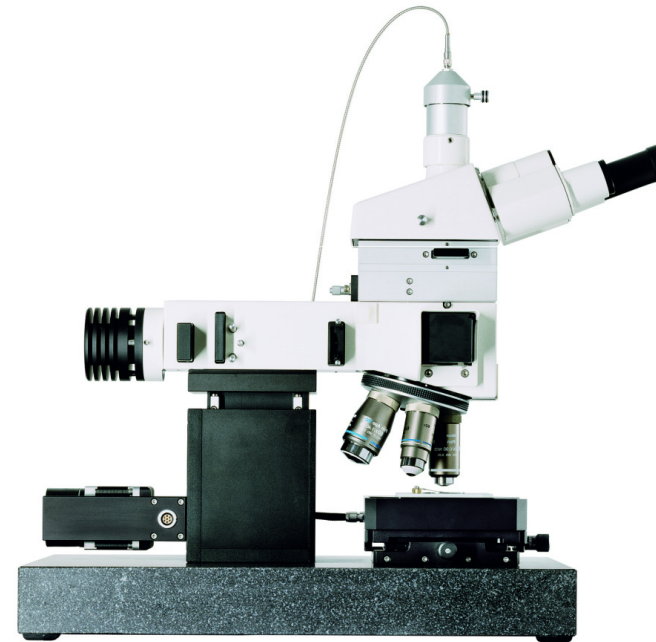
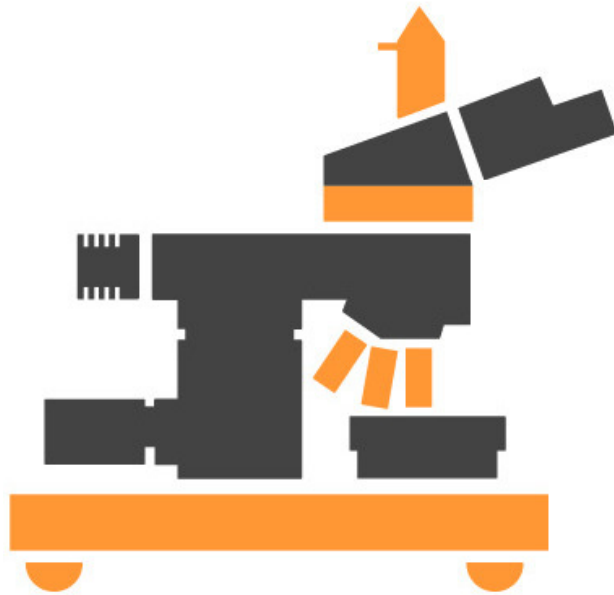
Microscopio Confocal + Espectroscopia Raman

3-D Imaging with chemical sensitivity

- take a complete Raman spectrum at every image pixel
- extract the relevant information (peak intensity, width, position etc.)
- display this information as image

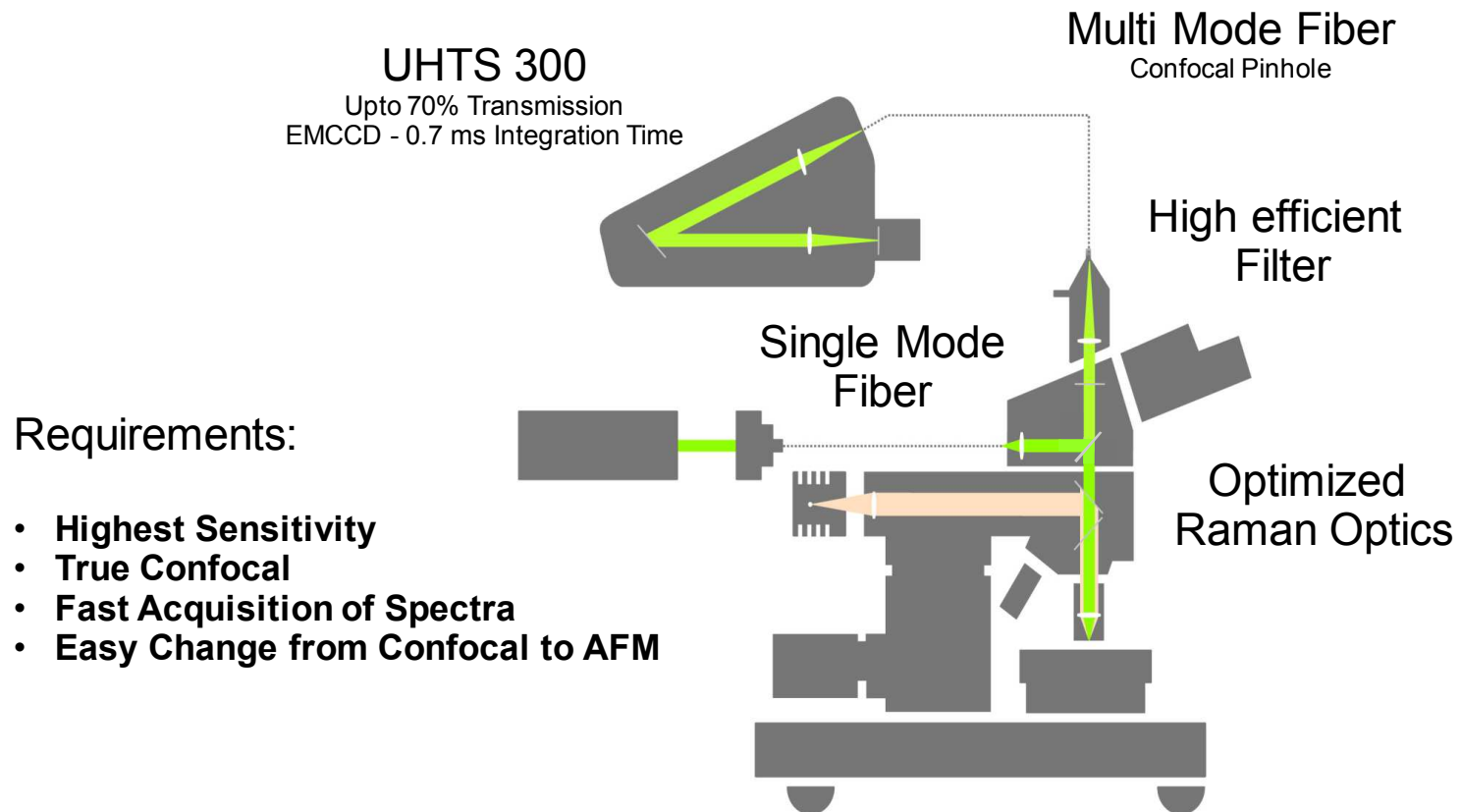


3. Microscopio Confocal + Espectrómetro Raman



Imaging with chemical sensitivity

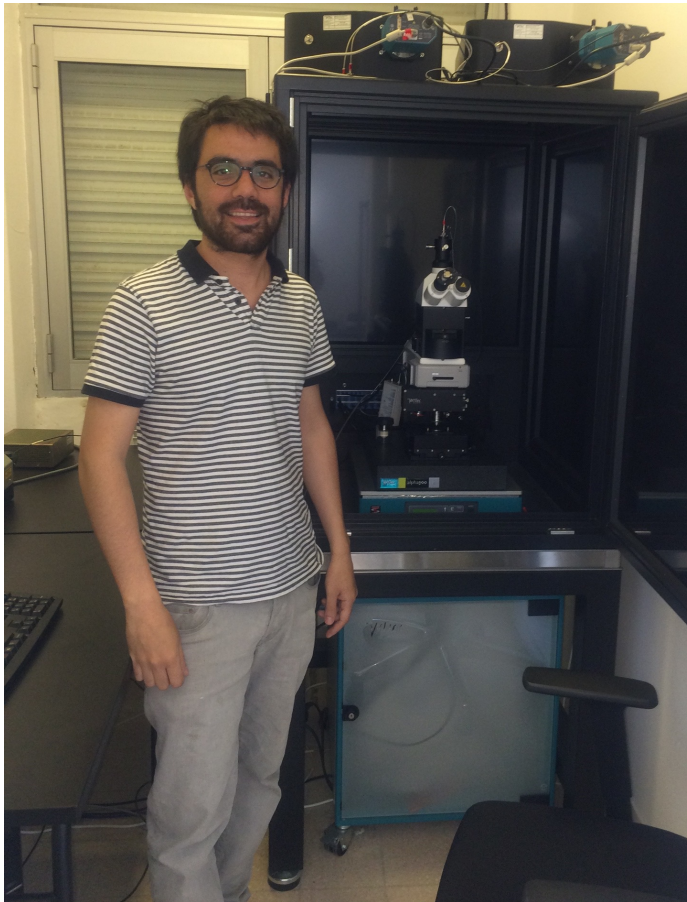
Esquema



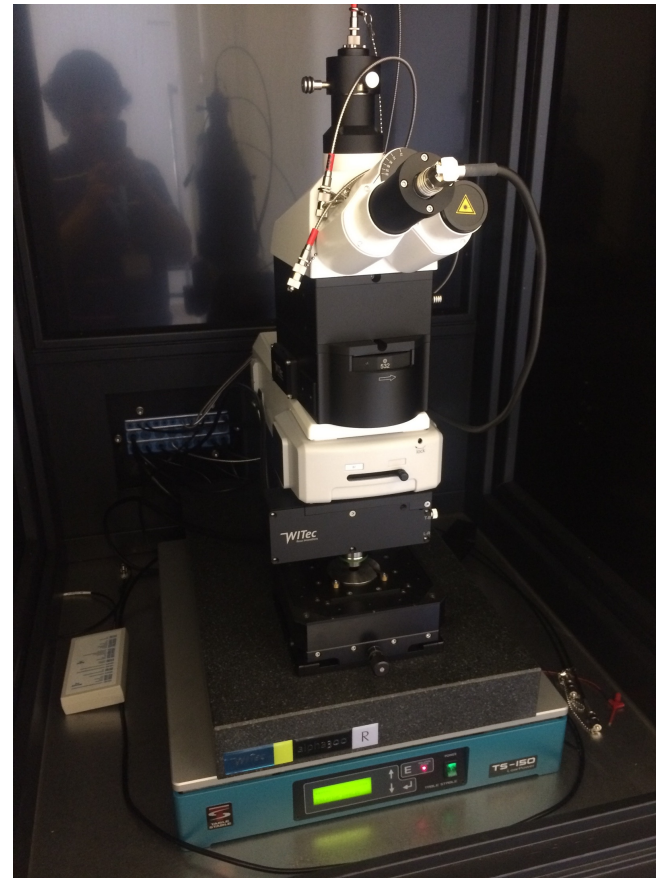
Características de la Técnica

- ❑ No Destructivo
- ❑ Identificación Química Directa
- ❑ Identificación de Propiedades de Materiales
- ❑ Fácil o nula preparación de muestra
- ❑ Sólidos cristalinos, sólidos amorfos y Líquidos
- ❑ Detección 3-dimensional
- ❑ Variación de Temperatura (Linkam)
- ❑ Combinación de detección química con AFM (EFM & MFM)

Disposición

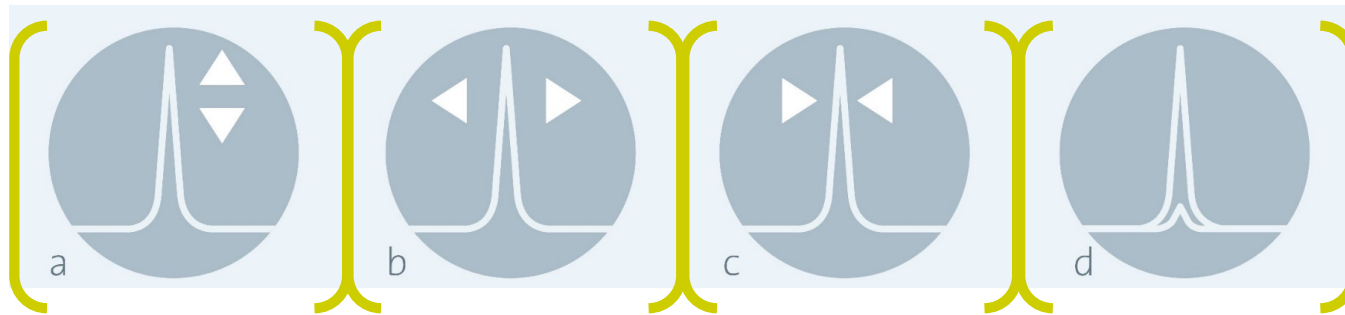


Octubre de 2023



28

Información Adicional:



**Ident.
Química**



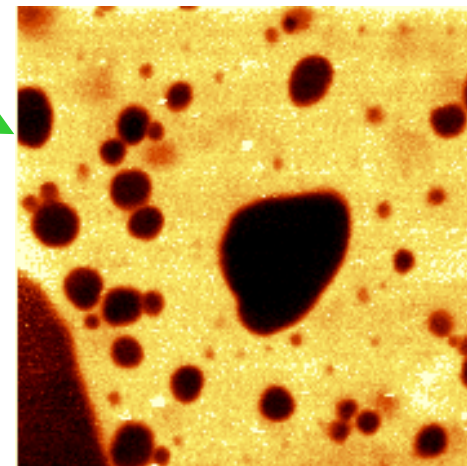
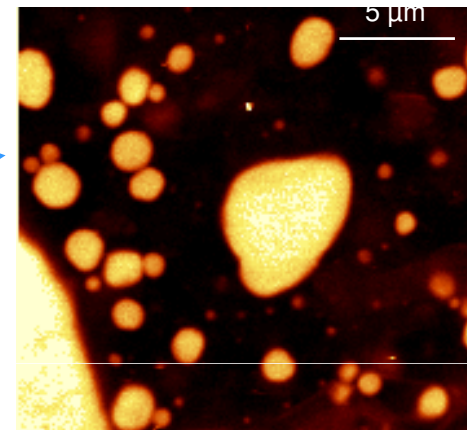
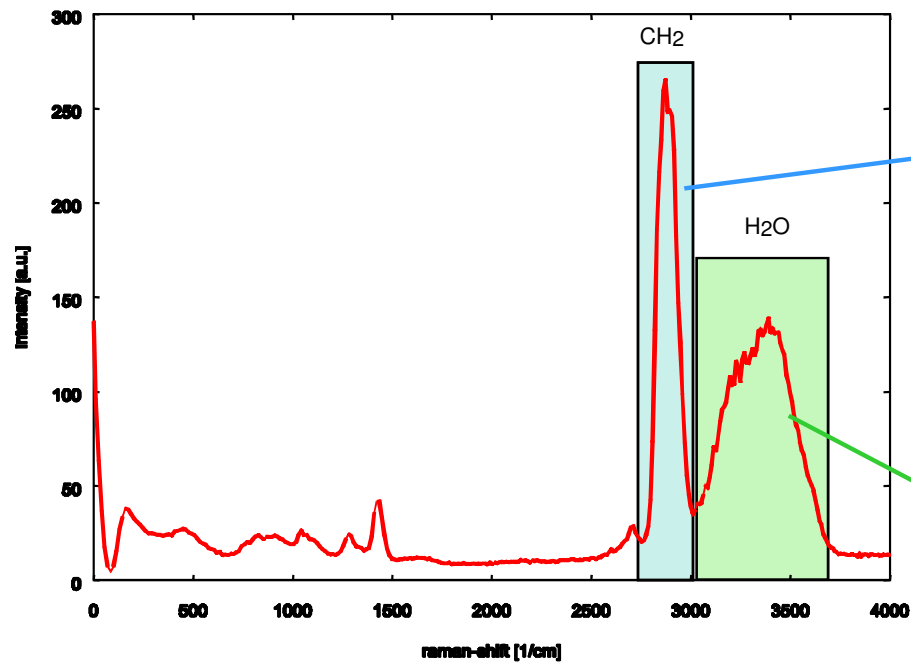
- a) Intensidad de pico: proporcional a concentración
- b) Corrimiento de pico: Identificación de stress y strain.
- c) Ancho de pico: Degree of crystallinity
- d) Polarización: simetría cristalina y orientación

Aplicaciones



Microscopia Raman Confocal

Imagen Raman: dispersión agua-aceite



Body lotion, imaging parameters:

2 mW excitation @ 532nm, 100x oil, NA=1,25

20 x 20 μm, 180 x 180 pixels (= 32400 spectra)

50ms/spectrum

Octubre de 2023

Reconstrucción Imagen Raman 3D

Sample: Oil/Water Emulsion

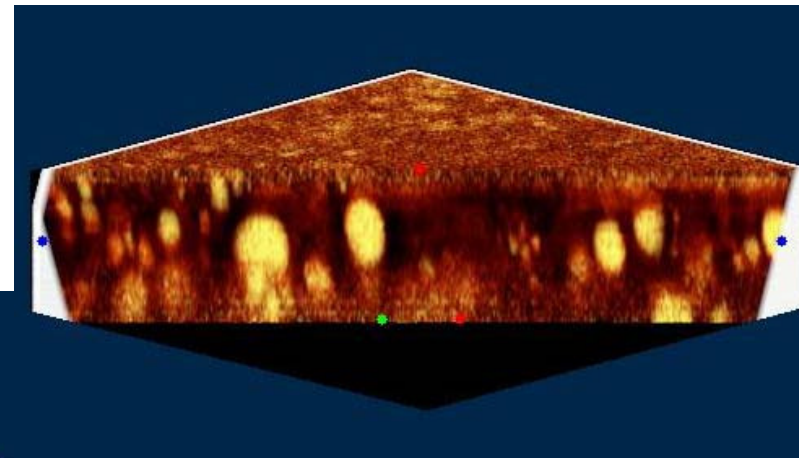
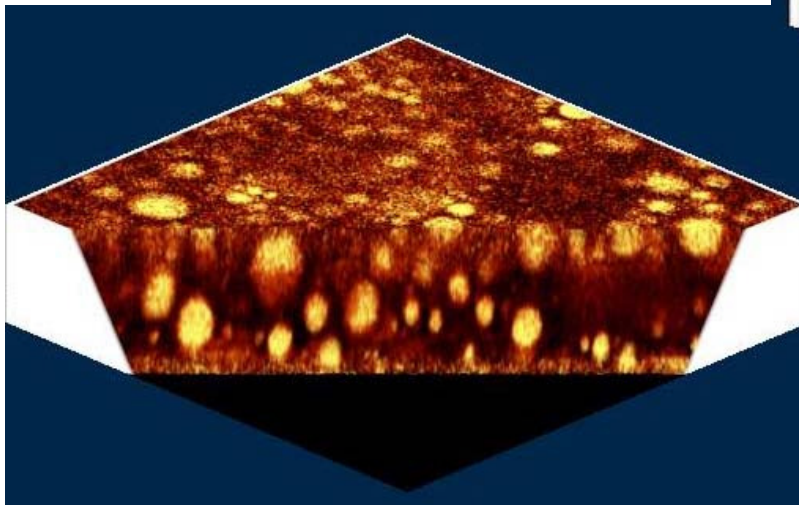
Evaluated Band: CH-Stretching

Excitation: 532nm

Scan Range XY: 100x100 μm

Scan Range Z: 30 μm /32 layers

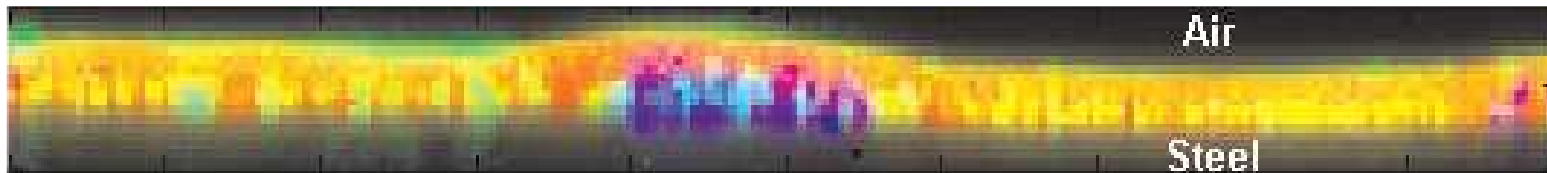
3-D Reconstruction



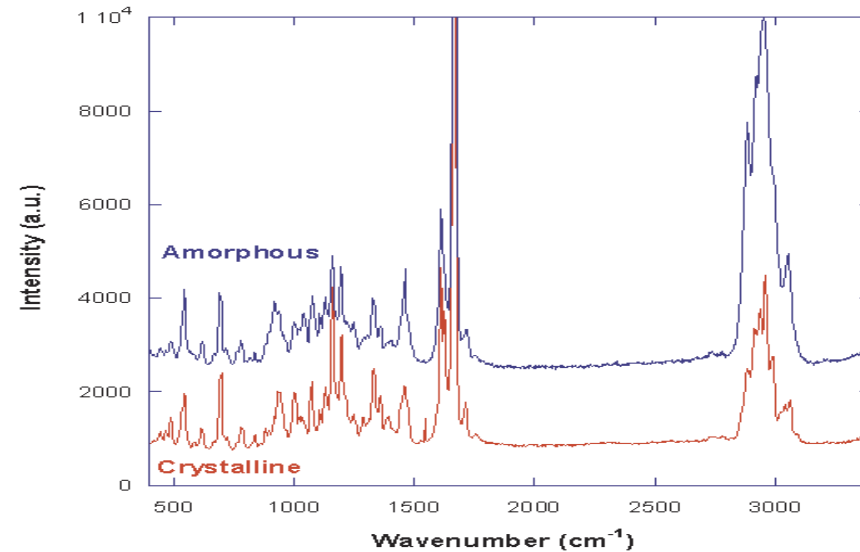
Total number of Spectra: 517 500

Total Acquisition Time: 23 min

Stents para Drug Delivery



Raman cross-sectional image
of a coating of a drug plus
polymer on a stent.
Polymer (red),
amorphous drug (green),
crystalline drug (blue),
polymer + amorphous drug (yellow).
100x 10 microns scan.



Geociencias

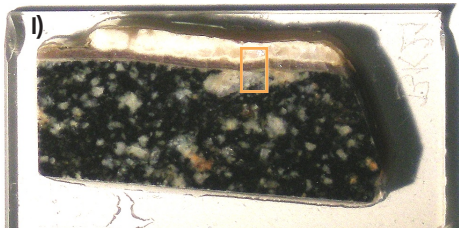


Fig. 1: I) Orange rectangle indicates scan position on polished rock section.

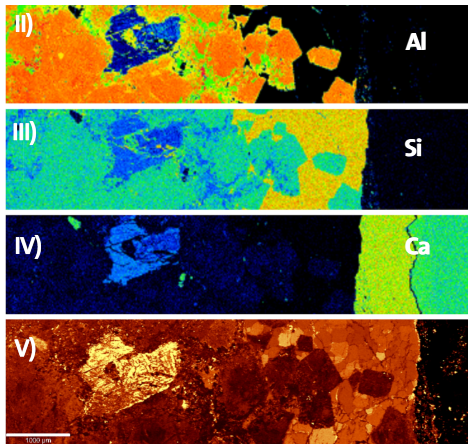


Fig. 1: II-IV) EMP maps showing elemental distribution; V) corresponding Raman image, integrated intensity 140-300 rel.1/cm.

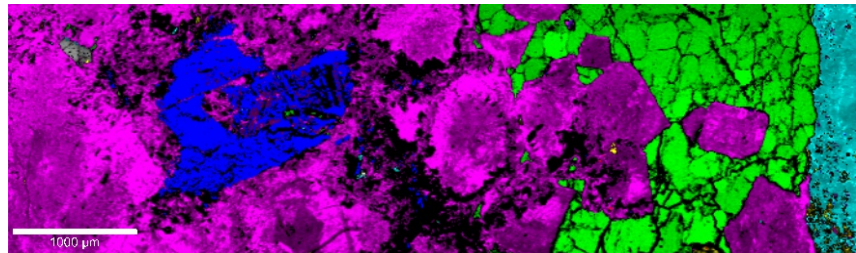
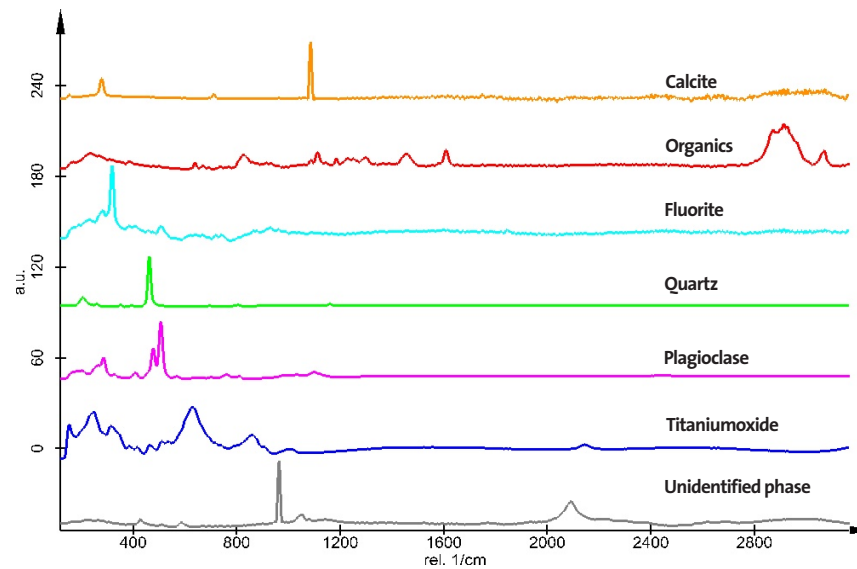
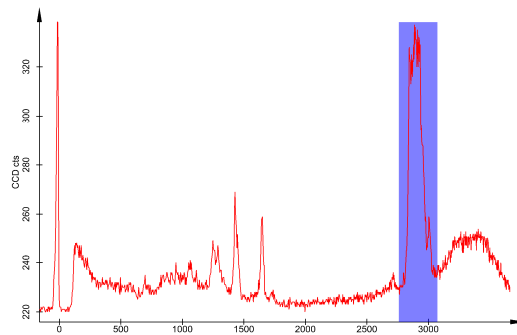
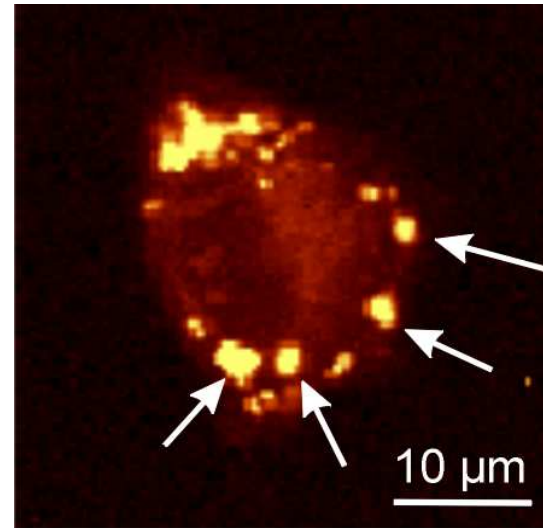
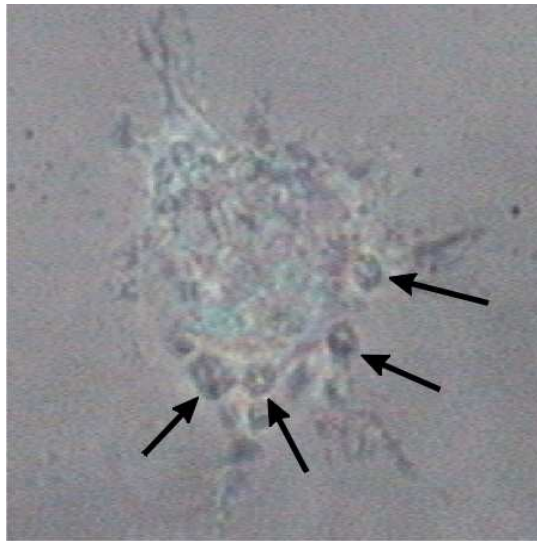


Fig. 2: I) Combined false color image and the corresponding color-coded spectra.



Células epiteliales en Buffer

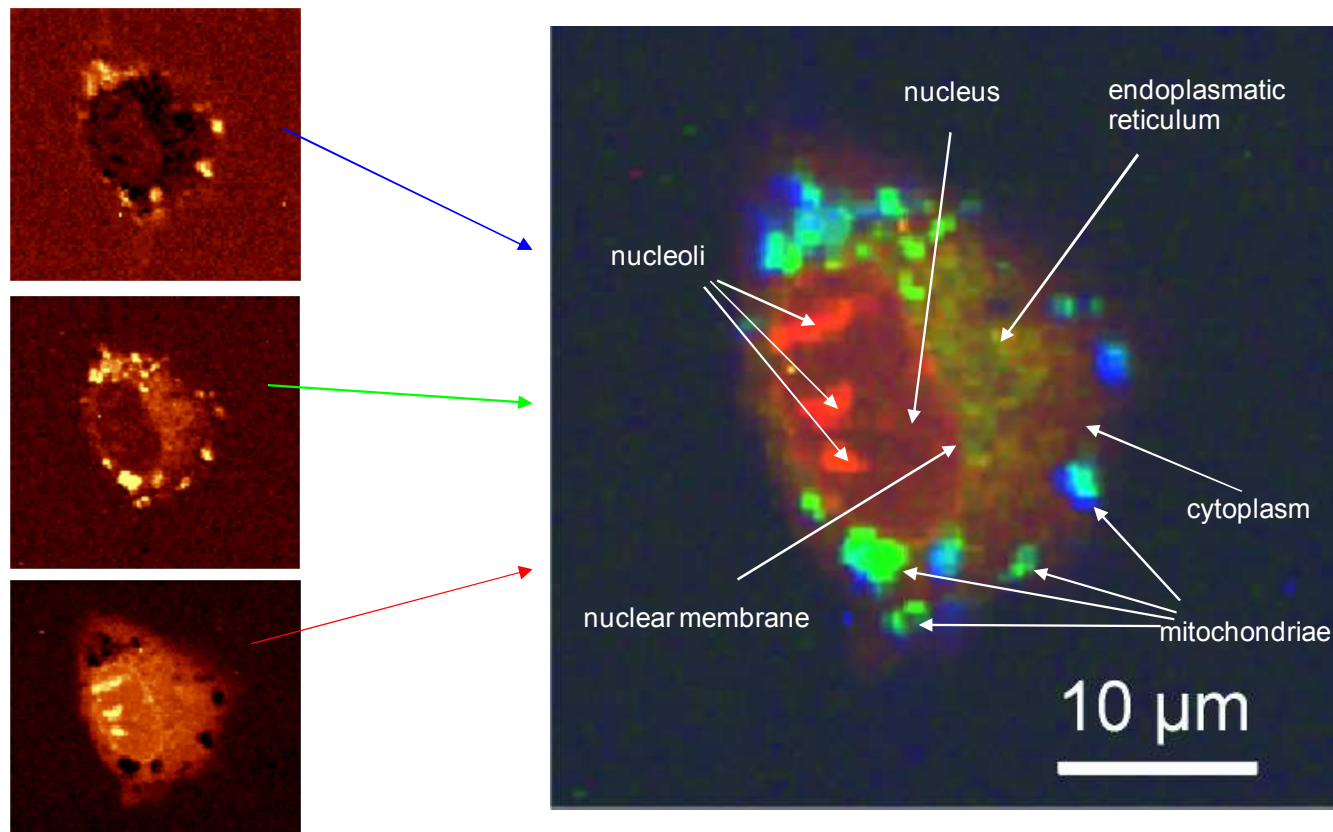


Intensity in the CH 2 stretching band
(2800 - 3000 / cm)

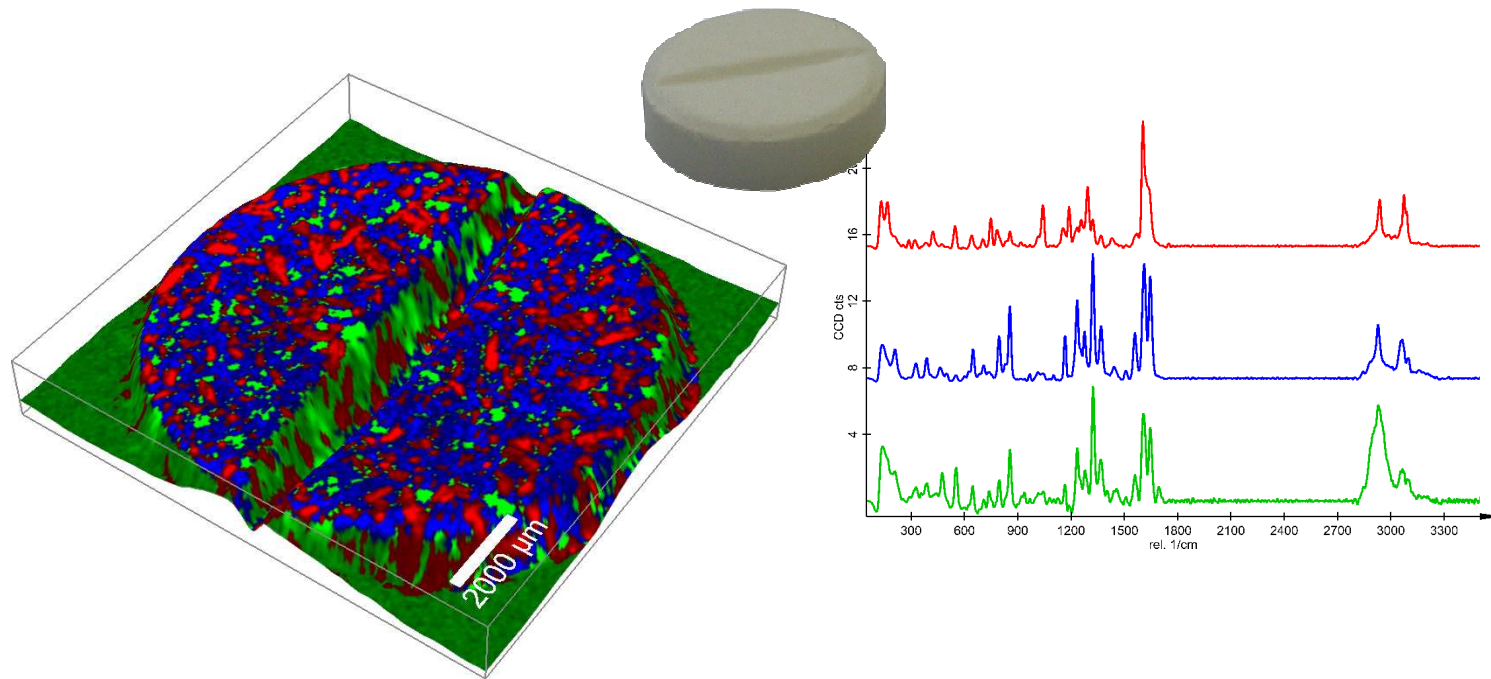
100 x 100 pixel = 10000 spectra
objective: 60x, NA = 1.0 water
532 nm excitation, 5 mW
100 ms / spectrum => 17 min.

Octubre de 2023

Imagen reconstruida



Tabletas: API y Excipientes

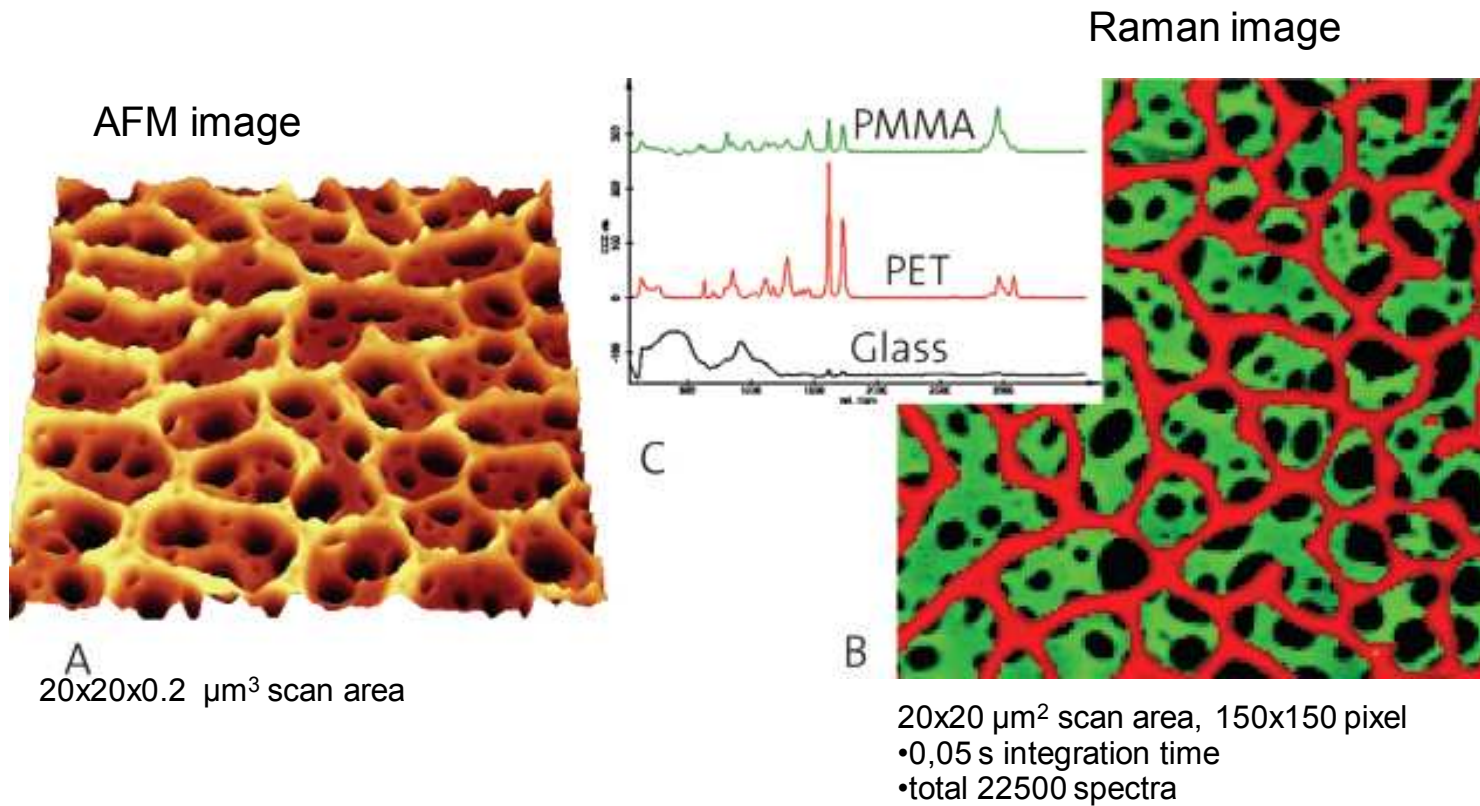


Confocal Raman Image overlaid
on the topography

Corresponding Spectra
Red & Blue : API
Green : Excipient

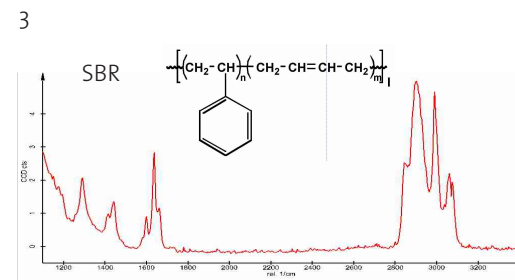
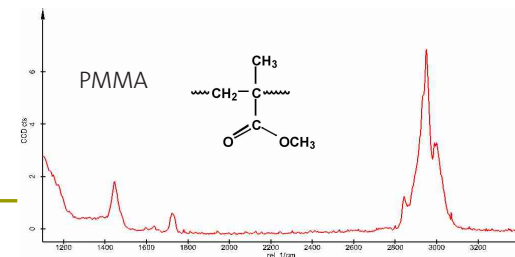
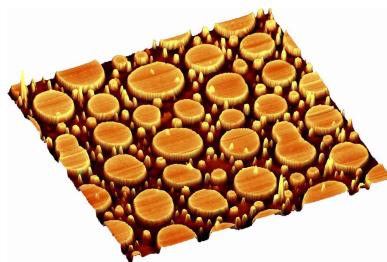
Polímeros

Thin Film of PMMA - PET



Blend PMMA+SBR

Fig. 5: Overview AFM image of a PMMA-SBR blend.



Octubre de 2023

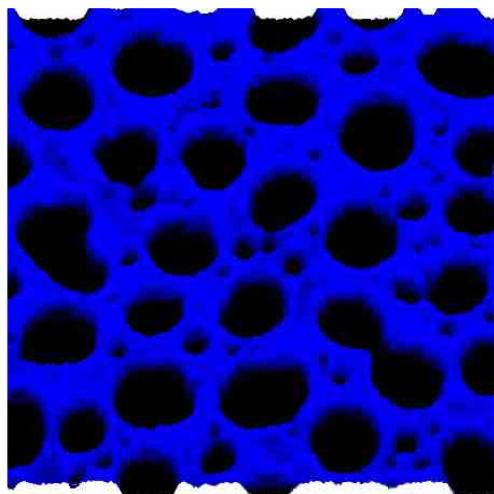


Fig. 9: Color coded Raman image of SBR resulting from the integral intensity of each spectrum.

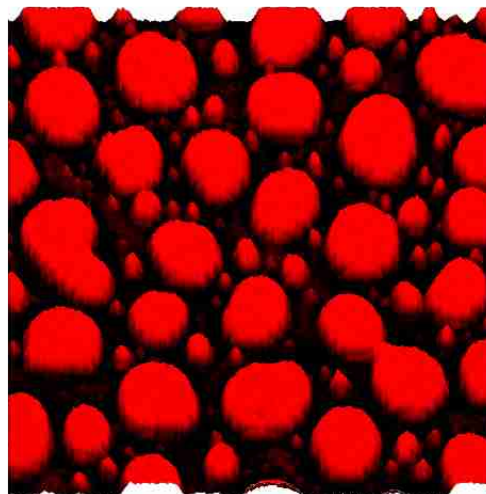


Fig. 10: Color coded Raman image of PMMA resulting from the integral intensity of each spectrum.

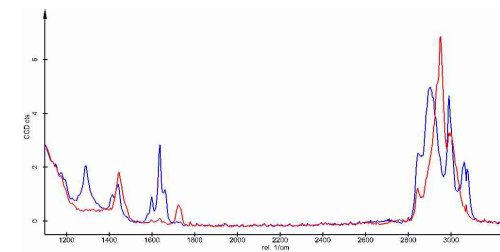


Fig. 11: Corresponding spectra: SBR (blue) and PMMA (red).

Alime

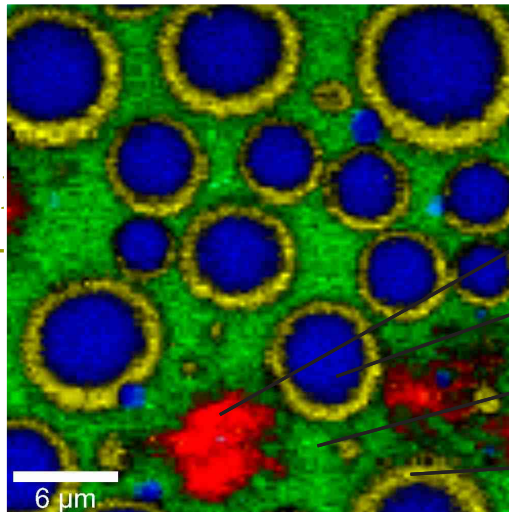


Fig. 2a: Raman Image of an emulsion used in food production. Scan range: 30 μm x 30 μm, 120x120 pixels (=14400 spectra, 532 Nd:Yag Laser for excitation, 100 x oil objective (NA=1.25)). Red + green: fatty matrix, blue: aqueous phase, yellow: emulsifier PGPR

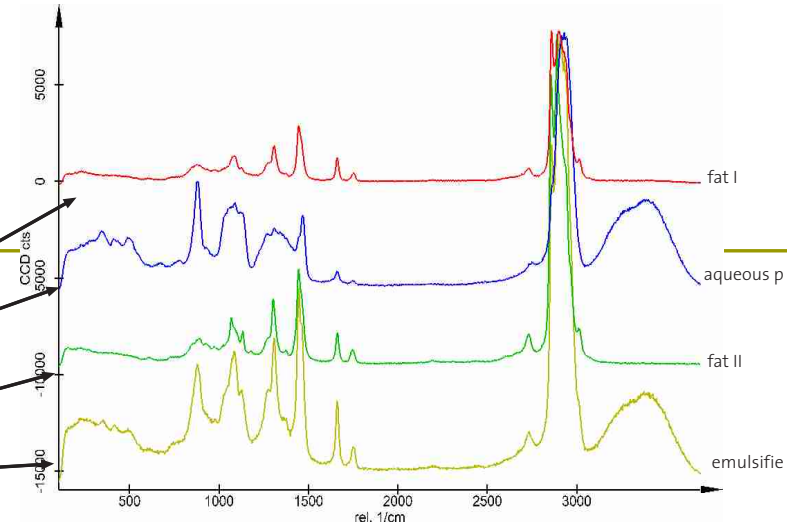
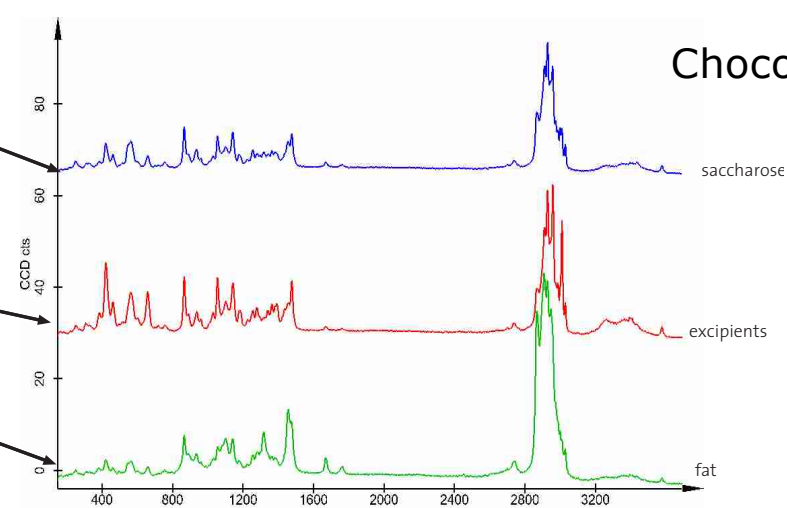
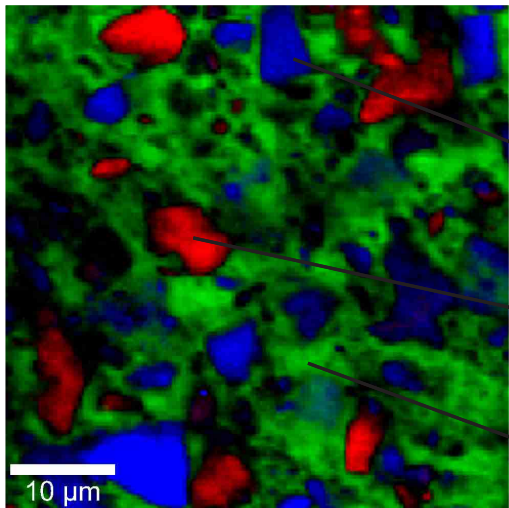


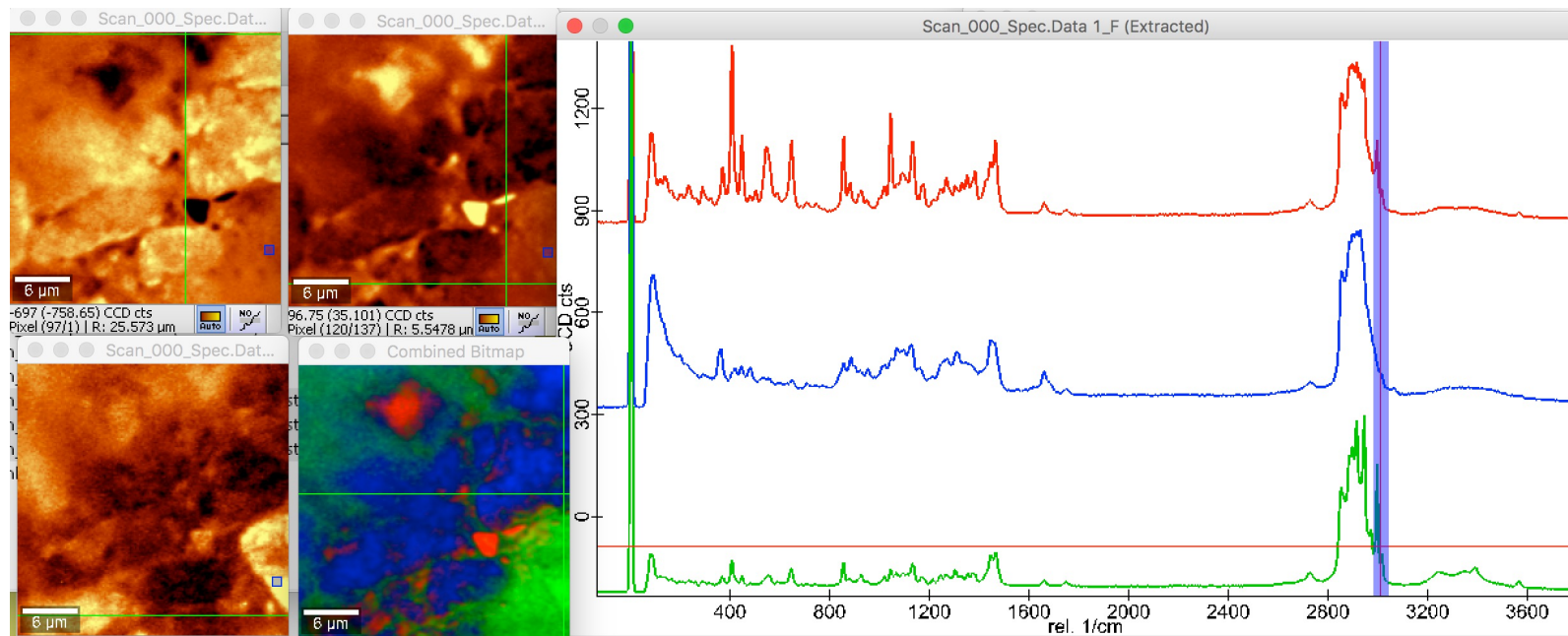
Fig. 2b: Corresponding spectra of the compounds contained within the emulsion.



Chocolate blanco

Chocolate Blanco (toblerone)

▣ Sacharose, Maltose and Fats



Experiencias

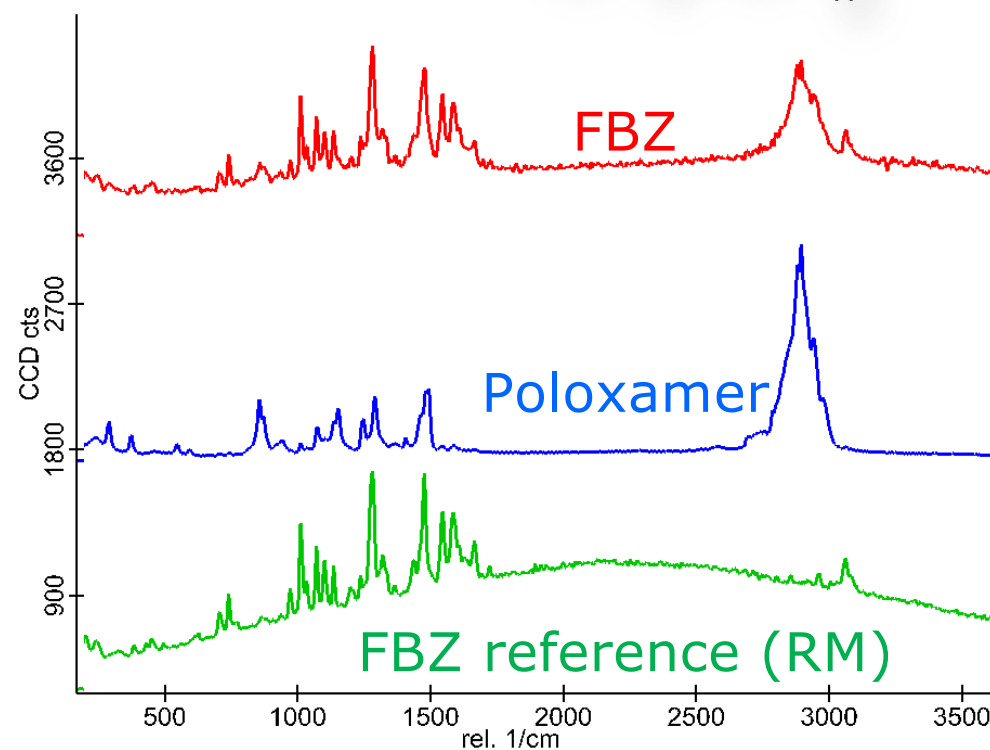
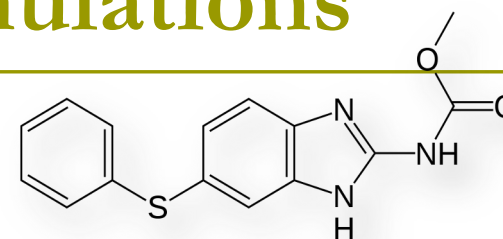


WITec alpha300RA

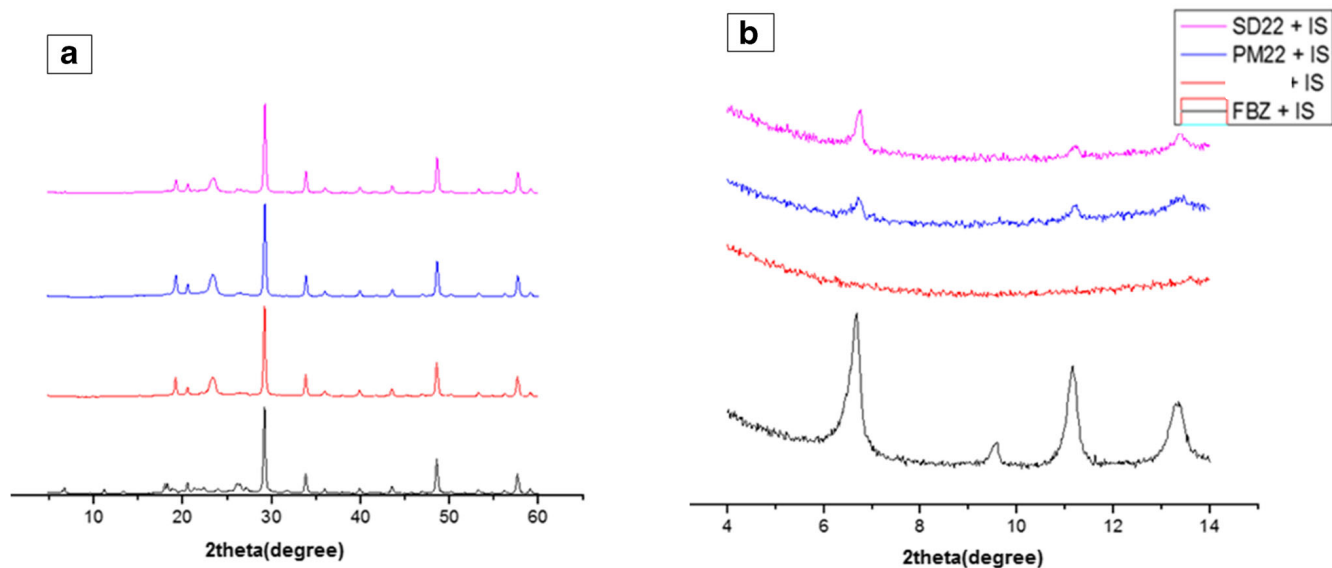
Example 1: Pharmaceutical Formulations

- ❑ Preparation of solid dispersions
- ❑ Formulation of Fenbendazole (FBZ) + Poloxamer (excipient)
 - API: FBZ (Raw Material, RM)
 - Excipient: Poloxamer
- ❑ Evaluation:
 - Physical Mixture (PM)
 - ❑ Grinding of Drug+Polymer

Fenbendazole (FBZ)

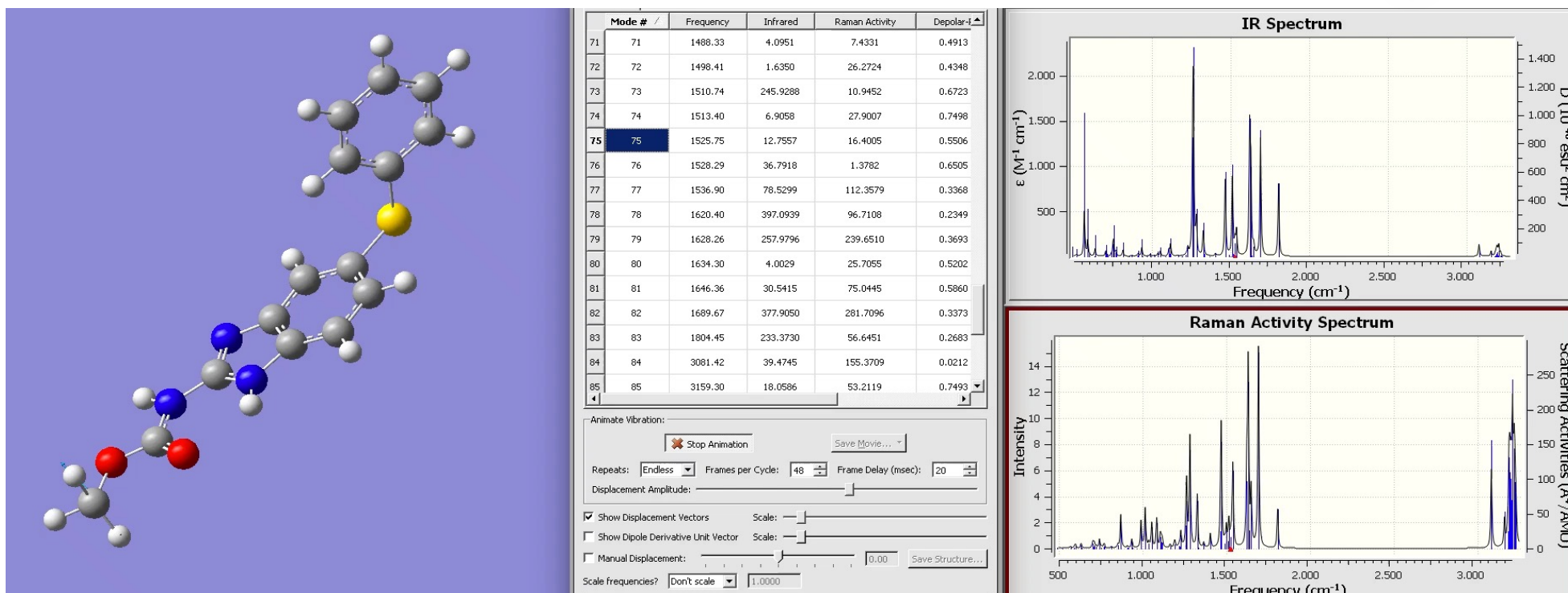
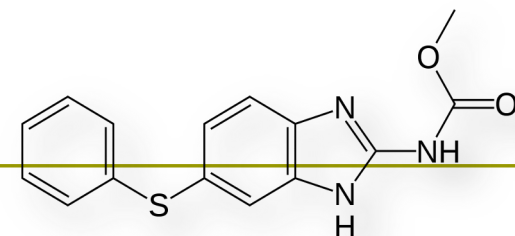


Example 1: Pharm. Formulations

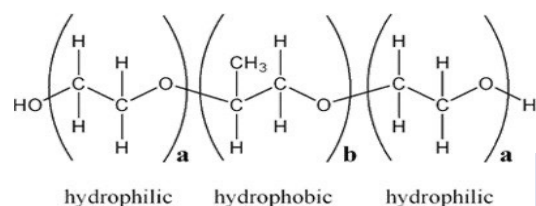


- XRPD: Internal standard for crystallinity determination
- The degree of crystallinity of FBZ remains equal.
- The degree of crystallinity of poloxamer become reduced for SD in comparison to PM.

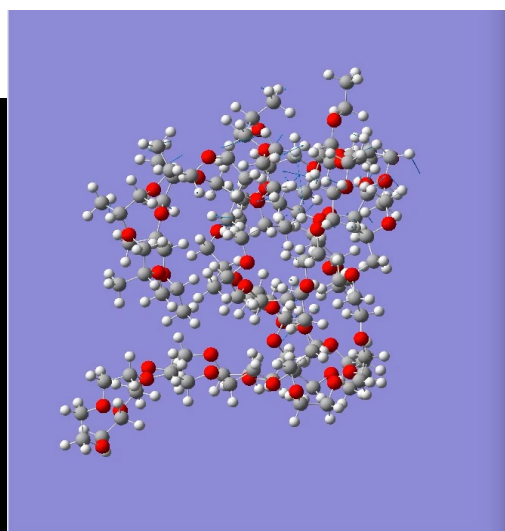
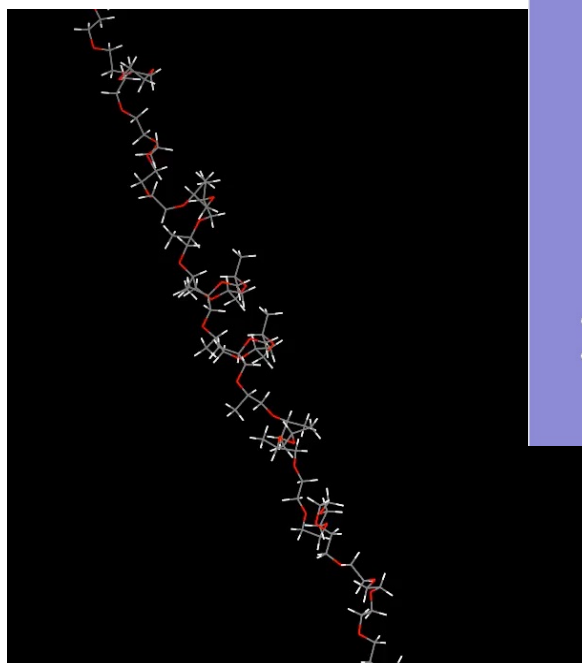
Example 1: FBZ Simulation



Example 1: Poloxamer P184

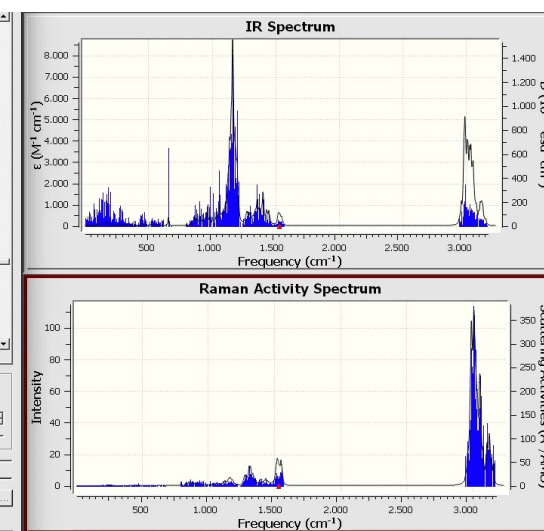


Classical MD → Structural Optimization ab initio Calculations
→ Phonons and polarizabilities determination



Mode #	Frequency	Infrared	Raman Activity	Dipole
910	1531.73	2.5095	21.6991	0.731
911	1533.41	4.4399	14.3750	0.746
912	1533.54	15.6748	18.5910	0.734
913	1534.14	4.0442	10.9234	0.743
914	1534.85	8.9271	18.0199	0.722
915	1535.13	8.2443	3.7176	0.700
916	1535.28	2.3846	9.3043	0.694
917	1536.93	11.6751	7.7106	0.756
918	1537.16	6.4488	14.3888	0.743
919	1537.67	3.8143	18.7628	0.725
920	1537.80	14.7864	6.7754	0.641
921	1537.93	0.2661	2.1380	0.705
922	1538.18	3.1835	8.7477	0.712
923	1538.40	9.8712	6.1394	0.756
924	1538.88	13.3642	9.3387	0.743

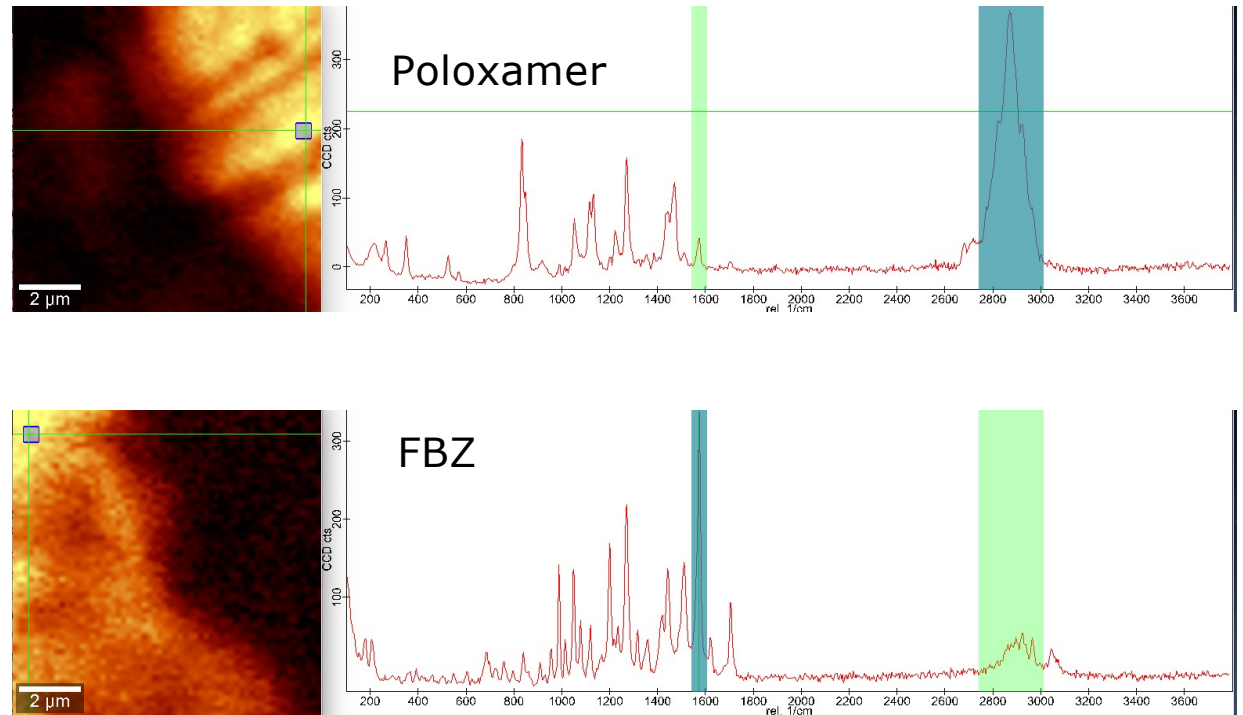
☐ Animate Vibration
☒ Stop Animation
 Repeats: Frames per Cycle: Frame Delay (msec):
 Displacement Amplitude:
☒ Show Displacement Vectors Scale:
☐ Show Dipole Derivative Unit Vector Scale:
☐ Manual Displacement:
 Scale frequencies?



poly(ethylene oxide)/poly(propylene
oxide)/poly(ethylene oxide)
Molecular Dynamics Simulation

Example 1: PM colocalization of FBZ

- **Physical Mixture (PM)**
- Very well defined regions of drug and excipient in the Physical Mixture (PM).
- Very poor dispersion.



Example 1: SD colocalization of FBZ

- **Solid Dispersion (SD)**
- Reduced Particle size for FBZ in the SD
- Better dispersion of FBZ.

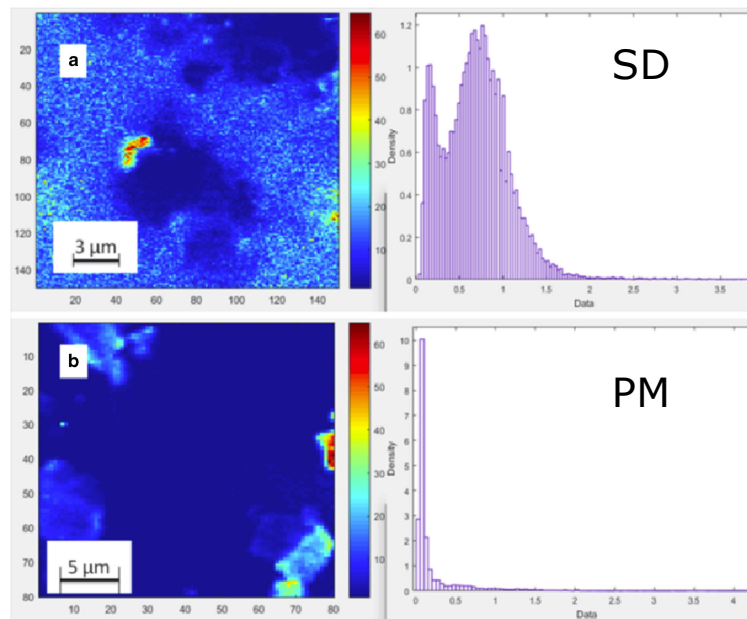
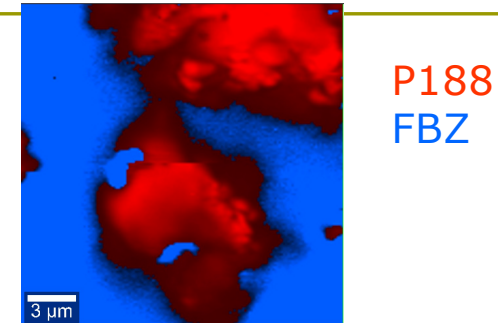


Fig. 7 XY confocal Raman microscopy mapping of FBZ/P407 SD22 (a) and PM22 (b); their corresponding histograms are shown on the right. Scale bars indicate the intensity ratio I_{1590}/I_{866} . All the images include the number of the grid points in X and Y axis, and the corresponding bar length

Problems with point-to-point variations (surface planarity).

- Data reduction using Intesity ratio plot & distribution:
- I_{1590}/I_{866}

Example 1: Conclusions

- ❑ PM: poor distribution of FBZ and Poloxamer.
- ❑ SD: better distribution of FBZ, with reduced particle size.
- ❑ FBZ keeps crystallinity.
- ❑ Poloxamer reduces its crystallinity when going from PM to SD

Journal of Pharmaceutical Innovation
<https://doi.org/10.1007/s12247-017-9306-9>

ORIGINAL ARTICLE



The Impact of Solid Dispersion on Formulation, Using Confocal Micro Raman Spectroscopy as Tool to Probe Distribution of Components

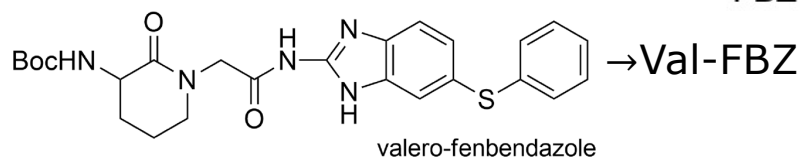
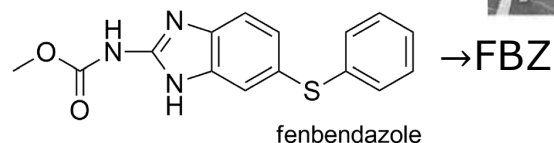
M. Elisa Melian¹ • A. Beatriz Munguía¹ • Ricardo Faccio² • Santiago Palma³ • Laura Domínguez¹



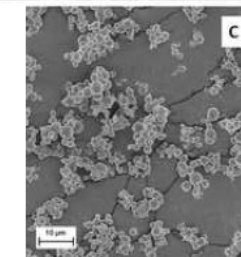
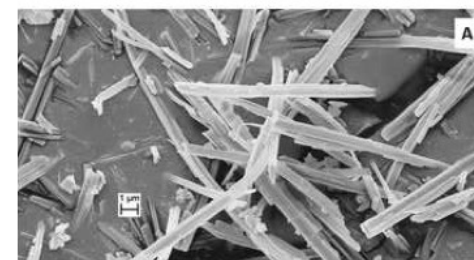
Example 2: Self dispersive Nanocrystals

□ Formulation of API + Poloxamer

- API: FBZ and Val-FBZ
- Excipient: Poloxamer
- Preparation of Nanocrystals
 - Wet Milling
 - Spray Drying



FBZ



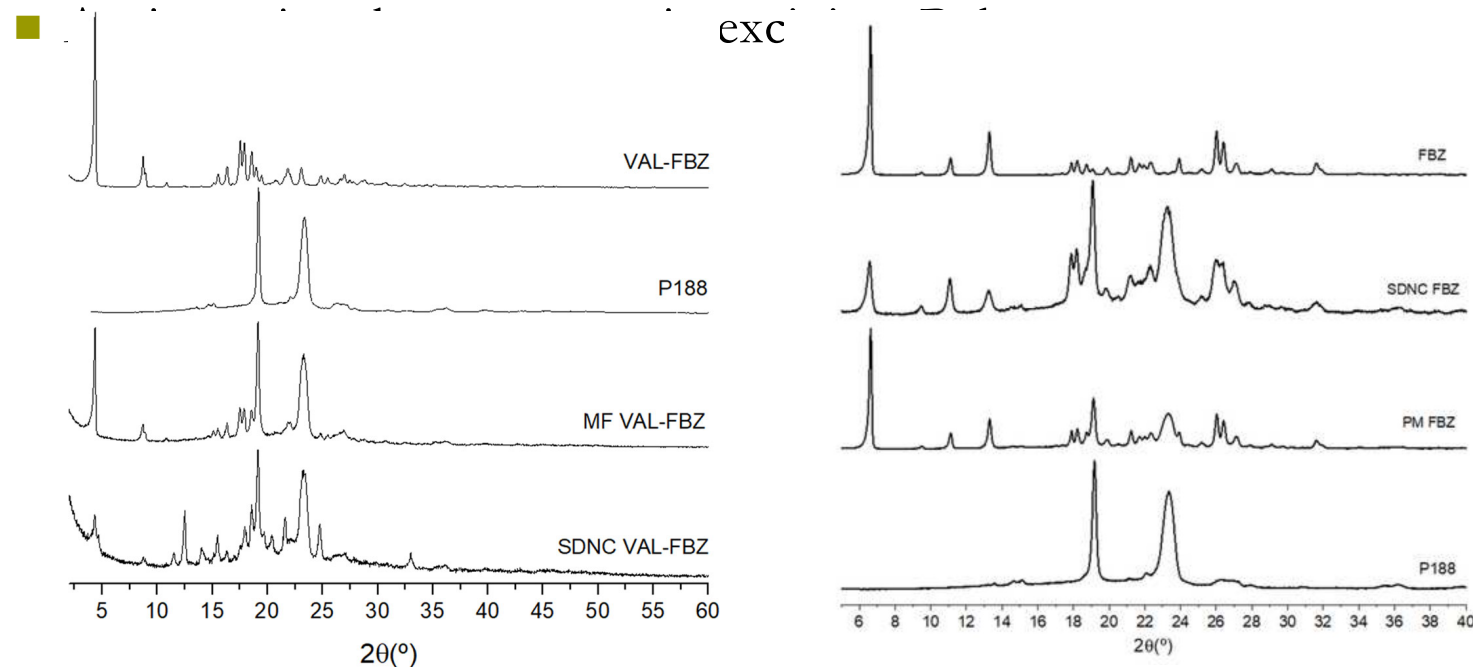
□ Evaluation:

- Performance of
- Solid Dispersion (SD) vs Nanocrystals (NC)

Example 2: Pharm. Formulations

□ XRD:

- No major changes upon treatment.
- FBZ and Val-FBZ keeps its crystallinity



Example 2: Raman for PM vs NC

□ API:

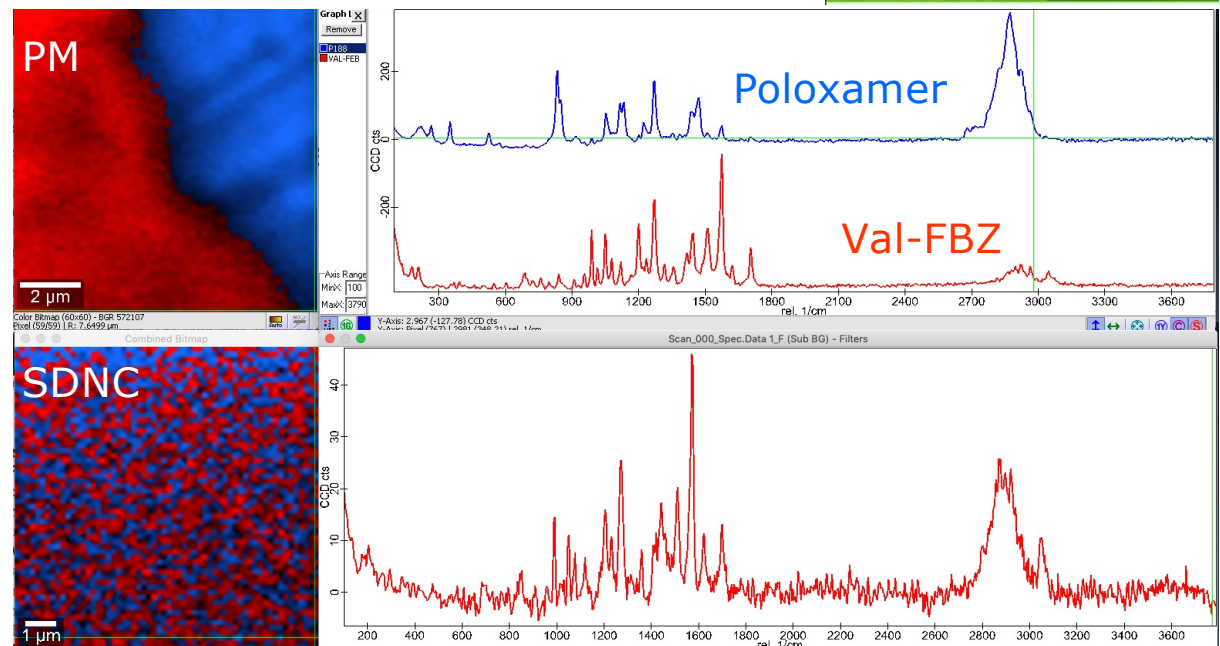
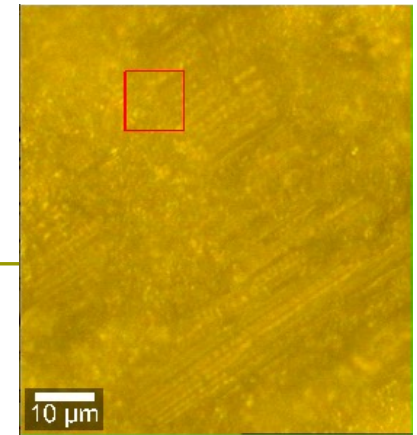
- Synthetic Drug
 - Val-FBZ

□ PM:

- Well defined regions for API and Excipient.

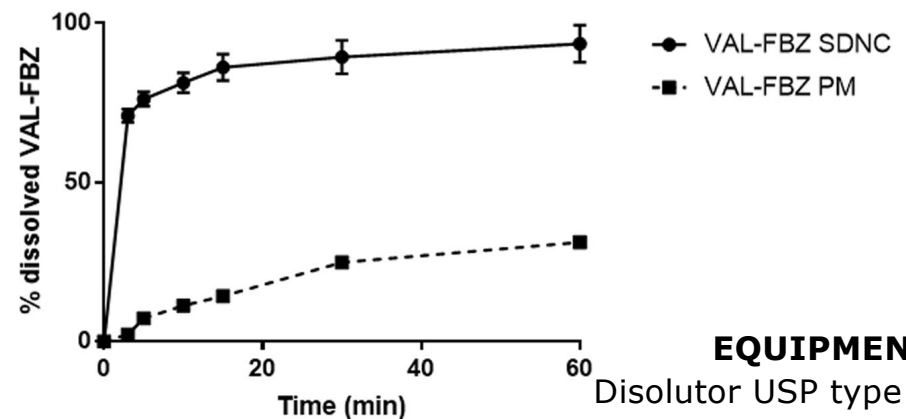
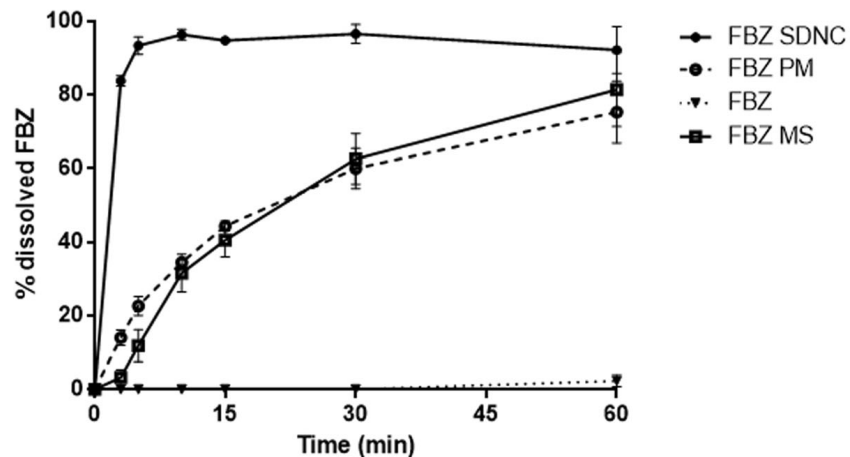
□ SDNC:

- Very good distribution and homogeneity.
- It is not possible to differentiate them (at the optical limit).



Example 2: Raman for PM vs NC

□ Dissolution Profile (in-vitro)



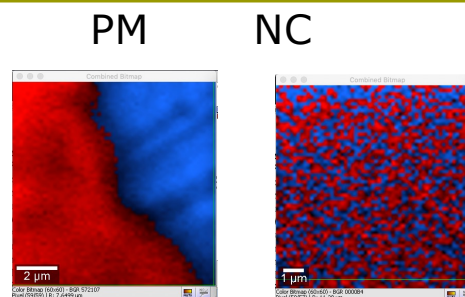
- Markedly increased dissolution rate compared with the simple mixture of the components.

EQUIPMENT
Disolutor USP type 2
SOLVENT
900 mL of HCl 0.1 N
TEMP.
37 ° C
VELOCITY
50 RPM
SAMPLE:
5 mg OF FBZ (SINK)

Example 2: Nanocrystals

□ Conclusions

- We obtained Nanocrystals (NC)
- API's keep their crystallinity
- Poloxamer reduces its crystallinity.
- Better dispersions API/EXC in NC
- Improvement in Dissolution Rate Profile.

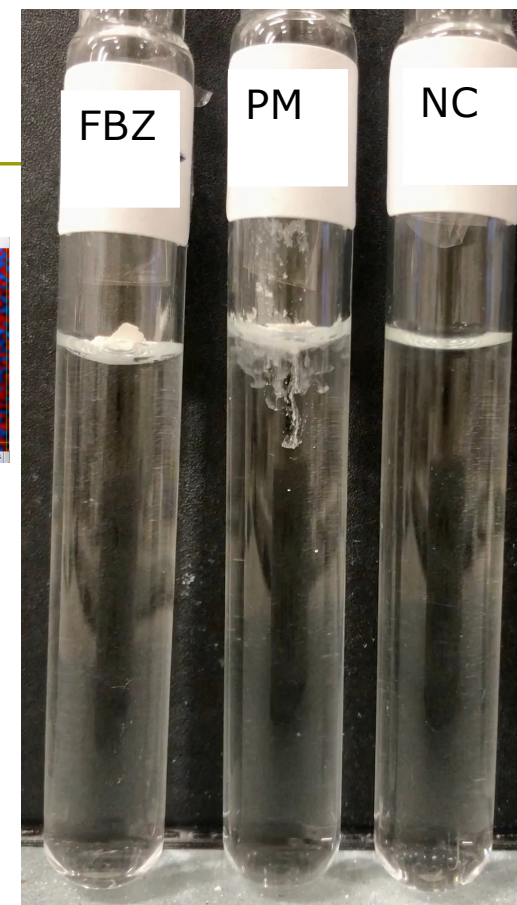


AAPS PharmSciTech (2020) 21:237
DOI: 10.1208/s12249-020-01777-y

Research Article

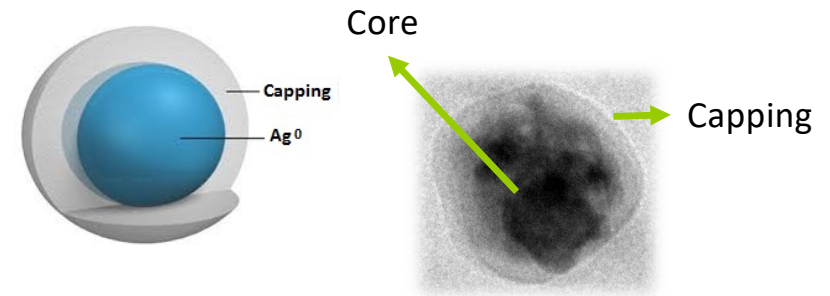
Nanocrystals of Novel Valerolactam-Fenbendazole Hybrid with Improved *in vitro* Dissolution Performance

Maria Elisa Melian,¹ Alejandro Paredes,² Beatriz Munguía,¹ Maximiliano Colobbio,³ Juan Carlos Ramos,³ Ramiro Teixeira,¹ Eduardo Manta,^{3,4} Santiago Palma,² Ricardo Faccio,⁵ and Laura Domínguez^{1,6}

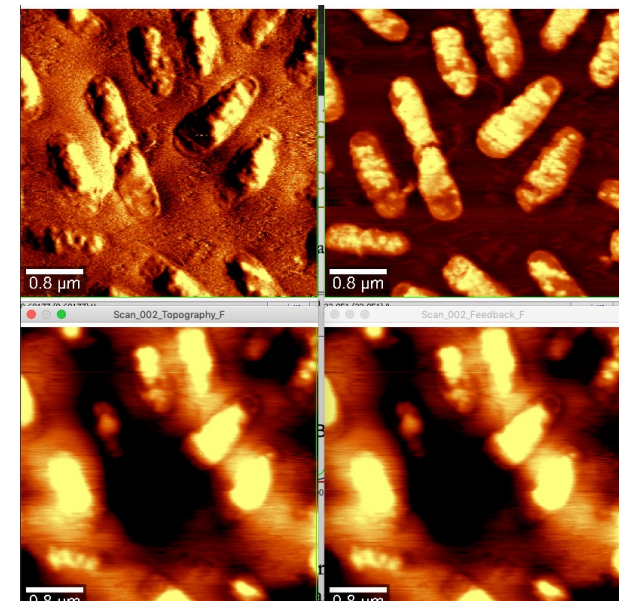


Example 3: Biogenic Synthesis of Ag NP

- Biogenic Synthesis Provides a green route for Ag NP
 - It uses extracellular matrix for chemical reduction of Ag^+ ions.
 - Additionally, provides a capping to the Ag-NP that can present.
 - The capping present difference in the performance of the Ag NP in the biofilm eradication.
- CRM and Ag NP:
 - SERS relevant for the capping characterization
 - SERS relevant for the analysis of the effect of Ag NP on the lysis of bacterias.

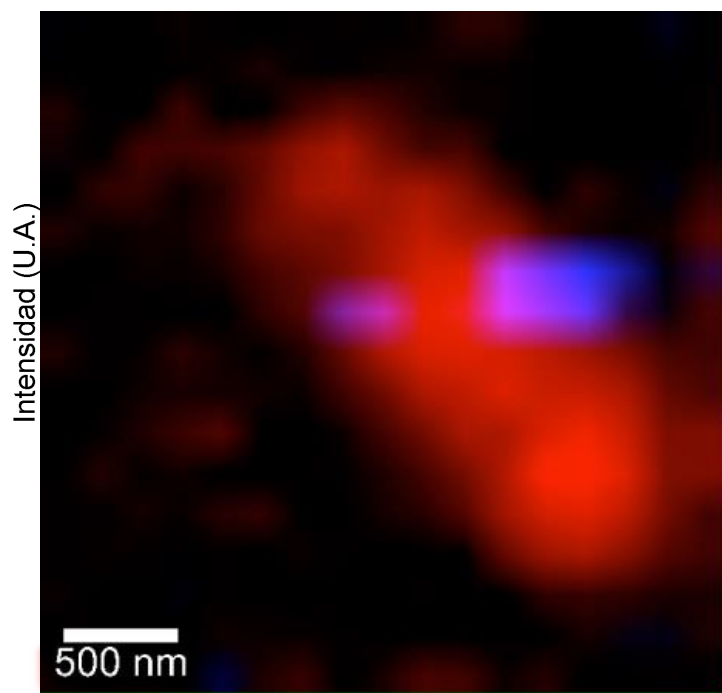
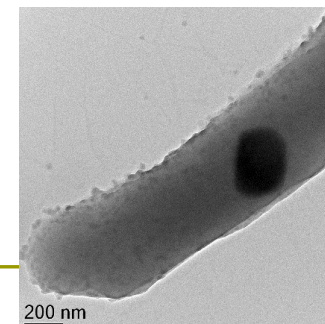


AFM NC Mode



Ag-NPs + *Escherichia coli*.

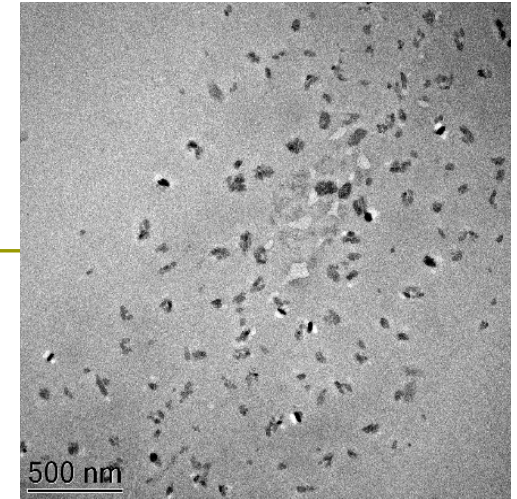
Ag-NPs+ Microorganism: Pch *Escherichia coli*.



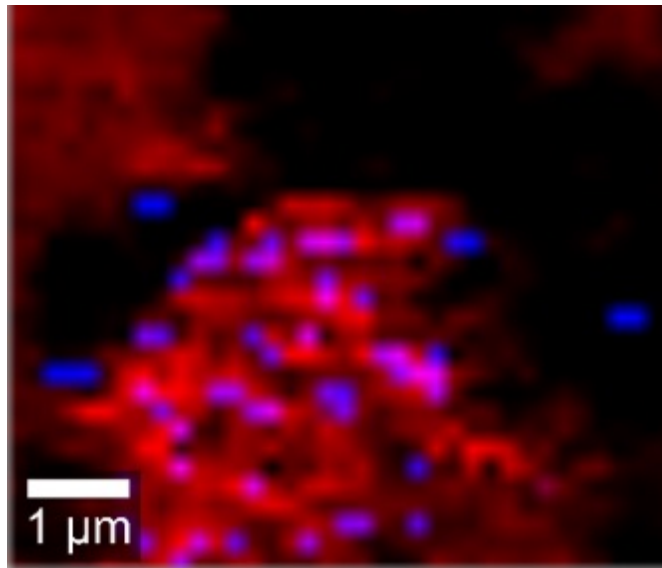
Raman Shift (cm ⁻¹)	assignment	Changes between control and treated cells
624	Nuclei acids	↓
810	Amino acids	↓
982	polysaccharides	=
1186	polysaccharides	↓
1243	polysaccharides	↓
1308	amino acids or nucleic acids	↓
1344	amino acids or fatty acids	=
1593	Fatty acids	↓

Raman assignment from: De Gelder *et al.*, *J Raman Spectroscopy*, 2007, 38(9), 1133-1147

Ag-NPs + *Staphylococcus aureus*



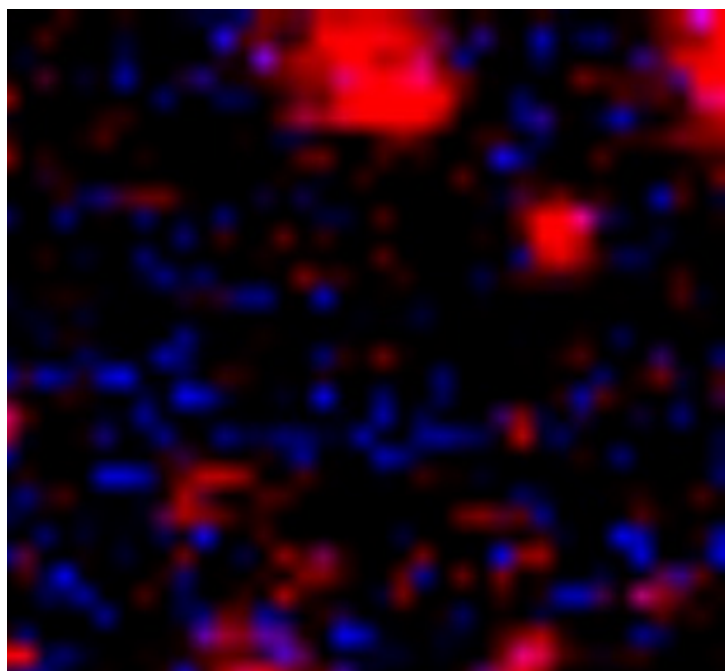
CRM



Raman Shift (cm ⁻¹)	Assignment	Changes between control and treated cells
663	Nuclei acids	↓
790	amino acids	↓
879	amino acids	↓
929	polysaccharides	↓
1116	polysaccharides	↓
1325	amino acids or nucleic acids	↓
1538	Fatty acids	↓

Raman assignment from: De Gelder *et al.*, *J Raman Spectroscopy*, 2007, 38(9), 1133-1147

Example 3: Ag-NPs + *Candida albicans*.



Raman Shift (cm ⁻¹)	Assignment	Changes between control and treated cells
670	nucleic acids	↓
788	Amino acids	↓
885	Amino acids	↓
999	polysaccharides	↓
1100	polysaccharides	↓
1319	amino acids or nucleic acids	↓
1596	fatty acids	↓

Raman assignment from: De Gelder *et al.*, *J Raman Spectroscopy*, 2007, 38(9), 1133-1147

Example 3: Microbial cells + AgNPs

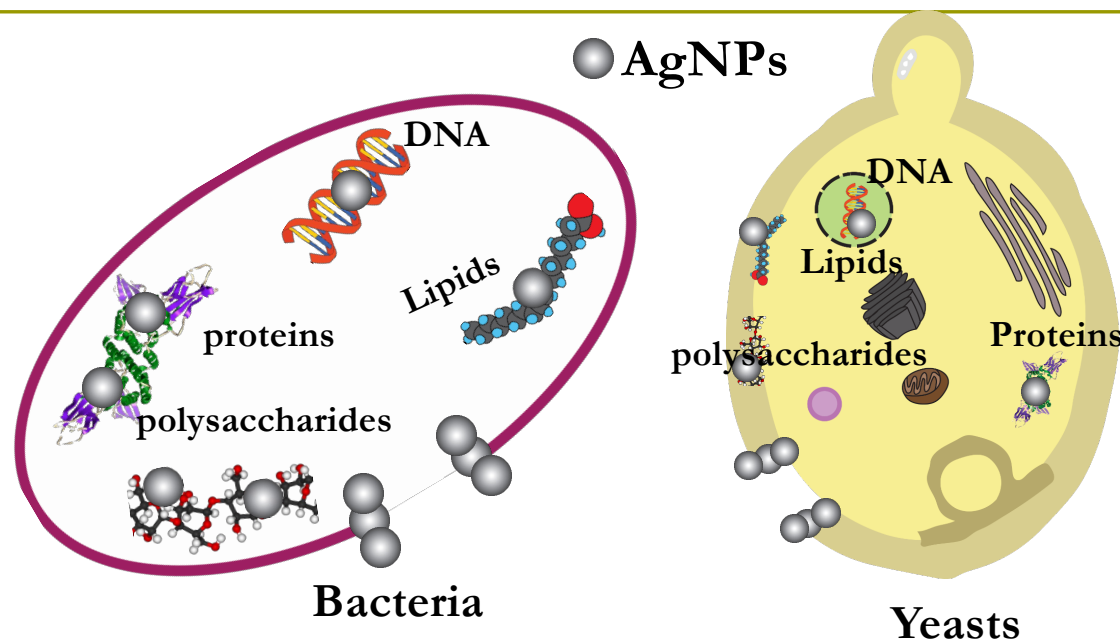


Table 2. Raman band assignment of microbial cells, control and treated cells with PeNPs. The band assignment was made based on previous work [15].

Microorganism	Raman Shift (cm ⁻¹)	Band Assignment	Effect on treated cells
<i>E. coli</i>	624	Nucleic acids	Disappear
	810	Amino acids	Diminishes
	982	Polysaccharides	Increases
	1186	Polysaccharides	Disappear
	1308	Amino acids or nucleic acids	Increases
<i>S. aureus</i>	993	Polysaccharides	Diminishes
	1092	Polysaccharides	Diminishes
	1327	Amino acids or nucleic acids	Diminishes
	1587	Fatty acids	Diminishes
<i>C. albicans</i>	698	Nucleic acids	Diminishes
	750	Amino acids	Diminishes
	991	Polysaccharides	No changes
	1318	Amino acids or nucleic acids	No changes
	1597	Fatty acids	Diminishes

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<https://doi.org/10.1088/2053-1591/ab6636>

Materials Research Express

PAPER

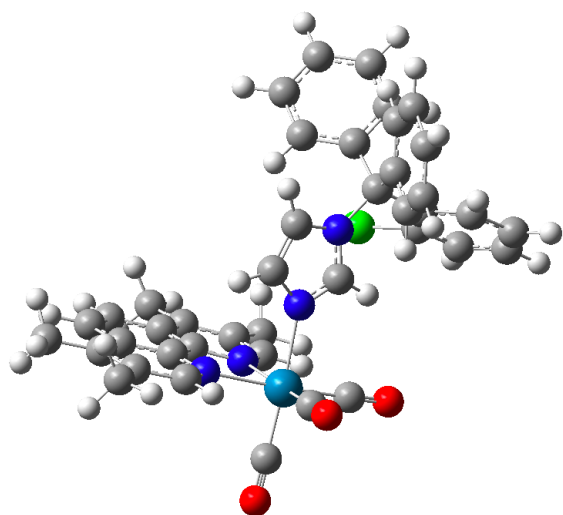
Biogenic silver nanoparticles: understanding the antimicrobial mechanism using Confocal Raman Microscopy

María Belén Estevez¹, Scott G Mitchell^{2,3}, Ricardo Faccio^{4,5} and Silvana Alborés^{1,5}

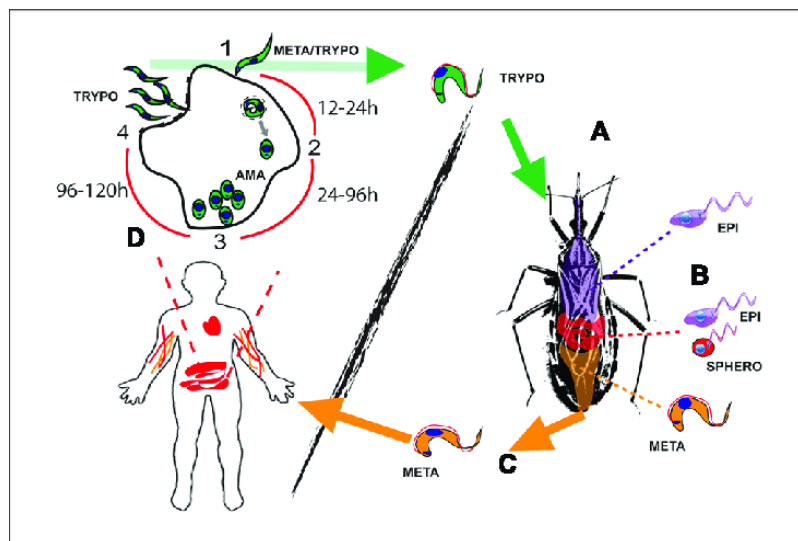
- Damage to bacterial and fungal cells changes in: polysaccharides, lipids, proteins and nucleic acids.
- Attachment of Ag=NP to the surface of the bacteria accompanied by a corresponding decrease in the integrity of the cell wall .

Example 4: Protozoan parasite *Trypanosoma cruzi*

- Chagas' disease (American Trypanosomiasis) is an ancient and endemic illness in Latin America caused by the protozoan parasite *Trypanosoma cruzi*
- Organometallic compounds (Re-based) for the treatment of Chagas disease



Re-based compounds

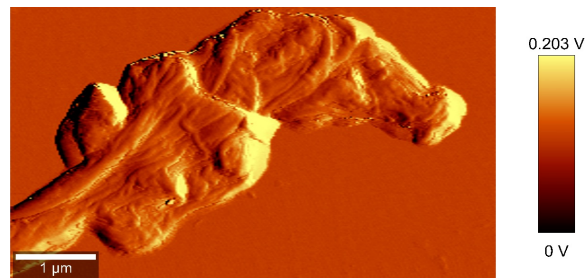


IC_{50} : concentration of drug needed to inhibit a given biological process to half of the maximum. Provides a measure of the effectiveness of a compound

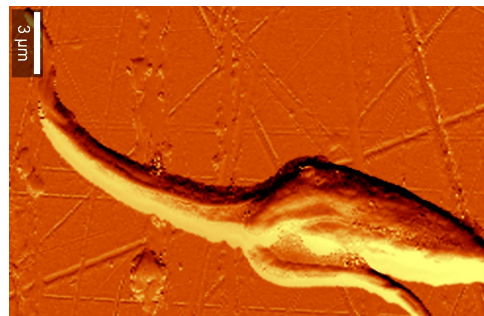
Example 4: Parasite *Trypanosoma cruzi*

Incubation of parasite with the drug in different concentrations and times

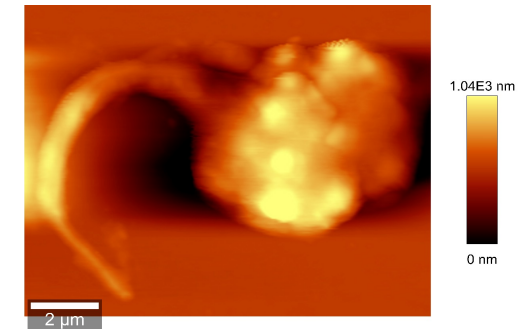
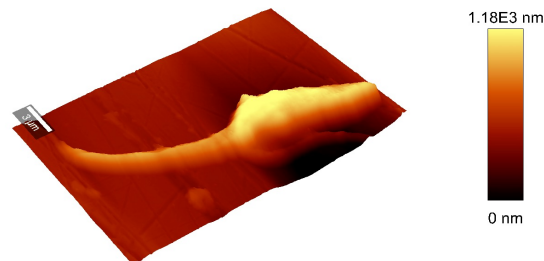
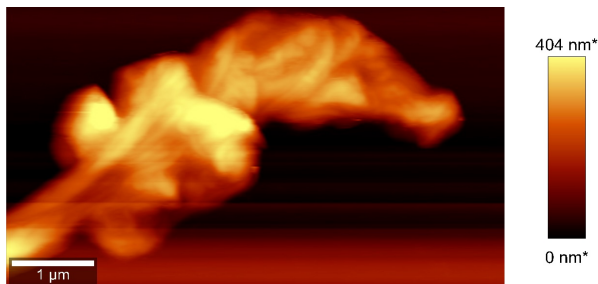
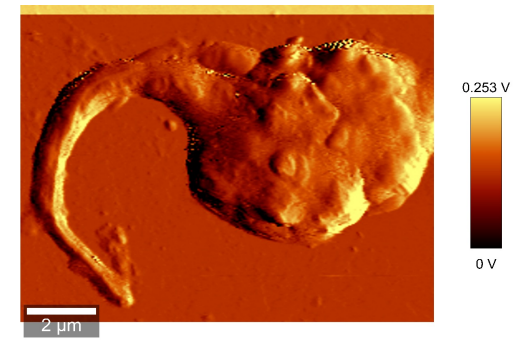
Control



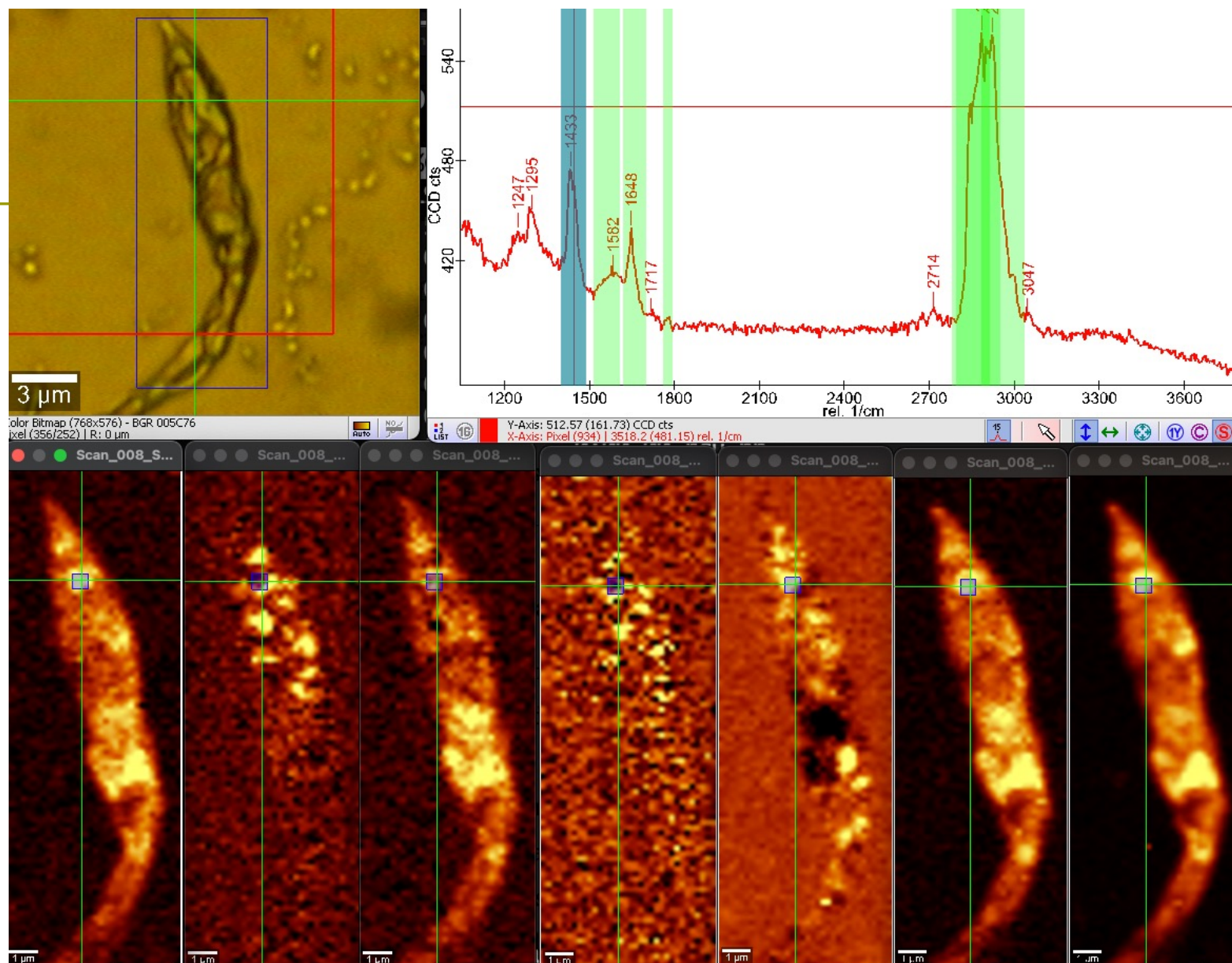
10xIC₅₀ t=4 hs



10xIC₅₀ t=24 hs



AFM morphology characterization, topography and amplitude contrast imaging



Octubre de 2023

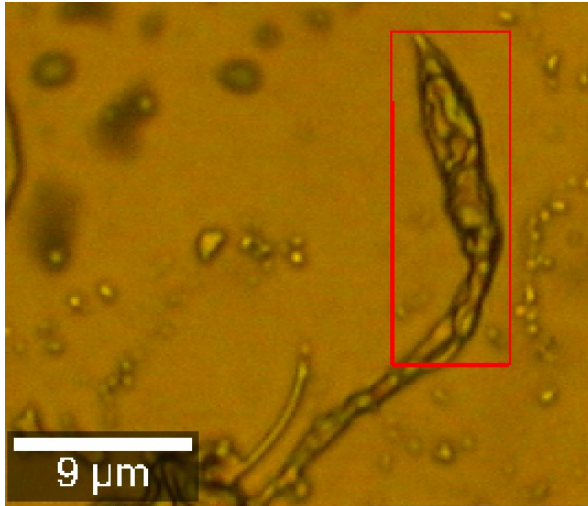
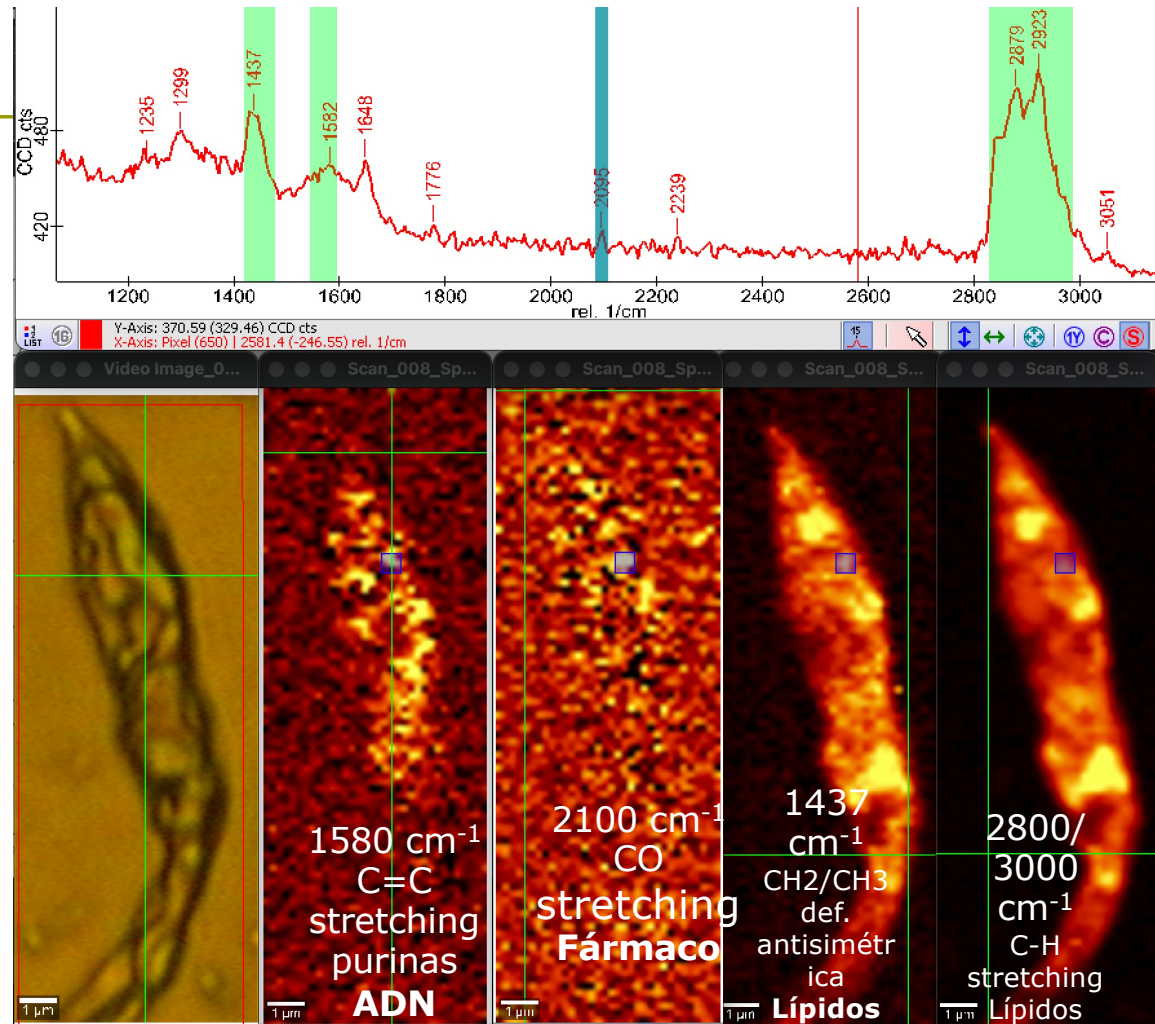
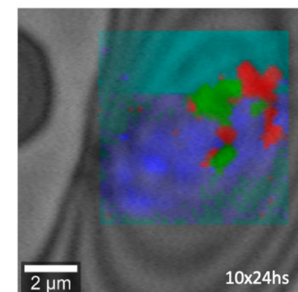
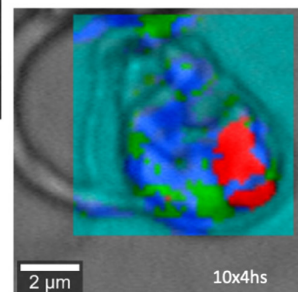
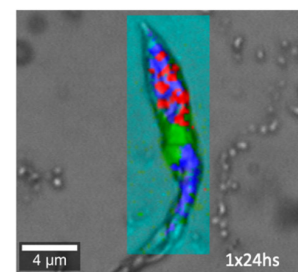
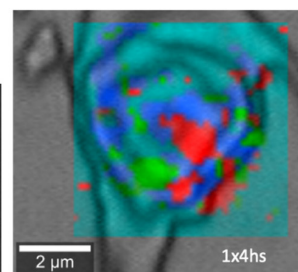
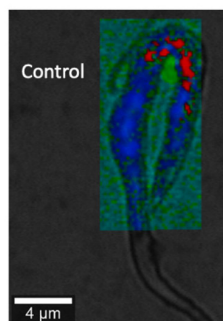
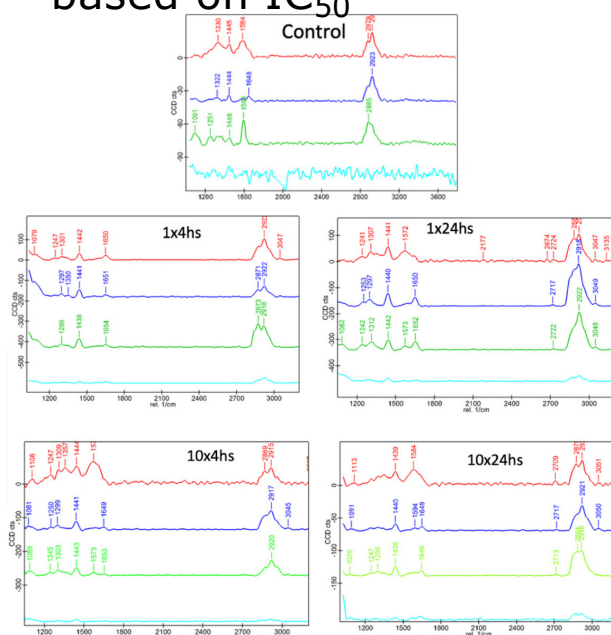


Imagen Raman para
tres bandas
características

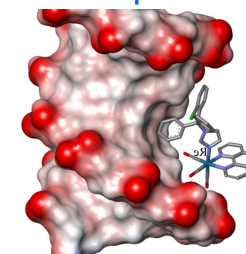


Example 4: Parasite *Trypanosoma cruzi*

1x and 10x refers to the corresponding concentration factors based on IC₅₀



Red:
mitochondrial
DNA
Green: nuclear
DNA
Blue: Lipids



Drug-DNA mechanism

Signals associated to DNA suffer important changes in their localization along the parasite, indicating a possible weak interaction with the Re-based compound

Issue 6, 2023



From the journal:
Dalton Transactions

Multifunctional organometallic compounds for the treatment of Chagas disease: Re(i) tricarbonyl compounds with two different bioactive ligands†

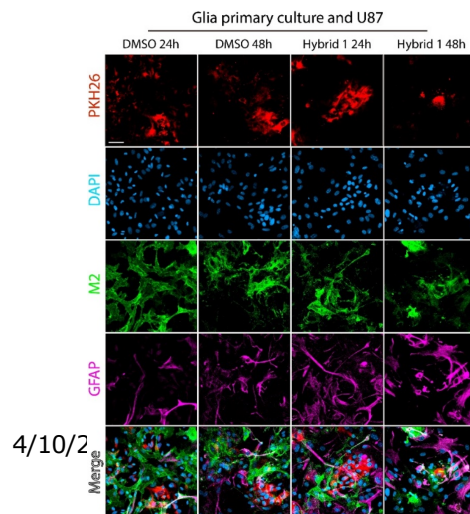
Mariano Soba, ^{a,b} Gonzalo Scalese, ^b Federico Casuriaga, ^a Nicolás Pérez, ^b Nicolás Veiga, ^a Gustavo A. Echeverría, ^b Oscar E. Piro, ^b Ricardo Faccio, ^d Leticia Pérez-Díaz, ^b Gilles Gasser, ^f Ignacio Machado ^{b,g} and Dinorah Gambino ^{b,*a}



Example 5: Tumor Cells

- System: Glioblastoma (GBM) is the most central nervous system (CNS) intractable disease.
- Drug: Boron-rich selective epidermal growth factor receptor (EGFR)-inhibitor **hybrid 1**, as a potential drug for GBM treatment

Human glioblastoma cell line
U87 MG (ATCC HTB-14)



ACTIVITY of HYBRID 1 in GLIOBLASTOMA CANCER

Inhibits GBM in an *in vivo* xenograft mouse model

Encouraging *in vitro* BNCT proof-of-concept trial

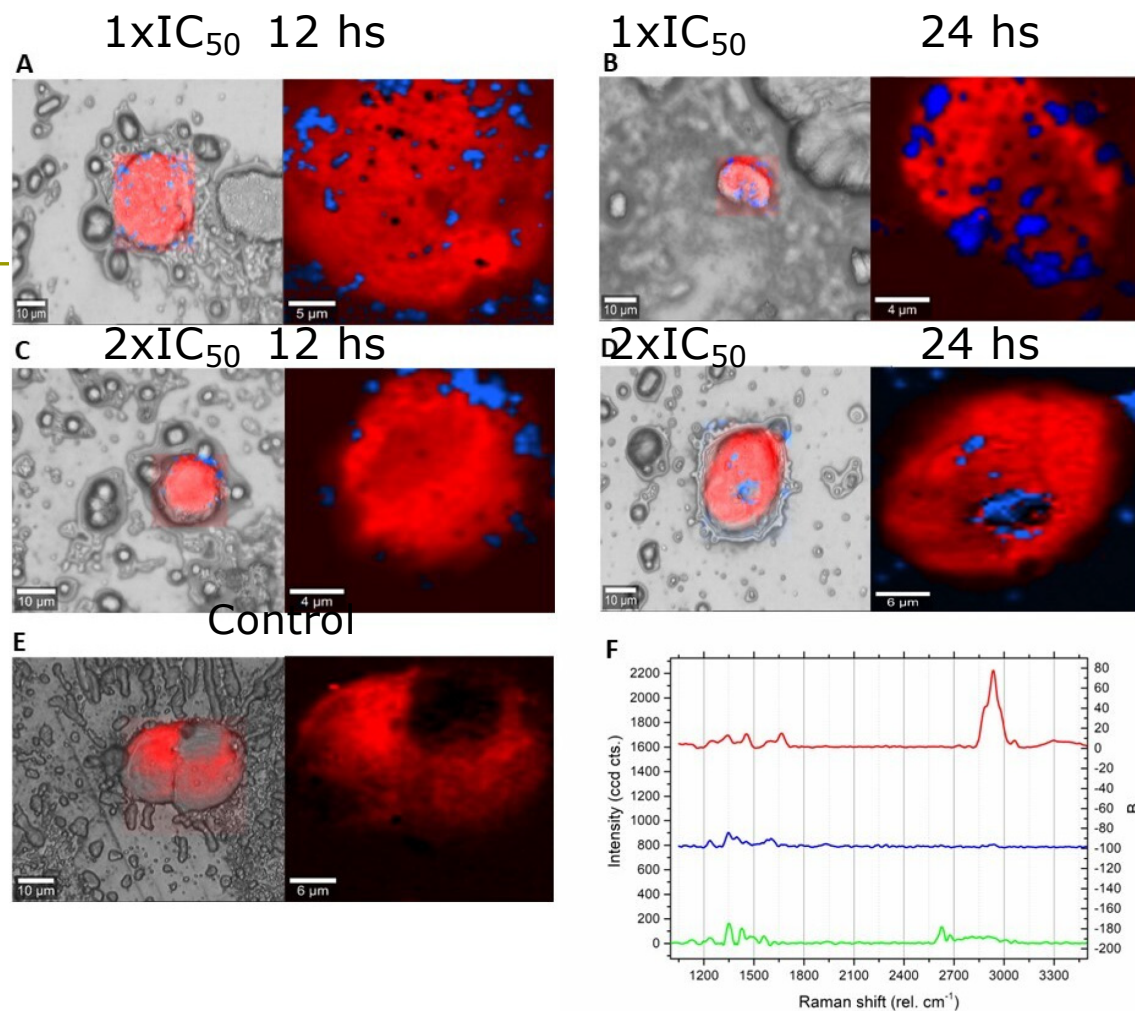
Absence of mutagenicity and high oral LD₅₀



Induces late-apoptosis in U87 MG cell line

EGFR_{WT}, IC₅₀ = 2.3 nM
EGFR_{T790M}, IC₅₀ = 1.1 μM

Selectively kills GBM tumor cell in a tumor microenvironment model



Red: Treated Cell

Blue: $\nu(\text{B-H})$ bonds of the carboranyl clusters appear $\sim 2550\text{--}2630\text{ cm}^{-1}$

Green: pure drug (as reference)

Conclusions

- Uniform boron distribution in the cell periphery at shorter times and within the cell at longer times
- boron accumulating in the proximity of the cell nucleus is much more efficient in cell killing than the same amount of boron

molecular
pharmaceutics 2023

pubs.acs.org/molecularpharmaceutics

Article

A Potential Boron Neutron Capture Therapy Agent Selectively Suppresses High-Grade Glioma: *In Vitro* and *In Vivo* Exploration

Catalina Alamón,[▽] Belén Dávila,[▽] María Fernanda García, Susana Nieves, María Alejandra Dagrosa, Silvia Thorp, Mariángela Kovacs, Emiliano Trias, Ricardo Faccio, Martín Gabay, Nidal Zeineh, Abraham Weizman, Francesc Teixidor, Clara Viñas, Moshe Gavish,^{*} Hugo Cerecetto,^{*} and Marcos Couto^{*}

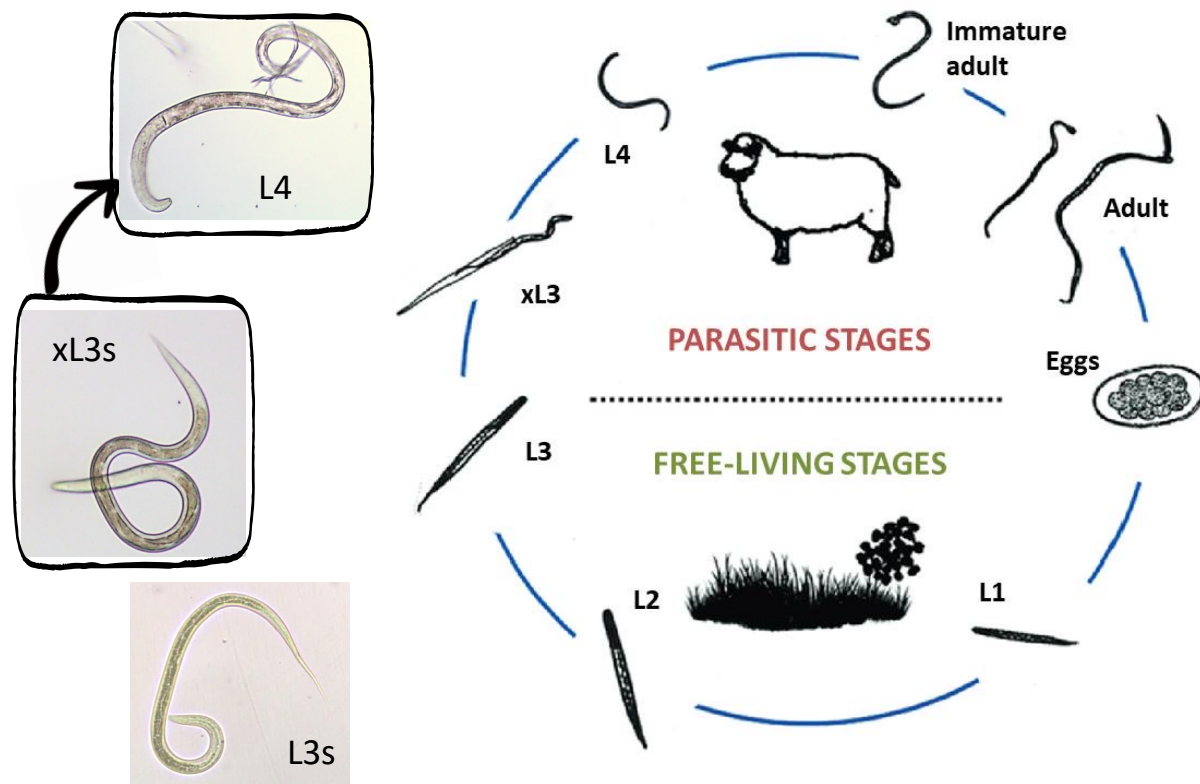
Cite This: *Mol. Pharmaceutics* 2023, 20, 2702–2713

Read Online



Example 6: *Haemonchus contortus*

Haemonchosis: infection caused by the nematode *Haemonchus contortus*



- One of the most important parasitic diseases of productive species
- Affects several millions of small ruminants
- Thus generating substantial economic losses.

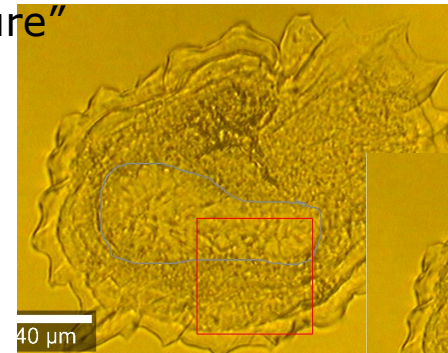
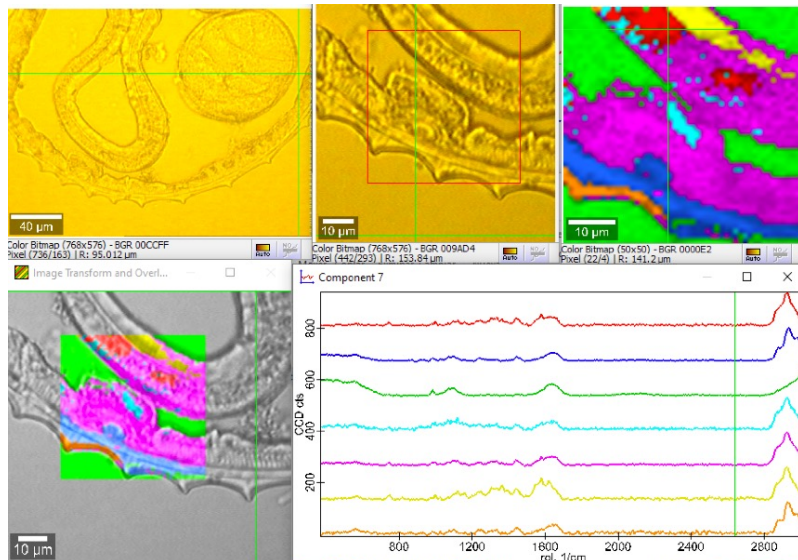
Example 6: *Haemonchus contortus*

The warm *H. Contortus* is too large (several mm long)

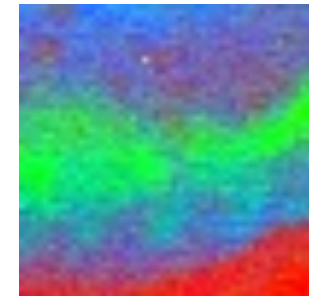
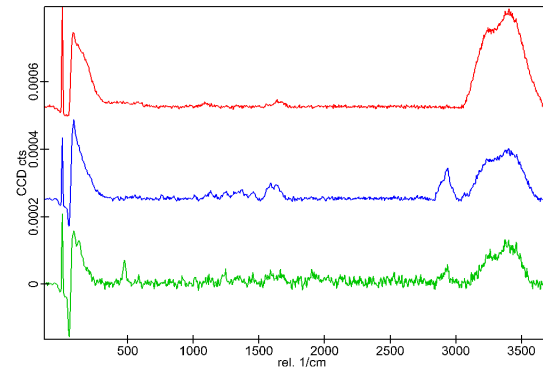
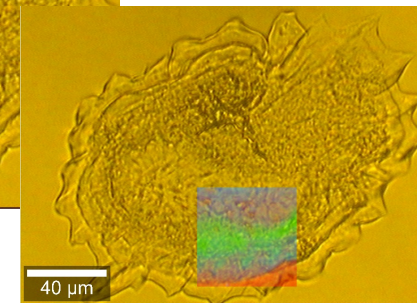
- Sample preparation: "optimal cutting temperature" (OCT) cryosectioning.

Control:

In the image we can see several organs: intestine, cuticle and ovaries



Treated *H. contortus*



On going work! Dra. Beatriz Munguía & Dra. Elisa Melián

Drug located close to Intestine
Advance in the action mechanism

Example 7: Microplastics Characterization

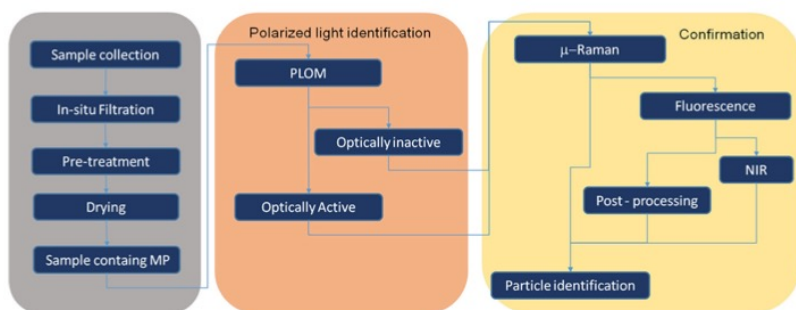


Fig. 1 General workflow of analysis for polarized light microscopy identification and further chemical confirmation

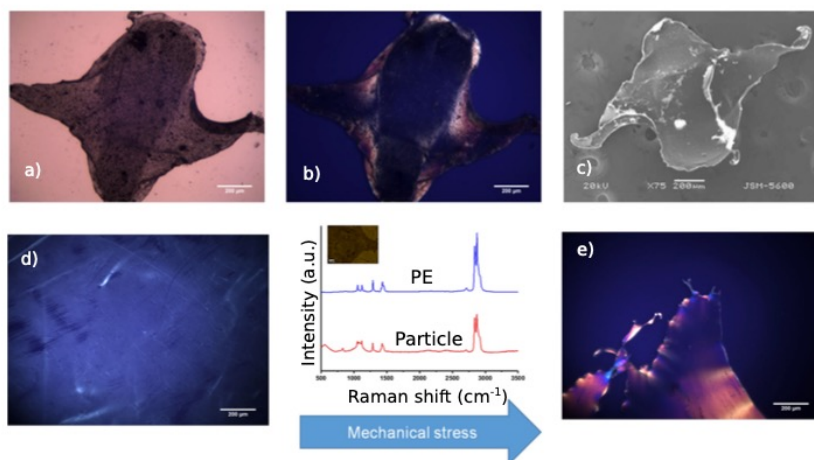


Fig. 2 Birefringence of MP of PE film (a) without and (b) with polarized-light under PLOM and corresponding (c) SEM photography. Film bag before (d) and after (e) stretching showing the effect of mechanical stress to the exhibited birefringence

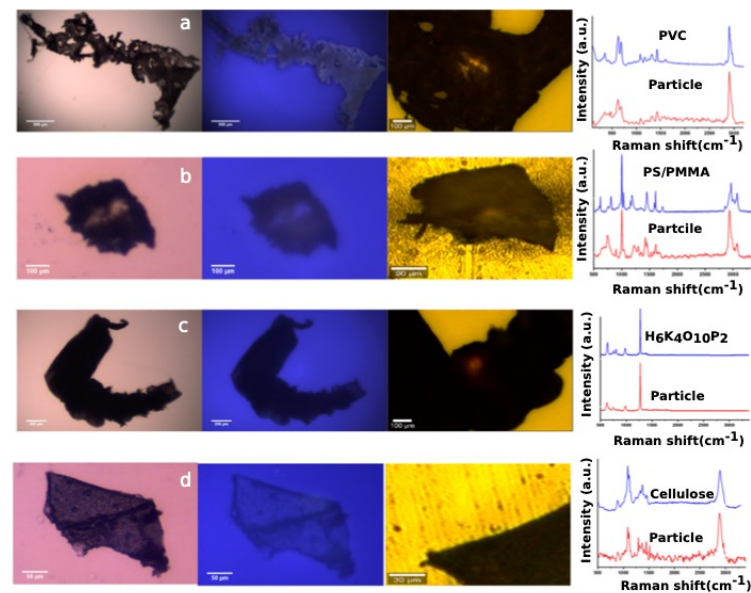


Fig. 4 Selected examples of non-optically active particles (OAPs) confirmed as microplastics. (a) particle without and with polarized light, and its confirmation as PVC by Raman spectra (b) particle without and with polarized light, and its confirmation as PMMA by Raman spectra (c) particle without and with polarized light, and its confirmation as non target inorganic particle and (d) cellulose (paper) by Raman spectra

Environmental Science and Pollution Research
<https://doi.org/10.1007/s11356-019-07011-y>

RESEARCH ARTICLE

Identification of microplastics in wastewater samples by means of polarized light optical microscopy

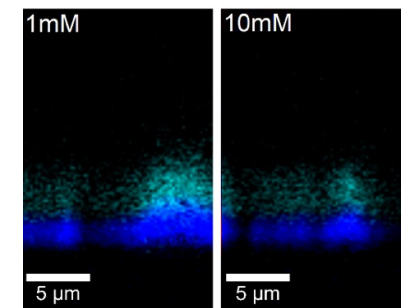
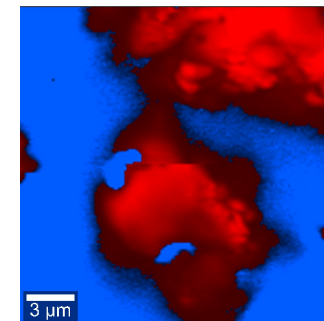
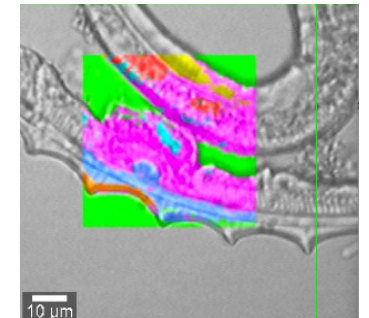
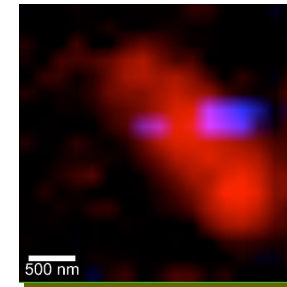
Ignacio Sierra¹ · Mauricio Rodríguez Chialanza² · Ricardo Faccio³ · Daniel Carrizo⁴ · Laura Fornaro¹ · Andrés Pérez-Parada¹



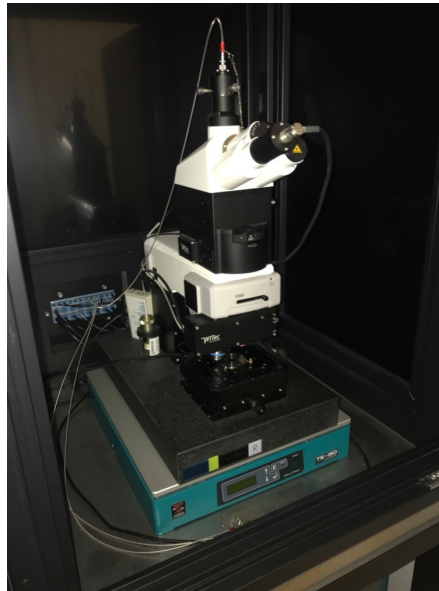
Yet, lot of work to do!

Resumen: Micro Raman Confocal

- ❑ No Destructivo
- ❑ Identificación Química Directa
- ❑ Identificación de Propiedades de Materiales
- ❑ Preparación sencilla de muestra
- ❑ Sólidos y Líquidos
- ❑ Detección 3-dimensional
- ❑ Combinación de detección química con AFM
- ❑ Variación de Temperatura (Linkam)

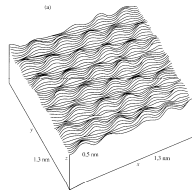
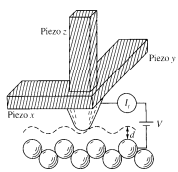


Microscopía de Fuerza Atómica



Introducción

■ Historia



Microscopio de efecto túnel (STM)

Microscopio de fuerza atómica (AFM)

Premio Nobel Física 1986

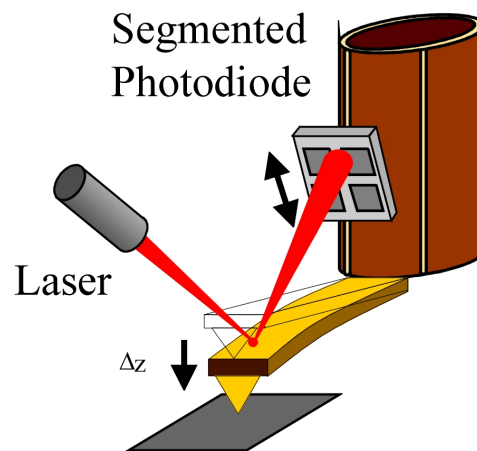


G. Binnig



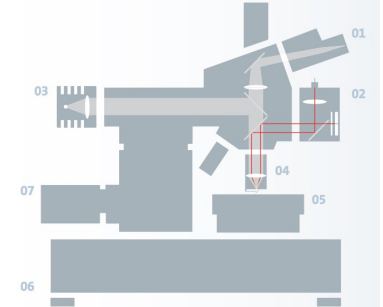
H. Rohrer

Octubre de 2023

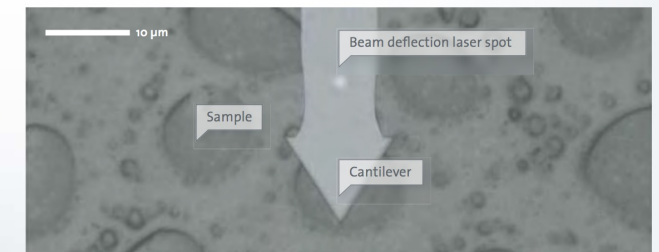


✕ BEAM PATH

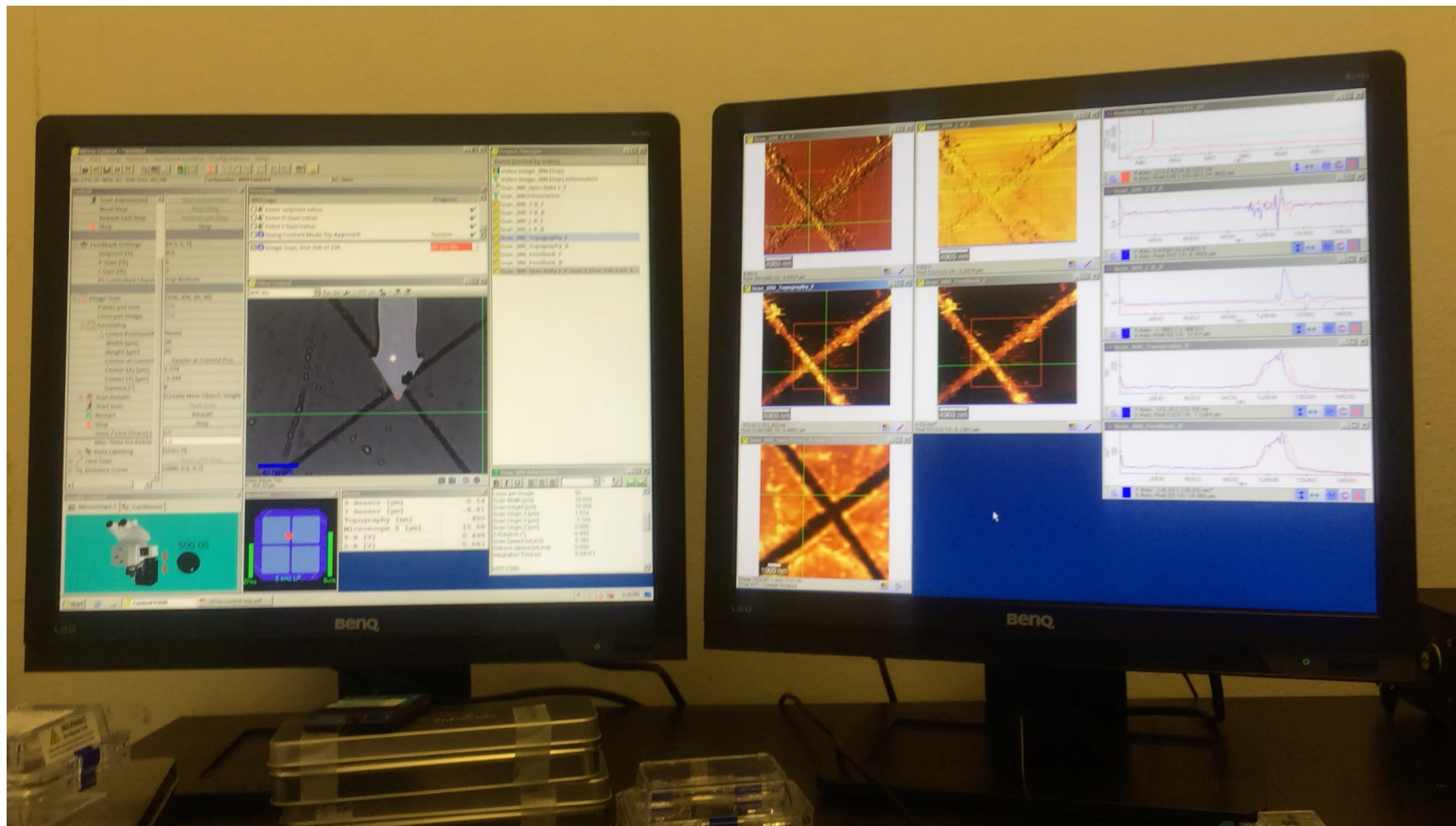
- 01 Video camera system
- 02 Beam deflection unit
- 03 LED white light illumination
- 04 AFM objective with cantilever
- 05 Scan-stage
- 06 Active vibration-isolation table
- 07 Z-stage for focusing



✕ SIMULTANEOUS CANTILEVER AND SAMPLE VIEW



Introducción: Raman & AFM Dual



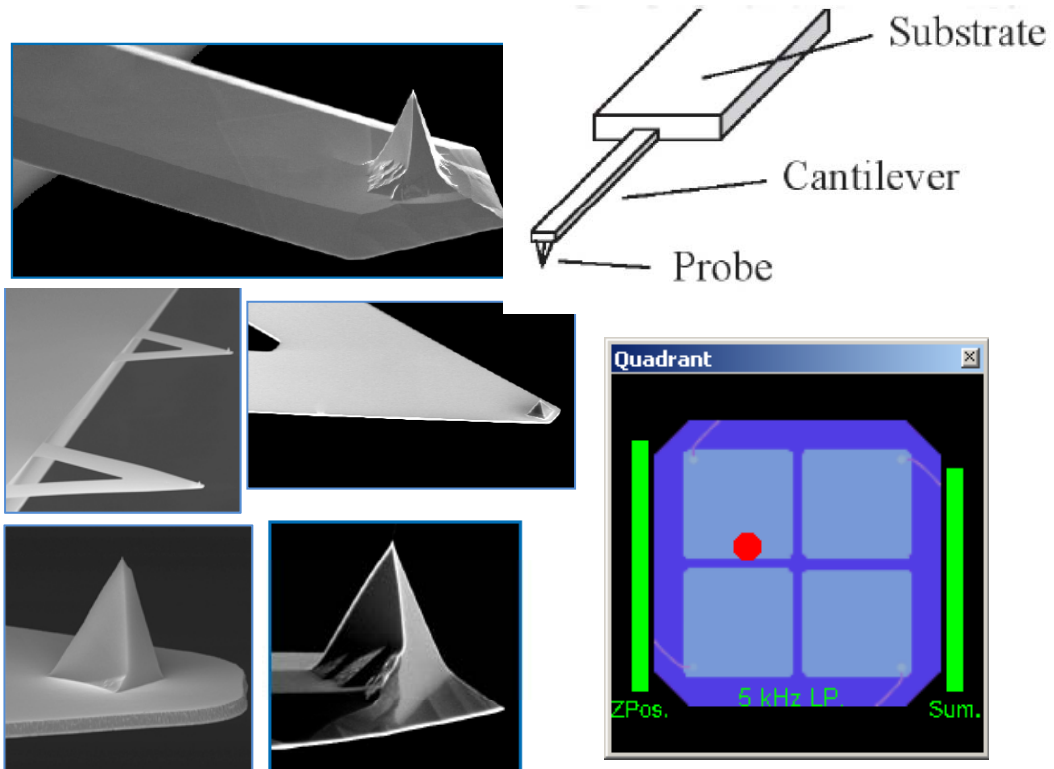
Octubre de 2023

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6. Introducción

■ Partes relevantes:

- Sustrato
- Cantilever
- Probe
- Fotodiodo

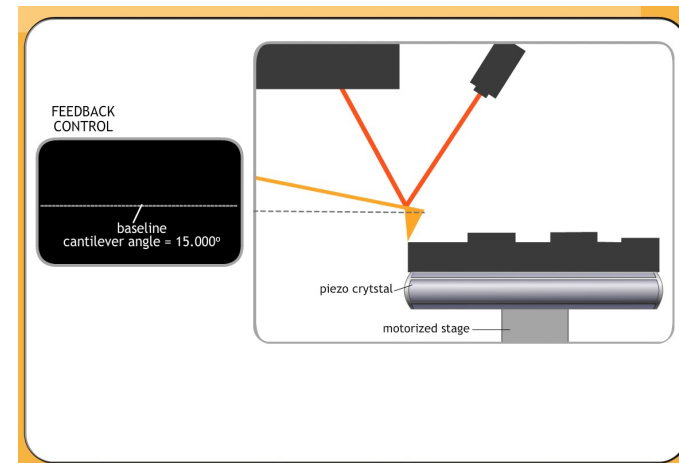
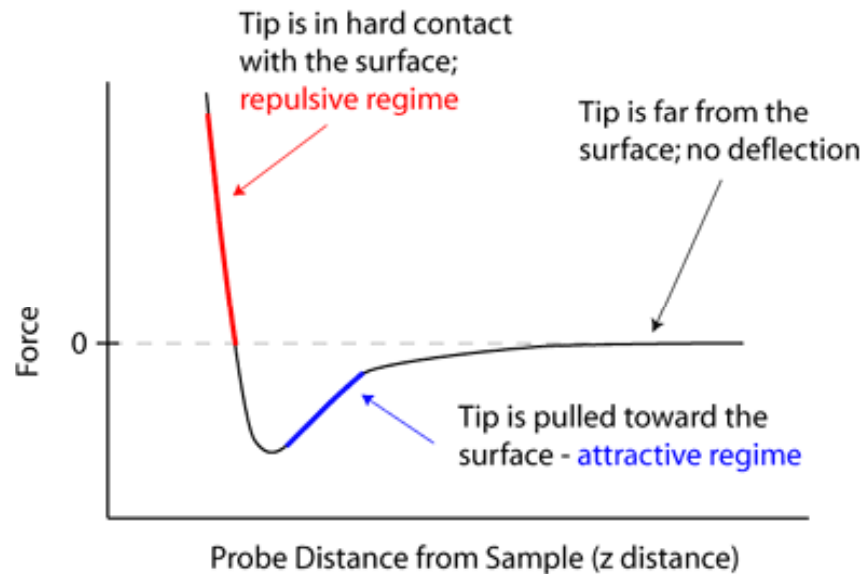


Modos de Medida

- ❑ Contacto Continuo entre Punta y Muestra
 - Modo Contacto (contact mode)
 - Medida de Fuerzas (Curva de Fuerza)
- ❑ Contacto intermitente entre punta y muestra
 - Contacto Intermitente (Tapping Mode)
 - Contraste de Fase (Phase Contrast mode)
- ❑ Otros Modos:
 - Fuerza Magnética (MFM)
 - Fuerza Eléctrica (EFM)
 - Potencial de Superficie (SPM)
 - Fuerzas Químicas (CPM)

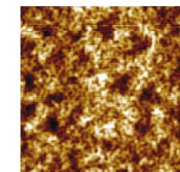
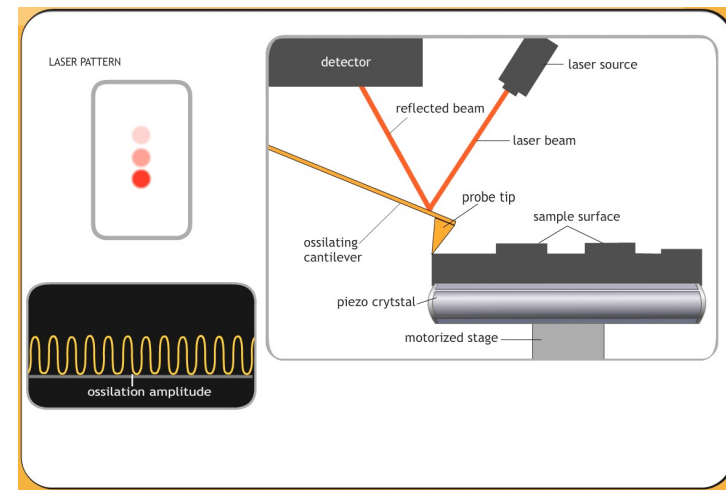
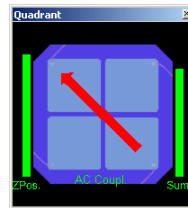
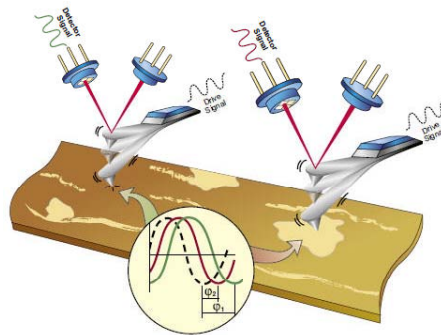
Modo Contacto

- ❑ Topografía
- ❑ Afectada por la respuesta mecánica del material
- ❑ Permite distinguir zonas con diferente comportamiento mecánico



Modo No Contacto e Intermitente

- Contacto Intermitente (Force Modulation):
 - El cantilever oscila, interaccionando con la muestra
- No Contacto
 - El cantilever oscila, sin Interacción con la Muestra



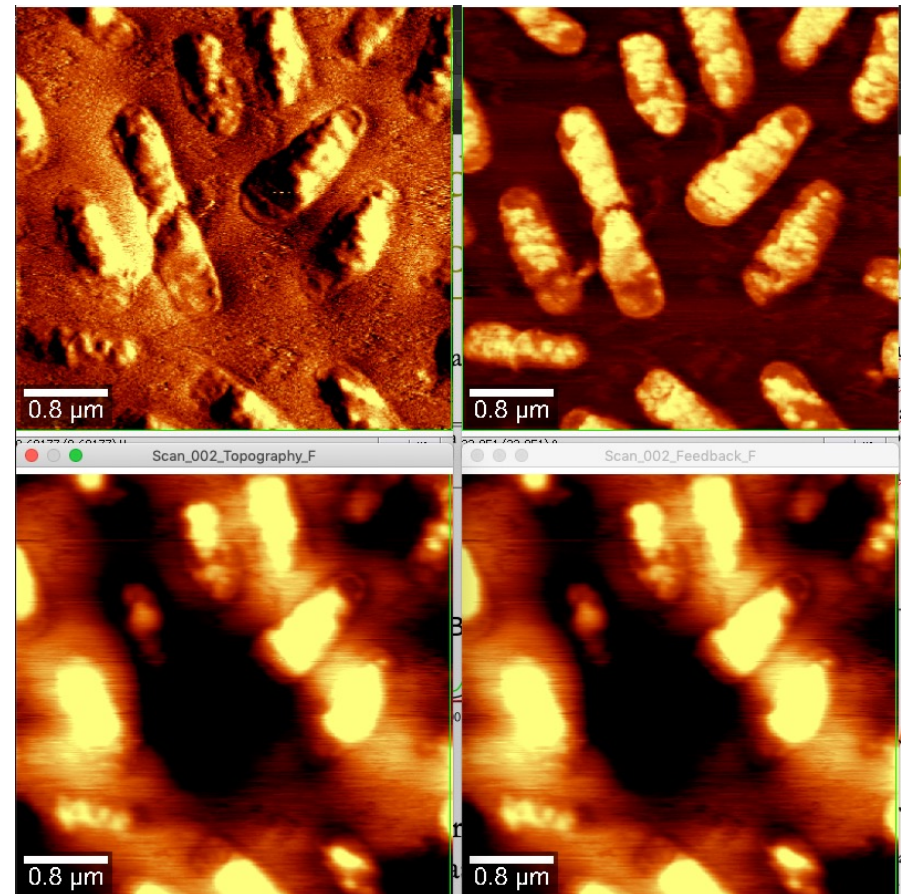
Topografía



Fase

Modo No Contacto e Intermitente

- Elección del Cantilever:
 - Tapping Mode (Force Modulation):
 - Resonancia: 65-85 kHz
 - Constante $k=2.8 \text{ Nm}^{-1}$
 - Algo de Interacción con la muestra
 - Modo No Contacto
 - Resonancia: 230-300 kHz
 - Constante $k=42 \text{ Nm}^{-1}$
 - Muestras muy “blandas”
 - (ej.: Polímeros, geles, microorganismos, etc). El Objetivo es la Phase Contrast image.
- Imágenes de Fase:
 - Propiedades Viscoelásticas.
 - Potencial de Adhesión.



Modo No Contacto e Intermitente

- Selección del Modo de Medida
- Ajuste del Set-Point:
 - Amplitud amortiguada del tip, debe ser inferior a la de la amplitud libre.
- Tapping Mode (Intermitente)
 - Set Point ↓
- Modo No Contacto
 - Set Point ↑

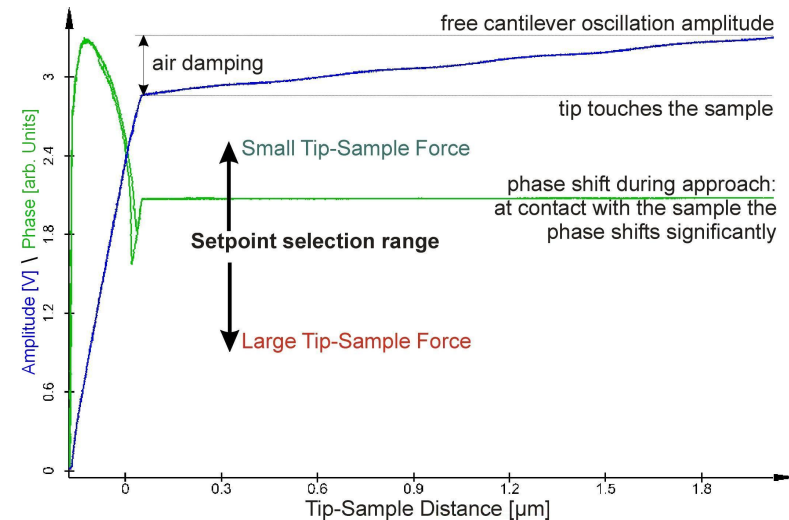
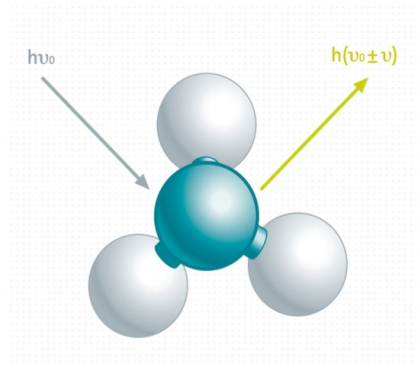
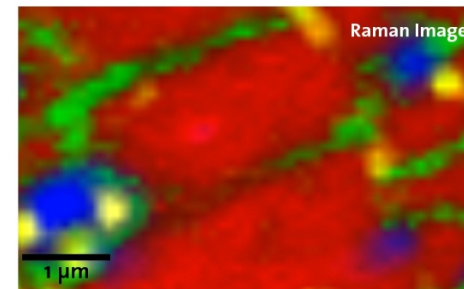
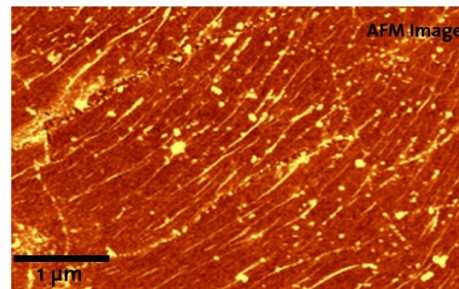
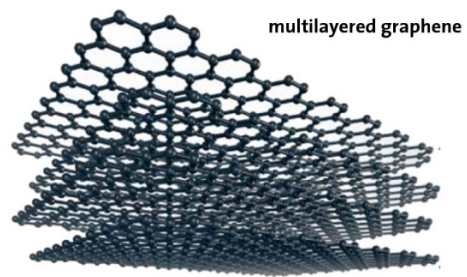


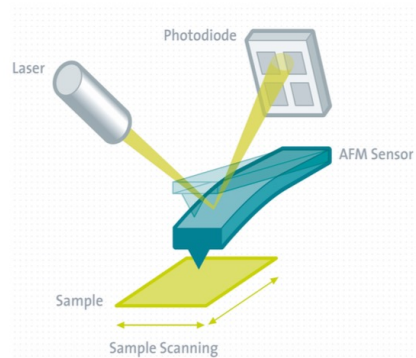
Fig. 1: Phase (green) and Amplitude (blue) as a function of tip→sample distance. The effect of the setpoint selection is also indicated.

Micro Raman AFM Dual

- Alpha 300R → Alpha 300RA
 - FSE_1_2014_1_102008 - ANII



Raman Effect

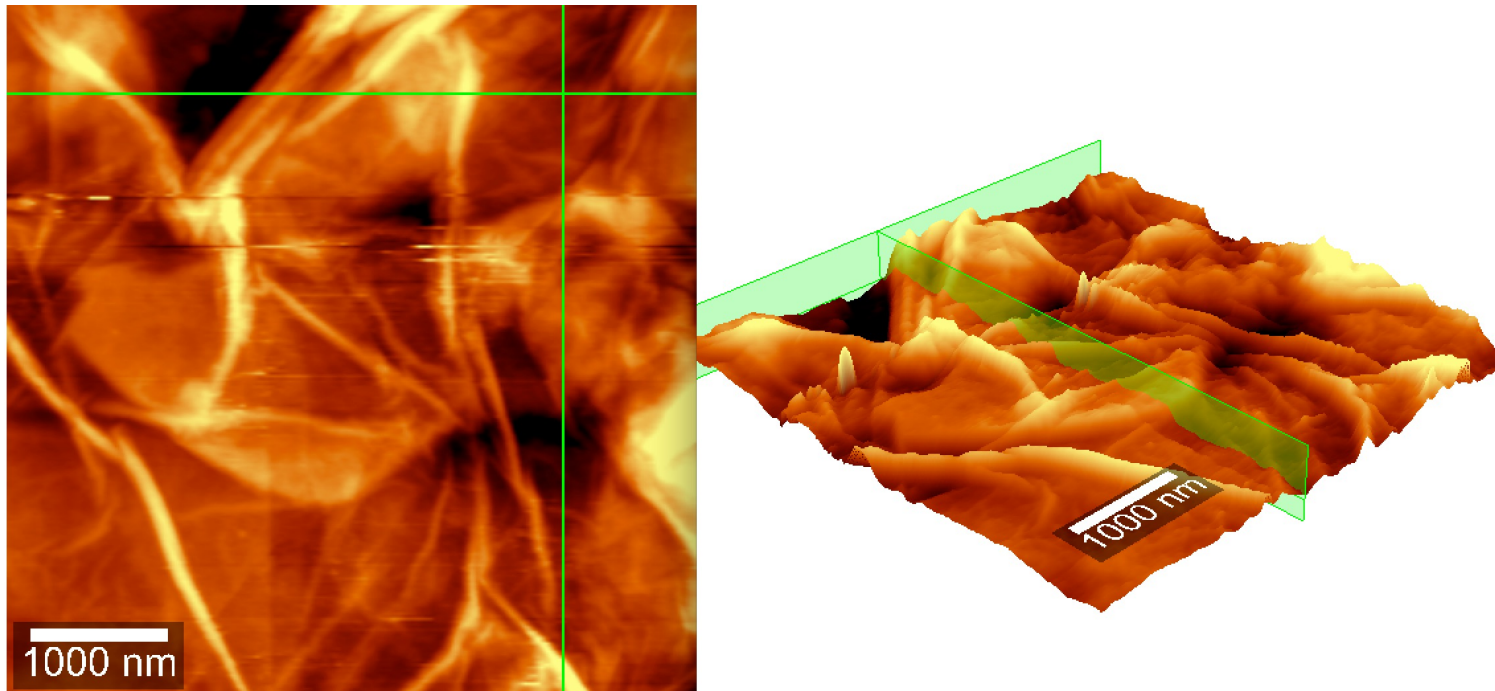


AFM Principle



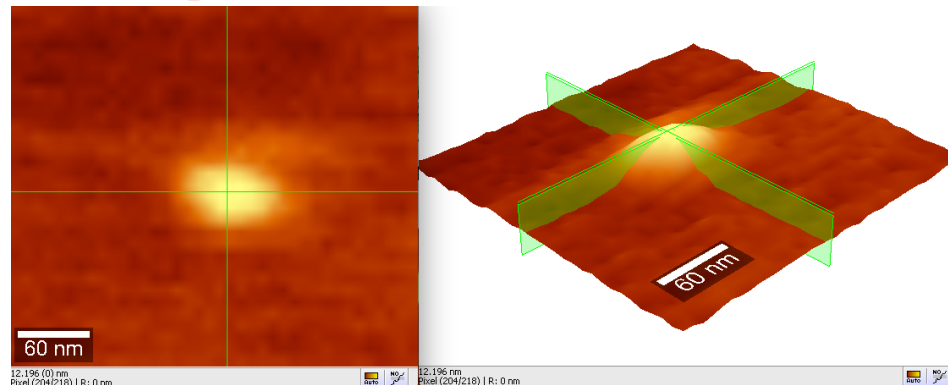
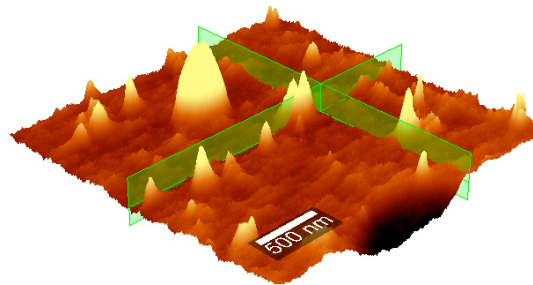
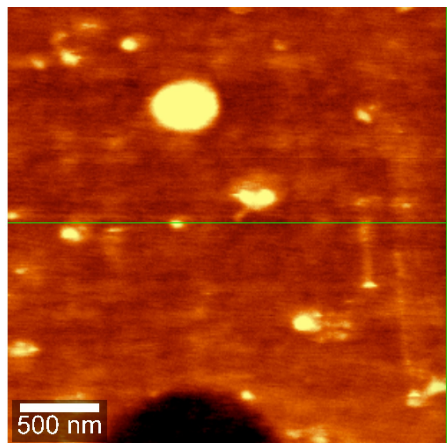
Ejemplos: Modo No Contacto

▣ Reduced Graphene Oxide

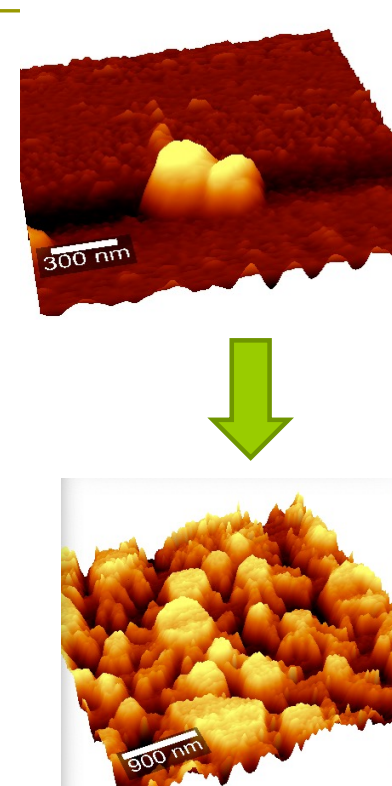
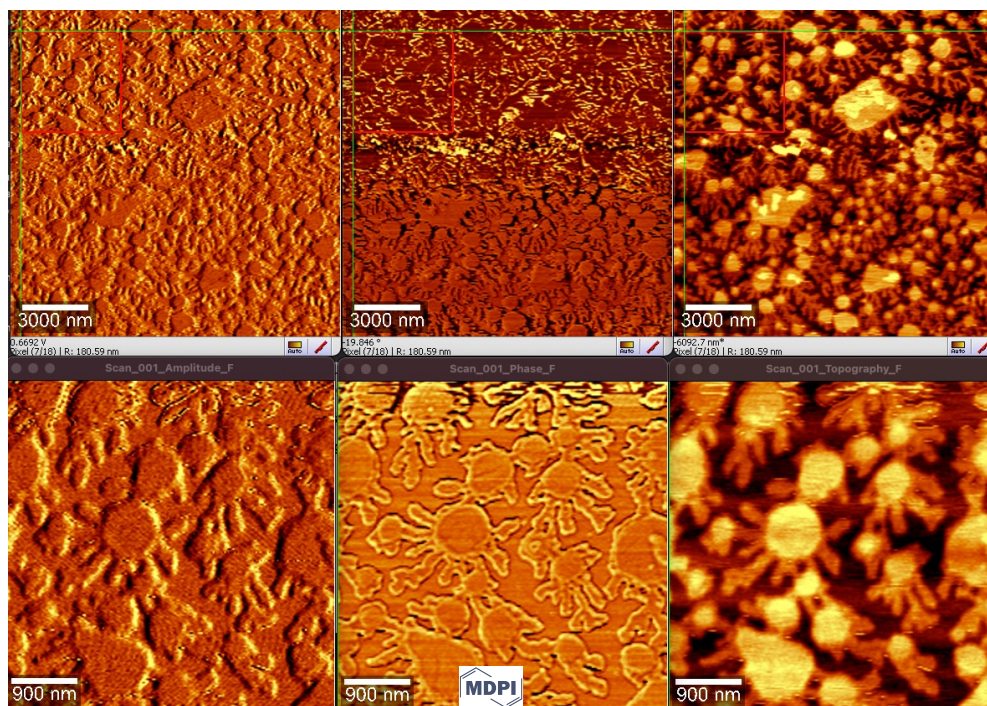


Ejemplos: Modo No Contacto

- GOQD sobre sustrato de silicio
- 60 nm de diámetro y 13 nm de alto



Nanopartículas lipídicas funcionalizadas



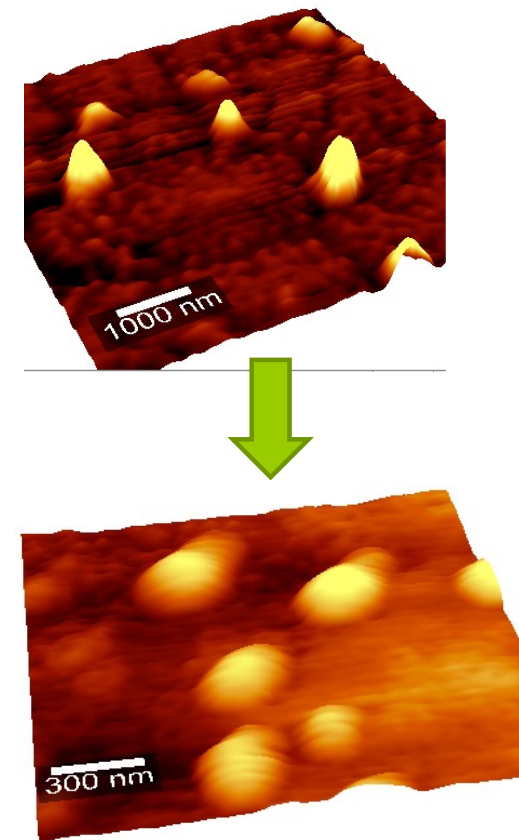
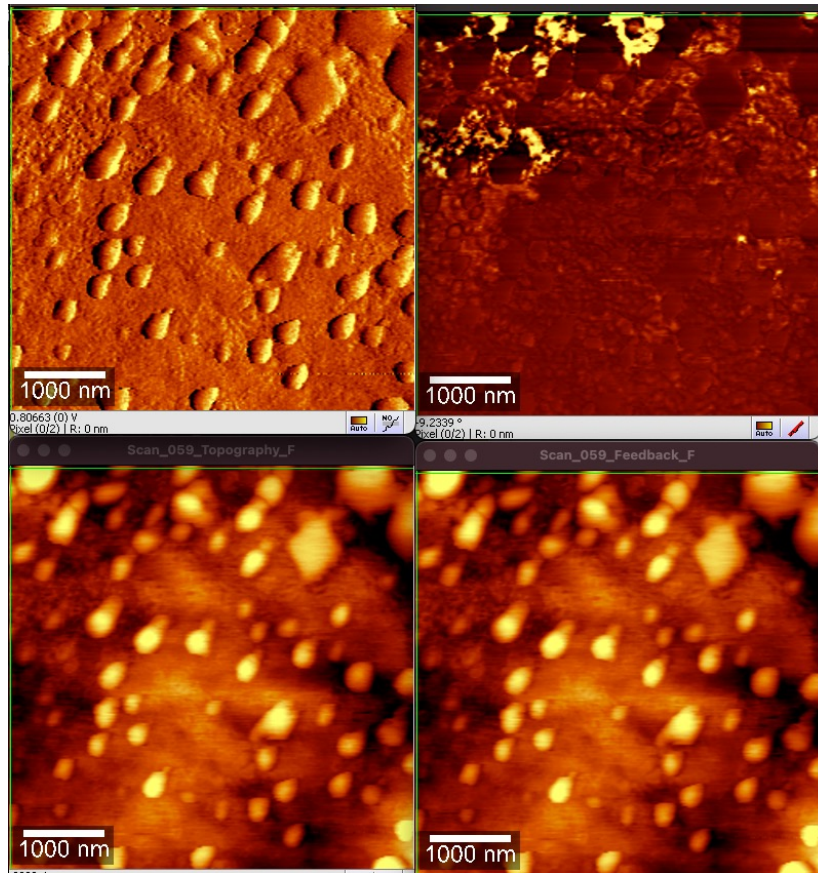
Article

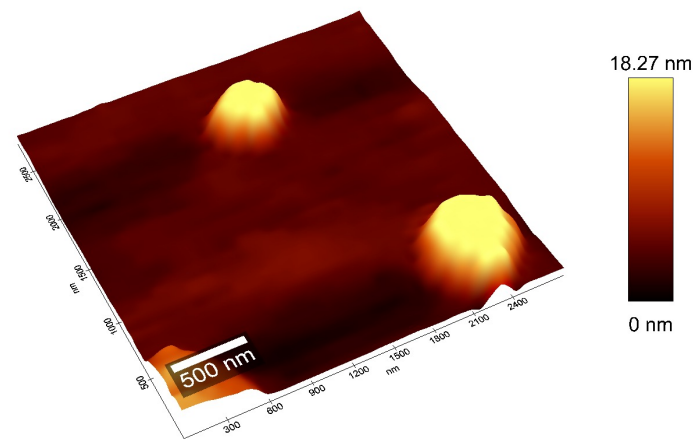
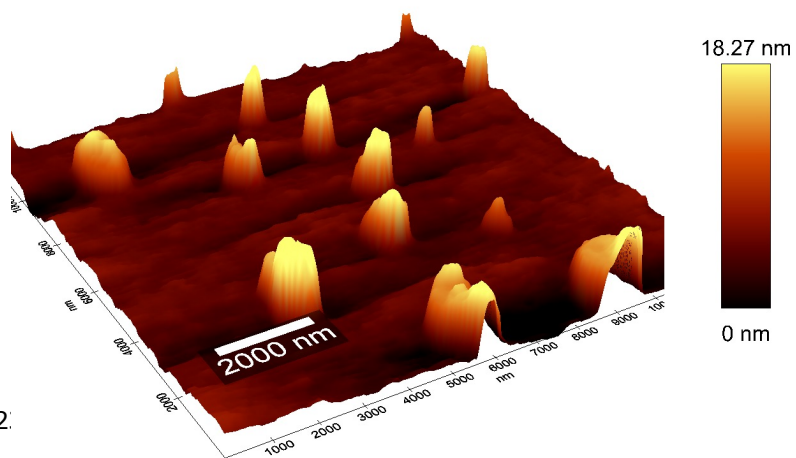
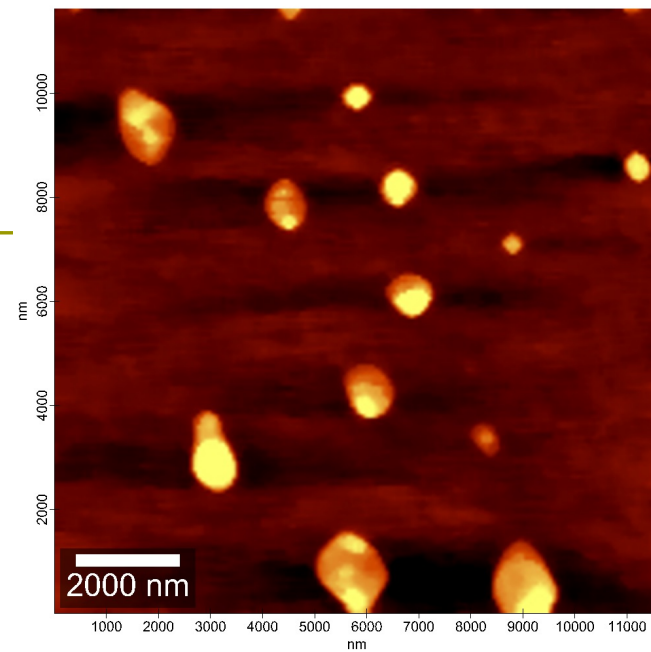
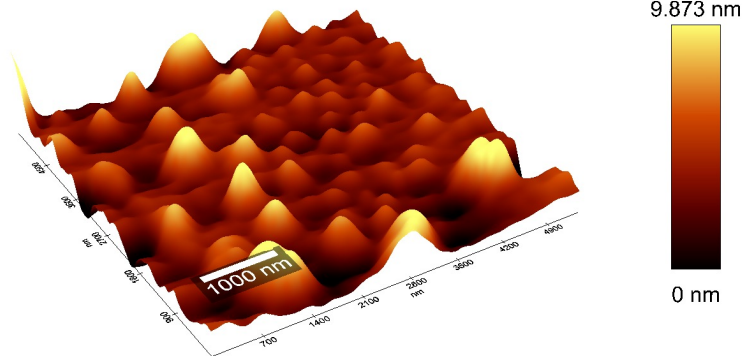
T908 Polymeric Micelles Improved the Uptake of Sgc8-c Aptamer Probe in Tumor-Bearing Mice: A Co-Association Study between the Probe and Preformed Nanostructures

Romina Castelli ¹, Manuel Ibarra ², Ricardo Faccio ³, Iris Miraballes ⁴, Marcelo Fernández ⁵,
Albertina Moglioni ⁶, Pablo Cabral ¹, Hugo Cerecetto ^{1,*}, Romina J. Glisoni ^{7,*} and Victoria Calzada ^{1,*}

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Liposomas funcionalizados





Octubre de 202.

Resumen: AFM/EFM/MFM

□ Diferentes Modos de Medida:

- *Contacto*
- *No Contacto e Intermitente (Tapping)*
- *Liftmode*

□ AFM/EFM/MFM

□ Topología y Propiedades Físicas:

- *Dureza*
- *Viscosidad*
- *Adhesión*

□ Posibilidad de medir en Líquidos

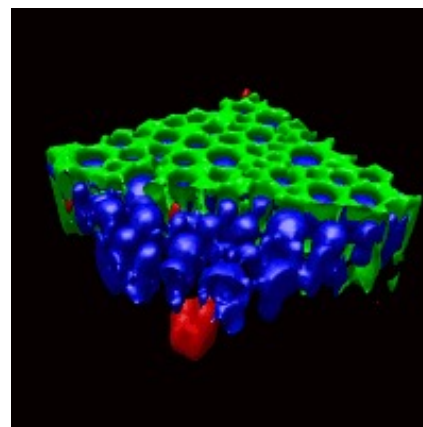
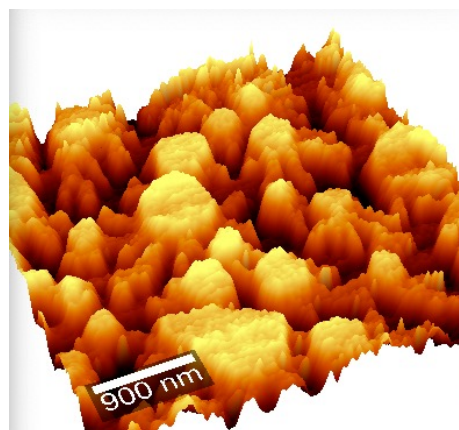
Gracias!



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