

Nanotecnología y biomateriales para salud, medio ambiente y alimentos

Caracterización de bio-nanomateriales



Dr. Ricardo Faccio
Área Física-DETEMA
 Facultad de Química
 Universidad de la República
rfaccio@fq.edu.uy

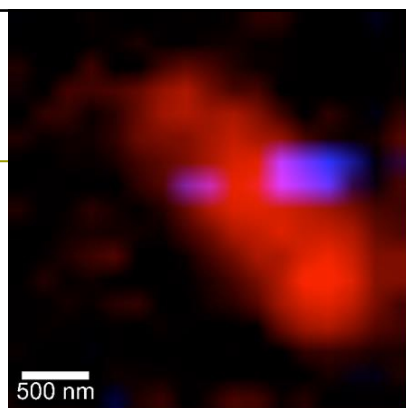


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Punteo

- ▣ Introducción
- ▣ Espectroscopia Vibracional Raman
- ▣ Técnicas de Imagenología.
 - Microscopia Raman
 - Microscopia de Fuerza Atómica
- ▣ Difracción de Rayos X
- ▣ Conclusiones



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1. ¿Qué es la Nanotecnología?

- Es el estudio y control de la materia, a una escala entre 1 y 100 nm

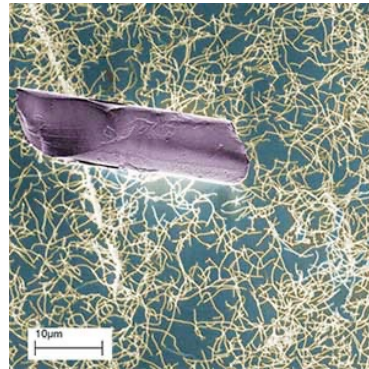
$$1 \text{ nm} = 1/1\,000\,000\,000 \text{ m}$$

$$1 \text{ nm} = 1 \times 10^{-9} \text{ m}$$

La nanociencia es el estudio de fenómenos y manipulación de materiales a escalas atómica, molecular y macromolecular; donde las propiedades difieren significativamente de aquellas a gran escala.

La nanotecnología es el diseño, la caracterización, la producción y la aplicación de estructuras, dispositivos y sistemas para controlar la forma y tamaño a escala nanométrica.

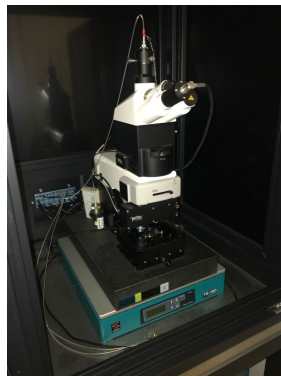
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2. Microscopia Raman Confocal

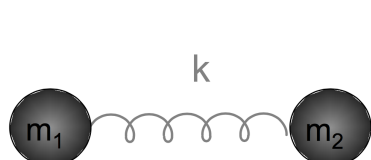


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2. Modos Vibracionales



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

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

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

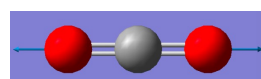
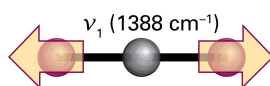
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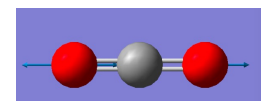
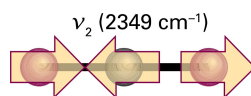
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Modos Vibracionales del CO₂

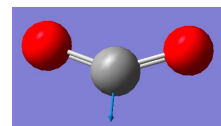
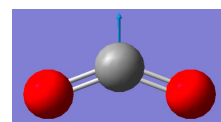
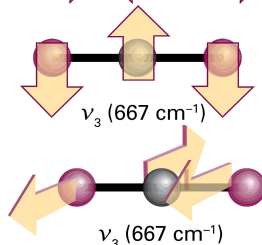
Estiramiento
simétrico



Estiramiento
antisimétrico



Deformación
degenerada



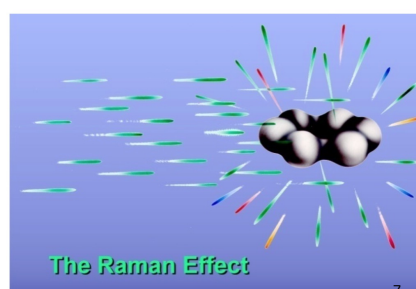
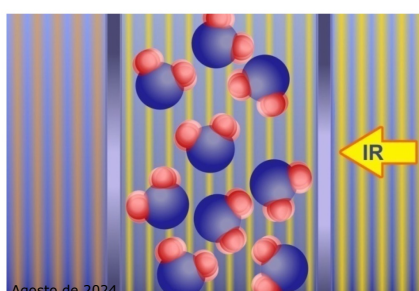
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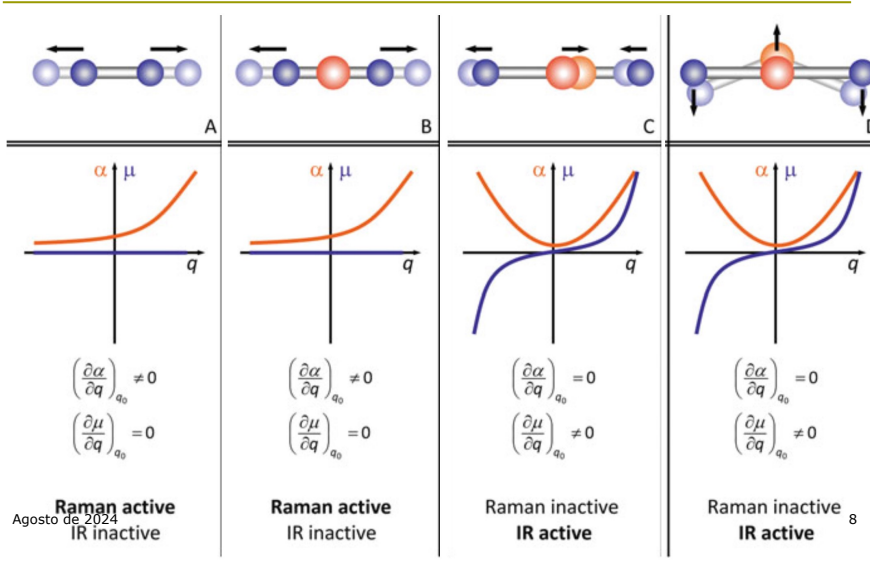
2. Reglas de Selección

- 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.



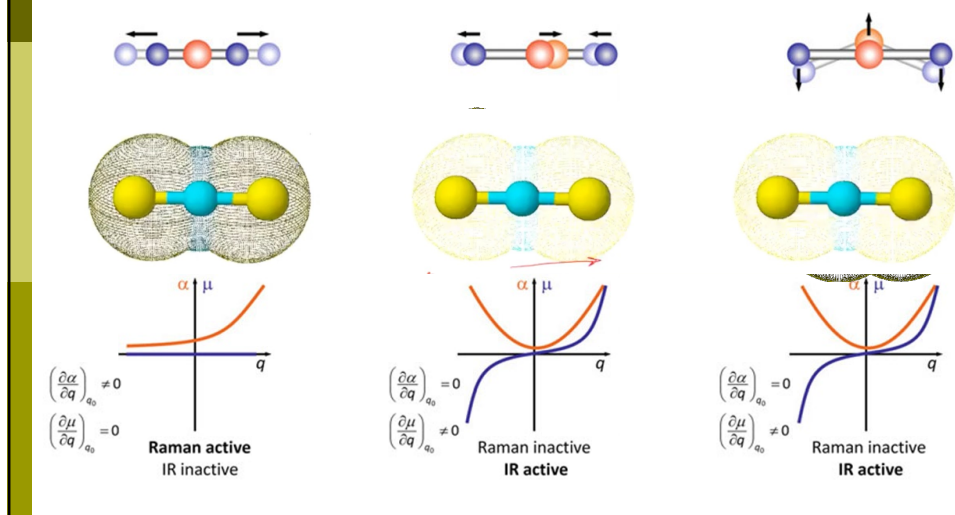
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2. Reglas de Selección: IR vs Raman



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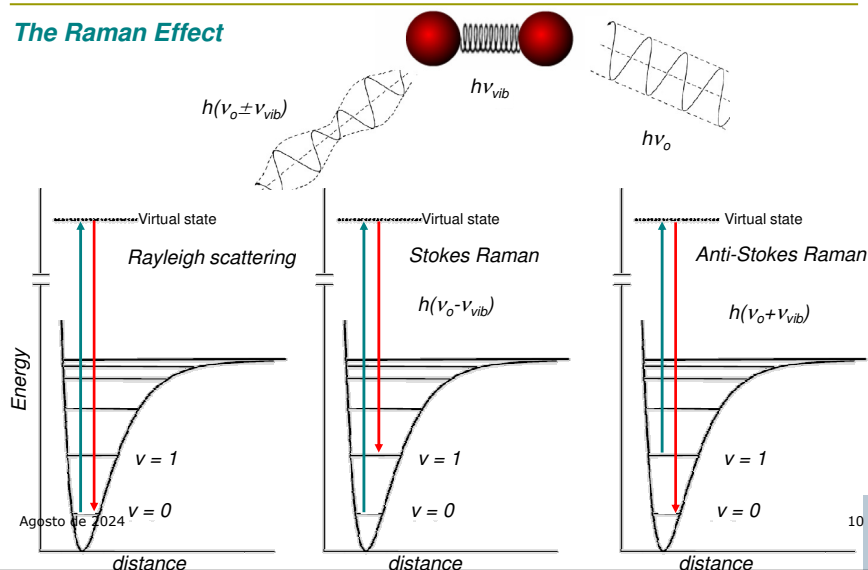
1. Reglas de Selección: IR vs Raman



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2. El efecto Raman

The Raman Effect

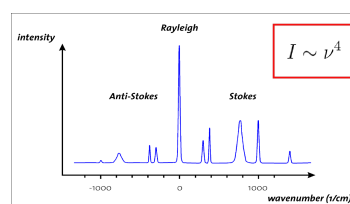
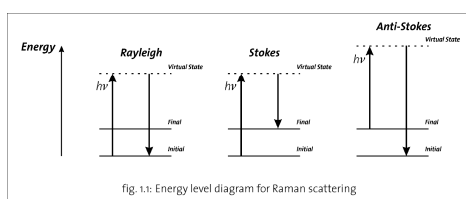
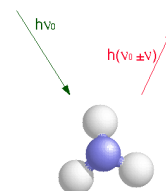


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2. El efecto Raman

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



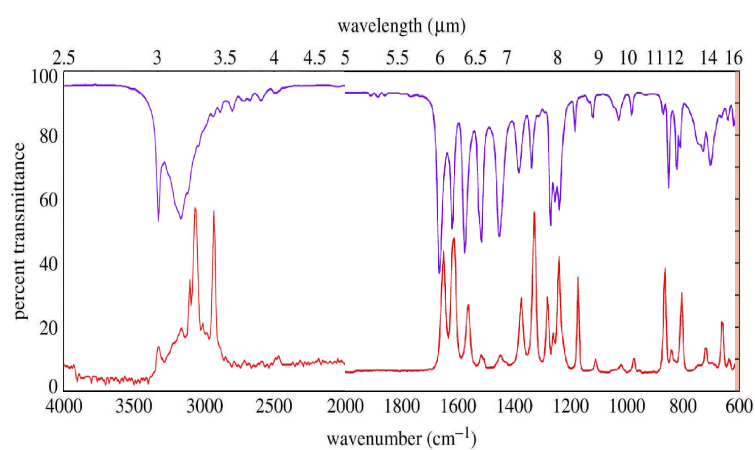
- theoretical prediction 1923 by A. Smekal
- experimentally discovered 1928 by Sir Chandrasekhara Raman, Nobel Prize 1930

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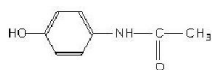
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2. Infrarrojo vs. Raman



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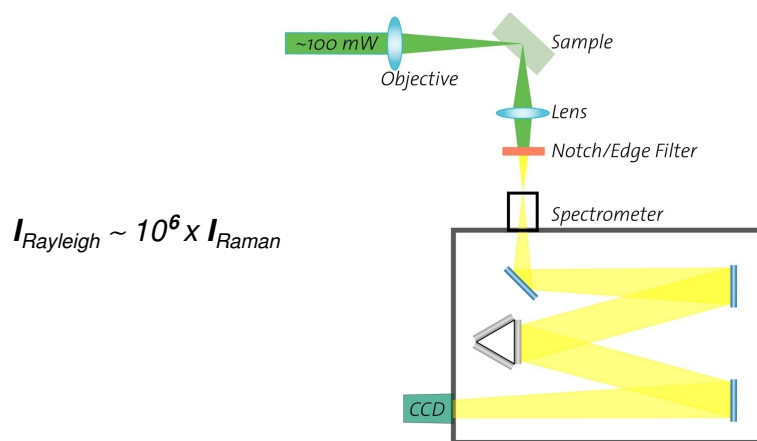
Paracetamol



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5. Esquema Típico de Experimento



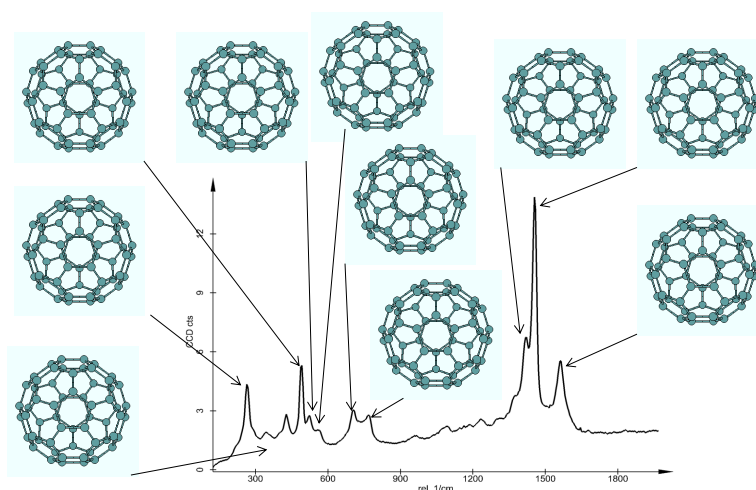
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- typical integration times: seconds - several minutes

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1. Espectroscopia Raman



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2. 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

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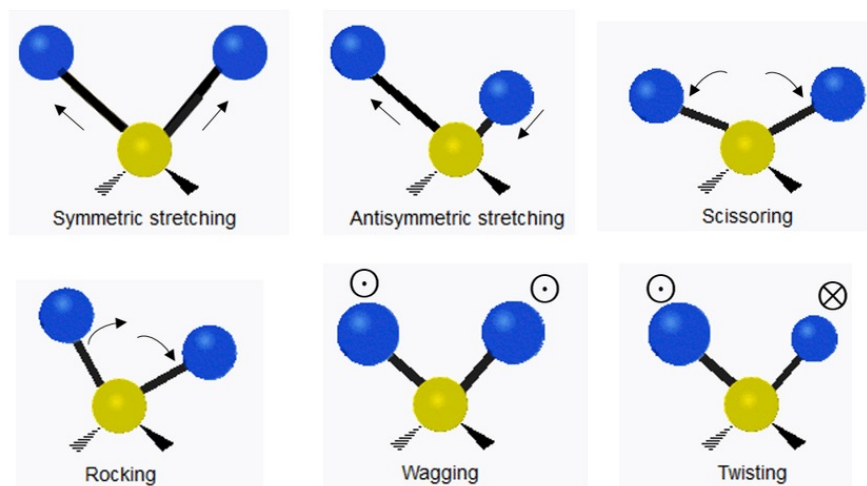
$\nu(\text{CC})$ alicyclic, aliphatic chain vibrations	600 - 1300 cm^{-1}	medium	Medium
$\nu(\text{C=S})$	1000 - 1250 cm^{-1}	strong	weak
$\nu(\text{CC})$ aromatic ring chain vibrations	*1580, 1600 cm^{-1}	strong	medium
	*1450, 1500 cm^{-1}	medium	medium
	*1000 cm^{-1}	strong/medium	weak
$\delta(\text{CH}_3)$	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
$\nu(\text{C-(NO}_2\text{)})$	1340 - 1380 cm^{-1}	strong	medium
$\nu(\text{C-(NO}_2\text{)})$ asym	1530 - 1590 cm^{-1}	medium	strong
$\nu(\text{N=N})$ aromatic	1410 - 1440 cm^{-1}	medium	-
$\nu(\text{N=N})$ aliphatic	1550 - 1580 cm^{-1}	medium	-
$\delta(\text{H}_2\text{O})$	~1640 cm^{-1}	weak broad	strong
$\nu(\text{C=N})$	1610 - 1680 cm^{-1}	strong	medium
$\nu(\text{C=C})$	1500 - 1900 cm^{-1}	strong	weak
$\nu(\text{C=O})$	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
$\nu(\text{-S-H})$	2550 - 2600 cm^{-1}	strong	weak
$\nu(\text{C-H})$	2800 - 3000 cm^{-1}	strong	strong
$\nu(\text{=C-H})$	3000 - 3100 cm^{-1}	strong	medium
$\nu(\text{=C-H})$	3300 cm^{-1}	weak	strong
Agosto de 2024 $\nu(\text{N-H})$	3300 - 3500 cm^{-1}	medium	medium
$\nu(\text{O-H})$	3100 - 3650 cm^{-1}	weak	strong

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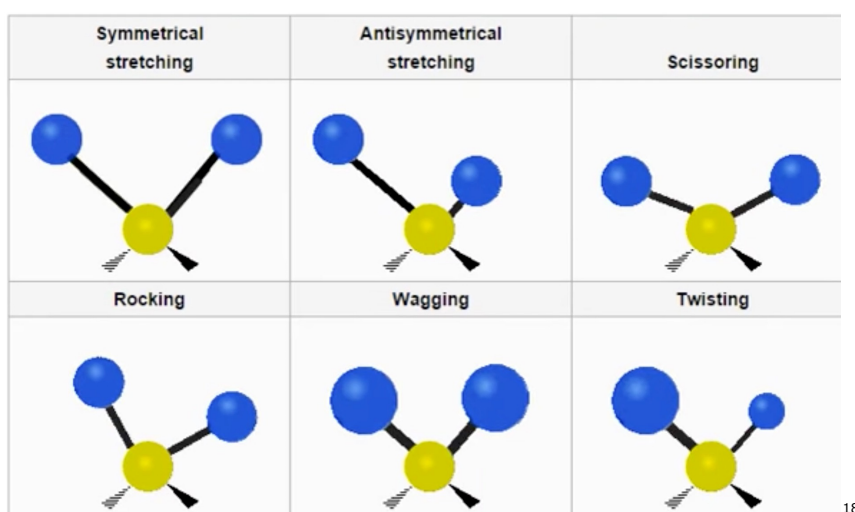
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Coordenadas Internas



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Coordenadas Internas



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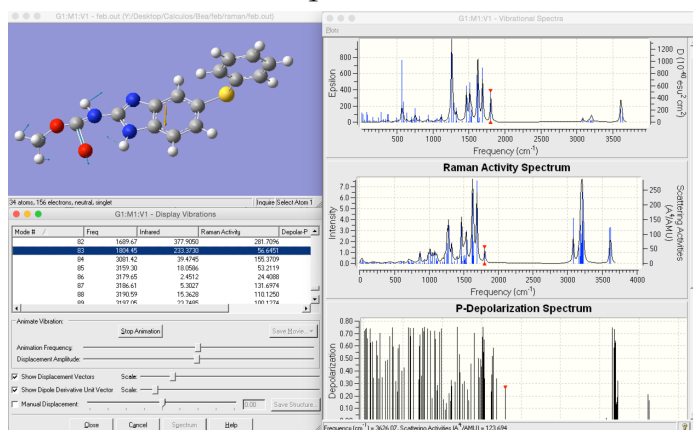
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2. Métodos Teóricos

■ Cálculos *ab initio* o *Primeros Principios*

■ DFT

■ Gaussian

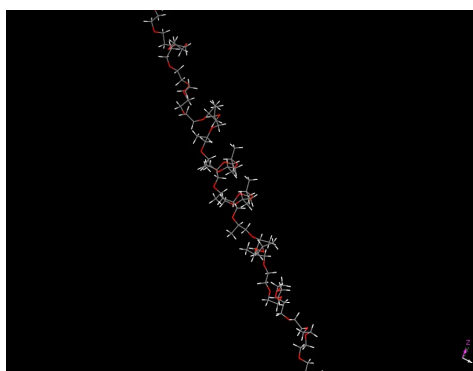
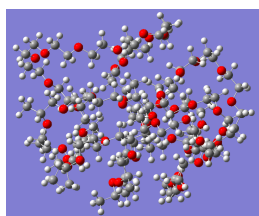
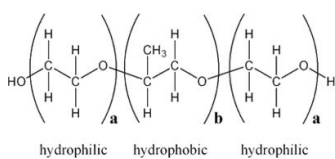


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Poloxamer P184

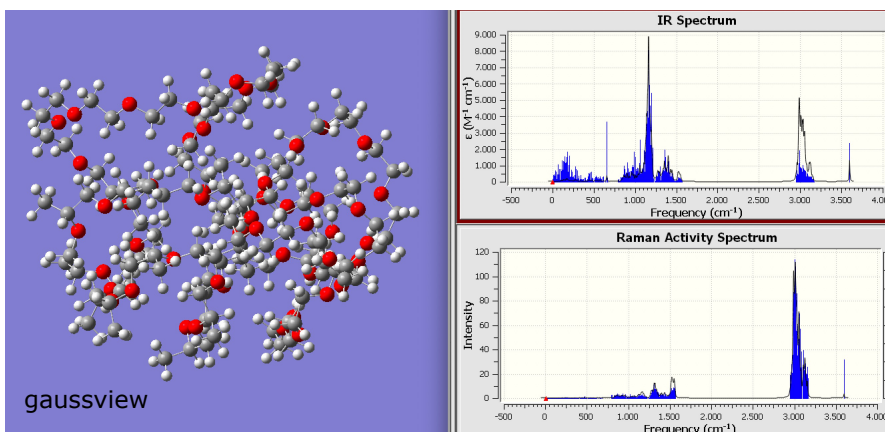


poly(ethyleneoxide)/poly(propyleneoxide)/poly(ethylene oxide)
Molecular Dynamics Simulation

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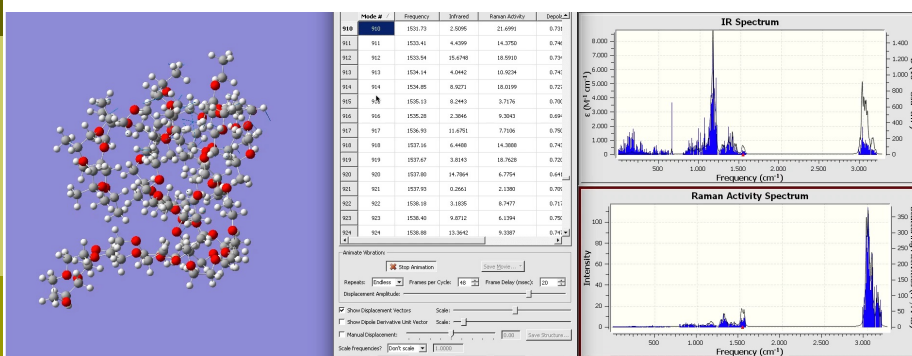
Poloxamer P184 Simulation



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Poloxamer P184 Simulation

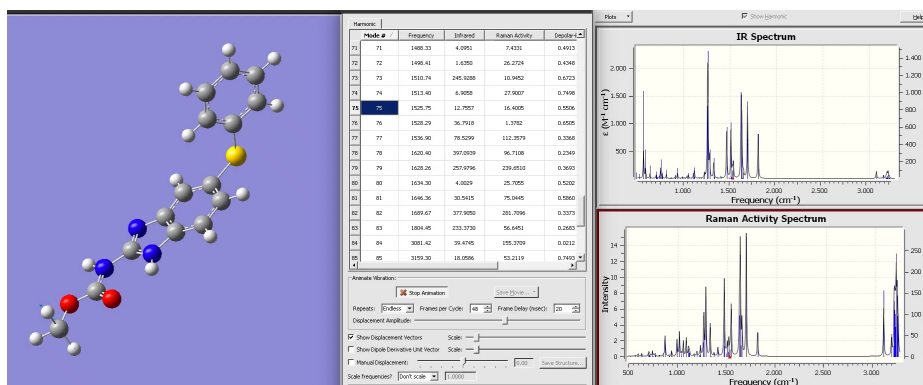


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FBZ Simulation



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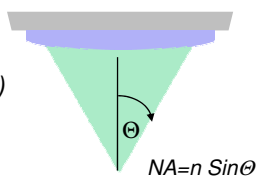
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3. Microscopia Óptica Clásica

What is the resolution limit of a classical optical microscope?

Ernst Abbé (1840-1905): diffraction theory

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



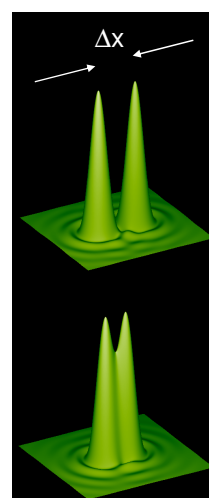
air: $NA \leq 0,95$ (340nm @ 532nm exc.)

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

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

$\text{resolution limit} \approx \lambda / 2$

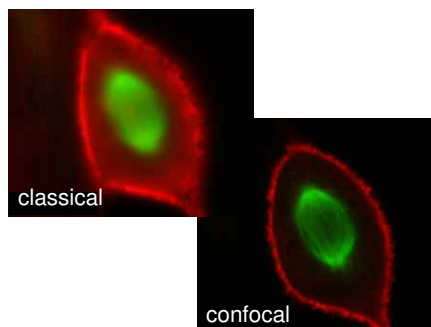
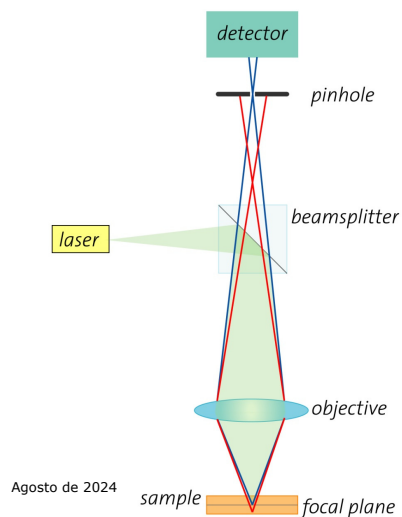
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3. Microscopia Confocal

confocal microscopy



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

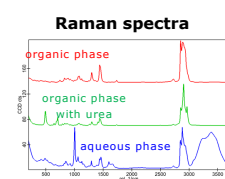
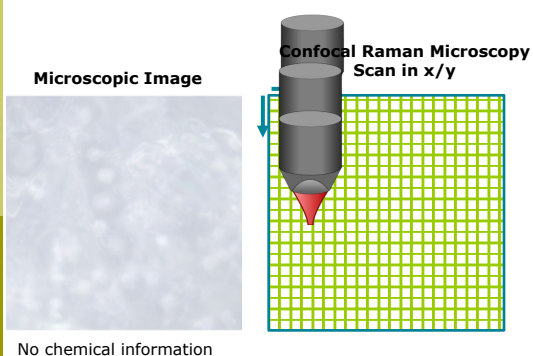
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spatial, but NO chemical information

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Confocal Raman imaging (2D)

- Example: Emulsion (hand cream with urea)

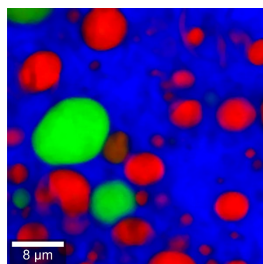


Chemical information

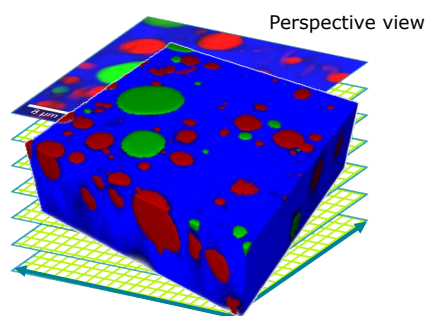
Chemical information & spatial resolution

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Raman imaging – 3D



2D Raman image



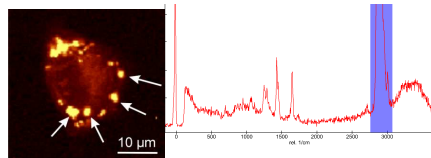
3D Raman image

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3. 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

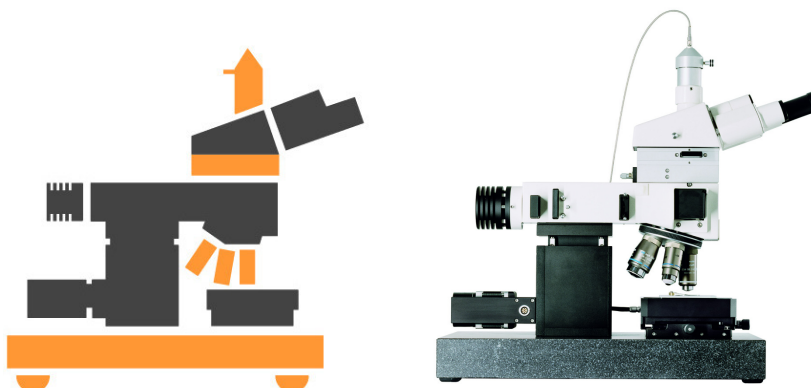


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 ANII- Proyecto EQC_X_2012_1_14

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3. Microscopio Confocal + Espectrómetro Raman



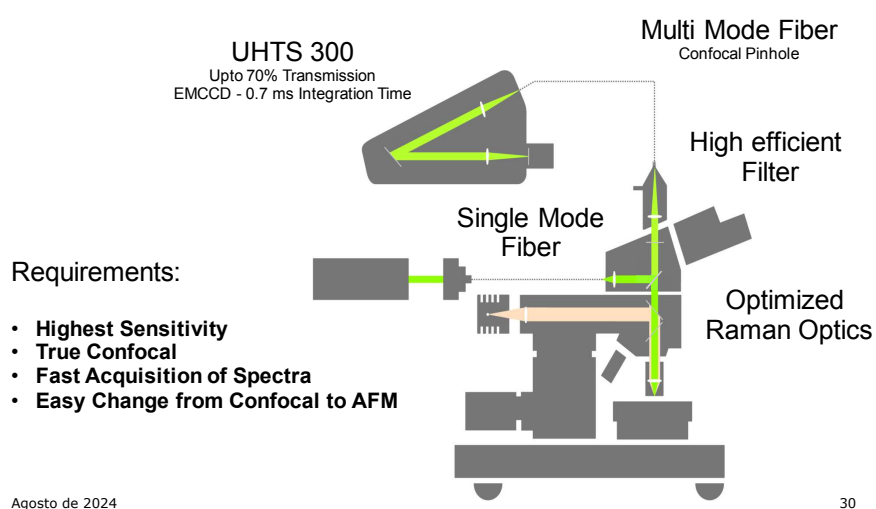
Imaging with chemical sensitivity

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3. Esquema

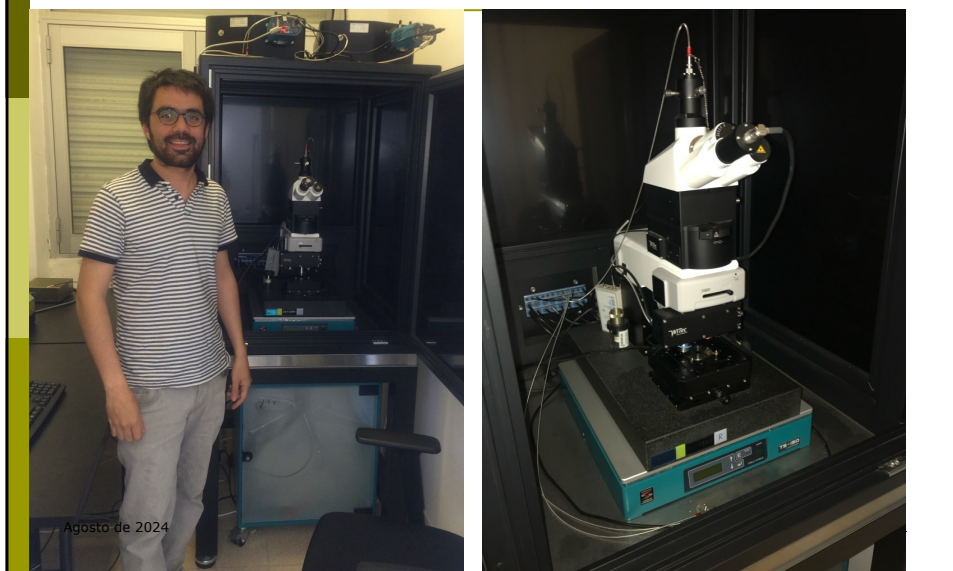


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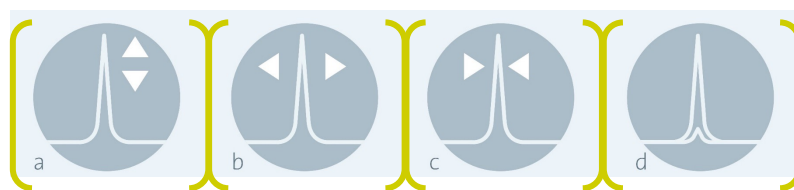
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3. Disposición



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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

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3. Aplicaciones

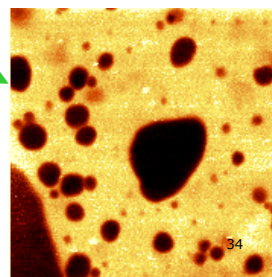
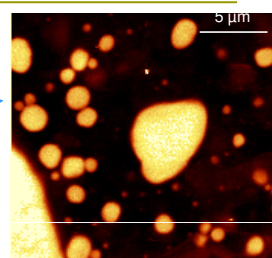
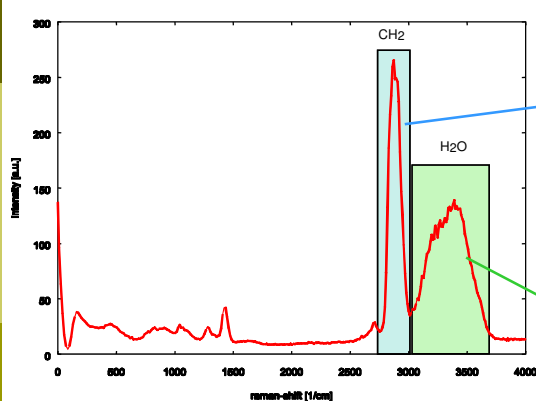
Microscopia Raman Confocal

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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)

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50ms/spectrum

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Reconstrucción Imagen Raman 3D

Sample: Oil/Water Emulsion

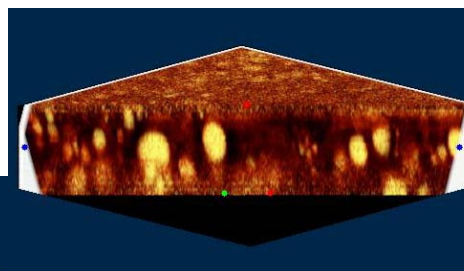
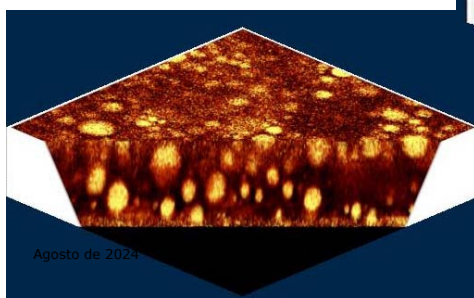
Evaluated Band: CH-Stretching

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

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Blend PMMA+SBR

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

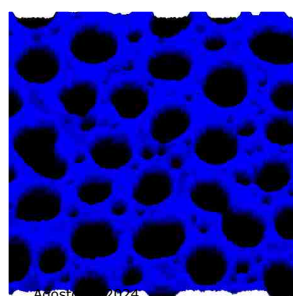
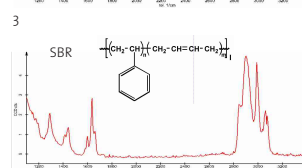
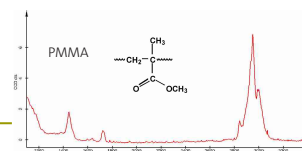
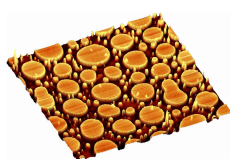


Fig. 9: Color coded Raman image of SBR resulting from the integral intensity of each spectrum.

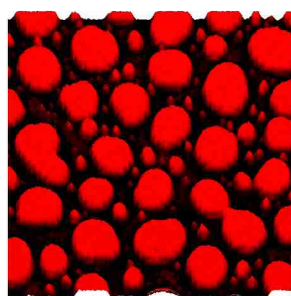


Fig. 10: Color coded Raman image of PMMA resulting from the integral intensity of each spectrum.

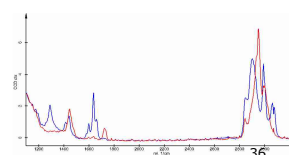
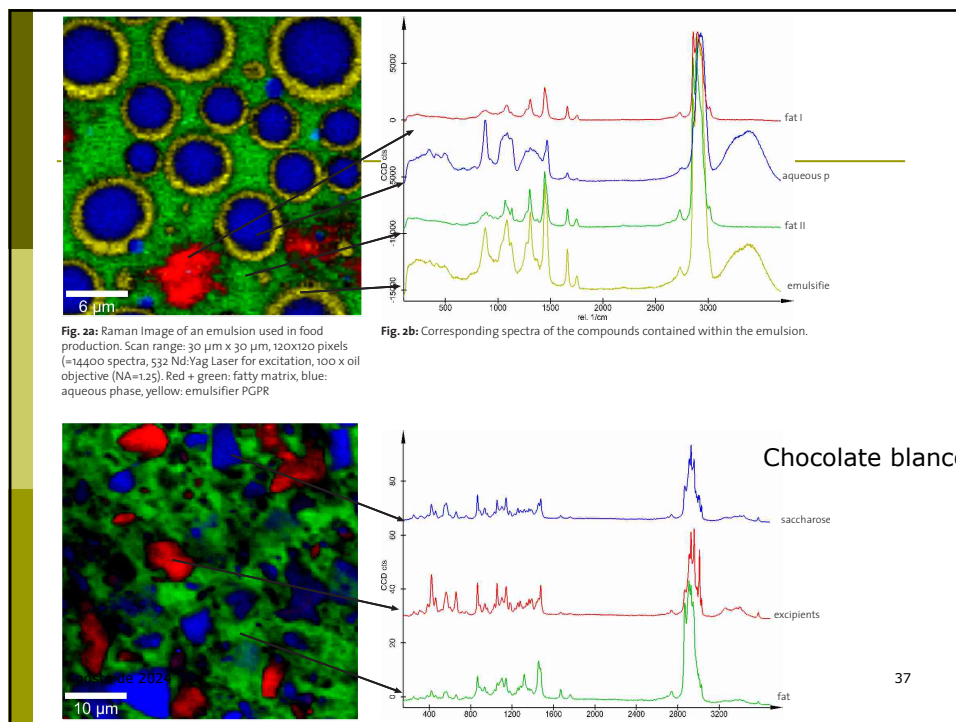


Fig. 11: Corresponding spectra: SBR (blue) and PMMA (red).

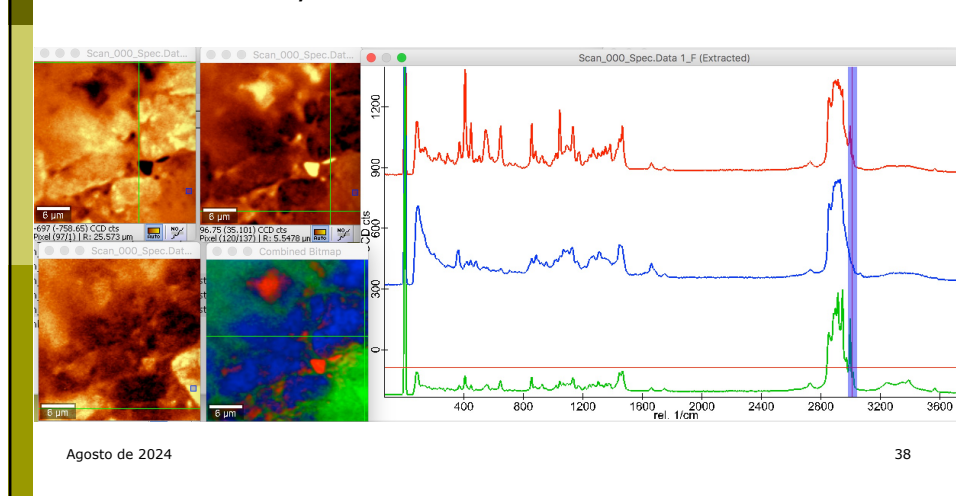
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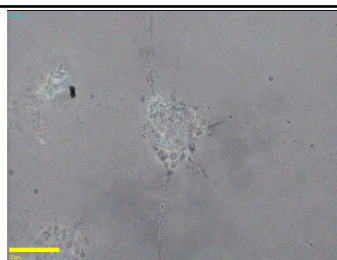
Chocolate Blanco (toblerone)

■ Sacharose, Maltose and Fats

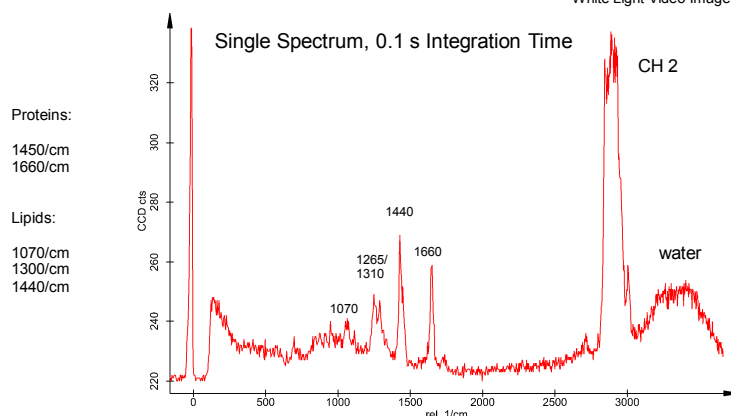


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Células Vivas: células epiteliales de Rata

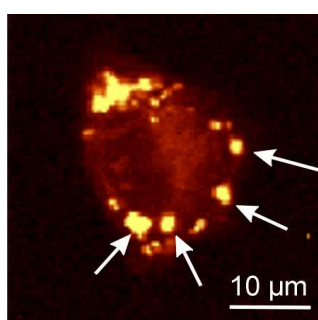
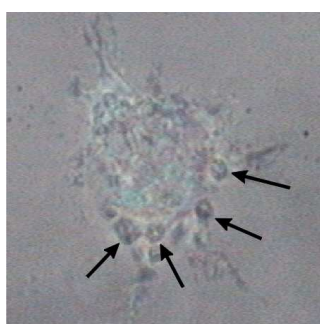


White Light Video Image

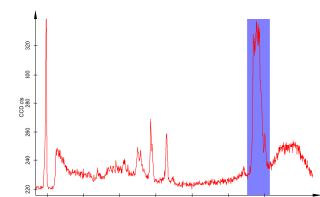


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Células epiteliales en Buffer



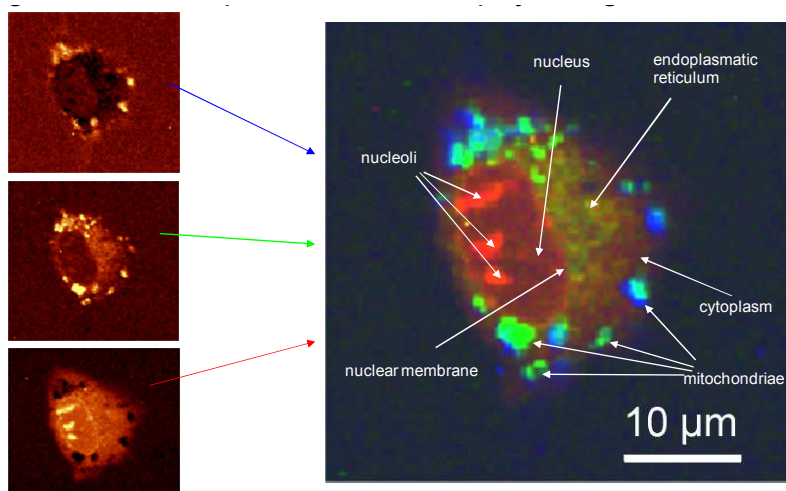
Intensity in the CH 2 stretching band
(2800 - 3000 / cm)



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

40

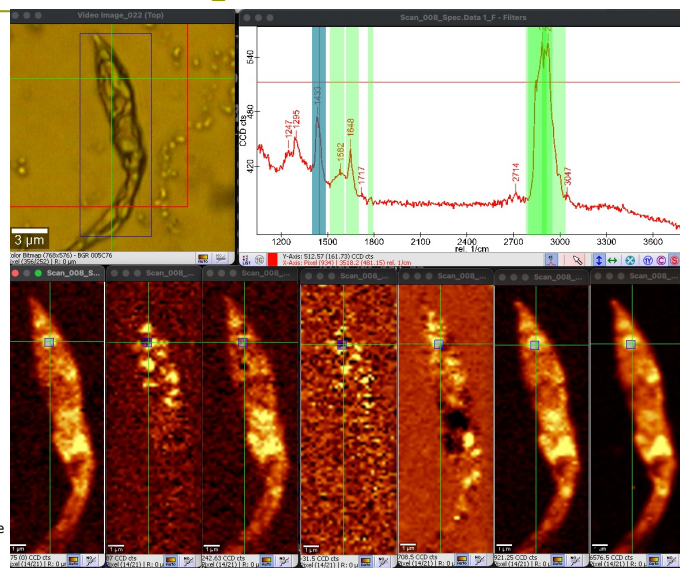
Imagen reconstruida



41

T. Cruzi: unpublished

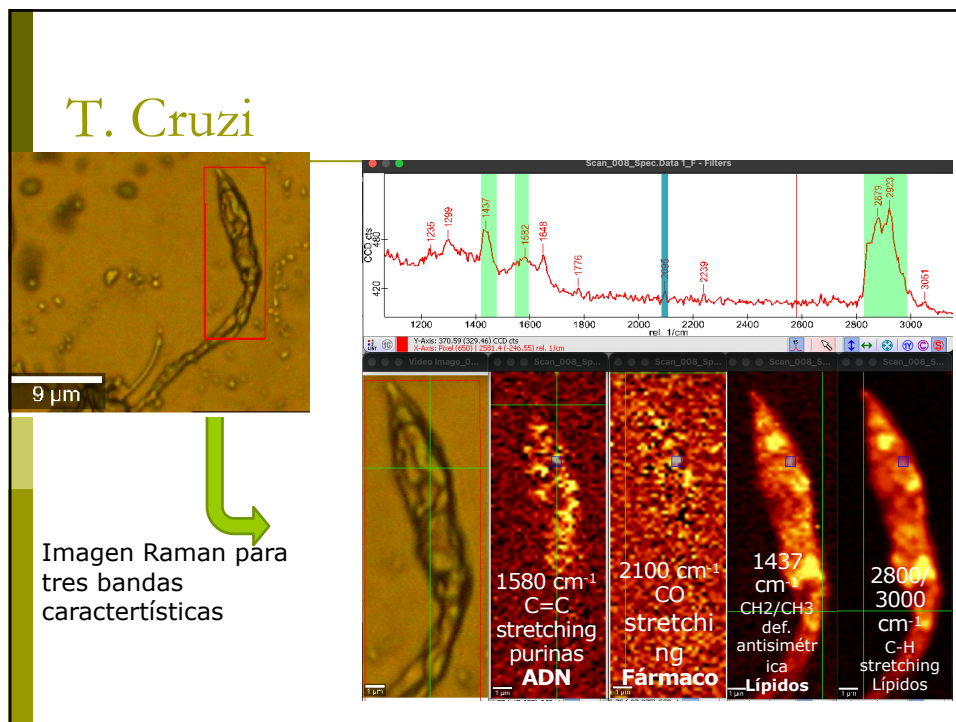
Dr. D. Gambino



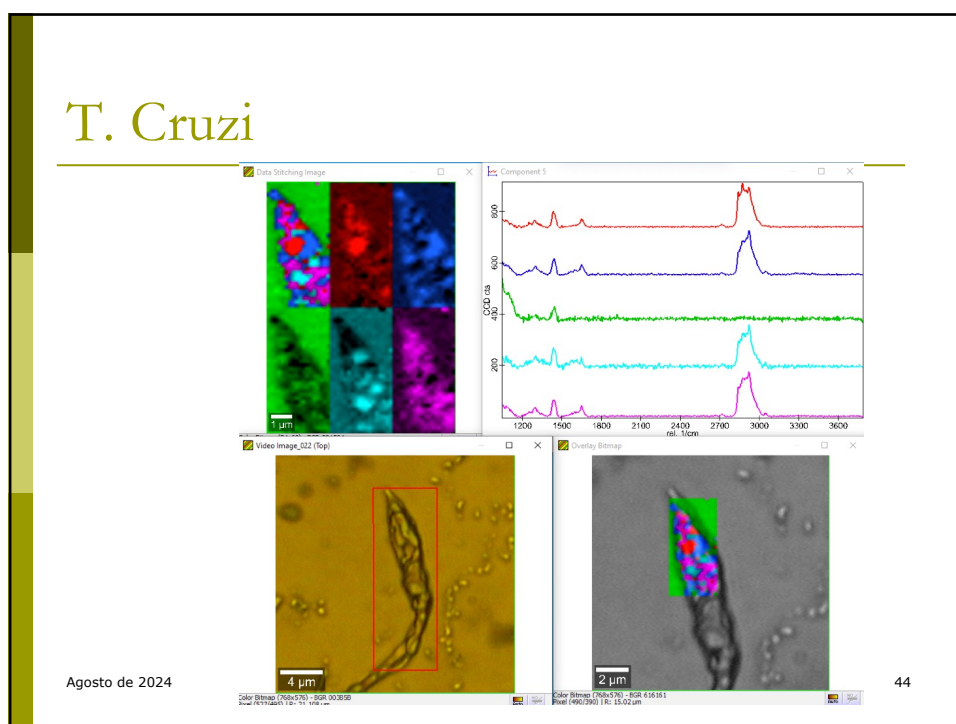
Agosto de

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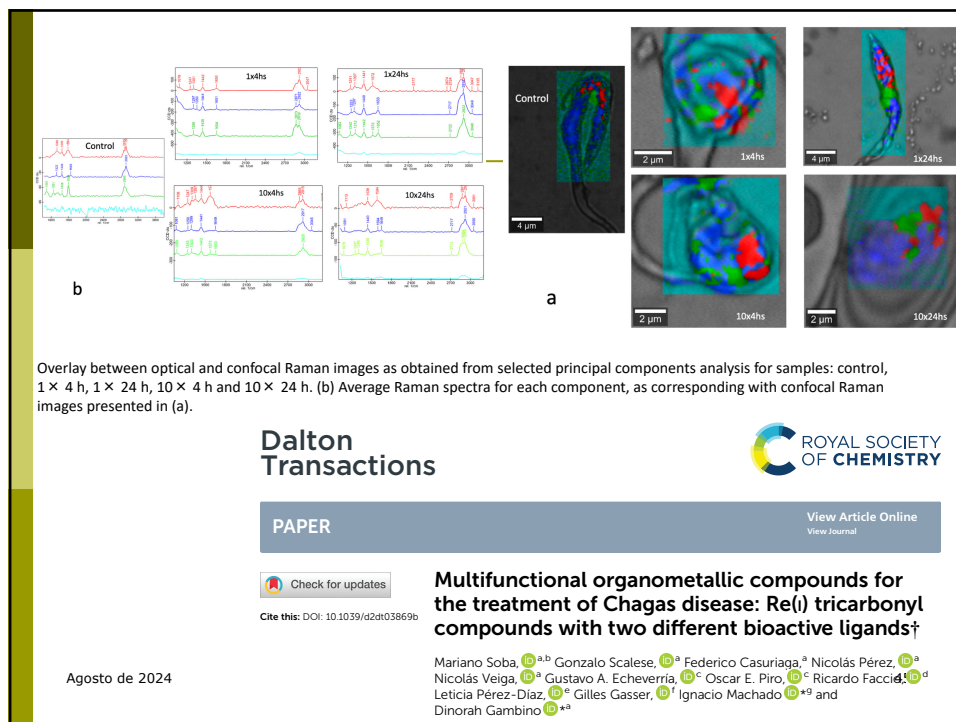
42



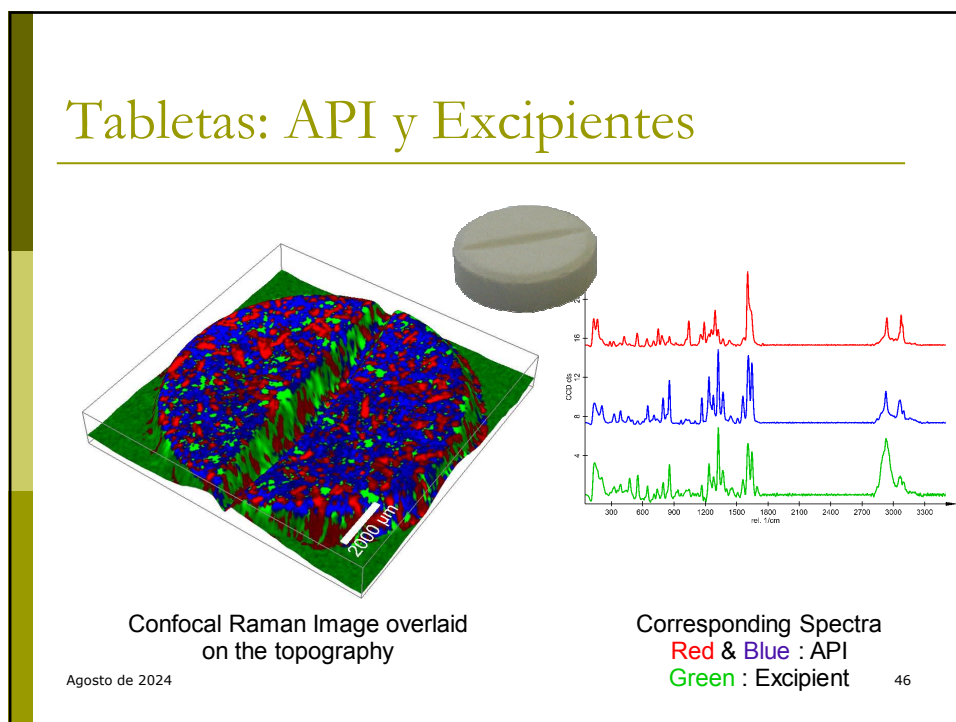
43



44



45



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Experiencias

WITec alpha300RA

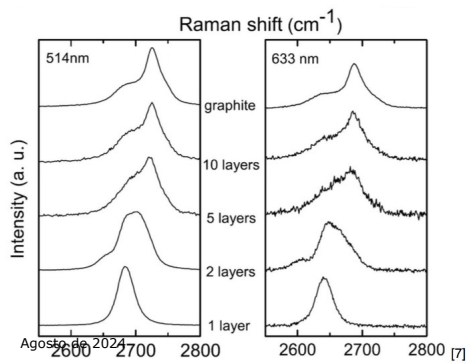
Agosto de 2024

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Ejemplo 4: Grafeno

"Position and fwhm of the G and 2D peak(...)confirm presence of single-layer graphene"

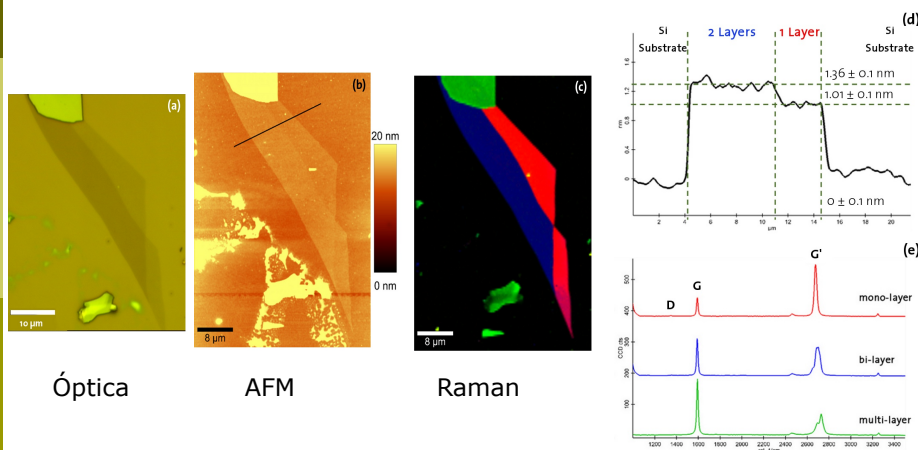


- Position of 2D peak depends on number of layers
- Additional layers increase FWHM of 2D peak (more e^- states for resonant scattering)
- Occurrence of peaks at 1350 cm^{-1} (D) and 1620 cm^{-1} (D') reveal defects of graphene sample

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Ejemplo 4: Grafeno y MLG

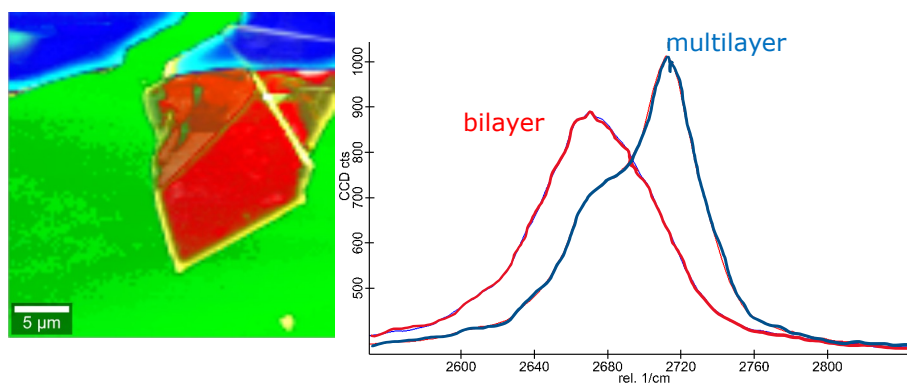


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Ejemplo 4: Grafeno

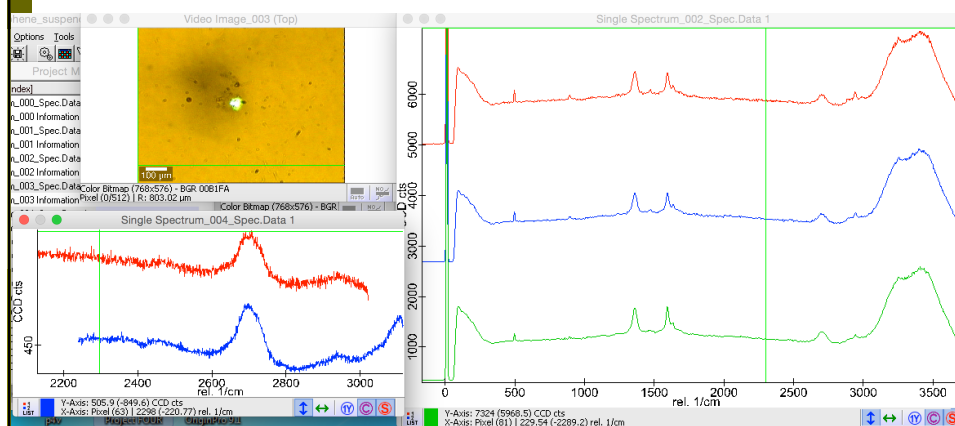


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Ejemplo 4: Grafeno en Solución



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Ejemplo 5: Nanotubos de Carbono

Fig. 5.1 Different carbon allotropes with nanometer-scale phases ranging from 0D C60 fullerenes, 1D carbon nanotubes, 2D graphene, to 3D diamond and graphite

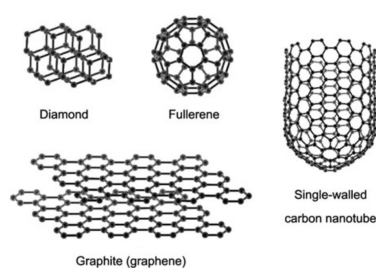
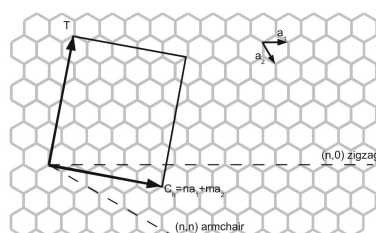


Fig. 5.2 The vector C_h describes how a sheet of graphene is rolled up to create a carbon nanotube [6]



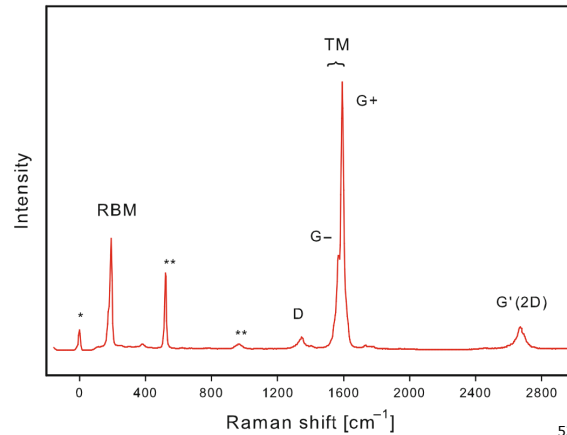
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Ejemplo 5: Nanotubos de Carbono

Fig. 5.3 Typical Raman spectrum of an individual single-walled carbon nanotube recorded with a confocal micro-Raman spectroscope using a green 532 nm laser. The feature marked “*” is the attenuated elastically scattered Rayleigh line. Features marked “**” come from the Si support

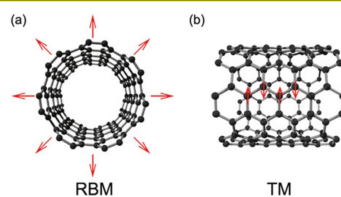


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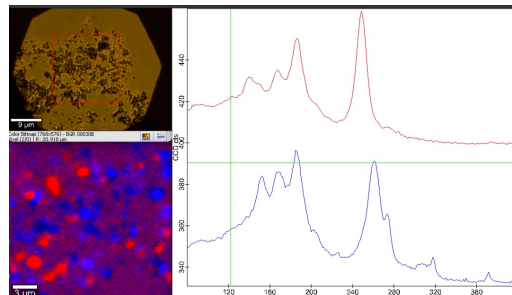
53

Ejemplo 5: Nanotubos de Carbono



$$\omega_{\text{RBM}} = \frac{c_1}{d_t} + c_2$$

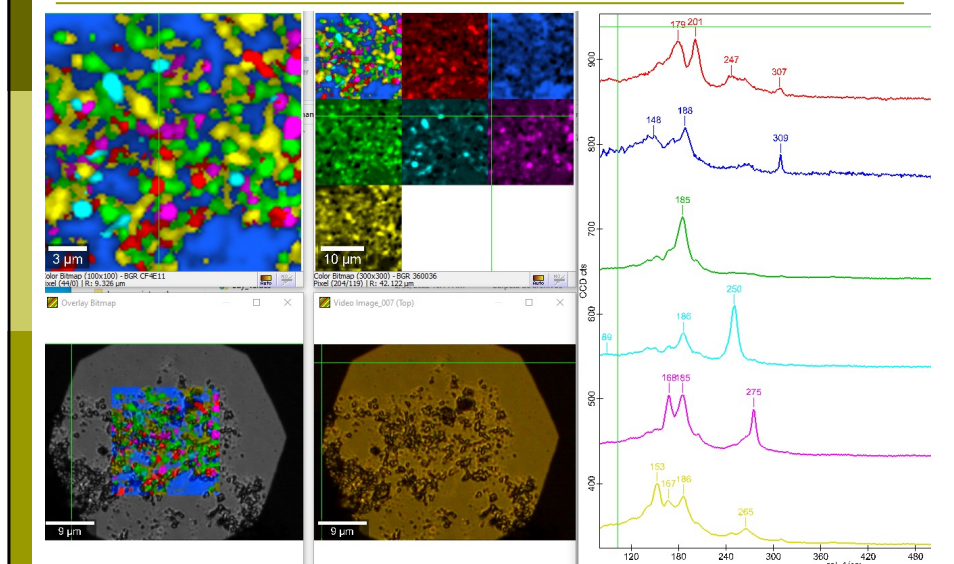
Fig. 5.4 Raman-active vibrational modes of carbon nanotubes. (a) The so-called radial breathing mode (RBM) is a prominent feature of the tubular nanostructure and not present in planar graphite. The Raman shift of the RBM is approximately inversely proportional to the tube diameter. (b) An example of a tangential mode (TM). In analogy to graphitic Raman modes it is often also called G-mode or high-energy mode (HEM)



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MCR: CNT y RBM



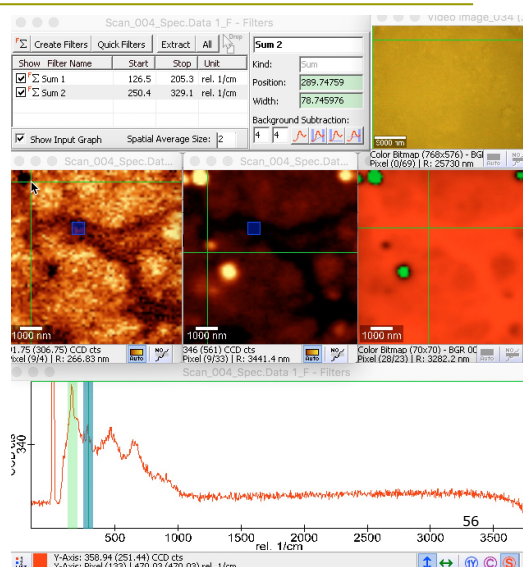
55

Ejemplo 7: Caracterización de NT

Ejemplo de Funcionamiento:

- Muestra de NT calentados a $T=200^{\circ}\text{C}$.
- Presencia de Nanopartículas de Anatasa en muestra de NT.

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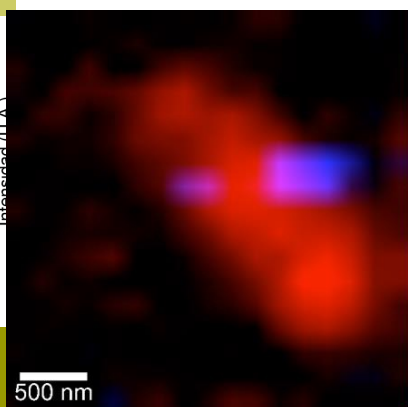
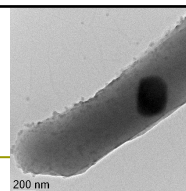
56

Ejemplo 8: Interacción de Bacterias con Ag-NP

Evaluación de la Interacción PchNPs-Microorganismo.

***Escherichia coli*.**

Microscopía Raman Confocal



Corrimiento Raman (cm^{-1}) ($\pm 4\text{cm}^{-1}$)	Asignación de bandas	Comparación con células tratadas con PchNPs
624	Ácidos nucleicos	Disminuye
810	Aminoácidos	Disminuye
982	Polisacáridos	Igual
1186	Polisacáridos	Disminuye
1243	Polisacáridos	Disminuye
1308	Aminoácidos o ácidos nucleicos	Disminuye
1344	Aminoácidos o ácidos grasos	Igual
1593	Ácidos grasos	Disminuye

Asignación según De Gelder et al., J Raman Spectroscopy, 2007, 38(9), 1133-1147

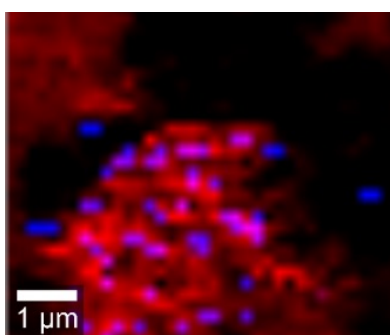
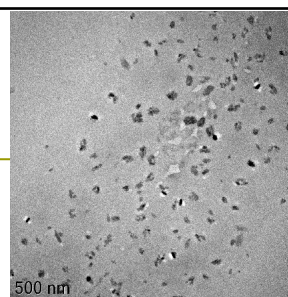
57

Ejemplo 8: Interacción de Bacterias con Ag-NP

Evaluación de la Interacción PchNPs-

Microorganismo. ***Staphylococcus aureus*.**

Microscopía Raman Confocal



Corrimiento Raman (cm^{-1}) ($\pm 4\text{cm}^{-1}$)	Asignación de bandas	Comparación con células tratadas con PchNps
663	Ácidos nucleicos	Disminuye
790	Aminoácidos	Disminuye
879	Aminoácidos	Disminuye
929	Polisacáridos	Disminuye
1116	Polisacáridos	Disminuye
1325	Aminoácidos o ácidos nucleicos	Disminuye
1538	Ácidos grasos	Disminuye

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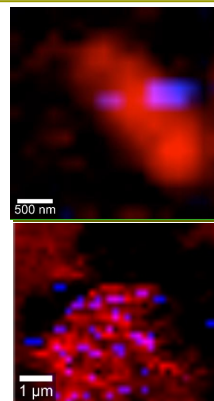
Asignación según De Gelder et al., J Raman Spectroscopy, 2007, 38(9), 1133-1147

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Ejemplo 8: Interacción de Bacterias con Ag-NP

Table 1. Raman band assignment of microbial cells, control and treated cells with PchNPs. The band assignment was made based on previous work [26].

Microorganism	Raman Shift (cm ⁻¹)	Band Assignment	Effect on Treated Cells
<i>E. coli</i>	624	Nucleic acids	Diminishes
	810	Aminoacids	Diminishes
	982	Polysaccharides	No changes
	1186	Polysaccharides	Diminishes
	1243	Polysaccharides	Diminishes
	1308	Aminoacids or nucleic acids	Diminishes
	1344	Aminoacids or fatty acids	No changes
<i>C. albicans</i>	1593	Fatty acids	Diminishes
	670	Nucleic acids	Diminishes
	788	Aminoacids	Diminishes
	885	Aminoacids	Diminishes
	999	Polysaccharides	Diminishes
	1100	Polysaccharides	Diminishes
	1319	Aminoacids or nucleic acids	Diminishes
	1596	Fatty acids	Diminishes



M. B. Estevez, S. G. Mitchell, R. Faccio, S. Alborés,
Mater. Res. Express 6 (2019) 1250

M. B. Estevez, S. Raffaelli, S. G. Mitchell, R. Faccio, S. Alborés,
Molecules 2020, 25, 2023

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Ejemplo 8: Microbial cells + AgNPs

● AgNPs

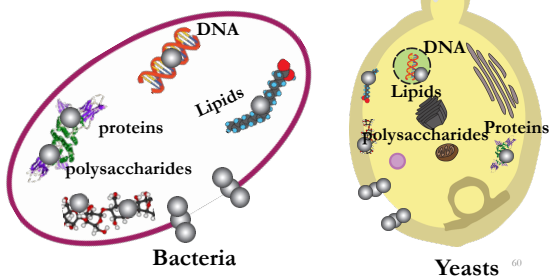


Table 2. Raman band assignment of microbial cells, control and treated cells with PeNPs. The band assignment was made based on previous work [15].

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
<i>S. aureus</i>	1308	Amino acids or nucleic acids	Increases
	993	Polysaccharides	Diminishes
	1092	Polysaccharides	Diminishes
	1327	Amino acids or nucleic acids	Diminishes
<i>C. albicans</i>	1587	Fatty acids	Diminishes
	698	Nucleic acids	Diminishes
	750	Amino acids	Diminishes
	991	Polysaccharides	No changes
	1318	Amino acids or nucleic acids	No changes
	1597	Fatty acids	Diminishes

M. B. Estevez, S. G. Mitchell, R. Faccio, S. Alborés, **Mater. Res. Express 6 (2019) 1250**

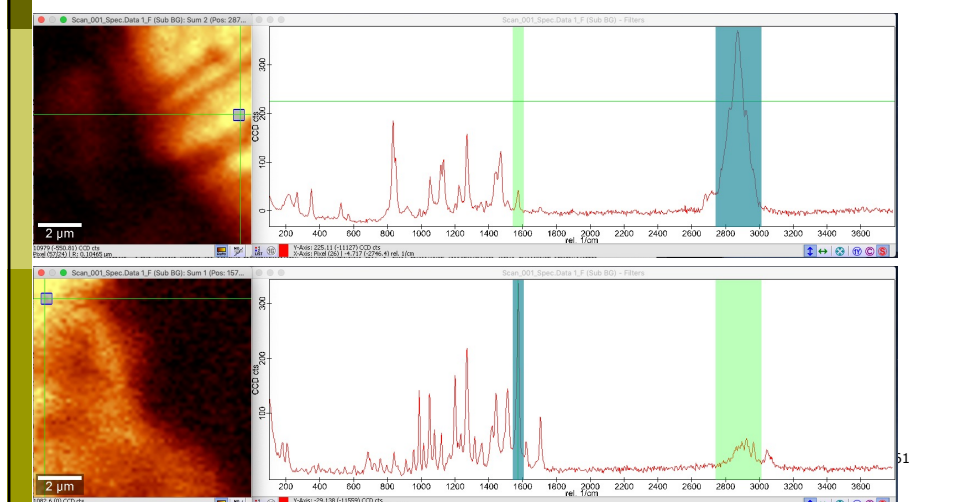
27/8/24

M. B. Estevez, S. Raffaelli, S. G. Mitchell, R. Faccio, S. Alborés,
Molecules 2020, 25, 2023

60

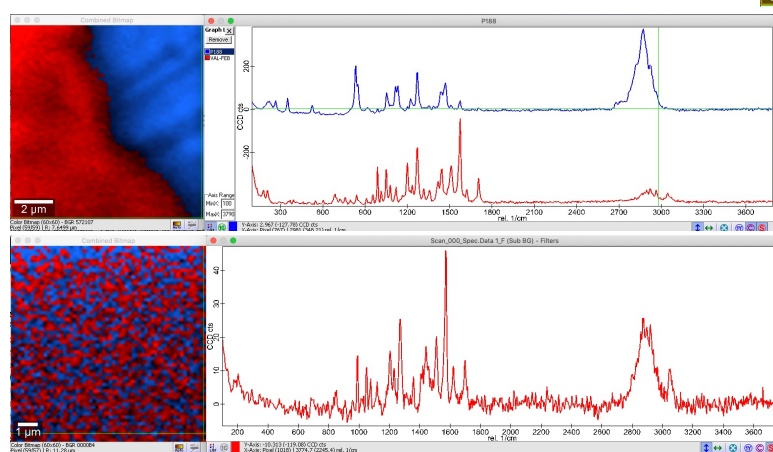
Ejemplo 9: Formulaciones Farmacéuticas

Val-Feb Mezcla Física con P188



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Ejemplo 9: MF vs Dispersión



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Ejemplo 9: MF vs Dispersiones

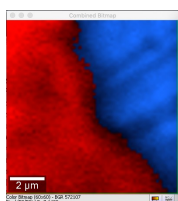
Journal of Pharmaceutical Innovation
<https://doi.org/10.1007/s12247-017-9306-9>

ORIGINAL ARTICLE



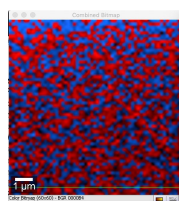
The Impact of Solid Dispersion on Formulation, Using Confocal Micro Raman Spectroscopy as Tool to Probe Distribution of Components

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



MF

Agosto de 2024



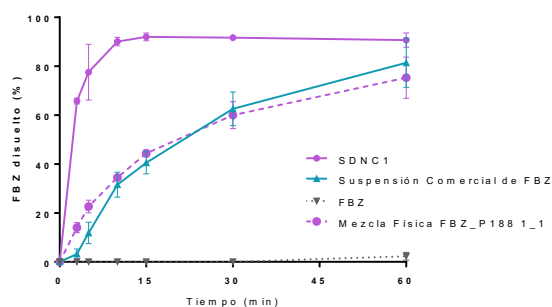
Dispersion de NC

Nanocrystals of novel valerolactam-fenbendazole hybrid with improved biopharmaceutical behavior. **Elisa Melian et al**, AAPS Pharm SciTech 2020 21(7):237

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Example 9: Raman for PM vs NC

□ Dissolution Profile (in-vitro)



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

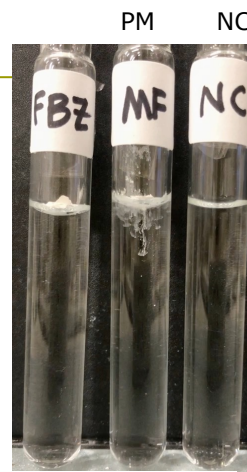
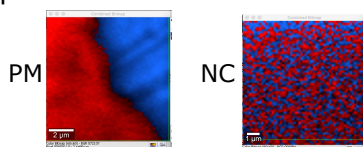
- Markedly increased dissolution rate compared with the simple mixture of the components.

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Example 9: Nanocrystals

Conclusions

- We obtained Nanocrystals (NC)
- FBZ keeps cristallinity
- Poloxamer reduces cristallinity.
- Better dispersions API/EXC in NC
- Improvement in Dissolution Rate Profile.



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

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

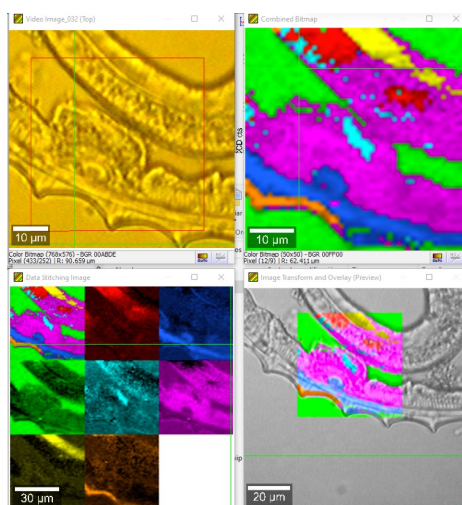
27/8/24

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}

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Nematodo: *H. contortus*

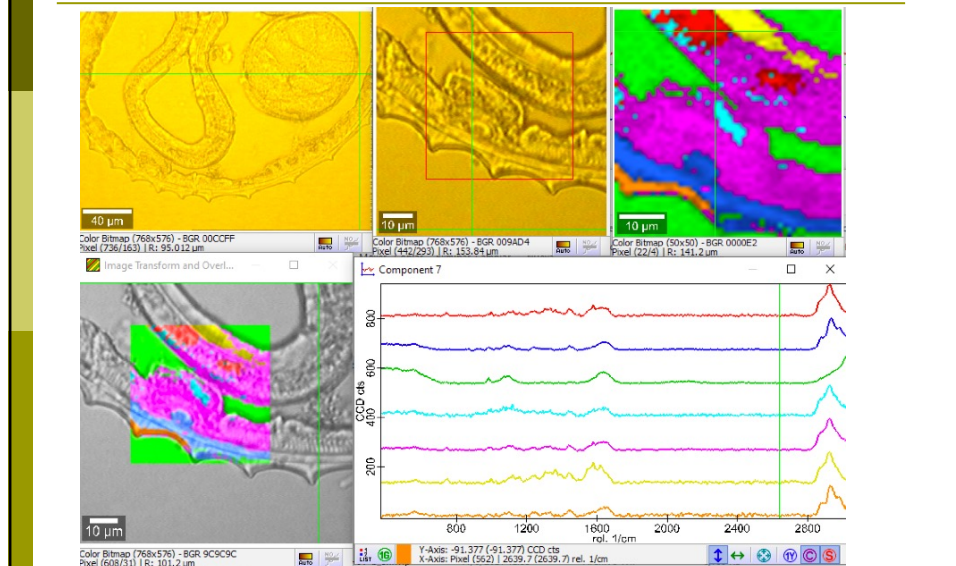
Corte Transversal: cutícula e intestino



Dra. Beatriz Munguía
Q.F. Elisa Melián
Área Farmacología, CIENFAR, FO

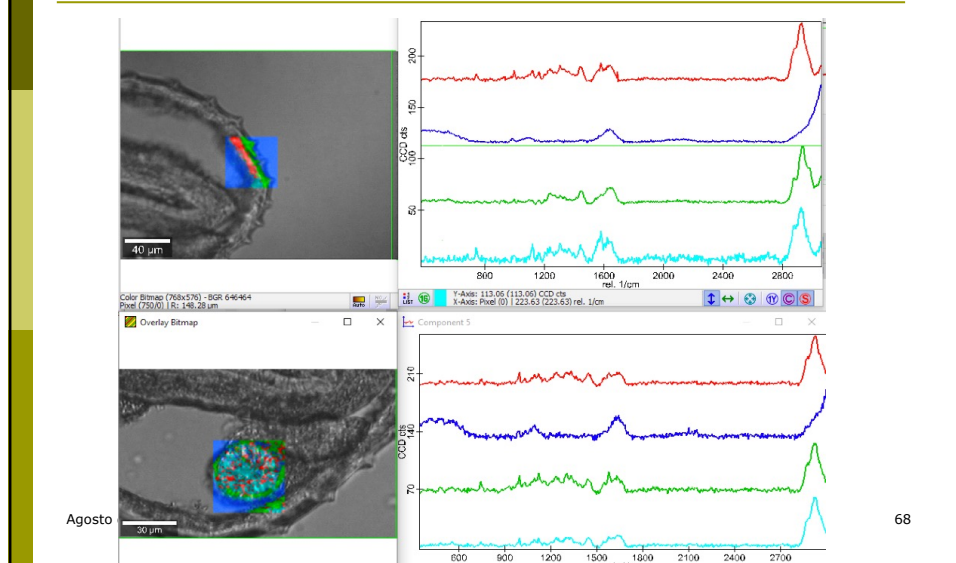
66

Nematodo: *H. contortus*



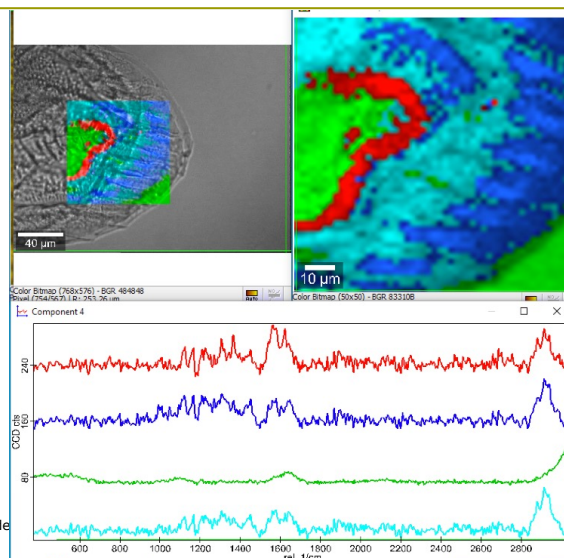
67

Nematodo: *H. contortus*



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Nematodo: *H. contortus*



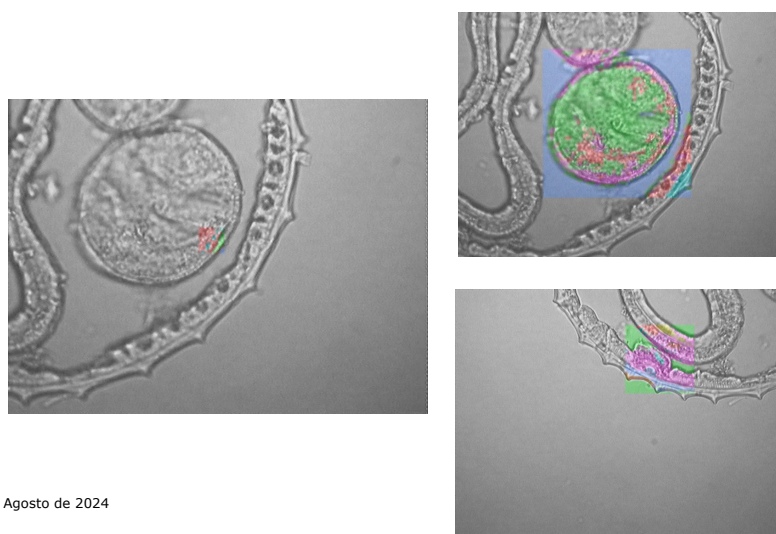
Burza:
aparato
reproductor
masculino

Agosto de

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Nematodo: *H. contortus*

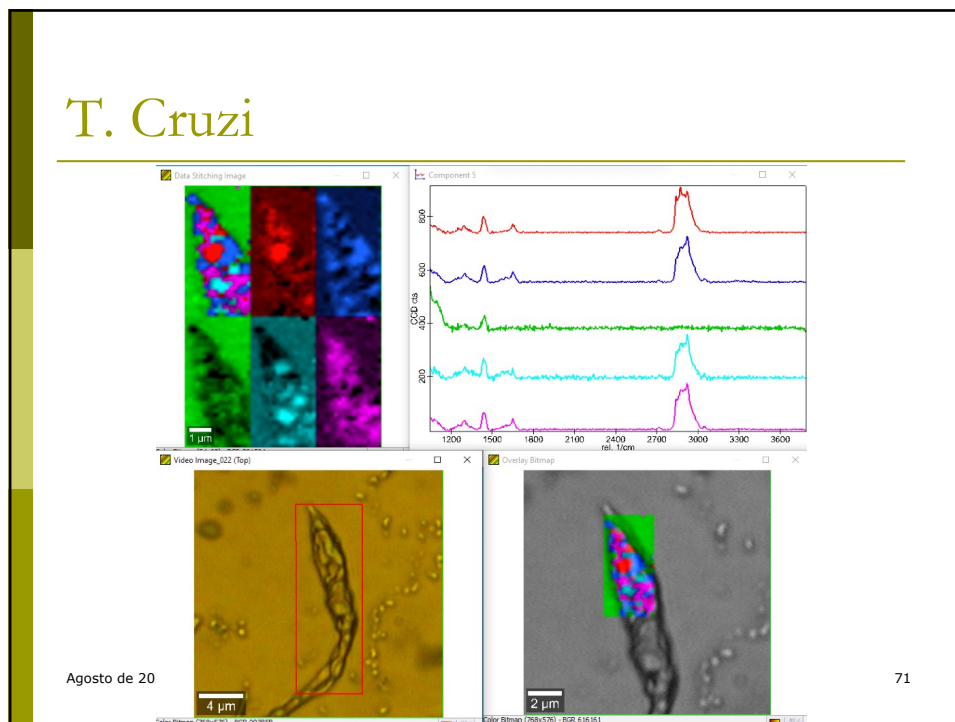


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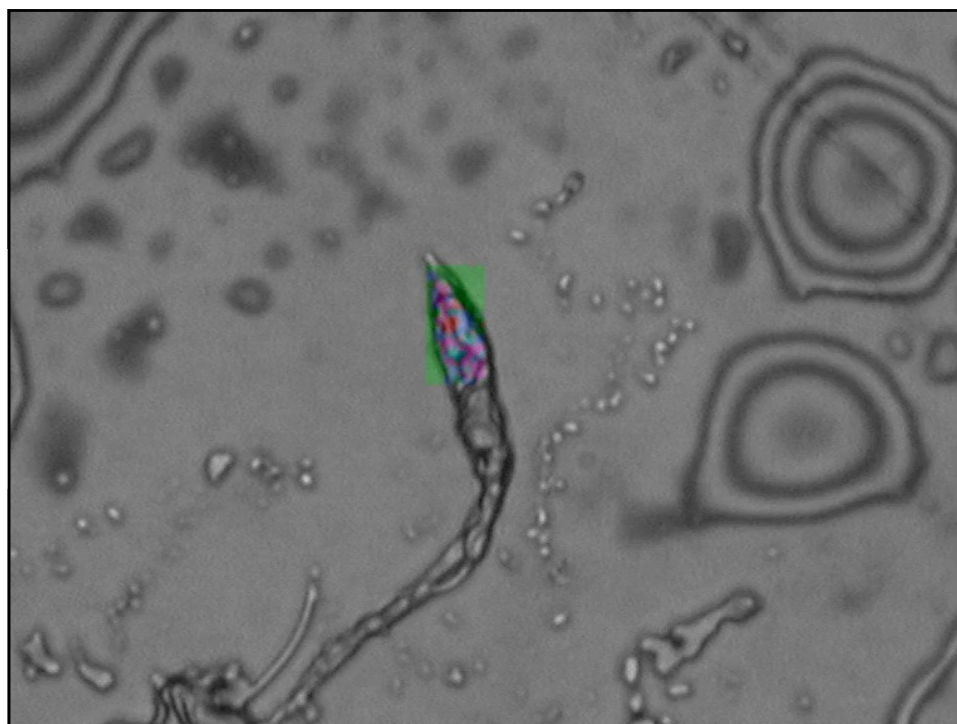
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T. Cruzi



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Resumen: Micro Raman Confocal

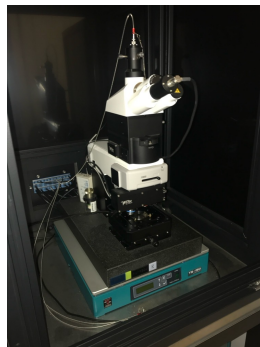
- ▣ No Destructivo (en principio)
- ▣ Identificación Química Directa
- ▣ Identificación de Propiedades de Materiales
- ▣ Preparación sencilla de muestra
- ▣ Sólidos y Líquidos
- ▣ Detección 3-dimensional
- ▣ Combinación de detección química con AFM

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4. Microscopía de Fuerza Atómica



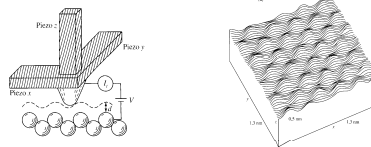
Agosto de 2024

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4. Introducción

■ Historia



Microscopio de efecto túnel (STM)

Microscopio de fuerza atómica (AFM)

Premio Nobel Física 1986



G. Binnig



H. Rohrer

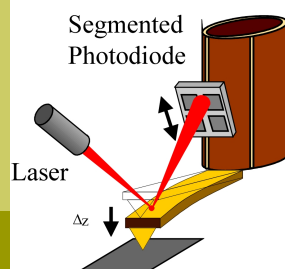
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4. Introducción

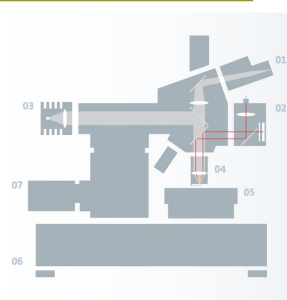
■ Esquema



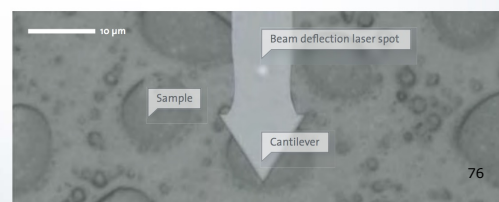
Agosto de 2024

✖ 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



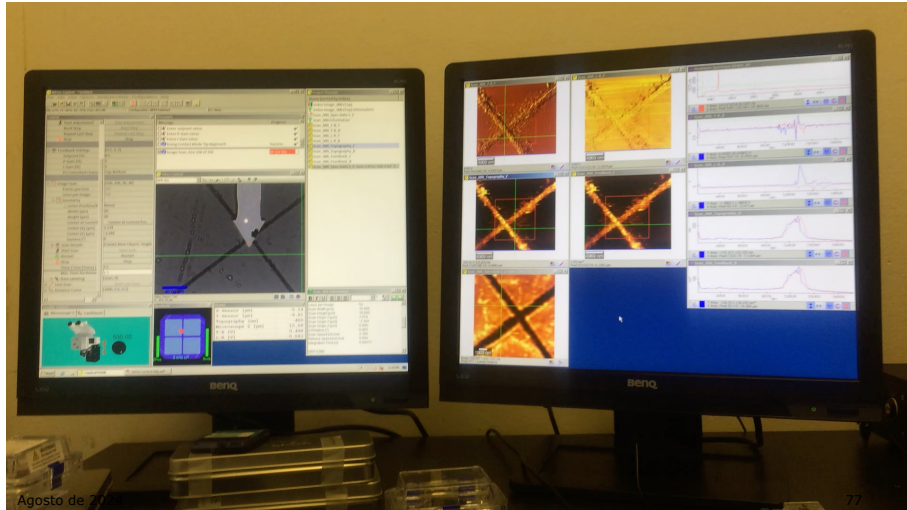
✖ SIMULTANEOUS CANTILEVER AND SAMPLE VIEW



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4. Introducción: Raman & AFM Dual

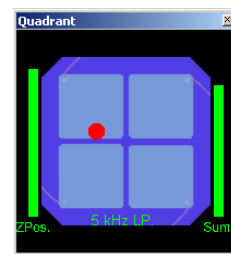
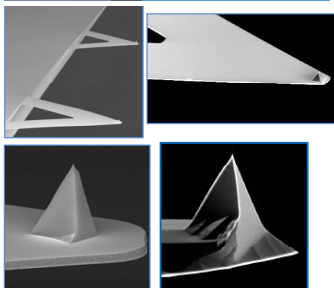
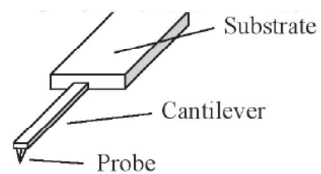
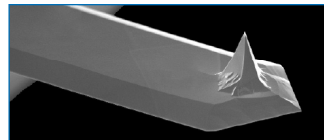


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6. Introducción

■ Partes relevantes:

- Sustrato
- Cantilever
- Probo
- Fotodiodo



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6. 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)

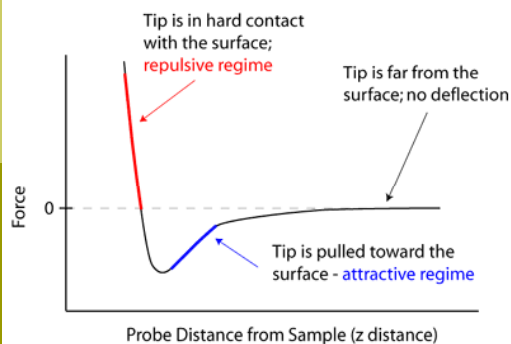
Agosto de 2024

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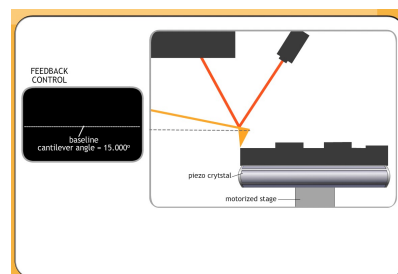
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6. Modo Contacto

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



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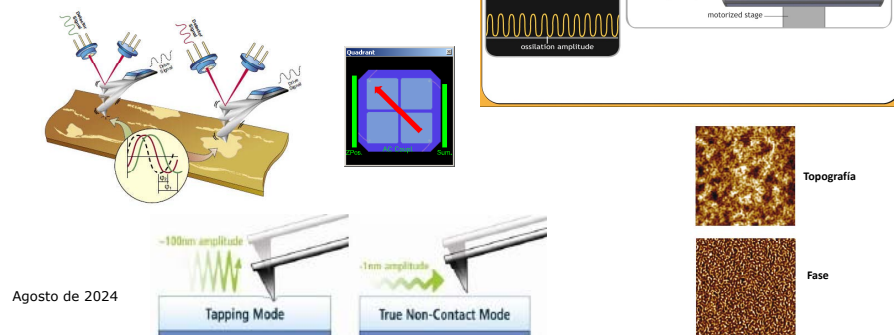
80

http://virtual.itg.uiuc.edu/training/AFM_tutorial/

80

6. 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

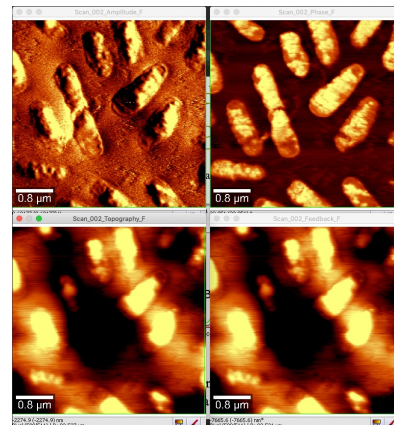


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6. Modo No Contacto e Intermitente

- 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.



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6. 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 ↑

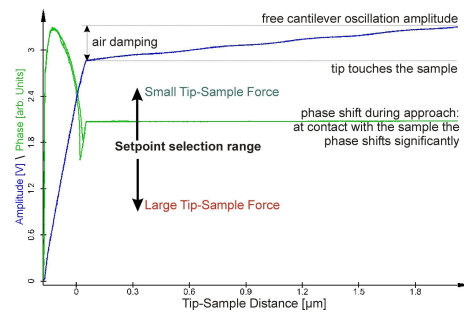


Fig. 1: Phase (green) and Amplitude (blue) as a function of tip→sample distance. The effect of the setpoint selection is also indicated.

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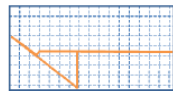
83

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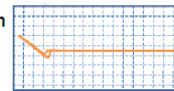
6. Medidas de Fuerza y Adhesión

- ❑ La adhesión se determina localmente mediante curvas de Fuerza.
- ❑ Topografía mecánico.

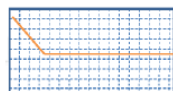
Fuerza de adhesión elevadas



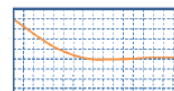
Fuerzas de adhesión pequeñas



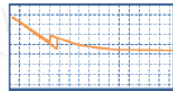
Muestra rígida



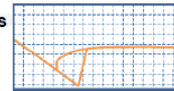
Muestra blanda



Presencia de fuerzas repulsivas de largo alcance



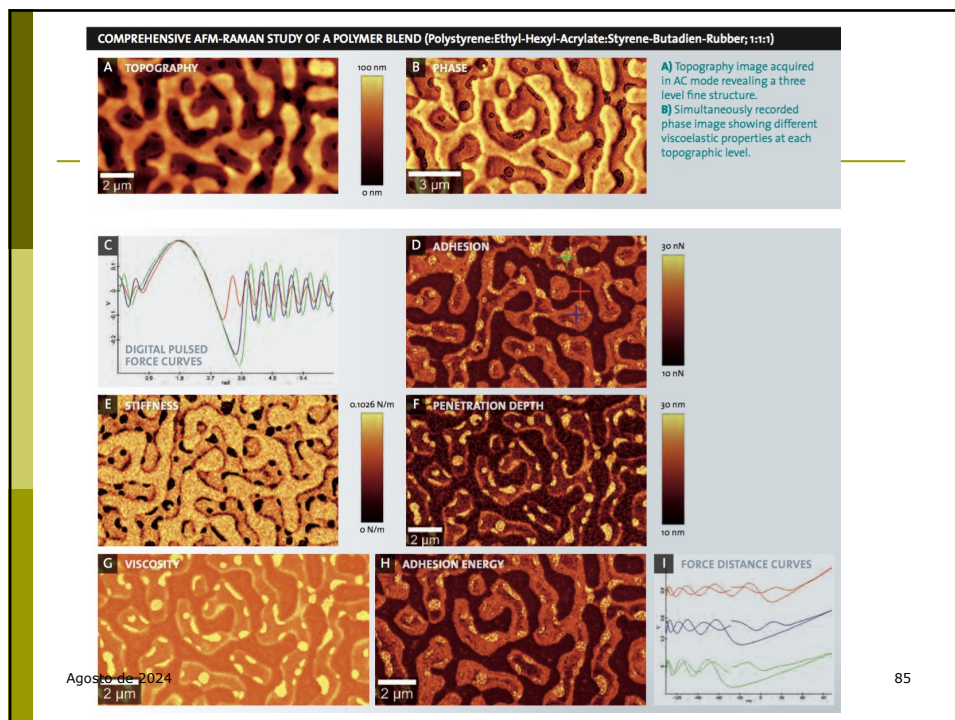
Presencia de fuerzas atractivas de corto alcance



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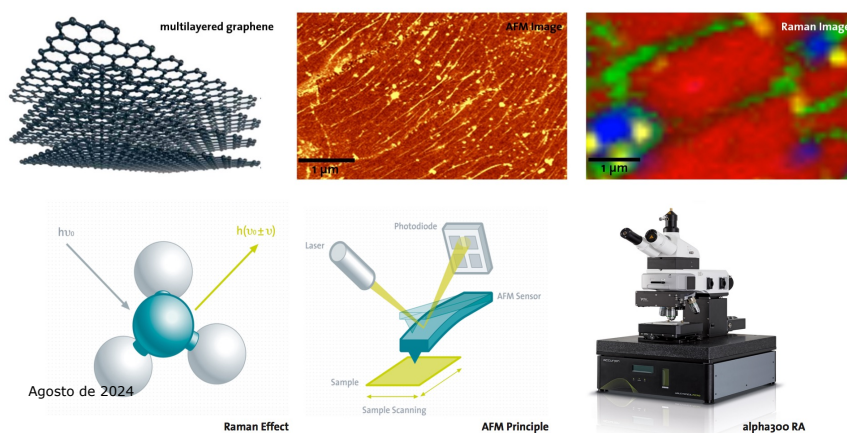
84



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Micro Raman AFM Dual

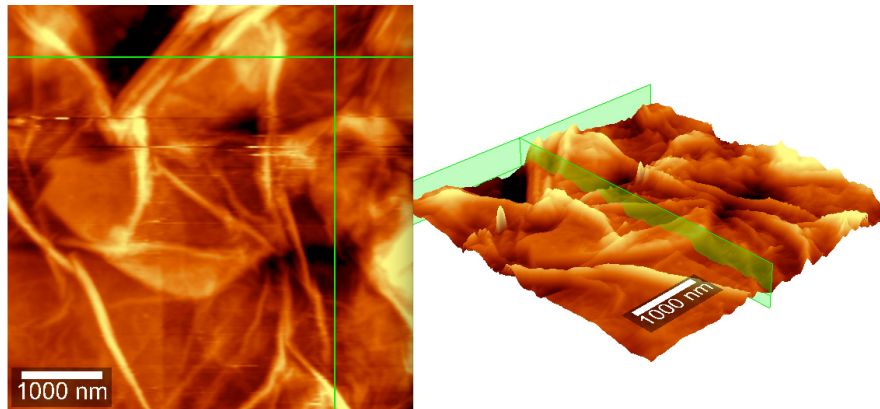
- ▣ Alpha 300R → Alpha 300RA
- FSE_1_2014_1_102008 - ANII



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6. Ejemplos: Modo No Contacto

▣ Reduced Graphene Oxide



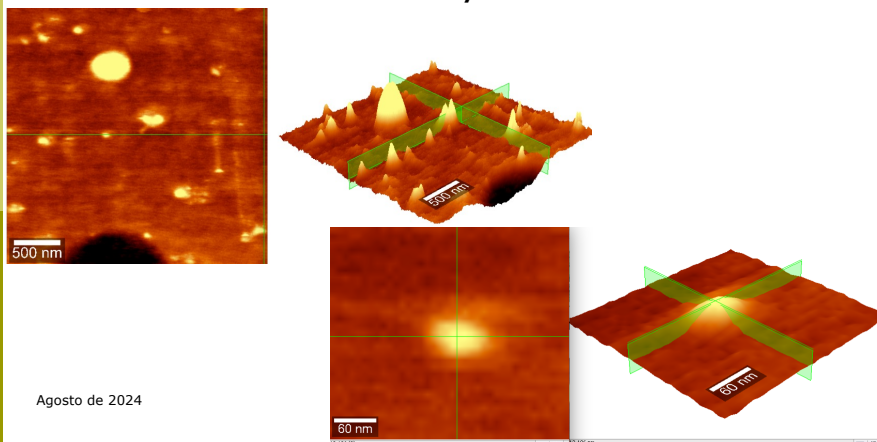
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6. Ejemplos: Modo No Contacto

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



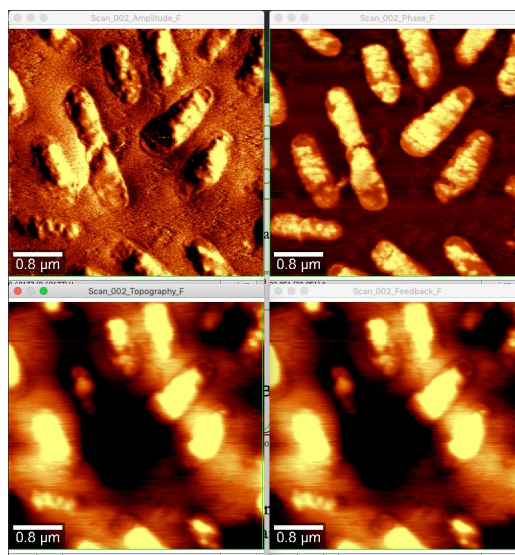
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6. Ejemplos: Bacterias con Ag-NP

■ NC AFM

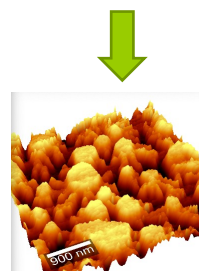
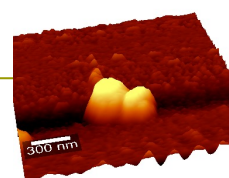
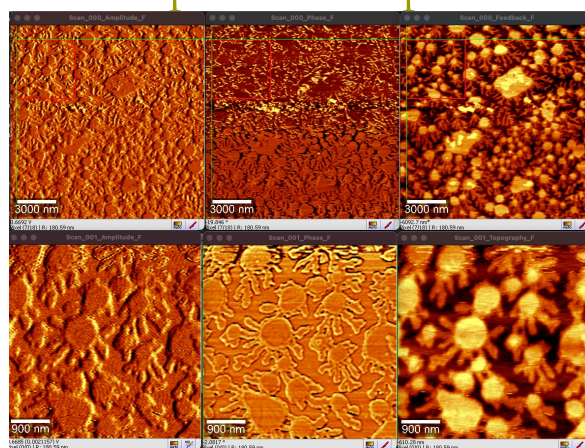
- Amplitud
- Fase
- Topografía
- Feedback



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5. 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

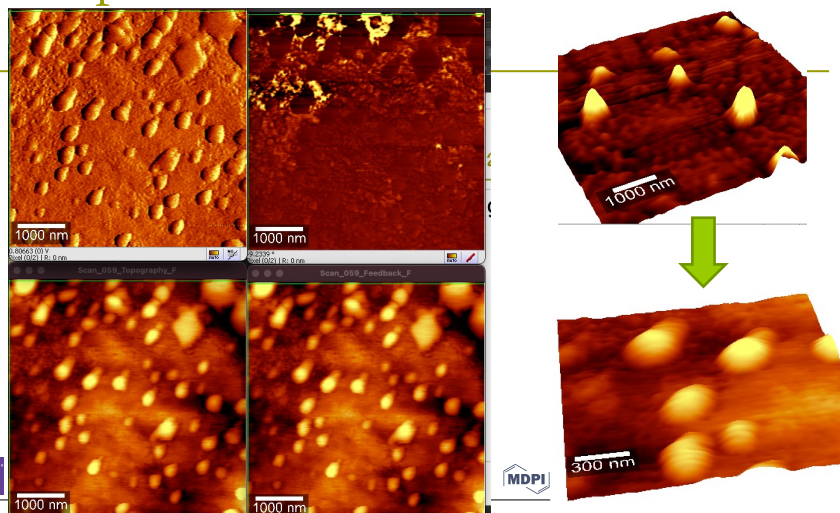
Romina Castelli ¹, Manuel Ibarra ², Ricardo Faccio ³, Iris Miraballes ⁴, Marcelo Fernández ⁵, Albertina Mogliani ⁶, Pablo Cabral ¹, Hugo Cerecetto ^{1,*}, Romina J. Glisoni ^{7,*} and Victoria Calzada ^{1,*}

Pharmaceuticals **2022**, *15*, 15. <https://doi.org/10.3390/ph15010015>

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5. Liposomas funcionalizados



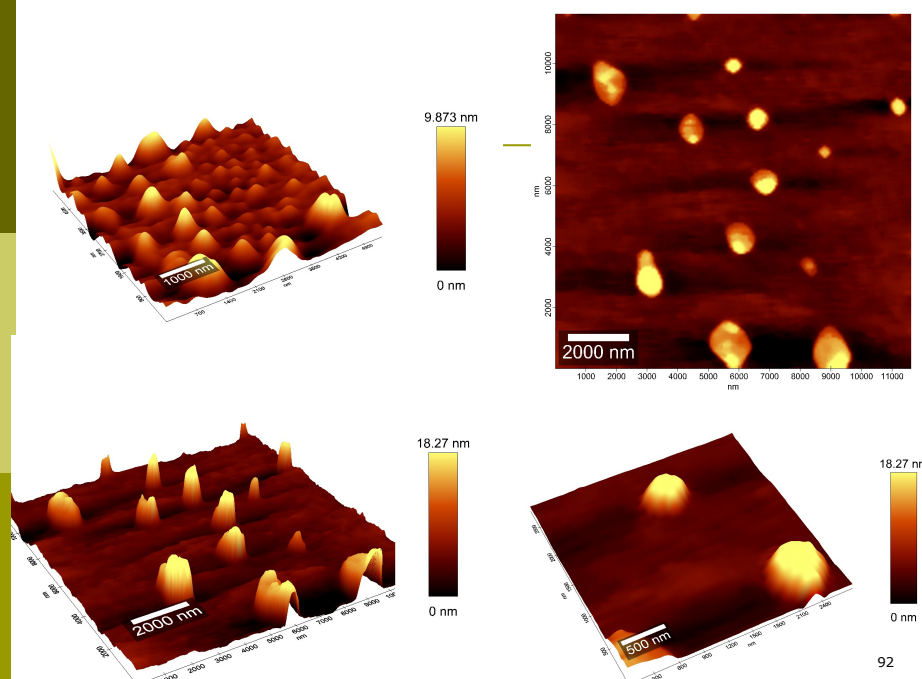
Article
T908 Polymeric Micelles Improved the Uptake of Sgc8-c Aptamer Probe in Tumor-Bearing Mice: A Co-Association Study between the Probe and Preformed Nanostructures

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,*}

Pharmaceuticals 2022, 15, 15. <https://doi.org/10.3390/ph15010015>

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6. 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

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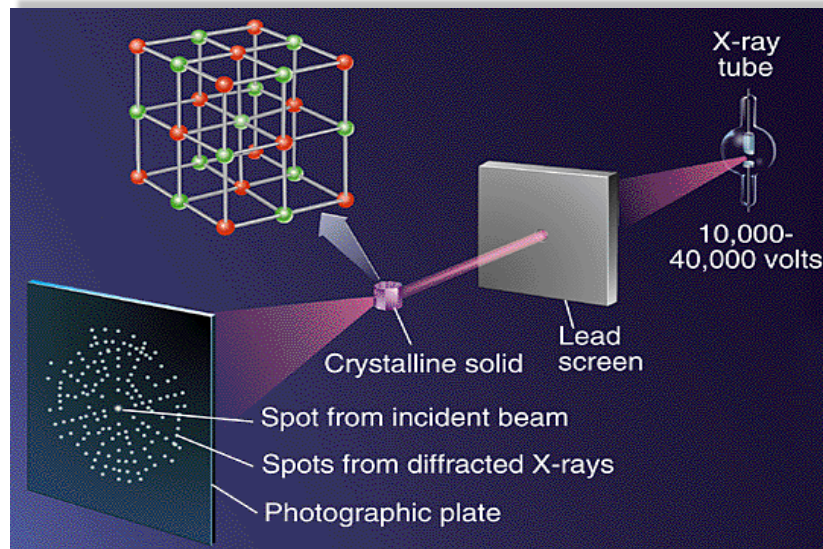
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Difracción de Rayos X



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Experimento de Difracción



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Difracción de Monocristal



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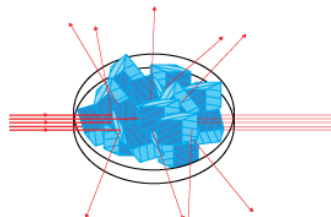
¿Qué es polvo policristalino?

- ▣ Conjunto muy numeroso (infinitos) de cristales microscópicos (infinitesimales) idénticos (esféricos) ordenados aleatoriamente.
 - Ejemplos: Azúcar impalpable, sal fina.
 - La coherencia interna en cada cristal es la propiedad a observar, por lo tanto los metales generalmente son policristalinos.

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¿Qué papel juega cada cristal?

- ▣ Cada cristal en el polvo actúa de forma idéntica a un cristal único, generando su propio patrón de difracción.
- ▣ La presencia de infinidad de cristales produce superposición de patrones observándose un nuevo tipo de diagrama.
- ▣ La información contenida en el patrón de difracción es la misma, pero la superposición de muchos cristales.



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Difracción de Monocristal



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Difracción de Polvo



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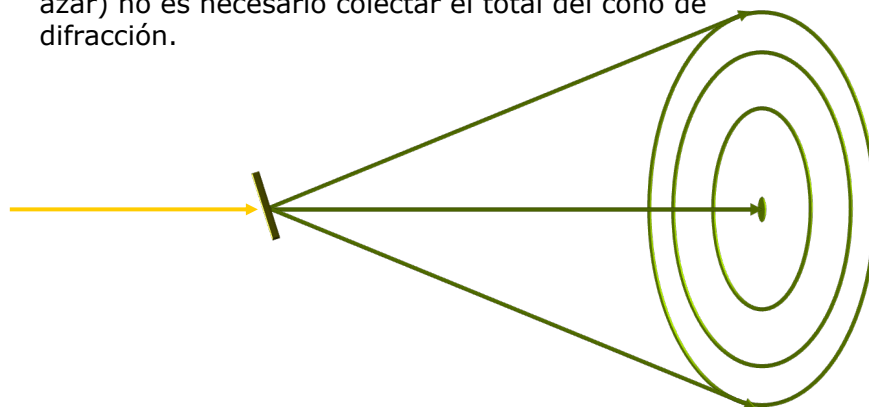
¿Qué es polvo policristalino?

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 - Ejemplos: Azúcar impalpable, sal fina.
 - La coherencia interna en cada cristal es la propiedad a observar, por lo tanto los metales generalmente son policristalinos.

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Condiciones para XRPD

- ▣ Si el polvo policristalino cumple con todas las condiciones del principio (infinitud de cristales uniformes orientados al azar) no es necesario coleccionar el total del cono de difracción.



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Difractómetro: Rigaku Ultima IV



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Geometrías de Haz de Rayos X

▣ Geometría Para focalizante Bragg-Brentano



▣ Geometría de Haz paralelo, Cross-Beam-Optics CBO



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Difractómetro: Rigaku Miniflex

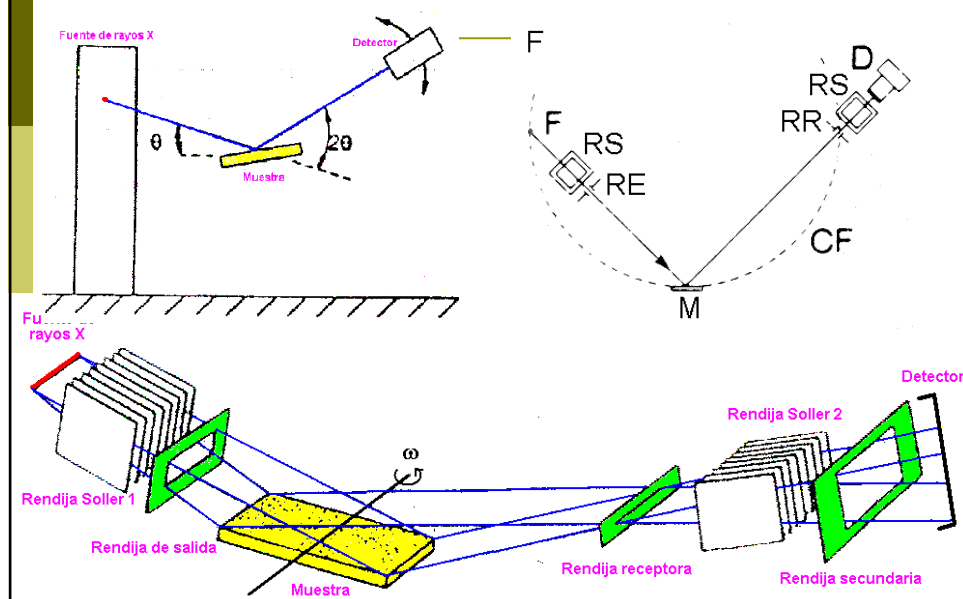


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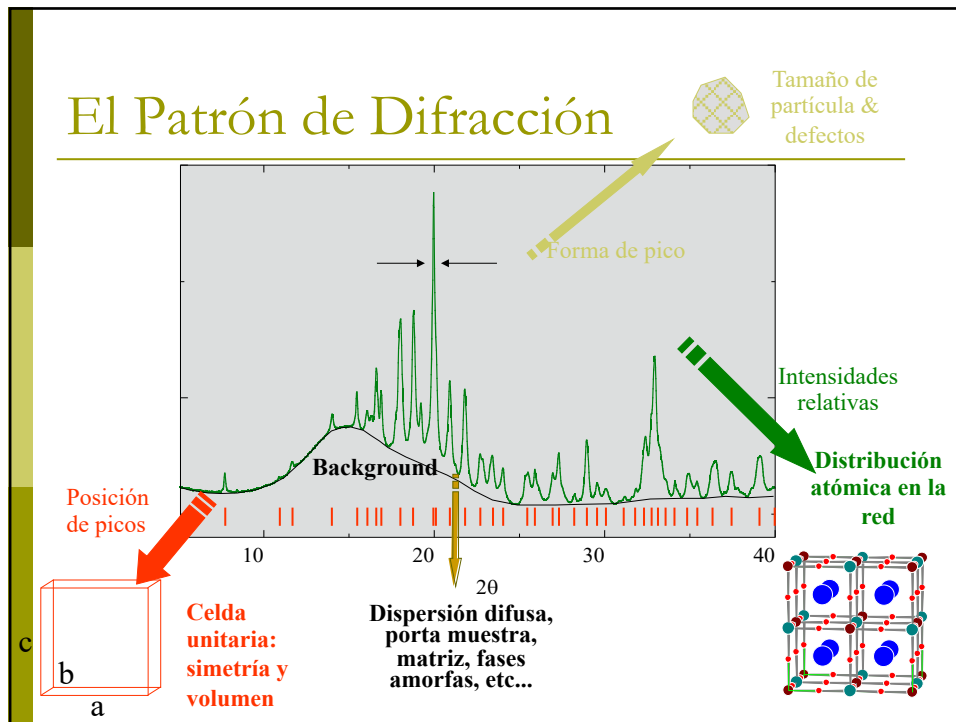
105

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Geometría Bragg-Brentano o para-focalizante.



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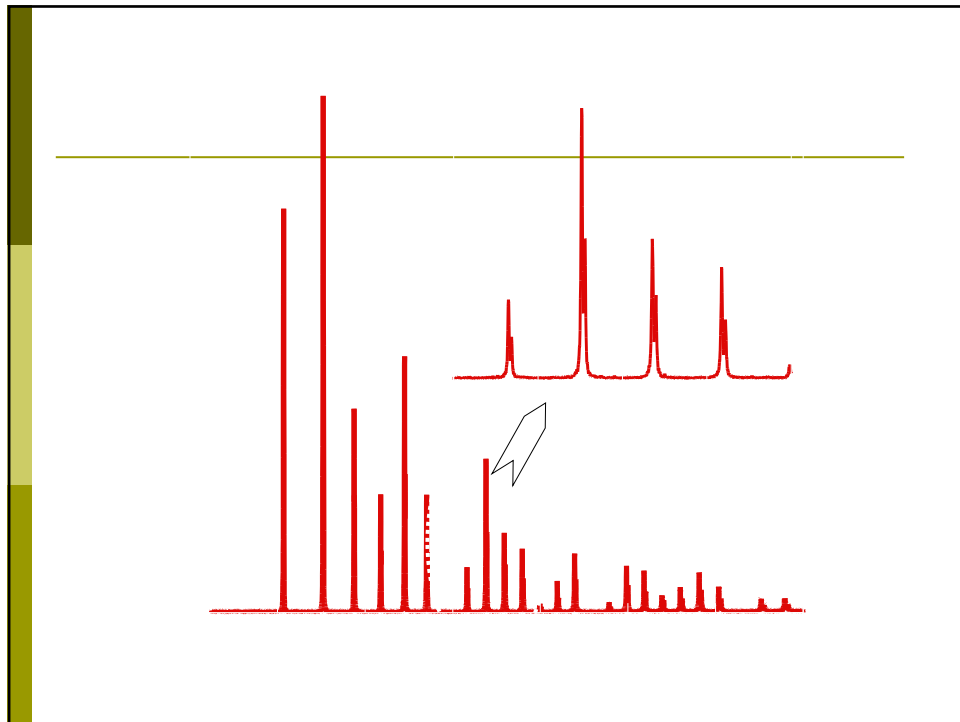


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Información contenida en un diagrama de difracción de polvo

- Posiciones de los picos
 - Sistema Cristalino
 - Grupo Espacial de Simetría
 - Simetría Traslacional
 - Dimensiones de la celda unidad
 - Información Cualitativa de fases
- Intensidades
 - Contenido de la Celda Unidad
 - Simetría Puntual
 - Fracción cuantitativa de fases
- Forma y ancho de picos
 - Tamaño de cristales (2-200 nm)
 - Tensiones internas no uniformes
 - Presencia de defectos de cristales (fallas de apilamiento, dislocaciones, inclusiones, gradientes de fase, etc.)

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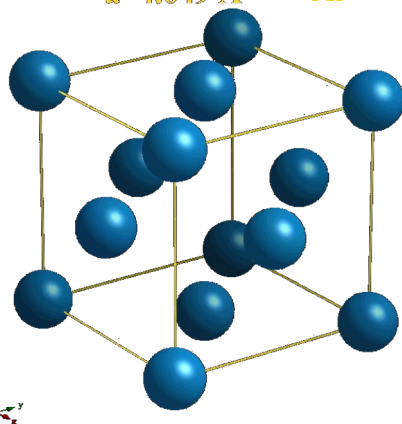
109

Dimensiones y contenidos de la celda (ejemplo cúbico)

$$d_{hkl} = \frac{a}{\sqrt{h^2 + k^2 + l^2}}$$

$$a = 4.049 \text{ \AA}$$

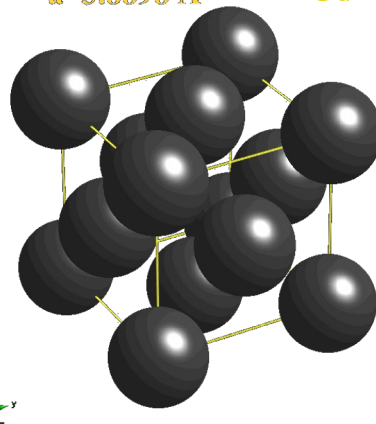
Al



$$n\lambda = 2d_{hkl}\sin\theta$$

$$a = 3.8898 \text{ \AA}$$

Pd

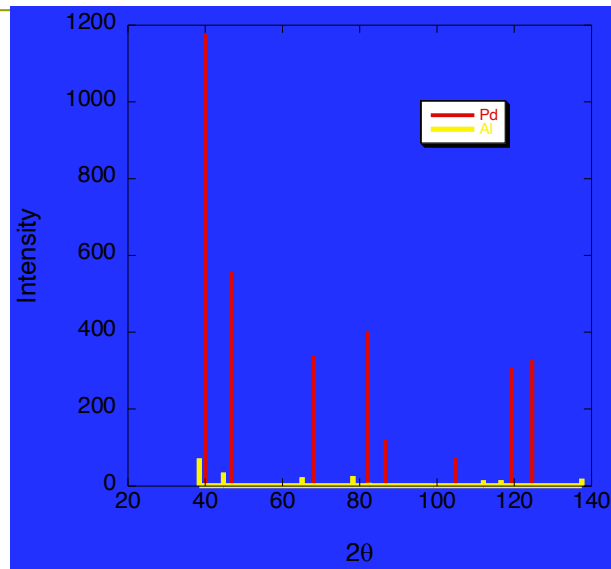


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Dimensiones de la Celda Unidad

$$d_{hkl} = \frac{a}{\sqrt{h^2 + k^2 + l^2}}$$

$$n\lambda = 2d_{hkl}\sin\theta$$

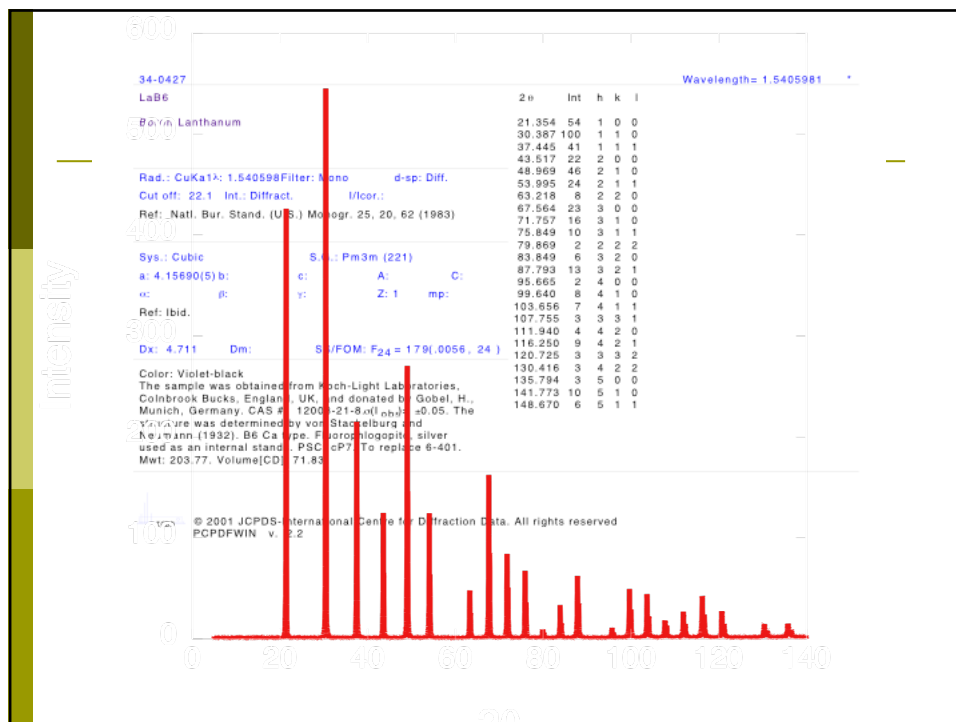


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Análisis Cuantitativo

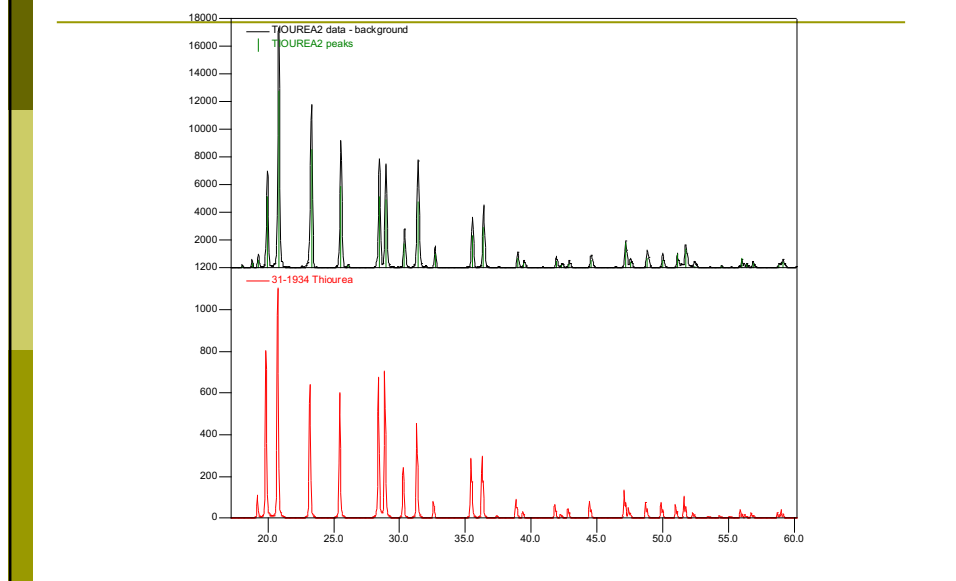
- ▣ El diagrama de difracción de polvo de una fase es "la huella digital del compuesto" y puede ser utilizado para su identificación cuali-cuantitativa.
- ▣ Algoritmos de búsqueda-ajuste son utilizados para comparar diagramas experimentales con otros almacenados en una base de datos (ICDD) de compuestos ya estudiados. $I_{100}/I_{100_corundon}$
- ▣ Puede ser utilizado para mezclas complejas de fases.
 - Standard Interno
 - Full Profile Pattern Refinement: Rietveld Method
- ▣ Puede ser utilizado para identificar polimorfos de un mismo compuesto.

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Software: Search-Match



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Tarjetas JPCD-ICDD

31-1934 Quality: *

CAS Number: 62-56-6

Molecular Weight: 76.12

Volume[CD]: 360.31

Dx: 1.403 Dm:

Sys: Orthorhombic

Lattice: Primitive

S.G.: Pnma [62]

Cell Parameters:

a 7.664 b 8.559 c 5.492

 α β γ

SS/FOM: F30=76(.0081, 49)

I/lor: 0.91

Rad: CuK α 1

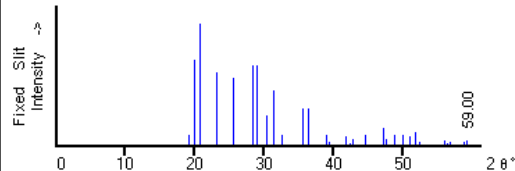
Filter: Graph

d-sp:

CH₄N₂S

Thiourea

Ref: Natl. Bur. Stand. (U.S.) Monogr. 25, 17, 83 (1980)



2 θ	Int-f	h	k	l	2 θ	Int-f	h	k	l	2 θ	Int-f	h	k	l
19.196	9	0	1	1	37.361	1	1	3	1	51.611	11	4	1	1
19.846	70	1	0	1	38.836	9	3	0	1	52.271	3	4	2	0
20.737	100	0	2	0	39.348	3	2	3	0	53.429	1	3	2	2
23.181	60	2	0	0	41.786	7	2	1	2	54.288	1	0	4	2
25.443	55	2	1	0	42.215	2	0	4	0	55.062	<1	4	2	1
28.383	65	2	0	1	42.781	5	2	3	1	55.881	4	1	2	3
28.890	65	1	2	1	44.439	8	3	2	1	56.230	2	0	5	1
30.262	25	2	1	1	47.069	14	1	4	1	56.710	3	2	1	3
31.306	45	2	2	0	47.400	5	4	0	0	58.699	3	4	0	2
32.570	8	0	0	2	48.702	9	2	4	0	59.000	4	3	3	2
35.423	30	0	3	1	49.871	8	3	1	2					
36.267	30	1	1	2	50.968	7	0	1	3					

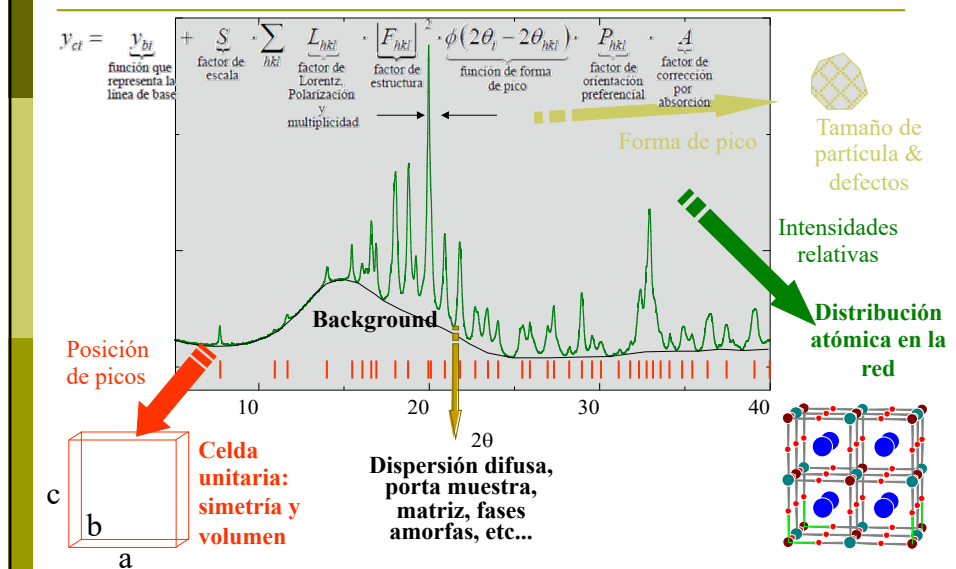
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¿Qué sucede en el polvo real?

- ▣ Pueden no cumplirse una de las 3 condiciones fundamentales:
 - Orientación aleatoria de los cristales
 - ▣ Orientación Preferencial.
 - Tamaño de cristales entre 1 y 10 μm .
 - ▣ Ensanchamiento por tamaño (size-broadening)
 - ▣ Intensidad falsa de máximos (spikey peaks)
 - Forma regular de los cristales
 - ▣ Ensanchamiento anisotrópico (anisotropic-broad.)

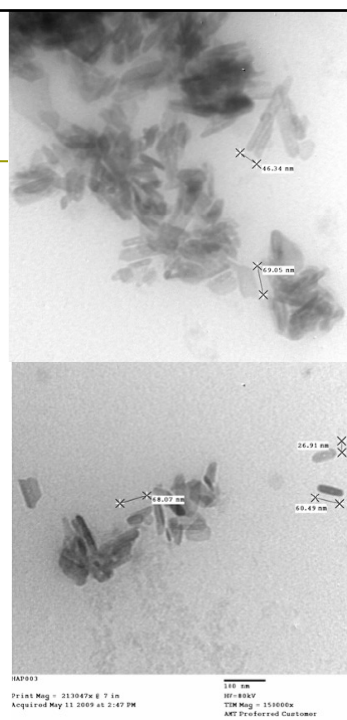
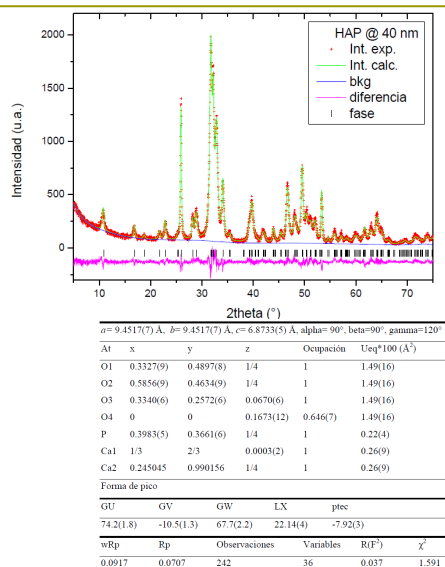
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Otras plicaciones generales:



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Nano-HAP



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Dispersión a Bajo Ángulo (SAXS)

Generalidades

Intensidad de dispersión:

$$I = \phi P(q) S(q)$$

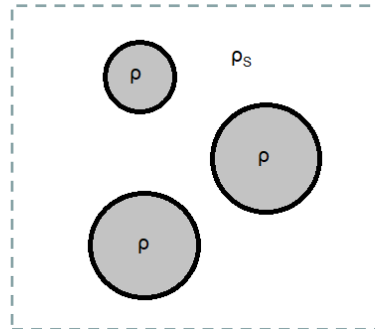
con:

ϕ (fracción en volúmen)

$P(q) = [\rho(r) - \rho_s]^2 \int |F(qr)|^2 dr$ (función intrapartícula)

$S(q) = 1 + 4\pi n \int [g(r) - 1] \frac{\sin(qr)}{qr} r dr$ (función interpartícula)

siendo: $g(r)$ (función de distribución de pares)



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Dispersión a Bajo Ángulo (SAXS)

Generalidades

$$I = \phi P(q) S(q)$$

Sistemas diluidos:

$$S(q) \sim 1$$

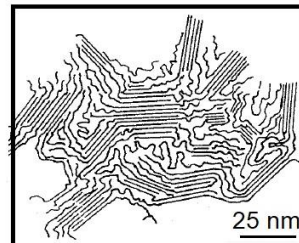
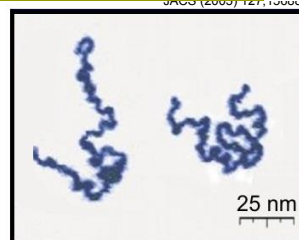
Sin interacciones entre polímeros

Sistemas concentrados:

$$S(q) = 1 + 4\pi n \int [g(r) - 1] \frac{\sin(qr)}{qr} r dr$$

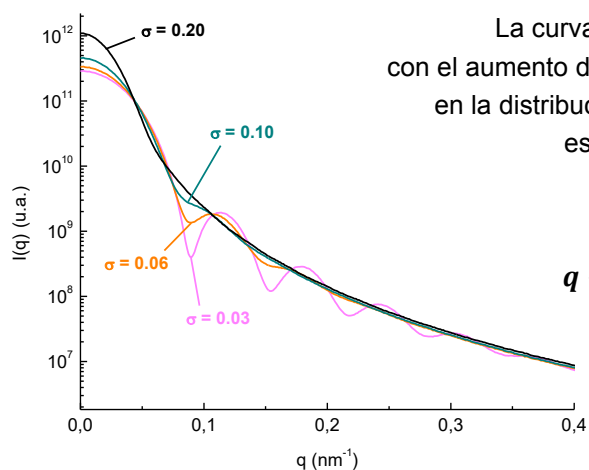
Interacciones entre polímeros

Ej: atractivas, repulsivas, etc.



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Dispersión a Bajo Ángulo (SAXS)



La curva de SAXS se suaviza con el aumento de la polidispersión (σ) en la distribución de tamaño de los espacios vacíos o fillers

Función de Guinier:

$q \rightarrow 0$ (ej: $q < 0.1 \text{ nm}^{-1}$)

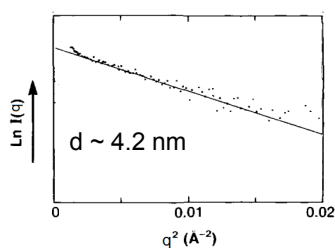
$$I(q) \sim G e^{-\frac{q^2 s^2}{3}}$$

(no interactuantes)

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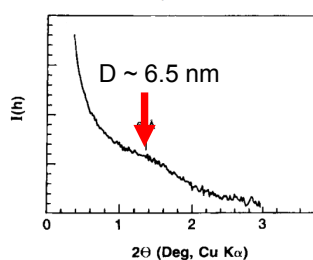
Dispersión a Bajo Ángulo (SAXS)

Estructura laminar



Dominio laminar

$$\ln[I(q)] \sim -\frac{q^2 s^2}{3}$$



Distancia de correlación

$$D = \frac{2\pi}{q_{\max}}$$



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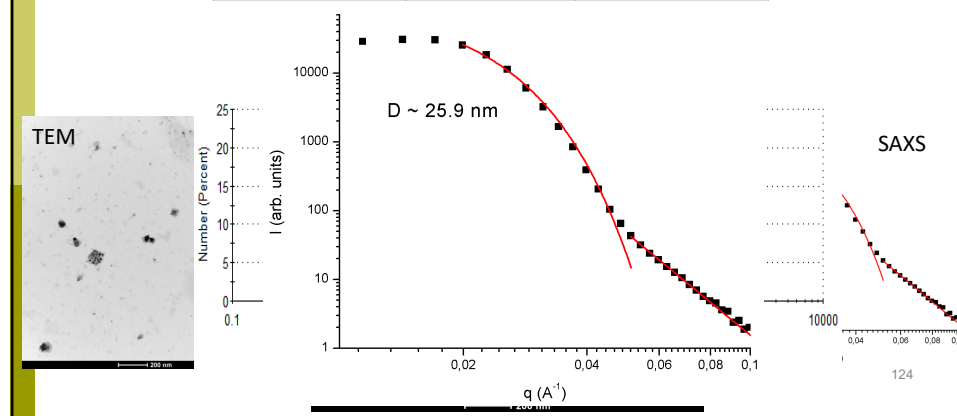
Ejemplo: NPs de Ag por Biosíntesis

Tesis de Doctorado de la B.C.
Belén Estevez

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Caracterización de PchNPs: Tamaño

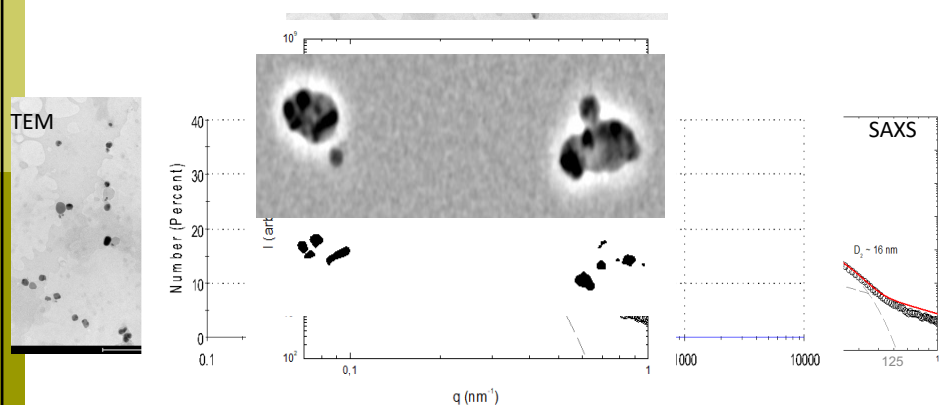
TEM	DLS	SAXS
17(7) nm	43(15) nm PDI: 0,3	26(1) nm



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Caracterización de PeNPs: Tamaño

TEM	DLS	SAXS
32(11) nm	16(4) nm PDI: 0,2	16(1) nm 40(1) nm



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Conclusiones

- ❑ No hay una única técnica para analizar nanomateriales.
- ❑ No hay una única técnica para concluir sobre algún aspecto de la caracterización de nanomateriales.
- ❑ Se trata una tarea compleja, de la cual se debe concluir luego de un análisis amplio y de consenso.

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U D E L A R



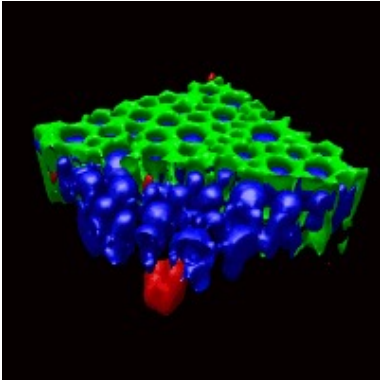
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QUÍMICA

Gracias!



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