



UDelar
FQ
FACULTAD DE
QUÍMICA

Microscopía Raman Confocal & Microscopia de Fuerza Atómica



UNIVERSIDAD
DE LA REPÚBLICA
URUGUAY

Dr. Ricardo Faccio



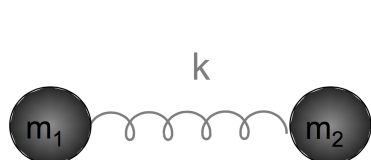
Centro NanoMat
Facultad de Química
Universidad de la República
rfaccio@fq.edu.uy



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

1. Modos Vibracionales



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

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

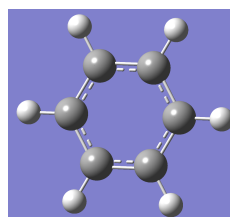
Bond	Bond Energy [kcal (kJ)]	Stretching Frequency (cm ⁻¹)
<i>Frequency dependence on bond energies</i>		
C—C	83 (350)	1200
C=C	146 (611)	1660
C≡C	200 (840)	2200
	stronger bond	$\bar{\nu}$ increases
Bond	Bond Energy [kcal (kJ)]	Stretching Frequency (cm ⁻¹)
<i>Frequency dependence on atomic masses</i>		
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

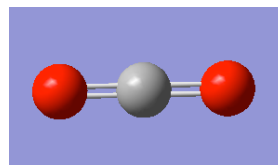
■ Moléculas no Lineales

- ▣ N° modos = $3N - 6$
- ▣ Ejemplo: Benceno
 - 30 modos



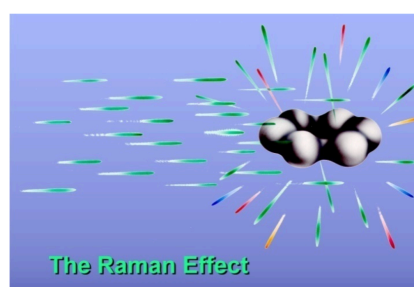
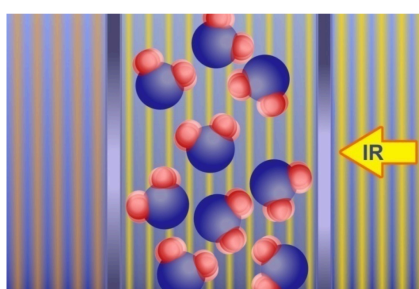
■ Moléculas Lineales

- ▣ N° modos = $3N - 5$
- ▣ Ejemplo: CO₂
 - 4 modos

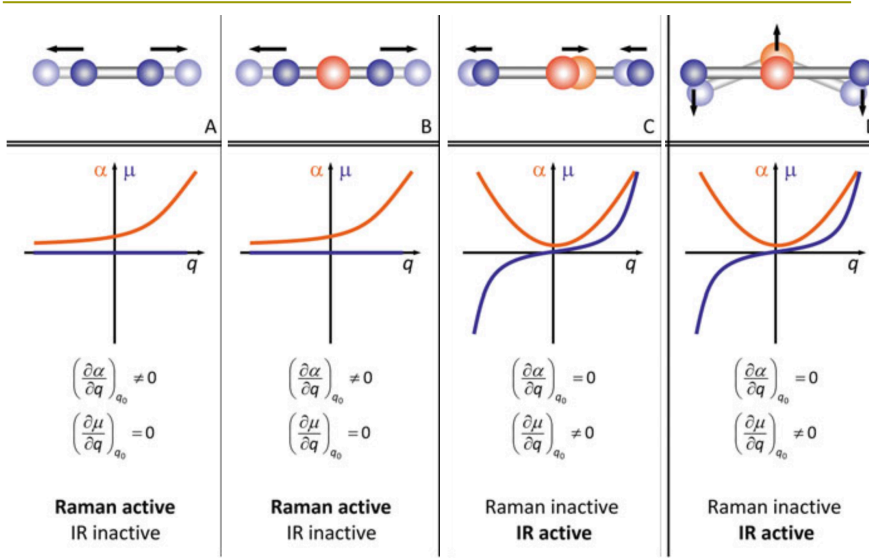


2. Reglas de Selección

- 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.

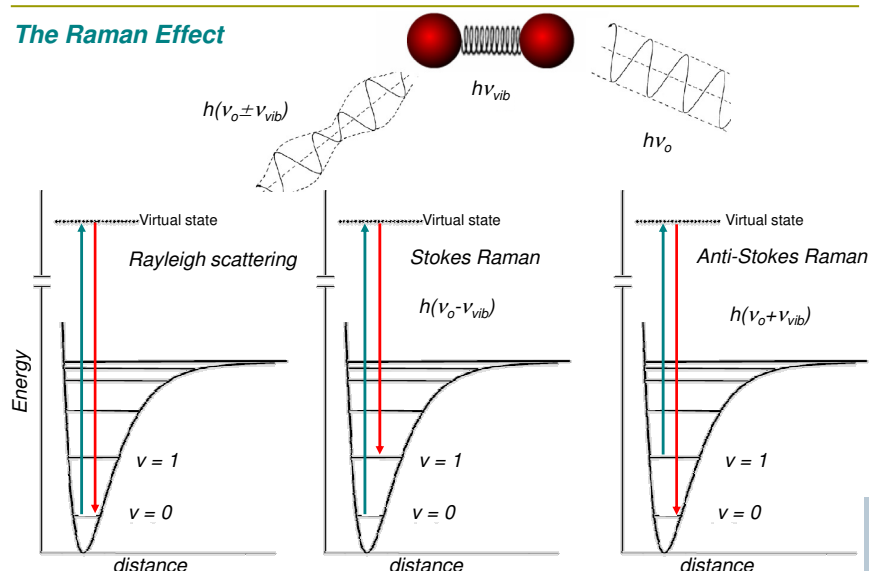


2. Reglas de Selección: IR vs Raman



2. El efecto Raman

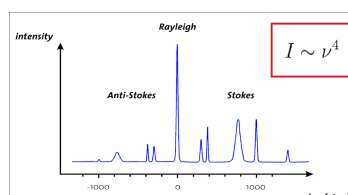
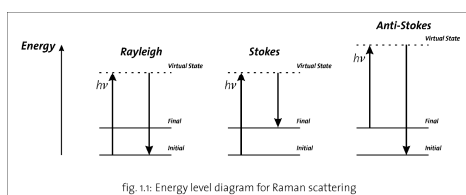
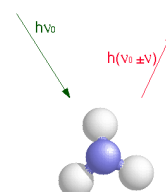
The Raman Effect



2. El efecto Raman

The Raman Effect

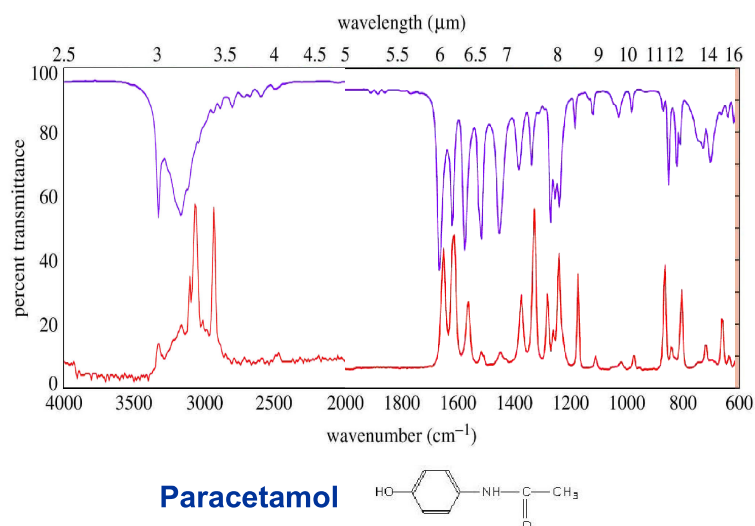
- non-resonant excitation (Stokes) or annihilation (Anti-Stokes) of a vibrational quantum
- energy shift between the exciting and scattered photon is characteristic for the molecules involved in the scattering process



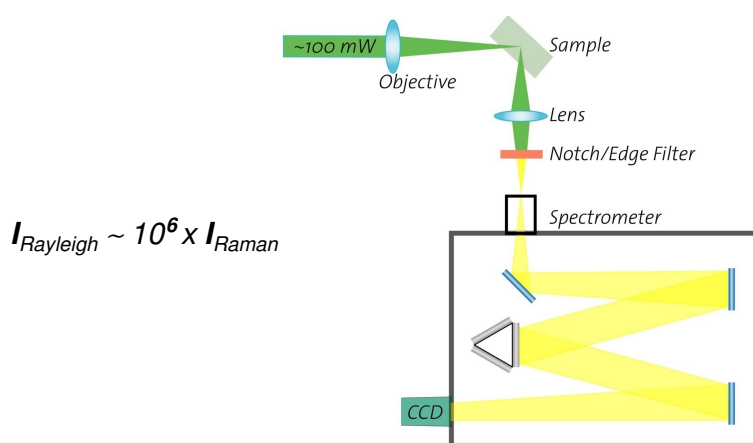
- theoretical prediction 1923 by A. Smekal
- experimentally discovered 1928 by Sir Chandrasekhara Raman, Nobel Prize 1930



2. Infrarrojo vs. Raman

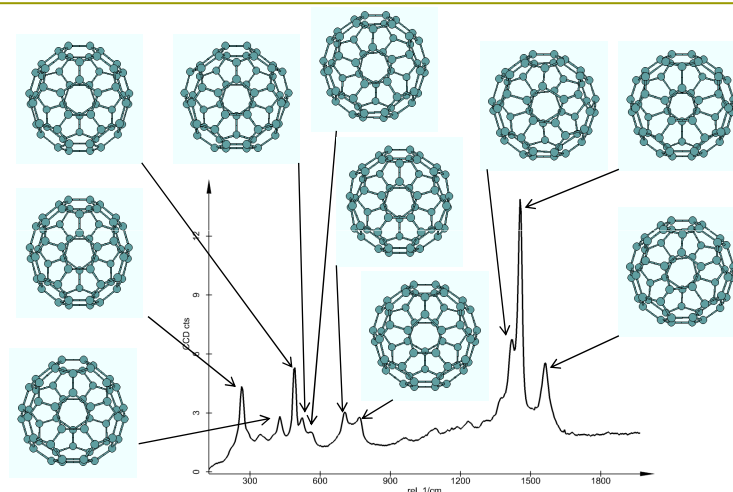


2. Esquema Típico de Experimento



- typical integration times: seconds - several minutes

2. Espectroscopia Raman



chemical, but NO spatial resolution

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

$\nu(\text{CC})$ 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

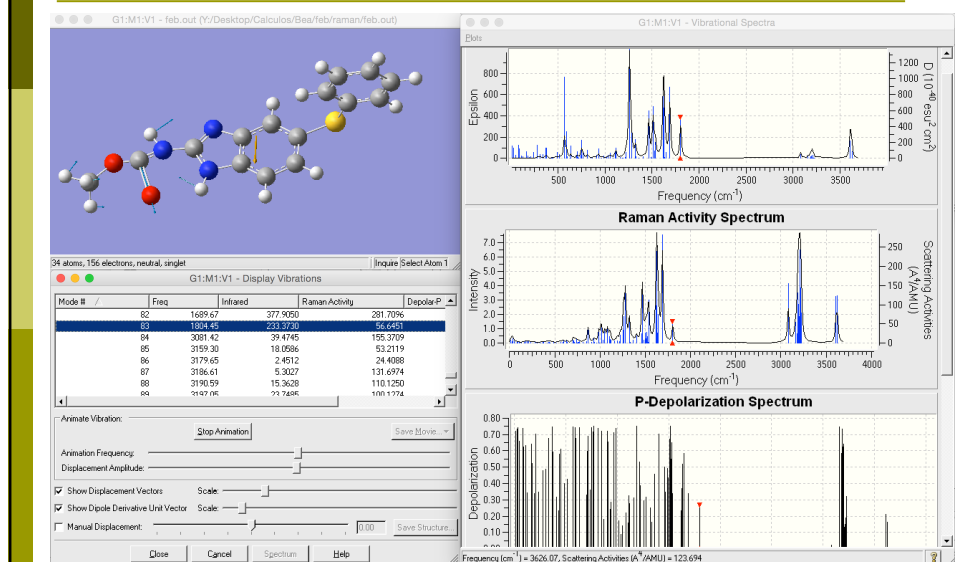
2. Métodos Teóricos

▣ Cálculos *ab initio* o *Primeros Principios*

▣ *DFT*

■ *Gaussian*

2. Simulación de IR y Raman

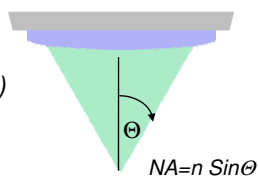


3 Microscopia Óptica Clásica

What is the resolution limit of a classical optical microscope?

Ernst Abbé (1840-1905): diffraction theory

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

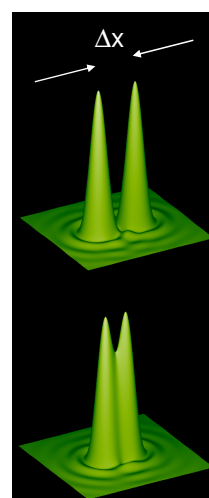


air: $NA \leq 0,95$ (340nm @ 532nm exc.)

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

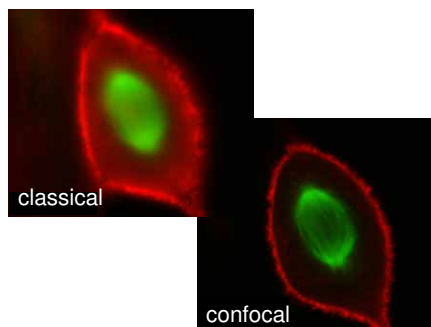
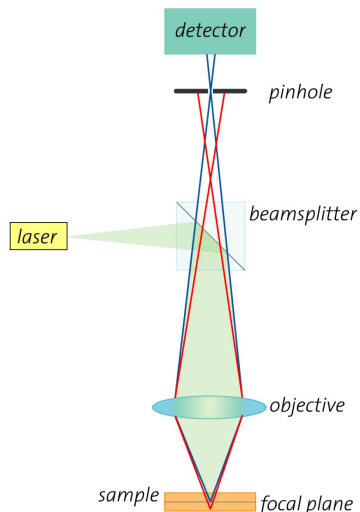
oil: $NA \leq 1,4$ (230nm @ 532nm exc.)

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



3. Microscopia Confocal

confocal microscopy



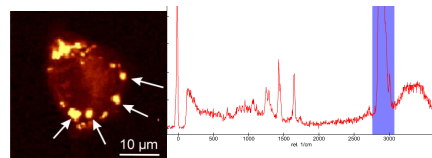
- much smaller background
- 3-D information
- slightly higher resolution possible

spatial, but NO chemical information

3. Microscopia Confocal + Espectroscopia Raman

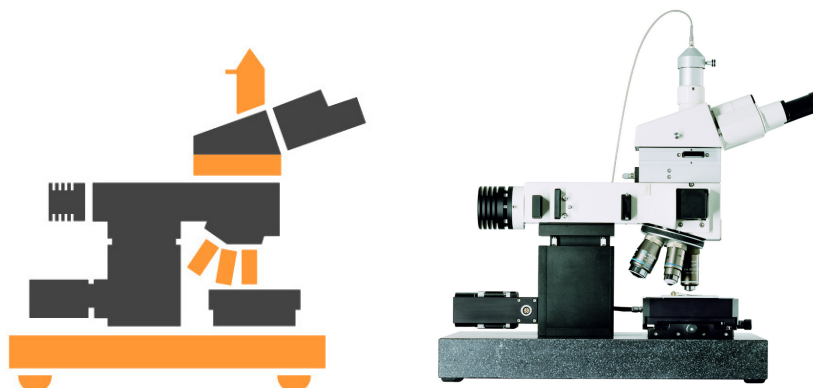
3-D Imaging with chemical sensitivity

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



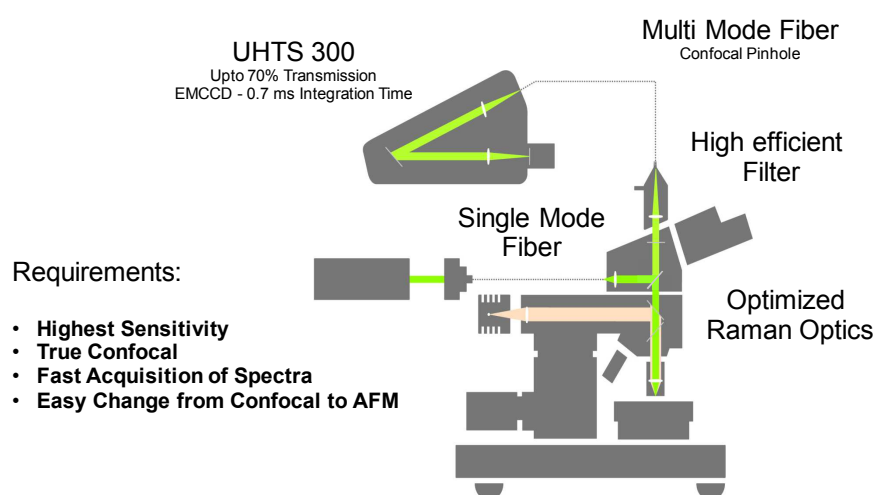
**Parque Científico y Tecnológico de Pando (PCTP) /
Instituto Polo Tecnológico de Pando (IPTP)
ANII- Proyecto EQC_X_2012_1_14**

3. Microscopio Confocal + Espectrómetro Raman



Imaging with chemical sensitivity

3. Esquema



3. 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)

3. WITec Alpha 300 RA

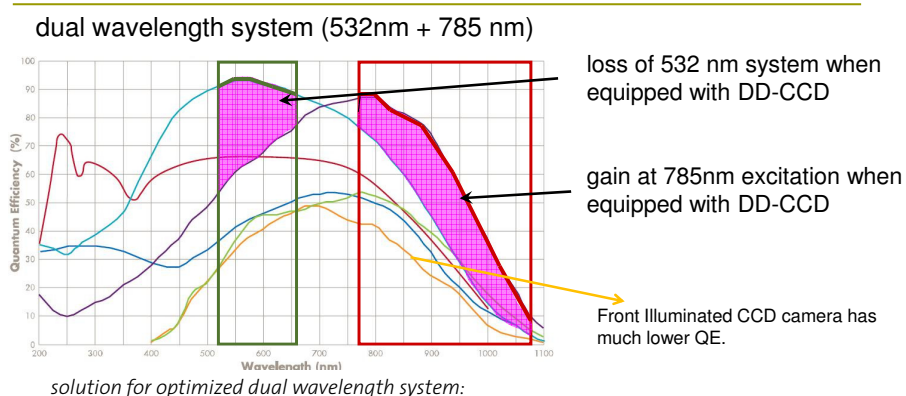
WITec Confocal Raman Microscope System Alpha300R de alta sensibilidad para longitudes de onda de excitación de 532 nm y 785 nm.

- ❑ Imagen Espectral Raman: adquisición de espectros Raman completos en cada pixel (mapping)
- ❑ Adquisición de espectros Raman en áreas seleccionadas (micro-Raman)
- ❑ Imágenes 3D Raman confocal.
- ❑ Microscopía confocal de Reflexión
- ❑ Resolución Óptica: Límite de difracción, típico 300 nm @ 532 nm de longitud de onda de excitación (objetivo NA 1.4)
- ❑ Tamaño de la muestra: máx. 120 mm x e y dirección, 25 mm de altura
- ❑ **Dos espectrómetros y detectores CCD optimizados, uno para IR cercano y otro para rango Visible.**
- ❑ Criostato para micro-confocal rango T= -196 C a 600 C (Linkam)

3. Plataforma Piezoeléctrica

- ❑ Plataforma piezoeléctrica para el barrido de muestras.
- ❑ Rango continuo de barrido de hasta 200µm en ejes X e Y y 20 µm en eje Z.
- ❑ Dispositivo de barrido lineal con feedback de loop cerrado
- ❑ Resolución de barrido:
 - Precisión de posicionamiento:
 - < 2 nm en eje ejes X e Y
 - < 0.2 nm (2 Å) en eje Z
- ❑ AFM, MFM y EFM
 - FSE_1_2014_1_102008 Fondo Sectorial de Energía - ANII
 - “Nanomateriales para almacenamiento de energía: nueva serie de cá todos para baterías de ion-Litio”

3. Doble CCD optimizada para cada región del espectro



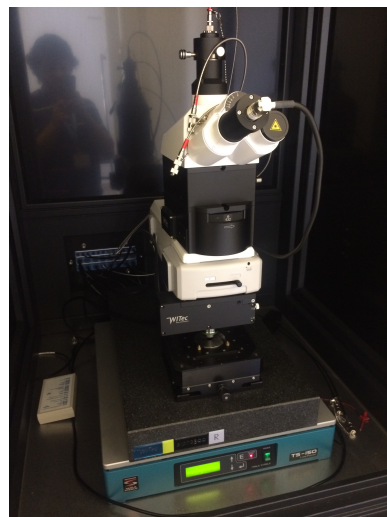
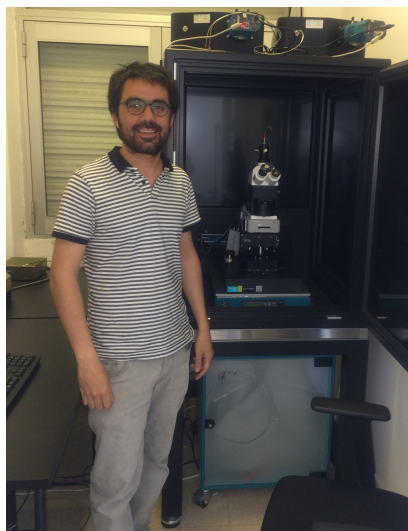
solution for optimized dual wavelength system:

=> 2 spectrometers + 2 cameras

532nm excitation: 500nm blaze, standard BI-CCD

785nm excitation: 750nm blaze, DD-CCD

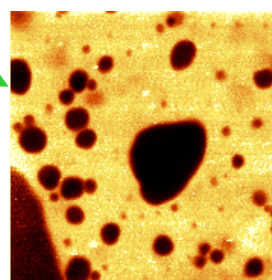
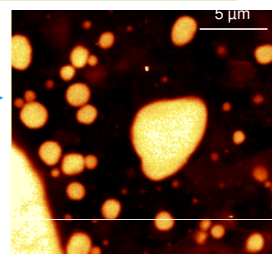
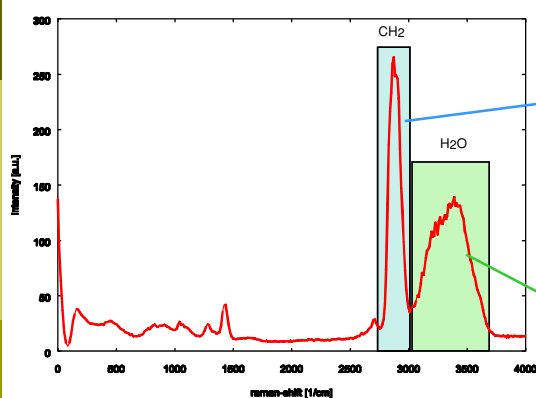
3. Disposición



4. 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

Reconstrucción Imagen Raman 3D

Sample: Oil/Water Emulsion

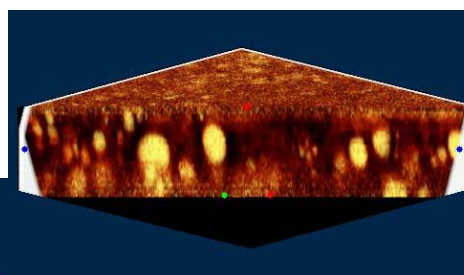
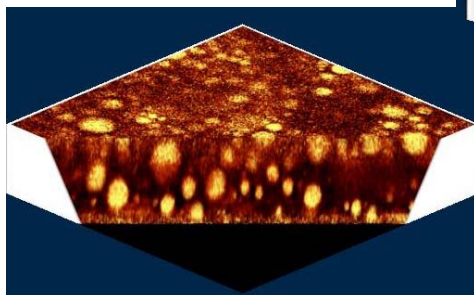
3-D Reconstruction

Evaluated Band: CH-Stretching

Excitation: 532nm

Scan Range XY: 100x100 μm

Scan Range Z: 30 μm/32 layers



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.

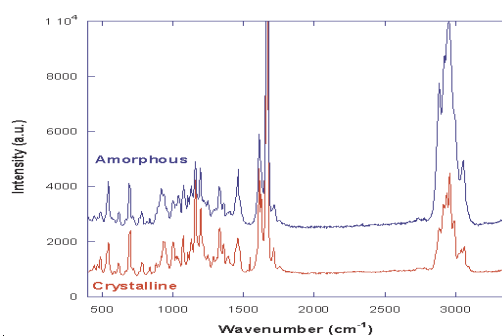


Image Courtesy:
 Dr. Klaus Wormuth, SurModics (www.surmodics.com)

Geociencias

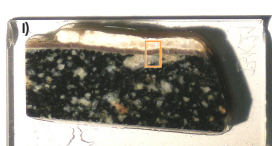


Fig. 1: I) Orange rectangle indicates scan position on polished rock section.

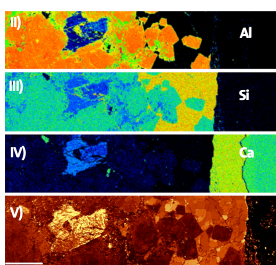


Fig. 2: II-IV) EMP maps showing elemental distribution; V) corresponding Raman image, integrated intensity 140-300 rel.1/cm.

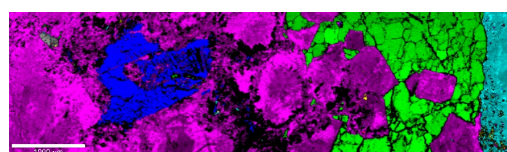
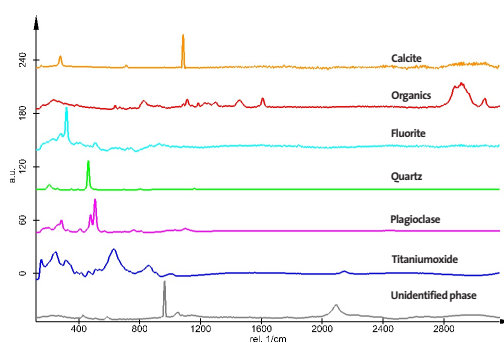
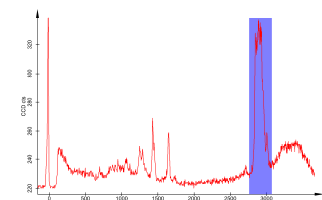
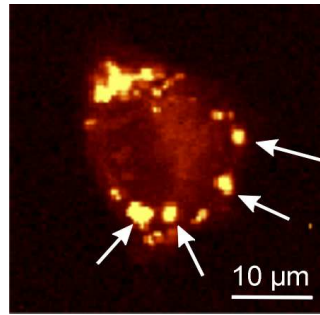
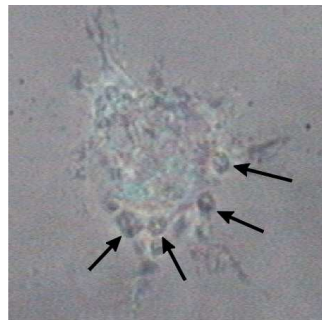


Fig. 2: I) Combined false color image and the corresponding color-coded spectra.



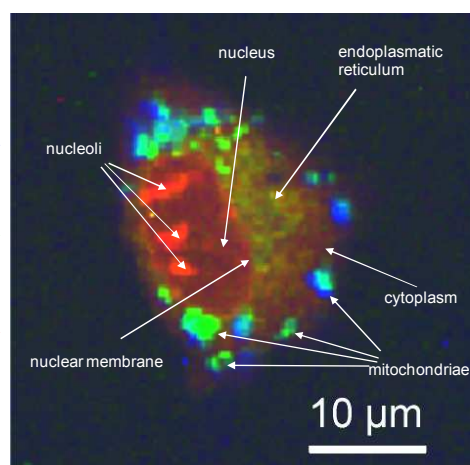
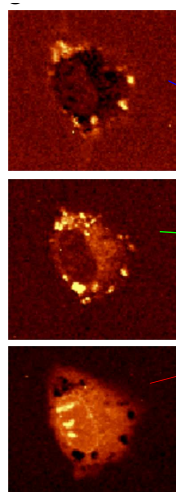
Células epiteliales en Buffer



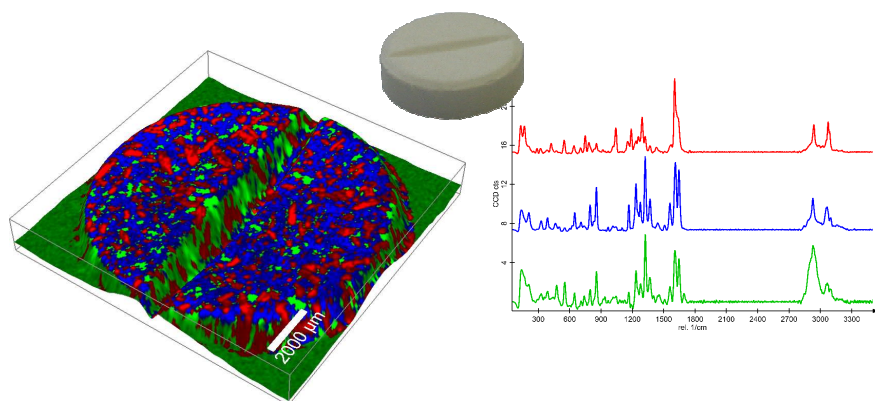
Intensity in the CH 2 stretching band
(2800 - 3000 / cm)

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

Imagen reconstruida



Tabletas: API y Excipientes

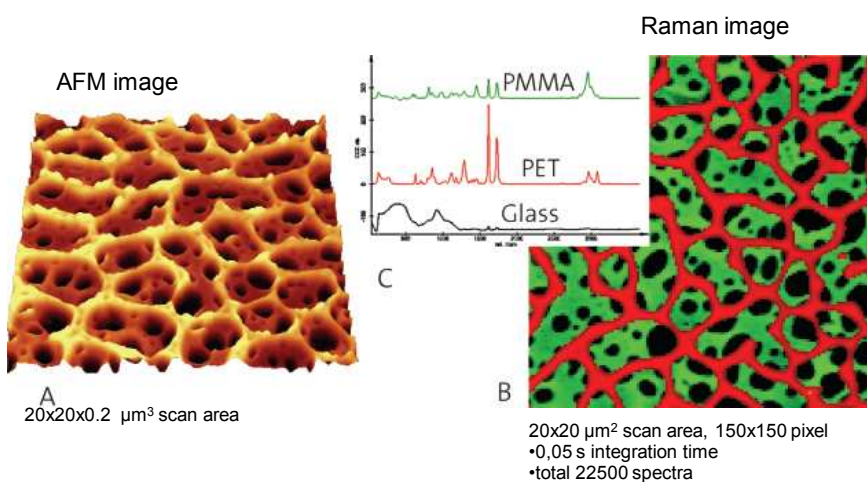


Confocal Raman Image overlaid
on the topography

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.

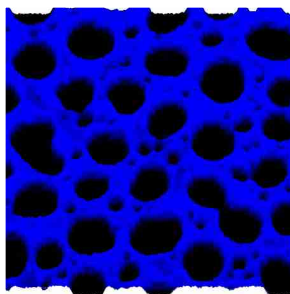
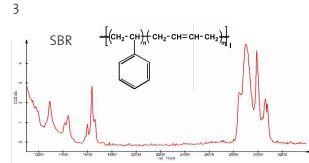
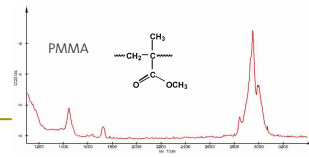
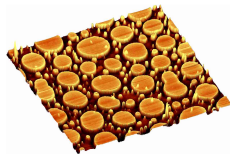


Fig. 9: Color coded Raman image of SBR resulting from the integral intensity of each spectrum.

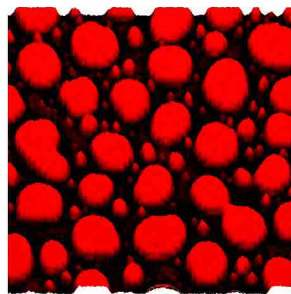


Fig. 10: Color coded Raman image of PMMA resulting from the integral intensity of each spectrum.

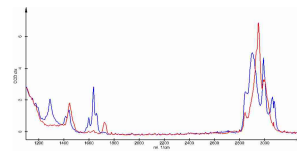


Fig. 11: Corresponding spectra: SBR (blue) and PMMA (red).

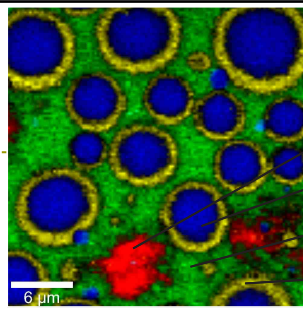


Fig. 2a: Raman Image of an emulsion used in food production. Scan range: 30 μm x 30 μm, 120x120 pixels (=14400 spectra, 532 Nd:Yag Laser for excitation, 100 x oil objective (NA=1.25). Red + green: fatty matrix, blue: aqueous phase, yellow: emulsifier PGPR

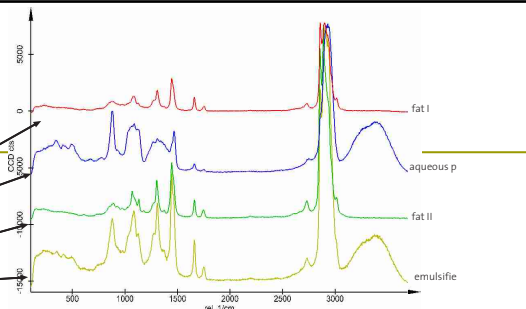
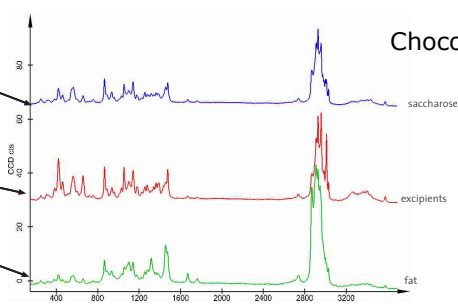
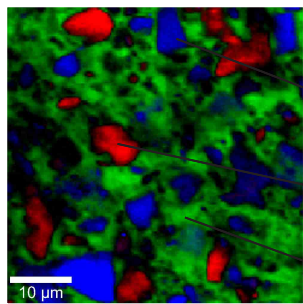


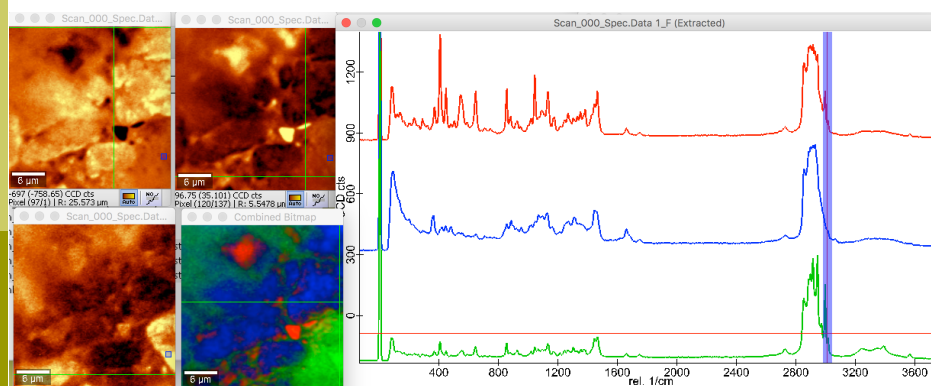
Fig. 2b: Corresponding spectra of the compounds contained within the emulsion.



Chocolate blanco

Chocolate Blanco (toblerone)

■ Sacharose, Maltose and Fats



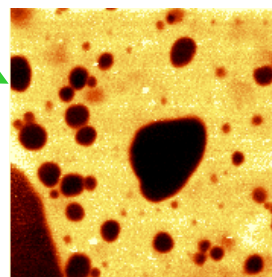
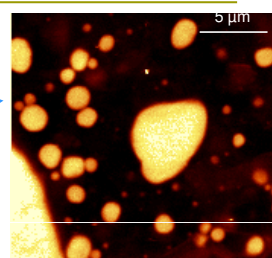
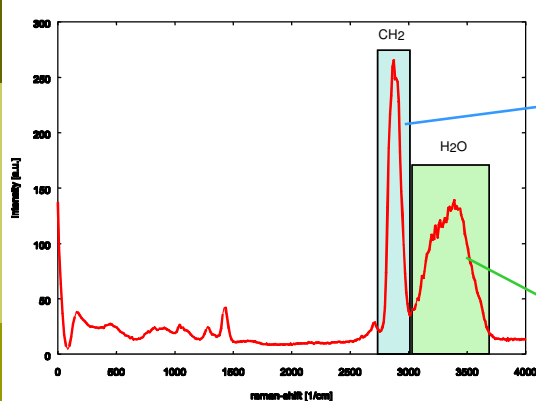
Experiencias

WITec alpha300RA

4. 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

Reconstrucción Imagen Raman 3D

Sample: Oil/Water Emulsion

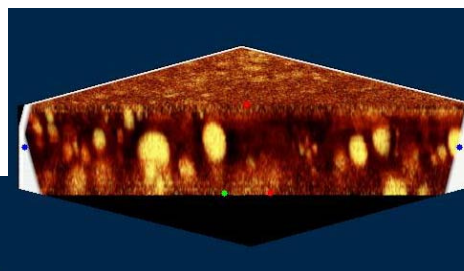
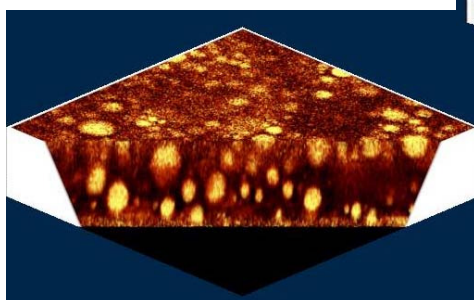
3-D Reconstruction

Evaluated Band: CH-Stretching

Excitation: 532nm

Scan Range XY: 100x100 μm

Scan Range Z: 30 μm /32 layers



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.

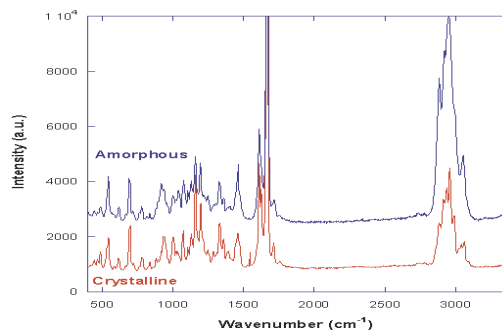
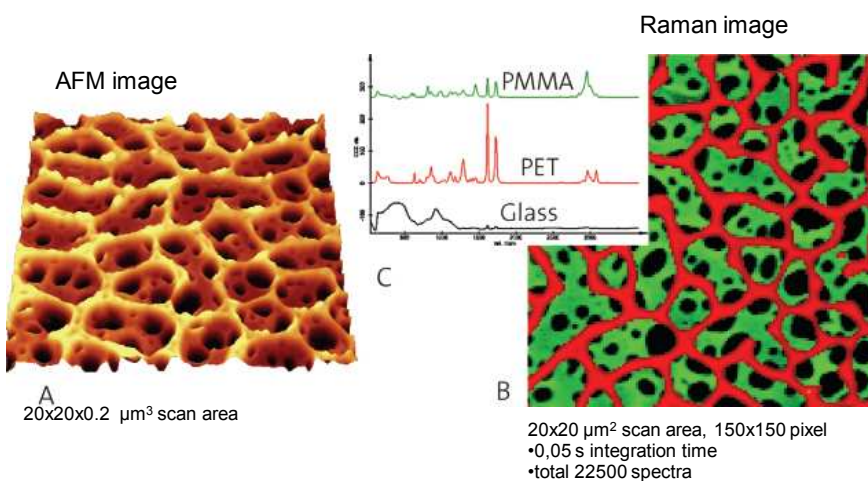


Image Courtesy:
Dr. Klaus Wormuth, SurModics (www.surmodics.com)

Polímeros

Thin Film of PMMA - PET



Blend PMMA+SBR

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

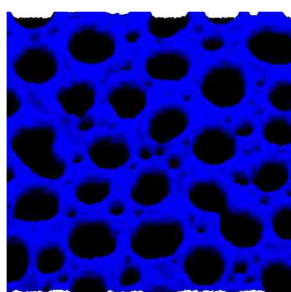
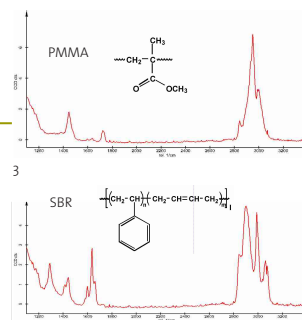
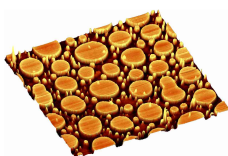


Fig. 9: Color coded Raman image of SBR resulting from the integral intensity of each spectrum.

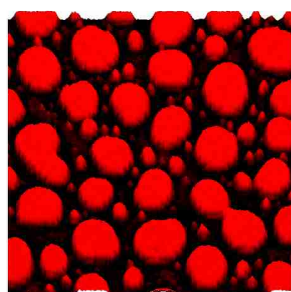


Fig. 10: Color coded Raman image of PMMA resulting from the integral intensity of each spectrum.

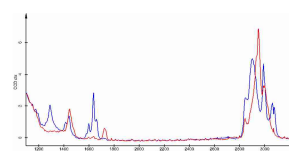
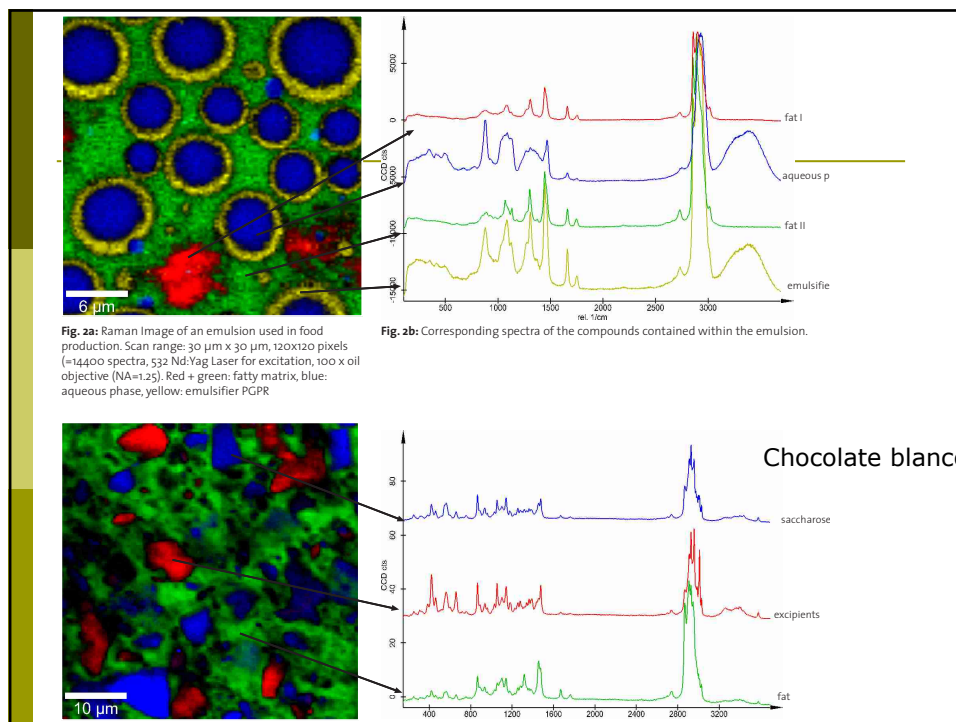
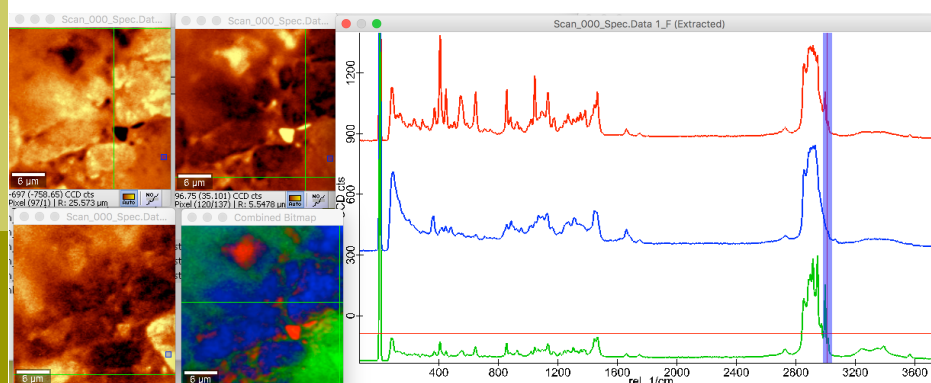


Fig. 11: Corresponding spectra: SBR (blue) and PMMA (red).



Chocolate Blanco (toblerone)

■ Sacharose, Maltose and Fats

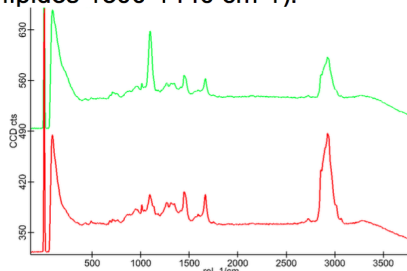


Experiencias

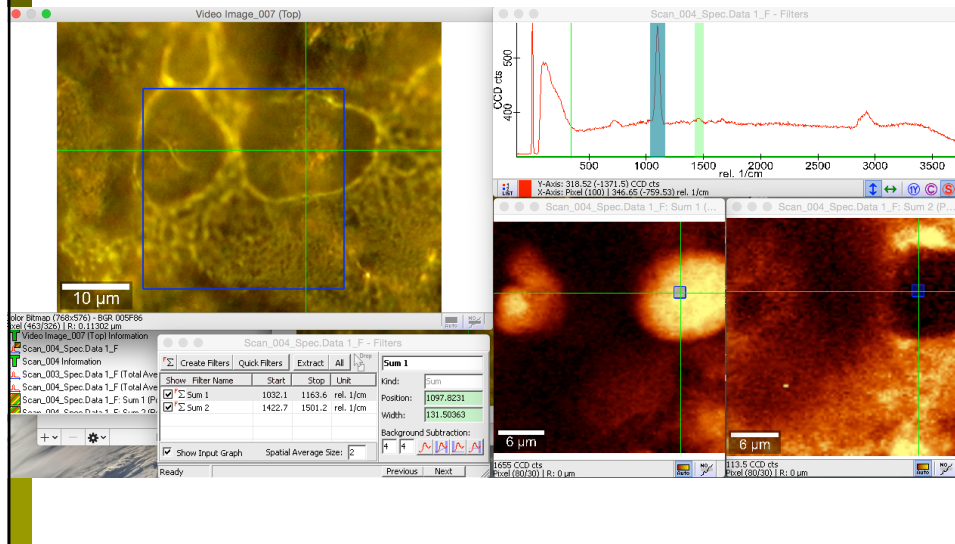
WITec alpha300RA

Ejemplo 1: *Mesocestoides vogae* larva tetrathyridea

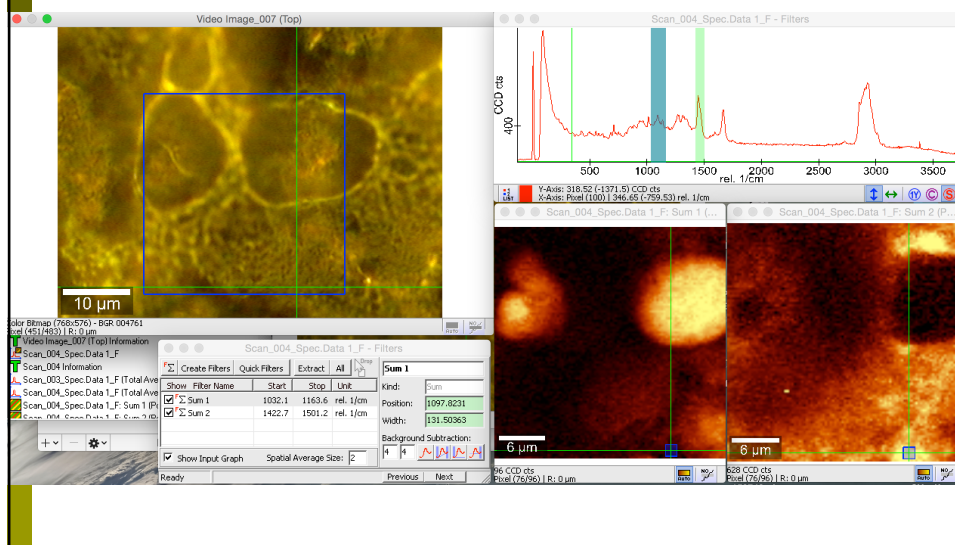
Identificación monohidrocalcita (verde) en *Mesocestoides vogae* (Lab. Farmacología/LEA Fac. de Química), gracias a la presencia de la banda ubicada a 1100 cm^{-1} , notar la imagen con contraste (verde). Por otra parte, las bandas restantes permiten identificar diferentes regiones de la membrana (bandas C-H $2700\text{-}3000\text{ cm}^{-1}$, proteínas $1660\text{-}1450\text{ cm}^{-1}$, lípidos $1300\text{-}1440\text{ cm}^{-1}$).



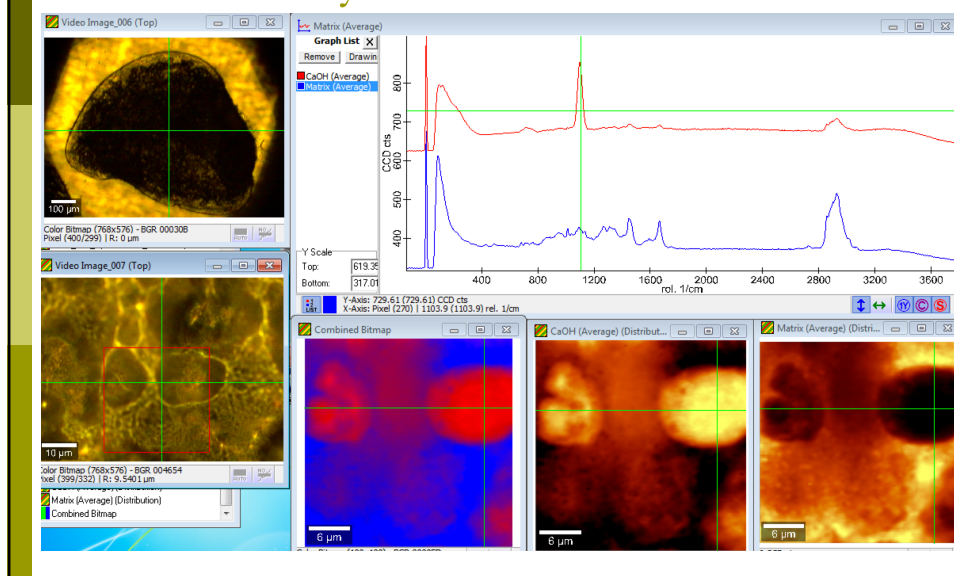
Ejemplo 1: Mesocestoides vogae larva tetrathyridea



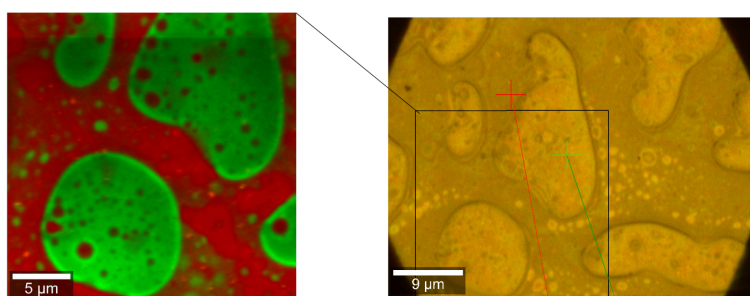
Ejemplo 1: Mesocestoides vogae larva tetrathyridea



Ejemplo 1: Mesocestoides vogae larva tetrathyridea

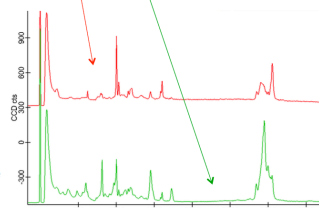


Ejemplo 2: Blend PMMA+PS

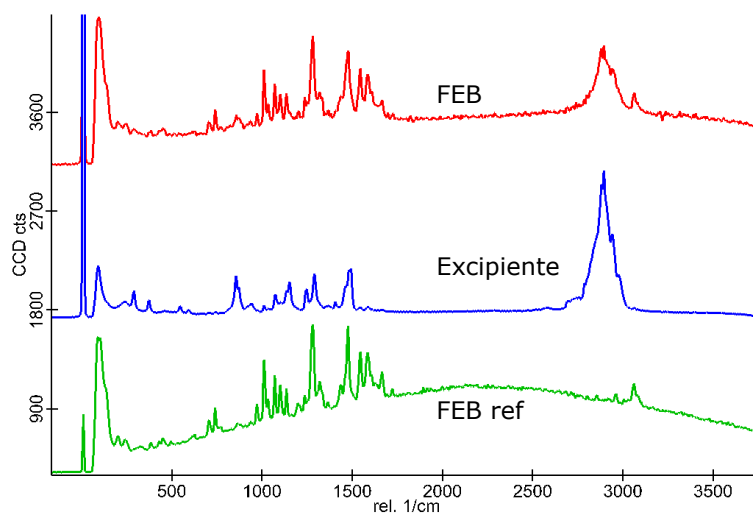


Mezcla de Poliestireno (PS) con polimetilmetacrilato (PMMA). Se identifican dos zonas (roja y verde), se obtiene un espectro raman en ambos lugares. El espectro indica que la zona marcada en verde es PMMA y la roja PS

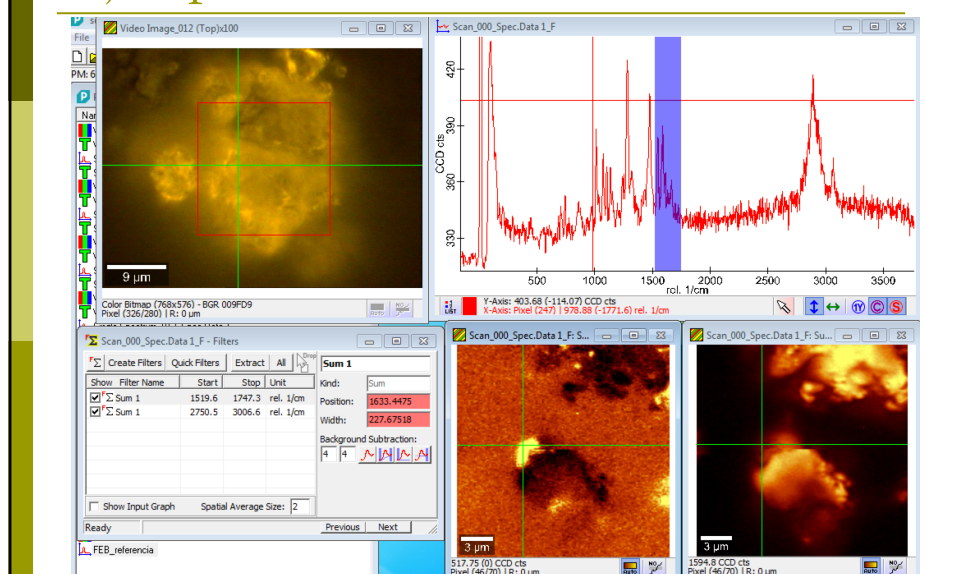
Observando las diferencias en el mapping correspondiente al recuadro negro, donde se obtiene la imagen combinada de ambos componentes.



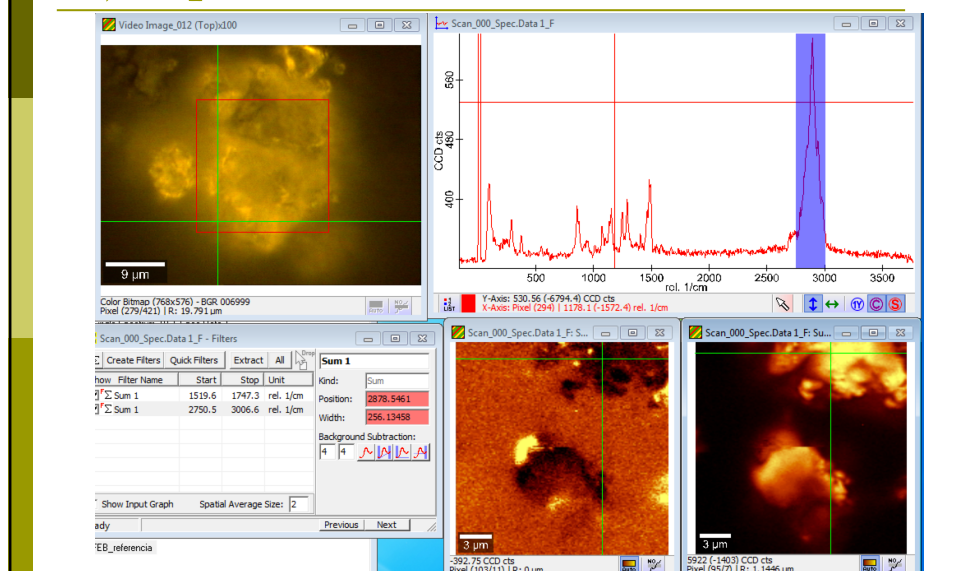
Ejemplo 3: Formulaciones



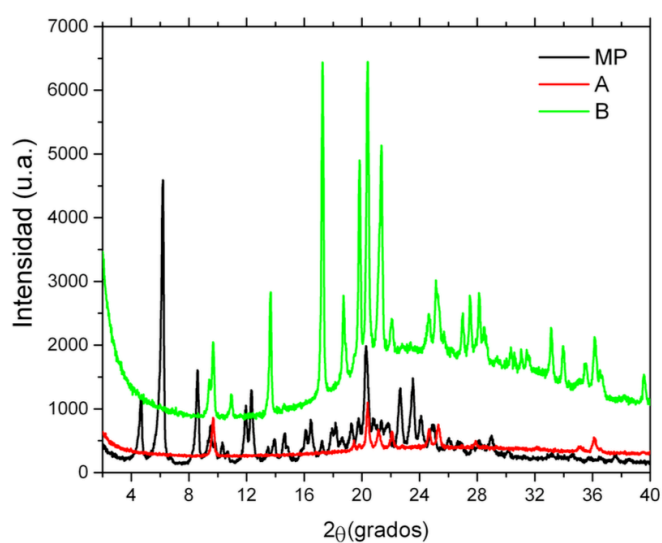
Ejemplo 3: Formulaciones



Ejemplo 3: Formulaciones

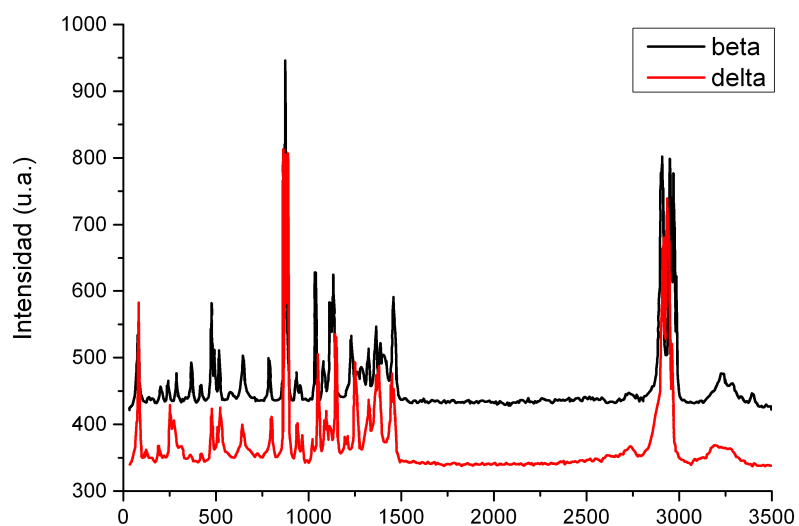


Ejemplo 4: Asesoramiento muestra A, B y Materia Prima

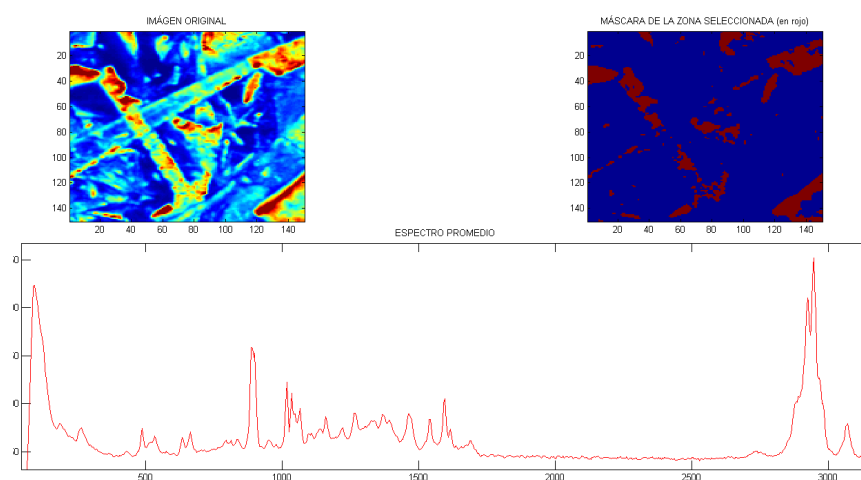


- Diagrama de Difracción para muestras de materia prima (MP), muestra A y muestra B.

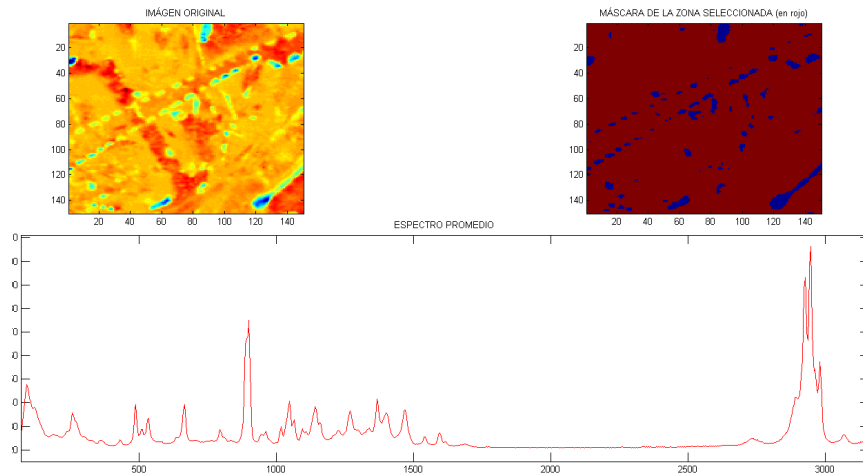
Ejemplo 4: Espectros Raman de Mannitol



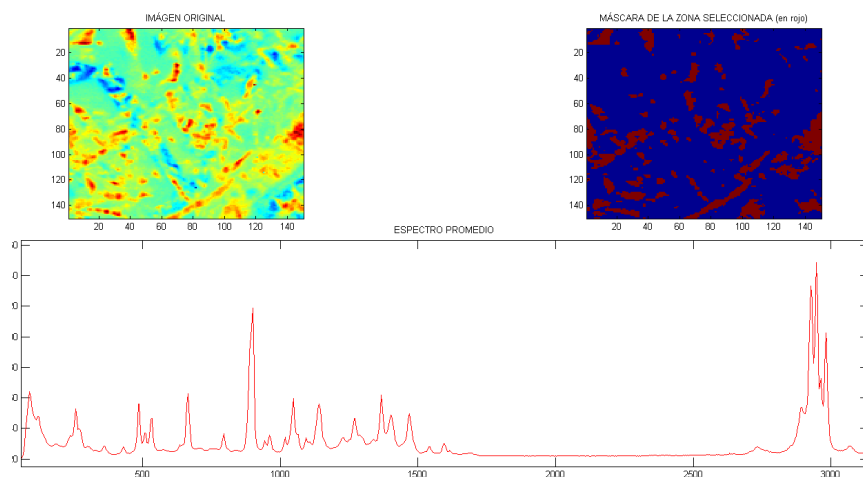
Ejemplo 4: PCA Componente 1



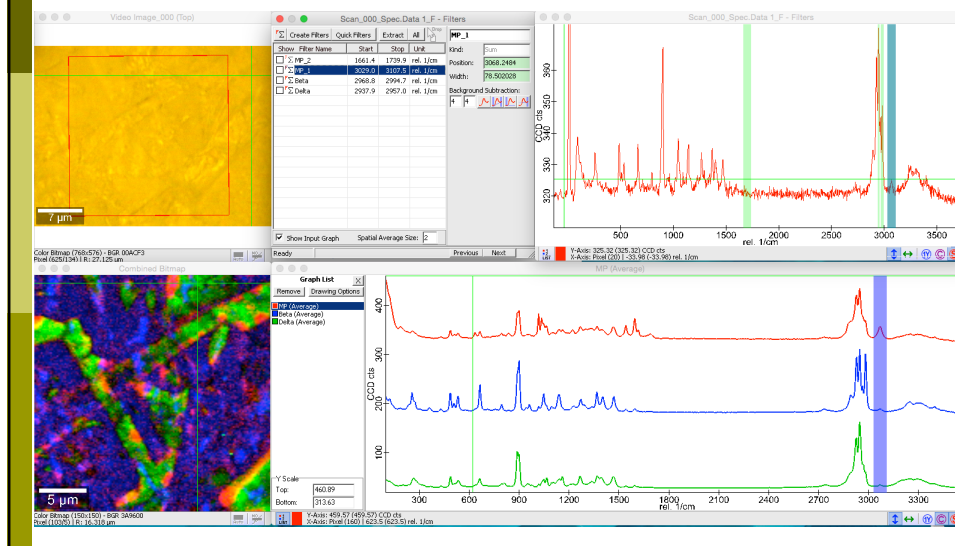
Ejemplo 4: PCA Componente 2



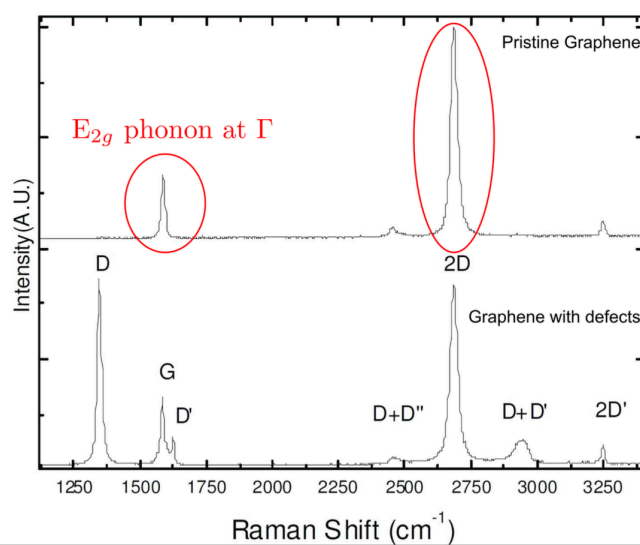
Ejemplo 4: PCA Componente 3



Ejemplo 4: Formulaciones: Manitol+ API

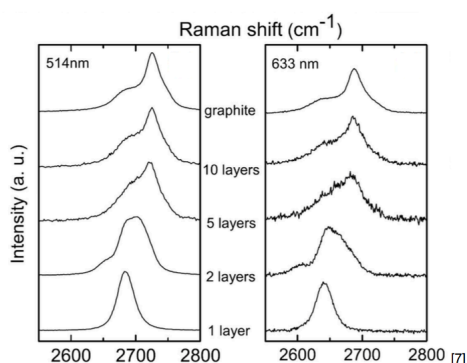


Ejemplo 5: Grafeno



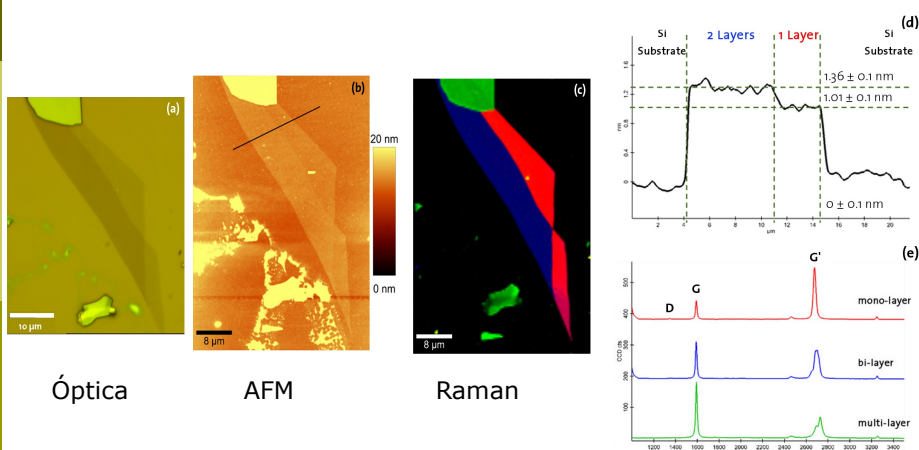
Ejemplo 5: Grafeno

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

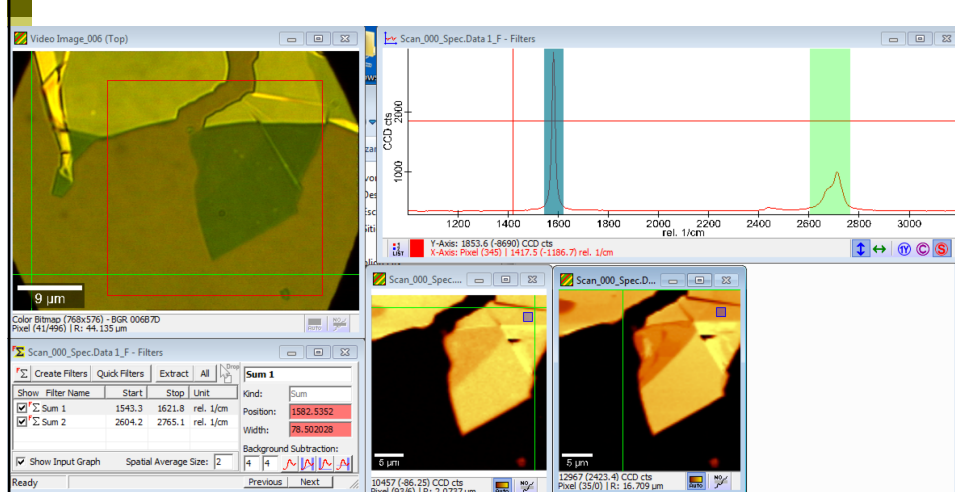


- Position of 2D peak depends on number of layers
- Additional layers increase FWHM of 2D peak (more e^- states for resonant scattering)
- Occurrence of peaks at 1350 cm^{-1} (D) and 1620 cm^{-1} (D') reveal defects of graphene sample

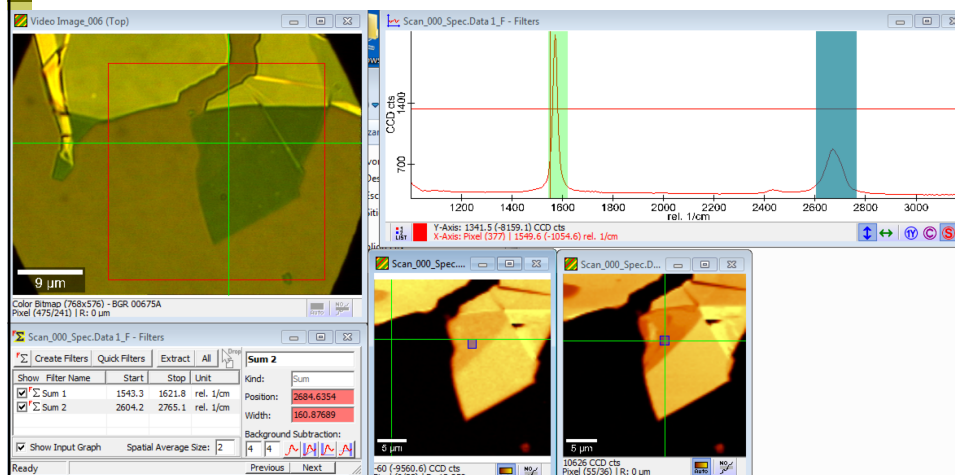
Ejemplo 5: Grafeno y MLG



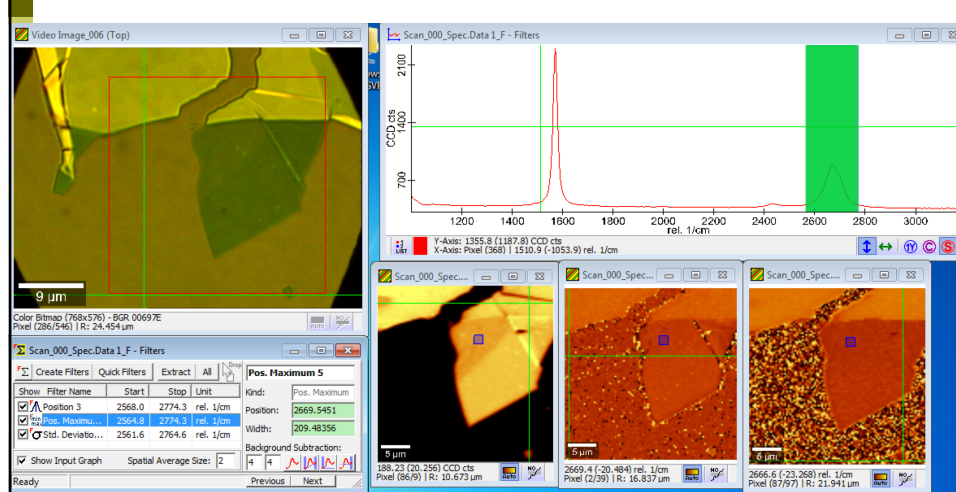
Ejemplo 5: Grafeno



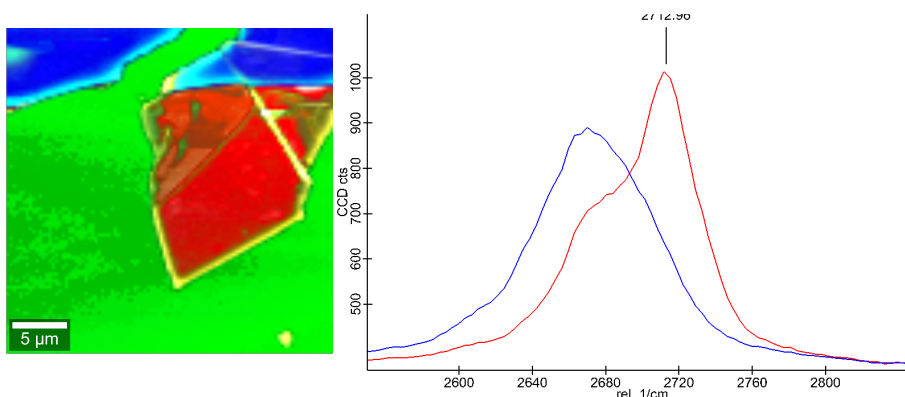
Ejemplo 5: Grafeno



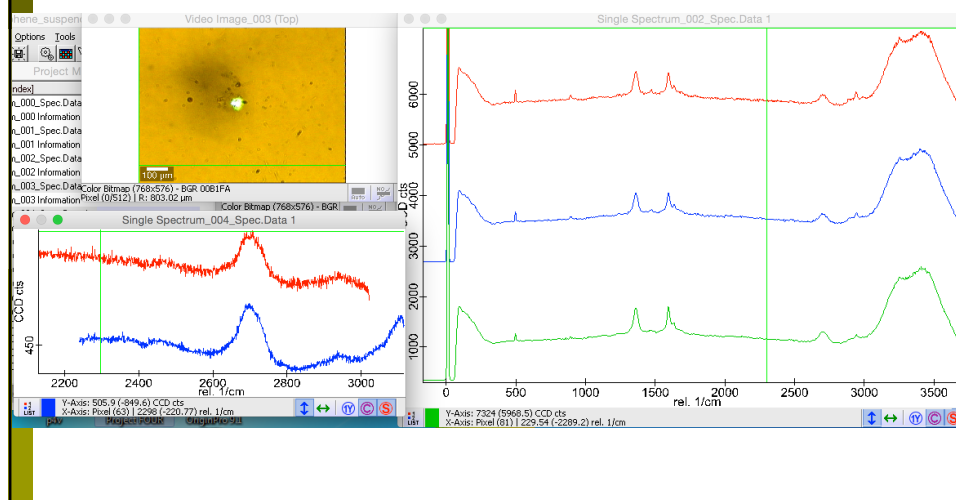
Ejemplo 5: Grafeno



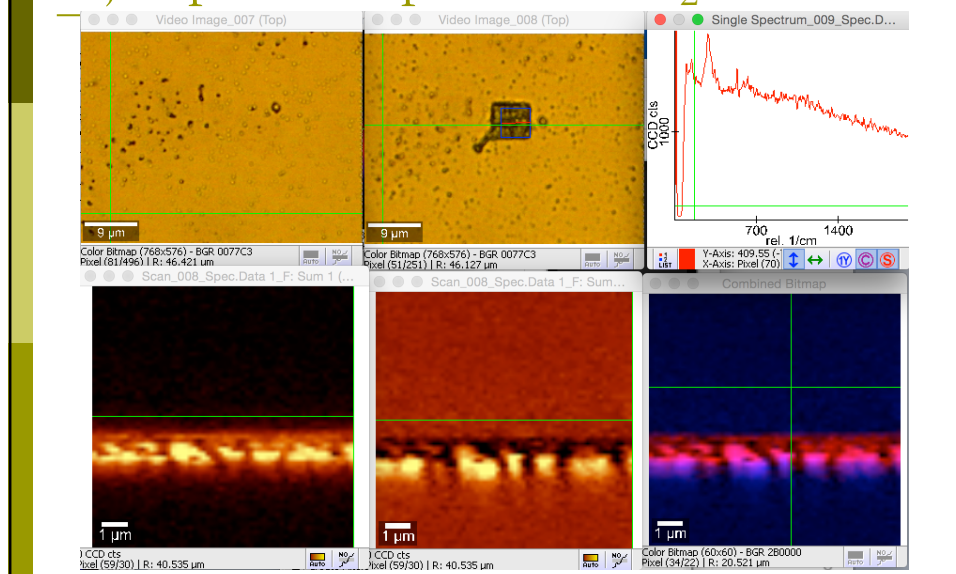
Ejemplo 5: Grafeno



Ejemplo 5: Grafeno en Solución



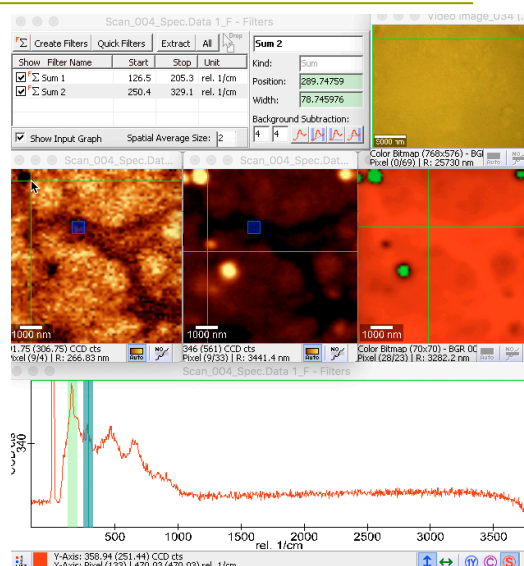
Ejemplo 6: Depth Scan Cu_2O



Ejemplo 7: Caracterización de NT

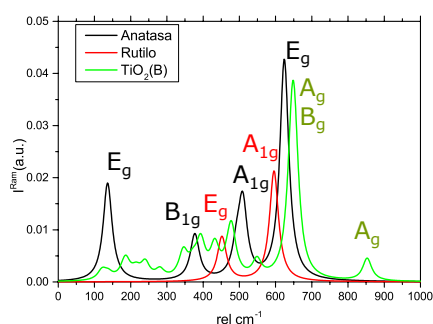
Ejemplo de Funcionamiento:

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



Modelado: Espectros Raman de polimorfos de TiO_2

Espectros Raman Calculados

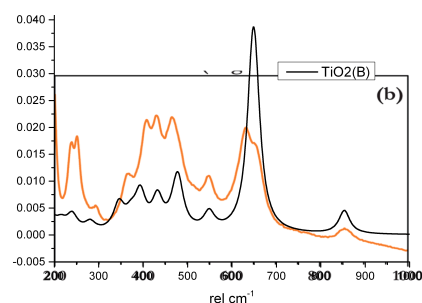


Cálculos DFT: Código VASP

Ecut=400 eV

Ondas Planas

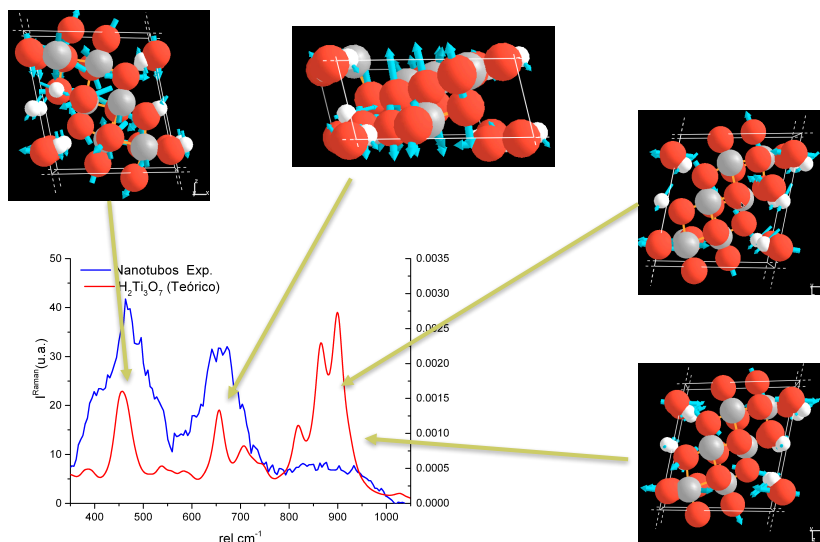
DFPT fonones



Espectros Raman Calculados y Experimental:

Dylla et al, J. Phys. Chem. Lett. 2012, 3, 2015 2019

Modelado: Espectros Raman de Titanatos de H y Na (Teóricos)

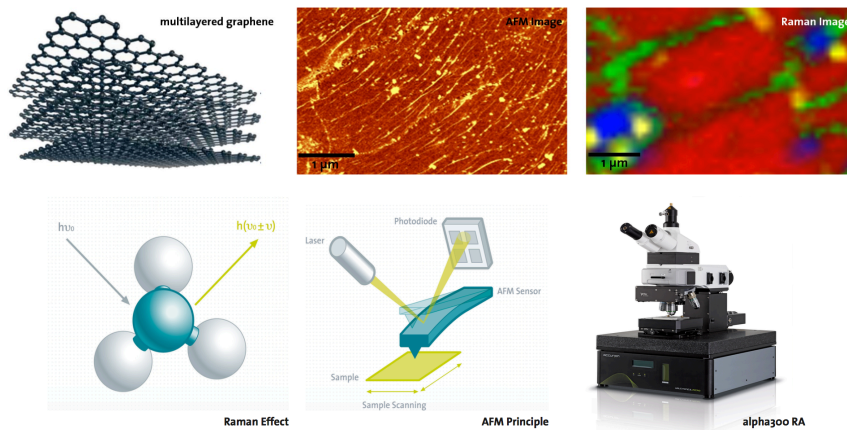


Resumen: Micro Raman Confocal

- ▣ 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)

Micro Raman AFM Dual

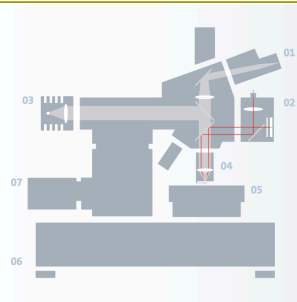
- ▣ Alpha 300R → Alpha 300RA
- FSE_1_2014_1_102008 - ANII



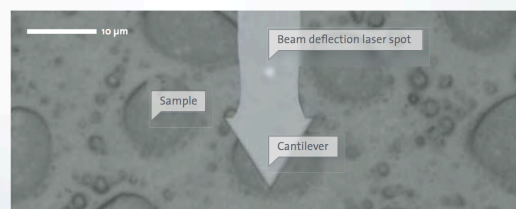
AFM y Raman

BEAM PATH

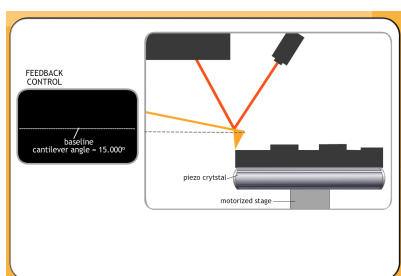
- 01 Video camera system
- 02 Beam deflection unit
- 03 LED white light illumination
- 04 AFM objective with cantilever
- 05 Scan-stage
- 06 Active vibration-isolation table
- 07 Z-stage for focusing



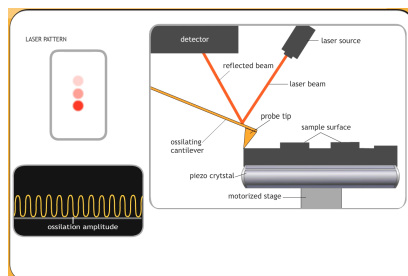
SIMULTANEOUS CANTILEVER AND SAMPLE VIEW



Métodos de Medida

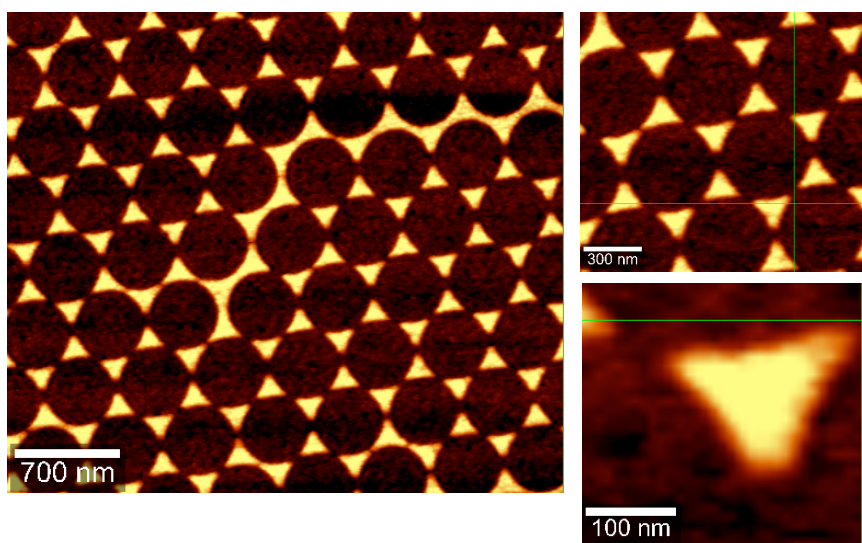


Contacto

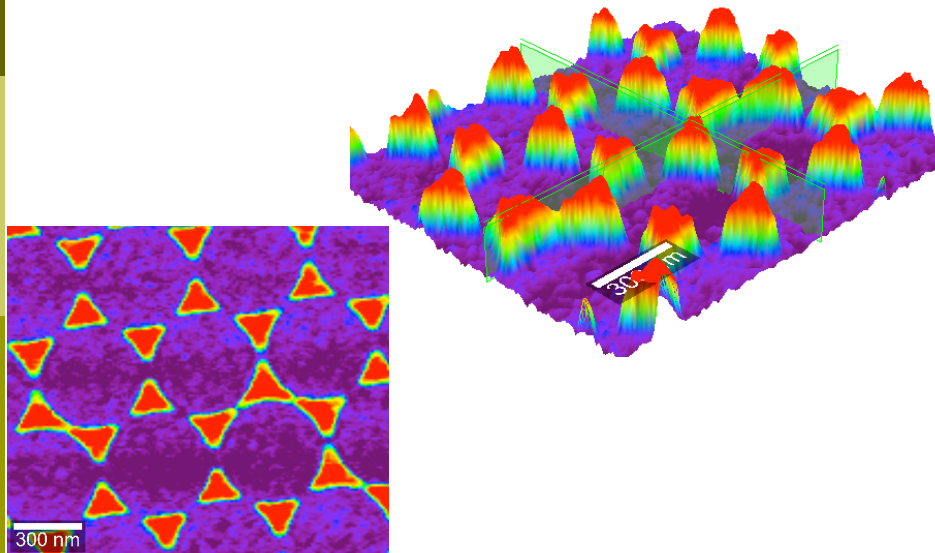


AC Mode

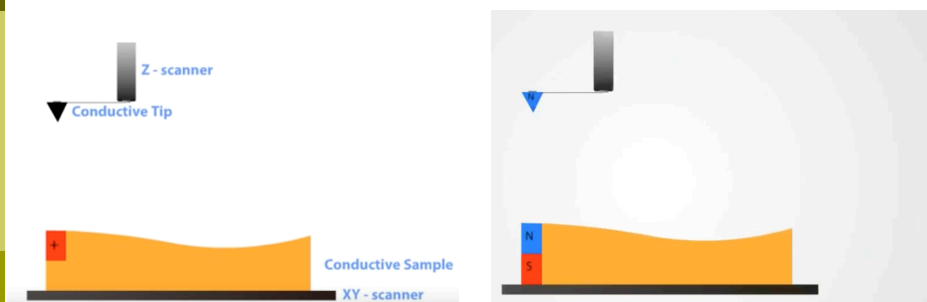
Contact mode: Triángulos de Al



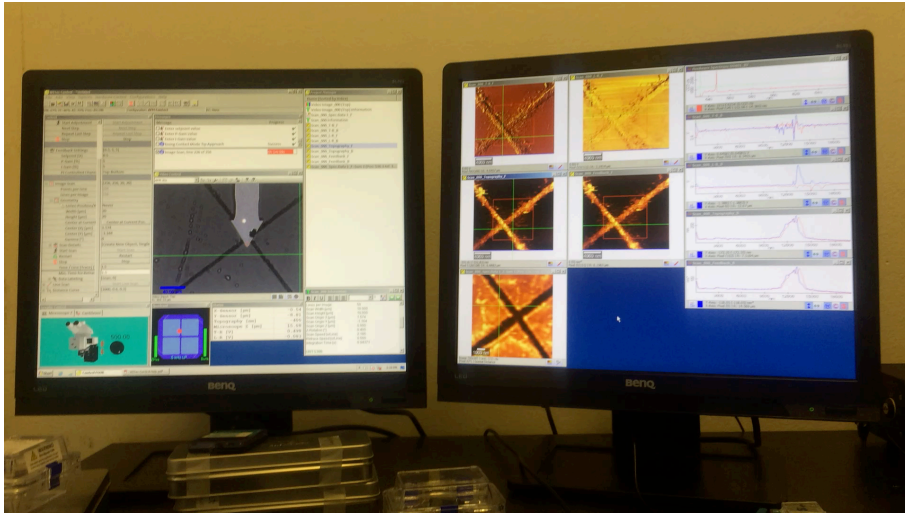
Contact mode: Triángulos de Al



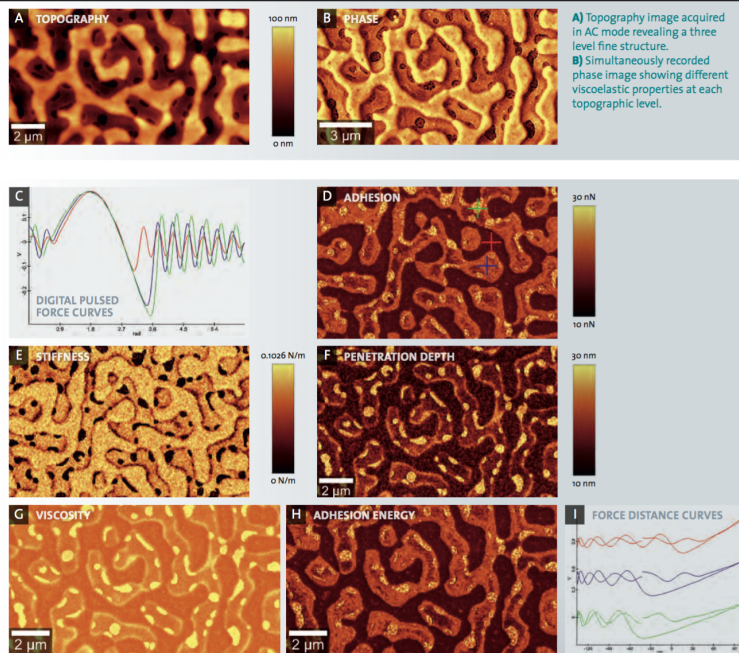
EFM & MFM



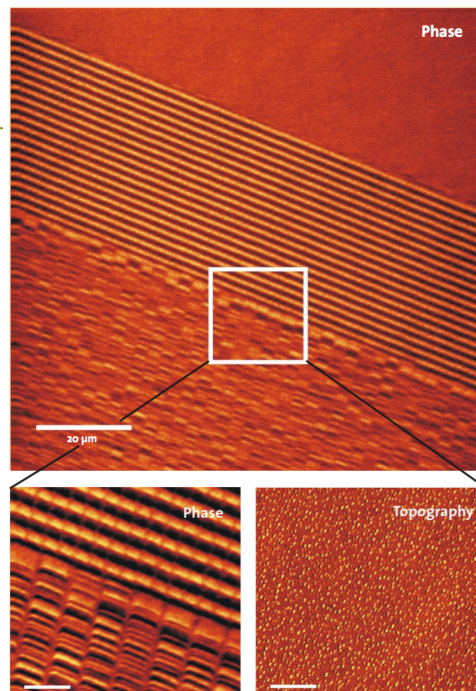
Raman & AFM Dual



COMPREHENSIVE AFM-RAMAN STUDY OF A POLYMER BLEND (Polystyrene:Ethyl-Hexyl-Acrylate:Styrene-Butadien-Rubber, 1:1:1)



□ MFM



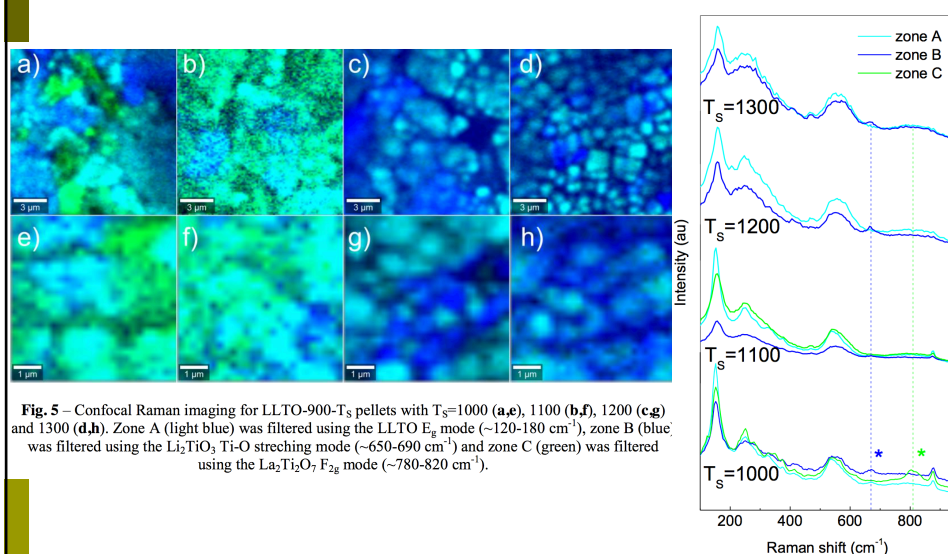
Resumen: AFM/EFM/MFM

- Diferentes Modos de Medida:
 - *Contacto*
 - *No Contacto*
 - *Liftmode*
- AFM/EFM/MFM
- Topología y Propiedades Físicas:
 - *Dureza*
 - *Viscosidad*
 - *Adhesión*
- Posibilidad de medir en Líquidos

Nanomateriales Para Energía

- ▣ Celdas Solares
 - Nanoestructura de TiO₂ para DSSC.
- ▣ Baterías de Ion-Litio (FSE 2011/2014)
 - Electrolitos Sólidos y Poliméricos.
 - Cátodos de LiFePO₄.

LLTO: Cerámico



LLTO-PMMA:

Microscopia Raman Confocal:

- LLTO muestra muy buena afinidad con grupos nitrato

Fig. 3 – Confocal Raman spectroscopy for LLTO (red), PMMA (blue) and LiNO_3 (black) sample references (a) and LiPMMA-LX with X=0 (b), 1 (c), 2(d) and 3(e). Raman spectra ascribed to LLTO-rich, PMMA-rich and LLTO-PMMA interface zones are represented with red, blue and black lines, respectively.

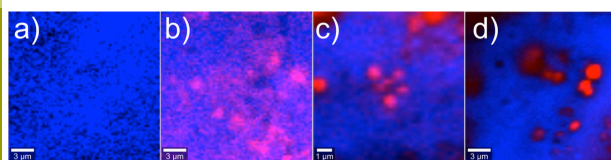
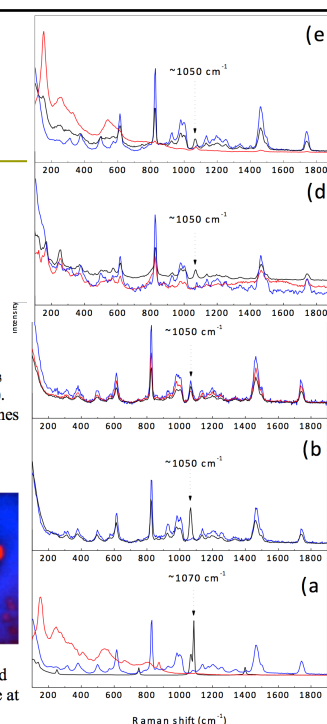


Fig. 4 – Confocal Raman microscopy for LiPMMA-LX with X=0 (a), 1(b), 2(c) and 3(d). The filtering of the LLTO A_{1g} mode at $\sim 145 \text{ cm}^{-1}$ and the PMMA $\nu(\text{C}=\text{O})$ mode at $\sim 1725 \text{ cm}^{-1}$ are shown in red and blue, respectively.



Agradecimientos



AGENCIA NACIONAL
DE INVESTIGACIÓN
E INNOVACIÓN

- Parque Científico y Tecnológico de Pando (PCTP) / Instituto Polo Tecnológico de Pando
- ANII- Proyecto EQC_X_2012_1_14.
- ANII-FSE_1_2014_1_102008 Fondo Sectorial de Energía.
 - Microbiología, DEPBIO – FQ.
 - Grupo de Farmacología & LEA, CIENFAR - FQ
 - Instituto Nacional de Donación y Trasplante - INDT
 - Grupo Física del Estado Sólido– IF-FING
 - Cátedra de Química Inorgánica –FQ
 - Cátedra de Análisis Instrumental – FQ
 - Cátedra de Fisicoquímica -FQ
 - Laboratorio de Biomateriales – FCIEN



