# *ALBA synchrotron: new tools for materials characterization*

## The soft X-rays microscope at Mistral beamline

# *Outline*

- **The transmission soft X-rays microscope (TXM)**

  *** Basic concepts**

  *** Inside the TXM**

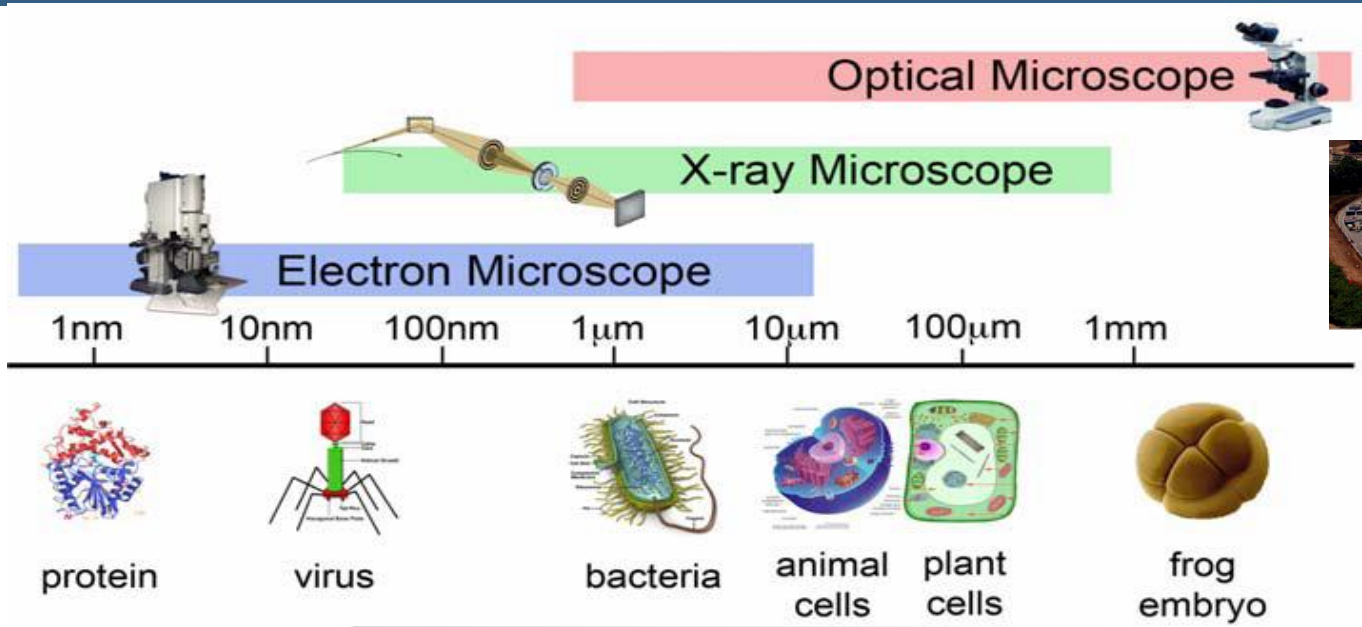
- **Techniques and applications**

  *** Cryo tomography**

  *** Magnetic contrast imaging**

  *** Spectroscopic imaging**

# Why X-ray Microscopy?




***A massive microscope to study the structure and the interior of matter***

## *Advantages*

- **Fully hydrated thick specimens in biomedical application**
- **Inherent natural contrast of light elements of wet samples**
- **Quantitative (elemental composition, oxidative state *in situ*)**
- **Better than 25-30 nm resolution – isotropic**
- **Fast - collect tomographic data set**
- **Spectroscopic imaging**

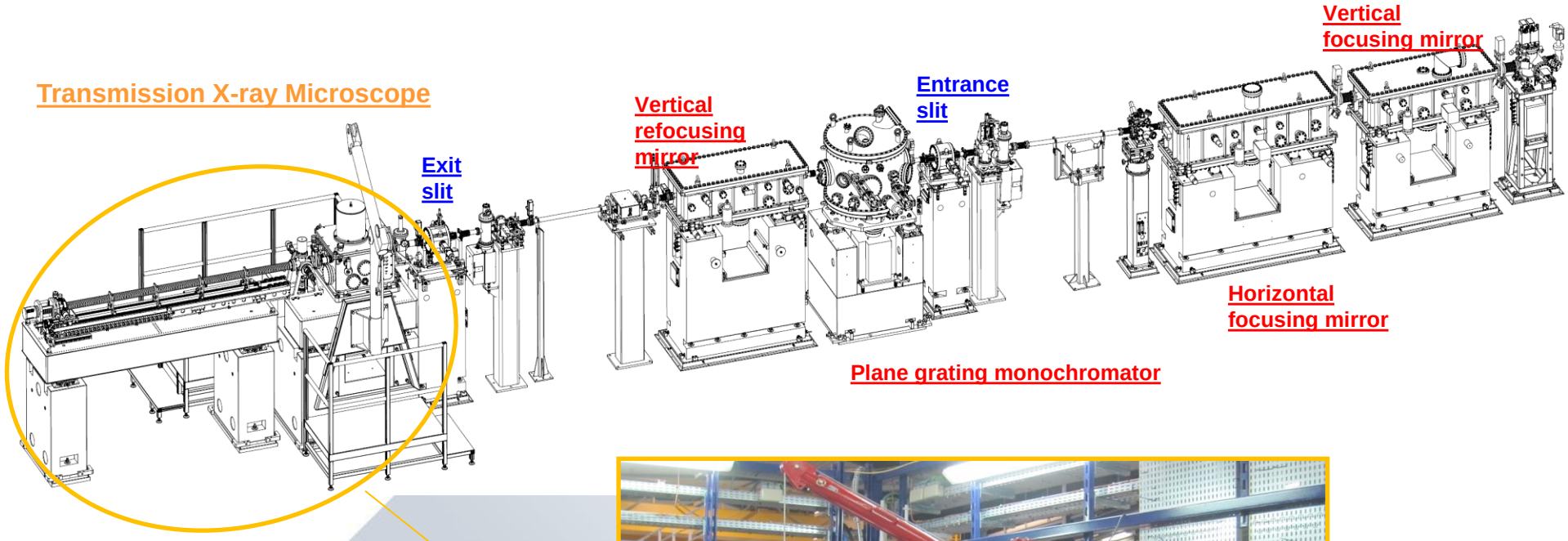
# Soft X-ray microscopy- a tool to image inherent contrast of intact materials

- X-ray crystallography solving the macromolecular structure- advantage of the atomic resolution resulting from the small wavelength of X-rays. On the macro scales of the clinical research X-rays are used by benefit of the high penetration depth. In between these two extremes is *X-ray microscopy* - a new and yet exceptional *tool in the imaging*.

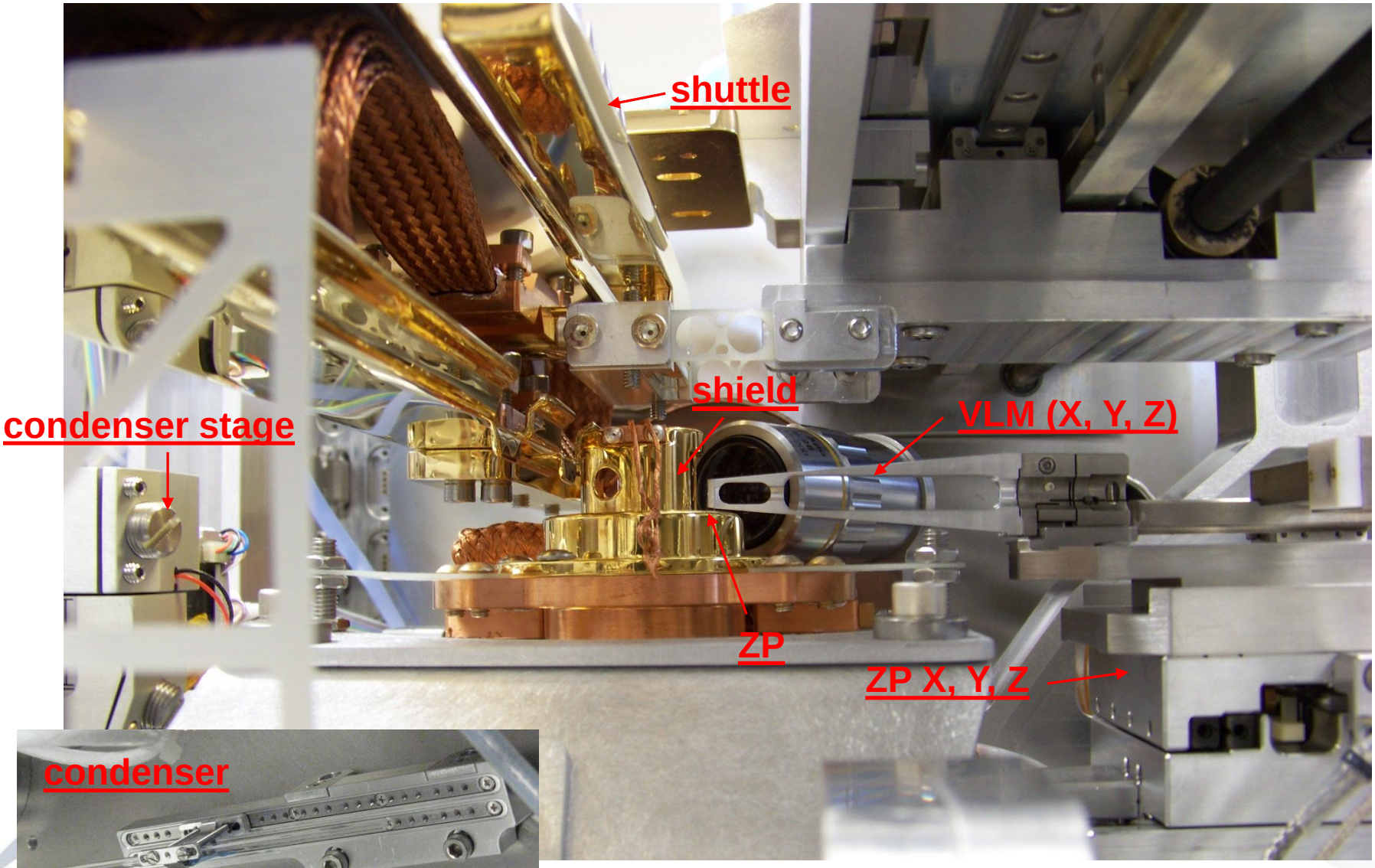
- Soft X-ray cryo-tomography is a *new complementary approach* in bio-medical and life science fields that, which in combination with fluorescence microscopy, can provide answers at medium resolution on the organelles organization in whole, unstained, un-sectioned cells.

- MISTRAL cryo-TXM at the ALBA Synchrotron Light Source opened for users in February 2013. It is devoted mostly to *soft X-ray microscopy of large cellular volumes mostly in the water window energy range.*

# What do we call the microscope at Mistral

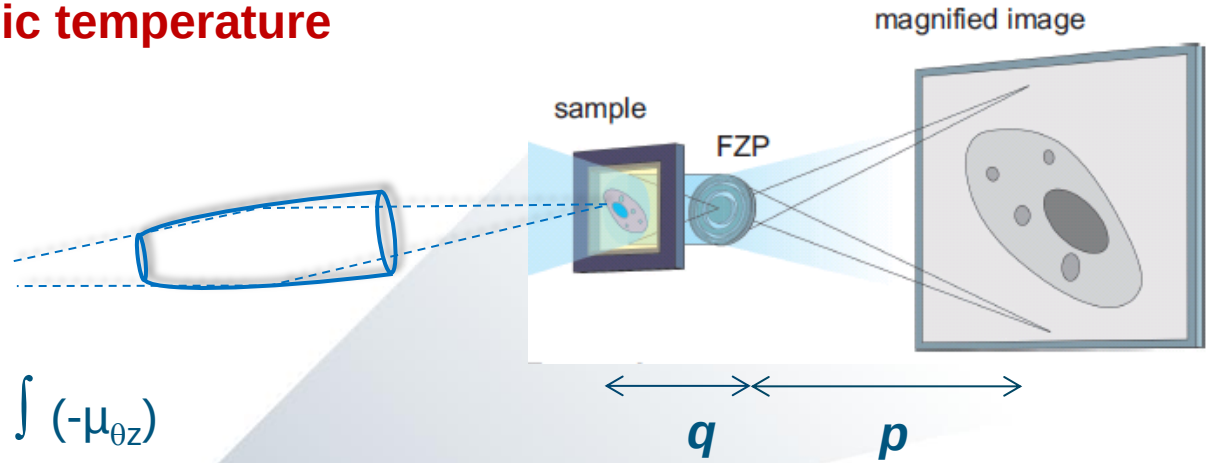



## *Inside the TXM*



# The transmission soft X-rays microscope

- **25-30 nm resolution**
- **covered E range in 1st phase: 275-1200 eV**
- **3D and spectroscopic imaging**
- **vacuum system**
- **cryogenic temperature**




$I_\theta = I_0 \exp \int (-\mu_{\theta z})$

Magnification assuming a "thin lens"

***M=p/q , p>>q → M>>1***

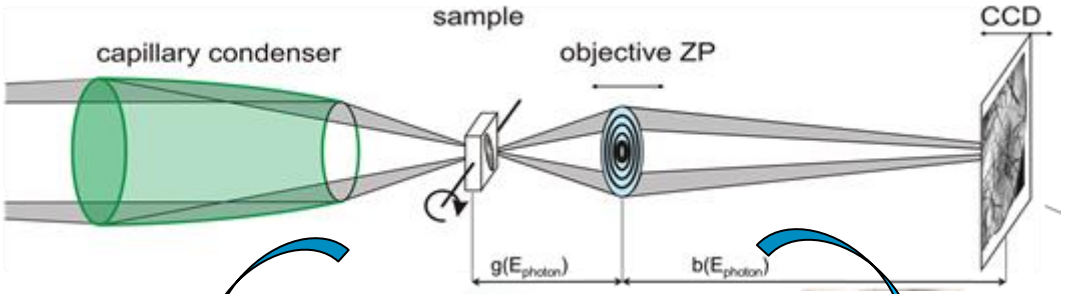
***→ $P_{eff}$=P/M "small enough"***

Resolution of the imaging system limited by the objective lens:

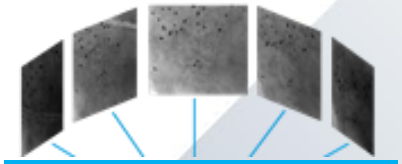
***$r_s \sim \lambda/Na$***

***$\lambda$ wavelength***
***Na "numerical aperture"***

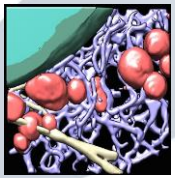
# Applications: 3D & spectroscopic imaging




***Tomography***



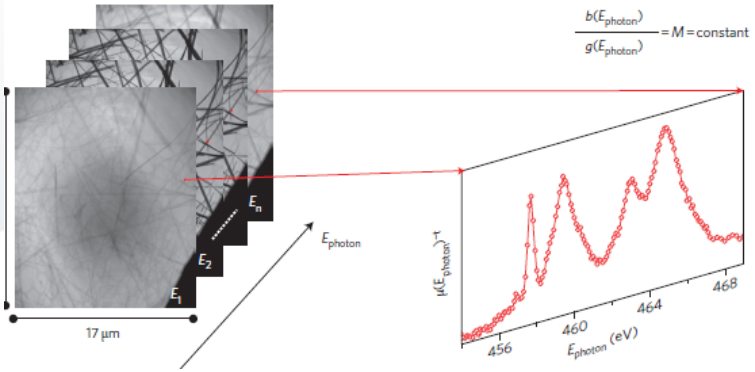
***Reconstruction and segmentation***



*From Perez et al., unpublished*

**3D imaging to reveal structure**

***Spectroscopy***




*From Guttmann et al., 2012, Nature Photonics 6: 25-29*

**Spectroscopic imaging to map chemical elements**

*e.g.* light elements in living cells: H, C, N and O constitute 96% (w/w)

Na, Mg, P, S, Cl, K and Ca make up the remaining 4%

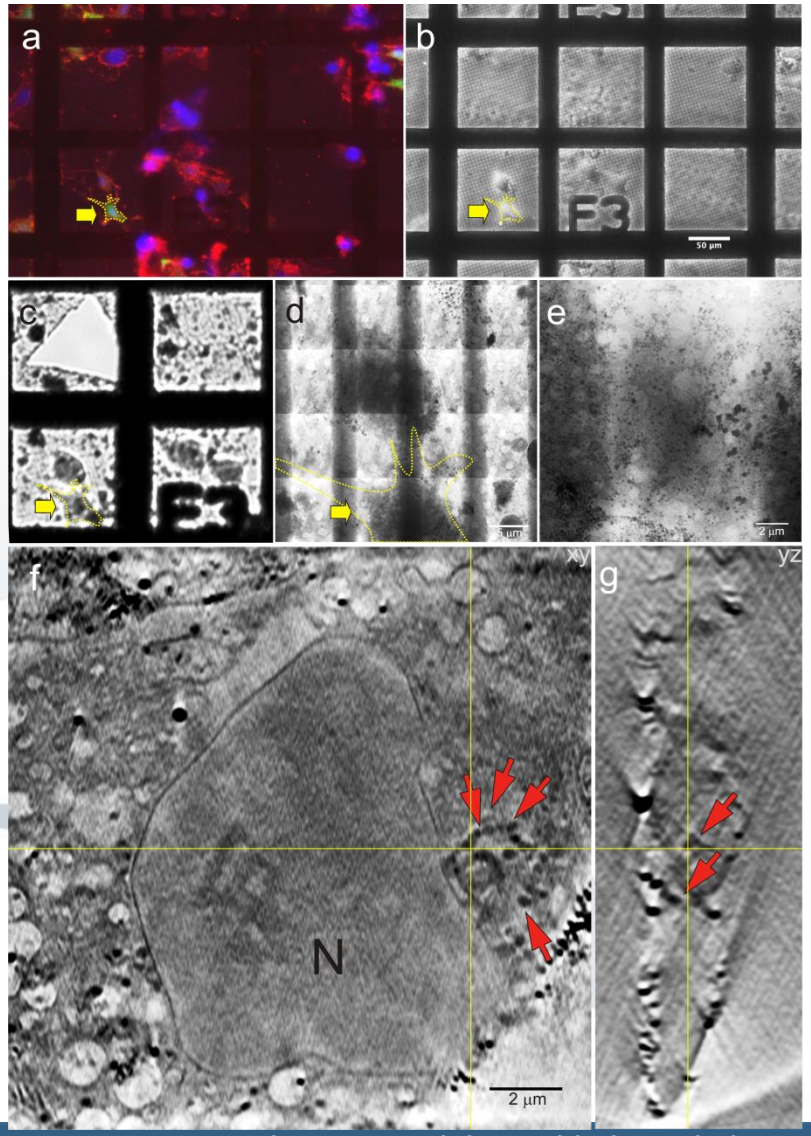
# Experiments workflow: from sample preparation to X-ray imaging

### *Principe of the correlative imaging: optical light and x-ray microscopy*

The soft X-ray cryo tomography used to reconstruct in three dimensions whole infected Ptk2 host cells at different post-infection times to study the cellular rearrangements caused by the Vaccinia virus infection.

*The correlative microscopy of the Ptk2 cells infected with MVA-C-DF1C (GFP). a) and b) in vivo light microscopy of the infected cells growing*
*c) Cryo-light microscopy in line with the Transmission X-ray Microscope of vitrified grid corresponding to the same area in a and b.*
*d) mosaic composition on cryo transmission soft X-ray microscopy images of the same area of the grid. e) 0º soft X-ray projection image of the same cell at higher magnification. f) and g) virtual slices of the reconstructed soft X-ray cryo-tomogram xy and yz planes respectively.*

*The group at CNB-CSIC Madrid: Dr. Chichón and Prof. Carrascosa.*

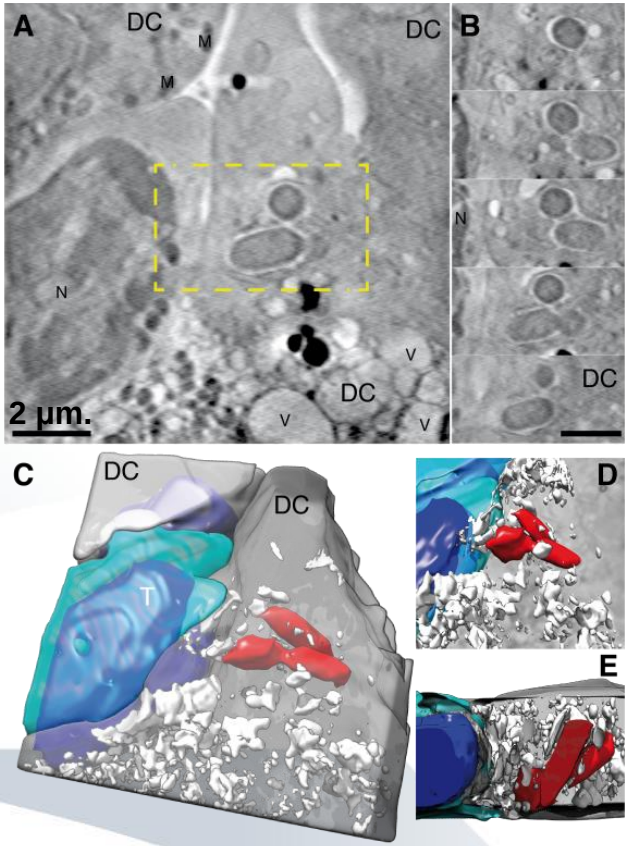


# Example I:

## *X-ray microscopy of Bacteria Trans-infect T lymphocytes through the Immunological Synapse*

- During infections several bacterial species survive phagocytosis and disseminate systemically through infected antigen presenting cells such as dendritic cells (DC). It has been proposed that T cells could also serve as bacterial reservoir during infections in mice.
- Taken into account that primary T cells are infected poorly *in vitro*, the route bacteria invade T cells remains unknown.
- Here was demonstrated that T cells take up bacteria from infected DCs through the Immunological Synapse. T cells acquire bacteria more efficiently from infected DCs carriers than by direct exposition to bacteria, in a process remarkably enhanced by antigen recognition .

*Project of Dr. E.Veiga at CNB-CSIC and Instituto de Investigación Sanitaria Princesa at Hospital de la Princesa (Madrid).*




*Virtual slice of a tomogram of an infected dendritic cells DC exposing internal bacteria. N - the nucleus of the T cell and V - vesicles. Bacteria are visible in the dashed square. (B) Consecutive virtual slices every 460 nm showing the proximity of the three bacteria, in the square of A. (C, D and E) Volumetric representations of the tomogram in A and B: the T cell – cyan, the nucleus – blue, DC- grey and the bacteria- red*
(Cruz et al., 2014. Cell Host & Microbe, 15: 611-622) .

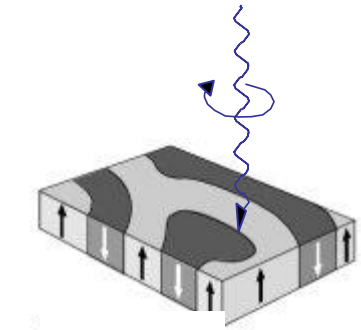
# Example II:

## *Magnetic imaging of 60 nm diameter magnetic bubbles*

- At the TXM there is the possibility of high resolution imaging of domains using the microscope and the circular magnetic dichroic absorption contrast.
- Thanks to the microscope performance and the beamline optics, it is possible to achieve detailed information on the changes of a specific magnetic domain when a pulsed magnetic field is applied.
- Depending on the magnitude of the pulsed field, either maze, bubbles or intermediate situations have been observed.
- The samples - thin films (80 nm) of Co5Nd alloys deposited on Si3N4 membranes.
- The pulses of magnetic fields were created with a microcoil developed at Alba. They could achieve +/- 1 T as a maximum amplitude and had a duration of about 15 ms.

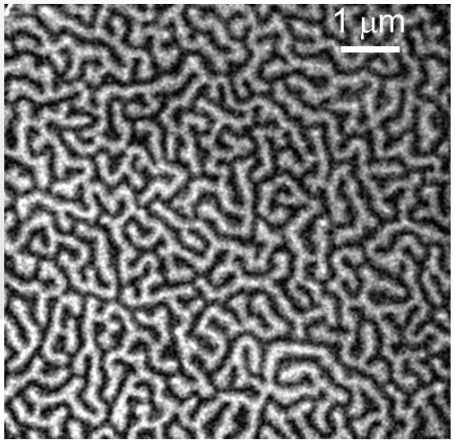
# Magnetic imaging

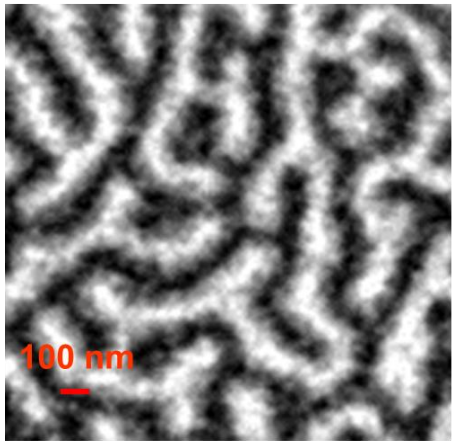
***If the circularly polarized X-rays is used, the absorption coefficient will depend on the projection of the magnetization onto the photon propagation direction.***



***"Magnetic contrast"***

**Co/Pt multilayer of 50 nm thickness at 778 eV ( Co $L_3$ edge), t=20s**

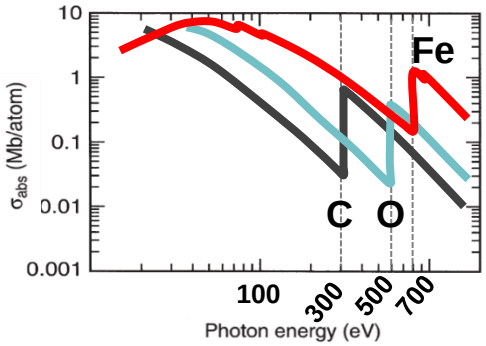


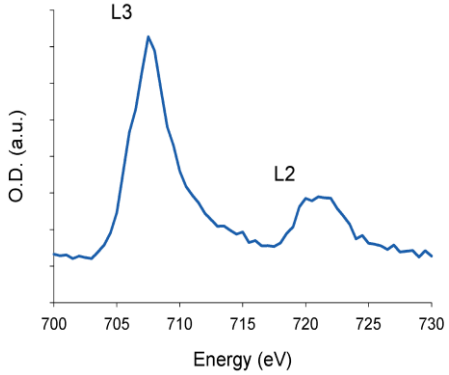




*Alba team: E. Pereiro, A. Sorrentino ,S. Ferrer R. Valcarcel, J. Avila and O. Matilla in collaboration with Universidad de Oviedo: Carlos Quiros , Cristina Blanco , Maria Velez and Jose M. Alameda, and CNM : Jaume Esteve and Marta Duch.*

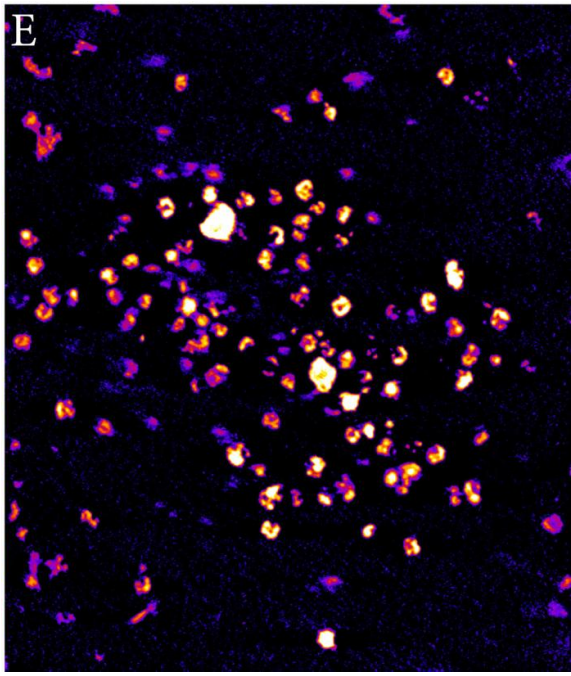
# Example III:

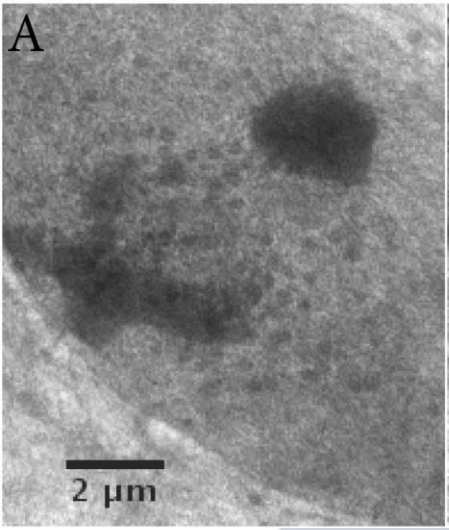
## Spectroscopic imaging

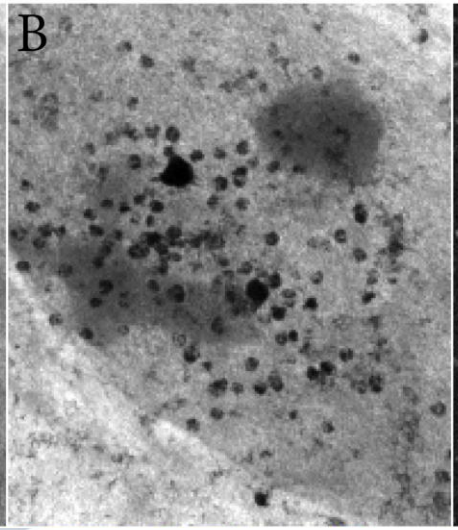


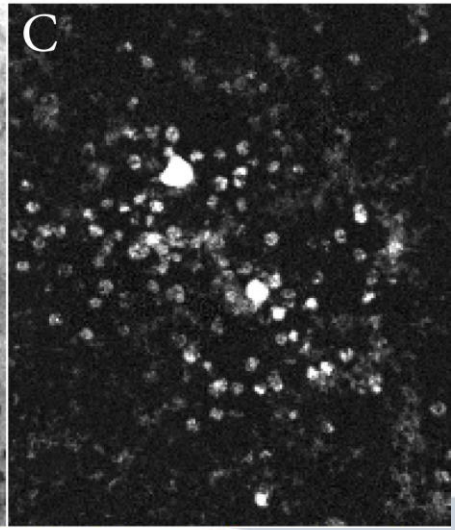




SPION uptake in MCF-7 cancer cells




0 deg projection before L3 Fe edge

after L3 Fe edge

0 deg edge map

central slice of the BgART reconstructed volume

JJ Conesa et al., unpublished

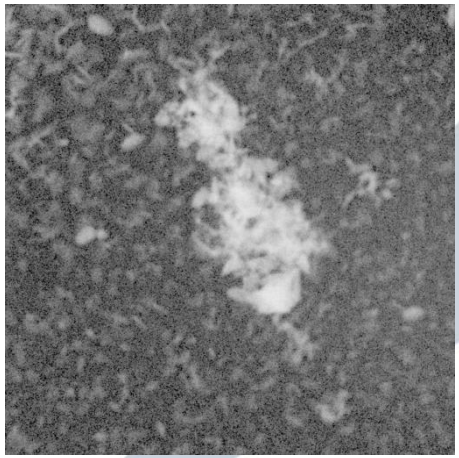
FJ Chichón, JJ Conesa, M. Chiappi (CNB-CSIC), E. Pereiro (ALBA)

# Soft x-ray microscopy of lithium-oxygen batteries

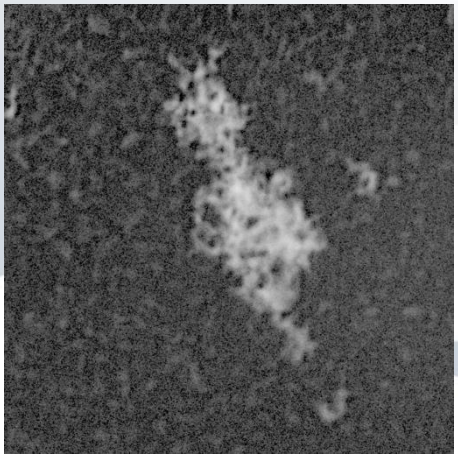
- The performance of lithium air batteries in organic electrolytes depends on the composition and the morphology of the discharge products, which in the ideal case consist of lithium peroxide. This is deposited within pores of a material that is typically carbonaceous.
- Soft X-ray microscopy is able to provide unique access to light elements, and separately visualize the host porous network and the deposited oxide.
- Additionally the spectroscopy around O-edge can provide valuable information on the chemical state of O, giving clear hints on the composition of the discharge product actually present.

*2Li → 2Li+ + 2e- (anode)*
*O2 + 2Li+ + 2e- → Li2O2↓ (cathode)*
*2Li + O2 → Li2O2↓ (overall).*

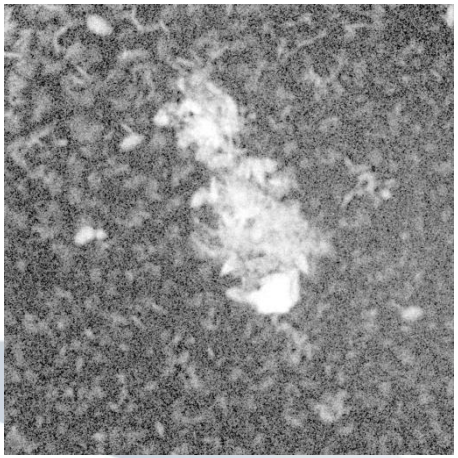
***Preliminary results of the group D. Tonti, UAB***



**520 eV**

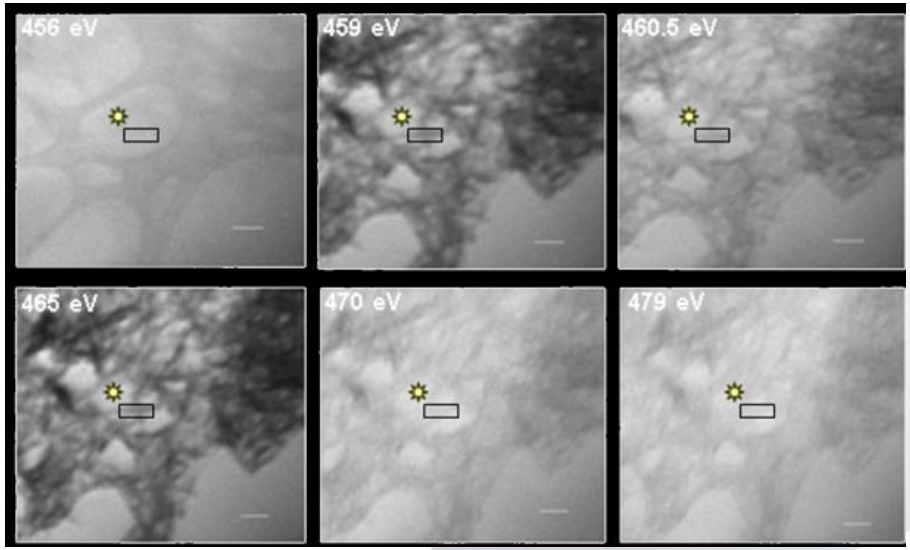


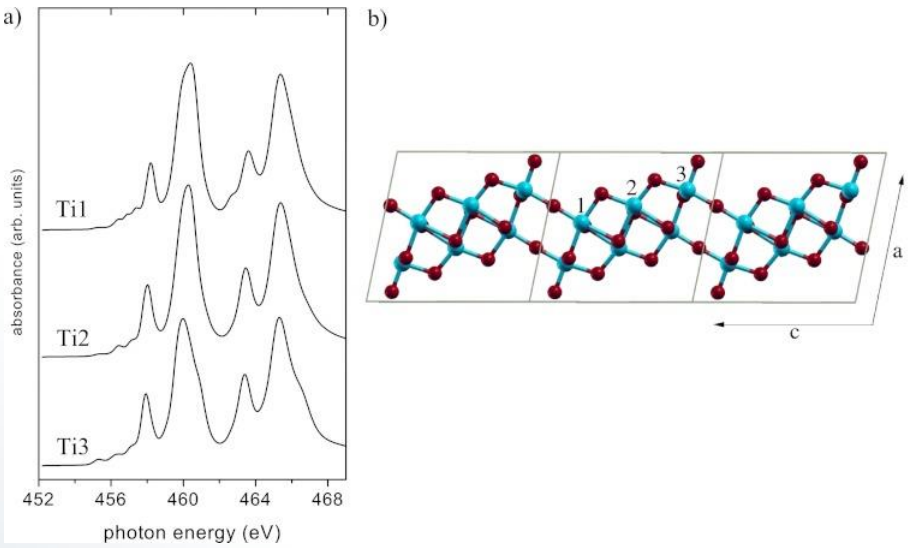
**540 eV**

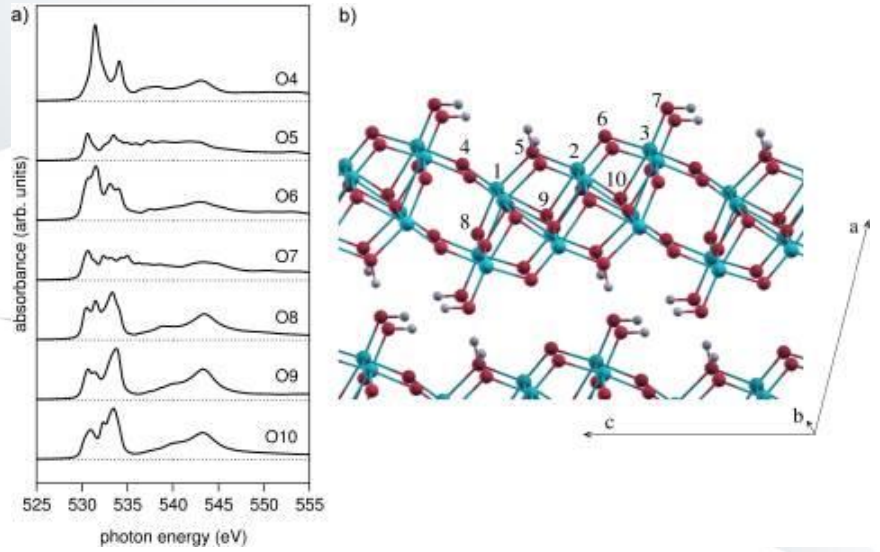


**I(540)-I(520)**

# Towards atomic resolution in sodium titanate nanotubes using near-edge X-ray-absorption fine-structure spectro-microscopy




*From Bittencourt et al., 2012.*
*Beilstein J. Nanotechnol. 3:789-97*

# Conclusions

- Each microscopy technique has its own advantages and restrictions: **the multimodal combination** of all techniques enables to get a deeper understanding of molecular changes.

- **Combination of 2- and 3-D X-ray microscopic techniques** for a deeper cellular and molecular insight into modification on the structural/cellular level during the chemical changes.

- **Spectro-microscopy imaging approach** allow to elucidate subcellular structures in bio-medical samples and elemental specification at the nanometer scale which could be used for the investigation of different samples *in situ*.

# Mistral team:

**E. Pereiro,**
**A. J. Perez,**
**M. Rosanes,**
**A. Sorrentino,**
**R. Valcárcel,**
**T. Ducic**

***Thank you!***