# MIXING THE MICROSCOPIES

*An Insight Over the Capabilities of the Correlative Microscopies*


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# SUMMARY

- Correlative Microscopy – a definition
- CLEM – Correlative Light to Electron Microscopy
- CPEM – Correlative Probe and Electron Microscopy
- Correlative Raman and Electron Microscopy
- Correlative X-ray Tomography and Electron Microscopy
- Towards a multi-scale 3D reconstruction

# CORRELATIVE MICROSCOPY – The definition

**DEFINITION**

*Analysis of the same Region of Interest (ROI) through different types of microscopy*

**OBJECTIVE**

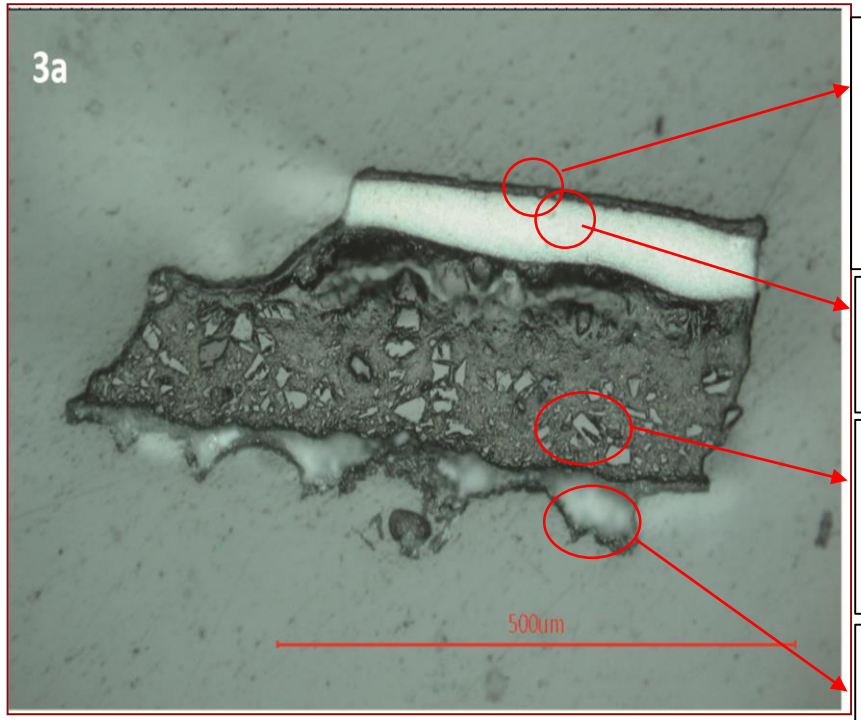
*To correlate on the same ROI the distinct information obtained from each microscopy*

# CLEM (Correlative Light – Electron Microscopy)

*Optical Microscopy*


*Electron Microscopy*

# CLEM (Correlative Light – Electron Microscopy)

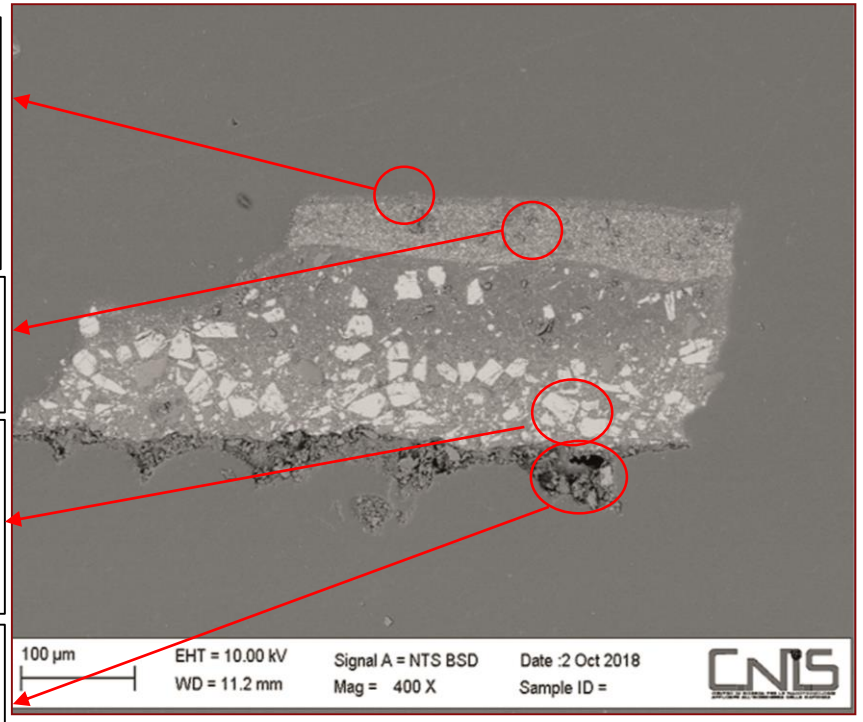



Surface earthy layer and black pigment of vegetable origin

White containing Zn, Ti and Ba

Imprimitura containing $BaSO_4$ grains

Preparatory layer made of glue and chalk




*Pictorial stratigraphy of a contemporary picture from GNAM (Roma) – Courtesy of Dr. M.P. Sammartino (Dip. Chimica, Univ. La Sapienza)*

# CLEM (Correlative Light – Electron Microscopy)

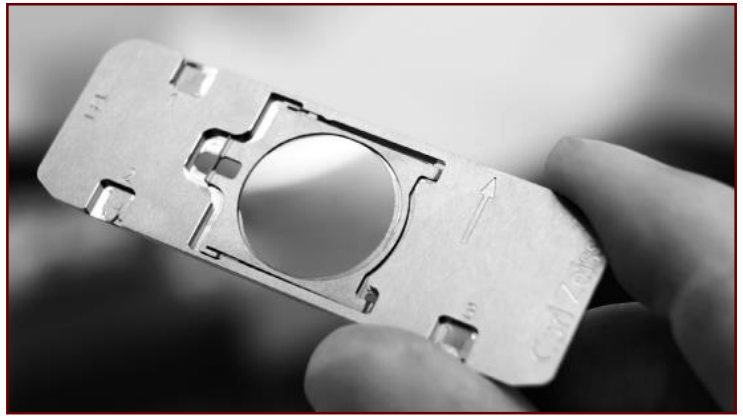

*Fluorescence Microscopy*

*Electron Microscopy*

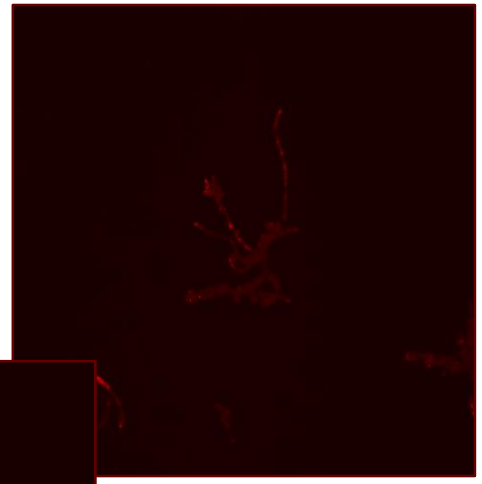
*Information about the cellular function*

*Information about ultrastructure*

# CLEM (Correlative Light – Electron Microscopy)



Zeiss Shuttle & Find Sample Holder



Candida albigans' Hyphae
*(prof.ssa D. Uccelletti – Dip. Biologia e Biotecnologie, Università La Sapienza Roma)*

# CPEM (Correlative Probe – Electron Microscopy)

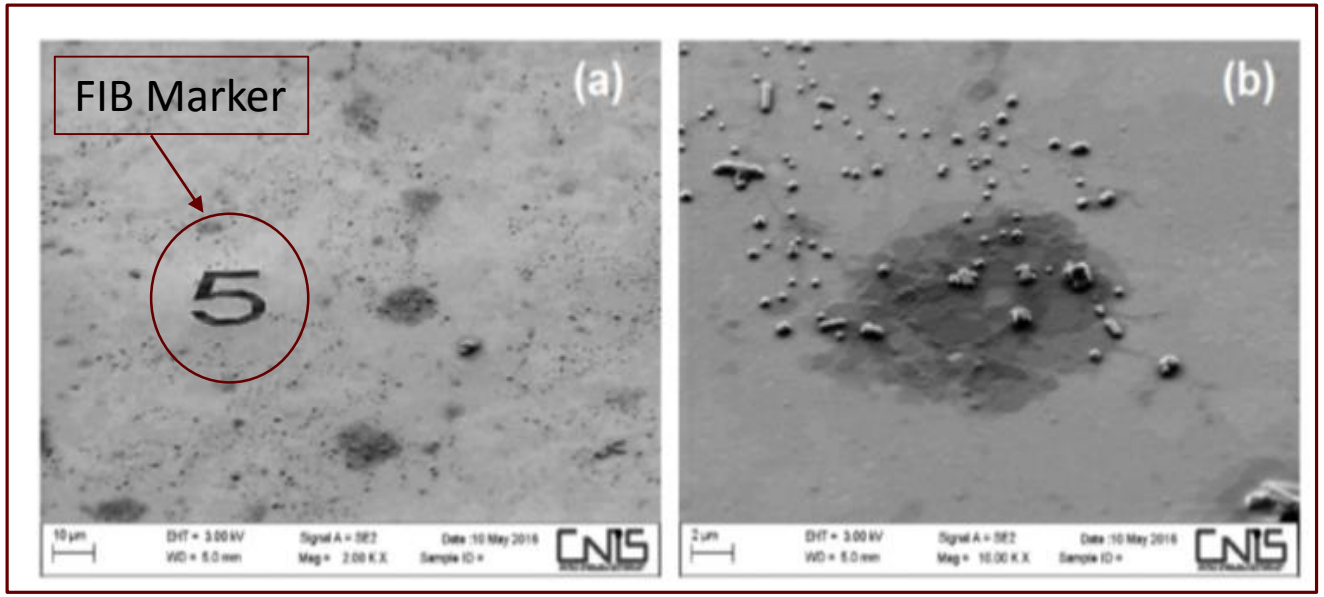

*Atomic Force Microscopy*

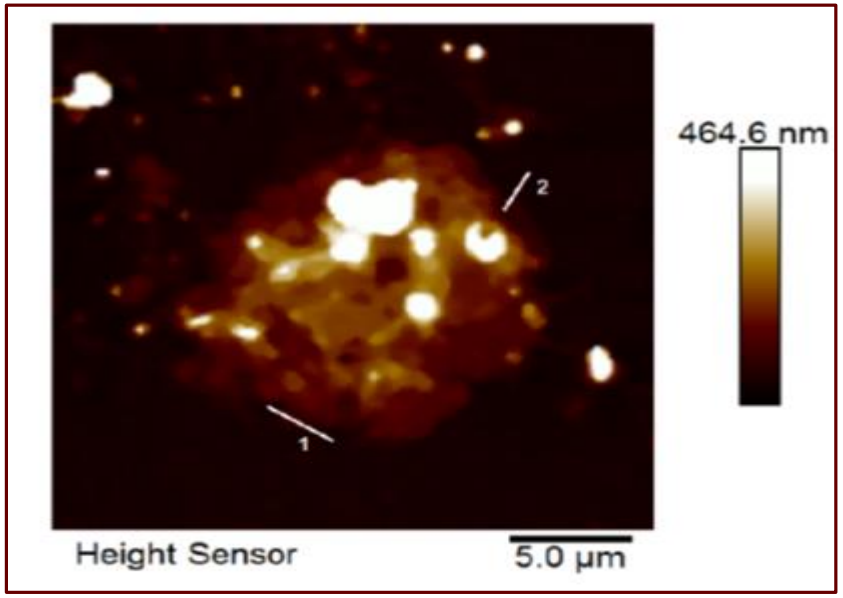
*High resolution on Z*
*Information about mechanical, electrical and magnetic properties*


*Electron Microscopy*

*High resolution range (mm – nm) & Fast Imaging*
*Element Analysis (EDX)*

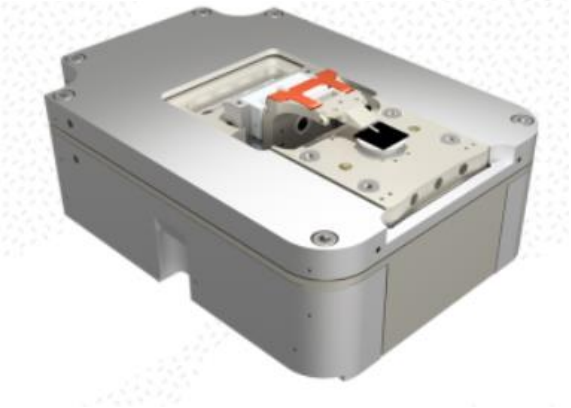
A simple way to correlate the two microscopies is to draw a large maker near the ROI by the Focus Ion Beam




Giant Unilammelar Vescicles (GUV)
*(Dr.ssa A. Capocefalo – Dip. Fisica, Università La Sapienza)*

LiteScope™



NenoVision

*LiteScope™ is a small AFM module mountable on the SEM through an adaptor*

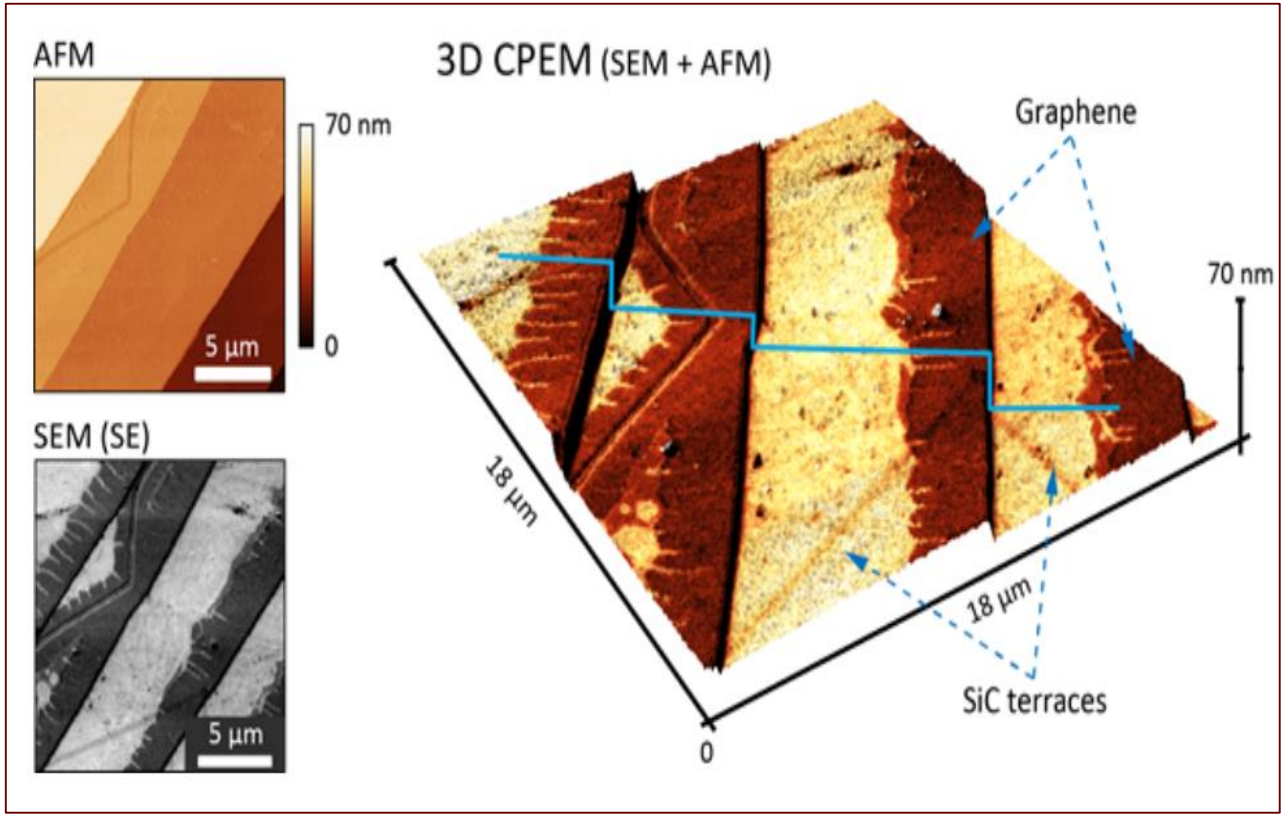
| Material mechanical properties | |
|---|---|
| Technique | Application |
| **Atomic Force Microscopy** (AFM) | topography |
| **Energy dissipation** | local elastic properties (tapping mode) |
| **Force Modulation Microscopy** (FMM) | local elastic properties (contact mode) |
| **Force-distance curves** | local elastic properties (non-topographic) |
| **Nanoindentation** | depth-dependent material characterization |
| **Nanomanipulation** | various in-situ operations |

| Material electrical properties | |
|---|---|
| Technique | Application |
| **Conductive AFM** (C-AFM) | conductivity map |
| **Conductive CPEM** (C-CPEM) | conductivity map including insulated areas |
| **Force Modulation Microscopy** (FMM) | local elastic properties (contact mode) |
| **Kelvin Probe Force Microscopy** (KPFM) | local surface potential |
| **Electrical spectroscopy** | local electrical properties (non-topographic) |
| **Scanning Tunneling Microscopy** (STM) | sub-nanometer topography |

| Material electro-mechanical properties | |
|---|---|
| Technique | Application |
| **Piezoresponse Force Microscopy** (PFM) | piezoelectric domain imaging |

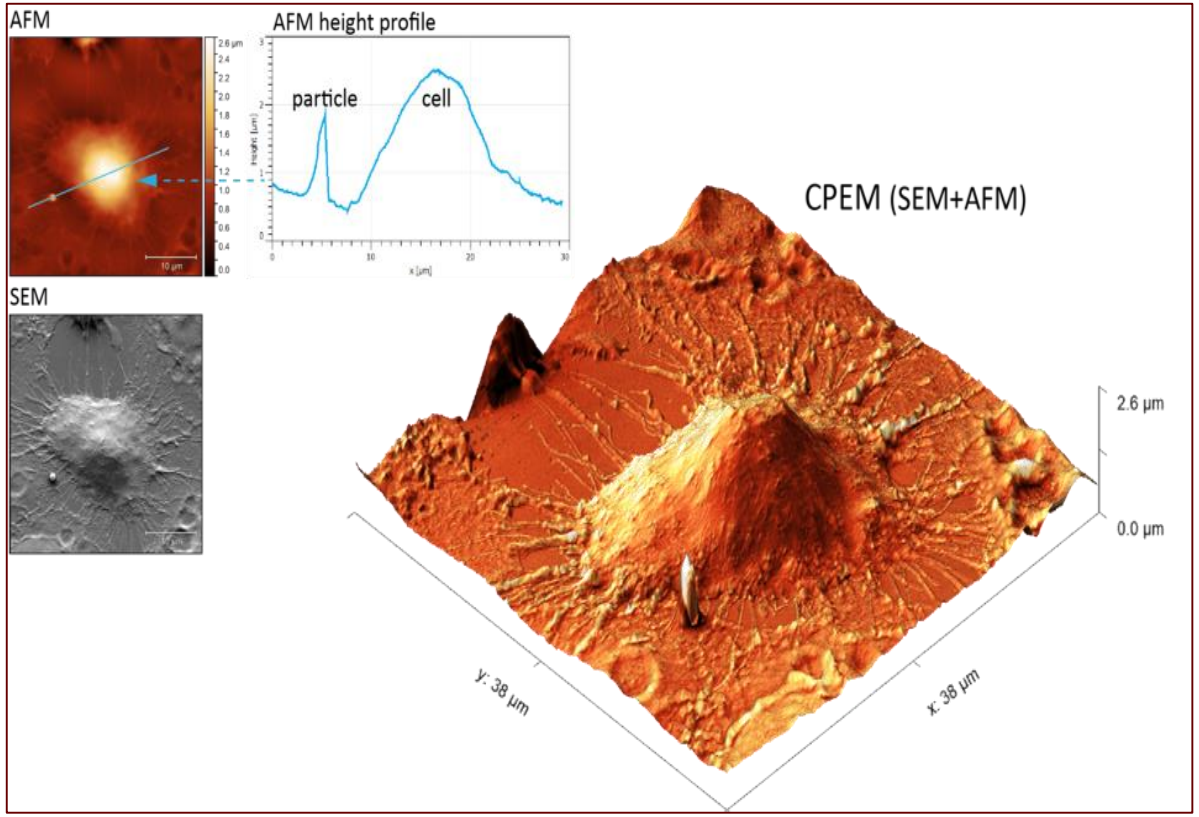
# CPEM (Correlative Probe – Electron Microscopy)

*Graphene on Silicone Carbide*




*Jan Kunc, MFF UK, Czechia*

*Cells conjugated with nanoparticles*




*Marco Cassani, ICRC-FNUSA, Czechia*

# RAMAN – Electron Microscopy

*Raman Spectroscopy*

*Identification of molecules, allotropes and polymorphs*
*Determination of the level of cristallinity and orientation*

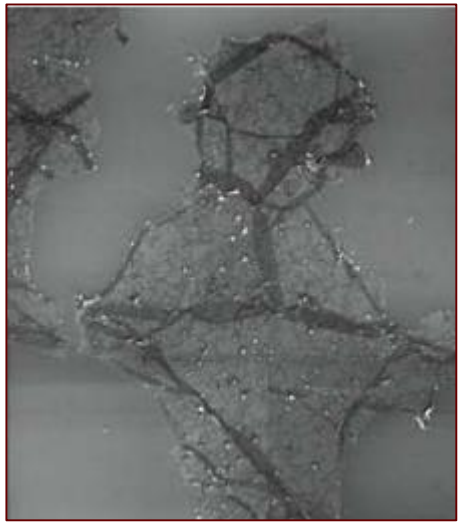

*Electron Microscopy*

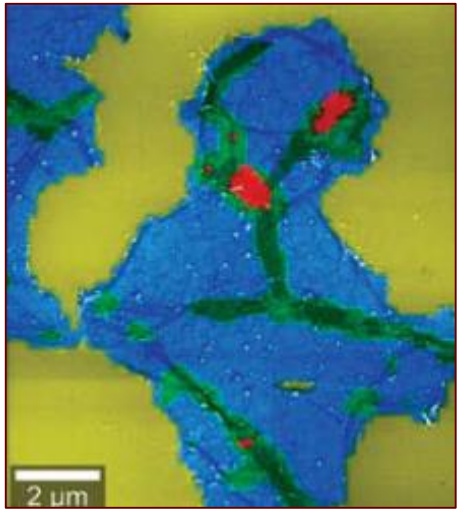
*High resolution range (mm – nm) & Fast Imaging*
*Element Analysis (EDX)*

# RISE™ Microscopy

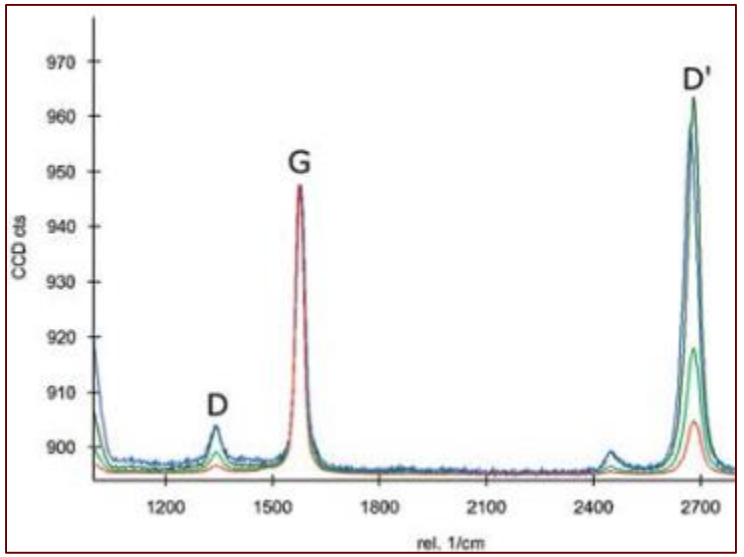
Correlative Molecular and Ultrastructural Imaging

WITec
focus innovations






*RAMAN in SEM characterization of the Graphene*




*Hollricher et al., Microscopy Today 22 (2014) pp. 36-39*

# X-Ray Tomography – Electron Microscopy



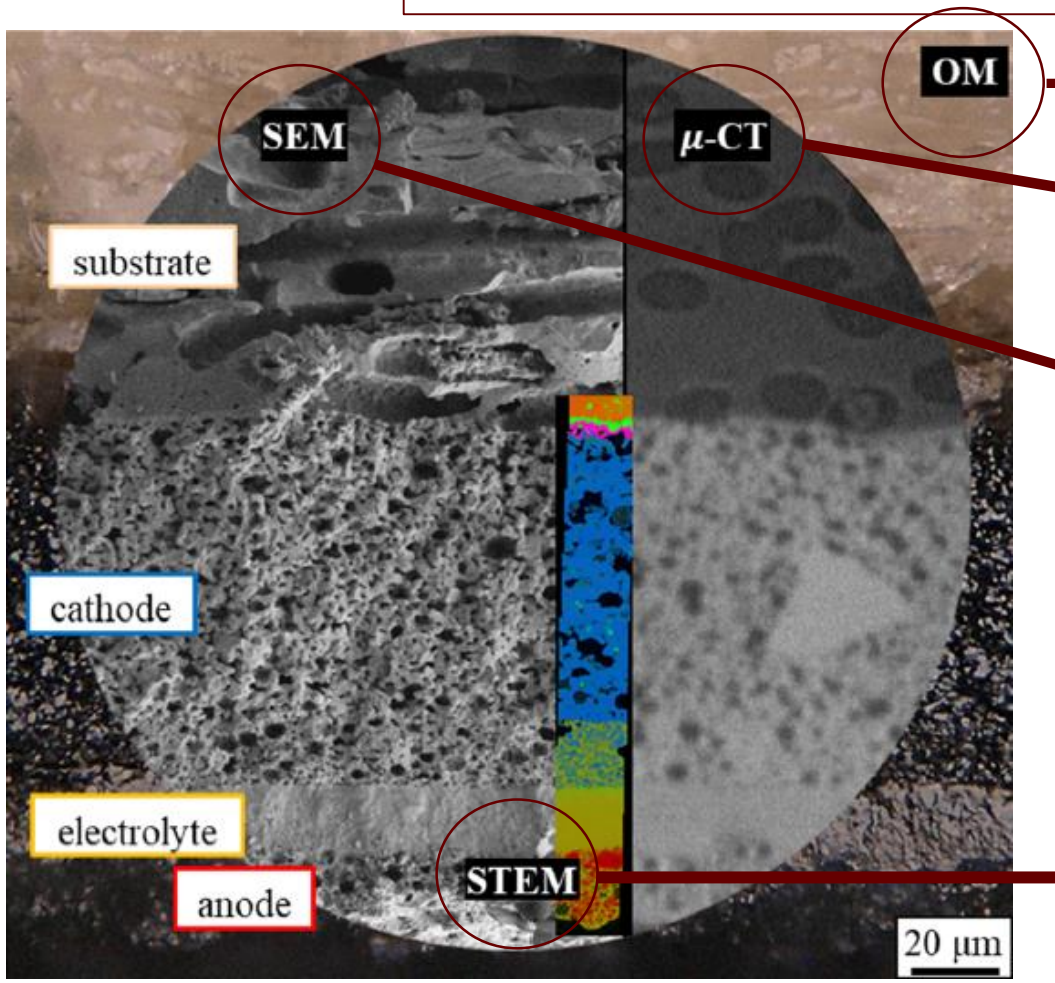
*X-Ray Tomography*

*Electron Microscopy*

*Non-destructive*
*Large field of view*
*Voxel resolution until 40 nm*

*High resolution*
*Element Analysis (EDX)*
*Focused Ion Beam*

*Multiscale characterization of a ceramic solid oxide fuel cells*




*True color images and localization of macroscopic failures*

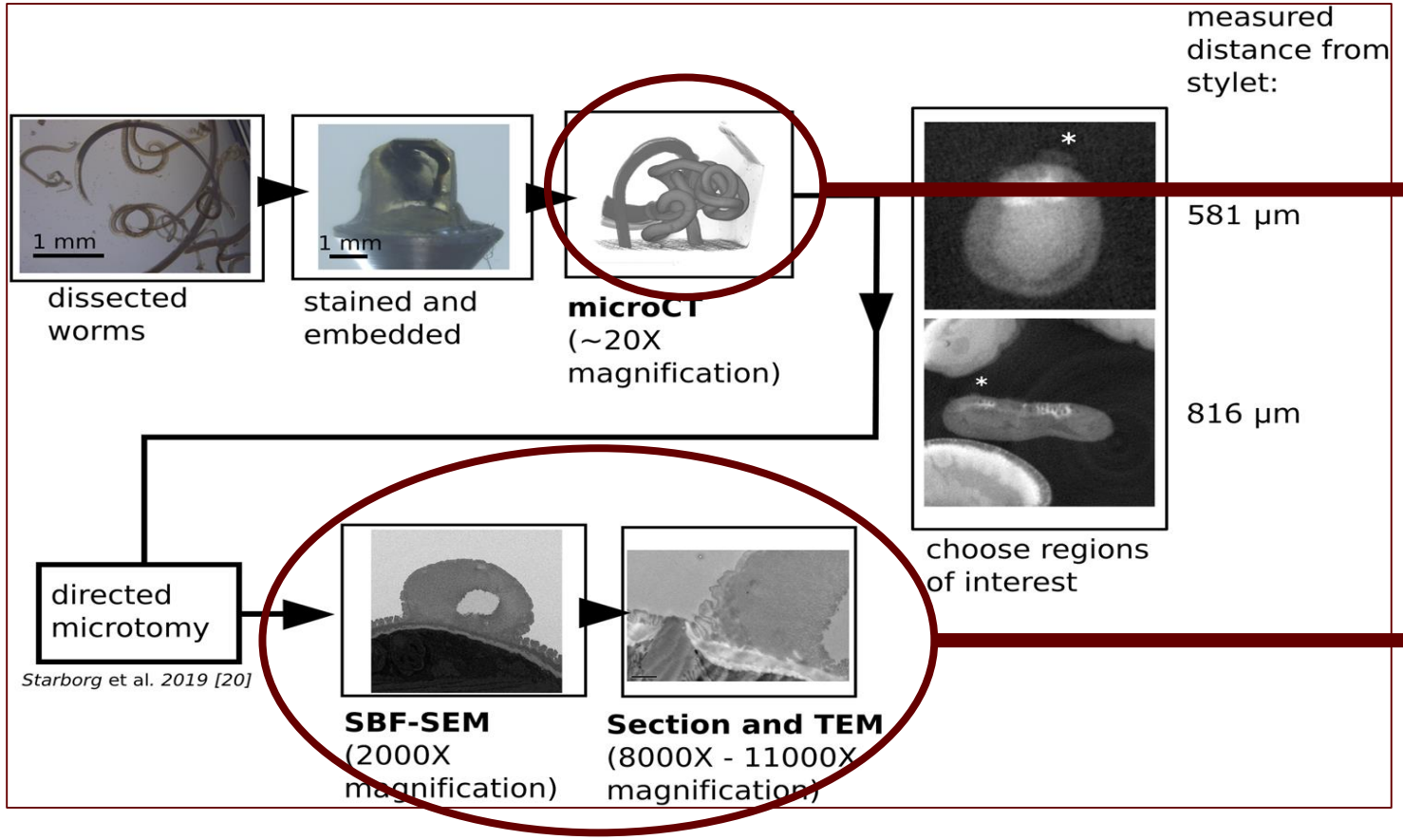
*Localization of the porous system and all the functional layers. Porous fraction and tortuosity can be also computed.*

*Identification of the three phases of the anode (Ni – YSZ – pores). Detailed 3D reconstruction by FIB-SEM tomography.*

*EDX and phase identification by SAED analysis.*

*Wankmüller et al., J Mater Sci (2020) 55:11120–11136*

*Characterisation of cuticular inflation development and ultrastructure in Trichuris muris*

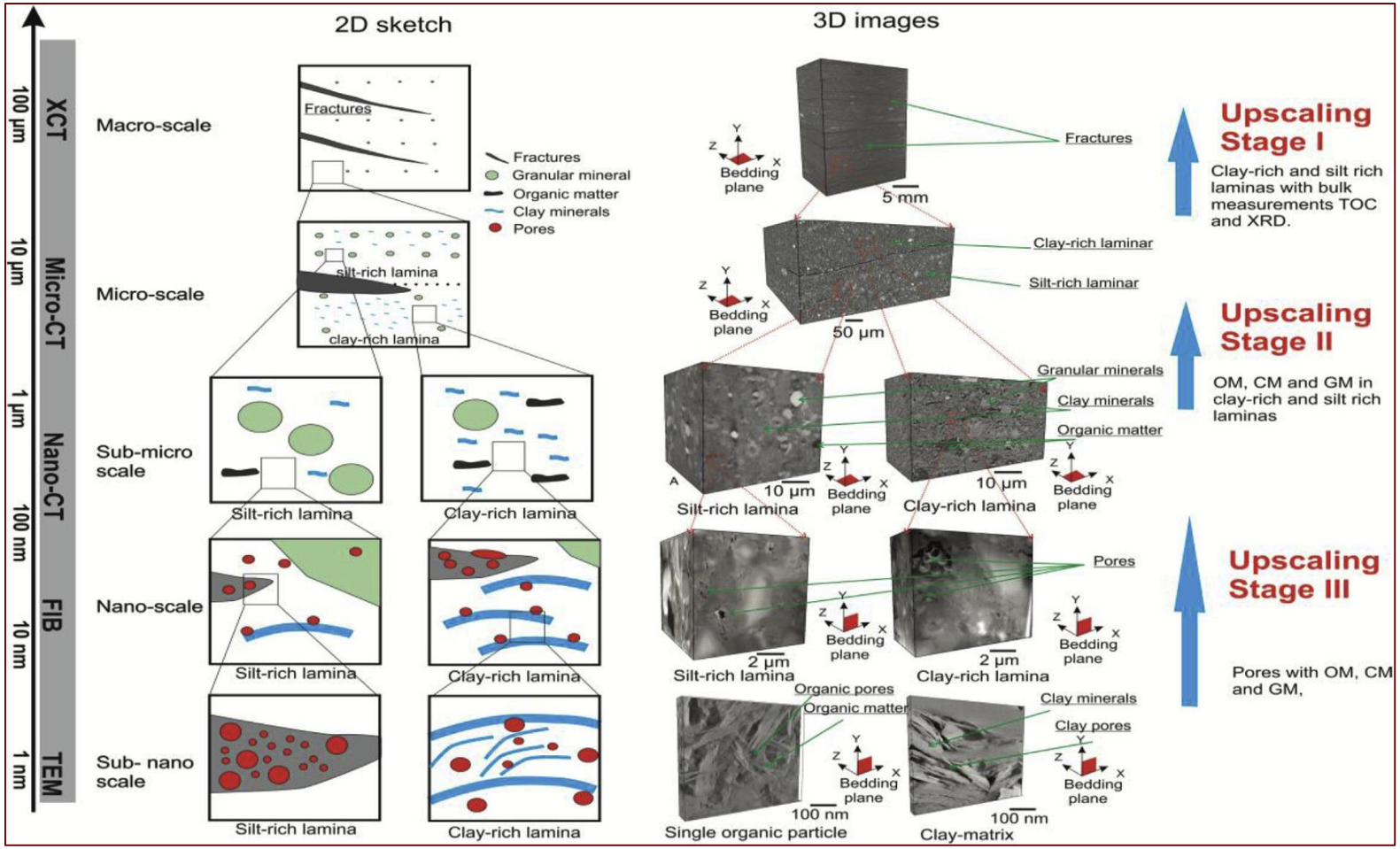



*Research of the ROI through the MicroCT*

*The ROI is trimmed to the ultramicrotomy and the ultrastructure is analyzed by SEM and TEM*

*O'Sullivan et al., Scientific Reports 10 (2020) 5846*

*Characterisation of the porosity for a heterogeneous shale rock*




*It's possible to correlate not only a 2D ROI, but also expand the analysis to a volume*

*Ma et al., Energy 181 (2019) 1285-1297*

nanomaterials

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