

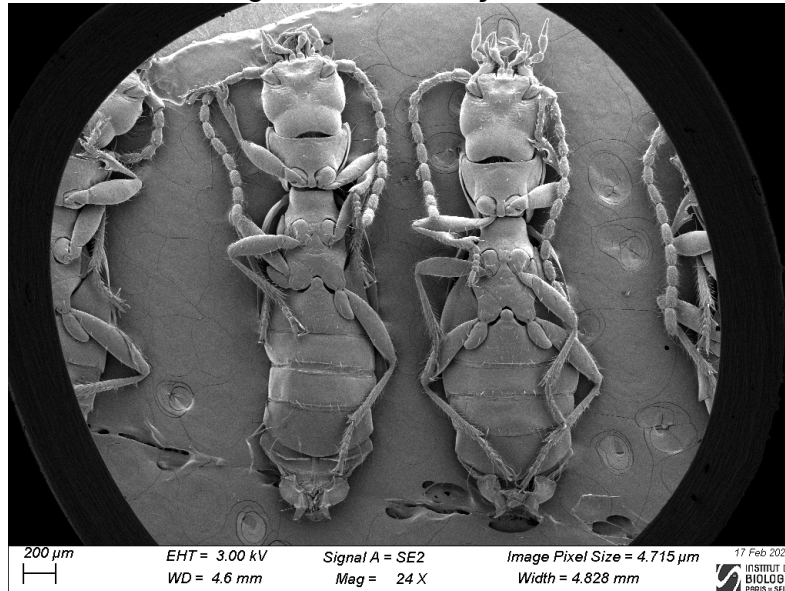
Préparation et observation des échantillons biologiques « mous » par Microscopie Électronique

Alexis Canette, Audrey Geeverding et Michaël Trichet

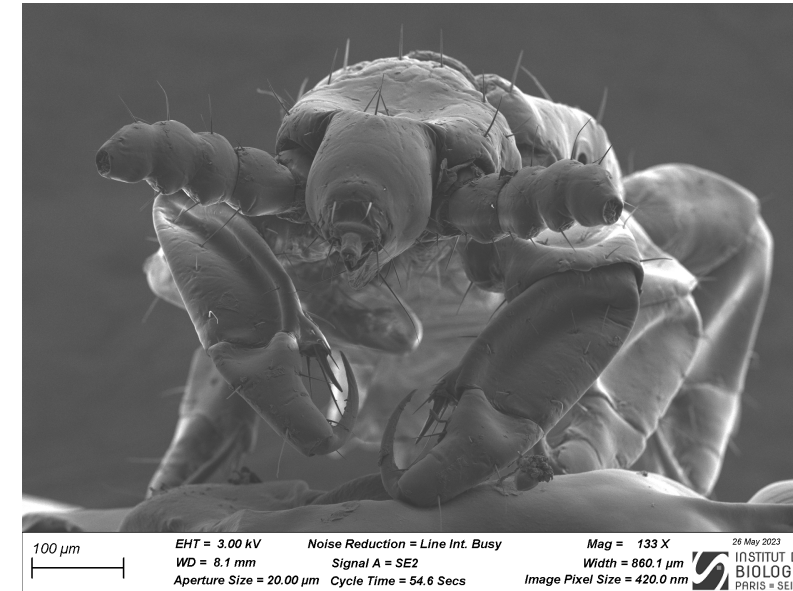
SEM : used as a stereomicroscope



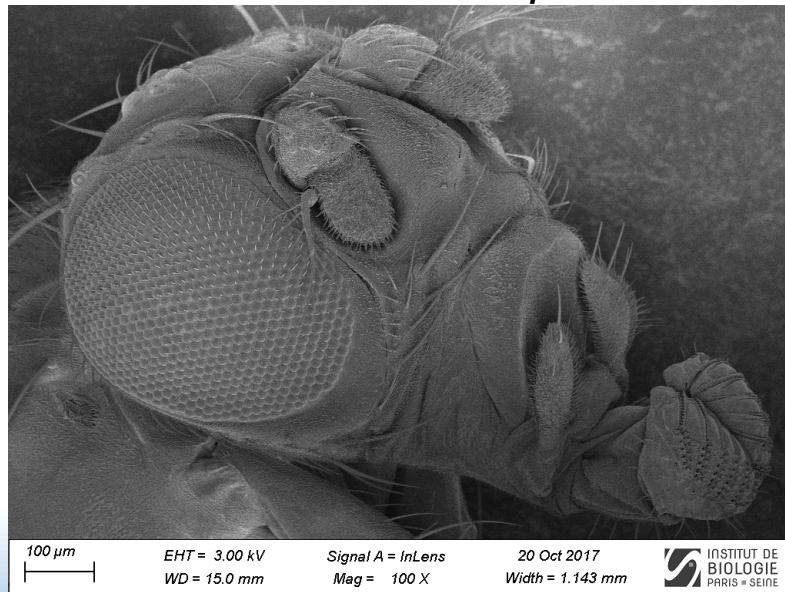
M. Jager @ ISYEB. *Dytiscidae*



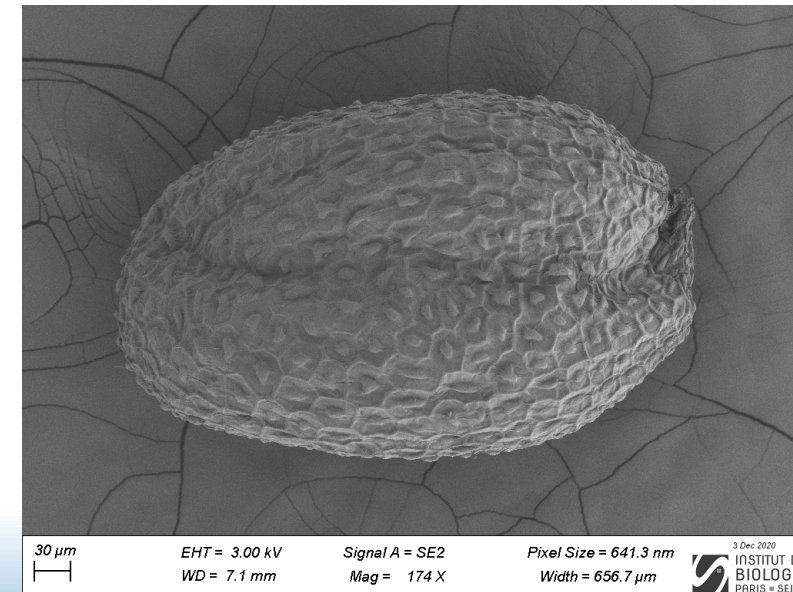
M. Trichet @ IBPS. *Pulex*



V. Bazin @ IBPS. *Drosophila*



A. Guivarch @ iEES. *A.thaliana* seed

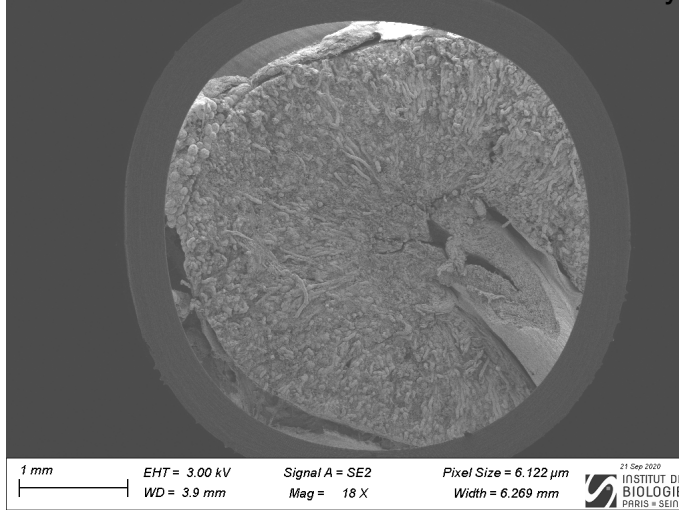


SEM : a wide and continuous range of magnification ...

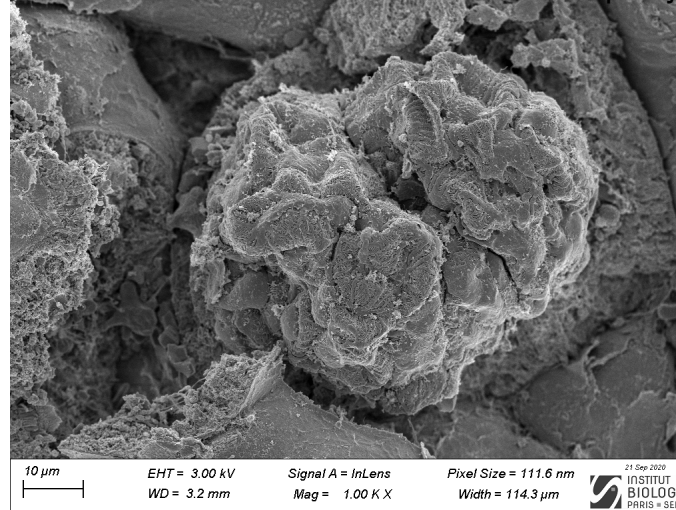


JJ. Candelier @ IAL

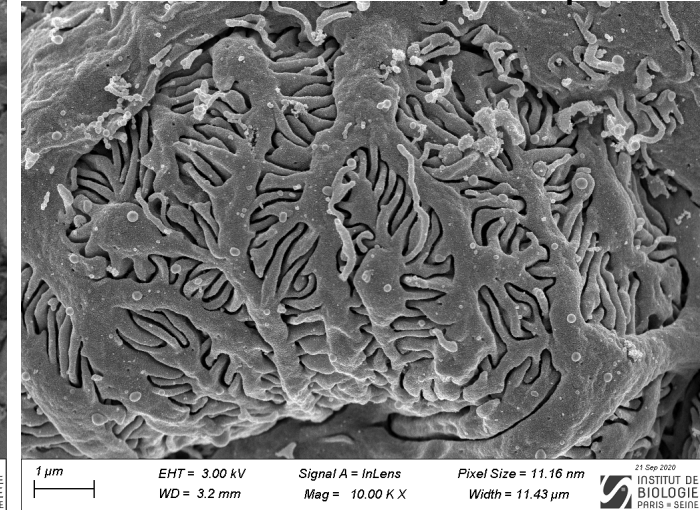
Kidney



Glomerulus capillary



Podocyte with pedicels



organ



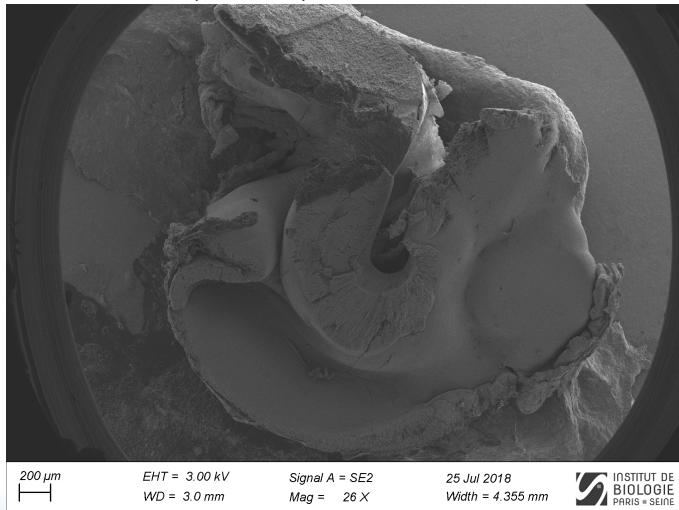
tissue



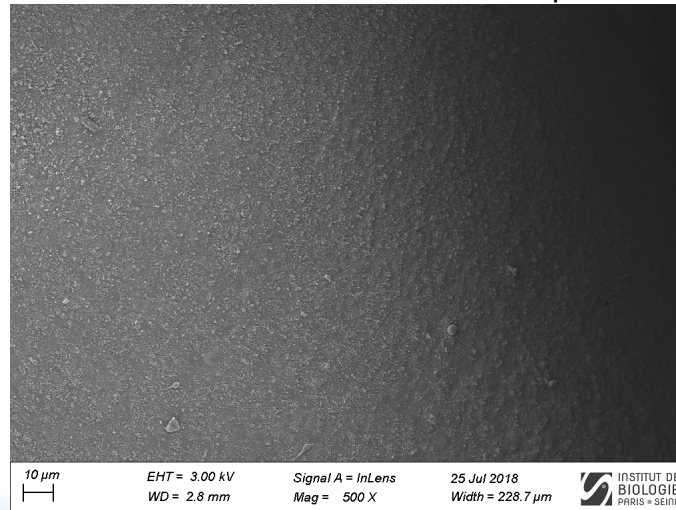
cell

Audreu et al., J.NbD, 2021

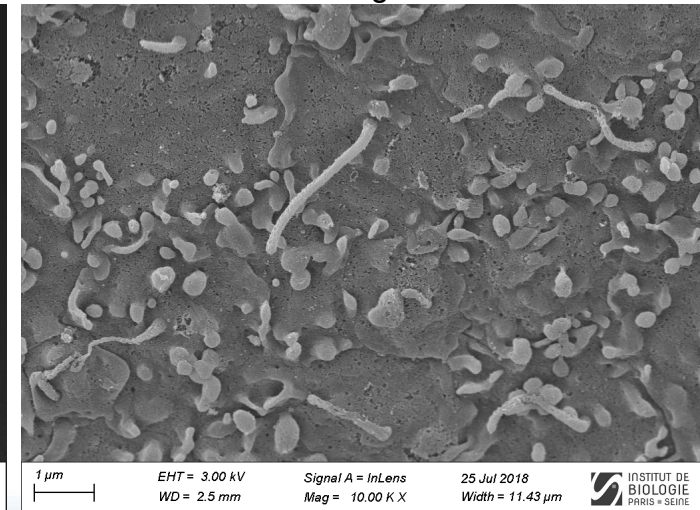
Brain



Forebrain ventricular epithelium

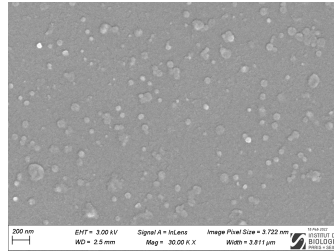


Progenitor cells with cilia

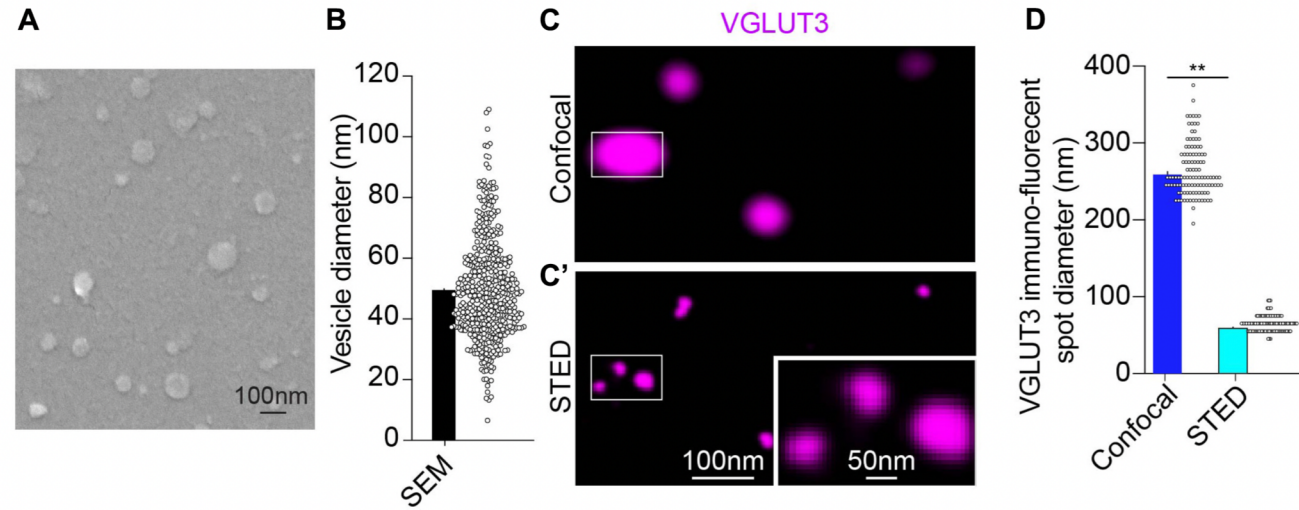


SEM : ... up to high resolution

synaptic vesicles

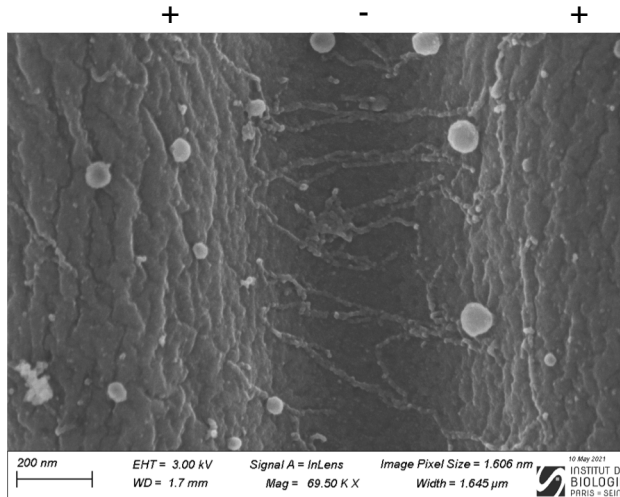


on glass slide



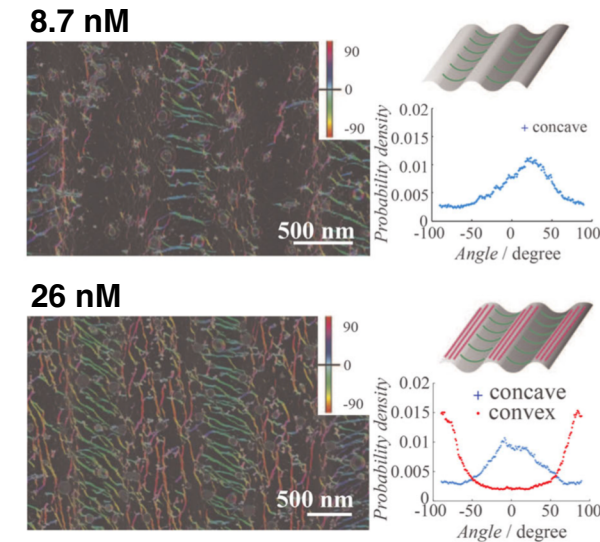
Cristofari et al., Front.Mol.Neurosci., 2022

septin filaments 8.7 nM

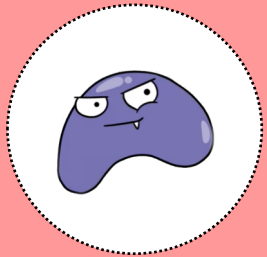


on lipid bilayers supported by undulated solid substrates

segmented filaments
colored according to
their orientations

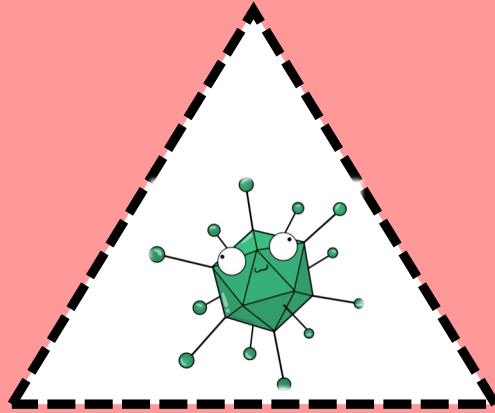


Nakazawa et al., JCS 2023



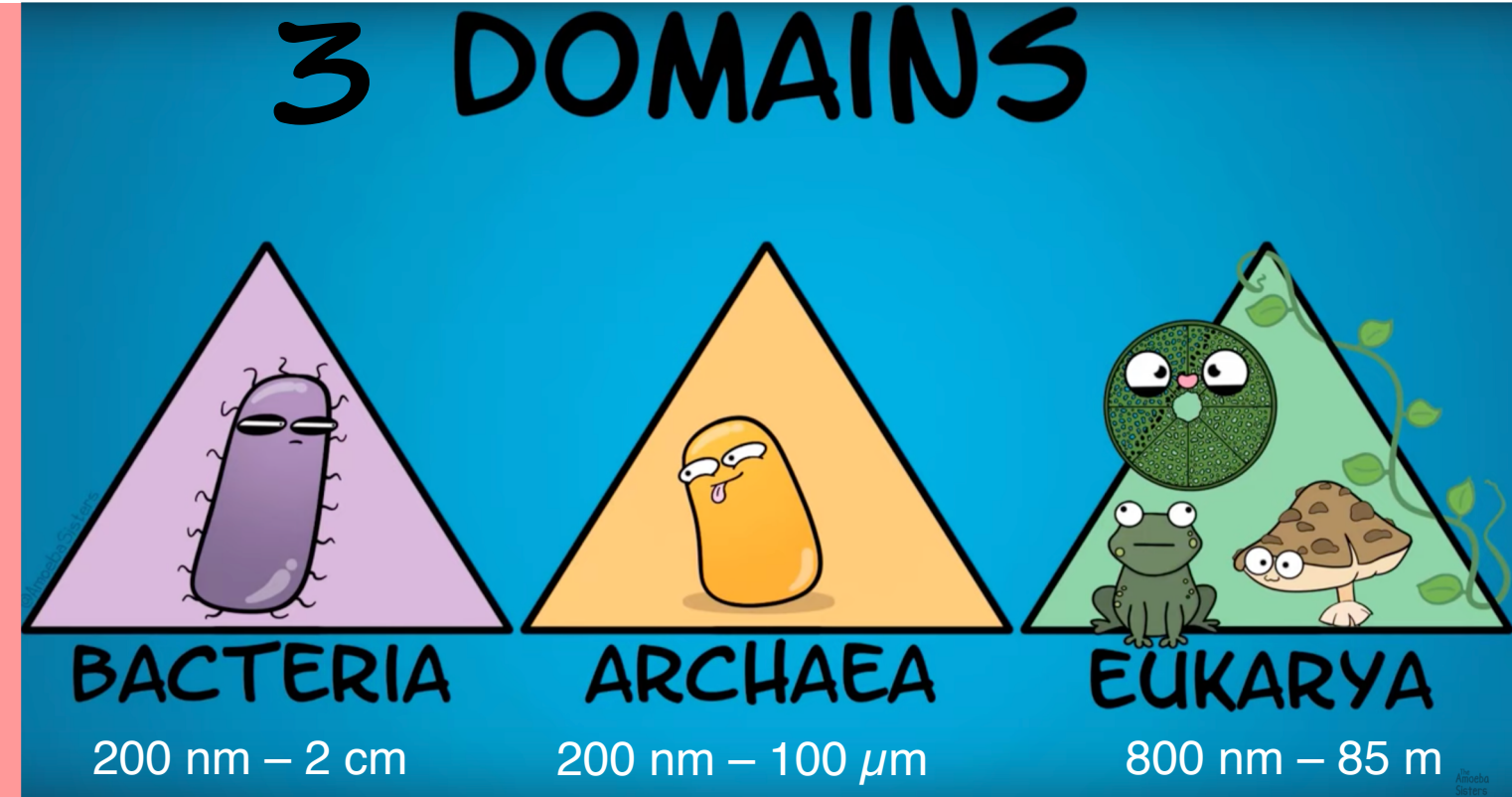
PRIONS

10 nm – 20 nm



VIRUSES

20 nm – 3 μ m



INFECTIOUS PARTICLES / AGENTS

PROKARYA
single-cell organisms

single-cell to
multicellular organisms

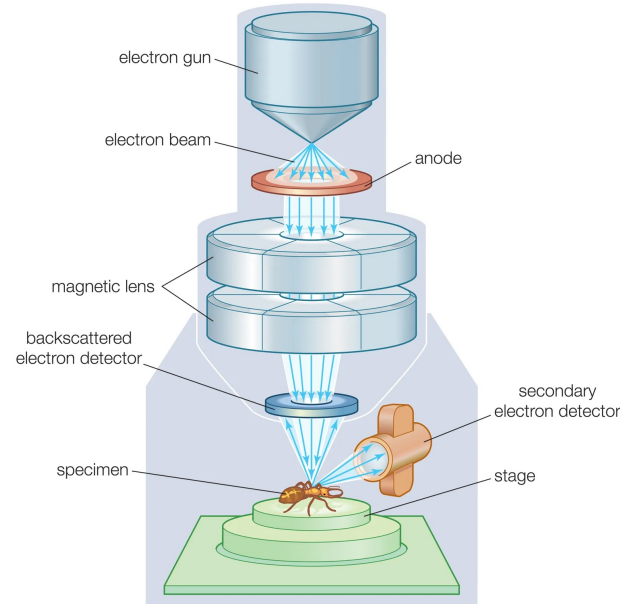
Observe biological samples in a SEM



Pseudomonas aeruginosa

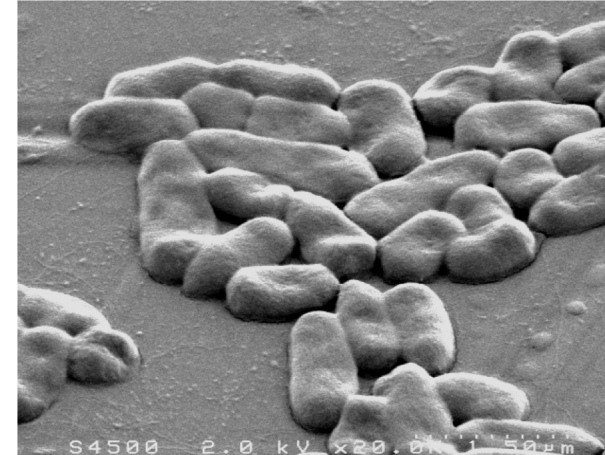


Artist's impression



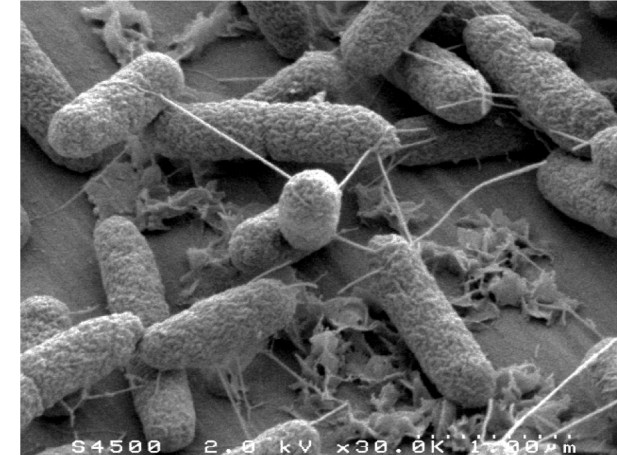
© 2012 Encyclopædia Britannica, Inc.

Pseudomonas aeruginosa



SEM

Pseudomonas aeruginosa



SEM

In natura:

- Hydrated
- Few electron-dense
- Non-conductive
- Variable size
- Free
- Sensitive: osmolarity, pH, temperature, ions, radiation, mechanical constraints ...

In the microscope:

- Stable under **vacuum**
- Contrasted
- Conductive
- Reduced size
- Held on a support
- Kept close to initial state / stable under the **beam**

KITCHEN
NIGHTMARES

TOP
CHEF

Conventional preparation for SEM



Chemical fixation

GA, PFA

Post fixation

OsO₄

Dehydration

ethanol

Drying

CPD or HMDS

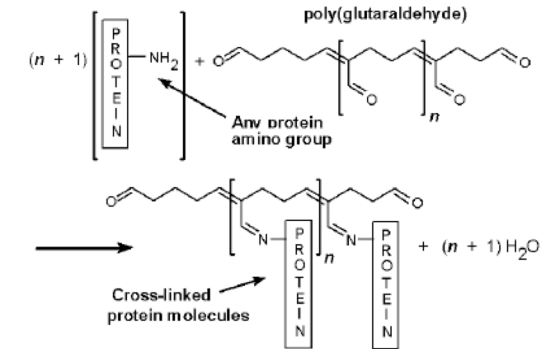
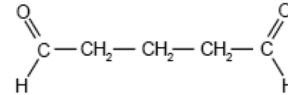
Mounting

gluing on stub

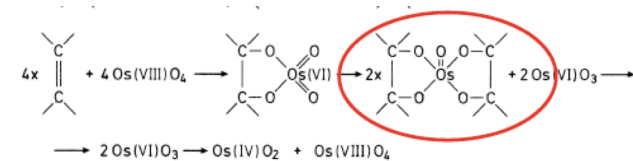
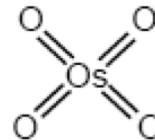
Coating

Au, Pd, Pt, W, Cr, Ir, C...

Glutaraldehyde



Osmium tetroxide



in a buffer solution to maintain osmolarity, pH and [ions]

Conventional preparation for SEM



Chemical fixation

GA, PFA

Post fixation

OsO₄

Dehydration

ethanol

Drying

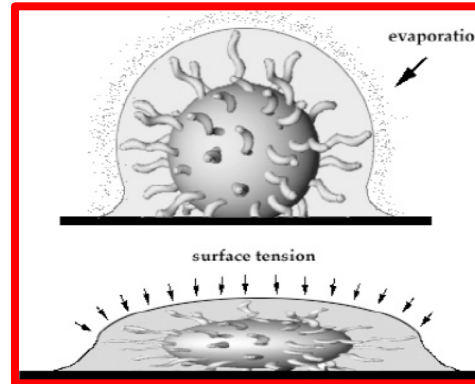
CPD or HMDS

Mounting

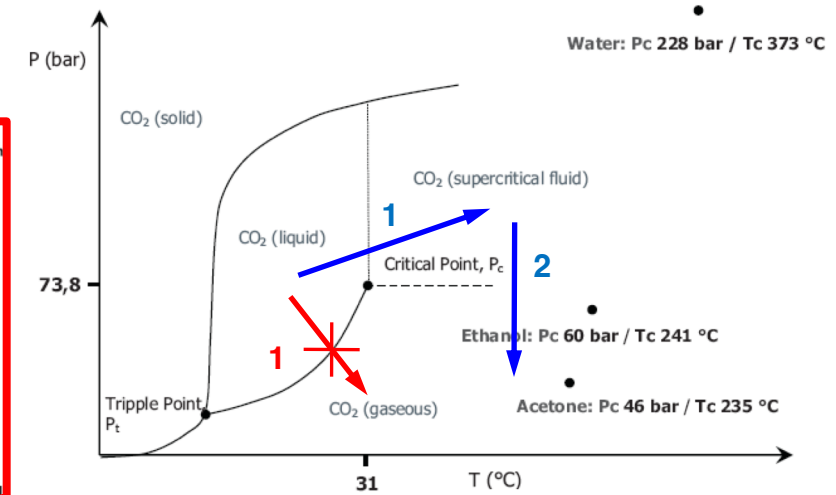
gluing on stub

Coating

Au, Pd, Pt, W, Cr, Ir, C...



CO₂ phase diagram



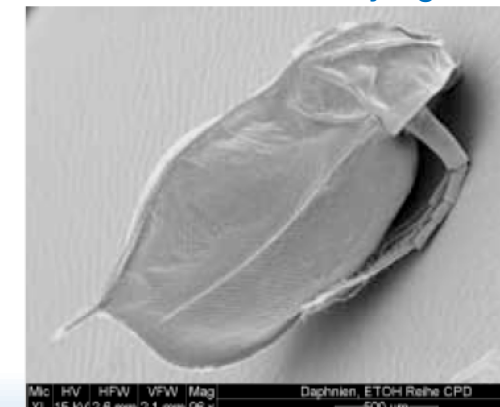
Leica CPD300



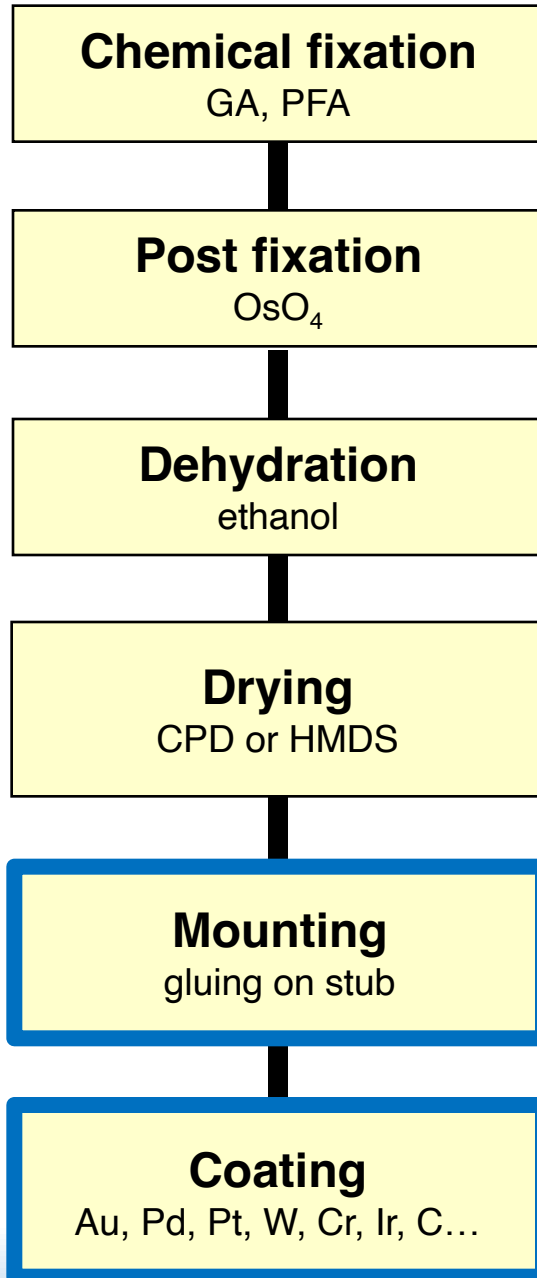
Air drying



Critical Point Drying



Conventional preparation for SEM



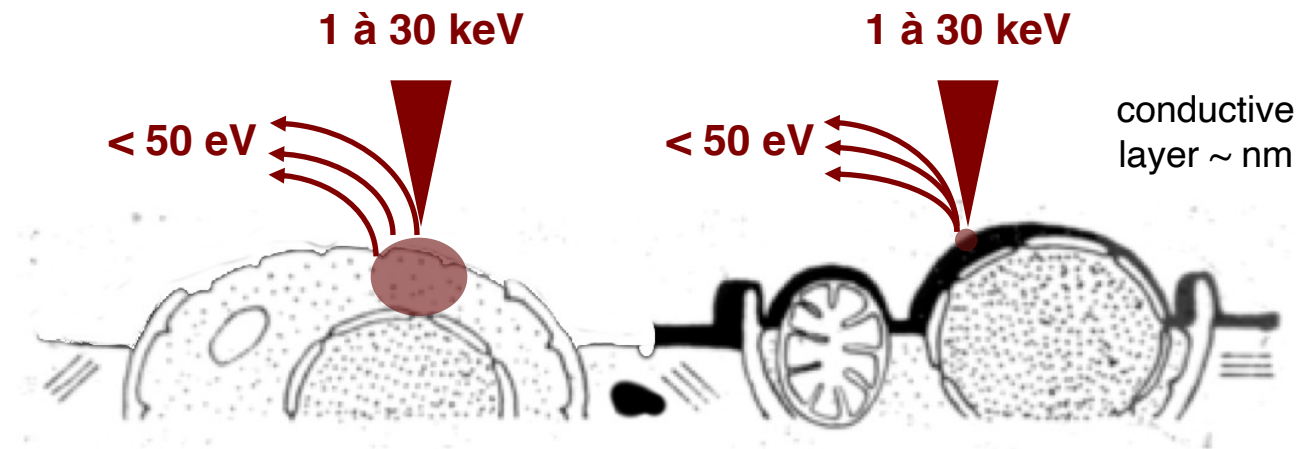
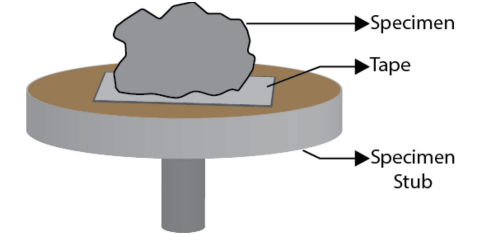
Stubs (Al)



Tabs (C), tapes (Cu)



Cements and paints (Ag, C)

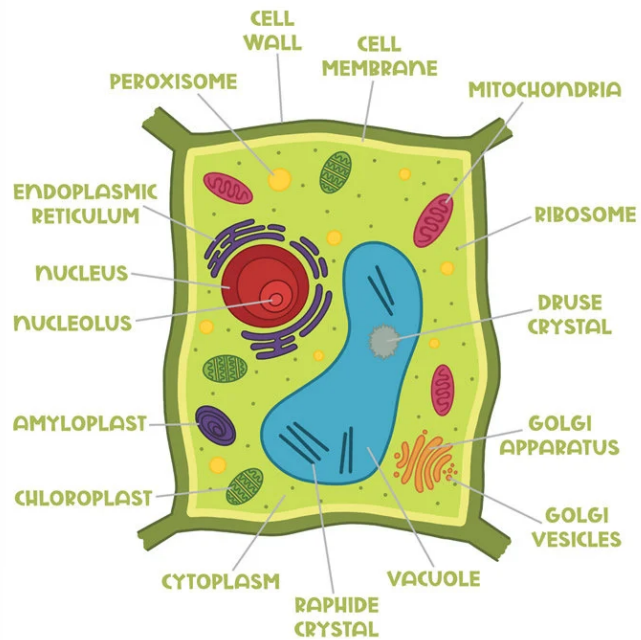


e⁻ accumulation → charge

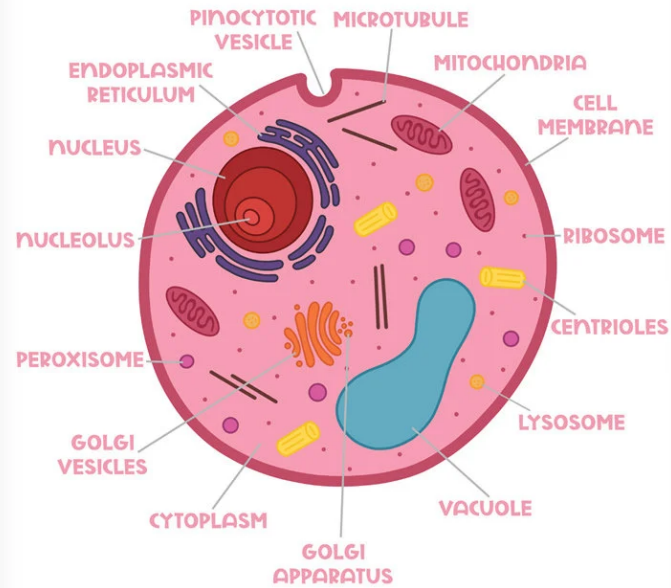
e⁻ dissipation
se⁻ prod. at surface → z-resolution
se⁻ yield → SNR
sample protected from beam damages

EUKARYA

PLANT CELL

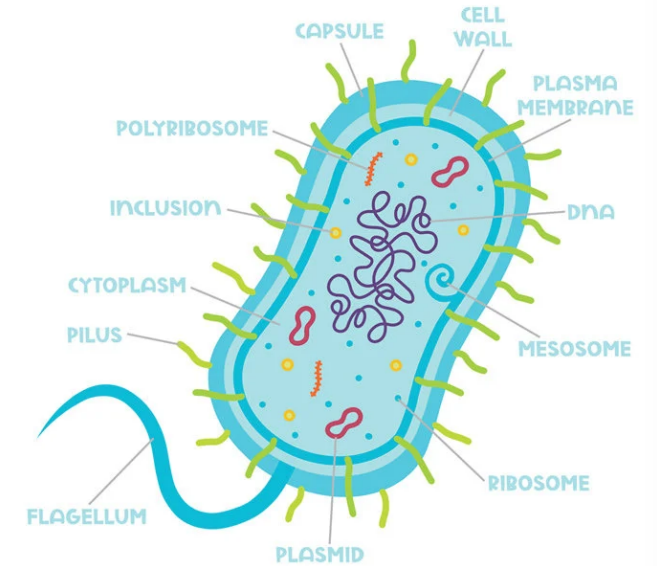


ANIMAL CELL



PROKARYA

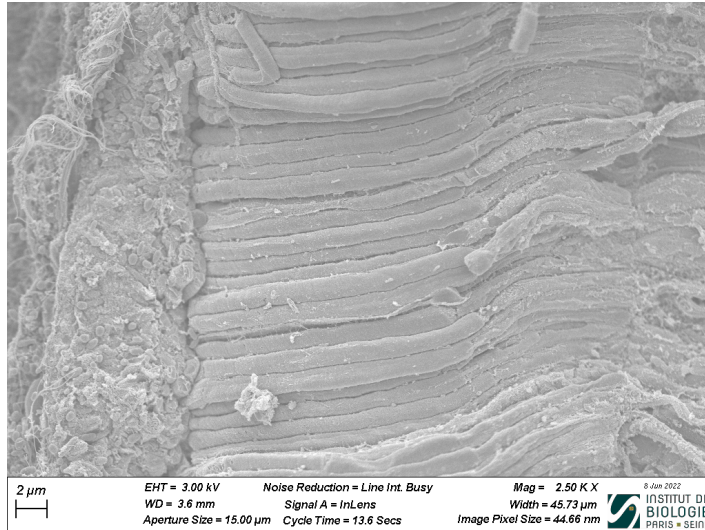
BACTERIA CELL



Methods to reach intracellular structures

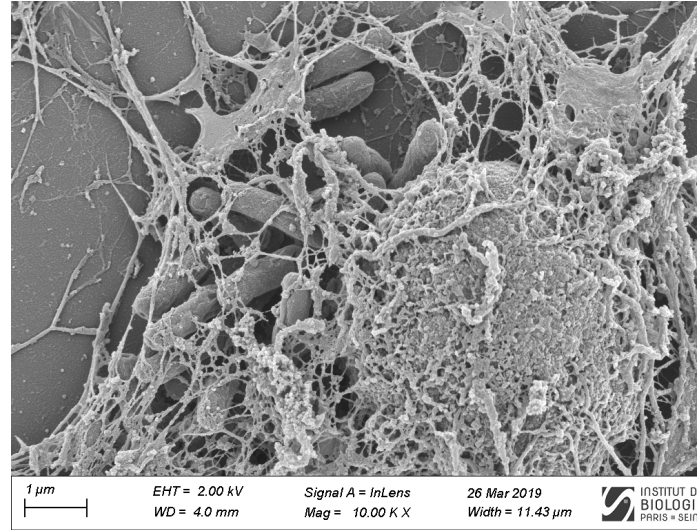


mechanical : **dissection** → SEM



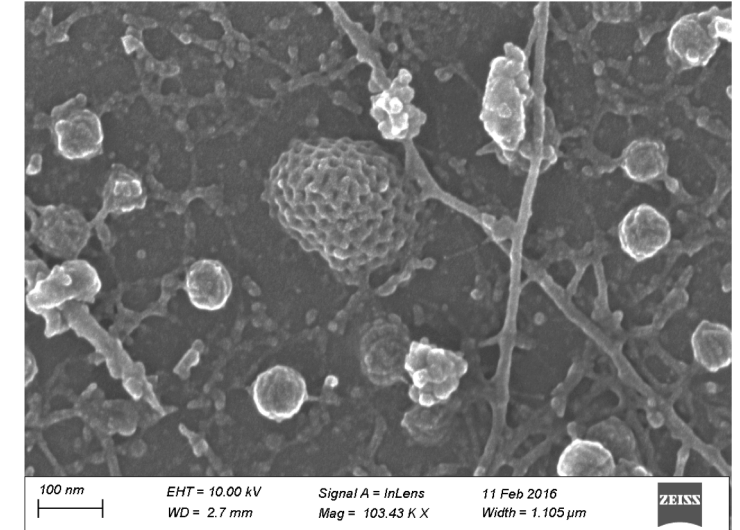
E. Clerin @ IDLV. **Rods** in mouse retina

chemical : **digestion** → SEM



V. Prudent @ CIRB. *E.coli* bacteria in a **macrophage**

mechanical : **sonication** → SEM



S. Vassilopoulos @ Inst. Myologie. **Clathrin** cage

mechanical : **section** → TEM and SEM « TEM-like »
surfacing → SBF-SEM



mechanical : **cryo-fracture** → cryo-SEM
cryo-fracture + replica → TEM

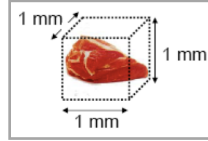


irradiation : **milling** → SEM and FIB-SEM



**Chemical fixation
and staining**

0.5 mm / hour
tissue infiltration



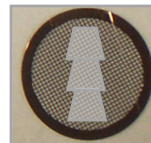
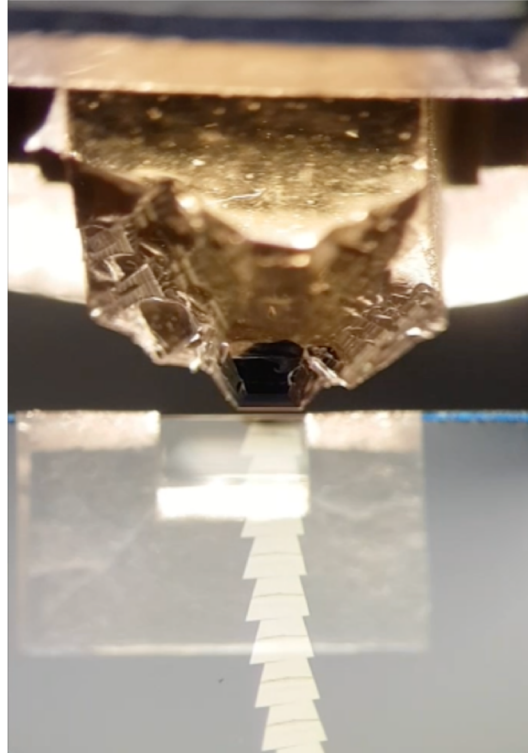
Dehydration

**Embedding
in resin**

Ultramicrotomy

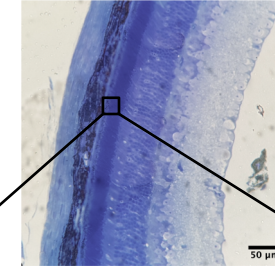
**Deposit on
a support**

Staining

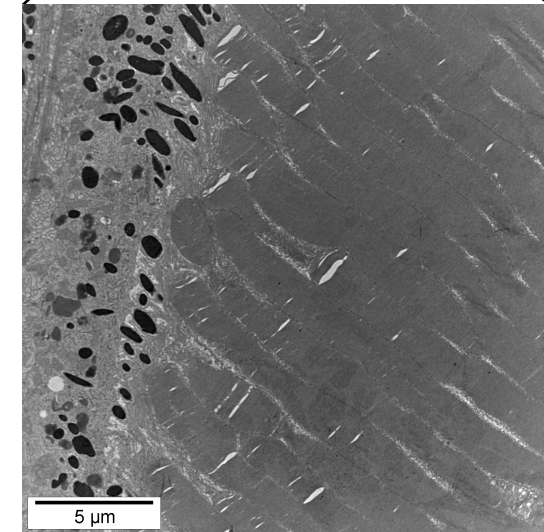


3 mm

Retina LM



500 nm
semithin section



80 nm
ultrathin section

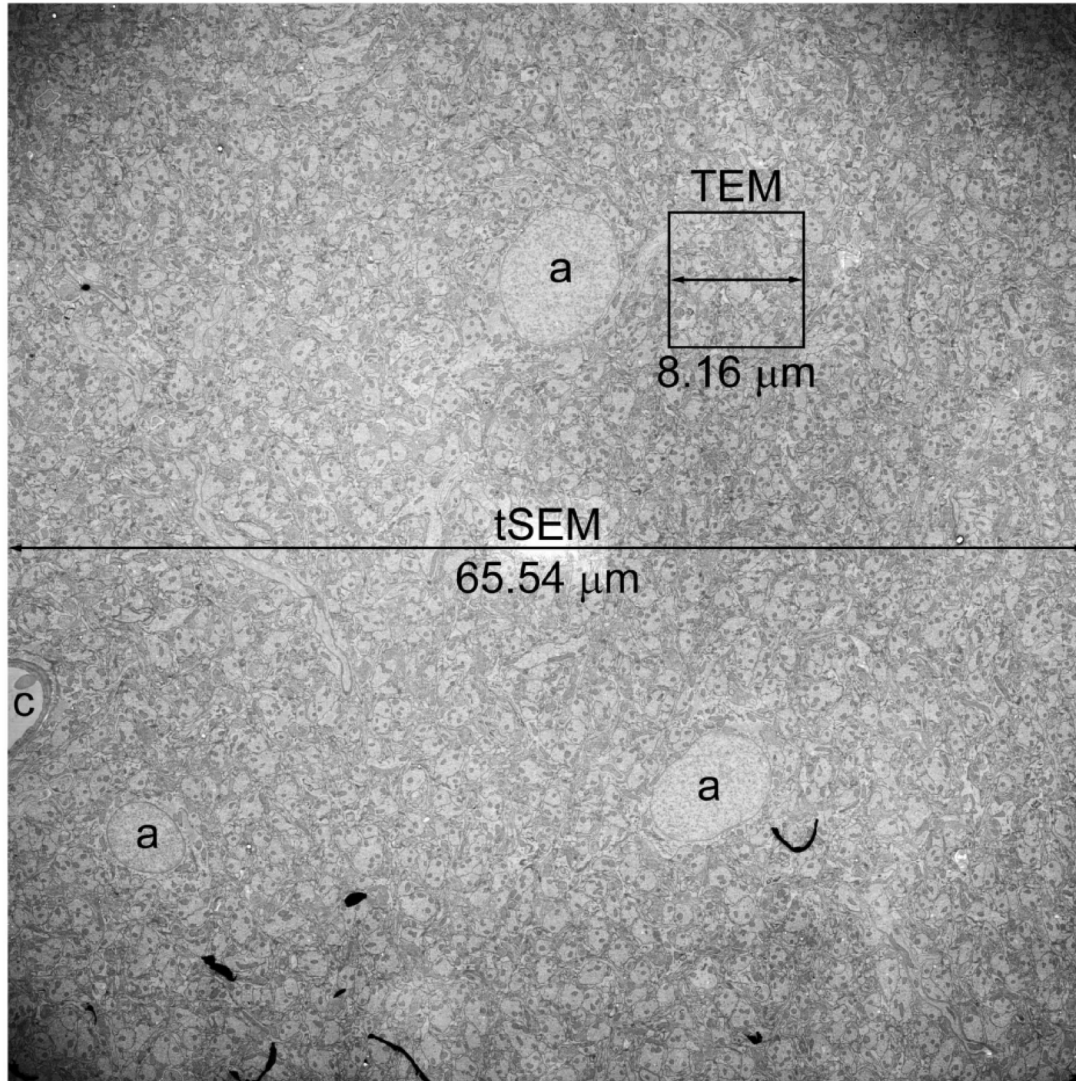
E. Nandrot @ IDLV

TEM

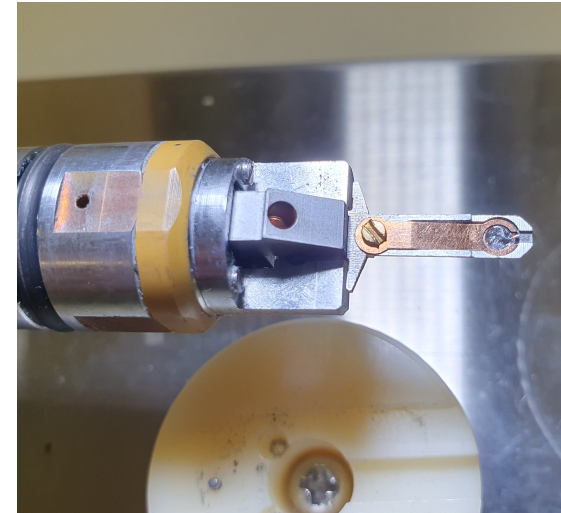
TEM vs SEM for ultrastructure in sample sections



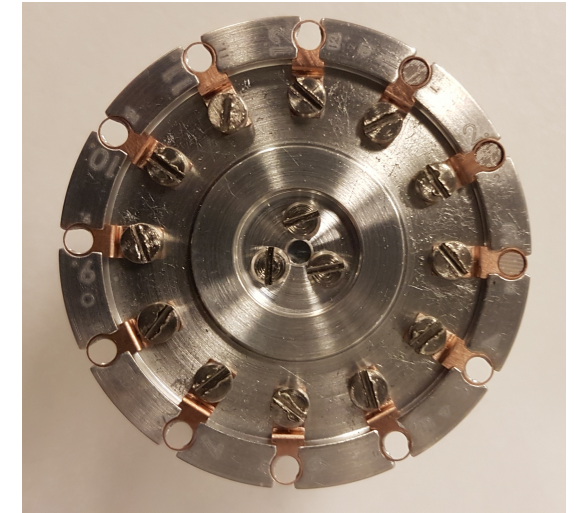
Max Field (no mozaic) at 2 nm/pix with a 4 080 pix TEM camera vs a 32 768 pix SEM scan



Kuwajima et al., PLoS ONE, 2013



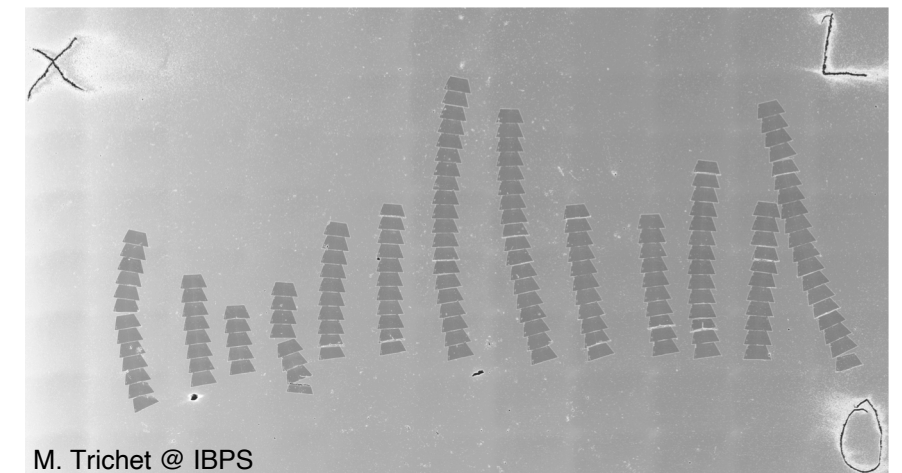
→ TEM



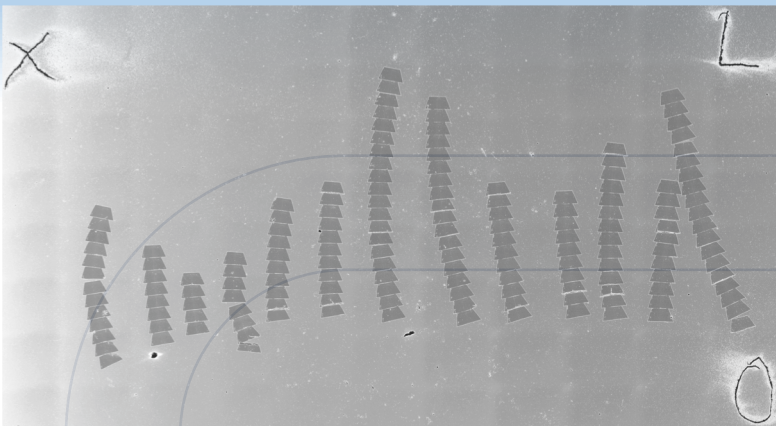
→ STEM-SEM



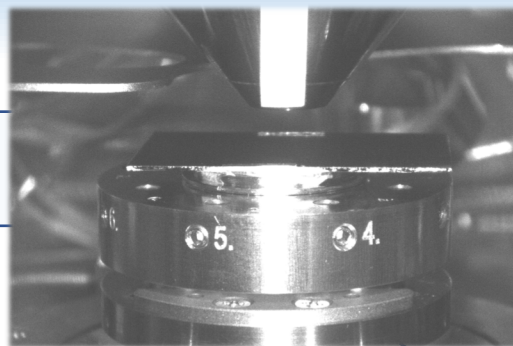
3 mm



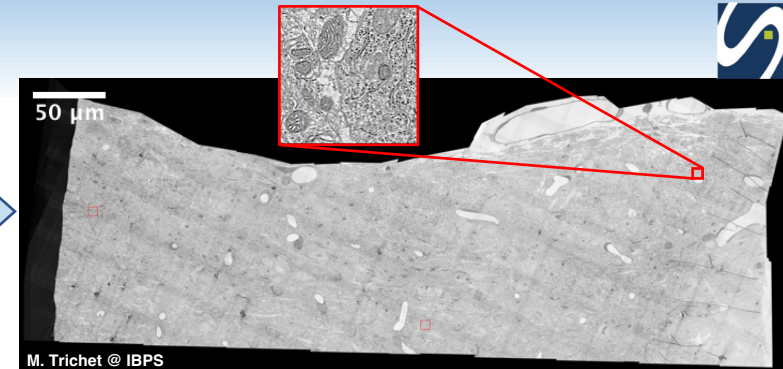
M. Trichet @ IBPS



sections on wafer



large field of view :
mosaic of images



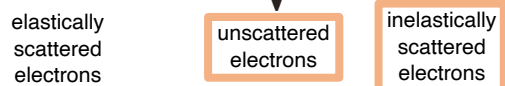
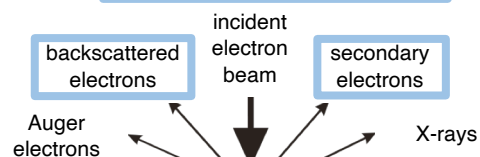
M. Trichet @ IBPS
olfactory nerve layer

3D :
Array Tomography



A. Trembleau @ IBPS
axons of olfactory sensory neurons

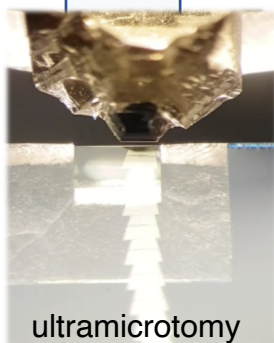
In column detection



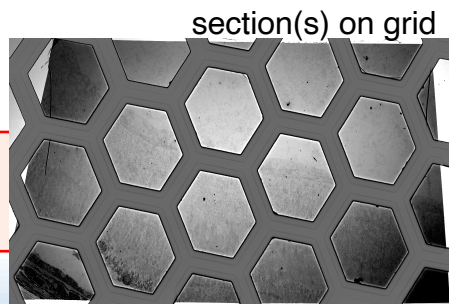
STEM detection



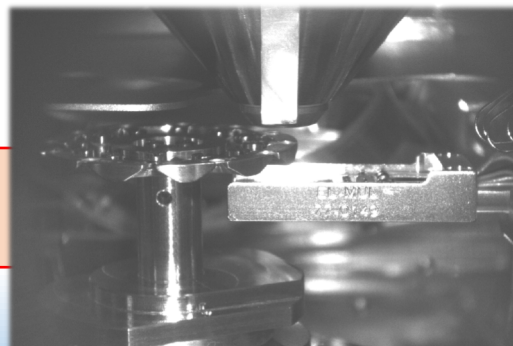
SEM



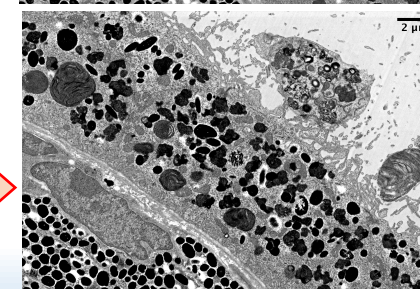
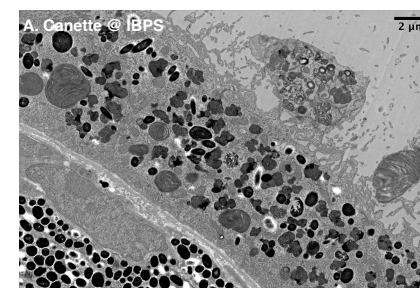
ultramicrotomy



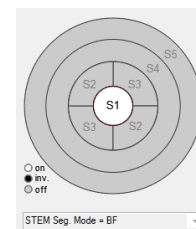
section(s) on grid



high contrast :
5-quadrant



retinal pigment epithelium of mouse

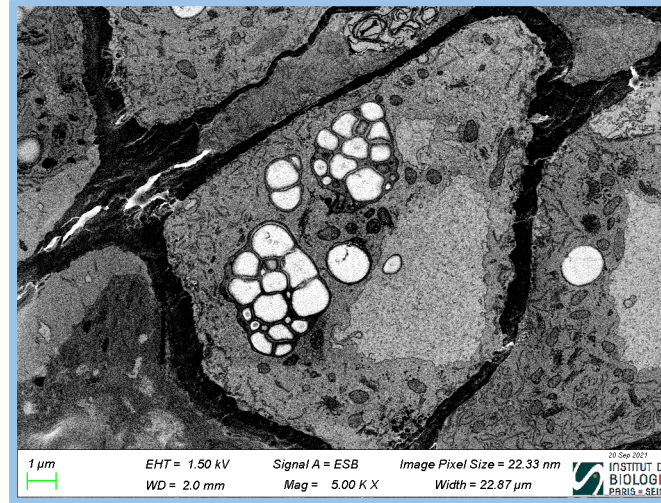


SEM for ultrastructure in sample sections

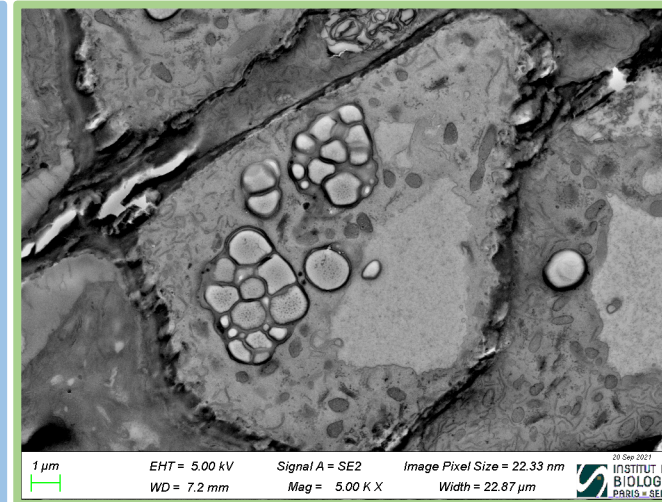
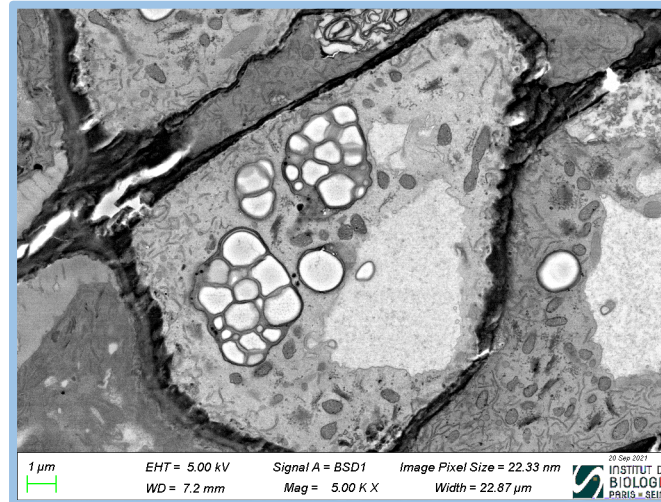


C. Boulogne @ I2BC and
A. Canette @ IBPS.
Cells in a **A. thaliana** root.

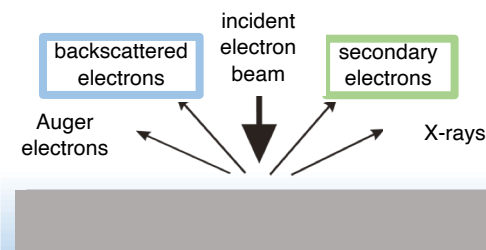
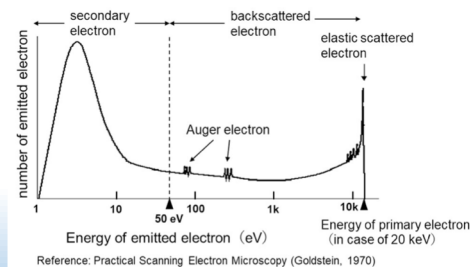
250 nm thin section
+ 5 nm carbon coating



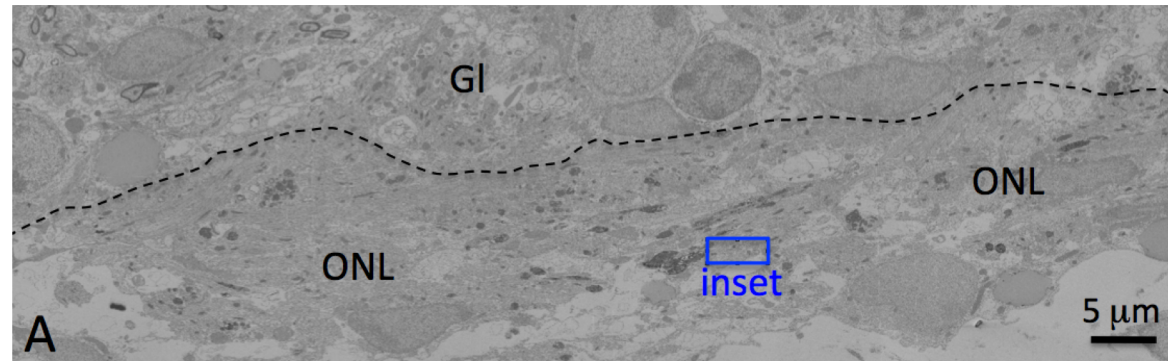
In column detection



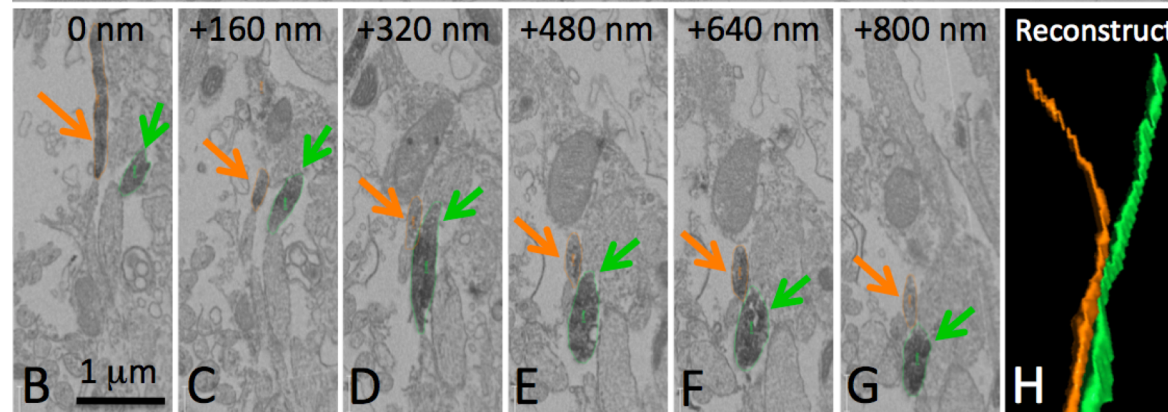
In chamber detection



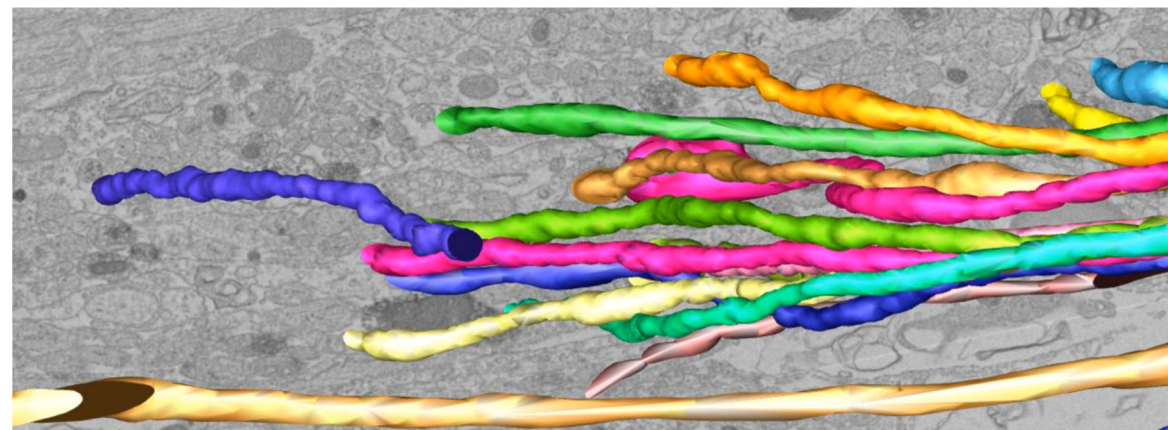
SEM for ultrastructure and 3D in sample sections



SEM 2D



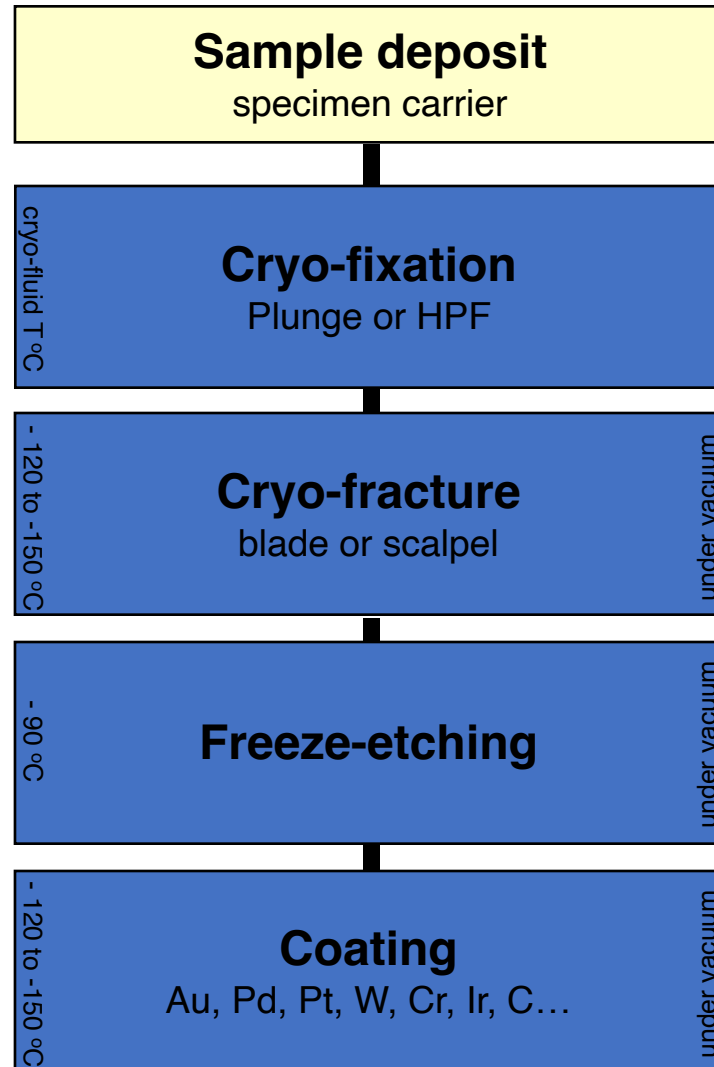
SEM 3D : aTomo



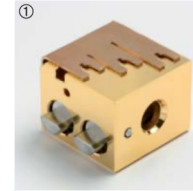
3D reconstruction

M. Trichet and A. Trembleau @ IBPS.
Axons in neuronal olfactory tissue.

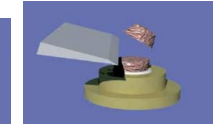
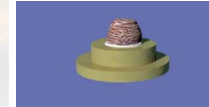
Cryo-SEM : our workflow for biological samples



specimen carriers holder



Leica VCT100



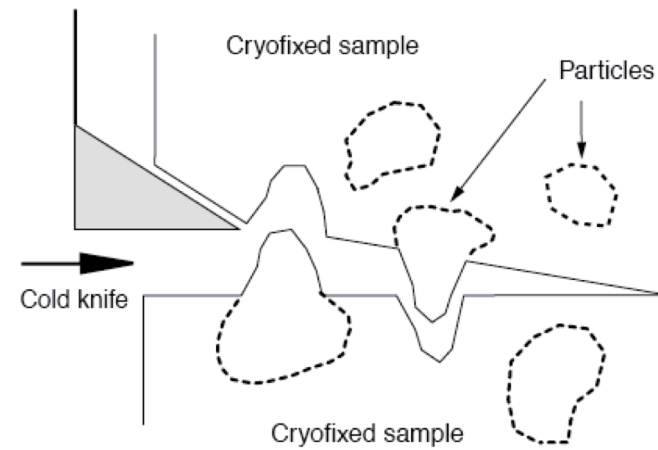
Leica ACE600



specimen transfer shuttle



cryo-fracture/etching/coating

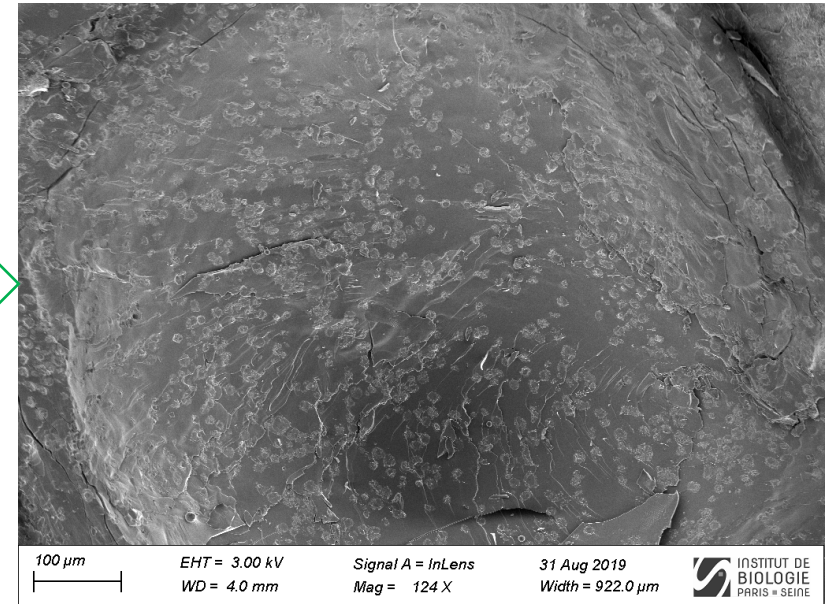
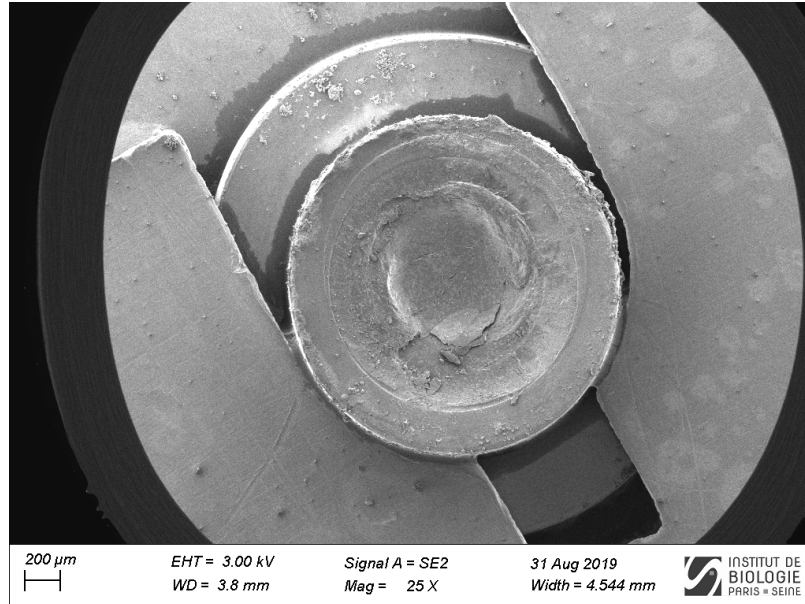


Zeiss Gemini500

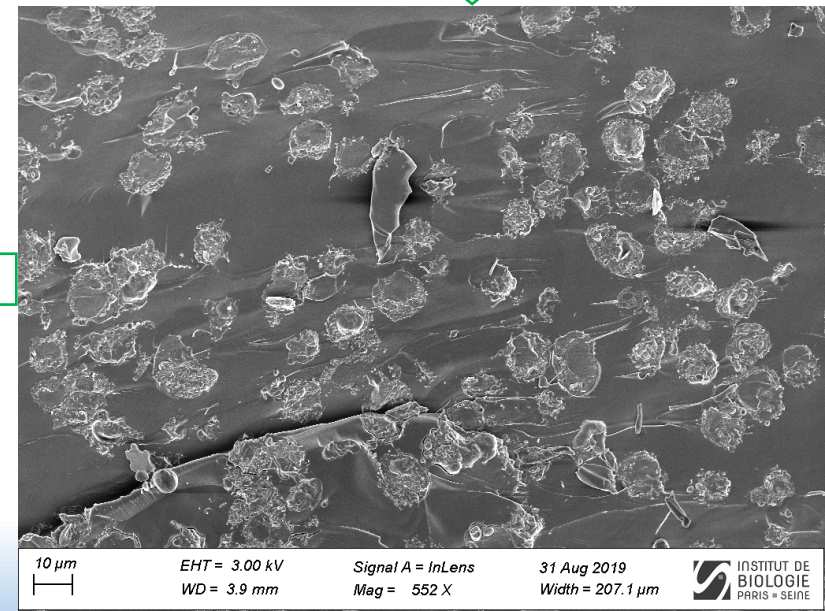
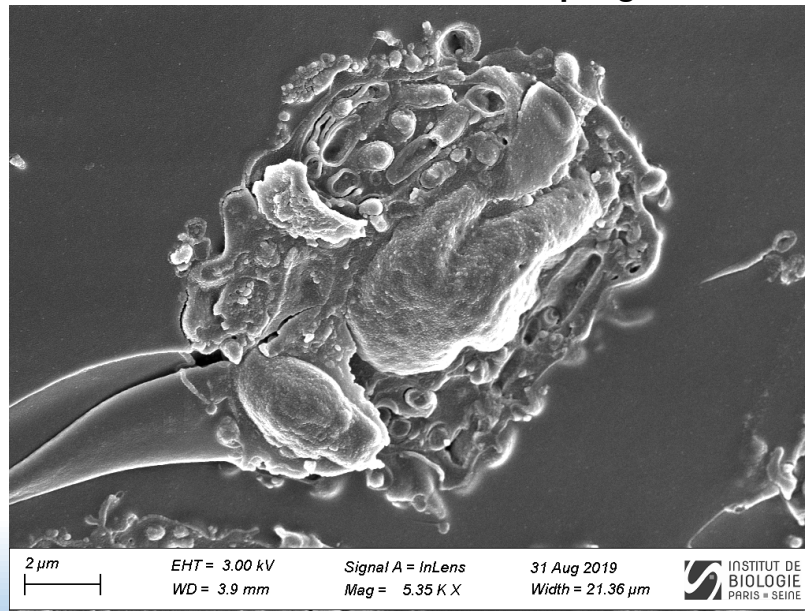
Observation

-120 to -150 °C
under vacuum

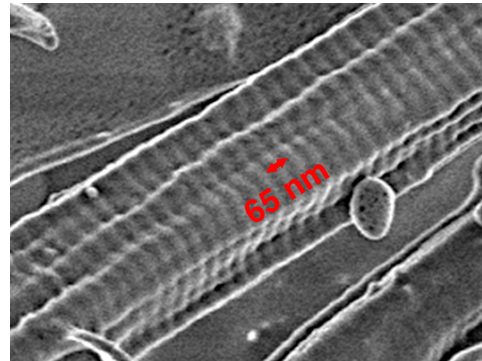
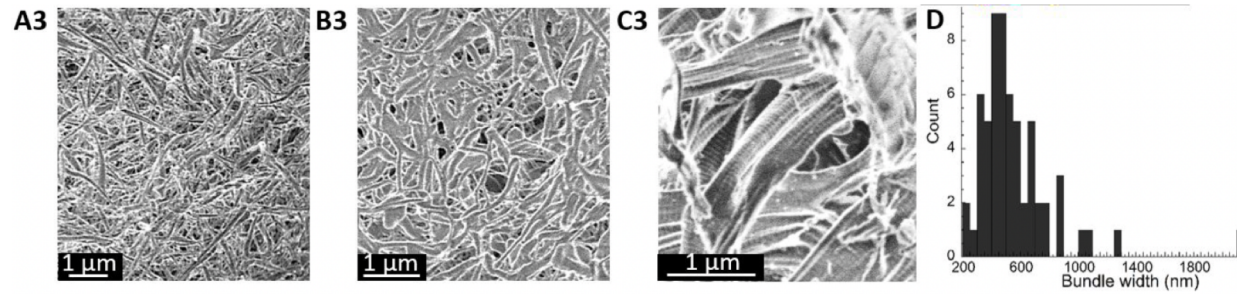
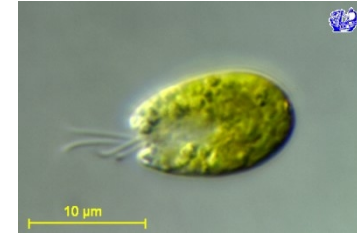
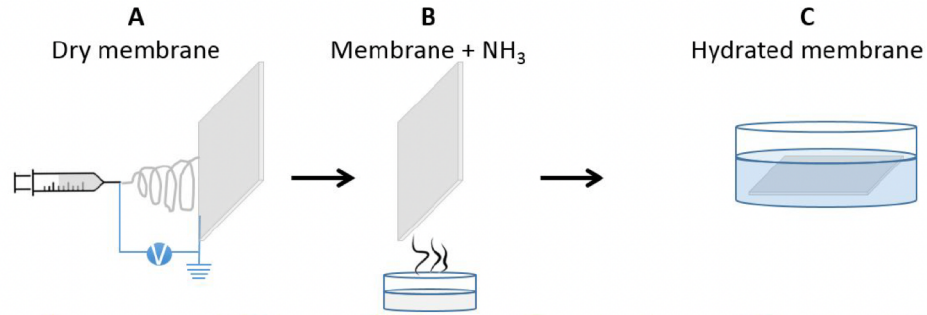
mechanical : **cryo-fracture** → cryo-SEM



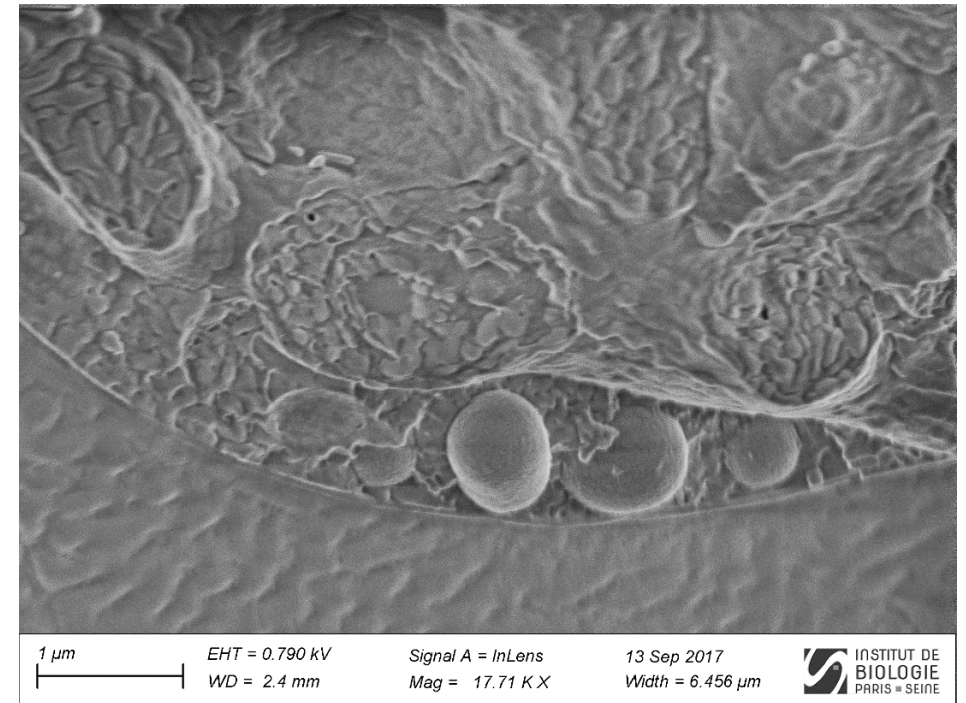
V. Prudent @ CIRB. **Macrophage**



Cryo-SEM : examples of studies



Dems et al., ACS Appl. Bio Mater, 2020. Electrospinning of **collagen**



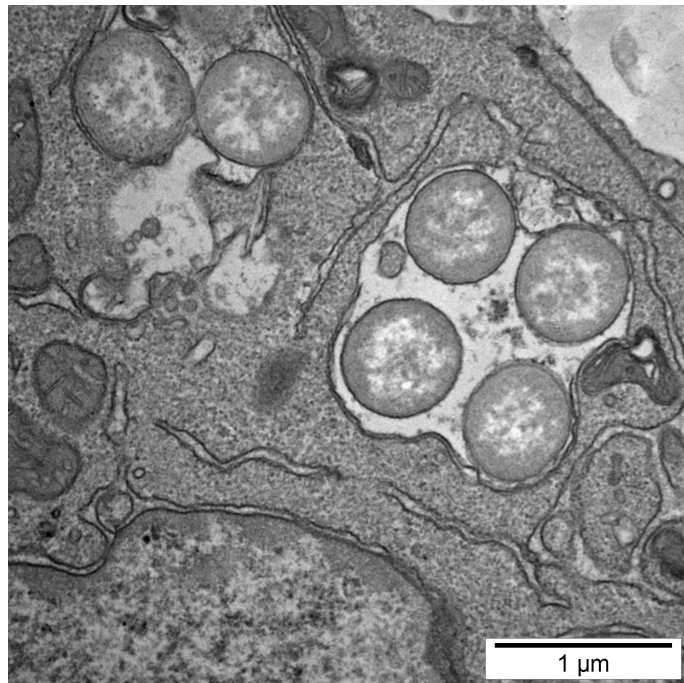
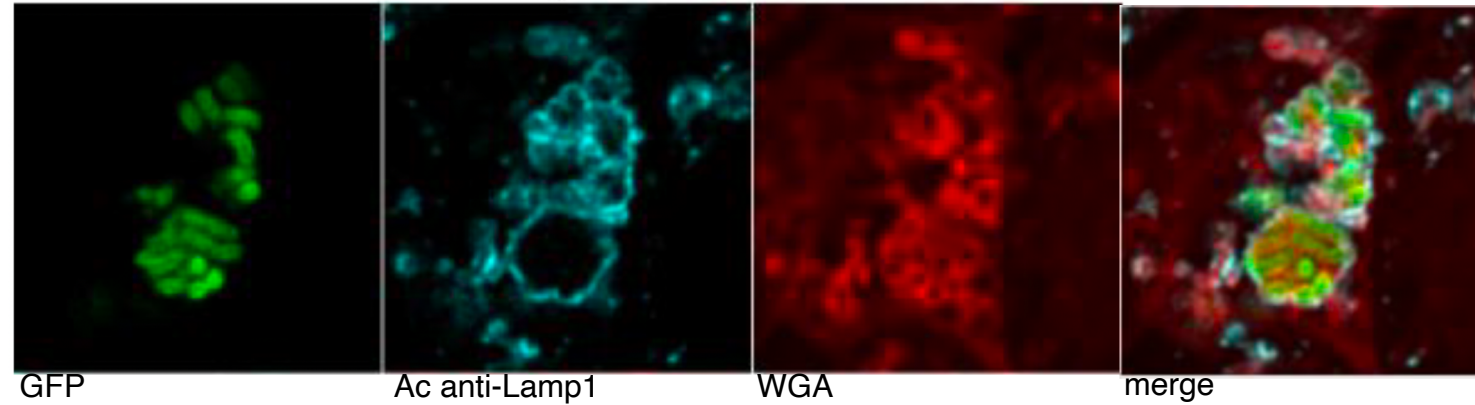
K. Benzerara @ IMPMC. Carbonates in ***Tetraselmis***

An example of a multi-approach study

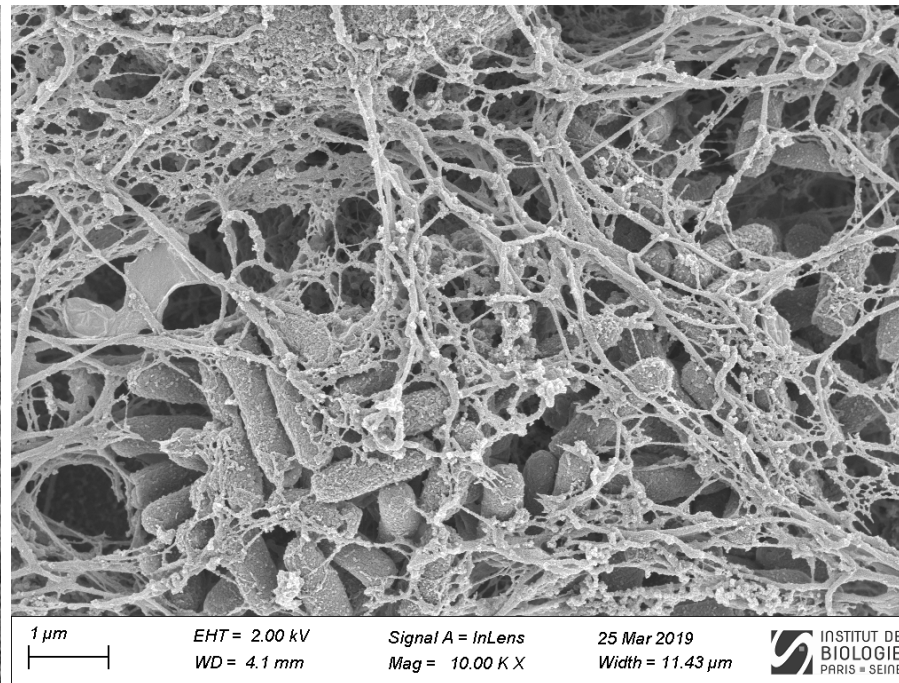


V. Prudent @ CIRB. *E.coli* bacteria in a macrophage

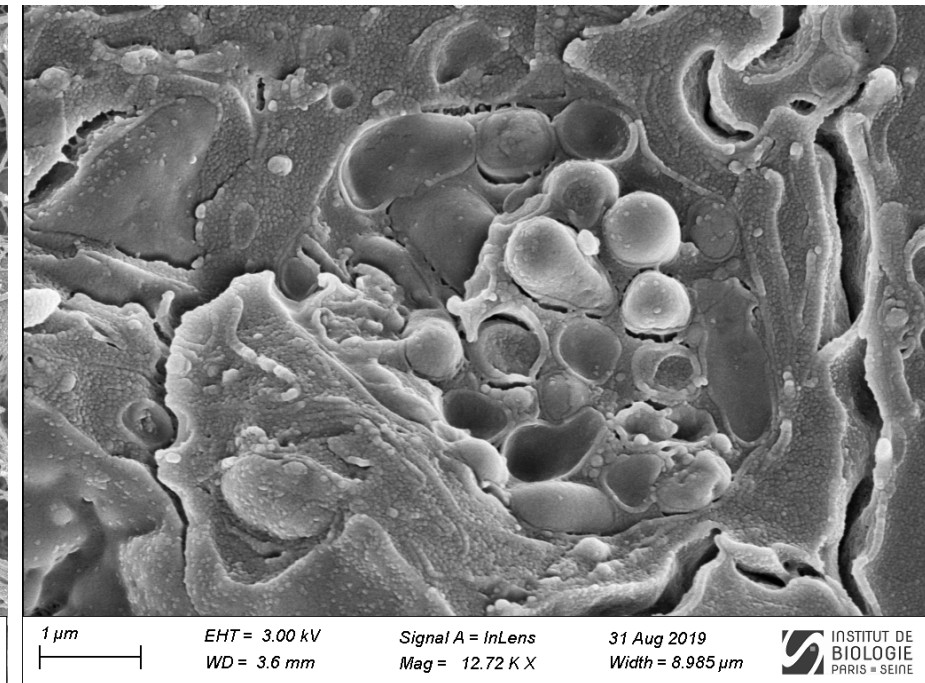
STED



Section → **TEM**



Chemical digestion → **SEM**



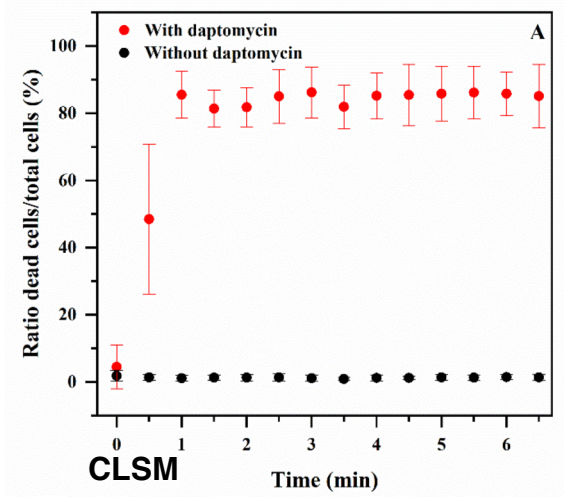
Cryo-fracture → **Cryo-SEM**

A. Canette @ IBPS

Examples of a multi-modal studies



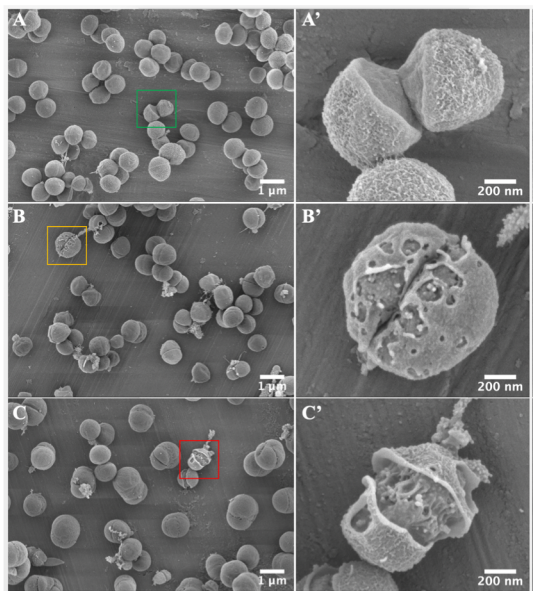
An antibiotic against *Staphylococcus aureus*



cell death measurements
with temporal resolution

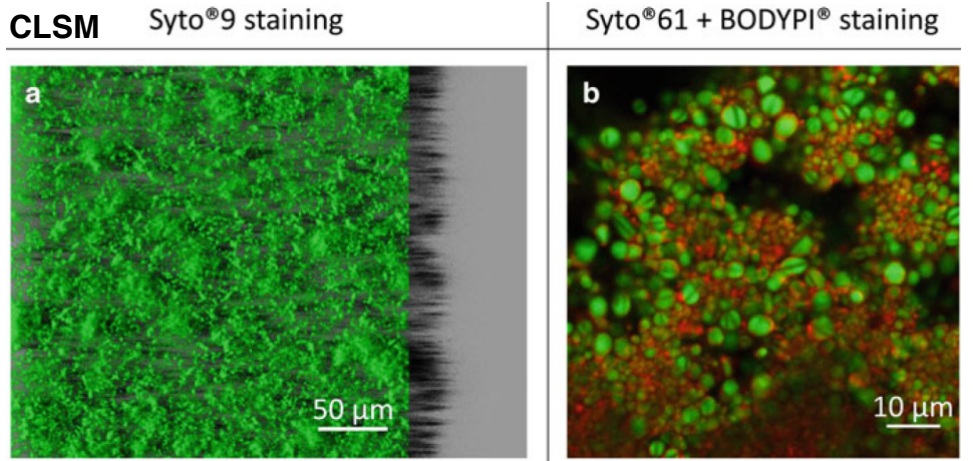
cells degradation
visualization

Control
30 min
treatment
3 hours
treatment



SEM Canette et al., BMC, in revision

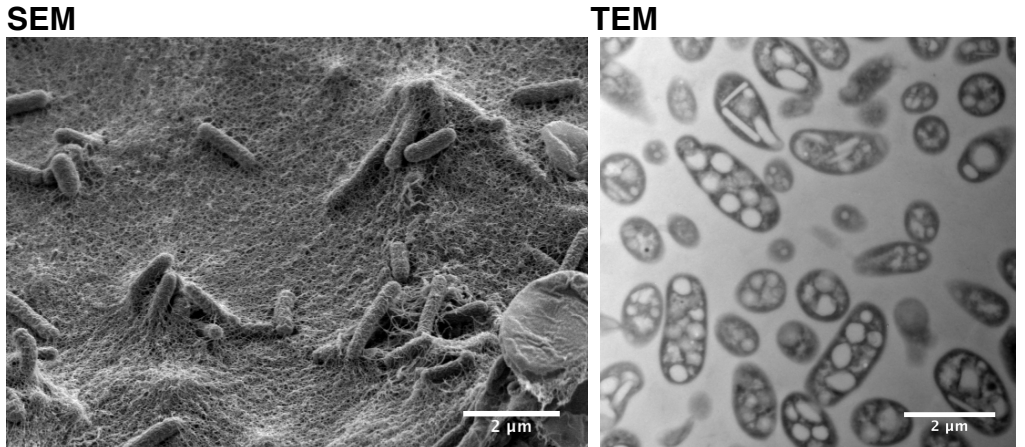
Marinobacter hydrocarbonoclasticus on solid alkane



components
labelling

Canette et al., Springer Protocols Handbooks, 2015

visualization
of cells
arrangement
and
intracellular
compartments

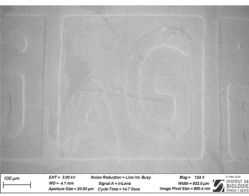


Branchu et al., Microbiology, 2017

Correlative microscopy and Immunolabeling with SEM

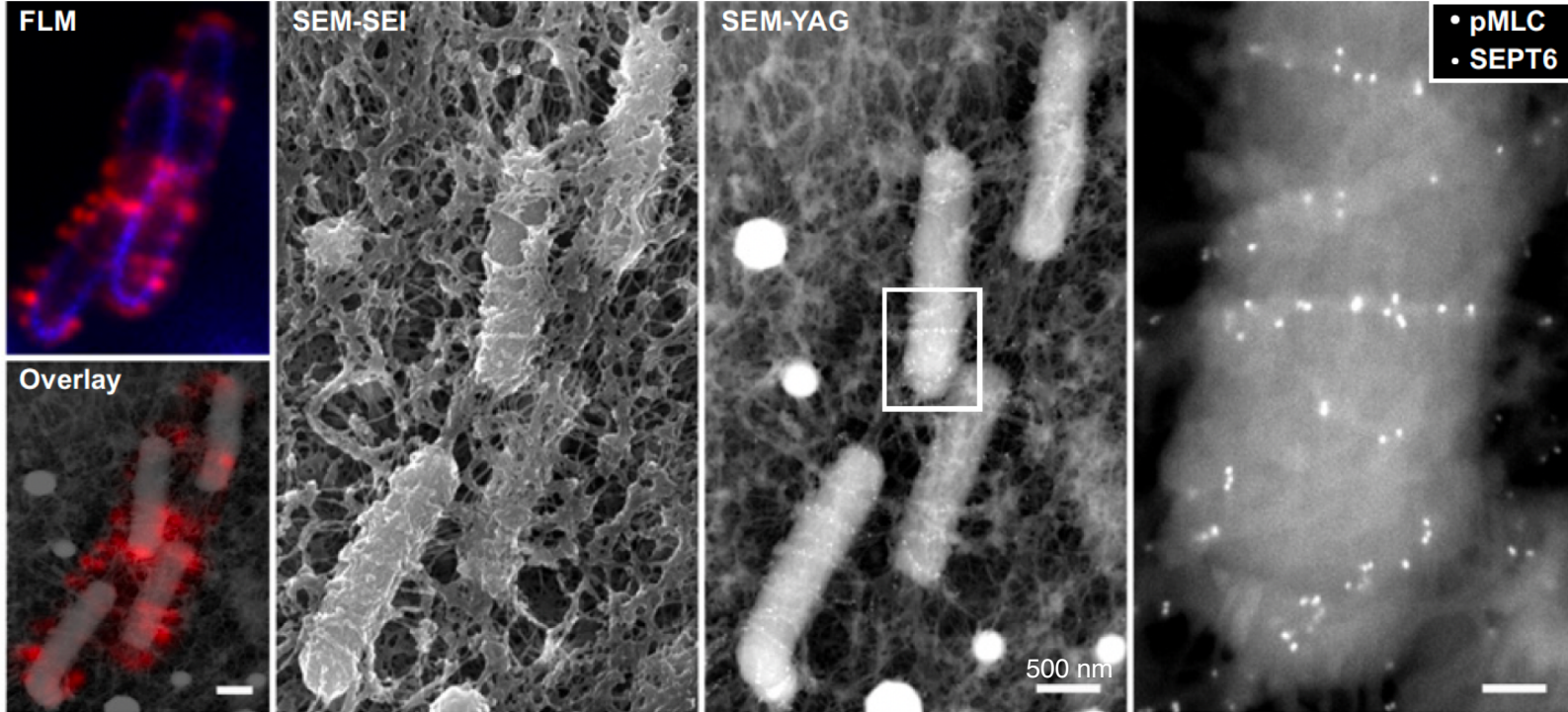


patterned dish

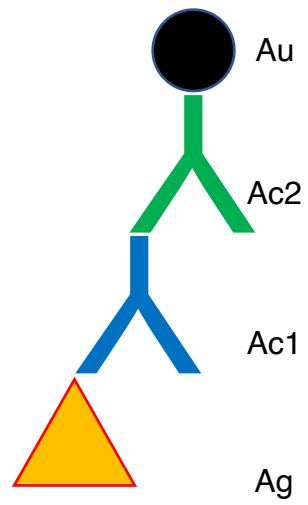


Shigella flexneri bacteria in a HeLa cell

Mostowy et al., Cell Host & Microbe, 2010

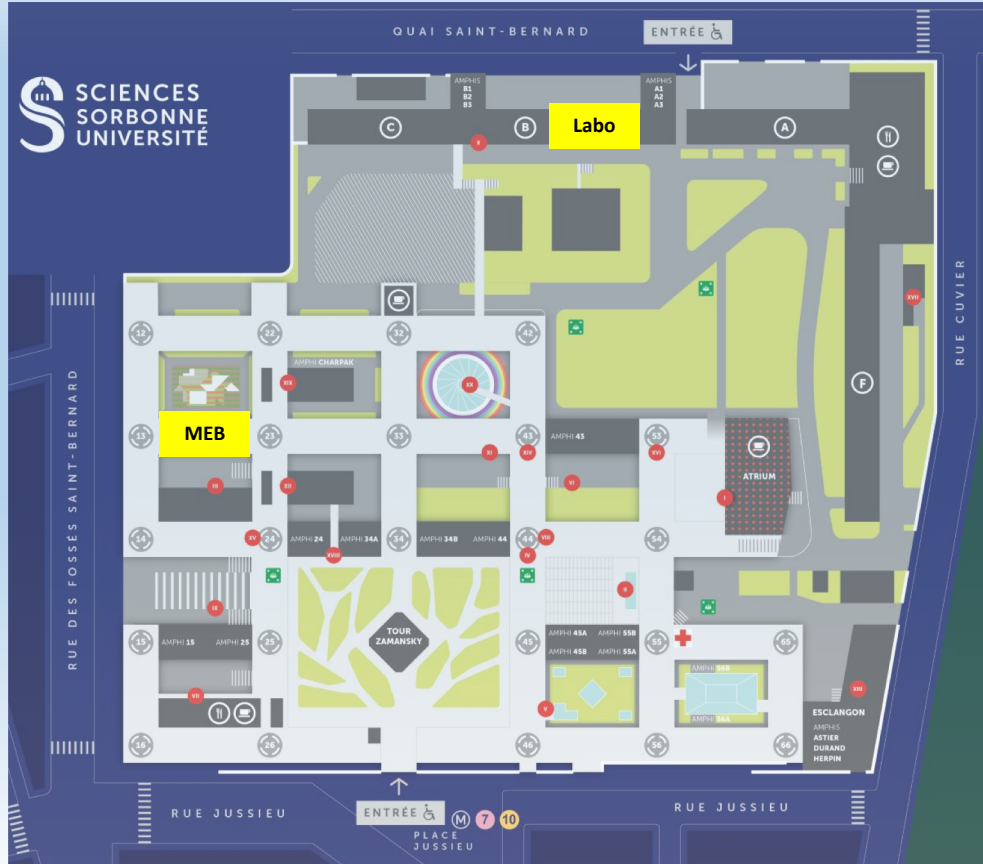


↑ digestion



immunogold principle

Merci pour votre attention



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