

Scanning Electron Microscopy: a new staple for nanoscopic cell biology?

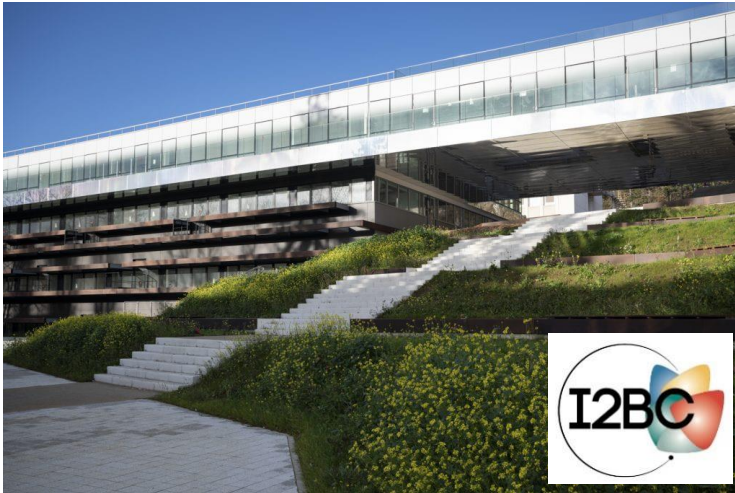
Claire Boulogne

5 juillet 2023

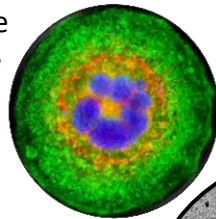
GN-MEBA – Rouen



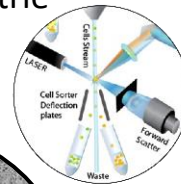
Imagerie-Gif core facility



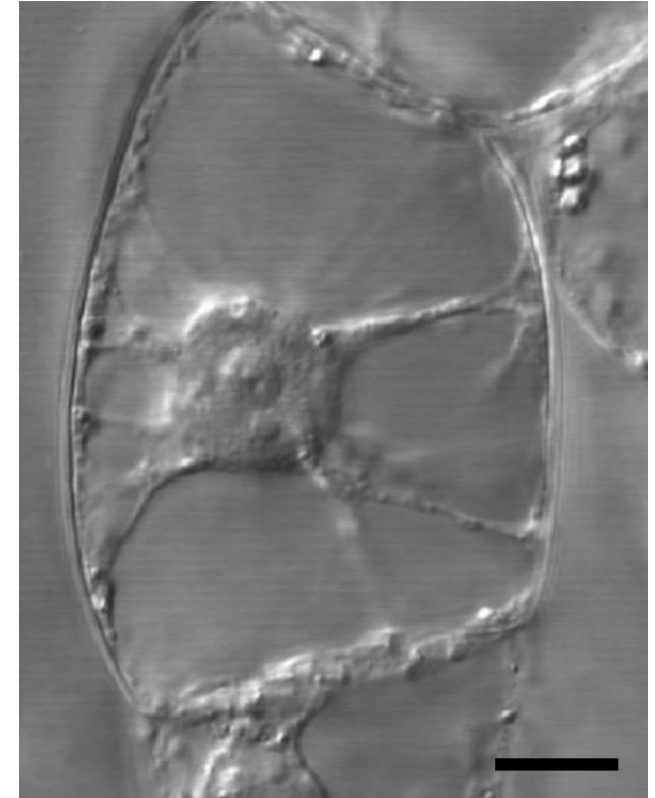
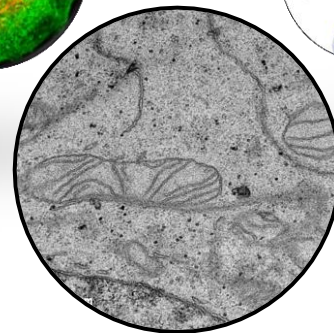
Microscopie
photonique



Cytométrie

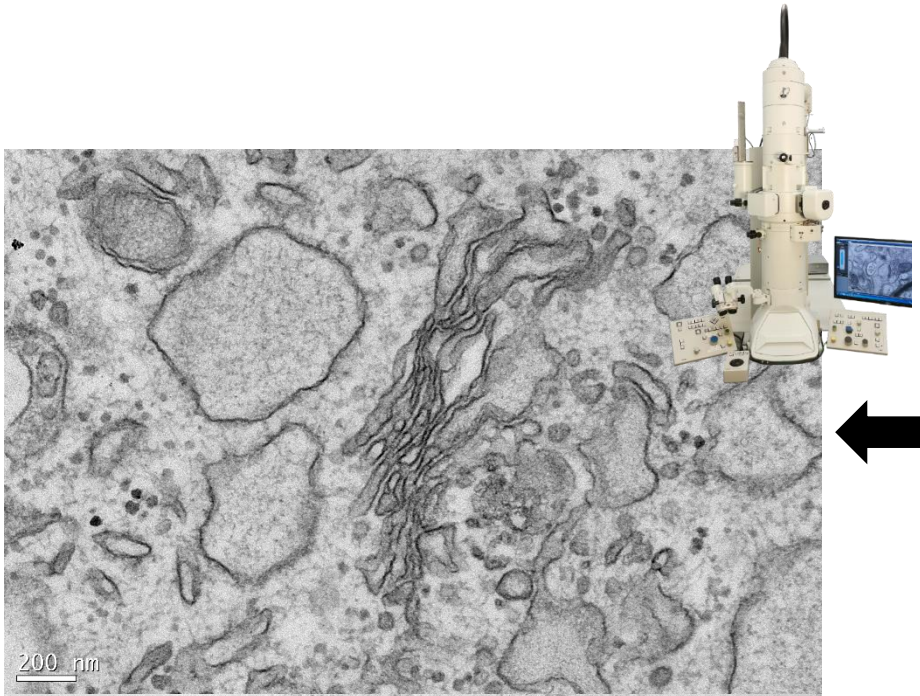


Microscopie
électronique

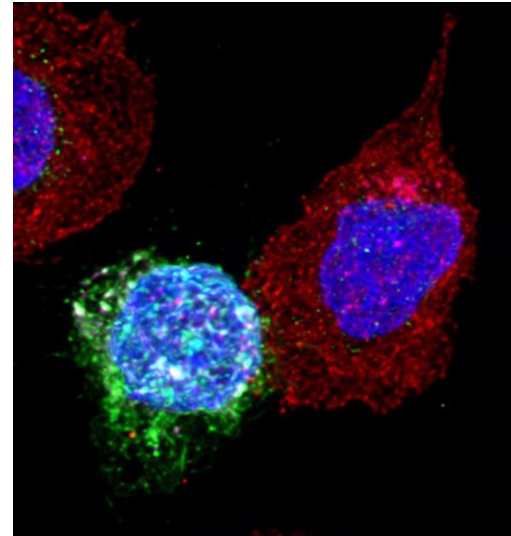


BY-2 cell, DIC contrast, scale bar = 10µm

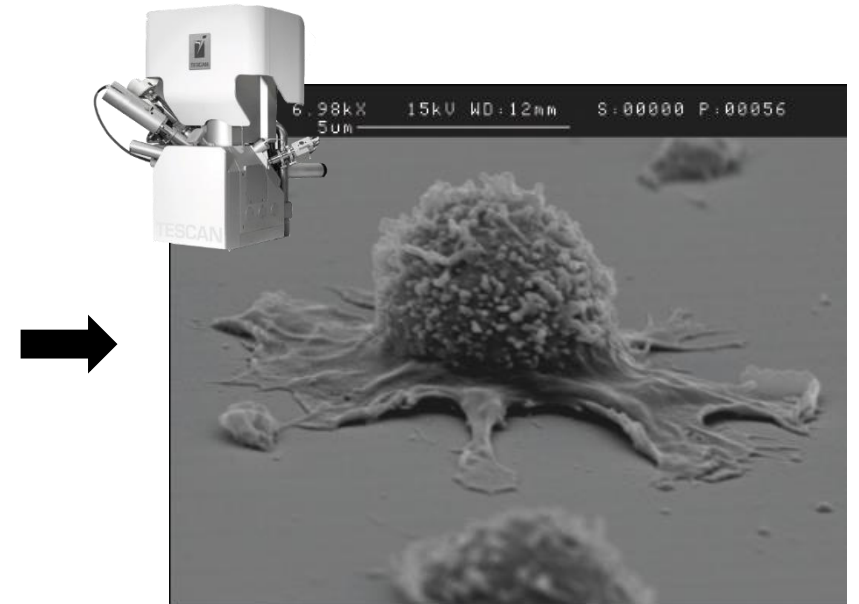
Transmission Electron microscopy: the reference tool for nanoscopic imaging of cells



Transmission electron microscopy



Fluorescence microscopy



Scanning electron microscopy

Sample preparation for TEM

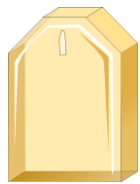


Fixation or Cryo-fixation

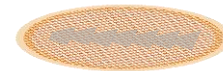
Post-fixation and contrast

Dehydration

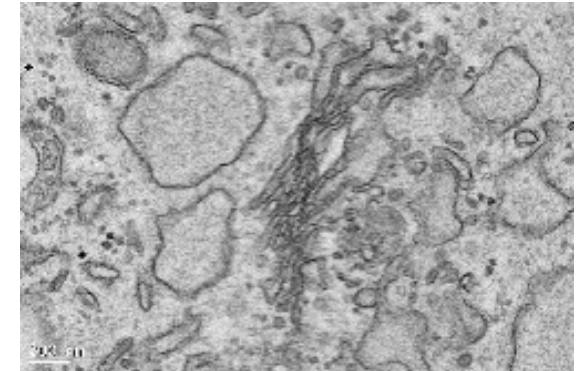
Resin embedding



Sectioning



TEM observation



TEM limitations for cell analysis

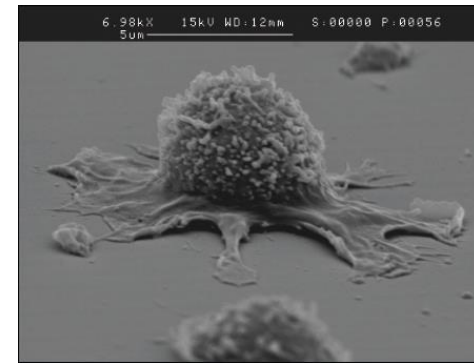
3D structure is lost by sectioning



Sample before
sectioning



Sample after
sectioning

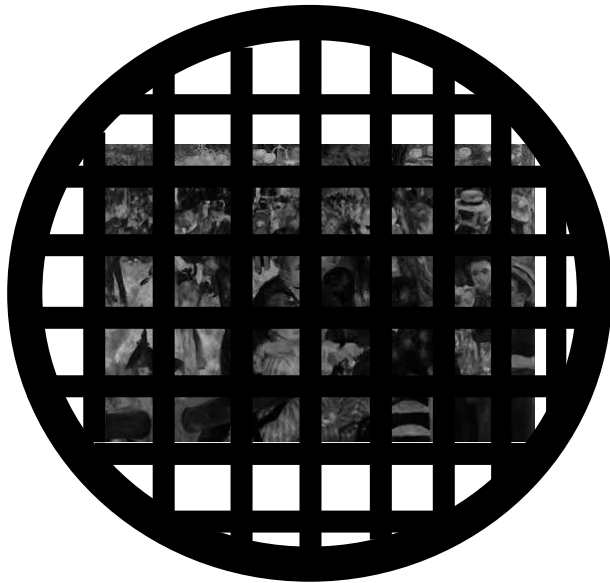


For a $5\mu\text{m}$ cell
 \Rightarrow 100 sections of 50nm.

\Rightarrow Some cells are more
than $100\mu\text{m}$ length...

TEM limitations for cell analysis

**Grid bars limit
the field of view**



Grid bars avoid
visualisation a cell
context in a tissue

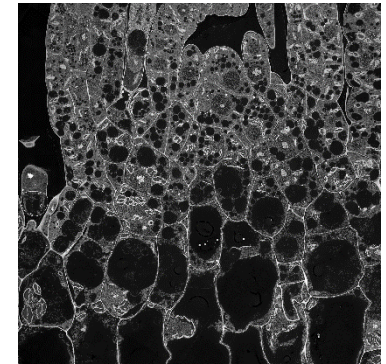


Slot grids without grid bars
=> Fragiles

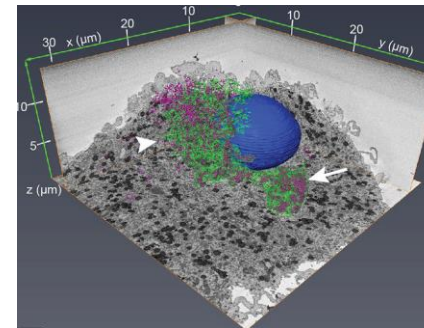
Acquisition of a new SEM @Imagerie-Gif



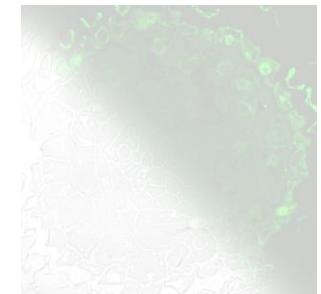
Applications



Mosaic



Volume acquisition



Correlative (CLEM)

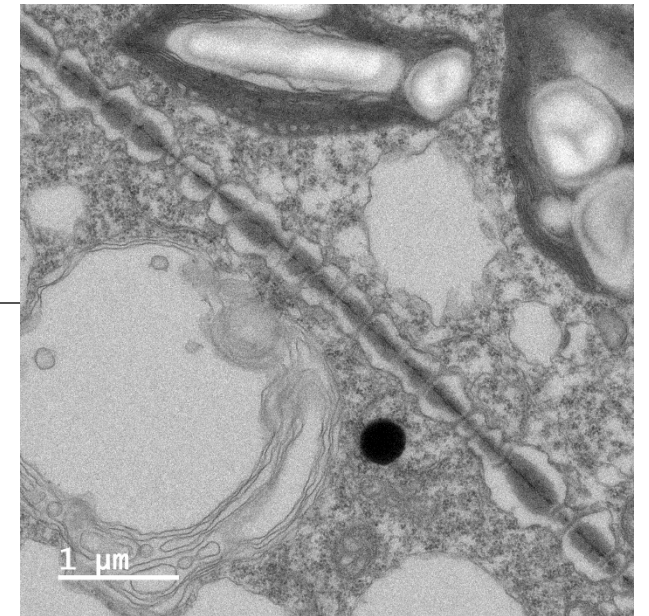
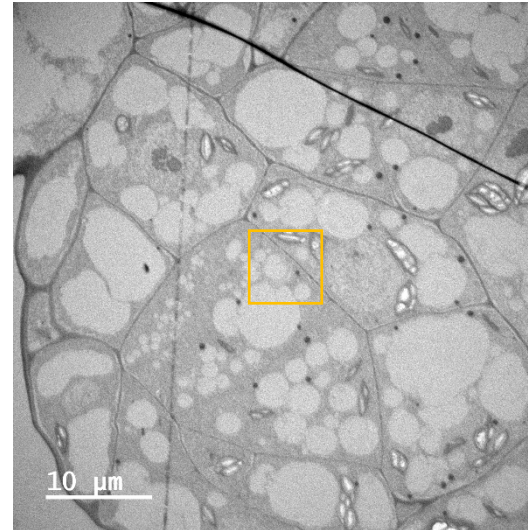
- Delivered and installed in October 2022
- Training since January 2023



Example 1: Mosaic for large field acquisition



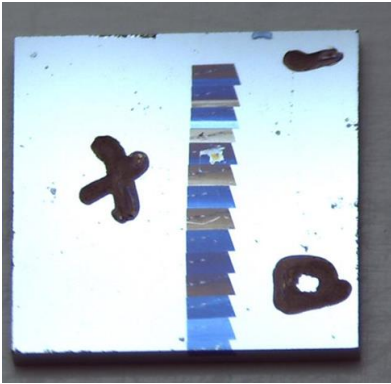
Longitudinal section of Phycostrella



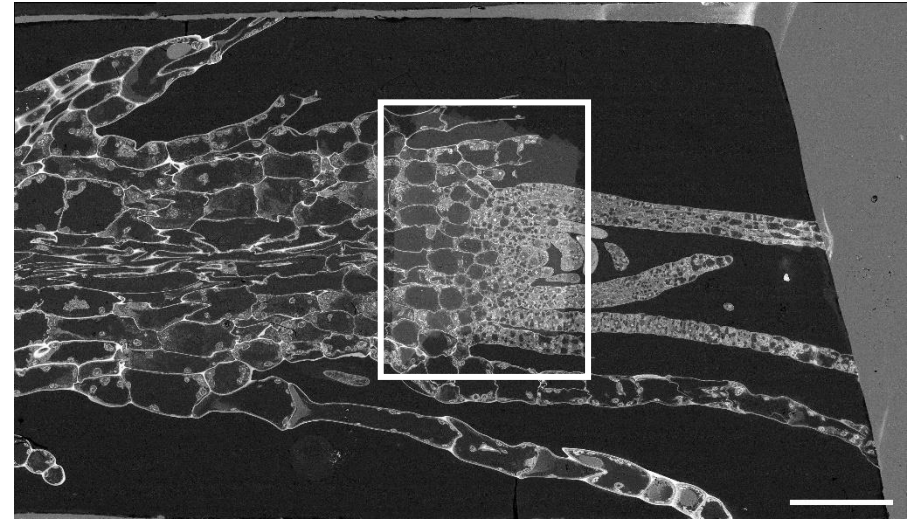
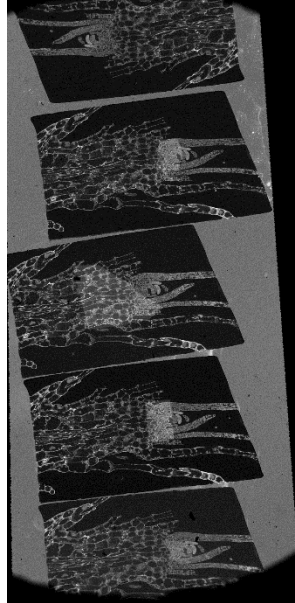
Objectif : imager les plasmodesmes sur toute la longueur la tige pour modéliser les flux hydriques
=> Acquisition de mosaïques.

Example 1: Mosaic for large field acquisition

Sections on silice wafer

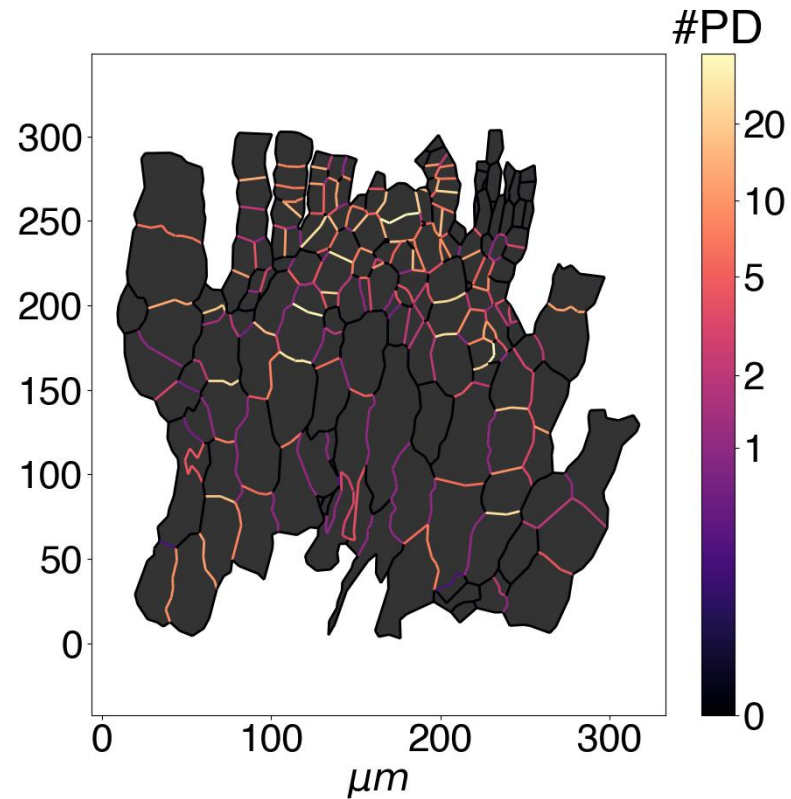
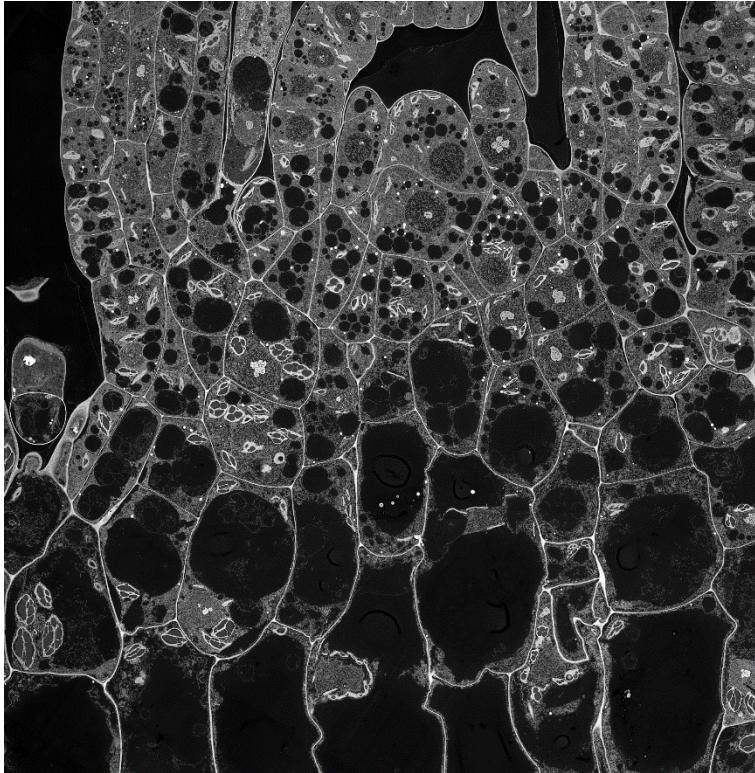


Observation with BSE detector (BSD4)



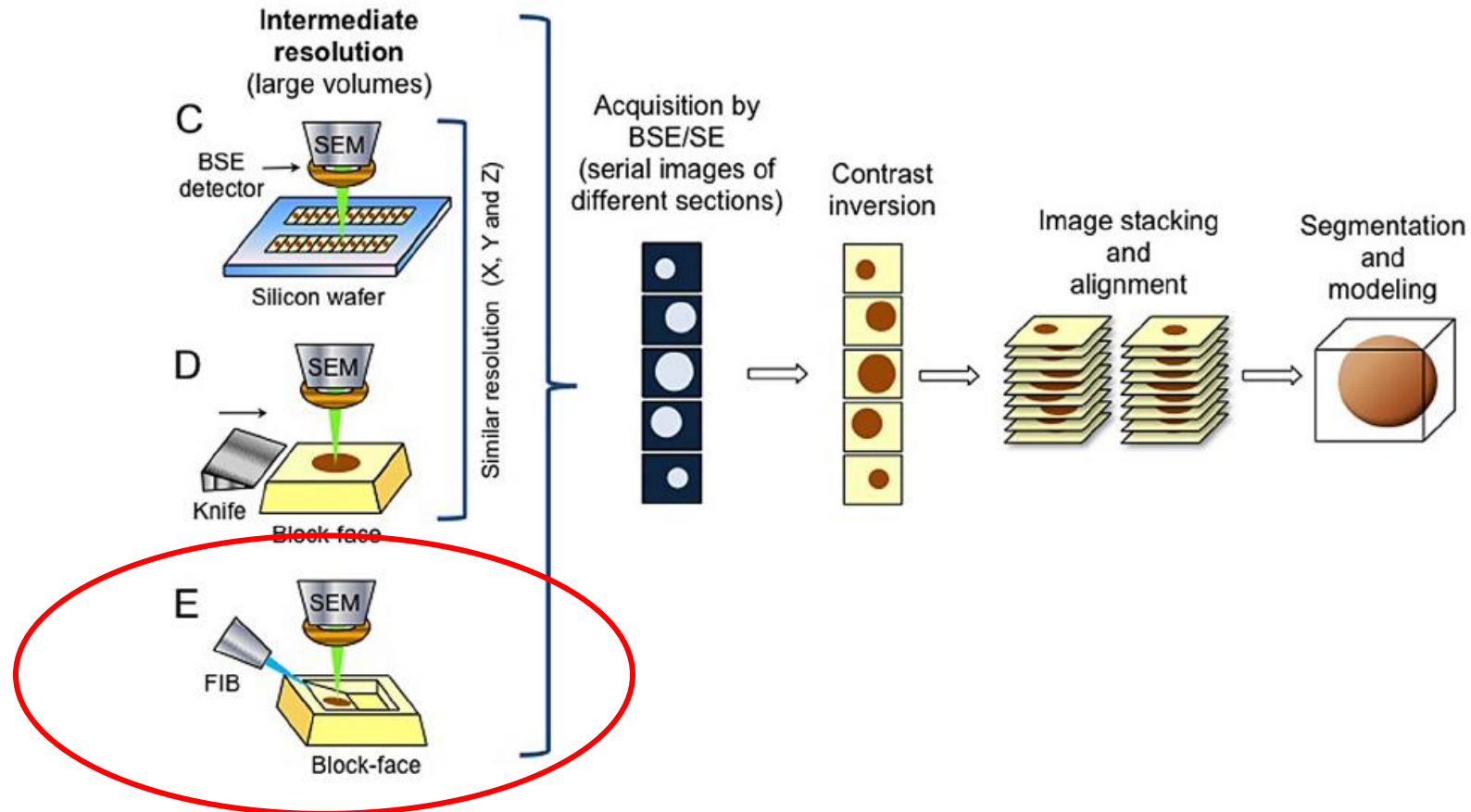
- ⇒ Imaging at 5kV - 500pA
- ⇒ Pixel size 2nm – FOV 200 μ m – 90 tiles – acquisition time 30h

Advantages of SEM for mosaic acquisition



- Wafers are more convenient than grids for large field
- Large tiles
- Fast reconstruction

SEM: the 3D revolution in Biology



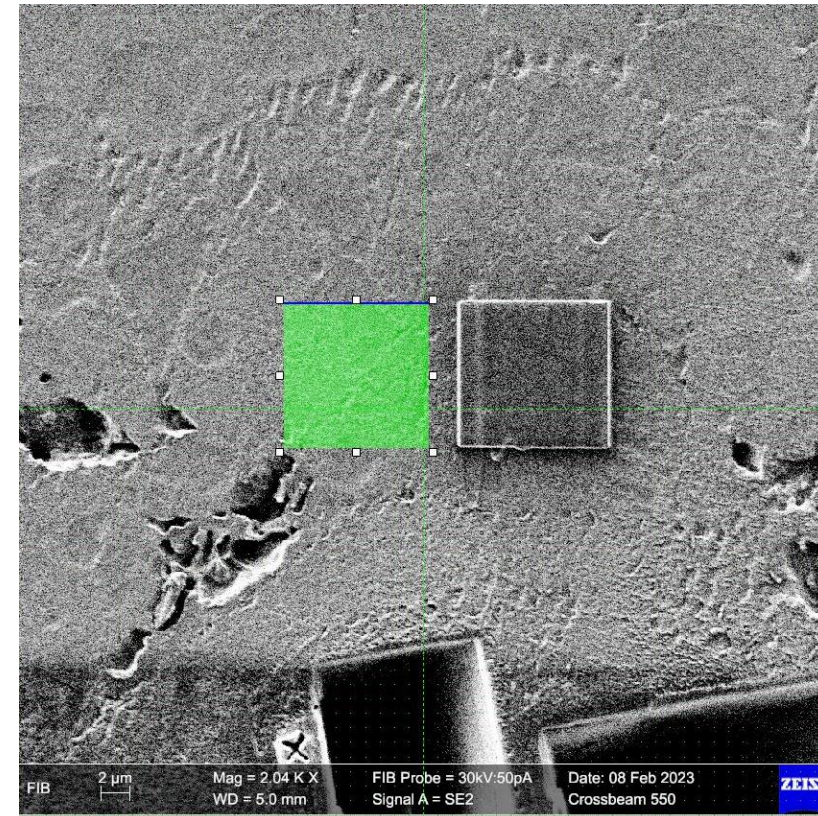
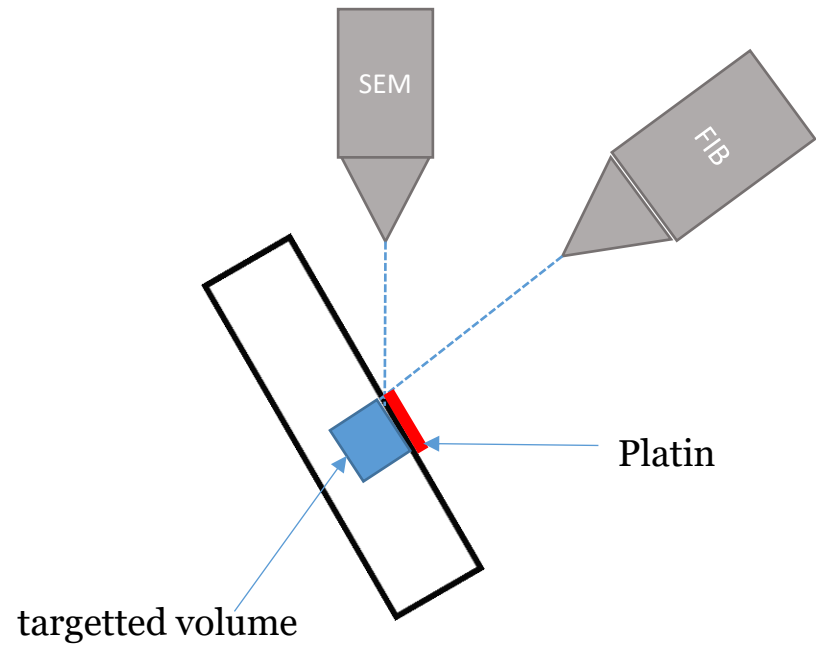
Miranda *et al.*, 2015

FIB-SEM acquisition



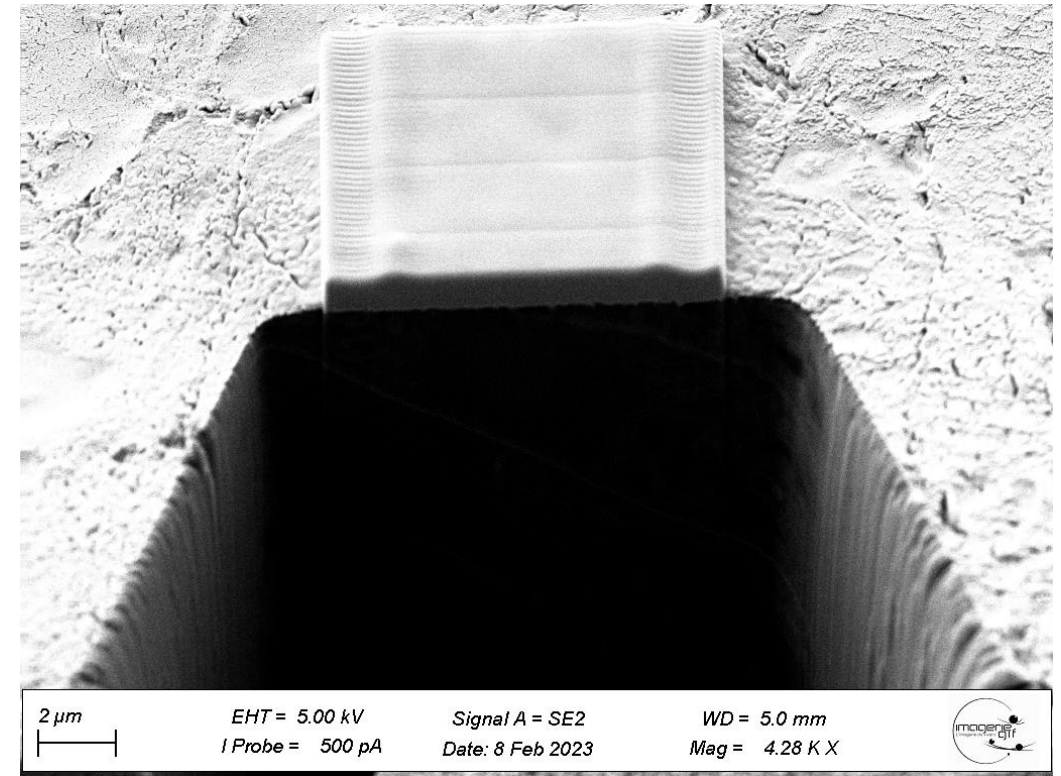
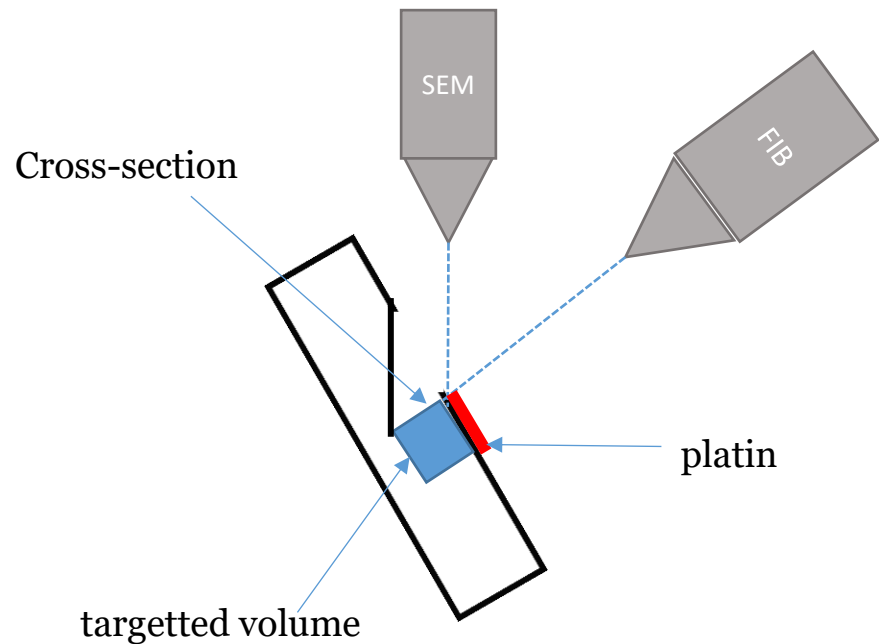
Workflow for FIB-SEM acquisition on resin block

Platin deposition on the acquisition area



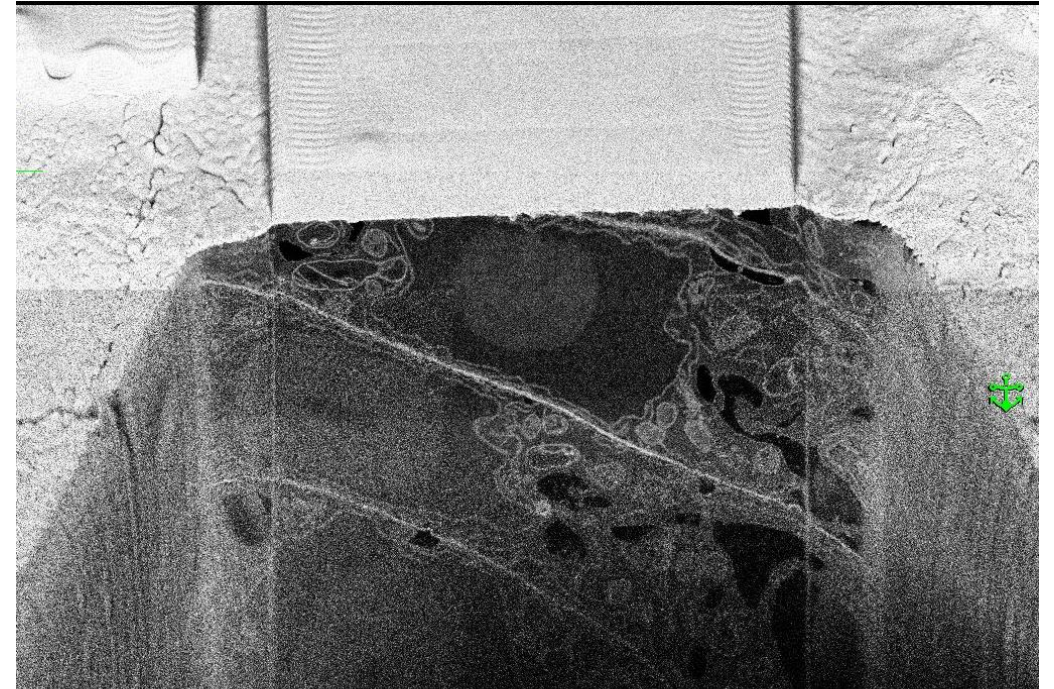
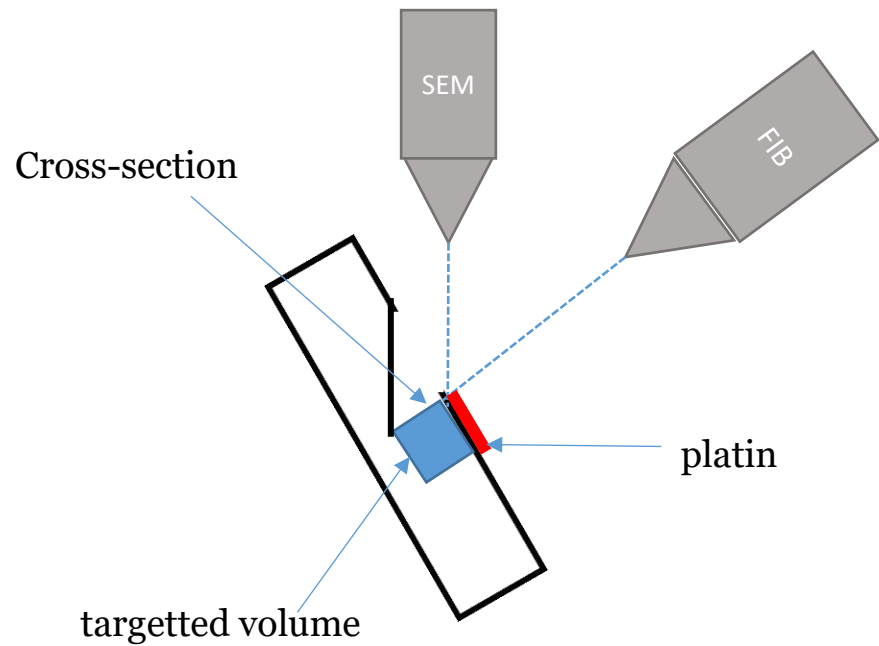
Workflow for FIB-SEM acquisition on resin block

Milling of the resin to make a cross-section



Workflow for FIB-SEM acquisition on resin block

Milling of the resin to make a cross-section



2 μ m



EHT = 2.00 kV
I Probe = 1.0 nA

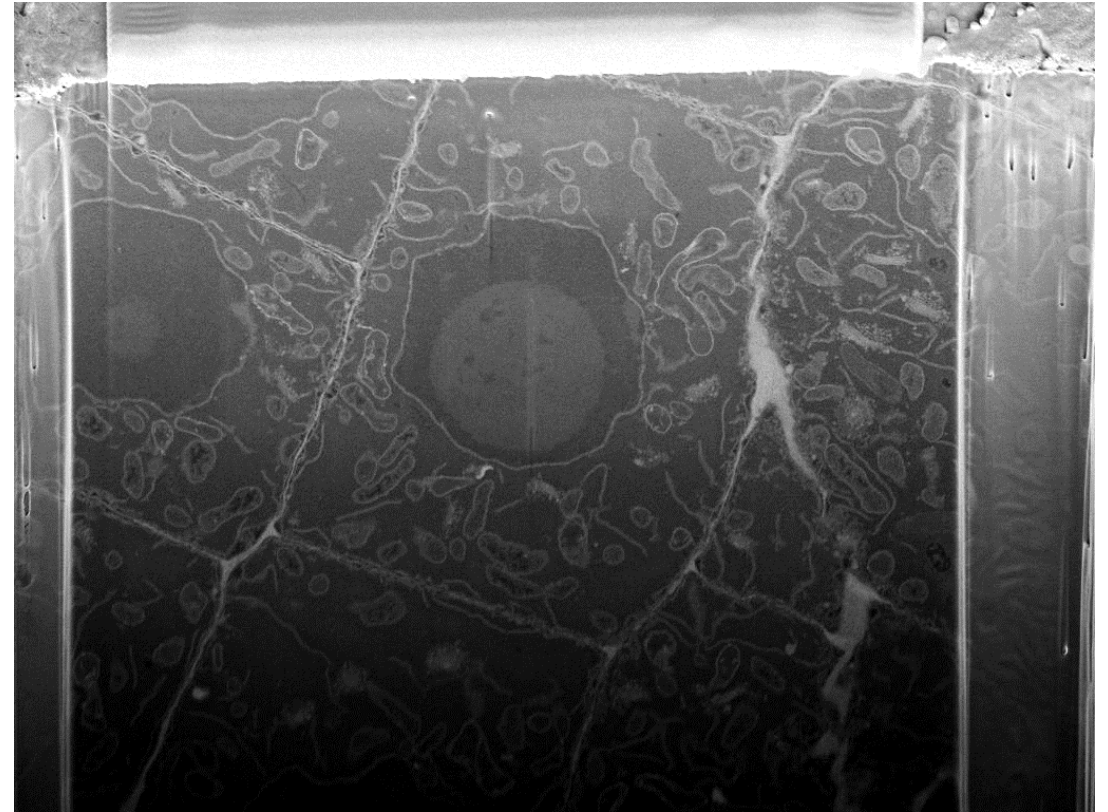
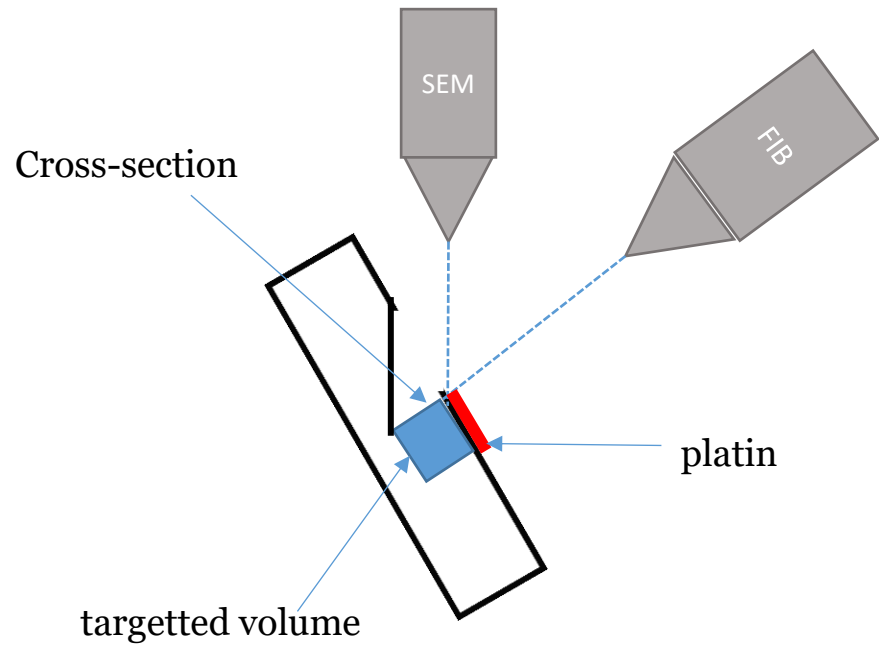
Signal A = ESB
Date: 8 Feb 2023

WD = 5.1 mm
Mag = 5.58 K X

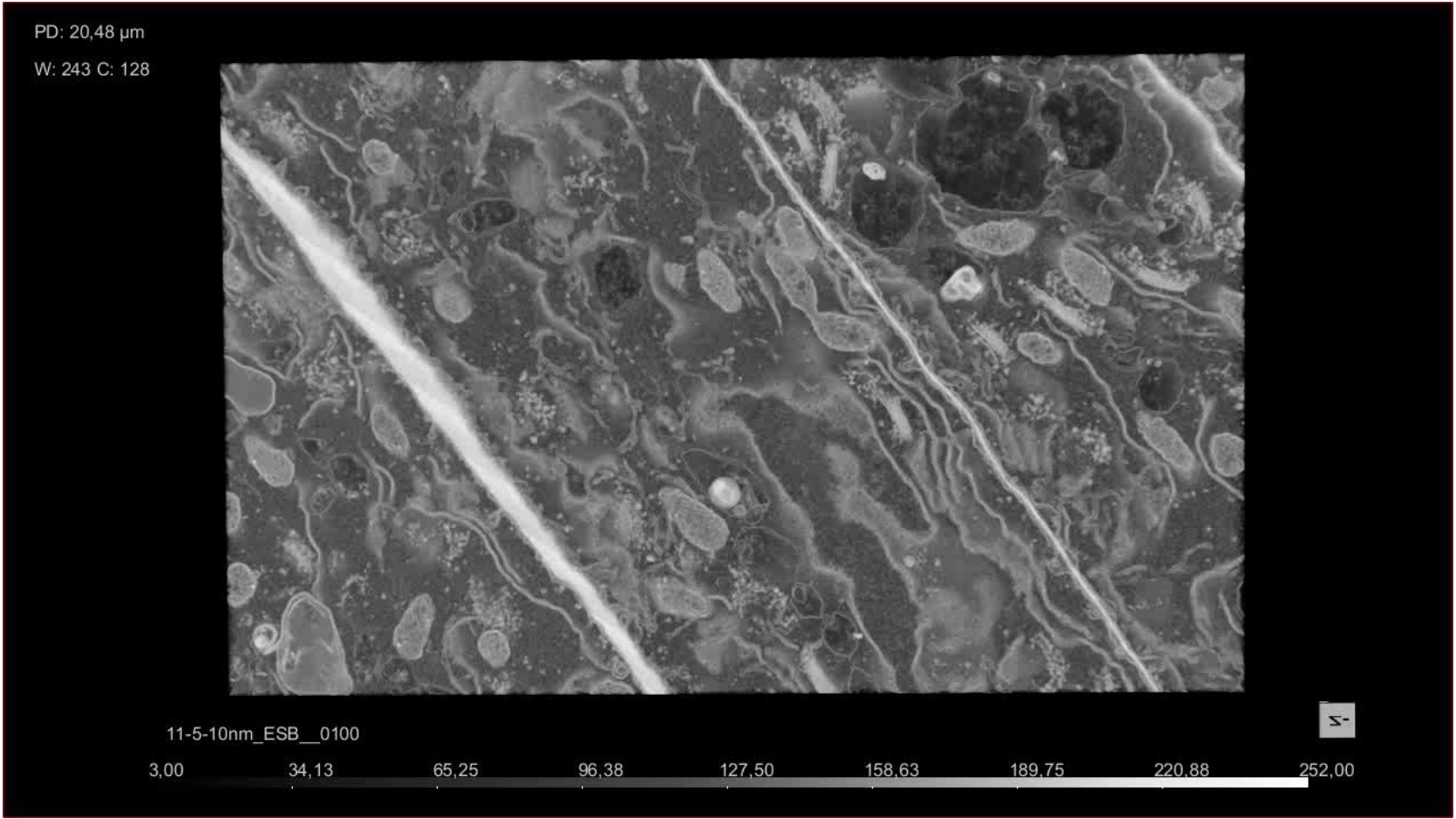


Workflow for FIB-SEM acquisition on resin block

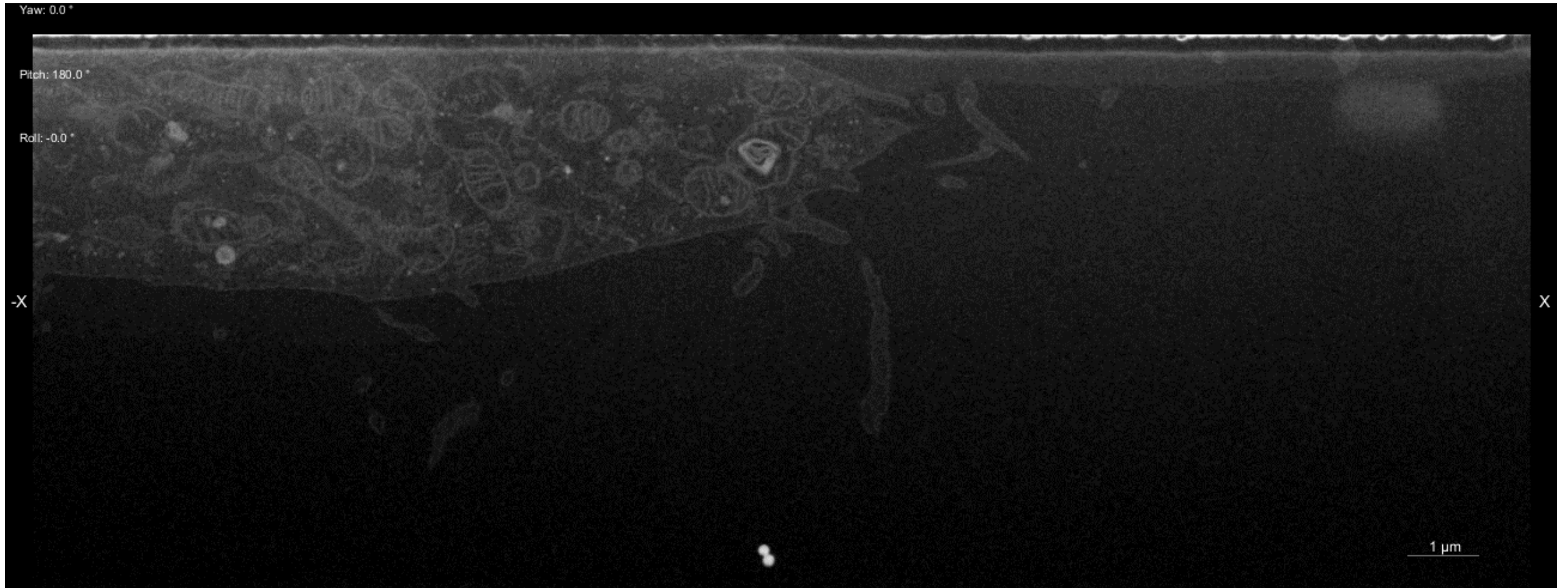
Milling of the resin to make a cross-section



Volume rendering



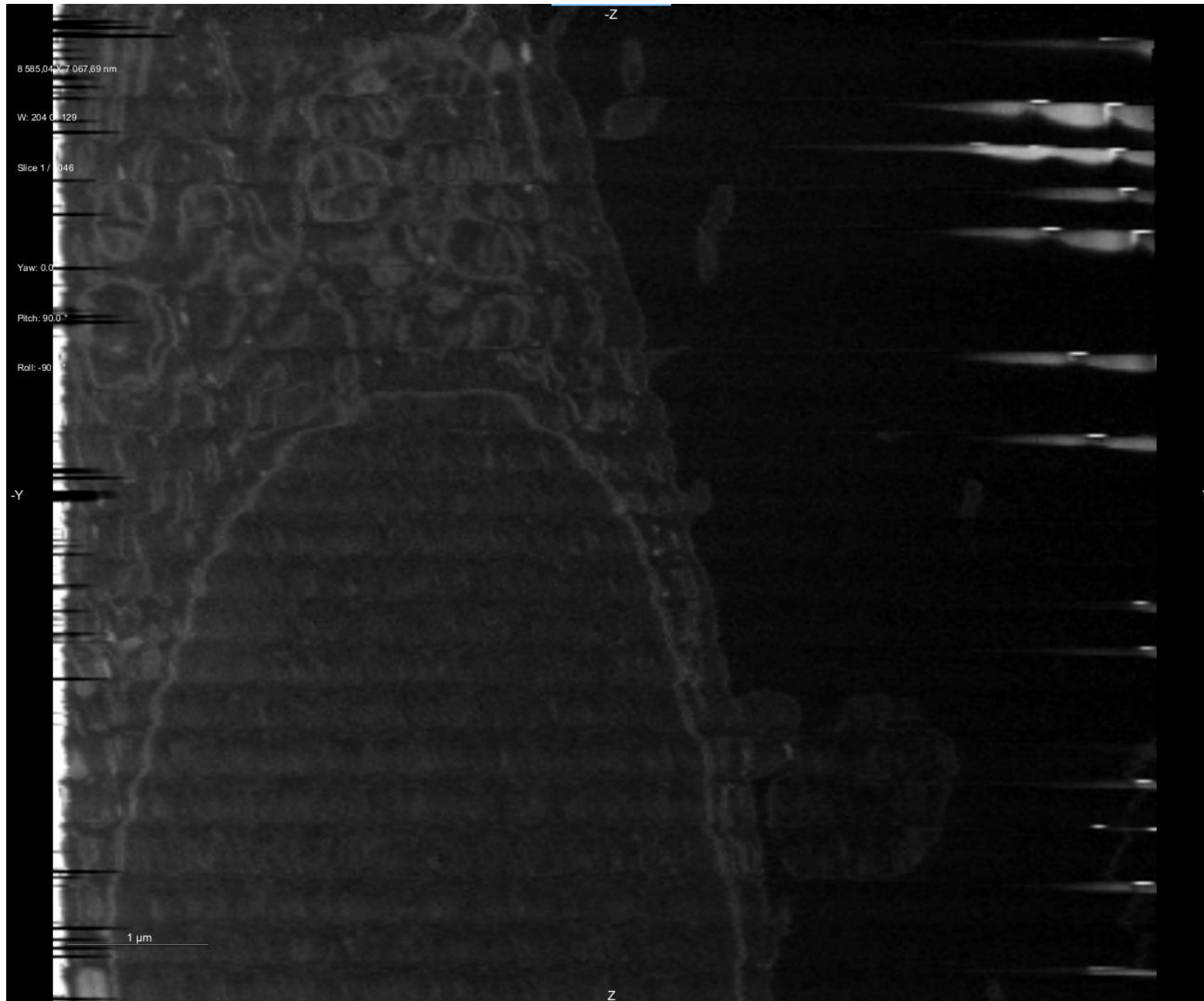
Difficulties for resin embedded samples



Adherent cell (HeLa) contrasted with osmium and embedded in araldite resin.

FIB setting => FIB current 700pA – FIB HT 30kV – slice thickness 20nm

SEM settings => EHT 2kV – I current 2nA – Pixel size 20nm



Cause:

- High electron dose to improve the signal (low contrasted sample).
- Electron accumulates in the resin
- Damage of the resin => « melting » and instability under the Gallium beam.

Solutions:

- Decrease electron dose
- More resistant samples

Solutions to improve sample stability

More resistant sample

- More resistant resins
 - Epoxy resin
 - Araldite
 - Durcupan => more used for SEM

• Conductive resins

- Metal particles in the resin

Nguyen *et al.*, 2018, *Frontiers in Neural Circuits*,
"Methodological Improvements With Conductive Materials for
Volume Imaging of Neural Circuits by Electron Microscopy"

- Conductive resins

Heiligenstein and Lucas, 2022, *Frontiers in Cell and
Developmental Biology*, "One for All, All for One: A Close Look
at In-Resin Fluorescence Protocols for CLEM"

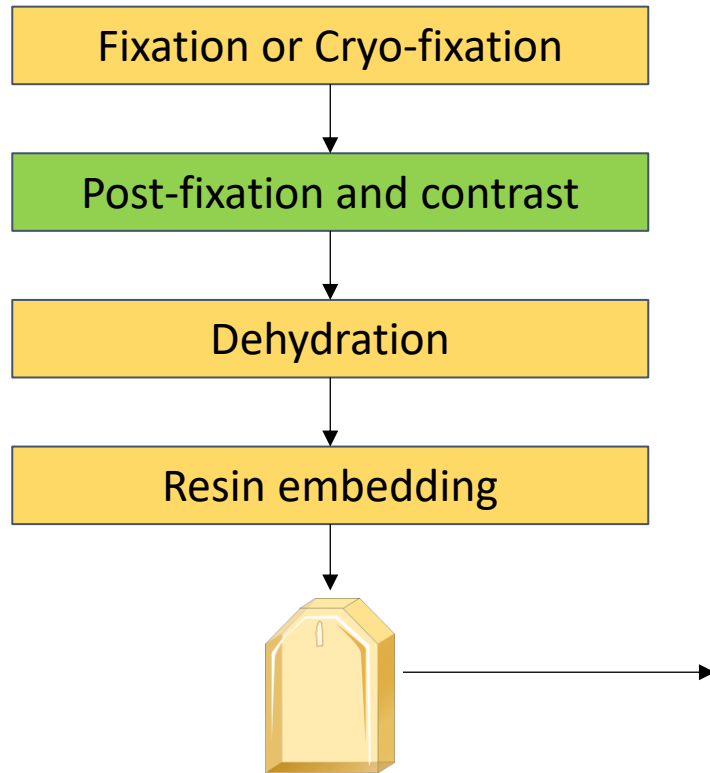
Decrease electron dose

- Decrease the imaging volume or the resolution
- Decrease electron current and imaging time.

=> Decrease signal-to-noise ratio

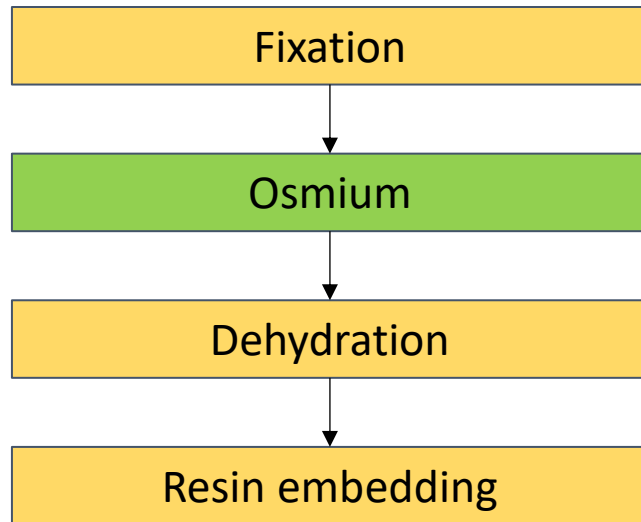
- Increase the contrast of the sample in previous steps.

Improve the contrast of the sample

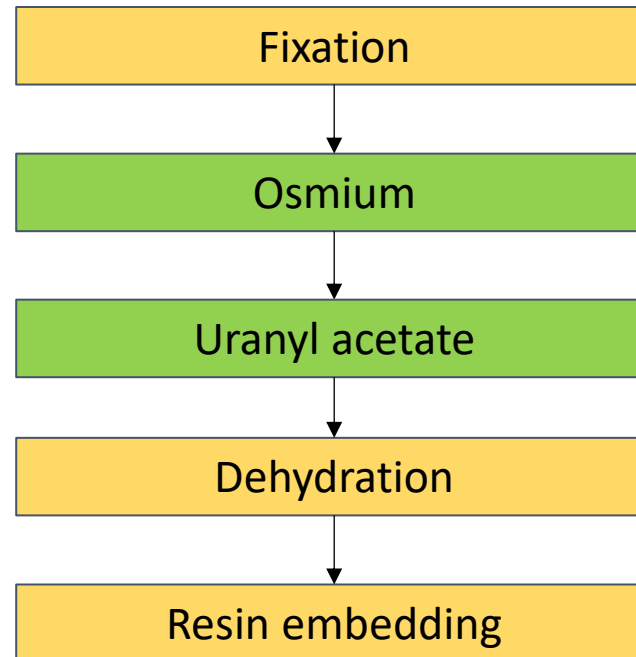


Comparison of 3 contrasting protocols

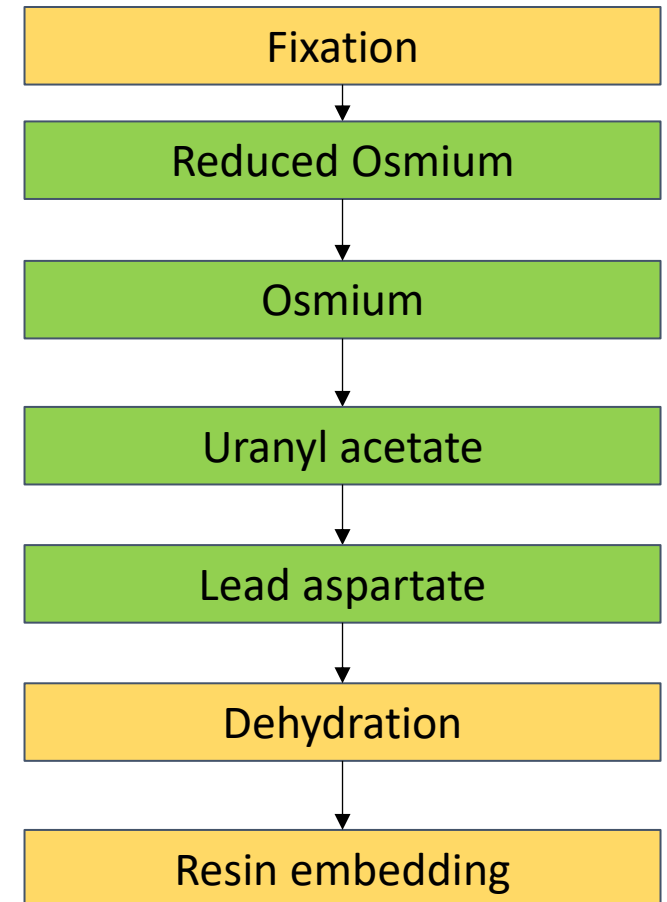
Protocol 1 (minimum)



Protocol 2 (classical)

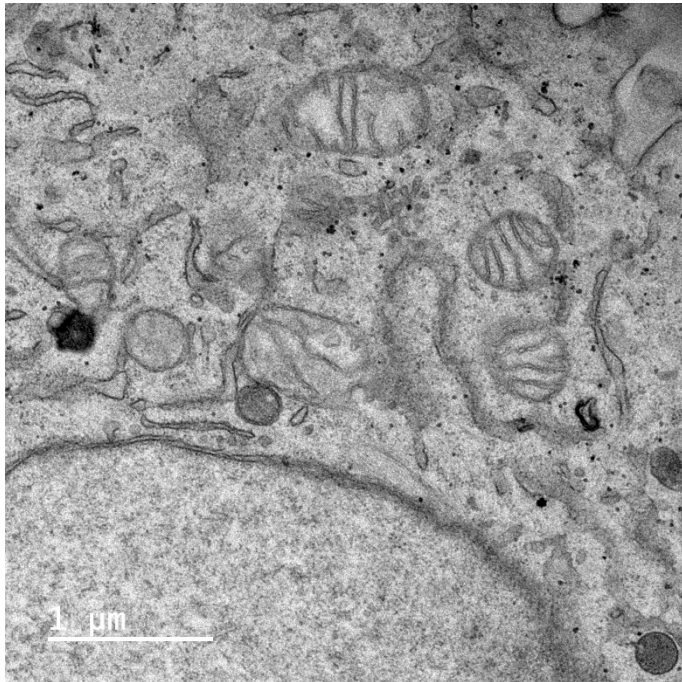


Protocol 3 (Deerinck, NCMIR)

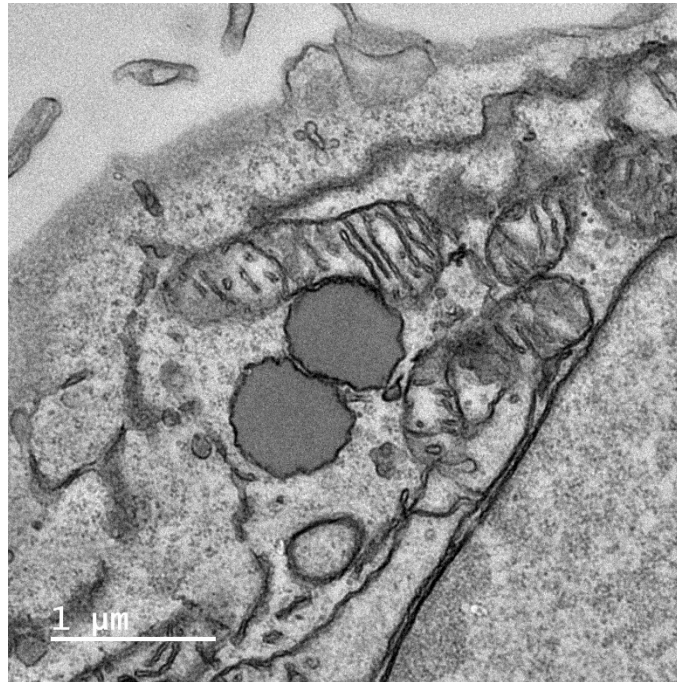


Comparison of 3 contrasting protocols

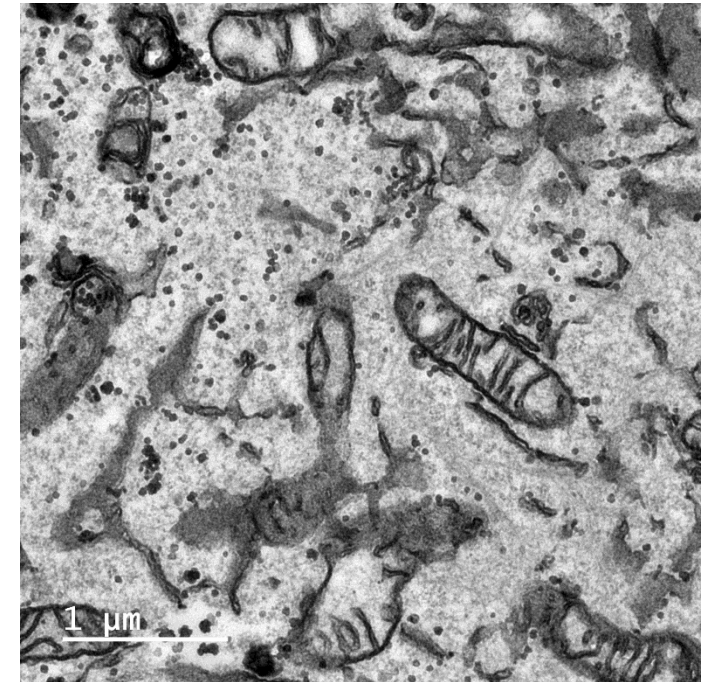
Protocol 1 (minimum)



Protocol 2 (classical)



Protocol 3 (Deerinck, NCMIR)

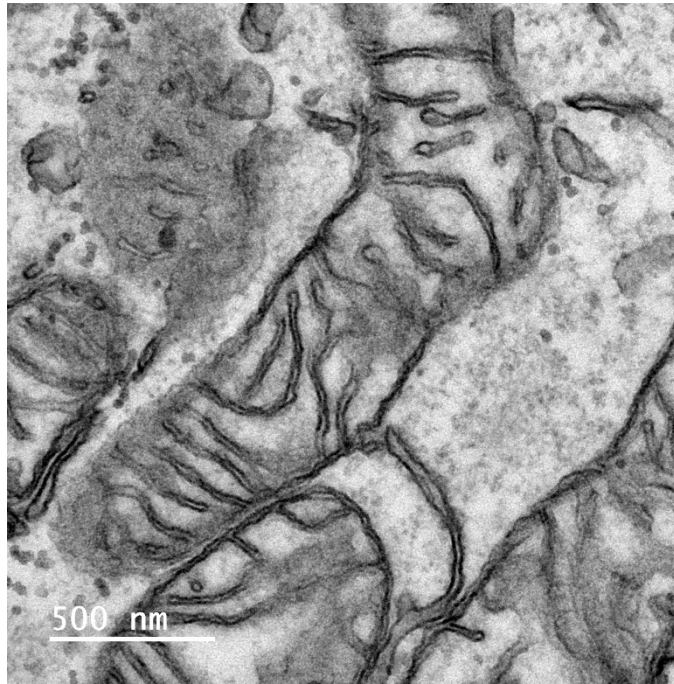


Comparison of 3 contrasting protocols

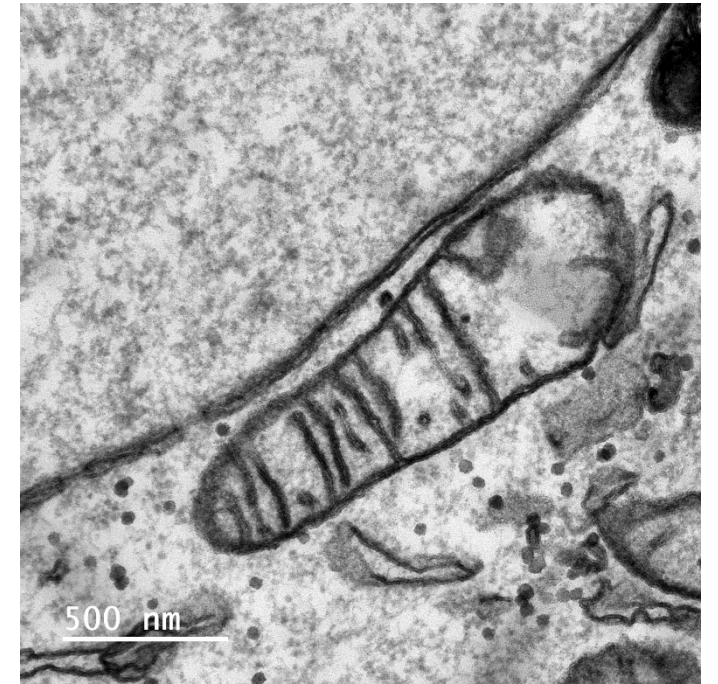
Protocol 1 (minimum)



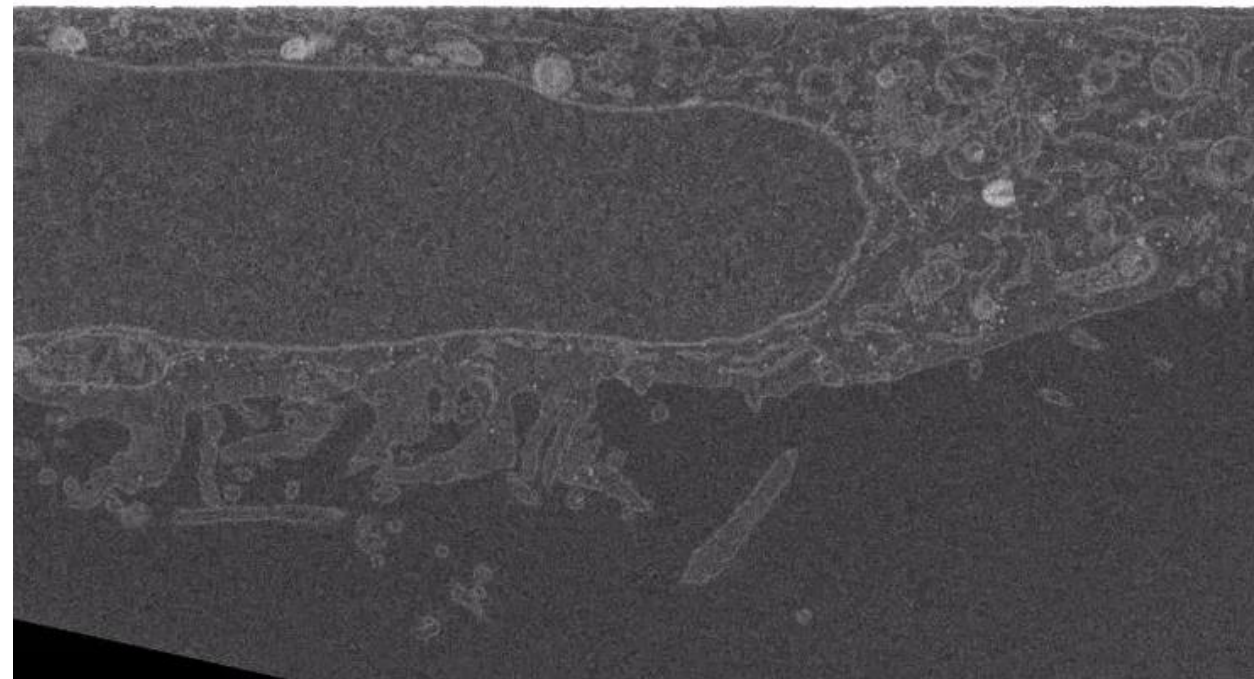
Protocol 2 (classical)



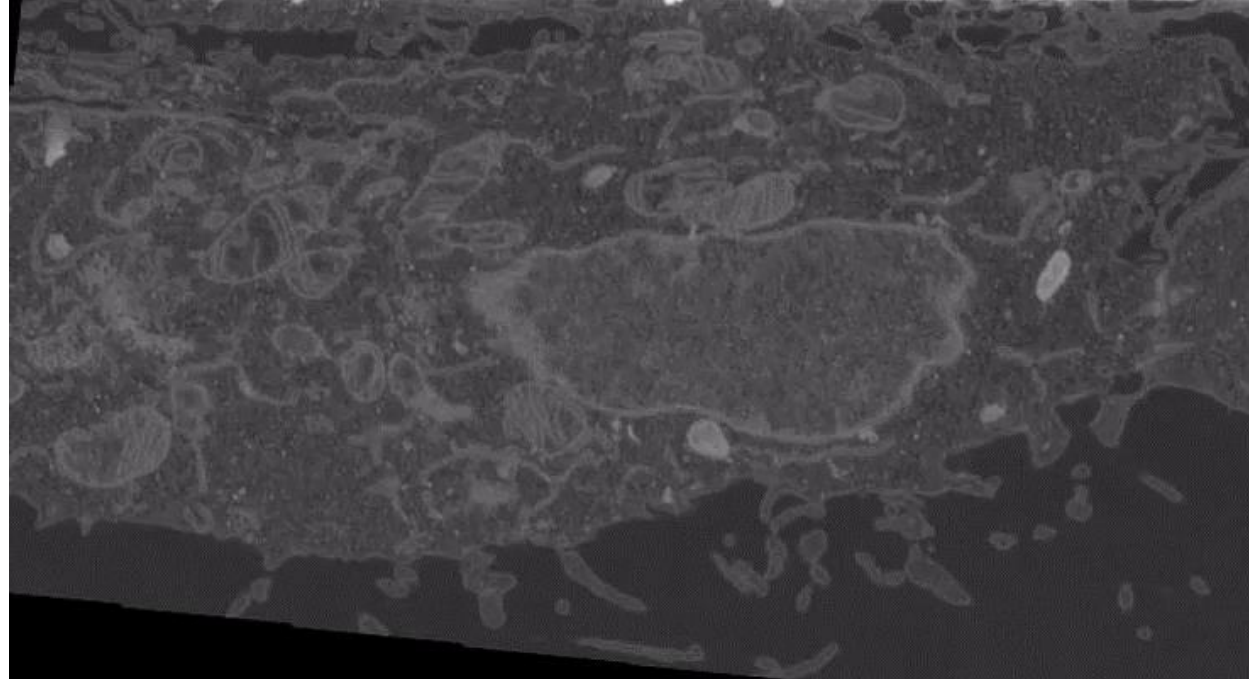
Protocol 3 (Deerinck, NCMIR)



FIB-SEM acquisition with or without uranyl

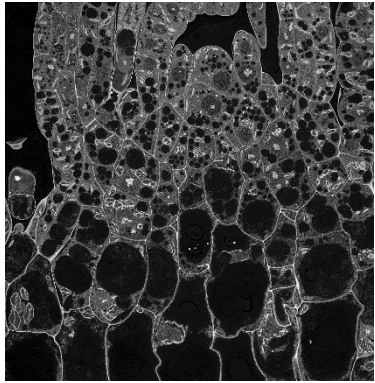


Protocol 1 (osmium)

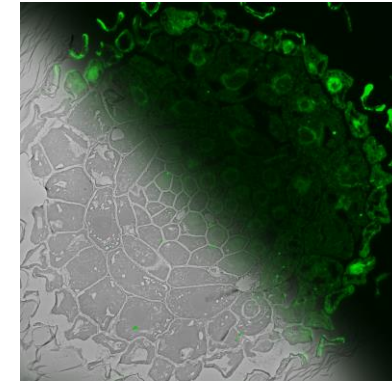


Protocol 2 (osmium + uranyl)

Conclusion: multiple applications for SEM in Cell Biology

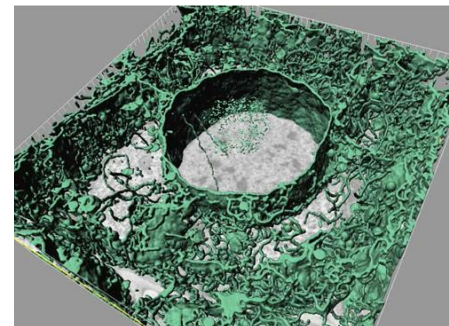
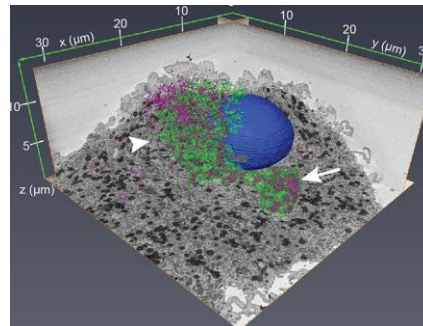
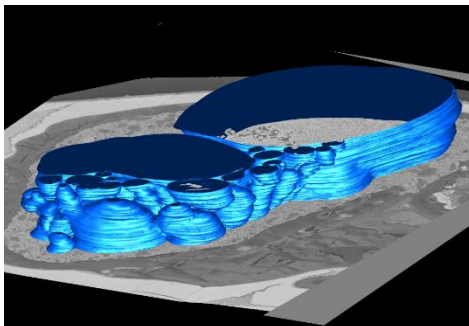


mosaic



Correlative Light and Electron microscopy

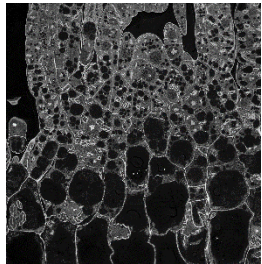
3D acquisitions



Aknowledgements



Cynthia Gillet
Cynthia Dupas
Sandra Sara (master student)



Phycostrella project:

Equipe Reproduction et développement
des plantes (Université Claude Bernard
Lyon)
Yoan Coudert
Jeanne Abitbol (PhD student)

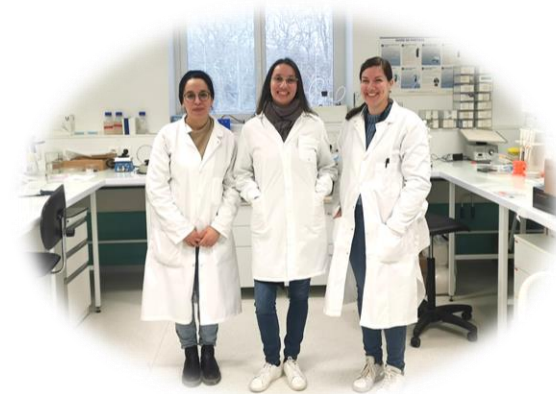


Training and help:
Christophe Depagne
Sebastian Schaedler



FIB projects: ReMemER consortium

- Autophagy and development (R. Legouis)
- Autophagy and antiviral immunity (A. Esclatine)
- Replication and assembly of poxviruses (E. Quemin)
- Lipid trafficking and membrane contact sites (F. Giordano)



Les FIBettes:
Naïma El Khallouki
Céline Largeau
Eva Hernandez