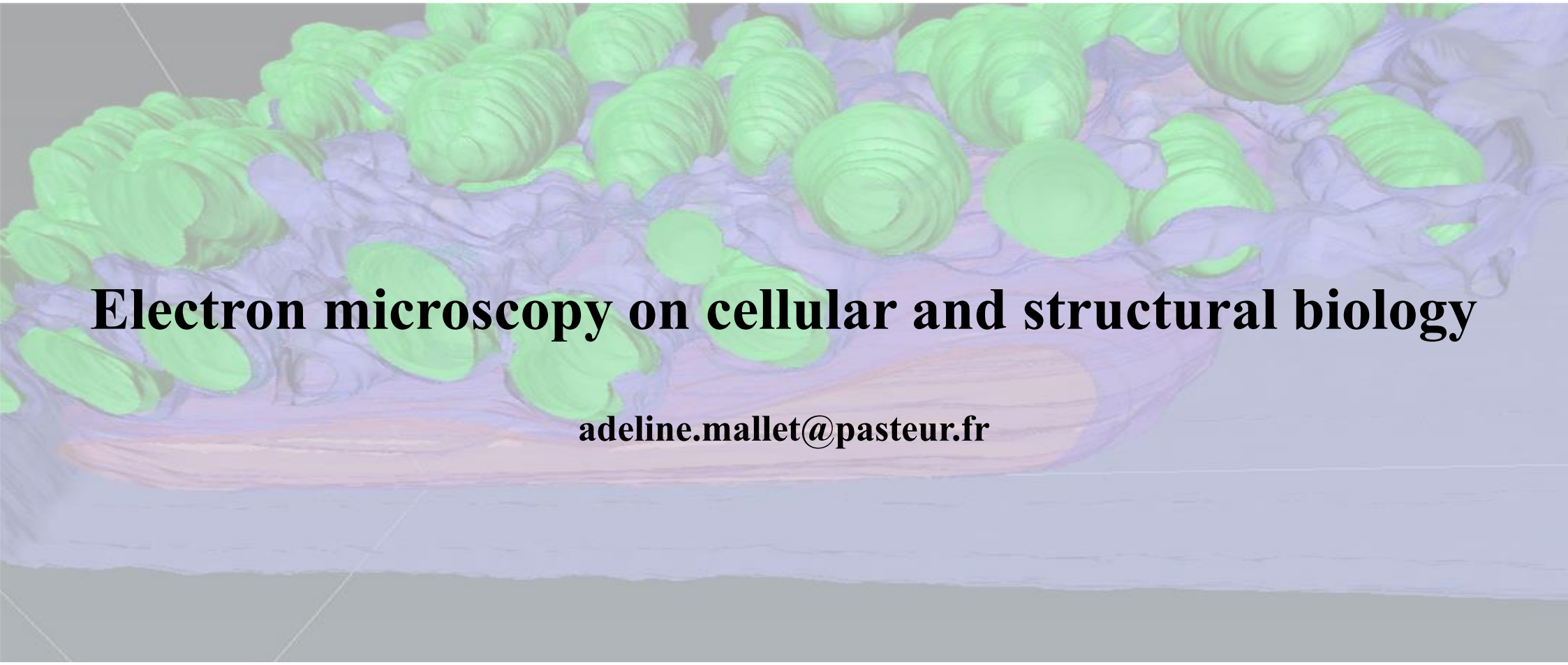

Electron microscopy on cellular and structural biology

adeline.mallet@pasteur.fr

Ultrastructural BioImaging Core Facility (UBI) - Institut Pasteur

Ultrastructural BioImaging Core Facility (UBI)

Affiliations : Center of Research and Technological Ressources / Cell Biology and Infection

Ultrastructural BioImaging Core Facility

Head: Adeline MALLET

Administration: Cidalia DA AGRA

Cryo- Methods (HPF and Tokuyasu)

Olivier GORGETTE
Catherine THOUVENOT

3D Applications (FIB-SEM)

Perrine BOMME
Esthel PENARD
Alice MARTEIL (M1)

Scanning and Transmission Electron Microscopy (SEM-TEM)

Maryse MOYA-NILGES
Christine SCHMITT
Nadège CAYET

Cryo-Transmission Electron Microscopy and Electron Tomography

Gérard PEHAU-ARNAUDET
Anastasia GAZI
Cyril SCANDOLA

Our equipement

Sample preparation systems

- 3 ultramicrotomes
- 3 cryo-ultramicrotomes
- 2 cryo-substitution systems (AFS)
- 1 plunge freezer
- 1 High pressure freezer
- 1 Critical point dryer
- 2 Metal coaters (Pt, Au/Pd, C)
- 1 cryo-light microscope (Correlative applications)
- and Carbon coaters, evaporators

Ultramicrotomy

5 Electron microscopes

- 1 Scanning Electron Microscope (SEM)
- 1 FIB-SEM (3D applications)
- 1 Transmission Electron Microscope 120kV (TEM, T12)
- 1 Cryo-transmission Electron Microscope 120kV (TEM, T12)
- 1 Cryo-Transmission Electron Microscope 200kV (TEM, F20)


Transmission Electron Microscope

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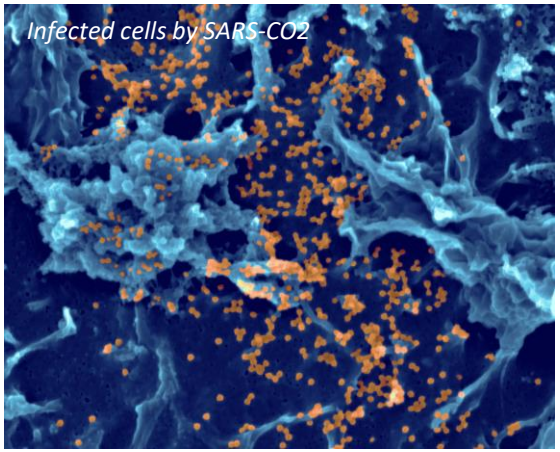
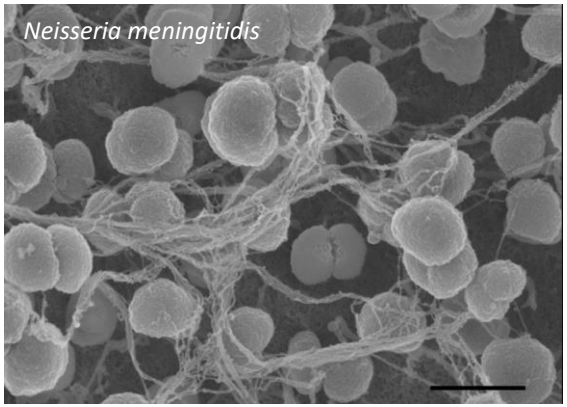
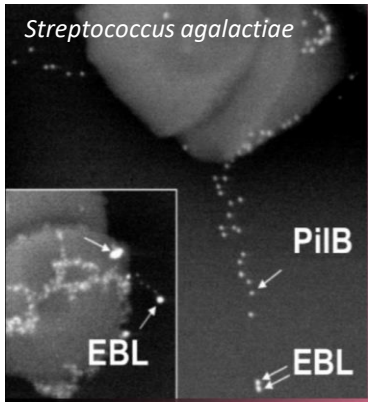
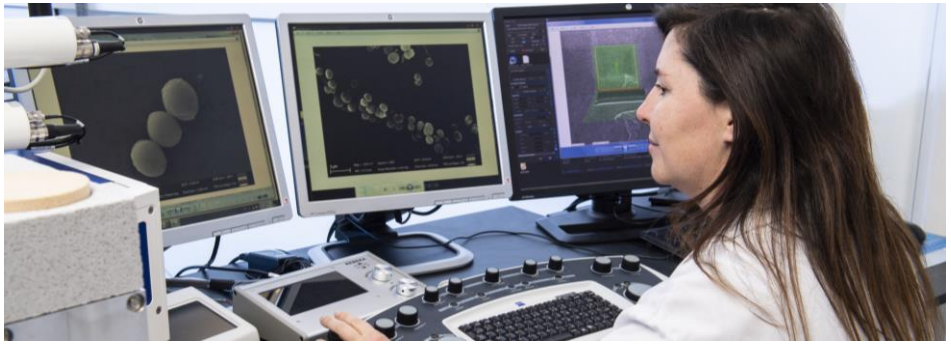
Electron Microscopy on biological samples



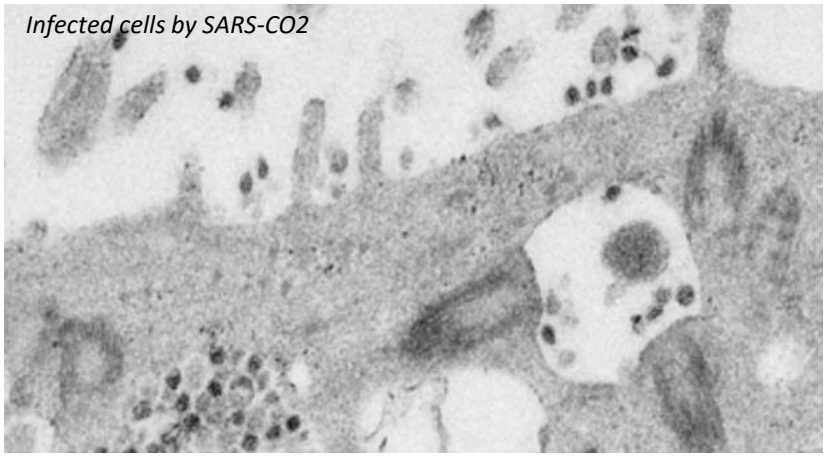


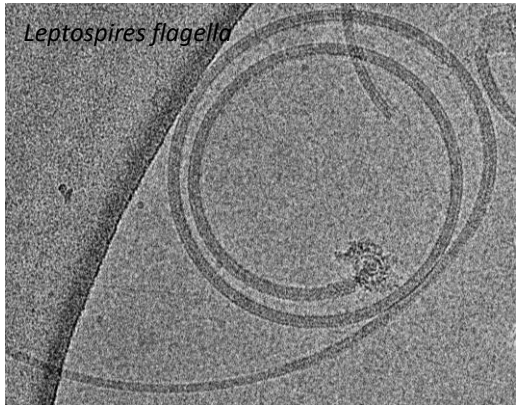


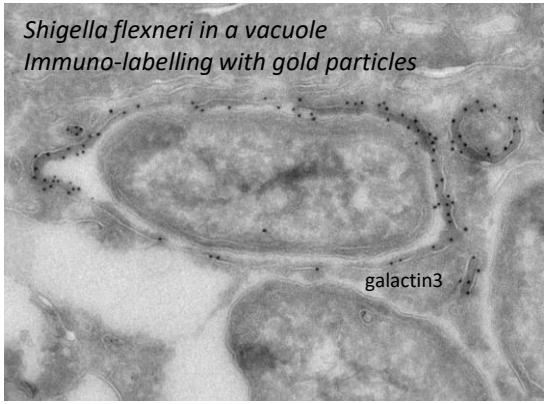


Scanning Electron Microscopy

Correlative Microscopy / Cryo / 3D methods







Transmission Electron Microscopy

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Our activities : The major research challenges

- ✓ Introduction : CLEM & 3D EM
- ✓ 3D Electron microscopy
  - ssTEM
  - CLEM FIB-SEM
  - Project highlighted : Sars-CoV-2
- ✓ Structural Biology Application
- ✓ Conclusion & Perspectives

Perrine Bomme

Esthel Penard

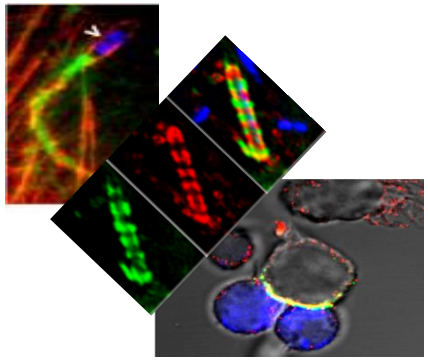
Institut Pasteur

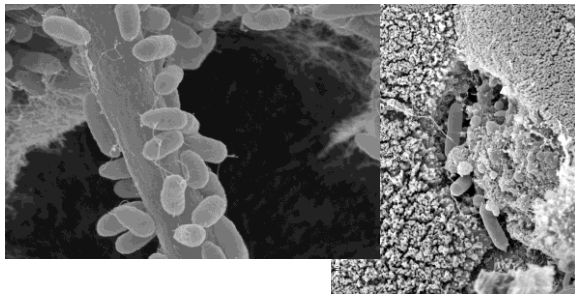
Correlative Light & Electron Microscopy

Confocal Microscope

Scanning Electron Microscope

CLEM





Fonctional and dynamic analysis

Structural information & high resolution

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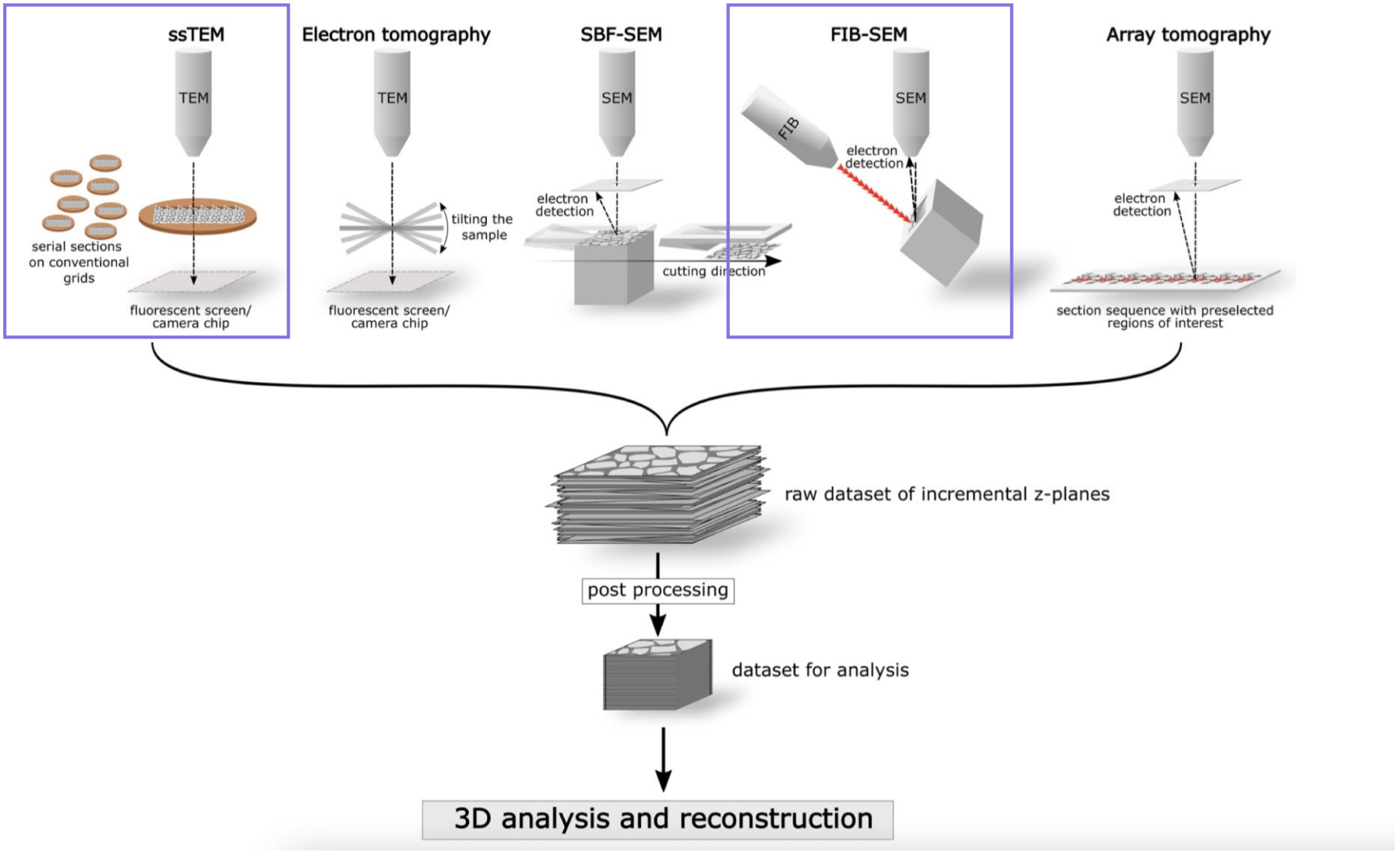
3D Electron Microscopy

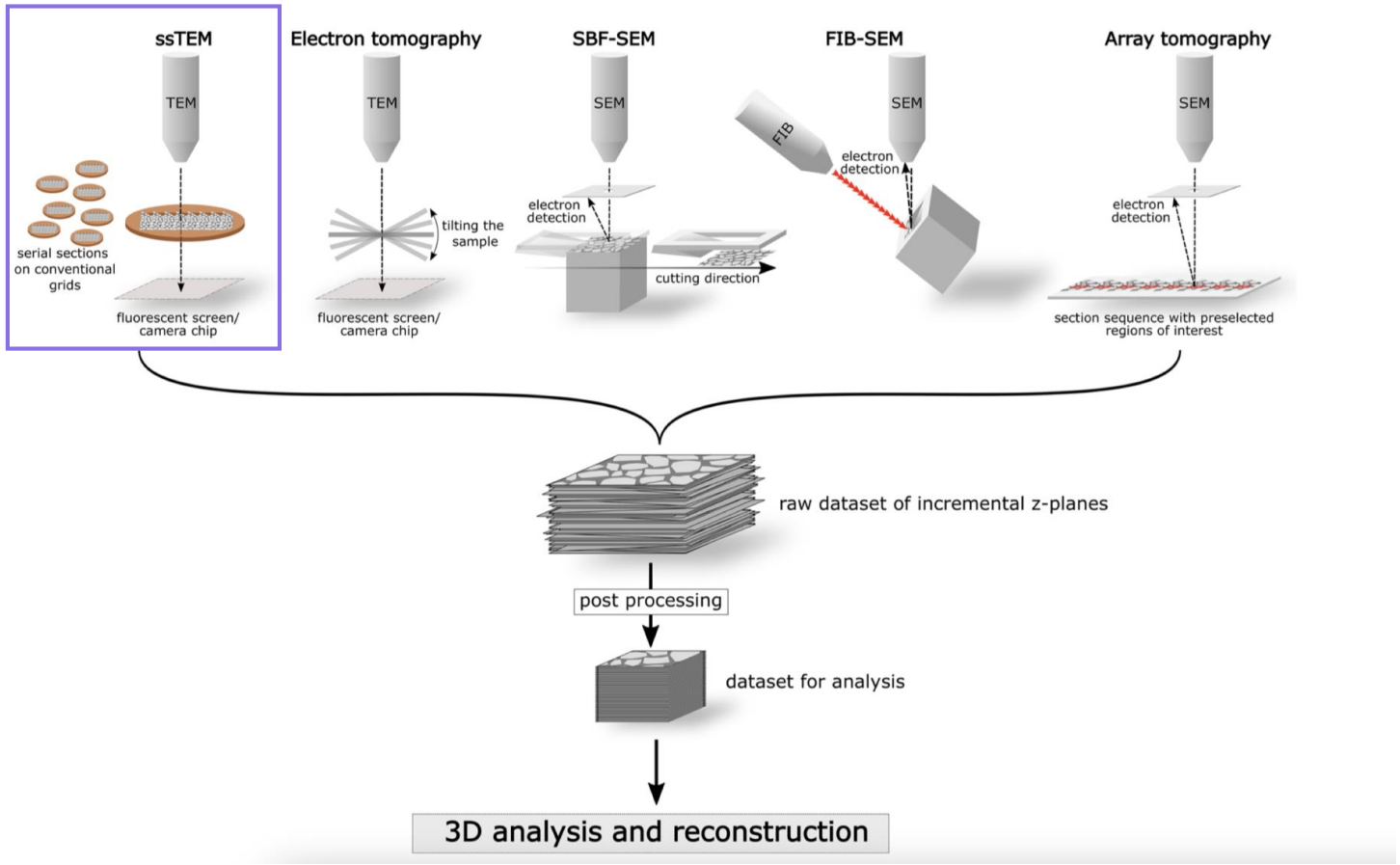


Schneider, J. P., Hegermann, J. & Wrede, C. Volume electron microscopy: analyzing the lung. *Histochem. Cell Biol.* **155**, 241–260 (2021)

3D CLEM with intracellular informations

Light microscopy

Electron microscopy

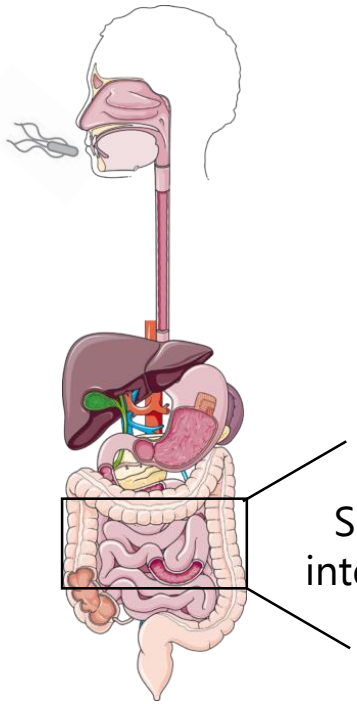


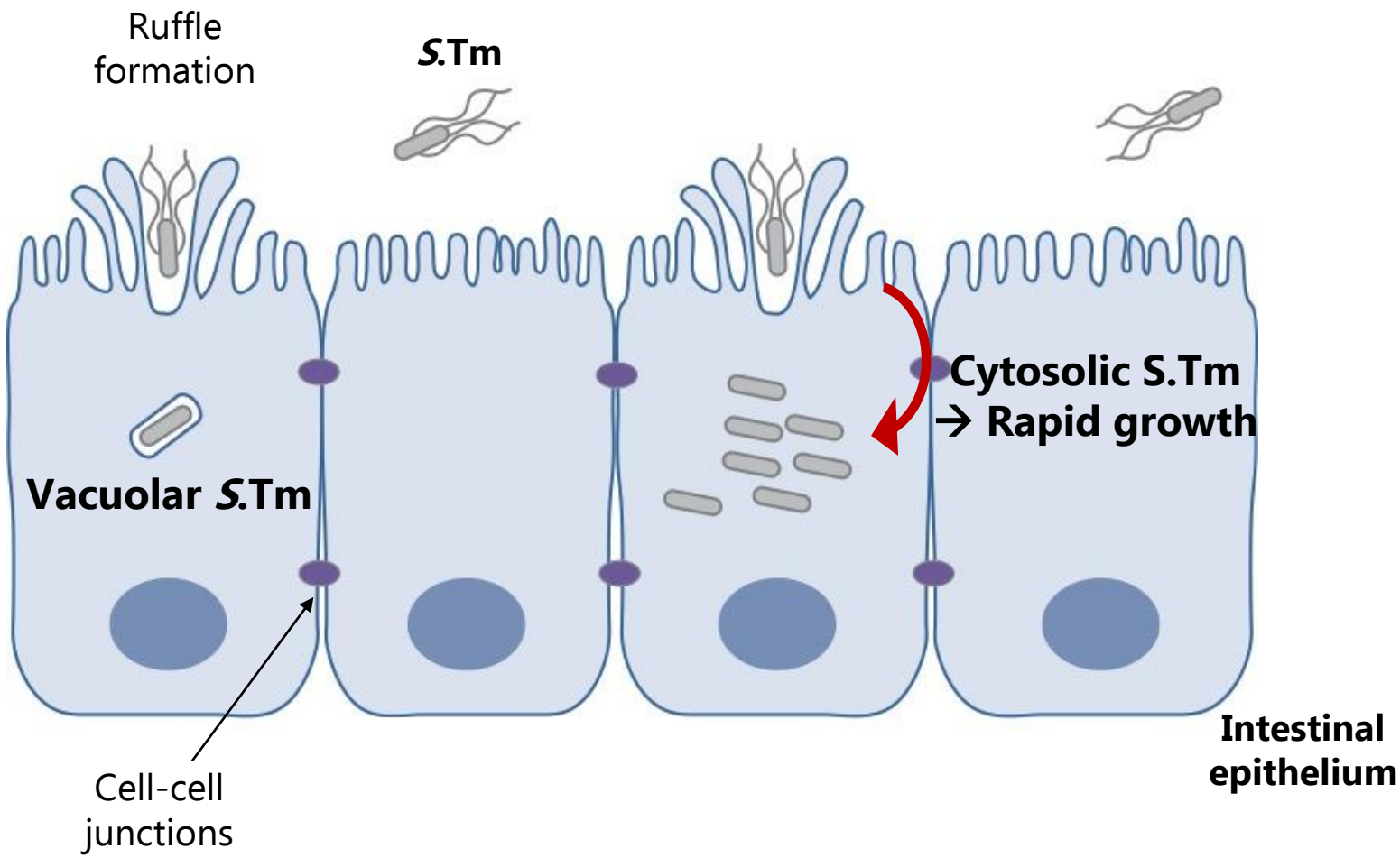
Schneider, J. P., Hegermann, J. & Wrede, C. Volume electron microscopy: analyzing the lung. *Histochem. Cell Biol.* **155**, 241–260 (2021)

Salmonella infections

Perrine Bomme

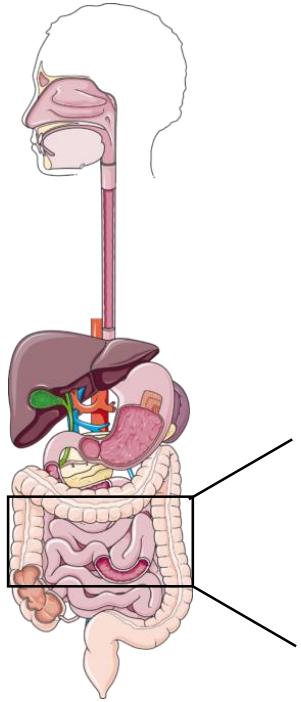
Collaboration: Jost Enninga, Léa Swistak

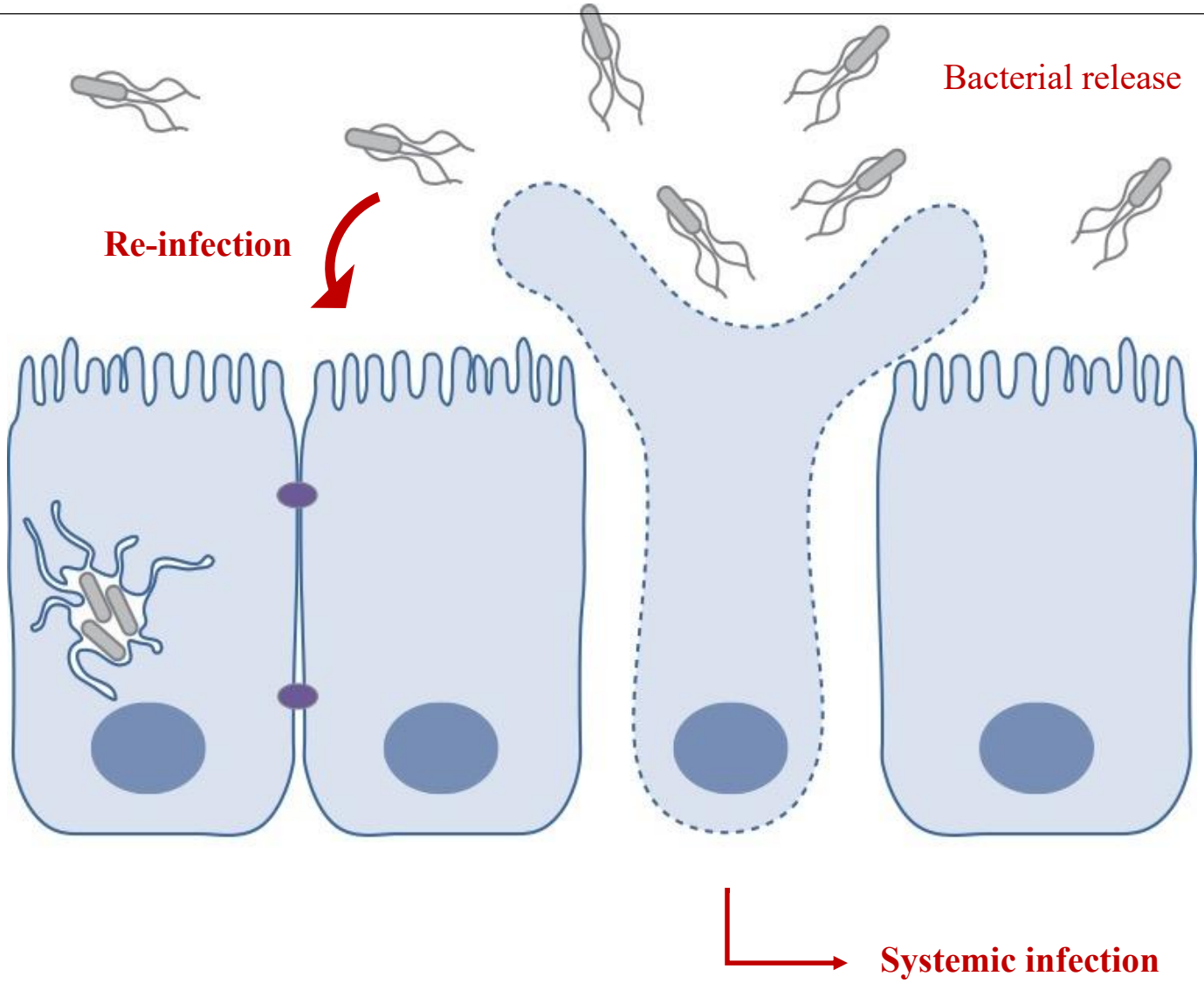




Adapted from Hume 2017, LaRock 2015 by **Lea Swistak**

Salmonella infections





Adapted from Hume 2017, LaRock 2015 by **Lea Swistak**

Perrine Bomme

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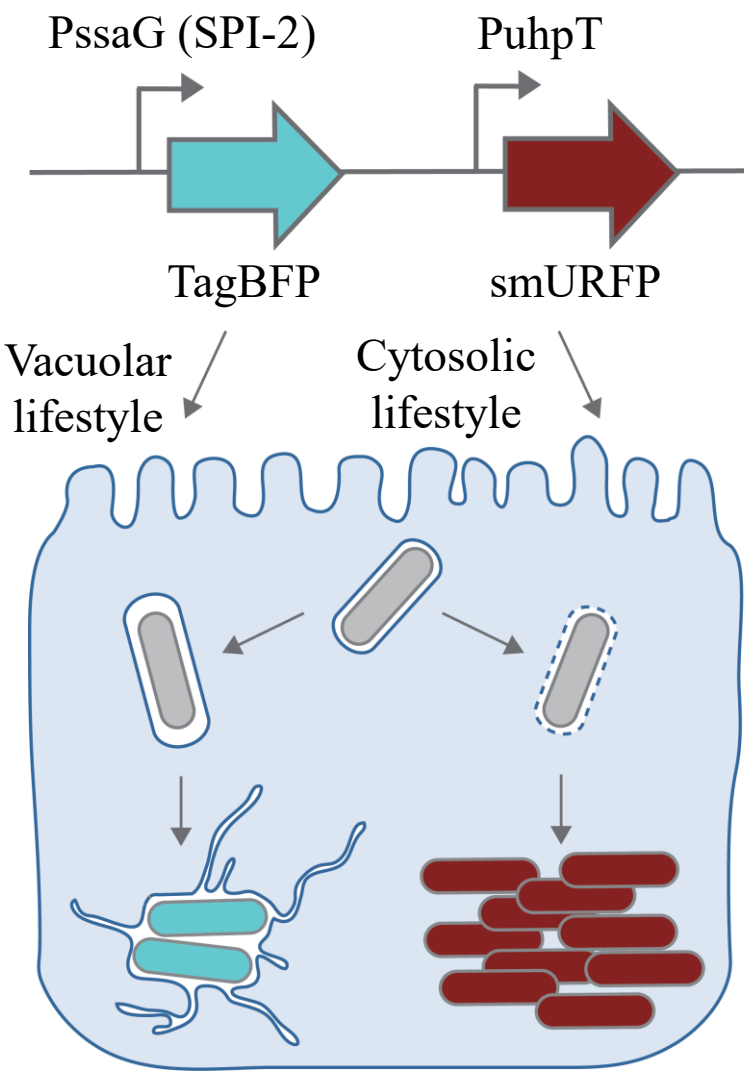
Multiplex fluorescent reporter of S.Tm intracellular niches

Intracellular localization



Adapted from Hume 2017, LaRock 2015 by **Lea Swistak**

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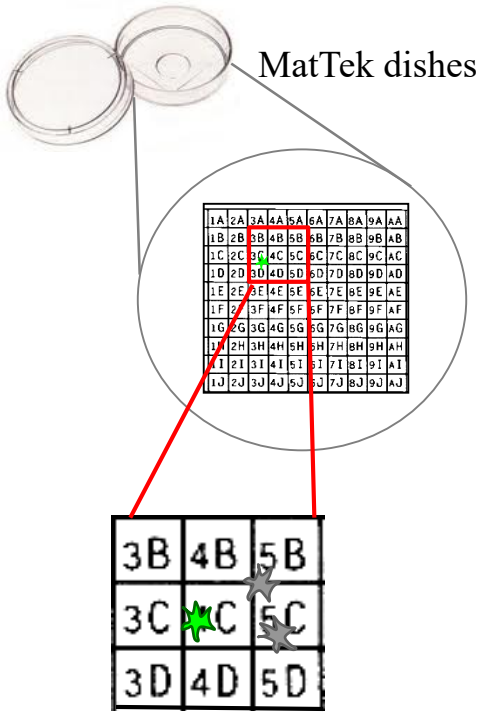
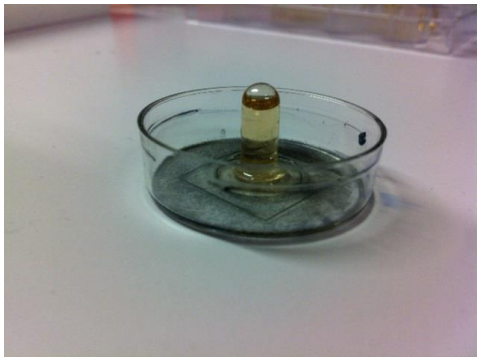
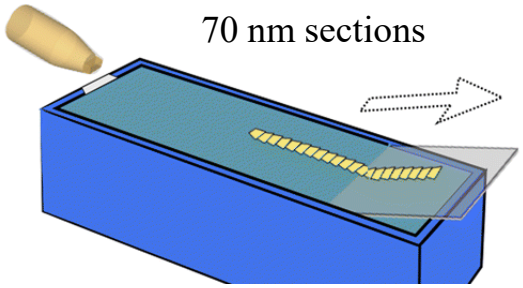
ssTEM : from preparation to observation

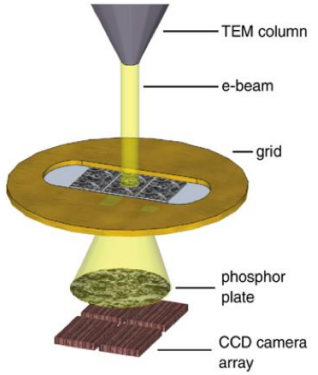
Cell culture + FLM → EM sample preparation → Ultramicrotomy → TEM



Osmium post-fixation
Dehydration
Epoxy-Resin Embedding
Polymerization
Sample mounting
Gold/palladium coating







Adapted from Briggman KL et al

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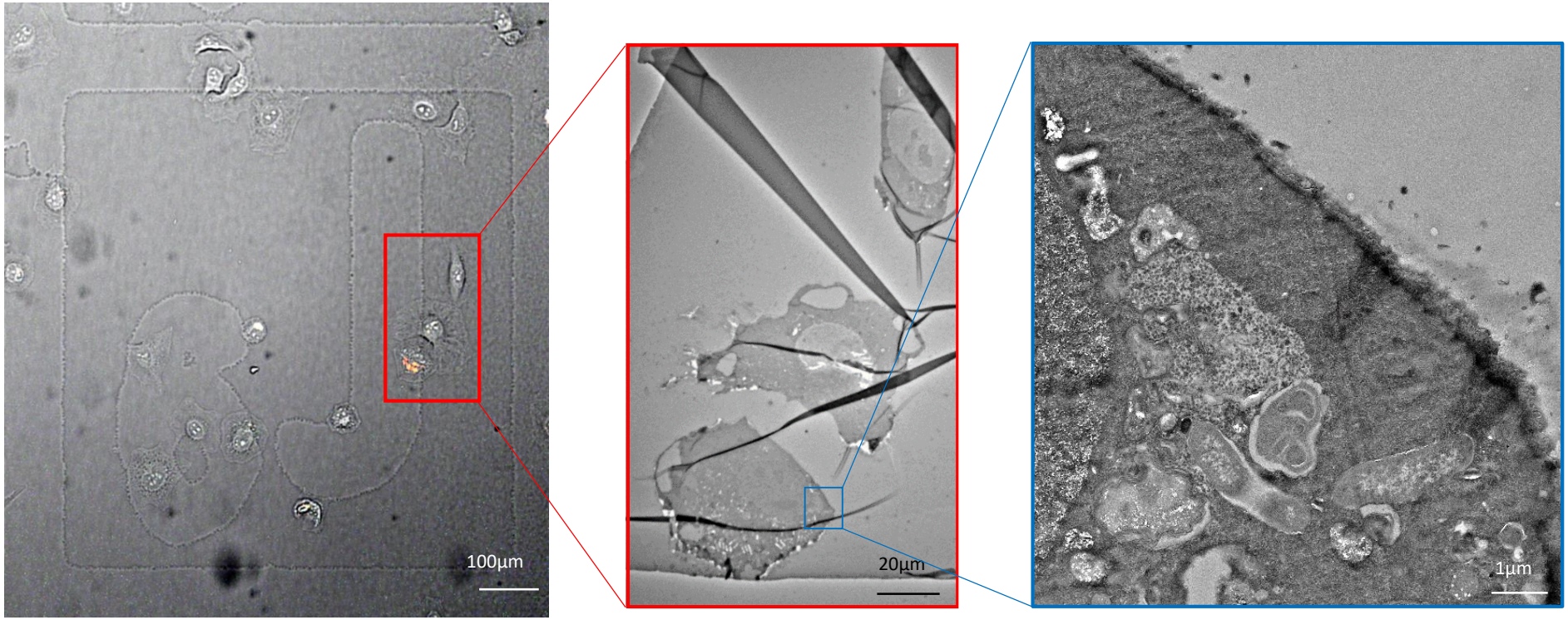
Find the region of interest (ROI)

Perrine Bomme

FLM-MatTeck

TEM-section 70nm



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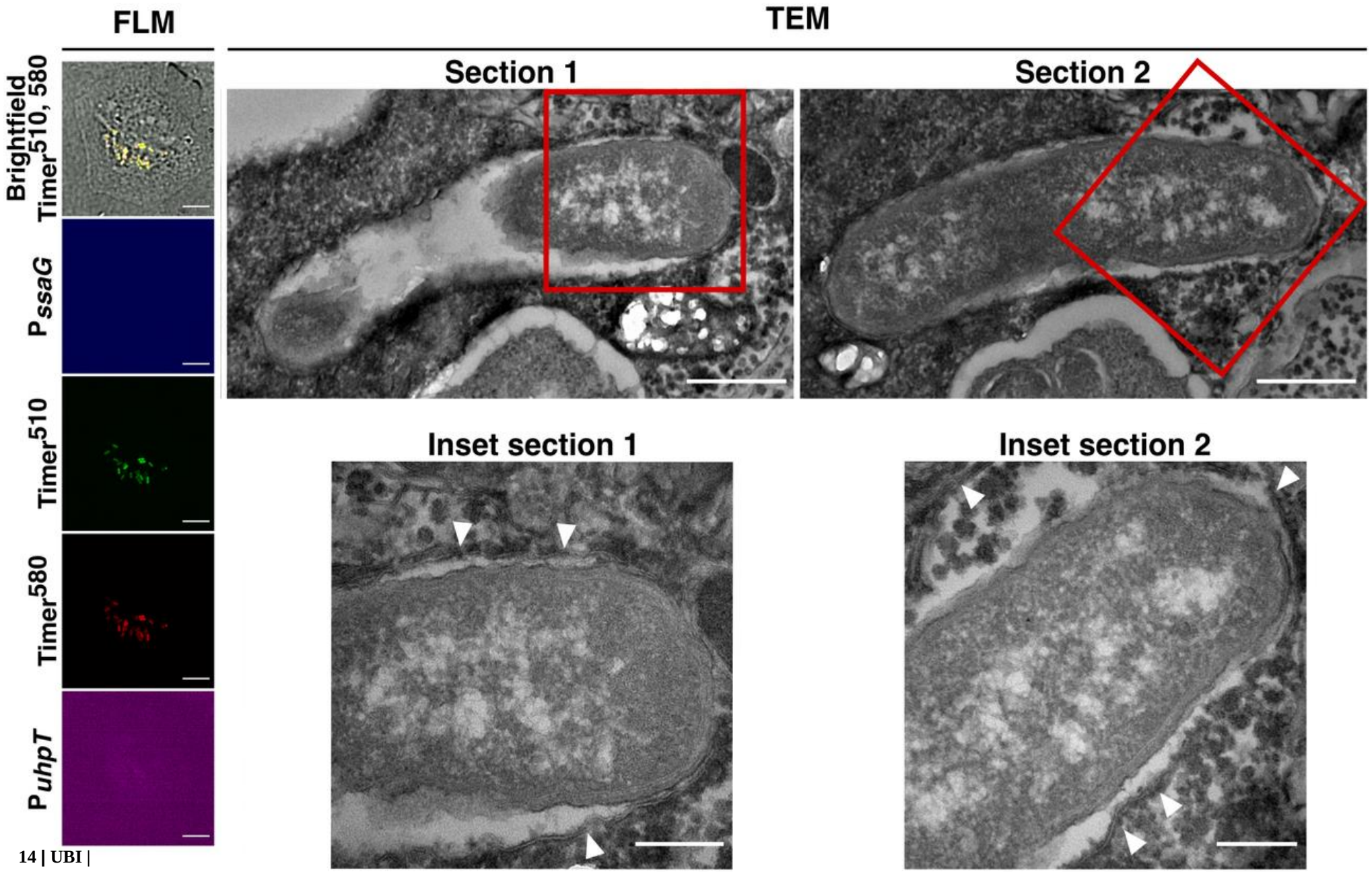
ssTEM : Results

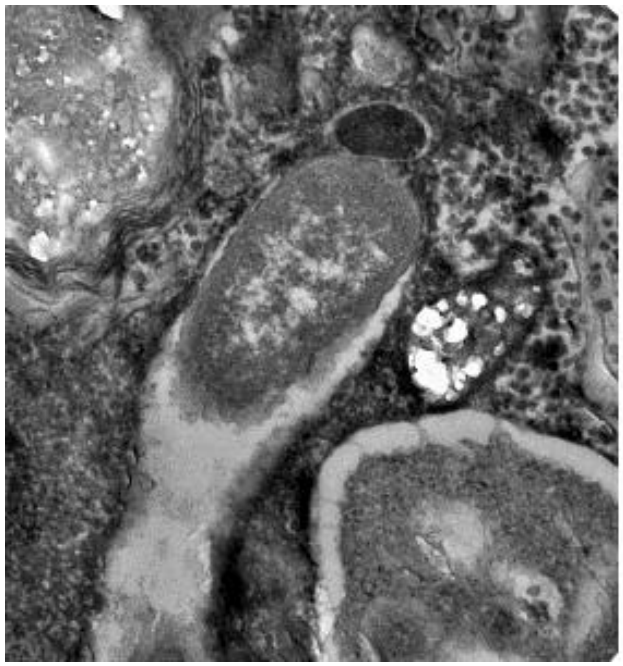
Perrine Bomme



ssTEM : Results


Perrine Bomme

Section#1



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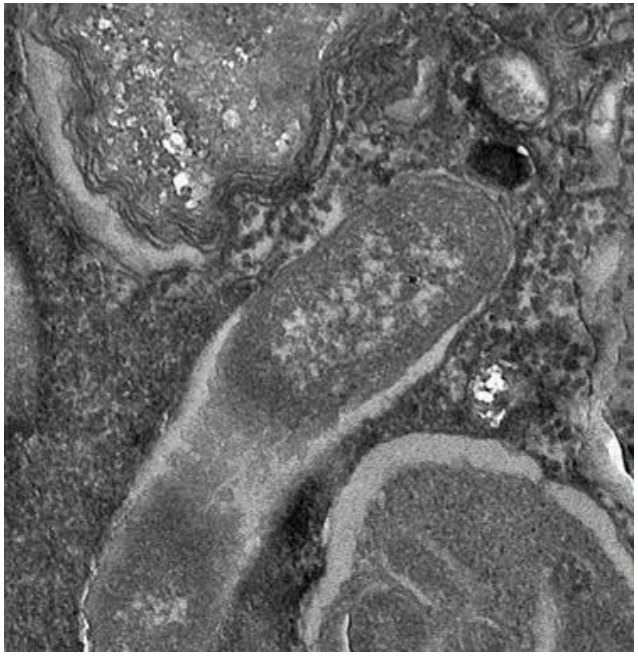
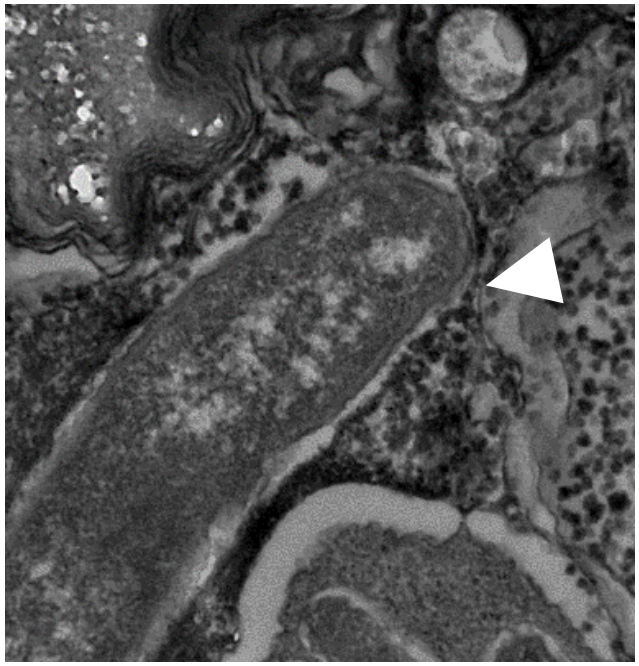
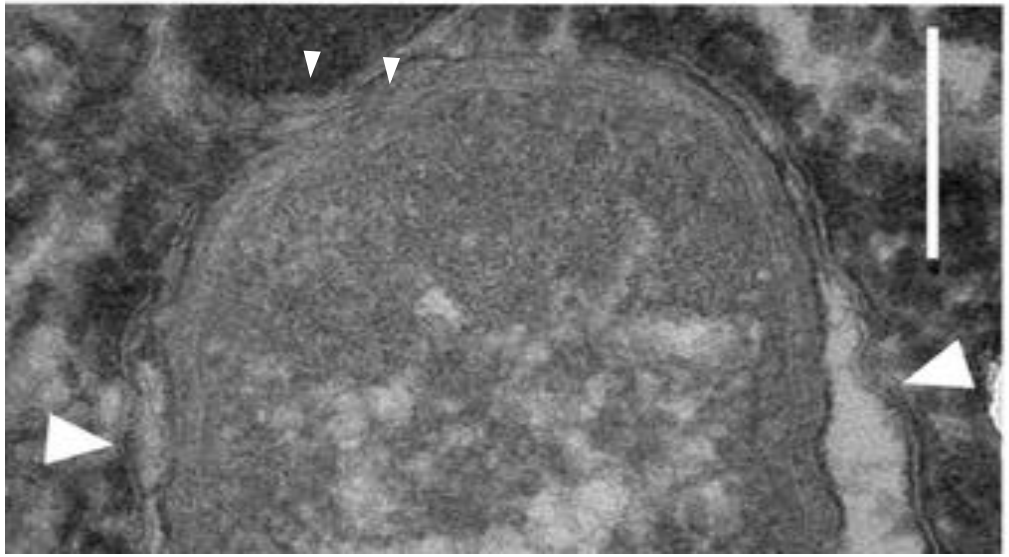


Section#3





3 sections → 70 nm
= 210nm

→ Intracellular informations
ssTEM : Not in a large volume

Collaboration with Lea Swistak, Jost *Enninga, Jakson Luk (Cricks Institute) Jackson et al. Plos Path (2021)*

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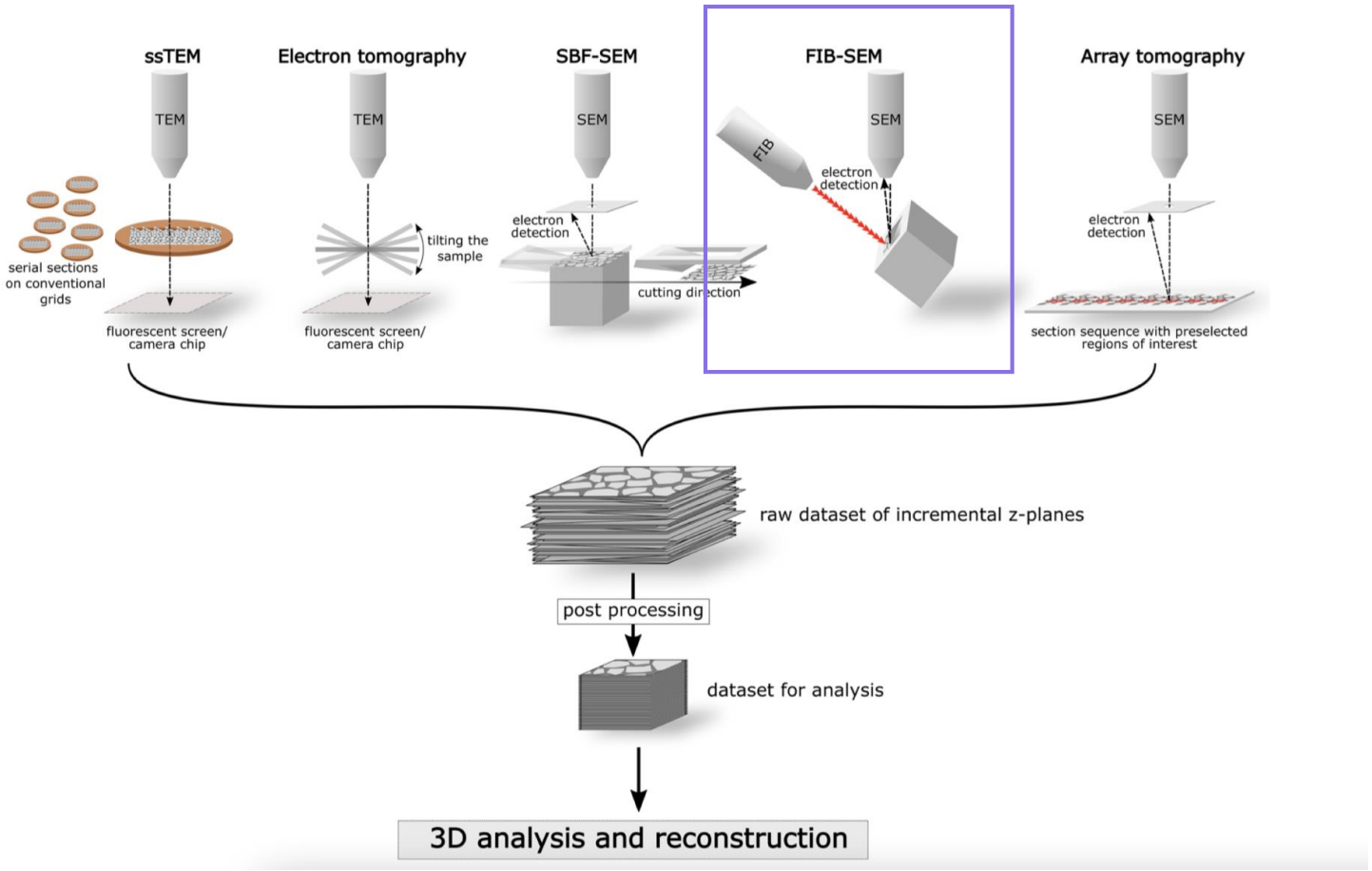
3D CLEM with intracellular informations

Light microscopy

Electron microscopy



Schneider, J. P., Hegermann, J. & Wrede, C. Volume electron microscopy: analyzing the lung. *Histochem. Cell Biol.* **155**, 241–260 (2021)

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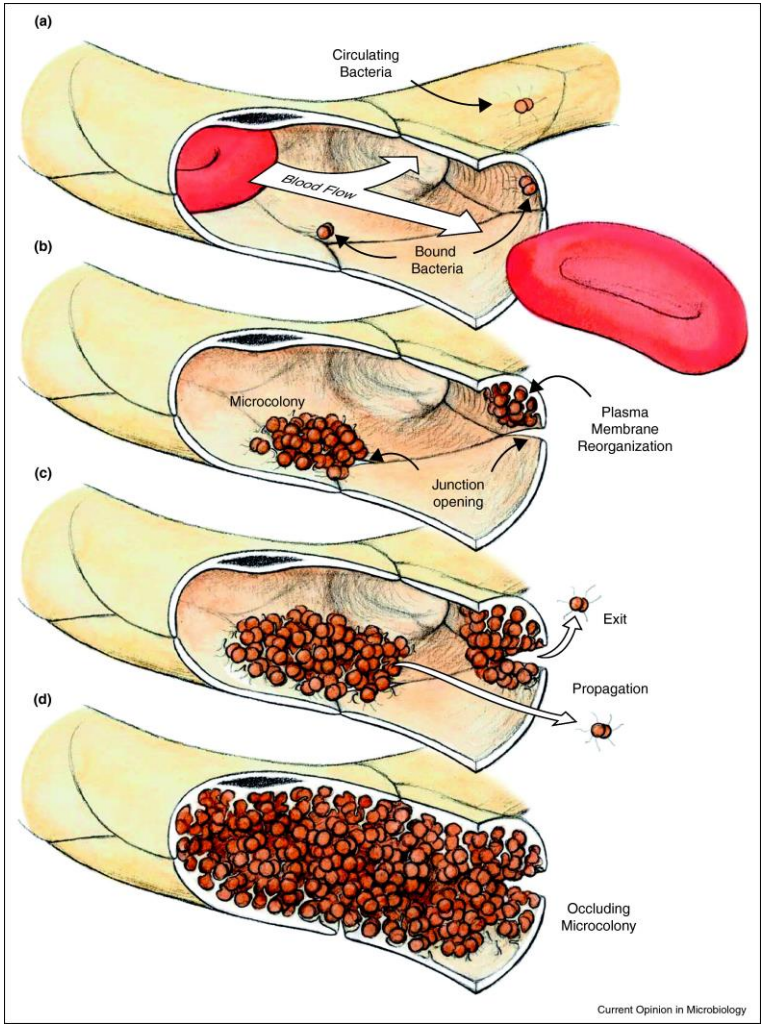
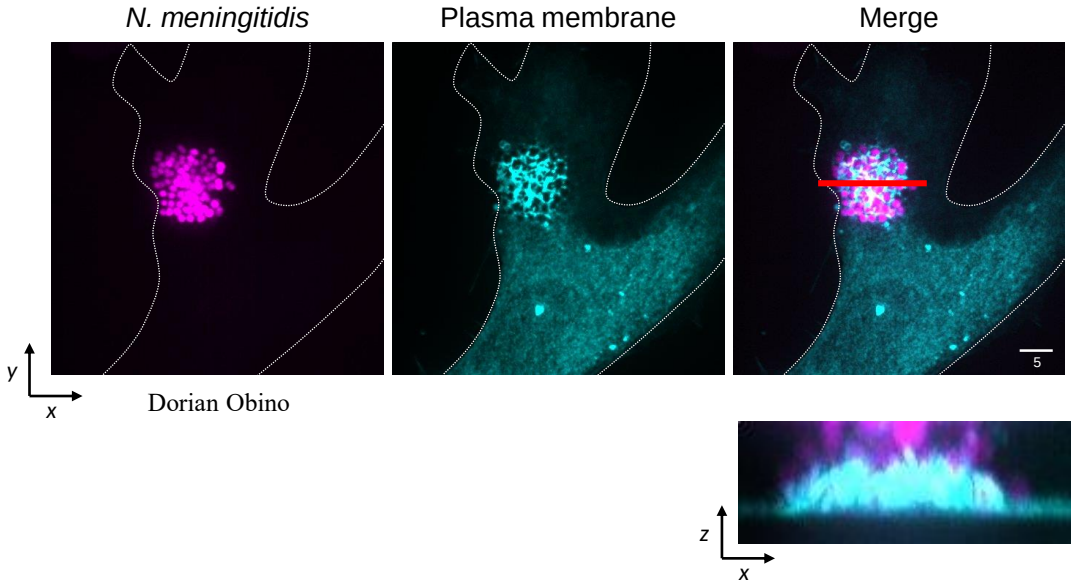
Vascular colonization by *Neisseria meningitidis*

Collaboration: Guillaume Dumenil, Dorian Obino



Melican K. et al., ***2012.***



- Plasma membrane Reorganization
- No ultrastructural information

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Correlative Light & 3D Electron Microscopy

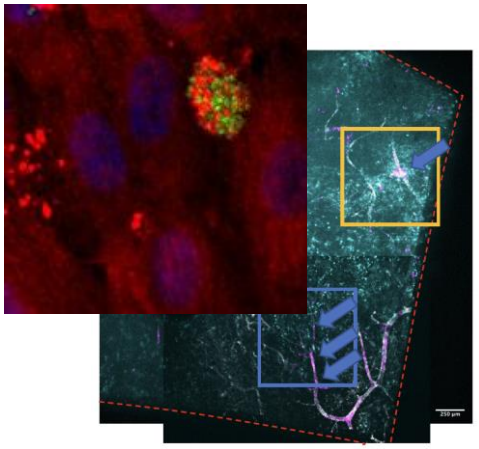
Confocal microscopy

CLEM

FIND A ROI

FIB-SEM

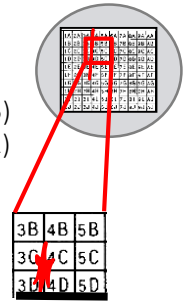
Focused ion Beam–
Scanning Electron Microscopy



Low magnification
Fluoresence

1 Cells in culture

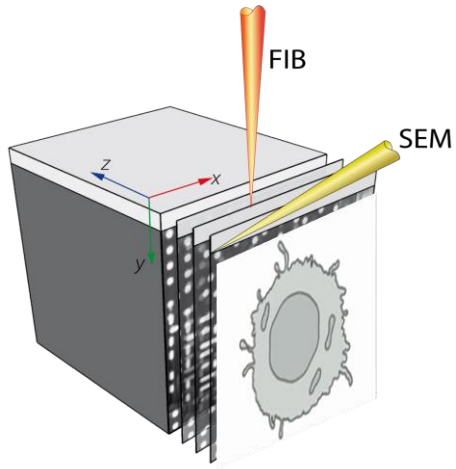
Luk CH *et al* (2021)
Santos JC *et al* (2015)
Mostowy *et al* (2011)



2 Tissue sample

?

Dehydration
Epoxy-Resin Embedding
Polymerization
Sample mounting
Gold/palladium coating



Large Volume
Isotropic resolution

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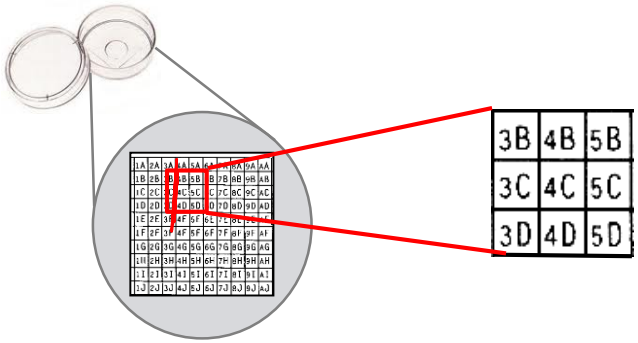
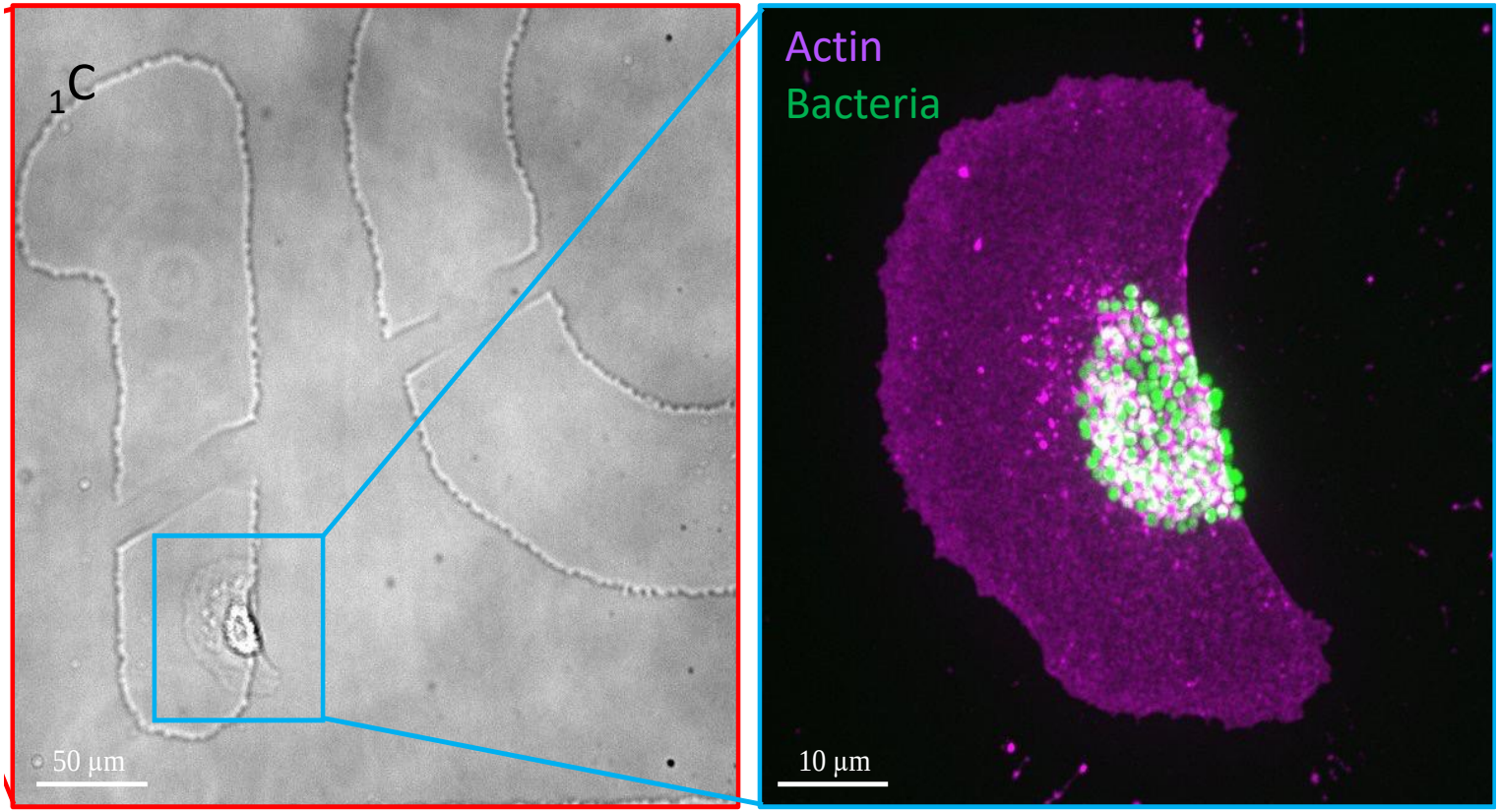
1) Infected cells acquisition in correlative FIB-SEM



Light microscopy

HUVEC infected by *Nm*, fixed 2hpi.



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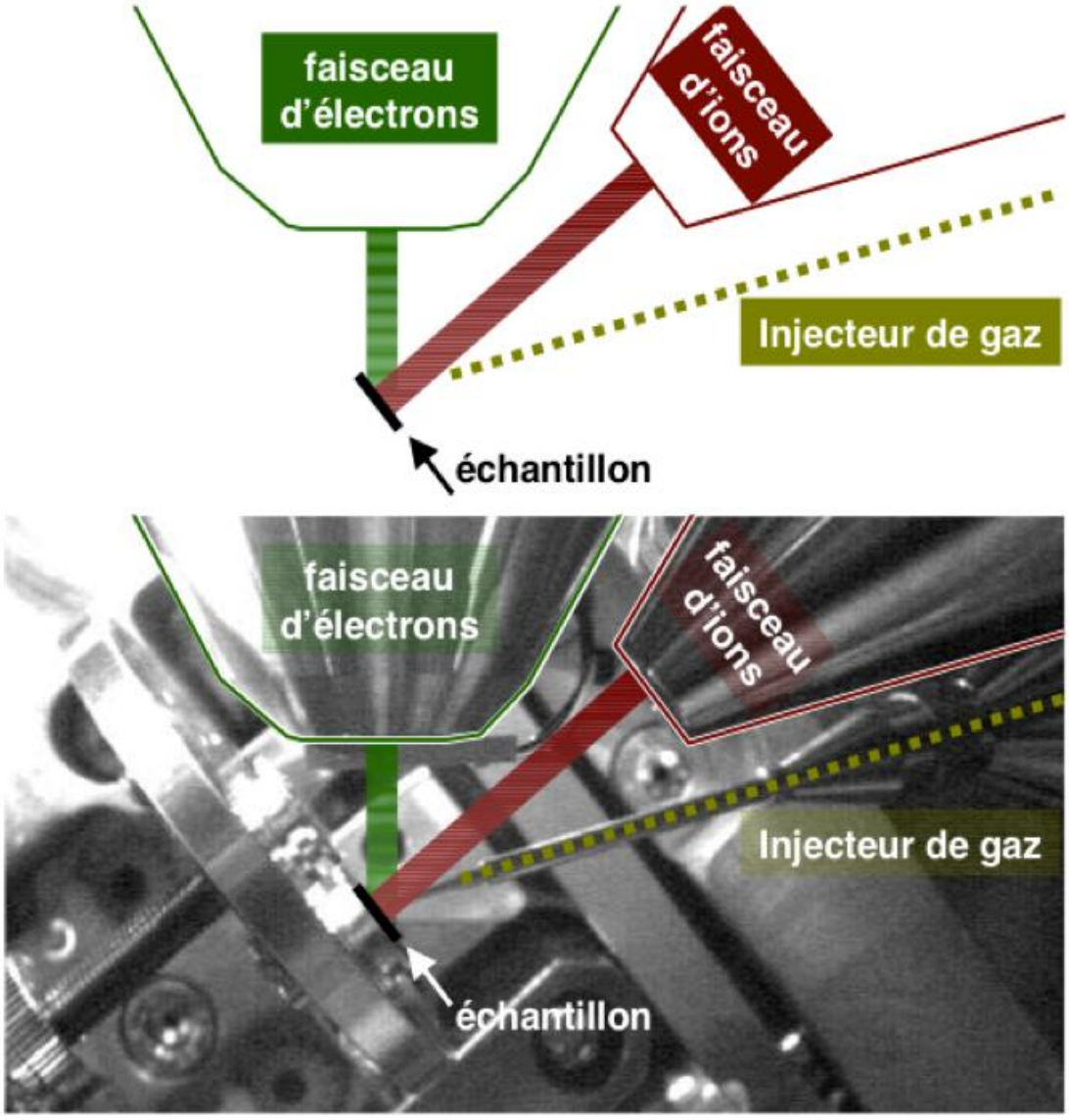
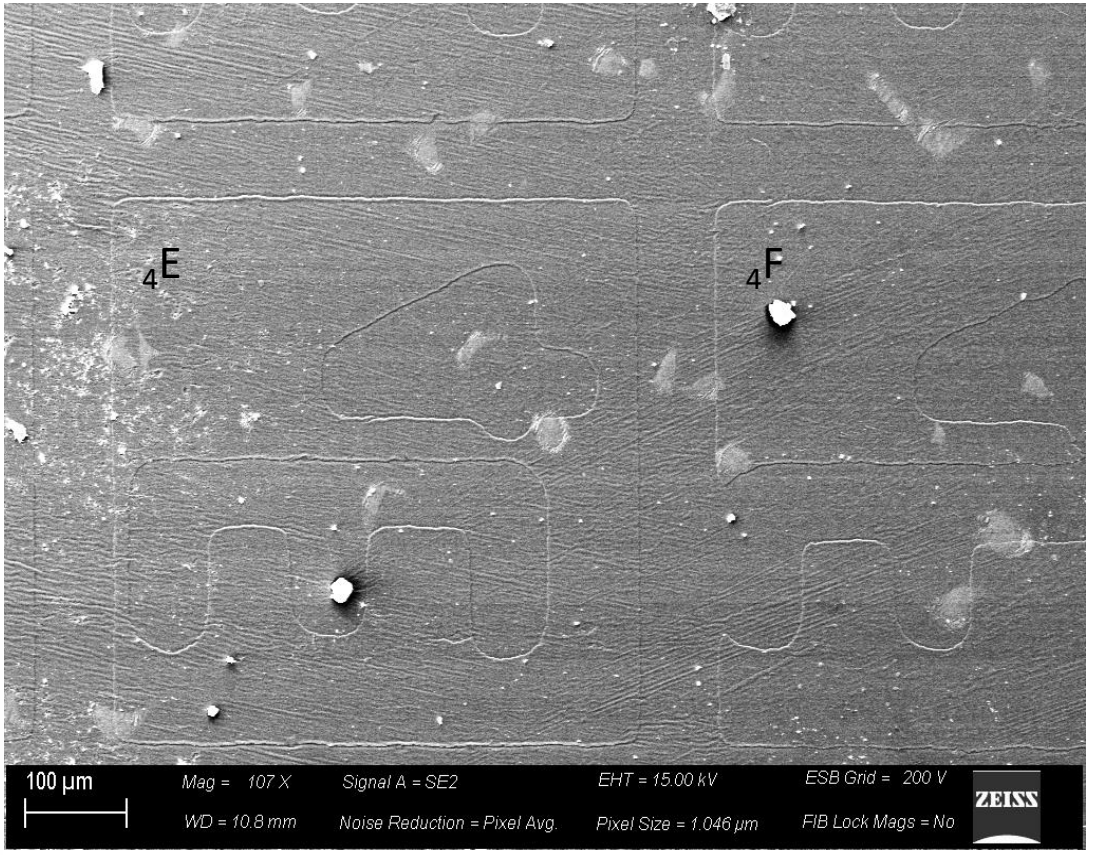
FIB-SEM parameters





$_4C$ $_4D$ $_4E$ $_4F$

$_3C$

$_2C$

$_1C$

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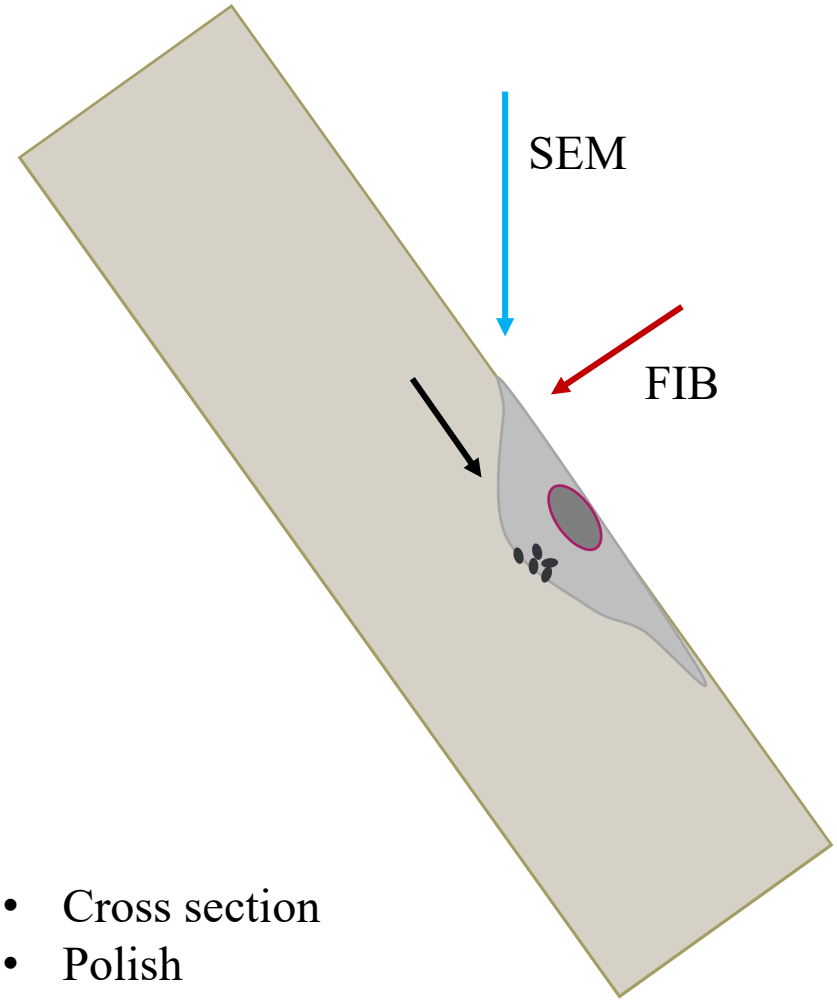
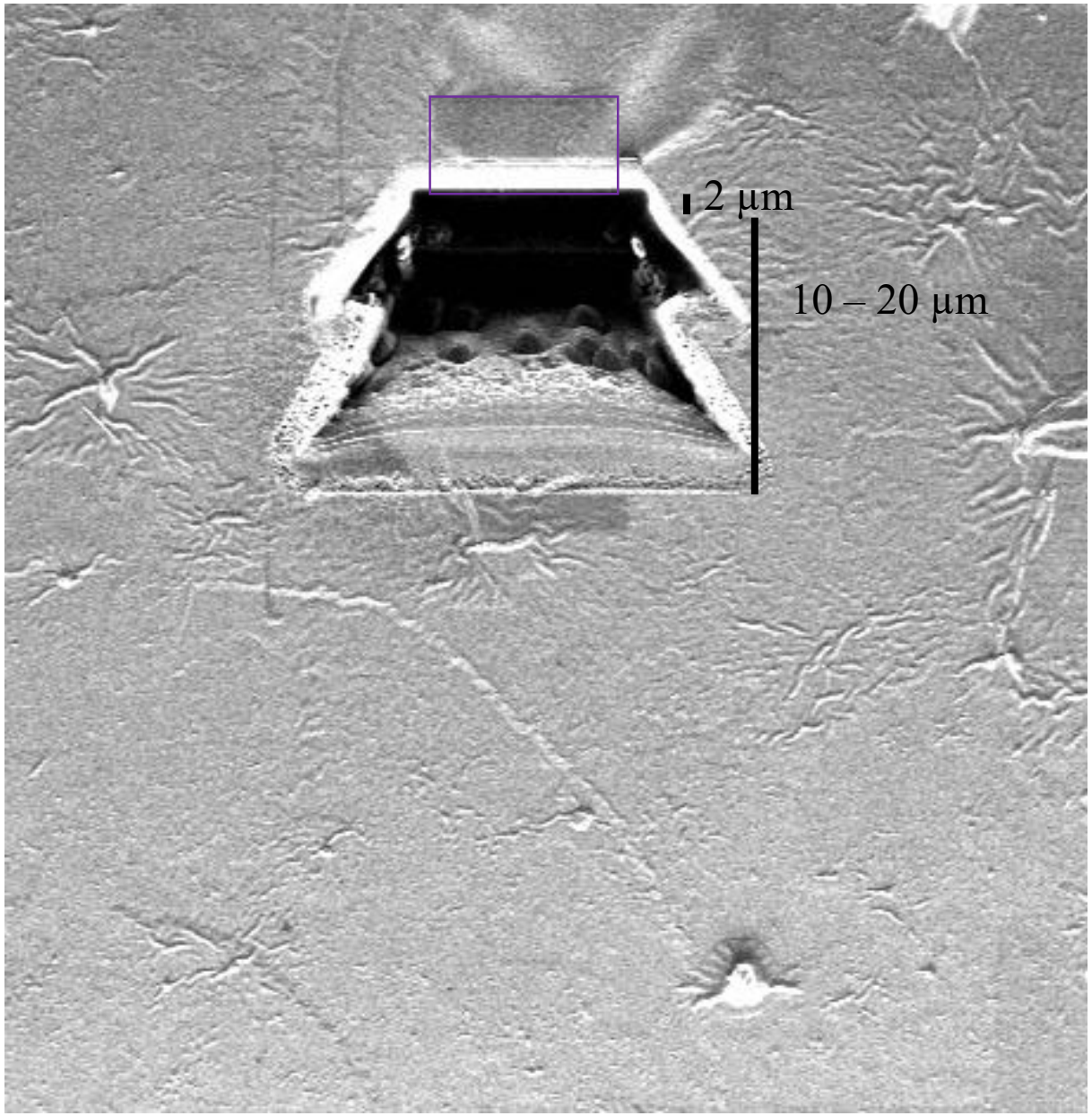
FIB-SEM parameters



- Cross section
- Polish



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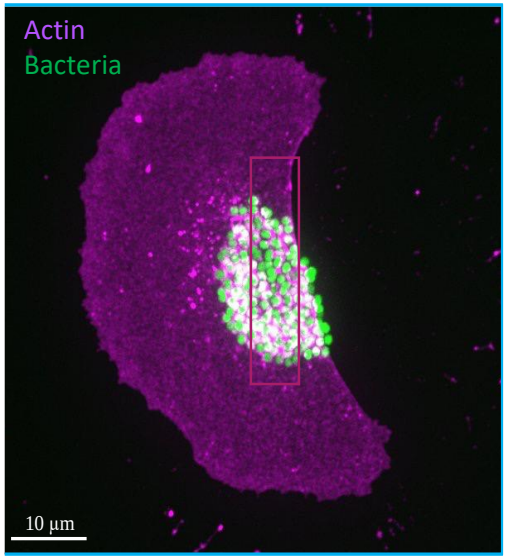
FIB-SEM acquisition

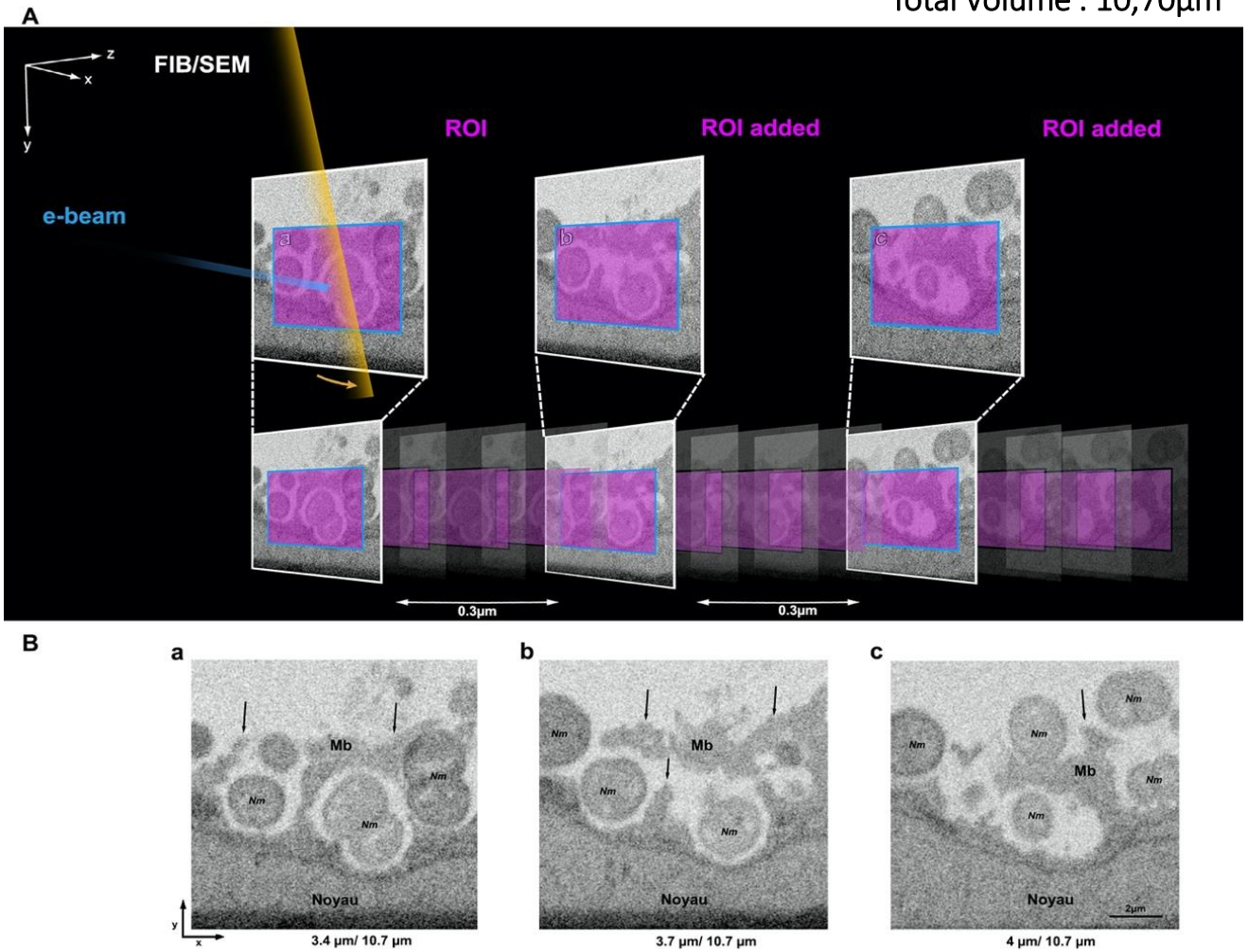
Light microscopy

FIB-SEM

HUVEC infected by *Nm*, fixed 2hpi.



Total Volume : 10,70µm



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Correlative FIB-SEM on infected cells

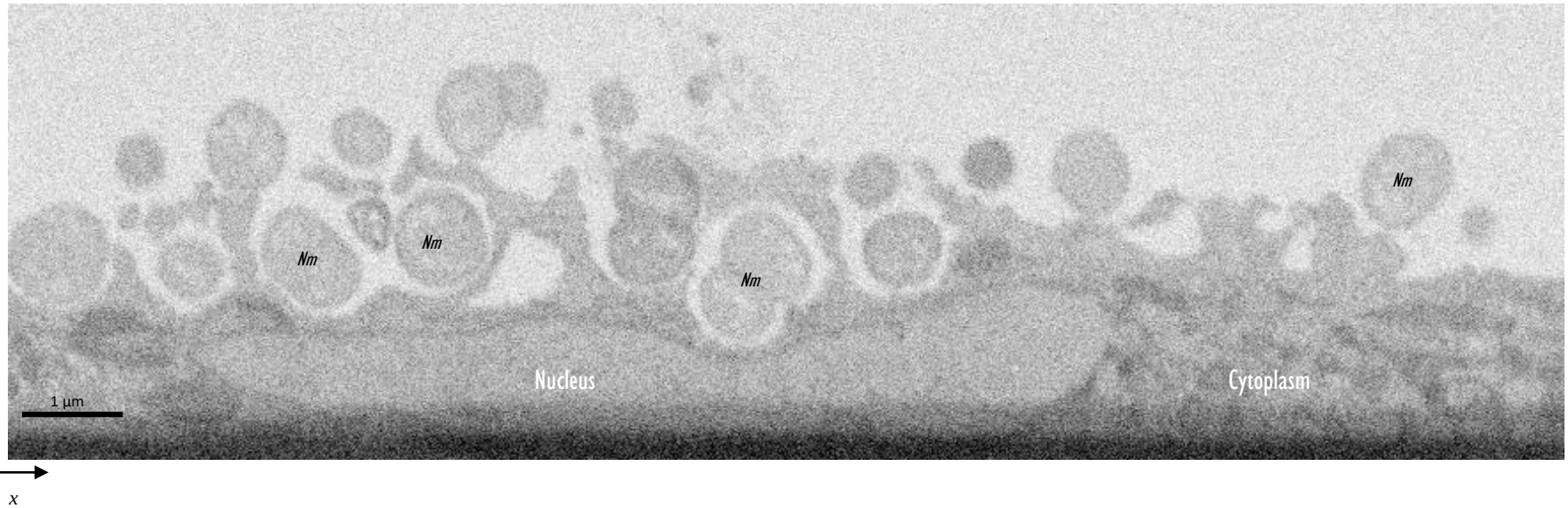
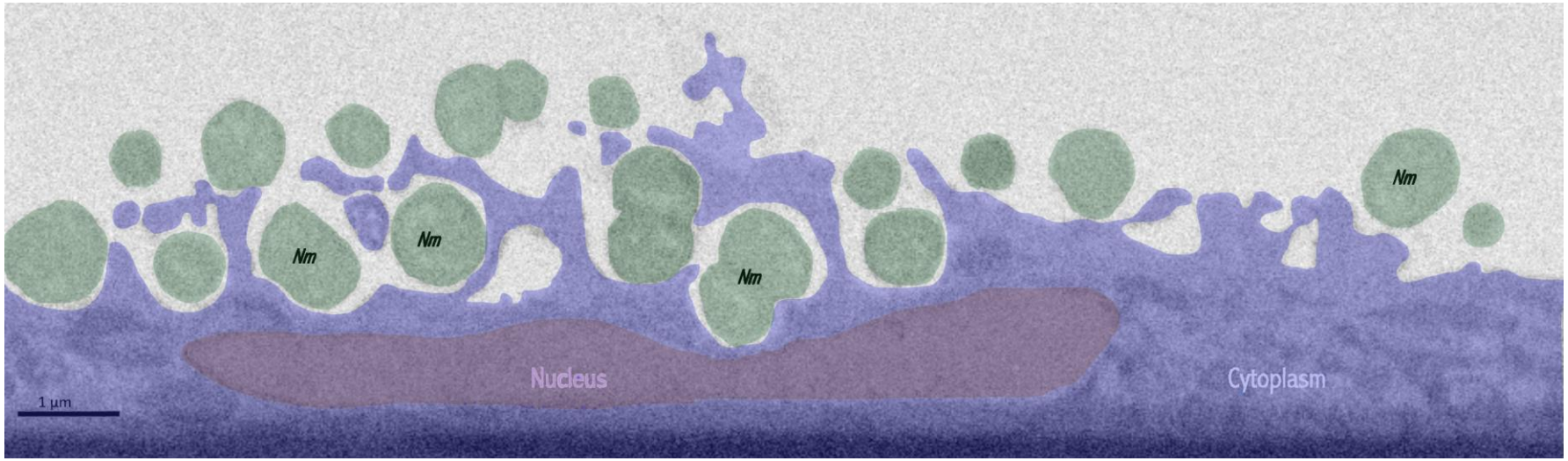


Image extracted from the stack



Bacteria
Plasma Membrane
Nucleus

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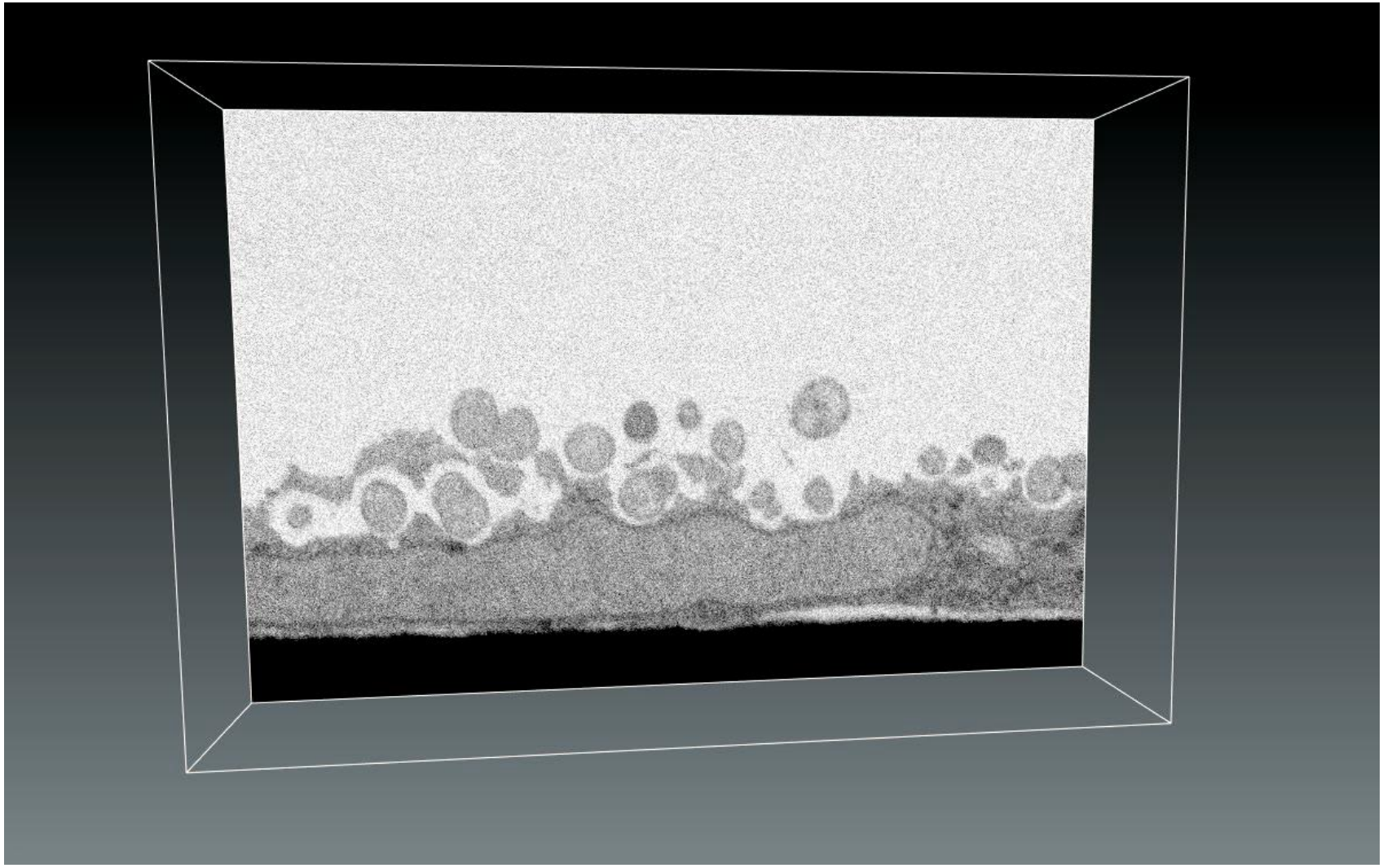
Correlative FIB-SEM on infected cells



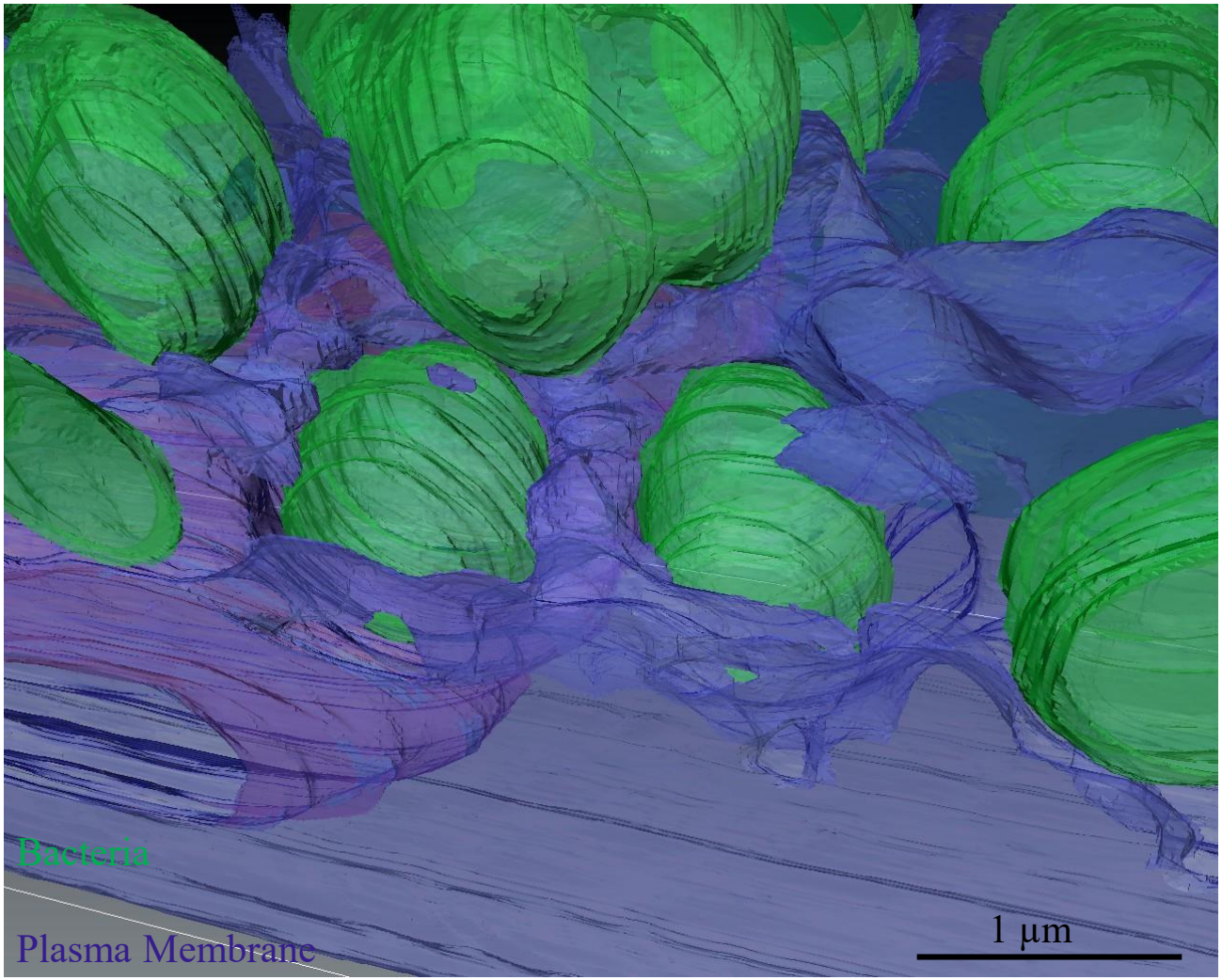
3D Reconstruction

Amira®
3D Visualization & Analysis
Software For Life Sciences

Bacteria
Plasma Membrane
Nucleus

Institut Pasteur

Results : CLEM FIB-SEM on infected cells



⇢ Plasma membrane rearrangement around bacteria : niche

⇢ Bacteria are not intracellular

What is the impact of vascular colonization by *Neisseria meningitidis* on a ***tissue environment ?***

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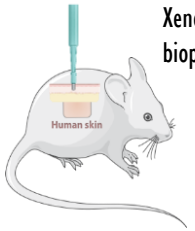
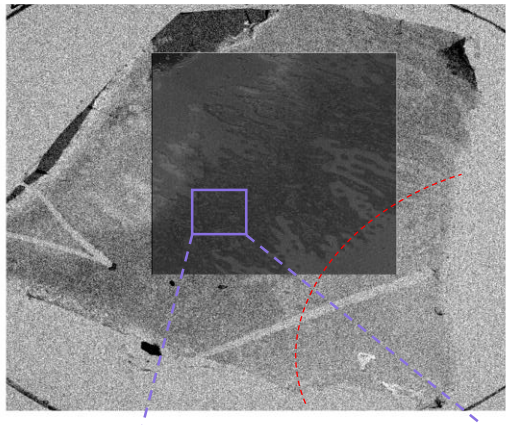
2) Workflow : CLEM FIB-SEM *on tissue samples*

Biopsy Landmarks → **Confocal microscopy** → **Sample preparation & Ultramicrotomy** → **Scanning Electron Microscopy : Map** → **FIB-SEM & 3D analysis**

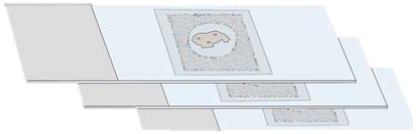


Xenograft biopsy

Infected humanized mouse



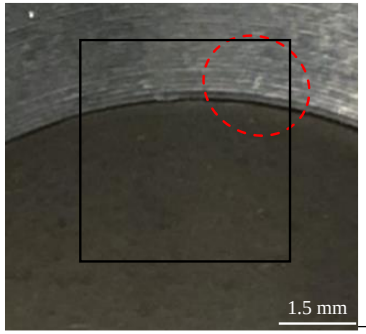
vibratome sections

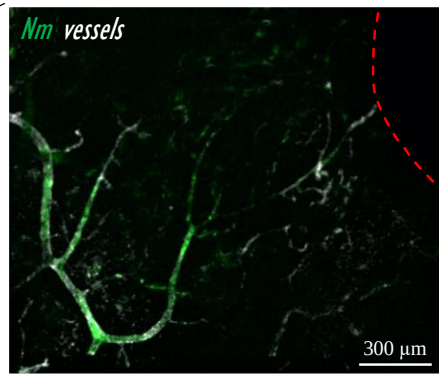


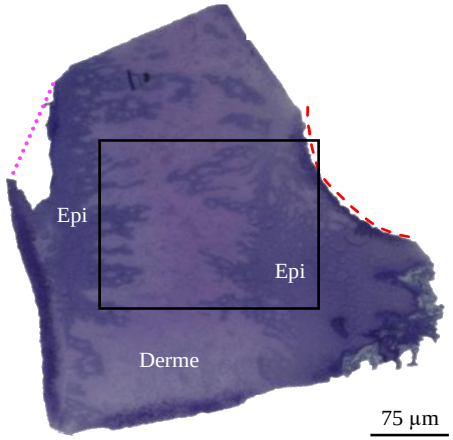
Localisation of region of interest, and check the presence of infected blood vessels.

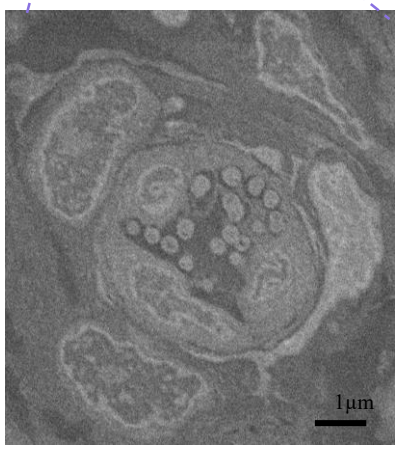
150nm thickness

landmark









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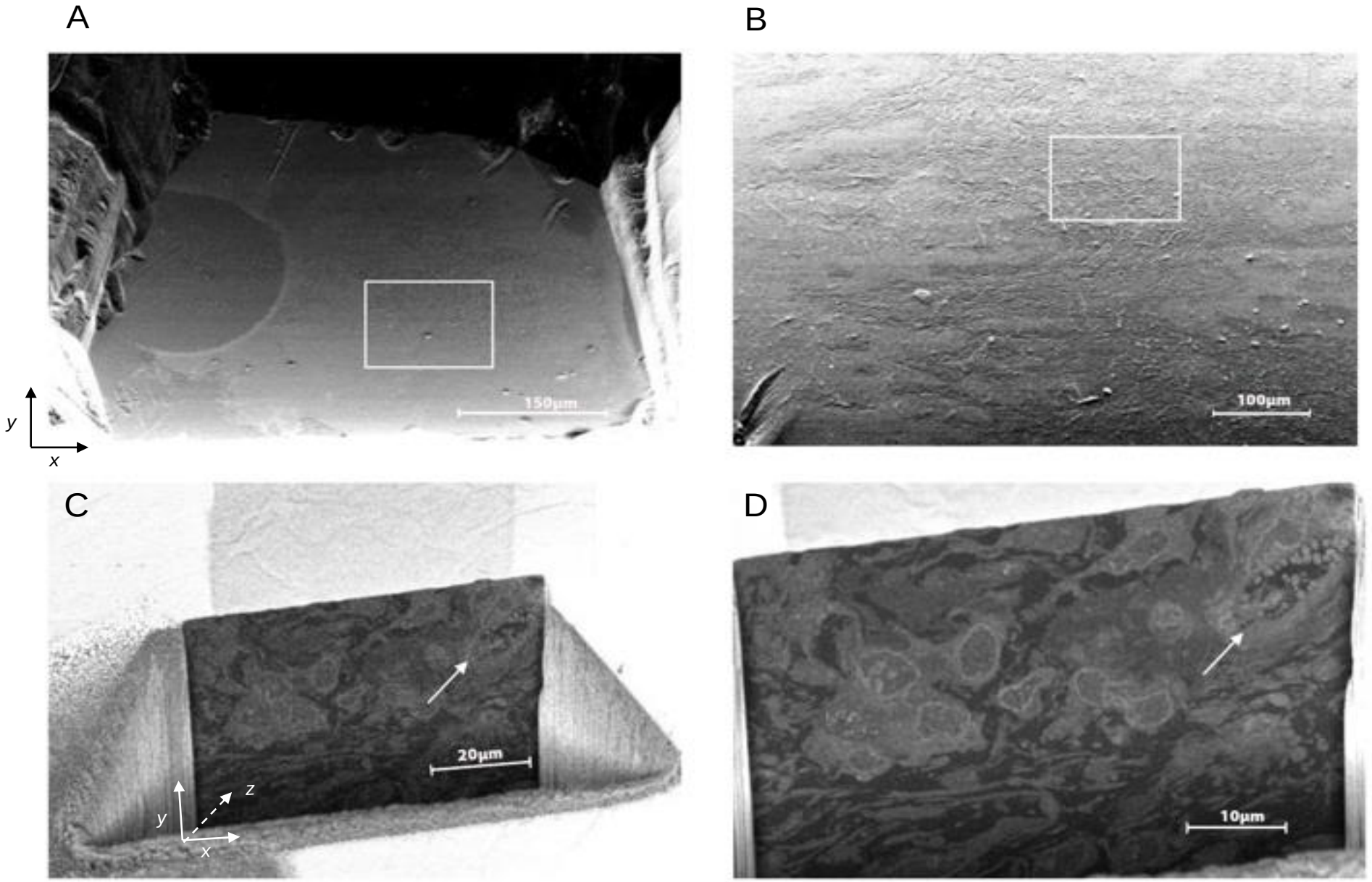
Correlative FIB-SEM on tissue sample



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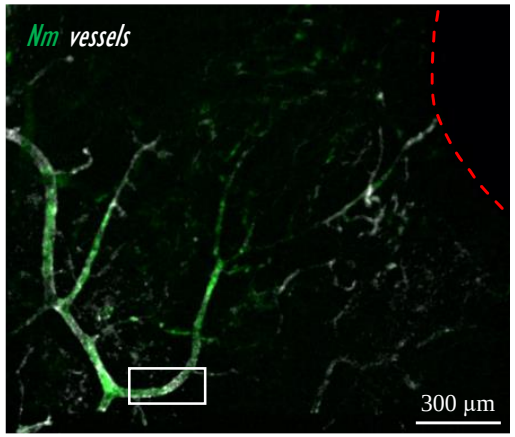
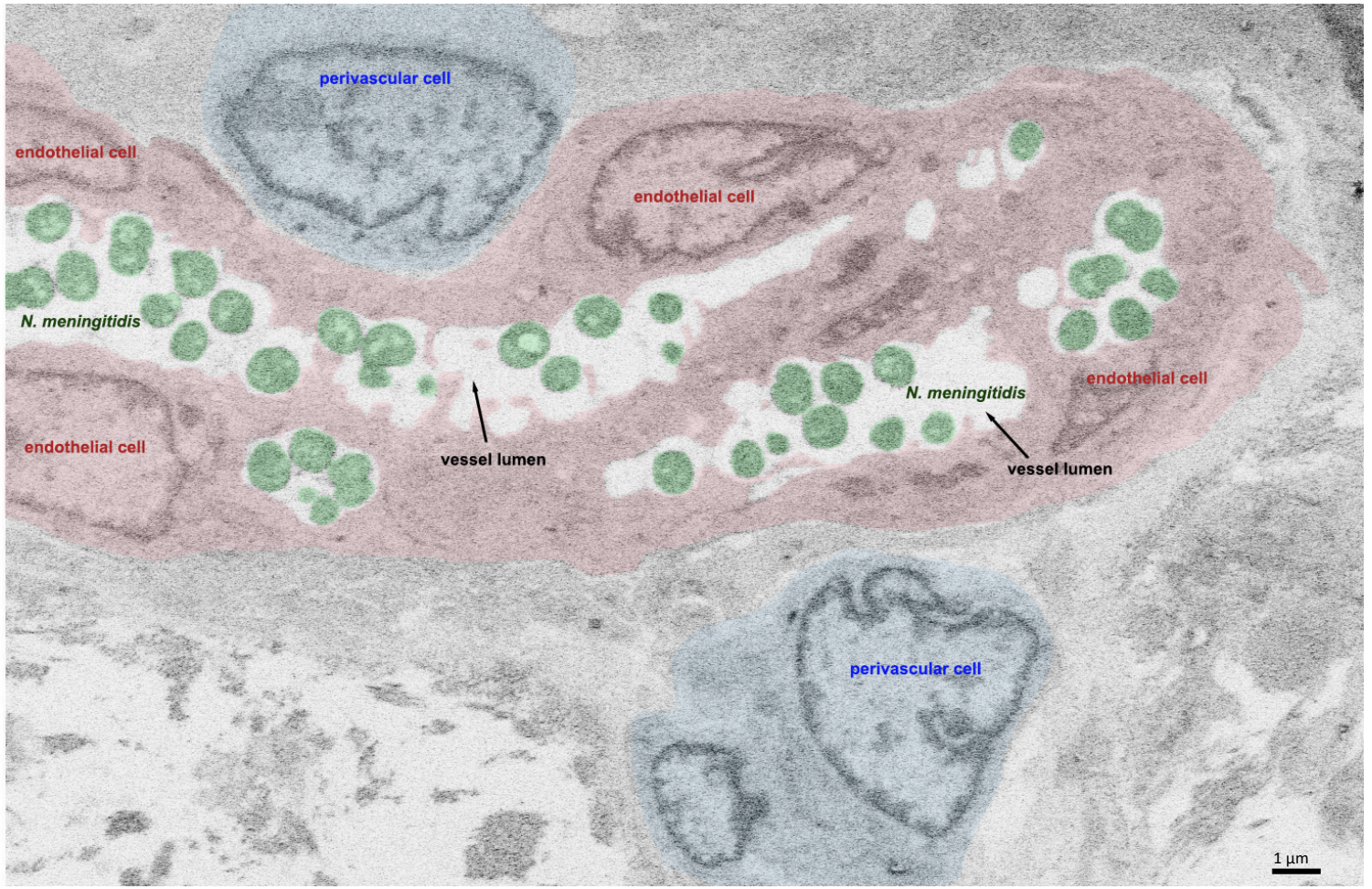
Ultrastructure of infected blood vessel in CLEM FIB-SEM



Total volume = 5 µm

Experiment from mice model infected by *Nm*, fixed 3hpi.



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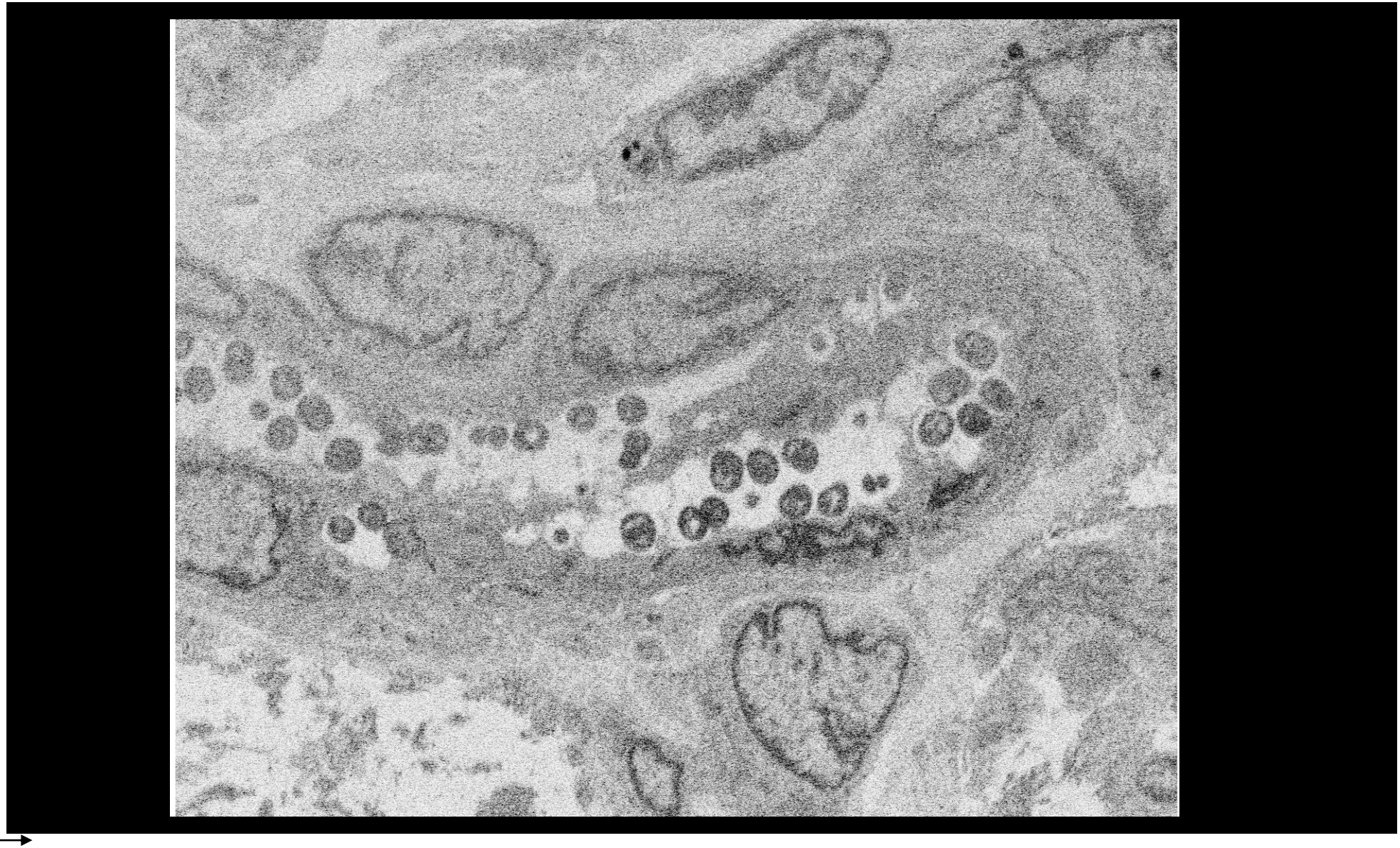
Ultrastructure of infected blood vessel in CLEM FIB-SEM

⇢ First 3D acquisition of the vascular colonization



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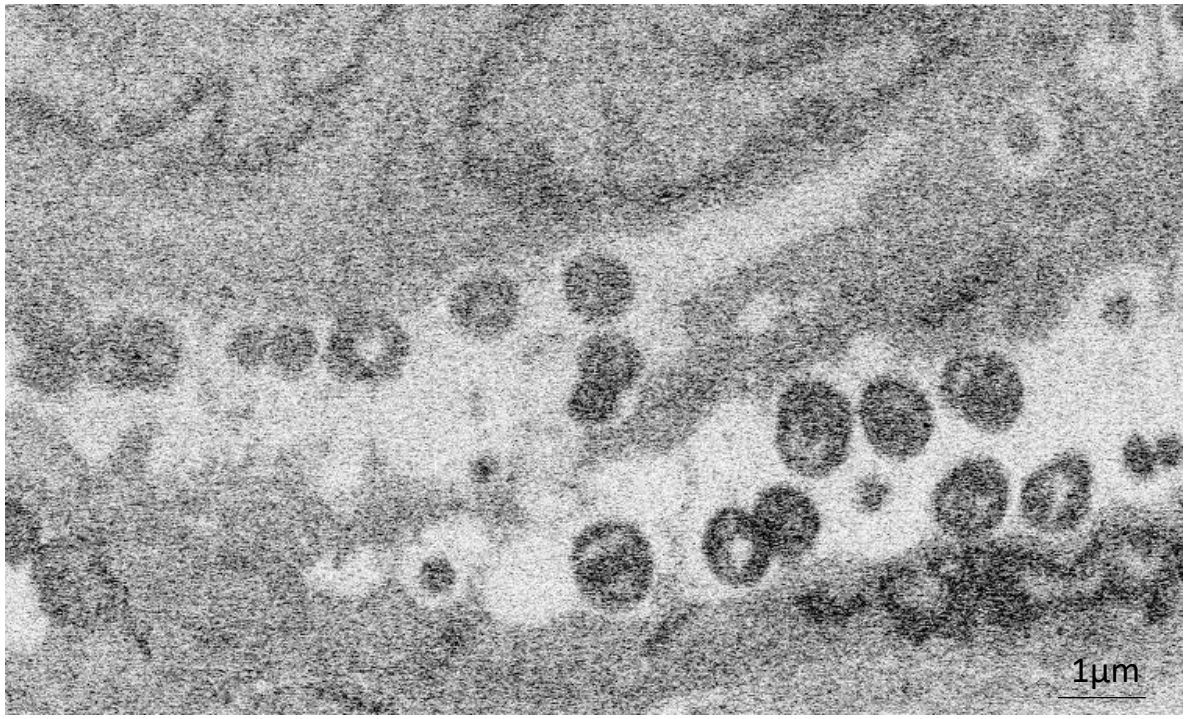
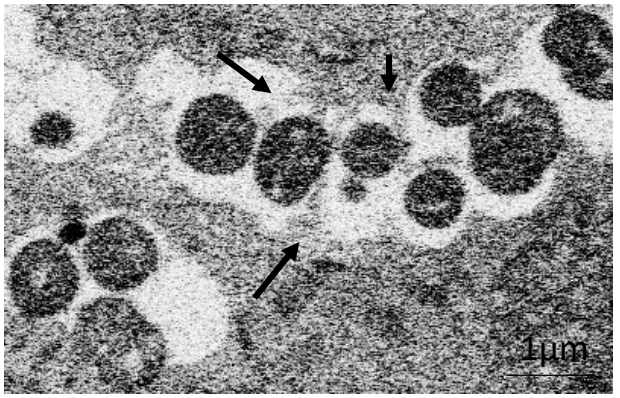
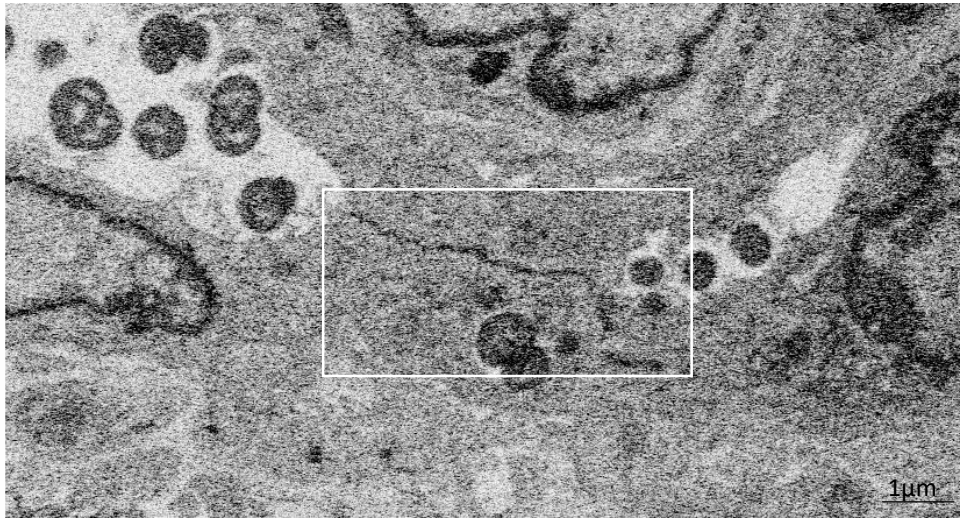
Ultrastructure of infected blood vessel in CLEM FIB-SEM

Vascular colonization





⇢ Plasmic membrane rearrangement around bacteria.



⇢ cellular junction

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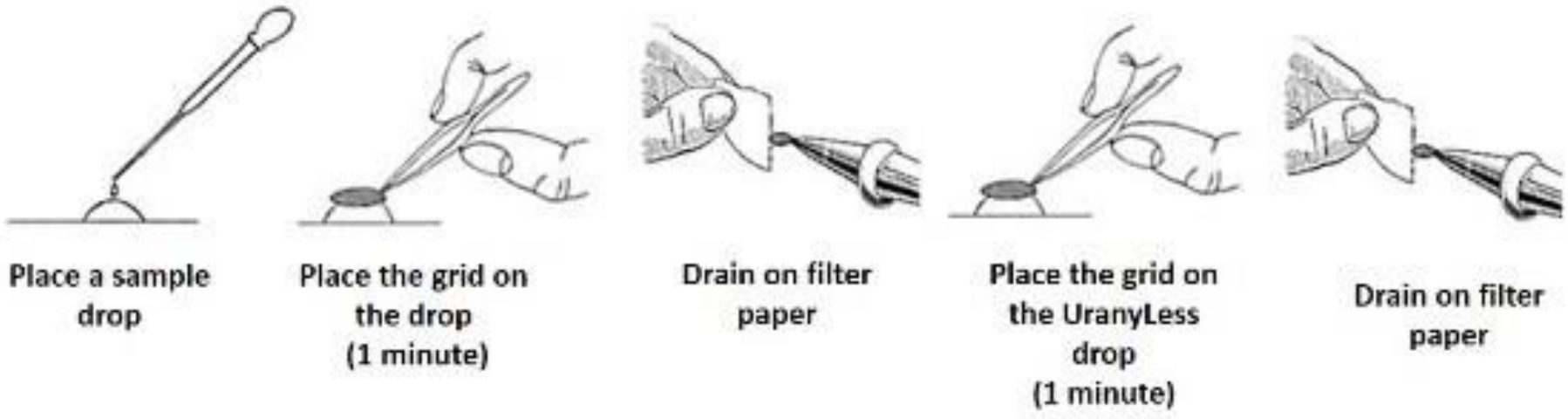
Structural Biology Applications

Negative staining on single particles

- Virus
- Proteins
- Complexes

→ Small samples !



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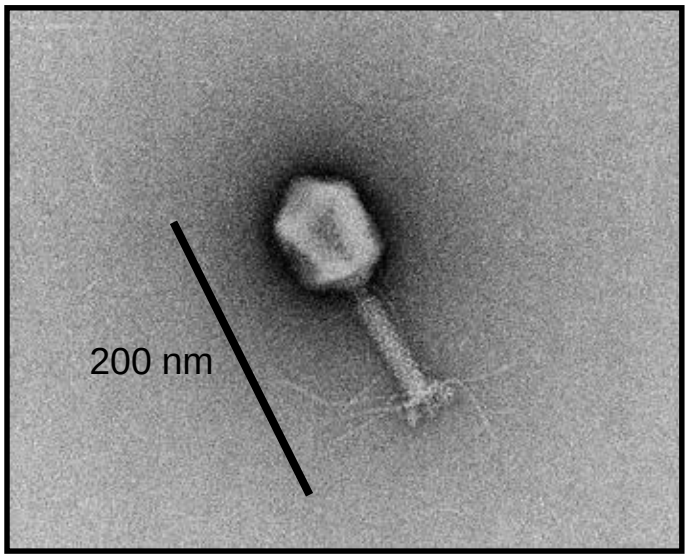
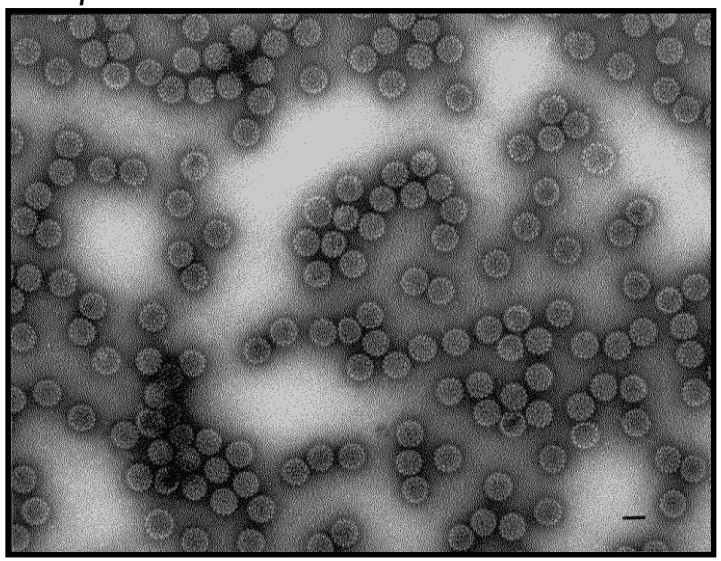
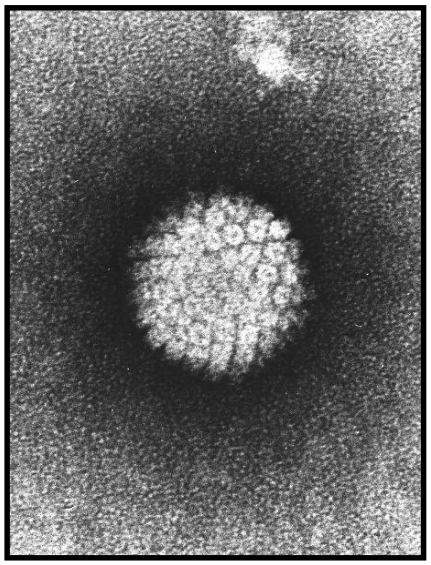
Structural Biology Applications

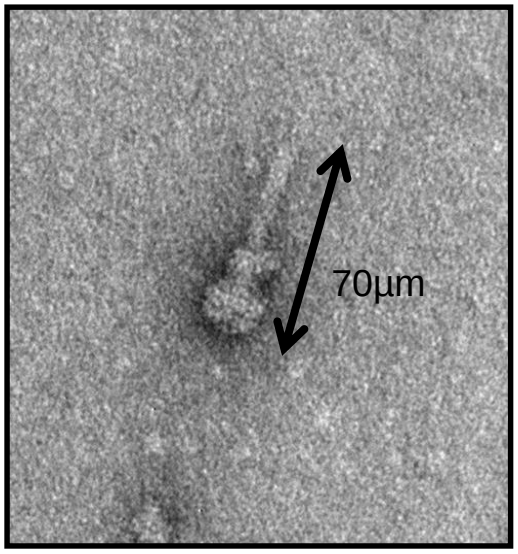
Papillomavirus humain



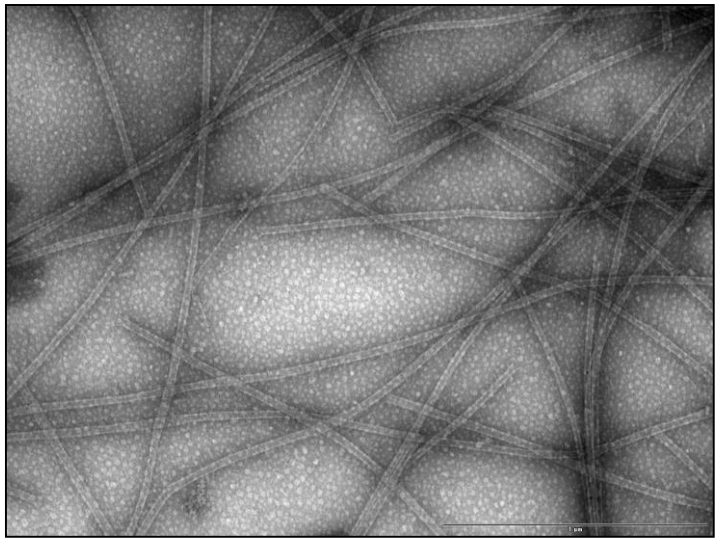




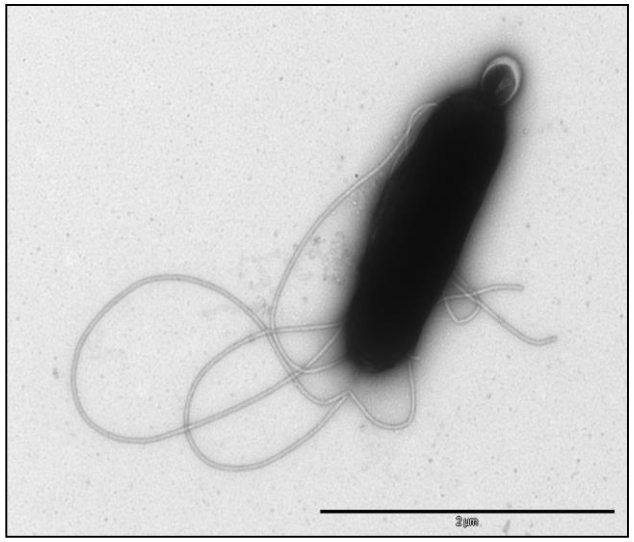
Appareil TypeIII



Microtubules (HCV)



E.coli



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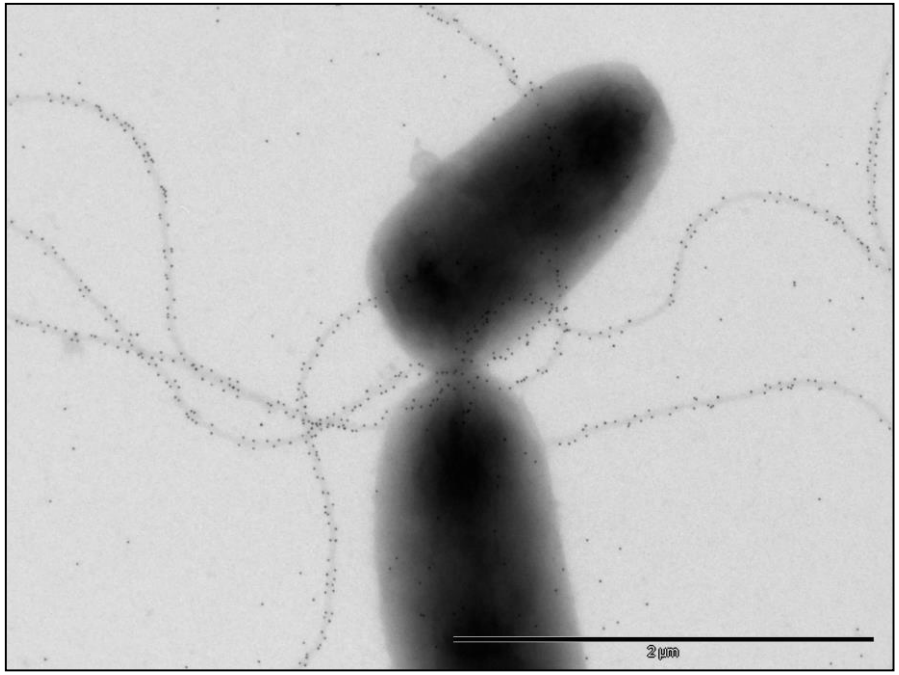
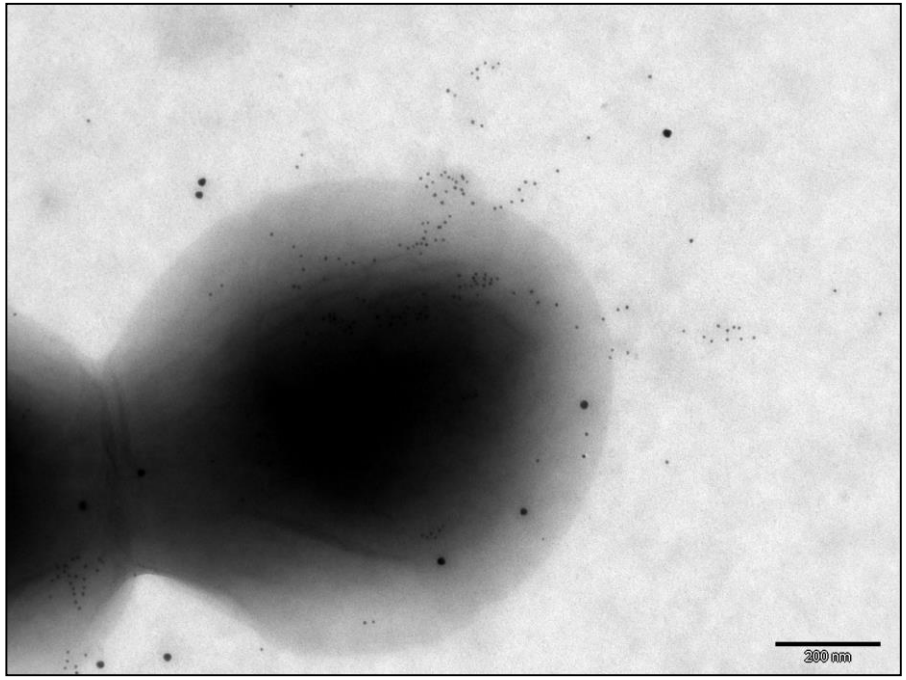
Structural Biology Applications

E.coli



Streptococcus



Cryo EM
NanoImaging Core
Facility

- Cryo TEM
- Sub tomogram averaging
- Cryo FIB (Cryo lamella)

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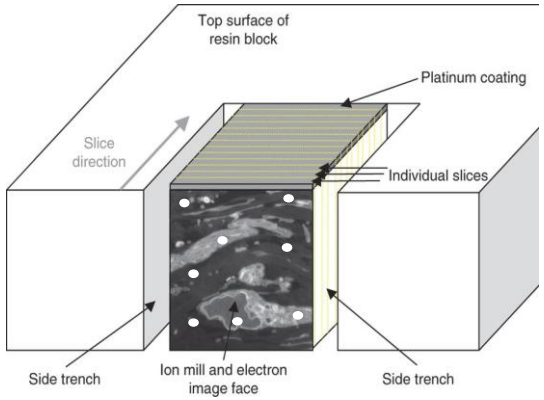
Conclusion & Perspectives

3D EM :
- ssTEM
- FIB-SEM

CLEM

Sample preparation
Multi scale
Multi Information

- Development of **CLEM approaches**
- **3D**
- **Ultrastructural details**



→ Zeiss Crossbeam laser FIB-SEM

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& Thanks you for your attention!