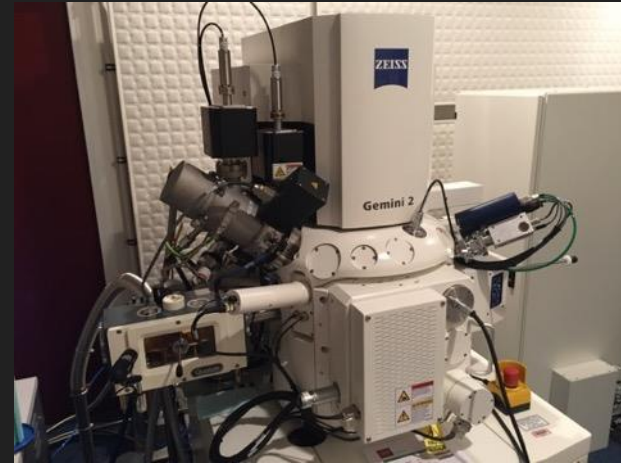


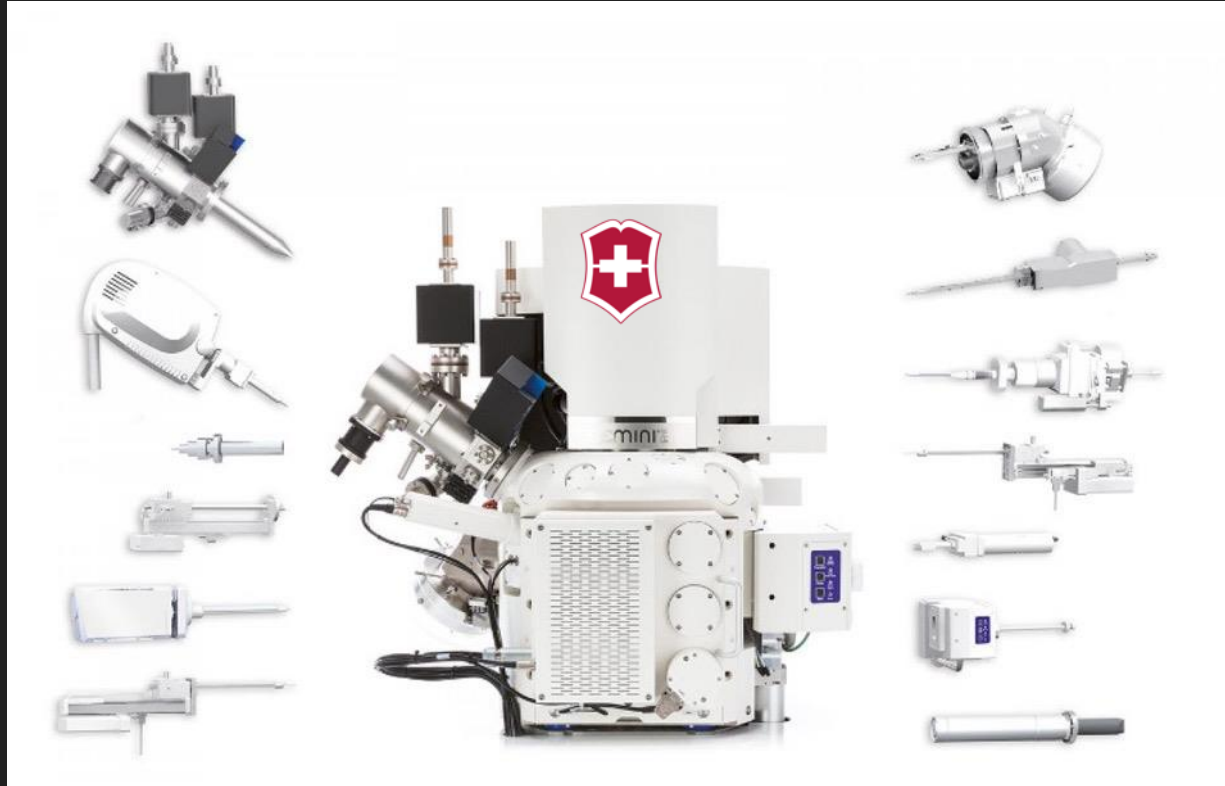
# Le MEB-FIB « cryo » Véritable couteau suisse pour le biologiste



Benoit Gallet, IBS Grenoble  
Décembre 2023, GN-MEBA



# MEB-FIB: microscope double faisceau ...



# MEB-FIB

# MEB

FIB

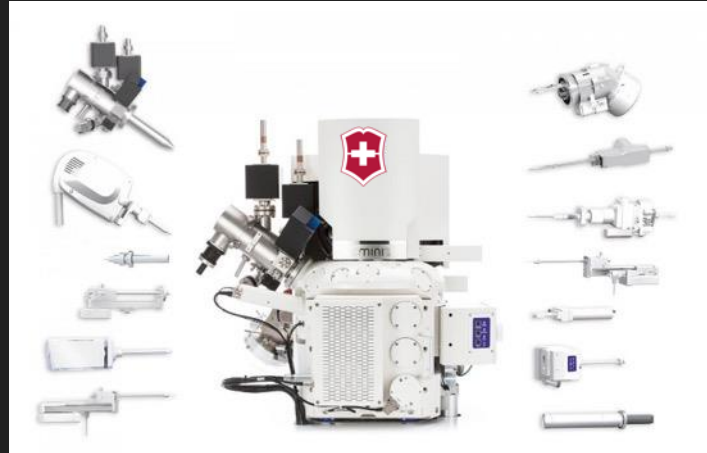
Plasma  
cleaner

EsB/SE

EDS

BSD

GIS  
Métallisation



Compensation  
de charge

STEM detector

Tandem decel

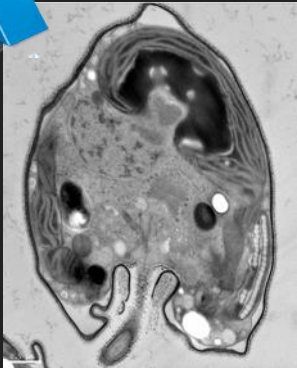
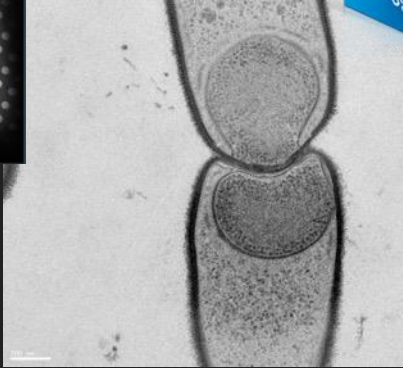
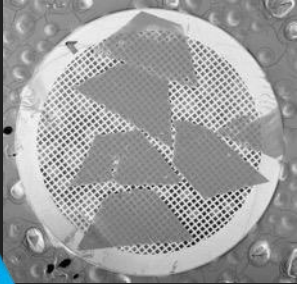
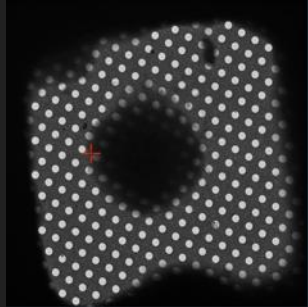
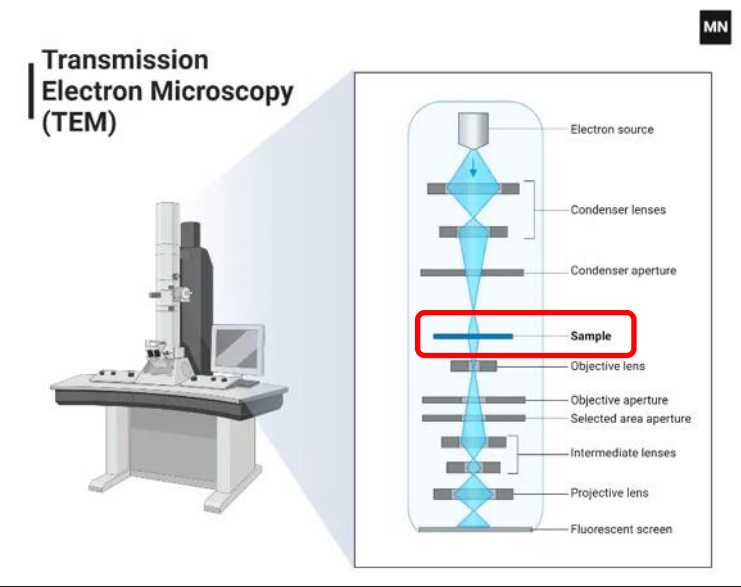
Micro-  
manipulation

Ablation laser

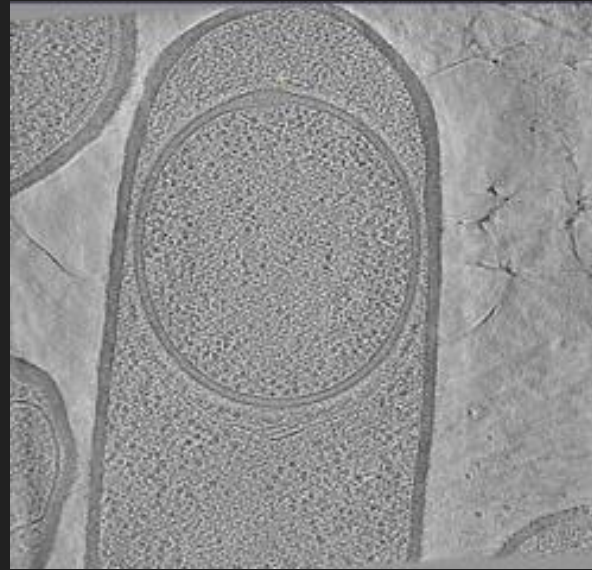
# Couteau Suisse...



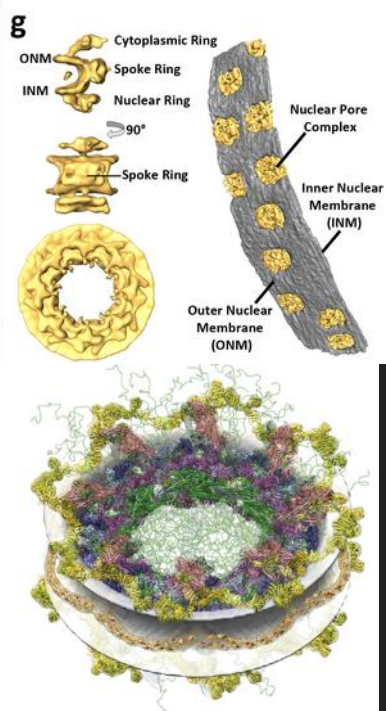
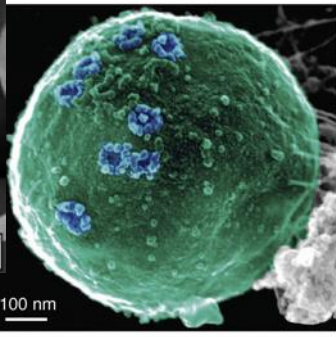
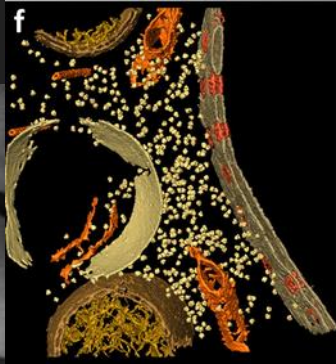
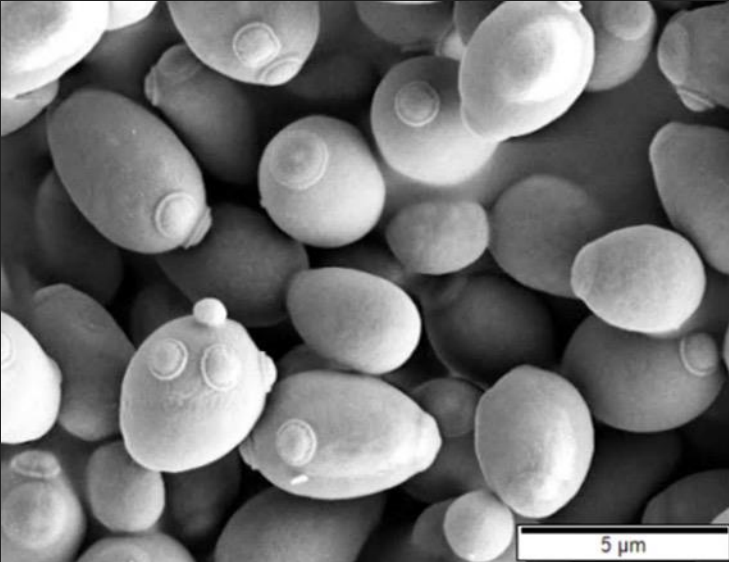
# Microscopie électronique cellulaire



# Microscopie électronique cellulaire



# Enjeu de la biologie intégrative



# Objectif de la biologie structurale intégrative :

1. Résolution d'imagerie : MET + volume = **Tomographie électronique**
2. Echantillon en condition native : **Cryo**
3. Observation dans le context cellulaire : **In situ**
4. Technique de preparation/imagerie => ***coupes* ~100nm**

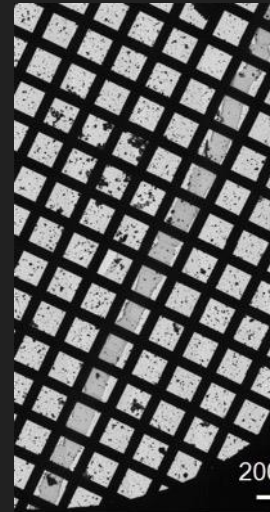
**=> Cryo Tomographie Electronique In Situ**

# Réalisation de cryo-coupes

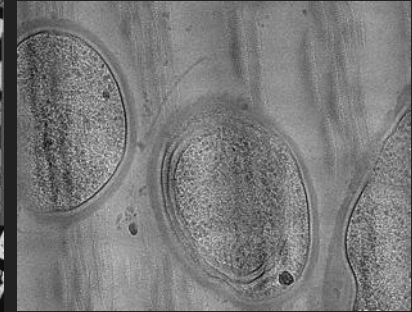
- CEMOVIS
  - Preservation close-to-native state
  - $< 100$  nm section = high SNR
  - Coupes séries
  
- Cryo-MEB-FIB



Ultramicrotome



Ribbon on EM grid

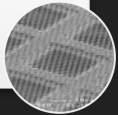


*B. subtilis* cells

# Cryo-tomographie électronique « in-situ »



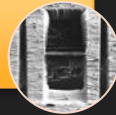
Préparation  
grille



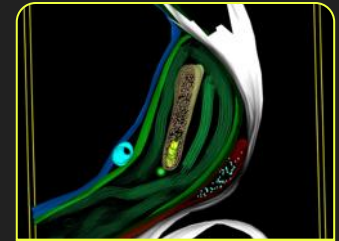
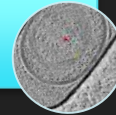
Cartographie



Cryo-FIB-SEM



Tomographie

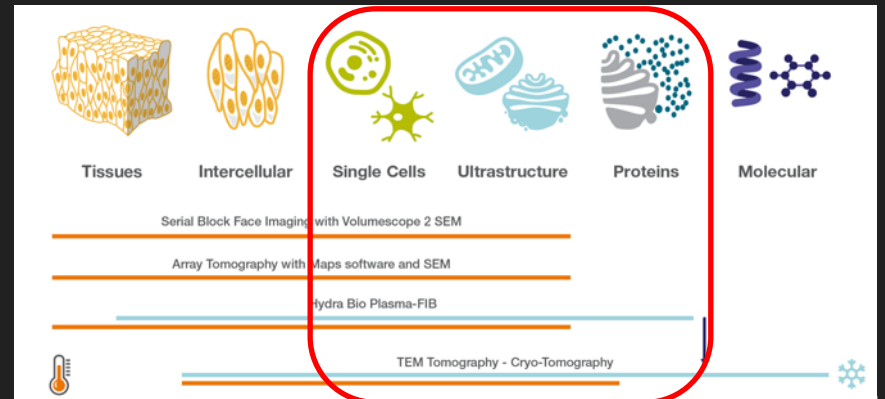
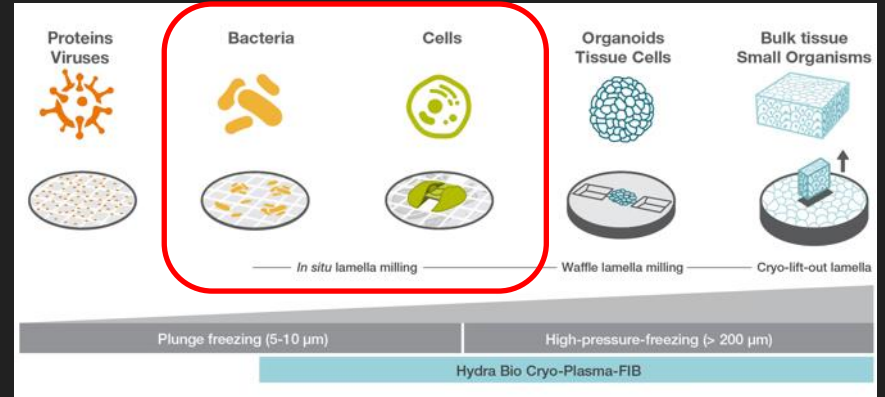


Processing  
Analyse



# MEB-FIB: applications (science du vivant)

- MEB
  - Imagerie (Pression variable)
- Préparation d'échantillons
  - Lamelles cryo-TEM
- (Micro-)Analyses
  - EDX
- Imagerie 3D
  - (Cryo-) Slice & view



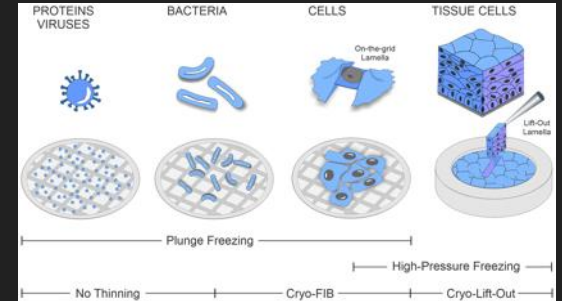
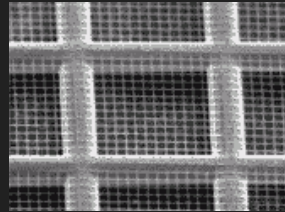
# Préparation d'échantillon

- Production des échantillons
  - Culture : Bactéries, cellules
  - Prélèvement : tissu, échantillons environnemental

- Concentration
- Dépôt sur grille
- Vitrification
  - Plunge freezing
  - HPF (Waffle method, bulk method)



2 hours



*Klumpe et al. 2022*

**=> Echantillon vitrifié sur grille**

# Caractérisation / contrôle

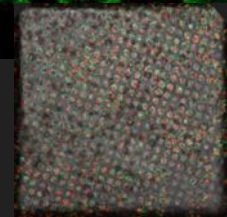
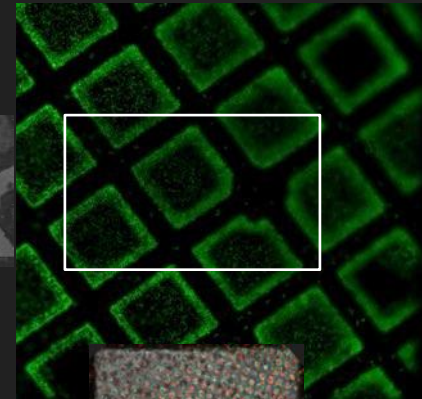
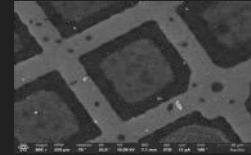
- Etape facultative
- Permet de
  - Vérifier la qualité de l'échantillon (état et concentration des cellules)
  - Vérifier l'état de la glace (pas de craquelures)
  - Localiser une région d'intérêt (ROI)
  - Enregistrer les coordonnées des ROIs



Cartographie



- Cryo-fluorescence/Cryo-TEM

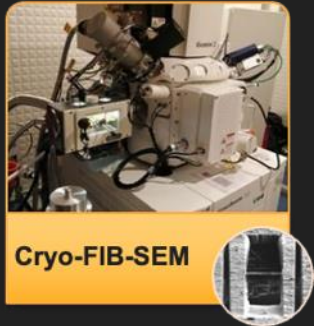


2-4 heures

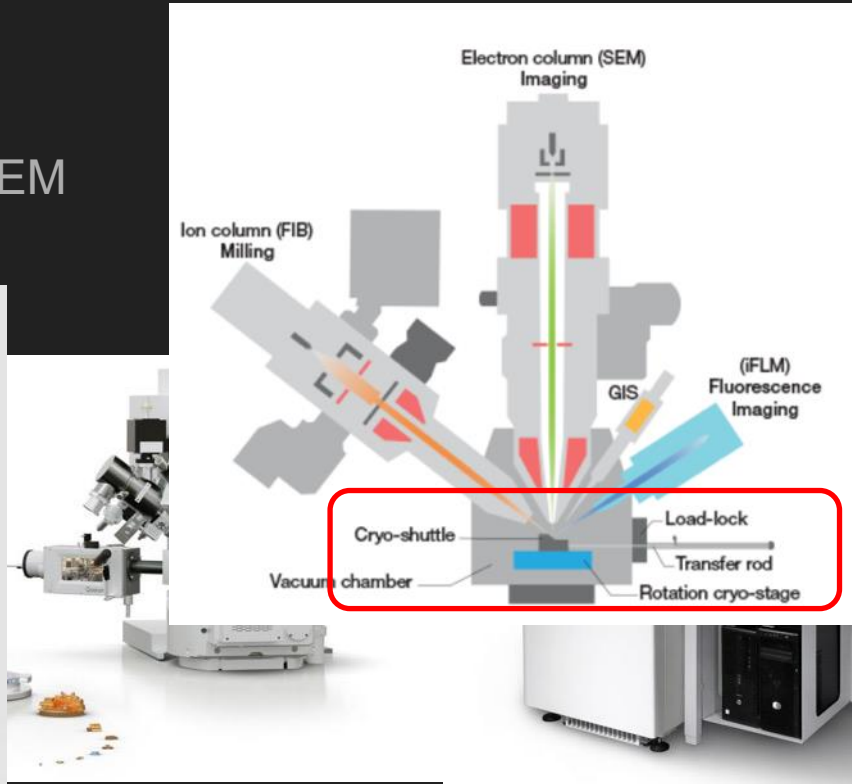
**=> Choix des meilleures grilles**  
**=> coordonnées des ROIs**

# Coupes = cryo-lamelles

- Utilisation Cryo-FIB-SEM



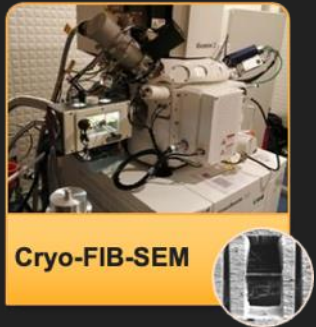
1-2 jours



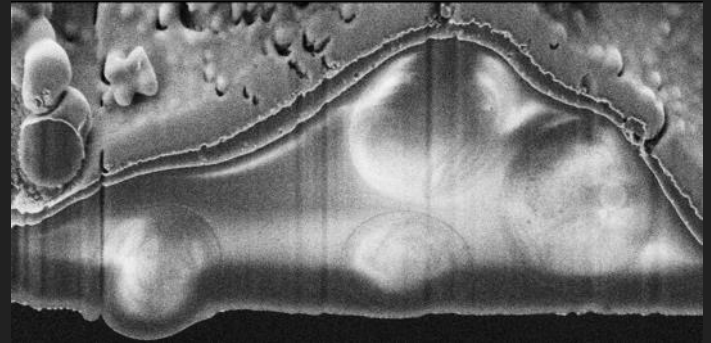
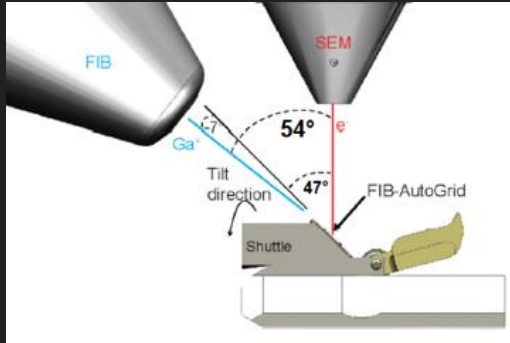
Ga ou Plasma...

# Coupes = cryo-lamelles

- Utilisation Cryo-FIB-SEM
- Métallisation (10nm Pt)
  - GIS / sputter coater (cryo)
- Angle /FIB :  $\sim 10^\circ$

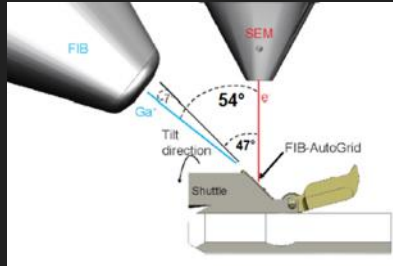
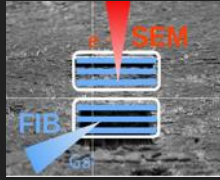


1-2 jours

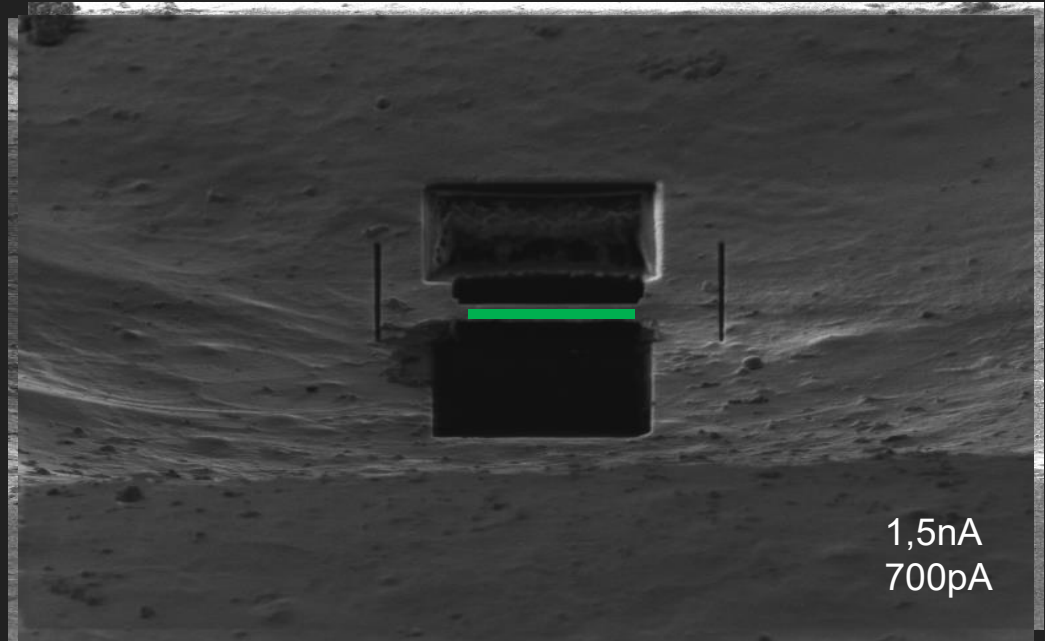


# Coupes = cryo-lamelles

- Utilisation Cryo-FIB-SEM

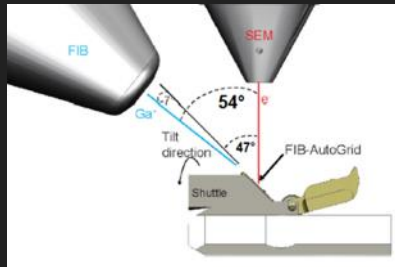
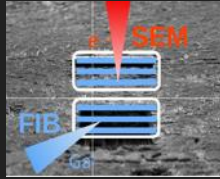


1-2 jours

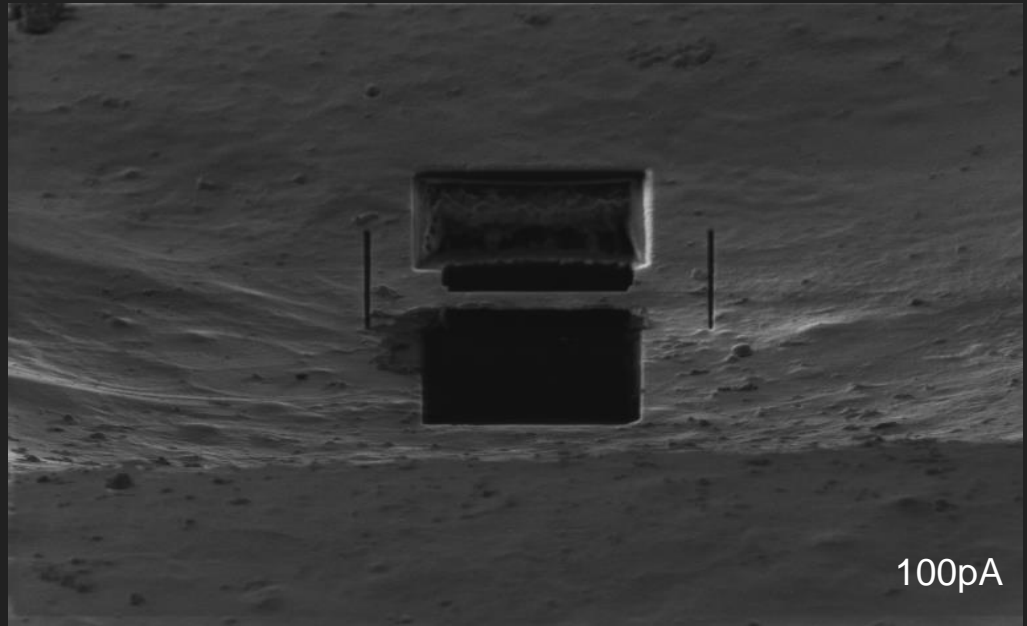


# Coupes = cryo-lamelles

- Utilisation Cryo-FIB-SEM

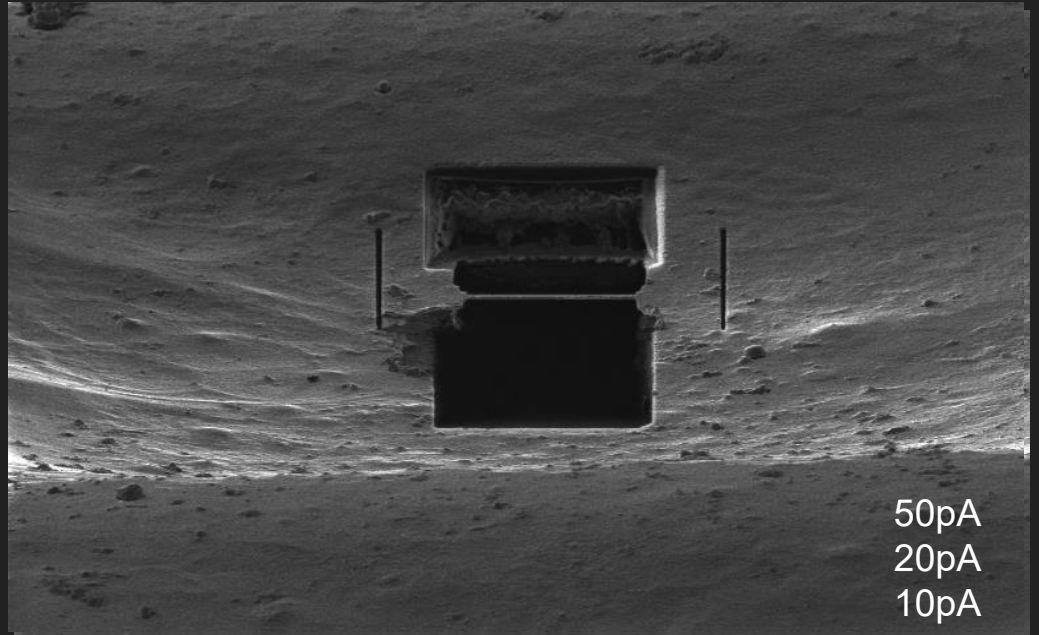
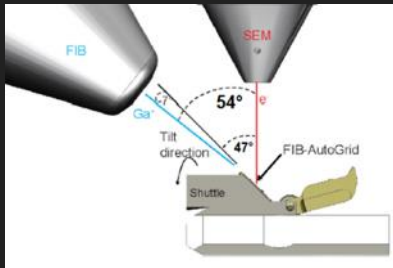
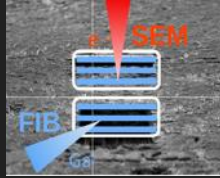
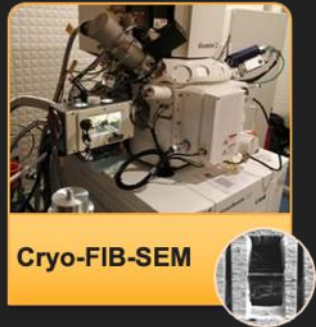


1-2 jours



# Coupes = cryo-lamelles

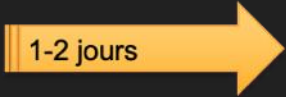
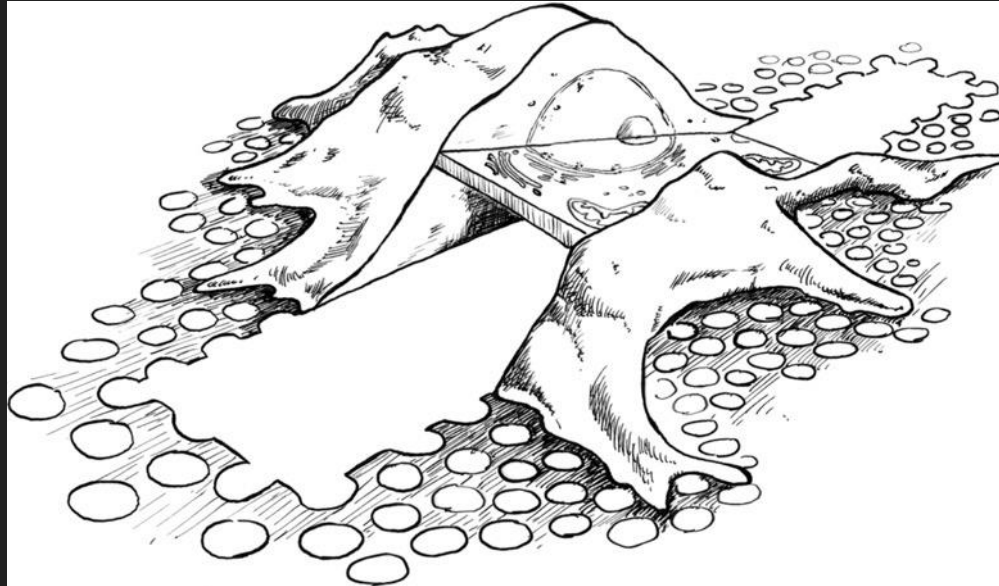
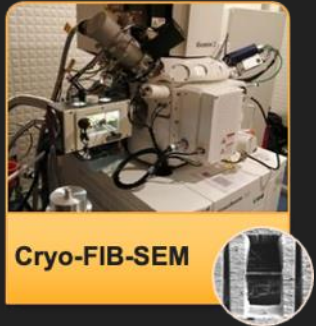
- Utilisation Cryo-FIB-SEM



1-2 jours

# Coupes = cryo-lamelles

- Utilisation Cryo-FIB-SEM



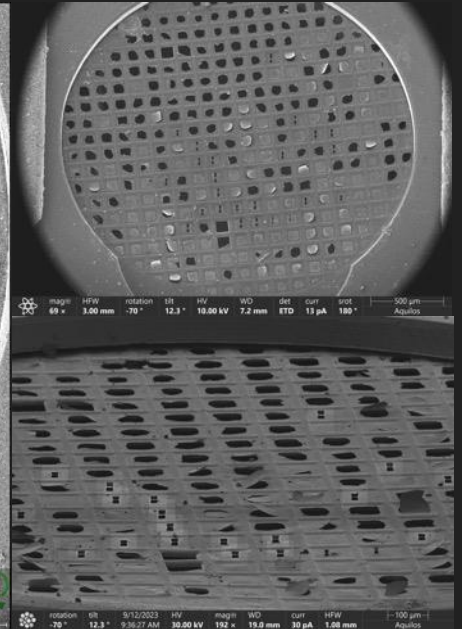
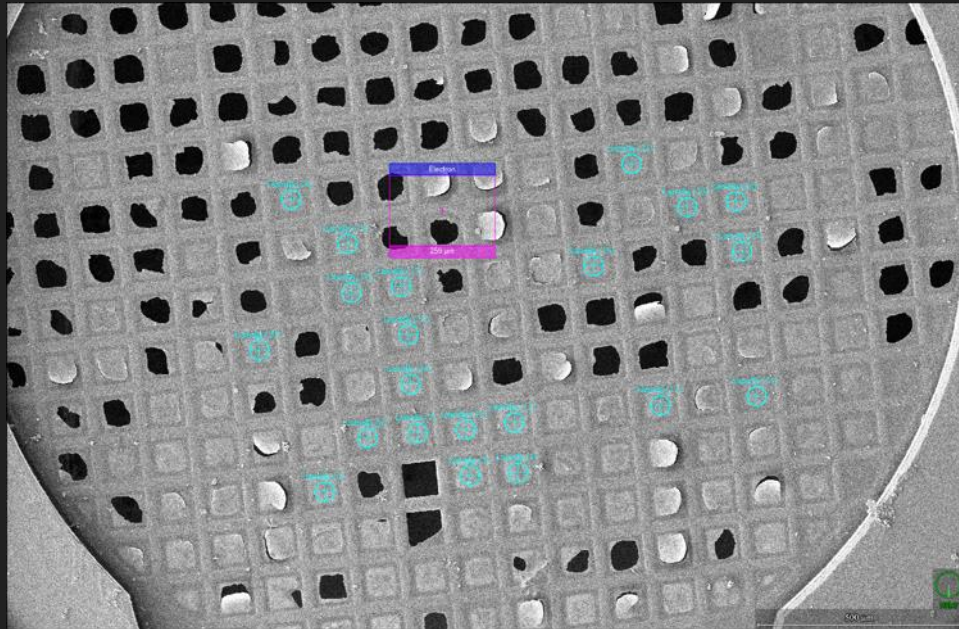
# Coupes = cryo-lamelles

- Utilisation Cryo-FIB-SEM



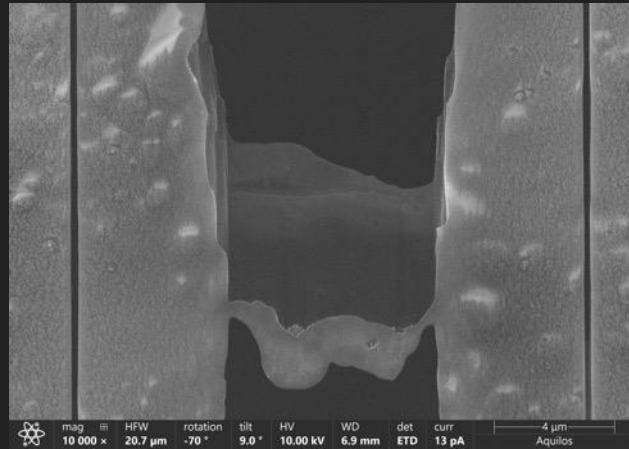
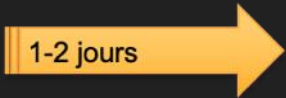
1-2 jours

A yellow arrow pointing to the right, indicating the time required for the process.

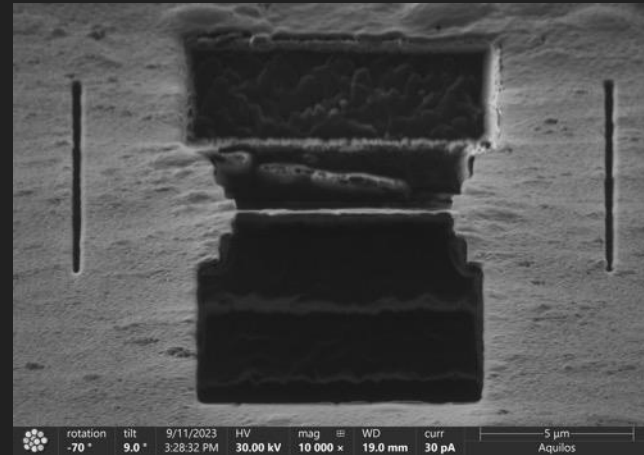


# Coupes = cryo-lamelles

- Utilisation Cryo-FIB-SEM



MEB



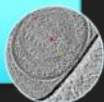
FIB

# Imagerie cryo-MET

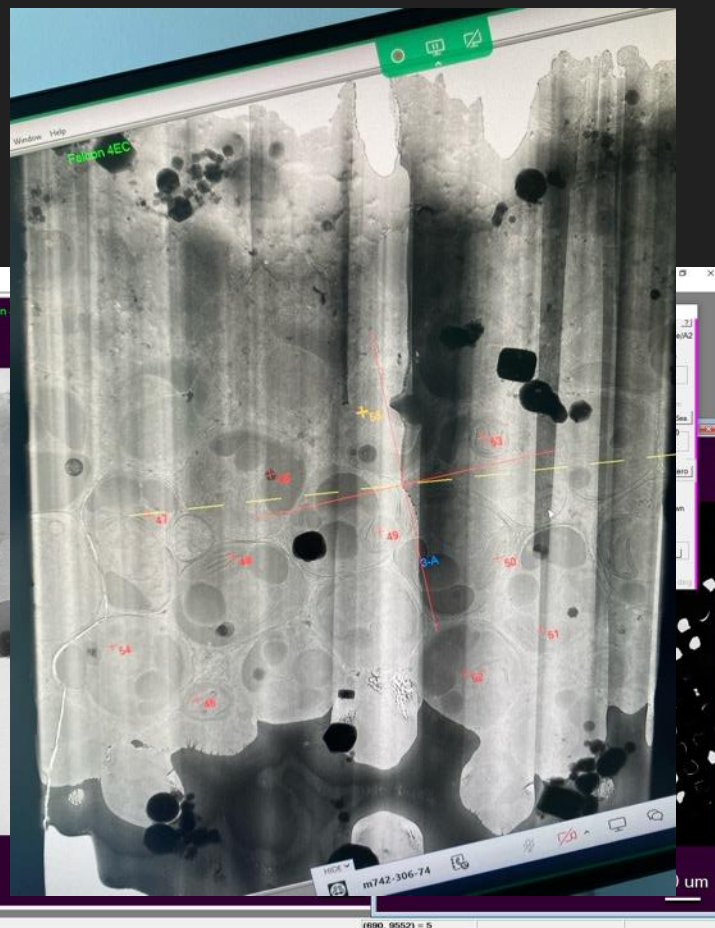
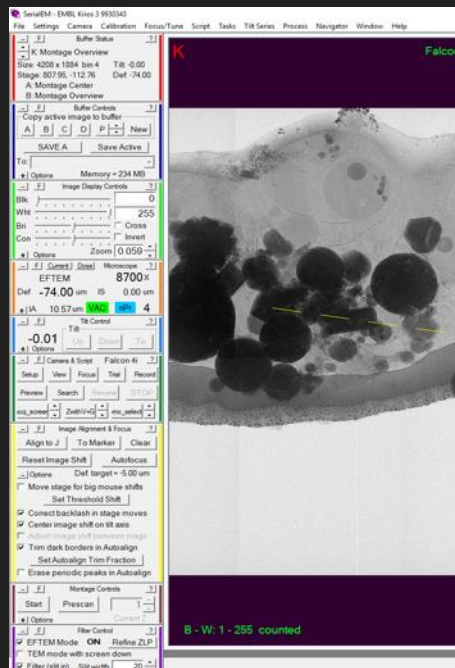
- 300kV + filtre + DD



Tomographie



1-2 Jours

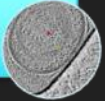


# Imagerie cryo-MET

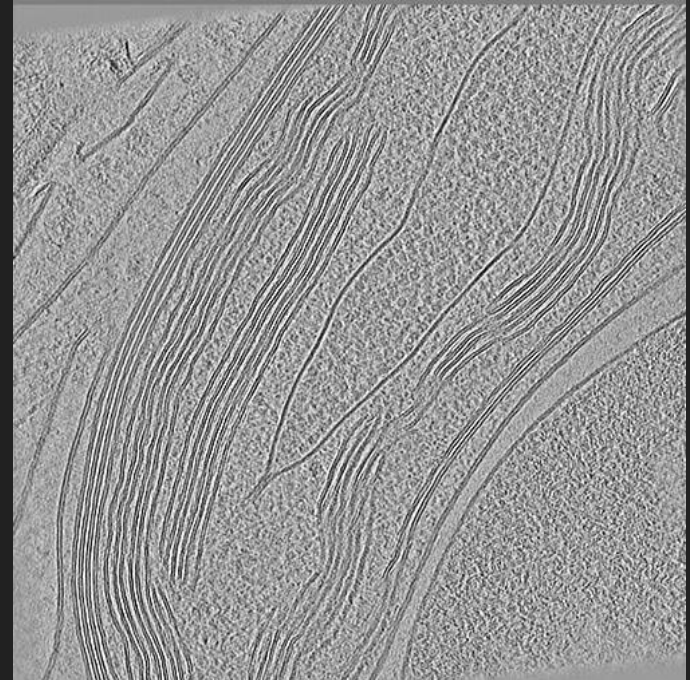
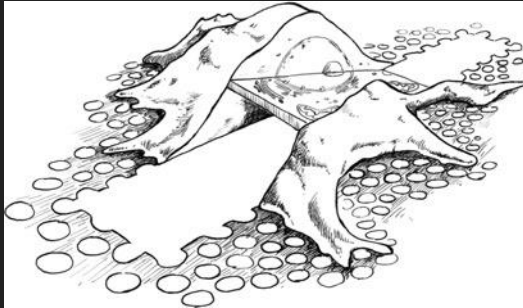
- Enregistrement de
  - Séries tiltées
  - $-60^\circ \leftrightarrow +60^\circ$
  - A partir de l'angle de la lamelle



Tomographie



1-2 Jours

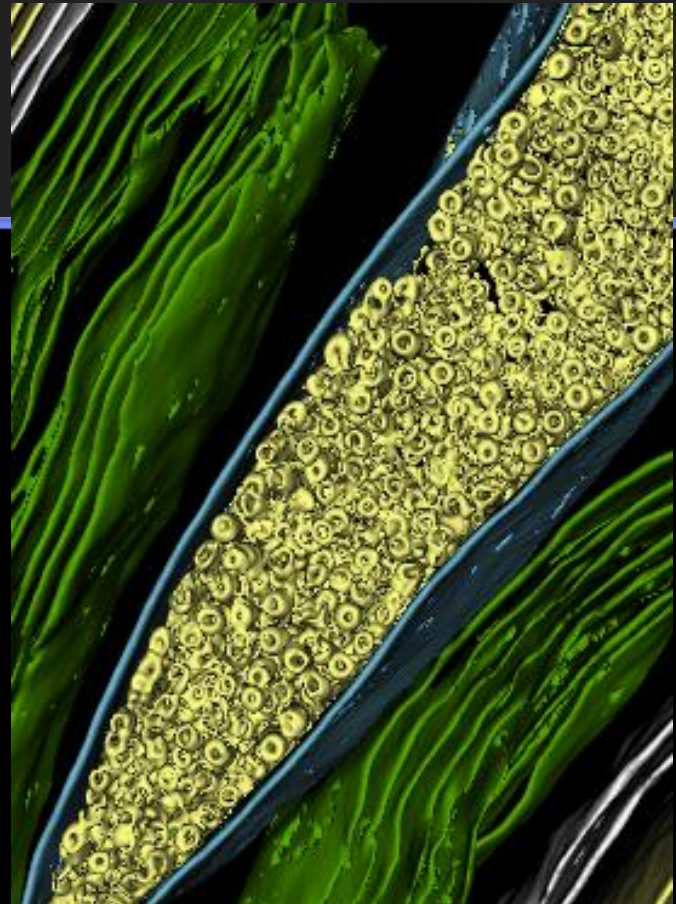
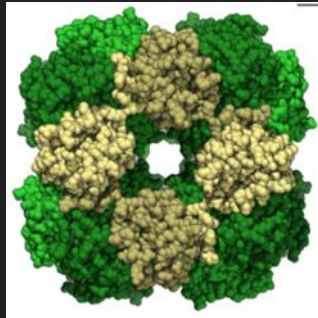


# Analyse

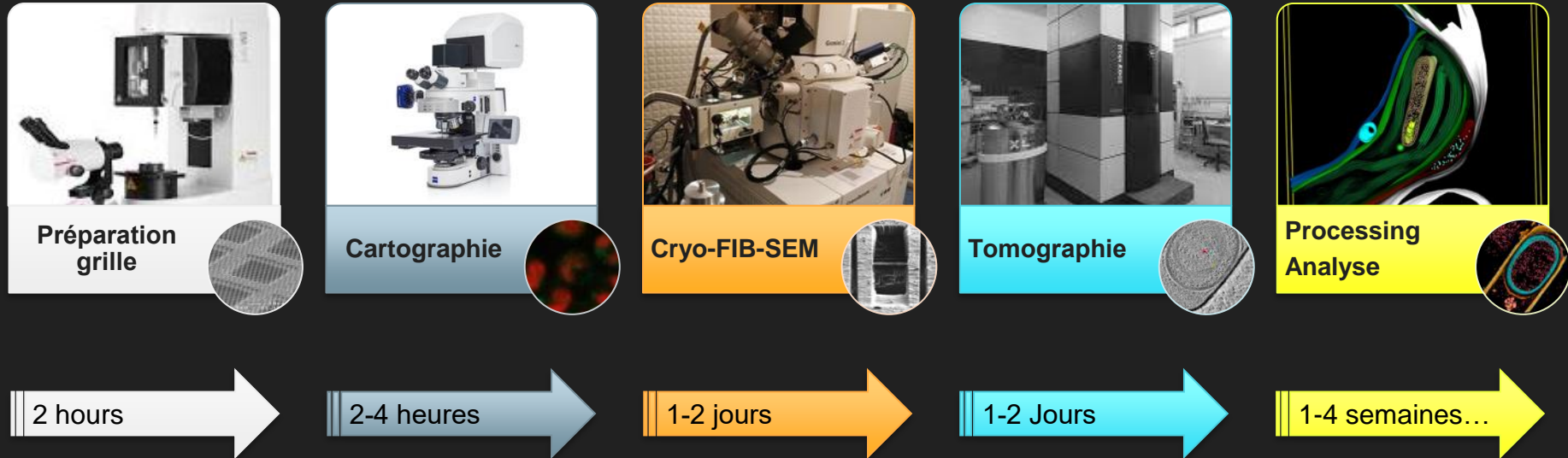
- Reconstruction du volume
  - IMOD / AreTomo
  - +/- automatique
- Segmentation
  - Mise en valeur des objets
  - +/- automatique



1-4 semaines...



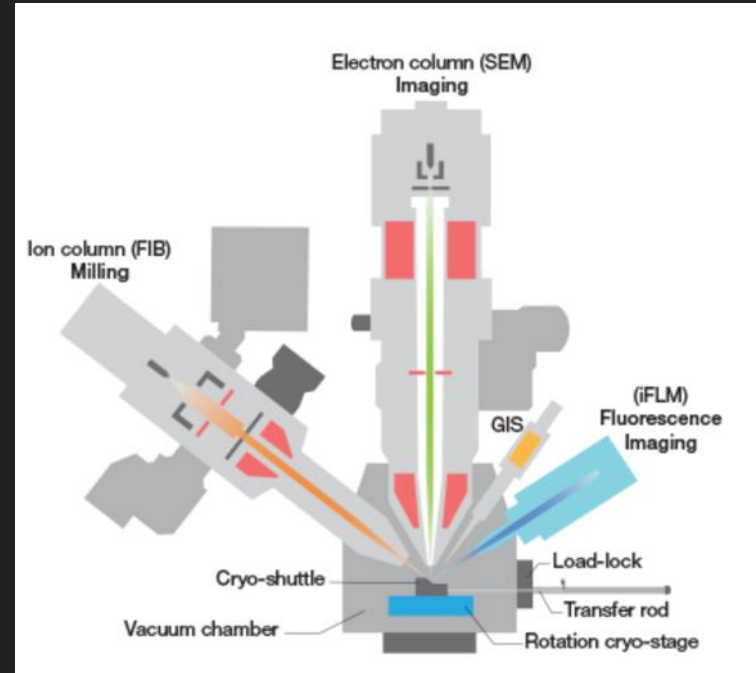
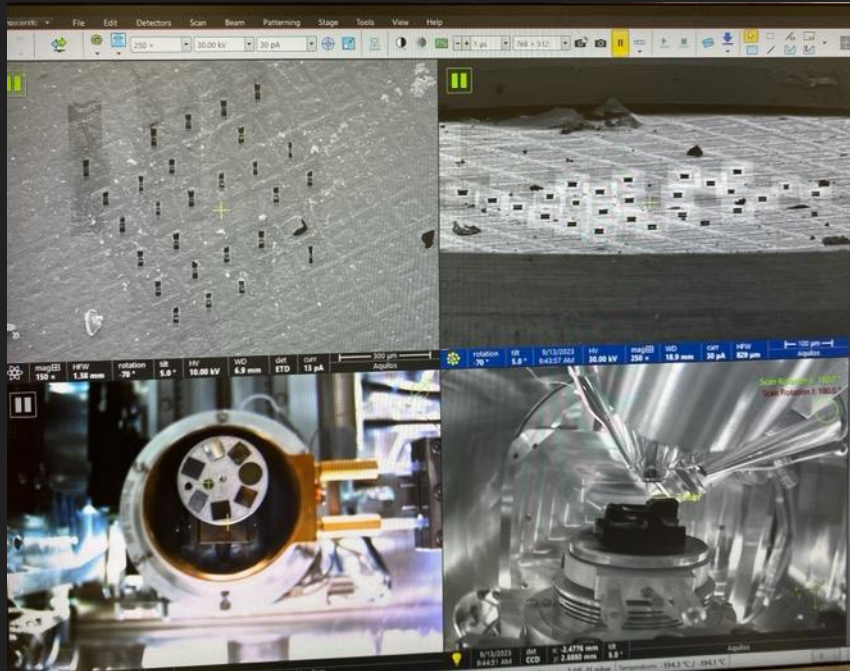
# Cryo-tomographie électronique « in-situ »



# Cryo-MEB-FIB pour la biologie

- Cryo-MEB
  - Imagerie MEB d'échantillons congelés
  - Echantillons hydratés mais métallisés
- Cryo-FIB 3D
  - Slice & view
  - Faible contraste mais échantillons natif
- ...

# Questions ?







# MEB-FIB: applications (science du vivant)

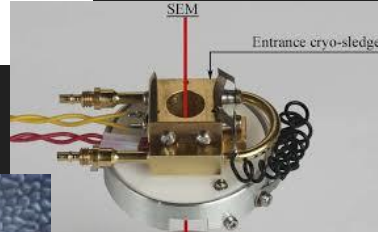
- Preparation d'échantillon :
  - MEB = Vide + irradiation



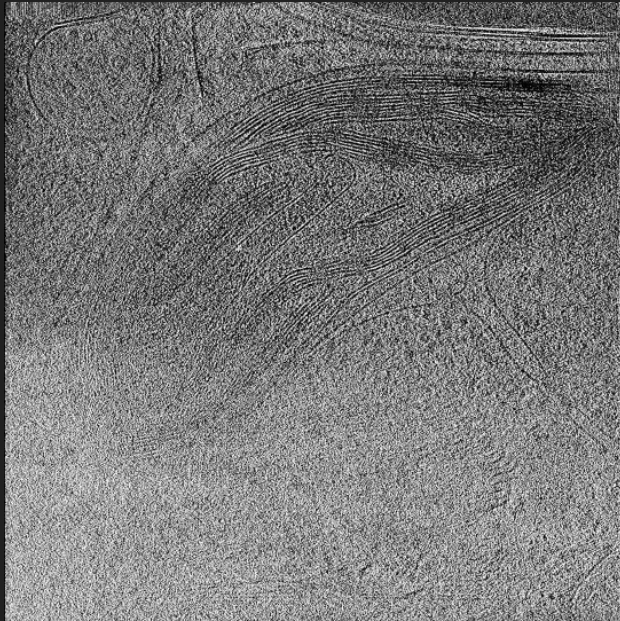
# MEB-FIB : en version cryo !!

Quorum

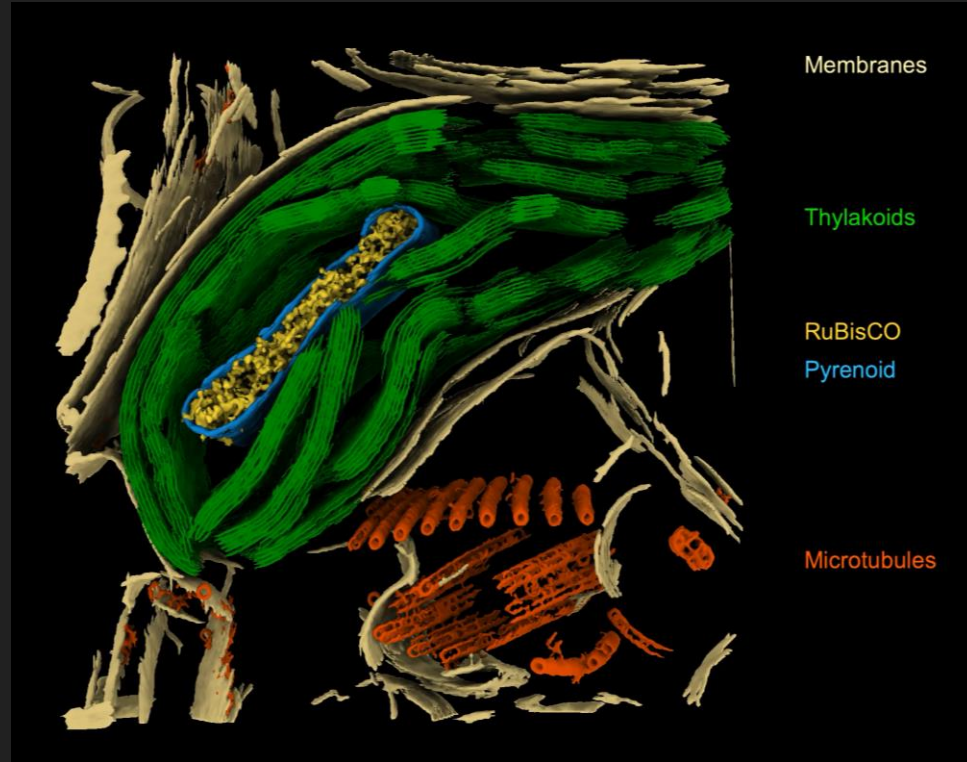
*Leica*  
MICROSYSTEMS



# Chloroplast morphology: data processing



- Tomogram reconstruction = volume



- Segmentation of features of interest