



Center for Innovation
and Technological
Research



Institut Pasteur

CRYO SEM: Au plus proche de l'état natif

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GnMeba 30 Nov – 1 Dec 2015

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ULTRA⁺Pole

PLAN

- Ultrapôle
- cryo-SEM en biologie
- Equipement
- Etude VIH
- Développement méthode corrélative en cryo
- Conclusion

ULTRAPOLE

10 Ingénieurs et techniciens
Responsable: Jacomine Krinjse Locker



3 TEM : 120KV et 200kV tecnai_FEI

Analyses 2D
CRYO-TEM
Immunolocalisation
CEMOVIS
Tomographie



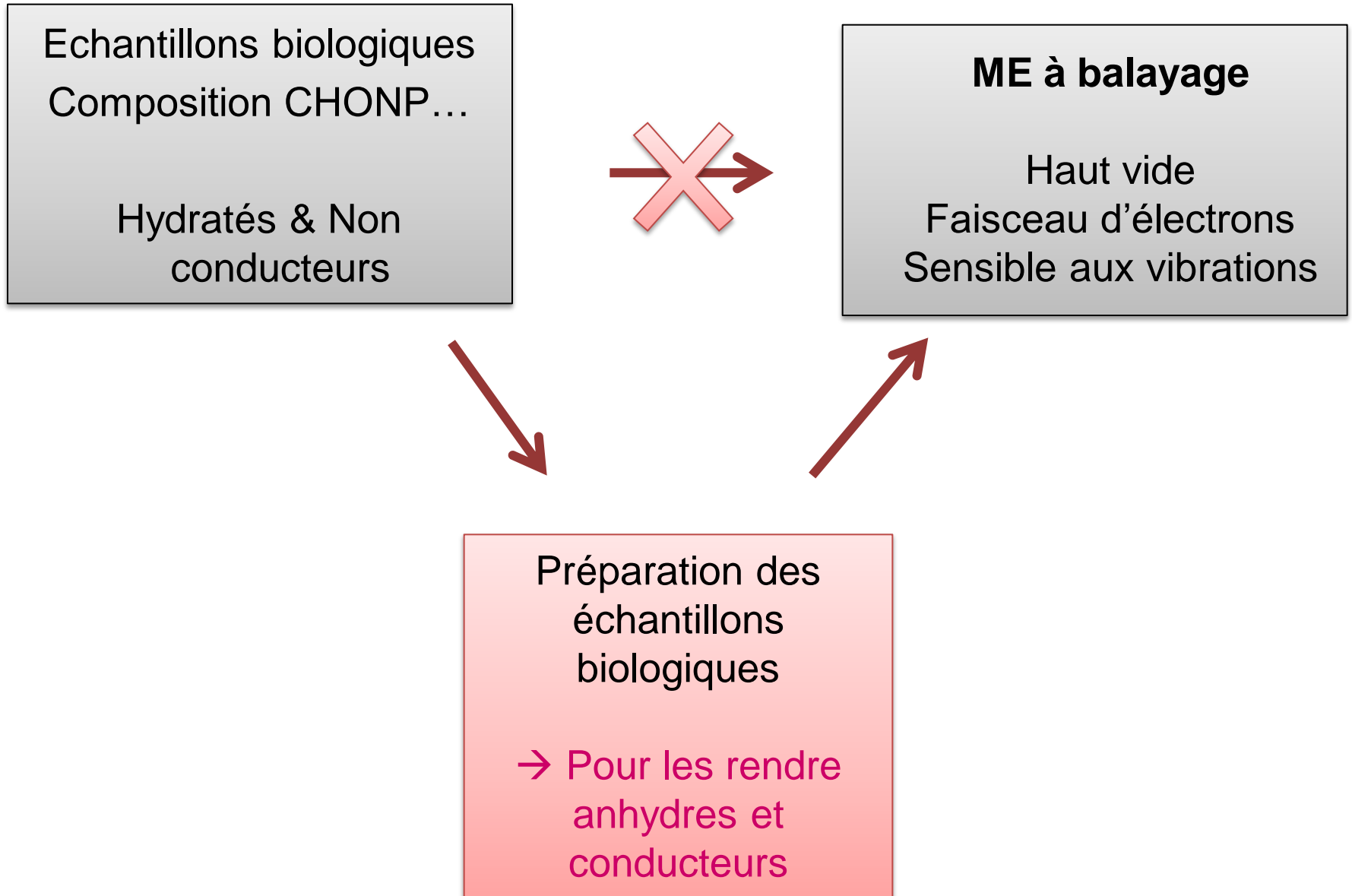
JEOL 6700F et Zeiss-Auriga

- Analyses morphologiques
- Immunolocalisation
- Cryo-SEM
- STEM
- FIB/SEM



Etude des interactions hôte-pathogène

Contraintes liées à l'étude d'échantillons biologiques



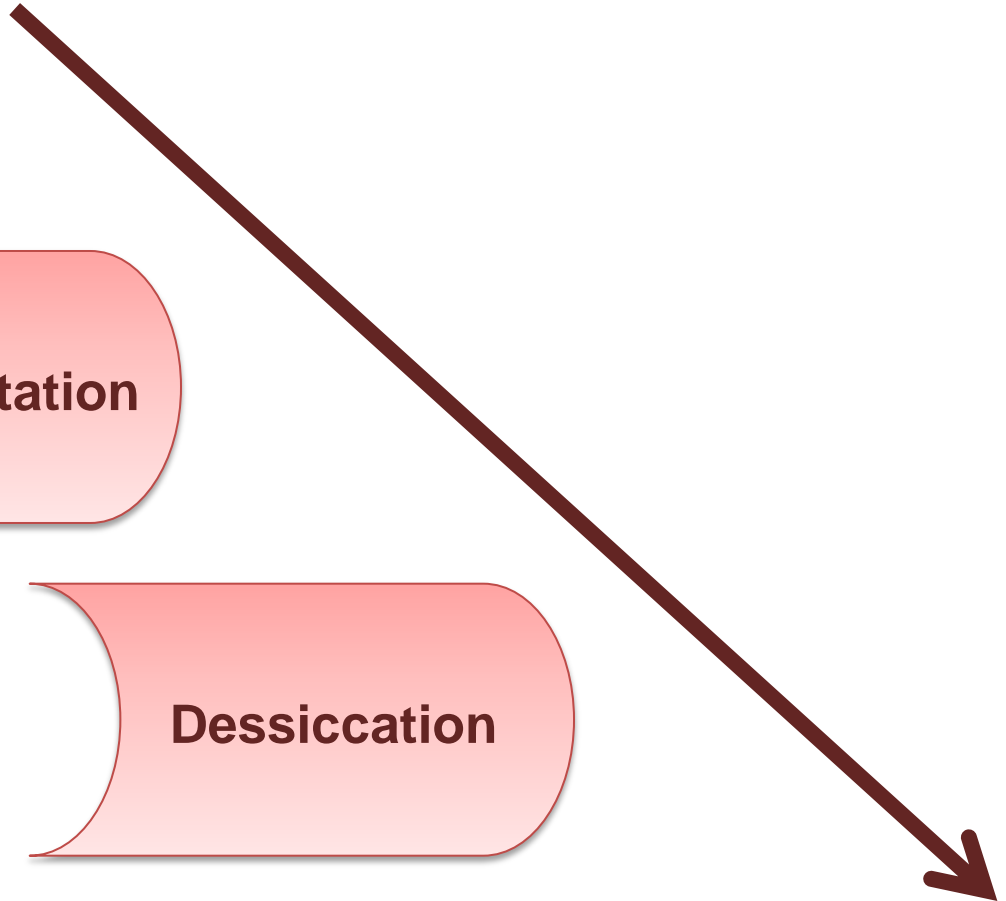
Préparation d'échantillons biologiques

**Fixation
chimique**

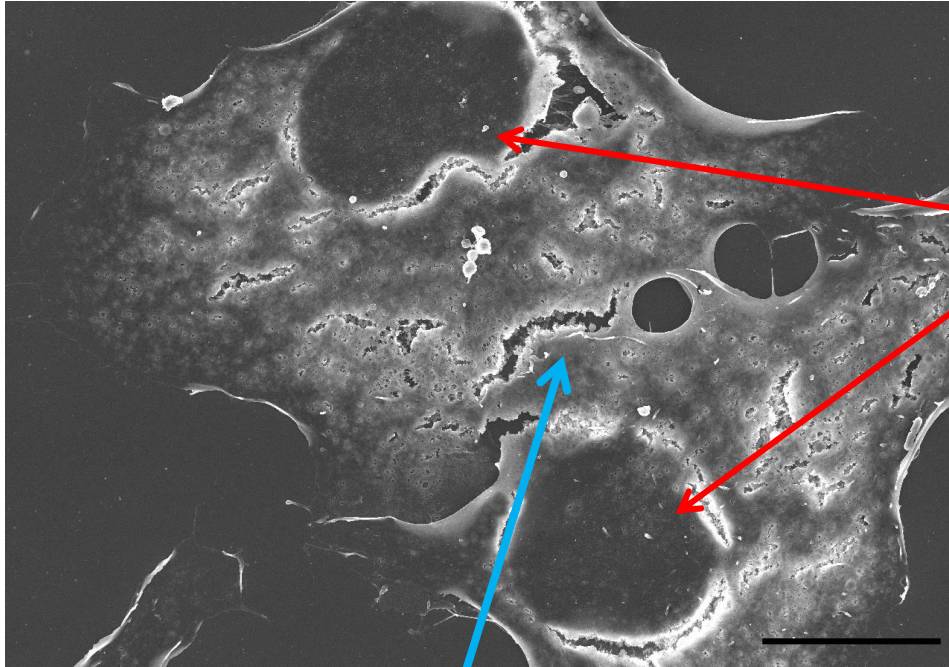
Déshydratation

Dessiccation

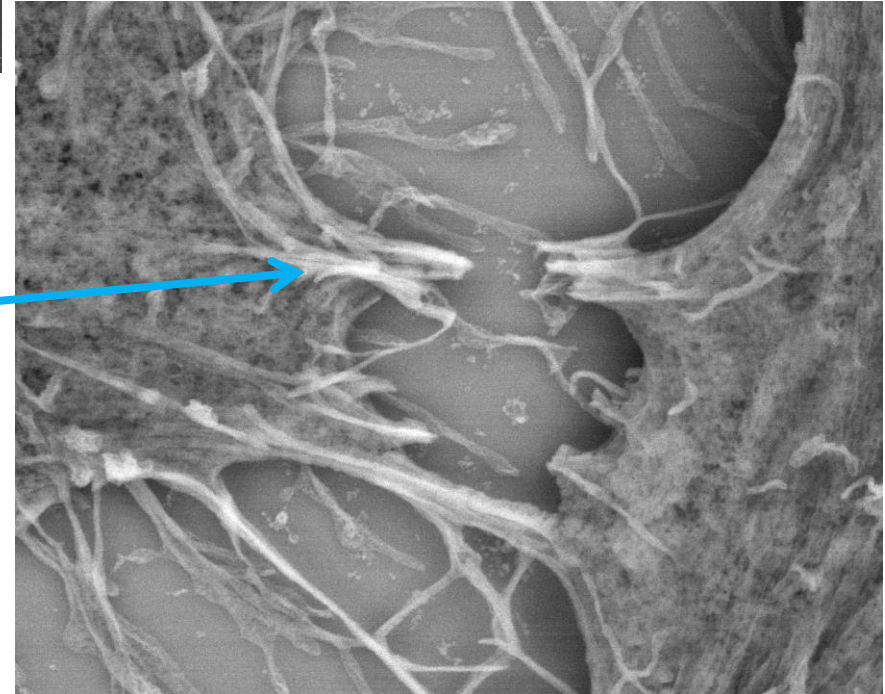
Métallisation



Artefacts



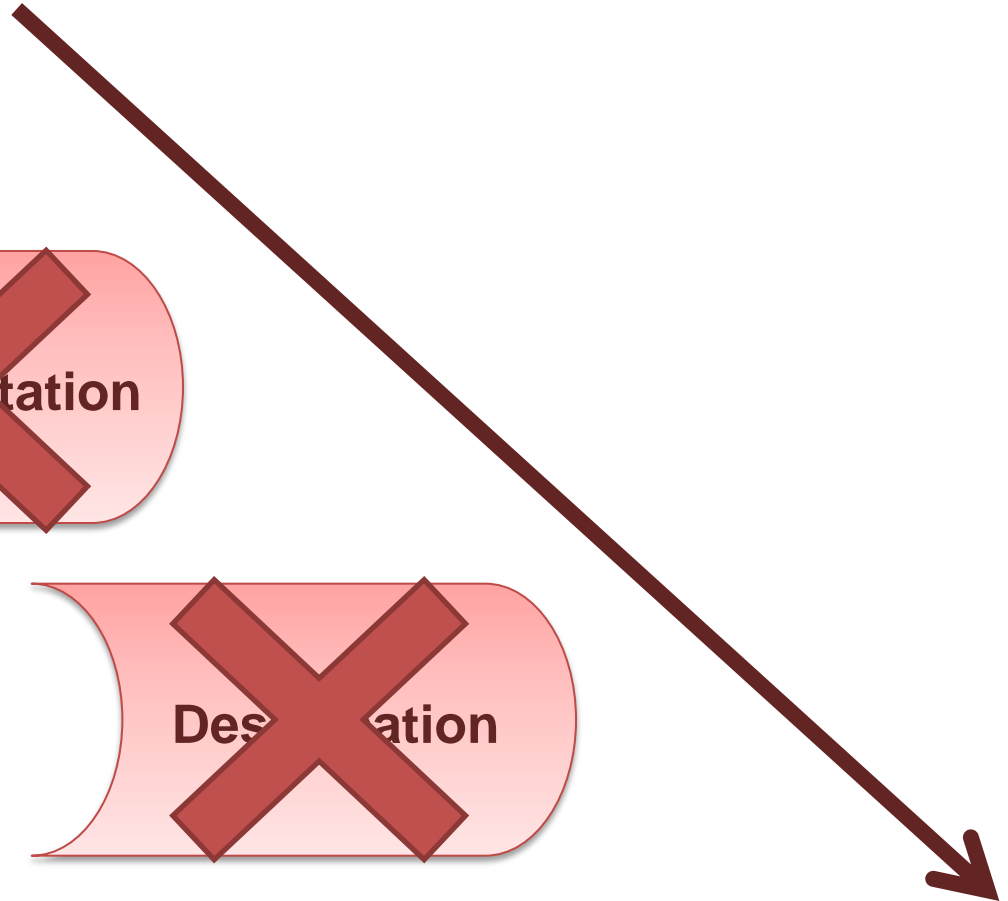
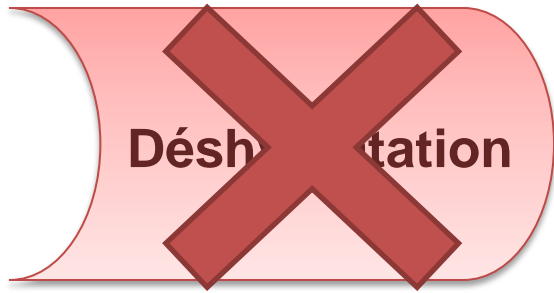
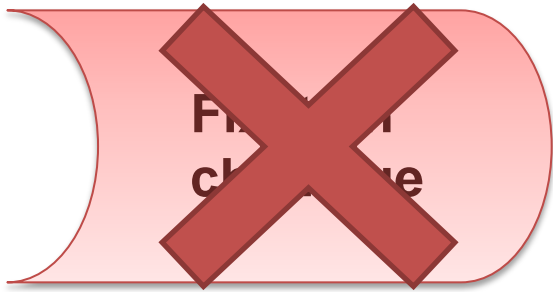
Affaissement des structures



Ruptures membranaires

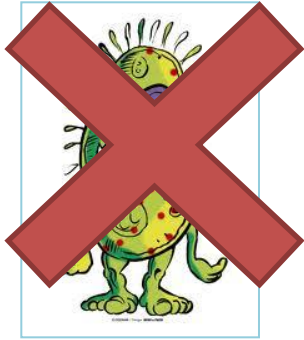
Scale bar: 10 μ m

Cryo SEM: Méthode



Cryo SEM: Méthode

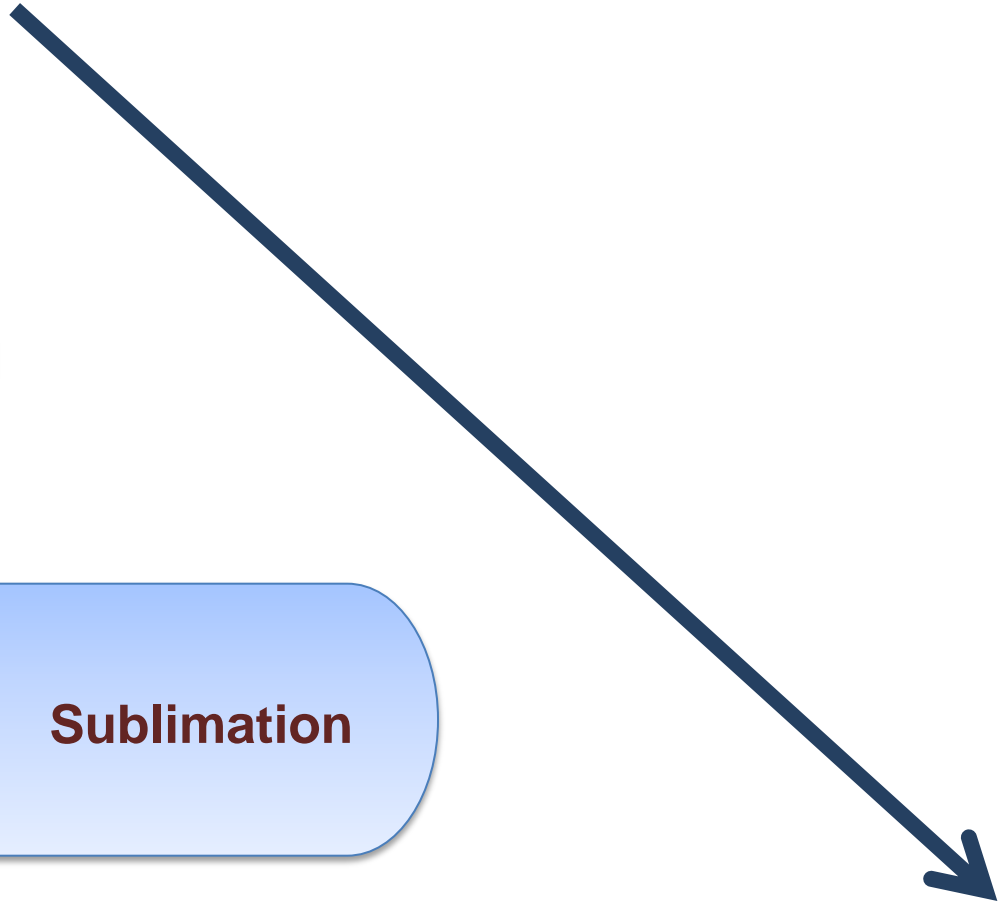
Fixation chimique



Cryo Fixation

Sublimation

Métallisation



Cryo SEM: Equipement

*Pointe Field Emission Gun FEG
à Cathode Froide
Résolution du microscope :
quelques nm à 5kV*

*Détecteur d'électrons
secondaires :*

-semi in lens SEI

-dans la chambre LEI

*Détecteur d'électrons
rétrodiffusés type YAG*



Cryo SEM: Equipement

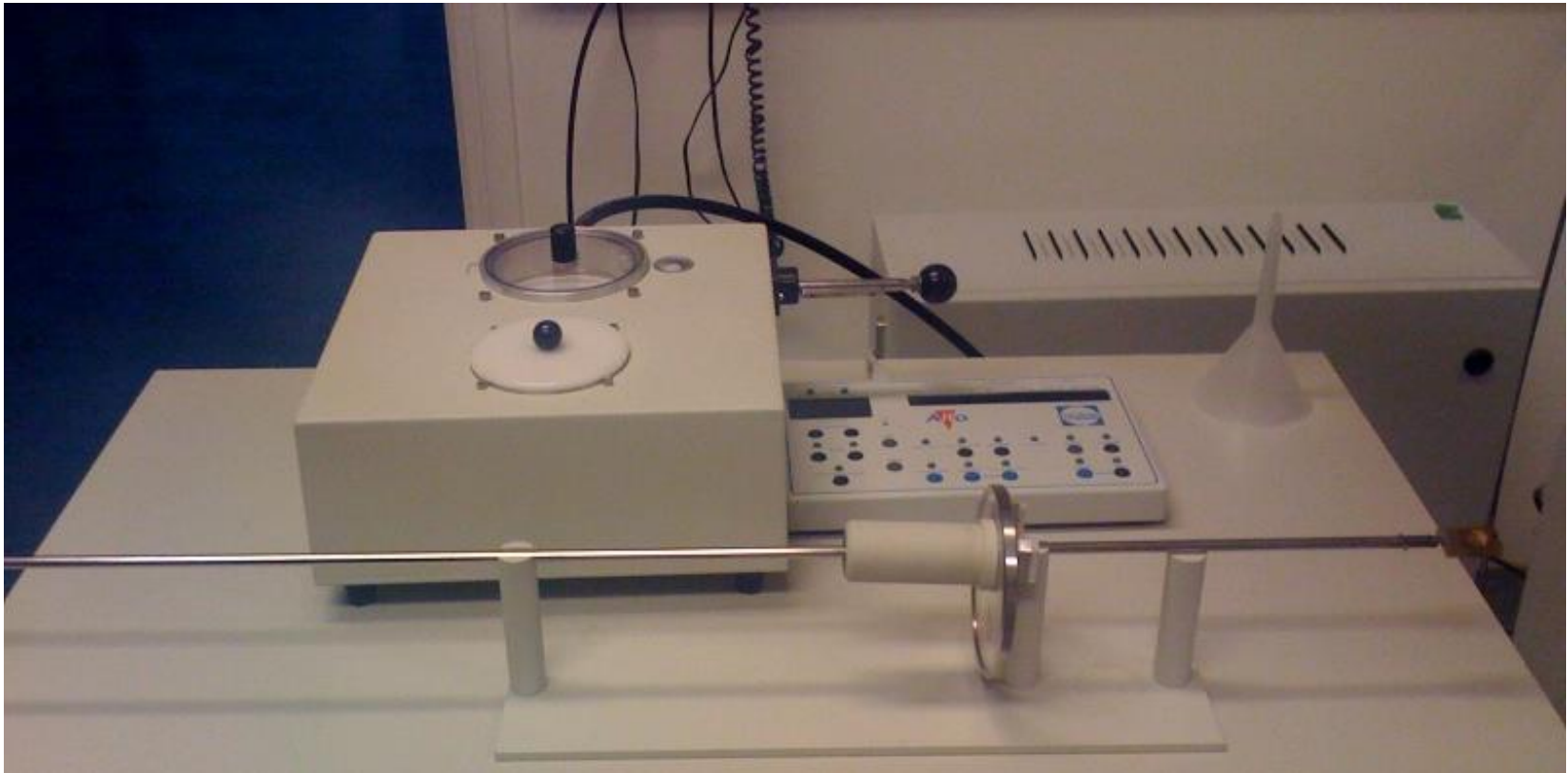
Systeme cryo transfert ALTO 2500



Cryo SEM: Equipement

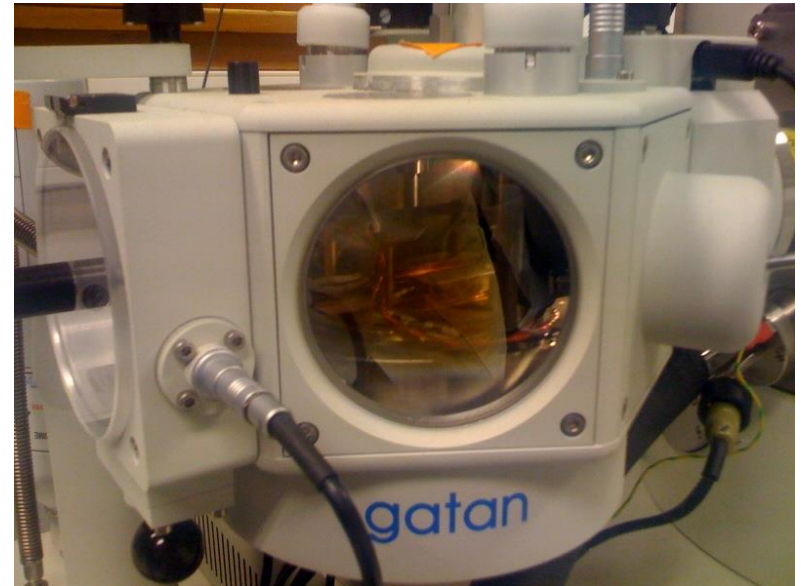
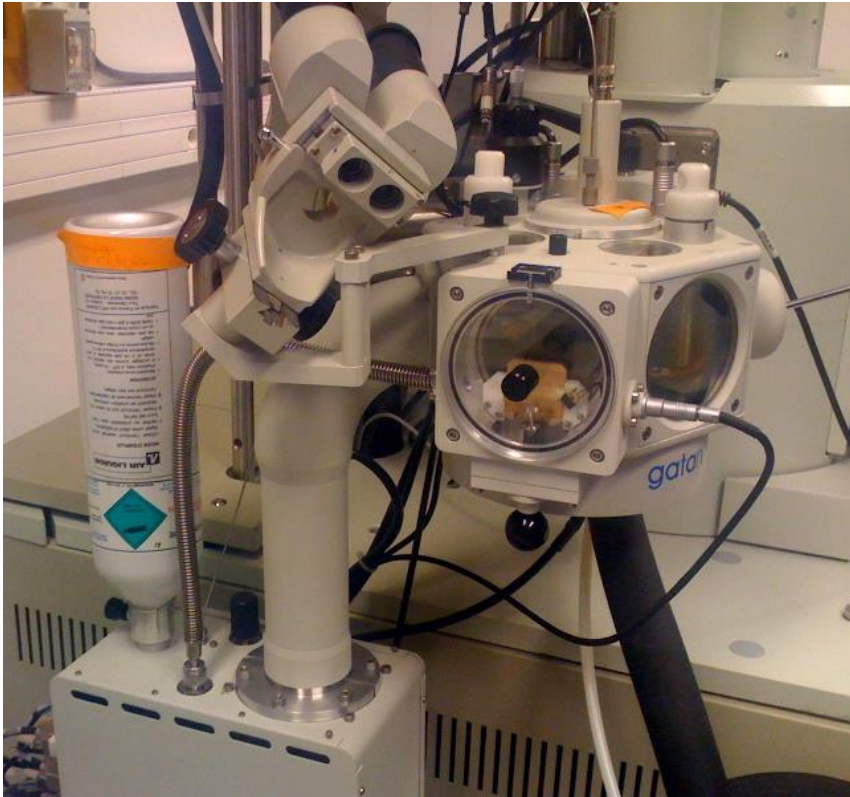
Module de préparation des échantillons

Cryo Fixation



Cryo SEM: Equipement

Chambre de transfert des échantillons



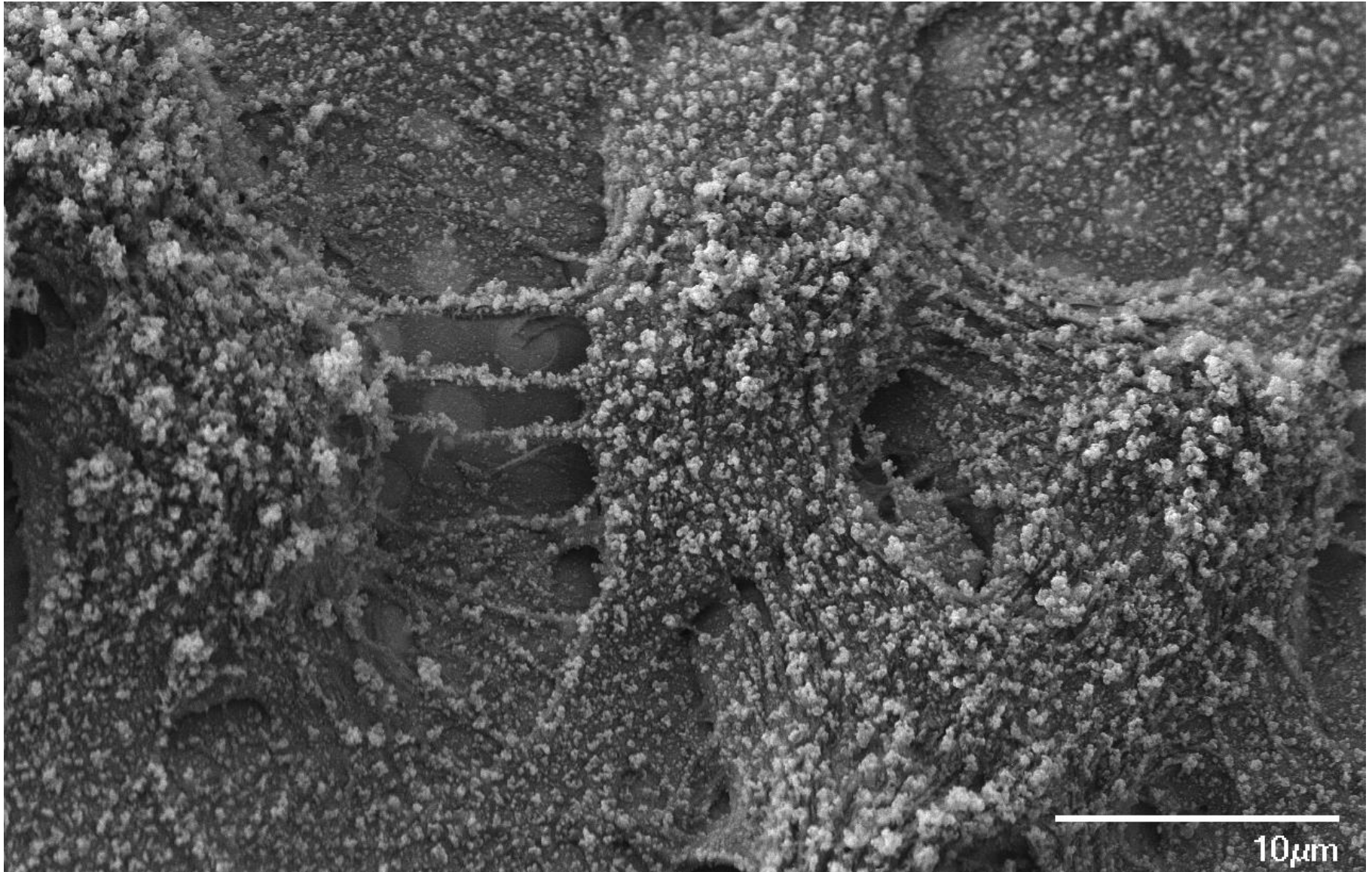
Sublimation



Métallisation

Cryo SEM: Artefact

Formation cristaux de glaces à la surface

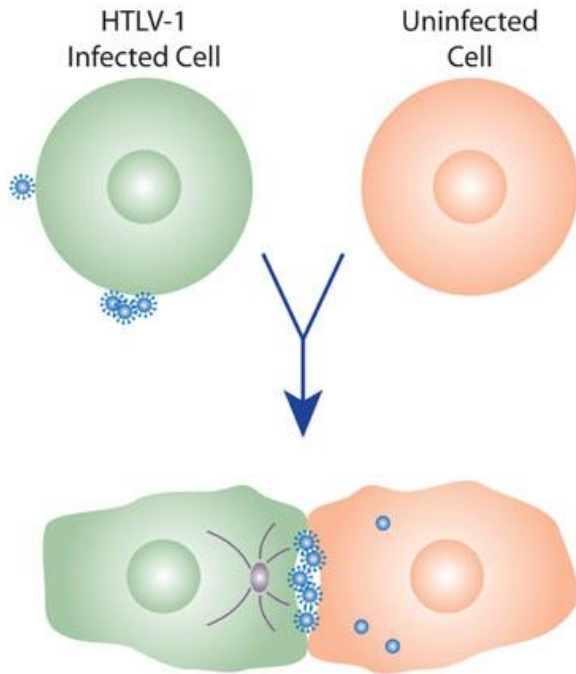


Etude du VIH

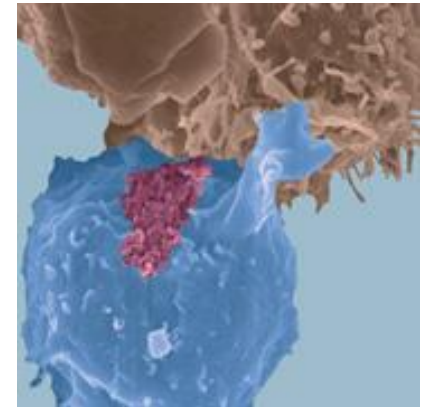
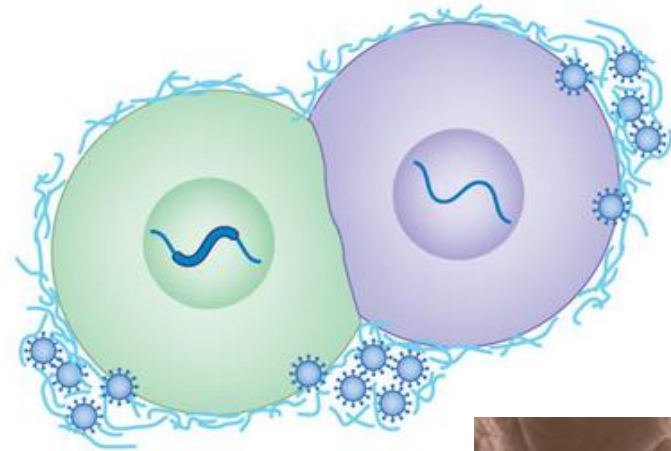
+ 25 millions de morts depuis 1980
Transmission par fluides corporels

Aucuns traitements curatifs
Cible les cellules du système immunitaire

1) Synapse virologique

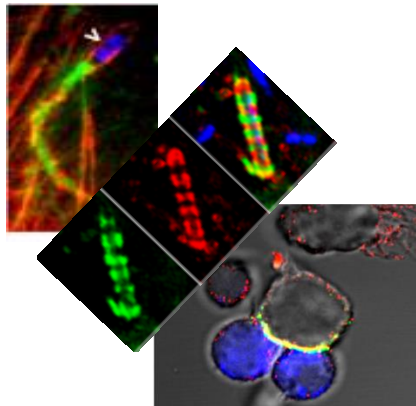


2) Biofilm viral



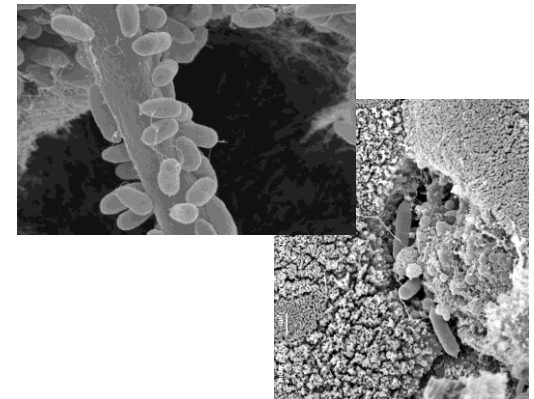
Méthode « CLEM »: Principe

Light Microscope (LM)



Analyses fonctionnelles,
quantitatives, données
dynamiques

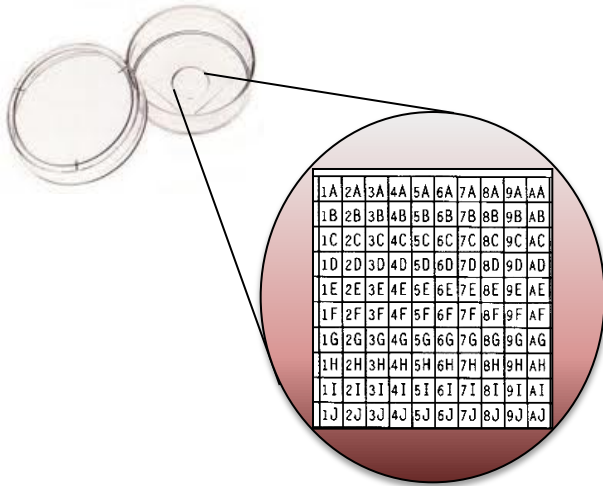
Electron Microscope (EM)



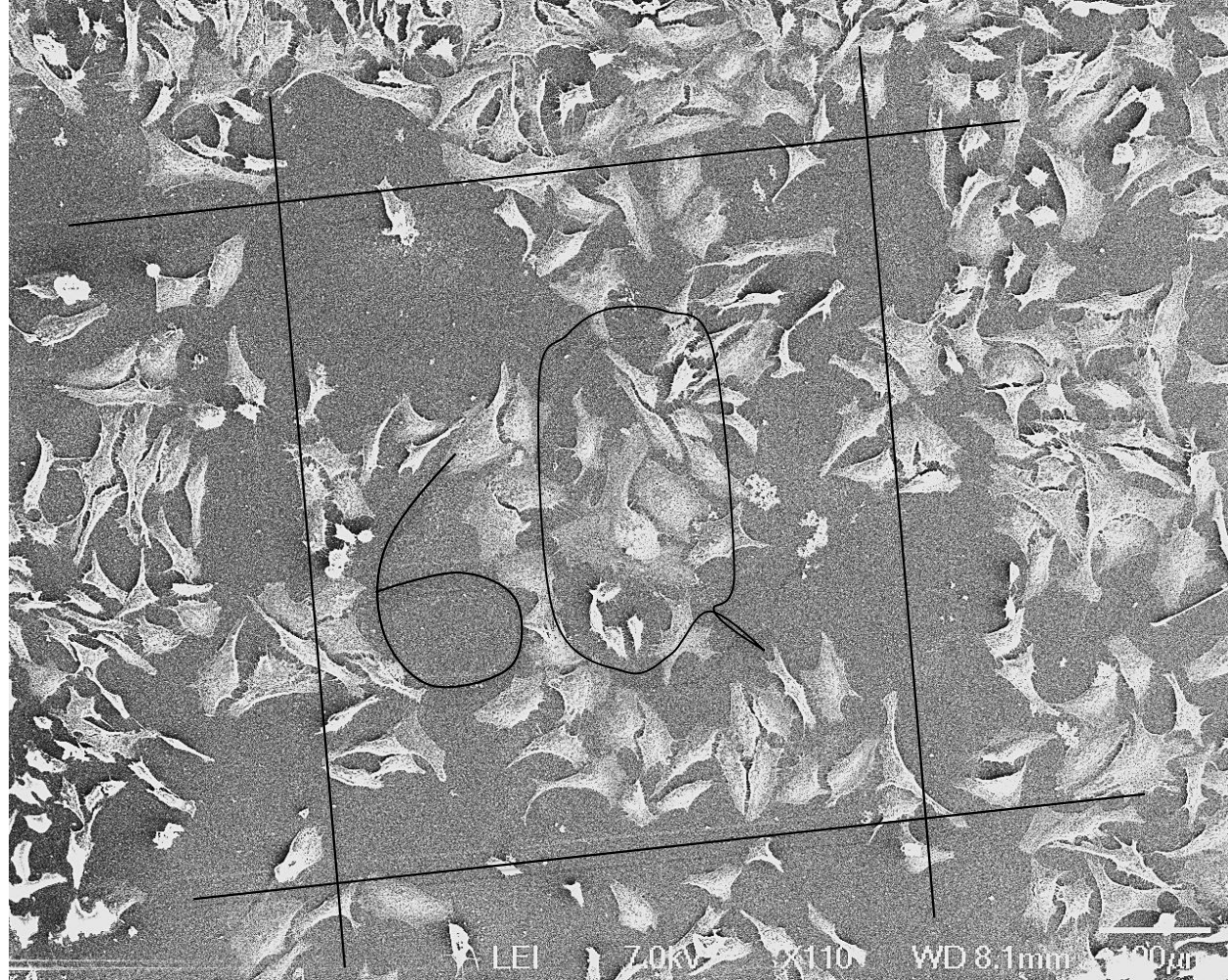
Informations de structure,
haute résolution, localisation
de protéines de surface

Méthode « CLEM »

Correlative Light and Electron Microscopy



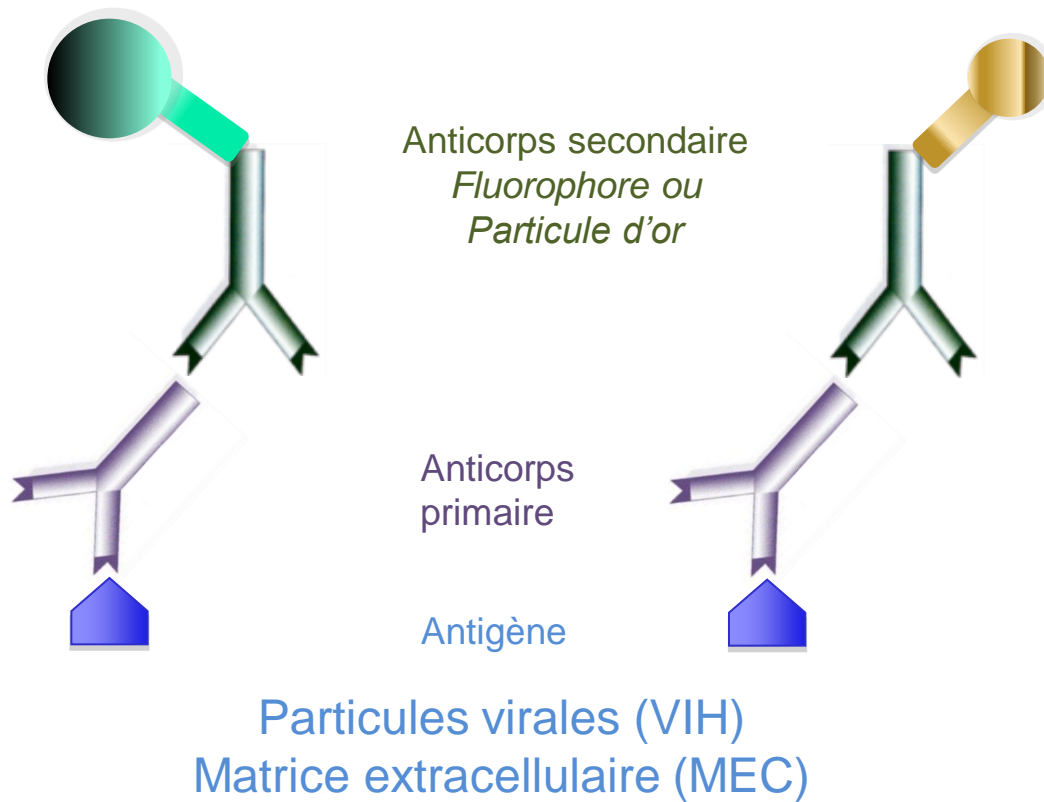
3B	4B	5B
3C	4C	5C
3D	4D	5D



Etude du mode de transmission du VIH de cellules à cellules

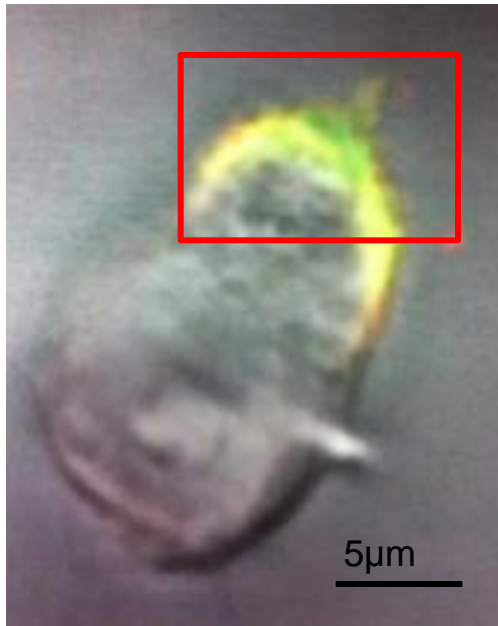
L'échantillon lymphocytes infectés par le VIH

Immuno-marquage

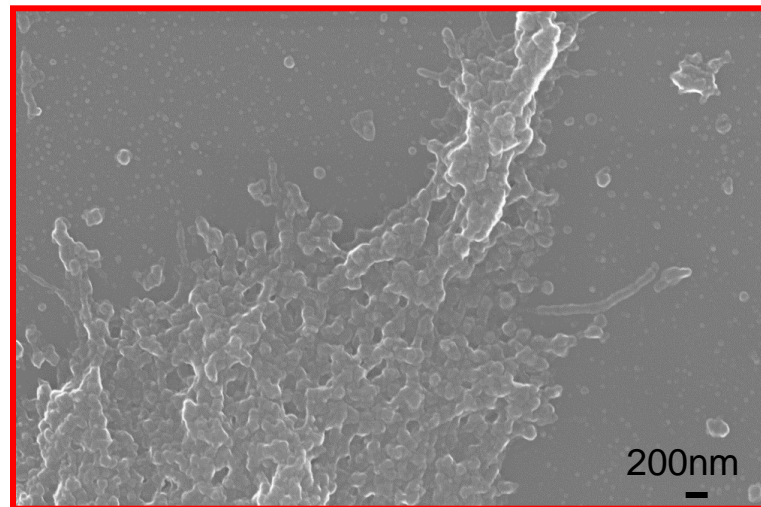
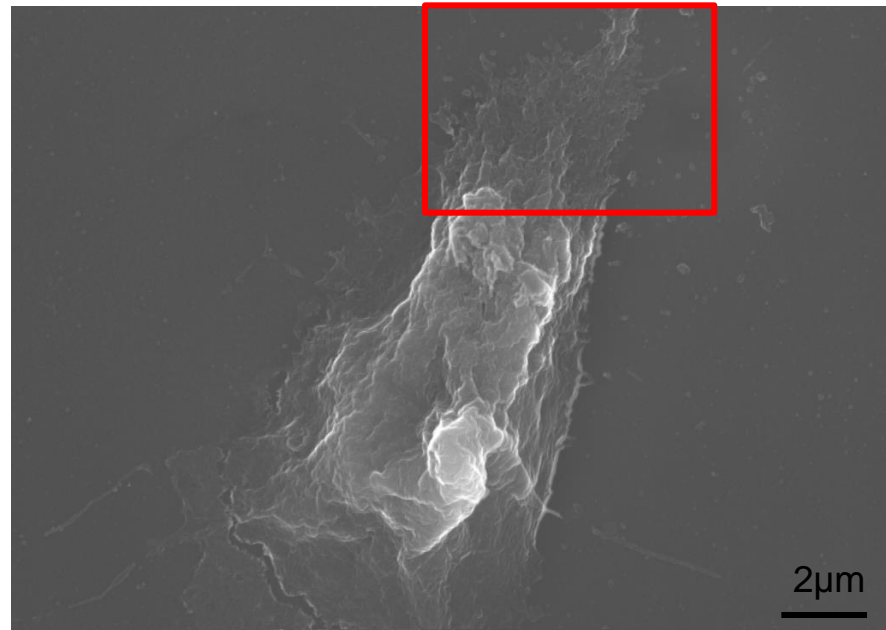


RT CLEM: retrouver la cellule d'intéret

FLM



SEM

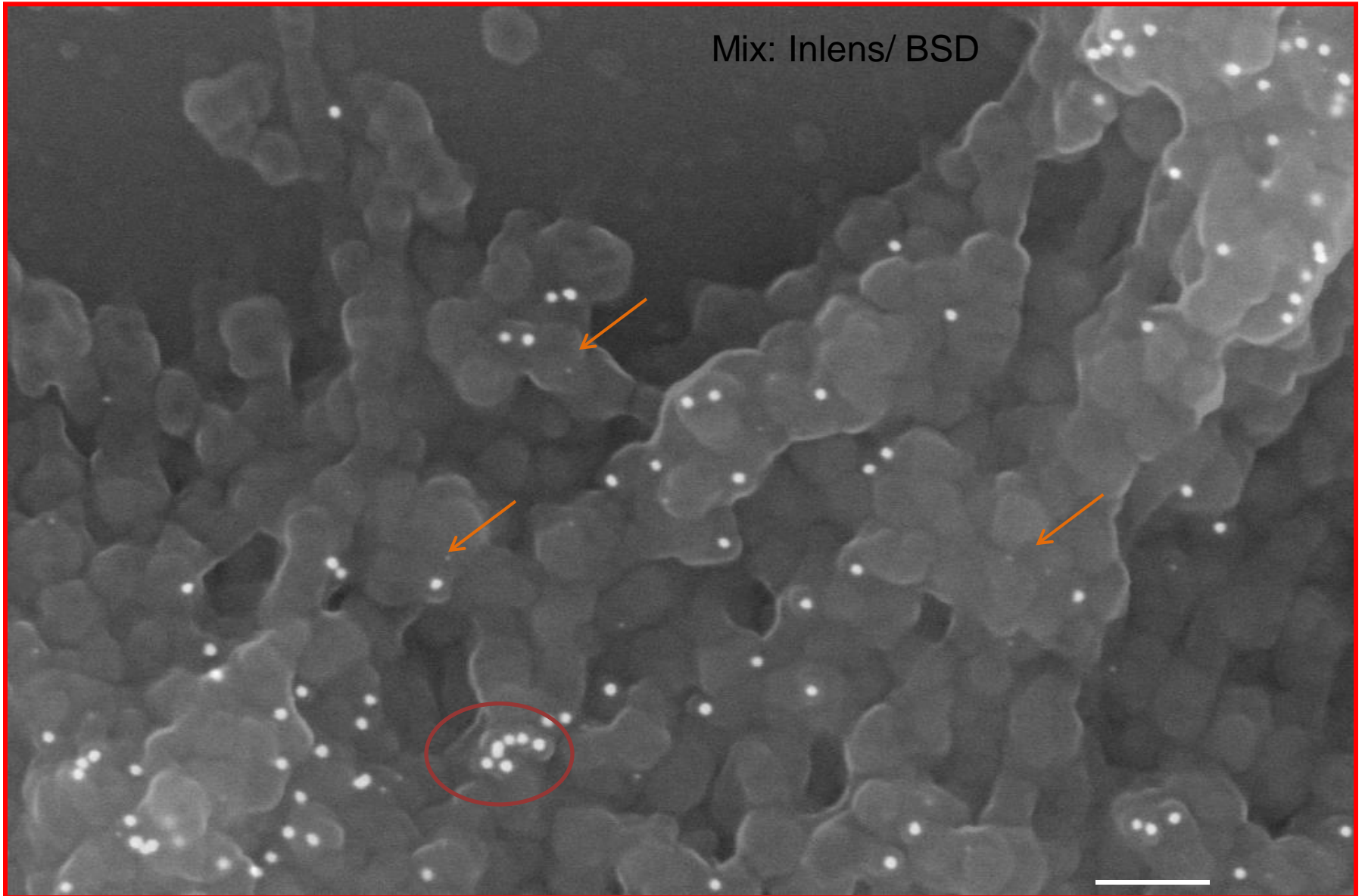


20nm: anti-MEC

10nm: anti-HIV

ACH2 cell line

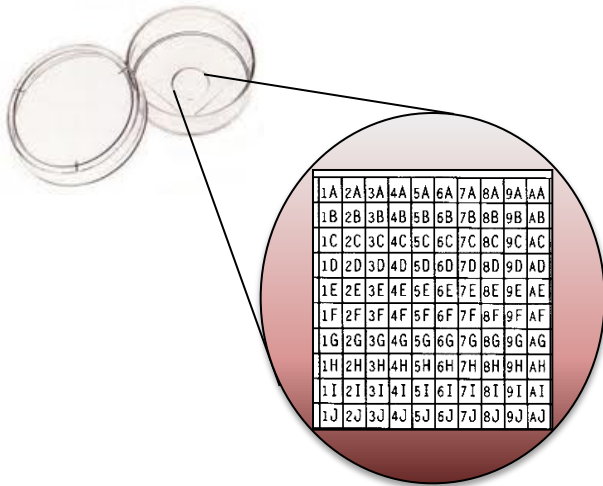
RT CLEM: immuno-localisation des particules virales



20nm: anti-MEC

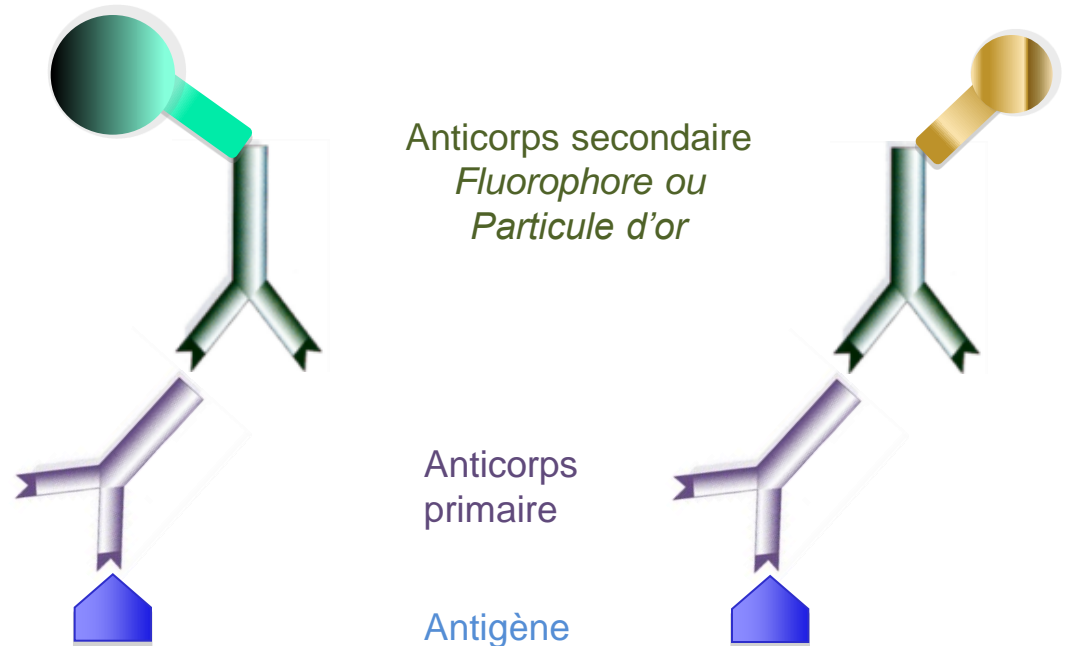
10nm: anti-HIV

CRYO_SEM



3B	4B	5B
3C	4C	5C
3D	4D	5D

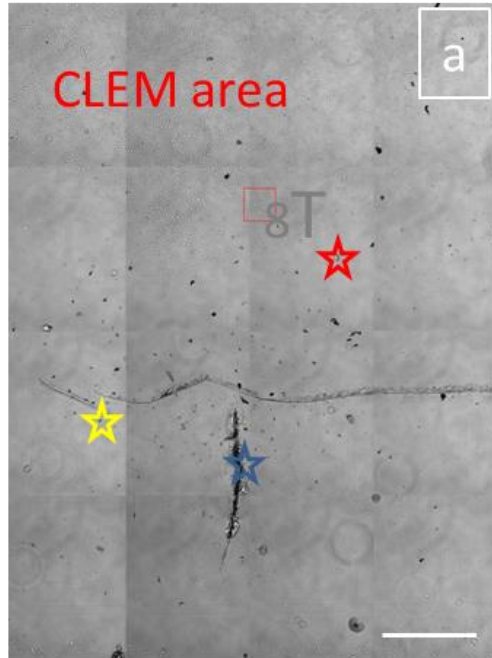
Immuno-marquage



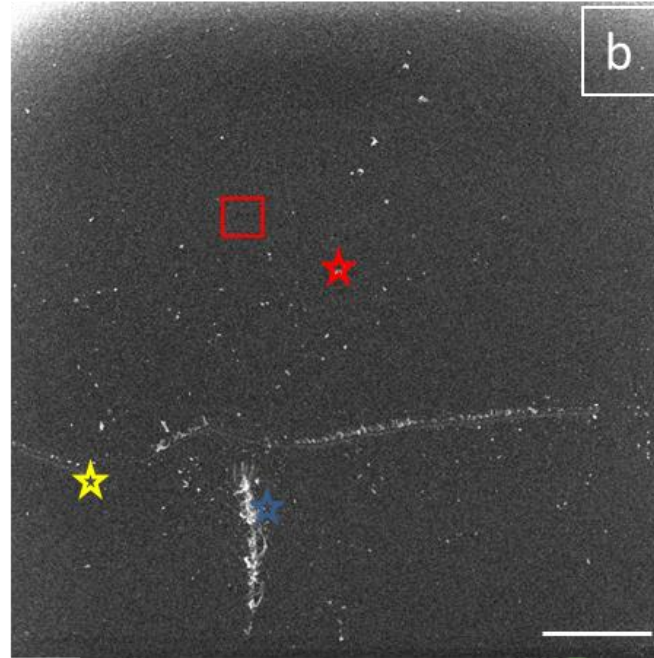
Particules virales (VIH)
Matrice extracellulaire (MEC)

CRYO_SEM: méthode

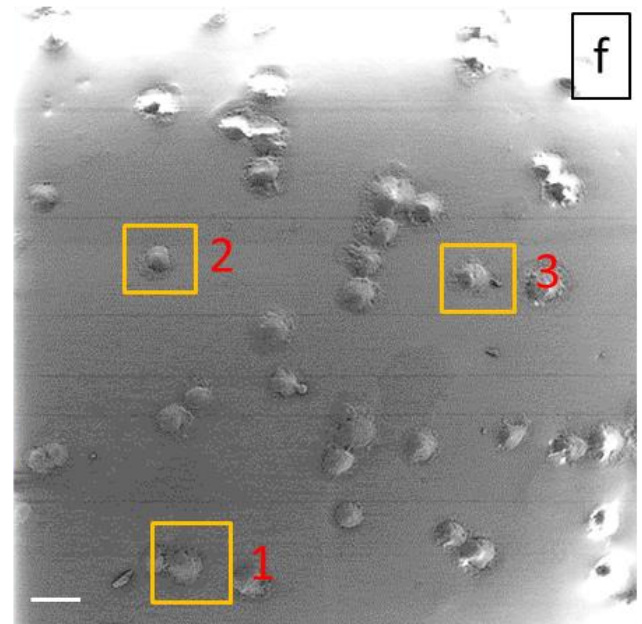
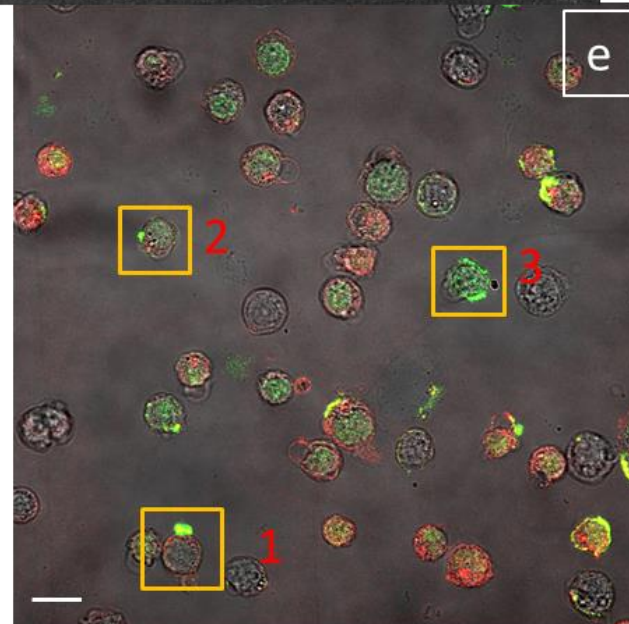
FLM: Mosaic



Cryo-SEM: overview

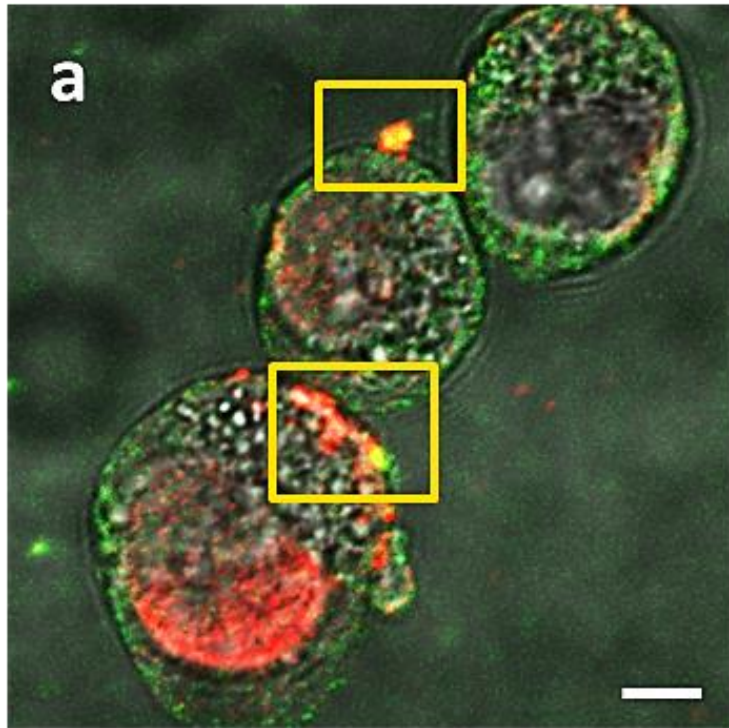


APPLY
TURBOREG

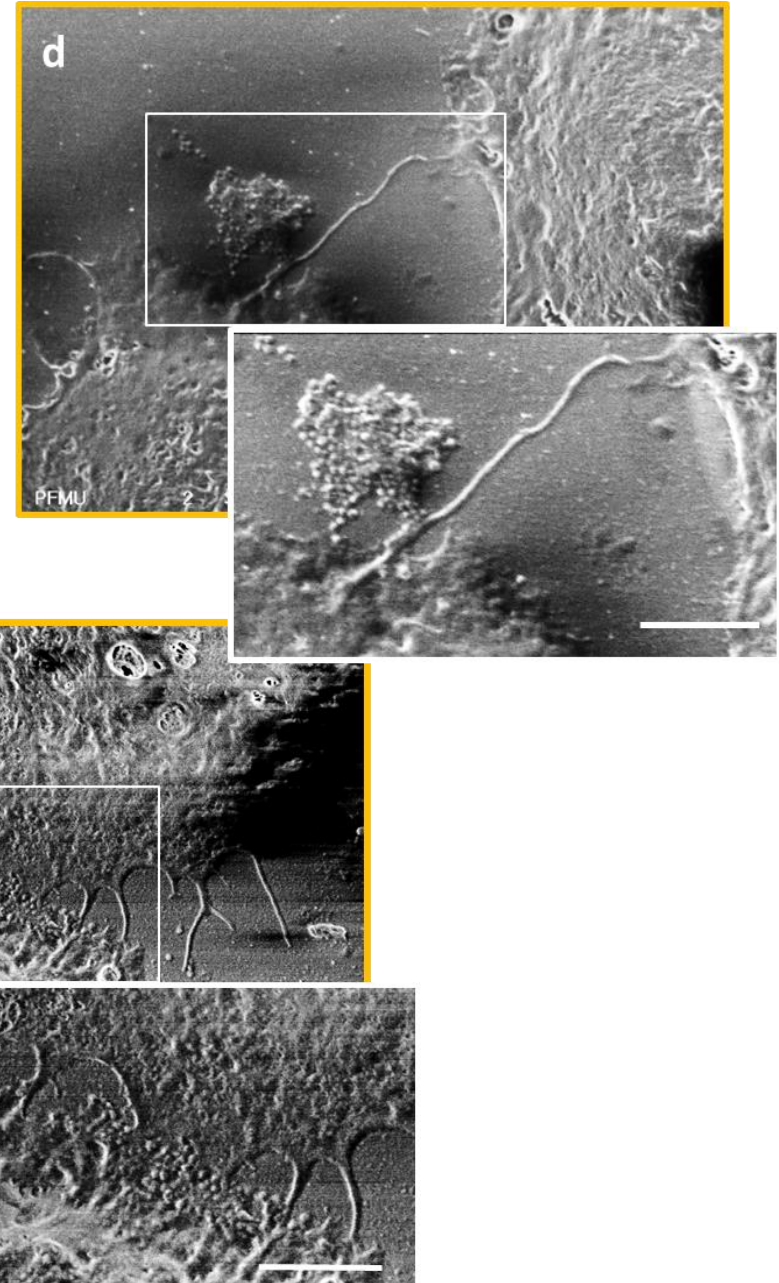


Cryo CLEM: retrouver la cellule d'intéret

FLM

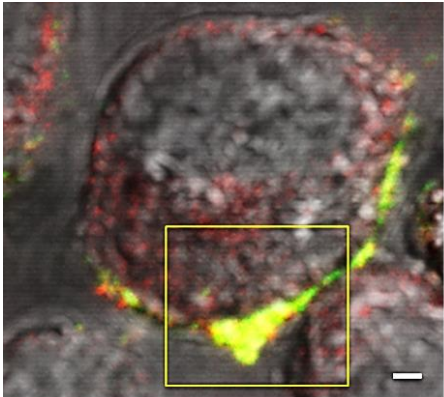


Cryo SEM

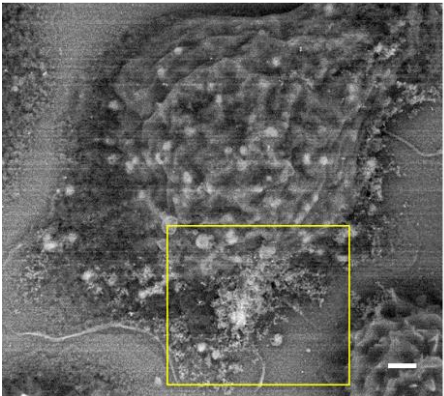
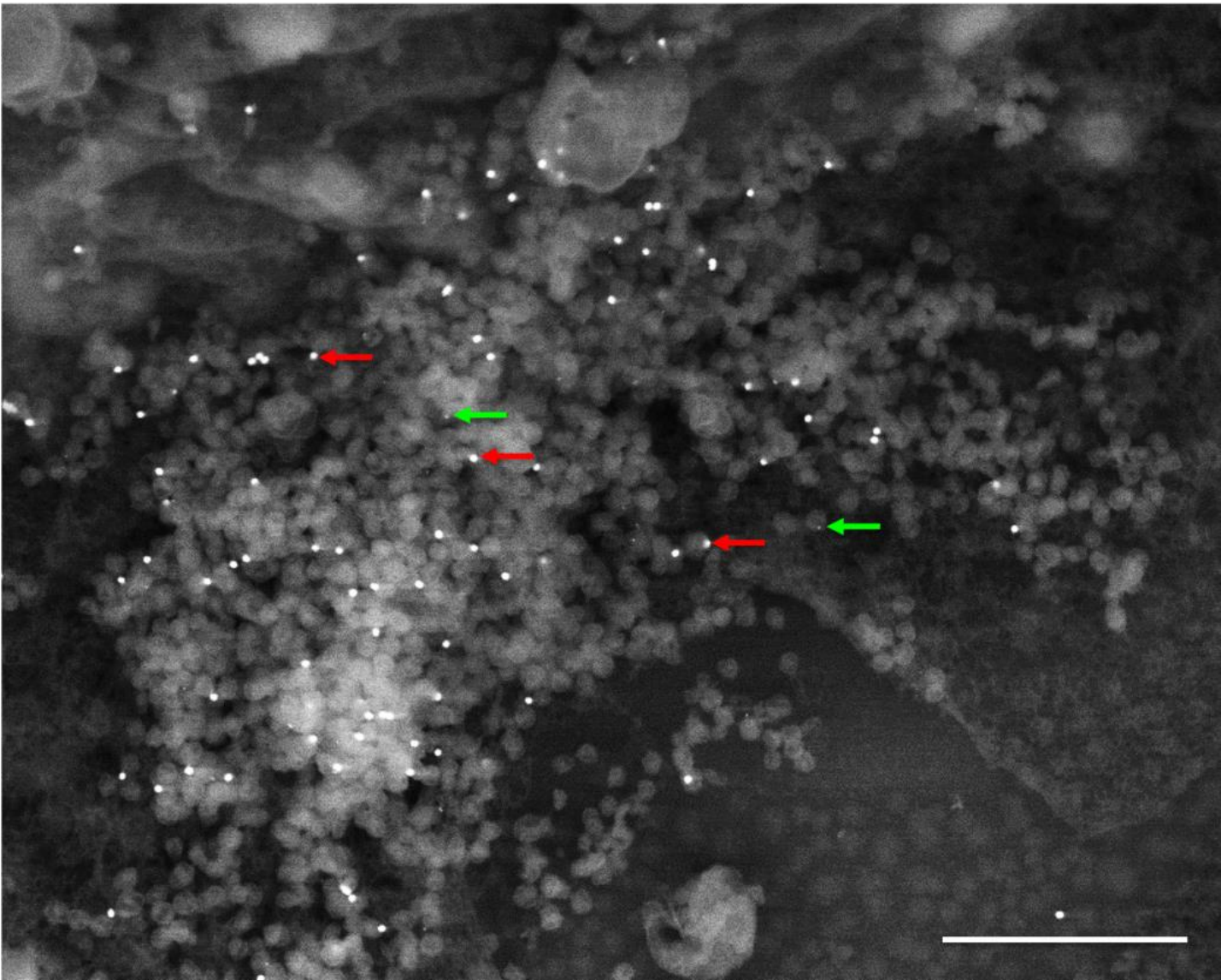


Cryo CLEM: immuno-localisation des particules virales

FLM



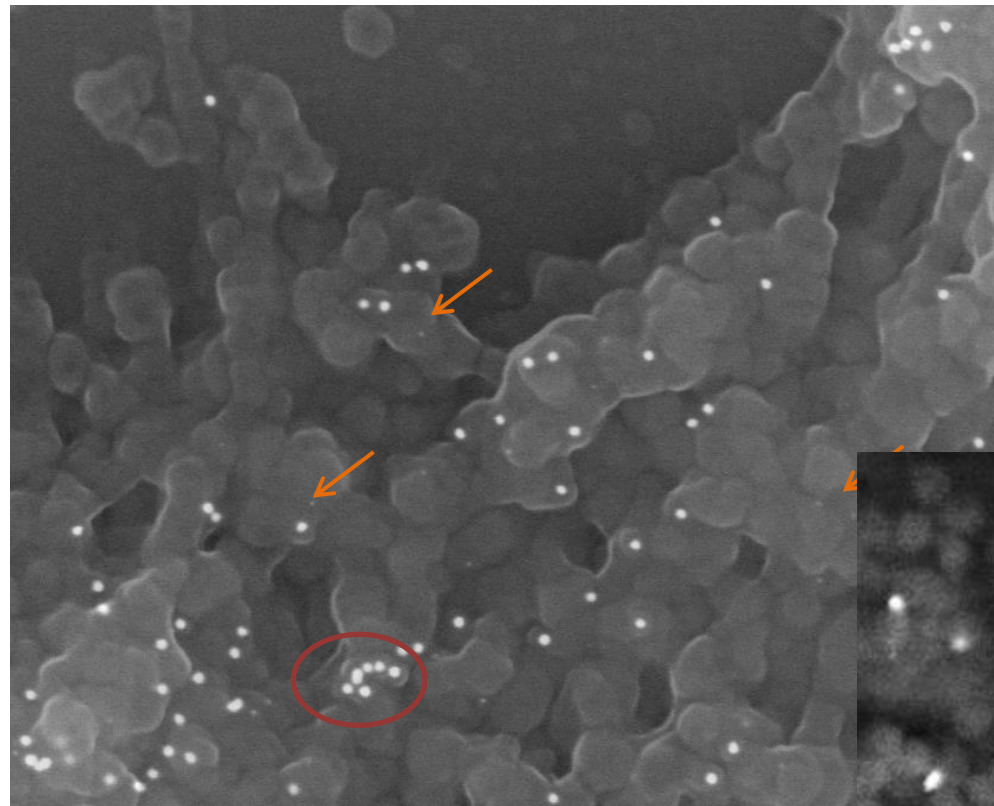
Cryo SEM



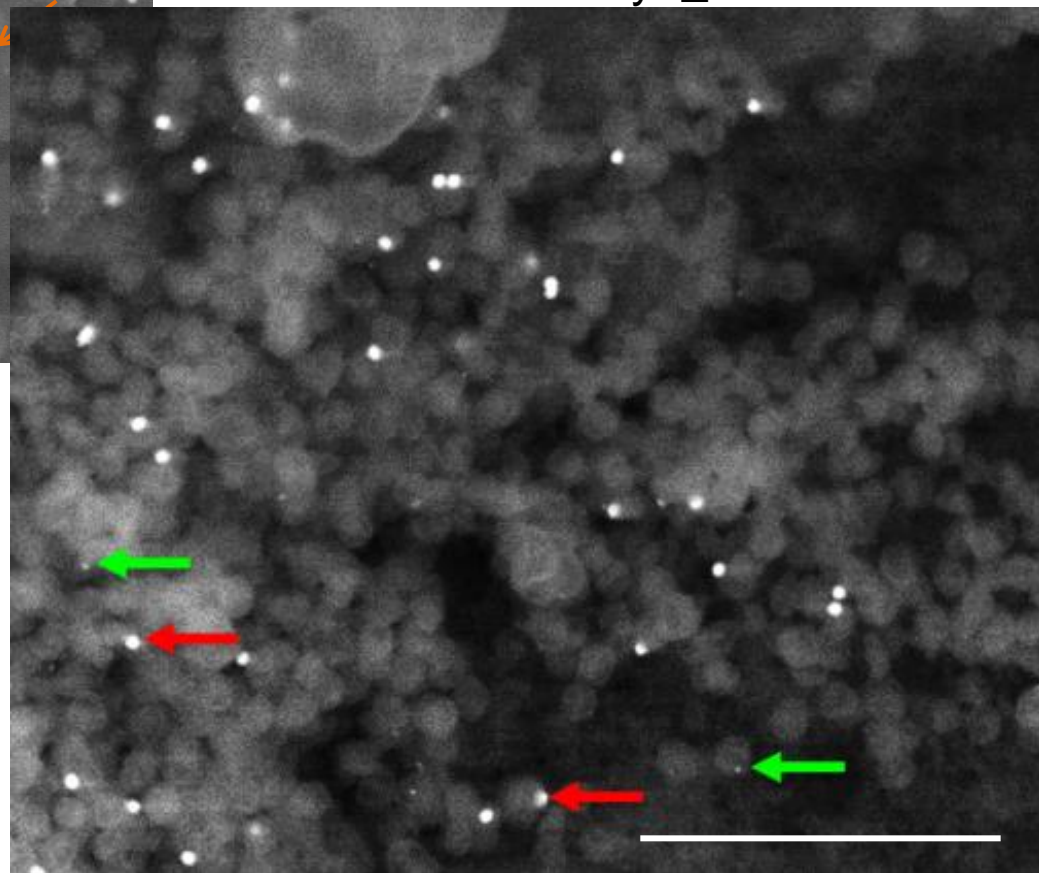
20nm: anti-MEC

10nm: anti-HIV

Comparatif



Cryo_SEM



Conclusion

Avantages

- L'observation de structures biologiques au plus proche de l'état natif
- Une observation très rapide de l'échantillon
- Cryofractures possible

Inconvénients

- Techniquement plus délicat
- L'échantillon est perdu après observation

Remerciements



Unité de Virologie Structurale
Marie Isabelle Thoulouze
Catherine Inizan



FRANCE-BIOIMAGING