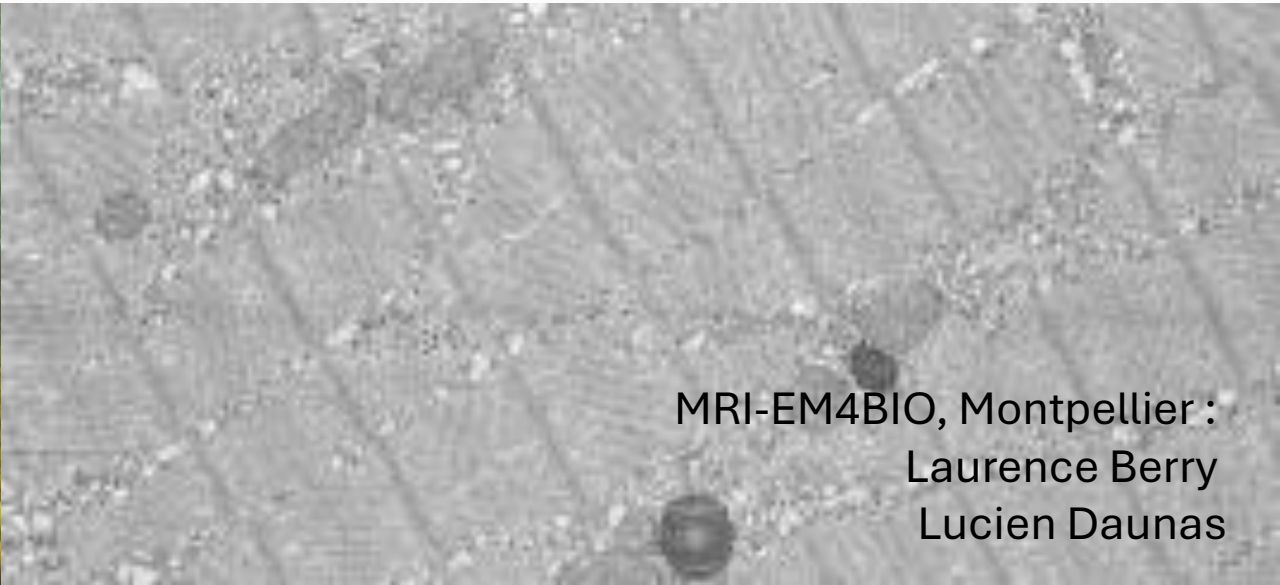


Etude des interactions mitochondries-réticulum sarcoplasmique dans le muscle squelettique de truite par microscopie électronique à balayage par coupes sériées.

Angelina Campagna

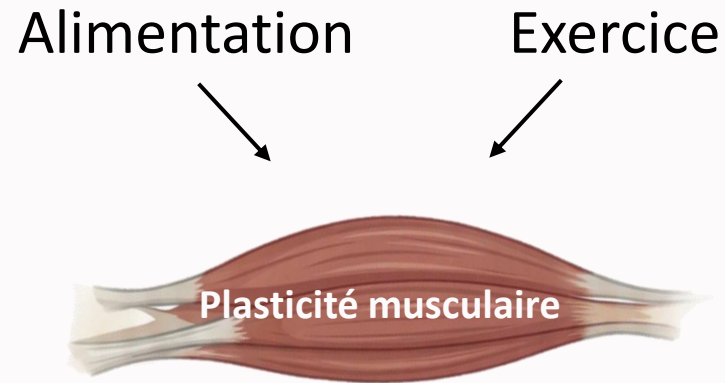


DMEM, Montpellier :
Béatrice Chabi,
Vincent Ollendorff,
LPGP, Rennes :
Jean-Charles Gabillard

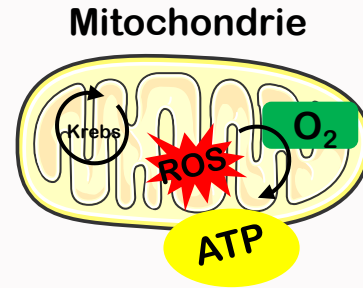


MRI-EM4BIO, Montpellier :
Laurence Berry
Lucien Daunas

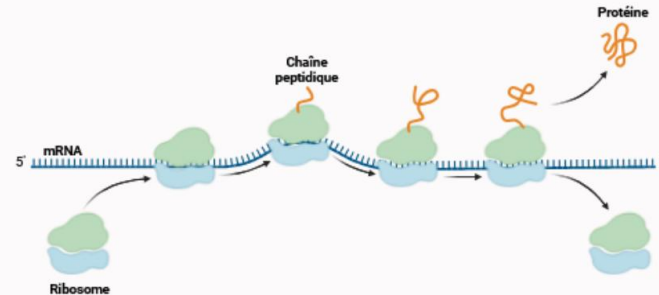
Régulation des interactions entre les mitochondries et la synthèse protéique dans le muscle squelettique



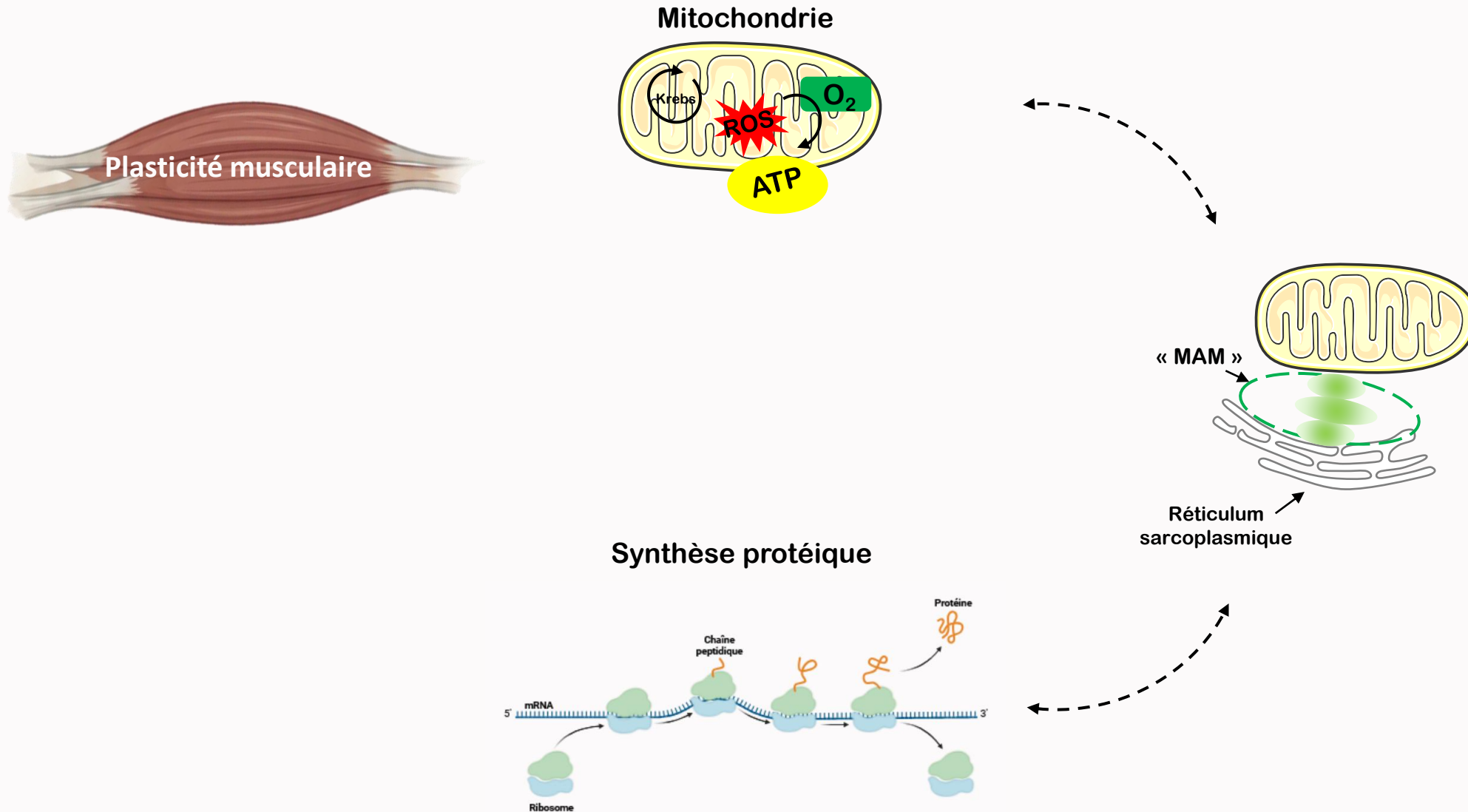
Régulation des interactions entre les mitochondries et la synthèse protéique dans le muscle squelettique



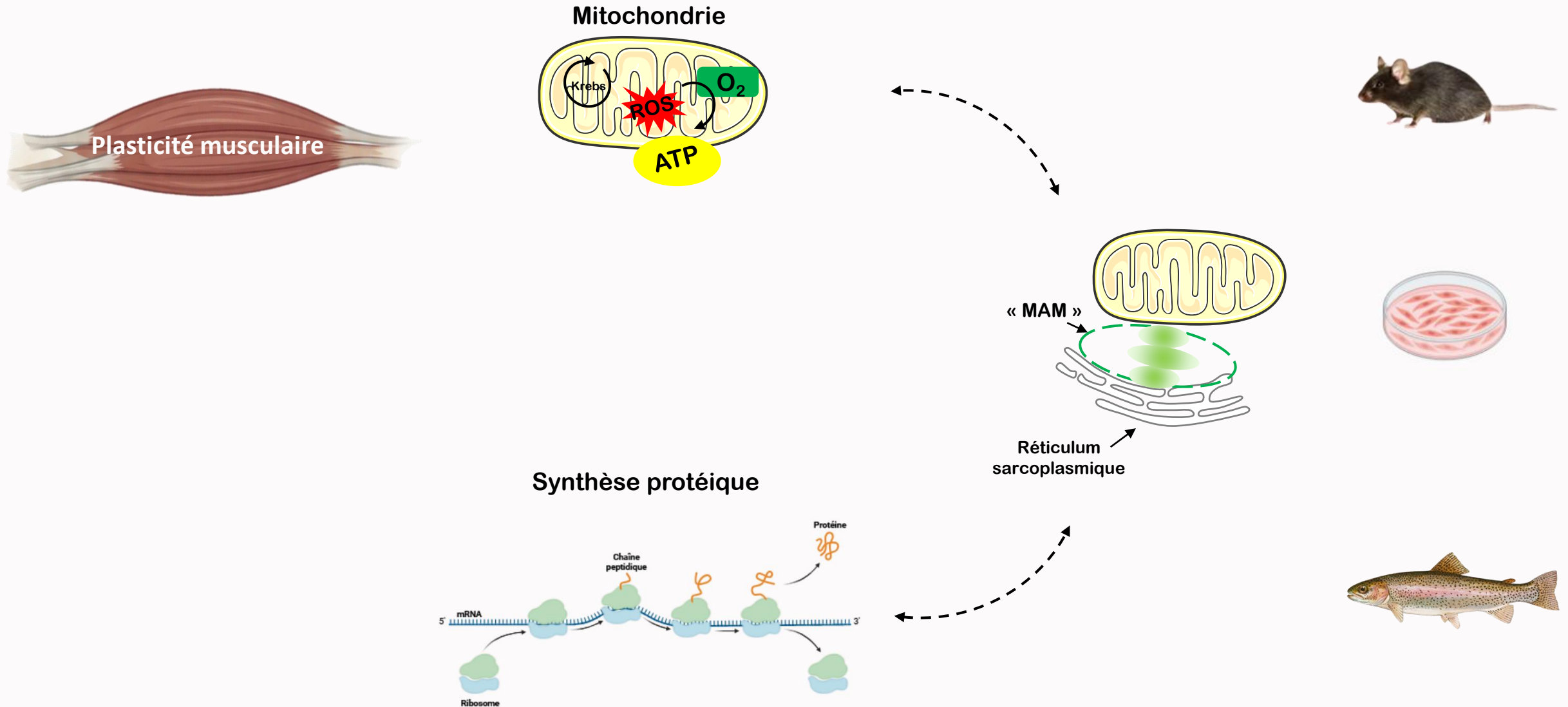
Synthèse protéique



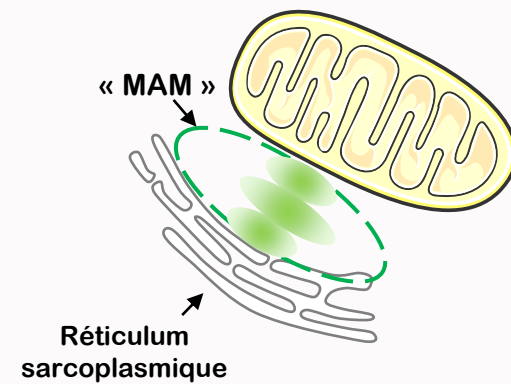
Régulation des interactions entre les mitochondries et la synthèse protéique dans le muscle squelettique



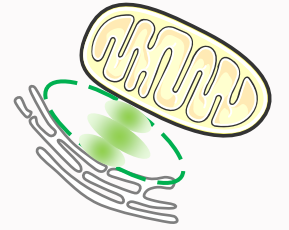
Régulation des interactions entre les mitochondries et la synthèse protéique dans le muscle squelettique



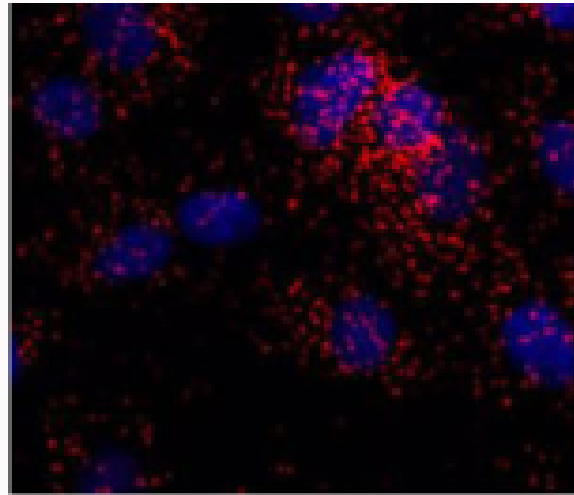
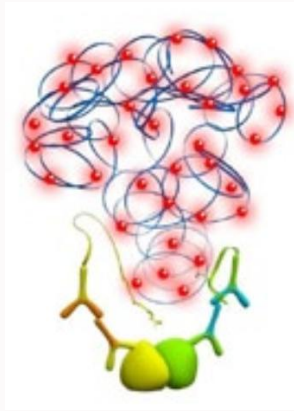
Comment quantifier ces zones d'interactions ?



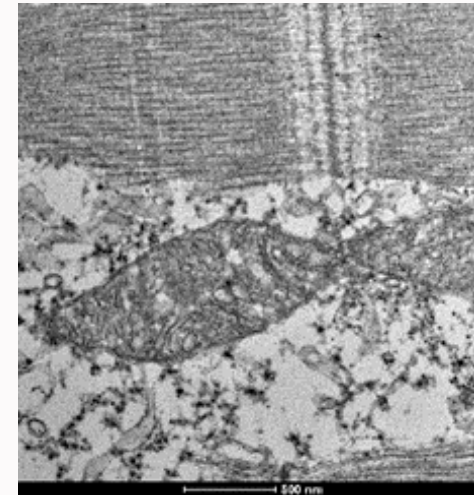
Méthodes de quantification des interactions Mitochondrie/RS sur échantillons fixés



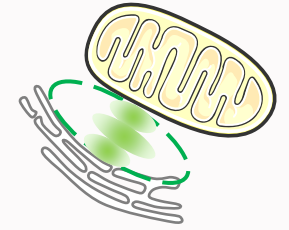
Proximity ligation assay (PLA)



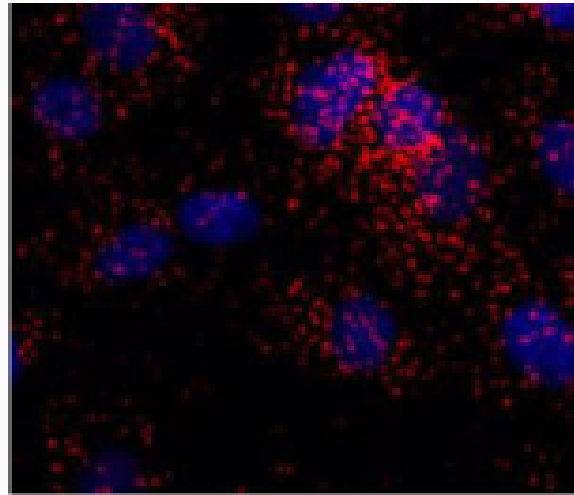
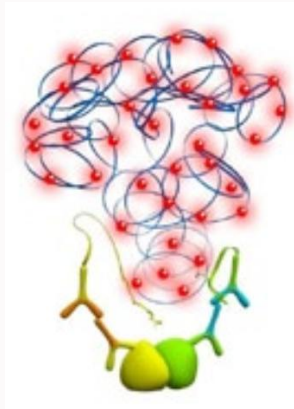
Microscopie électronique



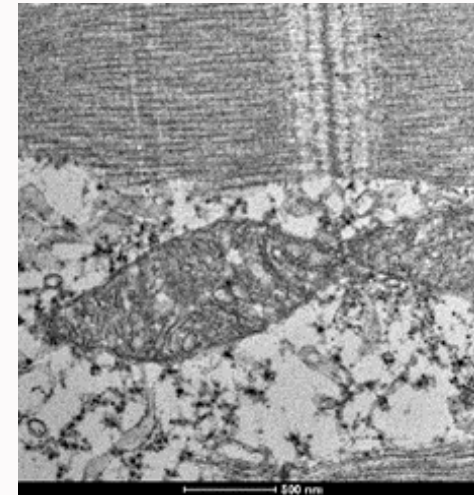
Méthodes de quantification des interactions Mitochondrie/RS sur échantillons fixés



Proximity ligation assay (PLA)



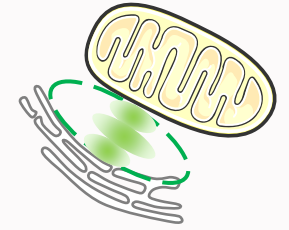
Microscopie électronique



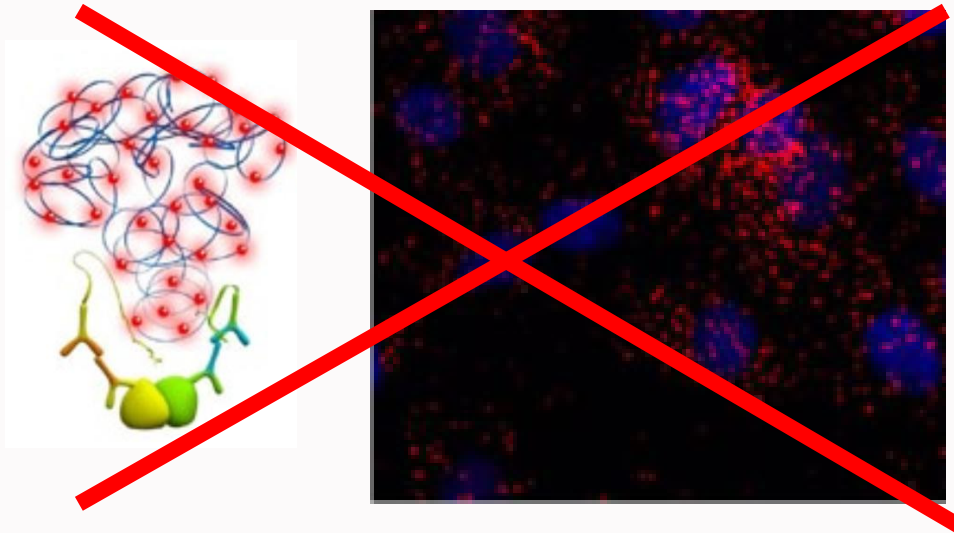
Limites du PLA :

- Distances précises des MAMs ?

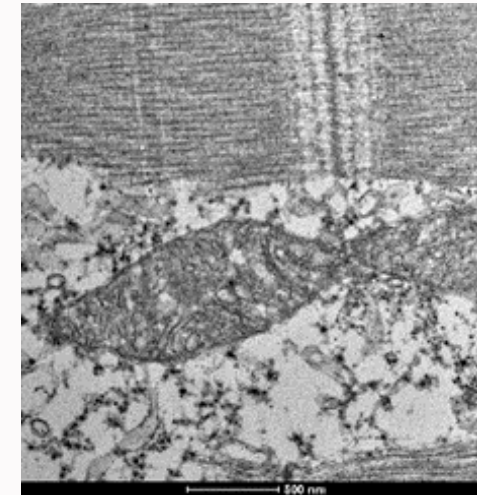
Méthodes de quantification des interactions Mitochondrie/RS sur échantillons fixés



Proximity ligation assay (PLA)



Microscopie électronique

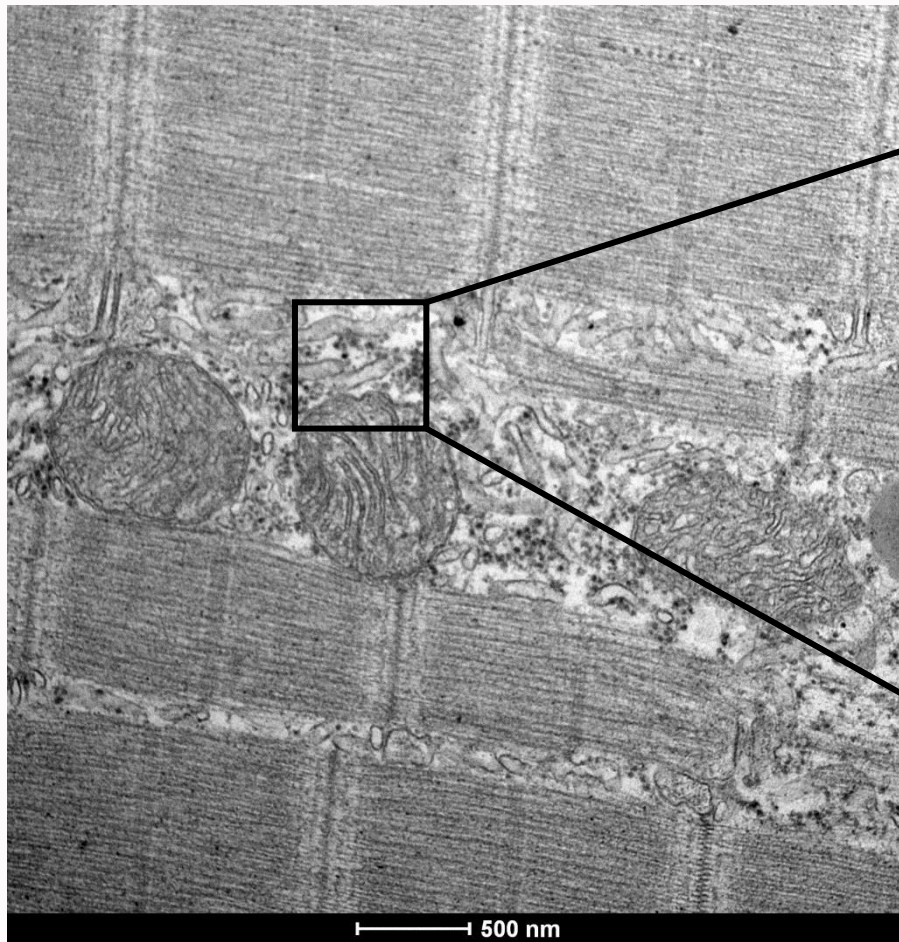
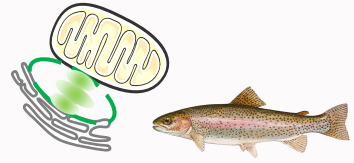


Limites du PLA :

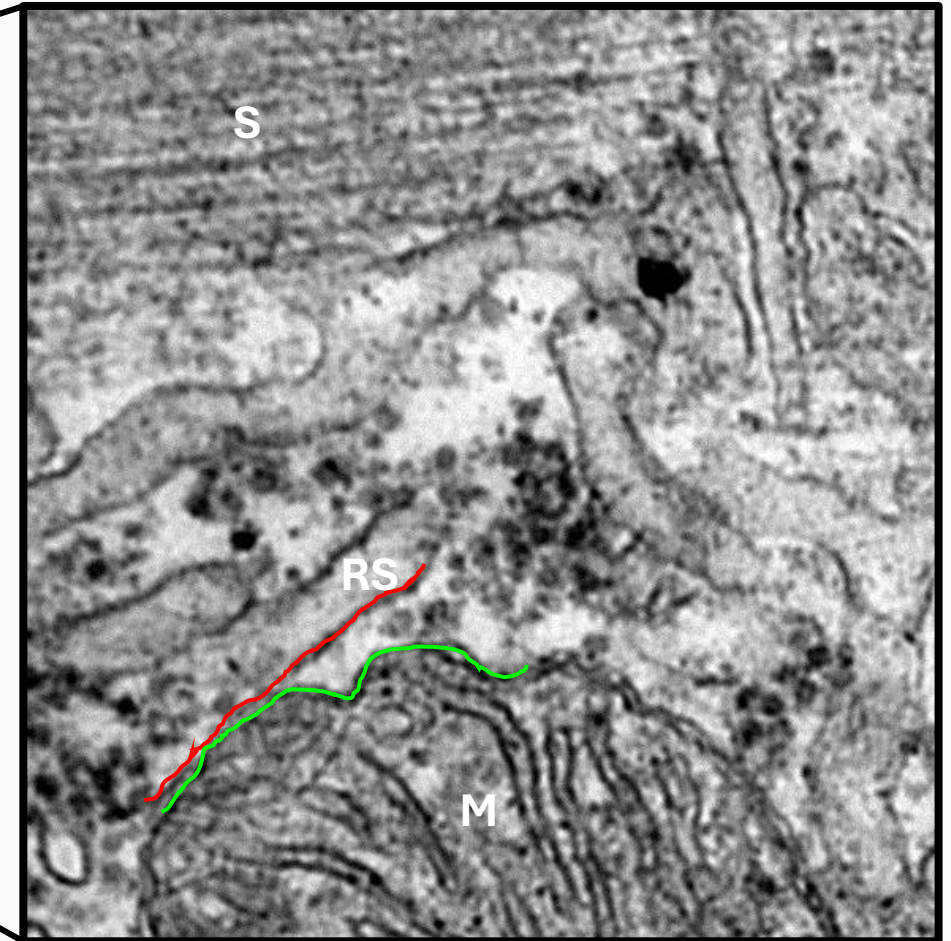
- Distances précises des MAMs ?
- Anticorps pas spécifique pour la truite arc en ciel



Interactions entre les mitochondries et le RS en MET 2D

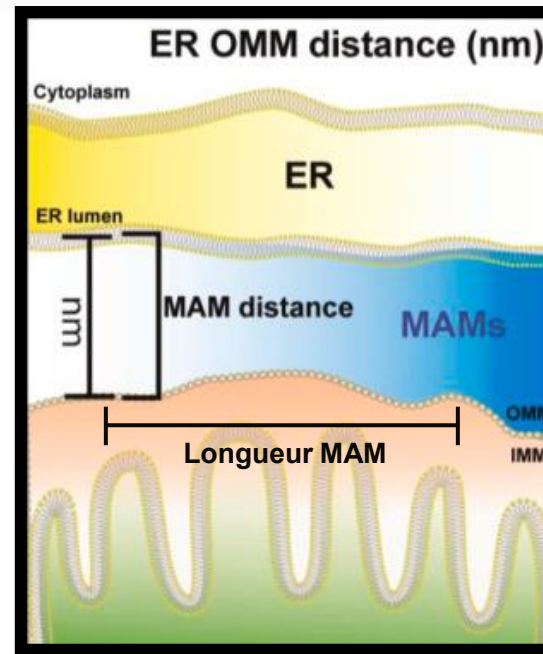
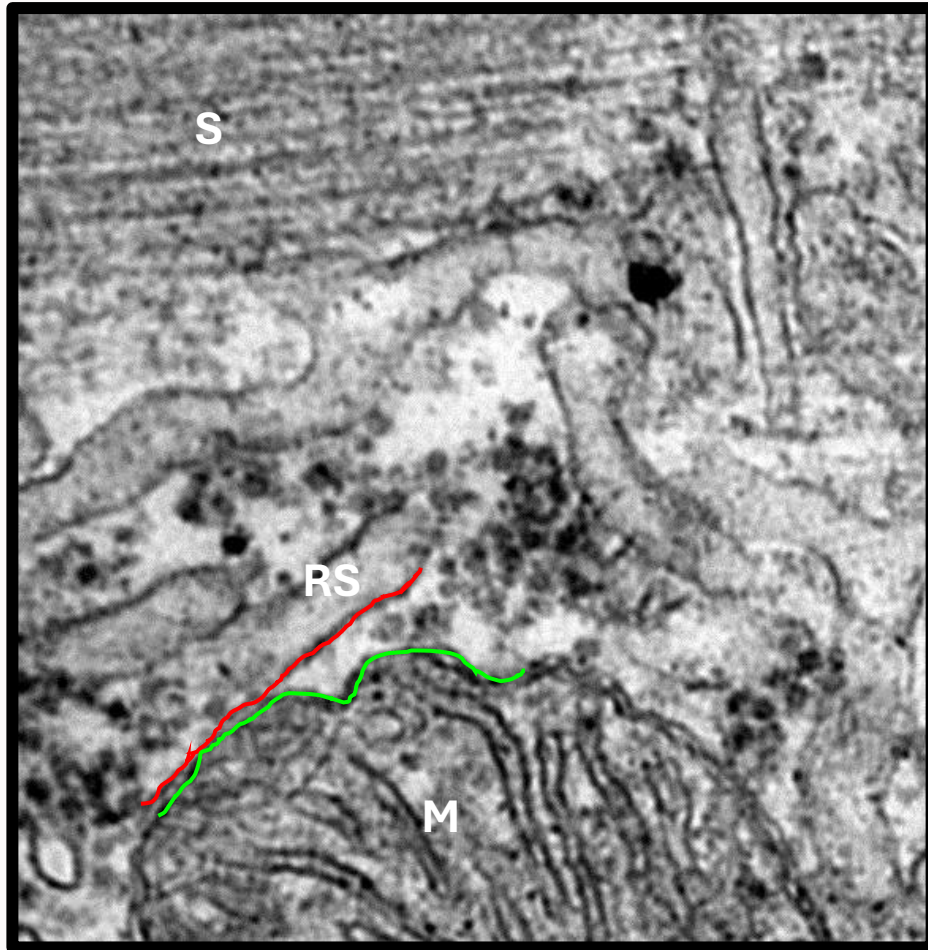
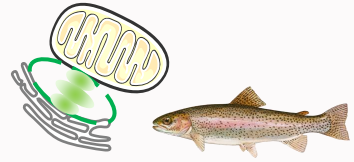


Chantal Cazevielle, Plateau INM COMET



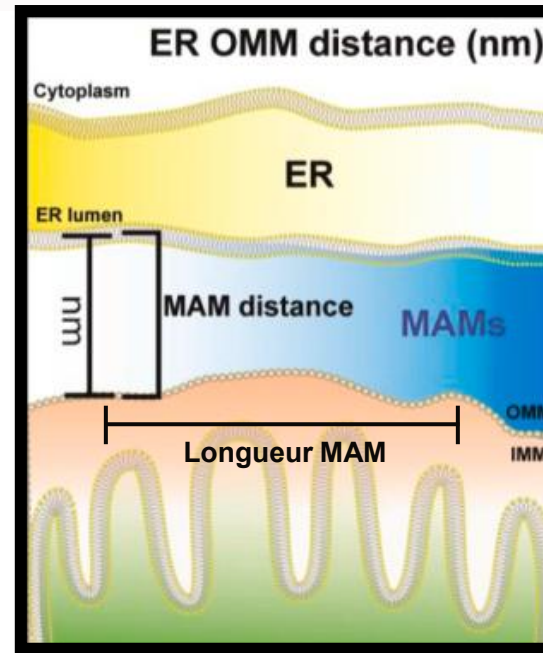
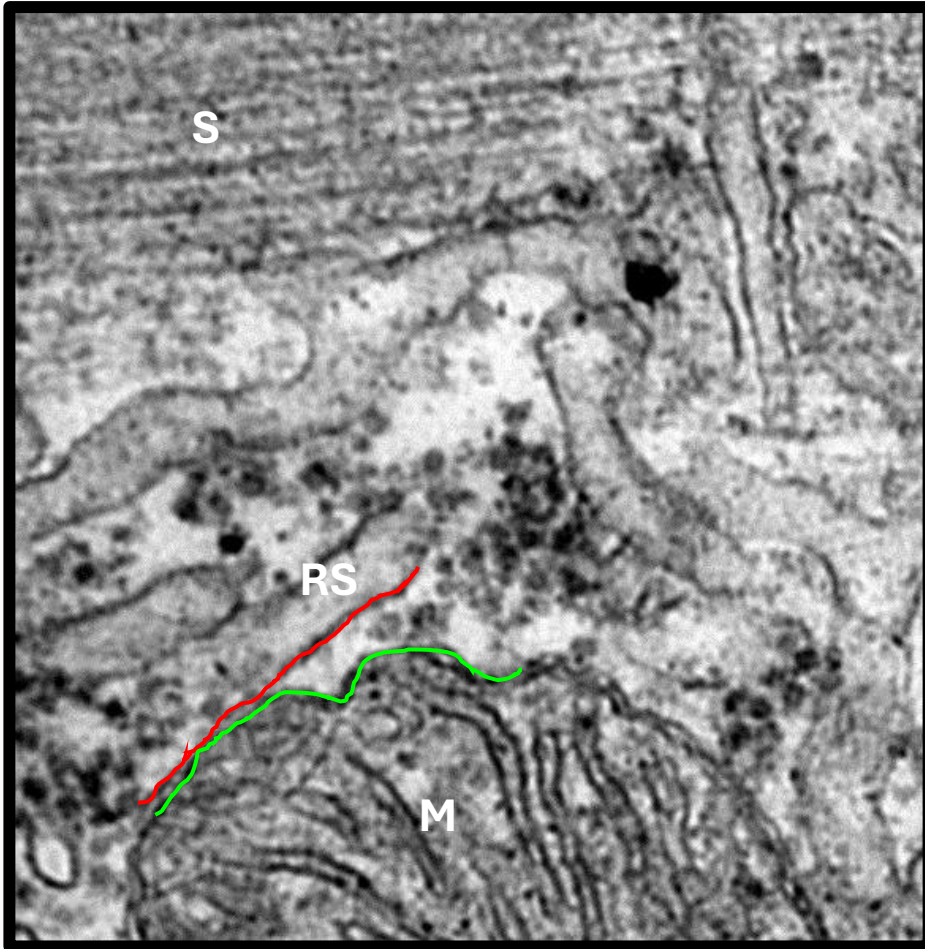
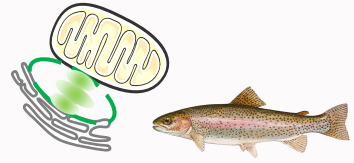
S : Sarcomères
RS : Réticulum Sarcoplasmique
M : Mitochondrie

Interactions entre les mitochondries et le RS en MET 2D



Adapté de Neikirk et al., Eur J Cell Biol 2023

Interactions entre les mitochondries et le RS en MET 2D

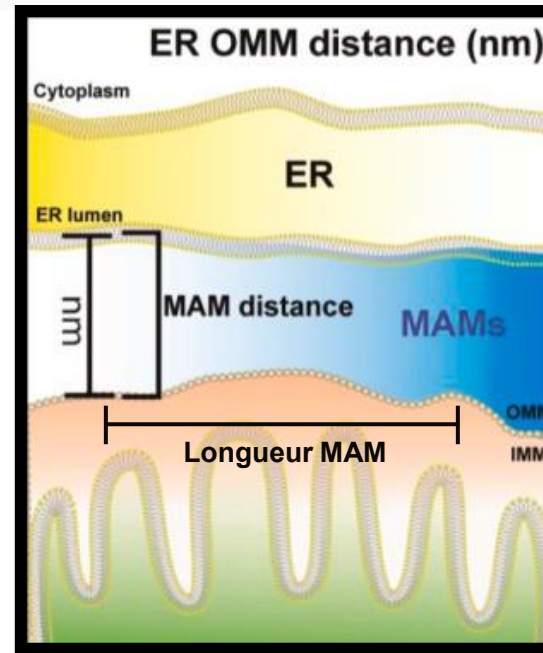
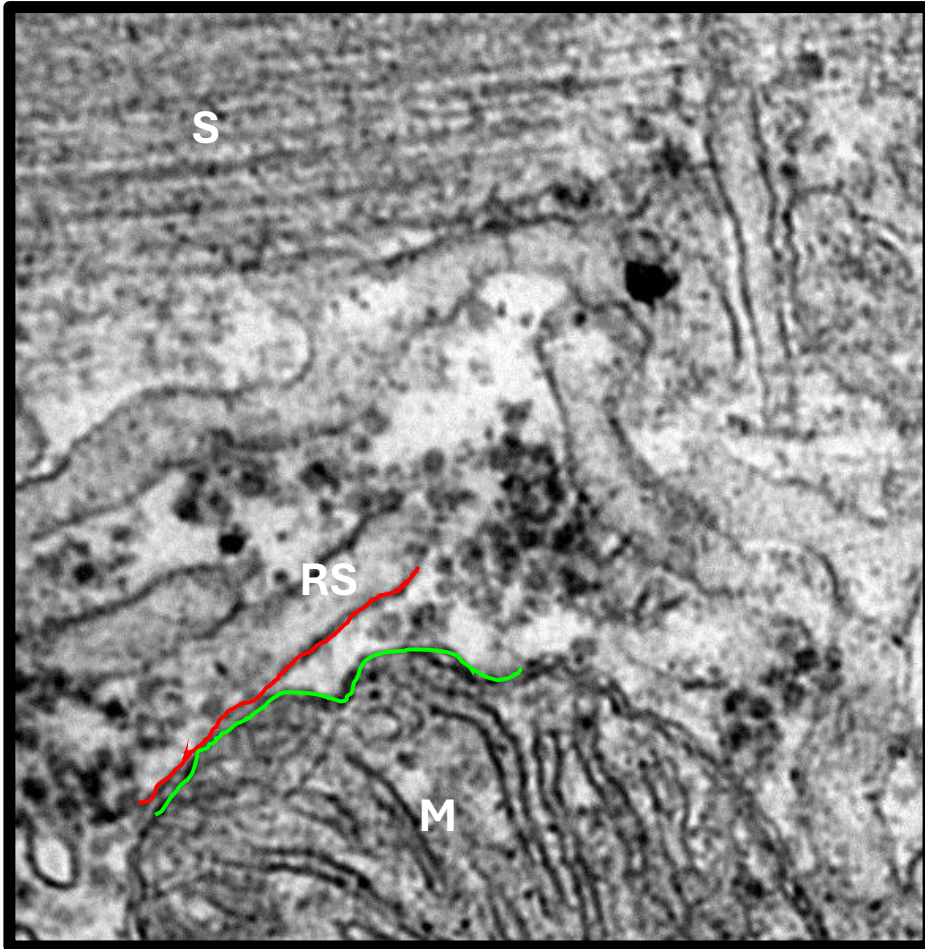
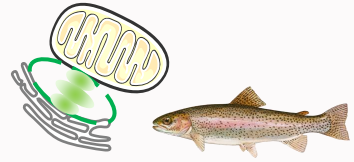


Adapté de Neikirk et al., Eur J Cell Biol 2023

Limites en microscopie 2D :

- Réseaux mitochondriaux et du RS ?
- Volumétrie des mitochondries ?
- Surface de recouvrement des MAMs ?

Interactions entre les mitochondries et le RS en MET 2D



Adapté de Neikirk et al., Eur J Cell Biol 2023

Limites en microscopie 2D :

- Réseaux mitochondriaux et du RS ?
- Volumétrie des mitochondries ?
- Surface de recouvrement des MAMs ?

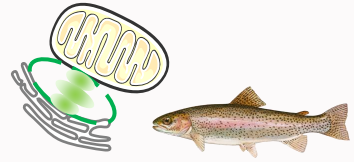
Microscopie Electronique 3D



MRI
Montpellier Resources
Imagerie

MRI-EM4BIO

Interactions entre les mitochondries et le RS en SEM 3D

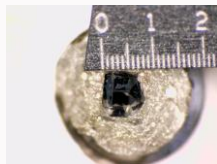


MRI-EM4BIO



REDISSECTION/FIXATION

POST-FIXATION/
ENROBAGE RÉSINE



ULTRAMICROTOME

Bloc final

Fixation

Glutaraldéhyde/Paraformaldéhyde

Contraste

Double couche d'Osmium
Acétate d'uranyle
Aspartate de plomb

Déshydratation

Acétonitrile

Enrobage Résine

Epon Hard

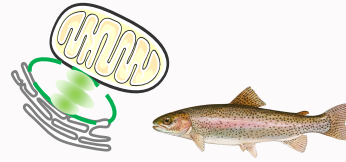
Taillage du bloc

Manuellement (lame de rasoir)
Ultramicrotome



**GEMINI 360 ZEISS
VOLUTOME**

Interactions entre les mitochondries et le RS en SEM 3D

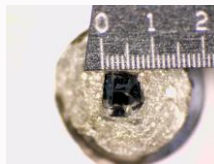


MRI-EM4BIO



REDISSECTION/FIXATION

**POST-FIXATION/
ENROBAGE RÉSINE**

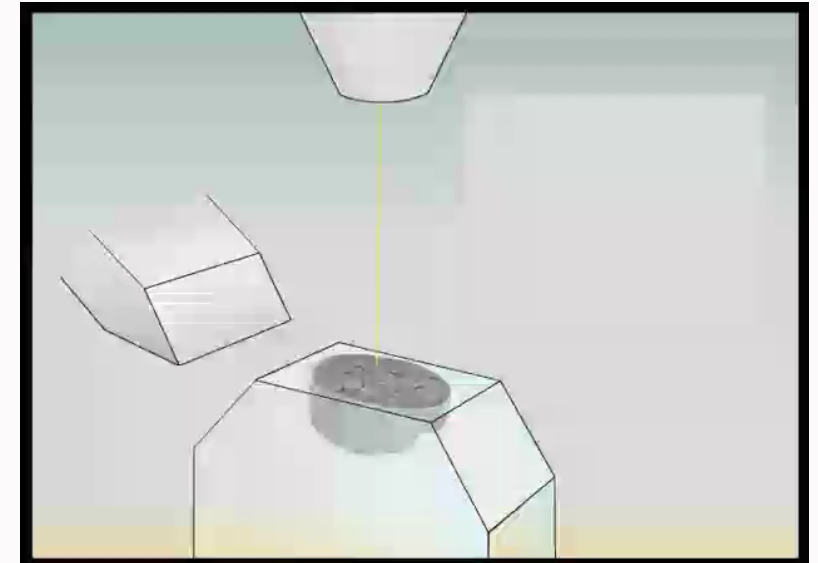


ULTRAMICROTOME

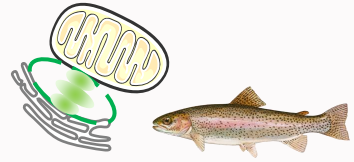
Bloc final



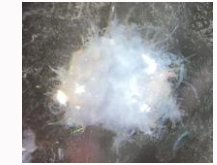
**GEMINI 360 ZEISS
VOLUTOME**



Interactions entre les mitochondries et le RS en SEM 3D

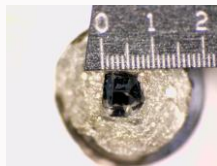


MRI-EM4BIO



REDISSECTION/FIXATION

POST-FIXATION/
ENROBAGE RÉSINE

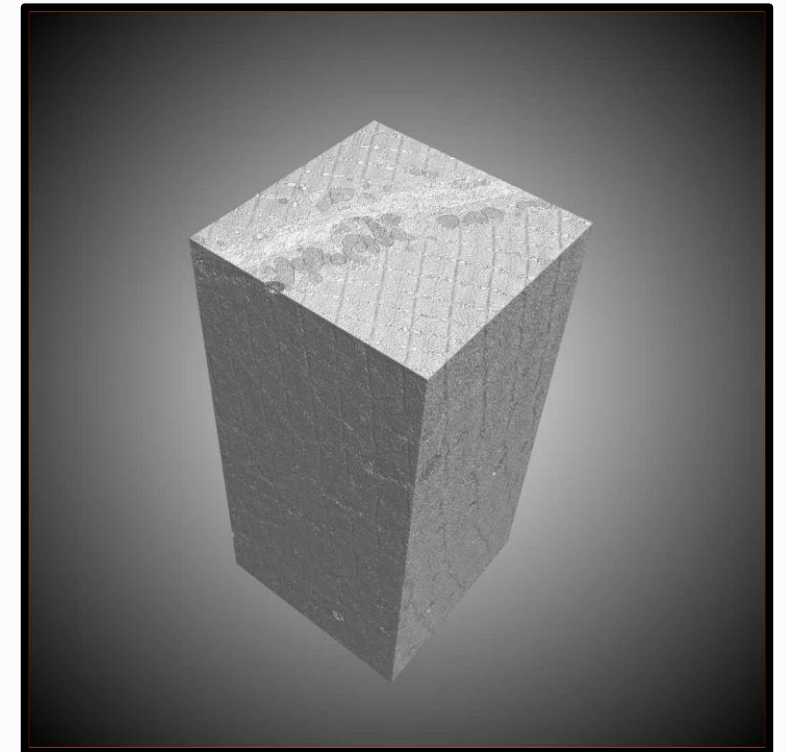


ULTRAMICROTOME

Bloc final



**GEMINI 360 ZEISS
VOLUTOME**

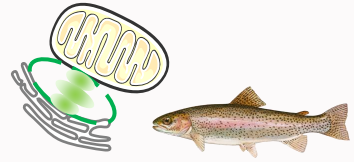


500 sections sériées

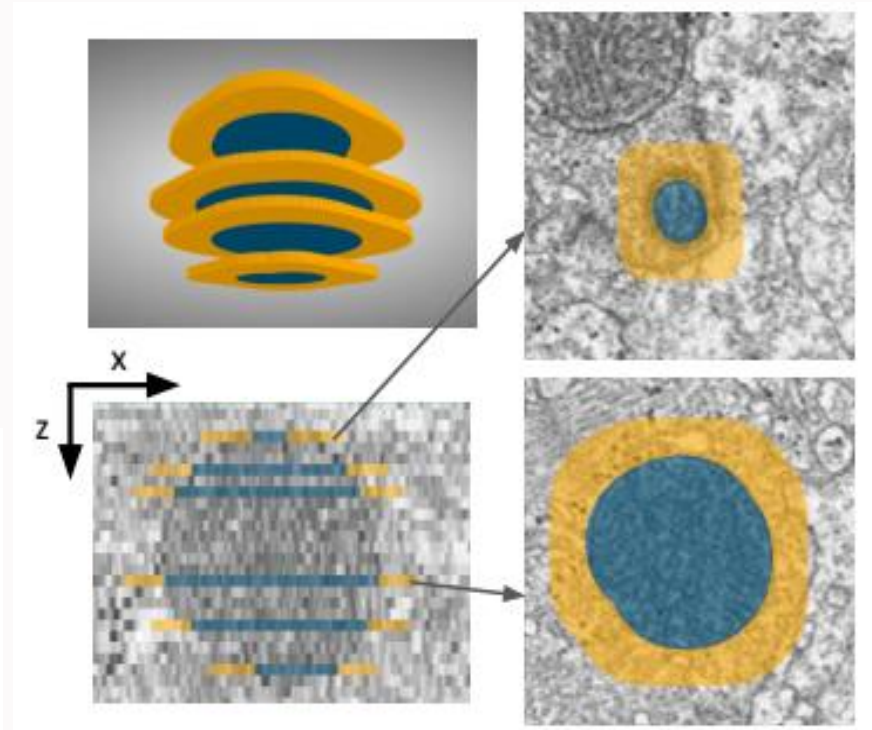
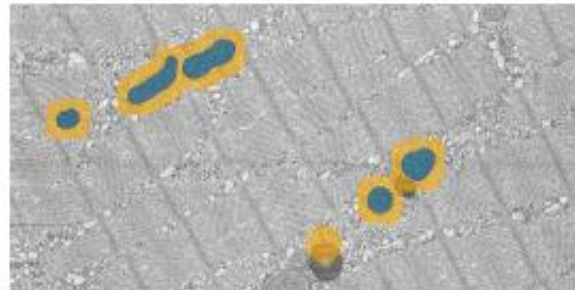
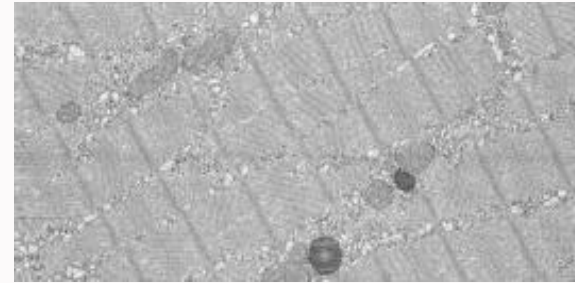
Résolution (x,y,z) : 6 x 6 x 50nm

Résolution max (x,y,z) : 3 x 3 x 25nm

Interactions entre les mitochondries et le RS en SEM 3D

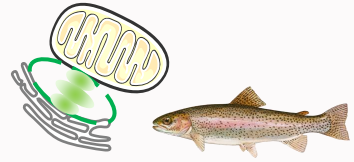


Phase d'Entrainement



~ 100 structures cibles + Background

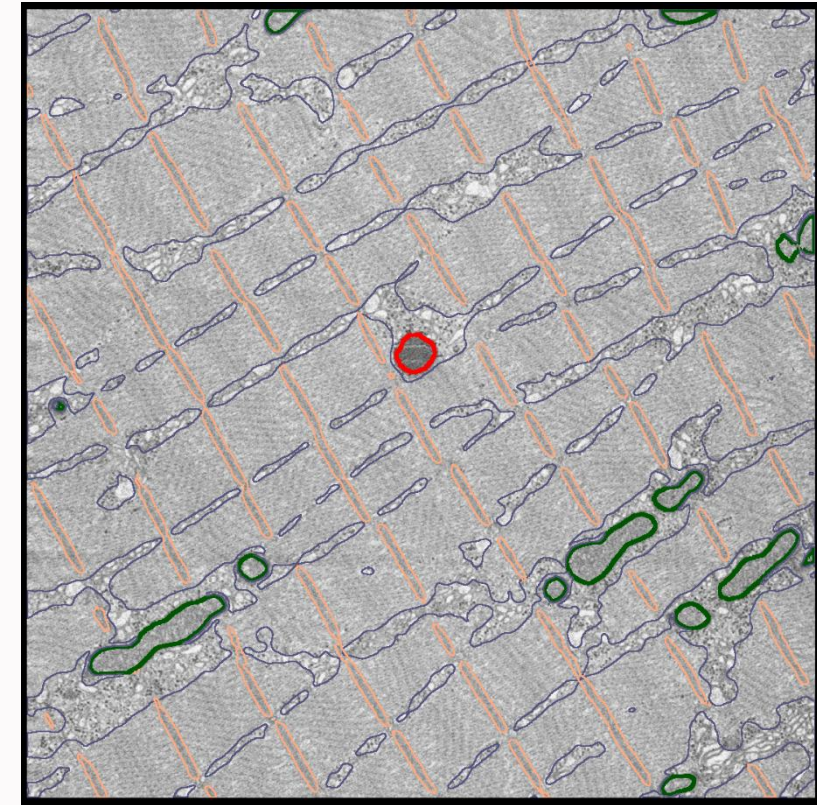
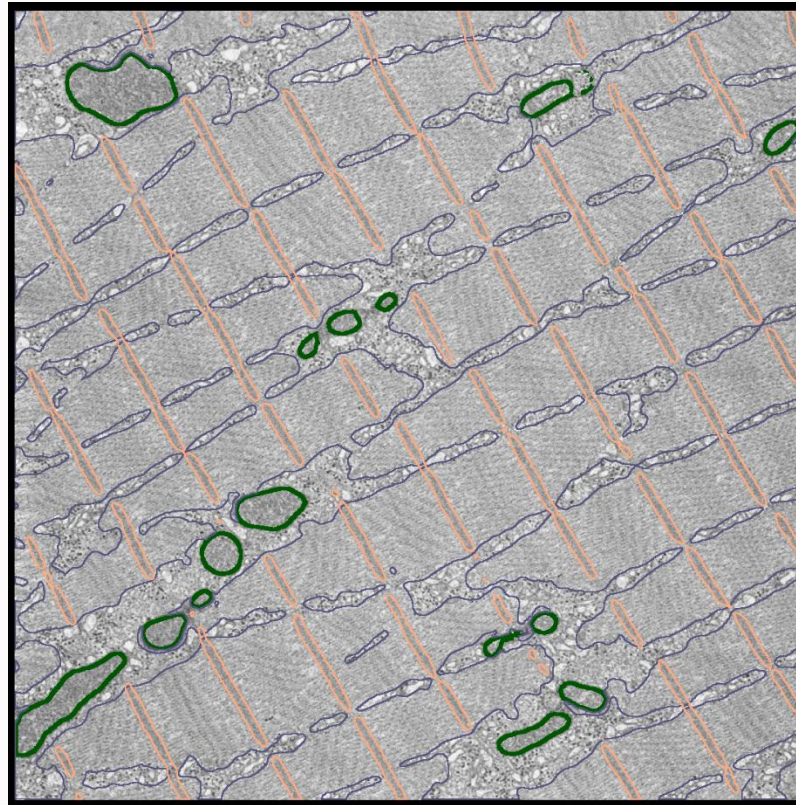
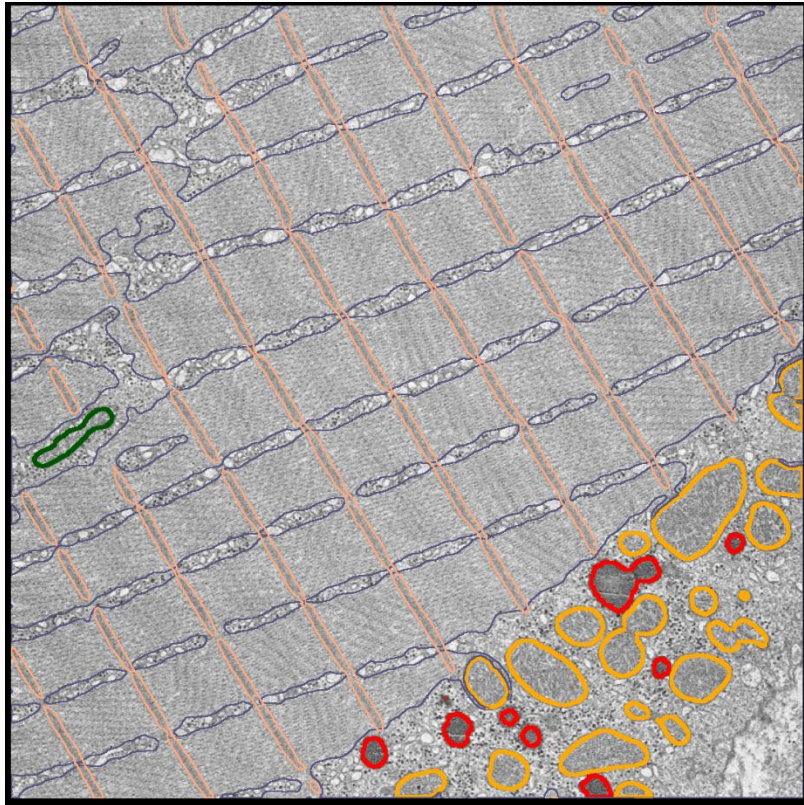
Interactions entre les mitochondries et le RS en SEM 3D



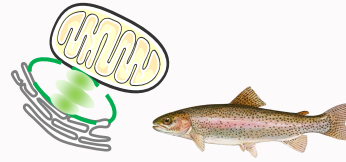
MRI-EM4BIO



Phase de Validation



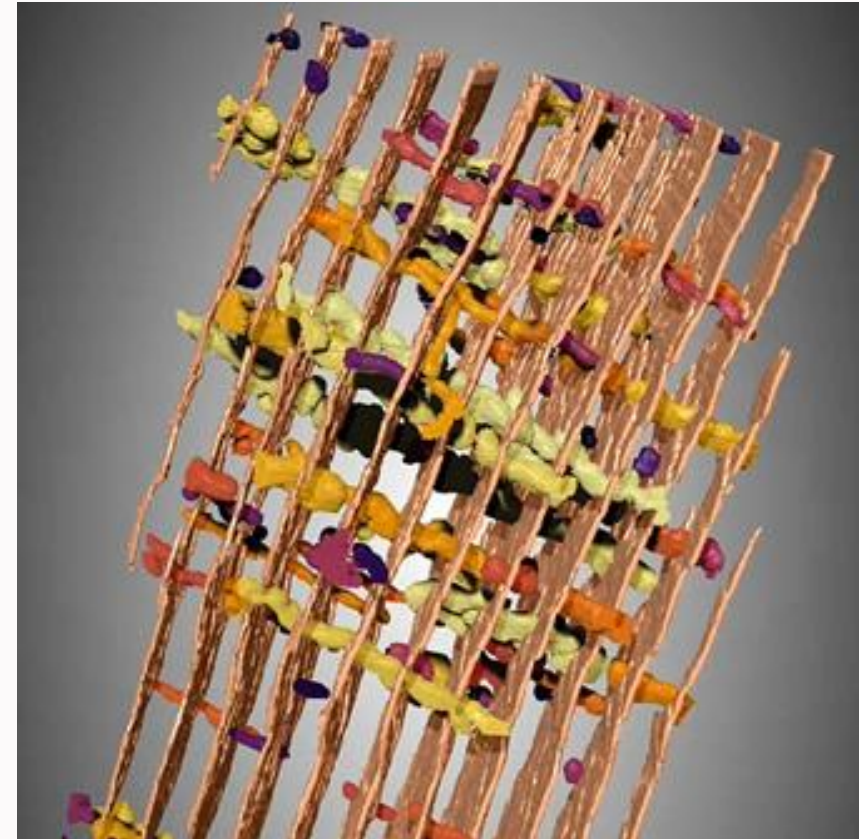
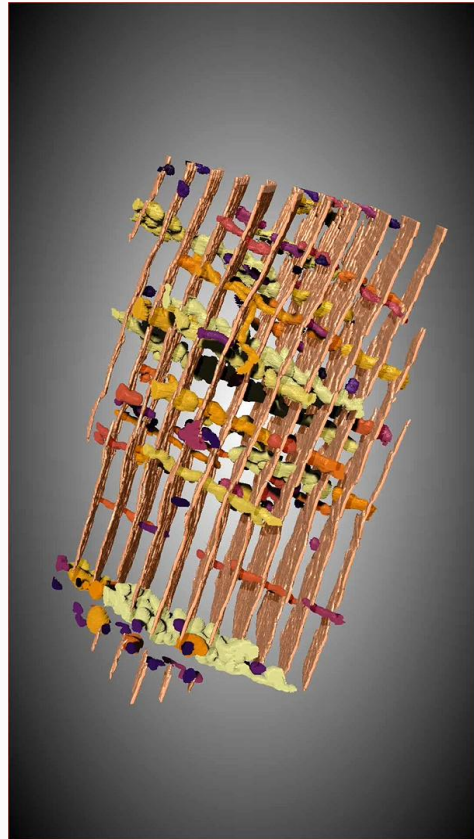
Interactions entre les mitochondries et le RS en SEM 3D



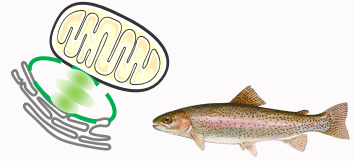
MRI-EM4BIO



Reconstruction 3D



Interactions entre les mitochondries et le RS en SEM 3D



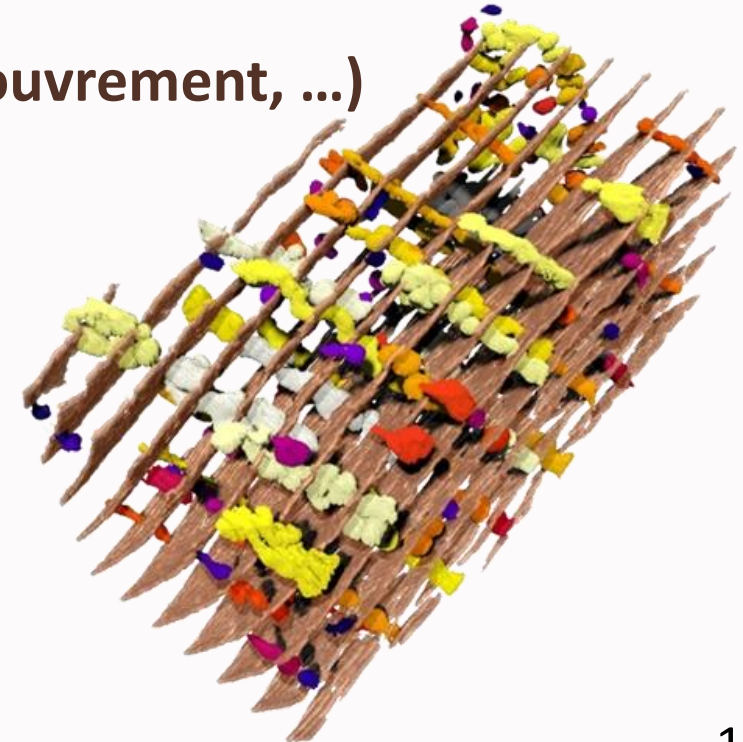
MRI-EM4BIO

Prochaines étapes :

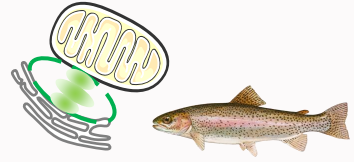
- Segmentation fine du réticulum sarcoplasmique + reconstruction 3D
- Analyses d'images (Distances, Volumétries, Surfaces de recouvrement, ...)



Quantifications des MAMs



Interactions entre les mitochondries et le RS en SEM 3D



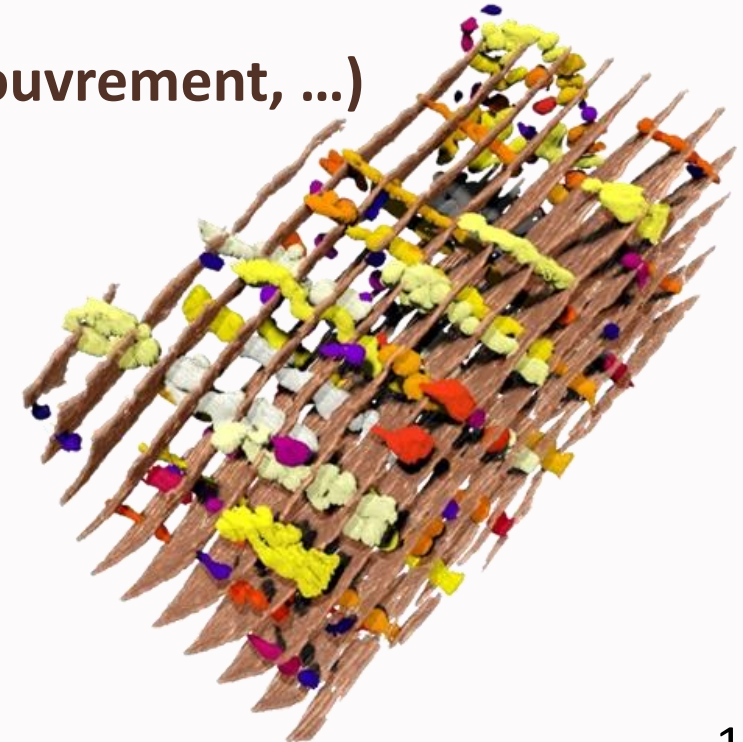
MRI-EM4BIO

Prochaines étapes :

- Segmentation fine du réticulum sarcoplasmique + reconstruction 3D
- Analyses d'images (Distances, Volumétries, Surfaces de recouvrement, ...)



Quantifications des MAMs



→ Plus d'accès, Coût de licence

Remerciements



Chantal Cazevieille



MRI-EM4BIO



Laurence Berry



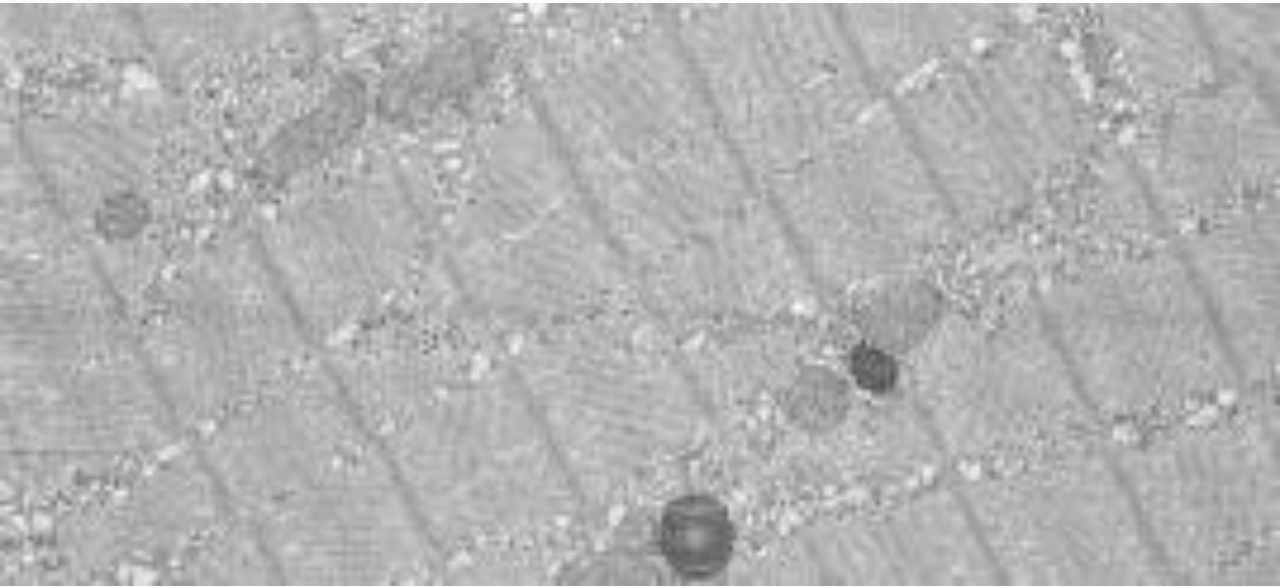
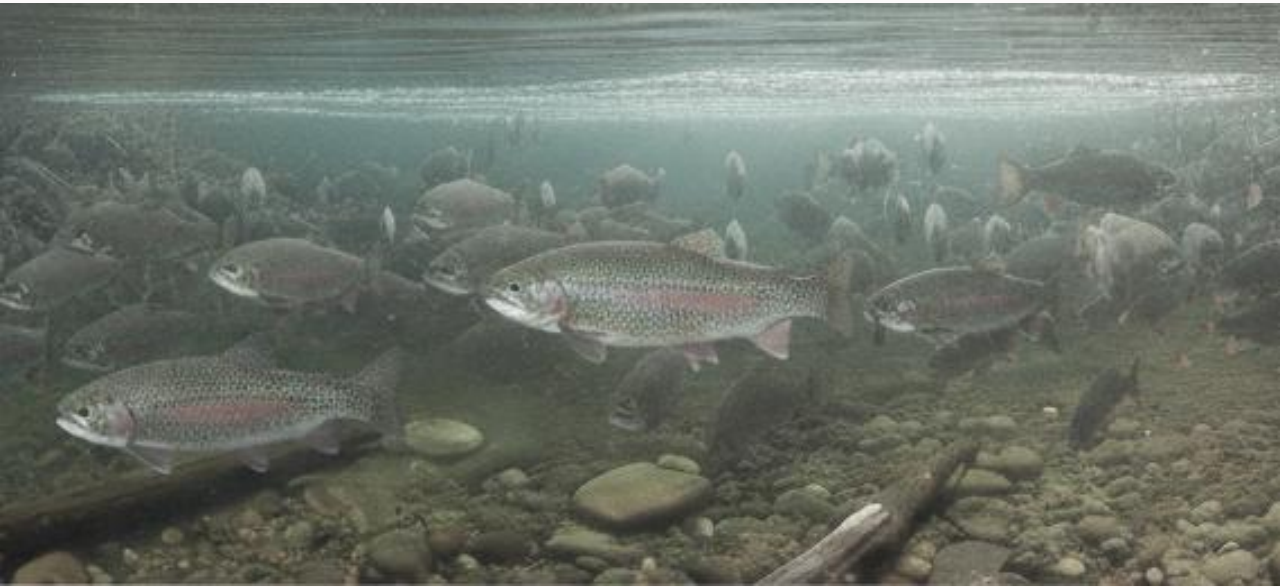
Lucien Daunas



Financement



Merci de votre attention



Résumé :

- Préparation des échantillons : Fixation → Contraste → Déshydratation → Enrobage résine → Taillage,
- MEB : Image → coupe → 500 sections sériées,
- Dragonfly : Entraînement → Validation → Modèle 3D → Analyses

Angelina CAMPAGNA ; angelina.campagna@inrae.fr

Béatrice CHABI, Laboratoire DMeM, Montpellier, France
Vincent OLLENDORFF, Laboratoire DMeM, Montpellier, France

