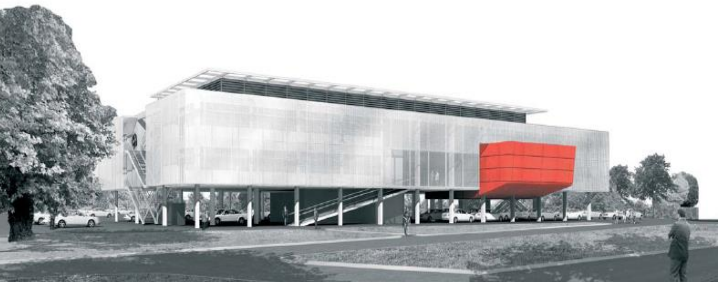


UMS 3420

cnrs advancing the frontiers

université de BORDEAUX

Inserm
Institut national
de la santé et de la recherche médicale

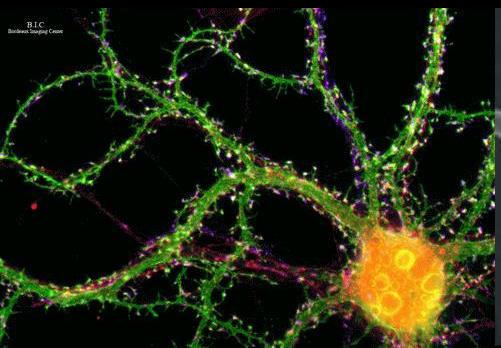
US 4

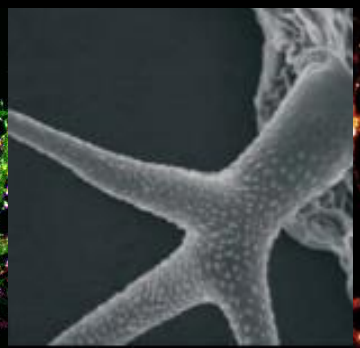
FRANCE-BIOIMAGING

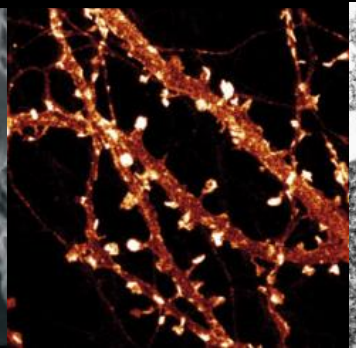
IBiSA

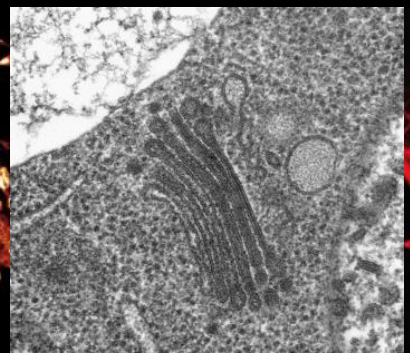
BIC

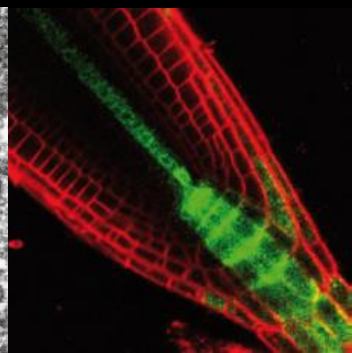
Bordeaux Imaging Center











What is the BIC ?

Cell Imaging core facility of Aquitaine

⇒ *Gather ressources in light and electron microscopy.*

⇒ *In the health and plant domain.*

⇒ *Infrastructure in health biology and agronomy (IBISA) label*

BIC
Bordeaux Imaging Center

Location

Bordeaux Imaging Center

Location of Bordeaux Imaging Center

Campus
of Bordeaux University

Electronic Imaging Unit

Hospital

Faculty of Sciences

BIC
Bordeaux Imaging Center

What is the BIC ?

Our missions :

- ***Service packages***
  - *Devices providing*
  - *Sample preparation*
- ***Training***
- ***R&D***

Bordeaux Imaging Center

BIC – Devices

14 Microscopes:

1 STED microscopes,

3 épifluo., 1 video-FRAP, 3 confocal, 3 multiphotons, 1 macroscope, 1 « spinning disk » confocal, 1 speed multiphoton (dev)

Laser et autres

➢3 femtosecondes laser

➢FLIM, FCS, patch-clamp

Image analyse

➢ 4 Images analyse station

4 Electron Microscopes :

- TEM FEI – Spirit 120kV (tomography & cryo-observation)
- TEM FEI Tecnai 12 120kV (EDS & tomography)
- TEM Hitachi H7650 120kV
- SEM FEI Quanta 200 (with environemental mode & EDS)

Sample prep devices :

- 5 ultramicrotomes
- 1 microwaves automate for sample preparation Leica EM-AMW
- 2 cryo-congélation hyperbare Leica EM-Pact and 1 Leica EM-HPM100
- 3 Leica EM-AFS (automated freeze substitution)
- 1 Immunolabelling automate Leica EM-IGL
- 1 sputter coater Cressington 108
- 1 critical point dryer Leica EM-CPD

BIC Bordeaux Imaging Center

Future Devices in 2016

MEB FEG

High resolution
Cryo transfer system
Serial block face
X Analysis System
Coordinate tracking system (CLEM)

MET 200kV Lab6

High resolution
Cryo transfer system
Electron Tomography
X Analysis System
STEM module

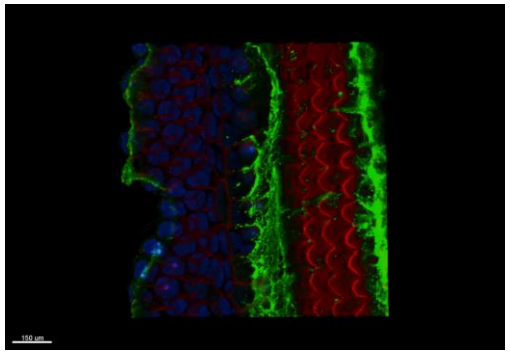
Bordeaux Imaging Center
Structure

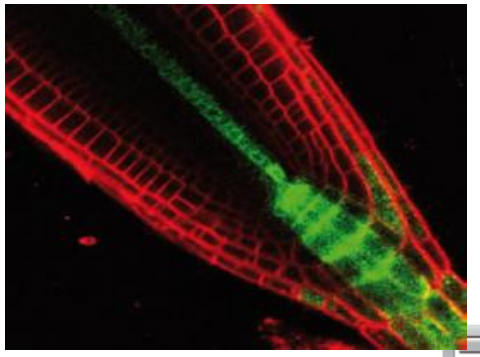
3 Units
15 Engineers

Director Daniel Choquet
Co-Director : Marc Landry & Patrick Moreau

Electronic	Photonic	Plant
Etienne Gontier	Christel Poujol	Lysiane Brocard
Melina Petrel Sabrina Lacomme Isabelle Svahn Hugo LeGuenno	Sebastien Marais Fabrice Cordelières Matthieu Ducros	Catherine Cheniclet Brigitte Batailler Valérie Rouyère

Jennifer Petersen: R&D





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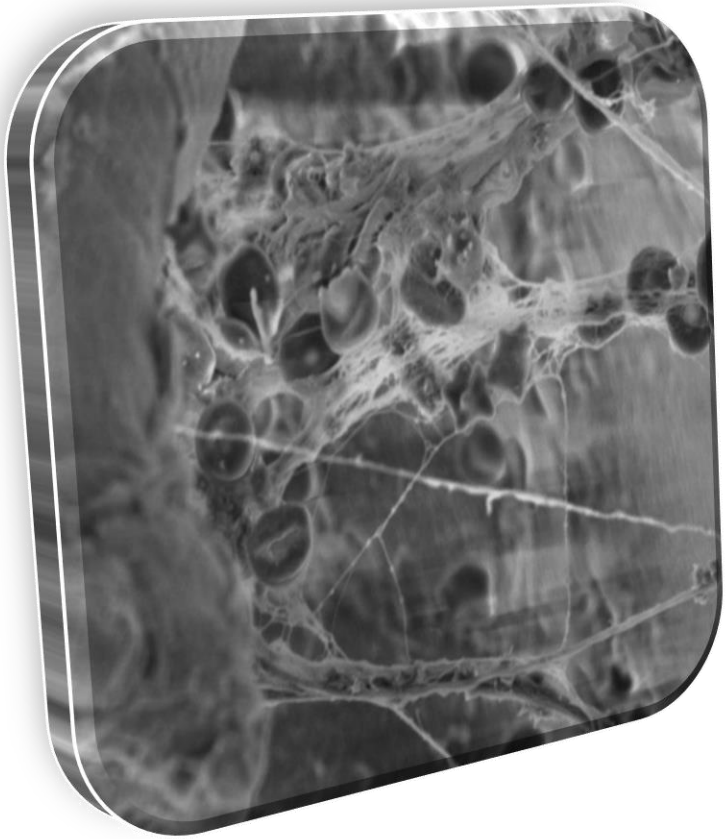
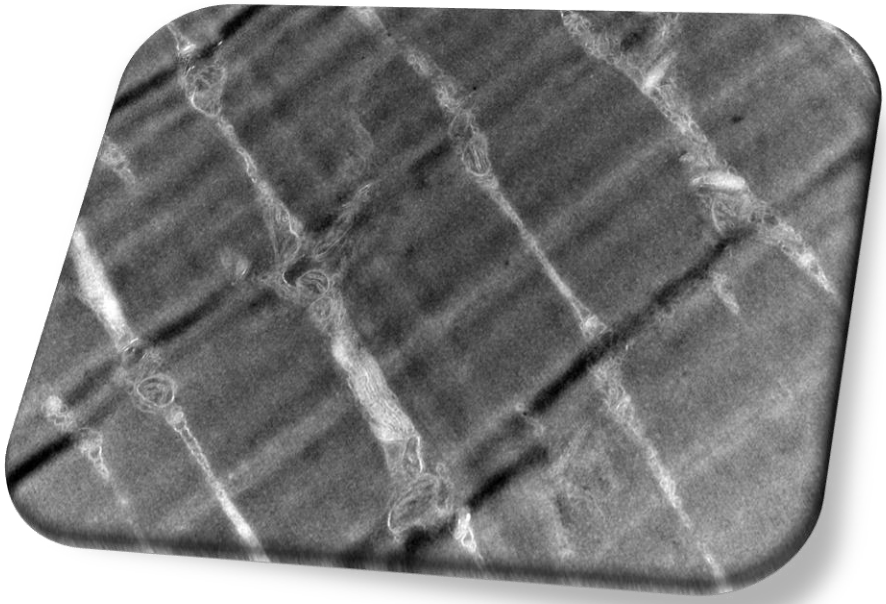
Electronic imaging unit of the Bordeaux Imaging Center





Etienne.gontier@u-bordeaux.fr

BIC
Bordeaux Imaging Center

Electronic Imaging Team

Marc Landry

Scientific Manager.

Etienne Gontier

Technical Manager.

In charge of the TEM activity

Sabrina Lacomme

In charge of the cryo-preparation / immunostaining.

Melina Petrel

In charge of the SEM activity.

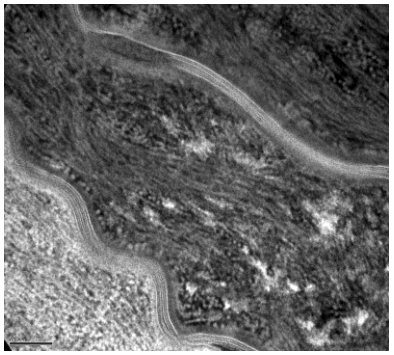
Isabelle Svahn

Technician in sample preparation and participates in the development

Hugo Le Guenno (CDD)

BIC Bordeaux Imaging Center

Business Areas



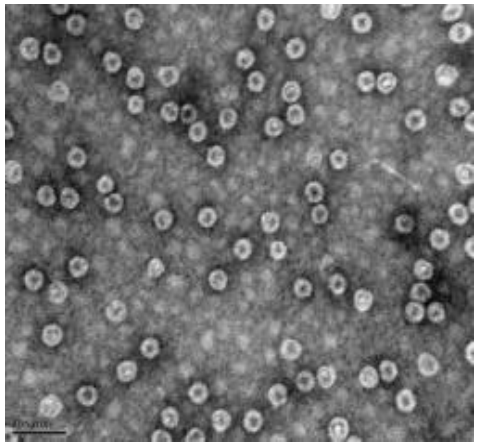
⇒ We work on transmission electron microscopy and scanning

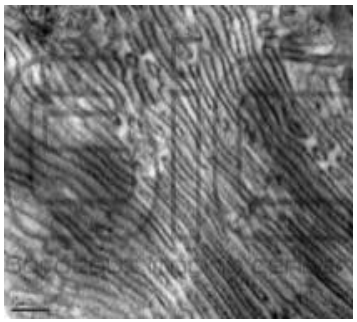
⇒ Major Axis : Biological (Animal / Plant) :

* Whole tissue
* Isolated cells or in culture
* cell suspensions, organelles or membrane fractions
* micro-organismes (Virus, Levures...).

But also we open the activity to

* Materials
* Bio-materials
* Polymers





BIC
Bordeaux Imaging Center

Electronic Imaging Division

The electronic imaging division proposes activities according to three main axis:

Service and maintenance:

- we are a **Core facility** in EM imaging and preparation equipment
- we offer **Sample preparation**
- we offer full service by **Engineers**
- we make **Assistance and advice**

Training:

- ❖ we train in the use of equipment
- ❖ we provide theoretical and practical training on sample preparation methods

Development:

- ▪ we develop new imaging techniques and provide state-of-the-art equipment and sample preparation to users.

Our expertise

⇒Sample preparation

⇒About biology and materials

Cryo-préparation :

- High pressure freezing, Freeze -substitution and low temperature resin inclusion
- Cryo-ultramicrotomy (Material and Biology)

Correlative microscopy:

- Dedicated support and preparation
- Localization of the ROI and ultrastructural observation

Immuno-staining :

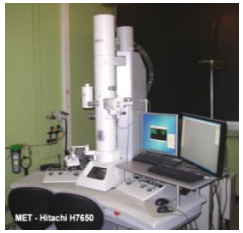
- By pre-embedding or on section
- By Tokuyasu

Devices of sample preparation

– *1 robot for conventionnal preparation (AMW)*
– *1 High pressure freezer (HPM100)*

– *3 robots for cryosubstitution*
– *3 Ultra-microtomes*
– *1 Cryo-ultramicrotome (UC7)*

SEM

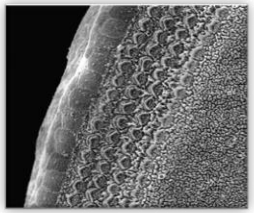


TEM

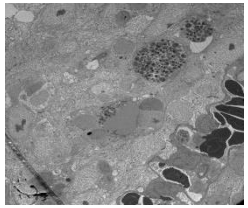
IHC

HPF

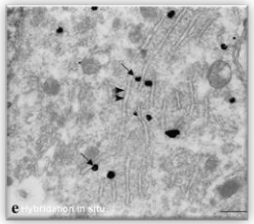
Tomography



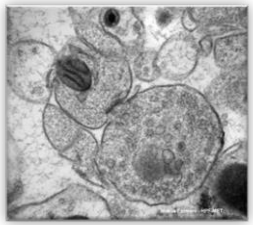
Topography



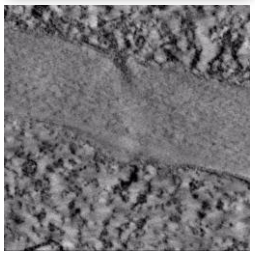
2D ultrastructural imagery



Immunolabelling 2D ultrastructural imagery



HPF 2D ultrastructural imagery

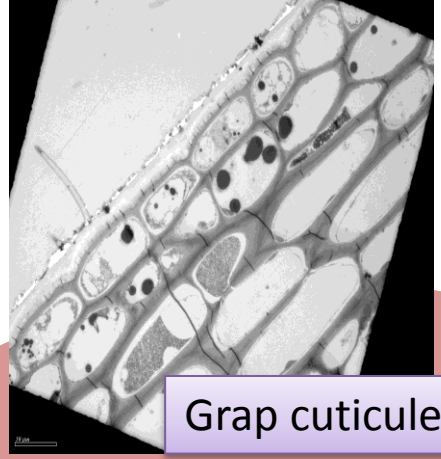


3D ultrastructural imagery

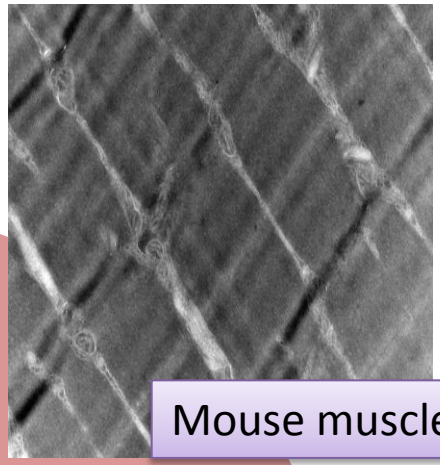
Bordeaux Imaging Center

EM and Biology

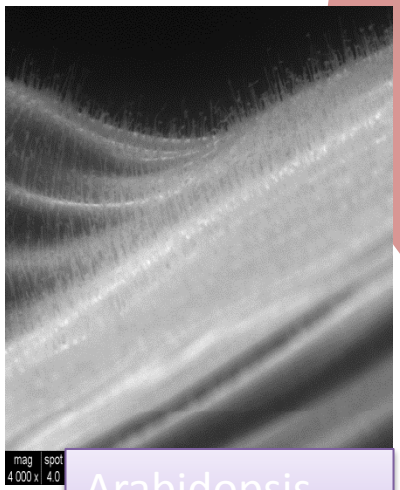
Sturgeon egg



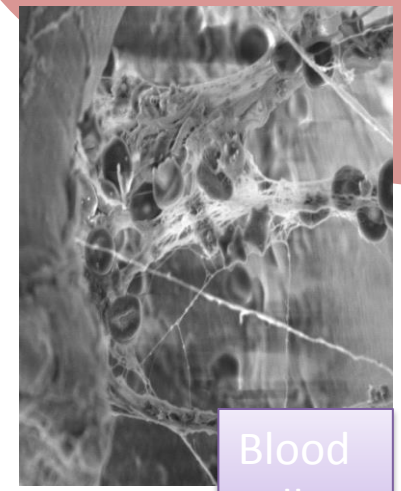
Grap cuticule



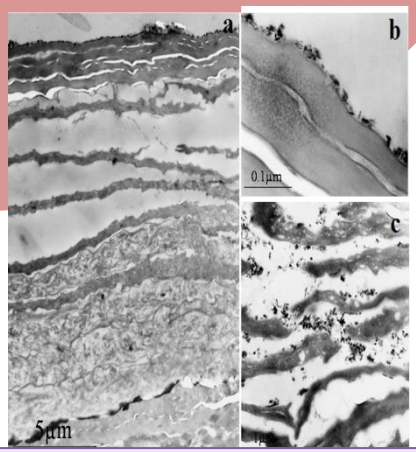
Mouse muscle



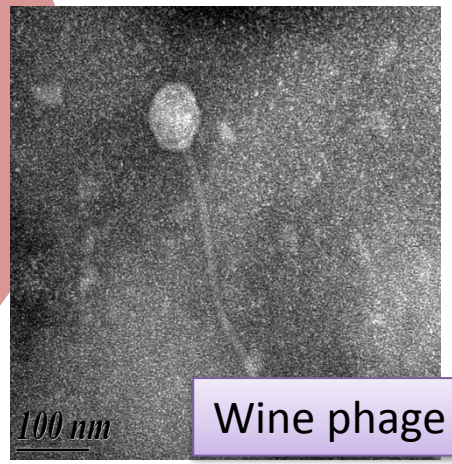
Arabidopsis stem



Blood cell



Effect of sunscreen on skin



Wine phage

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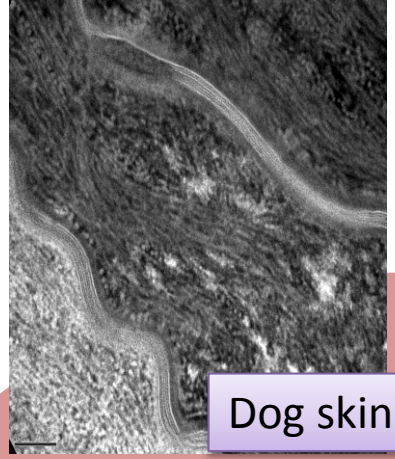
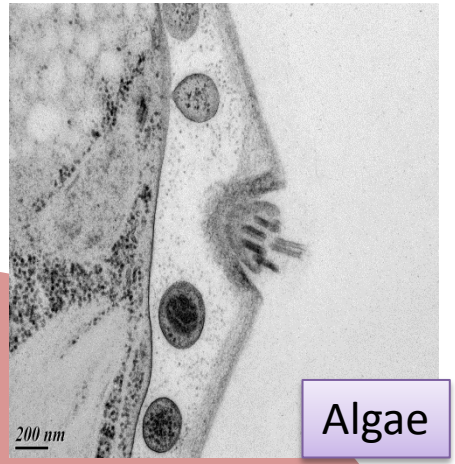
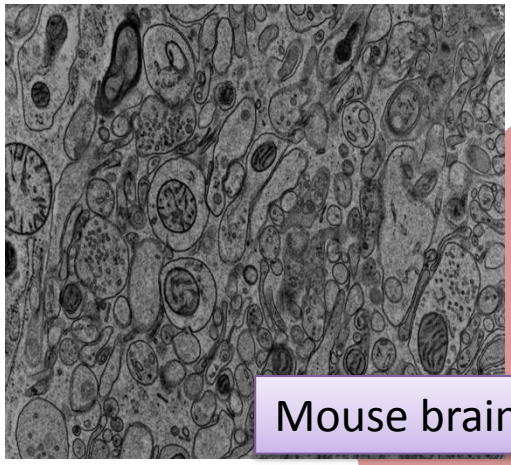
EM and Biology



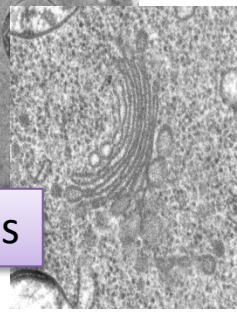
Dog skin



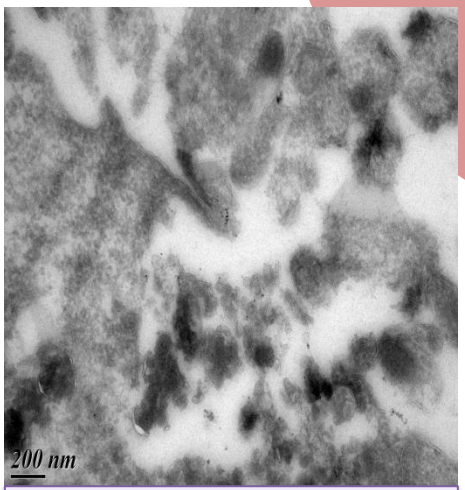
Algae



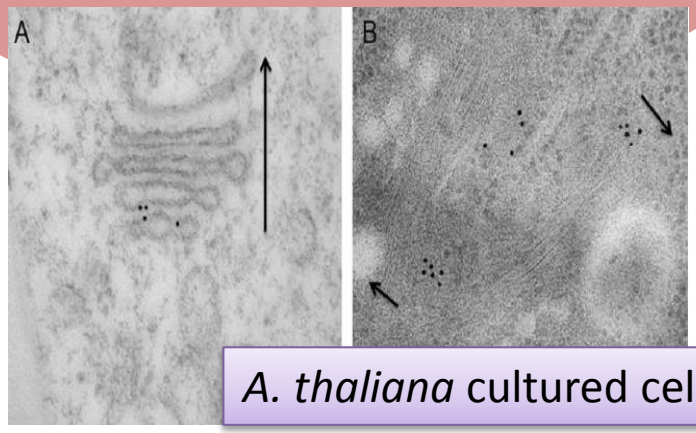
Mouse brain



Tabaco BY2 cells



Exosome in neuron cells



A. thaliana cultured cells

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Physical demands of ***TEM sample preparation***

Biology

Aqueous/hydrated

Soft

Light elements
(C, O, H, N, S, P etc.)

"Large"

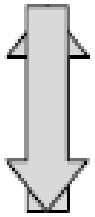
Not suitable for EM

Electron microscope

High vacuum

Electron beam

Sensitive to vibration
(High magnifications)



Biological samples need to be transferred into a solid state...

...which preserves the structures as a function of the living state…

…and not as a function of specimen preparation

Resistant to high vacuum

Resistant in electron beam

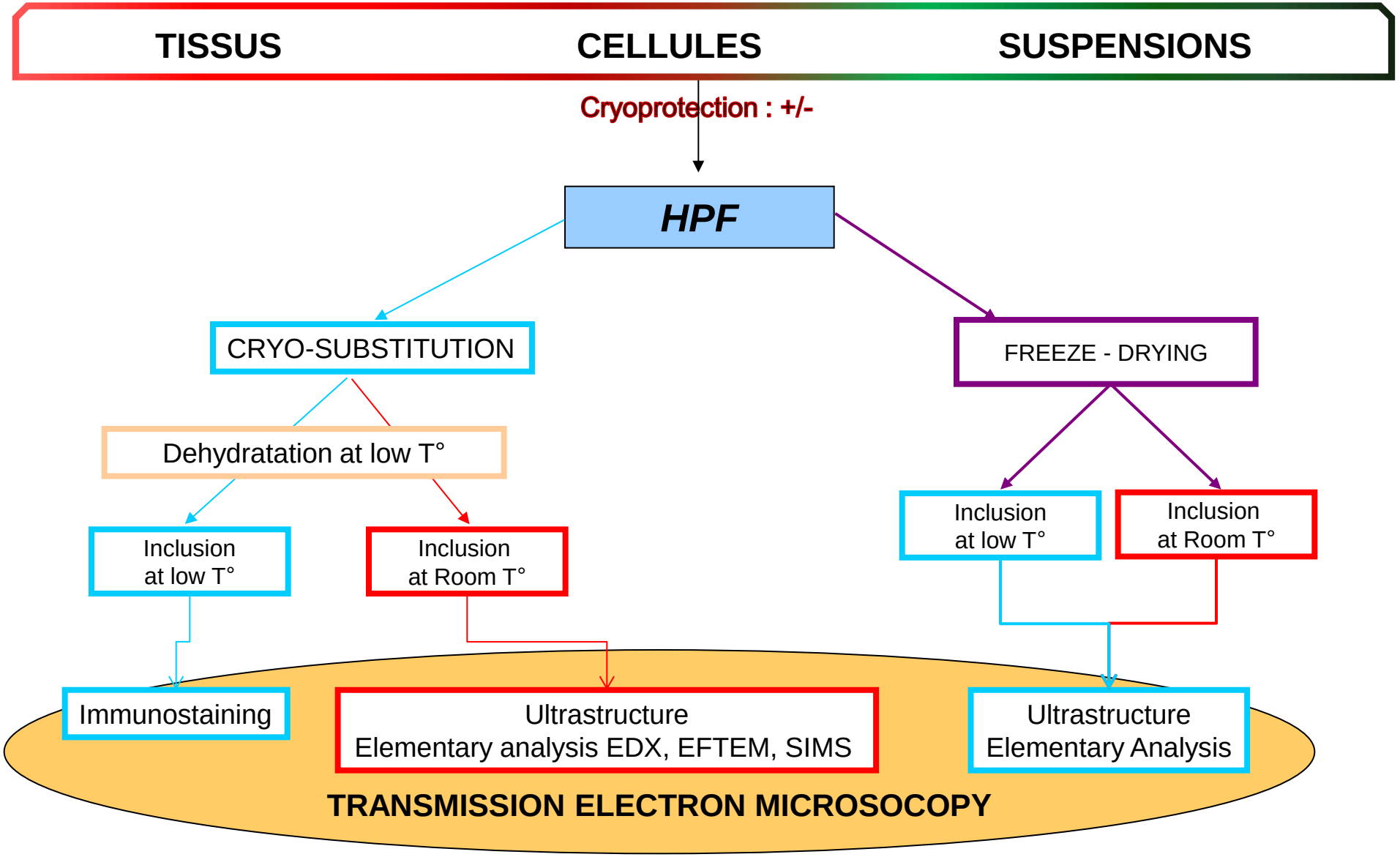
Thin – permeable for electrons
(for TEM)

Contrast

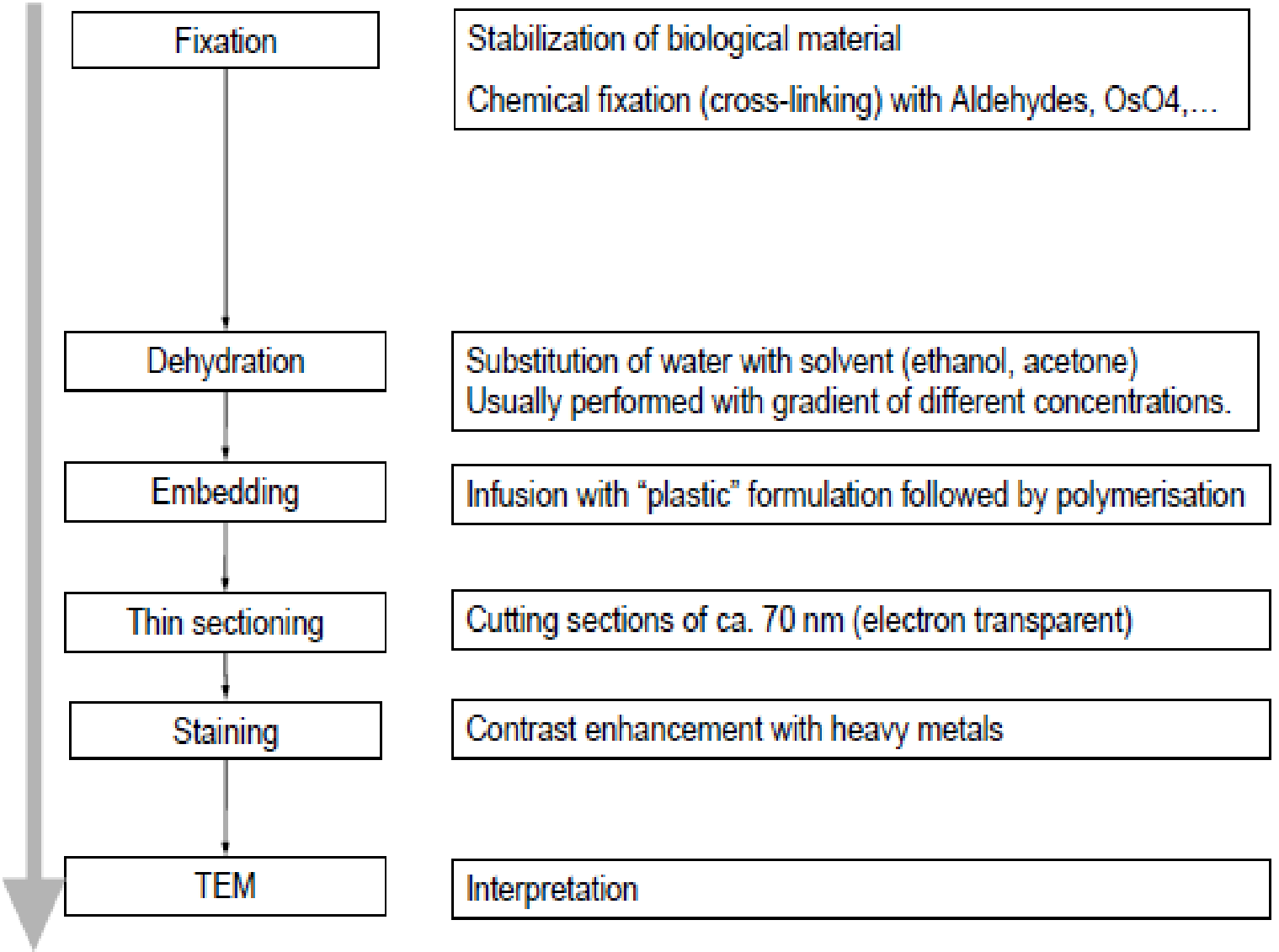
TEM sample preparation

For biological samples → Fixation.
→ Embedding in resin.
→ Section.
(→ immunolabelling.)
→ Staining.

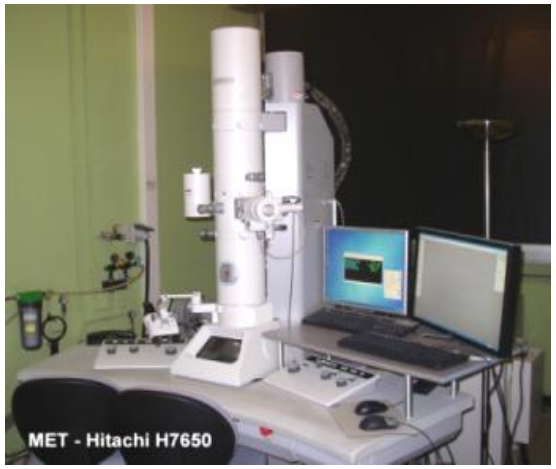
✓ 2 types of preparation : **Conventional or by cryomethods**



Room temperature processing for TEM



Transmission Electron Microscopy (TEM)



Hitachi - H7650 – 120kV

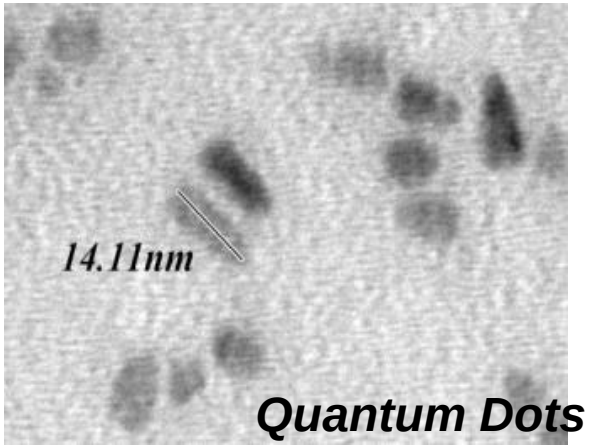
Interests :

- Resolution 1 nm
- It's **User friendly**
- Ultrastructural information and particle characterization

=> Conventional 2D ultra-structural imaging at high resolution

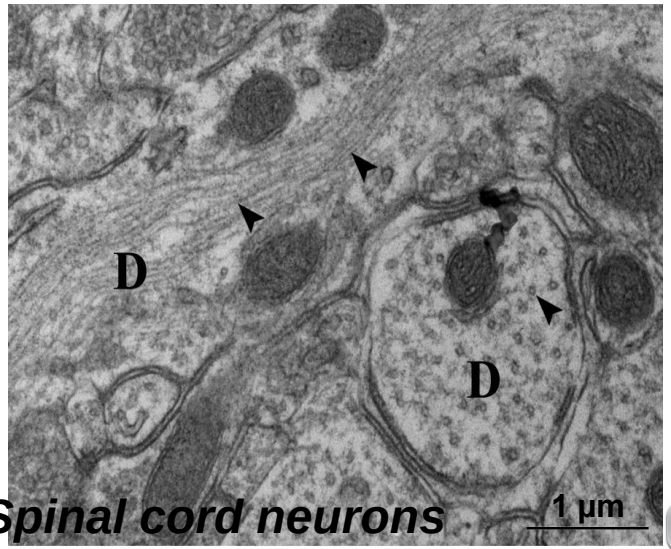
2 types of observation are possible:

High resolution for materials



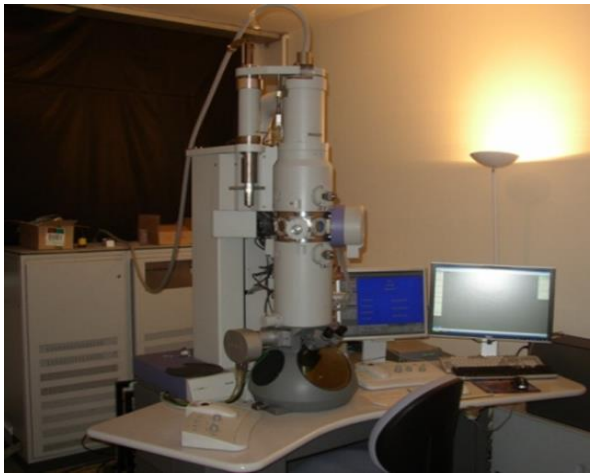
Quantum Dots

High contrast for biology



Spinal cord neurons

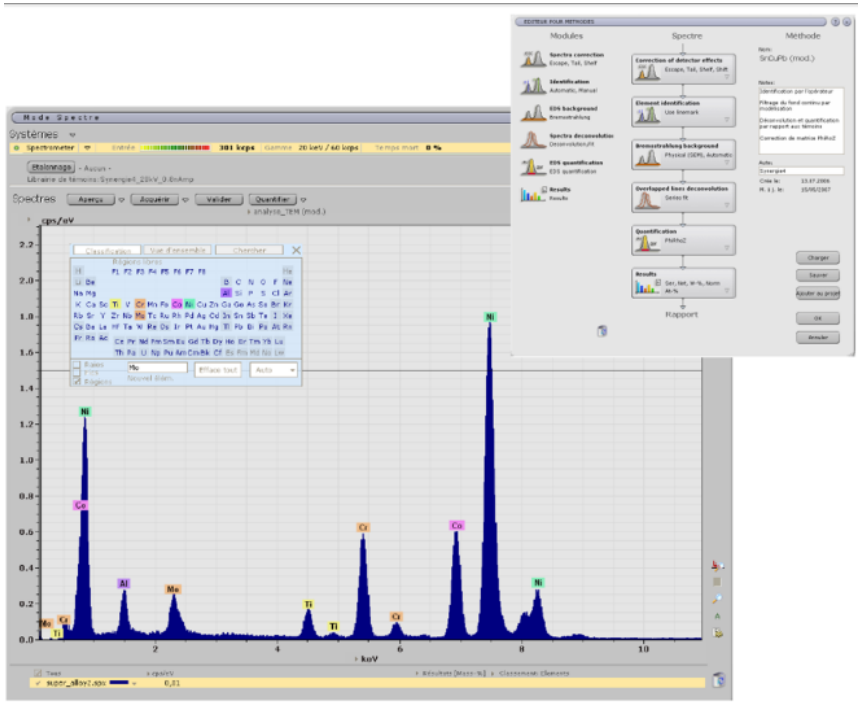
Microscopie Electronique en Transmission

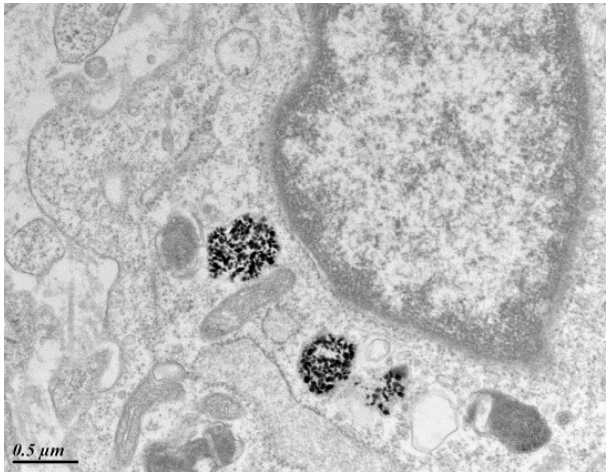


MET 120 kV Tecnai12

X-rays microanalysis

XFlash 6I60T





BIC
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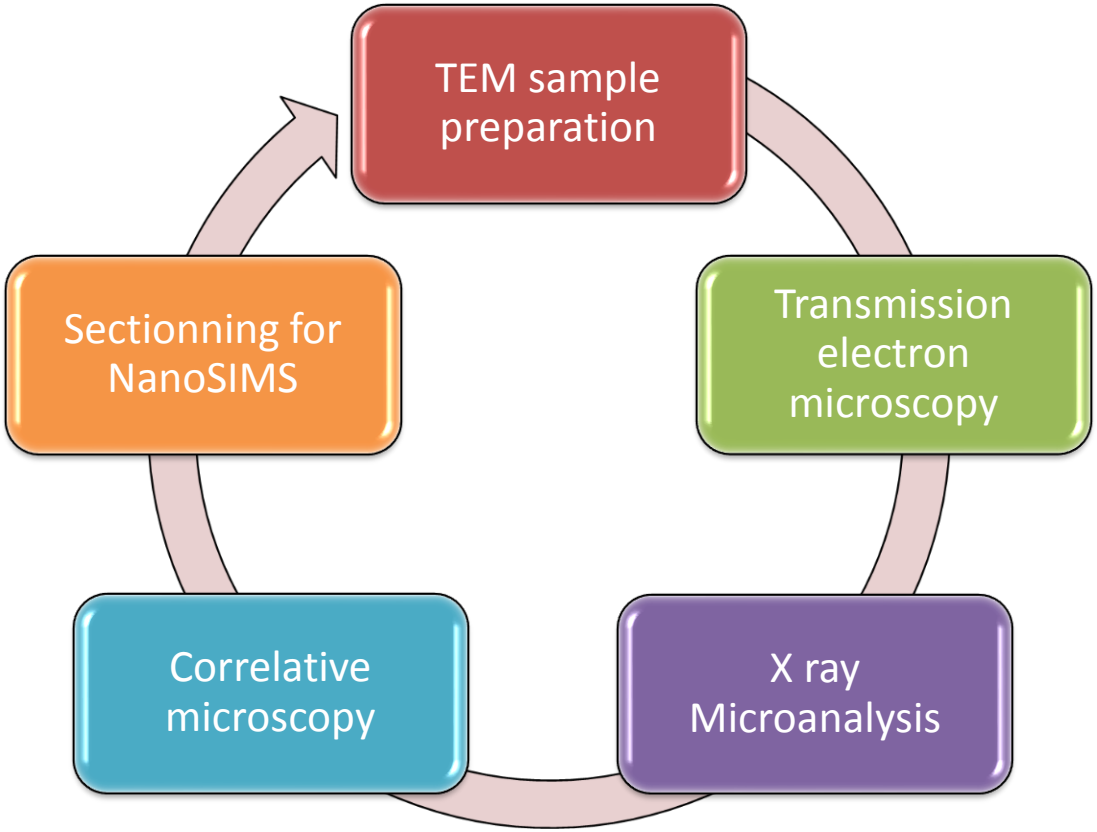
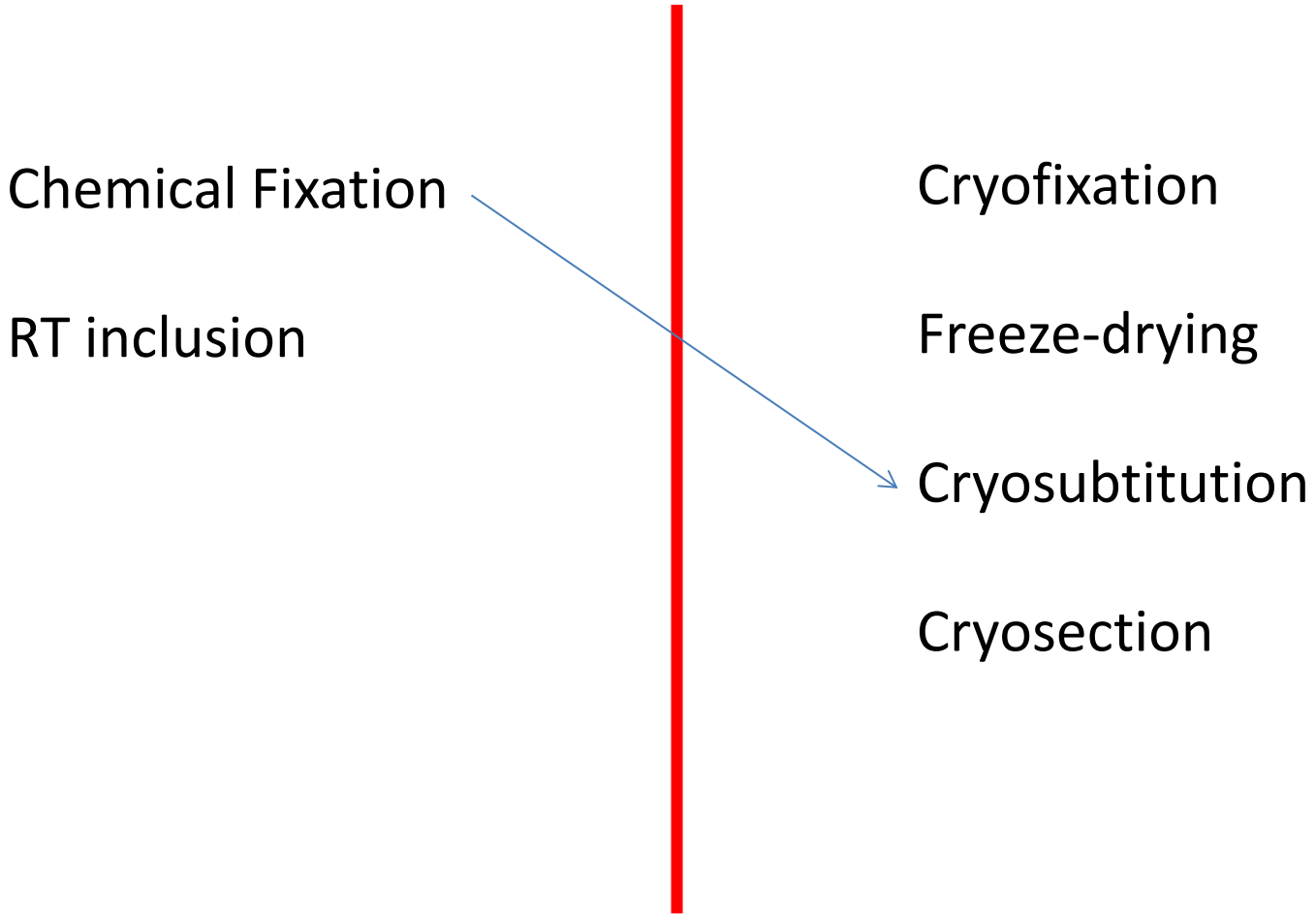
BIC Field of work for AQUAMAPMET



Bordeaux Imaging Center

Sample preparation possible for the project



First Results for TEM

Liver and gills of common carp (Cyprinus carpio)

RT method:

After 3-4 days, samples were prepared according to the following protocol :

1. Infiltration by glutaraldehyde 4-5% in PBS (phosphate buffer) 0.1M (over night)
2. Washing during 10 min with PBS
3. Fixation with OsO4 1% for 1 hour
4. Washing
5. Dehydration steps:

1 ml Aqua dest + 0.5 ml Aceton (=30%)	10min
take out 300 µl, add 300 µl Aceton	10min
take out 600 µl, add 600 µl Aceton	10min
take out 1 ml, add 1ml Aceton	10min
take the whole volume out and refill with Aceton	2hrs

6. Resin (using EMbed-812 kit) infiltration steps:

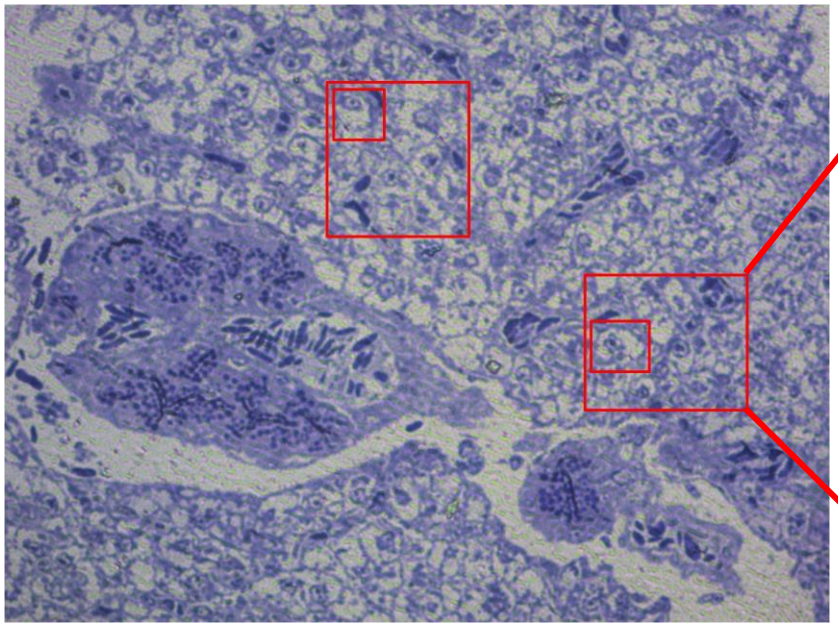
1:1 Aceton:Resin	30min
1:3 Aceton:Resin	1hrs
100% Resin	over night

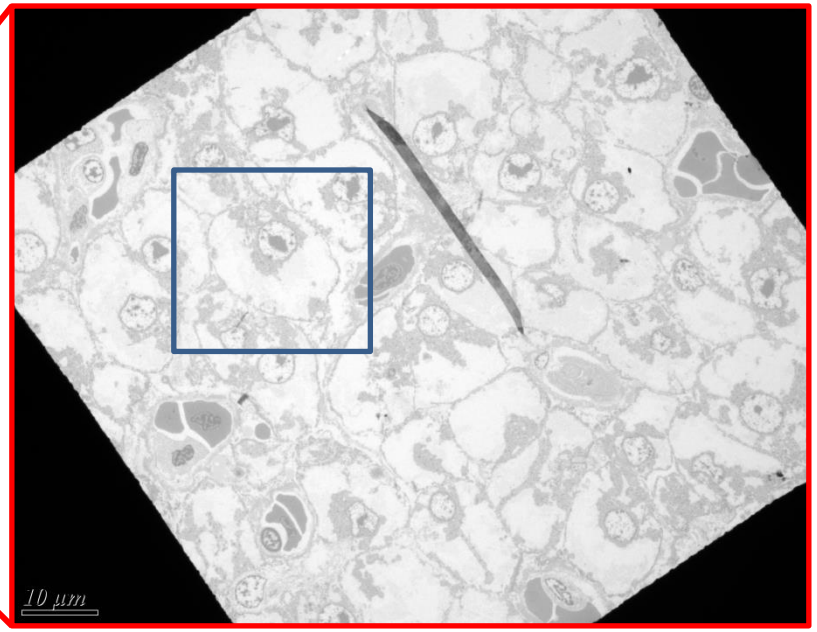
7. Put the samples in moulds and bake out for 24hrs at 60°C

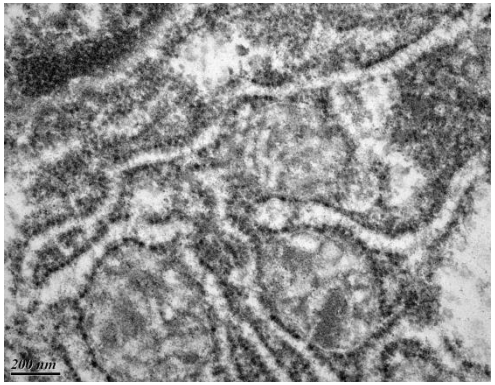
JETRA = LIVER

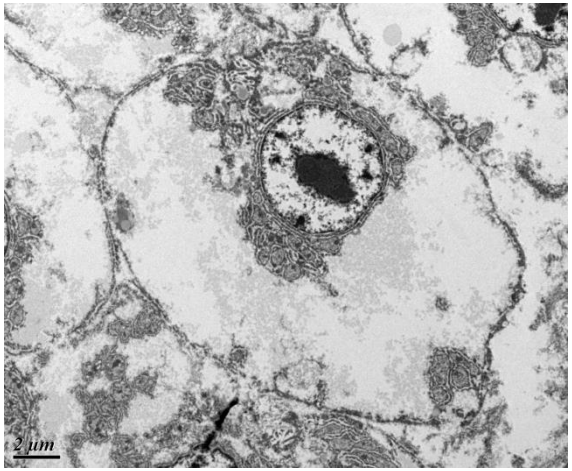
Semithin section

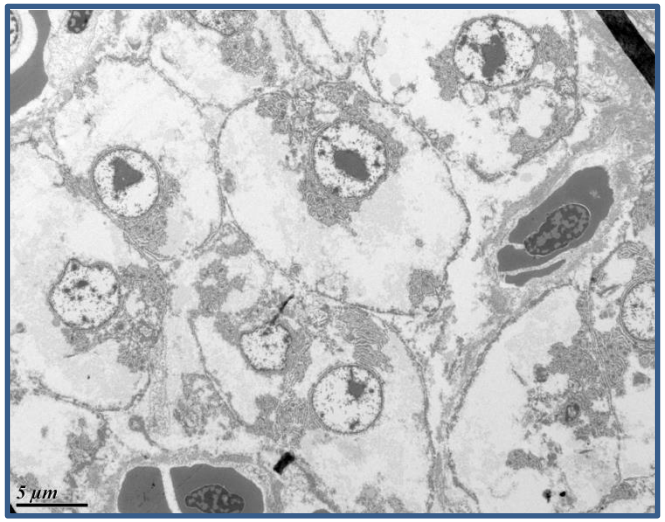
Ultrathin section

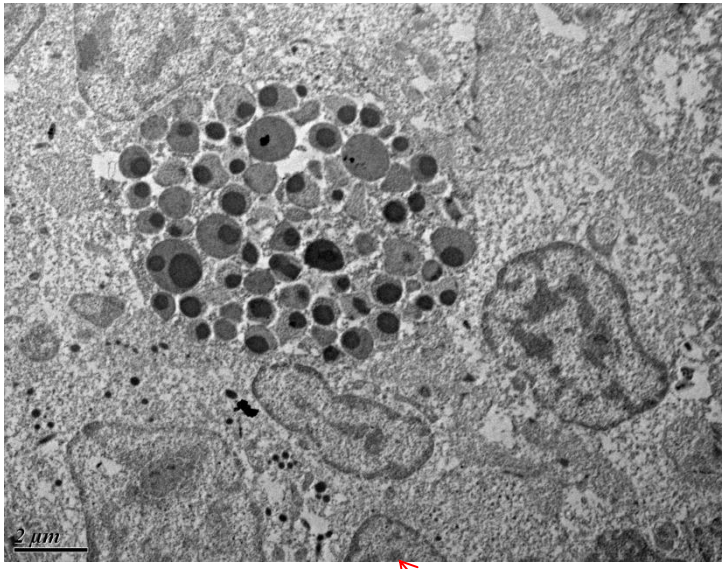


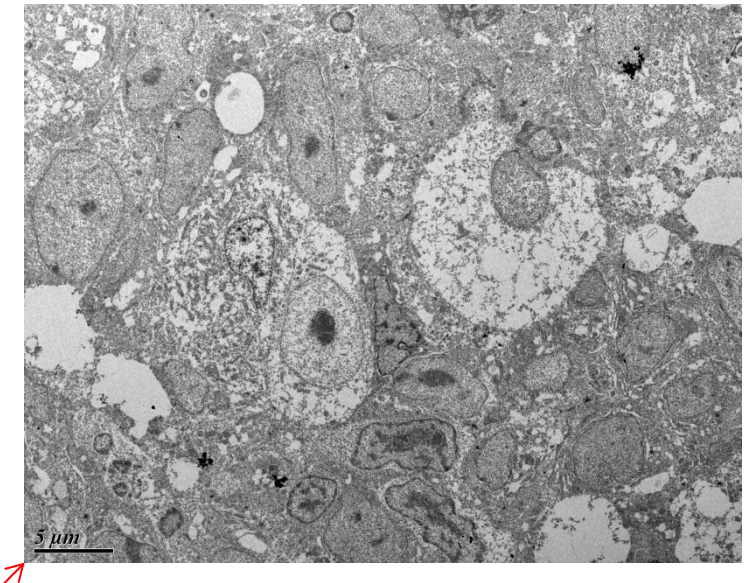


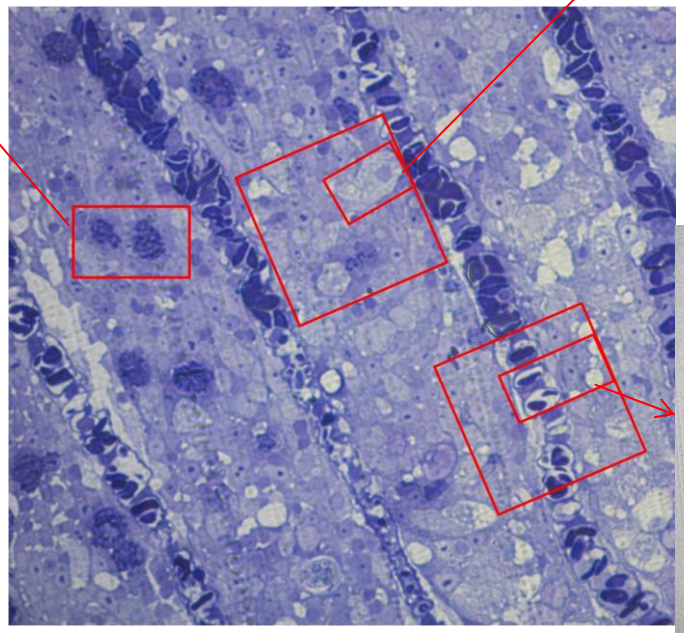


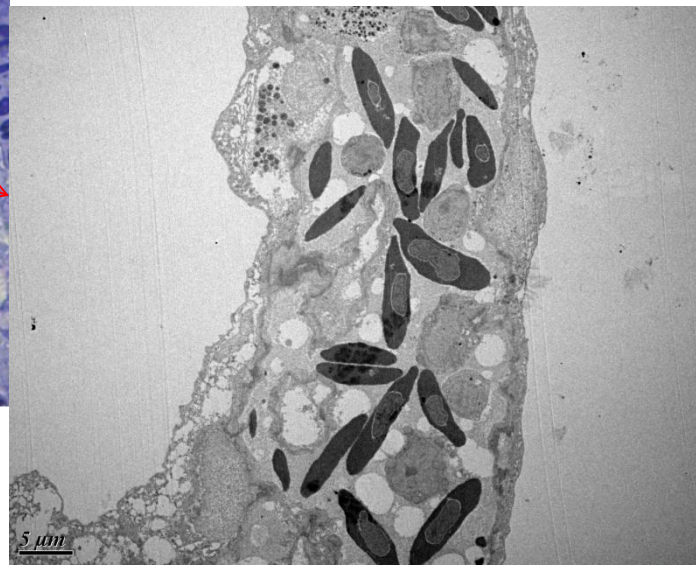












ŠKRGE = GILLS.

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THANK YOU FOR YOUR ATTENTION

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