NanoSIMS imaging of biological samples: technique and challenges for sample preparation

Dirk Schaumlöffel

Université de Pau et des Pays de l'Adour / CNRS
Institut des Sciences Analytiques et de Physico-Chimie
pour l'Environnement et les Matériaux, UMR 5254 IPREM, Pau, France

WRAP-UP MEETING
Accumulation, Subcellular Mapping and Effects of Trace Metals in Aquatic Organisms (AQUAMAPMET) **Zagreb, 2-3 December 2019**


Paris
Pau


Photo Mariano

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IPREM - Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les Matériaux

Staff

135 permanent

140 non permanent

3 scientific poles

- **Analytical Chemistry, Physical Chemistry, Theoretical Chemistry**
- **Chemistry and Microbiology of the Environment**
- **Physical Chemistry of Surfaces and Polymer Materials**

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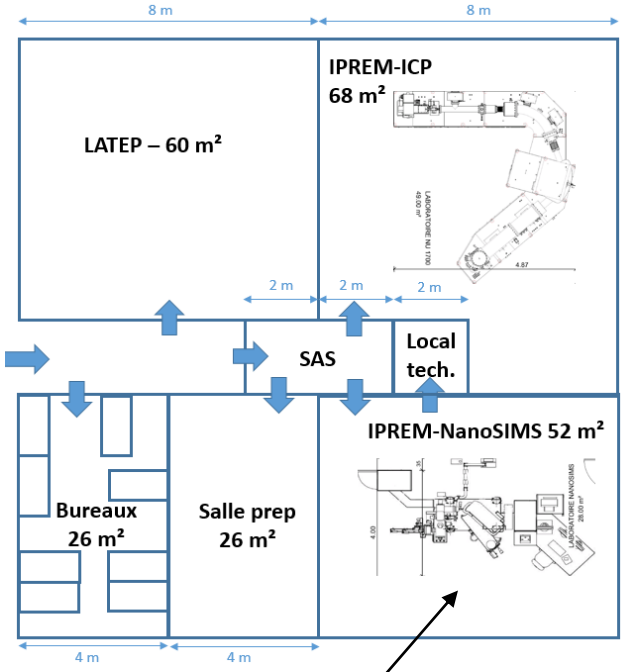
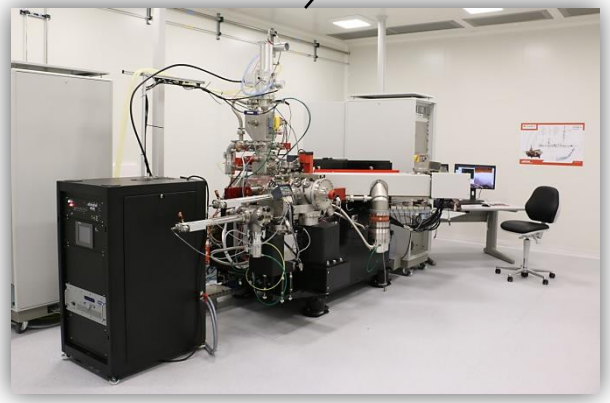
IPREM → **Halle-MARSS**

installation of the NanoSIMS



Halle MARSS



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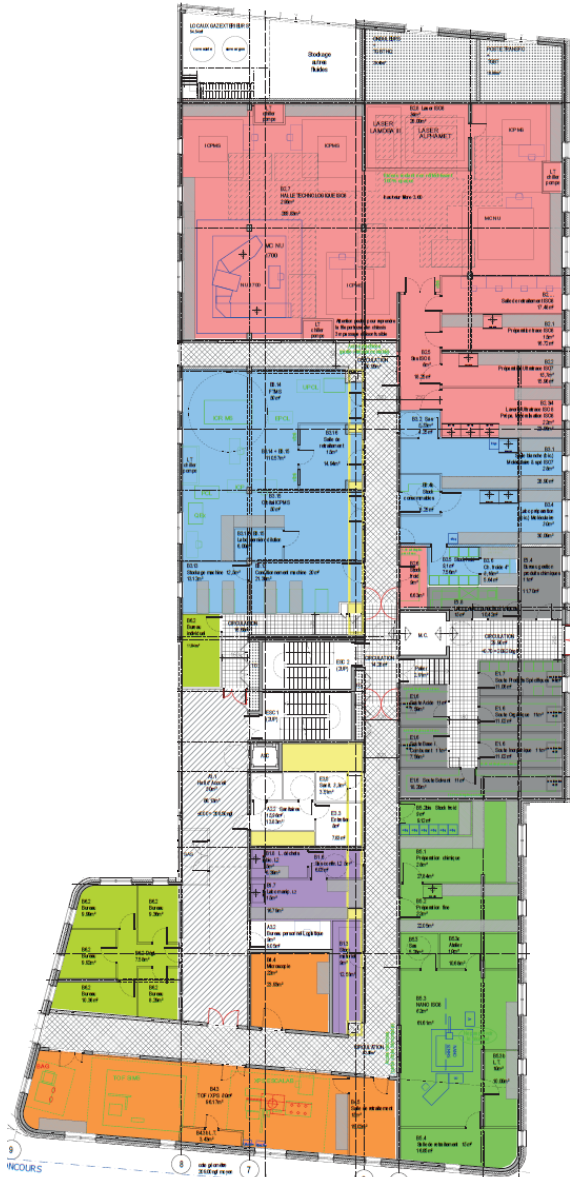
IPREM 1

IPREM 2

2021-2022

IPREM 2



Outline

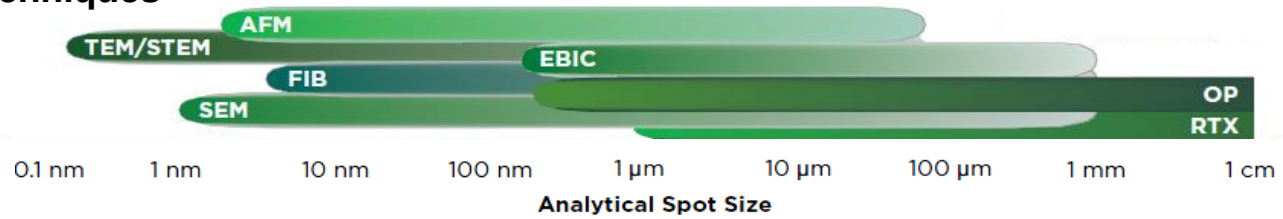
1 Introduction

2 NanoSIMS technique

3 Challenges for sample preparation

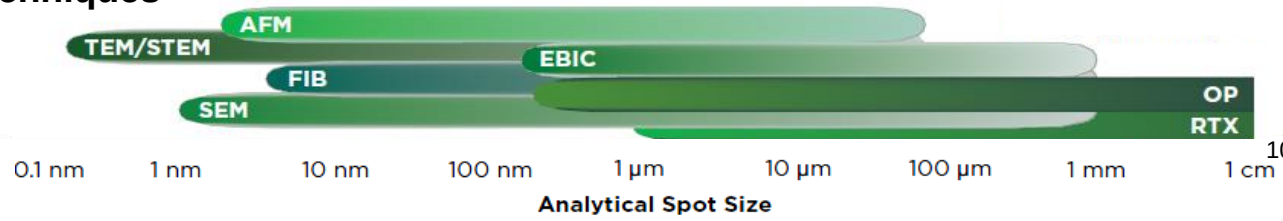
Combining microscopy/imaging with …

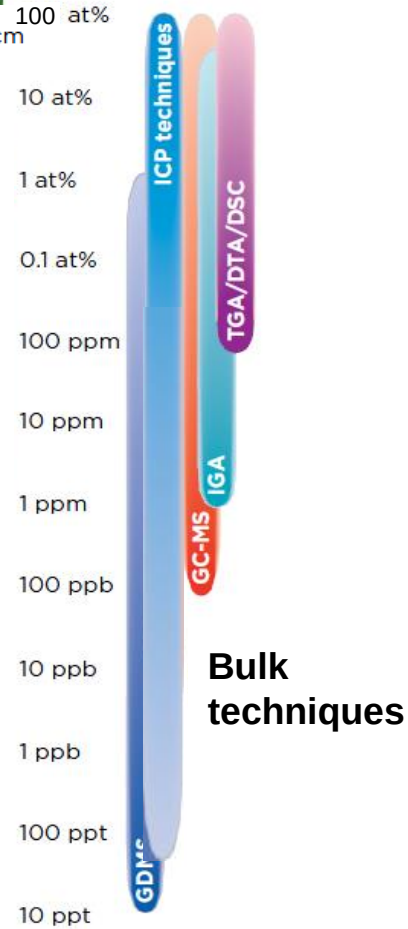
Imaging techniques



Combining microscopy/imaging with element/molecule specific techniques

Imaging techniques

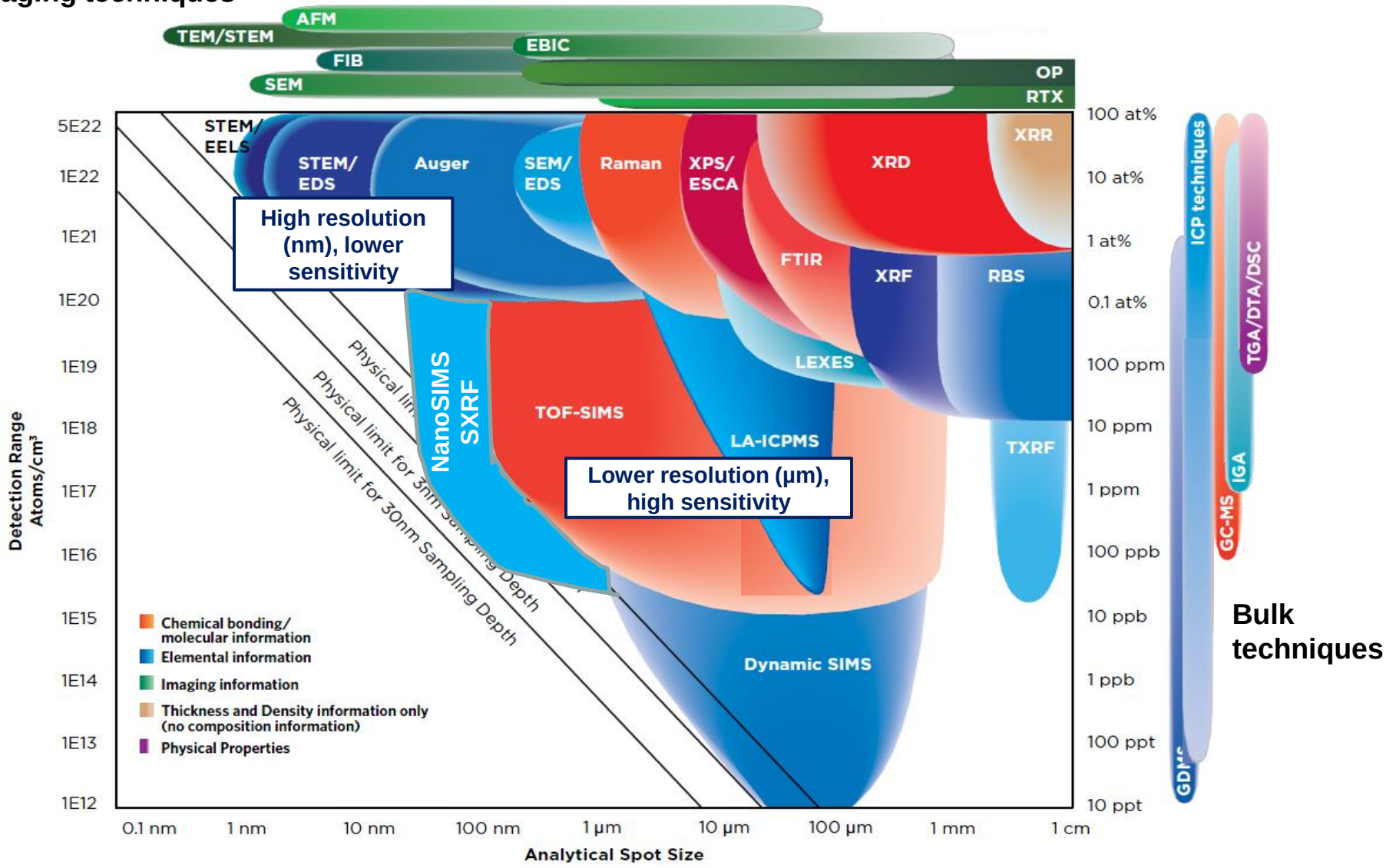




Bulk techniques

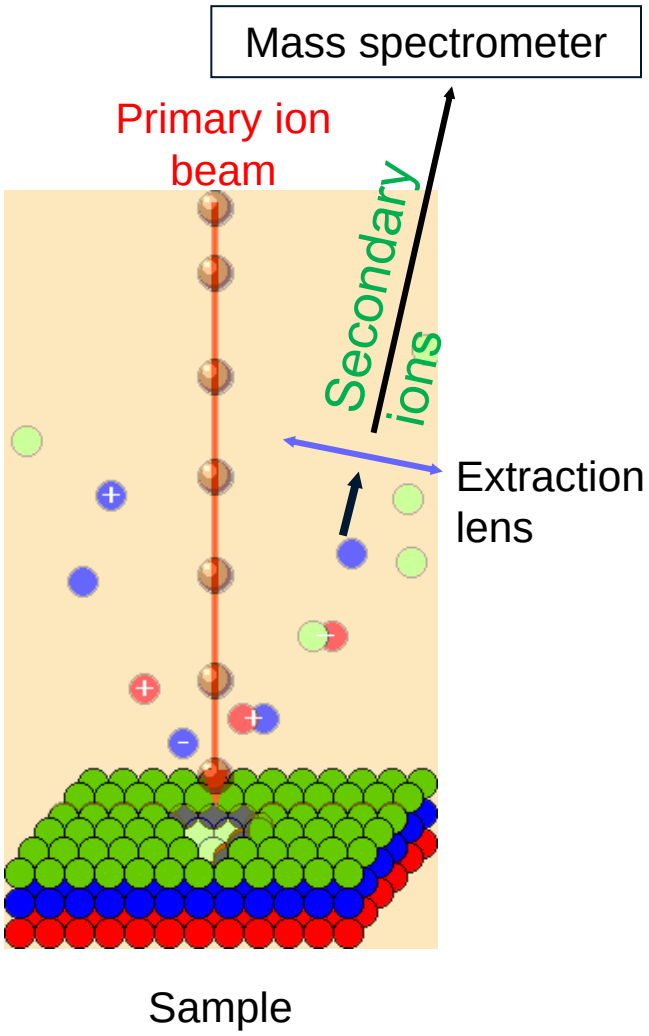
Combining microscopy/imaging with element/molecule specific techniques

Imaging techniques

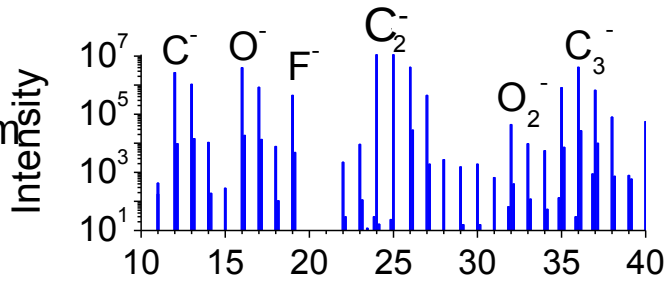


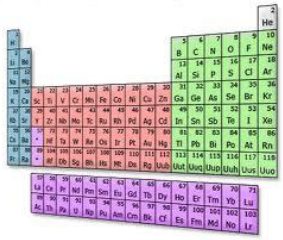
Bulk techniques

SIMS : Secondary Ion Mass Spectrometry



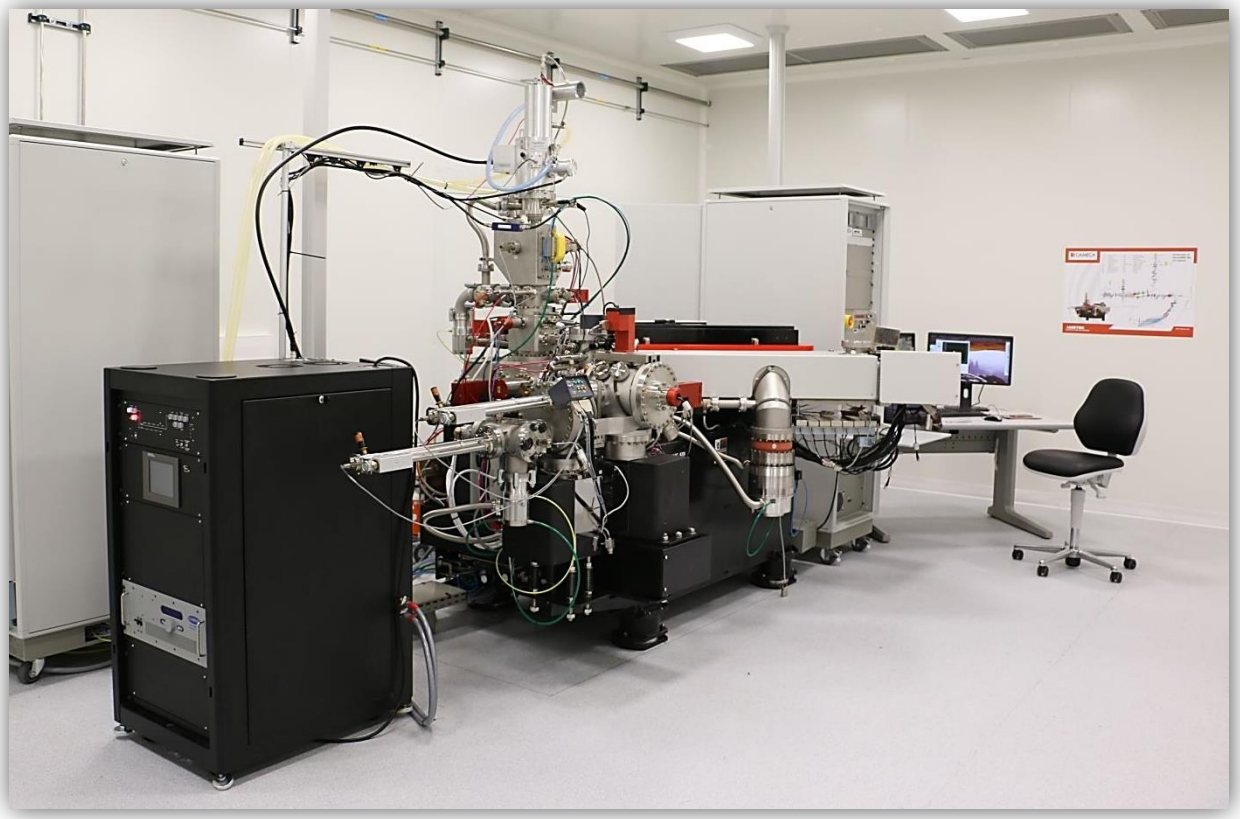
Mass spectrum





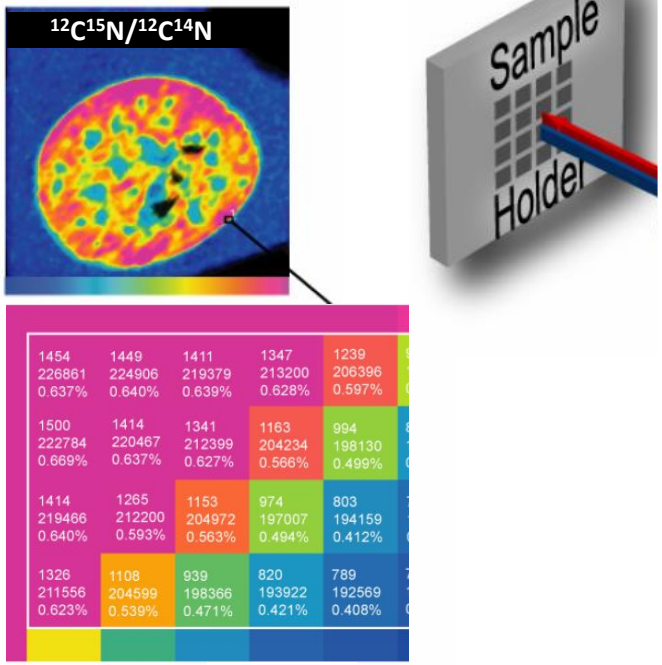
Nano Secondary Ion Mass Spectrometry (NanoSIMS)

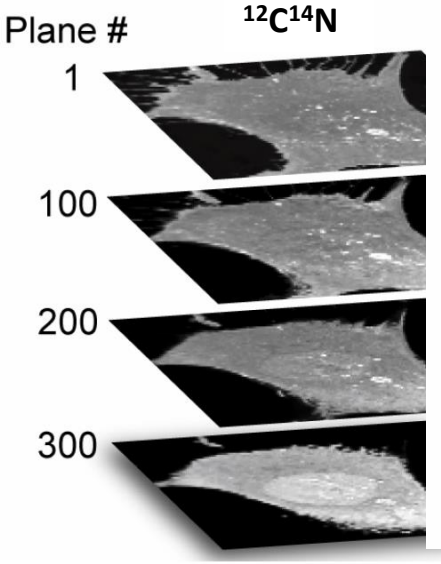
The NanoSIMS 50L instrument
part of the Mass Spectrometry Center in Pau, France **(MARSS)**

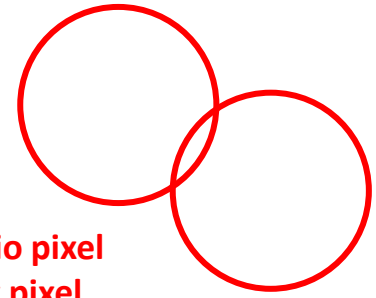


- **High lateral resolution: 50nm in Cs^+, 40nm in O^-**
- **High Sensitivity *together with* High Mass Resolution *and* small spot size**
- **Parallel Detection: 7 masses**

The NanoSIMS: a scanning Ion Microprobe with a multicollection mass spectrometer



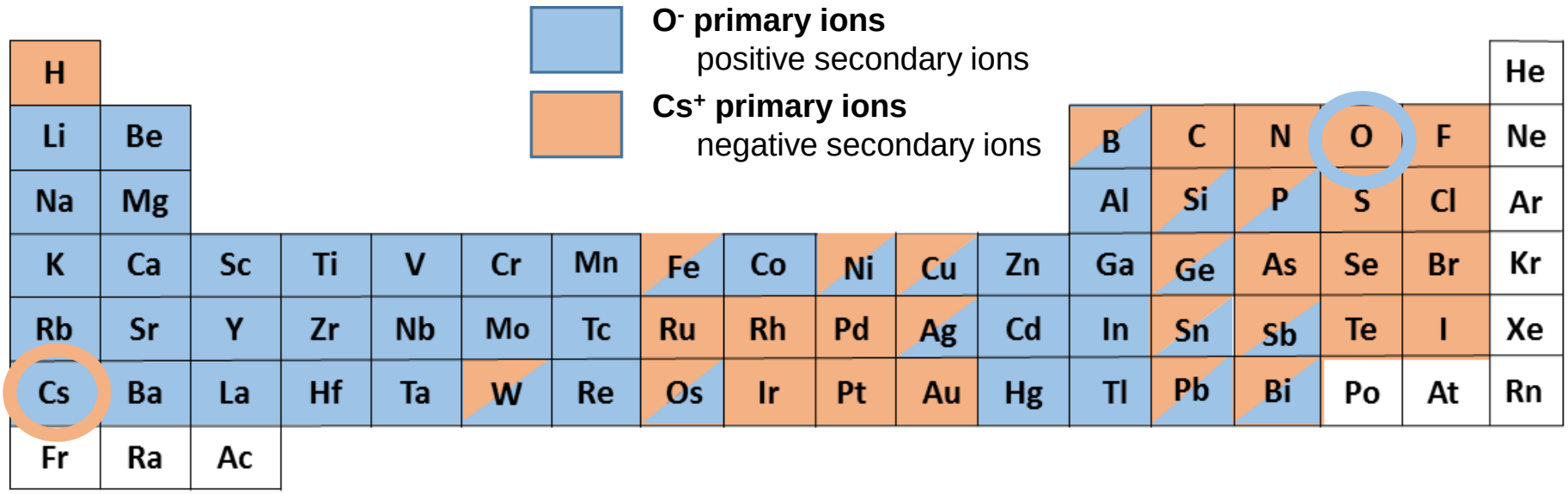




Ratio pixel by pixel

Schematics from: M. Steinhauser et al., Nature, Vol 481, 26 January 2012

Primary Ion Beam - Secondary Ion Yields



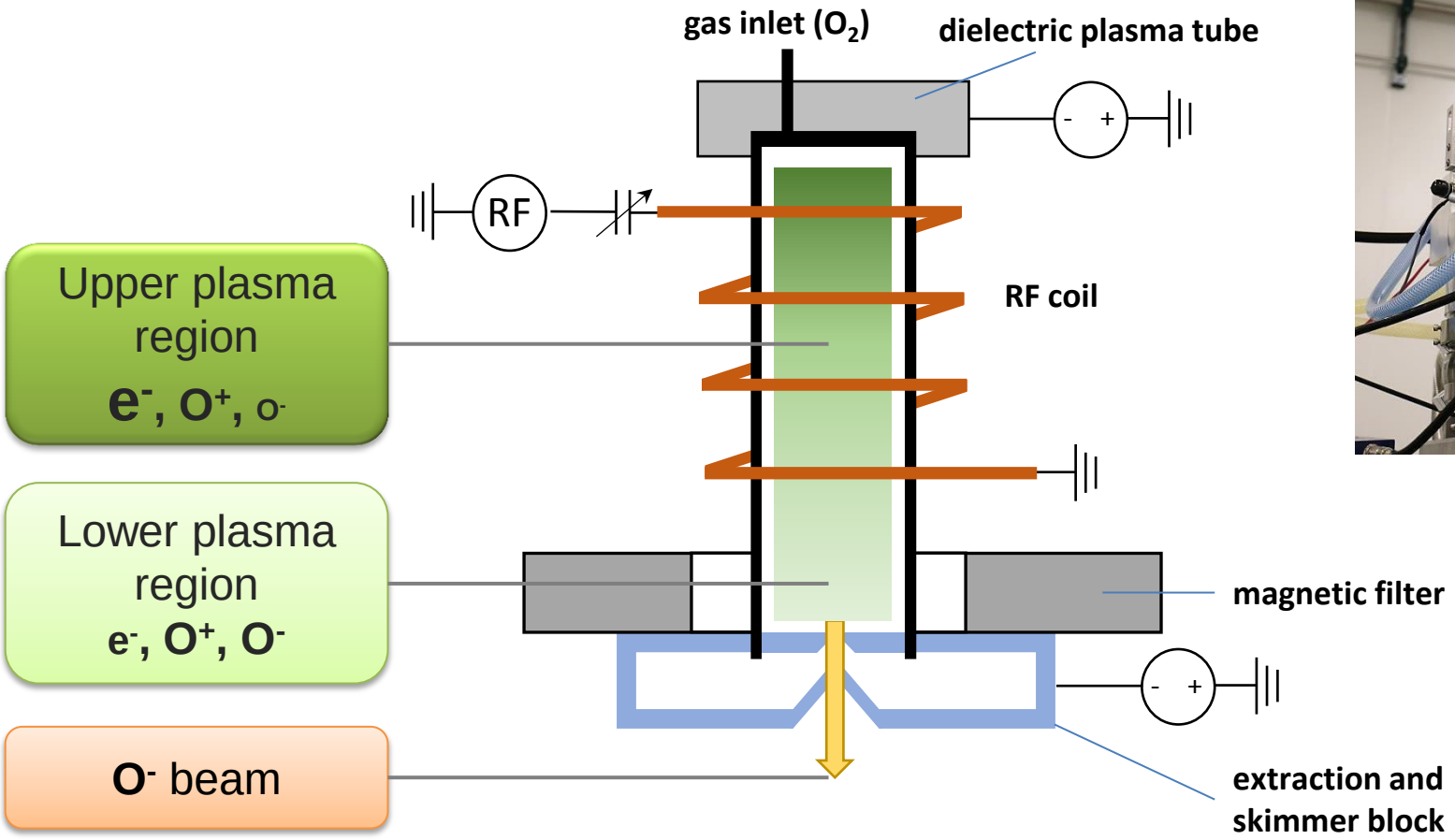
Cs^+ primary ion source

Classic NanoSIMS application (e.g. cell imaging):
C, N (via CN^-), **O, S, P, Se** and their stable isotopes for tracer studies.

O^- primary ion source

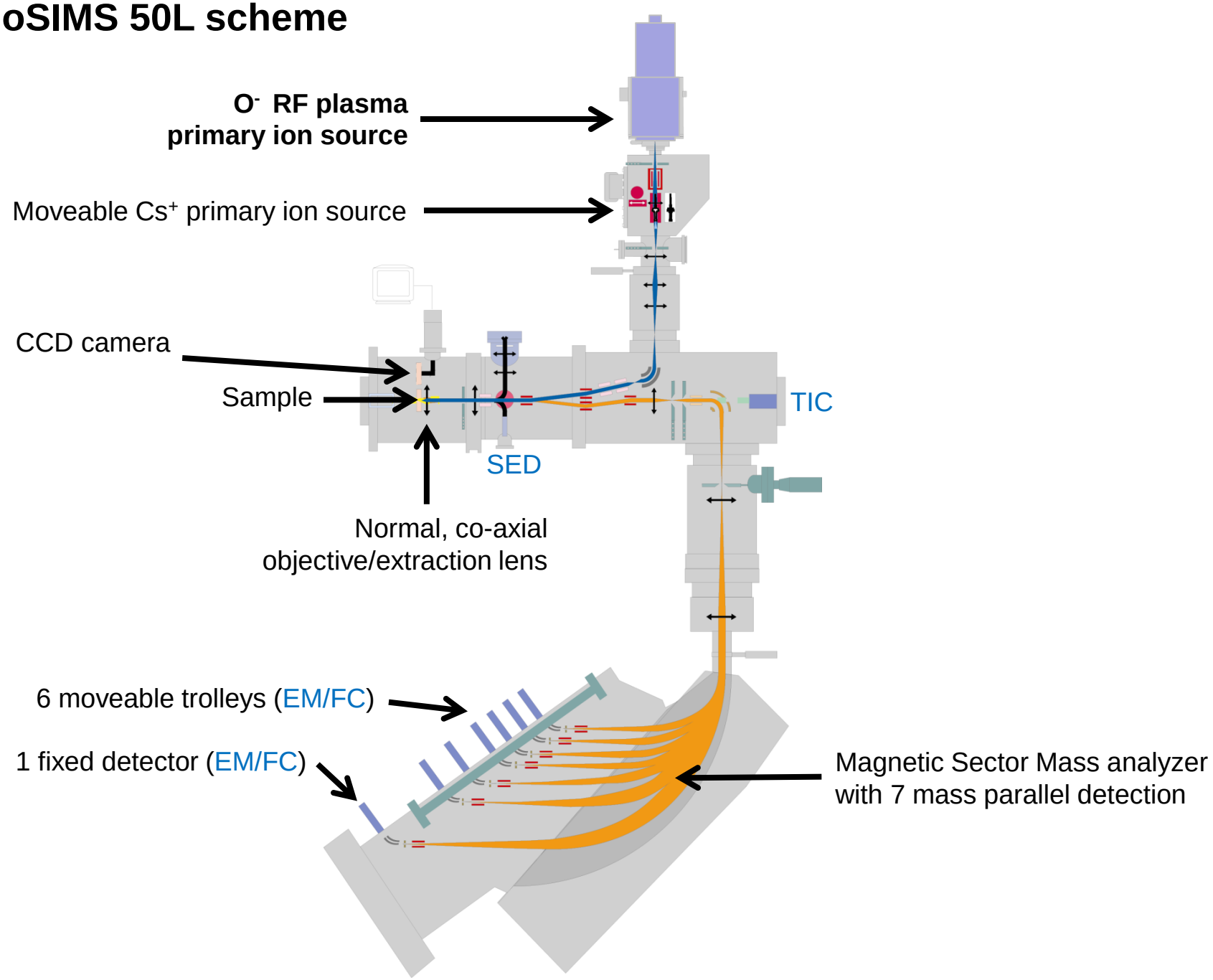
Imaging of major and trace metals is possible:
Ca, Mg, Al, Mn, Cr, Cu, Fe, Ni …

New O^- RF plasma primary ion source on NanoSIMS



- **Higher beam density** = better sensitivity for (trace) metals (Ca, Fe, Cu, Mn….)
- **Higher lateral resolution** : **40 nm**
  = sharper images enabling the observation of smaller details
- **Long term stability** – less maintenance

NanoSIMS 50L scheme



Characteristics of NanoSIMS

- **Allmost all Elements** (from H, D, T,… up to Pu), but with different sensitivity
- **High Sensitivity**: down to ppb in spot analysis, ppm in imaging,
- **High resolution imaging**: down to **40** nm lateral resolution, access to **3D** analysis with depth resolution of 10-15nm.
- **Isotopic analysis:** e.g. metabolic pathways and activity in biology

- **Quantification difficult**: matrix effects
- **Sample preparation** for biological samples is challenging

Preparation of (biological) samples for NanoSIMS

NanoSIMS analyses require :

- **Flat samples** to avoid artifact during ionization
- **Dehydrated samples** stable in ultra-high vacuum (10^{-11} mbar)
- **Conductive sample surfaces** to avoid charging effects from the ion beam

How these requirements can be compatible with biological cells or tissue ?

Sample preparation methods for transmission electron microscopy can be adapted for NanoSIMS

Biological sample preparation (similar to TEM)

Analysis at room temperature **under vacuum**: sample must be **dehydrated and fixated**

Chemical fixation

Glutaraldehyde
Formaldehyde
Osmium tetroxide

Cryofixation

high pressure freezer
tissues
(up to 6 mm diameter,
200 µm thick)

Dehydration

Solvent baths (acetone or ethanol/water)
with increasing solvent concentrations

Dehydration

Cryo-substitution
lyophilization

Resin embedding

Solvent baths with increasing
resin concentrations

Resin embedding

Solvent baths with increasing
resin concentrations

Ultramicrotomy

300 nm sections for NanoSIMS
70 nm sections for TEM/X-EDS

Equipment at Bordeaux Imaging Center

Biological sample preparation (similar to TEM)

Analysis at room temperature **under vacuum**: sample must be **dehydrated and fixated**

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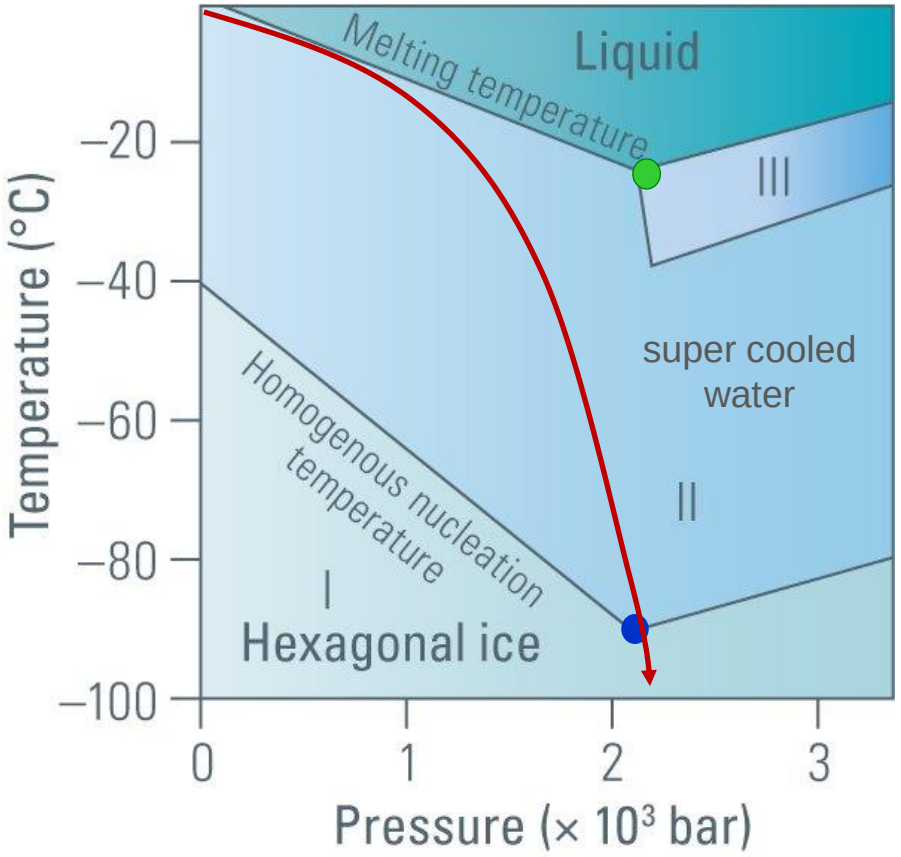
Cryofixation by high pressure freezing

States of water depending on pressure and temperature



At a pressure of 2045 bar the melting point of water is lowered to -22 °C ● and the temperature for homogenous nucleation is reduced to -92 °C.●

Kanno H et al. Science 189: 880–881 (1975)

High pressure freezing allows synchronized pressurization (2100 bar) and cooling of the sample within **20 ms** in a highly reproducible manner:

(1) lowering of the freezing point,
(2) reduction in the rate of ice crystal formation, and
(3) slowing of the growth of ice crystals

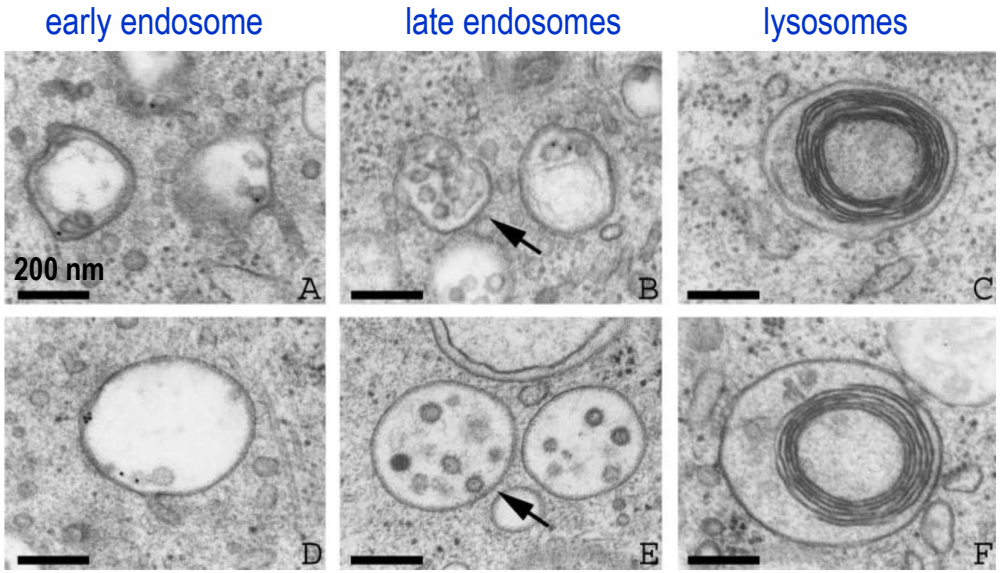
At 2100 bar water is 1500 times more viscous than at atmospheric pressure. This reduced considerably formation of ice crystals. Amorphous ice is formed.

⇨ Water is transformed in the vitreous state (amorphous ice) and thus the **cellular ultrastructure is fixed** and preserved.

Why cryofixation?

- Reduced Fixation Artifacts
  - Membrane blisters
  - Mesosomes
  - Nuclear equivalent
- Reduced Shrinkage

EM



Chemical fixation

Cryo fixation

J. Murk et al., J. Microsc. 212 (2003), 81–90

- Reduced extraction of cellular components
  - Lipids
  - Proteins
  - Proteoglycans
  - Metals

Biological sample preparation (similar to TEM)

Analysis at room temperature **under vacuum**: sample must be **dehydrated and fixated**

less redistribution of highly diffusable trace metals !

Chemical fixation

Glutaraldehyde
Formaldehyde
Osmium tetroxide

Cryo fixation

high pressure freezer
tissues
(up to 6 mm diameter,
200 µm thick)

Dehydration

Solvent baths (acetone or ethanol/water)
with increasing solvent concentrations

Dehydration

Cryo-substitution
lyophilization

LIMITATION of NanoSIMS:
Direct analysis of frozen hydrated samples not possible

Resin embedding

Solvent baths with increasing
resin concentrations

Resin embedding

Solvent baths with increasing
resin concentrations

Ultramicrotomy

300 nm sections for NanoSIMS
70 nm sections for TEM/X-EDS

Equipment at Bordeaux Imaging Center

Preparation of (biological) samples for NanoSIMS

NanoSIMS analyses require :

- **Flat samples** to avoid artefacts during ionization

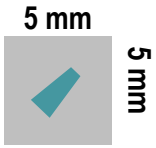
  Sections prepared with an ultramicrotome or polishing

- **Dehydrated samples** stable in ultra-high vacuum (10^{-11} mbar)

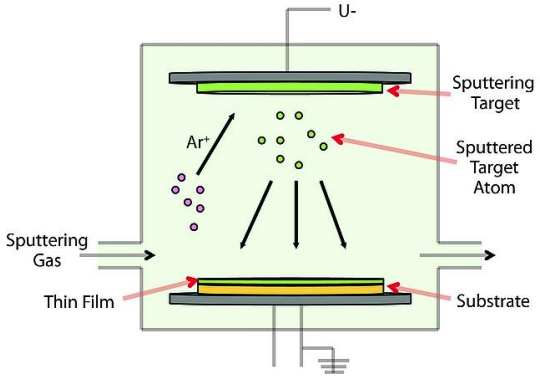
  Dehydrated and embedded in epoxy resin

- **Conductive sample surfaces** to avoid charging effects from the ion beam

  Ultrathin sections (< 500 nm) placed on conductive silicon wafer pieces



Thicker samples are metal (Au, Pt) coated with sputter coater (nm), similar to SEM



Biological sample preparation in the AQUAMAPMET project

Chemical fixation due to the geographical distance between sampling (Croatia) and preparation (BIC, Bordeaux, France)

Chemical fixation

Glutaraldehyde
Formaldehyde
Osmium tetroxide

Cryo fixation

high pressure freezer
tissues
(up to 6 mm diameter,
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Solvent baths (acetone or ethanol/water)
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Ultramicrotomy

300 nm sections for NanoSIMS
70 nm sections for TEM/X-EDS

Equipment at Bordeaux Imaging Center

Detailed protocol for sample preparation in the AQUAMAPMET project

Sampling

Croatia: Krka river, Ilova river, Visovac and Brljan lake
contaminated and reference site; fish and parasite tissues

Fixation

At sampling sites in Croatia

Paraformaldehyde 2% + Glutaraldehyde 2.5%
Phosphate buffer (PB) 0.1 M
Fixation during 3-4 h at RT
Conservation at 4°C possible, if not immediately mailed

Express mail to BIC

Samples cooled at 4°C

Rinsing

At BIC, France

3 x 5 min in phosphate buffer 0.1 M

Post-Fixation

Osmium tretroxid 1% + Paraformaldehyd 1%
Phosphate buffer (PB) 0.1 M
Fixation during 1 h at RT

Rinsing

3 x 5 min in phosphate buffer 0.1 M

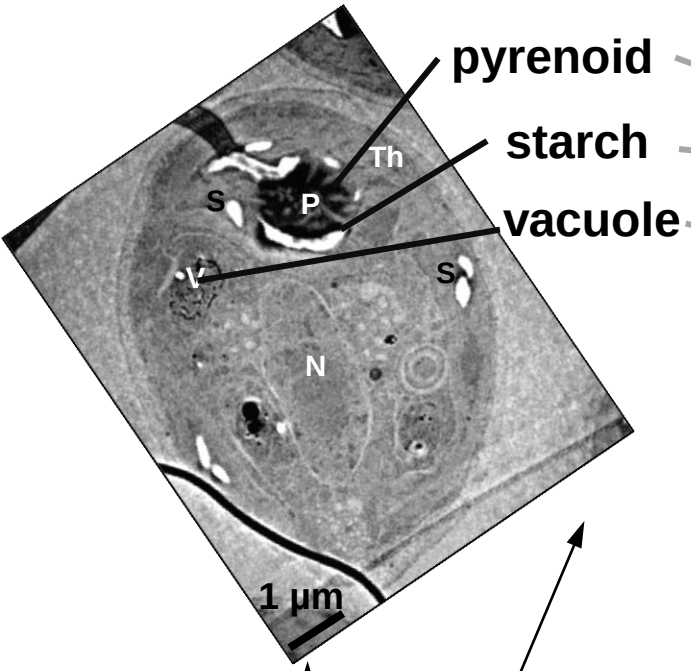
Dehydration and Impregnation

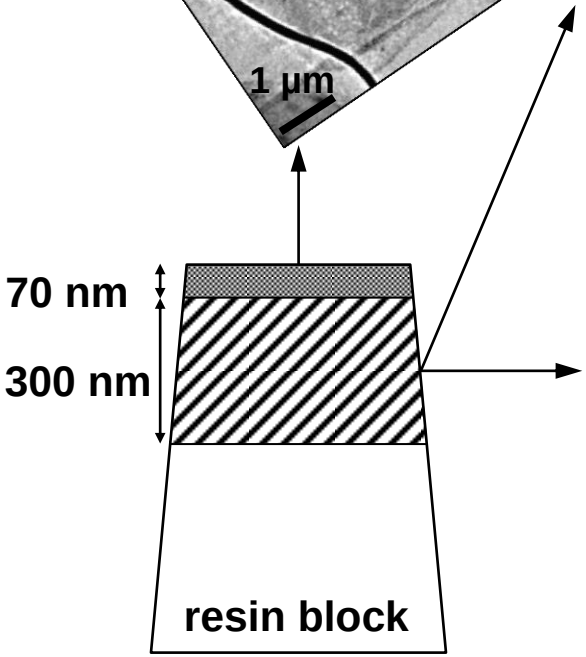
Leica EM AMW - Tissue Processor
50, 70, 95,100% ethanol; 19 min, 37°C
Ethanol:Epon resin 3:1, 1:1, 1:3, pure; 55 min, 40-50 °C

Polymerization

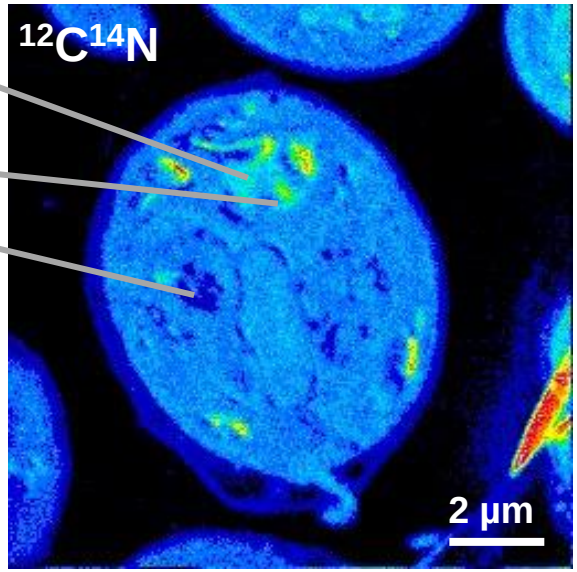
50-63 °C, 5 min; 63-75°C, 5 min; 75-83°C, 15min
83°C during 105 min

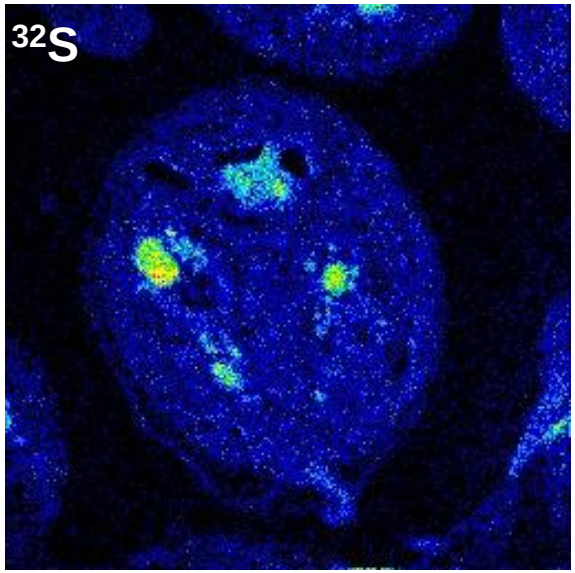
Correlative imaging: TEM and NanoSIMS

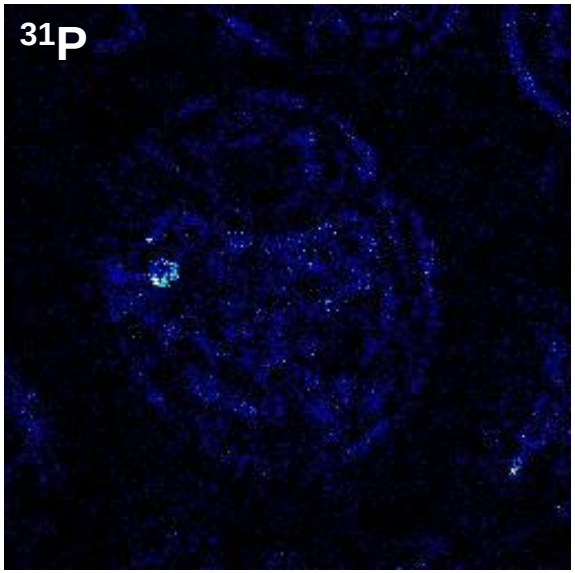


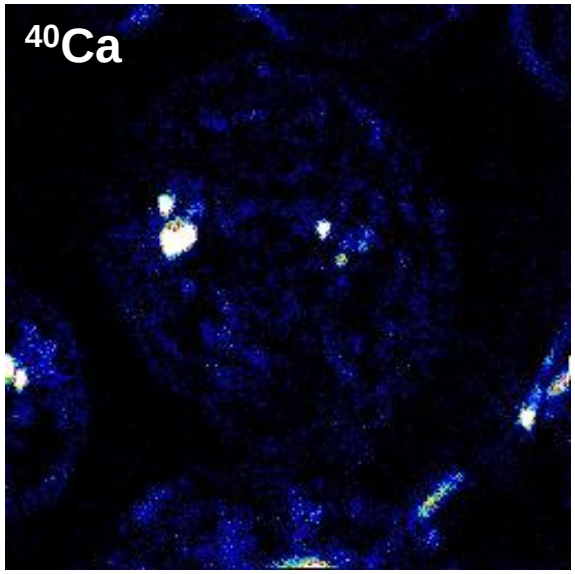


Cs source









Max

Min

O source


Thank you for
your attention !