Optimizing cells preparation for correlative single-cell imaging

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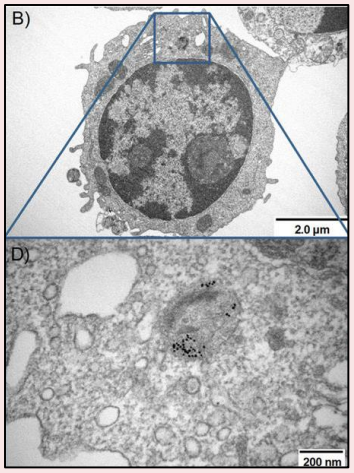
lcbm
Laboratoire de Chimie et Biologie des Métaux

Single-cell imaging techniques (non-exhaustive list)

Electron Microscopy



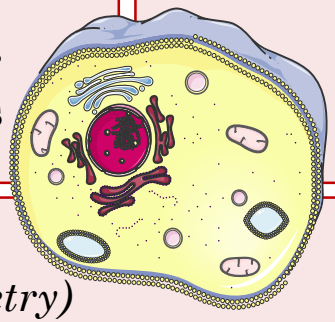
Gamrad et al. Sci. Rep. (2016)

TEM, STEM, SEM…

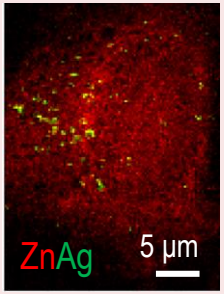
Ultrastructure

Spatial resolution
< 1 nm.

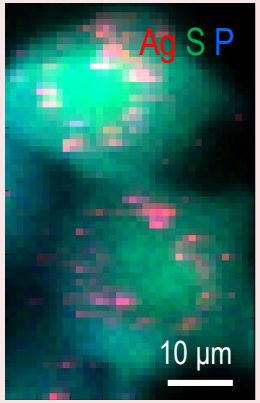
→ Subcellular compartments



Nano/micro XRF imaging



ID16B



ID21

ID16A, ID16B, ID21 @ESRF

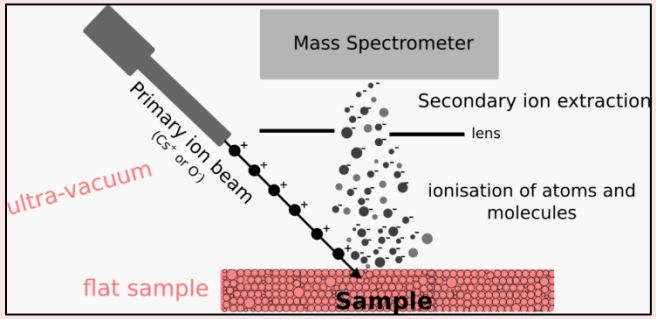
Elemental composition

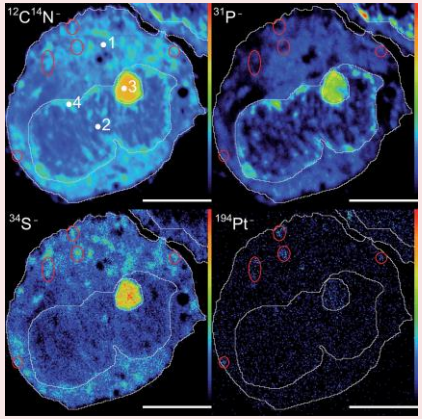
Resolution: 20nm to 1µm
Detection limit: sub-ppm
Optimized for transition metals

→ Trace elements

NanoSIMS

(Secondary Ion Mass Spectrometry)





Legin et al. Chem. Sci. (2014)

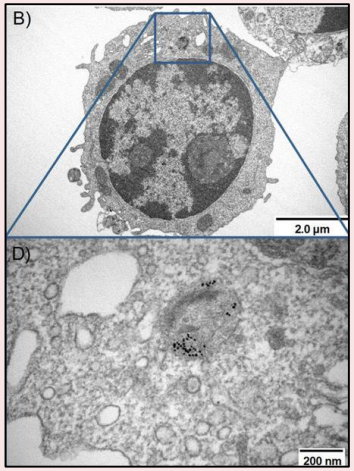
Molecular and elemental distribution
Sensitive to isotopes

Resolution: 50 nm
Detection limit: sub-ppm, optimized for light elements and molecular groups

→ Macronutrients (proteins, lipids..)

Experimental constraints

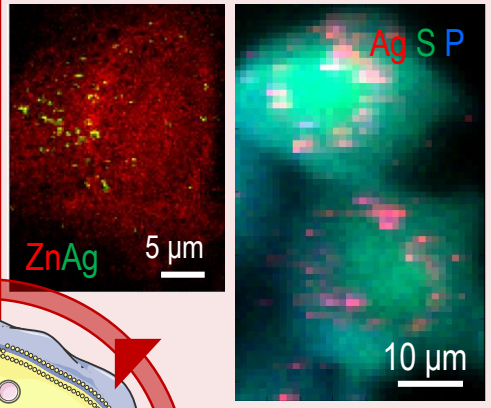
Electron Microscopy



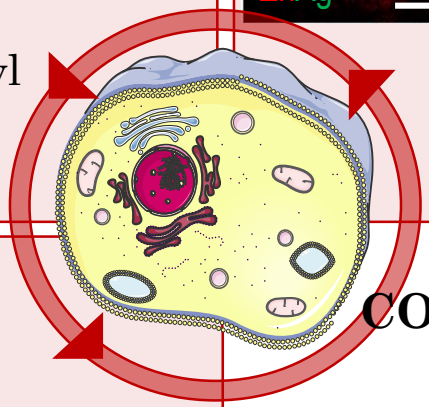
Gamrad et al. Sci. Rep. (2016)

- Resin embedded
- Thin sections for TEM (~100 nm)
- Preserve ultrastructure (no freeze-drying)
- Staining: OsO_4, Uranyl acetate, Pb-citrate

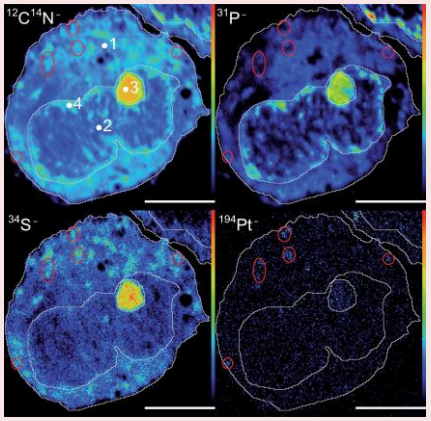
Nano/micro XRF imaging



- Avoid exogenous metals: NO staining
- Avoid labile ion leaching: NO chemical fixation



NanoSIMS



Legin et al. Chem. Sci. (2014)

- Room temperature
- Flat surface
- Avoid labile ion leaching: NO chemical fixation

CORRELATIVE IMAGING
On the same cell

- Measure at room temperature
- Thin sections: 200-400 nm
- No chemical fixation: High-pressure freezing + freeze substitution.
- Staining: No U, Pb.
  Use OsO_4, or tannic acid, or post-stain.

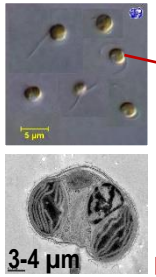
Scientific cases

Algal remodeling in planktonic photosymbiosis

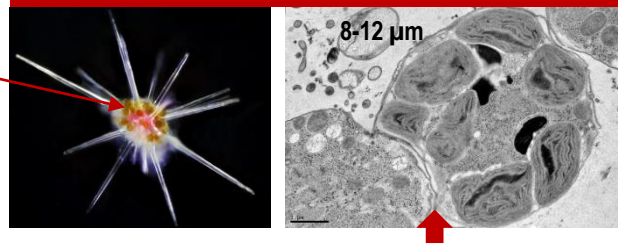
*PI: **Johan Decelle**, Plant and Cell Physiology lab (PCV) - CNRS/UGA/INRA/CEA Grenoble.*

Photosymbiosis between unicellular organisms

Free-living microalgae



Microalgae in host



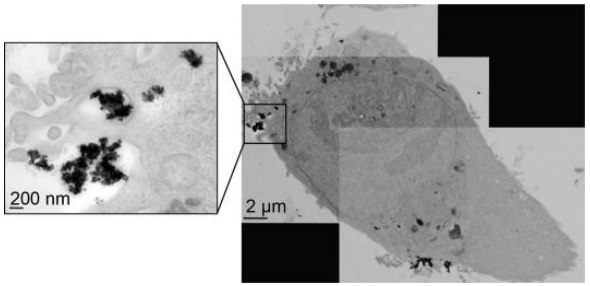
➡ Morphological reconfiguration

- Metabolic reconfiguration?
- **Trace metal exchanges?**
- Role of Fe (involved in photosynthesis)

Silver nanoparticle induced impairment of hepatocyte functions

*PI: **Aurélien Deniaud**, Laboratory of Chemistry and Biology of Metals (CBM) - CNRS/UGA/CEA Grenoble.*

Silver nanoparticles (AgNPs), used as biocides in medical devices, are toxic to hepatic cells



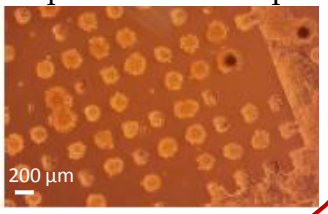
AgNPs enter cells and release toxic Ag^+ ions

- **Ag^+ trafficking?**
- Excretion?

⬇

Development of 3D cell cultures: liver-like model

Spheroids on chip



- Interest in sub-cellular compartments → thin sections
- Samples are "rare" → one preparation for all techniques
- Limited access to experimental techniques → easy-to-store samples

1. Cryo-fixation: high pressure freezing

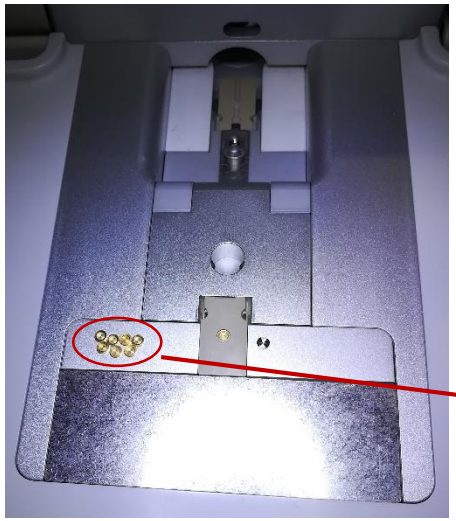
Vitrification = transformation of water in the sample into AMORPHOUS ICE.

High pressure (> 2000 bar)
Fast cooling (~ 20 ms)
No ice crystal nucleation

All sample preparation performed by
***Benoit Gallet**, Institut de Biologie Structurale (IBS), UGA/CNRS/CEA Grenoble.*

Cryo-fixation vs chemical fixation:
L. Perrin et al JAAS (2015), 30, 2525.

HPM100, Leica



2. Dehydration: freeze substitution

EM ASF2, Leica

Freeze substitution = dehydration at low temperature by dissolving frozen water with an organic solvent.

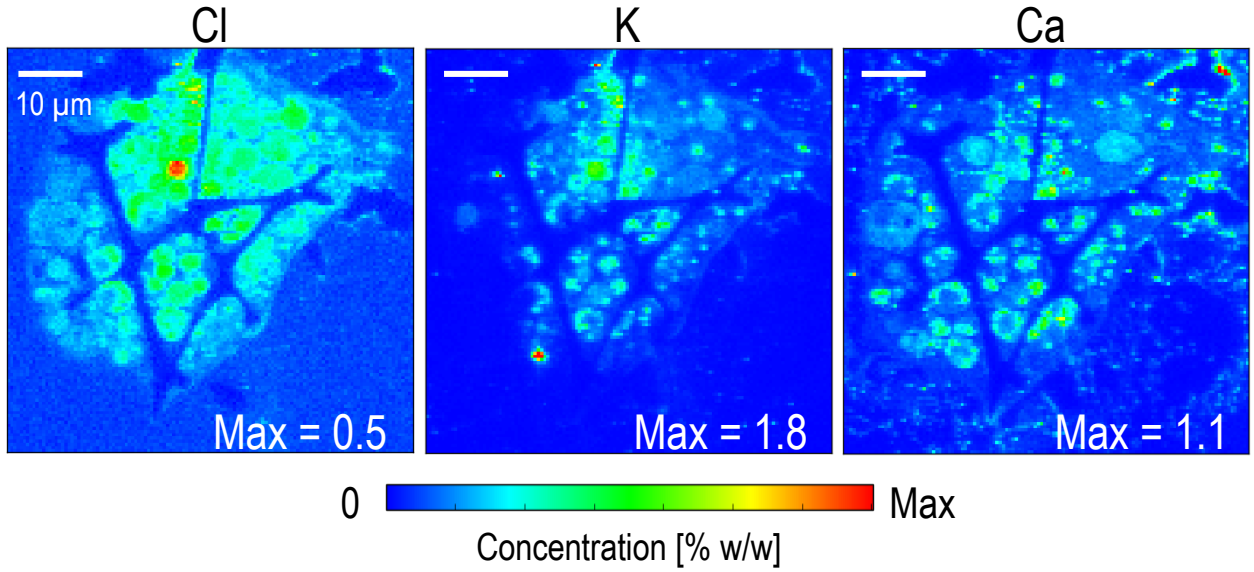
Chemical fixatives are often used

Ex: -90°C to -30°C for 5 days with Acetone + OsO_4

REVIEW: C. Quintana. Micron (1994) 25, 63. "Cryofixation, Cryosubstitution, Cryoembedding for Ultrastructural, Immunocytochemical and Microanalytical Studies "

Preserves the mobile ion content

@ ID21
E = 7.3 keV



3. Preparation: final steps

3.1 Embed in resin

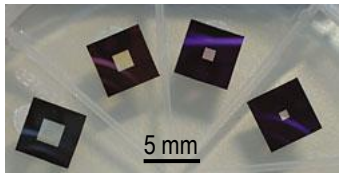
- Araldite, Epoxy (from PubChem database: Epoxy resin $C_{21}H_{25}ClO_5$ ρ=1.12 g/cm^3)
- Graded resin/acetone (v/v) series, each step lasting 2 h at increased temperature.

Decelle et al. (2019) Current Biology.

3.2 Prepare sections

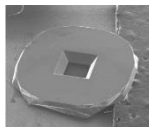
- ~ Adjacent sections for different techniques:
  60-80 nm for TEM, 300-400 nm for XRF and SEM
- One section for different techniques: Semi-thin, 100-200 nm

3.3 Lay on Si_3N_4 membranes

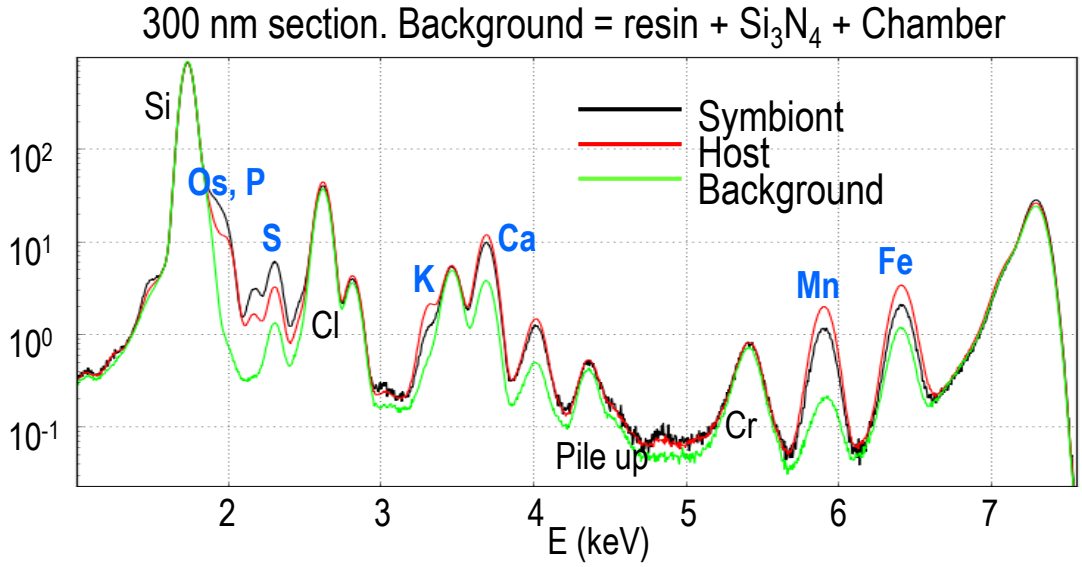


http://www.silson.com/

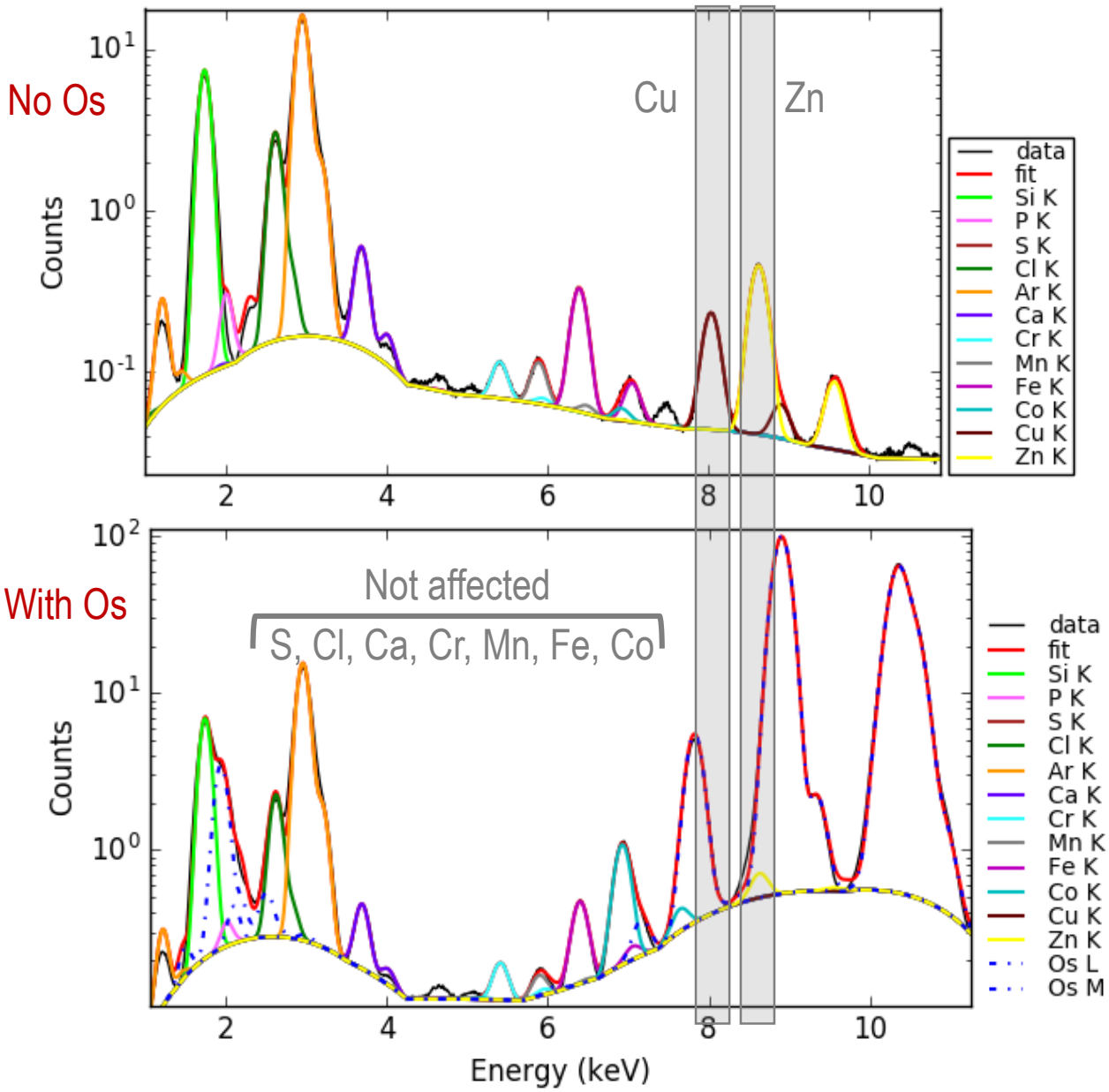
200-500 nm thickness



OR 50 nm thickness Si_3N_4 from *OXFORD instruments* if you want to measure the same section in XRF and TEM



High pressure freezing and freeze substitution on algal samples.
With vs without OsO_4



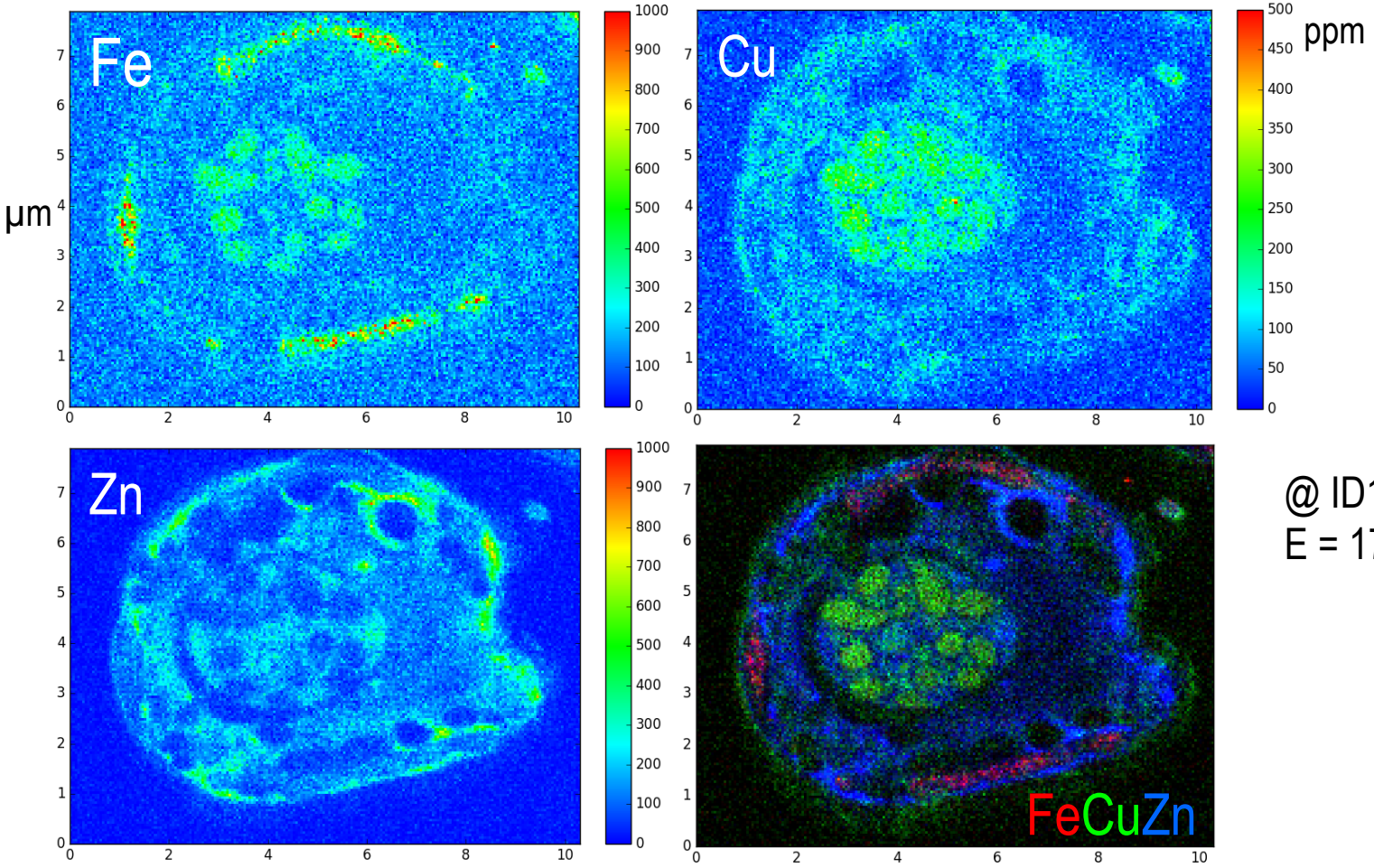
[Ca] = 1100 ppm (= 0.11%)
[Mn] = 40 ppm
[Fe] = 150 ppm
[Cu] = 70 ppm
[Zn] = 130 ppm

[Ca] = 900 ppm
[Mn] = 40 ppm
[Fe] = 210 ppm
[Cu] = 0 !!
[Zn] = 90 ppm

OsO_4 preparation not efficient for detection of Cu and Zn in trace concentration

No Os

(1. high-pressure freezing. 2. freeze substitution with **no fixative**)



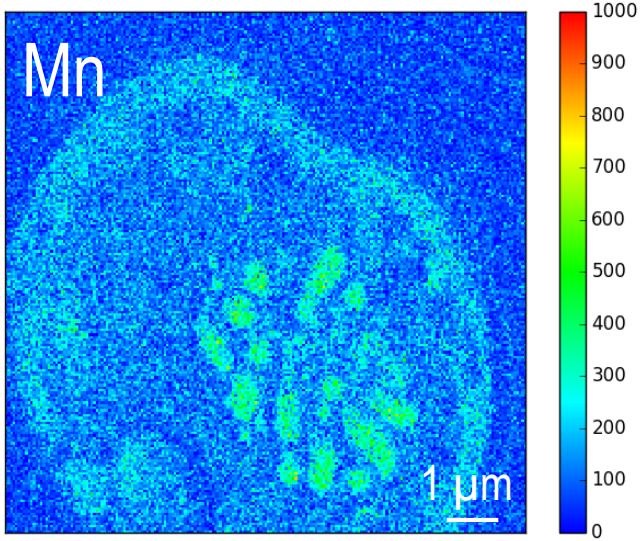
The ultrastructure is not well preserved. → Add glutharaldehyde.
Kashiv et al. *Scientific Reports*, **2016**, *6*, 21437.

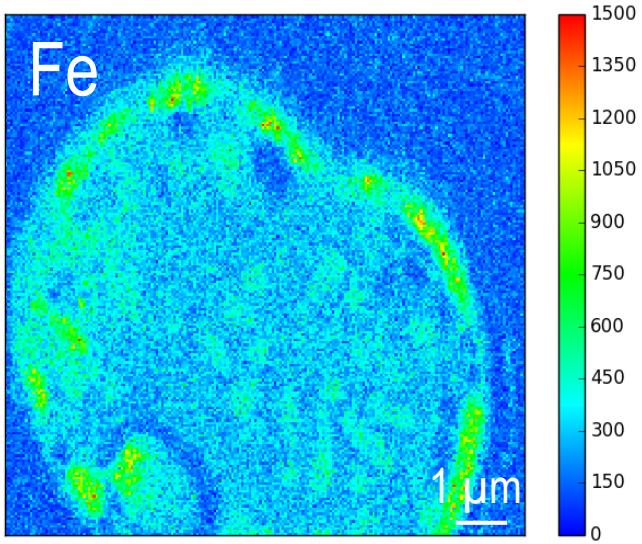
Not compatible with EM → Stain with tannic acid. (Kashiv et al.)
Or post-stain with OsO_4

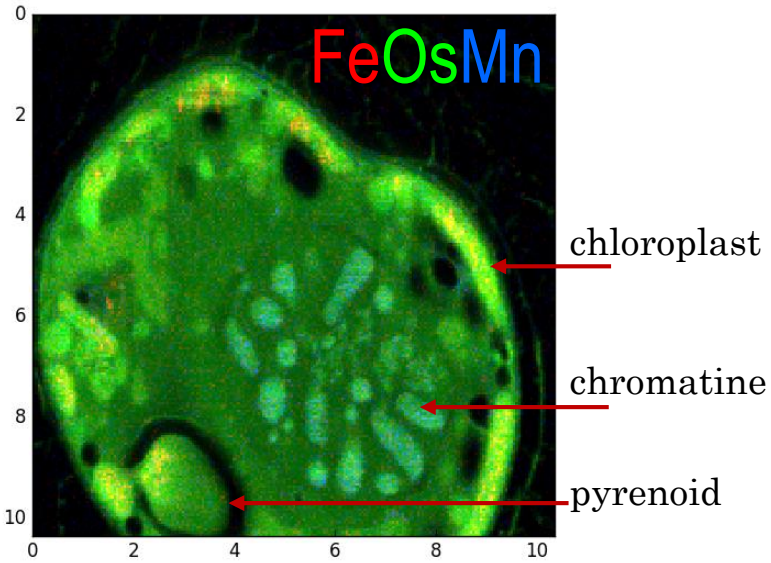
No direct visualization of subcellular compartments

With Os

(1. high-pressure freezing. 2. freeze substitution with **1% OsO_4**)







OsO_4 **fixes** tissues by cross-linking lipids. → It provides **contrast** at the membranes in elemental imaging.

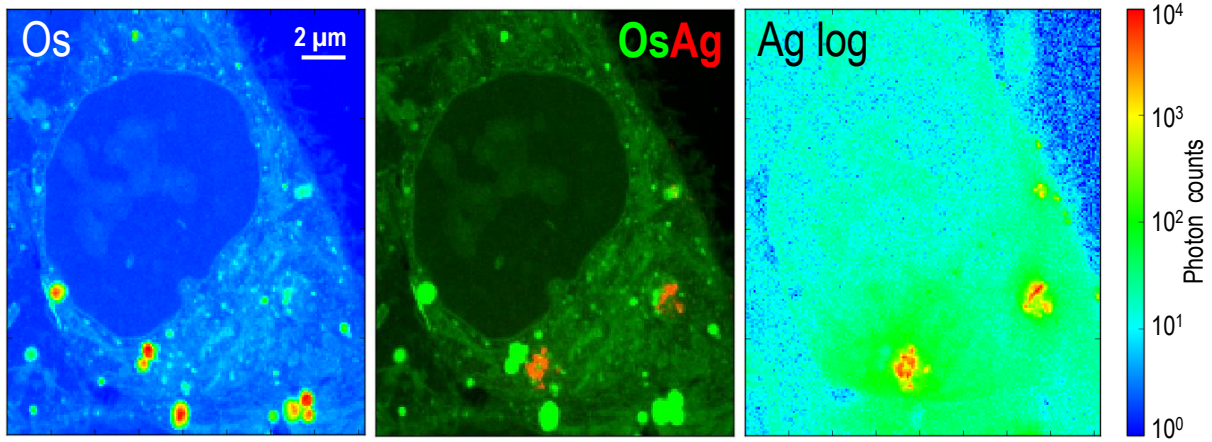
1. Ultrastructure preserved.
2. Identification of cellular compartments from XRF
3. Compatible with EM

@ ID16B-NA
E = 17.5 keV

Identification of subcellular compartments

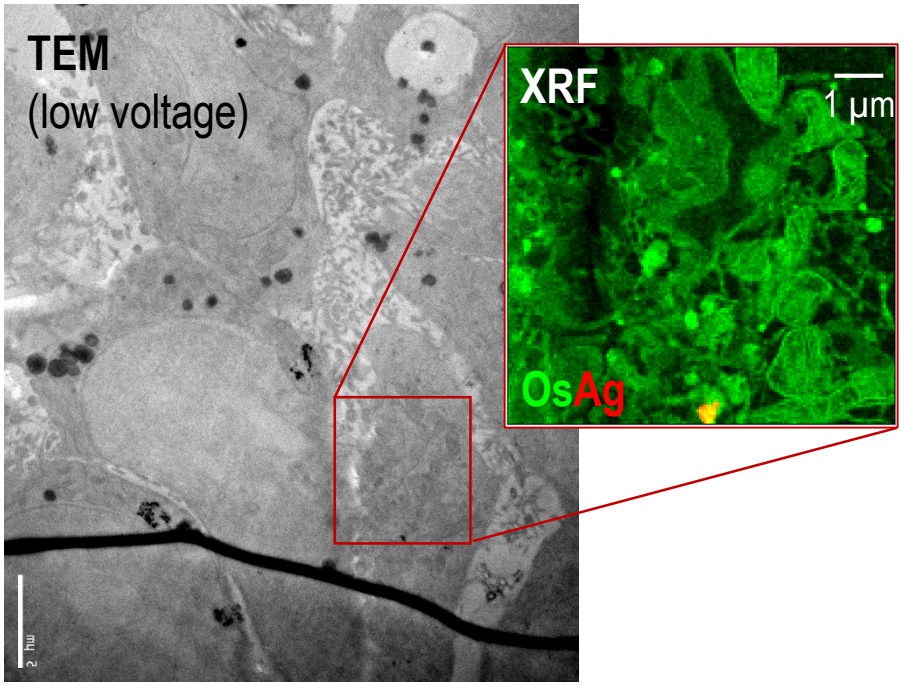
High-energy setup for high sensitivity to *4d* metals
→ NO contrast in cells

Os staining is needed to visualize the cells.

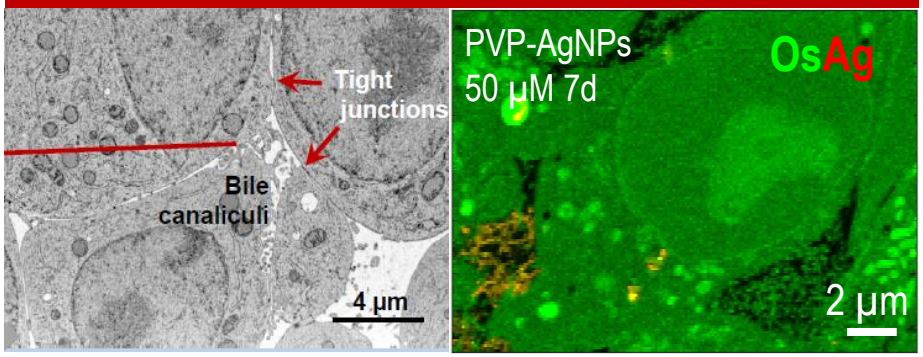


Preparation: 1. high-pressure freezing. 2. freeze substitution with 1% OsO_4

@ ID16B-NA
E = 26.7 keV

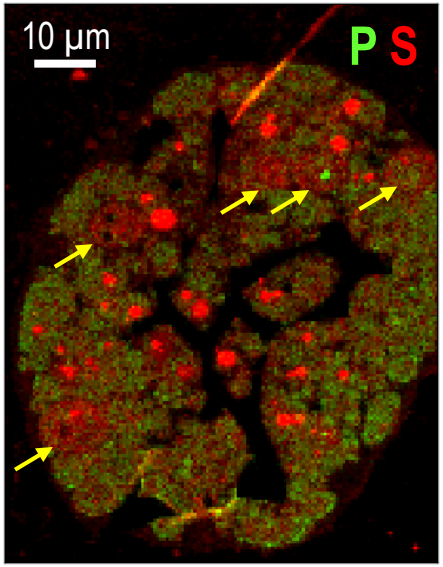


3D cell cultures – bile canaliculi

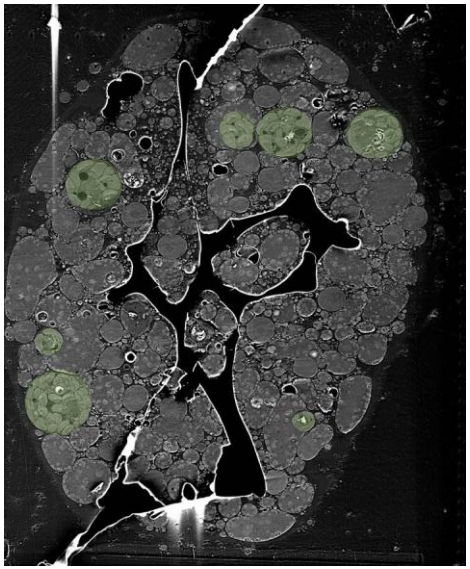


Collaboration with
***Vanessa Tardillo Suarez**, ID16B.*

Example of correlative microscopy



@ ID21 E = 7.3 keV
Pixel size 0.5 x 0.5 µm^2
Flux ~ 10^{10}ph/s, 0.5 s/pt

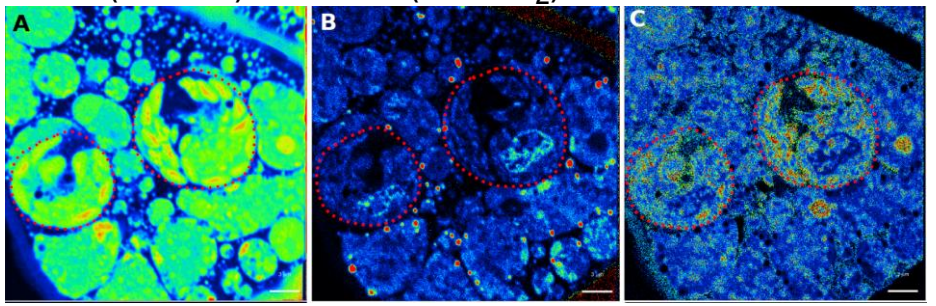


SEM
After SR-XRF

Symbiont vs host

<< P → metabolic control, no division.
>> N → provided by the host:
investment in energy acquisition.

N ($^{12}C^{14}N$) P ($^{31}P^{16}O_2$) N/P

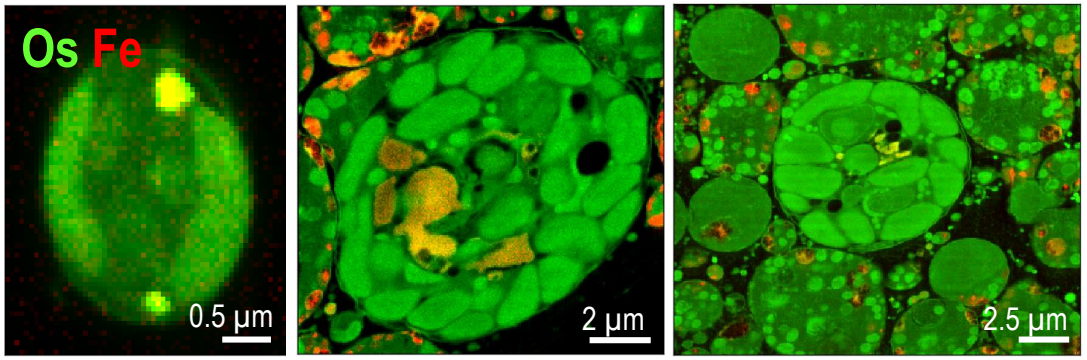


NanoSIMS @Helmholtz Center Leipzig

Free living vs symbiotic

>> Fe → provided by the host:
enhance photosynthesis.
Metal-storage vacuoles.

Free living Symbiotic Host + Symbiont



@ ID16B-NA. E=17.5 keV. Pixel size 50 x 50 nm^2

Decelle et al. (2019) Current Biology.

Conclusions

1. Feasibility of **multimodal approach** to single-cell imaging techniques
2. Sample preparation based on high-pressure freezing (cryo-fixation) and freeze-substitution (dehydration)
3. The **preparation** must be **adapted to the scientific case**
4. Information obtained: morphology, trace elements, macronutrients, trafficking, metabolism….

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Giovanni Finazzi

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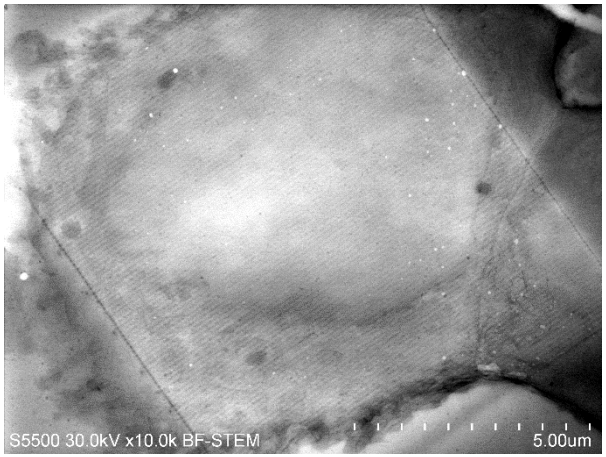
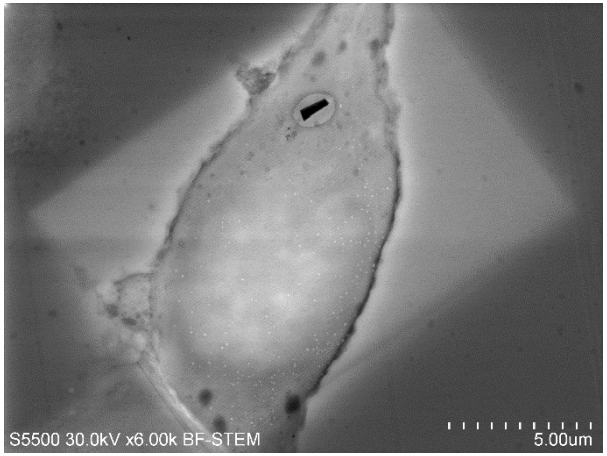
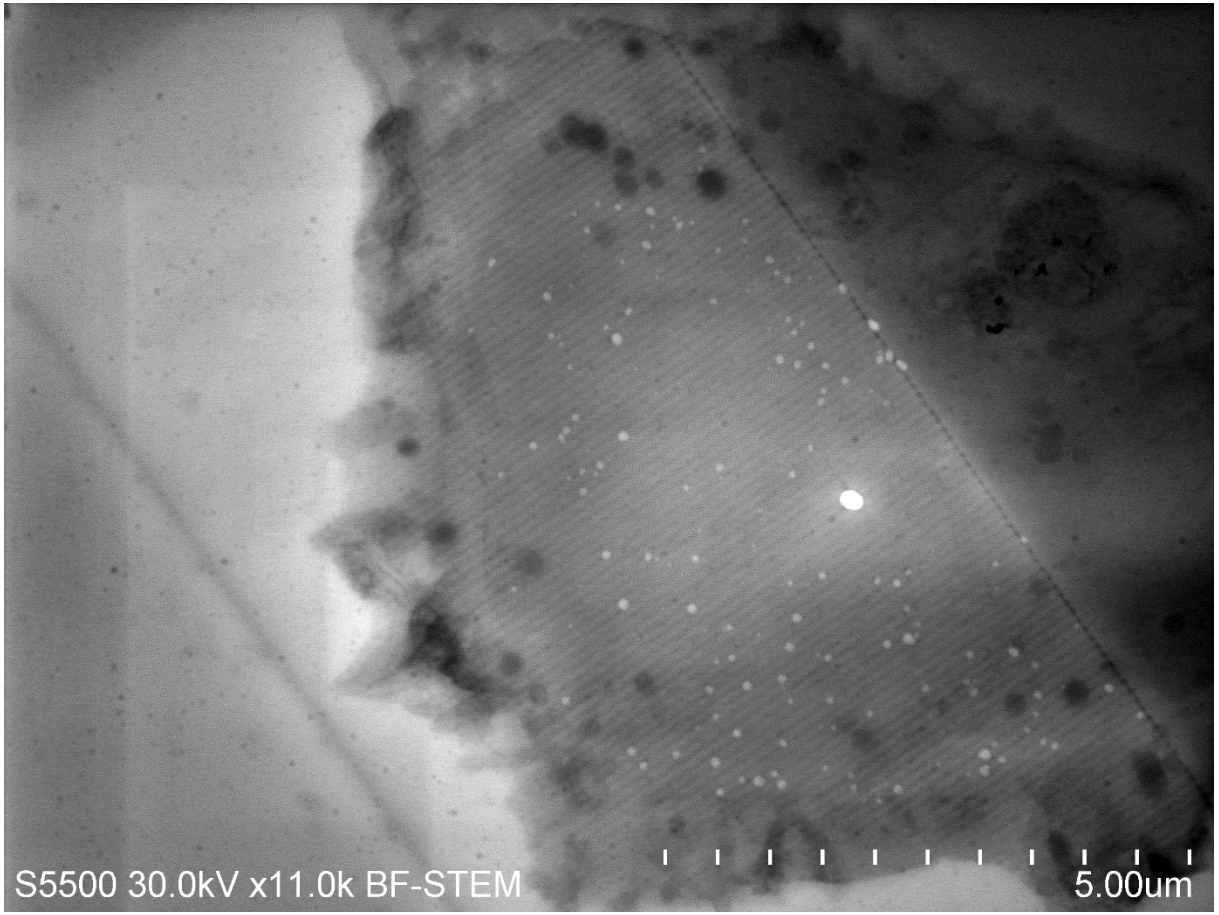
THANK YOU FOR YOUR ATTENTION

Radiation damage







SEM after nano-XRF @ ID16B-NA

E = 26.7 keV, Flux ~ $2 \cdot 10^{11}$ ph/s.

Pixel size 100 x 100 nm^2. Dwell time 500 ms/pt.