Preparation of tissues for µXRF/ µXAS analyses

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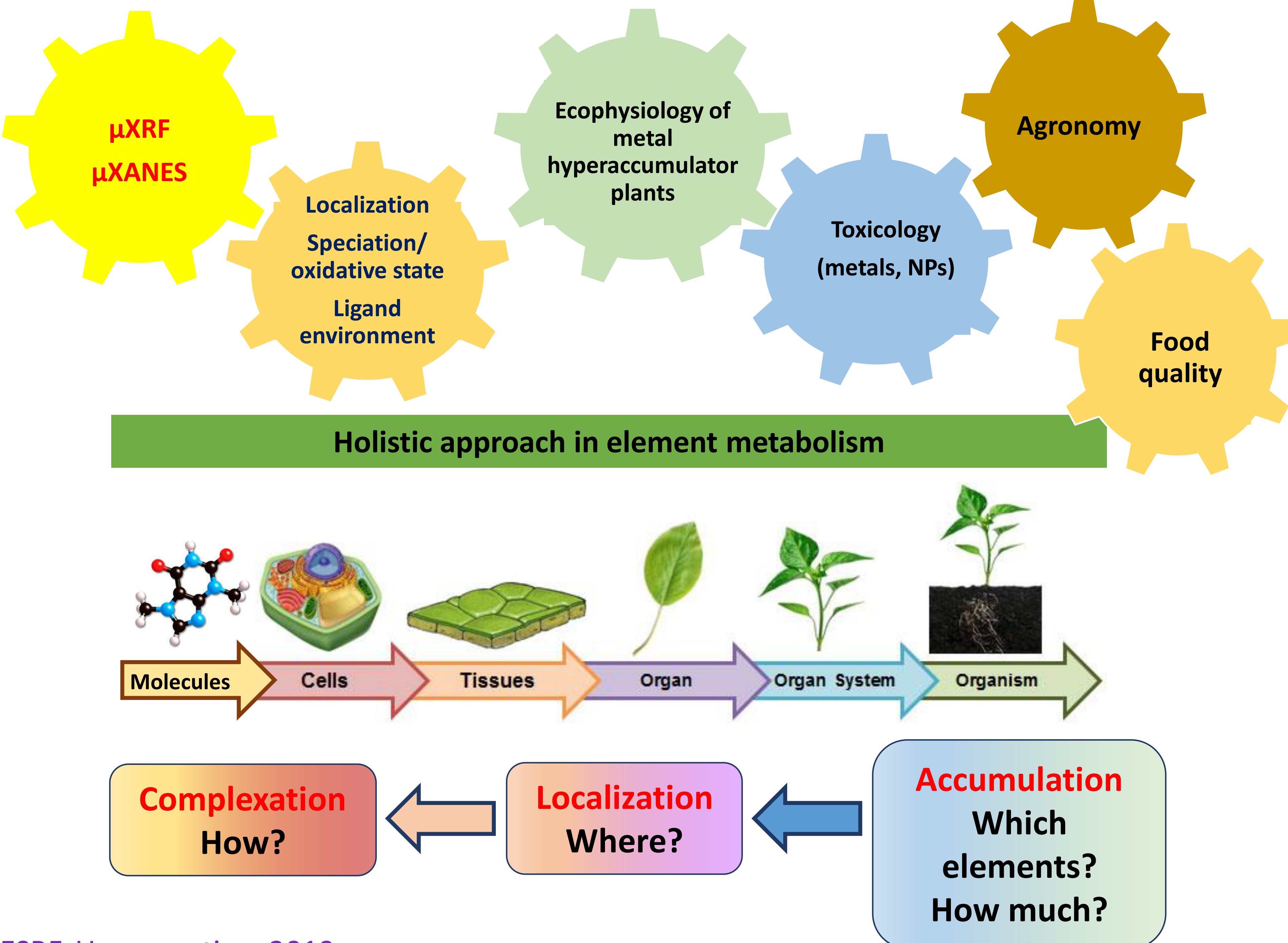

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Sample preparation for imaging & µ-XAS

- **The main goals to be achieved during sample preparation:**

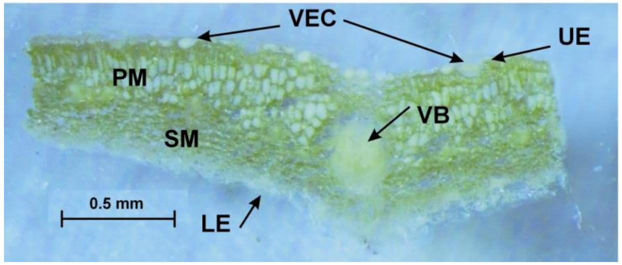
➢ **preserve local distribution of elements in tissues**

➢ **preserve sample morphology and anatomy**

➢ **preserve metal ligand environment as similar as "in vivo" stage**

➢ **Vaccum compatible samples (for LE-XRF, µPIXE)**

Changes in tissue morphology and chemical redistribution must be limited to dimensions that are smaller than the resolution of the microprobe.



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Sampling

- **Good/normal physiological state of an organism** (depending on the experimental conditions)

Case study 1: Changes in elementome during water deprivations

Case study 1: Accumulation of Cd in metal hyperaccumulating plant

- **Short time between organ excision and fixation (activation of degradation enzymes -** proteases, cellulases, pectinases, catechol oxidases)

Sampling

- **Laboratory experiments**

➢ keeping plants in good conditions

➢ prevent wilting

- **Field experiments**

➢ small plants – excavation together with the roots

➢ Bigger plants (trees) fixation in the field

Sample preparation

Sample

Organ level

Tissue level

Cell level

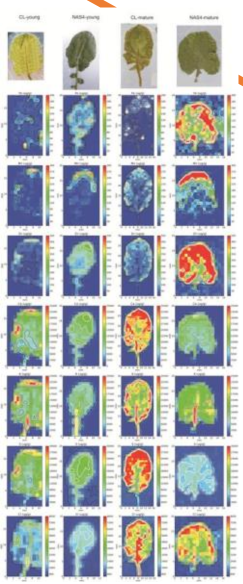
Direct measurements

Tissue & cell exposure

- „in air“ HE-µXRF
- Fast acquisition system (MAIA detector), dwell time >10 ms/px

PROBLEMS

- ➢ Radiation damage (water, ROS)
- ➢ Metabolic changes
- ➢ XRF signal coming from different depths
- ➢ Combined information across different depths/ physiologically distinct tissues



Fixation

- Vaccum compatibility (µLE-XRF, µPIXE)

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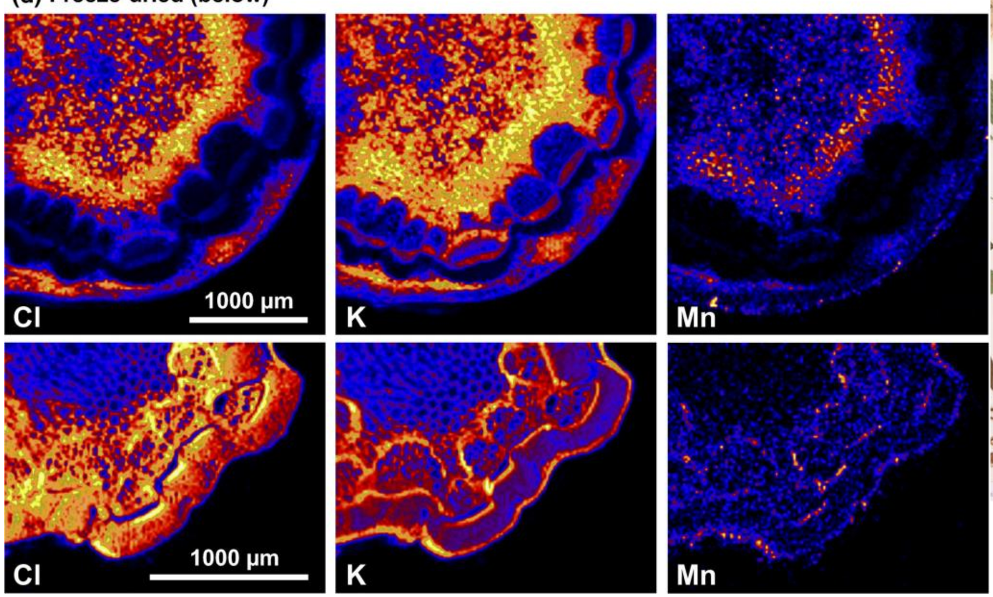
Ecophysiology - analysis of herbarium specimens

- **Air-drying of plant material NOT SUITABLE!**

(d) Freeze-dried (below)



(e) Air-dried (herbarium, above)

- Distorted morphology & element localization

New Phytologist

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X-ray elemental mapping techniques for elucidating the ecophysiology of hyperaccumulator plants

Antony van der Ent, Wojciech J. Przybyłowicz, Martin D. de Jonge, Hugh H. Harris, Chris G. Ryan, Grzegorz Tylko, David J. Paterson, Alban D. Barnabas, Peter M. Kopittke, Jolanta Mesjasz-Przybyłowicz

First published: 10 October 2017 | https://doi.org/10.1111/nph.14810 | Cited by: 10

Fixation

Chemical fixation (TEM)

Fixatives
- **PFA**
- **Ethanol**
- **methanol**

dehydration

embedding

Sectioning (<20 um)
Ultrathin cuttings

Cryofixation

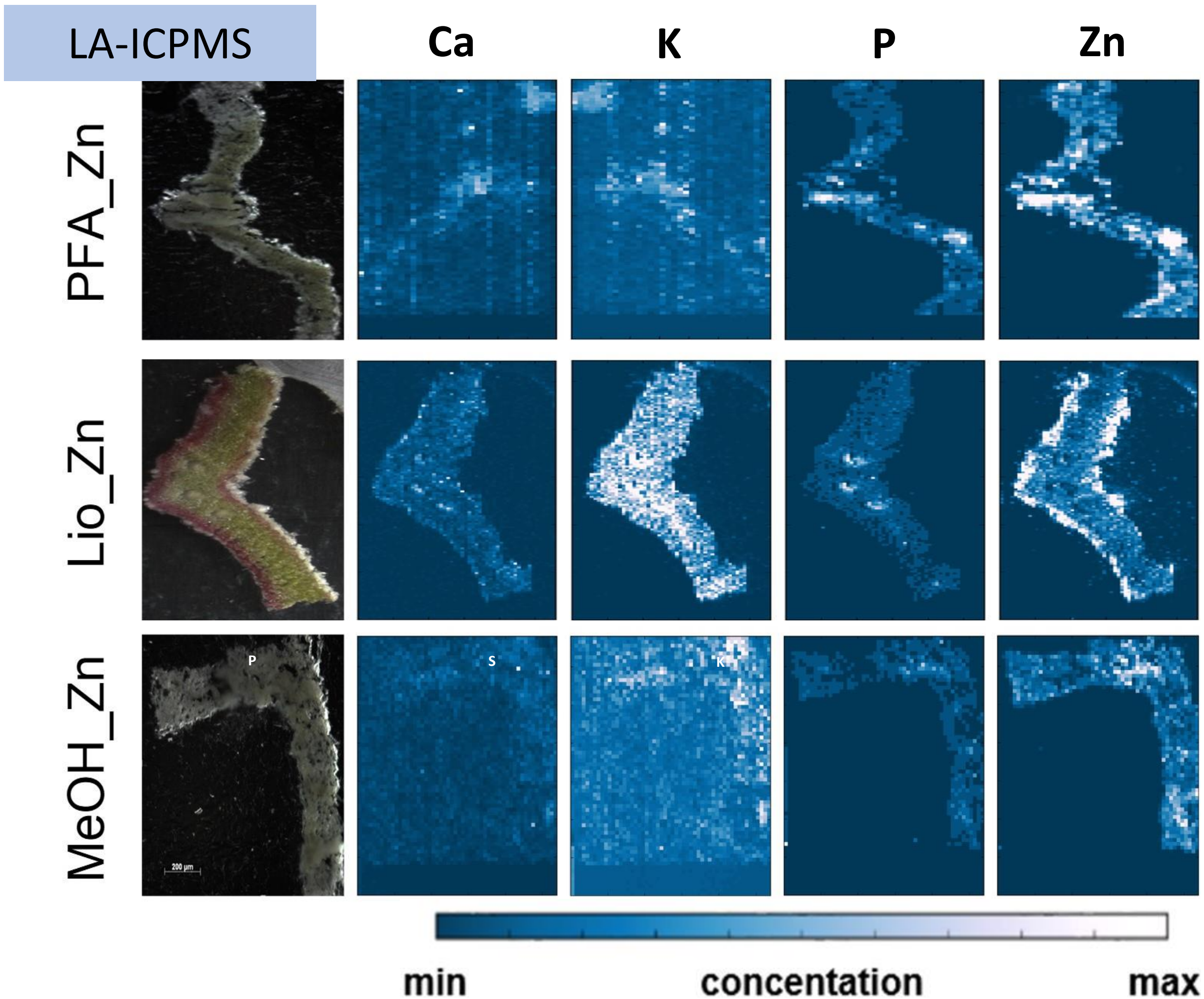
Freezing

Sectioning (Cryostat)

Direct measurements
Frozen-hydrated state

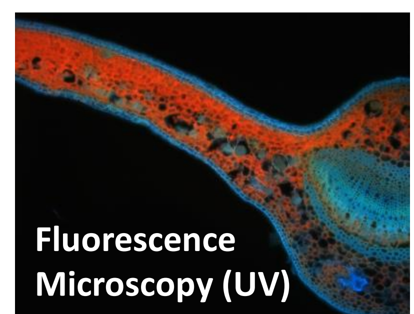
liophylization

Comparison between chemical and cryofixation



Chemical fixation:
- **Element redistribution**
- **Contamination**

Cryofixation
- **Preservation of structure and function**



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Fixation

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Fixatives
- PFA
- Ethanol
- methanol

dehydration

embedding

Sectioning (<20 um)
Ultrathin cuttings

Cryofixation

Freezing

Sectioning
(Cryostat)

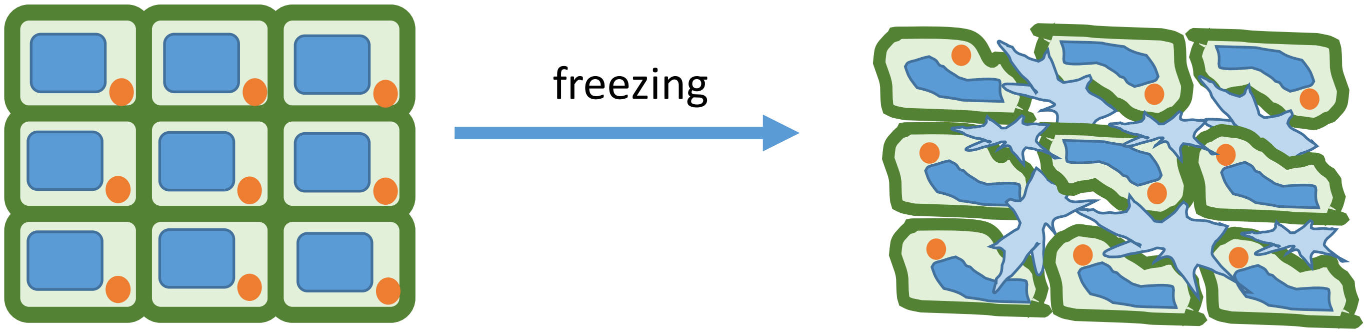
Direct measurements
Frozen-hydrated state

Liophylization
(Freeze-drying)

PROBLEMS
- ice-crystal formation, tissue and cell damage
- In FH – water absorbs LE-XRF, dilution (10x)
- In FD - shrinking during freeze-drying
- In FD Water deprivation may alter ligand environmet

Cryofixation

- Freezing



Environmental conditions

Ice crystal formation and tissue disintegration

➢ **Prevent ice crystal formation** and tissue and cell damage – increase freezing speed - vitrification

➢ Freezing in LN2 is not suitable

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Freezing

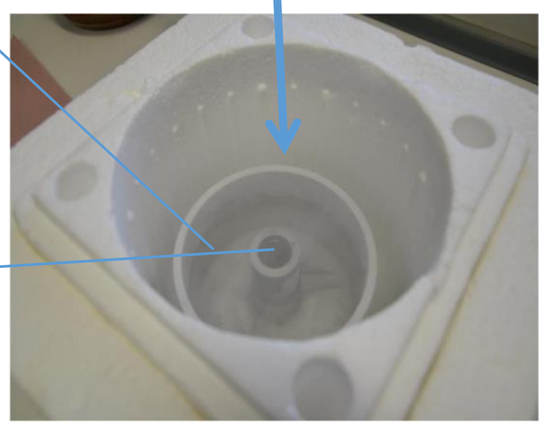
Metal mirror freezing

- Plunge freezing - cryogens
- Metal mirror freezing (slam freezing)
- High pressure freezing

Al thermoblock

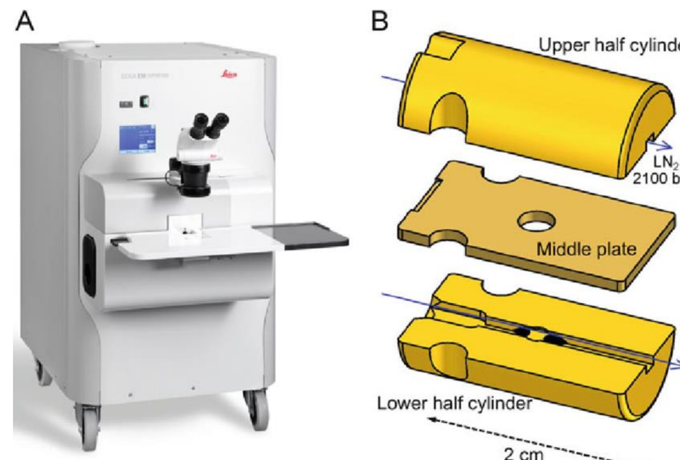
Liquid N_2

Liquid propane – freezing point -188°C



Also ethane, isopentane

Plunge freezing



High pressure freezing

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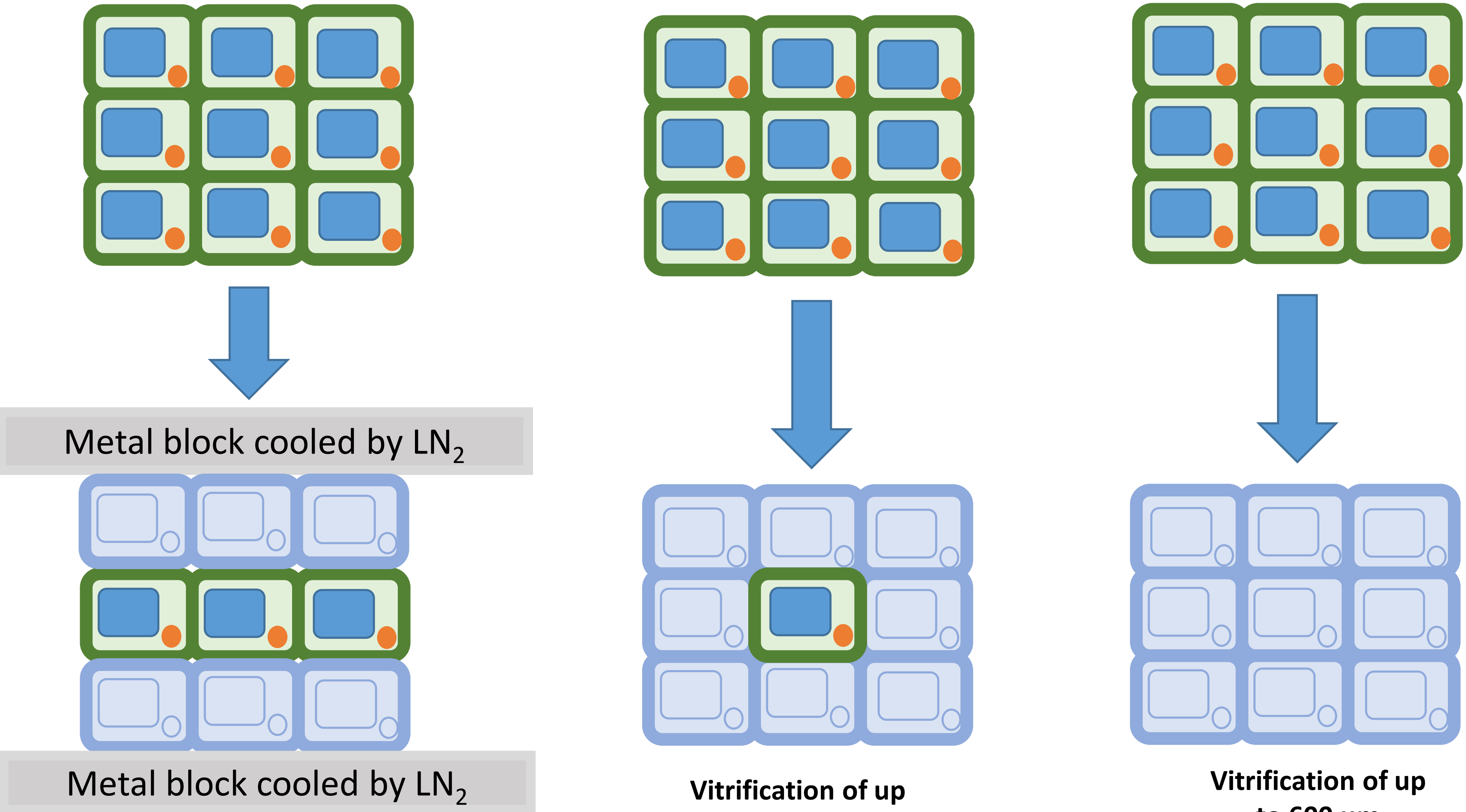
Metal mirror freezing

Plunge freezing in propane

High pressure freezing (NO pre-treatment)



Vitrification of up to 100 µm

Vitrification of up to 600 µm

Zone of vitrification cca. 10-20 µm

Efficient only with very small pieces (<100 µm)

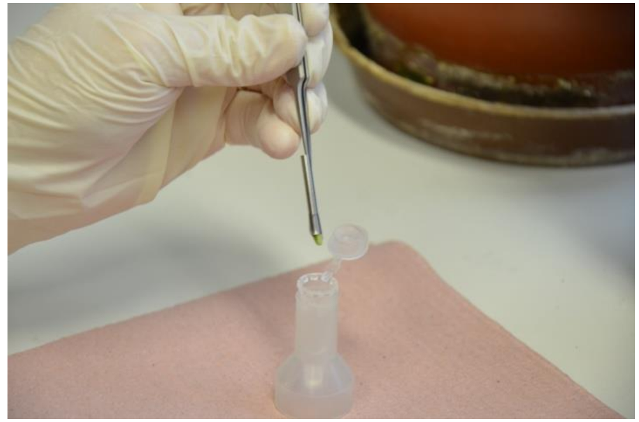
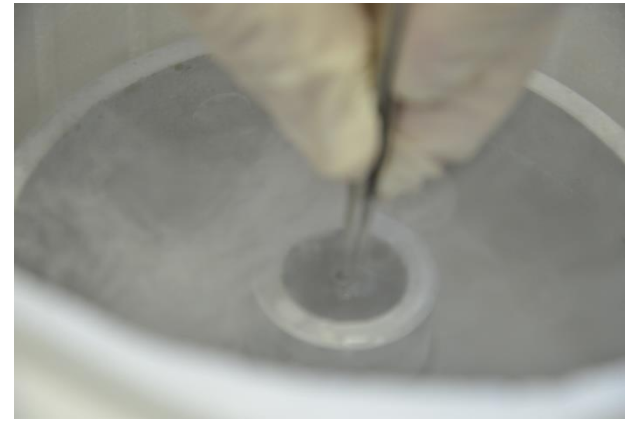
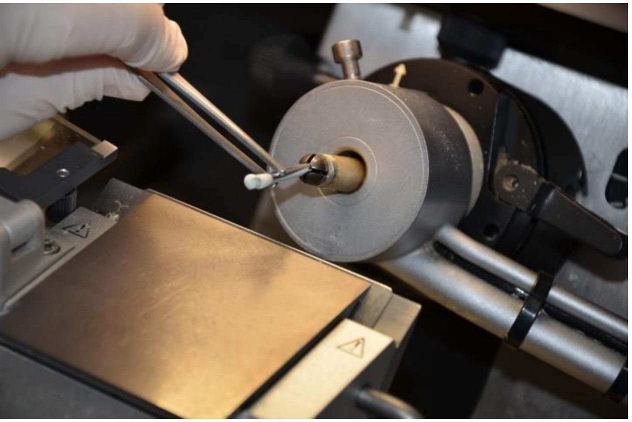
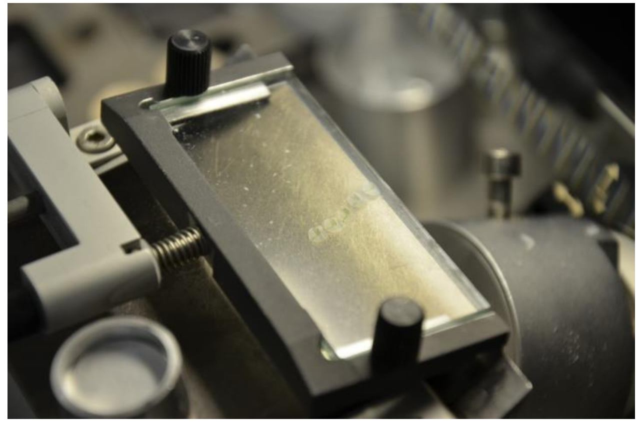
International Journal of PIXE | Vol. 24, No. 03n04, pp. 217-233 (2014) | Proceedings of the 8th Int...

Micro-PIXE elemental mapping for ionome studies of crop plants

Katarina Vogel-Mikuš, Paula Pongrac and Primož Pelicon



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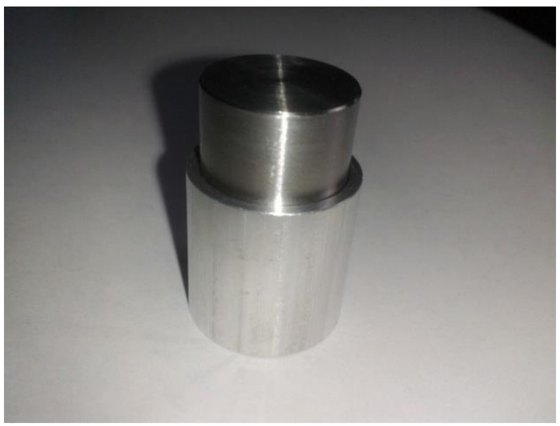
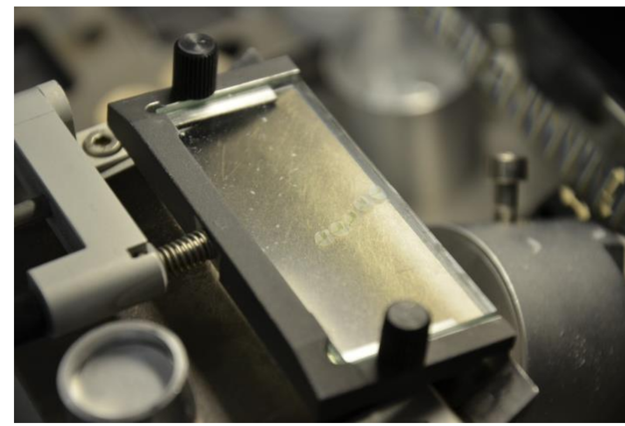
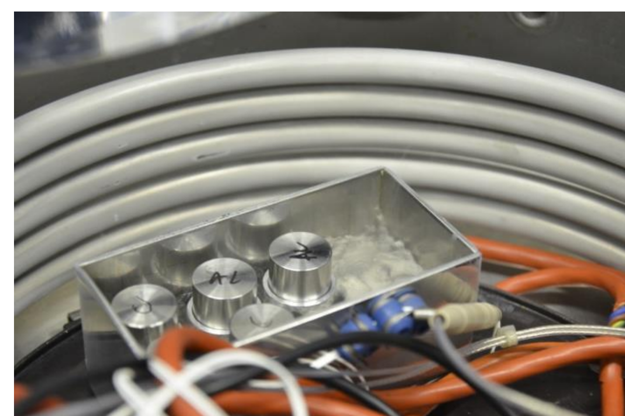
Freeze-drying

- Should be performed gradually from -196°C to 25°C and slowly to prevent shrinking of the specimens
- Can alter ligand environment (µXAS)





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Freeze-drying

- Should be performed **gradually and slowly** (few days) from -196°C to 25 °C to prevent shrinking of the specimens
- Computer assisted
- Improvised ☺

Shelf temperature

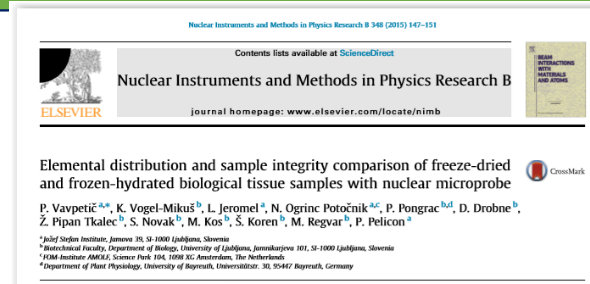
3rd day – transfer the samples to the highest position – adjustment to room temperature, 24 h

2nd day - transfer the samples to the higher position, 24 h

1st day – pour LN2 into the lowest shelf to cool it down, put in the box with samples, leave for 24 h

Results – PIXE – tissue/ cell level

N. praecox from natural environment



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Elemental distribution and sample integrity comparison of freeze-dried and frozen-hydrated biological tissue samples with nuclear microprobe

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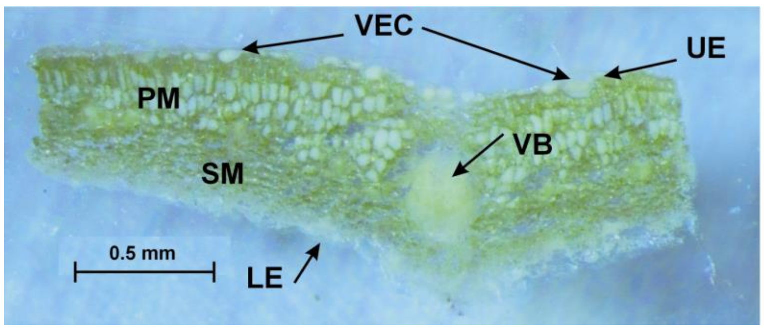
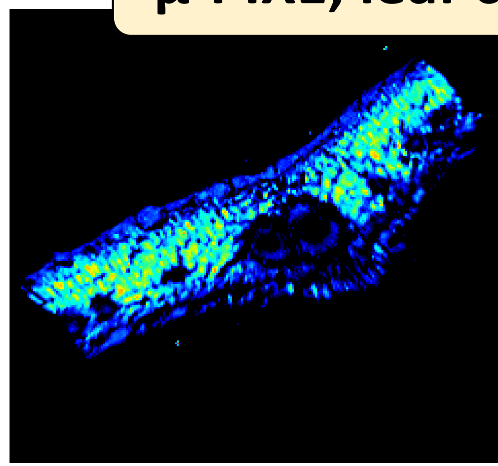
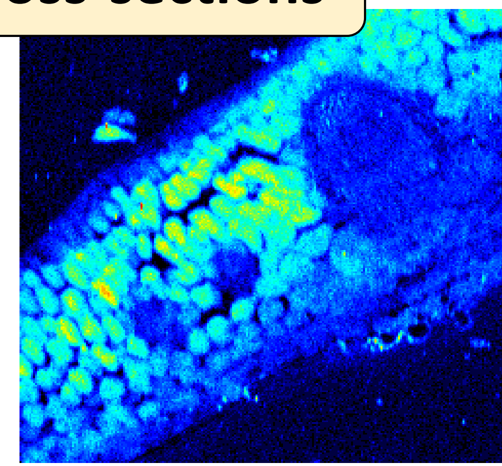
- Well retained morphology and element distribution

µ-PIXE; leaf cross-sections



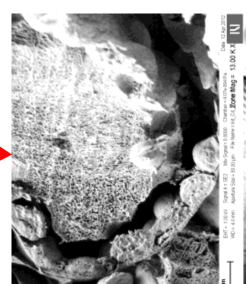


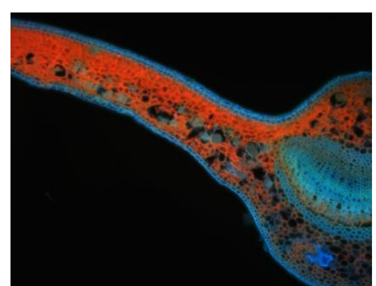
Ca – freeze-dried



Ca – frozen hydrated

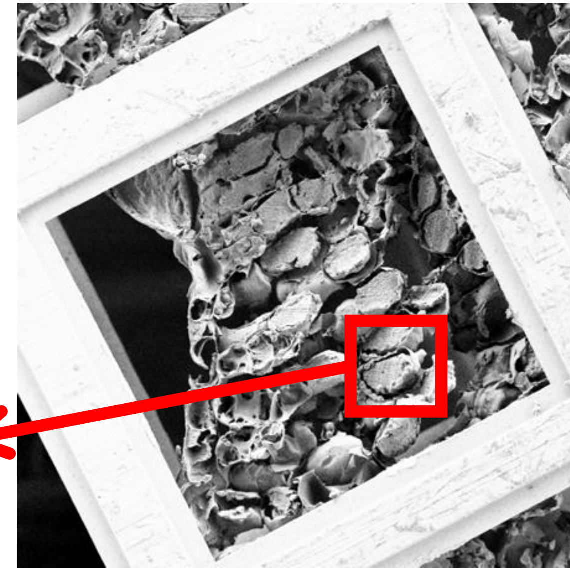
SEM

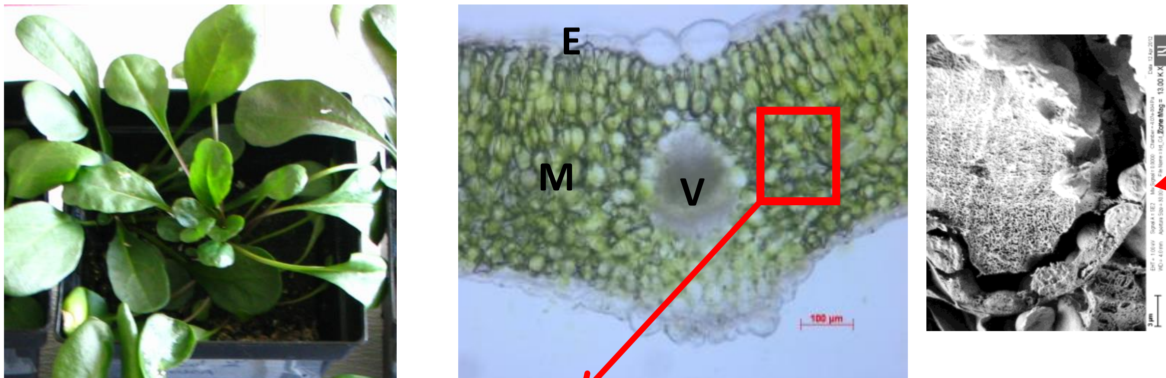


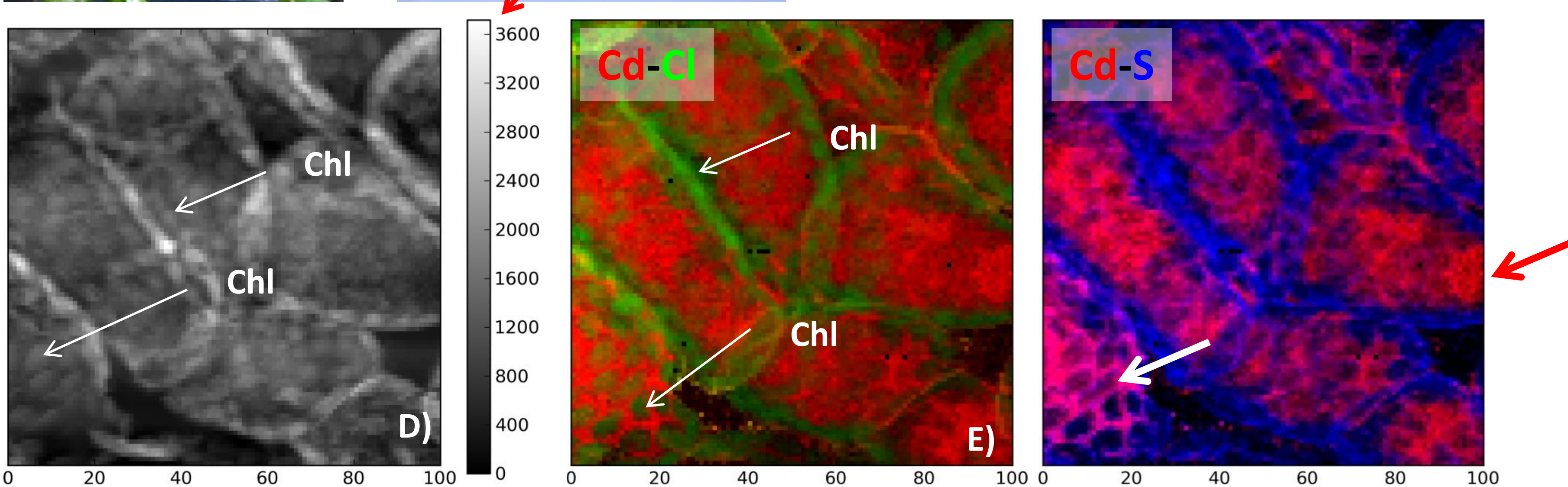


Fluorescence Microscopy (UV)

SR-µXRF - the cell level
T. praecox in hydroponics, Cd





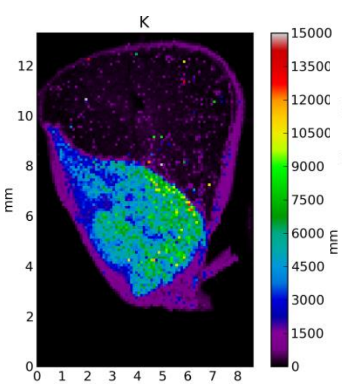


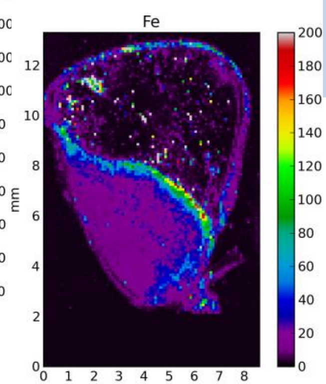
(µXRF, E=3.55 keV, 0.3 x 0.7 µm beam), ID 21, ESRF

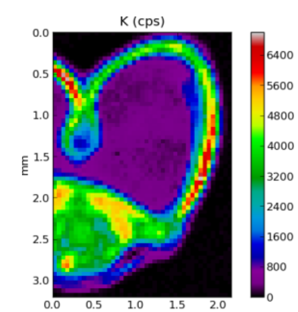
Hand cutting – organ and tissue levels (seeds)

Fresh plant material → Hand-cuttings → Put between two layers of Al foil → Freezing → Freeze-drying → Mounting to the holders

soaking at 4°C

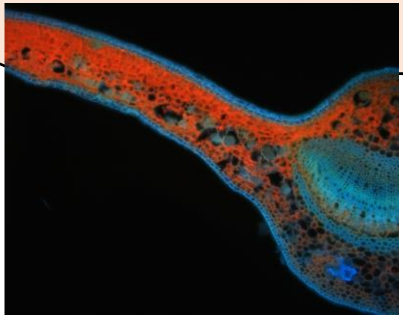


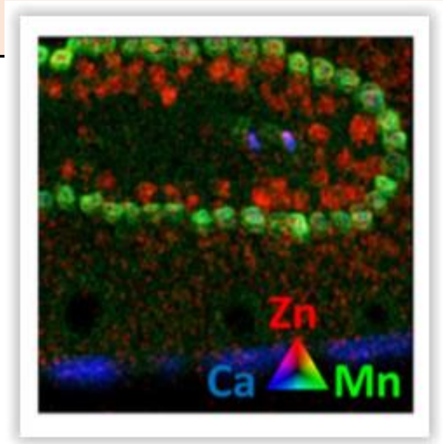


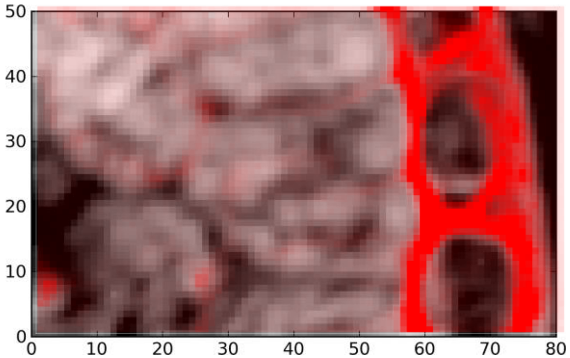


Conclusions

- Cryofixation is the most suited for µXRF/ µXAS
- LN_2 is not suitable cryogen
- Measurements in frozen hydrated state better resemble *in vivo* state, especially for µ-XAS
- Herbarium, air-dried and chemically fixed specimens are not suitable for µXRF/µXAS







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ESRF elettra

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- ✓.....

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