Biophysical and physicochemical methods for analyzing plants *in vivo* and *in situ* (III): X-ray spectroscopy for localising&quantifying metals and for investigating metal ligands

X-ray spectroscopy on biological samples
General comments on sample preparation techniques

a) chemical fixation and resin embedding

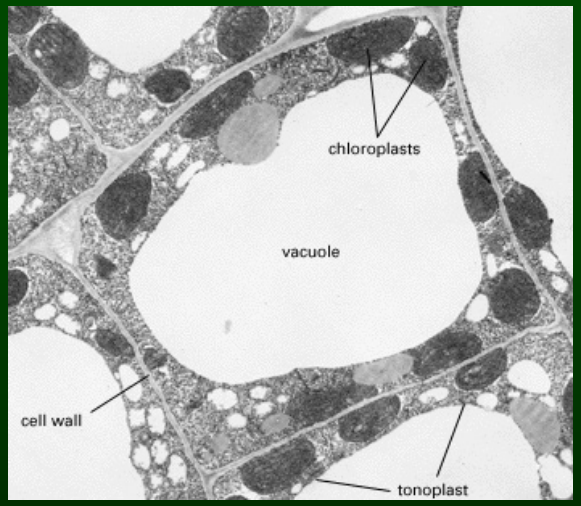
→ Advantages: over many years best established procedure in many laboratories

→ Disadvantages: Metals will inevitably be re-distributed → ARTEFACTS

b) freeze substitution or freeze drying

→ Advantages: less element re-distribution than in (a)

→ Disadvantages: still at least intracellular (vacuole→ wall)
re-distribution artefacts inevitable



c) frozen-hydrated tissues

→ Advantages: hardly any element-redistribution → METHOD OF CHOICE!

→ Disadvantages: Required rapid-freeze techniques and cryostage
(→ expensive)

e) non-frozen fresh tissues

→ Advantages: NO preparation necessary, "*in vivo*" situation

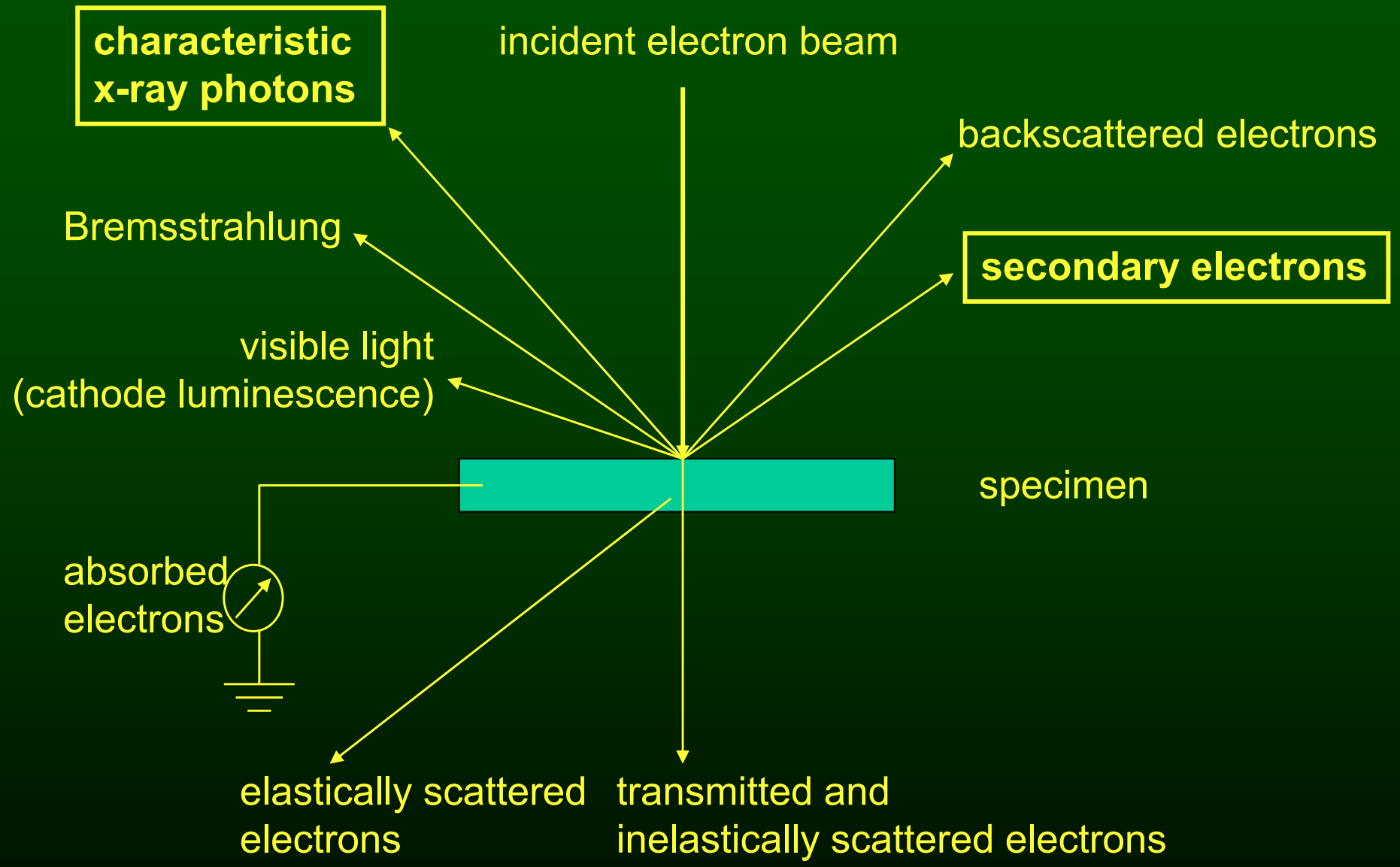
→ Disadvantages: Strong beam damage → MORE artefacts than in (c)!

(1) X-ray emission spectroscopy

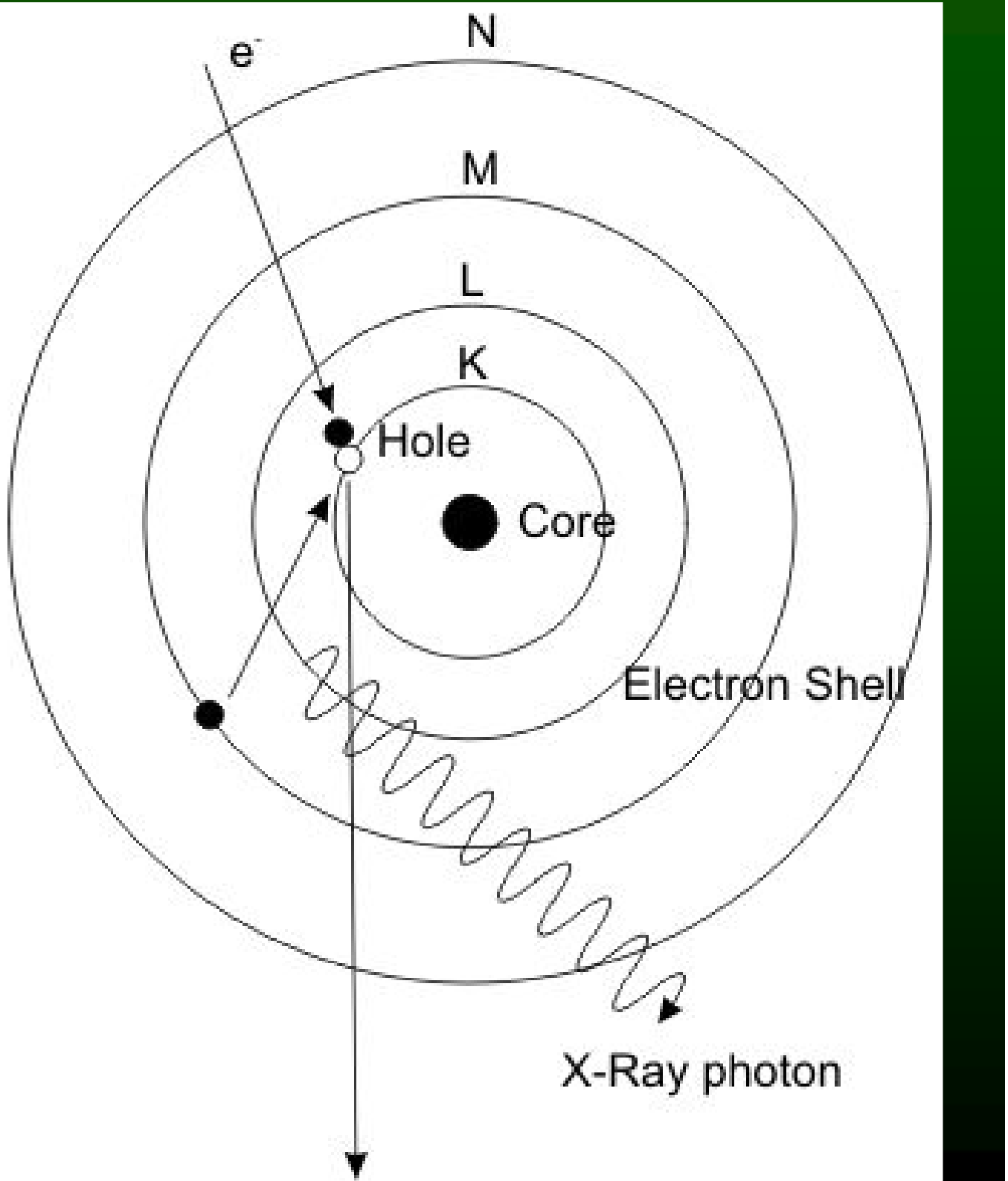
(a) Energy Dispersive X-ray Analysis (EDXA)

Use of an electron microscope as an X-ray spectrometer

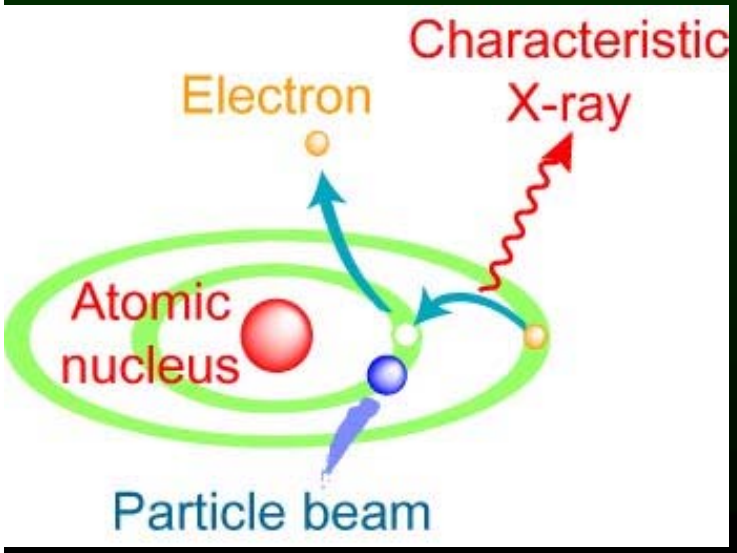
Signals generated in the scanning electron microscope (SEM)



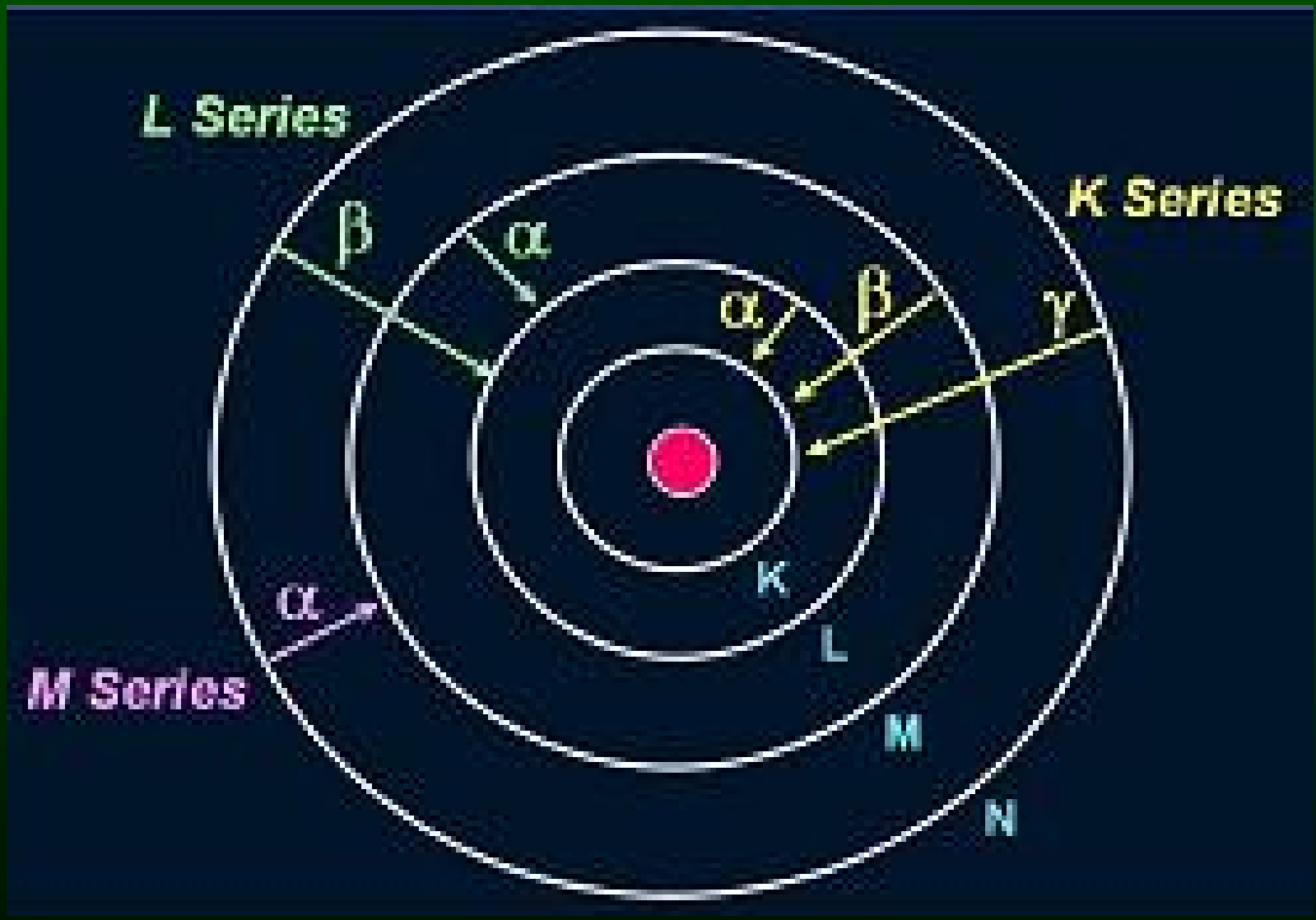
Principle of Energy Dispersive X-ray Analysis (EDXA)



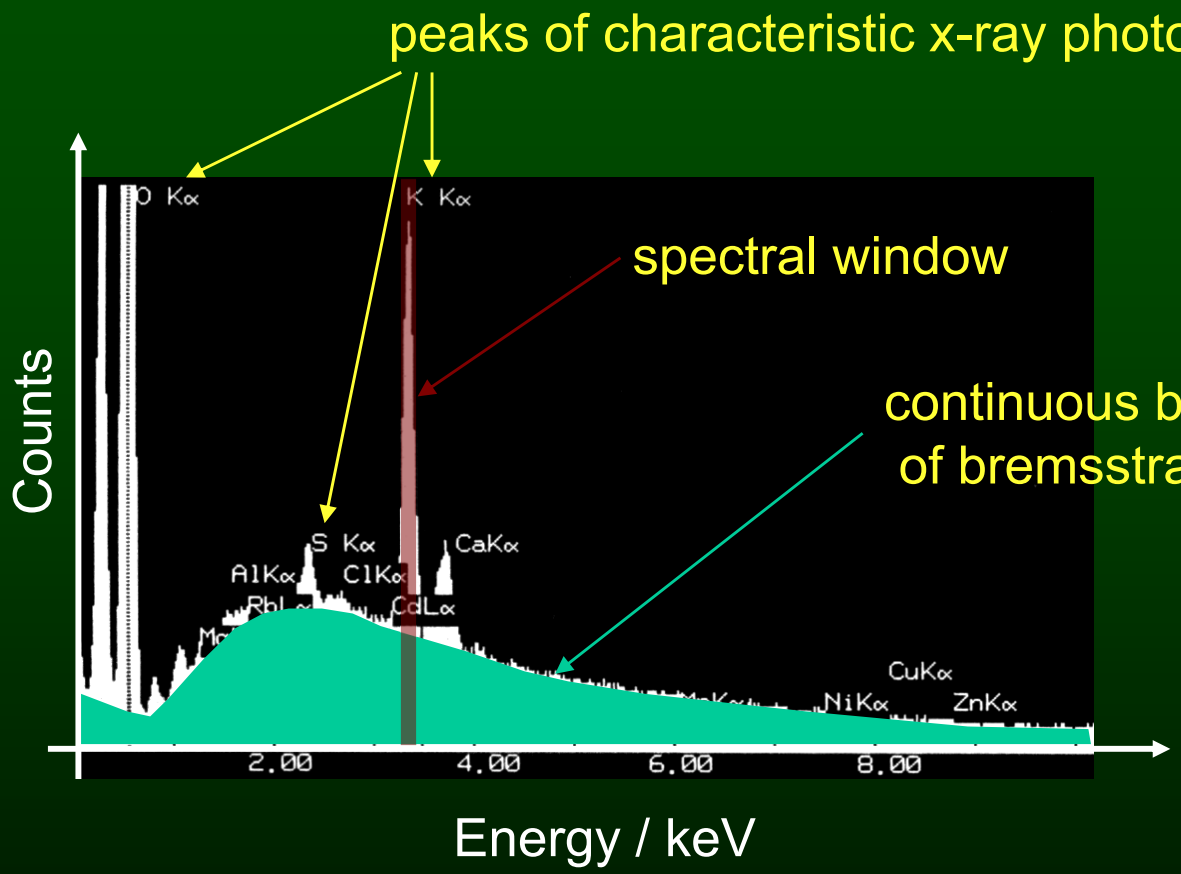
Principle of Particle Induced X-ray Emission (PIXE)



The origin of the different lines in an EDXA spectrum

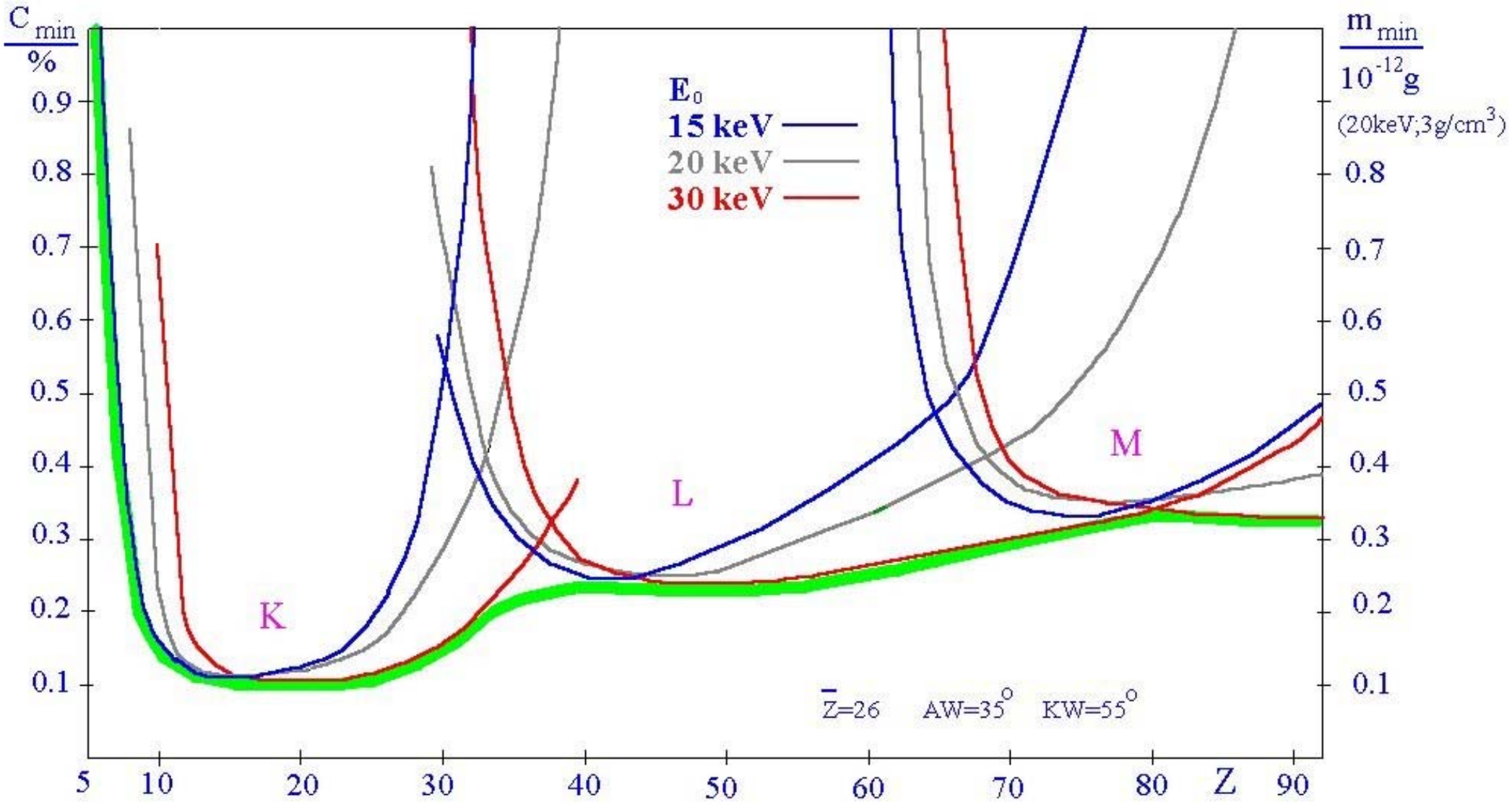


Analysis of EDXA spectra



Analysis: a) recording of complete spectrum, subtraction of background
--> quantification of peak areas by comparison to internal standard
b) recording of counts in spectral window
--> dot maps, line scans

Detection limits of EDXA



Methods of plant analysis using EDXA

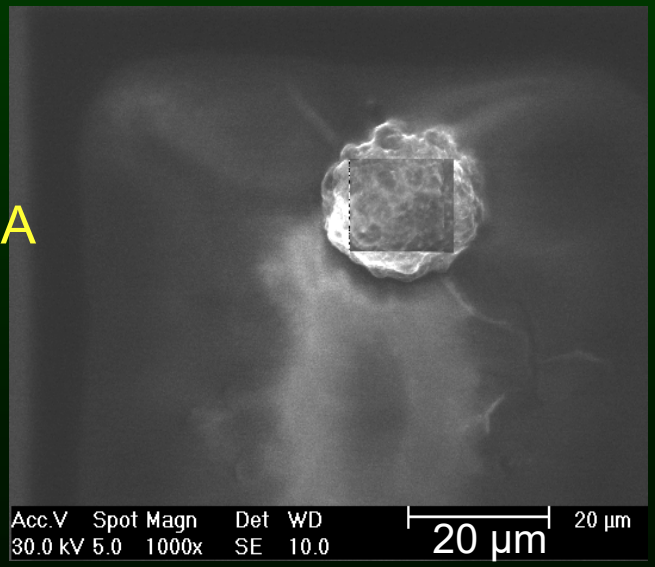
Sampling of single-cells saps with micropipettes

micropipette filled with silicon oil, connected to air-filled syringe for controlling pressure difference

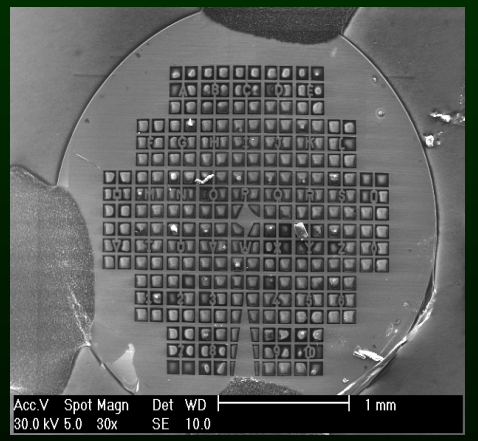
turgor pressure of punctured cell fills pipette with 5-20 picolitres (10^{-12} l) of cell sap

Sample preparation:
1) transfer to storage grid, addition of internal standard (e.g. RbF) and matrix (e.g. mannitol)
2) transfer to analysis grid, drying with isopentane

Analysis:
1) recording of EDXA spectra in SEM
2) data processing



typical dried sample



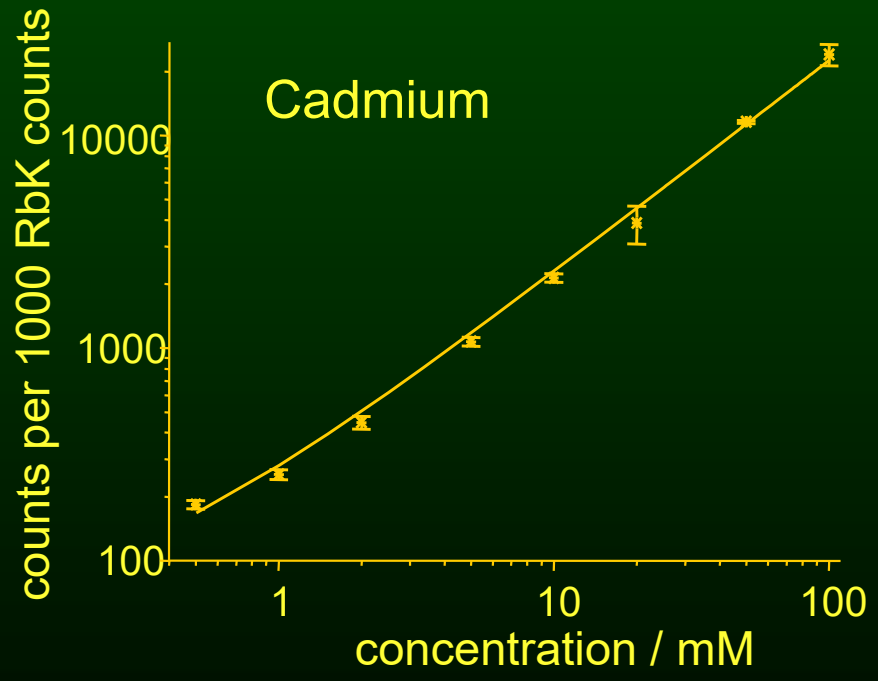
analysis grid

Küpper H, Zhao F, McGrath SP (1999) Plant Physiol 119, 305-11

Methods of plant analysis using EDXA

Quantification of elements in single-cells saps

1) net peak area is normalised by internal standard (an element not naturally present in the sample, e.g. Rb)
2) ratio obtained from 1) is quantified using calibration curve



Evaluation of the method

Advantages:

- potentially very accurate
- enables measurement of small concentrations

Disadvantages:

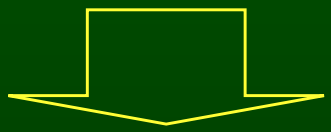
- only few types of cells are accessible to sampling with micropipettes
- risk of preparation artefacts
- no distinction between cytoplasm and vacuole, measurement of cell walls impossible
- very difficult to obtain information about heterogeneity of element distribution inside the analysed tissue

Küpper H, Zhao F, McGrath SP (1999) Plant Physiol 119, 305-11

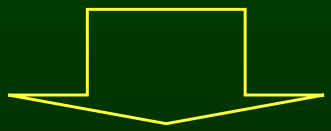
Methods of plant analysis using EDX or XRF

Freeze-fracturing

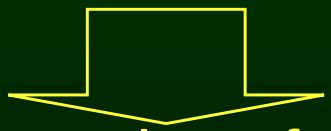
Excise sample from plant, mount in/on sample holder (e.g. stub or vice). The EDX/XRF spectrum of the holder must not interfere with that of the sample!



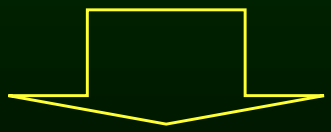
Shock-freeze the sample, e.g. in **melting nitrogen slush (NOT regular liquid nitrogen!) or supercooled isopentane**,
transfer to cooled (-170°C) preparation chamber



Fracture / cut sample



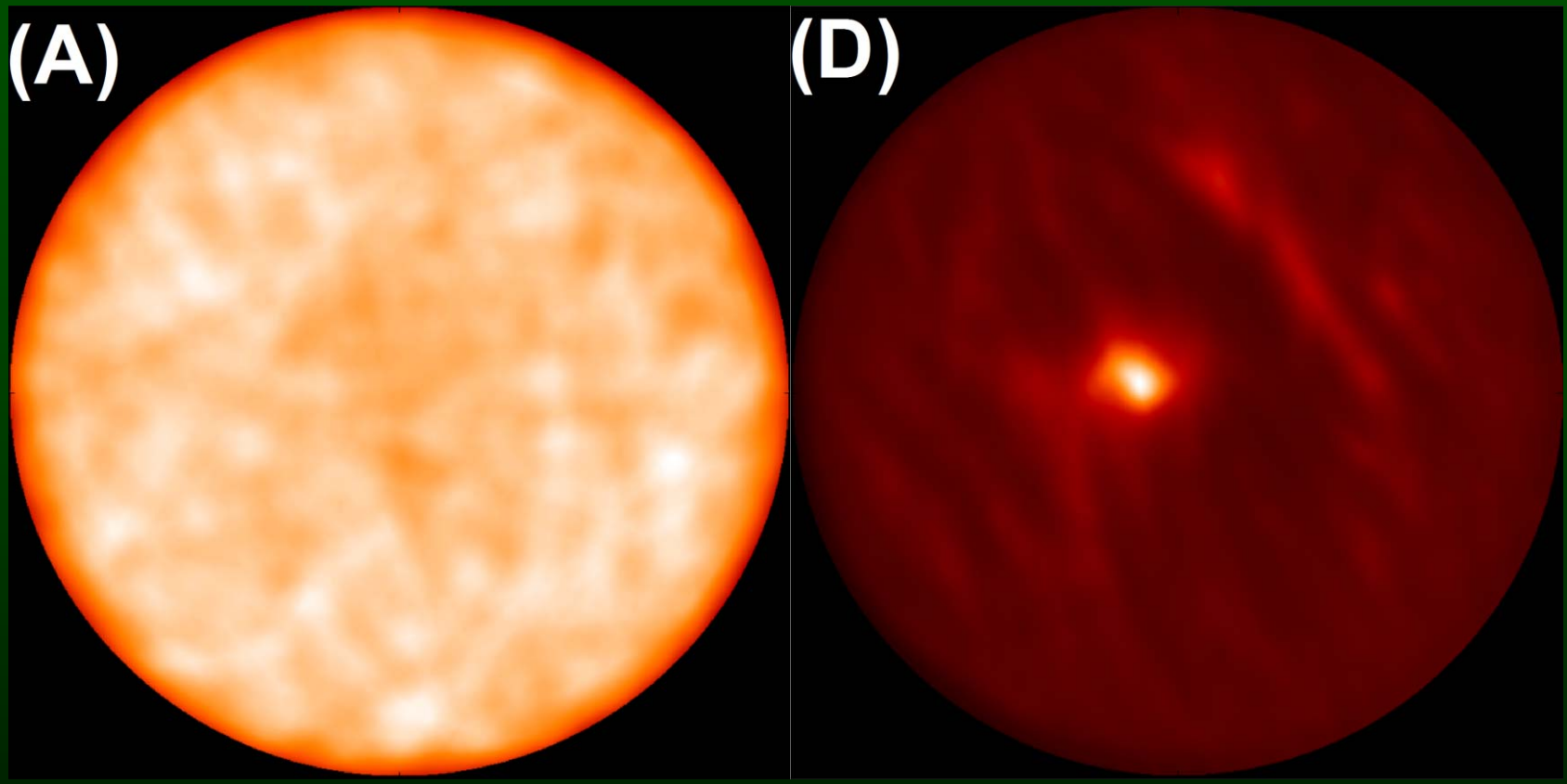
For EDX: Produce conductive sample surface by evaporating carbon wire



Transfer to cooled (-150°C) sample stage (= cryochamber) or cryostream, analyse

modified from Küpper H, Zhao F, McGrath SP (1999) Plant Physiol 119, 305-11

Tomographic X-ray emission spectroscopy (μ-XRF): Artefacts of slow freezing

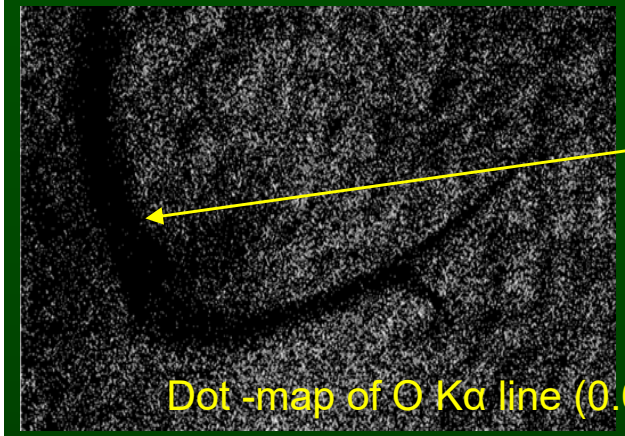


→ shock-freezing in supercooled isopentane led to homogeneous standards (left), freezing in LN_2 was too slow due to gas layer and led to de-mixing during the freezing process (right)

Mishra S, Wellenreuther G, Mattusch J, Stärk H-J, Küpper H (2013) Plant Physiology 163, 1396-1408

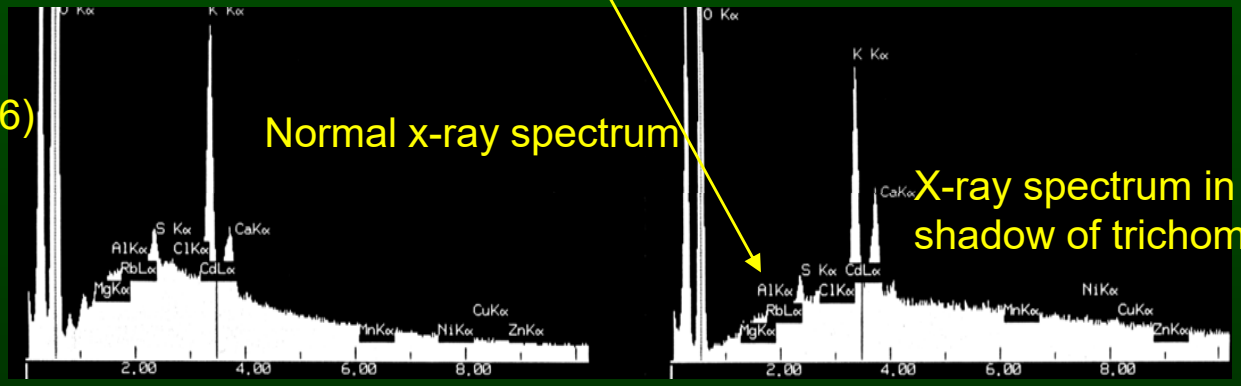
Methods of plant analysis using EDXA

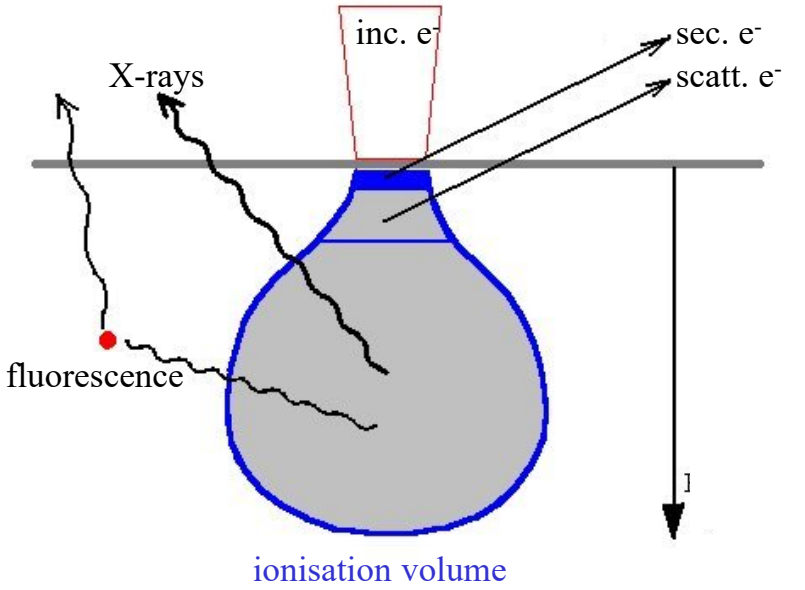
Analysis of bulk-frozen samples



Effect of shading

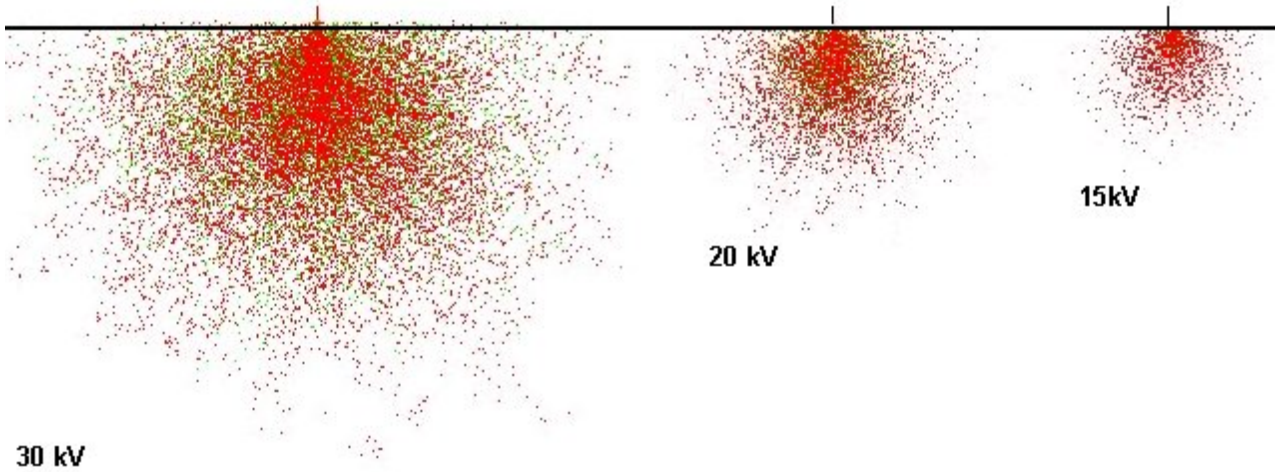
shading inside a sample leads to absorption of low-energy x-rays





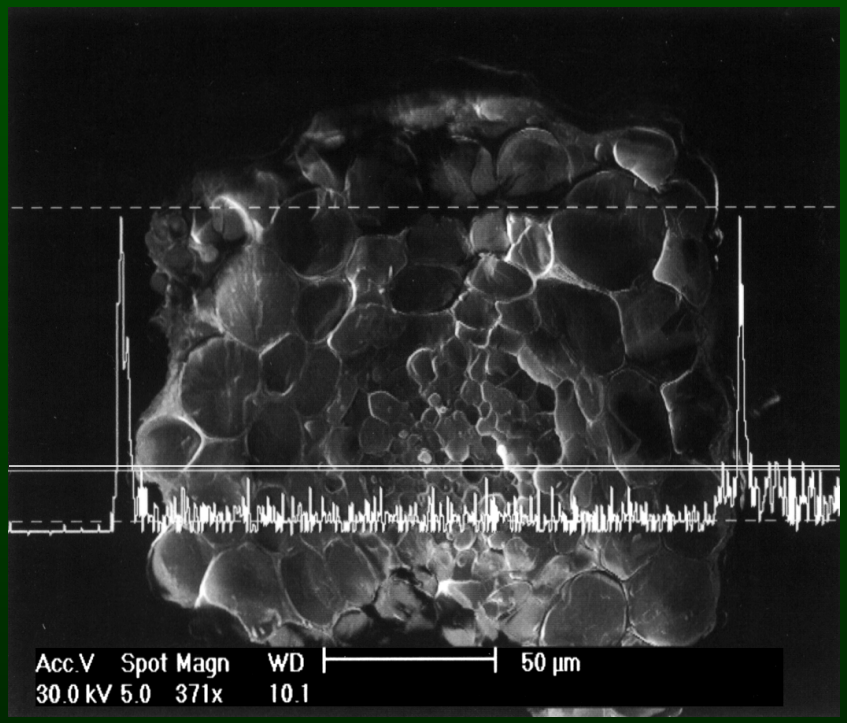
Effect of acceleration voltage

high acceleration voltage leads to deeper penetration into the sample!

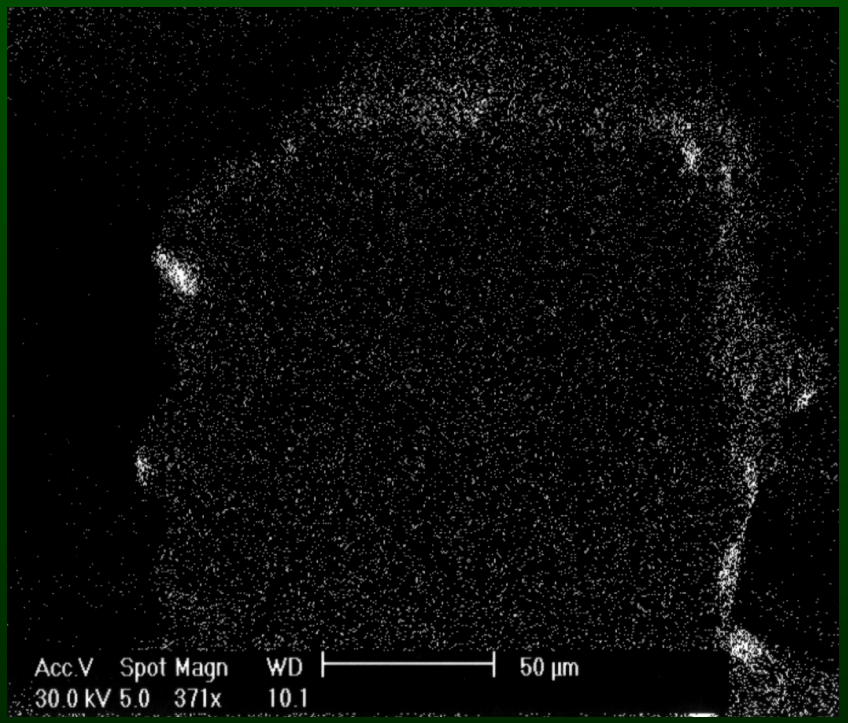


Methods of plant analysis using EDXA

Qualitative and semi-quantitative analysis of bulk-frozen samples



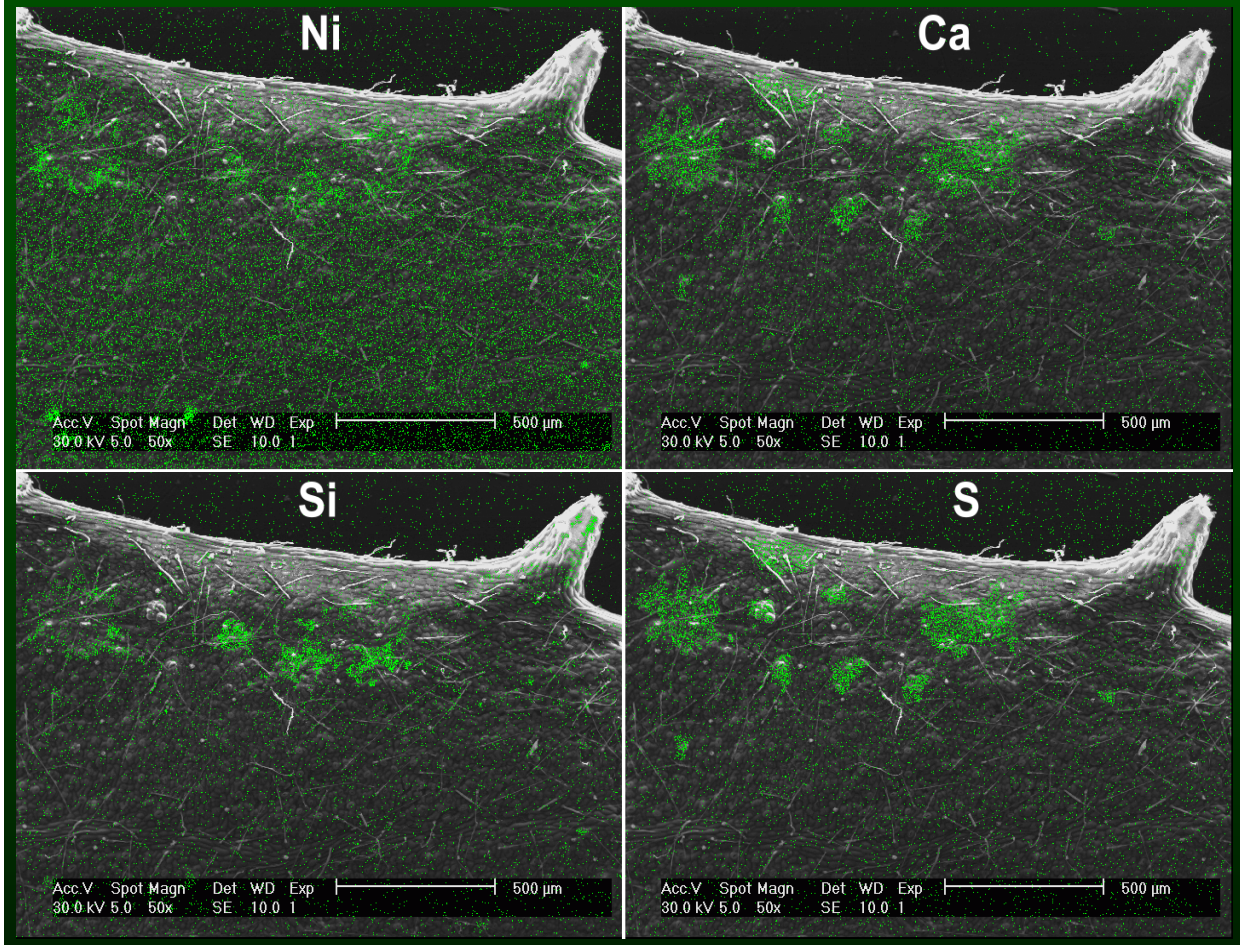
Line scans
Scan of the Zn K alpha line (0.6x half width) along the straight line. Amplitude represents the counts/s inside the selected spectral window.



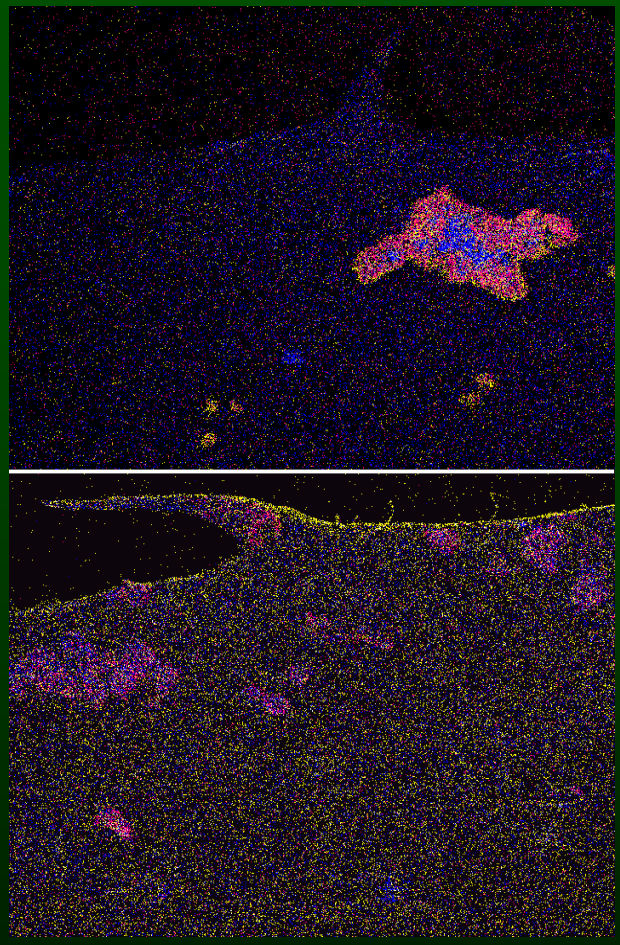
Dot maps
Scan of the Zn K alpha line (0.6x half width) over the whole image. Each dot represents one x-ray count inside the selected spectral window.

Küpper H, Lombi E, Zhao FJ, McGrath SP (2000) Planta 212, 75-84

EDXA imaging application example:
Ni silicate accumulation in cell walls of *Berkheya coddii*



Dot maps (K α lines) of the upper side of a *Berkheya coddii* leaf. Quantitative relation between Si and Ni in metal accumulation spots: 3.5 (± 1) Si / Ni (P = 0.0055)



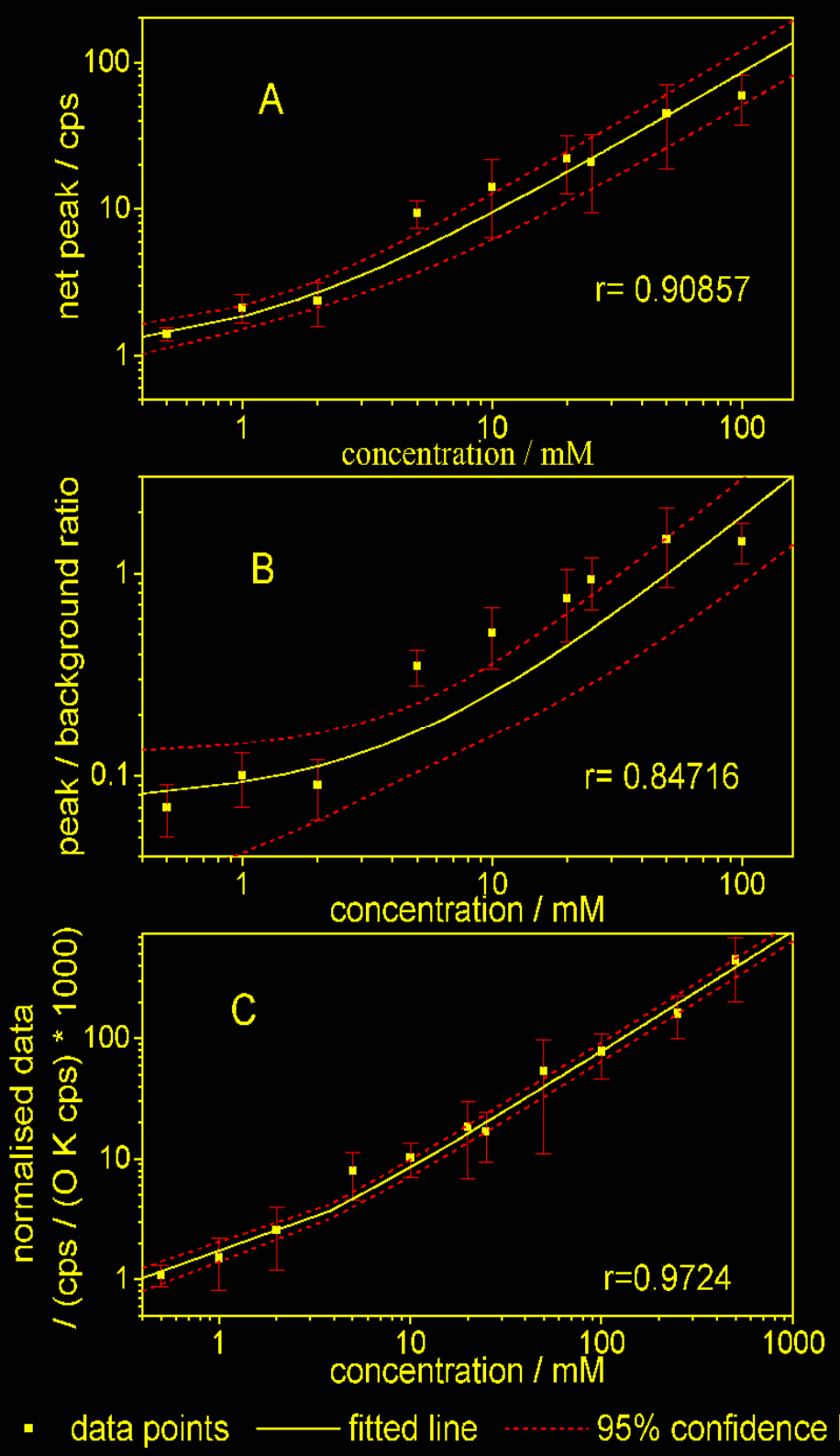
Dot maps (K α lines) showing the development of metal accumulation spots.
Up: senescent leaf,
down: young-mature leaf;
blue: Ca, *red:* Mn, *yellow:* Ni

Küpper H (2001, doctoral thesis). UFO Atelier für Gestaltung und Verlag, Allensbach (ISBN 3-935511-07-8)4

EDXA

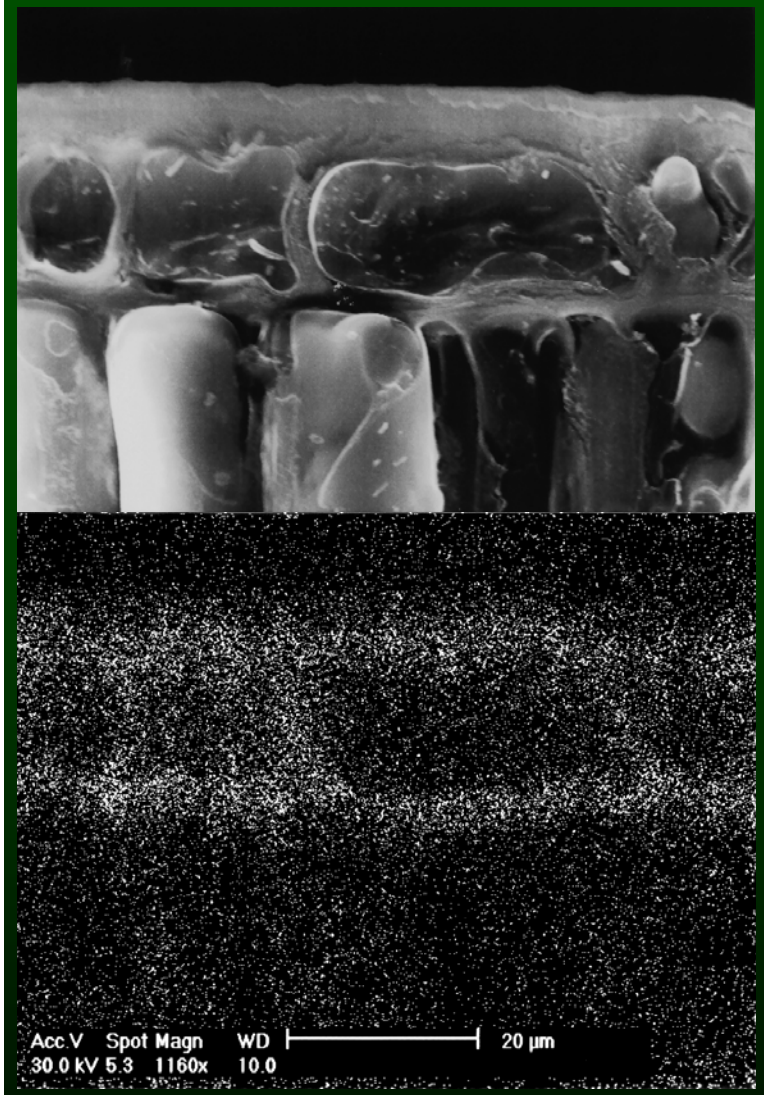
Quantitative analysis of bulk-frozen samples

Counts in spectra (A) can be normalised to either the background **(B)** or an internal standard. The oxygen Kα line has proven to be a reliable internal standard in bulk-frozen samples, in particular in aqueous compartments like vacuoles **(C)**.

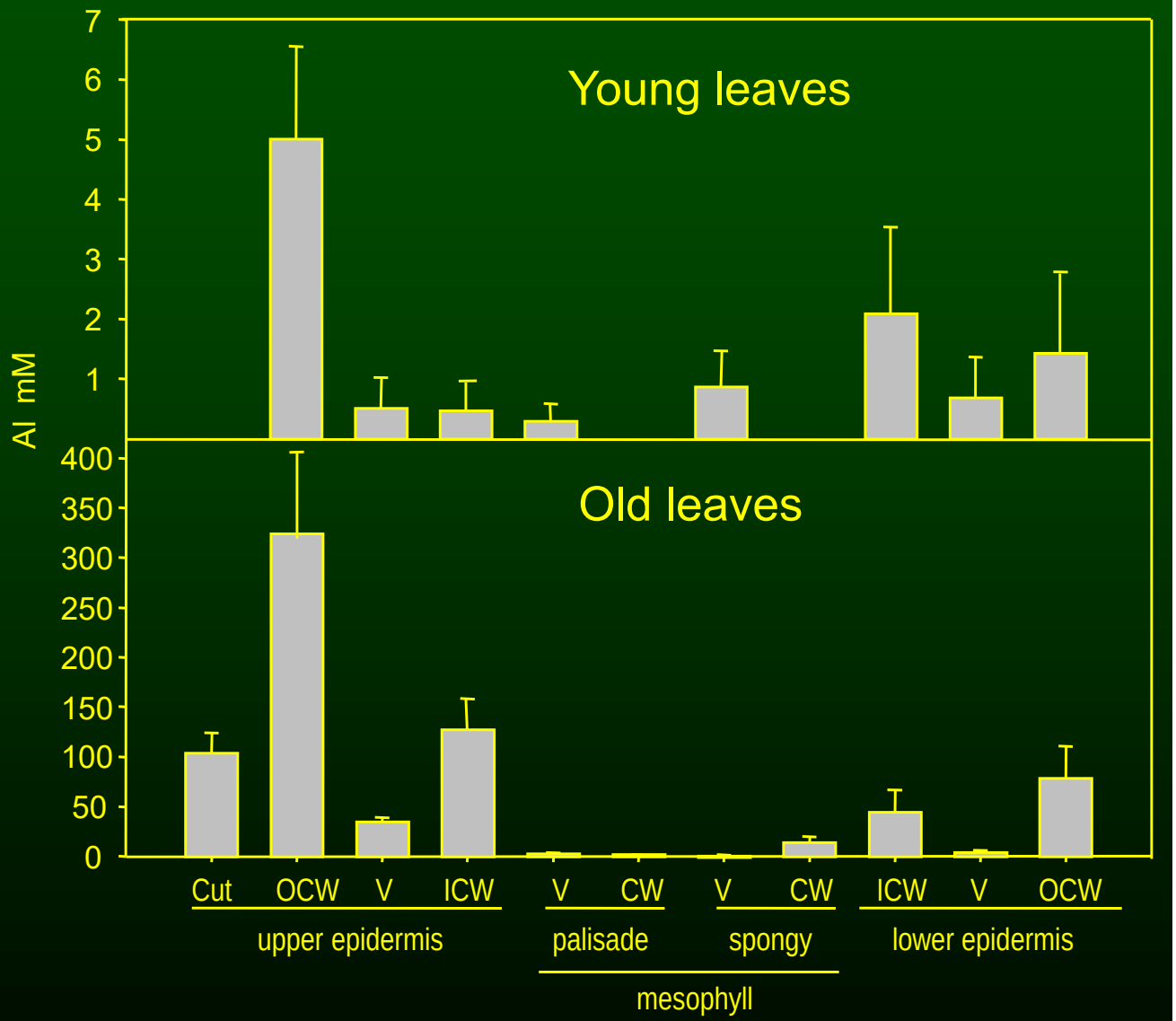


Küpper H, Lombi E, Zhao FJ, McGrath SP (2000) Planta 212, 75-84

EDXA quantification application example:
Al accumulation in epidermal cell walls of tea (*Camellia sinensis*)



Electronoptic picture of an old *C. sinensis* leaf (upper epidermis) and dot map of the Al K α line



Carr HP, Lombi E, Küpper H, McGrath SP, Wong MH* (2003) Agronomie 23, 705-710

Methods of plant analysis using EDXA

Analysis of bulk-frozen samples

Evaluation of the freeze-fracturing method

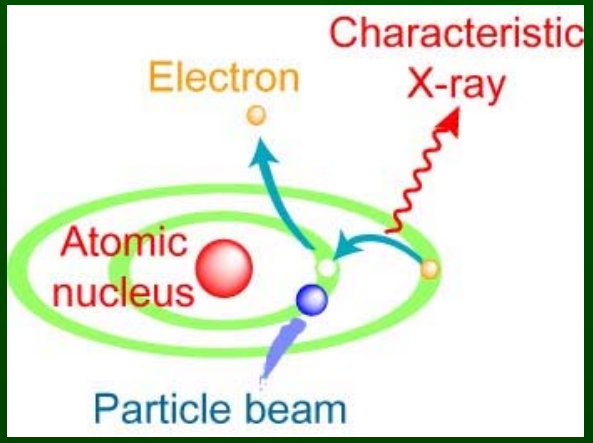
Advantages:

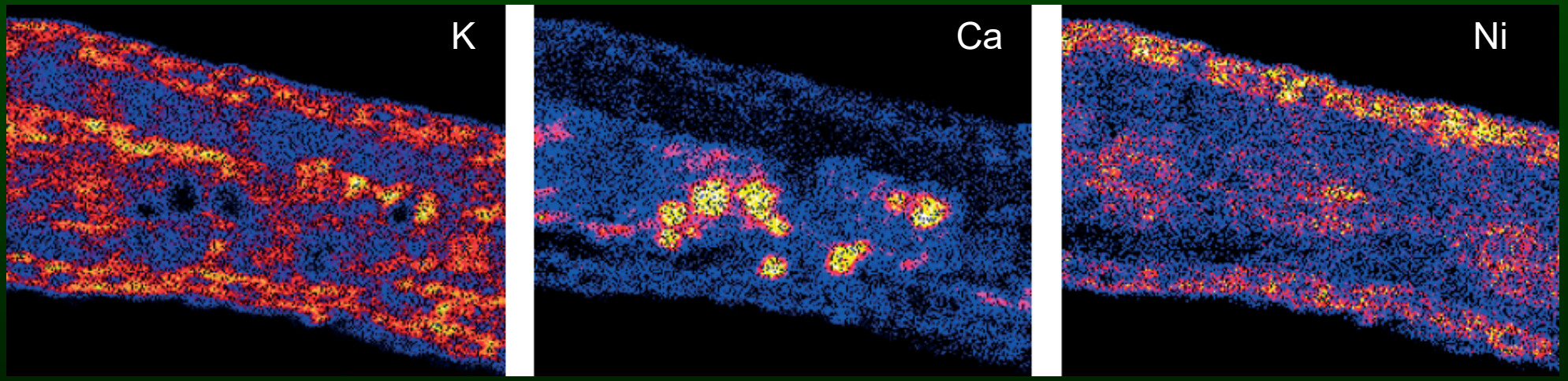
- All types of cells and tissues can be analysed
- *In situ*-analysis with very little risk of preparation artefacts
- Easy analysis of the heterogeneity of element distribution, by use of dot-maps even in an imaging way

Disadvantages:

- Limited sensitivity (min. 1mM) and accuracy (shading)
- Elements in dead tissues with low water content cannot be reliably quantified

(1) X-ray emission spectroscopy
(b) Proton induced X-ray emission (PIXE) imaging





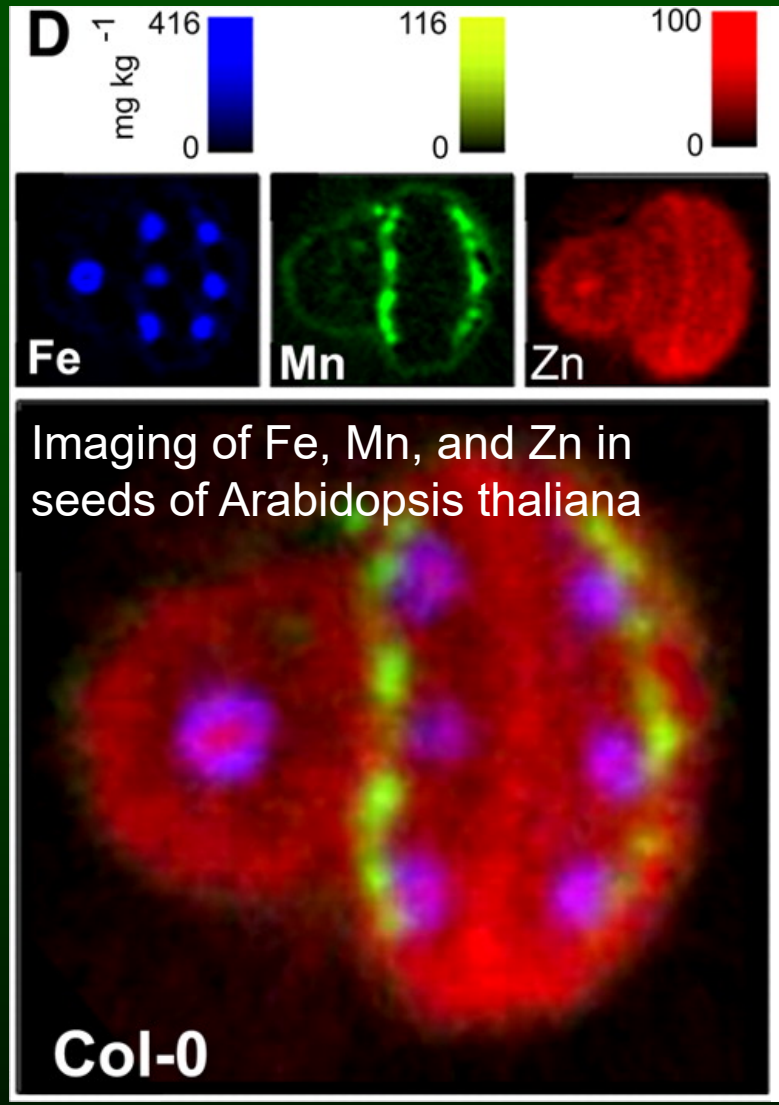
From:Siegele R, Kachenko AG, Bhatia NP, Wang YD, Ionescu M, Singh B, Baker AJM, Cohen DD, 2008, X-ray spectrometry 37, 133-6

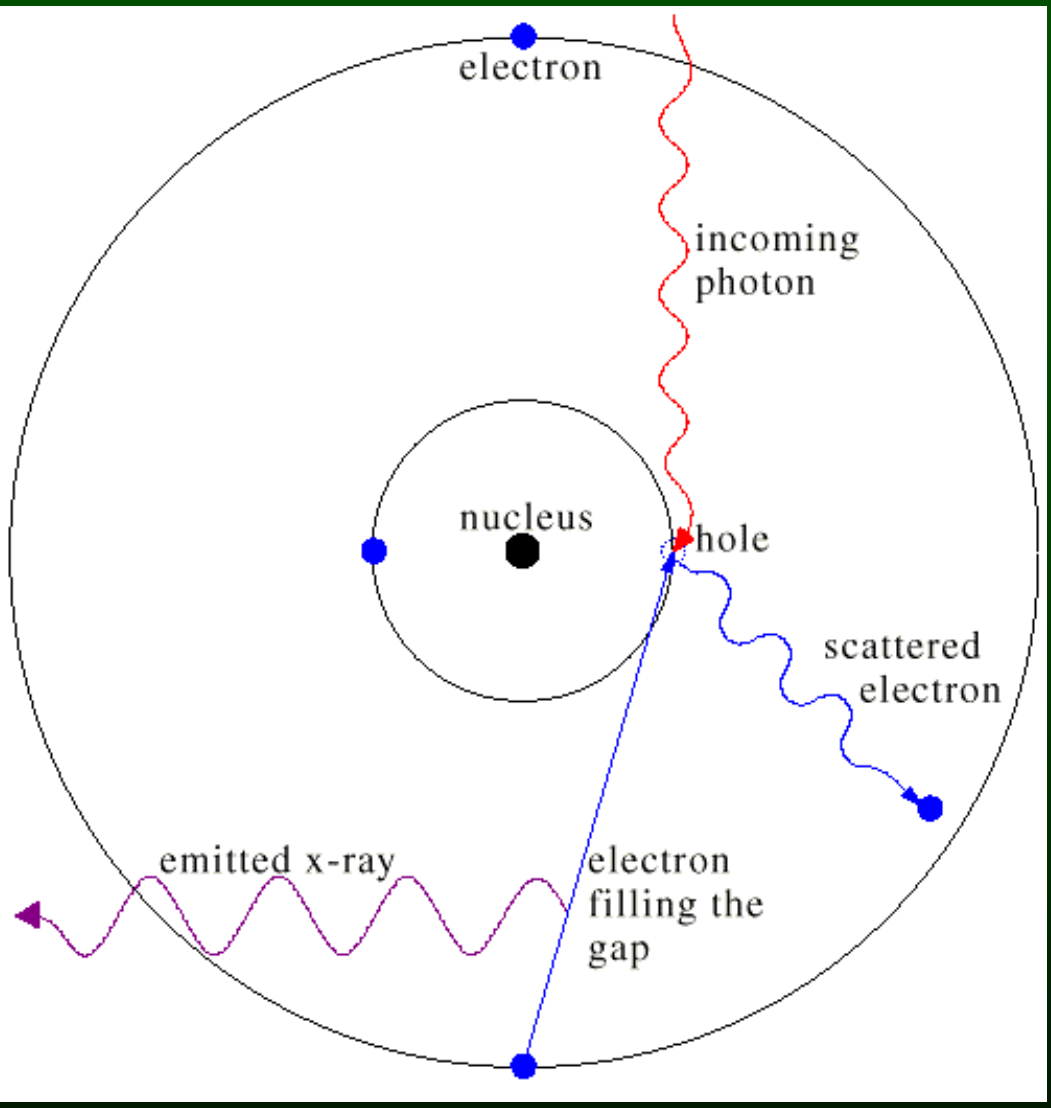
Imaging of potassium, calcium and nickel in a leaf of Hybanthus floribundus

→ more sensitive than EDX, but no observation of frozen-hydrated samples (samples have to be freeze-dried) → increased risk of artefacts

Tomographic X-ray emission spectroscopy:
principle of X-ray fluorescence imaging (XRF)

From:
Kim SA, Punshon T, Lanzirotti A, Li L, Alonso JM, Ecker JR, Kaplan J, Guerinot ML, 2006, Science 314, 1295-8





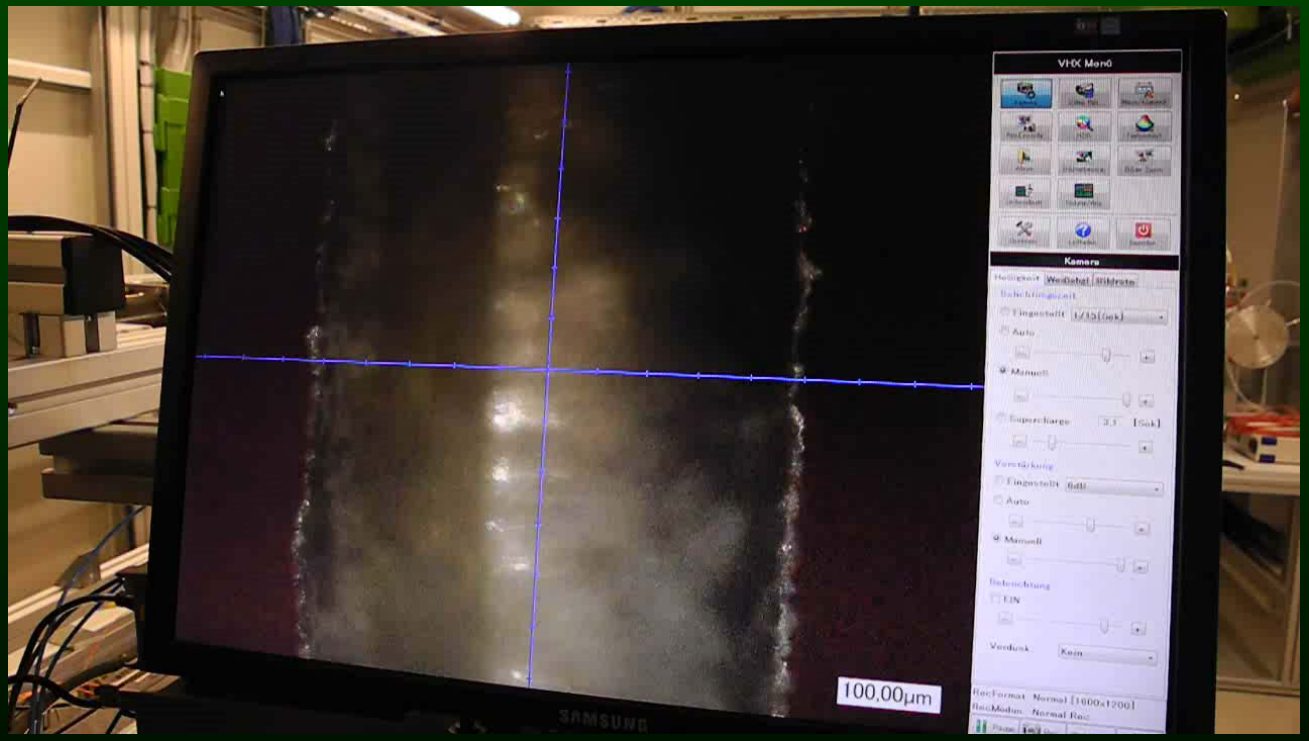
→ MUCH more sensitive than EDX and PIXE, but in contrast to EDX it requires a synchrotron for excitation at resolutions <15µm (limit of most commercial lab sources)
→ limitation of beamtime

Tomographic X-ray emission spectroscopy:
Why is tomography better than 2D mapping for biological samples?

→ MAIN advantage for our purposes: no thin sectioning necessary for large samples (e.g. roots, leaves)

→ drastic reduction of sample preparation artefacts

→ analysed optical slice of sample is protected by surrounding sample and mounting capillary, therefore less damage during storage, transport and analysis

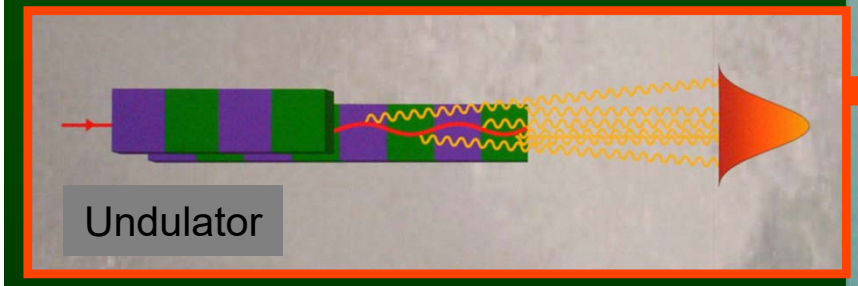


From work of Mishra S, Alfeld M, Sobotka R, Andresen E, Falkenberg G, Küpper H on As-stress in *Ceratophyllum demersum* measured at the the PETRA III P06 beamline of DESY (Hamburg)

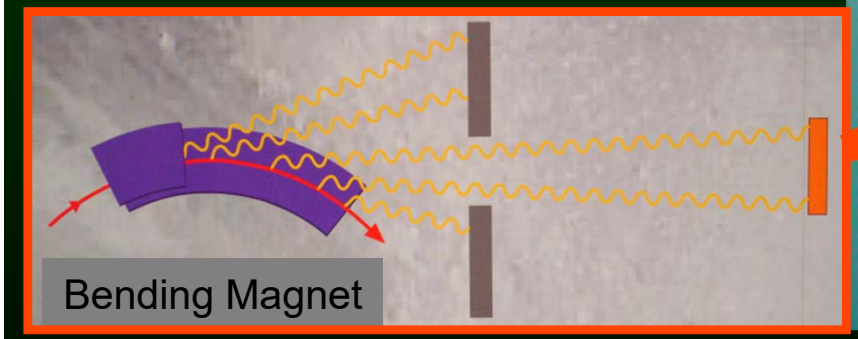
→ For small samples (e.g. microalgae): 3D analysis possible

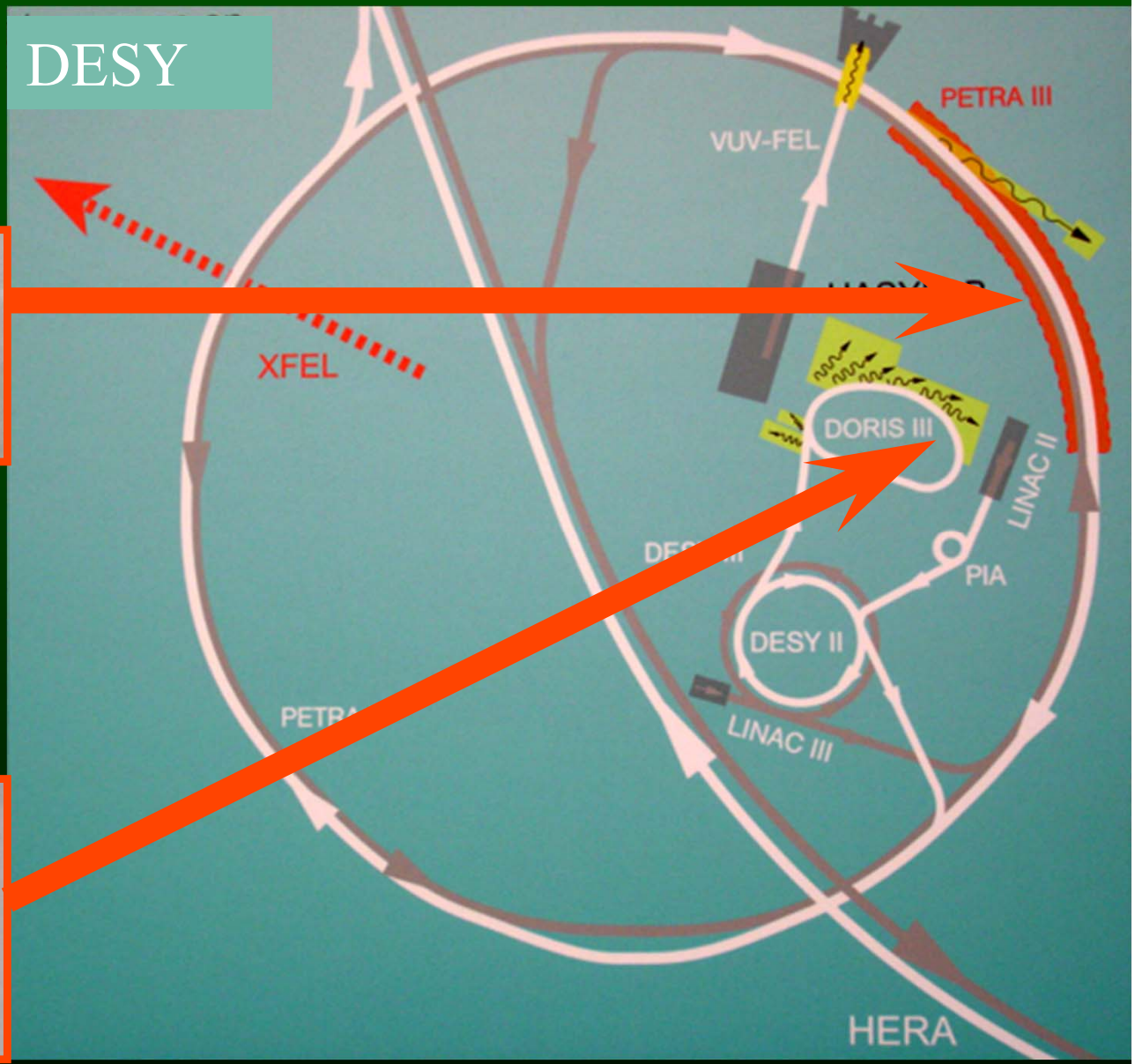
Where we measure (I): Synchrotrons

P06 beamline at PETRA III, microprobe, focussing with KB mirror system, 0.6 µm beam size, large area detectors for large solid angle of detection



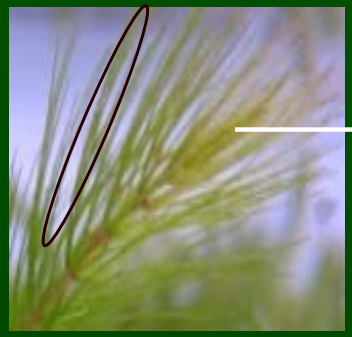
Beamline L at DORIS, focussing with single-bounce capillary, 10 µm beam size, 2 Vortex SDD detectors

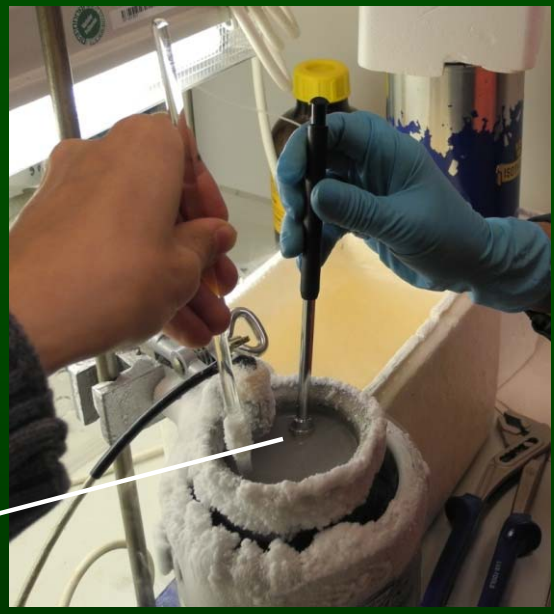




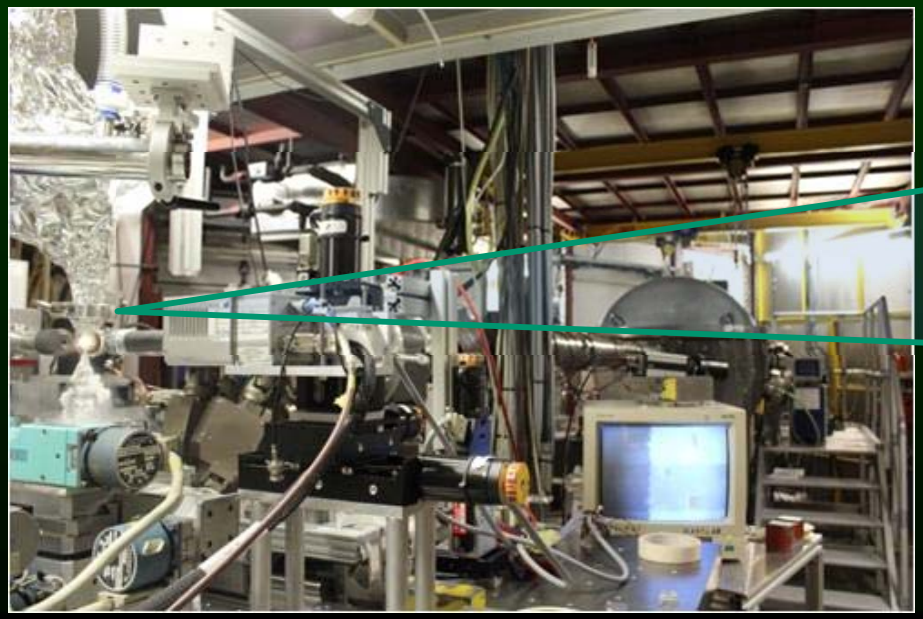
(1) X-ray emission spectroscopy

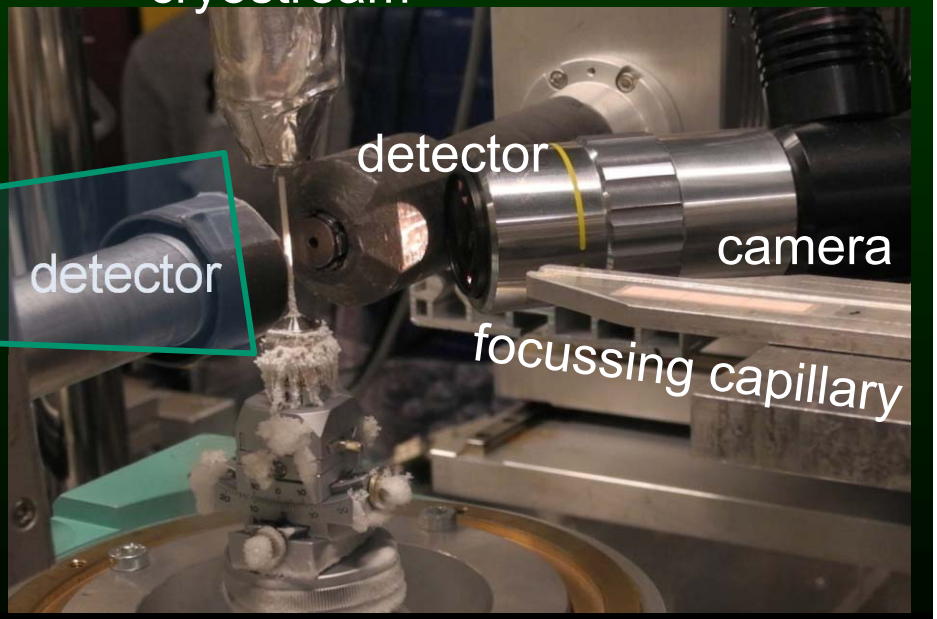
(c) µ-XRF: Sample preparation and measurement



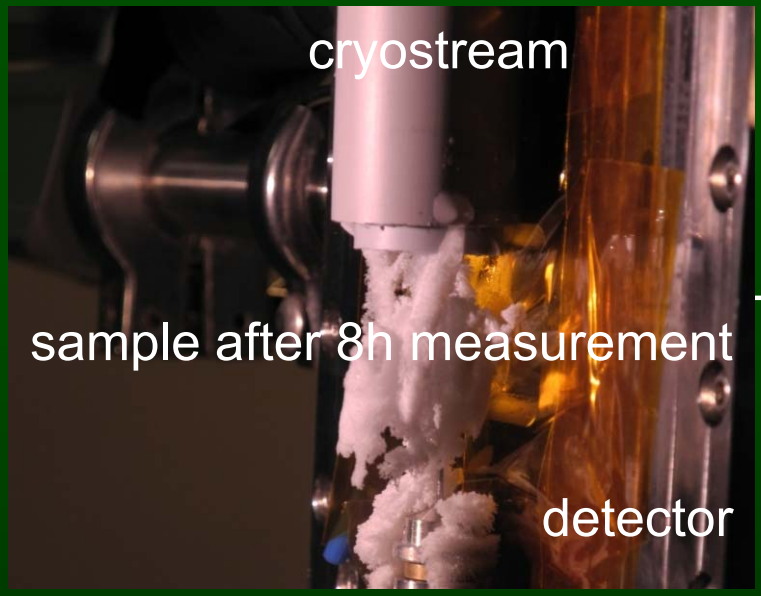


From work of Mishra S, Wellenreuther G, Küpper H
on As-stress in *Ceratophyllum demersum*
measured at the DESY (Hamburg)

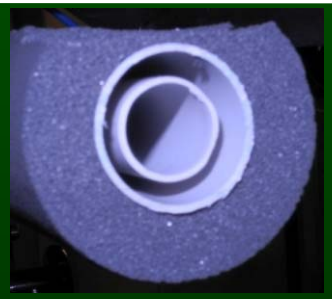


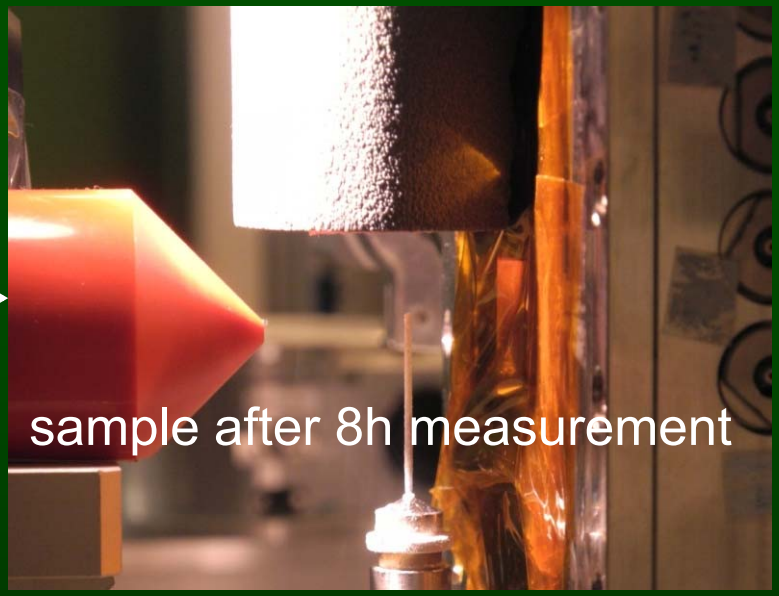


Tomographic X-ray emission spectroscopy (μ-XRF): Cryostream extension for a bulky MAIA detector

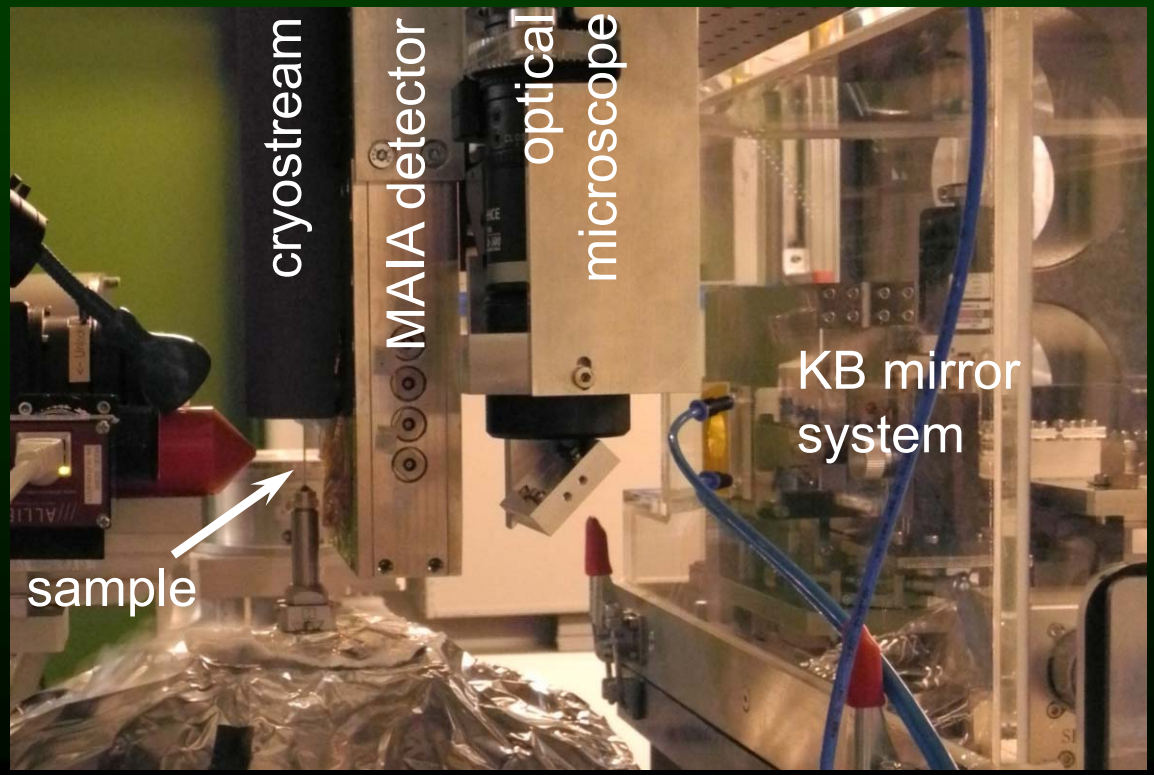


improved flow and insulation of cryostream extension

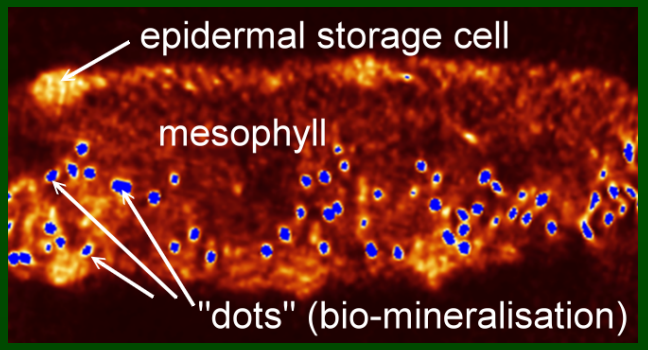




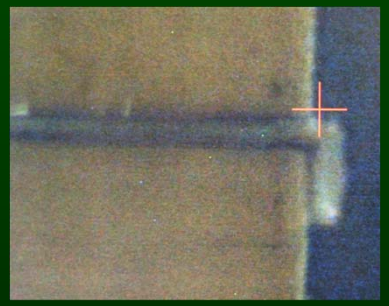
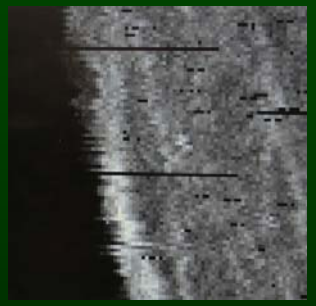
From work of Mishra S, Alfeld M, Sobotka R, Andresen E, Falkenberg G, Küpper H on As-stress in *Ceratophyllum demersum* measured at the the PETRA III P06 beamline of DESY (Hamburg)



μ-XANES **tomography**: Problems of the state of the art



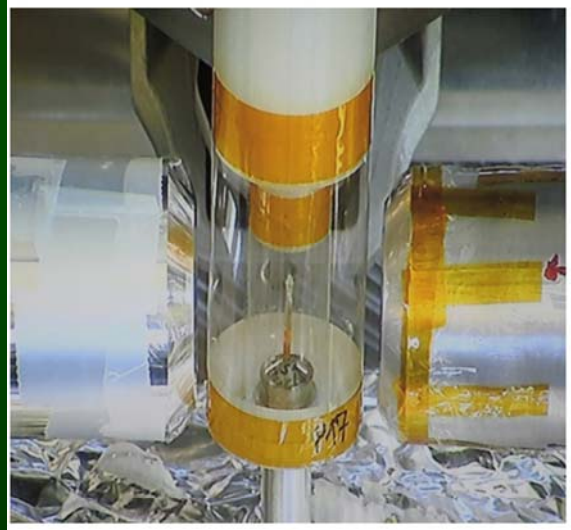
1) Limitations of the measurement
   a) limited resolution because of required dwell time
   b) limited resolution because of slow readout
   c) beam damage to structure and ligands by inefficient detection (high dose of incoming radiation required for each detected fluorescence photon)

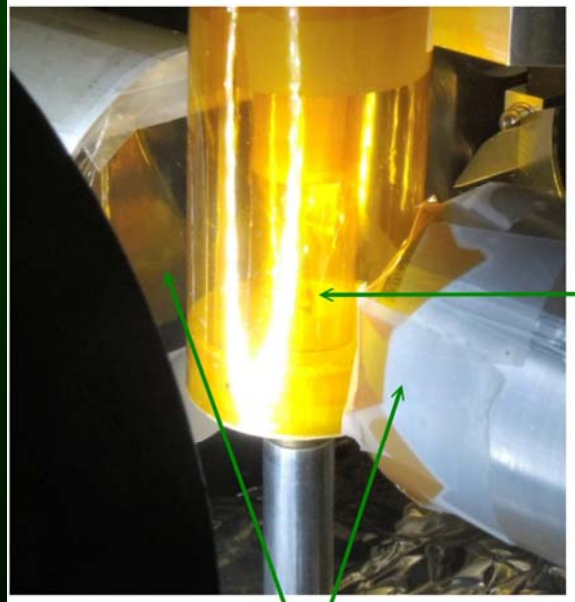



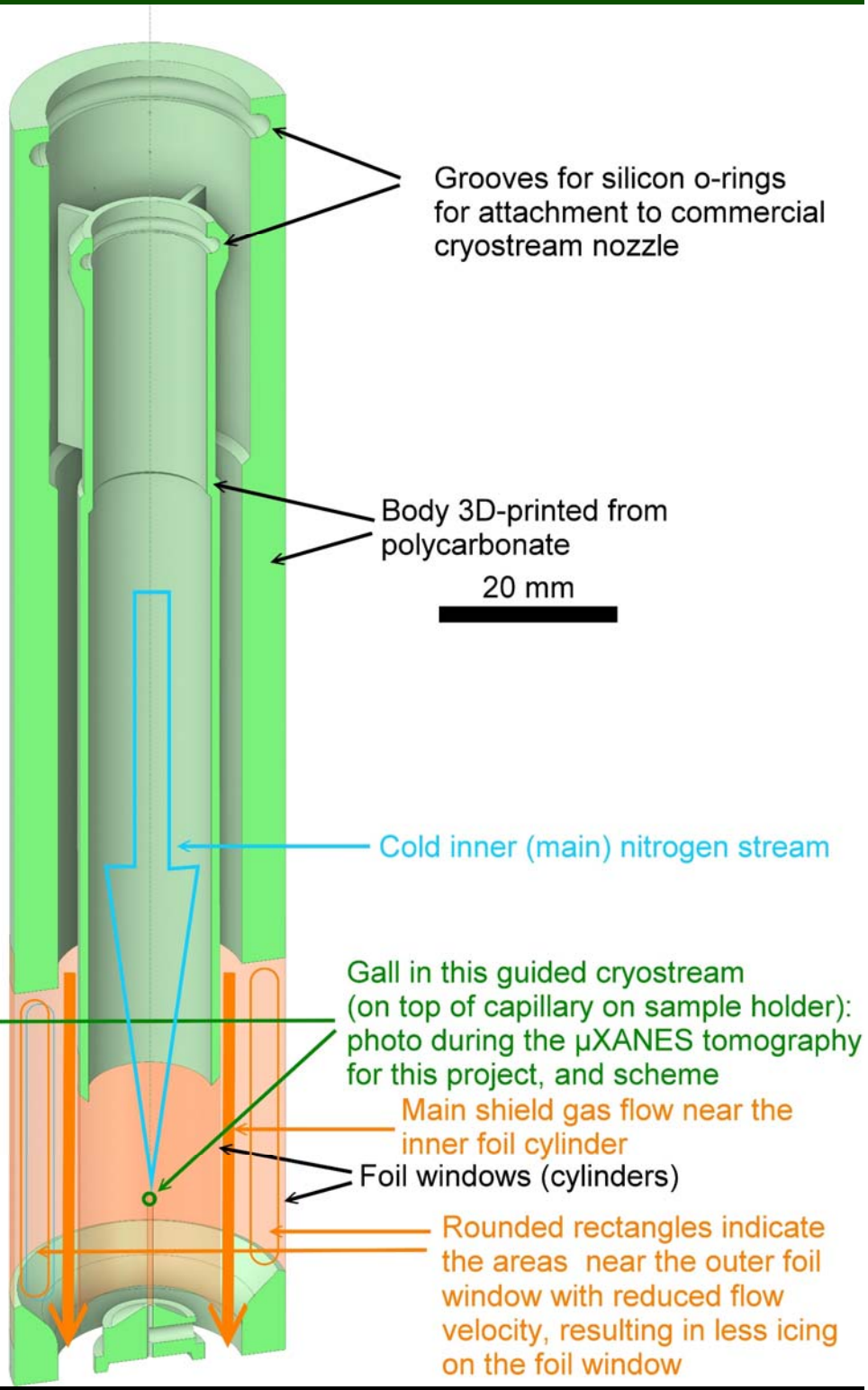
2) Problems of sample mounting
   a) cryostreams lead to vibrations if flow not laminar
   b) cryostreams lead to icing of the sample+detector when flow not laminar
   c) cryochambers reduce problems 2a+2b, but make problems 1a+1c worse because of very limited space for detectors with large solid angle
   d) cryochambers make sample changes very slow because of the need of vacuum and transfer chambers

Mijovilovich A, Mishra A, Brückner D, Spiers K, Andresen E, Garrevoet J, Falkenberg G, Küpper H (2019)
Spectrochimica Acta B 157, 53-62

Tomographic X-ray emission spectroscopy (μ-XRF): Guided Cryostream for minimising icing, drift and vibrations

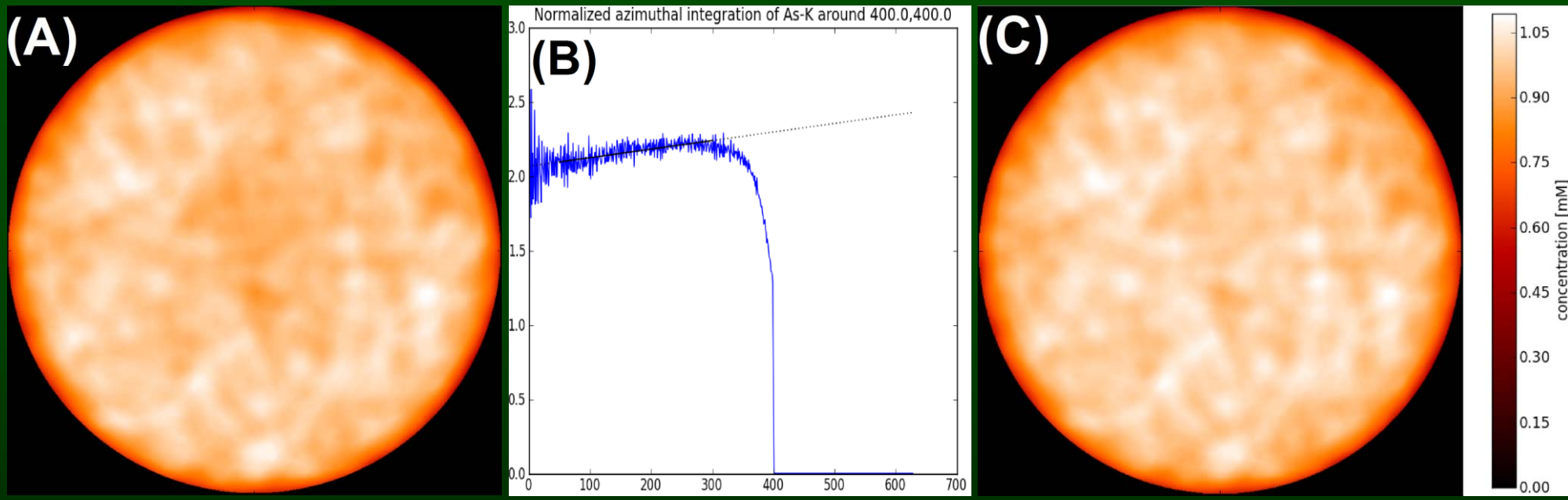






From: Morina F, Kuvelja A, Brückner D, Mojović M, Nakarada Đ, Bokhari SNH, Vujić B, Falkenberg G, Küpper H (2025) How eriophyid mites shape metal metabolism in leaf galls on Tilia cordata. New Phytologist 246, 2222-42 https://doi.org/10.1111/nph.70103

Tomographic X-ray emission spectroscopy (μ-XRF): Quantification with correction of self-absorption



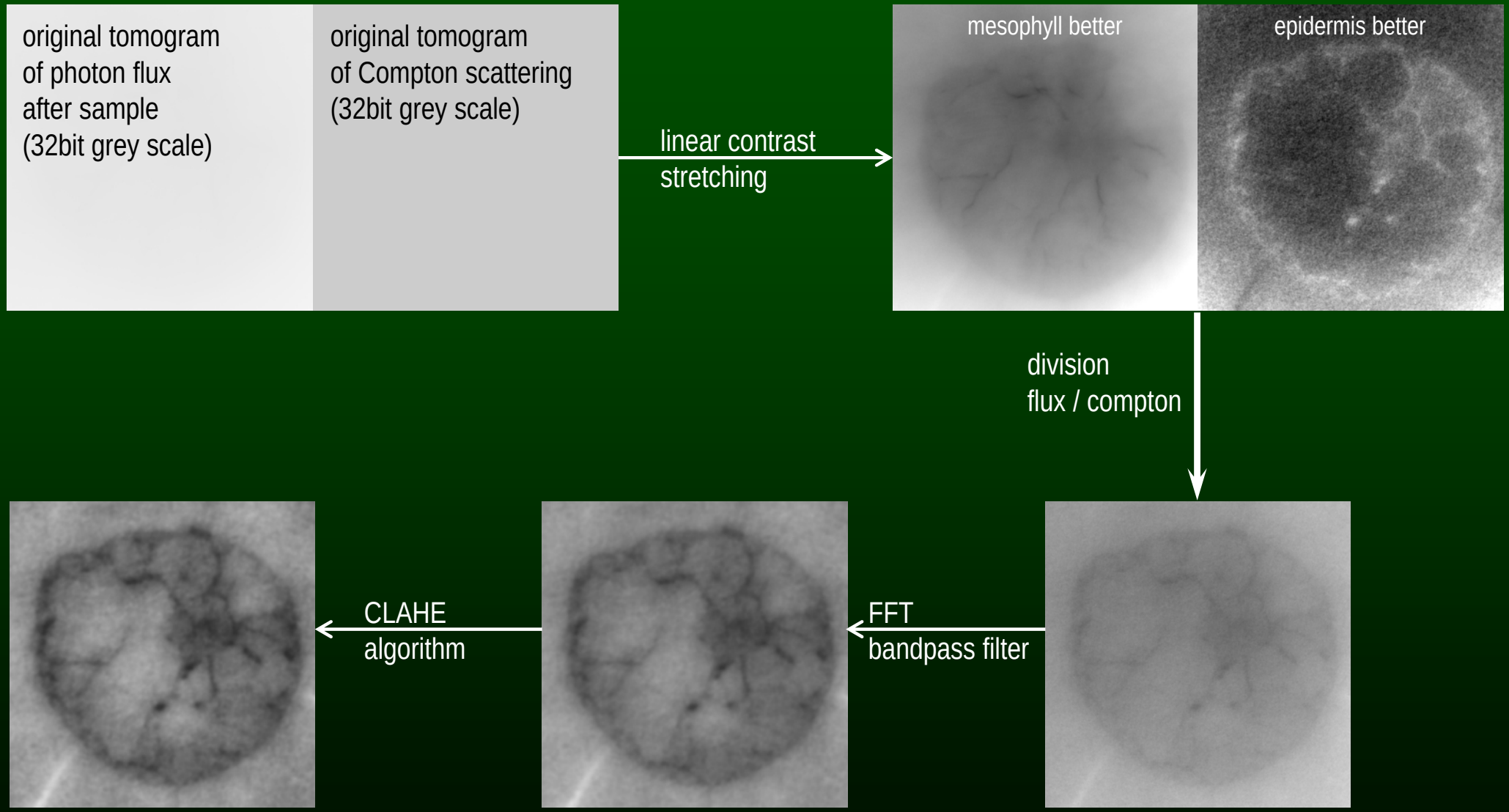
→ intensity artefacts due to absorption inside the sample were corrected by taking the fluorescence intensity distribution in homogeneous standards resembling the shape of the sample as a reference

→ for minimising disturbance by background and neighbouring emission lines, full spectral deconvolution/fitting was used (in PyMCA for SDD data, Geopixe for Maia data).

→ for minimising reconstruction artefacts, the MLEM (maximum likelihood expectation maximization) algorithm was applied

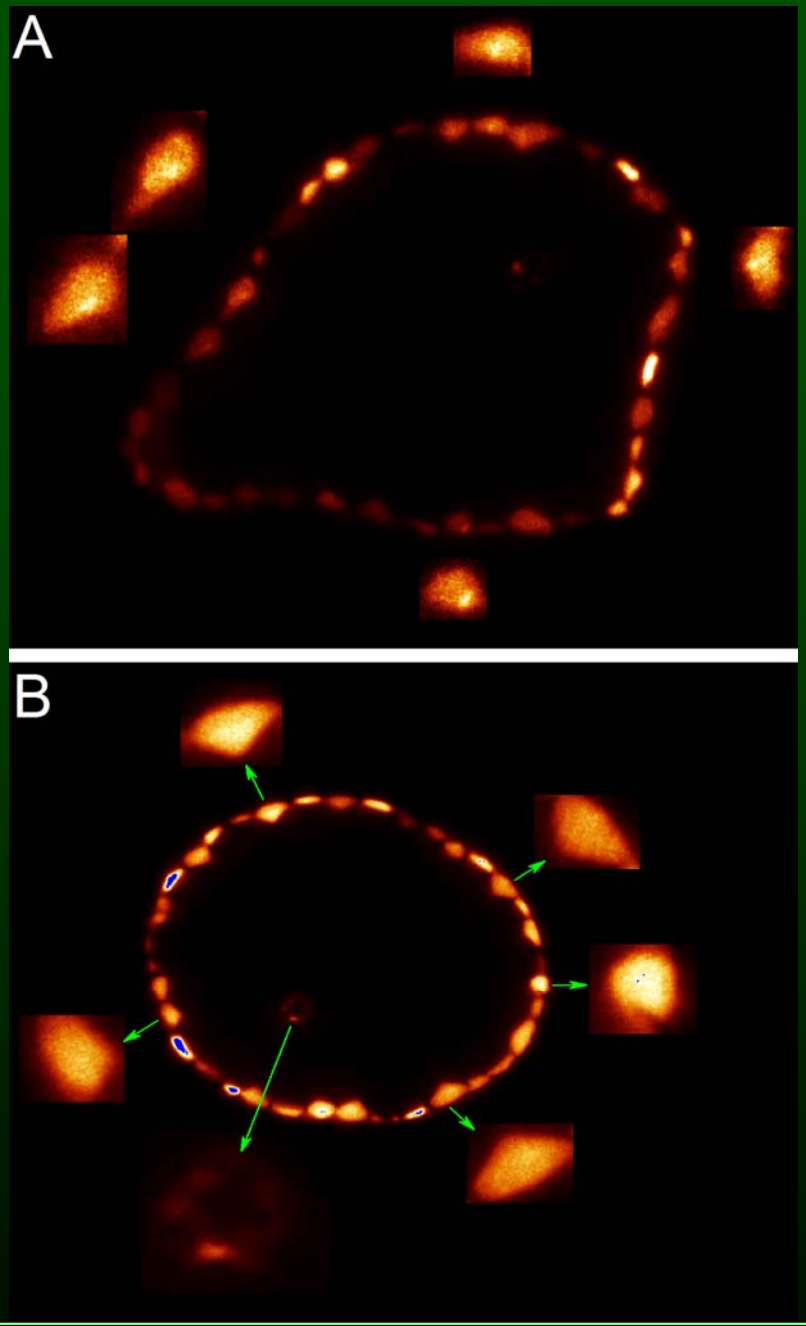
Mishra S, Wellenreuther G, Mattusch J, Stärk H-J, Küpper H (2013) Plant Physiology 163, 1396-1408

Tomographic X-ray emission spectroscopy (μ-XRF): Using Flux and Compton tomograms for showing tissue structures in frozen-hydrated plant samples



Mishra S, Alfeld M, Sobotka R, Andresen E, Falkenberg G, Küpper H (2016) Journal of Experimental Botany 67, 4639-4646

Sub-cellular distribution of As in *C. demersum* leaves



2 phase response to As toxicity

A) Initially, at sublethal concentrations, As is accumulated mainly in the nucleus
→ genotoxicity (besides inhibition of Chl biosynthesis)

B) At lethal concentration, As fills the whole cell
→ various types of damage

Mishra S, Alfeld M, Sobotka R, Andresen E, Falkenberg G, Küpper H (2016) Journal of Experimental Botany 67, 4639-4646

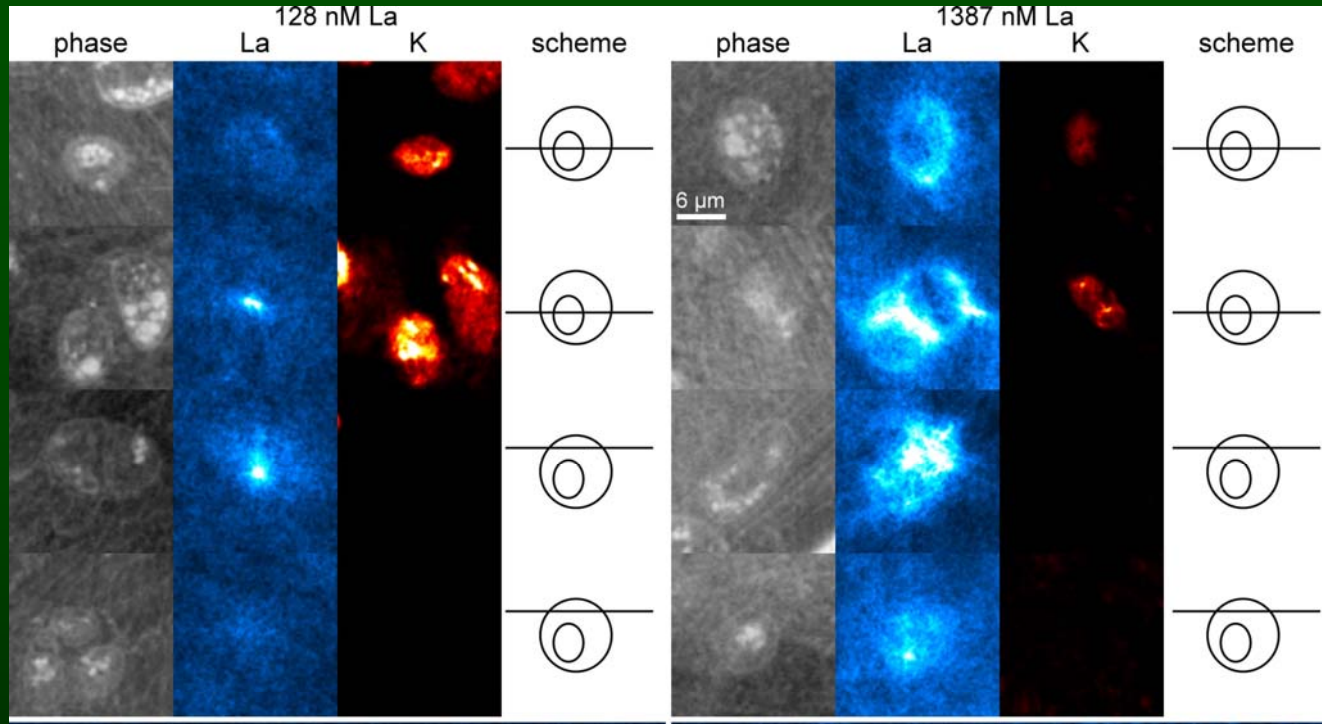
Sub-cellular distribution of La

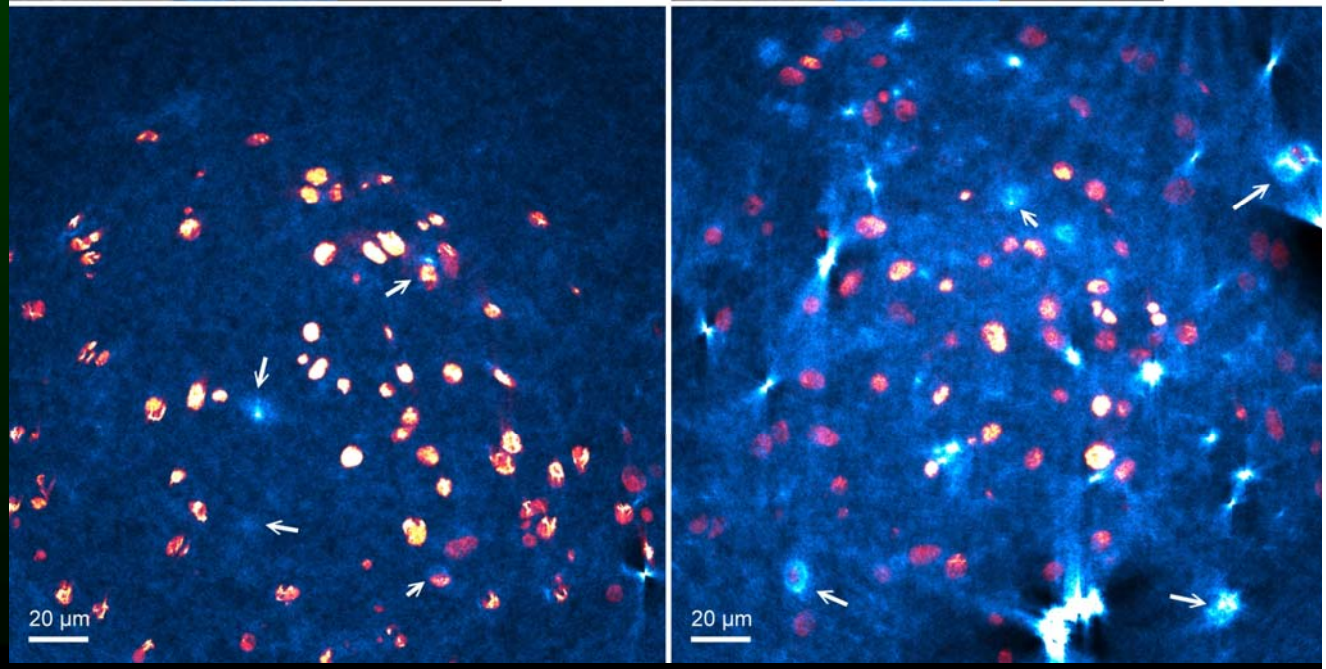
(measured at La Kα with 0.2µm resolution at beamline ESRF ID16A)

Response to La toxicity

A) At sublethal La concentrations, La is usually accumulated inside the cells, often in small spots (biominerals?)

B) At lethal concentration, essential elements become released through leaky membranes, La binds in the whole cytoplasm





Ashraf N, Vitova M, Cloetens P, Mijovilovich A, Bokhari SNH, Küpper H* (2021) Effect of nanomolar concentrations of lanthanum on Desmodesmus quadricauda cultivated under environmentally relevant conditions. Aquatic Toxicology 235, 105818, https://doi.org/10.1016/j.aquatox.2021.105818

Where we measure µXRF (II): At home

Customised Bruker Tornado M4 machine in our lab

- **Purpose: Imaging measurement of element distribution (Z≥11 Na) with tissue-level (15-20µm) resolution *in vivo***

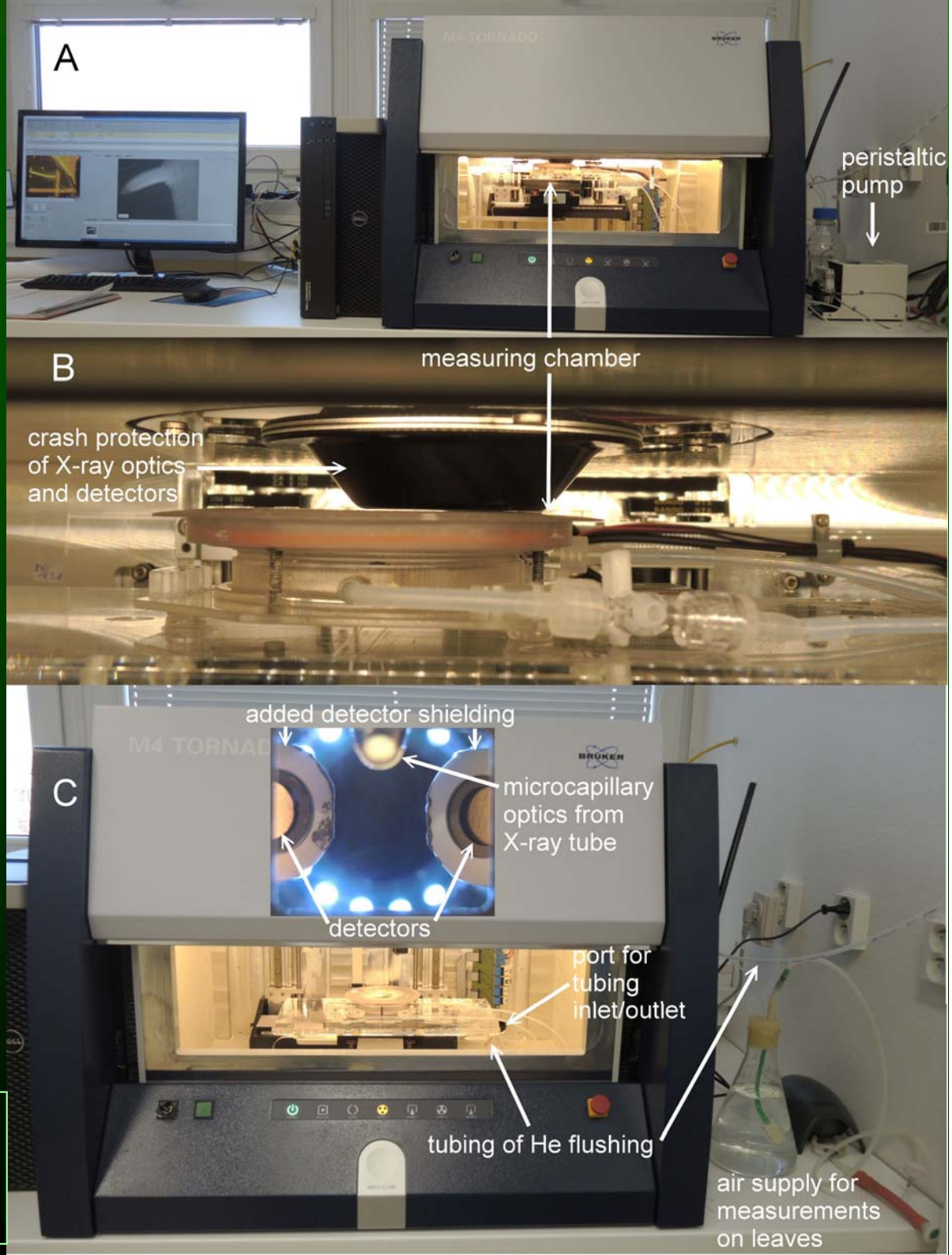
- **Special features:**

→ biology-optimised configuration with 2 SDD detectors of together 120 mm² active area and fast readout electronics

→ custom-made special shielding to reduce background counts in the range of the trace elements that are important for our work

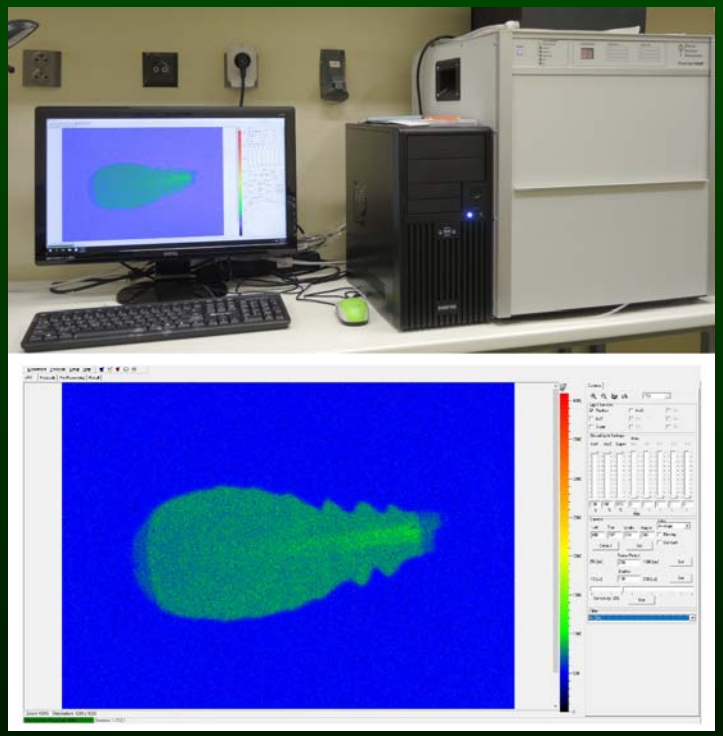
→ custom-made measuring chamber for keeping samples alive

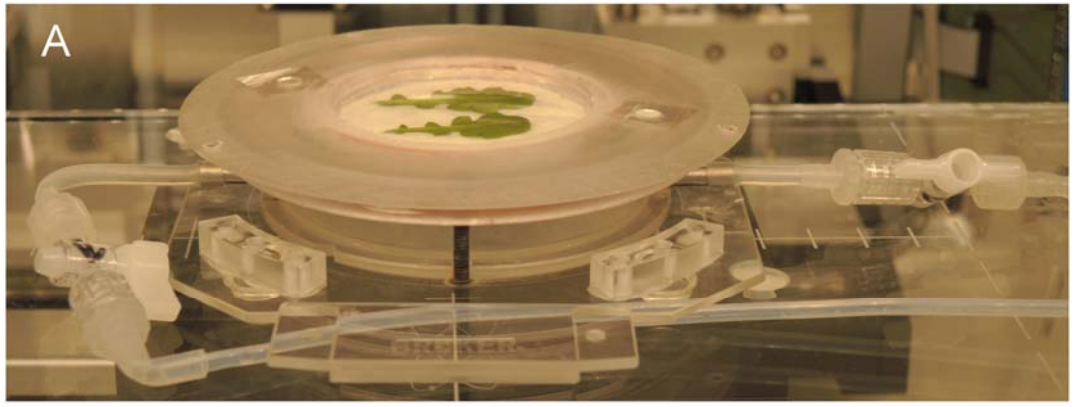
Mijovilovich A, Morina F, Bokhari SNH, Wolff T, Küpper H* (2020) Analysis of trace metal distribution in plants with lab-based microscopic X-ray fluorescence imaging, Plant Methods, DOI: 10.1186/s13007-020-00621-5

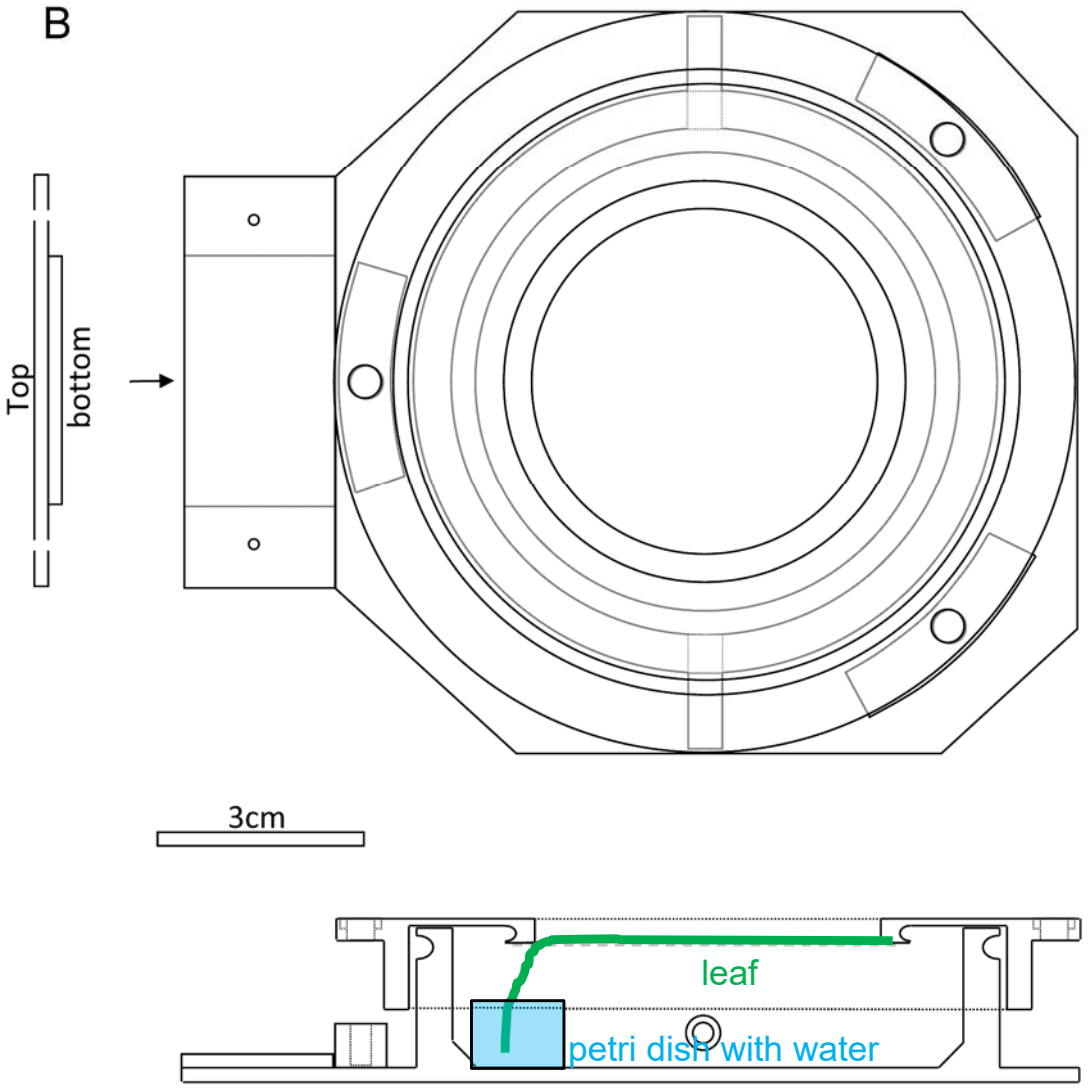


Where we measure µXRF (I): Customised Bruker Tornado M4 in our lab

Sample preparation and photosynthesis measurement



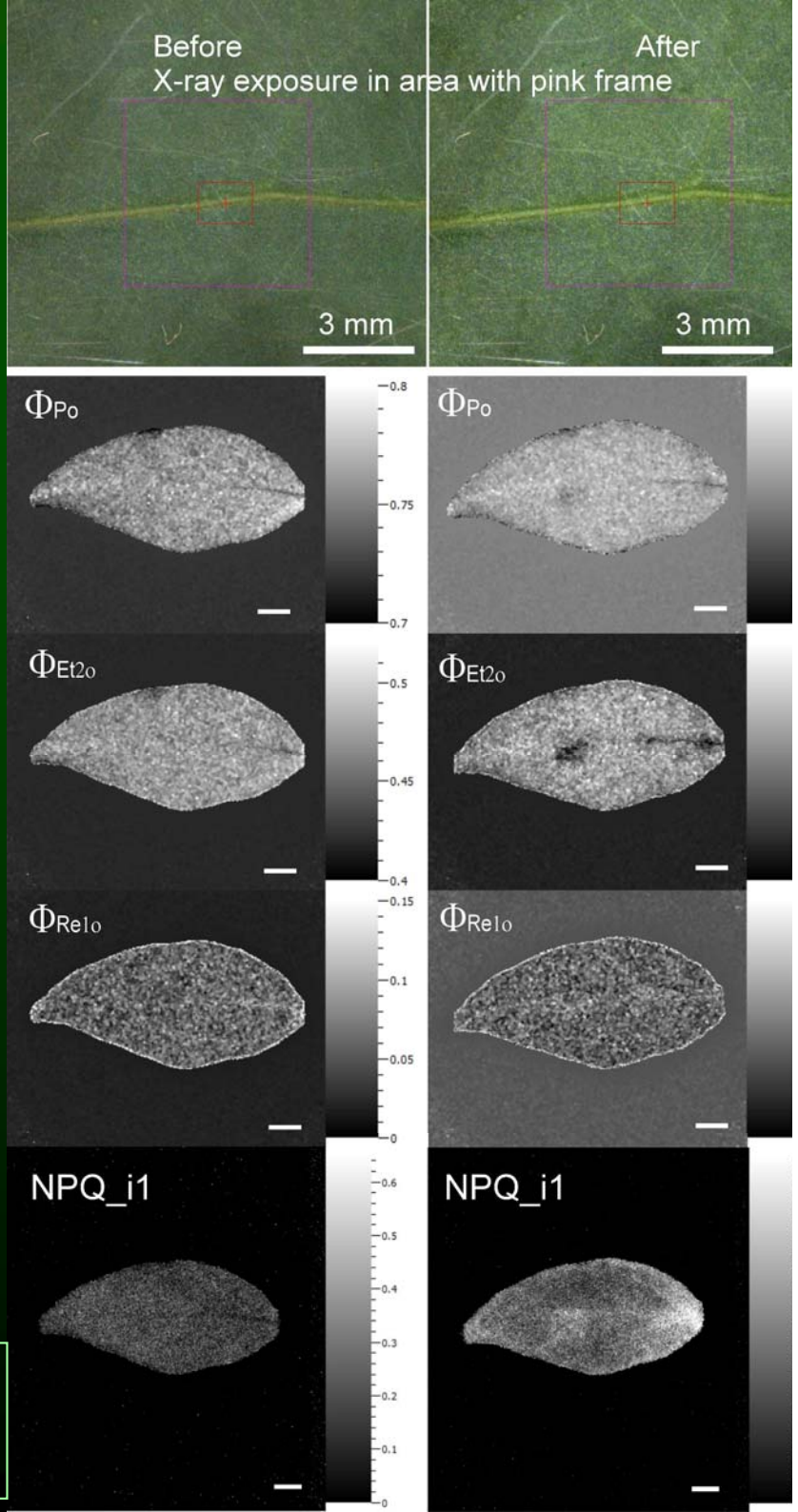




OJIP: Küpper H, Benedikty Z, Morina F, Andresen E, Mishra A, Trtílek M (2019) Plant Physiology 179, 369-381.

µXRF: Mijovilovich A, Morina F, Bokhari SNH, Wolff T, Küpper H* (2020) Plant Methods, DOI: 10.1186/s13007-020-00621-5

Verification of sample vitality during measurement in the in-house X-ray fluorescence microscope for analysis of metal localization in living tissues



Measurement of photosynthetic activity by direct fast imaging of OJIP chlorophyll fluorescence kinetics

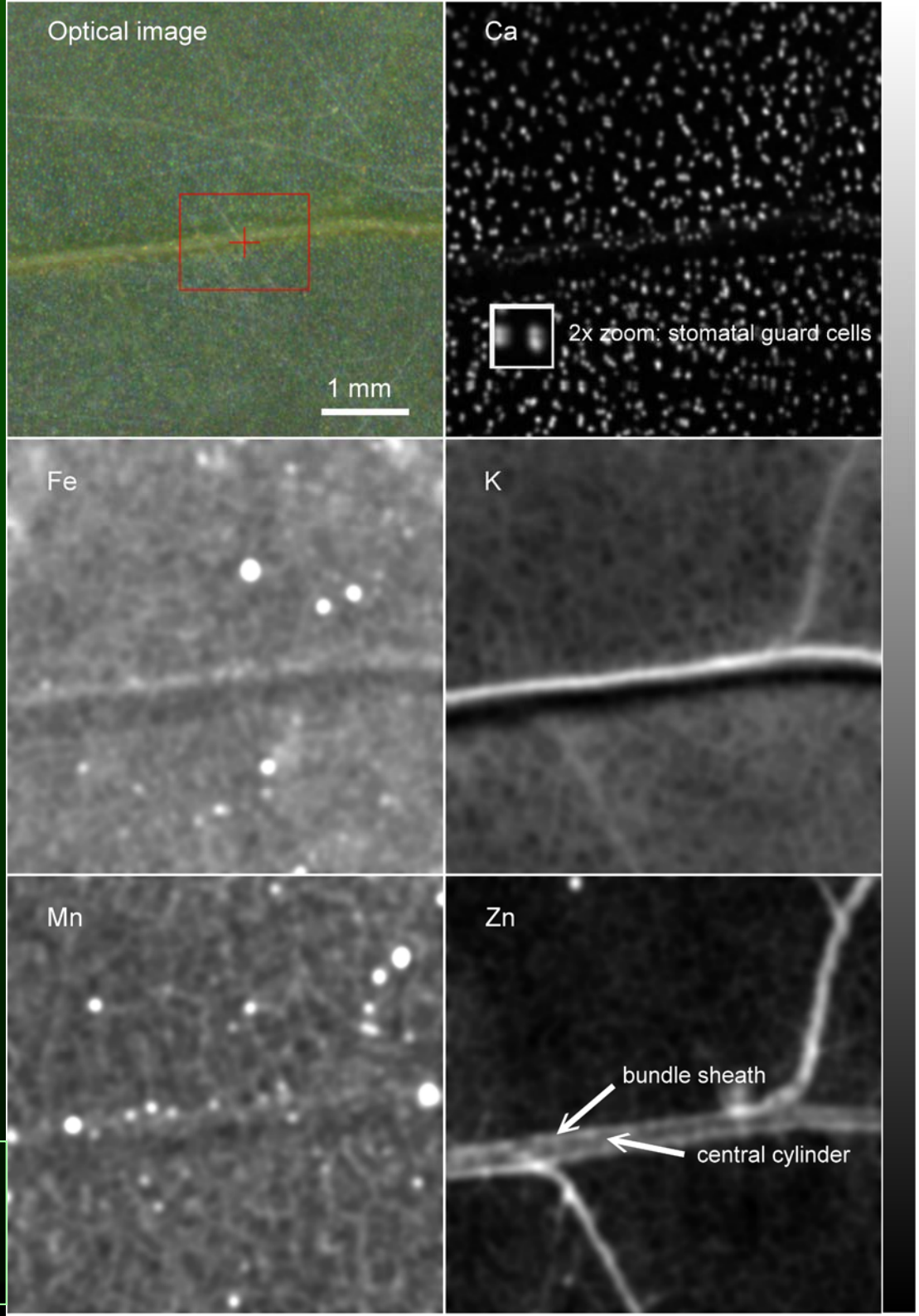
→ Samples stay vital even after 20h total measurement time, but small effects on electron transport and non-photochemical quenching can be seen

Mijovilovich A, Morina F, Bokhari SNH, Wolff T, Küpper H* (2020) Analysis of trace metal distribution in plants with lab-based microscopic X-ray fluorescence imaging, Plant Methods, DOI: 10.1186/s13007-020-00621-5

Examples of the use of the in-house X-ray fluorescence microscope for analysis of metal localization in living tissues: *Capsicum annuum* (pepper) leaves

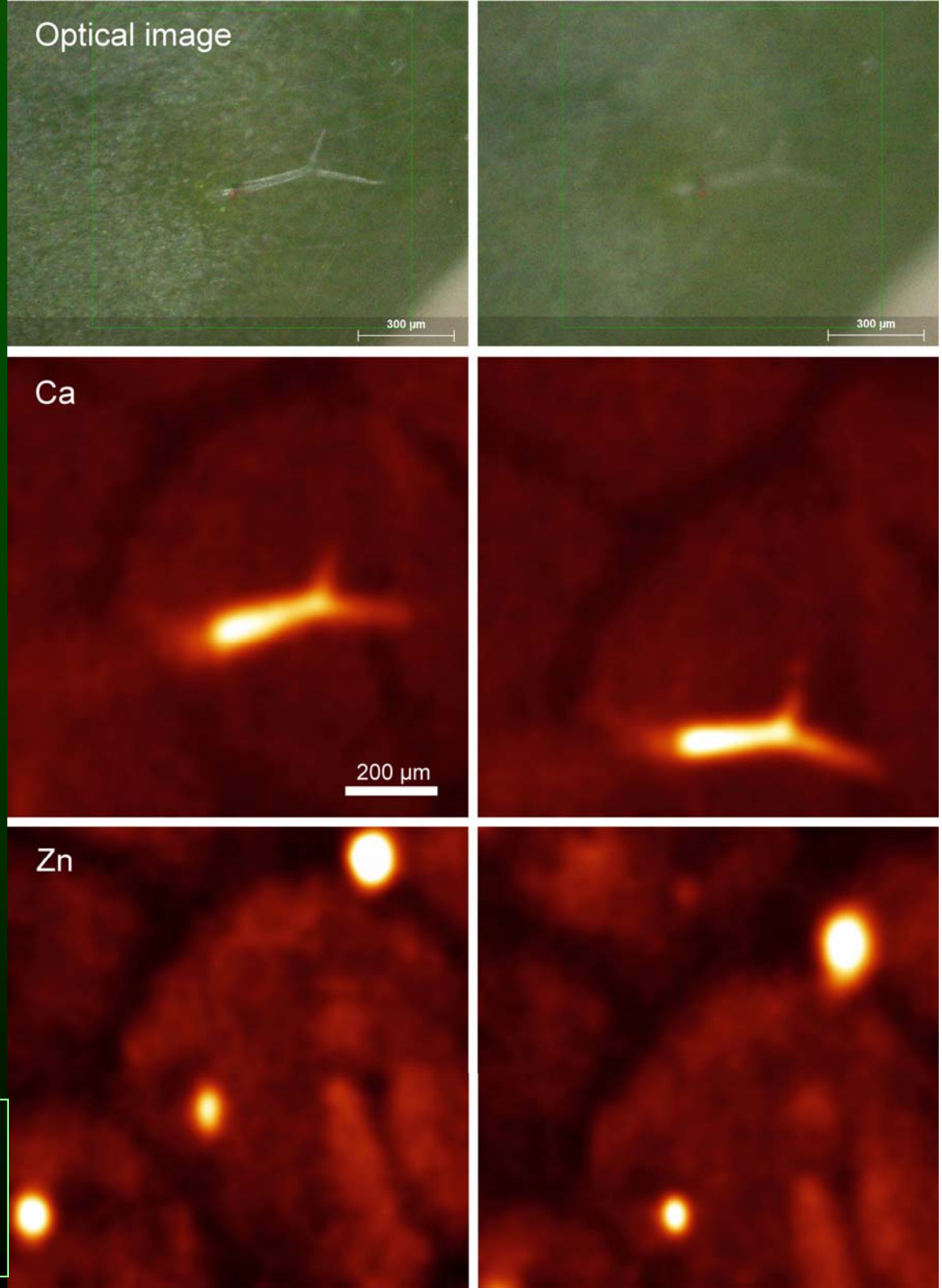
→The sensitivity of the machine is sufficient for visualising trace metals in non-accumulator crop plants

→The spatial resolution (15µm beam size) is sufficient for imaging metal distribution between tissues and larger cells



Mijovilovich A, Morina F, Bokhari SNH, Wolff T, Küpper H* (2020) Analysis of trace metal distribution in plants with lab-based microscopic X-ray fluorescence imaging, Plant Methods, DOI: 10.1186/s13007-020-00621-5

Examples of the use of the in-house X-ray fluorescence microscope for analysis of metal localization in living tissues: *Arabidopsis halleri* leaves

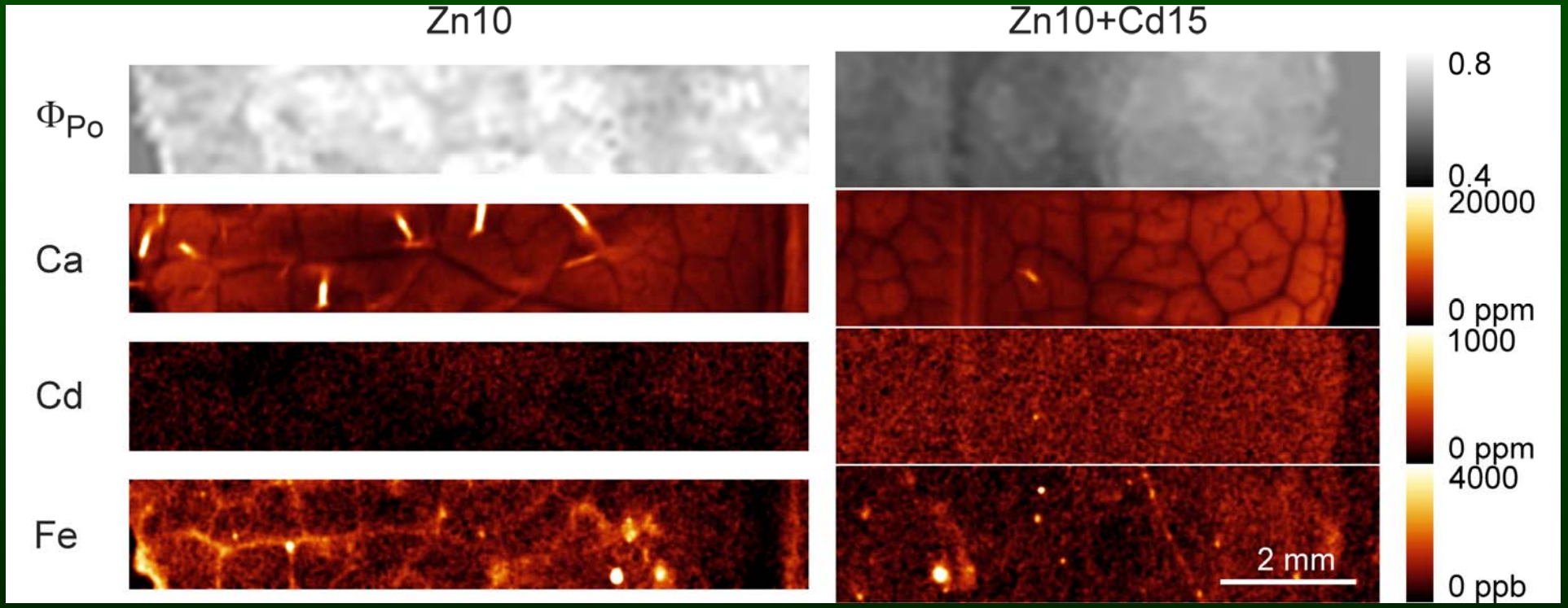


→de-focussing affects the resolution of the optical image more than the µXRF maps

→Due to the geometric arrangement of the optical camera vs. the X-ray optics (polycapillary), de-focussing leads to a shift of the µXRF maps relative to the optical image.

Mijovilovich A, Morina F, Bokhari SNH, Wolff T, Küpper H* (2020) Analysis of trace metal distribution in plants with lab-based microscopic X-ray fluorescence imaging, Plant Methods, DOI: 10.1186/s13007-020-00621-5

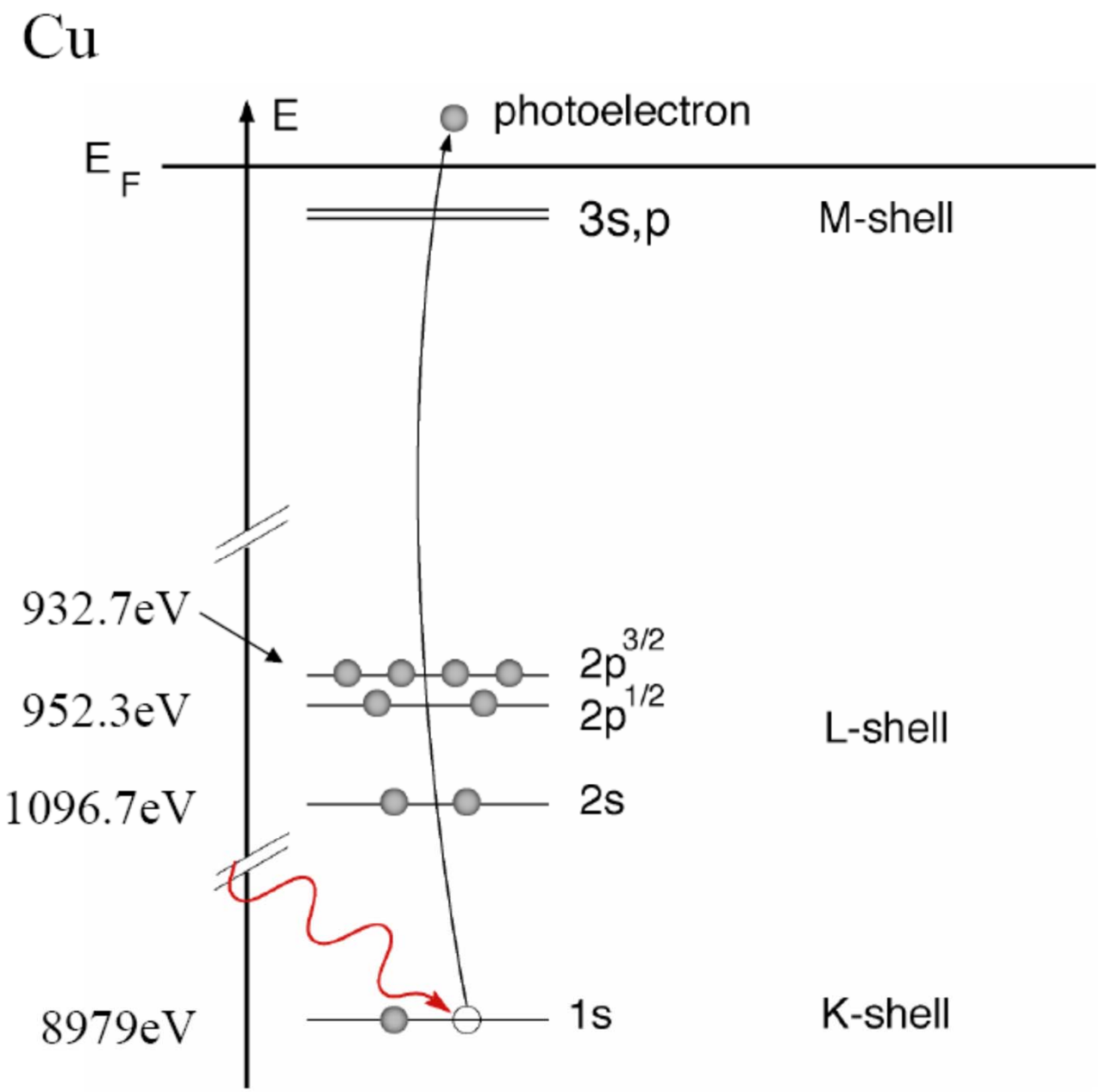
Examples of the use of the in-house X-ray fluorescence microscope for analysis of metal localization in living tissues: Cd accumulation vs. PSII efficiency in *Arabidopsis halleri* leaves



→ **In vivo measurement allows for direct correlation between physiology and metal accumulation!**

Morina F, Küpper H* (2020) Direct inhibition of photosynthesis by Cd dominates over inhibition caused by micronutrient deficiency in the Cd/Zn hyperaccumulator Arabidopsis halleri. Plant Physiology and Biochemistry 155_252-261 (DOI: https://doi.org/10.1016/j.plaphy.2020.07.018)

X-ray absorption (I)



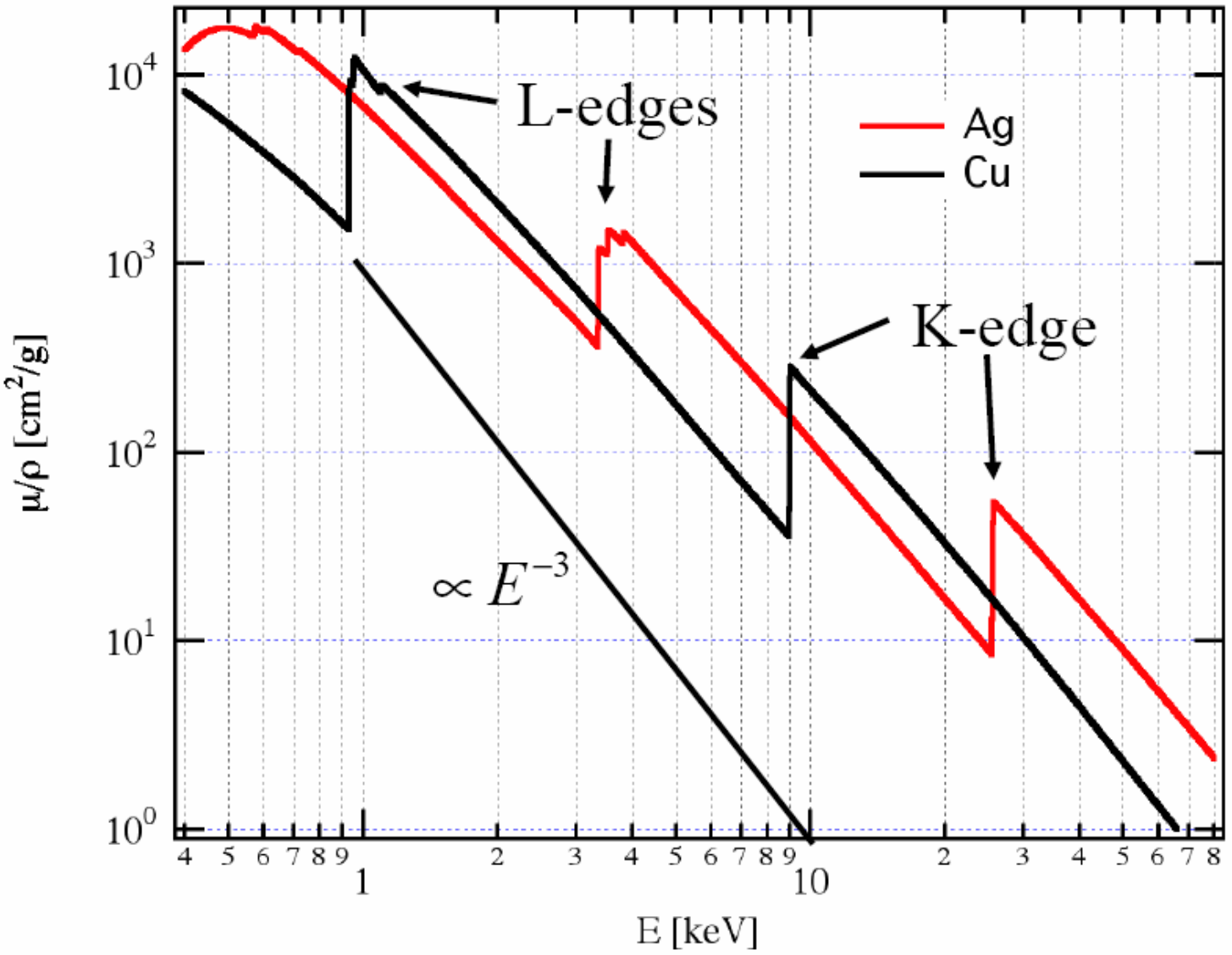
- Electrons in atoms are arranged in shells with different atom-specific binding energies: K, L, M
- Atomic electron can absorb x-rays if:

$$E_{photon} > E_{ionization}$$

(Pauli-principle)

X-ray absorption (II)

μ(E): linear absorption coefficient



- mainly atomic effect
- strong dependence on energy:

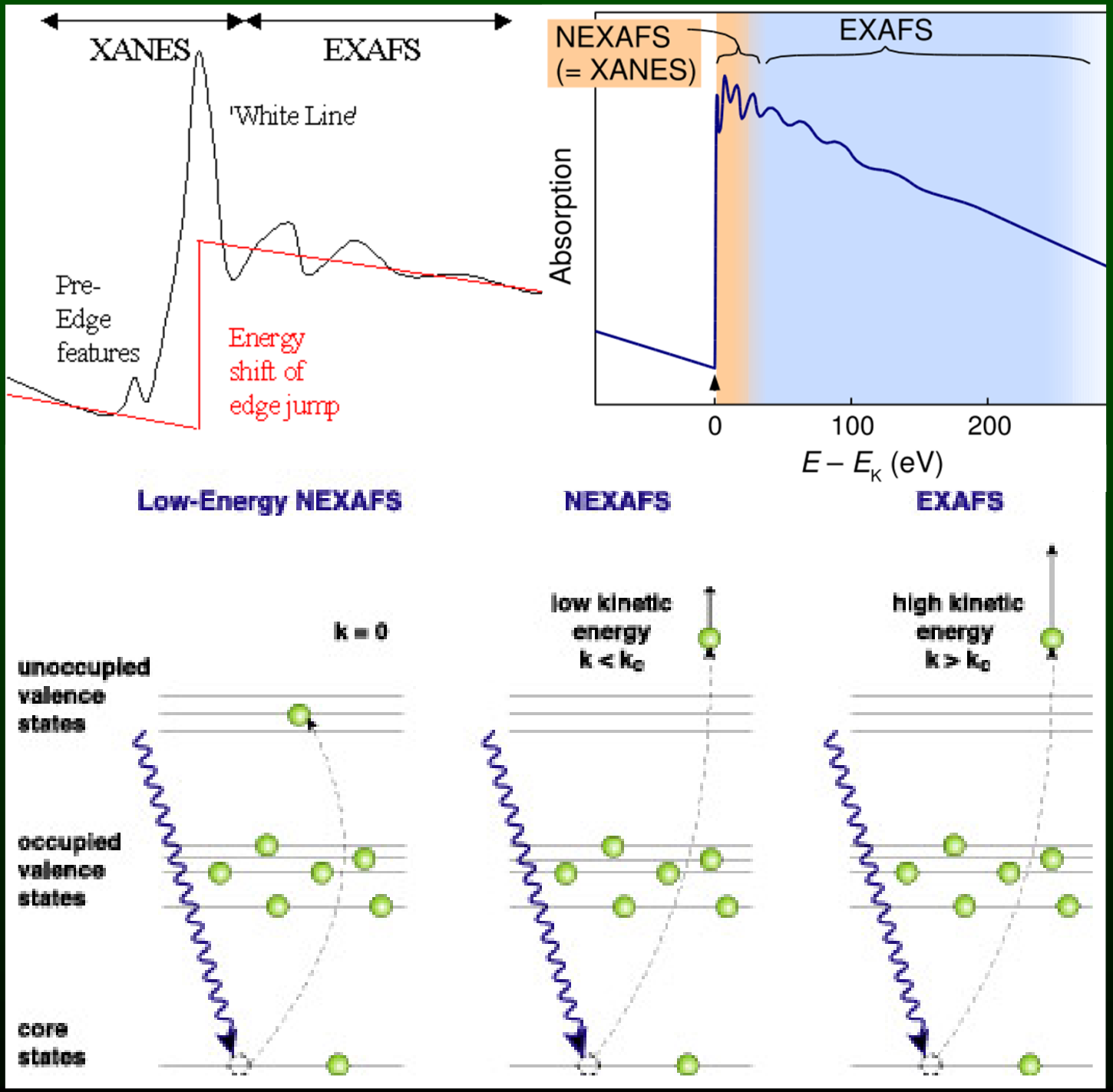
$$\propto E^{-2.78}$$

- strong dependence on atomic number:

$$\propto Z^{2.7}$$

- inner shell electr. contribute most strongly

XAS techniques



What can we learn from XAS?

Three different characteristics:

- edge position:

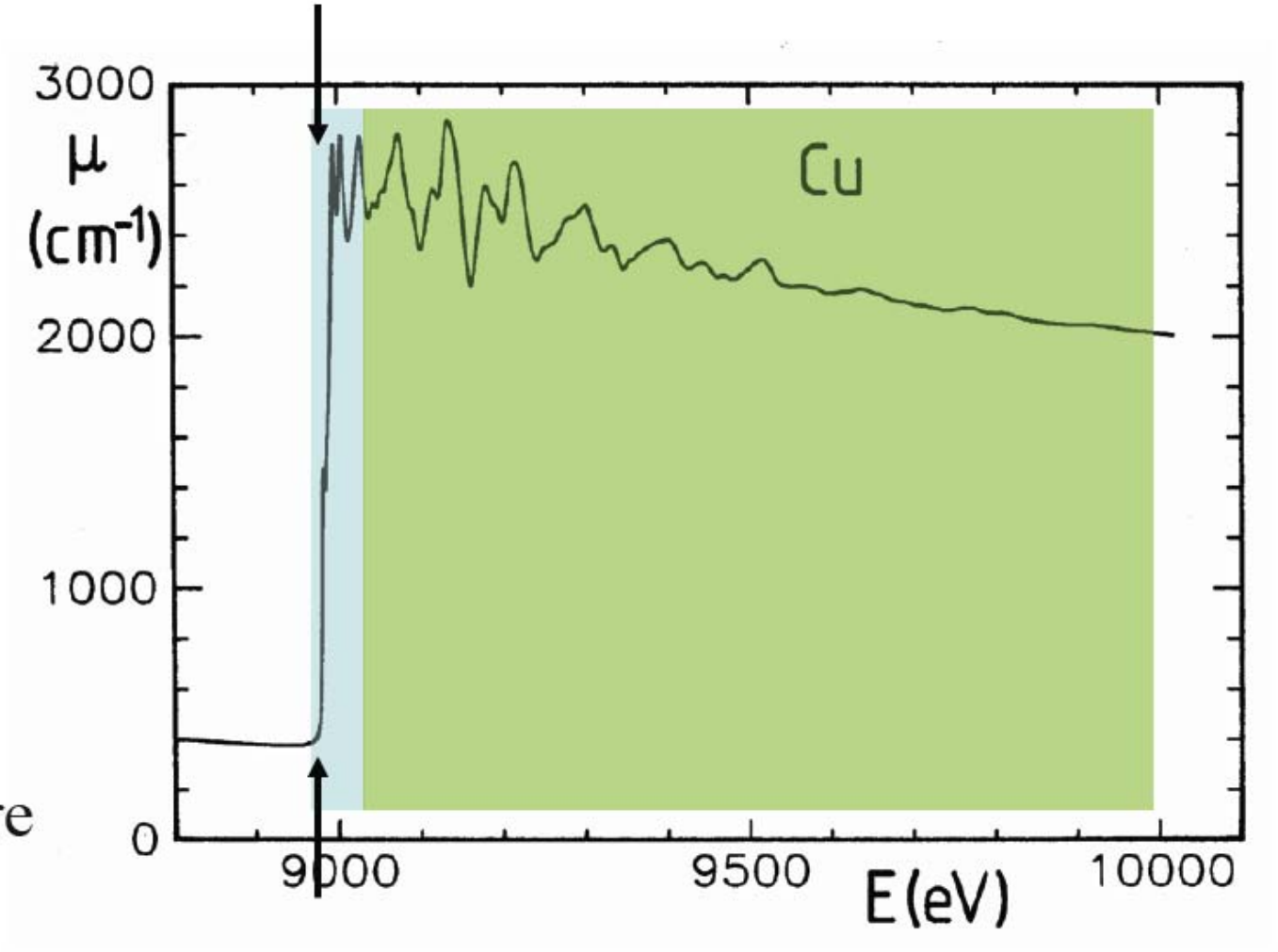
  oxidation state

- near edge spectrum (XANES):

  local projected density of states

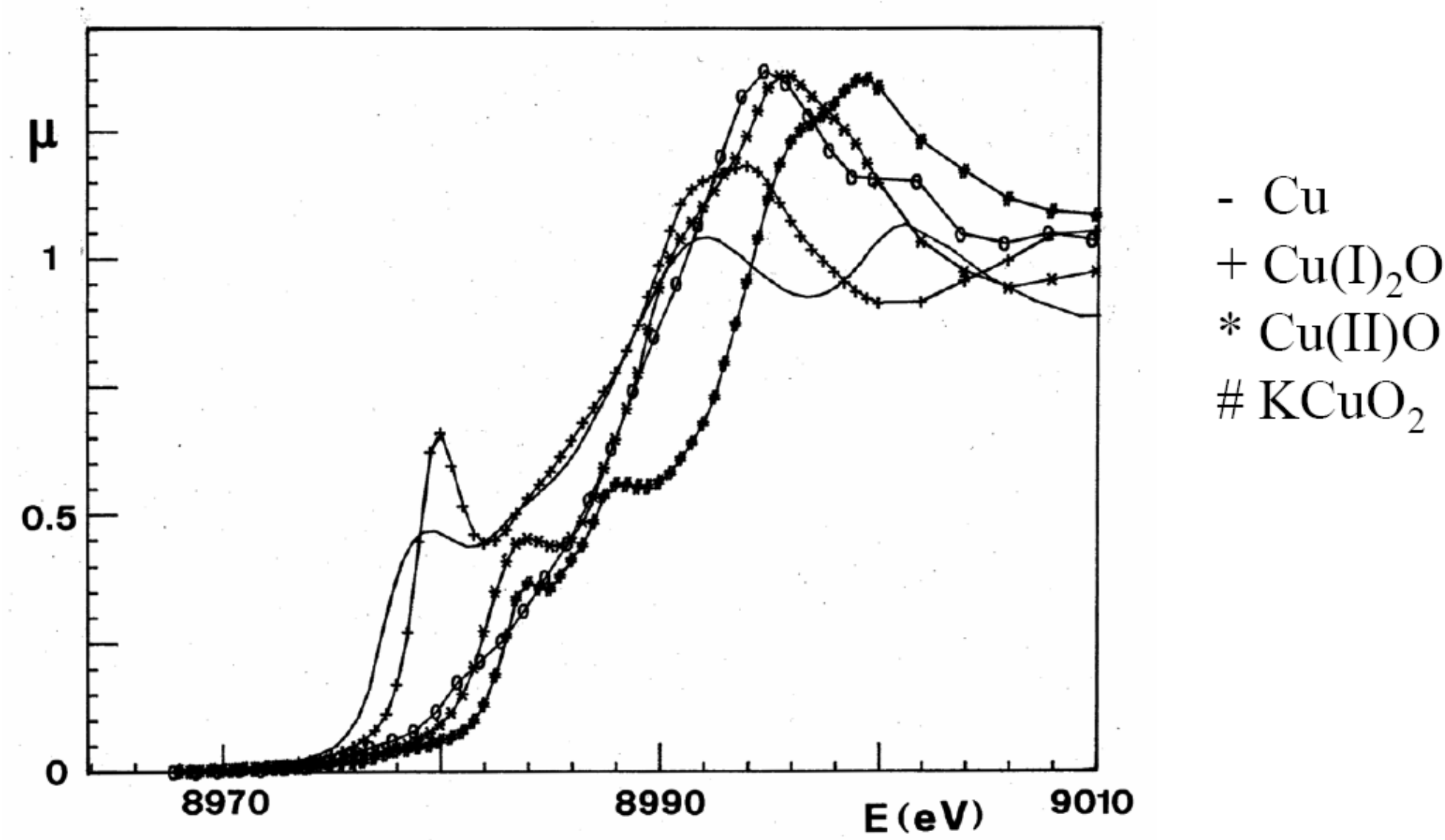
- extended fine structure (EXAFS):

  local neighborhood of atomic species



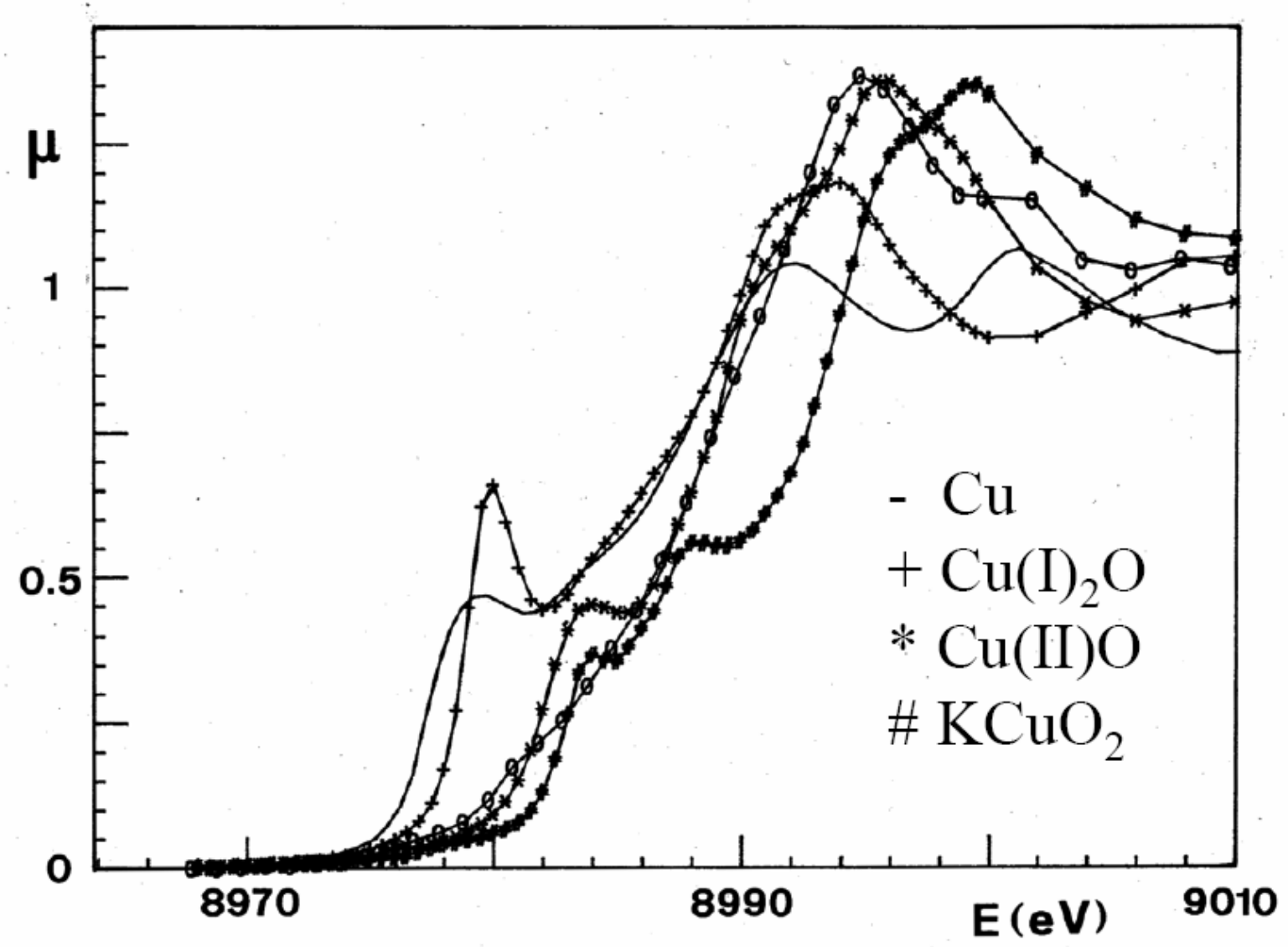
Example of what can we learn from XANES (I)

Edge is shifted to higher energy with increasing formal valence:



Example of what can we learn from XANES (II)

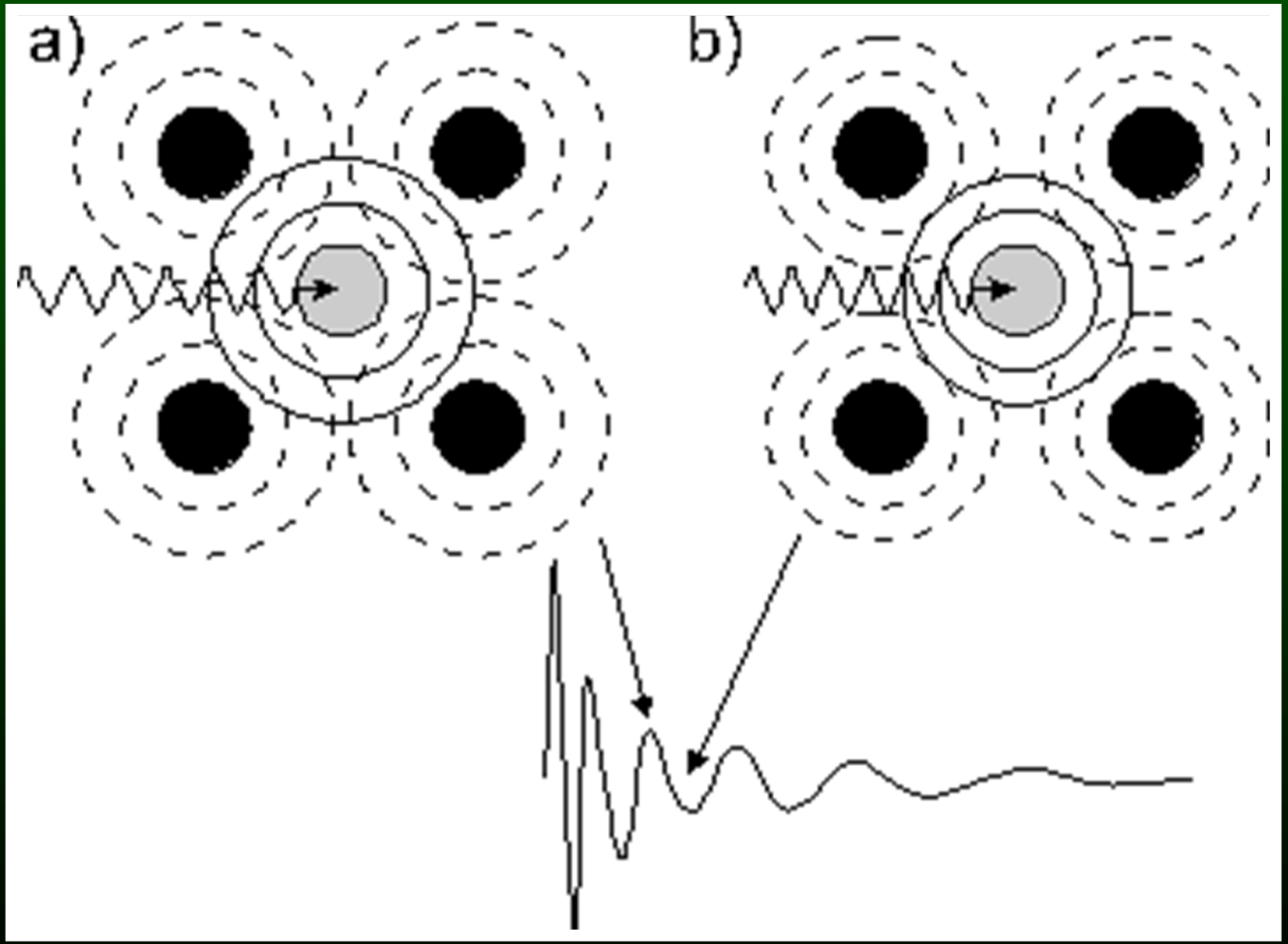
Finger print for chemical state of element of interest:



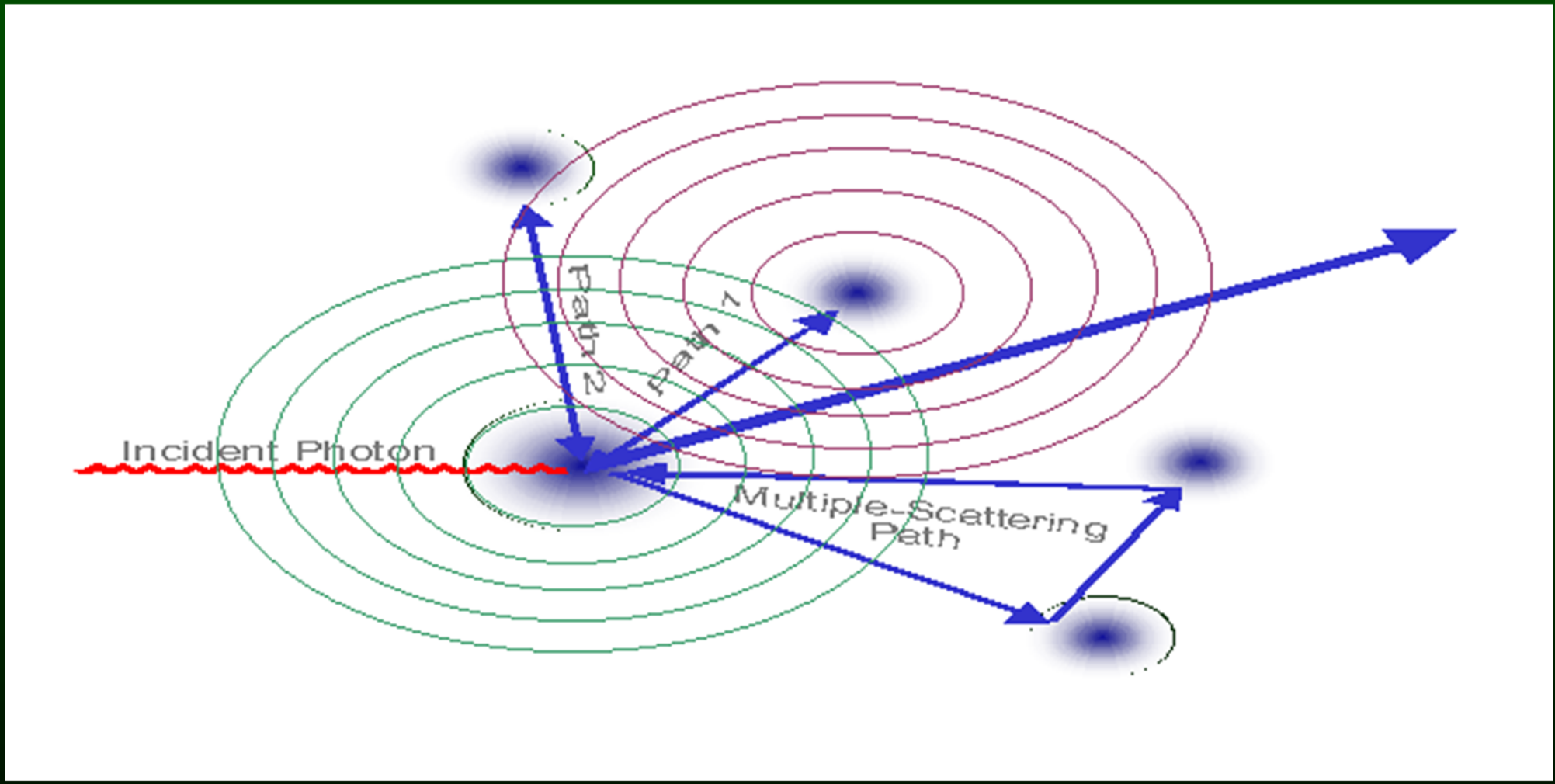
determine concentration of chemical compounds in mixtures

example: inhomogeneous specimens

Principle of Extended X-ray Absorption Fine Structure (EXAFS)



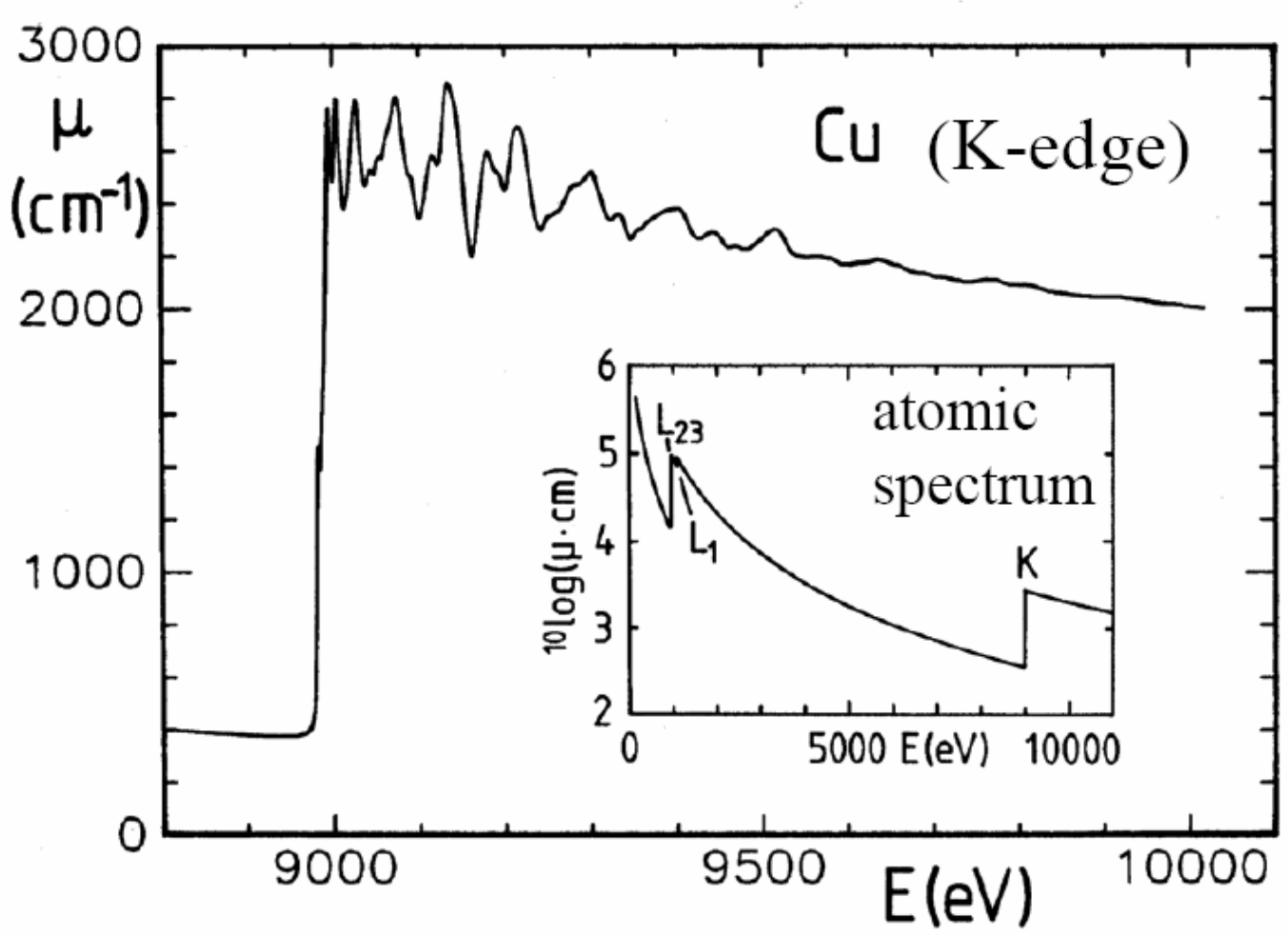
Principle of single vs. multiple scattering contributions in EXAFS



Effects of single vs. multiple scattering contributions in EXAFS

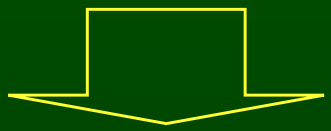
μ(E): linear absorption coefficient

metallic Cu:

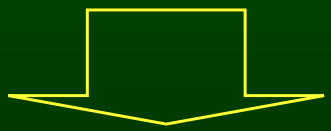


Preparation of plant material for XAS (EXAFS and XANES)

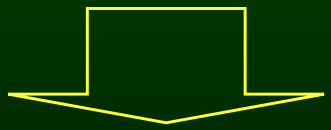
Excise sample from plant



Freeze the sample in melting nitrogen slush



grind sample in mortar cooled by dry ice



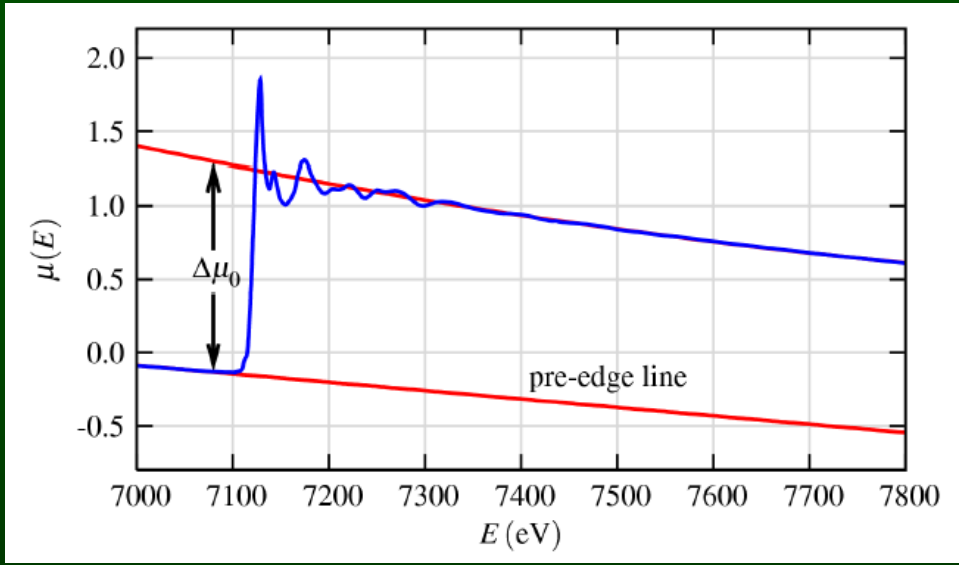
fill the still frozen-hydrated powder into an EXAFS cuvette, seal with Kapton tape

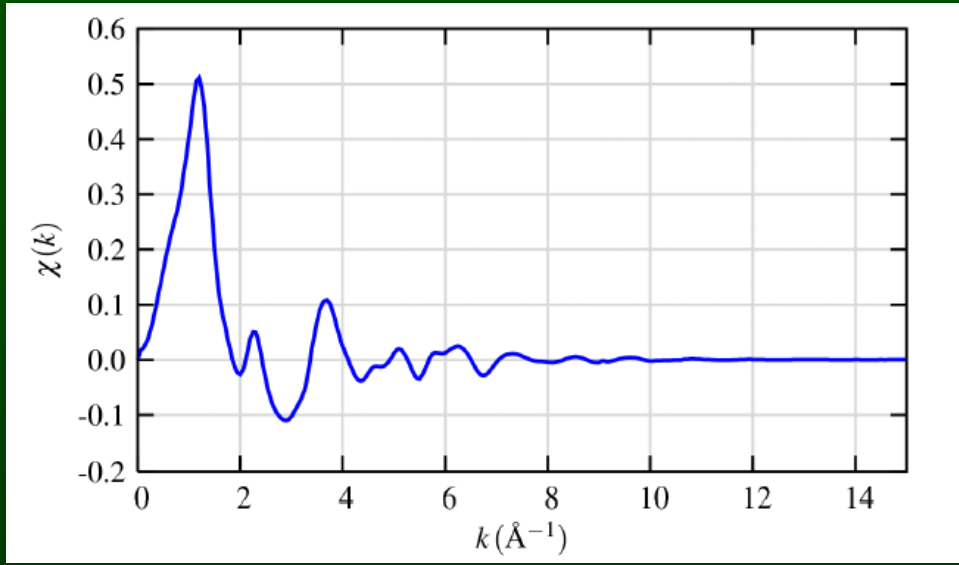
The EXAFS spectrum of the cuvette must not interfere with that of the sample!

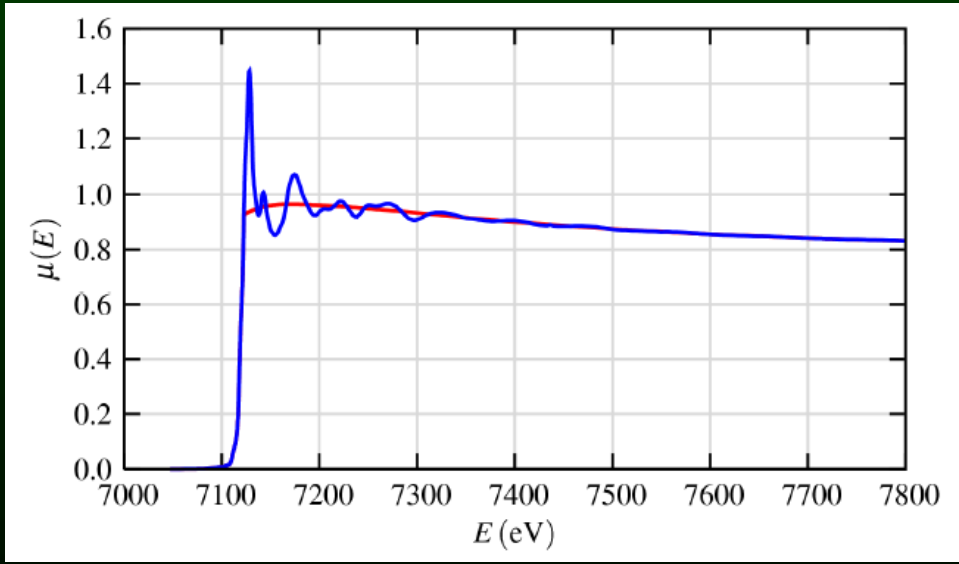
Transfer to cooled (20 K) sample holder of beamline, analyse

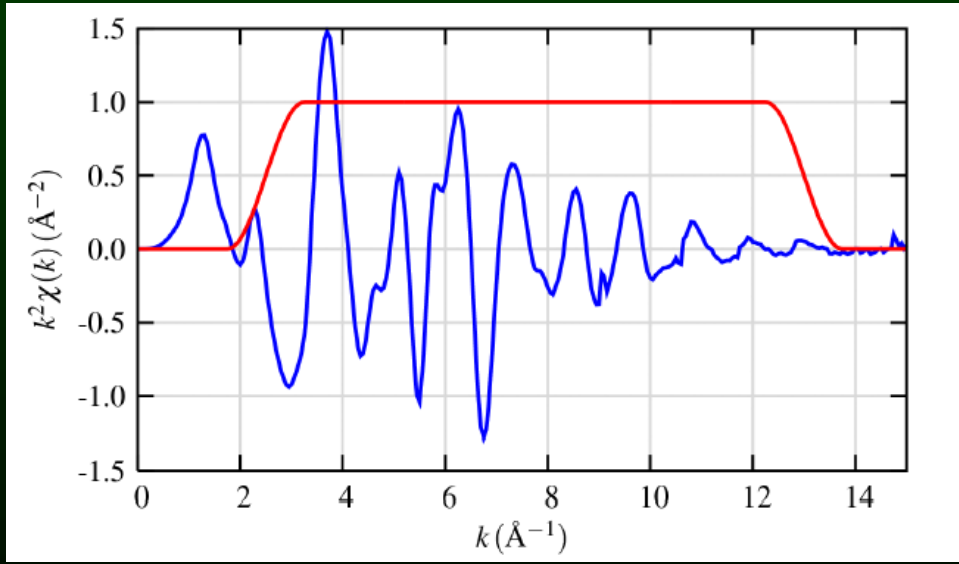
Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH (2004) Plant Physiology 134 (2), 748-757

Analysis of EXAFS data (I)

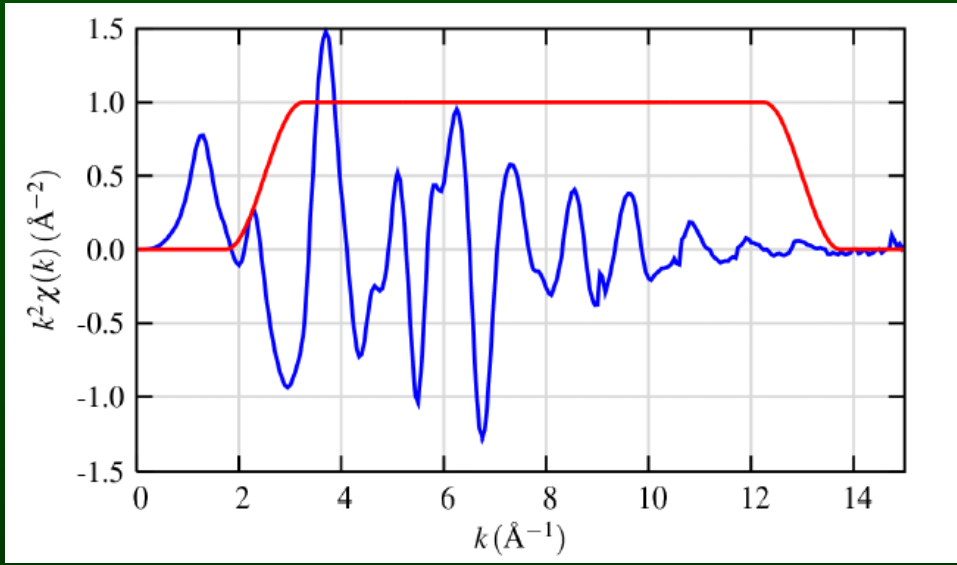


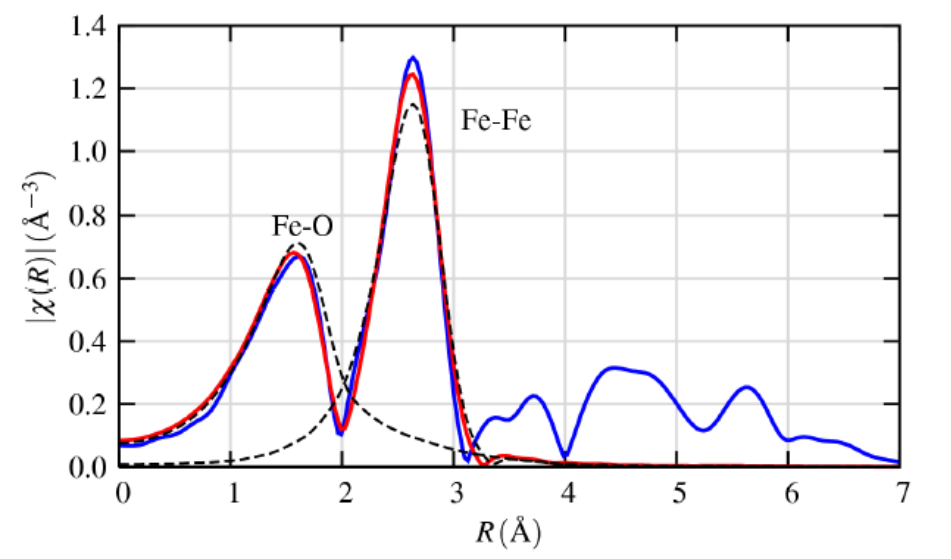


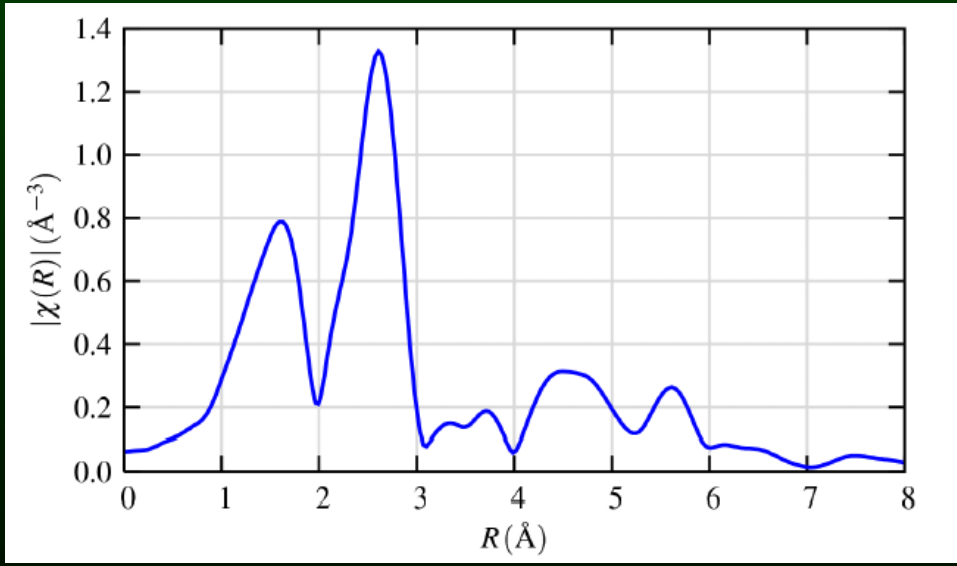


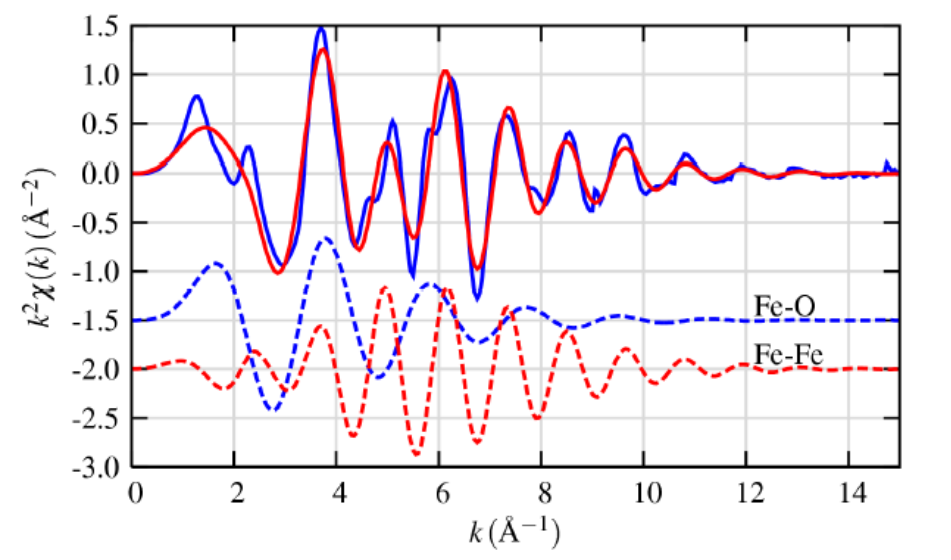


Analysis of EXAFS data (II)



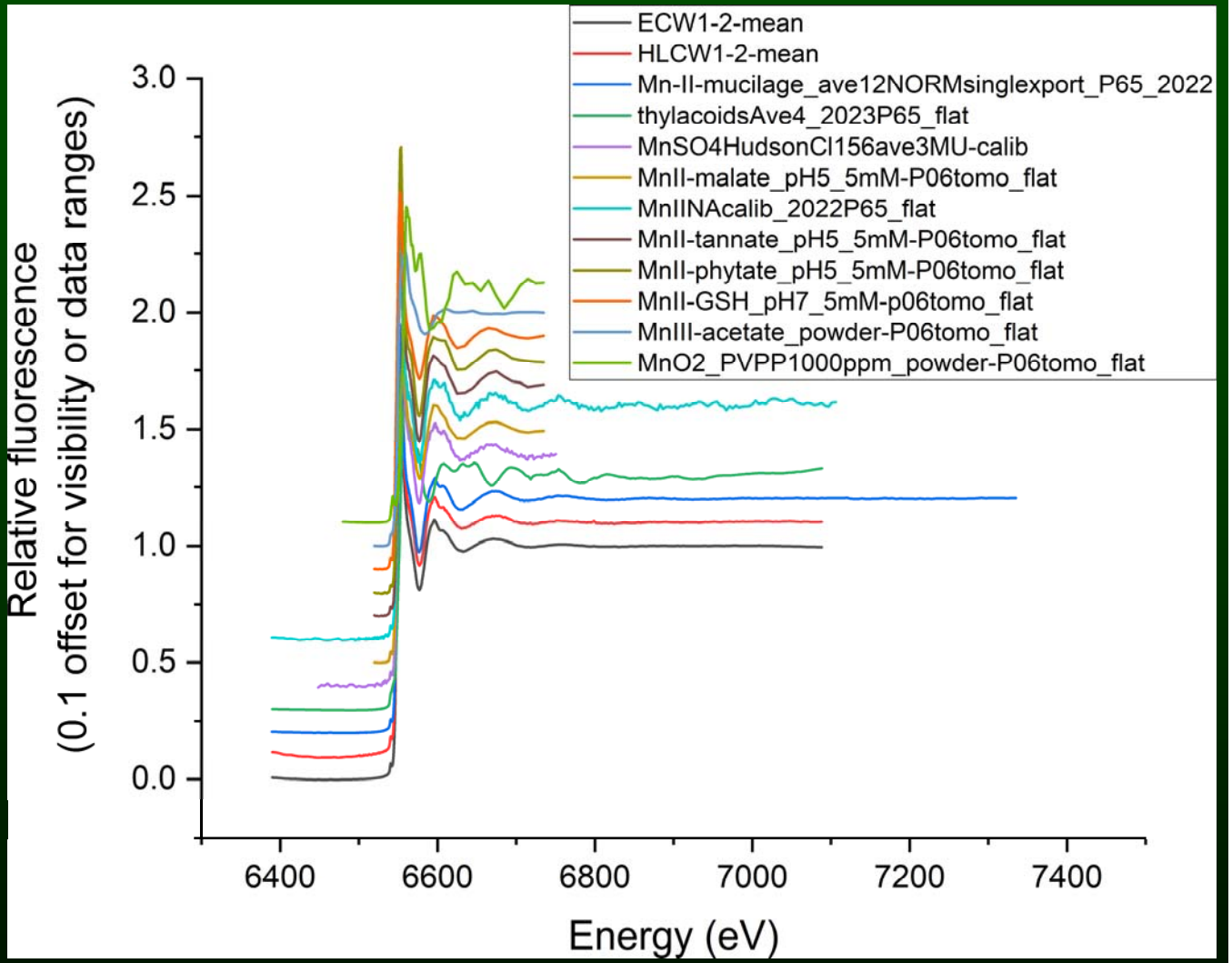






Analysis of XANES data: problems with data reduction

→ **Data for a project originate from several measuring campaigns and different beamlines and were in part recorded for different purposes (e.g. XANES vs. EXAFS), so that the data range is different (data shown here after individual data reduction for each spectrum)**

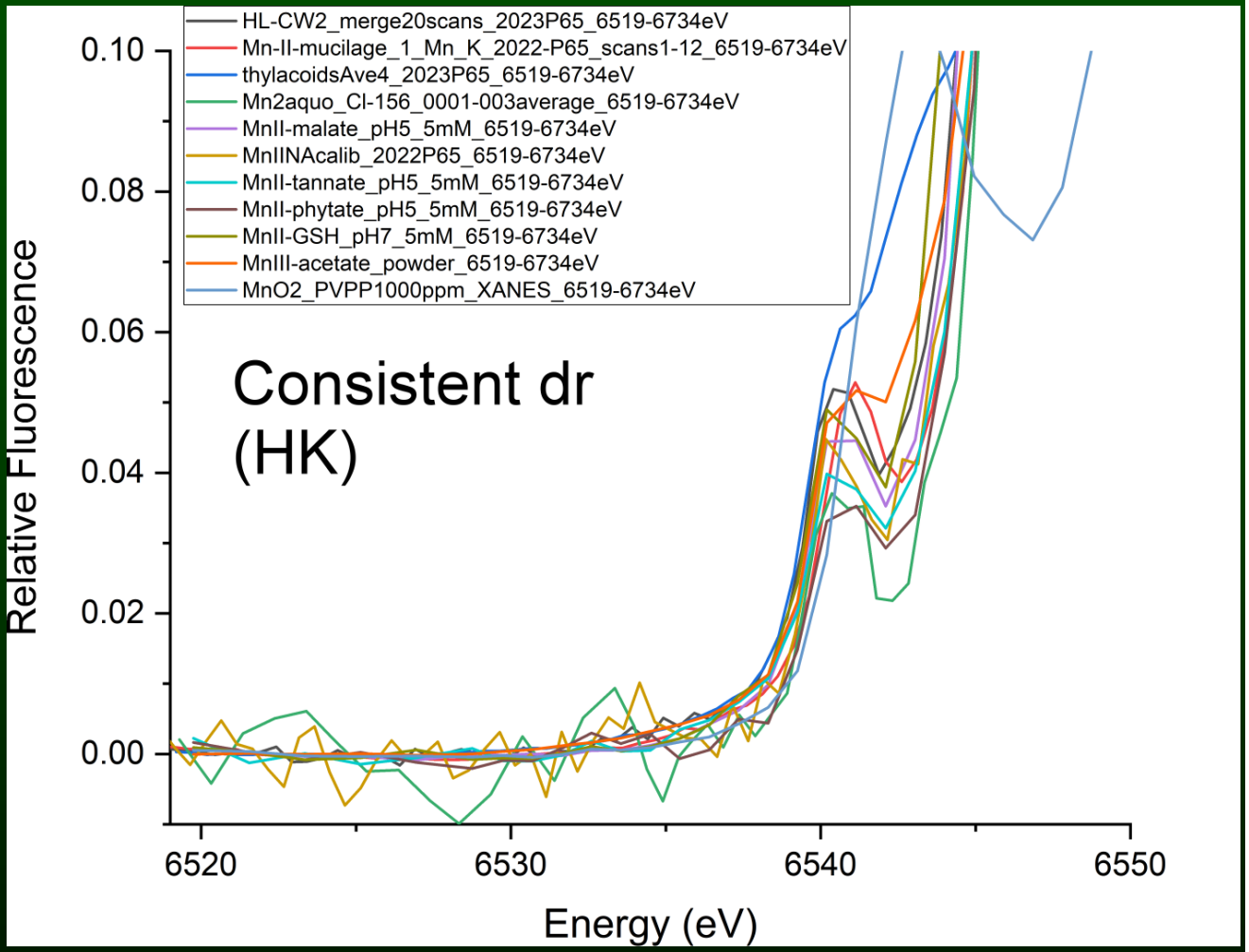


From: Morina F, Kuvelja A, Brückner D, Mojović M, Nakarada Đ, Bokhari SNH, Vujić B, Falkenberg G, Küpper H (2025) How eriophyid mites shape metal metabolism in leaf galls on Tilia cordata. New Phytologist 246, 2222-42 https://doi.org/10.1111/nph.70103

Analysis of XANES data: problems with data reduction

Consistent data reduction

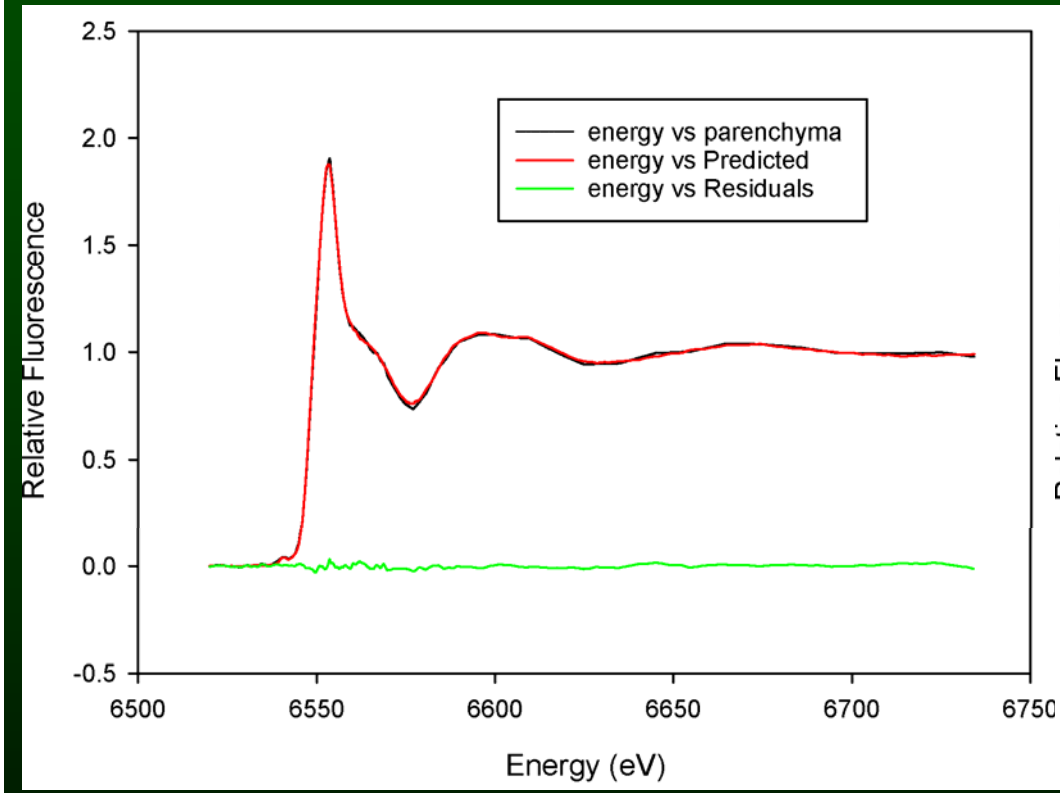
1) **The data range is different**
   → **The data were truncated to the consensus range that is available for all datasets to prevent bias in fitting the background**
2) **Data are noisy**
   → **Robust but spectroscopically still correct standard energy ranges for pre-edge linear fit, post-edge polynomial and post-edge spline were identically applied to all spectra. NO smoothing!**



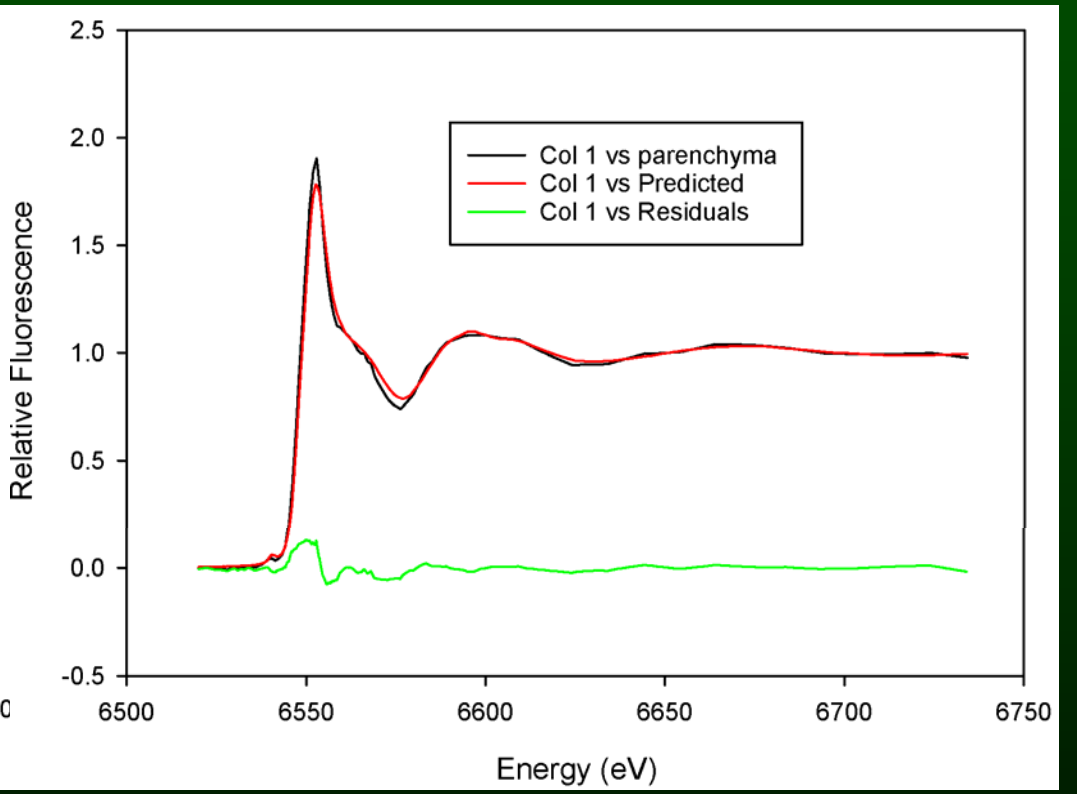
From: Morina F, Kuvelja A, Brückner D, Mojović M, Nakarada Đ, Bokhari SNH, Vujić B, Falkenberg G, Küpper H (2025) How eriophyid mites shape metal metabolism in leaf galls on Tilia cordata. New Phytologist 246, 2222-2242

Fit quality: Consistent vs. single-spectrum data reduction for XANES data

Consistent data reduction

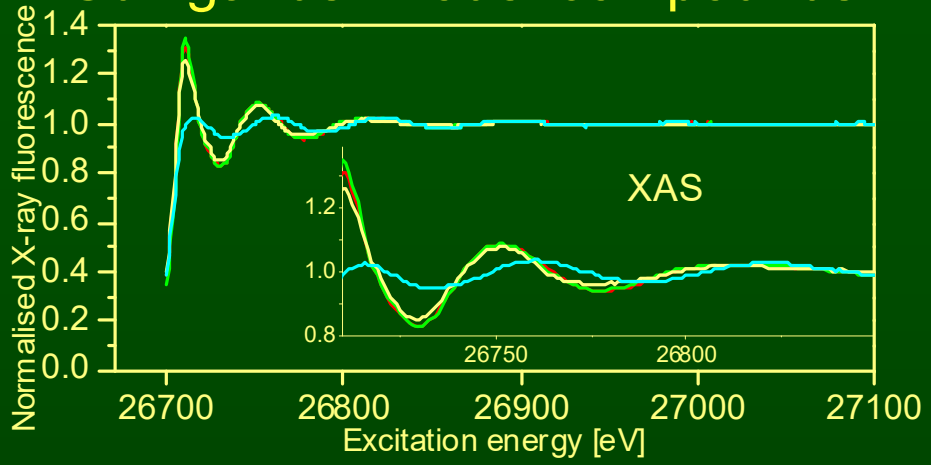


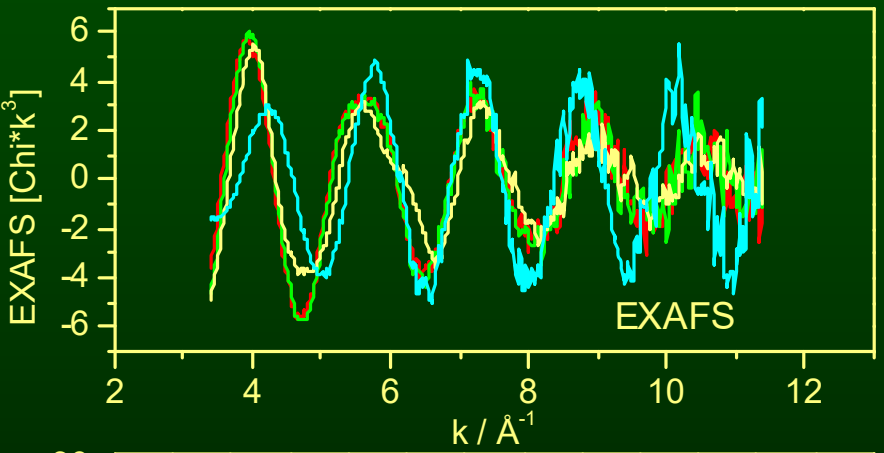
Single-spectrum data reduction

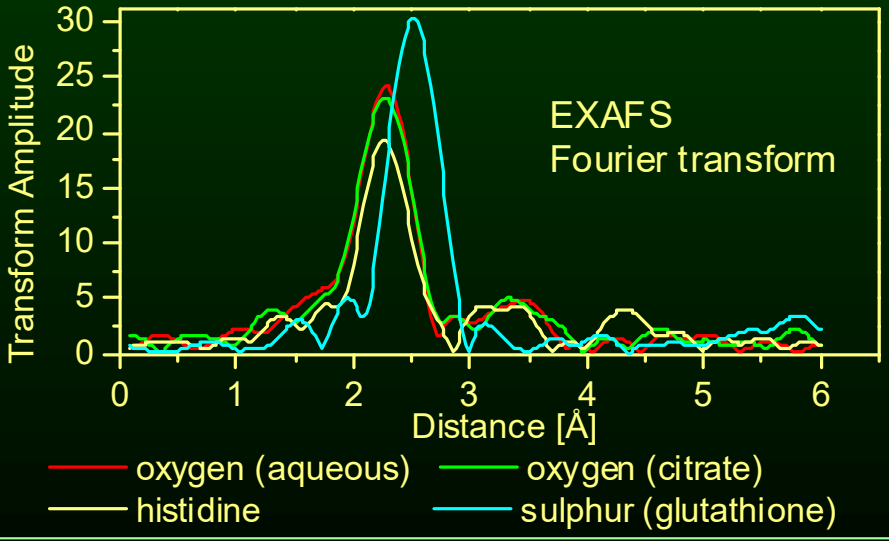


From: Morina F, Kuvelja A, Brückner D, Mojović M, Nakarada Đ, Bokhari SNH, Vujić B, Falkenberg G, Küpper H (2025) How eriophyid mites shape metal metabolism in leaf galls on Tilia cordata. New Phytologist 246, 2222-2242

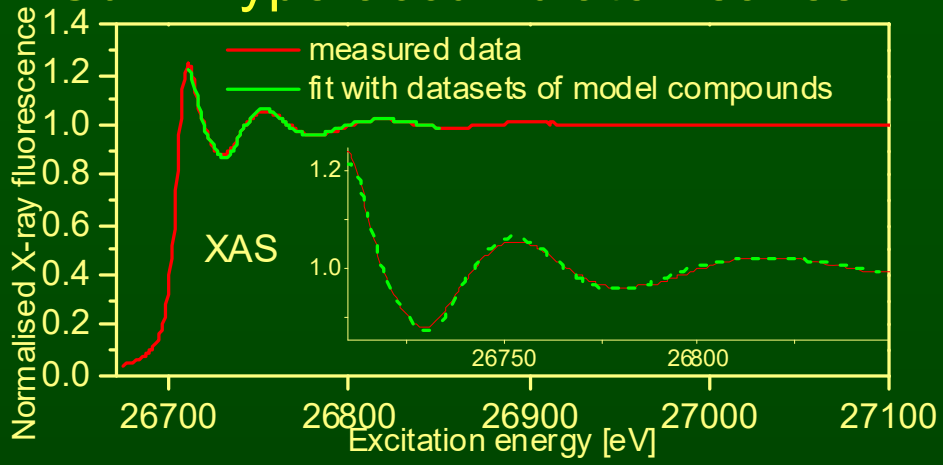
Cd-ligands: model compounds

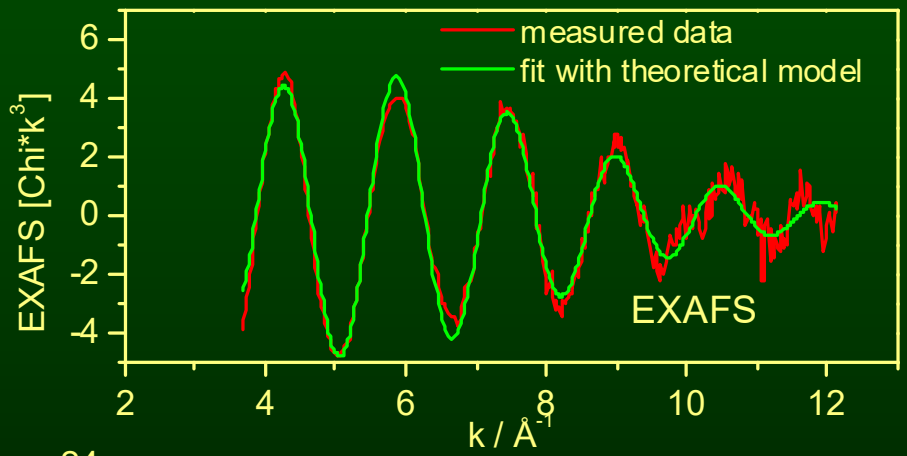


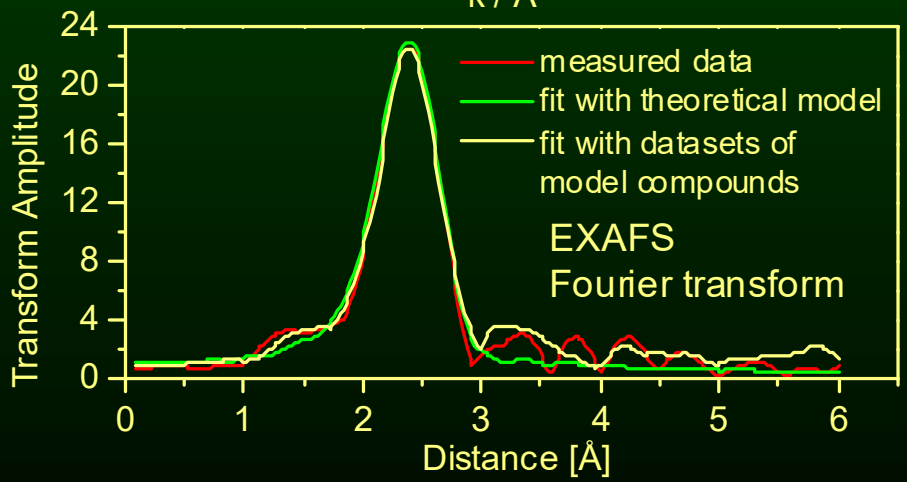




Cd in hyperaccumulator leaves

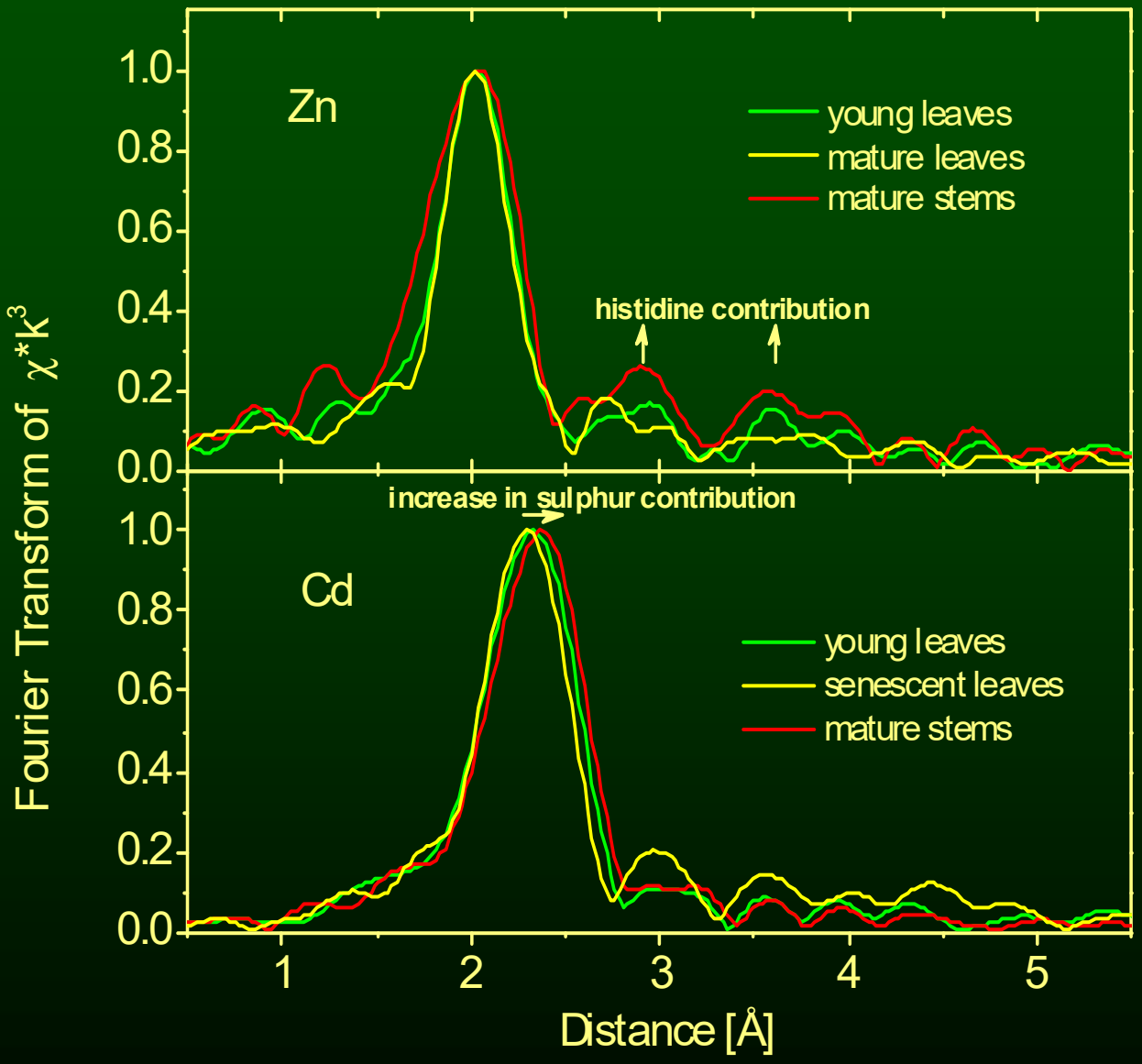






Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH (2004) Plant Physiology 134 (2), 748-757

Application example: Speciation of cadmium and zinc hyperaccumulated by *Thlaspi caerulescens* (Ganges ecotype)



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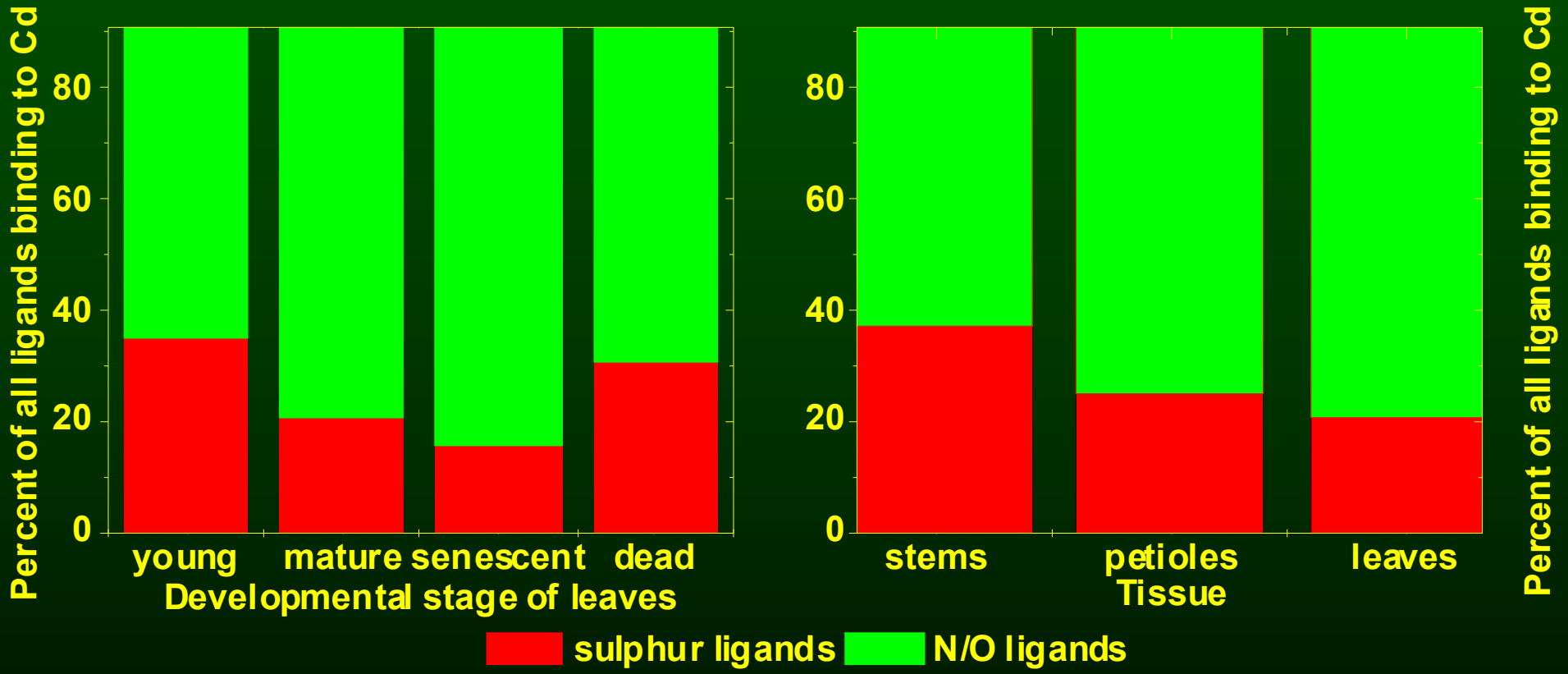
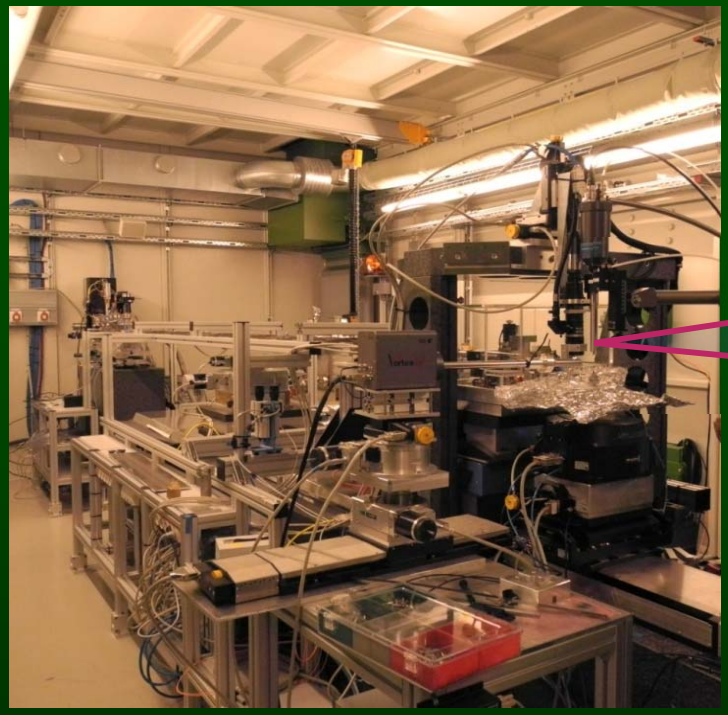
Application example: Speciation of cadmium and zinc hyperaccumulated by *Thlaspi caerulescens* (Ganges ecotype)

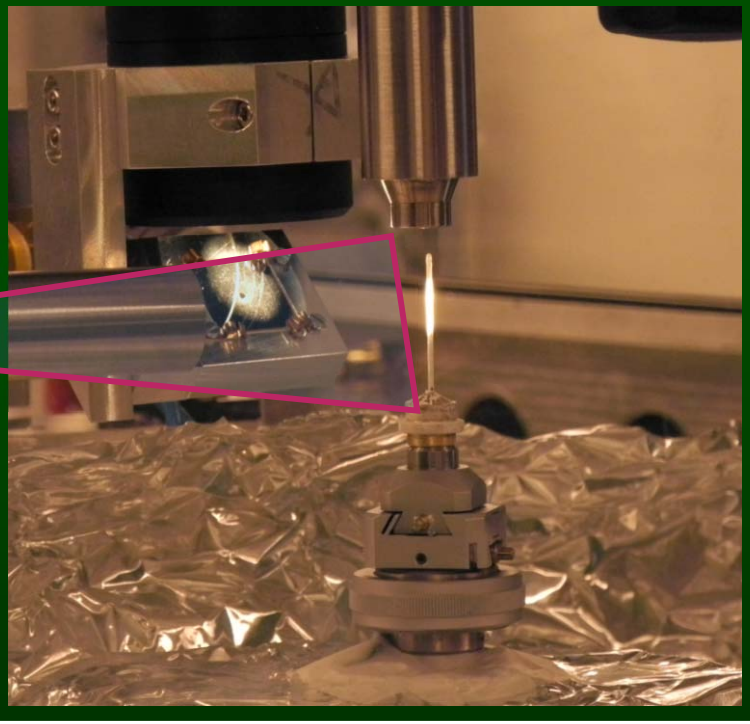


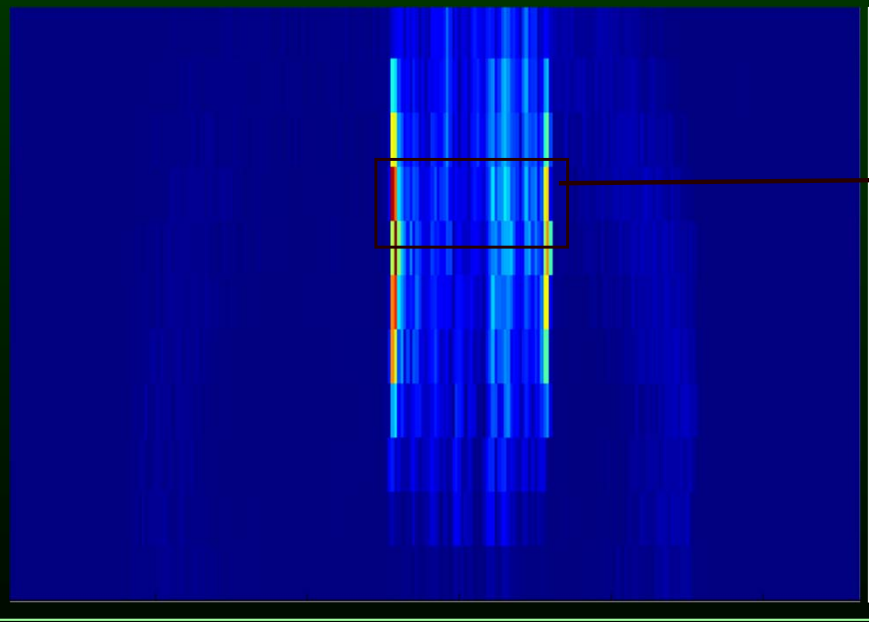
Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH (2004) Plant Physiology 134, 748-757

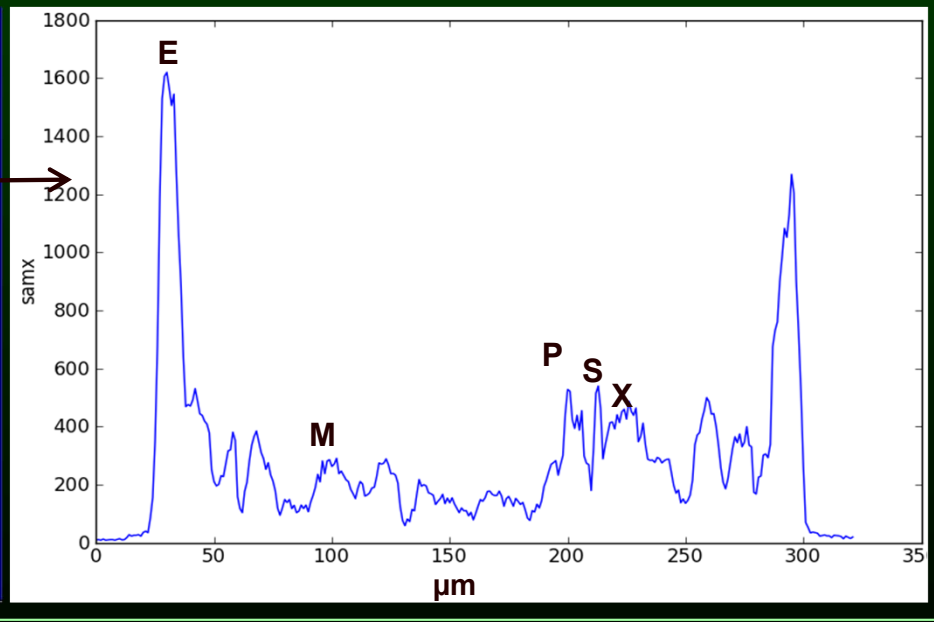
Microscopic X-ray absorption spectroscopy

Confocal µ-XANES: Sample mounting and measurement



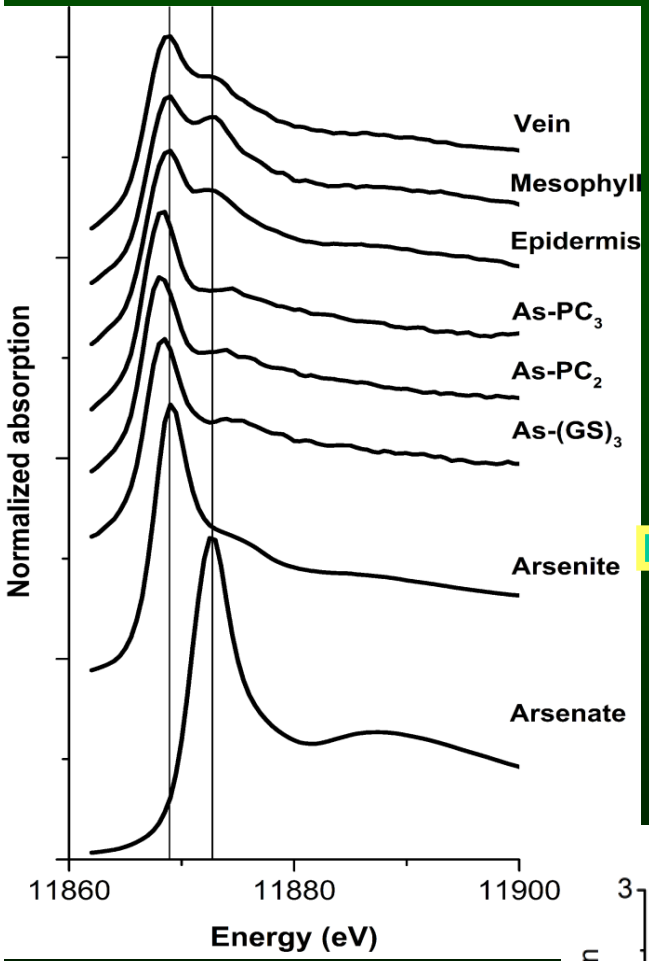


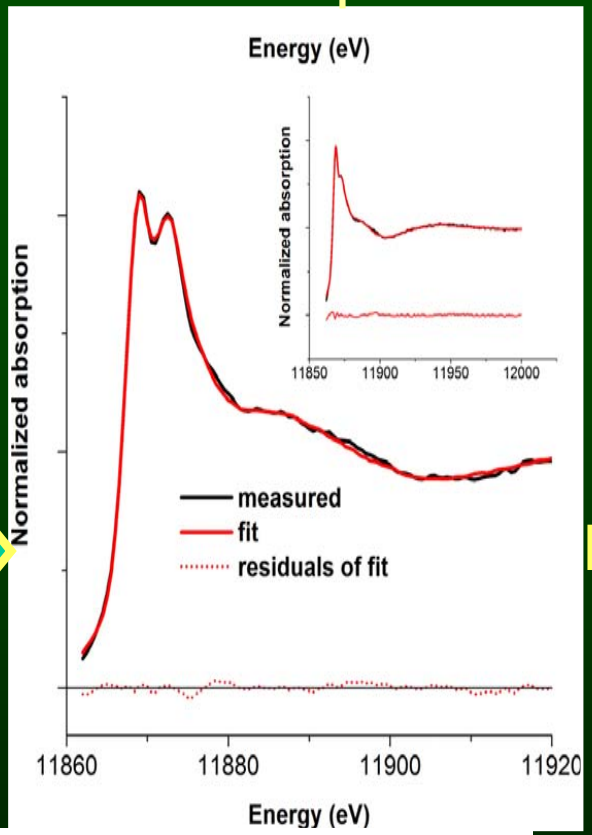


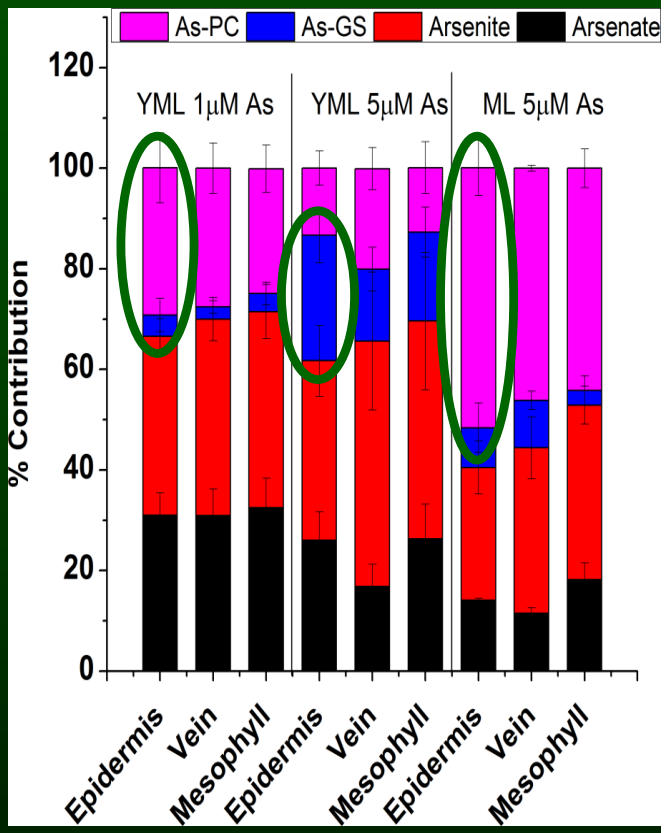


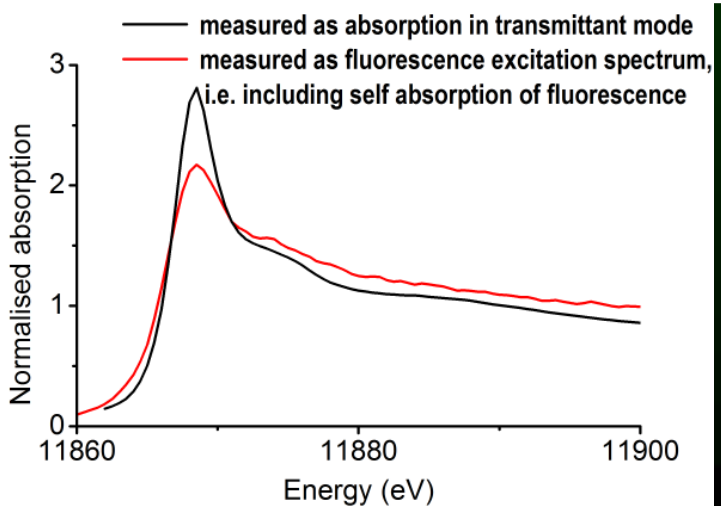
Mishra S, Wellenreuther G, Mattusch J, Stärk H-J, Küpper H (2013) Plant Physiology 163, 1396-1408

Tissue specific As speciation through confocal µ-XANES: linear combination fitting with correction for absorption and baseline drift







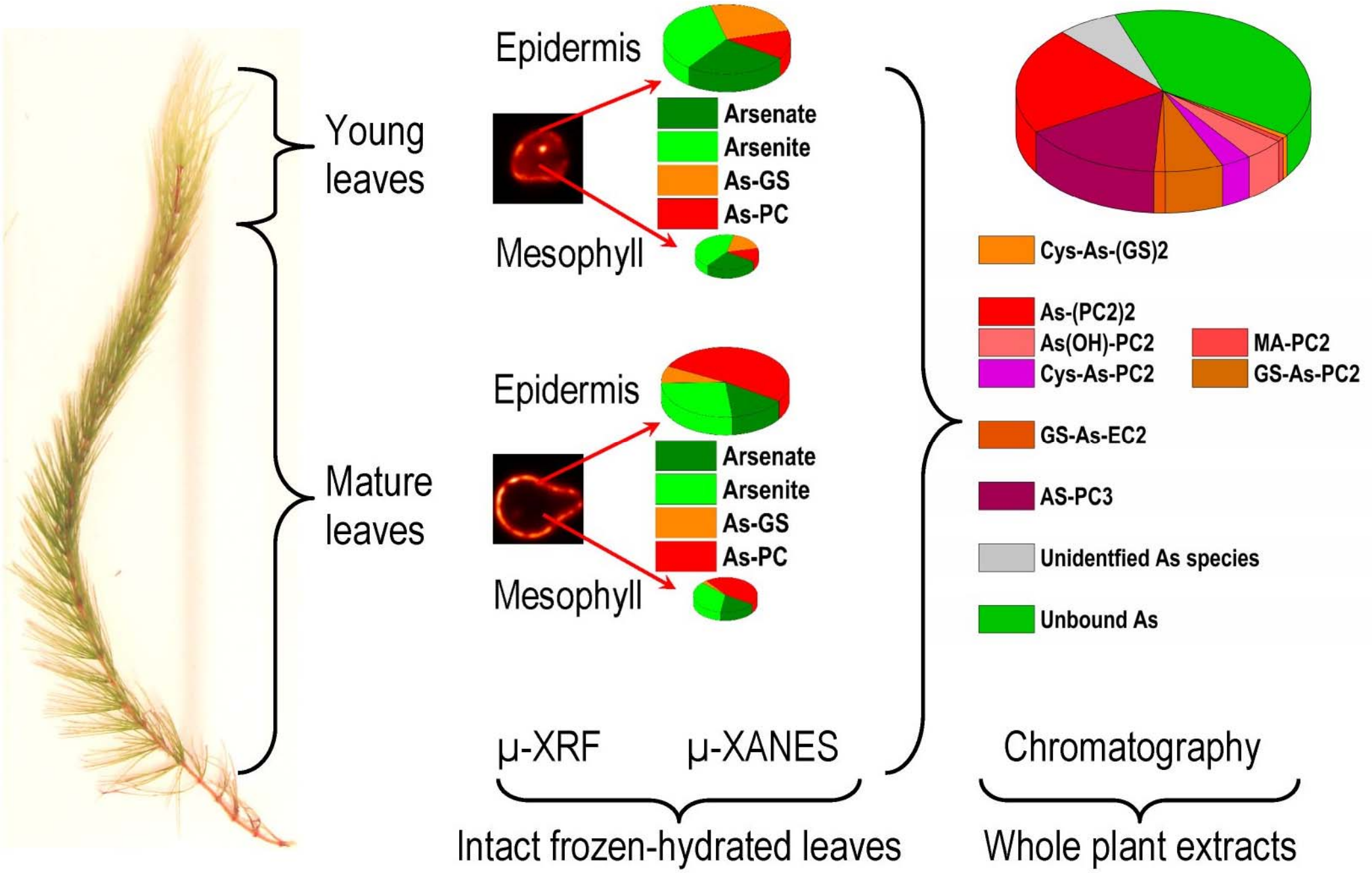


Mishra S, Wellenreuther G, Mattusch J, Stärk H-J, Küpper H (2013) Plant Physiology 163, 1396-1408

→highest As-PC in the epidermis of young-mature leaves at 1µM As and mature leaves at 5µM

→ highest As-GS in epidermis of young-mature leaves at 5µM As

Arsenic: comparison of μXRF&μXANES with chromatography

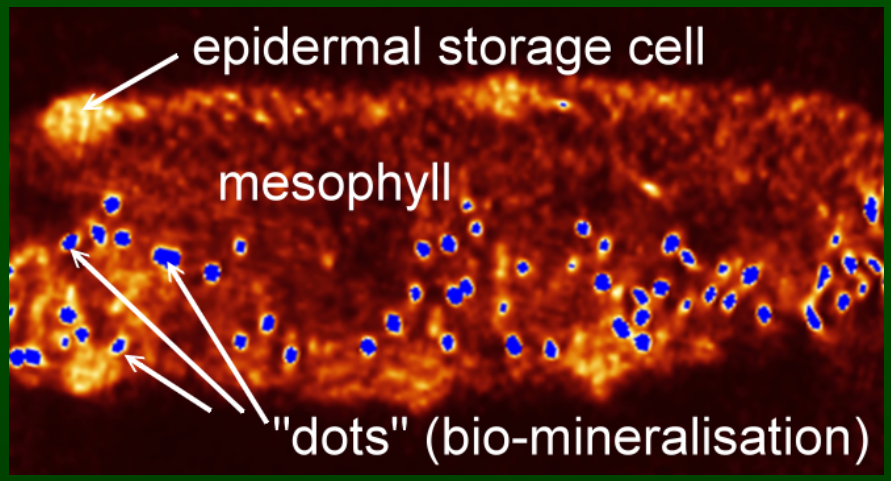


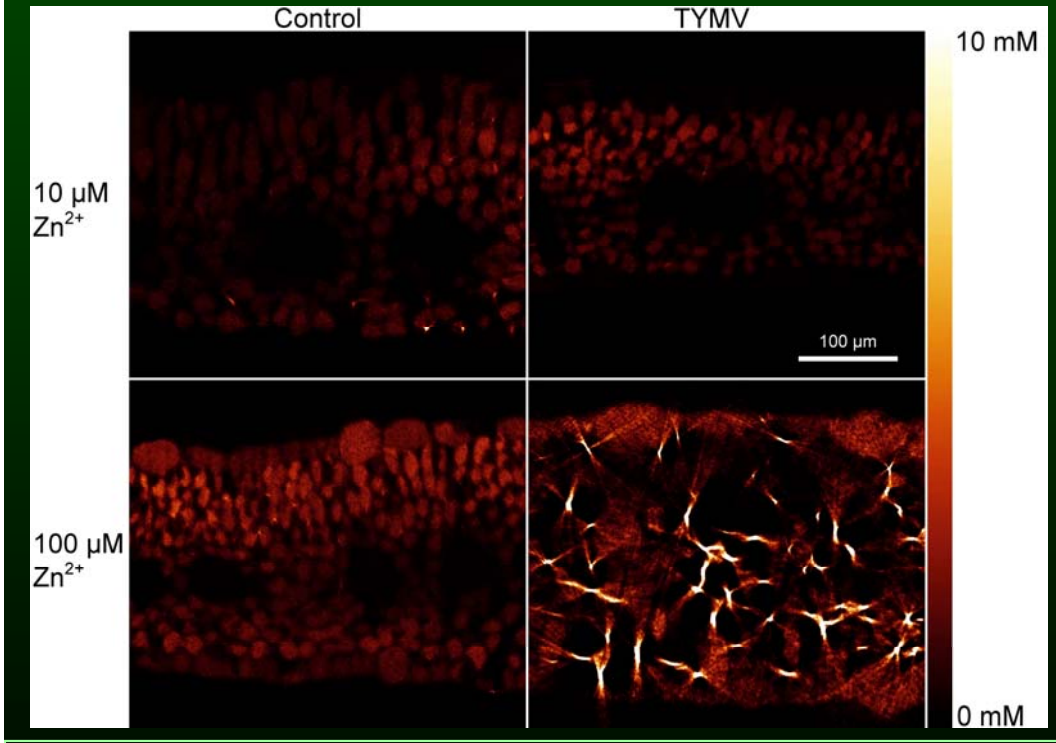
Mishra S, Wellenreuther G, Mattusch J, Stärk H-J, Küpper H (2013) Plant Physiology 163, 1396-1408

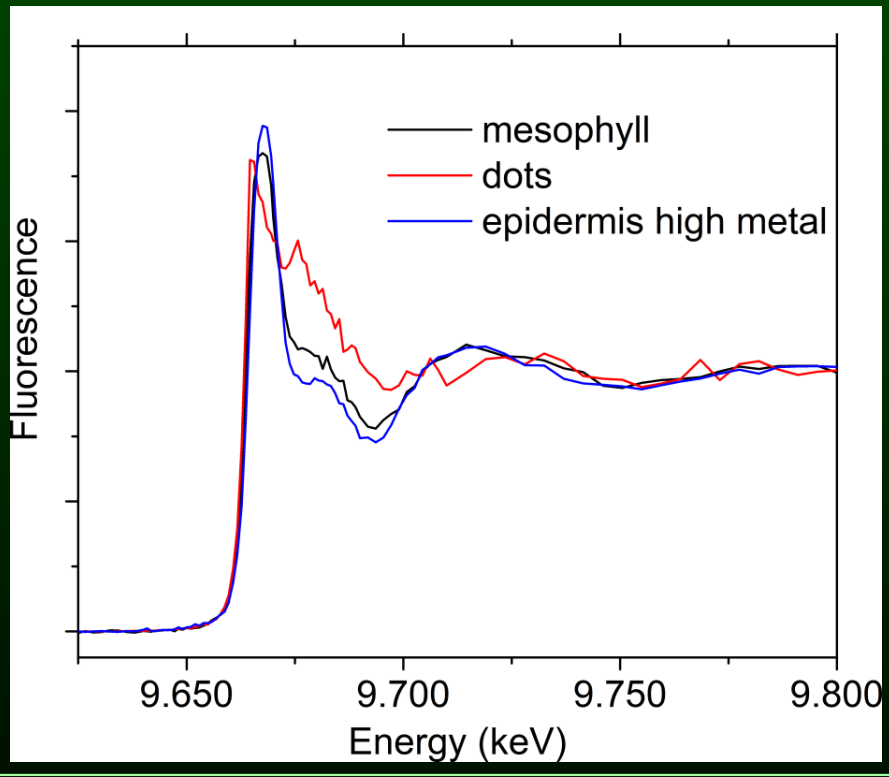
Tissue specific Zn speciation through µ-XANES **tomography**

Biomineralisation in response to virus infection

→ Infection with Turnip Yellow Mosaic Virus leads to enhanced biomineralization as revealed by µXRF and µ-XANES tomography



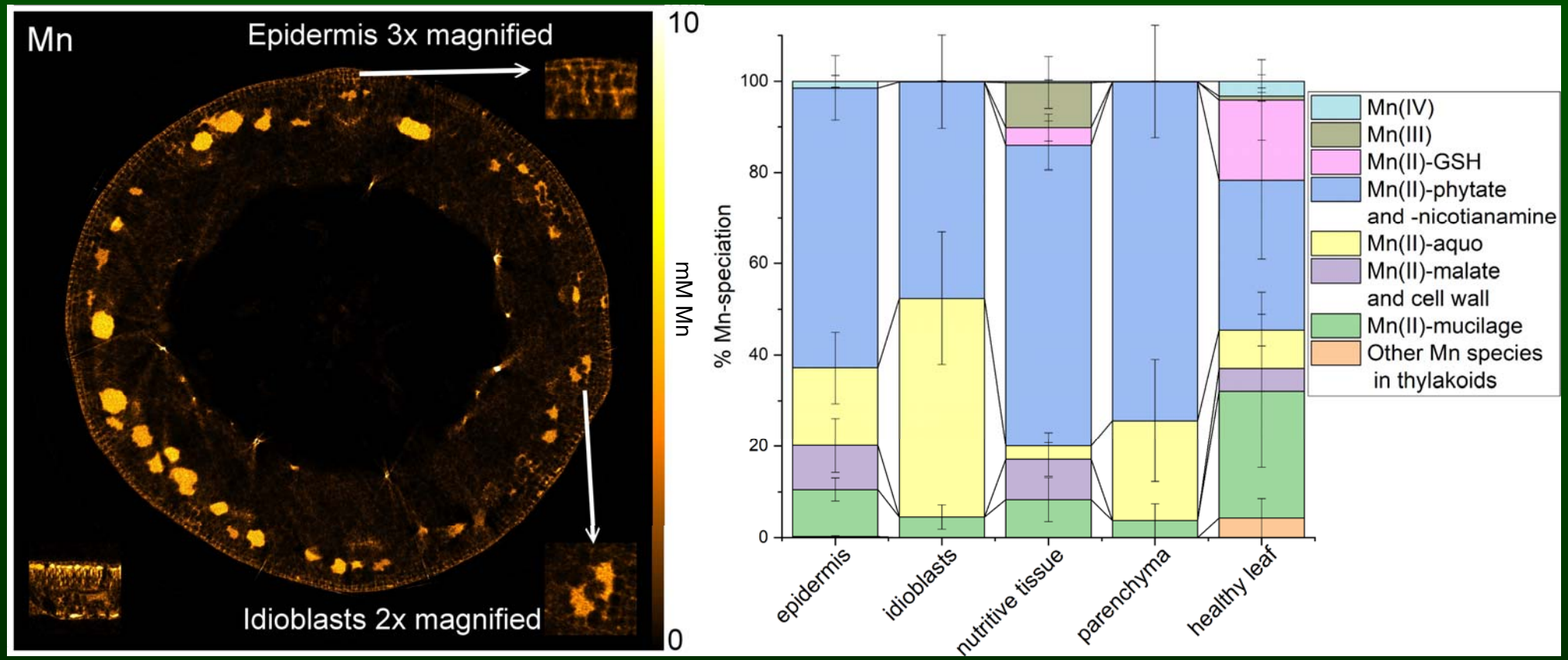




Morina F, Mishra A, Mijovilovich A, Matoušková Š, Brückner D, Špak J, Küpper H. (2020) Frontiers in Plant Science. DOI: https://doi.org/10.3389/fpls.2020.00739

Mijovilovich A, Mishra A, Brückner D, Spiers K, Andresen E, Garrevoet J, Falkenberg G, Küpper H (2019) Spectrochimica Acta B 157, 53-62

Tissue specific Mn speciation through µ-XANES **tomography**



Quantitative analysis of XANES and EXAFS showing the percentage contribution of different Mn species in gall tissues and healthy leaf

Higher contribution of Mn(II)aquo in the idioblasts
Higher contribution of Mn(III), Mn(II)-GSH and Mn(II)-phytate/NA in the nutritive tissue
Higher contribution of mucilage, Mn(II)-GSH and thylakoid bound Mn in the healthy leaf

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X-ray spectroscopy on biological samples
General *technical* conclusions

a) Sample preparation

→ For analysing metals in biological samples, organisms need to be treated with metal concentrations they encounter in real life if meaningful results should be obtained

→ Samples should be prepared by shock-freezing and kept frozen-hydrated to minimise the risk of artefacts of element re-distribution and changes in speciation

b) Measurement

→ Compared to mapping of thin sections, tomography of larger samples minimises sample preparation (sectioning) and therefore the risk of artefacts

→ Detectors with large solid angle increase the ratio of signal / beam damage

c) Data analysis

→ Standards having the same shape and average composition as the samples allow for good correction of absorption effects

All slides of my lectures can be downloaded from my workgroup homepage

Biology Centre CAS → Institute of Plant Molecular Biology → Departments → Department of Plant Biophysics and Biochemistry,

or directly

http://webserver.umbr.cas.cz/~kupper/AG_Kuepper_Homepage.html