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IAEA International Atomic Energy Agency

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X-ray Microscopy and X-ray Fluorescence: applications in cultural heritage and environmental sciences

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X-ray Microscopy and X-ray Fluorescence: applications in cultural heritage and environmental sciences

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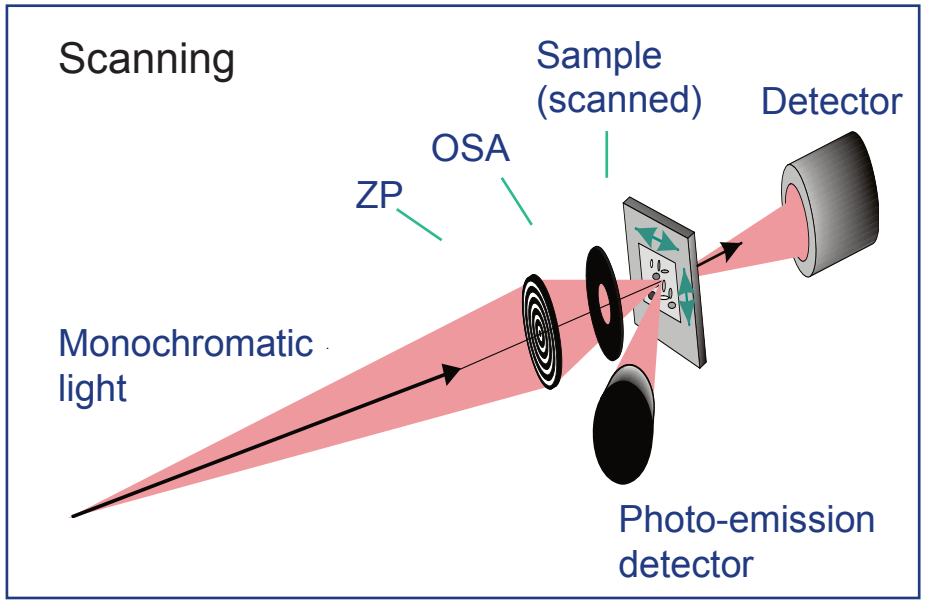
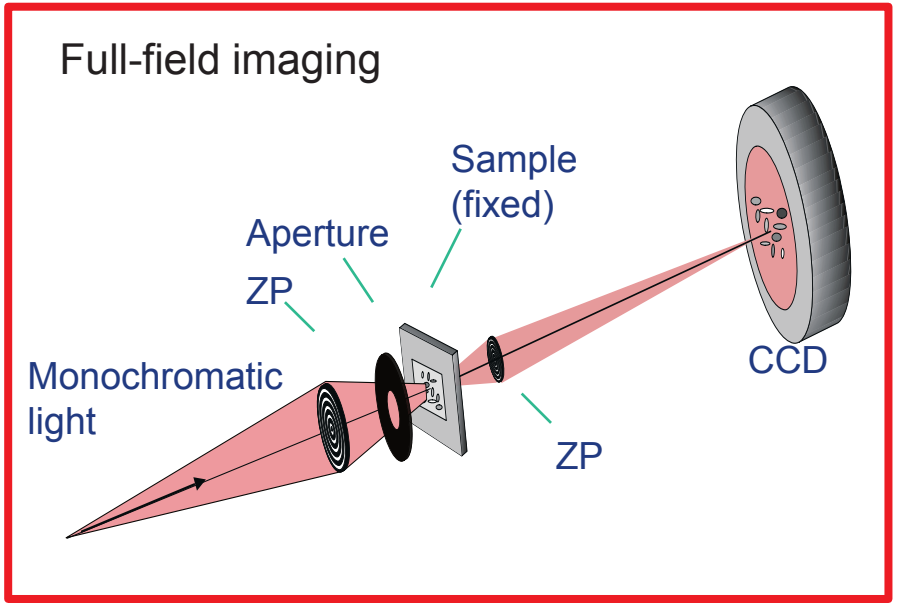
Background info: X-ray microscopy types





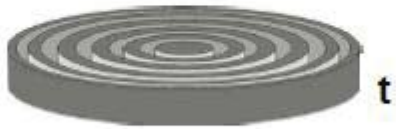
+ versatile detectors can run simultaneously;
+ easier optics set-up;
- long exposure time;
- complex electronics.

Ideal for spectromicroscopy

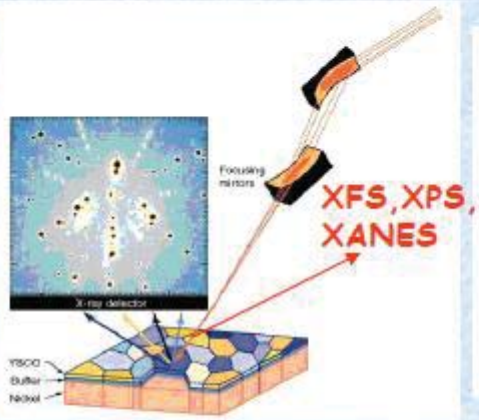
+ short exposure time;
+ higher resolution
- static system;
- complex optical alignment.

Ideal for dynamic studies and tomography

Focusing optics: zone plates, mirrors, capillaries



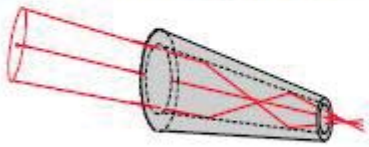
Zone Plate optics: from ~ 200 to ~ 8000 eV
Resolution: 30 nm in transmission



KP-B mirrors each focusing in one direction: soft & hard: ~ 1000 nm
Soft & hard x-rays!
chromatic focal point, easy energy tunability, comfortable working distance
Resolution ~ 1000 nm

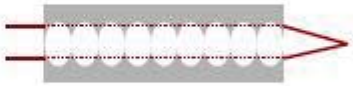
Normal incidence: spherical mirrors with multilayer interference coating (Schwarzschild Objective)
not tunable, E < 100 eV
Resolution: best ~ 100 nm

Capillary: multiple reflection concentrator



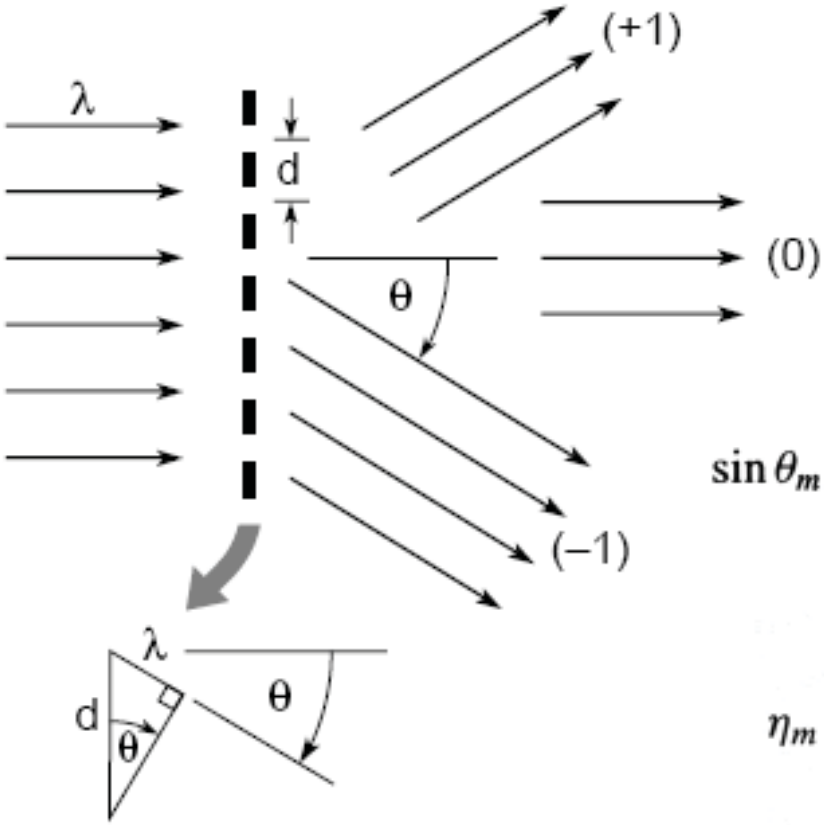
Hard x-rays ~ 8-18 keV
Resolution: > 3000 nm

Refractive lenses



Hard x-rays ~ 4-70 keV
Resolution: > 1000 nm

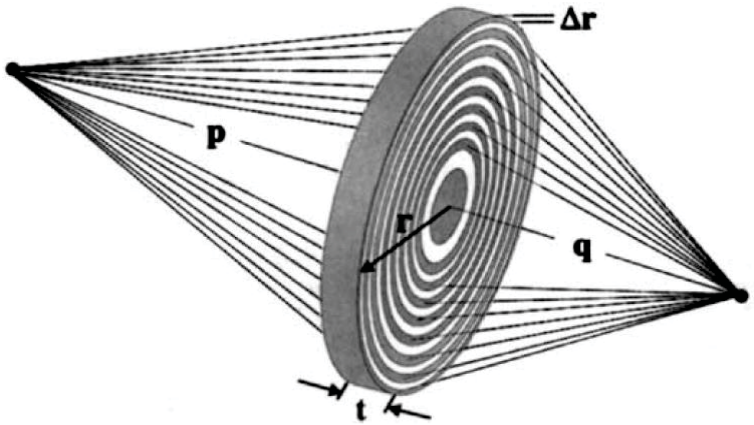
Background info: Diffraction by a grating



$$\sin\theta_m = \frac{m\lambda}{d}\,; \quad m = 0,\ \pm1,\ \pm2,\ \pm3, \ldots \qquad (9.2)$$

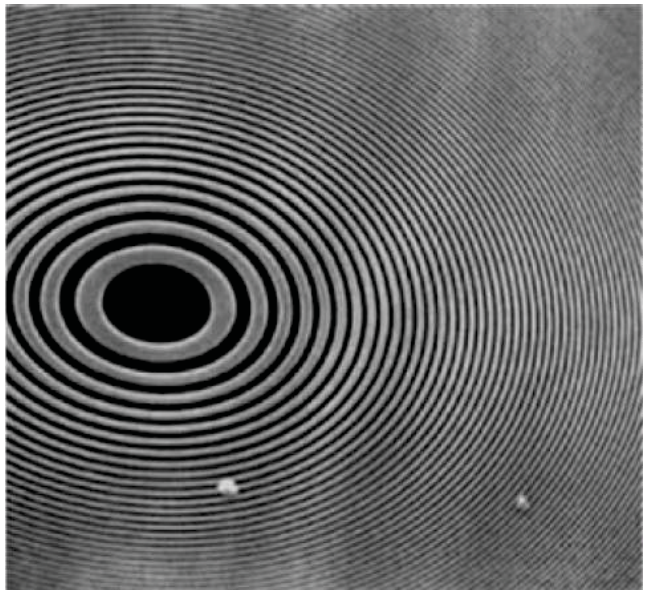
$$\eta_m = \begin{cases} \frac{1}{4} & m = 0 \\ 1/m^2\pi^2 & m \text{ odd} \\ 0 & m \text{ even} \end{cases} \qquad (9.24)$$

(50% absorbed)



Zone plate (ZP) is a circular diffraction grating with radially increasing line density

$$\frac{1}{f} = \frac{1}{p} + \frac{1}{q}$$ if n > 100; $$f = \frac{2r\Delta r}{\lambda}$$



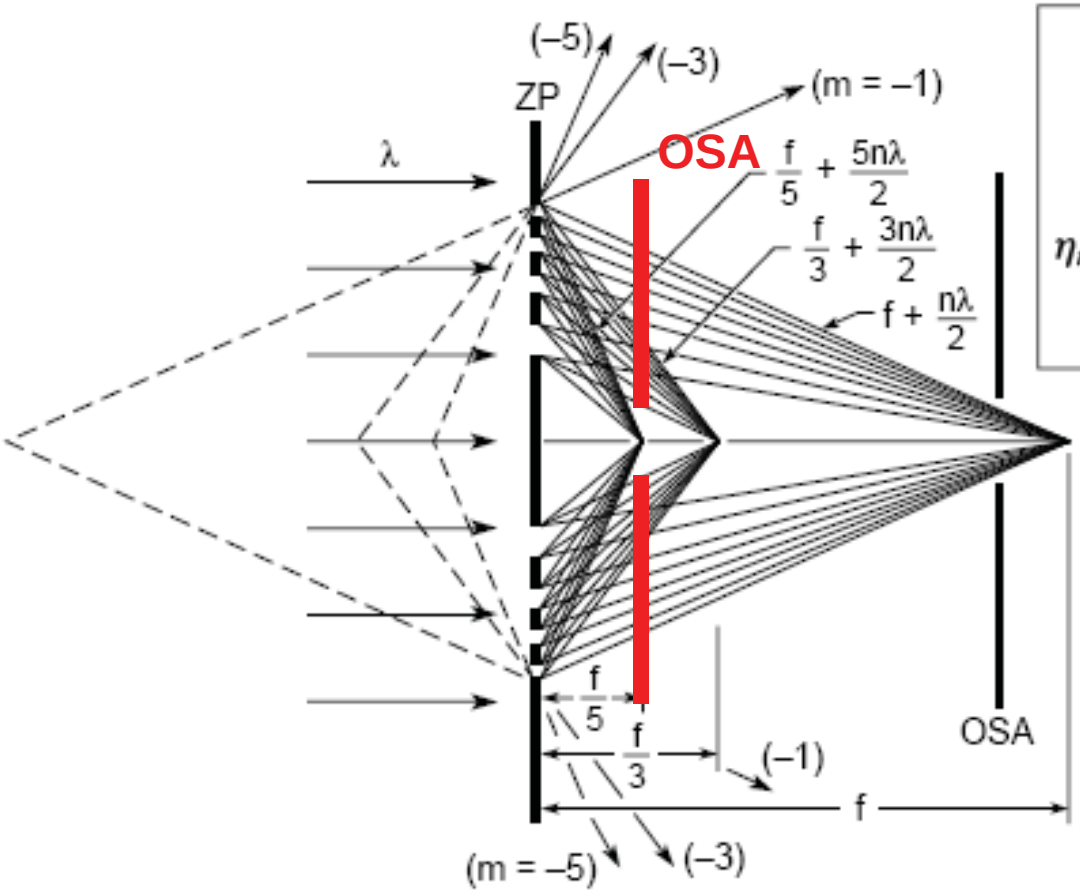
Lateral resolution of a ZP (Rayleigh):

$$NA \equiv \frac{r}{f} = \frac{\lambda}{2\Delta r}$$

$$\partial_{Rayleigh} = \frac{0.61\lambda}{NA} = 1.22\Delta r$$

(not smaller than the diffraction limit of the wavelength)

Background info: Diffraction by a grating



$$r_n^2 \simeq mn\lambda f_m \qquad (9.19)$$

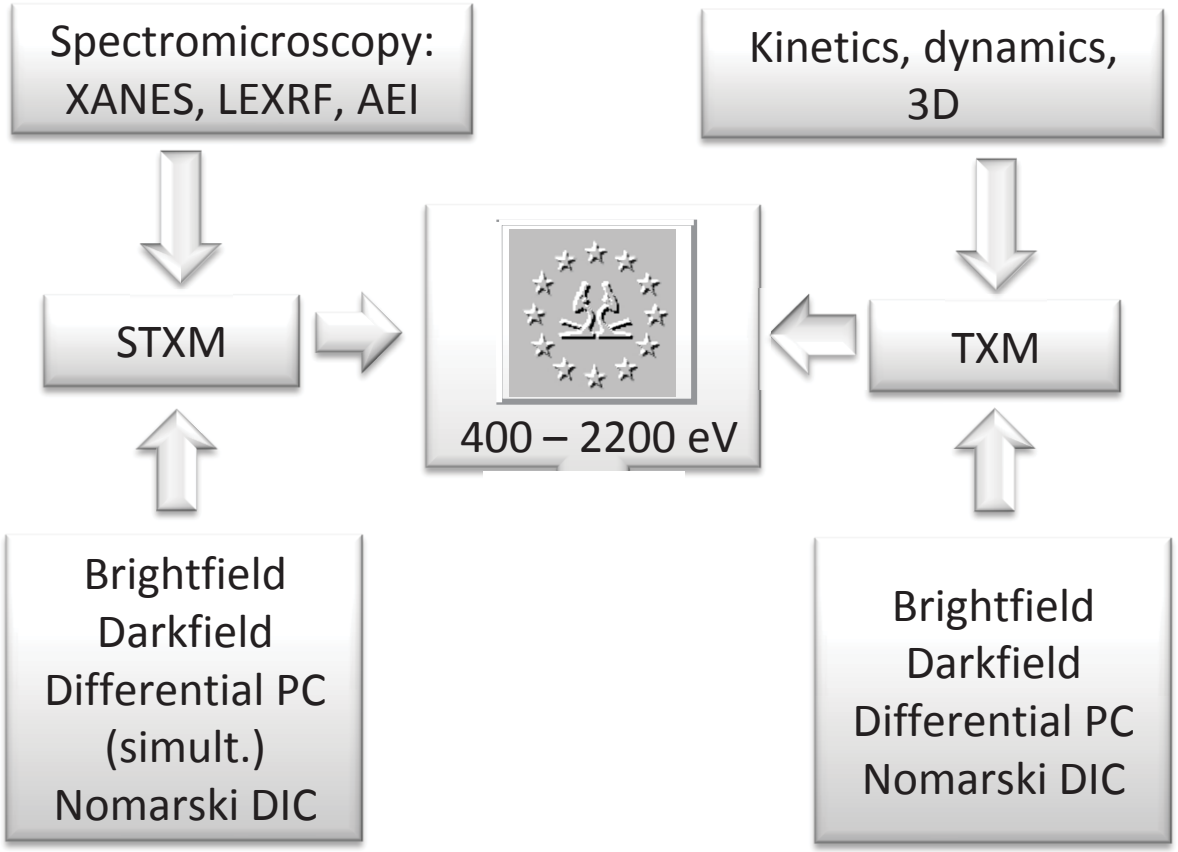
$$\eta_m = \begin{cases} \frac{1}{4} & m = 0 \\ 1/m^2\pi^2 & m \text{ odd} \\ 0 & m \text{ even} \end{cases} \qquad (9.24)$$

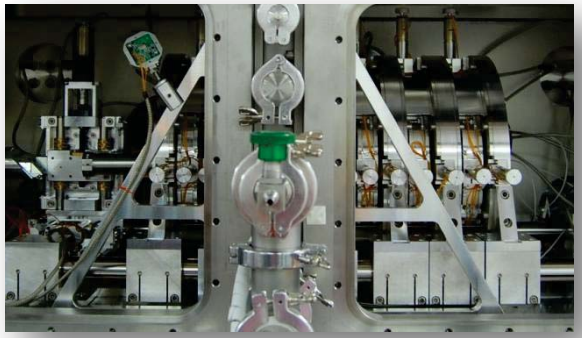
$$f_m = \frac{1}{m}\,\frac{r_N^2}{N\lambda}$$

$$f_m = \frac{1}{m}\,f_1$$

The TwinMic microscope at ELETTRA

Integrating both modes into a *single* instrument?

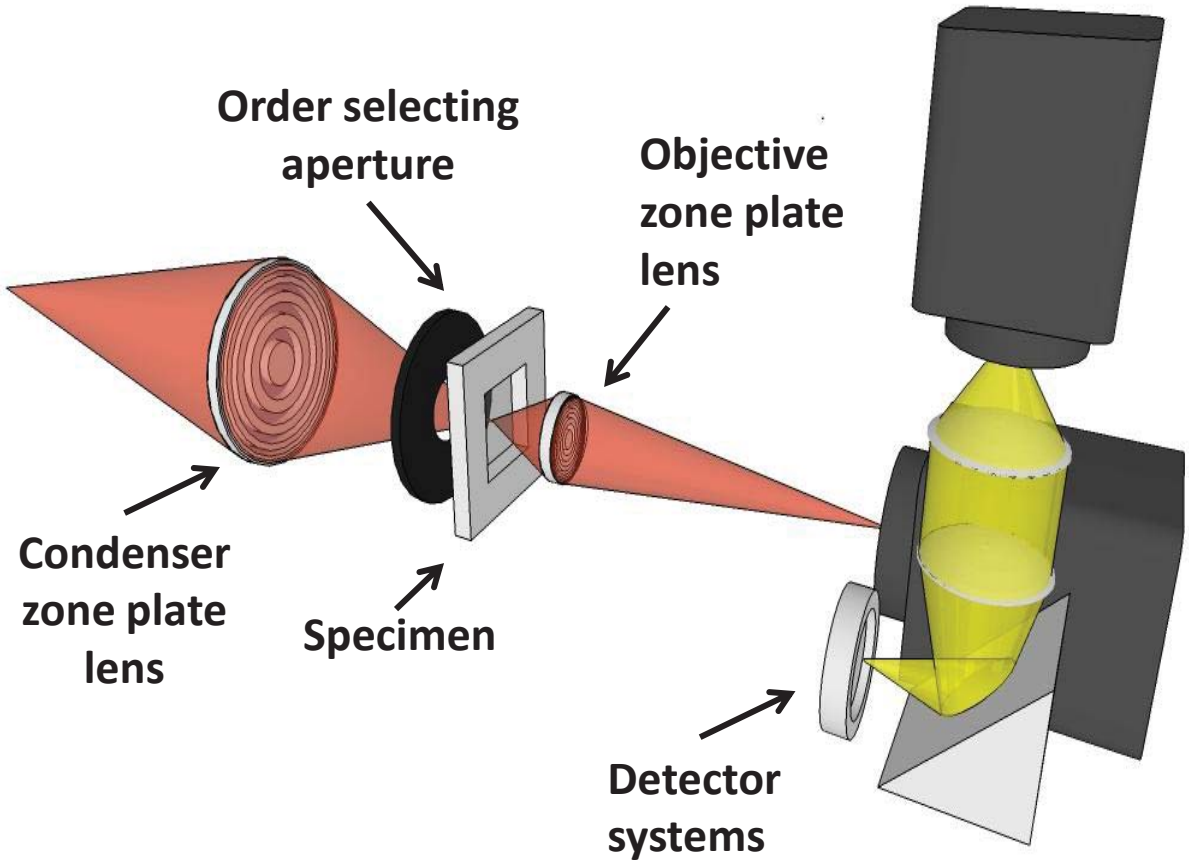




- Biotechnology
- Nanotechnology
- Environment
- Geochemistry
- Food Science
- Medicine
- Pharmacology
- Cultural Heritage
- New Materials

Imaging modes in X-ray microscopy

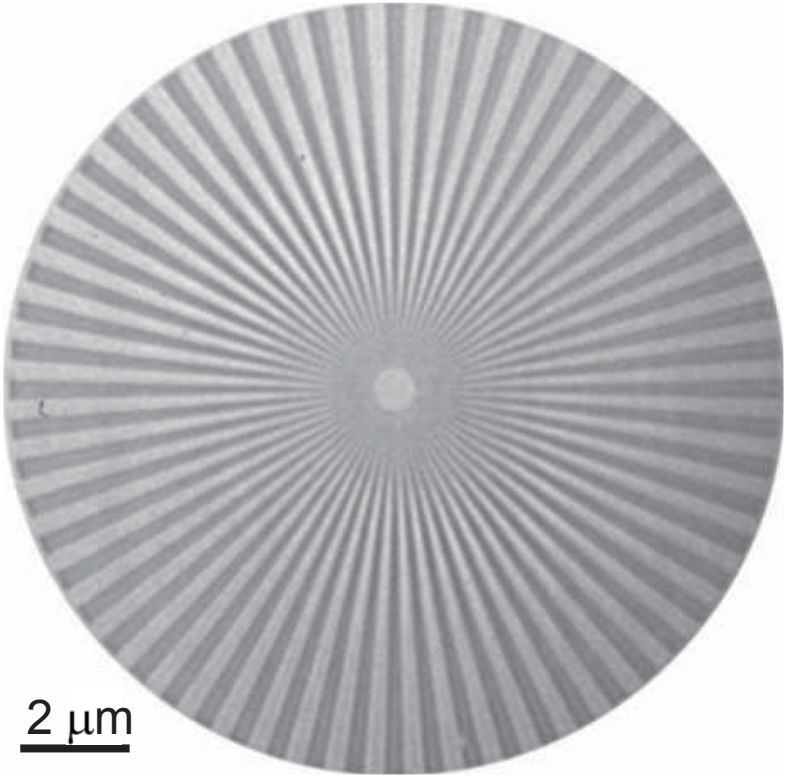
The full-field imaging mode



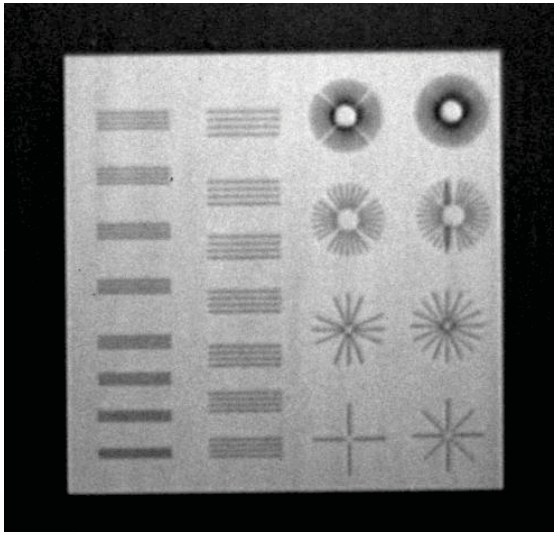
Gunther Schmahl

- Similar to conventional visible light microscope
- Analysis of morphology in transmission
- Fast imaging, dynamics, microtomography

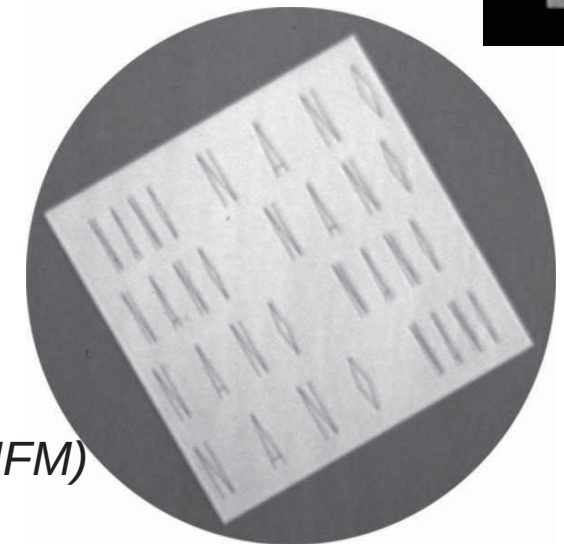
Resolution tests of a ZP



ZP parameters:
110 μm diameter
50 nm outer zones
f=3.2 mm @ 720 eV
fabricated by TASC/ INFM



2 μm

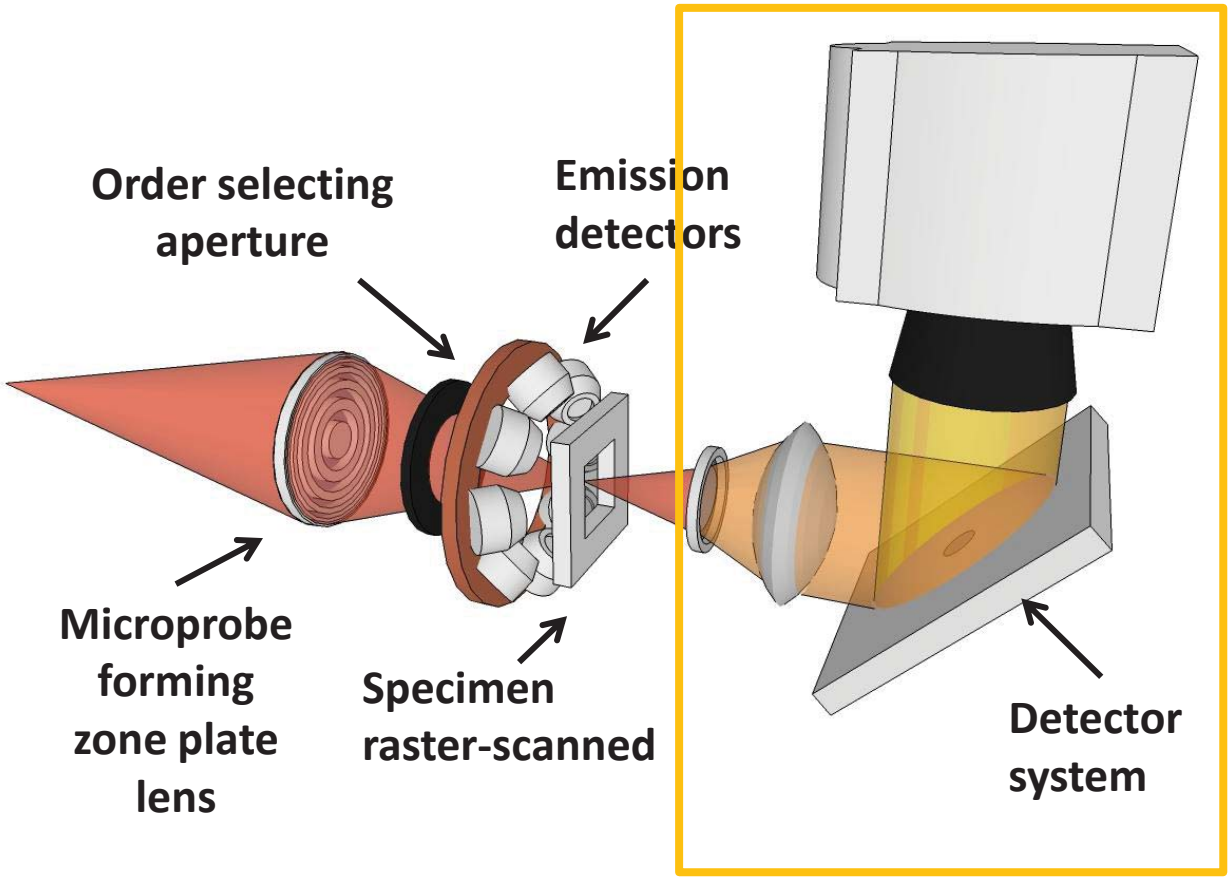


Test pattern with 30 nm features (fabricated by TASC/ INFM)

Experiment performed by M. Prasciolu and D. Cojoc, TASC/ INFM)

Imaging modes in X-ray microscopy

The scanning transmission and emission mode

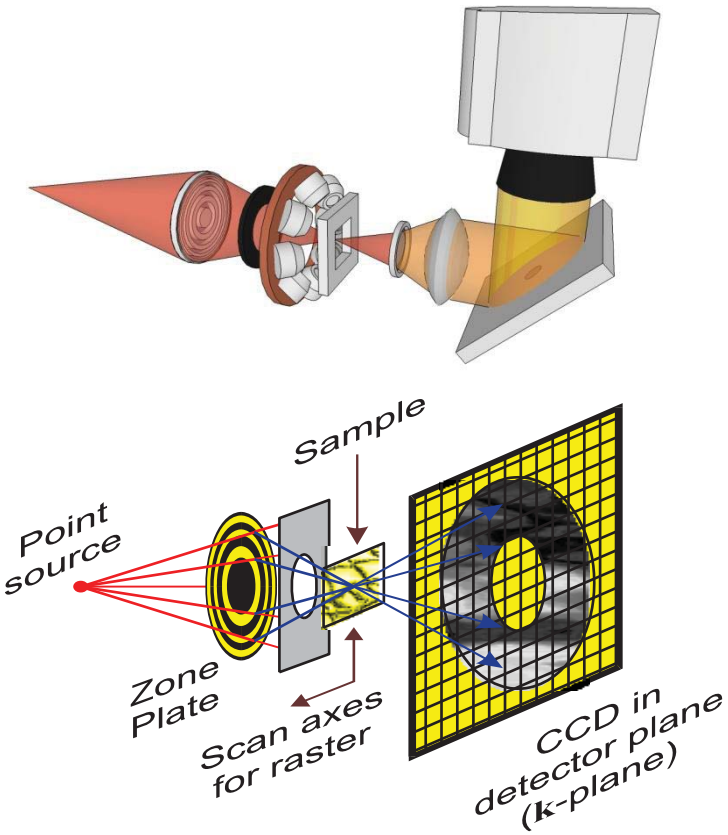


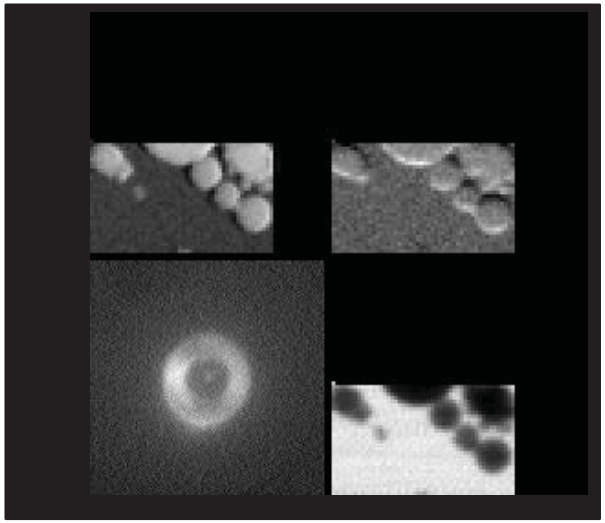
Janos Kirz

- Simultaneous acquisition of different transmission and emission signals
- Elemental mapping and chemical analysis
- Slower and more complex data acquisition

Scanning transmission mode

Differential phase contrast with a fast read-out CCD camera





Simultaneous acquisition of:

- Absorption or transmission
- Differential phase contrast
- Darkfield images

Optics based:

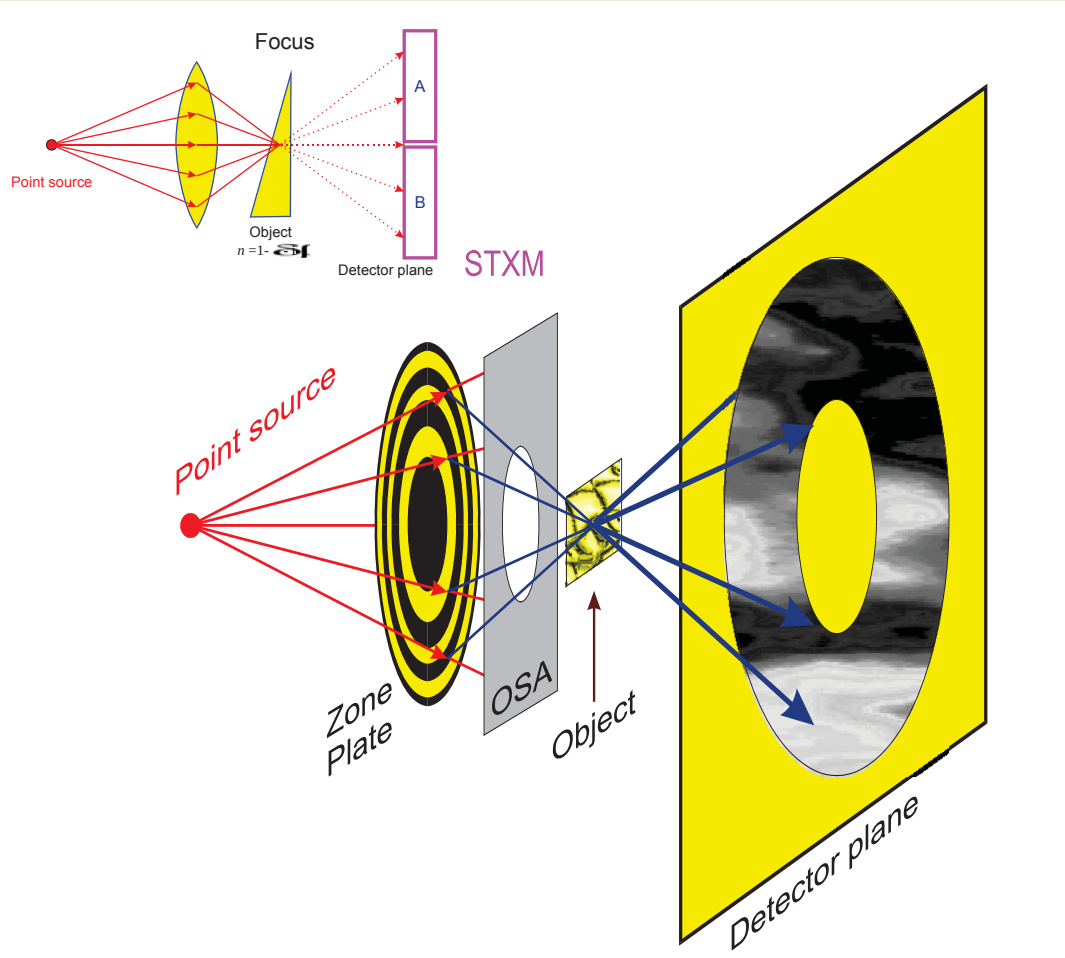
- Zernike phase contrast (Schmahl et al.)
- Differential interference contrast (Wilhein et al., Kaulich et al.)

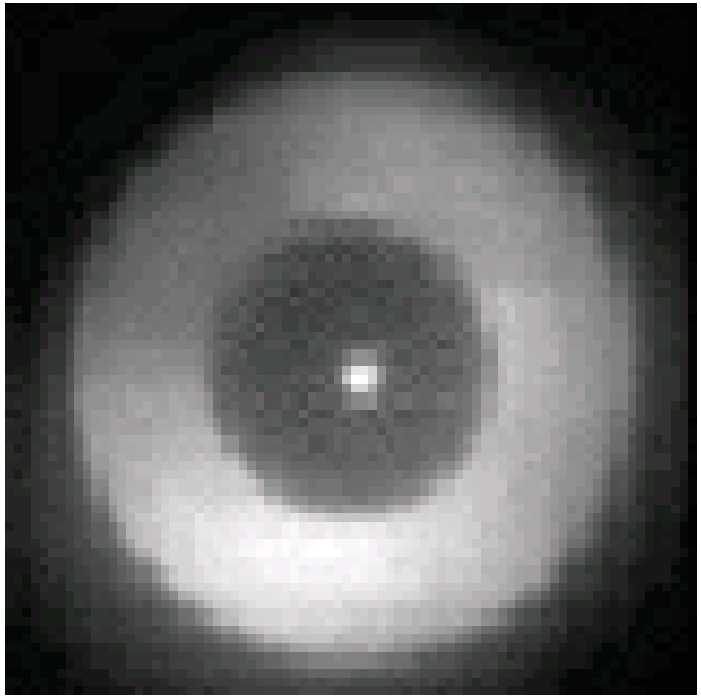
Detector based:

- Differential phase contrast (Morrison et al., Feser et al., Hornberger et al.

*B. Kaulich et al., JOSA A **19** (4), 797-806 (2002)*

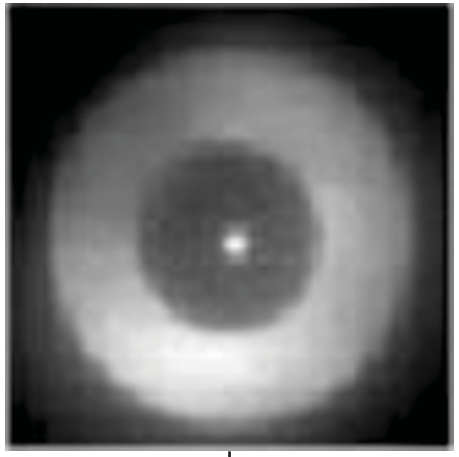
Detector based contrast technologies in scanning X-ray microscopy:





Acquired with Andor Ixon DV860A
Frame transfer back-illuminated Electron Multiplying CCD with shutter and light converting system (128x128px, 5 Mhz, 110f/s)

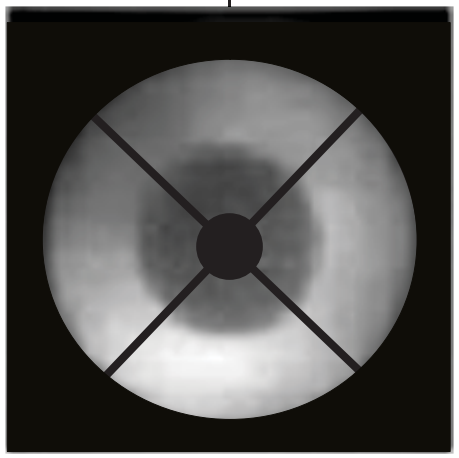
Computational extraction of contrasts by masking:



Raw data acquisition of first diffraction order image for each pixel of the raster scan

Applying different masks

Bright field

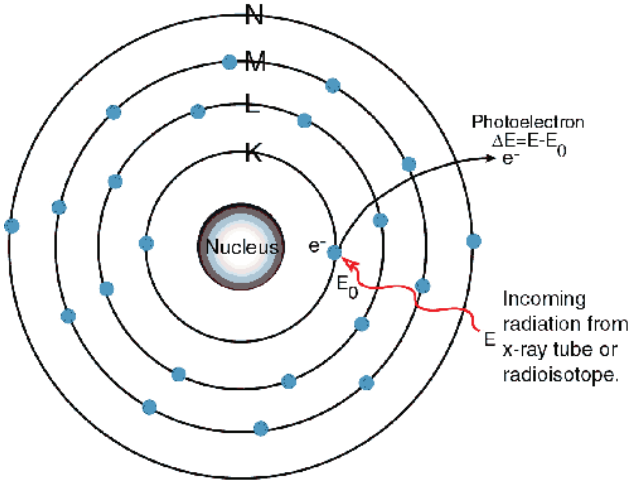


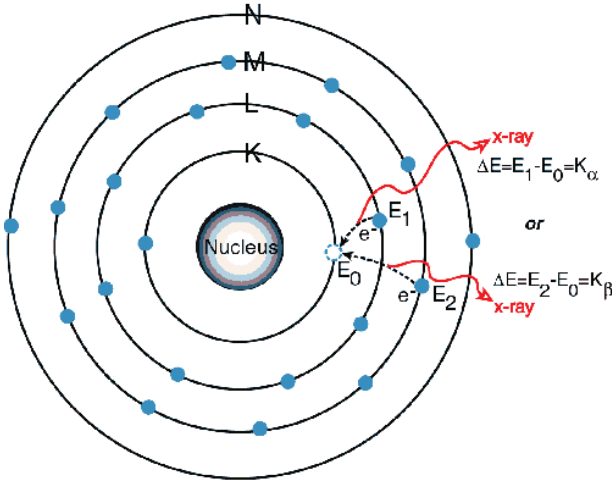
Differential phase and absorption

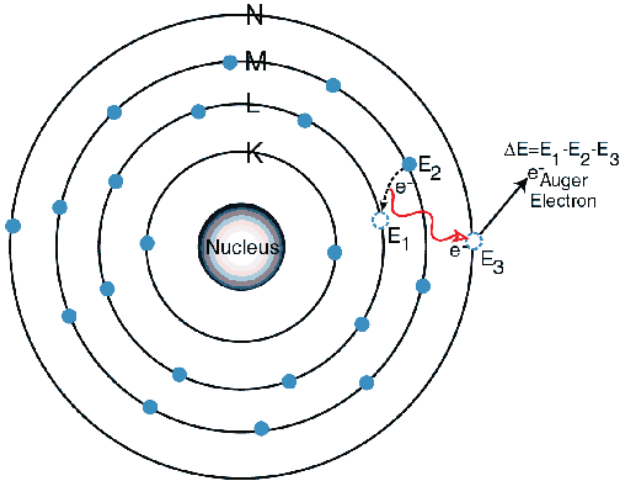
Darkfield

A. Gianoncelli et al., Appl. Phys. Lett. 2006

Photoionization







X-ray absorption
(through photoelectric effect)

The primary X-ray photon causes the ejection of electrons from the inner shells, creating vacancies

X-ray Fluorescence

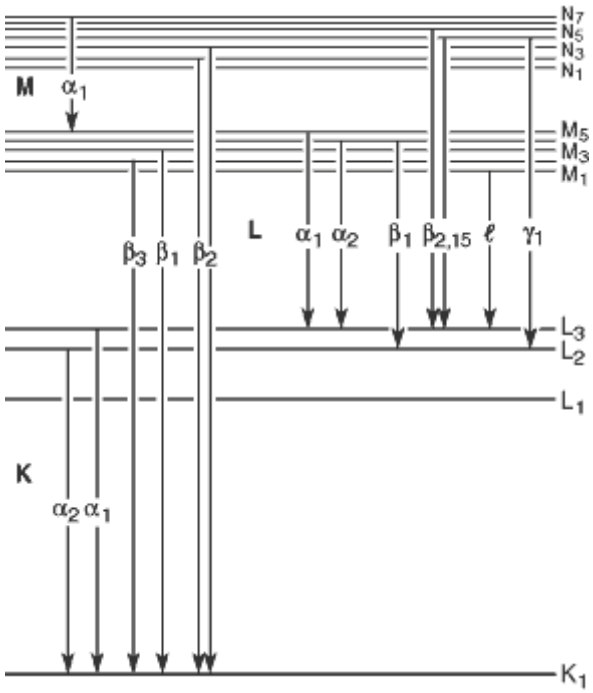
The vacancy created by the primary X-ray photon is filled by an electron coming from an outer shell causing the emission of a characteristic X-ray photon whose energy is the difference between the two binding energies of the corresponding shells

Auger effect

The vacancy created by the primary X-ray photon is filled by an electron coming from an outer shell and the energy is transferred directly to one of the outer electrons, causing it to be ejected from the atom.

http://www.amptek.com/xrf.html

X-ray Fluorescence (XRF)



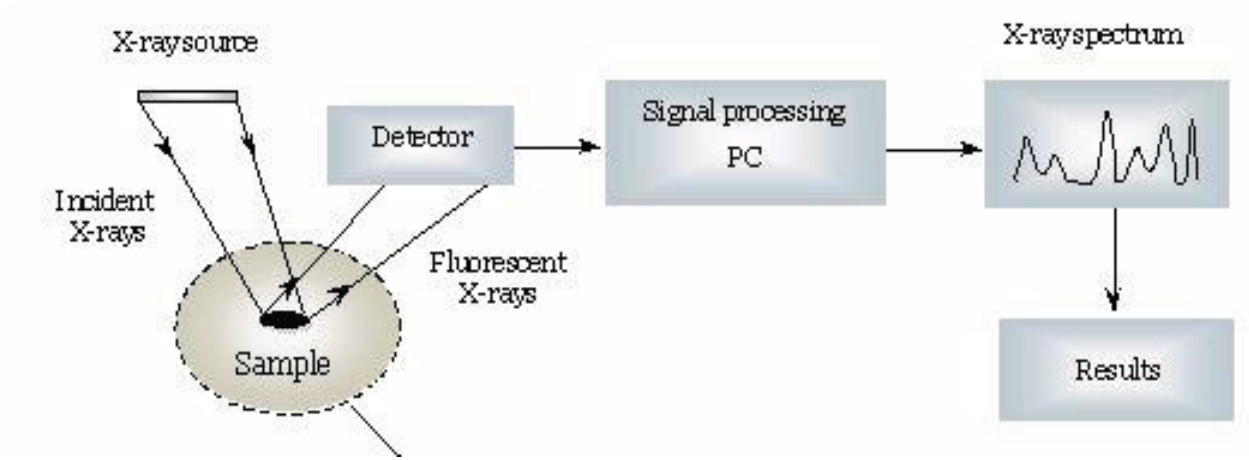
- **X-ray fluorescence (XRF)** is the phenomenon where an electron from an outer shell then drops into the unoccupied orbital, to fill the hole left behind. This transition gives off an X-ray of fixed, characteristic energy that can be detected by a fluorescence detector.
- The energy needed to eject a core electron is **characteristic of each element**, and so is the energy emitted by the transition. The transition of an L shell electron dropping into the K shell is termed a Kα transition, while an M shell electron dropping into the K shell is a Kß transition.

- Elemental analysis technique
- Non destructive (no sample preparation, in air, no damages on the sample)
- Analysis of the surface sample layers (the XRF photons must be able to exit the sample and reach the detector)

http://xdb.lbl.gov/xdb.pdf

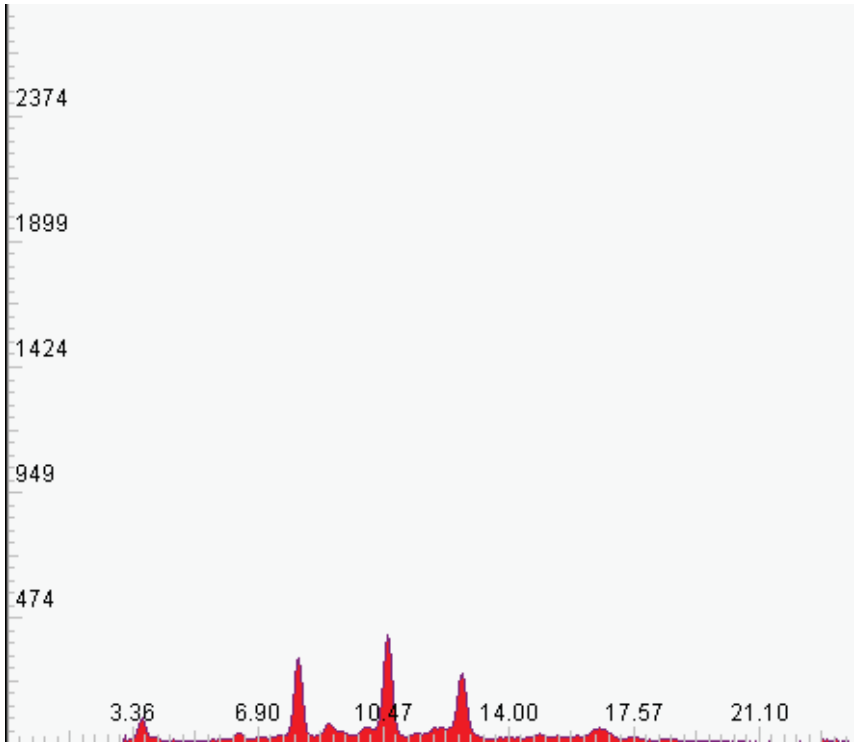
Bearden J.A. (1967) *Rev. Mod. Phys. 39:78*

XRF system



- Source: X-rays
- Detector: solide state (Ge, Si), photomultiplier
- Signal processing: preamplifier + filter shaping + multichannel analyser
- PC + analysis software (qualitative and quantitative)

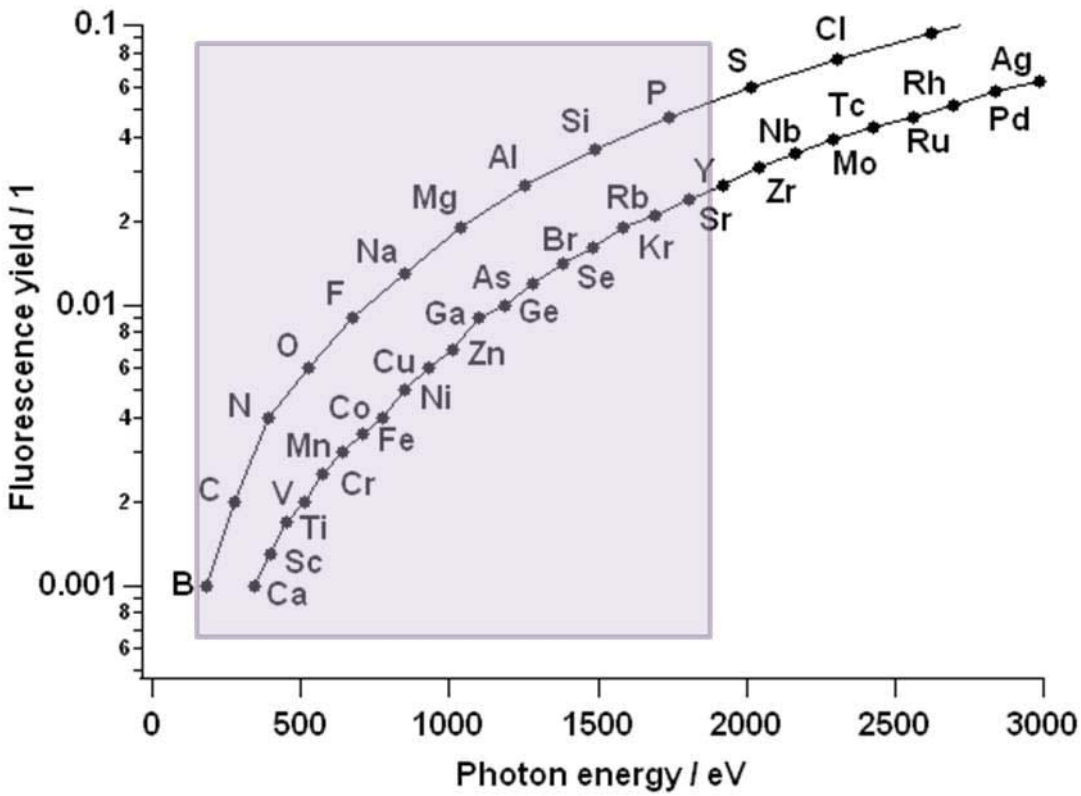
XRF spectrum



- Histogram coming from the multichannel analyser (ADC convertor)
- Calibration with a known sample
- Determination of chemical elements by using known tables (databases)

A novelty: Low-energy X-ray fluorescence

Simultaneous acquisition of absorption, differential phase contrast and LEXRF ?



Detecting trace elements:

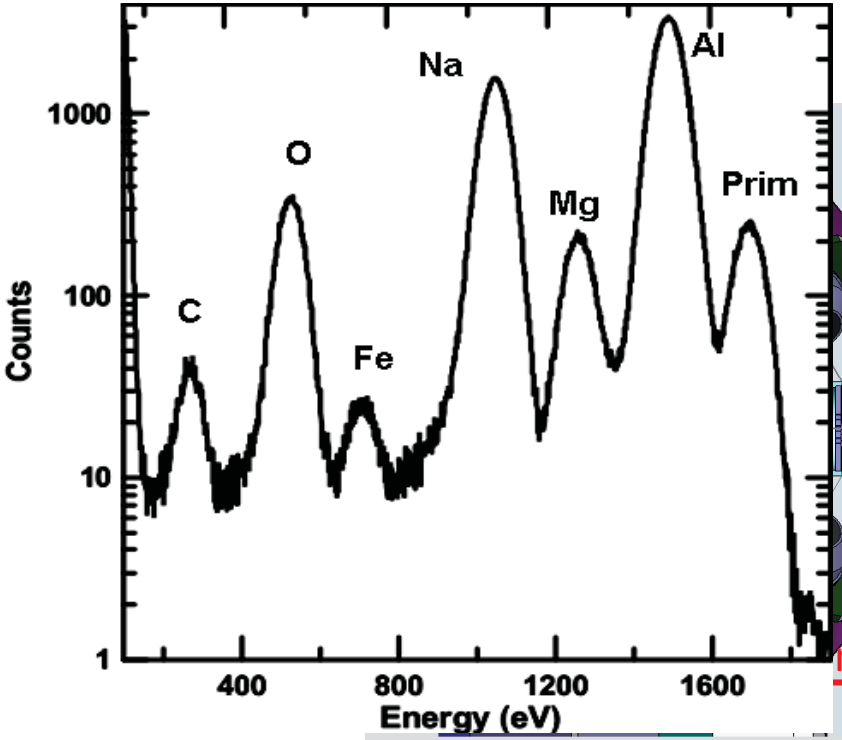
X-ray fluorescence: **~1000x better sensitivity** than electrons for trace elemental mapping (ion concentrations etc.). Parts per billion!

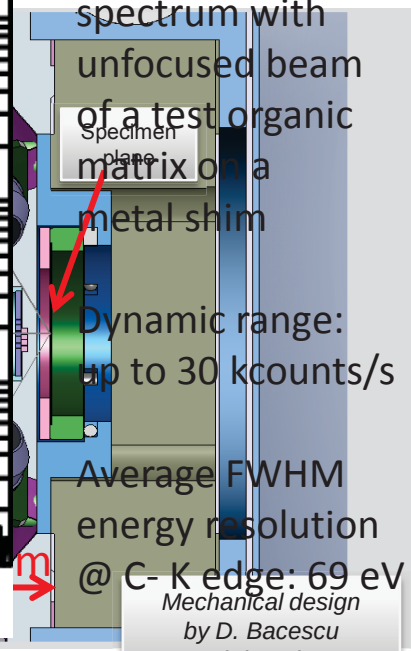
Low fluorescence yields for soft X-rays:

Is the signal sufficient for detecting trace elements in the ppm or ppb range?

A novelty: Low-energy X-ray fluorescence

Simultaneous acquisition of absorption, differential phase contrast and X-ray micro-fluorescence in STXM mode!



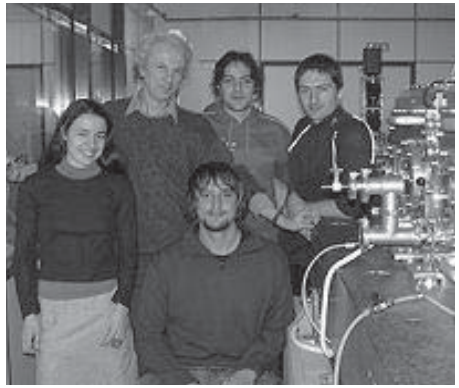


TwinMic LEXRF spectrum with unfocused beam of a test organic matrix on a metal shim

Dynamic range: up to 30 kcounts/s

Average FWHM energy resolution @ C- K edge: 69 eV

Mechanical design by D. Bacescu (Elettra)



A. Longoni et al., Politecnico Milano/ INFN and ELETTRA

- Novel compact SDD technology
- Customized read-out electronics optimized to the TwinMic station
- Superior performance at low energies

Anal. Chem. **2008**, *80*, 6436–6442

Visualization of a Lost Painting by Vincent van Gogh Using Synchrotron Radiation Based X-ray Fluorescence Elemental Mapping

Joris Dik,[*,†] Koen Janssens,[‡] Geert Van Der Snickt,[‡] Luuk van der Loeff,[§] Karen Rickers,[∥] and Marine Cotte[⊥,#]

- Van Gogh (1853-1890) is one of the fathers of modern picture and was known for his vivid colors, the intense paint brushes dipinti and for his big productive career.

- His productivity is higher than we think since often he was re-utilising some older painting as base for a new painting.

Visible light

Radiography **IR Riflettography**

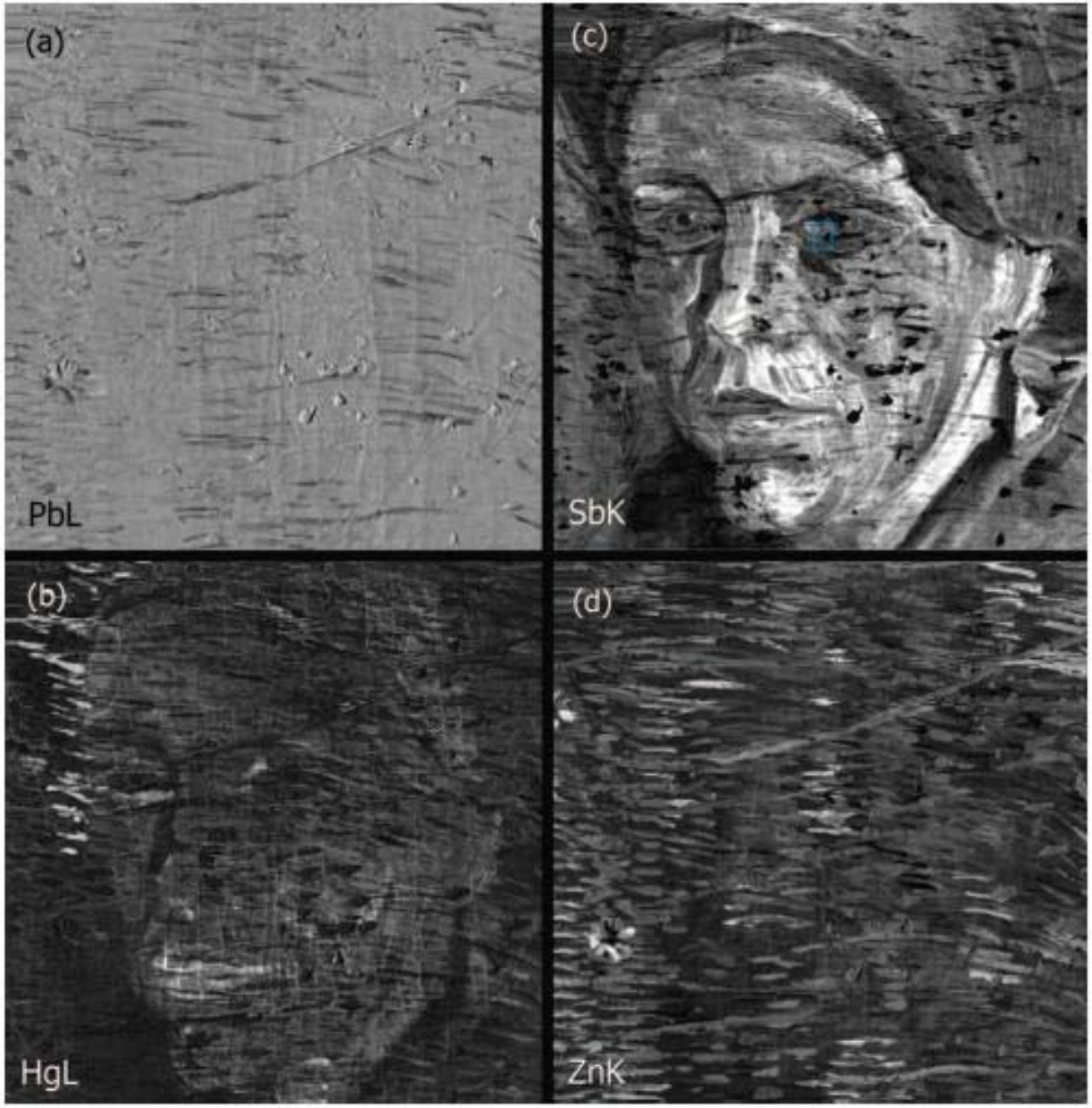
HASYLAB (Hamburg, Germany)

Beam (0.5 × 0.5 mm^2) at 38.5 keV

17.5 × 17.5 cm^2
dwell time: 2 s per pixel
total scan time ~ 2 days

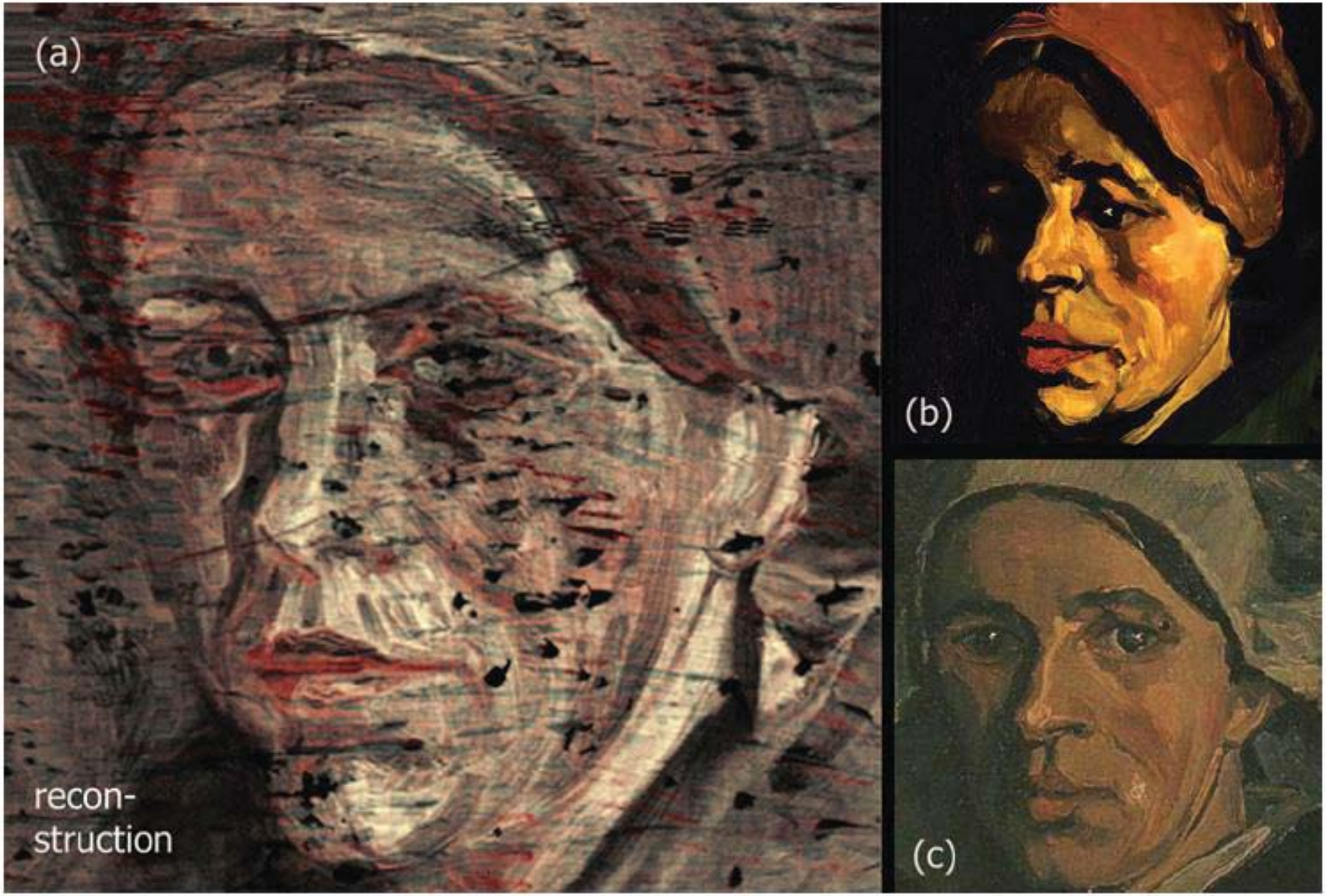
The XRF scan revealed a number of elemental distributions that mostly correspond with the surface painting.
Its main elemental components include transition metals such as Mn, Cr, Co, Fe, Cu, Zn, As, and Ba.

Pb Distribution **Sn Distribution**



Hg Distribution **Zn Distribution**

J Dik et al. "Visualization of a Lost Painting by Vincent van Gogh Using Synchrotron Radiation Based X-ray Fluorescence Elemental Mapping" Anal. Chem. 2008, 80, 6436–6442

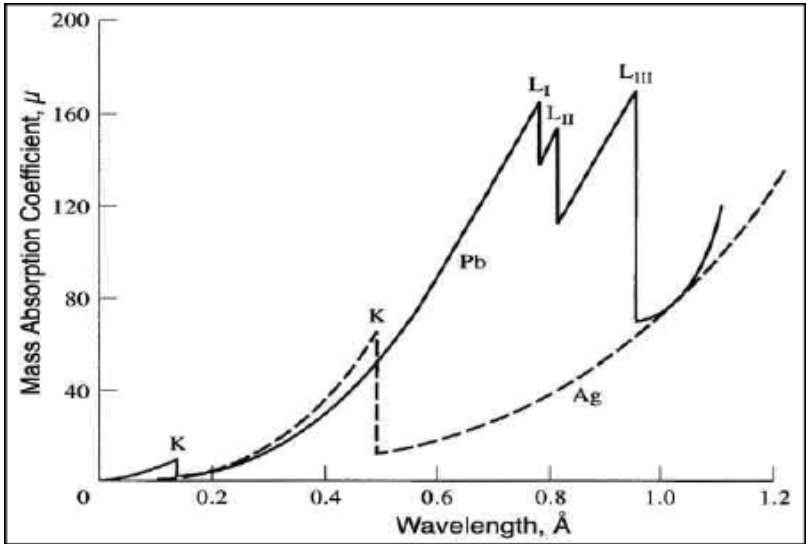


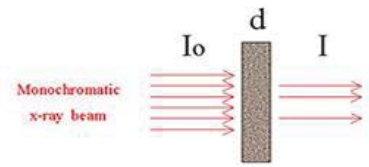
(b) Detail from Head of a Woman, Nuenen, 1884-85, oil on canvas, 42 cm × 33 cm, Kröller-Müller Museum, Otterlo
(c) Detail from Head of a Woman, Nuenen, winter 1884-85, oil on canvas, 42 cm × 34 cm, Van Gogh Museum, Amsterdam.

J Dik et al. "Visualization of a Lost Painting by Vincent van Gogh Using Synchrotron Radiation Based X-ray Fluorescence Elemental Mapping" Anal. Chem. 2008, 80, 6436–6442

X-ray Absorption Spectroscopy

- Element specific
- Applicable under extreme conditions (high-pressure, high temperature, in-situ)
- Applicable to gasses, liquids and solids
- Combination with microscopy





$$I = I_0 e^{-\mu d}$$

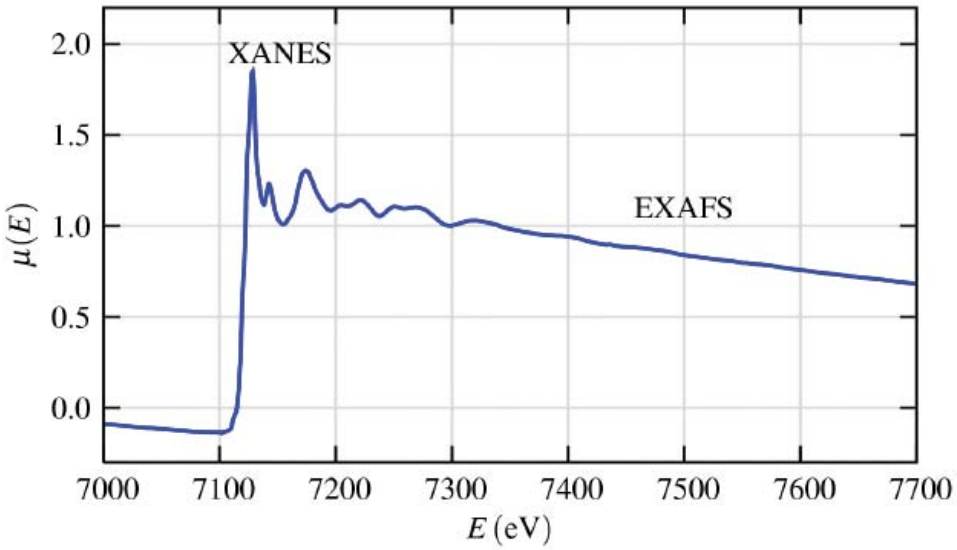
- The primary X-ray photons are absorbed when their energy is at least equal to the binding energy of the electrons.
- For lower energy the photons are easily transmited by the sample
- When the X-ray photon reaches the electron binding energy absorption occurs
- By progressively increasing the energy the absorption efficiency decreases

X-ray Absorption Fine Structure

- Spectroscopic technique
- Element-specific (x-rays are chosen to be at and above the binding energy of a particular core electronic level of a particular atomic species).
- XAFS can study elements with Z>15 (all but the lightest elements have core-level binding energies in the x-ray regime).
- An energy-tunable x-ray source is needed to measure XAFS (synchrotron).
- The x-ray absorption spectrum is typically divided into two regimes: x-ray absorption near-edge spectroscopy (XANES) and extended x-ray absorption fine-structure spectroscopy (EXAFS).

XANES is strongly sensitive to formal oxidation state and coordination chemistry (e.g., octahedral, tetrahedral coordination) of the absorbing atom

EXAFS is used to determine the distances, coordination number, and species of the neighbors of the absorbing atom



XANES is region of x-ray absorption spectrum within ~50eV of the absorption edge.

XANES on a paint sample

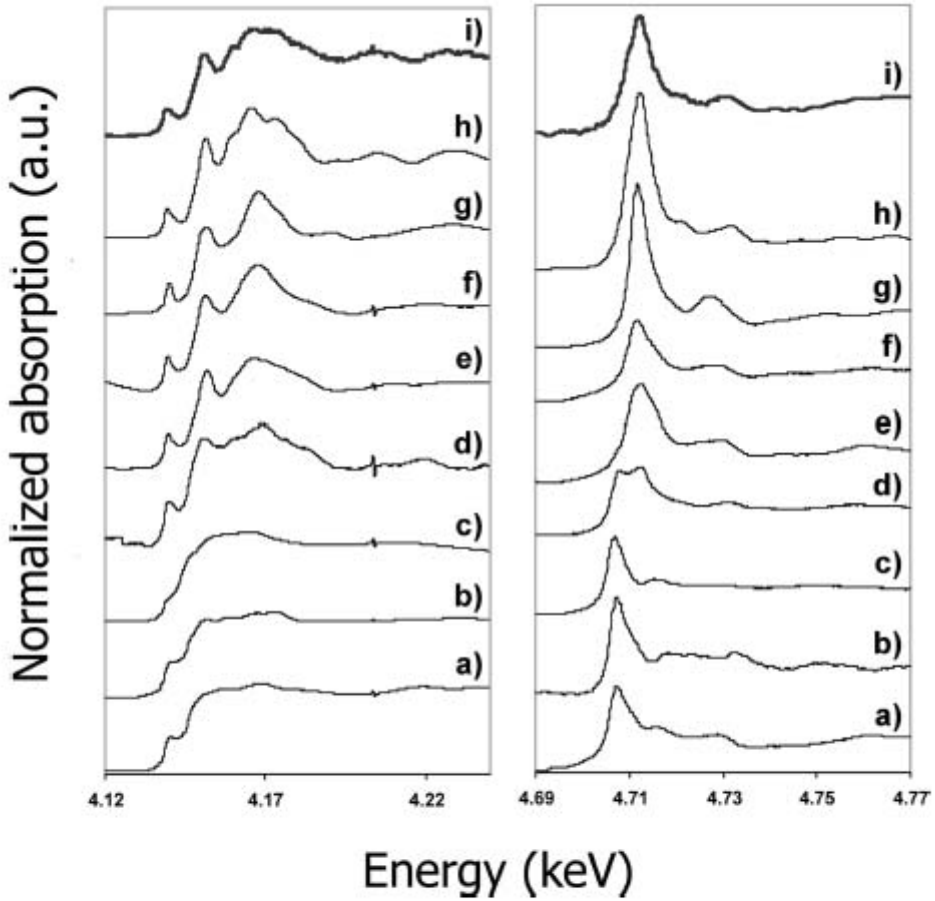


Figure 6. XANES spectra at the Sb-L_{III} edge (upper spectrum) and at the Sb-L_I edge (lower spectrum). Reference antimony compounds: Sb_2O_3 as (a) valentinite and as (b) senarmontite; (c) Sb_2S_2O, kermesite; (d) Sb_2O_4; (e) Sb_3O_6OH, stibiconite; (f) $KSbO_3 \cdot 3H_2O$; (g) $NaSbO_3OH \cdot 3H_2O$; (h) Naples yellow; and (i) Sb pigment in the cross section of the Van Gogh painting (Figure 5).

the best agreement of spectra at the two L-edges corresponds to the Naples yellow spectra

(the same was verified on the K-edge directly on the painting)

STXM imaging coupled with XRF and XANES

Case study: **blackening of Pompeian Cinnabar Paintings** (M. Cotte, J. Susini, N. Metrich, A. Moscato, C. Gratziu, A. Bertagnini, M. Pagano "Blackening of Pompeian Cinnabar Paintings: X-ray Microspectroscopy Analysis", Anal. Chem. 2006, 78, 7484-7492.)

- The Pompeian red painting are suffering from degradation
- From the exavation (started in 1988 in Villa Sora) the vivid red painting started to darken progressively
- The darkening and degradation process is often attributed to the formation of black metacinnabar (through photoinduced conversion)

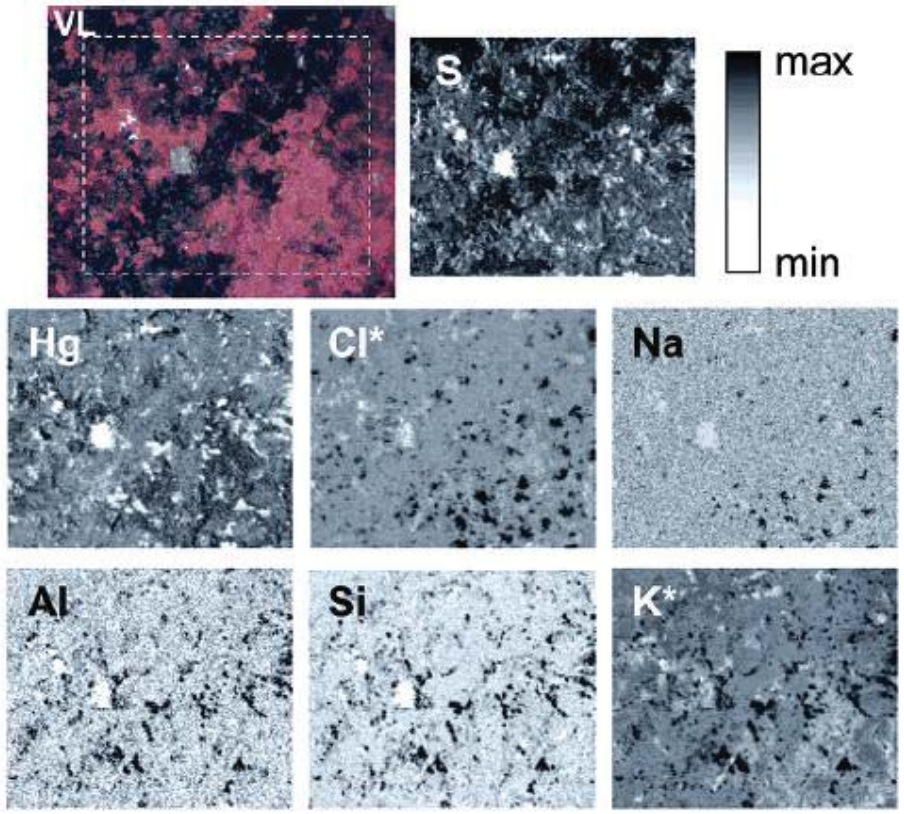
- Different samples with different degradation levels were selected
- Syncrhrotron radiation was used in order to have:
  - micro-spot
  - low detection limit
  - high chemical sensitivity

- X-ray microscopy
- XRF mapping to identify spatial distribution of chemical elements of interest
- XANES spectroscopy to probe local chemical speciation

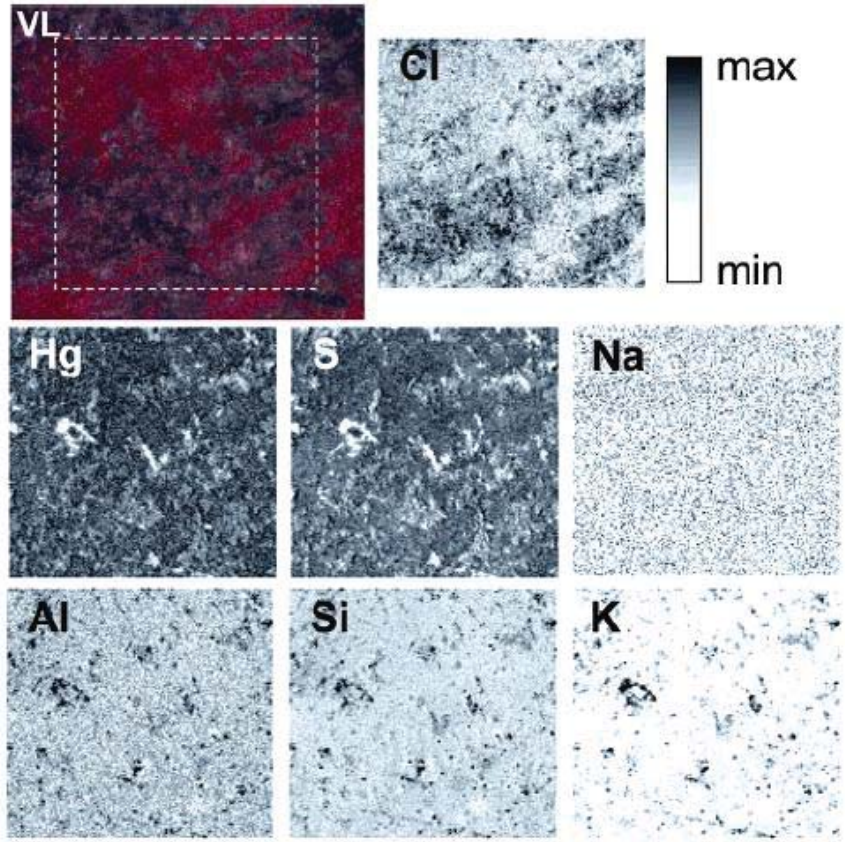
M. Cotte, et al., Anal. Chem. 2006, 78, 7484-7492.)

STXM imaging coupled with XRF and XANES

XRF showed peculiar ditributon of Cl and S



8mm x 7mm, pixel size 50x50μm^2

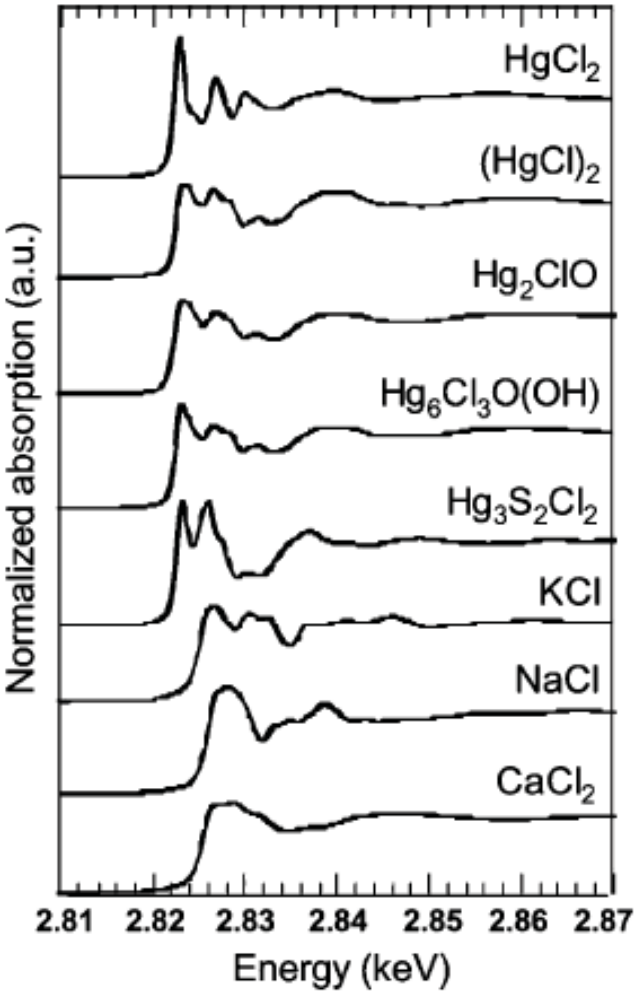


7mm x 7mm, pixel size 50x50μm^2

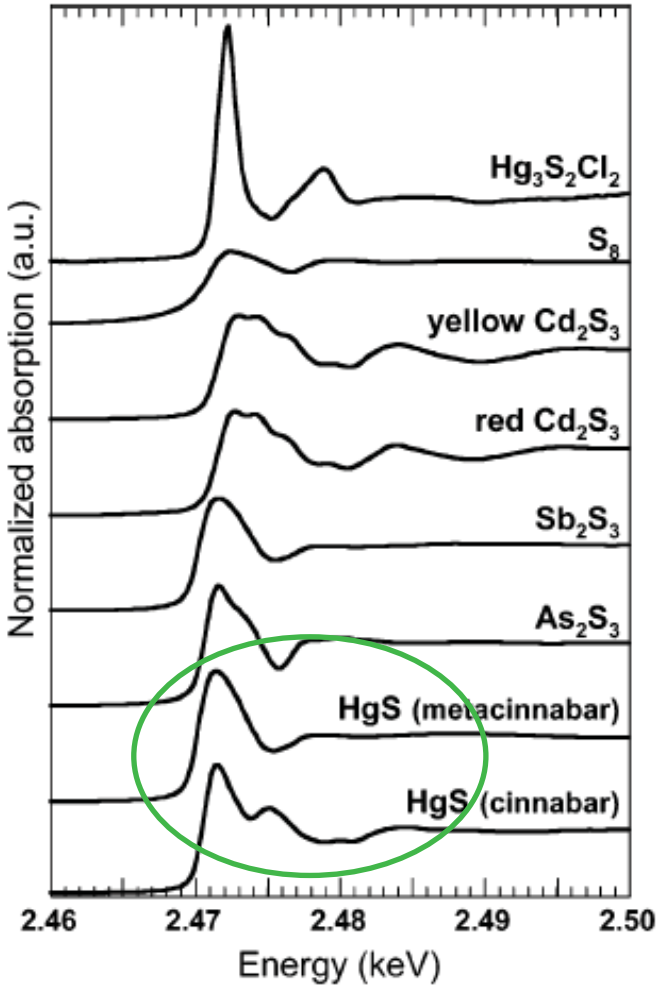
M. Cotte, et al., Anal. Chem. 2006, 78, 7484-7492.

STXM imaging coupled with XANES

Acquisition of reference compound spectra for calibration



Chlorinated references

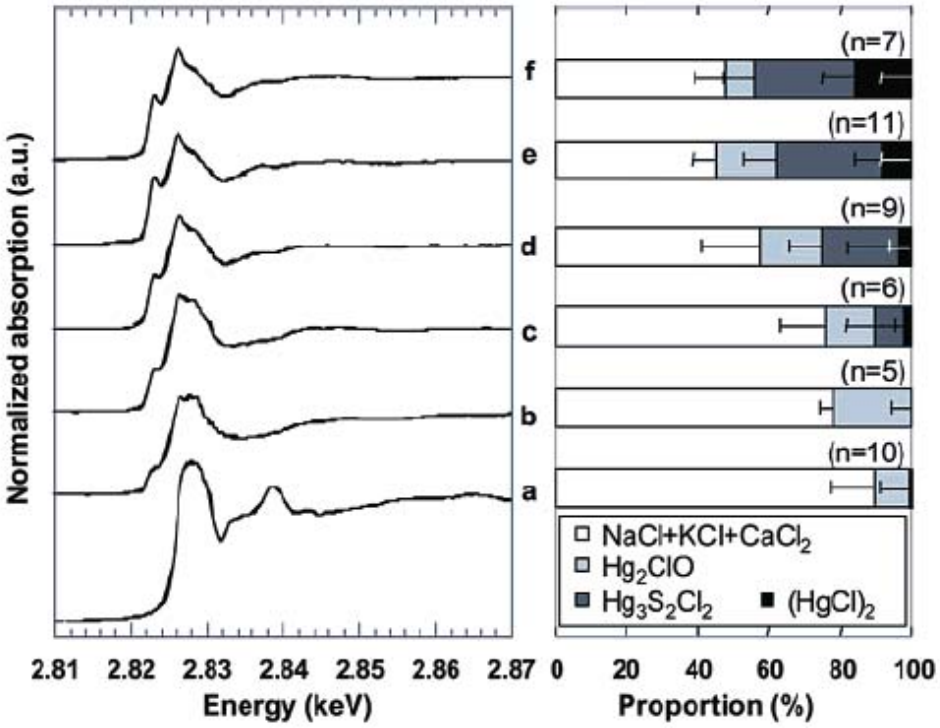


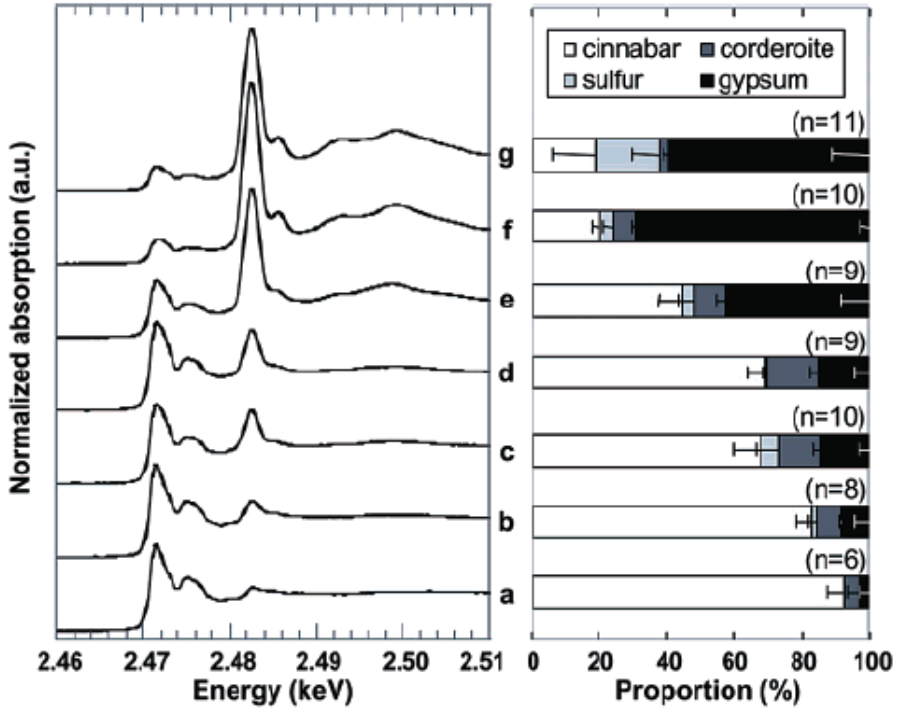
Sulfur references

M. Cotte, et al., Anal. Chem. 2006, 78, 7484-7492.

STXM imaging coupled with XANES

By fitting the XAS with a linear combination of the reference comound spectra in order to identify the proportion of the different components in the altered zones





- **Cl K-edge**

  In red and rust parts less than 20% of Hg is coordinated with Cl

  In gray and black areas the Hg species are around 40-50%

- **S K-edge**

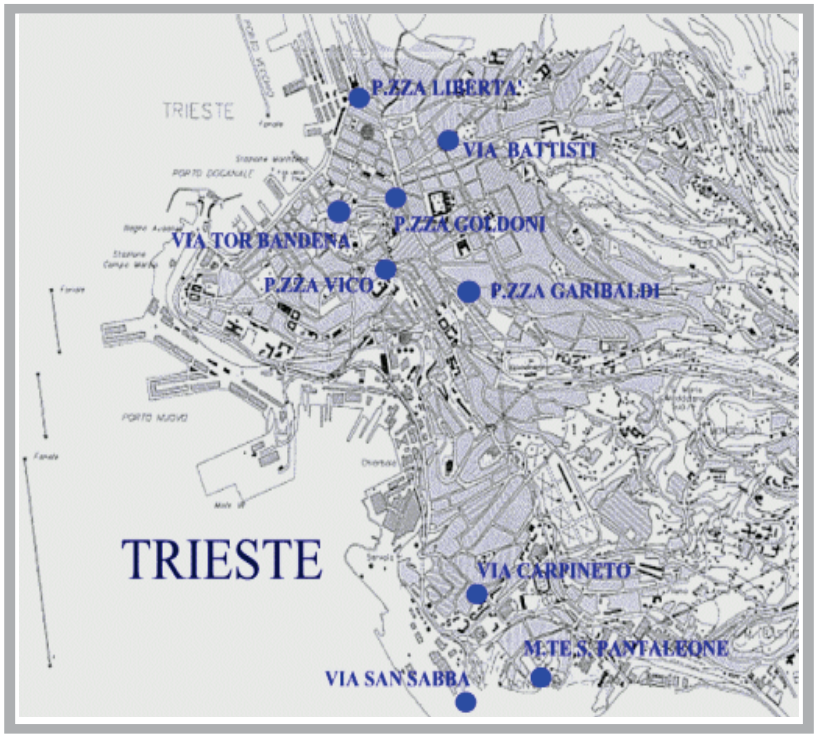
  In one sample the amount of sulfate increases dramatically in dark areas (>50%)

  In both samples high sulfate concentrations in white/orange spots

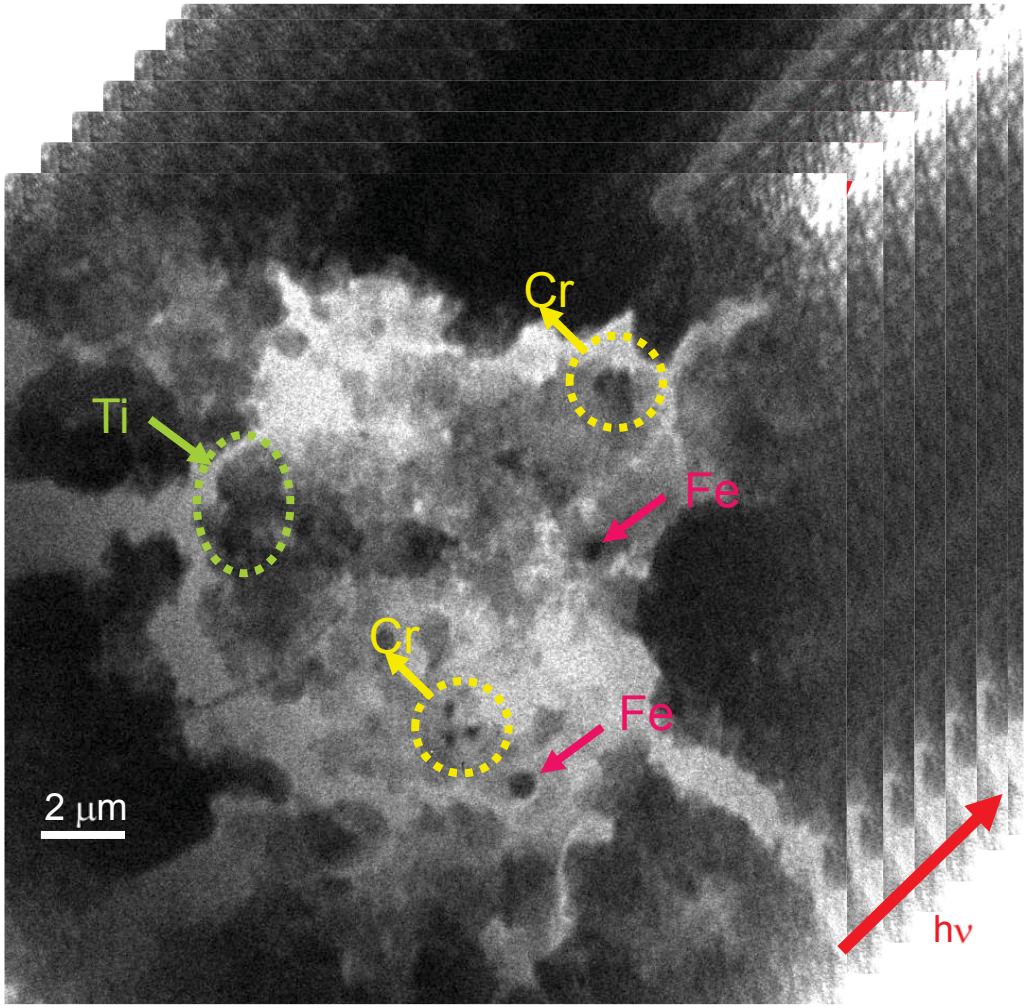
  No contribution of metacinnabar

M. Cotte, et al., Anal. Chem. 2006, 78, 7484-7492.

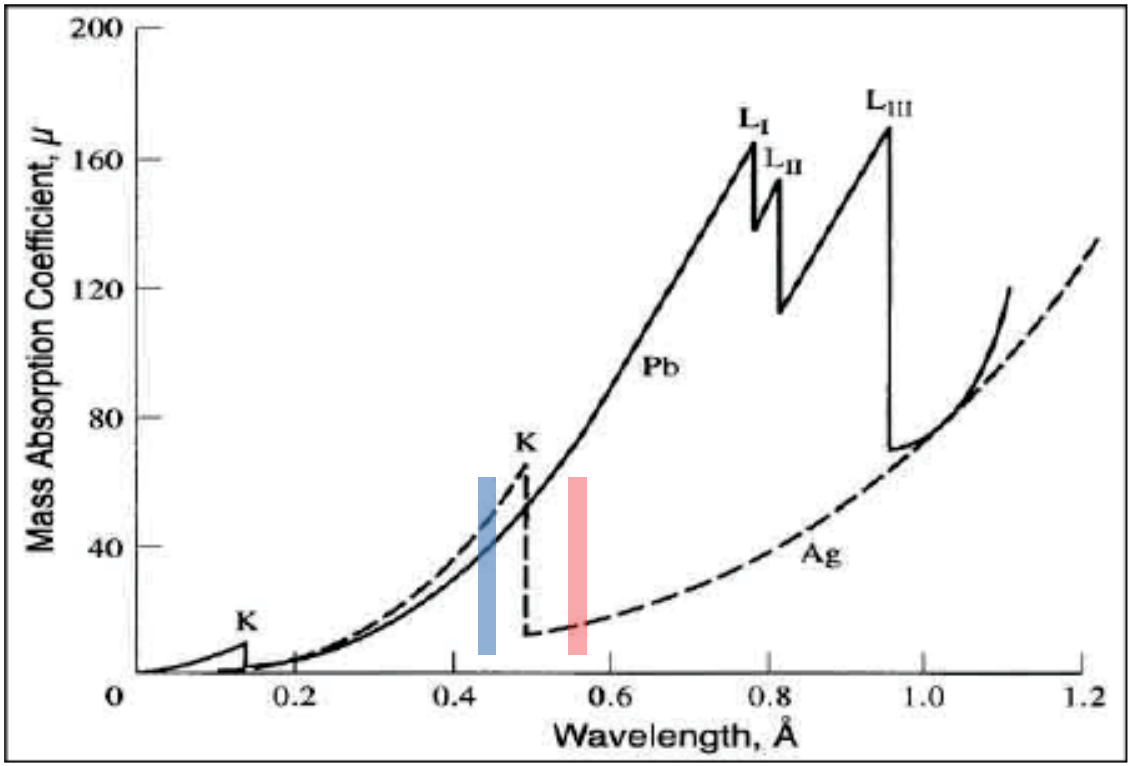
Environmental science: Analysis of air particulate matter



P. Barbieri et al.,
Dept. of Chem., Univ. Trieste, I

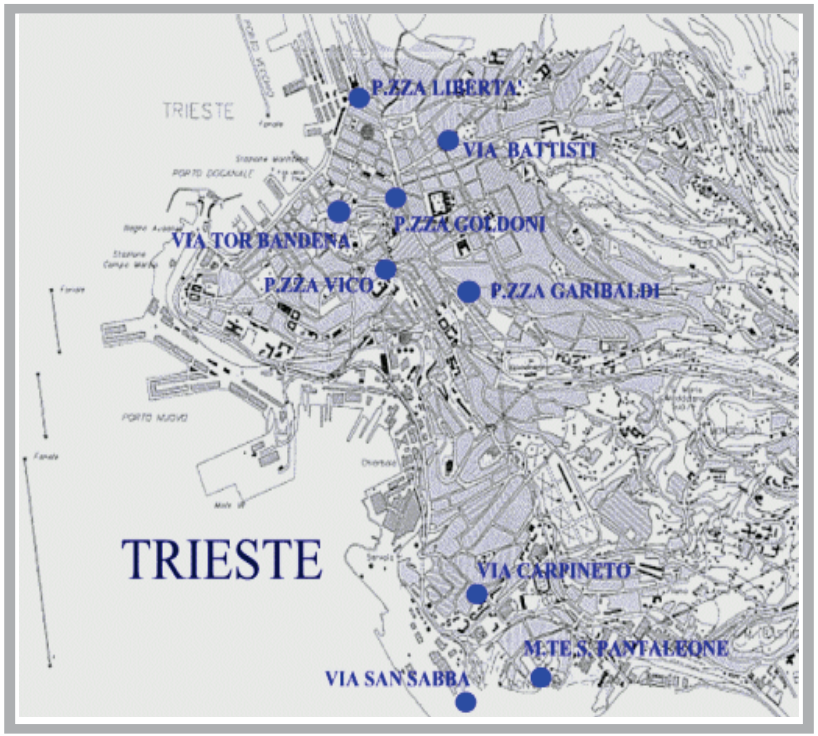


Across Edge Imaging

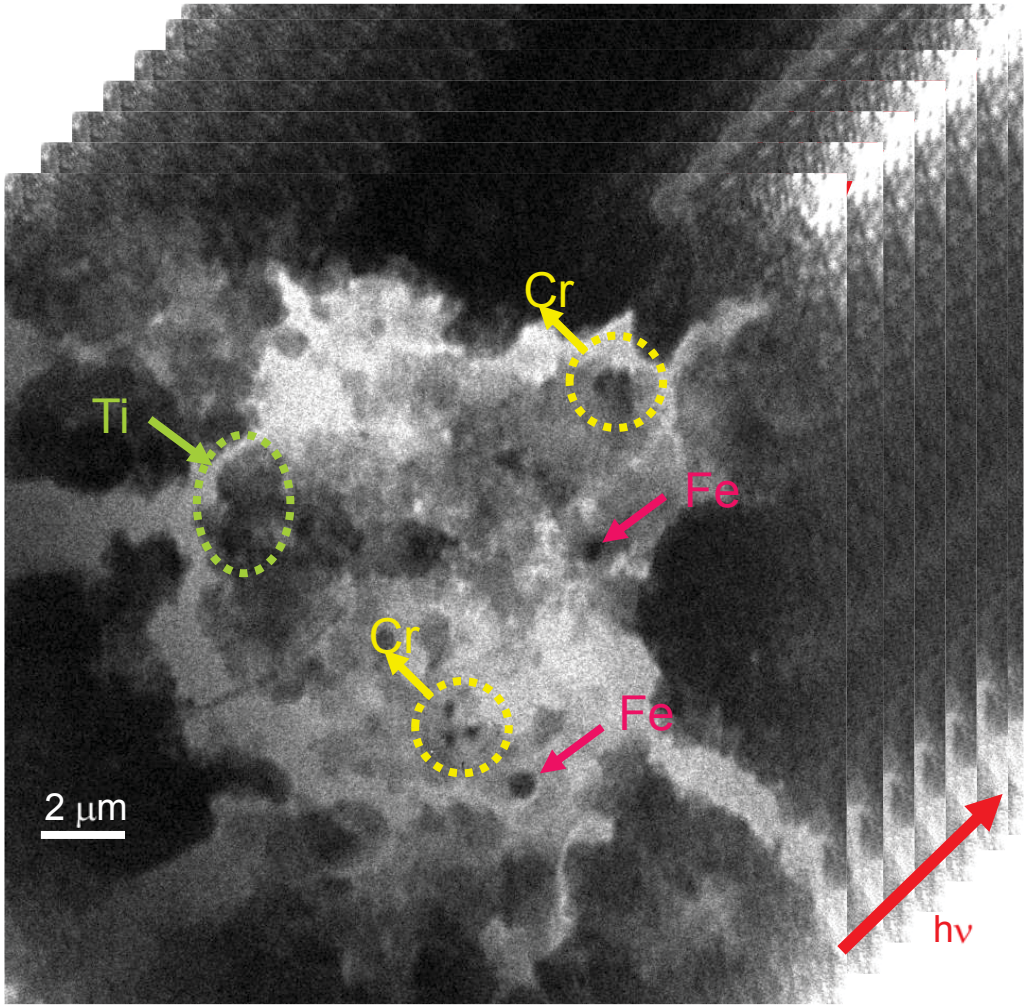


Acquisition of two images: Above and Below the K-edge

Environmental science: Analysis of air particulate matter

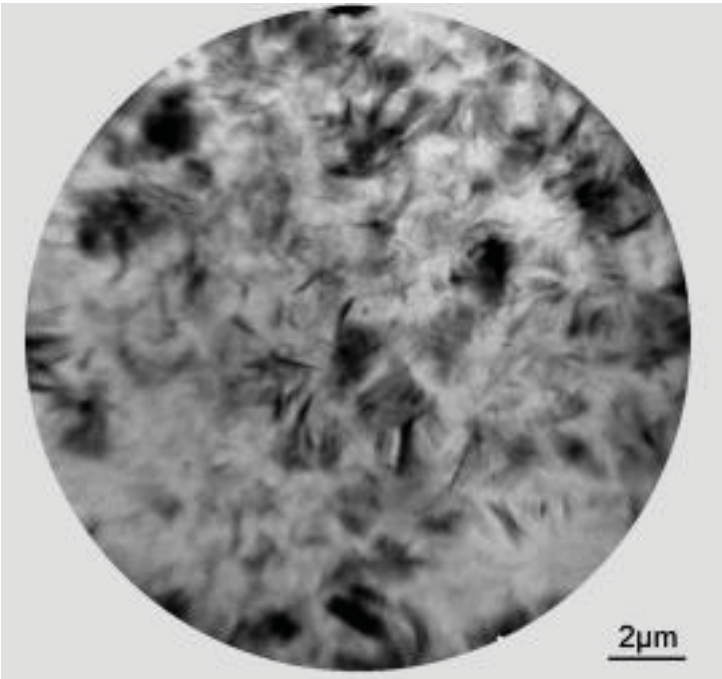


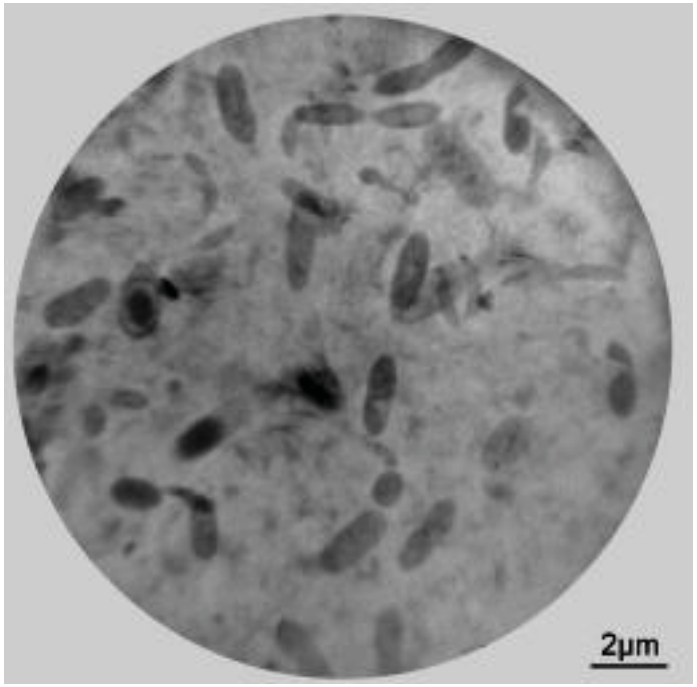
P. Barbieri et al.,
Dept. of Chem., Univ. Trieste, I



Environmental science: Imaging in liquids

Bacteria and clay dispersion: Destruction of associations of clay particles by soil microbes

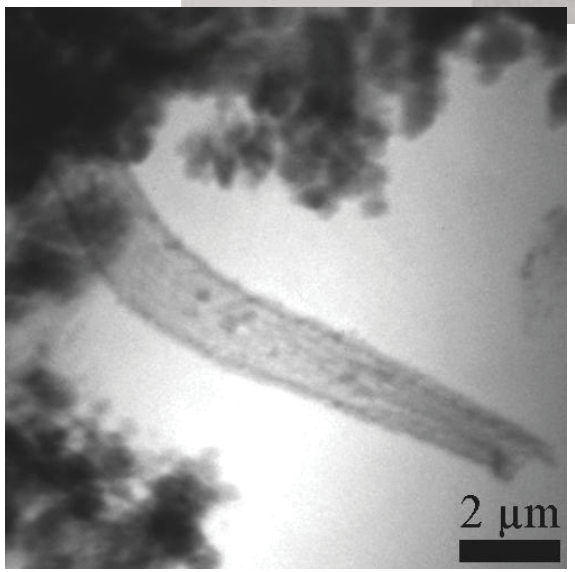




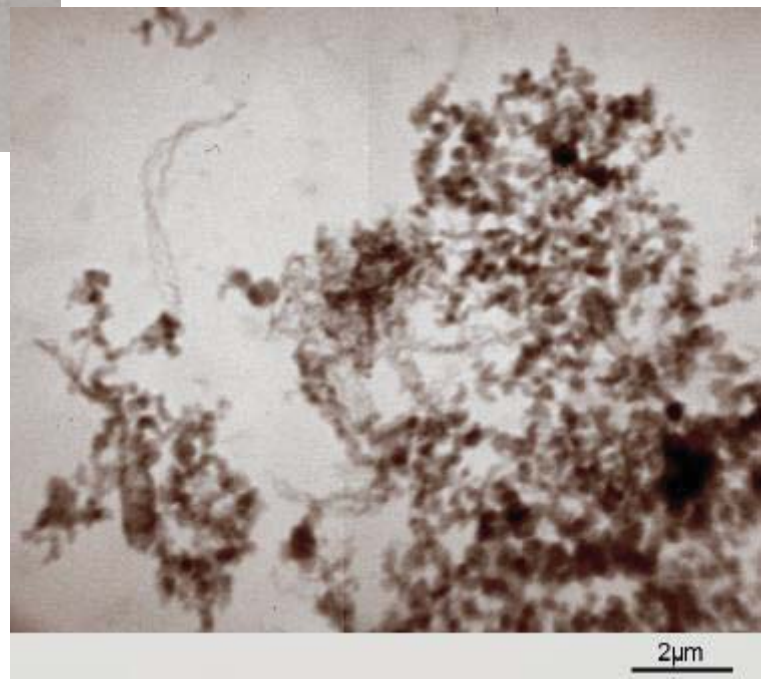
X-ray images acquired with the full-field imaging microscope at BESSY I @ 520 eV

J. Thieme et al., IRP, Uni Goettingen / G. Machulla, Uni Halle, D

Environmental science: Imaging in liquids

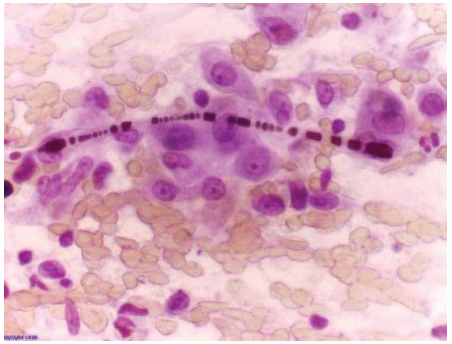


Iron precipating bacteria:
Biological removal of Fe for preparation of drinking water



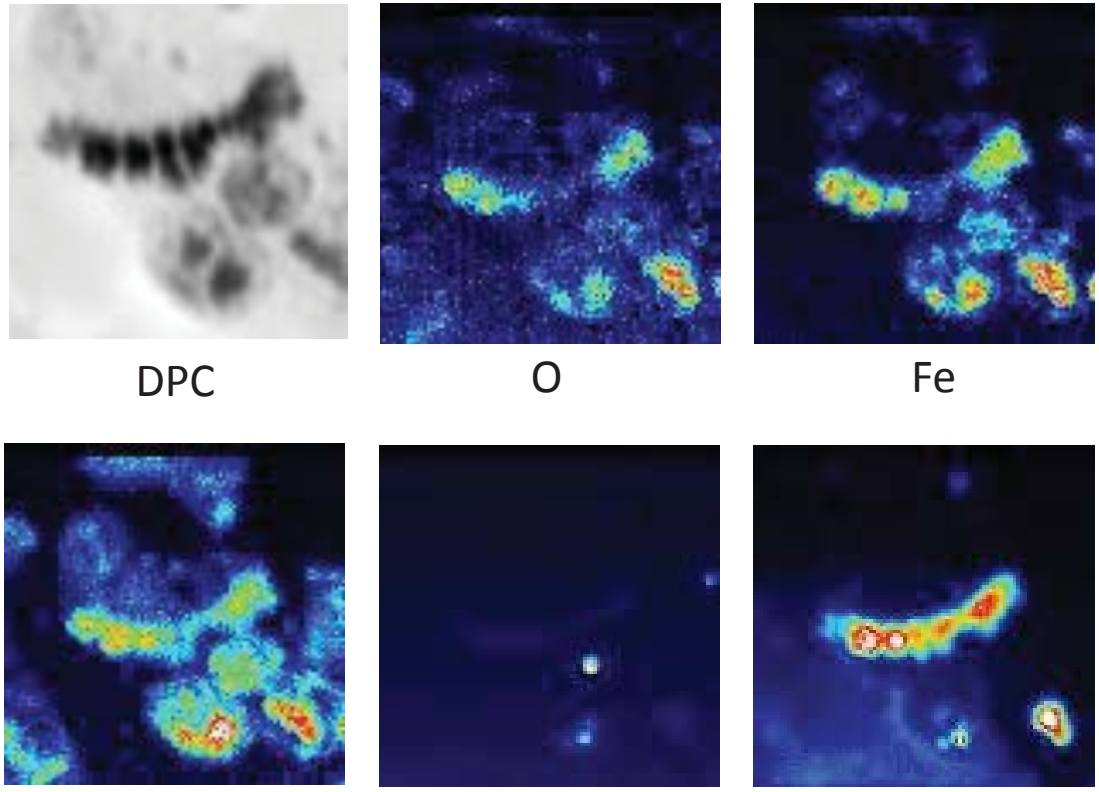
J. Thieme et al., IRP, Uni Goettingen, D

Clinical medicine: Asbestos in lung tissue



M. Melato,
Monfalcone Hospital
L. Pascolo,
Sincrotrone Trieste

Cancer, death and differentiation of lung tissue due to asbestos



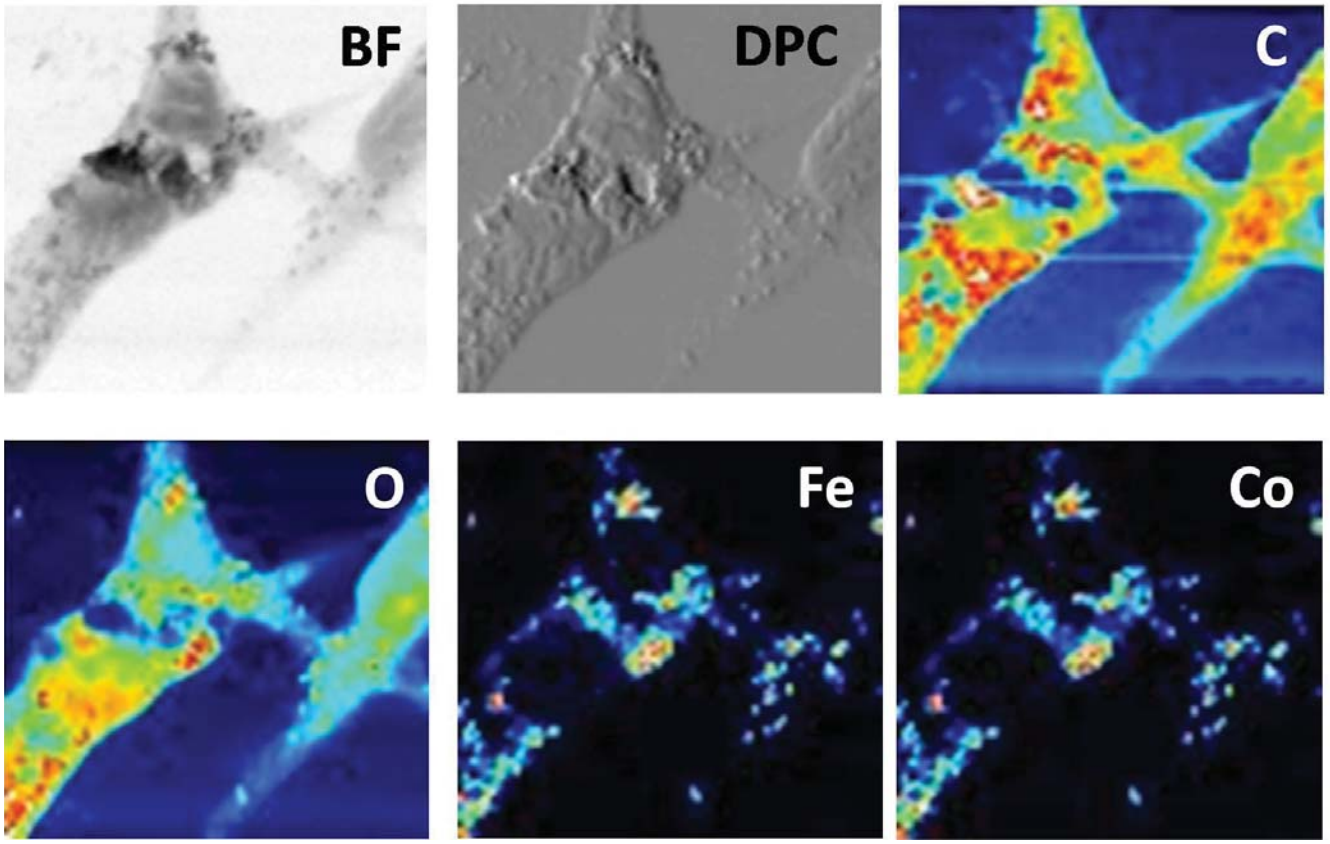
E=2019 eV, 50um x 50 um, 100 x 100 pixels

M. Regvar, D. Eichert, B. Kaulich, A. Gianoncelli, P. Pongrac, K. Vogel-Mikus, I. Kreft, New insights into globoids of protein storage vacuoles in wheat aleurone using synchrotron soft X-ray microscopy, Journal of Experimental Botany, Vol. 62, No. 11, 3929–3939, 2011.

Nanotoxicology: $CoFe_2O_4$ ENPs

G. Ceccone,
P. Marmorato, EC Joint Research Center, Ispra, I

Localization of engineered nanoparticles (ENPs) inside a cell and on the possible effects on the cell metabolic behaviour



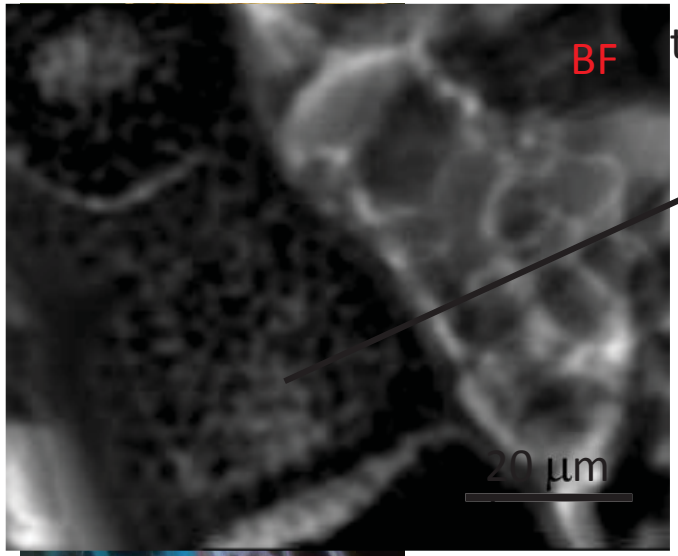
$CoFe_2O_4$ in mouse 3T3 fibroblast cells,
E=2019 eV, 25um x 35 um, 50 x 70 pixels, 15s/pixel

P. Marmorato, G. Ceccone, A. Gianoncelli, L. Pascolo, J. Ponti, F. Rossi, M. Salomé, B. Kaulich, M. Kiskinova, Cellular distribution and degradation of cobalt ferrite nanoparticles in Balb/3T3 mouse fibroblasts, Toxicol. Lett. 207, (2011), 1218-136.

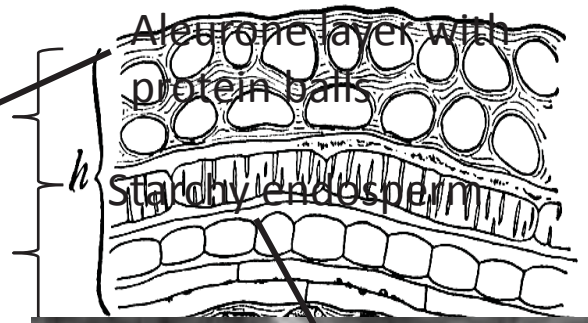
Biogenetics and Food Science: Inside the wheat

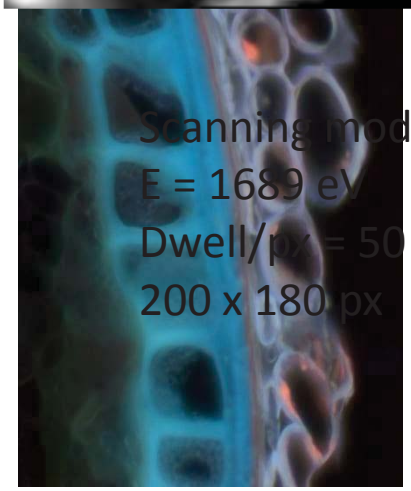
Ivan Kreft,
University Ljubljana

Functionality and toxicity of Zn in wheat and buckwheat analyzed on sub-cellular level

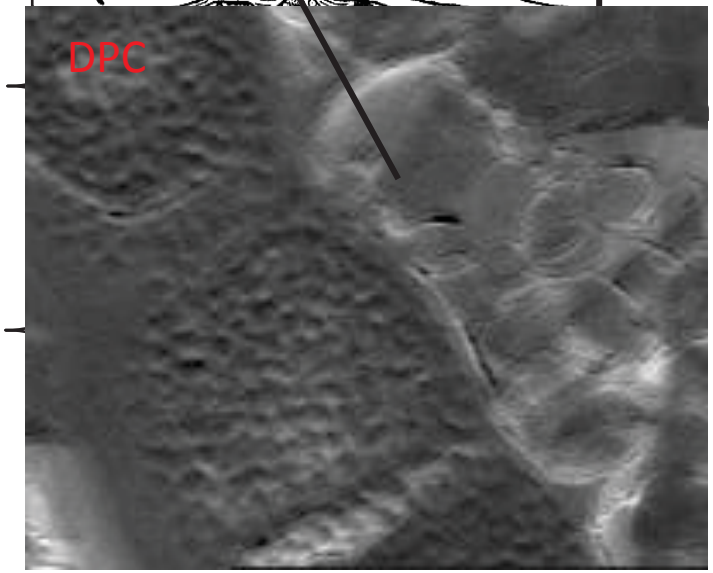


X-ray micrographs of structure of a wheat grain





Endosperm with starch granules



M. Regvar, D. Eichert, B. Kaulich, A. Gianoncelli, P. Pongrac, K. Vogel-Mikus, I. Kreft, New insights into globoids of protein storage vacuoles in wheat aleurone using synchrotron soft X-ray microscopy, Journal of Experimental Botany, Vol. 62, No. 11, 3929–3939, 2011.

Biogenetics and Food Science: Inside the wheat

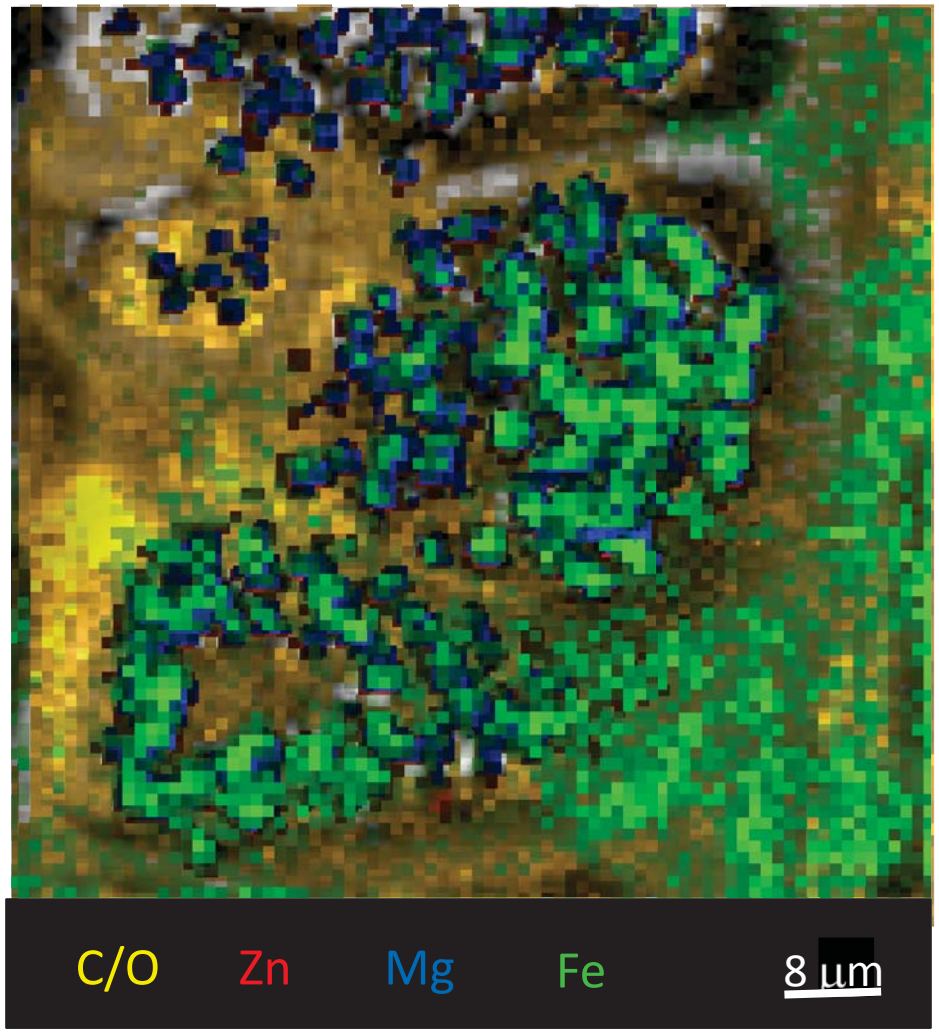
Ivan Kreft,
Fac. of Biotechnology,
University Ljubljana

Functionality and toxicity of Zn in wheat and buckwheat analyzed on sub-cellular level

Healthy control wheat

E=1686 eV
80 x 80 μm^2
80 x 80 px
8 s dwell/ px
1 µm resolution
4 detectors
New STXM optic

Specimen preparation by Paula Pongrac and Katharina Vogel, Uni Ljubljana, Slo



M. Regvar, D. Eichert, B. Kaulich, A. Gianoncelli, P. Pongrac, K. Vogel-Mikus, I. Kreft, New insights into globoids of protein storage vacuoles in wheat aleurone using synchrotron soft X-ray microscopy, Journal of Experimental Botany, Vol. 62, No. 11, 3929–3939, 2011.

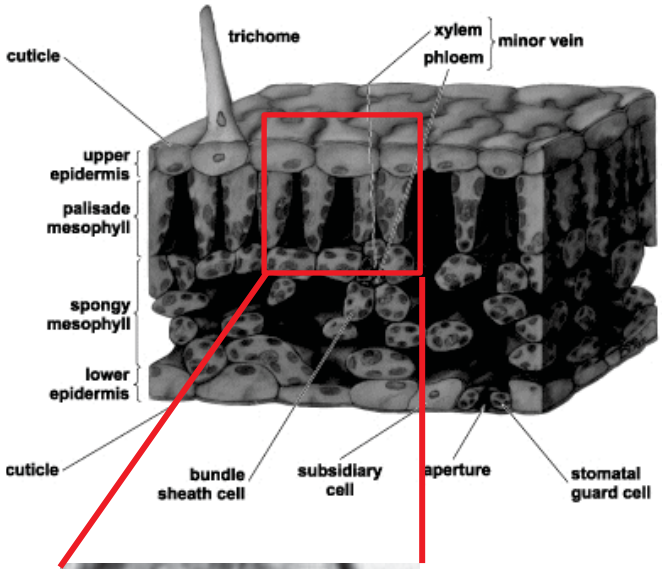
Biotechnology: Al in tea leaves

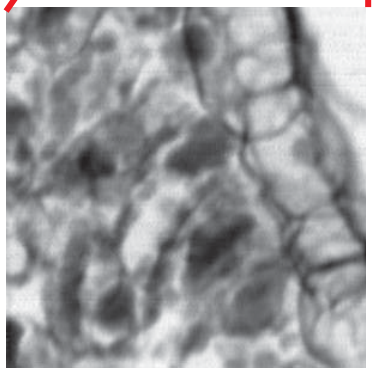
C. Poschenrieder, Uni Barcelona, ES

Katharina. Vogel, University Ljubljana, SI

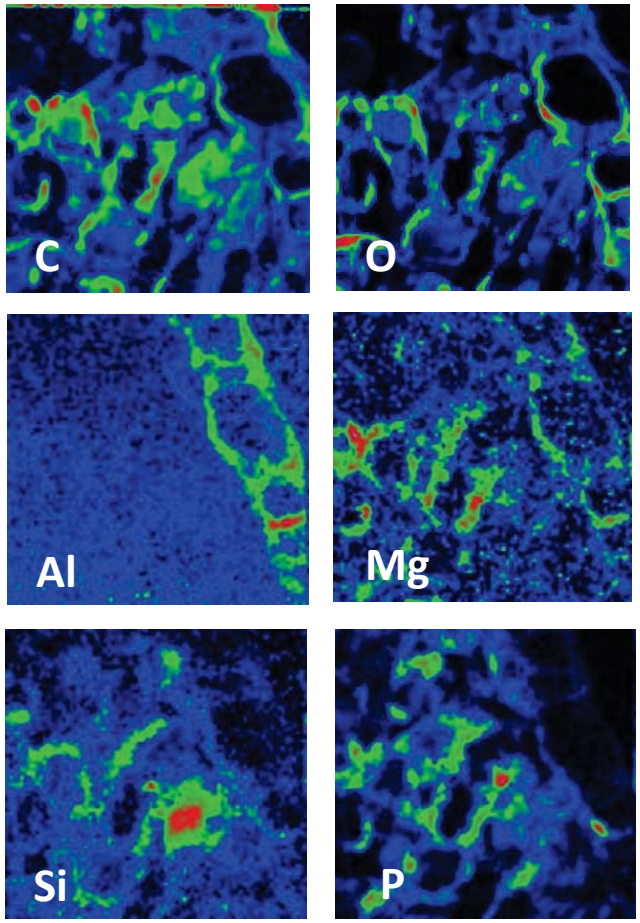
Functionality and toxicity of Al in tea leaves analyzed on sub-cellular level

Cross-section of a leaf





E=2.19 keV
80 x 80 μm^2
80 x 80 px
12s /px



R. Tolra, K. Vogel-Mikus, R. Hajiboland, P. Kump. P. Pongrac, B. Kaulich, A. Gianoncelli, V. Babin, J. Barcelo, M. Regvar, C. Poschenrieder, *Localization of aluminium in tea (Camellia sinensis) leaves using low-energy X-ray fluorescence spectro-microscopy*, J Plant Research 124, 165-172.

Phytoremediation

• **Phytoremediation** consists of mitigating pollutant concentrations in contaminated soil, water or air by means of plants able to contain, degrade, or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives

• Phytoremediation is an emerging technology that employs the use of plants for the clean-up of contaminated environments.

• Progress in the field is however handicapped by limited knowledge of the biological processes involved in plant metal uptake, translocation, tolerance and plant–microbe–soil interactions

• A better understanding of the basic biological mechanisms involved in plant/microbe/soil/contaminant interactions would allow further optimization of phytoremediation technologies

M. Nečemer et al. Spectrochimica Acta Part B 63 (2008) 1240–1247 ; J. A. Howe et al. Environ. Sci. Technol. 2003, 37, 4091-4097.

Localization and Speciation of Chromium in Subterranean Clover

- In order to optimize of phytoremediation as a potential remediation strategy and to assess potential health hazards with this process, it is helpful to understand the mechanisms of Cr uptake, translocation, tolerance, and bonding by plants and the conditions under which Cr is maximally absorbed and/or immobilized.

- The oxidation state of Cr significantly affects its absorption and translocation by plants.

- SXRF microprobe spectroscopy was used to localize Cr in the leaf

- XANES spectroscopy was used to determine the oxidation state of Cr

- XRF and XANES experiments performed at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory, NY, beamline X-26A

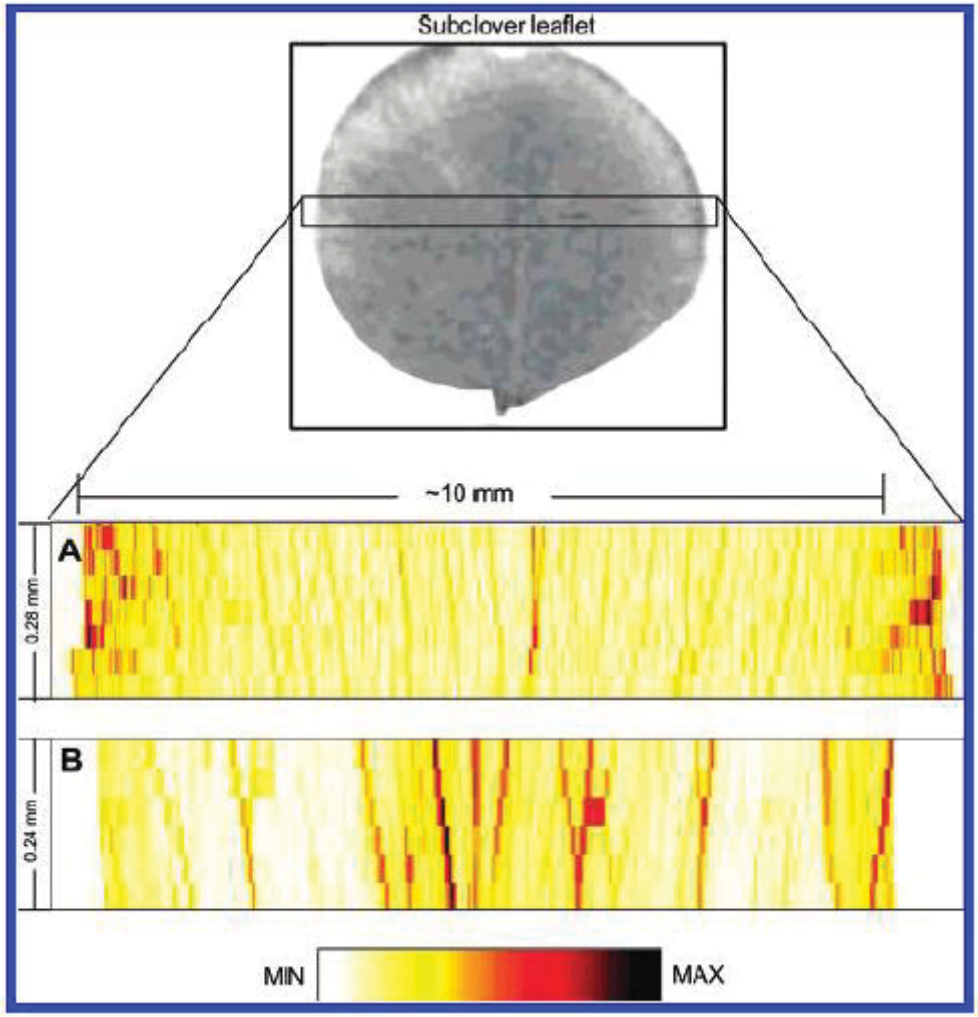
J. A. Howe et al. Environ. Sci. Technol. 2003, 37, 4091-4097.

XRF spectroscopy

Beam size:
10µm x 12µm

Leaves of plants grown in 0.04 mmol Cr(VI) L^{-1} for 21 days

Leaves of plants grown in 1.6 mmol Cr(VI) L^{-1} for 4 days

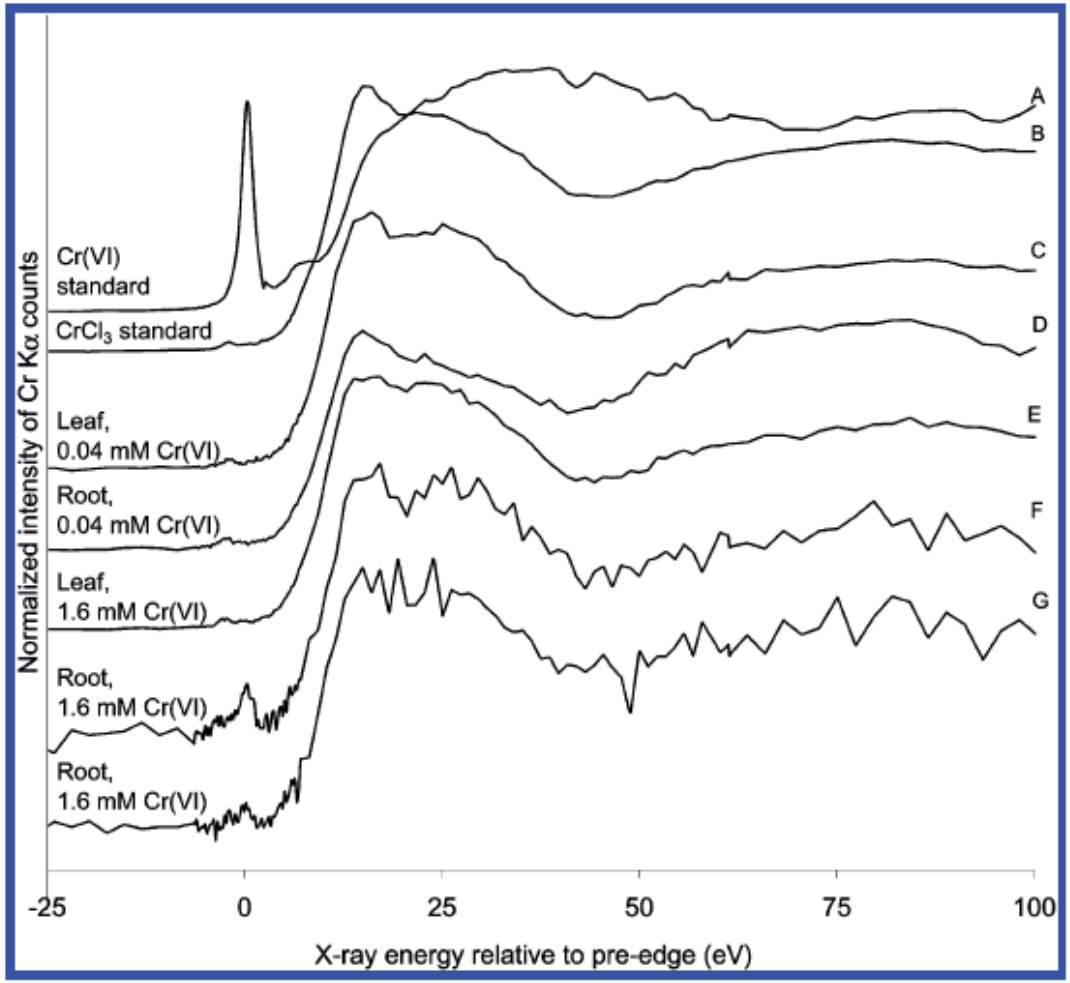


Accumulation Cr at the leaf margins and slightly elevated concentrations in the veins

Highest levels of Cr at the veins, with only slight accumulations at the leaf margins and in areas surrounding the midvein

J. A. Howe et al. Environ. Sci. Technol. 2003, 37, 4091-4097.

XANES spectroscopy



XANES spectra of 5% (w/w) standards of Cr(VI) and Cr(III) and fresh tissue from subclover plants grown with 0.04 and 1.6 mmol Cr(VI) L^{-1}. Spectra F and G are successive scans at the same position on the root.

J. A. Howe et al. Environ. Sci. Technol. 2003, 37, 4091-4097.

XRF and XANES results

- The visual symptom of Cr toxicity of subclover plants grown in 1.6mmolCr(VI) L-1 for 4 days was distinctly different from that at the lower concentration of Cr(VI).
- Plant death occurred within 7-10 days after initiation of the Cr(VI) treatment. The higher concentrations of Cr(VI) apparently interfered with transport processes, which might have contributed to plant death.

- XANES: relatively rapid reduction of Cr(VI), which continued following excision of the tissue from the plant. The **influence of the X-ray beam** on the reduction of Cr(VI) is not known and also might have contributed to the observed reduction of Cr(VI).

- XANES: reduction of Cr(VI) to Cr(III) occurred in or on the roots of subclover plants grown in Cr(VI).
- **Low** treatment **concentrations** of Cr(VI): the plant appears to have been able to quantitatively reduce Cr(VI) to Cr(III).
- **Higher concentrations**: some of the Cr(VI) is still present in the roots. The reduction of Cr(VI) to Cr(III) is an important mechanism of detoxification by the plant.

Conclusions

- Synchrotron radiation offers a big range of analytical techniques for material analysis
- SR offers higher brightness (shorter acquisition time, more sample analysed, better statistics...) and collimated beams
- But limited access to users
- X-ray microscopy with simultaneous acquisition of absorption, phase contrast can be a powerful complementary tool to traditional techniques, especially when combined with XRF and XANES spectroscopies
- XRF analysis can provide useful information related to the distribution of the chemical elements
- XANES spectroscopy combined with X-ray microscopy allows identifying oxidation states of chemical elements
- Suitable sample preparation is crucial