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The TwinMic spectromicroscopy beamline at Elettra: recent achievements and future perspective

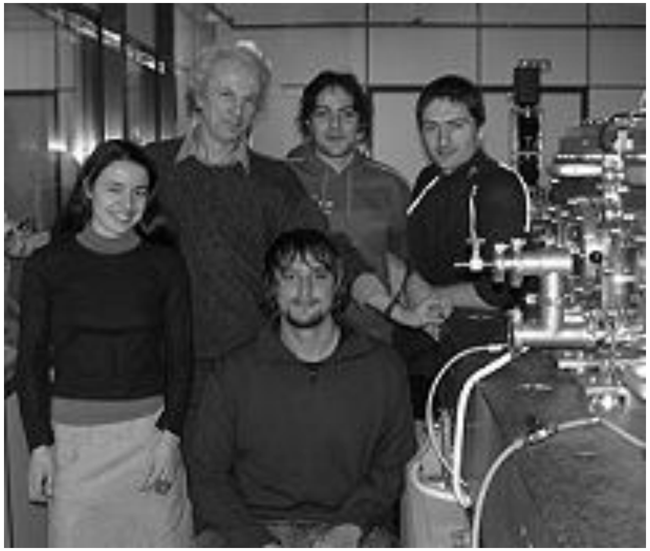
Alessandra Gianoncelli

alessandra.gianoncelli@elettra.eu

Outline

- Brief beamline description
- End-station description
- Life science applications: a few brief examples
- Other application: Material Science and New Imaging Techniques
- Future Upgrades

Open to users in late 2007



The team of the TwinMic project (EC FP5; 2001 – 2004):
ESRF: J. Susini, M. Salome and O. Dhez (F)
SLS: C. David, T. Weitkamp, F. van der Veen (CH)
TASC/ INFM: E. Di Fabrizio, S. Cabrini and D. Cojoc (I)
KCL: G. R. Morrison, P. Charalambous, A. Gianoncelli (UK)
RAC: T. Wilhein and U. Vogt (D)
UNI Goettingen: J. Thieme (D)
IJS: J. Kovac (SLO)

The team that build the TwinMic BL at ELETTRA:
D. Cocco, D. Bacescu, A. Bianco, G. Sostero and D. Lonza

The team that implemented low-energy X-ray emission:
A. Gianoncelli, B. Kaulich (Elettra)
A. Longoni, R. Alberti, T. Klatka et al. (Politecnico Milano)
G. Margaritondo, V. Gajdosik, C. Poitry-Yamate et al. (EPFL Lausanne)

And many many others ...

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TwinMic: Integration of both imaging modes into a single instrument

Spectromicroscopy:
XANES, LEXRF, AEI

↓

STXM →

Brightfield
Darkfield
Differential PC
(simult.)
Nomarski DIC

↑ STXM

400 – 2200 eV

Kinetics, dynamics, 3D

↓

← TXM

Brightfield
Darkfield
Differential PC
Nomarski DIC

↑ TXM

The European team that initiated the project

- Morphological analysis, XANES and AAEI
- Different contrasts incl. brightfield, differential phase and interference contrast, darkfield, etc
- Versatile specimen environment

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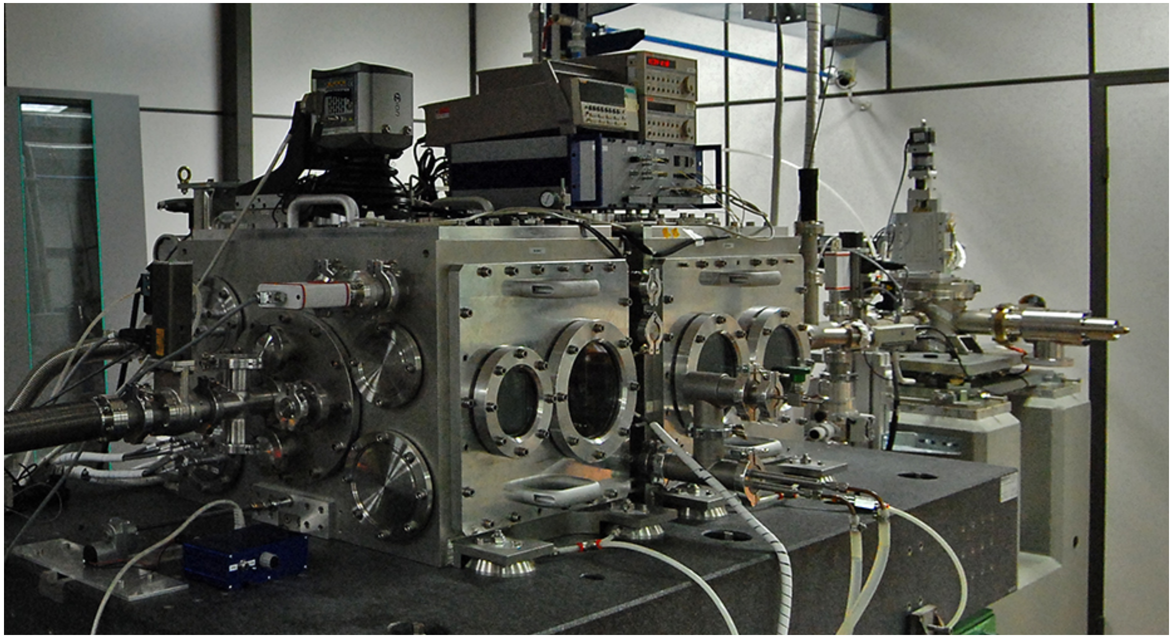
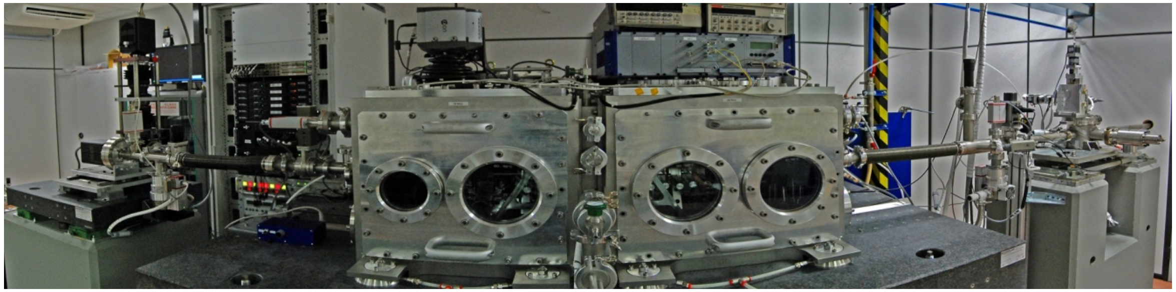
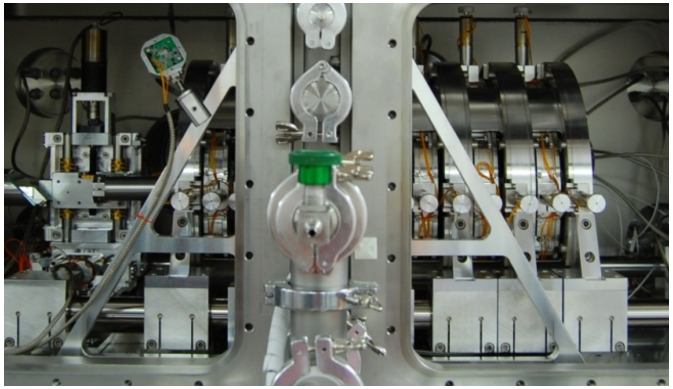
TwinMic microscope

400 – 2200 eV

TwinMic – Combination of scanning and full-field imaging in a single instrument







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

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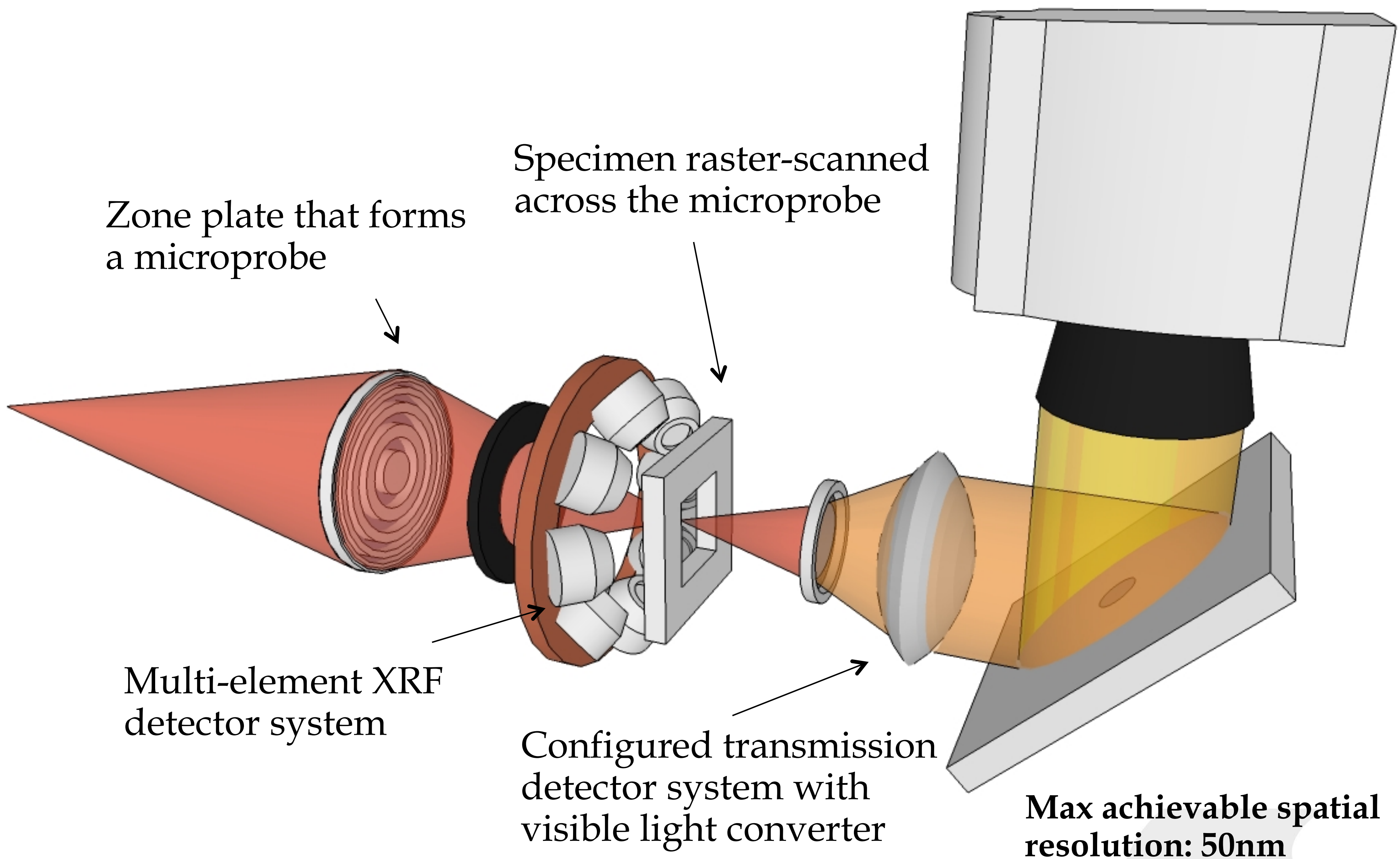
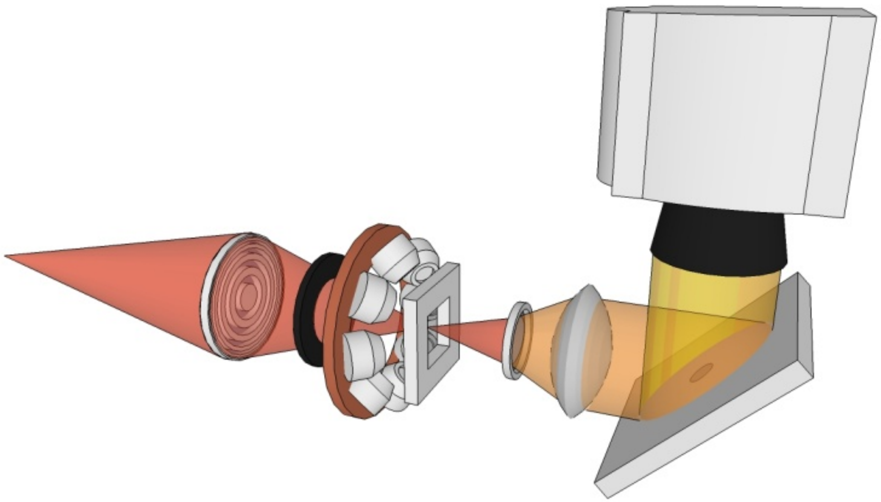
Scanning X-ray microscope (STXM)

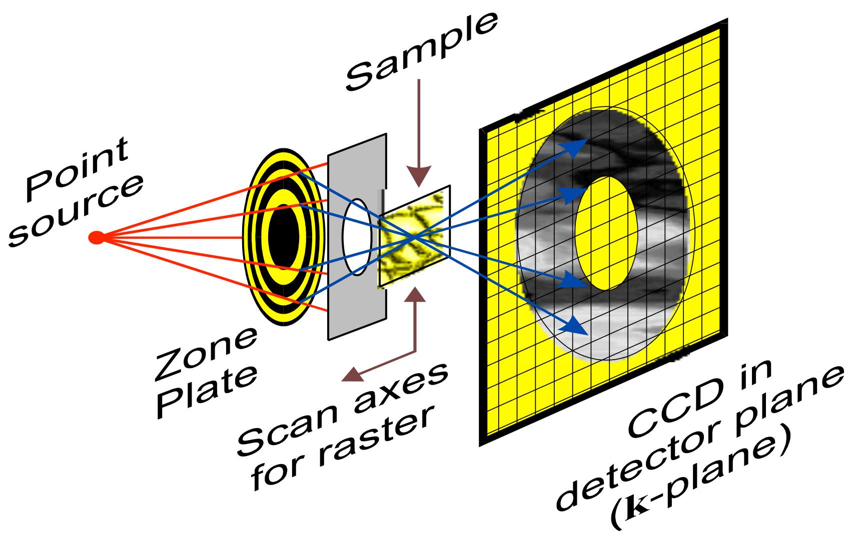


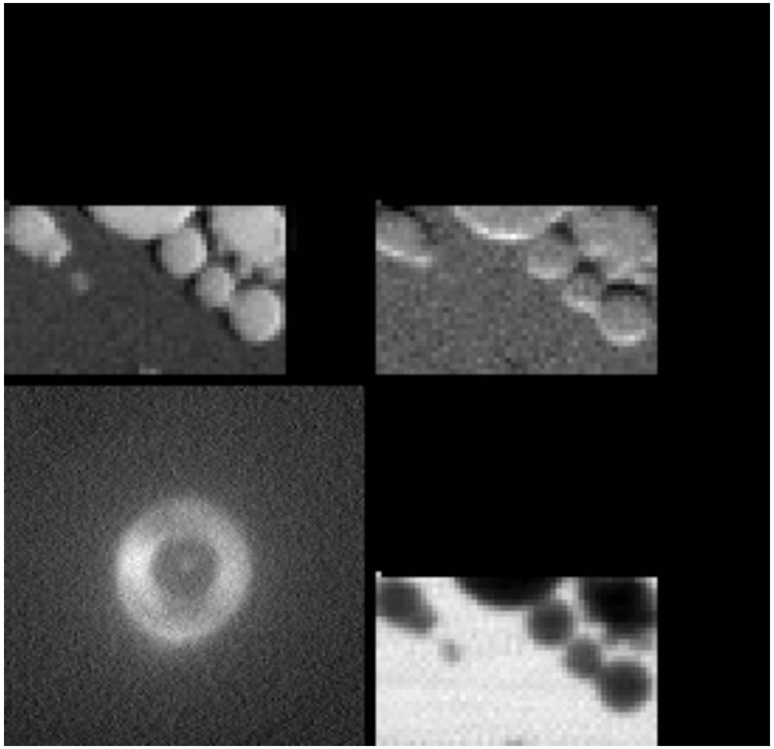
Max achievable spatial resolution: 50nm

STXM mode

Differential phase contrast with a fast read-out CCD camera







Simultaneous acquisition of:

- Absorption or transmission
- Differential phase contrast
- Darkfield images

Morrison, G. et al., IPAP Conf. Series 7, 377-379 (2006)
Gianoncelli A. et al., Appl Phys Lett 89, 251117 (2006)

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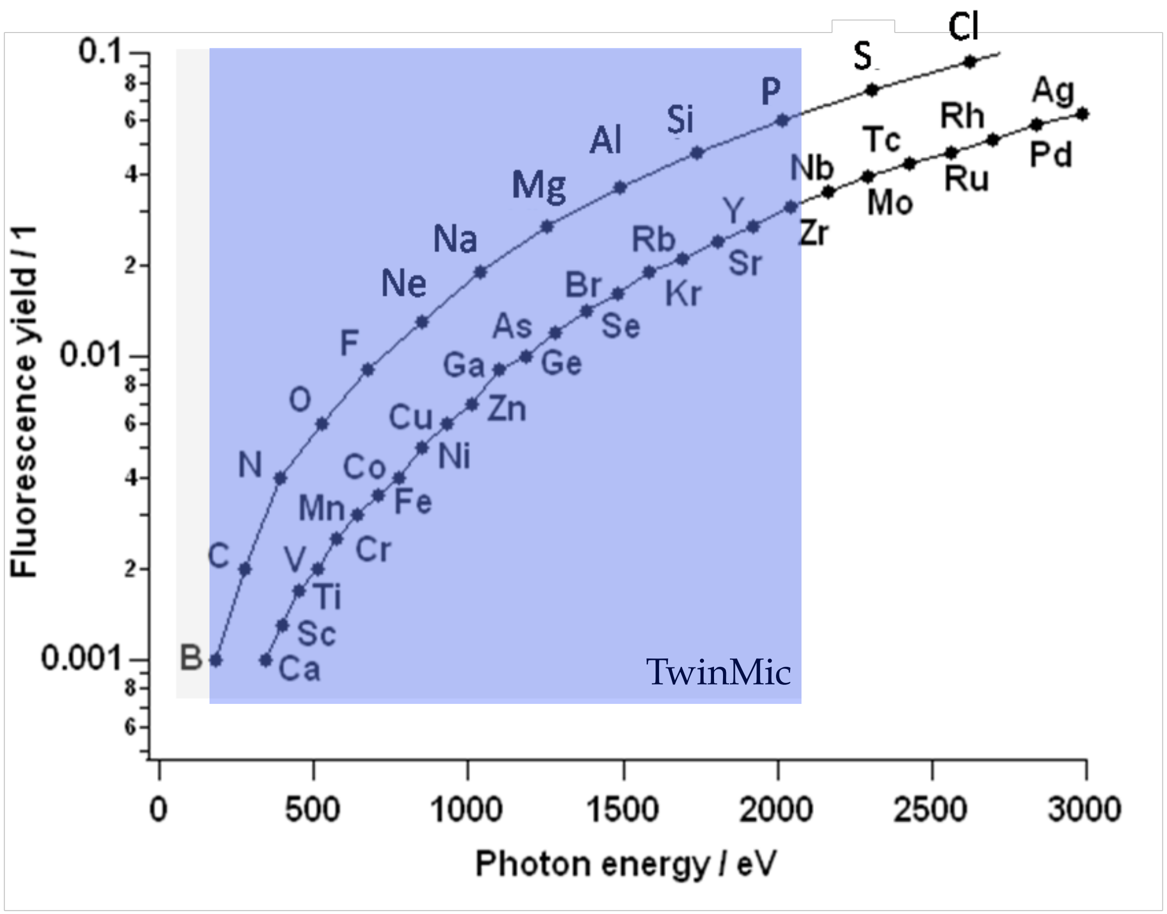
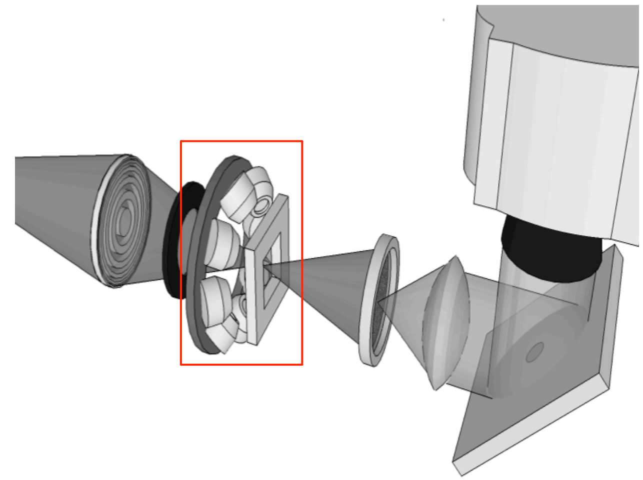
LEXRF

Low-energy X-ray fluorescence for elemental analysis:





Detecting trace elements:

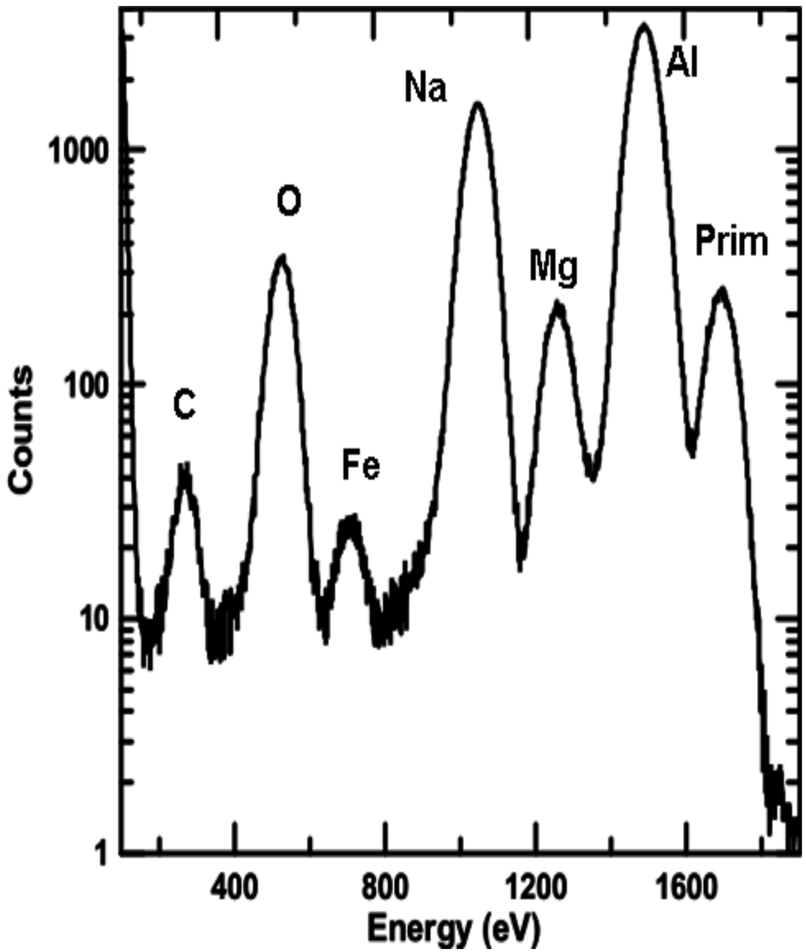
X-ray fluorescence: ~1000x better sensitivity than electrons for trace elemental mapping (ion concentrations etc.).

Low fluorescence yields for soft X-rays! !!

A. Gianoncelli, B. Kaulich, M. Kiskinova, R. Alberti, T. Klatka, A. Longoni, A. de Marco, A. Marcello, Simultaneous Soft X-ray Transmission and Emission Microscopy, Nucl. Instr. and Meth. A 608 (1), 195-198

LEXRF

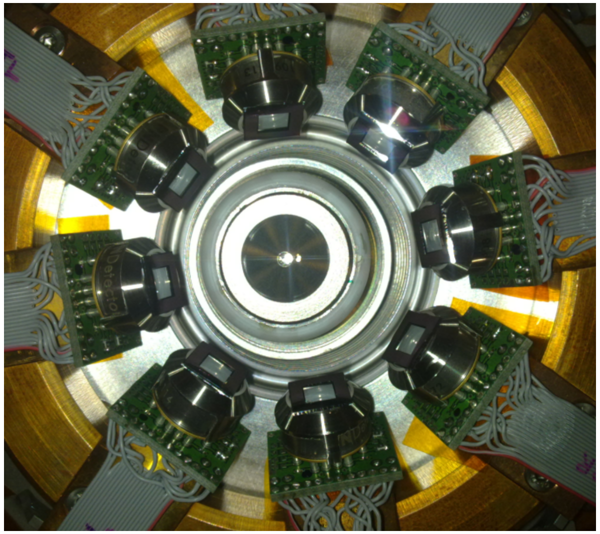
Low-energy X-ray fluorescence:

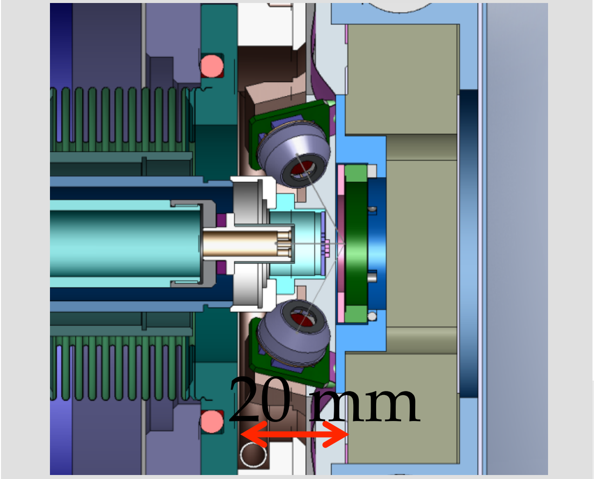


TwinMic LEXRF spectrum with unfocused beam of a test organic matrix on a metal shim

Dynamic range: up to 30 kcounts/s

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





A. Gianoncelli, B. Kaulich, M. Kiskinova, R. Alberti, T. Klatka, A. Longoni, A. de Marco, A. Marcello, Simultaneous Soft X-ray Transmission and Emission Microscopy, Nucl. Instr. and Meth. A 608 (1), 195-198

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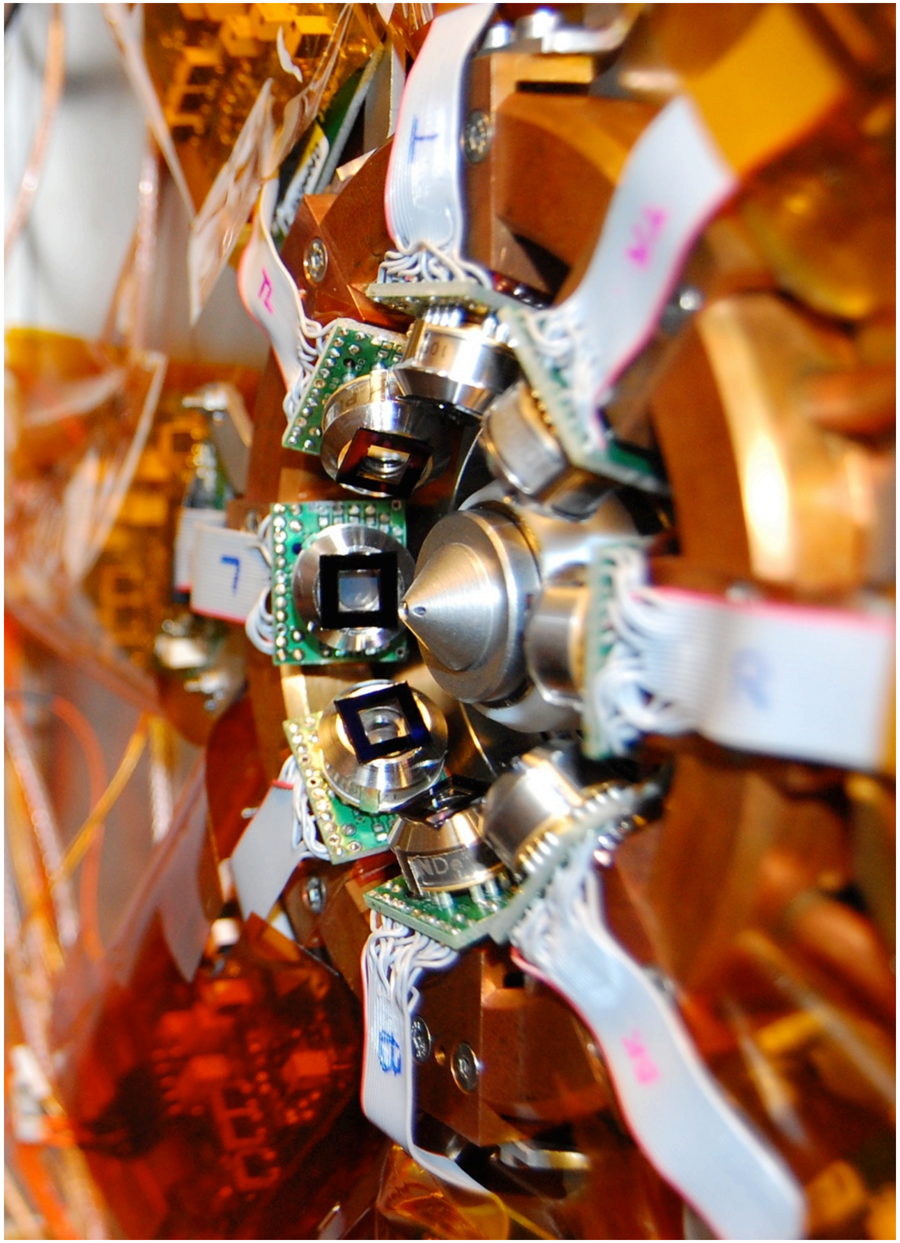
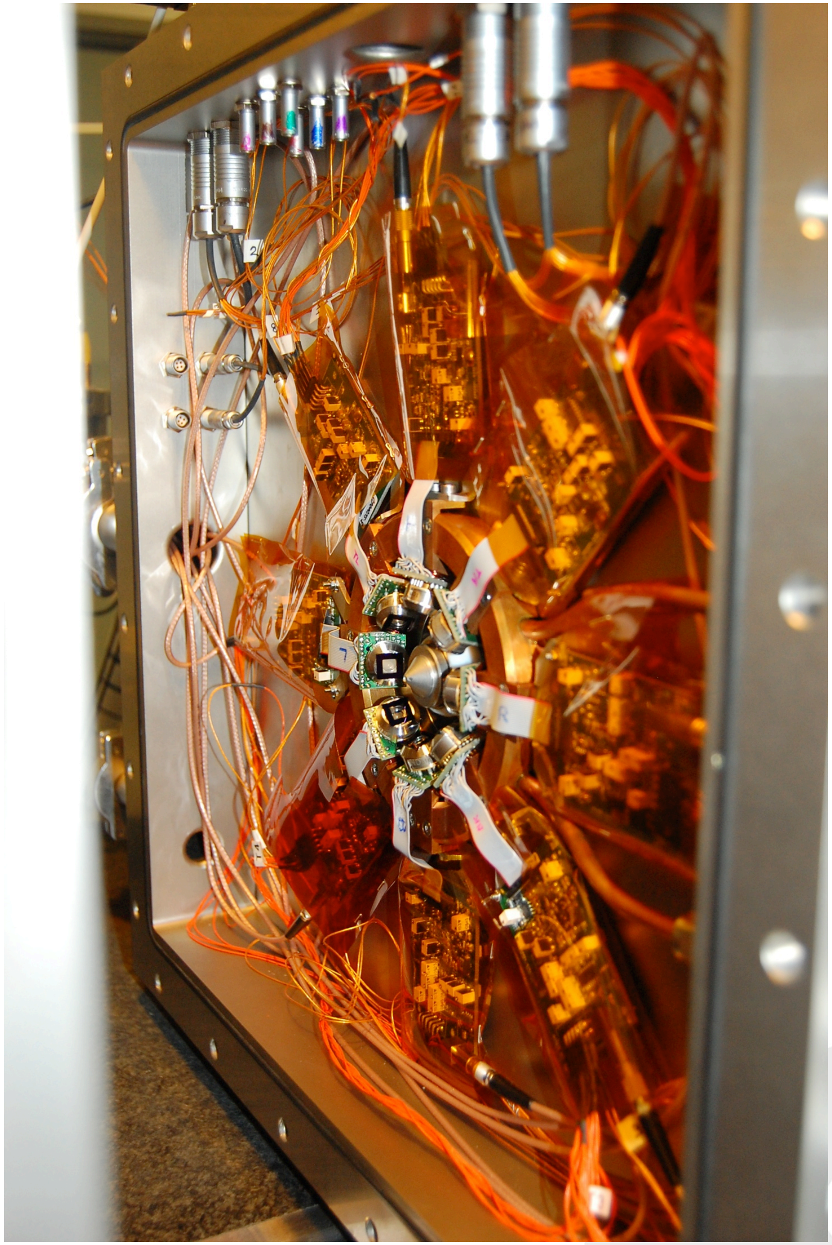
LEXRF





Aluminium toxicity

Soluble Al – “the most important growth-limiting factor for plants in most strongly acid soils and mine spoils” **Foy (1984)**

Acid soils occupy ~ 40 billion hectares (~ 30 %) of the world's ice free land area **von Uexküll and Mutert (1995)**

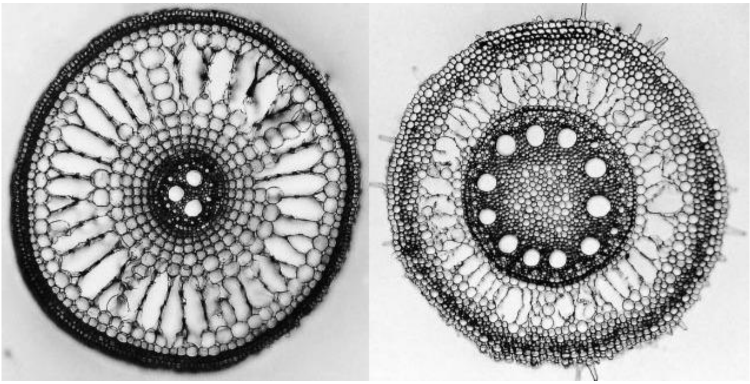
In Australia alone, acid soils cost $1.5 billion p.a. in lost productivity

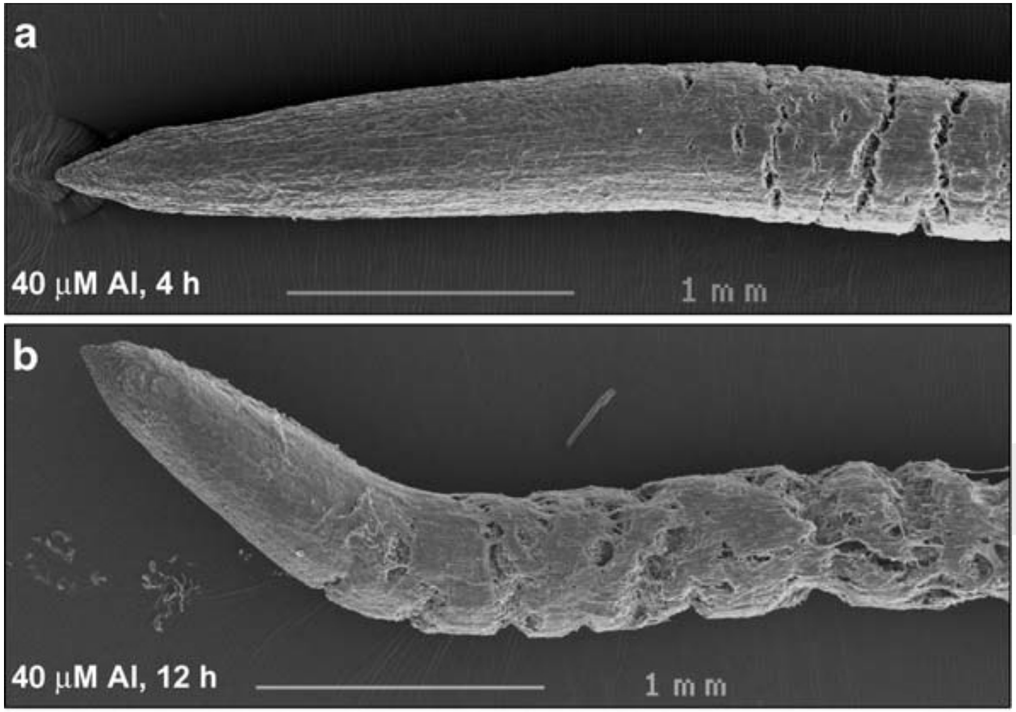
Aluminium toxicity

Soluble Al – "the most important growth-limiting factor for plants in most strongly acid soils and mine spoils" **Foy (1984**)

Although known since 1904 that Al is the primary factor causing a reduction in plant root growth in acid soils, the mechanism by which Al is toxic remains unclear

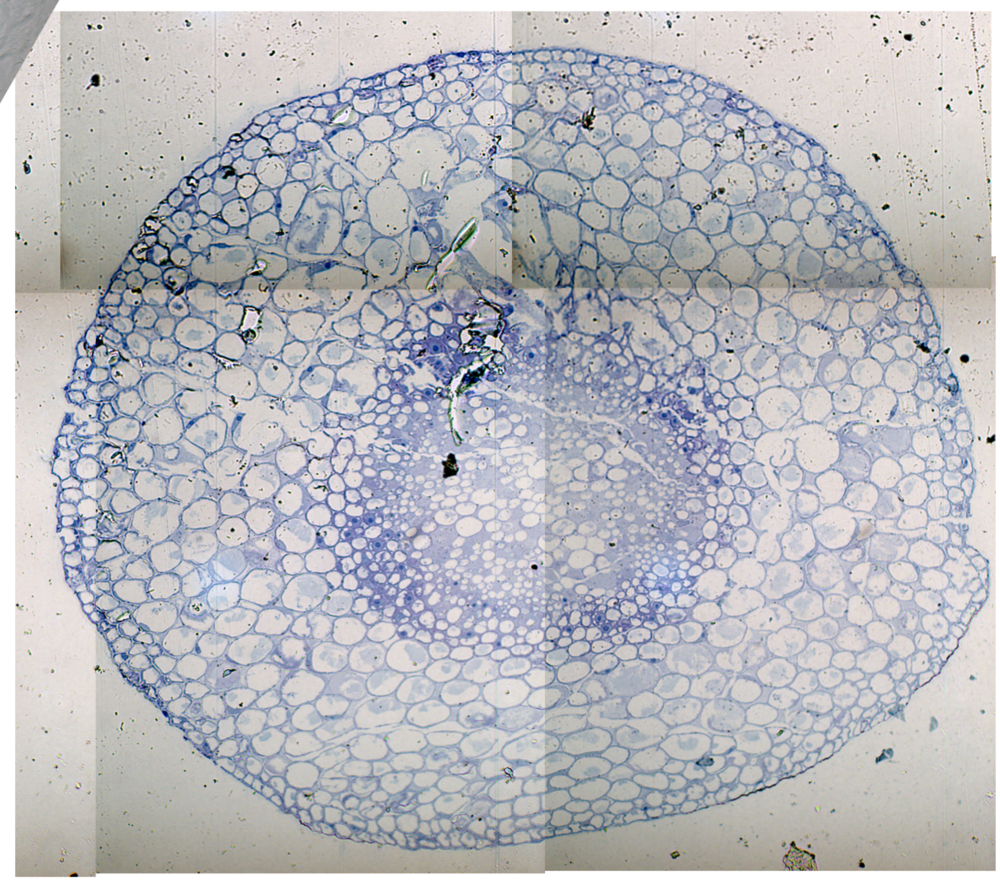
Recent research (2014) has shown that Al exerts its toxic effects very quickly, reducing root growth in ≤ 30 min. Therefore, a crucial step in elucidating how Al exerts its toxic effects is to examine where the Al is accumulating within the roots





Aluminum toxicity

30 minutes, 6 mm, Sample 1



P. M. Kopittke, K. L. Moore , E. Lombi et al, "**Identification of the Primary Lesion of Toxic Aluminum in Plant Roots**" Plant Physiology, 2015, 167, 140

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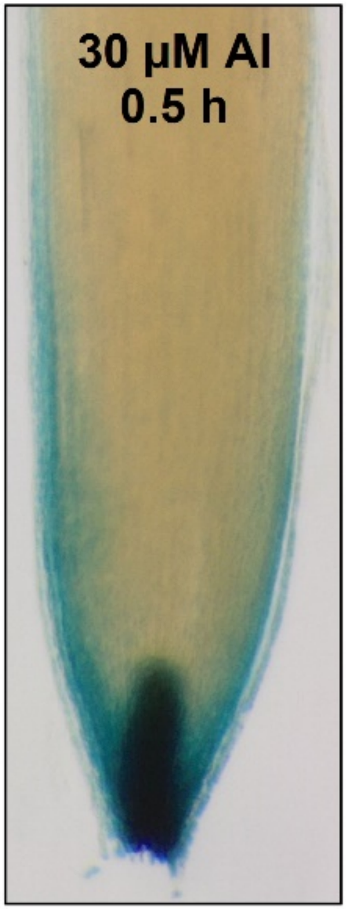
7 μm-thick transverse cross section of soybean roots

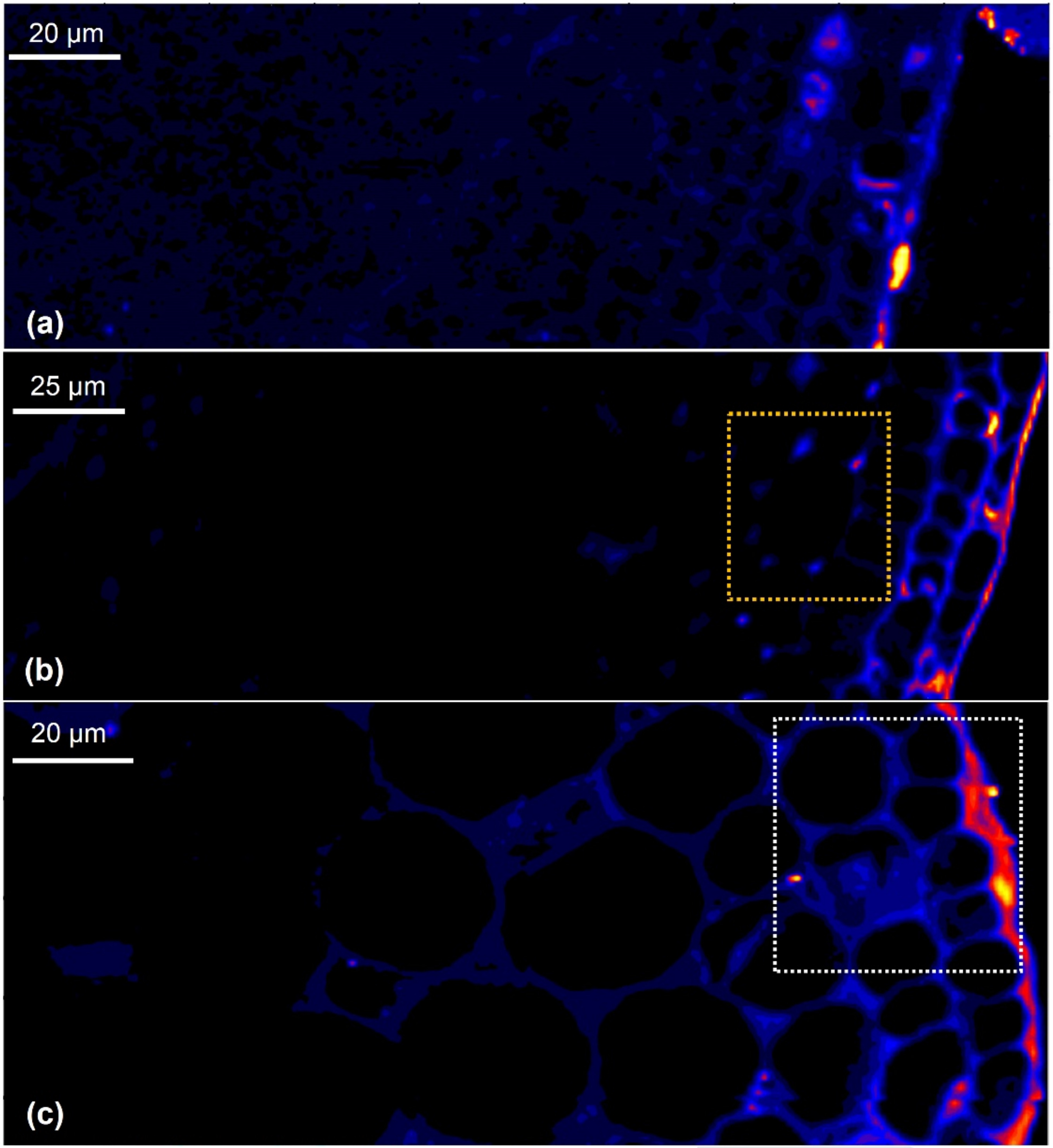
Exposed to 30 μM Al for 0.5 h.



c) 6mm from apex

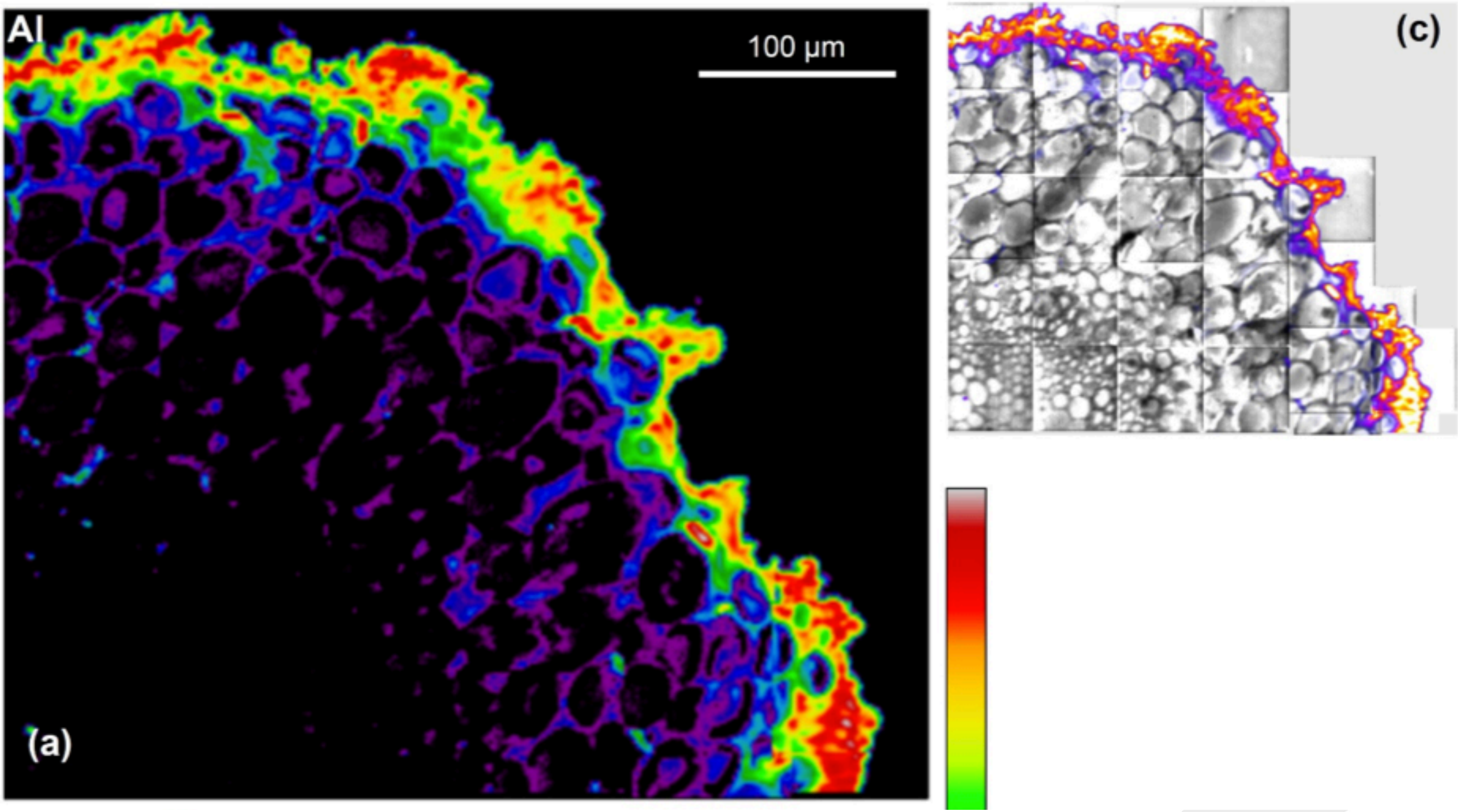
b) 2mm from Apex

a) 0.5 mm from Apex



P. M. Kopittke, K. L. Moore , E. Lombi et al, "**Identification of the Primary Lesion of Toxic Aluminum in Plant Roots**" Plant Physiology, 2015, 167, 140

Soybean roots exposed to 30 μM Al for 0.5 h



P.M. Kopittke et al "**Identification of the Primary Lesion of Toxic Aluminum in Plant Roots**" Plant Physiology 2015, 167, 140

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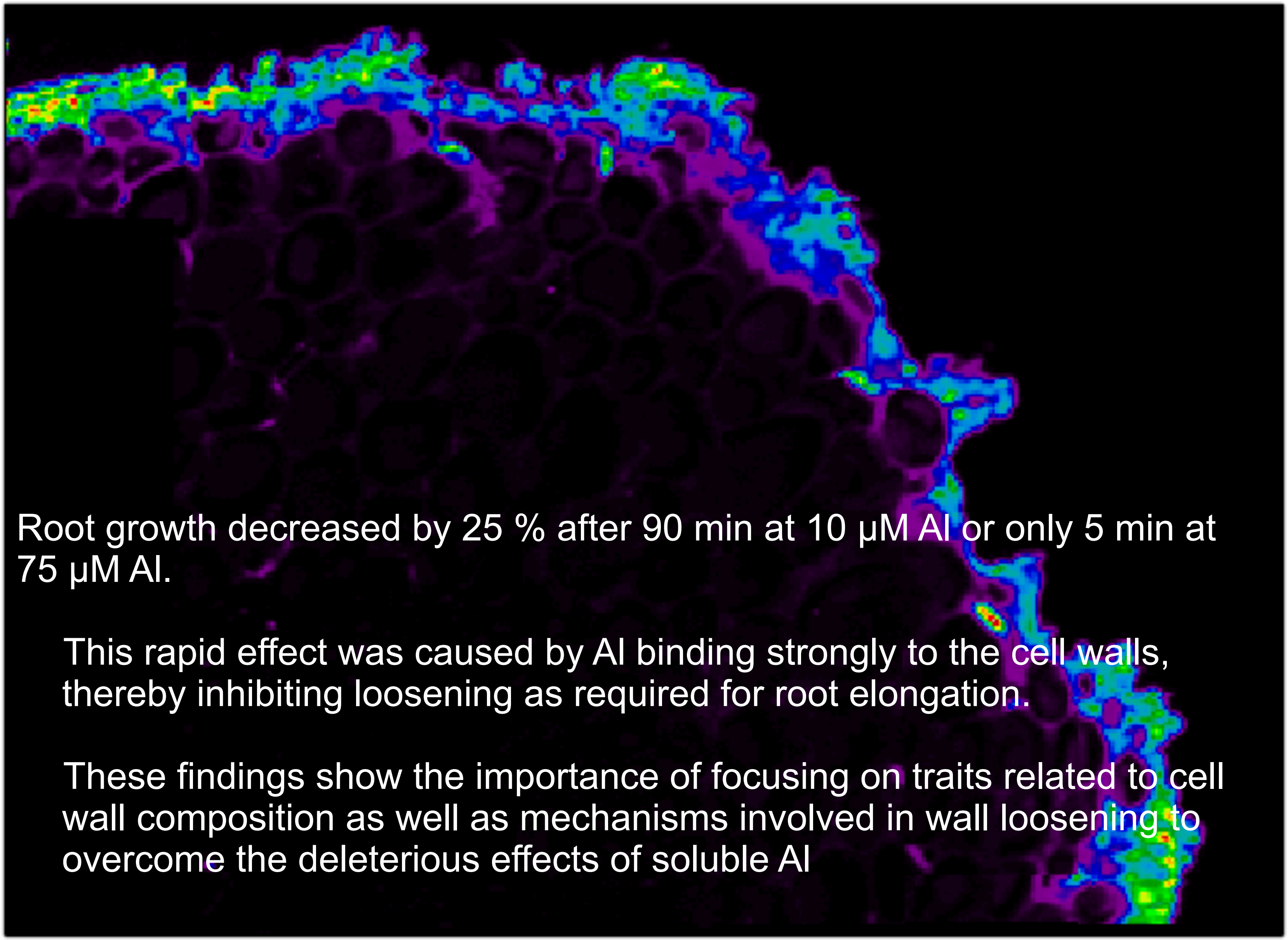
Soybean roots exposed to 30 µM Al for 0.5 h

Al



Root growth decreased by 25 % after 90 min at 10 µM Al or only 5 min at 75 µM Al.

This rapid effect was caused by Al binding strongly to the cell walls, thereby inhibiting loosening as required for root elongation.

These findings show the importance of focusing on traits related to cell wall composition as well as mechanisms involved in wall loosening to overcome the deleterious effects of soluble Al

P.M. Kopittke et al “**Identification of the Primary Lesion of Toxic Aluminum in Plant Roots**” Plant Physiology 2015, 167, 140

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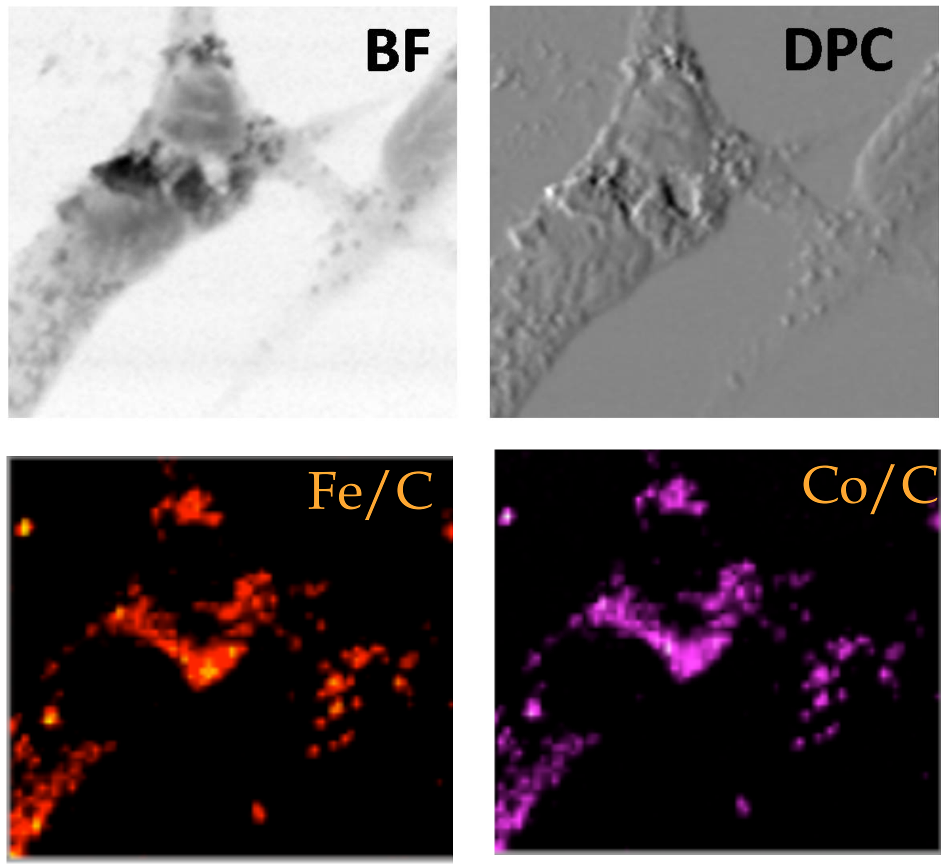
Nanotoxicology

Cellular distribution and degradation of $CoFe_2O_4$ NPs in Balb/3T3 Fibroblast cells

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

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



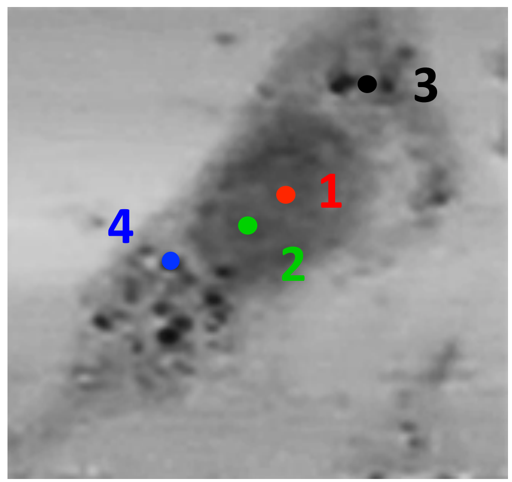
$CoFe_2O_4$ in mouse 3T3 fibroblast cells, E=2019 eV, 60um x 60 um

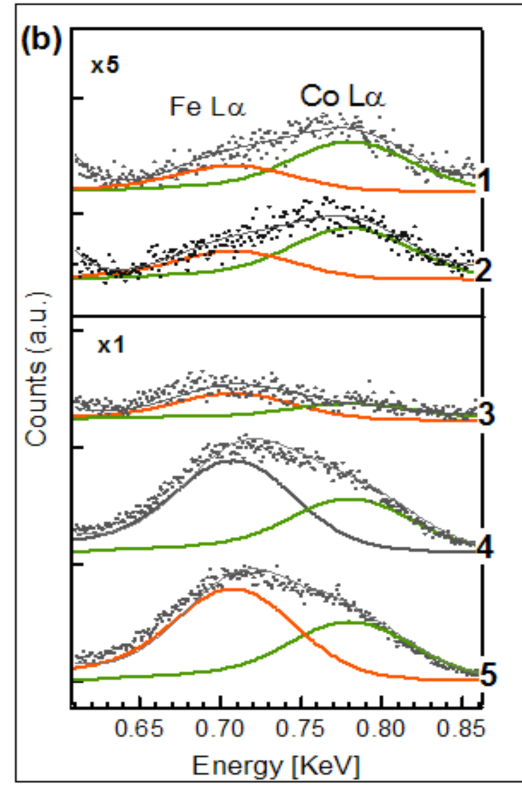
P. Marmorato, G. Ceccone, A. Gianoncelli, L. Pascolo, J. Ponti, F. Rossi, M. Salomé, B. Kaulich, and M. Kiskinova, *Cellular distribution and degradation of Cobalt Ferrite Nanoparticles in Balb/3T3 Fibroblasts*, Toxicology Letters, 2011, 207 - 2, 128-136.

Balb/3T3 exposed to 1000mM

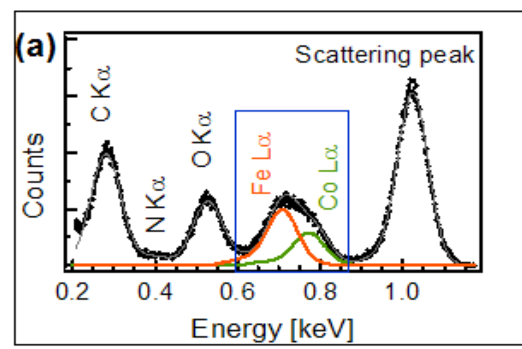
Fe/Co~0.8

Fe/Co~1.5





Similar behaviour (but less evident) in the nuclear region for 500µM concentration



P. Marmorato, G. Ceccone, A. Gianoncelli, L. Pascolo, J. Ponti, F. Rossi, M. Salomé, B. Kaulich, and M. Kiskinova, *Cellular distribution and degradation of Cobalt Ferrite Nanoparticles in Balb/3T3 Fibroblasts*, Toxicology Letters, 2011, 207 - 2, 128-136.

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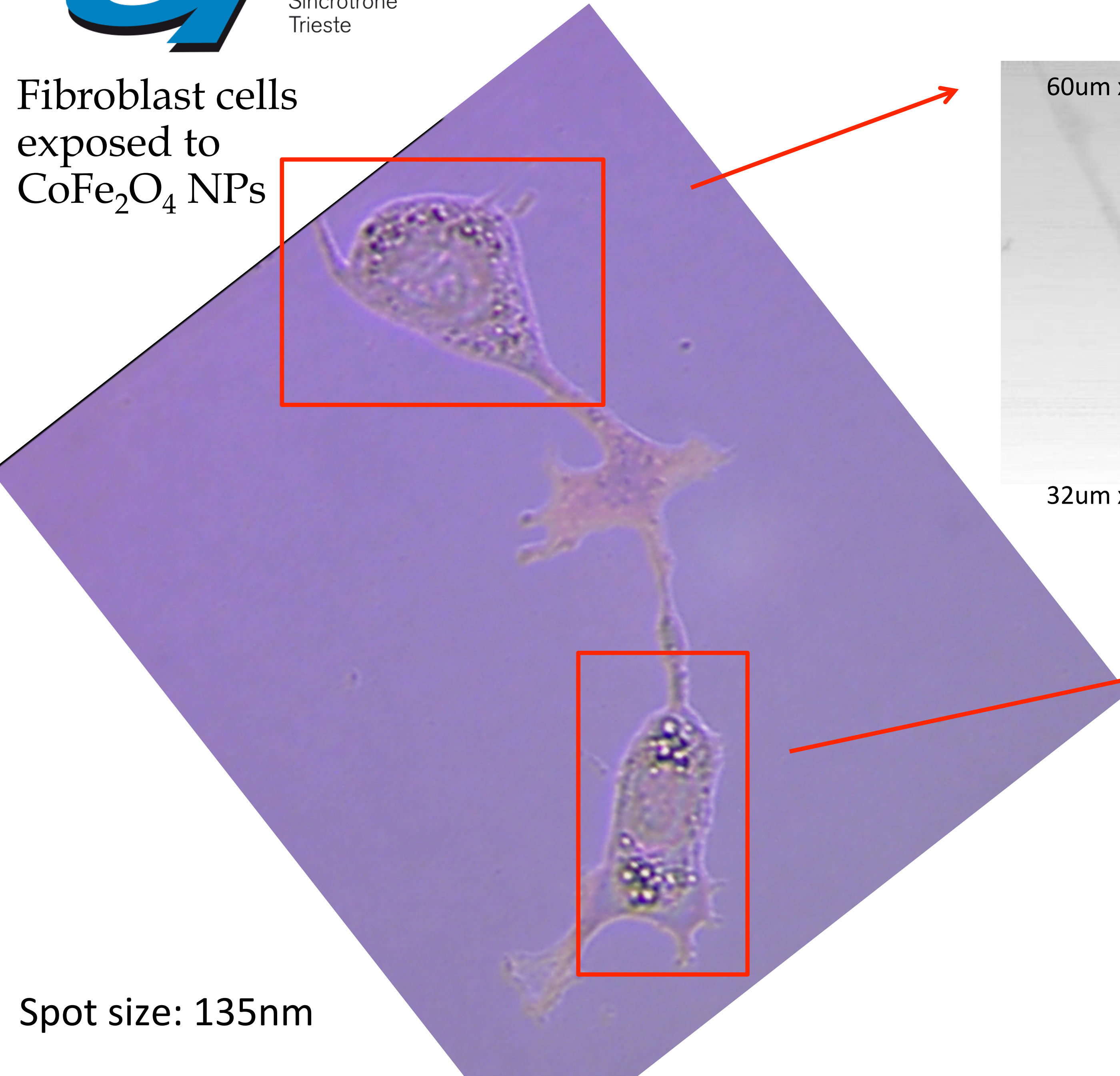
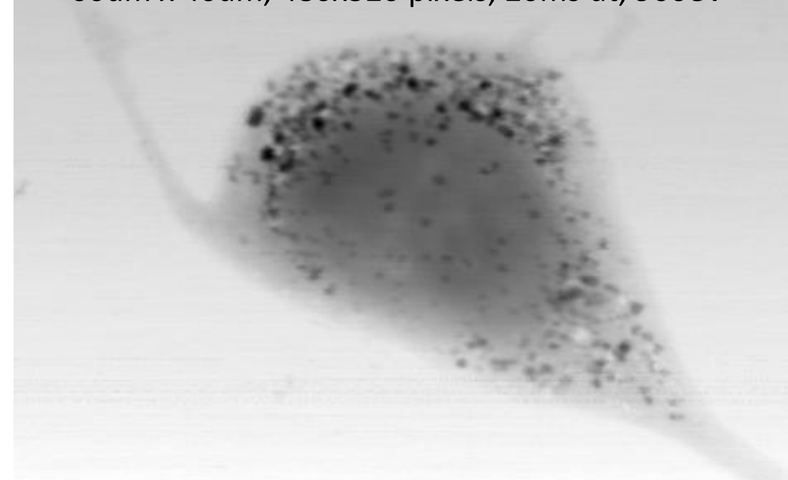
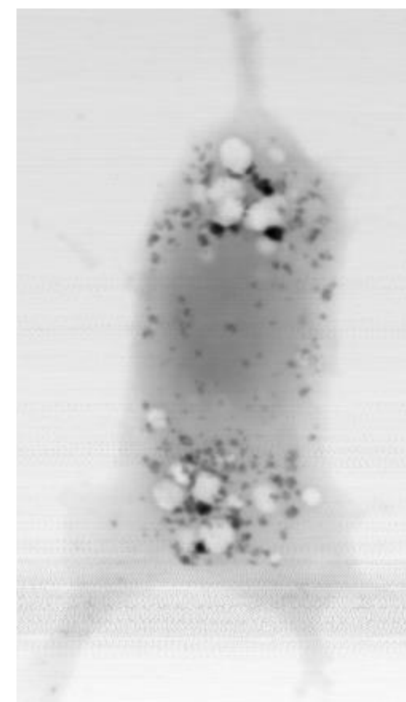
Fibroblast cells exposed to $CoFe_2O_4$ NPs



60um x 40um, 480x320 pixels, 20ms dt, 900eV



32um x 60um, 256x480 pixels, 20ms dt, 900eV



Spot size: 135nm

High-resolution scanning transmission soft X-ray microscopy for rapid probing of nanoparticle distribution and sufferance features in exposed cells Kourousias G, Pascolo L, Marmorato P, Ponti J, Ceccone G, Kiskinova M, Gianoncelli A *X-Ray Spectrometry (2015)*

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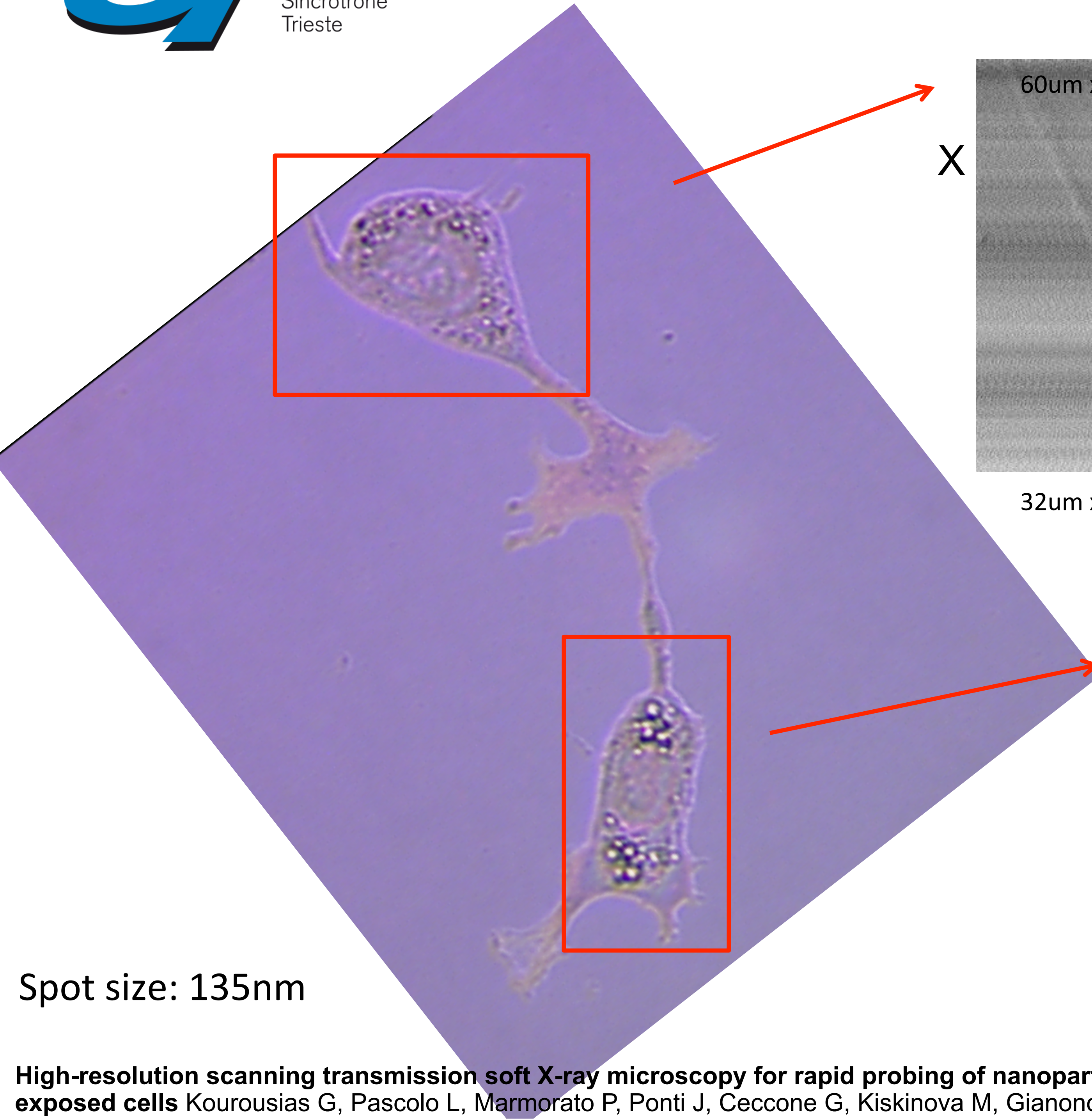
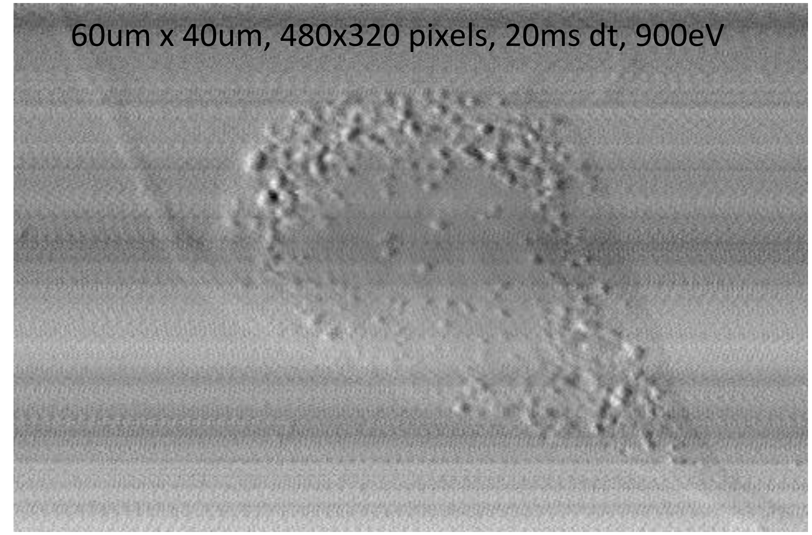
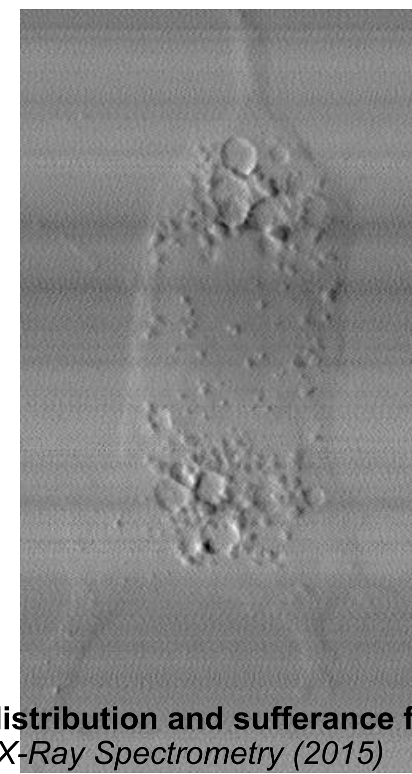




32um x 60um, 256x480 pixels, 20ms dt, 900eV



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Red oil specifically stains lipids

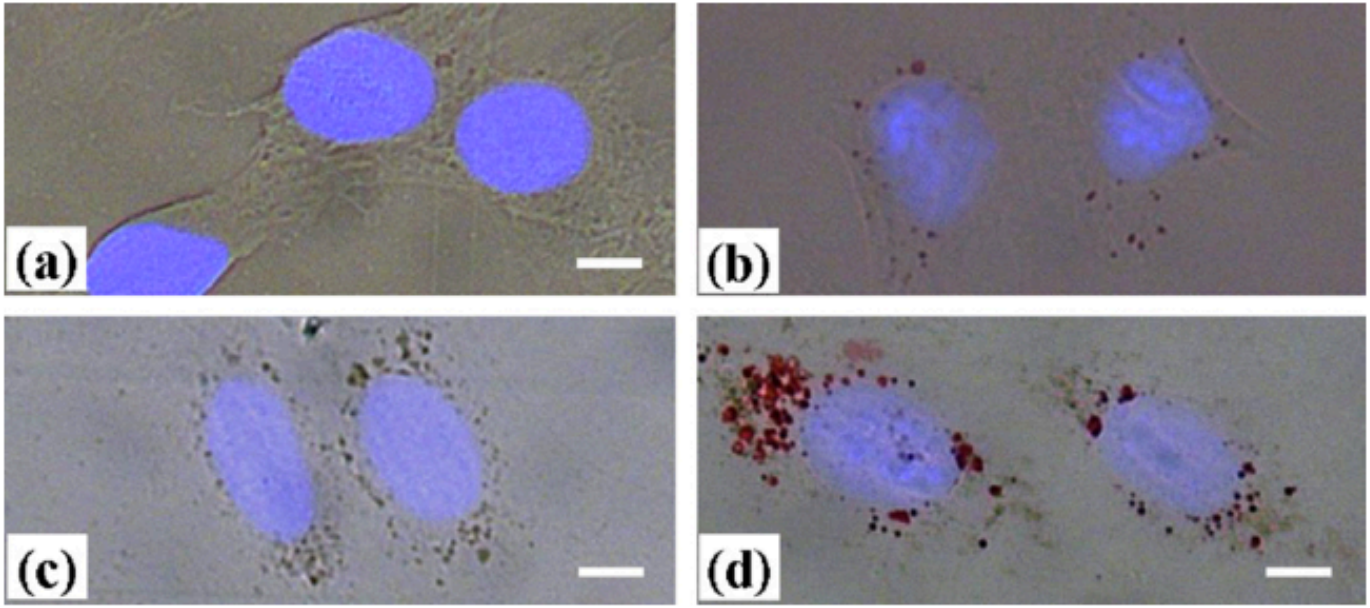
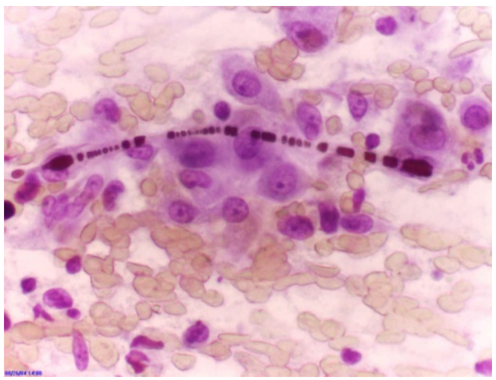


Fig. 6. Optical images of Balb/3T3 control cells (a and b) and incubated for 24 h with 500 µM $CoFe_2O_4$ NPs suspension (c and d). Red spots represent lipids stained by Red Oil O solution (b and d) whilst nuclei are stained in blue by Hoechst. Bar = 10 µm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

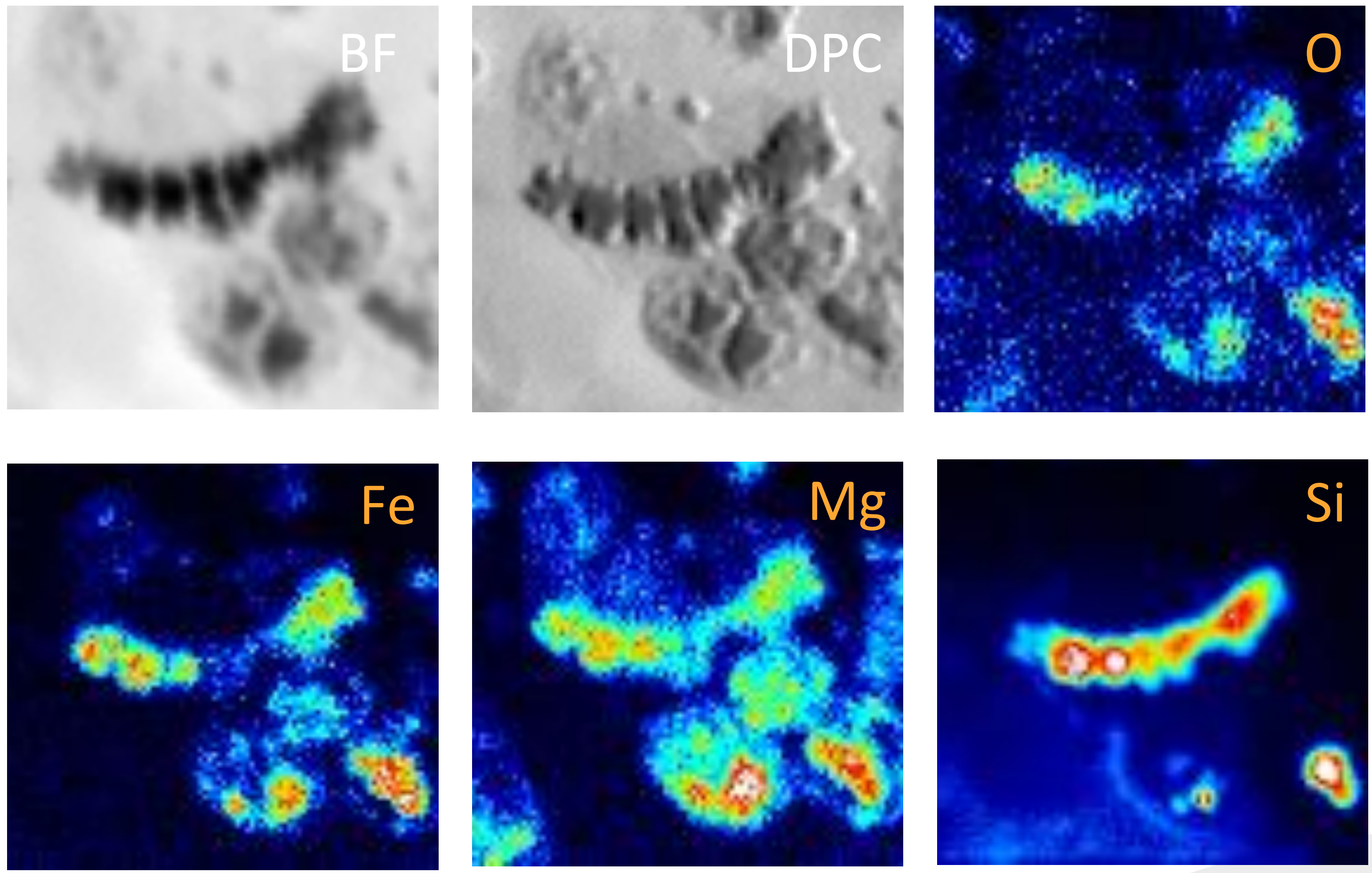
P. Marmorato, G. Ceccone, A. Gianoncelli, L. Pascolo, J. Ponti, F. Rossi, M. Salomé, B. Kaulich, and M. Kiskinova, *Cellular distribution and degradation of Cobalt Ferrite Nanoparticles in Balb/3T3 Fibroblasts*, Toxicology Letters, 2011, 207 - 2, 128-136.

Exposure to Asbestos



L. Pascolo, M. Melato, Burlo Hospital, Trieste, Italy

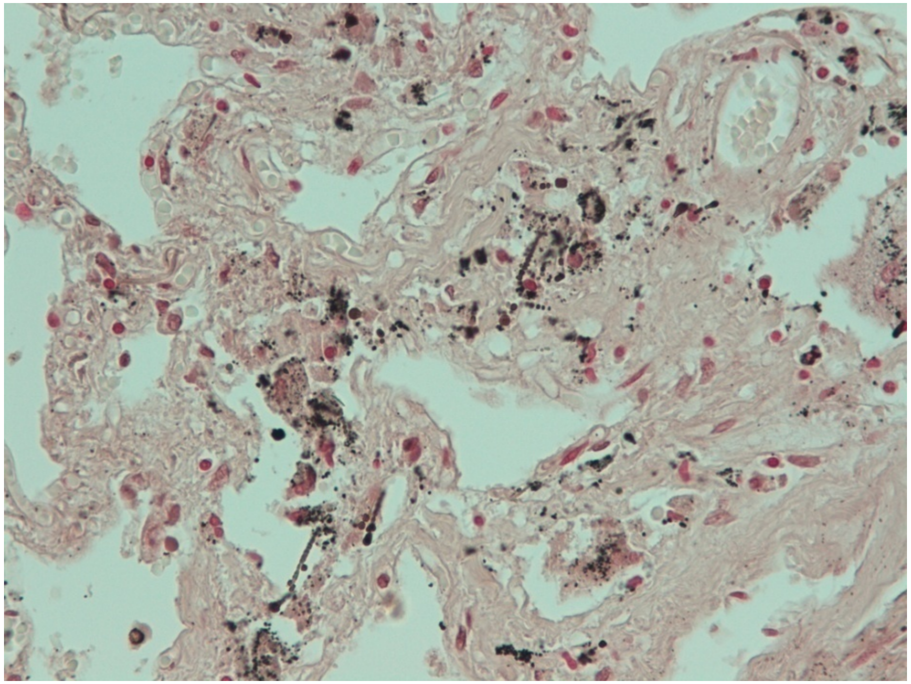
Mesothelioma and differentiation of lung tissue due to asbestos; the role of Mg

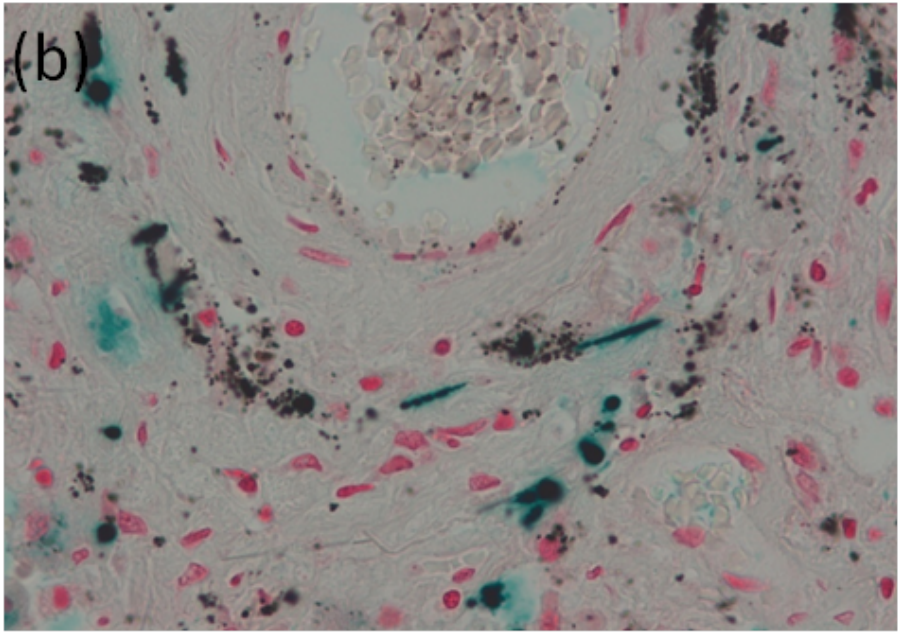


E=2019 eV, 50mm x 50 mm, 100 x 100 pixels, 15s/pixel LEXRF, 4 SDDs

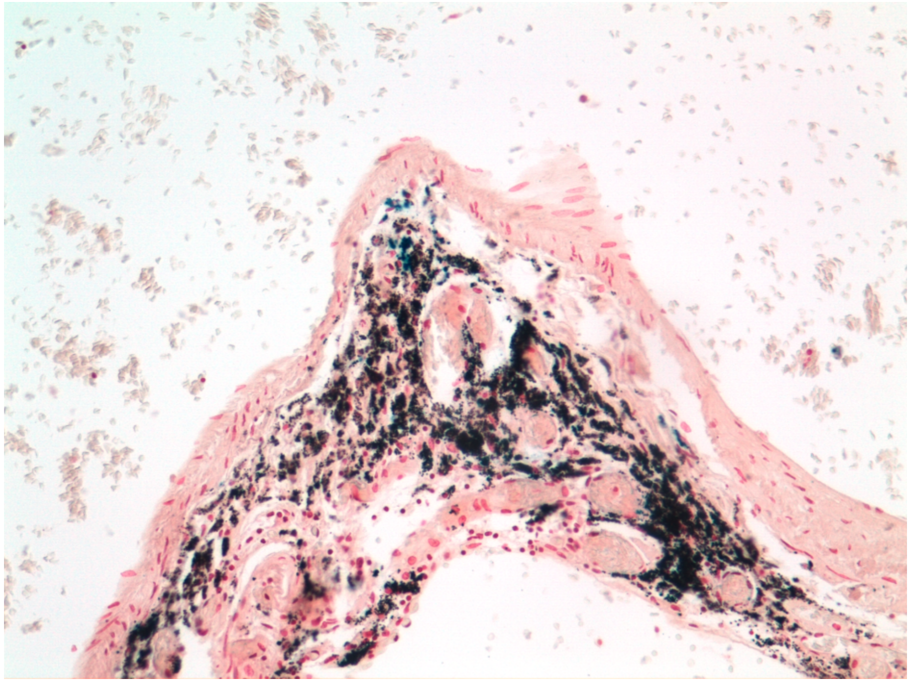
L. Pascolo, A. Gianoncelli, et al. Particle and Fibre Toxicology 2011, 8:7.
L. Pascolo, A. Gianoncelli, et al. Scientific Reports 2013, 3.

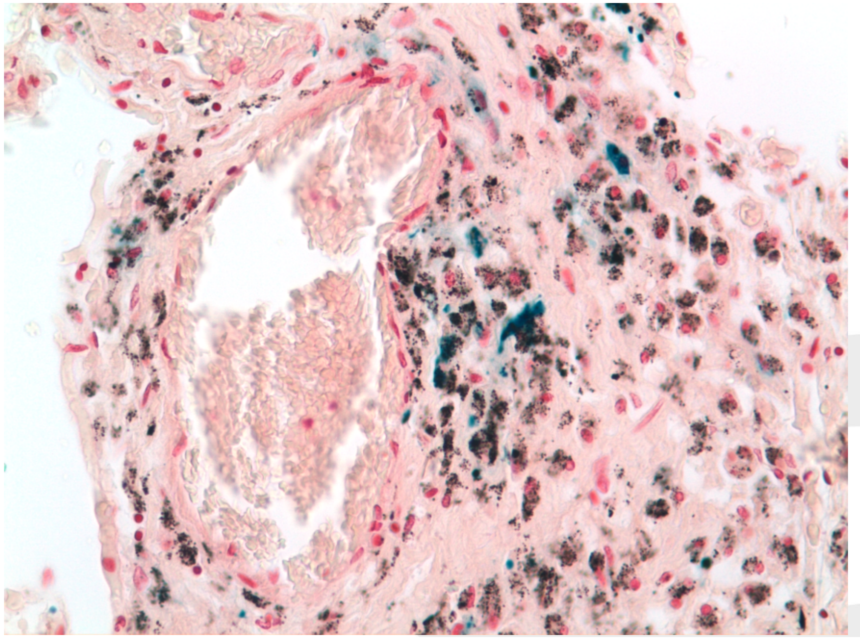
Asbestos and anthracosis



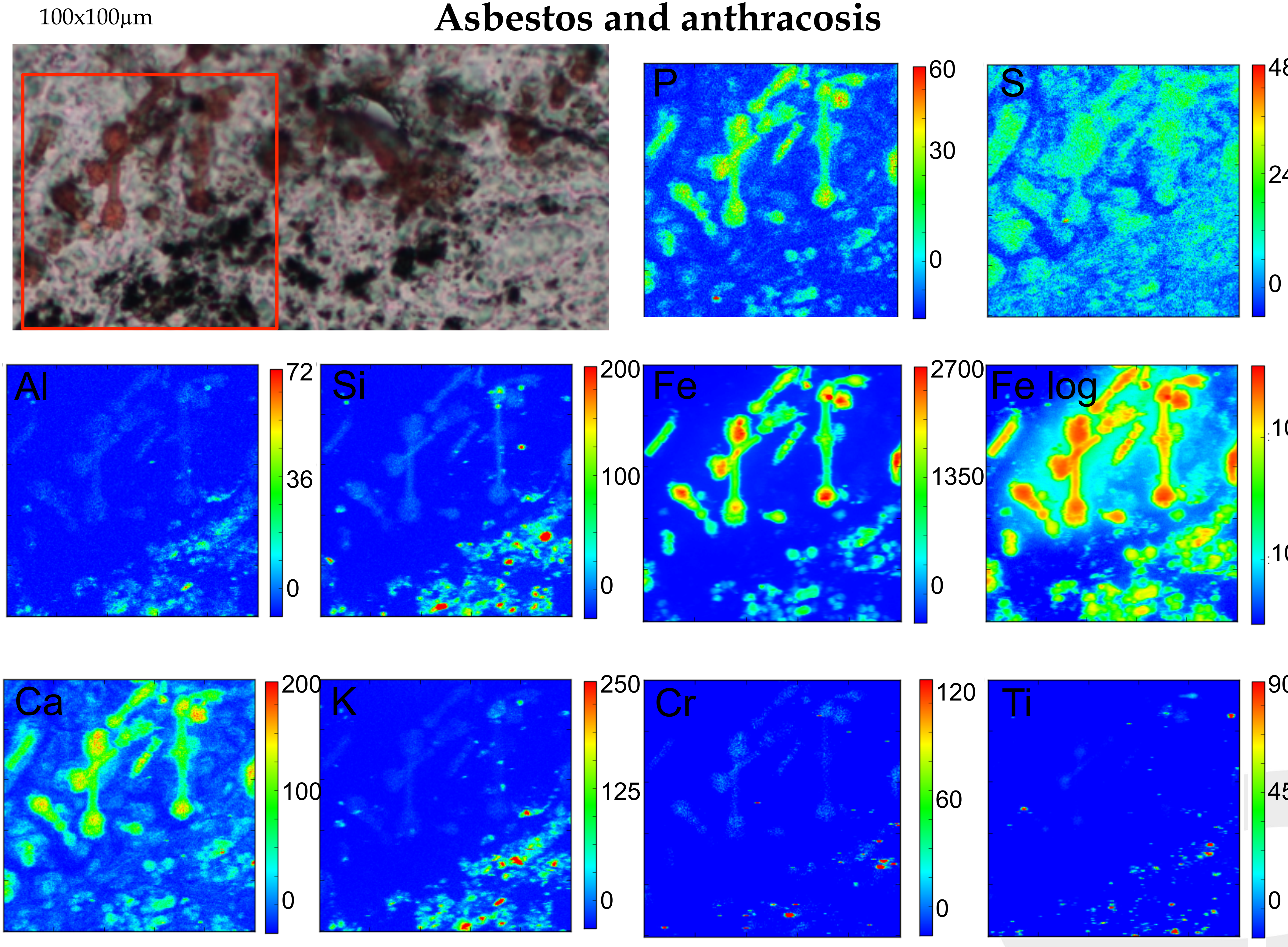


Anthracosis

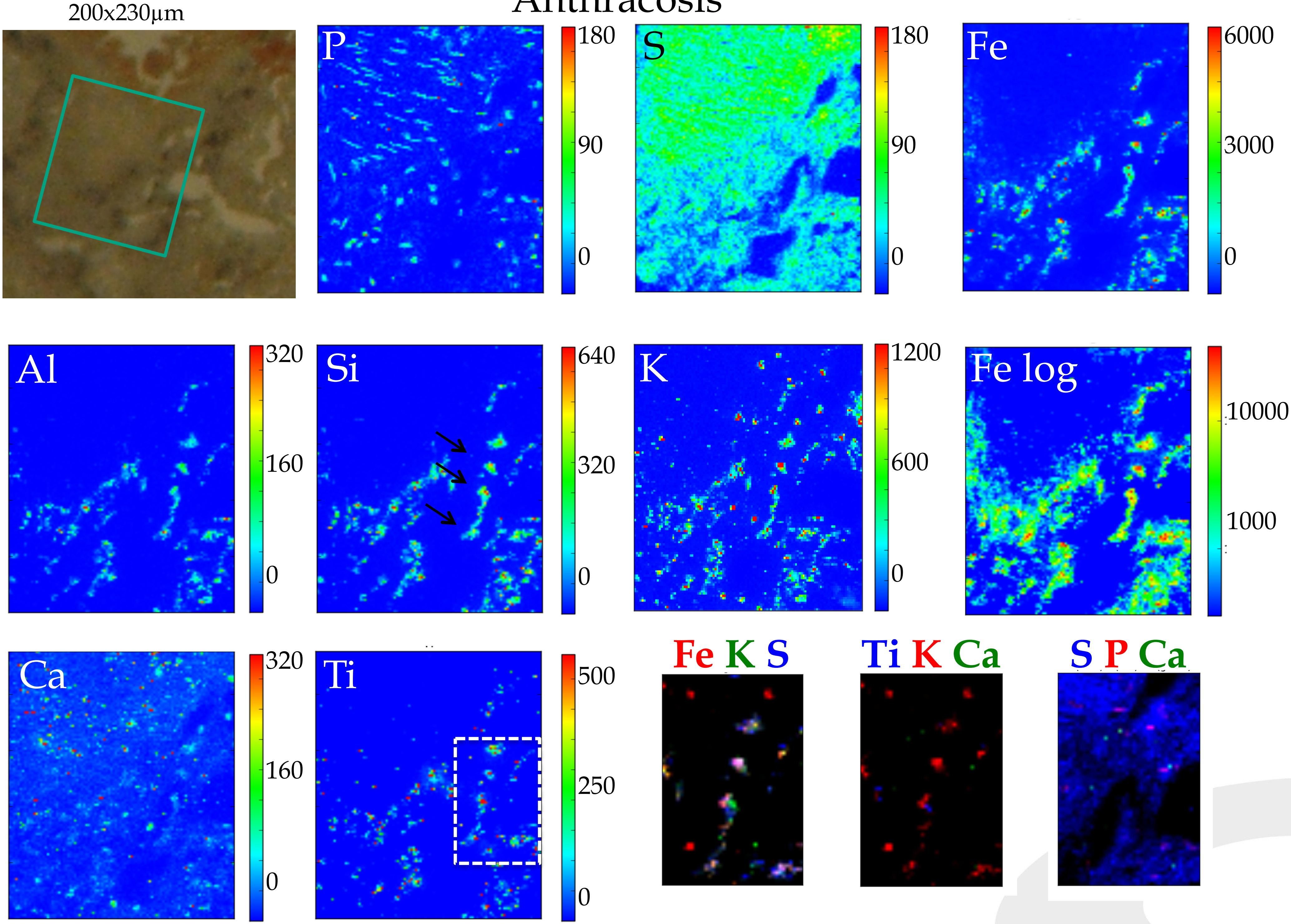




Asbestos and anthracosis



Anthracosis



L. Pascolo, V. Borelli, V. Canzonieri, A. Gianoncelli et al, **Differential protein folding and chemical changes in lung tissues exposed to asbestos or particulates**, Scientific Reports Vol. 5, pp. 12129 (2015)

FTIR spectro-microscopy

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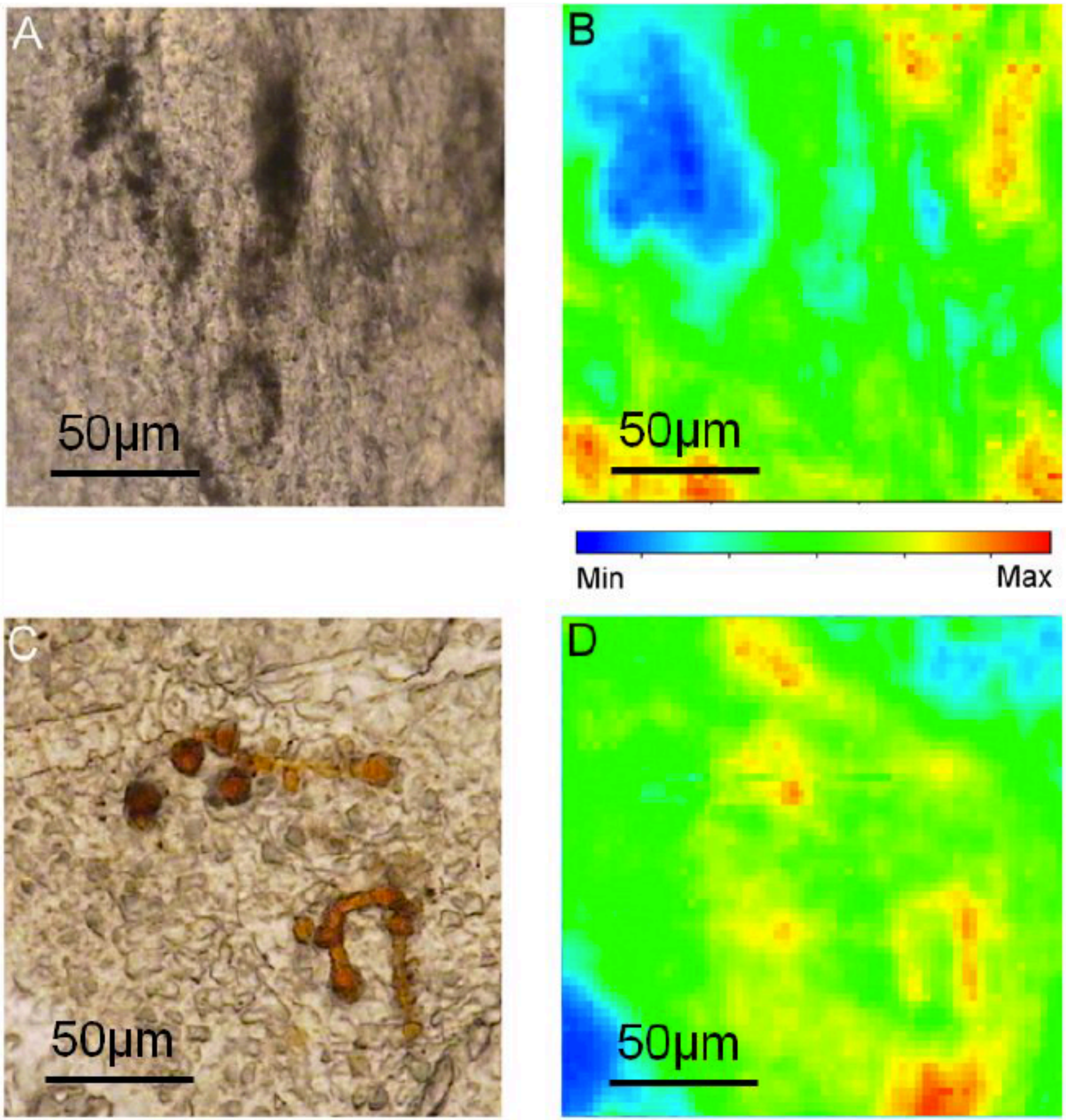


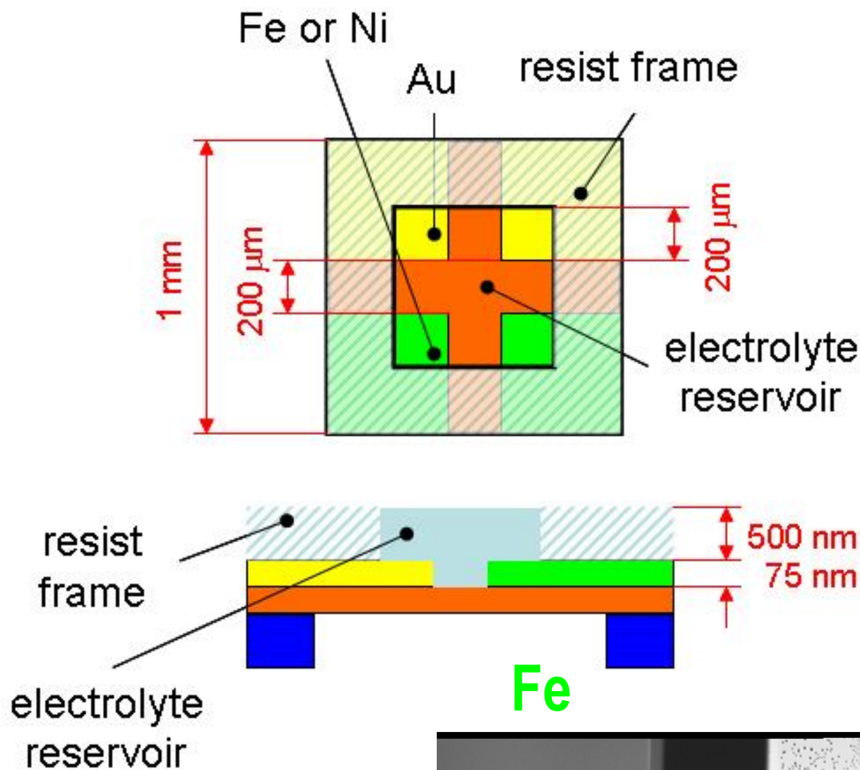
Sensitive, label-free and non-damaging technique for the characterization of biomolecules

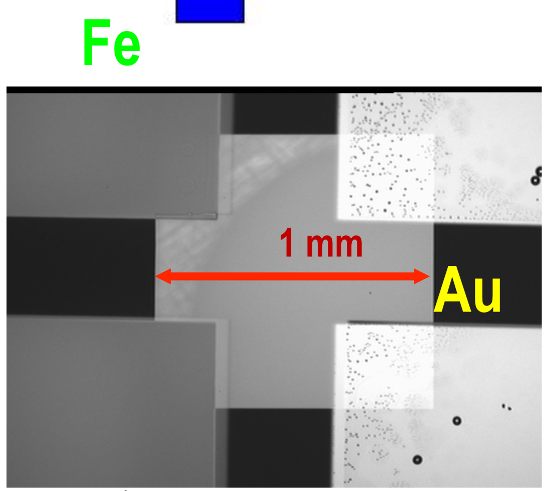
Particularly useful for the study of proteins, the so called Amide I (~ 1700-1600 cm^{-1}) and Amide II (~ 1580-1480 cm^{-1}) bands, from the C=O stretching and N-H bending vibrations of the peptide backbone

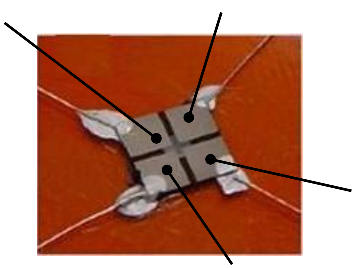
L. Pascolo, V. Borelli, V. Canzonieri, A. Gianoncelli et al, **Differential protein folding and chemical changes in lung tissues exposed to asbestos or particulates**, Scientific Reports, Vol. 5, pp. 12129 (2015)

Vacuum compatible Electrochemical cells

Through-mask evaporation

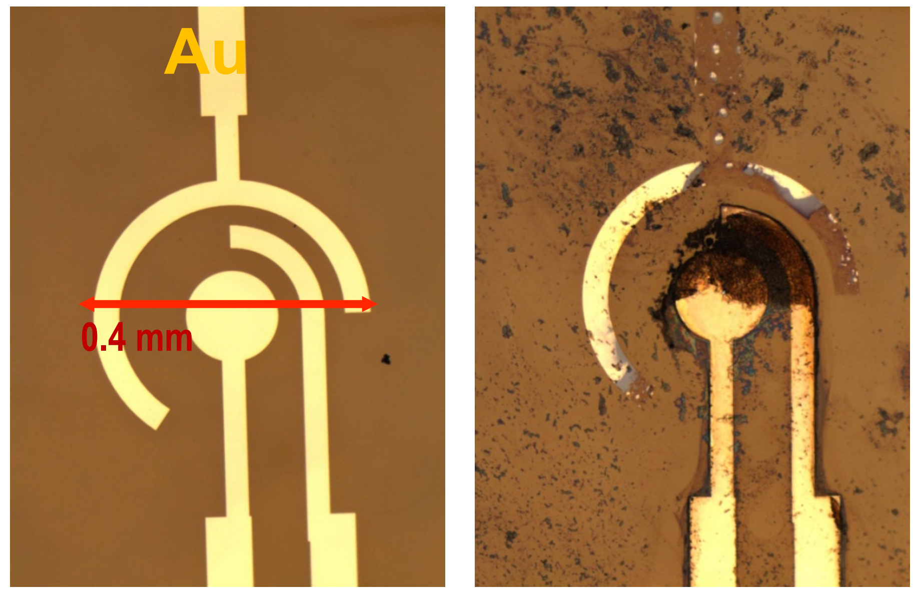






spare electrode

Electron-beam lithography



1) Improve the current density distribution
2) Localisation of the electrochemical processes

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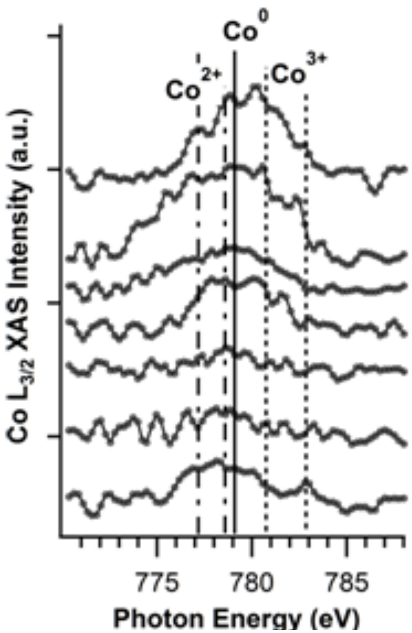
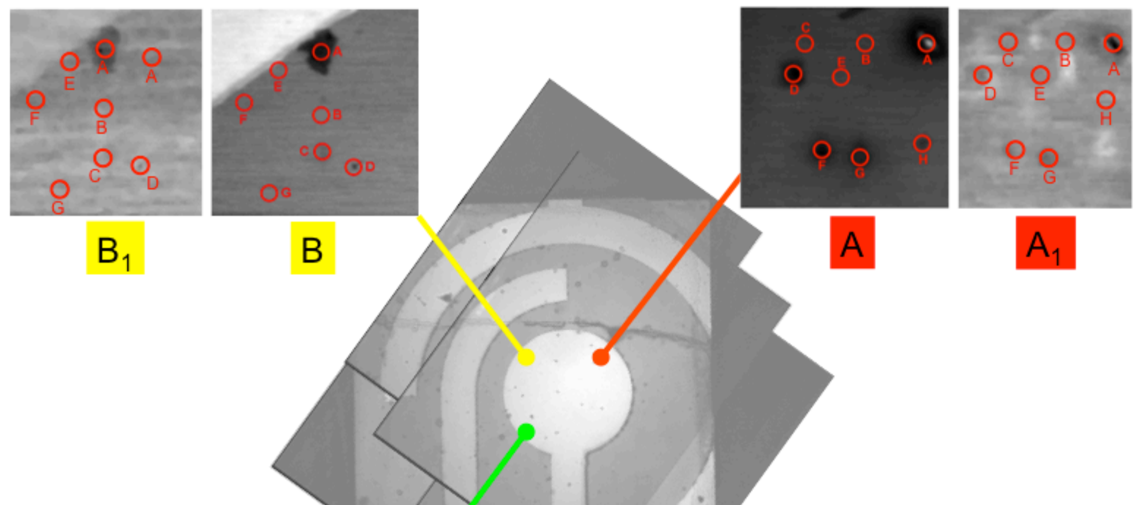
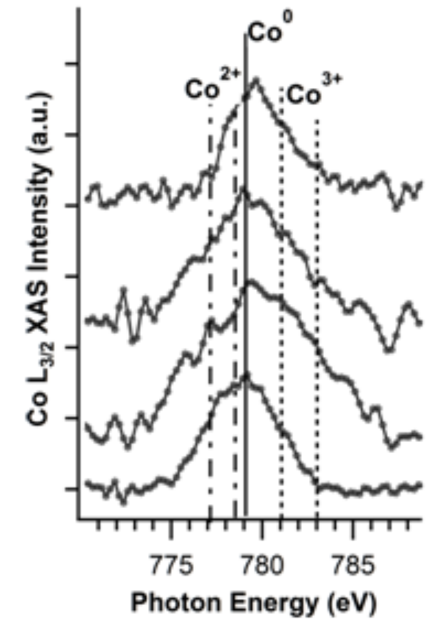
Definition of Co chemical state and distribution: micro-XAS

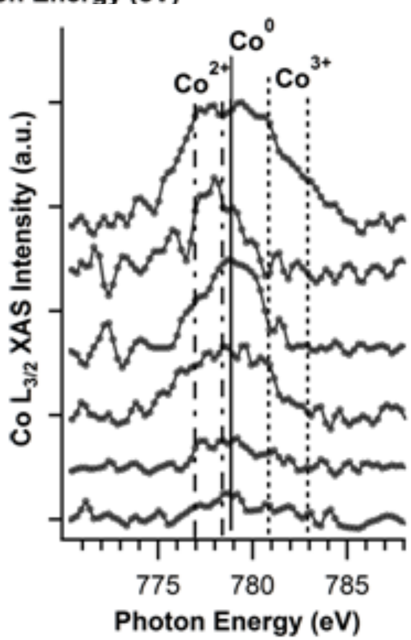


intermediate-c.d.

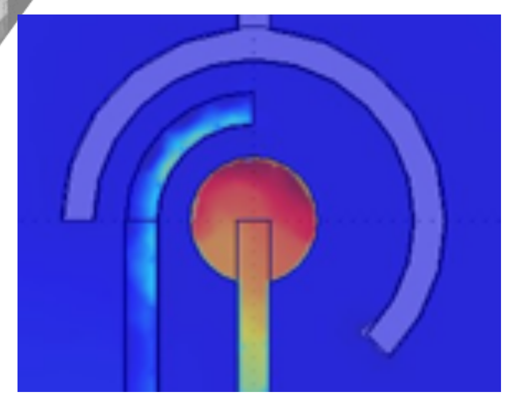
high-c.d.







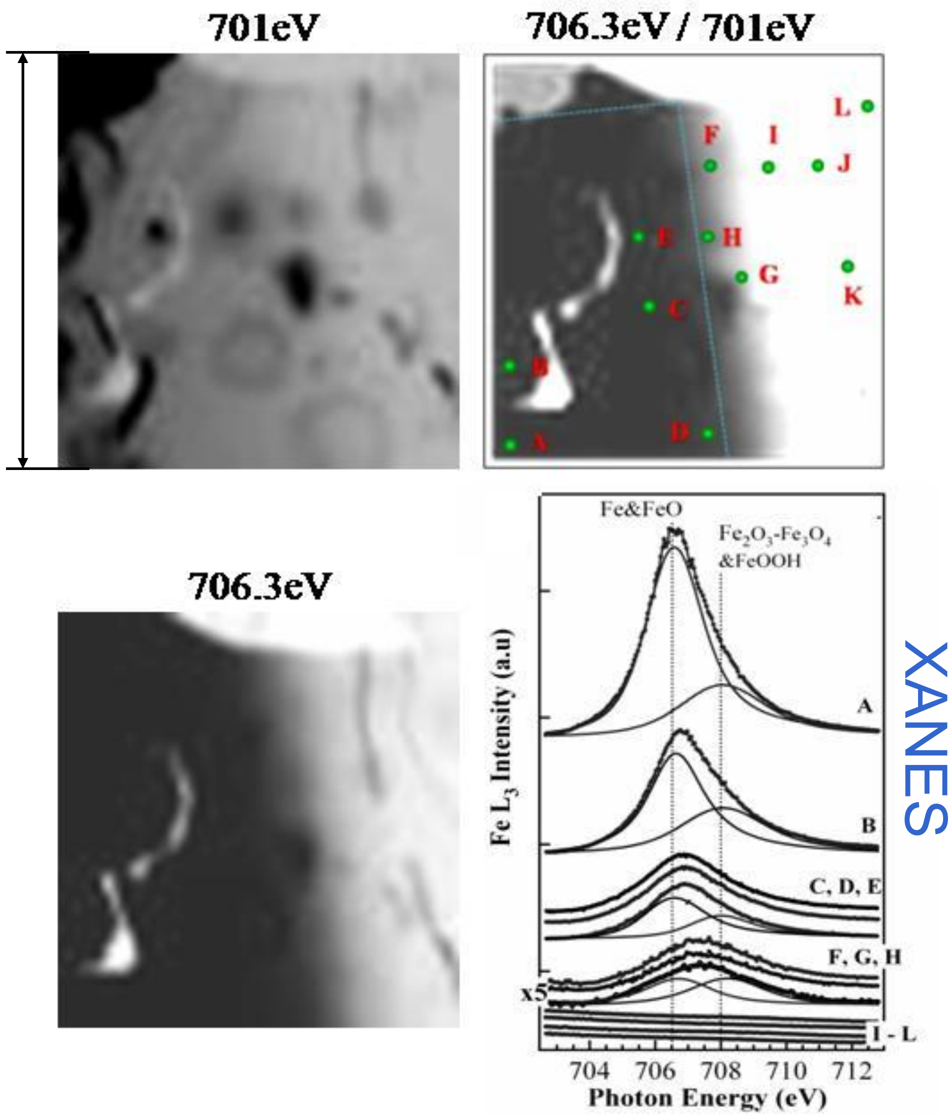
low-c.d.

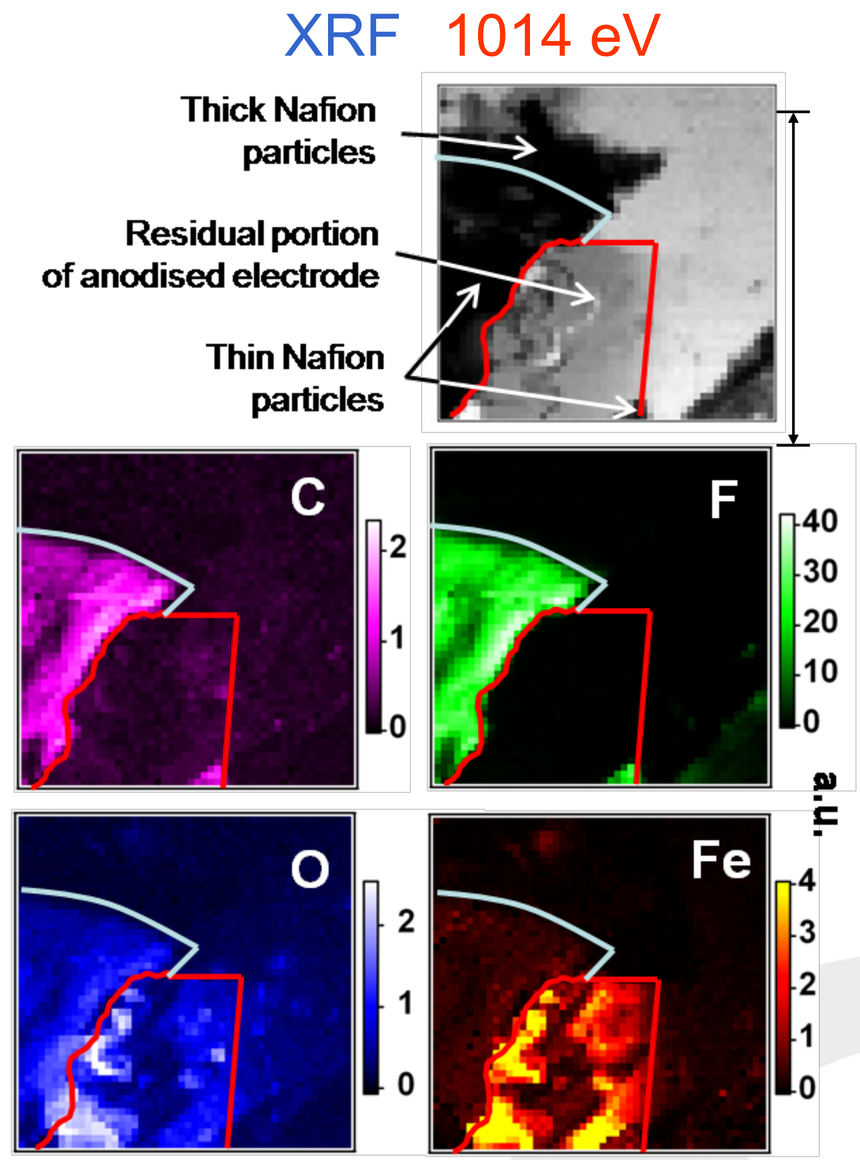


Bozzini B, Gianoncelli A, Bocchetta P, Dal Zilio S, Kourousias G , Analytical Chemistry (2014), 2014, 86 (1), 664

(ii) Combinatorial capability

FC durability - Bipolar plate (Fe) corrosion and Nafion poisoning





Bozzini et al. *ChemSusChem* , 7 (2010) 846

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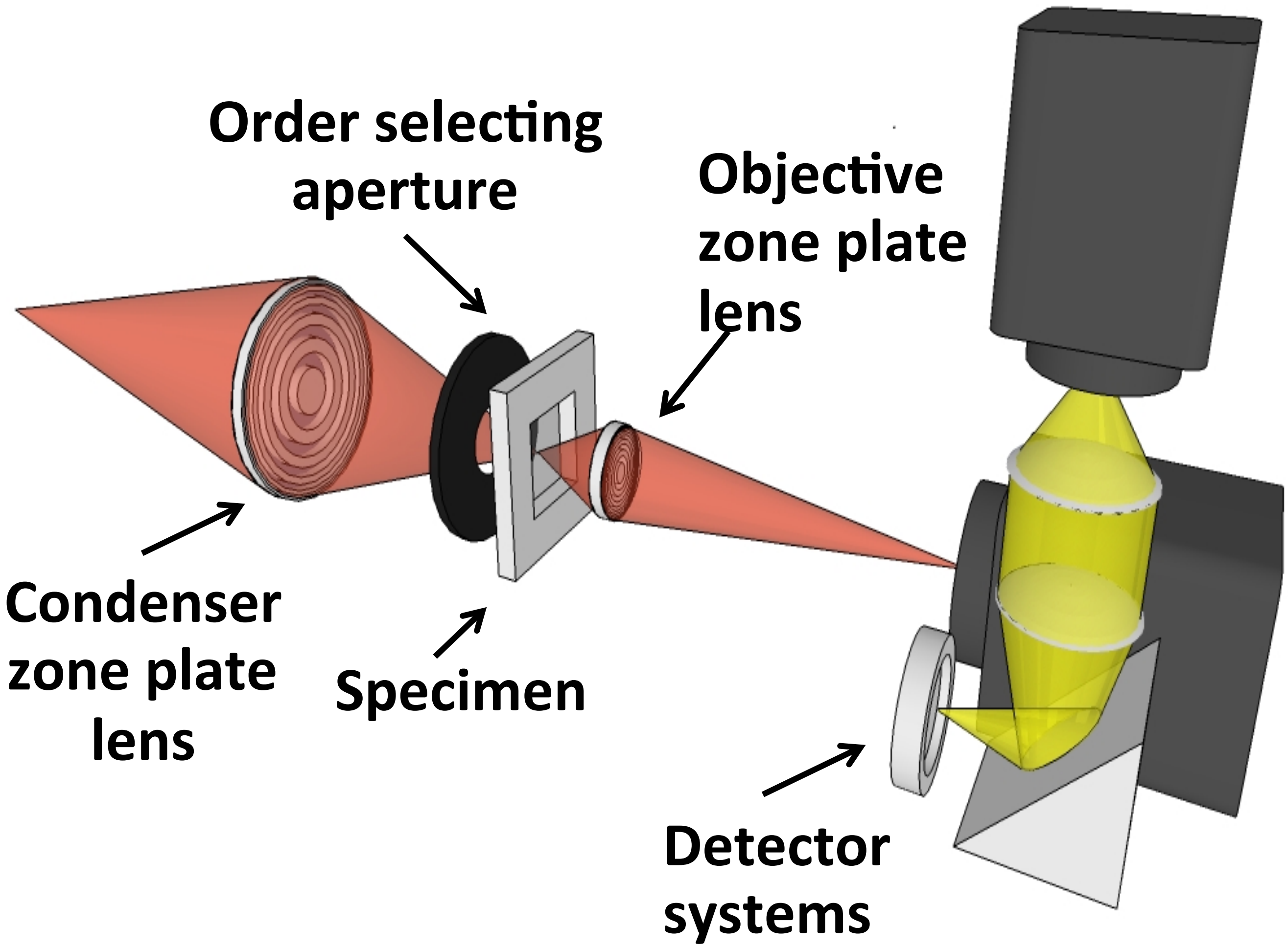
Full field Imaging mode



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

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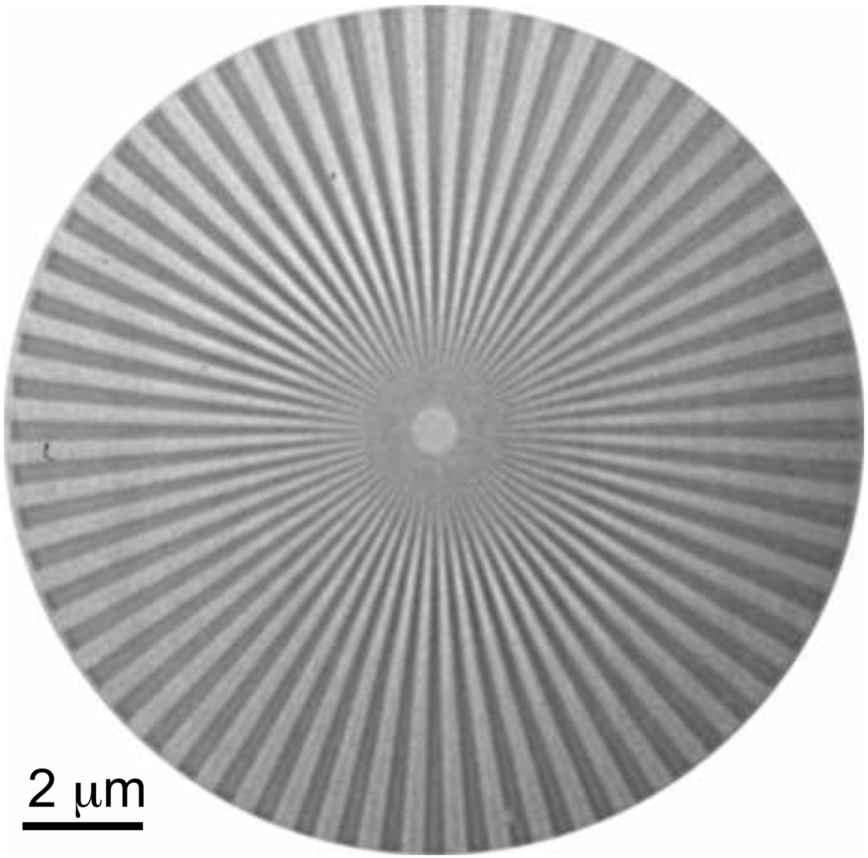
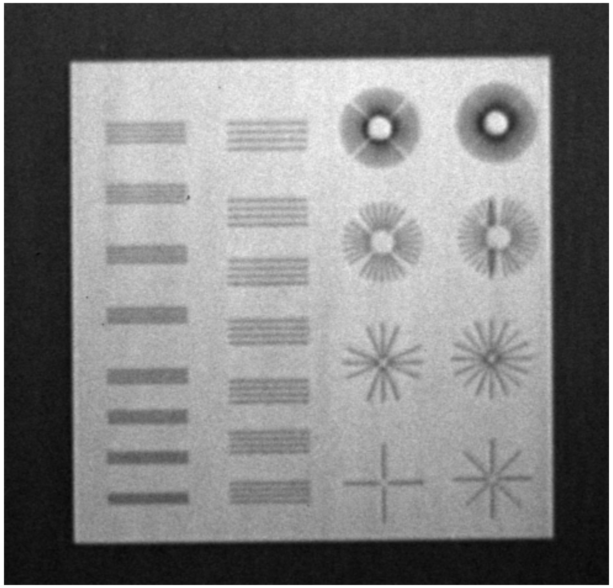
Resolution tests in full-field imaging mode:

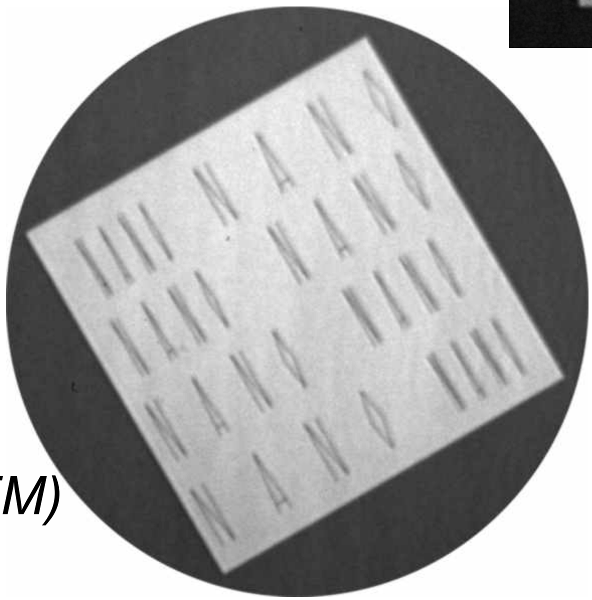


2 μm

<u>ZP parameters:</u>
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. Prascioli and D. Cojoc, TASC/ INFM)

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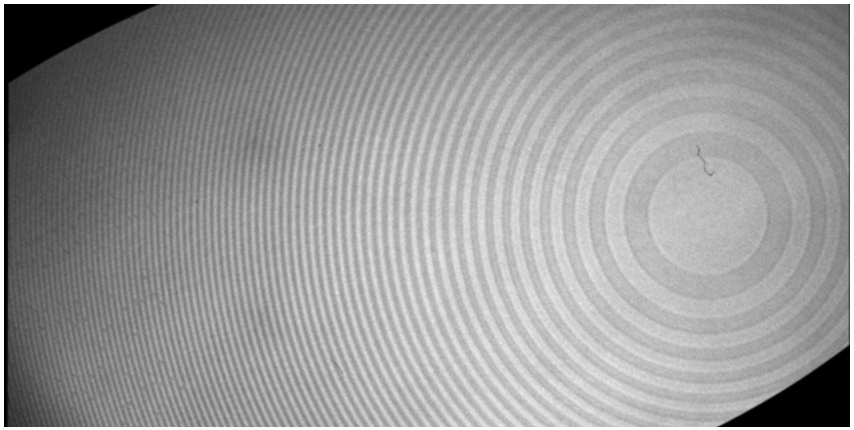
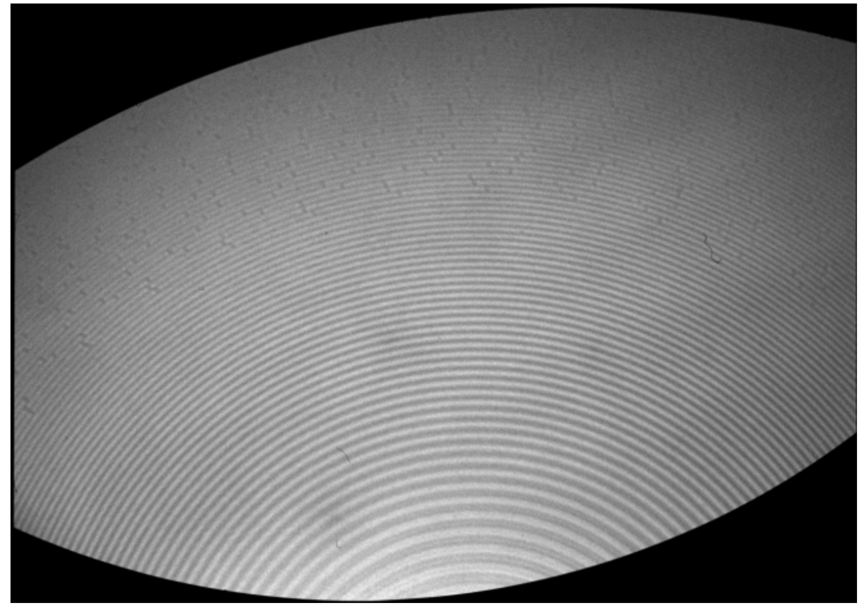
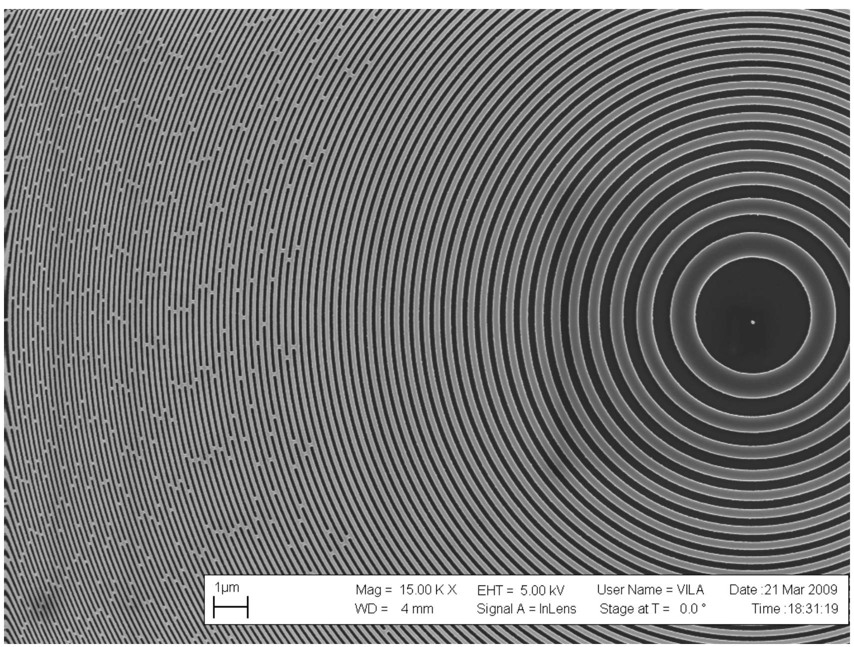
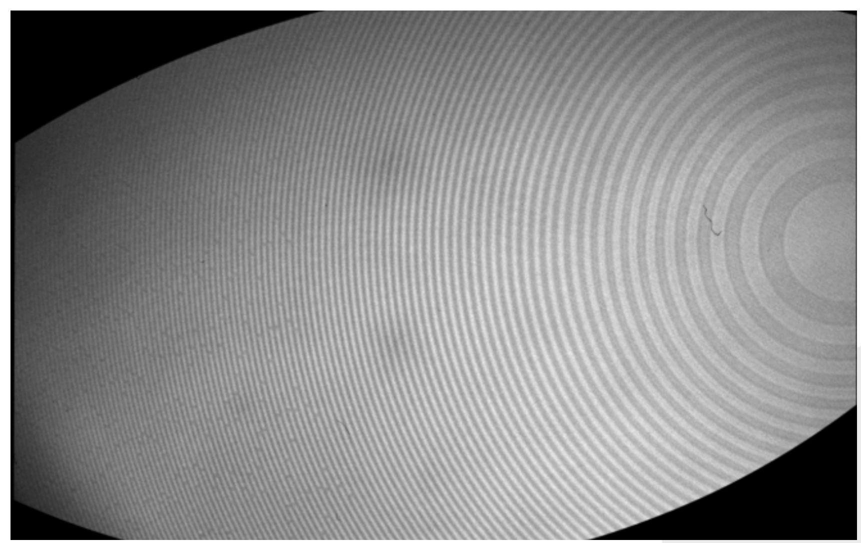
Experiment performed by
J. Vila-Comamala, Paul Scherrer Institute, *Switzerland*

Resolution tests in full-field imaging mode:









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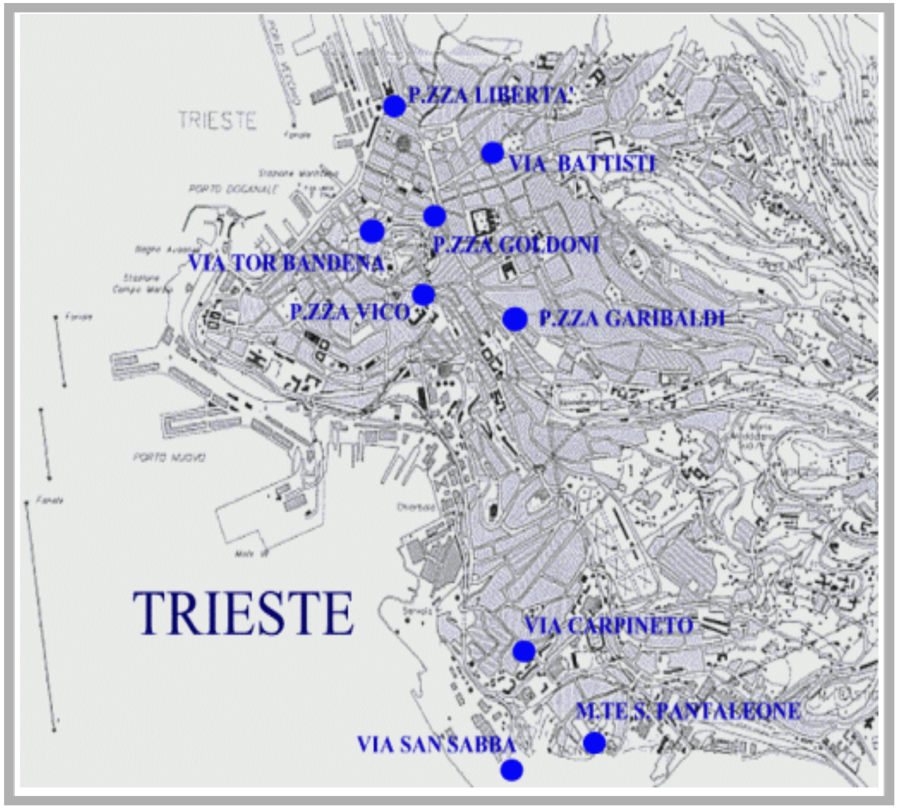
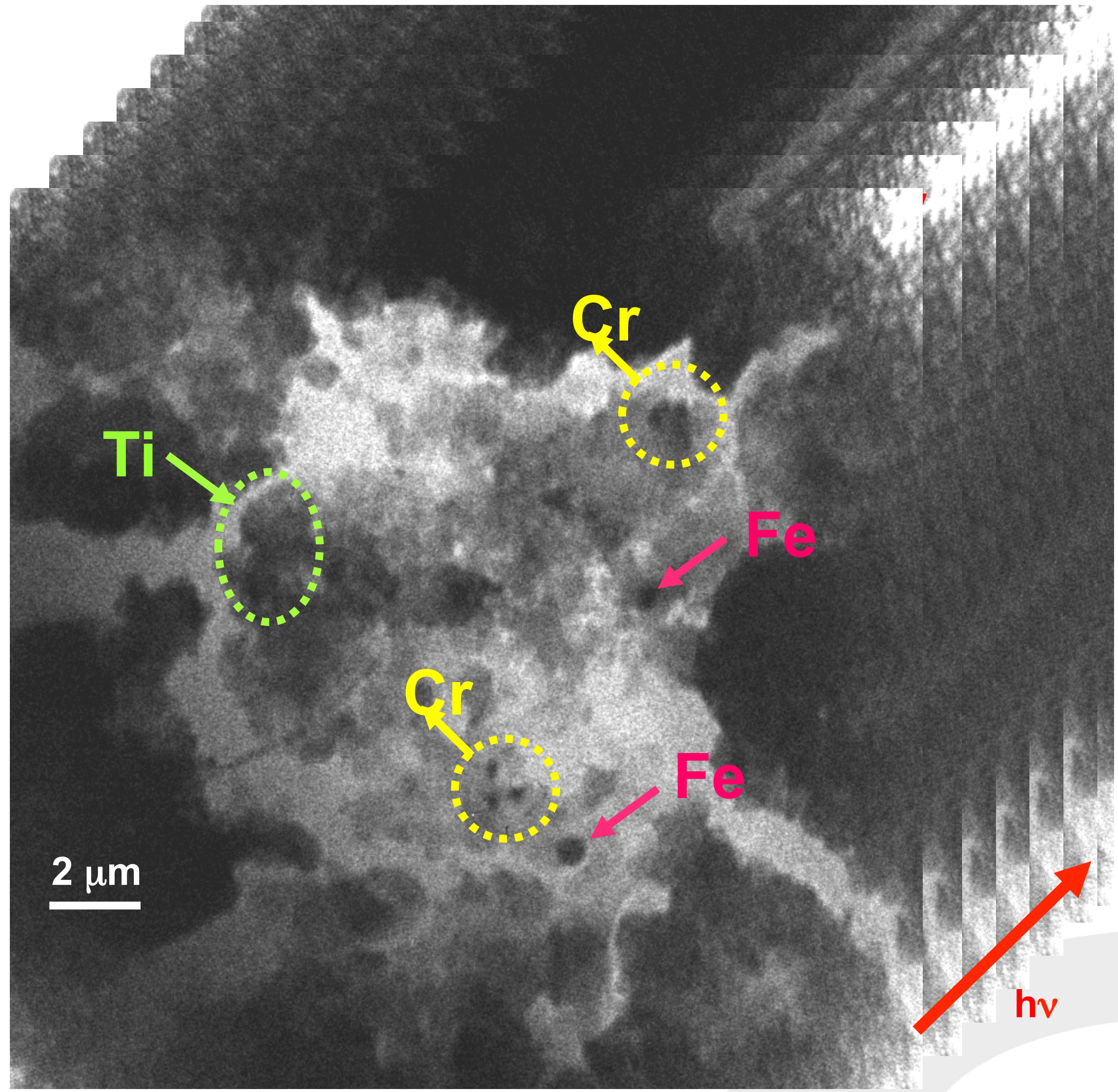
Environmental science: Analysis of air particulate matter





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

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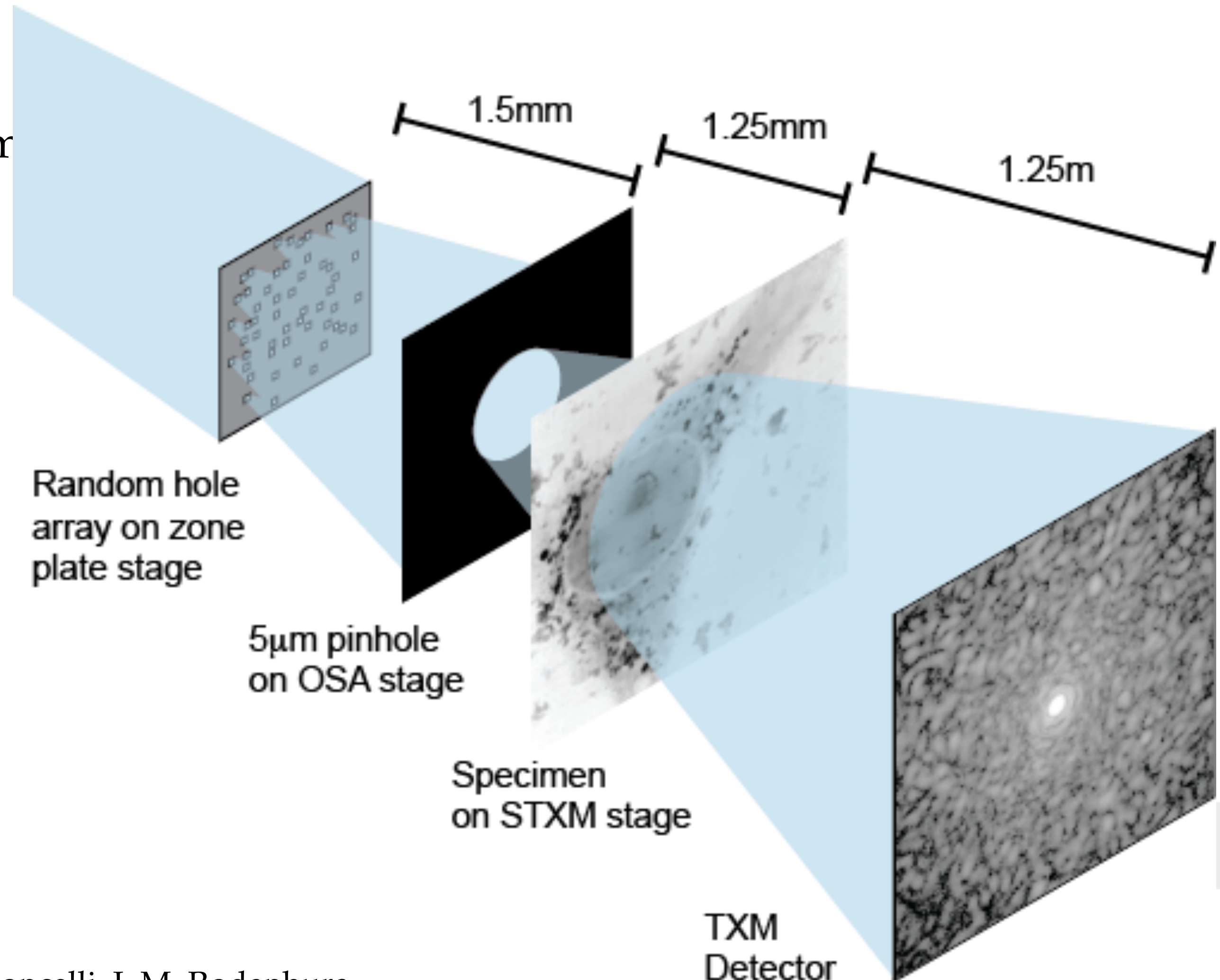
Soft X-ray spectromicroscopy using ptychography with randomly phased illumination

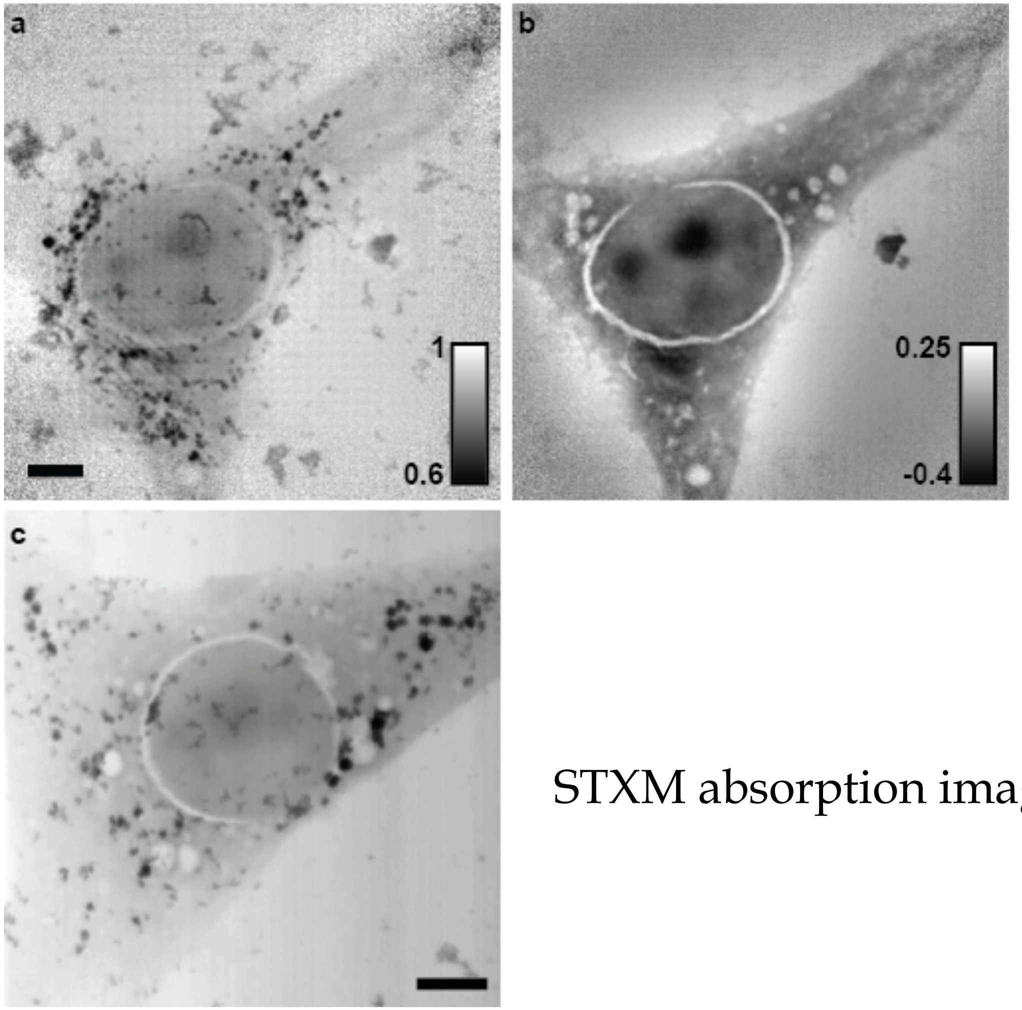
- Illuminated area defined by a 5 µm pinhole
- Sample was scanned with raster steps ~1 µm



To spread the illumination across the detector

A. M. Maiden, G. R. Morrison, B. Kaulich, A. Gianoncelli, J. M. Rodenburg, *"Soft X-ray spectromicroscopy using ptychography with randomly phased illumination"*, Nature Communications 4, 1669, (2013)

Soft X-ray spectromicroscopy using ptychography with randomly phased illumination



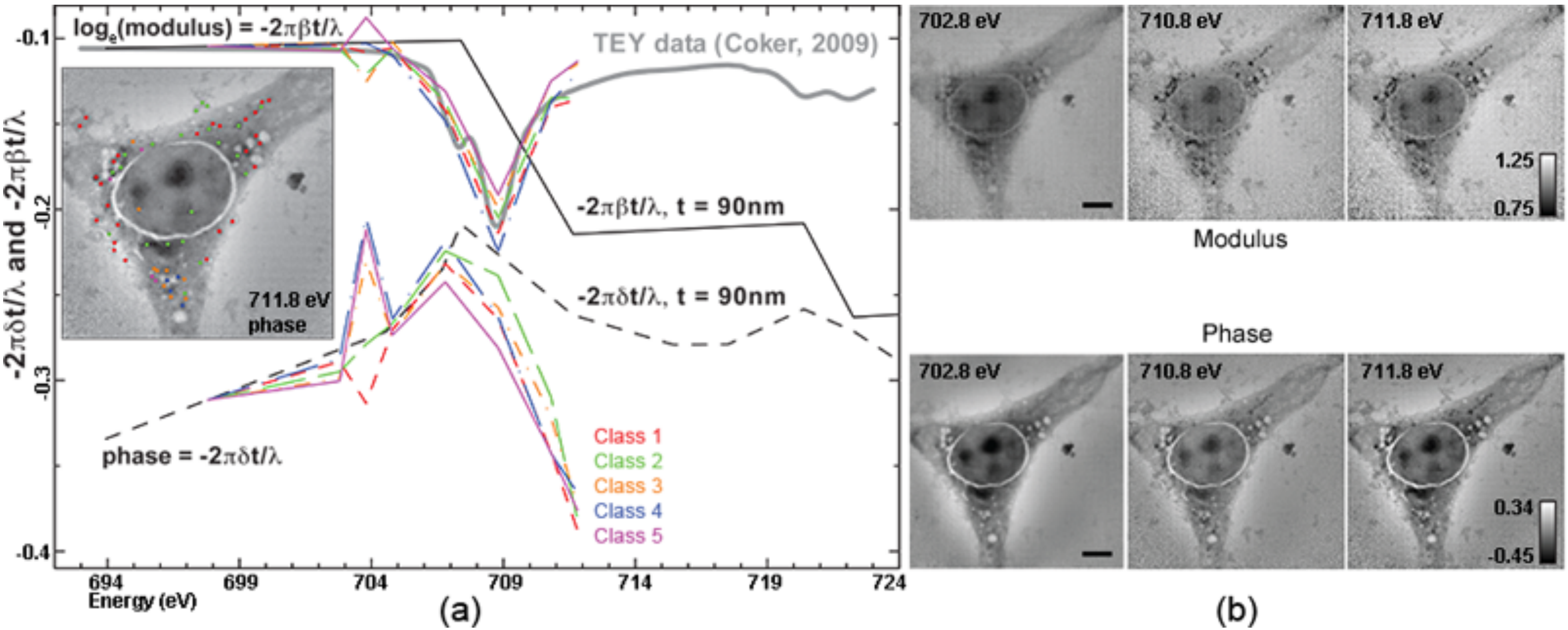
Balb/3T3 mouse fibroblast cells that had been exposed to cobalt ferrite ($CoFe_2O_4$) nanoparticles

Ptychography reconstruction
using the ePIE algorithm (Uni of Sheffiled)

STXM absorption image

A. M. Maiden, G. R. Morrison, B. Kaulich, A. Gianoncelli, J. M. Rodenburg, *"Soft X-ray spectromicroscopy using ptychography with randomly phased illumination"*, Nature Communications 4, 1669, (2013)

Soft X-ray spectromicroscopy using ptychography



First direct measurements from cobalt ferrite nanoparticles of the phase variations across the iron L_3 edge, with the phase variations showing stronger and clearer features than the modulus data provided

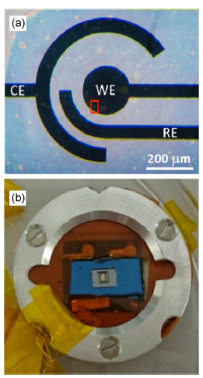
The observed variations in modulus contrast across Fe L_3 edge are consistent with estimates based on total electron yield (TEY) made by Dr V.S. Coker (Manchester University, UK)

A. M. Maiden, G. R. Morrison, B. Kaulich, A. Gianoncelli, J. M. Rodenburg, *"Soft X-ray spectromicroscopy using ptychography with randomly phased illumination"*, Nature Communications 4, 1669, (2013)

Electrodeposition dynamics tracked *in situ*

Ptychography across Mn edge (stack of images)

The cell was monitored in its pristine state and after biasing, across Mn and Co edge, elements present in the electrolyte solution



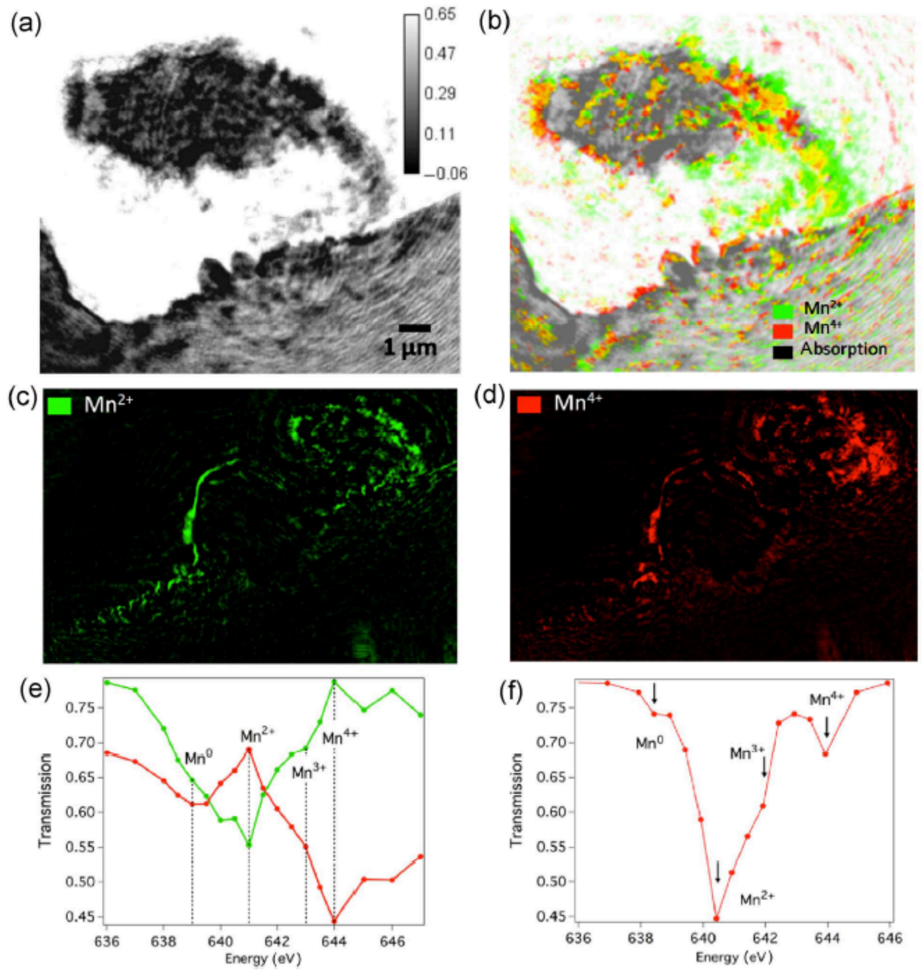


Figure 6 Spectroscopic analysis of the WE/electrolyte interface highlights the spatially resolved Mn species. (a) Absorption image acquired at 636 eV (below the Mn L absorption edge) for a sub-region of the WE electrode. (b) The same area where the distribution of the Mn^{2+} and Mn^{4+} states are indicated by red and green, overlapping the absorption contrast dominated by Mn species. The results are based on scans at 18 different energies, ranging from 636 to 647 eV. (c) and (d) show the same information as (b) over a larger electrode area. (e) shows the average absorption spectra for the region where the Mn^{2+} state dominated (green plot) compared with that where Mn^{4+} state dominated (red plot), and (f) shows the average absorption spectrum collected over the entire area shown in (a).

As in classical scanning X-ray transmission microscopy, ptychography can provide elemental and chemical information by recovering the phase and amplitude functions from two images obtained using photon energies above and below the absorption edges

Kourousias G, Bozzini B, Gianoncelli A, Jones MWM, Junker M, van Riessen G, Kiskinova M *Shedding light on electrodeposition dynamics tracked in situ via soft X-ray coherent diffraction imaging* **Nano Research**, Vol. 9 - 7, pp. 2046-2056 (2016)

LEXRF new detector

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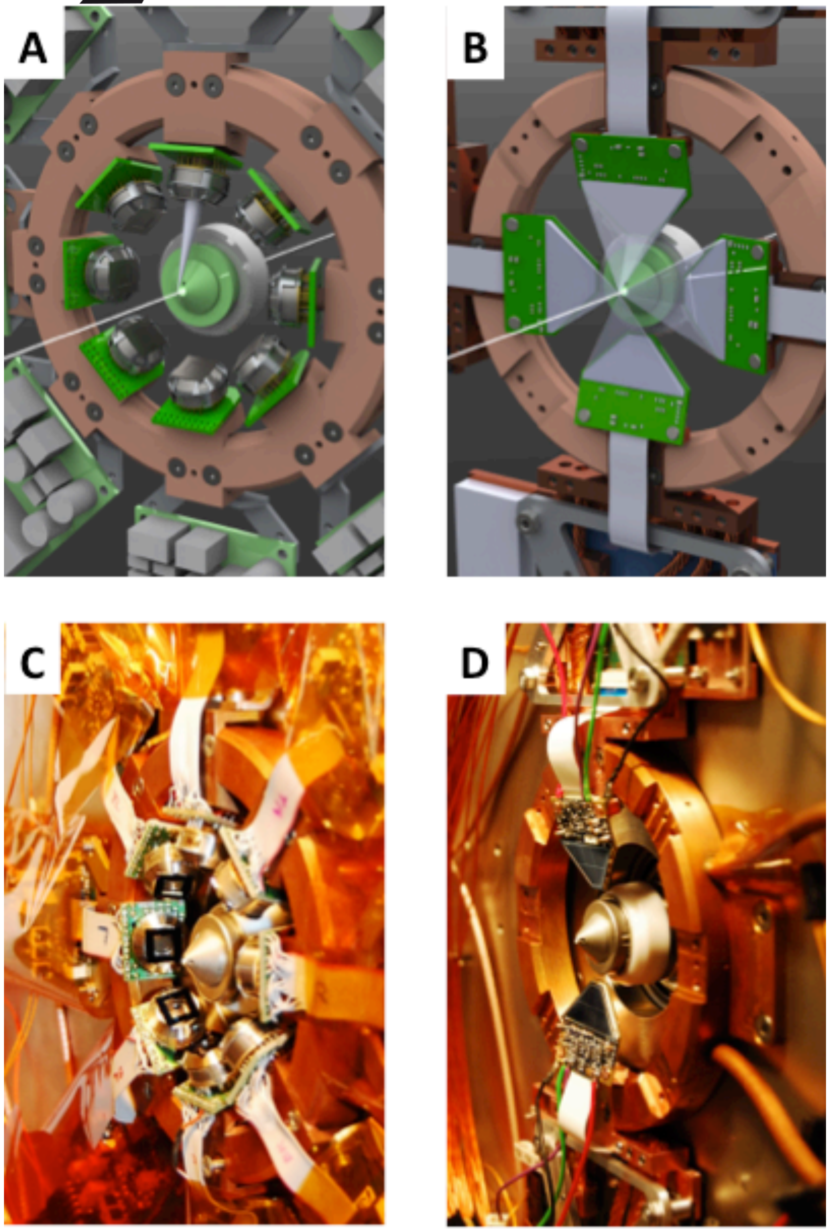
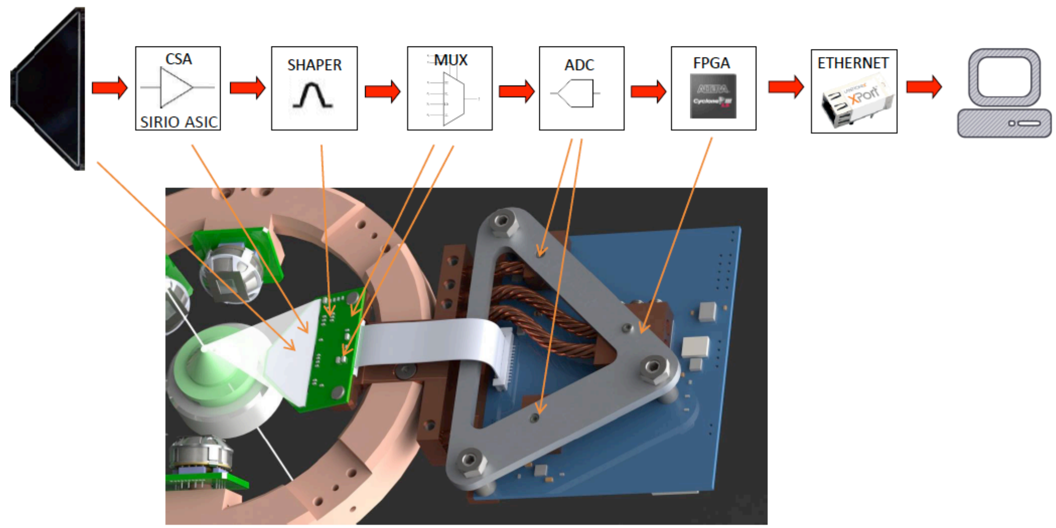


- Based on SDDs
- Bigger solid angle



A. Gianoncelli, J. Bufon, M. Ahangarianabhari, P. Bellutti, G. Bertuccio, S. Carrato, G. Cautero, S. Fabiani, G. Giacomini, D. Giuressi, G. Kourousias, R. Hendrik Menk, C. Piemonte, I. Rashevskaya, A. Rachevski, A. Stolfa, R. Borghes, M. Altissimo, G. Zampa, N. Zampa,, A. Vacchi, "A new Detector System for Low Energy X-ray Fluorescence coupled with Soft X-ray Microscopy: first Tests and Characterization A new Detector System for Low Energy X-ray Fluorescence coupled with Soft X-ray Microscopy: first Tests and Characterization" *in preparation*

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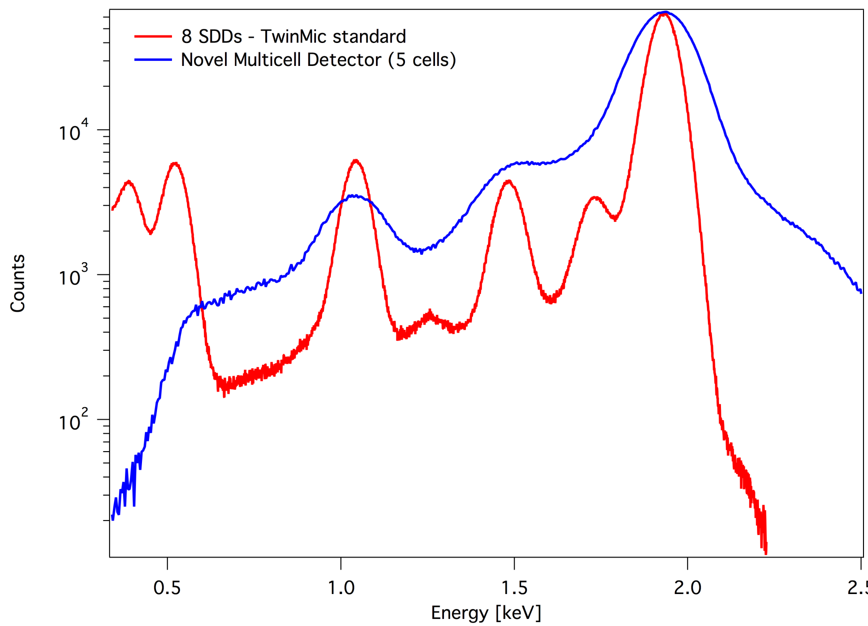
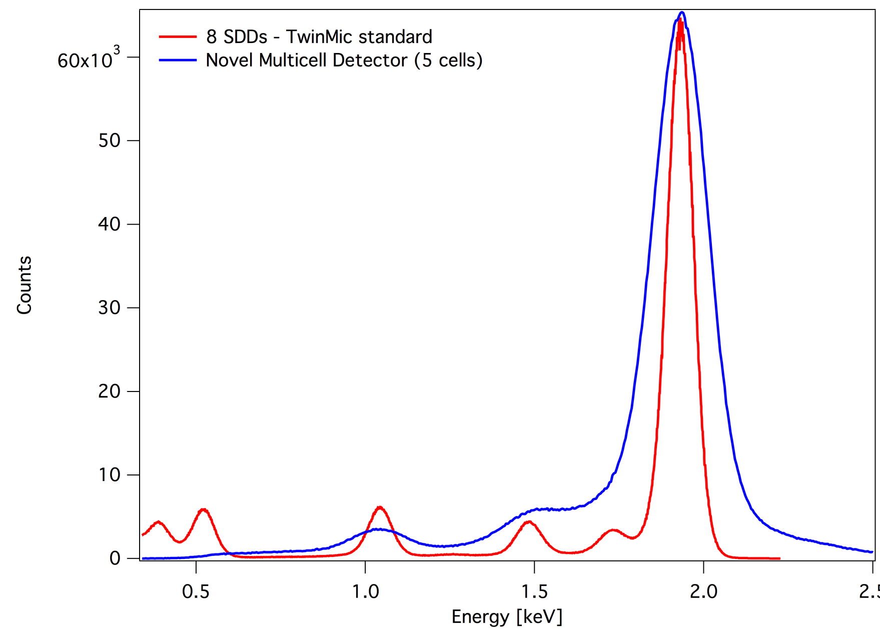
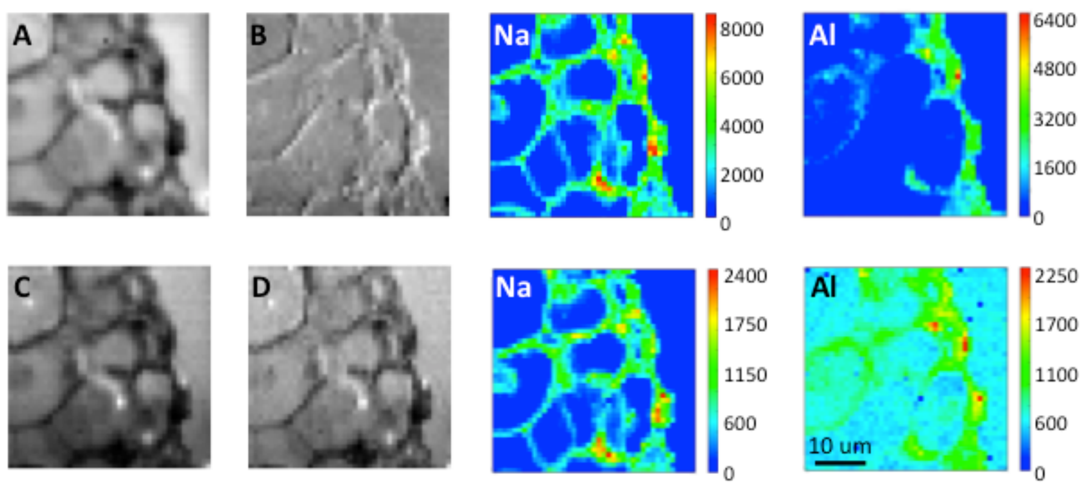
Spectra comparison

(acquired on soybean root)



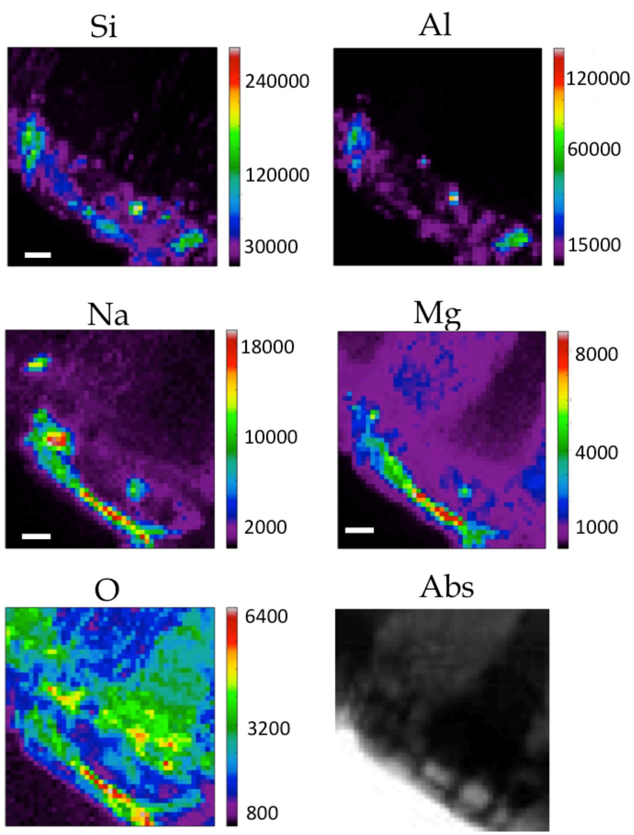




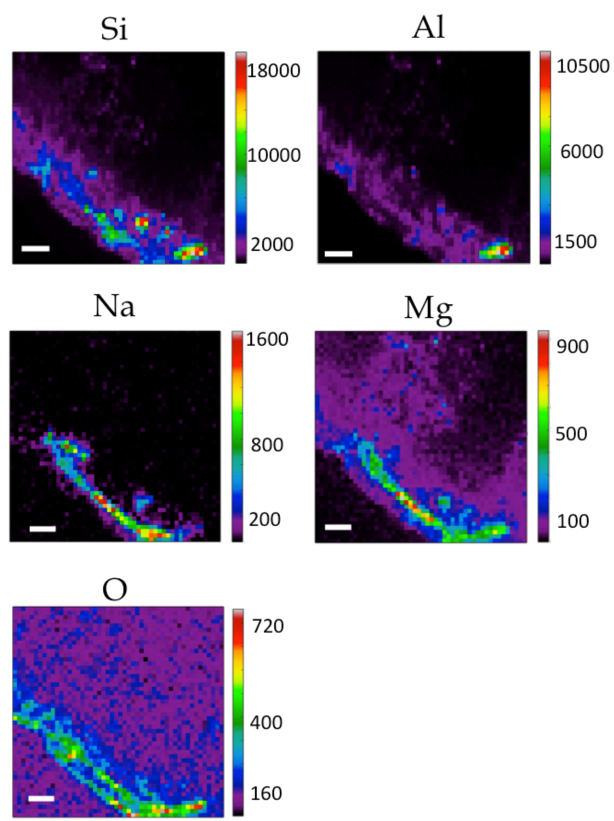
A. Gianoncelli, J. Bufon, M. Ahangarianabhari, P. Bellutti, G. Bertuccio, S. Carrato, G. Cautero, S. Fabiani, G. Giacomini, D. Giuressi, G. Kourousias, R. Hendrik Menk, C. Piemonte, I. Rashevskaya, A. Rachevski, A. Stolfa, R. Borghes, M. Altissimo, G. Zampa, N. Zampa,, A. Vacchi, "A new Detector System for Low Energy X-ray Fluorescence coupled with Soft X-ray Microscopy: first Tests and Characterization A new Detector System for Low Energy X-ray Fluorescence coupled with Soft X-ray Microscopy: first Tests and Characterization" *in preparation*

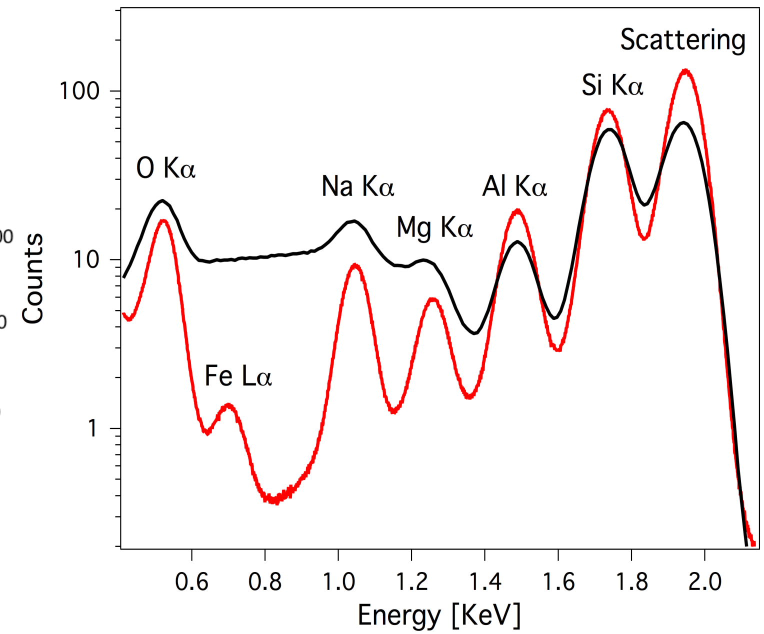
New XRF detector development

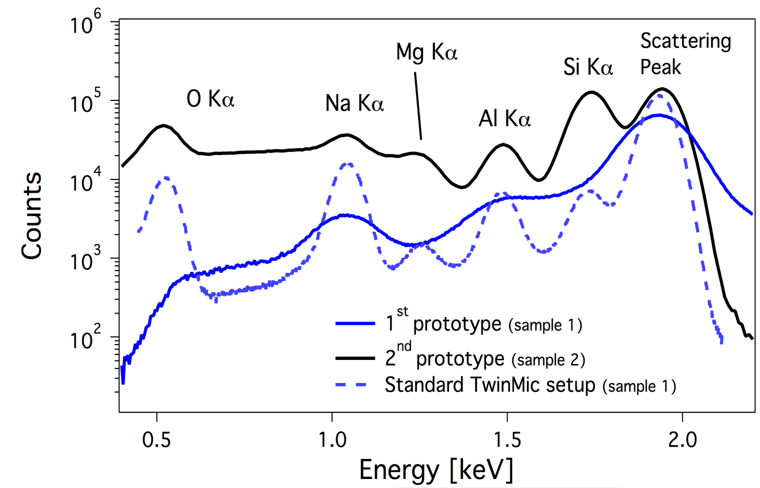
Standard TwinMic set up



New detector set up







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Summary and outlook

- Simultaneous soft X-ray transmission and emission microscopy can be a suited complementary tool especially for life science applications

- X-ray spectro-microscopy has proven to be a valuable tool for exploring morphologically complex matter, including biosystems at cellular & sub-cellular level

- In situ SXM with resolution ~100 nm proved able to probe morphological and chemical effects of electrochemistry, in some cases, dynamically. We developed microfabricated cells with nm-thick electrodes and electrolytes: sealed for aqueous electrolytes; open with spun IL-based electrolytes

  Possibility of dosing electroactive gases in the analysis chamber.

Correlative research is essential (FTIR, PIXE, EM, lab-related bulk analysis…)

All of this con be achieved only through continuos techniques and tecnology developments:

- Evaluation of the possibility of a monolitich array of XRF detectors

- New efficient X-ray Optics

Home | Program | Sponsors | Satellite Workshop CH MA-XRF | Registration | Abstract Submission | Venue | Accommodation | Contact

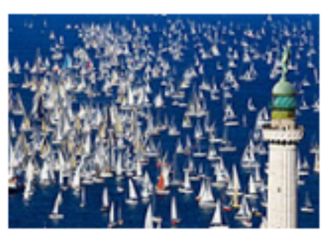
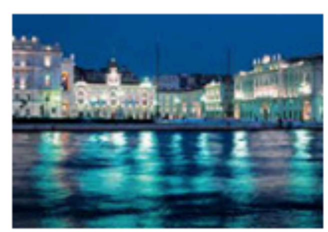
The ICXOM conference series is an international symposium for the discussion of new developments and advances in instrumentation, methods and applications in the fields of X-ray micro- and nano-analysis.
This conference series started in 1956 in Cambridge, UK, in response to the need for physical scientists, instrument developers and those using X-ray microscopes and electron probe analysers to come together every three (and now two) years to exchange experiences, learn about new developments in the field and gain new ideas for further research.
Following the trend of the last decade, the conference is focused on synchrotron radiation. Recent developments in laboratory instrumentation are also highly welcome. Besides micro-beam X-ray fluorescence and absorption spectroscopy, different methods based on diffraction and full-field imaging are covered, together with their applications in Life Sciences, Material Science, Earth and Environmental Sciences, and Cultural heritage.

Topics Include

- Optics for Microanalysis
- Scanning Microscopy
- Full-field Imaging
- Coherent Diffractive Imaging
- Microdiffraction
- X-ray Fluorescence
- Absorption spectroscopy
- Data Analysis
- Detectors

This edition of ICXOM conference series will include a satellite workshop on MA-XRF (Macro-XRF) applied to Cultural Heritage on Monday 25th of September

TRIESTE CITTÀ DELLA CONOSCENZA
TRIESTE CITY OF KNOWLEDGE

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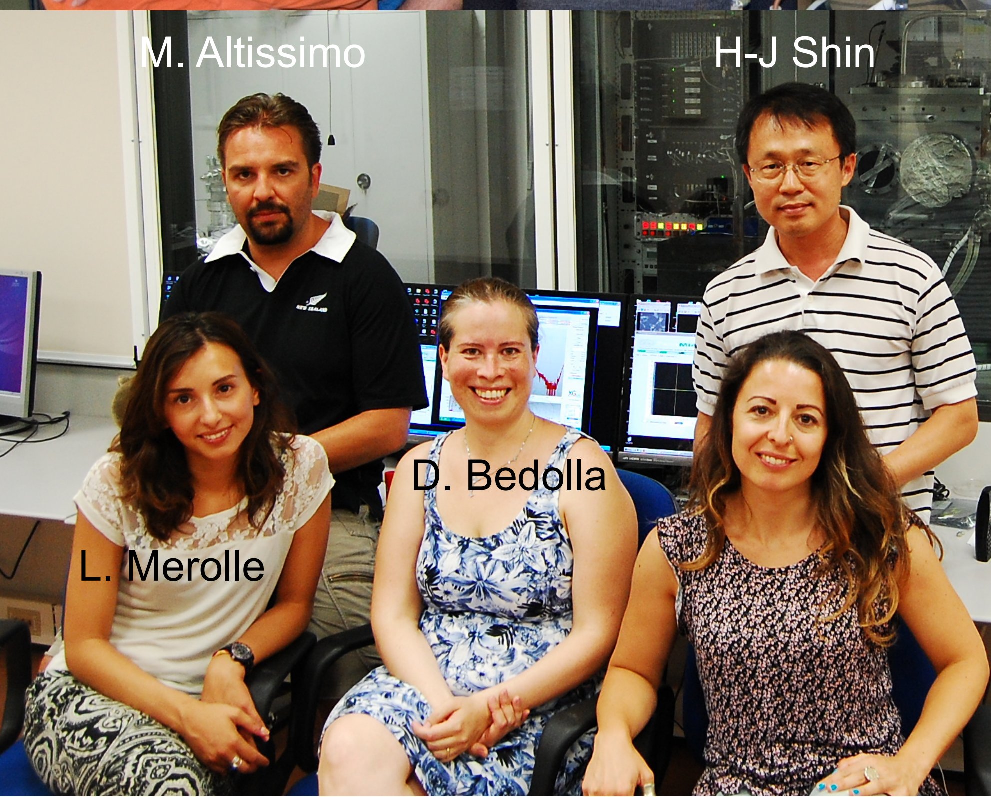


Acknowledgements:

G. De Giudici, D. Medas, University of Cagliari, Italy

I. Kreft et al. (Josef Stefan Institute, Ljubljana, Slovenia)

K. Vogel-Mikus, M. Debeljak et al. (Josef Stefan Institute, Ljubljana, Si)

E. Lombi, Uni of South Australia

P. Kopittke, Uni of Queensland

L. Pascolo (Ospedale Burlo, Trieste)

E. Malucelli, S. Iotti, S. Lagomarsino (Uni Bologna, Italy)

C. Poitry-Yamate (EPFL, Switzerland)

M. Jones, G. Van Riessen, M. Junker et al, La Trobe University, Melbourne, Australia

G.R. Morrison et al (University College London, UK)

B. Bozzini et al (Universita' del Salento, Lecce, Italy)

G. Ceccone, P. Marmorato et al. (JSRC, Ispra)

D. Paterson, M de Jonge, Australian Synchrotron, Melbourne, Australia

and many many others.

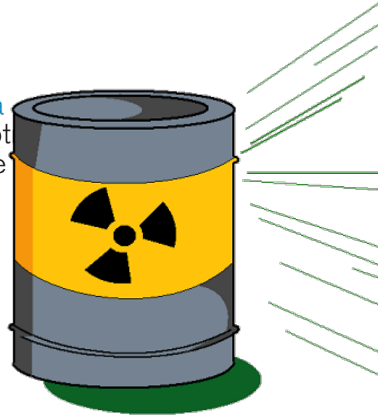
B. Kaulich
Diamond Light Source

M. Kiskinova
ELETTRA

Thank you!

www.elettra.eu

X-ray Radiation Damage



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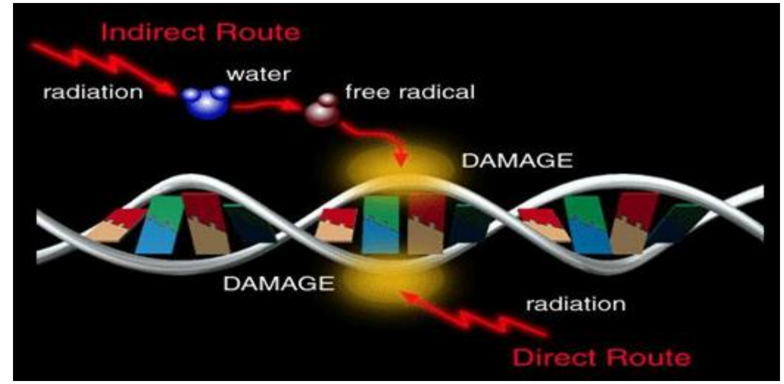
with SISSI and CNR-IOM



AFM
Morphology

FTIRM
Biochemistry

X-ray microscopy
Density

XRF
Elemental analysis

Step 0 — Cell growth on 50 nm Si_3N_4 membranes
Cell fixation with PFA 4% and air drying

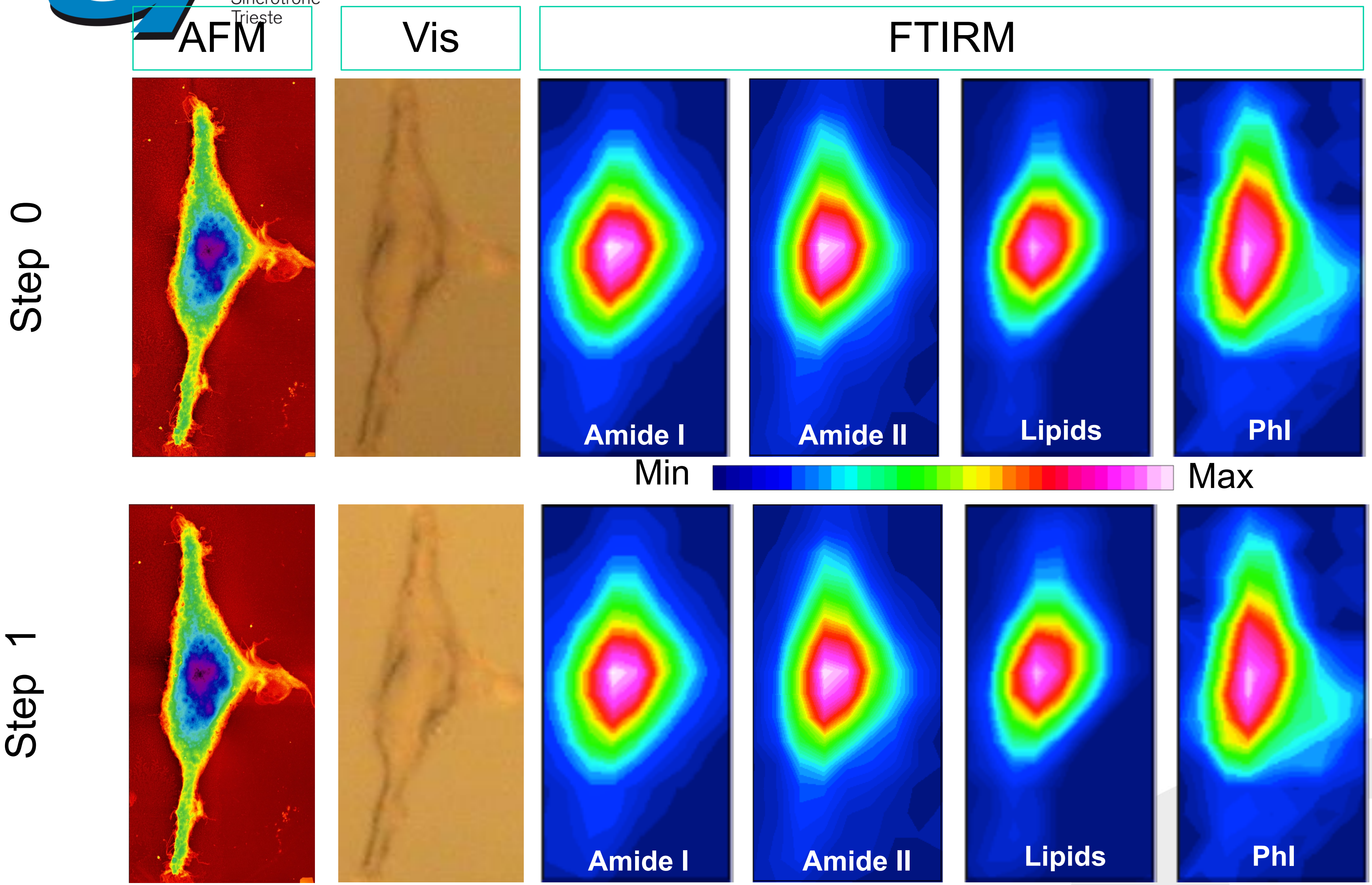
Step 1 — Cell drying in vacuum @ TwinMic
(PRESSURE<10^{-5} mbar) for 1:30 hour

Step 2 — LD STXM mapping @ TwinMic, 1 keV
Estimated dose: $2*10^6$ Gray

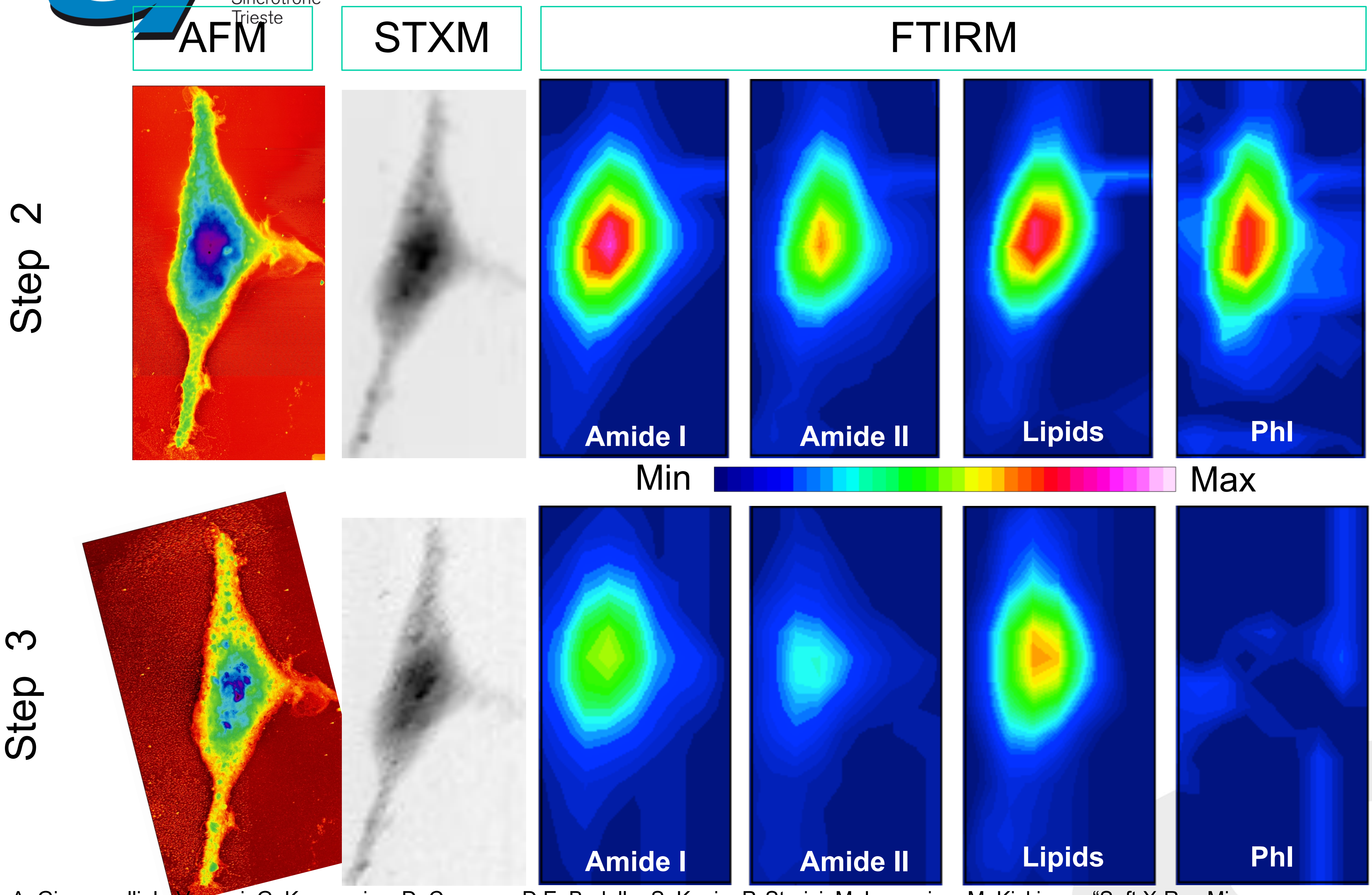
Step 3 — HD STXM mapping @ TwinMic, 1 keV
Estimated dose: $2*10^7$ Gray
Cumulative dose: $2.2*10^7$ Gray

Step 4 — XRF mapping @ TwinMic, 1 keV
Estimated dose: $6*10^8$ Gray
Cumulative dose: $6.2*10^8$ Gray

A. Gianoncelli et al. “Soft X-Ray Microscopy Radiation Damage On Fixed Cells Investigated With Synchrotron Radiation FTIR Microscopy” **Scientific Reports** 5:10250 2015



A. Gianoncelli, L. Vaccari, G. Kourousias, D. Cassese, D.E. Bedolla, S. Kenig, P. Storici, M. Lazzarino, M. Kiskinova "Soft X-Ray Microscopy Radiation Damage On Fixed Cells Investigated With Synchrotron Radiation FTIR Microscopy" **Scientific Reports** 5:10250 2015



A. Gianoncelli, L. Vaccari, G. Kourousias, D. Cassese, D.E. Bedolla, S. Kenig, P. Storici, M. Lazzarino, M. Kiskinova "Soft X-Ray Microscopy Radiation Damage On Fixed Cells Investigated With Synchrotron Radiation FTIR Microscopy" **Scientific Reports** 5:10250 2015

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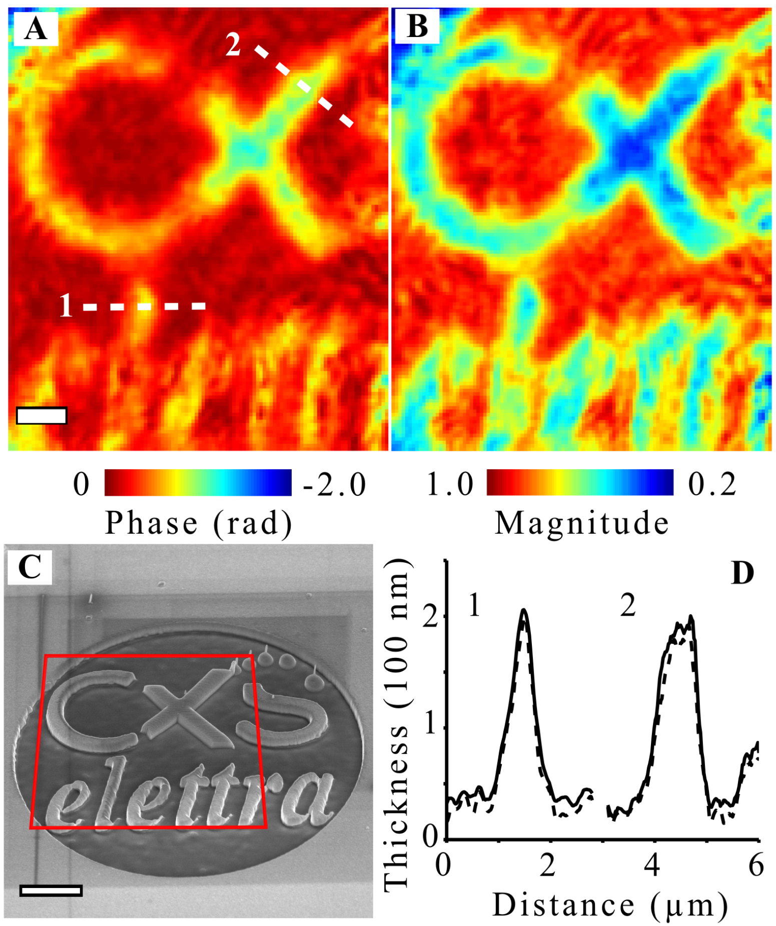
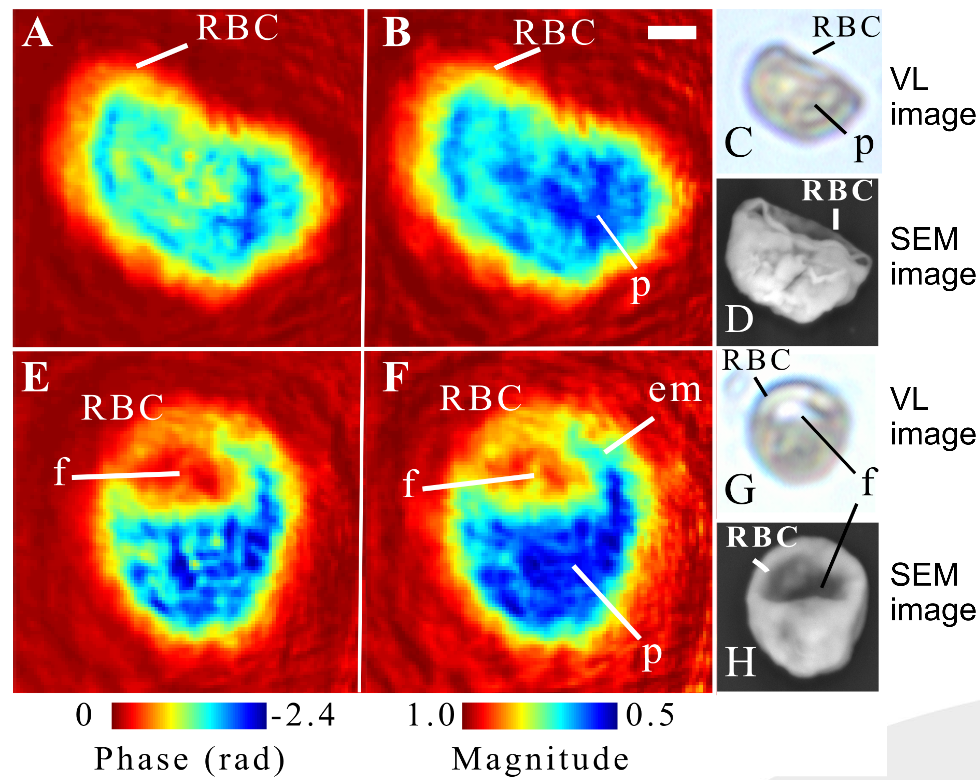
fCDI imaging at TwinMic

M.W.M. Jones, B, Abbey, G. Van Riessen, La Trobe University

CXS – ELETTRA test pattern



Blood cells infected with the malaria parasite *P. falciparum*



Jones MWM, Abbey B, Gianoncelli A, Balaur E, Millet C, Luu MB, Coughlan HD, Carroll AJ, Peele AG, Tilley L, van Riessen GA "Phase-diverse Fresnel coherent diffractive imaging of malaria parasite-infected red blood cells in the water window" Optics Express, 21(26), 32151 (2013)