Applications to metal detection in biological samples

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Outline

Importance of metals in biological systems

Why synchrotron microprobe ?

metals detection in cells and tissues

High-resolution cellular chemical imaging

Importance of metals in biological systems

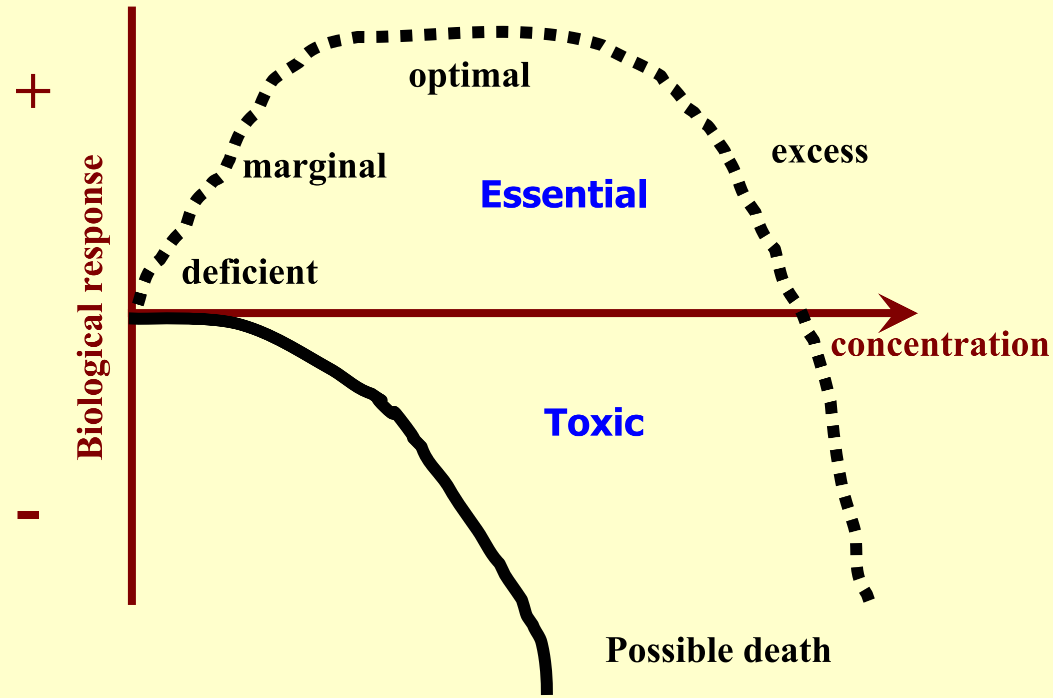
An organism must regulate transcription, translation, proper assimilation and incorporation of the necessary metal to function.

Metal are essential element in cell

double-edge nature of metals in living systems

3D high-resolution magnetic resonance angiogram of a human foot

Image: EPIX Medical, Schering AG, and Berlex Lab



But importance of their chemical form
And their sub-cellular localisation??

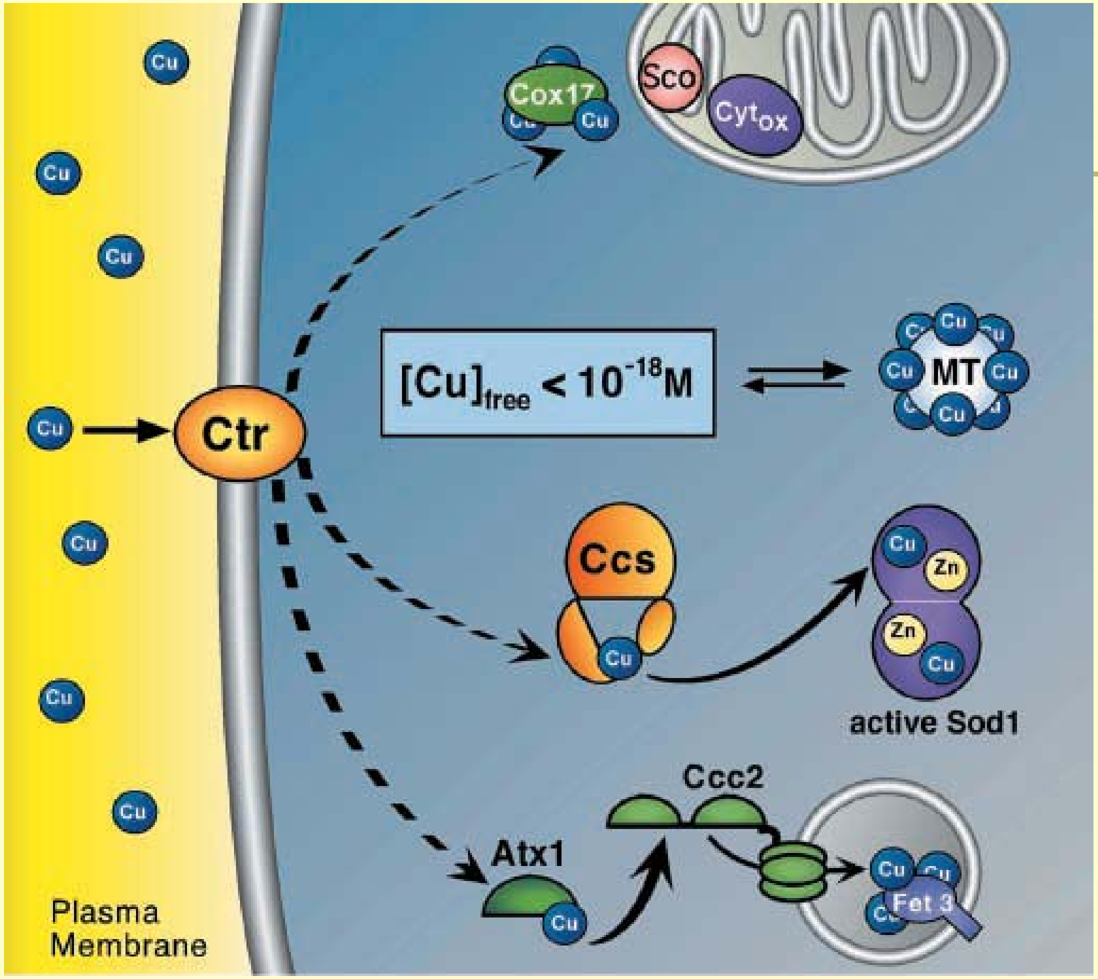
Metal are essential element in cell Ca, Mn, Fe, Cu, Zn, Se…

Homeostasis of metals : Complex regulatory systems

genes, metallochaperones, metallothioneins …

Metal as co-factor of numerous enzymes: Catalase (Fe), urease (Ni), Glutathione peroxydase (Se), DNA polymerase (Zn), Nitrate reductase (Mo), Vitamin B12 coenzyme (Co) …

But metal can also catalyse cytotoxic reactions



Copper trafficking pathways in eukaryotes. Known pathways for the delivery of copper in yeast are depicted. Copper uptake, mediated in part by the cell surface copper transporter (*Ctr*), is eventually deployed to mitochondrial cytochrome oxidase ((*Cytox*) via a pathway involving Cox17 and Sco), to cytosolic SOD1 (via a pathway involving CCS), or to the copper transporter CCC2 and the multicopper oxidase Fet3 in the secretory pathway (involving ATX1). Cytosolic concentrations of free copper are typically maintained at exquisitely low levels (,10218 M) by metal scavenging systems including metallothioneins (*MT*) – **Reprinted from O'Halloran et al. JBC (2000) Vol. 275, No. 33**

Metals are essential elements in cell Ca, Mn, Fe, Cu, Zn, Se…

traces metals

enzymatic systems
various other proteins

Implicated in large number of metabolic processes
(ADN replication, proteins synthesis…),

Participate to the regulation of primordial functions (reproduction, growth, cellular differentiation, brain function …)

Immune defence, ROS detoxification

Aberrations in the cellular metal ion concentrations may lead to cell death and severe diseases

Metals and Medicines

^{111}**In**-DTPA, brain imaging

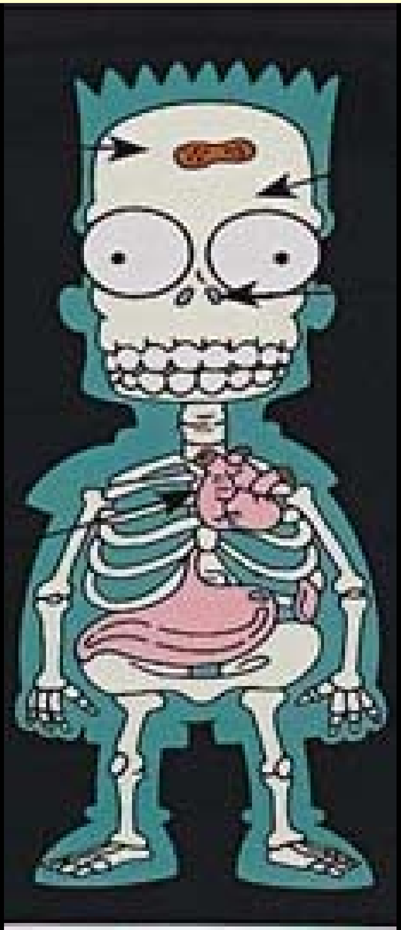
^{153}**Sm**-EDTMP, pain palliation for bone cancer

Ga compounds, bone cancer treatment

Gd-BOPTA, magnetic resonance imaging

Pt compounds, gonad cancer treatment

Au, arthritis treatment



Li$_2$CO$_3$, manic depression treatment

^{67}**Ga**-citrate, clinical diagnosis of neoplasma

^{99m}**Tc** heart function diagnostic

Bi-nanoparticles, X-ray CT

BaSO4, gastrointestinal X-ray contrast enhancement

and others…

Metals and toxicity

~ 30 elements are potentially toxic for human

- Heavy metals : Pb, Hg, Cd

- Importance of the chemical form : Fe(II)/Fe(III); Cr(III)/Cr(VI); As(III)/As(V) …

Various Target

- **Pb: CNS**, [Blood lead concentrations, even those below 10 µg/dl, are inversely associated with children's IQ scores at age three and five, declines in IQ are greater at these concentrations than at higher concentrations , Canfield RL, *et al. N Engl J Med*. 2003.], **Haematopoiesis**
- **Hg: CNS, heart**
- **Ni & Cr: Allergy, lung**
- **Cd: kidneys, prostate**

Example of some average elemental composition

Human ovarian adenocarcinoma cells: Mn (9.5 ppm), Fe (143 ppm), Cu (12 ppm), Zn (212 ppm)…

Human brain tissues - grey matter: P (15500 ppm), S (4600 ppm), Ca (380 ppm), Mn (1.2 ppm), Fe (250 ppm), Cu (22 pm), Zn (78 ppm), Se (0.2 ppm)…

Prostate tissue – cancerous sections: Cr (7 ppm), Mn (8 ppm), Fe (1370 ppm), Zn (17 ppm), Se (11.5 ppm)…

Lead in bone : Tibia Pb ~ 30 ppm

As in hair : As < 1 ppm

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Why synchrotron microprobe?

ELEMENTAL MICROANALYSIS	Analytical depth	Limit of detection	Spatial Resolution	Selectivity	Quantification	Biological samples
Electron microscope EDS (X-ray Energy Dispersive Spectrometry) STM-EELS (Electron Energy Loss Spectroscopy)	0,1 to 1 µm < 50 nm	100 to 1000 µg/g (ppm) 100 ppm	0,02-1 µm 1 nm	Multielement (Z≥6) Multielement(Z≥6) Chemical species	+ +	Hydrated frozen Dehydrated
Nuclear Microprobe	10 to 100 µm	1 to 10ppm	1 µm	Multielement (all Z)	+++	Dehydrated
Synchrotron Radiation Microprobe µ-XAS (X-ray Absorption Spectroscopy)	> 100 µm > 100 µm	< 0,1 ppm 100 ppm	0,1 to 1 µm 1 µm	Multielement(Z≥6) Imaging - 3D Chemical species	++ +	Hydrated frozen
Laser microprobe mass spectrometry (LMMS)	100 nm	< 0,1 ppm	1 µm	Multielement Isotopes	+	Hydrated
Secondary Ion Mass Spectrometry (SIMS)	100 nm	< 0,1 ppm	0,05 µm	Multielement Isotopes	+	Dehydrated (thin sections)

Why synchrotron microprobe?

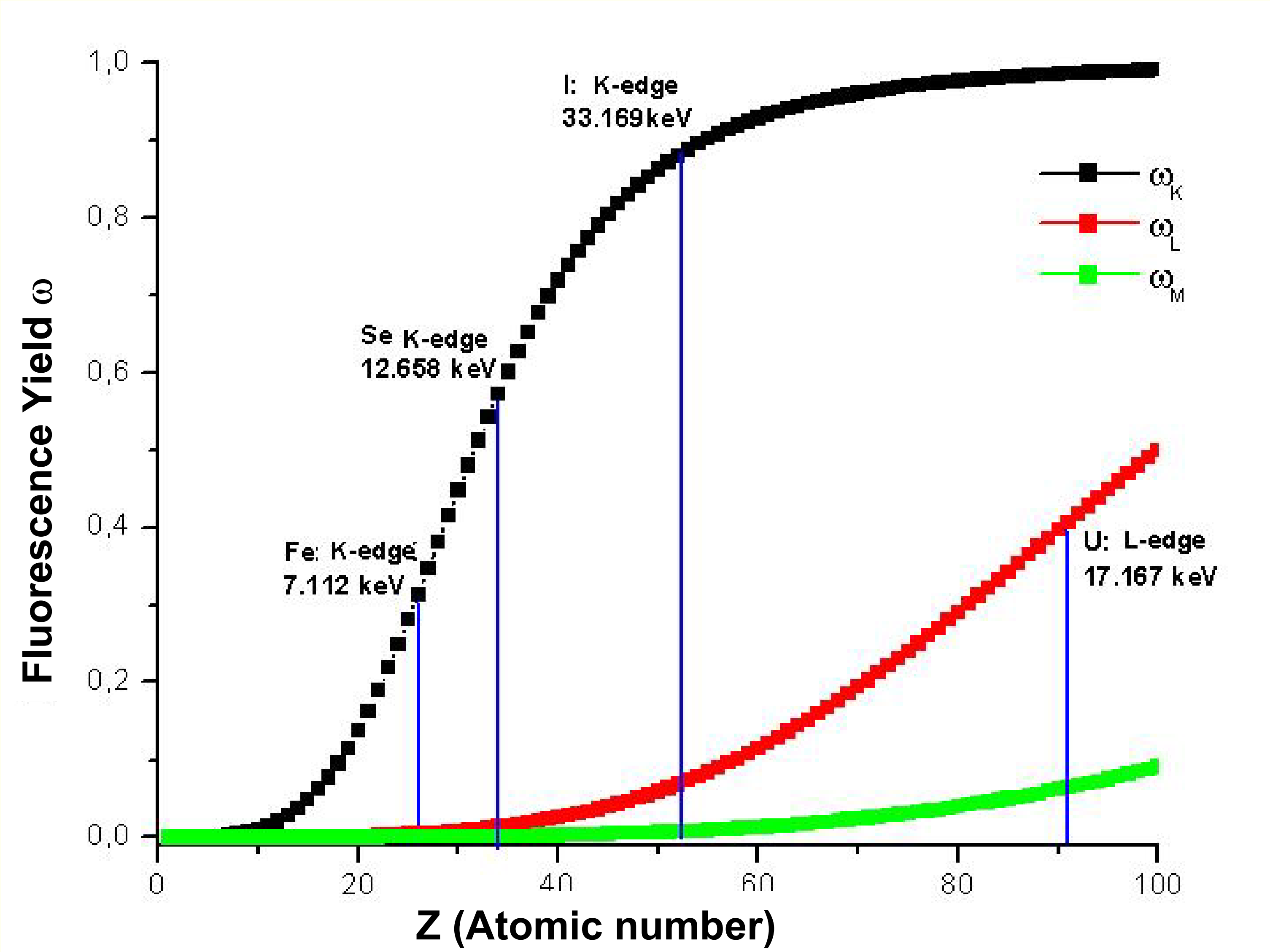
- **Photons best projectiles for XRF:** $\sigma_f^{\ \nu} >> \sigma_f^{\ e^-,p}$

- **Synchrotron source : small, coherent, high brilliance, horizontal polarisation**

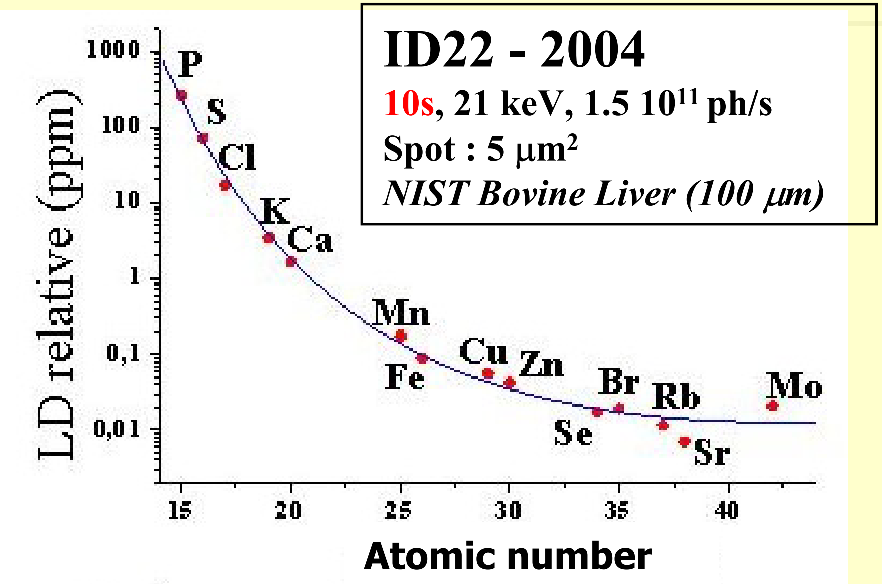
  + Energy tunability

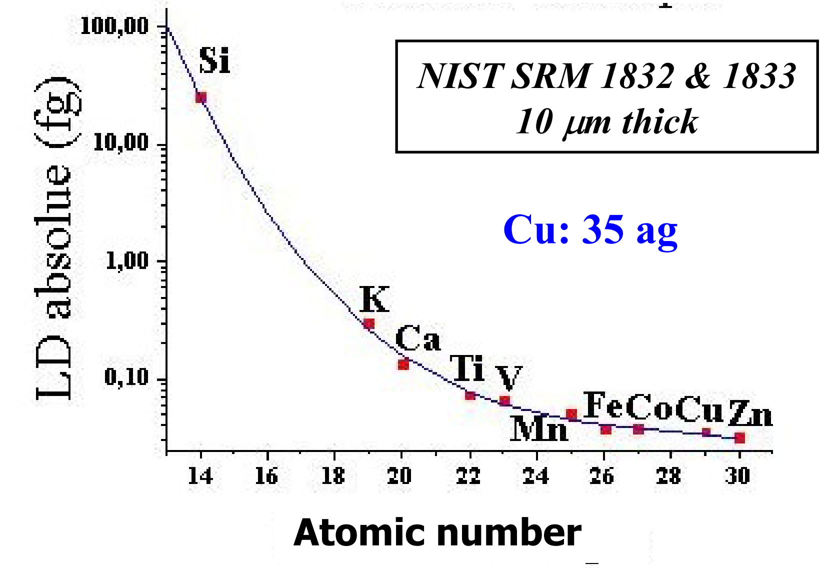
- **X-ray microprobe possible: synchrotron X-ray source properties + X-ray optics developments**

Why synchrotron microprobe?



Why synchrotron microprobe?





Why synchrotron microprobe?

- High sensitivity: ~ ppm (Z>Fe) in a few μm^3 sample
- In-air analysis, no sample staining
- Substantial reduction in radiation damages compare to PIXE, SIMS, EM
- Less constrains for sample preparation
- Quantification
- Selective chemical imaging (X-ray spectroscopy)
- Large analytical depth (10 – 100 μm) with high spatial resolution
- Large sampling area possible
- Multimodality (Fluorescence, spectroscopy, diffraction, X-ray imaging)

Powerful method to study trace metals in biological structures

Non-destructive?

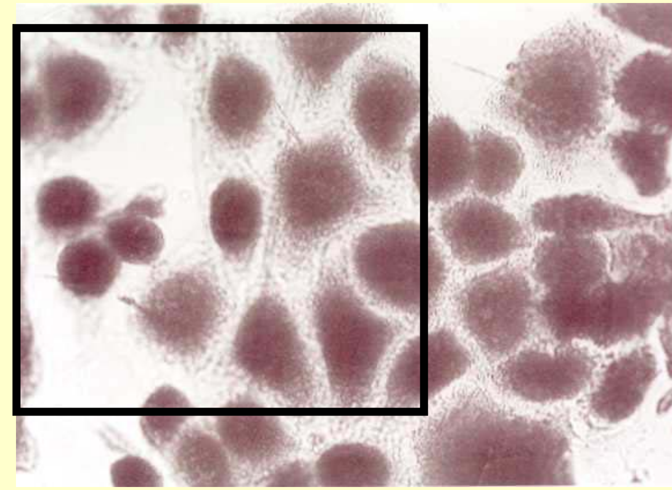
IGROV-1 cells, Freeze-dried
E =14 KeV, Flux=1.4.1011ph/s

beam ~1.3x3.5 μm, Map. 100x160 μm^2
3 sec/pixel : size 0.5x2 μm

No observed damages in Soft X-ray cryo-microscopy till 10^{10} Gy (total) [*Jacobsen et al. Nature Structural Biology, 5,(1998)*]

ID22 10^{+11} **ph/s/μm^2 ~ 3.10^7Gy/s**

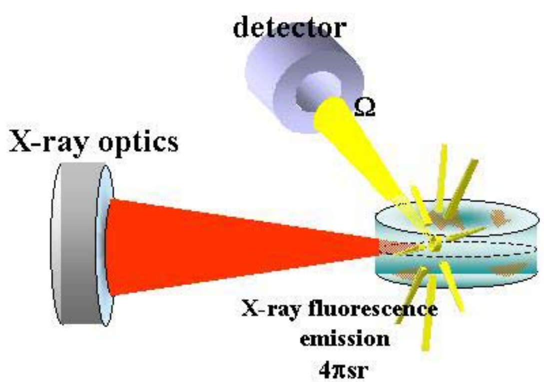
ID22NI > 10^{+13} **ph/s/μm^2 > ~3.10^9 Gy/s, @ 17 keV**



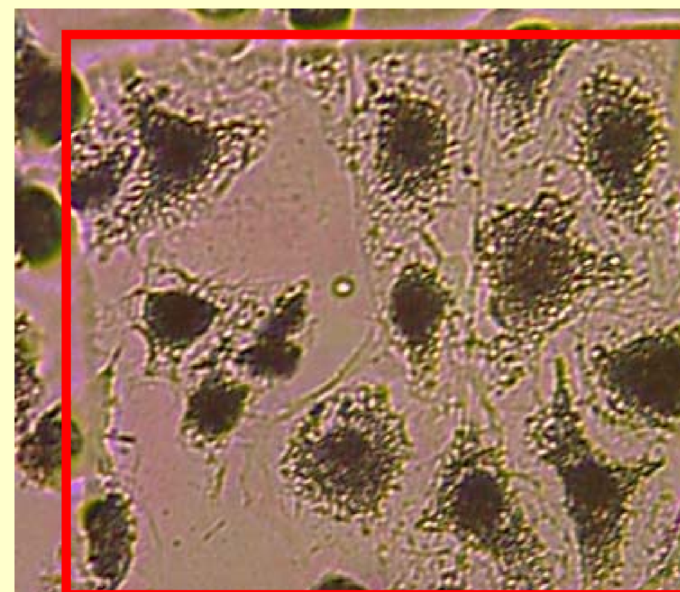
Dehydrated samples: problems appearring

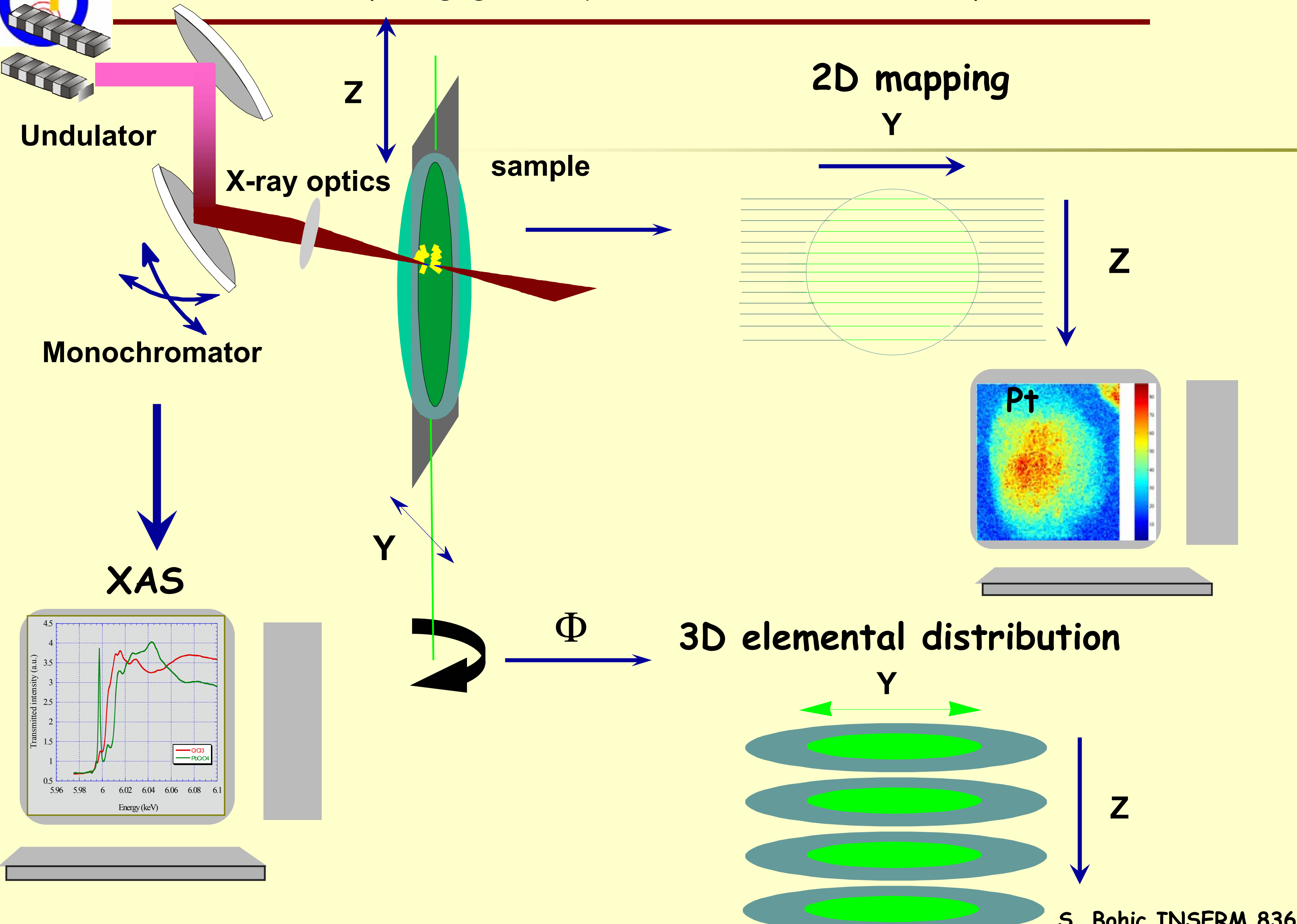
Approaching the limits for frozen-hydrated samples

Fast scan required (ex:@ 0.1μm, 0.1s/pixel, ~2h acquisition = area~25x25 μm^2)



XRF signal collected: only 5-6% of 4πSr





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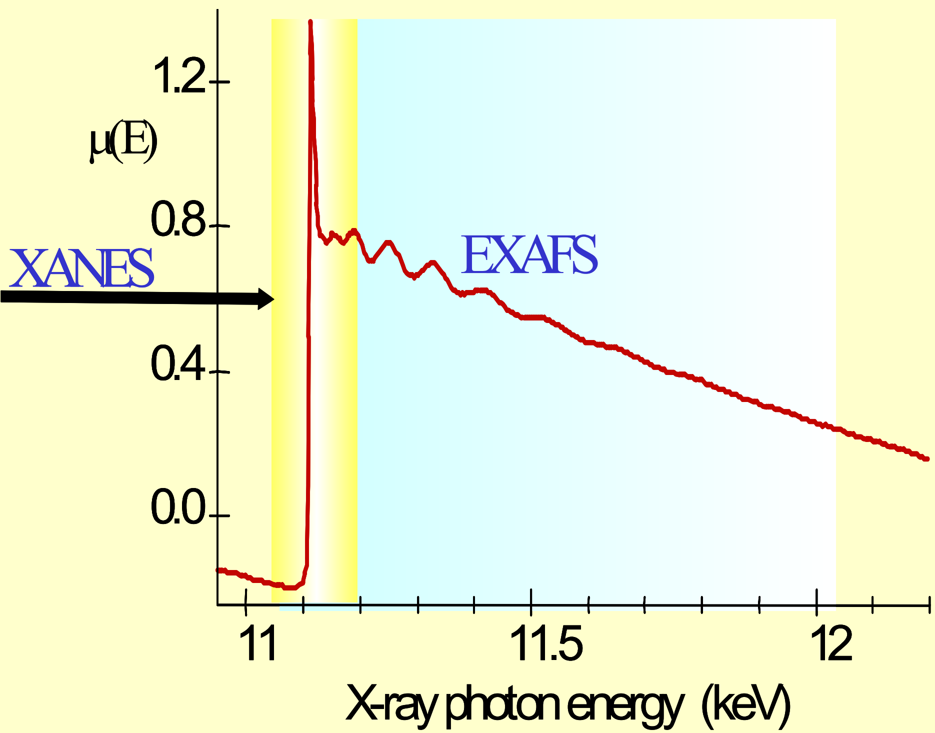
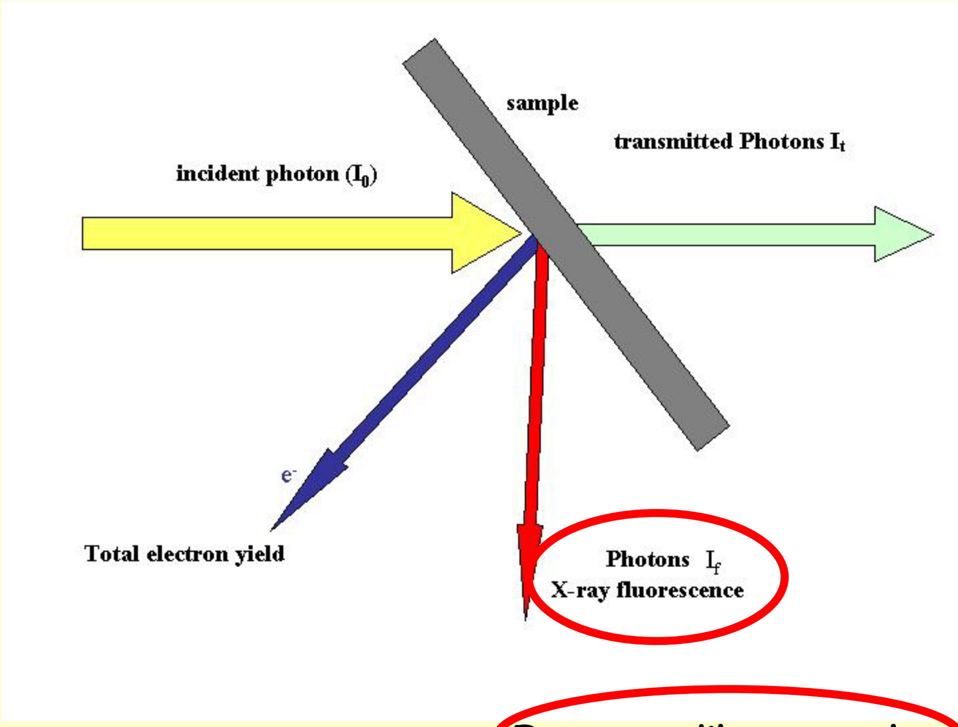
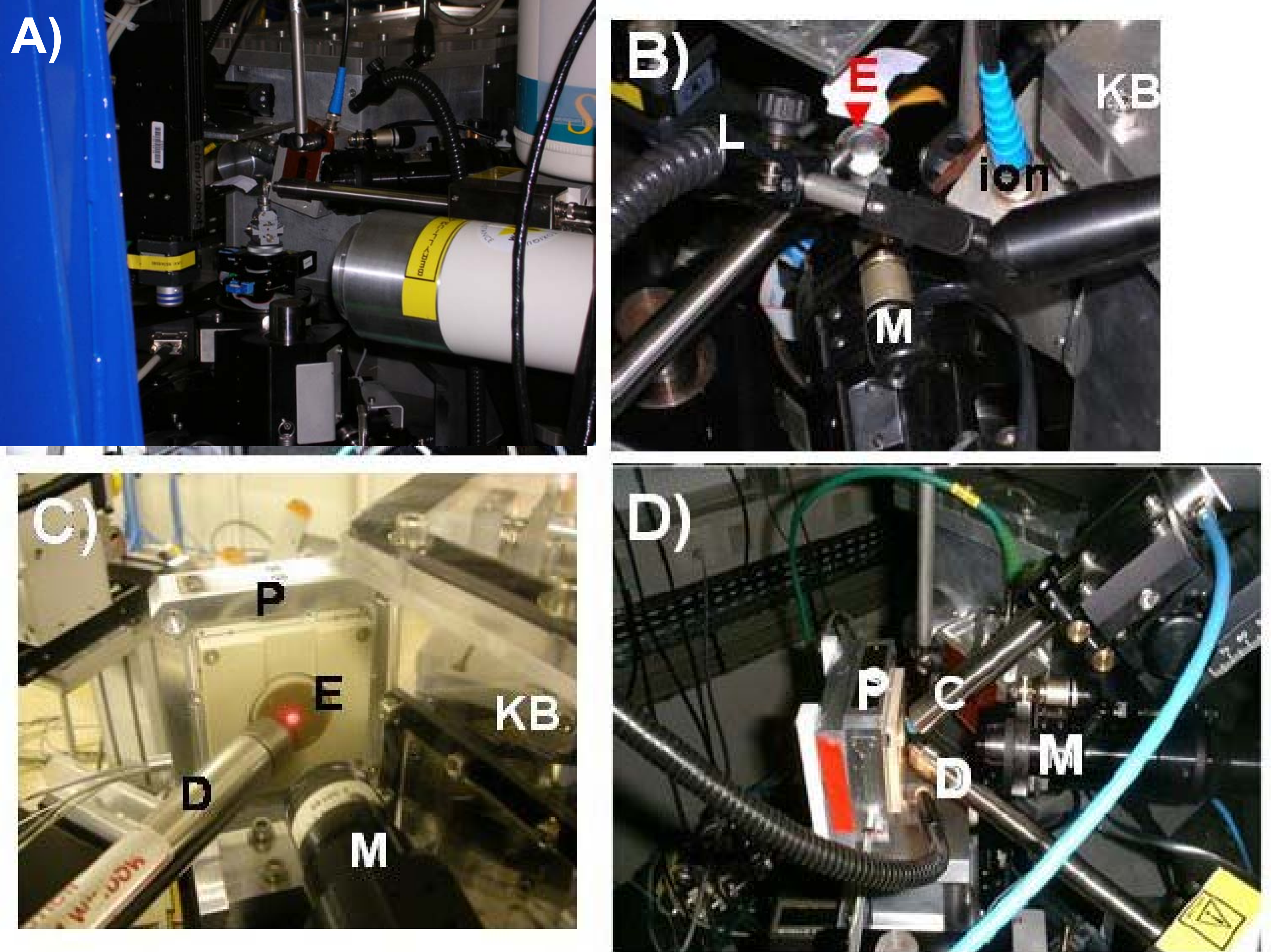
Additional info: X-ray absorption spectroscopy





For very dilute samples

Info: oxidation state, number and type of neighbour atoms, interatomic distance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Outline

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Metals detection in cells and tissues

Anticancer drugs – Cellular localisation

Metals detection in cells and tissues (Ga-drugs)

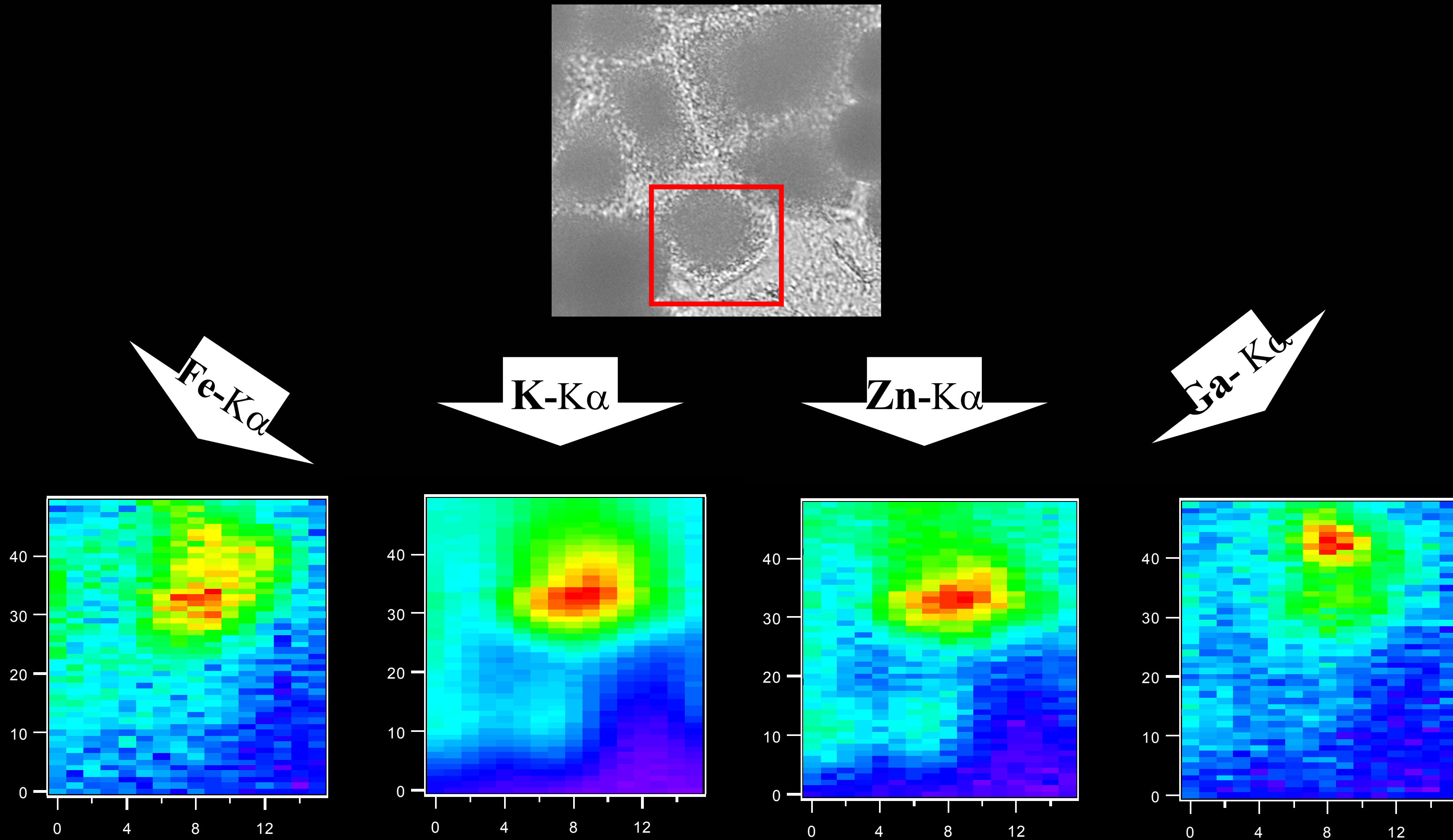
Ga: second most used metal in chemotherapy with Pt-based drugs.
$GaNO_3$ clinical efficacy in hypercalcemia and bone cancer

Ga^{3+}: similar solution and coordination chemistry to those of Fe^{3+}

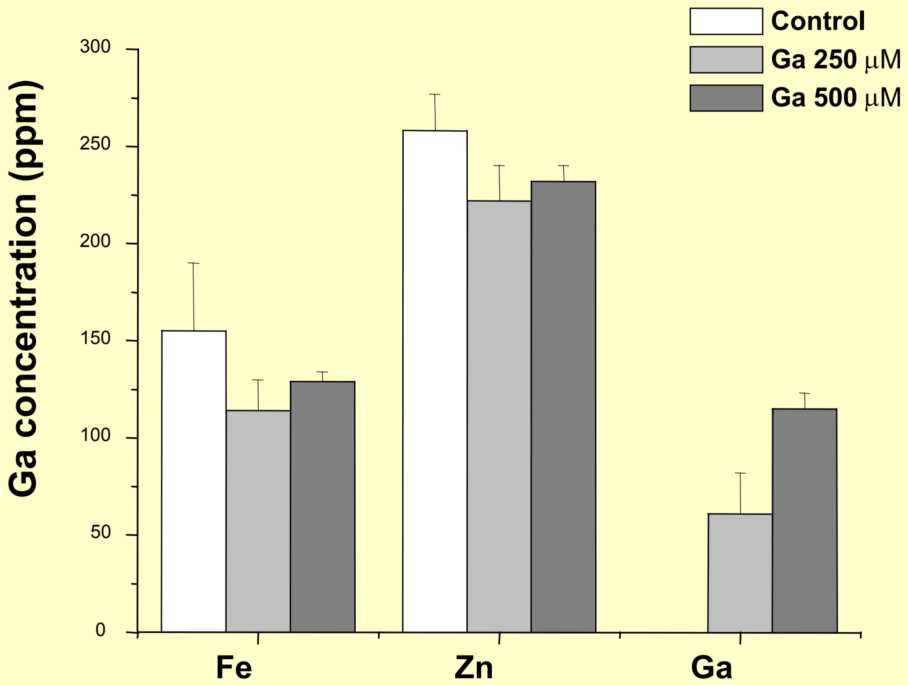
Gallium can be complexed to transferrin receptors and released from lysosomes.

Cellular mechanisms for Ga antitumor activity still not fully elucidated.

Metals detection in cells and tissues (Ga-drugs)



Metals detection in cells and tissues (Ga-drugs)



- Ga localisation in cells found mainly uniform, few % of cells Ga and Fe found in ~5μm structures in the perinuclear region - possibly lysosomes

- Results found correlated with PIXE microanalysis

- No nuclear localisation, Ga may interfere with iron homeostasis

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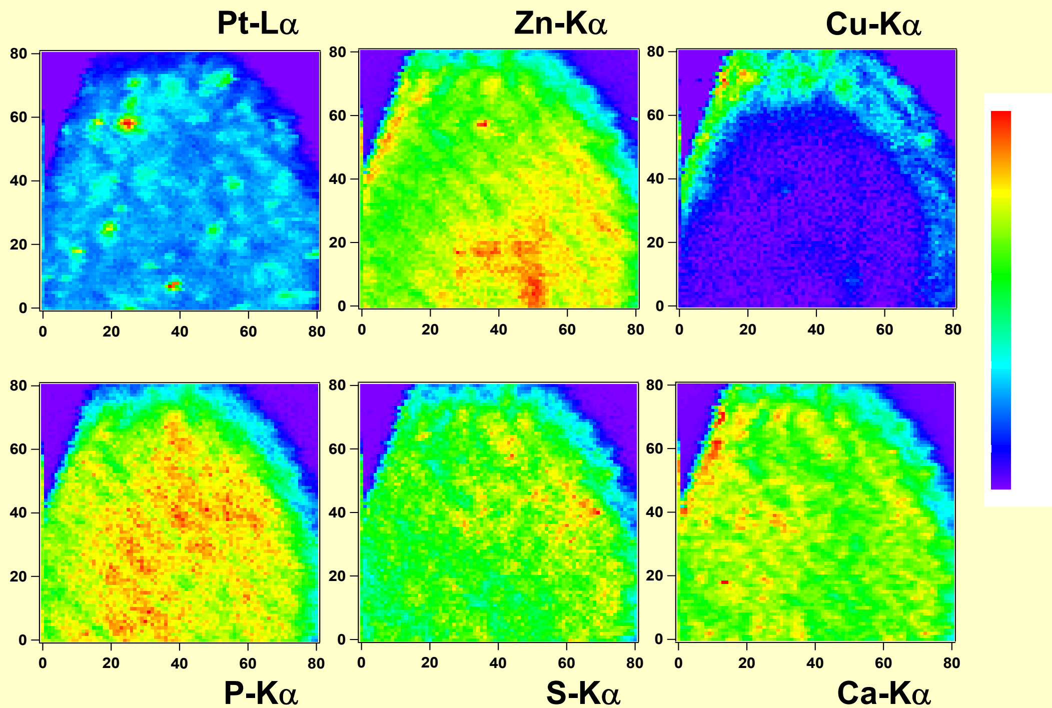
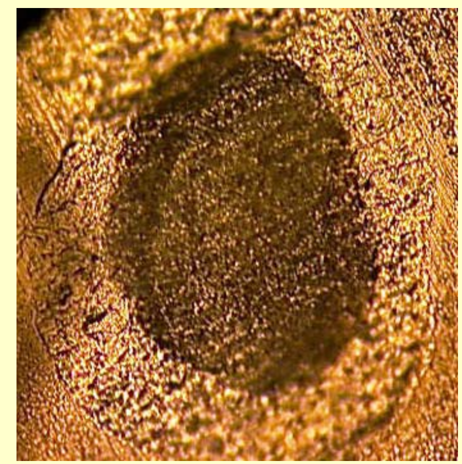
Metals detection in cells and tissues (Pt-drugs)

Alderden, R.A., 2005. PhD Thesis. School of Chemistry; University of Sydney.

Centre for Heavy Metals Research,



elemental distributions in a segment of a cisplatin treated spheroid (24 hr, 50 μM,). pixel is 3 x 2 μm (H x V).



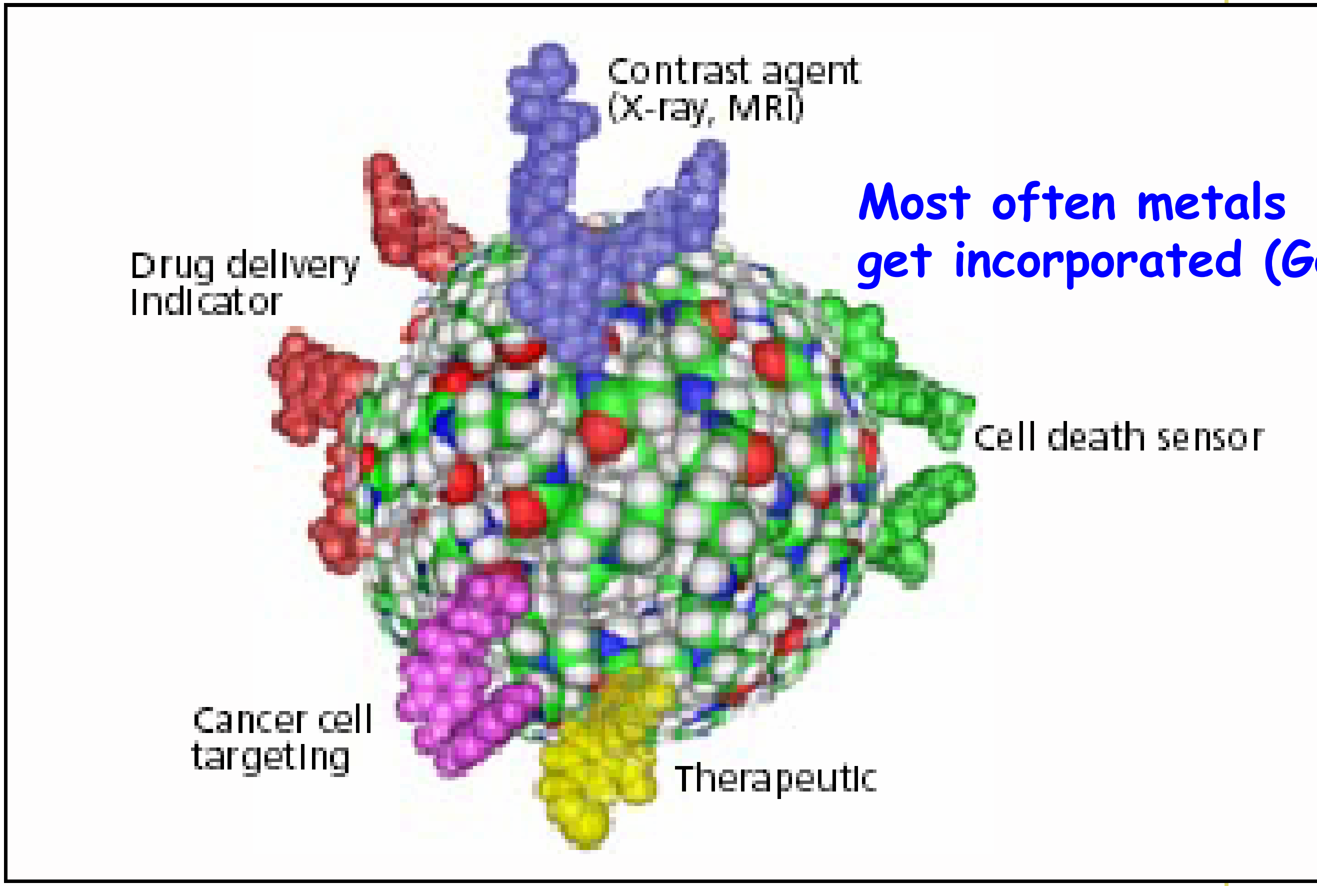
Microscope image (x10): cisplatin treated spheroid, formalin fixed/ paraffin embedded, 20 μm section

- S-XRF useful to study intracellular pharmacology of newly synthesized Pt-based anticancer drugs *in vitro*

Metals detection in cells and tissues

Multifunctional agent

Multifunctional agents



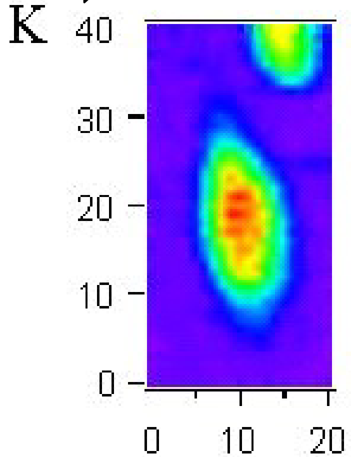
Most often metals get incorporated (Gd, Fe,Ti…)

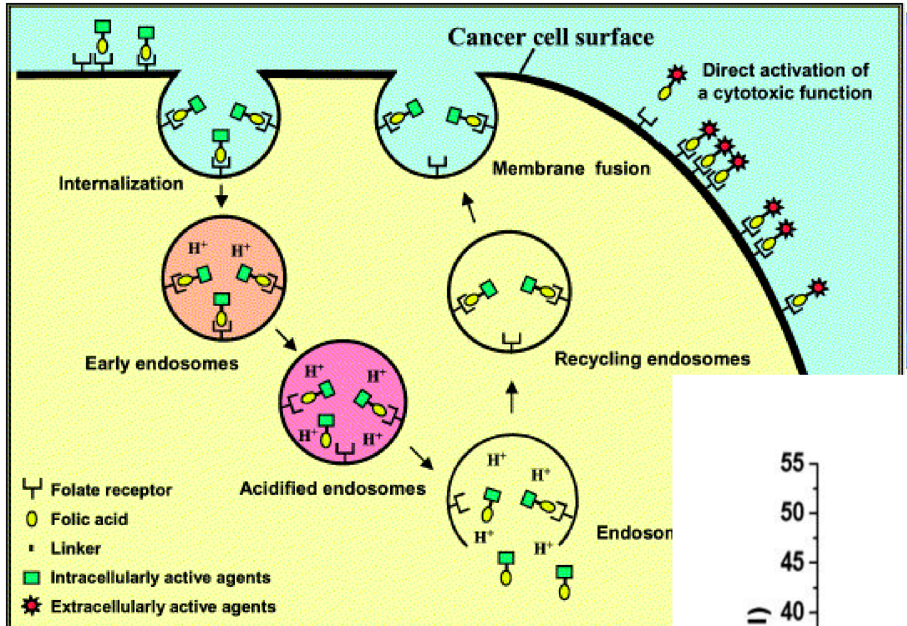
nanoscale platforms for creating multifunctional devices capable of detecting cancer and drugs delivery.

Tumour cell specific MRI contrast agent

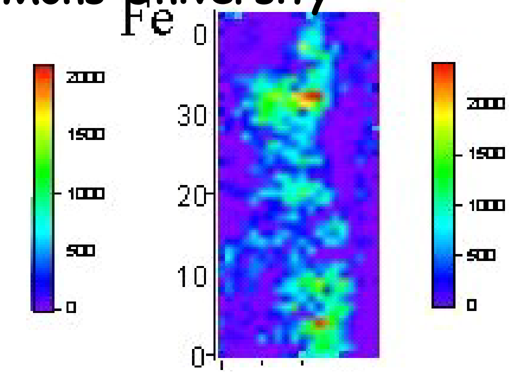
Prof. RN Muller,
Mons University

Cell CHO-K1





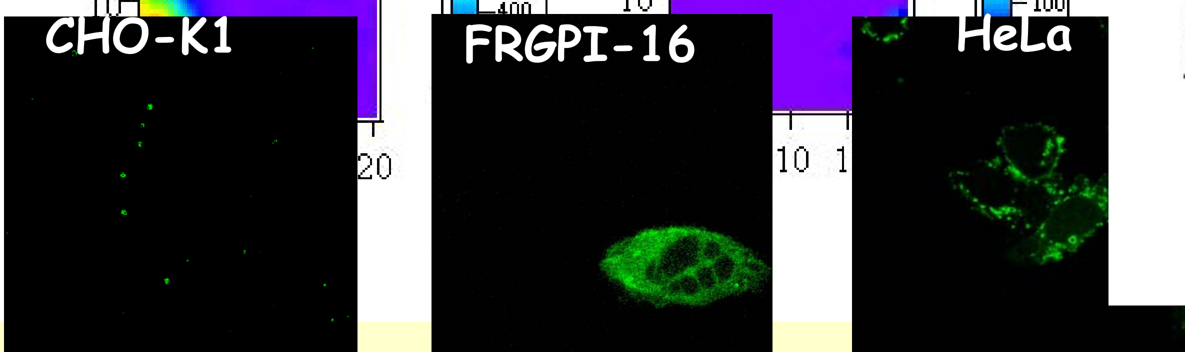
Cell FRGPI16

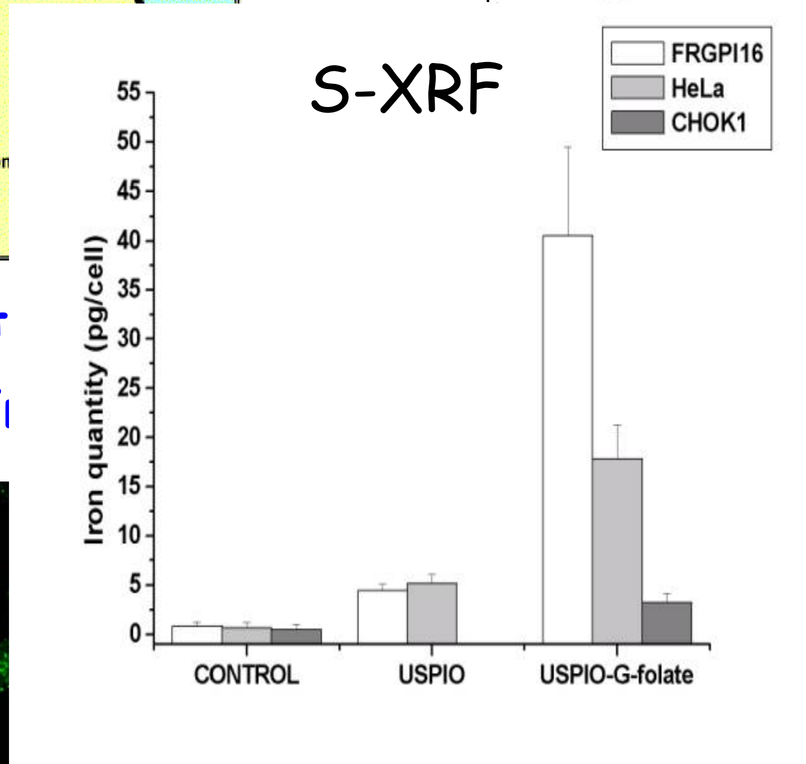


Cell H

Confocal microscopy – 3 h. incubation wit

USPIO = Ultra small superparamagnetic i

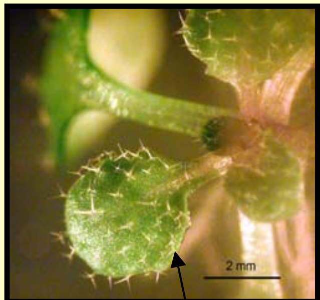




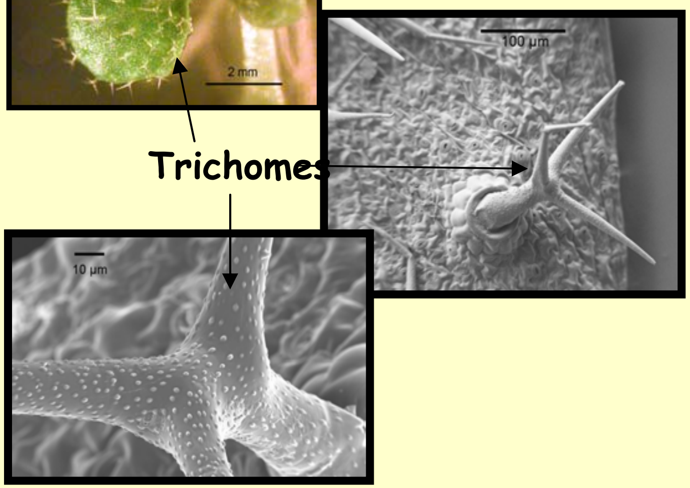
Heavy metal and plant physiology
Phytoextraction

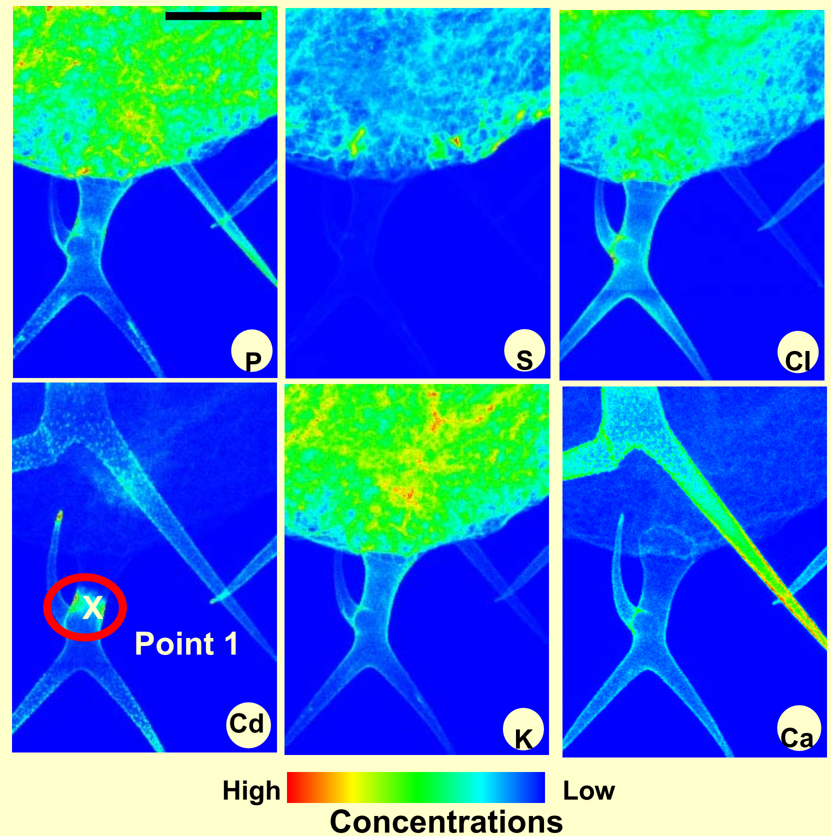
Metals detection in cells and tissues

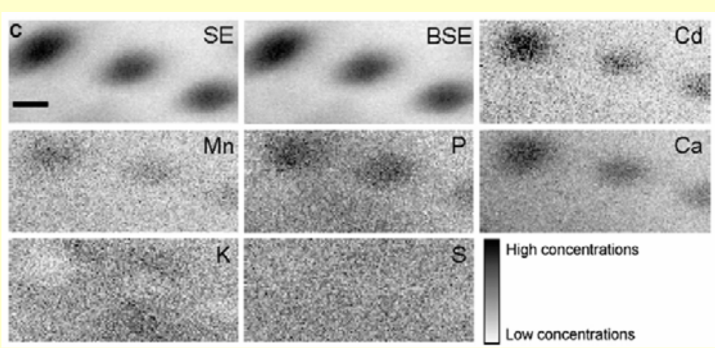
Localization and chemical form of Cd in the model plant *Arabidopsis thaliana*



Beamline ID21 (ESRF)
beam ~ 1 μm²



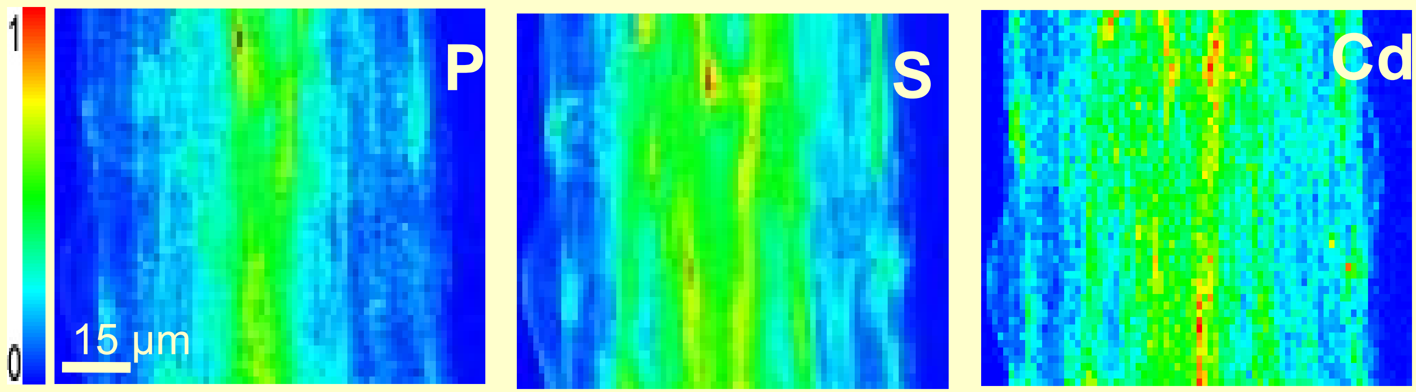


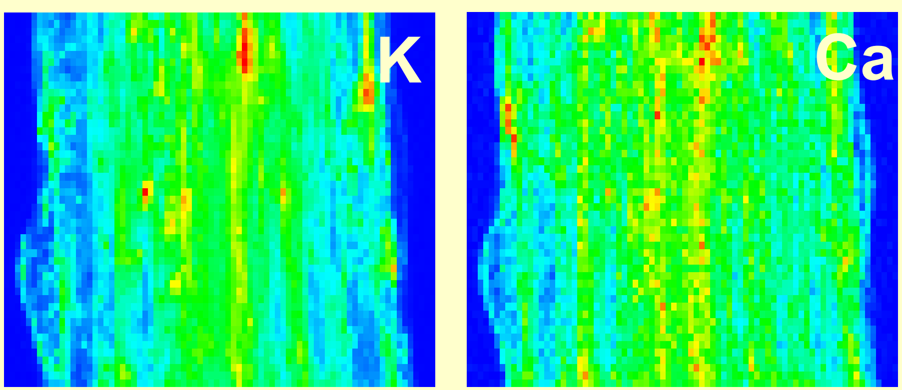


(Isaure et al., Spectrochim. Acta B., 2006)

Metals detection in cells and tissues

Localization of cadmium in roots by µ-xrf





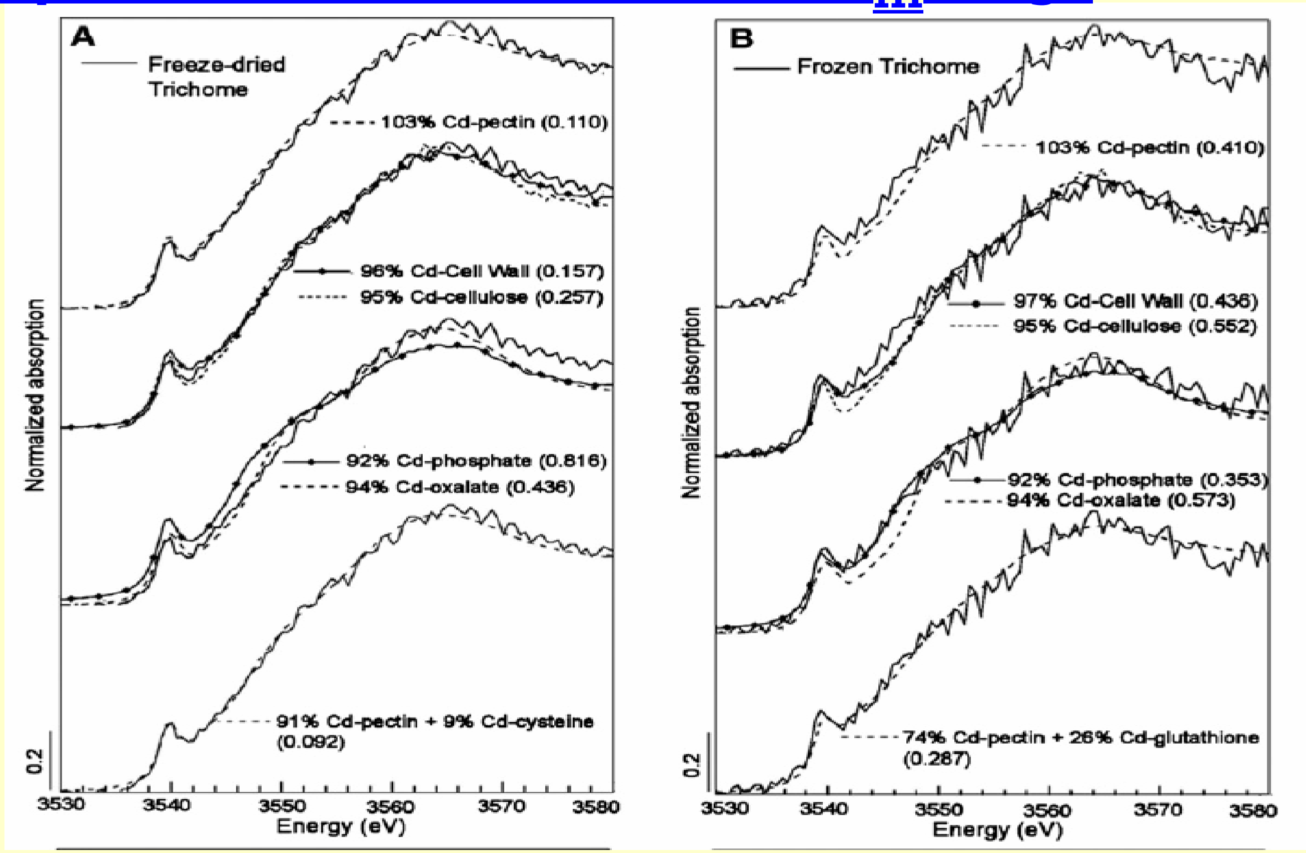
Elemental maps of a root obtained by µXRF. Maps were normalized by the incident energy I_0.
Step size = 1µm, dwell time = 500 ms/pixel @ 3.55 keV and 250 ms/pixel @4.1 keV

Cd is preferentially localized in vascular bundles of the root. Co-localization with S.

(Isaure et al., Spectrochim. Acta B., 2006)

Metals detection in cells and tissues

cadmium speciation – microXANES @ Cd L_{III} -edge



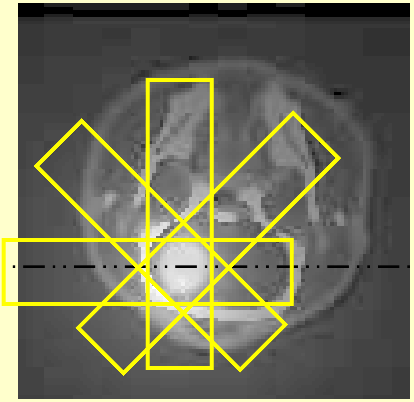
- Cd is associated with O/N ligands in the trichome.
- Best spectral agreement found for Cd adsorbed on cell wall: Cd is complexed to carboxyl and/or hydroxyl groups belonging to the trichome cell wall

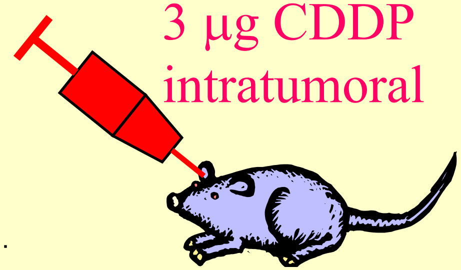
(Isaure et al., Spectrochim. Acta B., 2006)

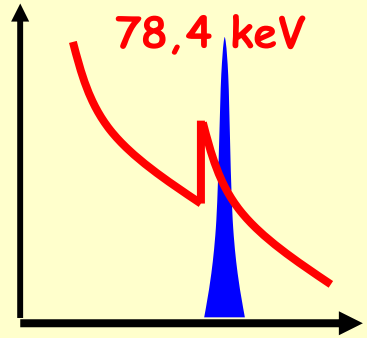
Metals detection in cell and tissues

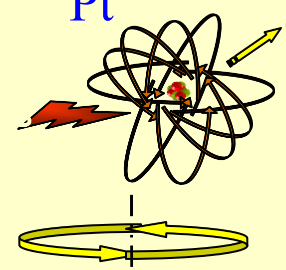
Cisplatin detection in brain tumor –Synchrotron photoactivation

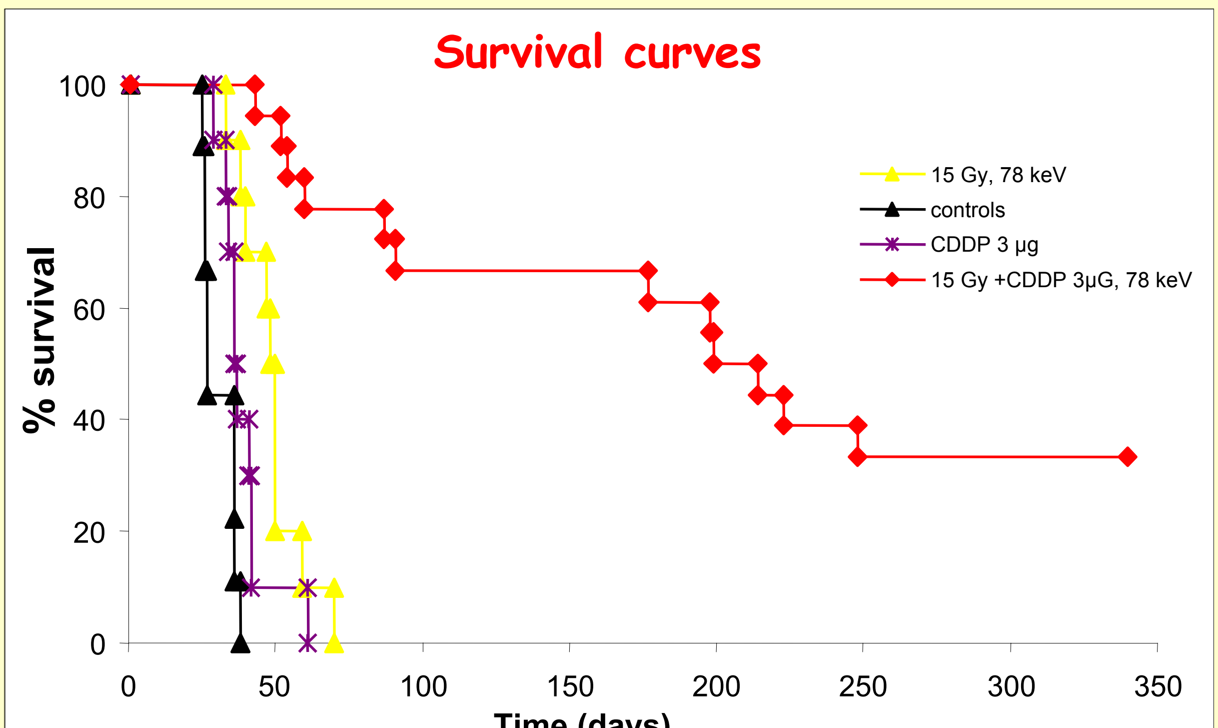
Metals detection in cell and tissues









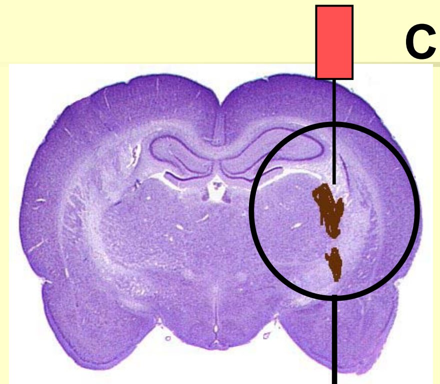


<u>Biston et al. *Cancer Res* (2004) 64</u>

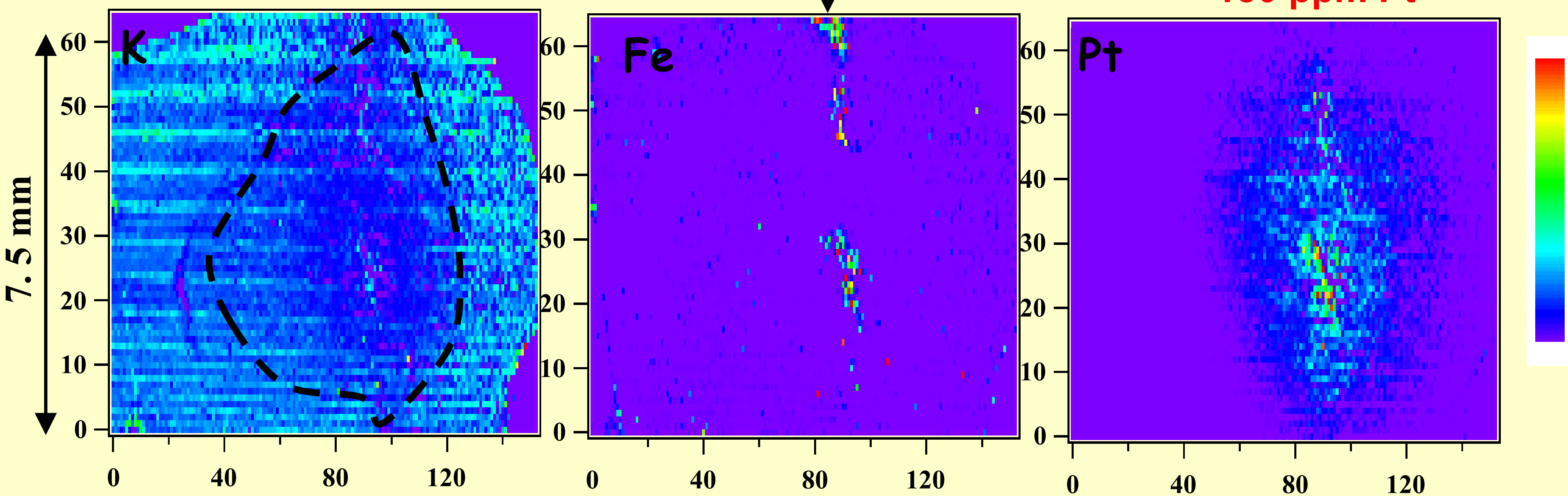
Metals detection in cell and tissues

Cisplatin

(3 µg)



< ~150 ppm Pt >



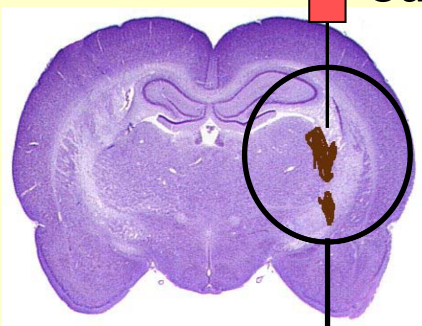
Spot 10 µm, stepsize 50 µm

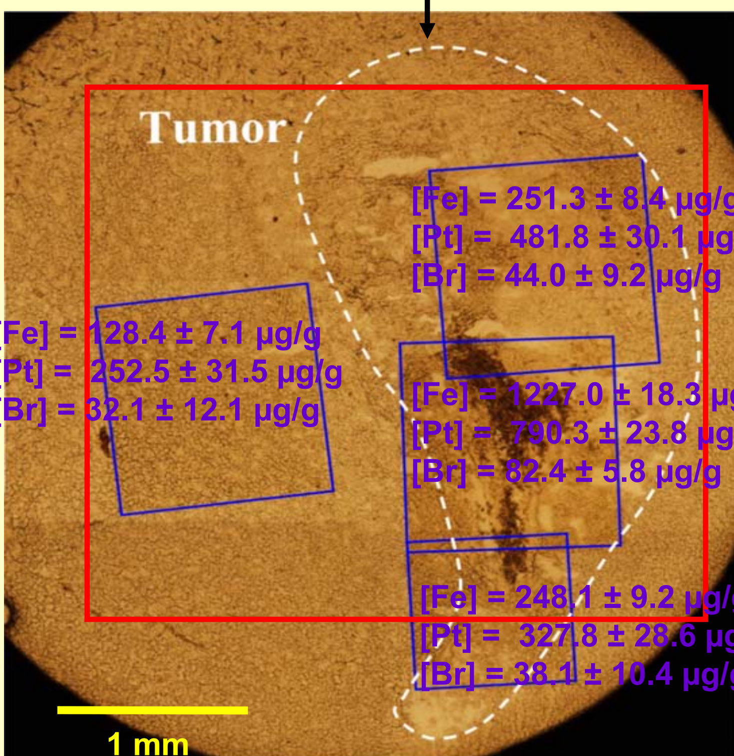
Biston et al. *Cancer Res* (2004) 64

Metals detection in cell and tissues

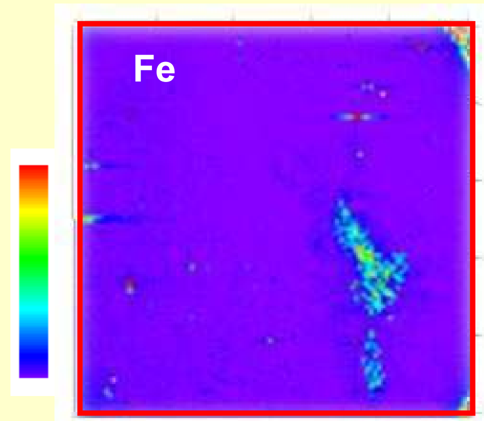
Carboplatin

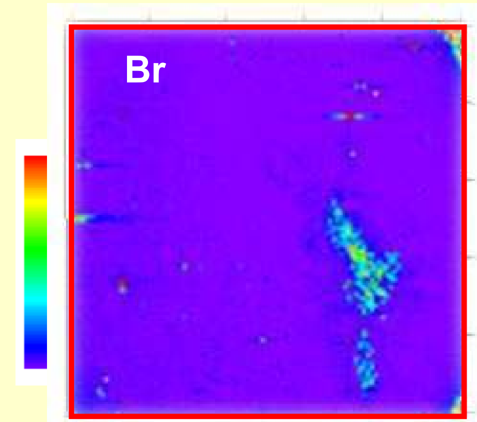
(40 µg)

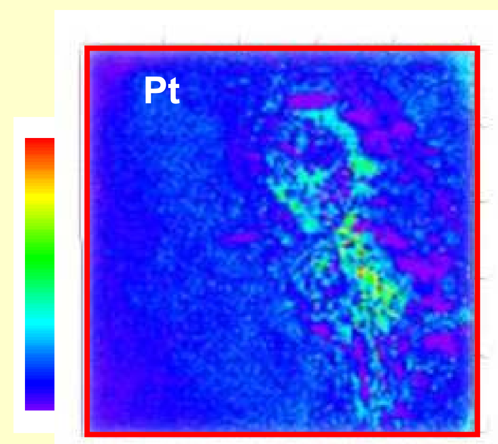




PIXE







1 mm

< ~ 610 ppm Pt >

SXRF

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Metals detection in cell and tissues

As-based drug against acute leukeamia–
Hair microanalysis

Metals detection in cell and tissues

Localisation and speciation of arsenic in hair of leukaemic patient treated with low pharmacological concentration (<1 μmol/l) As_2O_3

Pharmacological doses of As_2O_3 $\Rightarrow$ Is the drug trapped in the hair before or after being metabolised in its active form?

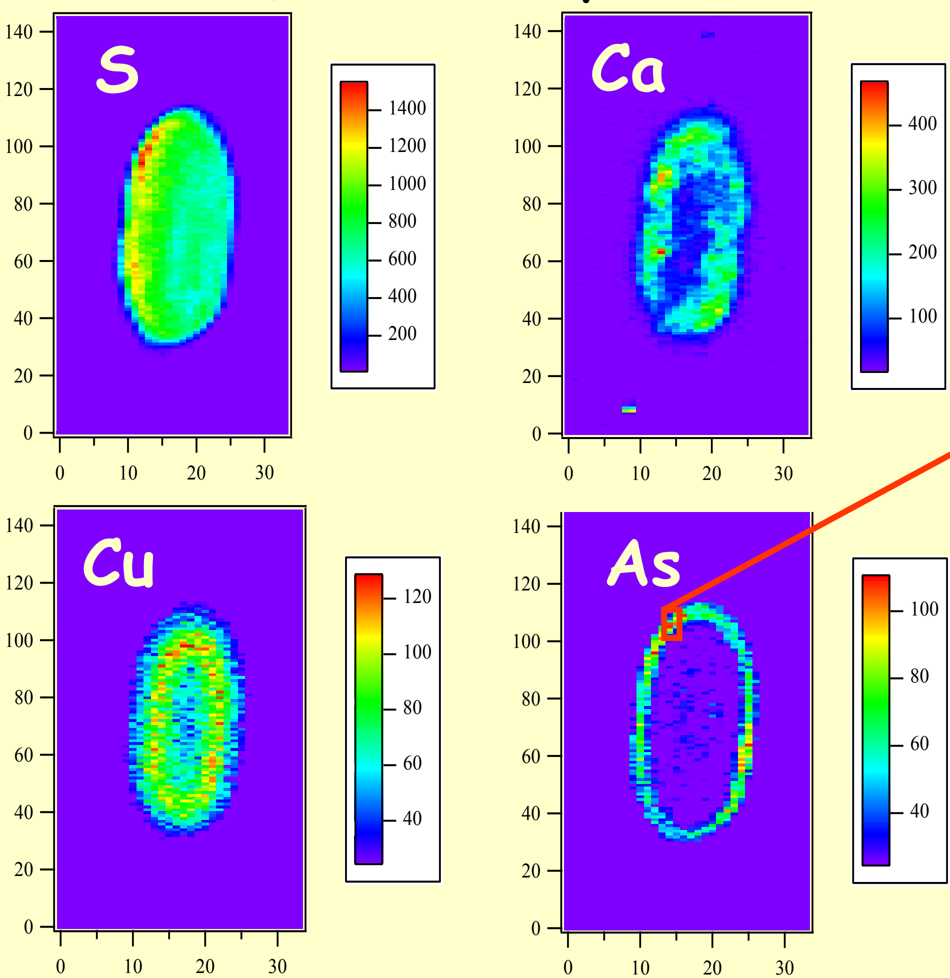
Hair growth 350 μm/day: 3-4 months history, As content vs. treatment?

Hair storage, released? kinetic?

Relevant biological system – easily sampled stored and transported

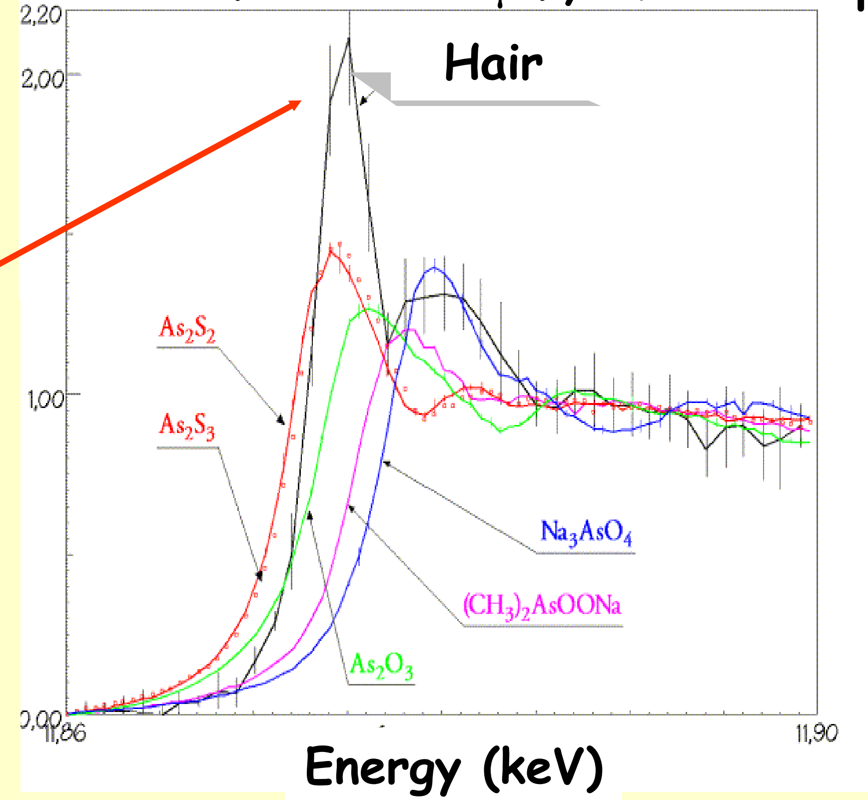
Metals detection in cell and tissues

μ-SXRF mapping of hair from patient treated with As_2O_3 <1 μmol/l section 15 μm thick; Map 120 x140 microns, Dwell time: 3sec./pts



μ-XANES

Beam size 1x3 μm, 0.5 eV step



Metals detection in cell and tissues

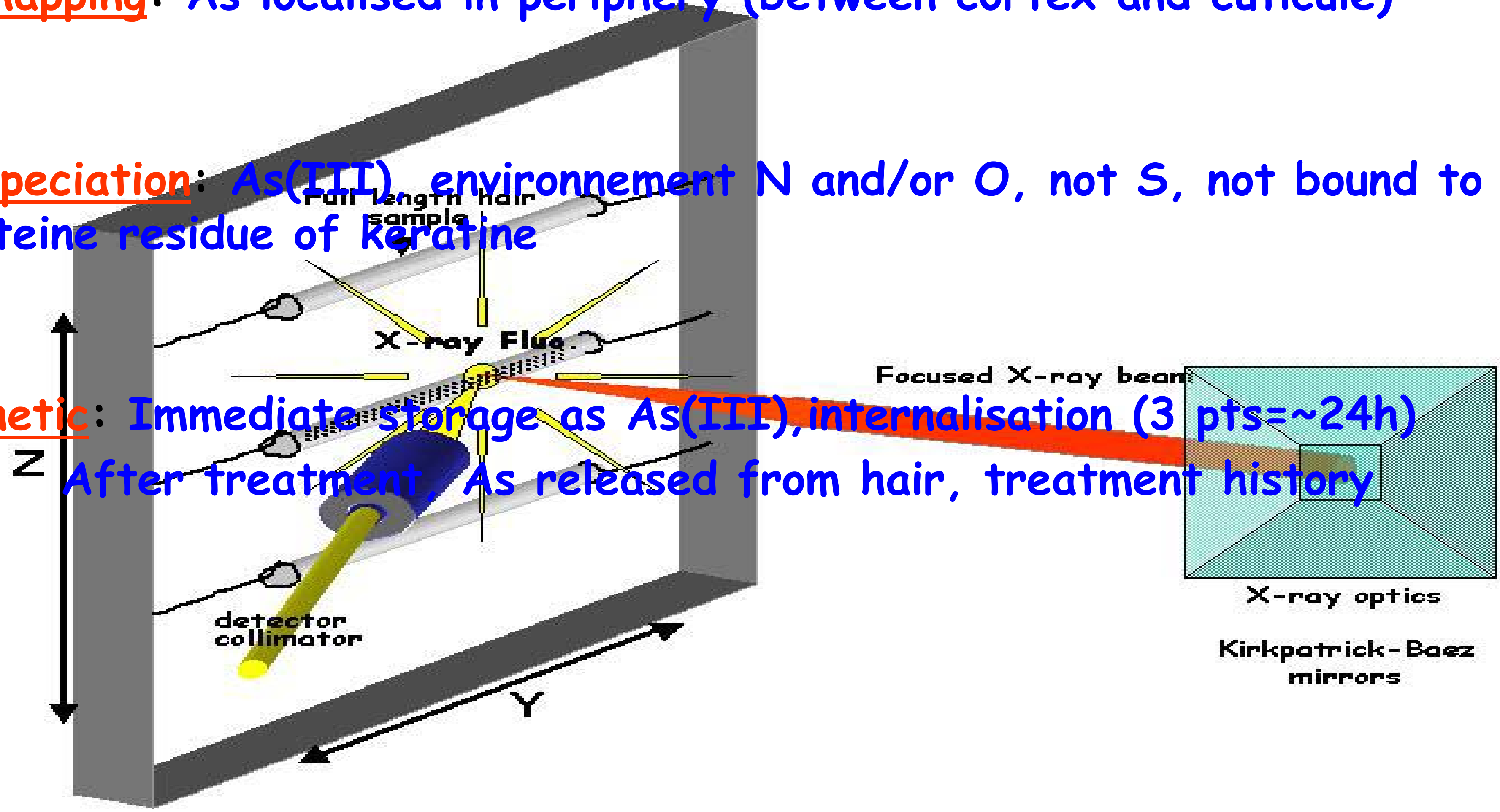
- Follow-up of As in patient's hairs

- Average value over 3 lines: 100 µm step (~ 7h hair growth)

- Mapping: As localised in periphery (between cortex and cuticule)

- Speciation: As(III), environnement N and/or O, not S, not bound to cysteine residue of keratine

- Kinetic: Immediate storage as As(III), internalisation (3 pts=~24h)
After treatment, As released from hair, treatment history



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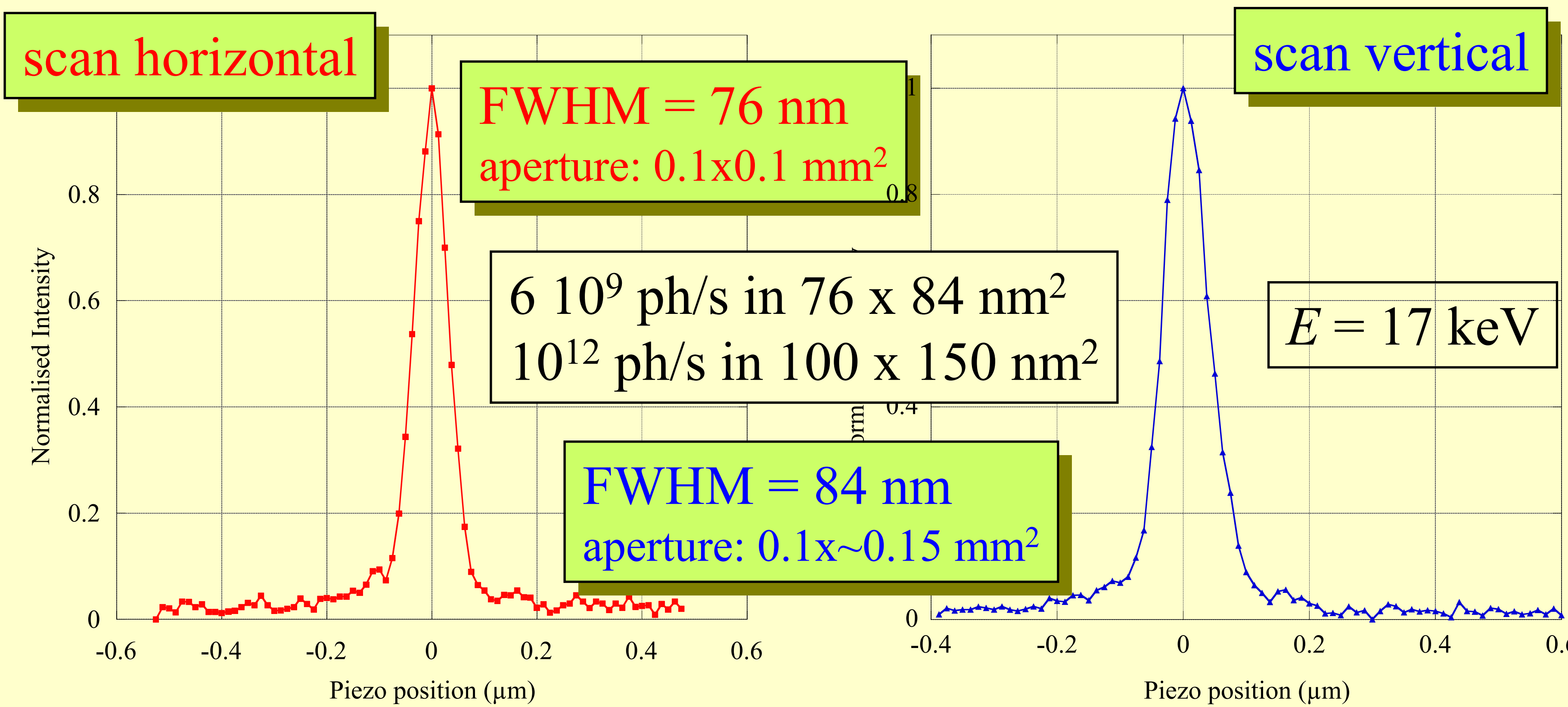
Why synchrotron microprobe ?

metals detection in cells and tissues

High-resolution cellular chemical imaging

Synchrotron Nanoprobe

(ESRF Project – P. Cloetens & ID22 – X-ray imaging group)



ID22NI, February 2006

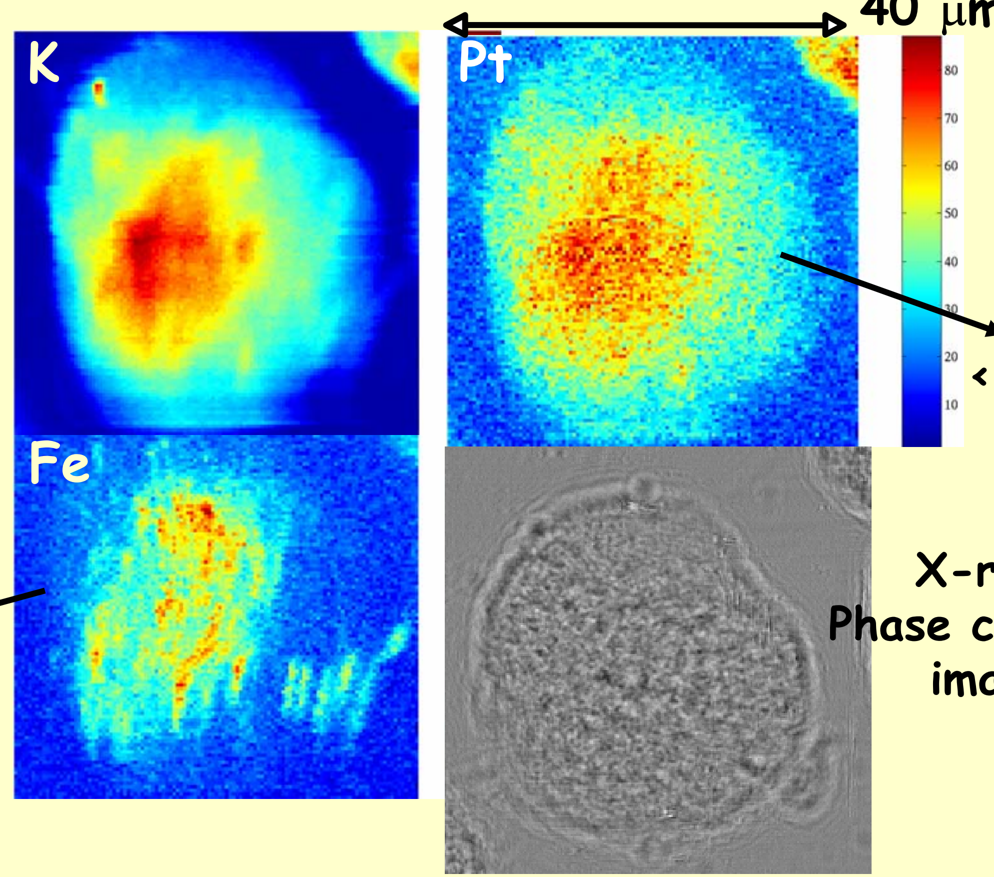
S Bohic, P Cloetens, W Ludwig, R Tucoulou

High-resolution cellular chemical imaging

intracellular pharmacology of Pt antitumor drugs

IGROV1-p cells
Cryofixed/lyophilised

Cisplatine 10 μg/ml
Spatial resolution 0,3μm



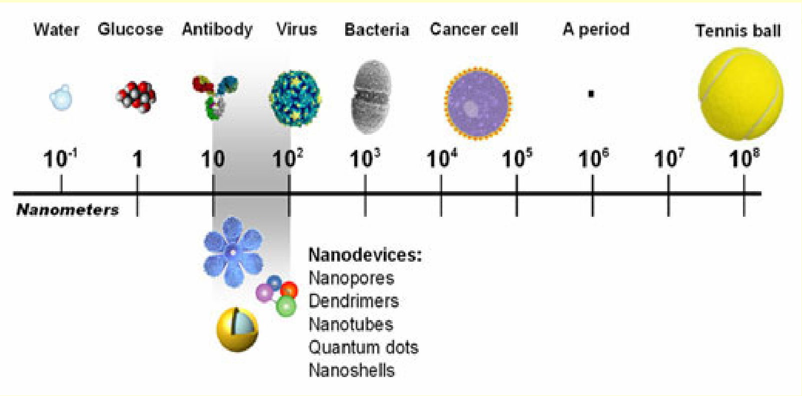
<500 ppm>

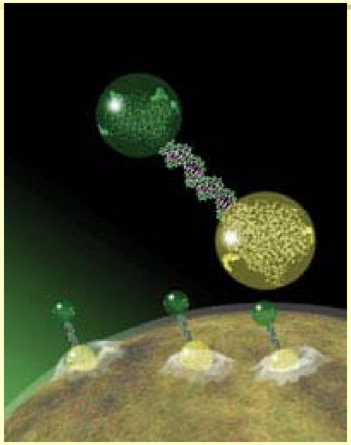
<200 ppm>

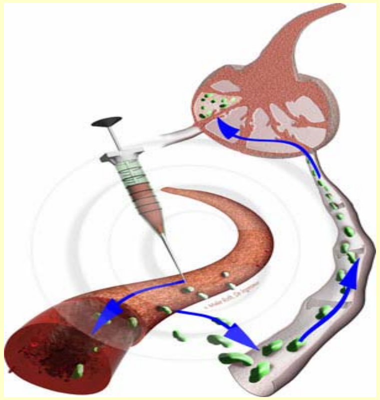
X-ray
Phase contrast
image

Complementary data on Pt-drugs pharmacology
Information input for models on X-ray/Pt interaction in DNA

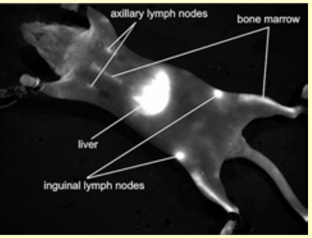
Metal-based nanoparticles– Nanomedicine

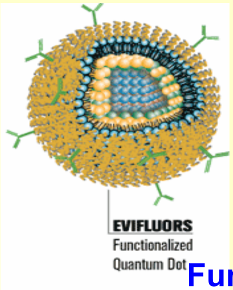


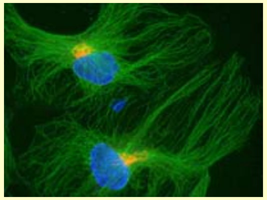




Drugs



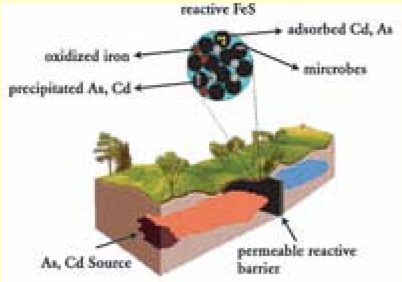




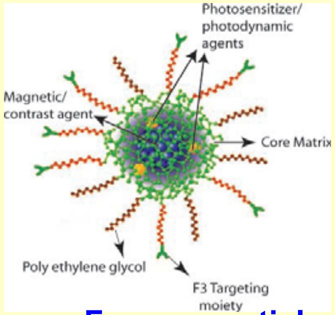
Functionalized Quantum dot

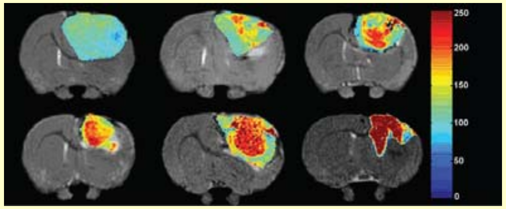
Au-nanoparticles (tumor treatment)

Cosmetics



Waste removal





Fe-nanoparticles + PDT (tumor treatment)

High-resolution cellular chemical imaging

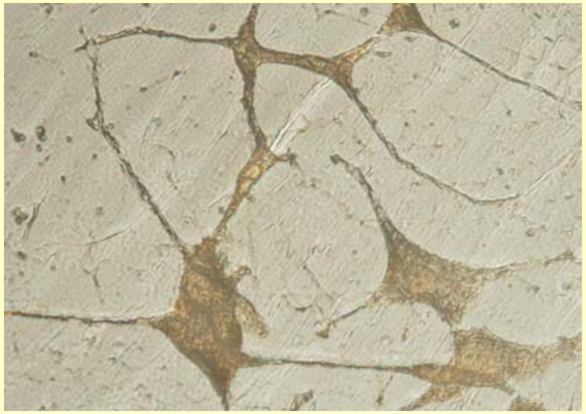
Fe et neurodegenerescence

R. Ortega (CNRS-Bordeaux); P. Cloetens (ESRF-ID19)

- Occupational exposure to specific metals, such as aluminium, manganese, lead, mercury, zinc, copper, and iron appears to be a risk factor for PD.

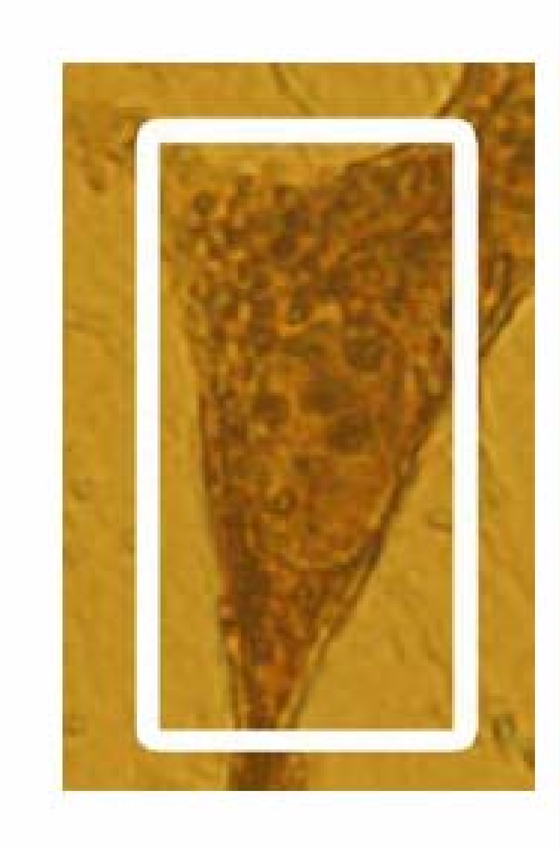
- Recent evidence suggest that a disruption of copper and iron homeostasis is implicated in the overproduction of free radicals, and is suggested as a possible causal factor in the death of nigral cells associated to PD.

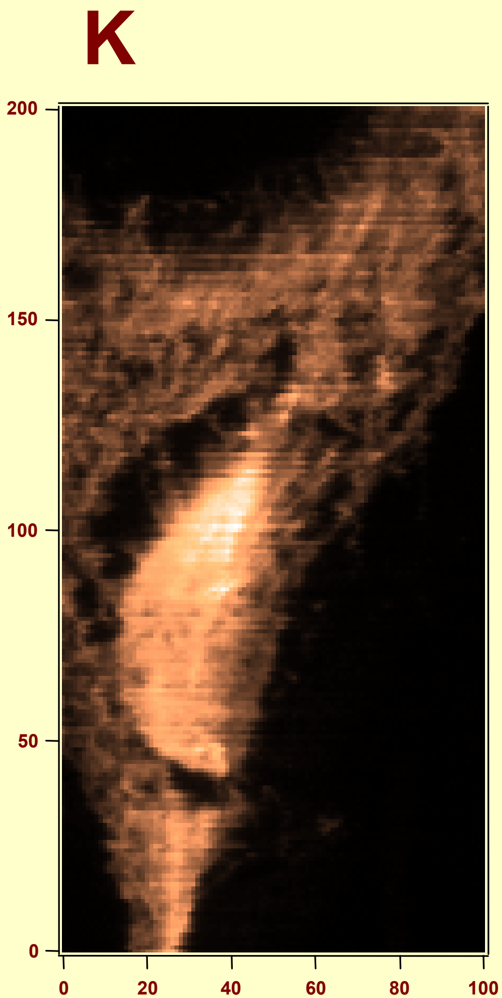
Nano-analysis of cellular models of dopaminergic cells
Metal concentrations and distributions

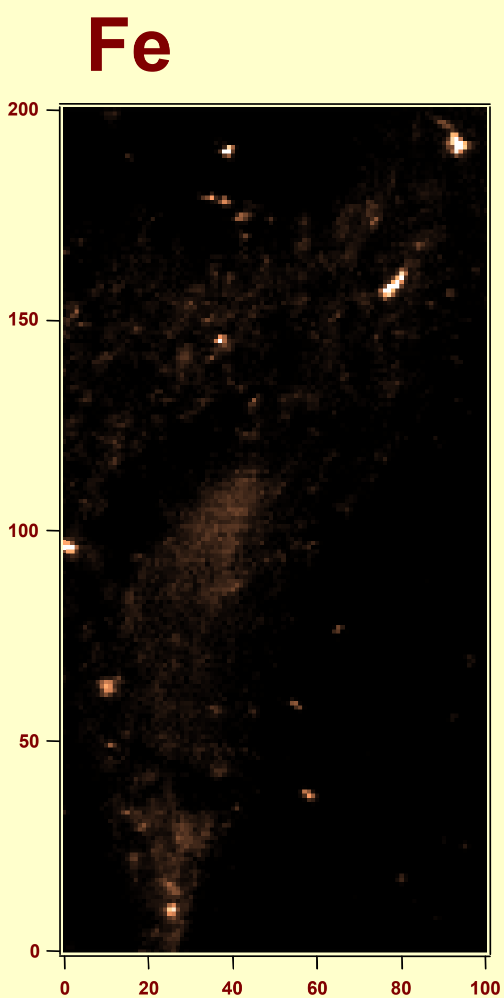


High-resolution cellular chemical imaging

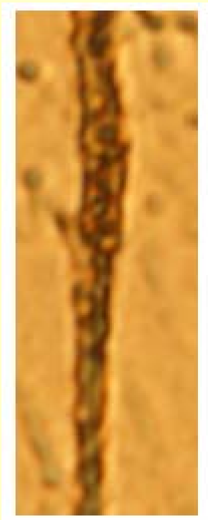
PC12 cells
control



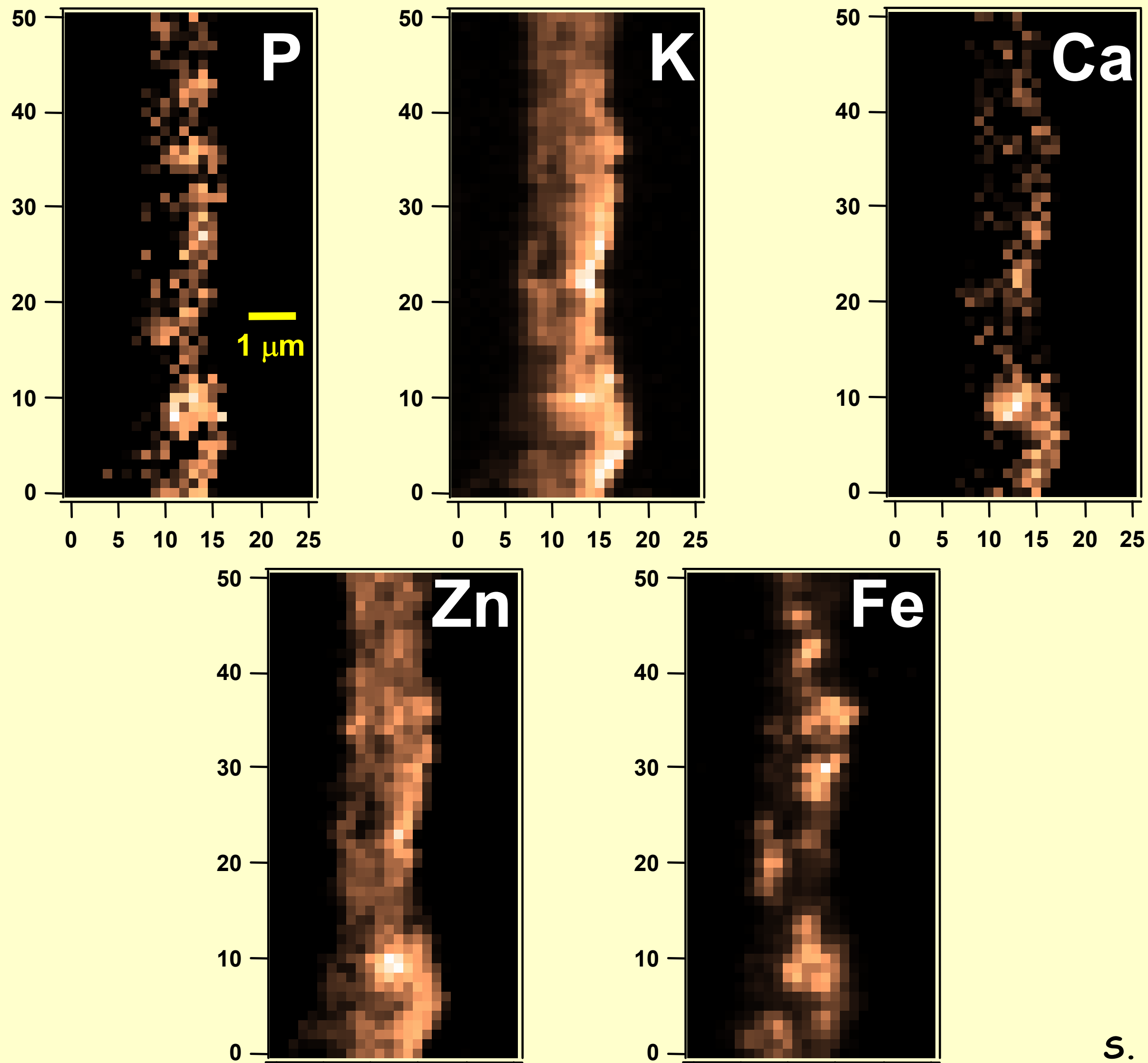




High-resolution cellular chemical imaging



Neurite PC12 cells



Hope this will contribute to foster further collaboration between biologists – chemists – physicists and physicians around synchrotron microprobe techniques

Any questions?