X-ray Fluorescence Microscopy for Biology and Bionanotechnology: Challenges and Unique Opportunities

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Outline

- Trace element analysis in biological systems
- X-ray Fluorescence Microscopy
- Nanocomposites for Nanomedicine and Theranostics
- High speed is a ‘game changer’
- Sensitivity, radiation damage, and speed
- Outlook

Imaging with elemental contrast:
Trace metals in the life sciences

- Trace elements (metals) are fundamental, intrinsic components of biological Systems. estimated: 1/3 of all known proteins contain metalcofactors as integral, catalytic components, often with regulatory functions, e.g.,
  - Zn in Zinc finger proteins: transcription factors in the cell nucleus
  - Fe in Haemoglobin; and necessary in Chlorophyll synthesis
- Metals are linked to diseases
  - Endogenous dysregulation, e.g., Alzheimer's, ALS, Wilson disease (Cu accumulation)
  - Exogenous uptake, e.g., Pb, As, Hg (or lack thereof: e.g., Se deficiency)
  - Bio-remediation
- Metals are used in therapeutic drugs and diagnostic agents
  - Cis-platin in chemotherapy
  - Gd in Magnetic resonance imaging (MRI)
  - Novel bio-inorganic nanoparticles, in particular Nanomedicine: multifunctional nanovectors ideally combining targetting, therapy (e.g., Pt, TiO2) and diagnosis (e.g., Gd)

Zinc plays an unexpected role in oocyte maturation, Kim *et al*. Nat Chem Biol. 2010 6(9):674-81

Recent reviews of **XFM applications**:

Imaging: T. Paunesku *et al*., J Cell Biochem **99**(6), 2006

Spectroscopy: C. Fahrni, Curr Opin Chem Biol **11**(2), 2007

Review of **XFM tomography**:

M. de Jonge & S. Vogt, Curr Opin Struct Biol **20**(5), 2010

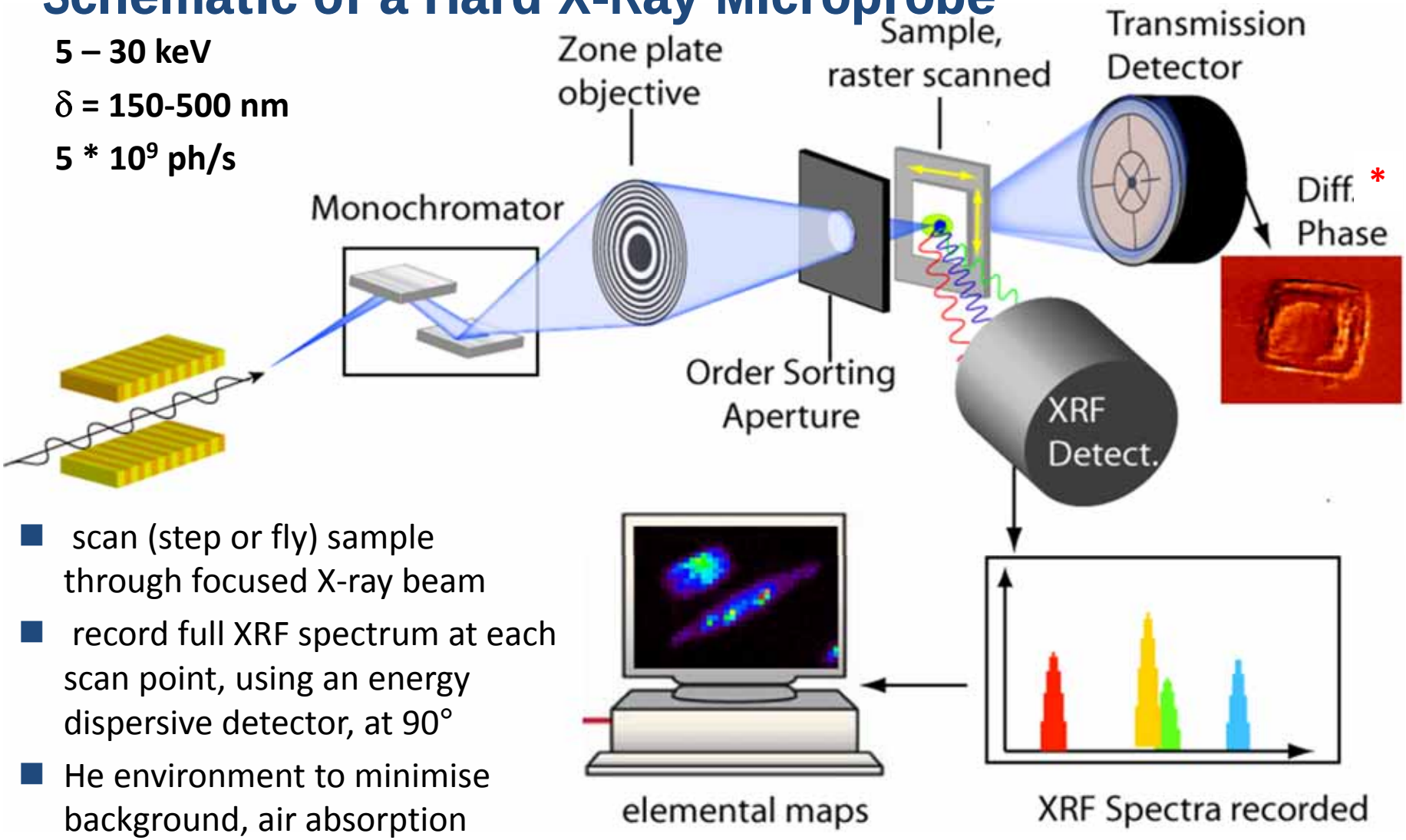
Schematic of a Hard X-Ray Microprobe

schematic NOT to scale !!

5 – 30 keV

δ = 150-500 nm

5 * 10^9 ph/s



- scan (step or fly) sample through focused X-ray beam
- record full XRF spectrum at each scan point, using an energy dispersive detector, at 90°
- He environment to minimise background, air absorption
- Fit data at every pixel or use PCA
- Data acquisition: Epics, visualization: IDL / MAPS

* B. Hornberger *et al*, J Synchrotron Radiat **15**(Pt 4), 2008
* de Jonge *et al*, Phys Rev Lett **100**(16), 2008

Periodic table highlighting X-ray fluorescence

K-line Fluorescence typically used

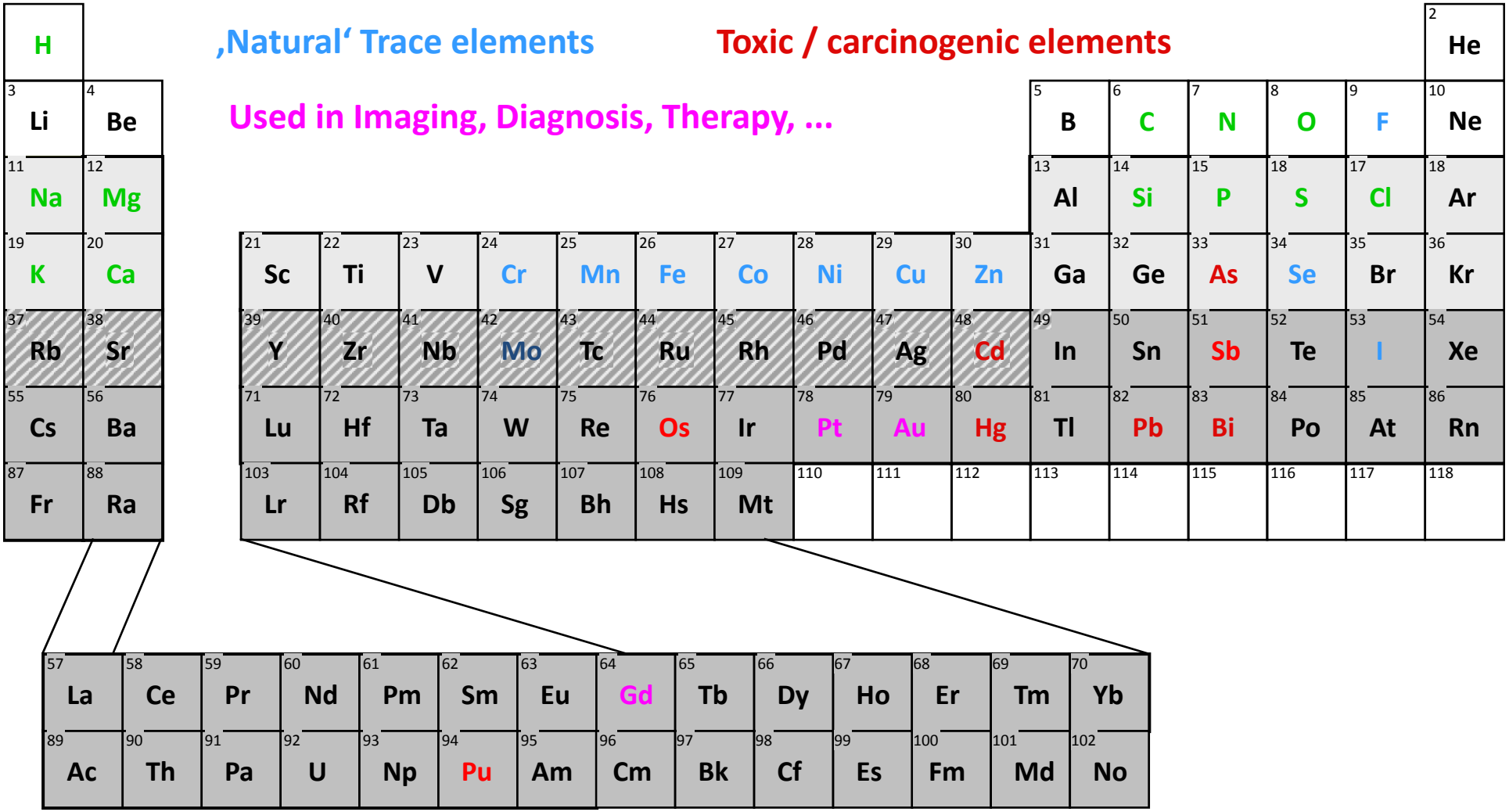
L-line Fluorescence typically used

Major/minor elements in Biological Systems

‚Natural' Trace elements

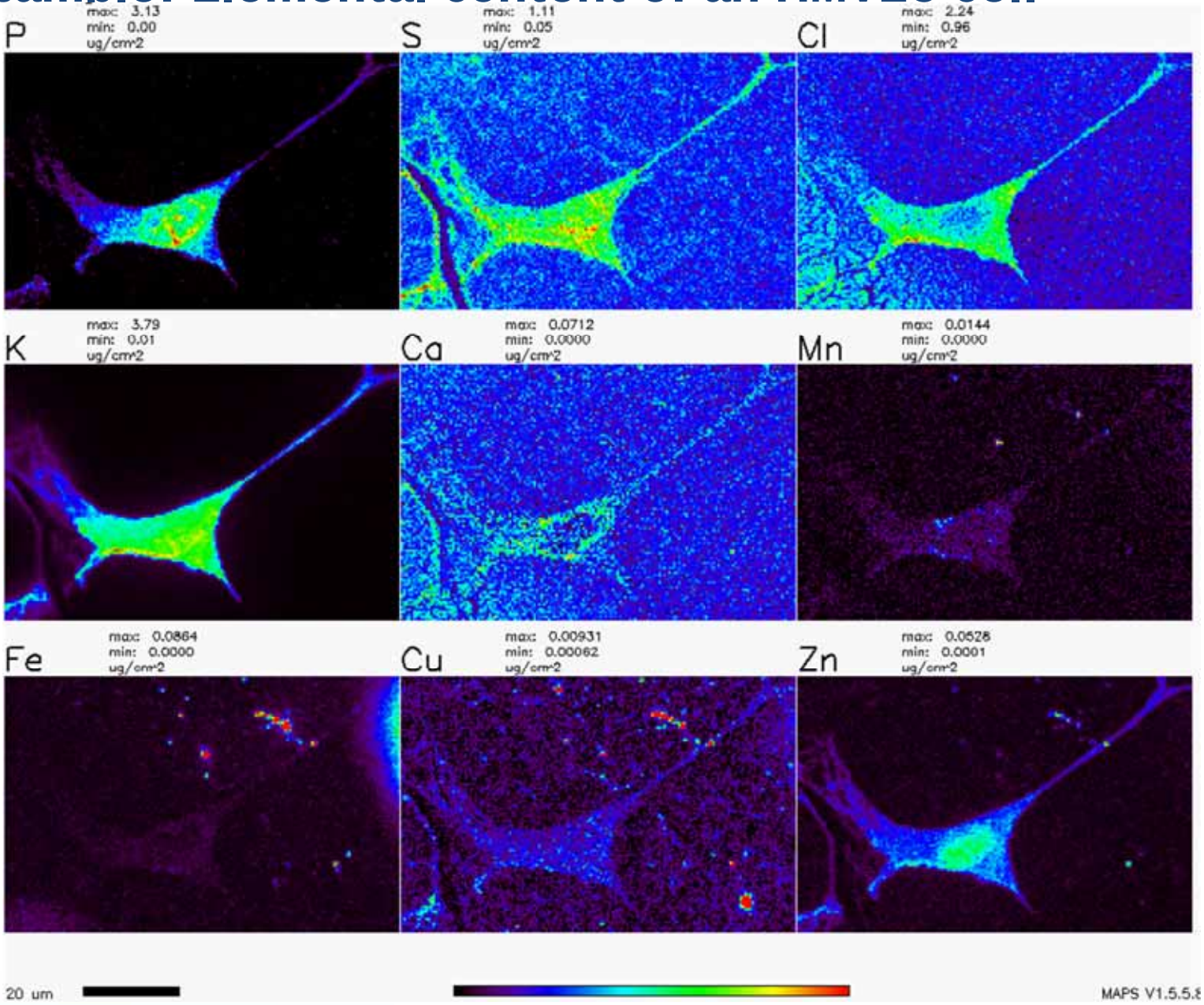
Toxic / carcinogenic elements

Used in Imaging, Diagnosis, Therapy, ...



A typical sample: Elemental content of an HMVEC cell

Overview Image of a full HMVEC cell (plunge frozen in liquid ethane, freeze dried), 2 hours after initiating angiogenesis. Cu is localised strongly to areas outside of the cell, comparison to other timepoints suggests the Cu is transported out of the cell, and after a few hours back into the cell.



See also: L. Finney et al, PNAS **104**(7): 2247-52. (2007)

Great tool, but is it the right tool for the job ?

from
http://www.cartoonstock.com/

Comparison of some techniques for trace element mapping:

	Spatial Resol.	object thick.	Res. Limit.	Advantages/Disadvantages
Light-microsc.	200 nm	30 µm	Wave-length	+ changes in living cells can be monitored, but competition w. proteins +/- only see ions (in solution), and not total content - need dyes - quantification difficult
Hard X-ray-micropr.	**200 nm-20nm**	**10 µm**	**Curren-tly Optics**	**+ no dyes, visualize total elemental content** **+ very high sensitivity, low background, selective excitation** **+ simultaneously detect >10 elements** **+ µ-XANES for chemical state mapping / - slow**
Analytical Electron-micropr.	20 nm	0.1 µm	object thickn.	+ high spatial resolution + simultanously detect >10 elements - thick samples very difficult, sectioning necessary - slow - radiation damage
EELS/ EFTEM	2 nm	0.005 - 0.05 µm	Rad. Damage	+ very high spatial resolution - require ultrathin sections - only some elements readily accessible (e.g., P, Fe) -co-registration can be difficult (EFTEM), slow (EELS)
Proton Micropr. (PIXE)	~1um	~50um	Rad. damage Flux limit	+ simultanously detect >20 elements + high sensitivity - slow - radiation damage

B. Twining *et al*, J Eukaryot. Microbiol. 55(3), 2008

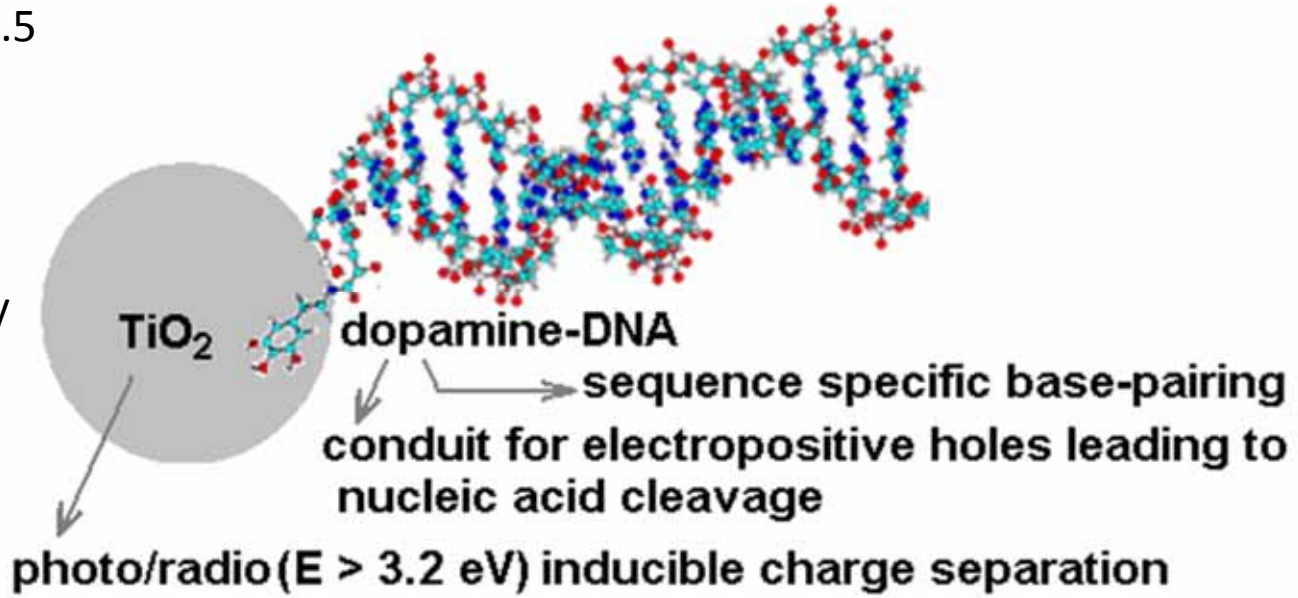
Nanocomposites for Nanomedicine / Theranostics

- Numerous developments to create functional nanocomposites that combine properties for
  - medical imaging (e.g. Gd as a contrast agent for MRI)
  - therapy (e.g., kill cancer cells)
  - targeting (e.g., bind only to DNA of cancer cells)
- For example:
  - nanocomposites that target specific oncogenes in cancer cells
  - can destroy the gene or the cell
  - be visualised by MRI (*in vivo*!)
- But: before being able to test on subject, need to confirm *in vitro*:
  - Do the nanocomposites enter the cells ?
  - Do they 'find' the right target ?
  - Do they ONLY interact with the right target (e.g., toxicity) ?
  - Do different components remain joined ?

Tatjana Paunesku, Gayle Woloschak, *et al*

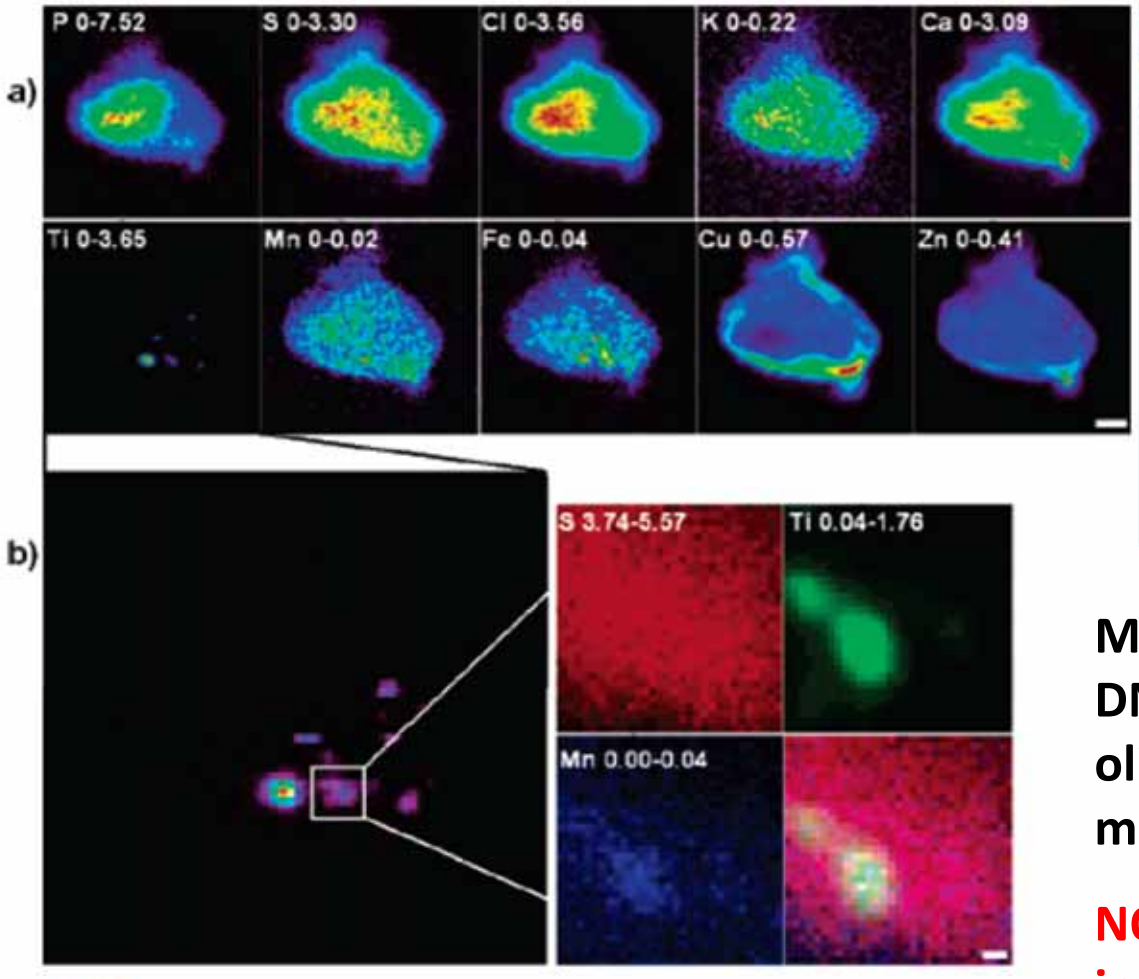
Ti nanocomposites as intracellular probes and tools

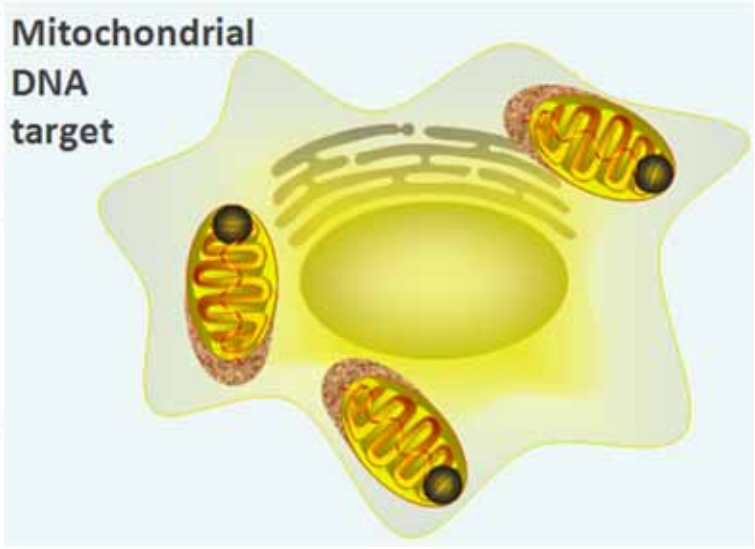
- attach TiO_2 nanoparticle (4.5 nm diameter) to DNA
  - May include Gd or Fe3O4 core for MRI
- combine DNA biochemistry with semiconductor properties of TiO_2
- ➔ carrier-particle that can bind to a specific chromosomal region w/ ability to cleave it upon illumination



See also, T. Paunesku *et al*, "Biology of TiO2 –oligonucleotide nanocomposites", Nature Materials 2, 343-346 (01. May 2003)

DNA-TiO_2 nanoconjugates targeting of mitochondrial DNA in MCF7 cells



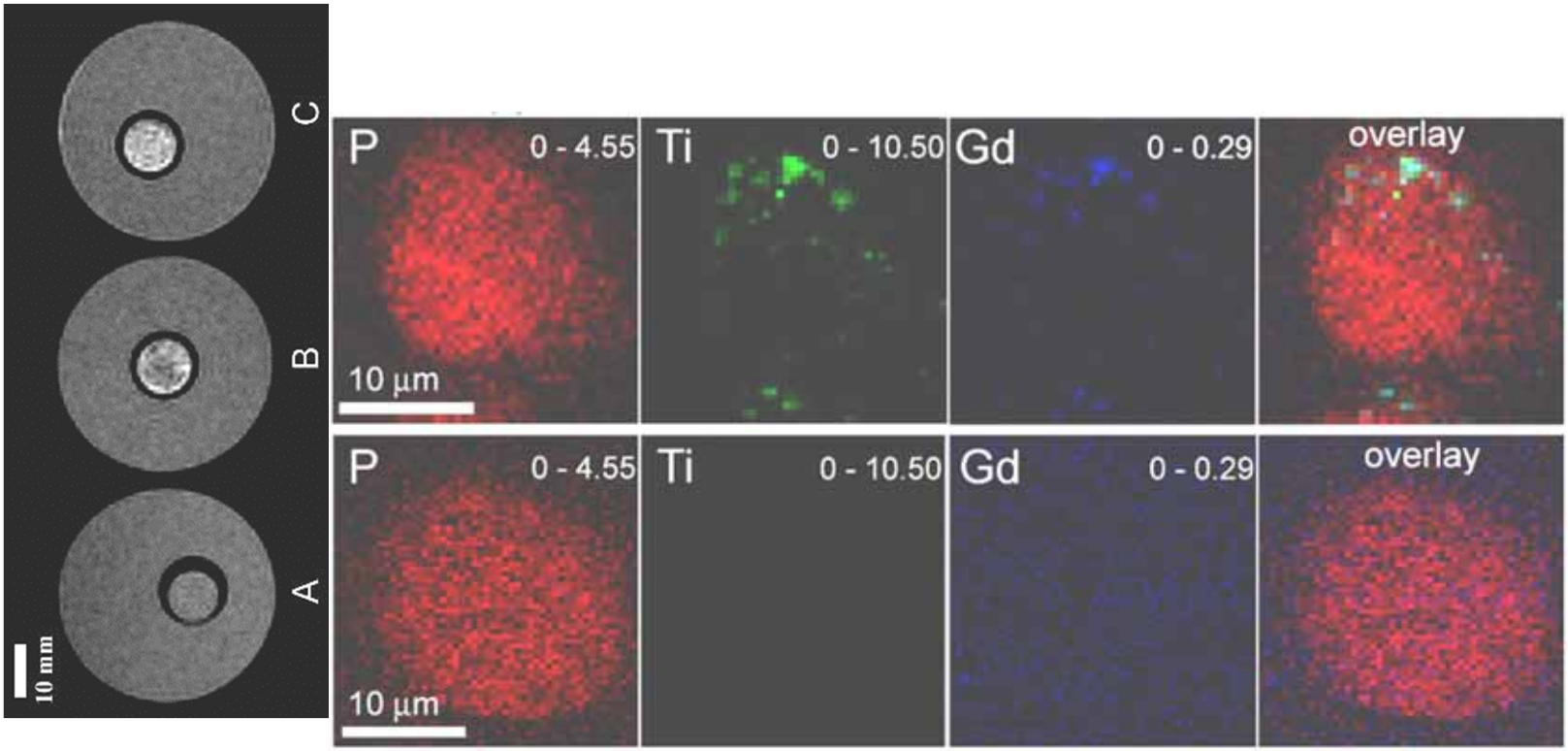


Mitochondria – mitochondrial DNA targeting: using DNA oligonucleotide as a targeting moiety

NOTE that natural presence of Mn indicates mitochondria

Paunesku, et al. Nano Letters 2007

Imaging TiNC by X-ray fluorescence—TiO_2 conjugated to mitochondrial targeting oligonucleotide and Gd contrast agent



MRI (left) and XFM (right) of treated (top) and control (bottom) cells.

Diagnostic labeling molecules can be bound to TiO_2 surface as a scaffold to make nanoconjugates visible to diagnostic equipment.

Endres *et al*. JACS 129(51): 15760-1. 2007

Speeding things up ….

- Step scans, ‘routine’ to do, but slow with motor and control system overhead (eg, 150 ms per pixel)
- Fly scans: move sample continuously with regards to the beam, acquire data continuously – typically ‘no’ data acquisition overhead
  - Correct approach requires hardware triggering & specialized hardware
- A Simple implementation with Epics, without hardware triggering achieves down to ~ 20 ms dwelltime per pixel

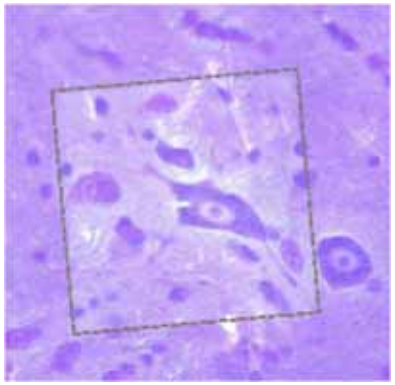
- With hardware triggering, down to 1 ms dwell time per pixel

- Australian Synchrotron / BNL collaboration (MAIA detector with fast readout): down to 50 microseconds!

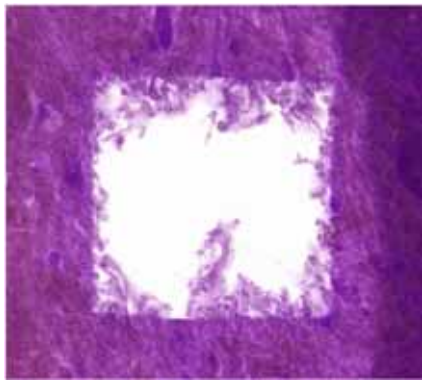
Sensitivity, spatial resolution and radiation damage:

- Exciting optics developments: <10 nm spatial resolution seems achieveable, but what about radiation damage ?
- From soft X-ray microscopy, Limit is ~ 10^{10} Gy, corresponding to: focused photon density of 10^{13} ph/μm^2 at 10keV (current have flux density 10^{11} ph/s/μm^2)

Fixed (p-formaldehyde), paraffin, scanned, rehydrated



Freeze dried (unfixed), scanned, rehydrated

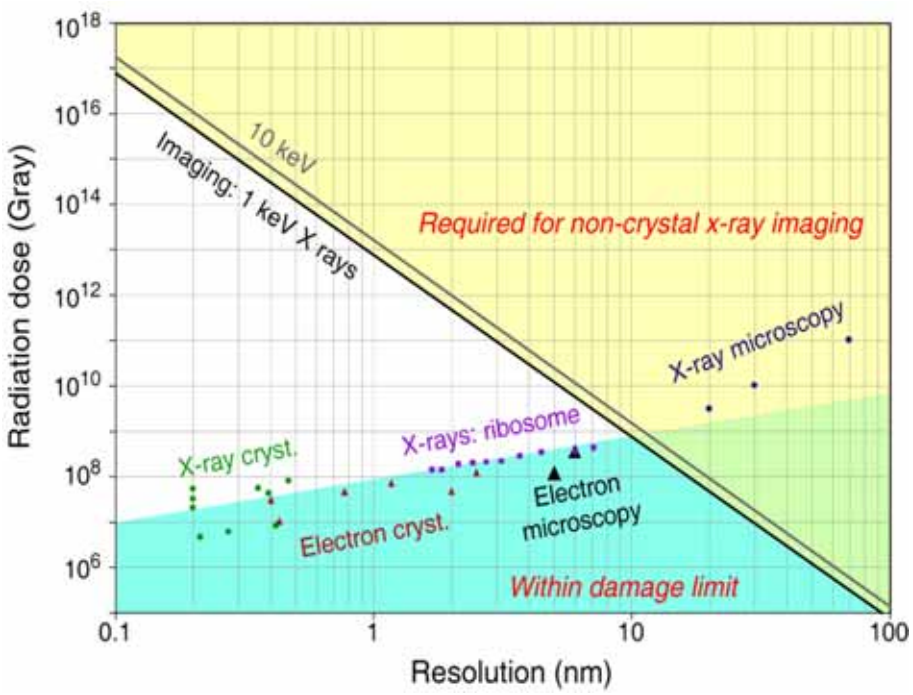


APS Today (100 mA, 3.0 nm,UA, L=2.4 m), 6% of 4πSR detected, at 10 keV incident beam energy, for a biological sample in water (frozen hydrated) => minimum detectable Zn [#atoms], in 1s or limited by rad damage:

	Spot size		
sample thickness [um]	**200 [nm]**	20 [nm]	5 [nm] (0.1s)
0.1 [um]	**3500**	35	15
10 [um]	**26000**	260	60

Depth of field:

10keV	200 [nm]	20 [nm]	5 [nm]
DOF +/-[um]	433	4.3	0.3



This plot: Howells *et al.*, *J. Electr. Spectr. Rel. Phen.* **170**, 4 (2009). See also Shen *et al.*, *J. Sync. Rad.* **11**, 432 (2004).

Sensitivity & spatial resolution:

Today at APS:

- (100 mA, 3.0 nm,UA, L=2.4 m)
- XRF detector collects 6% of 4πSR

	Spot size		
sample thickness [um]	200 [nm]	20 [nm]	5 [nm] (0.1s)
0.1 [um]	3500	35	15
10 [um]	26000	260	60

Future ?

- ERL: 100x more coherent flux
- plus assume XRF detector collects 30% of 4πSR

	Spot size		
sample thickness [um]	200 [nm]	20 [nm] (15ms)	5 [nm] (0.5ms)
0.1 [um]	180	6	4
10 [um]	1800	50	25

10 keV incident beam energy, biological sample in water (frozen hydrated)

minimum detectable Zn [#atoms], limited by rad damage:

For materials sciences samples, radiation damage less of an issue

Source vs detectors & optics ?

- Most of the improvement in sensitivity comes from detector and optics improvement. What role does APS (ring & ID upgrade) play ?

SPEED !!! - only this will make most experiments feasible

- Example scan of a 10x10 micron area in x-ray fluorescence (e.g., cell, or part of a semiconductor structure)

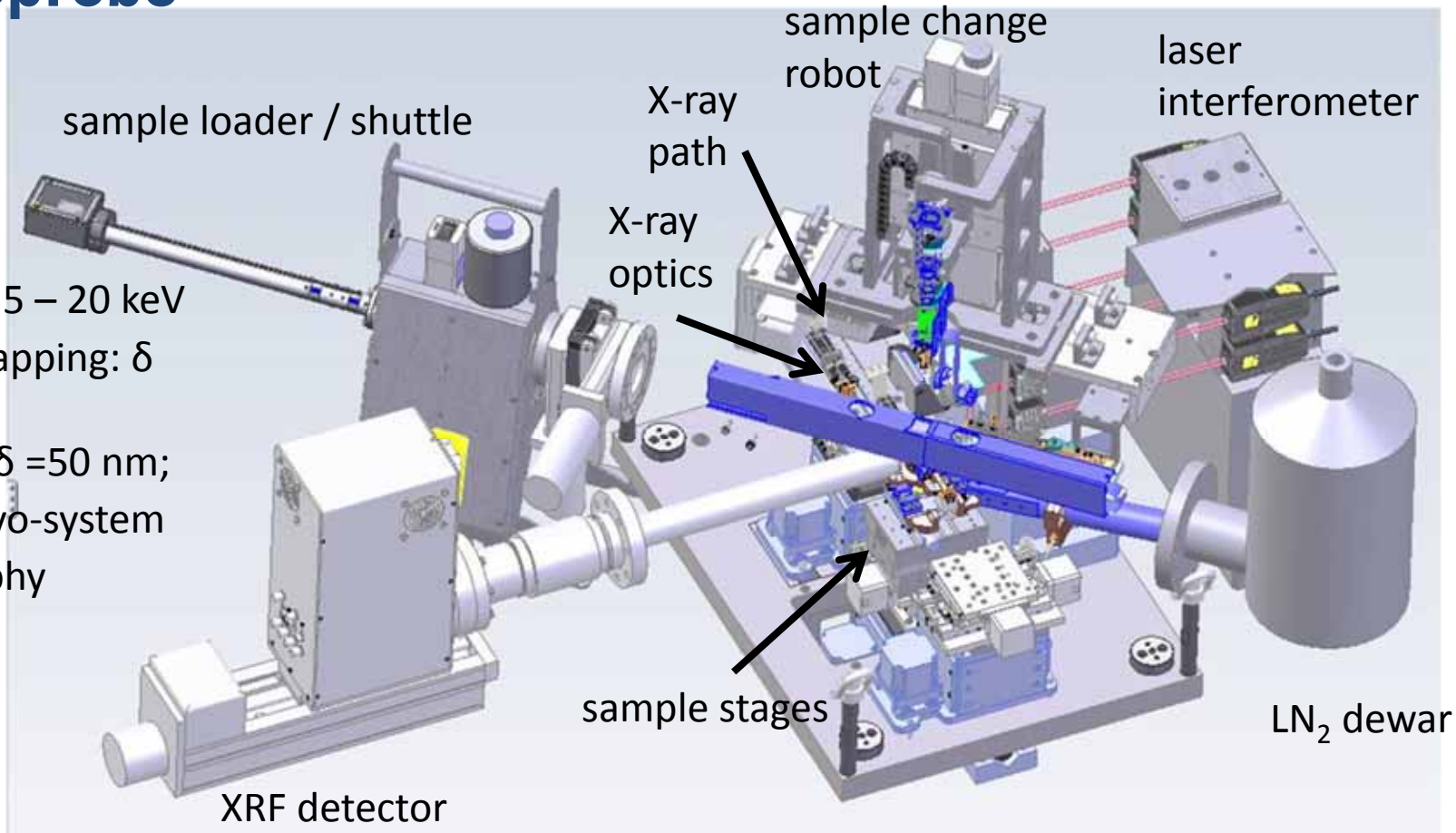
- Today (100 mA, 3.0 nm,UA, L=2.4 m)
- XRF detector collects 6% of 4πSR

dwell time [s]	resolution [nm]	scan time [h]
1	200	1
1	20	70
0.1	5	122

- ERL: 100x more coherent flux
- plus assume XRF detector collects 30% of 4πSR

dwell time [ms]	resolution [nm]	scan time [h]
250	200	0.2
15	20	0.5
0.5	5	0.6

BioNanoprobe



- Energy range: 5 – 20 keV
- resolution: Mapping: δ <=30 nm;
  Spectroscopy: δ =50 nm;
- in vacuum, cryo-system
- Fast tomography

NIH/NCRR funding (PI: G. Woloschak, Northwestern University). BioNanoprobe was built by Xradia, installed at APS: LS-CAT (sector 21), commissioning w/o xrays: summer 2011, w. xrays fall

co-Investigators:

Si Chen, S. Vogt (tech lead), T. Paunesku, K. Brister (LS-CAT tech lead), B. Lai, J. Maser, C. Jacobsen, W. Anderson, *et al*

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xradia insight in 3D

BioNanoprobe

Successful factory
acceptance tests at x-radia

Now installed at LS-CAT at APS
Stability tests w/o X-rays

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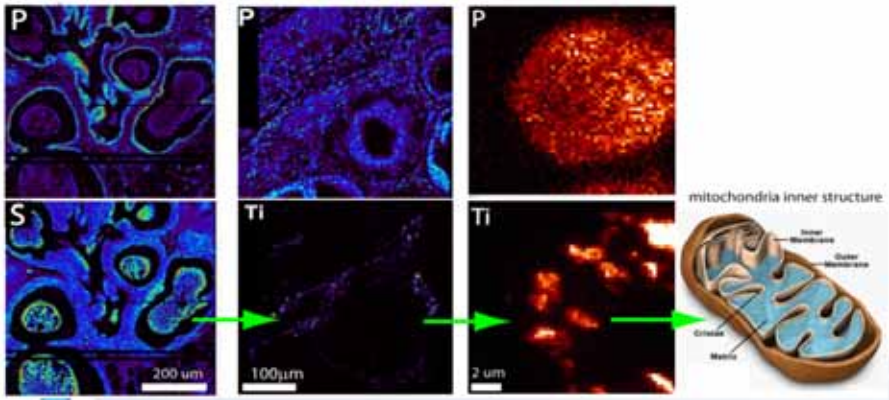
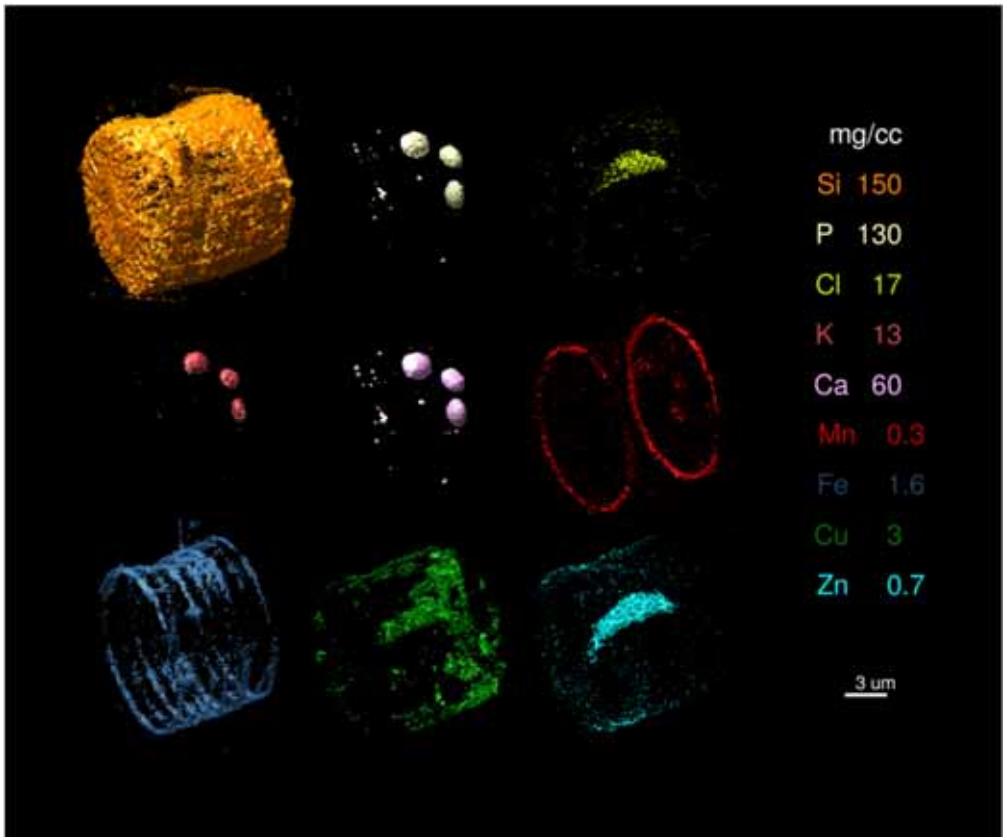
xradia insight in 3D

Outlook & Future:

- Exciting optics, detector, instrumentation developments: 10-20 nm spatial resolution seems achievable, with sensitivity down to <10 Zn atoms for THIN specimens (Limiting factor: radiation damage)
- Routine XRF-tomography, w/ dose fractionation
- **Significant speed increases in elemental mapping are a game changer.**
- Need 10-100x more focussed flux into a small focus, to enable experiments that require simultaneously:
  - high spatial resolution
  - Extended sample / many samples
  - High sensitivity
- e.g. detect and map single nanovectors in tissues, cells and organelles, to understand their properties





3D reconstruction of a single diatom at 300 nm resolution, de Jonge et al, PNAS, 107(36): 15676, 2010

Thank you for your attention!