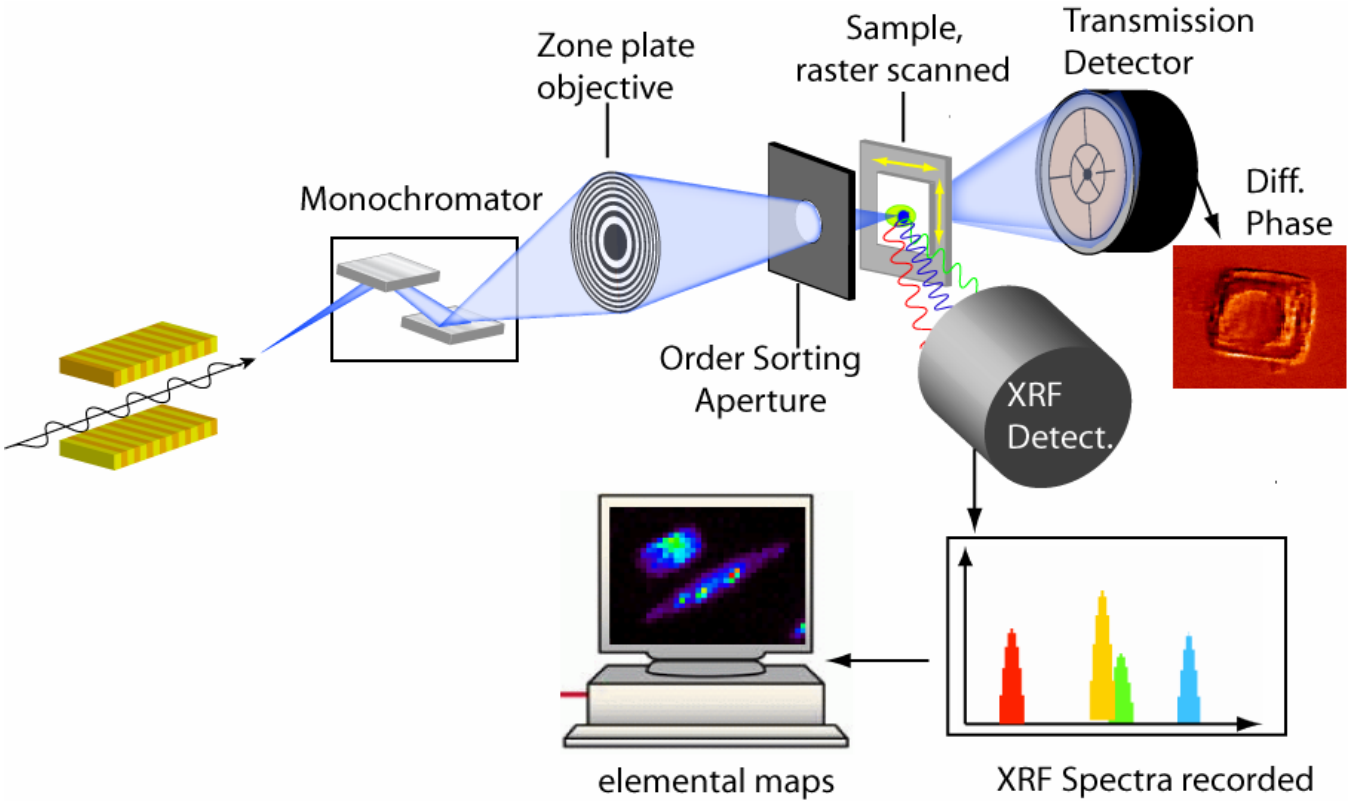
Imaging and probing trace elements: scanning X-ray fluorescence microscopy



S. Vogt, B. Lai, G. Woloschak, C. Jacobsen, T. O'Halloran, K. Kemner, Q. Shen, and many more

Current status: Hard X-ray microprobes

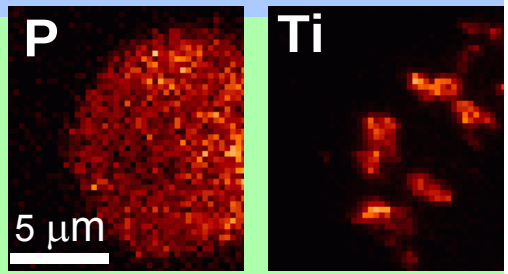
Energy range: 5 – 30 (10-13) keV; Flux:10^9-10^{10} ph/s
High spatial resolution: 30nm (KB) - 150 nm (ZP); routine: 200nm (ZP)
Low spatial res.: 1-5 μm Flux: 10^{12} ph/s
μ-XANES (spatial resolution, maybe 300 nm ?)
EPICS based controls software, a few different data analysis packages
Energy dispersive detectors (Ge -> Silicon Drift; single vs. multi element)

WHAT ARE THE LIMITS?

Spatial resolution; spectroscopy at high spatial resolution, limited detector NA; no routine cryo instrument

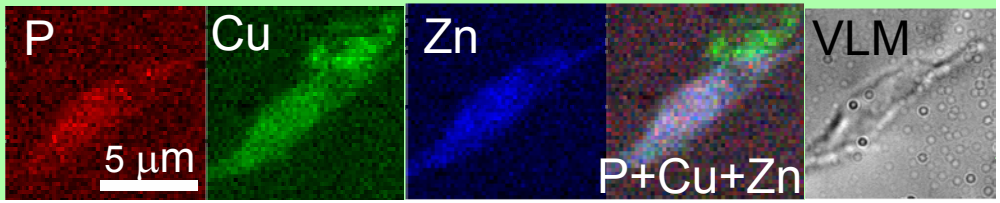
Research Examples

- Investigating Bio-inorganic nanovectors: can 'see' clusters of nanoparticles, but not SINGLEs, nor RESOLVE subcellular targets.



Paunesku *et al*, Nano Lett. 2007; Paunesku *et al*, Nat. Mat. 2003

-Natural trace elements: In a model of angiogenesis, the growth of new blood vessels critical to cancer, 80 to 90% of cellular copper moves to the tips of tendril-like projections and across the cell edge



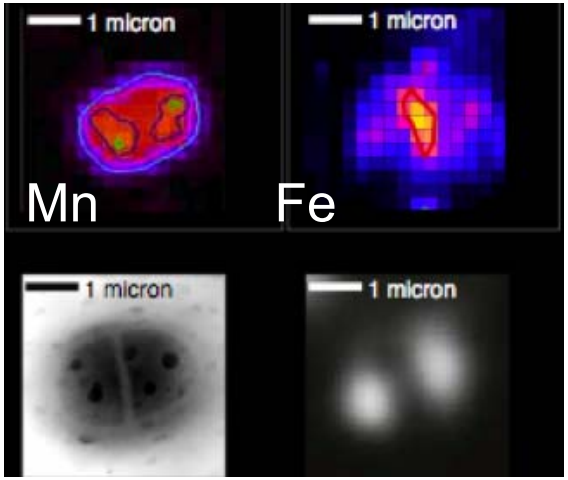
Finney *et al*, PNAS 2007

Key future science applications

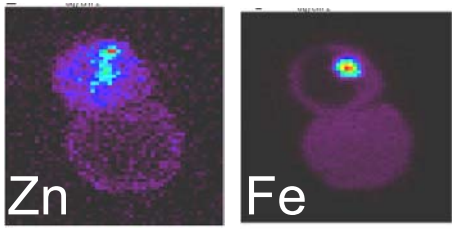
Biology and Life Sciences

Map 'natural' metals within organelles.
Potential to detect and localise individual or at least small clusters of metalloproteins in cells, providing a very exciting tool for cell/molecular biology

- in particular: could now probe interactions at cell interfaces and membranes



Malaria infected RBC (top) vs RBC (bottom)



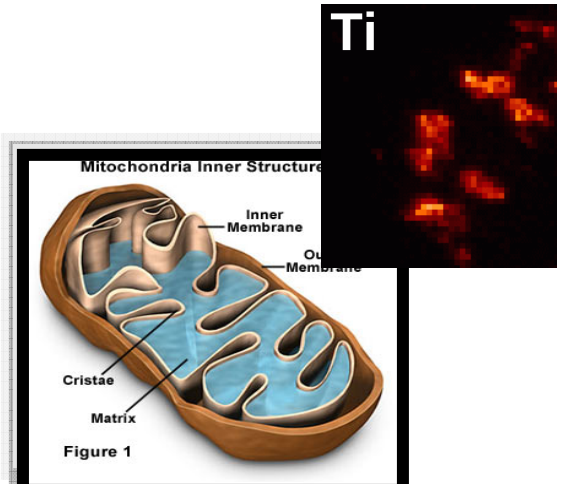
Biomedical – infectious diseases

Probe elemental content of cytoplasma (host cell), vesicles (phagosome), as well as parasites, to significantly improve our understanding of infectious diseases

Nanomedicine

Enable future experiments, that detect and map **single nanovectors** in tissues, cells and organelles. In particular, multifunctional nanovectors that combine targeting (e.g., DNA), therapy (e.g., Pt, TiO2) and diagnosis / imaging (e.g., Gd)

- *Verify functionality, study mode of action, side effects / toxicity, ….*



The ideal XRF microprobe:

- Energy range: 2-30 (10-13) keV
- Beam sizes: <=10nm, AND ~100nm, AND ~ 1um
  - K-B mirrors (achromatic, high efficiency, long working distance) vs zoneplates (in line, ease of use, stability)
- Detectors:
  - need large solid angle XRF detector to mitigate radiation damage (30% of 4πsr)
    - Space around sample is tight; problematic to get XRF out (at 90º) for high NA lens, without loss in sensitivity
    - Multi element SDD, fast readout, compatible with fast scans with full spectral information. (dev. P. Siddons BNL, C. Ryan, CSIRO)
  - Need to visualize specimen structure (low Z), e.g., using differential phase contrast in transmission (collab. with C. Jacobsen et al)
- Specimen Environment & Preparation
  - Must have Cryogenic specimen environment
  - Should have sample prep facilities (ideal: high pressure freezing)
- data acquisition and data analysis (semi-automation, good user interface, …)
  - Make sure beamline scientists / users are involved in development !!!
- Correlative experiments with other techniques (IR, visible light, EM, soft X-rays..)
  - Should be discussed at the outset!

The ideal XRF microprobe FACILITY (pt2):

Laboratories and ancillary facilities

- Wet lab, with basic equipment (e.g., biol. work bench (BSL2), incubator, ...)
- Light microscopy (good research fluorescence microscope)
- Sample prep, in particular freezing (high pressure freezing: $250k)
- Access to correlated (same mounts ?) electron, IR, and soft x-ray microscopies

Adequate staffing !!!!!!

- Including people with significant background in biol sample prep.
- Only supporting beamline ops is insufficient, need to support whole experiment (planning, sample prep, data analysis, interpretation)!

Quality of Life

(disclaimer: I am a BL scientist….)

- Offices for beamline staff NEAR beamline
- Labspace near beamline
- Sufficient floor space at beamline, AND office like controls environment
- Lunchroom (dare one ask for a real coffee machine ???)
- ‘full’ remote control of beamline
- Facilities that allow remote users to participate in the experiment
    - but there should usually be some on site users (no mail in service ☺)

Last comments:

<10 nm spatial resolution seems achievable, with sensitivity down to <5 Zn atoms, for thin biological samples. (Limiting factor: radiation damage)

- I believe this will have a significant impact in Life Sciences and Bio-medical Sciences.

- However:
  - There must also be lower resolution, ‘routine’ instruments available
  - Need critical mass (20% of time on nanoprobe beamline insufficient)