X-Ray Fluorescence and Spectro-Microscopy for Biomedical Applications

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1. X-ray fluorescence microprobe
   → Motivation: Trace metal detection
   → Instrumentation

2. Exogenous Metals
   → Cellular transformation of chromium carcinogen (Cr)
   → Cisplatin and derivatives in cancer cells (Pt)
     • Microbe-metal interaction in planktonic bacteria & biofilms (Cr)
     • Transfection of TiO$_2$ nanoparticle-oligonucleotide composites (Ti)
     • Metal complexes of anti-inflammatory drugs (Cu, Ni, Co)

3. Endogenous Metals
   → Host-parasite interaction in mycobacterial infection (Fe)
   → Cell differentiation mediated by metalloproteins (Zn)
     • Quantifying trace elements in marine protists (Fe, Cu, Zn)
Fluorescence Mapping and Spectroscopy
with hard x-rays excitation ($E_{\text{exc}} = 5 – 30 \text{ keV}$)

Constituents of Earth's crust
- Elements intrinsic to biological systems
- Elements with biological activity

K excitation
L excitation
Fluorescence Spectroscopy
Fluorescence Mapping

D. Legnini, Oct/01
Why study metal concentrations in biol. cells?

• Some metals are integral components of numerous classes of proteins. These proteins often have regulatory functions
  – Ca in Calcium-binding proteins: second messenger pathways, e.g. Troponin C in muscle
  – Zn in Zinc finger proteins: transcription factors
• By studying the metal concentrations conclusions can be drawn about regulatory functions of these proteins.

left: zinc finger motif from yeast transcription factor SWI5
[from Encyclopedia of Molecular Biology]
Why use x-ray-excited fluorescence to study trace metals?

- Higher fluorescence cross sections
- Better signal/background ratio
  ⇒ sub-ppm (part-per-million) sensitivity
  ⇒ quantitative
- Less radiation damage
- Better resolution for sample >1 µm thick
- Selectively excite one particular element
- Map chemical states by XANES
- SIMPLE SAMPLE PREPARATION !
  ⇒ no fluorescence markers needed
  ⇒ no staining nor thinning
  ⇒ can study hydrated “natural” samples

Detection Limit for Transition Elements for 1 sec. acquisition time, 0.2 x 0.2 µm² spot, E=10 keV

- Zn
- Fe
- Ti
- K
- Cu
- Co
- Mn
- V
- Ca

Detection Limit (atoms) vs. Fluorescence Energy (keV)

Attogram (10⁻¹⁸ gm)
Schematic of Scanning X-Ray Microprobe

Undulator

Crystal monochromator

Zone plate objective lens

Aperture

Sample, raster scanned

Transmission detector

X-ray fluorescence detector

Spectra

Computer monitor
Performance of 2-ID-D X-Ray Microprobe

Spatial Resolution = 150 nm FWHM
Efficiency = 20-25% (with two Au ZPs)
Flux density = $2 \times 10^{11}$ photons/sec/$\mu$m²/0.01%BW
Flux density gain = $3 \times 10^4$
Main branch 2-ID-D: \[ E = 5 - 30 \text{ keV}, \quad \delta = 150 \text{ nm} \leftrightarrow 2 \cdot 10^9 \text{ phot/s} \]
Side branch 2-ID-E: \[ E = 7 - 15 \text{ keV}, \quad \delta = 250 \text{ nm} \leftrightarrow 5 \cdot 10^8 \text{ phot/s} \]
Integrated epi-fluorescence microscope
2-ID-D/E Hard X-ray Microprobe Facility

sample

Epifluorescence Microscope
Cellular Uptake and Cellular Metabolism of Chromium Carcinogens

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Chromium Carcinogenesis

- Cr(VI) is a known carcinogen causing cancers in the respiratory tract.
- It is predominantly encountered in the workplace.
- Recent attention has focused on environmental exposure to Cr(VI), resulting from the poor disposal practices of Cr(VI) into unlined ponds.

Commonly Encountered Carcinogens in the Workplace

- Chromium 25%
- Asbestos 15%
- Nickel 10%
- Benzene 4%
- PAH 3%
- Arsenic 3%
- Cadmium 3%
- Others 26%
Preparation of Whole Cell Samples

- Cells were treated for 4 hr in cell growth medium.
- The medium was removed and the cells were washed to remove extracellular material.
- The cells were harvested, deposited on a formvar support in ammonium acetate solution and freeze-dried using liquid nitrogen cooled isopentane.

Distribution of V79 cells on a 3 mm diameter gold grid
Preparation of Thin Sectioned Cells

• Cells are treated and harvested as for whole cell preparation.

• Cells are centrifuged to a pellet, washed, dehydrated, set in Spurr’s resin, and microtomed to 1-µm thin sections.
Effect of Oxidation State on Cr Genotoxicity

Cr Uptake Analysis of V79 Whole Cells

Cr(III)-Treated Cell

Cr(VI)-Treated Cell

30 µm
Micro-SRIXE and GFAAS Determination of Cr Uptake into V79 Cells

Micro-SRIXE of individual cells, n=5

GFAAS of 10^6 cells
K-Edge XANES Spectra of Cr Standards

![Graph showing K-Edge XANES Spectra of Cr Standards](image)

- [Cr(glygly)$_2$]$^-$
- [CrO(ehba)$_2$]$^-$
- [CrO(mampa)]$^-$
- Cr(VI)

Relative Intensity vs. Monochromator Distance (mm)
K-edge XAS Spectra of Cr-Treated Cells

Thin-Section of V79 Hamster Lung Cell Following Exposure to Cr(VI)

Scan dimensions = 11 × 11 µm; Beam size = 300 nm diameter.

Cellular Metabolism of Chromium

- [SO$_4$]$^{2-}$
- [HPO$_4$]$^{2-}$

**Mitochondria**
- Damaged RNA

**Cytoplasm**
- Reduction via vitamin C, GSH
- Intermediates {Cr(V), Cr(IV), radicals}
- Reduction

**Nucleus**
- Cr(III)
- Damaged DNA
- Intermediates

**Cr(III)**
- Reduction

**Cr(VI)**
- Reduction via vitamin E

**Intermediates**

**Cr(III)**
Workshop on Biological Applications of X-ray Microbeams

Recent studies in cell biology, environmental science, and microbiology using hard X-ray microscopes have yielded promising results. This led to a workshop on existing biological applications, benefits, and future needs of high-resolution X-ray microscopy. About 50 researchers attended the workshop held May 14-15, 2001, at Argonne National Laboratory.

Hard X-ray microscopes have been used for several years (Fig. 1) [1]. Broadly speaking, the applications can be classified as analyses of tissues, eukaryotic cells and microorganisms. The high elemental sensitivity of X-ray fluorescence and the high spatial resolution offered by the X-ray microscope are crucial for trace-element mapping and analysis in these systems. Current applications include medical studies in the areas of pathogenesis, carcinogenesis, and drug efficiency, and studies in marine biology and ecology. Examples include cellular that address elemental imaging for drug metabolism (Pt and Cu) in human tissue, metal metabolism (Cu-induced carcinogenesis); trace-metal concentrations in microorganisms from mineral surfaces; elemental concentrations in marine and freshwater microbes; and cellular processes, such as changes in metal distribution during differentiation, cell cycle progression, etc. Several groups are labeling cellular and subcellular structures with antibodies bound to metals or with TEG nanoparticles with bound DNA or peptides. Expansion of these approaches to other metals would increase the number of intracellular structures that could be resolved. A noninvasive use of the hard X-ray microscope is microradiography, which has been used to study bone ultrastructural changes. Soft X-ray tomography has already led to 3D imaging of cryopreserved specimens providing intracellular structures of single cell preparations and some cellular substructures (such as nuclei, nucleoli, and others).

These types of studies, as well as classic biological applications, which are predominantly observations of natural events, will likely continue to be important. An example is the study of intracellular parasites and the influence of metals on the progression and course of microbial infections. Mycobacteria species, for example, are phagocytosed and survive in macrophage vesicles while utilizing cellular metals. Trace-element mapping in the macrophage subcellular compartments and in the phagocytosed bacteria themselves will provide new insights into the pathogenesis of this response. Another application involves analysis of metal-binding proteins and metal-binding processes in neurologic diseases, many of which have critical errors in metal metabolism. Patients with some forms of familial amyotrophic lateral sclerosis (Lou Gehrig's disease) exhibit abnormalities in protein structure of the enzyme Cu, Zn superoxide dismutase (SOD1). Although an abnormal copper release was detected in certain SOD1 mutants, the relationship of this defect to the disease is not yet understood [2]. Copper dystrophy has also been found in some patients with Alzheimer's and Parkinson's disease [3]. Calcium- and zinc-permeable receptor channels were implicated in selective neurodegeneration in cases of global ischemia, Alzheimer's disease, and amyotrophic lateral sclerosis [4]. Hard X-ray elemental mapping and spectroscopy is likely to shed some light on the role of metals in the pathogenesis and progression of these diseases and may also permit imaging of the intracellular inclusions created from metal-binding proteins. Finally, recent studies have demonstrated that many oncogenes (genes that become deregulated in cancer) encode proteins that are transcription factors that bind Zn or encode proteins that bind other metals [5]. Hard X-ray elemental mapping with improved resolution will permit new studies of the role of metal binding on the intracellular function of these important proteins and may also permit measuring of intracellular concentration of these proteins.
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| - No fluorescence dyes or markers are needed  
- Minimal sample prep, allow hydrated/in situ studies  
- Parallel acquisition of ~ 10 elemental images  
- Quantification to ppm level for most metals  
- 200 nm spatial resolution (≈ optical, better than EM for thick specimens)  
- Substantially less radiation damage than EM  
- Reveal oxidation state by micro-XANES | - Long integration time (1-2 hrs)  
- Non-specific to binding partners |
Future Enhancements

• Install multilayers monochromator
  – gain of 20x in flux
  – wider energy bandwidth

• Design a second generation fluorescence microprobe
  – higher resolution scanning stages
  – monitor ZP and sample position with interferometer
  – increase stiffness, reduce thermal drift

• Option to run in vacuum
  – facilitate detection of low-Z elements (Na, Mg, P, S…)
  – reduce scattering from air or helium
  – increase acoustic and thermal isolation

• Implement plunge freeze and vacuum dried (no fixation)
• Develop a cryo sample holder (frozen hydrated samples)
• Future, full-field imaging fluorescence microscope….
Contributors

**Microprobe**

**Beamline**

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