Overview

- Why image sulfur?
- Imaging methods
- Example 1: Imaging total sulfur
  - Mercury accumulation in zebrafish larvae
- Example 2: Imaging sulfur forms
  - Sulfur in intact tissues
- Next steps

Sulfur XAS of Biological Tissues

- Sulfur X-ray absorption spectroscopy is a unique *in situ* probe for biological systems
  - Looks at all sulfur in sample
    - solid, solution, gas etc.
  - Chemical species information
  - No pretreatment necessary
  - Non-destructive (at least in principal)
  - Ideal for complex samples such as whole tissues
XAS of Biological Samples

X-ray absorption spectroscopy can probe molecular form (speciation) of sulfur in a variety of biological samples.

- Purified proteins
- Isolated organelles
- Cell cultures
- Tissue sections
- Intact organs
- Intact organisms

Can also study environments such as soil or water, food, etc...

XAS Imaging of Biological Samples

- Most biological samples are structured

- We would like to obtain spatial information about the distributions of elements, including sulfur

We would like to do this when levels are dilute, and on intact living specimens.

Questions we may want to answer:

- How is sulfur distributed?
- What is the chemical form of sulfur in a particular location?
- How is a sulfur chemical species distributed?

- We would like to do this when levels are dilute, and on intact living specimens
**X-ray Fluorescence Imaging**

- If X-ray energy is above an element’s absorption edge, the element will “fluoresce” X-rays.
  - Each element has a characteristic energy
  - Can image total sulfur, along with other elements, using hard X-ray beamline

- Also called:
  - X-ray fluorescence microprobe
  - μ-XRF (μ-X-ray fluorescence)
  - SRIXE (synchrotron radiation induced X-ray emission)

**Introduction**

Basic arrangement for microprobe and XAS-imaging

- Transmittance detector
- Sample
- Raster scan sample
- Microscope
- Small X-ray beam
- Fluorescence detector
X-ray Fluorescence Imaging

At each pixel, fluorescent line intensities yield elemental quantities.

Sample

Raster scan sample

Small X-ray beam

Fluorescence detector

Fluorescence emission spectrum (per pixel)

Elemental maps

Micro-XAS

- Following fluorescence imaging, select a pixel of interest and collect a spectrum.
- Micro-XAS spectrum can then be analyzed in a similar way to a bulk spectrum.
- This is also known as:
  - μ-XAS, μ-XANES, μ-XAFS, ...
- HOWEVER, beam dwells on sample a long time, therefore beam damage an issue.

Spectrum from specific spot gives chemical information.

Collect spectrum by scanning incident energy, monitor fluorescence.

Sample

Using elemental map, choose interesting spot on sample.

Small X-ray beam, scan energy (needs good energy resolution).
**XAS-Imaging**

- Use the sensitivity of the near-edge to generate maps of chemical species for a given element.
- Works best for species with large contrast in the edge, such as sulfur.
- Also known as Chemically-Specific imaging, XANES-imaging, oxidation-state imaging etc.
- Need small beam with very good energy resolution at the energy of interest (S K-edge).

---

**XAS Imaging vs. X-ray Fluorescence Imaging**

Differences lie in the incident energy.

---

**XAS Imaging – Method**

- Use two or more energies to distinguish chemical species.
- Obtain quantitative maps of each species.

---
Simultaneous Spatial and Chemical Information
Two routes to information

- Micro X-ray Fluorescence (Image above edge) → Elemental Map → Micro-XAS at pixels → Chemical information
- XAS-Imaging (Image at edge) → Chemical Maps

Choice of XAS Imaging

- Micro X-ray fluorescence plus micro-XAS:
  - Gives entire XAS spectrum at selected points
  - May miss spatial detail
  - Longer dwell time at those pixels
- XAS Imaging:
  - Need to know which species to look for
  - Need good spectral contrast between species
  - Shorter dwell times
  - Gives quantitative spatial maps of each species

Special Issues at Sulfur K-edge

Compared with hard X-ray measurements:

- Attenuation is substantial
  - Use thin or no windows
  - Reduce air path or use He
- Beam damage is HIGHER with lower energy beam!
  - Beam is absorbed in very short pathlength
Example 1: Total Sulfur Imaging
Mercury Accumulation in Zebrafish Larvae

Proc. Natl. Acad. Sci. 105(34), 12108-12112

Zebralsh as model vertebrate in toxicology

Advantages of zebrafish:
- high fecundity
- easy maintenance
- readily treated with exogenous agents
- well characterized staging series

Our use: To study accumulation of Hg species in vertebrates
Emission of characteristic (fluorescence) X-rays by atoms in a sample as a result of their interaction with primary X-rays each element produces fluorescence X-rays at a unique set of energies.

### X-ray Fluorescence Imaging

- Sample is raster scanned with X-ray microbeam
- X-ray emission spectrum is collected at each pixel

### X-ray Fluorescence Imaging

- S
- Ca
- Zn
- Hg
Methylmercury uptake in live zebrafish

3.5 day-old larvae exposed to 100 μM CH₃Hg-S-(L-Cys) for 24 hours

Methodology

X-ray fluorescence imaging

Histological analysis

Tissue uptake of organic mercury

3.5 day-old larvae exposed to 100 μM organic mercury for 24 hours

Methylmercury uptake on cellular level

3.5 day-old larvae exposed to 100 μM CH₃Hg-S(L-Cys) for 24 hours

- Mercury-rich layer is ~ 7 μm thick
- High sulfur region correlates with the eye lens nucleus


The eyes have it

Lens epithelium
Lens core (cells devoid of nucleus and organelles)

Organic mercury accumulates preferentially in actively dividing lens epithelial cells

Summary and Future Studies

- X-ray fluorescence imaging of zebrafish is a sensitive system to study the fate of elements in vertebrates
- Sulfur distributions may give important insights into biochemistry
- Future studies include:
  - Studying fates of different mercury chemical forms
  - Testing treatments (e.g. chelation agents)
  - Continuing personnel: Gosia Korbas, Tracy MacDonald

Example 2: Imaging of Sulfur Species in Whole Cells


Why Study Sulfur in Whole Cells?

- Sulfur is an essential biological element
  - Many roles
  - Diverse biochemistry
- Sulfur is "spectroscopically silent"
  - Sulfur biochemistry only partly understood because there are so few tools for studying it in biological systems
- Having a probe of the total sulfur in cell cultures could help study
  - How drugs or toxic metals interact with cells
  - How apoptosis is important in cancer and AIDS

Sulfur K-edge Spectrum

- Observed variations:
  - Oxidation states: -2 to +6
  - Energy of peak: 14 eV
- Spectrum sensitive to local structure
  - Can be used as "fingerprint" of "class" of species present

Sulfur K near-edge spectrum is rich
Sulfur K-edge XAS Imaging

- Ongoing research program aimed at studying sulfur in mammalian cells
  - However, these are very challenging (small size, fragile, low concentration sulfur)
- Start with more tractable samples
  - Use as stepping stone and proof of principal

Sulfur forms in onion

Onion Chemistry

The stored precursor and the enzyme Allinase are mixed upon cell breakage. This results in release of the lachrymatory factor.
Onion Chemistry

**Sulfoxide**

Highly characteristic spectrum due to low-lying excited state.

Onion section, washed

After bruising, sulfoxide decreases, LF increases

---

**Where in onion is the precursor located?**

- Conventional analysis cannot answer this
- As soon as the cells are broken, the precursor is destroyed

**Use sulfur K-edge XAS imaging**
Energies for Fluorescence XAS Imaging

- Thiol/thioethers (RSH/RSR)
- Disulfides
- Sulfoxide
- Sulfate (x0.4)

Disulfides RSR/RSH Sulfoxides Sulfate Total sulfur

Quantitative maps - amounts of sulfur species

- Disulfides
- RSR/RSH
- Sulfoxides
- Sulfate
- Total sulfur
Sulfur XAS Imaging Setup

SSRL setup:
- Tapered metal monocapillary
- ~10µm spot

Sulfur XAS Imaging Setup

Spectromicroscopy of Sulfur in Onion

Green Onion (Scallion)
- Transverse section through leaf

Disulfides (0.1)
- RSR/RSH (0.5)
- Sulfoxides (0.4)
- Sulfate (0.05)
- Total (1)

Amounts:

Fractions:

Optical:

• Spring Onion TS
- 15 µm stepsize
Spring Onion TS

Identify 3 regions:
1. Exterior (epidermis) – high sulfoxide
2. Intermediate layer – RSR/RSH
3. Interior (cortex) – sulfoxides within cells

Single Pixel Fluorescence Spectra

High Sulfoxide

46% sulfoxide, 46% methionine, 6% disulfide, 2% sulfate

Low Sulfoxide

22% sulfoxide, 71% methionine, 7% disulfide
Red Onion – Transport Vessel

Identify three regions:
- Center – disulfide-rich
- Sheath – RSR/RSH-rich
- Remainder (cortex) – sulfoxide-rich cell interior, disulfide-rich cell walls

Sulfoxide Disulfide RSR/RSH

Top: Relative Amounts

Bottom: Fractions

1 mm x 1 mm
10 micron step

Optical:
Red Onion – Transport Vessel

Fractions

Identify three regions:
- Center – disulfide-rich
- Sheath – RSR/RSH-rich
- Remainder (cortex) – sulfoxide-rich cell interior, disulfide-rich cell walls

Future Work:
Imaging Sulfur at Different Length Scales

Imaging Sulfur in Brain

- Conventionally, microprobe developments push for smaller and smaller beams
- While this is valuable in many cases, sometimes this is not helpful
- Example: Human brain is too large to image at micron resolution in a tractable amount of time...
Imaging Metals in Human Brain

Section of “normal” human brain imaged to show iron and zinc

Fe=red
Zn=blue


Combine Different Imaging Length Scales

Three resolutions – analogous to microscope objectives

Macro imaging: Large organs or surveys
100 µm pixel

Micro imaging: Small organs or organisms
1-5 µm pixel

“Nano” imaging: Subcellular resolution
200 nm pixel

Summary

- Total Sulfur Imaging
  - Use hard X-ray microprobe beamline
  - Good for studies of co-location
- Speciation of Sulfur
  - Use microprobe beamline at S K-edge (e.g. BL 14)
- Low Energy Challenges
  - More attenuation
  - More beam damage
Group Members

Ruwandi Andrahennadi
Soo In Yang
Jake Pushia
Limei Zhang
Ian Coulthard
Dongmei Liu
Gossa Korbas
Cheryl Wiramanadath
Juxia Fu
Satya Singh

Not pictured: Justin Tse, Tracy MacDonald, Meki Gallego

Acknowledgements

Canada Research Chairs Program
University of Saskatchewan
Province of Saskatchewan
CF, NSERC, NIH, CIHR, SHRF

Canadian Light Source
(NSSC, NRC, CIHR,
and U. Saskatchewan)

Stanford Synchrotron Radiation Laboratory
(U.S. DOE and NIH)

Canadian Light Source
(NSSC, NRC, CIHR,
and U. Saskatchewan)

Synchrotron health training program at the University of Saskatchewan and the Canadian Light Source
Cross-disciplinary training in XAS, XRF-imaging, protein crystallography and biomedical imaging as applied to health
Money available for MSc, PhD & postdoctoral fellows
I am program leader – see me for details!

http://artsandscience.usask.ca/thrust
thrust@artsandscience.usask.ca