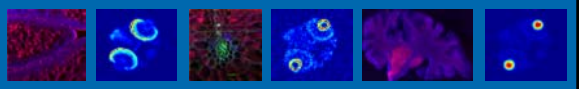

X-ray Fluorescence Imaging of Sulfur: Application to Biology

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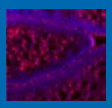
SSRL Structural Molecular Biology Low-Z XAS Summer School 2010
July 20, 2010

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

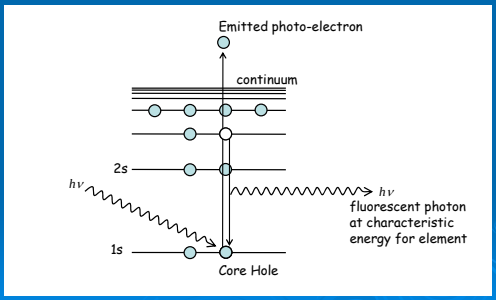
- Most biological samples are structured

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

XAS Imaging of Biological Samples

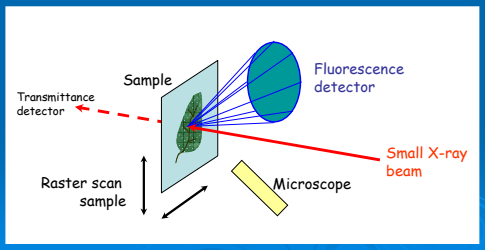
- Questions we may want to answer:
  - How is sulfur distributed?
  - What is the chemical form of sulfur in a particular location?
  - How is a sulfur chemical species distributed?
- We would like to do this when levels are dilute, and on intact living specimens

X-ray Fluorescence Imaging



Introduction

Basic arrangement for microprobe and XAS-imaging

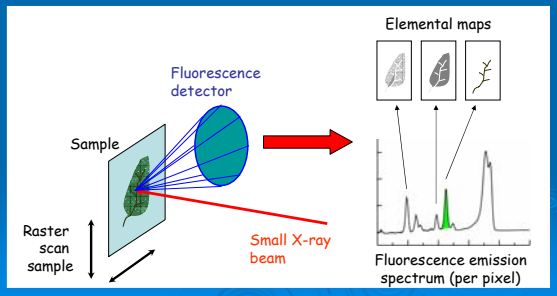


X-ray Fluorescence Imaging

- 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)

X-ray Fluorescence Imaging

At each pixel, fluorescent line intensities yield elemental quantities

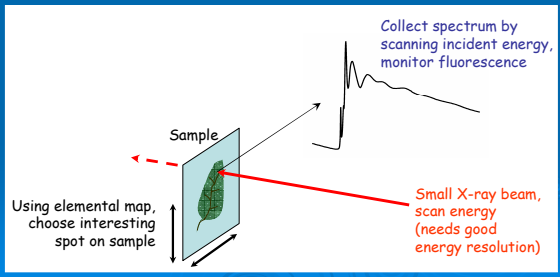


Micro-XAS

- 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

Micro-XAS

Spectrum from specific spot gives chemical information

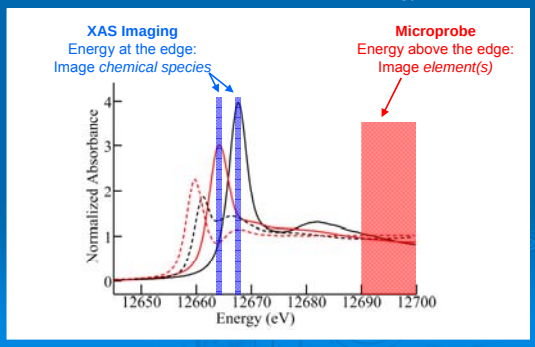


XAS-Imaging

- 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)

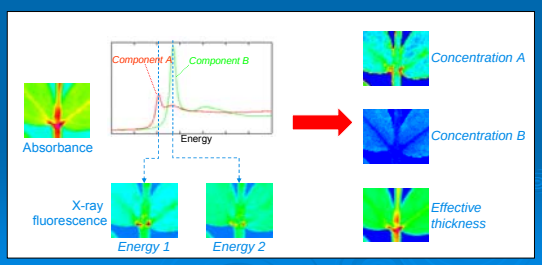
XAS Imaging *vs.* X-ray Fluorescence Imaging

Differences lie in the incident energy



XAS Imaging – Method

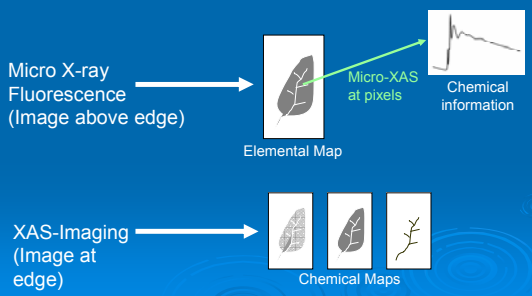
- Use two or more energies to distinguish chemical species
- Obtain quantitative maps of each species



Pickering et al., *PNAS* **97**(20) 10717-10722

Simultaneous Spatial and Chemical Information

Two routes to information



Choice of XAS Imaging

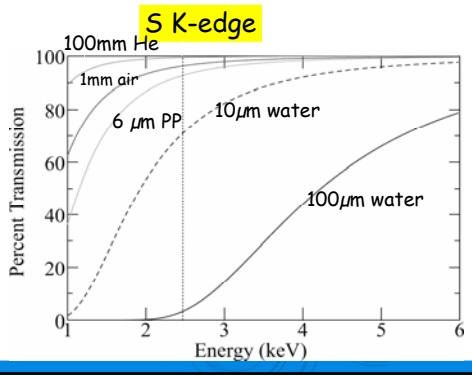
- Micro X-ray fluorescence plus micro-XAS:
  - ✓ Gives entire XAS spectrum at selected points
  - × May miss spatial detail
  - × Longer dwell time at those pixels
- XAS Imaging:
  - × Need to know which species to look for
  - × Need good spectral contrast between species
  - ✓ Shorter dwell times
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

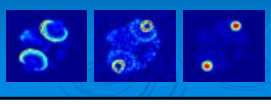
Attenuation



Example 1: Total Sulfur Imaging

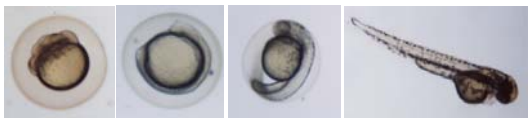
Mercury Accumulation in Zebrafish Larvae

M Korbas, SR Blechinger, PH Krone, IJ Pickering & G. N. George (2008) *Proc. Natl. Acad. Sci.* **105**(34), 12108-12112



Zebrafish as model vertebrate in toxicology

hpf/dpf – hours/days post fertilization



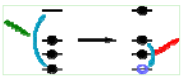
2 hpf 12 hpf 24 hpf 3 dpf

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

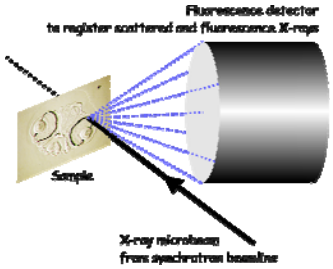
Our use: To study accumulation of Hg species in vertebrates

X-ray Fluorescence Imaging

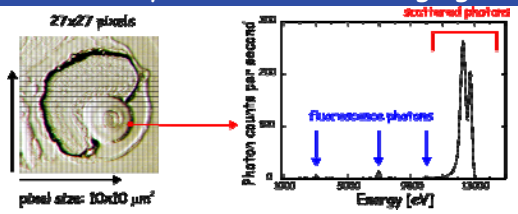
Emission of characteristic (fluorescence) X-rays by atoms in a sample as a result of their interaction with primary X-rays



each element produces fluorescence X-rays at a unique set of energies

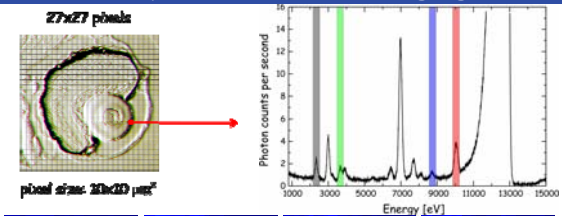


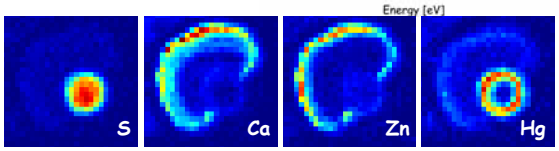
X-ray Fluorescence Imaging



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

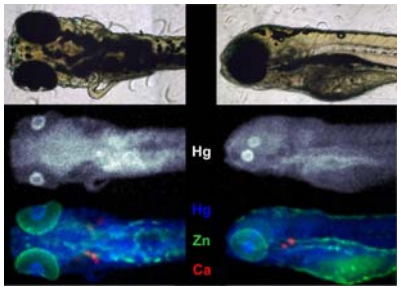
X-ray Fluorescence Imaging





Methylmercury uptake in live zebrafish

3.5 day-old larvae exposed to 100 µM CH_3Hg-S(L-Cys) for 24 hours

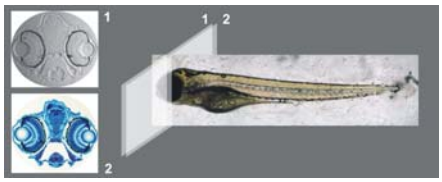


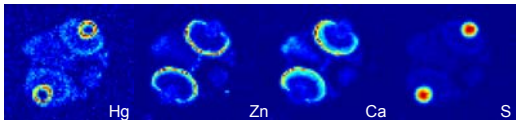
Korbas et al., *Proc. Natl. Acad. Sci.* **105**(34), 12108-12112 (2008)

Methodology

X-ray fluorescence imaging

Histological analysis



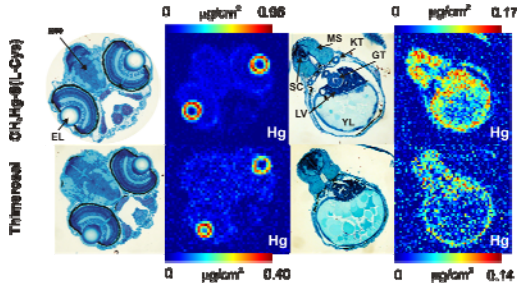


low high

Korbas et al., *Proc. Natl. Acad. Sci.* **105**(34), 12108-12112 (2008)

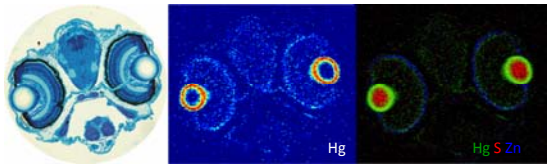
Tissue uptake of organic mercury

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



Methylmercury uptake on cellular level

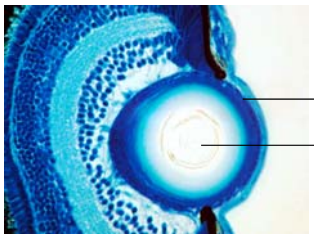
3.5 day-old larvae exposed to 100 µM CH_3Hg-S(L-Cys) for 24 hours



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

Korbas et al., *Proc. Natl. Acad. Sci.* **105**(34), 12108-12112 (2008)

The eyes have it



120 hpf

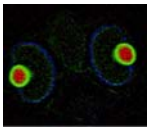
Lens epithelium

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

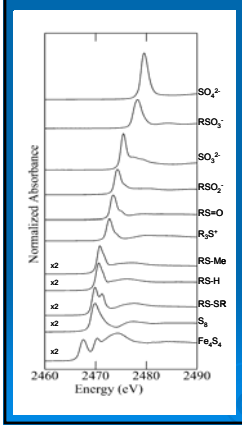
Next paper: M Korbas, PH Krone, IJ Pickering & GN George, *J. Biol. Inorg. Chem.* (published online)

Example 2: Imaging of Sulfur Species in Whole Cells

IJ Pickering, EY Sneeden, RC Prince, E Block, HH Harris, G Hirsch & GN George (2009) *Biochemistry*, **48:** 6846-6853

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

Sulfur K near-edge spectrum is rich

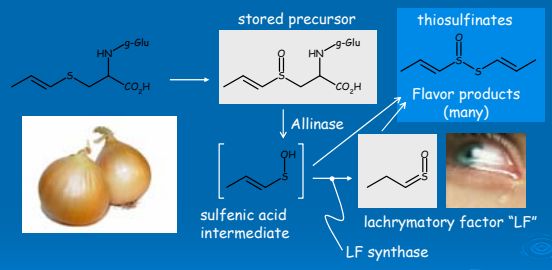
- Observed variations:
  - Oxidation state: -2 to +6
  - Energy of peak: 14 eV
- Spectrum sensitive to local structure
  - can be used as "fingerprint" of "class" of species present

Sulfur K-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

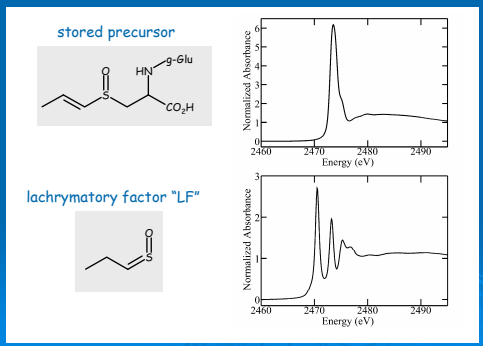
Sulfur forms in onion

Onion Chemistry

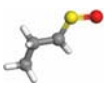


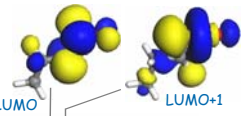
The stored precursor and the enzyme Allinase are mixed upon cell breakage. This results in release of the lachrymatory factor.

Onion Chemistry

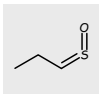


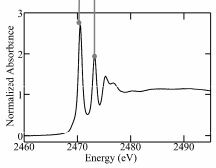
Onion Chemistry





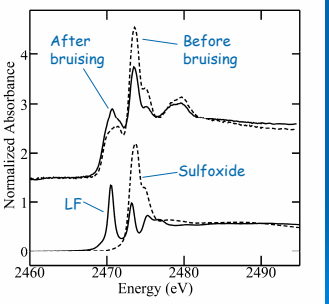
lachrymatory factor "LF"





Highly characteristic spectrum due to low lying excited state

Onion Chemistry



Onion section, washed
After bruising,
sulfoxide decreases,
LF increases

← Onion

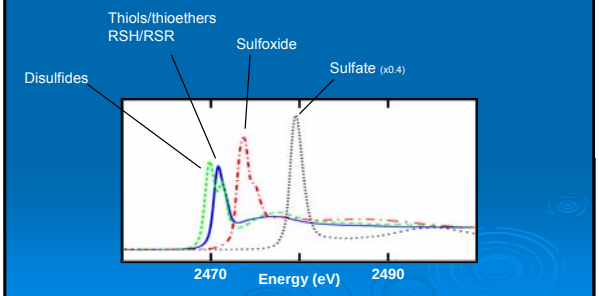
← Models

Onion Chemistry

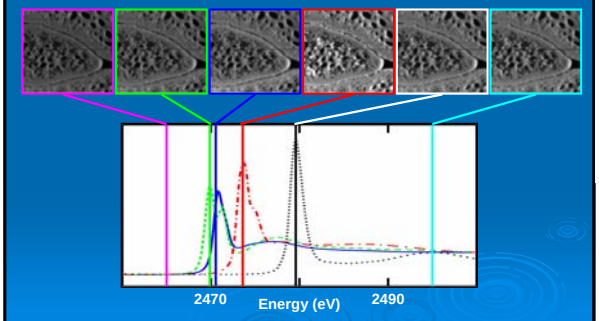
- Where in onion is the precursor located?
  - Conventional analysis cannot answer this
  - As soon as the cells are broken, the precursor is destroyed
- Use sulfur K-edge XAS imaging

IJ Pickering, EY Sneeden, RC Prince, E Block, HH Harris, G Hirsch & GN George
Biochemistry, 48: 6846-6853

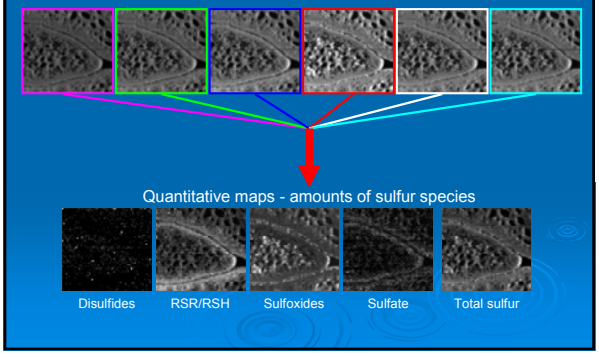
Energies for Fluorescence XAS Imaging



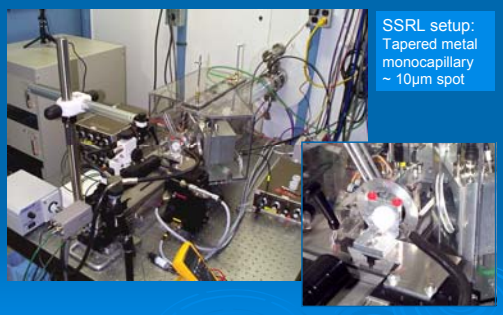
Energies for Fluorescence XAS Imaging



Energies for Fluorescence XAS Imaging



Sulfur XAS Imaging Setup



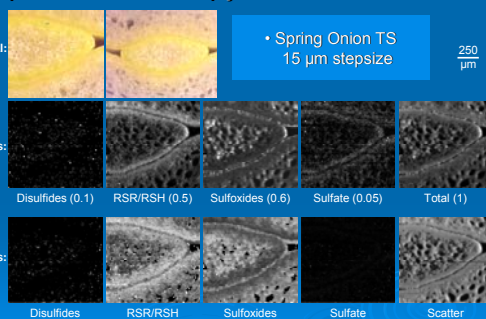
SSRL setup:
Tapered metal monocapillary
~ 10μm spot

Spectromicroscopy of Sulfur in Onion

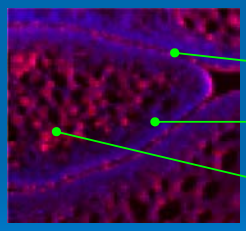
Green Onion (Scallion)

Transverse section through leaf

Spectromicroscopy of Sulfur in Onion



Spring Onion TS



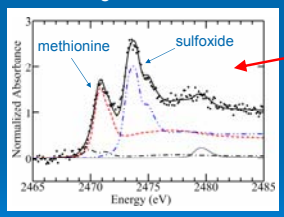
Identify 3 regions:

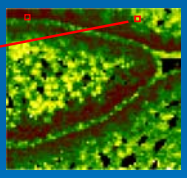
1. Exterior (epidermis)
   – high sulfoxide
2. Intermediate layer
   – RSR/RSH
3. Interior (cortex)
   – sulfoxides within cells

Sulfoxide RSR/RSH

Single Pixel Fluorescence Spectra

High Sulfoxide

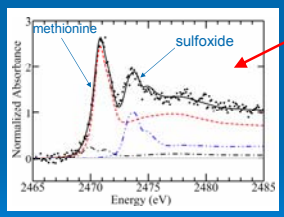


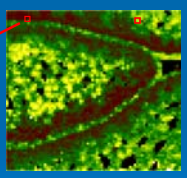


46% sulfoxide, 46% methionine, 6% disulfide, 2% sulfate

Single Pixel Fluorescence Spectra

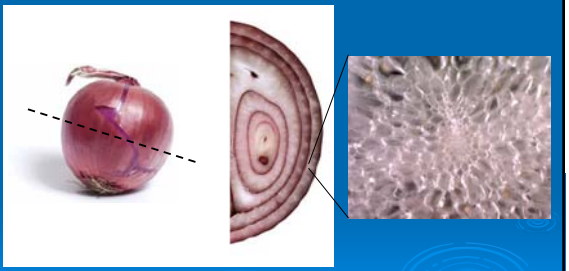
Low Sulfoxide



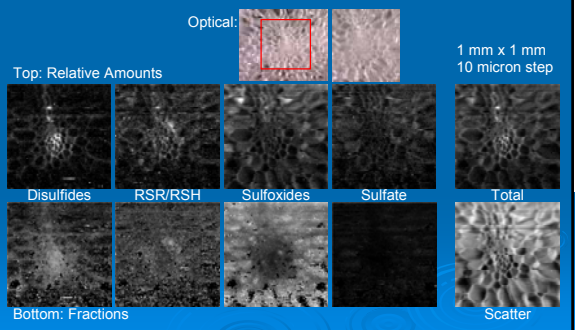


22% sulfoxide, 71% methionine, 7% disulfide

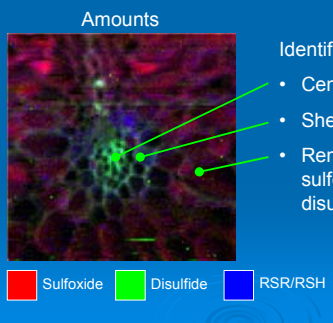
Red Onion – Transport Vessel



Red Onion – Transport Vessel



Red Onion – Transport Vessel

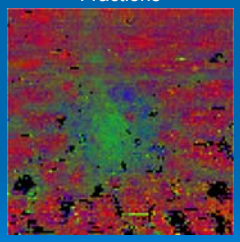


Identify three regions:

- Center – disulfide-rich
- Sheath – RSR/RSH-rich
- Remainder (cortex) – sulfoxide-rich cell interior disulfide-rich cell walls

Red Onion – Transport Vessel

Fractions

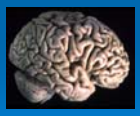


Identify three regions:

- Center – disulfide-rich
- Sheath – RSR/RSH-rich
- Remainder (cortex) – sulfoxide-rich cell interior disulfide-rich cell walls

Sulfoxide Disulfide RSR/RSH

Future Work: Imaging Sulfur at Different Length Scales

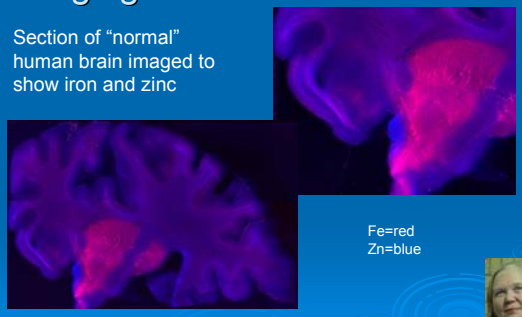


Imaging Sulfur in Brain

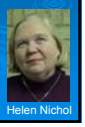
- Conventionally, microprobe developments push for smaller and smaller beams
- While this is valuable in many cases, sometimes this is not helpful
- Example: Human brain is too large to image at micron resolution in a tractable amount of time...

Imaging Metals in Human Brain

Section of "normal" human brain imaged to show iron and zinc



Fe=red
Zn=blue

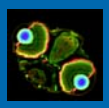


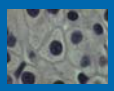
Helen Nichol

Popescu et al. (2009) "Mapping metals in Parkinson's and normal brain using rapid-scanning X-ray fluorescence." *Phys. Medicine Biol.* **54**:651-663

Combine Different Imaging Length Scales

Three resolutions – analogous to microscope objectives





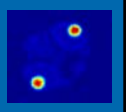
Macro imaging:
Large organs or surveys
100 µm pixel

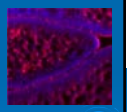
Micro imaging:
Small organs or organisms
1-5 µm pixel

"Nano" imaging:
Subcellular resolution
200 nm pixel

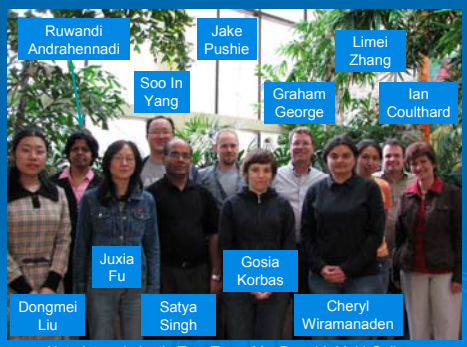
Summary

- 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



Not pictured: Justin Tse, Tracy MacDonald, Meki Gallego

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University of Saskatchewan
Province of Saskatchewan
CFI, NSERC, NIH, CIHR, SHRF

CIHR - THRUST

CIHR Training grant in Health Research Using Synchrotron Techniques

- 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!

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