LIGHT-SOURCE-BASED X-RAY IMAGING FOR BIOLOGY: FROM TRACE ELEMENTS TO CELLULAR STRUCTURE

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ACKNOWLEDGEMENTS

Teams from the 5 DOE light sources
Numerous users
Sponsors: DOE BES & BER, NIH institutes, …
Near continuous sources with high average brightness, wide tunable energy range and high stability enable:

- **Imaging** processes on the 100ps – days timescale
- Balanced flux on sample to follow processes (interact but don’t destroy)
- Diverse, highly optimized, multiplexed endstations for a wide range of scientific communities and numerous user groups

Pulsed sources with ultra-high peak and average brightness with full spatial coherence enable:

- Pump probe: Resolving ultrafast processes (<=ns)
- Near-instantaneous snapshots of processes in isolated areas (‘diffract before destroy’)
- A small number of endstations addressing carefully selected, high profile problems
INFRARED SPECTROMICROSCOPY
INFRARED SPECTROMICROSCOPY

Chemical Imaging of Biological Systems
- Biogeochemistry and Environment
- Health and Medicine
- Bioenergy
- Interfacial Phenomena

Nano-spectroscopy
- < 25 nm spatial resolution, wavelength independent

Microbial Biofilms in Boiling Hot Springs

Micro-spectroscopy
- 2-10 um spatial resolution, diffraction limited

Alzheimer’s disease is characterized by the accumulation of plaques in the brain.
- Plaques are misfolded Abeta protein.
- IR microspectroscopy can image misfolded proteins within tissue based on increased amyloid (β-sheet) structure.

Hoi-Ying Holman
hyholman@lbl.gov


Holman et al. unpublished (2017)


work done at ALS

work done at NSLS
X-rays have energies comparable to binding energies of electrons in atoms

As you cross an absorption edge, x-rays have enough energy to kick out another bound electron, and absorption increases significantly.

Some Binding Energies (eV)

<table>
<thead>
<tr>
<th>Element</th>
<th>Binding Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H 1s</td>
<td>13.6</td>
</tr>
<tr>
<td>O 1s</td>
<td>545</td>
</tr>
<tr>
<td>Fe 1s</td>
<td>7112</td>
</tr>
<tr>
<td>Pb 1s</td>
<td>88005</td>
</tr>
<tr>
<td>Pb 2p3/2</td>
<td>13043</td>
</tr>
</tbody>
</table>

Enables:
- Tuning the contrast for detecting a particular element.
- Spectroscopy to detect chemical state.

Courtesy M. Newville
TRANSMISSION X-RAY MICROSCOPY (SOFT X-RAYS)

- Natural contrast between protein and water in the so called water window
- Spatial resolution not limited by wavelength

- *Drosophila melanogaster* cell, in vitrified ice, imaged @ 0.5 keV @ BESSY

**Legend:**
- Cy: cytoplasm
- V: vesicle
- M: nuclear membrane
- N: nucleus

**Graph:**
- X-rays and electrons penetration distance vs. energy
- X-ray energy (eV) on the x-axis, penetration distance (μm) on the y-axis
- Electrons energy (keV) on the x-axis, penetration distance (μm) on the y-axis
- Various materials' (carbon, oxygen, water, protein) penetration characteristics are shown
Solution X-Ray Scattering and X-Ray Tomography Revealed Bacterial-Chromatin Packing Across the Nanoscale and Mesoscale

**Soft X-ray Tomography (SXT)**

*Image showing E. coli cells with chromatin, cytosol, cell wall, and capillary marked.*

1. HU multimerization shift controls nucleoid compaction.

2. Nucleoloid remodeling during environmental adaptation is regulated by HU dependent DNA bundling
   Remesh SG et al. 2020 Nature Communications (in revision)

**Small Angle X-ray Scattering (SAXS)**

*Graph showing intensity vs. q (Å⁻¹) with peaks at 70, 60, and 42 Å.*

National Center for X-ray Tomography Supported by NIH-NIGMS and DOE-BER

work performed at ALS
BIO IMAGING WITH HARD X-RAYS

- **Diffract and scatter**  
  - Structure determination

- **Imaging and Tomography**  
  - Hard x-rays penetrate matter deeply  
  - Visualization of structure in ‘thick’ samples (>1um), over large field of view  
  - Live imaging possible at reduced resolution  
  - Nanotomography down to 20 nm 3D resolution, lensless imaging down to 10 nm resolution

- **(Trace) Element Imaging via X-ray Fluorescence**  
  - Quantitative ion distributions at physiological concentrations, metal homoestasis, metal-linked diseases …  
  - Therapeutic (metal-based) drugs and diagnostic agents, theranostics, …  
  - Low background, no labeling required  
  - Visualization of chemical state

- **Radiation Damage Mitigation:**  
  - Cryogenic temperatures  
  - Reduction of X-ray dose

Uptake of TiO$_2$ nanoparticles (blue) into liver tumor cells one hour after injection. Sulfur (green) indicates cytoplasm, phosphorous (red) DNA.  
(Trace) Element Imaging via X-ray Fluorescence.  
(Part of NCI Cancer Close Up 2017)  
https://visualsonline.cancer.gov/
(DIRECT) IMAGING REGIMES WITH (COHERENT) X-RAYS

Adapted from Prof. Oleg Shpyrko (UCSD)

X-ray in vivo microtomography of embryonic evolution

During gastrulation: series of dramatic, coordinated cell movements drive reorganization of a simple ball or sheet of cells into a complex multi-layered organism.

**Scientific Goal:**
Understand the behavior of cells during development by imaging—in vivo and with subcellular resolution.

**Method:**
Phase contrast image before damage, Series of tomograms every 10 min, Tomogram = 1200 projections in 18 s

3D time-lapse series of *X. laevis* embryo during mid-gastrulation

*J. Moosmann et al., Nature 497, 394 (2013)*
Brain mapping currently occurs at orders of magnitude disparate resolutions and volumes from nanometer reconstructions of small volumes of brains with electron microscopy to mm³ voxel resolution maps of whole brains with MRI. Large gap remains in our understanding of brain anatomy at the mesoscale – detailing the cellular compositions of entire brains along with the trajectories of the vasculature and the long distance projections of neurons between and within brain regions. X-rays today can bridge the gap between those lengthscales.

- tomographic cross-section of a full mouse brain at 1 μm resolution (left: one extracted cross section of a full 3D dataset)
- nano-tomographic datasets down to ~30 nm resolution (right) and 10s of um field of view

Planned upgrades and developments are expected to extend technique to image at ~10 nm 3D resolution across mm sized volumes.
Trace elements (metals) are fundamental, intrinsic components of biological systems. Estimated: 1/3 of all known proteins contain metal cofactors as integral, catalytic components, often with regulatory functions, e.g.,
- Zn in Zinc finger proteins: transcription factors
- 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 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 targeting, therapy (e.g., Pt, TiO2) and diagnosis (e.g., Gd)

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:

Periodic table highlighting X-ray fluorescence

Major/minor elements in Biological Systems

- "Natural" "Trace" elements
- Toxic / carcinogenic elements

Used in Imaging, Diagnosis, Therapy, ...

K-line Fluorescence typically used
L-line Fluorescence typically used
SCANNING PROBE IMAGING: X-RAY ABSORPTION SPECTROSCOPY IMAGING

- Provide world-class micro-to-macro XAS/EXAFS spectroscopy capabilities with elemental mapping and imaging – enables speciation information
- Facilities cover a wide range of energies (2-25 keV) and spot sizes (2-500 µm) to meet requirements of a wide variety of science

After B Hedman
ROLE OF SULFUR SPECIES IN STROKE

Change in concentration, speciation and location as a function of stroke

Sulfate ester

Sulfonylic Acid

Sulfate ester

Sulfonylic Acid

CA = pyramidal neurons and dendrites
CC = White Matter

Work done at SSRL
5.2keV, 70nm ZP, 167x151 Cartesian grid
0.5s exposure, 6.5h measurement
white spots beam damage (not careful)
~20 nm resolution
=> Beautiful structural visualization, strong contrast
Extended to 3D

P

Ca

Cl

K

S

Ptycho

work done at APS

submitted
LBTS AS “GFP” FOR 3D NANOSCALE X-RAY IMAGING

- GFP and YFP are ubiquitous for imaging individual proteins within cells and tissues with fluorescence microscopy
- Limitations to GFP tags:
  - large size: limits protein transport
  - use of visible light imaging can limits the spatial resolution of the technique

Lanthanide-Binding Tags (LBTs)
- Analogous application for nanoscale X-ray fluorescence microscopy
- LBTs are small peptides and easily fused to proteins and transported
- X-ray fluorescence microscopy improves the spatial resolution to ~10 nm in 3D

Lisa Miller (BNL), Karen Allen (Boston Univ), Barbara Imperiali (MIT)

Work performed at HXN beamline at NSLS-II and BNP beamline at APS

SAMPLE PREPARATION, SENSITIVITY, SPATIAL RESOLUTION AND RADIATION DAMAGE:

- Strongly depends on actual application. Higher resolution carries radiation damage implications
- mm sized samples, typically um resolutions
- 10s-100s of micron sized samples: sub um resolution, typically down to 20-30 nm
- For high resolution, high sensitivity (trace metals), thin, trace element-clean substrates
- X-ray microscopy, Limit is ~ $10^{10}$ Gy for frozen hydrated samples, ~10 nm structural resolution limit
- Discuss with beamline staff!

**APS Today** => minimum detectable Zn [#atoms]

<table>
<thead>
<tr>
<th>Spot size</th>
<th>200 [nm]</th>
<th>20 [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 [um]</td>
<td>3500</td>
<td>35</td>
</tr>
<tr>
<td>10 [um]</td>
<td>26000</td>
<td>260</td>
</tr>
</tbody>
</table>

THE LCLS X-RAY LASER AT SLAC PROVIDES HIGH-RESOLUTION, DAMAGE-FREE, ROOM TEMPERATURE STRUCTURES AND DYNAMICS

- Very high brightness, short pulse X-ray source
- 2 dedicated instruments for structural biology
- Major upgrade underway (120 Hz to 1 MHz), marking a step-change in relevance to bioscience

**High resolution structures**
- Particularly suited to delicate and small (µm) crystals and low protein consumption (e.g. GPCRs)
- No crystal harvesting, and fast (days) optimization time
- Native-like membrane environment

**Molecular dynamics**
- Enzyme dynamics via “mix and inject” on µs to ms timescales
- Photo-excitation of proteins with chromophores on ps to µs timescale
- Structural dynamics (e.g. retinal, rhodopsin) on sub-ps timescale
IMAGING STRUCTURAL DYNAMICS WITH ULTRAFAST X-RAYS

- Radiation damage-free structural determination
  - High resolution metalloenzymes structure prior to photoreduction
  - Drug discovery: GPCRs in complex with ligands

- Structural dynamics at physiological conditions
  - Enzymatic reactions at physiological conditions
  - Antibiotic binding dynamics
  - Ligand binding to adenine riboswitch

- Multi-scale imaging in combination with cryo-EM
  - LCLS and cryo-EM combine to provide imaging from cells to molecules


Olmos et al., BMC Biology 16, 59, (2018)
COMBINING SMALL ANGLE X-RAY SCATTERING AND CRYO-EM TO STUDY STRUCTURE OF KATANIN CATALYSIS SUBUNIT

Cells constantly assemble and disassemble their microtubule cytoskeleton. Katanin is a microtubule-severing enzyme that generates internal breaks in microtubules, thus modulating their dynamics and organization. Owing to a lack of 3D structures, the mechanism of microtubule severing by this enzyme has remained poorly understood.

Bio-SAXS structure
For full length

CryoEM structure
For core only

Flexible sequences are missed in crystal and cryo-EM structures, while they show in BioSAXS structures.

BioSAXS measurements performed at 12-ID-B
Using Inline FPLC-SAXS and a home-designed temperature-controlled (~4C) flow cell

X-Ray Footprinting: a Solution State Method for Protein Structure

- Uses water locations to reveal the changes in protein conformation as a function of time or as a function of interactions
- Residue-specific resolution, both on protein surface and inside channels and cavities

Where to perform the method

Currently two synchrotron beamlines in the United States are dedicated to X-ray footprinting:

- Beamline 3.2.1 at the ALS
  alsfootprint.snappages.site
- Beamline 17-BM at the NSLS-II
  case.edu/medicine/csb/

Highlighted Publications

Overview of current instrumentation
Analytical Chem. 2020, 92, 1, 1565.

GPCR structure elucidation
Cell. 2019 May 16;177(5):1232.

Carotenoid protein structure and dynamics

Protein-metal interactions

Supported by NIH-NIGMS
HOW CAN YOU MAKE USE OF THESE RESOURCES?

- Beamtime is available on most beamlines at most synchrotrons to outside users through a competitive proposal process.
- Proposal submission deadlines typically 2 or 3 times a year.
- Typically 80% or more of ‘beamtime’ on any beamline is distributed

Some types of proposal:
- General User Proposals
  - Open to anyone, just have to write a good proposal. Proposals get reviewed by committee, assigned based on scores. Proposals that don’t quite make the score, ‘age’ so that they have a better chance next time.
  - Users typically come for experiments 3-4 days (9-12 shifts), carry out experiments with help of beamline scientist
  - No cost for beamtime, the expectation is that results will be published.
- Proprietary Experiments
  - Are also possible, generally not published, but cost recovery of beamtime is required

Most importantly: try to identify possible beamlines in advance, and contact the beamline scientist well before writing the proposal.

Feel free to contact me (svogt@anl.gov) (or point of contacts – later in slide), for help on general feasibility and potential beamlines/lightsources for a specific project.
SUMMARY

- Infrared Microscopy: chemical imaging of biological systems, based on differences in IR spectra (eg, lipids, proteins, protein folding, …). Resolution a few microns down and 10s of nm using near field methods.

- Transmission X-ray microscopy (TXM) (can be combined with tomographic approaches)
  - Soft X-ray range: typically to image cellular structure exploiting natural contrast between water and proteins, lipids, etc. resolution down to 30ish microns, < 10 um thickness.
  - Can be combined with spectroscopy (eg, STXM) for chemical imaging
  - Hard x-ray range: typically exploiting phase contrast resolution down to 20nm, thicker samples
    - Typically requires chemical fixation or cryogenic sample preservation

- Tomography / radiography – micron resolution, fast, can image live samples at reduced resolution. Phase contrast provides significantly increased contrast for biological (soft) samples
SUMMARY

- XAS Imaging / X-ray fluorescence microscopy / microspectroscopy
  - Macro to micro to nanoprobe, covering mm sized samples to 10s of micron sized samples, with resolutions from microns to 10s of nm. Typically fairly slow experiments.
  - Sensitivities down to ppm for trace element imaging using X-ray fluorescence (e.g., P, ..., Zn, ...)
  - Can combined with lensless imaging methods (e.g., ptychography) to push structural resolution down to 10 nm
  - High resolution requires sample preservation (chemical fixation or cryo)
  - Can combine with spectroscopy to image chemical state (e.g., Fe²⁺ vs Fe³⁺, ...)
- Small Angle X-ray Scattering to ‘image’ protein shape
- Macromolecular Crystallography to ‘image’ protein structure
- X-ray Free Electron Laser (LCLS) to ‘image’ ultrafast structural dynamics

- Combinations of these techniques as well as visible light microscopy and (cryo-) EM to address multimodal problems
- The future is bright: NSLS-II was just built, upgrades under way at LCLS and APS, planned for ALS, ...
  - Many of these techniques can expect gains of 2 orders of magnitude
Also happy to discuss / direct any other question …

THANK YOU !!!