Workshop on Biological Imaging and Spectroscopy at the NSLS II

Informal Report

Workshop held September 11–12, 2008

Washington, D.C.

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Prepared on behalf of the participants in the Workshop
by the
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Introduction

The National Synchrotron Light Source (NSLS) is located at the Brookhaven National Laboratory in Upton, New York. It opened in the early 1980s and has been a highly productive national user facility, operated by Brookhaven for the U.S. Department of Energy (DOE). Scientists from around the world make use of the facility for research in a wide range of scientific disciplines.

Planning began in 2003 for a replacement for the NSLS, which is being called the NSLS-II. This facility is being designed and received approval of the DOE Critical Decision-3, Start of Construction, in January 2009. It is expected to begin full operation in 2015.

Planning for experimental stations at the NSLS-II has been going on for several years, and workshops have been held to produce information about the value of different proposed beamline technologies for the disciplines that could make use of them. A summary of capabilities anticipated for the life sciences is at:

http://www.bnl.gov/nsls2/sciOps/LifeSci/bioMed.asp

Early in 2008 a panel of experts in synchrotron technologies for the life sciences and their application in different areas of biological and medical research was organized by units of the U.S. National Institutes of Health (NIH). The panel met on April 29, 2008, to provide recommendations to NIH regarding the types and numbers of experimental stations that NIH should support at the NSLS-II. The panel report provided specific information regarding stations for macromolecular crystallography and x-ray scattering. The panel did not, however, discuss in detail the value of NSLS-II capabilities in imaging or spectroscopy.

Thus it was decided to hold a workshop devoted to these techniques. The purpose of the workshop, held on September 11–12, 2008, was to assess the potential value to four major life science research communities of the imaging and spectroscopy capabilities planned for the NSLS-II. The workshop was organized jointly by program staff at DOE and NIH. The intention was that the workshop would focus very specifically on how the NSLS-II capabilities could enable progress in fields of biological research that are essential to the agencies' missions. A list of attendees is included at the end of this report.

This meeting was not held to determine funding or to decide policy, and it was not an official advisory committee meeting. It was an effort to obtain information about techniques to be developed at the new light source for three communities: biological and biomedical scientists; developers of the NSLS-II; and Federal agency personnel. The information developed during the course of the discussions is being made publicly available in this informal report so that all of these communities can benefit from the input that the attendees provided.

There is a wide range of imaging and spectroscopy techniques already available at synchrotron light sources, and new ones will be developed over the years leading up to the expected opening of the NSLS-II. Thus an analysis in fine detail was not requested of the attendees, as such details would inevitably change with time.

Instead, the attendees identified representative needs for new approaches to imaging and characterization in their fields prior to the workshop, and then assessed at the meeting the potential that each of these needs could be addressed by one or more of the techniques planned for the NSLS-II.

The agenda for the workshop included presentations about the capabilities being planned for the NSLS-II by scientists involved with the project. The attendees then met in four subgroups to discuss the needs listed by that subgroup, and how the NSLS-II techniques might help meet any of them. These subgroups were:

- Cell and organismal biology
- Diagnostic medicine
- Microbiology and geomicrobiology
- Plant biology

Each subgroup included scientists carrying out research in the field of biology, experts in the synchrotron techniques (including scientists with the NSLS-II project), scientists involved in forefront research in non-synchrotron imaging and spectroscopy technologies, and program staff at DOE and NIH.

A plenary session followed the subgroup meetings and enabled the participants to report on their discussions and to clarify questions about techniques and their applications.

Following the meeting the written inputs from the subgroups and the plenary session were regrouped by synchrotron technique for inclusion in this report. The six sections cover the following experimental groupings:

- Coherent diffraction imaging
- Near and far infrared microspectroscopy

- Phase contrast/diffraction enhanced x-ray imaging
- Scanning transmission x-ray microscopy
- Transmission x-ray microscopy
- X-ray spectroscopy, x-ray microspectroscopy and x-ray fluorescence microscopy

These statements were circulated to the attendees for comment and a number of revisions and corrections were made.

This document is not a formal report. As stated above it represents an informal collection of needs for four important areas of biomedical science and discussions of potential applications of the NSLS-II to meet those needs. The collection of needs is far from comprehensive; the assessment of applications is preliminary and subject to change as the plans for the experimental stations at the NSLS-II are developed in detail; and the contents of the statements, while considered generally appropriate by those involved in their discussion, were not formally approved by the attendees or any subgroup.

That said, the statements that follow are the result of discussions among experts both in the biological disciplines and in the application of advanced technologies to biological problems. They have identified many possible applications of this major new facility.

The participation of these scientists in the meeting and their contributions to the discussions are greatly appreciated by the organizers of the workshop at DOE and NIH. It is hoped that the contents of this informal report will be helpful to all who will be involved in planning for biological imaging and spectroscopy at the NSLS-II in the coming years.

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Coherent diffraction imaging

Coherent diffraction imaging (CDI) is a 'lensless' technique for 2D and 3D imaging at high-resolution of nanoscale structures such as viruses and cellular organelles, and of macromolecules associated with whole cells or groups of cells. In CDI, the coherent X-ray diffraction pattern of a specimen is recorded and then directly phased to obtain an image. Effectively, the objective lens in a typical microscope is replaced by oversampling iterative algorithms to invert the reciprocal-space diffraction pattern to a real-space image. The advantages in avoiding the use of lenses are that i) it has a greater depth of focus than other forms of X-ray microscopy; ii) the final image is aberration-free; iii) it can be implemented in both the soft X-ray and hard X-ray regions; and iv) the spatial resolution is ultimately limited by the X-ray wavelengths and radiation damage to biological specimens. Furthermore, by exploiting the abrupt change of the X-ray atomic scattering factors in the vicinity of absorption edges, CDI can be used to perform element and chemical state specific imaging of biological specimens.

Biological need to be addressed	Potential Value of Technique
Cell and organismal biology: Fixed cells and tissues: Improved resolution in Z is essential to take advantage of X-Y resolution	The highest resolution currently achieved by CDI is ~5 nm. In biological applications, the spatial resolution is limited by the radiation dose imposed on biological specimens. By using cryogenic technology, CDI can be used for quantitative 3D imaging of whole cells and cellular organelles at a resolution of 10 nm. CDI is ideally suited for quantitative 3D imaging of whole cells and cellular organelles at high resolution. By labeling specific macromolecules, CDI can be used to quantify macromolecular complexes inside cells as well as outside cells, such as the cellusome.
Cell and organismal biology: Fixed cells and tissues: Higher resolution in the X-Y plane	1–10 nm resolution may be possible (CDI is the highest resolution X-ray imaging modality currently available). While 5 nm resolution has been achieved with CDI, the ultimate resolution is only limited by X-ray wavelengths and radiation damage to biological structures.
Cell and organismal biology: Fixed cells and tissues: Adjustable resolution scales. Large field of view with low resolution and high resolution on the same instrument; coupling to light microscope	The resolution and the field of view of CDI can be relatively easily changed by adjusting the distance between the specimen and the detector. The ratio of the field of view to the desired resolution is limited by the number of pixels of the detectors.

Cell and organismal biology: Fixed cells and tissues: Precise quantification of local concentration of specific molecules	If one can tag specific macromolecules, CDI could be used to quantify supermolecular structures inside cells in three dimensions.
Cell and organismal biology: Fixed cells and tissues: Less shrinkage and distortion of samples.	For 3D imaging of whole cells and cellular organelles, samples could be kept in frozen-hydrated states to reduce the radiation damage effect.
Geomicrobiology: High resolution (micron scale) elemental mapping in three dimensions of complex and heterogeneous environmental matrices that include microbial cells and mineral particles over scales of cubic millimeters to probe, for example, redox transitions at interfaces. Need mainly for higher molecular weight elements, especially metals & radionuclides (Fe, Mn, U, Tc, Pu, Cr, Hg, As, Se).	10 nm to μm scale resolution. By avoiding the use of lenses, CDI has a greater depth of focus than other forms of x-ray microscopy, and can be applied to 3D imaging of micron-scale biological specimens at a resolution of 10 nm. Elemental mapping has recently been demonstrated. Does not require ordered structure. Suitable for 3D mapping of complex and heterogeneous structures.
Geomicrobiology: Ultra-high resolution (nanometer scale) microscopy, elemental mapping and spectroscopy (i.e., XANES[1], or EXAFS[2]) to resolve metal and radionuclide contaminant speciation (oxidation state, bonding environment, etc.) and mineral structure within microbial cells and at microbe-mineral interfaces. High resolution spectroscopies needed for speciation to probe biogeochemical transformation mechanisms and predict element reactivity and behavior (ideally, one would like to be able to do this on hydrated samples).	CDI could be used to perform element specific imaging by recording coherent X-ray diffraction patterns below and above specific absorption edges, which has recently been demonstrated. In order to achieve the highest possible resolution, the samples have to be kept in the frozen-hydrated state. Sensitivity is lower than with X-ray fluorescence.
Geomicrobiology: Microscopy and spectroscopies to map distributions of light elements (C, N, P, S) at the micron scale in soil/sediment aggregates (scale of tens to hundreds of millimeters) and to provide information on element chemistry such as carbon functional group distribution, degree of aromaticity etc. within organic matter (humic materials).	Elemental mapping has been demonstrated, but only for heavier elements (see Song *et al.,* "Nanoscale Imaging of Buried Structures with Elemental Specificity by Using Resonant X-ray Diffraction Microscopy" *Phys. Rev. Lett.* 100, 025504 (2008)).
Microbiology: Highly resolved cell biology for discerning supramolecular structures such as the ribosome, DNA polymerase, membrane transport systems which are difficult to see with other techniques (especially the latter). This method needs to be able to interrogate many examples of such structures in a single cell and devise a picture of the distribution of different conformational states of these complex machines.	For radiation hard biological materials, ~1 nm resolution may be achievable, and for radiation sensitive specimens, 10 nm can in principle be obtained. By rapid freezing of extra cellular polymeric materials at different stages, CDI can be used for performing structure-function studies. By labeling specific macromolecules, CDI can be used to quantify macromolecular complexes inside cells.
Microbiology: X-ray and electron damage as a tool for mapping cellular damage.	Coherent X-ray diffraction patterns can be acquired as a function of radiation dose, which can be used to study cellular damage.

[1] X-ray Absorption Near Edge Structure
[2] Extended X-Ray Absorption Fine Structure Spectroscopy

Microbiology: Role of viruses. Host-pathogen interactions. Phage. Eukaryotic cell-virus interactions.	A recent experiment has shown that CDI can be used to perform quantitative and high-contrast imaging of a single, unstained virion with a resolution of 22 nm. [See Song *et al.* "Quantitative Imaging of Single, Unstained Viruses with Coherent X-rays", *Phys. Rev. Lett.* **101**, 158101 (2008)]This work could be extended to the studies of host-pathogen interaction.
Plant biology: To quickly and routinely generate at greater than 10 nm resolution three dimensional native structure (including macromolecular complexes) for any cell type of the plant. Higher resolution through heroic efforts.	CDI is suitable for quantitative 3D image of thicker specimens. For radiation resistant plant cells, sub-10 nm resolutions are potentially achievable.
Plant biology: To overlay three dimensional native structure at both low and high resolution with information about molecular composition (e.g. proteins, polysaccharides, lipids, etc.), intercalation of ions into structure, etc. (structures detected must have phase or chemical contrasts against their surroundings).	By labeling specific molecules, CDI can be applied to quantifying macromolecular complexes inside plant cells.

Near and far infrared microspectroscopy

Infrared microspectroscopy[3] (IRMS) is a technique that combines infrared spectroscopy with microscopy, such that the chemical makeup of materials can be probed through their vibrational spectra on a microscopic scale. Synchrotron infrared (IR) light is an ideal source for IRMS and imaging due to the combination of its high brightness and broadband nature. Through a 10-micron pinhole, a synchrotron source is 100–1000 times brighter than a conventional globar source. The improvement in spatial resolution over globar sources is especially important for spectroscopic imaging of biological materials with high spatial resolution.

Biological need to be addressed	Potential Value of Technique
Cell and organismal biology: Fixed cells and tissues: Precise quantification of local concentration of specific molecules	Stable isotopes could be used to track molecules through tissue/cells with minimally invasive label.
Cell and organismal biology: Fixed cells and tissues: Higher resolution in x-y plane	Will be 0.5 to 1 μm. For a fixed tissue a key advantage will be correlating with x-ray fluorescence and spectroscopy. Can get contrast between lipid and water.
Cell and organismal biology: Fixed cells and tissues: Improved resolution in z axis to take advantage of x-y resolution	No resolution expected in z; sample thickness will determine spectrum
Cell and organismal biology: Fixed cells and tissues: Adjustable resolution scales, with large field of view at low resolution and high resolution on the same instrument; coupling to light microscope	Routine from 1 to 50 μm; coupling possible with epifluorescence, etc.
Cell and organismal biology: Living cells and tissues: Tracking specific molecules without staining	Should be useful within certain limits (0.5–1 μm scale tracking, thickness < ~ 20 um)
Cell and organismal biology: Living cells and tissues: Quantification of molecular concentration as a function of time (preferably without staining)	Should be possible, detection sensitivity will depend on the molecule(s) being tracked
Cell and organismal biology: Living cells and tissues: Chemical speciation on micron scale	Identify regions within cells and/or specific cells using conventional markers; characterize composition using IR.
Diagnostic medicine: How to accurately combine functional and structural information acquired from patients	Ex vivo tomographic distribution of enzymes, metals, pollutants, etc.
Geomicrobiology: High resolution (micron scale) elemental mapping in three dimensions of complex and heterogeneous environmental matrices that include microbial cells and mineral particles over scales of cubic millimeters to probe, for example, redox transitions at interfaces. Need mainly for higher molecular weight elements, especially metals & radionuclides (Fe, Mn, U, Tc, Pu, Cr, Hg, As, Se).	Yes: for mapping and spectroscopy (i.e. species identification) of organics and minerals at 2–5 micron resolution; sample thickness is limited to approx <20 microns; can be performed on hydrated samples since IR does not cause radiation damage.

[3] Also called "spectromicroscopy" and "Fourier Transform Infrared [FTIR] Spectromicroscopy"

Geomicrobiology: Microscopy and spectroscopies to map distributions of light elements (C, N, P, S) at the micron scale in soil/sediment aggregates (scale of tens to hundreds of millimeters) and to provide information on element chemistry such as functional group distribution, degree of aromaticity etc. within organic matter (humic materials).	Yes: for mapping and spectroscopy (i.e. species identification) of organics and minerals at 2–5 micron resolution; sample thickness is limited to approx <20 microns; can be performed on hydrated samples since IR does not cause radiation damage.
Geomicrobiology: Fungal degradation of plant polymers. Maturation of soil/ sedimentary organic matter. Interactions between macro- and microorganism interactions (commensalism, symbiosis)	Yes: for mapping and spectroscopy (i.e. species identification) of organics and minerals at 2–5 micron resolution; sample thickness is limited to approx <20 microns; can be performed on hydrated samples since IR does not cause radiation damage.
Geomicrobiology: Simultaneous information on microbial community composition (phylogeny of individuals) using FISH[4], etc. and chemistry of plant, soil, aquatic organic matter being processed. Possible stable isotope (^{13}C, ^{15}N) applications to track specific compounds into cells & various organic fractions?	Yes: FTIR[5] microscopes are easily equipped with conventional light microscopy tools (e.g. DIC, epi-fluorescence, polarizers). FTIR is sensitive to isotopic substitution and could be used to track stable isotopes in samples. Minimum detection sensitivity needs to be explored.
Microbiology: To link or couple in situ hydridization microscopy techniques along with other imaging methods	Yes: FTIR microscopes are easily equipped with conventional light microscopy tools (e.g. DIC, epi-fluorescence, polarizers).
Microbiology: Polymerization/depolymerization of organic matter by fungi, soil microbes. Implications for national biofuels initiative.	Yes: for mapping and spectroscopy (i.e. species identification) of organics at 2–5 micron resolution; sample thickness is limited to approx <20 microns; can be performed on hydrated samples since IR does not cause radiation damage.
Microbiology: Structure-function of extracellular polymeric materials. Spatial distribution, interactions with metals and radionuclides.	Yes: same comments as immediately above, and combined with micro-XAS[6]
Microbiology: Applications for extreme environments. Microbial dynamics at high temperature that impact national biofuels initiatives. Hydrocarbon processing, hydrogen production under aerobic/ anaerobic conditions.	Yes: for mapping and spectroscopy (i.e. species identification) of organics at 2-5 micron resolution; sample thickness is limited to approx <20 microns; can be performed on hydrated samples since IR does not cause radiation damage.
Plant biology: To obtain three-dimensional structure of any tissue or tissue system with at least 1 μm resolution (to resolve shapes of individual cells).	Yes on sections with spectroscopy, which will generate chemical maps
Plant biology: To overlay three dimensional native structure at both low and high resolution with information about molecular composition (e.g. proteins, polysaccharides, lipids, etc.), intercalation of ions into structure, etc. (structures detected must have phase or chemical contrasts against their surroundings).	Yes at several μm resolution for broad biochemical differences without radiation damage; question about hydration; 3D not possible to date

[4] Fluorescence in-situ Hybridization

[5] Fourier Transform Infrared Spectroscopy

[6] X-ray Absorption Spectroscopy

Phase-contrast / Diffraction Enhanced X-ray Imaging

Diffraction enhanced imaging (DEI) is a novel radiography method that introduces fine selectivity for the angular deviation of x-rays traversing the subject. It uses collimated x-ray beams and an analyzer crystal positioned between the subject and the detector. The angular sensitivity of DEI measures the 'ultra-small-angle scattering' from the subject. Since DEI's contrast mechanism does not rely on the absorption of the subject, it is ideally suited for soft-tissue imaging. Moreover, at high energies, the radiation dose to the subject is significantly reduced.

Biological need to be addressed	Potential Value of Technique
Cell and organismal biology: For fixed cells and tissues: Improved resolution in z is essential to take advantage of x-y resolution	Early stage tumor development; effect of treatment on tissue. Much higher contrast than other x-ray imaging methods. Work required to correlate DEI contrast with conventional histology. Useful for animal model studies.
Cell and organismal biology: For fixed cells and tissues: Imaging from cells through thick tissues; and determination of molecular concentrations in living animals.	Higher contrast at NSLS-II will permit faster measurements (follow changes in internal structure on biologically relevant timescales - i.e., breathing, heartbeat) or will permit measurements with lower (non-lethal) doses.
Diagnostic Medicine: How to improve visualization of pathologic changes even earlier in the development of disease so that therapeutic interventions might be more effective	Ex vivo human and in vivo animal and development for clinical applications
Diagnostic medicine: How to most accurately combine functional and structural information acquired from patients	Same as above
Diagnostic medicine: How to reduce the radiation exposure to the population	Same as above
Diagnostic medicine: Testing the efficacy of drugs in development	In vivo animal
Diagnostic medicine: Monitoring of therapy	In vivo animal
Diagnostic medicine: Tissue Engineering: Emerging need for imaging stems cells and their fate in tissue engineering and transplant, and imaging inter-organ migration and repopulation of autologous stem cells.	In vivo animal

Geomicrobiology: Fungal degradation of plant polymers.	Imaging of plant ultrastructure based on small density differences; can be done on whole plants in 3D; resolution is ~10 microns
Microbial ecology: Interactions between macro- and microorganism interactions (commensalism, symbiosis)	Imaging of plant ultrastructure based on small density differences; can be done on whole plants in 3D; resolution is ~10 microns
Microbiology: Run small animal models (e.g., flies, worms, zebrafish, medaka) variously colonized or not with genetically different intestinal microbes to see what difference the mercury metabolizing genotype of the commensals makes on the distribution and speciation of Hg from the diet or environment, for understanding what Hg (and other toxic metals) are doing to eukaryote organisms in general terms and also specifically how toxic metals act in perturbing essential metal storage and trafficking.	Yes: imaging of organ ultrastructure based on small density differences which could be correlated with x-ray fluorescence trace-metal imaging; can be done on whole animals in 3D; resolution is ~10 microns
Plant biology: To obtain three-dimensional structure of any tissue or tissue system with at least 1 μm resolution (to resolve shapes of individual cells).	Yes on thicker (few mm to cm) samples but questionable about getting to 1 μm resolution (10 microns can be achieved today); detector is a factor
Plant biology: To map vascular system through a whole plant 10 cm tall at 10 μm resolution	Yes, including living plants

Soft X-Ray Scanning Transmission Microscopy

Soft x-ray microscopy uses x-rays with an energy of 200–1500 ev, or a wavelength of about 1–10 nm. The wavelength is much smaller than that of visible light, giving the potential for high spatial resolution imaging. The photon energy is well matched to inner-shell electron energies in low-z elements. In particular, by operating between the k edges of carbon and oxygen, one has good intrinsic contrast between organic material and water and good penetration in micrometer-thick specimens. Soft x-ray microscopy is therefore well suited to the study of single biological cells and similar specimens. Scanning transmission x-ray microscopy (stxm) enables spectroscopic imaging at each point in the scan.

Biological need to be addressed	Potential Value of Technique
Cell and Organismal Biology Fixed cells and tissue: Improved resolution in z is essential to take advantage of x-y resolution	Potential to image cellular structure with much lower dose, if the contrast mechanism is based on a bulk element. Probably limited to low z elements.
Diagnostic medicine: How to improve visualization of pathologic changes even earlier in the development of disease, so that therapeutic interventions might be more effective	Would help with ex vivo human and in vivo animal studies and development of clinical applications
Diagnostic medicine: Biomarkers to track metabolism, etc. (perhaps with nanoparticles); tracking of metals	In vivo animal and in vitro studies envisioned, microbeam applications
Diagnostic medicine: Monitoring of cell migration	Potential for in vivo animal and in vitro studies considered, but radiation damage is high and cryo-samples would have to be used.
Geomicrobiology: Ultra-high resolution (nanometer scale) microscopy, elemental mapping and spectroscopy (i.e., xanes[7], exafs[8]) to resolve metal and radionuclide contaminant speciation (oxidation state, bonding environment etc.) and mineral structure within microbial cells and at microbe-mineral interfaces. High resolution spectroscopies needed for speciation to probe biogeochemical transformation mechanisms and predict element reactivity and behavior (ideally, one would like to be able to do this on hydrated samples).	Yes: for mapping and spectroscopy (i.e. species identification) of organics and minerals at nanoscale resolution (down to 30 nm today); sample thickness is limited to approx <1 micron; cryo-cooling required for hydrated samples.

[7] X-ray Absorption Near Edge Structure
[8] Extended X-ray Absorption Fine Structure

Geomicrobiology: • Simultaneous information on microbial community composition (phylogeny of individuals) using FISH[9], etc., and chemistry of plant, soil, aquatic organic matter being processed. Possible stable isotope (^{13}C, ^{15}N) applications to track specific compounds into cells & various organic fractions? • Fungal degradation of plant polymers. • Maturation of soil/ sedimentary organic matter. • Interactions between macro- and microorganism interactions (commensalism, symbiosis, for example)	Yes: for mapping and spectroscopy (i.e. species identification) of organics and minerals at nanoscale resolution (down to 30 nm today); sample thickness is limited to approx <1 micron; cryo-cooling required for hydrated samples.
Microbiology: Highly resolved microbial cell biology for discerning supramolecular structures such as the ribosome, DNA polymerase, membrane transport systems which are difficult to see with other techniques (especially the latter). This method should be able to interrogate many examples of such structures in a single cell and devise a picture of the distribution of different conformational states of these complex machines not possible with any other methods	Might be able to provide the spatial resolution necessary (<10 nm) to see these macromolecules, but how to distinguish them from the remaining cellular components is unclear; radiation damage will be an issue
Microbiology: Polymerization/depolymerization of organic matter by fungi, soil microbes, with implications for national biofuels initiative.	Yes: for mapping and spectroscopy (i.e., species identification) of organics and minerals at nanoscale resolution (down to 30 nm today); sample thickness is limited to ~1 μm; cryo-cooling will be required for hydrated samples
Microbiology: Role of viruses. Host-pathogen interactions. Phage. Eukaryotic cell-virus interactions.	Yes: for mapping and spectroscopy (i.e. species identification) of organics and minerals at nanoscale resolution (down to 30 nm today); sample thickness is limited to approx <1 micron; cryo-cooling required for hydrated samples.
Microbiology: Structure-function of extracellular polymeric materials. Spatial distribution, interactions with metals and radionuclides.	Yes: for mapping and spectroscopy (i.e. species identification) of organics and minerals at nanoscale resolution (down to 30 nm today); sample thickness is limited to approx <1 micron; cryo-cooling required for hydrated samples.
Microbiology: X-ray and electron damage as a tool for mapping cellular damage.	Yes: for mapping and spectroscopy (i.e. species identification) of organics and minerals at nanoscale resolution (down to 30 nm today); sample thickness is limited to approx <1 micron; cryo-cooling required for hydrated samples.

[9] Fluorescence in-situ Hybridization

Microbiology: Applications for extreme environments. Microbial dynamics at high temperature that impact national biofuels initiatives. Hydrocarbon processing, hydrogen production under aerobic/ anaerobic conditions.	Possible: same comments as above, but can only be done in frozen samples
Plant Science: To quickly and routinely generate at greater than 10 nm resolution three dimensional native structure (including macromolecular complexes) for any cell type of the plant. Higher resolution through heroic efforts	Yes on sections with spectroscopy, which will generate chemical maps
Plant Science: To overlay three dimensional native structure at both low and high resolution with information about molecular composition (e.g. proteins, polysaccharides, lipids, etc.), intercalation of ions into structure, etc. (structures detected must have phase or chemical contrasts against their surroundings).	Yes for broad biochemical differences (protein, lipid, C, N, O, redox etc) at 10 nm in sections – NSLS-II will greatly enhance this; 3D not possible to date
Plant biology: To obtain three-dimensional structure of any tissue or tissue system with at least 1 μm resolution (to resolve shapes of individual cells).	Yes on mm size samples
Plant biology: To map vascular system through a whole plant 10 cm tall at 10 μm resolution	Yes on mm size samples, depending on the required resolution, cryo-cooling is likely necessary

Transmission x-ray microscopy

Soft x-ray microscopy uses x-rays with an energy of 200–1500 eV, or a wavelength of about 1–10 nm. The wavelength is much smaller that that of visible light, giving the potential for high spatial resolution imaging. The photon energy is well matched to inner-shell electron energies in low-Z elements. In particular, by operating between the K edges of carbon and oxygen, one has good intrinsic contrast between organic matter and water and good penetration in micrometer-think specimens. Soft x-ray microscopy is therefore well suited to the study of specimens such as single biological cells.

Biological need to be addressed	Potential Value of Technique
Cell and organismal biology: For fixed cells and tissues: Improved resolution in z is essential to take advantage of x-y resolution	Location of specific molecules within a cell. Global information about cell. Partitioning of molecules (tagged with inorganic labels; correlated with GFP, etc.) into different compartments. Location of molecules in context (context from C/N absorption contrast; localization from tag).
Cell and organismal biology: For fixed cells and tissues: Precise quantification of local concentration of specific molecules	Precise quantification of biological macromolecules may be possible if they can be tagged.
Diagnostic medicine: Monitoring of cell migration	Potential for in vivo animal/in vitro/microbeam is minimized as radiation damage is too high and cryo-samples must be used
Geomicrobiology: Ultra-high resolution (nanometer scale) microscopy, elemental mapping and spectroscopy (i.e., XANES[10], EXAFS[11]) to resolve metal and radionuclide contaminant speciation (oxidation state, bonding environment etc.) and mineral structure within microbial cells and at microbe-mineral interfaces. High resolution spectroscopies needed for speciation to probe biogeochemical transformation mechanisms and predict element reactivity and behavior. (Ideally, one would like to be able to do this on hydrated samples).	Yes- for mapping of organics at nanoscale resolution (down to 30 nm today); little chemistry information, but data acquisition is fast; sample thickness is limited to approx <10 microns; cryo-cooling required for hydrated samples.
Microbial ecology: Interactions between macro- and microorganism interactions (commensalism, symbiosis)	Yes- for mapping of organics at nanoscale resolution (down to 30 nm today); little chemistry information, but data acquisition is fast; sample thickness is limited to approx <10 microns; cryo-cooling required for hydrated samples.

[10] X-Ray Absorption Near Edge Structure
[11] Extended X-ray Absorption Fine Structure

Microbiology: Role of viruses. Host-pathogen interactions. Phage. Eukaryotic cell-virus interactions.	Yes- for mapping of organics in 3D at nanoscale resolution (down to 30 nm today); little chemistry information, but data acquisition is fast; sample thickness is limited to approx <10 microns; cryo-cooling required for hydrated samples.
Microbiology: X-ray and electron damage as a tool for mapping cellular damage.	Yes- for mapping of organics in 3D at nanoscale resolution (down to 30 nm today); little chemistry information, but data acquisition is fast; sample thickness is limited to approx <10 microns; cryo-cooling required for hydrated samples.
Plant biology: To quickly and routinely generate at greater than 10 nm resolution three dimensional native structure (including macromolecular complexes) for any cell type of the plant. Higher resolution through heroic efforts	Yes, sections with high throughput

X-ray spectroscopy, x-ray microspectroscopy and X-ray fluorescence microscopy

X-ray absorption spectroscopy (XAS) is widely used to characterize the atomic structure of metal centers in metalloproteins including metal oxidation state, spin state, and metal-ligand distances. Data can be obtained on samples in the powder, crystalline, or solution state. In solution, this technique can provide element-specific structural information for reaction intermediates of protein samples that are difficult to isolate or crystallize. XAS can be used independently, or to substantiate the structural information for intricate protein systems derived from x-ray crystallographic or nuclear magnetic resonance experiments. Determination of high resolution structure and interaction mechanisms at the metal-containing active site of a protein can provide a structural basis for drug design. In *x-ray microspectroscopy,* XAS is combined with a highly focused x-ray beam to probe the chemical state of elements of interest at very specific locations, such as organelles or vesicles. In *x-ray fluorescence microscopy,* the sample raster is scanned through a highly focused x-ray beam. Characteristic x-rays are emitted for the elements in the sample and are detected using energy-dispersive detectors, and used to determine location and quantity of the elements of interest with perfect registration and high sensitivity (down to the attogram level). This technique is used, for example, to acquire elemental maps of biological systems in the natural state as well as in diseased states.

Biological need to be addressed	Potential Value of Technique
Cell and Organismal Biology Fixed cells and tissue: Improved resolution in z is essential to take advantage of x-y resolution	Image single cell in 3 dimensions with 20 nm spatial resolution and detection of all elements with atomic sensitivity. Able to image individual nanoparticles. Example: Detailed characterization of individual plaque; degeneration of individual neurons; effect of environmental toxins in individual cells; uptake of metal-based chemotherapies and imaging agents.
Cell and Organismal Biology Fixed cells and tissue: Precise quantification of local concentration of specific molecules	*Fluorescence detected:* Determination of elemental concentrations in subcellular compartments. Fundamental studies of metal homeostasis *Absorbance detected:* Same potential, for low z elements
Cell and Organismal Biology Fixed cells and tissue: Less shrinkage and distortion of samples	*Fluorescence detected:* Quicker measurements may avoid some sample degradation (although probably not radiation damage, since total dose is the same)
Diagnostic medicine: How to improve visualization of pathologic changes even earlier in the development of disease so that therapeutic interventions might be more effective	For detection of scattering in vivo animal.

Diagnostic medicine: How to most accurately combine functional and structural information acquired from patients	Ex vivo tomographic distribution of enzyme metal cofactors, pollutants, toxins, etc.
Diagnostic medicine: How to reduce the radiation exposure to the population	Exploratory for contrast agents/in vivo animal
Diagnostic medicine: Biomarkers: track metabolism, etc. (perhaps with nanoparticles)	In vivo animal
Geomicrobiology: High resolution (micron scale) elemental mapping in 3-dimensions of complex and heterogeneous environmental matrices that include microbial cells and mineral particles over scales of cubic millimeters to probe, for example, redox transitions at interfaces. Need mainly for higher molecular weight elements: metals & radionuclides (Fe, Mn, U, Tc, Pu, Cr, Hg, As, Se).	Yes: elemental mapping down to 0.5 microns with current sources; improvement in optics and source could yield 50 nm resolution in future; hydrated samples will require cryo-cooling; can also do spectroscopic mapping for oxidation state information, but time-consuming with current sources and detectors; 3D tomographic mapping is also an heroic effort today (time-consuming), but brighter sources and better detectors will improve this technique dramatically.
Geomicrobiology: Ultra-high resolution (nanometer scale) microscopy, elemental mapping and spectroscopy (i.e., XANES[12], EXAFS[13]) to resolve metal and radionuclide contaminant speciation (oxidation state, bonding environment etc.) and mineral structure within microbial cells and at microbe-mineral interfaces. High resolution spectroscopies needed for speciation to probe biogeochemical transformation mechanisms and predict element reactivity and behavior. (Ideally, one would like to be able to do this on hydrated samples).	Yes: same comments as above; typical resolution today is >300 nm, but improvements in focusing optics are expected to improve resolution to <50 nm in the future with bright source.
Microbial ecology: Interactions between macro- and microorganism interactions (commensalism, symbiosis, for example)	Yes, same comments as above.
Geomicrobiology: Simultaneous information on microbial community composition (phylogeny of individuals) using FISH[14], etc. and chemistry of plant, soil, aquatic organic matter being processed. Possible stable isotope (^{13}C, ^{15}N) applications to track specific compounds into cells & various organic fractions?	Possible: micro-XRF for elemental mapping and speciation has been combined with epifluorescence
Microbiology: Run small animal models (e.g., flies, worms, zebrafish, medaka) variously colonized or not with genetically different intestinal microbes to see what difference the mercury metabolizing genotype of the commensals makes on the distribution and speciation of Hg from the diet or environment, for understanding what Hg (and other toxic metals) are	Yes: elemental mapping down to 0.5 microns with current sources; improvement in optics and source could yield 50 nm resolution in future; hydrated samples will require cryo-cooling; can also do spectroscopic mapping for oxidation state information, but time-consuming with current sources and detectors; 3D tomographic mapping is

[12] X-ray Absorption Near Edge Structure
[13] Extended X-ray Absorption Fine Structure
[14] Fluorescence in-situ Hybridization

doing to eukaryote organisms in general terms and also specifically how toxic metals act in perturbing essential metal storage and trafficking.	also a heroic effort today (time-consuming), but brighter sources and better detectors will improve this technique dramatically.
Microbiology: Role of viruses. Host-pathogen interactions. Phage. Eukaryotic cell-virus interactions.	Same as above
Microbiology: Structure-function of extracellular polymeric materials. Spatial distribution, interactions with metals and radionuclides.	Same as above, plus potential for combination with micro FTIR.
Microbiology: Applications for extreme environments. Microbial dynamics at high temperature that impact national biofuels initiatives. Hydrocarbon processing, hydrogen production under aerobic/ anaerobic conditions.	Same as above
Microbiology: To link or couple in situ hybridization microscopy techniques along with other imaging methods	Possible: micro-XRF has been combined with epifluorescence
Plant biology: To obtain three-dimensional structure of any tissue or tissue system with at least 1 μm resolution (to resolve shapes of individual cells).	Yes for metals
Plant biology: To overlay three dimensional native structure at both low and high resolution with information about molecular composition (e.g. proteins, polysaccharides, lipids, etc.), intercalation of ions into structure, etc. (structures detected must have phase or chemical contrasts against their surroundings).	Yes for metals and metal redox at 10-100 nm
Plant biology: Map specific molecules with tagged and engineered metal probes	Yes, depending on probe development and in vivo expression
Plant biology: To quickly and routinely generate at greater than 10 nm resolution three dimensional native structure (including macromolecular complexes) for any cell type of the plant. Higher resolution through heroic efforts	Yes for metals with sections

Attendees for the National Synchrotron Light Source II Imaging and Spectroscopy Workshop

Washington, DC
September 11–12, 2008

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