Argonne NATIONAL LABORATORY

... for a brighter future

Life Science Team Report
APS Renewal

LS Team

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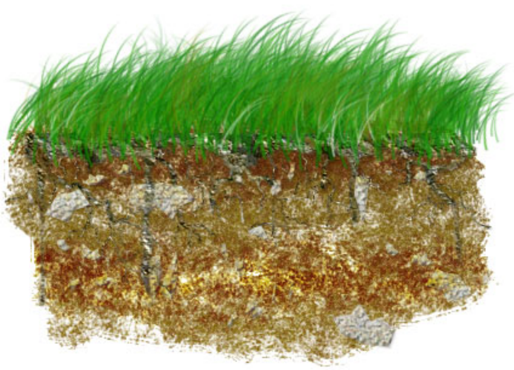


U.S. Department of Energy

THE UNIVERSITY OF CHICAGO

A U.S. Department of Energy laboratory managed by The University of Chicago

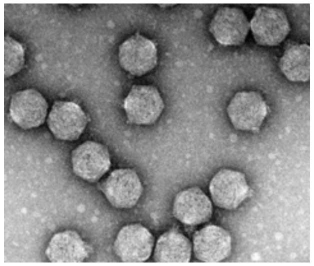
- Macromolecular crystallography has had a huge impact on the biological sciences, leading to many significant insights into the function of a myriad of biological systems.
- The impact of other x-ray techniques on biology is developing more slowly due to factors including limited availability of appropriate experimental facilities.
- America has been slow to develop facilities dedicated to scattering or imaging of biological specimens, and synchrotrons in Europe and Asia have a considerable advantage in these areas.

Summary

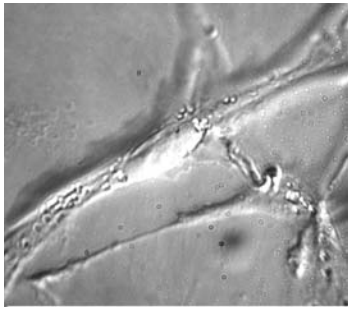
- ***Life science/synch community usage has been growing rapidly in recent years***
  - ***driven by advances in x-ray science***
  - ***need for new approaches to solving the multitude of questions arising from the explosion of biological information coming out of –omics projects***
  - ***growing realization of capabilities of x-ray sources***
- ***Slow adaptation of x-ray techniques [beyond crystallography]***
  - ***high perceived energy barrier for utilization of x-ray facilities***
  - ***need for specialized software to analyze/interpret data***
  - ***perception that light and electron imaging is generally superior***
- ***Life scientists often share synchrotron facilities with physical scientists***
  - ***increases perceived energy barrier for utilization of methods***
  - ***utilize multi-purpose beamlines that are a compromise between needs of different communities using them***
  - ***this has limited the efficiency of experiments and impact of the work.***
- ***Development of dedicated (single technique) beam lines [optimized for life science needs] will be required for further progress.***
- ***Impact of experiments enabled by this development will be substantial and wide spread.***

- A variety of factors make this an important time for development of new x-ray facilities for the study of biological materials.
  - First, development of novel x-ray optics is paving the way for development of the bio-nanoprobe (BNP) [among others]
  - Second, convergence of computational and experimental techniques is greatly enhancing the amount of information that can be extracted from imaging and scattering data.
  - Third, development of the field of bio-nanotechnology as an interface between inorganic and organic sciences is providing new tools for probing intracellular structures (contrast agents – molecular imaging).
- Such technological advances have increased the sensitivities, spatial and temporal resolutions of x-ray imaging of biological structures by orders of magnitude.

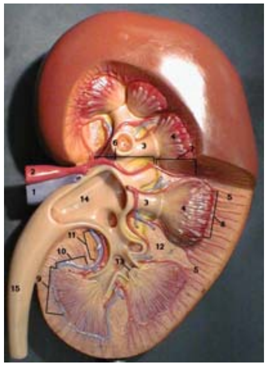
Imaging Biological systems at multiple length scales



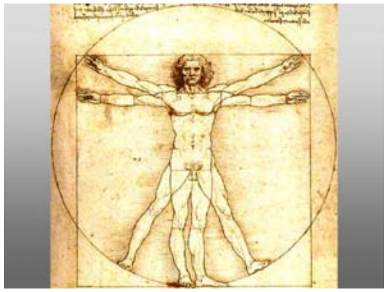
Scanning microscopies



scattering



full field microscopies



Synchrotron Radiation can impact our understanding of biological systems at all length/time scales

Science Drivers are everywhere…

- *Evolutionary biology*
  - *How did the two predominating biomineralization systems (calcium carbonate, calcium phosphate) develop, in particular, the apatite-collagen bio-nanocomposite system in bone?* [increased penetration; resolution]
- *Quantitative analysis of energy-supply systems (metabolism)*
  - *How do birds and insects achieve one-way flow through their valve-less respiratory systems?* [time and spatial resolution – penetration]
- *Environmental stress tolerance*
  - *Extreme environmental stresses are always associated with major structural changes*
- *Tissue mechanics*
  - *Nanocomposite-based biological support structures (e.g. bone, tendons, teeth, chitin, xylem, phloem, spider silk) exhibit combined strength and elasticity far exceeding engineered materials.*

Synchrotron Radiation can impact our understanding of biological systems at all length/time scales

- *In-vivo, longitudinal biomedical imaging*
  - *Effects of asthma drugs on tracheal pathways and surrounding muscles in mouse models may be observed quantitatively, leading to better numerical models of these processes and to more effective treatment for asthma and other airway constrictive diseases.*
- *Metals in biology*
  - *Understanding of the redistribution of metals accompanying the development of different degenerative diseases (such as Alzheimer's Disease, Lou Gehrig's Disease, and others) is limited even though metals and metal chelators represent an increasingly important class of drugs*
- *Bio-nanotechnology*
  - *Observing nanovectors (hybrid organic-inorganic particles) "in action" at the site of their activity in cells will yield critical data that can be used to streamline and guide nanovector design*
- *Fibrous biomaterials*
  - *Molecular architecture of ligno-cellulose (biomass)(energy applications); connective tissue; muscle contraction (biomedical applications)*
- *Molecular structure and dynamics*
  - *Scattering approaches to characterizing molecular structure and dynamics to provide insight into macromolecular function; macromolecular assemblies; membrane protein structure and function*

What is the role of copper in angiogenesis?

- Angiogenesis – the growth of new blood vessels – is critical to tumor growth – which cannot take place in the absence of metabolites supplied by the blood stream
- Depleting copper disrupts angiogenesis, making both it and its macromolecular partners important drug targets in cancer therapy
- Can we learn anything about the role of copper in angiogenesis using x-ray imaging?

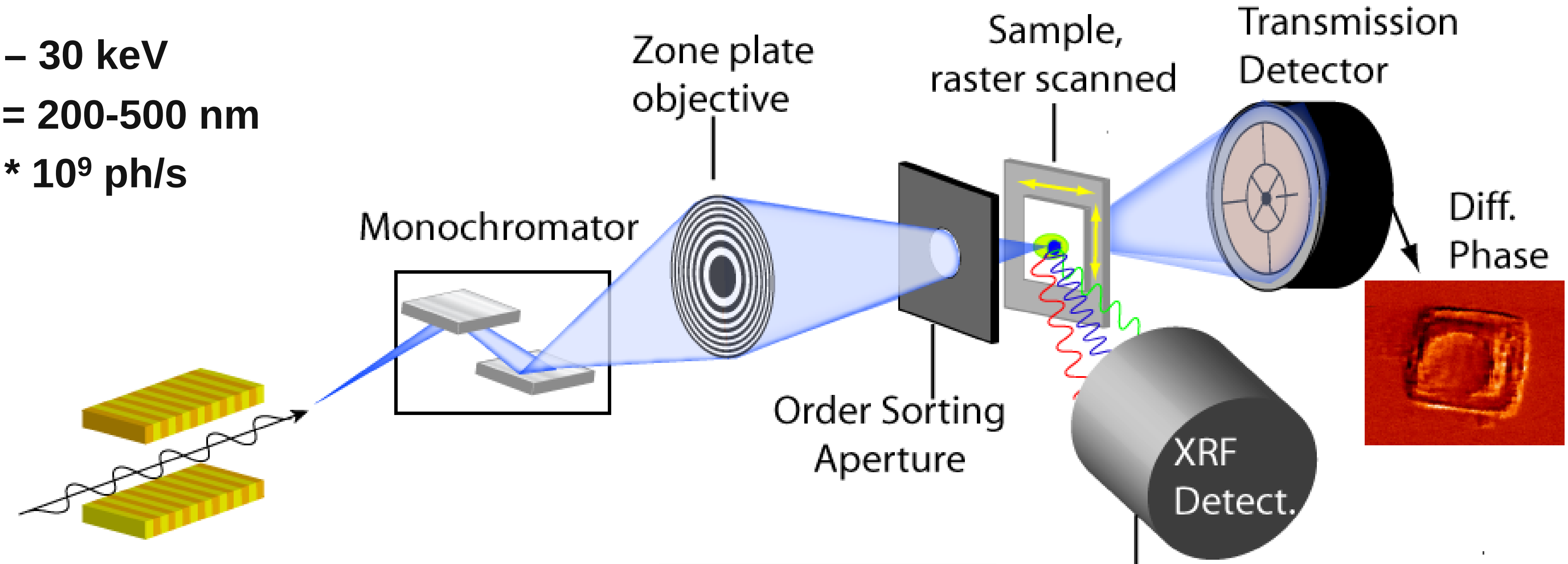
Schematic of a Hard X-Ray Microprobe

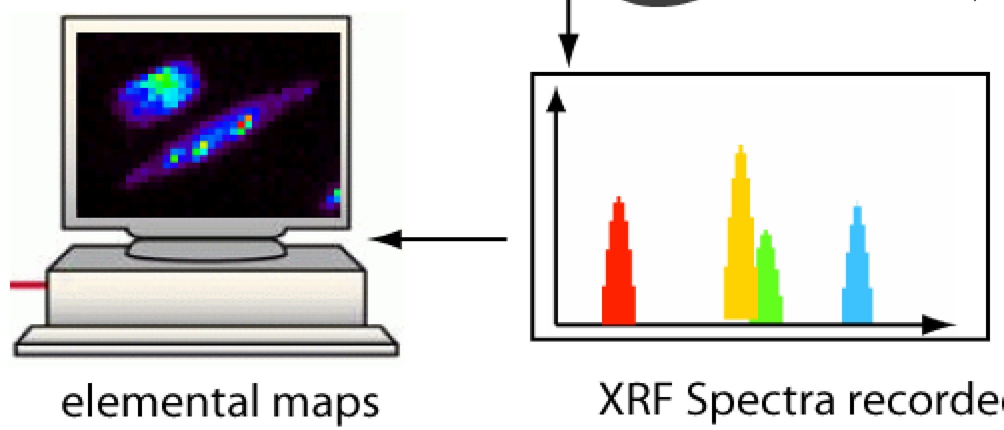
schematic NOT to scale !!

5 – 30 keV

δ = 200-500 nm

5 * 10^9 ph/s

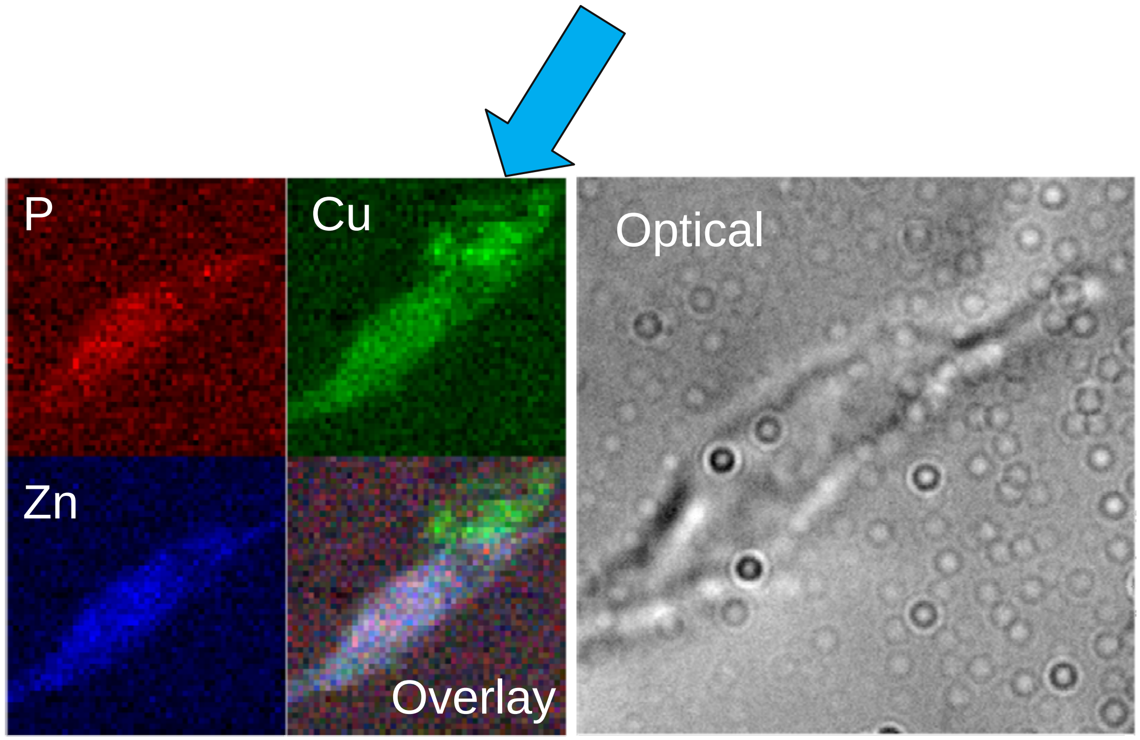




- stepscan sample through focused X-ray beam
- record full XRF spectrum at each scan point, using an energy dispersive detector, at 90°

- Straightforward quantification: compare specimen counts/spectra to calibration curve, to quantify to area density

Cells Forming Blood Vessels Send Their Copper to the Edge



"X-ray fluorescence microscopy reveals large-scale relocalization and extracellular translocation of cellular copper during angiogenesis"

L. Finney, *et al*, PNAS 104(7): 2247-52. (2007)

How could improvements to the APS further inform this work?

- Enhanced resolution – determine precise location of copper secretion from cells
- Enhanced sensitivity – determine full range of copper distribution in extracellular media
- Increased scan speed – increased field of view/number of pixels
- Enhanced penetration – tissue section studies ?

How can we design nanoconjugates that modulate cellular function?

- Synthetic DNA can be conjugated to inorganic nanoparticles
- DNA will target genomic sequences complimentary in sequence
- Can we determine the location of these genomic sequences using nanoconjugates with specific sequences?
- Can we use these nanoconjugates to modulate biological functions?

DNA-TiO_2 nanoconjugates targeting of genomic DNA

overla
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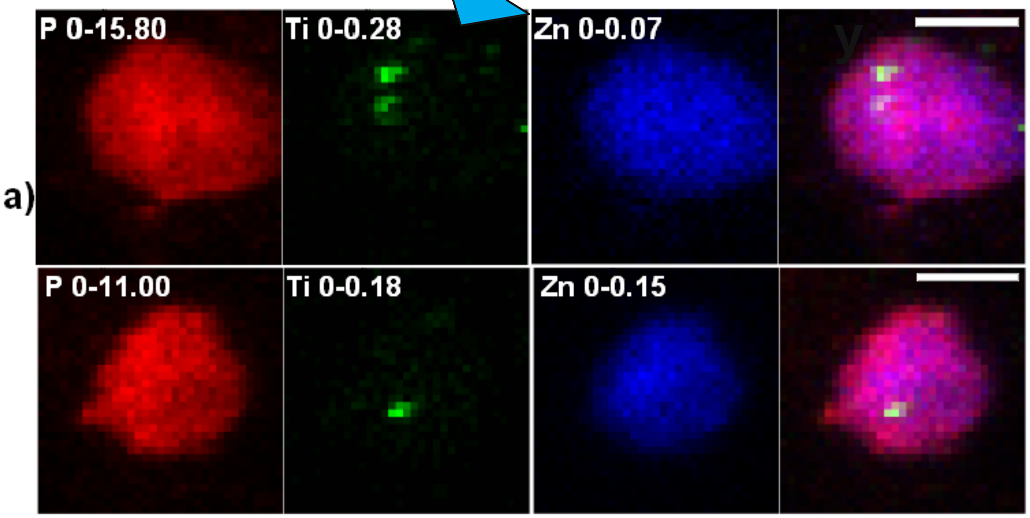
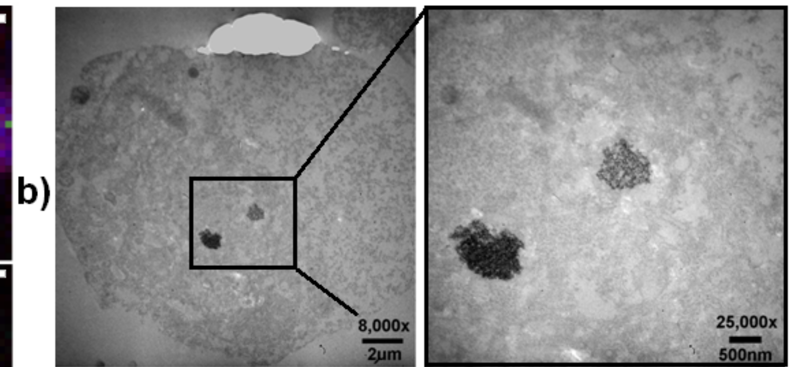
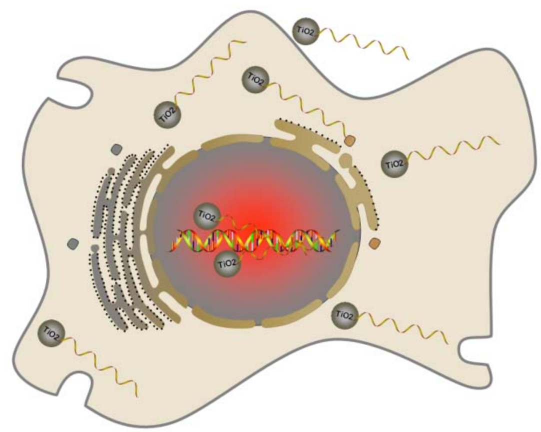




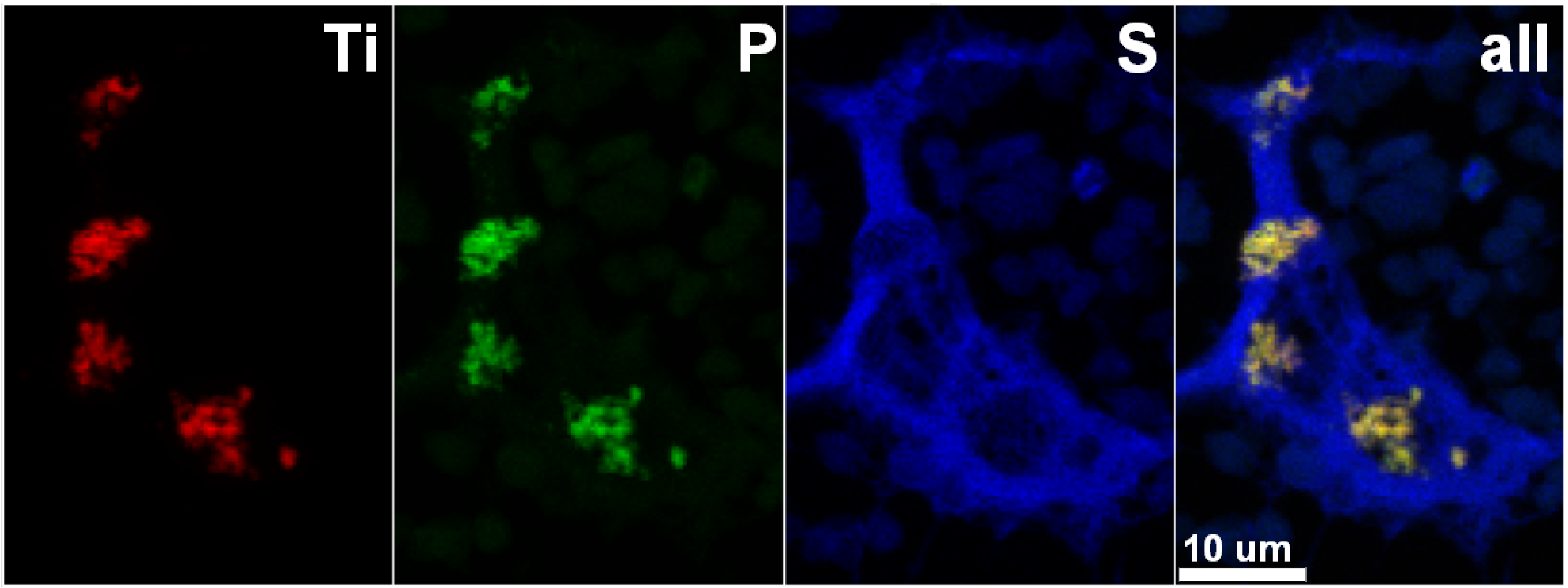


Nucleolus – genomic DNA targeting

XFM (left) and TEM (right) of TiNC treated cells.

Paunesku et al. Nano Letters 2007 7(3):596-601

A single cell of the VX2 tumor tissue with TiNCs imaged by XFM



Titanium is a component of the nanoparticles; phosphate coats large portion of the nanoparticle surface and is present in cellular nuclei. Sulfur is present in viral and cellular proteins, outlining the cytoplasm.

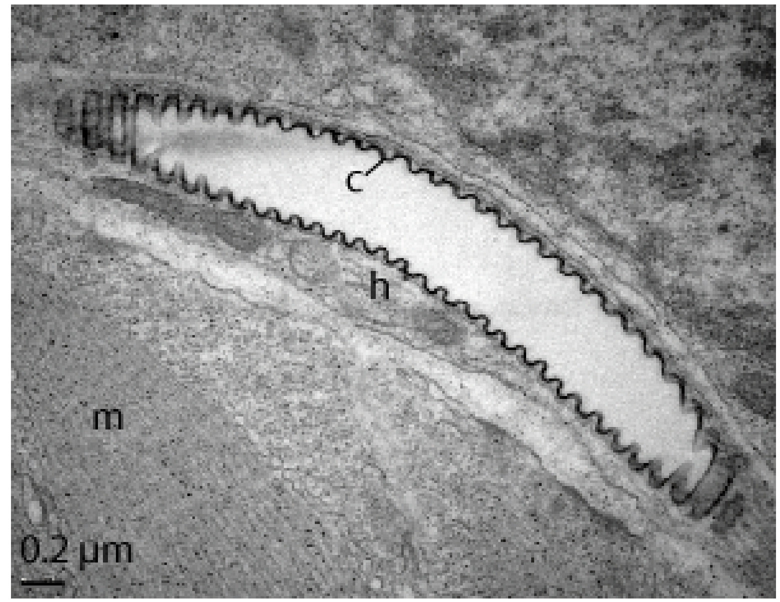
How could improvements to the APS further inform this work?

- Increased spatial resolution
- Increased speed of scans (larger field of view/number of pixels)

How can x-ray imaging answer fundamental questions about insect tracheal system physiology?

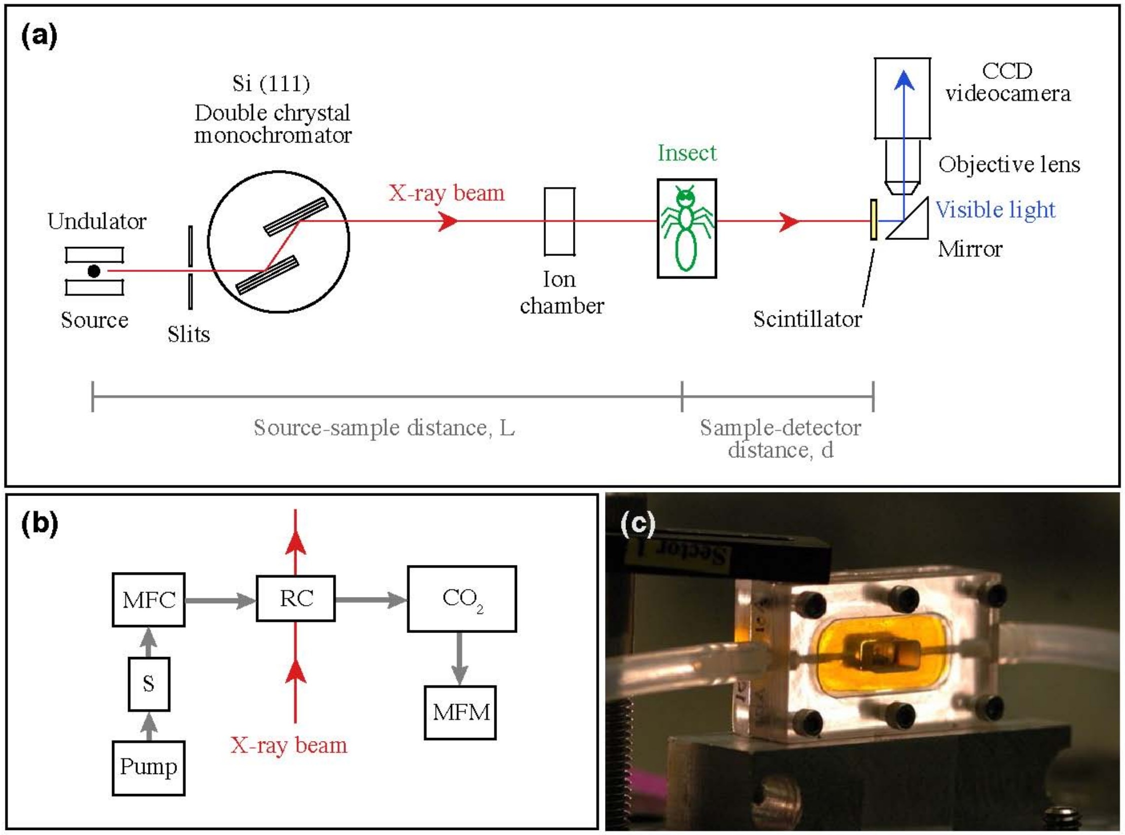
- How are oxygen and CO_2 transported?
  - (diffusion vs. convection; how distributed among tissues?)
- What are mechanisms by which gas exchange is regulated? How do insects achieve 100x variation in gas exchange?
  - (relative importance of spiracles, varied convection, fluid level in tracheoles?)
- How is tracheal structure regulated?
  - (what pathways control morphological development and plasticity?)
- Does the tracheal system limit insect size?
  - (or is it possession of an exoskeleton that keeps insects small?)

C. 2nd instar, 31,000x, class I trachea (tracheole)



Light microscopy images of tracheae in grasshopper leg.

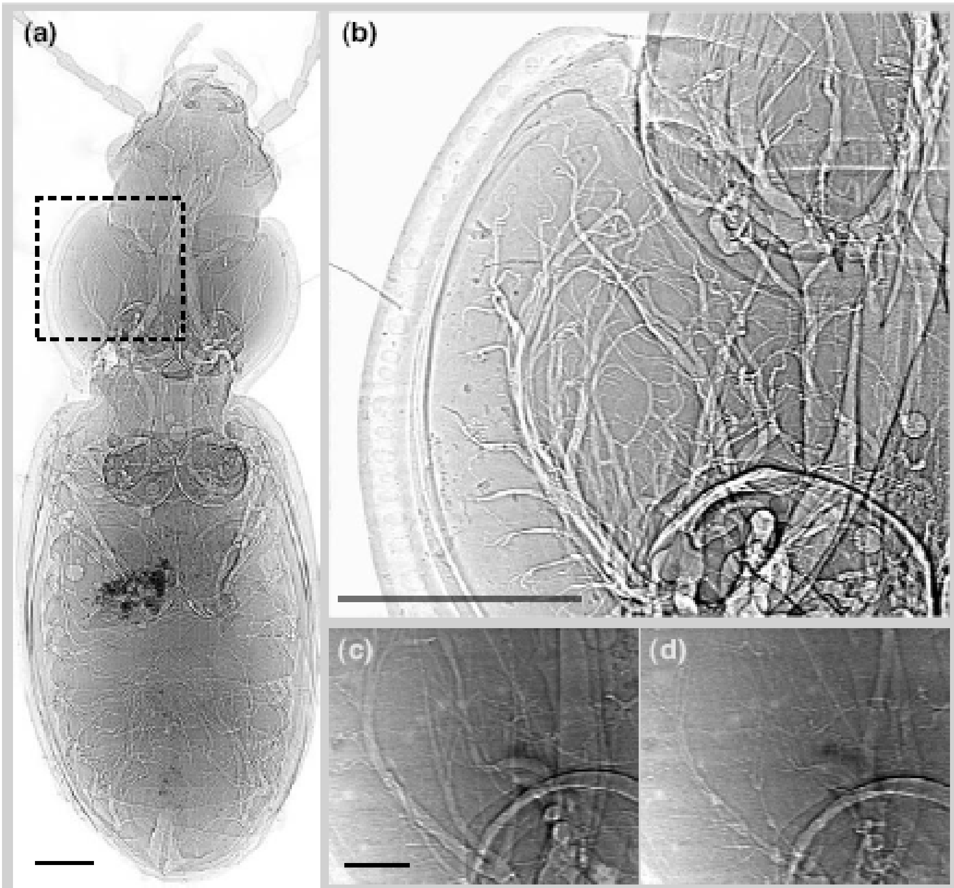
Transmission electron microscopy image of grasshopper tracheoles

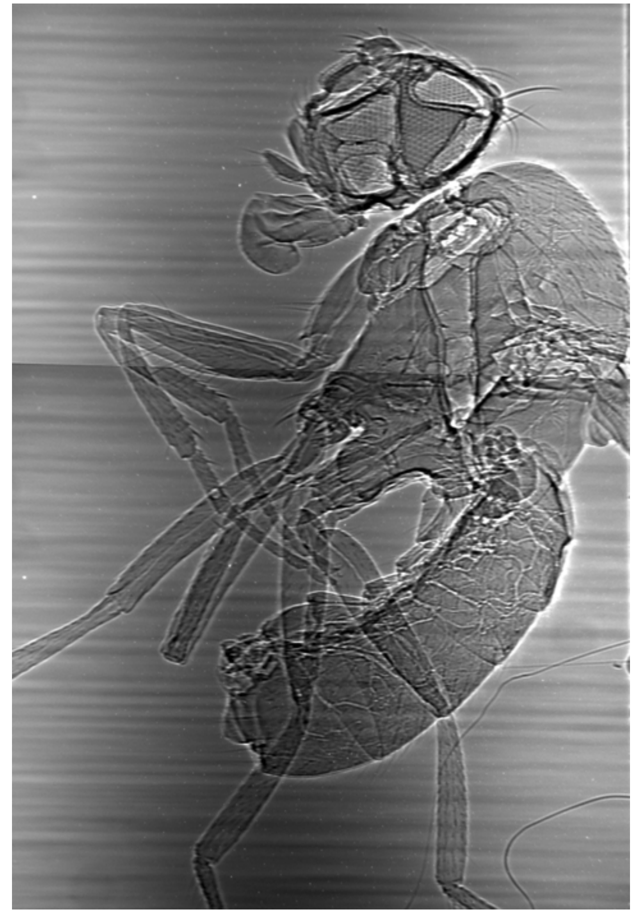


XOR-32ID

FOV:
2.4 x 3. 2 mm

Socha, J.J., Westneat, M.W., Harrison, J.F., Waters, J.S., and Lee, W.K. 2007. Real-time phase-contrast x-ray imaging, a new technique for the study of animal form and function. BMC Biology 5:6.





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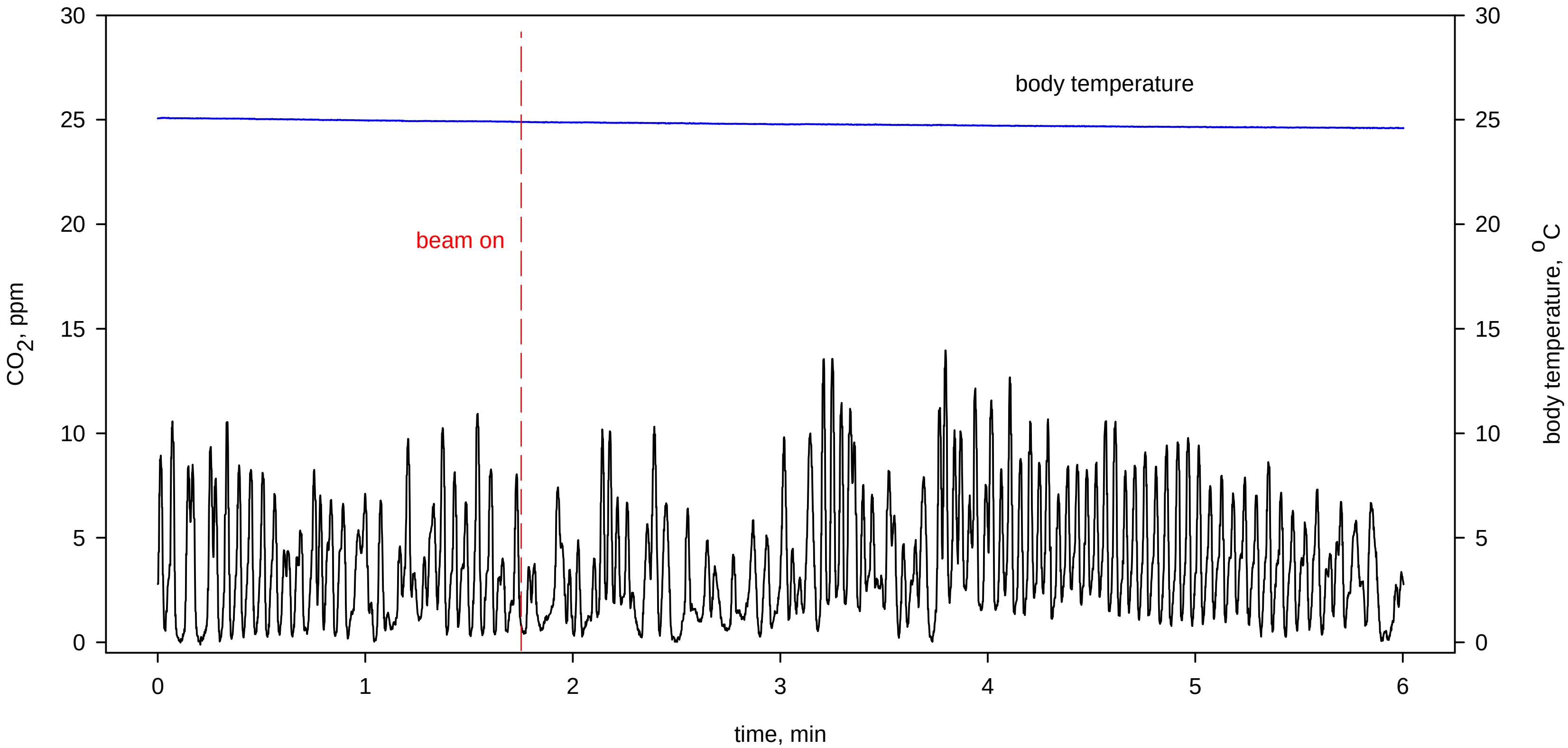
Fluctuating gas exchange is common

CO_2 emission by a grasshopper



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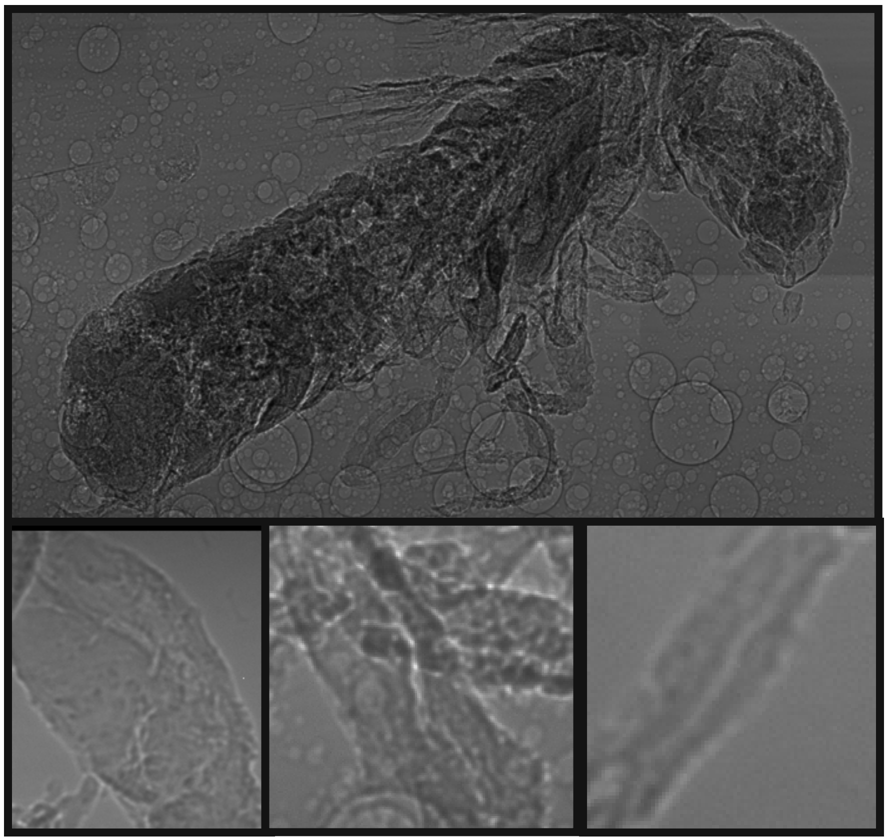
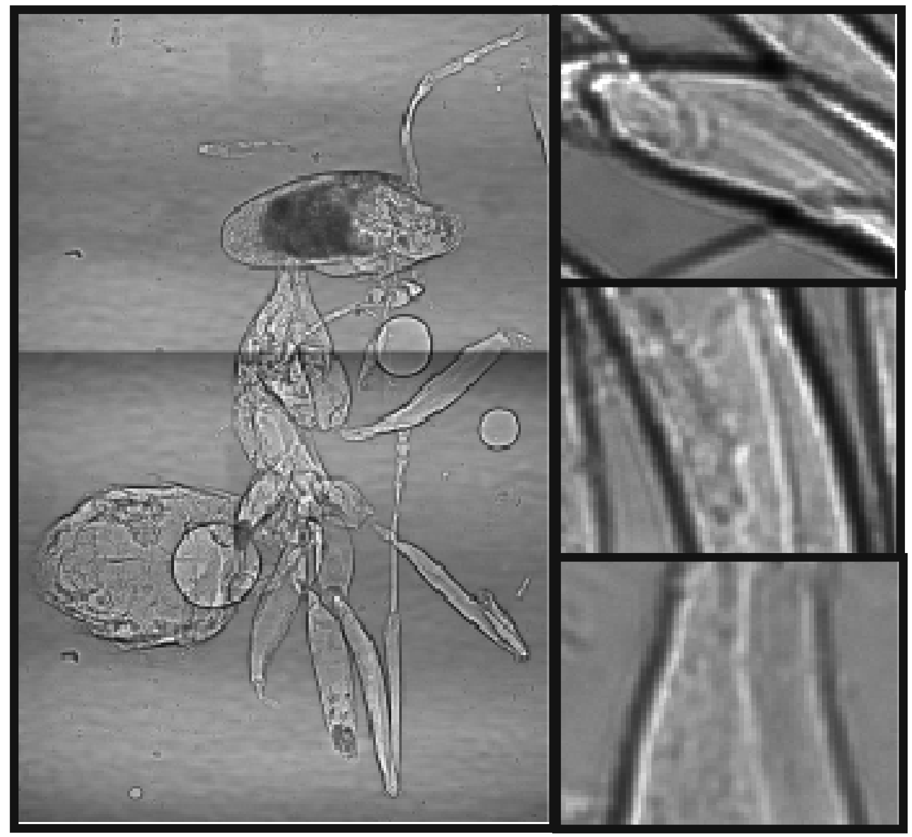
Fossil tracheae can be imaged for insects in amber, suggesting that tracheal diameter/leg length could provide a proxy for assessing historical atmospheric oxygen levels



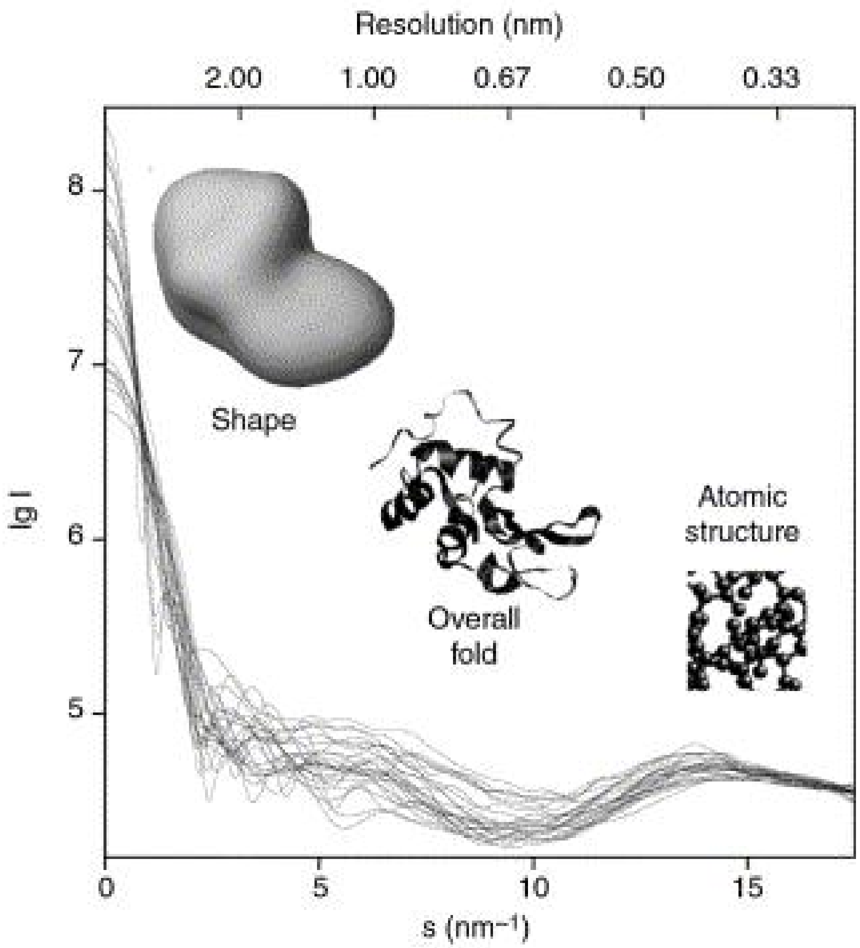


How could improvements in APS impact these studies?

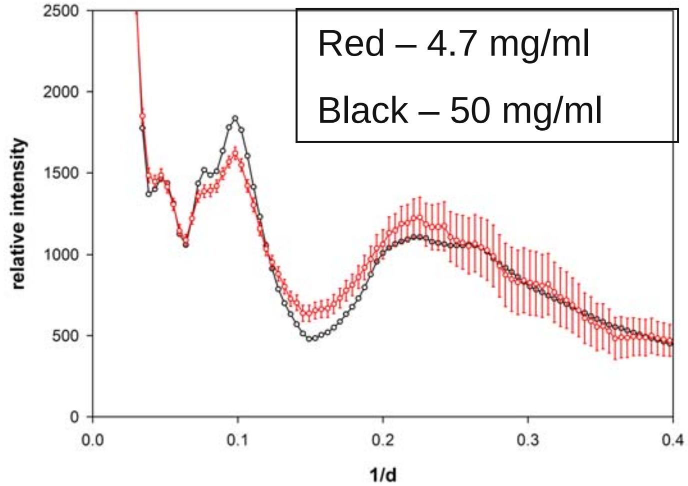
- Larger filed of view – studies of larger samples (e.g. biomedically important animal models like mice)
- Enhanced resolution – smaller structural changes/features
- Enhanced sensitivity – e.g. fluid flow without contrast agents
- Enhanced penetration – larger samples

What can we learn about the structure of large macromolecular complexes that cannot crystallize?

- SAXS
- Software to obtain shape of macromolecular structures in solution is greatly enhancing the utility of solution scattering approaches to the problem of macromolecular structure and function
- WAXS may contain adequate information to provide a short list of possible folds for a protein

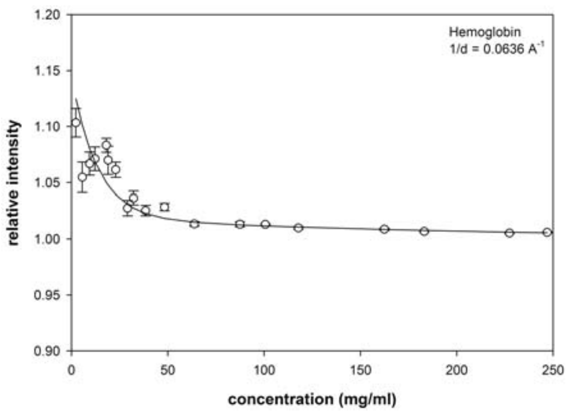


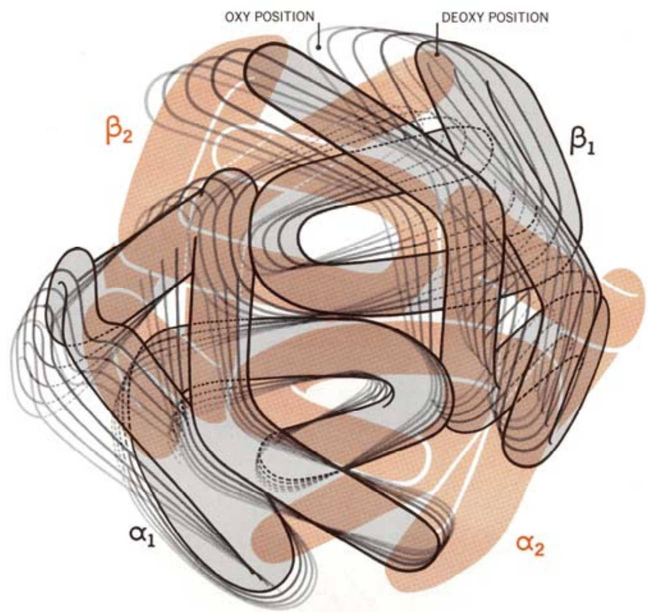
Dynamics of hemoglobin in solution



Change of intensity as a function of concentration demonstrates that high concentrations damp out protein breathing

Increased breathing at low concentrations is comparable to the structural changes that hemoglobin undergoes during allosteric changes





What can APS enhancements do for use of SAXS/WAXS for study of macromolecular structures?

- HT specimen changing (using flow chambers to minimize radiation damage)
- Detectors for TR SAXS/WAXS
- Dedicated beam line(s) to maximize efficiency
- Tunability for A-SAXS and A-WAXS (MADMAX) studies

Capabilities needed

- *Bionanoprobe*
  - *x-ray fluorescent imaging – current resolution ~0.1 m; future resolution ~ 30 nm.*
- *Phase Imaging Capabilities*
  - *Need increased field of view for whole animal studies (model organisms – rodents); whole organ/tissue*
  - *Need increased spatial and temporal resolution*
- *Coherent Diffraction Imaging*
  - *Enhanced coherence fraction to impact imaging capabilities*
  - *Used for imaging ot crystalline material in situ;*
- *SAXS/WAXS/Fiber*
  - *Stable, small tunable source;*
- *Cryopreservation*
  - *Critical for minimizing rad damage*
- *Detectors*
  - Fast detectors for TR studies

Life Science Breakout Session

- Stuart Stock (Northwestern)
  - 3D x-ray imaging of structures at multiple spatial scales
- Andrew Resnick (Case)
  - What would I do with the APS?
- Stephen Baines (Stony Brook)
  - Biological causes and climatic consequences of variability in the elemental composition of marine microbes
- Kendra Greenlee (North Dakota State)
  - Using synchrotron imaging to visualize insect tracheal system structure and function
- Thomas O'Halloran (Northwestern)
  - Subcellular Transition Metal Trafficking in Infectious Disease, Cancer and Neurological Disorders
- Mark R. Chance (Case)
  - Interdisciplinary Biophysics at Synchrotron Sources: Opportunities and Challenges
- Lee Makowski (Argonne)
  - SAXS/WAXS for structure and dynamics studies of macromolecules