Soft x-ray spectromicroscopy: chemical imaging in cells

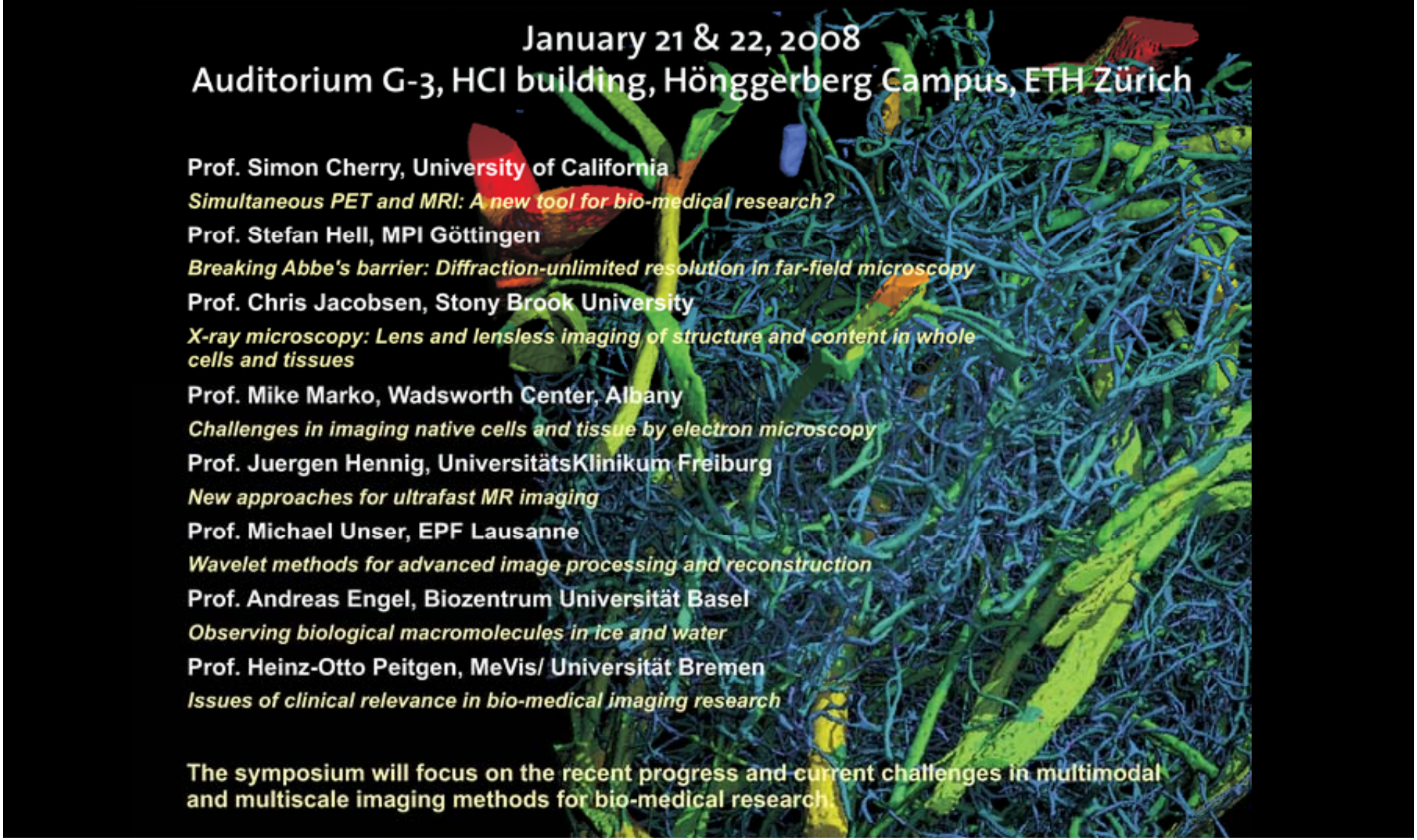
Chris Jacobsen

Stony Brook

Zurich Center for Imaging Science and Technology (CIMST)

Imaging: Pushing the Limits in Bio-medical Research

January 21 & 22, 2008
Auditorium G-3, HCI building, Hönggerberg Campus, ETH Zürich



Prof. Simon Cherry, University of California
Simultaneous PET and MRI: A new tool for bio-medical research?

Prof. Stefan Hell, MPI Göttingen
Breaking Abbe's barrier: Diffraction-unlimited resolution in far-field microscopy

Prof. Chris Jacobsen, Stony Brook University
X-ray microscopy: Lens and lensless imaging of structure and content in whole cells and tissues

Prof. Mike Marko, Wadsworth Center, Albany
Challenges in imaging native cells and tissue by electron microscopy

Prof. Juergen Hennig, UniversitätsKlinikum Freiburg
New approaches for ultrafast MR imaging

Prof. Michael Unser, EPF Lausanne
Wavelet methods for advanced image processing and reconstruction

Prof. Andreas Engel, Biozentrum Universität Basel
Observing biological macromolecules in ice and water

Prof. Heinz-Otto Peitgen, MeVis/ Universität Bremen
Issues of clinical relevance in bio-medical imaging research

The symposium will focus on the recent progress and current challenges in multimodal and multiscale imaging methods for bio-medical research.

Scientific Need

Complementary microscopies:

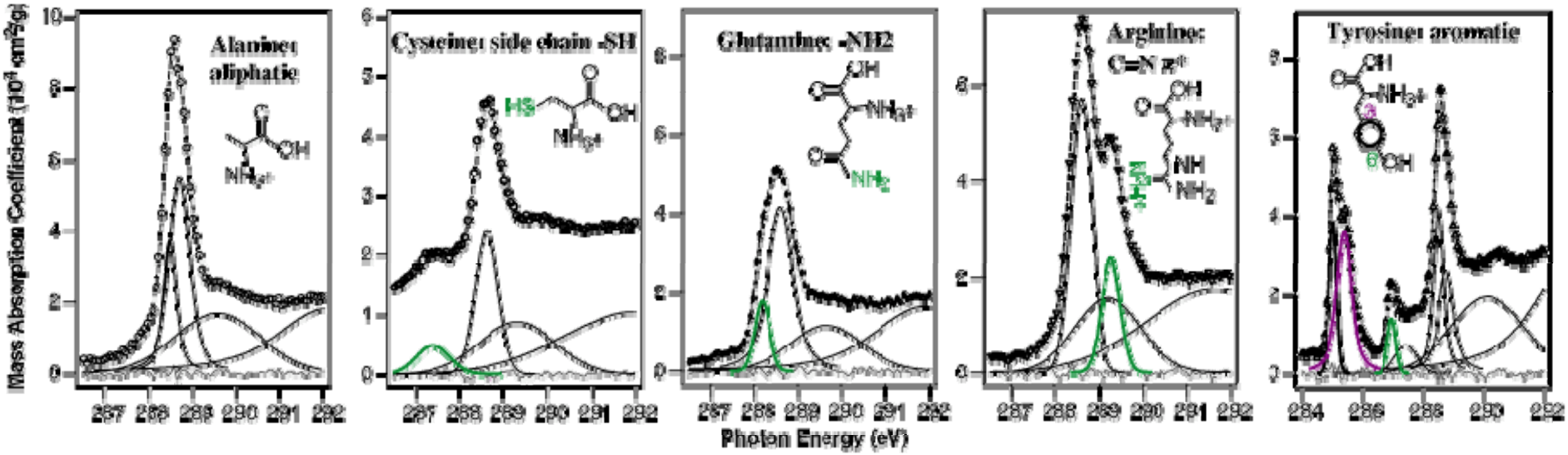
- Fluorescence microscopy: can tag some *pre-selected* proteins. Single molecule detection in some cases; very powerful!
- Tomography can reveal overall density.
- Spectromicroscopy can look at *overall* chemical organization. Organic functional group resonances in infrared, ultraviolet (*e.g.*, CARS), soft X ray.

Soft x-ray spectromicroscopy:

- Zone plate focus to 10-30 nm, multiple images over a spectroscopically interesting energy range.
- Data is complex (~10^5 XANES spectra!), so sophisticated analysis methods are required.
- Specimen must be stable over 10-100 images: cryo required for best results.
- Requires fast scanning.
- Can be combined with tomography for 3D chemical speciation! Demonstrated by Johansson *et al., J. Synch. Rad.* **14**, 395 (2007) at ALS.

C-XANES of amino acids

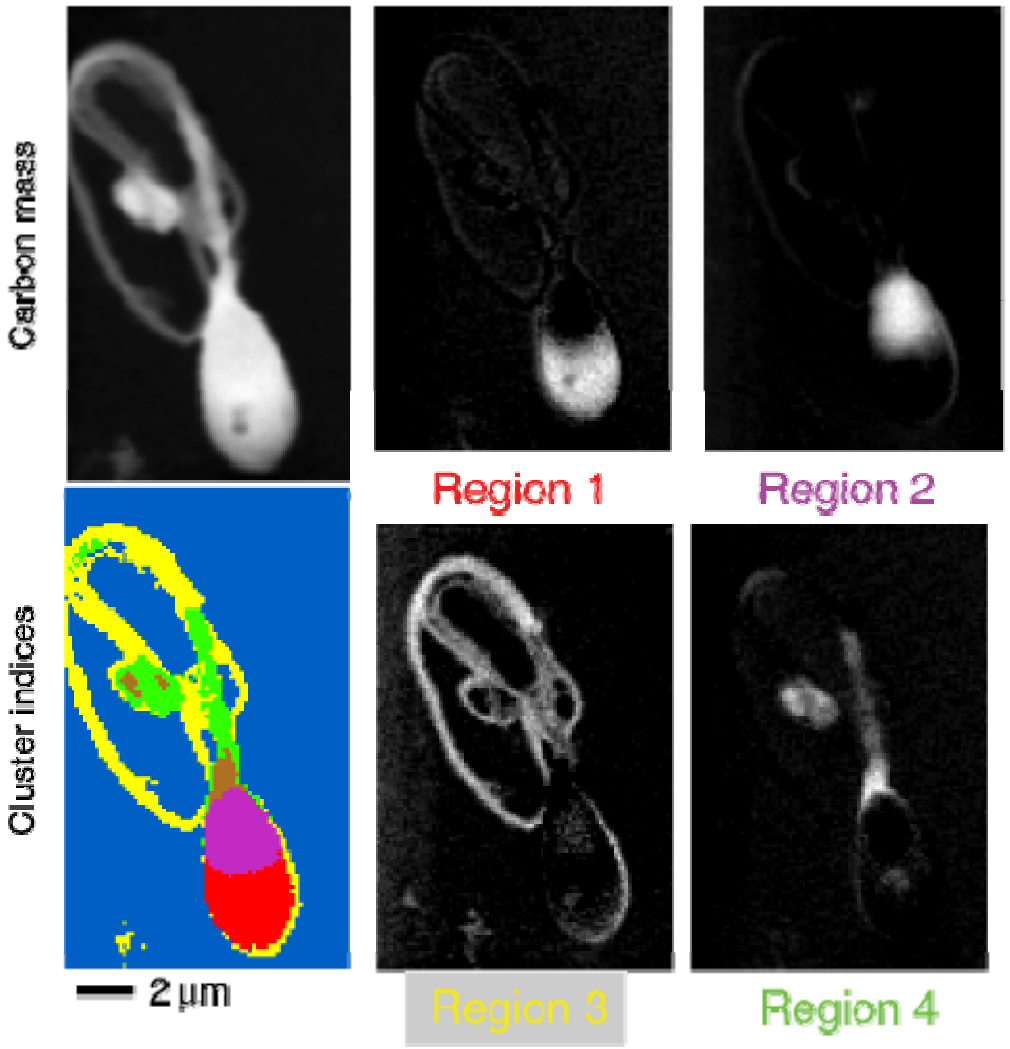
- K. Kaznacheyev *et al.*, *J. Phys. Chem.* **A 106**, 3153 (2002)
- Experiment: K. Kaznacheyev *et al.*, Stony Brook (now CLS)
- Theory: O. Plashkevych, H. Ågren *et al.*, KTH Stockholm; A. Hitchcock, McMaster



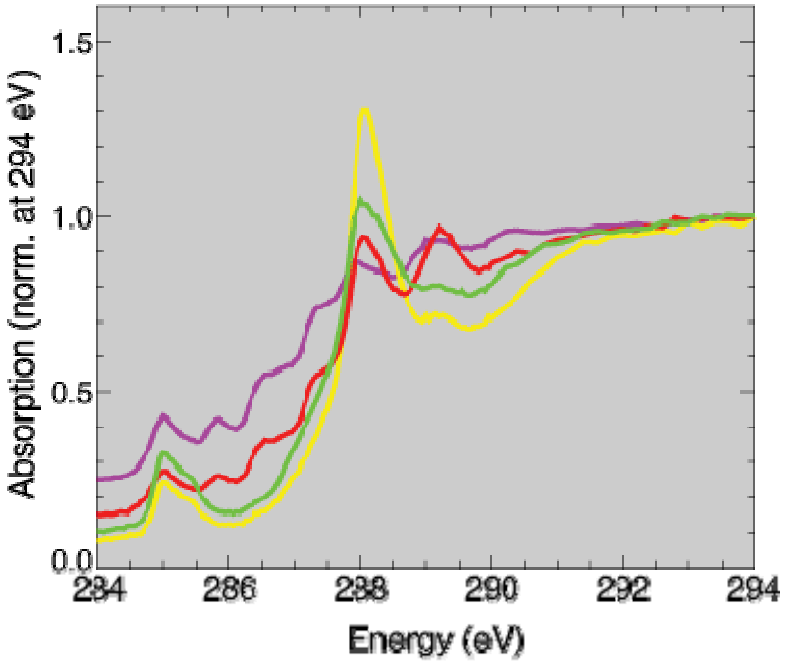
Polymers: see e.g., Dhez, Ade, and Urquhart, *JESRP* **128**, 85 (2003)

Sperm analysis

Biochemical organization of sperm revealed directly from data: enzyme-rich region, DNA, mitochondria and flagellar motor, lipid

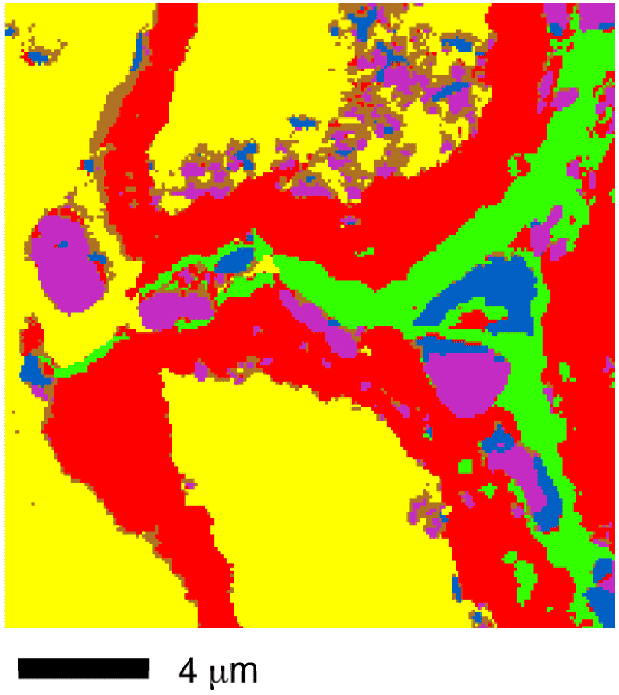


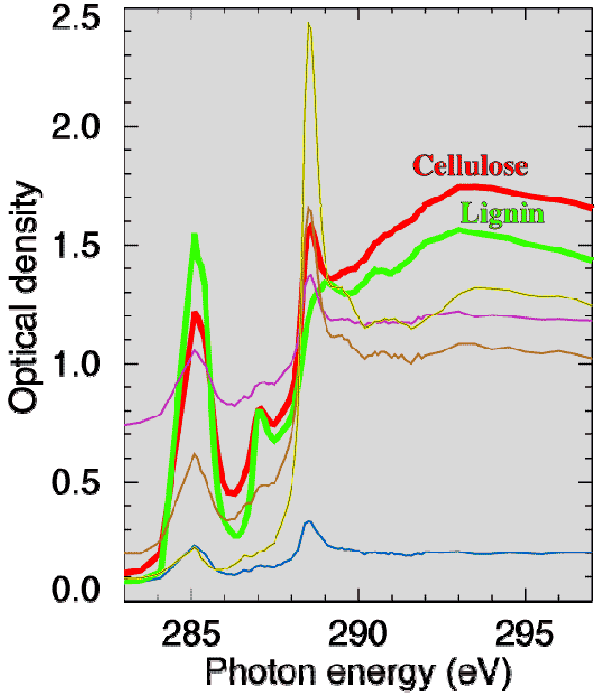
H. Fleckenstein, M. Lerotic, Y. Sheynkin *et al*., Stony Brook. Human sperm, air-dried.



Dealing with complexity

- Spectrum-per-pixel (Jacobsen *et al.*, *J. Micros.* **197**, 173 (2000) : ~10^5 spectra so can't do it by hand!
- One approach: cluster analysis, or unsupervised pattern recognition. Lerotic *et al.*, *Ultramicroscopy* **100**, 35 (2005).
- Another approach: non-negative matrix factorization. Fleckenstein *et al.*, unpublished.
- Problems are mathematically similar to Google searches, and Amazon's "If you liked that, then try this" suggestions.





Lignin and cellulose in 400 million year old chert: Boyce *et al.*, *Proc. Nat. Acad. Sci.* **101**, 17555 (2004), with subsequent pattern recognition analysis by Lerotic *et al.*, *Ultramicroscopy* **100**, 35 (2004).

User Demand

- Soft x-ray spectromicroscopy under high demand at NSLS; *very* high demand at ALS; new facilities at SLS; under development at Shanghai, Soleil, Diamond, Australia, BESSY II…
- Present NSLS bio users include Haraszti, Grunze *et al.* (EMBL/Heidelberg); Di Masi *et al.* (NSLS)…
- Related to biology, there is great potential in bioenergy research for things like mapping lignin versus cellulose (Michette *et al.*, King's College, London)…

Soft x-ray spectromicroscopy at NSLS II

- **“First 6” soft x-ray beamline:** scanning microscope as a frequent endstation.
- **Additional undulator beamline devoted to bio/soft matter/organic environmental science with cryo:** for 100% access, fixed location for complicated cryo apparatus. Requires undulator; otherwise lower performance than NSLS I! Allows for more routine use, and technique/technology development.
- **Complementary, off-line capabilities:** cryo specimen transfer for tomography using zone plates or diffraction, ultraviolet studies using CARS (Coherent Anti-Stokes Raman Spectroscopy), and EELS (electron energy loss spectroscopy) in electron microscopes.

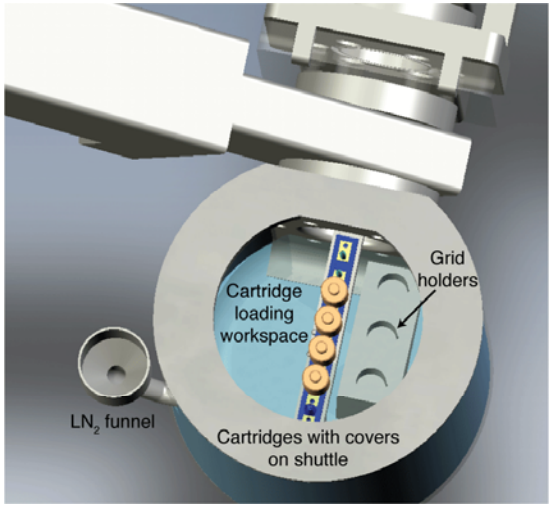
Current NSLS Programs

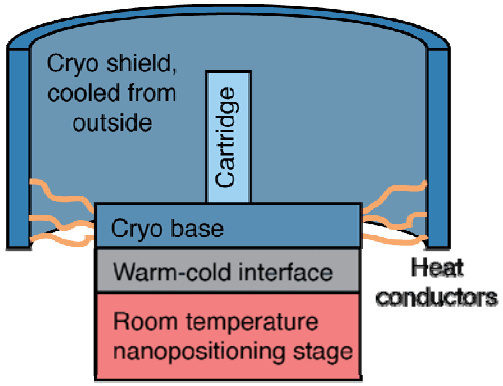
- Well-established user program at X1A, though without cryo capabilities as required for biological studies. One of two beamlines incorporates NSLS monochromator upgrade.
- Local experimenters with experience in scanning microscope instrument development; spectromicroscopy analysis methods; phase contrast imaging (at APS); cryo transfer system design (at ALS, Xradia); zone plate fabrication (but no facilities at BNL's CFN!).

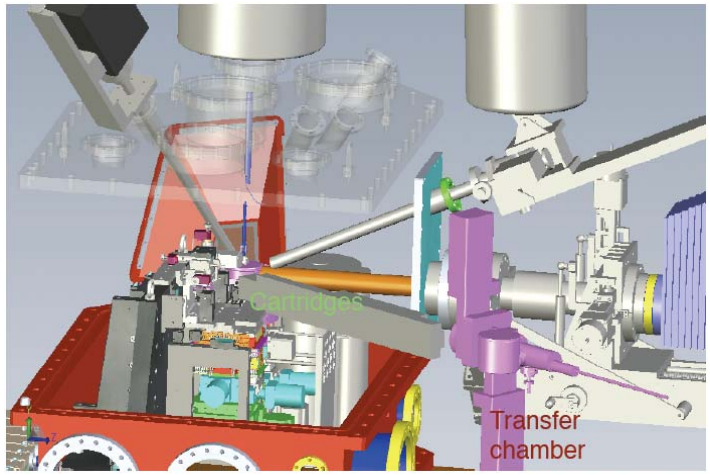
Upgraded/New NSLS Programs

- **Upgraded program:** soft x-ray cryo scanning microscope. Testbed for fast scanning as required for NSLS II, cryo specimen preparation and transfer, and phase contrast tomography. Would also serve spectromicroscopy, environmental science, soft matter studies. Directly transferable to NSLS II.
- **New program:** spectromicroscopy using cryo full-field microscope with zone plates on a monochromator beamline. 10-20x higher radiation dose, but 1000x higher throughput. Directly transferable to NSLS II.
- **New complementary program:** cryo ultraviolet CARS microscopy (lower damage so higher resolution, experience in cryo specimen preparation and handling). Regarding cryo light microscopy, see Schwartz *et al., J. Micros.* **227**, 98 (2007); Sartori *et al.*, *J. Struct. Bio.* **160**, 135 (2007).

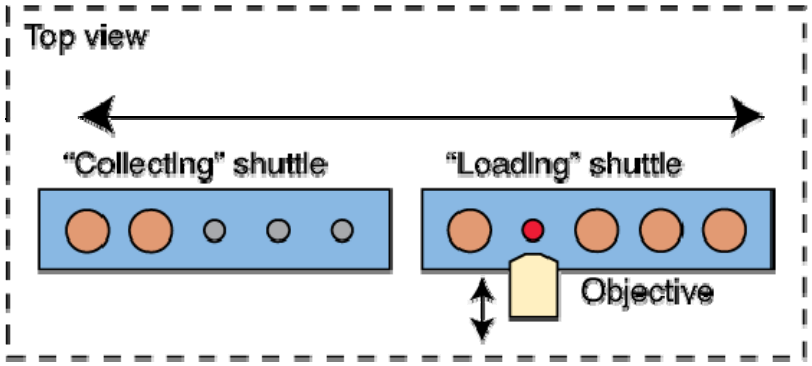
Cryo system: Xradia example

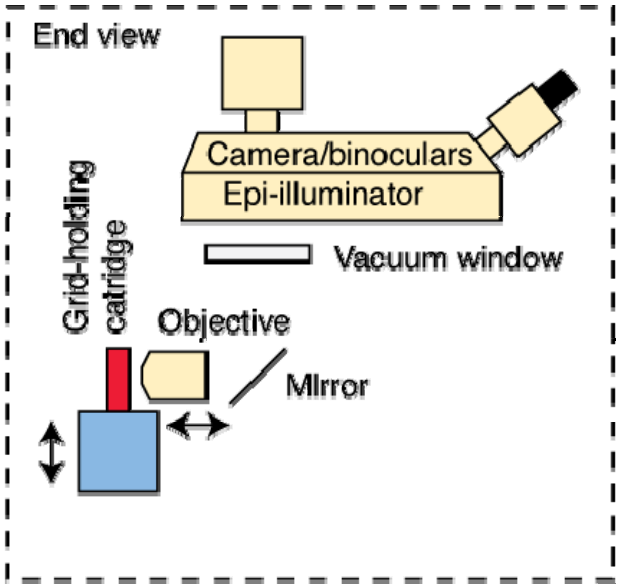






- Mount fragile grid in cartridge once.
- Transfer cartridge between visible light and various X-ray microscopes (including scanning, tomography).
- Robotic sample insertion in microscope.





Optics needs

- NSLS II has selected two optics types for R&D: multilayer Laue lenses, and Fresnel lenses.
- Multilayer Laue: hard X-rays only (absorption), not very tunable (chirped Bragg condition).
- Fresnel lenses: chromatic aberration goes like $(\text{energy})^2$, absorption limits low-energy use, have non-$2m\pi$ phase jumps as energy is tuned.
- Need for other optics for soft x-ray spectromicroscopy! High resolution mirror optics and/or zone plates.

Cryo specimen preparation

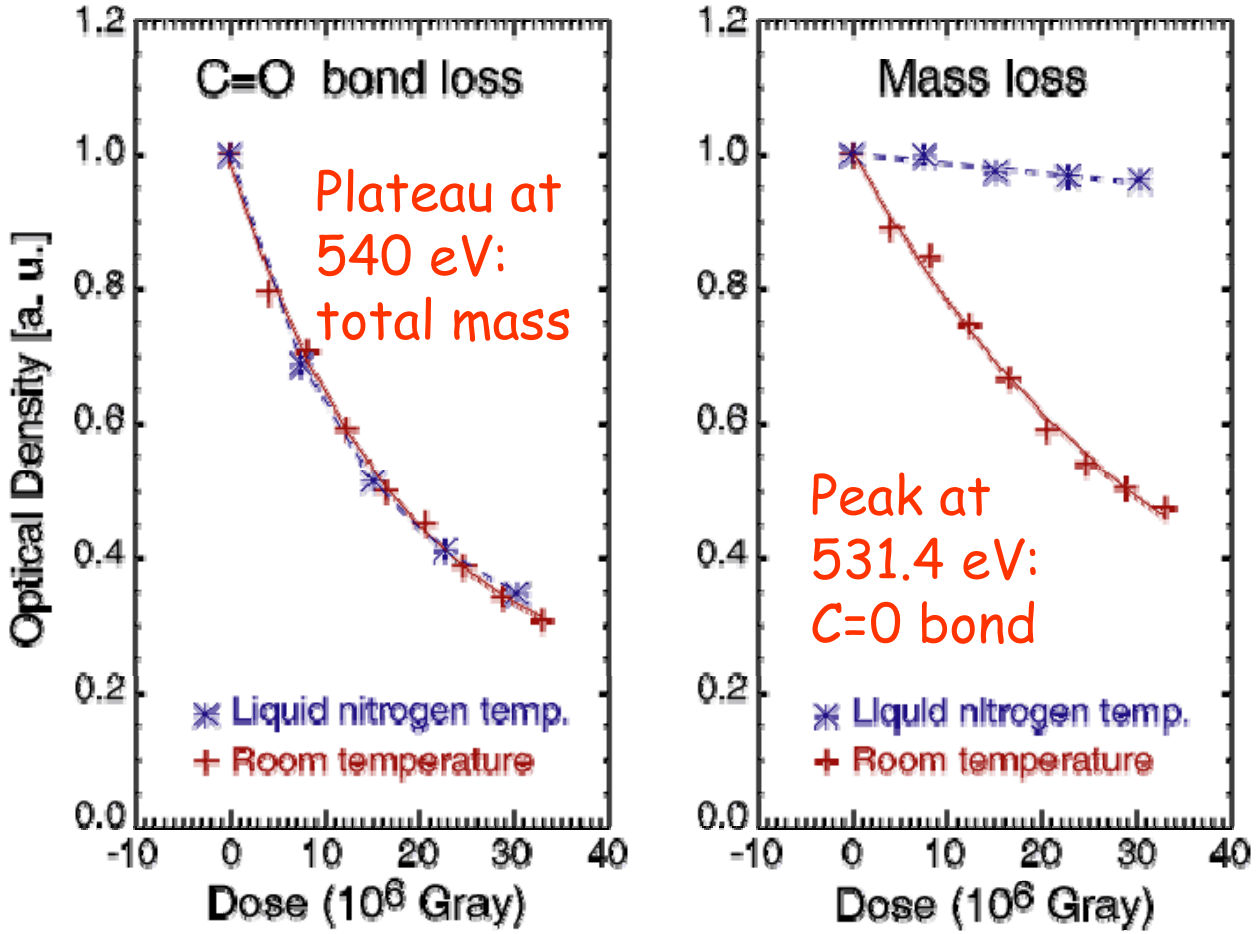
- A full prep lab should include cryo plunger, high pressure freezer, and cryo ultramicrotome.
- Evaluation of specimen quality: cryo light microscopy (gives new science opportunities!), lab x-ray source for checking for ice crystallization diffraction rings.
- Specimen preselection: cryo light microscopy with indexing to x-ray microscopes and nanoprobes.
- Integrated cryo specimen system for x-ray, visible light microscopy being developed at Xradia.

Funding

- Program could serve biology, soft condensed matter, environmental science. Joint funding?
- At present: mix of NIH, NSF, NYSTAR
- NIH NCRR: Bio imaging resource?
- DoE BER?

Cryo and XANES

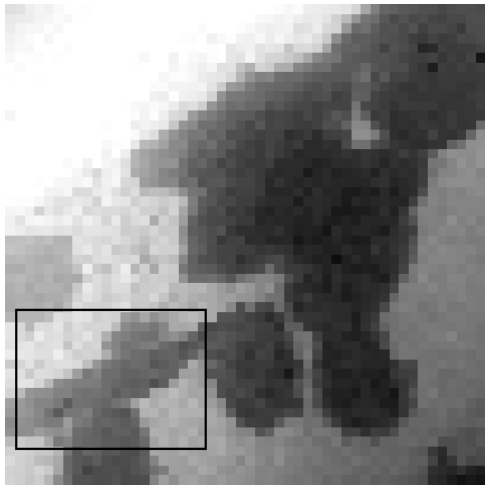
LN_2 temp: protection against mass loss and shrinkage, but not against breaking bonds (at least C=0 bond in dry PMMA)



Beetz and Jacobsen, J. Synchrotron Radiation **10**, 280 (2003)

Radiation damage on (initially) living cells

- Chick embryo fibroblasts. Reflux of culture medium every 20 min to keep unexposed cells alive.
- Makes it hard to view living cells!

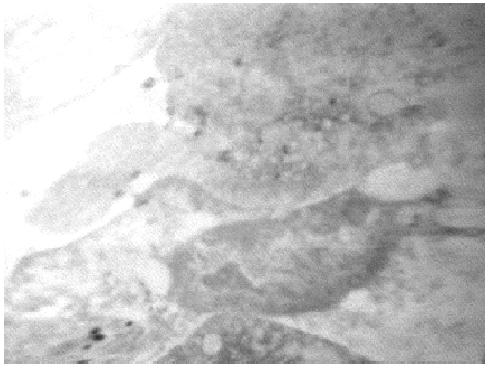


10 μm
$6.0 \cdot 10^2$ Gray, ET=2 min.

Experiment by V. Oehler, J. Fu, S. Williams, and C. Jacobsen, Stony Brook using specimen holder developed by Jerry Pine and John Gilbert, CalTech. Never properly published, but see Kirz *et al*, *Q. Rev. Biophys.* **28**, 33 (1995)

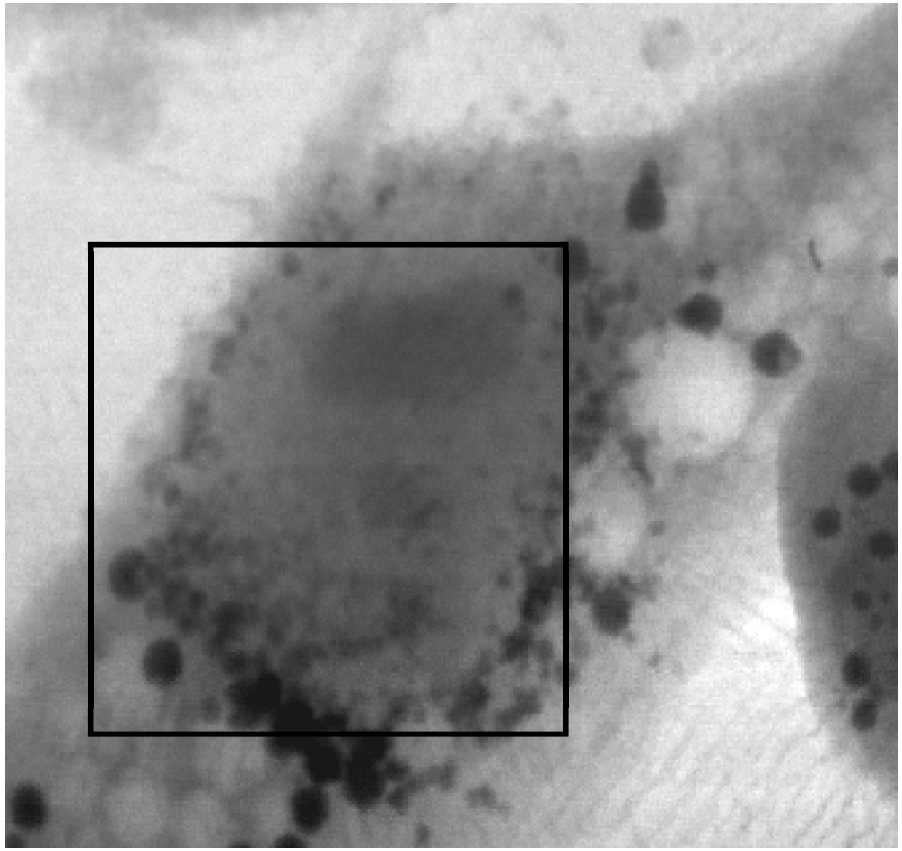
5 μm
$1.2 \cdot 10^5$ Gray, ET=9.5 min.

5 μm
$2.4 \cdot 10^5$ Gray, ET=17 min.



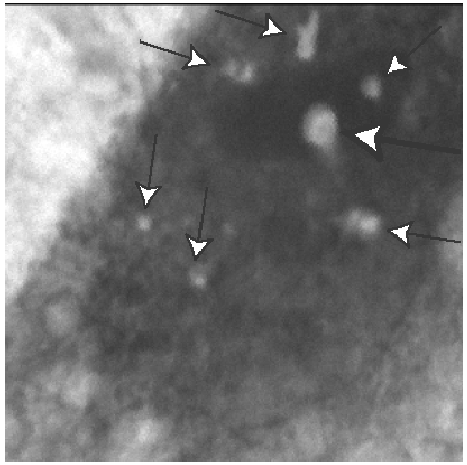
5 μm
$3.7 \cdot 10^5$ Gray, ET=24.5 min.

Radiation damage resistance of wet specimens at liquid nitrogen temperature



Frozen hydrated image after exposing several regions to ~10^{10} Gray

After warmup in microscope (eventually freeze-dried): holes indicate irradiated regions!



Maser et al., *J. Micros*. **197**, 68 (2000)