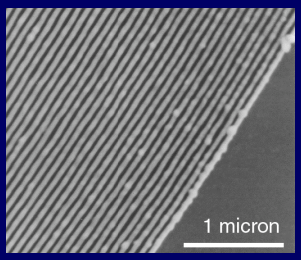
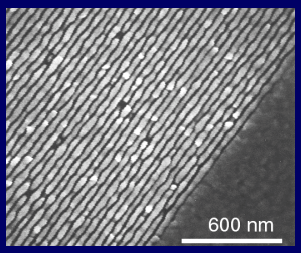
Zone plate microscopy

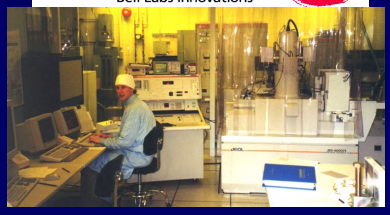


40 nm zones in 120 nm Ni (have also done 45 nm in 180 nm Ni)



20 nm zones in 60 nm Ni

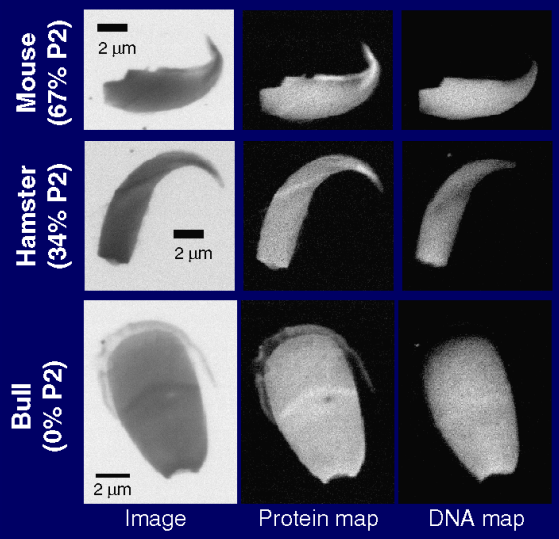
- SUNY Stony Brook: A. Stein, C. Jacobsen (past: S. Spector)
- Bell Labs: D. Tennant
- Image resolution essentially equal to outermost zone width
- JEOL JBX-9300FS: 1 nA into 4 nm spot at 100 keV, 500 μm field



S. Spector, C. Jacobsen, D. Tennant, *J. Vac. Sci. Tech. B* **15**, 2872 (1997)

Spectromicroscopy of DNA packing in sperm

- X. Zhang, R. Balhorn, J. Mazrimas, and J. Kirz, *J. Structural Biology* **116**, 335 (1996)
- Use XANES/NEXAFS resonances for chemical state mapping
- Each sample imaged at N=6 energies; images aligned by hand
- Conclusion: protamine II replaces protamine I, rather than binding to protamine I complex (implications for infertility)

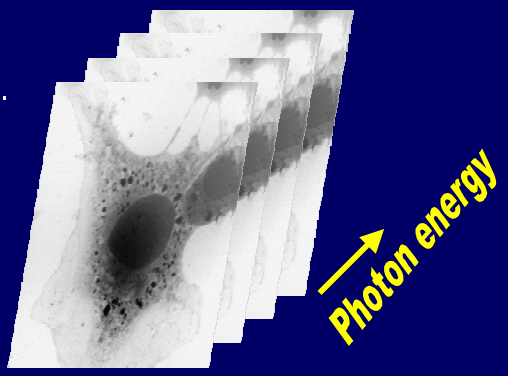


Spectromicroscopy by image stacks

- Acquire sequence of images over XANES spectral region; automatically align using Fourier cross-correlations; extract spectra.
- Full data set to exploit with multivariate statistics (e.g., principal component analysis)
- C. Jacobsen *et al.*, *J. Microscopy* **197**, 173 (2000).

Images at N=150 energies are common.

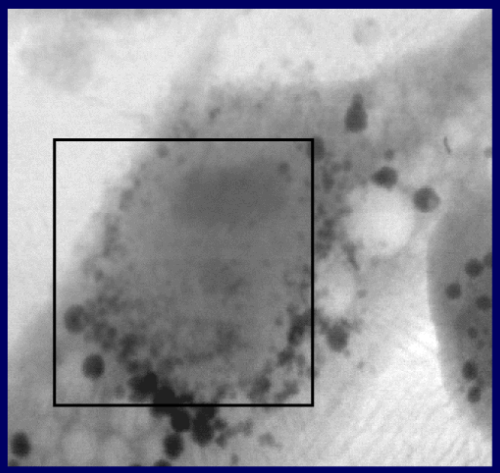
Total acquisition time at NSLS X-1A (10^{18} brightness): 3-10 hours for a 10 μm field at 50 nm resolution



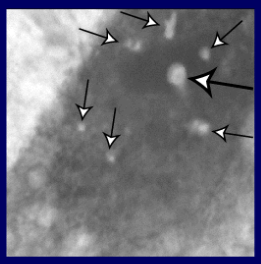
Cryo specimens can withstand multiple images

Left: frozen hydrated image *after* exposing several regions to ~10^{10} Gray (about 10^4 times single image dose)

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

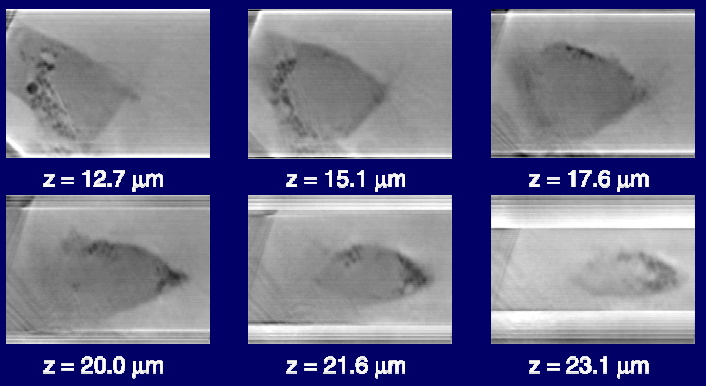


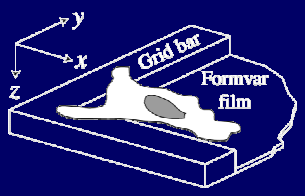
Maser *et al.*, *J. Microscopy* **197**, 68 (2000)



Tomography of a fibroblast

- Y. Wang *et al.* (Stony Brook), *J. Microscopy* **197**, 80 (2000)
- 3T3 fibroblast, extending over ~12 μm depth, 100x100x250 nm minimum feature size
- Required ~30 hours to acquire data set at NSLS X-1A
- Future efforts aimed at higher resolution without a depth-of-field limit by using diffraction tomography



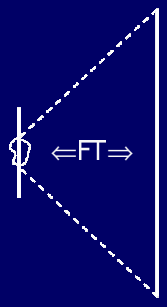


Imaging using x-ray diffraction from non-periodic specimens

- Diffraction pattern can be recorded with no optics-imposed resolution limits
- Proposed by Sayre (in Schlenker, ed., *Imaging and Coherence Properties in Physics*, Springer-Verlag, 1980)
- Previous experiments by Sayre, Yun, Chapman, Miao, Kirz
- Reconstruction: iterate between real and Fourier space

Real space:

- Finite support: object fills only part of the field
- Positivity?

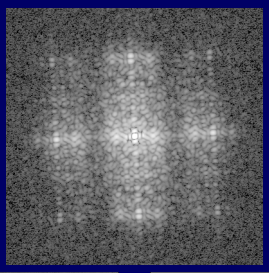


Fourier space:

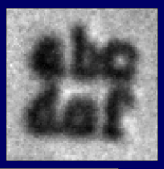
- Re-impose the measured intensities while letting the phases evolve

Imaging using soft x-ray diffraction from non-periodic specimens

- Reconstruction: λ=1.8 nm diffraction pattern, plus optical micrograph for low spatial frequencies
- Miao, Charalambous, Kirz, Sayre, *Nature* **400**, 342 (1999).



Soft x-ray diffraction pattern (left) with low-angle information from optical micrograph (below)



Scanning electron micrograph of object

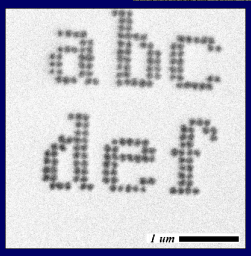
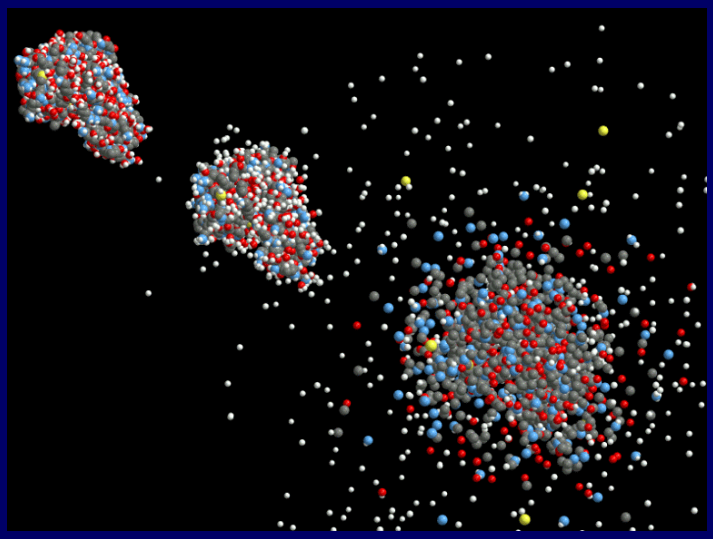


Image reconstructed from diffraction pattern (θ_{max} corresponds to 80 nm)

Single molecule imaging?

- R. Neutze *et al.*, *Nature* **406**, 752 (2000)
- For macromolecules that can't be crystallized, collect many single molecule diffraction patterns from fast x-ray pulses, and reconstruct
- Lysozyme explodes in ~50 fsec



Single molecule imaging: what's needed?

- Lots of coherent photons in a short pulse!
- R. Neutze *et al.*, *Nature* **406**, 752 (2000)
- LCLS (Stanford), TESLA (Hamburg) X-FEL experiment proposals led by J. Hajdu (Uppsala)