Soft and Hard X-Ray Microscopy

David Attwood
University of California, Berkeley
and
Advanced Light Source, LBNL

Cheiron School
October 2010
SPring-8
The short wavelength region of the electromagnetic spectrum

\[ \hbar \omega \cdot \lambda = hc = 1239.842 \text{ eV nm} \]

\[ n = 1 - \delta + i\beta \quad \delta, \beta \ll 1 \]
Two common soft x-ray microscopes

Full-Field Microscope

- 10–20 nm spatial resolution
- Modest spectral resolution
- Seconds exposure time
- Bending magnet radiation
- Higher radiation dose
- Flexible sample environment (wet, cryo, labeled magnetic fields, electric fields, cement, ...)

Scanning Microscope

- 10–20 nm spatial resolution
- Least radiation dose
- Best spectral resolution
- Requires spatially coherent radiation
- Minutes exposure time
- Flexible sample environment
- Photoemission, fluorescence imaging
A Fresnel zone plate lens for x-ray microscopy

Erik Anderson, LBNL
λ = 1.52 nm (815 eV)

Δr = 15 nm

N = 500

D = 30 μm

f = 300 μm

σ = 0.38

0.8 Δr = 12 nm

Cr/Si test pattern (Cr L₃ @ 574 eV)

(2000 X 2000, 10⁴ ph/pixel)
Novel zone plates for specific functionality

New x-ray lenses: Improving contrast and resolution for x-ray microscopy

Courtesy of Anne Sakdinawat, UC Berkeley
High resolution zone plate microscopy

- Well engineered
- Sample indexing
- Tiling for larger field of view
- Pre-focused
- High sample throughput
- Illumination important
- Phase contrast

\[ \text{Res} \approx \frac{1}{2} \frac{\lambda}{\text{NA}} \]

\[ \text{DOF} \approx \pm \frac{1}{2} \frac{\lambda}{\text{NA}^2} \]
The water window for biological x-ray microscopy
Fast freeze cryo fixation strongly mitigates radiation dose effects

Helium passes through LN, is cooled, and directed onto sample windows

W. Meyer-Ilse, G. Denbeaux, L. Johnson, A. Pearson (CXRO-LBNL)
Organelle details imaged with cryogenic preservation and high spatial resolution

Cryo x-ray microscopy of 3T3 fibroblast cells

C. Larabell, D. Yager, D. Hamamoto, M. Bissell, T. Shin (LBNL Life Sciences Division)
W. Meyer-Ilse, G. Denbeaux, L. Johnson, A. Pearson (CXRO-LBNL)
Bending magnet radiation used with a soft x-ray microscope to form a high resolution image of a whole, hydrated mouse epithelial cell

\[ hw = 520 \text{ eV} \]

32 µm x 32 µm

Ag enhanced Au labeling of the microtubule network, color coded blue.

Cell nucleus and nucleoli, moderately absorbing, coded orange.

Less absorbing aqueous regions coded black.


Courtesy of C. Larabell and W. Meyer-Ilse (LBNL)
Bio-nanotomography for 3D imaging of cells

Nanotomography of Cryogenic Fixed Cells

![Diagram of nanotomography setup](image)

- ALS Bending Magnet
- Plane mirror
- Condenser zone plate
- Pinhole
- Cryogenic He gas
- Rotation with stepper motor
- Micro zone plate
- Sample in Capillary
- CCD

λ = 2.4 nm

Soft X-Ray Nanotomography of a Yeast Cell

![Image of soft X-ray nanotomography](image)

Courtesy of G. Schneider (BESSY)

Courtesy of C. Larabell (UCSF & LBNL) and M. LeGros (LBNL)
Bio-nanotomography for 3D imaging of cells

Nanotomography of Cryogenic Fixed Cells

\[ \lambda = 2.4 \text{ nm (517 eV)} \]
\[ \Delta r = 35 \text{ nm} \]
\[ N = 320 \]
\[ NA = 0.034 \]
\[ D = 45 \mu\text{m} \]
\[ f = 650 \mu\text{m} \]
\[ \sigma = 0.64 \]
Resolution = 60 nm

Soft X-Ray Nanotomography of a Yeast Cell

\[ \lambda = 2.4 \text{ nm} \]

Courtesy of C. Larabell (UCSF & LBNL) and M. LeGros (LBNL)
Nanoscale 3-D biotomography

Mother daughter yeast cells just before separation

2-D slice from 3-D Tomogram. Images every 2°, 180° data set, several minutes. $\Delta r = 45$ nm

Color coding identifies subcellular components by their x-ray absorption coefficients

Courtesy of Carolyn Larabell, UCSF/LBNL.
Applications of soft x-ray microscopy

Biotomography at 60 nm resolution

- Cryofixation
- $2^\circ$ angular intervals
- Depth of focus limits resolution
- New XM-2 dedicated to biological applications, will become major facility worldwide to draw biologists to this evolving capability

Courtesy of C. Larabell (UCSF & LBNL)
Magnetic X-Ray Microscopy

- High spatial resolution in transmission
- Bulk sensitive (thin films)
- Complements surface sensitive PEEM
- Good elemental sensitivity
- Good spin-orbit sensitivity
- Allows applied magnetic field
- In-sensitive to capping layers
- In-plane and out-of-plane measurements

Courtesy of P. Fischer, (MPI, Stuttgart) and G. Denbeaux (CXRO/LBNL)
Magnetic domains imaged at different photon energies

FeGd Multilayer

Contrast reversal

$\hbar \omega = 704 \text{ eV}$
below Fe L-edges

$\hbar \omega = 707.5 \text{ eV}$
Fe L$_3$-edge

$\hbar \omega = 720.5 \text{ eV}$
Fe L$_2$-edge

P. Fischer, T. Eimueller, M. Koehler (U. Wuerzberg)
S. Tsunashima (U. Nagoya) and N. Tagaki (Sanyo)
G. Denbeaux, L. Johnson, A. Pearson (CXRO-LBNL)
Magnetic recording of nanomagnetic patterns to 15 nm spatial resolution

CoCrPt alloy
Co L$_3$-edge at 778 eV (1.59 nm)

Courtesy of Peter Fischer (LBNL)

Time resolved studies of vortex dynamics in patterned permalloy thin films

Pump and Probe setup requires:
- **Pump**: Current pulse to "pump" sample
- **Probe**: X-ray pulses (70ps) from ALS 2 Bunch mode
- **Perfect repeatability of dynamics**

**Sample:**
- 50 nm thick 2µmx4µm permalloy (Ni$_{80}$Fe$_{20}$)
- 100nm thick gold waveguide
  (ΔI along waveguide generates field to pump sample)

Environmental Consequences of Portland cement

1.5 billion ton of cement

Generates 1.5 billion ton of CO₂
Responsible for 7% CO₂ production in the world

Problem!

Courtesy of Professor Paulo Monteiro, CEE, UC Berkeley
Nanoscale x-ray imaging of cement processes: early hydrates forming during the pre-induction period

C3S hydrated for 34 min. in saturated lime and calcium sulfate at w/c = 5, 1 s exposure time, 516 eV, scale bar 1µm.

Early hydrates (Sheaf of wheat)

Grain

Courtesy of Professor Paulo Monteiro, CEE, UC Berkeley
Nanoscale x-ray imaging of cement processes

Orth $C_3A$

Orth $C_3A + 1\% \text{CaCl}_2$

Orth $C_3A + \text{accelerator}$

C: carbon
Ca: calcium
A: alumina ($\text{Al}_2\text{O}_3$)
S: silica ($\text{SiO}_2$)

520 eV, 40 nm - spatial resolution

Courtesy of Professor Paulo Monteiro, CEE, UC Berkeley
Spectromicroscopy: high spatial and high spectral resolution of surface and thin films

Zone plate focusing lens

Detector

Raster scan sample

\( h_\nu \)

Scanning Soft X-Ray Microscope

ALS beamline 11.0.2
395 eV; \( \lambda/\Delta\lambda \approx 6000 \)
240 \( \times \) 240 pixels
1.2 \( \mu m \times 1.2 \mu m \)
2 ms dwell time
Biofilm from Saskatoon River

RESULTS
- Ni, Fe, Mn, Ca, K, O, C elemental map, (there was no sign of Cr.)
- Different oxidation states for Fe and Ni

Protein (gray), Ca, K

Different oxidation states (minerals) found for Fe & Ni

Tohru Araki, Adam Hitchcock (McMaster University)
Tolek Tyliszczak, LBNL
Sample from: John Lawrence, George Swerhone (NWRI-Saskatoon), Gary Leppard (NWRI-CCIW)
Patterned polymer photoresists

M.K. Gilles, R. Planques, S.R. Leone
LBNL
Samples from B. Hinsberg, F. Huele
IBM Almaden

Exposure to UV light results in loss of carbonyl peak

Map chemical spectra taken of pure samples onto a sample containing both components

Courtesy of Mary Gilles, LBNL
Hard x-ray zone plate microscopy

- Shorter wavelengths, potentially better spatial resolution and greater depth-of-field.
- Less absorption ($\beta$); phase shift ($\delta$) dominates, higher efficiency.
- Thicker structures required (e.g., zones), higher aspect ratios pose nanofabrication challenges.
- Contrast of nanoscale samples minimal; will require good statistics, uniform background, dose mitigation.
Challenges for achieving nm scale resolution:

- High resolution objective lens: limiting the ultimate resolution
- High numerical aperture condenser lens:
- Detector: high efficiency for lab. source and high speed for synchrotron sources
- Precision mechanical system
Xradia nanoXCT: Sub-25 nm Hard X-ray Image

Xradia Resolution Pattern
- 50 nm bar width
- 150 nm thick Au
- 8keV x-ray energy
- 3\textsuperscript{rd} diffraction order


Xradia nano-XCT 8-50S installed at NSRRC, Taiwan

400 nm
Elemental contrast by tuning energy across the copper absorption edge (Guan-Chian Yin et al.)

Intensity difference between E = 8.4 keV and 9.5 keV
Tomography of a Tungsten plug with “keyhole” at ~60 nm spatial resolution

Xradia and NSRRC

APL. 88, 241115 (2006)
Scanning x-ray fluorescence microprobe (µ-XRF)

Kirkpatrick-Baez (KB) mirror pair

- Crossed cylinders at glancing incidence
- Photon in / photon out, low noise background
- Femtogram and part per billion (ppb) sensitivity
- Micron focus (1988), now ~25 nm (Yamauchi, Mimura and colleagues, Osaka U./SPring-8)

X-ray microprobe at SPring-8

Courtesy of K. Yamauchi and H. Mimura, Osaka University.

Sub-cellular elemental analysis using the hard x-ray fluorescence microprobe at SPring-8


Courtesy of K. Yamauchi and H. Mimura, Osaka University.
Breaking the 10 nm barrier in hard x-ray focusing

In-situ phase compensation

Piezo-electric phase compensator

Focusing mirror with phase error

H. Mimura et al., Nature Physics, 6, 122 (2009)
Optical configuration for active phase compensation

Synchrotron-based art conservation at ESRF

µ-XRF, µ-XRD, µ-XANES

Courtesy of Marine Cotte (ESRF, Grenoble, France)
Examples of $\mu$-XANES K-edge spectra occurring in art materials

Courtesy of Marine Cotte (ESRF, Grenoble, France)
18th Dynasty Egyptian glass vase studied for an understanding of color and opaqueness in antiquity

1st production of glass objects Egypt (1500 B.C.), opaque, colored, nanoscale calcium antimonate

Courtesy of Marine Cotte (ESRF, Grenoble, France)
Synchrotron radiation x-ray tomographic microscopy (SRXTM)

Double crystal monochromator (8-45 keV) (variable resolution vs throughput)

Sample

Nearby collimated, monochromatic x-rays

Rotation stage (1500 projections, 180° total, 8 hours)

X-ray scintillator

Visible light CCD

Visible light

High-NA visible light microscope objective

Mirror

Swiss Light Source

2.9 T Superbend, broad spectrum x-rays with 11 keV critical photon energy

Courtesy of Marco Stampanoni, Swiss Light Source.
TOMCAT Microscope

1 micron @ 10% MTF reached routinely

Courtesy of Marco Stampanoni, Swiss Light Source.
TOMCAT Microscope

1 micron @ 10% MTF reached routinely

Courtesy of Marco Stampanoni, Swiss Light Source.
Hard x-ray 3D x-ray tomography: microvascular architecture of a mouse brain

SRXTM, 25 keV, 15 µm resolution
Tomcat Beamline, Swiss Light Source

M. Stampanoni, T. Krucker et al.,
Tomographic reconstruction of a 500 million year old fossilized embryo from Southern China

*Markelia hunanensis* relative of modern roundworms and arthropods

SRXTM, 17.5 keV, 15 µm resolution
Tomcat Beamline, Swiss Light Source


100 microns

Courtesy of Marco Stampanoni, Swiss Light Source.
A lens is not necessarily required

\[ \Delta r_{\text{resol.}} = k_1 \frac{\lambda}{\text{NA}} \]

“Lensless” coherent diffraction imaging (CDI) is being aggressively pursued.
Coherent diffractive imaging (CDI)

Start: Combine measured diffraction intensity pattern with random phases

Satisfy known constraints (support) in real space

Fourier transform to k-space

Satisfy Fourier (k) space constraints (replace with measured intensity pattern, but keep new phases)

Inverse Fourier transform to real space
Coherent diffractive imaging (CDI) examples

Femtosecond diffractive imaging with a free electron laser

Flash FEL, $\lambda = 32$ nm (39 eV)
25 fs, $10^{12}$ photons/pulse
62 nm resolution


Synchrotron based CDI of 100 nm Au spheres

$\lambda = 1.66$ nm (750 eV)

CDI
ALS/9.0.1

STXM
NSLS/X1
$\lambda = 2.3$ nm
(540 eV)
42 nm resolution

Tilted 3°
Tilted 4°

Synchrotron based CDI of a freeze dried yeast cell

HHG, $n = 27$, $\lambda = 29$ nm (43 eV)
94 nm spatial resolution


Synchrotron based CDI of 100 nm Au spheres

$\lambda = 0.083$ nm (15 keV),
5 nm “resolution”

Shapiro, et al. PRL (2005)

$\lambda = 32$ nm (39 eV)

HHG, $n = 27$, $\lambda = 29$ nm (43 eV)
94 nm spatial resolution


Flash FEL, $\lambda = 32$ nm (39 eV)
25 fs, $10^{12}$ photons/pulse
62 nm resolution

Lensless imaging of magnetic nanostructures by x-ray spectro-holography

Lectures online at www.youtube.com

Amazon.com

UC Berkeley
www.coe.berkeley.edu/AST/sxreuv
www.coe.berkeley.edu/AST/srms
www.coe.berkeley.edu/AST/sxr2009