Biological X-ray Microscopy

New ways of imaging cells and tissues

Carolyn Larabell
Using microscopes to see things we can’t otherwise see
Resolution

Shortest distance between two points at which they can still be distinguished as separate entities
Magnification vs. Resolution
Examples
Imaging Cells with Electron Microscopy

Examples
Imaging Cells with Soft X-ray Microscopy

- Whole cells up to 10 μm thick
- Fully hydrated specimens
- Inherent contrast of organic material
- Quantitative - use linear absorption coefficient
- Better than 50 nm resolution - isotropic
- Localization of proteins and multi-protein complexes
- Fast - collect tomographic data set in < 5 min
• Whole cells up to 10 μm thick

• Fully hydrated specimens

• Inherent contrast of organic material

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### X-ray Microscopy:

Whole Cells up to 10 um thick

<table>
<thead>
<tr>
<th>Light Microscopy</th>
<th>Electron Microscopy</th>
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<tr>
<td>• Hundreds of microns thick</td>
<td>• 50-100 nm thick sections (standard TEM)</td>
</tr>
<tr>
<td></td>
<td>• 1-3 um thick (high voltage TEM)</td>
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X-ray Microscopy:

Fully Hydrated Specimens

Light Microscopy

- Yes - chemically fixed and live cells

Electron Microscopy

- Cryofixed cells up to 0.5 μm thick
- Frozen sections
  - 50-100 nm thin in standard TEM
  - 1-3 μm thick in high voltage (300-1 MeV) TEM
Consequences of dehydration

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X-ray Microscopy:

Inherent contrast of organic material

Light Microscopy
- Phase contrast
- Dyes/labels

Electron Microscopy
- Heavy metal stains
X-rays: Image in Water Window - Natural contrast

Between K shell absorption edges of oxygen (543 eV; 2.3 nm) & carbon (284 eV; 4.4 nm)

2.4 nm $\lambda$ (517 eV)
Cryo X-ray Microscopy of NIH 3T3 Fibroblasts

No chemical fixatives or contrast enhancement reagents

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X-ray Microscopy:

Quantitative

Light Microscopy
• No

Electron Microscopy
• No
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X-ray Microscopy:

Better than 50 nm Resolution

**Light Microscopy**

- 200 nm
- ‘Super resolution techniques’
  - approaching 50 nm; limited to very thin specimens (50 nm)

**Electron Microscopy**

- ~ 5 nm in plastic sections
- Requires extensive processing (fix, dehydrate, stain, embed in plastic, section)
- Frozen sections
- 3-5 nm with cryo electron tomography - must be less than 0.5 μm thick
- 0.3 nm of single particles
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Tomographic Reconstruction

±90°, 2° rot  ±60°, 2° rot  ±90°, 5° rot  ±60°, 5° rot

Baumeister et al., Trends in Cell Biology. 1999
Cryo X-ray Microscopy of NIH 3T3 Fibroblasts

Multiple images - requires frozen specimens

Room temperature (hydrated)  Cryofixed

Cryofixation avoids artifacts of radiation damage

Nuclear envelope of NIH 3T3 fibroblast

- Specimen must be frozen to prevent radiation damage
- Must maintain low temperature (77 K)

Cryo-preserved specimens tolerate hundreds of images (one sec/image) without apparent radiation damage

Yeast
Cryo X-ray Tomography


Specimen at atmospheric pressure
Cryo X-ray Tomography

A) Harmonic drive rotation motor for tomography
B) XYZ stages for coarse sample positioning
C) Precision bearing
D) Picomotor alignment stage
E) X-ray CCD Camera

Specimen at atmospheric pressure

Cryo X-ray Tomography

A) Harmonic drive rotation motor for tomography
   - XYZ stages for coarse sample positioning

B) Precision bearing
   - Micrometer alignment stage
   - X-rays from source

C) Liquid nitrogen temperature Helium gas
D) X-ray CCD Camera
E) 10μm

Yeast Cells

- Small - 5 µm diameter
- Important model system
  - genome sequenced
  - knockouts and GFP cell lines exist
- Hardy - tolerate technology development demands
- Grow in suspension - image 180°
Tomographic Reconstruction

±90°, 2° rot  ±60°, 2° rot  ±90°, 5° rot  ±60°, 5° rot

Baumeister et al., Trends in Cell Biology. 1999
Saccharomyces cerevisiae

45 images collected at 4-degree intervals

Projection images
(60 nm gold balls as fiducial markers)

Sections through the reconstructed data

Yeast bud = 3 microns diameter

Saccharomyces cerevisiae

Light Microscopy

X-ray Tomography

Yeast bud = 3 microns diameter
Saccharomyces cerevisiae

E. Coli

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Environmental Biology

Microbial Communities
Caulobacter crescentus

M. A. Le Gros, D. W. Parkinson, and C. A. Larabell; LBNL
Tomographic Reconstruction

±90°, 2° rot  ±60°, 2° rot  ±90°, 5° rot  ±60°, 5° rot

Baumeister et al., Trends in Cell Biology. 1999
Multilamellar vesicles

Lipid bilayers $\approx 5$ nm thick

90 images collected at 2-degree intervals
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Protein Localization

25,000 genes

250,000 proteins

Protein Function??

Biochemistry
Molecular Biology
Mass Spec
Chips/Arrays
Yeast 2-hybrid
Western blots

Apoptosis pathway

Location, Location, Location!!
Localization of Proteins - Light Microscopy

Immunofluorescent Labeling

Primary antibodies recognize the protein

Secondary antibodies recognize the primary antibodies; tagged with fluorescent molecule
Localization of Proteins - X-ray Microscopy

**Immuno-Labeling**

Primary antibodies recognize the protein

Secondary antibodies recognize the primary antibodies; tagged with *X-ray Probe*
Microtubules in Mammary Epithelial Cells

Secondary antibody tagged gold particles, enhanced with silver to make deposits large enough to see in x-ray microscope.

Nuclear Pore Complex Proteins

secondary label - 1.4 nm gold particles, enhanced with silver

Localization of Splicing Factors

secondary label - 1.4 nm gold particles, enhanced with silver

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Biological Research Projects
Schizosaccharomyces pombe

Cell Division Cycle of fission yeast

Copyright 1996 by Frans Hochstenbach

Schizosaccharomyces pombe
Schizosaccharomyces pombe

Red = actin in contractile ring
labeled with rhodamine phalloidin

Cyan = new septum proteins
labeled with calcofluor
Schizosaccharomyces pombe


New cell

Adult cell

Dividing cell (early)

Dividing cell (late)
Schizosaccharomyces pombe

Cell wall dissolution

A  B  C

D  E  F

G2
X-ray Tomography

Room for improvements!

- More images
  - Automated cryo-rotation stage enables collection of hundreds of images - will yield better resolution
- Better resolution zone plates
Soft X-ray microscopy at a spatial resolution better than 15 nm

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