SOFT X-RAY MICROSCOPY IN BIOLOGY

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SOFT X-RAY MICROSCOPY IN BIOLOGY

(present status)

1. Advantages of soft X-ray microscopy in biology
2. Theoretical consideration of limitations
3. Soft X-ray microscopes and examples of images (our study)
4. Application to study human chromosome fibers
5. Application to observe mouse macrophages
# Background

## Comparison of microscopes

<table>
<thead>
<tr>
<th></th>
<th>Optical</th>
<th>Soft X-ray</th>
<th>Electron</th>
</tr>
</thead>
<tbody>
<tr>
<td>resolution</td>
<td>≥200 nm</td>
<td>~10 nm</td>
<td>~0.1 nm</td>
</tr>
<tr>
<td>transmittance</td>
<td>high</td>
<td>intermediate</td>
<td>low</td>
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</tbody>
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**Soft X-ray microscopy:**

- **Wavelength:** mainly 1-10 nm
- **Specimens:** thickness 1-10 μm (hydrated condition OK)
Advantages and Limitations of soft X-ray microscopy in biology

1. Potential to observe **physiologically natural specimens** in a hydrated condition at the **resolution higher** than optical microscopy

2. **Higher penetration depth** than electron microscopy

3. Possibility for imaging and analysis of the distribution of elements and chemicals (**Spectromicroscopy**)

-Limitations-

**Radiation damage** and thermal diffusion (**Brownian motion**)
Radiation damage and Brownian motion

Radiation damage:

$8 \times 10^9$ DNA double strand breaks/cell for 10 nm resolution at $\lambda = 2.36$ nm

Image blurring by Brownian motion:

$3.8 \mu$sec for the movement of 10 nm by a 10 nm particle
To observe hydrated specimens

To avoid the problem of radiation damage and Brownian motion
(Damaged site also moves by Brownian motion)

Short exposure (< Brownian motion)

or

< 3.8μsec for 10 nm

Freeze; Fix; Dry
SOFT X-RAY MICROSCOPES
and
EXAMPLES OF IMAGES
Types of Soft X-Ray Microscopy

**Contact microscopy**
Resolution: ~10 nm

**Imaging microscope**
Resolution: ~20 nm
Directly magnified image
Real time observation possible

**Scanning microscope**
Resolution: ~25 nm
Use for Spectromicroscopy

Image from Howells et al., 1991
Example 1: Contact microscopy

Human chromosome fibers (dry)

Observed at PF, Japan

Shinohara et al., 1990
Example 2: Contact microscopy with an electronic zooming tube (system)

Resolution: >0.2-0.5 µm