How to study cells

Tools used to answer biological questions. The methods are important, but it's what we discover with them that's really interesting.

1. Microscopy (chap 1)
   **Role of microscopy in Cell Biology**
   - Invention of light microscopes in or just before the 1600s - cells became visible for the first time
   - sole tool to study cells for centuries
   - light microscopy is still a valuable tool to cell biologists

   **What microscopes do:**
   - designed for magnification, resolution, & contrast
   - wavelength of light limits resolution
   - sizes of cells and cell components:

   ![Diagram of microscopy scales](image)

   - **Types of microscopy:**
     1. **light microscopy**
        a. conventional wide field
        b. phase contrast, differential interference contrast
        c. darkfield

   - staining: another important method for generating contrast
   - preparing cells/tissues for staining:
     - fixation
     - sectioning

d. **fluorescence microscopy**
   - fluorescent dyes (see figure) can be used to identify molecular structures.
   - Viewing fluorescent cells:
     1. Epi-fluorescence illumination (see figure)
     2. Laser-scanning confocal microscopy (see figure)
   - Immunofluorescence - use of antibodies
   - Indirect immunofluorescence
   - Fluorescent proteins
**fluorescein**

![Fluorescein chemical structure]

**epifluorescence microscopy**

![Epifluorescence microscopy diagram]

**confocal microscopy**

(A) excitation

Confocal pinholes are used to focus the light onto the sample. A laser illuminates the sample, and a dichroic mirror reflects the excitation light while allowing the emission light to pass through.

(B) emission

The fluorescent specimen is illuminated with a focused point of light from a pinhole. The emitted light from the in-focus point is focused at the pinhole and reaches the detector.

(C) emitted light

Emitted light from out-of-focus point is cut out of focus at pinhole and is largely excluded from detector.

*Figure 9-12, Molecular Biology of the Cell, 4th Edition.*
2. Electron Microscopy
- TEM (Transmission electron microscopy)
- SEM (Scanning electron microscopy)

**Figure 9-22. Molecular Biology of the Cell, 4th Edition.**

**Figure 9-29. Molecular Biology of the Cell, 4th Edition.**
Comparing limits of resolution

<table>
<thead>
<tr>
<th>Light Microscopy</th>
<th>Typical Wavelength</th>
<th>Limit of Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional wide field</td>
<td>500 nm</td>
<td>200 nm</td>
</tr>
<tr>
<td>Epifluorescence</td>
<td>500 nm</td>
<td>200 nm</td>
</tr>
<tr>
<td>Laser scanning confocal</td>
<td>500 nm</td>
<td>200 nm</td>
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<tr>
<td><strong>Electron Microscopy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEM</td>
<td>0.01 nm</td>
<td>1-2 nm</td>
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<tr>
<td>SEM</td>
<td>0.01 nm</td>
<td>10-20 nm</td>
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<tr>
<td><strong>X-Ray Crystallography</strong></td>
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<tr>
<td>Diffraction analysis</td>
<td>0.15 nm</td>
<td>0.1 nm</td>
</tr>
</tbody>
</table>

2. Isolation and growth of cells
- Direct manipulation of cells
- Cell culture: primary cultures, cell lines

3. Isolating cellular components
Cellular components can be studied in isolation
- breaking cells
- centrifugation
3 methods of centrifugation:

(1) differential centrifugation
4. DNA, RNA & Protein methods

DNA & RNA methods such as reverse transcription, cloning, PCR, etc. learned in 103 & 220 are important tools for studying cells; will not be reviewed here. Protein methods will be discussed in the next lecture.