Cell Theory

(Please activate your clickers)
Cell Theory

- Cells are the simplest bits of living material, i.e. of material that has all the characteristics of life.

- All organisms are cells, are composed of cells, or can be subdivided into cells.

- All cells come from pre-existing cells.

- Most cells are too small to see (50 micrometers, μm, $10^{-6}$ meters in diameter).
A light microscope combines an ocular lens, an objective lens, and often several intermediate lenses.
**Light Microscopy**

The objective lens gives a real image.
**Light Microscopy**

The ocular lens gives a virtual image.

\[ I = \frac{H}{h} \]

\[ I = \frac{250}{F} + 1 \]

Diagram showing the relationship between object, lens, and image distances.
A light microscope combines an ocular lens, an objective lens, and often several intermediate lenses.

Total magnification is the product of magnification by each lens and can be up to 1000X.
Light microscopy

Advantages: Simple; can be used for live, functioning cells (though not at 1000x)

Disadvantages: Resolution limited by
(a) light-scattering from thick subjects,
(b) lack of contrast,
(c) wavelength of light (natural limit of resolution--
1/2 wavelength used, ca. 0.2 μm with blue light).

Solutions:
(a1) fixation, embedding and sectioning (thin slices),
(b1) staining, fluorescent tags
(b2) phase contrast or differential interference-contrast
(c) advanced localization techniques (STED, etc.)
There are several variations of light microscopy

Goal: improved contrast, minimized scattering
With STED microscopy, the position of a fluorescing spot can be localized more precisely than the fluorescence from the spot.

Microtubular network within a cell: Resolution ~ 80 nm (0.08 µm)
With atomic force microscopy, a moving “finger” traces the shape of an object. The resolution of the finger is not limited by the wavelength of light.

STM and AFM imaging of pentacene on Cu(111)
L. Gross et al., Science 325, 1110 -1114 (2009)
With atomic force microscopy, a moving “finger” traces the shape of an object. The resolution of the finger is not limited by the wavelength of light.
An electron microscope replaces the light beam with a beam of electrons and the glass lenses with charged or magnetic coils that bend electron movement.

![Diagram of an electron microscope](image)
**Electron Microscopy**

Use magnets to bend an electron beam, i.e. light with shorter wavelength, ca. $10^{-4} \ \mu m$

**Advantage:**
High resolution (shorter wavelength, ca. $10^{-4} \ \mu m$)

**Disadvantages:** Electron beam limited by:
(a) air;
(b) thick subjects;
(c) lack of contrast

**Solutions:**
(a) High vacuum: requires chemical fixation or freezing of subject;
(b1) Fixation and embedding (plastic) or freezing, followed by slicing (for transmission electron microscopy);
(b2) Scanning electron microscopy;
(c) Staining (osmium tetroxide, uranyl acetate).
Cell fractionation isolates quantities of cell parts
**Cell Fractionation**

**Strategy:**
Grind up cells (break open without destroying parts), separate parts by size (e.g. centrifugation)

<table>
<thead>
<tr>
<th>Spin</th>
<th>Sediment</th>
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<tbody>
<tr>
<td>2000 rpm x 10 min</td>
<td>10 μm diameter piece</td>
</tr>
<tr>
<td>10000 rpm x 10 min</td>
<td>1</td>
</tr>
<tr>
<td>30000 rpm x 60 min</td>
<td>0.01</td>
</tr>
<tr>
<td>70000 rpm x 300 min</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Analyze chemical activity and composition of pieces

**Advantage:** attribute function to isolated parts

**Disadvantage:** lose structure of cell and any functions that depend on coordination between different structures)