

Chapter 2

Introduction to Eukaryotic Autotrophs and Osmotrophs

Objectives

Use of the microscope. Identify the parts of the compound transmitted-light microscope and understand their functions. Know the available magnifications. Define resolution. Understand the difference between magnification and resolution. Compare sizes of various biological structures that are mentioned in this chapter.

Plant cells. Prepare a wet mount. Observe plant cells. Identify plant-cell components that are common to eukaryotic cells and those¹ that are unique to photoautotrophs. Contrast plant-cell and animal-cell structure.

Cytoplasmic streaming. Explain the role of and molecular basis for cytoplasmic streaming.

Plasmolysis and Osmosis. Define plasmolysis and explain osmosis.

Plastids. Outline the function of chloroplast and amyloplasts, relying on notes from prerequisites as appropriate. Explain the concept of staining and outline the procedure used to visualize starch.

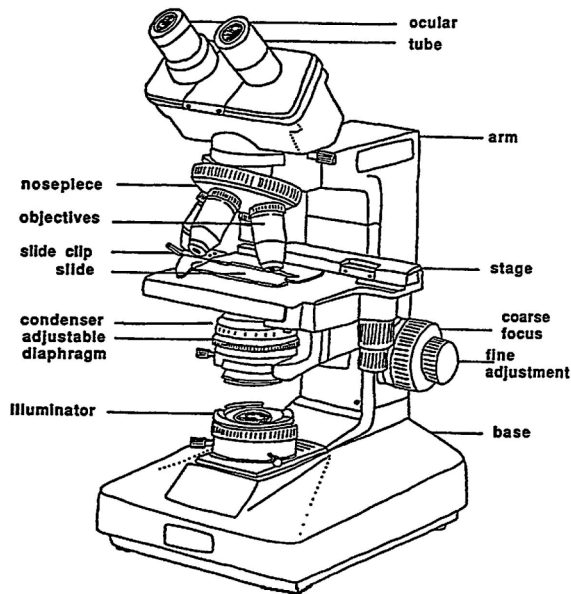
Cell Cycle. Explain the general functions and outcomes of mitosis and meiosis. Describe the stages of mitosis sufficiently to identify them on the basis of chromosome appearance. Distinguish mitotic metaphase and meiotic metaphase I.

Use and Care of the Light Microscope

The most obvious function of the microscope is to magnify small objects that are otherwise invisible. Various innovations have been made in light microscopy, but generally resolving power is the most useful property. Resolving power permits the user to distinguish two parallel lines. Limited by physics, the best ordinary modern compound microscopes allow distinction of objects that are as close as $0.2 \mu\text{m}$ ($0.2 \times 10^{-6} \text{ m}$). In other words, if two lines are $< 0.2 \mu\text{m}$ apart, they will appear as one line. Instructional microscopes are not this good, of course, but have a resolution of $\sim 1 \mu\text{m}$. Remember, the resolving power—not raw magnification—is the important parameter; magnification without resolution is empty. Given BOT 3015 prerequisites, this section will provide only a brief review and pertain only to ordinary transmitted-light microscopes.

Microscope components. A base supports the microscope and houses the light source (see figure). Light from the source is concentrated on the stage by a condenser lens, and passes through the specimen placed on the stage. Next, the light passes through the objective lens, which is housed on the nosepiece, and provides most of the magnification. Light then passes

¹ The singular does not exclude the plural and the plural does not exclude the singular. In other words, obtuse grammatical constructions will not be made in order to be strictly correct with regard to number.



through the body tube, into the eyepiece lens (or ocular), and eventually forms an image on one's retina.

Use of the instructional microscope. This refresher section applies specifically to the BOT 3015L microscopes and also provides a baseline for the care of any scientific instrument.

1. Retrieving the microscope. Remove the dust cover (which should always be on the microscope in storage). Grip the microscope

with one hand holding the arm and the other, the base.

2. Routine cleaning. Develop a habit of cleaning the lens each time the microscope is used. Only use lens paper. (Other products are not lint-free and may scratch the lens.)
3. Powering the microscope. Plug in the microscope and switch on the light source.
4. Initial Settings. Rotate the nosepiece until the detent for the low-magnification lens (4x) is seated. (The other objectives provide 10x and 40x magnification, respectively.) Place the slide on the stage and secure it using stage clamps. The lens must be a certain distance ("working distance") from the object. At the working distance, a property of the optical system of the particular microscope, the object is in focus. Observing from the side of the microscope, slowly turn the coarse adjustment until the objective is near the slide. Use care to avoid ramming the objective onto the slide. Then, looking through the eyepiece, turn the coarse focus knob to move the objective away from the stage until the object comes into focus. Use the fine focus adjustment knob to sharpen the focus.
5. Light adjustments. Adjust light intensity by altering the aperture of the diaphragm, which is mounted in the condenser housing. The condenser is set properly after a specimen on the stage is in focus. Then, place a thin object, such as a dissecting needle, on top of the illuminator and raise or lower the condenser until the object is in sharp silhouette.
6. Increasing magnification. Focus with the low-power objective and then—watching from the side to avoid damage to the microscope—rotate the nosepiece until the higher-power objective clicks into position. The specimen should be approximately in focus ("parfocal"). Bring the specimen into sharp focus by adjusting the fine-focus knob. Total magnification is approximately equal to the product of the magnification due to the objective and the magnification of the eyepiece.

Cells of a Plant, An Advanced Eukaryotic Autotroph

Eukaryotic cells contain a double-membrane-bound nucleus and membrane-bound organelles. These structures are suspended in cytoplasm, which is delimited by a plasma membrane.

Plant cells contain the basic components of a typical eukaryotic cell. In addition, they contain several specific structures². Several types of plant cells will be examined in this exercise. Each type exemplifies a characteristic of plants such as the cell wall, plastids (chloroplasts and amyloplasts), and a central vacuole.

For ordinary transmitted-light microscopy, material must be mounted in a medium and covered with a flat cover slip to prevent optical distortions and preserve resolution. The medium for permanent slides in BOT 3015 is resin, and for temporary, student-prepared slides, water (“wet mount”).

Preparation of wet mount—specimen originally suspended in aqueous solution. A water drop containing the specimen (such as microscopic algae) is placed onto a slide that is maintained in a horizontal position. Use of a coverslip inhibits drying and flattens the preparation (an optical necessity, and also allows lateral movement of the slide without contacting the objective). The coverslip should be placed onto the drop of water by lowering one side of the coverslip so that it touches one side of the drop at a 45° angle first. This procedure minimizes the trapping of air bubbles under the coverslip. (Viewed through the microscope, air bubbles are round and have a dark outline.) If the preparation begins to dry before the observation is completed, add water to the edge of the coverslip. Conversely, blotting the edge of the coverslip with tissue will remove water.

Preparation of wet mount—specimen not originally suspended in aqueous solution. A water droplet is first placed on the slide and the specimen (such as an epidermal peel) is placed onto the water. If the specimen does not sink, a small droplet of water is added on top of it. Otherwise, the procedures are as described above.

<p>Specimen 1: <i>Hydrilla</i> Leaves (observation of a living plant cell, cell walls³, chloroplasts⁴, and cytoplasmic streaming)⁵</p>
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1. Wet mount. Remove a young *Hydrilla* leaf and make a wet mount of the whole leaf as described above. Place the slide on the microscope stage and observe under low magnification.
2. Observation of cell wall. Observe the rows of rectangular cells. Note that each cell is surrounded by a thick cellulosic cell wall. The wall is outside the plasma membrane, which is below the resolution of light microscopes.

The cell wall serves several functions (strength and structure, intercellular transport, communication, and a barrier against invasion by pathogens).

3. Observation of chloroplasts. Observe and draw the cells and chloroplasts at all magnifications. Note that some chloroplasts are moving because the cytoplasm is streaming and carrying the chloroplasts along.
4. Label cells, chloroplasts, and cell wall.

² Fig. 3-3, Fig. 3-7, Fig. 4-1

³ pp. 52-58

⁴ pp. 41-44

⁵ pp. 38-39

A chloroplast is a type of plastid, an endosymbiotically derived ~2- μm organelle that converts light energy into stable chemical energy, primarily through the reduction of CO_2 to organic form.

Cytoplasmic streaming is common in plants and mixes the cytosol, thus facilitating transport. The mechanism that drives cytoplasmic streaming includes two commonly known proteins, actin and myosin.

Specimen 2: Onion⁶ Epidermal Cells (observation of cell walls and vacuole⁷; and inference of the plasma membrane by plasmolysis)⁸

1. Wet Mount. Cut a red onion bulb into eighths and remove one of the fleshy-leaf pieces. Break the leaf by bending backward. With forceps, remove an epidermal strip and make a wet mount.
2. Observations. Observe the red, rectangular cells under the low-power objective. Note, as before, the cell wall and imagine the plasma membrane that is appressed to it. Observe the nucleus, which appears as a dense body in the translucent cytoplasm. Draw as under 10x and label the large red-stained central vacuole and cell wall.

A central vacuole is a hallmark of plant cells and sometimes occupies > 90 % of the total volume of the cell. The vacuole is a metabolically inaccessible compartment: it sequesters toxins; stores sugars, ions, and other substances; digests some substances; and buffers the cytosol against fluctuations of Ca^{2+} , a signal ion. A red onion is used in this exercise for illustration as the vacuoles contain anthocyanins, which also impart color to flowers. The vacuolar membrane is called the tonoplast, but it is too thin for resolution by light microscopy.

3. Experimental plasmolysis. Remove the slide from the microscope and remove the coverslip. Using forceps, transfer the epidermal tissue to the surface of a strong salt solution (1M KCl) that is in a Petri dish. After 2 min, transfer the onion epidermal tissue back to the slide, and observe and draw it under the microscope at 10x. Remember to record, in your notebook, the procedure you performed to obtain the sample.

Water potential⁹ is a physical-chemical term that allows one to quantify the propensity for net diffusive water movement. Typically, two forces are involved. First, water moves from a region of lower ratio of solutes:water to a region of higher ratio. Second, water moves from a region of higher hydrostatic pressure to a region of lower hydrostatic pressure. The net effect of these forces determines the direction of water movement. In the present case, the very high ratio of external solutes: water (KCl solution) “drew” water out of the cells. At first, higher hydrostatic pressure inside the cell also contributed to water egress, but once the membrane pulled away from the wall (plasmolysis), the pressures inside the cell and outside were equal, so only solute content was a factor. (Again, use of a pigmented vacuole allows easy observation of the cell’s shrinkage.)

⁶ Fig. 25-43; p. 577

⁷ p. 46

⁸ pp. 74-77

⁹ Fig. 4-5

Specimen 3: Potato Tuber¹⁰ Cells (observation of cell walls and amyloplasts)¹¹

1. Wet Mount. Using a razor blade, make a very thin slice of potato tuber and place the slice on a slide. Add the coverslip as usual and observe. Blot some of the water off of the slice, add a few drops of I₂KI onto the slice, observe at 10x, and draw at 40x.
2. Observations. Observe and label the cell walls, as before. Observe and label amyloplasts, the brownish purple plastids, organelles, that store starch.

Staining a specimen is a common procedure in microscopy. A stain (like I₂KI here) selectively increases the contrast of a selected cellular component or activity (like starch here).

Cell Division in Plants¹²

The cell cycle¹³. Growth of multicellular organisms such as plants results from nuclear division (≡mitosis¹⁴), cell division (≡cytokinesis, which is discussed in BOT 3015) and cell expansion. Cell multiplication follows a prescribed sequence, the cell cycle, which has two phases: interphase and mitosis. Mitosis and cytokinesis (which are generally synchronous in plants) result in the formation of two identical daughter cells. Haploid, diploid, triploid, tetraploid . . . cells can divide mitotically.

Meiosis¹⁵ and syngamy. Sexual reproduction involves the fusion of two haploid gametes (≡syngamy) to form a diploid zygote. Thus, the essence of sex is alternating meiosis (≡reduction division—one diploid cell forms four haploid cells; one tetraploid cell forms four diploid cells . . .) and karyogamy (≡nuclear fusion, to restore the diploid condition). The marvelous outcome is segregation of traits and independent assortment¹⁶, Mendel's two principles. Although the meiotic mechanism itself is generally similar among sexual organisms, the timing of meiosis and karyogamy varies dramatically¹⁷. BOT 3015L does not address the mechanism of meiosis (see BSC 2010/2011) in detail.

Mitosis. During interphase, each chromosome doubles so that each comprises two identical sister chromatids, which are joined at the centromere. Mitosis, which usually requires less than 1 h, begins when the chromosomes condense and thus become visible when stained. The replicated bipartite mitotic chromosomes are divided equally to daughter cells, as implied above. In the following procedures, observe the characteristic stages of mitosis in a root tip. Importantly, note that mitosis is a continuous process, but the observations are static¹⁸

¹⁰ Fig. 25-42, pp. 576-577

¹¹ Fig. 3-12, pp. 42-43

¹² Summary comparison: p. 161

¹³ pp. 58-60

¹⁴ pp. 61-67

¹⁵ pp. 141-143

¹⁶ Fig. 3-39, Fig. 3-40

¹⁷ In BOT 3015 and in subsequent units of BOT 3015L, three basic sexual life cycles will be studied. Now, study thoroughly Fig. 12-15.

¹⁸ An excellent animated graphic of the dynamic process in onion root tip can be seen at http://www.biology.arizona.edu/cell_bio/activities/cell_cycle/cell_cycle.html

Specimen 4: Growing root tips of onion (observation of mitosis)

1. Wet Mount. Remove a healthy white root from a green onion. Then, cut off and discard all except 1-2 mm of the apical end, which is retained on the slide. Add 1 drop of 1 M HCl (!!!) and heat gently for 1 min to fix the cells. Blot the root tip with tissue, add a drop of toluidine-blue to stain chromosomes, and gently heat the preparation again for 30 s. Blot away excess stain, and rinse the section with a drop of water. Make a wet mount. Then apply gentle pressure to the coverslip with a pencil eraser to squash and disperse the tip to essentially a monolayer of cells.
2. Observations. Observe the preparation under the 10x objective. (Recall, always set up the microscope with the lowest-magnification objective, then—step-by-step—increase the magnification.) Locate a dividing cell and observe and draw it using the 40x objective. Label chromosomes, cell wall, and stage of mitosis. Repeat until high-quality observations and drawings of cells in at least two of the following stages have been completed¹⁹:
 - a. Prophase—condensation of the chromosomes into microscopically discernable bodies, loss of nuclear membrane.
 - b. Metaphase²⁰—chromosomes are aligned on the equatorial plane.
 - c. Anaphase—chromosome division (sister chromatids separate, each becoming a chromosome of the respective nascent daughter nuclei).
 - d. Telephase—distinct daughter nuclei.

Review Questions

1. What plant-cell component(s) affect plant-cell shape, and how?
2. Which organelle in plant cells is primarily responsible for autotrophy? Give a brief description of the process in plants that makes them autotrophs.
3. If a plant and animal cell were both put into pure water, what do you expect to happen to the cells? What differences do you expect between the effects on the cell types and why?
4. Compare one chromosome of a mother cell to a daughter cell of mitosis and compare one chromosome of a mother cell to a daughter cell of meiosis, how are the chromosomes in these comparisons different between mitosis and meiosis?

¹⁹ Mitotic stages are diagramed on p. 148. Note, as indicated in BOT 3015, *slight* variations (e.g., timing of nuclear membrane disintegration) in mitosis exists among eukaryotes. The descriptions here apply to onion.

²⁰ Note the key difference in metaphase in mitosis in which homologous chromosomes are not paired and metaphase I in meiosis in which pairing of homologous chromosomes facilitates crossing-over. See Fig. 8-7