Laboratory 2: Microscopy and Observation of Cells
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Corresponding Readings:
Campbell Ch. 4

BIOL-100L

Safety Information:
- We will be using laboratory glassware such as microscope slides. Please wear closed-toed shoes to protect your feet from broken glass. You will not be allowed to enter the lab room wearing sandals, slippers, or flip-flops, so please remember to dress appropriately for lab.
- We will be staining cells with methylene blue stain. You are required to wear goggles and gloves when working with methylene blue. If you don’t know where these are, ask.

Learning Objectives
1. Identify the components of a compound microscope and describe the function of each.
2. Use proper technique to prepare and observe cells with a compound microscope.
3. Draw, identify, label and estimate sizes of cells and cell structures using a compound microscope.
4. Construct and label a graph with all required elements.

Introduction:
Cells are considered the fundamental units of life because an individual cell exhibits all the basic characteristics of all living things. Objects that are smaller than cells are important to the study of biology, but these smaller structures are not living. In this introductory lab exercise, we will observe both animal and plant cells.

Most cells are too small to be seen with the naked eye. Cells sizes are so small that they are usually measured in units of microns (um). One inch equals approximately 25 millimeters (mm), and one micron is a thousandth of a millimeter. Cells typically measure from one to several hundred microns. We will use compound light microscopes to observe cells; A light microscope can be used to observe objects as small as a single micron.

Cells exhibit amazing diversity. Different cells within the same organism may be very different from each other. These differences are the result of specialization for different functions. Cells of different kinds of organisms may also be very different. Differences in basic cell structure between organisms have been used to help classify organisms into groups. All cells have a thin plasma membrane that forms the outer boundary of the living cell. Some cells have thicker, rigid cell walls outside of the plasma membrane. Cells can be classified as being either eukaryotic (larger cells containing membrane-bound organelles such as nuclei, chloroplasts or mitochondria) or prokaryotic (smaller cells lacking membrane-bound organelles). The compound light microscope allows us to observe cell-sized structures and some of the larger structures within cells. Only the largest organelles such as the nucleus and chloroplasts are easily visible through a light microscope that magnifies to 400X. Smaller cellular structures can only be seen with an electron microscope.

Living cells can be observed using wet mounts, in which the living cells are immersed in a liquid. Samples of cells can also be preserved and placed on prepared slides using fixatives; these are called fixed specimens. Since many cells look transparent under the light microscope, it is sometimes necessary to use a stain to increase the contrast. Today, you will observe some of these techniques and become familiar with the use of the compound light microscope. To receive full credit for this lab, you must demonstrate your ability to find an object on a microscope slide using the light microscope. See the instructor to get this requirement checked off before leaving lab.
Exercise 3.1: Parts of a compound light microscope

Begin by familiarizing yourself with the parts and functions of the compound microscope, which uses multiple lenses to magnify an image. The compound microscope is expensive and relatively fragile. Please be sure that you never move the microscope unless you are supporting it by its base or its arm.

The light source is located at the base of the instrument. There is usually a control near the light source for varying the intensity of light. Too much light can wash out the image you are looking at, while too little will not give you a clear enough image. When you begin looking at a specimen, try turning the light down and then raise the light intensity until the image is clear.

The stage is the platform on which the sample is placed. There is usually a spring-loaded arm on the stage which holds the slide in a fixed position.* The stage can be moved using two knobs which move it backwards and forwards or left and right. The stage can also be moved up and down using the coarse and

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*Note: The image of the microscope and the labeled diagram are not a part of the natural text. They are described in the instruction.
**fine focus adjustment knobs.** Altering the distance between the lenses and the specimen brings the specimen into focus. The adjustment should be done with care so that the specimen does not come too close to the objective.

*NOTE: The clips on the stage hold the slide in place by applying pressure to the sides of the slides. Please do not try to force the slide under the clips. It is unnecessary and will eventually damage the instrument. Pull the clip towards you, position the slide into the corner of the stage, and then gently release the clip to hold the slide in place.

The **objectives** are lenses that gather light from the specimen and magnify the image. Objectives are expensive: treat them with care. On the side of the objective barrel, a set of labels describes various qualities of the objective. The **magnification** (e.g. "4X", 10X, or "40X") describes the power by which the image is magnified by the objective.

A second objective is the **eyepiece objective**. This is often a 10X objective. Multiply the magnifying power of the eyepiece objective (10X) by the power of the objective lens you are using (either 4X, 10X, or 40X) to get the total magnification. For example, if you were viewing a specimen using a 40X objective with a microscope that has a 10X eyepiece, the total magnification would be the product of the magnification values for the individual objectives, 40X times 10X = 400X. So you are observing the specimen 400X larger than how you would see it with the naked eye.

Identify the light source, light adjustment control, stage, objective lenses, eyepiece objective, coarse adjustment, fine adjustment, and stage knobs. Plug it in and turn on the light source.

**Exercise 3.2: Using a compound light microscope**

Obtaining a good image using the compound light microscope can take some practice. Before you begin looking at cells, go through the following exercise to familiarize yourself with how to correctly use a compound microscope.

1. Obtain a compound microscope, being careful to support the base. Review the descriptions above and the figure on page 42. Use the **coarse adjustment knob** to move the stage away from the objectives. Rotate the objectives so that the **lowest-power objective (4X)** is in the viewing position. This is usually the shortest objective and is colored red on many of our microscopes.

2. Obtain a slide with a printed letter 'e' on the slide. Open the spring loaded arm on the stage and place the slide with the letter 'e' facing right-side-up, into the corner position on the stage and return the clip arm so that it presses and holds the slide into the corner position. Use the **stage controls** to move the stage sideways, toward you, or away from you, to center the 'e' within the light shining through the stage.

3. Look through the eyepiece and use the **course adjustment knob** to raise the stage up towards the 4X objective lens until you see the "e". Bring it into focus as much as possible using the **coarse adjustment knob**. Then use the **fine adjustment knob** to bring the 'e' into the clearest focus possible. It is common that partners may need to slightly move the **fine adjustment knob** to accommodate for their own vision.

**If you have difficulty finding an object on a slide, try the following techniques:**

- Always start on the lowest objective power (i.e.4X), if you lose sight of your object go back down to a lower powered objective – equivalent to zooming out.
- Position the slide so the **edge of the coverslip** or slide is directly under the lens, and centered over the light coming through the hole in the stage.
- Use the coarse adjustment knob to raise the stage so it is as close to the lens as possible without touching the lens. Most microscopes have been preset to prevent the slide from touching the lens at the lowest power.
While looking through the eyepiece, use the course focus knob to slowly lower the stage down you see the edge of the coverslip (it should look like a solid line).
- Bring the edge line into focus with the coarse adjustment and then with the fine adjustment knobs.
- Move the stage controls away from the coverslip edge to look for the object you want to find.

5. Draw a sketch of the 'e' as you observe it through the microscope under medium power. Record the objective power used.

6. Does the 'e' appear right-side-up or upside down? Forward or backward?

7. If you move the stage to the right, which way does the microscope image appear to move?

8. Now you will move to a higher magnification: Look into the eyepiece. Center letter "e" in the middle of the field of view. Carefully rotate the objectives clockwise so that the next higher-powered objective lens (10X) is in the viewing position. Use the fine adjustment knob to bring the "e" into clear focus. NOTE Only use the fine adjustment knobs with the high powered objective lenses as they are longer and can crack the slides.

9. Did the field of view of the “e” become dimmer or brighter when you used the higher-power lens?

10. Now use the light adjustment dial to readjust for the optimum amount of light.

Exercise 3.3: Observing a Wet Mount of Human Cheek Cells

1. Mix a drop of water and a drop of methylene blue on a clean microscope slide.

2. Use a toothpick to rub the inner lining of your cheek. The best way is to brush the broad side of a toothpick across the inside of your cheek, do not gauge with the point or scrape a raw spot into your cheek.

3. Swirl the side of the toothpick with the cells in the water/methylene blue mixture. Obtain a coverslip and slide one edge up against the liquid so the liquid wicks across the coverslip edge, gently lay the coverslip down at an angle over the stain and cell mixture. This helps to avoid introducing air bubbles to the liquid. Air bubbles under the coverslip look like perfectly round, black circles. These are not cells, nuclei, or anything else you might be interested in.

4. Observe the wet mount under the microscope. Start with lowest magnification and work your way to higher magnifications. The nucleus, plasma membrane and cytoplasm should be visible.

5. Have the instructor check your work to make sure you are observing cheek cells. Draw several cells you observe and label the cell membrane, the nucleus, and any other cellular structures that you can see under high power.

6. Use the pointer visible in the eyepiece to estimate the size of a typical cheek cell. Your instructor will provide a scale for calculation. Record this value beside your drawing of the cell.

7. Place the slides and toothpicks in the designated beaker of bleach water when finished.

Exercise 3.4: Observing Osmosis in Elodea Water Plant Cells

1. Place a drop of water on a slide. Using forceps, pull off a small, young leaf from the tip of an Elodea plant in fresh water (0.0M solution) and place it on the drop of water. Lower a cover slip over the leaf.
2. Use proper microscopic technique to observe the Elodea cells under high power. Note that there are two layers of cells. Focus so a single cell layer is distinct. Observe the cells; you may be able to observe “cytoplasmic streaming” – the movement of cytoplasm and suspended chloroplasts. The cell membra

3. **Draw several cells. Label the cell wall, plasma membrane, chloroplasts and vacuole.** The plasma membrane in healthy plant cells is pressed against the inside of the cell wall and may not be visible. Estimate the length and width of a typical Elodea cell and record this by your drawing.

4. Repeat the procedure to observe Elodea from a 0.4M sucrose sugar solution. Osmosis occurs in cells placed in solutions that have a higher solute concentration than cell cytoplasm. Water moves out of the cell and causes the plasmalemma to shrink away from the cell wall (a process called plasmolysis). **Draw several plasmolyzed cells. Label the cell wall, plasma membrane, chloroplasts and vacuole.**

5. Determine the percent of cells that are plasmolyzed in the 0.4M sucrose solution by counting the total number of cells in one field of view under high power and the number of cells that are plasmolyzed.

6. Obtain Elodea leaves from 0.1M, 0.2M and 0.3M sucrose solutions. Use the same procedure to determine the percent of cells plasmolyzed in each of these solutions.

7. **Construct a graph of percent plasmolysis of Elodea cells as a function of solute concentration.** Discuss your results.

**Exercise 3.5: End-of-lab Quiz**

When you have completed all drawings and assignments, signal your instructor or teaching assistant. S/he will examine your drawings and graph and assign a score. Each student will then be quizzed on microscopy. You will individually be asked to identify parts of the microscope and their function, and to use proper microscopic technique to focus on an object under high power. You will be assigned another score based upon your performance.

**Lab Cleanup:**
- Use lens paper to clean objective lenses on microscope. Wind up electrical cord and return microscope to cabinets.
- Return “e” and colored thread” slides to box
- Wipe off your area of the lab bench with disinfectant and paper towels.
- After you are done, wash your hands before leaving lab.