Biology 196 Light Microscopy

Prior to arriving in lab, bring in a sample of *stagnant water*. Good sources of water teeming with live organisms; ponds, lakes, birdbath, water filter in your fish tank. Not tap or flowing water.

Objectives:

- Learn the basic parts of a compound light microscope and their function.
- Learn how to focus and view specimens using a light microscope.
- Examine preserved specimens from all 6 Kingdoms.
- View living specimens by preparing wet mounts.
- Read Campbell's Biology concept 6.1, Appendix D and E (9^{th} edition)

Introduction

The world is full of things too small to be seen by the naked eye; this exercise will acquaint you with these unseen creatures. Microscopes are tools used to enlarge small objects so they can be studied. The compound light microscope is an instrument containing two lenses for magnification, and a variety of knobs to resolve (focus) the image. In this lab, we will learn about the proper use and handling of the microscope. This will prepare you for future work with microscopes throughout your academic career.

PART 1 - THE COMPOUND LIGHT MICROSCOPE

A. Identification of parts

Identify and learn the function of each part on your microscope. Find all of the parts listed in the table below on your assigned microscope, using Figure 1 as a guide.

Table 1. Compound Light Microscope Parts and their Functions.

Microscope Part	Function
Eyepiece	Contains the ocular lenses. This is the part you look into.
Ocular Lenses	Our microscopes have 10X or 15X oculars. All of our microscopes are **binocular**, since they are equipped with two ocular lenses.
Diopter ring	Allows for the possible inconsistencies of our eyesight. If your microscope has this, you may twist the ocular lens on the right to focus it separately.
Head	Upper part containing the oculars and rotating nose piece.
Arm	Narrow, vertical part connecting the head and the base. Provides a carrying handle.
Nosepiece	Revolving device that holds objective lenses. The viewer can rotate the nose piece to change objective lenses.
Mechanical stage	Flat, movable area that holds and supports microscope slides.
Stage clips	Keeps the slide in place on the stage. The clips are spring loaded.
Stage control	Knob that allows the mechanical stage to move up/down and left/right
Iris diaphragm lever	Found just beneath the opening in the stage. Controls the amount of light used to illuminate the object. It also can change the angle of the light rays creating contrast, making transparent items more visible.
Condenser	Below the stage. It features B for brightfield, D for darkfield and various other settings that bend or dim the light.
Condenser adjustment knob	Allows the viewer to position the condenser. Start with it closest to the stage.
Coarse adjustment knob	At the base of the arm. Will raise and lower the stage, changing its distance from the lens. Used to bring object into initial focus; used only with the scanning (4X) lens
Fine adjustment knob	Raises and lowers the stage in smaller increments, used to bring object into final adjustment once the initial focus has been made. Used with any lens.
Lamp	Light source for illumination of the specimen.
Rheostat	Dial that adjusts the light intensity.
Base	Supportive, flat surface of the microscope that rests on the table.
Objective lenses	
Scanning objective	4X magnification. ALWAYS used for initial focusing of the slide. Should be in place before the slide is inserted and again before it is removed. Used to view a large portion of the slide (large field of view, low magnification).
Low power objective	10X magnification. Longer than and used after the scanning power objective.
High dry power	40X magnification. Used to view an object in even greater detail.
Oil immersion objective	100X magnification. Designed to be used only in conjunction with immersion oil to view specimens with the greatest magnification.

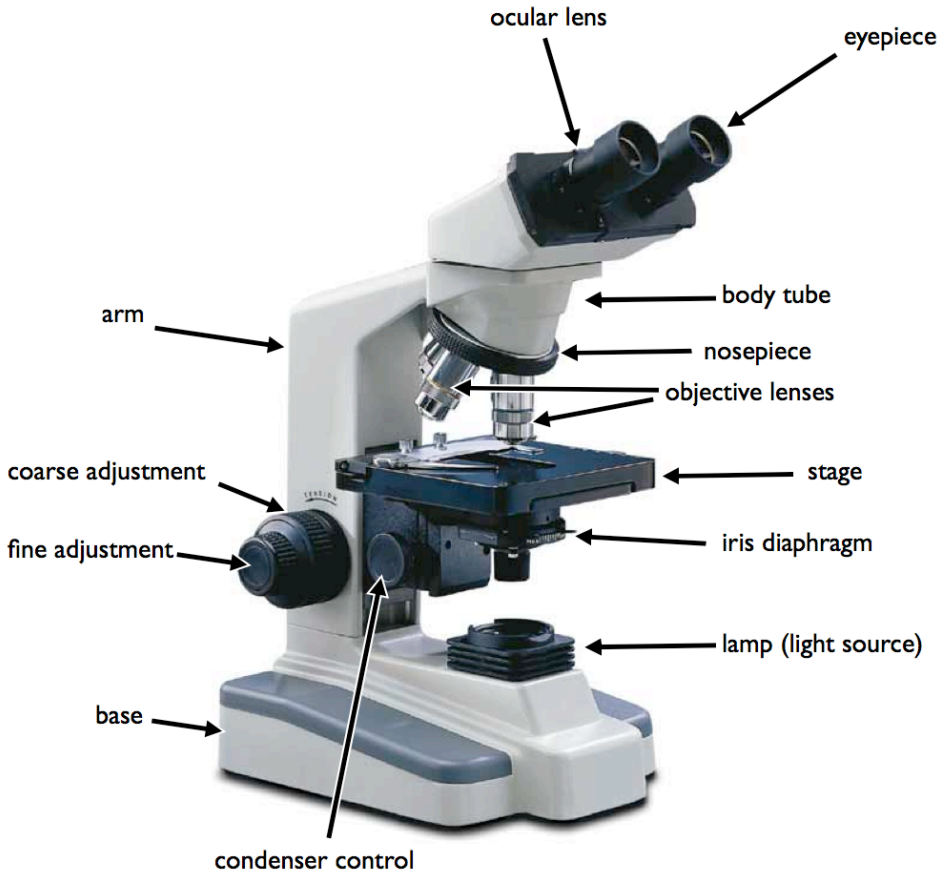

Figure 1- Typical Binocular Light Microscope

B. Proper Care and Handling of the Microscope

1) Always carry the microscope in front of you with one hand under the base and the other grasping the arm.
2) Do not remove parts of the microscope
3) Report any malfunctioning microscope to your instructor. You will not be penalized. Also report broken slides and slides in which you can't find the specimen.
4) Do not tilt the microscope. Keep it flat on the bench top.
5) Keep the stage clean and dry to prevent rust.
6) Do not use the oil immersion objective without oil. It won't work properly. If you use oil, be sure to clean the slide and the objective lens after use.
7) The ocular lenses, objective lenses and the microscope stage can be cleaned using Kimwipes with glass cleaner on the paper. Do NOT spray cleaner onto the scope directly. Slides are also cleaned this way. Paper towels can scratch glass.
8) The scanning objective (shortest, 4X) should be in position at the beginning ***and*** the end of use.

C. Focusing the Microscope

1) Keep both eyes open when looking through the eyepiece. Closing one eye can result in eyestrain and headaches. Remember to adjust the distance between the oculars if you do not see light through *both* eyes when looking through the oculars.
2) With the coarse adjustment knob, drop the stage to its lowest point.
3) Make sure the 4X objective is in place; rotate the nosepiece until the scanning power objective is in place over the stage and you hear it click into position.
4) Place the letter "e" slide in the microscope specimen side up. Use the stage clips to secure it.
5) Use stage control knobs to move the slide until the "e" is centered over the light.
6) Raise the stage with the course adjustment knob until the slide and objective lens are at their closest point. While doing this, watch from the side, NOT by looking into the eyepiece.
7) Looking into the eyepiece, adjust the light intensity using the rheostat dial (~5-7). Overly bright light may hide the object and cause eyestrain.
8) While looking through the oculars, slowly turn the coarse adjustment knob (moving the stage downward) until the specimen is in focus.
9) Slowly turn the fine focus adjustment knob to sharpen the image.
10) Holding the rotating nose piece (not the lens), rotate the nose piece to the next lens (10X) until it clicks in place.
11) Slowly turn the fine focus knob ONLY to focus the specimen at this higher magnification. Compound light microscopes, such as ours, are **parfocal**. That is, once the object is in focus with the scanning objective (4X), it should also be in reasonable focus at medium and high powers. Once the initial focusing is completed at scanning power, the course adjustment knob is not used again.
12) The light intensity may need to be increased as the magnification is increased.
13) If the specimen is no longer visible at 10X or 40X, you will need to refocus the specimen starting with the scanning (4X) lens. Do not yet attempt to use the 100X oil immersion lens.
14) Notice the change in the **working distance** (the distance from the slide to the objective lens) as you change from one objective lens to another.

Microscope trouble shooting hints:

- If you see a grainy texture, try moving the slide with the stage controls. If the slide moves but the texture doesn't, your condenser is out of focus and you are seeing a lighting artifact.

- If you see a perfect clear circle, you are probably looking at an air bubble.

- If you only see part of a circular field, the nosepiece is probably not in proper position. Make sure the objective is clicked into position.

- The lowest power objective (4x, scanning) should be about 1cm, or the width of your pinky, above the slide when the object begins to come into focus.

15) Practice using the different settings on the condenser and try moving it up and down. Generally, you will get best results with the diaphragm all the way up and the condenser in “B” (brightfield) position.

16) Draw the letter "e" as it appears on the slide *without* the aid of the microscope.

17) Now draw the letter “e “while peering through the microscope at 4X. In what ways has the appearance of the letter "e" changed?

Viewed by naked eye

Viewed with scanning lens

Move the slide to the right as you are looking through the ocular lenses. Which way does the “e” move? _____

Move the slide toward you. Which way does the “e” move? _____

It may take some time to get adjusted to these movements. Practice until it becomes easy to move the “e” in the direction you desire.

Now change to the low power (10X) objective. On your drawing of the letter e as it was observed with the scanning power, *draw a circle around the portion of the letter that you can now see with high power magnification*.

18) When you have finished your observations of this slide (or any slide), rotate the nosepiece until the scanning objective (4x) clicks in place and then remove the slide from the stage.

D. Magnification and Field of View

Total magnification refers to the magnification of the specimen when viewed through the microscope compared to the unaided eye. A compound microscope uses two magnifying lens simultaneously. Total magnification is calculated by multiplying the magnification of the ocular lens by the magnification of the objective lens. Calculate the total magnification of the following:

TABLE 2 **Ocular mag x Objective mag = total mag**

	Ocular Magnification	Objective Magnification	Total Magnification
Scanning objective	Hint: 10X or 15X		
Low power objective			
power objective			
Oil immersion			

Resolution: the shortest distance between two points on a specimen that can still be distinguished by the observer or microscope as separate points.

Describe the relationship between magnification and resolution:

_____

Field of view is the area of the slide that can be viewed when peering through the oculars. Field of view decreases any time an object is magnified.

Describe the relationship between magnification and field of view:

_____

Consider the high dry power objective lens.

Is the field of view *larger or smaller* than the low power objective lenses?

Is the working distance *greater or lesser* than the low power objective lenses?

Describe the relationship between the working distance and total magnification.

_____

E. Depth of field

Depth of field: the vertical distance that remains in focus at one time.

1) Obtain a colored thread prepared slide. With the scanning power, find a point where the threads cross. Slowly focus up and down. Notice that when one thread is in focus, the other seems blurred. Determine the order of the threads.
2) Switch to low power and notice that the depth of field is more shallow. There is less distance in focus at one time.
3) Switch to high dry power and repeat the process, recording your results.

Thread position	Under Scanning lens	Under High Power
Top		
Middle		
Bottom		

PART 2 – SURVEY OF CELLS IN ALL 6 KINGDOMS

Introduction

Cells, the basic unit of life, are found in every living organism. Each individual cell is a complex system designed to perform specific functions. Some organisms are multicellular while other organisms are unicellular, or only made of a single cell.

Cells are divided into two major categories; prokaryotic cells and eukaryotic cells. **Prokaryotic cells** are found in the domains Eubacteria and Archaea. These cells lack membrane-bound organelles, but do contain DNA and ribosomes. Most prokaryotic organisms are unicellular. **Eukaryotic cells** are found in the domain Eukarya, and are typically more complex than prokaryotic cells. These cells have membrane-bound organelles, each with its own specific structure and function. Most eukaryotic organisms are multicellular, with one large exception being the protists.

Now that you have perfected your microscope skills, use them to visualize cells in all 6 kingdoms of organisms, making note of cell size, shape, and any visible intracellular structures.

A. Method – Oil Immersion Lens

In order to view smaller specimens, such as Prokaryotic cells, the 100X Oil immersion lens will need to be used.

When a light ray travels through a glass slide it comes into contact with air and bends. This is called **refraction.** Refraction will distort the image of the specimen. The use of immersion oil reduces this refraction, giving a clearer view of the specimen. Oil immersion is used only at very large magnifications that require high resolving power, such as when using the 100X objective lens.
Before using the 100X oil immersion lens for the first time, let your instructor know so they can observe.

1) Follow standard procedures to obtain a focused image using the 40X objective.
2) Rotate the nose piece so that the specimen is between the high dry power (40X) and oil immersion lens.
3) Place a drop of oil on the slide where the light is visible.
4) SLOWLY rotate the oil immersion lens over the specimen. The lens must hit the oil,

otherwise the stage might be moved, ruining the focus of the specimen.

5) The light intensity may need to be increased.
6) Use ONLY the fine focus adjustment knob to bring the specimen into focus.
7) If the specimen is lost from the field of view, you MUST remove the slide properly, clean it of oil, and start over. If you try to change lenses with the oil present, it will get onto the other lenses, potentially ruining them.
8) To clean, blot the excess oil with kimwipes. Once no more oil is visible, spray cleaner solution onto a kimwipe and use it for a final, gentle cleaning. Remember to clean the lenses, slide, and stage.

B. Method – Wet Mount

In order to view living organisms, **wet mounts** must be prepared on microscope slides. Many living cells are colorless; therefore to view them under a microscope, stain must be used.

1) Obtain a clean slide. Add a drop of specimen, if it is in solution. If the specimen is dry, first add the specimen, then add 1-2 drops of water.
2) Take a clean coverslip and contact the edge of it to the edge of the water drop. Slowly lower the coverslip at an angle down over the specimen. Performing this slowly will help ensure the absence of air bubbles.
3) Wick the excess fluid if necessary by placing a folded kimwipe at the edges of the coverslip. Do not press on the coverslip.
4) When finished viewing under the microscope, make sure you dispose of the slide in either the glass disposal bin, or the biohazard glass disposal bin if so directed by your instructor.

C. Method – Staining Using Methylene Blue

Certain cell structures may be highlighted by using stains such as methylene blue or IKI (Iodine-Potassium Iodide). Methylene blue is useful for viewing the otherwise nearly invisible nucleus of the cell.

1) Locate the Coplin stain bottle and rinse jar filled with distilled water.
2) Place the specimen on your slide and allow to air dry and/or heat fix if directed by your instructor.
3) Carefully place 1-2 drops of the stain directly on the specimen.
4) Carefully lower a coverslip at an angle over the wet sample to minimize air bubbles.
5) If there is excess stain around the coverslip, wick dry with blotting paper, being

careful not to disturb the specimen. Make sure you replace the lid on the stain.

6) For plant specimens, allow the slide to sit for ~10 minutes before viewing. This allows the stain to permeate the cell wall. Animal cells can be viewed immediately. If the stain is too dark for easy observation, carefully add a drop of water along one edge of the cover slip. Place the edge of a kimwipe on the opposite edge of the cover slip. The kimwipe will wick the stain from under the cover slip and pill the water under the cover slip. Typically animal and plant cells are visible using 40X-100X magnification. (Remember how to calculate magnification?)

A survey of different kingdoms and the types of cells found in each.

Make drawings of various cell types. You should find all the cells on 4x (scanning) but draw them using the 40x (high dry) or 100x (oil immersion lens) for bacteria. The goal is to compare cell types and recognize structures. *Label all your drawings with the source of the specimen, total magnification, and any visible structures*. Your instructors may ask you to turn in these drawings or make similar drawings for next week's quiz, so put some effort into it.

Start with the largest cells that are easiest to find. Then move down the kingdoms and domains to smaller, evolutionary precursors of our own cells. Pond water is difficult and likely to take the most time, so view all other slides first. When preparing your own slides, more is NOT better. Putting big chunks of tissue on slides makes it impossible to detect single cells.

D. Animal cells – source: you. Domain: Eukarya Kingdom: Animalia

1) Using a clean toothpick, gently scrape cells from the inside of your cheek. Smear the cells onto the slide. Set aside and allow to air dry. Handle only your own cells.

2) Add a small drop of methylene blue and a coverslip. Observe under the microscope. Make use of the iris diaphragm to increase contrast.

3) Draw 2-3 cheek cells in the space provided on the next page. Label the sketch and note the magnification used (10x or 40x objectives are best). Label all visible cellular structures.

4) **Disposal of cheek cell preparations.** These are cells and fluids from the human body and considered **biohazardous.** Throw toothpicks and slides into the biohazard *sharp* disposal containers. Decontaminate your microscope with a kimwipe and Windex to kill germs and place in the biohazard bin.

E. Plant cells – source: onion. Domain: Eukarya Kingdom: Plantae

1) With a scalpel, strip a small, **very, very thin** transparent layer of cells from the inside of an onion. Place it gently on a clean glass slide.

2) Add a drop of methylene blue. Cover with a cover slip let sit for 10 minutes prior to viewing. Locate the cell wall and the nucleus near the cell wall. *Note some obvious differences (i.e. size, shapes, location of nucleus, etc.) between the plant cell and the animal cell. Make a labeled sketch in the space provided below.* If available, an individual *Elodea* leaf can be viewed as well.

Plant leaves - source: prepared slide, cross section of *Ligstrum* or privet leaf.

3) Find the following tissues. For help, refer to your text or a poster in the lab.

Epidermal tissue - Epidermis - This protective layer may contain specialized cells (guard cells) that function to open and close pores in the leaves (stomata). These pores allow gas exchange and are found on the undersurface of leaves.

Ground tissue - Palisade parenchyma - the upper most layer of the ground tissue contains the cells that are actively involved in photosynthesis. These cells contain chloroplasts.

Vascular tissue - Vein - contains both xylem and phloem cells.

Drawing of animal cell with parts labeled and magnification noted.

Drawing of plant cells with parts labeled and magnification noted.

F. Protists – source; pond water. Domain: Eukarya Kingdom: Protista

1) Prepare wet mounts of living specimens, either those available in lab or those brought by the student. Students often collect the best samples.
2) Use a disposable pipette to remove organisms from the bottom of the container. Gently squeeze the bulb before putting the pipette into the container. Siphon up a few drops directly from the bottom of the container, **without stirring** the water around. If the container is stirred, living organisms are much harder to find.
3) If the specimen is alive and moving quickly, such as an organism in pond water, add proto-slo (or detain). This is a viscous liquid and slows their movements. Do NOT stain pond organisms if you want to view them moving.
4) Add a coverslip and observe. Locate the organisms first using the scanning objective. Center them in the field of view, and go to higher powers.
5) Either darkfield "D" or phase (green or red triangles) on your condenser work best for finding and viewing unstained protists. Living organisms are either green and/or moving under their own power. This should help you distinguish them from air bubbles, dust, dead things, etc.
6) Make notes on what you observe; their behavior, colors, speed, etc. Note: Not all of the organisms you are viewing are necessarily protists. You can draw protists that your classmates find as well.

G. Fungi. Domain: Eukarya Kingdom: Fungi

1) View the typical structure of fungus on the posters in lab. Fungal cells are often filamentous. They have a cell wall made of chitin that surrounds their cell membrane. They reproduce by releasing spores.
2) View the prepared slides of *Rhizopus* sporangia & *Aspergillus* conidiophores. READ the label on your slide, you may be viewing a fungus growing on a plant.
3) If time allows, prepare a slide from the mushroom cap provided. Cut the mushroom in half and start from the center of the cap. With a scalpel, strip a **very thin** layer of cells from the mushroom. Place it gently on a clean glass slide. View with the **dissecting** microscope.

   Draw the gill portion and spores if visible.

H. Bacteria. Domain: Prokarya Kingdom: Eubacteria

Bacteria are the only prokaryotic cells you will look at today. The only non-membrane bound organelle they contain is the ribosome, which cannot be seen with a light microscope. Due to their small size, bacteria are difficult to view with light microscopes.

1) Obtain a prepared slide of *E. coli* and/or *S. pneumonia.* These are some abnormally large bacterial cells. They are visible at scanning power, and are more easily distinguished with oil immersion.

Drawing of bacterial cells. (Remember to label the total magnification.)

Clean-Up

When you have completed your observations, clean all the objectives and ocular lenses with cleaner (Windex) soaked kimwipes. Wipe the stage and the slides. Return the slides to the correct slide tray. Discard your wet mounts in the glass disposal bin. Lower the microscope stage and point the 4x objective down over the stage. Wrap up the cord, apply the dust cover and return the microscope. **Your instructor may take away some or all points you earned today for not cleaning your microscope properly.**

Remember the Objective: Learn the basic parts of a compound light microscope and their function. Subsequently use the microscope for the discovery and recording of cells and structures too small to be seen by the naked eye.

Study Checklist

To perform well on the next quiz, you should have a thorough knowledge of the following:

- Rules of microscope use.
- Identify the various parts of the microscope and their functions.
- Know the 4 different objective lenses by name and power, and how they affect the total magnification.
- Know how to focus the microscope. How is the image of the letter "e" affected by magnification and movement of the slide.
- Define the following vocabulary words, and describe the relationships between them: **magnification, depth of field, field of view, resolution, parfocal, working distance.**
- Procedure for making wet mounts.
- Why do we use stains?
- Differences and similarities between prokaryotic and eukaryotic cells
- Differences and similarities between all 4 kingdoms of eukaryotic cells. Make special note of visible organelles and cell appendages.
- Create an evolutionary tree with domain and kingdom names and list the examples you worked with.

MICROSOCOPE APPENDIX, additional info

Check the magnification of the ocular lenses on your microscope (10X or 15X).

The nosepiece holds 4 objective lenses. **4x** *scanning* is the shortest and has red writing on it. **10x** *low power* is the next longest and has yellow text. **40x** *high dry* has blue text. **100x** called *oil immersion* can only be used with oil on the microscope slide.

The diaphragm is fitted with a wheel with various markings. **B** is for brightfield, which allows all the light from the light source to pass directly through the object. It is used most frequently. This is great for stained, fixed cells and specimens.

D is for darkfield, which makes the background black and the objects white. This is great for finding live, unstained organisms that may be moving. The other settings are for phase contrast (green or red triangles); they increase the resolution of the microscope by bending the light from the source before it passes through the specimen. These are very helpful functions but require you to experiment to get the best image.

The diaphragm can be raised and lowered using the silver knob under the stage. It is best if you raise the diaphragm all the way, and then FORGET that it is movable. Moving the diaphragm for beginning students only complicates focusing.

Also on display are dissecting scopes, they do not magnify as greatly as the compound scope, but allow you to see entire organisms without killing or preparing them.

Dissecting microscope