

EXERCISE 4

Microscopy & Cells

LEARNING OBJECTIVES

- Learn to use a compound microscope
- Make basic slide preparations (wet mounts).
- Distinguish between plant and animal cells based on microscopic observations of their structures.
- Microscopic observation of bacterial cells
- Observation of some other eukaryotic cells: microscopic algae and protists.

Answer these questions before you come to lab:

1. What are the three components of the cell doctrine?

2. Name the two types of cells found in the living world.

3. State three differences between these types of cells

4. State the four structures that are common to all cells.

5. State three differences between plant and animal cells.

PART 1: THE COMPOUND MICROSCOPE

Care and maintenance of a microscope

- Always treat your microscope with care. When moving the microscope, use two hands. Grasp the arm of the microscope with one hand and support the base with the other hand. ***Do not swing it!***
- The lens surfaces must be treated with great care, as they can easily be scratched or chipped. Use ***ONLY*** lens paper to remove dust if needed. ***NEVER use paper towels, handkerchiefs, shirt tails, Kleenex, alcohol, or blow moist air onto any lens as a way of cleaning it.***
- At the higher magnifications, always refocus with the fine adjustment ***only***. Never use the coarse adjustment at high power. If you do, you run the risk of smashing the objective into the slide and causing irreparable damage to both! Course adjustment should only be used during initial focusing with the low power objective.
- **Do not force** anything-lenses, knobs, levers- ask for help.
- Before storing the microscope, turn the objectives to the ***lowest power***. Clean all lenses with lens paper. Remove all slides from the stage. Secure the power cord, and replace the microscope in the cabinet with the eyepieces facing ***inwards***.

A. Identifying the Parts of the Microscope

This type of microscope is called a compound light microscope. Compound, because it uses two lenses to magnify and show details of specimens that are too small to observe with the naked eye. The purpose of a microscope is mainly magnification. One of the lenses is in the eyepiece and the other is the objective lens. The microscope uses light as the source of illumination of the specimen.

Locate all the parts of the microscope as your instructor describes them:

Eyepieces: The eyepiece contains the ocular lens system and is one of the two lenses used for magnification. Engraved on the side of the ocular lens you will see its magnification. What is the magnification of your ocular lens system? _____

Nosepiece: This is a revolving circular mechanism that holds the different objective lenses. Rotating the nosepiece changes the objective lens. The objective lens is in place when it is directly over the stage and you will hear a click when it is in place.

Objective lenses: These are individual lenses attached to the nose piece. A magnification number is indicated on each objective lens. Your objective magnification values are _____, _____, _____, and _____.

Total Magnification: To obtain the total magnification produced by both the objective and ocular lenses, you simply multiply the two values.

Eyepiece Magnification	Objective Lens Magnification	Total Magnification
X	Scanning X	X
X	Low power X	X
X	High power X	X
X	Oil immersion X	X

Note: The oil immersion lens is not used in an introductory biology lab. **DO NOT ATTEMPT TO USE IT AND DO NOT PUT IT IN PLACE.**

Stage: Also called the mechanical stage. This is the surface that supports and secures the slide with the help of the **stage clips**.

Stage Controls: these are usually located on the side of the stage. Front/back controls move the slide front to back and vice versa. Side/side controls move the slide from side to side.

Condenser: This is located under the stage and focuses the light from the lamp through a hole in the stage and onto the specimen. It can be used to adjust the quality and amount of light passing through the specimen. There is a **condenser adjustment knob** that may be used to raise or lower the condenser.

Iris diaphragm: Located under the condenser, this is used to adjust the intensity of light passing through the specimen. It is opened or closed using the **iris diaphragm lever**.

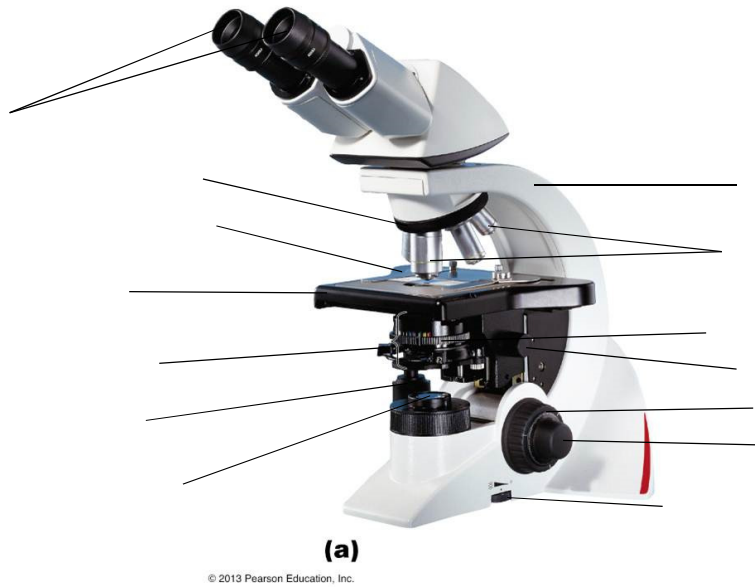
Coarse-Adjustment Knob: This large knob, located on the arm, adjusts the distance between the stage and the objective lens in large increments. It is used initially to bring the specimen into focus. It is dangerous to use this knob when the objective lens is already near the slide. It should be turned very slowly to avoid breaking the slide.

Fine-Adjustment Knob: This is the small knob attached to the coarse-adjustment knob. It adjusts the distance between the stage and the objective in small increments. It is typically used after the objective lens is already near the slide and the specimen is almost in focus. It should be turned very slowly to avoid breaking the slide.

Lamp: Light source located under the condenser. There is a switch to turn it on and off.

Rheostat: Regulates the intensity of the light ('light dimmer').

B. Label the parts of the microscope:



C. Learning to focus the microscope

1. Plug in the microscope, turn on the light, open the iris diaphragm and raise the condenser. Rotate the nosepiece so that the scanning objective (4X) is in place over the stage.
2. Obtain a prepared slide of the letter **e**.
3. Place the slide on the stage so that the label is on your left. What is the orientation of the **e**? Is it upside down or the right way it? (Look at it on the stage on, we are not looking down the microscope yet).
4. Using the stage controls, move the slide so that the **e** is directly in the center of the circle of light.
5. Using the coarse adjustment, while looking down the microscope through the eyepiece (use both eyes), bring the stage up towards the objective until you can see the **e**. Bring it

into as sharp a focus as you can with the coarse adjustment knob and then fine tune with the fine adjustment knob.

6. What is the orientation of the **e** now? Move the slide to the right. Which way does it appear to move? This is called **inversion** and refers to the fact that the image you see under the microscope is not only inverted but also reversed.

Sketch the **e** in the space below: Magnification?

7. **Increasing magnification:** The system of objectives is constructed so that they are **parfocal**. **Parfocal** means that an object remains relatively in focus when you change objectives. The area of the microscope slide that can be viewed through the microscope is the **field of view**. When you switch from a lower magnification objective to a higher magnification objective, the size of your field of view under the microscope is greatly reduced. Therefore, always center the object under observation in your microscope field of view before changing objectives. This will reduce the chances that you "lose" your specimen somewhere outside the field of view. **Note:** The greater the magnification, the smaller the field of view.
8. Move your **e** until it is centered in the field of view and in focus, with the 4X objective in place. Next, carefully rotate the nosepiece until the low power (10X) objective is in place. Focus.

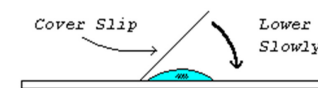
Sketch its appearance in the space provided: Magnification?

9. Center the **e** again and move the nosepiece so that the high power (40X) is in place. Focus. What is the magnification of your specimen? Describe what you see and explain why this is all that you can see at this magnification.

10. **Depth of focus:** Obtain a slide of colored threads. Find a point with the low power where the threads intersect. Slowly focus up and down. Notice that when one thread is in focus, the others seem blurred. The vertical distance that remains in focus at one time is called the **depth of focus**. Switch to high power and notice that the depth of focus is more shallow (decreases) with high power than with low power. Determine the order to the threads and complete the chart below.

Order of threads

Depth	Thread color
Top	
Middle	
bottom	



- The specimen is now ready to place on stage for observation with the microscope.
- View under all magnifications, starting with 4X.
- Sketch what you observe under 100X or 400 X total magnifications in the space below. You only need to draw **four or five cells accurately**. Label the cell wall, chloroplasts, and the cytoplasm.

PART 2: OBSERVATION OF CELL STRUCTURE

Now that we know how to use the microscope we will use it to observe some different types of cells. Many of the organelles in cells are much too small to be seen with the magnifications available on these microscopes. Observe and record all of the structures that you can see in each of the cells. Also note any differences between the cells that you observe. To view the cells under the microscope we first have to place the specimen on a slide by making a temporary wet mount.

Exercise 1: Plant Cells

Making a Temporary Wet Mount of Elodea (water plant)

- Place a drop of water in the center of a microscope slide.
- Using tweezers pick up a leaf of *Elodea* and place it in the drop of water.
- Hold a cover slip between your thumb and index finger to one side of the specimen at about a 45° angle. Slide the cover slip toward the drop of water and specimen until it contacts the drop of water.
- Gently lower the cover slip onto the specimen and the drop of water, trying to avoid large air bubbles. Air bubbles appear as black rings under the microscope and are often mistaken for something more exciting! More water may be added at the edge of the cover slip if needed to fill in any air spaces.

100X

400X

Onion cells

- Make a temporary wet mount of small piece of onion skin. Use skin only do not include any of the tissue from the onion. The thinner your specimen, the better the cells will be seen. Use a drop of iodine to make the mount.
- Sketch what you observe under 100X or 400 X total magnifications in the space below. You only need to draw **four or five cells accurately**. Label the cell wall, cytoplasm, and the nucleus.

100X

400X

How are these two kinds of plant cells different?

How are they the same?

Why are chloroplasts absent in the red onion cells?

IMPORTANT NOTE: Wear gloves. Human cheek cells experiment – Toothpicks and coverslips should be disposed in sharps container. Slides are to be placed in 10% bleach solution.

Exercise 2: Human cheek cells

1. Gently scrape the inside of your mouth with a toothpick to obtain epithelial cells.
2. Swirl the toothpick in a drop of drop of methylene blue on a clean slide.
3. (Dispose of toothpick in Sharps container and cheek cell slide in 10% bleach solution.)
4. Carefully add a cover slip as described above and examine the mount at all levels of magnification.
5. Draw four or five cells at highest magnification, labeling the cell membrane, cytoplasm, and nucleus.

400X

How are these cells **similar to** and **different from** the onion cells and the *Elodea* cells?

Exercise 3: Prokaryotic Cells-Bacteria

Prokaryotic cells, for example, bacteria are extremely small ranging in size from 1μ to 20μ.

(How many micrometers (μ) are there in 1 millimeter? _____)

Like, plants most bacteria possess a rigid cell wall that surrounds the cell membrane and helps the cell maintain its shape and prevents it from bursting. Inside the cell membrane, the cytoplasm contains ribosomes, DNA region, and storage granules. However, they have no membrane-bound organelles like eukaryotic cells.

Bacteria are often classified according to their shape:

1. **Coccus** (spherical cells): *Streptococci*, which consist of chains of spherical cells, are associated with strep throat. (plural, cocci)
2. **Bacillus** (rod-shaped): *Escherichia coli*, found in the intestines of humans.
3. **Spirillum** (spiral-shaped): *Borrelia burgdorferi*, cause of Lyme disease.

Since bacteria are so small in order to view them we have to magnify them 1000x. Observe the demonstration microscopes and draw the shapes of the bacterial cells below.

Coccus 1000x

Bacillus 1000x

Spirillum 1000x

Exercise 4: Survey mixture

Place a drop of the mixture of microscopic algae on a slide with a cover slip and observe. Sketch the appearance of three different types of cells and identify them using the chart provided.

REVIEW QUESTIONS

1. What happens to the field of view and depth of field when you increase magnification?

2. If the ocular had a magnifying power of 10X and the objective had a magnifying power of 10X, what would be the total magnification of an object viewed through these lenses be?

3. Why is it necessary to center the specimen in the field of view before switching to a higher power objective lens?

4. Which adjustment knob is never used with the high power objective in place (fine or coarse)? _____
Why not? _____
5. List four things you should do before putting away your microscope away.

6. Which organelles did you see with the magnification available?

7. You are trying to view a specimen under the microscope and have the following problems. Describe ***specifically how you would adjust the microscope*** to get a better view of the specimen in each case:

There is not enough light

The specimen is blurred

The specimen is not in the center of the field of view

8. From your **observations**, give 3 differences between a cell of *Elodea* and an onion cell.
Use comparable features!

Elodea cell

Onion cell

i.

ii.

iii.

9. Give 3 differences (**observed**) between the Elodea cell and the cheek cell.

Elodea cell

Cheek cell

i.

ii.

iii.

10. Why did we have to use the 100X objective to view the bacterial cells but not the others?