Microscopes and Cell Diversity

Learning Goals

Students should be able to:

1. define the following terms: magnification and resolution.
2. identify the parts of a microscope and describe their function.
3. properly focus and illuminate a specimen on the microscope.
4. prepare a wet mount.
5. identify the distinguishing features of various cells.
6. properly clean and store the microscope after use.

Introduction

Science and technology progress hand in hand. The microscope is arguably one of the most important tools ever developed in the biological sciences and remains a fundamental tool in most classrooms and laboratories.

The most common microscopes used in teaching classrooms are binocular compound light microscopes. Binocular means the microscope has 2 eyepieces. Compound indicates the microscope has 2 or more lenses or mirrors that participate in image formation. "Light" indicates that the microscope uses visible light (not electrons or UV light) as the source of illumination.

Terminology - Concepts

Magnification: Magnification means the microscope enlarges the appearance of the specimen. It makes the specimen appear bigger. Magnification is calculated by multiplying the magnifying ability of the ocular times the magnifying ability of the objective. If the microscope has a 10X ocular, when the 4X objective is being used, the total magnification of the specimen is 40X. In other words, when viewed through the microscope, the specimen will appear 40 times larger than in real life.

Resolution: Resolution is the ability to distinguish any two components of the specimen as separate. Resolution is determined by the source of illumination (visible light) and the quality of the optical elements. In light microscopy, the limits of resolution are about 1500X. What that means is that you can't magnify an image more than 1500X and see any details.

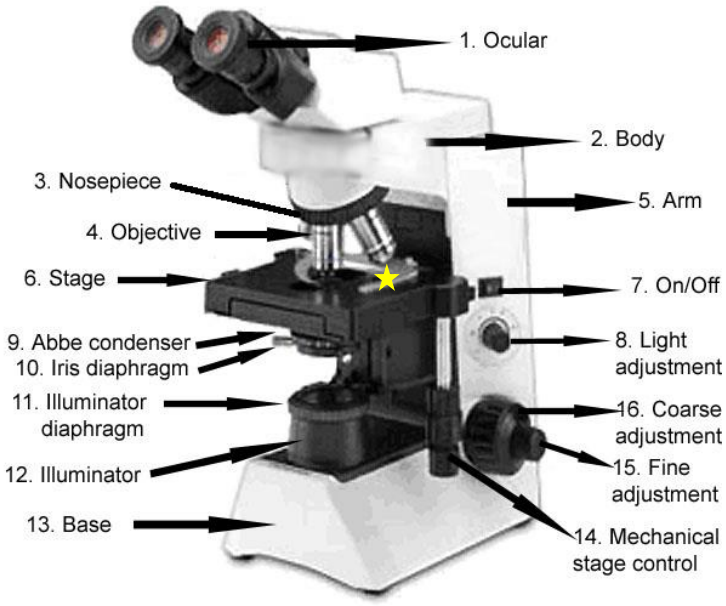
Parfocal: If a microscope is parfocal, then you only need to do coarse adjustment to focus the specimen **once**. The image will remain in good focus as you change objectives. Therefore, parfocal means that once a specimen is in focus it remains in focus regardless of the magnification.

Field of View: Field of view is the circle of light or the area of the slide/specimen that you see when you look through the microscope.

Depth of Field: Depth of field refers to the thickness of the specimen and the ability to focus the microscope at different levels within the specimen.

Working Distance: Working distance is the space between the end of the objective lens and the stage. As the magnification increases, the length of the objective lens increases and the working distance decreases.

Parts of the Microscope and Their Functions



1. **Ocular (eyepiece).** The oculars or eyepieces magnify the image. The ocular has a 10X magnification. They are adjustable. When using the microscope, you should have both eyes open and near the oculars. Keep both eyes open; squinting into the microscope or using just one eye to look into the microscope will lead to headaches. Grab the eye tubes and adjust (move the oculars closer together or further apart) the oculars until you see a single circle of light.
2. **Body.** The body contains the mirrors and lenses that bend the light that forms the image.
3. **Nosepiece.** The nosepiece holds the objectives. It rotates and positions the objectives over the stage. When rotating the objectives, an audible click can be heard when the objective snaps into position over the stage.
4. **Objective:** The objectives magnify the specimen. The common objectives are 4X, 10X, 40X and 100X. The 4X objective is also called the scanning objective. The 40X objective is sometimes called the high dry objective. The 100X objective is also called the oil immersion lens.
5. **Arm:** The arm is the back support for microscope. The microscope is always carried by holding the microscope by the arm and the base.
6. **Stage:** The stage is the flat, black platform that holds the slide.
7. **On/Off switch:** This turns the light on and off.
8. **Light adjustment:** This knob increases and decreases the brightness of the illuminator.
9. **Abbe condenser:** The condenser is a series of lenses that focuses the light through the specimen. The condenser can be raised and lowered to increase and decrease image contrast. Always start with the condenser fully raised.

10**. Iris diaphragm:** The iris diaphragm opens and closes to allow light to pass through the slide. It is controlled by a lever. Light level control is important to visualizing the specimen. More light is not always better. Too much light bleaches out the specimen and removes contrast. Turn on the light. While looking at the stage from the side, move the lever so that the diaphragm is all the way open and then all the way shut. Notice the difference in the amount of light passing through the slide. Move the lever to a position about halfway open.

11. **Illuminator diaphragm**: The illuminator diaphragm controls light levels from the illuminator. By turning the diaphragm light levels increase or decrease.
12. **Illuminator:** The illuminator is the light bulb found in the base.
13. **Base**: The base is the bottom of the microscope.
14. **Mechanical stage control**: The mechanical stage control moves the stage from right to left and front to back. This control allows the user to position the slide over the light source and to make slight adjustments to the position of the slide while viewing.
15. **Fine adjustment knob**: Movement of this knob brings the knob into sharp focus.
16. **Coarse adjustment knob**: The coarse adjustment know is used bring the specimen into focus.

★ The yellow star is on the mechanical stage or stage clip. The stage clip holds the slide.

Activities:

1. Complete wet mounts of cheek cells, onion epidermis, and leaf epidermis. Draw the specimens.
2. View and draw slides of paramecium, blood and dried bone.

How to view a slide using a microscope.

1. Place microscope on the bench. Always lift and place the microscope. Do not drag it along the surface of the bench.
2. Plug in the microscope and turn on the light.
3. Adjust the iris diaphragm to the halfway point. Turn the light adjustment knob all the way on. Make sure the illuminator diaphragm is open and light is passing through.
4. Pull open the stage clip and position the slide on the stage. The slide should be pushed firmly against the stage clip. Release the clip.
5. Use the mechanical stage controls to move the slide over the light coming through the stage.
6. Position the 4X objective over the stage. It should click into place.
7. Move the stage to its highest position (closest to the objective).
8. Look into the oculars. Grab the coarse adjustment knobs and slowly focus down until the specimen begins to come into focus.
9. Once the specimen is in focus. Grab the fine adjustment knobs and finely adjust the focus.
10. Adjust the light using the iris diaphragm lever.
11. Locate a cell or structure of interest and move that to the center of your field of view. This is an essential step when changing objectives. If you do not center the image, when you increase the magnification your 'structure' seems to disappear.
12. Grab the nosepiece and rotate the 10X over the stage until the objective clicks into place. DO NOT TOUCH THE COURSE ADJUSTMENT KNOBS, DO NOT MOVE THE STAGE DOWN.
13. Use the fine focus knob to bring the image into sharp focus.
14. If you are going to view the specimen at 400X, use the mechanical stage controls to center the specimen in your field of view. Now rotate the 40X objective over the stage. Remember, do not move the stage down. Rotate the fine adjustment knobs to sharpen the image.

Use these directions for all the slides you will be viewing in this exercise.

Putting your microscope away

1. Turn off your light.
2. Use the coarse adjustment knob to lower the stage completely.
3. Wrap and secure the power cord.
4. Use a paper towel to wipe any liquids from the stage.
5. If the oculars or objectives are wet or dirty, remove a sheet of lens paper and lens cleaner from the materials box on the bench. Dampen the lens paper with the lens cleaner and rub the oculars and objectives. **The optical elements of the microscope should be cleaned only with lens cleaner and lens paper! Do not use regular paper towels on the optical elements of the microscope.**

Materials

Wet mounts

Onion	Coverslips	0.9 % NaCl
Leaves	Methylene blue	Toothpicks
Hay infusion	Iodine	
Slides	Water	

Prepared slides

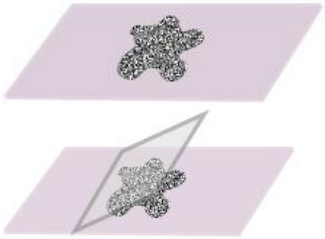
Paramecium	Blood (peripheral, Wright stain)	Dried bone

Procedure

Wet mount of cheek cells

1. Remove a clean slide and coverslip from the materials box on the bench.
2. Add a small drop of 0.9% NaCl to the center of the slide.

3. Use the blunt, rounded end of a toothpick to gently rub the inside surface of your cheek.
4. Stir the toothpick into the drop of NaCl and spread the drop to the size of a nickel. Don't worry if you don't see anything in the drop. There are plenty of cells in the drop.
5. Pick up the coverslip and slide it across the slide at a 60^0 angle. Once the coverslip hits the drop, let the coverslip drop on to the specimen. Angling the coverslip, rather than dropping the coverslip flatly onto the smear minimizes the air bubbles that form under the coverslip.



6. Add a drop of methylene blue to the slide at the edge of the coverslip, not on the coverslip.
7. Apply a piece of paper towel to the opposite side of the coverslip to pull the stain across the slide.
8. Place the slide on the microscope. Start with the scanning objective (4X). Cheek cells will look like blue dust.

9. Continue increasing magnification until you reach 400X. Don't forget to adjust the light levels as you increase magnification.
10. Draw a clump of at least 3 cells in your lab report. Remember to exaggerate the size of the cells. Label the nucleus, plasma membrane and cytoplasm. Make sure to include the magnification.

Wet mount of onion cells

1. Remove a clean slide and coverslip from the materials box on the bench.
2. Add a small drop of water to the center of the slide.
3. Peel a small piece of the inner epidermis of a slice of onion. The inner epidermis is the thin skin on the inside curve of the onion.
4. Suspend the skin in the drop.
5. Pick up the coverslip and slide it across the slide at a 60^0 angle. Once the coverslip hits the drop, let the coverslip drop on to the specimen. Angling the coverslip, rather than dropping the coverslip flatly onto the smear minimizes the air bubbles that form under the coverslip.
6. Add a drop of iodine to the slide at the edge of the coverslip, not on the coverslip.
7. Apply a piece of paper towel to the opposite side of the coverslip to pull the stain across the slide.
8. Place the slide on the microscope. Start with the scanning objective (4X). Onion epidermal cells will look like stacked rectangular boxes.
9. Continue increasing magnification until you reach 400X. Don't forget to adjust the light levels as you increase magnification.
10. Draw at least 3 cells in your lab report. Remember to exaggerate the size of the cells. Label the nucleus, cell wall, plasma membrane, and cytoplasm. Make sure to include the magnification.

Plant cells, unlike animal cells have a thick cell wall. The plasma membrane is present but is not as obvious as the cell wall.

Wet mount of leaf epidermal cells

1. Remove a clean slide and coverslip from the materials box on the bench.
2. Add a small drop of water to the center of the slide.
3. Peel or carefully slice a small piece from the underside of a leaf. This can be challenging, but you only need a very small piece.
4. Suspend the slice in the drop of water.
5. Pick up the coverslip and slide it across the slide at a 60^0 angle. Once the coverslip hits the drop, let the coverslip drop on to the specimen. Angling the coverslip, rather than dropping the coverslip flatly onto the smear minimizes the air bubbles that form under the coverslip.
6. Add a drop of iodine to the slide at the edge of the coverslip, not on the coverslip.
7. Apply a piece of paper towel to the opposite side of the coverslip to pull the stain across the slide.
8. Place the slide on the microscope. Start with the scanning objective (4X). Leaf epidermal cells vary in shape. They typically have geometric shapes, rectangular, hexagonal, etc. Special cells, called guard cells look like lips embedded within the leaf. These are the only epidermal cells to contain chloroplasts.

9. Continue increasing magnification until you reach 400X. Don't forget to adjust the light levels as you increase magnification.
10. Draw at least 3 cells in your lab report. Remember to exaggerate the size of the cells. Label the nucleus, cell wall, plasma membrane, chloroplasts, stoma, guard cell and cytoplasm. Make sure to include the magnification.

Plant cells, unlike animal cells have a thick cell wall. The plasma membrane is present but is not as obvious as the cell wall. Guard cells allow gases to pass into and out of the leaf. The gases pass through the opening between the guard cells. The opening is called the stoma.

Wet mount of hay infusion
1. Remove a clean slide and coverslip from the materials box on the bench.
2. Add a small drop of the hay infusion/water sample to the center of the slide.
3. Pick up the coverslip and slide it across the slide at a 60^0 angle. Once the coverslip hits the drop, let the coverslip drop on to the specimen. Angling the coverslip, rather than dropping the coverslip flatly onto the smear minimizes the air bubbles that form under the coverslip.
4. Place the slide on the microscope. Start with the scanning objective (4X).
5. Continue increasing magnification until you reach 100X. Don't forget to adjust the light levels as you increase magnification.
6. Draw least 3 cells in your lab report. Remember to exaggerate the size of the cells. Hay infusions/water samples contain a variety of organisms. There is no telling what you will find. You will see algae, protozoans, bacteria, copepods and others.

Prepared slides
Paramecium is a protozoan, a member of the Kingdom Protista (Domain Eukarya). It is a unicellular organism that moves by cilia. Paramecia live in freshwater. There are probably hundreds of species of paramecia.
1. Obtain a prepared slide of Paramecium.
2. Place the slide on the microscope. Start with the scanning objective (4X).
3. Continue increasing magnification until you reach 400X. Don't forget to adjust the light levels as you increase magnification.
4. Draw at least 3 cells in your lab report. Remember to exaggerate the size of the cells. Label the nucleus, plasma membrane, cilia, and cytoplasm. Make sure to include the magnification.

Blood is a connective tissue. Plasma is the liquid component. Red blood cells and white blood cells are suspended in the plasma. Most of the cells in a normal blood smear are red bloods cells. The cells with nuclei are white blood cells. There are 5 different types of white blood cells. You might not see all 5. Platelets will also be presents. Platelets are cell fragments that are critical to blood clotting. Platelets look like little flecks of blue dust.
1. Obtain a slide of a prepared blood smear.
2. Place the slide on the microscope. Start with the scanning objective (4X).
3. Continue increasing magnification until you reach 400X. Don't forget to adjust the light levels as you increase magnification.
4. Draw the field of view in your lab report. Make sure to include the magnification.

Bone is also a connective tissue. Apatite is the hard-bony matrix. Bone cells, osteocytes are found in the pits, or lacunae, you see in the slide. Bone is organized in bullseye-like structures called osteons which are made of layers of bone. Canaliculi are the grooves extending from the lacunae.
1. Obtain a slide of a prepared dried bone slide.
2. Place the slide on the microscope. Start with the scanning objective (4X).
3. Increase magnification to 100X. Don't forget to adjust the light levels as you increase magnification.
4. Draw an osteon in your lab report. Label a lacuna, canaliculi and osteon. Make sure to include the magnification.

Return all of your slides to the correct trays. Clean your microscope and return to its storage cabinet.

Lab Report Microscope and Cell Diversity Name _____

1. What is the total magnification achieved when using the 4X objective? _____
2. What is the total magnification achieved when using the 10X objective? _____
3. What is the total magnification achieved when using the 40X objective? _____

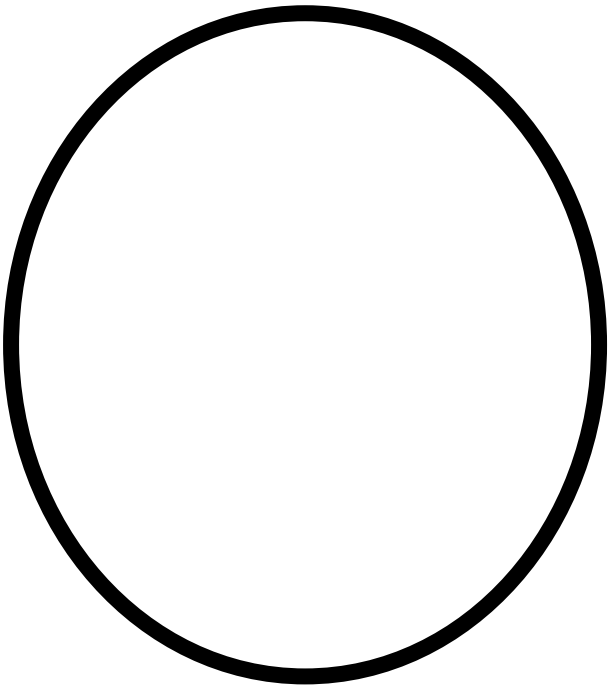
4.. Define resolution.

5. Define magnification.

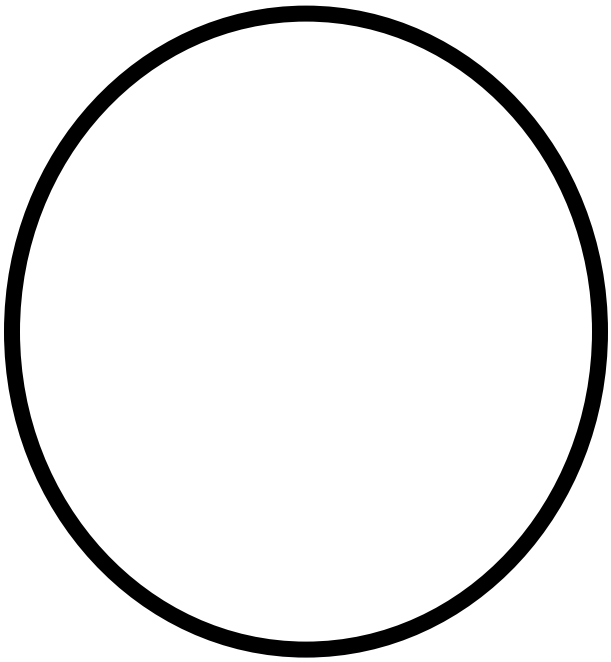
6. What is the purpose of using stain?

7. Label the microscope below.

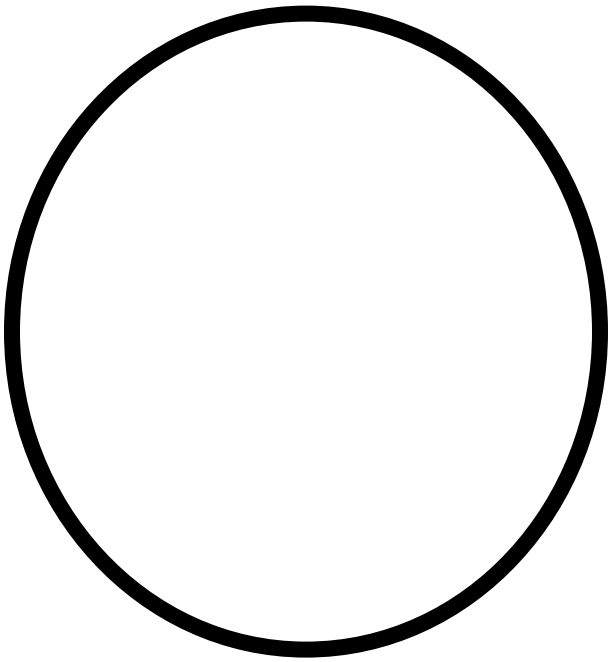
Cheek Cells – label at least 3 structures
Magnification _____



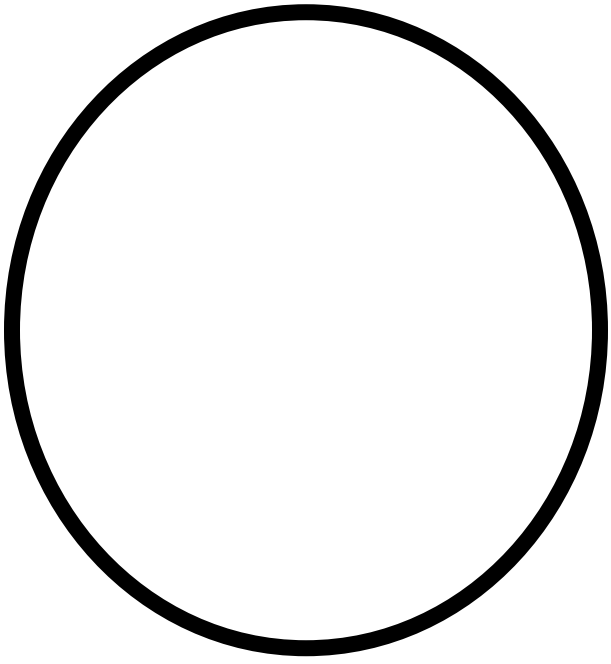
Onion Cells – label at least 3 structures
Magnification _____



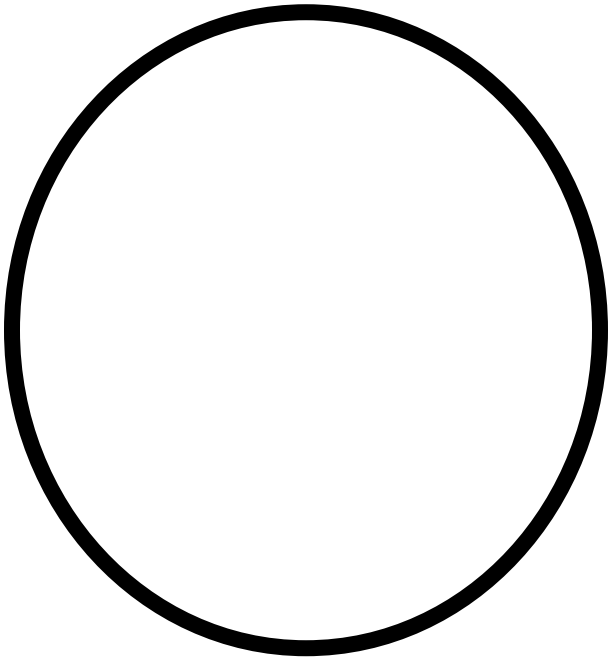
Leaf Epidermal cells – label at least 3 structures
Magnification _____



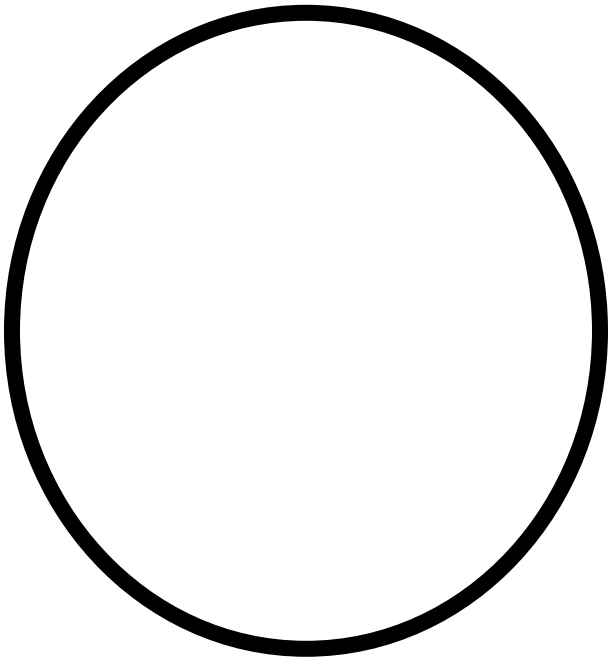
Hay infusion – draw and label at least 2 cells
Magnification _____



Paramecium – label at least 3 structures
Magnification _____



Blood – Draw and label a field of view
Magnification _____



Bone – Draw and label an osteon
Magnification _____