

Exercise 2

The Compound Light Microscope

INTRODUCTION:

Student Learning Objectives: After completing this exercise students will:

- a. Demonstrate proficient use of the microscope using low, high dry, and oil immersion lenses.
- b. Recognize the relative size differences between bacteria and common object such as a punctuation period.
- c. Recognize the different shapes of bacteria under the microscope and draw a picture of them to demonstrate that you understand their differences.
- d. Observe, describe, and draw microorganisms in a wet mount using hay infusion and/or pond water and yeast culture.
- e. Observe and describe human blood cells.

Activities for today:

- Disinfect your working area (table) and get ready for lab.
- Introduction to the use of the microscope.
- Learn the functions of the parts of the microscope.
- Assign microscopes to students.
- Observe prepared specimens and objects, and compare sizes and appearance.
- Prepare and observe a wet mount using hay infusion and/or pond water.
- Prepare and observe a wet mount of yeast cells.
- Observe a prepared blood smear under oil immersion.
- Observe prepared bacterial smears, and note the different morphology

Materials

Microscope

Glass slides

Coverslips

Water

Medicine dropper

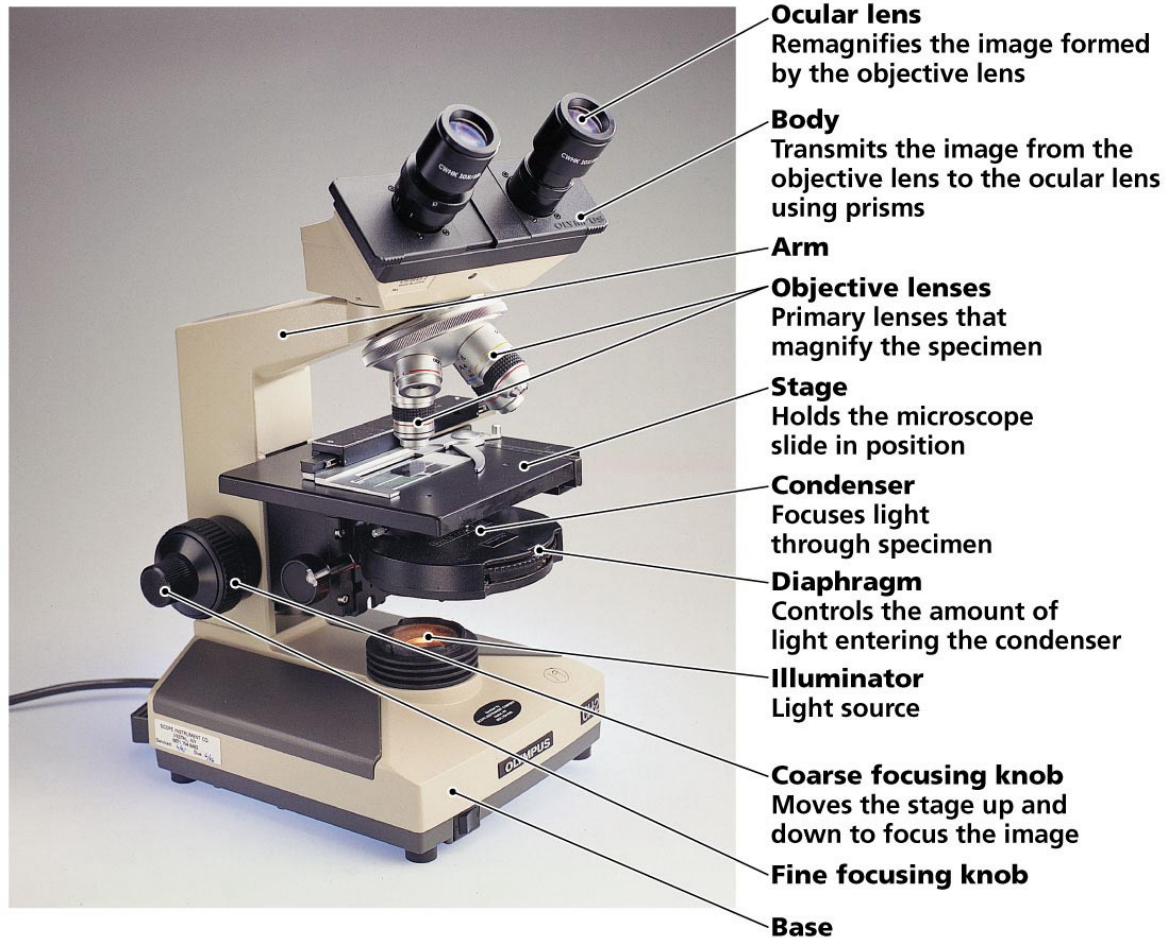
Letter "e" and "." Sheet

Scissors or razor blade

Prepared slides: Blood smear, Bacterial types

Hay infusion, pond water, yeast culture suspension

PLEASE KEEP THE 40X OBJECTIVE DRY!! THE 100X OBJECTIVE IS THE OIL IMMERSION OBJECTIVE.



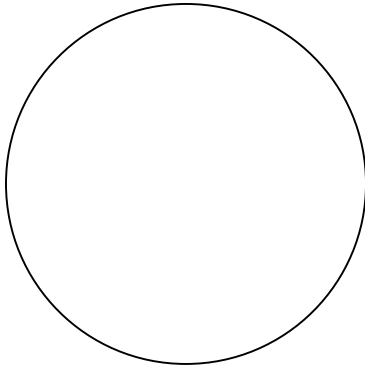
(a)

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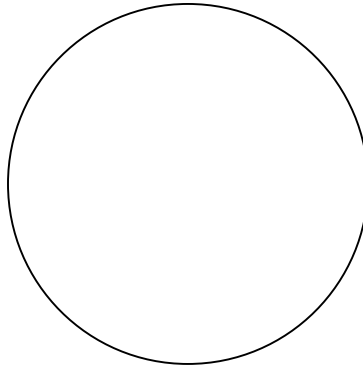
1. The Compound Light Microscope

Eyepiece (ocular) Magnification	Objective Magnification	Total Magnification
10 X	4 X Scanning	
	10 X Low	
	40 X High Dry	
	100 X OIL Immersion	

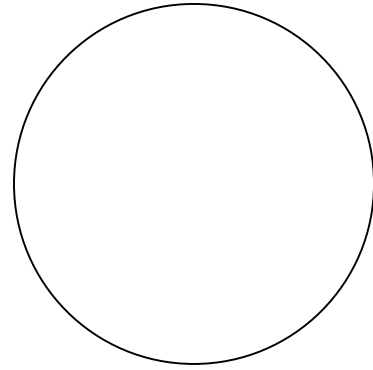
2. Observe the slide of the letter "e". Make a slide by cutting out a letter "e" and placing it on a slide (as you would read it normally), and adding a drop of water and a cover slip. View these under 40X, 100X and 400X. Draw each magnification.



40X



100X

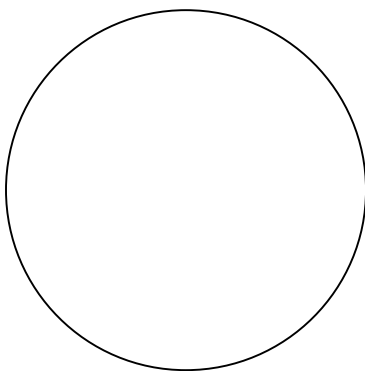


400X

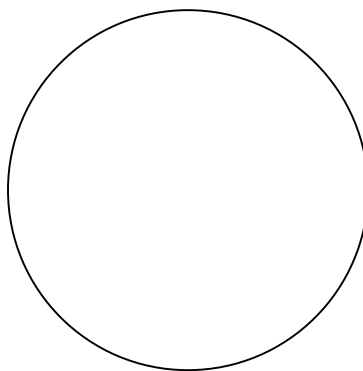
- a. What is the first thing you notice about the letter "e"?

- b. Now move the stage to the left, and then to the right, up and then down. What is happening to the image as you do this?

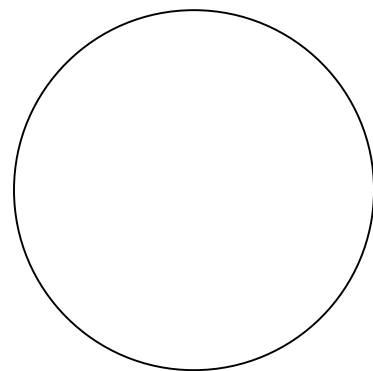
3. Make a slide of a period by cutting one out of the sheet provided, placing it on a slide, and adding a drop of water and a cover slip. View these under 40X, 100X and 400X. Draw each magnification.



40X



100X

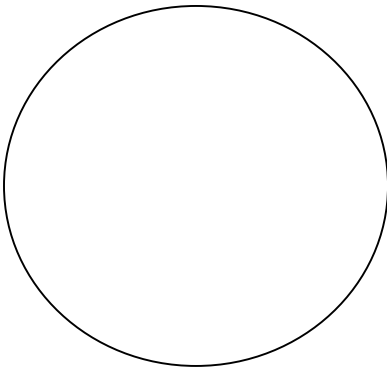


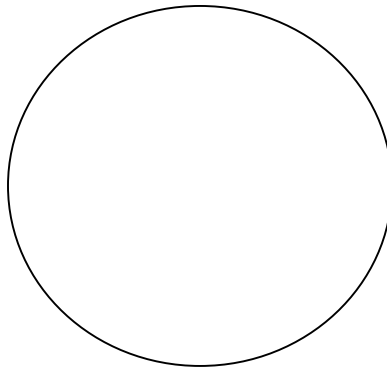
400X

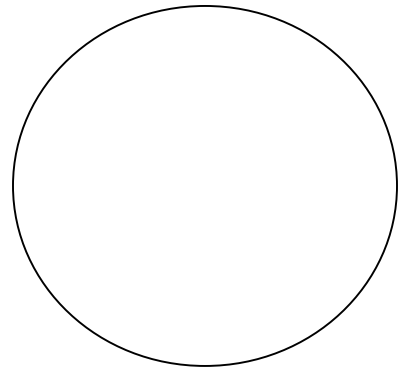
Note the relative size of the period in the viewing fields above. You will be comparing these to other objects soon.

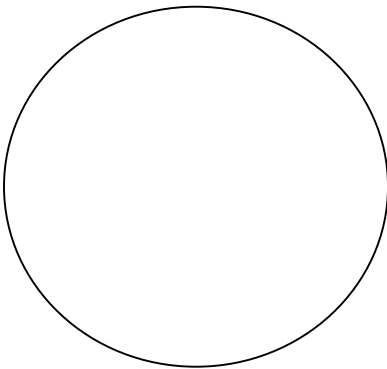
4. Preparation and observation of a wet mount using hay infusion and/or pond water.

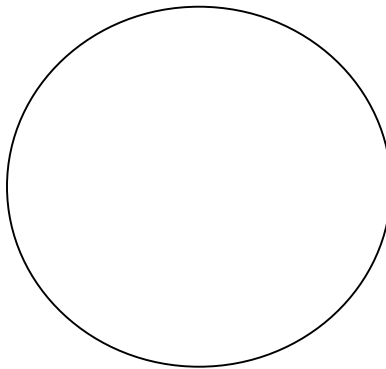
1. Using a medicine dropper, place a drop of hay infusion on a clean microscope slide and slowly cover the drop with a thin cover slip.
2. Using the low power objective, and using the coarse adjustment knob as well as the fine adjustment knob, bring the specimen into focus.
3. Rotate the turret to the high dry objective, and using the fine adjustment knob, bring the organisms into focus. Since the objectives are *parfocal*, you may need to do minor adjustments to keep the specimen in focus.
4. As you increase the magnification, you will have to make adjustments to the amount of light entering the specimen by using the diaphragm lever as well as the light intensity knob. You will also notice that the diameter of the field will decrease with increased magnification.
5. Observe the field for different microorganisms and draw at least 5 different ones in the circles below. If possible, try to identify the organisms you see, or describe their mode of locomotion.

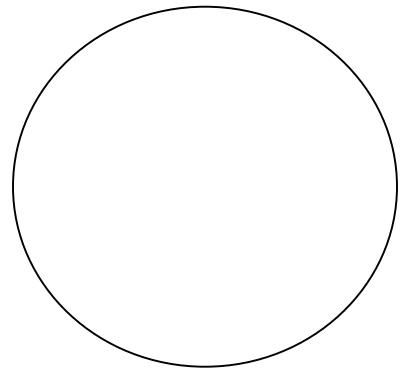






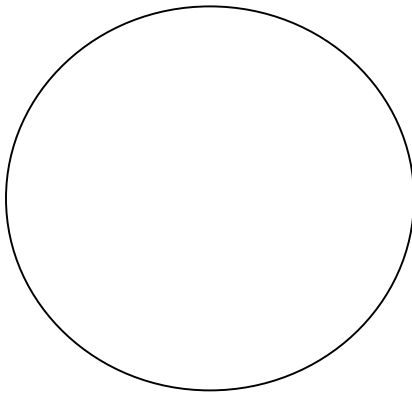




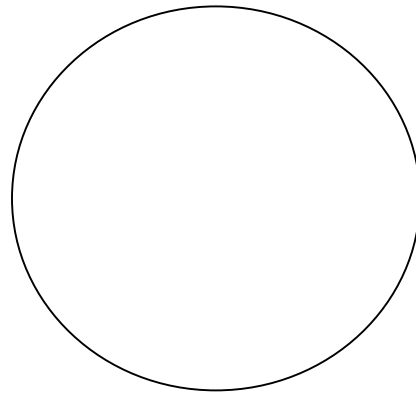


5. Preparation and observation of a wet mount of a yeast culture.

1. Obtain a suspension of yeast cells.
2. Using a medicine dropper, place a drop on a clean slide and place a cover slip on it.
3. Using the low power objective, observe the specimen, and adjust the light intensity as needed.
4. Using the high dry objective, observe and draw a group of yeast cells. Do you see any unusually shaped cells with "bumps" sticking out?



Yeast cells at 100X

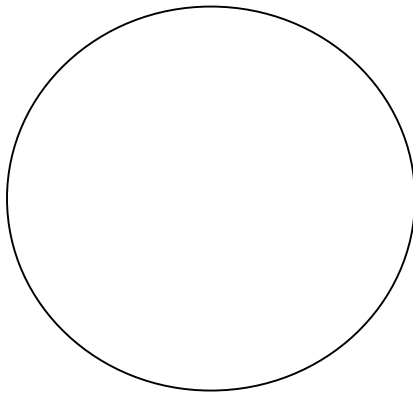


Yeast cells at 400X

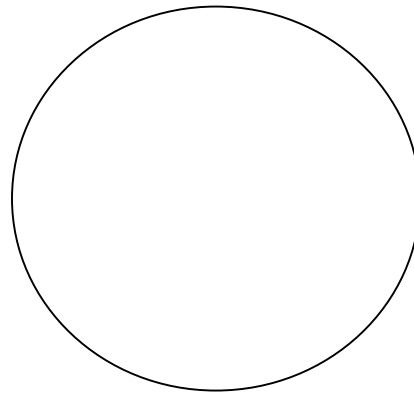
6. Observation of a prepared blood smear under oil immersion.

1. Obtain a prepared slide of a blood smear from the tray provided. These slides have been stained with a special stain allowing the observation of red and white blood cells.
2. Place the slide on the microscope stage and lock into place.
3. Using the low power objective, observe the specimen and note the different types of cells in the viewing field. Draw and label these cells.
4. Using the high dry objective, observe the specimen and bring a white blood cell (preferably a PMN) into the center of the field.
5. Move the turret so that the space between the high dry and the oil immersion objectives is above the specimen, and place a drop of immersion oil on the cover slip.

6. Slowly, rotate the turret until the oil immersion objective clicks into position. Very little if any focal adjustments will be needed to focus the specimen, since the objectives are *parfocal*.
7. Observe and draw the white blood cell in view.
 - How many nuclei does this cell have? _____
 - Do human red blood cells have a nucleus? _____
8. Label the cells in your drawings as RBCs and WBCs.



Blood smear at 100X

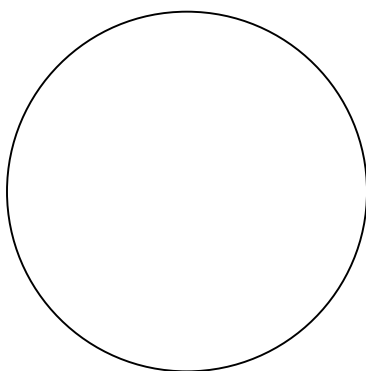


Blood smear at 1000X

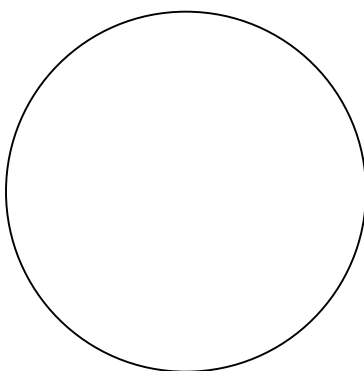
PLEASE KEEP THE 40X OBJECTIVE DRY!! THE 100X OBJECTIVE IS THE OIL IMMERSION OBJECTIVE.

Observation of prepared bacterial slides

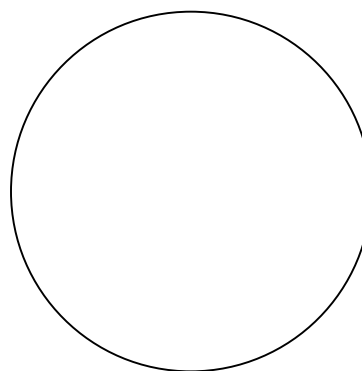
1. Be sure to view a slide of a coccus, rod and spirillum.
2. Draw each under oil immersion. When you are finished with your observations, place the slides in the appropriate containers. If using prepared slides with coverslips, please wipe the surface clean.



Cocci 1000X



Rods 1000X



Spirilla 1000X

- Compare the relative sizes of a gram positive coccus and a period at 100X; how many cocci would fit across the diameter of one period? _____
- What unit of measurement is used when measuring the size of objects viewed with a compound light microscope? _____
- How many of the above unit are present in a millimeter? _____
- Are there any Bacteria or Archaea that are large enough to be seen without the aid of a microscope? (find out the name and where it lives)

At the conclusion of your lab activities:

- Please clean up the prepared slides as instructed by your professor, and place them in the appropriate trays.
- Clean the oil immersion objective using lense paper so as not to scratch the lenses.
- Clean the stage and stage clips.
- Rotate the turret until the lowest power objective is in position.
- Turn down the voltage (light intensity) knob down to the lowest setting and turn the power switch OFF.
- Unplug the microscope, put the protective cover on it, and return it to its appropriate location in the microscope cabinet.

REMEMBER TO DISINFECT THE SURFACE OF YOUR WORK AREA BEFORE LEAVING LAB