Microscopes

Objectives

In this lab, you will learn to:

1. Identify the parts of the microscope.
2. State the function of the microscope parts.
3. Properly focus the microscope with all objective lenses.
4. Explain differences between the compound and dissection microscopes, and determine when to use each.

Introduction

Learning to properly use microscopes is one of the most important skills that you will learn in the General Biology Lab. Most cells and the details of many tissues cannot be observed without aid of the microscope. You will use the microscope throughout this course and many of you will take other biology courses such as Anatomy & Physiology and Microbiology, in which the microscope will also be used.

While all mircoscopes are designed to magnify small objects, they do differ in form and function. Microscopes that you will use in this course include the compound light microscope and the stereoscopic, or dissecting, microscope. Compound microscopes have multiple magnifying lenses and generally magnify objects in the range of $40\times$ to $1000\times$. Compound light microscopes use visible light to illuminate their subject. Stereoscopic microscopes also utilize visible light and generally magnify in the range of $7\times$ to $30\times$. Often, subcellular structures are not visible with these types of microscopes and require the use of electron microscopes. Because electron microscopes are expensive and often require extensive sample preparation, we will not use one in this course; however, you may ocassionally see images taken with electron microscopes.

This lab exercise is meant to introduce you to the compound light microscope and dissecting microscope. You will learn names and functions of the parts of these microscopes, how to properly care for and use these microscopes, and when to appropriately use each type of microscope.

Compound Microscope

Microscope Care and Use

The following are important rules when using the microscope:

1. Always carry the microscope using two hands. There is a handle on the back of the microscope and an undercut in the front for this purpose.
2. Clean the stage before and after each use.
3. Only use lens paper to clean the microscope lenses. Tissues or paper towels can scratch the lenses, which are very expersive.
4. When cleaning lenses, moisten lens paper with a few drops of distilled water. If necessary, alcohol may be used to remove clinging dirt. Please inform your instructor if you think that alcohol may be necessary. Acetone, xylene, or nitro-containing thinners should NEVER be used to clean the lenses.
5. Slides should only be placed on or removed from the stage when the scanning objective ($4\times$) is in place.
6. Start viewing a new slide using the scanning power objective and work your way up to higher levels of magnification, each time adjusting the focus as needed and centering the object in your field of view.
7. Always rotate the nosepiece by using the knurled nosepiece ring. Do not rotate the nosepiece by holding the objective lenses.
8. There should be no need to turn the fine focus knob for several revolutions. If you turn the fine focus knob two full revolutions and the specimen is not in focus, return to low magnification and refocus using the coarse focus knob.
9. Never use the coarse focus knob when the high power ($40\times$) or oil immersion ($100\times$) lenses are in place because you may break the slide or scratch the lens.
10. Use both eyes. Adjust the interpupular distance so that one image is visible.
11. If you wear eye glasses, you may continue to wear them while using the microscope; however, you may want to fold down the rubber eyeguards.
12. When returning the microscope to the cabinet, make sure that all slides are removed, the stage is lowered, the scanning objective is in place, the microscope is clean, the cord is neatly placed around the cord wrap on the back of the microscope, and the dust cover is on.

Bright Field Microscopy with the Compound Light Microscope

Materials

- compound light microscope
- microscope slide

- cover slip
- newspaper clipping with the letter ``e''
- dropper from your lab kit

Procedure

1. Clean the lenses of your microscope with lens paper only.
2. Make a wet mount of the letter ``e'' following these directions:
    1. Always use clean slides and cover slips. Slides can be cleaned with water and a paper towel. However, you should not attempt to clean a cover slip. If it is too dirty, dispose of it in the broken glass container and get a new cover slip.
    2. Place a drop of water from a glass dropper or pipette onto a clean slide.
    3. Place the letter ``e'' in the drop of water.
    4. Place another drop of water on the letter ``e'' (note: generally 2 drops are not necessary when making a wet mount, but in this case, it prevents the coverslip from falling off)
    5. Place a cover slip over the drop of water by holding the cover slip at a 45° angle to the slide. Move the lower edge of the cover slip into the water and then lower the top edge maintaining contact with the water. (This procedure prevents the formation of air bubbles under the cover slip. Under the microscope air bubbles appear as circles with heavy black edges.)
3. Place the slide on the stage with the letter ``e'' in the correct orientation and centered over the aperture in the stage. The slide is held in place by the spring-loaded finger on the mechanical stage. The slide should NEVER be placed under the mechanical stage.
4. The letter ``e'' is illuminated by transmitted light from the built-in light source. The illumination is behind the specimen and passes through it to the microscope lens. An object to be viewed by transmitted light must be thin enough to be transparent.
5. While viewing from the side of the microscope, completely raise the stage using the coarse adjustment. After these steps have been completed, look through the ocular or eyepiece and with the coarse adjustment knob slowly focus downward. Stop when the object first comes into focus. Never focus upward. There is a danger that the objective may be racked up too far, breaking the slide and possibly damaging the lens.

6. When the object first comes into focus, stop the adjustment and move the slide about until the specimen is in the center of the field of view. Focus with the coarse adjustment until the object becomes distinct, then use the fine adjustment knob to bring the object into fine, sharp focus.

7. Rotate the condenser's iris diaphragm lever to a level that provides appropriate balance between contrast (higher when closed) and resolution (lower when closed). Contrast provides differences in brightness on the specimen. High contrast, for example, is useful when trying to observe flagella on protozoans. Resolution is the ability to distinguish two points as separate, rather than as one.

8. Observe whether or not the light is too bright and adjust the illumination dial to a comfortable level.

9. Focus the condenser by raising it as far as it will go and then lower it until the field of view looks grainy. Then raise the condenser just to the point where the grainy appearance goes away.

10. After viewing the specimen with scanning power, turn the revolving nosepiece slowly until the low power objective ($10\times$) ``clicks'' into position. Move your slide so that the specimen is in the center of the field. Refocus so that you have a distinct image. Repeat these steps and go to high power ($40\times$). Your microscope is parfocal, which means when the object is in focus with one objective it is also in focus with the other objectives. However, because critical observations are made under high power, it is usually necessary to make slight focusing changes with the fine adjustment. Always make focusing adjustments under high power with only the fine adjustment knob. When in focus, the high power lens is so close to the slide that the movement of the coarse adjustment knob in an upward direction may break the slide or damage the lens.

11. Notice that only a small portion of the letter ``e'' can be seen under the high power as compared with low power. If you cannot see anything, you may be looking at a white area around the letter ``e.'' One reason for focusing first with scanning power is that the field of view is much larger and the object is easier to locate.

12. What changes occur in the intensity of illumination as you change from low to high power?

13. Can you think of any reasons why the amount of light reaching your eyes changes as you move to higher magnifications?

14. In addition to differences in illumination, you should see differences in the area of your field of view. The image below represents areas of the field of view using the $4\times$, $10\times$, and $40\times$ objective lenses. In Figure 1 on page , label each area with the appropriate objective lens magnification.

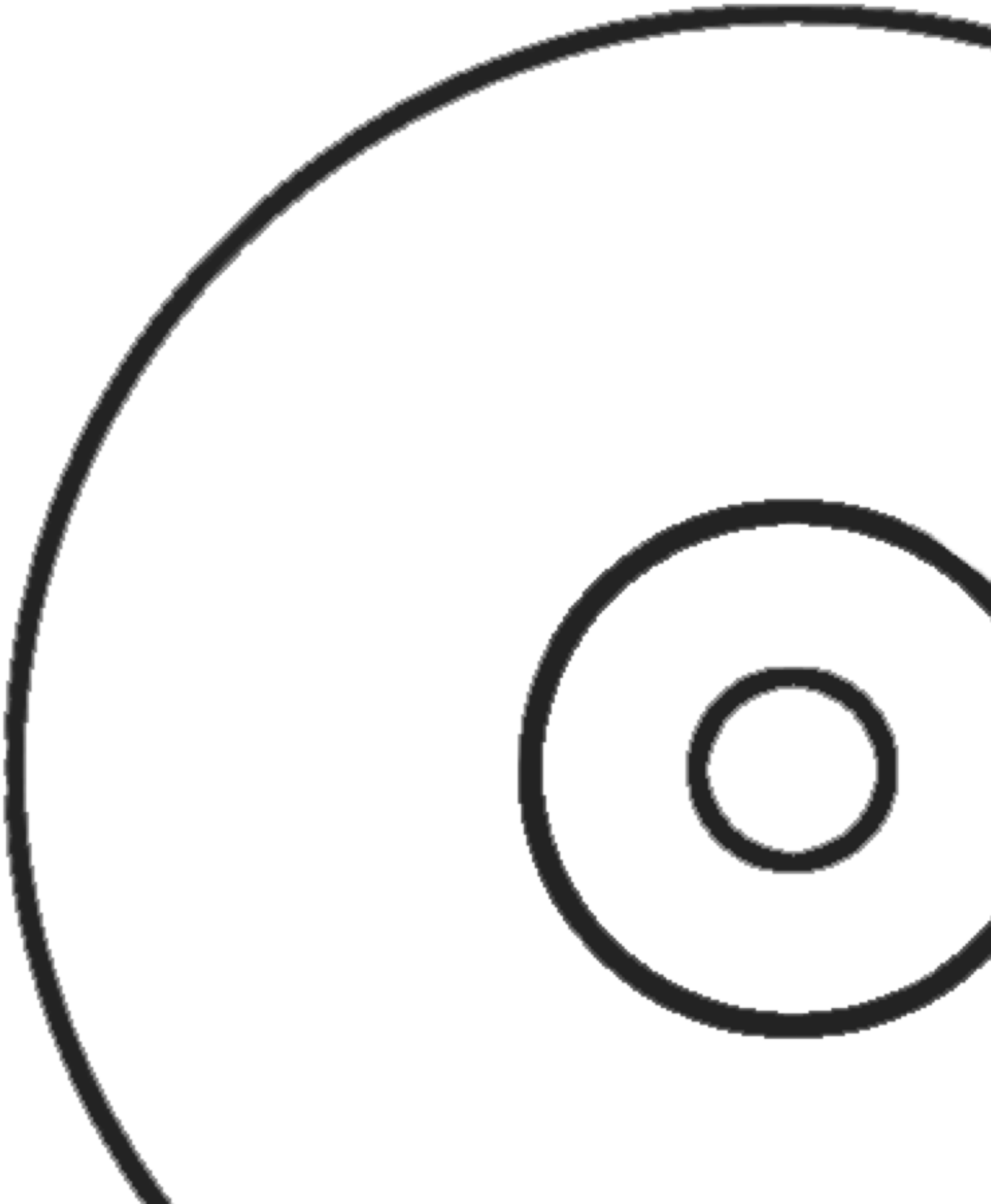


Figure 1: Areas of the field of view as magnification changes.

15. Observe the orientation of letter ``e'' as it sits on the stage without aid of the microscope. When viewed through the microscope, how does its orientation or position differ?

16. With the letter ``e'' centered in the field of view, observe it under the microscope while you move the slide slowly to the left. In which direction does the letter ``e'' appear to move when viewed through the microscope?

17. Move the slide toward you. In which direction does the object appear to move when viewed through the microscope?

18. With the low power ($10\times$) objective in place, focus on the letter ``e'' and draw it in the field of view shown in Figure 2 as you see it through the microscope.

Figure 2: Drawing of the letter ``e'' as seen with the compound light microscope at low power.

19. Calculate the total magnification of this field by multiplying the number on the ocular times the number on the objective. Record the total magnification.

Magnification:

20. Save this slide of the letter ``e'' for view under the dissection microscope

Depth of Field

Materials

- compound microscope
- microscope slide
- cover slip
- 2 hair samples
- dropper

Procedure

1. Using a dropper, place one drop of water near the center of a clean microscope slide. Take two hairs, one dark and one light and cut them about 1 cm (1/2 inch) in length. Place them in the drop of water in the form of an X. Carefully place the cover slip over the drop of water so that the hairs remain crossed.
2. Follow the proper procedures for focusing. Focus on the intersection of the crossed hairs under high power. Focus up and down with the fine adjustment to view the top hair and the bottom hair.
3. When the top hair is in sharp focus, is the bottom hair visible?

4. If it is visible, is it in sharp focus?

5. What is the effect on depth of field when changing from low power to high power?

Stereoscopic Microscopy with the Binocular Dissection Microscope

Introduction

The dissection microscope has several features making it different from the compound microscope. Among them, you will notice that the magnification is less and that it has the ability to use reflected, rather than transmitted light. While light trasmitted from the base may be used with very thin specimens, it is rarely used with this type of microscopy due to the lower magnification. Rather, a light source from above shines onto the specimen. Two particularly important features of the dissection microscope are that the image is not inverted and there is ample space for manipulation and dissection of objects.

Materials

- Dissection (stereoscopic) microscope
- slide of letter ``e'' from first part of lab
- budding Hydra slide

Procedure

1. Adjust the binocular eyepieces so that you can see one image through both oculars.
2. Set the magnification knob on top of the microscope on the lowest setting.

3. Place the slide of the letter ``e'' on the white field of view. The slide can be held in place using the stage clips.
4. Turn the focusing knobs on the sides of the microscope until the letter ``e'' comes into focus.
5. Increase the magnification by turning the knob on top of the microscope.
6. In Figure 3 on page , draw the letter ``e'' as you see it through the dissection microscope with the magnification knob on top of the microscope set at ``1''.

Figure 3: Drawing of the letter ``e'' as seen with the dissection microscope.

7. Calculate the total magnification of this field by multiplying the number on the ocular times the magnification number on top of the microscope. Record the total magnification.
8. Examine a prepared slide of a budding hydra under the lowest and highest magnification of the dissection microscope. Hydras live in fresh water attached to an object such as a leaf or log by their base called a basal disc. On the opposite end of the Hydra are long slender tentacles housing stinging cells used to capture food. At the base of the tentacles is a small opening, the mouth, which opens into the hollow body. This hollow area which comprises most of the Hydra is called the gastrovascular cavity. Hydras reproduce asexually by budding small hydras off the column or body wall. Label the basal disc, column, tentacles, bud, gastrovascular cavity, and mouth. Draw and label a hydra in the space provided in Figure 4.

Figure 4: Drawing of the budding Hydra

Living Organisms

Materials

- Dissection microscope
- compound microscope
- living organisms tube with mechanical stage adapter or hay infusion: This culture may contain single-celled organisms such as Amoeba proteus, Paramecium, Peranema, Euglena, Euplotes or multi- cellular rotifers.

Procedure

1. View the live organisms with the dissection microscrope using transmitted and then reflected light. Using which light source do you see the highest number of organisms?

2. Move the live organisms to the compound microscope using the adapter holder for the mechanical stage or if using hay infusion, prepare a wet mount. Observe with the scanning, low, and high power objectives.
3. Observe the size, shape, movement and features of the organisms.
4. After you have examined the live organisms, spend 3-5 minutes looking at the cellular detail, movement, and behavior of each organism. Make drawings and identify the three organisms you have drawn. Record observations on movement and behavior for each organism.

Organism 1 Drawing and Observations:

Organism 2 Drawing and Observations:

Organism 3 Drawing and Observations:

Review Questions

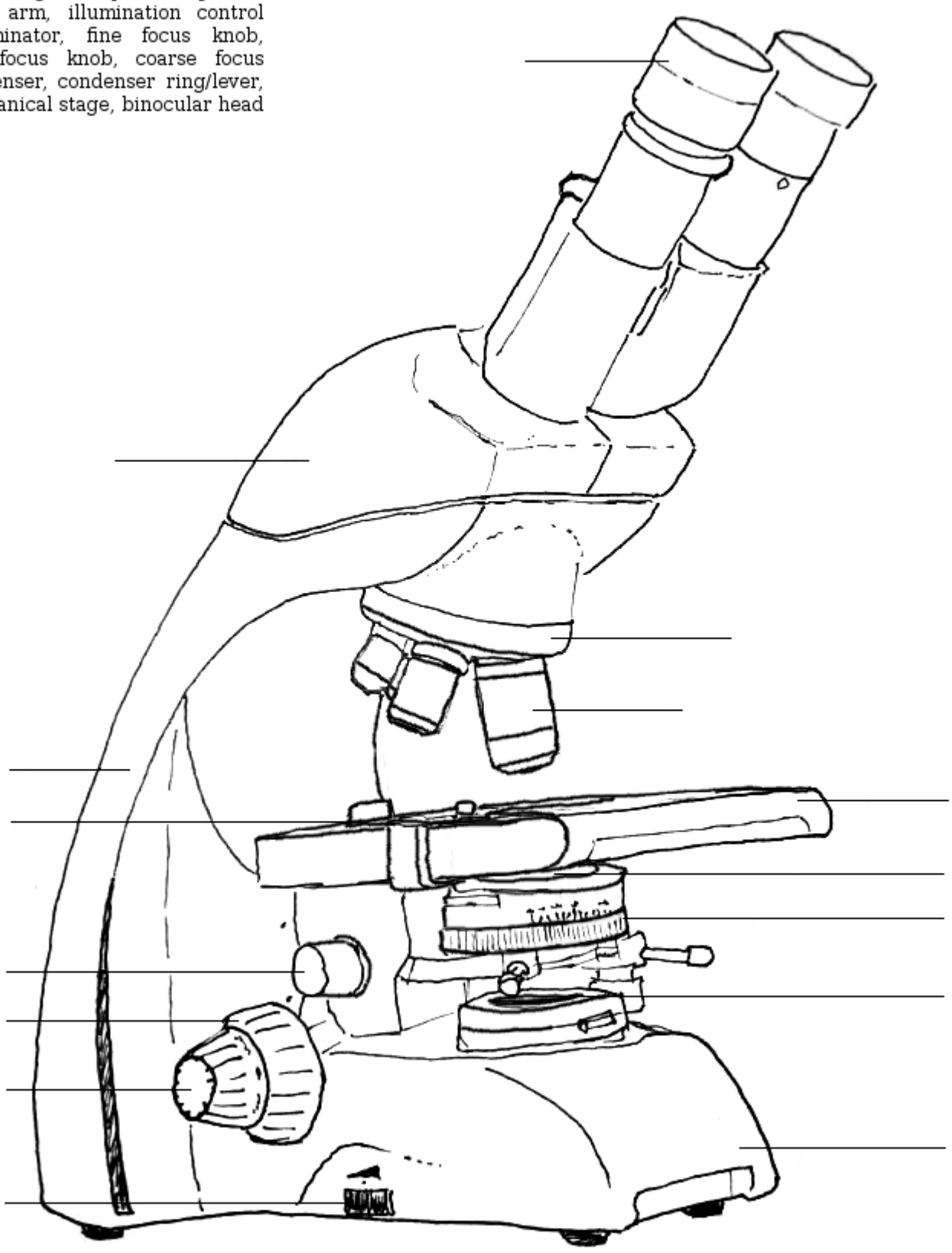
1. Give the function of the following parts of a compound microscope:
    1. condenser
    2. iris diaphragm
    3. objective
    4. ocular
2. In which microscope is the image of the specimen inverted?

3. Which microscope uses transmitted light?

4. Which microscope uses reflected light?

5. For which types of specimens or situations might you use each these microscopes?

Label the following microscope parts: ocular, revolving nose piece, objective lens, base, arm, illumination control knob, illuminator, fine focus knob, condenser focus knob, coarse focus knob, condenser, condenser ring/lever, stage, mechanical stage, binocular head



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Microscope

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