Care and Use of the Compound Microscope

Objectives

After completing this lab students should be able to

1. properly clean and carry a compound and dissecting microscope.
2. focus a specimen using all objectives of a compound microscope.
3. focus a specimen using a dissecting microscope.
4. identify the parts of the compound and dissecting microscopes and explain the function of each part.
5. define magnification and resolution.
6. estimate the size of an object when viewed through a compound microscope.
7. adjust the diopters to fit one's own eyes.
8. prepare and view wet mounts using the compound microscope.

Introduction

During this course you will need to view biological structures and organisms too small to be seen by the unaided eye. You will have two types of microscopes to assist you in viewing these specimens. They are the compound binocular microscope and the dissecting microscope. The unaided eye has the ability to distinguish between two items if they are at least 0.1mm apart (resolving power) (Vovopich and Moore, 1999). By using a light microscope we are able to distinguish between two items that are at least 0.1µm apart (Vovopich and Moore, 1999)

Care and Cleaning of the Compound Binocular Microscope

Since all microscopes are delicate precision instruments they must be well cared for. It is important to lift them from the cabinet with the use of two hands. One hand should grab the microscope by its arm and the other hand should be place under the base of the microscope to ensure the microscope is firmly held. Since the microscope you will be using is also used by several other students it is important to clean the ocular lens and the objective lens each lab period before and after use. To do this take a piece of lens paper and dampen it with the lens cleaning solution found at each table. **It is important to only use lens paper and not paper towels, since they will scratch the lens.**

Observation and Identification of the Parts of the Compound Binocular Microscope

Now that you have the microscope carefully placed on your lab table and you have cleaned the ocular lens and objective lens, it is time to become familiar with the microscope's parts and their functions. To assist you in doing this you will need to find each part described below on the microscope and then write its name on the appropriate line in Fig. 1.1. We will start at the base of the microscope and work our way up to the oculars.

1. The **light source** is found in the **base** of the microscope (which bears the weight of the microscope). It is activated by turning on the **light switch** at the back of the microscope. The intensity of the light is adjusted by turning the **light intensity control knob** on the base.

2. The **iris diaphragm** is located just above the light source on the bottom side of the stage. Using the lever attached to it you can increase or decrease the amount of light reaching the specimen.

3. Between the stage and the iris diaphragm is the **condenser**. The condenser further aids in the focusing of the light onto the specimen. It can be moved up and down by the black knob called the **condenser knob** that is located on the right side of the stage. Take a moment to move the condenser up and down and then position it up close to the stage. For the purpose of this class we do not need to change the position of the condenser. If you have a problem focusing your specimen always check the position of the condenser before calling over the instructor.

4. Above the condenser lies the **stage**. It is mounted at a right angle to the arm and positioned just below the nosepiece. The stage is where you will place your specimen. It is through the movement of the stage up and down that you will bring your specimen into focus.

5. Resting on top of the stage is the **mechanical stage**. This contains a spring clip that will hold the slide in place. To the right of the mechanical stage are two **control knobs** that allow you to move the slide left and right and backwards and forwards. This will enable you to look at all areas of the specimen.

6. At the back of the stage is the **arm** of the microscope that supports the head of the microscope. It is connected to the base and is a good place for you to grab hold of the microscope when you need to carry it or lift it out of its storage cabinet.

7. Attached to the arm are the coarse and fine adjustment knobs. These knobs move the stage up and down for the purpose of focusing the specimen. The **course adjustment knob** moves the stage a large visible distance with a single turn and as such should be used only with 4X and 10X objectives. It should **NEVER** be used with the 40X and 100X objectives. You run the risk of damaging these objectives and breaking the slide if you do not heed this warning. The **fine adjustment knob** is used to move the stage up or down only very slightly. Since these scopes are **parfocal** (all the objective lens focus the image in the same plane), once you have focused your specimen at the 4X or 10X then when you progress to the next objective you will only need to use the fine focus to make the minor adjustment needed for the specimen to be in focus.

8. Above the stage and attached to the **rotating nose piece** are the four objective lenses. They are called objective lenses because they are closest to the object or

specimen you are looking at. The magnification of the lenses are **4X** marked by a red ring, **10X** marked by a yellow ring, **40X** marked by a blue ring and **100X** marked by a white ring. Generally, 4X is referred to as the scanning lens, 10X as the low power lens and 40X as the high power lens. The 100X is the oil immersion lens and must be used by placing a drop of immersion oil on the slide before clicking it into place. The 100X lens will NOT be used in this course. Please note that the length of the lens increases as their power of magnification increases.

9. The image magnified by the objective lens in use is passed up through the body tube into the oculars. Each **ocular** contains two lenses for a total magnification of 10X. The total magnification of the microscope is the product of the ocular lens and the magnification of the objective lens in use. This means that if one has the 10X objective lens in place the total magnification that you will see is the product of 10 x 10 or 100X. It is this combined magnifying power that makes this microscope a compound microscope. What would be the total magnifying power of the scope if the 40X objective was used?

Calibration of the Binocular Compound Microscope

1. Using a piece of lens paper clean the ocular lens, each of the four objectives, the condenser (through the opening in the stage) and the light source. If the 4X objective is not currently in line with the body tube rotate the 4X objective until it clicks into place. Plug in the microscope and turn on the light source.

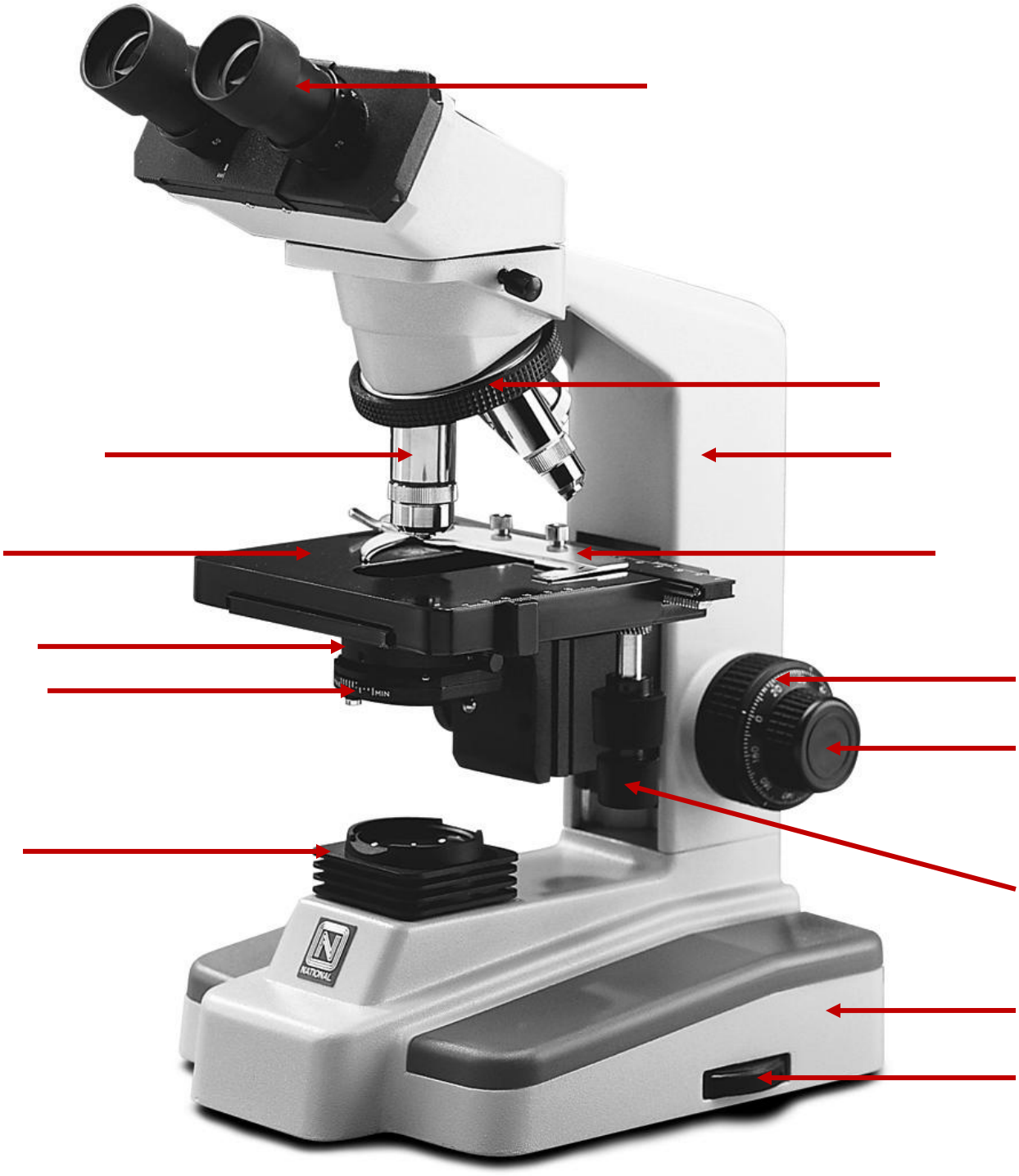
2. Obtain a slide of a prepared specimen. Place the slide cover slip side up in the spring clip on the stage and find the specimen with the 10X objective.

3. Adjust the eyepieces by looking through the oculars and slide them in and out until you see one image. This interocular distance will correspond to your interpupillary distance. Record this setting in mm here _____.

4. Adjust the focus point of the ocular on the right eyepiece to correspond to the number written in the above blank by turning the ring at the base of the ocular until the white scale is set to that number. Close your left eye and look through your right eyepiece. Focus the specimen using the fine focus.

5. Cover your right eye and look through the left eyepiece. **DO NOT ATTMEPT TO FOCUS WITH THE FINE FOCUS**. Instead focus the specimen by turning the ring at the base of the left ocular. Once you have it in focus look through both eyepieces to ensure that you have the eyepieces properly calibrated. Write the appropriate number here: R:_____. L:_____.

6. Always set the microscope to these values before you use it to observe your specimens.



©2003 National Optical & Scientific Instruments, Inc

Fig. 1.1. Photograph of the microscope used in Biology 1107.

Viewing a Specimen

1. Using a piece of lens paper clean the ocular lens, each of the four objectives, the condenser (through the opening in the stage) and the light source.

2. If the 4X objective is not currently in line with the body tube rotate the 4X objective until it clicks into place.

3. Plug in the microscope and turn on the light source.

4. Either on your lab bench or on the table are some letter "e" slides. Open the spring clip and place one of these slides on the stage so that you can read the "e" as you look at the stage. Using the stage control knobs move the slide until the "e" is positioned over the opening above the condenser lens and is illuminated by the light.

5. Raise the stage up as close as it will go to the objective using the coarse adjustment knob. The microscope has an automatic stop built in to prevent the slide from hitting the 4X (please note this stop will not prevent the slide from hitting the 40X or 100X objectives). As you look through your ocular lens use the coarse adjustment knob to move the stage away from the objective until the object is in focus. If nothing comes into view after several turns of the coarse adjustment knob you will need to check for the following errors: 1) the letter "e" was not positioned over the opening in the stage, 2) you lowered the stage too quickly and missed the letter "e", or 3) you have not lowered the stage far enough to see the letter "e". It may be necessary to repeat steps 4-5 to avoid any of the above three errors.

6. Once you have the letter "e" in focus use the mechanical stage control knobs to move it into the center of your field of view. If the "e" still needs a minor focusing adjustment use the fine focus knob to complete the focusing. If the field of view is too bright you can decrease the light by closing the iris diaphragm.

7. Draw the letter "e" as it appears in your field of view _____.
   Draw the letter "e" as it appears when you look at it on the stage _____.
   Does the letter appear different when viewed through the microscope?_____.
   If so how?_____.
   Is the letter larger or smaller when viewed through the microscope?_____.
   While looking through the oculars move the slide away from you. Which way did the letter move in your field of view?_____.
   While looking through the oculars move the slide to the right. Which way did the letter appear to move in the field of view?_____.
   Is it possible to bring the entire letter "e" into clear focus with the fine adjustment?_____, or is the outer edge slightly out of focus when the center is clear?_____.

8. Now move the 10X objective into line with the body tube. Since this microscope is parfocal you should only need to make minor adjustments to the focus using the fine focus adjustment knob. You may find that you now need to open the iris diaphragm to let more light in.
Why do you think that is needed? _____.
Is the letter larger or smaller when viewed through the microscope?_____.
While looking through the oculars move the slide away from you. Which way did the letter move in your field of view?_____.
While looking through the oculars move the slide to the right. Which way did the letter appear to move in the field of view?_____.
Is it possible to bring the entire letter "e" into clear focus with the fine adjustment?_____, or is the outer edge slightly out of focus when the center is clear?_____.

9. Now move the 40X objective into line with the body tube. Since this microscope is parfocal you should only need to make minor adjustments to the focus using the fine focus adjustment knob. You may find that you now need to open the iris diaphragm to let more light in.

Is it possible to bring the entire letter "e" into clear focus with the fine adjustment?_____, or is the outer edge slightly out of focus when the center is clear?_____.

Estimating the Diameter of the Microscope Field

1. Obtain a grid slide from the instructor's bench. The grid on this slide is composed of 2 mm length sides. Place the slide on the stage and position the grid over the opening in the stage. Put the 4X objective in line with the body tube and use the coarse adjustment knob to bring the grid into focus.

2. Using the stage adjustment knobs move the slide until one grid line touches the edge of the field of view on one side. Now count the number of squares you can see across the diameter of the field of view. If at the edge only part of a square is in view estimate what part of the millimeter is represented by that partial square. Record the value here in mm and then convert the value to µm: _____mm_____µm.

Repeat the process for the 10X recording your results here:
10X: _____mm_____µm.

Formula for estimating the diameter of an unknown field (B) based on a known field (A).

$$\text{Diameter of field B} = \frac{\text{diameter of field A} \times \text{total magnification of field A}}{\text{Total magnification of field B}}$$

So if you know that the diameter of field A is 3 mm and the total magnification is 60 X, you would compute the diameter of field B with a total magnification of 90 X as follows:

Field B = (3 mm X 60)/90
Field B =2 mm

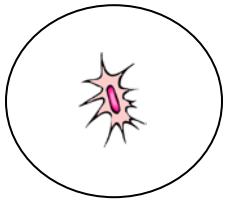
Based on this formula and your data for 10X estimate

40X: _____mm_____µm.

100X:_____mm_____µm.

3. Estimate the length (longest dimension) of the following microscopic objects. Base your calculations on the field sizes you have determined for your microscope.

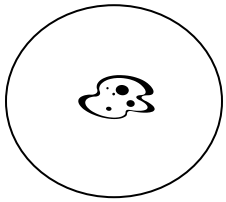
a. Object seen in low-power field:



approximate length:

_____ mm

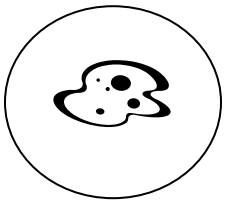
b. a. Object seen in low-power field:



approximate length:

_____ mm
or _____µm.

c. Object seen in oil immersion field:



approximate length:

_____µm.

The Dissecting Microscope

When a biologist needs to look at a specimen too large or thick to be viewed by a compound binocular microscope and too small to be viewed in sufficient detail with the naked eye they will use a dissecting microscope or stereoscopic microscope.

1. Use the dissecting microscope on your lab bench to locate the following parts and write the names on the appropriate line in Fig. 1.2.

2. **Adjustment focus knob** allows you to focus the objective on the specimen.

3. The image magnified by the objective lens in use is passed up through the body tube into the oculars. Each **ocular** contains two lenses for a total magnification of 10X.

4. **Light control switches:** There are two switches that control the light on this scope. One of them turns on either the top light or the bottom light or both. The other knob controls the intensity of the light.

5. **Magnification adjustment knob:** On this scope instead of individual objectives you can adjust the amount of magnification by turning this knob.

6. **Two light sources:** On this scope you have an in base light source and an upper light source. You may use either one or both depending on the type of specimen you are observing.

Fig. 1.2 Dissecting microscope that will be used in Biology 1107.

Preparing and Viewing Wet mounts

1. Obtain a clean slide and a cover slip from the slide box and the cover slip box found on your lab table. Take them to the instructor's bench and place a drop of pond water on the clean slide.

2. Holding the cover slip at an angle on the edge of the drop of water slowly lower the cover slip down on top of the drop of water. If correctly done there should be no air bubbles. However, if you should have air bubbles you can gently tap them out using the erasure side of a pencil.

3. Return to your microscope and view the contents of your drop of pond water. Practice using your mechanical stage to move the slide around and locate all of the microscopic life thriving in your single drop of pond water.