



## The Microscope



### EXERCISE 2A: INTRODUCTION TO THE MICROSCOPE

#### 2A INTRODUCTION

**M**any structures in the human body are too small to see with the naked eye. The invention of the microscope made it possible for scientists to see the diversity of subunits that comprise the human body. As microscopes improved, smaller and smaller objects could be seen. The powerful electron microscope enabled us to see molecules (e.g., DNA) and infectious particles (e.g., viruses). We will use the compound light microscope to study cells and tissues.

A microscope magnifies the image of an object. Microscopes operate on the same principle as a magnifying glass, except that compound light microscopes have two magnifying glass lenses. These lenses are constructed so that light is bent as it travels through the glass, increasing the size of the image that is ultimately focused on the retina of the eye. This increase in image size is called **magnification**. It is expressed as a figure followed by an x, for example, 10x means that the image is magnified 10 times. The x indicates the number of times the original size of the object's diameter is magnified.

**Resolution** refers to the ability to distinguish between two adjacent points. The resolution limit of the human eye is about 100 micrometers. Resolution is limited in the light microscope by the quality of the microscope lenses and the properties of light. The particles that make up light are called **photons**. Some objects are too close to be resolved by a light microscope. The compound light microscope has a resolution limit of about 0.2 micrometers under ideal conditions. Electron microscopes use electrons to form images. Photons and electrons travel in waves. It is the size of the wave that determines resolution. Since electrons travel in very short wavelengths, they are capable much better resolution than photons. "That is why electron microscopes have a much greater resolution capacity than light microscopes.



Fig 2.1 Ocular lenses.

The **ocular lens** and the **objective lens** are the two kinds of magnifying lenses on your microscope. The **total magnification** of the microscope is a multiple of the power of the ocular lens (Fig 2.1) and the objective lens (Fig 2.2). For example, if the objective lens in use is 4x and the ocular lens is 10x the total magnification is 40x. This means that the specimen appears 40 times larger through the lenses than it does when viewed with the naked eye. You will learn how to



Fig 2.2 Objective lenses.

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determine the total magnification with each of the objective lenses on your microscope.

### 2A OBJECTIVES

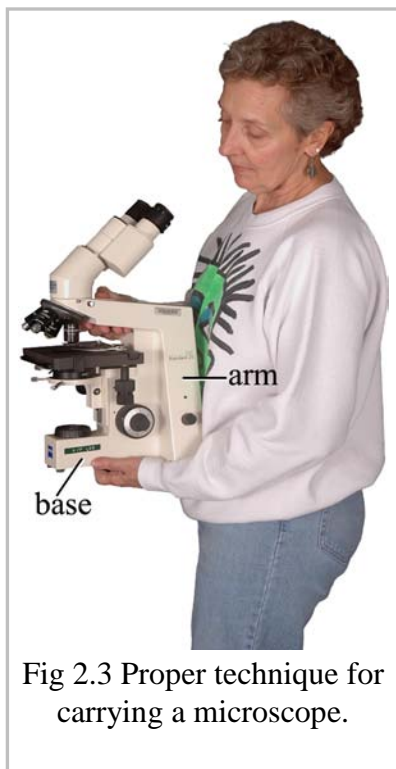
- To appreciate the microscope as an important laboratory tool.
- To define and understand the difference between magnification and resolution.
- To learn how to use and care for a microscope.

### 2A MATERIALS

1. Compound light microscope
2. Lens paper and lens cleaner

### 2A PROCEDURE

1. Care of the microscope. Microscopes are precision instruments and must be handled with care. A set of guidelines is presented below and in Laboratory Exercises 2C and 2D. Learning how to care for and properly use a microscope will enhance your experience in the laboratory. In this class, you will view cells and tissues of human organs. Master the following guidelines for microscope use and care and follow them when you operate a microscope. Refer to Fig 2.5 to locate features on your microscope.



2. When carrying a microscope from one location to another, always hold it upright and use both hands. One hand should be placed under the **base**, the other should be gripping the **arm** (Fig 2.3). Avoid jarring the microscope when you set it down.

3. It may be necessary for you to clean the lenses or the glass slide containing the specimen. To clean microscope components, always use lens paper and lens cleaner. Never clean a lens with a paper towel, tissue, or your shirttail, as these materials can scratch the lens.

Fold the lens paper into fourths. Place several drops of lens cleaner on the lens paper. Swipe the lens once lightly in one direction to remove superficial particles. Do not rub the glass with the lens paper as this may scratch the glass. Repeat the swipe in a circular motion with unused areas of the lens paper until the lens is clean. Repeat this procedure to clean specimen slides.

4. The **light source** of your microscope is located in the base. Before you turn on the light, adjust the light to its lowest level by turning the **brightness control knob** fully counter-clockwise. This precaution extends the life of the halogen light bulb. The toggle switch in the cord, or on the microscope arm turns the light on.



## EXERCISE 2B: THE ANATOMY OF THE MICROSCOPE

### 2B INTRODUCTION

Now you are ready to learn to use a microscope. The first step is learning the basic components of a compound light microscope (Fig 2.5). The microscope components will also be pointed out by your instructor.

There are three kinds of lenses in most light microscopes: the objective lenses, the ocular lenses, and the **condenser lens**. The objective lenses are mounted on the **turret** of the nosepiece. There are usually four objective lenses: the **wide-field (or scanning lens)**, the **low-power lens**, the **high-power lens**, and the **oil immersion lens**. Refer to Table 2.1 for the magnification power and uses of these lenses. Note that as the power of the lens increases, so does its length. **Working distance** (Fig 2.8) is defined as the distance between the bottom of an objective lens and the top of the coverslip on a specimen slide. The working distance decreases as the magnification power and length of the objective lens increases.

**Table 2.1 Characteristics of standard objective lenses for the light microscope.**

NAME OF THE LENS	POWER OF THE LENS	SOME APPLICATIONS
wide-field or scanning	3.2x or 5x	Used to scan a large specimen, or locate a specimen on a slide.
low-power	10x	Used to establish Köhler illumination and focus on the specimen with the coarse focus knob. A frequently used lens.
high-power	40x	Used to view a specimen in more detail. A frequently used lens. With this lens in place, only use the fine focus knob.
oil immersion	100x	Used to view a specimen with the highest magnification possible in a compound light microscope. Requires the use of immersion oil. With this lens in place, <i>only use the fine focus knob</i> .

Ocular lenses are located in the eye pieces. Their magnification is fairly low, usually 10x to 15x range. These lenses should be cleaned frequently. Ocular lenses are adjustable, like binoculars. Always adjust the eyepieces so that when you look through them you see a round, lighted area called **the field** or **the field of view**. If your specimen is properly located, it will appear in the field. Some ocular lenses contain a **pointer** that may be used to indicate specific structures on the specimen slide (Fig 2.4).

The condenser lens is part of a major component of the microscope called the **condenser**. The condenser is located below the **stage**. As its name implies, the condenser gathers the light from the source in the base and focuses it on the specimen. Many condensers have an additional lens called the **condenser front (or accessory) lens**. This lens improves resolution.

Light coming through an opening can be controlled using an **iris diaphragm**. An iris diaphragm can be opened to allow more light through, or closed to reduce the amount of light. There are two iris diaphragms on a standard compound light microscope. One, called the **field or substage diaphragm**, controls the amount of light leaving the light source in the base of the microscope. The second, called the **condenser diaphragm**, controls the amount of light passing through the condenser.

Other structures on the microscope that you are responsible for are listed in the procedure below.

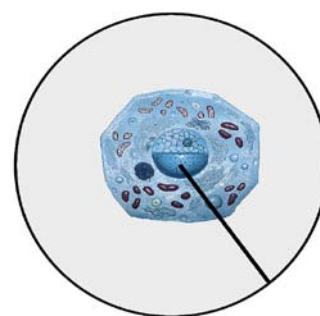


Fig 2.4 Microscope field with pointer.

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### 2B OBJECTIVES

- To be able to identify and name the basic components of the light microscope.
- To understand field of view and working distance.

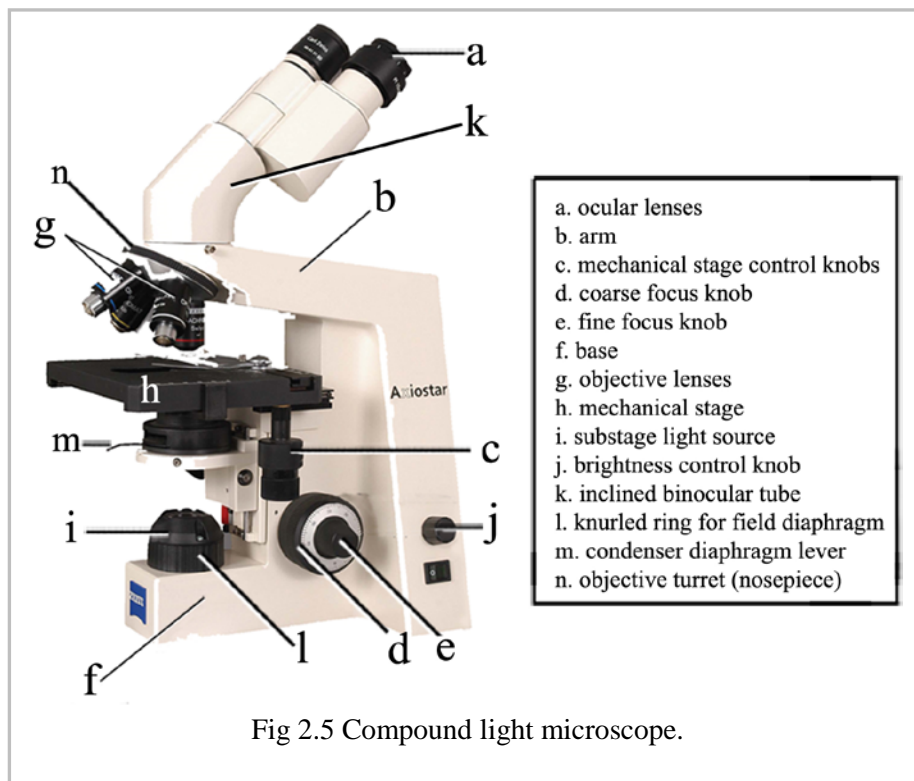
### 2B MATERIALS

1. Compound light microscope
2. Slides

### 2B PROCEDURE

1. Obtain a microscope and transport it to your desk following the procedure specified above. As your instructor identifies the components of the microscope, locate them on Fig 2.5 and on your microscope.
2. When you complete your study of the microscope you should be able to identify the following components:

- a. eyepieces (with ocular lenses)
- b. arm
- c. mechanical stage control knobs (x- and y-axes)
- d. coarse focus knob
- e. fine focus knob
- f. base
- g. objective lenses (four magnifications)
- h. mechanical stage
- i. substage light source
- j. brightness control knob
- k. inclined binocular tube
- l. knurled ring for field (substage) diaphragm
- m. condenser diaphragm lever
- n. objective turret (nosepiece)
- o. condenser control knob (not shown in Fig 2.5)
- p. condenser front (accessory) lens swing out control (not shown in figure)
- q. condenser lens (not shown in figure)
- r. condenser centering screws (not shown in figure)



## EXERCISE 2C: THE FUNCTIONS OF THE MICROSCOPE

### 2C INTRODUCTION

Each microscope component has a specific function. In this section, you will learn how to properly set up and use a microscope.

**Illumination:** The first step in using a microscope is to optimize the ability of the instrument to magnify and resolve specimens. We will use a method called **Köhler illumination** to achieve this goal. Köhler illumination aligns microscope elements to maximize illumination and resolution.

**Focusing:** When focusing a compound microscope, always begin with the **low power (10x) lens** in place. After achieving “coarse” focus with the **coarse focus knob**, you may rotate a higher power lens into place over the specimen. If you have focused properly with the 10x lens, you will only have to adjust slightly with the **fine focus knob** when you switch to the 40x lens. Microscope objectives designed to stay in focus on the sample when changing from one lens to another are said to be **parfocal**. This characteristic is found in better microscopes and helps protect both the lens and slides from damage. Remember to use the coarse focus knob with the low power lens. Use only the fine focus knob with higher power lenses.

**Depth of field:** Another attribute of a microscope is **depth of field (DOF)**. Depth of field is the distance above and below the specimen that is in focus. Depth of field is greatest at lower magnifications. A specimen has dimension in length, width, and height. Most specimens are more than one cell layer thick. So, at lower magnifications, more than one cell layer may be in focus, while at higher magnification fewer layers of cells will be in focus. By racking through fine focus, you can be in sharp focus on the deepest layer, the middle layer, or the most superficial layer of a specimen. You will use a prepared microscope slide with overlaying colored threads to explore depth of field.

**Oil immersion:** Once you have learned how to focus with the scanning lens, the low power lens, and the high power lens, you will learn how to use the 100x oil immersion lens. This lens is capable of the highest magnification of any of the objective lenses. It is designed to be used with **immersion oil**, which improves the resolution of the image. It is the only lens that is used with oil. The other lenses are often called “dry” lenses because of this difference. *You must be sure to clean both the lens and the slide after you have used oil.*

Finally, you will learn the use of the mechanical stage. This device enables you to move the slide in the horizontal or x-axis and the vertical or y-axis with precision.

## 2C OBJECTIVES

- To be able to establish Köhler illumination.
- To learn how to focus the microscope using the coarse and fine focus knobs.
- To learn how to operate the iris diaphragm of the condenser.
- To learn how to use the mechanical stage.
- To learn how to calculate working distance.
- To understand Köhler illumination and depth of field.
- To learn how to use the oil immersion lens (100x).

## 2C MATERIALS

1. Compound light microscope
2. Lens paper and lens cleaner
3. Immersion oil
4. Prepared microscope slides:
  - a. letter “e”
  - b. colored threads
  - c. a tissue slide
5. Millimeter rulers

**2C PROCEDURE**

1. You should establish Köhler illumination at the beginning of each microscope session. Once you have established Köhler illumination, the microscope will remain in that setting. Follow the steps outlined below.
  - a. Prepare the microscope.
    - 1) Clean all the lenses.
    - 2) Turn on the microscope.
    - 3) Open the iris diaphragm on the condenser and the field diaphragm.
    - 4) Rack the condenser to its top position by using the condenser control knob.
  - b. Focus on the specimen.
    - 1) Select a stained slide and place it in the mechanical stage.
    - 2) Focus on the specimen using the 10x objective lens.
  - c. Focus on the on the image of the field diaphragm.
    - 1) Close the iris of the field diaphragm.
    - 2) Focus the image of the field diaphragm on the slide by adjusting the condenser with the condenser control knob. The image of the iris diaphragm should have a blue glow around its rim and be hexagonal in shape (Fig 2.6).
    - 3) If you followed the directions in step a, focusing the image of the field diaphragm should require you to lower the condenser slightly.
  - d. Center the image of the field iris diaphragm with the condenser centering screws. Then open the field iris diaphragm until the field is just cleared.
  - e. Slowly close the condenser iris diaphragm in small increments until the glare is reduced and contrast is satisfactory.
2. Learn to focus the microscope, and explore depth of field. Practice operating the coarse and fine focus knobs, using objective lenses of different magnifications, until you understand their functions. Once you have the specimen in focus, observe the effects on a specimen of opening and closing the iris diaphragm of the condenser.
  - a. Obtain a slide with colored threads.
  - b. Using the low power objective, focus on the colored thread slide at the location where the threads cross.
  - c. Focus down on the threads, using the coarse adjustment knob, until all of the threads are out of focus. Then focus upward slowly, noting which color of thread comes into focus first. This should be the lowest, or most inferior, thread. Continue focusing upward. Record your observations in your Laboratory Review.
  - d. Practice focusing on the threads in a similar manner, using increasing levels of magnification. Notice that you may have to increase light when changing to a higher magnification lens.
  - e. Practice opening and closing the iris diaphragm on the condenser. This diaphragm can increase or decrease contrast in the specimen. Understanding its use will help you view many of the specimens used in this course.
3. Learn about field of view and use of the mechanical stage.
  - a. Return the thread slide and obtain a letter “e” slide.
  - b. Orient the slide so the “e” is right side up, then place the slide on the mechanical stage.
  - c. Using the lowest power lens, view the “e” again. Observe the orientation of the letter as viewed through the microscope
  - d. As you look through the microscope, move the slide to the right and left using the x-axis control on the mechanical stage. Then move the slide away from you and toward you with the y-axis control on the mechanical stage.

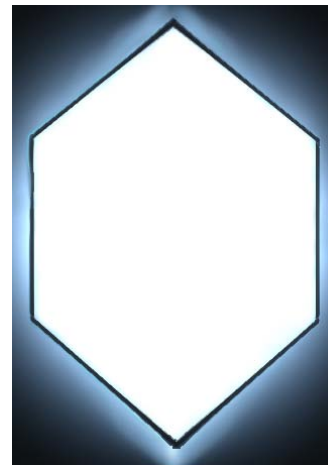


Fig 2.6 Image of the field diaphragm.

- e. Measure the diameter of the field by placing a millimeter ruler on the stage in the same position as your slide (remove the slide before you replace it with the ruler) (Fig 2.7). View the ruler at each magnification, counting the number of millimeter marks that are visible.
  - f. Record your observations in your Laboratory Review.
4. Measure the working distance for each of your objective lenses (Fig 2.8).
    - a. Obtain a specimen slide and place it in the mechanical stage.
    - b. Focus on the specimen using your low power objective lens.
    - c. Use a millimeter ruler to measure the distance between the bottom of the objective lens and the top of the coverslip
    - d. Rotate the next objective lens into place. Use the fine focus knob to sharpen the focus. Measure the working distance.
    - e. Measure working distance for each of your objective lenses.
    - f. Record your results in your Laboratory Review.
  5. Learn how to use the oil immersion lens.
    - a. Focus on a slide using the “dry” lenses, ending up in focus with the 40x objective.
    - b. Locate the portion of the specimen you wish to examine and center it in your field of view.
    - c. Rotate the turret holding the objective lenses so the 40x objective is removed and the oil immersion lens is ready to be placed in the light path.
    - d. Look at the surface of the slide and locate the spot where light is focused by the condenser on the slide.
    - e. Place one drop of oil on the spot of light.
    - f. Swing the oil immersion lens into place.
    - g. If you need to, touch up the focus a bit using only the fine focus knob.
    - h. When you have finished your study of the specimen, be sure to clean the lens and the slide, in the manner described in Laboratory Exercise 2A.

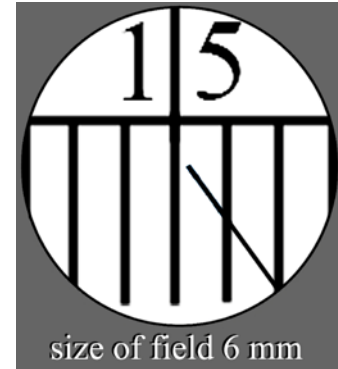


Fig 2.7 Establishing size of the field.

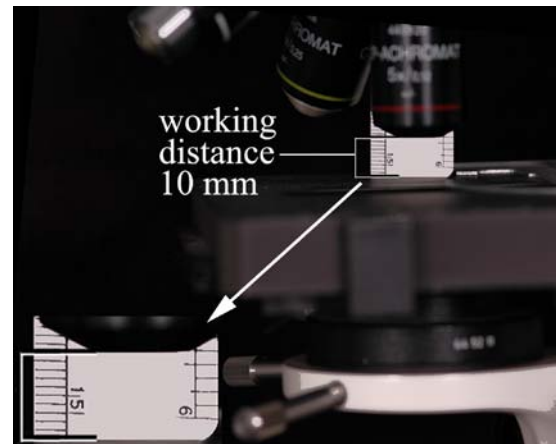


Fig 2.8 Working distance.

Note: As explained in the “To the Student” section at the beginning of this manual, you must complete any of the Review sections that require use of laboratory materials that are not available to you outside of the laboratory, such as a microscope and slides. It is advisable to check the Review sections periodically to see what activities should be completed in the laboratory.



## EXERCISE 2D: CARE OF THE MICROSCOPE

### 2D INTRODUCTION

Some of the procedures important to the care of a microscope were introduced in Laboratory Exercise 2A so that you could practice using your microscope. Those procedures are summarized here.

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Additional procedures important to microscope care will be introduced. You will be expected to follow these procedures every time you use the microscope.

### **2D OBJECTIVES**

- To review procedures on microscope care.
- To review cleaning procedures for microscope slides and microscope components.
- To learn to prepare a microscope for storage.

### **2D MATERIALS**

1. Compound light microscope
2. Lens paper and lens cleaner
3. Microscope slide box
4. Microscope cover
5. Rubber band

### **2D PROCEDURE**

1. When transporting a microscope, hold it upright and use both hands (Fig 2.3).
2. When focusing a compound microscope, begin with the low power lens in place. After achieving “coarse” focus, you may rotate a higher power lens into place over the specimen. The coarse focus knob should only be used with the low power lens. Use only the fine focus knob with higher power lenses.
3. When cleaning microscope components, use only lens paper and lens cleaner. Clean all lenses when you are finished using the microscope. This is particularly important if you have used the oil immersion lens. Clean each slide that you use and return the slide to its correct slot in your slide box.
4. As you prepare your microscope for storage:
  - a. Remove the glass slide containing the specimen. Return the slide to the correct slide box.
  - b. Lower the mechanical stage, and put the lowest power lens in place.
  - c. Dim the light (turn the knob fully counter-clockwise) and turn off the light source.
  - d. Fold the cord and rubber band it. NEVER wrap the cord around the base! This can damage the connection to the power source and the cord.
  - e. Replace the dust cover before you return the microscope. Your instructor will indicate where to place the microscope when you are finished.

**Remember! Complete the laboratory review for these exercises**



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12. In what direction do you turn the brightness control knob to adjust it to its lowest light level position? \_\_\_\_\_

13. Where is the on/off switch located on your microscope? (the position varies) \_\_\_\_\_

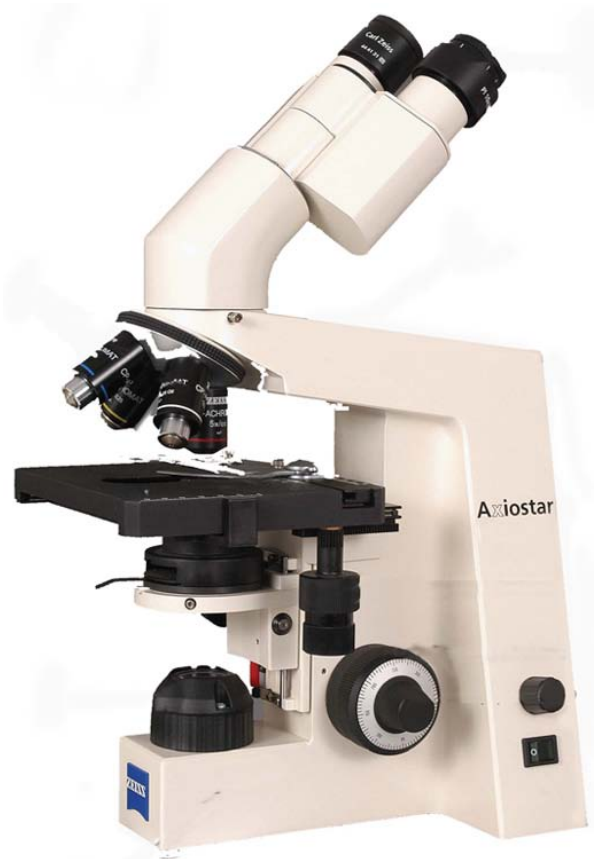


### REVIEW 2B: THE ANATOMY OF THE MICROSCOPE

#### 2B RESULTS AND OBSERVATIONS

Label Review Fig 2.1 below with the appropriate numbers from the following list:

- a. base
- b. brightness control knob
- c. substage light source
- d. mechanical stage
- e. mechanical stage control knobs
- f. arm
- g. coarse focus knob
- h. fine focus knob
- i. condenser diaphragm lever
- j. objective turret (nosepiece)
- k. objective lenses
- l. ocular lenses
- m. inclined binocular tube
- n. knurled ring for field diaphragm



Review Fig 2.1



### REVIEW 2C: THE FUNCTIONS OF THE MICROSCOPE

#### 2C RESULTS AND OBSERVATIONS

Fill in the blanks.

#### Total Magnification

14. Notice the length of the objective lenses. Which lens is shortest? \_\_\_\_\_

15. Which lens is longest? \_\_\_\_\_

16. As the magnification increases, what do you observe about the length of the lenses?  
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17. Calculate the total magnification possible with each of the objective lenses. Show your calculations. \_\_\_\_\_  
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**Depth of Field**

18. When you are focusing on the colored threads at low power, is it possible to get all three in focus at the same time? \_\_\_\_\_

19. What happens to the appearance of the colored threads when you increase the magnification? \_\_\_\_\_

**Field of View****Table 2.1: Letter “e”.** Fill in the blanks.

Sketch the letter “e” as you see it on the slide with your naked eye.	Sketch the letter “e” as it appears through the lowest power lens.
20.	21.

**Table 2.2: Field diameter.** Fill in the blanks.

POWER OF OBJECTIVE LENS	DIAMETER OF THE FIELD IN mm
lowest power objective lens	22.
10x objective lens	23.
40x objective lens	24.

**Working Distance**

25. Working distance is the space between the lens and the specimen. What happens to the working distance as the magnification increases? \_\_\_\_\_

26. As you increase the magnification, does the light become brighter or dimmer? \_\_\_\_\_

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27. Is it better to increase or to decrease the light when increasing magnification?

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### REVIEW 2D: CARE OF THE MICROSCOPE

#### *2D RESULTS AND OBSERVATIONS*

Fill in the blanks.

28. When focusing a compound microscope, always begin with which objective lens in place?

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29. Which focus knob should be used with the 40x objective lens? \_\_\_\_\_

30. At the end of a session using the microscope, what items should be cleaned?

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31. List the things that should be done before a microscope is stored. \_\_\_\_\_

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