

## Characteristics of the Light Microscope

### Objectives:

- To become familiar with the parts of the microscope and their function
- To determine total magnification
- To develop skill in focussing the microscope
- To determine field of view for each objective lens
- To understand some of the characteristics of microscope images
- To learn how to stain objects for view under the microscope

### Materials:

Light microscope	slide	coverslip	eyedropper	IKI stain
Onion	water	newspaper	ruler	scissors

### Procedures:

#### Part 1: Parts and Functions

Obtain a microscope and record its number. You and your partner will use this microscope from now on. Use the information in Appendix C (Reference: page 10) in your textbook to label the microscope diagram on page 3 of this package. Also write the function of each part on the same page.

#### Part 2: Total Magnification

The object's image gets magnified two times: once as it goes through the objective lens and again as it passes through the ocular lens. The *lens power* is stamped on each of the 3 or 4 lenses of your microscope. Write the power of each lens. If you do not have an oil immersion lens, find someone who has that lens.

ocular \_\_\_\_\_  
 objective: low \_\_\_\_\_ medium \_\_\_\_\_ high \_\_\_\_\_ oil immersion \_\_\_\_\_

1. The *magnification* of any object is the power of the ocular lens times the power of the objective lens.

$$\text{Magnification} = \text{ocular power} \times \text{objective power}$$

Calculate the magnification under low, medium, high and oil immersion powers. Show your work.

low \_\_\_\_\_ medium \_\_\_\_\_  
 high \_\_\_\_\_ oil \_\_\_\_\_

#### Part 3: Characteristics of Microscopic Images

1. Prepare a wet mount slide of the letter "e" by placing a small section of newspaper on a clean slide (hold the slide only at the edges). Add a drop of water and, holding a clean cover slip with your thumb and forefinger, allow one of the free edges to touch the drop of water. Now let the cover slip fall on to slide. Try to avoid air bubbles.
2. Make sure that the **LOW POWER** objective lens is in place. Place the slide on the stage with the "e" in the middle. Use the **COARSE ADJUSTMENT KNOB** to raise the stage all the way to the top. While looking through the ocular lens, slowly lower the stage using the coarse adjustment knob. The illuminated circle you see is called the **FIELD OF VIEW**. When the letter comes into view, use the **FINE ADJUSTMENT KNOB** to sharpen the image. Adjust the **IRIS DIAPHRAM** to see how changing the amount of light changes the image.
3. Look at the "e" on the slide and then look through the ocular lens. What happens to the e? Move the slide to the right? What happens? What happens if you move the slide to the left, then towards you and then away from you? Write your observations in the space below.

- Now, without moving the stage, flip to **MEDIUM** power (look from the side to make sure the lens and slide do not hit each other). The "e" should still be in focus and you only need to use **FINE ADJUSTMENT KNOB**. Note how the image has changed. Adjust the **IRIS DIAPHRAM** to illuminate the "e" more. **Do NOT use the coarse adjustment knob on medium, high or oil immersion power!!!** You could break the lens and slide!
- Without moving the slide, rotate to **HIGH** power (look from the side to make sure the lens and slide do not hit each other). Use only a slight rotation of the **FINE ADJUSTMENT KNOB** to bring the "e" into focus. Note how the image has changed. Again, adjust the **IRIS DIAPHRAM** to illuminate the "e" more. If you lose sight of your "e", go back down to low power and start again. Do not go to oil immersion yet. Rotate back to **LOW POWER** and remove the slide and cover slip and clean and dry them.

### Part 5: Field of View

Observe the millimeter markings on a plastic ruler under **LOW** power and measure how far the distance is across the circle. This is the diameter of the **FIELD OF VIEW**. Write down the diameters of the field of view for the low, medium and high power lenses (be careful with the high power lens). Scientists use the measurement micrometers ( $\mu\text{m}$ ) to express the size of light microscope images. Remember:  $1 \text{ mm} = 1000 \mu\text{m}$ . **Fill in the table below.** If a cell covers  $1/5$  of medium power field of view, how big is it?

	Field of view (mm)	Field of view ( $\mu\text{m}$ )
<b>LOW POWER</b>		
<b>MEDIUM POWER</b>		
<b>HIGH POWER</b>		
<b>OIL IMMERSION</b>		

### Part 6: Depth of Field

Prepare a wet mount of two strands of different color hair or thread (about 2 cm each). Make a cross with the two strands of hair and place the cover slip over the cross. Focus on the cross under low power, then medium and high power (use care!). You will notice that it is not possible to get both hairs in focus at the same time on high power because the cross of threads is 3-dimensional. Move the fine adjustment to view the various depths or "planes" of the image.

What does depth of field mean? Write answer below.

### Part 7: Staining

Get a small sample of onion skin and place it on a slide. Add a drop of water and a cover slip. Put a drop of IKI at one end of the coverslip. Touch a paper towel to the end of the coverslip opposite to where you put the IKI in order to draw out the stain across the onion skin and soak up excess IKI. Observe onion under low and medium powers. On the blank white paper provided, draw an onion cell & calculate its length under both low and medium powers. Use "Making a Biological Drawing" handout and follow format.

### Part 8: Live specimen

If you have time, obtain a *ceriodaphnia* (water flea) from the beaker at the front. Do **NOT** put a coverslip over it. Draw off excess water with a small piece of paper towel (leave a bit of water around the water flea). Observe it under your microscope. Be careful using high power. What parts can you see?