Introduction to Using the Light Microscope

I. Determining Diameter of Field

1. Rotate the nosepiece to low-power magnification. Place a clear plastic ruler on the microscope stage and focus on the millimeter divisions along the edge of the ruler.

a) Measure and record the diameter of the field of view by counting the number of millimeter divisions. Estimate to the nearest 0.5 mm.

b) Convert millimeters to micrometers (μm). (1 mm = 1000 μm.)

2. The field of view for both medium and high-power magnification can be determined indirectly by calculating the ratio quotient of the high-power objective lens to the low-power objective lens. Calculate the ratio quotient for each lens.

c) Ratio quotient = $\frac{\text{magnification of desired lens}}{\text{magnification of low power lens}}$

3. Calculate the diameter of the field of view for both the medium and high-power objectives (use micrometers).

d) Desired lens diameter = $\frac{\text{low-power field diameter}}{\text{ratio quotient}}$

II. Observing a Specimen

4. Obtain a 2 cm x 1 cm section of a scale from an onion (make sure the onion has first been peeled).

5. Hold the piece of onion so that the concave surface faces you. Then snap it backwards so that you can see the thin, transparent epithelial cells. Using a scalpel or forceps, remove a single layer of epithelium from the scale. Be sure that the layer is thin enough to be transparent to light before you continue. It should look like skin peeling after a sunburn. If it is not that thin, try again.

6. Prepare a wet mount of the section of onion tissue, being careful to not let the skin double over. (See *Nelson Biology,* page 38). If the section folds over, use a scalpel or forceps to unfold it.

7. Stain the slide with iodine. To do this, place a drop of iodine on one edge of the cover slip. Touch the opposite edge with the edge of a paper towel. This will draw the iodine stain onto the specimen.

8. Focus the cells under low power. As you focus on the specimen under low power, view the stage from the side so you can be careful not to allow the lens to touch the slide. It is easier on your eyes if you keep both eyes open while looking through the ocular lens (this takes some practice). Select a group of cells and move the slide so that the cells are in the middle of the field of view.

f) If the slide is moved to the right, in which direction do the cells appear to move?

9. With the cells centered in the field of view and in focus, rotate the nosepiece so that the medium power objective lens is in place. Bring the cells into focus using the fine adjustment knob.

g) How does the size of the cells seem to change?

10. With the cells centered in the field of view and in focus, rotate the nosepiece so that the high power objective lens is in place. Bring the cells into focus using the fine adjustment knob.

h) Do you see more or less of the cells?

i) Under which magnification is the image brought closer to the eye?

j) Which magnification would be most suited for scanning several objects?

k) Which magnification provides the widest angle for viewing?

11. Draw a group of four cells as they appear under high power. Label nucleus, cytosol, cell wall, and vacuole. You might have to refer to a textbook to help you identify some structures.

12. Estimate the width of a single onion cell in micrometers. Show your calculations.

Questions

1. When centering an object under the microscope you move it from left to right, which way does it appear to move? When you move it away from you, how does it appear to move?
2. Why is it easier to locate objects under low rather than high power?
3. Why is it a good idea to center a specimen in the field of view before switching to a higher power?
4. If you were trying to estimate the diameter of a very small specimen, which magnification would you use?
5. Explain why microscopes are stored with the low power lens in position?
6. Why should the coarse adjustment focus not be used with a medium or high power lens?
7. A thicker lens is often necessary for greater magnification, but results in a loss of resolving power. Explain why resolving power decreases as the thickness of the lens increases.
8. Why is the microscope called a compound microscope?
9. What is the proper way to carry a microscope?
10. How do you determine total magnification?
11. What is the total magnification of the specimen when you are looking through each of the objective lenses on your microscope?
12. Why is the built in pointer a useful feature of the microscope?
13. Why are micrometers rather than millimeters used for microscopic measurements?
14. Why is the field of view brighter under low power?

Application Questions

15. A student switches from the low- to the high-power objective lens of a microscope. The object being viewed disappears, even after careful focusing. Indicate why the object cannot be seen, and suggest a technique that would help eliminate this problem.
16. An oil immersion lens is often used to view very tiny objects. If an oil immersion lens has a magnification of 100x, calculate its field of view.
17. A correcting lens can be placed into the microscope to make objects appear in their normal (non-reversed) position. Suggest reasons why this would be useful.
18. Why is it important to measure the size of microscopic objects?