EXERCISE 3

The Microscope

If students have already had an introductory biology course in which the microscope has been introduced and used, there might be a temptation to skip this exercise. We have found that most students need the review, so we recommend spending this time early in the course to make sure they are all comfortable with the microscope, as it is used extensively throughout the laboratory manual.

Time Allotment: 2 hours.

Solutions:

Bleach Solution, 10%
Measure out 100 milliliters of household bleach. Add water to a final volume of 1 liter.

Methylene Blue Solution (Loeffler's)
Weigh out 0.5 gram methylene blue. Add 1 milliliter 1% potassium hydroxide solution, and 30 milliliters of absolute ethanol to 100 milliliters distilled water. Warm the water to about 50 degrees C, stir in methylene blue; filter.

Physiologic Saline (Mammalian, 0.9%)
Weigh out 9 grams of NaCl. Add distilled/deionized water to a final volume of 1 liter. Make fresh just prior to experiment.

Laboratory Materials

Ordering information is based on a lab size of 24 students, working in groups of 4. A list of supply house addresses appears in Appendix A.

24 compound microscopes, lens cleaning solution, lens paper, immersion oil
24 millimeter rulers
24 slides of the letter *e*
24 slides with millimeter grids
24 slides of crossed, colored threads (threads should cross at a single junction)
Filter paper or paper towels
1 box of microscope slides
1 box of coverslips
1 box of flat-tipped toothpicks
8–12 dropper bottles of physiologic saline
8–12 dropper bottles of methylene blue stain (dilute) or iodine
24 slides of cheek epithelial cells
10% bleach solution
Autoclave bag, disposable

Advance Preparation

1. Provide each student with a compound microscope, millimeter ruler, bottle of immersion oil, lens paper, and millimeter grid slide. A supply of glass cleaner, such as Windex™, should be available for lens cleaning.
2. Have available slides of the letter *e* and slides of crossed, colored threads. Some instructors prefer to have slides for an entire semester available in individual boxes, which can be handed out to students. Others prefer to keep the slides on trays to be distributed as needed.

3. Set up an area for wet mount supplies, including clean microscope slides and coverslips, flat-tipped toothpicks, *physiologic saline*, methylene blue stain or iodine, and filter paper, or set out prepared slides of cheek epithelial cells.
4. Set up a disposal area containing a beaker of *10% bleach solution* and an autoclave bag. Note: Detailed instructions for treatment and disposal of materials used in labs involving human tissue and excretions are found in the preface of this *Instructor's Guide*.
5. If the microscopes are binocular rather than monocular, give additional instructions on focusing.
   a. After the parts of the microscope have been identified, turn on the light, and adjust the interpupillary distance so that a single circle of light is visible through the eyepieces. This is difficult for some students, usually because they are moving back and forth and changing their eye position. Have each student record his/her own interpupillary distance for later use.
   b. For a microscope with an adjustable left eyepiece, focus the microscope as directed, using the right eye only.
   c. Focus using the left eyepiece with the right eye closed. Both eyepieces should now be focused on the specimen. (Reverse the directions if the right eyepiece is adjustable.)
6. The directions for perceiving depth (p. 33) are for microscopes with objective lenses that advance and retract during focusing. If the stage moves during focusing, the superior thread will come into focus first if these directions are followed. Alter instructions if necessary.

Comments and Pitfalls

1. Be sure to have the students check the orientation of the letter *e* on the slide before putting the slide on the microscope. If they forget to check, they will miss the point of the exercise.
2. Beware of common focusing problems: dirty lenses, inverted slide, objective lens not securely in place, and wrong lens in position (oil immersion instead of high-power).
3. It is difficult to use a millimeter ruler to measure the working distance of the high-power and oil immersion lenses on some microscopes. A best estimate is usually sufficient.
4. Many students have difficulty with the section on determining the size of the microscope field. The direct measurement is usually no problem, although some students measure area rather than diameter, and some students will have both the letter *e* slide and the grid on the stage at the same time. Emphasize that direct measurement should be done using only one lens. Otherwise, measuring discrepancies cause confusion. The problem is often with the math involved. It is probably worthwhile to stop the class and work through the use of the formula (p. 32) when you see that most students are at this point in the exercise.
5. Clarify what is meant by "detail observed" in the chart on p. 31.
6. Students may forget safety precautions when preparing the wet mount. Emphasize the importance of following directions for safe disposal of toothpicks and proper cleanup of glassware.
7. Many students forget to adjust the iris diaphragm and may end up using the light at its highest intensity, which is hard on the bulb. Remind students that the iris diaphragm should be adjusted so that the field is just filled with light when observed with the ocular lens removed. In practice, it may be necessary to adjust the iris diaphragm for best contrast, although some resolution may be lost.

Answers to Pre-Lab Quiz (p. 27)

1. d, stage
2. b, The slide should be almost in focus when you change to higher magnifications.
3. 350 ×
4. c, with special lens paper and cleaner
5. False

Answers to Activity Questions

Activity 2: Viewing Objects Through the Microscope (pp. 30–31)

5. Answers will vary depending on the lenses used. Working distance decreases as lens power increases. The *e* appears upside down and backwards.
6. The image moves toward you. The image moves to the right.
7. and 8. Grains begin to appear and are very visible with the high-power lens.

   The image is much larger.

   The entire *e* is visible with the low-power lens, but less than ½ of the letter is probably visible with the high-power lens.

   The field is smaller.

   The object must be centered so that it falls into the field of the higher power lens.

   The light to the field is reduced as the iris diaphragm is closed.

   The light intensity often must be increased when changing to a higher magnification, as the lens has a smaller diameter and therefore lets in less light. In practice, if the microscope does not have a variable light intensity adjustment, the iris diaphragm should be adjusted to obtain the best contrast.
9. Grains are very visible. Yes.

   The working distance is less than that of the high-power lens.

   It is desirable to begin focusing with a low-power lens because the field is larger, making it easier to find the specimen on the slide, and the working distance is larger, reducing the chance of hitting the slide with the lens.

Activty 3: Estimating the Diameter of the Microscope Field (pp. 32–33)

3. Answers depend on the field diameter of lenses used. For lenses with field diameters of 1.8 millimeters, 0.45 millimeter, and 0.18 millimeter, respectively, the estimated lengths are about 1.2 millimeters, 0.14 millimeter, and 1.8 millimeters.
4. No. The entire length of the object cannot be seen in one field. The estimate should be made with a lower-power objective lens.

Activity 4: Perceiving Depth (p. 33)

2. When the stage descends, the first clearly focused thread is the bottom thread; the last clearly focused thread is the top one. Answers depend on the order of the threads on the particular slides used.

Activity 5: Preparing and Observing a Wet Mount (p. 34)

8. A cheek epithelial cell is about 80–100 micrometers (0.08–0.1 millimeter) in diameter. Most of the cells are separated from each other rather than in a continuous sheet.

10. A cheek epithelial cell is about 80–100 micrometers (0.08–0.1 millimeter) in diameter. The cells on a prepared slide are easier to measure because they are in a sheet of cells and are professionally prepared.

REVIEW SHEET

EXERCISE 3

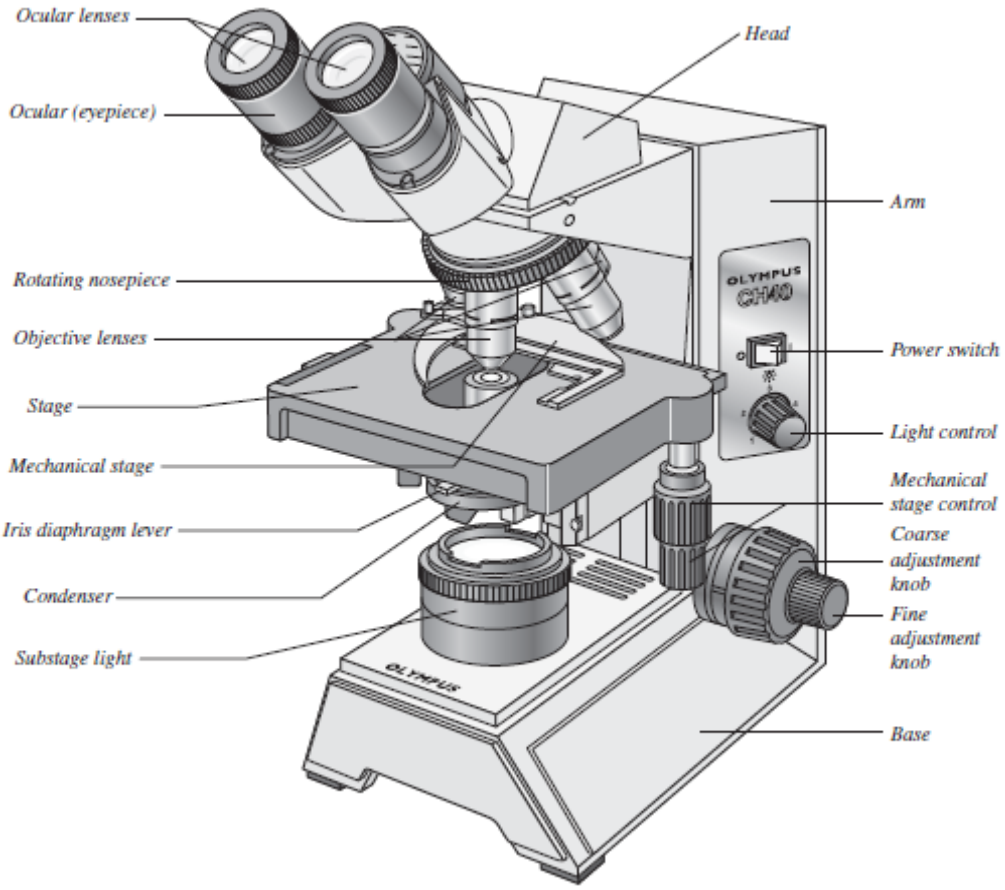
The Microscope

Name _____

Lab Time/Date _____

Care and Structure of the Compound Microscope

1. Label all indicated parts of the microscope.



2. Determine whether each of the following statements is true or false. If it is true, write *T* on the answer blank. If it is false, correct the statement by writing on the blank the proper word or phrase to replace the one that is underlined.

with grit-free lens paper 1. The microscope lens may be cleaned <u>with any soft tissue</u>.

low-power or scanning 2. The microscope should be stored with the <u>oil immersion</u> lens in position over the stage.

T 3. When beginning to focus, the <u>lowest-power</u> lens should be used.

away from 4. When focusing, always focus <u>toward</u> the specimen.

T 5. A coverslip should always be used <u>with wet mounts and the high-power and oil lenses</u>.

3. Match the microscope structures given in column B with the statements in column A that identify or describe them.

	Column A	**Column B**
i	1. platform on which the slide rests for viewing	a. coarse adjustment knob
h	2. lens located at the superior end of the body tube	b. condenser
e	3. secure(s) the slide to the stage	c. fine adjustment knob
b	4. delivers a concentrated beam of light to the specimen	d. iris diaphragm
c	5. used for precise focusing once initial focusing has been done	e. mechanical stage or spring clips
f	6. carries the objective lenses; rotates so that the different objective lenses can be brought into position over the specimen	f. movable nosepiece
d	7. used to increase the amount of light passing through the specimen	g. objective lenses
		h. ocular
		i. stage

4. Explain the proper technique for transporting the microscope.

Carry with two hands—one supporting the base, the other holding the arm.

5. Define the following terms.

real image: *An image formed by the objective lens that is inverted, reversed from left to right, and larger than the object*

resolution: *Ability to discriminate two closely situated objects as separate*

Viewing Objects Through the Microscope

6. Complete, or respond to, the following statements:

working distance
1. The distance from the bottom of the objective lens in use to the specimen is called the _____.

to the left
2. Assume there is an object on the left side of the field that you want to bring to the center (that is, toward the apparent right). In what direction would you move your slide?

Field
3. The area of the specimen seen when looking through the microscope is the _____.

95
4. If a microscope has a 10× ocular and the total magnification at a particular time is 950×, the objective lens in use at that time is _____×.

increases contrast
5. Why should the light be dimmed when you are looking at living (nearly transparent) cells?

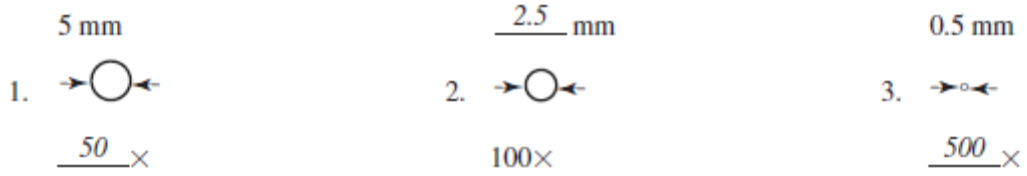
Parfocal
6. After focusing in low power, you find that you need to use only the fine adjustment to focus the specimen at the higher powers. The microscope is therefore said to be _____.

0.75
7. You are using a 10× ocular and a 15× objective. If the field size is 1.5 mm, the approximate field size with a 30× objective is _____ mm.

0.4
8. If the size of the high-power field is 1.2 mm, an object that occupies approximately a third of that field has an estimated diameter of _____ mm.

7. You have been asked to prepare a slide with the letter *k* on it (as shown below). In the circle below, draw the *k* as seen in the low-power field.

8. The numbers for the field sizes below are too large to represent the typical compound microscope lens system, but the relationships depicted are accurate. Figure out the magnification of fields 1 and 3, and the field size of 2. (Hint: Use your ruler.)



9. Say you are observing an object in the low-power field. When you switch to high power, it is no longer in your field of view.

Why might this occur? *The field decreases proportionately as magnification increases. Therefore, unless the object is centered at low power, it might be outside the higher-power field.*

What should you have done initially to prevent this from happening? *Center the object that you wish to view.*

10. Do the following factors increase or decrease as one moves to higher magnifications with the microscope?

resolution: *increases (to a point)* amount of light needed: *increases*

working distance: *decreases* depth of field: *decreases*

11. A student has the high-dry lens in position and appears to be intently observing the specimen. The instructor, noting a working distance of about 1 cm, knows the student isn't actually seeing the specimen.

How so? *The working distance for the h.p. lens is closer to 1 mm.*

12. Describe the proper procedure for preparing a wet mount.

Place the specimen on the slide with a medicine dropper or place a drop of water or saline on the slide. Mix specimen into drop using a toothpick. If staining, add a drop of stain and mix with a toothpick. Hold a coverslip with forceps so that the coverslip touches one side of the specimen drop, and then ***slowly*** *and* ***carefully*** *lower the angled coverslip onto the specimen.*

13. Indicate the probable cause of the following situations arising during use of a microscope.

a. Only half of the field is illuminated: *The lens is not correctly rotated into place.*

b. Field does not change as mechanical stage is moved: *The slide is not correctly positioned in the clamp on the mechanical stage and does not move when the mechanical stage moves.*