STUDENT LABORATORY — Onion Cell Staining and Cell Size

Grade: [ ] Lab Credits: [ ]

Full Name: ___________________________ Lab Date: __________
Lab Section: _______ Lab Instructor: ___________________________ Credit: 1 lab period

Objectives:
To create a wet-mount slide and use a draw-trough staining technique to examine cell structures
To determine the field diameter of a microscope and calculate average onion epidermis cell size

Pre-lab: [ ] (4 pts.)
Read Dragonfly (pp. 174, 1063, 1065) or Parrot (pp. 191, 207-7, A-10, A-15).

One millimeter (mm) contains 1,000 microns (µm). Microns are also known as micrometers. The SI symbol for micro (one millionth of a meter) is the Greek letter µ ("Mu"). Answer the following questions. Show your work and include unit symbols!

1. A carrot cell is 100 microns. Express this as millimeters. ________

2. A small ant measures 5 mm in length. How long is the ant in microns? ________

3. In the diagram to the right, the average size of each cell is 0.25mm. What is the field diameter in millimeters? In microns?
   ________    ________

4. Express the length of this biological specimen in millimeters and in microns.
   ________    ________

LABORATORY EXERCISE
*Note — This lab is due at the end of the lab period or as directed by your instructor. Your instructor may modify the lab based on time.

Materials:
Microscope, lens paper, slides, cover slips, water dropper bottle, tweezers, onion, Lugol’s iodine, clear metric ruler slides.

Procedure: [ ] (11 pts.)

Onion Wet Mount:
1. Get a clean glass slide and cover slip.
2. Obtain a piece of onion.
3. Use your fingers (nails work well), or forceps, to carefully peel off a small piece of skin from the inner or concave side of the onion chunk. This piece should be thin and translucent, looking much like a piece of scotch tape.
4. To prepare a wet mount of the onion with distilled water, lay the onion skin flat on a glass slide. Make sure the skin doesn’t fold over on itself (this can be tricky). Add one or two drops of water from the dropper bottle on top of the onion epidermis.
5. Place one side of the coverslip just to the edge of the water at a 45-degree angle. Gently lower the coverslip onto the drop (*Hint—you may want to use a forceps to position the coverslip). Using this procedure helps prevent air bubbles from being trapped under the coverslip.

6. Clean and adjust your slide: If water runs out from the edges of the coverslip, you may have added too much water. If there is an air space under the coverslip, you may have not added enough water, or you may have placed the coverslip on the slide improperly. You may want to use a piece of paper towel to soak up extra water, or add a drop of water to the edge of the coverslip to displace air bubbles. You can also gently tap the coverslip with a pencil tip or forceps to drive some air bubbles out.

7. Observe the onion under low (100x) power. Do not draw the onion yet.

Stain the onion cells using the Draw-Through® method:
   1. Precisely place a drop of Lugol’s iodine at one edge of the coverslip. Hold a small piece of paper toweling at the opposite edge with forceps to draw off the water and allow the iodine to seep through. Look at the picture on the right.
   2. Observe the stain advancing across the field of view. Allow 1-3min for the stain to seep across. (*Hint — focus on cells located near the edge of the coverslip where you added the stain. These cells will get stained first.)
   3. Observe the onion cells under low power.
   4. Draw two adjacent onion cells. Identify and label the cell wall, cell membrane, nucleus, and nucleolus, if visible.
   5. Remove the slide from the stage and save it for later. (4 pts for each drawing)

Determining the Field Diameter:
   1. Determine the diameter of the field of vision, or field diameter, using the metric ruler slide. Do this under low power (yellow, 10x objective). Move the metric ruler slide on the stage until it is centered while looking through the eyepiece. Draw what you see in the space provided above.
   2. Estimate the field diameter. The space between a pair of black lines is one millimeter. What is the diameter of the field of vision in millimeters? In microns? Record both below (2 pts):

<table>
<thead>
<tr>
<th>Field Diameter of Low Power (100X)</th>
<th>Millimeters (mm)</th>
<th>Microns (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>_____ mm</td>
<td>_____ µm</td>
</tr>
</tbody>
</table>

Measuring Onion Cells:
   1. Remove the ruler slide from the stage, and place the onion slide on the stage.
   2. Examine your onion cells under low power. Align the slide so that the cells run lengthwise in a straight line across the center of your field of view.
   3. Count the number of cells lengthwise along the diameter, the widest part of the circle. Record the number in the table below. (1 pt)

| Number of cells that fit across field of view | Lengthwise _____ |
## Analysis and Conclusions:  

(5 pts.)

1. Estimate the average length of an onion cell in mm and then in microns. (*Hint: In your calculations, divide the field diameter of your microscope by the number of cells that fit across.)*

<table>
<thead>
<tr>
<th>Field diameter under low power (100x)</th>
<th>_____ mm</th>
<th>_____ µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells that fit across the field of view <em>lengthwise</em></td>
<td>_____</td>
<td></td>
</tr>
<tr>
<td>Average length of an onion cell</td>
<td>_____ mm</td>
<td>_____ µm</td>
</tr>
</tbody>
</table>

Show your calculations:

*Note — Hand this lab in at the end of the lab period or as directed by your lab instructor.*