Pre-Lab Questions: Microscope Lab: Estimating Size and Calculating Magnification

1. Draw a sketch of the microscope in your lab notebook. Label the parts of the microscope.
Microscope Lab: Estimating Size and Calculating Magnification

IB Internal Assessment of................. DCP and CE

Part 1: Estimating Size of Specimens under the Microscope

Purpose: To determine an approximate field diameter for each of the objective lenses on our microscopes.

Materials:

1. a microscope with 4x, 10x and 40x objectives
2. a small plastic ruler
3. a cotton bud
4. a dropping pipette
5. fine tweezers
6. two slides and cover slips
7. live material of moss leaves, and/or onion cells
8. Iodine
9. Methylene blue

Background Information:
When viewing a small organism through the microscope, it's usually necessary to have some idea of its size. Therefore, you need to have some means of estimating the size. When someone is standing near a doorway, you can estimate their height by comparing them to the doorway. In the same way, you can estimate an organism's length by comparing it to the field of view that you are using.

Example: If the "doorway is 10 units, how high is the stick person?"

Answer: Approximately 6 units high.
Procedure:
Calculate the diameter of the field of view for low and medium power.

1. Copy the following 2 tables into your lab notebook under the “Observations” section of your lab report.

<table>
<thead>
<tr>
<th>Magnification of Microscope</th>
<th>Field Diameter (mm)</th>
<th>Field Diameter (μm)</th>
<th>Calculated Constant (FD xMagnification)</th>
<th>Average Constant for Microscope</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW (______X)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium (______X)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (_______X)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Magnification of Microscope</th>
<th>Field Diameter (mm) (Class Mean)</th>
<th>Field Diameter (μm) (Class Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW (______X)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium (______X)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (_______X)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Calculate the total magnification of the low power objective lens by multiplying the magnification of the ocular lens by the magnification of the objective lens. The magnification of the lenses is etched on the sides of the actual lens holders. Record the Magnification of all power levels for your microscope in table 1.

Example: Low power: Objective lens = 10X  Ocular lens = 10X

Total magnification at Low Power = 10X (10X) = 100X

1. Set up a microscope on the x4 objective and focus it on the ruler scale.

Figure 1
2. Move the microscope stage until the 0.0mm of the ruler touches the left-most point of the field of view.

3. Read off the diameter (in mm) and record your result neatly. (note how accurate you have been, e.g. +/- 0.5mm)

4. Change the objective to x10 and repeat steps 2 & 3. The field of view should now be smaller (less than half the x4).

5. Estimate the x40 objective's field of view by dividing the x4 diameter by 10. (Use mm units).

Discussion Questions Part 1: Use the information from your data table 2 (class means of field diameter) to answer the following questions.

1. Many ponds often have a green scum on the surface. This scum is a tangled mass of stringy algae filaments. Looking at a filament under high power shows four cells arranged end to end across the field of view.

Algae Filaments from "Pond Scum"

![Algae Filaments Image]

a. What is the diameter of your high power field of view in micrometers?
b. How long is each cell approximately?

2. Given the following information, estimate the approximate actual size of the organisms in each case in micrometers. Your answers should be rounded to a convenient number. (They are only estimates).

a. A bug stretches ½ way across the low power field.
b. A cell stretches ¼ way across the medium power field
c. Twenty cells fit across the high power field
d. Fifteen plant cells stretch across the medium power field
e. A bug stretches 2/3 way across the medium power field
f. An insect stretches ¾ way across the high power field.
g. Five micro bugs fit across the low power field.
h. Half a worm fits across the low power field.
Part 2: Calculating Magnification

Much of the time you will be asked to draw what you see under the microscope. These drawings will be much larger than your specimen. You need to indicate, somehow, approximately how much larger than life your drawings (or photographs) are. The general formula for calculation magnification is:

\[
\text{Magnification} = \frac{\text{Drawing size}}{\text{Actual size}} = \frac{D}{A}
\]

You must ALWAYS use the same units for drawing size and actual size for this equation to work!

Part 2 Discussion Questions:

1. To practice calculating magnification, copy the following chart into your lab report and in the table. (be careful with the units!)

<table>
<thead>
<tr>
<th>Actual Specimen Size</th>
<th>Drawing Size</th>
<th>Drawing Magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mm</td>
<td>2 cm</td>
<td></td>
</tr>
<tr>
<td>200 μm</td>
<td>1 cm</td>
<td></td>
</tr>
<tr>
<td>40 μm</td>
<td>2 cm</td>
<td></td>
</tr>
<tr>
<td>100 μm</td>
<td>5 cm</td>
<td>200X</td>
</tr>
<tr>
<td></td>
<td>4 cm</td>
<td>100X</td>
</tr>
</tbody>
</table>

2. A student draws a leaf and labels it \( \frac{1}{2} \times \). What does this label mean?

3. A student, observing a micro-organism under a magnification of 40X, calculates that it is about 100μm long.
   a. If she then draws the micro-organism 2 cm long, what is the magnification of her drawing?
   b. If her partner draws the micro-organism at a magnification of 1000X, how long will the drawing be?

4. You observe that an object stretches across \( \frac{3}{4} \) of the low power field. What is its approximate length? What would be the magnification if you drew it 10cm long?

5. If five cells fit across the high power field, what is their average length? If you draw one cell at the magnification of 500X, how long will your drawing be?
6. A paramecium swims across the medium power field in 15s. How fast is it swimming in micrometers per minute?

Part 3: Drawing, estimating and comparing the size of cells

Preparing slides of cells

Creating your own slides - Cheek Cells: Read the procedure carefully.

Procedure:
1. Before you begin, make sure your slide and cover slips are clean. You don't want lint or fingerprints on your slide. If the slide is dirty, rinse it off and dry it well with a paper towel.
2. Put a drop of methylene blue on a clean slide. Caution: Methylene blue will stain clothes and skin.
3. Gently scrape the inside of your cheek with the flat side of a toothpick. Scrape lightly. If you see something on the toothpick after the scraping, you did something wrong. Since cells are microscopic, you should not be able to see them!
4. Stir the end of the toothpick into the stain and throw the toothpick away.
5. Place the cover slip at a 45-degree angle on the edge of the water/saliva mix. Allow the liquid to spread down the edge of the cover slip. Once it has spread, carefully lower the cover slip over the liquid.
   - If you have a lot of air bubbles regardless of size, rinse of your slide and start over. It's important that you make a good slide.
6. Use low power objective. Cells should be visible, but they will be small and look like irregular shaped objects. Once you think you have located a cell, switch to high power and refocus. (Remember: DO NOT use the coarse adjustment knob at this point!) Draw a picture of your cheek cell.

Creating your own slides - Plant Cells:
1. Peel a translucent piece of tissue from the onion. The smaller the piece the better. (Translucent means that you can see light through the specimen, but it is not transparent.)
2. Place the piece of onion on a glass slide and add a drop or two of the iodine solution.
3. Cover the slide with a cover slip using the same technique that you used for your cheek cells.
4. Observe the onion cell under both low and high power. Draw a diagram of the onion cell.
Estimating the size of the cells

1. Focus your microscope on the x4 objective onto your cells
2. Once in focus turn the objectives to x10 and refocus USING FINE FOCUS.
3. Identify one cell which is clearly visible.
4. Estimate how many cells will fit into one diameter of your field of view.
5. Record this raw result in your data table. Use Figure 3 to then calculate the size of one cell. You should have two values, how many cells and the size of one cell.
6. Repeat steps 1-5 with another biological specimen. (You need results for two different cells)

The field of view when using the 10x objective (100x total magnification) is 2 mm. If 8 plant cells extend across the field of view (2 mm), then each cell is 2/8 or 0.25 mm long. Remember that the diameter of the field of view changes depending on the power of the objective.

7. Collect Class Data and complete an IB Data Collection & Processing (DCP) & Conclusion and Evaluation (CE) for Part 3: Estimating the size of the cells only!!!! (Aim: Compare the size of two different cells.)
8. Use IB Biology Internal Assessment Rubric & All other helpful documents given in class.

Cleaning Up

1. Rinse off your slides and cover slips. Dry them with a paper towel and return them to the center of the lab table where they were found.
2. Make sure the stain and medicine droppers are at the center of the lab table next to the slides and cover slips. Make sure all tops are secure.
3. Turn off the microscope, unplug it, and put the cover on it.