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.

2. Create a table and list microscope parts and their functions.

3. Summarize how to properly use a microscope. Helpful websites:
   - http://shs.westport.k12.ct.us/mjvl/biology/microscope/microscope.htm
   - Youtube video: http://www.youtube.com/watch?v=jP9HtcAvGDk

4. Cornell Notes-Powerpoint: Microscope and Magnification
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. Compound light microscope with 4x, 10x and 40x objectives
2. a small plastic ruler
3. a cotton bud
4. a dropping pipette
5. fine tweezers
6. three slides and cover slips
7. Onion cells, Elodea leaves, yeast solution
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 1:**

<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>
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<td></td>
</tr>
</tbody>
</table>

**Table 2:**

<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](image-url)
2. Move the microscope stage until the 0.0mm of the ruler touches the left-most point of the field of view.
3. Read of 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.

   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 \( \frac{1}{2} \) way across the low power field. _________
   b. A cell stretches \( \frac{1}{4} \) 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 \( \frac{2}{3} \) way across the medium power field _________
   f. An insect stretches \( \frac{3}{4} \) 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:

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

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 \( \frac{1}{2} \) X. 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 - Onion Plant 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. 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.)
3. Place the piece of onion on a glass slide and add a drop or two of the iodine solution.
4. Place the cover slip at a 45-degree angle on the edge of the iodine solution. 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.
5. Use low power (4X) objective to focus the onion cells.
6. Switch to 10X and focus using only the fine adjustment knob.
7. Using a sharp pencil and the clean template paper provided, draw precisely what you see under low power, labeling each visible structure. You do not need to draw every visible cell, but a representative group.
8. Using the "Estimating the size of the Cells" section below, estimate the size of one cell and also record how many cells would fit across the field of view—both values should be entered into your "raw data table."
9. Switch to high power and use only the fine adjustment knob to refocus. Draw another diagram that contains all visible structures appropriately labeled.

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

Elodea, (also known as Elodea densa, or "waterweed") is an aquatic plant in the family Hydrocharitaceae. Its leaves are only two cells thick, making it ideal for viewing cells and organelles.

1. Pick an Elodea leaf. Put it in the middle of a slide with a drop of pond water and cover with a coverslip as indicated above in the Onion slide instructions.
2. Locate one cell (usually the edge of the leaf works best) to examine more closely. Draw the cells under low (4X) and medium (10X) magnification. If possible also draw the cells under high power (40X). Remember you only need to draw a representative cell or group of cells, not every cell that you see.
3. Label all visible structures, including the cell wall, cytoplasm, chloroplasts, central vacuole (you cannot see the edges of the vacuole, but can infer that it is there because the chloroplasts are found and move only around the outer edges of the cell. Try to locate the larger, narrow, transparent nucleus.
4. Use the fine adjustment knob to focus up and down the central vacuole, and look for chloroplasts that are moving in a circular motion, indicating that cyclosis (cytoplasmic streaming) is occurring, as it often does in leaf cells.
5. Using the "Estimating the size of the Cells" section below, estimate the size of one cell and also record how many cells would fit across the field of view—both values should be entered into your “raw data table.”

Creating your own slides – Elodea Plant Cells: Read the procedure carefully.

Baker's yeast Saccharomyces cerevisiae (Domain Eukaryota, Kingdom Fungi) converts carbohydrates to carbon dioxide and alcohols. Like all fungi yeasts may reproduce sexually or asexually-- the most common mode is asexual reproduction by budding, as shown below:

1. Place one drop of yeast solution on the center of the slide and add a coverslip.
2. Examine your slide with the 4x, 10x, and 40x objectives (using only fine adjustment knob with 10x and 40x objectives). 3. Carefully focus up and down with the fine adjustment to observe the fact that these cells are three-dimensional (adjustment of the iris diaphragm and rheostat may help you to see this better. Yeast cells should be fairly oval in shape. How much size variation can you see? Do you see any cells with reproductive buds attached? Can you see any of the organelles within the cells? Yeast cells do have a thin cell wall and clear cytoplasm. The nucleus cannot be seen unless special staining techniques are used.
3. After observing the cells unstained, add a small drop of methylene blue by removing the slide from the microscope and dropping a drop of methylene blue next to one edge of the coverslip. Again, examine under each power.

4. Draw what you see under medium and high power. What difference(s) does the methylene blue make in the “visibility” of the yeast cells or their organelles? Note any other observations (for example, have all of the cells taken up the dye equally?).

5. Using the “Estimating the size of the Cells” section below, estimate the size of one cell and also record how many cells would fit across the field of view—both values should be entered into your “raw data table.”

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)

![Figure 2](image2.png)

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 and visible ultrastructure of three 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 by grasping plug, not pulling on wire!**, and put the cover on it.

**Sources:** The above lab procedures were modified from:

- [http://biology.clc.uc.edu/courses/bio114/cells%20intro.htm](http://biology.clc.uc.edu/courses/bio114/cells%20intro.htm)

**Appendix 1: Sample Raw Data Table Format.**

<table>
<thead>
<tr>
<th>Organism (Scientific Name)</th>
<th>Objective Used</th>
<th>FOV size</th>
<th>How many cells fit across FOV</th>
<th>Length of one cell in fraction form</th>
<th>Length of one cell (include correct units)</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>
Appendix 2: Sample Class Average Data Table Format (Need 3 tables total-one for each species of cell).

<table>
<thead>
<tr>
<th>Organism (Scientific Name)</th>
<th>Organism (Scientific Name)</th>
<th>Organism (Scientific Name)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Length (include correct units)</td>
<td>Cell Length (include correct units)</td>
<td>Cell Length (include correct units)</td>
</tr>
</tbody>
</table>

From this data table you will calculate statistics to appropriately graph the class data.