Part 1: Estimating Size of Specimens Under the Microscope

a. Determination the approximate field diameter for each of the objective lenses by using ruler

**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:**
To calculate the diameter of the field of view for low (40X) and medium (100 X) power.
1. 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.

**Example: Low power: Objective lens = 4X       Ocular lens = 10X**

Total magnification = magnification of eyepiece × magnification of objective lens
Total magnification at Low Power = 10 × 4 = 40X

2. Take a clear plastic ruler and examine the millimetre scale under low power.
3. Place the centre of one of the scale marks along the edge of the field as shown below.
4. Count the whole number of millimetre spaces. If there is part of a spacing, estimate (in decimals) the size of the millimetre portion that shows. Record the field diameter in millimetres.

Example:

\[
\text{Millimeter Ruler under Low Power}
\]

\[
\text{The distance across this field of view is 4.2mm.}
\]

5. Convert the field diameter for low power into micrometers and record this number. \((1 \mu m = \frac{1}{1000^{th}} \text{mm})\)
6. Repeat steps #3-5 for the medium power objective lens. \textbf{Do not use the ruler with high power.}

To calculate the field diameter for high power and the oil immersion lens.
The field diameter for high power cannot be measure directly using your millimetre ruler because this field diameter is LESS than one millimetre. Therefore, we must calculate the field diameter a different way.

\textbf{The diameter of the field of vision under high power is calculated by}

\textbf{1. using the information from the lower power objective.} There is an inverse relationship between magnification and field of vision. As the magnification increases, the size of field decreases. To calculate the field of vision at high power, use the following formula.

\[
\frac{\text{high power magnification}}{\text{low power magnification}} = \frac{\text{low power field diameter}}{\text{high power field diameter}}
\]

\textbf{Example}

Say the diameter of the 40X field is 2 mm. You can compute the diameter of 100X field as

\[
\frac{100}{40} = \frac{2 \text{ mm}}{\text{high power field diameter}}
\]

\[
80 = 100 \times \text{high power field diameter}
\]

High power field diameter = 0.8 mm or 800 \(\mu\text{m}\)
2. using a “constant” number that can be calculated:

Field diameter × total magnification = a constant

Once you know what the constant is for your microscope, you can use it to solve for the field diameter.

1. Calculate the constant for both low and medium power. The “constant” that you calculate will probably not be the same since we have been ESTIMATING the field diameter, and there is bound to be some error.
2. Take the average of the two constants calculated. Record this number in your notebook. From now on, it is the AVERAGE constant that you will use for your calculations. (All four powers will have the same average constant).
3. Calculate the field diameter for the high power and the immersion lens using the formula: \( \text{field diameter} \times \text{total magnification} = \text{constant} \).
4. Substitute the known values for the total magnification and the constant (the average) and then solve the equation for field diameter.

Example:
The average constant for my microscope at home is 145. High power magnification is 400X. What is the field diameter for my microscope at high power?

Field diameter × total magnification = a constant
Field diameter × 400X = 145
Field diameter = 145/400
Field diameter = 0.36 mm = 360 \( \mu \)m

5. Compare your result with the rest of the class. Record your field diameters on a common chart at the front of the room.
6. Calculate the class average field diameter for each power and record this information in your notebook. We will use these values from now on when we are estimating the size of specimens under the microscopes.

<table>
<thead>
<tr>
<th>Magnification of Microscope</th>
<th>Group 1 Field Diameter (( \mu )m)</th>
<th>Group 2 Field Diameter (( \mu )m)</th>
<th>Group 3 Field Diameter (( \mu )m)</th>
<th>Group 4 Field Diameter (( \mu )m)</th>
<th>Group 5 Field Diameter (( \mu )m)</th>
<th>Group 6 Field Diameter (( \mu )m)</th>
<th>Average Field Diameter (( \mu )m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW (_____X)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEDIUM (_____X)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIGH (_____X)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OIL (_____X)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Problems:
1. Consider the class averages of field diameter are 4100µm, 2000µm, 455µm for low, medium and high powers respectively to estimate the approximate actual size of the organisms in each case in micrometers. Round your answers to a convenient number.
   a. A copepod stretches ½ way across the low power field.
      Approximately 4100µm / 2 = 2050µm
   b. A cell stretches ¼ way across the medium power field
      Approx. 2000µm / 4 = 500µm
   c. A copepod stretches 2/3 way across the medium power field
      Approx. 2000µm x 2/3 = 1333.3µm
   d. An ciliate stretches ¾ way across the high power field.
      Approx. 455µm x ¾ = 341.3µm
   e. Half a worm fits across the low power field.
      Approx. 4100µm x 2 = 8200µm = (8.2 mm)

If you see several object in your field, the size of a single object can be calculated by using the formula below:

\[
\text{size of object} = \frac{\text{diameter of the field of view (µm)}}{\text{Number of times objects fits across the field view}}
\]

f. Twenty cells fit across the high power field
   Approx. 455µm / 20 = 22.8µm

g. Fifteen plant cells stretch across the medium power field
   Approx. 2000µm / 15 = 133.3µm

h. Five protozoan fit across the low power field.
   Approx. 4100µm / 5 = 820µm
b. Determination the size of objects by using ocular micrometer

The size of microscopic objects can be accurately measured with an ocular micrometer, a scale that has been etched in glass disk and inserted within the ocular of the microscope. You can also estimate the size of an object, with lesser degree of accuracy, by comparing the object's size to that of the diameter of the microscopic field. Directions for both methods, which are applicable to both compound and dissecting microscopes, follow.

Calibration of Ocular Micrometer

You will need two scales: the ocular micrometer, a glass disc bearing an arbitrary scale of 50 to 100 divisions (upper scale shown in Fig.), and a stage micrometer, a stage micrometer, a glass slide etched with known scale of 1 mm or 2 mm usually subdivided into units of 0.1 mm and 0.01 mm (Fig., lower scale). Because the ocular micrometer scale is arbitrary, the scale value differs for each objective, and therefore must be calibrated against a standard scale on the slide micrometer.

1. Place the stage micrometer on the microscope stage and bring it into sharp focus at the lowest power magnification.
2. Adjust the ocular and stage micrometer scales so that they are parallel and their zero lines coincide, as shown in Figure.
3. Locate the point toward the right where lines form the two scales also coincide.
4. Count the number of lines on the stage micrometer (SM) and the number of lines on the ocular micrometer (OM) between the zero point and the point where the lines coincide exactly.
5. Then divide the number of SM lines by the number of OM lines.
6. Because the number of SM lines represents a real value (in millimetre), this division reveals the value of one OM unit (OMU) for that specific objectives lens.
7. Repeat the procedure for all objective lenses.

In the example shown in the figure, 70 division on the OM scale are equal to 24 divisions (= 0.24 mm) on the SM. The division SM/OM = value of each OMU, or

0.24 mm/70 = 0.0034 mm = 3.4 µm

1 OMU = 3.4 µm.

Two OMU = 6.8 µm, 5 OMU = 17 µm, and so on.
Part 2: Calculating Drawing 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:

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

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

Problem

Practice calculating magnification, by copying the following table into your lab report and complete the missing values. (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>???</td>
<td>200X</td>
</tr>
<tr>
<td>???</td>
<td>5 cm</td>
<td>100X</td>
</tr>
<tr>
<td>???</td>
<td>4 cm</td>
<td>50X</td>
</tr>
</tbody>
</table>

Homework Questions:

1. A microscope has an eyepiece lens with a power of 5X. The objective lens being used has a power of 10X. How much is the total magnification?
2. With a low power lens magnification of 40X and a high power lens magnification of 200X, what would the calculated high power field diameter be if the lower power field diameter was previously determined to be 3700 µm?
3. If five cells fit across the high power field (field diameter of \(455\,\mu m\)), what is their average length? If you draw one cell at the magnification of 500X, how long will your drawing be?
4. 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 (Figure shown to right). If the diameter of your high power field of view in micrometers is \(455\,\mu m\) How long is each cell approximately?
5. A student draws a leaf and labels it \(\frac{1}{2} X\). What does this label mean?
6. A student, observing a micro-organism under a magnification of 40X, calculates that it is about 100µm long.
   a. If he then draws the micro-organism 2 cm long, what is the magnification of her drawing?
   b. If his partner draws the micro-organism at a magnification of 1000X, how long will the drawing be?
7. A paramecium swims across the medium power field in 15s. How fast is it swimming in micrometers per minute? Field diameter at medium power = 2000µm