5. Measurement of the sizes of objects using microscope

The light microscope is a common instrument for measuring the sizes of microscopic objects, such as cells and organelles. This may be carried out rather coarsely by using a transparent ruler, or more precisely with a micrometer graticule.

A. By using a transparent ruler

The size of the field of view under the microscope decreases proportionately when the magnification is increased. If the diameter of the field of view under the lowest magnification is known, the relative field diameters of other magnifications can be calculated.

Procedure
1. Place a transparent ruler (in mm divisions) on the stage of the microscope. Use the lowest magnification to measure field diameter 1.

   Field diameter 1 = (A) mm
   Magnification 1 = (B)

2. Switch to a higher magnification.

   Magnification 2 = (D)

3. Calculate field diameter 2 of the higher magnification using the formula as follow:

   \[
   \text{Field diameter 1} \times \text{Magnification 1} = \text{Field diameter 2} \times \text{Magnification 2}
   \]

   \[
   \text{Field diameter 2} = \frac{(A) \times (B)}{(D)} \text{ mm} = \frac{(A) \times (B)}{(D)} \times 10^3 \text{ µm}
   \]
4. Repeat the procedure for other magnifications and enter the calculations in the form of a table.

<table>
<thead>
<tr>
<th>Objective Magnification</th>
<th>Magnification 2</th>
<th>Field Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>x 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>x 10</td>
<td></td>
<td></td>
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<tr>
<td>x 25</td>
<td></td>
<td></td>
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<tr>
<td>x 100</td>
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</tbody>
</table>

5. Once the field diameter in micrometers is known, it is possible to estimate the size of an object by comparing its size to the field diameter. Consider the size of *Paramecium* (Fig. 2) shown below:
Suppose that you are using low power magnification where field diameter is 1500 µm. The length of *Paramecium* is about 1.5 divisions out of 10 imaginary divisions, which is equal to 0.15, or 15%. The estimated size would thus be:

\[
0.15 \times 1500 \, \mu m = 225 \, \mu m
\]

**B. By using the Harris-type micrometer graticule**

The Harris graticule is recommended for use in schools because it is inexpensive and reasonably accurate. This graticule is presented on a strip of 35 mm photographic film. The scale is 10 mm long, numbered at 1 mm intervals with 0.1 mm sub-divisions (Fig.3).

*Preparation*

1. Preparing a stage micrometer
   - Cut a micrometer scale from the filmstrip as shown in Fig.4.
   - Mount the scale on a slide under a cover slip using Canada Balsam (Fig.5).
2. Preparing an eyepiece micrometer
   • Cut another micrometer scale from the filmstrip.
   • Unscrew the eye lens of the eyepiece, and place the scale on the diaphragm within (Fig.6 & 7). Hence the scale is positioned between the field lens and the eye lens of the eyepiece.

**Note**
1. For detailed instructions, please refer to the manual that comes with the micrometer graticule.

**Procedure**
1. Calibrating the eyepiece micrometer
   • Place the stage micrometer on the stage of the microscope.
   • Rotate the eyepiece (containing the eyepiece micrometer) and move the stage micrometer until the two scale lines overlap each other.
   • An example illustrating the calculation is given below.
   The stage micrometer lines up exactly at 10 and 81 marks of the eyepiece micrometer.

\[
\begin{align*}
\Box \quad \text{Actual distance} & = 0.3 \text{ mm (300 } \mu \text{m)} \\
\Box \quad \text{No. of eyepiece divisions to cover } 300 \mu \text{m} & = 81 - 10 \\
& = 71 \\
\text{The size of each eyepiece micrometer division} & = 300 \div 71 \mu \text{m} \\
& = 4.23 \mu \text{m}
\end{align*}
\]
2. Measuring the size of an object
   - Replace the stage micrometer with the slide containing the object (e.g. a piece of fibre) to be measured.
   - The actual measurement is illustrated as shown below.

   ![Diagram](image)

   - No. of eyepiece divisions = 16 – 4
     = 12
   - Thickness of the fibre = 12 x 4.23 µm
     = 50.76 µm