

Calibration of microscope

In order to measure the size of a structure on a microscope slide it is necessary to calibrate the microscope. Inside the eyepiece of the microscope there is an eye piece graticule. It is graduated 1-10 with 10 subdivisions between each number therefore the eyepiece graticule has 100 eyepiece units [epu] along its length.



With different magnifications, the divisions on the eyepiece graticule will cover different actual lengths of the specimen on the slide.

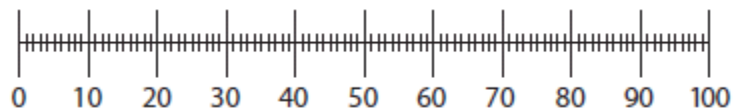
A stage micrometer is used to measure the length of each division at different magnifications. There are two types of stage micrometer available, check which you are using.

Either

The stage micrometer is a slide with a line **1 mm** long on it. The line is also marked for tenths and hundredths of a mm. There are 100 stage micrometer units [smu] on the 1 mm line.
Each stage micrometer unit = 0.01 mm or 10 μm .

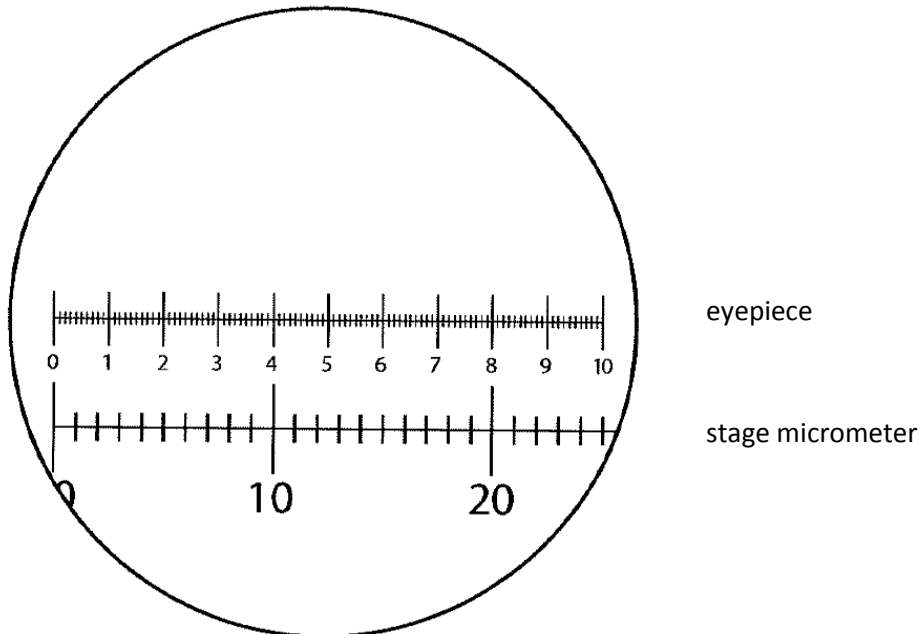
Or

The stage micrometer is a slide with a line **10 mm** long on it. The line is also marked for tenths and hundredths of a mm. There are 100 stage micrometer units [smu] on the 10 mm line. Each stage micrometer unit = 0.1 mm or 100 μm .



To calibrate the microscope

- Line up the zero of the eyepiece graticule and the zero of the stage micrometer.
- Make sure the scales are parallel.
- Look at the scales and see where they are in line again.



Using this x40 objective lens, 20 stage micrometer units make up 80 eyepiece units.

80 eyepiece units = 20 stage micrometer units

If 1 stage micrometer unit = 0.01 mm

$$1 \text{ eye piece unit} = \frac{20}{80} = 0.25 \text{ stage micrometer units}$$

$$1 \text{ stage micrometer unit} = 0.01 \text{ mm}$$

$$\begin{aligned} 1 \text{ eye piece unit} &= 0.25 \times 0.01 \text{ mm} \\ &= 0.0025 \text{ mm or } 0.0025 \times 1000 \mu\text{m} \\ &= 2.5 \mu\text{m} \end{aligned}$$

If 1 stage micrometer unit = 0.1 mm

$$1 \text{ eye piece unit} = \frac{2}{80} = 0.025 \text{ stage micrometer units}$$

$$1 \text{ stage micrometer unit} = 0.1 \text{ mm}$$

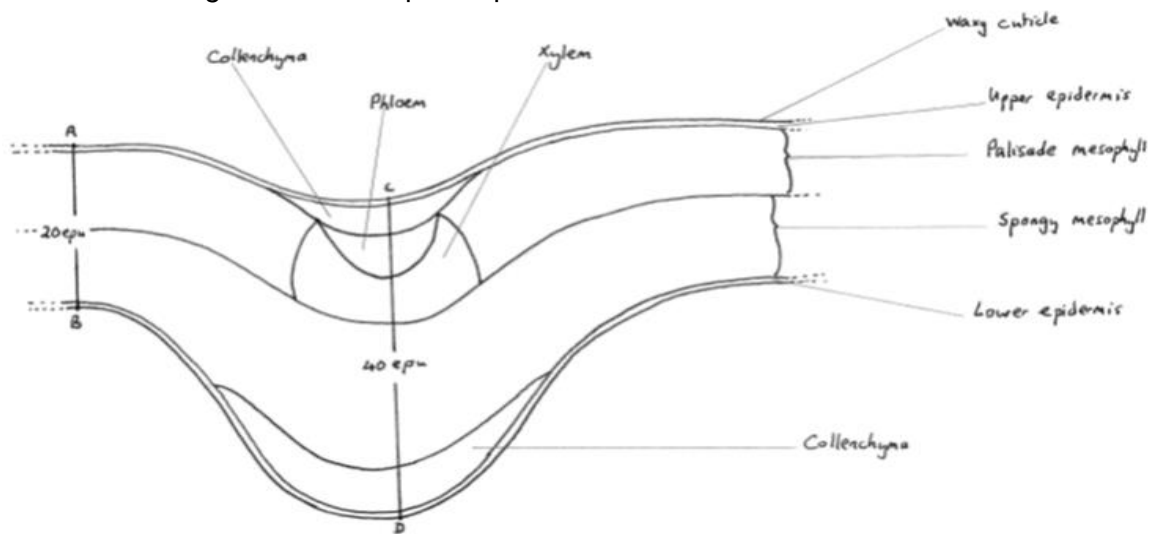
$$\begin{aligned} 1 \text{ eye piece unit} &= 0.025 \times 0.1 \text{ mm} \\ &= 0.0025 \text{ mm or } 0.0025 \times 1000 \mu\text{m} \\ &= 2.5 \mu\text{m} \end{aligned}$$

Microscope drawing

Low power plan

This shows the distribution of tissues in a transverse section (TS) or longitudinal section (LS) of a structure.

T.S. Leaf of *Ligustrum* – Low power plan



It is not always necessary to draw a plan of the entire structure but if a part is drawn it should be indicated that it is a part of a structure. This is usually done by drawing dotted lines to show where the tissues continue.

When completing low power plans, you should:

- use a sharp pencil.
- not use any shading
- not draw any individual cells
- make your drawing at least half a page of A4 in size and position the labels to the side of the drawing
- make all lines clear, complete and not overlapping
- draw label lines with a ruler to the centre of the tissue layer, they should not cross each other
- ensure tissue layers are all drawn to the correct proportion
- draw a line across two tissues and give the width of this line in eyepiece units. If one line across tissue A has been given 48 epu and the second line across tissue B has been given 12 epu, the correct proportion should show that tissue A is 4 times the width of tissue B at that point.
- check tissue boundaries by using a higher objective lens than that being used to draw the plan

High power drawing of individual cells

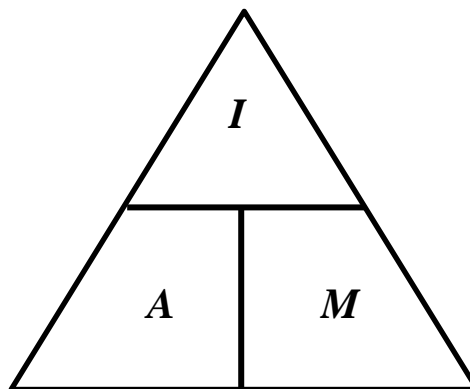
When completing high power drawings of individual cells, you should:

- use a sharp pencil
- not use any shading
- draw two or three cells
- make your drawing at least half a page of A4 in size and position the labels to the side of the drawing
- make all lines clear, complete and not overlapping
- use single lines to represent the tonoplast membrane or the cell membrane. A double line should be used to represent the cell wall
- calculate the actual length or diameter of the cells
- not draw structures which you cannot see for example details of the structure of the chloroplast or mitochondria using a x40 objective

Magnification of a drawing

Magnification shows us the size of a drawing or image in relation to the size of the actual object.

The magnification, size of object or size of image can be calculated using the triangle method.



I = Size of image

A = Actual size of object

M = magnification.

Cover what you wish to calculate and the equation is given.

$$I = A \times M.$$

$$M = \frac{I}{A}$$

$$A = \frac{I}{M}$$

Check that the units for the size of the object and image are the same.