

## **Calibration of the light microscope at low and high power, including calculation of actual size of a structure and the magnification of a structure in a drawing.**

**Specification reference:** 1.2

### **Cell structure and organisation**

#### **Introduction**

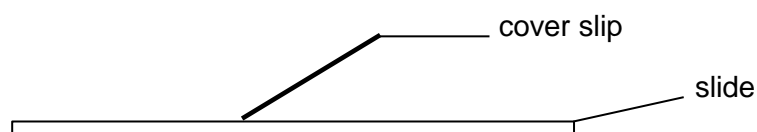
This practical is a simple introduction to the use of a microscope and how it can be used to take measurements.

#### **Apparatus**

Microscope fitted with an eye piece graticule  
Stage micrometer  
Microscope slide  
Microscope slide cover slip  
Paper towel

#### **Method**

1. Using the method given in the guidance section, calibrate your microscope at low, medium and high power.
2. Take one human hair and place it on a microscope slide.
3. Put a small drop of water on the hair.
4. Hold a cover slip on the slide as shown in the diagram.



5. Gently lower the cover slip onto the hair and press it gently with a piece of paper towel.
6. Use the x40 objective to measure the width of the hair in eye piece units and use the method given in the guidance section and your calibration to calculate the actual size.
7. Draw a section of the hair.
8. Calculate the magnification of your drawing by using the method given in the guidance section.

## Risk Assessment

Hazard	Risk	Control measure
Microscope lamp/ bulb is hot	Could burn skin when trying to move lamp/ microscope	Leave lamp to cool before moving

## Teacher/ Technician notes

Microscopes must be fitted with eye piece graticules. These can be bought separately and fitted to most microscopes retrospectively.

Stage micrometers can be bought ready made or can be made by buying film reticules from scientific suppliers and fixing them to microscope slides. Stage micrometers come in two sizes and the calibration of both are given below.

Please make sure that students are aware that there are **100 eyepiece** units in the eyepiece graticule and they should **not** be working in **fractions** of an eyepiece or stage micrometer unit e.g. 2.8. instead it should always be **whole numbers**, e.g. 28.

There is space for the students to record the calibration for their microscopes in this practical. It is best if they use the same microscope for each piece of microscope work, there is then no need for them to calibrate the microscope every time. Instead they can refer to that page in their lab book for the calibration for that objective. The microscopes can be labelled for ease of identification.

## **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.

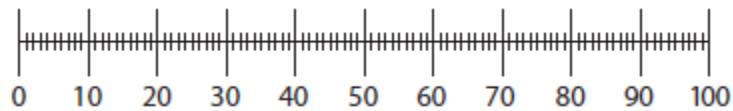
A stage micrometer is used to measure the length of each division at different magnifications. There are two types of stage micrometer available, check which you are using.

**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  $\mu\text{m}$ .

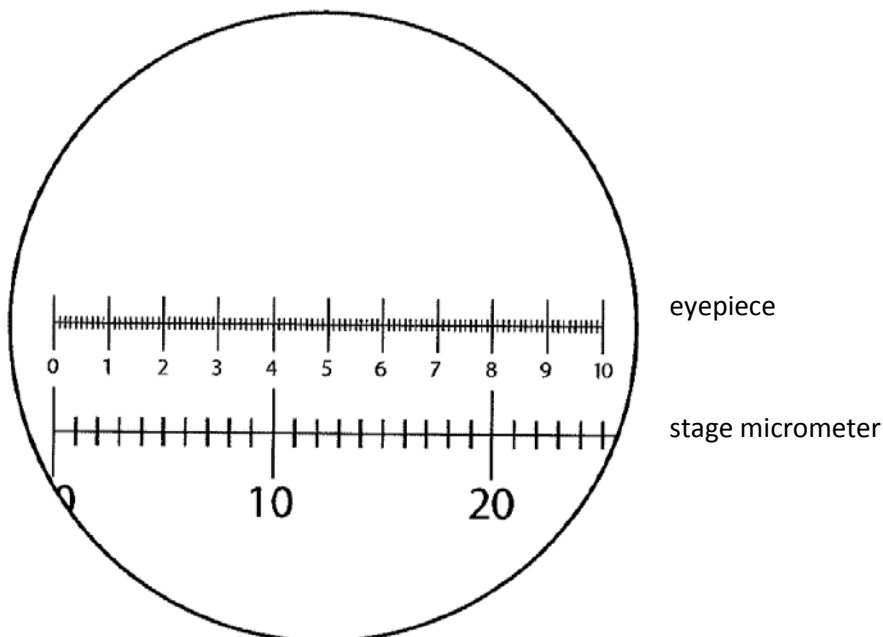
**Or**

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  $\mu\text{m}$ .



**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**

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

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

$$\begin{aligned} 1 \text{ eye piece unit} &= 0.25 \times 0.1 \text{ mm} \\ &= 0.025 \text{ mm or } 0.025 \times 1000 \mu\text{m} \\ &= 25 \mu\text{m} \end{aligned}$$

### Practical techniques

- use of light microscope at high power and low power, including use of a graticule