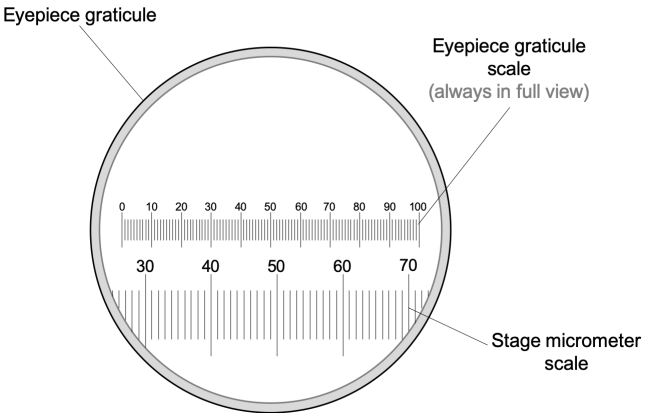
WJEC Wales Biology A Level

S.P 1.2: Calibration of a light microscope

Practical notes

Introduction

Light microscopes are used to increase the **magnification** and **resolution** of an image. They must be **calibrated** to enable the accurate measurement of the size of a specimen. An **eyepiece graticule** and **stage micrometer** are used to do this.



Equipment

- Light microscope
- Eyepiece graticule *(square grid fitted into eyepiece, size of graduations calibrated)*
- Stage micrometer *(slide with divided scale, used to calibrate eyepiece graticule)*
- Microscope slide

Risk assessment

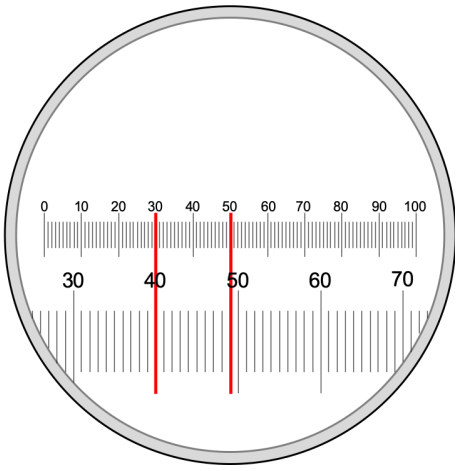
Hazard	Risk	Precaution	Emergency
Broken glass	Cuts	Keep glassware away from the edge of the desk; handle microscope slides carefully	Dispose of broken glassware carefully; elevate cuts; do not remove glass from cuts; seek medical assistance

Calibrating a microscope

1. Place the stage micrometer under the clips on the microscope stage

2. Turn the **lowest power objective lens** on the nose piece

3. Align the scales on the eyepiece graticule and stage micrometer so that they are parallel and there are **two points of intersection** (see diagram)



4. Stage micrometer is 1 cm long and divided into 100 divisions
   ∴ each division: 1 ÷ 100 = 0.01 cm = **100 µm**

5. 20 eyepiece graticule divisions = 9 stage micrometer divisions
   ∴ 20 eyepiece graticule divisions: 9 × 100 = 900 µm
   ∴ 1 eyepiece graticule division: 900 ÷ 20 = **18 µm**

6. Process repeated with the other objective lenses to find a calibration factor for each lens

Method

1. Using the method above, calibrate the microscope for all three objective lens magnifications
2. Place the microscope slide containing a specimen under the clips on the microscope stage
3. Turn the **lowest power objective lens** on the nose piece
4. Turn the **coarse adjustment knob** to move the stage close to the lens
5. Look down the microscope and turn the **coarse adjustment knob** to **focus** the image
6. Turn the **fine adjustment knob** until the best image is obtained
7. Rotate to the **medium power objective lens** and focus using the **fine adjustment knob**
8. Rotate to the **high power objective lens** and focus using the **fine adjustment knob**
9. Using the high power objective lens, make an annotated scientific drawing of the specimen and calculate the **magnification** of the drawing (see below)

Scientific annotated drawings

Low power plan drawings (×4 or ×10 objective lens)

Show the distribution of tissues but **not** individual cells

High power plan drawings (×40 objective lens)

Show **individual cells** (only draw a few cells)

Tips for biological drawings

- Drawing should fill at least half of the provided space
- Only draw what you can see
- Use a **sharp pencil**
- Ensure lines are **single**, **complete** and **non-overlapping**
- Do **not** use shading or colour
- Create **straight lines** for labels using a ruler
- Label lines should **not** have arrow heads
- Label lines should **not** intersect
- Include a scale in terms of **eyepiece units**
- Include a title and objective lens power
- Include a **magnification**

Magnification of drawings

$$\text{magnification} = \frac{\text{size of image}}{\text{size of object}}$$