Microscopy Skills

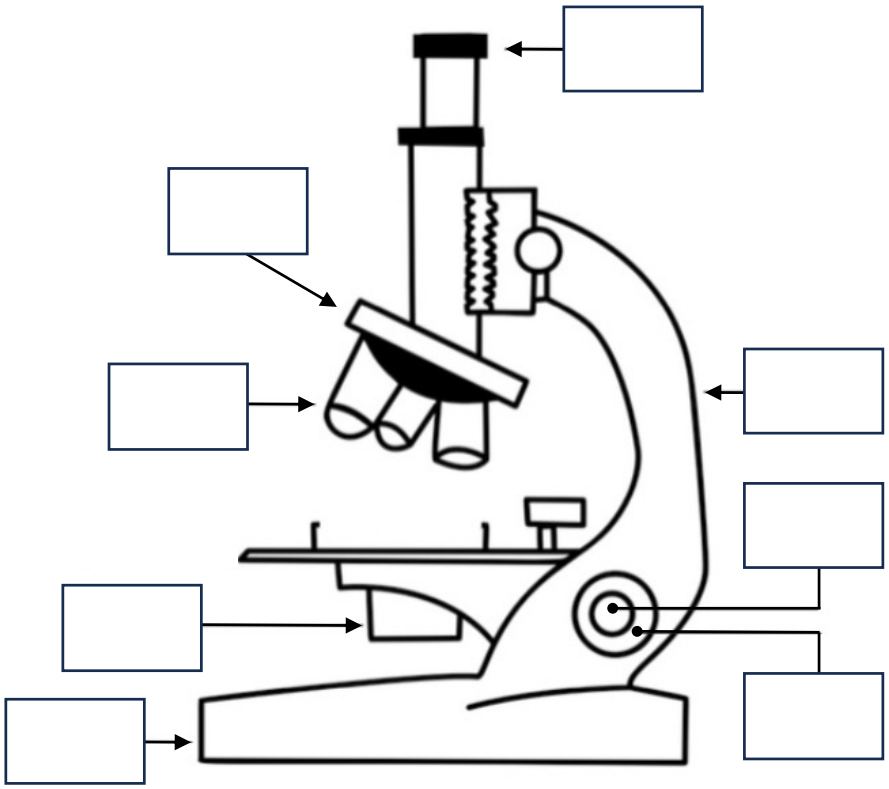
Introduction

The various organelles that are found in typical cells may not always be visible in a given cell type and may require staining to resolve the structure. The choice of stain is important, as specific chemicals will target particular structures. As cells and their sub-cellular components are very small and not normally visible to the naked eye, microscopes can be used to magnify images and enable us to see these tiny structures.

Light Microscopes

Light microscopes use glass lenses to bend light and magnify images. They can be used to view living specimens in their natural colours (although stains can be employed to highlight structures).

1. Label the parts of a light microscope



Key:

1. Base
2. Eyepiece
3. Fine focus
4. Nosepiece
5. Arm
6. Light source
7. Objective lens
8. Coarse focus

Important Safety Points:

- When lifting the microscope, put one hand on the arm and put the other hand under the base
- Always start by focusing with the lowest objective lens, then increase the lenses once focused.
- Make sure to remove any slides from the stage after use (do not leave a glass slide in place).
- The light source has an adjustable aperture – change the light intensity if object can't be seen.
- Always use microscopes on flat surfaces and do not leave it too close to the edge of a bench.

Aim

To observe the different characteristics of various specimens viewed under a light microscope.

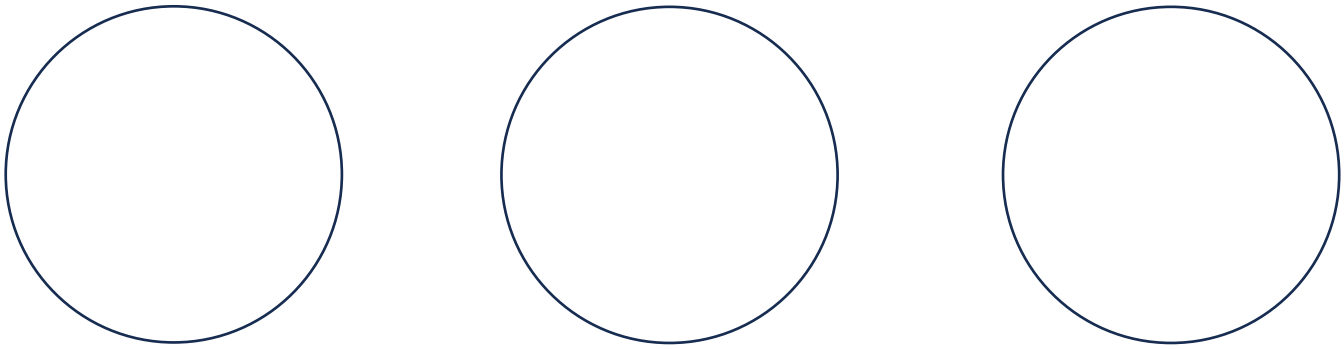
Materials

- 1 × light microscope
- 3 × glass slides
- 2 × plastic coverslips
- 1 × epithelial slide
- 1 × scalpel
- 1 × cutting board
- 1 × transfer pipette
- 1 × newspaper clipping
- 1 × sample of *Elodea*
- 1 × potato
- 1 × iodine solution
- 1 × paper towel

EXPERIMENT 1: Using the Microscope

- Place a newspaper clipping on a slide and secure it onto the microscope stage using the clips
- Carefully move the slide until you can see a single letter, then focus at different magnifications
- Calculate magnification by multiplying the magnification of the eyepiece lens and objective lens

2. Sketch the size of the selected newspaper letter under each of the three objective lenses used.



Magnification can be calculated by dividing the image size (drawing) by the actual size (letter).

- Using a ruler, measure the width of the letter (actual) and the width of your drawing (image)

3. Compare the magnification calculated based on your drawing with the actual magnification.

	Red lens	Yellow lens	Blue lens
Total Magnification			
Your Calculation			

Instead of using rulers, specimens can be measured using digital callipers for improved accuracy.

4. Define accuracy.

EXPERIMENT 2: Preparing Slides

- Using the scalpel, take a thin scraping of potato skin and place it on top of the glass slide
- Apply a drop of water (using pipette) and place a coverslip on the sample (water forms a seal)
- Place a drop of iodine on one side of coverslip and use paper towel to draw through sample
- Observe the sample under the microscope – try to identify the cell walls and starch granules

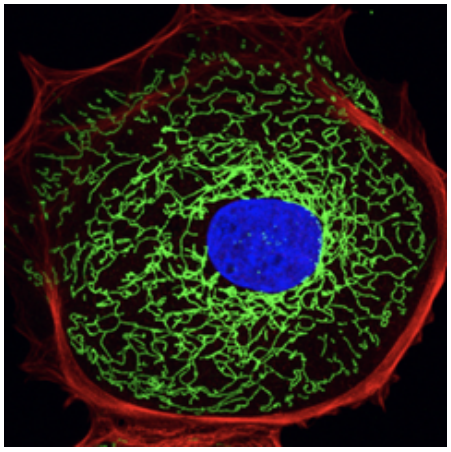
- Place a sample of *Elodea* onto a second glass slide and place a coverslip over the sample
- Observe the sample under the microscope – try to identify the chloroplasts and sap vacuoles

5. Draw a labelled diagram of the two samples, as observed under the highest magnification.

6. Observe the pre-prepared epithelial slide under the microscope and outline the differences that can be identified between the epithelial cells (animal) and the *Elodea* or potato sample (plant).

Cell structures can be identified using a technique called immunofluorescence (see image below).

7. Describe how immunofluorescence was used to stain the endoplasmic reticulum (green stain).



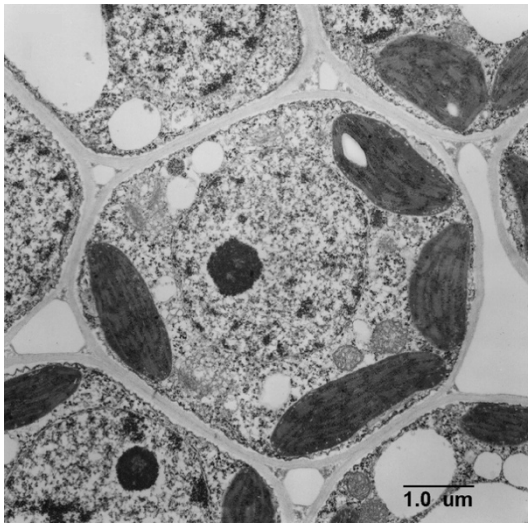
EXTENSION: Electron Microscopes

8. State one advantage and one disadvantage of using electron microscopes to view cells.

9. Distinguish between *scanning* electron microscopy and *transmission* electron microscopy.

Below is a micrograph of a eukaryotic cell taken using transmission electron microscopy (TEM).

10. a) Identify three cellular structures shown in the micrograph and describe their role in the cell.
b) Determine the cell type shown in the micrograph and provide a justification for your choice.



11. Outline how freeze fracture can be used to show internal cellular structures at high resolution.