

Using a light microscope

Use a light microscope to observe, draw and label a selection of plant and animal cells. A magnification scale must be included.

For AQA GCSE Biology 8461 and Combined Science: Trilogy 8464. Teacher answers start on a new page.

Name

Date

Aim

To prepare slides of plant and animal cells, observe them with a light microscope, and make a labelled drawing with a magnification.

Background

A **light microscope** shines light through a thin specimen. The light passes through an **objective lens** and then the **eyepiece lens**, which each magnify the image. Most cells are almost transparent, so a **stain** is added to make the sub-cellular structures easier to see. Iodine solution stains onion cells and methylene blue stains cheek cells.

Plant and animal cells share a nucleus, cytoplasm and cell membrane. Plant cells also have a cell wall and a permanent vacuole. With a light microscope you can see the nucleus, cytoplasm and cell wall clearly, but the cell membrane is very thin and hard to see. Structures such as ribosomes are too small to see because a light microscope has a lower resolving power than an electron microscope.

The **total magnification** is the magnification of the eyepiece lens multiplied by the magnification of the objective lens. The magnification of a drawing is found with the equation: $\text{magnification} = \text{size of image} \div \text{size of real object}$. The image and the real object must be in the same units. $1 \text{ mm} = 1000 \mu\text{m}$. Your drawing is not the same size as the image you see, so its magnification is not the microscope's total magnification. To find it, estimate the real size of a cell (for example from the width of the field of view) and divide the size on your drawing by it.

Equipment

- Light microscope with a built-in lamp, an eyepiece lens ($\times 10$) and objective lenses ($\times 4$, $\times 10$ and $\times 40$)
- 2 clean microscope slides and 2 cover slips
- Small piece of onion scale leaf (the fleshy leaf from inside the bulb)
- Forceps and a mounting needle
- Dropping pipette and water
- Dilute iodine solution
- Dilute methylene blue stain
- Clean cotton bud (one per student)
- Paper towel
- Sharp HB pencil, eraser, ruler (mm) and plain white paper
- Beaker of disinfectant for used slides and cotton buds
- Eye protection

Risk assessment

Hazard	Risk	Precaution
Glass slides and cover slips	They can break and cut the skin.	Handle with care. Tell the teacher about any breakages and put broken glass in the glass bin, never in the ordinary bin.
Iodine solution and methylene blue stain	They stain skin and clothes and can irritate the eyes.	Wear eye protection. Wipe up spills at once and wash any splashes off the skin with water.
Cheek cells (body fluids)	Saliva could pass on an infection if cotton buds or slides are shared.	Use only your own cotton bud. Put used cotton buds and slides straight into disinfectant. Wash your hands afterwards.
Microscope lamp and mains lead	Electric shock if the equipment gets wet.	Keep water away from the microscope and its lead. Carry the microscope with two hands.

Method

1. Place a clean slide on the bench. Use the dropping pipette to put one drop of water in the middle of it.
2. Use forceps to peel a thin, see-through layer of epidermis from the inside of a piece of onion scale leaf.
3. Lay the peel flat on the drop of water so that it does not fold over. Add one drop of iodine solution on top of it.
4. Hold a cover slip at an angle with one edge touching the liquid. Use a mounting needle to lower it slowly so that no air bubbles are trapped. Blot any liquid from the edges with paper towel.
5. Clip the slide on to the stage. Turn the nosepiece to the lowest-power objective lens ($\times 4$). Looking from the side, turn the coarse focusing knob until the lens is close to the slide but not touching it.
6. Look through the eyepiece. Turn the coarse focusing knob to move the lens away from the slide until the cells are roughly in focus. Then use the fine focusing knob to make the image sharp.
7. Switch to the $\times 10$ objective lens and refocus using the fine focusing knob only. Then switch to the $\times 40$ objective lens and refocus again. Record the eyepiece and objective magnifications and work out the total magnification.
8. Choose a few neighbouring cells. Use a sharp pencil to draw them with clear, unbroken lines. Do not shade or colour. Make the drawing large, taking up at least half a page.
9. Label the structures you can see (such as cell wall, cytoplasm, nucleus and vacuole) with ruled label lines that touch the structure and do not cross. Write a title beside the drawing.
10. Estimate the real length of one cell. Switch back to the $\times 4$ objective, place a clear plastic ruler on the stage and measure the diameter of the field of view in mm. Count how many cells fit end to end across it, then divide the diameter by the number of cells. Measure the same cell on your drawing in mm and calculate the magnification of the drawing: $\text{length on drawing} \div \text{real length}$. Write it beside the drawing.
11. Make a second slide of animal cells. Gently rub the inside of your cheek with a clean cotton bud, then smear it on to a drop of water on a new slide. Put the cotton bud straight into disinfectant.
12. Add one drop of methylene blue stain to the smear and lower a cover slip over it at an angle.

- L3 . Focus on the cheek cells in the same order as before (lowest power first, then fine focusing only). Draw and label two or three cells. Estimate their real size in the same way and write the magnification of the drawing beside it.
- L4 . Put the used slides and cover slips into disinfectant. Turn the objective back to the lowest power, lower the stage, switch off the lamp and put the cover on the microscope.

Results

Estimating the real size of a cell and the magnification of the drawing (total magnification of the microscope: _____)		
Measurement	Onion cells	Cheek cells
Diameter of the field of view with the $\times 4$ objective (mm)		
Number of cells across the diameter		
Estimated real length of one cell (mm)		
Estimated real length of one cell (μm)		
Length of the same cell on the drawing (mm)		
Magnification of the drawing		

Exam questions 10 questions · 27 marks

- 1** A student uses an eyepiece lens of $\times 10$ and an objective lens of $\times 40$. Calculate the total magnification. [2 marks]

Answer =

- 2** A student draws a cell. The drawing is 36 mm long. The real cell is 90 μm long. Calculate the magnification of the drawing. Show your working. [3 marks]

Answer =

- 3** Explain why you should always start by focusing with the lowest-power objective lens. [2 marks]

4 The onion epidermis used on the slide must be very thin. Explain why. **[2 marks]**

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5 A stain such as iodine solution is added to the onion cells. Explain why. **[2 marks]**

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6 The cover slip is lowered on to the slide at an angle using a mounting needle. Explain why. **[2 marks]**

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7 Give three rules a student should follow when making a biological drawing from a microscope. **[3 marks]**

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8 A student looks at cells from an onion and from the inside of a cheek. Name two structures seen in the onion cells that are not seen in the cheek cells. **[2 marks]**

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9 A cell in a drawing is 27 mm long. The magnification of the drawing is $\times 100$. Calculate the real length of the cell in micrometres (μm). Show your working. **[3 marks]**

Answer =

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10 Describe how you would prepare a slide of onion epidermis cells, observe the cells with a light microscope and make a labelled drawing that includes a magnification. **[6 marks]**

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Teacher answers

Required practical 1: Using a light microscope. The example results are practice data, not real measurements.

Example results

Example results (practice data): onion epidermis cells, viewed at $\times 40$, $\times 100$ and $\times 400$ (eyepiece $\times 10$)	
Measurement	Onion cells
Diameter of the field of view with the $\times 4$ objective (mm)	4.5
Number of cells across the diameter	18
Estimated real length of one cell (mm)	$4.5 \div 18 = 0.25$
Estimated real length of one cell (μm)	250
Length of the same cell on the drawing (mm)	50
Magnification of the drawing	$50 \div 0.25 = \times 200$

Conclusion

The onion epidermis cells are rectangular and about 0.25 mm (250 μm) long. The drawing has a magnification of $\times 200$. Each shows a cell wall, a nucleus stained brown by the iodine, cytoplasm and a large permanent vacuole. The cheek cells are rounder and have no cell wall and no large vacuole, but they have a nucleus, cytoplasm and cell membrane. This shows that plant cells and animal cells have some sub-cellular structures in common and some that are different. The cell sizes vary, so a mean of several cells gives a better estimate of the real size.

Errors and improvements

Error	Effect on the results	Improvement
The field of view is measured with a ruler to the nearest 0.5 mm and the cells across it are counted roughly (random error).	The estimated real size of a cell is uncertain, so the magnification of the drawing is only approximate.	Count the cells across the field of view in two directions at right angles and take a mean, or use an eyepiece graticule calibrated with a stage micrometer.
The cells are not drawn accurately, for example they are changed in size or shape (systematic error).	The measured length does not match the real cell, so the real size and the magnification are wrong.	Look back at the image while measuring and drawing, and draw only a few cells carefully.
The onion peel is too thick or folded, or air bubbles are trapped under the cover slip.	Cells overlap or are hidden, so the structures cannot be seen or drawn clearly.	Use a thinner peel, lay it flat and lower the cover slip slowly at an angle.
The focus is changed with the coarse focusing knob at high power.	The objective lens may hit and crack the slide, and the image is lost.	Only use the fine focusing knob after changing to a higher-power lens.

Mark scheme

One mark for each point unless a maximum is given. Words in brackets are not needed for the mark.

1. [2]

- 10×40 (1)
- $\times 400$ (1) allow 400

2. [3]

- converts units, for example $36\text{ mm} = 36\,000\text{ }\mu\text{m}$ or $90\text{ }\mu\text{m} = 0.09\text{ mm}$ (1)
- magnification = size of image $\div$ size of real object, so $36\,000 \div 90$ or $36 \div 0.09$ (1)
- $\times 400$ (1) allow 400 for a correct answer with no working

3. [2]

- it gives the widest field of view / makes it easier to find the specimen (1)
- the high-power lens is long and could hit and crack the slide / damage the lens (1)

4. [2]

- light has to pass through the specimen (1)
- so that cells are not on top of each other and can be seen clearly (1) allow one cell thick

5. [2]

- cells / structures (such as the nucleus) are colourless / transparent (1)
- the stain makes structures easier to see / increases contrast (1)

6. [2]

- to avoid trapping air bubbles (1)
- bubbles would hide the cells / be mistaken for cells (1)

7. [3]

- use a sharp pencil / clear single unbroken lines (1)
- no shading or colouring (1)
- make the drawing large / fill the space (1)
- label lines drawn with a ruler that touch the structure and do not cross (1)
- add a title and the magnification (1)
- Max 3

8. [2]

- cell wall (1)
- (large) permanent vacuole (1)
- reject chloroplasts (onion epidermis cells do not contain chloroplasts)

9. [3]

- real size = image size $\div$ magnification (1)
- $27 \div 100 = 0.27\text{ mm}$ (1)
- $270\text{ }\mu\text{m}$ (1) allow 270 for a correct answer with no working

10. [6]

Level 3 5-6	A clear, logical method covering the slide preparation, the focusing procedure and the drawing, with the magnification included. Most steps are in the correct order and show good practical detail.
Level 2 3-4	A method covering at least two of slide preparation, focusing and drawing, with some correct detail. There may be gaps in the order or detail.
Level 1 1-2	A few relevant points about the preparation, focusing or drawing, with little detail or order.

Indicative content

- put a drop of water on a clean slide
- peel a thin layer of epidermis with forceps and lay it flat on the water
- add a drop of iodine solution as a stain
- lower a cover slip at an angle with a mounting needle to avoid air bubbles
- clip the slide on the stage, start with the lowest-power objective lens and focus with the coarse then fine focusing knob
- change to a higher-power lens and refocus using the fine focusing knob only
- draw with a sharp pencil using clear, unbroken lines, no shading, and labelled with straight lines that do not cross
- write a title and the magnification of the drawing ($\text{size on drawing} \div \text{real size}$) or add a scale bar

Exam tips

- Always write the magnification or add a scale bar on a drawing. A drawing without one loses a mark.
- Convert units before you calculate: $1 \text{ mm} = 1000 \text{ } \mu\text{m}$. Most wrong magnification answers come from mixing mm and μm .
- Say that you focus with the fine focusing knob after changing to a higher-power lens. Using the coarse knob at high power is a common mistake.
- For a drawing, examiners want a sharp pencil, no shading, a large drawing and label lines that do not cross.
- Remember what the stain does: it makes colourless structures show up. Do not say that it kills the cells or makes the image bigger.