

Using a light microscope

Investigate biological specimens using microscopes, including magnification calculations and labelled scientific drawings from observations

For Edexcel GCSE Biology 1BI0 and Combined Science 1SC0. Teacher answers start on a new page.

Name

Date

Aim

To prepare a slide of onion epidermis, observe the cells with a light microscope, make a labelled scientific drawing and calculate magnification and the real size of the cells.

Background

Most cells are too small to see with the naked eye. A light microscope uses lenses to magnify a specimen so that cells and some of their parts (such as the nucleus, cytoplasm, cell wall and vacuole) can be seen. The specimen must be thin enough for light to pass through it.

Onion epidermis is a good specimen because it peels off as a layer one cell thick. Stains such as iodine solution make the parts of the cells easier to see, because the nucleus and cell wall take up the stain more strongly than the rest of the cell.

The total magnification of a microscope is the eyepiece lens magnification $\times$ the objective lens magnification. The magnification of a drawing is different: it depends on how large you draw the cells. The equation $\text{magnification} = \text{size of image} \div \text{size of real object}$ lets you work out any one of the three values if you know the other two. In this practical you find the magnification of your drawing by comparing the size of the circle you draw with the real size of the field of view.

Cells are measured in micrometres (μm). $1 \text{ mm} = 1000 \mu\text{m}$, so you must convert units before you calculate.

Equipment

- Light microscope with a $\times 10$ eyepiece lens and $\times 4$, $\times 10$ and $\times 40$ objective lenses
- Onion (a small piece of the inner layer of a bulb scale)
- Microscope slides and coverslips
- Forceps and mounted needle
- Scalpel and cutting tile
- Dropping pipette
- Iodine solution (a few drops in a small labelled container)
- Water
- Paper towel
- Transparent 30 cm ruler marked in millimetres
- Sharp HB pencil, eraser and plain white paper

Risk assessment

Hazard	Risk	Precaution
Iodine solution	Irritates eyes and skin and stains skin and clothes.	Wear eye protection. Use only a few drops. Wipe up spills at once and wash hands if iodine gets on skin.
Scalpel	Cuts to fingers.	Cut away from the body on a cutting tile. Keep fingers behind the blade. Carry the scalpel with the blade covered or pointing down.
Glass slides and coverslips	Cuts from broken glass.	Handle by the edges. If glass breaks, tell the teacher and do not pick it up with bare hands.
Microscope lamp and electrical lead	Trailing leads can cause trips. A hot lamp can burn.	Keep leads tidy and away from the table edge. Do not touch the lamp housing. Carry the microscope with two hands.

Method

1. Put a drop of water on a clean slide. Wear eye protection.
2. Cut a piece of onion about 1 cm square. Use forceps to peel off a thin, transparent layer from the inside curve of the piece (the epidermis).
3. Lay the epidermis flat on the drop of water on the slide. Use the mounted needle to spread it out so that it does not fold.
4. Add one drop of iodine solution to the specimen.
5. Hold a coverslip at an angle against the edge of the drop. Lower it slowly with the mounted needle so that air bubbles are not trapped. Blot any extra liquid from the edge with paper towel.
6. Clip the slide on the stage. Turn the objective lens to the lowest power ($\times 4$) so that the total magnification is $\times 40$.
7. Look from the side and use the coarse focus to move the lens close to the slide. Then look through the eyepiece and turn the coarse focus slowly to bring the cells into focus. Use the fine focus to make the image sharp.
8. Take the slide off the stage and put the transparent ruler there instead. At the same $\times 40$ total magnification, count how many millimetres fit across the field of view (about 4.5 mm). Convert this to μm . Then put the slide back and refocus.
9. Move to the $\times 10$ objective lens (total magnification $\times 100$) and focus again, using the fine focus only.
10. Move to the $\times 40$ objective lens (total magnification $\times 400$). Use the fine focus only. The magnification is now 10 times greater than at $\times 40$, so the diameter of the field of view is 10 times smaller (about 0.45 mm). Choose a small group of clear cells to draw.
11. Draw a circle of diameter 90 mm on plain paper to represent the field of view. Draw the cells in the circle to the same scale: if a cell stretches a quarter of the way across the field of view, draw it a quarter of the way across the circle. Use a sharp pencil and clear, continuous lines. Do not shade or colour.
12. Label the cell wall, cytoplasm, nucleus and vacuole with straight lines drawn with a ruler. The lines must touch the part they name and must not cross. Give the drawing a title.

- L3 . Calculate the magnification of your drawing: diameter of circle on drawing ÷ real diameter of field of view at ×400 (both in mm). Measure the length of three cells on your drawing in mm.
- L4 . Use the magnification to calculate the real length of each cell in μm. Calculate the mean.
- L5 . Switch off the lamp, return the objective lens to the lowest power and clean up as instructed.

Results

Diameter of field of view at ×400 = _____ mm. Diameter of circle on drawing = 90 mm. Magnification of drawing = $90 \div \text{_____} = \times \text{_____}$

Cell	Length of cell in drawing (mm)	Real length of cell (μm)
Cell 1		
Cell 2		
Cell 3		
Mean		

Exam questions 9 questions · 26 marks

- 1** A microscope has an eyepiece lens of ×10 and an objective lens of ×40. Calculate the total magnification. [2 marks]

Answer =

- 2** A student draws a plant cell. The cell is 60 mm long in the drawing. The magnification of the drawing is ×250. Calculate the real length of the cell. Give your answer in micrometres (μm). [3 marks]

Answer =

- 3** A different student draws a cell that has a real length of $160\text{ }\mu\text{m}$. The cell is 64 mm long in the student's drawing. Calculate the magnification of the drawing. **[3 marks]**

Answer =

- 4** A student drew three cells at a magnification of $\times 300$. The lengths of the cells in the drawing are in the table. Calculate the mean real length of the cells in μm . **[3 marks]**

Lengths of cells in the drawing

Cell	Length in drawing (mm)
1	36
2	42
3	39

Answer =

- 5** The student used the lowest power objective lens first, before changing to a higher power objective lens. Give two reasons for this. **[2 marks]**

- 6** When the student changed to the $\times 40$ objective lens, the image went out of focus. State which focusing knob should be used and explain why the other knob should not be used. **[2 marks]**

Teacher answers

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

Example results

Example results (practice data). Diameter of field of view at $\times 400 = 0.45$ mm. Diameter of circle on drawing = 90 mm. Magnification of drawing = $90 \div 0.45 = \times 200$

Cell	Length of cell in drawing (mm)	Real length of cell (μm)
Cell 1	24	120
Cell 2	30	150
Cell 3	27	135
Mean	27	135

Conclusion

The onion epidermis cells are about 135 μm long on average (0.135 mm), so a microscope is needed to see them and their structure. Example working for cell 1: real length = image size $\div$ magnification = $24 \text{ mm} \div 200 = 0.12 \text{ mm} = 120 \mu\text{m}$. The cells fit together with no gaps and each has a cell wall, cytoplasm, a nucleus and a large vacuole. No chloroplasts are visible, because the inner layers of an onion bulb grow underground, do not receive light and do not photosynthesise.

Errors and improvements

Error	Effect on the results	Improvement
Measuring the cell length on the drawing with a ruler to the nearest millimetre (random error).	A 1 mm error on a 24 mm length changes the calculated real length by about 4%, so the real size is only approximate.	Draw larger, measure to the nearest 0.5 mm, measure several cells and calculate a mean.
Measuring the diameter of the field of view with a millimetre ruler (random error).	The field of view is only about 4.5 mm across at $\times 40$, so an error of 0.5 mm is about 11%. This error is carried into the $\times 400$ value, the magnification and every real size calculated from it.	Measure the field of view several times and use the mean, or use a stage micrometer (a slide with a scale marked in 0.01 mm divisions).
Air bubbles trapped under the coverslip, or the specimen folded over.	Cells are hidden or overlap, so the length of the cell may be measured wrongly.	Lower the coverslip slowly at an angle and spread the specimen out flat with a mounted needle.
Choosing cells that are not typical, for example the three largest.	The mean length does not represent the cells in the specimen.	Measure cells from several areas of the specimen, and measure more cells.
Drawing from memory or drawing cells that look tidier than they are.	The drawing and its measurements do not match the specimen.	Look at the specimen while drawing, draw only what is seen and draw the cell outlines at their true proportions.

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) allow eyepiece lens magnification $\times$ objective lens magnification
- $\times 400$ (1) allow 400; do not accept 400 with a unit such as mm
- Correct answer with no working gains 2 marks

2. [3]

- real size = image size $\div$ magnification, or $60 \div 250$ (1)
- 0.24 (mm) (1)
- 240 μm (1)
- Correct answer with no working gains 3 marks
- Allow 2 marks for 0.24 mm given as the final answer

3. [3]

- converts to the same units: 64 mm = 64 000 μm , or 160 μm = 0.16 mm (1)
- magnification = image size $\div$ real size, or 64 000 $\div$ 160, or 64 $\div$ 0.16 (1)
- $\times 400$ (1) allow 400
- Correct answer with no working gains 3 marks
- Allow 1 mark for 0.4 or 2.5 (units not converted)

4. [3]

- mean length in drawing = $(36 + 42 + 39) \div 3 = 39$ mm (1)
- $39 \text{ mm} \div 300 = 0.13$ mm (1)
- 130 μm (1)
- Allow converting each cell separately (120, 140, 130 μm) and then finding the mean = 130 μm
- Correct answer with no working gains 3 marks

5. [2]

- the field of view is larger, so the specimen is easier to find (1)
- the lens is further from the slide, so there is less risk of the lens hitting and breaking the slide (1)
- allow the image is easier to focus on low power (1)
- Max 2

6. [2]

- the fine focus (1)
- the coarse focus moves the lens a long way / could make the lens hit and crack the slide (1)
- ignore 'the coarse focus is less accurate' alone

7. [3]

- drawn in sharp pencil with clear, continuous lines (1)
- no shading or colouring (1)
- large drawing that fills most of the space (1)
- only draws what can be seen / cell parts in correct proportion (1)
- labels drawn with a ruler as straight lines that touch the structure and do not cross (1)
- has a title / magnification written on it (1)
- Max 3

8. [2]

- the onion bulb grows underground / the cells do not receive light (1)
- so they do not photosynthesise / do not need chloroplasts (1)
- do not accept 'the iodine stain hides the chloroplasts'

9. [6]

Level 3 5-6	A clear, logical and complete description of how to prepare the slide and focus the microscope, with at least one precaution given (for example avoiding air bubbles or using the fine focus only on high power). The steps are in a sensible order.
Level 2 3-4	A description that includes most of the main stages of preparing a slide and focusing, but with some missing detail or steps out of order.
Level 1 1-2	A basic description with a few separate points and little order.

Indicative content

- place a drop of water on a clean slide; peel a thin layer of epidermis with forceps and lay it flat on the drop; add a drop of stain such as iodine solution; lower a coverslip at an angle using a mounted needle to avoid air bubbles; place the slide on the stage and secure with clips; select the lowest power objective lens; use the coarse focus to bring the cells into focus and then the fine focus to make it sharp; change to a higher power objective lens and refocus using the fine focus only.

Exam tips

- Always show your working in magnification questions. Write the equation, substitute the numbers and give the units. Examiners give marks for the method even if the final answer is wrong.
- Convert to the same units before you divide. Most mistakes happen when mm and μm are mixed. Multiply mm by 1000 to get μm .
- In a drawing question, marks are given for sharp continuous lines, no shading, drawing large, correct proportions and labels drawn with a ruler that do not cross. Learn these as a list.
- Never say you use the coarse focus on high power. Use the fine focus to sharpen the image, and the lowest power objective lens first.
- Magnification is a ratio, so it has no unit. Write $\times 200$, not 200 mm.