Using a light microscope

Answers, marking points and revision notes | AQA Combined Science 8464 and AQA GCSE Biology 8461

GCSE science required practicals

This practical online

Answers

1 Give **two** reasons why only a very thin layer of onion skin is used on the slide. (2 marks)

Light has to pass through the sample to reach the lens, so a thin layer lets light through. A thin layer is only one cell thick, so the cells do not lie on top of each other and each cell can be seen clearly.

Marking points

- light needs to pass through the specimen
- cells are not overlapping / only one layer of cells so individual cells can be seen

2 Kofi lowered the coverslip onto the slide slowly, holding it at an angle.

a. What problem does this prevent?

b. Why is iodine solution added to the onion cells? (2 marks)

a. It stops air bubbles being trapped under the coverslip.
b. It stains the cells so parts such as the nucleus and cell wall are easier to see.

Marking points

- (a) stops air bubbles forming / being trapped
- (b) stains the cell so the nucleus / structures show up more clearly

3 Kofi wants to compare the sizes of his five cells fairly. Give **two** things he must keep the same when measuring each cell. (2 marks)

He must keep the same total magnification for every cell, and he must measure every cell in the same direction, along its longest side.

Marking points

- the same magnification / same objective lens
- measure each cell along the same axis (e.g. longest side) / use the same ruler

4 Describe how to bring the onion cells into focus, starting with the slide clipped onto the stage. (3 marks)

Select the lowest power objective lens first. Watching from the side, turn the coarse focusing knob so the stage moves up close to the lens without touching it. Then look through the eyepiece and turn the coarse knob to move the stage away until the cells appear, and finish with the fine focusing knob to make the image sharp.

Marking points

- start with the lowest power objective lens
- move the stage up close to the lens while watching from the side (so the lens does not hit the slide)
- look through the eyepiece and focus with the coarse knob, then the fine knob to sharpen

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5 Give **one** hazard in this practical and explain how the risk from it can be reduced. (2 marks)

Iodine solution can irritate the eyes. Wearing eye protection and adding it with a dropping bottle reduces the chance of it getting into the eyes.

Marking points

- a hazard named, e.g. iodine solution is an irritant / scalpel is sharp / coverslips can break
- a matching control, e.g. wear eye protection / cut away from the body / handle coverslips by the edges

6 The microscope had an eyepiece lens of x10 and Kofi used an objective lens of x40. Calculate the total magnification. (1 mark)

x400

total magnification = eyepiece magnification × objective magnification

total magnification = 10 × 40

total magnification = x400

Marking points

- 10 × 40 = x400

7 Use the table and the magnification of x400 to calculate the real length of cell C in micrometres (μm).

1 mm = 1000 μm. Show your working. (3 marks)

Real length = 126 ÷ 400 = 0.315 mm, which is 0.315 × 1000 = 315 μm.

real size = image size ÷ magnification

real size = 126 mm ÷ 400

real size = 0.315 mm

0.315 mm × 1000 = 315

real length of cell C = 315 μm

Marking points

- uses real size = image size ÷ magnification
- 126 ÷ 400 = 0.315 (mm)
- converts to 315 μm

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8 Calculate the mean real length of the five cells in micrometres (μm). Show your working. (3 marks)

Mean image length = (113 + 97 + 126 + 104 + 118) ÷ 5 = 558 ÷ 5 = 111.6 mm. Real length = 111.6 ÷ 400 = 0.279 mm = 279 μm.

total of image lengths = 113 + 97 + 126 + 104 + 118

total = 558 mm

mean image length = 558 ÷ 5

mean image length = 111.6 mm

mean real length = 111.6 mm ÷ 400

mean real length = 0.279 mm

0.279 mm × 1000 = 279

mean real length = 279 μm

Marking points

- adds the five lengths and divides by 5 to get 111.6 mm
- divides by the magnification: 111.6 ÷ 400 = 0.279 mm
- 279 μm

9 **Higher tier**

The real length of cell D is 260 μm. Give this length in metres, in standard form.

1 m = 1 000 000 μm. (2 marks)

260 ÷ 1 000 000 = 0.00026 m = 2.6×10^{-4} m

length in m = 260 μm ÷ 1 000 000

length in m = 0.00026 m

length = 2.6×10^{-4} m

Marking points

- 260 ÷ 1 000 000 = 0.00026 (m)
- 2.6×10^{-4} m

10 In a textbook photograph of a cheek cell, a scale bar 25 mm long is labelled 50 μm. Calculate the magnification of the photograph. (2 marks)

25 mm = 25 000 μm, and 25 000 ÷ 50 = x500.

image size = 25 mm = 25 × 1000 = 25 000 μm

magnification = image size ÷ real size

magnification = 25 000 μm ÷ 50 μm

magnification = x500

Marking points

- converts to the same unit: 25 mm = 25 000 μm
- magnification = 25 000 ÷ 50 = x500

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11 **Higher tier**

Leila uses a different method to estimate cell size. At x400 the circle of light she can see (the field of view) is 0.45 mm across. Exactly three onion cells, lying end to end, fit across the middle of the field of view.

Estimate the length of one cell in µm. Show your working. (3 marks)

0.45 mm = 450 µm. Three cells fill this width, so one cell is 450 ÷ 3 = 150 µm long.

field of view = 0.45 mm × 1000 = 450 µm

length of one cell = field of view ÷ number of cells

length of one cell = 450 µm ÷ 3

length of one cell = 150 µm

Marking points

- converts 0.45 mm to 450 µm
- divides the field of view by the number of cells
- 150 µm

12 Kofi measured the cells on the screen with a ruler marked in mm.

a. Explain why he measured five cells rather than one.

b. Suggest a better way of measuring the cells and say why it is better. (3 marks)

a. Cells of the same type vary in size, so a mean of several cells gives a more representative value than one cell, which might be unusually large or small.

b. Use an eyepiece graticule that has been calibrated against a stage micrometer. The cells are then measured against a scale of known small divisions inside the microscope, which gives a more accurate length than holding a ruler against a screen.

Marking points

- (a) cells differ in size so a mean of several is more representative / one cell may be untypical
- (b) use an eyepiece graticule calibrated with a stage micrometer
- (b) the scale is seen with the cell, with known small divisions, so the measurement is more accurate

13 **Higher tier**

Kofi could see the nucleus of each cell but could not see any ribosomes, even when he increased the magnification. Explain why. (2 marks)

A light microscope has a limited resolution, so two points closer together than about 0.2 µm cannot be seen as separate. Ribosomes are far smaller than this, so magnifying the image more just makes it bigger and blurrier without showing extra detail. An electron microscope, with much higher resolution, is needed.

Marking points

- the resolution of a light microscope is too low / it cannot separate very small or close structures
- ribosomes are smaller than the resolution limit, so more magnification only gives a bigger blurred image (an electron microscope is needed)

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14 Plan an investigation to find out whether a leaf has more stomata on its upper surface or on its lower surface.

You should use a light microscope. Clear nail varnish can be painted onto a leaf, left to dry and peeled off with clear sticky tape to give a thin copy of the leaf surface.

(6 marks)

Take one leaf from a plant, for example a privet or a geranium. Paint a small patch of clear nail varnish on the upper surface and a patch the same size on the lower surface, and leave them to dry. Press clear sticky tape onto each patch, peel off the varnish layer and stick each one onto its own labelled slide. Put the upper surface slide on the stage, start on the lowest power objective and focus with the coarse then fine knob, then switch to the x40 objective and refocus with the fine knob. Count the number of stomata in the whole field of view. Move to four more areas of the same peel and count again, then work out a mean number per field of view. Repeat for the lower surface slide using exactly the same magnification, so the field of view is the same area. Keep the leaf type and age the same and take both peels from the same leaf, and repeat the whole thing with several leaves from the same plant. Compare the mean counts: the surface with the higher mean has more stomata. If the field of view diameter is known, the number per mm^2 can be calculated using πr^2 for the area.

Marking points (any 6, with the method in a logical order)

- nail varnish painted on upper and lower surfaces of the same leaf and allowed to dry
- peel removed with clear tape and stuck onto a labelled slide
- focus on low power first, then move to a high power objective and use the fine knob
- count the stomata in the field of view
- count in several different fields of view and calculate a mean
- use the same magnification for both surfaces so the area counted is the same
- control variables: same leaf / same type and age of leaf / same plant
- repeat with several leaves to check repeatability
- compare the means, or work out stomata per mm^2 from the field of view area

How it is marked

- 5-6 marks: a full, logical plan that someone could follow: making the peels from both surfaces, focusing correctly, counting stomata in several fields of view at the same magnification, taking means, naming controls and saying how the results are compared.
- 3-4 marks: a workable plan with the main steps (peels, microscope, counting on both surfaces) but missing some detail such as repeats, means, same magnification or controls.
- 1-2 marks: a few relevant points, such as using nail varnish or looking at the leaf under a microscope, without a clear order or a way of comparing the two surfaces.

Revision notes: the method

Aim: To prepare a slide of plant cells, observe them with a light microscope, and use magnification to work out the real size of the cells.

Equipment

- light microscope with x4, x10 and x40 objective lenses
- glass microscope slide and coverslip
- onion
- forceps
- scalpel or small knife
- iodine solution in a dropping bottle

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- mounted needle
- paper towel
- ruler marked in mm (or an eyepiece graticule)
- pencil and plain paper for a drawing

Method

1. Cut a small square of onion and peel off the very thin, see-through layer of skin from the inner surface using forceps.
2. Put a drop of water on the middle of a clean slide and lay the onion skin flat on it, smoothing out any folds.
3. Add one or two drops of iodine solution to stain the cells so the nucleus and cell wall show up.
4. Hold the coverslip at an angle at the edge of the liquid and lower it slowly with a mounted needle so that no air bubbles are trapped.
5. Soak up any extra liquid at the edges with a piece of paper towel.
6. Clip the slide onto the stage and select the lowest power (x4) objective lens.
7. Looking from the side, use the coarse focusing knob to raise the stage until it is close to the lens without touching it.
8. Looking through the eyepiece, use the coarse knob to move the stage away until the cells come into focus, then sharpen the image with the fine focusing knob.
9. Switch to a higher power objective and refocus with the fine knob only.
10. Measure the length of several cells (with an eyepiece graticule, or on a screen image), work out the real size of each and calculate a mean.
11. Make a clear pencil drawing of a few cells, label the cell wall, cytoplasm and nucleus, and write the magnification next to it.

Variables

- **Independent:** The cell being looked at (a different cell is measured each time)
- **Dependent:** The length of the image of each cell, measured in mm and converted to a real length in micrometres (μm)
- **Control:** the same total magnification for every cell measured; every cell measured along its longest side; cells all taken from the same layer of the same onion; the same ruler or graticule used for every measurement

Hazards

Hazard	What could happen	How to reduce the risk
Iodine solution	can irritate the skin and eyes and stains skin and clothes	wear eye protection, use a dropping bottle and wash off any splashes straight away
Sharp scalpel and thin glass coverslips	cuts to the fingers, especially if a coverslip snaps	cut away from the body on a tile, handle coverslips by the edges and tell the teacher about any broken glass
Microscope lamp or mirror	using a mirror in direct sunlight can damage the eye	use the built-in lamp, or point the mirror at a window but never at the Sun

Key points

- Magnification = size of image ÷ size of real object; learn it in all three arrangements
- Get both sizes in the same unit before dividing: 1 mm = 1000 μm

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- Total magnification = eyepiece lens magnification × objective lens magnification
- Always start on the lowest power objective, then move up and use only the fine knob at high power
- Lower the coverslip at an angle to stop air bubbles, which look like black-edged circles
- Pencil drawings: clear single lines, no shading, labels with ruled lines, magnification written on

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