Chapter 1: Microscopy

Chapter outline

This chapter relates to Chapter 1: Cell structure in the coursebook.

In this chapter, learners will complete practical investigations on:

- 1.1 Making a temporary slide and drawing cells
- 1.2 Measuring cells, using an eyepiece graticule and stage micrometer
- 1.3 Comparing animal cells and plant cells

Practical investigation 1.1: Making a temporary slide and drawing cells

Learning objective: 1.1(a)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (l), (m), (n), (o) Successfully collecting data and observations: (a), (g)
PDO	Recording data and observations: (f) Layout of data or observations: (h)

Duration

This practical is likely to take around 1 h. Learners who are not familiar with using a microscope may need a little longer.

Preparing for the investigation

- Most learners will have used a microscope before, but some may not. It is also likely that the microscopes available for them to use in the ASAL course are different from those used in previous courses. You may like to demonstrate the use of the microscopes before asking learners to explore their own.
- It is generally a good idea to allocate a particular microscope to each learner, or to a pair of learners, and for them to use the same microscope each time. This can help them to become very familiar with it, and also encourages them to be responsible for taking good care of it. However, if learners will eventually be taking their practical examination in an unfamiliar laboratory using unfamiliar microscopes, you may prefer to ensure that each learner has experience using a different kind of microscope from time to time.
- For assessment of the learners' drawings in Part 2, you could provide a list of criteria and ask learners to exchange drawings with each other and assess the drawings against the list. See Table 1.1 for a sample assessment sheet. This encourages them to think hard about these criteria, and involves them more closely in their own learning than if you simply mark their drawings and provide feedback. Later, you can add your own assessments to their work.
- Note that this diagram is a high-power detail, showing individual cells and the structures within them. It is suggested that you do not introduce this term until later, when learners learn how to draw low-power plans that show tissues only, with no individual cells.

Equipment

Each learner or group will need:

- a light microscope, preferably a high quality A level-type microscope equipped with a ×10 eyepiece and at least two objective lenses
- a source of illumination (this could be built into the microscope, or a lamp, or bright light from a window)
- two or three microscope slides
- two or three coverslips

- a dropper pipette
- a mounted needle or seeker
- forceps (tweezers)
- sharp scissors or a blade (safety razor or scalpel)
- filter paper or paper towel
- tile
- some pieces cut from an onion bulb
- a medium-hard (HB) pencil
- a good quality eraser

Access to:

- iodine in potassium iodide solution

Feature of drawing	How well was this achieved?	Comment
takes up at least half of the space available		
drawn using a sharp pencil		
all lines are clear and single, with no overlaps and no gaps		
cell walls are shown as double lines		
proportions of different structures are correct		
there is no shading		
label lines are drawn with a ruler and touch what they are labelling		
labels are written clearly and do not overlap the drawing		

Table 1.1

Additional notes and advice

- You could either provide whole onion bulbs for learners to use, or you could cut sections from the layers in the bulbs and place these in beakers of water, ready for them to use.
- Microscopes should be the best quality that your school can afford. It is frustrating for learners to have to use microscopes that do not focus clearly. Ensure that microscopes are regularly serviced and checked.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- Learners should be shown how to handle a sharp blade safely.
- Iodine in potassium iodide solution generally contains ethanol as a solvent and may therefore be flammable. Learners should wear eye protection and wash off any spills on their skin or clothes. (Unlike solid iodine, this solution does not produce iodine vapour.) Low hazard.

Carrying out the investigation

- It is unlikely that any major problems will occur. Learners may have difficulty in spreading out the epidermis in the drop of water without folding it, but with practice this is generally achieved by most.
- It is possible that the onion cells may contain starch grains; if so, these will stain dark blue when the iodine is added. However, starch grains are not always present, so learners should not be surprised if there are none.
- There is a strong tendency for many learners to be so focused on 'getting it right' that they may draw an idealised diagram of the epidermis, rather than drawing what they can see. It is important to emphasise that they are being asked to **observe carefully** and to **record their observations**. This exercise is all about developing these practical skills, not their recall of the structure of plant cells. If possible, move around and look at the learners' slides through their microscopes, and compare their drawings with what they can see.

Learners who finish within the allocated time, and have made diagrams that you consider to be good quality, could be provided with a prepared slide of TS root and asked to draw three or four cortex cells.

Sample results

Method

Part 2

3

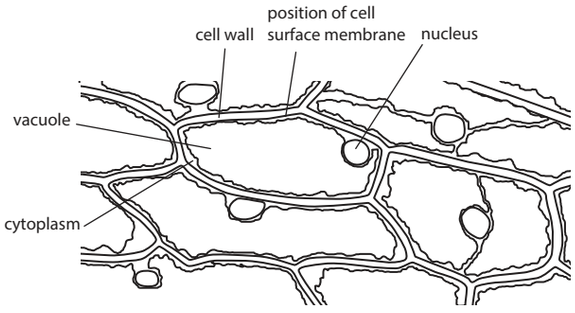


Figure 1.1

4 See Figure 1.1.

Part 3

3 It is possible that the onion cells may contain starch grains; if so, these will stain dark blue when the iodine is added. However, starch grains are not always present, so learners should not be surprised if there are none.

Practical investigation 1.2: Measuring cells, using an eyepiece graticule and stage micrometer

Learning objective: 1.1(b) and (c)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (l), (p) Successfully collecting data and observations: (a), (h)
PDO	Recording data and observations: (c)
	Displaying calculations and reasoning: (b)
ACE	Interpreting data or observations and identifying sources of error: (a), (j)

Duration

This practical is likely to take 1 h. You may like to extend this time to provide more practice in using a graticule and micrometer.

Preparing for the investigation

- Learners often become confused when using an eyepiece graticule and stage micrometer. It could be helpful to use Exercise 1.5, in the Cambridge AS & A level Biology workbook, *Using an eyepiece graticule and stage micrometer*, to practise this skill theoretically before doing this investigation. On the other hand, some learners may understand the procedures more easily when they are actually handling the graticule and micrometer themselves.
- Eyepiece graticules can be obtained relatively cheaply, but stage micrometers tend to be expensive. Cambridge Assessment International Education supply a low cost kit for you to make your own. You can download the publications list from the cambridgeinternational.org website. Click on the 'support for teachers' tab. This list also includes many different prepared slides.
- The instructions ask learners to make their first set of measurements using a prepared slide of a transverse section through a leaf. This ensures that they are looking at some clearly visible cells, and also may be more interesting for them than spending yet more time looking at onion epidermis cells. However, you (and they) may prefer to make the measurements using the onion epidermis cells first. Either sequence is fine.
- If learners always use the same microscope, then once they have calibrated a particular objective lens, they can use this calibration for all future measurements using that same objective lens and same eyepiece. Note, however, that a new calibration will be required for each of the objective lenses.

Equipment

Each learner or group will need:

- a microscope, with a graticule in the eyepiece
- prepared slide of section through a leaf
- onion epidermis slide from Practical investigation 1.1 (or a new one can be made)

Access to:

- a stage micrometer

Safety considerations

- Learners should have read the safety guidance section of the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- There are no additional significant safety issues associated with this investigation.

Carrying out the investigation

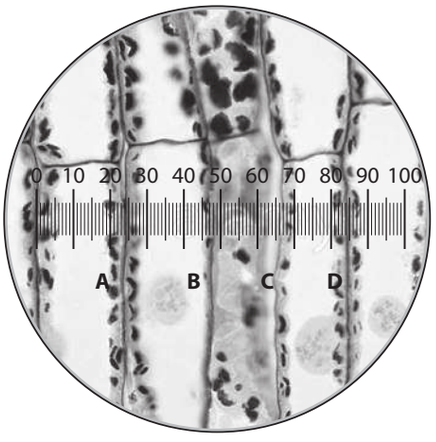
Some learners may become confused about which scale they are looking at. Swivelling the eyepiece causes the eyepiece graticule scale to move but not the stage micrometer scale.

Learners can often carry out each step in the measurement and calibration when following instructions, but find it difficult to remember what to do when they need to think about this for themselves. Step-by-step instructions are provided for measuring the palisade cells, but not the onion epidermis cells. You could provide a 'help sheet' to take learners through, step-by-step, when measuring the onion epidermis cells. For some learners, it could be valuable to repeat this several times, measuring different types of cell each time.

Some learners may have difficulty converting mm to µm, or may forget to do so when calculating magnification. See *Exercise 1.1, Units for measuring small objects*, and *Exercise 1.2, Magnification calculations*, in the Cambridge AS & A level Biology workbook, for guidance and practise in doing this.

Sample results

Part 1



Answers to the workbook questions (using the sample results)

Part 1

5 Four palisade cells measure 84 graticule units.

Part 2

3 Alignments at 0,0 and 80, 0.24.

4 24 small divisions on the stage micrometer scale = 24 × 10 µm = 240 µm

This equals 80 divisions on the eyepiece graticule scale.

So 1 division on the eyepiece graticule scale = 240 ÷ 80 = 3 µm.

5 Using the answer to Part 1 Question 5, 84 graticule units = 3 × 84 µm = 252 µm.

6 This was the width of 4 cells, so the mean width of one cell is 252 ÷ 4 = 63 µm.

7 Sample value for the width of 6 onion epidermis cells = 156 graticule units.

8 156 graticule units = 156 × 3 = 468 µm

So the mean width of one onion epidermis cell = 468 ÷ 6 = 78 µm.

Part 3

1 The answer will depend on the learner's drawing.

2 The answer will depend on the width of the drawing measured in 1. Their calculation here should be:

magnification = size of drawing ÷ size of actual cell(s)

The answer should be given as a number with a **x** sign in front of it, and no units, for example, **×40**.

Practical investigation 1.3:
Comparing animal cells and plant cells

Learning objective: 1.1(a)

Skills focus

The following skills are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (l), (m), (n), (o), (p) Successfully collecting data and observations: (a), (g), (h), (j)
PDO	Recording data and observations: (c), (f) Displaying calculations and reasoning: (b) Layout of data or observations: (h), (i)
ACE	Interpreting data or observations and identifying sources of error: (a), (j), (k)

Duration

This practical is likely to take about 1 h.

Preparing for the investigation

- Learners should be quite confident using a microscope by now, and should also be beginning to build confidence in using an eyepiece graticule and stage micrometer to make measurements. They can now use these skills to compare the appearance of three types of cell.
- If learners move straight into this activity from Practical investigation 1.1 or 1.2, they will already have temporary slides of onion epidermis. If not, they will need to make a new slide.

Equipment

Each learner or group will need:

- a microscope, with a graticule in the eyepiece
- prepared slide of section through a leaf
- onion epidermis slide from Practical investigation 1.1 (or a new one can be made)
- clean microscope slides and cover slips
- dropper pipette
- iodine in potassium iodide solution
- methylene blue stain
- cotton bud or similar

Access to:

- a stage micrometer

Additional notes and advice

- Check local regulations about the use of human cheek cells. If this is not possible, then you can try scraping cells from the inner surface of a trachea from an animal such as a sheep (obtainable from a butcher).
- Cotton buds are compacted cotton wool attached to the end of a short plastic stick (Figure 1.3). They can generally be obtained from pharmacies or supermarkets, where they are sold for use with cosmetics or for baby bathing.

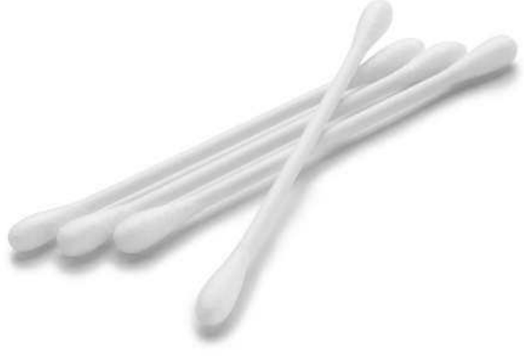


Figure 1.3

- Methylene blue stain can be bought from biological suppliers. It is very commonly used as a vital stain (i.e. one that is taken up by living cells) in temporary slides. If you have difficulty obtaining it from a biological supplier, you may be able to buy it from a shop selling fish for people to keep in ponds or tanks, where it is used as a treatment for fungal infections of fish. However, the concentration of the stain from this source may not be ideal for staining cells.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.

- Ensure that cotton buds are placed into a container of disinfectant (e.g. Lysol) immediately after use. This prevents the unlikely event of pathogenic bacteria being transferred from the mouth of one learner to another.
- Make sure that learners understand that they should simply run the cotton bud gently over the inner surface of their cheek. They should **not** dig the bud into the skin. There will be loose cells on the cheek surface which will readily adhere to the cotton wool.
- Methylene blue is classed as harmful (it is a reducing agent, and has various uses in medicine). Learners should avoid getting it on their skin or clothes, and should wear eye protection. If it does stain fingers, however, it is very unlikely to cause any harm.

Carrying out the investigation

- When making the cheek cell mount, learners may be puzzled that they cannot see anything on the microscope slide when they have rubbed the cotton bud onto it. They should continue with adding the stain; in most cases, cells will then become visible.
- Learners may have difficulty in locating the stained cells on the slide. They should be reminded to begin with the lowest power objective lens, and search the slide systematically until they see something. They can then move onto a higher power lens to look for individual cells.
- Some learners may construct their comparison table using what they **know** about cells, rather than using what they can **observe and measure**. This will become obvious if they mention structures such as cell membranes or endoplasmic reticulum, which they will not be able to see.

Some learners may need more support in constructing the comparison table. You could consider having a 'help sheet' available with an outline table for them to copy or complete, perhaps with one or two rows already filled in for them.

Learners who require a challenge could work in a group to research the functions of these three types of cell, and consider how each is adapted to its functions.

Sample results

Part 1

5

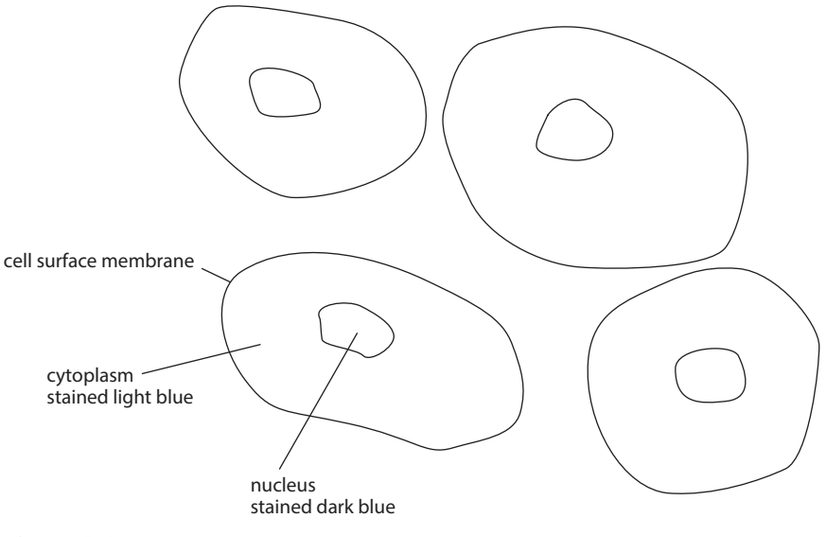


Figure 1.4

Answers to the workbook questions (using the sample results)

Part 1

6 Width of cheek cells in eyepiece graticule units: 14, 11, 13

Mean width of one cheek cell = 12.7 eyepiece graticule units

7 Using the same conversion factor as in Practical investigation 1.2, one division on the eyepiece graticule scale = 3 µm.

8 Therefore the mean width of one cheek cell = 38.1 µm.

9 Learners should measure each of the three cheek cells they have drawn along exactly the same positions as they made the measurements on the microscope slide. The measurement should be made in mm.

They should then calculate the mean width of the three cheek cells they have drawn, in mm.

The calculation is then:

$$\text{magnification of drawing} = \frac{\text{mean width of cheek cells in drawing in mm} \times 1000}{\text{mean width of cheek cells in answer to 9}}$$

The magnification should be given as a whole number, preceded by the **×** sign, and with no units.

Part 2

Answers will depend on the learner's observations and measurements. Some possible table entries are given in Table 1.2:

Feature	Onion cells	Palisade cells	Cheek cells
mean diameter / µm	78 µm	63 µm	38.1 µm
cell wall present	yes	yes	no
cytoplasm present	yes	yes	yes
chloroplasts present	no	yes	no
nucleus present	yes	yes	yes
position of nucleus	near the side of the cell	near the side of the cell	approximately central
shape of cell	approximately rectangular	approximately rectangular	approximately circular
large vacuole present	yes	yes	no
cell grouping	cells joined to one another	cells joined to one another	cells not joined

Table 1.2