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Chapter 1

Microscopy

CHAPTER OUTLINE

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

In this chapter, you 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

In this activity, you will practise using a light microscope. You will also make a temporary mount of plant tissue, observe it using the microscope and make a drawing of some of the cells.

YOU WILL NEED

Equipment:

• a light microscope • a source of light (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

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- Take care when using a sharp blade to cut the onion epidermis.

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Method

Part 1: Making a temporary slide and viewing it through a microscope

Figure 1.1 shows the parts of a microscope.

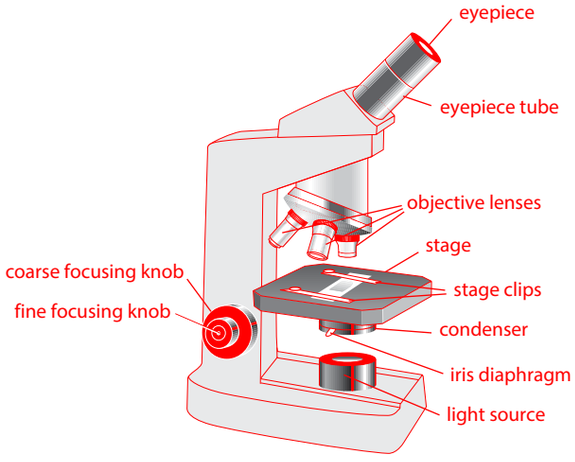

Figure 1.1: A light microscope.

1 Set up your microscope on the bench. Look for each of the parts that are labelled on the diagram.

2 You are now going to make a slide that you can view through your microscope.

- Take a piece of one of the layers from inside an onion bulb. Using scissors or a sharp blade, cut out one piece measuring approximately 1 cm × 1 cm.
- Using a dropper pipette, place a drop of water onto the centre of a clean microscope slide.
- Using forceps, gently peel away the very thin layer of epidermis on the inside surface of the piece of onion. *Immediately* place the epidermis into the drop of water on the slide. Use a mounted needle or seeker to gently spread out the epidermis, so that it is not folded over and is covered by water. You may need to add another drop of water to it.
- Gently lower a coverslip onto the slide, to cover the onion epidermis. It's a good idea to use a mounted needle (see Figure 1.2) as this helps to avoid trapping any air bubbles. If any air bubbles do occur, you should ignore these when making drawings – they will resemble car tyres under the microscope.

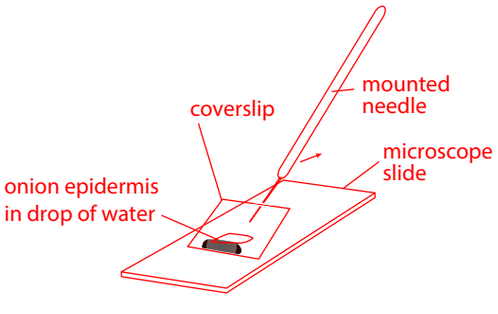

Figure 1.2: Lowering a coverslip.

TIP

Your microscope will almost certainly not be the same as the one in Figure 1.1. For example, it may have a mirror instead of a light source.

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- Use filter paper to gently remove any water from the top of the coverslip or on the surface of the slide.

3 Now you can look at your slide through the microscope.

- Turn the objective lenses so that the smallest one is over the hole in the stage.
- Look down through the eyepiece and make sure that you can see light. If you cannot see light, adjust the light source or the mirror.
- Place your slide on the microscope stage, with the epidermis over the hole.
- Looking from the side of the microscope, turn the coarse focusing knob to lower the objective lens, until the objective lens is almost touching the slide.
- Look down the eyepiece again. Slowly turn the coarse focusing knob the other way, to raise the objective lens. Stop when you can see the epidermis. It will probably not look clear.
- Now turn the fine focusing knob until you can see the epidermis clearly. You should be able to see something similar to Figure 1.3.

TIP

If you leave water on the surface of the slide, this may get onto the objective lens. Over time, deposits may form on the lens.

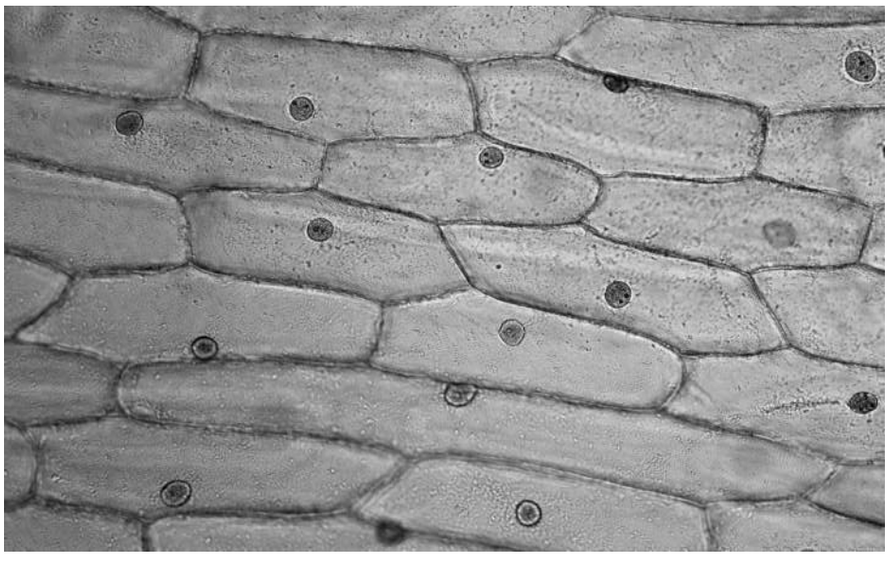

Figure 1.3: Micrograph of epidermal cells.

TIP

With some microscopes, it is possible to lower the objective lens so much that you can crash into the slide and break the coverslip. If you look from the side, it is less likely you will do this.

Part 2: Making a high-power drawing of onion epidermis

1 Focus on the onion epidermis using the lowest power objective, as described previously. Carefully swing the objective lenses around until the next largest one is over the slide. Focus using the fine focusing knob.

2 Decide which objective provides the best view of the epidermis. If you have an even higher power objective lens, you could try that one as well.

3 Make a drawing of the epidermis in the space that follows.

- Use a medium–hard (HB), sharp pencil.
- Use a high-quality eraser, so that you can completely remove any mistakes in your drawing.

TIP

It is sometimes a good idea to keep changing the objective lens as you do your drawing. For example, you may decide to use the lowest power lens, but occasionally change up to the higher power lenses to check on the detail.

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- Your drawing should be large, using at least 50% of the space available – but make sure you leave enough space around it for your labels.
- Take care to get the shapes and proportions of the cells correct.
- All lines should be single and clear. Do not leave any gaps, however small, in the lines.
- Always show the cell walls with two lines – cell walls have thickness.
- Do not shade anything at all in your drawing.
- Draw what you can see, not what you think you should see.

4 Label the cytoplasm, nucleus and cell wall on your drawing.

- Use a pencil for the label lines. You may also like to use a pencil to write the names.
- Use a ruler to draw the label lines. Make sure that the end of the label line touches the part that you are labelling.
- Keep label lines separate from each other.
- The label lines can go in any direction, but the written labels should be horizontal.

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Part 3: Adding a stain to a temporary slide

You are going to add some iodine in potassium iodide solution to your onion epidermis slide. This will stain (colour) any starch grains in the onion cells blue–black.

1 Place a small drop of iodine solution on the microscope slide, touching the edge of the coverslip.

2 Very carefully place one edge of a piece of a filter paper against the *opposite* side of the coverslip, as shown in Figure 1.4. The water underneath the coverslip will soak into the filter paper, bringing through the iodine solution.

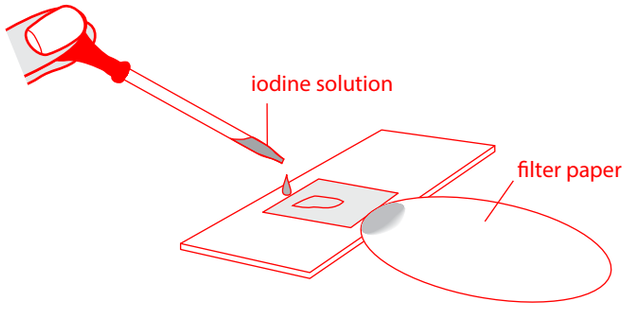

Figure 1.4: Adding iodine solution.

3 Clean the slide, and then observe the stained onion epidermis through the microscope. Describe any differences you can see in the stained cells compared with their appearance before staining.

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

In this activity, you will use an **eyepiece graticule** and **stage micrometer** to measure two types of plant cell. An eyepiece graticule is a little scale that fits inside the eyepiece of your microscope. When you look through the eyepiece, you can see the scale on the graticule at the same time as the object on the microscope stage. You can measure the size of the object in 'eyepiece graticule units'.

You then need to **calibrate** these graticule units. You do this using a stage micrometer. This is a slide with a scale with very small divisions on it, which you place on the microscope stage. The markings on this scale are very precisely drawn, and we know exactly how far apart they are.

KEY WORDS

eyepiece graticule: small scale that is placed in a microscope eyepiece

stage micrometer: very small, accurately drawn scale of known dimensions, engraved on a microscope slide

calibrate: convert the readings on a scale to a standard scale with known units

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YOU WILL NEED

Equipment:

• microscope, with a graticule in the eyepiece • prepared slide of section through a leaf • onion epidermis slide from Practical Investigation 1.1

Access to:

• a stage micrometer

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- There are no significant safety issues for this practical investigation.

Method

Part 1 : Measuring cells using an eyepiece graticule

1 Place a prepared slide of a transverse section through a leaf onto the stage of your microscope.

2 Check that there is an eyepiece graticule inside the eyepiece of your microscope. Look down through the eyepiece and turn it around. You should see the scale on the eyepiece graticule turning around.

3 Using the smallest objective lens, focus on the leaf section. Move the slide until you can see palisade cells. If necessary, change to a different objective lens, until you can see a group of palisade cells clearly. Move the slide until the cells are placed vertically.

4 Turn the eyepiece graticule until the scale lies horizontally across the group of cells, as shown in Figure 1.5.

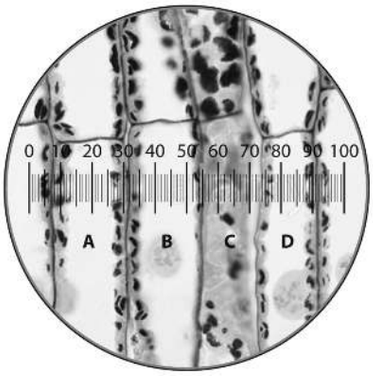

Figure 1.5: Micrograph of palisade cells seen using an eyepiece graticule.

5 Move the slide until the 0 on the graticule scale lies exactly over the cell wall of one cell. Use the scale to measure the width of three or four cells in eyepiece graticule units.

..... palisade cells measure ..... graticule units.

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Part 2: Calibrating the eyepiece graticule

1 Keeping the same objective lens over the slide, remove the slide from the stage and replace it with a stage micrometer.

2 Look down the eyepiece and focus on the stage micrometer scale. Move the eyepiece and/or the slide until the eyepiece graticule scale and the stage micrometer scale lie exactly next to each other, as shown in Figure 1.6.

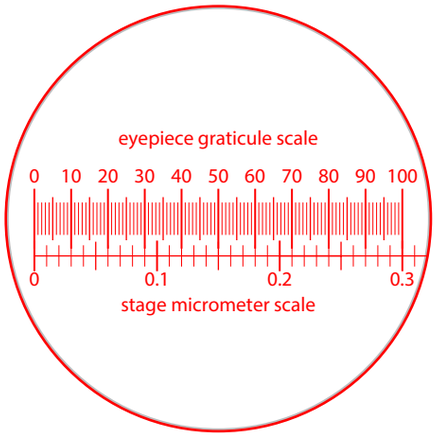

Figure 1.6: Stage micrometer seen using an eyepiece graticule.

3 Look for a good alignment of marks on the two scales, as far apart as possible. In the example in Figure 1.6, there is alignment at 0, 0 and at 80 on the eyepiece graticule scale and 0.24 on the stage micrometer scale.

Write down the alignments on your scales:

4 The large divisions on the stage micrometer scale are 0.1 mm apart. The small divisions are 0.01 mm apart.

$$0.01\,\text{mm} = 0.01 \times 10^{3}\,\text{m} = 10\,\mu\text{m}$$

Use this information to calculate how many μm are represented by one small division on the eyepiece graticule scale.

1 small eyepiece graticule unit = ... μm

TIP

If you get confused about which scale is the eyepiece graticule, and which is the stage micrometer, just turn the eyepiece. The scale that goes round is the eyepiece graticule scale.

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5 Go back to the measurement you made at the end of Part 1, where you measured the width of three or four cells in eyepiece graticule units.

Convert this measurement to μm.

..... palisade cells measure ..... μm.

6 Divide this value by the number of cells, to find the mean width of one palisade cell.

Mean width of one palisade cell = ... μm

7 Remove the stage micrometer from the microscope. Place a slide of onion epidermis cells onto the stage. *Using the same objective lens* as you did for the palisade cells, measure the width of a group of cells in graticule units.

..... onion epidermis cells measure ..... eyepiece graticule units.

8 Convert this measurement to μm, and then calculate the mean width of one onion epidermis cell.

Mean width of one onion epidermis cell = ... μm

Part 3: Calculating the magnification of a drawing

The **magnification** of an image is the number of times larger it is than the actual object.

magnification = size of image ÷ size of actual object

KEY WORD

magnification: the number of times larger an image of an object is than the real size of the object

1 Measure the width of the group of onion cells in your drawing in Practical Investigation 1.1. Record your answer in mm, and then multiply by 10^3 to convert it to μm.

Width of ..... cells in the drawing is ..... mm = ... μm.

2 Use your answer in Step 8 in Part 2 of this practical investigation to calculate the magnification of your drawing.

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Practical Investigation 1.3: Comparing animal cells and plant cells

In this activity, you will prepare a temporary slide of human cheek cells, and compare their size and structure with palisade cells and onion epidermis cells.

YOU WILL NEED

Equipment:

• microscope, with a graticule in the eyepiece • prepared slide of section through a leaf • onion epidermis slide from Practical Investigation 1.1 (or you can make a new one) • clean microscope slides and coverslips • dropper pipette • iodine in potassium iodide solution • methylene blue stain • cotton bud or similar

Access to:

• a stage micrometer

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- There is a very small risk of pathogenic organisms in the saliva and cheek cell sample on the cotton bud. Place the bud in a container of disinfectant immediately after use.

Method

Part 1: Observing, recording and measuring cheek cells

1 Gently rub a cotton bud around the inside of your cheeks, as shown in Figure 1.7.

2 Rub the cotton bud onto the centre of a clean microscope slide. Note: you will not be able to see very much on the slide, but there should be a few cheek cells present. Place the bud in a container of disinfectant immediately after use.

3 Add a small drop or two of methylene blue stain to the part of the slide where you rubbed the cotton bud. This stain is absorbed by living cells. More is taken up by the nucleus than by the cytoplasm, so it makes the nucleus look dark blue and the cytoplasm pale blue.

4 Carefully lower the coverslip onto the slide (see Figure 1.2), trying to avoid trapping air bubbles. Clean the slide and coverslip using filter paper.

Figure 1.7: Method for taking a sample of cheek cells.

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5 Look at the slide through the microscope. These cells are much smaller than the plant cells you have looked at earlier, so you may need to use a larger objective lens to view them.

In the space below, make a large labelled drawing of three or four cheek cells.

6 Use the eyepiece graticule to measure the diameter of three cheek cells in graticule units. (The cells will not be arranged in a neat row as in Practical Investigation 1.2, so you will have to measure each one separately.) Calculate the mean diameter of one cheek cell, in eyepiece graticule units.

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