

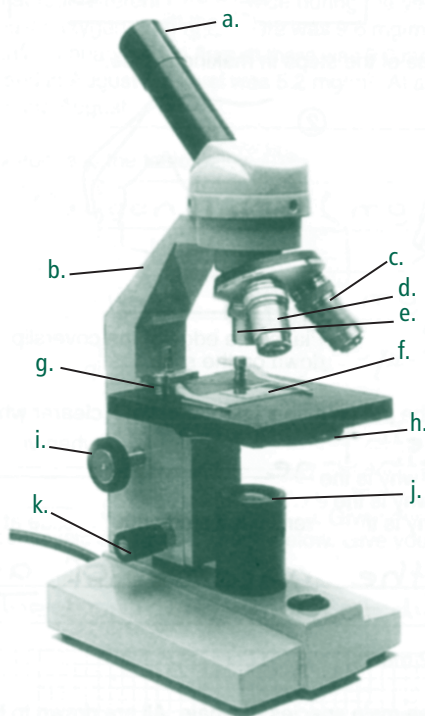
Investigate biological material at the microscopic level

Using a microscope and making a slide

Cells were first observed nearly 350 years (by Hooke in 1665) when the first simple microscopes were developed. Today, biology students get their first view of cells using standard **compound microscopes**.

Unit 1A: Compound microscope

- Complete the table to give the name and the function of each of the parts of the compound microscope.



Part	Name	Function
a.		
b.		
c.		
d.		
e.		

2. Compound microscopes have two sets of lenses, eyepiece (or ocular) and objective. The magnification of an object on the stage is given by:

Always give the (overall) magnification with any biological drawing, e.g. $150\times$

High power (HP): _____ \times _____ = _____

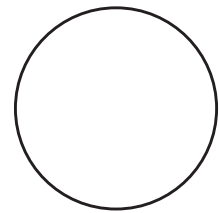
1. Rewrite the procedure for focussing a microscope as a *series of steps* that another student could follow to focus your microscope. You will need to use 8–10 steps.

2. Suggest a reason why it is necessary to:
- look from the side while putting the high-power lens in place

 - use *only* the fine focus when using the high-power lens

 - adjust the iris diaphragm when the high-power lens is in place

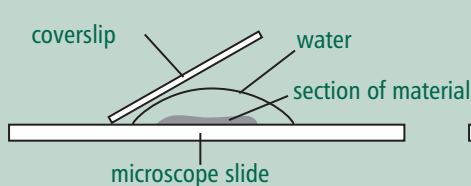
3. Practise using your microscope by getting a piece of newsprint and finding the word **the**.
- Put the newsprint on the stage exactly as you read it. Try to focus on the **e** under LP, MP and HP. Draw what the letter looks like (MP is probably best).
 - Under what power do you see more of the **e**? _____
 - Under what power is the **e** biggest (i.e. most magnified)? _____
 - Give two changes to the **e** as it is viewed under the microscope:
(1) _____ (2) _____



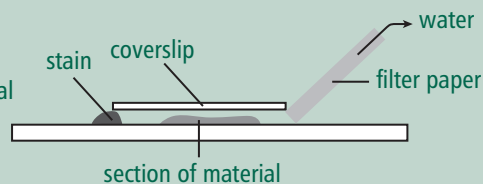
Making a slide

Material for viewing under the microscope is placed on a (microscope) **slide**. Your ability to make a slide is assessed in AS 91160 (Bio 2.8). In preparing a (temporary) slide, a 'wet' mount is done. This involves getting a thin layer of the material (the **section**) and placing it on the middle of the slide. Cover the section with one or two drops of water or stain and lower a coverslip over the section. Alternatively, the coverslip may be placed over the section then the water or stain drawn under the coverslip from one side.

Two commonly used stains in school labs are *iodine* (stains cell walls and nucleus orange-brown) and *methylene blue* (stains nucleus blue). A useful stain for plant sections is *toluidine blue* – stains cell walls purple and lignified cell walls (e.g. xylem and fibres) blue.



Making a slide of a section



Drawing stain across a section

For material larger than sections, *cavity slides* may be used – these have a small indentation in the slide to take more liquid and/or large material such as small organisms (e.g. used in viewing samples of pond water). *Cellulose* may be added to the water to trap or slow down movement (e.g. of unicellular organisms) so they are more easily viewed. Fibres from cotton wool added to liquid in cavity slide also can assist with this.

Unit 1C: Slide making

1. Rewrite the procedure for making a slide as a *series of steps* that another student could follow to make the slide. You will need to use three or four steps.

2. Suggest a reason why it is necessary to:
 - a. make sure the section is thin _____

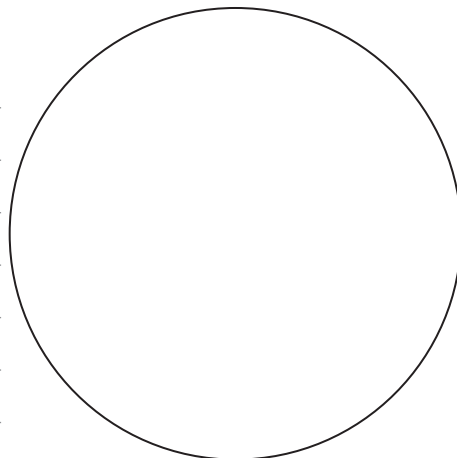
 - b. make sure the section is wet _____

 - c. use a stain such as iodine _____

 - d. use a coverslip _____

 - e. lower the coverslip carefully over the section _____

3. Practise by making a slide of starch grains then focussing on the grains. Cut off a small piece of potato and add one or two drops of iodine to the cut surface. Leave for about a minute. Smear or squeeze the fluid from the cut surface onto a slide and add a coverslip. Focus under LP, MP and HP.
 - a. *Show your slide under HP to your teacher to get feedback on the quality of your slide and focussing.*
 - b. Draw your starch grains under HP. Include a title and magnification with your diagram. Show your drawing to your teacher for feedback.
 - c. Describe what the grains look like.



Biological drawings

A *biological drawing* shows cells and tissues as viewed under the microscope. Usually, only a *few* cells need to be drawn. Your ability to make a biological drawing is assessed in AS 91160 (Bio 2.8). The following are the requirements of a quality drawing.

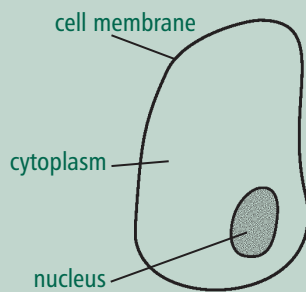
- Have a *title* describing the section which includes the *magnification* (e.g. 'Cross-section of geranium stem, 400 \times magnification'). Name any *stain* used.
- Done in *pencil*, with any errors erased.
- *Large and clear* (e.g. half a page or bigger).
- All lines clear (not sketchy) and entire (no gaps or overlaps).
- *Labelled* – straight lines are ruled from the label to the object; the lines do not cross each other. Labels must be horizontal and arranged neatly around the diagram.
- Representative of the material viewed – draw the section as you look down the eyepiece and *not* from memory.

The following need to be drawn correctly and accurately:

- shape of the cells (e.g. square / rectangular / circular / irregular)
- sizes or proportions of the cells or layers
- cell contents / components present (e.g. nucleus / vacuoles / chloroplasts / cell wall / cell membrane – if you can't see it, don't draw it)
- location and numbers of organelles (e.g. central / near cell wall or membrane; one / few / many)
- arrangement of cells (e.g. close packed / rows / columns).

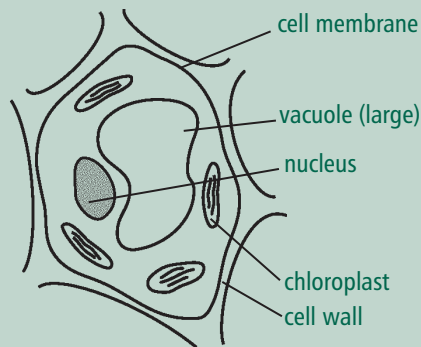
Your drawing will be assessed together with the section on display under your microscope – this is to check that the drawing is representative of the material that you have prepared and focussed on.

Cell structure and function is studied in AS 91156 (Bio 2.4), but some background is needed here to allow for identification of cells and their features. The basic structure of an animal and a plant cell as seen under a (light) microscope like yours follows.



Animal cell

An animal cell viewed through a light microscope shows a nucleus, cell membrane and cytoplasm.



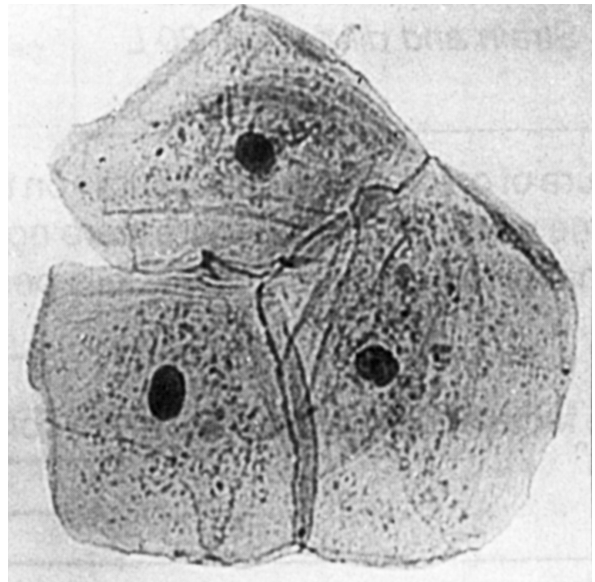
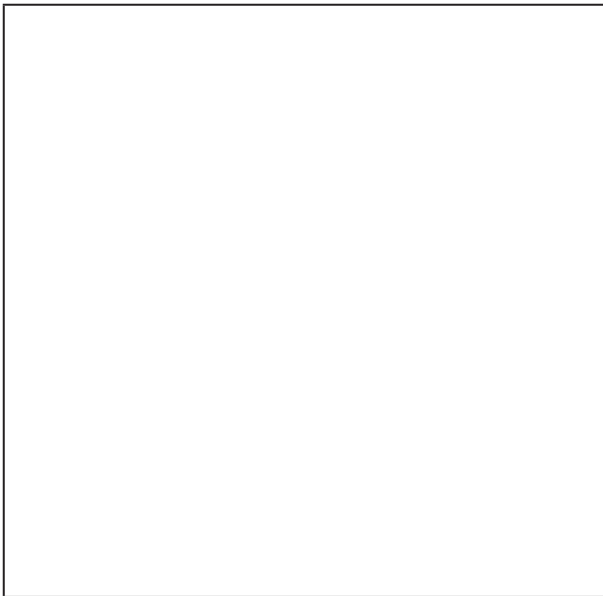
Plant cell

The light microscope shows that plant cells may also contain chloroplasts and large vacuoles. A cell wall is present outside the cell membrane.

All cells are enclosed in a (plasma) *membrane*; all plant cells have a (cellulose) *cell wall* around the membrane. The matrix inside the cell is the *cytoplasm*, which contains the *nucleus* and cell organelles. The nucleus contains the chromosomes with the genetic material, *vacuoles* are storage sacs, *chloroplasts* the site of photosynthesis. Vacuoles are typically large in plant leaf cells as they store the products of photosynthesis. The cell wall gives both shape to cells and support to tissues. Plant cells have a very regular arrangement (e.g. some plant tissue resembles a brick wall when viewed under the microscope).

Unit 1D: Biological drawings

1. The two pictures following are *photomicrographs* – photographs taken of sections as viewed under a microscope.
 - a. i. Make a biological drawing of the following section of cells from the lining of a human cheek, stained with methylene blue and magnified $400\times$, in the box alongside. Three structures should be labelled. Show your drawing to your teacher for feedback.



- ii. Give a reason why the cells were stained with methylene blue.

Demonstrate understanding of gene expression

Proteins and protein synthesis

Proteins are giant molecules (polymers) made of **amino acids** (monomers). A chain of amino acids is a **polypeptide** (bonds between amino acids are called **peptide** bonds); a protein molecule consists of one or more polypeptide chains of up to several thousand amino acids. Human proteins are made of 20 different amino acids (e.g. proline, valine, serine – usually shortened to *pro*, *val*, *ser*). The primary structure of a protein is the *sequence of amino acids*; each protein has a different sequence of amino acids (the number of different sequences of the 20 different amino acids that make up human proteins is vast) – it is this sequence that determines the properties of a protein. The information for the sequence of amino acids in a polypeptide is stored in a **gene**. Therefore, a gene may be defined as ‘that length of DNA which codes for a polypeptide’. When a mutation occurs in a gene it may result in an altered protein – this may not affect the functioning of the protein or it may result in a completely non-functional protein.

All the organic materials that make up an organism are proteins or substances made by protein catalysts (**enzymes**). As proteins are produced from the instructions of genes they are the *link* between genes and the body they build. Proteins come in two main kinds, depending on the shape of the molecule.

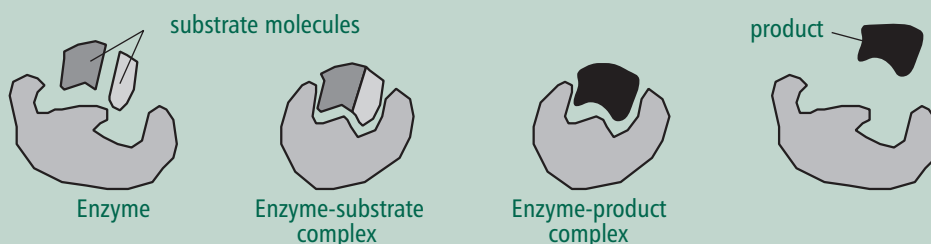
- **Fibrous proteins** – polypeptide chains form tough, rope-like bundles; e.g. *collagen* (forms essential connective tissue such as tendons, ligaments, muscles, and bones) and *keratin* (an essential component of the skin epidermis and its derivatives – hair, nails, claws, feathers). Typically, fibrous proteins have a mechanical function such as support and protection. Others are contractile for movement – e.g. *actin* and *myosin* in muscle fibres, *tubulin* in the spindle fibres of cell division.
- **Globular proteins** – polypeptide chains are irregularly folded into a globe-shaped molecule; e.g. enzymes, some hormones. Typically, globular proteins have a chemical function (e.g. haemoglobin for oxygen transport, antigens and antibodies in the immune response) or regulatory function (e.g. enzymes as catalysts, hormones in the endocrine system).

Although globular proteins are large molecules, their chemical activity usually resides in a small part – the **active site**.

As hydrogen bonds which help maintain protein shape are weak, the shape of a protein is destroyed (**denatured**) relatively easily (e.g. by high temperatures); the protein loses its chemical / biological function.

Enzymes take part in the reactions they catalyse by temporarily combining with the substance they act on (the *substrate*), forming an enzyme-substrate complex. The active site changes shape slightly to fit the substrate. This *induced-fit* mechanism is possible because the hydrogen bonds which contribute to protein shape are weak enough to allow some movement.

The basic shape of a protein is the linear sequence of amino acids held together by peptide bonds. Amino acids also contain chemical groups not involved in the peptide bond that interact with each other (using **hydrogen bonds**) such that the protein becomes twisted and folded. Hydrogen bonds are weak, and are easily broken.



If an enzyme loses its shape through denaturing or through a mutation changing the amino acid sequence, the active site may be lost so the enzyme can no longer catalyse its specific reaction. This can have serious consequences in metabolic pathways.

Unit 7A: Proteins

- Define a gene. _____
- Describe, with examples, the two main types of protein.
 - _____
 - _____
 - Describe the primary structure of a protein.

 - Explain, using examples, why proteins are so important.

 - Explain, using examples, why enzymes are so important.

Protein synthesis

DNA is found in the chromosomes in the nucleus of the cell. The base sequence of a DNA molecule codes for the amino acids that make up a protein (one gene codes for one polypeptide). A sequence of three bases in DNA (a **triplet**) codes for one amino acid.

Protein synthesis occurs in **ribosomes** in the cell cytoplasm. A carrier molecule, **mRNA** (messenger ribonucleic acid), is needed to take the code from DNA to the ribosomes. Ribonucleic acids (RNA) are nucleic acids like DNA but there are some key differences:

- three different forms of RNA exist – **messenger RNA** (mRNA), **transfer RNA** (tRNA), **ribosomal RNA** (rRNA)
- RNA is *single stranded* (DNA is double stranded)
- the sugar in RNA is **ribose** (DNA is deoxyribose)
- the base **uracil** (U) replaces the base thymine (T) found in DNA.

Unit 7B: DNA and RNA

Complete the following table, then write a paragraph underneath to compare the structure of DNA and RNA.

	DNA	RNA
Bases present		
Number of strands and relative length		
Sugar		
Location in cell		

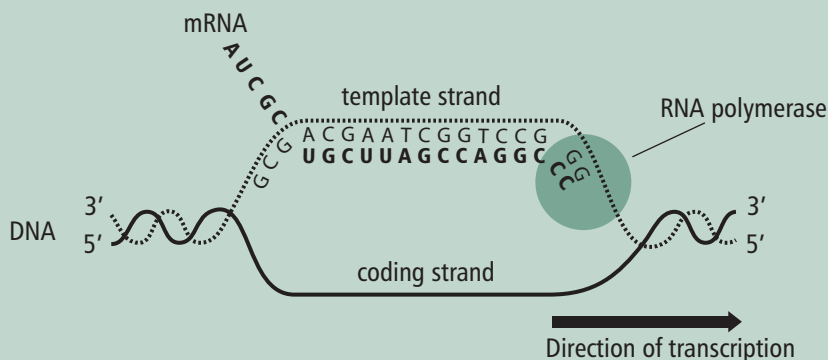
There are two main stages of protein synthesis – **transcription** and **translation**.

Transcription

Every gene has three regions:

- the **promoter** – turns a gene 'on' and 'off', and signals the site of transcription
- the **coding** region – has the base sequence for a protein
- the **terminator** – signals the end point of transcription.

In transcription, the enzyme *RNA polymerase* binds to the promoter and the DNA 'unwinds' and 'unzips'. Only one of the two strands of DNA, the **template strand**, is copied by mRNA (**transcription**); this is similar to the process of DNA replication and the base pairing rules are the same except that adenine (A) in DNA pairs with uracil (U) in mRNA. (The other strand of DNA, the 'coding strand', has the same base sequence as the mRNA transcribed, except U replaces T.) When RNA polymerase reaches the terminator sequence, transcription is completed; mRNA detaches, the two DNA strands re-join (*anneal*) and the helix reforms.

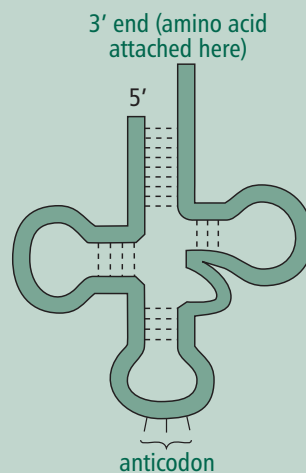


Translation

mRNA leaves the nucleus and travels to the ribosomes for **translation**. Ribosomes, made of protein and rRNA, move along the mRNA and 'read' the coded message, *linking the amino acids in the correct order to form a polypeptide*. Bases in mRNA are read in a sequence of three (a **codon**) by ribosomes; each codon corresponds to a DNA triplet, thus a DNA triplet codes for a particular amino acid. It is the function of the ribosomes and **tRNA** to decode mRNA to translate it into the correct amino acid sequence of the polypeptide.

tRNA:

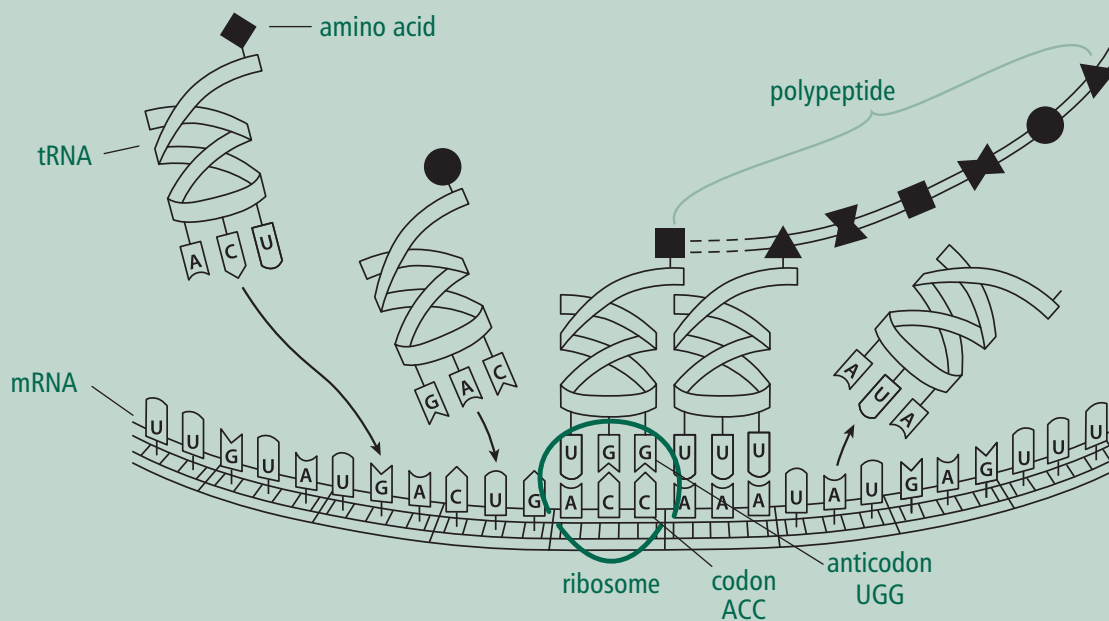
- small molecules (about 70–90 nucleotides), folded back on themselves, similar in shape
- has a three-base sequence of CCA at the outer (3') end to which an amino acid attaches
- has unpaired three-base sequence at the 'fold' (an **anticodon**) which bonds to the complementary codon of mRNA.



The 3-dimensional structure is more complex

Each of the 20 different amino acids in the cytoplasm is recognised by a specific tRNA molecule. tRNA attachment to an amino acid is enzyme-catalysed.

Ribosomes move along the mRNA from the *start* codon (AUG) until one of the *stop* codons (UAA, UAG or UGA) is reached. When the tRNA anticodon matches the complementary codon of the mRNA, the specific amino acid attached to that tRNA is bonded to the polypeptide chain being formed. Every time the ribosome moves along one codon, another amino acid is added to the polypeptide. Normally, several ribosomes move along the mRNA at any time (the whole complex is called a *polyribosome*), ensuring rapid synthesis of the needed protein. mRNA is broken down by RNAase enzyme when enough protein has been made. The complete polypeptide chain(s) coil(s) and fold(s) to form the protein.



Proteins are the products of **gene expression**:
DNA → mRNA → polypeptide → protein

ANSWERS

Achievement Standard 91160 (Biology 2.8)

Unit 1A: Compound microscope (page 1)

1.

Part	Name	Function
a.	eyepiece lens	Magnifies the section being viewed; combines with objective lens to give overall magnification
b.	arm	Used for carrying the microscope
c.	high-power objective lens	Combines with eyepiece lens to give greatest magnification of the section being viewed
d.	medium-power objective lens	Combines with eyepiece lens to give middle magnification of the section being viewed
e.	low-power objective lens	Combines with eyepiece lens to give lowest magnification of the section being viewed
f.	slide on stage	Platform for the material being viewed
g.	stage clips	Hold the slide in place
h.	iris diaphragm	Adjusts the amount of light for viewing
i.	coarse (focus) adjustment	Gives the first or rough focus of the section being viewed
j.	light source (or mirror)	Provides the light for viewing
k.	fine (focus) adjustment	Gives detailed focus of the section being viewed

2.

Answer will depend on microscope used. Typically, school microscopes have eyepiece lens of $10\times$ and objective lenses of $4\times$, $10\times$ or $40\times$.
These combinations give overall magnification of LP $40\times$, MP $100\times$ and HP $400\times$.

Unit 1B: Focussing a microscope (page 2)

1.

Typical steps, in order, for focussing material under highest power/magnification follow. The slide may be put on the stage first rather than third as given here.

- Switch on the light (or adjust the mirror); adjust the (iris) diaphragm.
- Put the lowest-power objective lens in place.
- Place the slide on the stage so it is centred under the lens.
- Using the coarse adjustment, wind down until the objective lens is as close to the slide as it will get without hitting the slide.
- Look through the eyepiece and slowly wind it back up until the section is in focus.

- Bring the section into sharp focus using the fine adjustment.
- Switch the medium-power objective lens in place and use the coarse and/or fine adjustment to focus as necessary.
- Switch the highest-power objective lens into place, while watching from the side. Focus using fine focus only.
- Use the iris diaphragm to give more light if necessary.

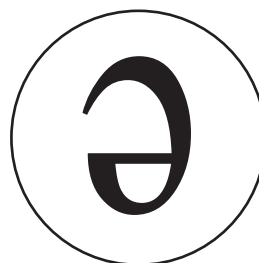
2. a. If the section/slide is too thick, then the large HP lens will not fit into place; forcing it may damage both lens and slide.

- b. The large lens will be almost touching the slide and focussing will only need a very small adjustment; the coarse adjustment gives a sufficiently large adjustment that the focus will be lost.

If focus is lost, go back to medium, even low power, and start again.

- c. The large HP lens is so close to the slide and so zoomed in on the section being viewed that usually more light is needed to allow the section to be clearly seen.

3. a.



- b. low power
c. high power
d. upside down, back to front

Unit 1C: Slide making (page 4)

1. Typical steps for making a slide follow.

- Place or smooth a thin layer of material on the centre of the slide.
- Add a drop of water or stain to the section, removing excess fluid.
- Place one side of a coverslip at one side of the drop of water or stain and let the coverslip gently 'fall' onto the drop of water or stain.

2. a. To allow light through for clear viewing.

- b. To prevent it drying out.

If dry, can no longer be viewed clearly.

- c. Stain gets taken up by parts of the section/cell (e.g. nucleus or cell wall); therefore these part(s) stand out so more clearly seen.
- d. Coverslip protects the large HP objective lens which otherwise might go into water or stain and get damaged or reduce clarity of viewing.
- e. To prevent/reduce formation of air bubbles in/on the section.

3. a. *Teacher feedback.*
 b. *Suitable title is: Starch grains from potato cells 400× magnification*
Drawing should show a few large oval or circular grains.
 c. Starch grains are (large) oval or circular bodies stained blue/purple/black by the iodine.
 This is effectively a starch test in action.

Unit 1D: Biological drawings (page 6)

1. a. i. *Teacher feedback.*
 ii. Methylene blue is taken up by / stains the nucleus so the nucleus shows clearly in each cell.
 b. i. *Teacher feedback.*
 ii. Iodine is taken up / stains the nucleus and the cell wall so both show clearly.
2. a. Title needs to state where section is taken from; shape of the cells not accurate; cell wall needs to be thicker; nucleus expected to be present in each cell. Overall, drawing is not sufficiently representative of tissue viewed.
 b. Title needs to state where section is taken from and give actual magnification used; cell wall needs to be thicker; nucleus not accurately drawn; label for nucleus needs to be horizontal line.
 c. Title needs to state where section is taken from and give actual magnification used; overall size of drawing too small; lines drawn not accurate enough as show both sketching and overlap and double lines have not been erased; onion cells do not have chloroplasts, so label not correct. Overall, drawing is not sufficiently representative of tissue viewed.
 d. Title needs to state where section is taken from; shape and arrangement of cells not accurate; lines not accurate as both sketching and overlap present and double lines not erased; nucleus needs to be oval; cell wall needs to be thicker; lines for labels need to be ruled. Overall, drawing not sufficiently representative of tissue viewed.

Unit 1E: Specialised features of cells and tissues (page 9)

1. *Teacher feedback.*
2. Explanation of special features could identify and link into a paragraph, e.g.:
- Close-packed arrangement of epidermal cells provides a complete unbroken 'skin' for leaf.
 - Cell walls for support of cells.
 - Stomata are open pores to allow entry/exit of (named) gases in photosynthesis / respiration / transpiration.
 - Guard cells are bean-shaped (with thickened inner walls) and are able to open and close the stomata (mechanism depends upon changes in turgor pressure) to control loss of water vapour.

Achievement Standard 91156 (Biology 2.4)

Unit 2A: Cell structure and function (page 11)

- 1.
- | Cell structure | Name | Function |
|----------------|-----------------|--|
| a. | plasma membrane | Boundary between cell and environment – a 'gatekeeper' maintaining stable cell environment |
| b. | cytoplasm | Cell matrix; contains the organelles |
| c. | mitochondrion | Site of aerobic respiration |
- d. Golgi body
Modifies proteins made by ribosomes, then secretes them
- e. endoplasmic reticulum
Transport network and involved in production of lipids (smooth ER) and proteins (rough ER)
- f. ribosomes
Site of protein synthesis
- g. vacuole
Storage sac (for various chemicals needed or produced by the cell)
- h. lysosome
Specialised vacuole containing enzymes
- i. nucleus
Control centre for cell; contains chromosomes / DNA / genetic material
- j. nucleolus
Site of RNA production
- k. centriole
Forms the spindle in cell division
- l. chloroplast
Site of photosynthesis
- m. cell wall
Rigid, supporting structure for cell (part of skeletal support system in whole plant)
2. Plants have a cell wall to give a rigid support structure to each cell which contributes to the overall support / skeletal system in the plant. Animals have a distinct skeletal system for support. Plant cells exposed to light have chloroplasts to photosynthesise and produce food in the form of glucose / starch – plants are autotrophs; animals are heterotrophs.
- Autotrophs are organisms that make their own food from inorganic materials using solar energy (plants) or chemical energy (bacteria). Heterotrophs are organisms that need to consume organic material for food – they can't make their own food.*
3. a. Sugars, amino acids, mineral ions, pigments, waste products from metabolism.
 b. Vacuoles in leaf cells store the (large amounts of) glucose made in photosynthesis. Vacuoles also contribute to the support of the cell by acting as a fluid 'skeleton'. The vacuole swells as water enters and exerts (turgor) pressure on the cell membrane so the cell becomes rigid.
4. a. i. Secretory cells synthesise substances (e.g. proteins are synthesised by ribosomes) and then release the substances through the plasma membrane to the exterior for use by cells elsewhere in the body.
- Secretion and excretion are two different processes. Excretion is the production and release of metabolic wastes by cells or organs, e.g. CO₂ from respiration.*
- ii. Ribosomes are the site of synthesis of proteins, e.g. enzymes.
- iii. (1) Pancreatic cells synthesise and secrete enzymes; enzymes are proteins.
 (2) White blood cells synthesise and secrete antibodies to attack invading pathogens; antibodies are proteins.
 (3) Pituitary gland cells synthesise hormones such as ADH, TSH, FSH; such hormones are proteins.
- b. i. Lipids are fats (including fatty acids and steroids) and oils. Some vitamins (e.g. A, D, E and K) are also classified as lipids.
 ii. Steroids include hormones such as oestrogen, testosterone, and progesterone. Therefore, ovary cells which produce and secrete oestrogen and progesterone, and cells of testes which produce testosterone, would have large amounts of smooth ER as this is associated with lipid synthesis. Vitamin D is also part of the steroid group, so dermal