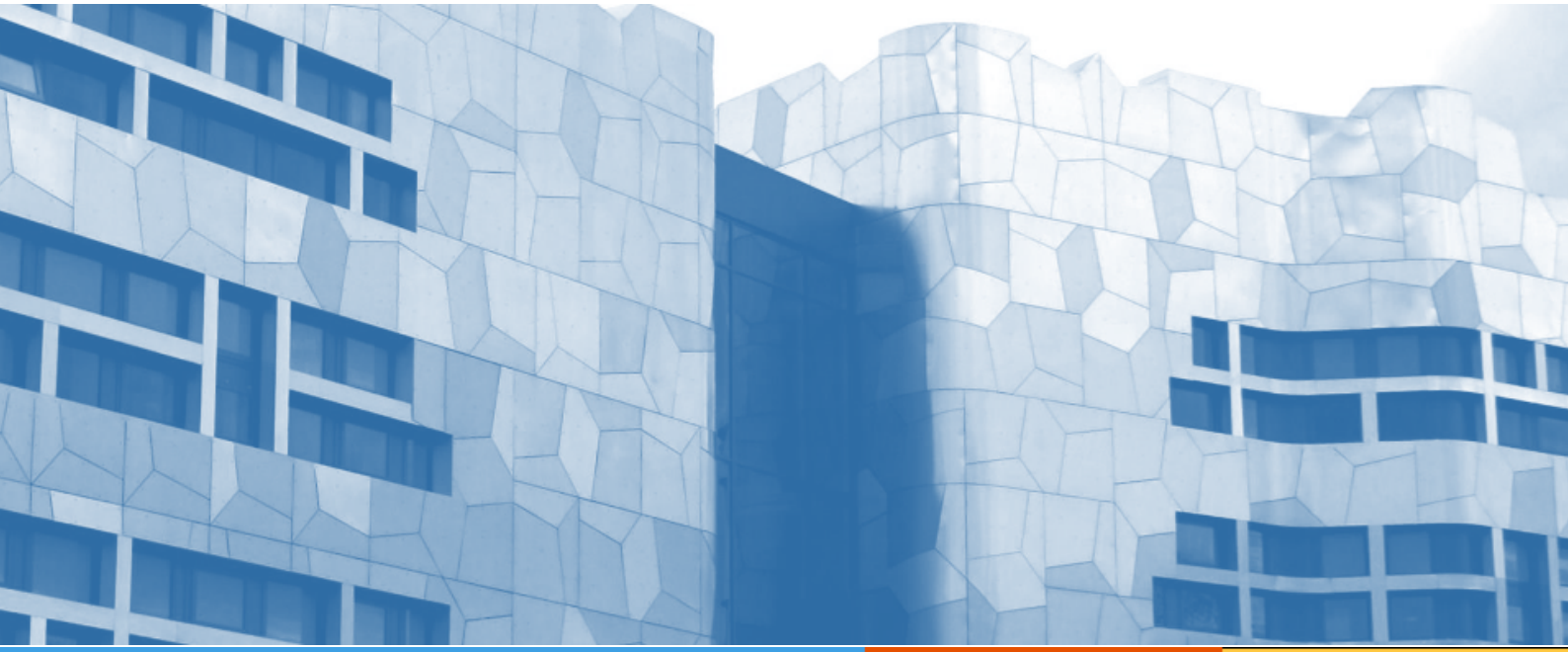
WJEC GCE BIOLOGY
SUMMER 2026 ASSESSMENTS

A2 Unit 5



GCE A LEVEL

1400U50-1C

BIOLOGY – A2 UNIT 5
Practical Examination

TOPIC AREAS FOR ASSESSMENT IN UNIT 5 IN SUMMER 2026

- 1.1 Chemical elements joined together to form compounds
- 1.3 Cell membranes and transport
- 3.2 Photosynthesis uses light energy to synthesise organic molecules
- 3.7 Homeostasis and the kidney
- 4.4 Variation and evolution

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Guidance Notes

There are three types of specified practical work in this specification

- Investigative work
- Microscopy
- Dissection

In general you should be able to:

- apply investigative approaches and methods to practical work and think independently when undertaking practical work;
- use a wide range of experimental and practical instruments, equipment and techniques appropriate to the knowledge and understanding included in the specification.

Experimental Design

You should be able to :

- identify the independent variable – the factor you will test/ change
- identify the dependent variable- the factor which you are measuring
- identify the controlled variables – the factors that you need to keep constant
- use the correct units for all your variables
- identify a suitable range for your independent variable, this would normally be at least five values
- explain why repeat readings would be needed – a mean is more reliable than an individual reading and it will help identify anomalous results
- design a suitable control experiment
- assess the main risks of your experiment

Hazard	Risk	Control measure

Hazard - an object or chemical and the nature of the hazard

Risk - an action in the method that can create a risk from the hazard

Control measure - must be practicable in the context of the practical

Table of results

Your table should have:

- correct column headings
- appropriate units in headings (not in body of table)
- columns for sufficient repeats
- appropriate recording of readings, time to the nearest second, same number of decimal places throughout table except 0

Exemplar table of results

Independent variable (unit)	Dependent variable (unit)			
	Trial 1	Trial2	Trial 3	Mean
Value 1				
Value 2				
Value 3				
Value 4				
Value 5				

Graphs

Your graph should have:

- the independent variable plotted on the *x* axis
- the dependent variable plotted on *y* axis
- the axes labelled correctly
- used at least half of the grid should have been used on both axes
- the correct units on both axes
- a suitable linear scale used on each axis, including a figure at the origin for both axes
- all plots accurately plotted
- the points accurately joined with a suitable line with no extrapolation. Point to point using a ruler through centres is advised for most graphs
- range bars correctly drawn

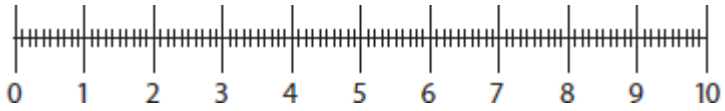
Analysis of results

You should be able to :

- identify a trend in the results
- comment on the consistency of the readings
- comment on the accuracy of the readings
- suggest improvements for any inaccuracies identified
- give an explanation of results using relevant and sound biological knowledge
- draw a suitable valid conclusion

Calibration of microscope

In order to measure the size of a structure on a microscope slide it is necessary to calibrate the microscope. Inside the eyepiece of the microscope there is an eye piece graticule. It is graduated 1-10 with 10 subdivisions between each number therefore the eyepiece graticule has 100 eyepiece units [epu] along its length.



With different magnifications, the divisions on the eyepiece graticule will cover different actual lengths of the specimen on the slide.

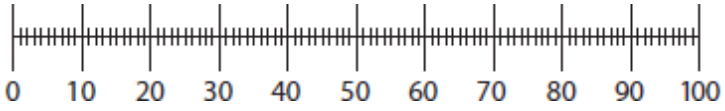
A stage micrometer is used to measure the length of each division at different magnifications. There are two types of stage micrometer available, check which you are using.

Either

The stage micrometer is a slide with a line **1 mm** long on it. The line is also marked for tenths and hundredths of a mm. There are 100 stage micrometer units [smu] on the 1 mm line.Each stage micrometer unit = 0.01 mm or 10 µm.

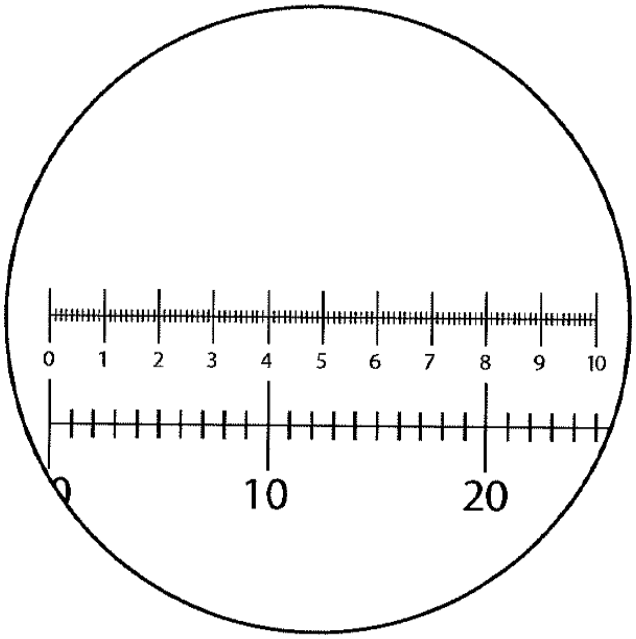
Or

The stage micrometer is a slide with a line **10 mm** long on it. The line is also marked for tenths and hundredths of a mm. There are 100 stage micrometer units [smu] on the 10 mm line. Each stage micrometer unit = 0.1 mm or 100 µm.



To calibrate the microscope

- Line up the zero of the eyepiece graticule and the zero of the stage micrometer.
- Make sure the scales are parallel.
- Look at the scales and see where they are in line again.



Using this x40 objective lens, 20 stage micrometer units make up 80 eyepiece units.

80 eyepiece units = 20 stage micrometer units

If 1 stage micrometer unit = 0.01mm

1 eye piece unit $= \frac{20}{80} =$ 0.01mm

1 stage micrometer unit = 0.01mm

1 eye piece unit = 0.25 x 0.01mm
= 0.0025mm or 0.0025 x 1000μm
= 2.5 μm

If 1 stage micrometer unit = 0.1mm

1 eye piece unit $= \frac{20}{80} =$ 0.25 stage micrometer units

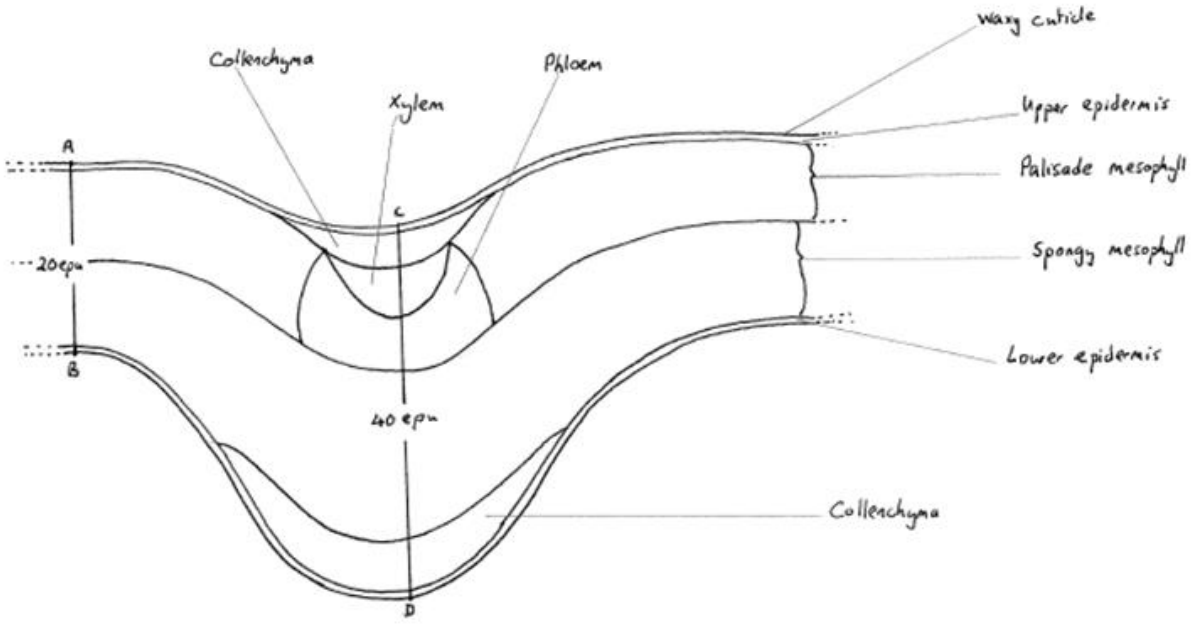
1 stage micrometer unit = 0.1mm

1 eye piece unit = 0.25 x 0.1mm
= 0.025 mm or 0.025 x 1000 μm
= 2.5 μm

Microscope drawing

Low power plan

This shows the distribution of tissues in a transverse section (TS) or longitudinal section (LS) of a structure.



T.S Leaf of *Ligustrum* – Low power plan

It is not always necessary to draw a plan of the entire structure but if a part is drawn it should be indicated that it is a part of a structure. This is usually done by drawing dotted lines to show where the tissues continue.

When completing low power plans, you should:

- use a sharp pencil.
- not use any shading
- not draw any individual cells
- make your drawing at least half a page of A4 in size and position the labels to the side of the drawing
- make all lines clear, complete and not overlapping
- draw label lines with a ruler to the centre of the tissue layer, they should not cross each other
- ensure tissue layers are all drawn to the correct proportion
- draw a line across two tissues and give the width of this line in eyepiece units. If one line across tissue A has been given 48 epu and the second line across tissue B has been given 12 epu, the correct proportion should show that tissue A is 4 times the width of tissue B at that point.
- check tissue boundaries by using a higher objective lens than that being used to draw the plan

High power drawing of individual cells

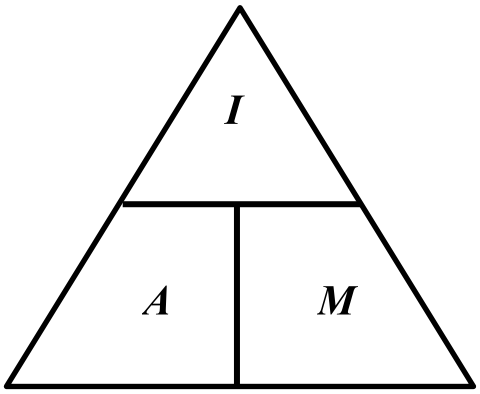
When completing high power drawings of individual cells, you should:

- use a sharp pencil
- not use any shading
- draw two or three cells
- make your drawing at least half a page of A4 in size and position the labels to the side of the drawing
- make all lines clear, complete and not overlapping
- use single lines to represent the tonoplast membrane or the cell membrane. A double line should be used to represent the cell wall
- calculate the actual length or diameter of the cells
- not draw structures which you cannot see for example details of the structure of the chloroplast or mitochondria using a x40 objective

Magnification of a drawing

Magnification shows us the size of a drawing or image in relation to the size of the actual object.

The magnification, size of object or size of image can be calculated using the triangle method.



I = Size of image
A = Actual size of object
M = magnification.

Cover what you wish to calculate and the equation is given.

$I = A \times M$

$M = \frac{I}{A}$

$A = \frac{I}{M}$

Check that the units for the size of the object and image are the same.

Microscopy

Throughout the A level course you will observe the following slides. Ensure that you are able to identify the structures and tissues given below.

Leaf e.g. Privet

cuticle; upper epidermis; palisade mesophyll/cells; spongy mesophyll; xylem; phloem; collenchyma; lower epidermis

Marram grass leaf

cuticle; adaxial epidermis/upper epidermis; fibres/sclerenchyma; palisade mesophyll; xylem; phloem; abaxial epidermis/lower epidermis; stomata

Water lily leaf

cuticle; adaxial epidermis/upper epidermis; palisade mesophyll; xylem; phloem; spongy mesophyll; air spaces; collenchyma; abaxial epidermis / lower epidermis; stomata

Root

epidermis/exodermis; cortex; endodermis; pericycle; xylem; phloem

Stem

Epidermis; cortex; medulla/pith; xylem; phloem; vascular bundle; cambium; collenchyma; sclerenchyma

Artery and Vein

Endothelium; tunica interna/tunica intima; tunica media; tunica externa/tunica adventitia; lumen

Ileum

Columnar epithelium; mucosa/lamina propria; muscularis mucosa/mucosal muscle; submucosa; circular muscles; longitudinal muscles; serosa; villus; goblet cells

Trachea

Ciliated epithelium; lamina propria; blood vessel/artery/vein/arteriole/venule; mucous glands; submucosa; muscle; cartilage; connective tissue; perichondrium

Lung

Bronchus/bronchiole with ciliated epithelium; alveoli with squamous epithelia; blood vessels

Tapeworm

Scolex with hooks and suckers; proglottids/segments; zone of proglottid formation behind scolex;

Anther

Epidermis; tapetum/inner wall; fibrous layer/outer wall; area of dehiscence/stomium; pollen sac; xylem; phloem; parenchyma

Testis

Seminiferous tubule; spermatozoa; spermatids; spermatogonia; area of Leydig cells; Sertoli cell

Ovary

Germinal epithelium; primary follicle; graafian follicle; secondary follicle; primary oocyte; secondary oocyte; blood vessels; stroma; corpus luteum; theca; cumulus cells; antrum

Spinal cord

Dorsal root; ventral root; grey matter; white matter; central canal; pia mater; dura mater; ventral median fissure; meninges

Kidney

Bowman's capsule; glomerulus; capillaries; distal / proximal convoluted tubule

FOOD TESTS

SPECIFICATION REFERENCE 1.1

CHEMICAL ELEMENTS ARE JOINED TOGETHER TO FORM BIOLOGICAL COMPOUNDS

Introduction

The chemical composition of different foods can be determined by performing chemical tests. There are five tests which you need to know and understand how to use:

Reducing sugars

- Non-reducing sugars
- Proteins
- Starch
- Fats and oils

These tests can be used on pure chemicals to demonstrate the positive results from each and then used to investigate the composition of different foods.

Apparatus and reagents

5 boiling tubes
2 test tubes
1 beaker
3 dropping pipettes
Benedict's reagent
Dilute hydrochloric acid (0.5mol dm^{-3})
Dilute sodium hydroxide / sodium bicarbonate
Biuret reagent
Iodine - potassium iodide solution
Absolute alcohol
Glucose solution
Sucrose solution
Albumen solution
Starch solution
Oil
Water bath

Method

Reducing sugars

1. Mix 2 cm^3 of the test solution with an equal volume of Benedict's reagent.
2. Heat the mixture in a water bath to between 70 °C and 90 °C for 5 minutes.
3. If the blue Benedict's has changed colour to an orange / red precipitate the test solution is a reducing sugar.

Non-reducing sugars

1. Mix 2 cm^3 of the test solution with an equal volume of Benedict's reagent.
2. Heat the mixture in a water bath to between 70 °C and 90 °C.
3. Observe and record colour change. If it is not a reducing sugar there will be no colour change and the solution will remain blue.
4. Put another 2 cm^3 of the test solution into a boiling tube, add 2 drops of hydrochloric acid and heat in a water bath to 70 °C and 90 °C for 2 minutes.
5. Add 2 drops of sodium hydroxide.
6. Add 2 cm^3 Benedict's reagent.
7. Heat the mixture in a water bath to between 70 °C and 90 °C for 5 minutes.
8. If the blue Benedict's has changed colour to orange / red precipitate, the test solution is a non – reducing sugar.

Proteins

1. Mix 2 cm^3 of the test solution with 2 cm^3 of Biuret reagent in a boiling tube.
2. Cover the top of the boiling tube and invert it once. If the solution turns purple the test is positive.

Starch

1. Mix 2cm^3 of the test solution with 2 drops of iodine in potassium-iodide solution.
2. If starch is present the solution will change colour to blue/ black.

Fats and oils

1. Mix the fat or oil with 5 cm^3 of absolute alcohol in a boiling tube.
2. Shake the tube.
3. Pour the mixture into another boiling tube half full of cold water.
4. If lipids are present a cloudy white emulsion will form.

Risk Assessment

Hazard	Risk	Control measure
Biuret is an irritant	Could splash onto hands or into eyes when transferring biuret to test tube	Wear gloves/ eye protection
Ethanol is flammable	Could catch fire if used near a Bunsen burner	Ensure all Bunsen burners are turned off before ethanol is used

Benedict's reagent and Iodine solution are classed as low hazard by CLEAPSS at these concentrations.

Teacher/ Technicians notes

Starch

Iodine is only sparingly soluble in water (0.3 g per litre); it is usual to dissolve it in potassium iodide solution (KI) to make a 0.01 M solution (by tenfold dilution of a 0.1 M solution) to use as a starch test reagent. Refer to CLEAPSS Recipe card 33.

Reducing/ non reducing sugar

Benedicts reagent can be purchased from a laboratory supplier or can be made
1 dm^3 of Benedicts reagent contains:
100 grams anhydrous sodium carbonate
173 grams sodium citrate
17.3 grams copper (II) sulphate pentahydrate

Protein

Biuret reagent can be purchased from a laboratory supplier or potassium hydroxide and dilute copper(II)sulphate solution can be used as an alternative

Fats and oils

Absolute alcohol should be used because if it has been diluted an emulsion of fats will be produced in the alcohol before it is poured into the water.

Further work

- Testing a variety of foods.
- The Benedict's test is semi-quantitative, 0.5g % concentration reducing sugar gives a green precipitate, 1g% concentration a yellow precipitate, 1.5g % an orange precipitate and above 2 g% a red precipitate. Students could assess the % concentration of reducing sugar by comparing the colour to standard solutions of reducing sugars. They would need to plan which variables needed to be controlled to carry out such a semi-quantitative study.
- The Biuret test is also semi-quantitative. The deeper the violet colour the more peptide bonds (hence protein) which is present.

Practical techniques

Use of qualitative reagents to identify biological molecules.

DETERMINATION OF WATER POTENTIAL BY MEASURING CHANGES IN MASS OR LENGTH

SPECIFICATION REFERENCE 1.3

CELL MEMBRANES AND TRANSPORT

Introduction

If two solutions of different water potentials are separated by a selectively permeable membrane, water will move into the solution with the lower water potential. The cytoplasm and cell sap are solutions and the tonoplast and cell membrane are selectively permeable. The cell can therefore be considered to be an osmotic system in which a solution is surrounded by a selectively permeable membrane. It will lose or gain water by osmosis depending on the water potential of the adjacent cell or bathing solution. Where there is no change in mass or length the water potential of the bathing solution is equal to that of the tissue.

A tissue sample, such as a cylinder of potato or fragment of leaf, contains millions of cells. If it gains water by osmosis, the mass increases. The cells will stretch by a small amount, until prevented from doing so by the cell wall, and so the length of a cylinder of tissue will increase. The converse is also true – if the tissue sample loses water, its mass decreases and the length of a cylinder of tissue decreases.

Apparatus

Vegetable large enough to extract 50 mm cylinders: potatoes, sweet potatoes, yams, beetroots, swede, turnip, parsnip and carrot are suitable.
Chopping board/ white tile
Cork borers: sizes 3 and 4 are suitable
Ruler graduated in mm
Fine scalpel
Fine forceps
5 x boiling tubes
Boiling tube rack
50 cm^3 measuring cylinder Distilled water
Sodium chloride solutions (0.2, 0.4, 0.6, 0.8 mol dm^{-3})

Method

1. Cut 15 cylinders of tissue, each approximately 50mm long, on the chopping board and use the scalpel to remove any periderm (skin) as its suberin makes it waterproof, and would prevent osmosis.
2. Place 30cm^3 of distilled water or solution into each test tube. Make sure you label each tube.
3. Using the scalpel and forceps, ensure the ends of the cylinder are at 90° to its length.
4. Measure the length of the cylinder to the nearest mm or the mass to the nearest 0.01 g.
5. Using the forceps, place 3 cylinders into each boiling tube.

6. Leave at room temperature for a minimum of 45 minutes, or overnight at 4 °C.
7. Gently blot the cylinders and re-measure the length or re-weigh the cylinders.
8. Record your results in a table.
9. Plot the mean percentage change against the concentration of solution.
10. Estimate the solute potential of the tissue.

Risk Assessment

Hazard	Risk	Control measure
Scalpel blades are sharp	May cut skin when cutting cylinders	Cut away from body onto a white tile
Cork borers are sharp	May cut skin when cutting cylinders	The cylinders of tissue must be cut on the chopping board with the force directed downwards

Technician's Notes

Making solutions – masses per dm^3 are given in the table below. The weighed solutes should be dissolved in a minimum volume of water and then the solution made up to 1 dm^3 with distilled water.

concentration of solution / mol dm^{-3}	mass sodium chloride per dm^3 solution / g
0	0
0.2	11.7
0.4	23.4
0.6	35.1
0.8	46.8

Alternatively, a 1 mol dm^{-3} solution may be made and diluted as needed. To make up a 1 mol dm^{-3} solution 58.5 g of sodium chloride is required.

Sample Results

Concentration of bathing solution mol dm^{-3}	Initial length / mm	Final length / mm	Length change / mm	% length change	Mean % length change
0	52	57	5	10	10
	49	55	6	12	
	50	54	4	8	
0.2	48	49	1	2	3
	50	52	2	4	
	50	51	1	2	
0.4	58	55	-3	-5	-2
	50	48	-2	-4	
	53	54	1	2	
0.6	50	48	-2	-4	-6
	52	48	-4	-8	
	50	47	-3	-6	
0.8	49	41	-8	-16	-13
	50	43	-7	-14	
	52	48	-4	-8	

When there is no change in length, the concentration of the cell contents is equal to that of the bathing solution, which is read at the x-intercept. The equivalent solute potential can be read from the table.

At incipient plasmolysis, $\psi_P = 0$, $\therefore \psi_{cell} = \psi_S$ $\therefore$ this figure gives the water potential of the cells.

Molarity / mol dm^{-3}	Solute potential / kPa
0.05	-130
0.10	-260
0.15	-410
0.20	-540
0.25	-680
0.30	-860
0.35	-970
0.40	-1120
0.45	-1280
0.50	-1450
0.55	-1620
0.60	-1800
0.65	-1980
0.70	-2180
0.75	-2370
0.80	-2580
0.85	-2790
0.90	-3000
0.95	-3250
1.00	-3500

- If cylinders are left for too short a time before re-measuring, results are still valid. The intercept would be the same but less water will have entered or left the cells, giving a smaller gradient. The error in measurement is proportionally greater and the experiment, therefore, less accurate.

- In biology, it is normal to join data points to construct a line on a graph. Where an intercept is to be read, a line/ curve of best fit may be used. This takes into account all the data, rather than only the two points either side of the intercept.

Further Work

- Different plant material may be used to test a hypothesis relating to dissolved sugar concentration and relative sweetness of the vegetables. An example would be to compare the intercepts of using cylinders of potato and sweet potato. It could be hypothesised that the line for sweet potato would intercept the horizontal axis at a higher concentration and lower solute potential because sweet potato has a higher concentration of dissolved sugars.

Practical Techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).

DETERMINATION OF THE SOLUTE POTENTIAL BY MEASURING THE DEGREE OF INCIPIENT PLASMOLYSIS

SPECIFICATION REFERENCE 1.3

CELL MEMBRANES AND TRANSPORT

Introduction

When the water potential of plant tissue and its surroundings are equal, there is no net movement of water in or out of the cell. The cell is neither turgid nor plasmolysed and is at incipient plasmolysis. The cell membrane is withdrawn from the cell wall in places and the cell contents exert no pressure on the cell wall.

In principle, when examined under the microscope, every cell in the tissue would be expected to show its cytoplasm withdrawn in places from the cell wall. Cells, however, show variation in their behaviour. Incipient plasmolysis is the point where plasmolysis just takes place and for practical purposes this is taken to be when half of the cells are plasmolysed and half are not plasmolysed.

The equation describing water potential is $\psi_{cell} = \psi_S + \psi_P$

At incipient plasmolysis, the pressure potential, $\psi_P = 0$

$\therefore \psi_{cell} = \psi_S + 0$

$\therefore \psi cell = \Psi_S$

Thus the water potential of the cells is equal to their solute potential.

The water potential of the cells is also equal to the solute potential of the bathing solution, which is known. Thus, ψ_S of the bathing solution = $\psi_{cell} = \psi_S$.

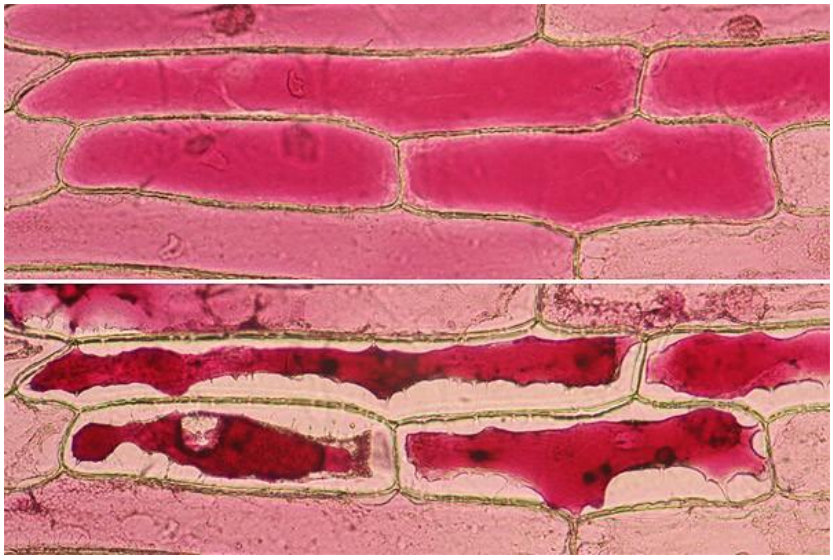
Apparatus

White tile
Fine forceps
Fine scissors
Rhubarb petioles or red onion
5 x 9 cm Petri dishes, 100 cm^3 beakers or watch glasses
Distilled water
Sodium chloride solutions 0.2, 0.4, 0.6, 0.8 mol dm^{-3}. (Instructions for making these solutions is given in the previous experiment.)
Stopclock
Microscope slides
Cover slips
Microscope
Dropping pipettes

Method

1. Set up five labelled Petri dishes/ small bottles each containing 10cm^3 of one of the following solutions: distilled water, 0.2, 0.4, 0.6, 0.8 mol dm^{-3} sodium chloride solution.
2. Insert the fine forceps' tip just under the upper epidermis of the onion leaf.
3. Keeping the forceps handles parallel with the epidermis, so as not to penetrate the underlying mesophyll, grip the epidermis and, maintaining the tension in the tissue, pull the epidermis off the mesophyll, away from you and place into distilled water.
4. When several square centimetres of epidermis have been peeled, place one square into each labelled petri dish/small bottle.
5. Leave at room temperature for a minimum of 30 minutes.
6. Carefully spread the tissue out on a microscope slide, so that it is not folded. Take a scalpel and rock the blade backwards and forwards over the tissue in order to cut out a 0.5 x 0.5 cm square.
7. Add two drops of bathing solution and apply a cover slip.
8. If any solution exudes from the cover slip, blot it with filter paper to dry the slide.
9. Using a x10 and then a x40 objective lens, examine all the cells in a field of view and count the number that are turgid and the number plasmolysed.
10. Repeat the counts at all concentrations of bathing solution.
11. Record your results in a table.
12. Plot a graph of % cells plasmolysed against the concentration of the bathing solution.
13. Using the graph, read the concentration of bathing solution that would produce plasmolysis in 50 % of the cells.
14. From the table given in the previous experiment, determine the solute potential of this solution. This is equal to the solute potential of the cells.

The photograph below shows the appearance of turgid and plasmolysed cells.



Turgid Cells

Plasmolyed Cells

Risk Assessment

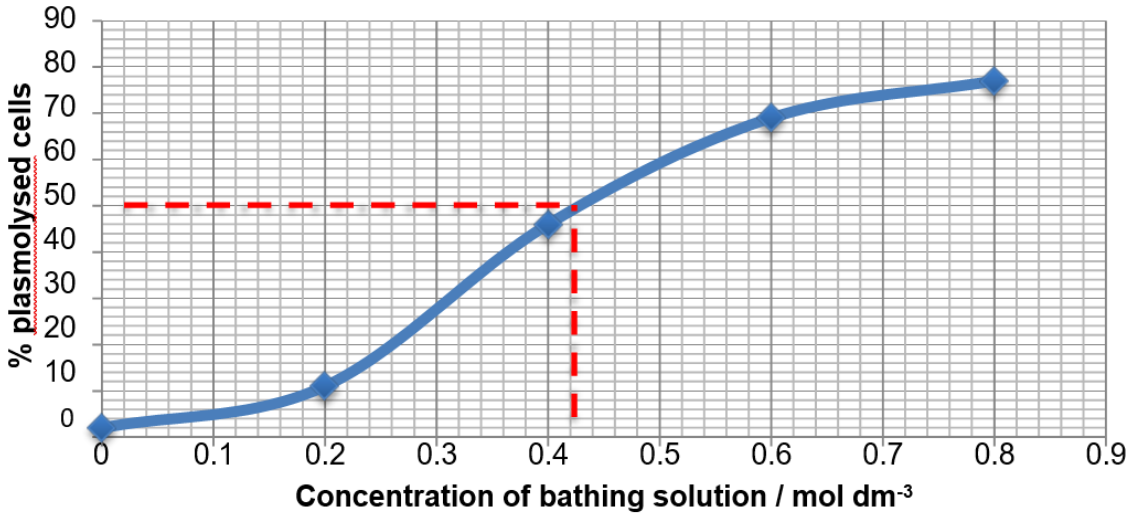
Hazard	Risk	Control measure
Scalpel blades are sharp	May cut skin when cutting cylinders	Cut away from body onto a white tile

Teacher / Technician's Notes

- To ensure that the same cell is not counted more than once, a system of tracking across the specimen may be used. The slide is always moved in the same direction across the field of view and successive fields of view are always separated from the previous one by the width of at least one cell.
- Cells occur on the edge of the field of view and are only partially visible. A rule should be made regarding their scoring, for example, a partially visible cell is never counted or a cell is scored if it is at least half visible.
- In biology, it is normal to join data points to construct a line on a graph. In this situation, a curve of best fit may be used. This takes into account all the data, rather than only the two data points either side of that representing 50% plasmolysis.

Sample Results

Concentration of bathing solution / mol dm^{-3}	Number of cells in field of view						Total		
	1		2		3				
	plasmolysed	turgid	plasmolysed	turgid	plasmolysed	turgid	plasmolysed	turgid	% cells plasmolysed
0	1	30	0	32	1	36	2	98	2
0.2	4	28	2	32	5	29	11	89	11
0.4	20	15	10	19	16	12	46	54	46
0.6	20	9	25	11	24	11	69	31	69
0.8	19	7	34	8	24	9	77	23	77



The solute potential of the equivalent concentration may be read from the table given in Experiment 4.

Alternatively, the % plasmolysed cells can be plotted against the solute potential, so that the solute potential of the cells may be read directly from the graph.

Further work

Make a scientific drawing of turgid and plasmolysed cells, labelled to show

- Cell wall
- Cytoplasm
- Position of cell membrane (it is too thin to actually see but the boundary of the cytoplasm marks its position)
- Vacuole
- Nucleus
- Position of bathing solution

Practical techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).

INVESTIGATION INTO THE PERMEABILITY OF CELL MEMBRANES USING BEETROOT

SPECIFICATION REFERENCE 1.3

CELL MEMBRANES AND TRANSPORT

Introduction

Cell membranes are fluid structures which control the exit and entry of materials into cells. Some substances cross the membrane through proteins, others diffuse through the phospholipid layer. The molecules within the membrane are constantly moving and their movement is greatly affected by temperature. Heating the membrane can cause gaps to form between the phospholipid molecules and the membrane will become more permeable. The protein in the membrane can be denatured by heat.

Beetroot cells contain betalain, a bright red, water soluble pigment, in the cell vacuoles. If the cell membranes are damaged the pigment can escape from the cells and can be detected in an aqueous medium around the tissue. Beetroot grows in soil at a temperature of between 10 – 15 °C.

Apparatus

Beetroot cylinders
White tile
10 test tubes
Scalpel
250 cm^3 beaker
Forceps
Water baths at (25, 35,45,55,65 °C)
Thermometer
Stop clock
Colorimeter with a blue filter / colour chart

Method

1. Cut 5 pieces of beetroot, 1 cm long, from the cylinders provided.
2. Wash under running water to remove the pigment released from cells during cutting.
3. Place a test tube containing 5 cm^3 of distilled water into each water bath to equilibrate for 5 minutes.
4. Place 1 piece of beetroot into each test tube for 30 minutes.
5. After 30 minutes, shake the test tubes gently to make sure any pigment is well-mixed into the water, then remove the beetroot cores.
6. Describe the depth of colour in each test tube. A piece of white card behind the tubes will make this easier to see. Arrange the tubes in order of temperature of the water bath.
7. If you have access to a colorimeter, set it to respond to a blue/ green filter (or wavelength of 530 nm) and to measure absorbance. Check the colorimeter reading for distilled water.
8. Measure the absorbance/percentage transmission of each tube and plot a graph of absorbance/percentage transmission against temperature.

Risk Assessment

Hazard	Risk	Control measure
Scalpels are sharp	Could cut skin when cutting cylinders	Cut away from body onto white tile

Teacher / Technicians' Notes

Beetroot must be raw, not cooked. Use a size 4 cork borer and cut with care using a cutting board. Cut enough cores to make eight 2 cm lengths per working group. Leave the cores overnight in a beaker of distilled water. The pigment from any cells that have been cut by the cork borer will leak into the water. Rinse away any pigmented water in the morning and replace with fresh water.

Each student should be given cylinders of the same diameter (5 – 8 mm. diameter) and enough to be able to cut 5 x 1 cm cylinders.

Thermostatically controlled water baths could be set up at the required temperatures or students could be given equipment to enable them to make their own water baths (tripod, gauze, beaker, Bunsen burner and thermometer).

If students are using a colorimeter it would be an advantage to have a simple set of printed instructions by the instrument.

Further details are available from:

http://www.nuffieldfoundation.org/practical-biology/investigating-effect-temperature-plant- cell-membranes

Sample Results

Here is a sample of results obtained with a colorimeter – measuring transmission of light at 530 nm (rather than absorbance).

Temperature (°C)	Observation	Colorimeter reading (% transmission of light)			
		Repeat 1	Repeat 2	Repeat 3	Mean
0	Clear	100	98.5	99.0	99.2
22	Very pale pink	93.9	95.0	96.0	95.0
42	Very pale pink	80.1	77.0	76.9	78.0
63	Pink	26.3	29.9	31.0	29.1
87	Dark pink	0.7	0.7	1.0	0.8
93	Red	0.0	0.1	0.0	0.0

Further work

- Students could extend their investigation by finding the effect of alcohol or detergents on membrane permeability.
- The procedure allows for students to identify systematic and random variables. It is a good opportunity to practice graphical treatment of results, including range bars to assess the variation in repeats.

Practical techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).
- Use appropriate instrumentation to record quantitative measurements, such as a colorimeter or potometer.

INVESTIGATION INTO THE SEPARATION OF CHLOROPLAST PIGMENTS BY CHROMATOGRAPHY

SPECIFICATION REFERENCE 3.2

PHOTOSYNTHESIS USES LIGHT ENERGY TO SYNTHESIS ORGANIC MOLECULES

Introduction

Chloroplast pigments, located on the membranes of the thylakoids and grana, harvest light in the light-dependent reactions of photosynthesis, and transfer its energy into the light- independent reactions, in the synthesis of complex organic molecules.

In flowering plants, there are two major groups of chloroplast pigments:

- The chlorophylls
  - Chlorophyll *a* is the commonest and is found in all photosynthetic organisms studied.
  - Chlorophyll *b* is found in flowering plants.
  - Phaeophytin, a breakdown product of chlorophyll *a* molecule, lacking the central magnesium ion is seen in flowering plants and in purple sulphur bacteria.

- Carotenoids comprise
  - Carotenes: α- and β-carotene are orange but lycopene, found in tomatoes, is bright red.
  - Xanthophylls, such as lutein and zeaxanthin, appear yellow.

Chloroplast pigments will be extracted from the leaves of an angiosperm, separated by chromatography and identified. The distance each pigment travels on the chromatogram depends on

- its solubility in the solvent – more soluble pigments travel further
- its absorption by the paper or silica gel – pigments that are absorbed less travel further

Apparatus

Dark green leaves e.g. spinach, stinging nettle
Chromatography paper or silica gel chromatography plates
Scissors
Pencil
Sand
Ruler
Pestle and Mortar
Capillary tube
Hair dryer
Propanone
2 x Boiling tube
Distilled water
2 x Stopper
Pipette
Petroleum ether
Vial

Method

Preparing the pigment solution

1. Chop 2g of the leaf material finely with scissors and place in the mortar.
2. Add a pinch of sand and 5 cm^3 of propanone.
3. Grind the leaf fragments to a slurry.
4. Place slurry in a boiling tube.
5. Add 3 cm^3 distilled water, shake vigorously and stand for 8 minutes.
6. Add 3cm^3 petroleum ether mix by gentle shaking and allow layers to separate.
7. Collect the upper, petroleum ether layer, which contains the chloroplast pigments, in a pipette and transfer to a vial.

Preparing the chromatography paper

1. Draw a pencil line across the chromatography paper or plate approximately 2 cm from one end.
2. Draw chloroplast pigment solution into a capillary tube and put a small spot in the centre of the pencil line. Ensure that the capillary tube does not pierce or tear the chromatography paper.
3. Dry the spot as quickly as possible, preventing its spread.
4. Repeat steps 2 and 3 until there is a small but intense spot of pigment.

Running the chromatogram

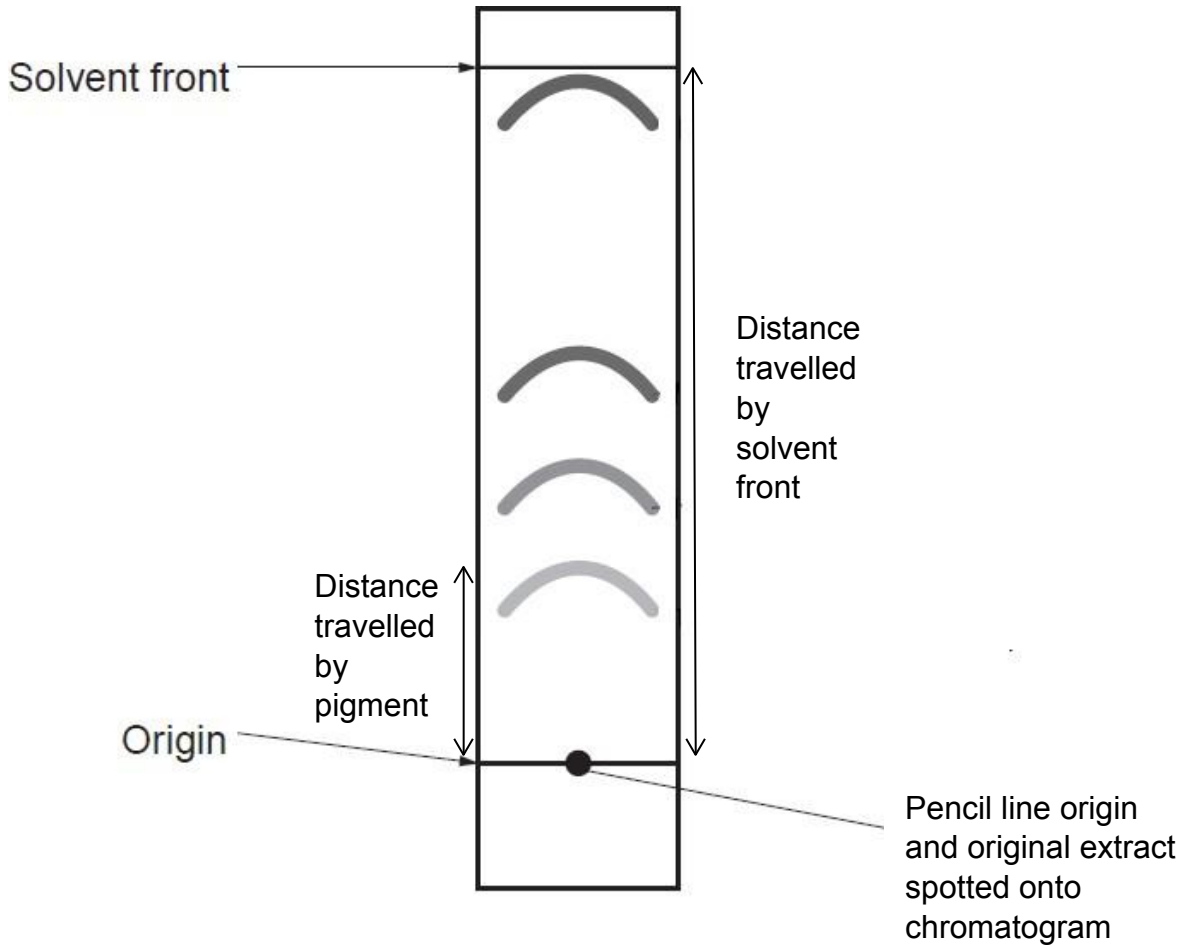
1. Place freshly-made 1:2 propanone : petroleum ether solvent mixture in a boiling tube until it is approximately 5 mm deep.
2. Without touching the sides of the boiling tube, slide the chromatography paper into the boiling tube so that its end is below the surface of the ethanol but the spot is above, and not touching it.
3. Hold the chromatography paper in place with the stopper, folding the paper over the rim of the boiling tube at the top.
4. Leave the boiling tube ensuring that it is not moved, until the solvent has climbed up the paper to within 10 mm of the top.
5. Remove the chromatography paper from the boiling tube and immediately, draw a pencil line across the paper to mark the solvent front.
6. Mark the position of the top of each pigment spot with a pencil.

Identifying the pigments

1. Use a ruler to measure the distance from the origin to the solvent front.
2. Measure the distance from the origin to the top of each pigment spot.
3. Calculate R for each pigment, where $R_f = \frac{\text{distance travelled by pigment}}{\text{distance travelled by solvent front}}$

4. Published data allow the pigments to be identified as R_f is constant for each pigment in a solvent. The table here shows data for separation in 1:2 propanone: petroleum ether.

Spot colour	Pigment	R_f
yellow	ß-carotene	0.96
grey	phaeophytin	0.70
blue-green	chlorophyll *a*	0.58
green	chlorophyll *b*	0.48
yellow-brown	xanthophyll	0.44 (TLC) 0.75 (paper chromatography)



Risk Assessment

Hazard		Risk	Control measure
Propanone Petroleum ether	May cause eye damage	Macerating leaf material; Pouring solvent for chromatography	Eye protection
	May degrease the skin	Macerating leaf material; Pouring solvent for chromatography	Wear gloves
	Inhalation may exacerbate respiratory problems, including asthma	Macerating leaf material; Pouring solvent for chromatography	Work in fume cupboard
	Fire hazards	Accidental ignition	Work in fume cupboard

Teacher / Technician's notes

This method uses chromatography paper. The method in the link below gives simple instructions for the use of TLC. Either is acceptable.

http://www.saps.org.uk/secondary/teaching-resources/189-investigation-of- photosynthetic-pigments-in-green-plants

Further Work

- Use different solvents or solvent proportions.
- Compare pigments in young and senescent deciduous leaves e.g. *Acer* (sycamore, maple).
- Separate pigments in coloured leaves e.g. *Coleus.*
- Compare the pigments in sun and shade leaves of e.g. *Quercus robur* (English oak), *Urtica dioica* (stinging nettle).

Practical techniques

- Separate biological compounds using thin layer/ paper chromatography or electrophoresis.

INVESTIGATION INTO THE EFFECT OF LIGHT ON THE RATE OF PHOTOSYNTHESIS

SPECIFICATION REFERENCE 3.2

PHOTOSYNTHESIS USES LIGHT ENERGY TO SYNTHESIS ORGANIC MOLECULES

Introduction

Photosynthesis can be summarised as:

$$\text{carbon dioxide + water} \xrightarrow{\text{Light}} \text{glucose + oxygen}$$

To measure its rate, in principle, the mass of carbon dioxide or water used, the light energy absorbed or the mass of sugar or oxygen produced could be assessed. But in practice, an easier method involves assessing the colour change in a pH indicator. pH increases when carbon dioxide is absorbed from solution in the photosynthesis of aquatic organisms, i.e. becomes less acidic.

When carbon dioxide dissolves in water the carbonic acid produced dissociates releasing hydrogen ions, which lower the pH of water:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

As photosynthesis removes carbon dioxide from the solution, the concentration of hydrogen ions decreases and so the pH increases. This can be visualised by noting the colour change of hydrogen carbonate indicator:

$$\underset{\text{Yellow}}{\text{Acid}} \rightleftharpoons \underset{\text{Red}}{\text{Neutral}} \rightleftharpoons \underset{\text{Purple}}{\text{Alkali}}$$

Scenedesmus quadricauda, a photosynthetic protoctistan, immobilised in alginate beads, is a suitable experimental material. If it turns the indicator purple, its rate of photosynthesis exceeds its rate of respiration; if it turns yellow its respiration exceeds its photosynthesis; if it remains red, photosynthesis and respiration are occurring at equal rates and the algae are at the compensation point.

Apparatus

For making algal balls

5 cm^3 *Scenedesmus quadricauda* culture
Glass rod
3 cm^3 sodium alginate solution (3 %)
Beakers
10 cm^3 syringe without needle
Distilled water
200 cm^3 calcium chloride (2 g /100 cm^3)
Tea strainer

For running the experiment

Algal balls
Glass vial + stopper 10 cm^3
Hydrogen carbonate indicator
Colour chart for indicator (*School Science Review* **85** (312) 37–45) or colorimeter with 550 nm filter
Metre ruler
Fluorescent lamp
Timer

Method

Making algal balls

1. Stir a mixture of 5 cm^3 *Scenedesmus* culture and 3 cm^3 3 % sodium alginate solution gently with the glass rod until they are well mixed.
2. Draw the mixture into a 10 cm^3 syringe barrel.
3. With constant pressure on the plunger, drop the mixture, one drop at a time, into 200 cm^3 calcium chloride solution.
4. Leave the balls for 20 minutes.
5. Strain the balls through the tea strainer.
6. Return the balls to the beaker and swirl them in distilled water.
7. Repeat steps 5 and 6 twice more.
8. Use immediately or store at 4 °C, but bring to room temperature for approximately 20 minutes before use.

Running the experiment

1. Place 20 algal balls in a vial.
2. Add 10 cm^3 hydrogen carbonate indicator.
3. Place the vials at a distance from a light source.
4. After a given time assess the pH of the indicator in the vial using the colour chart or read its absorbance at 550 nm in a colorimeter.

Risk assessment

Hazard	Risk	Control measure
Solid calcium chloride is an irritant to skin and eyes and if inhaled	Making calcium chloride solution	Solid to be weighed in fume cupboard; Students to be given solution.
Gas accumulation in culture vessel could cause the glass to break	During period of algal culture	Ensure cotton wool stopper allows ventilation; Stand culture vessel in deep tray
Excess heat from lamp may cause burns	When decanting from culture vessel	Ensure no contact with skin

Teacher/ Technician's notes

Growing your alga: Prepare a culture of green alga such as unicellular *Scenedesmus quadricauda*. Make up a solution of algal enrichment medium, and subculture the alga into this. Aerate gently and keep at temperatures between 18–22 °C. Constant illumination ensures faster growth of the alga. After 3–4 weeks, the culture should have a green 'pea soup' colour. Subculture the alga again to maintain a healthy culture. You could use other algae, but *Scenedesmus* should produce 2 to 3 litres of dark green 'soup' in about 4 weeks from 50 cm^3 of original culture. (Details from SAPS Sheet 23).

Preparing solutions to make alginate beads (Refer to Recipe card 2):

- Dissolve 3 g of sodium alginate in 100 cm^3 of cold, pure water. Stir with a spatula every half hour or so. Leave overnight and stir in the morning.
- Dissolve 4 g of calcium chloride-6-water in 200 cm^3 of pure water in a 250 cm^3 beaker.

Hydrogencarbonate indicator: Refer to Recipe card 34 and Hazcard 32. Low hazard once made; must be made fresh by qualified staff using fume cupboard. The indicator is very sensitive to changes in pH, so rinse all apparatus with the indicator before use. Avoid exhaling over open containers of the indicator. Make up a 'standard colour scale' of reaction bottles containing buffers from pH 7.6 - pH 9.2 with hydrogencarbonate indicator if students will not have access to a colorimeter.

Lamps: You need a brighter light than a standard 40 W or 60 W bench light. Low energy bulbs produce too limited a spectrum of light for full activity. 150 W tungsten or halogen lamps are best. 150 W portable halogen lamps have a stand and handle separate from the body of the lamp which makes them safer to handle. But they do produce heat, so you will need a heat filter for the investigation.

Heat filter: Use a large flat-sided glass vase or a medical 'flat' filled with water. With a high power lamp, the small volume in a medical 'flat' may get too hot for comfort.

Making alginate beads:

- When making up the alginate or diluting the algal culture it is essential to use pure water; otherwise calcium ions in the water will cause the alginate to 'set' prematurely.

Students can run all distances from the lamp at the same time if they 'fan' the vials out in front of the light source.

It takes at least an hour for colour changes to happen – so students will need to return to the lab at break or after lessons to 'read' the results.

More details are available on the link below:

http://www.nuffieldfoundation.org/practical-biology/investigating-photosynthesis-using-immobilised-algae

Sample results

Distance of vial from lamp / cm	Colour of indicator after 1 hour	Absorbance after 1 hour / a.u.	pH after 1 hour
10	purple	0.92	9.0
30	magenta	0.81	8.8
50	red	0.56	8.4
70	orange	0.39	8.2
90	yellow	0.25	8.0

Plot the pH or the absorbance against the distance from the lamp source.
As the light intensity falls in proportion to $\frac{1}{d^2}$, the pH or absorbance could be plotted against $\frac{1}{d^2}$.

Further work

- More than one vial at each distance should be used so that a mean can be calculated from replicate readings.
- Instead of placing vials at different distances, light intensity may be varied by covering the vials with neutral density filters and maintaining the same distance from the lamp. In this way, any potential heating effect has a consistent effect.
- Vials may be covered with coloured filters to expose the algae to different wavelengths. In order that the algae receive the same light intensity, readings must be taken with a light meter with the filters over the probe, to find distance for each wavelength which has the same light intensity, where the vials should be placed.

Practical techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).
- Use appropriate instrumentation to record quantitative measurements, such as a colorimeter or photometer.

INVESTIGATION INTO THE ROLE OF NITROGEN AND MAGNESIUM IN PLANT GROWTH

SPECIFICATION REFERENCE 3.2

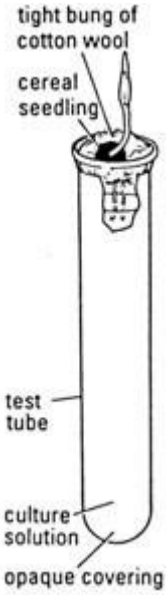
PHOTOSYNTHESIS USES LIGHT ENERGY TO SYNTHESIS ORGANIC MOLECULES

Introduction

Plants require nitrogen in the form of nitrate ions absorbed by the roots to make amino acids, chlorophyll and nucleotides. Magnesium ions are also absorbed by the roots of a plant and are required by the plant as a component of chlorophyll. The effect of a lack of nitrate ions or magnesium ions on plant growth can be demonstrated by comparing seeds grown in a culture solution containing all of the plants essential nutrients with seedlings grown in a culture solution lacking nitrate and also to seedlings grown in a culture solution lacking magnesium.

Apparatus and chemicals

Sach's complete water culture solution
Sach's water culture solution lacking nitrate ions
Sach's water culture solution lacking magnesium ions
Test tubes (1 per culture solution)
Cotton wool
Aluminium foil
Dropping pipette
Germinated barley seedlings



Method

1. Select equal size barley seedlings.
2. Set up 5 test tubes, as shown in the diagram, with Sach's complete culture solution.
3. Set up another 5 test tubes with Sach's culture solution lacking nitrate.
4. Set up a further 5 test tubes with Sach's culture solution lacking magnesium.
5. Solutions should be topped up when necessary and completely replaced weekly.
6. All 15 barley seedlings should be placed in the same conditions for example light and temperature.
7. After a month examine the seedlings, record any differences between them and measure the length of the roots and shoot.
8. Dry the seedlings in an oven and record the dry mass.

Risk assessment

Hazard	Risk	Control measure
Sach's culture solution can be an irritant	Very low risk of irritation to eye or skin.	Wear goggles. Wash skin thoroughly if solution comes into contact with skin.

Teacher/Technician's notes

It is advised that this is set up as a class demonstration.

Solid media for Sach's water culture solutions are available from laboratory suppliers. It can be cheaper, and certainly easier to buy the ready-prepared nutrient solutions.

Sach's culture solution

Dissolve the following in 1 dm^3 of distilled water.
0.25 g calcium sulphate (VI)-2-water
0.25 g calcium phosphate (V)-2-water $CaH_4(PO_4)_2.2H_2O$
0.25 g magnesium sulphate (VI)-7-water
0.8 g sodium chloride
0.7 g potassium nitrate
0.005 g iron(III)chloride-6-water.
For Sach's culture solution with nitrogen deficiency replace potassium nitrate(V) with 0.52 g potassium nitrate.
For Sach's culture solution with magnesium deficiency replace magnesium sulphate (VI) with 0.17g potassium sulphate (VI)

The barley seeds should be germinated about a week before use in a layer of damp vermiculite in a margarine tub.

There are a variety of alternative seedlings which can be used such as tomato. More information is available on the link below:

http://www.nuffieldfoundation.org/practical-biology/investigating-effect-minerals-plant-growth

Sample results

Lacking in nitrogen - poor growth, plants short and spindly and chlorosis (yellowing) especially in the older leaves. The young leaves at the tip may be green but small.

Lacking in magnesium - interveinal chlorosis.

Practical techniques

- safely and ethically use organisms to measure
    - plant or animal responses
    - physiological functions
- use ICT such as computer modelling, or data logger to collect data, or use software to process data.

DISSECTION OF A MAMMALIAN KIDNEY

SPECIFICATION REFERENCE 3.7

HOMEOSTATIS AND THE KIDNEY

Introduction

The urinary system consists of two kidneys, two ureters, the bladder and urethra. The kidney's functions are to remove nitrogenous wastes, such as urea from the body and to maintain the water content, and thus the pH and ion balance of the blood. The wastes constitute urine, which moves from the kidneys, through the ureters to the bladder, from where it leaves the body through the urethra.

By dissecting a mammalian kidney, you will recognise aspects of the gross structure e.g. the cortex and medulla, pyramids and pelvis, and will understand how collecting ducts join to drain urine into the ureter.

Lambs' kidneys from the butcher are suitable to show the kidney's anatomy.

Apparatus

Kidney
Chopping board
Scalpel
Scissors
Forceps
Lens on a stand

Method

These instructions are written for people who are right-handed. If you are left-handed, you may wish to work with left and right reversed.

1. Observe the outside of the kidney.
    - Note if it is covered in fat. Mammals tend to lay down fat around internal organs and fat around the kidney is common.
    - Note if blood vessels and the ureter are attached to the kidney, and note the connective tissue that sheaths and binds them. They emerge at a recess in the kidney called the hilum.
    - Note that the kidney, like all other organs, is covered in a thin membrane. This is the tough and fibrous renal capsule.
    - Organs bought from a butcher may have approximately 1 cm deep slashes which show that they have been examined by meat inspectors, as is required by law.
2. Remove the fat from the outside of the kidney. It can be removed by hand.
3. Place the kidney flat on the chopping board with the hilum on the right hand side.

4. Keeping the blade of a fine scalpel horizontal, pierce the kidney on the right hand side in of the hilum, and make repeated small cuts, bringing the blade towards you each time. Rotate the kidney anti-clockwise after every few cuts, so that you cut right round the organ.

5. Extend the cuts through to the centre so that the kidney can be separated into two halves.

6. Note that the cortex is red-brown and that the medulla is deeper red. Note the pyramids and the pelvis. Note that the pelvis extends into the ureter.

7. Using one half of the kidney, place on the chopping board so that the place where the ureter emerges from the kidney is towards you. With forceps in your left hand, lift the connective tissue and cut through it with fine scissors, away from you, to uncover tubules leading into the pelvis.

8. Continue cutting towards the cortex, exposing finer tubules. More can be seen if the dissection is done through a lens on a stand. The tubules are continuous with the microscopic collecting ducts of the nephrons.

9. Further detail can be observed by taking a small sample from the cortex and medulla and crushing them under a coverslip. The kidney tubules can be seen using the x10 objective lens.

Risk assessment

Hazard	Risk	Control measure
Dissecting instruments are sharp	Can pierce or cut the skin	Care with use

Teacher/ Technician's notes

Safety of handling butcher material: Anything that a butcher sells must have been passed as ‘fit for human consumption’, but may be carrying food-poisoning bacteria. It is not necessary to wear gloves during the dissection (although the students will probably be happier doing so), but it is necessary to wash hands thoroughly after handling the material and before leaving the laboratory.

Cleaning equipment and work surfaces: Dissecting instruments are best autoclaved after use because most disinfectants attack metal instruments. Clean contaminated equipment such as dissecting boards with hot water and detergent and soak for at least 10 minutes in a freshly-prepared 1% solution of Virkon disinfectant. Wipe down working surfaces with 1% Virkon, and leave wet for 10 minutes before drying off. If you perform dissections on layers of newspaper you may protect the bench surface enough for it not to need to be disinfected.

A virtual kidney dissection is available on the link below:

http://www.bristol.ac.uk/anatomy/media/elearning/internet/letsdissect/letsdissectkidneytutor/index.html

Students should be encouraged to make records of each stage of their dissection either by drawings or photographs which can then be annotated.

Practical techniques

- Safely use instruments for dissection of an animal organ, or plant organs
- Produce scientific drawing from observation with annotations.

INVESTIGATION OF A CONTINUOUS VARIATION IN A SPECIES

SPECIFICATION REFERENCE 4.4

INHERITANCE

Introduction

Polygenic characters often show continuous variation, which can be demonstrated by plotting a frequency histogram, producing an approximately normal curve. Counts or measurements of samples are made and if their distribution is approximately normal, their means may be compared using Student's t test.

Apparatus

Ruler in mm
15 ivy leaves from each of two contrasting sites e.g. growing in bright sun and growing in the shade.

Method

1. Measure the maximum width of each leaf.
2. Calculate the mean width of each sample.
3. Plot frequency histograms for the two samples, to determine whether they are approximately normally distributed.
4. Calculate the standard deviation for each sample

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

5. Test that the means are different using Student's t test.

$$t = \frac{\left|\bar{x}^1 - \bar{x}^2\right|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

Where,

$\left|\bar{x}^1 - \bar{x}^2\right|$ = the difference in mean values of sample 2 and sample 2

s_1^2 and s_2^2 are the squares of the standard deviation of the samples

n_1 *and* n_2 are the number of readings in each sample.

Sample 1	Width (mm)	Deviation from the mean $(x - \bar{x})$	Deviation squared $(x - \bar{x})^2$
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
Mean			$\Sigma =$
Sample 2			
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
Mean			$\Sigma =$

Degrees of freedom	p= 0.1	p= 0.05	p = 0.02	p = 0.01	p = 0.002	p = 0.001
1	6.314	12.706	31.821	63.657	318.310	636.620
2	2.920	4.3030	6.965	9.925	22.327	31.598
3	2.353	3.182	4.541	5.841	10.214	12.924
4	2.132	2.776	3.747	4.604	7.173	8.610
5	2.015	2.571	3.365	4.032	5.893	6.869
6	1.943	2.447	3.143	3.707	5.208	5.959
7	1.895	2.365	2.998	3.499	4.785	5.408
8	1.860	2.306	2.896	3.355	4.501	5.041
9	1.833	2.262	2.821	3.250	4.297	4.781
10	1.812	2.228	2.764	3.169	4.144	4.587
11	1.796	2.201	2.718	3.106	4.025	4.437
12	1.782	2.179	2.681	3.055	3.930	4.318
13	1.771	2.160	2.650	3.012	3.852	4.221
14	1.761	2.145	2.624	2.977	3.787	4.140
15	1.753	2.131	2.602	2.947	3.733	4.073
16	1.746	2.120	2.583	2.921	3.686	4.015
17	1.740	2.110	2.567	2.898	3.646	3.965
18	1.734	2101	2.552	2.878	3.610	3.922
19	1.729	2.093	2.539	2.861	3.579	3.883
20	1.725	2.086	2.528	2.845	3.552	3.850
21	1.721	2.080	2.518	2.831	3.527	3.819
22	1.717	2.074	2.508	2.819	3.505	3.792
23	1.714	2.069	2.500	2.807	3.485	3.767
24	1.711	2.064	2.492	2.797	3.467	3.745
25	1.708	2.060	2.485	2.787	3.450	3.725
26	1.706	2.056	2.479	2.779	3.435	3.707
27	1.703	2.052	2.473	2.771	3.421	3.690
28	1.701	2.048	4.467	2.763	3.408	3.674
29	1.699	2.045	2.462	2.756	3.396	3.659
30	1.697	2.042	2.457	2.750	3.385	3.646
40	1.684	2.021	2.423	2.704	3.307	3.551
60	1.671	2.000	2.390	2.660	3.232	3.460
120	1.658	1.980	2.358	2.617	3.160	3.373
∞	1.645	1.960	2.326	2.576	3.090	3.291

Risk assessment

Hazard	Risk	Control measure
Ivy leaves may be allergenic and generate contact dermatitis	Skin contact on handling may occur	Avoid skin contact
Berries are toxic	If leaves are collected in autumn, berries may be present	Avoid ingesting berries

Teacher / Technician's notes

Make sure the maximum width of each leaf is measured. ICT packages can be used to manipulate the data, but students must understand each step in the statistical process.

Sample data

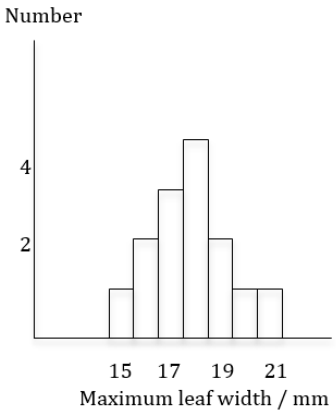
Maximum width of ivy leaf (mm) grown in	
shade	sun
17	7
16	11
18	8
21	8
19	9
20	10
17	9
19	10
18	9
17	10
18	10
18	11
16	12
15	18
18	10
mean = 17.8	mean = 10.1

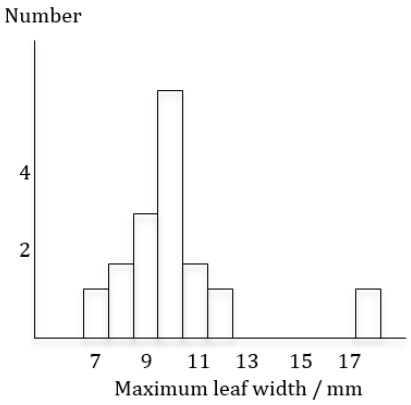
The data can be placed in size classes and frequency histograms plotted. In this example, a size class of 1 mm is suitable.

Maximum width of ground ivy leaf (mm) growing in shade	
15	1
16	2
17	3
18	4
19	2
20	1
21	1

Maximum width of ground ivy leaf (mm) growing in sun	
7	1
8	2
9	3
10	5
11	2
12	1
18	1

The frequency histograms show distributions that are approximately normal and so that data can be analysed with a Student t test.





The Student t test

This test can be used as the sample sizes are suitable (15-30) and the data are approximately normally distributed.

1. Formulate a null hypothesis: there is no significant difference between the mean maximum widths of the two populations of ground ivy leaves growing in the sun and shade.
2. Calculate the standard deviation, s.
   - (i) First calculate the differences from the mean. As a check on your arithmetic, remember that, allowing for rounding errors, the sum of these deviations from the mean, $(\bar{x} - x) = 0$.
   - (ii) Then square those values and add them.
   - (iii) Then divide by the number in the sample -1.
   - (iv) Then find the square root of this figure.

Ivy leaves growing in shade			Ivy leaves growing in the sun		
Maximum width / mm	Deviation from mean $(\bar{x} - x)$	Deviation from mean^2 $(\bar{x} - x)^2$	Maximum width / mm	Deviation from mean $(\bar{x} - x)$	Deviation from mean^2 $(\bar{x} - x)^2$
17	0.8	0.64	7	3.1	9.61
16	1.8	3.24	11	-0.9	0.81
18	-0.2	0.04	8	2.1	4.41
21	-3.2	10.24	8	2.1	4.41
19	-1.2	1.44	9	1.1	1.21
20	-2.2	4.84	10	0.1	0.01
17	0.8	0.64	9	1.1	1.21
19	-1.2	1.44	10	0.1	0.01
18	-0.2	0.04	9	1.1	1.21
17	0.8	0.64	10	0.1	0.01
18	-0.2	0.04	10	0.1	0.01
18	-0.2	0.04	11	-0.9	0.81
16	1.8	3.24	12	-1.9	3.61
15	2.8	7.84	18	-7.9	62.41
18	-0.2	0.04	10	0.1	0.01
mean = 17.8		sum = 34.4	mean = 10.1		sum = 89.75
		s_{shade} = 1.57			s_{sun} = 2.53

3. Calculate the test statistic, t, using the standard deviation and the mean:

In this example, t is calculated using the equation $t = \frac{|\bar{x}_{shade} - \bar{x}_{sun}|}{\sqrt{\frac{s^2_{shade}}{n} + \frac{s^2_{sun}}{n}}}$

where $\bar{x}_{shade}$ = mean reading for ivy growing in the shade
$\bar{x}_{sun}$ = mean reading for ivy growing in the sun
s^2_{shade} = for ivy growing in the shade
s^2_{sun} = for ivy growing in the sun n = number in sample
n = number in sample

Substituting into the equation $t = \frac{|17.8 - 10.1|}{\sqrt{\frac{6.41}{15} + \frac{2.46}{15}}} = \frac{7.7}{0.77} = 10.0$

4. For n = 15 in each sample, the number of degrees of freedom (df) = (15-1) + (15-1) = 28.
5. For df = 28 and level of significance, p = 0.05, the critical value of t = 2.048
6. Comparing the critical value with the calculated value: the calculated value is greater than the critical value so the null hypothesis is rejected at the 5% level of significance, or p = 0.05.
7. Formulating the conclusion: the maximum mean widths of the two populations are significantly different at the 5% level of probability. The maximum width of ivy leaves is greater growing in the shade than growing in the sun.

Further work

The table below shows other suitable examples of continuous variation, of which the means may be tested with the t test.

Location	Example of character	Example of comparison to be tested	Notes
In school	length of beans	lengths of beans can be compared e.g. broad beans with kidney beans	
	student height	heights of Year 7 and Y13 students	use either all girls or all boys
		heights of Year 13 students	compare mean height of girls with boys
Coast	length of periwinkle	lengths of periwinkles growing at high water mark compared with those at low water mark	slipping is a hazard
	length of marram grass leaves	compare leaf length in grey dunes with yellow dunes	
Woodland	height of woodsage or soft rush	height of plants growing in high light intensity compared with low light intensity	

Practical techniques

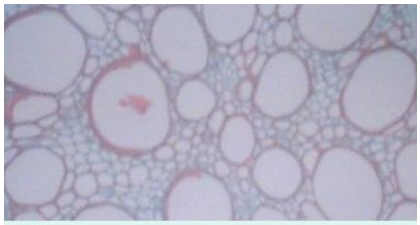
- Use sampling techniques in fieldwork
- Use ICT such as computer modelling, or data logger to collect data, or use software to process data.

MAGNIFICATION, ACTUAL SIZE AND MICROSCOPY

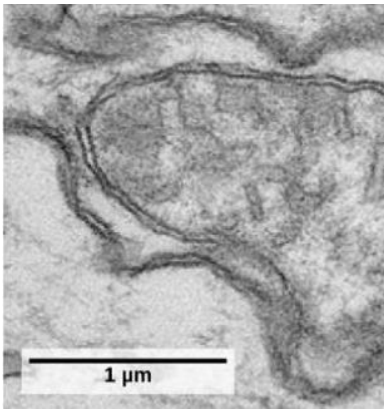
This resource covers practical skills you need to learn and use when dealing with actual size, image size and the magnification of images. These skills will be used in nearly all units at AS and A2.

MAGNIFICATION and ACTUAL SIZE

Drawings and images are often shown with their **magnification** given next to the image or stated in a caption or in the text.



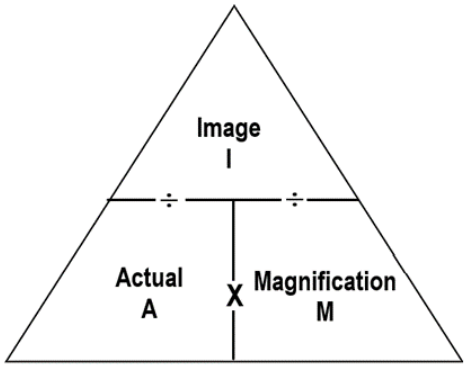
Dicotyledonous root [ts] x200



Sometimes the magnification is shown as a **scale bar** where a line is drawn to represent a certain length.

The information provided can be used to calculate the **actual size** of an object using the relationship

magnification = image size / actual size

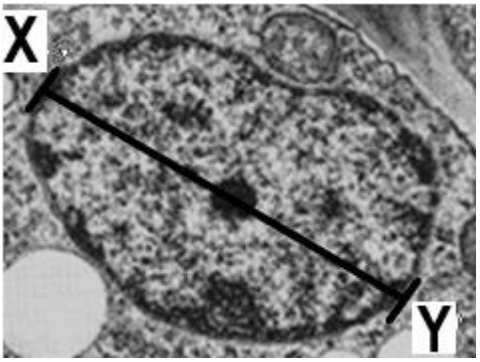


You need to be able to use this relationship to calculate any of the three values.

Actual size and Magnification

Calculating actual size:

Look at the electron micrograph of a nucleus.
This image is printed at a **magnification** of **x 10 000**,
ie., it is 10 000 times larger than the actual cell.



To find the actual size of the nucleus in micrometres (µm):

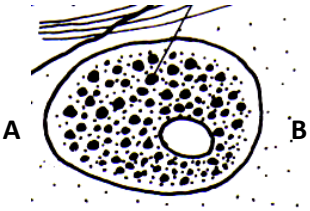
a. mark on the image where you are going to measure – in this case between **X** and **Y**

b. measure the length of the line in millimetres = **image size** = 110mm

c. convert to **micrometres** (µm) by multiplying by 1000 = 110 x 1000 = 110 000 µm

d. divide image size by the **magnification** to give the actual size in micrometres = $\frac{110\ 000}{10\ 000}$ = 11 µm

Finding the magnification:



When you have drawn a cell or tissue you need to know how much bigger is the drawing than the actual cell.

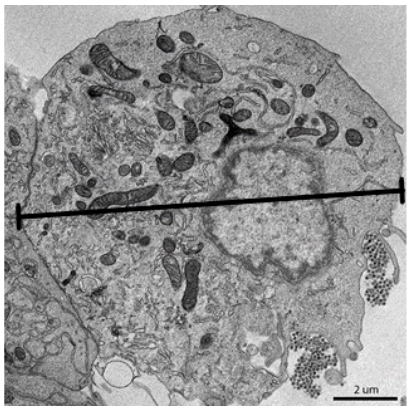
You know that the actual size of the cell in the drawing is 50 µm.

To find the magnification of the drawing:

a. mark on the drawing where you are going to measure – in this case between A and B
b. measure the distance in millimetres = 25mm
c. convert to **micrometres** (µm) by multiplying by 1000 = 25 x 1000 = 25 000 µm
d. divide by the **actual size** to give the magnification of the drawing = $\frac{25\,000}{50}$ = x 500

Using the scale bar

The scale bar can be used to calculate both magnification and actual size.



Magnification:

Finding the magnification:

a. measure the length of the scale bar = 15mm
b. convert to µm = 15 000µm
c divide the image length of the scale bar by the actual length = $\frac{15\,000}{2}$ = x 7 500

Actual size

a. mark on the drawing where you are going to measure
b. divide the length of the line you have drawn by the length of the scale bar = $\frac{115mm}{15mm}$ = 7.7
(ie., the line you have drawn is 7.7 times longer than the scale bar)
c. multiply this value by the actual length of the scale bar = 7.7 x 2µm = 15.4µm

IMPORTANT:

You must always remember to convert all lengths to the **same units**.

1cm	=	10 mm	cm →mm	x10	mm → cm	÷10
1mm	=	1 000µm	mm → µm	x 1000	µm → mm	÷ 1000
1µm	=	1 000nm	µm → nm	x 1000	nm → µm	÷ 1000

Practice Questions

1. For the image below:

(a) Calculate the magnification of the image using the **scale bar only**.

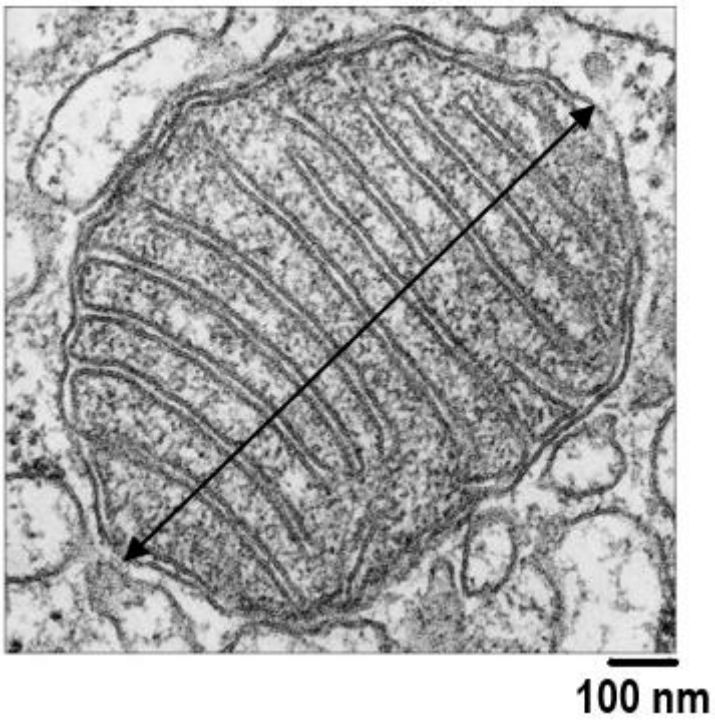
Working out:

Magnification of image = ☐

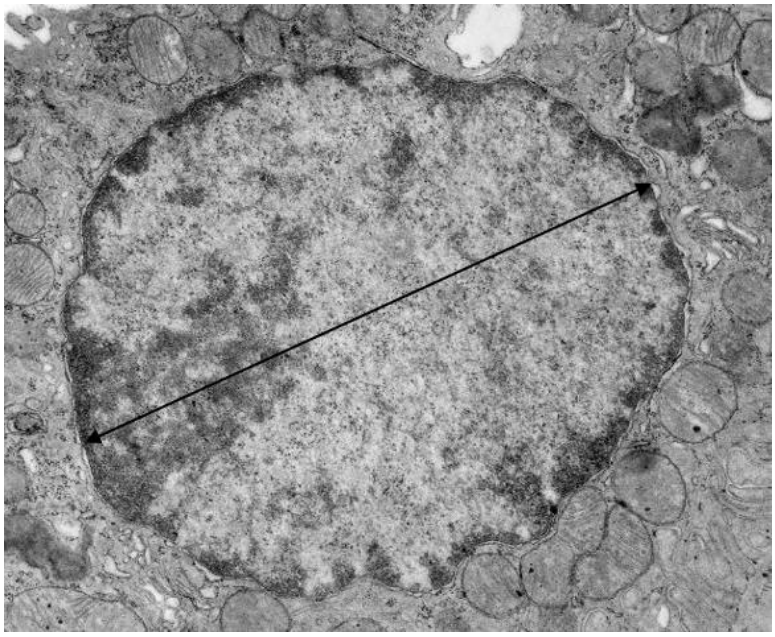
(b) Calculate the actual size of the organelle along the line shown on the image. Give your answer to the nearest micrometre.

Working out:

Actual length of line = ☐ μm



2. (a) The image below is printed at a magnification of x 10 000.

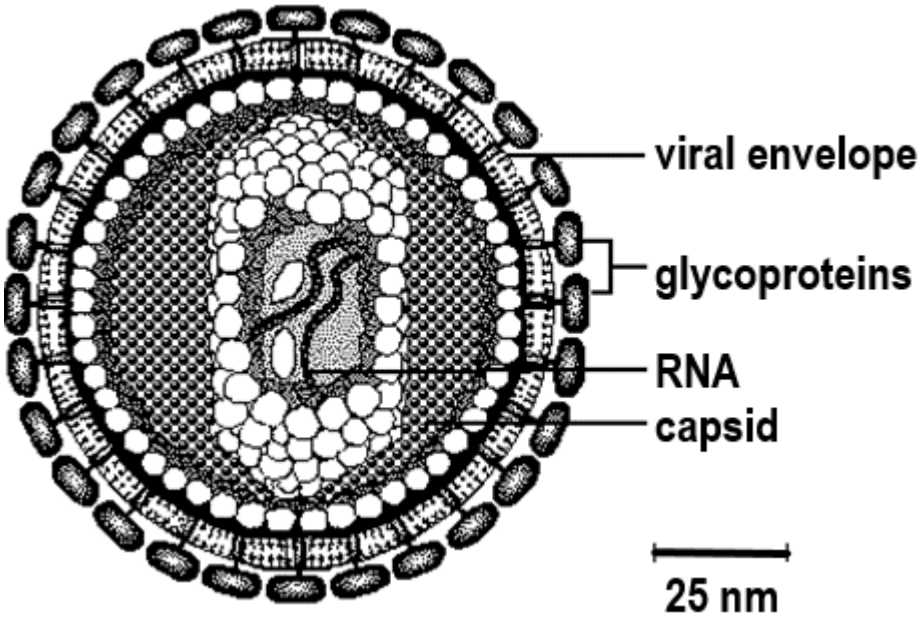


Calculate the actual size of the organelle along the line shown on the image. Give your answer to the nearest micrometre.

Working out:

Actual length of line = ____ μm

(b) The human immunodeficiency virus (HIV) infects cells involved in generating a specific immune response. The diagram below shows the structure of an HIV particle.

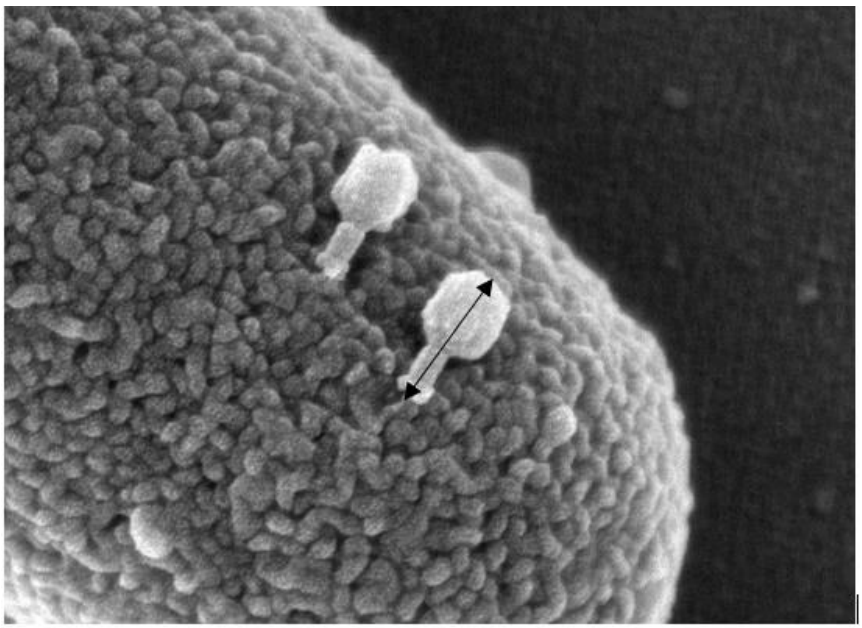


Calculate the diameter in nanometers of the virus shown above. Give your answer to two significant figures.

Working out:

Diameter of virus = _____ nm

(c) The electron micrograph below shows two bacteriophages (a type of virus) infecting a bacterium.



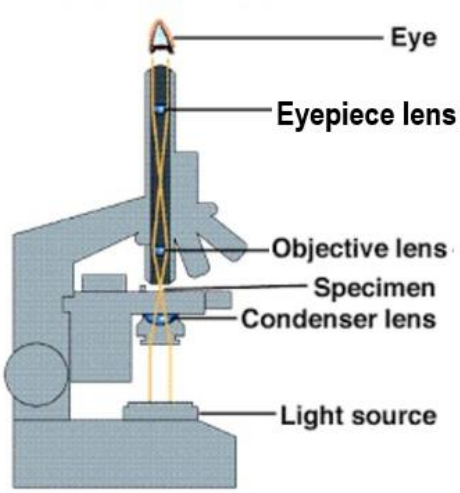
(magnification x 135 000)

Calculate the actual length of the bacteriophage along the line shown (⟷). Show your working and express your answer in standard form to two significant figures.

Working out:

Actual length of bacteriophage = _____ nm

MICROSCOPY



You will be asked to use images that have been produced using light microscopes and electron microscopes. The principles of these different types of microscope are similar and both are used to produce **magnified** images of objects which are too small to see with the naked eye.

Both types of microscope use a series of **lenses** to magnify the image seen by the eye.

The main differences between the microscopes are given in the table below.

Differences

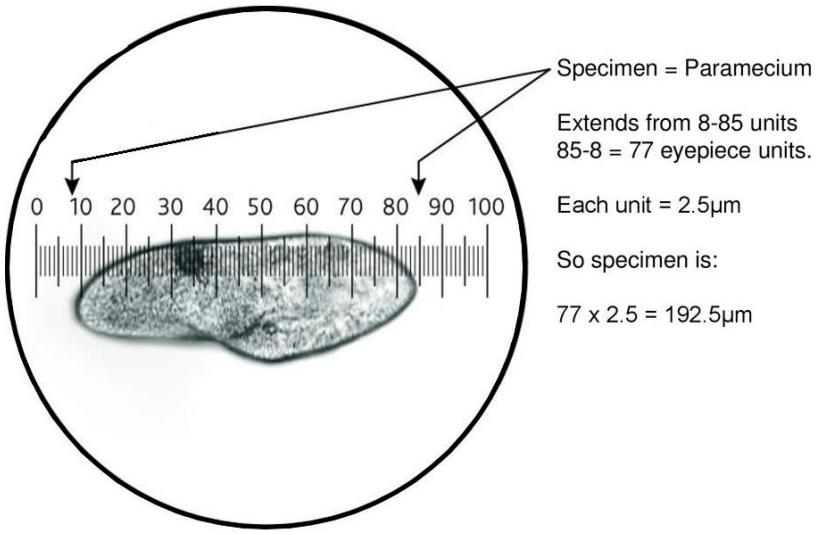
Light microscope	Electron microscope
Beam of light (longer wavelength)	Beam of electrons (shorter wavelength)
Small	Large and non portable
Relatively inexpensive	Expensive
Not a lot of training required to use	Training required
See colour images	Black and white images
Specimen can be alive and unharmed	Specimen must be dead
Lower resolving power	Greater resolution
Lower magnification	Greater magnification

Because electron microscopes use beams of electrons rather than light, they can:

- produce images at a **higher magnification**
- produce images which are clearer and with greater detail – they have **greater resolution**

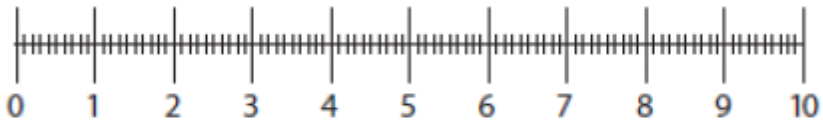
In both microscopes, **staining** is used to give more **contrast** between cell structures and make them easier to see. BUT, staining kills cells so cannot be used when observing live cells.

Your microscope may have a **ruler** in the eyepiece – called an **eyepiece graticule**.You use this to measure the dimensions of the object you are viewing. If you know the value of 1 eyepiece unit you can then calculate the actual size of the object.



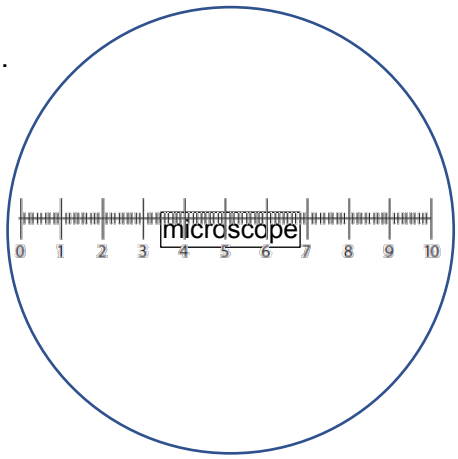
Calibration of a microscope

In order to measure the size of a structure on a microscope slide it is necessary to **calibrate** the microscope. Inside the eyepiece of the microscope there is an eye piece graticule. It is graduated 1-10 with 10 subdivisions between each number. Therefore, the eyepiece graticule has 100 eyepiece units [epu] along its length.

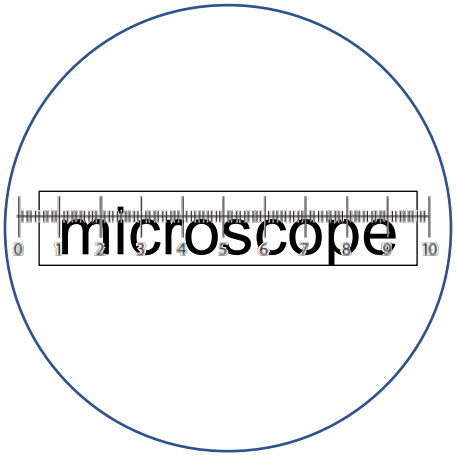


With different magnifications, the divisions on the eyepiece graticule will cover different actual lengths of the specimen on the slide.

Using a x10 eyepiece lens and a x 4 objective lens you would see an image similar to that on the right.



The **eyepiece graticule** covers only part of the image - the edges of the image are between 34 and 68 on the eyepiece graticule = 36 epu using the x4 objective.



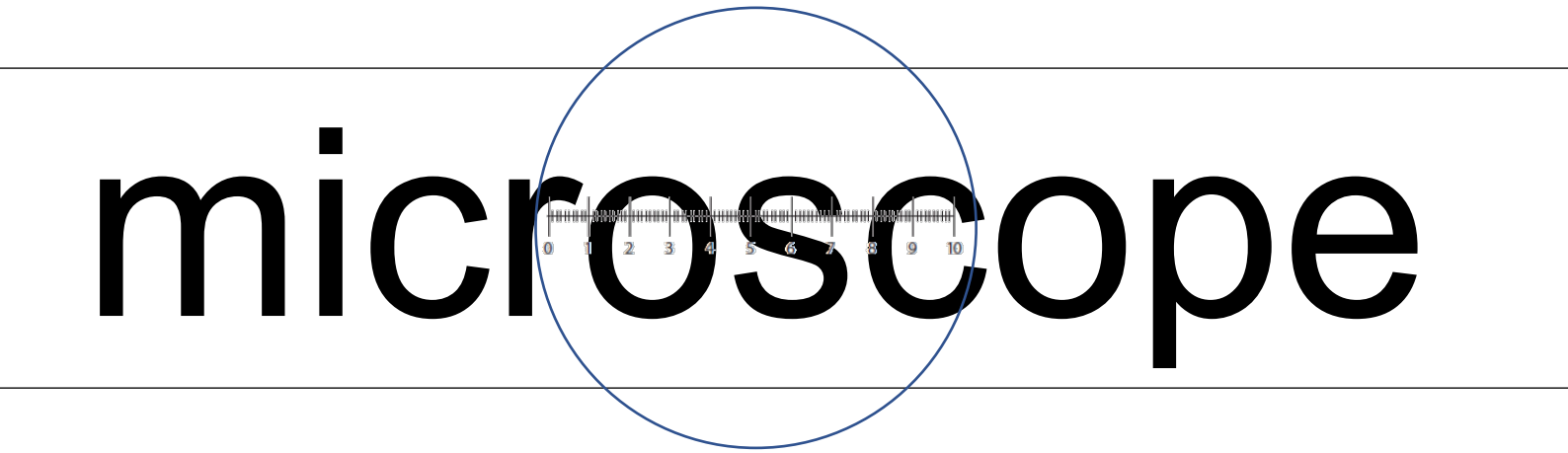
If the objective lens is changed to a x10 magnification the image appears **2.5 times larger** than using the x4 objective lens but might not be as clear (lower resolution).

The eyepiece graticule is the same size but now, the edges of the image are between 5 and 97 on the eyepiece graticule = 92 epu. (Allowing for resolution issues the size should be 90 epu.)

Using a x40 objective lens the image would appear to be **4 times larger** than using the x10 objective lens.

Not all the image fits into the field of view and resolution is worse – the image will be a

lot more blurred. From the **o** to the edge of **c** is 70 epu. Using the x10 objective lens this was 17 epu, 17 x 4 = 68 epu which, again, allowing for resolution issues is close.

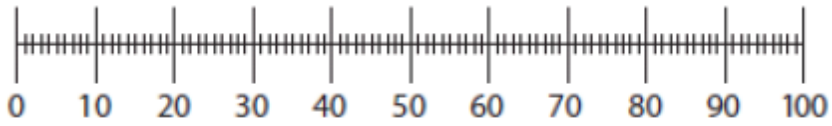


This means that your **eyepiece graticule** has to be **calibrated** for each objective lens on your microscope.

A **stage micrometer** is used to measure the length of each division of the eyepiece graticule at different magnifications.

The stage micrometer is a slide with a line **1 mm** long on it. The line is also marked for tenths and hundredths of a mm.

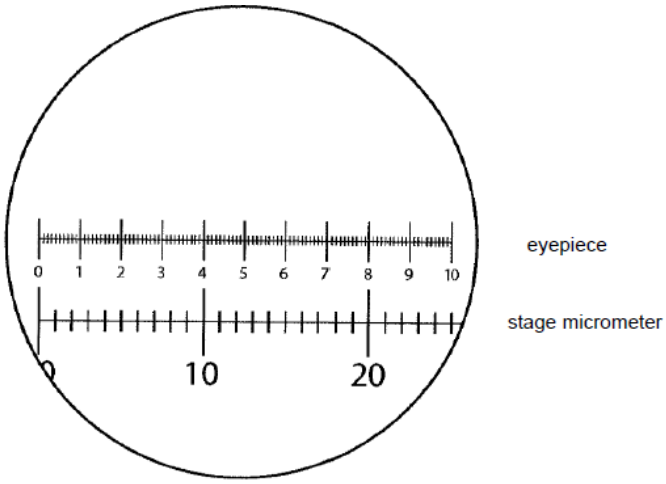
There are 100 stage micrometer divisions [smd] on the 1 mm line.
Each stage micrometer division = 0.01 mm or 10 µm.



To calibrate the microscope

- Line up the zero of the eyepiece graticule and the zero of the stage micrometer.
- Make sure the scales are parallel.
- Look at the scales and see where they are in line again.

Using this x40 objective lens, 80 eyepiece units = 20 stage micrometer units



Use the following pattern to calibrate a microscope using a particular objective lens.

x epu = **y** smd

1 epu = $\frac{\textbf{y}\ \text{smd}}{\textbf{x}\ \text{epu}}$

= **z** x 0.01mm (length of 1 smd)

1 epu = **z** x 0.01mm

1 epu = **z** x 0.01mm x 1000µm

If 1 stage micrometer unit = 0.01 mm

1 eye piece unit = $\frac{20}{80}$ = 0.25 stage micrometer units

1 stage micrometer unit = 0.01 mm

1 eye piece unit = 0.25 x 0.01 mm
= 0.0025 mm or 0.0025 x 1000µm
= 2.5µm

NOTE: There are different stage micrometers available so check the information given to find out the size of 1 smd. The pattern stays the same – just change the length of 1 smd in your calculation.

Calibration of eyepiece graticule using a x 4 objective lens

Learn this pattern and use it when asked to calibrate a microscope.

27 epu = 100 smd

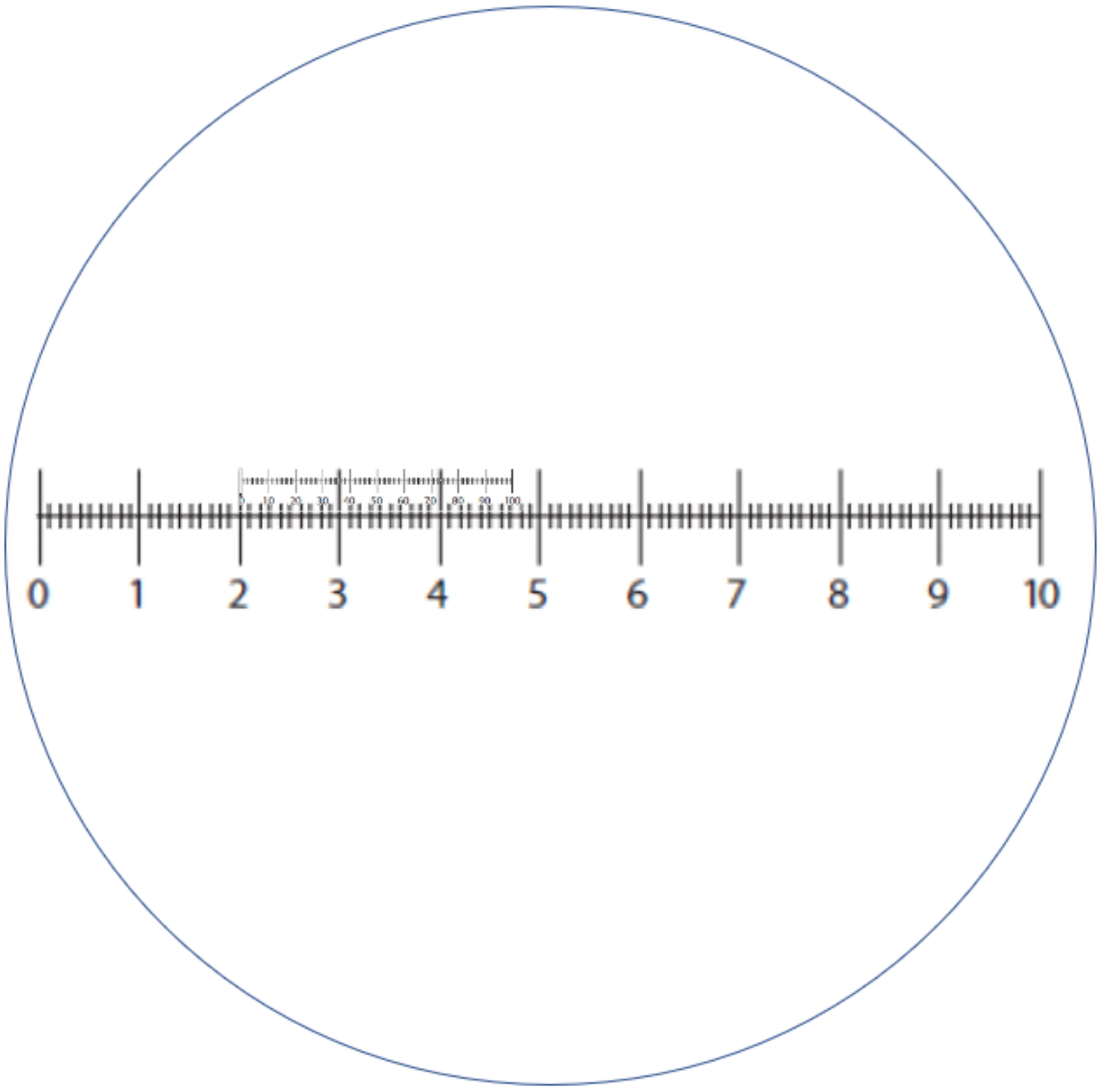
1 epu = $\frac{100 \text{ smd}}{27 \text{ epu}}$

1 smd = 0.01mm

1 epu = 3.7 x 0.01mm

= 0.037mm x 1000

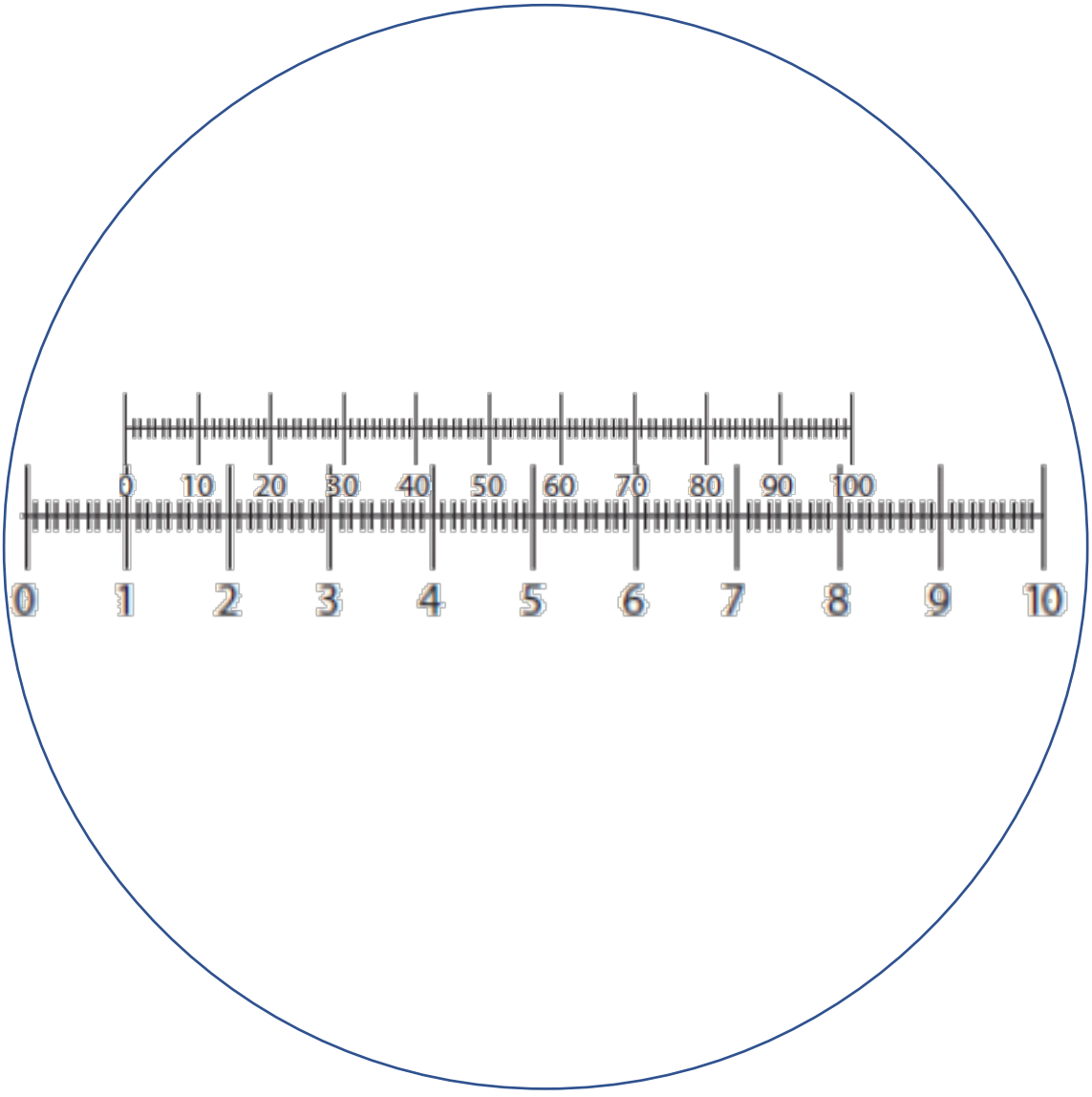
1 epu = 37.0μm



Calibration of eyepiece graticule using a x 10 objective lens

Follow the pattern for the x 4 objective lens to complete the calibration for the x 10 objective lens.

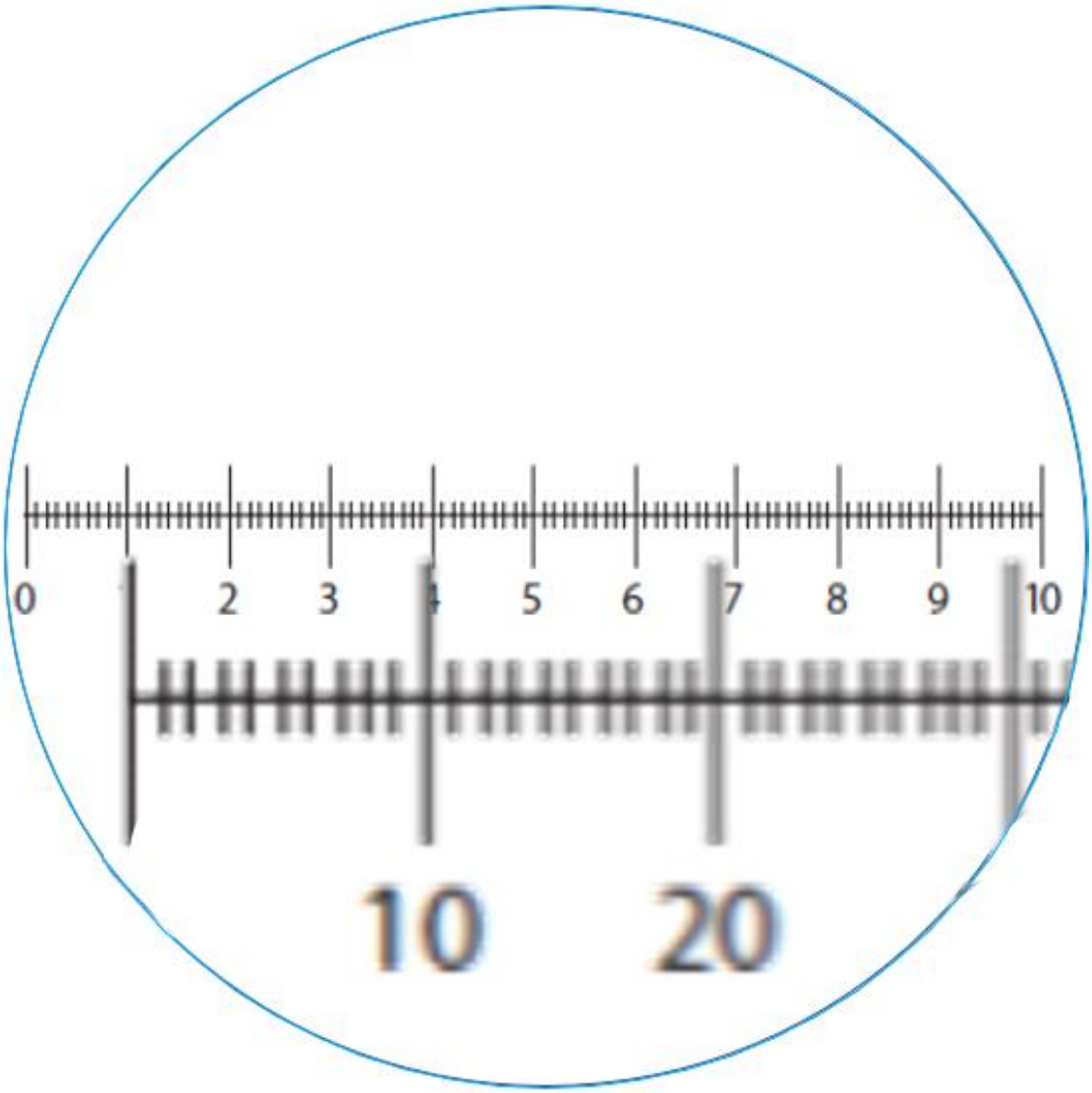
	epu	=		smd
1	epu	=	_____	smd
				epu
		=		
1	smd	=	0.01mm	
1	epu	=		X 0.01 mm
		=		mm
1	epu	=		μm



Calibration of eyepiece graticule using a x 40 objective lens

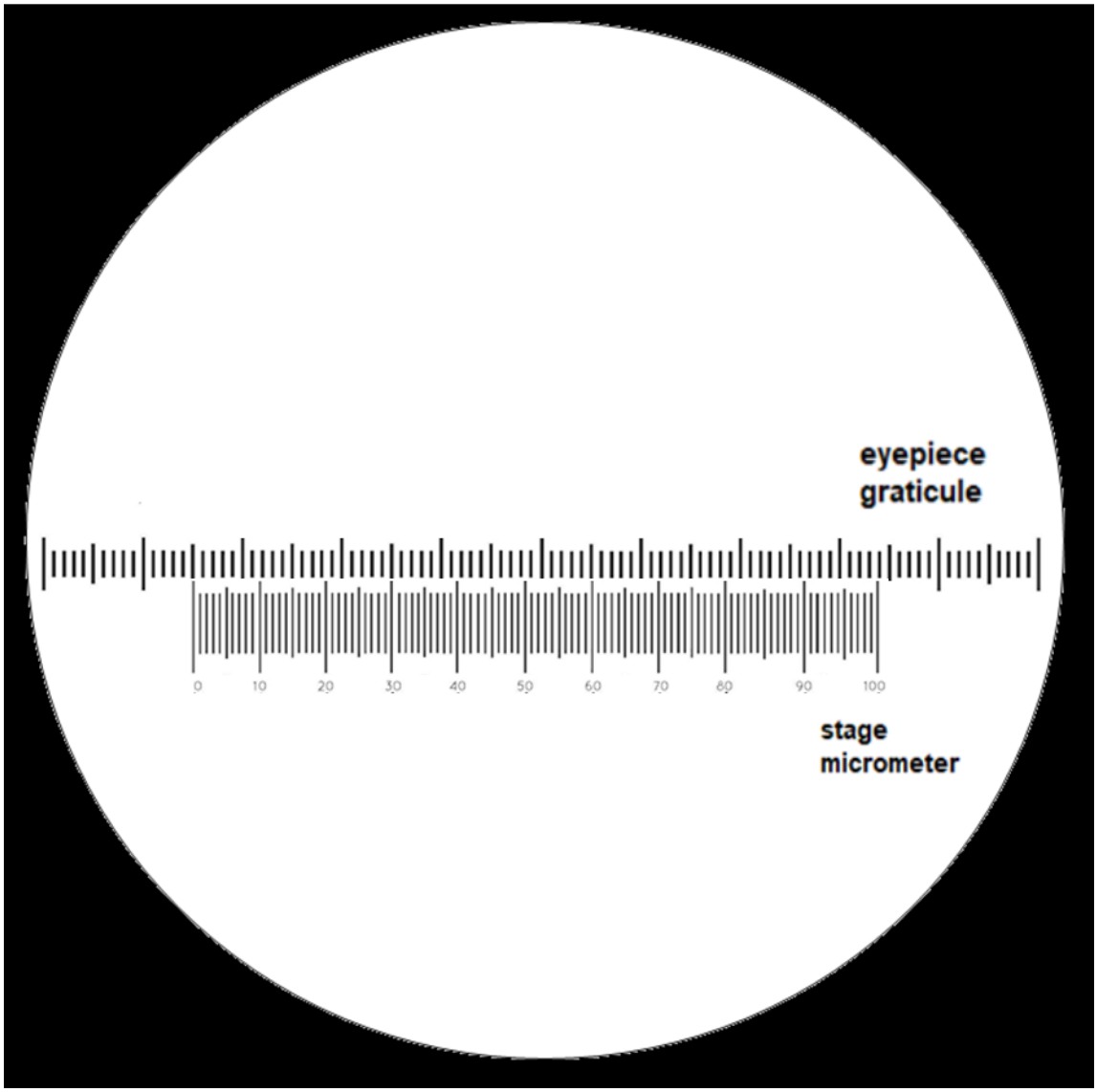
Follow the pattern for the x 4 objective lens to complete the calibration for the x 40 objective lens.

	epu	=		smd
1	epu	=	_____	smd
				epu
		=		
1	smd	=	0.01mm	
1	epu	=		X 0.01 mm
		=		mm
1	epu	=		μm



Practice Questions:

1. A student calibrated the x10 objective lens of a microscope using the eyepiece graticule and stage micrometer shown below.

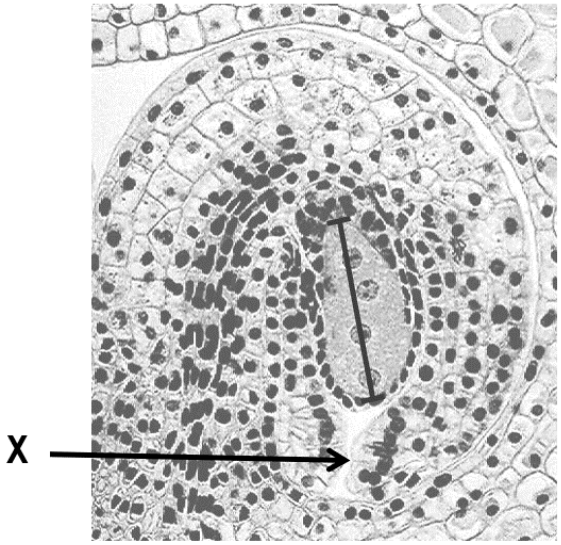


(a) Calculate the size of one eyepiece unit (epu) at this magnification. Give your answer in micrometers.
[1 smd = 0.01mm]

Working out:

1 epu using a x10 objective lens = _____ μm

(b) Using the same microscope, the student examined a slide showing a section through part of a developing plant ovary.

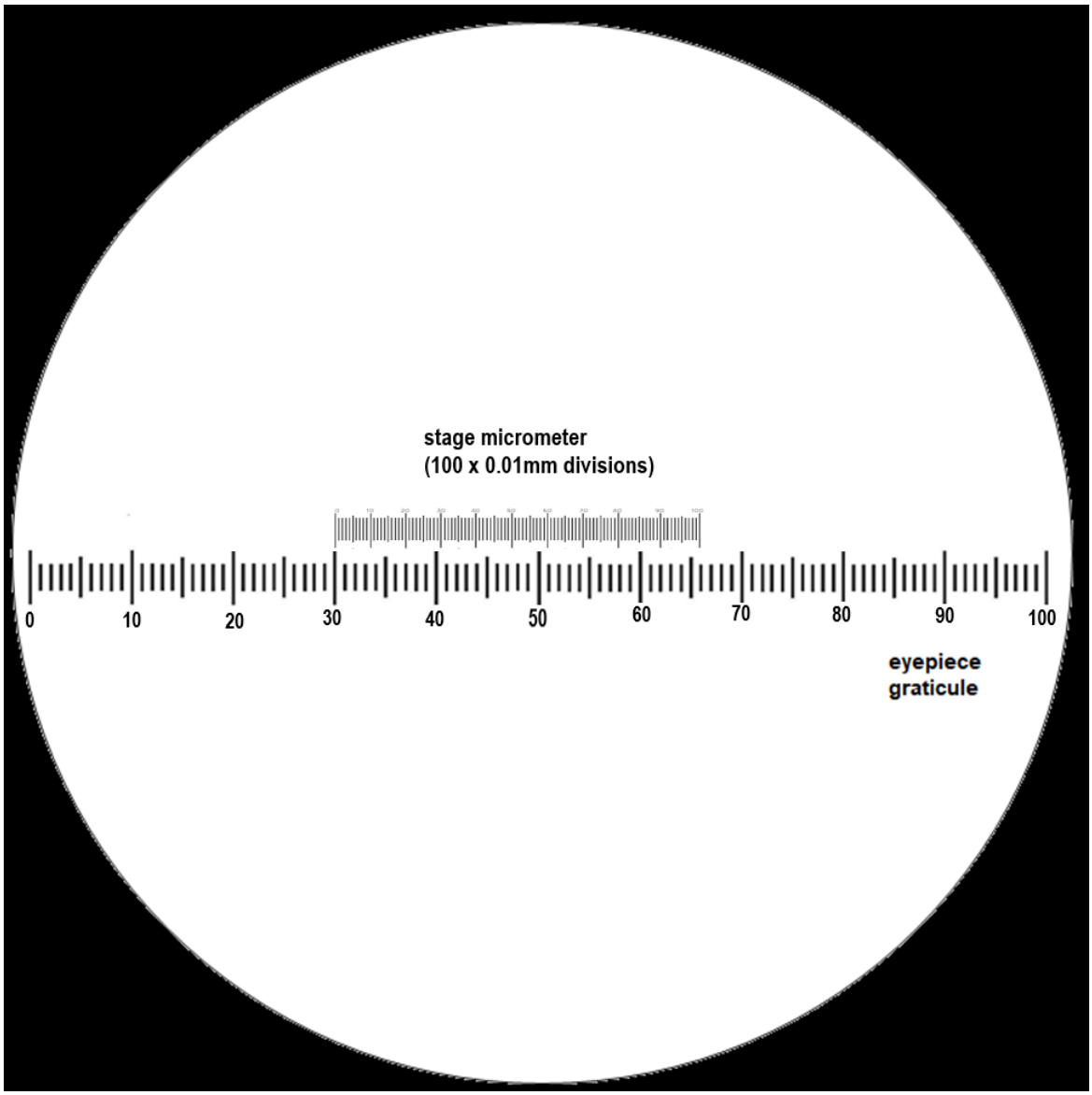


At the point indicated by the line the developing ovary was 31 epu long. Use your answer to (a) to calculate the actual length of the embryo sac.

Working out:

length of developing plant ovary = [ ] μm

2. Using a ×4 objective lens a microscope was calibrated using the eyepiece graticule and stage micrometer shown below.



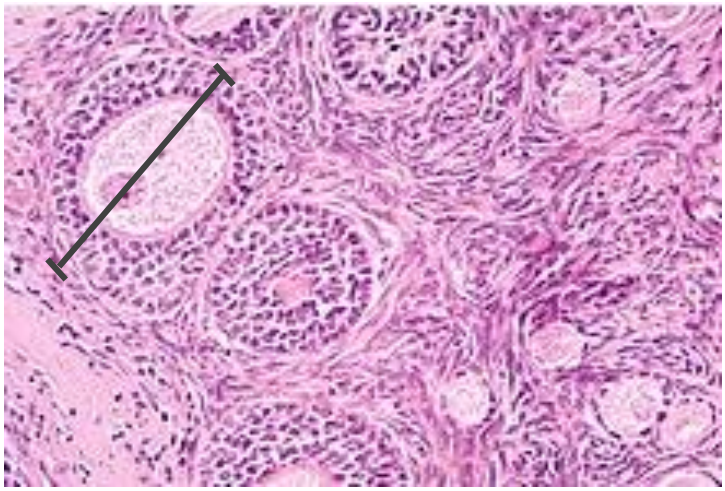
(a) Calculate the size of one eyepiece unit (epu) at this magnification. Give your answer to the nearest micrometre.

[1 smd = 0.01mm]

Working out:

1 epu using a x4 objective lens = _____ μm

(b) The image below shows a section through the ovary of a mammal.



One structure in the ovary had a diameter of 101 epu at the point indicated by the line on the image. Using your calibration of the microscope at this magnification, calculate the actual diameter of the structure at this point. **Express your answer in mm to 1 decimal place.**

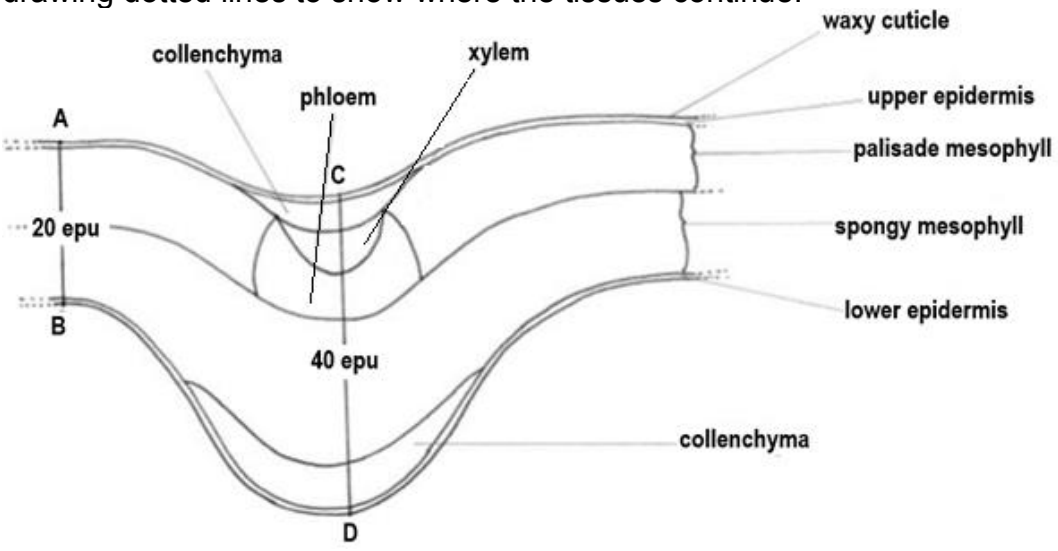
Working out:

diameter of structure = _____ mm

DRAWING CELLS AND TISSUES

Low power plan

This shows the distribution of tissues in a transverse section (TS) or longitudinal section (LS) of a structure. It is not always necessary to draw a plan of the entire structure but if a part is drawn it should be indicated that it is a part of a structure. This is usually done by drawing dotted lines to show where the tissues continue.



When completing low power plans, you should:

- use a sharp pencil.
- not use any shading
- not draw any individual cells
- make your drawing at least half a page of A4 in size and position the labels to the side of the drawing
- make all lines clear, complete and not overlapping
- draw label lines with a ruler to the centre of the tissue layer, they should not cross each other
- ensure tissue layers are all drawn to the correct proportion
- draw a line across two tissues and give the width of this line in eyepiece units.

Checking if the drawing is in proportion:

If one line across tissue A has been given 48 epu and the second line across tissue B has been given 12 epu, the correct proportion should show that tissue A is 4 times the width of tissue B at that point. The following equation can be used

$$\frac{\text{image size C-D (epu)}}{\text{image size A-B (epu)}} = \frac{\text{drawing size C-D (mm)}}{\text{drawing size A-B (mm)}}$$

Using the image above:

$$\frac{\text{C-D 40epu}}{\text{A-B 20 epu}} = \frac{\text{C-D 50mm}}{\text{A-B 25mm}}$$

$$2 = 2$$

Conclusion – this drawing **is** in proportion

Magnification of a drawing and Actual Size

Example:

Using the low power plan on the previous page:

measurements were taken using a x10 objective

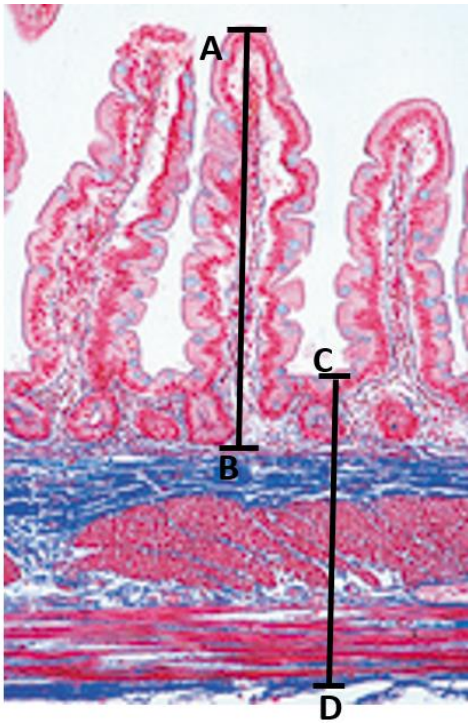
actual length 1 epu	=	18.5μm
image length C-D	=	40 epu
actual length C-D	=	40 x 18.5μm
	=	740μm

Magnification of drawing

drawing length C-D	=	50mm
	=	50 000μm
actual length C-D	=	740μm
magnification	=	50 000 ÷ 740
	=	67.56...
	=	x 70 (to 2 significant figures)

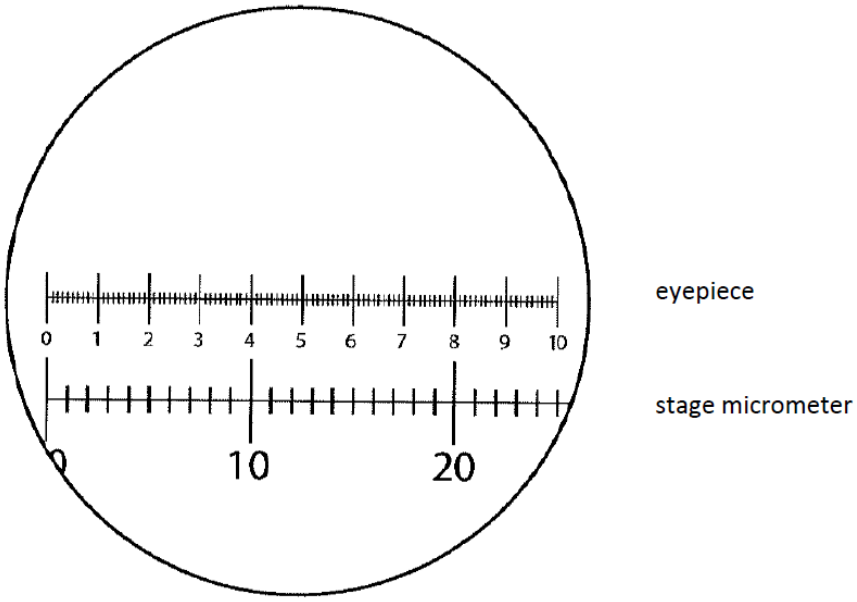
Practice Questions:

1. The photograph below shows a section through part of the digestive system.



Length A – B = 103 epu
Length C – D = 95 epu

(a) The microscope used to measure A-B and C-D on the image was calibrated using the x10 objective lens and a x10 eye-piece lens. The image below shows the eyepiece graticule and stage micrometer as they appeared using these lenses.



1 stage micrometer division (smd) = 10µm.

(i) Calculate the actual length of one epu at this magnification.

Working out:

1 epu = ____ µm

(ii) Using your calculation calculate the **actual** length of A- B shown in the image.

Working out:

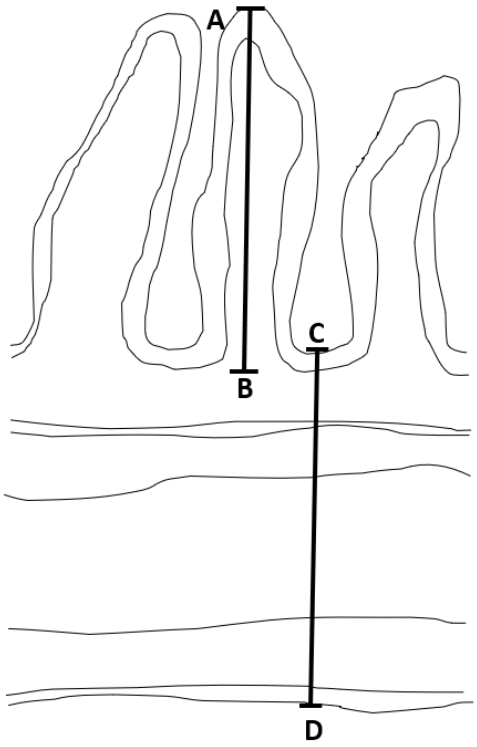
Actual length of A-B = ____ µm

(iii) Using a ruler, measure the length of A-B on the photograph and use this, together with your answer to (a) (ii) to calculate the magnification of the photograph.

Working out:

Magnification of photograph = ____ µm

(b) A low power plan was drawn of the photograph.



The ratio of the lengths of A and B in the photograph should be the same as in the low power plan for the plan to be in proportion to the actual size of the original section:

$$\frac{\text{size C-D in photograph (epu)}}{\text{size A-B in photograph (epu)}} = \frac{\text{drawing size C-D (mm)}}{\text{drawing size A-B (mm)}}$$

(i) Measure the lengths of A-B and C-D in the low power plan and use the relationship shown above to prove that the low power plan is **not** in proportion to the actual sizes of A-B and C-D.

Length A-B in plan =		**Length C-D in plan =**	
Working out:			
		Ratio =	≠

(ii) Rearrange the equation shown above to calculate the length that line A-B should have been drawn in the low power plan to make the plan proportional to the actual object sizes. [2]

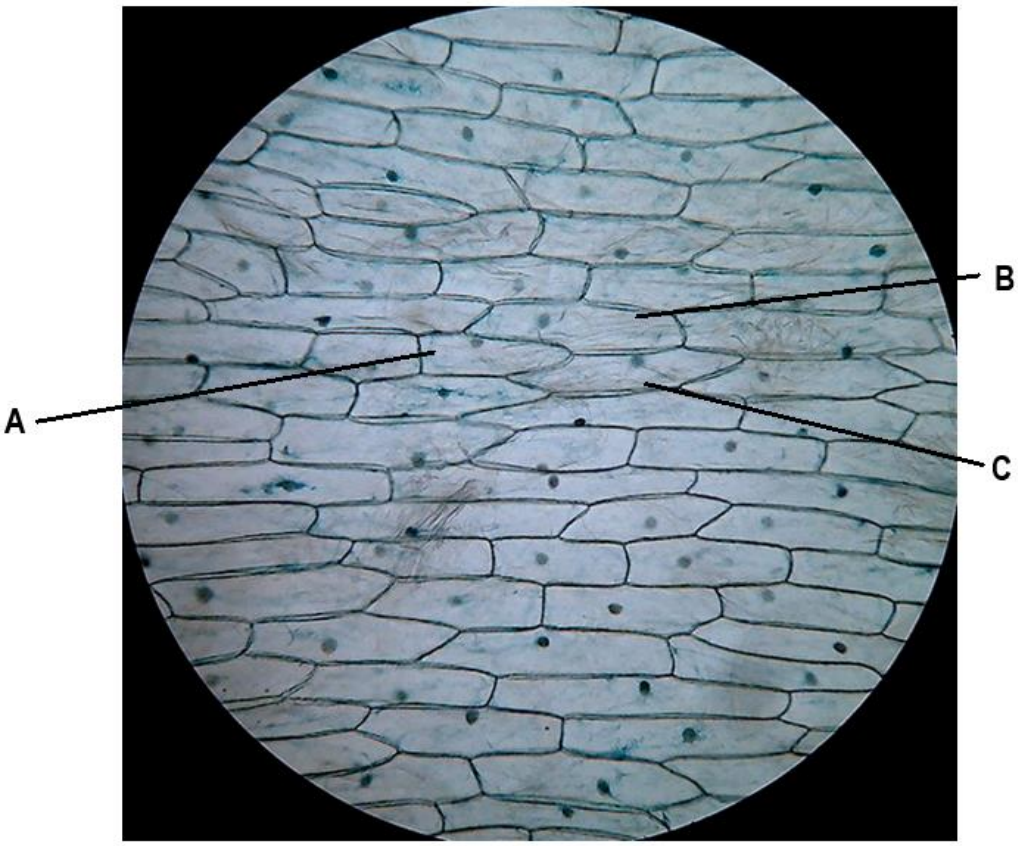
Working out:

Length of A-B in low power plan = _____ mm

Practical: Preparation and scientific drawing of a slide of onion cells

1. Place two drops of water onto a microscope slide.
2. Take a small piece of onion and using forceps peel off the membrane from the underside (the rough side).
3. Lay a piece of the membrane flat on the surface of the slide taking care that it is a single layer and not folded back on itself.
4. Add three drops of iodine solution.
5. Place one edge of a coverslip onto the slide and lower it gently using a mounted needle, making sure that there are no air bubbles.
6. Gently press the coverslip down using a piece of paper towel.
7. Using the x4 objective position the slide and focus on the section.
8. Swing the x10 objective into place and move the slide carefully until a clear area of cells are observed i.e. no large bubbles, no folds and a single layer of cells.
9. Draw a group of at least three cells in the correct proportion. Indicate the length of one cell in eye piece units on the drawing.
10. You should use the x40 objective to help you identify and label structures in the cells.
11. Calculate the actual size of one of your cells and the magnification of your drawing.

The photograph shows the appearance of onion epidermal cells using the x10 objective.



Cells **A**, **B** and **C** were measured using an eyepiece graticule:

Cell	Maximum width /epu	Maximum height / epu
A	27	9
B	38	9
C	40	8

(a) Draw a labelled diagram to show cells A, B and C as they appear in the photograph.

Use the measurements to make sure that your drawing is in proportion to the actual sizes.

(b) Use the widths of cells A and C and relationship between actual sizes and drawing sizes to determine if your drawing is in proportion

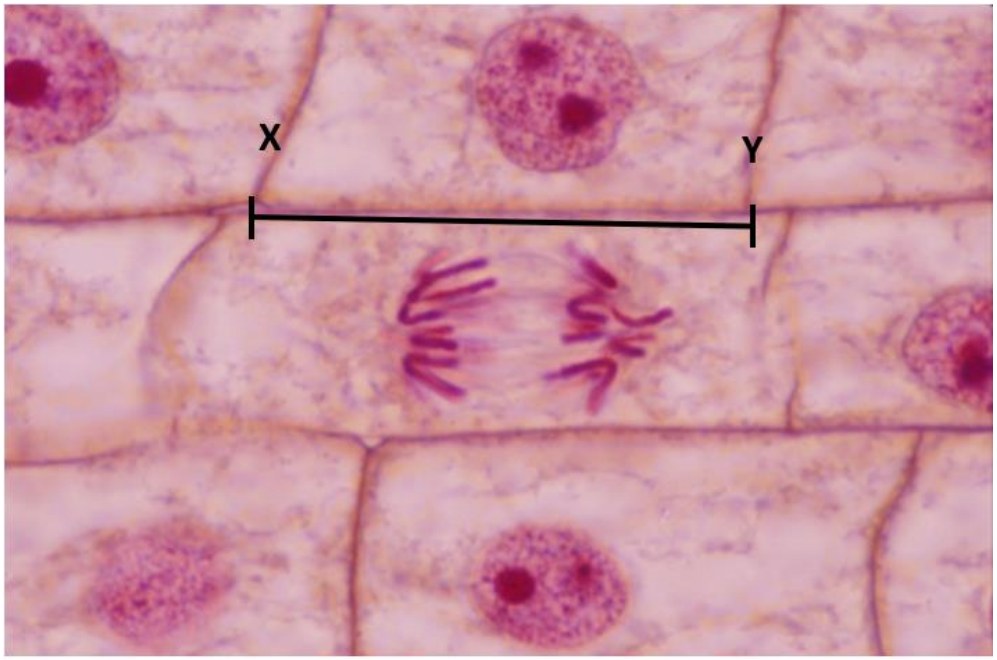
Width cell A in plan =		**Width cell C in plan =**	
Working out:			
		Ratio A : C =	=

(c) Use your calibration of the eyepiece graticule using a x10 objective lens to calculate the actual sizes of these cells in µm.

Cell	Actual maximum width /µm	Actual maximum height / µm
A		
B		
C		

Practice Question:

1. (a) The photomicrograph shows a section of a garlic root tip showing cells at different stages of cell division.



The length of one cell was measured using an eye-piece graticule along the line labelled **X—Y**.

(i) Suggest the magnification of the objective lens used to observe the cells shown in the photomicrograph.

(ii) The length of line **X—Y** was 64 epu. With the lenses used to observe the root tip, 1 epu = 2.5μm.

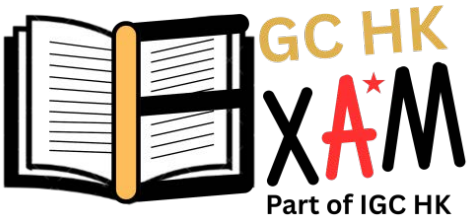
Calculate the actual length of line **X—Y** and use this to calculate the magnification of the photomicrograph. Express your answer to **one** significant figure.

Working out:

Actual length of **X-Y** = ____ μm

Magnification of the photomicrograph = ____

(b) The garlic root tip shown in the photomicrograph was stained. Explain why a stain was used and suggest why this technique could not be used to observe live cells.



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