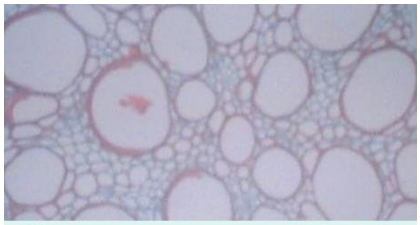
MAGNIFICATION, ACTUAL SIZE AND MICROSCOPY

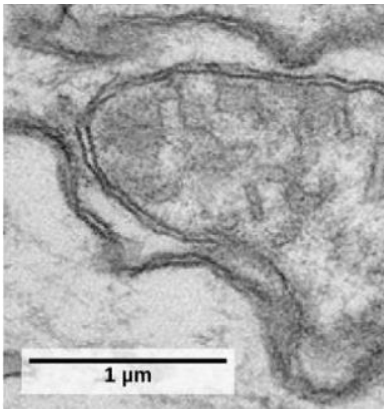
This resource covers practical skills you need to learn and use when dealing with actual size, image size and the magnification of images. These skills will be used in nearly all units at AS and A2.

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.



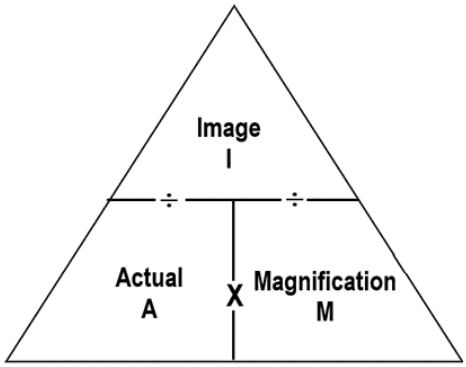
Dicotyledonous root [ts] x200



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

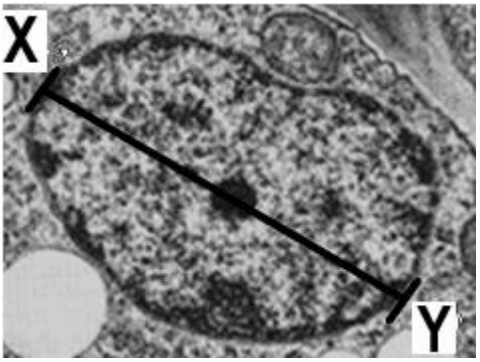


You need to be able to use this relationship to calculate any of the three values.

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**

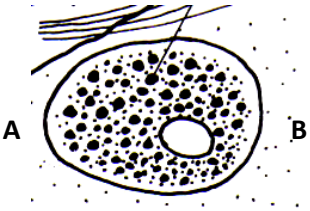
b. measure the length of the line in millimetres = **image size** = 110mm

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.



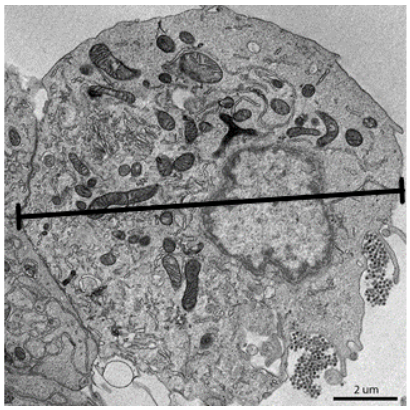
You know that the actual size of the cell in the drawing is 50 µm.

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:

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**.

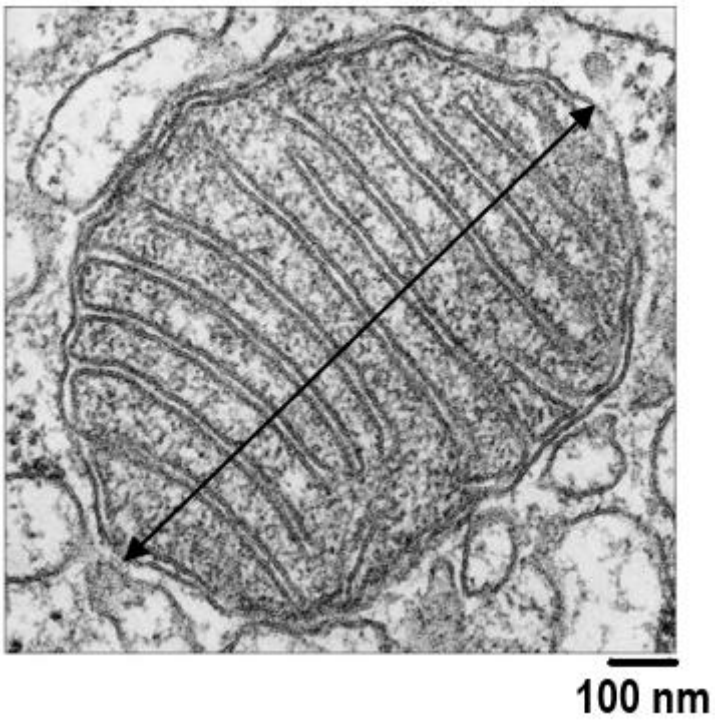
Working out:

Magnification of image = ☐

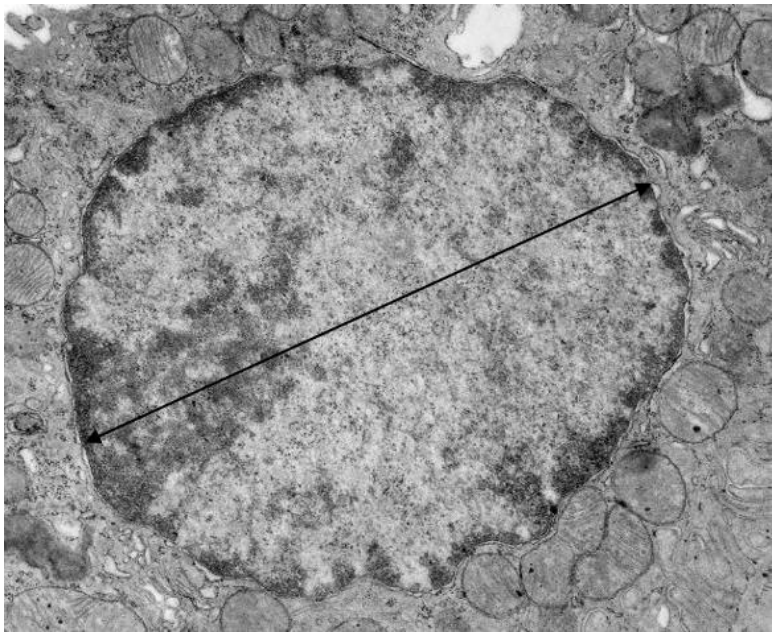
(b) Calculate the actual size of the organelle along the line shown on the image. Give your answer to the nearest micrometre.

Working out:

Actual length of line = ☐ µm



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

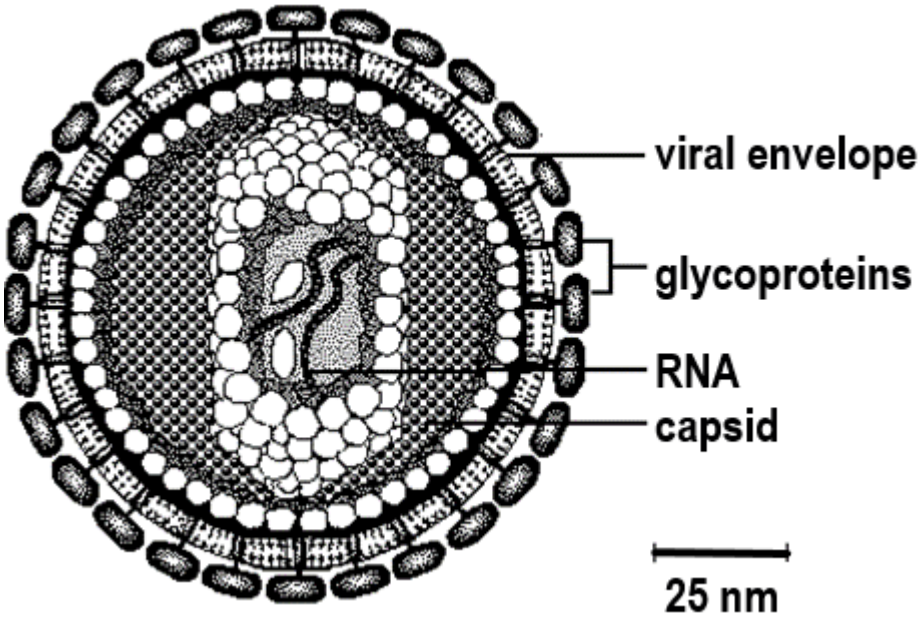


Calculate the actual size of the organelle along the line shown on the image. Give your answer to the nearest micrometre.

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.

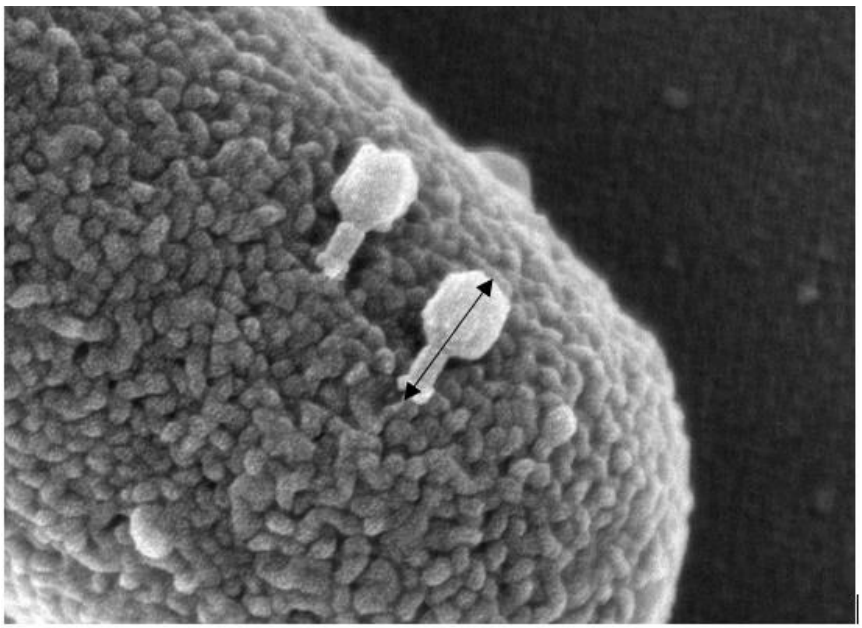


Calculate the diameter in nanometers of the virus shown above. Give your answer to two significant figures.

Working out:

Diameter of virus = _____ nm

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



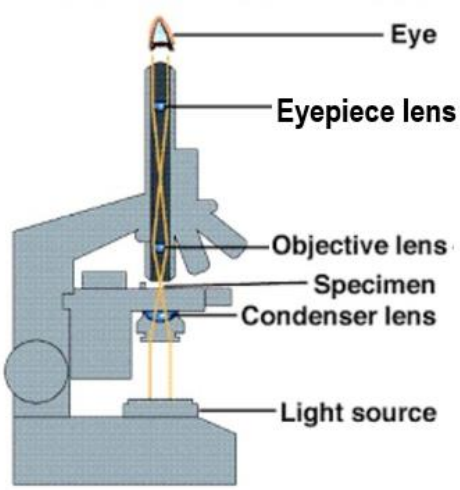
(magnification x 135 000)

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

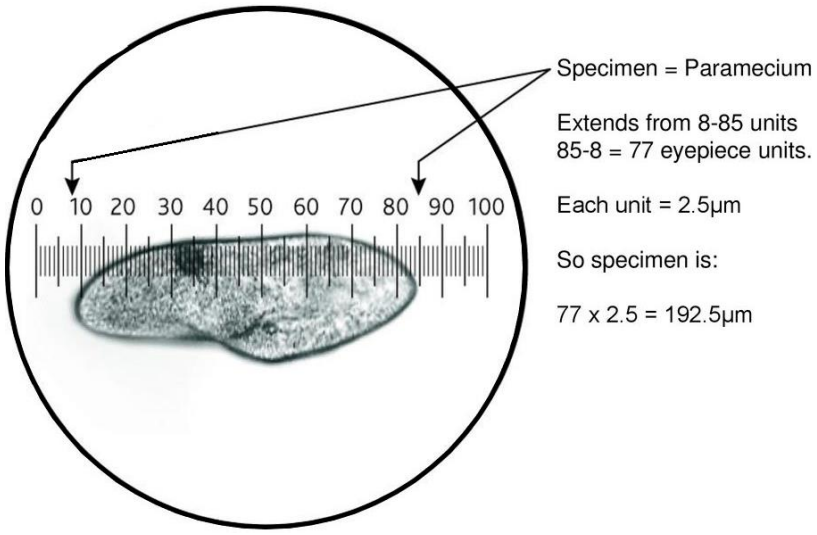
Light microscope	Electron microscope
Beam of light (longer wavelength)	Beam of electrons (shorter wavelength)
Small	Large and non portable
Relatively inexpensive	Expensive
Not a lot of training required to use	Training required
See colour images	Black and white images
Specimen can be alive and unharmed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

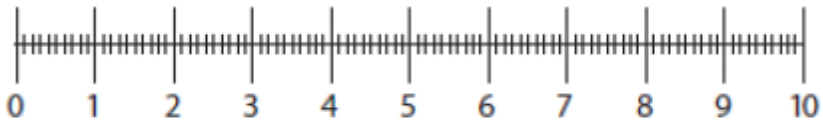
In both microscopes, **staining** is used to give more **contrast** between cell structures and make them easier to see. BUT, staining kills cells so cannot be used when observing live cells.

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.



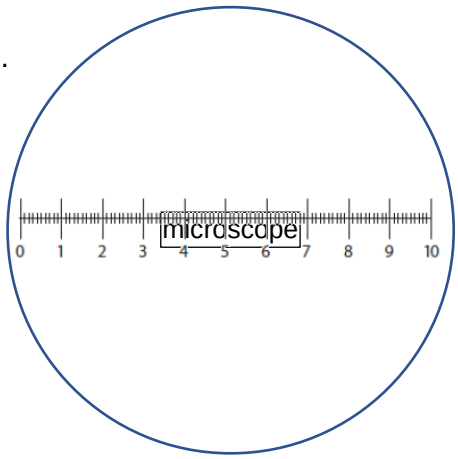
Calibration of a microscope

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.

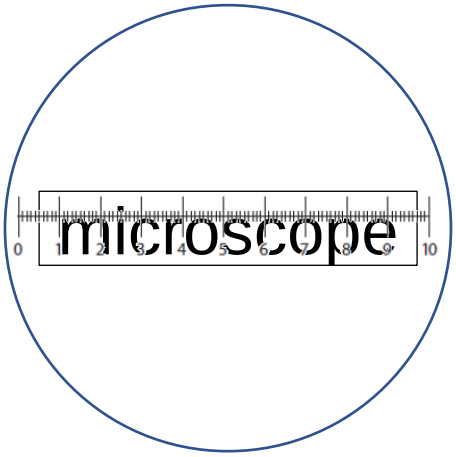


With different magnifications, the divisions on the eyepiece graticule will cover different actual lengths of the specimen on the slide.

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.



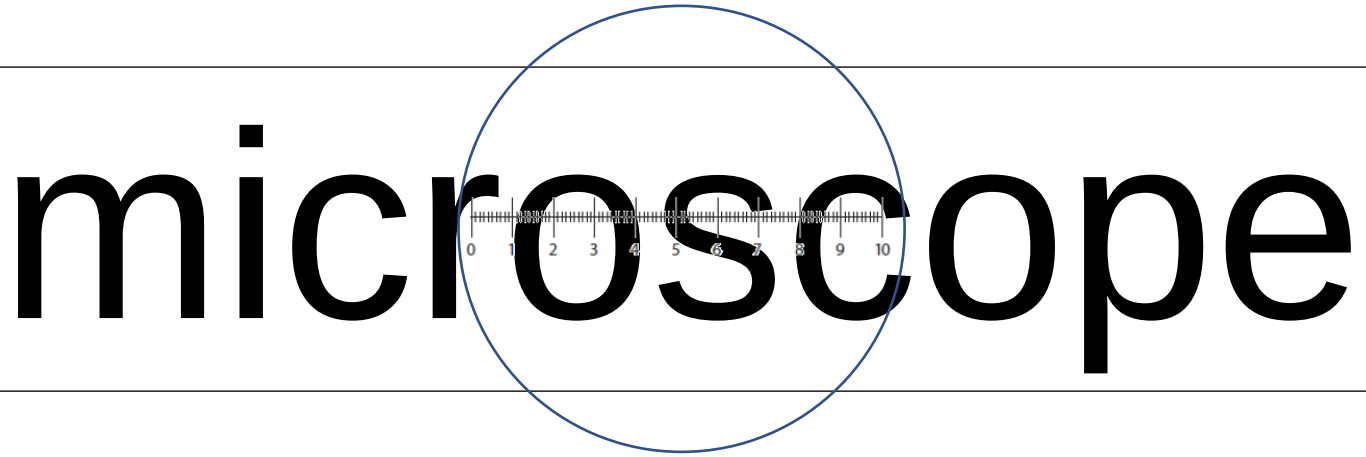
If the objective lens is changed to a x10 magnification the image appears **2.5 times larger** than using the x4 objective lens but might not be as clear (lower resolution).

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.

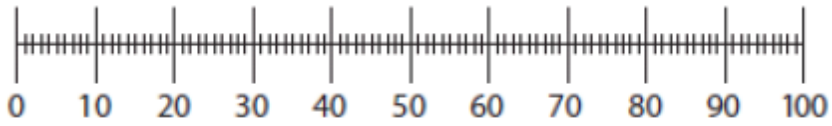


This means that your **eyepiece graticule** has to be **calibrated** for each objective lens on your microscope.

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.

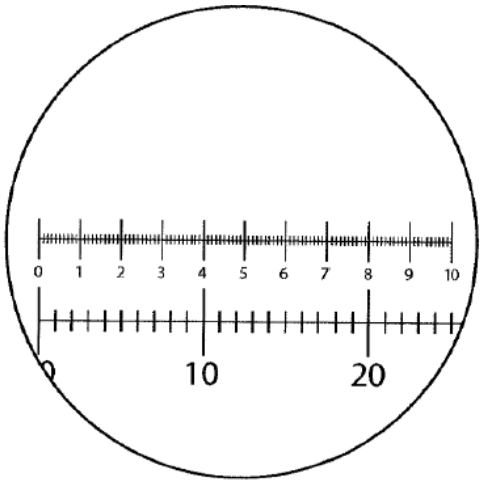
There are 100 stage micrometer divisions [smd] on the 1 mm line.
Each stage micrometer division = 0.01 mm or 10 µm.



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

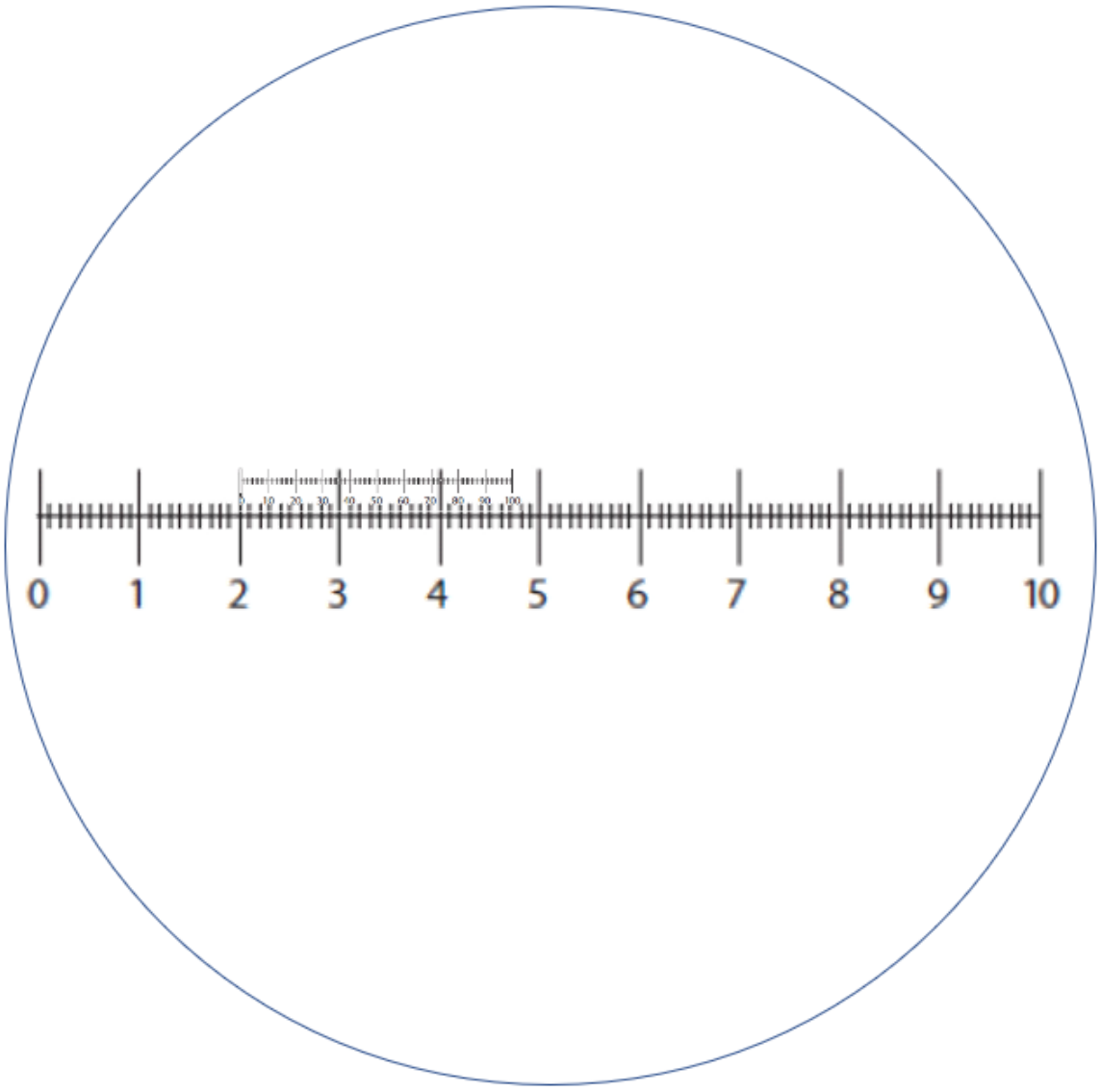
1 epu = $\frac{100 \text{ smd}}{27 \text{ epu}}$

1 smd = 0.01mm

1 epu = 3.7 x 0.01mm

= 0.037mm x 1000

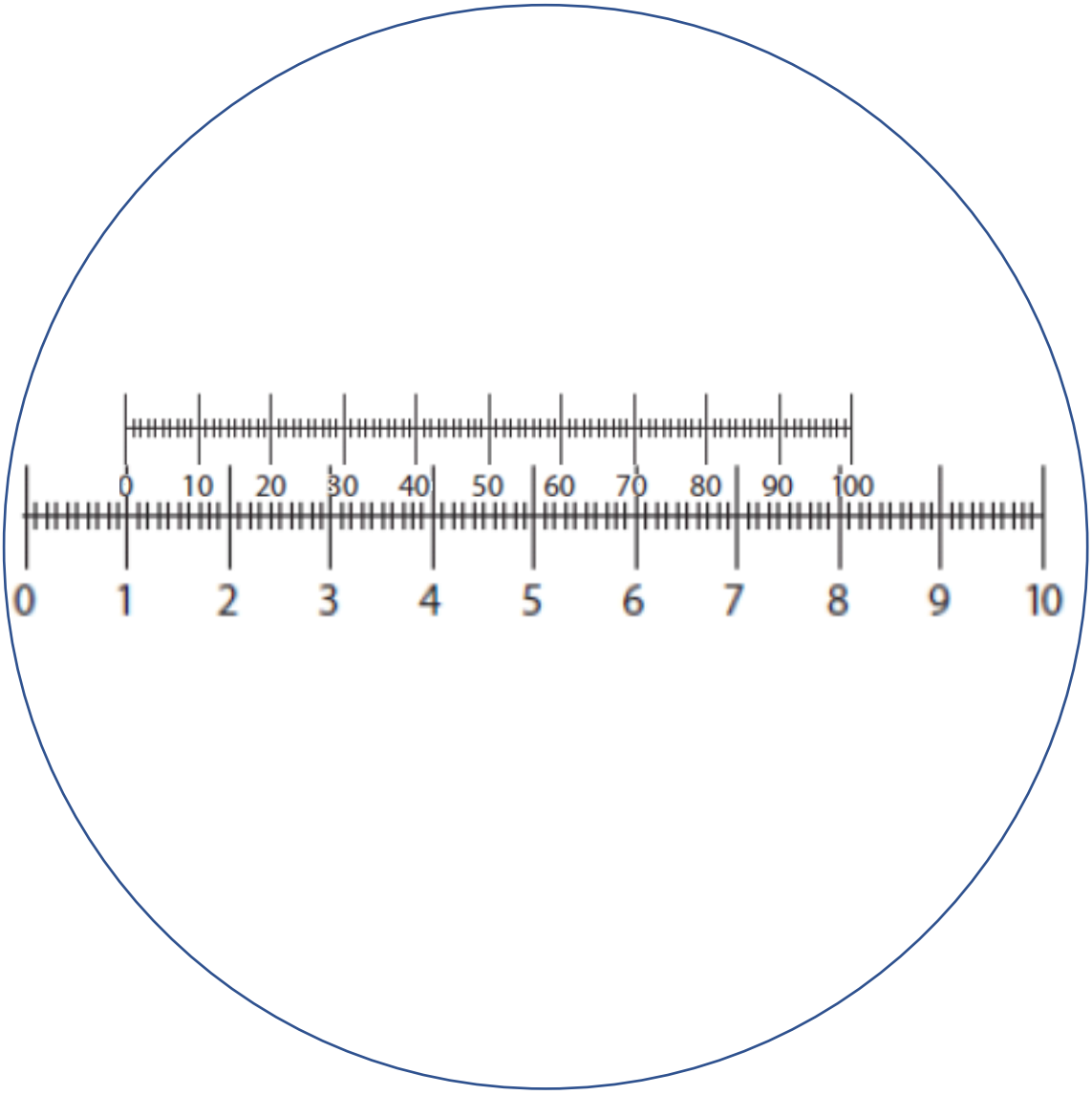
1 epu = 37.0µm



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.

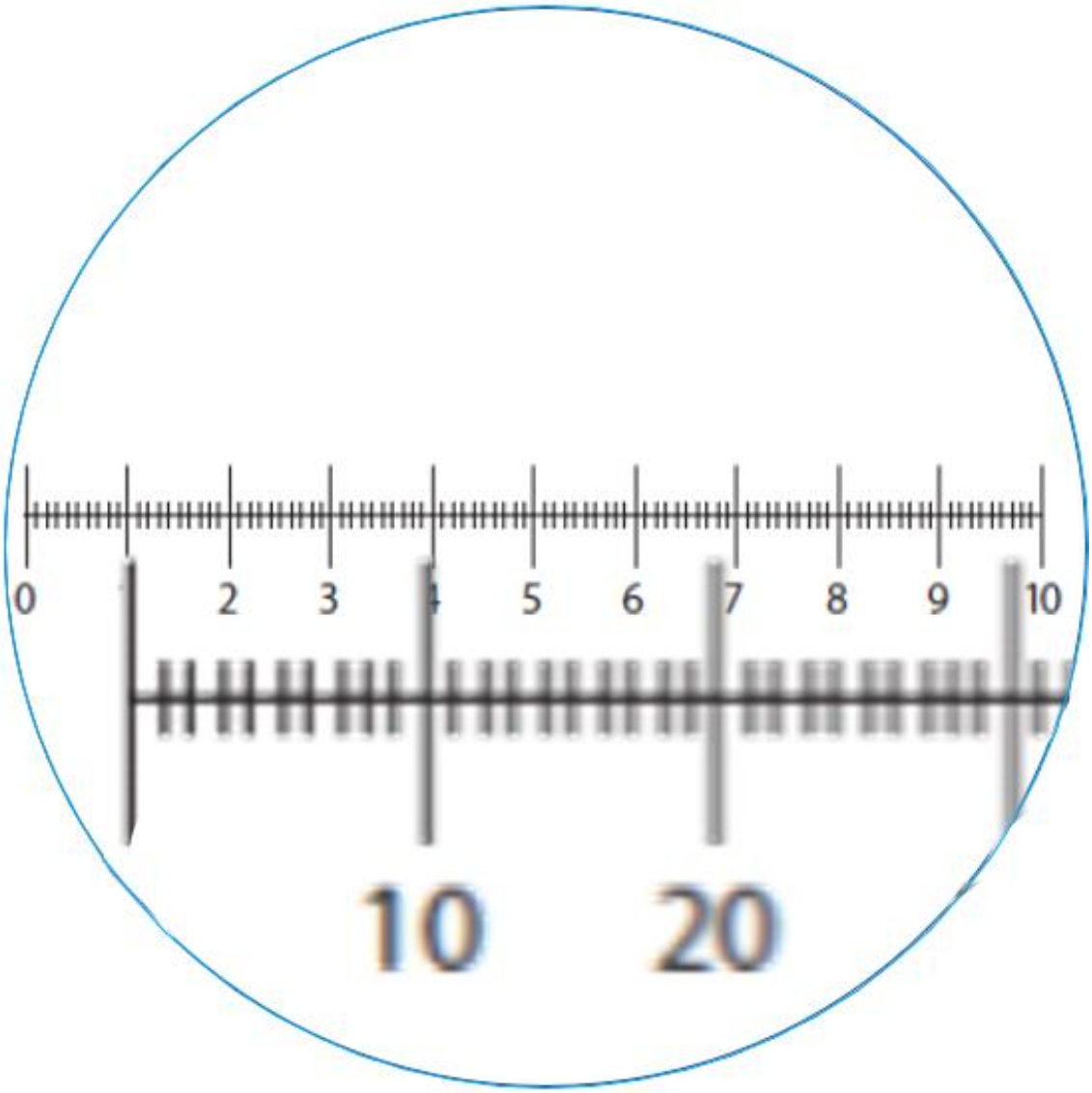
	epu	=		smd
1	epu	=	_____	smd
				epu
		=		
1	smd	=	0.01mm	
1	epu	=		X 0.01 mm
		=		mm
1	epu	=		µm



Calibration of eyepiece graticule using a x 40 objective lens

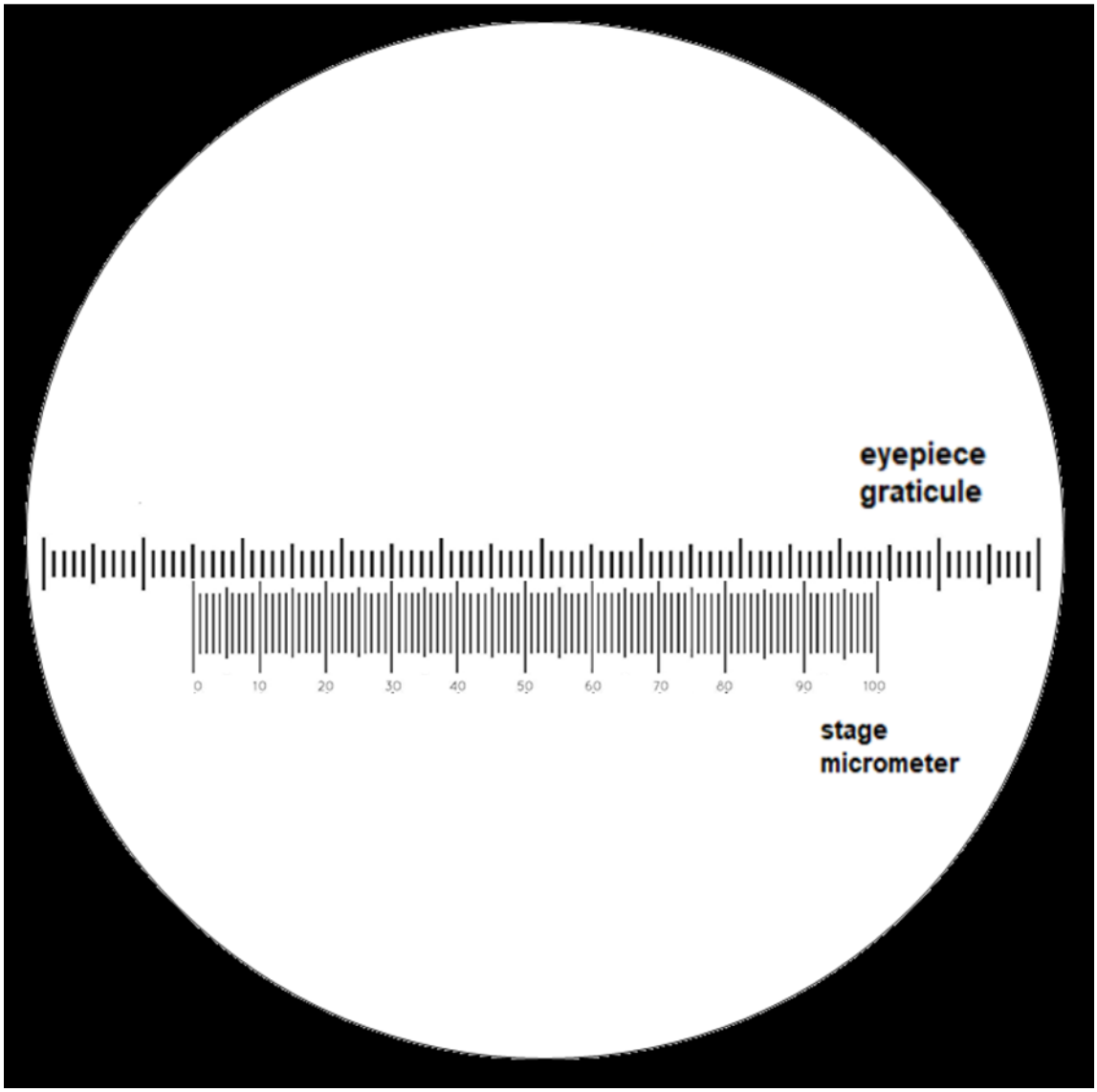
Follow the pattern for the x 4 objective lens to complete the calibration for the x 40 objective lens.

	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.

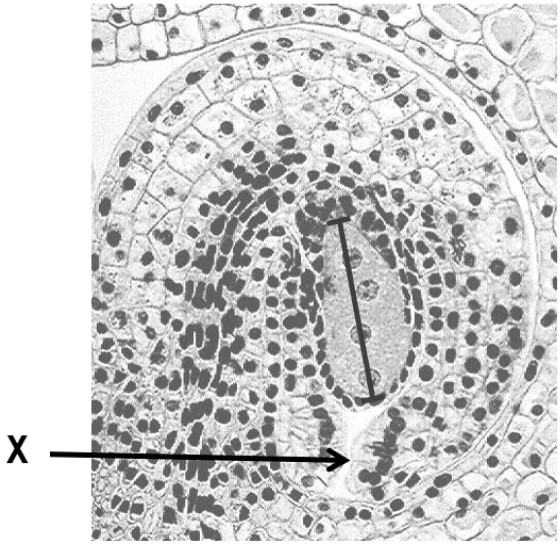


(a) Calculate the size of one eyepiece unit (epu) at this magnification. Give your answer in micrometers.
[1 smd = 0.01mm]

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.

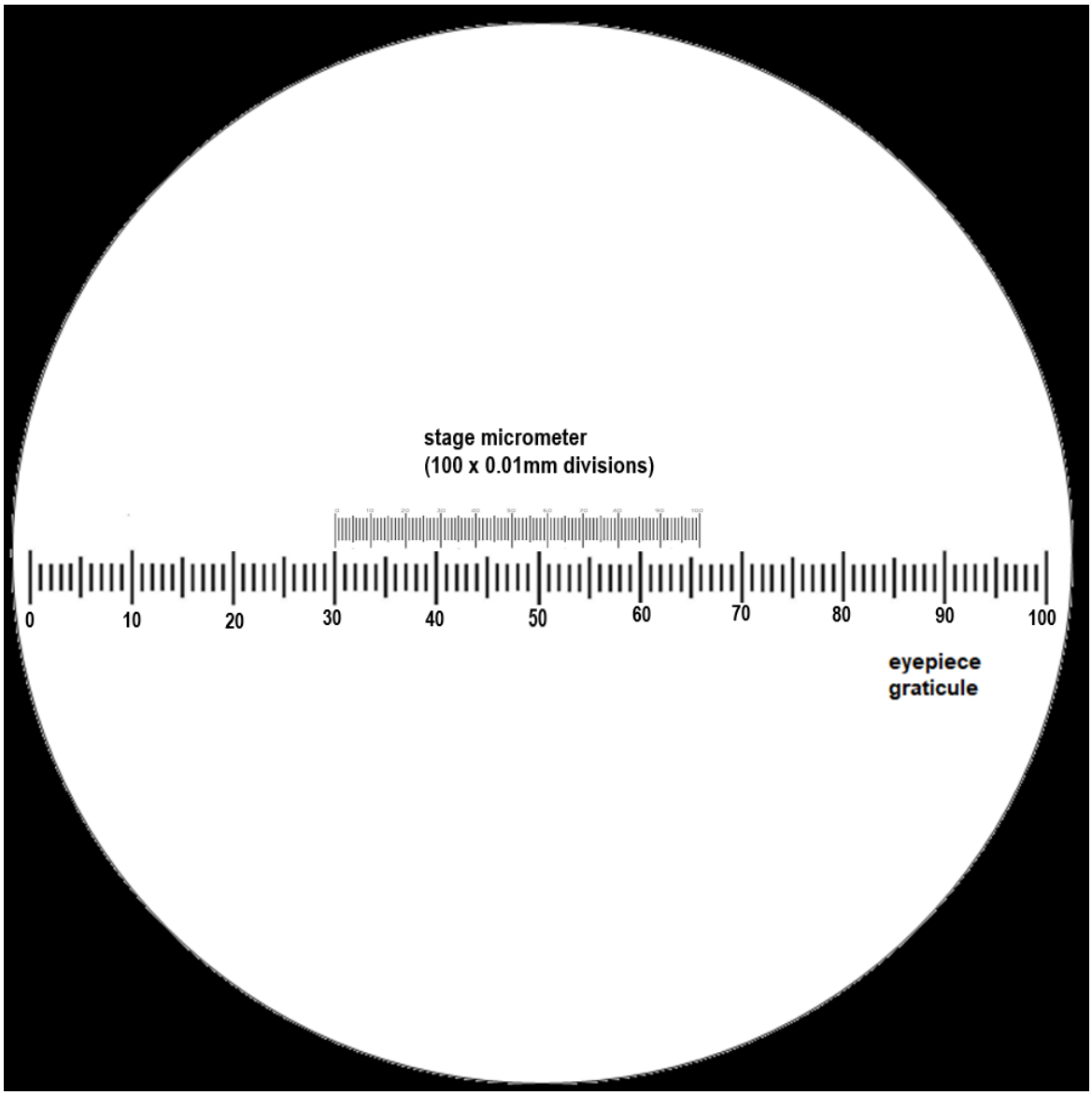


At the point indicated by the line the developing ovary was 31 epu long. Use your answer to (a) to calculate the actual length of the embryo sac.

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.



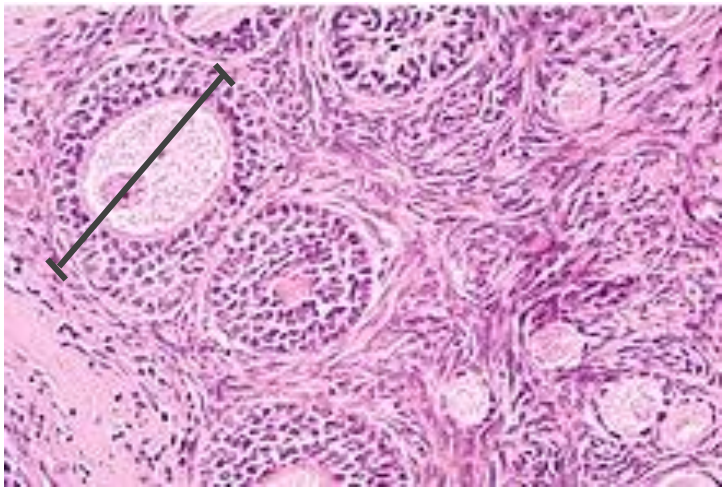
(a) Calculate the size of one eyepiece unit (epu) at this magnification. Give your answer to the nearest micrometre.

[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.**

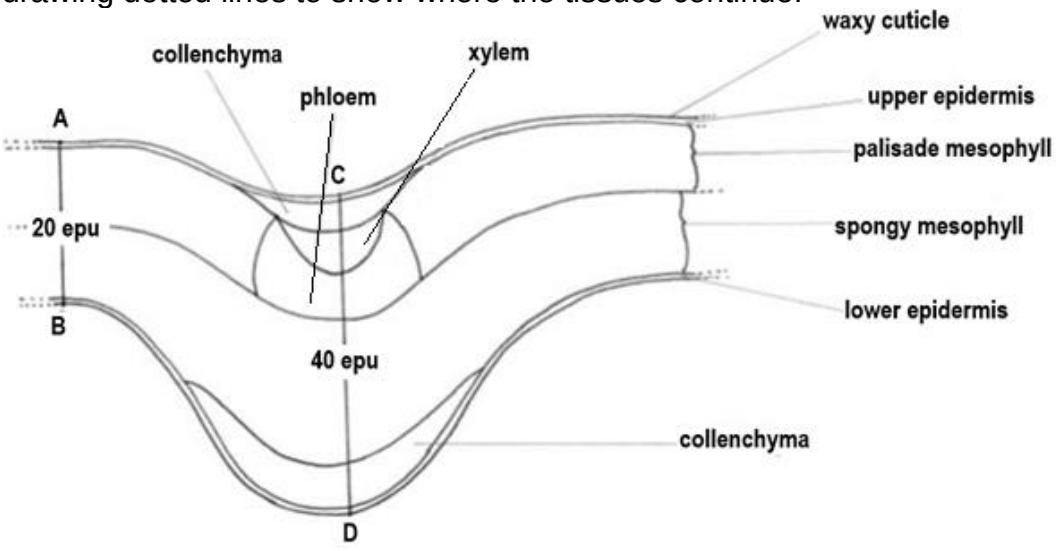
Working out:

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

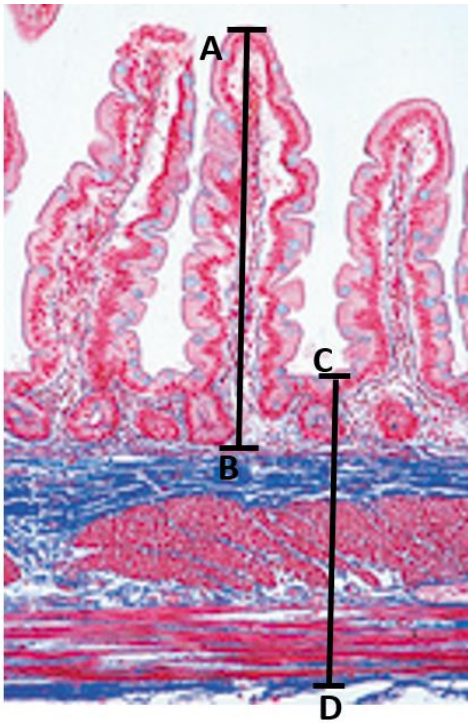
actual length 1 epu	=	18.5µm
image length C-D	=	40 epu
actual length C-D	=	40 x 18.5µm
	=	740µm

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)

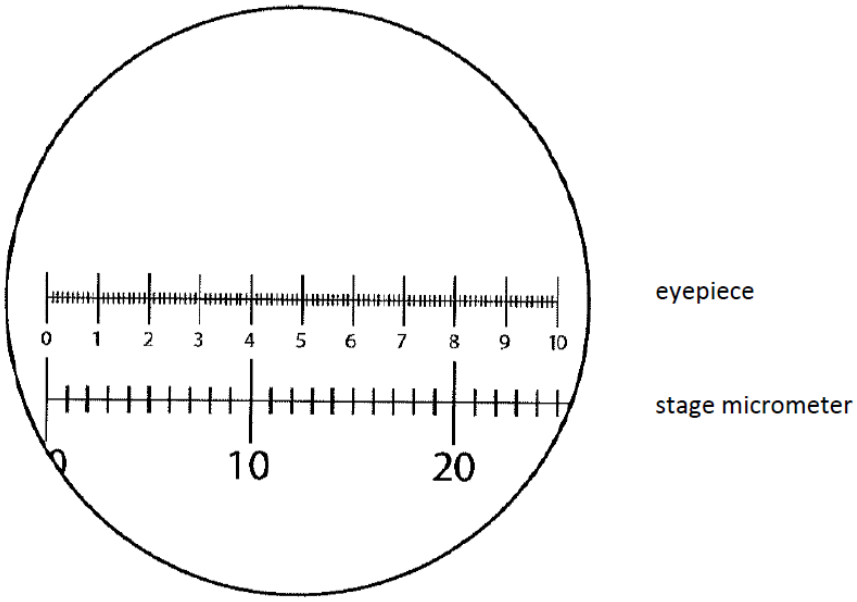
Practice Questions:

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:

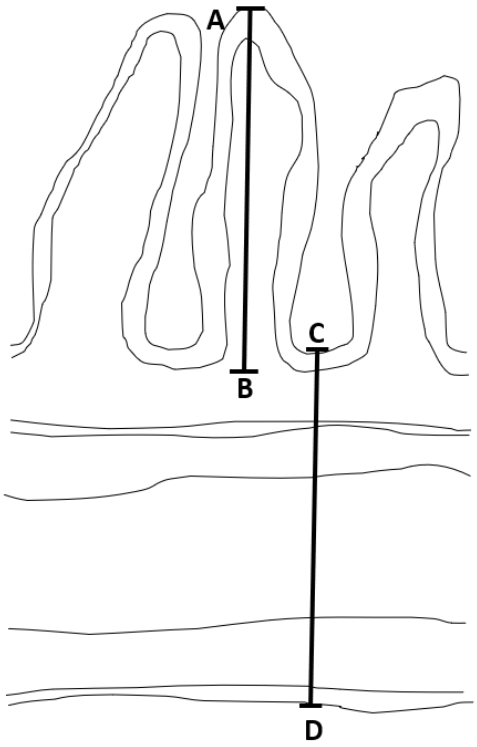
Actual length of A-B = [ ] µm

(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]

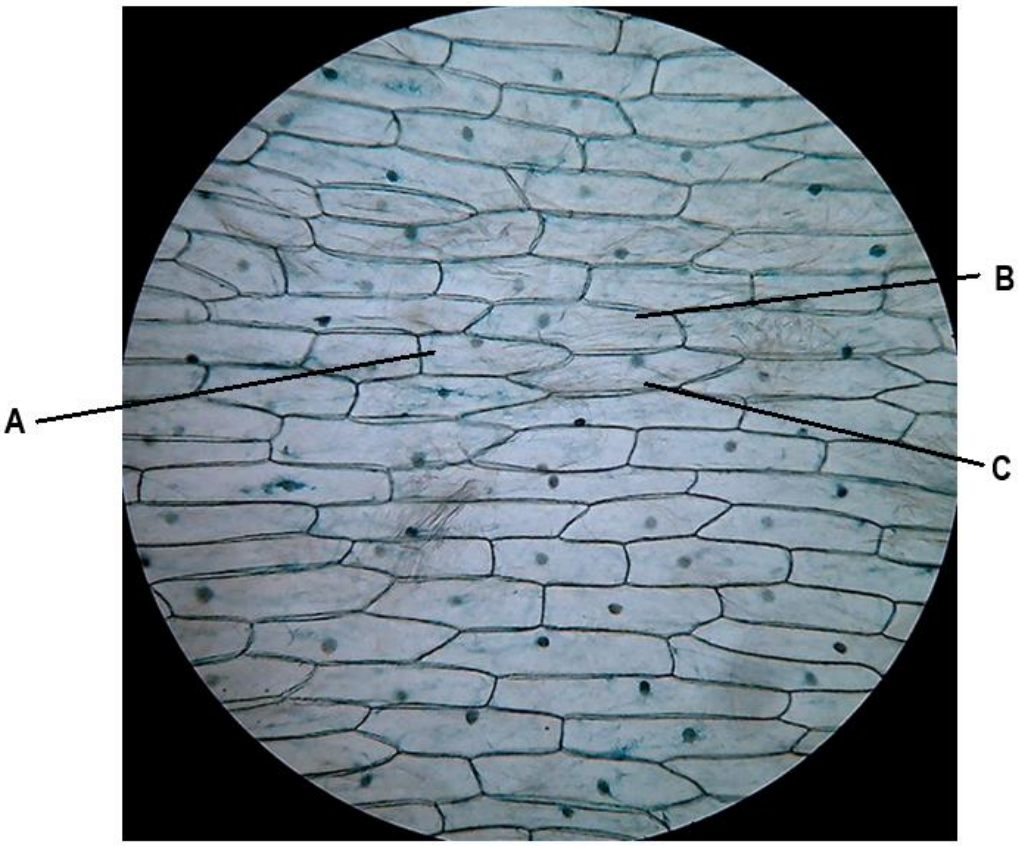
Working out:

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

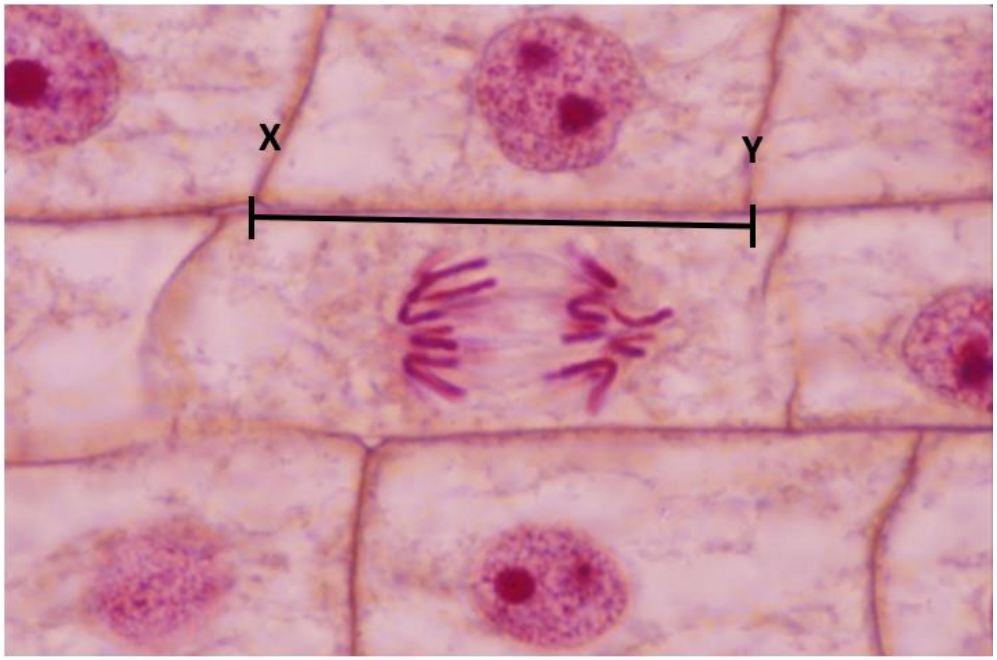
Width cell A in plan =		**Width cell C in plan =**	
Working out:			
		Ratio A : C =	=

(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.