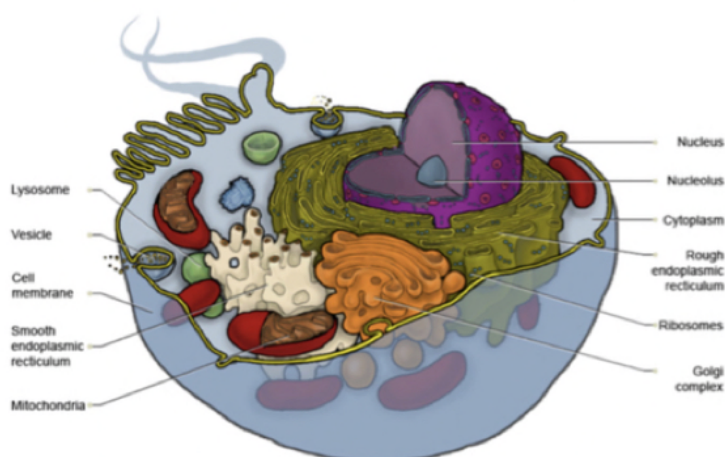


3.2.1 Cell Structure and Microscopy

Name **Teacher Booklet**



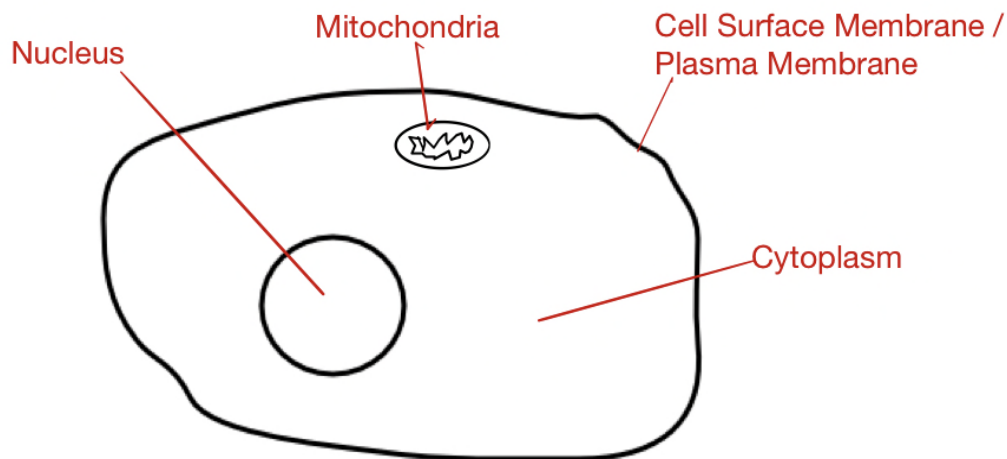
Reflection on learning outcomes	☺ or ☹	Target
Define what is meant by a eukaryotic cell.		
Explain the roles of different components and organelles within eukaryotic cells and interpret pictures, diagrams and electron micrographs to identify cell organelles.		
Identify and explain key adaptations of specialised eukaryotic cells.		
Be able to identify levels of organisation in terms of cells, tissues, organs and organ systems.		
Describe the structure of prokaryotic cells, explaining the purpose of Prokaryotic structures including plasmids, capsules and mesosomes.		
Describe the structure of virus particles and explain the functions of the structural components including the Genetic material, Capsid, Capsule and attachment proteins.		
Explain the difference between magnification and resolution.		
The principles and limitations of optical microscopes, transmission electron microscopes and scanning electron microscopes.		
How to prepare a slide to view under the Optical (Light microscope)		
Use the formula $\text{Magnification} = \frac{\text{Size of Image}}{\text{Size of Real object}}$		
Measuring the size of an object viewed with an optical microscope using an Eyepiece Graticule and a Stage micrometer.		
Principles of cell fractionation and ultracentrifugation as used to separate cell components.		

Outcomes:

- Define what is meant by a eukaryotic cell.
- Explain the roles of different components and organelles within eukaryotic cells and interpret pictures, diagrams and electron micrographs to identify cell organelles.

What do you remember from GCSE?

1. Label the **structures** on the simple diagram of an **animal cell** below and briefly describe the **function**
2. Add any other **Cell structures** you know of to this Cell diagram



Definition

Eukaryotic Cells: *Have a distinct nucleus and membrane bound organelles*

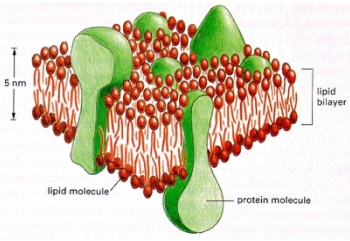
Task – Make a revision sheet.

1. Detach the last page from the back of this pack. On A3 paper, cut out and arrange your different organelles. Some organelles have more than one picture so you may wish to group these together when doing your revision sheet. Use your notes here to do the following;
2. Each organelle must be named and labelled with detailed structure.
3. Each must have a description of function. You must include an appropriate amount of detail.
4. Make it clear which organelles are only found in plant cells.

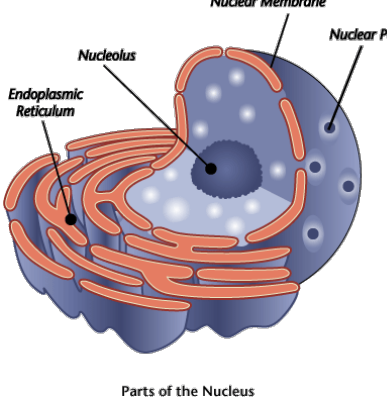


Cell structures and Organelles

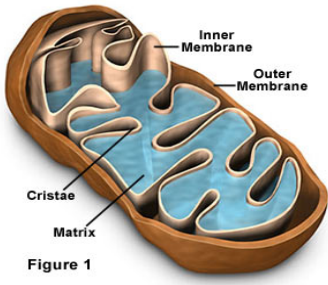
Cell Surface Membrane

Structure	Function
	- 7nm thick - plasma membrane
	- partially permeable membrane which controls exchange between the cell and its environment.

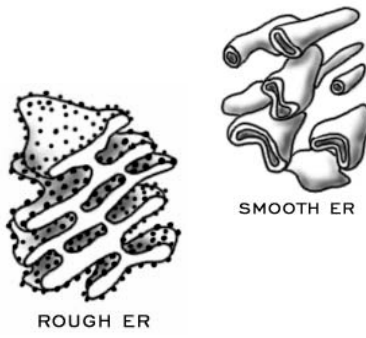
Nucleus

Structure	Function
	- Surrounded by 2 membranes known as the nuclear envelope. - The envelope has small holes or pores – nuclear pores, about 40-100nm across. - The nucleus contains linear DNA, chromosomes which are usually in a loosely coiled state known as chromatin. The nucleus contains a darker staining area, the nucleolus.
	- It controls the cells activities - Contains genetic material as DNA and chromosomes - Produces mRNA and tRNA - The nucleolus manufactures ribosomal RNA and ribosomes - Nuclear pores allow mRNA and ribosomes to leave the nucleus and nutrients and hormones to enter the nucleus - The nuclear envelope controls entry and exit of materials, and contains the reactions in it

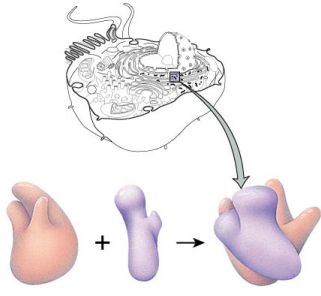
Mitochondria

Structure	Function
	- This organelle is surrounded by 2 membranes called an envelope - 1-10um in length - The inner membrane is folded to form projections called cristae (larger surface area) which project into the interior solution, the matrix - The matrix contains protein, lipids, ribosomes and DNA so they can produce their own proteins.
	- The later stages of aerobic respiration. - They make ATP.

Endoplasmic Reticulum

Structure	Function
	- extensive system of membranes, continuous with the nuclear membrane. - forms a system of flattened sacs called cisternae. - Ribosomes can be attached to the membranes = rough endoplasmic reticulum. - No ribosomes = smooth endoplasmic reticulum,
	<u>Rough ER</u> - Rough ER provides a large surface area for the synthesis of proteins and glycoproteins, and then transports these proteins through the cell. - Makes the golgi apparatus <u>Smooth ER</u> - synthesises, stores and transports lipids and carbohydrates.

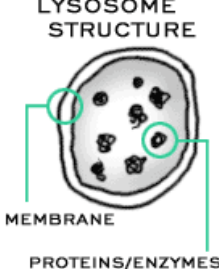
Ribosomes

	Structure - can be found free in the cytoplasm or attached to endoplasmic reticulum. - made of RNA and protein. - Found in two sizes: 80s in eukaryotic cells (25nm) and 70s in prokaryotes.	Function - Sites of protein synthesis.
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Golgi Apparatus

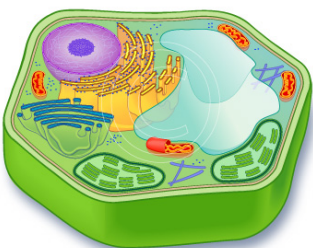
	Structure - stack of flattened sacs called cisternae. - formed from vesicles which bud off the rough ER, - broken down at the other end to form Golgi vesicles.	Function - Molecules, particularly proteins and lipids are collected, processed and sorted into golgi vesicles for transport - Transport, modify and store lipids - Make lysosomes
---	---	--

Lysosomes

	Structure - have a single membrane - Size up to 1.0 μm. - contain hydrolytic (digestive) enzymes e.g. proteases and lipases, and lysozymes.	Function - Digest old cell organelles and cells after they have died. - Hydrolyse material ingested by phagocytic cells e.g. white blood cells digest bacteria. - Release enzymes to the outside of the cell.
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Organelles in a plant cell

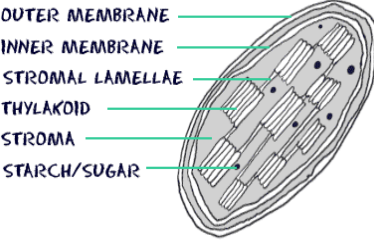
Cell wall

	Structure - Plant cells are surrounded by a relatively rigid cell wall made of polysaccharides such as cellulose embedded in a matrix. - The middle lamella is a thin layer marking the boundary between adjacent cell walls and cements cells together. - Algae cell walls are made up of cellulose or glycoprotein or a mixture. In fungi they are made of chitin.	Function - provides mechanical strength to the cell and the plant as a whole and prevents the cell from bursting when water enters by osmosis. - Allows water to pass along it contributing to movement of water through the plant. - freely permeable.
---	--	---

Large central vacuole

	Structure	Function
	- surrounded by a membrane called a tonoplast. - vacuole is fluid filled - Contains mineral salts, sugars and amino acids (temporary food store), oxygen, carbon dioxide, pigments (e.g. to colour petals).	- tonoplast controls the exchange of materials between the vacuole and the cytoplasm. - Make cells turgid to support herbaceous plants.

Chloroplasts

	Structure	Function
	- surrounded by 2 layers of membrane – envelope. - Around 2-10µm long. - Stroma a jelly like substance inside. - there are membranes forming flattened sacs called thylakoid membranes. - stacks of thylakoid membranes = grana. - Small starch grains and lipid droplets are present in the stroma.	- photosynthesis.

Exam Question

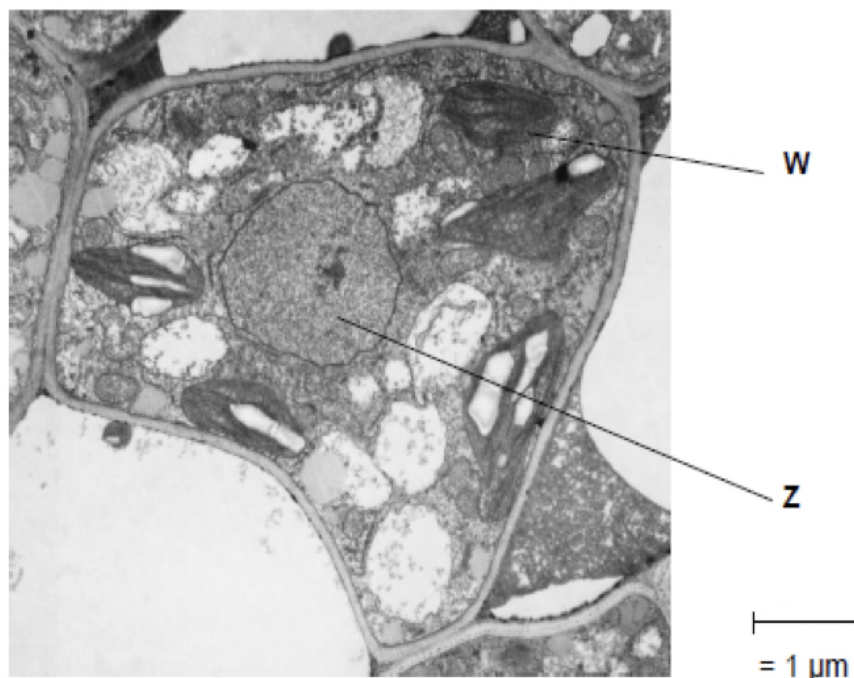
The figure shows a microscopic image of a plant cell.
Give the **Name** and **function** of structures W and Z.

Structure W Chloroplast

Function Site of Photosynthesis

Structure Z Nucleus

Function Contains DNA / Chromosomes / holds Genetic information of Cell



Check 1 - Which is the odd one out and why?

- Cell surface membrane, plasma membrane, **cell membrane**
Cell Membrane is not precise enough terminology at A-Level
- Rough endoplasmic reticulum, **smooth endoplasmic reticulum**, ribosomes
Smooth Endoplasmic reticulum not involved in Protein Synthesis
- Nucleus**, mitochondria, chloroplast
Contains linear DNA, unlike the Mitochondria and Chloroplast that possess Circular DNA
- Golgi body, rough endoplasmic reticulum, **lysosomes**
Lysosomes not involved in Protein Synthesis, Modification & Secretion
- Lysosomes, **mitochondria**, RER
Mitochondria contain DNA, the other two organelles don't possess DNA
- Nucleolus, chromatin, **ribosomes**
Ribosomes don't remain in the Nucleus, moving out into the Cytoplasm and RER

Check 2 – Answer these 4 synoptic questions

The diagram illustrates the hierarchical structure of cellulose. It begins with a green leaf, from which a section of the cell wall is magnified. A scale bar indicates 10 μm . This section shows cellulose microfibrils, with a further magnification showing a single microfibril with a scale bar of 0.5 μm . The microfibril is composed of multiple cellulose molecules. At the bottom, the chemical structure of cellulose is shown, consisting of a chain of α -glucose monomers linked by glycosidic bonds. The diagram includes four numbered questions and their answers:

- 1. Monomer?**
Alpha Glucose
- 2. Bond name?**
Glycosidic Bond
- 3. Bond name?**
Hydrogen bonds
- 4. Reaction that forms bonds?**
Condensation

Labels in the diagram include: Cell Wall, Cellulose microfibrils in a plant cell wall, Microfibril, Cellulose molecules, and 10 μm , 0.5 μm .

Eukaryotic Cells

Recap questions

1. Match the term to the definition:

Ultrastructure	Each type of organelle has a specific role within the cell
Eukaryotes	The detail of the inside of cells, as revealed by the electron microscope
Division of Labour	These cells have a distinct nucleus and membrane bound organelles

2. Suggest why:

a) chloroplasts are moved around plant cells.

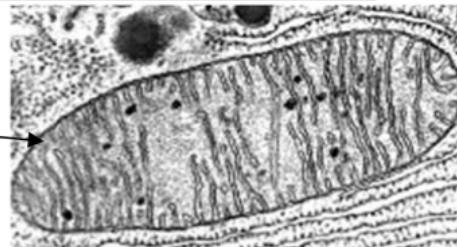
To maximise absorption of light energy for Photosynthesis

b) white blood cells need to be able to move.

To follow and engulf pathogens

3. a) Identify the organelle.

Mitochondria



b) Explain why there are many of these organelles in a muscle cell. (3)

Muscle cell needs a lot of Energy;

Mitochondria site of Aerobic Respiration

ATP produced

Energy Store

4. Cells that secrete enzymes contain a lot of rough endoplasmic reticulum (RER) and a large Golgi apparatus.

a) Describe how the RER is involved in the production of enzymes. (2)

Has Ribosomes;

Enzymes are Proteins and Ribosomes are the site of Protein Synthesis

b) Describe how the Golgi apparatus is involved in the **secretion of enzymes**. (1)

Modifies/packages (into vesicles)/transports proteins to cell surface

5. Complete the table to show three **differences** between plant and animal cell **structure**. Remember **comparative statements**.

Plant	Animal
- Has cell wall	No cell wall
- Has chloroplasts	No chloroplasts
- Has large central vacuole	No large central vacuole

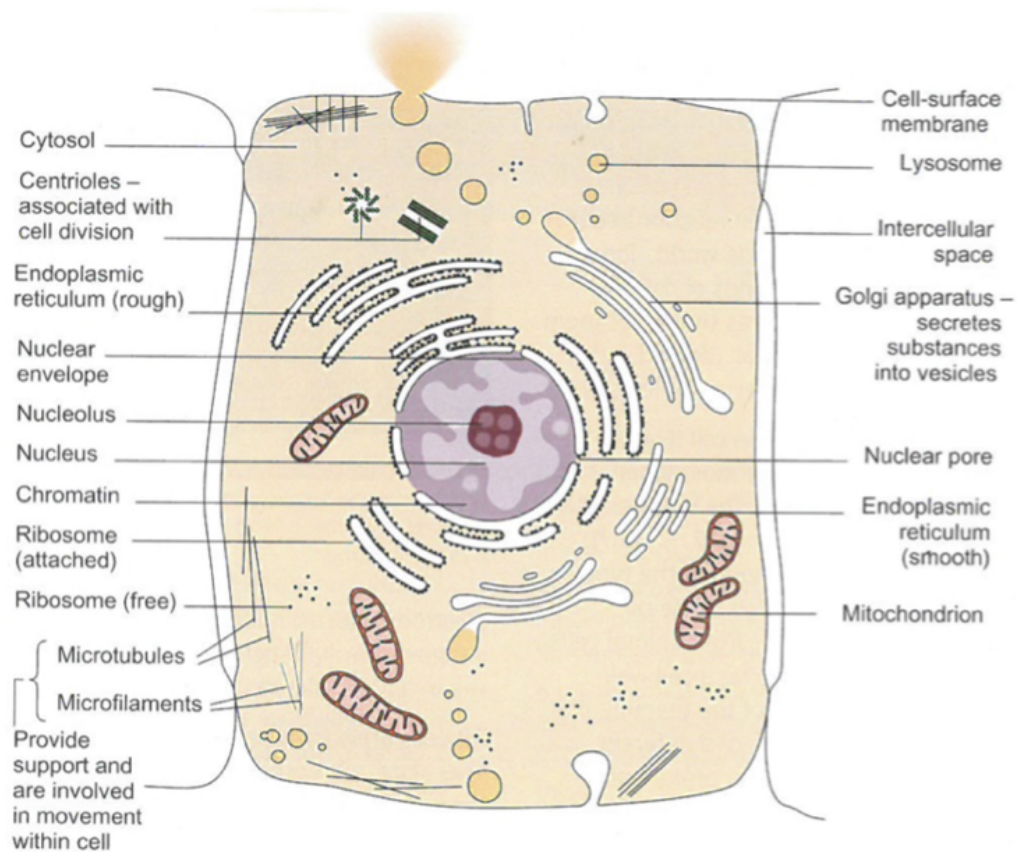
6. Now state three **similarities** between animal and plant cell structure.

Both e.g. ribosomes, nucleus, RER, SER, golgi, mitochondria

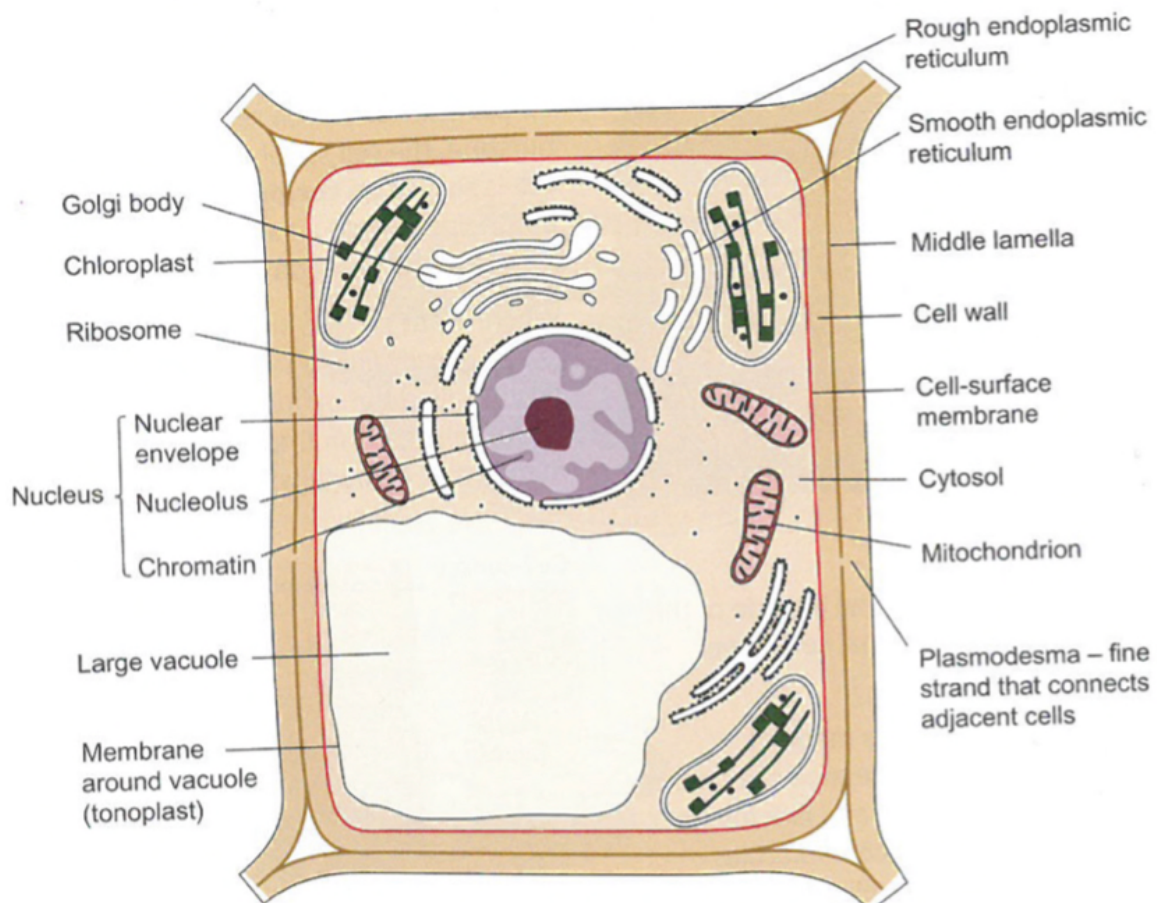
7. List the organelles which are membrane bound.

Nucleus, RER, SER, mitochondria, golgi apparatus, lysosomes, chloroplasts, vacuole

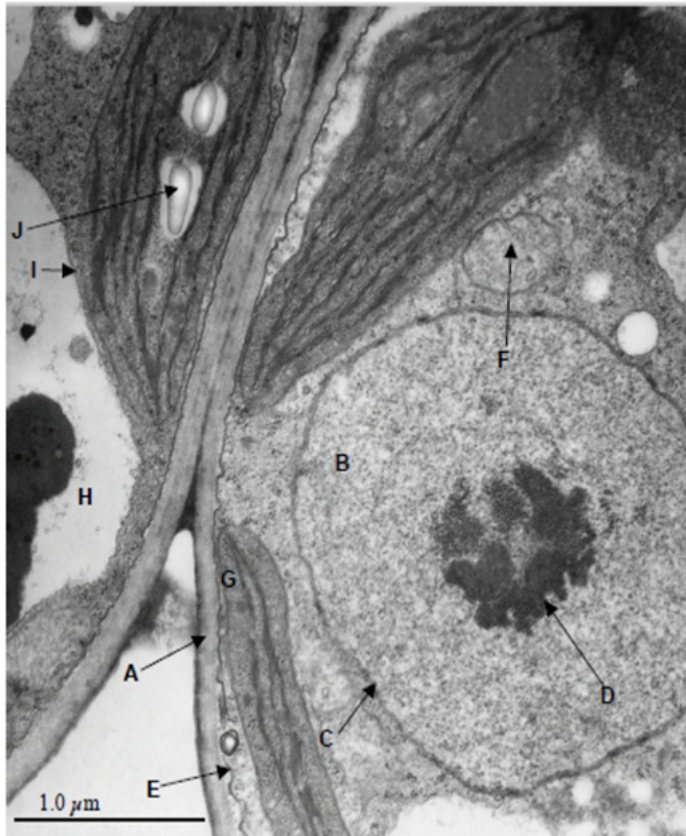
Ultrastructure of an Animal Cell



Ultrastructure of a Plant Cell



Identify the labelled structures and write a brief statement of their functions in the table below

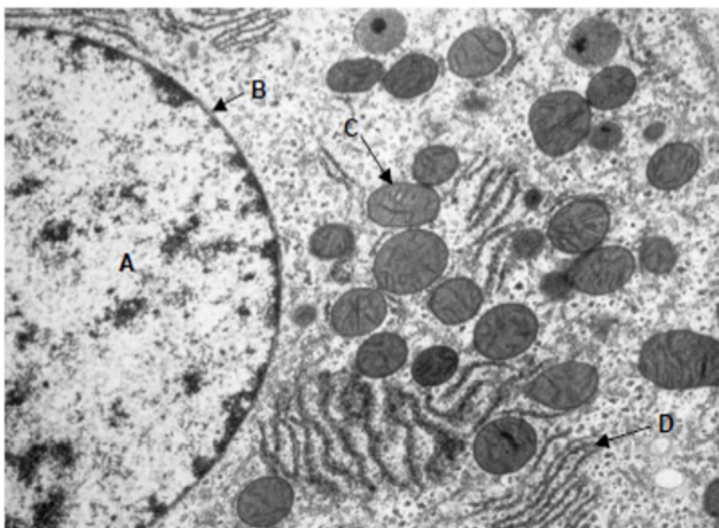


Label	Name of structure	Function
A	cell wall	Maintains the shape of the (plant) cell. Prevents the cell bursting.
B	nucleus	Contains the chromosomes/genetic material/DNA. The genetic code controls the activities of the cell.
C	nuclear envelope	Compartmentalises/separates the DNA/genetic material from the rest of the cell.
D	nucleolus	Contains DNA that controls the manufacture of ribosomes.
E	cell surface membrane	Controls which substances can enter and leave the cell; selectively permeable.
F	mitochondrion	Site of aerobic respiration. Organelle where (most) ATP is made.
G	chloroplast	Contains chlorophyll and is the site of photosynthesis. In the chloroplast grana/thylakoids, light-dependent reactions take place, producing reduced NADP and ATP. In the chloroplast stroma, light-independent reactions take place, producing carbohydrates.
H	sap vacuole	Storage of solutes in a plant cell.
I	tonoplast	Membrane around plant sap vacuole controlling which substances can enter and leave the vacuole.
J	starch grain	Storage of carbohydrate.

Q) Calculate the **magnification** of this image

$$\text{Scale bar} = 25\text{mm} = 25,000\mu\text{m}$$

$$25,000\mu\text{m} / 1.0\mu\text{m} = \times 25,000$$



1. Identify the labelled structure and write a brief statement of their functions in the table below.

Label	Name of structure	Function
A	nucleus	Contains the chromosomes/DNA/genetic material. Controls the activities of the cell.
B	nuclear envelope	Compartmentalises/separates the DNA/genetic material, from the rest of the cell.
C	mitochondrion	Site of aerobic respiration. Organelle where (most) ATP is made.
D	rough endoplasmic reticulum	The site of (most) protein synthesis, on the ribosomes.

2. The cell contains large numbers of the Structure labelled C. Explain why.

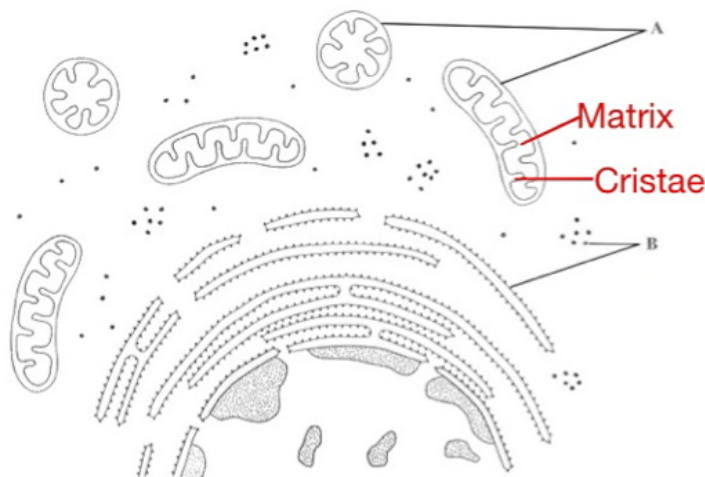
Mitochondria site of Aerobic Respiration;

Large numbers of Mitochondria enable large quantities of ATP to be produced

3. Measure the length of the structure labelled C on the Image. Use the magnification to find its real length.

Exam Questions

1. The diagram shows part of an animal cell as shown through an Electron Microscope.



- a) Name the **organelles** labelled A and B

A = Mitochondria

B = Ribosomes

- b) Explain why the shapes of the two organelles labelled A appear different.

Idea of sections of cuts, mitochondria in different positions, different 3D shapes (e.g. sausage shaped)

- c) Give the **function** of Organelle B

Protein Synthesis

- d) Label the internal structures visible in Organelle A

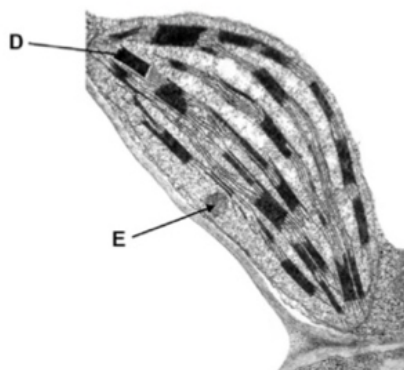
- e) State the **two** ways **organelle A** is adapted to carry out its **function**

Cristae for larger surface area

Matrix contains ribosomes and DNA for protein synthesis

Figure 3 is an electron micrograph of a chloroplast.

Figure 3



Identify structures labelled D and E.

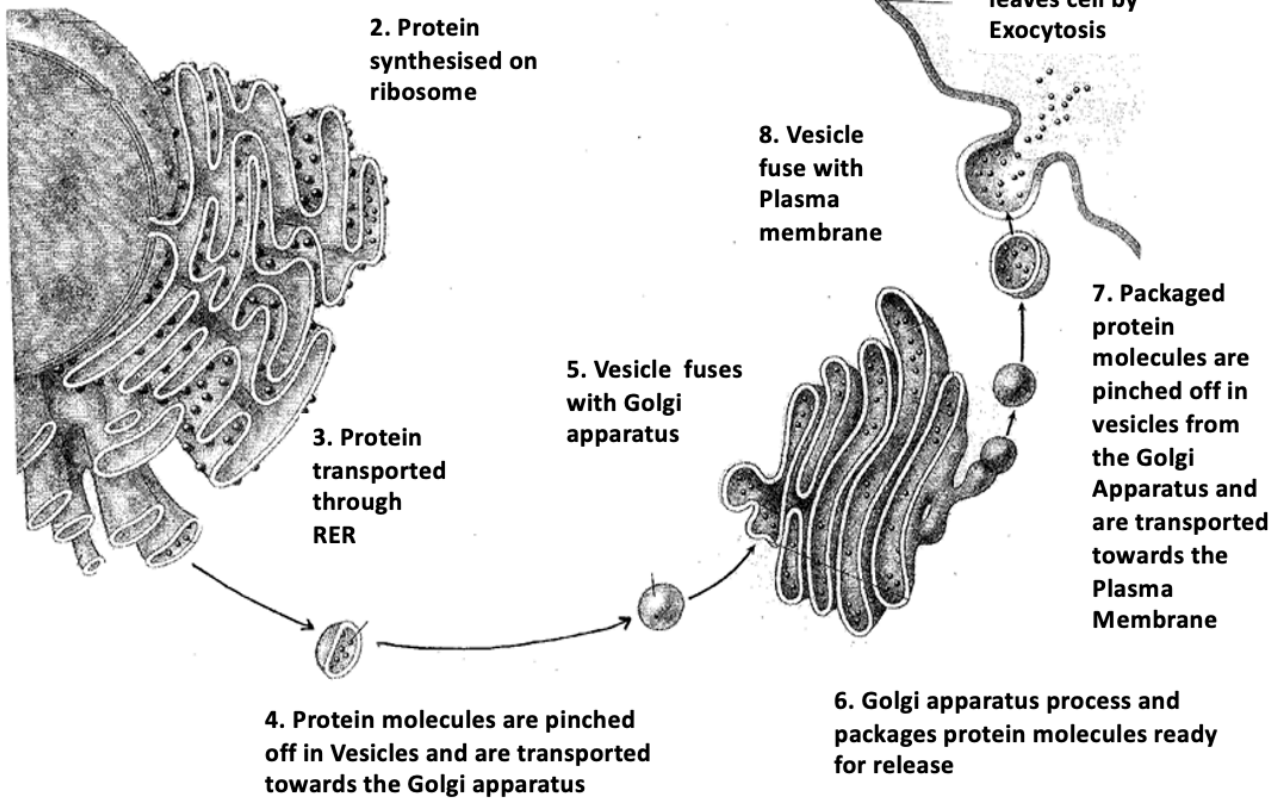
[2 marks]

D Thylakoids / Grana / Granum

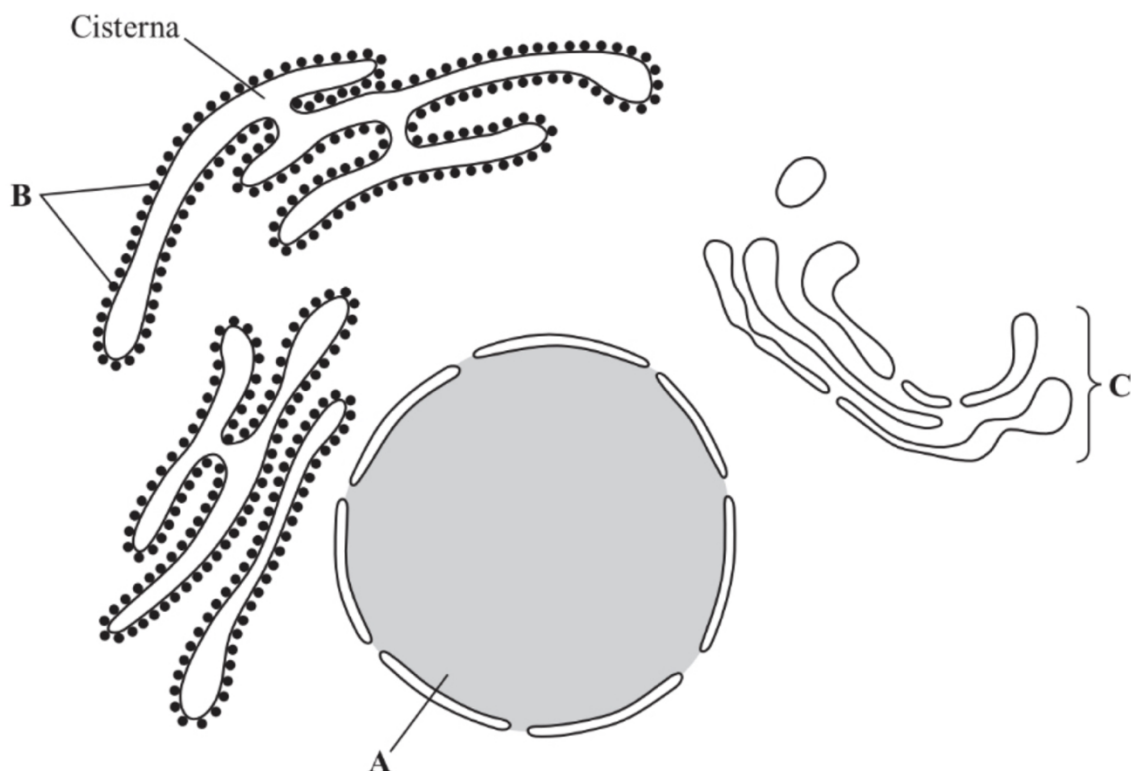
E Starch grain

1. DNA in nucleus contains instructions to make Proteins

Division of Labour – Protein Synthesis, Modification and Secretion



The diagram shows a section through part of a cell as it would appear when seen with an electron microscope.



- (a) This cell produces and secretes a protein. Describe the part played by organelles **A**, **B** and **C** in producing and secreting this protein.

- (a) **A** Carries the (genetic) code / genetic instructions / DNA / makes mRNA / transcription / makes ribosomes;
- B** Links amino acids / synthesises / makes protein;
- C** Involved in modifying / packaging protein / forms glycoproteins / forms vesicles;

3

- (b) The table shows information about the different parts of this cell.

Part of cell	Percentage of total cell volume	Number in the cell
Cytoplasm surrounding cell organelles	54	1
Mitochondria	22	about 1700
Nucleus	6	1
Lysosomes	1	about 300
Cisternae of rough endoplasmic reticulum	9	1

In the drawing there appear to be a number of separate cisternae in the rough endoplasmic reticulum. The table gives the approximate number of cisternae as one. Suggest an explanation for the apparent difference.

- (ii) With electron microscopes sections must be cut;
Cisternae are joined to each other;
Outside plane of section;

2 max

Outcomes:

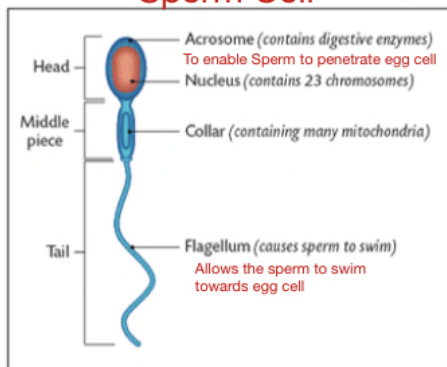
- Identify and explain key adaptations of specialised eukaryotic cells.

Specialised Cells

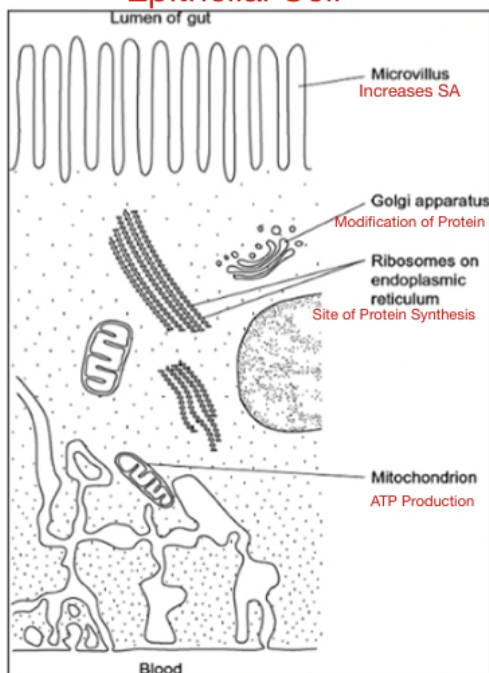
Here are some examples.

1. Name each cell or type of cell.
2. Label the function of any named structures.
3. In your pairs, come up with some rules to apply to any specialised cells e.g. microvilli for large surface area for more exchange

Sperm Cell



Epithelial Cell



Rules:

Microvilli

Increases SA

Flagellum

Provides Motility

RER/Ribosomes

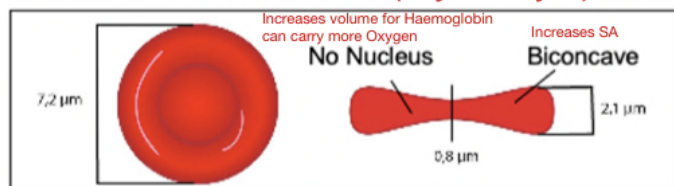
Site of Protein Synthesis

Anything else

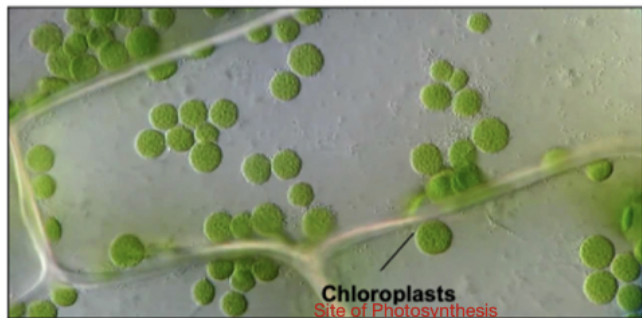
Mitochondria - ATP

through Aerobic Respiration

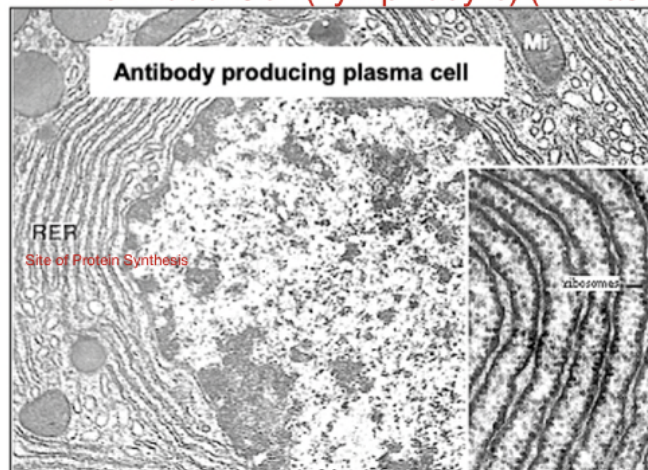
Red Blood Cell (Erythrocyte)



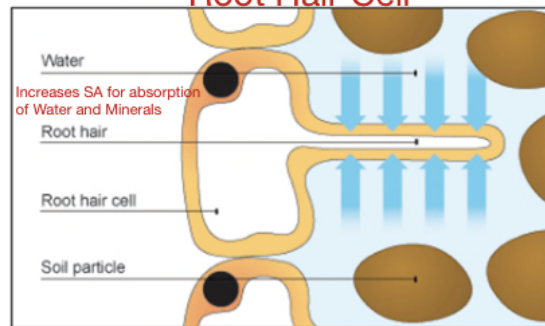
Palisade Cells



White Blood Cell (Lymphocyte) (B Plasma Cell)



Root Hair Cell

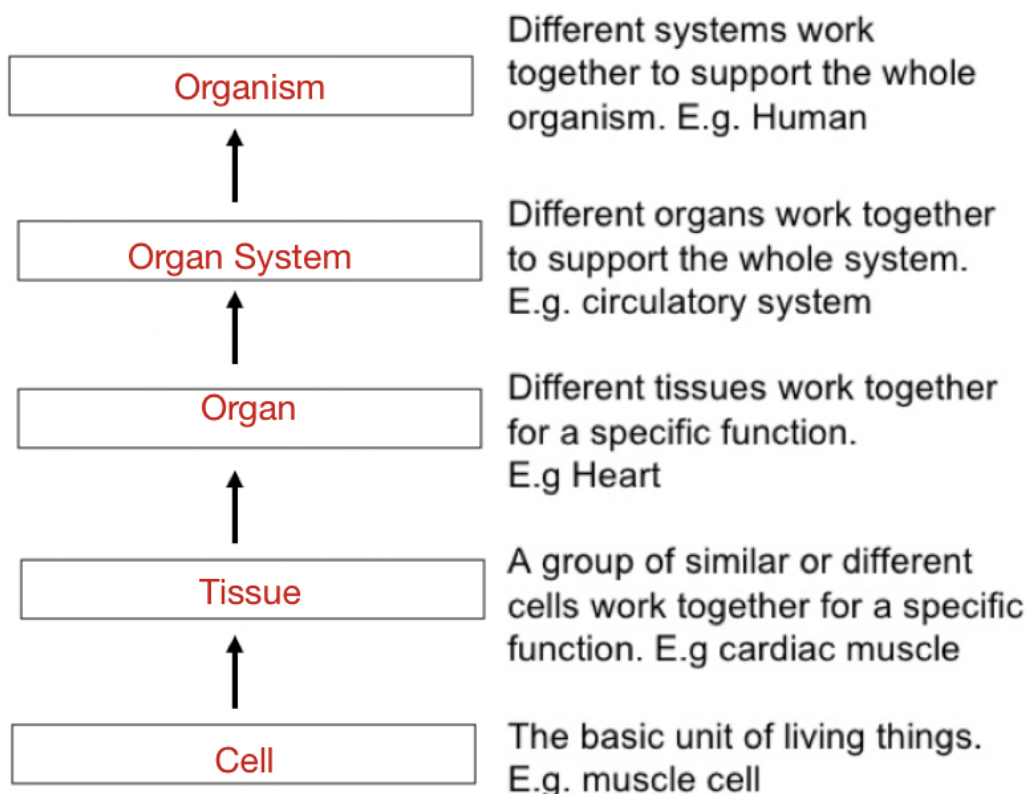


Outcomes:

- Be able to identify levels of organisation in terms of cells, tissues, organs and organ systems.

Organisation

Using page 73 and 74 of the Toole and Toole text book, pick from the following words to put in the boxes below to describe how cells are organised: **Tissue, Organ system, Cell, Organism, Organ**. Give an additional example next to the definitions e.g. next to heart, you could have pancreas, leaf etc.



Use the text book to answer the following questions:

1. Define the term **tissue**

A group of similar cells that perform a specific function

2. Explain why an artery is described as an **organ** whereas a capillary is not.

An artery is composed of different types of tissue performing different function e.g.

Muscular tissue, Endothelial tissue and Connective tissue, whilst a capillary is made up of only one type of tissue (Endothelial)

3. State whether each of the following is a tissue or an organ:

	Tissue	Organ
Liver		X
Lungs		X
Phloem	X	
Epithelium	X	
Salivary Glands		X
Trachea		X
Root		X

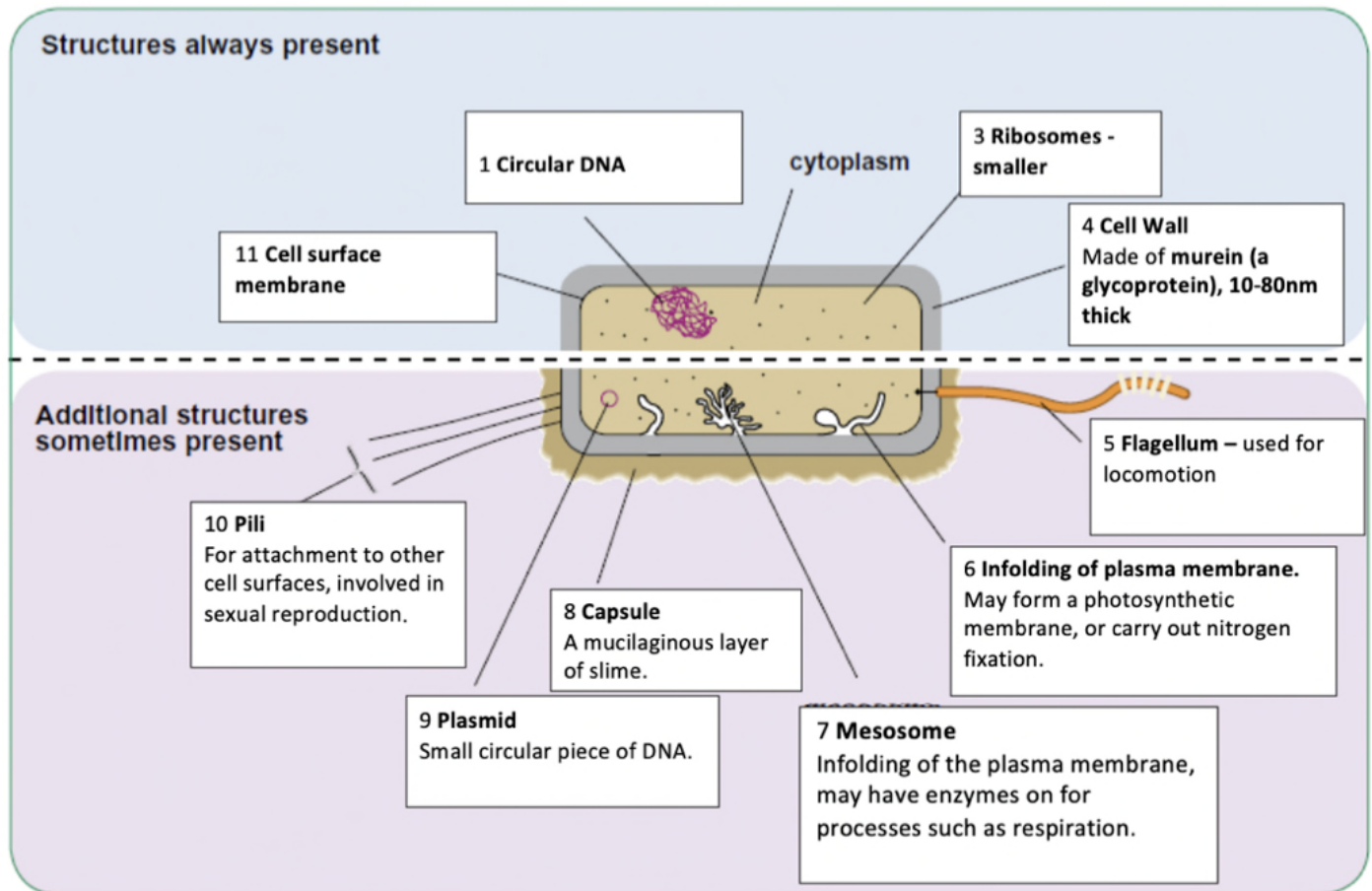
4. Using the information on the PowerPoint slide explain why **Xylem** is considered to be a **tissue**.
The cells of the Xylem are a collection of similar cell types that perform a specific function.
It is used to transport Water and minerals through the plant and gives mechanical support

Outcomes:

- Describe the structure of prokaryotic cells, explaining the purpose of Prokaryotic structures including plasmids, capsules and mesosomes.



Prokaryotic cells are smaller and have no nucleus or other membrane bound organelles.



The structure of a typical prokaryotic cell.

Which of the structures in a prokaryote are **only** present in a prokaryote, and not in a eukaryote?

Circular DNA, Mesosome, Capsule, Plasmid, Pili

Which of the structures in a prokaryote are **always** present in a eukaryote as well?

Cytoplasm, Cell Surface membrane (Plasma Membrane),

Match the Structure to the Function

Structure	Function
Cell Wall	Site of Protein Synthesis
Capsule	Lipid Storage. May be converted to carbohydrate and used in respiration
Plasma Membrane	Store Carbohydrates for breakdown during respiration
Lipid Droplets	Locomotion
Flagellum	Small circle of DNA which contains genes which help bacteria survive adverse conditions
Ribosomes	Made of murein. Physical barrier which prevents mechanical damage to cell
Circular DNA	Protection and helps bacteria stick together
Plasmids	Controls entry & exit of chemicals into/out of the cell
Glycogen Granules	Contains genetic information of the cell

Compare the Structure of Prokaryotes and Eukaryotes

Prokaryote	Structure	Eukaryote
Smaller	Diameter – smaller or larger?	Larger
Circular DNA not associated with Proteins	DNA organisation – circular or linear, and associated with proteins or not?	Linear DNA associated with Histone Proteins
Free in Cytoplasm	DNA location – nucleus or not?	Nucleus
Smaller	Ribosomes – smaller or larger?	Larger
No	Membrane bound organelles present?	Yes
Yes - Murein (Glycoprotein)	Cell Wall – if present, made of....	Animal Cell - No Plant - Yes (Cellulose) Fungi - Yes (Chitin)
No	Chloroplasts?	Yes
Yes	Flagella?	Some cells e.g. Sperm cells have flagella
Yes	Mesosome?	No
Yes	Pili?	No
Yes	Capsule?	No
Yes	Plasmids?	No

Exam Questions

a) Fig 1.1 is a diagram of a bacterium as seen under an Electron Microscope

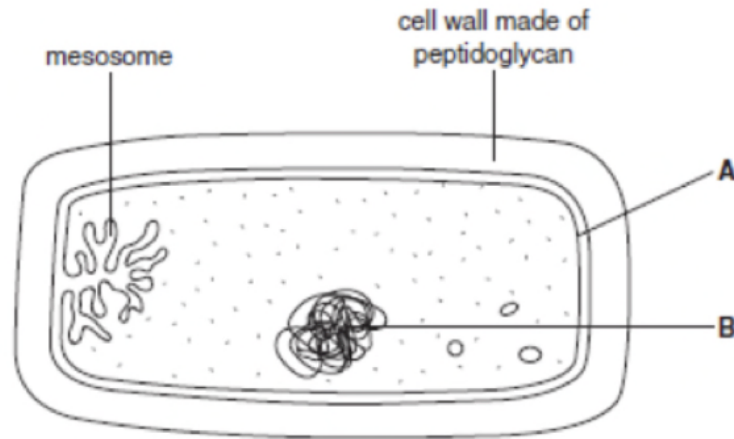


Fig.1.1

i) Name the structures labelled A and B

A Plasma Membrane

B Circular DNA (2)

ii) It has been suggested that the mesosome has the same role as Mitochondria in Eukaryotic Cells. Suggest the role of the mesosome in prokaryotic cells, such as bacteria.

Site of Aerobic Respiration where ATP is produced. Membrane is folded to provide a
Large Surface area for attachment of enzymes involved in Respiration (2)

iii) Eukaryotic cells, such as Euglena, contain membrane-bound organelles. Each organelle has a specific function in the cell.

State the **process** that is carried out in each of the organelles listed below.

Ribosome Protein Synthesis

Chloroplast Photosynthesis (2)

Fig 2.1 is a diagram of a cell showing the organelles involved in the production and secretion of an extracellular protein. The rough endoplasmic reticulum (RER) is shown enlarged at the side of the diagram.



Fig. 2.1

a) Name the structure labelled C, D and E.

C Vesicle

D Plasma Membrane / Cell Surface Membrane)

E Rough Endoplasmic Reticulum/ RER (3)

b) The cell shown in Fig. 2.1 is a eukaryotic cell.

i) Identify **two** features, **visible** in Fig 2.1, which would not be present in a Prokaryotic Cell.

Nucleus / Nuclear Membrane / Rough Endoplasmic Reticulum (RER) /

Golgi apparatus / Mitochondria (2)

ii) Name **one** feature that would be present in the cytoplasm of a prokaryotic cell that is not found in a Eukaryotic cell.

Circular DNA / Plasmid / Smaller Ribosomes

(1)

Outcomes:

- Describe the structure of virus particles and explain the functions of the structural components including the Capsid, Capsule and attachment proteins.



Virus

- Have no cellular structure – they are **acellular** (particles)
- They are **non-living**
- They can only reproduce when inside other cells (host cells)
- 50 times smaller than bacteria - **20nm to 300nm**

There are a thousand micrometers (μm) in a millimetre (mm).

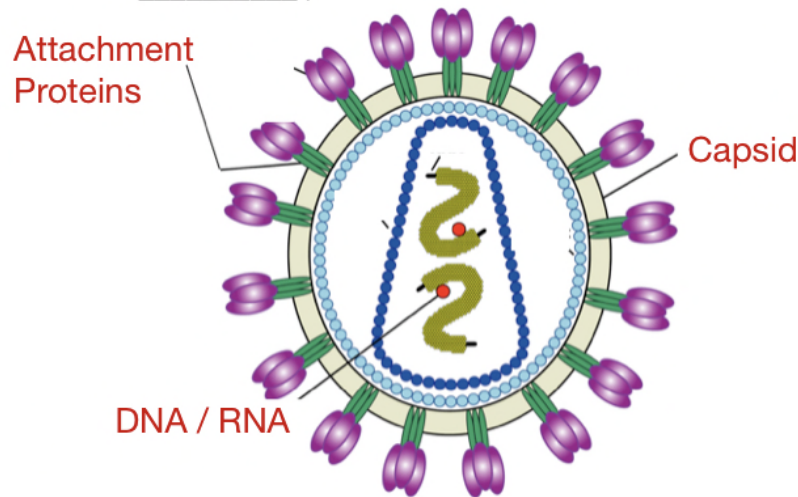
Convert the following to micrometers, b) convert the size of each cell in mm to standard form;

Prokaryotic cell: 0.006mm long = 6 μm , standard form $6 \times 10^{-3} \text{ mm}$

Eukaryotic cell: 0.04mm long = 40 μm , standard form $4 \times 10^{-2} \text{ mm}$

Convert the size of a virus to μm :

300nm $\longrightarrow$ 0.3 μm



- Nucleic acids** such as **DNA or RNA** as genetic material
- The **capsid** is a protein coat that encloses the nucleic acid.
- Some viruses have a lipid envelope.
- The lipid envelope, or if this is not present, the capsid, have **attachment proteins** which are essential to allow the virus to **identify and attach to a host cell**.

Q Think about the structures that a eukaryote or prokaryote has. Based on that, can a virus:

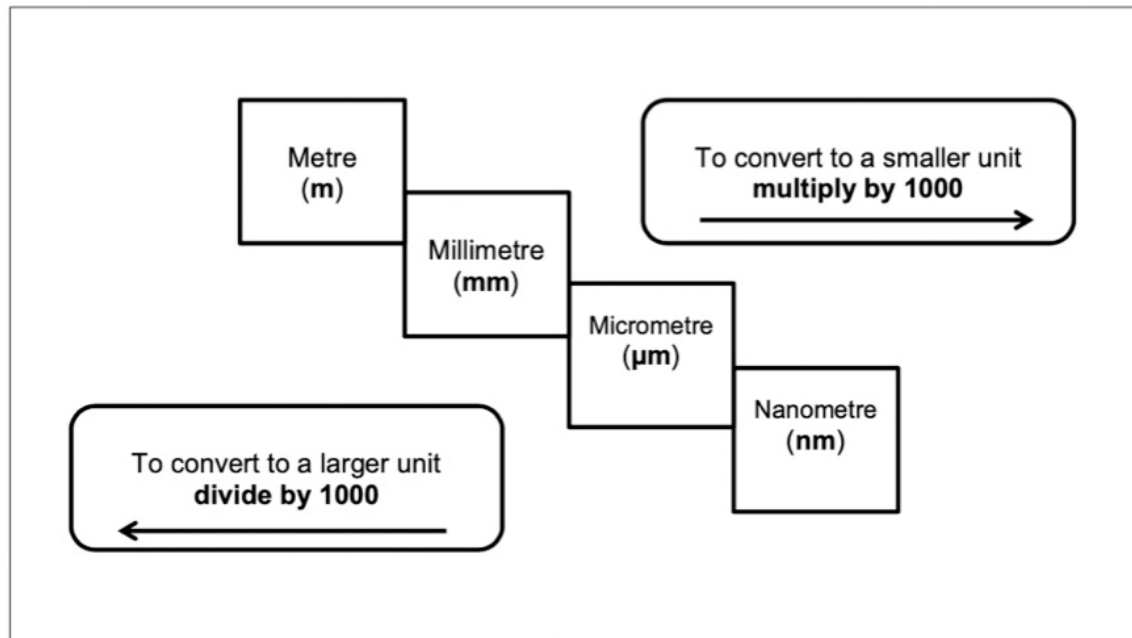
- a) Carry out respiration? No
- b) Carry out photosynthesis? No
- c) Carry out protein synthesis? No
- d) Modify proteins? No
- e) Make lipids? No

A virus injects its genetic information into a host cell and the host cell replicates it for them.

Outcomes:

- Explain the difference between Magnification and Resolution
- How to prepare a slide to view under the Optical (Light microscope)
- The principles and limitations of optical microscopes, transmission electron microscopes and scanning electron microscopes.

Magnification



$$10 \text{ m} = \underline{10,000} \text{ mm} \quad 20 \text{ mm} = \underline{20,000} \text{ µm} \quad 43 \text{ µm} = \underline{43,000} \text{ nm}$$

$$14 \text{ m} = \underline{14,000,000} \text{ µm} \quad 87 \text{ mm} = \underline{87,000,000} \text{ nm} \quad 296 \text{ mm} = \underline{296,000} \text{ µm}$$

$$80,000 \text{ µm} = \underline{80} \text{ mm} \quad 6532 \text{ nm} = \underline{0.006532} \text{ mm} \quad 74 \text{ mm} = \underline{74,000} \text{ µm}$$

**Definitions:**

- **Magnification** = how many times bigger the image is when compared to the object.
- **Resolution** = the minimum distance apart that two objects can be in order for them to appear as separate items. The higher the resolution, the greater the clarity (the image is more clear and precise).

Many **biological specimens are colourless** when they have been **cut into very thin sections** in preparation for viewing with a microscope and so stains are used. **Stains** are coloured chemicals that bind to the specimen. Some stains bind to specific organelles, e.g. iodine stains starch blue/black and methylene blue stains the nucleus dark blue and cytoplasm light blue in living cells.

How does a light microscope function?

- The **light** then **passes through two lenses** the **objective lens** and the **eyepiece lens** where it is **magnified** and **focussed** to form an **image**.
- The **areas that light can easily pass through** e.g. Cytoplasm **appear bright in the image**, while the **nucleus (heavily stained) appears dark** due to the **light finding it more difficult to pass through**.

Light Microscopes

- The maximum resolving power is 0.2µm.

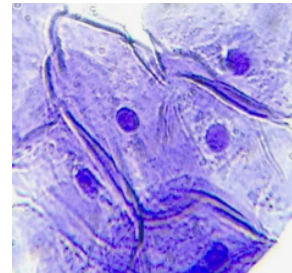


Preparing a slide

1. Add drop of water to (glass) slide;
2. Obtain thin section (of plant tissue) and place on slide / float on drop of water;
3. Stain with/add iodine in potassium iodide;
4. Lower cover slip using mounted needle;

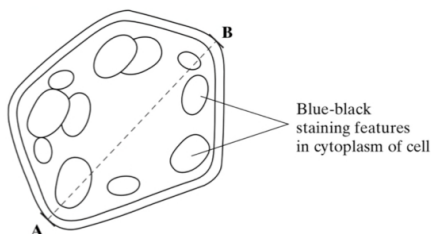
Exam Questions

Using this image of cheek cells seen under a light microscope explain why staining is important.

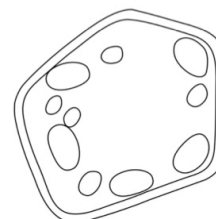


- The nucleus and cytoplasm is visible.
- There is increased contrast between the cytoplasm and surrounding solution and between the cytoplasm and nucleus.
- This allows identification of the nucleus and cytoplasm

Cells were scraped from the cut surface of a potato tuber. They were stained with iodine solution and examined with an optical microscope. The drawing shows one of these cells.



- (iii) The focusing knob of the microscope was adjusted slightly. The drawing below shows how the cell then looked.



- (i) Name **two** polysaccharides found in this cell which would not be found in a red blood cell.

1. . (i) Any two from:
2. . Cellulose / starch / amylose / amylopectin;
Do not allow starch with amylose or amylopectin.

1

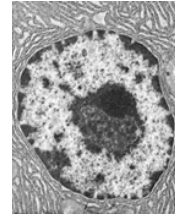
Explain the difference in appearance of the cell contents.

- (iii) (Focused) at different level / depth;
Looking at different structures / parts of structure;

2

The cell was stained with uranium salts in preparation for a transmission electron microscope. Explain how this stain caused the nucleus to show a dark shade compared with the light shade of the cytoplasm.

- **Stain** taken up by the **nucleus more than the cytoplasm**
- Electrons cannot pass through the stained parts of the nucleus so this appears in electron shade on the screen.
- **Electrons pass through the cytoplasm** and cause light emission (Fluorescence) on the screen



Electron microscopes - An electron micrograph is produced.

- Use a beam of electrons - **shorter wavelength than light**.

The resolution of an electron microscope is much **higher** than a light microscope. You can see internal structure of organelles that are too small to be seen under a light microscope e.g. ribosomes. The resolving power is **inversely proportional** to wavelength.

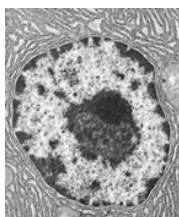
Why does an Electron microscope have a higher resolution than a light microscope?

- **Electrons** have a **smaller (shorter) wavelength** than **light**.
- Wavelength of light = 400nm, Wavelength of electrons = 1nm
- This means that unlike light, electrons can pass between two objects that are extremely close together.
- This means that **Electron Microscopes can distinguish two extremely close objects**.
- Two objects can be separated by as little as 0.1nm and still be **distinguished** as **separate objects** by a Transmission electron microscope.



1. TEM (transmission electron microscope)

Electron beam passes through a very thin section of the sample. **Stain** taken up by the **nucleus more than the cytoplasm**. Electrons cannot pass through the stained parts of the nucleus so this appears in electron shade on the screen. **Electrons pass through the cytoplasm** and cause light emission (fluorescence) on the screen. Image is **2D**. Higher magnification than both light microscope and SEM.



Resolution = 0.1nm. Why can't this sometimes be achieved?

- Difficulties preparing the specimen
- A higher energy electron beam is needed which may destroy the specimen.

2. SEM (scanning electron microscope)

Electron beam is directed on to the surface of the sample. The pattern of electrons being scattered is determined by the contours on the specimen's surface.

Image is **3D**. **Resolution = 20nm.**

Microscope comparisons

LIGHT (Optical)	SEM	TEM
Uses rays of light	Uses a beam of electrons	Uses a beam of electrons
Low resolution	High resolution= more detail	High resolution = more detail
Can observe both live and dead specimens	Can only observe dead specimens	Can only observe dead specimens
Maximum resolution of 0.2 micrometres (μm)	Maximum resolution of 0.0002 micrometres (μm) (0.2nm)	Maximum resolution of 0.0001 micrometres (μm) 0.1nm)
Maximum magnification = X 1500	Maximum magnification = X 15000000	Maximum magnification = X 15000000
Light is transmitted through a specimen	A beam of electrons is directed over the specimen. These electron 'knock off' electrons from the specimen's surface. The gathered 'knocked off' electrons are then used to form an image.	Electrons are transmitted through a specimen
Thin specimens required	Relatively thick specimens can be scanned	Thin specimens required
2D image: Image produced shows denser objects as darker ones.	3D image produced	2D Image produced shows denser objects as darker ones.

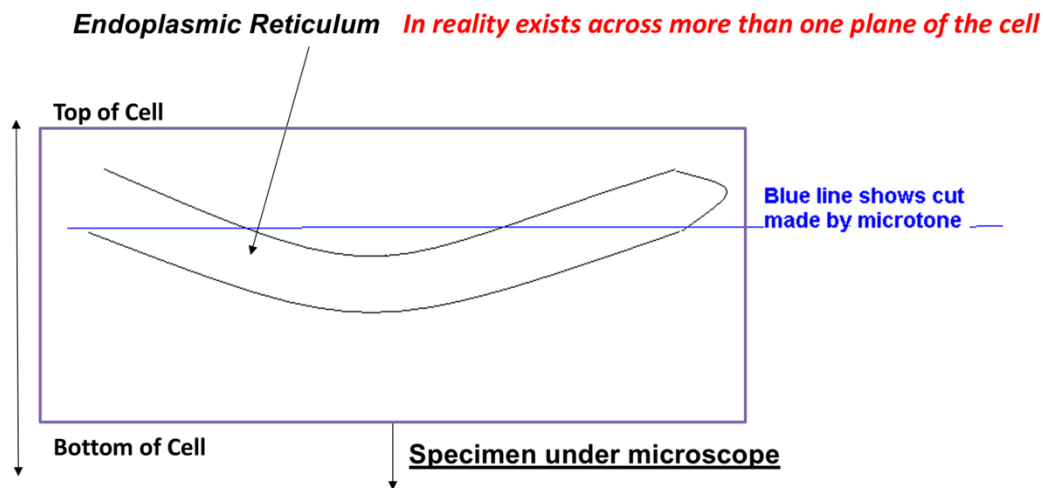
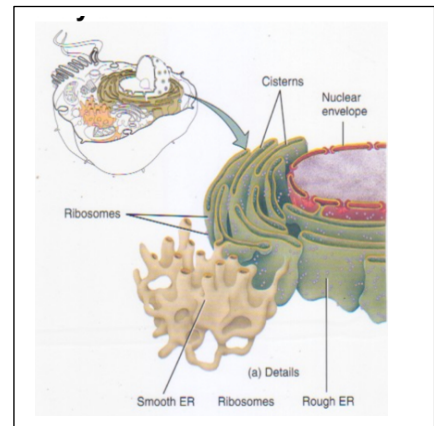
✔ Advantages and disadvantages of the different Microscopes

	Light	TEM	SEM
Advantage	Live specimens	High resolution	High resolution 3D image Can be thick specimen
Disadvantage	Poor resolution Need thin specimen	•Dead specimen only as it is in a vacuum •Needs very thin specimen •Difficulty preparing specimen can limit resolution •High energy electron beam may destroy specimen •Requires complex staining processes •Artefacts risen from preparation that are not part of the natural specimen.	As with TEM except that a thick specimen may be used as the electrons scatter rather than pass through.

Artefacts in Images

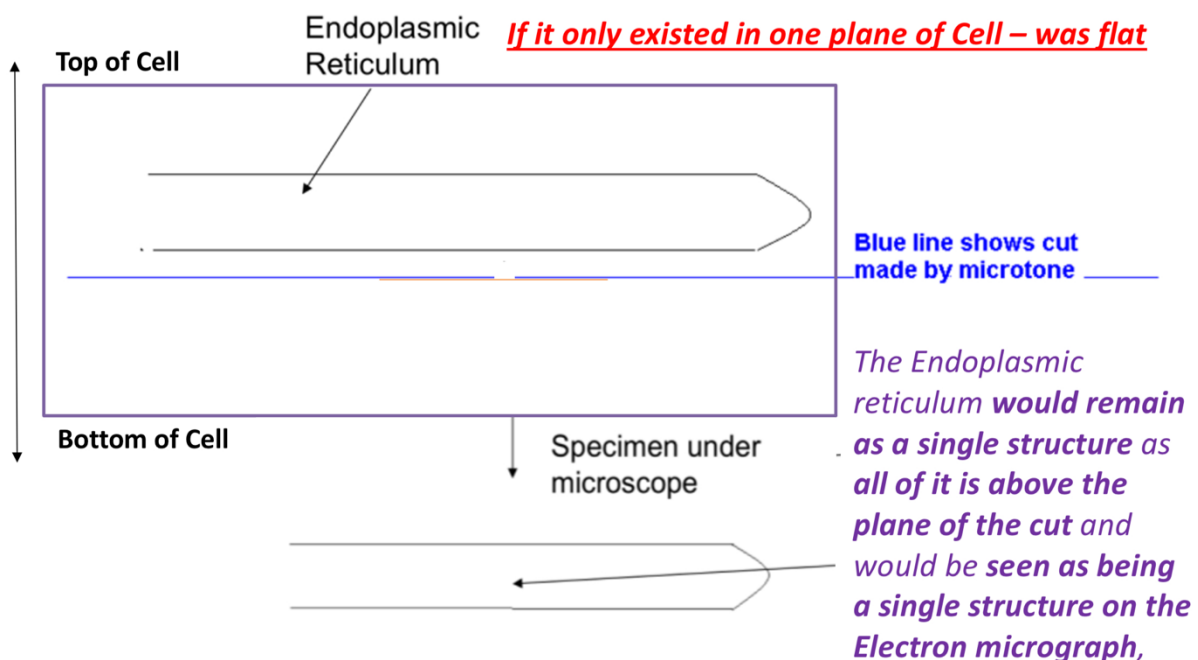
Because of the delicate cutting process required in preparing a specimen for the Transmission electron microscope, objects get fanned and broken up. This creates **artefacts**, which are structures that look different to how they originally did in the whole cell.

It would appear from the Micrograph that there are a large number of Smooth Endoplasmic cisternae in the cell, in fact there is only one. Can you explain this difference?



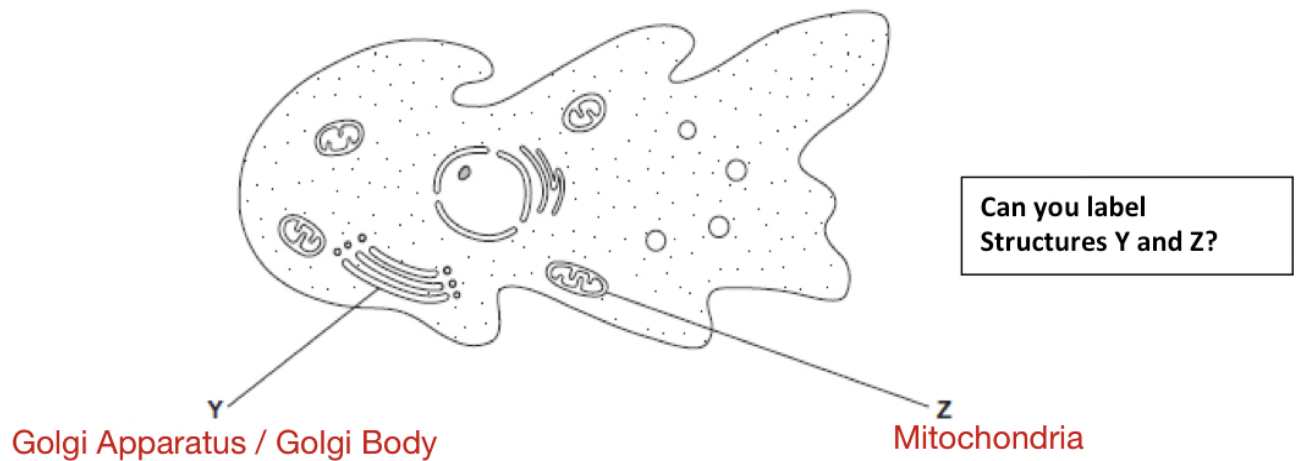
*The Endoplasmic reticulum is connected below the plane of the cut
Only the section above the line is viewed under the microscope, the lower half is disposed of!*

As a result the Endoplasmic Reticulum appears to be two separate structures on the Electronmicrograph



Exam Questions

An amoeba is a single-celled, eukaryotic organism. Scientists used a transmission electron microscope to study an amoeba. The diagram shows its structure.



The scientists used a transmission electron microscope to study the structure of the amoeba. Explain why.

- High resolution
- Shorter wavelengths
- To see internal structures/organelles/named organelles

Scientists use optical microscopes and transmission electron microscopes (TEMs) to investigate cell structure. Explain the advantages and limitations of using a TEM to investigate cell structure.

Advantage - small objects can be seen

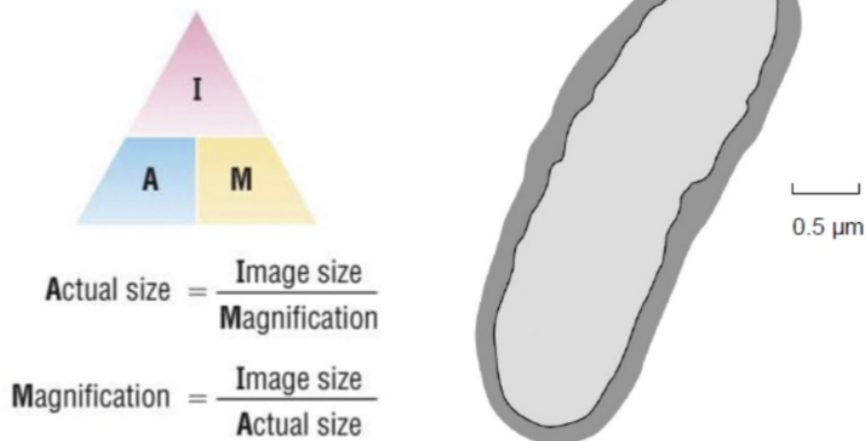
- TEM has high resolution
- Wavelength of electrons are shorter

Limitations – a not look at living cells

- Vacuum
- Thin sections
- Preparation may create artefact
- Doe not produce colour image

A bacterium is shown in **Figure 2**.

Figure 2



Calculate the magnification of the image.

[1 mark]

$$\text{Scale bar} = 10\text{mm} = 10,000 \text{ } \mu\text{m}$$

$$\text{Magnification} = \frac{10,000\mu\text{m}}{0.5 \text{ } \mu\text{m}}$$

$$\text{Magnification} = \underline{\quad \times 20,000 \quad}$$

Outcomes:

- Use the formula $\text{Magnification} = \frac{\text{Size of Image}}{\text{Size of Real Object}}$

Table 1 shows features of a bacterium and the human immunodeficiency virus (HIV) particle.

Complete **Table 1** by putting a tick (✓) where a feature is present.

[2 marks]

Table 1 shows features of a bacterium and the human immunodeficiency virus (HIV) particle.

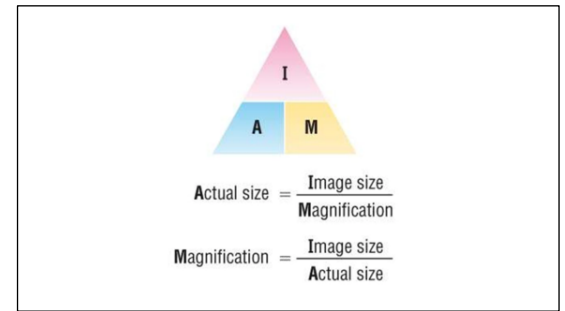
Complete **Table 1** by putting a tick (✓) where a feature is present.

[2 marks]

Feature	Bacterium	Human immunodeficiency virus (HIV) particle
RNA	✓	✓
Cell wall	✓	
Enzyme molecules	✓	✓
Capsid		✓

Magnification Calculations

- The use and manipulation of the magnification formula
Magnification = Measured size / Actual size
Actual Size = Measured size / Magnification

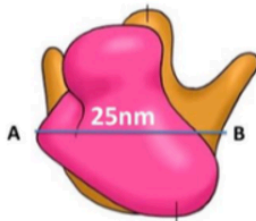


Example 1: When you are given the actual length of a Cell or Organelle (except for a ribosome* see example 2) and asked to calculate the magnification



1. Measure length of the Cell or Organelle in mm and then convert to μm ($\times 1000$)
2. You are given the Actual length of the Cell or organelle e.g. $1\mu\text{m}$
3. Magnification = $\frac{\text{Measured Length} - \text{of Cell or organelle } (\mu\text{m})}{\text{Actual length} - \text{of Cell or organelle } (\mu\text{m})}$

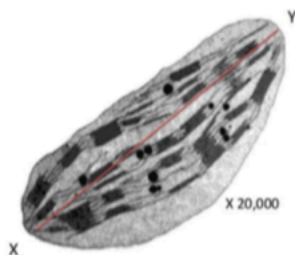
Example 2: If the Organelle is a Ribosome* - the only time you need to use nm



1. Measure length of the ribosome in mm and then convert to nm ($\times 1,000,000$)
2. You are given the Actual length of the ribosome e.g. 25nm
3. Magnification = $\frac{\text{Measured Length} - \text{of ribosome } (\text{nm})}{\text{Actual length} - \text{of ribosome } (\text{nm})}$

Top tip – Sometimes you may have to calculate the magnification of whole creatures, e.g. a Wasp. This may be measured in cm or mm. It's the same principle but obviously just keep the Measured length in mm, don't convert down to microscopic units of μm or nm as a wasp is not microscopic!

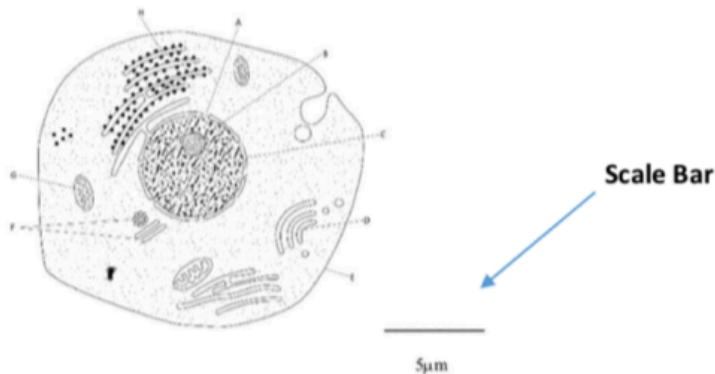
Example 3 : Calculating the Actual length of the Cell or Organelle except for a ribosome when you are provided with the magnification



1. Measure length of the Organelle or Cell in mm and then convert to μm ($\times 1,000$)
2. You are provided with the magnification of the Image e.g $\times 20,000$
3. Actual Length = $\frac{\text{Measured Length} - \text{of Cell or Organelle } (\mu\text{m})}{\text{Magnification}}$

Example 4: When you are provided with a Scale bar

E.g. Calculate the Actual length of Organelle G using the scale bar



Part A - Use the Scale bar to find Magnification

1. Measure the length of the scale bar in mm and then convert to μm ($\times 1000$)
2. Calculate the Magnification of the scale bar

$$\text{Magnification} = \frac{\text{Measured Length - scale bar } (\mu\text{m})}{\text{Actual length - scale bar } (\mu\text{m})}$$

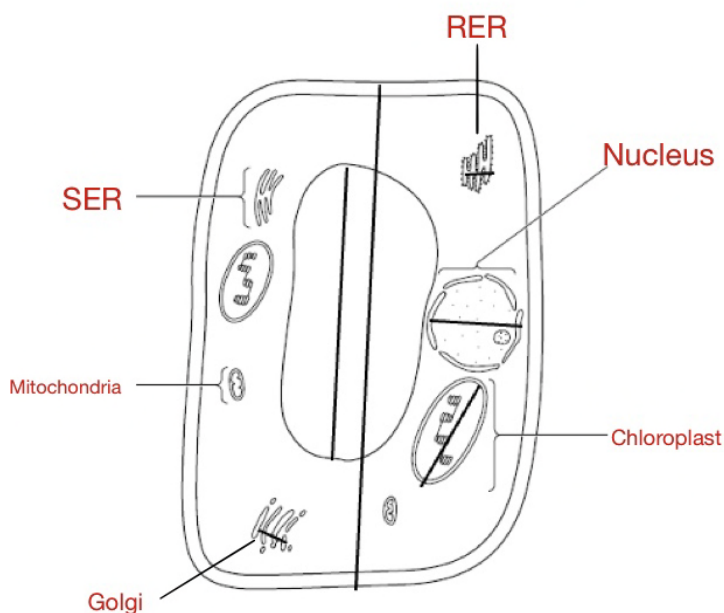
Part B - Use the Magnification calculated in Part A to find actual length of Cell or organelle

3. Measure the length of organelle G in mm and convert to μm ($\times 1000$)

$$\text{Actual length } (\mu\text{m}) = \frac{\text{Measured Length - organelle G } (\mu\text{m})}{\text{Magnification - scale bar from part 2}}$$

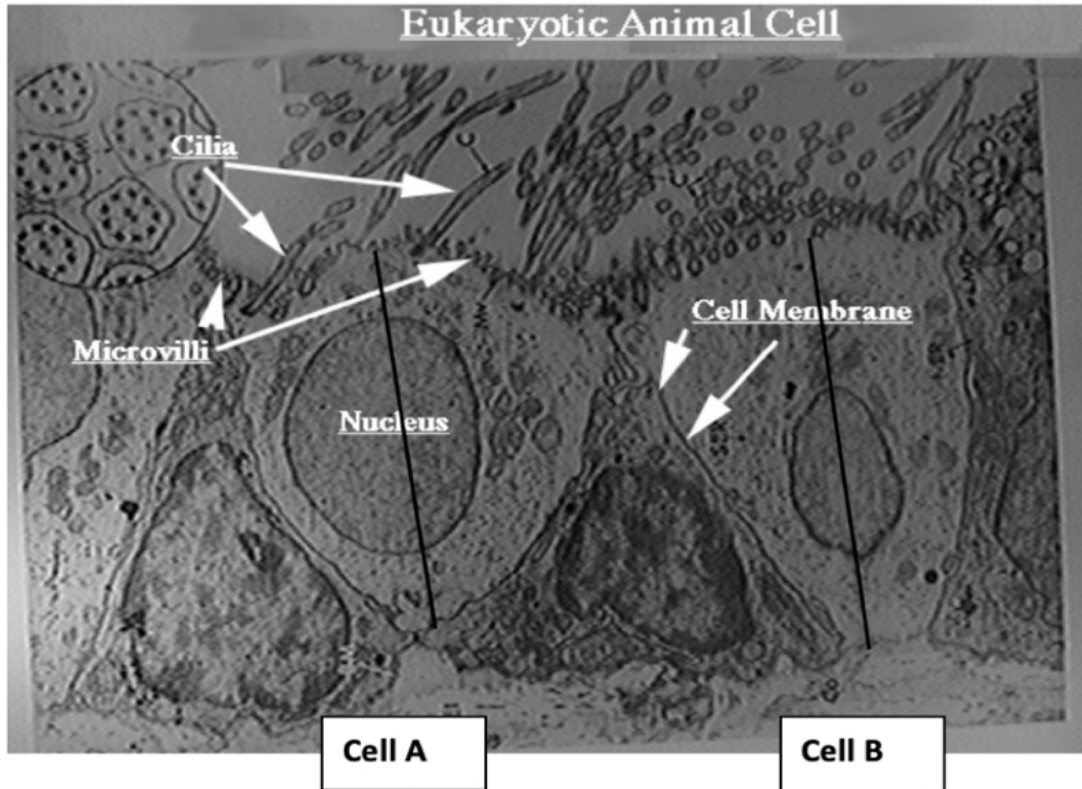
Q1) Magnification is **2300x**. Label and complete the table.

Q1) Magnification is **2300x**. Label and complete the table.



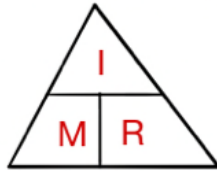
Part of Cell	Image Size (mm)	Actual Size (μm)
Chloroplast	20	8.7
Golgi (width)	5	2.2
Vacuole (length)	50	21.7
Nucleus	16	7.0
Cell Length	87	37.8
RER (width)	5	2.2

Q2) The real length of the nucleus in cell A is 6µm.



a) Calculate the magnification of this image

I = Image size
M = Magnification
R = Actual size



b) Calculate the real length of cells A & B

a) What do we know?
R = 6µm
I = length of nucleus in Cell A = 30mm
M = ?
Therefore:
 $M = \frac{30\text{mm}}{6\mu\text{m}}$ BUT need same UNITS.

So
I = 30mm X 1000 = 30 000µm

Now we can work out M
 $M = \frac{30\,000\mu\text{m}}{6\mu\text{m}} = \times 5000$

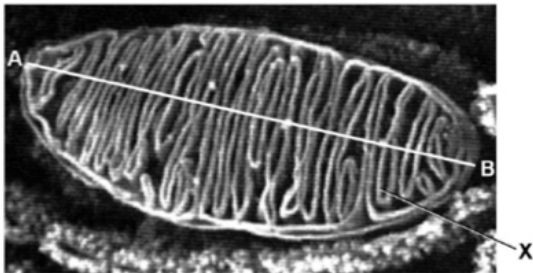
b) Real length of cell A
I = 49mm = 49mm X 1000 = 49 000 µm
M = x5000
R = ?
So
 $R = \frac{49\,000\mu\text{m}}{5000} = 9.8\mu\text{m}$

Real length of cell B
I = 53mm = 53mm X 1000 = 53 000 µm
M = x5000
R = ?
So
 $R = \frac{53\,000\mu\text{m}}{5000} = 10.6\mu\text{m}$

Q3)

Figure 2 is a photograph (micrograph) of a mitochondrion taken using a scanning electron microscope.

Figure 2



What is the evidence from Figure 2 that a scanning electron microscope was used to take this photograph?

[1 mark]

(Can see) 3D image;

Name the part of the mitochondrion labelled X in Figure 2.

[1 mark]

Crista/cristae;

1

Ignore matrix

The actual length of the mitochondrion between points A and B in Figure 2 is 4 µm.

What is the magnification of the mitochondrion in Figure 2?

Show your working.

[2 marks]

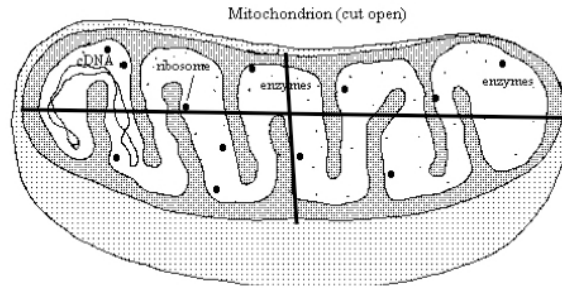
Value between 20,750 (83mm) and 21,250 (85mm) two marks;;

2

Formula given/used but calculation wrong, award 1 mark

Magnification = $\frac{\text{image size}}{\text{Object size}}$
(Large number divided by 4)

Q4) The real **length** of the mitochondria is 10µm.



a) Calculate the **magnification** of this image

a) What do we know?

$R = 10\mu\text{m}$

$l = \text{length of mitochondria} = 76\text{mm}$

$M = ?$

Therefore:

$M = \frac{76\text{mm}}{10\mu\text{m}}$ BUT need same UNITS.

So

$l = 76\text{mm} \times 1000 = 76\,000\mu\text{m}$

Now we can work out M

$M = \frac{76\,000\mu\text{m}}{10\mu\text{m}} = \times 7600$

b) Calculate the real width

$l = 24\text{mm} = 24\text{mm} \times 1000 = 24\,000\mu\text{m}$

$M = \times 7600$

$R = ?$

So

$R = \frac{24\,000\mu\text{m}}{7600} = 3.2\mu\text{m}$

b) Calculate the real **width**

Q5) The real size of mitochondria **D** is 5µm

a) Calculate the **magnification** of the image

$$\frac{12000}{5} = \times 2400$$

b) Calculate the real **width** of the nucleus

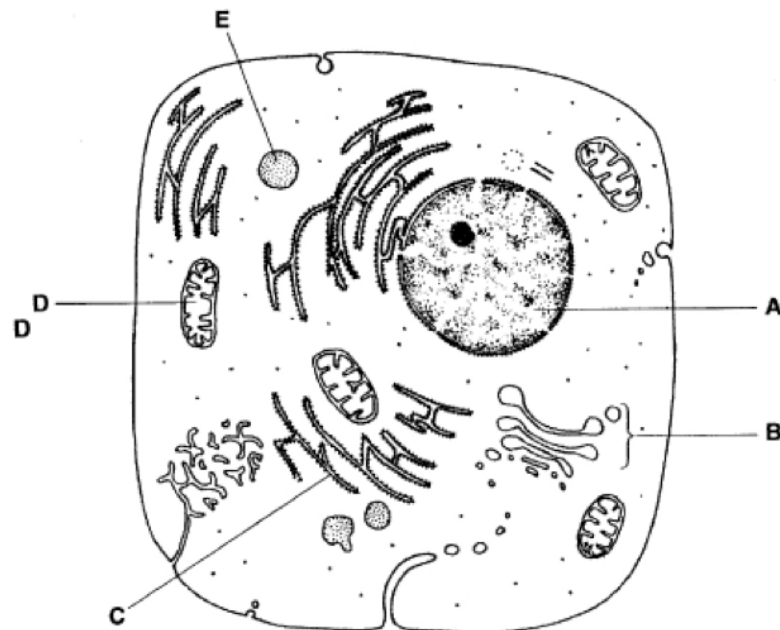
$$\frac{24000}{2400} = 10\mu\text{m}$$

c) Calculate the **width** of the Golgi apparatus

$$\frac{9000}{2400} = 4\mu\text{m}$$

d) Calculate the real **size** of the lysosome

$$\frac{5000}{2400} = 2\mu\text{m}$$



Practical Technique: Measuring an object under the Microscope

Outcome:

- Measuring the size of an object viewed with an optical microscope using an Eyepiece graticule and a Stage Micrometer

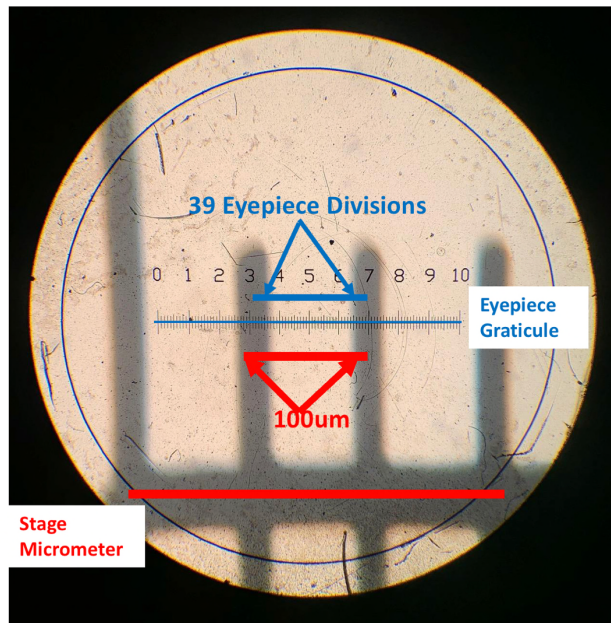
Stage A: Use a Stage micrometer slide to calibrate the Eyepiece Graticule

1. Place the **Stage Micrometer** slide onto the stage of the microscope
2. Select the **objective lens** that provides the desired level of **Magnification**
3. **Focus** the microscope so the **stage micrometer ruler** becomes **visible**.
4. Determine how many **small divisions** on the **Eyepiece graticule** there are between a **single division** on the **Stage Micrometer (a distance of 100µm)**
5. Divide **100µm** by the **number of small divisions** on the **Eyepiece graticule** to determine the **length of a single Eyepiece graticule division**.

Determine the length of a single Eyepiece Division

$$= \frac{100 \mu\text{m}}{39}$$

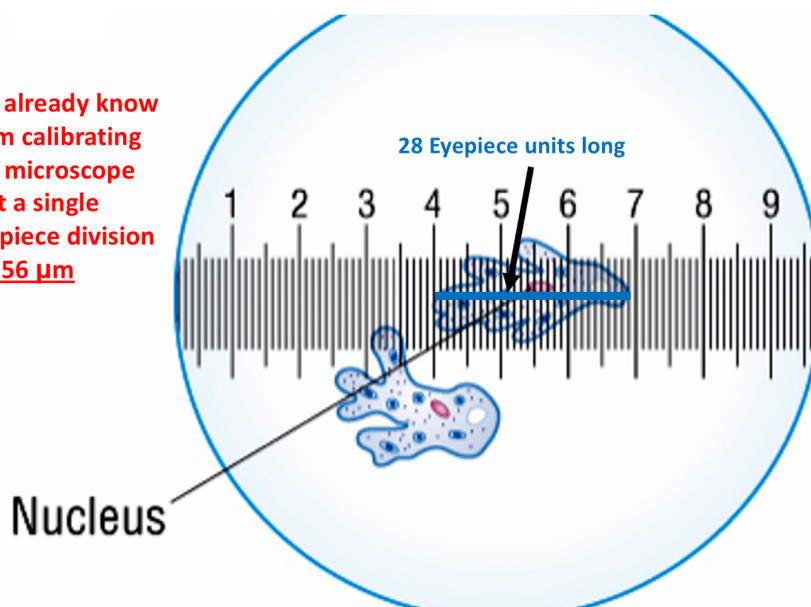
$$= 2.56 \mu\text{m}$$



Stage B: Use the calibrated Eyepiece Graticule to determine the actual length of the object

1. Remove the **Stage Micrometer Slide** and replace with the **slide** containing the **Specimen** you want to study (e.g. *Onion root tip cells*)
2. Use the **Eyepiece graticule** to measure the **number of small divisions** long the object is.
3. **Multiply** the **number of divisions** by the **length of a single division** (From Part A) to determine the **actual length** of the **object**.

We already know from calibrating the microscope that a single Eyepiece division = 2.56 µm



$$\begin{aligned} \text{Actual Length of Cell} &= \text{Number of Eyepiece units long} \times \text{Length of single Eyepiece Division} \\ &= 28 \times 2.56 \mu\text{m} = 71.7 \mu\text{m} \end{aligned}$$

Two devices are needed:

1) **An eyepiece graticule** – an eyepiece lens with a scale etched on the glass.

This is what you see if you look down the eye piece lens:

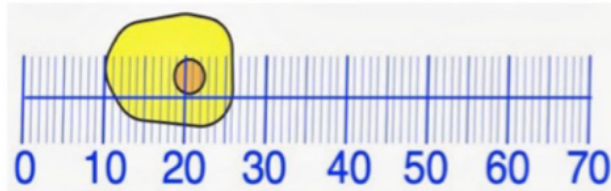


This scale has no units.

It is an arbitrary scale.

You can only measure organisms/cells in eye piece units – epu.

How long is the cell in epu? 16 epu.



Just count the lines, don't worry about the numbers on the scale.

To calibrate the eye piece graticule:

2) To do this a **stage micrometer** is needed. This is a glass slide with a scale of known dimensions on it. **1 division on the stage micrometer is 100 μm .**

The two scales must now be super-imposed on one another.



When the two scales are aligned, you can see that:

$$\underline{10} \text{ epu} = \underline{100} \mu\text{m}$$

$$\text{So, } 1 \text{ epu} = \underline{10} \mu\text{m}$$

If the cell above is 16 epu, how big is it in μm ?

$$\underline{160} \mu\text{m}$$

This calibration stays the same for the objective lens you have used. If you change the magnification you must re-calibrate the graticule.

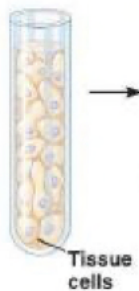
Outcome:

- Describe the processes of cell fractionation and ultracentrifugation and explain why the separation of cell components is important in studying cells and their components

Separating Cell Components

Use Toole and Toole pg 59-60 to complete the boxes here

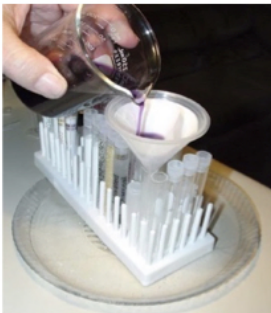
1. Chop up fresh tissue in ice cold, isotonic buffer solution. The ice stops Enzyme activity as proteases could damage organelles. The solution is isotonic to stop osmotic damage to the cells (bursting or shrinking) and the buffer maintains a constant pH so enzyme function and organelle structure are not affected.



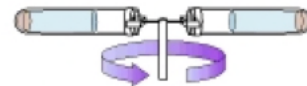
2. Put the chopped tissue in to a blender or homogeniser to break open the cells. What for?
Break open cells release organelles. What is the fluid produced called? Homogenate.



3. Filter the homogenate to remove the large debris, such as connective tissue and plant cell walls. These may interfere with results.



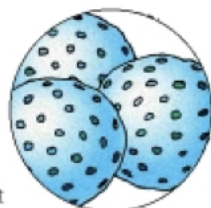
4. Pour the mixture in to tubes and spin very quickly in a Centrifuge. This is **Ultracentrifugation**. The organelles are separated based on mass and density.



5. The heaviest organelles, the Nucleus are forced to the bottom and form a sediment or pellet. The fluid left at the top is removed leaving just the sediment of nuclei. What is this fluid called? Supernatant



Nuclei sediment



6. The supernatant may then be spun again at a higher speed to produce a sediment containing Mitochondria (and chloroplasts if plant tissue), and at an even higher speed for other organelles (in order: lysosomes, rough ER, plasma membrane, smooth ER and lastly, ribosomes).

1. Spun at low speed



Nucleus

2. Spun at medium speed



Mitochondria

3. Spun at high speed



Lysosomes, membranes & ribosomes

Cell Fractionation Questions

Use Toole and Toole page 59-60 and previous worksheet

1. Why is **cell fractionation** carried out?

To study the structure and function of organelles in cells, you must obtain large numbers of isolated organelles.

2. Complete this sentence:

Organelles are separated out because they have different. Mass / Densities

3. Put a number (nice and big) next to each box to put them into the correct order to describe how cell fractionation is carried out.

2	1	4
Tissue is homogenised e.g. in a blender	Tissue is cut into pieces in cold, buffered, isotonic solution.	Filtrate is centrifuged at low speed
6	3	5
Supernatant is discarded	Homogenised suspension is filtered	Supernatant is decanted and re- centrifuged at higher speeds until desired organelle is separated.

4. (a) Draw a diagram of a **tube** after it has been **centrifuged**. Label



b)

i) Which is bigger - a ribosome or a nucleus? Nucleus

(ii) Which would be separated out into the sediment at the lower speeds on the centrifuge - ribosomes or nuclei? Nuclei

(c) Put the following organelles in order to describe which would be separated out first through to last:

Lysosomes	Chloroplasts	Nuclei	Rough ER	Mitochondria
4	2	1	5	3

d) What speed of centrifugation (in revolutions min⁻¹) is needed to separate out mitochondria?

5. Explain why the solution needs to be:

(a) **cold**; Ice-cold – Slows/stops enzyme activity to prevent digestion of organelles/mitochondria;

(b) **buffered**; Buffered – Maintains pH so that enzymes/proteins are not denatured;

(c) **isotonic**; Same water potential – Prevents osmosis so no lysis/shrinkage of organelles/mitochondria/C;

Cut and Stick page

