Edexcel International AS Biology

Your notes

Cell Structure & Organisation

Contents

Your notes

The Cell as a Unit of Life

- Until microscopes became powerful enough to view individual cells, no-one knew for certain what living organisms were made from
- A scientist called **Robert Hooke** is thought to be the **first person to view cells,** using the term 'cell' to describe these newly discovered structures
- Matthias **Schleiden** and Theodor **Schwann** were two other scientists who studied animal and plant cells
  - In 1837 they came up with the idea that **all living organisms are made of cells**
  - This idea is known as '**cell theory**'
  - The cell theory is a **unifying concept** in biology, meaning that it is **universally accepted**
- Cell theory includes **three main ideas**
  - **All living organisms** are made up of **one or more cells**
  - Cells are the **basic functional unit** in living organisms
  - **New cells** are produced from **pre-existing cells**
- The cells of all living organisms share some **common features**
  - Cell surface membrane
  - Cytoplasm
  - DNA
  - Ribosomes
- Beyond these common features different cell types contain **different structural elements** and combinations of organelles, e.g.
  - Prokaryotic cells have no internal membranes and smaller ribosomes
  - Eukaryotic cells have several internal membrane-bound organelles and larger ribosomes
- When examined under a **microscope** it is possible to see the **ultrastructure** of different cell types
  - Cell ultrastructure refers to the internal structures of the cell

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Multicellular Cell Organisation

- Cell theory states that cells are the basic functional unit of all living organisms
- Cells can become **specialised** for **specific functions,** e.g.
  - Epithelial cells in the small intestine are specialised to absorb food efficiently
  - Red blood cells are specialised to transport oxygen
  - Xylem cells in plants are specialised to allow the transport of water around a plant
- In multicellular organisms specialised cells of the **same type** group **together** to form **tissues**
  - A tissue is **a group of cells that work together to perform a particular function**, e.g.
    - Epithelial cells group together to form epithelial tissue the function of which, in the small intestine, is to absorb food
    - Muscle cells group together to form muscle tissue, the function of which is to contract in order to move parts of the body
- Different tissues can **group together** to form **organs**
  - An organ is **a group of tissues working together to perform a particular function**, e.g.
    - Many different tissues, including cardiac muscle tissue, blood vessel tissues and connective tissue, group together to form the **heart**, enabling it to function to pump blood around the body
    - Tissues including palisade mesophyll, spongy mesophyll, and vascular tissue, group together in plants to form **leaves**, enabling them to perform photosynthesis effectively
- Different organs **work together** to form **organ systems**
  - An organ system is **a group of organs working together to perform a particular function**, e.g.
    - The heart and blood vessels work together to form the **circulatory system**, the job of which is to allow blood to circulate around the body
    - The stomach, pancreas, small intestine, and large intestine work together to form the **digestive system**, the job of which is to digest food and absorb nutrients

Levels of Organisation Table

Level	Description
Organelles	A component within a cell that carries out a specific task
Cells	Basic functional and structural units in a living organism
Tissues	A group of cells of similar structure working together to perform a particular function
Organs	Made from a group of different tissues working together to perform a particular function
Organ systems	Made from a group of organs with related functions, working together to perform body functions within the organism

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Examples of Specialised Cells and their Associated Tissues, Organs and Organ Systems Table

Specialised cell	Tissue	Organ	Organ system
Epithelial cell	Epithelial tissue (made up of epithelial cells)	Stomach (made up of epithelial tissue, muscular tissue and glandular tissue)	Digestive system (made up of all the organs involved in the digestion and absorption of food, including the stomach, small intestine, large intestine and liver, as well as many others)
Muscle cell	Muscle tissue (made up of muscle cells)	Bladder (made up of muscle tissue, epithelial tissue, connective tissue and fatty tissue)	Urinary system (made up of the kidneys, ureters, bladder and urethra)
Neurones (nerve cells)	Nervous (neural) tissue (made up of neurones)	Brain (made up of gray matter tissue, white matter tissue and the tissues that make up the blood vessels in the brain)	Central nervous system (made up of the brain and the spinal cord
Rod cells and cone cells	Retina (made up of rods and cones)	Eye (made up of many tissues, including the retina, cornea, sclera and choroid)	Visual system (made up of the eyes, optic nerves and the visual cortex in the brain)

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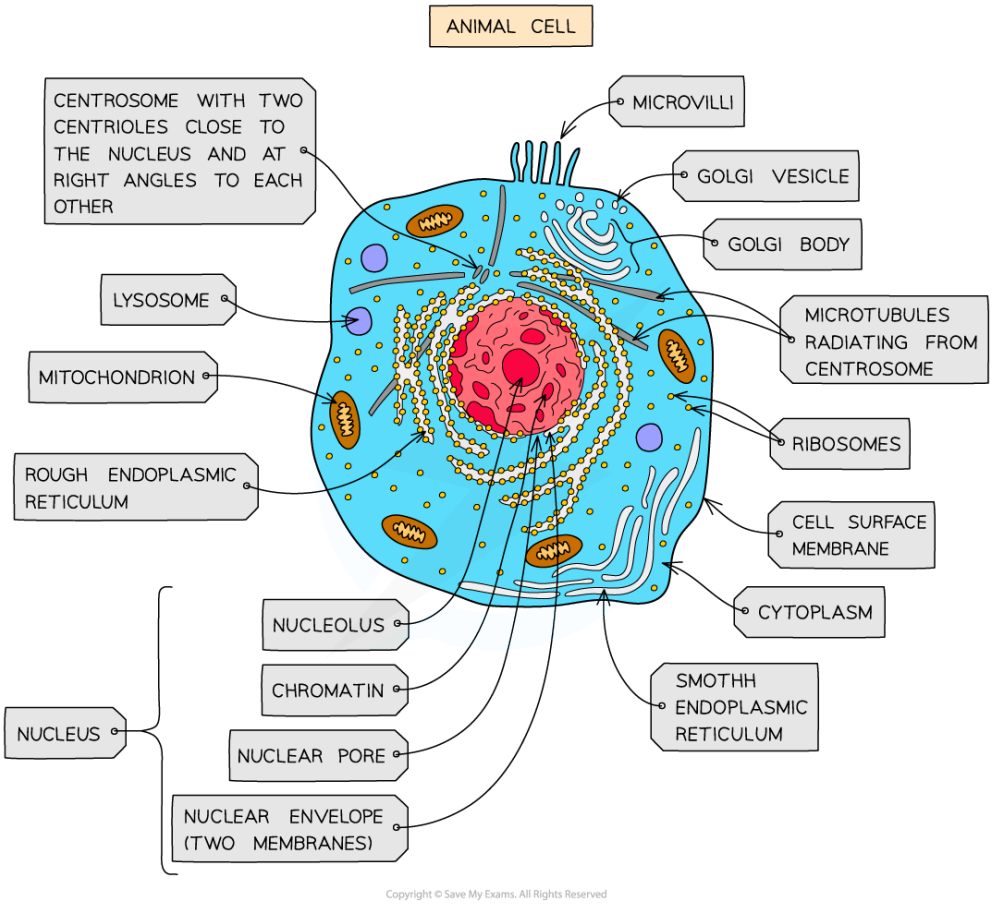
Organelle Structures

- Cells can be divided into two broad types; **eukaryotic** and **prokaryotic** cells
- Eukaryotic cells have a **more complex ultrastructure** than prokaryotic cells
  - The term ultrastructure refers to the **internal structure of cells**
- Eukaryotic cells are larger than prokaryotic cells
  - Eukaryotic cells range in diameter from around 10–100 μm
  - Prokaryotic cells range in diameter from around 0.1–5 μm
- The cytoplasm of eukaryotic cells is divided up into **membrane-bound compartments** called **organelles**
- Animal and plant cells are both types of eukaryotic cells that **share key structures** such as
  - Membrane-bound organelles, including a nucleus
  - Larger ribosomes known as 80S ribosomes
- **Key differences** between animal and plant cells include
  - Animal cells contain **centrioles** and some have **microvilli** while plant cells do not
    - Microvilli are folded regions of the cell surface membrane that increase cell surface area for absorption, e.g. in the small intestine
  - Plant cells have a **cellulose cell wall**, large permanent **vacuoles**, and **chloroplasts** while animal cells do not



Animal cells are a type of eukaryotic cell

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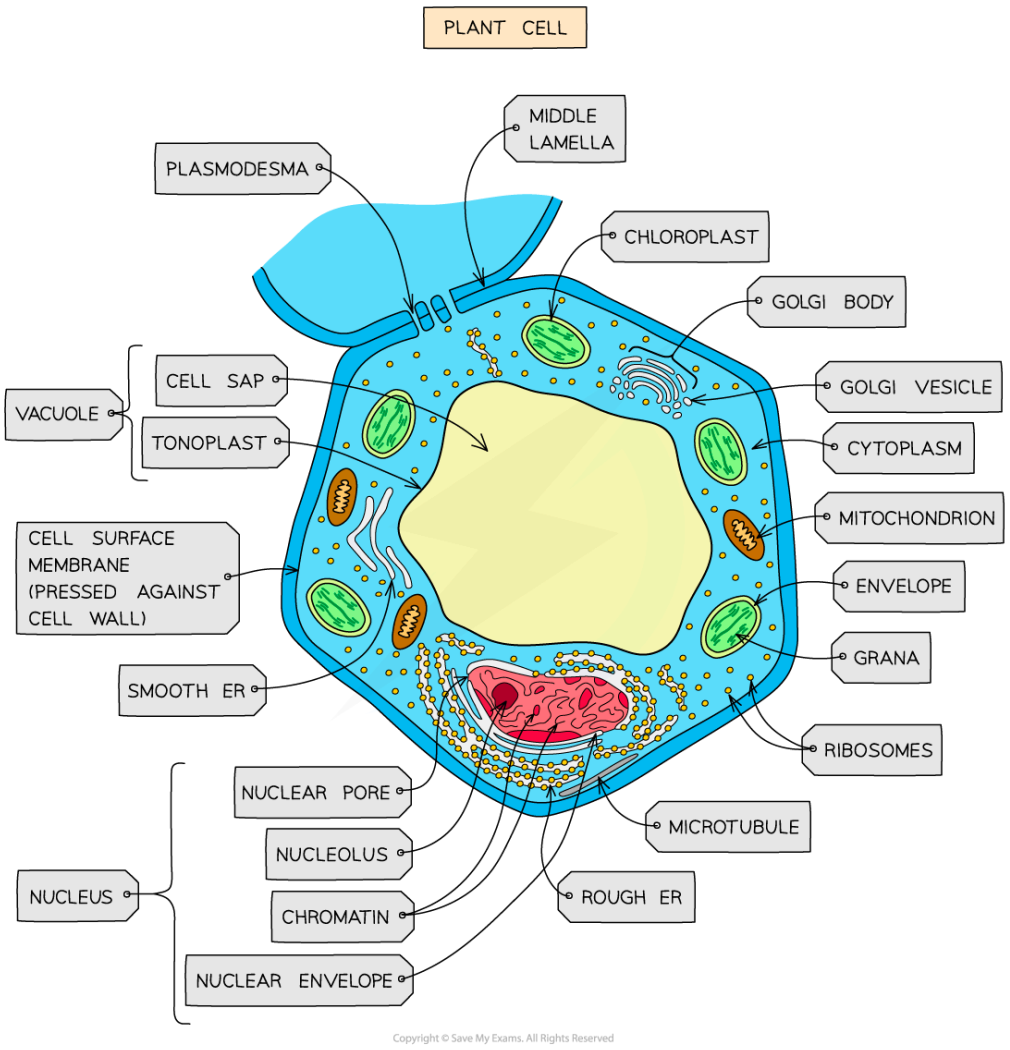

Plant cells are eukaryotic cells that have a cellulose cell wall, permanent vacuole, and chroroplasts

Organelle Functions

Cell surface membrane

- All cells are surrounded by a cell surface membrane which **controls the exchange of materials** between the internal cell environment and the external environment
  - The membrane is described as being **partially permeable**, meaning that some substances can pass through the membrane while others cannot
- Cell membrane is formed from a phospholipid **bilayer** spanning a diameter of around 10 nm
- Many organelles inside cells are surrounded by cell membrane, so when referring to the outer membrane of a cell it is always a good idea to refer to it as the cell **surface** membrane

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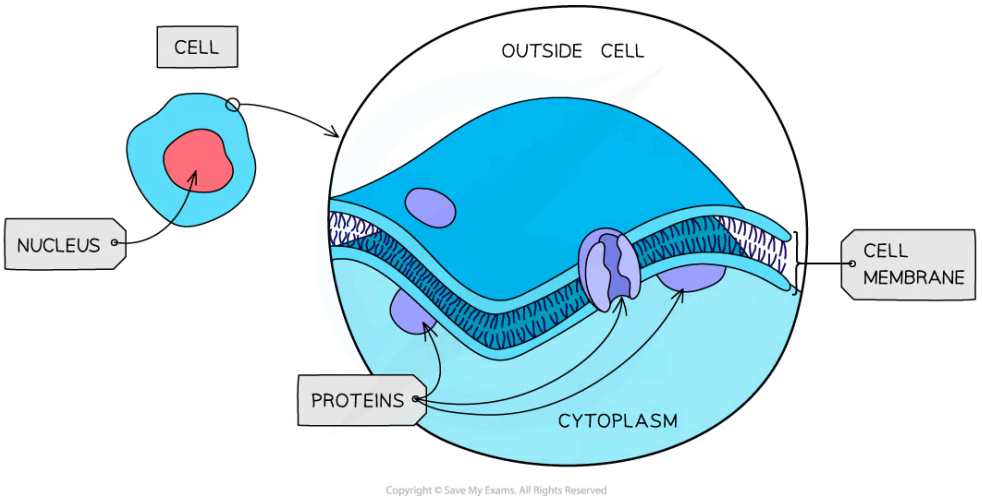
- The cell surface membrane can also be referred to as the **plasma membrane**



The cell surface membrane surrounds the cell, separating it from its external environment

Nucleus

- Present in all eukaryotic cells, the nucleus is relatively **large** and separated from the cytoplasm by a **double membrane** called the **nuclear envelope,** which has many pores
  - Nuclear pores are important channels for allowing mRNA and ribosomes to travel out of the nucleus, as well as allowing enzymes, e.g. DNA polymerases, and signalling molecules to travel in
- The nucleus contains **chromatin,** the material from which chromosomes are made
  - Chromosomes are made of sections of **linear DNA** tightly wound around proteins called **histones**
- Usually, at least one or more darkly stained regions of the nucleus can be observed under a microscope; these regions are individually termed **nucleolus** (plural nucleoli) and are the sites of **ribosome production**

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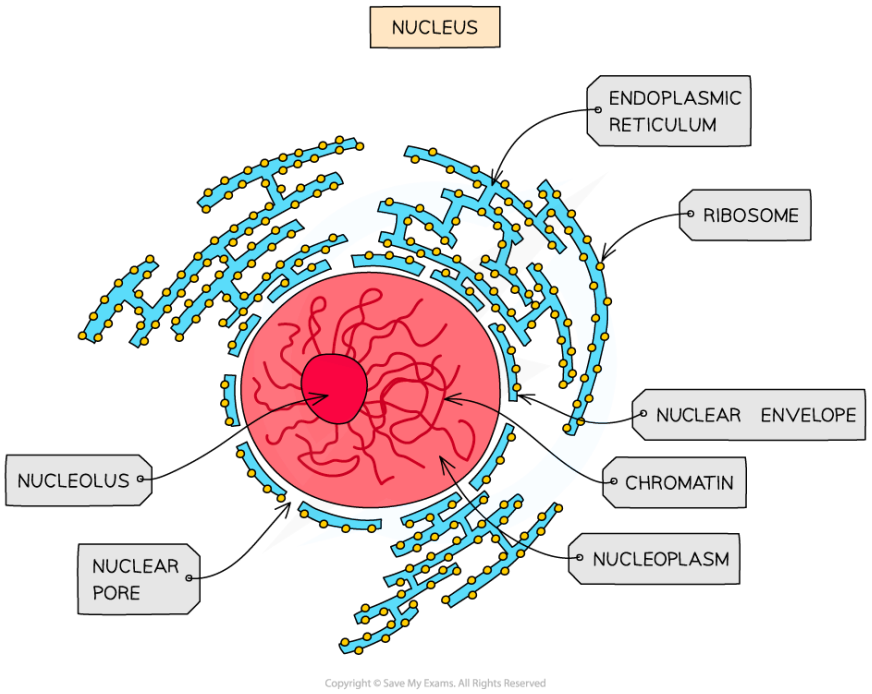

The nucleus of a eukaryotic cell is surrounded by the nuclear envelope and contains chromatin as well as a region called the nucleolus. Note that the nucleus is shown here surrounded by another organelle; the endoplasmic reticulum

Mitochondria

- The site of **aerobic** respiration within eukaryotic cells, mitochondria (singular mitochondrion) are just visible with a light microscope
- Mitochondria are surrounded by a **double-membrane** with the inner membrane folded to form structures called **cristae**
- The **matrix** of mitochondria contains enzymes needed for **aerobic respiration,** producing **ATP**
- Small circular pieces of **DNA,** known as mitochondrial DNA, and ribosomes are also found in the matrix
  - These are needed for replication of mitochondria before cell division

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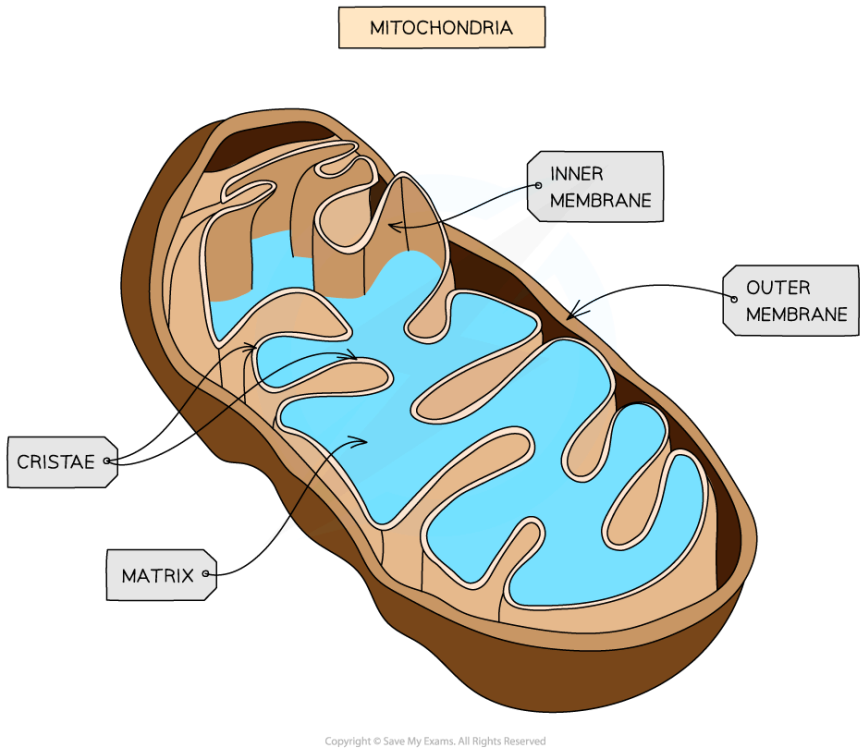

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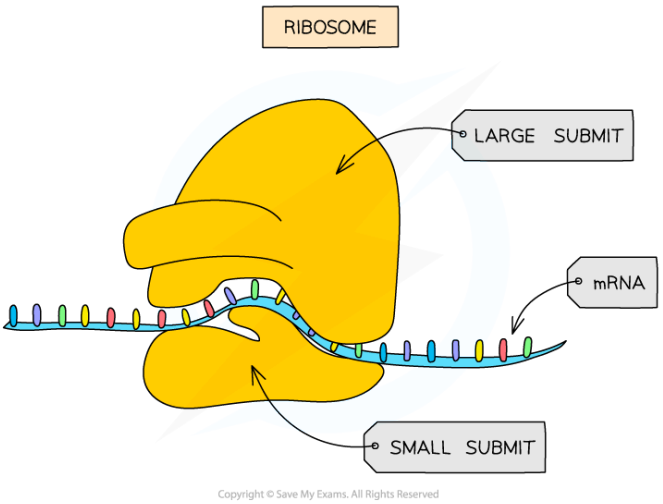
Mitochondria are the site of aerobic respiration in eukaryotic cells

Ribosomes

- Ribosomes can be found as free organelles in the cytoplasm of all cells or as part of the **rough endoplasmic reticulum** in eukaryotic cells
- They are not surrounded by a membrane
- Each ribosome is a complex of **ribosomal RNA (rRNA)** and **proteins**
- 80s ribosomes are found in eukaryotic cells
- 70s ribosomes are found in prokaryotes, mitochondria, and chloroplasts
- Ribosomes are the site of translation



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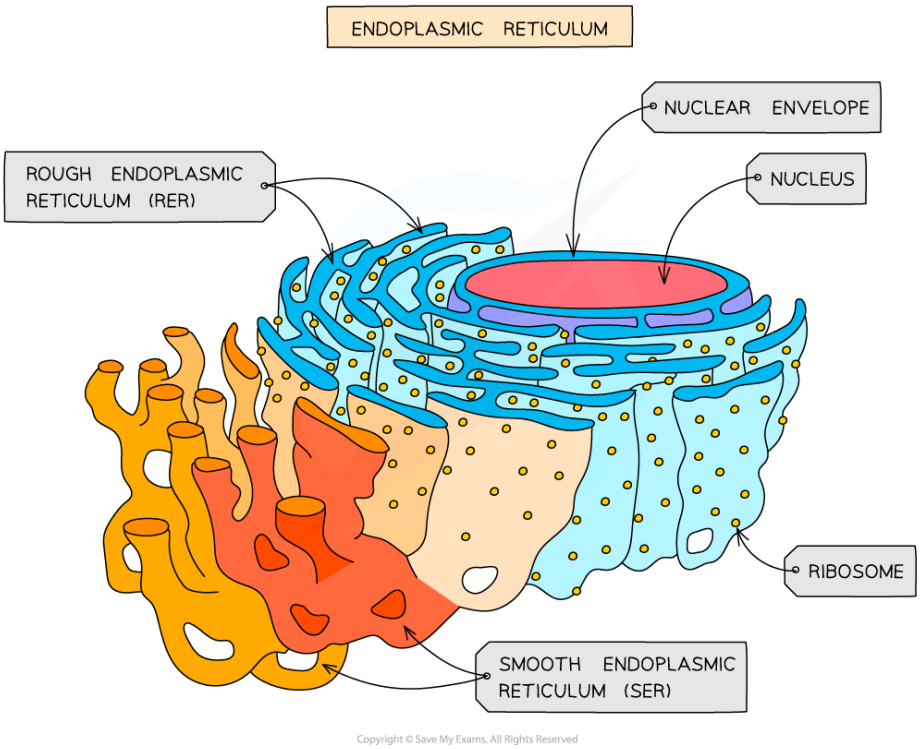
Ribosomes are formed in the nucleolus and are composed of almost equal amounts of RNA and protein

Endoplasmic reticulum

- There are two types of endoplasmic reticulum; rough and smooth
- **Rough Endoplasmic Reticulum (RER)**
  - RER is formed from folds of membrane continuous with the **nuclear envelope**
  - The surface of RER is covered in **ribosomes**
  - The role of the RER is to **process protein**s made on the ribosomes
- **Smooth Endoplasmic Reticulum (SER)**
  - SER is also formed from folds of membrane but its function is distinct from the RER, being involved in the production, processing and storage of **lipids, carbohydrates** and **steroids**
  - SER does not have ribosomes on its surface

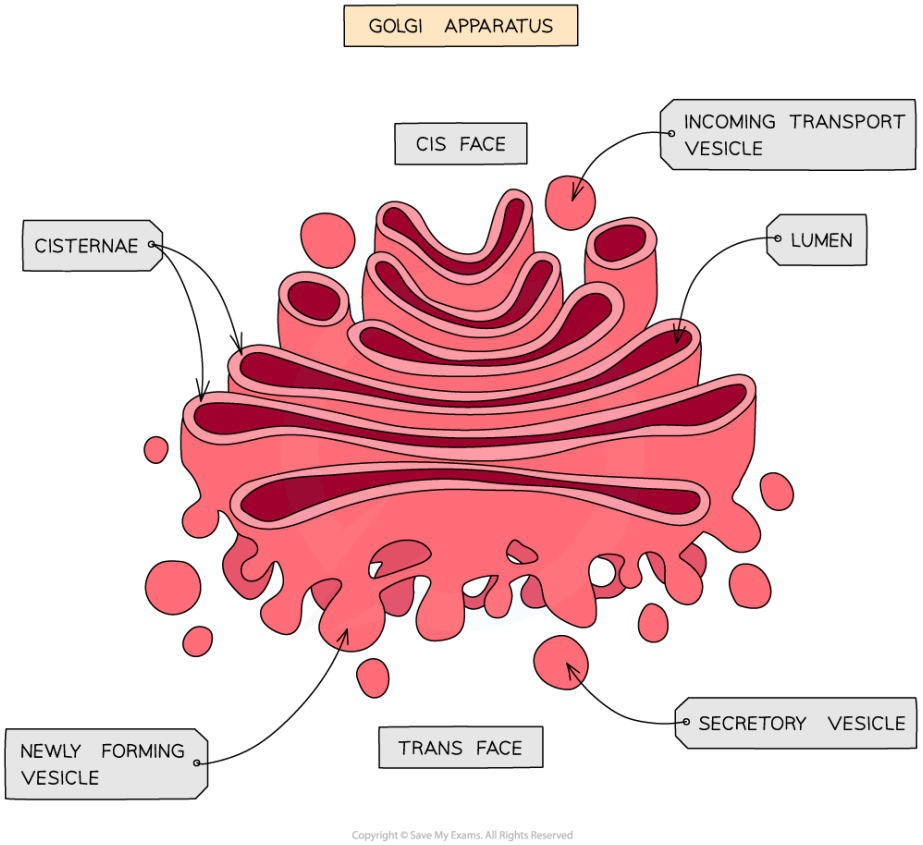


The RER and SER are visible under the electron microscope; the presence or absence of ribosomes helps to distinguish between them

Golgi apparatus

- The Golgi apparatus consists of **flattened sacs of membrane** similar in appearance to the smooth endoplasmic reticulum
  - The Golgi apparatus is sometimes known as the **Golgi body**
  - The Golgi can be distinguished from the SER by its **regular, stacked appearance**; it can be described as looking like a wifi symbol!
- The role of the Golgi apparatus is to **modify proteins and lipids** before **packaging** them into **Golgi vesicles**
  - The vesicles then **transport the proteins and lipids** to their required destination
  - Proteins that go through the Golgi apparatus can be
    - Exported from the cell, e.g. hormones such as insulin
    - Put into lysosomes, e.g. hydrolytic enzymes
    - Delivered to other membrane-bound organelles

Your notes

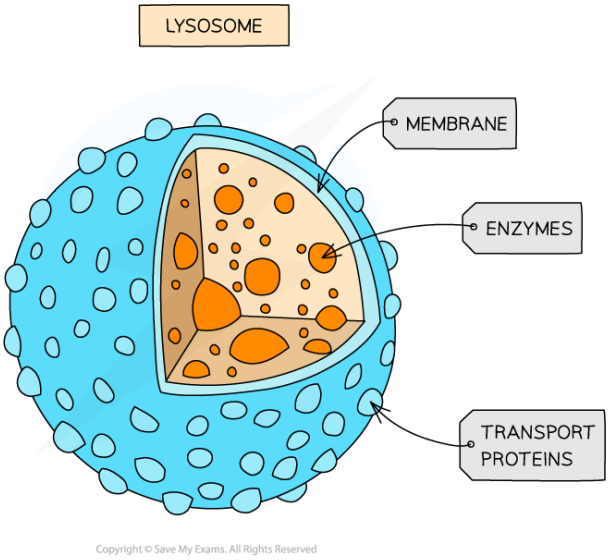


The Golgi apparatus; the cis face lies near the rough endoplasmic reticulum, while the trans face lies near the cell membrane

Lysosomes

- Lysosomes are specialist forms of vesicle which contain hydrolytic **enzymes**
- The role of lysosomes is to break down waste materials such as worn-out organelles,
  - Lysosomes are used extensively by cells of the **immune system** and in programmed cell death, known as **apoptosis**

Your notes

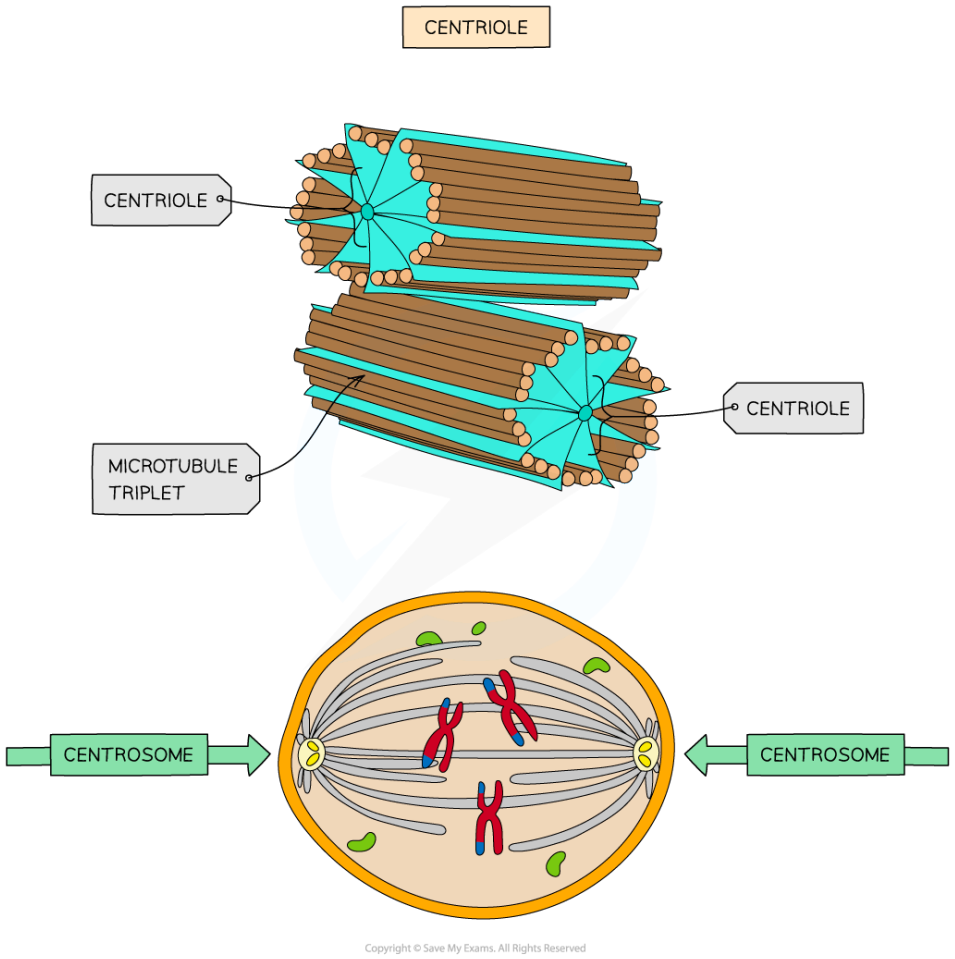


Lysosomes contain digestive enzymes

Centrioles

- Centrioles are made of hollow fibres knows as **microtubules**
  - Microtubules are filaments of protein that can be used to **move substances around** inside a cell, as well as to **support the shape of a cell** from the inside
- Two centrioles at right angles to each other form a **centrosome** which organises the spindle fibres during cell division
- Centrioles are not found in plants and fungi

Your notes



Centrioles are structures formed from microtubules; they are involved with the process of nuclear division in animal cells

Your notes


Your notes

Formation of Extracellular Enzymes

- In cells, **many organelles** are involved in the **production** and **secretion** of proteins
  - Organelles are **specialised parts of a cell** that carry out a particular function
  - Some organelles are membrane-bound, meaning that they are surrounded by membrane
- The **organelles** involved in protein synthesis include
  - Nucleus
    - Transcription of the DNA code occurs here
  - Ribosomes
    - Free ribosomes and those on the RER produce proteins in the process of translation
  - Rough endoplasmic reticulum (RER)
  - Golgi apparatus
  - Cell surface membrane
    - Proteins formed within the cell are secreted here

Rough endoplasmic reticulum

- Ribosomes on the RER produce proteins that can be **secreted out of the cell** or become **attached to the cell surface membrane**
- Proteins that have been passed into the lumen of the **rough endoplasmic reticulum** are **folded** and **processed** here
  - The term lumen refers to the inside space of the RER
- Note that free ribosomes found within the cytoplasm make proteins that stay within the cytoplasm rather than being moved to another organelle or being exported from the cell

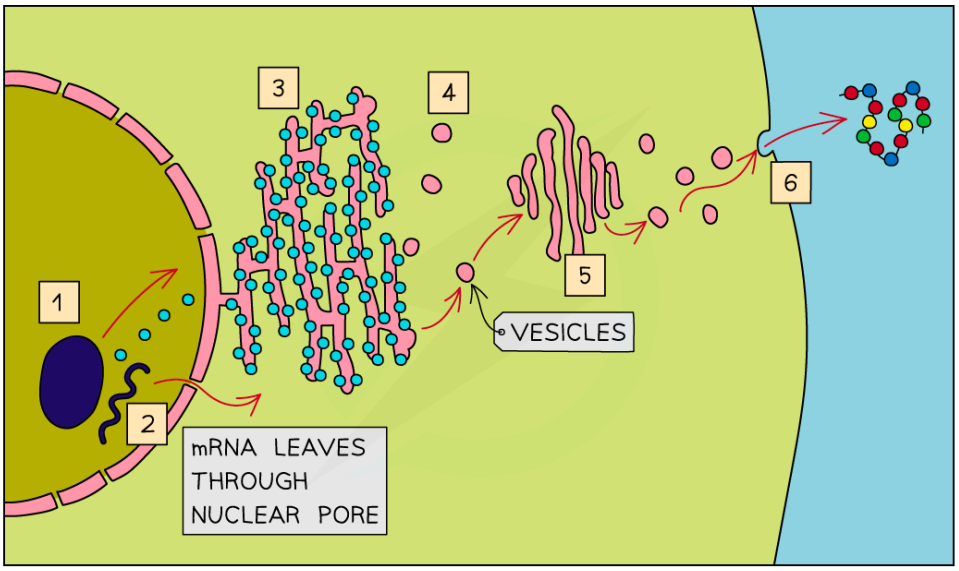
Golgi apparatus

- Processed proteins from the RER are transported to the **Golgi apparatus** in **vesicles** which fuse with the Golgi apparatus, releasing the proteins into the Golgi
  - The Golgi apparatus **modifies** the proteins, preparing them for **secretion**
- Proteins that go through the Golgi apparatus are usually
  - Exported, e.g. extracellular enzymes
    - The term extracellular refers to 'outside the cell'
  - Put into lysosomes, e.g. hydrolytic enzymes

- Delivered to other membrane-bound organelles

- The modified proteins then leave the Golgi apparatus in **vesicles**

Your notes



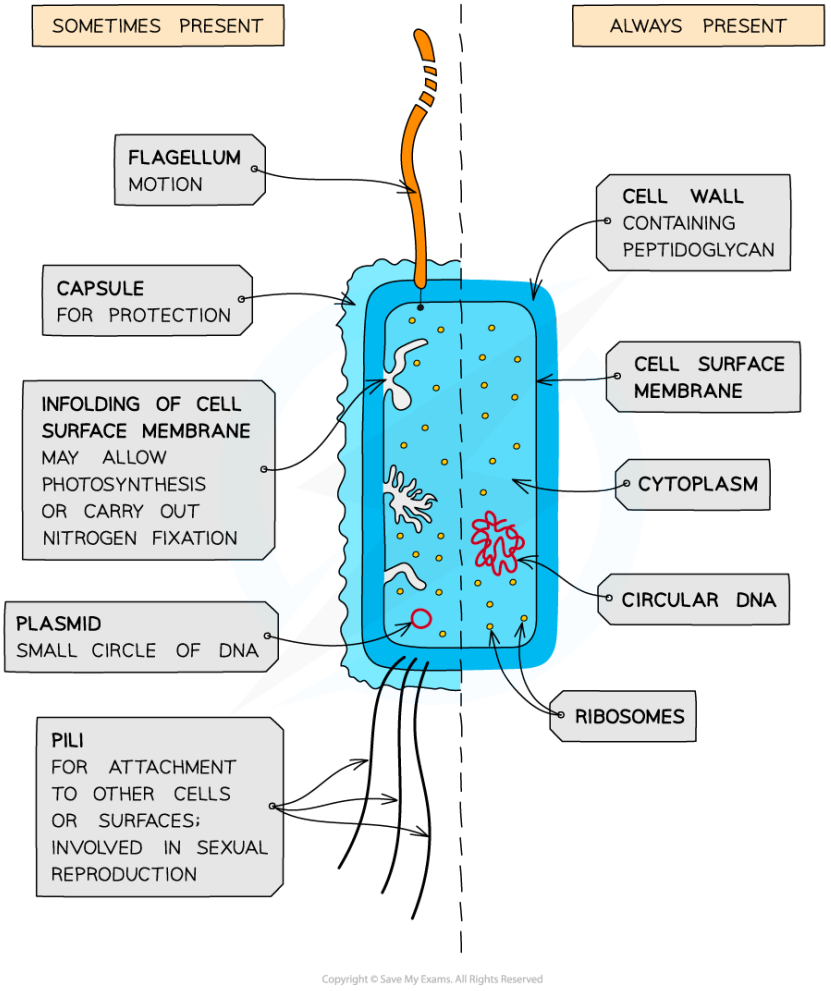
1	THE **NUCLEOLUS** MANUFACTURES **RIBOSOMES** FOR PROTEIN SYNTHESIS IN THE **RER** (ROUGH ENDOPLASMIC RETICULUM)
2	THE **NUCLEUS** MANUFACTURES mRNA, WHICH IS NEEDED BY **RIBOSOMES** TO MAKE PROTEINS
3	THE **RIBOSOMES** IN THE **RER** MAKE PROTEINS
4	THE **RER** PROCESSES THE PROTEINS WHICH ARE THEN SENT IN VESICLES TO THE GOLGI BODY
5	THE **GOLGI BODY** FURTHER PROCESSES THE PROTEINS AND SENDS THEM IN VESICLES TO THE **PLASMA MEMBRANE**
6	THE VESICLES FUSE WITH THE **PLASMA MEMBRANE** TO SECRETE THE FINISHED PROTEIN PRODUCT

The RER and Golgi apparatus are involved with producing, packaging and transporting proteins in a cell. This process can be used to produce and export extracellular enzymes.

Your notes

Cell Components: Structures

- Animal and plant cells are types of eukaryotic **cells**, whereas bacteria are a type of **prokaryote**
- Prokaryotic cells are much **smaller** than eukaryotic cells
- They also differ from eukaryotic cells in having
  - A **cytoplasm** that **lacks membrane-bound organelles**
  - **Ribosomes** that are smaller (70 S) than those found in eukaryotic cells (80 S)
  - **No nucleus,** instead having a **single circular bacterial chromosome** that is free in the cytoplasm and is **not associated with proteins**
  - A cell wall that contains the glycoprotein **murein**
    - Murein is sometimes known as **peptidoglycan**
- In addition, many prokaryotic cells also have the following structures
  - Loops of DNA known as **plasmids**
  - **Capsules**
  - Flagella (singular flagellum)
  - Pili (singular pilus)
  - A cell membrane that contains folds known as **mesosomes**



Bacteria are prokaryotic cells

Prokaryotes vs eukaryotes

- There are a number of important structural and physiological differences between prokaryotic and eukaryotic cells
- These differences affect their metabolic processes and how they reproduce

Comparison of Prokaryotic and Eukaryotic Cells Table

Your notes

FEATURE	PROKARYOTES	EUKARYOTES
SIZE	0.5–5 μm DIAMETER	UP TO 100 μm DIAMETER
GENOME	DNA CIRCULAR WITH NO PROTEINS, IN THE CYTOPLASM	DNA IS ASSOCIATED WITH HISTONES (PROTEINS) FORMED INTO CHROMOSOMES
CELL DIVISION	OCCURS BY BINARY FISSION, NO SPINDLE INVOLVED	OCCURS BY MITOSIS OR MEIOSIS AND INVOLVES A SPINDLE TO SEPARATE CHROMOSOMES
RIBOSOMES	70S RIBOSOMES	80S RIBOSOMES
ORGANELLES	VERY FEW NO MEMBRANE–BOUND ORGANELLES.	NUMEROUS TYPES OF ORGANELLES MEMBRANE–BOUND SINGLE MEMBRANES: LYSOSOMES, GOLGI COMPLEX, VACUOLES DOUBLE MEMBRANES: NUCLEUS, MITOCHONDRIA, CHLOROPLAST NO MEMBRANE: RIBOSOMES, CENTRIOLES, MICROTUBULES
CELL WALL	MADE OF PEPTIDOGLYCAN (POLYSACCHARIDE AND AMINO ACIDS) AND MUREIN	PRESENT IN PLANTS (MADE OF CELLULOSE OR LIGNIN) AND FUNGI (MADE OF CHITIN, SIMILAR TO CELLULOSE BUT CONTAINS NITROGEN)

Your notes

Examiner Tips and Tricks

You will need to know all the **differences** between prokaryotic and eukaryotic cells; remember that not all of the structures mentioned here are present in **all** prokaryotic cells

Remember that **size is not a structural feature** so if you are asked for a structural difference between a prokaryotic and eukaryotic cell don't include size in your answer.

Cell Components: Functions

- The **ultrastructure** of prokaryotic cells includes several features that are not found in eukaryotic cells, e.g.
  - Plasmids
  - Capsule
  - Flagella
  - Pili
  - Mesosomes
  - Circular DNA

- Ribosomes

- Some of these features are found in all prokaryotes, while some are only present in some

Your notes

Additional Prokaryotic Structures Table

Prokaryotic cell structure	Purpose of structure
Plasmids	Small loops of DNA that are separate from the main circular DNA molecule. Plasmids contain genes that can be passed between prokaryotes (e.g. genes for antibiotic resistance). Not present in all prokaryotes
Capsule	Some prokaryotes (e.g. bacteria) are surrounded by a final outer layer known as a capsule. This is sometimes called the slime capsule. It helps to protect bacteria from drying out and from attack by cells of the immune system of the host organism. Not present in all prokaryotes
Flagellum (plural = flagella)	A long, hair-like structure that rotates, enabling the prokaryote to move (a bit like a propeller). Some prokaryotes have more than one. Not present in all prokaryotes
Pilus (plural = pili)	Thread-like structures on the surface of some bacteria that enable the bacteria to attach to other cells or surfaces
Mesosomes	Infolded regions in the plasma membrane of some prokaryotic cells. They have many functions including aerobic respiration, cell wall formation and DNA replication
Circular DNA	The genetic material of prokaryotic cells mainly consists of a single circular strand of DNA that is not contained in a membrane-bound nucleus. The area in the prokaryotic cell where this circular DNA molecule is found is known as the nucleoid
Ribosomes	Prokaryotic ribosomes are 70S ribosomes and are smaller than the 80S ribosomes of eukaryotic cells. They are the site of protein synthesis in prokaryotic cells

Examiner Tips and Tricks

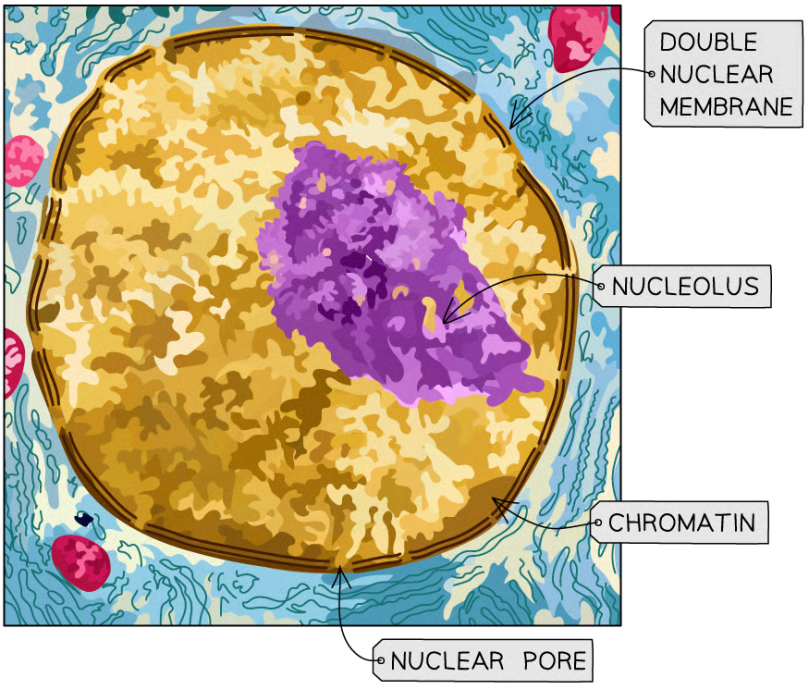
Note that the specification does not include the role of mesosomes in prokaryotic cells.

Your notes

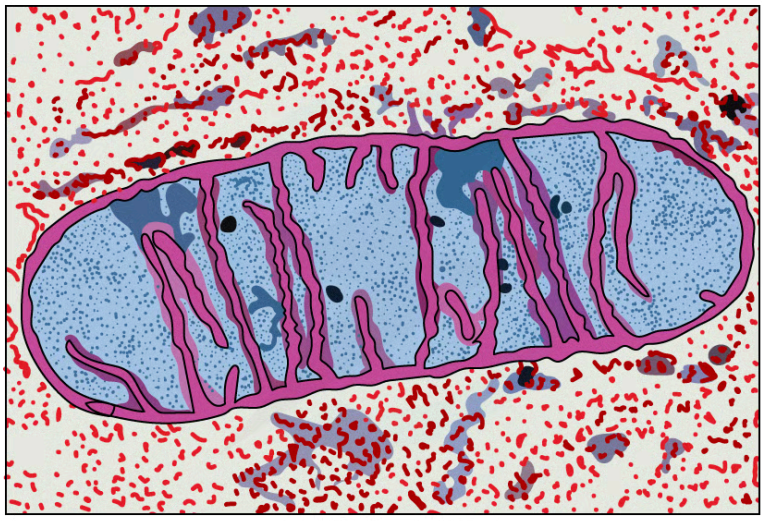
Electron Microscopy of Animal Cells

Organelles under the electron microscope

- There are two types of electron microscope
  - **Transmission** electron microscopes (TEMs)
  - **Scanning** electron microscopes (SEMs)
- **Transmission Electron Microscopes**
  - TEMs use electromagnets to focus a **beam of electrons**
  - This beam of electrons is **transmitted through** a thin specimen
  - Denser parts of the specimen absorb more electrons; these denser parts appear **darker** on the final image, producing **contrast** between different parts of the object being observed
  - The **internal structures** within cells, or even within organelles can be seen as a **2D image**
  - The **resolution** of these images is **very high**
- **Scanning Electron Microscopes**
  - SEMs scan a beam of electrons across a specimen
  - This beam **bounces off the surface of the specimen** and the electrons are detected, forming an image
  - This means SEMs can produce **3D images** that show the **surface** of specimens
  - Since they scan the outside surface it means that the specimen viewed **does not have to be thin**
  - The images they form are of a **lower resolution than TEMs**

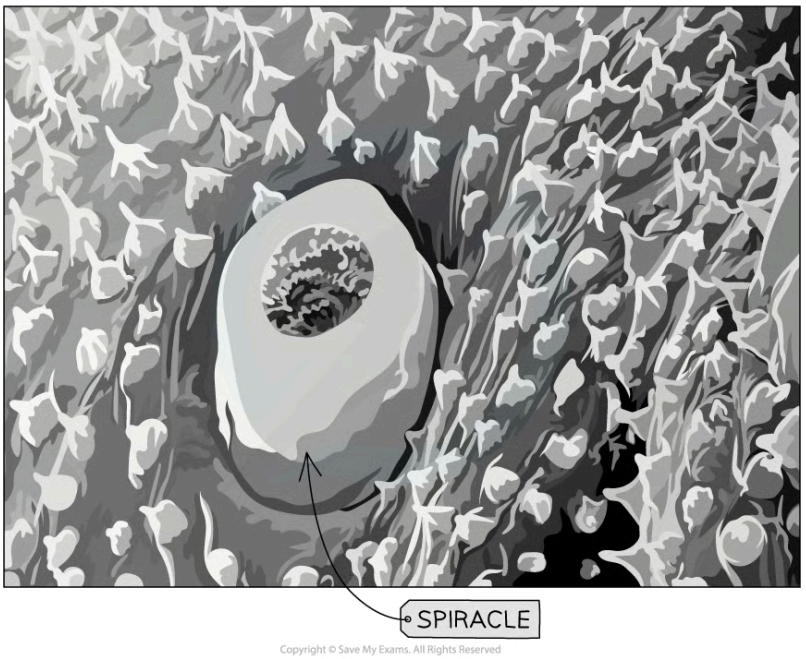


A stained TEM microscope of the nucleus. It is clear this is a TEM micrograph as the image is 2D and in high resolution; the inside of the nucleus would not be clear to view at low resolution.

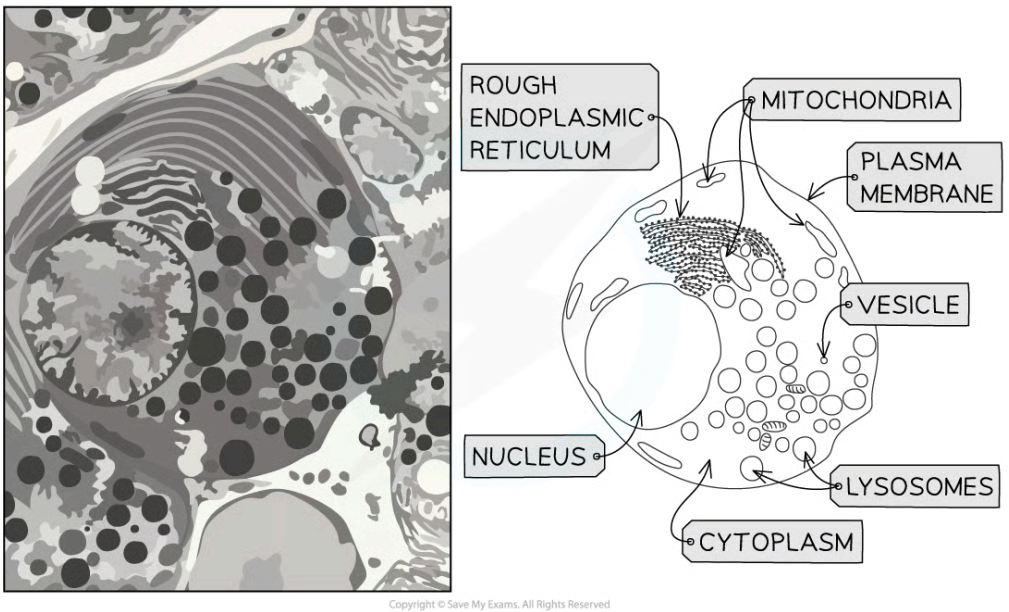


A stained TEM micrograph of a mitochondrion. It is clear this is a TEM micrograph as the image is 2D and in high resolution; the inside of a mitochondrion would not be clear to view at low resolution.

Your notes



A SEM of a spiracle (part of an insect). You can tell this is a SEM micrograph as the image is 3D.



Cell organelles can be identified in a micrograph produced using a TEM

Examiner Tips and Tricks

Your notes

You need to be able to recognise organelles from electron microscope images; cells in real life are not always as easy to observe as cells in diagrams, so be sure to get practice at looking at electron micrographs of cells

Generally, if you can see internal structures the image would have been taken with a TEM and if the image appears 3D then an SEM would have been used.

Your notes

Your notes

Magnification & Resolution

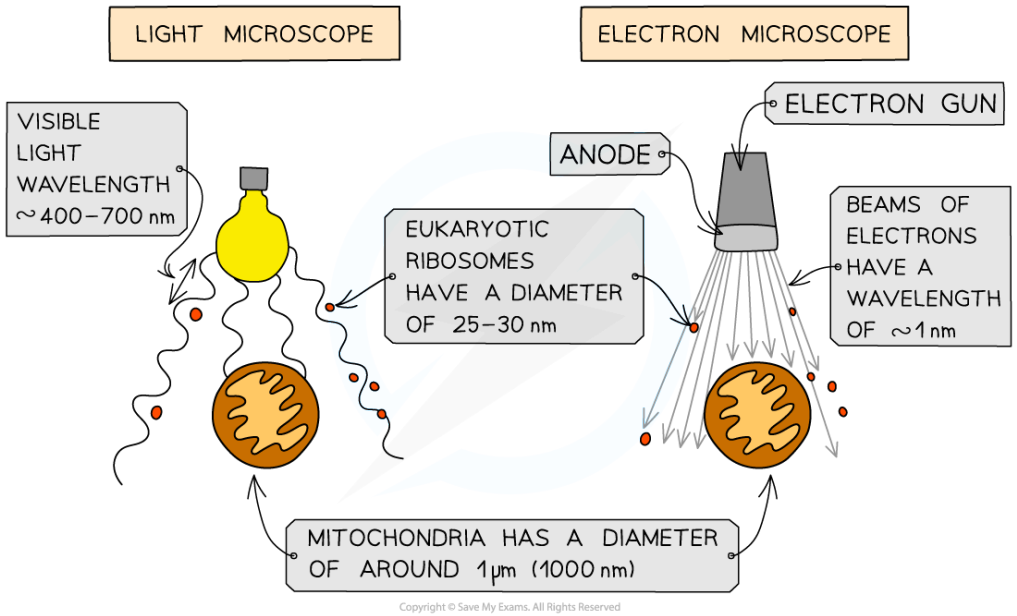
Magnification

- Magnification is how many times bigger the image of a specimen observed is in comparison to the actual, real-life size of the specimen
- A **light microscope** has two types of lens
  - An **eyepiece lens** which often has a magnification of x10
  - A series of, usually 3, **objective lenses**, each with a different magnification
- To calculate the **total magnification,** the magnification of the **eyepiece** lens and the **objective** lens are **multiplied** together

total magnification = eyepiece lens magnification x objective lens magnification

Resolution

- **Resolution**, or **resolving power,** is the ability to distinguish between two separate points
  - If two separate points cannot be resolved, they will be observed as one point and the image will be unclear
- The resolution of a microscope **limits the magnification** that it is capable of; there is no point in magnifying an image at low resolution as this will just result in a big blur rather than a small blur!
- The resolution of a light microscope is **limited by the wavelength of light;** the wavelength of light is too long to allow for high resolution
  - E.g. the phospholipid bilayer structure of the cell membrane cannot be observed under a light microscope
    - The width of the phospholipid bilayer is about 10 nm
    - The maximum resolution of a light microscope is 200 nm
    - Any points that are separated by a distance less than 200 nm cannot be resolved by a light microscope and therefore will not be distinguishable as separate points on an image
- **Electron microscopes** have a much higher resolution, and therefore magnification, than a light microscope as electrons have a much smaller wavelength than visible light



The resolving power of an electron microscope is much greater than that of the light microscope due to the smaller wavelength of electrons

Comparing electron & light microscopes

- **Light microscopes** are used for specimens **larger than 200 nm**
  - Light microscopes shine **light** through the specimen
  - The specimens can be **living,** and therefore can be moving, **or dead**
  - Light microscopes are useful for looking at **whole cells**, small plant and animal **organisms**, and **tissues within organs** such as in leaves or skin
- **Electron microscopes**, both scanning and transmission, are used for specimens **larger than 0.5 nm**
  - Electron microscopes fire a **beam of electrons** at the specimen
  - The electrons are picked up by an electromagnetic lens which then shows the image
  - Electron microscopy requires the specimen to be **dead**; this can provide a **snapshot** in time of what is occurring in a cell, e.g. DNA can be seen replicating and chromosome position within the stages of mitosis are visible
  - Electron microscopes are useful for looking at **organelles, viruses,** and **DNA,** as well as looking at whole cells in more detail

Light v Electron Microscope Table

Your notes

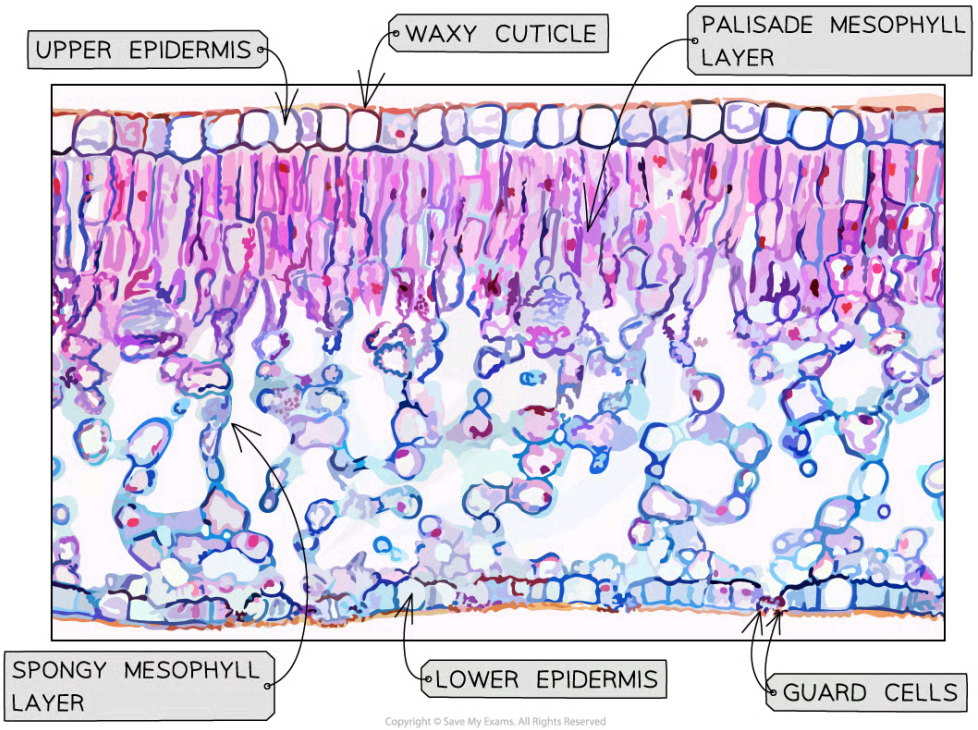
ELECTRON MICROSCOPE	LIGHT MICROSCOPE
LARGE AND INSTALLATION MEANS IT CAN'T BE MOVED	SMALL AND EASY TO CARRY
VACUUM NEEDED	NO VACUUM NEEDED
COMPLICATED SAMPLE PREPARATION	EASY SAMPLE PREPARATION
OVER x 500 000 MAGNIFICATION	UP TO x 2000 MAGNIFICATION
RESOLUTION 0.5 nm	RESOLUTION 200 nm
SPECIMENS ARE DEAD	SPECIMENS CAN BE LIVING OR DEAD

Your notes

Staining Specimens

- Specimens to be viewed under a microscope sometimes need to be **stained**, as the cytoplasm and other cell structures may be **transparent** or **difficult to distinguish**
  - Note that most of the colours seen in images taken using a light microscope are the result of added stains
    - Chloroplasts are the exception to this; they show up green, which is their natural colour
- The **type of stain** used is dependent on what type of specimen is being prepared and what the researcher wants to observe within the specimen
  - **Different molecules absorb different dyes** depending on their chemical nature
- Specimens or sections are sometimes stained with **multiple dyes** to ensure that several **different tissues** within the specimen show up; this is known as **differential staining**
- Some common stains include
  - Haemotoxylin
    - Stains plant and animal cell nuclei purple, brown or blue
  - Methylene blue
    - Stains animal cell nuclei blue
  - Acetocarmine
    - Stains chromosomes in dividing nuclei of plant and animal cells
  - Iodine
    - Stains starch-containing material in plant cells blue-black
  - Toluidine blue
    - Stains tissues that contain DNA and RNA blue
  - Phloroglucinol

- Stains a chemical called lignin found in some plant cells red/pink



Toluidine blue and phloroglucinol have been used to stain this tissue specimen taken from a leaf

Your notes

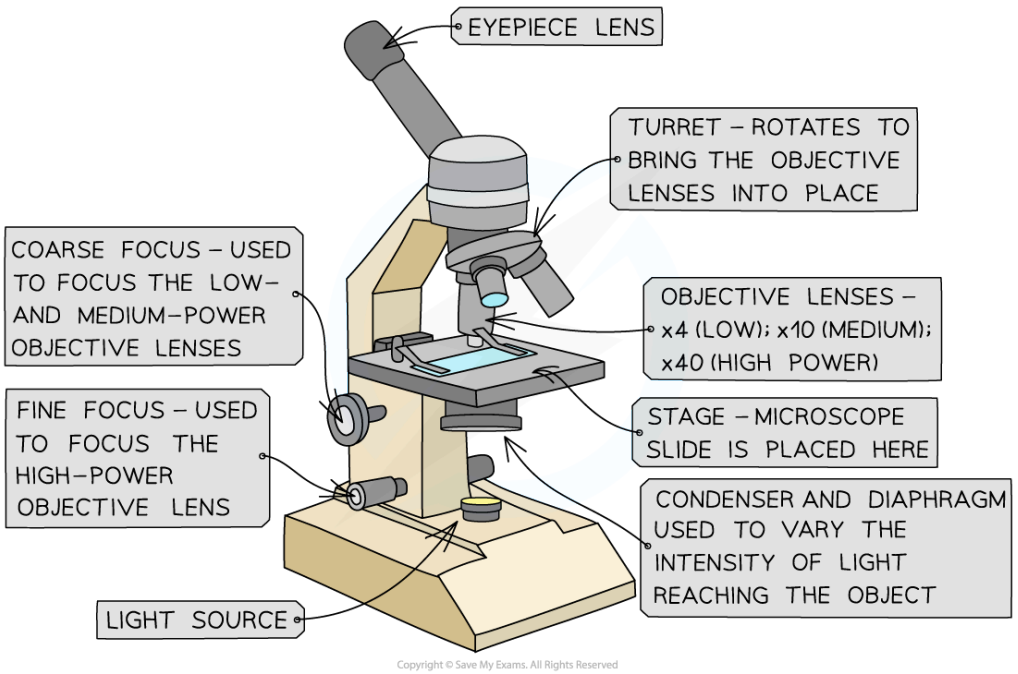
Your notes

Microscope Images

- Many biological structures are too small to be seen by the naked eye
- **Optical,** or **light, microscopes** are an invaluable tool for scientists as they allow for tissues, cells and organelles to be seen and studied
  - Light is directed through a thin layer of biological material that is supported on a glass slide
  - This light is focused through several lenses so that an image is visible through the eyepiece
  - The magnifying power of the microscope can be increased by rotating the higher power objective lens into place

Preparation of microscope slides

- The key components of an optical microscope are
  - The eyepiece lens
  - The objective lenses
  - The stage
  - The light source
  - The coarse and fine focus
- Other tools that may be used
  - Forceps
  - Scissors
  - Scalpel
  - Coverslip
  - Slides
  - Pipette
  - Staining solution



The components of an optical microscope

Method

- Preparing a slide using a **liquid specimen**
  - Add a few drops of the sample to the slide using a pipette
  - Cover the liquid / smear with a coverslip and gently press down to **remove air bubbles**
  - **Wear gloves** to ensure there is no cross-contamination of foreign cells
- Methods of preparing a microscope slide using a **solid specimen**
  - Take care when using sharp objects and wear gloves to prevent the stain from dying your skin
  - Use scissors or a scalpel to cut a small sample of the tissue
  - Use forceps to peel away or cut a **very thin layer** of cells from the tissue sample to be placed on the slide
    - The tissue needs to be thin so that the **light** from the microscope can pass through
  - Apply a stain to make cells more visible
  - Gently place a coverslip on top and press down to remove any air bubbles
- Some tissue samples need to be treated with **chemicals to kill cells or make the tissue rigid**

Your notes

- This involves **fixing the specimen** using the preservative formaldehyde, dehydrating it using a series of **ethanol** solutions, impregnating it with **paraffin or resin** for support and then cutting thin slices from the specimen
- The paraffin is removed from the slices and a **stain** is applied before the specimen is mounted and a coverslip is applied


Your notes

Slide Preparation Table

DRY MOUNT	WET MOUNT	SQUASH SLIDES	SMEAR SLIDES
SOLID SPECIMENS	WET SPECIMENS	SOFT SPECIMENS	BODY FLUID SPECIMENS
THIN SLICES CALLED SECTIONING. COVERSLIP PLACED ON TOP	SUSPENDED IN WATER OR IMMERSION OIL. COVERSLIP PLACED AT ANGLE	WET MOUNT SQUASHED BETWEEN SLIDE AND COVERSLIP.	THE EDGE OF THE SLIDE IS USED TO SMEAR THE SAMPLE, CREATING THIN EVEN COATING.
USES: HAIR, POLLEN, DUST, MUSCLE TISSUE, PLANT TISSUE	USES: AQUATIC SAMPLES AND OTHER LIVING ORGANISMS	USES: ROOT CELLS TO LOOK AT CELL DIVISION	USES: BLOOD SMEARS TO VIEW ERYTHROCYTES

Using a microscope

- When using an optical microscope always **start with the low power objective lens**
  - It is **easier to find** what you are looking for in the field of view
  - This helps to **prevent damage** to the lens or coverslip in case the stage has been raised too high
- **Preventing the dehydration** of tissue
  - The thin layers of material placed on slides can **dry up rapidly**
  - Adding a drop of water to the specimen beneath the coverslip can prevent the cells from being damaged by dehydration
- **Unclear or blurry images**
  - Switch to the lower power objective lens and try using the **coarse focus** to get a clearer image
  - Consider whether the specimen sample is **thin enough** for light to pass through to see the structures clearly
  - There could be **cross-contamination** with foreign cells or bodies

Limitations

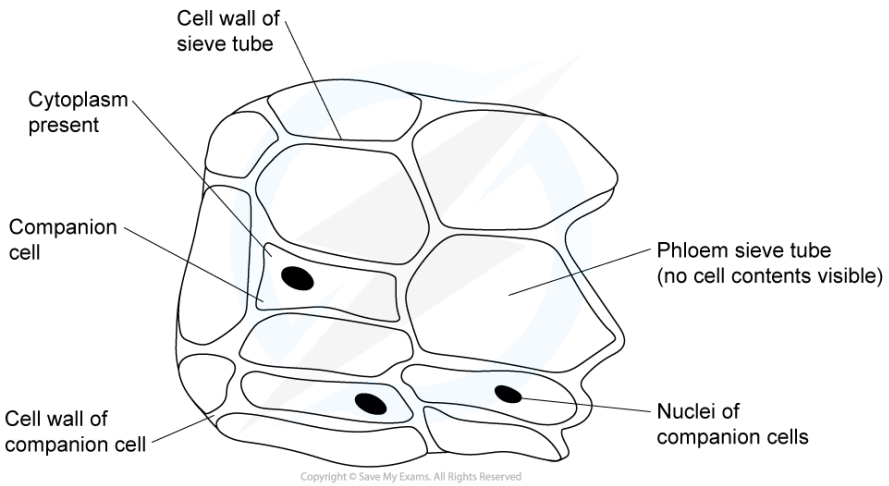
- The size of cells or structures of tissues may appear inconsistent in different specimen slides
  - Cell structures are 3D and the different tissue samples will have been **cut at different planes** resulting in this inconsistencies when viewed on a 2D slide
- Optical microscopes do not have the same magnification power as other types of microscopes and so there are some structures that cannot be seen

- The treatment of specimens when preparing slides could alter the structure of cells

Your notes

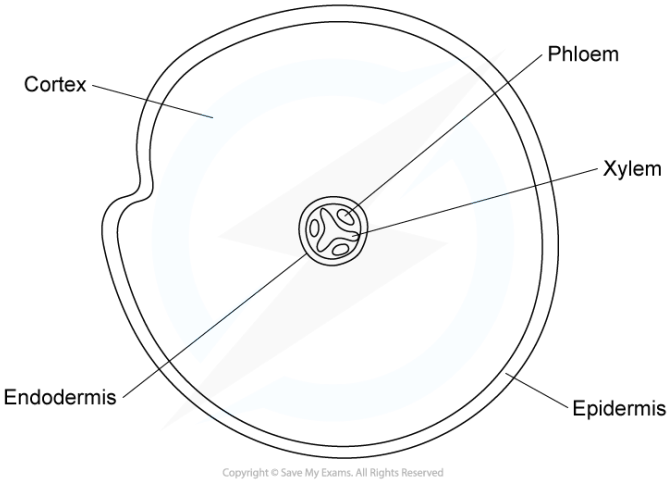
Drawing cells

- To record the observations seen under the microscope, or from photomicrographs taken, a **labelled biological drawing** is often made
  - Biological drawings are **line drawings** that show specific features that have been observed when the specimen was viewed
- There are a number of **rules or conventions** that are followed when making a biological drawing
  - The drawing must have a title
  - The **magnification** under which the observations shown by the drawing are made must be recorded
  - A **sharp pencil** should be used
  - Drawings should be on plain white paper
  - Lines should be **clear**, **single lines** with no sketching
  - **No shading**
  - The drawing should take up as much of the space on the page as possible
  - Well-defined structures should be drawn
  - The drawing should be made with **proper proportions**
  - **Label lines** should not cross or have arrowheads and should **connect directly** to the part of the drawing being labelled
  - Label lines should ideally be kept to one side of the drawing in parallel to the top of the page, and should be drawn with a **ruler**
  - Only visible structures should be drawn; not structures that the viewer thinks they should be able to see!
- Drawings of **cells** are typically made when visualizing cells at a **higher magnification power**



An example of a cellular drawing taken from a high-power image of phloem tissue

- **Plan drawings** that show the arrangement of cells within a tissue or organ are typically made using samples viewed under **lower magnifications**
  - Individual cells are never drawn in a plan diagram



An example of a tissue plan diagram drawn from a low-power image of a transverse section of a root. Note that there is no cell detail present.

Measurements of Microscopic Images

- **Magnification** is **how many times bigger** the image of a specimen observed is in comparison to the actual, real-life size of the specimen
- A **light microscope** has two types of lens:
  - An **eyepiece lens,** which often has a magnification of x10
  - A series of (usually 3) **objective lenses**, each with a different magnification

Your notes

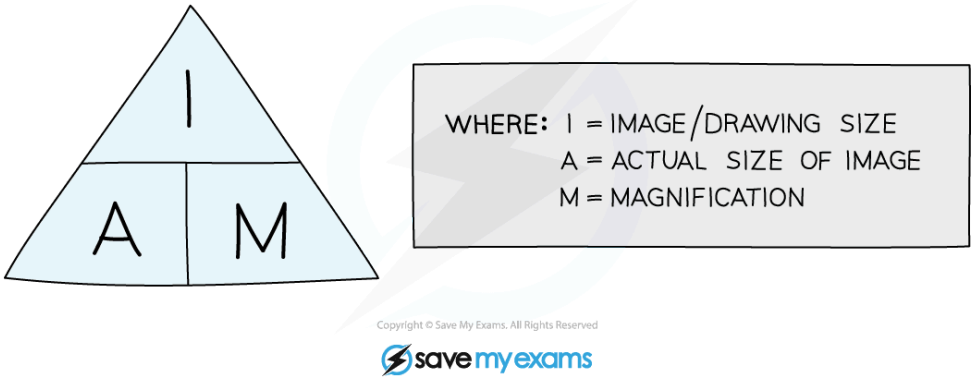
- To calculate the **total magnification,** the magnification of the **eyepiece** lens and the **objective** lens are **multiplied** together:

total magnification = eyepiece lens magnification x objective lens magnification

- The **magnification** (M) of an object can also be calculated if both the size of the **image** (I), and the **actual size** of the specimen (A), is known

magnification = image size ÷ actual size

- Remember to ensure that the image size (I) and the actual size (A) of the specimen have the **same units** before doing the calculation

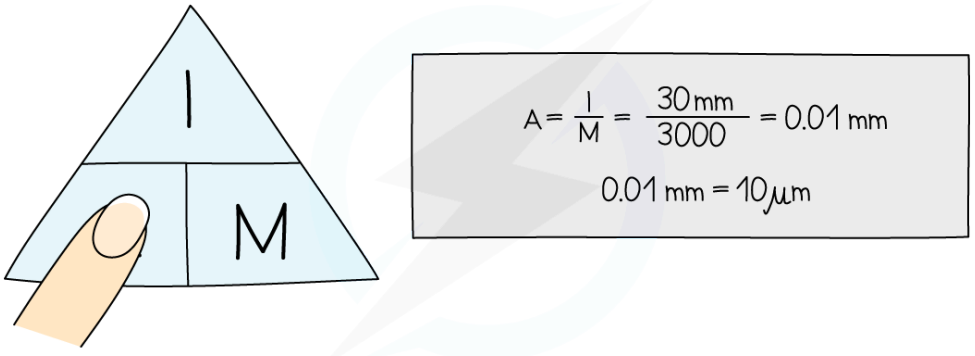


The equation for calculating magnification can be rearranged to calculate either actual size, image size, or magnification.

Worked Example

An **image** of an animal cell is 30 mm in diameter and it has been **magnified** by a factor of x3000.

What is the **actual** diameter of the cell?

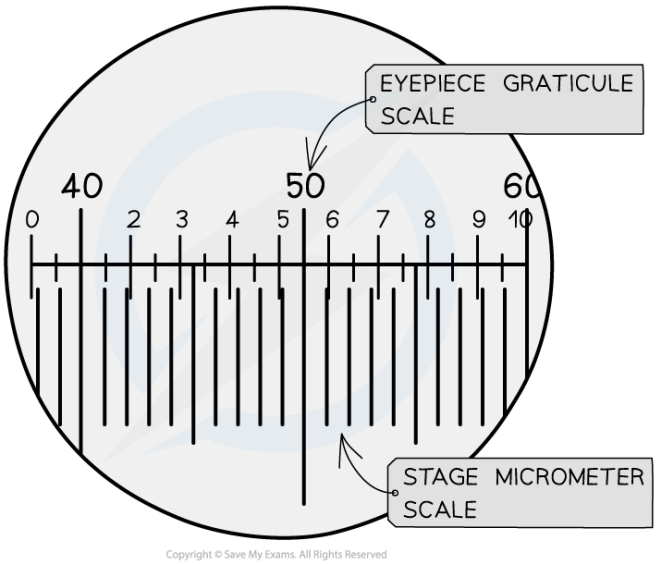


Your notes

Using an eyepiece graticule & stage micrometer

Your notes

- A **graticule** is a small disc that has an **engraved ruler**
- It can be placed **into the eyepiece** of a microscope to act as a ruler in the field of view, so is sometimes known as an **eyepiece graticule**
- As an eyepiece graticule has no fixed units it must be **calibrated** for the objective lens that is in use
  - The graticule in the eyepiece remains the same size when the magnification of the microscope is altered, so recalibration is needed at each viewing magnification
- Calibration of the eyepiece graticule is done a microscope slide with an engraved scale known as **a stage micrometer**
- By using the **eyepiece graticule and the stage micrometer together,** the size of each graticule unit can be calculated
  - After this is known the graticule can be used as a **ruler** to measure objects in the field of view

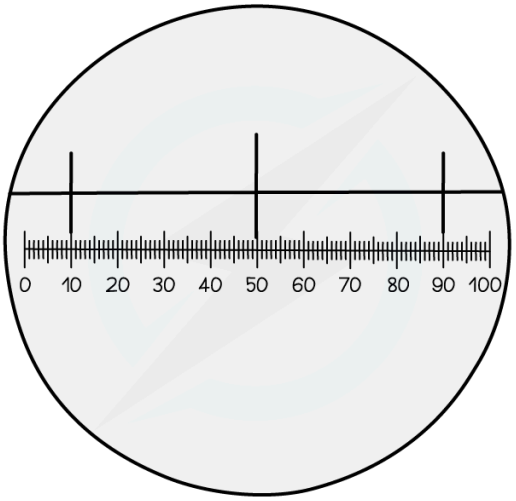


The stage micrometer scale is used to find out how many micrometers each eyepiece graticule unit represents

Worked Example

Calculate the size of the units of the eyepiece graticule in the image below.

Note that the large divisions in the top half of the image show the stage micrometer and that each stage micrometer division is 1 mm across.



Your notes

Answer:

Step 1: Observe the number of eyepiece unit divisions per micrometer unit

In the image, the stage micrometer has three lines

Each micrometer division has 40 eyepiece graticule divisions within it

Step 2: Calculate the size of each eyepiece graticule unit

40 graticule divisions = 1 mm (1000 µm)

1 graticule unit = 1000 ÷ 40 = 25 µm

- An object that spanned five eyepiece graticule units could therefore be measured as follows

5 x 25 µm = 125 µm

Examiner Tips and Tricks

The biggest pitfall with these kinds of calculations is forgetting to convert the units so that they match before embarking on a calculation. E.g. if image size is measured in mm but the actual size of an object is given in µm then both need to be converted into µm before using the equation triangle above.

To convert a measurement from mm into µm the measurement must be multiplied by 1000 (there are 1000 µm in 1 mm).