

Microscopy and measuring cells

Revision notes · Magnification and resolution in light and electron microscopes, why specimens are stained, and Core Practical 5 on drawing and measuring cells.

For Pearson Edexcel International AS Biology XBI11 and International A Level Biology YBI11. **A Level only** is IA2 content (Units 4 to 6), not needed for the International AS.

Microscopy and measuring cells Spec 3.7, 3.8

Magnification and resolution Spec 3.7

Magnification	How many times larger the image is than the real object.
Resolution	The smallest distance between two points at which they can still be seen as two separate points.

Magnification can be increased as much as you like, but the image only shows more detail if the **resolution** is good enough. Resolution is limited by the wavelength of the radiation used: two points closer together than about half the wavelength merge into one.

$$\text{magnification} = \text{size of image} \div \text{size of real object}$$

Light and electron microscopes compared			
Feature	Light microscope	Transmission electron microscope (TEM)	Scanning electron microscope (SEM)
Radiation	Light (wavelength about 400 to 700 nm), focused by glass lenses	Beam of electrons (very short wavelength), focused by electromagnets, in a vacuum	Beam of electrons scanned over the surface, in a vacuum
Maximum useful magnification	About $\times 1500$	Over $\times 1\,000\,000$	Up to about $\times 500\,000$
Resolution	About 200 nm (0.2 μm)	Roughly 0.1 to 0.5 nm	Roughly 3 to 10 nm
Specimen	Living or dead; thin section or whole mount	Dead, in a vacuum; extremely thin sections	Dead, in a vacuum; coated with a thin layer of metal
Image	Colour (if stained), 2D	Black and white, 2D, shows internal ultrastructure	Black and white, 3D appearance of the surface
Problems	Poor resolution; cannot see organelle detail	Preparation may create artefacts; cannot view living cells; expensive	Lower resolution than TEM; surface only

Electron microscopes have a much greater resolution than light microscopes because the **wavelength of electrons is much shorter** than the wavelength of light. This is why ribosomes, the Golgi apparatus and the inner membranes of mitochondria can only be seen with an electron microscope. Artefacts are

structures that appear in the image because of the way the specimen was prepared and are not present in the living cell.

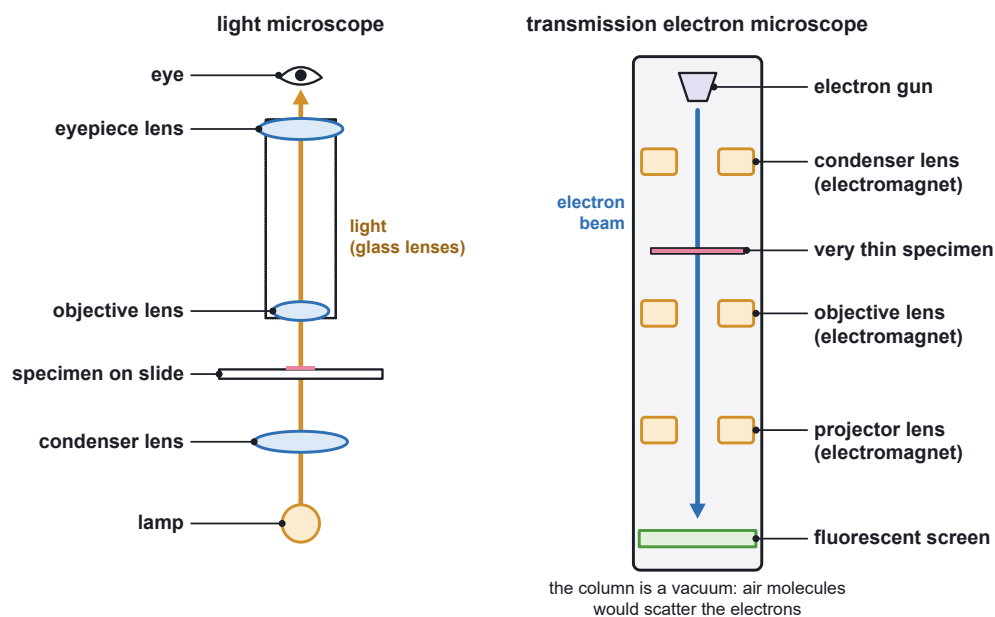
Calculating magnification

A drawing of a cell is 60 mm long. The actual length of the cell is 15 μm . Calculate the magnification of the drawing.

1. Convert both measurements to the same unit: 60 mm = 60 000 μm .
2. magnification = size of image $\div$ size of real object
3. magnification = 60 000 $\div$ 15

Answer: $\times 4000$

Maths skill: Write the formula, convert to the same units first, and give no unit for magnification (just $\times$). To find the actual size, rearrange: actual size = image size $\div$ magnification.



Resolution (same scale):

light microscope: about 200 nm
| TEM: about 0.5 nm (400 times smaller, so much more detail)

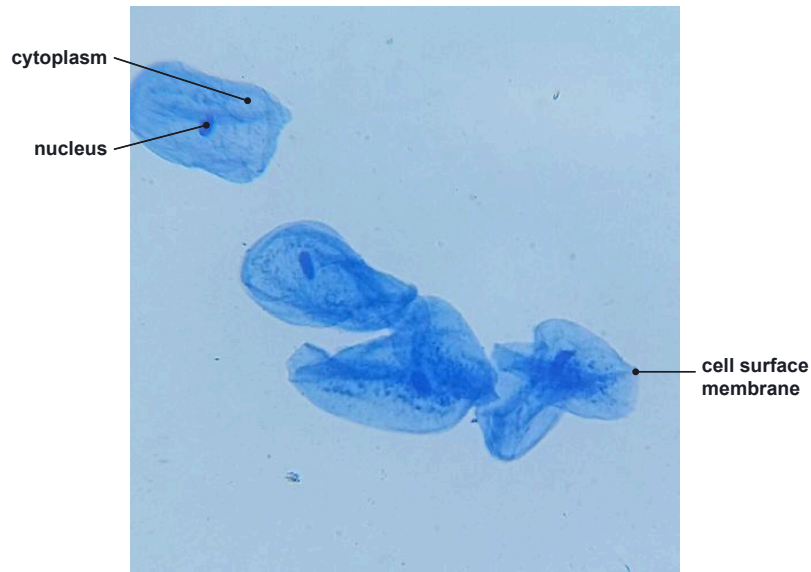
Electrons have a much shorter wavelength than light, so the TEM has a much higher resolution.

Staining specimens Spec 3.7

Cells are mostly water and are almost transparent, so structures inside them hardly absorb light and are difficult to see. **Staining** increases the contrast between structures, and different stains bind to different structures so they can be told apart.

- Light microscopy: coloured dyes absorb light and make structures visible. Examples are methylene blue (stains nuclei and cytoplasm blue), eosin (stains cytoplasm pink), iodine solution (stains starch blue-black) and acetic orcein or toluidine blue (stain DNA/chromosomes dark).
- Differential staining uses stains that bind to different structures, so that two tissues or two parts of a cell are different colours.
- Electron microscopy: the specimen is treated with salts of heavy metals, such as lead or uranium. These scatter electrons, so the stained parts look dark and the rest looks lighter. Different structures take up different amounts of metal, which gives the contrast that allows organelles to be identified.

Exam tip: The reason for staining is always contrast: structures are made visible and distinguishable. Say that, rather than 'to make it clearer'.



Cheek cells stained with **methylene blue**: the nucleus stains darker than the cytoplasm. Unstained, the cells are almost invisible.

Image: Fritzmann2002, CC BY-SA 4.0, via Wikimedia Commons

Core Practical 5: observing, drawing and measuring cells Spec 3.8

Core practical: Core practical 5: use a light microscope to make observations and labelled drawings of suitable animal cells, and use a graticule to make measurements and understand scale.

Preparing and viewing the slide

1. Wash hands. Use a clean cotton bud (or a sterile spatula) to gently scrape the inside of your own cheek.
2. Smear the cells onto a clean microscope slide in a drop of water or stain (for example methylene blue). Dispose of the swab in disinfectant.
3. Lower a coverslip at an angle with a mounted needle, to avoid trapping air bubbles. Blot excess stain with filter paper.
4. Start with the lowest-power objective lens, focus with the coarse focus and then the fine focus, and increase the magnification as needed.
5. Alternatively, use a prepared slide of an animal tissue, such as a section of liver or of squamous epithelium.

Making a labelled drawing

- Use a sharp pencil, a clear continuous line, no shading and no colouring.
- Draw only what you see. Make the drawing large (fill at least half of the space) and draw a few cells, not the whole field of view.
- Label with ruled lines that do not cross and touch the structure, and give the title, the magnification used and a scale bar.

Measuring with a graticule

An eyepiece **graticule** is a transparent scale with arbitrary divisions placed in the eyepiece. Its units have no fixed size, so it must be calibrated for each objective lens against a **stage micrometer**, a slide with a scale of known size (usually divisions of 10 μm).

1. Place the stage micrometer on the stage and focus on its scale.

2. Line up the zero of the graticule with the zero of the micrometer.
3. Find a point further along where two divisions line up again. Count the graticule divisions and the micrometer divisions between these points.
4. Calculate the size of one graticule division in μm .
5. Replace the micrometer with the specimen and measure the cell in graticule divisions. Multiply by the calibration value. Repeat for several cells and calculate a mean.

Calibrating a graticule

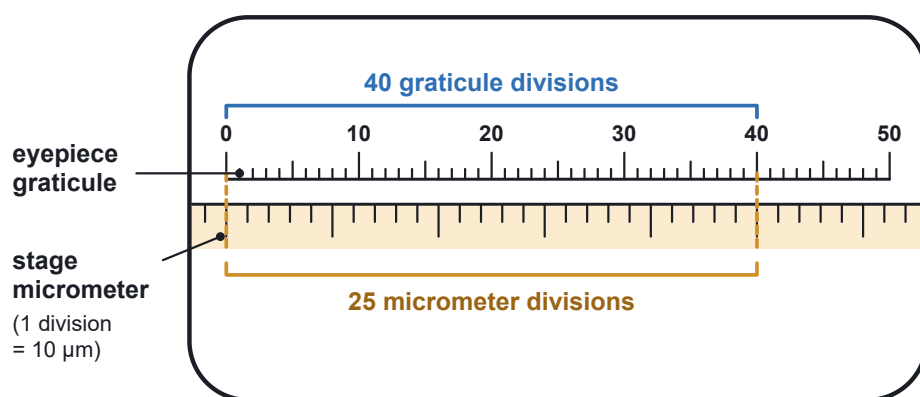
At $\times 400$, 40 graticule divisions are the same length as 25 divisions of a stage micrometer. Each micrometer division is $10\ \mu\text{m}$. A cell measures 12 graticule divisions. Calculate the actual length of the cell.

1. Length of 25 micrometer divisions = $25 \times 10 = 250\ \mu\text{m}$
2. One graticule division = $250 \div 40 = 6.25\ \mu\text{m}$
3. Cell length = 12×6.25

Answer: $75\ \mu\text{m}$

Maths skill: Recalibrate whenever the objective lens is changed. Measuring several cells and finding the mean makes the result more reliable. Work in μm and convert to mm by dividing by 1000.

Risks and controls: your own cheek cells should not be shared and must be disposed of in disinfectant, to prevent the spread of infection. Stains such as methylene blue can stain skin and clothes. Take care with glass slides and coverslips, which can break. Wear eye protection if the stain is an irritant.



$$40 \text{ graticule divisions} = 25 \times 10\ \mu\text{m} = 250\ \mu\text{m}$$

$$1 \text{ graticule division} = 250\ \mu\text{m} \div 40 = 6.25\ \mu\text{m}$$

(calibration for this objective lens only)

Calibrating the graticule: 40 graticule divisions = $250\ \mu\text{m}$, so 1 division = $6.25\ \mu\text{m}$.

Quick check

1. Define resolution.
2. Why do electron microscopes have a higher resolution than light microscopes?
3. Why are specimens stained?
4. What is the formula for magnification?
5. Why must an eyepiece graticule be calibrated?

Quick check answers

Microscopy and measuring cells

1. The smallest distance between two points at which they can still be seen as separate.
2. Electrons have a much shorter wavelength than light.
3. To increase contrast so that structures are visible and can be told apart.
4. Magnification = size of image $\div$ size of real object.
5. Its divisions have no fixed size; they depend on the objective lens, so they must be compared with a stage micrometer.