

Microscopy

Apart from a very few special cases, cells cannot be seen with the naked eye. Both light microscopes (LM) and electron microscopes (EM) are used to magnify them and their structures.

Size matters

Rarely, cells can be seen with the naked eye. The biggest bacterium known is about 0.3 mm (300 μ m), visible, just by comparison.
 • red blood cell = 5 μ m
 • a mitochondrion = 4 μ m

So, these are far too small to see without a microscope.

Magnification

Image size $\div$ actual size = magnification

If an image of a length is 3 cm wide at a magnification of $\times 500$, the actual width of the specimen would be $3 \div 500 = 0.006$ cm.

Magnification and resolution

It seems obvious that we must magnify small cells and structures so that they can be seen, but that is only part of the solution. The other important factor is **resolution** - being able to see two objects as distinct. The human eye has a resolution of about 0.1 mm; two objects closer together than this will look like one object. An LM can resolve to about 0.2 μ m and the EM to about 1 nm or less. This depends on the wavelength (λ), for light this is about 500 nm, for an electron beam less than 1 nm. Once you can resolve well it is worth magnifying a lot. Without high resolution, high magnification just gives something big, but still fuzzy.

Measurements

estimate (μ m)	estimate (nm)	estimate (μ m)	estimate (nm)	estimate (μ m)	estimate (nm)
10 μ m	10 μ m	10 μ m	10 μ m	10 μ m	10 μ m
100 μ m	100 μ m	100 μ m	100 μ m	100 μ m	100 μ m
1000 μ m	1000 μ m	1000 μ m	1000 μ m	1000 μ m	1000 μ m
10000 μ m	10000 μ m	10000 μ m	10000 μ m	10000 μ m	10000 μ m

Worked example

The electron micrograph image shows a ribosome.



Magnification $\times 4000$

Calculate its actual length between W and Y in μ m, to two significant figures. (2 marks)

The image length is 4.8 mm and the magnification is $\times 4000$. So, actual size = length $\div$ magnification $\times 4000$ = 1.2 μ m

Achieving greater magnification and resolution

Magnification: magnify a magnified image again, e.g. 10 times $\times$ 10 times = 100 $\times$

Resolution: use resolution (light or electron) of a shorter wavelength.

Empty magnification

If we magnify something beyond the resolving power no more detail is seen, the magnification is said to be empty.



Is reaching the Synapsis, reaching 1M magnification results in more detail.

Now try this

Explain why an EM has a much better resolving power than an LM. (2 marks)

Practical microscopy

In order to see as much detail as possible when looking at something through a microscope, preparation of the specimen is needed. Cutting of sections and staining are common techniques in light microscopy.

The light transmission microscope

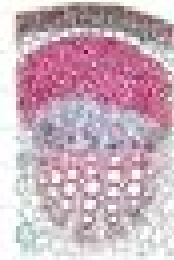
Light microscopy works when light passes through a specimen so that it can then enter the eye. This means the specimen must be thin. An onion root scale epidermis has a single layer of cells which can be peeled off and viewed easily. But most other structures need to be sectioned.



Light from the broad width of a cell enters.

Staining

If a thin section is obtained and put straight under the microscope the results can be disappointing. Most structures are colourless in their natural state. Staining is used to give structures colour and contrast and allow identification.



- Common stains used:
- iodine solution - stains starch blue/black
 - ethanolic crystal - stains DNA reddish-purple
 - methylene blue - stains nuclei and chromosomes blue
 - phosphotungstic - stains lignin, found in plant cells, red.
- Thick leaf section causing light to not pass a good view of the chloroplasts.

The electron microscope

The electron microscope (EM) has revolutionised our understanding of cells. It is not all good news, though.

- All specimens are examined in a vacuum so it is impossible to look at living material.
- The treatment is likely to result in artefacts that is things which are not really there but appear due to the preparation methods.
- EMs are still extremely expensive to buy, run and maintain.

Sectioning

A sharp razor blade can be used, with practice, to cut the sections.



Using a blade to cut the sections



Labels: eyepiece, objective, stage, base, condenser, slide, high power objective lens, low power objective lens, eyepiece, objective, stage, base, condenser, slide, high power objective lens, low power objective lens.

Worked example

Evaluate the strengths and weaknesses of EM and LM microscopes. (4 marks)

An EM has much better resolution and therefore magnification is not 'empty', up to very high values. However, specimens cannot be alive and there is so much harsh treatment in the preparation of materials it is not always clear what is real and what is artefact. An LM has limited resolution so magnification above about 1000 $\times$ is pointless. Many organisms are too small to see at this power. However, living material can be viewed and the problem of artefacts is much less severe.

Now try this

What eyes would you use to take the dimensions of onion root tip cells? (1 mark)

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A Loxley



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