



MICROSCOPY

An introduction to the microscope and magnification

MAGNIFICATION AND RESOLUTION

Because cells are too small to be seen with the naked eye, the **light microscope** was developed to produce enlarged and more detailed images of cells. The **magnification** of an image is how much bigger it appears under the microscope than it is in real life, and is worked out using the following formula:

$$\text{magnification} = \text{image size} \div \text{actual size}$$

unit	symbol	metres
metre	m	1
decimetre	dm	0.1
centimetre	cm	0.01
millimetre	mm	0.001
micrometre	μm	0.000 001
nanometre	nm	0.000 000 001
picometre	pm	0.000 000 000 01

However, magnification on its own does not increase the level of detail seen, it just increases the size. The term **resolution** refers to the ability to see two distinct points separately. For example, if the resolution of a light microscope is 200nm (0.2 μm), this means it can see any two different points as separate objects if they are 200nm apart or more; but if they are any closer than this amount, they appear as one object.

THE LIGHT MICROSCOPE

Light microscopes use a number of lenses to produce an image that can be viewed directly at the eyepiece. Light passes from a bulb under the stage, through a **condenser lens** and then through the specimen. This beam of light is passed through an **objective lens** and then the **eyepiece lens**. The light microscope usually has a number of objective lenses which can be rotated into position, these are x4, x10, x40 and x100 lenses. The eyepiece lens then magnifies the image again by x10. So the final magnifications the microscope is capable of producing are x40, x100, x400 and x1000.

$$\text{overall magnification} = \text{objective lens magnification} \times \text{eyepiece lens magnification}$$

You can view some specimens directly using the light microscope. Others have to be prepared to get around the issues involving the fact that biological material may not be coloured and so detail cannot be seen; also that some materials distort when you cut them into small sections:

- 1 staining** – coloured stains are chemicals that bind to chemicals on or in the specimen, this allows the specimen to be seen
- 2 sectioning** – specimens are embedded in wax – thin sections are then cut out without distorting the specimen – this is especially useful for making sections of soft tissue, such as brain

THE ELECTRON MICROSCOPE

Light microscopes have low resolution, of about 200nm (0.2 μm), so structures closer together than this appear as one object. A higher resolution can be achieved with an **electron microscope**. Electron microscopes generate a beam of electrons, which have a wavelength of 0.004nm. They can distinguish objects 0.2nm apart. There are two types:

A – Transmission Electron Microscope (TEM)

The electron beam passes through a very thin prepared sample, and the electrons pass through denser parts less easily, giving some contrast in the final **2D** image produced. Maximum possible magnification of x500,000

B – Scanning Electron Microscope (SEM)

The electron beam is directed onto a sample. The electrons don't pass through the specimen, they bounce off, producing a final **3D** image view of the surface of the sample. Maximum possible magnification of x100,000

Advantages	Limitations
The resolution is 0.1nm (2000x more than the light microscope)	Electron beams are deflected by air molecules, so the sample has to be placed in a vacuum
Can produce more detailed images of the structures inside cells	Electron microscopes are extremely expensive
The SEM produces a final 3D image not possible with the light microscope	Preparing samples and using the electron microscope both require a high degree of skill and training



Electron **micrographs** are sometimes shown in colour. The final image produced from an electron microscope is always in greyscale; the colours are added afterwards using specialised computer software. Such images will be labelled as **false-colour** electron micrographs.

