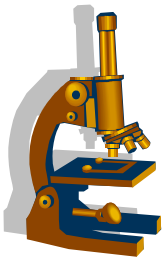
Microscopy



Of all the techniques used in biology **microscopy** is probably the most important. The vast majority of living organisms are **too small to be seen in any detail with the human eye** and cells and their **organelles can only be seen with the aid of a microscope**. Cells were first seen in 1665 by Robert Hooke (who named them after monks' cells in a monastery), and were studied in more detail by Leeuwenhoek using a primitive microscope.

Units of measurement:

Metre	m	= 1 m
Millimetre	mm	= 10^{-3} m
Micrometre	μm	= 10^{-6} m
Nanometre	nm	= 10^{-9} m

Magnification and Resolution

By using more lenses microscopes can achieve greater magnification, **but this does not mean that more detail can be seen.** The detail visible depends on the **resolving power (or resolution)** of a microscope, which is **the smallest separation at which two separate objects can be distinguished (or resolved).**

The resolving power of a microscope is **ultimately limited by the wavelength of light** (400-600nm for visible light). To improve the resolving power a shorter wavelength of light is needed, and sometimes microscopes have blue filters for this purpose (because blue light has a shorter wavelength).

Magnification is how much bigger a sample appears to be under the microscope than it is in real life.

Overall magnification = Objective lens x Eyepiece lens

Resolution is the ability to distinguish between two points on an image i.e. the level of detail

- Resolution is **limited by the wavelength of the radiation** used to view the sample.
- This is because when objects in the specimen are much smaller than the wavelength of the radiation being used, they do not interrupt the waves, and so are not detected.
- The **wavelength of light is much longer than the wavelength of electrons**, so the resolution of the light microscope is a lot lower.
- Using a microscope with a more powerful magnification will **not** increase this resolution any further. It will increase the size of the image, but objects closer than about 200nm (0.2μ) will still only be seen as one point.

Different kinds of Microscopes

Light Microscope. This is the oldest, simplest and most widely used form of microscopy. Specimens are illuminated with light, which is focussed using glass lenses and viewed using the eye or photographic film. **Specimens can be living or dead**, but often **need to be stained with a coloured dye** to make them visible. Many different stains are available that stain specific parts of the cell such as DNA, lipids, cytoskeleton, etc. All modern light microscopes are **compound microscopes**, which means they use several lenses to obtain high magnification. **Light microscopy has a resolution of about 0.2µ (= 200 nm),** which is good enough to see cells**, but not the details of cell organelles.**

Preparation of Slide Samples

- **Fixation:** Chemicals preserve material in a life like condition. Does not distort the specimen. This is often followed by **Dehydration, when water is removed from the specimen using ethanol. This is particularly important for electron microscopy** because water molecules deflect the electron beam which blurs the image.
- **Embedding:** Supports the tissue in wax or resin so that it can be cut into thin sections. Sectioning produces very thin slices for mounting. Sections are cut with a microtome or an ultra-microtome to make them either a few micrometres (light microscopy) or nanometres (electron microscopy) thick.
- **Staining: Most biological material is transparent and needs to be stained to increase the contrast between different structures**. Different stains are used for different types of tissues. Methylene blue is often used for animal cells, whilst I_2 / KI solution is used for plant tissues. Finally, the specimen is **mounted** on a slide to protect the material so that it is suitable for viewing over a long period.

Electron Microscopes.

These use a beam of electrons, rather than light, to "illuminate" the specimen. This may seem strange, but electrons behave like waves and can easily be produced (using a hot wire), focussed (using electromagnets) and detected (using a phosphor screen or photographic film). **A beam of electrons has an effective wavelength of less than 1 nm,** so **an em can be used to resolve even the smallest cellular details.** The development of the electron microscope in the 1950s revolutionised biology, allowing organelles such as mitochondria, ER and membranes to be seen in detail for the first time.

The main problem with the electron microscope is that specimens must be fixed in plastic and viewed in a vacuum, and must therefore be dead. Other problems are that the electron beam can damage the specimens and they must be stained with an electron-dense chemical (usually heavy metals like osmium, lead or gold). Initially there was a problem of **artefacts** (i.e. observed structures that were due to the preparation process and were not real), but improvements in technique have eliminated most of these.

There are two kinds of electron microscope:

Transmission electron microscopes (TEM) work much like a light microscope, transmitting a beam of electrons through a thin specimen and then focussing the electrons to form an image on a screen or on film. **This is the most common form of electron microscope and has the best resolution.**

Scanning electron microscopes (SEM) scan a fine beam of electrons onto a specimen and collect the electrons scattered by the surface. **This has poorer resolution, but gives excellent 3-dimentional images of surfaces.**

Transmission Electron Microscope (TEM)	Scanning Electron Microscope (SEM)
Pass a beam of electrons through the specimen. The electrons that pass through the specimen are detected on a fluorescent screen on which the image is displayed. **Ultra-thin sections are needed** for transmission electron microscopy, as the electrons have to pass through the specimen for the image to be produced. This is **the most common form of electron microscope** and **Has the best resolution**	Pass a beam of electrons over the surface of the specimen in the form of a 'scanning' beam. Electrons are **reflected off the surface** of the specimen as it has been **previously coated in heavy metals,** and then focussed on a fluorescent screen to make a visible image. Larger, thicker structures can thus be seen under the SEM, as the electrons do not have to pass through the sample in order to form the image. This gives **excellent 3-dimensional images of surfaces** but **Resolution is lower** than that of the TEM.

Comparison of the light and electron microscope

Light Microscope	Electron Microscope
Cheap to purchase (£100 – 1500)	Expensive to buy (over £ 50,000).
Cheap to operate.	Expensive to produce electron beam.
Small and portable.	Large and requires special rooms.
Simple and easy sample preparation.	Lengthy and complex sample preparation.
Material rarely distorted by preparation.	Preparation distorts material.
Vacuum is not required.	Vacuum is required.
Natural colour of sample maintained.	All images in black and white.
Magnifies objects only up to 2000 times	Magnifies over 500 000 times.
Specimens can be living or dead	Specimens always dead, as they must be fixed in plastic and viewed in a vacuum
Stains are often needed to make the cells visible	The electron beam can damage specimens and they must be stained with an electron-dense heavy metal salt (often osmium, lead or gold).
Limited resolution – about 0.2µ (200nm). Cannot see cell detail	**Transmission E-M** (**you MUST specify this**) has **excellent resolution** (about 1nm), so can see the most minute cell details