

Bio Factsheet



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Microscopes and Their Uses in Biology

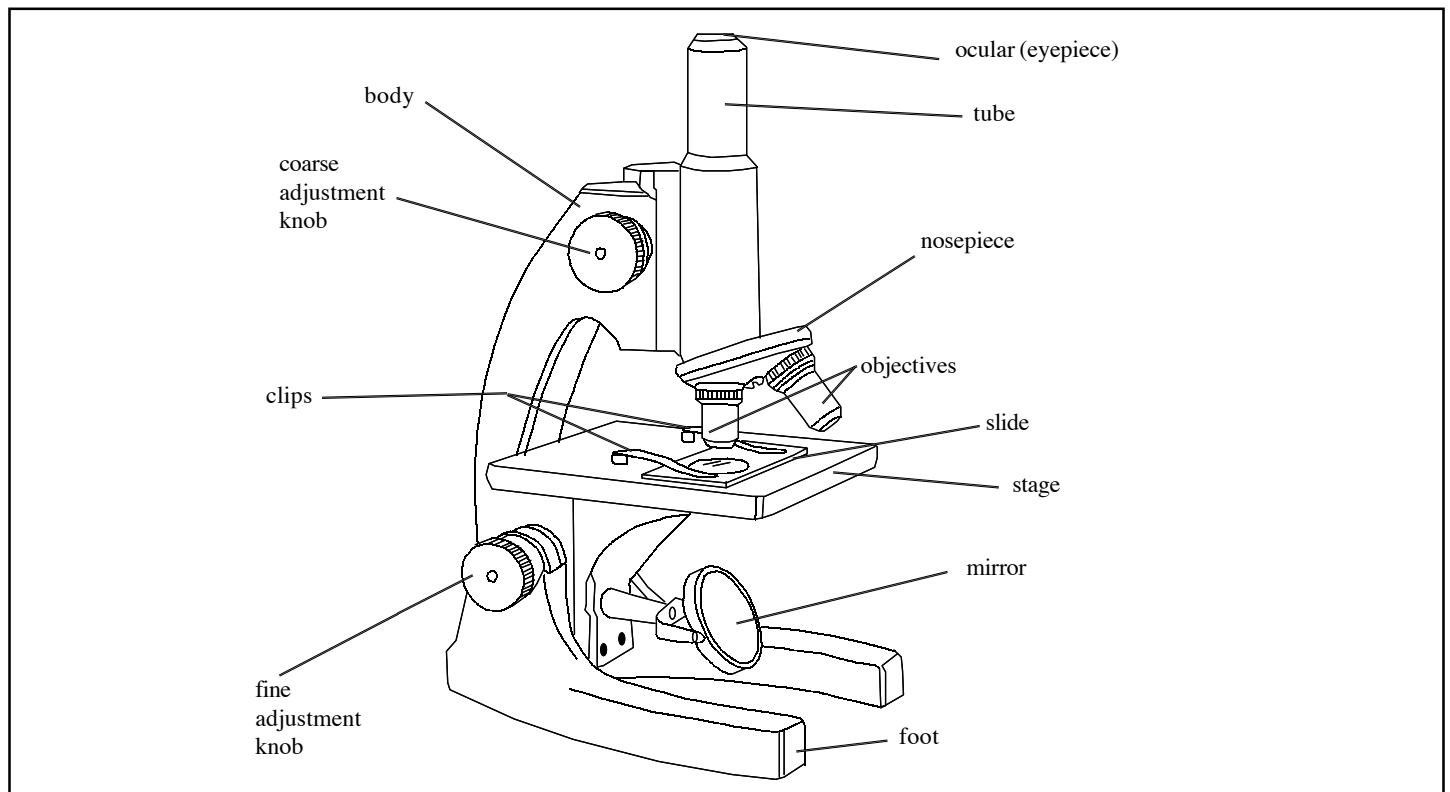
This Fact sheet is designed to introduce AS candidates to light and electron microscopes.

- Students must be aware of the limitations of microscopes.
- Students must know how to 'set microscopes up' correctly for optimum use.
- Candidates may be asked to interpret electron microscope pictures of cells and so should be aware of the capabilities of the electron microscope.
- Candidates must know the meaning of 'resolving power' and may be asked to calculate magnifications or absolute sizes of biological structures.

The optical(light) microscope

A basic optical microscope is shown in Fig 1.

Fig 1. An optical microscope



A basic optical microscope employs a mirror and condenser lens to illuminate a specimen by projecting a beam of light onto it. (In the above picture the condenser lens cannot be seen since it is hidden under the stage). The image is focussed and magnified by objective and eyepiece lenses. The total **magnification** is calculated by multiplying together the number on the eyepiece (eg x10) and the number on the objective lens (eg x40). In this case the image would have been magnified $\times 400$. The maximum magnification available with optical microscopes is about $\times 1500$.

However, creating an enlarged image does not necessarily provide the observer with more information. To achieve that, the image not only has to be enlarged but must also show detail.

How successfully this is achieved depends on the **resolving power (resolution)** of the microscope.

The **resolving power** of a microscope is its ability to distinguish between two points that are close together - a measure of the clarity of the image. No optical lens system can resolve two points closer together than about half (0.45) the wavelength of the light used to view them. The wavelength of visible light used by optical microscopes lies in the range 450 - 650 nm. This means that no light microscope can resolve two points closer than 0.45×450 nm apart. Thus any two points closer than about 200 nm apart will appear to be one point. Consequently, using short wave (bluish) daylight rather than longer wavelength (yellowish) artificial light will improve the resolving power of an optical microscope.

Remember – a nanometer (nm) is 10^{-9} of a metre. Micrometres (μm) may also be used in microscopy. One micrometre is 10^{-6} of a metre. Thus the limit of resolution of a light microscope is about $0.2\mu\text{m}$

The electron microscope

The specimen is illuminated in an electron microscope by an electron beam. Electrons are very small, negatively charged particles that show wave-like properties. The electron beam is focused using electromagnets arranged around the path of the electron beam. Electrons produce an image when focused onto a fluorescent screen. Modern electron microscopes can produce images with a magnification of up to $\times 500\,000$. Table 1. compares the basic components, advantages and disadvantages of optical and electron microscopes.

Table 1. advantages and disadvantages of a light and transmission electron microscopes

Feature	Light microscope	Electron microscope
radiation used	light rays	uses beams of electrons which require high voltages for generation
radiation source	light bulb which only requires low voltage	electron gun
nature of lenses	glass to focus light rays	electromagnets which can deflect and thus focus electron beams
lenses used	condensor, objective and eye piece lenses	condensor, objective and eyepiece lenses
image seen	directly by eye	a fluorescent (TV) screen is required
radiation medium	air	a vacuum is required since electrons are easily scattered by particles in air
magnification	up to $\times 1,500$	up to $\times 500,000$
limit of resolution	200 nm	0.2 nm
what it can show	structure and arrangement of tissues, microscopic organisms, living or dead, but it cannot show the cell ultrastructure	can only show dead structures. Will show cell ultrastructure including the fine structure of cell organelles.

Electron beams have wavelengths much shorter than the wavelengths of visible light. The resolving power of a microscope is inversely proportional to the wavelength of the radiation it uses. An electron microscope therefore has a much greater resolving power than an optical microscope.

There are two types of electron microscope, transmission electron microscopes (TEMs) and scanning electron microscopes (SEMs). The electrons pass through or past a thin section of the specimen in a TEM on their way to the fluorescent screen or photographic film. In SEMs the electrons are reflected off the prepared surface of the specimen. SEMs are very useful for detailed study of surfaces.

The extreme conditions that specimens are put into during preparation, staining and presentation for electron microscopy has led to some scepticism about the technique. The suggestion is sometimes made that some of the features seen using an electron microscope may well be artefacts which are consequences of the preparation techniques rather than what is really there in the living cell.

Functions of the parts of an optical microscope

The **condenser** control focuses the light onto the specimen and produces an image on the objective lens.

The condenser **iris diaphragm** controls the amount of light entering and leaving the condenser. It is adjusted using a lever. The diaphragm reduces the amount of glare and improves the contrast that can be achieved.

Focusing adjustments can be made with the **coarse and fine controls**. These alter the distance between the stage and the lens system. Generally use of the coarse adjustment control is restricted to focusing the low power objective lens.

The **objective lens** provides the first magnified image. The **eyepiece lens** magnifies that image and presents it to the viewer.

Setting up an optical microscope

- Carefully place the microscope into a position that is comfortable for you. Plug the illuminator into the power supply. Make sure that the lamp power setting is at a minimum. Switch the power on. Adjust the power setting to about two thirds maximum.
- Select the lowest power objective lens.
- Place a prepared slide (cover slip uppermost) onto the stage of the microscope. Viewing the specimen from the side, move the slide on the stage until the specimen is illuminated.
- Focus the image using the coarse adjustment and finally the fine adjustment.
- Close the iris diaphragm by moving its lever, then reopen it until you get to the point where the image does not get any brighter.
- Place the point of a sharpened pencil or the point of a mounted needle or something similar, on the centre of the light source. Focus the condenser so that the point and the specimen are both in focus.
- Review the specimen using the low power objective lens. Move the slide on the stage so that what you want to look at in particular is in the centre of the field of view. (Remember that what is at the top of the specimen will be at the bottom of the image and vice versa and when you move the slide physically to the left the image will move to the right.)
- Rotate a higher-powered lens into position. Listen for a distinct 'click' to make sure that the lens has located correctly. The part of the specimen you want to observe should still occupy a position near the centre of field of view under the new lens, but obviously appearing larger.
- Use of the fine focus control will bring the specimen clearly into focus. It is important to remember that high power lenses operate closer to the slide surface than low power lenses. It is essential therefore that you do not use the coarse adjustment control when working with high power lenses. A lapse of memory can result in the high powered objective being driven into the slide or stage. Even the slightest damage can reduce the quality of the image produced. It is therefore the most important part of the microscope and the most expensive to replace.
- The correct light setting for each lens will be different. Therefore repeat instructions 5 and 6.
- If necessary repeat 8, 9 and 10.
- When you have finished viewing a particular slide under high power, rotate the low power objective lens back into position and lower the stage, BEFORE you remove the slide from the stage. This will lessen the risk of damage to the high power lens.
- Turn the light control down to its minimum. Then switch off the power.
- While you are waiting for the lamp to cool down (it is less likely that the filament will be damaged if you allow it to cool before moving it) you should clean the microscope stage. Clean the eyepiece lens with a lens tissue. Give the objectives a visual check. Report any problems.
- Unplug the microscope and rewind the lamp cable. Replace the microscope's dust cover and return it to its storage box or station.

Remember – a microscope is an expensive piece of equipment and so should be cared for and used properly. It is a precision instrument and if set up carelessly will not yield the clarity and detail that it is capable of. For instance, students frequently omit step 6 of the directions above since they find it difficult to do. and this usually results in a big loss of resolving power.

Exam Hint - Candidates may be expected to do calculations to measure the absolute size or magnification of microscopical features.

Size and Magnification

Photomicrographs, electron-micrographs and biological drawings usually have either a statement of magnification (eg x100) or a linear scale of some description (a bar of defined length eg 50 nm). Actual size can be determined using the following equation:

$$\text{Actual size} = \frac{\text{Image size}}{\text{Magnification}}$$

Magnification can be determined using the same relationship, rearranged as follows:

$$\text{Magnification} = \frac{\text{Image size}}{\text{Actual size}}$$

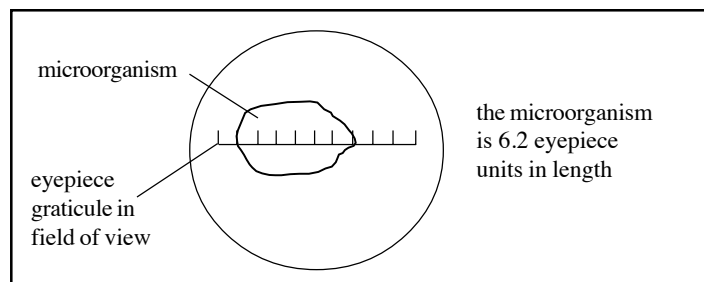
Estimation of size

It is possible to estimate the size of a structure seen with a microscope by comparing the image with a known linear scale. Two pieces of apparatus are commonly used:

- a graticule (eyepiece micrometer); and
- a stage micrometer.

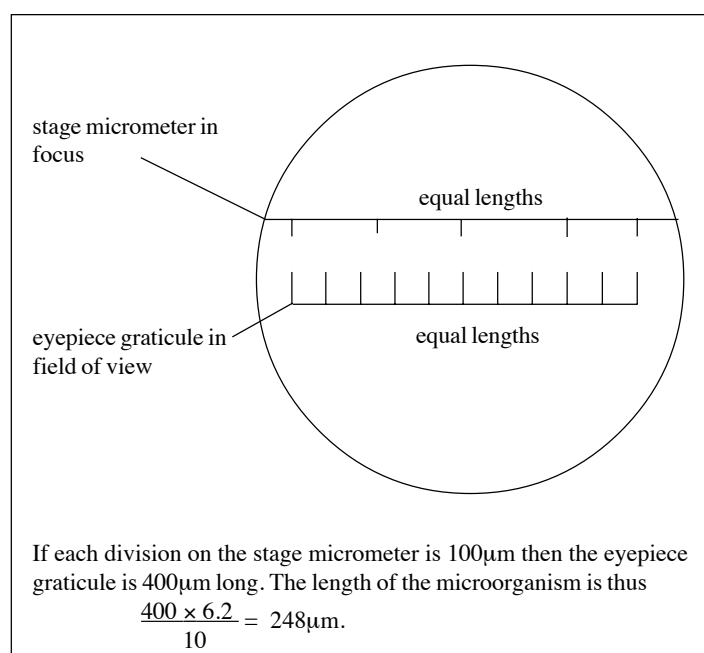
A stage micrometer is a slide with a fine scale of known dimension etched onto it. An graticule is a fine scale that fits inside an eyepiece lens. This is shown in Fig 3.

Fig 3. Using an eyepiece graticule to measure a microorganism



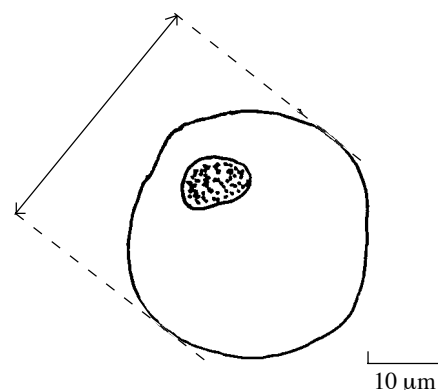
The graticule can be calibrated using a stage micrometer. This is shown in Fig 4.

Fig 4. Calibrating the graticule using a stage micrometer



Practice Questions

1. Explain the difference between magnification and resolving power(resolution) with respect to microscopy? 6
2. Suggest an explanation for each of the following:
 - (a) The resolution of an optical microscope is better using natural light than artificial light. 2
 - (b) Specimens have to be viewed in a vacuum in an electron microscope. 2
3. The diagram shows a drawing of a mammalian cell.



- (a) What is the actual diameter of the cell? 2
- (b) What is the magnification? 2

Mark scheme

Semicolons indicate marking points

1. magnification is the number of times a point has been enlarged; optical microscope maximum x1500; electron microscope x500 000; resolving power is the ability to distinguish between two points; limit of resolution 200 nm optical microscope; limit of resolution 0.2 nm electron microscope; 6
2. (a) blue light has a shorter wavelength; resolving power inversely proportional to wavelength; natural light has greater blue light component than artificial light. max 2
- (b) electrons are very small; easily scattered by particles in air; 2
3. (a) Scale: 10mm = 10µm; Drawing diameter 34mm therefore actual = 34µm; 2
- (b) Magnification = $\frac{34 \times 10^{-3}}{34 \times 10^{-6}}$; 2

Further Reference

See Factsheet 77 Using maths in Biology

Acknowledgements;

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