

### For use by students up to GCSE

Feature	Essential	Desirable	Points to consider
<b>Size</b>		Full size	Smaller size is cheaper, but lower performance is likely.
<b>Overall magnification</b>	x40, x100, x400	x1000 at GCSE	x100 oil immersion objective best used with Abbe condenser and iris diaphragm.
<b>Focusing</b>	Fine and coarse		Single coarse focus is adequate for most KS3 work, but maximum effective magnification is x200.
<b>Condenser</b>	In stage	Abbe at GCSE	Abbe for use with x100 oil immersion objective.
<b>Diaphragm</b>	Disc	Iris at GCSE	Iris for use with x100 oil immersion.
<b>Robustness</b>	Yes		An adjustable stop and/or slipping clutch and/or retractable high-power objective protects the slide. Fixed components are tamper proof.
<b>Graticule and stage micrometer</b>		At GCSE	
<b>Digital imaging</b>	Yes		For teacher demonstration.

### For use by students at A-level

Feature	Essential	Desirable	Points to consider
<b>Size</b>	Full size		
<b>Overall magnification</b>	x40, x100, x400, x1000		x100 oil immersion objective best used with Abbe condenser and iris diaphragm.
<b>Focusing</b>	Fine and coarse		
<b>Condenser</b>	Abbe		
<b>Diaphragm</b>	Iris		Essential with Abbe condenser.
<b>Robustness</b>		Yes	An adjustable stop and/or slipping clutch and/or retractable high-power objective protects the slide.
<b>Graticule and stage micrometer</b>	Yes		
<b>Digital imaging</b>	Yes		For teacher demonstration.

Feature	Explanatory notes
<p><i>For further guidance and information about training refer to the CLEAPSS website: <a href="http://www.cleapss.org.uk">www.cleapss.org.uk</a></i></p>	
<b>Size</b>	<p><b>Smaller <math>\frac{3}{4}</math> or <math>\frac{1}{2}</math> size</b> microscopes can be cheaper, but the smaller optical parts may reduce quality of results.</p>
<b>Overall magnification</b>	<p>Magnification of the objective x magnification of the eyepiece.</p>
<b>Focusing</b>	<p><b>Separate fine and coarse focus</b> controls are most common. Turning the control knob raises or lowers the body or stage, usually by a rack and pinion gear system which is straightforward to clean and regrease and usually lasts many years.</p> <p>Servicing other mechanisms can be more difficult.</p> <p>System movement is controlled by dovetail slides, or ball-bearings on wires with shim adjusters.</p> <p>The dovetail sliding mechanism works well if well-made and kept greased, but poor machining can cause the mechanism to become either too loose or too tight.</p> <p>The ball-bearing sliding mechanism has many parts, and can be very difficult to re-assemble if cleaning and re-greasing is needed.</p> <p>Some basic microscopes have a <b>single coarse focus control</b> which limits magnification to x200.</p> <p>Advanced microscopes may have a <b>co-axial fine and coarse focus</b> system - hard to take apart and re-assemble, and may also use a ball-bearing sliding mechanism.</p> <p>A <b>stop adjuster</b> prevents the sliding part from being lifted out, and can prevent the objective from being wound down onto the slide. Its position is usually adjusted by turning a nut to alter the length of a bolt.</p> <p>Some controls have <b>clutches</b> which will slip if excessive force is used when the sliding part is against the stop.</p>
<b>Condenser</b>	<p>A simple <b>in-stage condenser</b> consists of a single lens fixed in the stage.</p> <p>The advanced <b>Abbe</b> condenser has two lenses and can be adjusted by a helical or rack and pinion mount for better definition on high power objectives.</p>
<b>Diaphragm</b>	<p>On cheaper microscopes the condenser aperture is adjusted using a robust rotatable <b>disc diaphragm</b>; different-sized holes give limited control of aperture size.</p> <p>More advanced microscopes have an <b>iris diaphragm</b> which gives full control over aperture size. This mechanism is delicate and easily damaged, and repair is difficult and lengthy.</p>
<b>Robustness</b>	<p>An adjustable stop and/or slipping clutch and/or retractable high power objective prevents the high power objective being wound down too far, crushing the slide and scratching the objective lens.</p> <p>Components can be locked on, but cleaning can be harder.</p> <p>The eyepiece must be detachable to focus an Abbe condenser, adjust the iris diaphragm, and insert a graticule.</p>
<b>Graticule and stage micrometer</b>	<p><b>Graticules</b> are discs of glass with graduated scales placed in the focal plane of the microscope eyepiece.</p> <p><b>Stage micrometers</b> are needed to calibrate the eyepiece graticule.</p> <p>Both are expensive so here are cheap substitutes:</p> <ul style="list-style-type: none"> <li>• Graticule: strip of film marked with a scale placed in the eyepiece;</li> <li>• Stage micrometer: film or mm graph paper mounted on a slide.</li> </ul>

<p><b>Digital imaging</b></p>	<p>Use best-quality microscope, with magnification up to x1000, Abbe condenser, iris diaphragm, and lenses allowing the projected image to be fully in focus.</p> <p>The objective lenses need to be achromatic (to prevent colour distortion), and if possible semi-plan or plan.</p> <p>The semi-plan/plan lens allows a higher proportion of the field of view to be in focus. This is important for whole class viewing of images on screen.</p> <p>Variable lighting intensity allows the best light for the camera as well as the operator.</p> <p><b>Digital imagery</b> can be obtained in two ways.</p> <ul style="list-style-type: none"> <li>• <b>Camera integral to the microscope</b> will always be in optimal focusing position. However the camera software can soon become obsolete and may be difficult to update and the school would need to retain a compatible computer.</li> <li>• <b>Camera attached to a standard microscope</b> is cheaper and more flexible to update. However, it may be difficult to focus the camera, particularly if it has to be attached or inserted into the eyepiece after focusing the microscope. It is better if the microscope has an eyepiece exclusively for the camera.</li> </ul>
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## Other considerations

<p><b>Eyepiece</b></p>	<p>Most are <b>Widfield</b> with a larger field of view; <b>Huygens</b> with a narrow field of view is cheaper.</p> <p>Most are x10 magnification. Some will have pointers on springy circles of wire fitting the eyepiece.</p> <p>Eyepieces can be taken apart to clean, but take care to re-assemble correctly.</p> <p>Eyepiece needs to be detachable to focus an Abbe condenser, adjust an iris diaphragm, and insert a graticule.</p>
<p><b>Objectives</b></p>	<p>Objectives are <b>achromatic</b> unless otherwise stated.</p> <p>A higher quality and plan lens system can improve the field of view; a semi-plan lens system is cheaper, but the field of view is compromised.</p> <p>The <b>main objective lenses</b> are x4, x10, x40 and x100 (oil immersion, an optional extra).</p> <p>Higher power objectives are usually retractable (spring-loaded).</p> <p>Removable objectives make cleaning easier; fixed objectives are tamper proof.</p> <p>Numerical aperture (NA) is quoted on better quality objectives. A common set of magnifications and NA (in brackets) is x4 (0.10), x10 (0.25) and x40 (0.65) and for more advanced use with oil immersion x100 (1.25).</p> <p>In more expensive microscopes objectives are to DIN standard (DIN is the German Institute for Standardisation, or Deutsches Institut für Normung), implying a standard diameter, standard screw-thread and parfocal distance; useful if objectives are exchanged between microscopes.</p>
<p><b>Nosepiece</b></p>	<p>May hold three or more objectives.</p> <p>The rotating mechanism may consist of two rings of small ball-bearings which rotate as the nosepiece is turned round. If the nosepiece is difficult to rotate the ball bearings may need to be cleaned which can be time consuming.</p>
<p><b>Stand</b></p>	<p><b>'Traditional' style</b> with mirror; the whole body of the microscope, including the stage, can be angled for comfortable viewing.</p> <p>The <b>'modern' style</b> with electrical illumination; the body is fixed with a non-adjustable angled eyepiece tube and the stage and therefore slide will always be horizontal. There is usually a prism or mirror to bend the light. If the prism is dislodged it can be re-sited, but the light path must be at the correct angle for the eyepiece. A good quality prism is expensive.</p> <p>See technical notes below.</p>
<p><b>Stage</b></p>	<p>The stage should hold a standard microscope slide, usually with stage clips. Clips are easily lost, but also easy to bend back into place.</p> <p>Some clips can make viewing of eg Brownian motion cells difficult.</p> <p>Advanced microscopes may have a mechanical stage allowing the user to move the slide.</p>

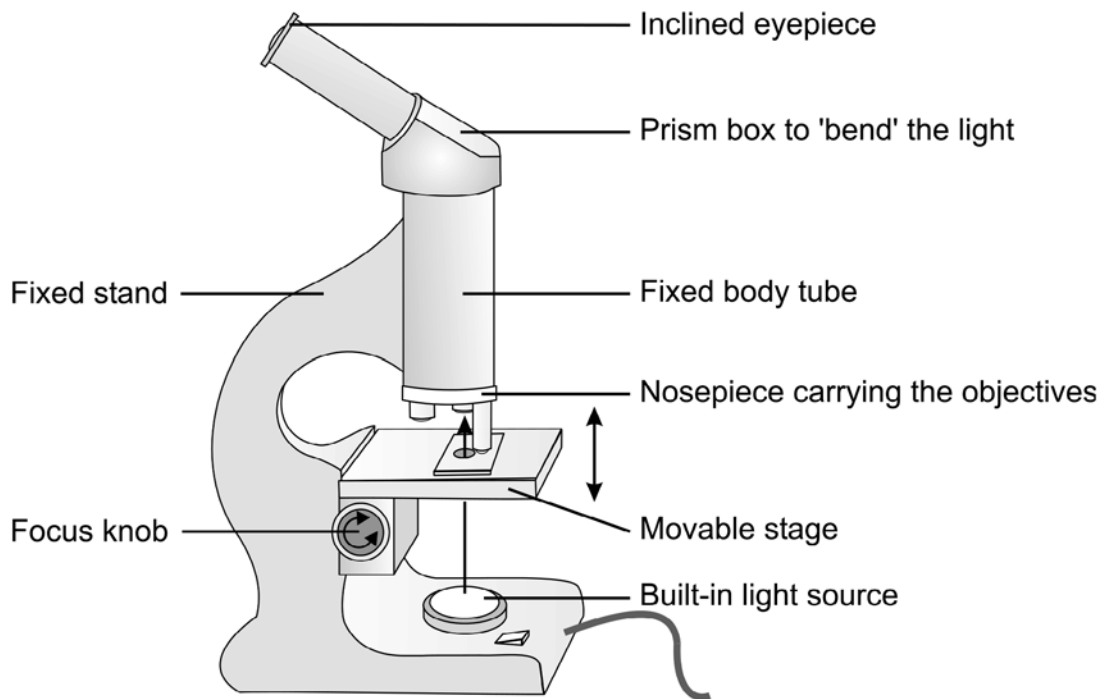
<b>Illumination</b>	<p>A <b>mirror</b> makes a microscope portable and cheaper. The light source is bench lamps or daylight. (Do not focus the rays directly from the Sun, which could damage eyes or cause blindness; use diffuse light from another part of the sky.)</p> <p><b>Tungsten</b> or <b>halogen bulbs</b> give a bright light, but can heat the metal in the microscope and warm the specimen. Replacement bulbs could become obsolete and difficult to obtain.</p> <p><b>LEDs</b> use low voltage and are cold and long-lasting, but more expensive. LEDs do not always give true colours, as the light source is not full spectrum.</p> <p><b>Fluorescent light sources</b> are full spectrum and cool to run.</p> <p>Some microscopes may have above stage illumination for viewing opaque specimens; some may have adjustable light intensity.</p>
<b>Power source</b>	<p><b>230V a.c. mains-operated and low voltage with transformer;</b> use of microscopes can be restricted by availability of 13-amp sockets.</p> <p>Power leads plugged directly into the mains are usually sturdier than those connected to a transformer. The connection of the former into the microscope also tend to be more robust. Mains-operated microscopes need to be PAT tested.</p> <p>Low voltage <b>rechargeable batteries</b> (microscope supplied with 230V charger) make the microscope portable and safer to use. It may be difficult to keep the batteries charged especially if microscopes are left turned on.</p>

**See Technical Notes on following page.**

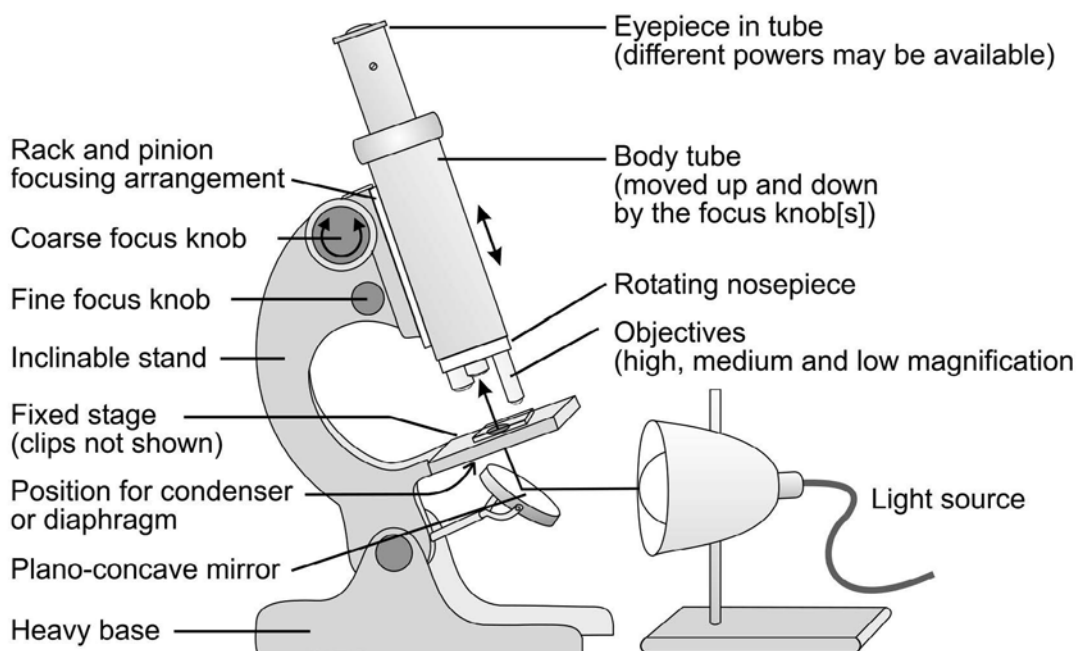
## Technical notes

### Parts of the microscope

#### 'Modern' microscope



#### 'Traditional' microscope



### Overall magnification and numerical aperture

A x20 eyepiece/ x20 objective combination could achieve a x400 magnification. However, this is only useful if it enables more detail to be distinguished. Suppose some cell structure appears to be a circle at low power. Using a higher-power eyepiece might make the circle larger, but fail to show any further detail such as the structure of the circular wall. This magnification without extra detail is called 'empty magnification'. The maximum useful magnification for a particular objective is calculated from its **numerical aperture** or **NA**.

This quantity is a number which specifies the ability of the objective to resolve detail; the higher the number the better the objective. While this resolution depends on the quality of the optics, ultimately it depends on the angle the objective presents to the object and the wavelength of the light used.

**Numerical aperture (NA)** is quoted on better-quality objectives thus: x40/0.64, meaning an objective of magnification x40 and an NA of 0.64. An oil-immersion objective might be: x100/1.25. No objective can have an NA greater than 1.0 unless it is designed for immersion in oil or water. (If numerical aperture numbers are not quoted for objectives, their quality may be suspect.)

Once the NA is known, the maximum useful magnification is calculated by applying the following 'rule of thumb':

Maximum magnification = 1000 x NA

Consequently, the x40/0.64 objective could be used to give a total magnification of x640, that is it can be used with any eyepiece of magnification up to:

$640/40 = 16$ .

On the other hand, an eyepiece of magnification x15 would give empty magnification with the x100/1.25 oil-immersion objective because the total magnification would be:

$15 \times 100 = 1500$ , while  $1000 \times NA = 1250$ .

### Why is immersion oil needed?

An image appearing sharp using the x40 objective will be indistinct if the x100 objective is swung into place, because the amount of light transmitted through the specimen into the lens is too small for accurate focusing. Filling the space between the slide and the lens with immersion oil allows the x100 objective to capture more light, and dramatically improves the resolution.



Bacterial cells with x100 objective,  
and no immersion oil



Bacterial cells with x100 objective,  
using immersion oil