

Optical microscope

The **optical microscope**, often referred to as the "**light microscope**", is a type of microscope which uses visible light and a system of lenses to magnify images of small samples. Optical microscopes are the oldest and simplest of the microscopes. Digital microscopes are now available which use a CCD camera to examine a sample, and the image is shown directly on a computer screen without the need for optics such as eye-pieces. Other microscopic methods which do not use visible light include scanning electron microscopy and transmission electron microscopy.

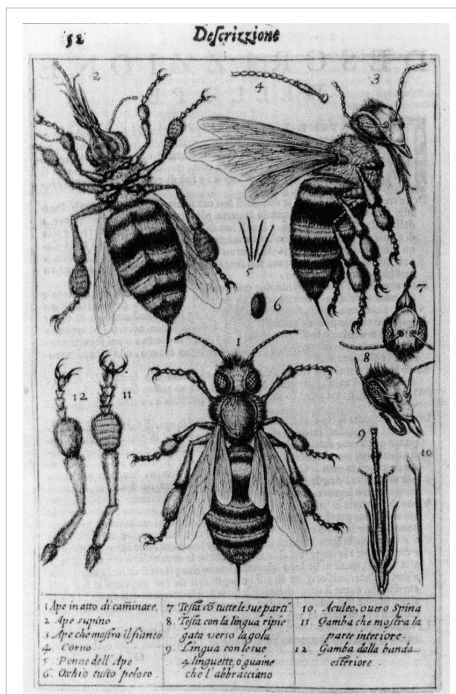
Optical configurations

There are two basic configurations of the conventional optical microscope in use, the simple (one lens) and compound (many lenses). Digital microscopes are based on an entirely different system of collecting the reflected light from a sample.

Light microscope

A *simple microscope* is a microscope that uses only one lens for magnification, and is the original light microscope. Van Leeuwenhoek's microscopes consisted of a small, single converging lens mounted on a brass plate, with a screw mechanism to hold the sample or specimen to be examined. Demonstrations^[1] by British microscopist have images from such basic instruments. Though now considered primitive, the use of a single, convex lens for viewing is still found in simple magnification devices, such as the magnifying glass, and the loupe. Light microscopes are able to view specimens in color, an important advantage when compared with electron microscopes, especially for forensic analysis, where blood traces may be important, for example.

History



The oldest published image known to have been made with a microscope: bees by Francesco Stelluti, 1630^[2]

It is difficult to say who invented the compound microscope. Dutch spectacle-makers Hans Janssen and his son Zacharias Janssen are often said to have invented the first compound microscope in 1590, but this was a declaration made by Zacharias Janssen himself during the mid 1600s. The date is unlikely, as it has been shown that Zacharias Janssen actually was born around 1590. Another favorite for the title of 'inventor of the microscope' was Galileo Galilei. He developed an *occholino* or compound microscope with a convex and a concave lens in 1609. Galileo's microscope was celebrated in the Accademia dei Lincei in 1624 and was the first such device to be given the name "microscope" a year later by fellow Lincean Giovanni Faber. Faber coined the name from the Greek words *μικρόν* (micron) meaning "small", and *σκοπεῖν* (skopein) meaning "to look at", a name meant to be analogous with "telescope", another word coined by the Linceans.^[3]

Christiaan Huygens, another Dutchman, developed a simple 2-lens ocular system in the late 1600s that was achromatically corrected, and therefore a huge step forward in microscope development. The Huygens ocular is still being produced to this day, but suffers from a small field size, and other minor problems.

Anton van Leeuwenhoek (1632-1723) is credited with bringing the microscope to the attention of biologists, even though simple magnifying lenses were already being produced in the 1500s. Van Leeuwenhoek's home-made microscopes were very small simple instruments, with a single, yet strong lens. They were awkward in use, but enabled van Leeuwenhoek to see detailed images. It took about 150 years of optical development before the compound microscope was able to provide the same quality image as van Leeuwenhoek's simple microscopes, due to timely difficulties of configuring multiple lenses. Still, despite widespread claims, van Leeuwenhoek is not the inventor of the microscope.

Components

1. ocular lens, or eyepiece
2. objective turret
3. objective lenses
4. coarse adjustment knob
5. fine adjustment knob
6. object holder or stage
7. mirror or light (illuminator)
8. diaphragm and condenser

All optical microscopes share the same basic components:

- The eyepiece - A cylinder containing two or more lenses to bring the image to focus for the eye. The eyepiece is inserted into the top end of the body tube. Eyepieces are interchangeable and many different eyepieces can be inserted with different degrees of magnification. Typical magnification values for eyepieces include 5x, 10x and 2x. In some high performance microscopes, the optical configuration of the objective lens and eyepiece are matched to give the best possible optical performance. This occurs most commonly with apochromatic objectives.
- The objective lens - a cylinder containing one or more lenses, typically made of glass, to collect light from the sample. At the lower end of the microscope tube one or more objective lenses are screwed into a circular nose piece which may be rotated to select the required objective lens. Typical magnification values of objective lenses are 4x, 5x, 10x, 20x, 40x, 50x and 100x. Some high performance objective lenses may require matched eyepieces to deliver the best optical performance.
- The stage - a platform below the objective which supports the specimen being viewed. In the center of the stage is a hole through which light passes to illuminate the specimen. The stage usually has arms to hold *slides* (rectangular glass plates with typical dimensions of 25 mm by 75 mm, on which the specimen is mounted).
- The illumination source - below the stage, light is provided and controlled in a variety of ways. At its simplest, daylight is directed via a mirror. Most microscopes, however, have their own controllable light source that is focused through an optical device called a condenser, with diaphragms and filters available to manage the quality and intensity of the light.

The whole of the optical assembly is attached to a rigid arm which in turn is attached to a robust U shaped foot to provide the necessary rigidity. The arm is usually able to pivot on its joint with the foot to allow the viewing angle to be adjusted. Mounted on the arm are controls for focusing, typically a large knurled wheel to adjust coarse focus,



Basic optical transmission microscope elements(1990's)

together with a smaller knurled wheel to control fine focus.

Updated microscopes may have many more features, including reflected light (incident) illumination, fluorescence microscopy, phase contrast microscopy and differential interference contrast microscopy, spectroscopy, automation, and digital imaging.

On a typical compound optical microscope, there are three objective lenses: a scanning lens (4 \times), low power lens (10 \times) and high power lens (ranging from 20 to 100 \times). Some microscopes have a fourth objective lens, called an oil immersion lens. To use this lens, a drop of immersion oil is placed on top of the cover slip, and the lens is very carefully lowered until the front objective element is immersed in the oil film. Such immersion lenses are designed so that the refractive index of the oil and of the cover slip are closely matched so that the light is transmitted from the specimen to the outer face of the objective lens with minimal refraction. An oil immersion lens usually has a magnification of 50 to 100 \times .

The actual power or magnification of an optical microscope is the product of the powers of the ocular (eyepiece), usually about 10 \times , and the objective lens being used.

Compound optical microscopes can produce a magnified image of a specimen up to 1000 \times and, at high magnifications, are used to study thin specimens as they have a very limited depth of field.

Operation

The optical components of a modern microscope are very complex and for a microscope to work well, the whole optical path has to be very accurately set up and controlled. Despite this, the basic operating principles of a microscope are quite simple.

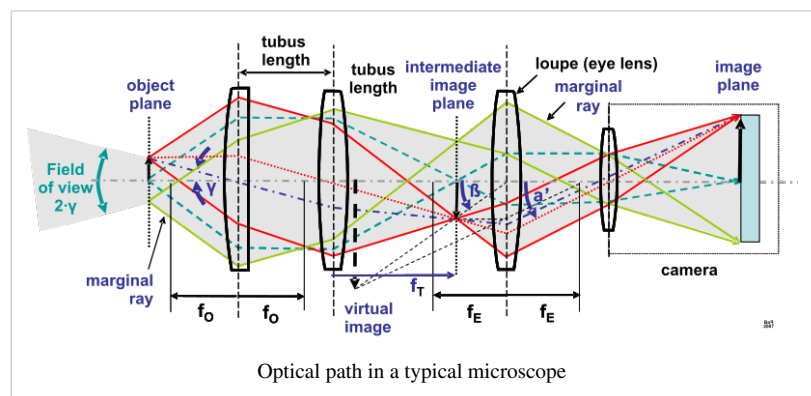
The objective lens is, at its simplest, a very high powered magnifying glass *i.e.* a lens with a very short focal length. This is brought very close to

the specimen being examined so that the light from the specimen comes to a focus about 160 mm inside the microscope tube. This creates an enlarged image of the subject. This image is inverted and can be seen by removing the eyepiece and placing a piece of tracing paper over the end of the tube. By carefully focusing a brightly lit specimen, a highly enlarged image can be seen. It is this real image that is viewed by the eyepiece lens that provides further enlargement.

In most microscopes, the eyepiece is a compound lens, with one component lens near the front and one near the back of the eyepiece tube. This forms an air-separated couplet. In many designs, the virtual image comes to a focus between the two lenses of the eyepiece, the first lens bringing the real image to a focus and the second lens enabling the eye to focus on the virtual image.

In all microscopes the image is viewed with the eyes focused at infinity (mind that the position of the eye in the above figure is determined by the eye's focus). Headaches and tired eyes after using a microscope are usually signs that the eye is being forced to focus at a close distance rather than at infinity.

The essential principle of the microscope is that an objective lens with very short focal length (often a few mm) is used to form a highly magnified real image of the object. Here, the quantity of interest is linear magnification, and this number is generally inscribed on the objective lens casing. In practice, today, this magnification is carried out by means of two lenses: the objective lens which creates an image at infinity, and a second weak tube lens which then forms a real image in its focal plane.^[4]

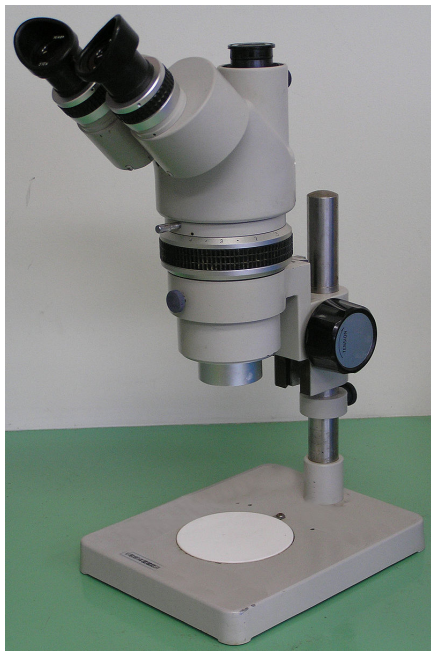


Applications

Optical microscopy is used extensively in microelectronics, nanophysics, biotechnology, pharmaceutic research and microbiology.^[5]

Optical microscopy is used for medical diagnosis, the field being termed histopathology when dealing with tissues, or in smear tests on free cells or tissue fragments.

Stereo microscope

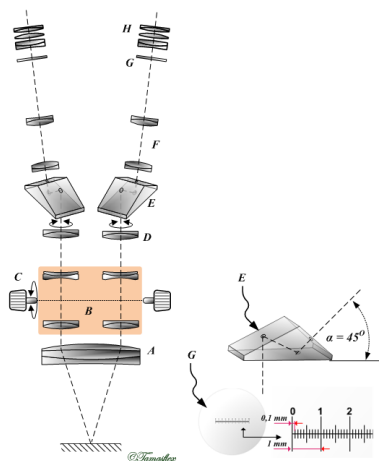


Stereo microscope

The **stereo** or **dissecting microscope** is designed differently from the diagrams above, and serves a different purpose. It uses two separate optical paths with two objectives and two eyepieces to provide slightly different viewing angles to the left and right eyes. In this way it produces a three-dimensional visualization of the sample being examined.^[6]

The stereo microscope is often used to study the surfaces of solid specimens or to carry out close work such as sorting, dissection, microsurgery, watch-making, small circuit board manufacture or inspection, and the like.

Unlike compound microscopes, illumination in a stereo microscope most often uses reflected (episcopic) illumination rather than transmitted (diascopic) illumination, that is, light reflected from the surface of an object rather than light transmitted through an object. Use of reflected light from the object allows examination of specimens that would be too thick or otherwise opaque for compound microscopy. However, stereo microscopes are also capable of transmitted light illumination as well, typically by having a bulb or mirror beneath a transparent stage underneath the object, though unlike a compound microscope, transmitted illumination is not focused through a condenser in most systems.^[7] Stereoscopes with specially-equipped illuminators can be used for dark field microscopy, using either reflected or transmitted light.^[8]



Modern stereomicroscope optical design.

A - Objective B - Galilean telescopes (*rotating objectives*) C - Zoom control D - Internal objective E - Prism F - Relay lens G - Reticle H - Eyepiece

Great working distance and depth of field here are important qualities for this type of microscope. Both qualities are inversely correlated with resolution: the higher the resolution (*i.e.* the shorter the distance at which two adjacent points can be distinguished as separate), the smaller the depth of field and working distance. A stereo microscope has a useful magnification up to 100 \times . The resolution is maximally in the order of an average 10 \times objective in a compound microscope, and often much lower.

There are two major types of magnification systems in stereo microscopes. One is fixed magnification in which primary magnification is achieved by a paired set of objective lenses with a set degree of magnification. The other is zoom or pancratic magnification, which are capable of a continuously variable degree of magnification across a set range. Zoom systems can achieve further magnification through the use of auxiliary objectives that increase total magnification by a set factor. Also, total magnification in both fixed and zoom systems can be varied by changing eyepieces.^[6]

Intermediate between fixed magnification and zoom magnification systems is a system attributed to Galileo as the "Galilean optical system" ; here an arrangement of fixed-focus convex lenses is used to provide a fixed magnification, but with the crucial distinction that the same optical components in the same spacing will, if physically inverted, result in a different, though still fixed, magnification. This allows one set of lenses to provide two different magnifications ; two sets of lenses to provide four magnifications on one turret ; three sets of lenses provide six magnifications and will still fit into one turret. Practical experience shows that such Galilean optics systems are as useful as a considerably more expensive zoom system, with the advantage of knowing the magnification in use as a set value without having to read analogue scales. (In remote locations, the robustness of the systems is also a non-trivial advantage.)

The stereo microscope should not be confused with a compound microscope equipped with double eyepieces and a binoviewer. In such a microscope both eyes see the same image, but the binocular eyepieces provide greater viewing comfort. However, the image in such a microscope is no different from that obtained with a single monocular eyepiece.



Scientist using a stereo microscope outfitted with a digital imaging pick-up

Digital display with stereo microscopes

Recently various video dual CCD camera pickups have been fitted to stereo microscopes, allowing the images to be displayed on a high resolution LCD monitor. Software converts the two images to an integrated anaglyph 3D image, for viewing with plastic red/cyan glasses, or to the cross converged process for clear glasses and somewhat better color accuracy. The results are viewable by a group wearing the glasses.

Digital microscopes

Low power microscopy is also possible with digital microscopes, with a camera attached directly to the USB port of a computer, so that the images are shown directly on the monitor. Often called "USB" microscopes, they offer high magnifications (up to about 200×) without the need to use eyepieces, and at very low cost. The precise magnification is determined by the working distance between the camera and the object, and good supports are needed to control the image. The images can be recorded and stored in the normal way on the computer. The camera is usually fitted with a light source, although extra sources (such as a fiber-optic light) can be used to highlight features of interest in the object. They also offer a large depth of field, a great advantage at high magnifications.



A miniature digital microscope.

They are most useful when examining flat objects such as coins, printed circuit boards, or documents such as banknotes. However, they can be used for examining any object which can be studied in a standard stereo-microscope. Such microscopes offer the great advantage of being much less bulky than a conventional microscope, so can be used in the field, attached to a laptop computer. Although convenient, the magnifying abilities of these instruments are often overstated; typically offering 200× magnification, this claim is based usually on 25× to 30× actual magnification PLUS the expansion of the image facilitated by the size of the available screen- so for genuine 200× magnification a ten-foot screen would be required.

Special designs

Other types of optical microscope include:

- the inverted microscope for studying samples from below; useful for cell cultures in liquid;
- the student microscope designed for low cost, durability, and ease of use;
- the petrographic microscope whose design usually includes a polarizing filter, rotating stage and gypsum plate to facilitate the study of minerals or other crystalline materials whose optical properties can vary with orientation.
- the polarizing microscope
- the fluorescence microscope
- the phase contrast microscope

Limitations

At very high magnifications with transmitted light, point objects are seen as fuzzy discs surrounded by diffraction rings. These are called Airy disks. The *resolving power* of a microscope is taken as the ability to distinguish between two closely spaced Airy disks (or, in other words the ability of the microscope to reveal adjacent structural detail as distinct and separate). It is these impacts of diffraction that limit the ability to resolve fine details. The extent of and magnitude of the diffraction patterns are affected by both by the wavelength of light (λ), the refractive materials used to manufacture the objective lens and the numerical aperture (NA) of the objective lens. There is therefore a finite limit beyond which it is impossible to resolve separate points in the objective field, known as the diffraction limit. Assuming that optical aberrations in the whole optical set-up are negligible, the resolution d , is given by:

$$d = \frac{\lambda}{2NA}$$

Usually, a λ of 550 nm is assumed, corresponding to green light. With air as medium, the highest practical NA is 0.95, and with oil, up to 1.5. In practice the lowest value of d obtainable is about 200 nm.

Other optical microscope designs can offer an improved resolution. These include ultraviolet microscopes, which use shorter wavelengths of light so the diffraction limit is lower, Vertico SMI, near field scanning optical microscopy which uses evanescent waves, and Stimulated Emission Depletion (STED) microscopy which is used for observing self-luminous particles. In the latter, non-self-luminous particle is illuminated by an external source, and thus this microscope is not diffraction limited by the Abbe's theory. Stefan Hell of the Max Planck Institute for Biophysical Chemistry was awarded the 10th German Future Prize in 2006 for his development of the Stimulated Emission Depletion (STED) microscope.^[9]

Several other optical microscopes have been able to see beyond the theoretical Abbe limit of 200 nm. In 2005, a microscope capable of detecting a single molecule was described as a teaching tool.^[10] A holographic microscope described by Courjon and Bulaboïs in 1979 is also capable of breaking this magnification limit, although resolution was restricted in their experimental analysis.^[11]

As a sensitivity improvement, a sarfus method was developed, which uses contrast-enhancing substrates and thereby allows to directly visualize films as thin as 0.3 nanometers.

Alternatives

In order to overcome the limitations set by the diffraction limit of visible light other microscopes have been designed which use other waves.

- Atomic Force Microscope (AFM)
- Scanning Electron Microscope (SEM)
- Scanning Ion-Conductance Microscope (SICM)
- Scanning Tunneling Microscope (STM)
- Transmission Electron Microscope (TEM)
- X-ray microscope

The use of electrons and x-rays in place of light allows much higher resolution - the wavelength of the radiation is shorter so the diffraction limit is lower. To make the short-wavelength probe non-destructive, the atomic beam imaging system (atomic nanoscope) has been proposed and widely discussed in the literature, but it is not yet competitive with conventional imaging systems.



A modern microscope with a mercury bulb for fluorescence microscopy. The microscope has a digital camera, and is attached to a computer.

STM and AFM are scanning probe techniques using a small probe which is scanned over the sample surface. Resolution in these cases is limited by the size of the probe; micromachining techniques can produce probes with tip radii of 5-10 nm.

Additionally, methods such as electron or X-ray microscopy use a vacuum or partial vacuum, which limits their use for live and biological samples (with the exception of ESEM). The specimen chambers needed for all such instruments also limits sample size, and sample manipulation is more difficult. Color cannot be seen in images made by these methods, so some information is lost. They are however, essential when investigating molecular or atomic effects, such as age hardening in aluminium alloys, or the microstructure of polymers.

See also

- Digital microscope
- Köhler illumination
- Microscope slide
- Objective

Further reading

- "Metallographic and Materialographic Specimen Preparation, Light Microscopy, Image Analysis and Hardness Testing", Kay Geels in collaboration with Struers A/S, ASTM International 2006.

External links

- A collection of early microscopes ^[12]
- Historical microscopes ^[13], an illustrated collection with more than 3000 photos of scientific microscopes by European makers (**German**)
- *Molecular Expressions* ^[14], concepts in optical microscopy
- Online tutorial of practical optical microscopy ^[15]
- OpenWetWare ^[16]
- Interactive Overview of a Light Microscope ^[17]

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