Iris Sorotsky
308668896

Microscopes

Microscope: “micro” - from Greek meaning small “scope” – from Greek meaning to look or see

By definition a microscope is:

1. An optical instrument consisting of a lens or combination of lenses for making enlarged images of minute objects.
2. A non-optical instrument (as one using radiation or using vibrations) for making enlarged images of minute objects.
3. An instrument for viewing objects that are too small to be seen easily by the naked eye.

The History of Microscopes

- **In 1590** - Two Dutch eye glass makers, Zaccharias Janssen and Hans Janssen experimented with multiple lenses placed in a tube. The Janssens observed that viewed objects in front of the tube appeared greatly enlarged, creating both the forerunner of the compound microscope and the telescope.
- **In 1609 –** Galileo Galilei developed a compound microscope with a convex and concave lens.
- **In 1625 -** The name microscope was coined by Giovanni Faber for Galileo Galilei’s compound microscope.
- **In the 1660s –** the extensive use of microscopes in research began in Italy, Holland and England.
- **In 1665 –** Robert Hooke, an English physicist looked at a silver of cork through a microscope lens, noticing “pores” or “cells” in it.
- **October 9th 1676 –** Antonie Van Leeuewenhoek, the father of microscopy, reported the discovery of micro-organisms. Van Leeuewnhoek also made the single lens microscope and in 1670 developed a method for grinding very small glass lenses, the lenses were so thin (in order of millimetres in diameter) they could magnify several hundred times.
- **In the late 17th century –** a Dutchman, Christiaan Huygens, developed a simple 2 lens ocular system that was achromatically corrected (a lens that is designed to limit the effects of chromatic and spherical aberration. Achromatic lenses are corrected to bring two wavelengths, typically red and blue, into focus in the same plane.) this was a big improvement in microscopes. The Huygens ocular is still being produced to this day although suffering from minor problems.
- **In 1893 –** August Kohler developed a key technique for sample illumination. The Kohler illumination is central to modern light microscopy.
- **In 1903 -** Richard Zsigmondy developed the ultramicroscope that was able to study objects below the wavelength of light. In 1925 he won a Nobel Prize in Chemistry.
- **In 1931 -** Ernst Ruska co-invented along with Max Knoll the electron microscope.

- **In 1932 –** Fritz Zernike invented the phase-contrast microscope that enabled the study of colourless and transparent biological materials. In 1953 he won a Nobel Prize in physics for this invention.
- **In 1981 –** the scanning tunnelling microscope was invented by Gerd Binnig and Heinrich Rohrer. Binnig and Rohrer won a Nobel Prize for this invention in 1986.

The Beginning – Glass Lenses

Like every good story our begins a long time ago in a far away land. Someone, somewhere, picked up a piece of transparent crystal. That particular crystal was thicker in the middle than at the edges. That someone noticed, amongst other discoveries about this magical piece of crystal, that it made things look larger then in reality. "Magnifying glasses" are mentioned in the writings of Seneca and Pliny the Elder, Roman philosophers during the first century A.D., but as it turns out, they were not used much until the invention of spectacles, near the end of the 13th century.
The name lenses, comes from the shape of the glass, since they are shaped like the seeds of a lentil.

The Earliest Type of Microscopes

The earliest, simple microscope was just a tube with a plate for the subject at one end and a lens which gives a magnification ten times the actual size.
Oddly enough, the compound microscope was invented before the single lens microscope. But the instruments and materials were so poor that much more could be seen with very small lenses of short focal length.
Somewhere around 1590 two Dutch eyeglass makers, Zaccharias Janssen and Hans Janssen (some also give credit to Hans Lippershey, the developer of the telescope) experimented with lenses in a tube and observed that nearby objects viewed through two lenses in line were magnified. However, their lenses were quite large and the magnification was only about ten times. None the less, their device was the first compound microscope!

The Birth of Light Microscopes – Single Lens Microscopes

"Light microscopes" is a type of microscope which uses visible light and a single lens or a system of lenses to magnify images of small samples.
The original design of light microscope is simple and consists of one lens used for magnification. Van Leeuwenhoek's microscope is made up of a small single converging lens (Biconvex meaning a lens with two convex surfaces or Plano-convex if one of the surfaces is flat and the other is convex) mounted on a brass plate, with a screw mechanism to hold the sample or specimen.
These types of microscopes were easy to develop and are popular because they use visible light so the sample can be directly observed by the eye. Van Leeuwenhoek made hundreds of microscopes and optical lenses over the years and many people copied them. Nine of Van Leeuwenhoek's original microscopes still exist today.


Leeuwenhoek Microscope (circa late 1600s)

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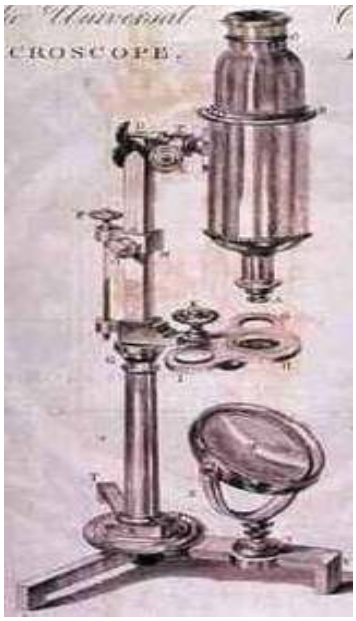
Compound Microscope

In general, a compound microscope is made of multiple lenses to collect light from the sample and then a separate set of lenses to focus the light into the eye or camera. As mentioned before the compound microscope was invented before the single lens. It took about 150 years of optical development before the compound microscope was able to reach the quality image of the simple, one lens, Van Leeuwenhoek's microscope. Most of the problems were due to difficulties in configuring multiple lenses.
The main advantages of multiple lenses are: improved numerical aperture, reduced chromatic aberration and exchangeable objective lenses to adjust the magnification.



Much more recently techniques in sample illumination developed to the high quality images seen today.

The first major development was made by August Kohler overcoming many limitations of older sample illumination techniques, for example a light bulb filament was always visible in the image of the sample.

Fritz Zernike received the Nobel Prize in physics in 1953 for his development of the phase contrast illumination, which allows imaging of transparent samples. By using interference rather than absorption of light, extremely transparent samples, can be imaged without having to use staining techniques. This was a great contribution to the research of live tissue.

Fluorescence Microscopy

In fluorescence microscopy the sample is illuminated with a narrow set of wavelength of light. This light interacts with fluorophores in the sample which then emit light of a longer wavelength. It is this emitted light which makes up the image. Modern biological microscopy depends heavily on this development for specific structures within a cell.

Electron Microscope

A light microscope, even one with perfect lenses and perfect illumination, simply cannot be used to distinguish objects that are smaller than half the wavelength of light. To see tiny particles under a microscope, scientists must bypass light altogether and use a different sort of "illumination," one with a shorter wavelength.

In the early 1900s a significant alternative to light microscopy was developed by Ernst Ruska and Max Knoll, using a beam of electrons to illuminate the specimen and produce a magnified image. (The invention and patent belong to Leo Szilard who declined to construct it)

electrons are speeded up in a vacuum until their wavelength is extremely short, only one hundred-thousandth that of white light and can achieve better than 50 pm resolution and magnifications of up to about 10,000,000 times. The electron microscope uses electrostatic and electromagnetic "lenses" ("lenses" are analogous to, glass lenses of an optical microscope) to control the electron beam and focus it to form an image.

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There are many types and variation of electron microscopes.

(TEM) Transmission Electron Microscope

The original form of the TEM uses a high voltage electron beam to create an image. The electron source is a tungsten filament cathode and the electrons are emitted by an electron gun. The electron beam is accelerated by an anode, focused by electrostatic and electromagnetic lenses then, transmitted through the sample. Part of the electrons scatter out of the beam so when the beam emerges from the sample it carries with it information regarding its structure. Part of the information the beam carries is spatial variation and this information can be viewed by projecting the magnified electron image. The image can also be photographed. The high resolution TEM (which is a hardware correction to the TEM) gives an image resolution of 50 pm and magnification of above 50 million times.

(SEM) Scanning Electron Microscope

The electron beam of a SEM does not, at any time, carry a complete image of the specimen. The SEM probes the specimen with a focused electron beam. When the beam interacts with the specimen, it loses energy. The lost energy converts to heat, light emission, low energy secondary electrons, x-ray emission, etc. this provides singles carrying information about the properties of the specimen surface. The image displayed by an SEM maps the varying intensity of any of these signals.

Scanning Electron Microscope image of a gall midge

Generally, the image resolution of an SEM is about an order of magnitude poorer than that of a TEM. However, since the SEM image relies on the surface processes rather than transmission, it is able to image volume samples up to many centimetres in size and has a great depth of field that results in an image that is a good representation of a 3D shape of the sample. A variation of the SEM called ESEM (environmental scanning electron microscope) can produce an image of sufficient quality and resolution even if the sample is wet or contained in low vacuum or gas.

(REM) Reflection Electron Microscope

In resemblance to the TEM the REM also uses an electron beam but instead of using the transmission like in TEM or secondary electrons like in SEM, the reflected beam of elastically scattered electrons is detected.

(STEM) Scanning Transmission Electron Microscope

The STEM is a type of TEM. The electrons pass through a sufficiently thin specimen. However, STEM is distinguished from conventional transmission electron microscopes (CTEM) by focusing the electron beam into a narrow spot which is scanned over the sample in a raster. The high resolution of the TEM is thus possible in STEM. The

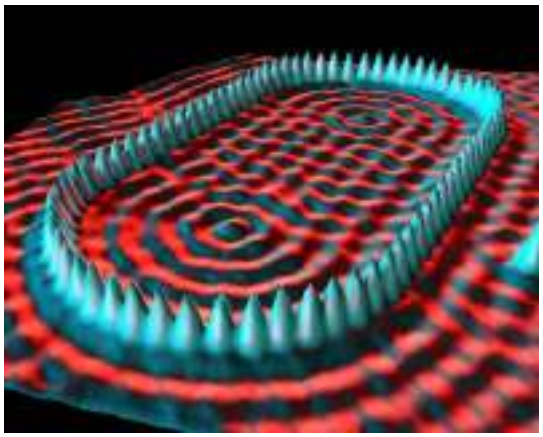
focusing action occurs before the electrons hit the specimen in the STEM, but afterward in the TEM.

(LVEM) Low Voltage Electron Microscope

The low voltage electron microscope is a combination of SEM, TEM and STEM in one instrument. Low voltage reduces the specimen damage by the incident electrons and increases image contrast.

(STM) scanning tunnelling microscope

The STM is an instrument for imaging surface at the atomic level, developed in 1981 by Gerd Binnig and Heinrich Rohrer who received the Nobel Prize in physics in 1986.



STM Iron on Copper

With STM individual atoms within the material are routinely imaged and manipulated. The STM unlike most electron microscopes can be used not only in vacuum but in air, water, and other various liquids and gases.

The STM is based on the concept of quantum tunnelling. When a conducting tip is brought very close to the surface of the sample, a voltage difference applied between the two can allow the electrons to tunnel through the vacuum between them. Information is acquired by monitoring the current while the tip's position scans across the surface, and is usually displayed in image form. STM is a challenging technique since it requires extremely clean and stable surfaces, sharp tips, vibration control and sophisticated electronics.

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