**HISTORY OF MICROSCOPY**

A microscopey (from the Ancient Greek: μικρός, mikrós, "small" and σκοπεῖν, skopeîn, "to look" or "see") is an instrument used to see objects that are too small to be seen by the naked eye. Microscopy is the science of investigating small objects and structures using such an instrument. Microscopic means invisible to the eye unless aided by a microscope. Although objects resembling lenses date back 4000 years and there are Greek accounts of the optical properties of water-filled spheres (5th century BC) followed by many centuries of writings on optics, the earliest known use of simple microscopes (magnifying glasses) dates back to the widespread use of lenses in eyeglasses in the 13th century. The earliest known examples of compound microscopes, which combine an objective lens near the specimen with an eyepiece to view a real image, appeared in Europe around 1620. The inventor is unknown although many claims have been made over the years. Several revolve around the spectacle-making centers in the Netherlands including claims it was invented in 1590 by Zacharias Janssen (claim made by his son) and/or Zacharias' father, Hans Martens, claims it was invented by their neighbor and rival spectacle maker, Hans Lippershey (who applied for the first telescope patent in 1608), and claims it was invented by expatriate Cornelis Drebbel who was noted to have a version in London in 1619. Galileo Galilei (also sometimes cited as compound microscope inventor) seems to have found after 1610 that he could close focus his telescope to view small objects and, after seeing a compound microscope built by Drebbel exhibited in Rome in 1624, built his own improved version. Giovanni Faber coined the name microscope for the compound microscope Galileo submitted to the Accademia dei Lincei in 1625 (Galileo had called it the "occhiolino" or "little eye").

Carl Zeiss binocular compound microscope, 1914

The first detailed account of the microscopic anatomy of organic tissue based on the use of a microscope did not appear until 1644, in Giambattista Odierna's L'occhio della mosca, or The Fly's Eye.

The microscope was still largely a novelty until the 1660s and 1670s when naturalists in Italy, the Netherlands and England began using them to study biology. Italian scientist Marcello Malpighi, called the father of histology by some historians of biology, began his analysis of biological structures with the lungs. Robert Hooke's Micrographia had a huge impact, largely because of its impressive illustrations. A significant contribution came from Antonie van Leeuwenhoek who achieved up to 300 times magnification using a simple single lens microscope. He sandwiched a very small glass ball lens between the holes in two metal plates riveted together, and with an adjustable-by-screws needle attached to mount the specimen. Then, Van Leeuwenhoek re-discovered red blood cells (after Jan Swammerdam) and spermatozoa, and helped popularise the use of microscopes to view biological ultrastructure. On 9 October 1676, van Leeuwenhoek reported the discovery of micro-organisms.

The performance of a light microscope depends on the quality and correct use of the condensor lens system to focus light on the specimen and the objective lens to capture the light from the specimen and form an image. Early instruments were limited until this principle was fully appreciated and developed from the late 19th to very early 20th century, and until electric lamps were available as light sources. In 1893 August Köhler developed a key principle of sample illumination, Köhler illumination, which is central to achieving the theoretical limits of resolution for the light microscope. This method of sample illumination produces even lighting and overcomes the limited contrast and resolution imposed by early techniques of sample illumination. Further developments in sample illumination came from the discovery of phase contrast by Frits Zernike in 1953, and differential interference contrast illumination by Georges Nomarski in 1955; both of which allow imaging of unstained, transparent samples.

**Electron microscopes**

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Electron microscope constructed by Ernst Ruska in 1933

In the early 20th century a significant alternative to the light microscope was developed, an instrument that uses a beam of electrons rather than light to generate an image. The German physicist, Ernst Ruska, working with electrical engineer Max Knoll, developed the first prototype electron microscope in 1931, a transmission electron microscope (TEM). The transmission electron microscope works on similar principles to an optical microscope but uses electrons in the place of light and electromagnets in the place of glass lenses. Use of electrons, instead of light, allows for much higher resolution. Development of the transmission electron microscope was quickly followed in 1935 by the development of the scanning electron microscope by Max Knoll. Although TEMs were being used for research before WWII, and became popular afterwards, the SEM was not commercially available until 1965. Transmission electron microscopes became popular following the Second World War. Ernst Ruska, working at Siemens, developed the first commercial transmission electron microscope and, in the 1950s, major scientific conferences on electron microscopy started being held. In 1965, the first commercial scanning electron microscope was developed by Professor Sir Charles Oatley and his postgraduate student Gary Stewart, and marketed by the Cambridge Instrument Company as the "Stereoscan". One of the latest discoveries made about using an electron microscope is the ability to identify a virus. Since this microscope produces a visible, clear image of small organelles, in an electron microscope there is no need for reagents to see the virus or harmful cells, resulting in a more efficient way to detect pathogens.

**Scanning probe microscopes**

From 1981 to 1983 Gerd Binnig and Heinrich Rohrer worked at IBM in Zurich, Switzerland to study the quantum tunnelling phenomenon. They created a practical instrument, a scanning probe microscope from quantum tunnelling theory, that read very small forces exchanged between a probe and the surface of a sample. The probe approaches the surface so closely that electrons can flow continuously between probe and sample, making a current from surface to probe. The microscope was not initially well received due to the complex nature of the underlying theoretical explanations. In 1984 Jerry Tersoff and D.R. Hamann, while at AT&T's Bell Laboratories in Murray Hill, New Jersey began publishing articles that tied theory to the experimental results obtained by the instrument. This was closely followed in 1985 with functioning commercial instruments, and in 1986 with Gerd Binnig, Quate, and Gerber's invention of the atomic force microscope, then Binnig's and Rohrer's Nobel Prize in Physics for the SPM. New types of scanning probe microscope have continued to be developed as the ability to machine ultra-fine probes and tips has advanced.

**Fluorescence microscopes**

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Fluorescence microscope with the filter cube turret above the objective lenses, coupled with a camera.

The most recent developments in light microscope largely centre on the rise of fluorescence microscopy in biology. During the last decades of the 20th century, particularly in the post-genomic era, many techniques for fluorescent staining of cellular structures were developed. The main groups of techniques involve targeted chemical staining of particular cell structures, for example, the chemical compound DAPI to label DNA, use of antibodies conjugated to fluorescent reporters, see immunofluorescence, and fluorescent proteins, such as green fluorescent protein. These techniques use these different fluorophores for analysis of cell structure at a molecular level in both live and fixed samples.

The rise of fluorescence microscopy drove the development of a major modern microscope design, the confocal microscope. The principle was patented in 1957 by Marvin Minsky, although laser technology limited practical application of the technique. It was not until 1978 when Thomas and Christoph Cremer developed the first practical confocal laser scanning microscope and the technique rapidly gained popularity through the 1980s.

**Super resolution microscopes**

Much current research (in the early 21st century) on optical microscope techniques is focused on development of superresolution analysis of fluorescently labelled samples. Structured illumination can improve resolution by around two to four times and techniques like stimulated emission depletion (STED) microscopy are approaching the resolution of electron microscopes. This occurs because the diffraction limit is occurred from light or excitation, which makes the resolution must be doubled to become super saturated. Stefan Hell was awarded the 2014 Nobel Prize in Chemistry for the development of the STED technique, along with Eric Betzig and William Moerner who adapted fluorescence microscopy for single-molecule visualization.

**X-ray microscopes**

X-ray microscopes are instruments that use electromagnetic radiation usually in the soft X-ray band to image objects. Technological advances in X-ray lens optics in the early 1970s made the instrument a viable imaging choice. They are often used in tomography (see micro-computed tomography) to produce three dimensional images of objects, including biological materials that have not been chemically fixed. Currently research is being done to improve optics for hard X-rays which have greater penetrating power.

**DIFFERENTIATE BETWEEN THE LIGHT MICROSCOPE AND ELECTRON MICROSCOPE**

* An electron microscope is very expensive to buy and requires special environments. This makes it expensive to maintain. In addition, it requires high technical skills to use and is therefore limited to specialized use such as research. A light microscope, on the other hand, is cheap to buy and maintain. It requires no special skills to use. As a result, it is suitable for most basic functions, and is very common in schools and other learning institutions.
* While a light microscope uses light to illuminate specimens and glass lenses to magnify images, an electron microscope uses a beam of electrons to illuminate specimens and magnetic lenses to magnify images.
* The resolution (the level of image detailing) is the main difference between these two microscopes. A light microscope has a resolution of up to 0.3µm, i.e. 3 micrometers. This limits it as two close objects cannot be seen as separate. In an electron microscope, the resolution is about 0.0001µm, i.e. 0.01 nanometers. This resolution can be used in situations that require greater details, such as studying cell nuclei.
* A light microscope can be used to view both dead and live specimens. This makes it important in studying live ones. By contrast, an electron microscope cannot be used to view living specimens as it uses electrons that are destructive to life.
* The size of a light microscope is relatively smaller and can be operated as a desktop instrument. When the size of an electron microscope is relatively larger due to separate systems such as cooling system, image processing system, vacuum system etc.
* It is possible to visualize the natural color of the specimen under a light microscope. But It is not possible to visualize the natural color of specimen under electron microscope
* Light microscope uses light ( approx 400-700 nm) as an illuminating source, an electron microscope uses electron beams (approx 1 nm) as an illuminating source.
* Light microscope as lower magnification than an electron microscope, an electron microscope as higher magnification
* Light microscope studying the detailed structure of an organism is difficult. An electron microscope 3D structure is obtained due to which it is easy to study the structural and other details of organisms.

 **DIFFERENCE BETWEEN THE SEM AND TEM**

* SEM is based on scattered electrons while TEM is based on transmitted electrons.
* SEM focuses on the sample’s surface and its composition whereas TEM provides the details about internal composition. Therefore TEM can show many characteristics of the sample, such as morphology, crystallization, stress or even magnetic domains. On the other hand, SEM shows only the morphology of samples.
* The sample in TEM has to be cut thinner whereas there is no such need with SEM sample.
* TEM has much higher resolution than SEM.
* SEM allows for large amount of sample to be analysed at a time whereas with TEM only small amount of sample can be analysed at a time.
* SEM is used for surfaces, powders, polished & etched microstructures, IC chips, chemical segregation whereas TEM is used for imaging of dislocations, tiny precipitates, grain boundaries and other defect structures in solids
* In TEM, pictures are shown on fluorescent screens whereas in SEM, picture is shown on monitor.
* SEM also provides a 3-dimensional image while TEM provides a 2-dimensional picture.
* The magnifications that TEMs offer are also much higher compared to SEMs: TEM users can magnify their samples by more than 50 million times, while for the SEM this is limited up to 1-2 million times.
* However, the maximum Field of View (FOV) that SEMs can achieve is far larger than TEMs, which users can only use to image a very small part of their sample. Similarly, the depth of field of SEM systems are much higher than in TEM systems.
* The two EM systems also differ in the way they are operated. SEMs usually use acceleration voltages up to 30 kV, while TEM users can set it in the range of 60 – 300kV.

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