MICROSCOPE IN DENTISTRY: A REVIEW ARTICLE

SUMAN MUKHERJEE1*, SHARMISTHA DASGUPTA2

1Department of Periodontics and Oral Implantology, Arogya Bardini Polyclinic, Purulia, West Bengal, India. 2Department of Periodontics and Oral Implantology, RHT Multispeciality Clinic, New Delhi, India. Email: smjee234@gmail.com

Received: 03 February 2021, Revised and Accepted: 24 February 2021

ABSTRACT

The microscope has been one of the oldest yet most exquisite inventions in human history. The lenses changed the future of medical science and its abstraction forever. Previously, humans never knew much about the source of disease, but today we know that the universe of microbes is vast and more limitless than it ever was. However, the microscope is not just limited to laboratory in vitro research and study; it has remodeled dentistry more today than ever. This article describes the various types of microscopes used in periodontics, endodontics, and oral pathology in dentistry.

Keywords: Microscope, Dark field, Electron, Viruses, Loupes, Microsurgery, Confocal, Compound, Microbes.

INTRODUCTION

The microscope has been one of the oldest yet most exquisite inventions in human history [1]. The lenses changed the future of medical science and its abstraction forever. Previously, humans never knew much about the source of disease, but today we know that the universe of microbes is vast and more limitless than it ever was. However, the microscope is not just limited to laboratory in vitro research and study; it has remodeled dentistry more today than ever. In periodontics, surgical microscopes and loupes have been playing a key role in microsurgery. Since, its advent in root coverage procedures and flap procedures, surgical loupes are gaining attention and interest. As such, we need to know the past of this novel discovery which changed the beliefs of medicine in general, and dental science in particular forever [2-4].

TIMELINE OF THE MICROSCOPE

• 1st century AD (year 100) – Romans invented Glass [5]
• 1300 A.D. – Concave and Convex lenses first came into general use for spectacles [6]
• Robert Bacon (1212-1294) – believed to be the first person to combine to lenses [6,7]
• 14th AD spectacles first made in Italy [8,9]
• 1590 – Two Dutch spectacle-makers and father-and-son team, Hans and Zacharias Janssen, create the first compound microscope [10-13]
• 1666 – Italian scientist Marcello Malpighi, called the father of histology by some historians of biology, began his analysis of biological structures with the lungs [14-16]
• 1667: Robert Hooke’s famous “Micrographia” is published, which outlines Hooke’s various studies using the microscope [17-20]
• 1675: Anton van Leeuwenhoek, who used a microscope with one lens to observe insects and other specimens. Leeuwenhoek was the first to observe bacteria [21,22]
• 18th century: As technology improved, microscopy became more popular among scientists. Part of this was due to the discovery that combining two types of glass reduced the chromatic effect [23]
• 1830: Joseph Jackson Lister discovers that using weak lenses together at various distances provided clear magnification [23-25]
• 1873: Ernst Leitz microscope was introduced with a revolving mount (turret) for five objectives [26,27]
• 1878: Oil immersion lens (cedar oil) were introduced that resulted in a homogeneous optical path [32]
• 1903 – Richard Zsigmondy won Nobel prize for his phase-contrast microscope
• 1904: The first commercial ultraviolet (UV) microscope by Zeiss [33-36]
• 1930: Fritz Zernike discovered that he could view unstained cells using the phase angle of rays. It took until 1941 to bring a commercial microscope to market
• 1931: Ernst Ruska co-invented an electron microscope for which he won the Nobel Prize in Physics in 1986
• 1937: First scanning electron microscope was built [37]
• 1939 – Siemens supplied the first commercially available electron microscope [38,39]
• 1953: Zernike was awarded the Nobel Prize for his phase-contrast work [40]
• 1981: Gerd Binnig and Heinrich Rohrer invented a scanning tunneling microscope that gives three-dimensional images of objects down to the atomic level. Binnig and Rohrer won the Nobel Prize in Physics in 1986 [41,42]
• 1982 – A scanning the probe microscope was invented that works by measuring current
• 1983 – Scanning laser confocal microscope was commercially available [43-45]
• 1986 – The atomic force microscope (AFM) was invented that measures force instead of current [46-48]
• 1986 – Gerard Binnig Heinrich Rohrer Discovered atomic microscope in 1981 and won Nobel prize for it in 1986
• Cryo-electron microscopy innovators win 2017 Nobel Prize in Chemistry.

The word “Microscope” is derived from the Ancient Greek word: μικρός mikrós, meaning “small” and, σκοπεῖν, skopeîn, meaning “to look” or “see”.

It was Giovanni Faber who coined the term microscope for the compound microscope (Galileo submitted to the Accademia dei Lincei in 1625 (Galileo had called it the “occhilino” or “little eye”).

A microscope can be defined as an instrument used to see objects that are too small for the naked eye. The science of investigating small objects using such an instrument is called microscopy. Microscopic means invisible to the eye unless aided by a microscope.

The ability to distinguish detail is called resolution or resolving power, and depends on the wavelength of light used and, on a value, called the
numerical aperture a characteristic of microscopes that determines how much light enters the lens.

The limit of resolution of an objective (d) is the distance between any two closest points on the microscopic object, which can be resolved into two separate and distinct points on the enlarged image.

\[ \text{Limit of resolution} = d = \frac{\lambda}{2 \text{ N.A.}} \]

**MAGNIFICATION POWER**

The magnification power measures how much larger an object appears after magnification. It is calculated by dividing the focal length of the scanning object (lens) by the focal length of the eyepiece.

A ×1 magnification power is a 100% increase in the magnified object’s size, for example, a 1-inch object at ×1 would appear to be 2 inches. At ×2 power, the same object would appear to be 3 inches. Magnification power is reported on scientific reports as a means of standardization.

There are three well-known branches of microscopy: (Tables 1 and 2)

- **Optical**
- **Electron**
- **Scanning probe microscopy.**

**Optical microscope**

Optical microscopy is a technique employed to closely view a sample through the magnification of a lens with visible light. This is the traditional form of microscopy, which was first invented before the 18th century and is still in use today [49].

**Binocular stereoscopic microscope**

It uses a magnification in the range of ×10–×100. It allows easy observation of 3D objects at low magnification [50].

**Polarizing microscope/petrographic microscope**

It has a magnification range of ×4–×100. It uses different light transmission characteristics of materials, such as crystalline structures, to produce an image. Materials that can be examined under a polarized microscope include minerals, ceramics, polymers, urea, and fungi. It is also used to study the property of collagen and amyloid [51-54].

**Differential interference contrast microscope**

It uses a magnification in the range of ×400–×1500. This microscope, similar to the phase contrast, is used to observe fine surface shapes or properties. It allows easy observation of 3D objects at low magnification. It uses a magnification in the range of ×10–×100. It allows easy observation of 3D objects at low magnification.

**Total internal reflection fluorescence microscope**

It uses an evanescent wave to only illuminate near the surface of a specimen. The region that is viewed is generally very thin compared to conventional microscopes. Observation is possible in molecular units due to reduced background light [59-63].

**Multiphoton excitation microscopy**

It uses multiple excitation lasers that reduce damage to cells and allows high-resolution observation of deep areas. It is used to observe nerve cells and blood flow in the brain [64-67].

**Structured illumination microscope**

It is a high-resolution microscope with advanced technology to overcome limited resolution found in optical microscopes that are caused by the diffraction of light [68-76].

**Scanning probe microscope/AFM**

It has a magnification of ×1,000,000. In 1986, Binning and Quate demonstrated for the 1st time the ideas of AFM, which used an ultra-small probe tip at the end of an cantilever. This microscope scans the surface of samples with a probe and this interaction is used to measure fine surface shapes or properties. The optical and electron microscopes can easily generate two-dimensional images of a sample surface. However, these microscopes cannot measure the vertical dimension (z-direction) of the sample, the height (e.g., particles), or depth (e.g., holes, pits) of the surface features. 111–113 AFM, which uses a sharp tip to probe the surface features by raster scanning, can image the surface topography with extremely high magnifications, up to ×1,000,000, comparable or even better than electronic microscopes. The measurement of an AFM is made in three dimensions, the horizontal X-Y plane, and the vertical Z dimension. Resolution (magnification) at Z-direction is normally higher than X-Y [77-84].

**Scanning near-field optical microscope (SNOM)**

NSOM/SNOM is a microscopic technique used for nanostructure investigation that breaks the far-field resolution limit by exploiting the properties of evanescent waves.

It is ideally suited to quickly and effortlessly image the optical properties of a sample with a resolution below the diffraction limit applied in nanotechnology research, nano-photronics, and nano-optics. In life science and materials research it is used for optical detection of the most minuscule surface. With SNOM, single-molecule detection is easily achievable. Dynamic properties can also be studied at a sub-wavelength scale. It provides a 70 times better resolution than an AFM [85-99].

**The compound microscope**

The word compound means multiple, mix, or a combination of both. A compound light microscope is a microscope with more than one lens and its light source. Because it contains its light source in its base, a compound light microscope is also considered a bright field microscope [100].

**Parts of a compound microscope**

- Mechanical parts – support and adjustment
- Magnifying parts – for enlargement of the specimen
- Illuminating parts – to provide light.

**WHAT CAN BE VIEWED**

Using stained prepared slides, you should see bacteria, chromosomes, organelles, protist or metazoa, smears, blood, negative stained bacteria, and thick tissue sections.

Utilizing unstained wet mounts for living preparations should enable you to see pond water, living protists or metazoa, and plant cells such as algae.

**USES/BENEFITS**

It can be used for blood analysis which is of great use in pathology labs to identify diseases. In forensic laboratories, it can be used to detect the
presence or absence of minerals or metals at a crime scene, thereby aid in the criminal investigation.

The phase-contrast microscope

The phase-contrast microscope can show components in a cell or bacteria, which would be very difficult to see in an ordinary light microscope. Frits Zernike (1888–1966) received a Nobel prize in 1953 for his discovery of phase contrast [101-105].

Altering the light waves

The phase-contrast microscope uses the fact that the light passing through a transparent part of the specimen travels slower and, due to this is shifted compared to the unilluminated light. This phase difference is not visible to the human eye. However, the phase change can be increased to half a wavelength by a transparent phase-plate in the microscope and thereby causing a difference in brightness. This makes the transparent object shine out in contrast to its surroundings.

"INVISIBLE CAN BE SEEN"

Transparent cells can be observed without staining them because the phase contrast can be converted into brightness differences. After all, it is not necessary to stain cells, cell division, and other processes can be observed in a living state.

Applications

- The sharp contrast in certain cases can only be seen through a phase-contrast microscope
- The high-contrast images of transparent specimens, such as microorganisms, thin tissue slices, living cells in culture, latex dispersions, lithographic patterns, glass fragments, and sub-cellular particles, such as nuclei and organelles, can be viewed in detail.

Darkfield microscope

This technique is used to observe unstained samples causing them to appear brightly lit against a dark, almost purely black, background. When light hits an object, rays are scattered in all azimuths or directions. The design of the dark field microscope is such that it removes the dispersed light, or zeroth order so that only the scattered beams hit the sample.

The introduction of a condenser and/or stop below the stage ensures that these light rays will hit the specimen at different angles, rather than as a direct light source above/below the object. The result is a "cone of light" where rays are diffractioned, reflected, and/or refracted off the object, ultimately, allowing you to view a specimen in a dark field [106-114].

Advantages of dark-field microscopy

- It is used unstained slides, is transparent, and absorbs little or no light
- The specimens often have similar refractive indices as their surroundings, making them hard to distinguish with other illumination techniques
- It is used to study marine organisms such as algae and plankton, diatoms, insects, fibers, hairs, yeast, and protozoa as well as some minerals and crystals, thin polymers, and some ceramics
- It is used in the research of live bacterium, as well as mounted cells and tissues
- It is useful in examining external details, such as outlines, edges, grain boundaries, and surface defects than internal structure.

Darkfield microscopy is often dismissed for more modern observation techniques such as phase contrast and DIC, which provide more accurate, higher contrasted images and can be used to observe a greater number of specimens. But recently, the dark field has regained some of its popularity when combined with other illumination techniques, such as fluorescence, which widens its possible use in certain fields.

Disadvantages

The darkfield microscopy images are prone to degradation, distortion, and inaccuracies. A specimen that is not thin enough or its density differs across the slide, may appear to have artifacts throughout the image. The preparation and quality of the slides can grossly affect the contrast and accuracy of a dark field image. One needs to take special care that the slide, stage, nose, and light source are free from small particles such as dust, as these will appear as part of the image. We have to use oil or water on the condenser and/or slide, it is almost impossible to avoid all air bubbles. These liquid bubbles will cause image degradation, flare, and distortion and even decrease the contrast and details of the specimen.

FLUORESCENT MICROSCOPE

It uses a magnification of range ×1500. On October 8, 2014, the Nobel Prize in Chemistry was awarded to Eric Betzig, William Moerner, and Stefan Hell for "the development of super-resolved fluorescence microscopy," which brings optical microscopy into the nano dimensions. It was British scientist Sir George G. Stokes who first described fluorescence in 1852.

In fluorescence microscopy, the sample you want to study is itself the light source. The technique is used to study specimens, which can be made to fluoresce.

The fluorescence microscope is based on the phenomenon that certain material emits energy detectable as visible light when irradiated with the light of a specific wavelength. A fluorescence microscope uses a much higher intensity light source which excites a fluorescent species in a sample of interest. This fluorescent species in turn emits a lower energy light of a longer wavelength that produces the magnified image instead of the original light source [115-119].

Applications

These microscopes are often used for:

- Imaging structural components of small specimens, such as cells
- Conducting viability studies on cell populations (are they alive or dead?)
- Imaging the genetic material within a cell (DNA and RNA)
- Viewing specific cells within a larger population with techniques such as FISH [120].

CONFOCAL MICROSCOPE

Confocal microscopy is a specialized form of standard fluorescence microscopy (also called wide-field fluorescence microscopy) that uses particular optical components to generate high-resolution images of material stained with fluorescent probes. It is rapidly gaining acceptance as an important technology because of its capability to produce images free of out-of-focus information. It provides a significant improvement in lateral resolution and the capacity for direct, non-invasive serial optical sectioning of intact, and thick living specimens.

Confocal microscopy offers several advantages over conventional optical microscopy, including shallow depth of field, elimination of out-of-focus glare, and the ability to collect serial optical sections from thick specimens.

In the biomedical sciences, a major application of confocal microscopy involves imaging either fixed or living cells and tissues that have usually been labeled with one or more fluorescent probes [121-126].

Applications

The broad range of applications available to laser scanning confocal microscopy includes a wide variety of studies in neuroanatomy and neurophysiology, as well as morphological studies of a wide spectrum of cells and tissues. Other applications include resonance energy transfer, stem cell research, photobleaching studies, lifetime imaging, multiphoton microscopy, total internal reflection, DNA hybridization, membrane and ion probes, bioluminescent proteins, and epitope tagging.
Many of these powerful techniques are described in these reviews. CLSM is widely-used in numerous biological sciences disciplines, from cell biology and genetics to microbiology and developmental biology.

**Electron microscope**

An electron microscope is a type of microscope that uses electrons to illuminate a specimen and create an enlarged image. It can magnify specimens up to 2 million times, while the best light microscopes are limited to magnifications of 2000 times. The greater resolution and magnification of the electron microscope are due to the wavelength of an electron, its de Broglie wavelength, being much smaller than that of a light photon, electromagnetic radiation [127].

**Transmission electron microscope (TEM)**

The way the image is created is similar to how a shadow is created with visible light. When the electron beam is transmitted through the sample, not all the electrons make it out. Some electrons are absorbed or deflected as they try to pass through the sample. The areas where more electrons made it through create bright spots on the screen below, and the areas where fewer electrons came through create darker spots. This, in turn, creates a magnified, shadow-like, black and white image of the sample.

SEM images are created by electrons that bounce off or are ejected from the sample. Because of this, the SEM gets surface images of the sample, whereas the TEM gets images of the internal composition of the sample. The downside of this is that the sample must be cut very thin for the electrons to pass through, making sample preparation much harder than that of a sample used in an SEM.

The main application of a TEM is to provide high magnification images of the internal structure of a sample. Being able to obtain an internal image of a sample opens new possibilities for what sort of information can be gathered from it.

ATEM operator can investigate the crystalline structure of an object, see the stress or internal fractures of a sample, or even view contamination within a sample through the use of diffraction patterns, to name just a few kinds of studies [128-133].

**Scanning electron microscope**

When an SEM fires, an electron at the sample you want to magnify several different signals can be given off as the electrons strike the sample. Among the various signals given off, three of the most important are backscattered electrons, secondary electrons, and X-rays. The backscattered electrons occur when the collision is elastic. The backscattered electrons are the electrons that were originally shot at the sample bouncing back off of it. Conversely, secondary electrons occur when the collision is inelastic. Unlike backscattered electrons, secondary electrons originate from the sample itself. They are electrons that have been jarred loose from inside the sample.

We use these two types of electrons to make an image of the sample by scanning a beam of the fired electrons across the whole sample, hence the “scanning” in the scanning electron microscope. As the electron beam is scanned across the whole sample, detectors inside the microscope pick up the signals given off by this interaction. The detectors then use these signals to create the magnified image of the sample. The secondary electrons produce the highest quality images with the greatest possible magnification in the SEM. The backscattered electrons produce a worse quality image but also give information about the sample’s composition.

The STEM provides structural and chemical information of a specimen at atomic-scale resolution and complements conventional transmission electron microscopy techniques. Mass measurements can now be performed routinely on a wide range of molecular and supramolecular structures using elastically scattered electrons. The recent progress in the acquisition and analysis of electron energy-loss spectroscopy data indicates that the scanning TEM is an efficient tool for mapping the chemical composition of biological samples [134-136].

**Reflection electron microscope (REM)**

In REM, the reflected beam of elastically scattered electrons is detected. It is used for looking at the microstructure of magnetic domains [137-139].

**Disadvantages of electron microscopy**

- It is expensive to buy and maintain
- Dynamic rather than static
- The specimen is specially prepared by sometimes lengthy and difficult techniques to withstand the environment inside an electron microscope.

**Modern day microscopes**

In the present day, the modest utility of the microscope as a tool in dental treatment has played a colossal role in building its usefulness.

Apotheker and Jako first introduced a commercial operating microscope to dentistry in 1981. Shanelec and Tibbetts took a step forward to introduce it in periodontics. Microscope in periodontics includes most commonly loupes, digital, and surgical microscopes. DinoLite is one of the original innovations of the 21st century, with a handy size of a fat pen that offers low power zoom capabilities with magnification up to ×500.

With the digital microscope, a live image transmission to a TV or computer can be done. Plain assimilation of a microscope and digital camera helped in advancement and revolutionizing microphotography.

The dental loupes are simple combinations of two or more lenses. It is available as simple, compound, and prism loupes. It helps to alleviate the eye strain by magnifying the image when you are working on tiny objects and you need precision in surgery. The approach and concepts of “Minimally Invasive Surgery” and “Microsurgery” are based on the utility of the microscope in surgery.

Dental microscopes, as a highly sophisticated structure of lenses, give magnification between ×4 and ×24.

The magnification recommended for periodontal surgery is between ×10 and ×20. The dental microscope provides an ergonomic working posture, optimal, coaxial lighting of the operation region, and quite freely selectable magnification levels.

During surgical intervention, the surgeon uses both hands to perform the treatment procedure. For this reason, a motor-driven magnification changer, operated by a foot pedal, seems to be more ergonomic.

Conversely, if the magnification needs frequent change, it can be accomplished faster with the manual changer. To visualize lingual or palatal sites that are difficult to access, the microscope must have sufficient maneuverability. Recent technical advancement has further enabled direct viewing of oral operation aspects.

**CONCLUSION**

It is safe to say that microscopes have played a central part in life sciences.

This has positively contributed to the enhancement of quality of life since a lot of discoveries directly contributed to the development of drugs and cures used in the treatment of diseases and conditions that were previously misunderstood or not well understood.

A cell is the single unit of life, and to understand and study it, a microscope is necessary. The discovery of cells and genes was major milestones in the medical sciences and was a great influence on the development of new effective cures and a reduction of mortality cases among populations.
REFERENCES
1. Clay RS, Court TH. The History of the Microscope: Compiled from Original Instruments and Documents, up to the Introduction of the
60. Matthyses AL, Simon SM, Rappoport JZ. Imaging with total internal reflection fluorescence microscopy for the cell biologist. J


