Microscopes in Conservative Dentistry and Endodontics Research

Over the past few decades, microscopes in endodontics have revolutionized routine as well as research-based clinical procedures. Magnification is chiefly driven by innovation and technology. It has now radically changed how endodontic research is currently being conducted.

Microscopy has three well-recognized branches:
- It is still common practice to utilize a conventional microscopy that relies on visible light and was developed before the eighteenth century
- Electron microscopy
- Scanning probe microscopy.

THE COMPOUND MICROSCOPE

A light source and a number of lenses make up a compound light microscope. A bright field microscope is also known as a compound light microscope since its light source is located at the bottom of the microscope.

Uses: In dentistry, used to study stained prepared slides for histopathological diagnosis, smears, blood analysis, etc.

**Binocular stereoscopic microscope/dissecting microscope**

It utilizes a magnification between ×10 and ×100. At low magnification, it makes it simple to observe 3D objects.

**Applications in dentistry**

The stereomicroscope is a valuable instrument in various fields of dentistry. Stereo and optical microscopy highlights aspects of external morphology, as well as root canal space of extracted teeth. *In vitro* studies have been conducted to evaluate apical microleakage around retrograde filling materials, studying the sealing abilities of root-end filling materials, for example, amalgam, mineral trioxide aggregate, and determination of working length during root canal procedures. Studying differences in canal configuration, lateral, foramina and auxiliary canals, and apical deltas at root apex is also very beneficial.

Stereoscope is valuable in studying palatine rugae patterns (Palatoscopy), a method used for identification in forensic odontology and in assessing cementum thickness and annulations in age estimation.

A stereomicroscope is a significant accessory tool that may be utilized for grossing of the biopsy specimens.

Stereoscopic examination of the specimen may offer us vital information about the nature of the tissue and proliferation type (papillary, presence of capsule or epithelium) which may not only facilitate in the precise orientation of the sample but also in histopathological diagnosis.

**The phase-contrast microscope**

With a phase-contrast microscope, light passing through a transparent object undergoes phase shifts, which are then converted to brightness shifts in the captured image. It may reveal parts of a cell or bacterium that would be very challenging to observe with a regular light microscope. Frits Zernike (1888–1966) was awarded the Nobel Prize for his work in 1953 on the phase-contrast microscope.

**Advantage**

- Since the phase contrast may be converted into brightness variations, transparent cells can be examined without being stained
- Cell division and other mechanisms may be seen when a cell is still alive, therefore, staining is not required.

**Applications**

- Only a phase-contrast microscope can reveal the extreme contrast in certain circumstances
- Viewing transparent specimens in detail includes viewing high-contrast pictures of microorganisms, thin tissue slices, live cells in culture, lithographic patterns, latex dispersions, sub-cellular particles, and glass pieces such as organelles and nuclei.

**Darkfield microscope**

Using this method, unstained samples may be seen on a dark, almost completely black background. Instead of a direct light source below or above the item, the addition of a condenser and/or impede below the stage assures that the light rays may strike the sample at various angles. The outcome is a “cone of light” where light is reflected, diffracted, and/or refracted off the item, enabling us to observe a specimen against a dark background.

**Advantages**

- It employs clear, translucent slides that hardly or never absorb light
- Using other methods of illumination, it might be tough to differentiate between the specimens because of their identical refractive indices
- In addition to certain crystals and minerals, thin polymers, and certain ceramics, it is utilized to
investigate marine life, including plankton and algae, insects, diatoms, hairs, fibers, protozoa, and yeast

• Both mounted cells and tissues and live bacteria are studied using this method
• It helps analyze surface defects, edges, grain boundaries, and other external features

**Disadvantages**

• Images obtained by darkfield microscopy are susceptible to deterioration, errors, as well as distortion
• Artifacts might appear all over a photograph if the specimen is not thin as much or the density of the slide fluctuates
• A darkfield image’s contrast and accuracy may be significantly impacted by the quality and preparation of the slides
• One must take extra care to ensure that slide, nose, stage, and light source are clear of microscopic particles like dust as they will be seen in the picture
• The condenser and/or slide need the use of oil or water since it is very difficult to eliminate air bubbles
• Such liquid bubbles may reduce contrast and specimen details, causing picture deterioration, distortion, as well as a flare.[7-11]

**FLUORESCENT MICROSCOPE**

It employs a ×1500 magnification range. Stefan Hell, William Moerner, and Eric Betzig awarded Nobel Prize in Chemistry for “the discovery of super-resolved fluorescence microscopy” in 2014 which advances optical microscopy to nanoscale.

In fluorescence microscopy, the object being studied serves as the light source. This method is utilized to examine samples that fluoresce under certain conditions. Based on the phenomena that occur when certain materials are exposed to the light of a certain wavelength, they release energy observable as visible light. A fluorescent sample in a sample of interest is excited by a considerably brighter light source used in a fluorescence microscope. This fluorescent species, rather than using the original light source, produces lower energy, longer wavelength light that enlarges the image.[12]

**Applications**
The microscopes found their applications in the following areas:

• Capturing the structural elements of tiny objects, like cells
• Evaluating the viability of cell populations to determine if they are dead or alive
• Depiction of the genetic material in a cell (RNA and DNA)
• Using methods like FISH to see particular cells within a bigger population.[13]

**CONFOCAL MICROSCOPE**

Confocal microscopy/wide-field fluorescence microscopy is a particular version of conventional fluorescence microscopy that produces high-resolution pictures of material labeled with fluorescent probes using certain optical components. The ability to directly, noninvasively, serially optically segment thick, intact living specimens and an appreciable gain in lateral resolution is also provided.

Compared to conventional optical microscopy, the benefits of shallow depth of field, the lack of out-of-focus glare, and the capacity to acquire serial optical slices from thick samples. The advantages of a confocal microscope are as follows:[14-17]

(a) Higher resolution can be obtained using a shorter wavelength
(b) Greater contrast can be achieved as compared to conventional microscopes
(c) The ability to obtain optical sections by changing the pinhole aperture
(d) 3D reconstruction of the images using the slices obtained in different focal planes.

**Applications**

Confocal microscopy is being used in the field of dentistry since the early 1990s. Its routine use includes:

1. Analysis of the surface roughness of dental materials, dental erosion
2. In studies involving the evaluation of microtensile bond strength
3. It has been extensively utilized to research surface topography and biofilm development on dental implants and hard dental tissues.

Numerous research in neurophysiology and neuroanatomy, and morphological analyses of a wide range of cells and tissues, are just a few of the numerous uses for laser scanning confocal microscopy. Other uses such as resonance energy transfer, lifetime imaging, epitope tagging, DNA hybridization, TIR, multiphoton microscopy, ion probes and membrane, stem cell research, photobleaching investigations, and photobleaching experiments.

Many biological sciences area as employ confocal microscopes, including genetics, cell biology, developmental biology, and microbiology.[14]

**Petrographic microscope/polarizing microscope**

It can magnify objects between ×4 and ×100. Minerals, ceramics, polymers, urea, fungus, collagen, and amyloid may all be seen using distinct light transmission qualities of material, including crystalline structures.[15,16]
Differential interference contrast microscope/ Nomarski microscopy
This optical microscopic approach increases the contrast in translucent, unstained materials such that small surface defects may be seen at a greater resolution of $\times 400$–$\times 1500$. However, the range of distinguishable specimen contrasts is limited by the use of polarized light.

Applications, imaging biological samples that are alive and unstained, including tissue culture smear. [17]

Total internal reflection fluorescence microscope
It only illuminates a specimen's surface or is close to it using an evanescent wave. Comparatively to traditional microscopes, the area that is seen is often relatively narrow. Due to the lower background light in molecular units, observation is feasible. [18]

Structured illumination microscope
As diffraction of light reduces the resolution in optical microscopes, this high-resolution microscope uses cutting-edge technology to tackle the problem. [19]

Scanning probe microscope/Atomic force microscopy
It can magnify objects at $\times 1,000,000$. The concept of a microscope that scans objects' surfaces with a very thin tip probe and uses the interaction of that probe to assess fine surface forms or attributes was first put on display by Binnig and Quate in 1986.

Disadvantage
These microscopes are unable to detect the sample's vertical dimension, height (i.e., particles), or the depth (i.e., pits or holes) of surface characteristics.

Advantage
It can provide images of the surface topography at magnifications up to 1,000,000 times higher than an electronic microscope, if not better. Unlike electron and optical microscopes that provide 2D pictures of a sample surface, an atomic force microscopy (AFM) makes measurements in three dimensions. [20,21]

Scanning near-field optical microscope
Using the characteristics of evanescent waves, the Scanning near-field optical microscope (SNOM)/near-field scanning optical microscopy microscopic approach for nanostructure analysis overcomes the far-field resolution restriction. Life science and material studies utilize it to detect minuscule surfaces.

Advantage
In nanotechnology, nanophotonics, and nano-optics, it is suitable for rapidly and easily imaging optical characteristics of samples. In the SNOM, single-molecule detection is simple. A subwavelength study of dynamic characteristics is also possible. It has a resolution of 70 times greater than an AFM. [20-22]

Electron microscope
In an electron microscope, electrons are used to light a specimen and generate an enlarged vision. When compared to the most powerful light microscopes, this microscope is capable of magnifying objects 2 million times. The lower de Broglie wavelength of an electron compared to a light photon account for the electron microscope’s superior magnification and resolution.

Transmission electron microscope
The image is formed like that of a shadow cast by direct illumination. Some of the electrons are lost when the electron beam is conducted through a sample. As they attempt to travel through the sample, some electrons are either absorbed or refracted. On the screen below, there are bright places where more electrons passed through, and in darker patches, there were fewer electrons passed through. This results in a black and white, enlarged, shadow-like picture of the sample.

Electrons that strike or are expelled from the material produce scanning electron microscope (SEM) pictures. As a result, the transmission electron microscope (TEM) obtains photos of the sample's internal composition, whereas the SEM obtains surface images of the specimen. The drawback of this with a TEM is that sample preparation is far more challenging than with an SEM since the sample should be cut extremely fine for the electrons to go through.

The most common use of a TEM is for providing high-resolution pictures of a sample's interior structure. A sample's internal picture may be obtained, which expands the types of information that may be extracted from it. A TEM operator, to name a few sorts of research, may look at an object's crystalline structure, assess the stress or internal fractures of a sample, or even use diffraction patterns to find impurities within a sample. [23-27]

Scanning electron microscope
Principal
Several signals may be emitted when an electron is fired at the sample we need to magnify. Three of the most significant signals are X-rays, backscattered, as well as secondary electrons. Electrons are backscattered when an elastic collision occurs. These are the electrons that were blasted at the sample and bounced back off of it that are causing the backscattered emissions. During inelastic collisions, secondary electrons are formed. Backscattered electrons are generated outside the sample, while...
secondary electrons are generated within the sample. They are electrons that were shaken free from the sample.

Endodontics uses SEM mostly for evaluating bacterial leakage, bacterial biofilm development, and fracture patterns of root posts and filling cement. Topographic analysis following various rotary devices and processes is another prevalent goal of study.[8-7]

Analyzing or measuring the gap between the filling material and the dentin wall in Endodontics is particularly significant use of SEM. In Endodontics, the use of SEM technology enables the viewing of root/dentin structures of varying heights without adjusting the focus. The color of dentin does not affect getting a proper focus in SEM images since they are in grayscale, while this is a restriction of optical stereomicroscopes.

In dentistry, it can be used for morphologic evaluation of titanium in Implants.

The “scanning” in the SEM refers to the process by which a beam of fired electrons is scanned over a sample to create a picture of the material. Signals from the electron beam’s interaction with the material may be detected by detectors within the microscope. They then utilize these signals to magnify the sample’s picture. Images produced by the SEM using secondary electrons are of the best quality and have the greatest magnification achievable. The picture quality is reduced because of the backscattered electrons, but the composition of the sample is shown.

SEM is mostly used in Endodontics to assess root canal bacterial leakage. At atomic-scale resolution, the SEM delivers structural and chemical information about a material using traditional transmission electron microscopy methods. A broad spectrum of molecular and supramolecular structures may now be regularly weighed using elastically dispersed electrons. Scanning TEM is an effective method for determining the chemical composition of biological specimen because of recent advances in the collection and processing of electron energy-loss spectroscopy data. SEM is mostly used in endodontics to analyze bacterial leakage in the root canal, bacteria.

Reflection electron microscope

Electrons that have been elastically dispersed are identified in reflection electron microscope. Magnetic domains may be studied using this technique.[22-26]

Drawbacks of electron microscopy

- Purchasing and maintaining it are both pricey
- Static, rather than dynamic
- For the specimen to tolerate the conditions within an electron microscope, appropriate preparation must be made using sometimes time-consuming and challenging processes.

The surgical operating microscope

Initially used in ophthalmology, the surgical operating microscope has gained popularity in endodontic and restorative procedures. It helps the operator in better visualization as well as documentation of cases which helps in predictable learning outcomes as well as in the subject teaching.

Shishir Singh
Department of Conservative Dentistry and Endodontics,
Terna Dental College, Navi Mumbai, Maharashtra, India

Address for correspondence:
Dr. Shishir Singh,
305/306, St Annes Apartments, Off Palimala Road, Bandra,
Mumbai, Maharashtra, India.
E-mail: drshishirs@gmail.com

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