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**DEPARTMENT: HUMAN ANATOMY**

**MATRIC NUMBER: 16/MHS01/044**

**LEVEL: 400**

**COURSE CODE: ANA 402**

**COURSE TITLE: ELECTRONI MICROSCOPIC TECHNIQUE AND ULTRASTRUCTURE**

**ASSIGNMENT TITLE: ELECTRON MICROSCOPY**

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**DATE: 29th APRIL 2020.**

**Question 1: Write an essay on the history of microscopy**

**Timeline of the Microscope:**

* c. 700 BCE — The "[Nimrud lens](https://en.wikipedia.org/wiki/Nimrud_lens)" of Assyrians manufacture, a rock crystal disk with a convex shape believed to be a burning or magnifying lens.
* 167 BCE — The Chinese use simple microscopes made of a lens and a water-filled tube to visualize the unseen.
* 13th century — The increase in use of lenses in [eyeglasses](https://en.wikipedia.org/wiki/Eyeglasses) probably led to the wide spread use of simple microscopes (single lens [magnifying glasses](https://en.wikipedia.org/wiki/Magnifying_glass)) with limited magnification.
* 1590 — earliest date of a claimed Hans Martens/[Zacharias Janssen](https://en.wikipedia.org/wiki/Zacharias_Janssen) invention of the [compound microscope](https://en.wikipedia.org/wiki/Optical_microscope#Compound_microscope) (claim made in 1655).
* After 1609 — [Galileo Galilei](https://en.wikipedia.org/wiki/Galileo_Galilei) is described as being able to close focus his telescope to view small objects close up and/or looking through the wrong end in reverse to magnify small objects. A telescope used in this fashion is the same as a [compound microscope](https://en.wikipedia.org/wiki/Optical_microscope#Compound_microscope) but historians debate whether Galileo was magnifying small objects or viewing near by objects with his terrestrial telescope (convex objective/concave eyepiece) reversed.
* 1619 — Earliest recorded description of a compound microscope, Dutch Ambassador [Willem Boreel](https://en.wikipedia.org/wiki/Willem_Boreel) sees one in London in the possession of Dutch inventor [Cornelis Drebbel](https://en.wikipedia.org/wiki/Cornelis_Drebbel), an instrument about eighteen inches long, two inches in diameter, and supported on 3 brass dolphins.
* 1621 — Cornelis Drebbel presents, in London, a compound microscope with a convex objective and a convex eyepiece (a ["Keplerian"](https://en.wikipedia.org/wiki/Refracting_telescope#Keplerian_telescope) microscope).
* c.1622 — [Drebbel](https://en.wikipedia.org/wiki/Cornelis_Drebbel) presents his invention in Rome.
* 1624 — Galileo improves on a compound microscope he sees in Rome and presents his occhiolino to Prince [Federico Cesi](https://en.wikipedia.org/wiki/Federico_Cesi), founder of the [Accademia dei Lincei](https://en.wikipedia.org/wiki/Accademia_dei_Lincei) (in English, The Linceans).
* 1625 — [Francesco Stelluti](https://en.wikipedia.org/wiki/Francesco_Stelluti) and [Federico Cesi](https://en.wikipedia.org/wiki/Federico_Cesi) publish Apiarium, the first account of observations using a compound microscope
* 1625 — [Giovanni Faber](https://en.wikipedia.org/wiki/Giovanni_Faber) of Bamberg (1574 - 1629) of the Linceans, after seeing Galileo's occhiolino, coins the word microscope by analogy with telescope.
* 1655 — In an investigation by [Willem Boreel](https://en.wikipedia.org/wiki/Willem_Boreel), Dutch spectacle-maker Johannes Zachariassen claims his father, [Zacharias Janssen](https://en.wikipedia.org/wiki/Zacharias_Janssen), invented the compound microscope in 1590. Zachariassen's claimed dates are so early it is sometimes assumed, for the claim to be true, that his grandfather, Hans Martens, must have invented it. Findings are published by writer [Pierre Borel](https://en.wikipedia.org/wiki/Pierre_Borel). Discrepancies in Boreel's investigation and Zachariassen's testimony (including misrepresenting his date of birth and role in the invention) has led some historians to consider this claim dubious.
* 1661 - [Marcello Malpighi](https://en.wikipedia.org/wiki/Marcello_Malpighi) observed capillary structures in frog lungs.
* 1665 — [Robert Hooke](https://en.wikipedia.org/wiki/Robert_Hooke) publishes [Micrographia](https://en.wikipedia.org/wiki/Micrographia), a collection of biological drawings. He coins the word cell for the structures he discovers in [cork](https://en.wikipedia.org/wiki/Cork_%28material%29) bark.
* 1674 — [Antonie van Leeuwenhoek](https://en.wikipedia.org/wiki/Antonie_van_Leeuwenhoek) improves on a simple microscope for viewing biological specimens.
* 1825 — [Joseph Jackson Lister](https://en.wikipedia.org/wiki/Joseph_Jackson_Lister) develops combined lenses that cancelled [spherical](https://en.wikipedia.org/wiki/Spherical_aberration) and [chromatic aberration](https://en.wikipedia.org/wiki/Chromatic_aberration).
* 1846 — [Carl Zeiss](https://en.wikipedia.org/wiki/Carl_Zeiss) founded [Carl Zeiss AG](https://en.wikipedia.org/wiki/Carl_Zeiss_AG), to mass-produce microscopes and other optical instruments.
* 1850s — [John Leonard Riddell](https://en.wikipedia.org/wiki/John_Leonard_Riddell), Professor of Chemistry at [Tulane University](https://en.wikipedia.org/wiki/Tulane_University), invents the first practical binocular microscope.
* 1863 — [Henry Clifton Sorby](https://en.wikipedia.org/wiki/Henry_Clifton_Sorby) develops a metallurgical microscope to observe structure of meteorites.
* 1860s — [Ernst Abbe](https://en.wikipedia.org/wiki/Ernst_Abbe), a colleague of [Carl Zeiss](https://en.wikipedia.org/wiki/Carl_Zeiss), discovers the [Abbe sine condition](https://en.wikipedia.org/wiki/Abbe_sine_condition), a breakthrough in microscope design, which until then was largely based on trial and error. The company of [Carl Zeiss](https://en.wikipedia.org/wiki/Carl_Zeiss) exploited this discovery and becomes the dominant microscope manufacturer of its era.
* 1928 — [Edward Hutchinson Synge](https://en.wikipedia.org/wiki/Edward_Hutchinson_Synge) publishes theory underlying the [near-field scanning optical microscope](https://en.wikipedia.org/wiki/Near-field_scanning_optical_microscope)
* 1931 — [Ernst Ruska](https://en.wikipedia.org/wiki/Ernst_Ruska) starts to build the first [electron microscope](https://en.wikipedia.org/wiki/Electron_microscope). It is a [transmission electron microscope](https://en.wikipedia.org/wiki/Transmission_electron_microscope) (TEM)
* 1936 — [Erwin Wilhelm Müller](https://en.wikipedia.org/wiki/Erwin_Wilhelm_M%C3%BCller) invents the [field emission microscope](https://en.wikipedia.org/wiki/Field_emission_microscope).
* 1938 — [James Hillier](https://en.wikipedia.org/wiki/James_Hillier) builds another [TEM](https://en.wikipedia.org/wiki/Transmission_electron_microscopy)
* 1951 — [Erwin Wilhelm Müller](https://en.wikipedia.org/wiki/Erwin_Wilhelm_M%C3%BCller) invents the [field ion microscope](https://en.wikipedia.org/wiki/Field_ion_microscope) and is the first to see [atoms](https://en.wikipedia.org/wiki/Atom).
* 1953 — [Frits Zernike](https://en.wikipedia.org/wiki/Frits_Zernike), professor of [theoretical physics](https://en.wikipedia.org/wiki/Theoretical_physics), receives the [Nobel Prize in Physics](https://en.wikipedia.org/wiki/Nobel_Prize_in_Physics) for his invention of the [phase-contrast microscope](https://en.wikipedia.org/wiki/Phase-contrast_microscope).
* 1955 — [George Nomarski](https://en.wikipedia.org/w/index.php?title=George_Nomarski&action=edit&redlink=1), professor of [microscopy](https://en.wikipedia.org/wiki/Microscopy), published the theoretical basis of [differential interference contrast microscopy](https://en.wikipedia.org/wiki/Differential_interference_contrast_microscopy).[[15]](https://en.wikipedia.org/wiki/Timeline_of_microscope_technology#cite_note-15)
* 1957 — [Marvin Minsky](https://en.wikipedia.org/wiki/Marvin_Minsky), a professor at [MIT](https://en.wikipedia.org/wiki/MIT), invents the [confocal microscope](https://en.wikipedia.org/wiki/Confocal_microscope), an optical imaging technique for increasing optical resolution and contrast of a micrograph by means of using a spatial pinhole to block out-of-focus light in image formation. This technology is a predecessor to today's widely used [confocal laser scanning microscope](https://en.wikipedia.org/wiki/Confocal_laser_scanning_microscope).
* 1967 — [Erwin Wilhelm Müller](https://en.wikipedia.org/wiki/Erwin_Wilhelm_M%C3%BCller) adds time-of-flight spectroscopy to the [field ion microscope](https://en.wikipedia.org/wiki/Field_ion_microscope), making the first [atom probe](https://en.wikipedia.org/wiki/Atom_probe) and allowing the chemical identification of each individual atom.
* 1981 — [Gerd Binnig](https://en.wikipedia.org/wiki/Gerd_Binnig) and [Heinrich Rohrer](https://en.wikipedia.org/wiki/Heinrich_Rohrer) develop the [scanning tunneling microscope](https://en.wikipedia.org/wiki/Scanning_tunneling_microscope) (STM).
* 1986 — [Gerd Binnig](https://en.wikipedia.org/wiki/Gerd_Binnig), Quate, and Gerber invent the [atomic force microscope](https://en.wikipedia.org/wiki/Atomic_force_microscope) (AFM)
* 1988 — [Alfred Cerezo](https://en.wikipedia.org/w/index.php?title=Alfred_Cerezo&action=edit&redlink=1), [Terence Godfrey](https://en.wikipedia.org/w/index.php?title=Terence_Godfrey&action=edit&redlink=1), and [George D. W. Smith](https://en.wikipedia.org/wiki/George_D._W._Smith) applied a position-sensitive detector to the [atom probe](https://en.wikipedia.org/wiki/Atom_probe), making it able to resolve materials in 3-dimensions with near-atomic resolution.
* 1988 — Kingo Itaya invents the [Electrochemical scanning tunneling microscope](https://en.wikipedia.org/wiki/Electrochemical_scanning_tunneling_microscope)
* 1991 — [Kelvin probe force microscope](https://en.wikipedia.org/wiki/Kelvin_probe_force_microscope) invented.
* 2009 — Dame [Pratibha Gai](https://en.wikipedia.org/wiki/Pratibha_Gai) invented the in-situ atomic-resolution environmental transmission electron microscope (ETEM). She decided not to patent her invention in order to further the advancement of science.

**Ancient History**

From ancient times, man has wanted to see things far smaller than could be perceived with the naked eye. Although the first use of a lens is a bit of a mystery, it’s now believed that use of lenses is more modern than previously thought. During the 1st century AD (year 100), glass had been invented and the Romans were looking through the glass and testing it. They experimented with different shapes of clear glass and one of their samples was thick in the middle and thin on the edges.

They discovered that if you held one of these “lenses” over an object, the object would look larger. These early lenses were called magnifiers or burning glasses. The word lens is derived from the Latin word lentil, as they were named because they resembled the shape of a lentil bean.

At the same time, Seneca described actual magnification by a globe of water. “Letters, however small and indistinct, are seen enlarged and more clearly through a globe of glass filled with water.” The lenses were not used much until the end of the 13th century when spectacle makers were producing lenses to be worn as glasses. Then, around 1600, it was discovered that optical instruments could be made by combining lenses.

**The First Microscopes**

The early simple “microscopes” which were only magnifying glasses had one power, usually about 6x – 10x. One thing that was quite common and interesting to look at, were fleas and other tiny insects, hence these early magnifiers called “flea glasses”.

Sometime, during the 1590’s, two Dutch spectacle makers, Zaccharias Janssen and his father Hans started experimenting with these lenses. They put several lenses in a tube and made a very important discovery. The object near the end of the tube appeared to be greatly enlarged, much larger than any simple magnifying glass could achieve by itself.

Their first microscopes were more of a novelty than a scientific tool since maximum magnification was only around 9x and the images were somewhat blurry. Although no Jansen microscopes survived, an instrument made for Dutch royalty was described as being composed of “3 sliding tubes, measuring 18 inches long when fully extended, and two inches in diameter”. The microscope was said to have a magnification of 3x when fully closed, and 9x when fully extended.

It was Antonie Van Leeuwenhoek (1632-1723), a Dutch draper and scientist, and one of the pioneers of microscopy who in the late 17th century became the first man to make and use a real microscope, and is often referred to as the father of microscopy. He made his own simple microscopes, which had a single lens and were hand-held. Van Leeuwenhoek achieved greater success than his contemporaries by developing ways to make superior lenses, grinding and polishing a small glass ball into a lens with a magnification of 270x, the finest known at that time (other microscopes of the time were lucky to achieve 50x magnification). He used this lens to make the world’s first practical microscope.

Leeuwenhoek’s microscope used a single convex glass lens attached to a metal holder and was focused using screws. Anthony Leeuwenhoek became more involved in science and with his new improved microscope was able to see things that no man had ever seen before. He saw bacteria, yeast, blood cells and many tiny animals swimming about in a drop of water. People did not realize that magnification might reveal structures that had never been seen before.

**Image of Leeuwenhoek’s microscope.**

In England, Robert Hooke re-confirmed Leeuwenhoek's discoveries of tiny living organisms in a drop of water. He replicated Leeuwenhoek's light microscope and proceeded to improve upon its design. Hooke was also an avid student of fossils and geology. As the first person to examine fossils with a microscope, he observed close similarities between the structures of fossil shells and petrified wood, as well as living wood and living mollusk shells. His examinations proved that dead wood could be turned to stone by the action of water that is rich in dissolved minerals. As the water seeped into the wood, the saturated solution would slowly deposit minerals throughout the wood. In his book Micrographia, published in 1665, Hooke concluded that the shell-like fossils he studied represented organisms that no longer existed on Earth. Two and a half centuries before Darwin, Hooke realized that the fossil record proves there is transformation among the life forms on the planet, and that species have both materialized and disappeared throughout the history of life on earth.

Another breakthrough, in the 18th Century, was the invention of the Achromat lens system by Chester Moor Hall. Around the year 1733, he managed to build the first achromatic objective, consisting of a combination of a convex crown glass and a concave flint glass. Hall tried to keep this a secret by having one type of glass manufactured by one company and the other by another company. Unknowing to him, both companies used the same lens shop which realized, of course, that the final customer was one and the same person. It took another 25 years until John Dollond picked up on the idea and got a patent for "a new method of making the object glasses of refracting telescopes by compounding mediums of different refractive qualities".

During the 19th Century, the exacting natural sciences experienced an enormous upswing in activity. In the 1820s and 1830s, the science of light and the theory of optical imaging were placed on a sound foundation. One of the most successful researchers in this field was Joseph von Fraunhofer (1787-1826). Astronomy was the main field of Fraunhofer's activities, and his most noted achievement is the first description of the dark lines of the solar spectrum as reference points for the measurement of refraction indexes. But most important to the history of microscopy, Fraunhofer also refined the achromat by using scientific and precise manufacturing methods, creating what is now known as the achromat system, the most commonly deployed optical lens system with chromatic aberration correction, and for basic advancement of knowledge on the diffraction of light. Early microscopists were hampered by optical aberration, blurred images, and poor lens design, which hampered high-resolution observations until late in the 1800s. Aberrations were partially corrected by the mid-19th century with the introduction of Lister and Amici achromatic objectives that reduced chromatic aberration and raised numerical apertures to around 0.65 for dry objectives and up to 1.25 for homogeneous immersion objectives.

**Microscopes go into Large Scale Production**

Within this period (the 1800s) of innumerable technical advances, a mechanic named Carl Zeiss began his own business in the German university town of Jena, Thuringia, with the goal of providing researchers with high-quality instruments. Between 1846 and 1866, microscopes of uniformly high quality were built in Zeiss' workshop in accordance with extremely strict rules of craftsmanship. In the beginning, these were quite simple instruments that were used as dissection microscopes, but in 1857 the Zeiss workshop produced the first genuine compound microscope (equipped with an eyepiece and an objective). The new instrument was called the Stativ 1, which combined practical functionality with the skilled optical refinement provided by a craftsman.

After almost 20 years, Zeiss was employing about 20 qualified staff members and took great pride in what had become a prosperous business. He knew that his instruments were good, but he refused to accept the trial and error method used at the time for the production of optics. Zeiss also was aware that competition from other microscope manufacturers would eventually bypass his accomplishments if he failed to continue to produce innovations. With the ultimate goal of creating reproducible products, Zeiss acknowledged that his manufacturing procedure had to be based on precise rules and strict guidelines, or as he once said: "The working hand should have no other function than to precisely implement the shapes and dimensions of all the design components determined beforehand by computation." For assistance in this endeavor, Zeiss formed a partnership with Dr. Ernst Abbe, a brilliant physicist and mathematician. Abbe was appointed as the research director of Zeiss Optical Works in late 1866. For the next six years, the team worked intensively to lay the scientific foundations for the design and fabrication of advanced optical systems. In 1869, they introduced a new illumination apparatus that was designed to improve the performance of microscope illumination. Three years later, in 1872, Abbe formulated his wave theory of microscopic imaging and defined what would become known as the Abbe Sine Condition. Several years later, Zeiss was producing a line of 17 different objectives, including three immersion systems, all featuring a level of image quality unknown until then. The construction of microscopes on a sound theoretical basis was possible at last, and still is today.

The Zeiss enterprise continued to push onward in the late 1800s. Abbe became an equal partner, and forward-thinking intelligence became the inherent capital of the young company. In his later years, Abbe became equally famous as a social reformist. Several problems still remained for Zeiss Optical Works however, since the quality of optical glass produced during the period was not sufficient to provide the theoretical resolution that was dictated by Abbe's sine condition. The glass used in the construction of microscope lenses was not homogeneous and it tended to undergo a phase separation during cooling, which led to a varying refractive index throughout the glass, and therefore, light waves passing through these lenses were refracted unpredictably. As a matter of fact, first-rate resolution was unattainable with the poor-quality glass.

Abbe first met Otto Schott, a glass chemist, in 1881. Over the next several years, Abbe and Schott developed several new glass formulas and adjusted the mixing and annealing process to eliminate internal defects and produce optical-grade glass with a uniform refractive index. In 1884, Schott, Abbe, and Zeiss formed a new company known as "Jenaer Glaswerk Schott und Genossen". Continued experimentation with glass recipes and preparation techniques yielded extraordinarily successful results, and in 1886, they introduced a new type of objective, the apochromat. By this time, an incredible 44 different types of optical glass were being produced. The creation of the apochromat objectives (with and without immersion media) eliminated color aberrations, which greatly assists bacteriologists in identifying infectious bacteria, and brought the resolving power of the microscope to the limit known today. The progress made in the development of objectives led to fields of view larger than anything ever achieved before. In the course of time, it also became evident that more attention would have to be paid to illumination. Professor August Köhler (1866-1948) became an early member of staff at Carl Zeiss in Jena, and in 1893 he published guidelines for an innovative scheme to illuminate microscope specimens. Köhler cleverly devised a microscope illumination system that made it possible to use the entire resolving power of Abbe's objectives. The Köhler illumination system provides homogeneously illuminated images. By incorporating a field iris diaphragm into the microscope illumination beam path, stray light was minimized and a simple procedure ensured proper positioning of the condenser for highest resolution and desired contrast (it is particularly beneficial that the aperture diaphragm in the condenser allows the image contrast and the resolving power to be balanced against each other without any loss in the consistency of the image brightness). Köhler's innovation was important in microphotography at the time of its development and has since become a highly significant method for virtually all forms of optical microscopy. Knowledge and observance of Köhler's rules and the associated settings of the microscope (whether automatically via personal computer and motorized functions or manually) are still essential today. Early in the twentieth century, microscope manufacturers began par focalizing objectives, allowing the image to remain in focus when the microscopist exchanged objectives on the rotating nosepiece. In 1924, Zeiss introduced a LeChatelier-style metallograph with infinity-corrected optics, but this method of correction would not see widespread application for another 60 years. Shortly before World War II, Zeiss created several prototype phase contrast microscopes based on optical principles advanced by Frits Zernike. Several years later the same microscopes were modified to produce the first time-lapse cinematography of cell division photographed with phase contrast optics. This contrast-enhancing technique did not become universally recognized until the 1950s and is still one of the methods of choice for many cell biologists today. Physicist Georges Nomarski introduced improvements in Wollaston prism design for another powerful contrast generating microscopy theory in 1955. This technique is commonly referred to as Nomarski interference or differential interference contrast (DIC) microscopy and, along with phase contrast, has allowed scientists to explore many new arenas in biology using living cells or unstained tissues. Robert Hoffman introduced another method of increasing contrast in living material by taking advantage of phase gradients near cell membranes. This technique is now termed Hoffman Modulation Contrast and is available as optional equipment on most modern microscopes.

**Electron Microscope Appears**

The use of visible light in microscopy limits the resolution that could be achieved, but this problem was overcome in 1931 when two German scientists Max Knoll and Ernst Ruska discovered that beams of electrons could be used instead of light. The electron microscope could be used to observe objects that were not visible using light microscopes.

Scientists working for corporations competed to develop the first commercial electron microscope and Ernst Ruska, working for Siemens, eventually achieved this in 1938. By the late 1930s, microscopes had been developed that could achieve resolutions as low as 10nm and by the mid-1940s, resolutions as low as 2nm had been achieved. The main competitors in Europe were Siemens, Philips and Carl Zeiss. In the late 1930s, the scientists in Japan formed the Japan Electron Optics Laboratory that eventually manufactured the greatest variety of electron microscopes among all of the companies.

The early versions of electron microscopes used transmission electron microscopy. The first scanning electron microscope hit the market in 1965, which revolutionized the world of material science.

**Question 2: Differentiate between the light microscope and electron microscope.**

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| --- | --- | --- | --- |
| **S.N.** | **Character** | **Light Microscope** | [**Electron Microscope**](https://microbenotes.com/electron-microscope-principle-types-components-applications-advantages-limitations/) |
| 1.       | Alternatively known as | Optical microscope | Beam microscope |
| 2.       | Invented by | It is believed that Dutch spectacles makers Zacharius Jansen and his father Hans were the first to invent the compound microscope in the 16th century. | In 1931 physicist Ernst Ruska and German engineer Max Knoll. |
| 3.       | Illuminating source | Uses light (approx wavelength 400-700 nm) to illuminate the objects under view. | Uses a beam of electrons (approx equivalent wavelength 1 nm) to make objects larger for a detailed view. |
| 4.       | Principle | The image formed by absorption of light waves. | The image formed by scattering or transmission of electrons. |
| 5.       | Structure | Light microscopes are smaller and lighter. | Heavier and larger in size. |
| 6.       | Lenses used | Lenses are made of glass. | Lenses are made of electromagnets. |
| 7.       | Vacuum | Not used under a vacuum | Operates under a high vacuum |
| 8.       | Specimen type | Fixed or unfixed, stained or unstained, living or non-living. | Fixed, stained and non-living. |
| 9.       | Specimen observed | Both live and dead specimens can be observed. | Only dead specimens are possible to be observed. |
| 10.    | Specimen preparation | Less tedious and simple. | It generally involves harsher processes, e.g. using corrosive chemicals. More skill required – both to prepare specimens and to interpret EM images (due to artifacts). |
| 11.    | Preparation time | Specimen preparation takes usually a few minutes to hours. | Specimen preparation takes usually takes a few days. |
| 12.    | Thickness of specimen | 5 micrometer or thicker | Ultra-thin, 0.1 micrometers or below |
| 13.    | Dehydration of Specimen | Specimens need not be dehydrated before viewing. | Only dehydrated specimens are used. |
| 14.    | Coating of specimen | Stained by colored dyes for proper visualization. | Coated with heavy metals to reflect electrons. |
| 15.    | Mounting of specimen | Mounted on the glass slide. | Mounted on the metallic grid (mostly copper). |
| 16.    | Focusing | Done by adjusting the lens position mechanically. | Done by adjusting the power of the electric current to the electromagnetic lenses. |
| 17.    | Magnification power | Low magnification of up to 1,500x. | High magnification of up to 1,000,000x. |
| 18.    | Resolving power | Low resolving power, usually below 0.30µm. |  The high resolving power of up to 0.001µm, about 250 times higher than the light microscope. |
| 19.    | Viewing of the image formed | Light microscope images can be viewed directly. Images are viewed by the eyes through the eyepiece. | Images are viewed on a photographic plate or zinc sulfate fluorescent screen. |
| 20.    | Nature of Image formed | Poor surface view | Good surface view and internal details |
| 21.    |  Image Color | Colored images. | Electron microscopes produce grayscale (sometimes called “black and white”) images (except “false-color” electron micrographs). |
| 22.    | Image dimension | Image plane “flat” (2D). | 2D only in a Transmission electron microscope (TEM); Scanning electron microscope (SEM) images give depth information which seems like 3D. |
| 23.    | Living processes | Visualization of living processes such as microscopic pond life in action and even cell division is possible. | Living processes cannot be viewed. |
| 24.    | Room settings | No special settings required. | It must be used in a room where humidity, pressure, and temperature are controlled. |
| 25.    | Simplicity in use | Simple to use | Users require technical skills |
| 26.    | Electric Current | No need for high voltage electricity. | High voltage electric current is required (50,000 V or above). |
| 27.    | Filaments | No filaments used. | Tungsten filaments used to generate electrons. |
| 28.    | Cooling System | Absent | Cooling system present to pacify the heat generated due to high voltage electric current. |
| 29.    | Radiation leakage | No radiation risk. | Risk of radiation leakage. |
| 30.    | Complexity | Less complex | Complex |
| 31.    | Expense | Cheap to buy and has low maintenance costs. | Very expensive to buy as well as to maintain. |
| 32.    | Suitability /   Practicality | Suitable for most basic functions, and is very common in schools and other learning institutions. | Limited to specialized use such as research. |
| 33.    | Advantages | * Easy to use
* Cheap
* True color but sometimes require staining
* Live specimens
 | * High resolution
* Provide detailed images of surface structures and interior structures
* High magnification
* 3D images
 |
| 34.    | Disadvantages | * Low resolution due to shorter wavelength of light (0.2nm)
* Low magnification
* The specimen used is thin.
 | * Expensive
* Requires extensive training
* Sample must be dead
* Black and white/false-color image
 |
| 35.    | Types/ Variants | * [Dark-field microscope](https://microbenotes.com/darkfield-microscopy/)
* [Phase-contrast microscope](https://microbenotes.com/phase-contrast-microscopy/)
* [Fluorescent microscope](https://microbenotes.com/fluorescence-microscope-principle-instrumentation-applications-advantages-limitations/)Confocal microscopePolarized microscope
* Differential interference contrast microscope
 | * Transmission electron microscope (TEM)
* Scanning electron microscope (SEM)
 |
| 36.    | Application | It is used for the study of detailed gross internal structure. | It is used in the study of the external surface, the ultrastructure of cell and very small organisms. |

**Question 3: Differentiate between the SEM and TEM.**

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| --- | --- | --- |
| **Properties** | **Scanning Electron Microscopy  (SEM)** | **Transmission Electron Microscopy (TEM)** |
| Light Source | SEM is based on scattered electrons, i.e. electrons emitted from the surface of a specimen. It is the EM analog of a stereo light microscope. | Electrons are used as “light source”. TEM is based on transmitted electrons and operates on the same basic principles as the light microscope. |
| Purpose | SEM provides detailed images of the surfaces of cells.  SEM focuses on the sample’s surface and its composition, so SEM shows only the morphology of samples. | Transmission electron microscope is used to view thin specimens (tissue sections, molecules, etc). TEM can show many characteristics of the sample, such as internal composition, morphology, crystallization, etc. |
| Sample Preparation | Sample is coated with a thin layer of heavy metal such as gold or palladium. | The sample in TEM has to be cut thinner (70-90 nm) because electrons cannot penetrate very far into materials. |
| Resolution | SEM can resolve objects as close as 20 nm. | TEM has a much higher resolution than SEM. It can resolve objects as close as 1 nm i.e. down to near-atomic levels. |
| Magnification | The magnifying power of SEM is up to 50,000X. | The magnifying power of TEM is up to 2 million times. |
| Processing of sample (s) | SEM allows for a large amount of sample to be analyzed at a time | With TEM only a small amount of samples can be analyzed at a time. |
| Image formation | Secondary or backscattered electrons arising from the interaction of electron beam and metal-coated specimen are collected and the resulting image is displayed on a computer screen. | Transmitted electrons hit a fluorescent screen giving rise to a “shadow image” of the specimen with its different parts displayed in varied darkness according to their density. The image can be studied directly by the operator or photographed with a camera. |
| 3D picture | SEM  provides a 3-dimensional image | TEM provides a 2-dimensional picture. |
| Current Applications | To study topography and atomic composition of specimens, process control and also, for example, the surface distribution of immuno-labels | To image the interior of cells (in thin sections), the structure of protein molecules (contrasted by metal shadowing), the organization of molecules in viruses and cytoskeletal filaments (prepared by the negative staining technique), and the arrangement of protein molecules in cell membranes (by freeze-fracture). |

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