THE FUNDAMENTALS OF ...

Electron Microscopes

Robert M. Dondelinger

The resolution provided by conventional optical microscopes is around 200 nanometers (nm or 10^{-9} meters), which is useful to magnify objects up to 2,000 times their size. This is sufficient for examining blood cells, human hairs, post-surgical tissue specimens, most bacteria, and most normal resident and transient flora. However, there exists a world beyond this, which cannot be seen by conventional microscopes. For instances where it is essential to see into this world, electron microscopes are employed. These devices provide resolution down to 50 picometers (pm or 10^{-12} meters) and magnifications up to about 10 million. This allows technicians in research laboratories to visualize microorganisms, cells, large molecules, viruses (such H2N2 and the next SARS), and crystals as clearly as blood cells and human hair.

Due to their size, cost of acquisition and operation, and plant requirements (such as physical space, electrical power requirements for the components, heating, ventilation, and air conditioning (HVAC) load, dimmable room lighting, utilities), electron microscopes are rarely found outside of research organizations and are rarely used as a diagnostic aid in healthcare facilities.

The world of electron microscopes breaks conveniently down into three types—transmission, scanning, and scanning/transmission electron microscopes (which are a combination of both types). They are commonly referred to as TEMs, SEMS, and STEMs. This article will cover TEMs in detail and only touch on the SEM since it is a derivative type. TEMs are the more common type and typically provide better images than either SEMS or STEMs.

Current Technology

To visualize small objects, an electron microscope employs a stream of electrons, the smallest unit of an atom. The design of the TEM is similar to an optical microscope, except that an electron beam "illuminates" the sample for viewing. It is the most common type and may be found in large medical centers and medical research facilities. Biomeds who are familiar with the operating principles of the cathode ray tube (CRT) found in old television

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sets and patient monitors will find the physical description of the TEM familiar.

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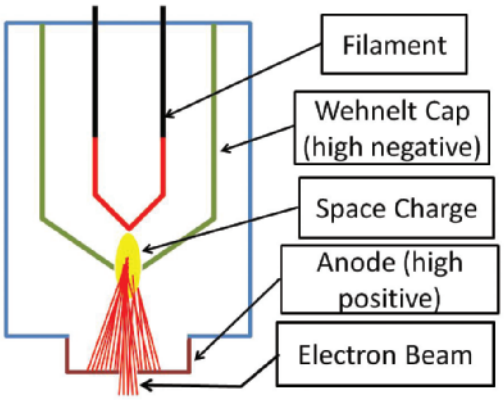

Figure 1. Basic Components of the Electron Gun

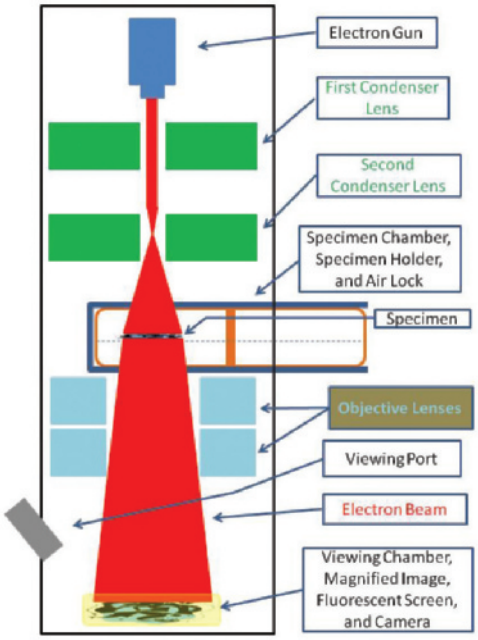

Figure 2. Basic Components of the Transmission Electron Microscope (TEM)

The electron gun is comprised of a filament, cathode, and anode—all housed in a single chamber. Like conventional vacuum tubes, the filament heats the cathode, which creates an electron cloud around the cathode. However, unlike the conventional vacuum tube, electrons from this cloud are forced to pass through a small (less than 1 mm) hole in the cathode (called a Wehnelt cap) by the high potential difference (voltage) between the filament/cathode and the anode. Because of this high voltage difference and the presence of the hole through the anode, a large number of electrons fly past the anode and exit through the anode hole. These electrons will eventually form the beam used to illuminate the specimen being examined. The electron gun can be adjusted to maximize the resolution by changing the cathode-anode voltage. By increasing the voltage, the electron speed increases and the wavelength becomes correspondingly shorter, resulting in a more consistent and better quality electron beam. This means a better quality and a higher resolution image.

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As the stream of electrons fly towards the target in the specimen chamber, the beam passes through the magnetic condenser "lenses," the first of several that compromise the imaging lens system. The condenser is an electromagnetic lens, similar to deflection coils around the neck of a cathode ray tube in a conventional television set or CRT computer monitor. As the name suggests, the condenser lens condenses the stream into a very small diameter electron beam while other lenses remove nonaligned, erratic, and slow electrons from the beam.

After passing through the condenser lenses, the concentrated beam enters the specimen chamber. Once the medical technician performs initial adjustments (high voltage, focusing, etc.) to the microscope, the high-voltage is turned off while the specimen cartridge is placed into the chamber through an air lock mechanism, providing a vacuum seal. To ensure the specimen remains nonvolatile and dry, the specimen chamber may employ a liquid nitrogen-cooled "cold trap" or anti-contamination device to reduce the release of contaminants that degrade the final image. The specimen cartridge contains a metal grid that holds the specimen for examination. The cartridge also contains adjustments permitting the technician to rotate the grid during the microscopic examination without breaking the vacuum seal. Once the specimen is in place and the vacuum has stabilized, the technician turns the high voltage

back on and the examination begins.

In a TEM, when the beam strikes a portion of the specimen, one of three things occurs. The electrons pass through, are absorbed by, or are scattered by the specimen. Just as with X-rays, sections of the sample that allow the electrons to pass through appear as light areas and sections that absorb electrons appear as dark areas when viewing the image of the specimen. The column liner absorbs scattered electrons to reduce image distortion.

Next, the electrons that pass through the specimen enter the imaging lens system. This system consists of three more types of electronic lenses, similar in design to the condenser lenses. The first of the three is the objective lens, which actually creates the image. Next, the electrons pass one or more intermediate lenses that magnify the image. The last is the projector lens, which casts the image onto the fluorescent screen located in the viewing chamber. The electrons excite the coating of the fluorescent screen, making it glow with a visible image. The screen is often capable of being tilted slightly to allow viewing though a shielded glass port.

A permanent record of the image is usually made with either a charge-coupled device (CCD) or conventional photographic film. Older designs employ a photographic film camera system—typically either 35 mm roll or sheet film—to provide a permanent record, while newer designs use the CCD camera alone. Some newer designs also offer a film camera as an optional system, while employing the CCD camera as the primary recording system. Most current designs have their photographic systems outside the vacuum system, but some manufacturers offer the photographic system inside the vacuum system. Other design variants the biomed may encounter include stereoscopic imaging systems, digital video recording, and display systems capable of both digital archiving and video conference viewing.

The vacuum system is critical to the operation of an electron microscope since it provides the vacuum necessary for electron gun operation. The vacuum created by the pump is as close as possible to absolute, typically as low as 10^{-6} torr (millimeter of mercury) or better. Normally, two pumps operate in tandem to achieve the ultralow vacuum required for operation. The first is usually a common rotary

ORIGIN AND EVOLUTION

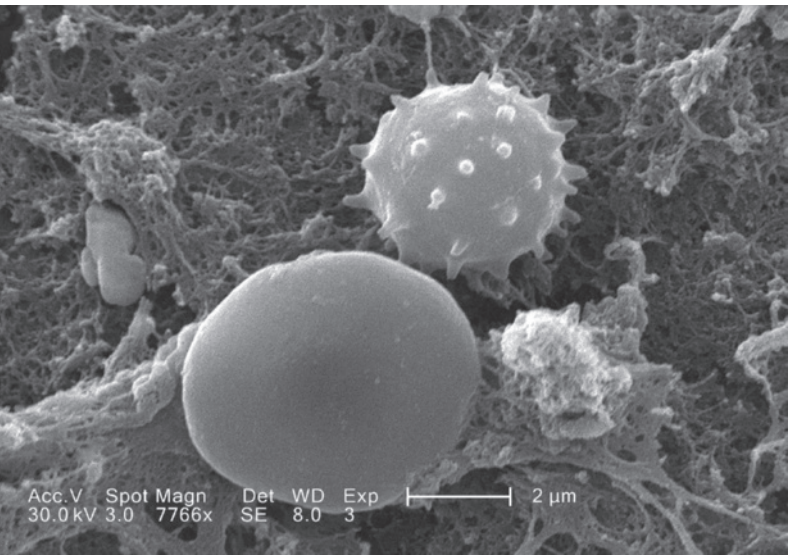
Since the early 1930s, scientists longed for a device that would be able to visualize objects smaller than 0.2 micrometers because they had reached the limit of optical microscope technology. They greatly desired to see into the interior of cells, to visualize the nucleus, mitochondria, and to visualize details that were, at that time, impossible to see. This would require a device capable of providing at least 10,000X magnification—an order of magnitude greater than the 1,000X magnification provided by visible light microscopes of the day.

Ernst Ruska, a German physicist, and Max Knoll, an electrical engineer, constructed the first a prototype electron microscope in 1931. Their prototype was only capable of 400X magnification, well within the range of optical microscopes of that time. However, their accomplishment proved the basic principles and design theorized by Hungarian physicist Leo Szilard, who patented the electron microscope but never built it. After two years of tinkering and refining their prototype, Ruska was able to construct a transmission electron microscope (TEM) that passed the optical microscope in both magnification and resolution.

Meanwhile, in May of 1931, the scientific director of Siemens-Schuckertwerke, Reinhold Rudenberg, acquired a patent for the TEM. His motivation for developing a better microscope was a personal one; family illness drove him to visualize the poliomyelitis virus. As a result, in 1932 Siemens built and operated their prototype TEM based upon the concepts contained in Rudenberg's patents. In 1937, Siemens-Schuckertwerke backed Ruska and several others in the development of applications, particularly biological ones, for their microscope. Also in 1937, while the Siemens engineers were pursuing their ideas, another German self-motivated physicist and experimenter, Manfred von Ardenne, developed the scanning electron microscope (SEM).

While these other developments were going on in Europe, at the University of Toronto Eli Franklin Burton and students Cecil Hall, James Hillier, and Albert Prebus worked with Siemens personnel to produce the first commercial TEM, in 1938. This was followed in 1942 by the debut of the first SEM, but due to the state of electronics technology, a commercial SEM was not introduced into the marketplace until 1965.

Although today's TEMs are capable of better than 10^6 magnification, the basic design has not changed from the prototype built by Ruska in 1931, which parallels the basic design of the optical microscope.



A scanning electron micrograph depicts red blood cells enmeshed in a fibrinous matrix on the luminal surface of a vascular catheter.

pump and provides a vacuum down to about 10^{-2} torr. The second, usually a water-cooled molecular diffusion vacuum pump employing either mercury or hot oil, further reduces the vacuum to achieve the required high vacuum. Its function, aside from providing a vacuum for the column down to and including the specimen chamber, is to remove oil, vapors, and other gasses from the column. This facilitates better image quality by eliminating molecules that may collide with the electrons, generating positively or negatively ionized particles. Moreover, the removed molecules are organic and can distort the image, contaminate the specimen, and degrade the image quality. Basically, the better the vacuum, the better the image.

Both the SEM and the STEM operate in the same manner as the TEM up to the specimen, but here is where the design takes an interesting turn. Once technology made it possible to deflect the electron beam, the SEM became a reality. The electron beam of the SEM "paints" or scans the surface of the specimen similar to the raster scan of a CRT monitor or television set. Typically, a secondary electron detector detects the reflected electrons. Its output is synched to the scanning pattern to acquire X-, Y- and Z-axis information. This information is reassembled into a surface image of the specimen. This technology eliminates the need for the imaging lens system as well as the photographic system and viewing chamber. The downside of the SEM is that the resolution is generally poorer than the TEM image. The upside is that the SEM is able to image samples up to several centimeters, has an excellent depth of field which allows it to provide good images of three dimensional samples, and can be used on wet specimens.

How to Manage Electron Microscopes

Due to their high cost, management of maintenance should be by serial number or locally assigned maintenance tracking number that is unique to the device. The maintenance management program used should capture the total cost of maintenance and facilitate lifecycle management of the microscope. The database should also be capable of producing a detailed maintenance history, showing both scheduled and remedial services performed, total costs, hours of labor, cost of repair parts, and cost of outside or contracted maintenance services.

Contrary to my oft-recommended advice to acquire a full-service contract for complex medical devices, especially laboratory equipment, I recommend billed service for electron microscopes. Although they are one-of-a-kind laboratory instruments, they are not as technically complex as other laboratory equipment. Additionally, unless regular updates of the image reconstruction software (primarily found only on the newest devices in the marketplace) are included in the service contract or agreement, there is little reason to enter into an annual or full-service contract. Seldom will the downtime of several days impair the laboratory or patient care to an extent where a service contract is worth the cost. In-house biomedical equipment technicians can remediate some problems.

Regulations

Normally electron microscopy would be considered high complexity under the Clinical Laboratory Improvement Amendments (CLIA) because of the detailed, complex, and exacting specimen preparation requirements. However, CLIA says that "research laboratories that test human specimens but do not report patient specific results for the diagnosis, prevention or treatment of any disease or impairment of, or the assessment of the health of individual patients" are exempt. Therefore, research facilities, where the majority of electron microscopes are found, are exempt from the complex provisions of CLIA. However, when they are used in ways that do not fit the above statement (from section 493.3(b)(2) of CLIA), such as large medical centers providing diagnoses that lead to treatments based upon electron microscopy results, their use would be considered of high complexity.

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Additionally, normal regulations from the Occupational Safety and Health Administration (OSHA) apply to electron microscopes. There are several risks inherent in the use of these microscopes because they generate high voltage to power the electron gun, often use liquid nitrogen, and use mercury to generate the high vacuum required for operation. Receiving an electric shock or a "cold burn," the potential to generate uncontrolled X-rays, and exposure to mercury vapor are just a few of the risks. Therefore, both the OSHA "general duty" clause (29 USC

§654,57 Sec 5(a)1) and numerous hazard-unique portions of OSHA regulations—depending on the particular design and features of the microscope—apply.

Risk Management Issues

Manageable risks are involved in using and maintaining electron microscopes. As mentioned above, exposure to mercury and liquid nitrogen can occur while replacing pump filters and both the operator and maintainer can be exposed to high voltages while adjusting and servicing the electron gun and related components. Additionally, if the electron gun is not adjusted properly, it may generate uncontrolled X-rays. Fortunately, most of these risks are minimized and managed through device interlocks and strict adherence to operating and maintenance procedures. This includes the use of personal protective clothing and equipment.

Troubleshooting

Most problems with electron microscopes are caused by either poor sample preparation or low vacuum. Sample preparation is totally dependent upon the skill of the laboratory technician. Low vacuum can be caused by poor operator maintenance, a leak within the vacuum system, or a hard failure of one or both vacuum pumps. The later can be resolved by a properly trained and experienced biomed. The other potential sources of trouble are the high voltage power supply (or supplies, depending on the design) and the camera system.

Training and Equipment

Model-specific training is essential if an organization is planning to maintain their electron microscope in-house. An experienced level III biomed should be the lead maintainer. Basic biomed hand tools and test equipment will be required, and, if a film camera is used, an in-depth knowledge of the film processing procedure will be necessary to help resolve some imaging issues. A high-voltage probe, similar to that used by television repair personnel, will be required for accurately measuring the cathode-anode voltage of the electron gun. Additionally, service equipment such as special tools, test equipment, service aids, etc., are model specific and can only be determined after thoroughly studying the service manual.

Future Development

Although the basic design of electron microscopes may remain unchanged for the near future, it is by no means considered mature technology. Many incremental improvements are expected over time. Improvements in TEM magnification, the availability of semiautomatic alignment, and easier-to-replace filaments are available from some manufacturers and will eventually become the industry standard. Additionally, improvements in the software used for the database functions and image filing options are on the horizon. Eventually, totally electronic image management is predicted to replace conventional film. These systems will handle acquisition by CCD camera, image display, storage, and retrieval, similar to the picture archiving and communication system (PACS) used in radiology today,

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With the rapid advance of electronics technology, both SEMs and STEMs are poised to take several quantum leaps. Electron detectors and detection technology will improve resolution to meet and possibly exceed the image quality provided by TEMs. Future software imaging applications will directly accept the electron detector information and provide better 3-D and rotatable images, similar to those provided by CT and MRI imaging systems. Lastly, new and improved sample preparation techniques, including the depositing of evaporated substances and noble metals, will provide enhanced sample detail. ■

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