Electron Microscopy

The maximum theoretical resolution of images created by light microscopes is ultimately limited by the wavelengths of visible light. Most light microscopes can only magnify 1000×, and a few can magnify up to 1500×, but this does not begin to approach the magnifying power of an **electron microscope (EM)**, which uses short-wavelength electron beams rather than light to increase magnification and resolution.

Electrons, like electromagnetic radiation, can behave as waves, but with wavelengths of 0.005 nm, they can produce much better resolution than visible light. An EM can produce a sharp image that is magnified up to 100,000×. Thus, EMs can resolve subcellular structures as well as some molecular structures (e.g., single strands of DNA); however, electron microscopy cannot be used on living material because of the methods needed to prepare the specimens.

There are two basic types of EM: the **transmission electron microscope (TEM)** and the **scanning electron microscope (SEM)** (Figure 2.21). The TEM is somewhat analogous to the brightfield light microscope in terms of the way it functions. However, it uses an electron beam from above the specimen that is focused using a magnetic lens (rather than a glass lens) and projected through the specimen onto a detector. Electrons pass through the specimen, and then the detector captures the image (Figure 2.22).



Figure 2.21 A transmission electron microscope (TEM).

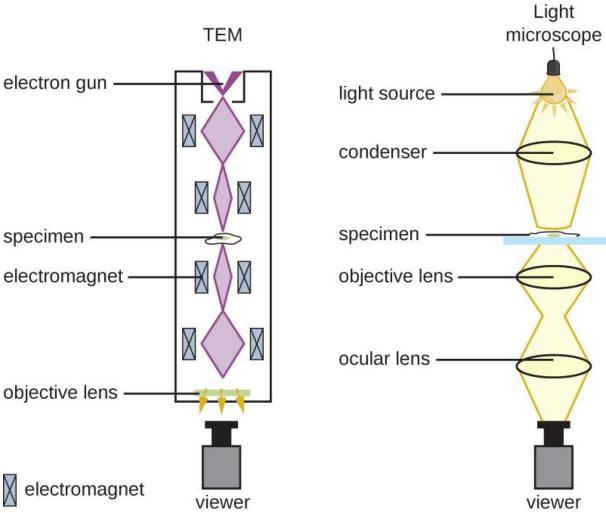


Figure 2.22 Electron microscopes use magnets to focus electron beams similarly to the way that light microscopes use lenses to focus light.

For electrons to pass through the specimen in a TEM, the specimen must be extremely thin (20–100 nm thick). The image is produced because of varying opacity in various parts of the specimen. This opacity can be enhanced by staining the specimen with materials such as heavy metals, which are electron dense. TEM requires that the beam and specimen be in a vacuum and that the specimen be very thin and dehydrated. The specific steps needed to prepare a specimen for observation under an EM are discussed in detail in the next section.

SEMs form images of surfaces of specimens, usually from electrons that are knocked off of specimens by a beam of electrons. This can create highly detailed images with a three-dimensional appearance that are displayed on a monitor (Figure 2.23). Typically, specimens are dried and prepared with fixatives that reduce artifacts, such as shriveling, that can be produced by drying, before being sputter-coated with a thin layer of metal such as gold. Whereas transmission electron microscopy requires very thin sections and allows one to see internal structures such as organelles and the interior of membranes, scanning electron microscopy can be used to view the surfaces of larger objects (such as a pollen grain) as well as the surfaces of very small samples (Figure 2.24). Some EMs can magnify an image up to 2,000,000×.¹

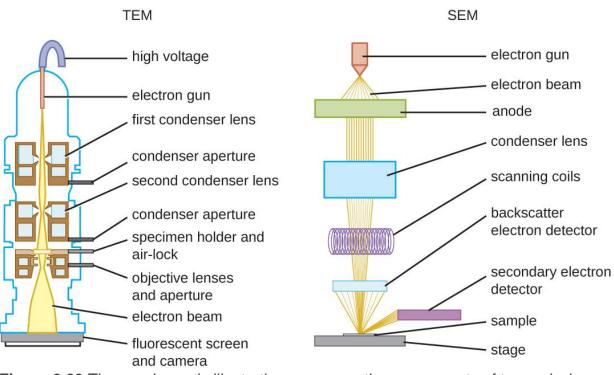


Figure 2.23 These schematic illustrations compare the components of transmission electron microscopes and scanning electron microscopes.



(a)



(b)

Figure 2.24 (a) This TEM image of cells in a biofilm shows well-defined internal structures of the cells because of varying levels of opacity in the specimen. (b) This color-enhanced SEM image of the bacterium *Staphylococcus aureus* illustrates the ability of scanning electron microscopy to render three-dimensional images of the surface structure of cells. (credit a: modification of work by American Society for Microbiology; credit b: modification of work by Centers for Disease Control and Prevention)

ELECTRON MICROSCOPES Magnification: 20–100,000× or more Use electron beams focused with magnets to produce an image.		
Microscope Type	Key Uses	Sample Images
Transmission (TEM)	Uses electron beams that pass through a specimen to visualize small images; useful to observe small, thin specimens such as tissue sections and subcellular structures. Example: <i>Ebola</i> virus	S.
Scanning (SEM)	Uses electron beams to visualize surfaces; useful to observe the three-dimensional surface details of specimens. Example: <i>Campylobactor jejuni</i>	