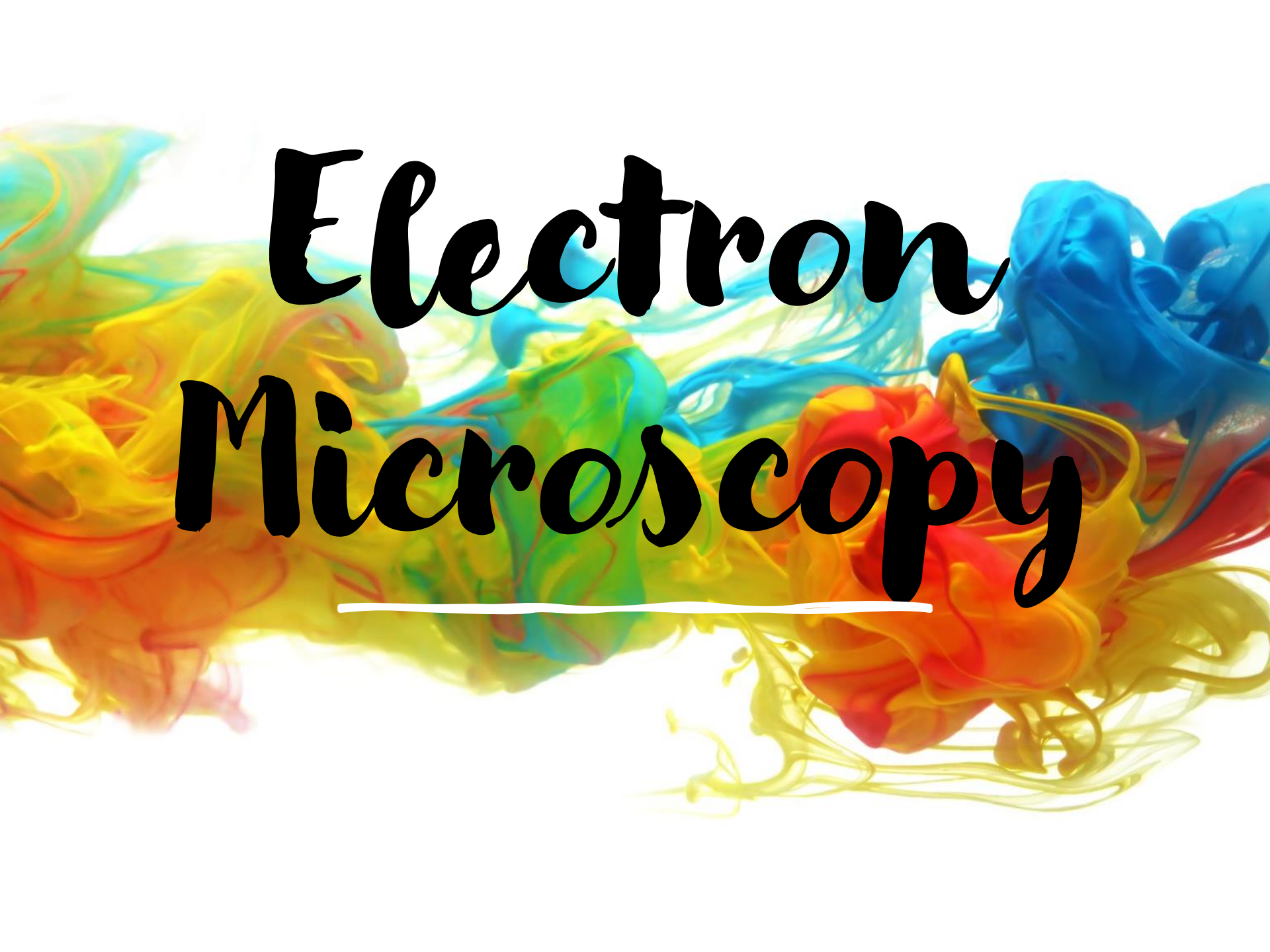

Electron
Microscopy

Two types of electron microscopy—transmission and scanning—are widely used to study cells. In principle, **transmission electron microscopy** is similar to the observation of stained cells with the bright-field light microscope. Specimens are fixed and stained with salts of heavy metals, which provide contrast by scattering electrons. A beam of electrons is then passed through the specimen and focused to form an image on a fluorescent screen. Electrons that encounter a heavy metal ion as they pass through the sample are deflected and do not contribute to the final image, so stained areas of the specimen appear dark.

Specimens to be examined by transmission electron microscopy can be prepared by either positive or negative staining. In positive staining, tissue specimens are cut into thin sections and stained with heavy metal salts (such as osmium tetroxide, uranyl acetate, and lead citrate) that react with lipids, proteins, and nucleic acids. Th
of cell structures, which consequently
1.33). Alternative positive-staining pr
specific macromolecules within cells.
electron-dense heavy metals (such as
determine the subcellular location of
scope. This method is similar to the u
cent dyes in fluorescence microscop
tures with resolutions of 2–10 nm car
of **electron tomography**, which gen
computer analysis of multiple two-
range of viewing directions.

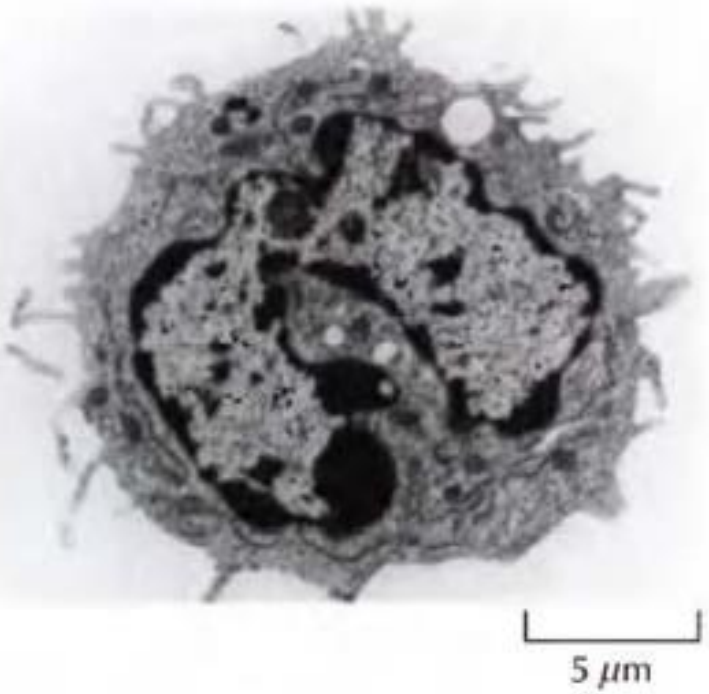


FIGURE 1.33 Positive staining
Transmission electron micrograph of a positively stained white blood cell. (Don W. Fawcett/Visuals Unlimited.)

Negative staining is useful for the visualization of intact biological structures, such as bacteria, isolated subcellular organelles, and macromolecules (Figure 1.34). In this method, the biological specimen is deposited on a supporting film, and a heavy metal stain is allowed to dry around its surface. The unstained specimen is then surrounded by a film of electron-dense stain, producing an image in which the specimen appears light against a stained dark background.

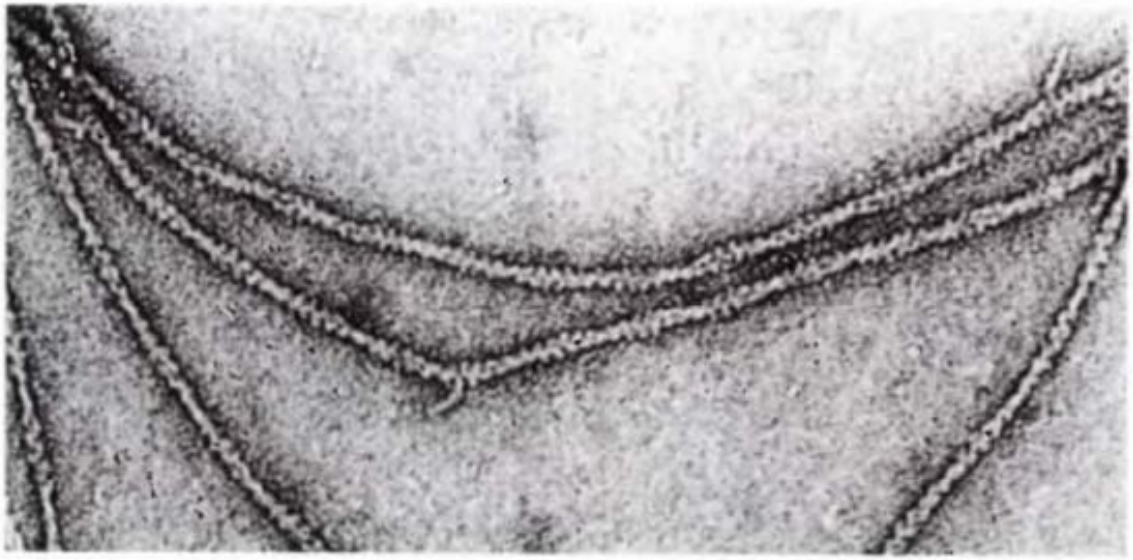


FIGURE 1.34 Negative staining Transmission electron micrograph of negatively stained actin filaments. (Courtesy of Roger Craig, University of Massachusetts Medical Center.)

stained dark background.

Metal shadowing is another technique used to visualize the surface of isolated subcellular structures or macromolecules in the transmission electron microscope (Figure 1.35). The specimen is coated with a thin layer of evaporated metal, such as platinum. The metal is sprayed onto the specimen from an angle so that surfaces of the specimen that face the source of evaporated metal molecules are coated more heavily than others. This differential coating creates a shadow effect, giving the specimen a three-dimensional appearance in electron micrographs.

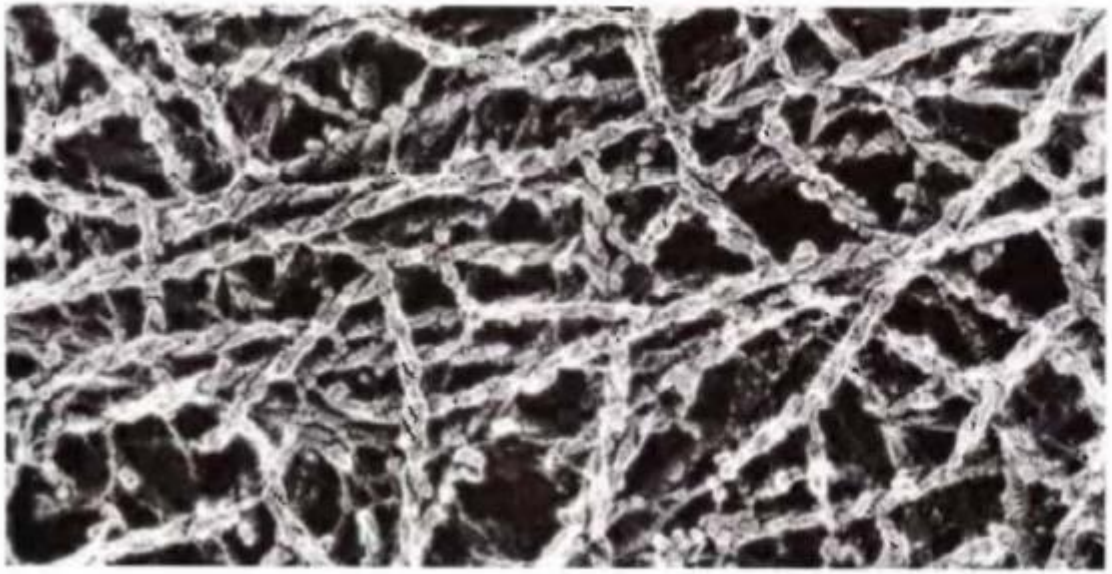
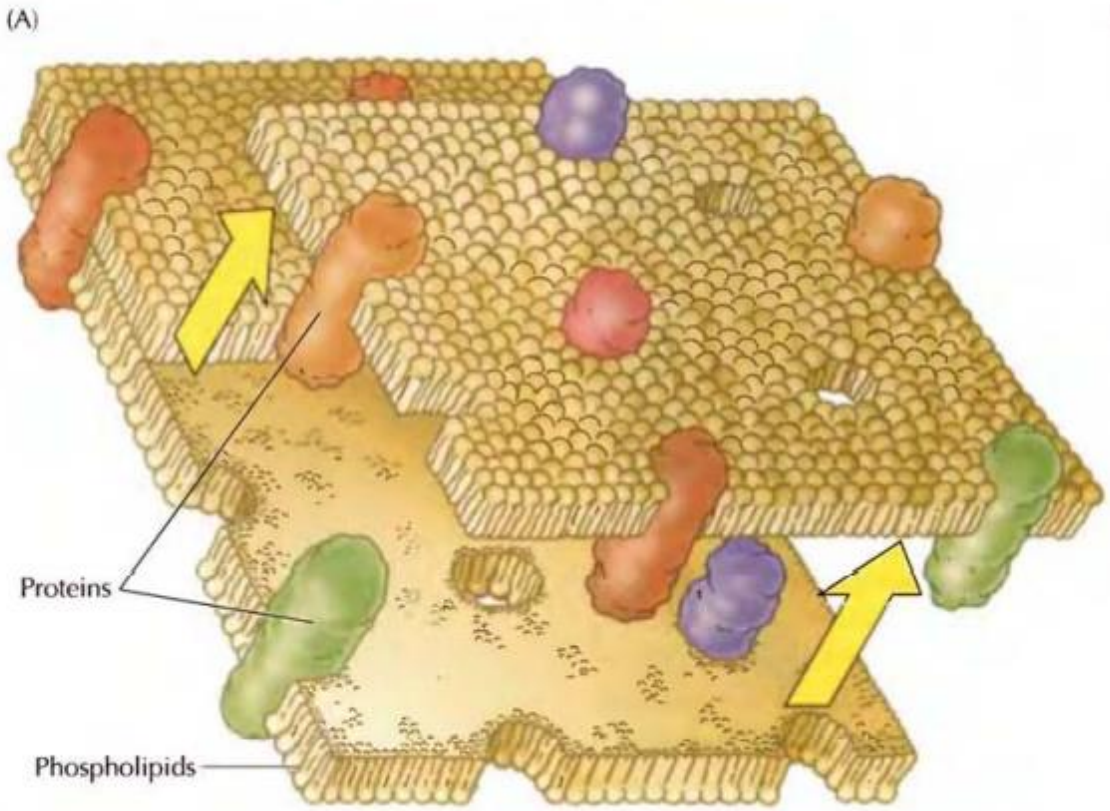


FIGURE 1.35 Metal shadowing
Electron micrograph of actin/myosin filaments of the cytoskeleton prepared by metal shadowing. (Don W. Fawcett, J. Heuser/Photo Researchers, Inc.)

The preparation of samples by **freeze fracture**, in combination with metal shadowing, has been particularly important in studies of membrane structure. Specimens are frozen in liquid nitrogen (at −196°C) and then fractured with a knife blade. This process frequently splits the lipid bilayer, revealing the interior faces of a cell membrane (Figure 1.36). The specimen is then shadowed with platinum, and the biological material is dissolved with acid, producing a metal replica of the surface of the sample. Examination of such replicas in the electron microscope reveals many surface bumps, corresponding to proteins that span the lipid bilayer. A variation of freeze fracture called freeze etching allows visualization of the external surfaces of cell membranes in addition to their interior faces.



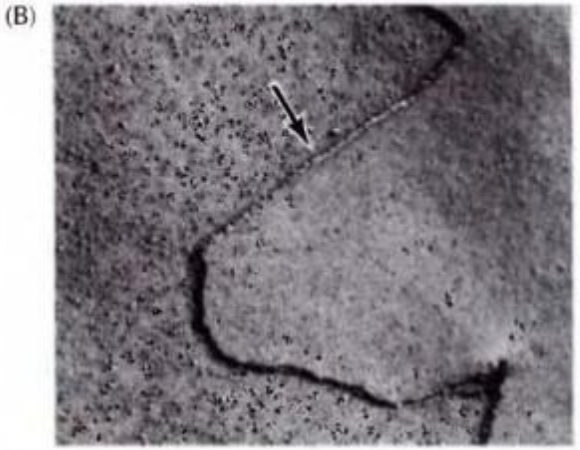


FIGURE 1.36 Freeze fracture
(A) Freeze fracture splits the lipid bilayer, leaving proteins embedded in the membrane associated with one of the two membrane halves. (B) Micrograph of freeze-fractured plasma membranes of two adjacent cells. Proteins that span the bilayer appear as intramembranous particles (arrow). (Don W. Fawcett/ Photo Researchers, Inc.)

The second type of electron microscopy, **scanning electron microscopy**, is used to provide a three-dimensional image of cells (Figure 1.37). In scanning electron microscopy the electron beam does not pass through the specimen. Instead, the surface of the cell is coated with a heavy metal, and a beam of electrons is used to scan across the specimen. Electrons that are scattered or emitted from the sample surface are collected to generate a three-dimensional image as the electron beam moves across the cell. Because the resolution of scanning electron microscopy is only about 10 nm, its use is generally restricted to studying whole cells rather than subcellular organelles or macromolecules.

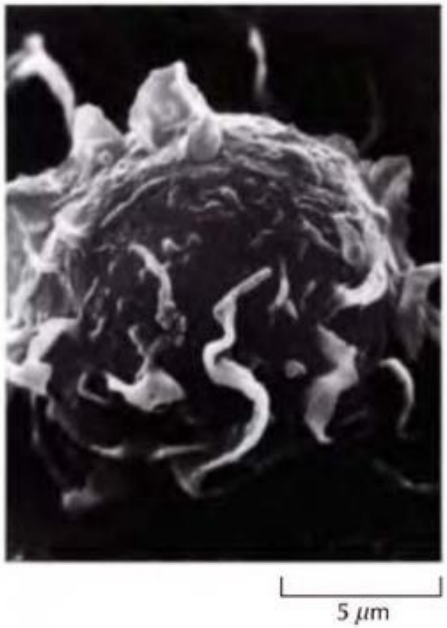


FIGURE 1.37 Scanning electron microscopy Scanning electron micrograph of a macrophage. (David Phillips/Visuals Unlimited.)