Electron Microscope

Transmission electron microscope (TEM)

- Electron beams behave like radiation and can be focused much as light is in a light microscope.
- If electrons illuminate the specimen, the microscope's resolution is normously increased because the wavelength of the radiation is around 0.005 nm, approximately 100,000 times shorter than that of visible light.
- The transmission electron microscope has a practical resolution roughly 1,000 times better than the light microscope; with many electron microscopes, points closer than 5 Å or 0.5 nm can be distinguished, and the useful magnification is well over 100,000.
- The value of the electron microscope is evident on comparison of the photographs.
- Microbial morphology can now be studied in great detail.

- A modern **transmission electron microscope (TEM)** is complex and sophisticated, but the basic principles behind its operation can be understood readily.
- A heated tungsten filament in the electron gun generates a beam of electrons that is then focused on the specimen by the condenser (**figure 2.23**).
- Since electrons cannot pass through a glass lens, doughnut-shaped electromagnets called magnetic lenses are used to focus the beam.
- The column containing the lenses and specimen must be under high vacuum to obtain a clear image because electrons are deflected by collisions with air molecules.
- The specimen scatters electrons passing through it, and the beam is focused by magnetic lenses to form an enlarged, visible image of the specimen on a fluorescent screen.
- A denser region in the specimen scatters more electrons and therefore appears darker in the image since fewer electrons strike that area of the screen.
- In contrast, electron-transparent regions are brighter.
- The screen can also be moved aside and the image captured on photographic film as a permanent record.

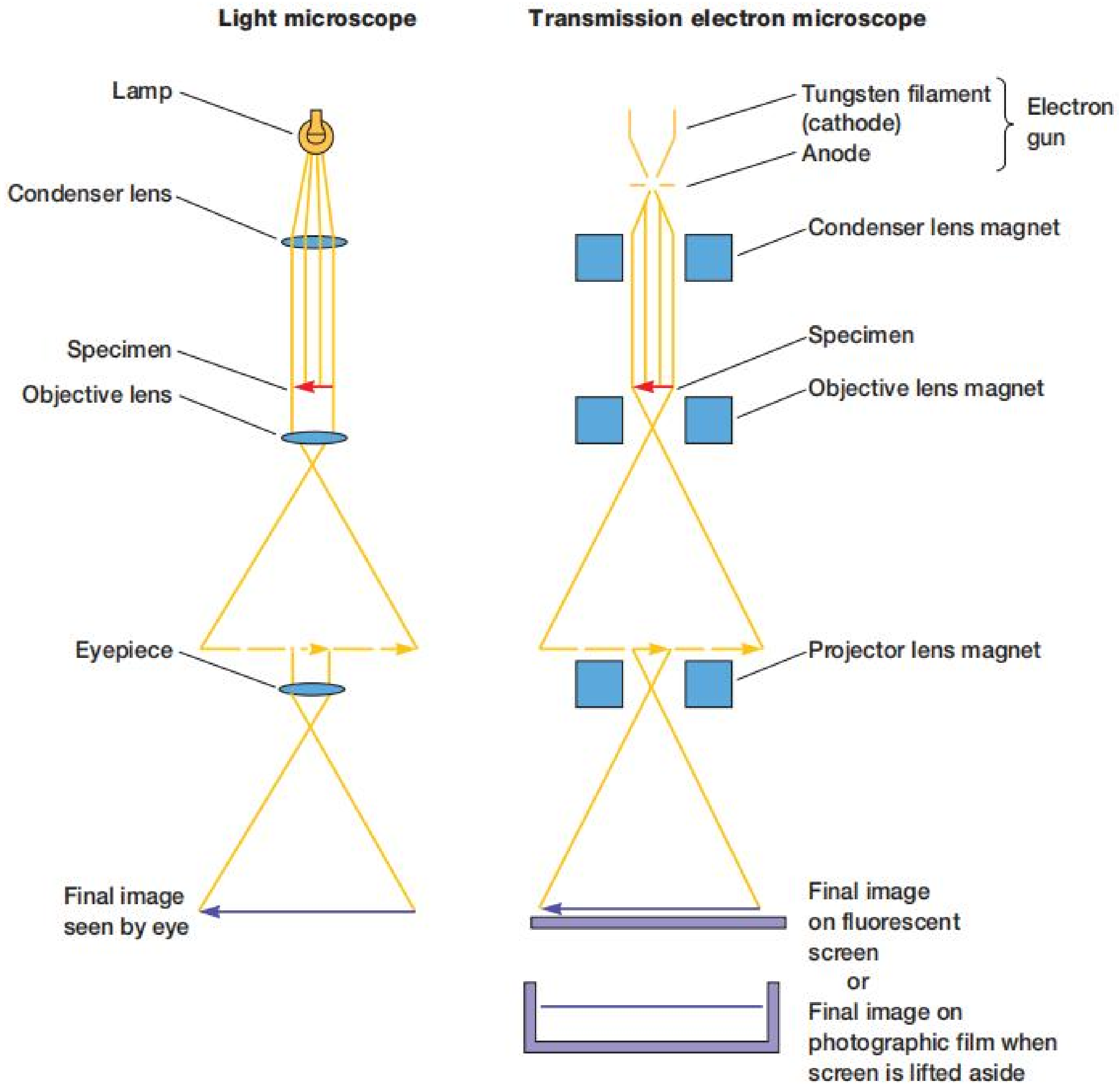

Figure 2.23 Transmission Electron Microscope Operation. An overview of TEM operation and a comparison of the operation of light and transmission electron microscopes.

The Scanning Electron Microscope

- **Scanning electron microscope (SEM)** has been used to examine the surfaces of microorganisms in great detail; many instruments have a resolution of 7 nm or less.
- The SEM differs from other electron microscopes in producing an image from electrons emitted by an object's surface rather than from transmitted electrons.
- Specimen preparation is easy, and in some cases air-dried material can be examined directly.
- Most often, however, microorganisms must first be fixed, dehydrated, and dried to preserve surface structure and prevent collapse of the cells when they are exposed to the SEM's high vacuum.
- Before viewing, dried samples are mounted and coated with a thin layer of metal to prevent the buildup of an electrical charge on the surface and to give a better image.

- The SEM scans a narrow, tapered electron beam back and forth over the specimen.
- When the beam strikes a particular area, surface atoms discharge a tiny shower of electrons called secondary electrons, and these are trapped by a special detector.
- Secondary electrons entering the detector strike a scintillator causing it to emit light flashes that a photomultiplier converts to an electrical current and amplifies.
- The signal is sent to a cathode-ray tube and produces an image like a television picture, which can be viewed or photographed.
- The number of secondary electrons reaching the detector depends on the nature of the specimen's surface.
- When the electron beam strikes a raised area, a large number of secondary electrons enter the detector; in contrast, fewer electrons escape a depression in the surface and reach the detector.
- Thus raised areas appear lighter on the screen and depressions are darker.
- A realistic three-dimensional image of the microorganism's surface with great depth of focus.
- The actual in situ location of microorganisms in ecological niches such as the human skin and the lining of the gut also can be examined.

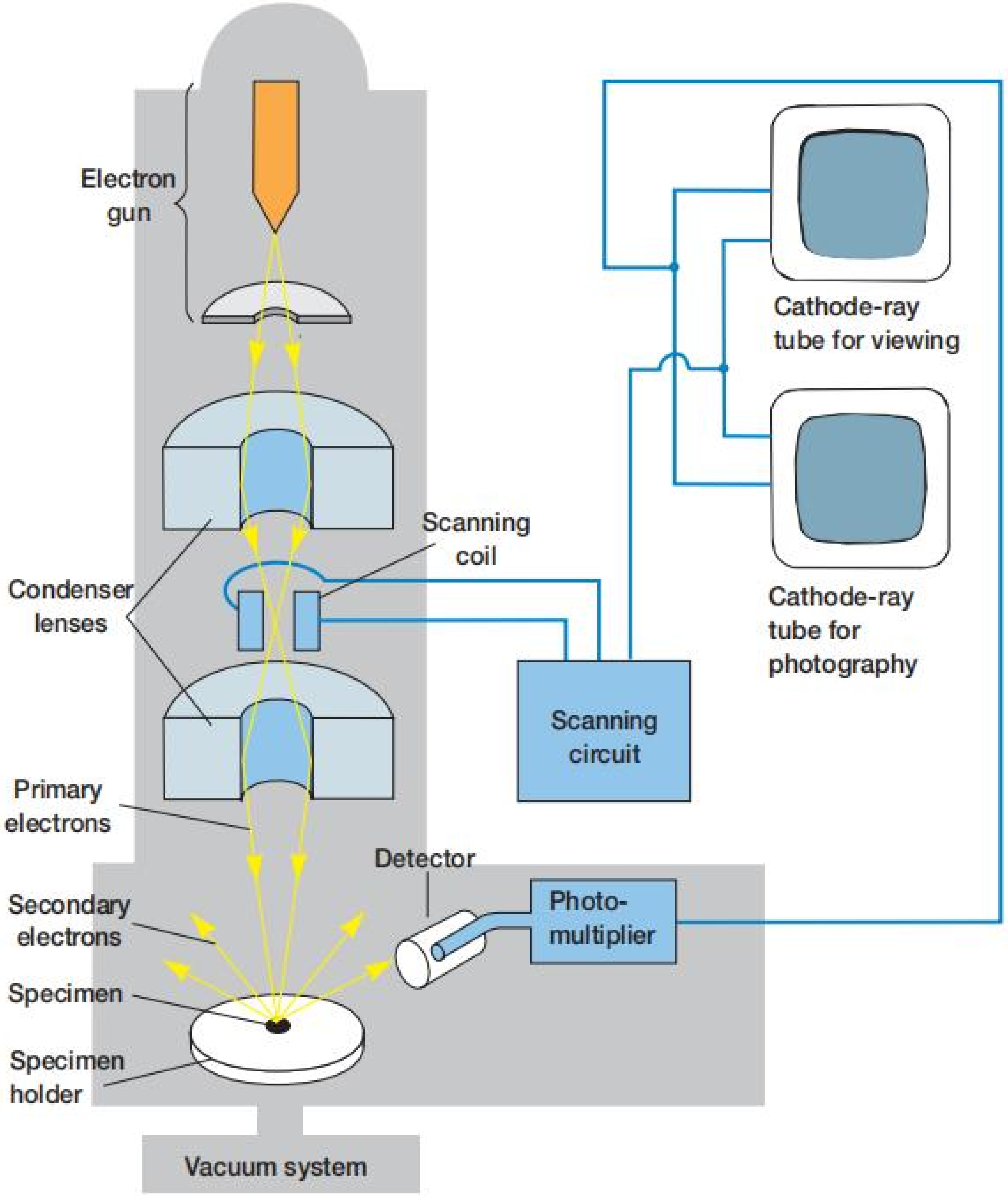

Figure 2.27 **The Scanning Electron Microscope.**