MODULE 4: Introduction to Light Microscopy

LEARNING OUTCOMES

1. Define basic terms and principles of brightfield microscopy.
2. Describe appropriate units of measurement for microorganisms.

INTRODUCTION

The pioneers of microscopy opened a window into the invisible world of microorganisms. Early microscopes, which used visible light to illuminate cells, continued to advance in the centuries that followed. The 20th century saw the development of microscopes that leveraged nonvisible light, such as fluorescence microscopy (which uses an ultraviolet light source) and electron microscopy (which uses short-wavelength electron beams). These advances led to major improvements in magnification, resolution, and contrast.

Brightfield Microscopy

The brightfield microscope is one of the most common types of light microscopes used in microbiology laboratories. It is a compound microscope, meaning that more than one type of lens is used to magnify an image. Visible light is the source of illumination and specimens are observed against a bright field or background. Some brightfield microscopes are equipped with special attachments that change the field to appear darker than the specimens being viewed. This is known as darkfield microscopy and is often helpful when viewing live microorganisms, such as protists, that might otherwise be killed if stained (Figure 4.1).

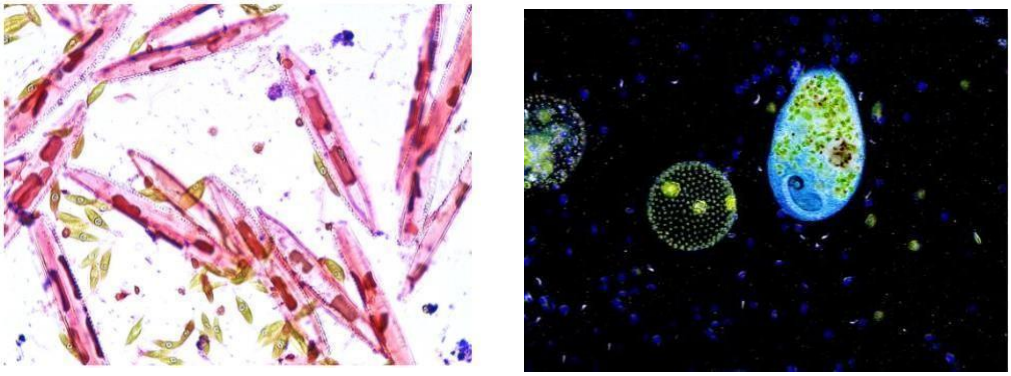

Figure 4.1: Gram stain of freshwater diatoms, euglenoids, and bacteria (left) and darkfield image of live protists (right).

The size of microbes can be hard to imagine because they are so small in comparison to what most people see day to day. Even when compared to plant or animal cells, microbes tend to be much smaller. The unit micrometer (µm), also known as a micron, is used when describing the size of bacterial cells. A micrometer is 1/1000 of a millimeter and 1/1,000,000 of a meter. To put it more tangibly, a typical cell of *Staphylococcus* bacteria measures one micrometer, or about 1/400 the size of the period at the end of this sentence.

Viruses, which are too small to be viewed with a light microscope and instead must be observed using a much more powerful electron microscope, are measured in nanometers (nm). One nanometer is 1/1000 of a micrometer. Most viruses range in size from 10 to 100 nanometers. See Figure 4.2 for a comparison of relative cell sizes.

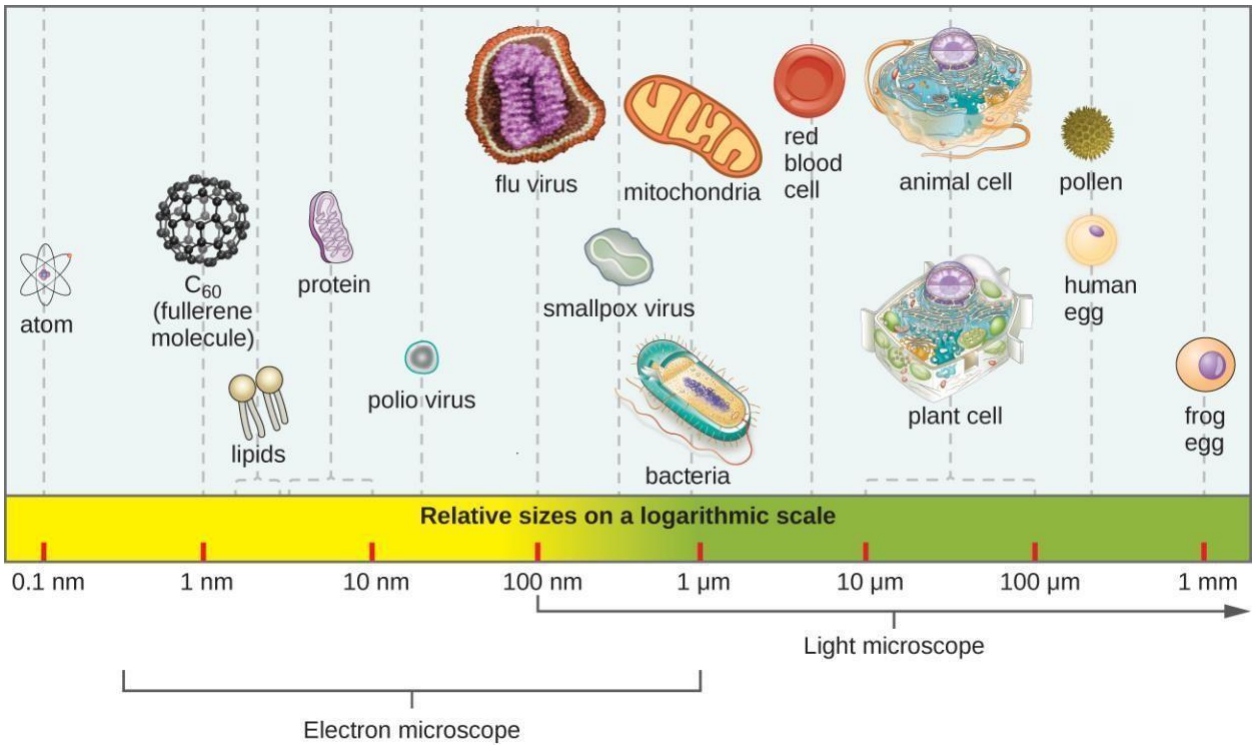

Figure 4.2: Relative sizes of various cellular and non-cellular structures. Bacteria and larger microorganisms such as protists and fungi are visible with a light microscope, while a more powerful electron microscope is required to observe most viruses.

In this module, you will use the light microscope to view bacterial smears that were previously prepared. The maximum magnification of the microscope, 1000X, will be used to observe all cells.