

## Cells and Microscopy

All living creatures are made of *cells*, the basic unit of an organism consisting of an aqueous solution of organic molecules surrounded by a membrane. These cells have the ability to copy themselves by a process of growth and division. The simplest life forms are made of single cells, whereas organisms are made up of many cells functioning together. The cell is the smallest unit that can be considered living. For example, viruses have some of the components of cells, but cannot reproduce without taking over control of a cell's reproductive machinery.

### Discovery of Cells

How did it become known that cells existed? In the 1600s the first microscopes were made, which led to the discovery of cells. In 1665 Robert Hooke examined a piece of cork and found it was composed of minute chambers that he called "cells." Actually, what he saw in the microscope was only the cell walls that remained after the living cells inside had died. In the 1800s Schleiden and Schwann published reports that cells were the universal building blocks of all living tissue. Through this and other works it became known that all living cells are formed by the division of the existing cells. This was later called the *cell theory*. It was the first indication that cells did not arise spontaneously. It took Louis Pasteur's classic experiments approximately 20 years later to prove this idea.

### Use of Microscopes

For hundreds of years the microscope has been used to learn more about cells. A light microscope requires three things in order to see a cell. First, it needs a light source that is focused on the specimen. Second, one needs a specimen which light can pass through. Third, a set of lenses, ocular and objective, focus the image of the cell in the eye. There are three types of light microscopes, bright-field, phase-contrast and differential-interference-contrast (DIC or Nomarski). All three can be obtained in the same light microscope, but one must interchange optical components in order to exploit differences in refractive index as light travels through different parts of a specimen. Other types of microscopy exist as well. For instance, fluorescence microscopy uses fluorescent dyes to stain parts of cells. The fluorescence is observed when light is passed through 2 sets of filters. The first excites the fluorescent dye and the other only allows the wavelengths emitted when the dye fluoresces. In addition, there are electron microscopes that use electrons and magnets instead of light and lenses to visualize a specimen. There are two types of electron microscopes: transmission and scanning. For transmission electron microscopy (TEM) the specimen must be very thin to allow the electrons to pass through it. Contrast is caused by staining the cell with heavy metals that absorb or scatter electrons. The magnification of a TEM microscope is about 2 nm. A scanning electron microscope (SEM) captures scattered electrons from the surface of a specimen coated with heavy metals and shows them on a video screen. This microscope creates beautiful 3D images of objects with a resolution between 3 and 20 nm.

A typical cell is about 5–20 micrometers ( $\mu\text{m}$ ) in diameter. If a cell is kept under the right conditions one can see in a light microscope particles moving within the cell and you can sometimes see a cell go through division. Visualizing the internal structures within a cell is difficult because cells are transparent and mostly colorless (except for colored pigments in various cells). To visualize these structures one can stain them with dyes that color particular components, or one can use optical tricks to enhance the slight differences in the refractive index of the components. Also, there is a particular form to cells. They have sharply defined boundaries that indicate that membrane exists in this position. There are two such boundaries, one for the cell membrane, which separates one cell from another, and the nuclear membrane, which separates the nucleus from the cytoplasm. Some other components can be visualized with a good light microscope, but anything smaller than 0.2  $\mu\text{m}$  can't be seen. Therefore, one must use the electron microscope, which has higher magnification.

A light microscope is essentially composed of a tube provided with two lenses (the objective and ocular) that amplify the image of the object. Magnification = ocular magnification multiplied by objective magnification, e.g. 10x ocular lens X 40x objective lens = 400X magnification. The function of a microscope is limited by the resolving ability because it is the capacity to distinguish between two distinct points. This property is called the limit of resolution and is defined as the minimum distance between two points that can be distinguished as separate points. This limit depends on the wavelength of light and the numerical aperture, which includes the refractive index of the material the light is passing through such as air or immersion oil.

To be seen very well, many cells require a series of processes to prepare them for the microscope, for example, fixation, embedding and cutting or staining. The type of process used depends on the type of microscope one uses. After these techniques are completed one can see the organelles and structures.

