EXPERIMENT 10 FAMILIARIZATION WITH MICROSCOPE

Structure

10.0 OBJECTIVES

After going through this experiment you will be able to:

- identify different parts of microscope;
- examine the morphology of microbes; and
- observe the motility of microbes under the microscope .

10.1 INTRODUCTION

The morphological study of bacteria requires the use of microscopes. Microscopy has come a long way since Leeuwenhoek first observed bacteria three hundred years ago using hand ground lenses. Historians credit the invention of the compound microscope to the Dutch spectacle maker, Zacharias Janssen, around the year 1590.

You have already performed the experiment of plating and incubating the microbes in a media and in the next experiment you will be guided to perform the staining of slides prepared from the culture. As the stained slides are ultimately seen under microscope, you should have the knowledge about the different parts of microscope and their utility.

10.2 MICROSCOPE

10.2.1 Parts and Specifications of Microscope

The compound microscope uses lenses and light to enlarge the image and is called an optical or light microscope (vs./ an electron microscope). The simplest optical microscope is the magnifying glass and is good to about ten times (10X) magnification. The **compound** microscope has two systems of lenses for greater magnification, 1) the ocular or eyepiece lens that one looks into and 2) the objective lens, or the lens closest to the object. Before purchasing or using a microscope, it is important to know the functions of each part.

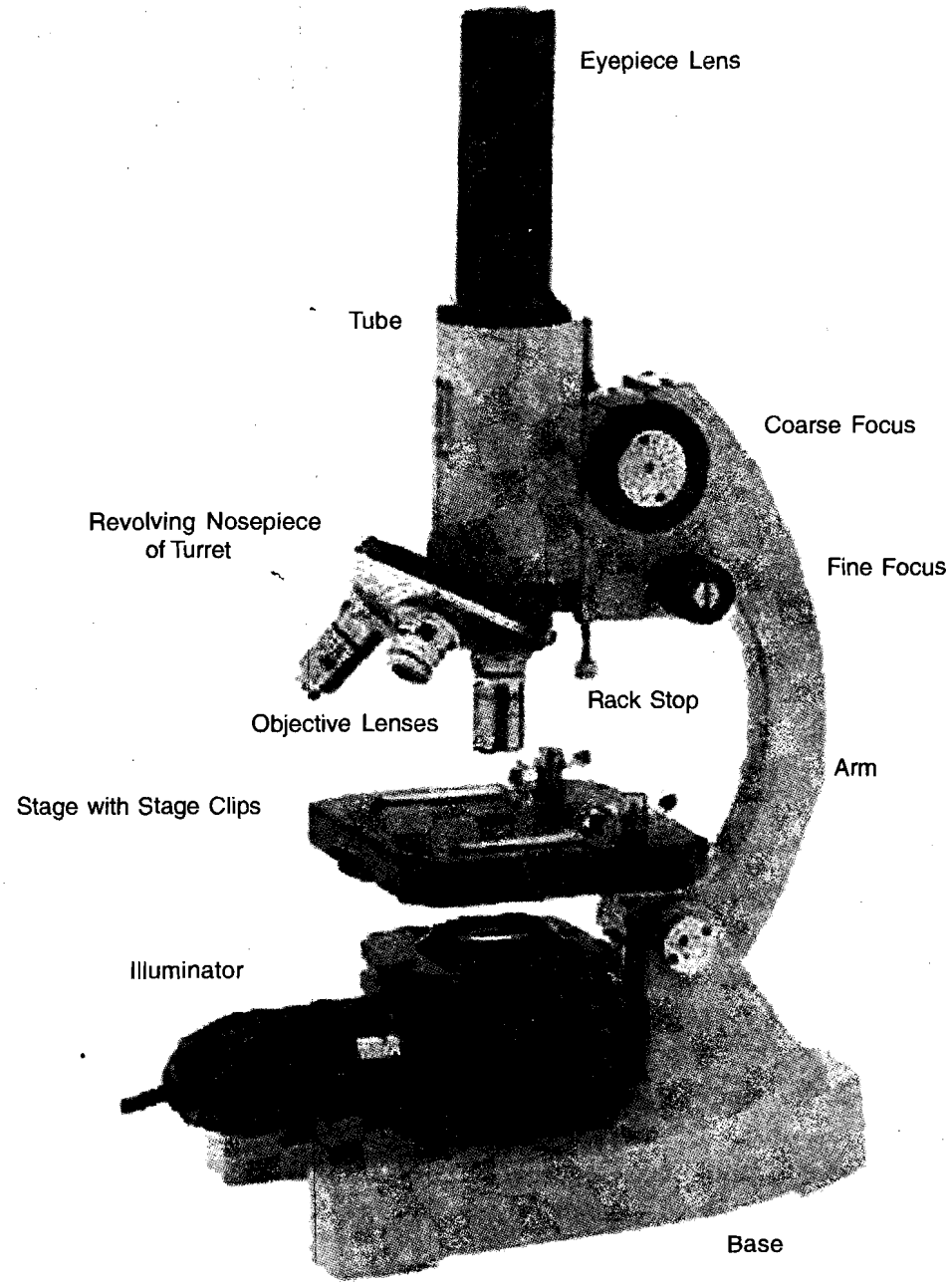

Fig. 10.1 Compound Microscope

Eyepiece Lens: The lens at the top that you look through. They are usually 10X or 15X power.

Tube: Connects the eyepiece to the objective lenses.

Arm: Supports the tube and connects it to the base.

Base: The bottom of the microscope, used for support.

Illuminator: A steady light source (110 volts) used in place of a mirror. If your microscope has a mirror, it is used to reflect light from an external light source up through the bottom of the stage.

Stage: The flat platform where you place your slides. Stage clips hold the slides in place. If your microscope has a mechanical stage, you will be able to move the slide around by turning two knobs. One moves it left and right, the other moves it up and down.

Revolving Nose piece or Turret: This is the part that holds two or more objective lenses and can be rotated to easily change power.

Objective Lenses: Usually you will find 3 or 4 objective lenses on a microscope. They almost always consist of 4X, 10X, 40X and 100X powers. When coupled with a 10X (most common) eyepiece lens, we get total magnifications of 40X (4X times 10X), 100X, 400X and 1000X. To have good resolution at 1000X, you will need a relatively sophisticated microscope with an Abbe condenser. The shortest lens is the lowest power; the longest one is the lens with the greatest power. Lenses are colour-coded and if built to DIN standards are interchangeable between microscopes. The high power objective lenses are retractable (i.e. 40XR). This means that if they hit a slide, the end of the lens will push in (spring loaded) thereby protecting the lens and the slide. All quality microscopes have achromatic, parcentered, parfocal lenses.

Rack Stop: This is an adjustment that determines how close the objective lens can get to the slide. It is set at the factory and keeps students from cranking the high power objective lens down into the slide and breaking things. You would only need to adjust this if you were using very thin slides and you weren't able to focus on the specimen at high power. (Tip: If you are using thin slides and can't focus, rather than adjust the rack stop, place a clear glass slide under the original slide to raise it a bit higher)

Condenser Lens: The purpose of the condenser lens is to focus the light onto the specimen. Condenser lenses are most useful at the highest powers (400X and above). Microscopes with in stage condenser lenses render a sharper image than those with no lens (at 400X). The Abbe condenser lens can be moved up and down. It is set very close to the slide at 1000X and moved further away at the lower powers.

Diaphragm or Iris: Many microscopes have a rotating disk under the stage. This diaphragm has different sized holes and is used to vary the intensity and size of the cone of light that is projected upward into the slide. The diaphragm setting depends on the transparency of the specimen, the degree of contrast you desire and the particular objective lens in use.

10.2.2 How to Focus a Microscope

The proper way to focus a microscope is to start with the lowest power objective lens first and while looking from the side, crank the lens down as close to the specimen as possible without touching it. Now, look through the eyepiece lens and focus upward only, until the image is sharp. If you can't get it in focus, repeat the process again. Once the image is sharp with the low power lens, you should be able to simply click in the next power lens and do minor adjustments with the focus knob. If your microscope has a fine focus adjustment, turning it a bit should be all that's necessary. Continue with subsequent objective lenses and fine focus each time.

10.2.3 Types of Microscope

The following types of microscope are being employed now.

i) Optical or Light Microscope

Bacteria may be examined under the compound microscope, either in the living state or after fixation and staining. Examination of wet films or 'hanging drops' indicates the shape, arrangement, motility and approximate size of the cells. But due to lack of contrast, details cannot be appreciated.

ii) Phase Contrast Microscope

Improves the contrast and makes evident the structures within cells that differ in thickness or refractive index. Also, the differences in refractive index between bacterial cells and

the surrounding medium make them clearly visible. Retardation, by a fraction of a wavelength, of the rays of light that pass through the object, compared to the rays passing through the surrounding medium, produces 'phase' differences between the two types of rays. In the phase contrast microscope, 'phase' differences are converted into differences in intensity of light, producing light and dark contrast in the image.

iii) Dark Field (Dark Ground) Microscope

Another method of improving the contrast is the dark field (dark ground) microscope in which reflected light is used instead of the transmitted light used in the ordinary microscope. The essential part of the dark field microscope is the dark field condenser with a central circular stop, which illuminates the object with a cone of light, without letting any ray of light fall directly on the objective lens. Light rays falling on the object are reflected or scattered on the objective lens, with the result that the object appears self-luminous against a dark background. The contrast gives an illusion of increased resolution, so that vary slender organisms such as spirochetes, not visible under ordinary illumination, can be clearly seen under the dark field microscope.

The resolving power of the light microscope is limited by the wavelength of light. In order to be seen and delineated (resolved), an object has to have a size of approximately half the wavelength of the light used. With visible light, using the best optical systems, the limit of resolution is about 300 nm. If light of shorter wavelength is employed, as in the ultraviolet microscope, the resolving power can be proportionately extended.