

EXERCISE

2

Other Microscopes

OBJECTIVES

At the conclusion of the exercise, you should...

1. understand the difference between brightfield, darkfield, phase, and fluorescent microscopy.
2. know the purpose and characteristics of electron microscopes (EM).
3. understand the difference between a compound microscope and a stereoscope.
4. practice using the stereoscope.
5. observe demonstrations of wet mount specimens with the darkfield microscope.
6. observe demonstrations of wet mount specimens with the phase contrast microscope.

INTRODUCTION

A variety of microscopes are available for different purposes in microbiology. The brightfield compound microscope is the most commonly used. In order to see a specimen clearly with a brightfield microscope, it has to have contrast. Contrast can be obtained by using stains. There are other types of microscopes that use more exotic means to generate contrast, such as darkfield and phase contrast. Unstained cells are more easily observed with these types of microscopes.

The stereo-type microscopes are commonly used as inspection instruments (stamps, coins, circuit boards) or as dissection microscopes. Their magnifying power is less than a standard compound microscope. The fluorescent microscope uses an ultraviolet source of light that causes the specially treated specimen to emit a fluorescent light. The fluorescent light observed is dependent on the type of fluorescent stain that is used.

In this exercise, you will observe examples of living microorganisms with darkfield and phase contrast microscopes. Later, the stereoscope will be used for observing the macroscopic structures of some fungi.

MATERIALS

Cultures:

Various living microscopic organisms
Petri dish with bacteria

Supplies:

Prepared slides of various organisms
Darkfield microscope with wet mount of protozoa or algae
Phase contrast microscope with wet mount of protozoa or algae

Technical Background

Darkfield microscopy is used to examine living microorganisms that are mostly invisible with a brightfield microscope. It uses a special condenser and reflected light. The reflected light causes the specimen to appear bright against a black background. It is most useful for viewing motile organisms that are difficult to stain.

Phase Contrast microscopy is also used to examine living microorganisms. It can help to distinguish the internal structures of some living specimens. It also uses a special condenser that allows both direct and reflected or diffracted light rays to come together to produce the contrasted image of the specimen.

Fluorescent microscopy is principally used to detect microbes in tissues or clinical specimens. It uses an ultraviolet light source that causes the microbes to give off a fluorescent light when stained with fluorescent stains

Stereoscope (dissecting) microscopy is used in microbiology to observe larger specimens, such as fungi. It can also be helpful to observe the colony morphology of bacterial cultures. It uses two eyepieces (binocular) and two objective lenses to create a three-dimensional image. These instruments come in fixed, variable, and zoom designs, usually with a maximum power of 100x. The most common ones are between 20x-40x, with a large working area between the objective lens and the stage area.

Electron Microscopy (EM) uses a beam of electrons instead of light. It is used to examine objects smaller than 0.2 μm (micrometer). **TEM** (*transmission electron microscope*) uses electrons to pass through a thin section of material. **SEM** (*scanning electron microscope*) provides three-dimensional views of specimens without sectioning them.

PROCEDURES

Working with Microscopes

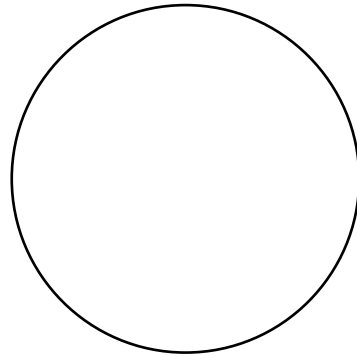
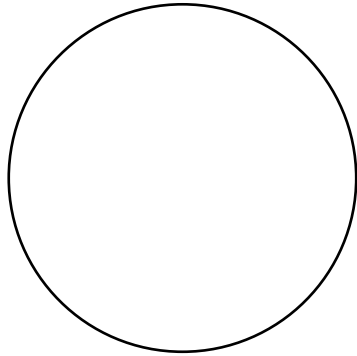
1. Use the darkfield microscope to observe the wet mounts prepared by the instructor.
2. Use the phase contrast microscope to observe the wet mounts prepared by the instructor.
3. Practice using the stereoscope at the lab bench by placing different objects on it.
4. Use the stereoscope to observe the plates with bacterial colonies.

EVALUATION OF RESULTS (EXERCISE 2: OTHER MICROSCOPES)

Purpose

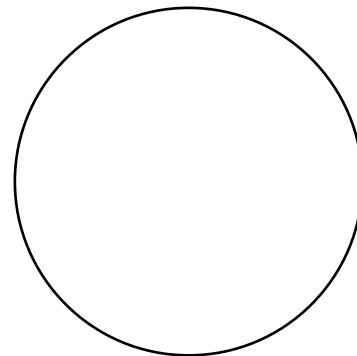
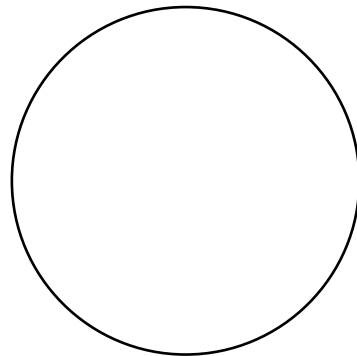
Observations and/or Data

Darkfield observations:



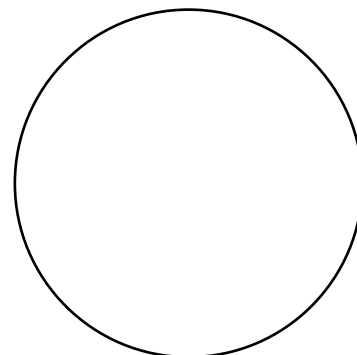
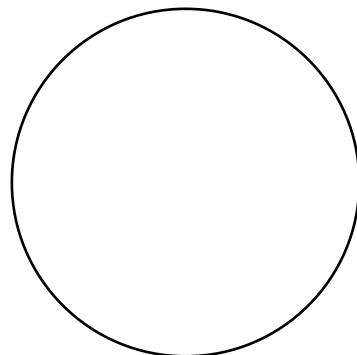
Specimen: _____ Total Mag. _____ Specimen: _____ Total Mag. _____

Phase Contrast observations:



Specimen: _____ Total Mag. _____ Specimen: _____ Total Mag. _____

Stereoscope observations:



Specimen: _____ Total Mag. _____ Specimen: _____ Total Mag. _____

CONCLUSIONS, DISCUSSIONS, AND QUESTIONS

1. What is the principle of, and principal use for the darkfield microscope?
2. What is the principle of and principal use for the phase contrast microscope?
3. Explain how the appearance of the various living microorganisms differed with the dark-field, phase contrast, and brightfield microscopes.
4. Explain how the stereoscope microscope is useful in microbiology.

