

EXERCISE

1

The Brightfield Microscope

OBJECTIVES

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

1. know the parts of the compound microscope and their purpose.
2. know how to safely transport the microscope.
3. be able to properly clean the microscope.
4. know how to store the microscope safely.
5. observe various specimens on microscope slides using the low, high, and oil immersion objectives.

INTRODUCTION

The microscope is one of the most important tools that anyone studying microbiology must learn to use. During this lab exercise, you will learn the proper use of the brightfield microscope and you will practice using the microscope with some prepared slides and, later, with living specimens.

MATERIALS

Materials for all exercises are listed per group (as determined by the instructor).

Supplies:

Prepared slides of various microorganisms
brightfield microscope. (See Figure 1.)

Technical Background

Parts of the Microscope: (Refer to Figure 1 illustrating a brightfield microscope.)

1. Ocular eyepieces: magnifies the object 10-15x
2. Diopter ring: for adjusting the focus to the user's eyes
3. Rotating nosepiece: for rotating the objectives
4. Objectives: 5x, 10x (low), 40x (high dry), and 100x (oil) lenses that magnify the specimen
5. Stage: platform that holds the microscope slide and slide holder
6. Condenser: lens that condenses the light before it passes through the specimen
7. Condenser aperture diaphragm lever (iris diaphragm): controls the amount of light that passes through the condenser lens

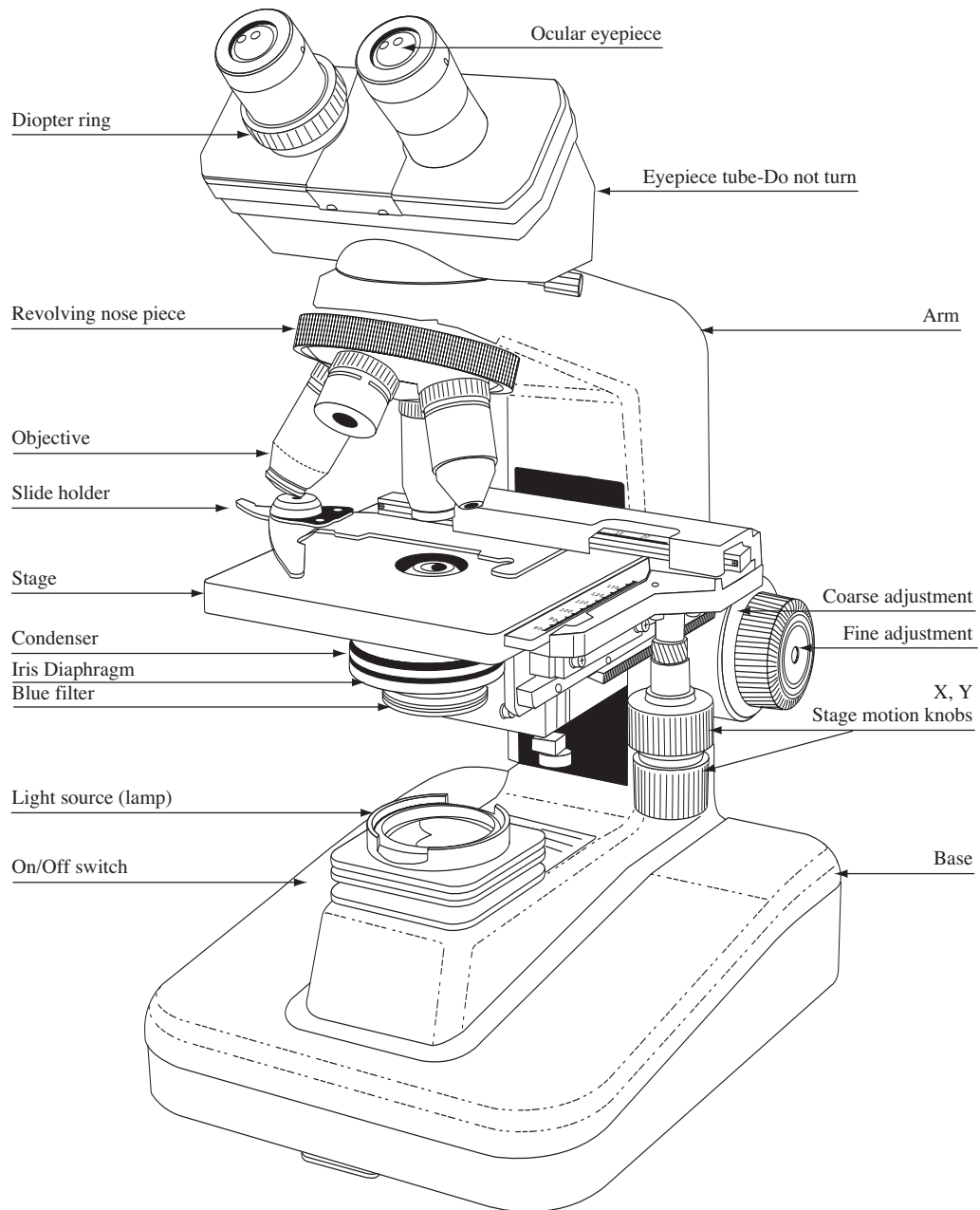


Figure 1. Brightfield Microscope.

8. Daylight filter: blue filter that provides a short wavelength for maximum resolution (omitted from some models of microscope)
9. Base
10. Light source: for power on and off, and controlling lamp brightness
11. X and Y stage travel controls: for moving the slide on the stage
12. Fine- and coarse-focusing knobs: for focusing the specimen
13. Arm: for carrying the microscope

Microscopic Terminology

Compound Microscope: uses two (or more) lenses between the eye and the object.

Brightfield Microscope: the object being observed is dark against a bright field.

Total Magnification: is calculated by multiplying the magnifying power of the objective times that of the eyepiece. The ocular eyepiece is usually 10x and the objectives are 5x, 10x, 40x, and 100x. For example, when using the 40x objective and a 10x ocular, the total magnification would be: $40 \times 10 = 400$. Total magnification = objective magnification \times eyepiece magnification.

Resolving Power: the ability of the lens to distinguish fine detail. Technically this refers to its ability to distinguish between two points a specified distance apart. The more minute the distance, the higher the resolving power of the optical system.

Numerical Aperture: used to determine the efficiency of objective lens to capture light. The larger the numerical aperture, the brighter and better resolved the image.

Refractive Index: a measure of the light bending characteristic of a medium. As light passes through the air between the objective lens and the slide, it is bent because the refractive indices of these two media are different.

Working Distance: the distance between the specimen and the tip of the objective lens. In general, the higher the magnification, the shorter the working distance.

Field of View: the area or diameter of the specimen that is in view. The higher the power of magnification, the smaller the field of view.

Depth of Field: the thickness of the object that is simultaneously in focus. The higher the power of magnification, the smaller the depth of focus.

Low Power Objective: 5x or 10x. The objective used to locate the object on the slide, using the coarse adjustment knob.

High-Dry Objective: 40x. The objective used for higher magnification without oil.

Oil Immersion Objective: 100x. The objective used for the highest magnification with oil.

PROCEDURES

Day 1: Brightfield Microscopy Using Low and High-Dry Objectives

Viewing a Specimen

1. Transport the assigned microscope to the lab bench, using both hands.
2. Plug in the microscope and turn on the power. Make sure the rheostat is turned all the way up to allow maximum light.
3. Clean the ocular lenses and objectives with lens paper before use.
4. Place a prepared slide with the coverslip up on the stage within the spring-loaded lever.
5. Turn the rotating nosepiece until the 10x is above the ring of light coming through the slide.
6. Move the slide using the X and Y stage travel knobs until the specimen is within the field of view.
7. Bring the condenser up to the bottom of the slide and then slightly back for maximum light.
8. Adjust the ocular distance for your eyes by sliding the eyepiece plates in or out.
9. Adjust the focus by looking first into the right eyepiece and focusing the image with the coarse and then fine focus knobs.
10. Look into the left eyepiece with the left eye, and focus the image by adjusting the diopter ring. The best focus is usually when the two white dots are aligned on the left ocular.
11. Rotate the revolving nosepiece to the high-dry objective.
12. Adjust the aperture diaphragm until there is sufficient light passing through the specimen. This will take practice. For a bright field, open the diaphragm completely. For a darker field, close the diaphragm slowly while observing the specimen. Generally, the higher the magnification, the more light will be needed to view the specimen.
13. Practice observing at least six prepared slides with the 10x and the 40x objectives.
14. When finished, remove the slide.
15. Clean the microscope oculars and objectives with lens paper. Remove any oil.
16. Rotate the 5x or 10x objective into place over the stage.
17. Lower the stage.
18. Turn off the power.
19. Unplug and wrap the power cord around the base under the stage.

PROCEDURES

Day 2: Brightfield Microscopy Using Oil Immersion objective

Technical Background

The most important objective used in microbiology is the oil immersion lens (100x). The oil is placed between the objective and the slide, and is used to prevent the loss of light due to the bending of light rays (refraction) as they pass through air. This enhances the **resolving power** of the microscope.

1. Follow the procedure on Day 1, Steps 1-13.
2. After focusing with the high-dry objective, turn the 40x objective away enough to place a drop of oil on the slide. **DO NOT LOWER THE STAGE**
3. Rotate the oil immersion objective (100x) into the oil, pass through, and return. This is to be sure there are no air bubbles between the objective and the oil.
4. Use only the fine focus to bring the object into focus.
5. Practice viewing the prepared slides at 10x, 40x, and then with oil.

6. DO NOT let oil get on any of the other objectives.
7. Always rotate the oil objective away before removing the slide.
8. When placing another slide on the stage, carefully start with the 10x or the 40x before going to oil.
9. Never use the coarse focus with the oil objective in place. The slide could break and the objective could get damaged.
10. Observe at least 6 slides with and without oil.
11. Clean the oil off the oil objective with lens paper. Always clean all the objectives with clean lens paper.
12. Replace the microscope the proper way.

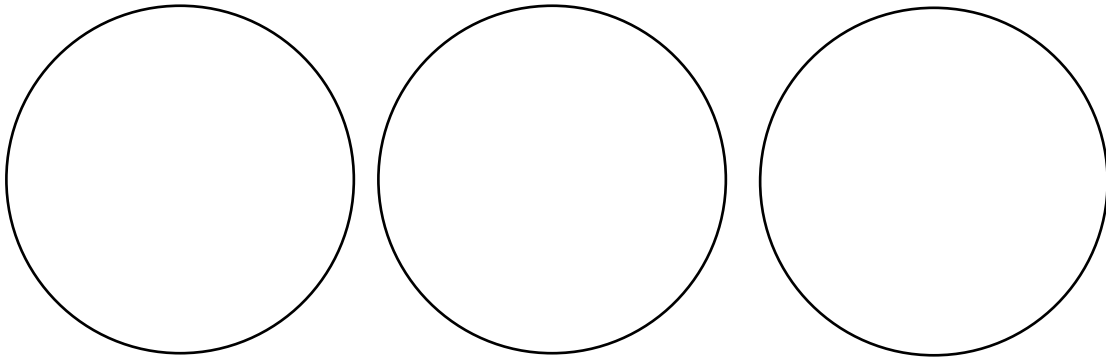
EVALUATION OF RESULTS

(EXERCISE 1: THE BRIGHTFIELD MICROSCOPE)

Purpose _____

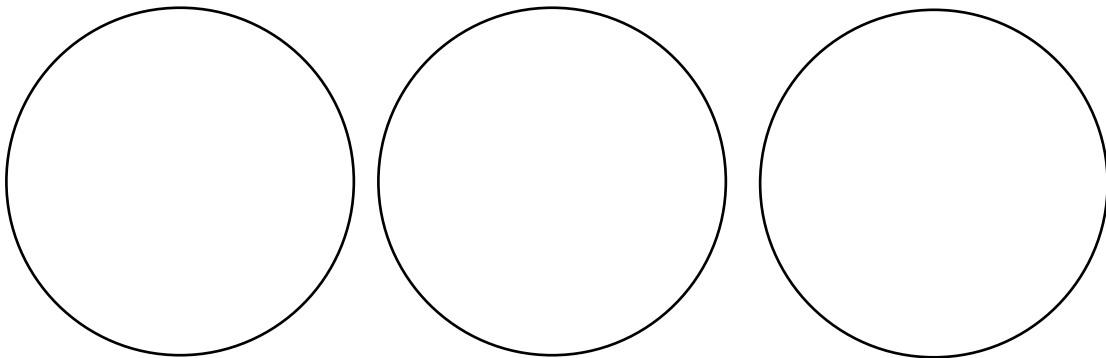
Observations and/or Data

Draw representative fields of the microscopic observations. Include the total magnification (Total Mag):



Specimen: _____

Total Mag: _____



Specimen: _____

Total Mag: _____

CONCLUSIONS, DISCUSSIONS, AND QUESTIONS

1. Why is oil necessary with the 100x objective?
2. If you are using an ocular that is 15x power and an objective of 40x, what is the magnification of the organism that you are viewing under the microscope?
3. List the steps necessary for storing the microscope.

