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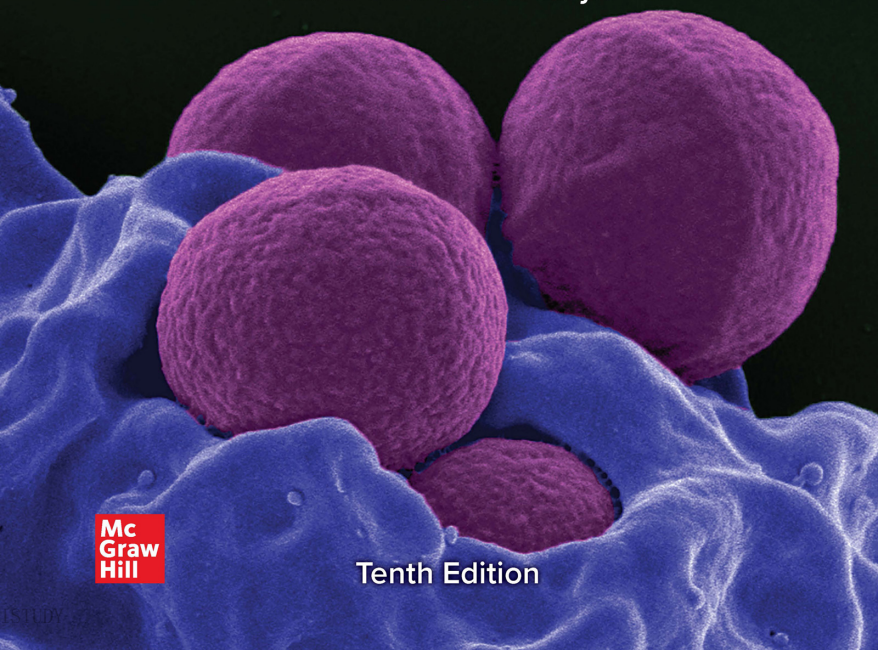
Microbiology Experiments

A HEALTH SCIENCE PERSPECTIVE

Anna Oller | John Kleyn



Mc Graw Hill

Tenth Edition

TENTH EDITION

Microbiology Experiments

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University of Central Missouri

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MICROBIOLOGY EXPERIMENTS

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PREFACE

To the Student

A microbiology laboratory is valuable because it actually gives you a chance to see and study microorganisms firsthand. In addition, it provides you with the opportunity to learn techniques used to study and identify these organisms. The ability to make observations, record data, and analyze results is useful throughout life.

It is very important to read the scheduled exercises before coming to class so that class time can be used efficiently. It is helpful to ask yourself the purpose of each step as you are reading and carrying out the steps of the experiment. Sometimes it will be necessary to read an exercise several times before it starts to make sense.

Conducting experiments in microbiology laboratories is particularly gratifying because the results can be seen in a day or two (as opposed, for instance, to plant genetics laboratories, which can take months). Opening the incubator door to see how your cultures grew and the results of the experiment is a pleasurable moment. We hope you enjoy your experience with microorganisms as well as acquire laboratory and critical thinking skills that will be valuable in the future.

To the Instructor

The manual includes a wide range of exercises—some more difficult and time-consuming than others. Usually more than one exercise can be done in a 2-hours laboratory period. In these classes, students can actually see the applications of the principles they have learned in the lectures and text. We have tried to integrate the manual with the text *Nester's Microbiology: A Human Perspective,* Tenth Edition, by Denise Anderson, et al.

The exercises were chosen to give students an opportunity to learn new techniques and to expose them to a variety of experiences and observations. We did not assume that the school or department has a large budget; thus, exercises were written to use as little expensive media and equipment as possible. The manual contains more exercises than can be done in one course so that instructors will have an opportunity to select the appropriate exercises for their particular students and class.

- An online Instructor's Manual is available from the publisher.
  - It lists equipment, cultures, media, and reagents needed for each exercise and has extensive information for storing cultures and making media.
- The Notes to the Instructor section gives suggestions for preparing and presenting the laboratory sessions.
- Additional questions that can be used to supplement those in the student manual are included.
- **We highly recommend that the instructors utilize the Instructor's Manual.** Contact your local McGraw Hill representative for the URL and password for this site.
- Revisions in the Tenth Edition include the following:
  - New exercise components:
    - Ex. 28 Bacteriophage Susceptibility was added to the titers.
    - Ex. 31 added Quorum Sensing to Epidemiology.
  - Changes to exercises:
    - Numerous Figures and Tables were added and updated throughout.
    - Numerous Lab Report tables were updated to guide students in recording lab results.
    - The Microscopy and Staining labs were moved to exercises 1–6, whereas Diversity and Aseptic Techniques were moved to exercises 7 and 8.
    - Additional tables were added to exercise 7 explaining media types.

- A more explicit explanation of dilution math was added to exercise 8.
- Exercises 16, 17, 18, and 19 were updated to minimize culturing pathogens from students.
- Demonstrations of representative microbes are included.
- Many graphics were updated to include color to differentiate and more clearly explain concepts.
- Measurement bars were added to most microscopy photos.
- Bacteriophage specificity was added to Prokaryotic Viruses in exercise 28.
- *Enterococcus* and MUG protocols were added to exercise 29 for those using it as the indicator organism.
- Using appropriate software to determine primers to the sequences was added to exercise 34.
- The culture media and reagents listing was updated to reflect the current exercises.
- An additional 11 color plates and many new figures were added to enhance student learning.
- Student objectives that meet suggested American Society for Microbiology (ASM) curriculum guidelines are designated by superscripted numbers.
- If a student has a special interest in microbiology and would like to do independent work, three projects are available online:
  - Methylotrophs
  - The UV-Resistant *Deinococcus*
  - Hydrocarbon-Degrading Bacteria

We hope these changes are helpful and that the manual contributes to the students' understanding of microbiology. We also hope both students and instructors enjoy their experience with a very interesting group of organisms.

Acknowledgments

We would like to thank Denise Anderson, Sarah Salm, and Deborah Allen for their text *Nester's Microbiology: A Human Perspective.* This text was the source of much of the basic conceptual material and figures for our laboratory manual.

We would also like to express our appreciation to Lauren Vondra, Erin DeHeck, Jeni McAtee, Beth Cray, David Hash, and all the others involved in publishing this manual.

LAB SAFETY RULES ASM 37

1. Place backpacks, coats, etc., in designated areas. Do not place backpacks or coats on bench tops.
2. At the beginning and end of each lab period, wipe the bench tops with disinfectant and allow to air dry/proper contact time. Make sure the entire surface comes into contact with the solution.
3. Upon entering and leaving the lab, wash your hands thoroughly—30–45 seconds minimum (ABCs, etc.).
4. Proper attire is crucial for your safety.
   a. Safety goggles and a lab apron or coat MUST BE worn when you are in the laboratory. Tie back long hair. Your instructor may require you to wear gloves. Remove them before leaving lab and store your apron/coat and goggles in the lab until the end of the course.
   b. Hats, sandals, and open-toed shoes are NOT allowed. If you drop a slide or test tube, you may be injured.
5. Report any accidents to the instructor(s) immediately.
6. If you have an open wound, inform the instructor and ask for a band-aid to prevent infection.
7. If you are tardy, you miss imperative safety information. Thus, you may not be allowed to enter the lab.
8. Do not smoke, eat, drink, or CHEW GUM. Do not apply cosmetics (chapstick), or insert contact lenses.
9. Do not chew on pens/pencils. Leave a pen/pencil in your lab drawer to avoid taking contamination home.
10. ALWAYS flame loops and needles BEFORE and AFTER obtaining ORGANISMS.
11. Do not place CONTAMINATED loops, needles, pipettes, etc. on bench tops or in lab drawers.
12. DO NOT LICK labels needing moistened. Use a water dropper/bottle to moisten. Do not pipette by mouth.
13. Do not work on top of the lab manual. If a spill occurs, it cannot be easily disinfected.
14. Loose papers are NOT permitted on the bench top. Secure in binders, use clips, etc., to prevent fires.
15. Use test tube racks or designated containers to carry and store cultures.
16. Hold the entire tube when handling cultures. Kimcaps can (and often do) drop and break on the bench top or floor when held by only the cap. Mix cultures gently to avoid creating aerosols.
17. Do not take lab items (plates, slides, etc.) outside of the lab, unless instructed to do so by your instructor.
18. Place contaminated slides, used staining reagents, broken glassware, etc., in the proper container(s).
19. You may only use items from your assigned area, regardless of how many students are in the class.
20. At the end of the lab period, place cultures, tubes, plates, etc., in designated areas. Push stools in fully.
21. Fill up water dropper bottles using distilled water. Restock labels, lens paper, etc., before leaving.
22. IF A CULTURE IS SPILLED/DROPPED:
   a. **Notify the instructor or lab technician immediately.**
   b. If you are NOT contaminated, move any lab books, pencils, sharpies, etc. out of the way.
   c. If you ARE contaminated, ask someone else to help move books, etc.
   d. Disinfect the bench area and any contaminated tubes or racks with disinfectant.
   e. Wash your hands immediately if they come into contact with any microbes or chemicals. Most chemicals need approximately 15 minutes of thorough washing to be completely removed.

23. When lighting Bunsen burners, open the gas jet and use a flint to light the burner. Do not leave the gas on for PROLONGED PERIODS OF TIME before lighting the flame. Turn off the burners (gas) when you are not using them. Make sure that paper, alcohol, the gas hose, and your microscope are not close to the flame.
24. You will be given an unknown culture. It is your responsibility to transfer it and place it in proper containers to ensure its survival. If you do not transfer your unknowns, your results may be questionable.
25. Make sure electronic devices (if allowed by your instructor) are enclosed in disposable/ disinfected plastic covers.
26. When the laboratory is in session, keep the doors and windows shut. Post a sign on the door indicating that a microbiology laboratory is in session.
27. If you are immunocompromised (including pregnancy), consult a physician before taking this class.
28. You are expected to stay the entire lab time. Ask the instructor if you are truly done with the day's lab.
29. Comply with all federal and state guidelines regarding microbial disposal and disinfection, and do not use microbes in any manner that could be threatening to others.
30. Improper behavior WILL NOT be tolerated, and you will be asked to leave. Improper behavior may include attitude, verbiage, clothing/dressing, and actions, which are safety violations.

I understand the above safety rules and regulations and agree to abide by them. I have also read the course syllabus and understand its contents. I also know where the following lab safety equipment is located and how to properly use it:

Safety Devices: Sink(s): Eyewash(es):
Fire Extinguisher(s): Fire Blanket(s):
MSDS Sheets:

_____ _____
Signature Date

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INTRODUCTION to Microbiology Stains & Culturing

When you take a microbiology class, you have an opportunity to explore an extremely small biological world that exists unseen in our own ordinary world. Fortunately, we were born after the microscope was perfected, so we can observe these extremely small organisms. We now also understand how the cell transfers the information in its genes to the operation of its cellular mechanisms so that we can study not only what bacteria do but also how they do it.

A few of these many and varied organisms are pathogens (capable of causing disease). Special techniques have been developed to isolate and identify them as well as to control or prevent their growth. The exercises in this manual will emphasize medical applications. The goal is to teach you basic techniques and concepts that will be useful to you now or can be used as a foundation for additional courses. In addition, these exercises are also designed to help you understand basic biological concepts that are the foundation for applications in all life science fields.

As you study microbiology, it is also important to appreciate the essential contributions of microorganisms as well as their ability to cause disease. Most organisms play indispensable roles in breaking down dead plant and animal material into basic substances that can be used by other growing plants and animals. Photosynthetic bacteria are an important source of the earth's supply of oxygen. Microorganisms also make major contributions in the fields of antibiotic production, food and beverage production, food preservation, and DNA technologies such as CRISPR. The principles and techniques demonstrated here can be applied to these fields as well as to medical laboratory science, nursing, or patient care. This course is an introduction to the microbial world, and we hope you will find it useful and interesting.

In the next few exercises, you will be introduced to several basic procedures: the use of the microscope and aseptic and pure culture techniques. These are skills that you will use not only throughout the course but in any microbiology laboratory work you do in the future. The exercises that meet the recommended ASM guidelines for laboratory skills have been designated in each lab. These are needed skills for future employment.

Note: The use of pathogenic organisms has been avoided whenever possible, and non-pathogens have been used to illustrate the kinds of tests and procedures that are actually carried out in clinical laboratories. In some cases, however, it is difficult to find a substitute, and organisms of low pathogenicity are used. These exercises will have an additional safety precaution.

Staining

Bacteria are difficult to observe in a broth or wet mount because there is very little contrast between them and the liquid in which they are suspended. This problem is solved by staining bacteria with dyes. Although staining kills bacteria so their motility cannot be observed, the stained organisms contrast with the surrounding background and are easier to see. The determination of the shape, size, and arrangement of the cells after dividing is useful in identifying an organism. These can be demonstrated best by preparing a smear on a glass slide from a broth culture, or a colony from a plate, then staining the smear with a suitable dye.

Examining a stained preparation is one of the first steps in identifying an organism. Staining procedures can be classified into two types: the simple stain and the multiple (or differential) stain. In the **simple stain**, a single stain such as methylene blue or crystal violet is used to dye the bacteria. The most commonly used simple stains are basic dyes, which are positively charged and make it possible to stain negatively charged bacteria.

The shape and arrangement of organisms can be determined, but all organisms (for the most part) are stained the same color. Another kind of simple stain is the negative stain. In this procedure, the organisms are

Table I.1.1 A Summary of Stains and Their Characteristics

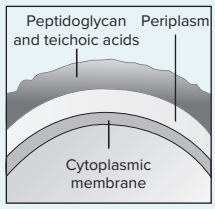
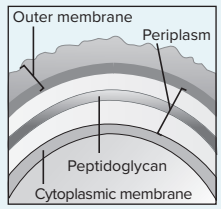
Stain	Characteristic
Simple Stains	A basic dye is used to stain cells. Easy way to increase the contrast between otherwise colorless cells and a colorless background. Allows visualization of shape and size. Examples include crystal violet, safranin, and methylene blue.
Differential Stains	A multistep procedure is used to stain cells and distinguish one group of microorganisms from another.
Gram stain	Used to separate bacteria into two major groups: Gram-positive and Gram-negative. The staining characteristics of these groups reflect a fundamental difference in the chemical structure of their cell walls. This is by far the most widely used staining procedure.
Acid-fast stain	Used to detect organisms that do not easily take up stains, due to mycolic acid on the cell wall, particularly members of the genus *Mycobacterium*.
Special Stains	A staining procedure used to detect specific cell structures.
Capsule stain	The common method darkens the background and a counterstain stains the bacterial cell, so the capsule stands out as a clear area surrounding the cell. Capsules are produced by *Klebsiella* and *Enterobacter* species.
Endospore stain	Stains endospores, which do not readily take up stains due to the presence of dipicolic acid and calcium. Members of the genera *Bacillus* and *Clostridium* are among the few species that produce endospores.
Flagella stain	The staining agent adheres to and coats the otherwise thin flagella, making them visible with the light microscope.
Fluorescent Dyes and Tags	Some fluorescent dyes bind to compounds found in all cells; others bind to compounds specific to only certain types of cells. Antibodies to which a fluorescent molecule has been attached are used to tag specific molecules. Flagella are seen on many bacteria, including *Escherichia coli* and *Spirillum volutans*.

mixed with a dye on a slide and the mixture is permitted to air dry. When the stained slide is viewed under the microscope, the organisms are clear against a dark background. The types of stains are shown in **table I.1.1**.

The multiple stain involves more than one dye. The most widely used example is the differential Gram stain. After staining, some organisms appear purple and others pink, depending on the structure of their cell wall, which are depicted in **table I.1.2**.

Special stains are used to observe specific structures of bacteria. Compared with eukaryotic organisms, prokaryotic organisms have relatively few morphological differences. Several of these structures, such as endospores, capsules, acid-fast cell walls, storage granules, and flagella, can be seen with differential stains. You will have an opportunity to stain bacteria with a variety of staining procedures and observe these structures over the next few labs.

Table I.1.2 Comparison of Features of Gram-Positive and Gram-Negative Bacteria

	 **Gram-Positive**	 **Gram-Negative**
Color of Gram-Stained Cell	Purple	Pink
Representative Genera	*Bacillus, Staphylococcus, Streptococcus*	*Escherichia, Neisseria, Pseudomonas*
Distinguishing Structures/Components		
Peptidoglycan	Thick layer	Thin layer
Teichoic acids	Present	Absent
Outer membrane	Absent	Present
Lipopolysaccharide (endotoxin)	Absent	Present
Porin proteins	Absent (unnecessary because there is no outer membrane)	Present; allow molecules to pass through outer membrane
General Characteristics		
Sensitivity to penicillin	Generally more susceptible (with notable exceptions)	Generally less susceptible (with notable exceptions)
Sensitivity to lysozyme	Yes	No

EXERCISE 1 Introduction to the Compound Light Microscope

Definitions

Compound microscope. A microscope with two lenses, often called a bright-field compound microscope.

Condenser. A structure located below the microscope stage that contains a lens for focusing the light rays on the specimen as well as an iris diaphragm.

Iris diaphragm. An adjustable opening that regulates the amount of light illuminating the specimen.

Magnification. The microscope's ability to optically increase the specimen size.

Objective lens. The rotating lenses that magnify the items from the slide, such as a 10× or 40×.

Ocular lens. The eyepiece looked through to view microscope specimens; usually has a 10× magnification.

Parfocal. Lenses that are on one plane to allow for fine adjustment between objective lenses of different magnifications.

Resolution. The smallest separation that two structural forms—two adjacent cilia, for example—must have in order to be distinguished optically as separate.

Viable. Alive.

Wet mount. A laboratory technique in which a microscopic specimen in liquid is added to the surface of a slide and covered with a coverslip.

Objectives

1. Describe the parts of the microscope and why they are important.[ASM 1]
2. Describe stained and unstained materials.
3. Explain the use and proper care of the microscope.[ASM 1]

Pre-lab Questions

1. Why should you carry a microscope in the upright position?
2. What is the only material that can be used to wipe an objective lens?
3. Why must you wipe off the ocular lens before storing the microscope?

Getting Started

Microbiology is the study of organisms too small to be seen with the naked eye, including a vast array of bacteria, viruses, protozoa, fungi, and algae. Van Leeuwenhoek, a Dutch merchant in the late seventeenth century whose hobby was lens making, was the first to see these previously unknown creatures. His microscope consisted of one simple lens, but it was enough to observe some of these tiny living things **(figure 1.1)**. Although he made drawings of some of these organisms, he did not suspect that they were essential for the existence of our world, or that a small percentage was responsible for contagious diseases. It was only after the improvement of the compound microscope almost 200 years later that it was possible to understand the role of microorganisms in disease. After the

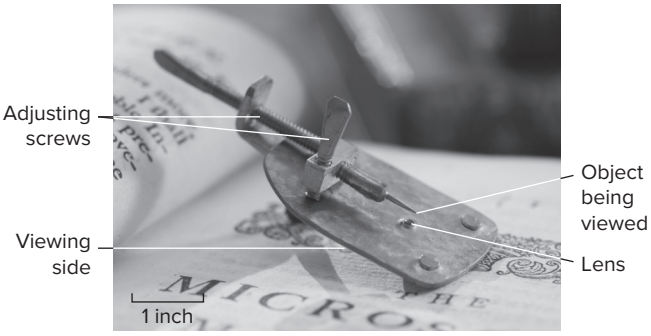


Figure 1.1 Model of a van Leeuwenhoek microscope. The original was made in 1673 and could magnify the object being viewed almost 300×. The object being viewed is brought into focus with the adjusting screws. This replica was made according to the directions given in the *American Biology Teacher* 30:537, 1958. Note its small size. © Tetra Images/Alamy Stock Photo.

microscope was perfected in the 1870s and 1880s, real progress was made in determining the actual cause of disease (see Appendix 6 for a history of the development of the microscope). In 1877, Robert Koch saw bacteria in the blood from an animal with anthrax. In combination with his postulates, Koch could see that bacteria caused the disease anthrax, and the disease was not caused by swamp gas or evil spirits.

The modern **compound light microscope** consists of two lens systems. The first is the **objective** lens, which is closest to the material on the slide, and the second is the eyepiece, or **ocular lens**, which magnifies the image formed by the objective lens. The total magnification is found by multiplying the ocular lens magnification by the objective lens magnification. For example, if the ocular lens magnification is 10×, and the objective lens magnification is 45×, the total magnification is 450 diameters.

Although it is possible to put additional magnifying glasses on top of the ocular lenses of the microscope, they would not improve the ability to see more detail. The reason is that the actual limiting factor of the light microscope is the **resolution**. This is the ability to distinguish two close objects as distinct from one another rather than as one round, hazy object. The resolution of a lens is limited by two factors: the angle of the lens and the wavelength of light entering the microscope. When using an objective lens of **magnification** 100× and light is optimal, a compound light microscope has a maximum magnification of about 1,000×.

A magnification of 1,000× is sufficient to easily visualize single-celled organisms such as algae and fungi. Bacteria, however, still appear very small (about the size of the letter *l* on a printed page), and their appendages, such as flagella, cannot be observed without special stains. Viruses are also usually too small to be seen in a light microscope.

To surmount the limitations of light and lens, the electron microscope, which uses electrons instead of light, was developed in the 1930s. It magnifies objects 100,000×, permitting the visualization of viruses and structures within cells. Because the electron microscope is a very large piece of equipment requiring specialized techniques, it is usually found only in universities or research facilities. More recently, many other microscopes have been developed but are usually for specific research applications.

In this exercise, you will have an opportunity to become familiar with a compound light microscope and learn how to use and care for it. You will prepare **wet mounts** of unstained organisms and learn to examine previously stained and unstained organisms. The microscope is an expensive and complex piece of equipment. Treat it with great care.

The Parts of the Microscope[ASM 1]

The eyepieces are the lenses at the top of the microscope. They usually have a magnification of 10× (see **figure 1.2**).

Objective lenses. Most microscopes have at least three objective lenses, and some have a fourth—4×. Each lens is color coded with a band around the objective. They include:

Low power (yellow band)	10×
High power (blue band)	40×
Oil immersion (white or red band)	100×

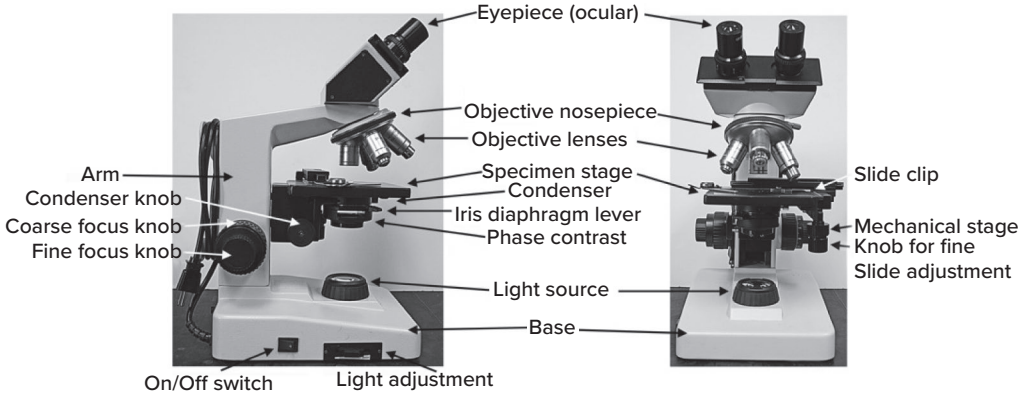


Figure 1.2 Modern bright-field compound microscope. Courtesy of Anna Oller, University of Central Missouri.

These lenses can be rotated and the desired lens clicked into place. The high power is often called high-dry, because it is considered the highest power without using oil (hence, dry).

Stage. The stage is below the objective lens. A slide clip keeps the microscope slide in place. It usually has a device called a *mechanical stage* for holding the slide, as well as knobs that permit the slide to be moved smoothly while viewing.

Condenser. Below the stage is the **condenser**, which focuses the light on the slide. If it is lowered, the amount of light is reduced, but the resolution is also lowered. For our purposes, the condenser should remain at its highest position under the stage.

Diaphragm. A lever on top of the condenser but under the stage controls the **iris diaphragm**. The diaphragm is important for adjusting the amount of light illuminating the slide. The higher the magnification, the greater the amount of light that is needed.

Coarse and Fine Focus Knob. The large course focus knob controls the large distance between the slide and the objective. It is used to bring a specimen into view on 10× or 40×. The fine focus brings a specimen into clear, or fine, focus using smaller increments than the coarse focus. The fine focus is important to see microbes viewed at 100×.

Light Source. The light source is at the base of most microscopes. Usually, the light source is set at maximum, and the amount of light on the slide is adjusted with the iris diaphragm. There is often a blue (or other color) filter held below the diaphragm to help correct the color that you see.

Precautions for Proper Use and Care of the Microscope[ASM 1]

1. Carry the microscope with both hands by the arm and base. Keep it upright. If the microscope is inverted, the eyepieces (oculars) may fall out.
2. Do not remove the objective or ocular lenses for any purpose.
3. ****Use only <u>one</u> hand to turn a course or fine focus knob.**** Using a hand on both sides of the focusing knobs can twist the knobs improperly, stripping the gears inside. This makes the microscope unable to properly focus, and costs money to repair.
4. If something seems stuck or you have problems making adjustments, do not apply force. Consult the instructor.
5. Never touch or wipe the objective or eyepiece lenses with anything but lens paper. Clean the lens by gently drawing a flat piece of lens paper across it. The presence of foreign particles can be determined by rotating the ocular lenses manually as you look through the microscope. A pattern that rotates is evidence of dirt. If wiping the lenses with lens paper does not remove the dirt, consult the instructor. It may be on the inside surface of the lens.
6. Before storing the microscope:
   If the microscope has an adjustable tube, rack it down so that the microscope can be stored more easily. Make sure the lowest objective (a blank, a 4×, or 10×) is clicked into place.

   Make certain the eyepiece lenses are clean. Sweat deposits from your eyes can etch the glass.

   Important: If you have used the oil immersion lens, be sure to wipe off all the oil. If not removed, it can leak into the lens and cause severe damage.

Materials

Prepared slides of a printed letter *e* with coverslips (optional)

Prepared stained slides of protozoa, baker's yeast, or other large cells

Suspension of protozoa and/or algae

Suspension of baker's yeast, *Saccharomyces cerevisiae*

PROCEDURE

Important note: In this introduction to the microscope, the 100× oil objective lens (usually labeled with a white band) will not be used. It is the most expensive lens, and its use with immersion oil will be explained in exercise 2. Be particularly careful not to hit this lens on the stage.

1. Place the microscope on a clear space on your bench, away from any flame or heat source. Identify the different parts with the aid of figure 1.2 and the Getting Started section, "The Parts of the Microscope."
2. Before using the microscope, be sure to read the Getting Started section, "Precautions for Proper Use and Care of the Microscope."
3. Obtain either a prepared slide with the printed letter *e* covered with a coverslip or a large stained specimen of protozoa, fungi, baker's yeast, or algae.
4. Place the slide on the mechanical stage, coverslip/specimen side up, and turn on the light at the base of the microscope.

   Tip: If a slide is placed upside down on the stage, the specimen will always appear fuzzy.
5. Move the ocular lenses apart, and then while looking through the lenses, push them together until you see one circle. This is the ocular width of your eyes. If you note the number on the scale between the lenses, you can set the microscope to this number each time you use it.
6. Rotate the low-power objective lens (10×) into place. When looking from the side, bring the lens as close to the slide as possible (or the slide to the lens depending on the microscope). Then when looking through the microscope, use the large coarse-adjustment knob, to raise the lens (or lower the stage) until the object is in focus. Increase and decrease the amount of light with the iris diaphragm lever to determine the optimal amount of light. Continue to focus until the specimen is in sharp view using the smaller fine-adjustment knob. Remember that the 10× objective lens should never touch the surface of the slide or coverslip.
7. Move the slide back and forth. When viewing objects through the microscope, the image moves in the opposite direction than the slide is actually moving. It takes a while to become accustomed to this phenomenon, but later it feels normal.
8. View the specimen at a higher magnification. Rotate the 40× objective lens into place (sometimes called the high–dry objective lens). Be very careful not to hit the slide or the stage with the oil immersion lens. Notice the change in the amount of light needed. Also note that the specimen is almost in focus. Use only the fine focus adjustment to bring the specimen into focus. Most microscopes are **parfocal**, meaning that the objective lens can be rotated to another lens and the slide remains in focus. View several prepared slides until you become comfortable with the microscope. Always start focusing with the low-power objective lens and then move to the high–dry objective lens.

Wet Mounts

1. Prepare a wet mount (**figure 1.3**). Clean a glass slide with a mild cleansing powder such as Bon Ami or as directed by your instructor.
2. Place one drop of suspension on the slide using a dropper. Carefully place a coverslip on edge next to the drop and slowly lower it so that it covers the drop. Try to avoid air bubbles. If the drop is so big it leaks out from under the coverslip, either (1) add a second coverslip to wick away the excess liquid, or (2) discard the slide into a container designated by the instructor and prepare another wet mount.
3. Examine the wet mount using the low-power objective lens and then switch to the high-power objective lens. When viewing unstained material, it is necessary to reduce the amount

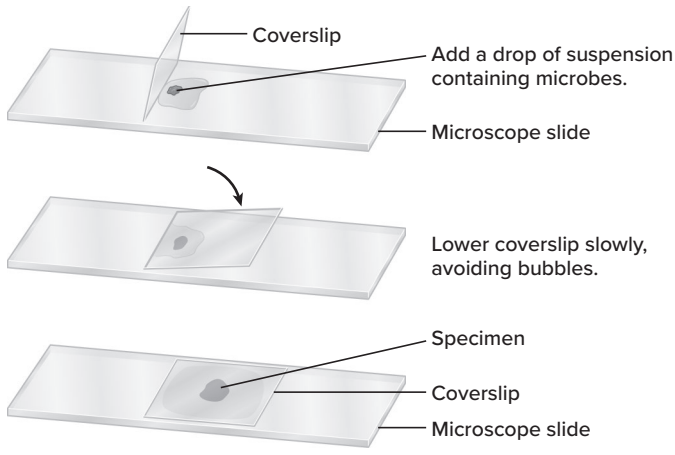


Figure 1.3 Preparation of a wet mount.

of light to increase the contrast between the cells and the liquid. If you are having difficulty focusing on the material, try to focus on the edge of the coverslip and then move the slide into view. Do not try to view the wet mount on the oil immersion lens, as the lens will push the liquid out from the coverslip and contaminate the microscope.

4. Draw the slides you viewed in the report. Indicate the **total** magnification.
5. Dispose your slides as directed by your instructor. The material on the slide is still **viable**, so the slides should be boiled, placed in bleach, or autoclaved.
6. Wipe the ocular lens (eyepiece) with lens paper. After clicking the proper objective into place, turn the microscope off. Then center the mechanical stage (so it does not stick out), rack the microscope down, and return your microscope carefully to its storage space.

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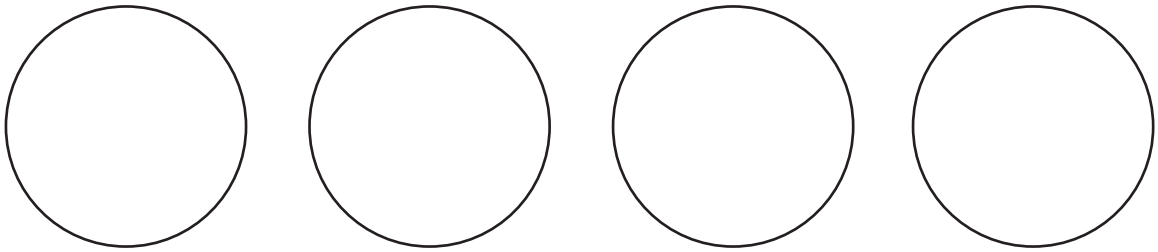
Name _____ Date _____ Section _____

EXERCISE 1 Laboratory Report: Introduction to the Compound Light Microscope

Results

1. Draw four fields of the specimens you observed. Label the objective power used and calculate the total magnification. If possible, show the same field or material at two different magnifications.



Specimen	_____	_____	_____	_____
Objective lens	_____	_____	_____	_____
Total magnification	_____	_____	_____	_____

2. On your microscope, what is the magnification of

   a. the ocular lenses? _____

   b. the low-power lens? _____

   c. the high–dry lens? _____

Questions

1. When you increase the magnification, is it necessary to increase or decrease the amount of light? Why?