

REVISED FIRST EDITION

BASIC AND PRACTICAL
MICROBIOLOGY

LAB MANUAL

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By Mette Prætorius Ibba and Katherine Elasky

Revised First Edition

BASIC AND
PRACTICAL
MICROBIOLOGY

Lab Manual

By Mette Prætorius Ibba and Katherine Elasky
The Ohio State University



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ATTENDANCE SHEET

Please collect your lab instructor's signature after each lab period.

Your lab instructor will sign off only if the following have been achieved:

- You have been present the entire lab period
- You have worked through and finalized all exercises
- You have properly cleaned your microscope
- You have answered the questions in the lab manual

Lab period	Date	Signature
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		

Lab period	Date	Signature
13		
14		
15		
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22		
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24		

Lab Safety, Microscopes, Microbial Diversity and Ubiquity

LAB SAFETY

When working in a microbiology lab—whether it is a teaching laboratory, a basic research laboratory, or a research laboratory in a clinical setting—it is extremely important to strictly practice lab safety by following all provided safety rules and guidelines. Microorganisms such as bacteria grow and divide quickly, and if they are introduced to an environment that provides suitable growth conditions and the necessary nutrients, they can easily settle down and make such an environment their new home. As you will learn in this course, we human beings are excellent hosts for many different microbial species.

Box 1 Clinical and Teaching Microbiology Labs Responsible for *Salmonella* Outbreak

Between August 2010 and June 2011, more than one hundred individuals in the United States were diagnosed with a *Salmonella* infection that was caused by the strain *Salmonella* Typhimurium. This particular *Salmonella* strain, which is commonly used in microbiology teaching labs, is a Biosafety Level 2 (BSL2) organism. Among the infected individuals who participated in the early examinations and investigations regarding the *Salmonella* outbreak were students of microbiology courses and children of healthy parents who attended microbiology courses. These initial results strongly suggested the possibility that the individuals' attendance in microbiology courses was responsible for the outbreak. This was further verified by results from additional investigations and genetic verification of the origin of the strain that caused the infection in the individuals. Throughout the entire time of the outbreak, infections were reported in thirty-eight states, with patients ranging in age from one to ninety-one years old. One death was reported.



FIGURE 1.1 *Salmonella typhimurium*

The symptoms of *Salmonella* infection are diarrhea, fever, and abdominal cramps that start 12–72 hours after the exposure that led to the infection. Healthy people will normally recover in 4–7 days without treatment. If *Salmonella* is given the opportunity to spread from the intestines to the bloodstream and other parts of the body, it can be life threatening, and immediate antibiotic treatments are crucial.

It is important to note that in this course we will work mostly with BSL1 organisms, and there will be limited exposure to BSL2 organisms in this lab. We will never work with an organism such as *Salmonella* Typhimurium. Nevertheless, we can potentially

contribute to the spread of other bacterial organisms, which may be a potential risk for elderly people, infants, young children, or individuals with impaired immune systems. Therefore, we cannot emphasize strongly enough the importance of strictly following the lab safety rules and regulations.

References

- Hayden, Erika C. "Salmonella Hits U.S. Teaching Labs." *Nature* 473, 132 (May 2011). doi:10.1038/473132a.
- "Human *Salmonella* Typhimurium Infections Associated with Exposure to Clinical and Teaching Microbiology Laboratories (Final Update)." Centers for Disease Control and Prevention, January 17, 2012, <http://www.cdc.gov/salmonella/2011/lab-exposure-1-17-2012.html>.
- You can get more information about current and past *Salmonella* outbreaks on the website of the Center for Disease Control and Prevention at: <http://www.cdc.gov/>.

In the lab, we will be working with bacterial organisms belonging to the level of low-risk microbes (Biosafety Level 1, **BSL1**). We will limit the use of potential pathogenic organisms (Biosafety Level 2 bacterial strains, **BSL2**). In contrast to pathogenic strains, the strains we will mostly be working with are unlikely to cause disease in healthy individuals. However, under certain circumstances, many bacterial strains can potentially cause disease (for instance, if bacterial cells in larger quantities come into contact with an immune-compromised individual: a person whose immune defense is weakened and thus is not capable of efficiently eliminating the microbes; such elimination is needed to overcome the bacterial infection). To minimize risk, protect the health of all students and educators in the teaching lab, and avoid spreading bacteria to communities outside of the lab, we are strictly following the rules and guidelines stated in the following exercise. The rules and guidelines that are followed in this course were developed by the American Society for Microbiology (ASM) Task Committee on Laboratory Biosafety. You can read more about the rules and regulations here: https://www.uab.cat/doc/teaching_lab_ASM.

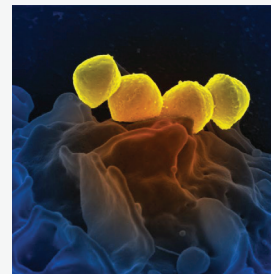
Box 2 Proper Hygiene Decreases Spread of Infectious Diseases

One of the most devastating incidents involving the spread of microbes—a spread mainly caused by the lack of handwashing—was the tragedy of puerperal fever (also called childbed fever). From the 1600s to the late 1800s, an enormous number of women suffered from puerperal fever just after giving birth and while still in the hospital. The number of postpartum mothers who lost their lives just after giving birth was immense. During much of this time, the **germ theory** did not exist, so finding the cause and preventing the disease were complicated and delayed. Ignaz Semmelweis (1818–1865) and Oliver Wendell Holmes (1809–1894), two medical doctors, were among the first to suggest that the spread of the disease among postpartum women was mostly caused by the fact that doctors did not clean their hands in between their different activities in the hospital. Dr. Semmelweis and Dr. Holmes had gained sufficient evidence to prove that, in fact, doctors transferred bacteria to the women giving birth from their previous work tasks in the hospitals, which often involved work such as examinations of cadavers. The bacteria on the hands and clothes of the doctors were directly transferred to the woman giving birth, leading to bacterial infections in the birth canals of the postpartum women. One of the doctors' strongest pieces of evidence was the fact that women giving birth who were only seen by midwives (who did not examine cadavers) did have a considerably lower risk of getting infected. Unfortunately, the two doctors' theory and evidence of proof were not easily acknowledged among their colleagues, and many years passed before proper action was taken to minimize the spread of the bacteria. It was not until the early to mid-1900s (when the germ theory was fully accepted and antibiotics were invented and widely used) that postpartum women suffering from puerperal fever could be effectively cured.

Many of the puerperal fever infections were caused by the bacterium *Streptococcus pyogenes*, but other bacteria such as *chlamydia*, *clostridium tetani*, *Clostridium Welchii*, *Escherichia coli*, *gonococci*, and *staphylococci* are also known for causing the disease. Due to the spread of bacteria to the blood, many women died from septicemia or peritonitis (inflammation of the abdominal lining).

Reference

Lane, Hilary J., Nava Blum, and Elizabeth Fee. "Preventing the Transmission of Puerperal Fever." *American Journal of Public Health* 100, no. 6 (June 2010).



Laboratory safety rules and procedures

Learning objectives

To understand and know how to follow microbiology safety rules.

Before you start working in a microbiology lab, it is very important that you familiarize yourself with all the rules and regulations that apply to working in such an environment.

Therefore, please read through the following rules carefully. For your safety, as well as the safety of everyone in the lab, you are expected to strictly follow each one of these rules.

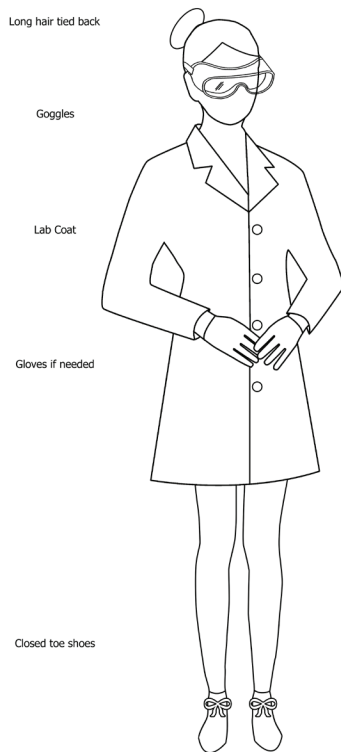


FIGURE 1.2 Lab Safety attire

- Wash your hands with soap and water when entering and leaving the lab.
- When indicated, always wear a long-sleeved lab coat to protect your clothes. Wear gloves during the entire lab if you have fresh cuts or abrasions or if you are immunocompromised. Use safety goggles or safety glasses while working with live microorganisms.
- Always wear closed-toed shoes that cover the entire foot.
- Tie back long hair (shoulder length or longer). If you wear a hat, the brim should face backwards; this is because we will be working with Bunsen burners.
- Do not wear dangling jewelry.
- Never bring food or drinks (including water and water bottles) into the lab. Do not chew gum or bite your nails in the lab.
- Never apply cosmetics or lotion, or insert or adjust contact lenses, in the lab.
- Know the location of (and how to operate) fire extinguisher(s), fire blanket(s), eyewash station(s), safety shower(s), and first aid kit(s) in the lab.

- Strictly follow the fire-safety regulations (detailed in the fire-safety presentation given by your lab instructor) and read through all fire-safety information posted in lab.
- Notify your lab instructor if you are immunocompromised (or living with or caring for an immunocompromised person), pregnant or may become pregnant, or have any known allergies to components we will be working with in the lab.

For the safety of all students working in the lab, as well as the TAs and instructors, it is very important to strictly follow the procedures described below.

- Wipe off the lab bench at the start and end of each lab period with the provided disinfectant and paper towels.
- Label all tubes and plates as indicated in the lab manual so organisms and growth medium can easily be identified.
- Be aware of your own Bunsen burner when it is lit, along with other Bunsen burners in your vicinity. The flames are not always easy to see. Shut off the Bunsen burner when not in use.
- Always inform your TA or lab instructor immediately of any accidents in the lab, including (but not limited to) fire, culture spills, or personal injury of any kind.
- If culture is spilled, first notify your TA or lab instructor. Then flood the area with disinfectant and use paper towels to wipe the area dry. Throw away the paper towels and broken glass in the biohazard bins, and wash your hands immediately, using soap and water.
- Be careful not to spill any culture on tablets or laptops that are used in the lab. Contaminated equipment cannot leave the lab until it is disinfected. It is recommended that you protect your tablet while working with cultures by enclosing it in a Ziploc bag or covering it entirely with Saran Wrap.

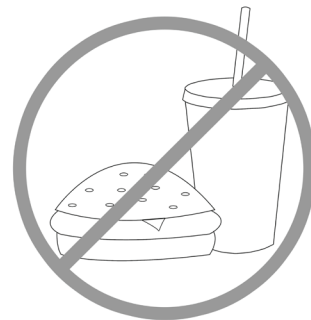


FIGURE 1.3 No food or drinks



FIGURE 1.4 Fire extinguisher and first aid kit

- Wear gloves when stated in the lab manual (or during the entire lab period if you have fresh cuts on your hands). Discard the gloves in the biohazard bins when the work has been completed and before leaving the lab.
- Return all shared materials to their designated locations when you are done using them.
- To avoid the spread of the microorganisms and to contain them in a safe place prior to their destruction (autoclaving), it is important to know exactly how to dispose of the microbes when you are done working with them:



FIGURE 1.5 Sharps and biohazard bin

- All live cultures on agar plates should always be disposed of in the biohazard bins. These bins are typically red and marked with a biohazard label.
- All microbes in culture tubes should be placed in racks designated for cultures to be autoclaved. Remove all tape or writing on the tubes.
- All material that has been in direct contact with microbial culture should be disposed of in the biohazard bins. This include gloves, microscope slides, pipet tips, serological pipettes, droppers (not the wrappers), swabs (not the wrappers), microcentrifuge tubes, and any other disposable plastic materials.
- Normal trash bins are also present in the lab. Please use these for all material that has not been in direct contact with the microbes, such as:
 - Paper towels used for wiping the benches before and after lab work
 - Gauze
 - Wrappers from swabs, droppers, and serological pipettes



FIGURE 1.6 Trash bin

Results and questions (R & Q)

Explain why drinking and eating are prohibited in the lab.

Where do we discard used gloves?

Explain why we have to remove the gloves when leaving the lab (e.g., when going to the restrooms or to the elevators).

Explain why students with a fresh cut should wear gloves for the entire lab period.

Why is it important to tie back long hair?

Describe three potential risks that could occur if lab safety rules are not followed.

Where do you dispose of used tubes with labels removed?

What goes where?

Draw a line from the item to the correct disposal location.



- Gloves
- Pipette Tips
- Slides
- Uncontaminated Paper Towels
- Contaminated Paper Towels
- Microcentrifuge tubes
- Wrappers
- Droppers
- Gauze
- Swabs



Laboratory Safety Sign-off Sheet

I have read and understood the rules and guidelines presented to me.

Signature: _____

Date: _____

Print name: _____

Lab day: _____

Lab time: _____

Room number: _____

TA names: _____

Semester: _____

Year: _____

MICROSCOPES, MICROBIAL DIVERSITY AND UBIQUITY

We can easily visualize microbial organisms with the naked eye when they grow up to form colonies on agar plates (solid media) or when they are grown in nutrient-containing broth (liquid media), transforming the media from clear to cloudy. However, we do need a microscope to see the individual microbial cells.

The most common of all microscopes is the compound brightfield microscope (compound microscopes use more than one lens), and this is the type of microscope that we will use most often in the lab. The history of the invention and development of microscopes goes back for centuries. In the 1590s, two Dutch lens grinders, Hans and Zacharias Janssen (1585–1638), developed the very first documented compound microscope. It was simple, consisting of a tube and two lenses, and it magnified images approximately three times. However, long before that, in the twelfth through the fourteenth centuries, the Romans developed the lens-grinding procedure; to improve people's eyesight, they also produced spectacles—a kind of one-lens microscope.

In the early 1600s, Galileo Galilei (1564–1642) further developed the compound microscope by adding convex and concave lenses. However, the first publication describing “objects” visualized with a microscope came from the English scientist Robert Hooke (1635–1703), who published his observations in his book, *Micrographia*, in 1665. Robert Hooke was also the first to name the basic unit of life a “cell.” About ten years later, Dutch cloth merchant and scientist Anton van Leeuwenhoek (1632–1723) was the first to actually observe and describe unicellular organisms, including bacterial cells, through a microscope. He had great expertise in lens grinding and created many very powerful lenses and microscopes. While using his very best lens, he observed a sample of pond water and discovered the world of the tiny microorganisms in their pond-water habitat. Leeuwenhoek reported his observations to the Royal Society in London in the late 1670s, and his reports included many detailed illustrations of (among other things) bacteria and algae. Throughout many years of his career, he continued to report his microscopic observations. He was the first person to improve the compound microscope by applying his powerful lenses, which could magnify objects 200–300 times. You can read more about the history of microscopes, Leeuwenhoek, and other pioneers of microscopy by following this link: <http://www.history-of-the-microscope.org/>.

Learn More

You can follow this link to get a peek at Hooke's *Micrographia*:

<https://ceb.nlm.nih.gov/proj/ttp/flash/hooke/hooke.html>

Similar to early microscopes, some of the newer, more sophisticated microscopes use visible light (light microscopes) to visualize the specimens, but other, even more powerful, microscopes use electron beams, and electromagnets instead of glass lenses (electron microscopes). The development of these

different types of microscopes, along with various related microscopic techniques, have contributed greatly to opening our eyes further and further to the stunning world of microorganisms and, in fact, of all living cells. The microscope not only has been, but still is and will be in the future, an essential instrument for a variety of research-area disciplines (from basic research to applied medical research) and for providing clinicians the necessary insight for the diagnosis of a variety of diseases. It is no wonder that quite a few Nobel Prizes have been awarded to scientists who have worked on microscope inventions and the improvements of these instruments.

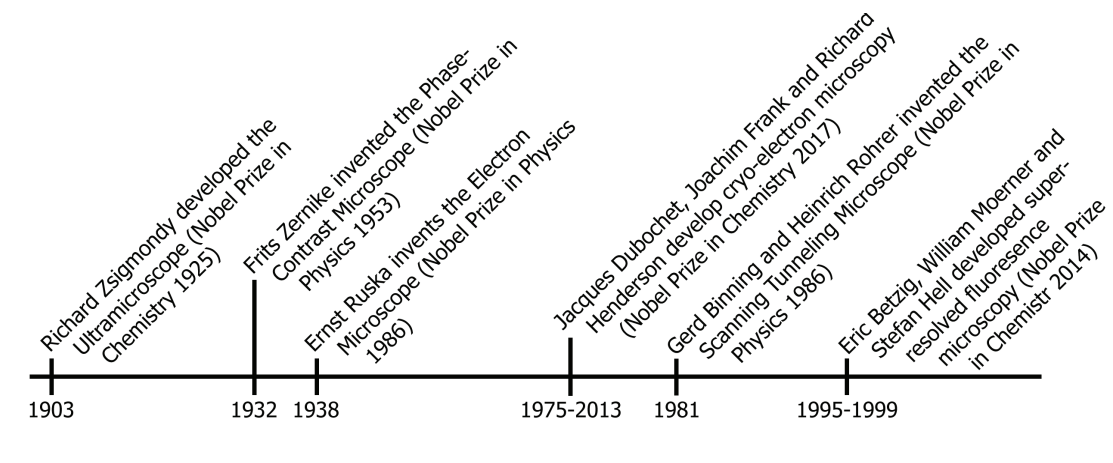


FIGURE 1.7 Timeline

Today's scientists can choose from a number of different types of microscopes to use for their examinations. Depending on their field of study, they might choose the **compound brightfield microscope** to examine morphology (shape) of live microbial cells, or "fixed specimens." When cells are fixed to a slide by heat, their shapes get distorted, and thus the microbes are no longer present in their native state. It is therefore not possible to examine the native shapes of these fixed microbial cells; however, other interesting characteristics of the microbial cells can be examined by staining the fixed cells (see Module 2 for more on staining). The brightfield microscope is the simplest and most common microscope, and it is found in many research labs. When using the brightfield microscope, specimens look dark against the bright (white) background created by the light. Since the contrast provided by the compound brightfield microscope is low and many bacterial cells are transparent, it is often necessary to stain the cells before performing microscopy of them.

When using a **darkfield microscope** or **phase contrast microscope**, there is no need to stain the specimens. These two microscopes provide better contrast, making it possible to observe more details within the living cells. In contrast to the brightfield microscope, species viewed in the dark-field microscope appear bright against a dark background. A microbiologist choosing to work with a

darkfield microscope or a phase contrast microscope would typically examine cell morphology, as well as motility, of living microbial cells in their native state. Also, bacteria, which are difficult to stain, will typically be examined using one of these two microscopes instead of the brightfield microscope.

Of all the three microscopes, the phase contrast microscope has the ability to provide the most detailed image of the species being examined. Thus, in addition to examining morphology and motility, scientists using the phase contrast microscope can also observe certain intracellular structures and inclusions, which are not possible to clearly see using the brightfield or the darkfield microscopes.

When viewing a specimen using a brightfield microscope, the light rays from the light source are transmitted through the specimen. Since the light is restricted upon passing through the specimen to the objective, it makes the cells appear darker or shaded against the bright background. In contrast, the darkfield microscope contains a special condenser, which ensures that only light that is reflected off the specimen will enter the objective. This gives rise to a bright image of the cells against the dark background (greater contrast) and therefore a more detailed image of the cells.

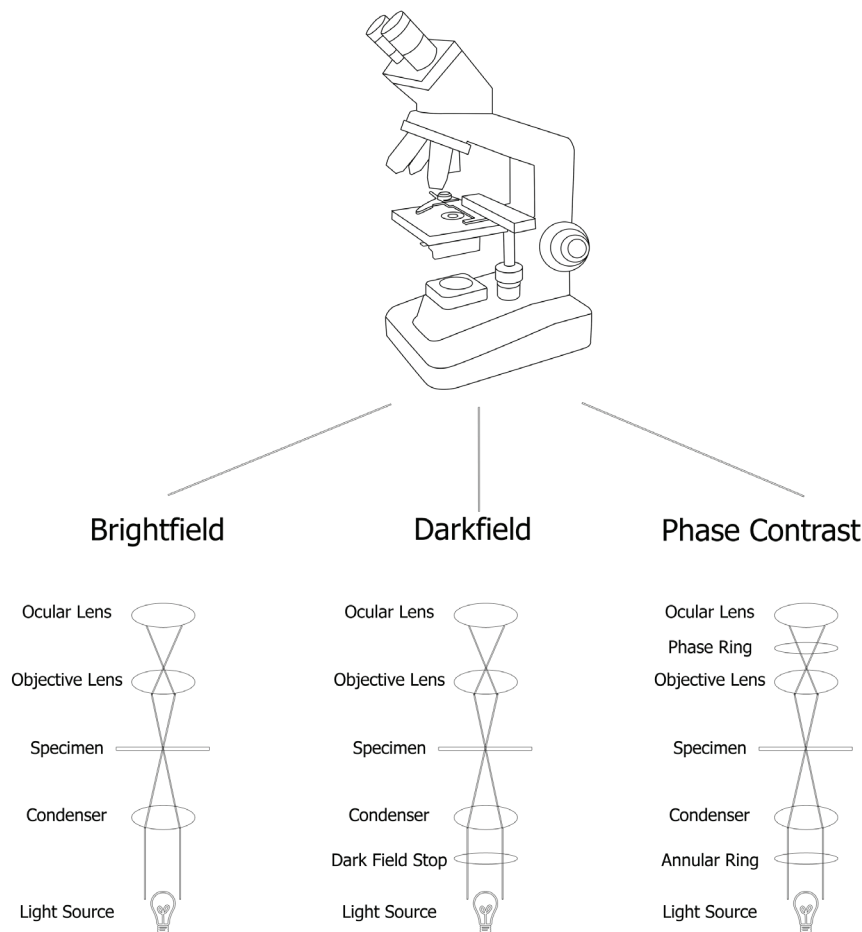
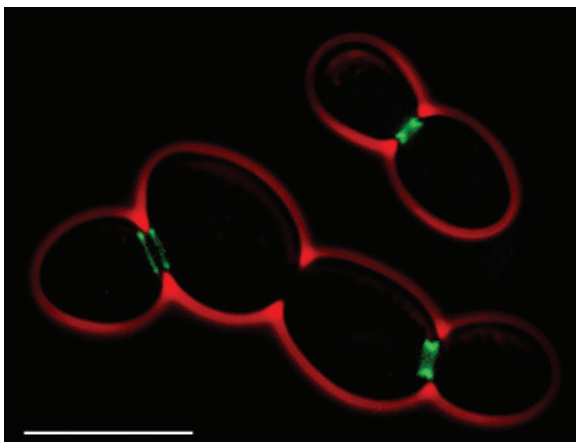


FIGURE 1.8 Light Microscopes

The phase contrast microscope differs from brightfield and darkfield microscopes in that it includes two special optical components: an **annular diaphragm** and a **phase-shifting element**. One of its features (provided by the annular diaphragm) ensures that the light transmitted from the light source is directed into a cone shape onto the specimen. Due to the different densities of the specimen, the light refracts differently. The other feature (provided by the phase-shifting element) is capable of shifting the waves of the refracted light, causing the waves to either add to or subtract from each other. Waves that add to each other are in phase and reinforce each other. This results in an increase in light intensity. Conversely, light waves that are out of phase cancel each other, result in no intensity, and appear dark. The resulting view is a specimen that appears as different levels of dark against a brighter background. The enhanced contrast gives rise to a more improved, detailed image of the cells.

Other microscopes worth mentioning (although they aren't used in this course) are the fluorescence microscope, the transmission electron microscope, and the scanning electron microscope. Whereas the light microscope magnifies specimens up to 1200–1300 times and the resolution is about 200 nm (i.e., we can see particles as close as 200 nm apart as individual particles), an electron microscope (such as the transmission electron microscope) can magnify images approximately 1,000,000 times, and the resolution is about 0.2 nm. The other type of microscope, the scanning microscope, can amplify images 100,000 times, with a resolution of approximately 3 nm.

The **fluorescence microscope** is also a light microscope; however, it differs from the other light microscopes in that it contains a high-energy mercury vapor lamp. This lamp produces shorter wavelengths of light (in the ultraviolet range), which is necessary for inducing fluorescence. Scientists use the microscope to examine specimens that can fluoresce, which means light of a special wavelength is



Fluorescent micrograph of *Saccharomyces cerevisiae* with GFP-tagged septins. Scale bar: 10 μ m

Source: Spitfire ch, https://commons.wikimedia.org/wiki/File:S_cerevisiae_septins.jpg. Copyright in the Public Domain.

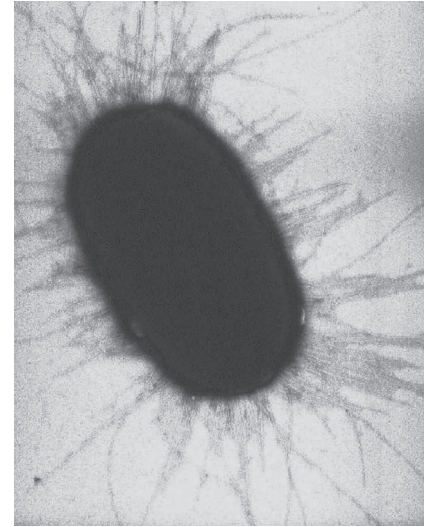
absorbed by the specimens (atoms or molecules) and reemitted as light of a higher wavelength (lower energy level). Some specimens and objects naturally fluoresce (e.g., minerals, rocks, and chlorophylls); however, in most cases, chemical dyes have to be applied in order for the specimens to fluoresce. This can be obtained by allowing the cells being examined to bind to, or take up, chemicals that fluoresce. Such chemicals, called fluorochromes or fluorophores (or chromophores), can also be conjugated to (bound to) antibodies that are used to recognize specific cell surface components (such as proteins and a variety of intracellular components). The technique is called immunofluorescence microscopy, and it is

a widely used and indispensable technique employed to study various areas of the medical and biological sciences. The microscope contains a darkfield condenser to improve contrast and several filters to adjust wavelengths of the emitted and reemitted light.

Electron microscopes are not light microscopes; instead of light, they employ electron beams, and they use electromagnets instead of lenses to create the magnified image of the specimen. A fluorescence screen is often applied to collect the image. The microscopes produce black-and-white images and, compared to the light microscopes, smaller objects can be viewed in much greater detail. The two different types of electron microscopes, the transmission and scanning electron microscopes, are capable of creating very different images: the transmission electron microscope (TEM) creates an internal view of the specimen (e.g., an internal view of the structures of a bacterial cell), whereas the scanning electron microscope (SEM) creates an external three-dimensional view (e.g., a surface view of a bacterial cell). The difference in the image produced relates to the way that the electrons are either transmitted through the specimen (TEM) or refracted (SEM). In order to view specimens with the electron microscope, they have to undergo lengthy processing, some of which includes fixation, production of very thin sections of the specimen, and staining to increase contrast between the different structures of the viewed specimens. The images appear as dark against a bright background. The development of the transmission electron microscope began in 1931, and in 1935, the scanning electron microscope was developed.

The most recent Nobel Prize within the microscopy field was awarded in 2017 to three scientists whose work helped develop the cryo-electron microscope. With their contributions, it is now possible to generate images of atomic resolution structures of biomolecules in solution. The technique involves freezing biomolecules in a way that they virtually retain their natural shapes. This method has been said "to move Biochemistry into a new era".

Another application of the transmission electron microscope is immunoelectron microscopy (IEM), which uses gold-labeled antibodies to visualize specific components of the cells. This technique is commonly used to precisely determine the localization of certain molecules being studied, and it can trace specific molecular processes within the cell. The images created are similar to those of TEM, but they add the dark, electron-dense spots at locations that have been bound by the gold-labeled antibodies.



Transmission electron micrograph of *Escherichia coli*



Colorized scanning electron micrograph of *Escherichia coli*

The compound brightfield microscope

Before we start to examine microbes using the compound brightfield microscope, there are some important terms and technical necessities that we need to go through before we are ready to work with the microscope.

Objective and ocular lenses and total magnification: we already learned that light microscopes contain two or more lenses. The compound microscope that we will work with in this course contains an **objective lens** (which is situated close to the specimen) and an **ocular lens** for each of the two eye pieces (which are situated closest to your eyes). The specimen is first magnified by the objective lens according to its magnification capability (4x, 10x, 40, or 100x magnification) and is then further magnified 10 times by the ocular lens (10x).

When calculating the **total magnification** of a given image, it is necessary to multiply the magnifications produced by the objective lens and the ocular lens. So, if you use the objective lens that magnifies 40 times and you know that the ocular lens magnifies 10 times, the total magnification of the specimen is 400 times:

$$\begin{array}{rcl} 40 \text{ times magnification} & \times & 10 \text{ times magnification} & = & 400 \text{ times magnification} \\ \text{(Objective lens)} & & \text{(Ocular lens)} & & \text{(Total magnification)} \end{array}$$

In creating a perfect image of the specimen, the **resolving power** (RP) or **resolution** is equally important, too. The resolving power is the ability of the microscope to distinguish two objects (or points) positioned closely together as being two separate objects (points) instead of one big object. The resolving power is determined by the wavelength of the light used and the **numerical aperture** (NA) of the objective lens, where the numeric aperture is a measure of the quality of the lens with regards to how well the lens can resolve fine details. The numeric value is written on the objective lens; this value reflects how much light can be brought into the lens: the higher the light-gathering ability is (i.e., the bigger the light cone brought into the lens), the higher the numerical aperture is and the better the resolution of the specimen will be.

Resolving power is calculated as:

$$\text{Resolving Power (RP)} = \frac{\text{wavelength of light used}}{2(\text{Numerical Aperture of the Objective})}$$

$$\frac{(500\text{nm})}{2(1.25)}$$

The size of the objective lens decreases with increasing magnification. This means that with higher magnification the amount of light that can be brought into the lens gets smaller. This makes the field appear darker. In addition, the amount of light that is able to gather in the objective lens also depends

on the refractive properties of the light, so more light will bend away as a result of the light rays moving faster through air than through the glass slide containing the specimen. The iris diaphragm or light rheostat can alter the availability of light when using the 4X, 10X, and 40X objective lenses, but when working with the highest magnifying lens (the 100X lens), **immersion oil** must be applied to the slide in order to increase the amount of light going into the objective lens and obtain a clear image, which otherwise would appear blurry. The reason is that the light rays travel at similar speed through the glass slide and the oil, and this prevents the light from bending away from the specimen, thereby providing sufficient light to view the specimen.

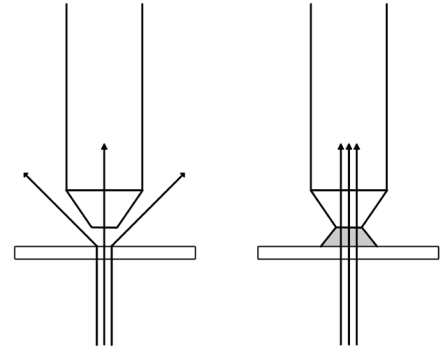


FIGURE 1.9 Light Rays with oil objective

Note: When using the 100X lens, immersion oil must always be applied. This lens is sealed against oil. However, the 4X, 10X, and 40X lenses are not sealed against the immersion oil. They can get damaged, and immersion oil should therefore never be used when working with these lenses. Please immediately clean off any immersion oil that may have unintentionally touched these lenses.

When you start working with the microscope, you will quickly realize that you can easily change from one objective (e.g., 10X) to another (e.g., 40X) and still have the specimen in focus or almost in focus. This feature, called **parfocality**, ensures that the specimens remain in focus while switching from one objective to another. It may be necessary to perform minor adjustments with the **fine focus knob**, though.

Although the contrast or definition is less when viewing the live microbes in the brightfield microscope (compared to dark field or phase contrast microscopes), it is still possible to examine **morphology** and **motility** of the microbes.

In the exercises to follow, we will be looking at prokaryote and eukaryote microbes such as the bacterial organism *Escherichia coli* and the yeast *Saccharomyces cerevisiae* (brewer's or baker's yeast), as well as samples of **hay infusions**, where we also can view protozoa—a unique selection of unicellular eukaryote organisms that normally live in pond water or creek water. We will also be comparing two very different fungi to each other.

When performing the exercises, you will be comparing and familiarizing yourself with the sizes of the different microbes that you are viewing, examining motility (the ability of certain microbes to self-propel through their environment using their **flagella**) and determining the microbes' cell and colony morphology (shape).

As you proceed with the exercises, you should be able to easily distinguish between prokaryote and eukaryote cells and to distinguish true motile organisms from **Brownian motion** and **current flow**. The movement of motile flagellated bacteria often appears directed in straight lines or as rotating tumbles. This should not be confused with Brownian motion, where the bacteria appear to just be vibrating in the same place instead of moving around in their environment. The occurrence of Brownian motion in the cell suspension is a result of random collisions of particle and/or cells. Another phenomenon that should not be confused with true motility of the microbial cell is current flow, which is the simultaneous movement, in one direction, of all particles, cells, etc., that are being viewed on the slide. Current flow is more commonly observed when the slides are starting to dry out.

Microscopes are valuable and very fragile equipment, so it is important to correctly handle and take good care of them. The microscope that you will be working with now is ready to use: the person who worked with it earlier has already cleaned it properly and positioned all of its parts correctly. After you are done working with the microscope, you need to do the same: make sure to clean it and position the parts correctly so the microscope is ready for the next person to use (see “Cleaning the microscope” below).

When carrying a microscope, use one hand to hold the arm of the microscope and place the other hand under the base of the microscope.

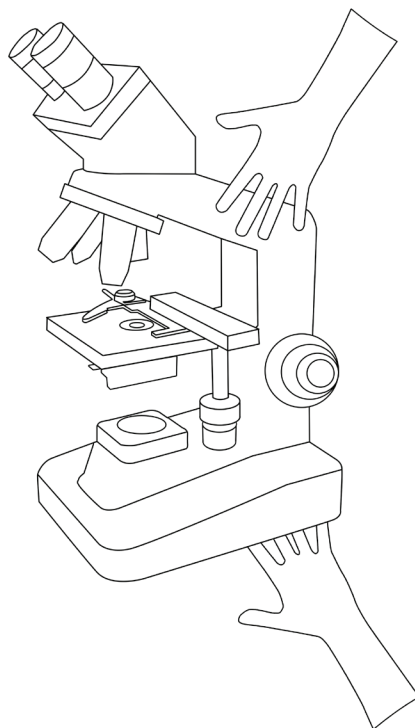


FIGURE 1.10 How to Carry the Scope

Where do the parts belong on the microscope?

Learning objective

To gain competence in working with light microscopes.

Procedure

To familiarize yourself with the brightfield microscope, use the descriptions of each of the parts of the microscope presented below to complete this exercise. A slide will be presented by your TA or lab instructor to check that you have gotten the answers right.

The parts of the compound brightfield microscope

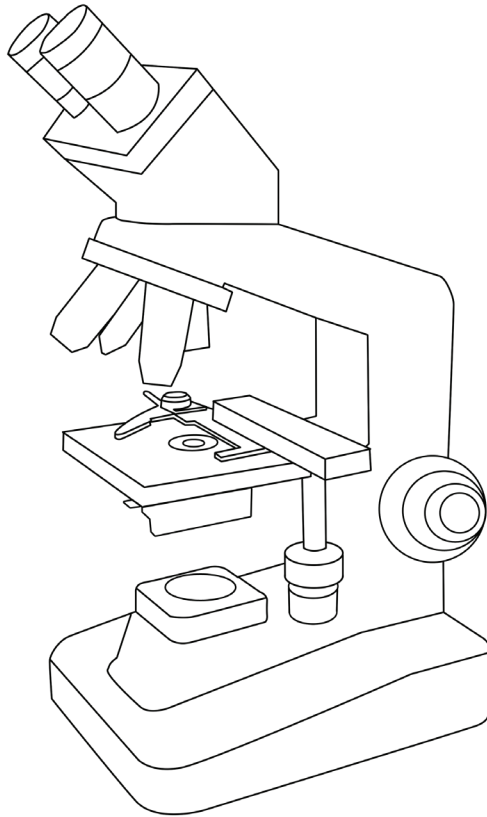
- **Arm:** The arm extends from the base of the microscope and connects the base to the lens systems and stage.
- **Stage:** The stage is the horizontal surface on which the slides containing the specimens are placed for viewing. The mechanical part of the stage includes a clip that holds the slide in place when moving the slide from side to side using the two control knobs located at the base of the mechanical stage.
- **Condenser:** The condenser lens focuses light from the light source onto the specimen. The condenser is positioned just below the stage.
- **Light source:** The light source includes the bulb (typically a halogen lamp) located near the base of the microscope below the condenser. The light rays are transmitted through the slide containing the specimen. The rheostat on the on/off switch is used to adjust the amount of light reaching the specimen.
- **Iris Diaphragm:** The iris diaphragm adjusts the amount of light reaching the specimen, which is done by moving the control lever left and right. The iris diaphragm is positioned between the condenser and the light source.
- **Objectives:** The objectives are the set of lenses which are located closest to the specimen that is being viewed and are attached to the nosepiece. The four objectives that typically are present on the brightfield microscope are the **4X objective** (scanning lens), the **10X objective** (low power lens or

low dry lens), the **40X objective** (the high power lens or high dry lens), and the **100X objective** (the oil-immersion lens).

- **Nosepiece:** The nosepiece is the revolving element that holds the objectives.
- **Coarse- and Fine focus Adjustment Knobs:** These are the two knobs that are used to move the stage in order to bring the specimen into focus. They are commonly located on either side of the stage. The coarse focus adjustment knob moves the stage much larger distances compared to the fine-focus adjustment, which moves the stage very small distances.
- **Oculars or Eyepieces:** The oculars, or eyepieces, are the lenses situated at the very top of the microscope and closest to your eyes. These lenses provide the final 10X magnification of the specimen being viewed. you can slide the oculars back and forth to adjust the distance between the lenses so you can see through both oculars at once; hence, it is a binocular microscope.

R & Q

Draw lines to connect the terms to the microscope.



Oculars

Arm

Objectives

Nosepiece

Stage Clip

Coarse Focus Knob

Fine Focus Knob

Stage Adjustment Knobs

Light Source