THE BRIGHT-FIELD MICROSCOPE: ITS PROPER USE AND CARE

The microscope is the basic instrument used to visualize cells and their structures. In the field of microbiology, the microscope is used to study organisms too small to be seen with the unaided human eye. Broadly, such organisms are termed microorganisms or microbes. In more specific terms, microbes include the prokaryotic bacteria (Fig. 1) and archaea, the acellular viruses and virus-like entities, and small eukaryotes like certain fungi, algae, and parasites. In this course, students will mainly confine their microscopic observations to the prokaryotes, though eukaryotic yeast cells will be examined during this exercise as well.

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand the principles of light microscopy, in particular the concepts of resolution, magnification, and depth of field/focus;
- Effectively use the microscope to view slides of different cell types;
- Correctly employ the oil immersion lens to view eukaryotic and prokaryotic cells;
- Appreciate the general size difference between prokaryotes and eukaryotes; and
- Recognize the various shapes of some common types of bacteria.

Principles

Success in the microbiology laboratory begins with, and may largely depend upon, the competent use of the compound bright-field light microscope. There are different types of light microscopes, e.g., fluorescent, phase-contrast, dark-field, etc. However, in this teaching laboratory, only the bright-field microscope will be used. This type of microscope shall be more than sufficient for a student to effectively view microorganisms.

The history of the light microscope began with the examination of specimens by early scientists using a simple, single lens (Fig. 2; see https://youtu.be/TJyOQmdwHhE). Subsequently, the compound microscope (i.e., two lenses aligned with one another) was developed, which provided greater flexibility and power. These early microscopes used, as do many present-day microscopes, visible light to illuminate the specimen being examined. In the early 20th century, the electron microscope was invented which uses electrons as the ‘illumination source’. However, the
electron microscope won’t be used in this laboratory due to its complex and expensive nature to operate and maintain.

A comprehensive study of the microscope and the field of microscopy will not be presented here. On their own, students should undertake a review of the microscope by reading the following article: [https://www.britannica.com/technology/microscope](https://www.britannica.com/technology/microscope). Rather, the ensuing discussion below shall focus on the overall mechanical function of the microscope and several key properties of the modern-day microscope.

Overview of the Bright-Field Microscope. The main microscope that will be used in the laboratory is the Olympus Model CH2 (Fig. 3). The instruction manual for this microscope can be downloaded ([http://www.alanwood.net/downloads/olympus-chs-cht-instructions.pdf](http://www.alanwood.net/downloads/olympus-chs-cht-instructions.pdf)). A few newer microscopes, the Olympus Model CX23, are available for use. The Model CX23 instruction manual can be downloaded as well ([https://brazosport.edu/Assets/pdfs/Mickey-Dufilho/Microbiology/Olympus microscope instructions.pdf](https://brazosport.edu/Assets/pdfs/Mickey-Dufilho/Microbiology/Olympus microscope instructions.pdf)). However, despite being slightly different in form, both microscopes are structural and operationally similar. A broad overview of the foundational structure and function of these microscopes is presented below.

Several of the important components of the microscope are shown in Figures 4 and 5. It is critical that students learn how these components function and how they are operated. Specific instructions on the basic use of the microscope, given below, shall depend upon students recognizing and appreciating the various parts of the microscope. Students should compare Figures 4 and 5 with the actual microscopes at their assigned bench seat.

**Please Note:** Remember that these are common microscopes being used by multiple students. Therefore, use great care in the use and handling of each microscope! Be considerate and clean the microscope after each use – this is a requisite part of being a good laboratory citizen.

- Arm and Base: These are the supporting structures for the entire body of the microscope. It is imperative when transporting a microscope that one hand be secured to the arm and the other placed under the base.

- Ocular lens(es): This is the eyepiece through which a student will view a specimen. It is binocular, i.e., two lenses, one for each eye. The oculars are designed such that near-sighted persons wearing glasses or contact lenses need not remove them before looking through the lenses. (Note: This is not true for persons who need corrected lenses for far-sightedness or astigmatism. Such individuals need to remove their glasses to properly use a microscope.) Also, one of the oculars is capable of being adjusted using the dioptr ring (not shown) so that any differences in focus from one eye to the other can be corrected. This aspect of the ocular will be covered in the procedure provided later in this document. Finally, the ocular lenses for the laboratory microscopes have a 10X magnification power, i.e., each lens can magnify objects ten-fold their actual size.

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**Figure 3. The compound bright-field microscope, the Olympus Model CH2.**
([https://commons.wikimedia.org/wiki/File:Compound_Microscope.png](https://commons.wikimedia.org/wiki/File:Compound_Microscope.png))
Objective lenses: Typically, a microscope possesses three or four lenses on a turret (rotating nosepiece) that in combination with the ocular lens provide the magnification and resolution necessary to visualize a specimen. (The principles of magnification and resolution shall be covered later in this document.) The four objectives on the microscopes in this laboratory have magnification powers are 4X, 10X, 40X, and 100X (though some models of microscopes differ in the magnification power of objective lenses). Note that the glass lenses of these objectives become progressively smaller in size as the magnification increases. When used in conjunction with the ocular lens, the total magnification per objective is 40X, 100X, 400X, and 1000X, respectively. The latter objective requires the use of immersion oil. The use of the oil shall be covered later, but its purpose is to help facilitate directing the photons from light source into the very small lens of this objective, thereby providing better resolution as well as illumination.

Please Note: Students must take special care with the objectives. Scratching the lenses by their inappropriate use and/or not removing all oil from any of these lenses can seriously damage them, thereby hindering the usefulness of the microscope. Moreover, immersion oil should only be used with the 100X objective, but the other objectives are sometimes contaminated with the oil due to carelessness.

Figure 4. Labeled image of an Olympus Model CH2 microscope. (http://faculty.ccbcmd.edu/courses/bio141/labmanual/lab14/focusing/microscope.html)
• Stage: This is the platform upon which the specimen slide will be placed. The stage also possesses the slide holder (described below) and a vertical (up/down movement) knob (not shown). Always be sure to clean the stage of any oil or liquids that may fall on it.

• Slide holder: Specimen slides are placed in this component located on the stage. Slides are held in place with a spring clip arm. Be sure not to “slam” the clip onto the slide which risks cracking the slide or creating small shards of glass. The slide holder is then moved along the stage surface in horizontal and vertical directions using mechanical controls (see below). Also, be sure to clean the slide holder of any oil or liquids that may contaminate it.

• Mechanical stage controls: Using the separate knobs, the slide holder can be used to locate a slide along the stage surface. These knobs move the stage in horizontal and vertical directions. Be careful not to over stress the knobs. See both Fig. 4 and 5 for details.

• Course focus knob: This knob (see both Fig. 4 and 5) is the larger, outside wheel of the focus knobs. This knob will move the objectives up and down in relatively large increments when focusing on a specimen. BE CAREFUL WHEN USING THIS KNOB. Operate this knob slowly! Moving an objective downward too quickly or forcefully can damage both the specimen and the objective.

• Fine focus knob: This knob (see both Fig. 4 and 5) is the smaller of the focus knobs. This knob also moves the objectives up and down, but in very small increments when focusing on a specimen. BE CAREFUL WHEN USING THIS KNOB. Operate this knob slowly! If an

![Figure 5. Labeled side view image of an Olympus Model CH2 microscope.](http://faculty.ccbcmd.edu/courses/bio141/labmanual/lab14/focusing/microscoperight.html)
objective is moved downward too quickly or forcefully, both the specimen and the objective can be damaged. The proper use of this knob is especially critical when using it with the oil immersion objective. In addition, when using this knob to finely focus on an object, an image’s depth of field can be visualized and perhaps provide unique details of a specimen.

- Iris diaphragm lever: This lever is critical to adjusting the amount of light passing through a specimen. It is a component of the condenser (not labeled; see both Fig. 4 and 5), the large unit under the stage. By moving the lever to one side or the other, the amount of light passing from the light source through the condenser lens can be regulated. In practice, the lever should be nearly closed when using the 4X objective, gradually opened with the higher power objectives, and opened fully when using the 100X objective. Regulating the intensity of light with this lever provides the needed viewing contrast.

- Condenser: This microscope component, which is not labeled in Fig. 4 and 5, possesses the iris diaphragm lever (described above). The condenser has a lens that fits in the hole of the stage. The condenser ‘concentrates’ light from its source through this lens before it passes through the specimen. There is a knob (not clearly shown or labeled) on the side of the condenser for moving it up and down below the stage. For most practical applications of the light microscope, the condenser should be moved fully upward. Do not overstress the knob when doing so and be sure to clean the lens of any oil or liquids. Moreover, scratches on this lens are problematic – be careful!

- On/off switch: Obviously, this is the switch that provides power for the light source. Before turning the switch to ‘on’, the voltage control dial (see below) should be turned down to minimum power. When work with the microscope is completed, be sure to turn the switch to ‘off’ rather than ‘pulling the plug’ on the outlet. If the microscope does not ‘power up’, inform the laboratory instructor. Always seek help to fix this problem.

- Voltage control dial: This dial controls the amount of energy that flows to the light source. By turning it in one direction or the other, the intensity of the light can be varied and used to provide proper contrast for viewing specimens. The dial may be used in conjunction with the iris diaphragm lever to regulate the amount of light passing through a specimen. Do not be afraid to experiment using both in order to properly view a specimen. When using the 100X objective, however, the dial should be initially in a position that provides the maximum amount of light. From there, the intensity of light can be adjusted to provide the proper contrast. As a caution, the voltage control dial should be turned down to minimum power when initially switching on the microscope.

Key Characteristics of the Modern Microscope. There are two key properties important to the usefulness of a microscope: magnification and resolution. Another concept to understand is depth of field/focus. The discussion that follows is a brief description of these three concepts. For greater detail and background information on these subjects (and more than is really needed to succeed in this exercise), go to MicroscopyU (https://www.microscopyu.com/).

Magnification is the enlargement of an image of a specimen as viewed through a microscope. The image of a specimen can be magnified, i.e., enlarged, either with the light microscope to a limited degree or with the electron microscope down to the level of the atom. However, magnification alone does not provide a clear, sharp image. The latter is dependent upon the property of resolution.

Resolution is the ability to distinguish between two or more closely laying objects. In bright-field microscopy, this property is dependent upon the wavelength. The smaller the wavelength
of light, the greater the resolution power of a microscope. Though a physics lesson is not intended here, the following illustrates the concept of resolution.

In his seminal observations using a microscope, as recorded in the publication of *Micrographia* (http://www.gutenberg.org/ebooks/15491) in 1665, Robert Hooke drew a remarkable and exquisitely detailed image of a flea (Fig. 6). The detail was possible due to the resolution of the lens he employed. Hooke was also fortunate that nature uses a wavelength of visible light that permits such detail to be observed. If the wavelength of light was different, then the image of the flea would be far less detailed. This concept is borne out in the exaggerated, but conceptually correct illustration presented in Fig. 7.

If wavelengths of light are represented as balls, then the larger balls (i.e., larger wavelength; Fig. 7a) employed would have a difficult time fitting tightly to the smaller areas outlining the image of a flea. The resulting image would appear somewhat blurry and having no real detail. However, smaller balls (i.e., shorter wavelength; Fig. 7b) would more snugly occupy the close areas of the flea thereby generating an image with far more detail. Hence, the smaller the wavelength, the greater the resolution of a microscope. This also explains how an electron microscope provides extreme detail. The ‘illumination’ source of electron microscopes is the electron. Electrons fired from a source are of a far, far smaller wavelength than light photons, thereby providing magnitudes of greater resolution.

Finally, depth of field (and focus) is a property that can be viewed by the fractional movement of the light microscope objective. Specimens, particularly large ones, have volume or thickness. In addition, if a coverslip is used, there is a small, but finite volume of medium between it and the slide. Hence, one can focus at different levels of view. This ‘depth of focus’ can facilitate viewing an image in three dimensions, but it can also be somewhat of an issue when initially looking for an object under the microscope. It is possible, actually very likely, that until a student becomes proficient at using a microscope, each will have difficulty in focusing on a specimen due to having focused instead on a speck of dust or dirt that is laying on the surface of a coverslip or within the milieu that exists between a coverslip and the slide.
Purpose
To be successful in this laboratory course, students must acquire a degree of mastery in the proper use of the microscope. Hence, the primary objective of this exercise is for the student to become comfortable and competent in the use of the microscope.

The following procedure, properly conducted, will provide students the opportunity to compare eukaryotic cells with the three basic prokaryotic cell types that exist: cocci (round ‘ball-like’ cells), rods (elongated cells), and spirilla (‘wavy/curly/spiral-like’ cells). This procedure will also test a student’s ability to properly manipulate the microscope.

Materials Required
The following materials are necessary to successfully conduct this exercise:

- Newspaper print
- Scissors
- Disposable plastic bulb pipette
- Sabouraud dextrose agar Petri dish culture (3-5 days old) of the yeast (single-celled fungus) *Saccharomyces kudriavzevii* (ATCC 2601; formerly designated as *Saccharomyces cerevisiae*) [abbreviated as *S. kudriavzevii*]
- Sterile toothpicks
- Prepared slides of different bacterial cell types (cocci, rods, and spirilla)

Procedures
Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

It is imperative that the procedure described below be taken seriously and that each student actively engage in this process. While a student may have previous hands-on familiarity with microscopy, it is nonetheless necessary to review the proper use and care for a microscope.

Different models of bright-field microscopes exist in teaching laboratories across the world. The following instructions apply to the proper handling and use of almost every light microscope. However, the particular directions described here are for the binocular microscopes that currently exist in the BIOL 3702 laboratory. It is critically important that students follow these general instructions not just for the responsible use and care of the microscope, but also so that requisite skills can be acquired and employed throughout the remainder of this semester as well as possibly beyond in future endeavors.

Additionally, it is expected that students become independently proficient in their microscopy skills. Initially, the laboratory instructor will assist students in the development of these skills. Yet, within a couple of weeks, the laboratory instructor will no long “hold hands” of students using a microscope. While peer assistance is a good and valuable thing, students should not count on it. Hence, the sooner a student learns how to effectively use a microscope, the student will not only fare better in the laboratory, but also will gain a better educational experience.

Part A: Preparing to Use the Microscope
1) From the cabinet beneath the laboratory bench, remove the microscope using both hands.
   Standard practice is to use one hand placed under the base of the microscope and the other
on the arm of the instrument. Carefully place the microscope on the bench top in an area clear of other materials.

Note: There is a chain that attaches the microscope to the cabinet to help prevent it from “walking away”. Be careful not pull out the microscope too quickly or the chain could cause it to be jerked from the hands (thereby crashing the microscope, possibly damaging it, and resulting in needless grief for the student and others).

2) Use lens paper only and wipe clean the ocular and objectives lenses. If necessary, use lens cleaning fluid to remove any oil on the lenses and other areas of the microscope. Discard the lens paper in the normal trash receptacle.

3) If needed, use a Kimwipe™ or a small piece of paper towel to absorb oil or liquid on other microscope parts. Discard the Kimwipe™/paper towel in the normal trash receptacle.

4) Holding the plug and not the cord, connect the microscope to an electric source.

5) Turn the condenser control to raise the condenser as far up as it will go. (Don’t force the control knob!)

6) Using the proper control, knob lower the stage as far down as it will go. (Don’t force the control knob!)

7) Be sure that the low power (4X) objective is set in place over light path that will be guided by the condenser lens.

Part B: Initial Observations Using a Microscope

1) Prepare a “wet mount” of newsprint as follows:
   a) Obtain a glass slide and place a tiny drop of water (about a quarter the size of a fingernail) in the center. Use a disposable plastic bulb pipette to obtain water from the labeled water bottle on the bench top.

   Note: Retain this pipette for use again in Part C of this exercise described below. If the pipette is wrapped in paper, discard the wrapper in the normal trash receptacle.

   b) Using scissors, cut a letter or two from the newspaper provided.

   c) With forceps, pick up the piece of newsprint and place it directly into the drop of water on the slide.

   d) Holding a coverslip at a 30-45° angle between the forefinger and the thumb, place one edge into the water drop, then gently lower the coverslip over the newsprint trying to avoid creating air bubbles. For the purposes of this portion of the exercise, a proper slide preparation will have the newsprint covered in water that reaches to the edge of the coverslip.

   • If the coverslip is “floating” over the newsprint, then too much water was applied to the slide. Use Kimwipe™ or a small piece of paper towel to absorb the excess water by touching it to the edge of the coverslip. Discard the Kimwipe™/paper towel in the normal trash receptacle.

   • Alternatively, if the water does not reach the edge of the coverslip, carefully place a drop of water along the edge to fill any empty spaces. If too much water is applied, remove it as above using a Kimwipe™ or paper towel piece. Discard the Kimwipe™/paper towel in the normal trash receptacle.
2) Place the newsprint slide prepared in Step 1 with the coverslip facing up into the stage slide holder of the microscope. Be sure the slide is snugly held in place by the spring clip of the slide holder.

3) Using the power switch, turn on the light source being sure to turn the voltage control dial to provide a lower light intensity.

4) With the mechanical stage controls, position the coverslip on the slide into the light path produced by condenser lens. The newsprint should be directly in the light path.

5) Adjust the distance between the ocular eyepieces such that only a single image of light is visible. If necessary, increase the light intensity by turning the voltage control dial.

Note: If a student wears eyeglasses or contacts for near-sightedness, the laboratory microscopes are constructed to be used with these items. Otherwise, students should remove their eyeglasses.

6) Looking from the side of the microscope, turn the course focus knob until the stage/specimen and the 4X objective are as close as possible. Note the direction that the course focus knob was turned.

7) While viewing the newsprint through the ocular lenses, slowly turn the course adjustment knob in the opposite direction to increase the distance between the objective and the specimen. A rough, out of focus image should appear as the knob is turned. The stage controls might need to be used to move the edge of the newsprint into the field of vision. If needed, adjust the light intensity using the condenser diaphragm lever such that the image is visible and well contrasted against the background.

8) Adjust the ocular lenses to conform with the visual acuity of each eye as follows:
   a) While looking through the ocular lenses at the newsprint, close the left eye.
   b) Slowly turn the fine focus adjustment until the newsprint image becomes sharp and clear. Again, adjusting the light intensity may be necessary.
   c) Open the left eye and close the right eye. Use the left eye-piece adjustment ring (i.e., diopeter adjustment ring) to focus the newsprint image for the left eye. Do not use the fine focus knob! Now, the image under the 4X objective should appear sharp and clear with both eyes open (though some additional fine focus may be needed using the fine focus knob).

The ocular lenses should remain fairly well-focused regardless of the objective used. However, students may occasionally re-check the ocular focus when using different objectives to ensure that both eyes are in the proper plane of focus.

9) Visually scan the newsprint using the fine focus adjustment as needed. Note that slightly moving the fine focus knob up and/or down permits one to see details at different levels, i.e., the depth of field/focus.

10) Rotate the nosepiece and click into place the 10X objective. Because the microscope possesses the characteristic termed parfocal, the newsprint image should be almost in focus already. Use the fine focus knob to sharpen the image, if needed, as well as adjusting.
increasing the light intensity to provide better contrast. Again, moving the fine focus knob up and/or down presents different depths of field/focus.

*Visually scan the newsprint at 100X magnification. Record any observations on the data report sheet attached to this document.*

11) Rotate the nosepiece and click into place the 40X objective. Again, the newsprint image should be almost in focus already. Use the fine focus knob to sharpen the image, if needed, as well as adjust the light intensity to provide better contrast (see Fig. 8). Take note of different fields of depth/focus.

*Visually scan the newsprint at 100X magnification. Record any observations on the data report sheet attached to this document.*

12) Now view the newsprint employing the 100X (oil immersion) objective as follows:

   a) Turn the nosepiece halfway between the 40X and 100X objectives.

   b) From the bottle of immersion oil, place a single drop of oil directly onto the coverslip spot that is to be viewed under the microscope.

   c) Move the slide using the mechanical knobs to locate the oil drop over the center of the condenser lens.

   d) Turn the nosepiece such that the 100X objective is set in place. If viewed from the side of the microscope, it will appear that the immersion oil “jumps” onto the objective lens.

   e) Increase the light intensity by moving the voltage control dial to full power and by adjusting the diaphragm lever to permit all this light to pass through the condenser.

   f) Looking through the ocular lenses, *carefully* and *slowly* turn the fine focus knob to produce a sharp image (see Fig. 8).

   **Caution:** Do not turn the focus knob so far such that the objective “crashes” into the coverslip (thereby, possibly scratching the lens).

   Note that adjusting the knob up and down will permit the depth of field/focus to be visualized. Depending upon the field of view, the light intensity may need adjustment using the voltage control dial, the diaphragm lever, lowering the condenser, or a combination these actions.

   g) *Visually scan the slide at 1000X magnification and record any observations on the data report sheet attached to this document.*

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**Figure 8.** Microscopic image of the letter ‘e’ from newsprint observed at 400X (left) and 1000X (right). (http://www.csun.edu/scied/7-microscopy/microscopy-techniques/microscopy_index.html)
13) When all observations have been completed, turn the nosepiece halfway between the 100X and 4X objective. Next, turn the coarse adjustment knob to raise the objective well above the stage. BE SURE TO PERFORM BOTH OF THESE ACTIONS BEFORE REMOVING THE SLIDE FROM THE MICROSCOPE STAGE.

14) Remove the slide and discard it in the sharps container.

15) Use lens paper to remove the oil from the 100X objective. Likewise, remove any oil that is present on the stage or other objectives using a Kimwipe™ or paper towel piece. **Discard the lens paper and/or Kimwipe™/paper towel in the normal trash receptacle.**

**Part C: Observing Eukaryotic Cells**

1) Prepare a “wet mount” of eukaryotic cells as follows:

   a) Obtain a glass slide and place a tiny drop of water (about a quarter the size of a fingernail) in the center. Use the disposable plastic bulb pipette from Part B.1.a. above (or acquire a new one) to obtain water from the labeled water bottle on the bench top. The water bottle on the bench top can serve as the source of water.

   b) Using a toothpick, remove the lid of the Petri dish containing the single-celled fungus (i.e., yeast), Saccharomyces kudriavzevii. Gently touch the toothpick to a single yeast colony growing on the surface of the medium in the petri dish. Very little cellular matter should be visible on the toothpick. Replace the petri dish lid.

   c) Place the end of the toothpick with the cells in the water drop on the slide. Twirl the toothpick to remove the yeast cells.

   d) Remove the toothpick and discard it in the bench-top waste disposal bin.

   e) Holding a coverslip at a 30-45° angle between the forefinger and the thumb, place one edge into the water drop, then gently lower the coverslip over the newsprint trying to avoid creating air bubbles. Avoid having your fingers become wet. For the purposes of this portion of the exercise, a proper slide preparation will one in which the water reaches to the edge of the coverslip.

   **Note:** Although *S. kudriavzevii* is not a human pathogen (think beer and wine!), it is not good practice to be needlessly exposed to any microbe. If your fingers or hands do become wet at any stage of this particular procedure, be sure to wash thoroughly.

   - If the coverslip is “floating” on the cell suspension, then too much water was applied to the slide. Use Kimwipe™ or a small piece of paper towel to absorb the excess water by touching it to the edge of the coverslip. Avoid having your fingers become wet with fluid from the Kimwipe™/paper towel. **If a Kimwipe™/paper towel is used, discard it in the waste disposal barrel.**

   - Alternatively, if the water does not reach the edge of the coverslip, use the bulb pipette to carefully place a drop of water along the edge to fill any empty spaces. If too much water is applied, remove it as above using a Kimwipe™ or paper towel piece. Avoid having your fingers become wet with fluid from the Kimwipe™/paper towel. **Discard the Kimwipe™/paper towel in the waste disposal barrel.**

   **At this point in the procedure, also discard the bulb pipette in the bench-top waste disposal bin.**

2) Place the “wet mount” slide of *S. kudriavzevii* cells in the slide holder on the microscope stage. Use the appropriate procedures that were described/practiced above to visualize the...
yeast cells using the 4X, 10X, 40X, and 100X objectives (total magnification 40X, 100X, 400X, and 1000X, respectively). Fig. 9 depicts a microscopic image of typical *S. cerevisiae* budding yeast cells.

*Visually scan the slide at each magnification and record any observations on the data report sheet attached to this document.*

3) When all observations have been completed, turn the nosepiece halfway between the 100X and 4X objective. Next, turn the coarse adjustment knob to raise the objective well above the stage. **BE SURE TO PERFORM BOTH OF THESE ACTIONS BEFORE REMOVING THE SLIDE FROM THE MICROSCOPE STAGE.**

4) Remove the slide and *discard it in the sharps container.*

5) Use lens paper to remove the oil from the 100X objective. Likewise, remove any oil that is present on the stage or other objectives using lens paper or Kimwipe™/paper towel, as appropriate. **Discard the lens paper/Kimwipe™/paper towel in the normal trash receptacle.**

Part D: Observing Bacterial Cell Types

1. Retrieve a prepared slide of three different, pre-stained bacterial cell types (Fig. 10).

   **Note:** When finished using this slide, **DO NOT DISCARD IT! Return it to the proper storage box.**

   If the slide has a layer of oil on it, use lens paper to gently wipe it clean. **Discard the lens paper in the normal trash receptacle.**

2. Hold the prepared slide up to the light and try to see any stained spots underneath the sealed coverslip. Typically, a small dark or bluish area near one end of the slide underneath the coverslip should be visible. This spot will serve as the initial guide to the positioning of the slide. If no spot can be viewed, estimate that the cells are centered about a third of the distance from the end of the slide.

2) Place the slide on the stage of the microscope using the slide holder to secure the slide. With the mechanical stage controls, move the bluish spot on the slide over the center of the condenser lens.

3) Employing the microscopic procedure previously detailed, use the low power (4X) objective to try to locate and focus on the bluish spot.

4) Rotate the nosepiece and click into place the 10X objective. Use the fine focus knob to sharpen the image, if needed, as well as adjusting the light intensity to provide better contrast.

5) Repeat the above step after rotating the nosepiece to click into place the 40X objective. Again, use the fine focus knob to sharpen the image, if needed, as well as adjusting the light intensity to provide better contrast. At this point in the procedure, single cells should be visible.

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6) Examine the cells under the 100X (oil immersion) objective by following the procedure detailed in above. Be sure to completely open the diaphragm and the illumination should be at maximum intensity. Again, use the fine focus knob to sharpen the image and, if needed, adjust the light intensity to provide better contrast.

*Visually scan the selected portion of the slide and record any observations of the one bacterial cell type (the cocci) on the data report sheet attached to this document.*

7) Using the mechanical stage controls, slowly move the slide to the middle portion of the slide looking for the second bacterial cell type. Additional immersion oil is usually not needed, but a drop can be placed on the slide if the proper amount of oil appears to be lacking. Looking through the ocular lenses, *carefully* and *slowly* turn the fine focus knob to produce a sharp image.

*Visually scan the selected portion of the slide and record any observations of the second bacterial cell type (the bacilli) on the attached data report sheet.*

8) Repeat the above procedure for the third portion of the slide to observe the third bacterial cell type (the spirilla).

*Visually scan the selected portion of the slide and record any observations on the data report sheet attached to this document.*

9) When all observations have been completed, remove the prepared slide from the microscope stage. The slide has immersion oil on it. The slide must be wiped clean with lens paper, NOT paper towel so as to avoid scratching the glass cover slip. *Discard the lens paper in the normal trash receptacle.*

10) **BE SURE TO RETURN THE PREPARED SLIDE TO THE PROPER STORAGE BOX. Do not otherwise discard the slide!**

**Part E: Storage of the Microscope**

When work is completed with the microscope: 1) turn the voltage control dial to its lowest setting; 2) use the on/off switch to cut the power to the light source; 3) holding the plug and not the cord, disconnect the microscope from the electrical outlet; 4) use lens paper, Kimwipe™, or paper towel, AS APPROPRIATE, to remove any oil or liquids from the lenses or other parts of the microscope (discard the lens paper, Kimwipe™, or paper towel in the normal trash receptacle); 5) wrap the power cord around the microscope (or in the case of the Model CX23, store it in the space at the back of its arm); and 6) using both hands (one on the arm and the other under the base), return the microscope to the storage cabinet.

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**Video Review:** The YouTube video available at the following URL ([https://youtu.be/-b3Eejf4rDQ](https://youtu.be/-b3Eejf4rDQ)) is an amusing, yet informative overview of the proper use of a microscope. Other than the re-use of microscope slides, essentially all that is presented in this video is applicable to this laboratory course. Every microbiology laboratory student is encouraged to watch this video.
HELPFUL HINTS AND PRECAUTIONS FOR THE PROPER USE AND CARE OF THE MICROSCOPE

- Do not force any adjustment knobs. If there is an issue, consult the laboratory instructor.
- Always clean the microscope, the oculars, and its objectives before and after use. Use lens paper and Kimwipe™/paper towel as appropriate!
- General rule:
  - Lower magnifications require less light (lower the condenser and/or move the iris diaphragm to provide less light and/or move the voltage control dial to provide less power to the light source).
  - Higher magnification requires more light (raise the condenser and/or move the iris diaphragm to provide more light and/or move the voltage control dial to provide more power to the light source).
- Always be sure that the slide is placed on the stage in the correct orientation:
  - The coverslip always faces the objective.
  - The specimen (stained side) always faces the objective.
- When repeated attempts to find and focus on a specimen fail, it is sometimes due to being on the improper depth of field/focus. Start from the beginning, but first try focusing using the low power objective on an air bubble (wet mount) or particle (prepared slide with a coverslip) or a surface contaminant (stained slide without a coverslip).
- Other hints for viewing stained specimens under the oil immersion objective:
  - Not enough oil was applied, thereby causing a “fuzzy” appearance.
  - There are bubbles in the oil. Properly remove the slide, wipe/blot away the oil, carefully replace the slide, add more oil, then refocus.
  - Be sure the specimen is centered.
  - Were enough cells placed/stained on the slide?
  - The proper side of the slide is not facing the objective.
  - Slowly move the fine focus knob up or down. If moved too quickly, the plane of focus can “fly by”.
  - Adjust the light intensity so that your eye sight is not blinded or the specimen “washed out” by too much light.
Student Name: __________________________

**DRAWINGS OF MICROSCOPIC IMAGES OF NEWSPRINT**

Magnification _______  Magnification _______  Magnification _______

**DRAWINGS OF MICROSCOPIC IMAGES OF YEAST CELLS**

Magnification _______  Magnification _______

Magnification _______  Magnification _______

Magnification _______  Magnification _______
Student Name: __________________________

**DRAWINGS OF MICROSCOPIC IMAGES OF BACTERIAL CELL TYPES**

- Cocci
- Bacilli
- Spirilla

**DISCUSSION QUESTIONS**

Answer the following questions in complete, grammatically correct sentences except where indicated.

1) What is the difference between ‘resolution’ and ‘magnification’?

2) What is the relationship between ‘depth of field’ of a specimen and the necessity to ‘focus’ on a specimen?
Student Name: ________________________________

3) Why is it necessary to use oil to improve the ability to see a specimen with the 100X objective? [This answer may require some background research. Be sure to cite your references!]

4) If the ocular lens was 5X instead of 10X, what would be the magnifications achieved with the:
   a) 4X objective? __________
   b) 10X objective? __________
   c) 40X objective? __________
   d) 100X objective? __________