

## THE COMPOUND BRIGHTFIELD MICROSCOPE

Microbiology is the study of microscopic organisms that are so small that they are below the limit of vision of the human eye. Bacteria are the smallest of microorganisms and usually measure no more than **2 micrometers (or 2 microns)**; the limit of vision for the human eye is approximately 200 micrometers. In order to observe bacteria a microscope must be employed.

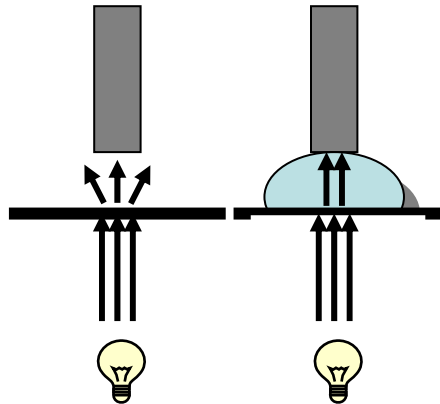
While there are a wide range of microscopes used in the study of microorganisms, the type of microscope used in most teaching labs is the **compound brightfield** microscope. Organisms viewed under this type of microscope appear dark against a bright background or field. Furthermore, these microscopes have two sets of lens. The specimen under observation is first magnified by an **objective lens** (the lens closest to the specimen); the **ocular** or eyepiece lens (the lens closest to the observer's eye) magnifies the specimen further. If one observes the specimen at an objective lens setting of 10X and an ocular lens setting of 10X, then the specimen has been magnified 10X by both lenses. For example, the objective lens first produces an image that is ten times larger than the specimen; the ocular lens enlarges this image another ten times to produce an image appears one hundred times larger than the specimen being viewed—this is the **total magnification**. One can calculate the total magnification produced by a given set of lenses by multiplying the magnification produced by the objective lens and the magnification produced by the ocular lens. **(10X)(10X) = 100X total magnification**. When one calculates the total magnification one should keep in mind that the X stands for the word *times*; it is not treated as a point of measure therefore one would not say that the total magnification is  $100X^2$ . Our microscope has four objective lens settings (scanning lens, 4X; low power lens, 10X; high dry power lens, 45X and oil immersion lens, 100X); the ocular lens is always set at 10X.

**Calculate the total magnification of a specimen if the objective lens were set at 45X.**

**What is the greatest possible total magnification of the compound brightfield microscope that we will be using in our lab exercises?**

The size of the lens is inversely proportional to the magnification of the lens. Therefore, at a higher magnification there is a smaller lens aperture for the light from the light source to pass through. As a result the field or background will appear darker. The **refractive properties of light** also reduce the amount of light that passes through the objective lens. This is because the light rays pass first through the glass containing the specimen in question and then through the air before it reaches the objective lens. As light travels faster through air than glass, the light that has been projected through the glass bends away from the lens as the light passes through the glass to the air before encountering the lens. When using the 4X, 10X and 45X objective lens setting one can increase the amount of light that reaches the objective lens by simply adjusting the **iris diaphragm**. The oil immersion lens is too small to accommodate the decrease in light by adjusting the iris diaphragm, at the 100X dry setting the light will simply bypass the lens. To circumvent this, one uses a special **immersion oil** when one is viewing a specimen

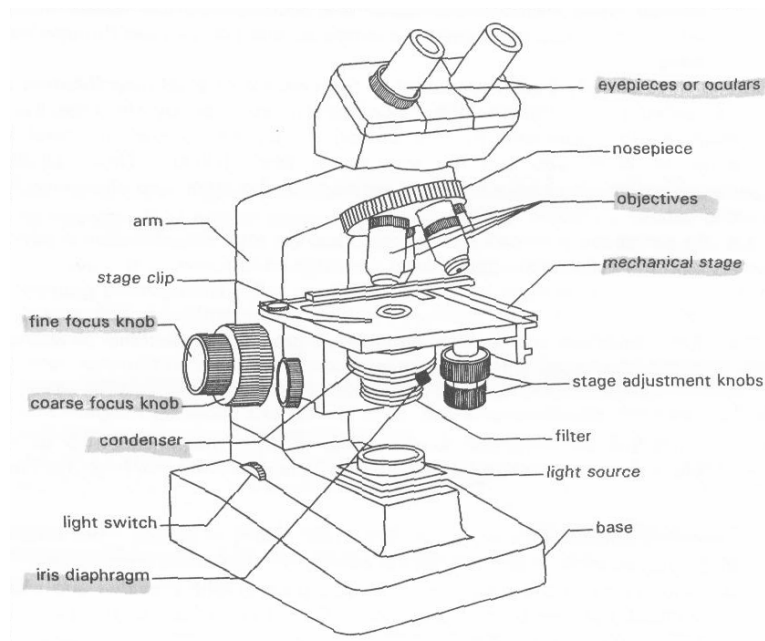
under the 100X magnification. Immersion oil is useful because its refractive index is almost the same as glass. The use of immersion oil obviates the passage of light through air, therefore, if immersion oil is used in conjunction with the 100X lens then the light will be directed into the lens.



**Figure 1.** The effect the refractive properties of light has on the amount of light that passes through the 100X objective lens with and without immersion oil.

Another feature of the microscope that we will be using in the lab is that it is **parfocal**. When one is using a microscope one first focuses on the specimen under the lowest objective, the parfocal nature of the microscope insures that the object in question will remain in focus as one switches to objective lens settings with higher magnifications. Usually, one only needs to make slight changes to the **fine focus knob** of the microscope to bring the image into clear focus at these higher magnifications.

The figure below shows the components of a compound brightfield microscope. Find each of these components on your microscope.



**Viewing fixed stained bacteria:** Now that you have identified the components on your own microscope, you will use the microscope to observe bacteria on a prepared slide. The bacteria in question is *Bacillus subtilis*. *B. subtilis* is a Gram positive rod; this specimen has been stained with crystal violet and will appear purple.

First clean all of the objective lenses using the lens paper provided in your drawer; start with the 4X objective and clean the 100X objective last.

Obtain one of prepared slides from the instructor and place it (specimen side face up) on the **mechanical stage** using the **stage clip** to secure the slide. Use the **stage adjustment knobs** to move the mechanical stage and to center the specimen over the **condenser**.

Use the low power objective ( \_\_\_X) to initially view the specimen. Focus the specimen by first using the **course adjustment knob** and then by using the **fine focus knob** to clearly focus your specimen.

**Draw what you observe under this magnification; what is the total magnification?**

Switch to the high power dry objective ( \_\_\_X) by rotating the **nosepiece** until you hear or feel the objective click into place, Do not remove the slide or the touch the course adjustment or fine focus knobs as you are changing objectives. Because the microscope is parfocal you should view your specimen first, then you can make any adjustments to bring the image into focus using the **fine focus knob** only.

**Draw what you observe under this magnification; what is the total magnification?**

Finally you will observe your specimen under the highest possible magnification. To do this rotate the nosepiece until it is positioned halfway between the 45X and 100X objectives, add a small drop of the immersion oil to the specimen ( again do not remove the slide or the touch the course adjustment or fine focus knobs). Now switch to the oil immersion objective ( \_\_\_X) by rotating the nosepiece until the objective clicks into place. You can again make any required adjustments by using the fine focus knob. If the field appears too dark to view the specimen you can increase the amount of light that reaches the objective by adjusting the **iris diaphragm**.

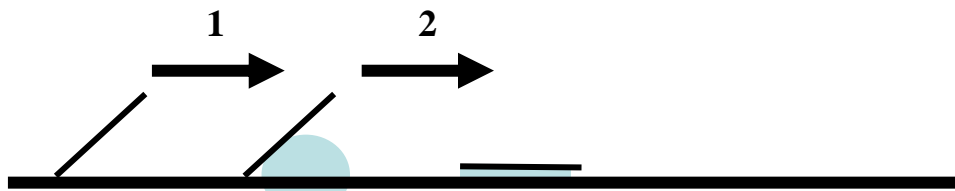
**Draw what you observe under this magnification; what is the total magnification?**

When you are finished viewing the specimen, rotate the nose piece to the 4X (\_\_\_\_\_ objective) **without going through the 45X and 10X objectives. This prevents the immersion oil from coming into contact with these objectives. This is important, because these lenses are not tightly sealed like the oil immersion lens and if oil gets into these lenses they will damage the lenses.** Remove the slip from the mechanical stage by pulling back the clip, return the slide to the instructor. Clean the lenses using the lens paper provided in your drawer. **Clean the oil immersion lens last.**

**Viewing live unstained microorganisms.** The observation of live microorganisms is somewhat more challenging than that of fixed and stained bacteria, but is still possible with the compound brightfield microscope. The instructor will provide a **hay infusion** such that you can gain experience in viewing live microorganisms. A hay infusion is hay that has been placed in nutrient broth such that the organisms present in the hay grow in the broth. A hay infusion mimics a pond-like habitat such that you can gain some idea of the diversity of microorganisms found in that environment. Furthermore, because you are observing live organisms, you will be able to see some of them move. In order to view the organisms you will prepare a **wet mount** of the hay infusion.

With a wax pencil place a small mark on the surface of the microscope slide; this will make it easier to focus on the microorganisms present in the wet mount. Use one of the droppers provided to place a **small** drop of the suspension onto a clean microscope slide. It is important the drop is a small one to prevent the cover slip from floating of the surface of the slide.

Hold a **coverslip** at a **45°** angle against the microscope slide as shown in **Figure 3**. Move the coverslip along the surface of the slide toward the drop of the hay infusion suspension until the inner surface of the coverslip contacts the liquid. When the liquid spreads across the bottom edge of the cover slip, drop the coverslip onto the microscope slide such that the cover slip covers the drop.



**Figure 3.** How to position and move a coverslip to prepare a wet mount.

Place the low power objective into position and place the slide on the mechanical stage with the coverslip side facing up.

Using the low power objective, focus on the small mark made with the wax pencil and use the fine focus knob to focus on the organisms present in the suspension. If you are

allowing too much light to enter the lens, it may be difficult to see the microorganisms, adjust the iris diaphragm to reduce the light level.

Because some of the organisms in this sample are eukaryotic and larger than bacteria, it should be possible to observe some of the organisms in the population under the low power (10X) objective. You should also see movement of the cells in the population. Identify cells that are 1. actively moving, 2. are moving due to current flow (organisms getting carried along with the liquid as the liquid moves) and 3. randomly bouncing around (Brownian motion).

**Draw what you observe under this magnification, describe how some of these cell types are moving:**

Now switch to the high power dry (45X) objective using the same steps that you used when viewing the stained fixed specimen. Change the fine focus knobs or the iris diaphragm as needed.

**Draw what you observe under this magnification: , describe how some of these cell types are moving:**

Finally, switch to the oil immersion (100X) objective to view live bacteria.

**Draw what you observe under this magnification in terms of bacterial shape; describe how some of the bacteria are moving:**

When you are finished; remove the slide as you did in the previous exercise and discard the slide in a container provided by the instructor. Clean the objective lenses, turn off the power and return the microscope to the cabinet.