Using a Compound Light Microscope

The Compound Light Microscope

The microscope is one of the most important instruments that biologists use daily to study microscopic organisms. Since the 1600s, researchers have been using primitive forms of microscopes in order to better study microscopic life that is invisible to the naked eye. The invention of the light microscope in the 1800s improved upon the primitive design, and allowed scientists to have a revolutionary view into the microscopic world. With the light microscope, the study of cells and microscopic bacteria became possible as previous models did not have the magnifying capacity to observe such small objects. Being able to observe these microscopic organisms gave insight into what was causing many common illnesses and enabled the development of barriers against these pathogens. The compound light microscope is the most common light microscope in use today and will be discussed in detail in this instruction set.

The compound light microscope increases our ability to see details and organisms by about 1000 times and allows us to see small objects separated by less than a micrometer. For perspective, a human hair has a width of about 10 micrometers. The ability of a microscope to distinguish detail is called the resolving power (RP), which depends on the wavelength of light that is used (shown by λ). Numerical aperture, or the amount of light entering the lens, also plays a role in one's ability to view a specimen. With a resolving power of 410 nm, you will be able to see two objects that are 410 nm apart as two distinct objects. If the objects become any closer than this, they will appear as a singular object which is not useful for scientific observation.

The process of using a compound light microscope begins with understanding the different components of the microscope. One should not operate a light compound microscope without a working knowledge of all its components. After taking proper precautions when setting up the microscope, the specimen must be located and focused, and then the image will be enhanced to achieve the clearest picture possible. If necessary, a microscopic slide will be prepared before observation.

For the best experience and most detailed results, a light microscope should be used in a laboratory setting with latex gloves and eye protection. The microscope should be used on a flat surface such as a lab bench to prevent defocusing of the lens. For the first time using this type of microscope, 30 minutes should be allocated to successfully complete the task. After becoming comfortable and familiar with the process, it can be completed in as little as 10 minutes.

Required Materials

You will need these materials and instruments to properly use a compound light microscope. Most of these items will be available in a typical biology laboratory.

- Compound light microscope
- Power source
- Glass microscope slides
- Latex gloves
- Eye protection
- Kimwipes
- Specimen for observation
- Sharps disposal container
- Coverslip

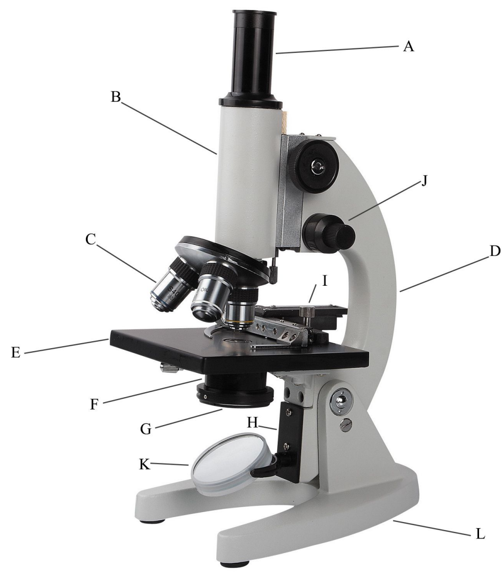

Figure 1. A typical compound light microscope. 18 in x 6 in.
Source: https://tinyurl.com/microscopesource
Edited by: Matthew Ryan

Parts of the Compound Light Microscope

Please refer to Figure 1 to reference the parts of the microscope that will be discussed in this section. While different brands/models will have slightly different features and compositions, most will have these basic parts described.

A. Ocular Lenses

The oculars are the lenses that you look through to see your specimen and can be monocular (single lense) or binocular (two lenses). Figure 1 illustrates a monocular model, however both are commonly used and function the same. The lenses may be rotated and moved for optimal viewing orientation as different people have different spacings between their eyes. If you are using a shared microscope, you may wish to rotate the lenses before use. This alteration is referred to as interpupillary adjustment and is necessary for a comfortable viewing experience. Moreover, oculars on different microscopes may have a different lower viewing magnification (4X or 10X). Some oculars may have a pointer on the lens which is useful in pointing out specific objects on the slide..

B. Body Tube

The light rays from the light source at the bottom of the microscope travel from the objective lens to the ocular through a series of magnifying lenses located within the body tube. In the microscope presented in Figure 1 and most other compound light microscopes, the oculars are positioned at an angle and the body tube contains a prism to bend the light rays to ensure they will pass through the ocular. Without this bend, the light rays would be unable to reach the ocular and thus render the process ineffective.

C. Objective Lenses

Almost all compound light microscopes used today have four objective lenses with different magnification values. These objective lenses are attached to a rotating nosepiece at the base of the body tube. The objective lenses function to focus the light that comes up through the specimen and transmit the image to the ocular. The length of the objective lens is related to its magnification, with the longest lenses have the greatest magnification.

- Scanning lens - 4X Magnification
- Low-power lens - 10X Magnification
- High-power lens - 45X Magnification
- Oil immersion lens - 100X Magnification

The magnification of each lens is printed on the barrel of the lens. Larger objects should be viewed with a weaker objective lens, while more microscopic objects should be viewed with a high power lens. Regardless of the object size, it is best to begin with a low power lens as it helps to first find the specimen. The total magnification while using a particular objective lens is equal to the power of the objective lens times the magnification of the ocular lens (typically 10X). For example, when using the scanning lens (4X), the total magnification is 40X (4*10).

D. Arm

The arm is used to support the stage and substage area. When carrying the microscope, it is important to grab hold of the arm with one hand and place your other hand under the base of the microscope.

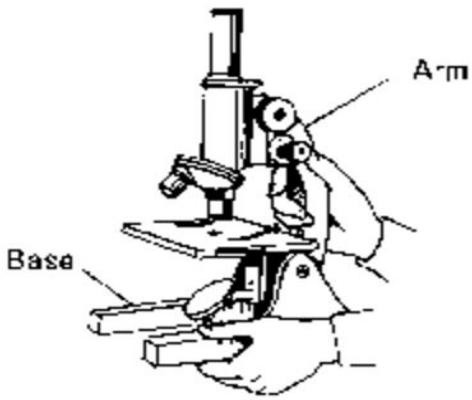

Figure 2. This demonstrates the proper way to hold a microscope.
CAUTION: Failure to do so can result in damage to the equipment.
Source: http://www.deftstudios.com/bioweb/images/blab03A.gif

E. Stage

The platform under the objective lenses and where the microscope slide is placed is referred to as the stage. The opening in the center of the stage is the stage aperture which is where the light travels through the slide. Most microscopes in use today have a movable stage with a device to hold the microscope slide in place.

F. Substage

The area under the stage is referred to as the substage and typically contains both a diaphragm and condenser.

G. Diaphragm

The diaphragm controls the amount of light that is passed from the lightsource through the specimen and through the lenses. The contrast between the medium and specimen can be controlled by adjusting the diaphragm to be wider or more narrow..
Note: This is especially useful when viewing thin, light-colored objects. The diaphragm is typically an iris, but in some models is annular. An iris consist of a circle of overlapping thin metal plates, which allows regulation of light entering the microscope with a lever from the side of the iris diaphragm.

H. Condenser

The condenser possesses several lenses that focus light on the specimen. Using a knob, it can be moved up or down. This knob should be positioned as close to the stage as possible.

I. Stage Adjustment Knobs

There are two knobs located on the top or side of the stage that control its movement. These knobs allow the user to move the slide vertically and horizontally.

J. Focal Adjustment Knobs

Perhaps the most important part of the microscope are the coarse and fine adjustment knobs that are used to focus the microscope. These are located near the bottom of the arm where it joins the base. These knobs function to raise or lower the stage in relation to the objective lenses. The coarse adjustment knob moves the stage a relatively large distance and should only be used with the scanning and low-power objective lenses. The fine adjustment knob moves the stage a much smaller distance and is used for focusing with the high-power and oil immersion lenses.
CAUTION: Do not use the coarse adjustment knob with the high-power lenses as you can break your slides and ruin the lens.

K. Light Source

In most compound light microscopes in use today there is an illuminator that is built in at the base of the microscope. There is typically an on/off switch located on the right side of the microscope. Higher light intensity is used for higher magnification observations.

L. Base

The base of the microscope acts as a stand for the microscope, and houses the illuminator (light).

Correct Handling and Care of the Compound Light Microscope

The microscopes that are used in most biology labs today are very delicate and expensive. When operating a compound light microscope, the following general guidelines should be followed to ensure the longevity of the microscope.

1. As noted earlier in Figure 2, when carrying the microscope from one area to another, it should be carried with one hand on the base and the other on the arm.
2. Before using the microscope, it is essential that the ocular and objective lenses are clean. Failure to do so will result in a foggy image that is not reliable for observation. If the lenses are not clean, use a Kimwipe or lens paper to remove any smudges or oil present.
   CAUTION: To avoid scratching the lens, use a circular wiping motion.
3. Always be sure to keep the microscope away from the edge of the lab bench to avoid destruction of the equipment from falling damage. Keep the electrical cord away from walkways.
4. Always be sure to use clean microscope slides as dirty slides will make viewing of the specimen futile.

5. Your eyelashes should never touch the ocular when viewing a specimen as oil from the lashes will smear the ocular lens.
6. As stated earlier, avoid breaking a microscope slide by first locating the specimen using a scanning/low-power objective. Only switch to the high-power lens after the specimen has been centered and located.
7. Always view the microscope from the side when changing objective lenses to avoid breaking the slides with the objective lenses. This helps to locate exactly how close the lens is to the slide.

Operating the Compound Light Microscope

1. Finding and Focusing the Specimen

The most important and often difficult part of viewing a specimen under a microscope is locating the specimen. Even after a specimen is located, there is often a very fine line between a crisp image and a blurry one.

a. Clean the ocular and objective lenses with a kimwipe to remove any smudges or oil that may prevent a clear view of the specimen. You should have a clear view through the ocular if done correctly.
b. Acquire a clean prepared microscope slide with your choice of specimen. These typically come packaged with the compound light microscope. If a pre-prepared slide is not available, you will have to prepare one (discussed in section 3).
c. Plug in the microscope to a working power outlet.
d. Flip the on/off switch to the “ON” position and fully open the diaphragm to its most open position. You will see light illuminating from the base of the microscope.
e. Place the slide across the stage and using the stage adjustment knobs, center the specimen over the stage aperture to ensure that light is directed through the specimen and into the lens.
   CAUTION: To avoid eye strain, keep both eyes open. Look at the specimen with one eye while keeping the other unfocused.
f. Adjust the light intensity using the diaphragm as needed. Check the intensity by looking through the ocular. If there is too much light, it will wash out the specimen. If it is too dark, the specimen will appear black.
g. Starting with the scanning objective lens, locate the specimen in the lens using the coarse adjustment knob to obtain the clearest image possible.
h. Once the specimen is located, switch to the low-power lens and readjust the focus using the fine adjustment knob to obtain a clearer image.
i. Continue using a higher power lens until desired magnification is achieved. A high-power or oil immersion lens may be required to view subcellular structures.
j. Re-center the specimen and adjust the diaphragm and condenser as needed in order to increase the contrast in relation to the specimen.
   Note: This process is difficult to master and takes practice. If possible, perform this the first time with somebody more experienced.

k. Dispose of the slide in the sharps container when finished.

2. **Improving Image Quality**

After locating your specimen under the highest magnification, there is still room to obtain a clearer image. The image that is viewed in the microscope is impacted by several factors such as orientation, depth of focus, and contrast of the specimen. The following tips will help to ensure that the clearest image possible is achieved.

a. Orientation: The orientation of the image is not the same as when being viewed directly with the human eye. Adjusting the stage knob “up” may actually be moving the stage down. Take note of which direction the stage moves.
b. Contrast: Without sufficient contrast, even the highest power magnification will not be enough to properly view the specimen. Contrast is necessary in order to obtain a sharp image. By increasing the diameter of the diaphragm and allowing more light through, the specimen will appear darker and contrast with the light background.
c. Depth of Focus: The microscope lenses provide a limited depth of focus. When viewing a specimen under the microscope, only a part of the specimen is in sharp focus at any given magnification. If you desire to view the three-dimensional structure of a specimen, viewing it as a series of focal depths is recommended.

3. **Preparing a Slide for Observation (if necessary)**

While many times a slide will already be prepared in the classroom, with laboratory work in the industry you will often need to make your own. If you have found a new type of plant cell that you would like to further analyze, you’ll need to take a part of the plant and view it under a microscope.

a. Using a pipet, add a single drop of water to a new microscope slide. Do not reuse an old slide as contamination from a previous specimen could impact the clarity of the image.
b. After the drop is on the slide, place the specimen in the water. The specimen should be small enough to fit within the drop.
c. Take a plastic square coverslip and place the edge of the coverslip on the side so that it is only touching the edge of the water.
d. To prevent the formation of air bubbles, slowly lower the coverslip over the specimen.

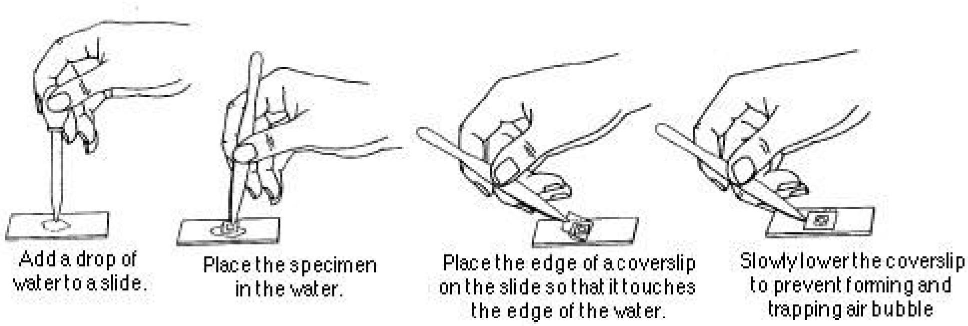

Figure 3 provides a visual demonstration for how to properly prepare a slide.
Source: https://cac-science8.wikispaces.com/Wet+Mount+Slide+Preparation

Troubleshooting Common Issues

- If the light source of the microscope is not turning on, try using a different power outlet. If it still does not work, then there may be an issue with the bulb.
- If during focusing you are having trouble finding the specimen, switch back to the scanning lens. Using a high powered lens can sometimes make locating a specimen difficult.
- If nothing is seen when looking through the ocular, ensure that the objective lens is clicked into place. If the objective lens is not centered over the ocular, nothing will be seen.
- Do not get frustrated if you are having a hard time obtaining a clear image of the specimen. The more you practice, the better you will become.