



Appendix A: Use and Care of Microscopes

Introduction

Human eyes are sensitive only to wavelengths in the middle of the electromagnetic spectrum known as visible light, and do not function well for objects at either extreme of the spectrum. One major reason for our success in analyzing the nature of the universe around us has been our ability to devise various means of overcoming these sense limitations. The invention of the light microscope was important for biological investigations as it renders objects as small as 0.2 micron distinct. The electron microscope further extends this to the limit of a few angstrom units ($1\text{\AA} = 0.1\text{ nm}$). It is important for you to be aware of the size of the objects you will be examining. If terms like micron, nanometer, and angstrom are unfamiliar to you, please consult your textbook for a description. In this laboratory, you will use the compound light microscope and the stereoscopic or dissecting scope.

Resolution and Magnification

Resolution of the image is critical to the study of a specimen. Resolution is the ability to see fine detail; specifically, it is the ability to separate two objects that are too close together to be distinguished by the unaided human eye. Resolution, or resolving power, is typically the limiting factor in any compound microscope. Although a microscope may provide increased magnification, the image can become fuzzy and indistinct as the magnification increases. The useful magnification of a microscope is that magnification at which the image is not only enlarged, but is also observable in fine detail. Magnification without resolution is of little value to investigations in the field of biology.

Related to resolution of the image is depth of focus. As you move the objective lens up and down, you can observe the specimen in several different planes of view. Each plane of view is an extremely thin "slice" through the specimen. As you focus on a specimen that is several cell layers in thickness, notice that different cells come into sharper view as the objective lens is moved up and down.

The Compound Microscope

A microscope is used so that you may observe objects or features that are too small to be seen by the unaided human eye. The compound light microscope is a principal tool for the study of biology. Accordingly, proficiency in the use of the compound microscope is a necessary skill. Follow the directions provided by your laboratory instructor to conduct

the prescribed tasks as a means of developing your ability to use this microscope effectively.

A compound microscope (Figure 1.A) consists of two separate lens systems that work together to increase, or compound, the magnification. The **ocular** lenses, or eyepieces, work in combination with the **objective** lenses to provide the desired magnification of the specimen that you are viewing. The combined ocular and objective lenses also produce an inverted image, or upside-down view, of the specimen on the slide. Each ocular lens has a magnifying power of 10X (the symbol "X" is used to designate "times life size;" 10X = ten times life size). Attached to the microscopes are three or four different objective lenses that provide, respectively, 4X, 10X, 40X, and 100X magnification. The 100X lens (oil immersion lens) will be attached to the microscope only during those laboratory exercises when you need the very high magnification that it provides. The revolving nosepiece holds the objective lenses: rotation of this nosepiece brings the desired objective lens into alignment over the specimen. Both the ocular and objective lenses are mounted such that the arm of the microscope holds these lenses in the correct position for viewing a specimen.

When using the compound microscope, you will place a glass slide with a specimen on the **mechanical stage**. A spring-loaded **slide clamp** holds the slide secure and in position. **Mechanical stage controls**, on the right-hand side of the stage, permit smooth positioning of the specimen beneath the objective lens that is in use. A beam of light from the **illuminator** passes through the **sub-stage condenser** and strikes the specimen. Light intensity is regulated by the controls in the **base** and by the **iris diaphragm** in the condenser. The **coarse** and **fine focus knobs** on the arm allow precise focusing of the specimen. Rotation of the **focus ring** on one of the ocular lenses provides additional clarity of focus. The lens systems are **parfocal**, meaning that the image remains in focus as you switch from one objective to another.

Parts of the Compound Light Microscope

Use Figure 1.A to locate the labeled parts on your compound microscope.

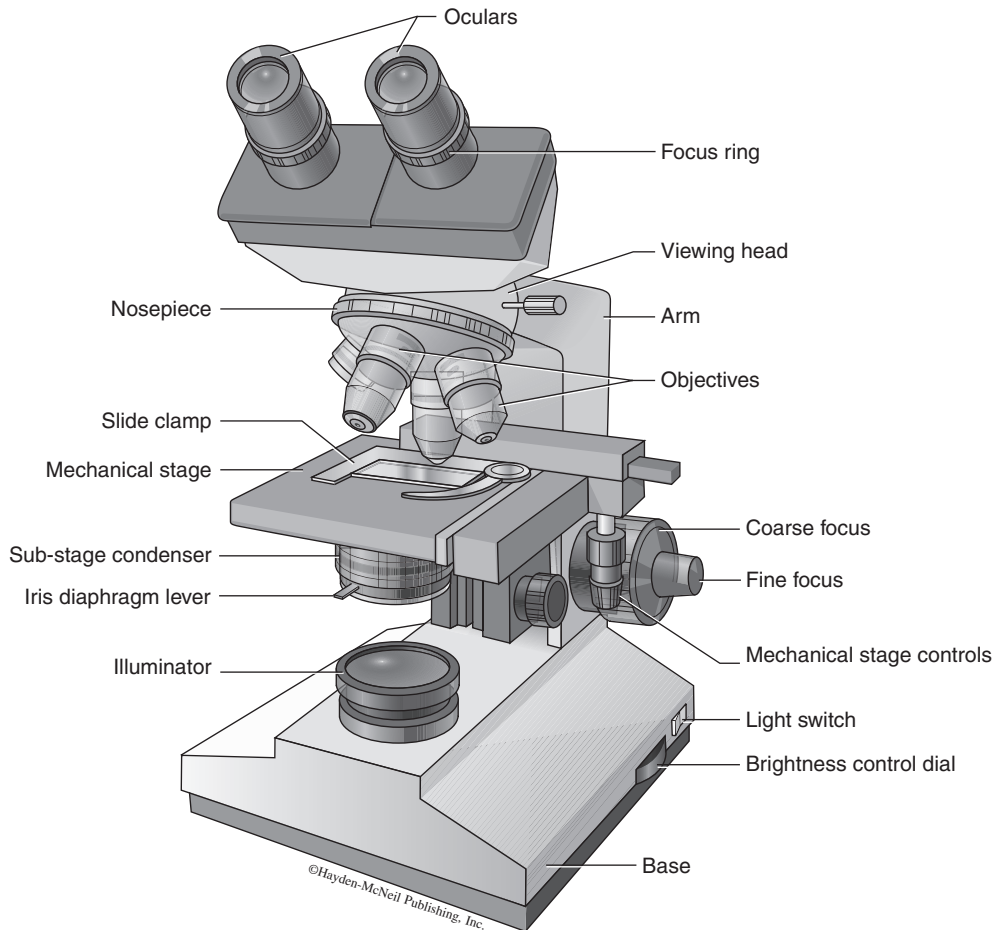


Figure 1.A. Student compound light microscope

The Stereoscopic Microscope

This microscope is said to be stereoscopic because it allows the operator to see a stereoscopic, or three-dimensional, image of the specimen. This image is not inverted. This is quite different from the image observed in the compound microscope. The principal reason that the stereoscopic microscope produces this three-dimensional image is that the two ocular lenses focus on the specimen from slightly different positions.

The stereoscope (Figure 2.A) provides much lower magnification than that of the compound microscope. This is advantageous in that specimens examined with the stereoscope are typically much larger than those viewed with a compound microscope. Furthermore, the specimen is usually opaque so that light does not pass through it; you observe only the surface and cannot see different "slices" by focusing up and down. You use the stereoscope in procedures that do not demand the higher magnification provided by the compound microscope. The stereoscope is often called a dissecting microscope, a name that reflects one of its frequent uses. While dissecting a plant or an

animal specimen, you may need some magnification in order to see structures clearly. Placing these types of specimens under the stereoscope provide the enhanced viewing that you require.



Figure 2.A. The stereoscopic microscope

The Electron Microscope

Although most courses will not use this type of scope in the laboratory, you should be aware that its greater resolving powers have enabled us to examine even organelles in great detail. The resolving power of a microscope is defined as the shortest distance between two points that allows them to be seen as two separate points. The resolving power of the electron microscope, because it uses much shorter electron waves instead of light waves to irradiate a specimen, can be almost 1000x greater than the best light microscope. In the electron microscope, traditional lenses are replaced by electromagnets which focus the electron rays. Electron microscopes are very costly, placing them well outside the budget of undergraduate courses.

Caring for the Compound Microscope

1. Always carry the microscope with both hands. One hand should support the microscope at the base while the other hand holds the arm.
2. Begin by viewing the specimen with the lowest power objective lens in place and then increase to the higher power objective lenses.
3. When ready to increase to a higher power objective lens, make sure that the specimen is centered in the field of view. This will ensure that the specimen will be in view when the new objective lens is in place.
4. Use only the fine adjustment knob to focus the microscope when using the higher power objective lenses.
5. When ready to put the microscope away:
 - Remove the slide from the stage
 - Return the lowest power objective lens into the position over the stage aperture
 - Lower the stage
 - Wrap the power cord around the base
 - Replace the dust cover
6. Clean the objective and ocular lenses regularly with a clean sheet of lens paper. Never use anything else to clean the lenses.
7. Report all breakages and malfunctions to your laboratory instructor.

