The Microscope

Contents

Objectives

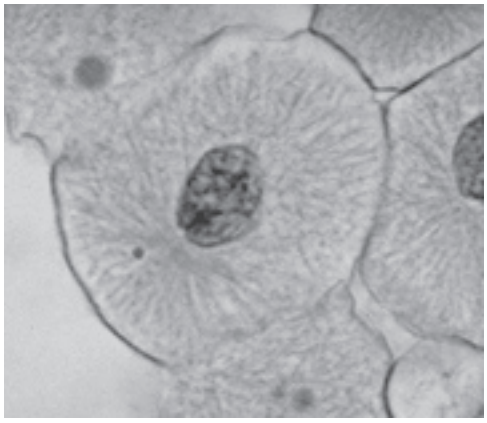
- Learn the basic parts of a light microscope
- Learn how to proficiently use the microscope
- Learn how to determine the magnification of a microscopic image

Introduction

The microscope allows one to see objects too small to be observed by the human eye. Although you will be limited to using a light microscope during this course, other types of microscopes are available which allow one to view much smaller objects in great detail. The most common of these alternative microscopes are the Transmission Electron Microscope (TEM) and the Scanning Electron Microscope (SEM). TEMs are used to examine very thin slices of tissues while SEMs are used to examine surfaces. These microscopes allow magnification in excess of 400,000 x compared to the light microscope's limit of approximately 1,000 x. Micrographs of cells taken with a transmission, scanning, and light microscopes are shown to the right. Compare the type and quality of information you get from these different images.

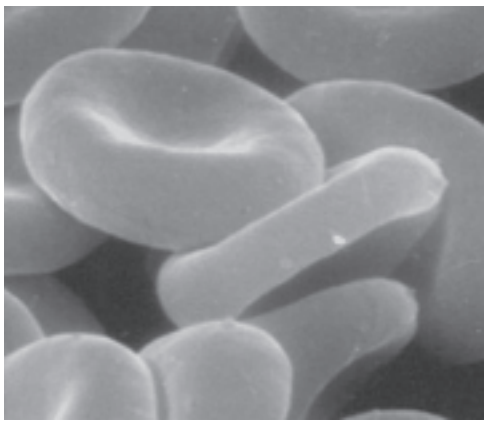
Light Microscope

Image of animal cell



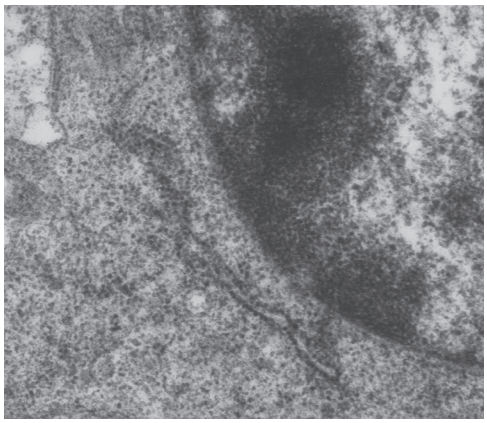
Scanning Electron Microscope

Image of red blood cells



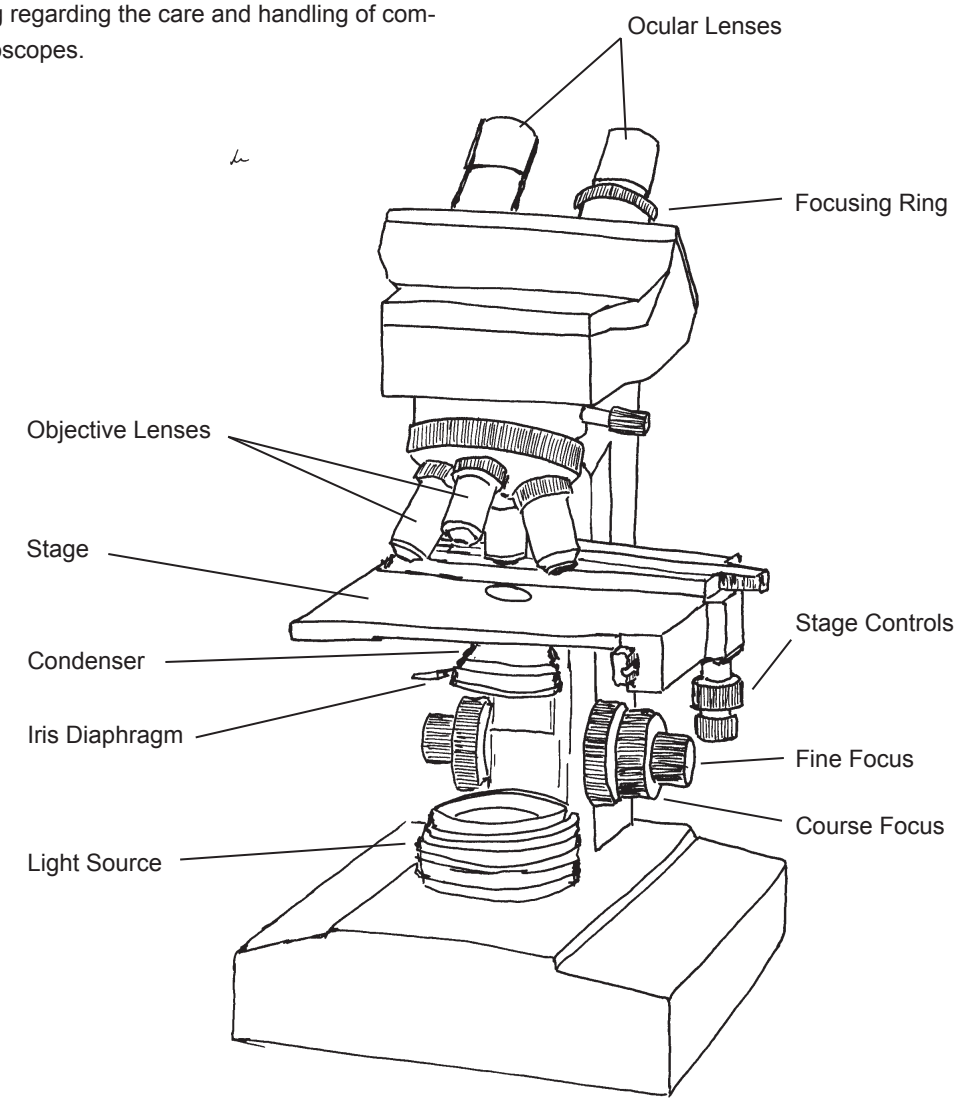
Transmission Electron Microscope

Image of cell nucleus (upper right)



Activity 2a.1
Care of Microscopes

Microscopes are delicate pieces of equipment. While you may not be using a real microscope in this course, you may have the opportunity to use one in the future. Note the following regarding the care and handling of compound microscopes.



Following these simple rules will maintain the microscopes and microscope slide in good condition.

1. Always carry the microscope with two hands.
2. Always start viewing the object with the lowest-power objective lens.
3. Always start by moving the stage as high as possible without touching the objective lens then use the coarse-focusing knob to focus down. Never use the coarse-focusing knob with higher powered objective lenses; only use the fine-focusing knob.
4. Only use lens paper to clean the lenses.
5. When finished, turn off the light source, return the objective lens to low power, remove the slide, wrap the cord around the microscope, and return the microscope to the cabinet using two hands.

Activity 2a.2
Parts of the Microscope

Before trying to observe any objects through the compound microscope, you should learn its various parts, their function and how they operate.

In the Microscope simulation on the BiologyOne DVD, the first screen (after the introductory page) reviews the basic parts of a light microscope. By clicking on the part's name the part will be identified and a short description of its function will pop up. Many parts of the simulated microscope are 'operational'. Try them out. Before going to the second simulation in this exercise be sure you are familiar with the microscope's parts and their functions.

Here is more about the microscope's major parts and hints for their use:

The ocular lens - The lens you look through. This is usually a 10 x magnification lens. Note that the oculars can be moved back and forth to accommodate the distance between each person's eyes. Also note the left ocular has a ring that can be turned to change its focus to accommodate differences between your eyes. To use this, while looking through the microscope, close your left eye and focus on an object using only your right eye. Then close your right eye and look through your left. Turn the ring on the left ocular to bring the object in sharp focus. Taking a minute to do this greatly improves image quality and reduces eye strain.

The objective lenses - Your microscope will have 3 or 4 objective lenses. They are marked with the magnification they provide. These will be 4 x, 10 x, 40 x, and 100 x if there's a fourth. The objective lenses are located on a turret which allows you to easily change from one magnification to another by rotating the turret. The objective lenses are parfocal which means that when you move to a higher power, only fine adjustment of the focus is necessary. The 100 x objective lens is specially designed for oil immersion to give better images at this high magnification. Because of this, without oil, image quality will be low using this lens.

The focus controls - There are two focus controls, a coarse focus adjustment and a fine focus adjustment. In most microscopes today, the focus controls move the stage up and down. When focusing, always start on the lowest power and turn the coarse focus knob until the image is as sharp as possible. When you find the object of interest, center it in the field of view, then move to a higher power, only using the fine adjustment knob. Please note that when at high power, the objective lens is very close to the slide. Moving the coarse focus knob can easily force the objective lens into the slide, breaking one or both.

The stage - The stage has clips to hold your slide in place. It is also equipped with gears which you can turn to easily move the slide under the objective lens.

The condenser and iris diaphragm - These are attached to the bottom of the stage. The condenser focuses the light on the object to be viewed. For our purposes, your best image will result if the condenser is moved to its highest position. If you are having trouble getting an image in focus, check this adjustment. The iris diaphragm controls the amount of light able to enter the microscope. The sharpest images result when the diaphragm is closed as far as possible. Your best results will occur if you start with the diaphragm fully closed and only open it as necessary.

The light source - The light source is an adjustable lamp located below the microscope's stage. Different microscopes will have the light switch in different locations, sometimes as part of the light adjustment knob, sometimes separate. Start with the light low and slowly increase its intensity as necessary as you look through the scope.

There are three slides for you to observe to practice using the microscope; one of the letter e, another of three threads and one of animal tissue. Note the orientation and movement of the image of the letter e. Adjust the fine focus when looking at the threads, noting which one is in focus. Can you determine which thread is on top of the others? Answer the questions in the Results Section.

Activity 2a.3
Viewing Images

The second screen of the Microscope simulation allows you to view images similar to the way you would through a real microscope. There are three slides for you to observe to practice using the microscope; one of the letter e, another of three threads and one of animal tissue. Be sure to click on the microscope's light before you begin.

1. Place the slide with the letter e on the microscope's stage by clicking on that slide. Start with the low objective lens in place. Move the focus knobs so that the stage moves up and down and observe how the image goes in and out of focus.

2. Next, use the stage controls to move the slide from side to side and front to back on the stage. As the slide moves to the left, note the direction the image of the e moves. The same motion occurs moving the slide front to back although you can't see the slide move (as the slide moves toward the front of the stage, the image move down and vice versa).

3. Move the objectives to a higher power by clicking on them. Note the change in magnification of the image. At the highest magnification, the objective lens is large enough that as the stage moves up it can force the slide into the objective lens. This will cause the slide to break and may damage the objective lens. When using a high power objective lens only use the fine focus when the slide is near the lens.

4. Change the slide to observe the slide of the threads by clicking on that slide. Note that as you move the focus up and down you can only get one thread in focus at a time. This is due to the limited depth of field of light microscopes. By moving the focus up and down try to identify which thread is on top, in the middle and on the bottom.

5. View the slide of the animal tissues. In future labs you will view various organisms and tissues 'through' the microscope. In these instances, you will not need to 'operate' a microscope, but you will be able to change the magnification of the image and move the specimen similar to the way you would with a real microscope.

Activity 2a.4
Magnification of Images

The microscope magnifies objects, but by how much? When using a real light microscope you can easily calculate the magnification of the image you are viewing. The total magnification that you see is the product of the magnification of the objective lens and the ocular lens. Thus, with a 10 x ocular and using the 4x objective lens, the total magnification power is 10 x 4 = 40 x. With a 10 x ocular and the 40x objective lens in place, the total magnification power would be 10 x 40 = 400 x.

Throughout the simulations on the BiologyOne DVD, magnification values are not given. Instead, magnifications of images are listed as low, medium or high. This is because screen resolutions of the various computers that may be used will vary causing the size of images to vary.

Lab Exercise *2a* **Name** _____

Results Section

Answer the following Questions

1. What is the orientation of the image of the e relative to its actual orientation on the microscope slide?

2. When you move the slide, what is the direction of movement of the image relative to the direction of movement of the slide?

3. When observing the threads, use the fine focus to determine which thread is on top, in the middle, and on the bottom. Write the order here.

   top _____
   middle _____
   bottom _____

4. If you are using a microscope that has 4x ocular lenses and your are using the 40x objective lens, what would be the total magnification of the image you are viewing?