

# Microscopes

## Notes 6.1

### **Microscopes are the gateway to the “invisible” world**

Microscopes allow us to see organisms and objects that we cannot see without our unaided (naked) eye. Microscopes help us see small objects and organisms by making them appear bigger, called magnification. However, this is not entirely useful to us. Think about a time you have tried to make a picture bigger on your computer. It became bigger but also became blurry.

Microscopes also increase resolution. Resolution is defined as the minimum distance two points can be apart and still be distinguished as two separate points. Microscopes make it easier to see two very close points.

Because objects in the microscopic world are so small, we must use a small unit to measure them. To measure small objects, we use a unit called the micron ( $\mu\text{m}$  or  $\text{um}$ ), also called a micrometer. There are 1,000 microns in a millimeter and 1,000,000 in meter! The human eye is able to distinguish points that are more than  $100\mu\text{m}$  (0.01mm) apart. Anything less than that is too small for a human to distinguish.

To increase resolution, microscopes use lenses. Different microscopes use different numbers of lenses. For example, a simple microscope uses one lens. A compound microscope uses two or more lenses. When there is more than one lens, each lens helps focus the image onto the next lens. In a compound microscope, the first lens focuses the image on the second lens, the second lens focuses on the third and so on until the image reaches your eye. By using multiple lenses, microscopes can resolve points that are separated by more than 200 nanometers<sup>1</sup> (nm).

Lenses come in a variety of shapes and sizes. This affects how well an image will be magnified and resolved. We measure this in powers. A power is how many times bigger the lens makes an object appear. A 5 power lens magnifies an image five times its actual size. This would be written as 5X; the X stands for times. The power of a single lens is called the magnification power.

If there are two lenses, you multiply the powers of each lens together to determine the total power. For example, you have a microscope with two lenses. The first lens has a magnification power of 4X and the second has a magnification power of 10X. The total power, or what you would see in the end, is 40X. The total power of two or more lenses combined together is called the power of magnification.

---

<sup>1</sup> A nanometer is 1/1,000,000,000 (one billionth) of a meter. It is 1,000 times smaller than a micron.

### There are a variety of microscopes

After the invention of the microscope, scientists began modifying it. As a result, there are many types of microscopes in use today. The most common is called a light microscope. The light microscope uses light and lenses to magnify, resolve, and view a specimen. There are several types of light microscopes, a few of which are mentioned below.

1. **Bright-Field Microscope.** The type we use in our classroom is called a bright-field microscope. A bright-field microscope shines light from below the specimen. The light passes through the specimen and to the objective lenses.
2. **Dark-Field Microscope.** The opposite of a bright-field microscope is called the dark-field microscope. A dark-field microscope shines light at an angle to the specimen instead of below it. The result is a very dark background and a much lighter colored specimen.
3. **Fluorescence Microscope.** A third type of light microscope is the fluorescence microscope. This requires a special type of microscope with lenses that only allow fluorescent light to pass through. This also requires a special chemical that will dye a specimen.

Light microscopes are great for magnifying and resolving certain objects and specimens. However, they can only magnify to a certain point. Beyond this point, the image will always be blurry and the resolution will be poor. We can't fix this even if we add more lenses. This is the result of how light works. In order to see the structures inside a cell we need to use an electron microscope.

Electron microscopes use electrons instead of light to see a specimen. Electron microscopes work by shooting electrons from an electron gun at the specimen. Electron microscopes do not use glass lenses. Electrons have mass which means they cannot pass through glass. It is similar to trying to throw a tennis ball through a window. Electron microscopes use magnets to direct the electrons to the specimen. The result is a very detailed image of the specimen.

There are two kinds of electron microscopes.

1. **Transmission Electron Microscope (TEM).** A beam of electrons is passed through the specimen. Electrons that make it through the specimen make the image. In order to do this, specimens must be cross-sectioned so that electrons can pass through it. This does not allow us to use living specimens. The result is a black and white image that is two-dimensional.
2. **Scanning Electron Microscope (SEM).** An electron beam is scanned across the surface of a specimen. It reveals the specimen in great detail. The specimen must be coated in a metal (typically gold). When the electrons hit the specimen, it is detected by instruments. These instruments create the image.

### Microscopes have many parts

Now that you have a basic understanding of what microscopes are and some of their inner workings, it is time to learn about the parts of the microscope. It is essential that you learn

the anatomy of the microscope because you will be interacting with it so often. Understanding the parts and their functions will help you obtain clear, detailed images and fix any problems you may have along the way.

The following parts are listed in order as covered in our lecture on microscopes. The numbers below match the diagram given to you in class.

1.  **Eyepiece (ocular).** This is where you look into the microscope to see the image of the specimen. It magnifies the image one last time before focusing it to your eye. Inside the eyepiece you may see an arrow. This is called the pointer. It allows you to share what you see with another person.
2.  **Body Tube.** This allows light to travel from the objective lens to the eyepiece. It also supports the eyepiece.
3.  **Nosepiece.** The nosepiece holds and rotates the three objective lenses.
4.  **Objective Lenses.** There are three objective lenses: low (red band, 4X), medium (yellow band, 10X), and high (blue band, 40X). These are attached to the nosepiece and vary in size. Their function is to magnify the specimen.
5.  **Arm.** The arm holds the up the upper portion of the microscope. It is also one of the safe carrying spots on the microscope.
6.  **Coarse Adjustment Knob.** This is a large knob found on the side of the microscope. The knob allows you to move the stage of the microscope so you can focus the specimen. You must be careful when using the coarse adjustment knob; it moves the stage and (un) focuses specimens quickly.
7.  **Fine Adjustment Knob.** This small knob is found under the coarse adjustment knob. It helps you make smaller adjustments to focusing your image. This should be used when focusing on medium or high power.
8.  **Slide.** This is not part of the microscope. However, it is required to use the microscope. The slide is a thin piece of glass used to hold the specimen.
9.  **Stage.** The stage is the large black square found under the objective lenses. Its purpose is to hold the slide. In the middle of the stage you will see the aperture and below it is the diaphragm. You will also have two stage clips on the stage.
10.  **Stageclips.** These hold the slide in place.
11.  **Aperture.** The aperture is a small, glass opening in the center of the stage. It allows light to pass from the lamp, through the stage, and to the objective lens.
12.  **Diaphragm.** This is a round disk found under the stage. There are five holes different-sized holes in the disk. It controls how much light passes through the aperture.
13.  **Lamp.** It generates light.
14.  **Base.** It holds up the microscope. It is the second safe carrying spot.

### Follow the rules of microscope use

Microscopes are expensive tools. They have a high initial cost, maintenance can be expensive, and replacing parts can also be expensive. Therefore, it is important to follow the rules of microscope care.

1.  **Never touch any lens.** The lenses need to be kept as clean as possible. Putting your finger on them will leave oils and other residues that will make it difficult to focus. If

your lens is dirty you need to use special paper to clean it. This prevents the lens from being scratched.

2. **Always start and finish on the lowest objective lens.** There are several reasons why you should start and finish on the lowest objective lens. First, starting there ensure you will have an easier time focusing. Second, finishing on the low objective prepares you shut down the microscope. Third, finishing on the low objective makes it easier and safer (for the microscope) to remove the slide.
3. **Always carry the microscope by the arm AND base.** Image the microscope as a baby: it's delicate, fragile, cute, but it doesn't make unusual smells. It is important to carry it correctly to prevent dropping it or damaging any of its parts.
4. **Clean the microscope when you are done with appropriate materials.** It's inevitable: the microscope will get dirty. Cleaning up any messes promptly will keep the microscope in better shape, reduce the wear and tear on the parts, and make it easier to operate. (Imagine minerals crystallizing in the diaphragm: it's happened and its hard to clean!) Only use approved materials to clean the microscope. There will be certain chemicals and papers used.
5. **Always keep the microscope away from the edge of any surface.** Gravity is mean to microscopes. Keeping a microscope near the edge is a chance for it to fall off and break.
6. **Only use the coarse adjustment knob on the low power objective lens.** Remember: the coarse adjustment knob moves the stage quickly. Using this on low power is fine because the stage will never hit the objective lens. However, the use of the coarse knob on medium and high power can result in the lens hitting the slide and causing damage. Plus, the fine adjustment knob is much easier to use on higher powers!
7. **Keep both eyes open when looking in the microscope.** Keeping both eyes open prevents fatigue and is easier to do. If you have trouble seeing in the microscope with both eyes open, cover one eye with a hand. It feels awkward, but it is worth it!

### **Proper microscope use requires following the correct procedure**

Following procedure is the best way to prevent any accidents with a microscope. The following five procedures are what we do most commonly with the microscopes.

#### **Getting your microscope ready**

1. Remove the dust cover first and plug it in second.
2. Set the nosepiece to the lowest objective.
3. Lower the stage as far as it can go.
4. Set the diaphragm to the lowest (1) setting.
5. Turn on the light.

#### **Placing the slide on the microscope**

1. Rotate the stage clips to the side of the stage.
2. Pick up the slide without touching the cover slip (hold the slide by its edges).
3. Place the slide with specimen directly over the aperture.
4. Secure the slide with the stage clips.

**Focusing**

1. Look into the eyepiece.
2. Rotate the coarse objective knob until the specimen comes into focus.
3. Use the fine adjustment knob to further focus the specimen. Adjust the diaphragm as needed.
4. Place yourself at eye-level with the slide, stage, and objective lenses.
5. Rotate the nosepiece so that the middle objective lens is used.
6. Focus the specimen using only the adjustment knob. Make adjustments as necessary.
7. Repeat steps 4-6 for going to the high power. You must have permission to go to high power.

**Removing a slide**

1. Return the nosepiece to the lowest objective lens. If you are on high power you must go to medium power and then low power.
2. Lower the stage as far as it can go.
3. Set the diaphragm to the lowest setting (1).
4. Remove the stage clips from the slide and rotate them to the side of the stage.
5. Remove the slide.

**Shutting down the microscope**

1. Set the nosepiece to the lowest objective lens.
2. Lower the stage as far as it can go using the coarse adjustment knob.
3. Set the diaphragm to the lowest setting (1).
4. Turn off the light.
5. Place the dustcover over the microscope.

**Practice Questions**

Answer the following questions for review.

1. What are two units used for measuring microscopic objects?
2. What is the difference between magnification and resolution?
3. What is the difference between magnification power and power of magnification?
4. How do you determine the power of magnification?
5. What is the difference between a simple and compound microscope?
6. What are the different types of light microscopes? Briefly describe each.
7. What are the different types of electron microscopes? Briefly describe each.
8. What are the parts of a microscope? What is the function of each?
9. Why is it important to follow the rules of microscope use?
10. What are the procedures for using a microscope?