

Electron Microscopes

Electron microscopes use beams of electrons to magnify the object. The beams are focused on a photographic plate by means of electromagnets. The **transmission electron microscope** is analogous to the compound light microscope. The object is ultra-thinly sliced and treated with heavy metal salts to improve contrast. Figure 2.5b is a micrograph produced by this type of microscope. The **scanning electron microscope** is analogous to the dissecting light microscope. It gives an image of the surface and dimensions of an object, as is apparent from the scanning electron micrograph in Figure 2.5c.

The micrographs in Figure 2.5 demonstrate that an object is magnified more with an electron microscope than with a compound light microscope. The difference between these two types of microscopes, however, is not simply a matter of magnification; it is also the electron microscope's ability to show detail. The electron microscope has greater resolving power. **Resolution** is the minimum distance between two objects at which they can still be seen, or resolved, as two separate objects. The use of high-energy electrons rather than light gives electron microscopes a much greater resolving power since two objects that are much closer together can still be distinguished as separate points. Table 2.2 lists several other differences between the compound light microscope and the transmission electron microscope.

Table 2.2 Comparison of the Compound Light Microscope and the Transmission Electron Microscope

Compound Light Microscope	Transmission Electron Microscope
1. Glass lenses	1. Electromagnetic lenses
2. Illumination by visible light	2. Illumination due to beam of electrons
3. Resolution \cong 200 nm	3. Resolution \cong 0.1 nm
4. Magnifies to 1,500 \times	4. Magnifies to 100,000 \times
5. Costs up to tens of thousands of dollars	5. Costs up to hundreds of thousands of dollars

Conclusions

- Which two types of microscopes view the surface of an object? *binoocular dissecting microscope + scanning electron microscope*
- Which two types of microscopes view objects that have been sliced and treated to improve contrast? *Compound, light microscope, transmission electron.*
- Of the microscopes just mentioned, which one resolves the greater amount of detail? *Transmission Electron microscope.*

Rules for Microscope Use

Observe the following rules for using a microscope:

1. The lowest power objective (scanning or low) should be in position, both at the beginning and end of microscope use.
2. Use only lens paper for cleaning lenses.
3. Do not tilt the microscope as the eyepieces could fall out, or wet mounts could be ruined.
4. Keep the stage clean and dry to prevent rust and corrosion.
5. Do not remove parts of the microscope.
6. Keep the microscope dust-free by covering it after use.
7. Report any malfunctions.

2.3 Binocular Dissecting Microscope (Stereomicroscope)

The **binocular dissecting microscope** allows you to view objects in three dimensions at low magnifications. It is used to study entire small organisms, any object requiring lower magnification, and opaque objects that can be viewed only by reflected light. It is also called a stereomicroscope because it produces a three-dimensional image.

Identifying the Parts

After your instructor has explained how to carry a microscope, obtain a binocular dissecting microscope and a separate illuminator, if necessary, from the storage area. Place it securely on the table. Plug in the power cord, and turn on the illuminator. There is a wide variety of binocular dissecting microscope styles, and your instructor will discuss the specific style(s) available to you. Regardless of style, the following features should be present:

Figure 2.6 Binocular dissecting microscope (stereomicroscope).

Label this microscope with the help of the text material.



1. **Ocular lenses (eyepieces):** What is the magnifying power of the ocular lenses on your microscope? 100X X 18X
2. **Body tube:** Holds nosepiece at one end and eyepiece at the other end; conducts light rays.
3. **Arm:** Supports upper parts and provides carrying handle.
4. **Nosepiece:** Revolving device that holds objectives.
5. **Objective lenses (objectives):**
 - a. **Scanning objective:** This is the shortest of the objective lenses and is used to scan the whole slide. The magnification is stamped on the housing of the lens. It is a number followed by an \times . What is the magnifying power of the scanning objective lens on your microscope? 4
 - b. **Low-power objective:** This lens is longer than the scanning objective lens and is used to view objects in greater detail. What is the magnifying power of the low-power objective lens on your microscope? 10X
 - c. **High-power objective:** If your microscope has three objective lenses, this lens will be the longest. It is used to view an object in even greater detail. What is the magnifying power of the high-power objective lens on your microscope? 40X
 - d. **Oil immersion objective:** (on microscopes with four objective lenses): Holds a $95\times$ (to $100\times$) lens and is used in conjunction with immersion oil to view objects with the greatest magnification. Does your microscope have an oil immersion objective? ~~100X~~ yes If this lens is available, your instructor will discuss its use when the lens is needed.
6. **Coarse-adjustment knob:** Knob used to bring object into approximate focus; used only with low-power objective.
7. **Fine-adjustment knob:** Knob used to bring object into final focus.
8. **Condenser:** Lens system below the stage used to focus the beam of light on the object being viewed.
9. **Diaphragm or diaphragm control lever:** Controls amount of illumination used to view the object.
10. **Light source:** An attached lamp that directs a beam of light up through the object.
11. **Base:** The flat surface of the microscope that rests on the table.
12. **Stage:** Holds and supports microscope slides.
13. **Stage clips:** Hold slides in place on the stage.
14. **Mechanical stage (optional):** A movable stage that aids in the accurate positioning of the slide. Does your microscope have a mechanical stage? YES
15. **Mechanical stage control knobs (optional):** Two knobs usually located below the stage. One knob controls forward/reverse movement, and the other controls right/left movement.

Focusing the Compound Light Microscope—Lowest Power

1. Turn the nosepiece so that the *lowest* power objective on your microscope is in straight alignment over the stage.
2. Always begin focusing with the *lowest* power objective on your microscope ($4\times$ [scanning] or $10\times$ [low power]).
3. With the coarse-adjustment knob, lower the stage (or raise the objectives) until it stops.
4. Place a slide of the letter *e* on the stage, and stabilize it with the clips. (If your microscope has a mechanical stage, pinch the spring of the slide arms on the stage, and insert the slide.) Center the *e* as best you can on the stage or use the two control knobs located below the stage (if your microscope has a mechanical stage) to center the *e*.

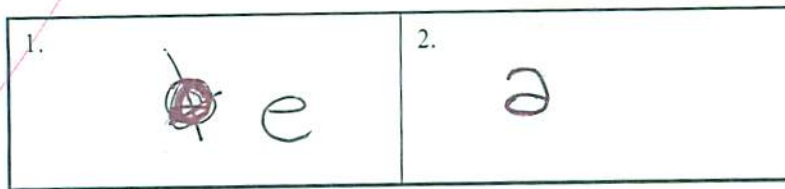
5. Again, be sure that the lowest-power objective is in place. Then, as you look from the side, decrease the distance between the stage and the tip of the objective lens until the lens comes to an automatic stop or is no closer than 3 mm above the slide.
6. While looking into the eyepiece, rotate the diaphragm (or diaphragm control lever) to give the maximum amount of light.
7. Using the coarse-adjustment knob, slowly increase the distance between the stage and the objective lens until the object—in this case, the letter *e*—comes into view, or focus.
8. Once the object is seen, you may need to adjust the amount of light. To increase or decrease the contrast, rotate the diaphragm slightly.
9. Use the fine-adjustment knob to sharpen the focus if necessary.
10. Practice having both eyes open when looking through the eyepiece, as this greatly reduces eyestrain.

Inversion

Inversion refers to the fact that a microscopic image is upside down and reversed.

Observation: Inversion

1. In space 1 provided here, draw the letter *e* as it appears on the slide (with the unaided eye, not looking through the eyepiece).



2. In space 2, draw the letter *e* as it appears when you look through the eyepiece.
3. What differences do you notice? rightside up with ^{up}aided eye and upside down with aided eye.
4. Move the slide to the right. Which way does the image appear to move? It goes left.
Explain. _____

Focusing the Compound Light Microscope—Higher Powers

Compound light microscopes are **parfocal**; that is, once the object is in focus with the lowest power, it should also be almost in focus with the higher power.

1. Bring the object into focus under the lowest power by following the instructions in the previous section.
2. Make sure that the letter *e* is centered in the field of the lowest objective.
3. Move to the next higher objective (low power [10×] or high power [40×]) by turning the nose-piece until you hear it click into place. Do not change the focus; parfocal microscope objectives will not hit normal slides when changing the focus if the lowest objective is initially in focus. (If you are on low power [10×], proceed to high power [40×] before going on to step 4.)
4. If any adjustment is needed, use only the *fine*-adjustment knob. (*Note*: Always use only the fine-adjustment knob with high power.) On your drawing of the letter *e*, draw a circle around the portion of the letter that you are now seeing with high-power magnification.
5. When you have finished your observations of this slide (or any slide), rotate the nosepiece until the lowest-power objective clicks into place, and then remove the slide.