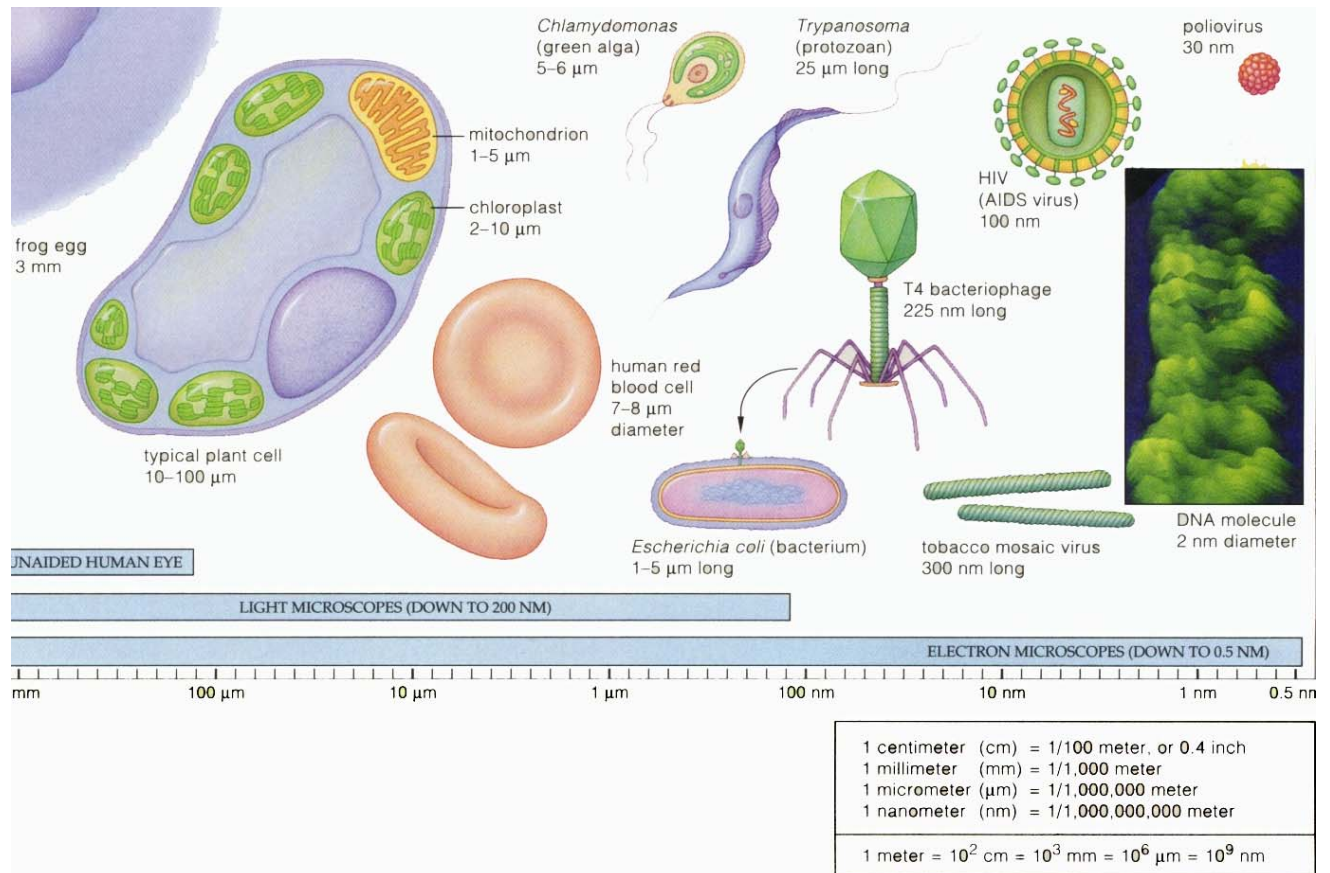


## Microscopes

Anton van Leeuwenhoek and Robert Hooke described cells in the 1600's using microscopes that magnified objects too small to be seen with the unaided eye. Van Leeuwenhoek was the first to observe single-celled organisms. Hooke is credited for giving cells the term we still use. The appearance of the cork reminded him of cells in a monastery, hence the name, cell. Today, many different types of microscopes are used in biological research and study. All microscopes magnify images so we can learn details about them that we could not otherwise see. Microscopes use a series of lenses to form enlarged images of the objects viewed. You will use microscopes throughout this course to assist you in your discovery of the biological world.

Magnification is just one characteristic of a microscope. The **resolution**, or resolving power, of the microscope is just as important as its ability to magnify an object. Resolving power is the distance apart at which two points in an object are seen as two distinct points rather than as one "blob". Details of the image seen are determined by the resolving power, while the magnification determines the size of the image seen. To give you an idea of what resolving power means, the human eye can distinguish two objects as separate from each other when they are at least 0.1 mm apart; a compound light microscope has the ability to resolve objects that are about 200 nm apart or 500 times closer than the human eye can. The most powerful microscope, the transmission electron microscope, can resolve objects that are just 0.2 nanometer apart. Incidentally, the ability to resolve detail is also dependent on the wavelength of the illumination; the shorter the wavelength, the finer the detail.



Units of Measurement used with Microscopes

## Exercise I: The Compound Light Microscope

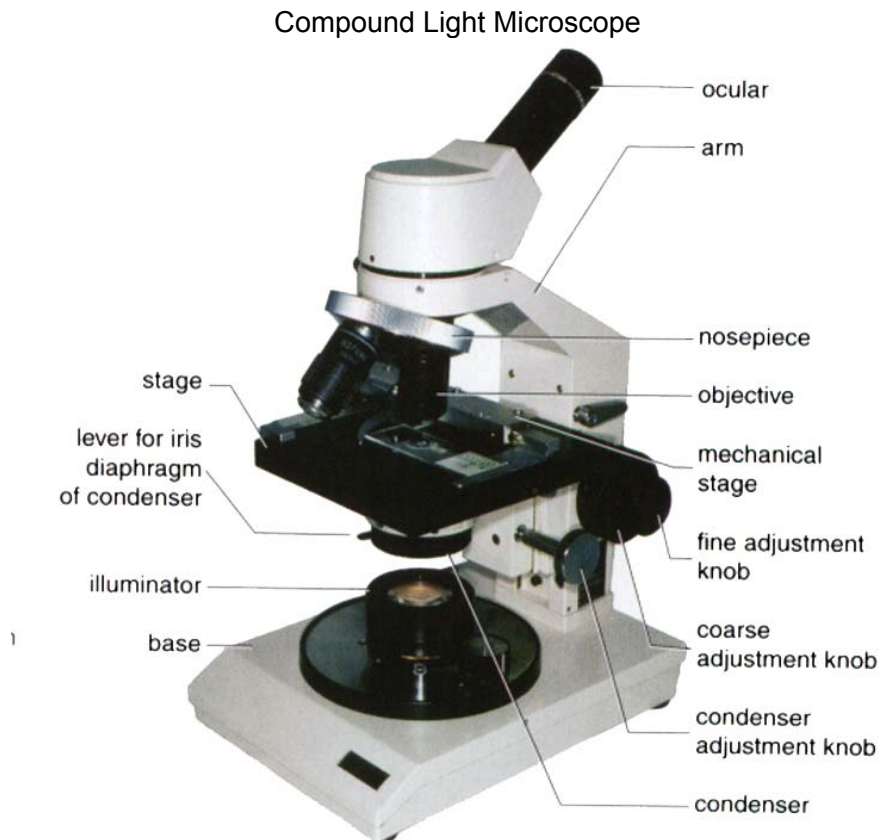
### Materials Required

- Compound Light Microscope
- Prepared slide of colored threads
- Prepared slide of leaf cross section
- Lens paper
- Clear plastic metric rulers
- Stage micrometers

The microscope used most frequently in the biology lab is the **compound light microscope**. The compound microscope is used to magnify and resolve fine detail within a transparent specimen (one through which light can pass). The compound microscope has two lens systems; an objective lens, located near the specimen, that magnifies the specimen a certain amount, and an ocular lens, or eyepiece, which further magnifies the image formed by the objective lens. The total magnification observed by the human eye is the product of the magnification of the two lenses (objective X ocular).

In order to use a microscope properly you must be familiar with the optical and mechanical parts of the microscope. You must also learn the appropriate care of this delicate (and expensive) instrument. The microscope gives you the opportunity to see a world of things you may never have available again, but to enjoy the experience, you need to know how to use it.

Remove a microscope from the storage cabinet. Carry it by its arm while supporting the base of the microscope with your free hand. The following diagram and list of terms and illustrations will help you to become familiar with the microscope. As you read through the list locate each of the parts on your microscope. If you don't understand how something functions or can't find it on your microscope, check with your instructor.



1. **Adjustment Knobs**

Your microscope has two adjustment knobs that are used to focus the specimen to be studied. The largest is the **coarse adjustment knob**. This is used for rapid (or coarse) focusing of the specimen when using the scanning objective lens. The coarse objective knob is rotated until the specimen is roughly in focus and then left alone. The **fine adjustment knob** controls precise focusing of the object. You should use just the fine adjustment knob should with the higher magnification objective lenses. Using the coarse adjustment knob with the higher magnification objective lenses may damage the lens and/or the slide you are observing. Moving the fine adjustment knob also helps you to determine the third dimension (depth) of the specimen you are studying.

2. **Stage**

The stage holds the slide to be observed. Moving the coarse and fine adjustment knobs moves the stage up or down to bring the specimen into focus. (In some microscopes the adjustment knobs move the nosepiece with the objective lenses rather than the stage.)

3. **Mechanical Stage**

Your slide is fixed into position on the stage with the mechanical stage. The slide is fastened into the mechanical stage by using a small lever located on the mechanical stage. Two knobs located on the side of the mechanical stage are used to move the slide around to locate your specimen; one knob moves the slide from side to side and the other moves the slide forward and backward. The mechanical stage permits precision movements of your slide, especially nice when using the higher magnification objectives.

4. **Condenser**

The condenser, located below the stage, contains a system of lenses that focuses light on your specimen. The condenser may be raised or lowered using the condenser knob. Most microscopes have a built-in light source. Use caution to avoid having the light cord hang where you might trip over it or catch an elbow in it.

5. **Iris Diaphragm**

The iris diaphragm is located on the condenser. The lever of the iris diaphragm is used to adjust the amount of light striking the object being studied. It is critical that you know the proper use of the condenser and iris diaphragm. A common problem with microscope use is having too much light, obliterating the object (more or less like trying to see something while looking directly at the sun).

6. **Objective Lenses**

The 4 objective lenses of your microscope and their magnifications are:

Scanning lens	4x magnification
Low Power Lens	10x magnification
High Power Lens	40 - 45x magnification
Oil Immersion Lens	100x magnification



The magnification of the objective lens is on the lens, along with a number of other lens properties. (See illustration above.)

**Note:** The magnification of the oil immersion lens requires using the lens with a special immersion oil for proper resolution. The procedure for using the oil immersion lens will be discussed later in this laboratory. **Never** use the oil immersion lens without immersion oil. Serious damage might result.

**7. Ocular**

The ocular lens, or eyepiece, further magnifies the image formed by the objective lens. It does not improve resolution. Your microscope may have a monocular system (one ocular lens) or an adjustable binocular system (two ocular lenses). The magnification of the ocular is 10x. The ocular may be equipped with a pointer or a numerical scale. They are useful in pointing out specific structures or determining the real dimensions of the specimen you are observing.

**Total Magnification**

Recall that the total magnification of the lens system is the product of the magnification of the ocular times the magnification of the objective lens being used (ocular X objective).

Record below the magnification for each of the lens systems of your microscope. Then record the total magnification achieved for each of the lenses.

Lens	Objective Magnification	Ocular Magnification	Total Magnification
Scanning			
Low Power			
High Power			
Oil Immersion			

**Additional Microscope Features**

In addition to the parts of the microscope you have just identified, the following are a few features of the microscope useful to know:

**Parfocal**

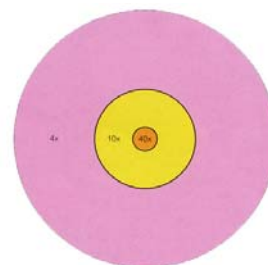
Most objective lens systems are **parfocal**, which means that once you have an object in focus at one magnification, it will stay reasonably in focus when you change objectives. When the microscope is parfocal only fine adjustment is required when changing magnification.

**Parcentered**

This term means that when you have your specimen in the center of the field of view at one magnification it will remain centered as you change magnification. It is most important to center your specimen when using the lower power objectives to avoid “losing” it from the field of view as you change to higher magnification.

**Field of View**

The visible area seen through the microscope when a specimen is in focus is the **field of view**. The greater the magnification, the smaller the field of view will be. The three circles in the diagram below demonstrate field of view at scanning, low power and high power magnification.

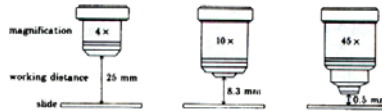


## Depth of Focus

The depth of focus is the thickness of the specimen which can be seen when in focus. As you increase magnification the depth of focus decreases. This can be readily illustrated by looking at a professionally prepared slide of colored threads. By focusing up and down with the fine adjustment knob at the high power magnification, you can observe the depth of focus as the individual threads come into and then out of focus.

## Working Distance

The space between the front mount (bottom) of the objective lens and the top of the coverslip of the microscope slide is the working distance. The working distance decreases as the objective lens magnification increases.



It is important to recognize that if a slide preparation is too thick it will be impossible to focus with the high power objectives because of the short working distance. In addition, there is a great risk of crushing the specimen, breaking the coverslip or slide, or damaging the objective lens with the shorter working distance. Accidents can be avoided if you never use the coarse adjustment knob while focusing with high power objectives.

## Estimating Size with the Microscope

You will often want to estimate the size of the specimens you observe with a microscope. To do this you must know the approximate diameter of your field-of-view for each of the objective lenses for your particular microscope. Once you have done these calculations, you can compare the size of a specimen against the known field diameter and make a reasonable estimate of the specimen's size.

### Determining the Microscope's Field-of-View Diameter

1. Obtain a clear metric ruler and position it on the stage of your microscope.
2. Bring the scale on the ruler into focus with the scanning objective lens.
3. The scale bars are in increments of 1 mm each. Each line is actually quite thick when viewed through the microscope. The distance from the left side of one line to the left side of the next line is one millimeter.
4. Adjust the ruler so that the left side of one line is just tangent to the lighted field-of-view at the edge of the diameter.
5. Starting at that edge, estimate how many bars and spaces it takes to cross the field-of-view. You will probably have to estimate the last fraction of a space or bar.
6. Record your microscope's field diameter with the 4X (scanning) objective in the table on the next page.
7. Now obtain a stage micrometer from the lab cart at the side of the room.
8. Bring the scale on the micrometer into focus with the low power (10X) objective lens.
9. The scale bars with the smallest distance between them are 0.01 mm (10  $\mu\text{m}$ ) apart. (Recall that 1000  $\mu\text{m}$  = 1 mm.)
10. Adjust the micrometer so that the left side of one line is just tangent to the lighted field-of-view at the edge of the diameter.
11. Starting at that edge, estimate how many bars and spaces it takes to cross the field-of-view. You will probably have to estimate the last fraction of a space or bar.
12. Record your microscope's field diameter with the 10X (low power) objective in the table on the next page.
13. Follow steps 10 – 12 for the high power and oil immersion objectives. Be especially careful with the oil immersion objective.
14. Record your measurements for each objective lens in the table. Be sure to fill in the column of the table indicating total magnification for each objective.

### Field of View Measurements for the Compound Microscope

Objective Lens	Total Magnification	Diameter of Visual Field	
		mm	µm
Scanning (4X)			
Low Power (10X)			
High Power (45X)			
Oil Immersion (100X)			

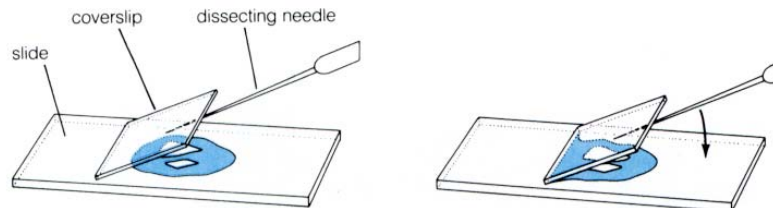
### Slide Preparation

Two types of slides are commonly used in biology classes: **permanent slides** (professionally prepared slides) and temporary **wet mounts** that you make.

#### 1. Wet Mounts

Wet mounts are temporary slides that you prepare yourself. When doing a wet mount follow the procedure outlined below:

- Place the specimen (mixed culture, tissue, etc.) on the center of a clean slide.
- Add a drop of water or designated stain if required. (Note: liquid cultures do not require adding water.)
- Place one edge of the coverslip on the slide near the specimen. Gently lower the coverslip on top of the specimen. You may want to use a probe or dissecting needle to help lower the coverslip. Try to avoid trapping air bubbles.
- Blot any excess fluid with lens paper before you place the slide on the stage of the microscope.
- After you have made your observations the slide and coverslip should be washed, dried and replaced in their appropriate locations.



#### 2. Permanent Slides

Permanent slides have been professionally prepared. They have often been stained to show better contrast of structure. A permanent slide may be a **whole mount** of the entire specimen or a **section** of the material. You should have only one permanent slide at your table at a time. Permanent slides should be cleaned only with lens paper.

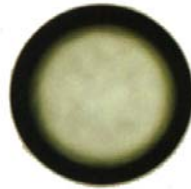
### Exercise II: Using the Compound Microscope

#### Materials Required

- Compound Microscope
- Clean microscope slides
- Coverslips
- Lens paper
- Immersion oil
- Sharp razor blades
- Cork
- Prepared slides of leaf tissue

**A. Making a Wet Mount**

1. Use a razor blade to shave several very thin fragments from a cork stopper.
2. Place the cork fragments on a clean microscope slide.
3. Add a drop of distilled water.
4. Cover the cork tissue with a coverslip using the method described above.
5. Position your slide on the microscope stage using the mechanical stage to hold the slide. Use the mechanical stage knobs to move the slide into the appropriate position on the microscope stage.
6. Use the scanning objective lens to locate and focus the cork tissue. Adjust the iris diaphragm to optimize the amount of light. Center the cork tissue in the field of view
7. Rotate the nosepiece to the low power objective and refocus and re-center the cork as needed. Once you have the cork in focus with the low power objective, move to the high power objective and observe the details of the cork tissue
8. By the way, you may see a number of air bubbles. It's easy to focus on a fascinating air bubble and think it is the object we are trying to find, especially if we haven't a clue what we are trying to find is supposed to look like. An excellent air bubble is shown here.



Air bubble (250X)

9. Make a sketch of your cork tissue. Recall that Robert Hooke's microscope had a magnification of 30X.

10. When you are finished, wash and dry your slide and coverslip and return them to the stock supplies on the side counter. Clean your objective lenses, stage and condenser surface with lens paper.

## B. Using the Oil Immersion Lens

The use of the oil immersion lens (with a total magnification of 1000x) allows you to see very fine detail. However, to have the needed resolution, light waves cannot be refracted by passing through the air space of the working distance. Air refracts (bends) light waves differently from the way a glass slide does. A slight distortion of the image is the result. To prevent this distortion, a film of immersion oil is placed on the coverslip of the slide, and the oil immersion lens will rest on the film of oil. (In other words, the working distance will be an oil-filled space rather than an air-filled space.) Immersion oil has the same "index of refraction" (bends the light in the same way) as the glass slide, so distortion will be minimized.

Obtain a prepared slide of a leaf section, lens paper and a bottle of immersion oil from the side counter or lab cart.

1. Locate and center a portion of the leaf with the scanning lens. Refocus and center the specimen as needed as you rotate to the low (10x) and high (45x) power objectives. Make sure that you have something in clear focus in the center of the field of view with the high power objective before proceeding.
  2. Rotate the nosepiece to a position half-way between the 45x objective and the oil immersion objective.
  3. Place a drop of immersion oil on the coverslip. Note: once you have added oil to the coverslip it is almost impossible to return to lower magnifications. Be sure that you have your specimen in good focus and location (centered in the field of view) before attempting to use the oil immersion lens. If something goes wrong, you will have to clean the oil from the slide and start over. (If you were doing a wet mount this might mean making a new slide.)
  4. **Slowly** rotate the oil immersion objective into position. It should be resting in the oil film. Carefully focus with the fine adjustment knob. You can move the slide around under the oil immersion objective, but you should **never** use the coarse adjustment knob to focus.
  5. Make a sketch of your leaf tissue as it appears at 1000X. Are there any similarities to the cork tissue?
- 
6. Referring back to the table on size estimate that you previously completed, what is the average size of the cells of the leaf?
  7. After you have completed your observations, remove the slide from the stage. Use lens paper to thoroughly remove **all** oil from the objective lens, the slide and coverslip. If you have any other places with oil spills, clean those too. You may need to use xylene or some other designated solvent to remove all traces of oil. Rotate the scanning objective into position when finished.

### Microscope Safety Cautions:

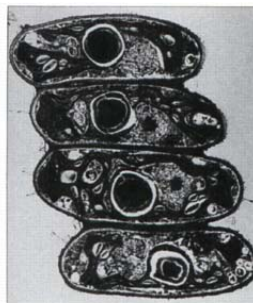
- Always carry the microscope in an upright position using both hands.
- Keep the microscope away from the edge of the table and place the cord out of the way where it will not catch on something.
- Use lens paper to clean your microscope. The objective lenses, ocular and top of the condenser, require frequent cleaning to remove smudges. Clean prepared microscope slides with lens paper as well. There is no such thing as using too much lens paper.
- It is especially important to remove all traces of immersion oil from the lenses, slides and microscope after using the oil immersion lens. Your instructor may provide an organic solvent (such as xylene) to help remove the oil films. You will discover that the most common cause of not being able to focus a specimen is a dirty lens or slide. A finger print that you may not be able to see can prevent a crisp focus.
- Keep the stage dry at all times. A wet stage will prevent the slide from being accurately positioned. Liquids may also corrode the microscope.
- When returning your microscope to its proper place in the cabinet **always**:
  - Remove the slide from the mechanical stage.
  - Clean all lens surfaces and the stage.
  - Rotate the nosepiece so that the scanning lens is in place.
  - Secure the cord so that it does not hang down.

### Using Different Microscopes

Often the microscope you use depends on what details you want revealed. Even when using the same magnification, different microscopes will illustrate different aspects of the object being viewed. The protist, *Scenedesmus*, is shown below viewed with three different kinds of microscopes, each at the same magnification (10 $\mu$ m). Each image shows different characteristics of the protist.



Light Microscope



TEM



SEM

### Exercise III: The Dissecting (Stereoscopic) Microscope

#### Materials Required

- Dissecting Microscope
- Prepared slide of flea or other insect
- Small culture dish containing moss
- Small culture dish containing salt crystals

The stereoscopic, or dissecting, microscope is the second microscope you will use in this course. The dissecting microscope allows you to magnify objects too large or too thick for the compound microscope. The effective magnification of the stereoscopic microscope is limited. Most provide magnifications in the range of 4x - 50x. In contrast to the compound microscope, the image viewed with the dissecting microscope is **not** inverted.

### Stereoscopic Dissecting Microscope



#### Using the Dissecting Microscope

1. Remove a stereoscopic microscope from the cabinet. It will be a binocular microscope, probably with a built-in light source. Some of the older dissecting microscopes lack built-in illuminators. For these you will also need to obtain a lamp from the back of the room.
2. Identify the parts of the dissecting microscope as shown in the diagram. Your microscope will have a **magnification knob** located on the side, with a magnification range of 0.7x to 3x, and a **focusing knob**. The oculars have a magnification of 10x. What is the range of total magnification possible with your dissecting microscope?
3. Adjust the interpupillary distance (the distance between the 2 eyepieces) for your eyes. Incidentally, dissecting microscopes work very much like binoculars in this way.
4. Observe the flea slide, moss specimen and salt crystals with the microscope. How does the stereoscopic microscope compare with the compound microscope? You may wish to examine other things. Skin, fingernails and strands of hair are always interesting to see, as is the image of the Lincoln Memorial on the US 5-dollar bill. When you have completed your observations return the microscope to the cabinet and the materials to their appropriate locations.

#### Electron Microscopes

Compound microscopes and dissecting microscopes are but two of many different types of microscopes used by biologists. Much research in biology involves working with electron microscopes. Electron microscopes focus a beam of electrons rather than light waves on the specimen to be studied. The electrons are focused by magnetic fields rather than lenses so that some electron microscopes can achieve resolution at much greater magnifications than light microscopes work. There are two types of electron microscopes: the transmission electron microscope (TEM) and the scanning electron microscope (SEM).

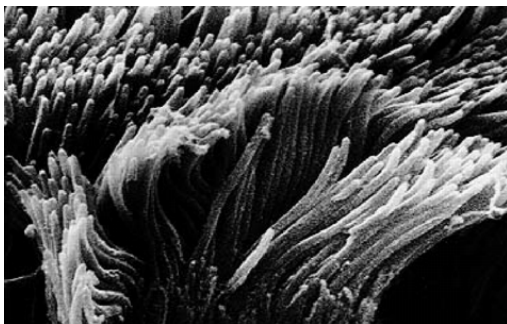
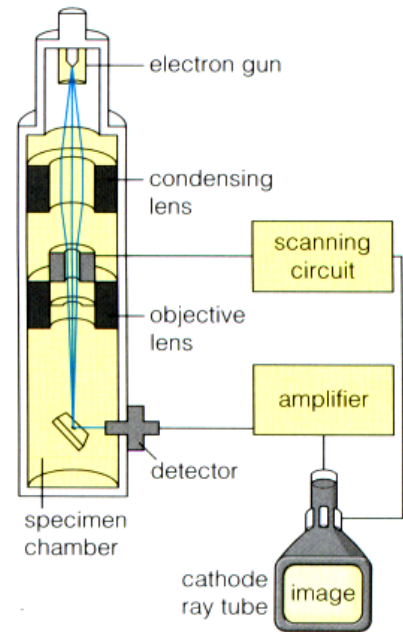
## Scanning Electron Microscope

BCC has a scanning electron microscope, donated by Richard and Jean Minch, shown below. Scanning electron microscopes provide three-dimensional images of the objects viewed. A scanning electron microscope beams electrons from a moving probe onto the surface of the specimen that has been coated with a metal, such as gold or platinum. The electrons reflected back from the specimen's surface are amplified and transmitted to a monitor where the image of the specimen can be viewed and photographed. Controls on the microscope allow one to move the specimen around, as well as change magnification. BCC's scanning electron microscope can magnify as much as 50,000X, although 5000 – 20,000X is typical.

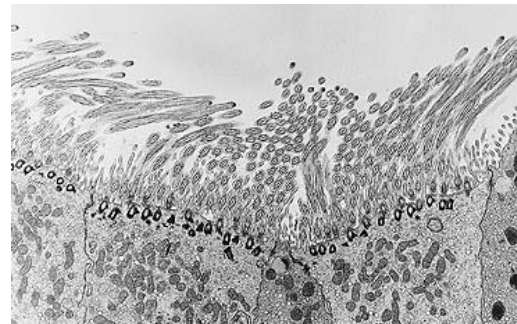
Students can do independent projects working with Life Science faculty using this microscope.



Richard and Jean Minch with BCC's Scanning Electron Microscope



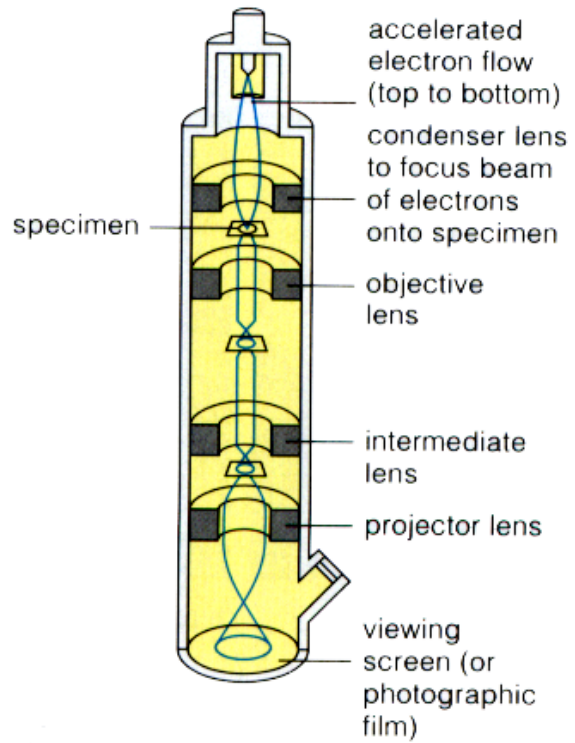
SEM of ciliated epithelium from rabbit trachea



TEM of ciliated epithelium from rabbit trachea

### Transmission Electron Microscope

A transmission electron microscope passes electrons through a thin specimen to reveal details of internal structure. Much of what we know about the structure of organelles of cells and membranes has been achieved using transmission electron microscopes. You will also observe many electron micrographs in the unit on cell structure and function. Preparation of specimens is crucial to success and involves slicing the specimen with extremely fine diamond "knives" along with coating the specimen with metals.



Transmission Electron Microscope