

Biology

Rules and Procedures for Using the Microscope

1. These are new and extremely expensive microscopes. We are very lucky to have such advanced microscopes to work with. Handle them with care and follow directions when using them!
2. You have been assigned a microscope. Use only the microscope that has been assigned for your use. You will be responsible for the microscope and its condition for this school year.
3. ALWAYS carry your microscope with 2 hands, one underneath to help support it!
4. NEVER wrap the microscope cord around the microscope! Simply coil the cord up and place the rubber band around it. If you are missing a rubber band notify your teacher so you can receive a new one.
5. When using prepared slides on the metal trays, you or your lab partner must pick up the slide that has the same number on it as your lab assignment. Such as: if you sit at lab table 3A, you got pick up the slide that is labeled 3A.
6. When returning these prepared slides you must return the labeled slide to its proper space on the metal tray.
7. When making your own slides, they must be washed, rinsed and dried thoroughly and then returned to the lab cart and place inside the proper slide container!
8. Cover slips must also be washed and dried before returning them to the cover slip container.

Focusing the Microscope

1. Always use the low (shortest 4X) power objective to begin focusing. The 4X objective should be directly over the slide. Start focusing by using the coarse adjustment knob (the larger focusing knob). Once you have the object focused and in view, you can use the fine adjustment knob (the smaller focusing knob) to make it clearer.
2. **YOU CAN ONLY USE THE COARSE ADJUSTMENT KNOB WHEN YOU ARE USING THE SHORTEST OBJECTIVE (LOWEST POWER 4X)**
3. Once you have brought the object into view and have focused the object as clearly as you can, you may then and only then, move the high objective (the medium length objective). **DO NOT TOUCH THE COARSE ADJUSTMENT KNOB ONCE YOU HAVE AN OBJECTIVE IN PLACE LARGER THAN LOW POWER (4X).**
4. Once you have brought the object into focus as much as you can, you may then move the high power objective(40X) into place over the slide and then begin using the fine adjustment knob to help focus. **DO NOT TOUCH THE COARSE ADJUSTMENT KNOB!**

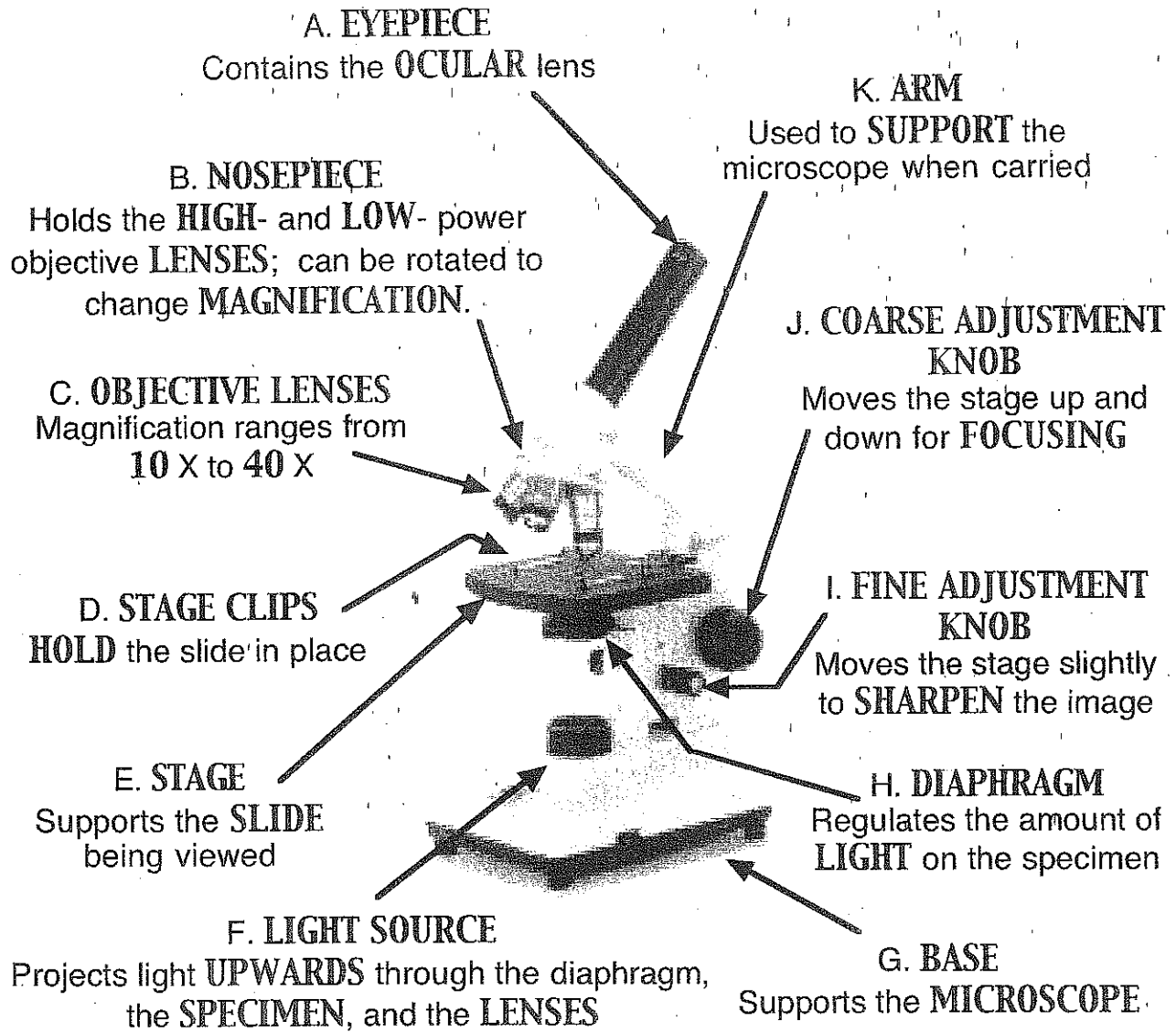
Remember that this takes a lot of practice. Try not to become frustrated! The microscope is **THE TOOL OF THE BIOLOGIST!** It just takes some patience and practice. Please feel free to ask for help. We want you to be able to actually see what is on the slide!

Laboratory Rules and Procedures

FOLLOW THESE RULES OR YOU WILL LOSE THE RIGHT TO PARTICIPATE IN LABS!

1. You must remain at your assigned lab table at all times unless instructed to move by your teacher. No more than 4 students may be at a lab table.
2. All students are responsible for making sure that all lab equipment is treated with care.
3. Any breakage of assigned or utilized lab equipment or materials will result in your repayment of the broken or damaged item.
4. Use only those materials that are directed to be used by the teacher.
5. All materials are to be returned to their proper place at the end of the lab. You are responsible for all equipment in your assigned lab drawer and cabinet. When using an item return it to its proper location.
6. Lab aprons, if required, must be folded neatly and returned to the drawer that they came from.
7. The lab must be completely clean, free from trash, etc. before your teacher will dismiss you from class.
8. Laboratory clean up will begin 10 minutes before the end of class. Keep your eye on the time.
9. All lab stools must be pushed in under the lab tables.
10. **NO** backpacks, purses, etc. are allowed in the lab area during lab activities.
11. All lab tables must be sprayed down with a disinfectant and dried thoroughly at the end of the lab before the teacher will dismiss you.
12. All lab tools or materials used during the lab must be returned to their proper spot before any student will be dismissed from class.
13. **EVERY** student is responsible for lab cleanup! No exceptions! We don't care how special you think you are ☺
14. **ABSOLUTELY NO** paper, trash, animal parts, etc. can be put in the sinks or any other area of the lab. Your teacher will check each sink before dismissal.
15. The laboratory must be absolutely clean before anyone is dismissed from class.
16. The teacher will dismiss you by lab tables after the teacher has had a chance to check each sink and lab area so it's up to you to clean up efficiently and have everyone working together to get you out in time!
17. **EVERY STUDENT MUST BE PREPARED FOR LAB AND CONDUCT ONESELF IN A PROFESSIONAL MANNER. YOU WILL BE GRADED ON YOUR LAB CONDUCT AS WELL AS YOUR WRITTEN LAB!**
18. **ANY STUDENT CHOOSING TO BREAK ANY OF THESE RULES WILL HAVE THEIR LABORATORY PRIVILEGES REVOKED!**

PARTS OF THE LIGHT MICROSCOPE

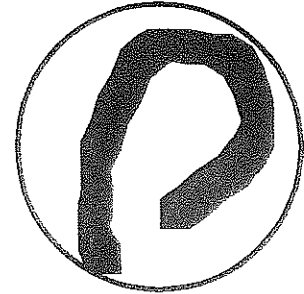
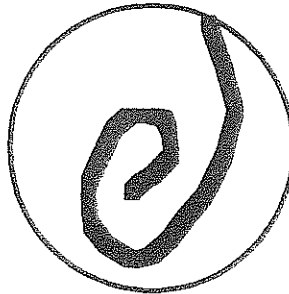
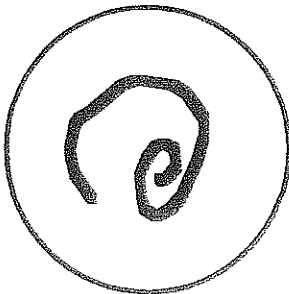


What happens as the power of magnification increases?

Power = $10\times 4\times = 40\times$

Power = $10\times 10\times = 100\times$

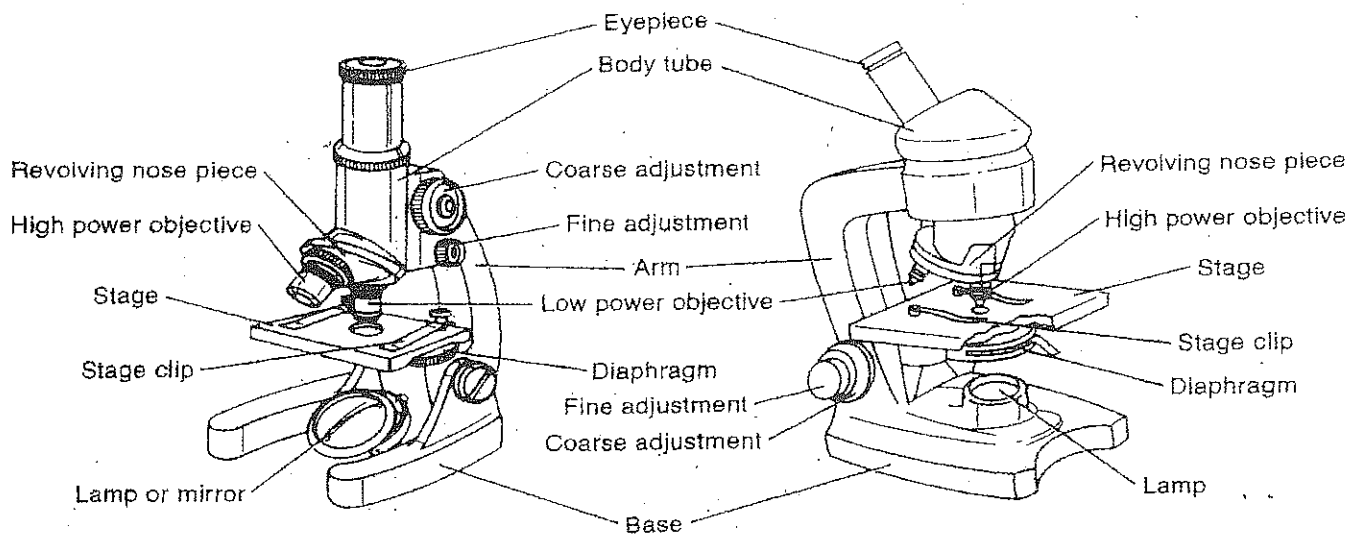
Power = $10\times 40\times = 400\times$



C. THE COMPOUND MICROSCOPE

Textbook reference: Sections 1-9

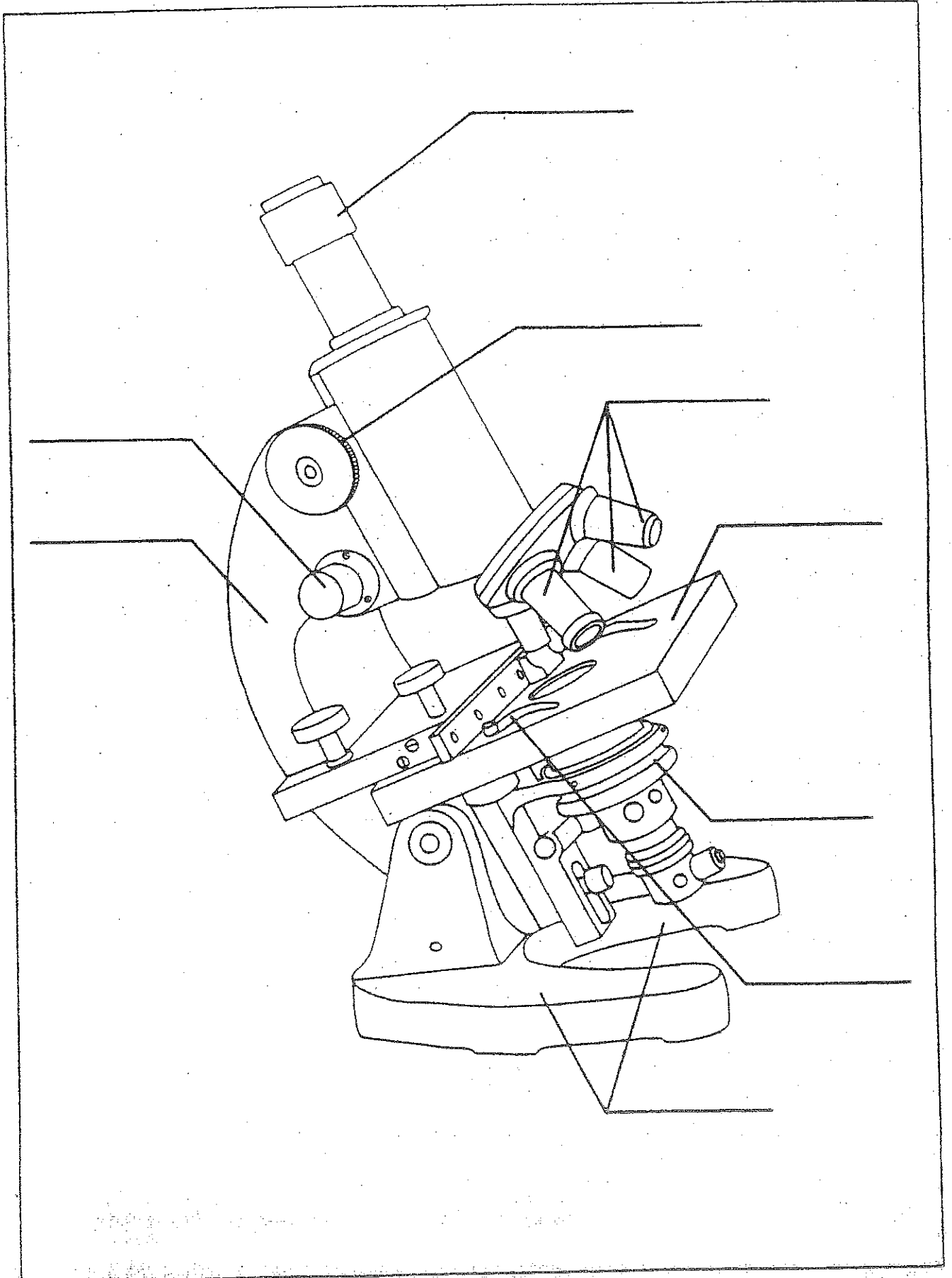
Many of the activities of biology require the use of a compound microscope. Each part of a compound microscope can be classified in one of four different categories, according to the function for which it is used: (1) optical parts, or lenses, for magnifying the object; (2) illuminating parts to provide light or to regulate the amount of light; (3) moving parts for raising, lowering, or revolving the lenses; and (4) supporting parts. On the chart below explain the function of each of the parts of a microscope, using one of the four categories listed above as a general guideline.



Microscope Part	Function
Arm	
Base	
Body tube	
Coarse adjustment	
Diaphragm	
Eyepiece	
Fine adjustment	
High-power objective	
Lamp	
Low-power objective	
Mirror	
Nosepiece	
Stage	
Stage clip	
Stage opening	

Name _____

Date _____



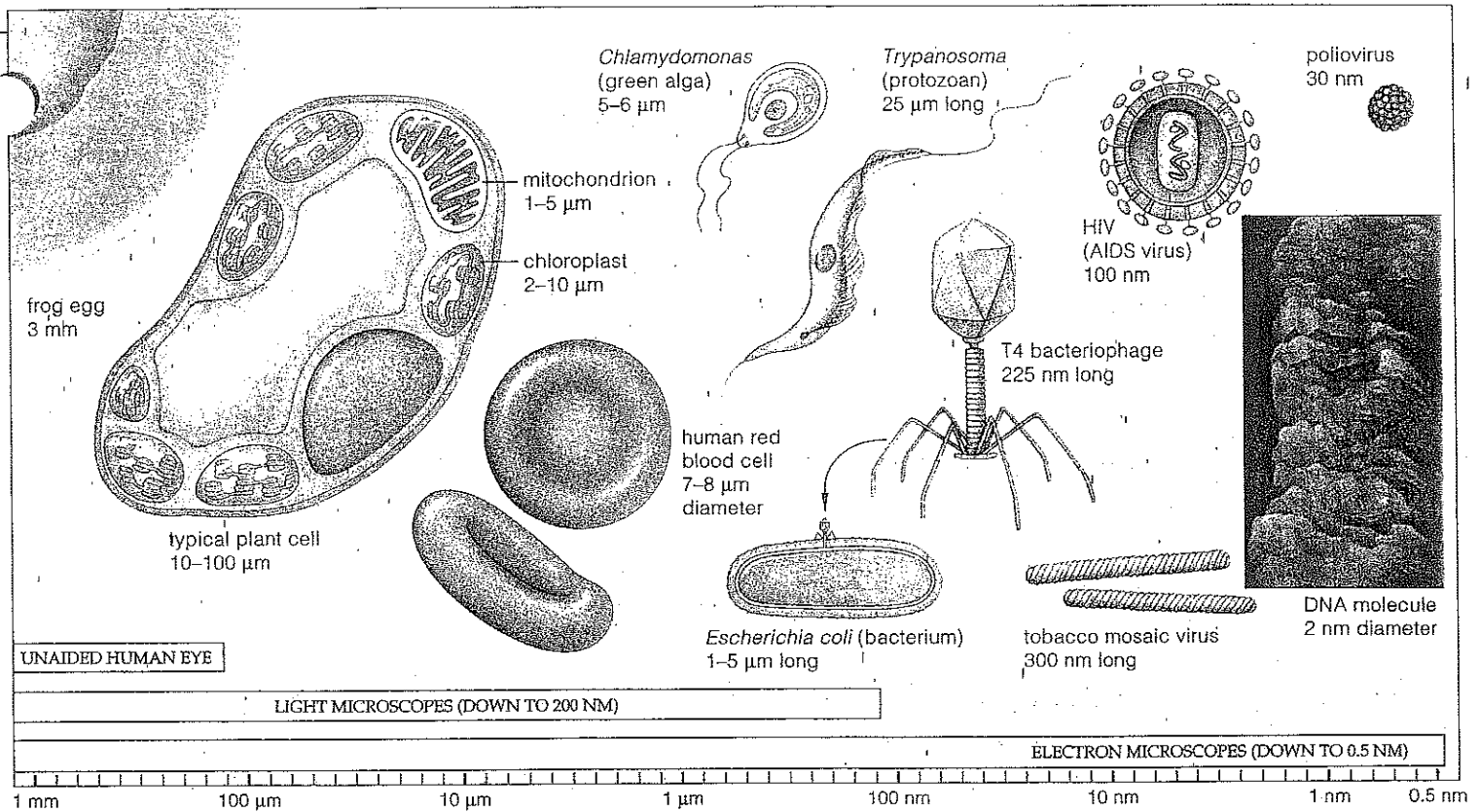


Figure 4.5 Units of measure used in microscopy. A *scanning tunneling microscope*, which can provide magnifications up to 100 million, gave us the photomicrograph of part of a DNA molecule. Its needlelike probe has a single atom at its tip. Voltage that is applied between the tip and an atom at a specimen's surface causes a detectable tunnel to form in the electron orbitals. As the tip moves over a specimen's contours, a computer analyzes the motion and creates a three-dimensional view of the surface atoms.

1 centimeter (cm) = 1/100 meter, or 0.4 inch
1 millimeter (mm) = 1/1,000 meter
1 micrometer (μm) = 1/1,000,000 meter
1 nanometer (nm) = 1/1,000,000,000 meter
1 meter = 10 ² cm = 10 ³ mm = 10 ⁶ μm = 10 ⁹ nm

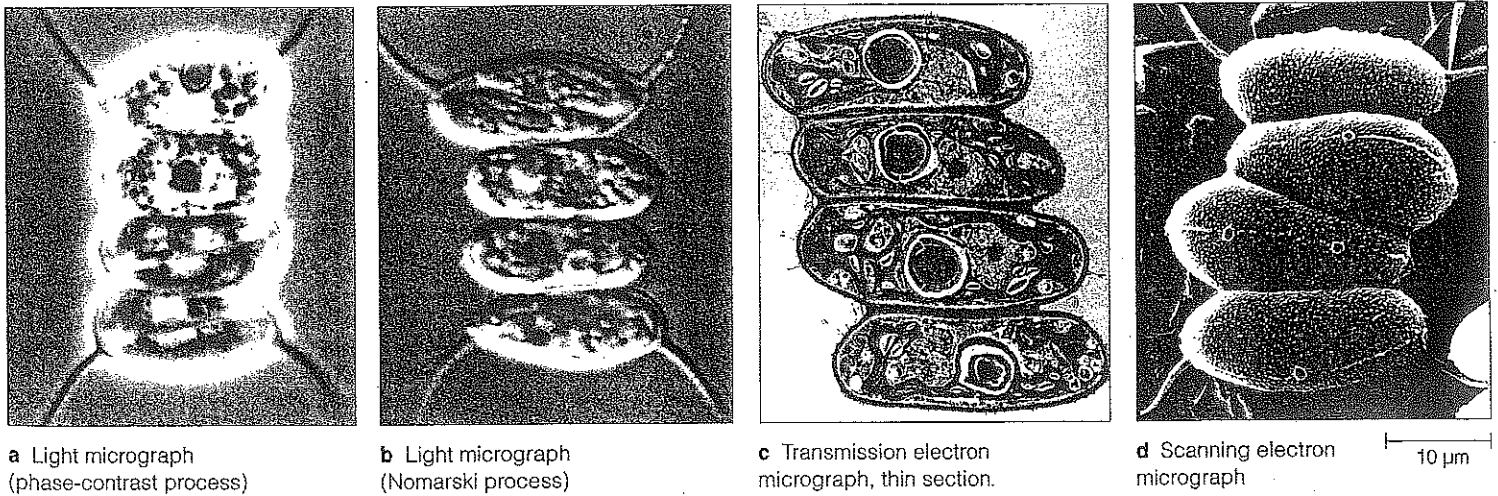
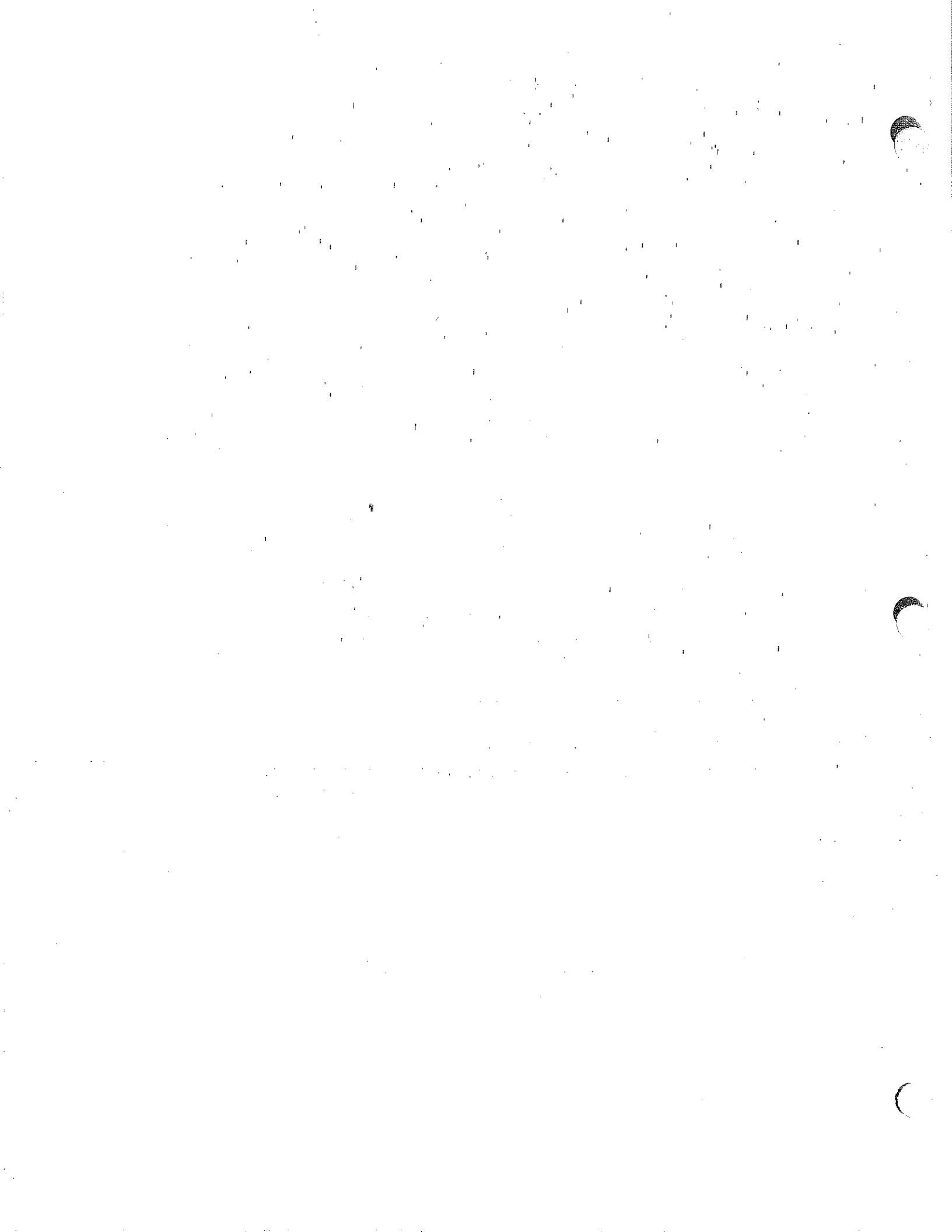


Figure 4.6 How different microscopes can reveal different aspects of the same organism—in this case, a green alga (*Scenedesmus*). The images of all four specimens are at the same magnification. The phase-contrast and Nomarski processes mentioned in (a) and (b) can create optical contrasts without staining the cells. Both processes enhance the usefulness of light micrographs.

As for other micrographs in the book, the short horizontal bar below the micrograph in (d) provides you with a visual reference for size. A micrometer (μm) is 1/1,000,000 of a meter.

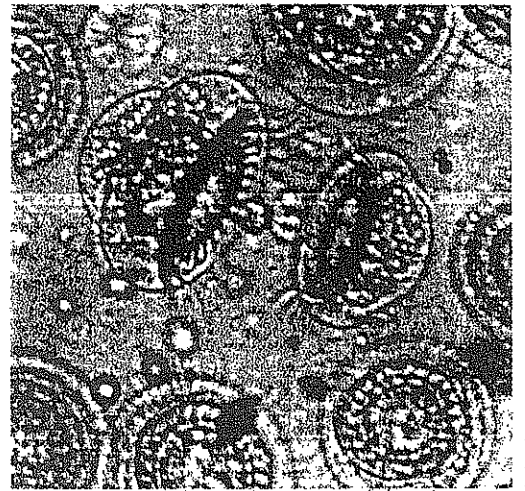


Before microscopes were first used about 330 years ago, no one knew for certain that living organisms were composed of cells. The first microscopes, like the ones you may have used in a biology laboratory, were light microscopes. A **light microscope (LM)** works by passing visible light through a specimen, such as a living *Trichodina* or a piece of animal or plant tissue. As Figure A shows, glass lenses in the microscope bend the light to magnify the image of the specimen and project the image into the viewer's eye or onto photographic film. A photograph taken through a microscope is called a **micrograph**. The notation "LM 109 \times " printed along the right edge of the micrograph of *Trichodina* in Figure A is in a form we use throughout this book. It tells you that the photograph was taken through a light microscope and that this image is about 109 times the actual size of the organism. *Trichodina* is actually about $\frac{1}{20}$ of a millimeter in diameter. This image could be magnified many more times than shown here. Beyond a certain point, though, the image would begin to blur, and additional magnification would only cause more blurring. Light microscopes can magnify objects only about 1000 times without causing blurriness.

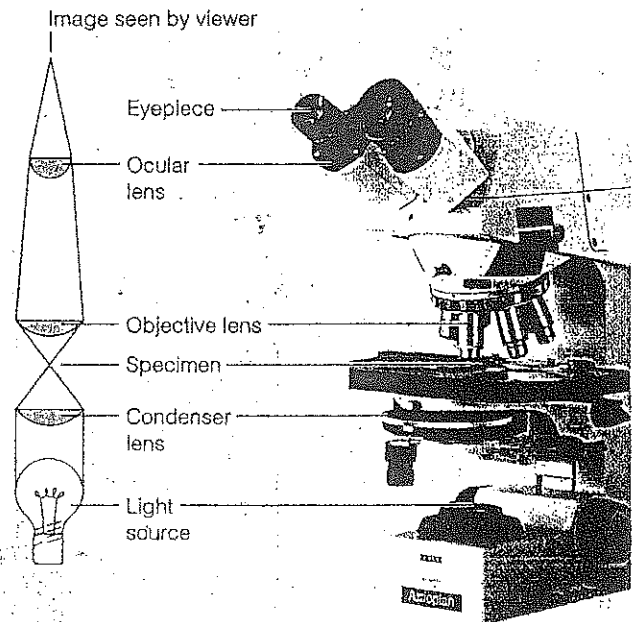
Magnification, the increase in the apparent size of an object, is only one important factor in microscopy (the use of a microscope). Also important is **resolving power**, a measure of the clarity of an image. Resolving power is the ability of an optical instrument to show two objects as separate. For example, what looks to your unaided eye like a single star in the sky may be resolved as two stars with the help of a telescope. Any optical device is limited by its resolving power. The light microscope cannot resolve detail finer than 0.2 micrometer (abbreviated μm ; $1 \mu\text{m} = \frac{1}{1000} \text{mm}$), about the size of the smallest bacterium. Consequently, no matter how many times its image of such a bacterium is magnified, the light microscope cannot show the details of the cell's internal structure. (The μ in the abbreviation for micrometer is the Greek letter mu.)

From the year 1665, when English microscopist Robert Hooke discovered cells, until the middle of this century, biologists had only light microscopes for viewing cells. But they discovered a great deal, including the cells composing animal and plant tissues, microscopic organisms—for example, *Trichodina* was discovered about 200 years ago—and some of the structures within cells. By the mid 1800s, these discoveries led to the **cell theory**, which states that all life is composed of cells and that all cells come from other cells.

Our knowledge of cell structure took a giant leap forward as biologists began using the electron microscope in the 1950s. Instead of light, the **electron microscope (EM)** uses a beam of electrons. The EM has a much higher resolving power than the light microscope. In fact, the most powerful modern EMs can distinguish objects as small as 0.2 nanometer (abbreviated nm ; $1 \text{nm} = \frac{1}{1,000,000} \text{mm}$), a thousandfold



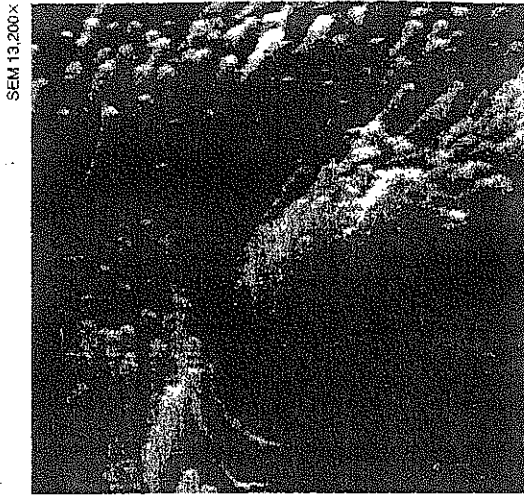
Light micrograph



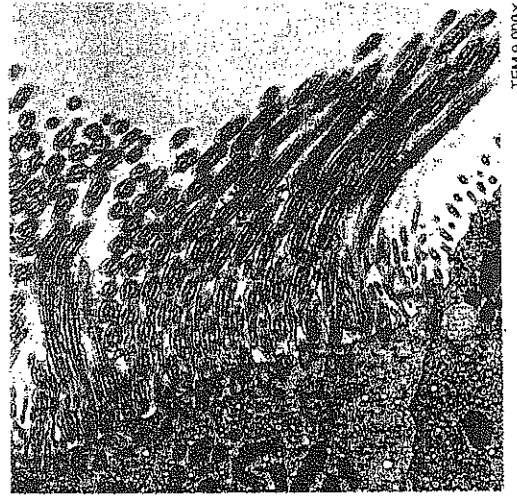
A. Light microscope (LM)

improvement over the light microscope. The period at the end of this sentence is about a million times bigger than an object 0.2 nm in diameter, which is the size of a large atom. Only under special conditions can EMs detect individual atoms. However, cells, cellular organelles, and even molecules like DNA and protein are much larger than single atoms. The highest-power electron micrographs you will see in this book have magnifications of about 100,000 times.

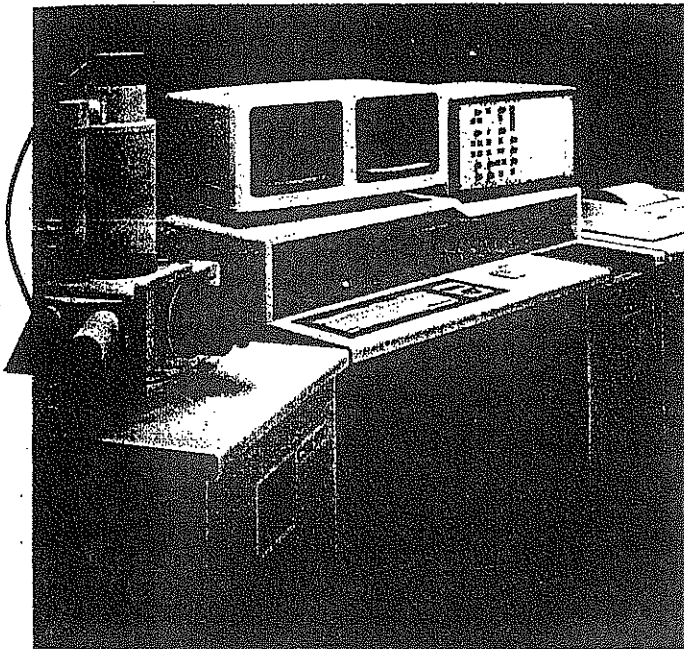
Figures B and C show two kinds of electron microscopes, along with images they have produced of cilia. (The cilia in the photographs are from cells of a rabbit, but



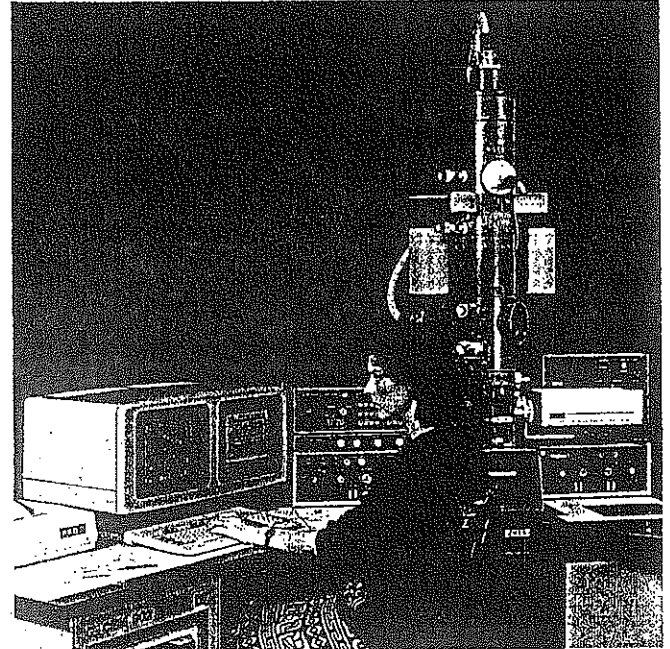
Scanning
electron
micrograph



Transmission
electron
micrograph



B. Scanning electron microscope (SEM)



C. Transmission electron microscope (TEM)

they are identical in structure to the cilia of *Trichodina* and other single-celled organisms.) Biologists use the scanning electron microscope (SEM) to study the detailed architecture of cell surfaces. The SEM uses an electron beam to scan the surface of a cell or group of cells that have been coated with metal. The metal stops the beam from going through the cells. When the metal is hit by the beam, it emits electrons. The electrons are focused to form an image of the outside of the cells. The scanning electron micrograph in Figure B shows the shapes and arrangement of cilia covering a cell. Many structural details of cell surfaces have been discovered using the SEM. As you can see, the SEM produces images that look three-dimensional.

The transmission electron microscope (TEM) is used to study the details of internal cell structure. Specimens are cut into extremely thin sections, and the TEM aims an electron beam through a section, just as a light microscope aims a beam of light through a specimen. However, instead of lenses

made of glass, the TEM uses electromagnets as lenses, as do all electron microscopes. The electromagnets bend the electron beam to magnify and focus an image onto a viewing screen or photographic film. The micrograph in Figure C shows internal details of the cilia of a rabbit cell as seen with the TEM.

Electron microscopes have truly revolutionized the study of cells and cell organelles. Nonetheless, they have not replaced the light microscope. One problem with electron microscopes is that they cannot be used to study living specimens because the specimen must be held in a vacuum chamber; that is, all the air and liquid must be removed. For a biologist studying a living process, such as the whirling movement of *Trichodina*, a light microscope equipped with a video camera might be better than either an SEM or a TEM. Thus, the light microscope remains a useful tool, especially for studying living cells. The size of a cell often determines the type of microscope a biologist uses to study it. The next two modules discuss the subject of cell size.