BME 1532-CELL BIOLOGY

Cellular Organelles and Microscopy Techniques

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Spring 2020

Last Week on BME 1532

- Cells as the Basic Units of Life
- Universal Features of All Cells
- Biomacromolecules
- Code of Life
- Gene Expression
- Common Structural Elements of All Cells
- Prokaryotes and Eukaryotes
- Ribosomes
- Endosymbiotic Theory

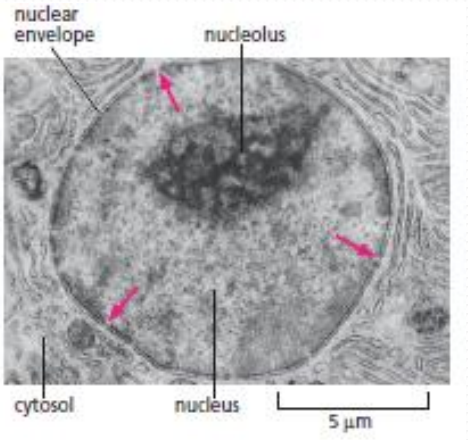
Membrane Enclosed Organelles in Eukaryotic Cells

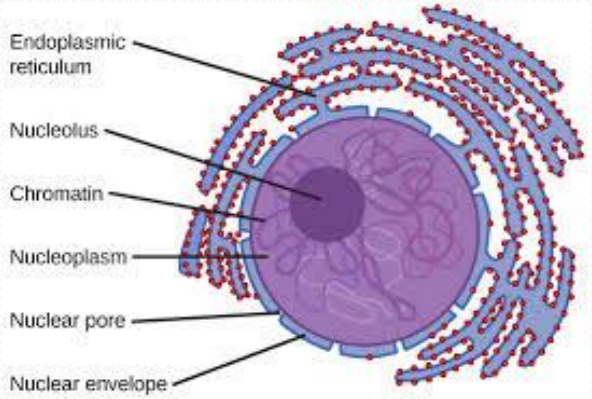
- Bacteria and archea consists of a single compartment enclosed by the plasma membrane, eukaryotic cells are elaborately subdivided by internal membranes.
- These structures that are enclosed by membranes are all distinct, membrane-enclosed organelles, each of which contains a unique set of large and small molecules and carries out a specialized function.
- These membranes also participate directly in the cell's metabolism, because many enzymes are found in the membranes.
- Furthermore, the cell's compartments provide different local environments that facilitate specific metabolic functions, so incompatible processes can go on simultaneously inside the same cell.
- If the cell did not have distinct membrane enclosed spaces for metabolic functions, both synthesis and degradation of biomacromolecules would occur in the same compartment (cytoplasm) and this would be very chaotic and harmful to the cell.

- Membranes of various kinds are fundamental to the organization of the cell.
- In general, biological membranes consist of a double layer of phospholipids and other lipids. In this lipid bilayer, proteins are embedded or attached to its surfaces.
- However, each type of membrane has a unique composition of lipids and proteins suited to that membrane's specific functions.

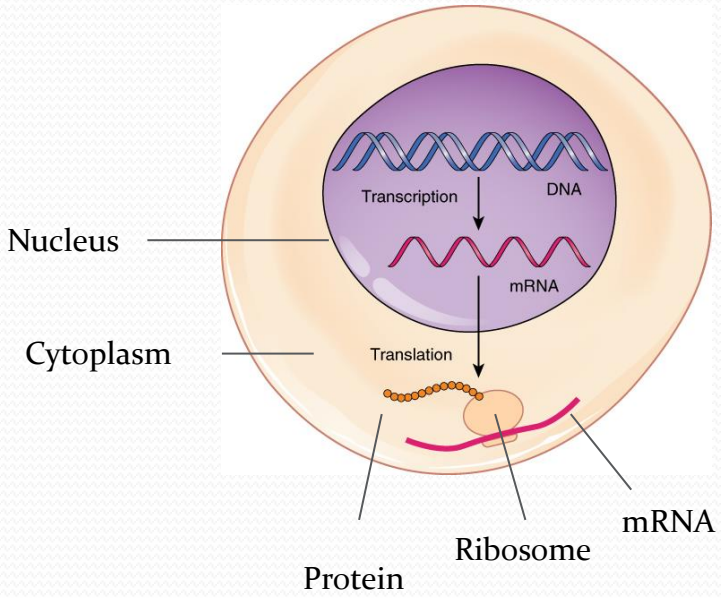
Nucleus

- The *nucleus* is generally the most prominent organelle in eukaryotic cells. It contains molecules of DNA.
- It has evolved to keep the DNA segregated from the physical and chemical chaos of the cytoplasm to allow delicate and complex control of the way the cell reads out its genetic information.
- It is surrounded by a double membrane, known as the *nuclear envelope*, and communicates with the cytosol via *nuclear pores* that perforate the envelope.
- Nuclear pores are protein complexes that regulate the entry and exit of certain large macromolecules and particles.
- Nucleolu is the dense region of nucleus in which ribosomal RNA (rRNA) are synthesized and ribosomes are assembled.



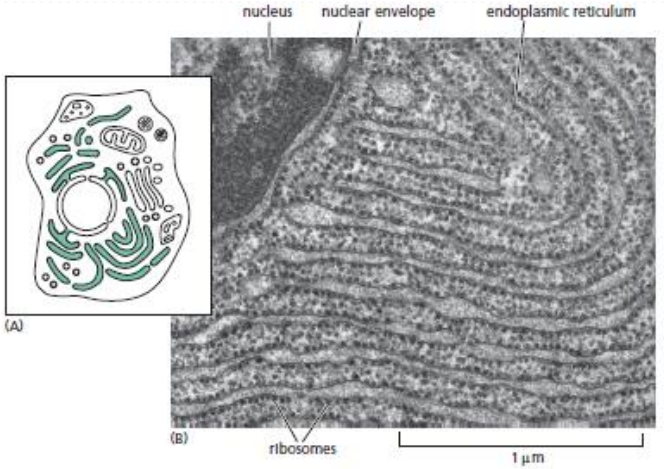


- The nucleus directs protein synthesis by synthesizing messenger RNA (mRNA) according to instructions provided by the DNA.
- The mRNA is then transported to the cytoplasm via the nuclear pores.
- Once an mRNA molecule reaches the cytoplasm, ribosornes translate the mRNA's genetic message into the primary structure of a specific polypeptide.



Endoplasmic Reticulum

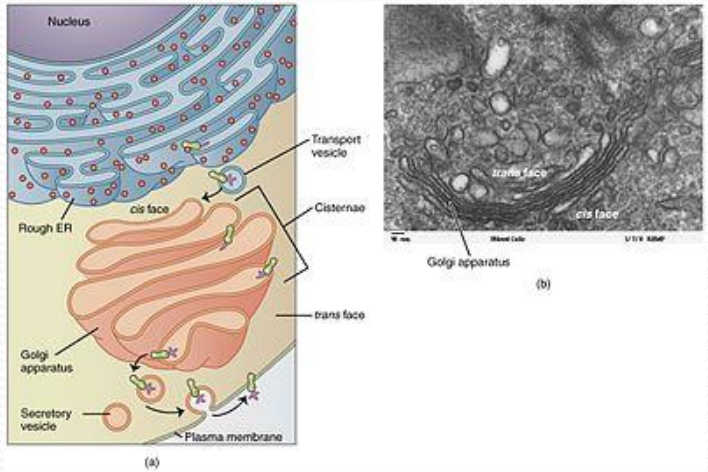
- Outer nuclear membrane is continuous with the membrane of the *endoplasmic reticulum* (*ER*), a system of interconnected sacs and tubes of membrane that often extends throughout most of the cell.
- The ER is the major site of synthesis of new membranes in the cell.
- Large areas of the ER have ribosomes attached to the cytosolic surface and are designated *rough endoplasmic reticulum* (*rough ER*).
- The ribosomes are actively synthesizing proteins that are delivered into the ER membrane or into the ER interior, a space called the *lumen*.



- The *smooth endoplasmic reticulum* (*smooth ER*) lacks ribosomes. It is the site of steroid hormone synthesis in some endocrine cells of the adrenal gland and the site where a variety of organic molecules, including alcohol, are detoxified in liver cells.
- In many eukaryotic cells, the smooth ER also sequesters Ca^{2+} from the cytosol; the release and reuptake of Ca^{2+} from the ER are involved in the rapid response to many extracellular signals.

Golgi Apparatus

- The *Golgi apparatus*, which is usually situated near the nucleus, receives proteins and lipids from the ER, modifies them, and then dispatches them to other destinations in the cell.
- Transport vesicles move material from the ER to the Golgi apparatus. Vesicles fuse to the membrane of the golgi apparatus where they are modified.
- Golgi products that will be secreted depart the Golgi inside transport vesicles that eventually fuse with the plasma membrane.



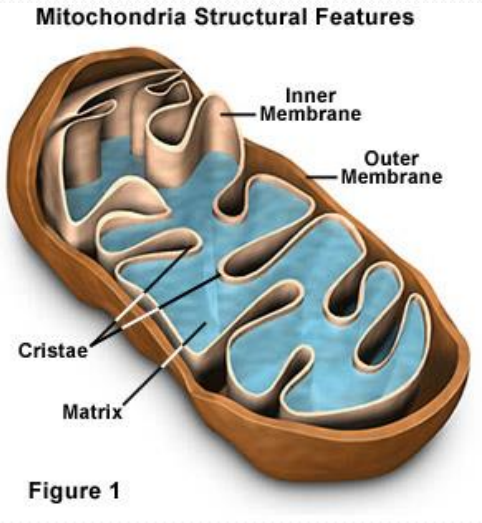
Lysosomes

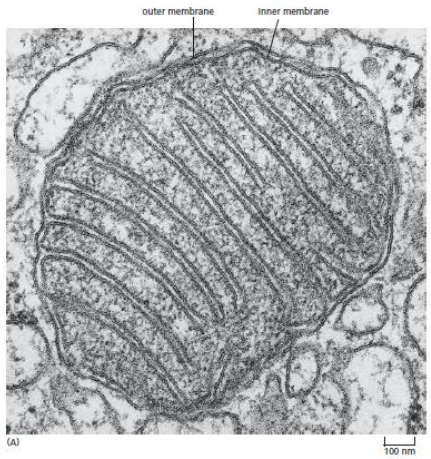
- A lysosome is a membranous sac of hydrolytic enzymes that a cell uses to digest all kinds of macromolecules.
- Inside lysosomes is acidic since lysosomal enzymes work best in the acidic environment.
- If a lysosome breaks open or leaks its contents. The released enzymes are not very active, because the cytosol has a neutral pH.
- However, excessive leakage from a large number of lysosomes can destroy a cell by autodigestion.
- Lysosomes carry out intracellular digestion in a variety of circumstances:
  - When the molecules are taken into the cell by phagocytosis, the vacuole formed in this way then fuses with a lysosome, whose enzymes digest the molecules. Digestion products, including simple sugars, amino acids, and other monomers, pass into the cytosol and become nutrients for the cell.
  - Lysosomes also use their hydrolytic enzymes to recycle the cell's own organic material. A damaged organelle or small amount of cytosol becomes surrounded by a membrane, and a lysosome fuses with this vesicle. The lysosomal enzymes dismantle the enclosed material and the organic monomers are returned to the cytosol for reuse.

- *Mitochondria* and (in plant cells) *chloroplasts* are each surrounded by a ***double membrane*** and are the sites of oxidative phosphorylation and photosynthesis, respectively; both contain internal membranes that are highly specialized for the production of ATP.

Mitochondria

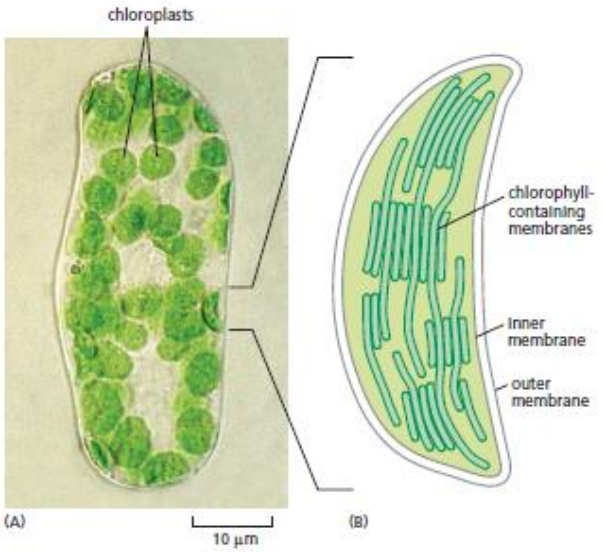
- Mitochondria have double membrane.
- Mitochondria are generators of chemical energy for the cell.
- They harness the energy from the oxidation of food molecules, such as sugars, to produce *adenosine triphosphate*, or *ATP*—the basic chemical fuel that powers most of the cell's activities.
- Because the mitochondrion consumes oxygen and releases carbon dioxide in the course of this activity, the entire process is called *cellular respiration*—essentially, breathing on a cellular level.
- Without mitochondria, animals, fungi, and plants would be unable to use oxygen to extract the energy they need from the food molecules that nourish them.
- Mitochondria contain their own DNA and reproduce by dividing in two.





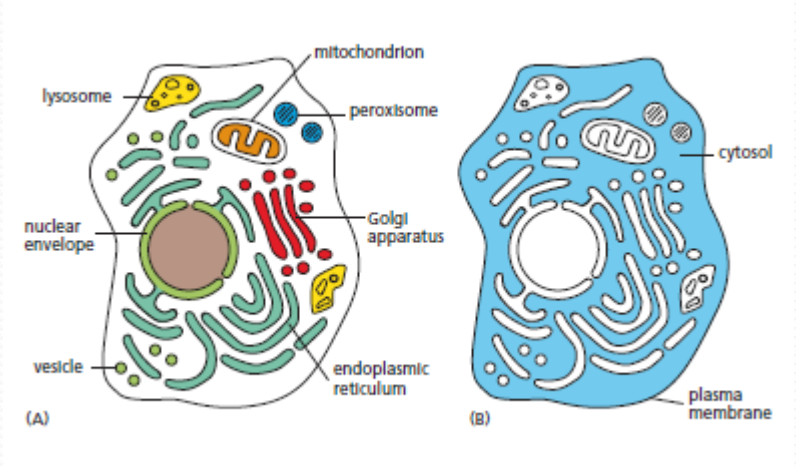
Chloroplasts

- Chloroplasts are large, green organelles that are found only in the cells of plants and algae, not in the cells of animals or fungi.
- These organelles have an even more complex structure than mitochondria: in addition to their two surrounding membranes, they possess internal stacks of membranes containing the green pigment *chlorophyll*.
- Chloroplasts carry out photosynthesis—trapping the energy of sunlight in their chlorophyll molecules and using this energy to drive the manufacture of energy-rich sugar molecules.
- In the process, they release oxygen as a molecular by-product.
- Plant cells can then extract this stored chemical energy when they need it, by oxidizing these sugars in their mitochondria, just as animal cells do.



- Chloroplasts thus enable plants to get their energy directly from sunlight. And they allow plants to produce the food molecules—and the oxygen—that mitochondria use to generate chemical energy in the form of ATP.
- Like mitochondria, chloroplasts contain their own DNA, reproduce by dividing in two.

- Membrane-enclosed morganelles are distributed throughout the eukaryotic cell cytoplasm.
- Cytosol is the part of the cytoplasm that is not contained within intracellular membranes.

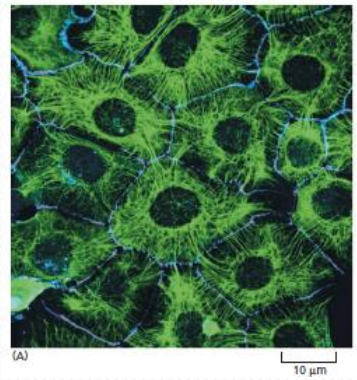


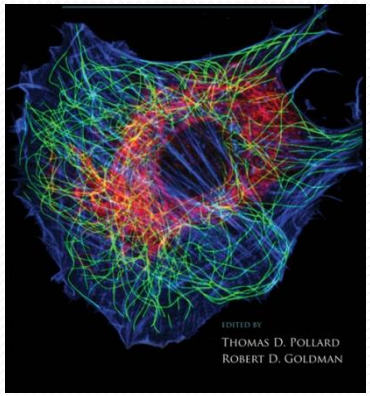
Cytoskeleton

- Many of the membrane-enclosed organelles, including the ER, Golgi apparatus, mitochondria, and chloroplasts, are held in their relative locations in the cell by attachment to the cytoskeleton, especially to microtubules.
- Cytoskeletal filaments provide tracks for moving the organelles around and for directing the traffic of vesicles between one organelle and another.
- These movements are driven by motor proteins that use the energy of ATP hydrolysis to propel the organelles and vesicles along the filaments.

The Cytoplasm Is Organized by the Cytoskeleton and Is Highly Dynamic

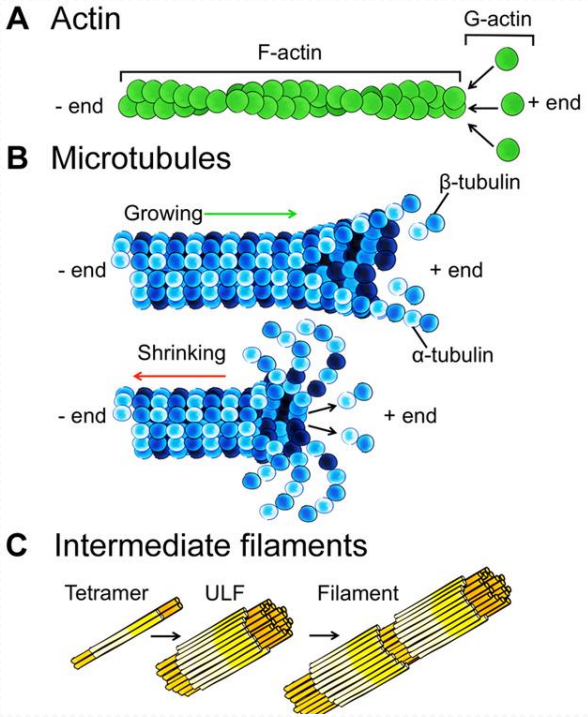
- There are several types of protein filaments crisscrossing the eukaryotic cell, forming an interlocking three-dimensional meshwork, the **cytoskeleton.**
- It helps cells **maintain their shape** and **internal organization**, and it also **provides mechanical support** that enables cells to carry out essential functions like **transport**, **division** and **movement**.
- There are three general types of cytoplasmic filaments—actin filaments, microtubules, and intermediate filaments —differing in width (from about 6 to 22 nm), composition, and specific function.

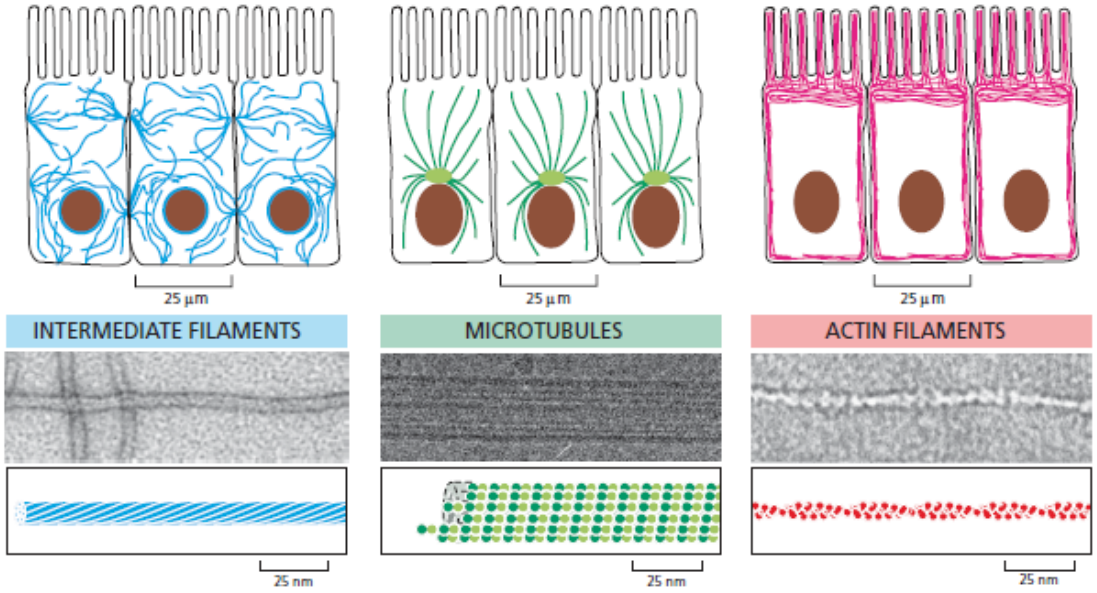




Cytoskeleton

- Each type of cytoskeletal component is composed of simple protein subunits that polymerize to form filaments of uniform thickness.
- These filaments are not permanent structures; they undergo constant disassembly into their protein subunits and reassembly into filaments.
- Their locations in cells are not rigidly fixed but may change dramatically with mitosis, cytokinesis, amoeboid motion, or changes in cell shape.
- The assembly, disassembly, and location of all types of filaments are regulated by other proteins, which serve to link or bundle the filaments or to move cytoplasmic organelles along the filaments.

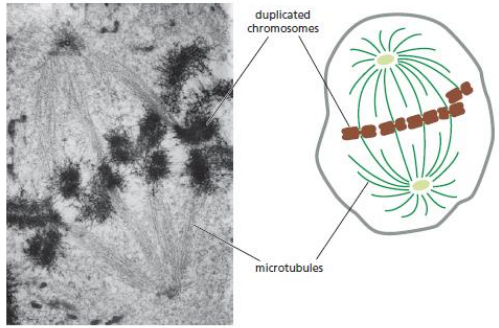




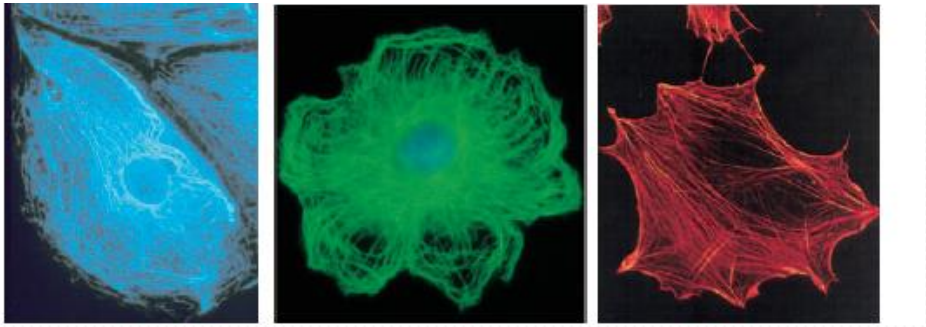
Intermediate filaments are ropelike fibers with a diameter of about 10 nm; they are made of fibrous intermediate filament proteins. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength and distributing the mechanical stresses in an epithelial tissue by spanning the cytoplasm from one cell–cell junction to another. Intermediate filaments are very flexible and have great tensile strength. They deform under stress but do not rupture. (Micrograph courtesy of Roy Quinlan.)

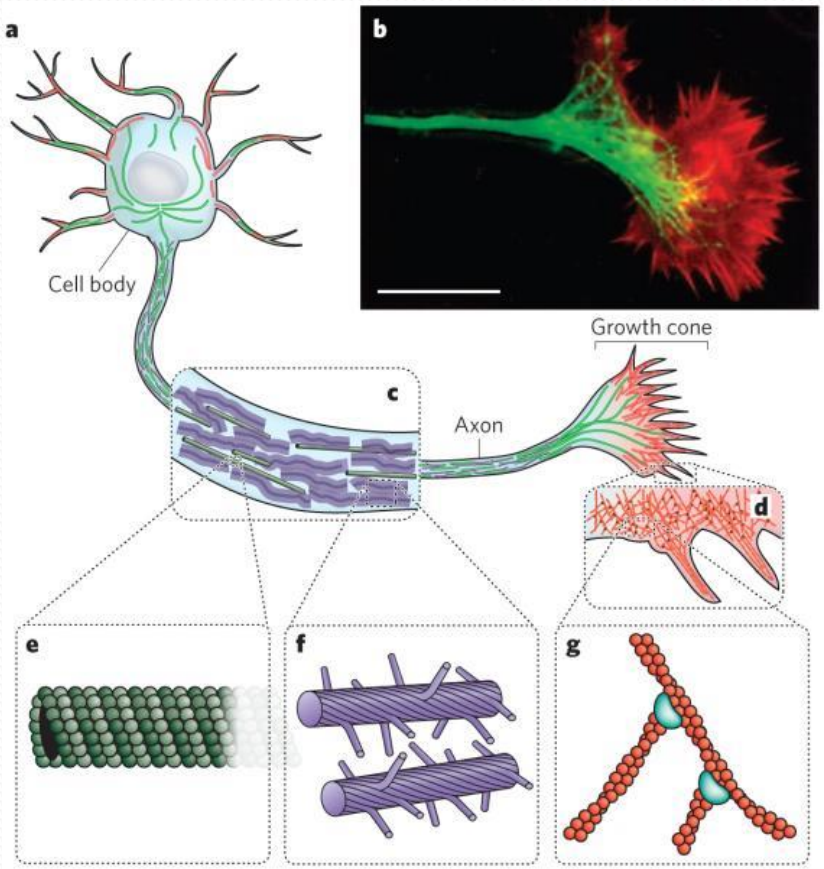
Microtubules are hollow cylinders made of the protein tubulin. They are long and straight and typically have one end attached to a single microtubule-organizing center called a *centrosome*. With an outer diameter of 25 nm, microtubules are more rigid than actin filaments or intermediate filaments, and they rupture when stretched. (Micrograph courtesy of Richard Wade.)

Actin filaments (also known as *microfilaments*) are helical polymers of the protein actin. They are flexible structures, with a diameter of about 7 nm, that are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, the layer of cytoplasm just beneath the plasma membrane. (Micrograph courtesy of Roger Craig.)



Microtubules help distribute the chromosomes in a dividing cell.

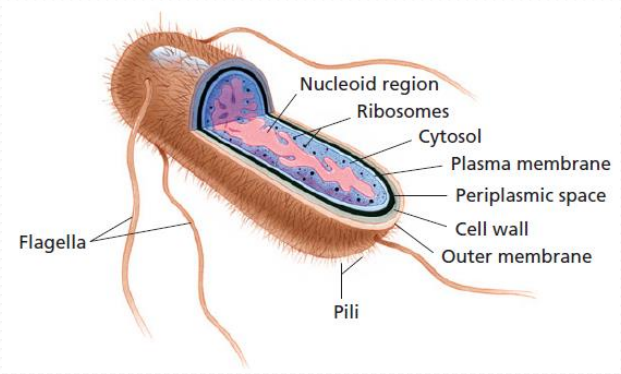




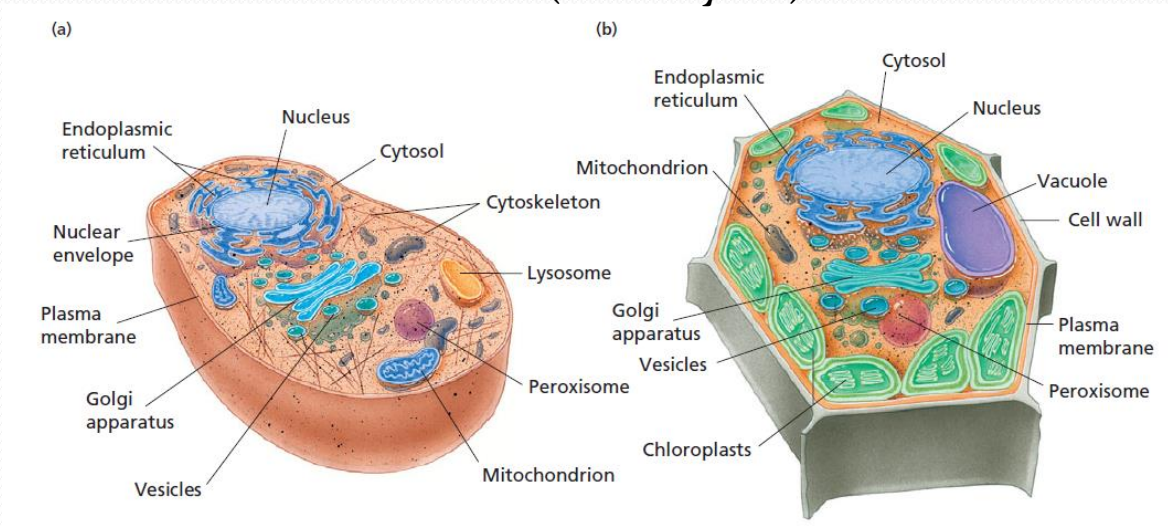
Microtubules
- Shape
- Organization
- Division
- Transport

Intermediate Filaments
- Shape
- Organization

Actin Filaments
- Shape
- Organization
- Movement
- Transport

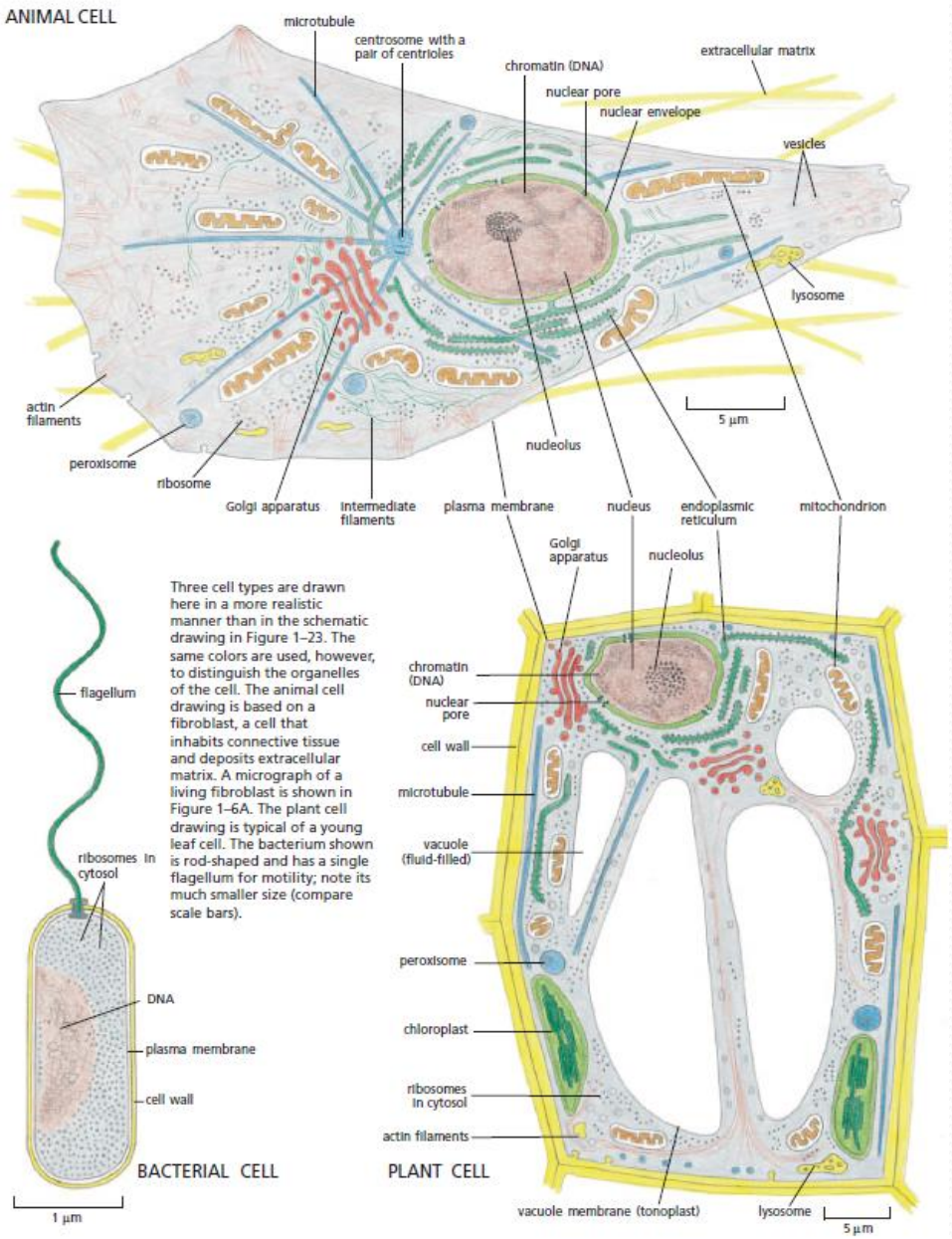


Bacteria (Prokaryote)



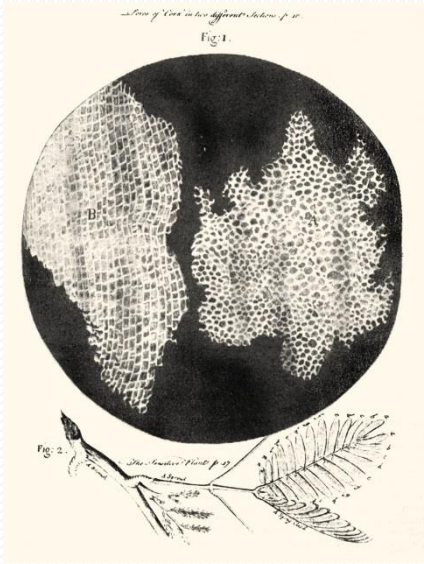
Animal Cell (Eukaryote)

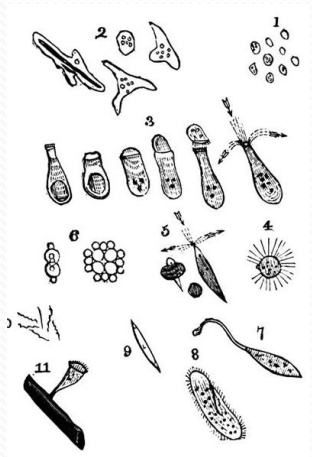
Plant Cell (Eukaryote)


ANIMAL CELL
microtubule
centrosome with a pair of centrioles
chromatin (DNA)
nuclear pore
nuclear envelope
extracellular matrix
vesicles
lysosome
5 µm
actin filaments
peroxisome
ribosome
Golgi apparatus
intermediate filaments
plasma membrane
nucleolus
nucleus
endoplasmic reticulum
mitochondrion
Three cell types are drawn here in a more realistic manner than in the schematic drawing in Figure 1–23. The same colors are used, however, to distinguish the organelles of the cell. The animal cell drawing is based on a fibroblast, a cell that inhabits connective tissue and deposits extracellular matrix. A micrograph of a living fibroblast is shown in Figure 1–6A. The plant cell drawing is typical of a young leaf cell. The bacterium shown is rod-shaped and has a single flagellum for motility; note its much smaller size (compare scale bars).
flagellum
ribosomes in cytosol
DNA
plasma membrane
cell wall
BACTERIAL CELL
1 µm
Golgi apparatus
nucleolus
chromatin (DNA)
nuclear pore
cell wall
microtubule
vacuole (fluid-filled)
peroxisome
chloroplast
ribosomes in cytosol
actin filaments
PLANT CELL
vacuole membrane (tonoplast)
lysosome
5 µm

Microscopical Techniques

- The development of the light microscope depended on advances in the production of glass lenses. By the seventeenth century, lenses were powerful enough to make out details invisible to the naked eye.
- Using an instrument equipped with such a lens, **Robert Hooke** examined a piece of cork and in 1665 reported to the Royal Society of London that the cork was composed of a mass of minute chambers.
- He called these chambers "cells," based on their resemblance to the simple rooms occupied by monks in a monastery.
- The name stuck, even though the structures Hooke described were actually the cell walls that remained after the living plant cells inside them had died.
- Later, Hooke and his Dutch contemporary **Antoni van Leeuwenhoek** were able to observe living cells, seeing for the first time a world teeming with motile microscopic organisms.





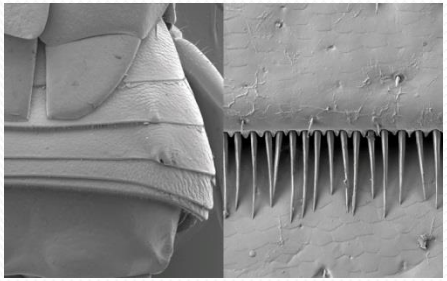
Microscope

- **Eyepiece (ocular)** is the lens the viewer looks through to see the specimen. The eyepiece usually contains a 10X lens.
- **Objective Lenses** are the primary optical lenses on a microscope. They range from 4x-100x and typically, include, three, four or five lenses on most microscopes.
- **Stage** is where the specimen to be viewed is placed.
- **Course Adjustment Knob** controls the height of the stage and rings the specimen into general focus.
- **Fine Adjustment Knob** fine tunes the focus and increases the detail of the specimen.
- **Diaphram** controls the amount of light reaching the specimen.
- **Illuminator** is the light source for a microscope, typically located in the base of the microscope.

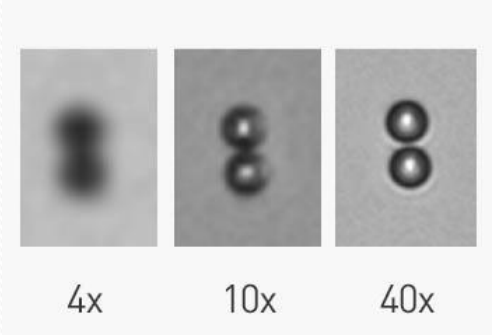
- An **inverted microscope** is a **microscope** with its light source on top and the objective lenses are below the specimen.
- It is generally used for cell culture studies.

- **Magnification** refers to the amount or degree of visual enlargement of an observed object.

Total Magnification = Ocular lens x Objective lens



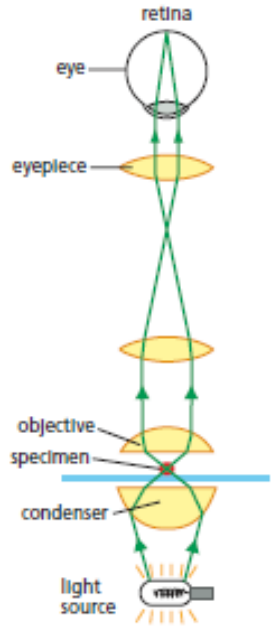
- **Resolution** is used to describe the ability of a microscope to distinguish detail.



Light Microscope

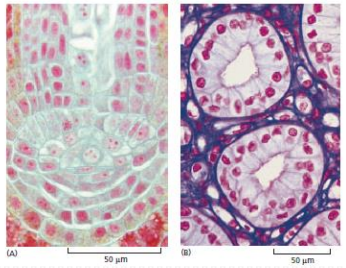
- If you cut a very thin slice from a suitable plant or animal tissue and view it using a light microscope, you will see that the tissue is divided into thousands of small cells.
- To see the internal structure of a cell is difficult, not only because the parts are small, but also because they are transparent and mostly colorless.
- One way around the problem is to stain cells with dyes that color particular components differently.
- Alternatively, one can exploit the fact that cell components differ slightly from one another in refractive index, just as glass differs in refractive index from water, causing light rays to be deflected as they pass from the one medium into the other.
- The small differences in refractive index can be made visible by specialized optical techniques such as phase contrast microscopy.

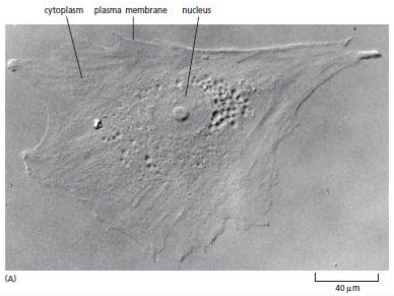
THE LIGHT MICROSCOPE



the light path in a light microscope

The light microscope allows us to magnify cells up to 1000 times and to resolve details as small as 0.2 µm (a limitation imposed by the wavelike nature of light, not by the quality of the lenses). Three things are required for viewing cells in a light microscope. First, a bright light must be focused onto the specimen by lenses in the condenser. Second, the specimen must be carefully prepared to allow light to pass through it. Third, an appropriate set of lenses (objective and eyepiece) must be arranged to focus an image of the specimen in the eye.

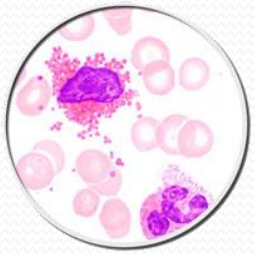


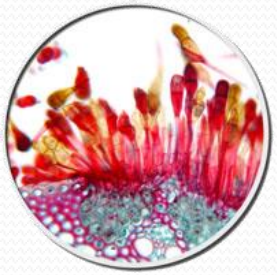


Types of Light Microscope

Brightfield Microscopy

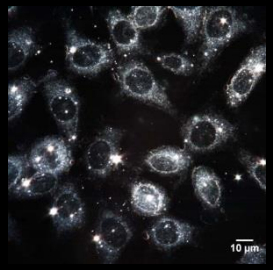
The object to be inspected is normally placed on a clear glass slide, and light is transmitted though the object. This makes the object appear against a bright background.





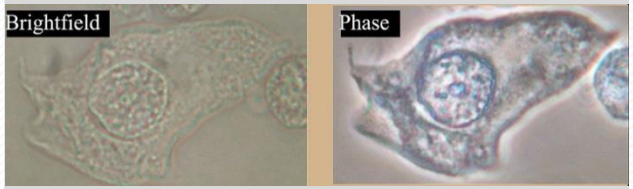
Darkfield Microscopy

It has an opaque disk that blocks the light which is not passing through the specimen so that only light that is scattered by objects on the slide can reach the eye. Thus, it shows the specimens bright on a dark background.



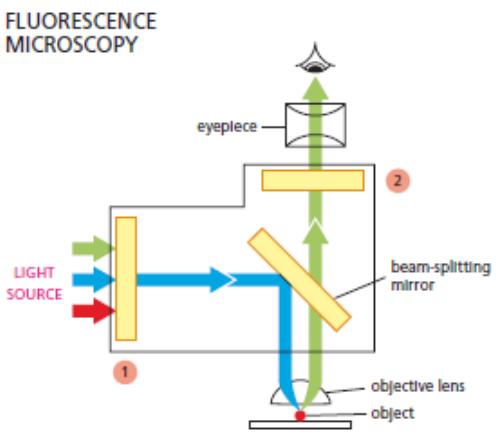
Phase-Contrast Microscopy

It enhances the phase difference between the specimen and its surroundings. Specimens which have a refractive index similar to their surroundings can be invisible in Brightfield, but are well defined in Phase Contrast.



Fluorescence Microscope

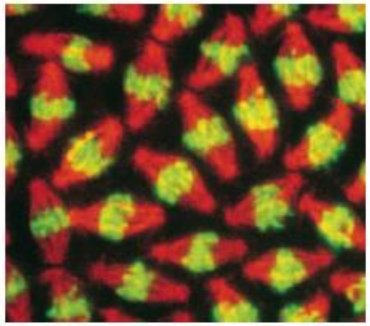
- Fluorescence Microscopy also uses light as the illumination source.
- However, it uses filters to filter the wavelength that will excite the specimen and then filter the wavelength that is emitted from the specimen.
- Fluorescent microscope can only visualize by tagging the molecules with fluorescent dyes or antibodies. These fluorescent substances absorb light and emit light at a specific wavelength.



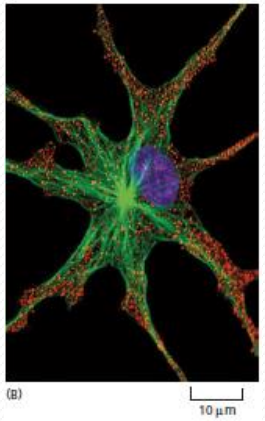
Fluorescent dyes used for staining cells are detected with the aid of a *fluorescence microscope*. This is similar to an ordinary light microscope except that the illuminating light is passed through two sets of filters. The first (1) filters the light before it reaches the specimen, passing only those wavelengths that excite the particular fluorescent dye. The second (2) blocks out this light and passes only those wavelengths emitted when the dye fluoresces. Dyed objects show up in bright color on a dark background.

FLUORESCENT PROBES

Dividing nuclei in a fly embryo seen with a fluorescence microscope after staining with specific fluorescent dyes.



Fluorescent dyes absorb light at one wavelength and emit it at another, longer wavelength. Some such dyes bind specifically to particular molecules in cells and can reveal their location when examined with a fluorescence microscope. An example is the stain for DNA shown here (*green*). Other dyes can be coupled to antibody molecules, which then serve as highly specific and versatile staining reagents that bind selectively to particular large molecules, allowing us to see their distribution in the cell. In the example shown, a microtubule protein in the mitotic spindle is stained *red* with a fluorescent antibody. (Courtesy of William Sullivan.)

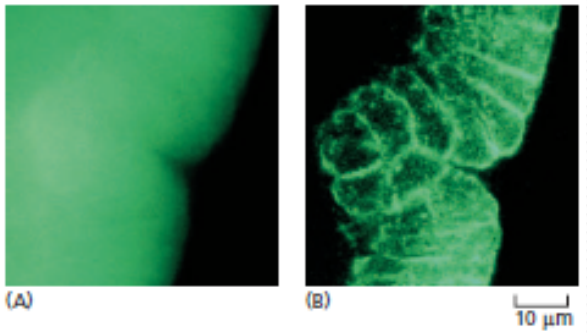


Confocal Microscope

- Confocal Microscopy uses lasers of specific wavelength as the illumination source.
- Only a single plane of focus is illuminated; out-af-focus fluorescence above and below the plane is subtracted by a computer which results in a sharp image.
- Sharper reflecting more details and could be observed in 3D.

CONFOCAL MICROSCOPY

A confocal microscope is a specialized type of fluorescence microscope that builds up an image by scanning the specimen with a laser beam. The beam is focused onto a single point at a specific depth in the specimen, and a pinhole aperture in the detector allows only fluorescence emitted from this same point to be included in the image. Scanning the beam across the specimen generates a sharp image of the plane of focus—an *optical* section. A series of optical sections at different depths allows a three-dimensional image to be constructed. An intact insect embryo is shown here stained with a fluorescent probe for actin filaments. (A) Conventional fluorescence microscopy gives a blurry image due to the presence of fluorescent structures above and below the plane of focus. (B) Confocal microscopy provides an optical section showing the individual cells clearly. (Courtesy of Richard Warn and Peter Shaw.)

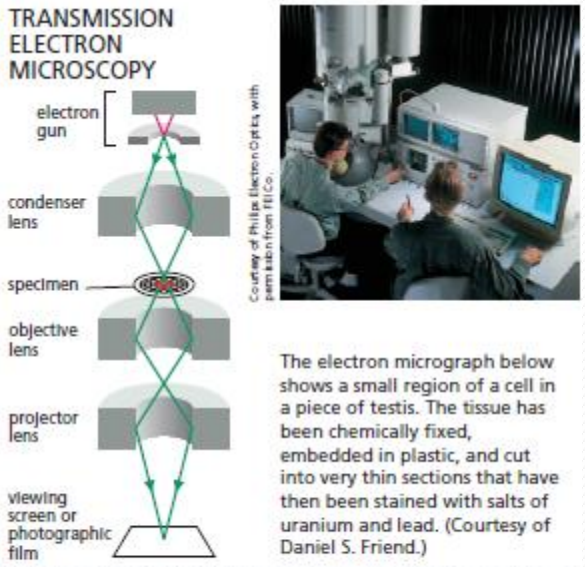


Electron Microscope

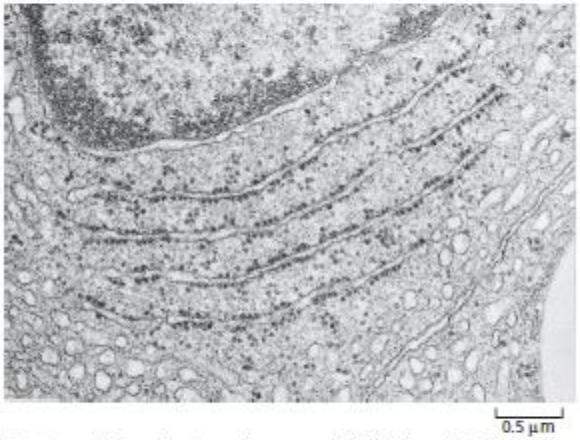
- Although cells were discovered by Robert Hooke in 1665, the geography of the cell was largely uncharted until the 1950s.
- Most subcellular structures, or organelles, are too small to be resolved by the light microscope.
- Instead of using light, the electron microscope (EM) focuses a beam of electrons through the specimen or onto its surface.
- Resolution is inversely related to the wavelength of the radiation a microscope uses for imaging, and electron beams have wavelengths much shorter than the wavelengths of visible light.
- Thus, an electron microscope can resolve objects that are very small, down to a few nanometers.

Transmission Electron Microscope

- The type of electron microscope used to look at thin sections of tissue is known as a *transmission electron microscope*. This is, in principle, similar to a light microscope, except that it transmits a beam of electrons rather than a beam of light through the sample.
- TEM used mainly mainly to study the internal ultrastructure of cells such as organelles, cytoskeleton, and DNA.



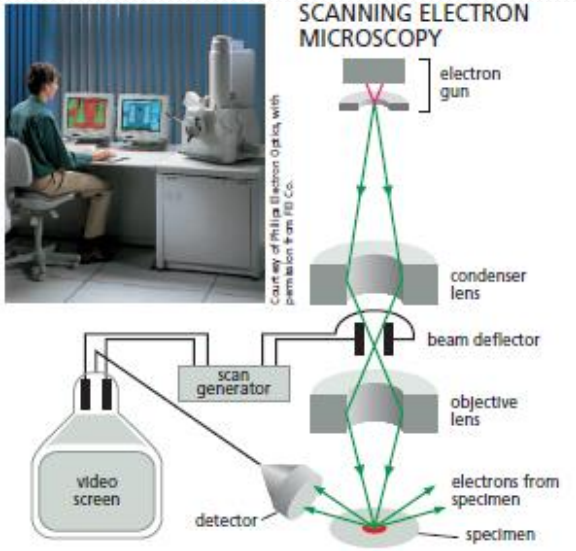
The electron micrograph below shows a small region of a cell in a piece of testis. The tissue has been chemically fixed, embedded in plastic, and cut into very thin sections that have then been stained with salts of uranium and lead. (Courtesy of Daniel S. Friend.)



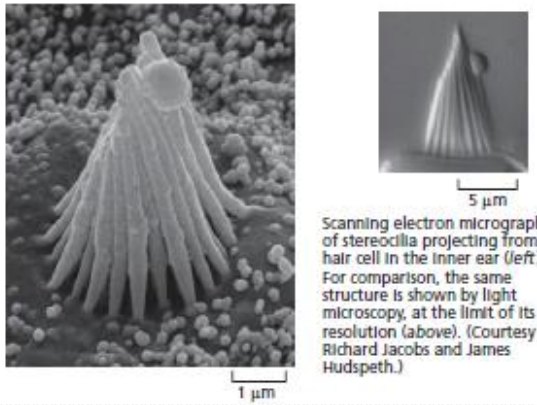
The transmission electron microscope (TEM) is in principle similar to a light microscope, but it uses a beam of electrons instead of a beam of light, and magnetic coils to focus the beam instead of glass lenses. The specimen, which is placed in a vacuum, must be very thin. Contrast is usually introduced by staining the specimen with electron-dense heavy metals that locally absorb or scatter electrons, removing them from the beam as it passes through the specimen. The TEM has a useful magnification of up to a million-fold and can resolve details as small as about 1 nm in biological specimens.

Scanning Electron Microscope

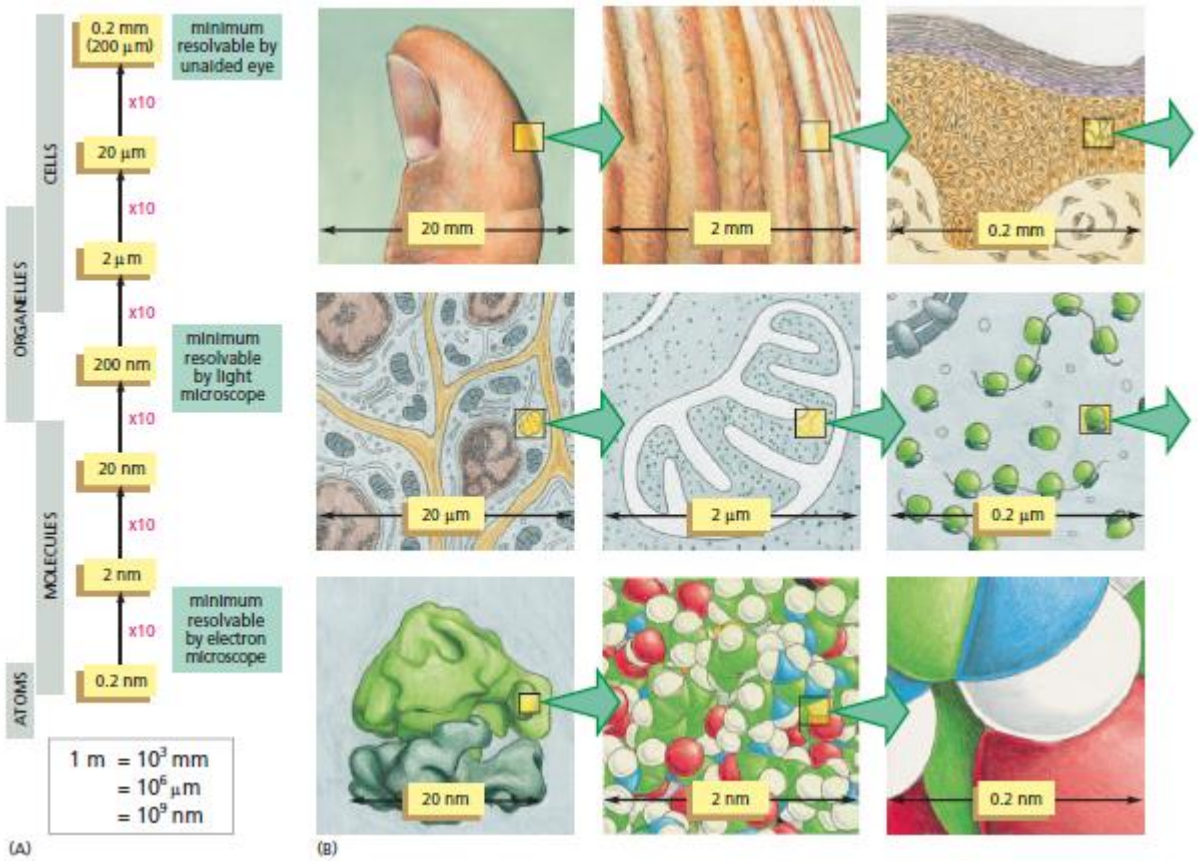
- Another type of electron microscope—the *scanning electron microscope*—scatters electrons off the surface of the sample and so is used to look at the surface detail of cells and other structures.
- Thus, SEM is especially useful for detailed study of the surface of a specimen.



In the scanning electron microscope (SEM), the specimen, which has been coated with a very thin film of a heavy metal, is scanned by a beam of electrons brought to a focus on the specimen by magnetic coils that act as lenses. The quantity of electrons scattered or emitted as the beam bombards each successive point on the surface of the specimen is measured by the detector, and is used to control the intensity of successive points in an image built up on a video screen. The microscope creates striking images of three-dimensional objects with great depth of focus and can resolve details down to somewhere between 3 nm and 20 nm, depending on the instrument.



Scanning electron micrograph of stereocilia projecting from a hair cell in the inner ear (left). For comparison, the same structure is shown by light microscopy, at the limit of its resolution (above). (Courtesy of Richard Jacobs and James Hudspeth.)



- Even the most powerful electron microscopes, however, cannot visualize the individual atoms that make up biological molecules.
- To study the cell's key components in atomic detail, biologists have developed even more sophisticated tools such as atomic force microscopy, nuclear magnetic resonance, X-ray crystallography etc.

Next Week on BME 1532

- DNA
- Higher Level Structures of DNA
- DNA Replication
- DNA repair