Lab #4: Microscopy & Structure and Function of Cells

OVERVIEW

The microscope is one of the most important and frequently used tools in the biological sciences. It allows the user to peer into the world of the cell, as well as discover the fascinating world of microscopic organisms. A typical compound microscope, similar to the one that we will use in today's activity, is capable of extending the vision of the observer more than a thousand to one million times. Since its invention more than 300 years ago, the microscope has greatly improved our understanding of the cell, tissues, disease, and ecology.

Microscopy Concepts

- **Magnification**: the act of enlarging something in appearance
- **Contrast**: difference in brightness and/or color that makes an object distinguishable
  - The ability to distinguish between specimen and background
- **Resolution**: clarity of an image; the ability to distinguish between two points

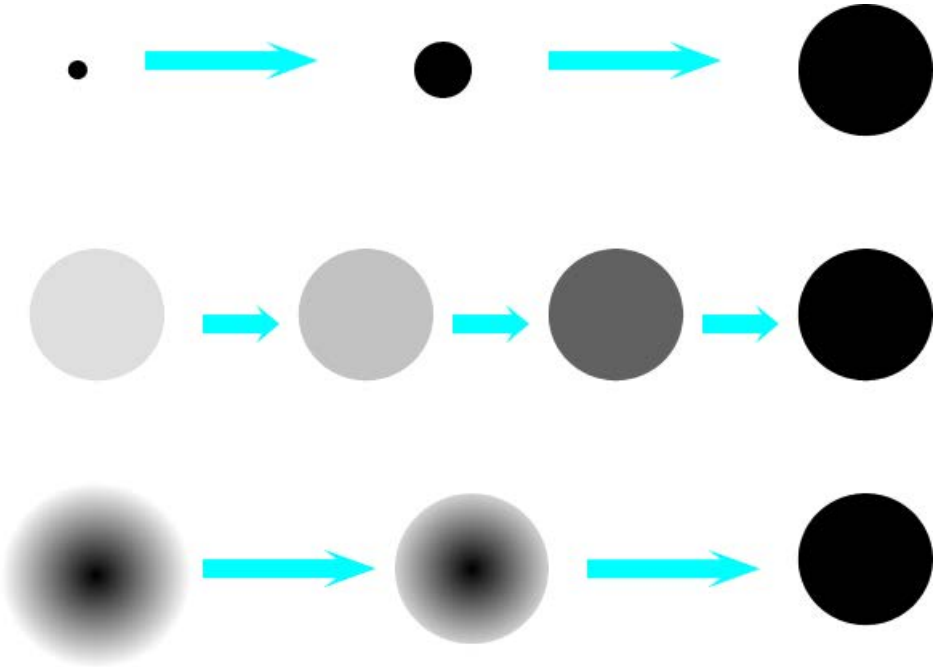

Figure 1. Microscopy concepts: magnification, contrast, resolution.

Microscope Comparisons

- **Light microscope**: uses any kind of light to view a specimen
    - **Simple light microscope**: have a single lens, similar to the early microscopes made by Anton Von Leeuwehoek.
    - **Compound microscope**: uses two sets of lenses to magnify an object
- **Compound bright field microscope**: capable of magnification range from 10-2000x and resolution of 300 nanometers (nm). Light is transmitted directly through the specimen, and the specimen generally appears as a dark object against a light background. This microscope is used to examine various types of cells, microscropic organisms, and tissues.
- **Dark field microscope**: similar to the brightfield microscope except that a special condenser causes light rays to reflect off the specimen at an angle, making the specimen appear bright against a dark background. This type of microscope is particularly useful when viewing specimens that lack contrast with a bright field microscope.
- **Fluorescence microscope**: uses ultraviolet light and fluorescent dyes to study specimens. This microscope is capable of magnifications from 10-3000x and has a resolution of 200nm. It is used in advanced biological laboratories and medical laboratories to study cells, antibodies, microscopic organisms, and tissues.
- **Phase contrast microscope**: uses regular light for illumination but possesses a special condenser to accent minute differences in the refractive index of structures within a specimen. As a result, it is useful in studying cellular components and microscopic organisms. It is capable of magnifications from 10-1500x and has a resolution of 200nm.
- **Electron microscopes**: utilize beams of electrons to magnify a specimen. They are capable of greater magnification than compound microscopes.
    - **Transmission Electron Microscope (TEM)**: uses extremely thin sections of specimens treated with heavy metal salts and is capable of magnifications from 200-1,000,000x. The TEM has a resolution of 0.1 micrometers (μm) because of the shorter wavelength of the electron beam. This instrument is used to study the ultrastructure of cells and certain biochemicals.
    - **Scanning Electron Microscope (SEM)**: provides three-dimensional views of objects and has a greater depth of focus. It is capable of magnifications from 10-500,000x and a resolution of 5.0 to 10.0 nm. The SEM is useful in studying the surface features of specimens.

Anatomy of Compound Light Microscope

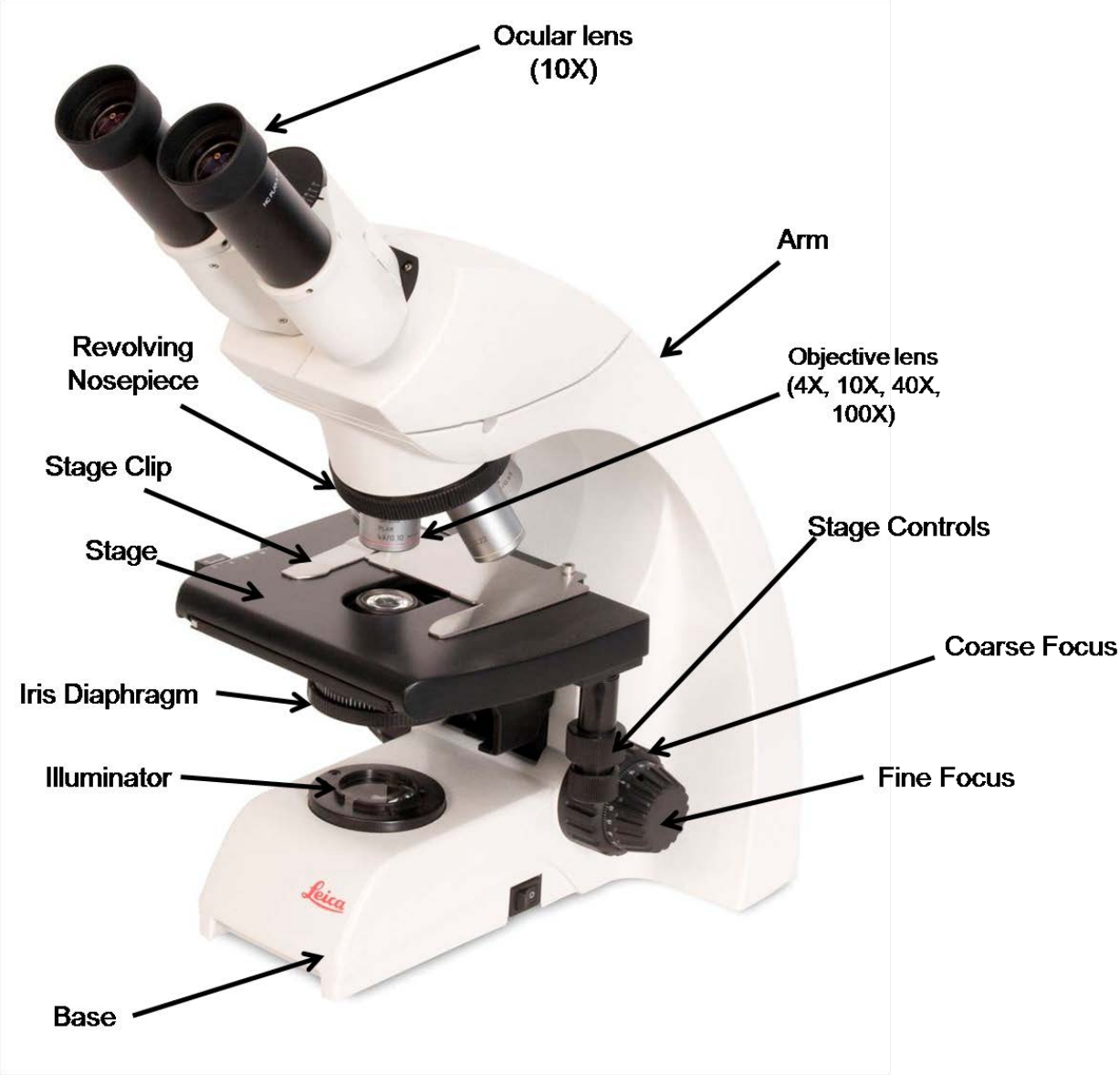

Figure 2. Compound Light Microscope

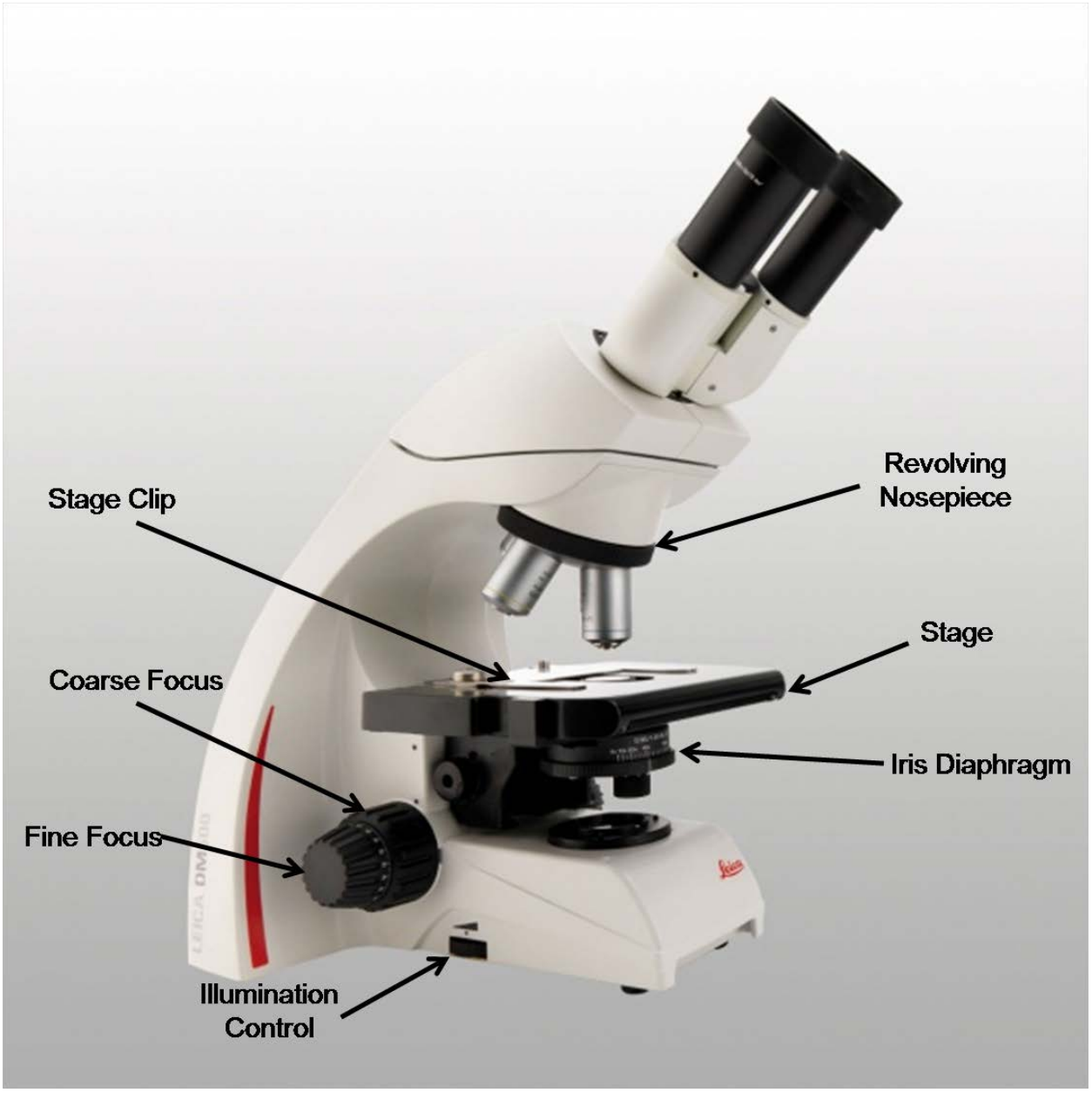

Figure 3. Compound Light Microscope

Properties	Definition
Parfocal	Once an objective has been focused, you can rotate another objective and the image will remain in coarse focus, requiring only slight movement of the fine focus knob
Parcentral	The center of the field of view remains about the same for each objective
Field of View	The circle of light you see when looking through the ocular lens
Working Distance	The space between the objective and the slide

Microscope Parts	Function
Ocular Lens	Magnifies specimen 10X; for viewing
Base	Supports the microscope
Arm	Serves as a handle
Revolving Nosepiece	Revolves and holds objectives
Objective Lens	Magnify specimen 4X, 10X, 40X, or 100X
Stage	Platform on which slides are placed
Stage Clip	Secures the slide
Stage Controls	Moves slide in the x & y-directions
Coarse Focus	Raises and lowers the stage for focusing
Fine Focus	Slightly moves the stage to sharpen image
Illuminator	Light source
Iris Diaphragm	Regulates the amount of light passing through the specimen as well as contrast of the specimen

USING THE MICROSCOPE

1. Ensure that your work area is clean and uncluttered.
2. After procuring your microscope from the cabinet, carry the microscope close to your body in an upright position with one hand holding the arm. Place the microscope directly in front of you on the table. Once in place, do not drag the microscope across the table for others to view. Doing so can damage the intricate optics and mechanisms of the microscope.
3. Examine the anatomy of the microscope.
4. Carefully clean the ocular and objective with *lens paper only*. If a smudge or scratch persists, consult the laboratory instructor.
5. Make sure that the microscope was stored with the scanning or low-power objective in place. Never begin a session with the high power objective in place. If necessary, use the revolving nosepiece to click the low-power objective in place.
6. Inspect the electrical cord, making sure that it is not frayed or damaged. Plug in the electric cord as instructed so it will not get in your way, trip other students, or damage the microscope. Turn the switch to the "on" position.
7. A good quality clean slide will be provided for observation. Carefully place your slide on the stage, and use the stage clips or the mechanical stage to hold it in place. Using the stage controls, center the specimen under the objective.
8. If using a binocular microscope, adjust the distance between the oculars to match the distance between your pupils. One of the oculars on some binocular microscopes can be focused individually for previsions. If using a monocular microscope, keep both eyes open to view the object, as closing one eye will result in eyestrain and a headache. If you are having difficulty keeping just one eye open, consult your lab instructor.
9. Use the coarse focus knob to focus the specimen. This knob is to be used to view specimens under scanning and low power only (for use with 4X objective lens ONLY!). Depending upon the brand of the microscope, either the nosepiece will move toward the stage or the stage will move toward the nosepiece. Practice your microscopy skills by viewing various parts of the slide. While viewing the slide, do not rest your hand on the stage.
10. Reposition the slide to attain the desired view. Use the iris diaphragm to focus and regulate the light entering the microscope. On scanning and low power, the fine focus knob may be used to fine-tune the specimen. Sketch and record the name and magnification of your specimen.
11. Using the revolving nosepiece, rotate the high power objective into position until you feel it click into place.
12. Many microscopes are **parfocal** (once the image is focused with one objective, it should be in focus with others) and require only minor adjustments in focusing. Using the **fine focus knob only**, focus your specimen. You may have to reposition your specimen carefully and adjust the iris diaphragm. Sketch and record the name and magnification of your specimen.

Understanding Magnification

Objective	Objective Magnifying Power (Obj)	Ocular Magnifying Power (Ocu)	Total Magnifying Power (Obj. x Ocu)
Low-power (scanning-power)			
Medium-power			
High-dry			
Oil-immersion (high-power)			

Understanding Image Orientation: Procedures

1. Place a prepared slide of the letter "e" on the stage of your microscope. Using low power, observe the slide. Sketch and describe your slide below.

   _____

   _____

   Question: What is the difference between the orientation of the letter between the unaided eye and the microscope?

   _____

   _____

2. Move the slide to the left and to the right. Describe the direction of the movement of the image.

   _____

   _____

Understanding Illumination

When using a microscope, proper illumination is essential as improper illumination can result in poor images and eyestrain. Most microscopes are equipped with an illuminator, attached to the base. The iris diaphragm is used to adjust the amount of light entering the objective lens by rotating a disc or moving an aperture adjustment control. The iris diaphragm can be used to regulate the light passing through an object, as well as the contrast of the object. Although closing the diaphragm results in poorer resolution, it can be useful in viewing certain specimens.

Procedures:

1. Carefully place a slide (instructor's choice) on the stage, and focus using low power.
2. Use the iris diaphragm to attain the best illumination for the specimen. Open and close the iris diaphragm and notice changes in the contrast of the specimen.
3. Follow the same procedure using the medium-power and high-dry objectives.
4. After you finish, return the slide to the slide tray.
5. Briefly describe the changes in illumination and contrast that you observed.

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_____

Using Oil Immersion

Many microscopes have an oil immersion objective for observing small organisms and the fine detail of specimens. It appears longer than the other objectives and is clearly marked. Oil immersion objectives are capable of magnification of 100x.

Procedures:

1. Obtain a prepared slide of human blood and place it on the stage of the microscope. The red blood cells, or **erythrocytes**, will appear as lightly stained biconcave discs with a lighter colored center. White blood cells, **leukocytes**, will appear large with a dark-stained nucleus.
2. Focus and view the specimen under low power.
3. Place a drop of immersion oil in the center of the viewing area of the slide. Do not use any other objectives with immersion oil and be careful not to get the oil on anything. Click the oil immersion objective into place. Focus on the specimen using the **fine focus knob ONLY**. Note: **DO NOT** use the coarse focus knob or this will break the slide and/or scratch the extremely expensive oil immersion objective lens!

4. Once the exercise is complete, clean the slide and objective thoroughly with lens paper. Lens paper with a drop of lens cleaner is often used to clean the objective and slide. Return the lowest power objective into place.

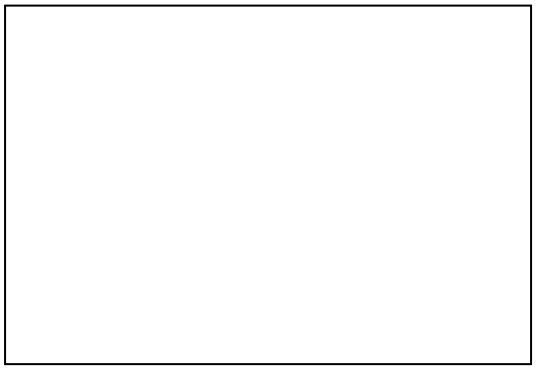
Preparing Wet Mounts

Wet mounts are often made in the laboratory to view fresh specimens.

Procedures:

1. Clean and dry a slide and coverslip thoroughly.
2. When mounting bits of tissue, place a small piece of the tissue to be studied in the center of the slide. Using a teasing needle, tease the tissue out flat. Add a drop of water, stain, or other reagent, and lower the coverslip on the tissue as discussed next. Your instructor will tell you what to use specifically. For pond culture studies, place a drop of the culture liquid in the center of the slide and lower a coverslip. Always place a coverslip over wet mounts.
3. Holding the coverslip at an angle, place its edge in the margin of the drop of liquid on the slide and release the coverslip slowly. A pair of forceps will help you lower the coverslip. The procedure will minimize air bubbles from forming beneath the coverslip.
4. If the coverslip floats on the liquid, it is caused by excess water on the slide. The excess water may be siphoned off with the edge of a paper towel. Too much liquid (water or stain) should be removed. If some of the reagents get on the microscope, they may corrode it and cause serious damage.
5. Many times, when observing living specimens, they appear to move rapidly. To slow them down, place a drop of a prepared slowing solution ("protoslo"). The result will be equivalent to a person swimming in a pool of molasses.
6. Prepare and observe a wet mount of pond water. Use the scanning, low-power and high-dry power objectives in this activity.
7. Sketch your observations below.

Pond Water



STEREOMICROSCOPE

A stereomicroscope is also known as a dissecting microscope. The stereomicroscope has two oculars and is capable of magnifications of 4x to 50x. Stereomicroscopes provide a significantly greater field of view and depth of field than compound microscopes. This type of microscope is advantageous when viewing larger objects and dissecting.

Procedures:

1. Choose an object to observe under the stereomicroscope from the options provided by the instructor.
2. Use a lamp, or other separate light source, to illuminate the object.
3. Use the focus adjustment knob to bring the object in focus.

STORAGE OF THE MICROSCOPE

1. At the completion of each laboratory experience, rotate the lowest power objective into place and remove the slide.
2. Clean the ocular and objectives with **lens paper only**.
3. If you have been using oil immersion, clean the slide and the objectives as instructed in the procedure for using oil immersion.
4. If you have been using wet mounts, clean the stage with a cleaning tissue or a clean cloth.
5. Turn off the light and unplug the microscope. Wrap the cord around the base as directed by the instructor. Place the dust cover over the microscope and carefully return it to the cabinet.

The Structure and Function of Cells

OVERVIEW

The **cell** is the smallest unit of biological organization that can undergo the activities associated with life, such as metabolism, response, and reproduction. The **cell theory** states that all living things are composed of cells and that the cell is the basic unit of structure and function of all living things.

Although cells vary in size from a bacterium 1 to 10 micrometers (μm) in diameter to a chicken egg larger than 1 centimeter (cm) in diameter, most cells are microscopic.

The inclusions and organelles within the cell are much smaller and are measured in nanometers (nm). The reason for the absence of giant cells is a matter of the surface area-to-volume ratio. If the surface area of a cell increases, the volume does not increase in direct proportion; the volume increases proportionally faster. Thus, the surface area could not support the metabolic needs of the increased volume. Some cells, such as frog eggs, chicken eggs, and ostrich eggs, can become large because they are not metabolically active until they begin to divide. Other cells, such as nerve cells, can possess extensions of more than a meter, but the extensions are narrow and have little volume.

Bacteria and protists, such as the green alga *Spirogyra* and the protozoan paramecium, are composed of one cell and called **unicellular**. Despite having just one cell, these organisms carry on all of the life processes efficiently. Several species of protists exist as colonies that are loosely connected groups or aggregates of cells. Examples of **colonial organisms** are the algae *Volvox* and *Scenedesmus*. Organisms such as an azalea, a mushroom, and a walrus, which are composed of many cells, are called **multicellular**. These organisms exhibit a division of labor and have a variety of specialized tissues.

Although innumerable forms of cells exist in mature, only two basic types of cells comprise life on Earth: prokaryotic and eukaryotic cells.

Prokaryotic Cells

- Lack a membrane-bound nucleus and organelles
- Much smaller than eukaryotic cells
- The cytoplasm of prokaryotic cells is surrounded by a plasma membrane
- The majority of prokaryotic cells are encased in a protective cell wall
- Prokaryotic organisms are placed within the kingdoms **Archaebacteria** and **Eubacteria**

Eukaryotic Cells

- More structurally complex and larger than prokaryotic cells
- Have a membrane-bound nucleus and organelles
- Members of the kingdoms **Protista**, **Plantae**, **Fungi**, and **Animalia** possess eukaryotic cells

PROKARYOTIC CELLS

1) **Archaebacteria**, or ancient bacteria, can be found living in extreme environments such as exceedingly salty habitats (extreme halophiles), exceptionally hot environments (extreme thermophiles), the anaerobic mud of swamps, and the gut of termites and many mammals (methanogens).
2) **Eubacteria**, or true bacteria, are better known to the general public. Although majority of eubacteria are harmless or helpful, such as *Lactobacillus acidophilus*, which is used in yogurt, there are several medically important species. Examples of these organisms are *Clostridium perfringens* (gangrene), *Helicobacter pylori* (ulcers), *Staphylococcus aureus* (boils), and *Bacillus anthracis* (anthrax).

Observing Cyanobacteria

The **cyanobacteria**, once classified as the blue-green algae, are photosynthetic eubacteria. These rather large prokaryotes do not possess chloroplasts, but have chlorophyll *a*. The cyanobacteria have a number of accessory pigments that can mask the green color of chlorophyll. As a result, species of cyanobacteria appear red, yellow, brown, or blue-green. The cyanobacteria are common and can be found in a number of environments including in the soil, on sidewalks, on the sides of buildings, on trees, and in bodies of water such as ditches.

Procedures:

1. Obtain prepared slides of *Gloecapsa*, *Nostoc*, *Oscillatoria*, and *Anabaena*.
2. Observe these slides with the high-dry objective. Sketch your observations below.

Gloecapsa

Nostoc

Oscillatoria

Anabaena

Observing Bacteria

Most bacteria are significantly smaller than the cyanobacteria. The bacteria are simple in form and anatomy and exhibit three basic shapes: **bacillus** (rod-shaped), **coccus** (spherical-shaped), and **spirillum** (spiral-shaped). An electron microscope is used to observe the anatomical detail of a typical bacterium.

Procedures:

1. Obtain a prepared slide of *E.coli*.
2. Observe the slide under oil immersion and sketch your observations below.
3. Clean off the slide and objectives as instructed in the procedure for using oil immersion
4. Obtain a small amount of plain yogurt on the tip of a toothpick. Rub the yogurt onto the central portion of a blank slide. Place one drop of water on the yogurt with a pipette, and mix with a toothpick. Gently place the coverslip on the water/yogurt mixture.
3. Observe the bacteria in the yogurt under oil immersion. The majority of bacterial cells in yogurt are *Lactobacillus acidophilus*.
4. Sketch your observations below.

E.coli *Lactobacillus*

EUKARYOTIC CELLS

Eukaryotes include the protists, fungi, plants, and animals. The cells of eukaryotes possess a membrane-bound nucleus and a variety of membrane-bound organelles.

Observing Protists

Protists include a diverse group of organisms. In fact, the former kingdom Protista is undergoing reorganization and one day will consist of several kingdoms. Presently, the protists can be separated into the plant-like protists (algae), fungi-like protists (slime and water molds), and animal-like proists (protozoans).

Procedures:

1. Obtain prepared slides of *Volvox*, *Amoeba*, *Spirogyra*, and *Paramecium*.
2. Observe these slides with the medium-power objectives
3. Sketch your observations below.

Volvox

Amoeba

Spiroygyra

Paramecium

Observing Plant Cells

Elodea is a common plant that lives in freshwater habitats such as ponds and lakes. It provides an excellent example for studying basic plant cell anatomy. The leaves of *Elodea* are only a few cells thick and allow light to pass through the leaf without special preparation techniques.

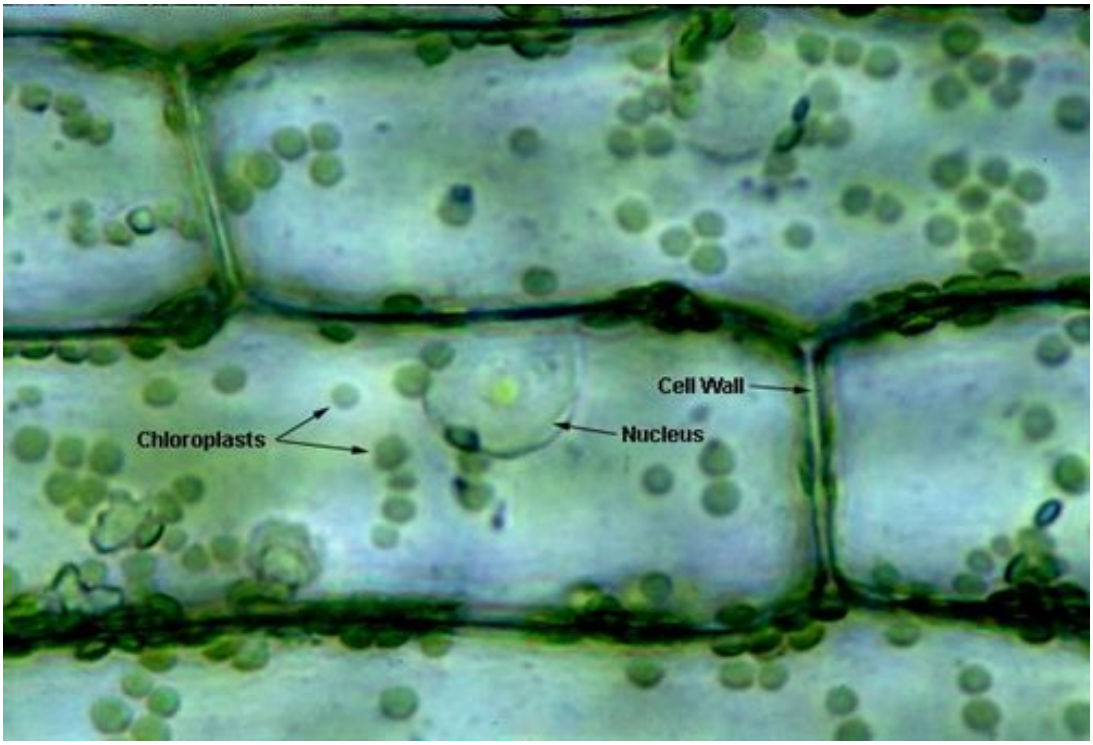


Figure 4. Magnified *Elodea* leaf

Procedures:

1. Prepare a wet mount of an *Elodea* leaf.
2. Examine the leaf surface with the scanning, low-power objective. Focus through the cell layers of the *Elodea* leaf.
3. Sketch the *Elodea* leaf at the scanning, low-power objective below.
4. Using the high-dry objective, examine a single cell of *Elodea*. Attempt to locate the following structures: cell wall, chloroplasts, nucleus, vacuole. Carefully notice of the cytoplasm and chloroplasts are moving. This process is called **cytoplasmic streaming**.
5. Sketch the *Elodea* leaf at the high-dry objective below.

Elodea at 40X total magnification

Elodea at 400X total magnification

Observing Animal Cells

A typical animal cell can be collected from the lining in your mouth. These simple cells, known as squamous epithelial cells, are flat and thing and possess on obvious nucleus. Epithelial cells appear in regions of wear and tear and are constantly being sloughed away. In this specimen, only the cell membrane, cytoplasm, and nucleus will be easily observed.

Procedures:

1. Obtain a clean blank slide, a coverslip, and a clean toothpick.
2. Using a clean toothpick, gently scrape the inside of your cheek.
3. Place a small drop of water on a blank slide. Gently roll and swirl the end of the toothpick with the epithelial scrapings into the drop of water. Discard the used toothpick into the designated container.
4. Carefully place a drop of methylene blue on the drop of water. *Avoid inhalation and skin contact with methylene blue. Rinse it off the skin with mild soap and water immediately, as methylene blue will stain clothing.*
5. Place a coverslip over the specimen and make your observations using the high-dry objective.
6. Sketch your observations below.

Cheek cells