jump | STEAM

AT HOME

DO TRY THESE AT HOME!

MICROSCOPY 103

The Anatomy of Cells

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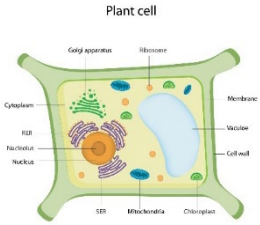
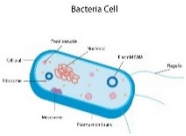
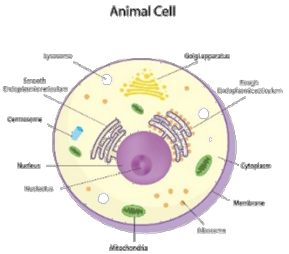
The Anatomy of Cells



It doesn't matter if you're an e Coli bacteria, redwood tree, or a human being, everything alive is made of cells. Regardless of the role in life or size, we are all made to carry out the same processes of life. Its called the cell theory of life and says that cells are the most basic functional unit of all living organisms.





CELL THEORY says:

1. Living things consist of cells.
2. Cells are the smallest basic units of structure and function in all living organisms.
3. All new cells arise from pre-existing cells.

CLASSIFYING LIFE

Once upon a time… all living organisms were EITHER plant or animal

- ✓ Animal kingdom could move but not make own food
- ✓ Plant kingdom could make own food but not move
- ✓ Mineral kingdom had non-living matter

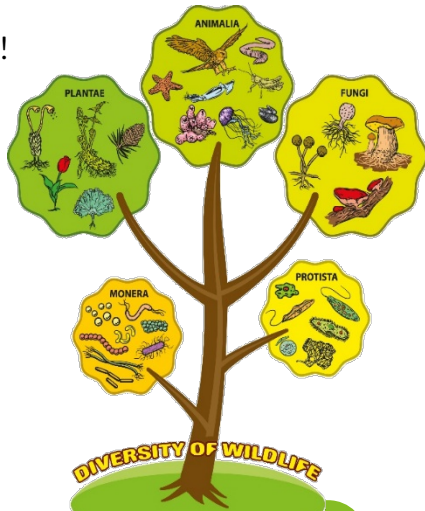
The discovery of microbes changed everything!

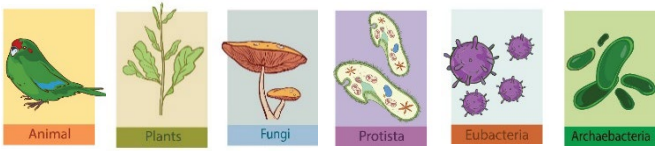
1960s a five kingdom system

1. Prokaryotes- no nucleus, single cell, no organelles, circular DNA
    1. Bacteria
2. Eukaryotes – have nucleus, compartmentalized organelles, linear DNA
    1. Everything else

1970's 3 domain – 6 kingdom system

❖ Bacteria, Archae, and Eukarya

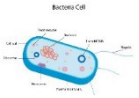


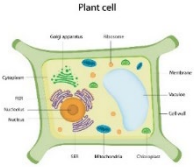


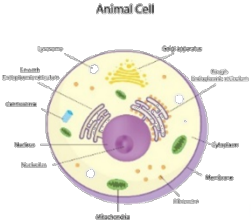
CELL BIOLOGY

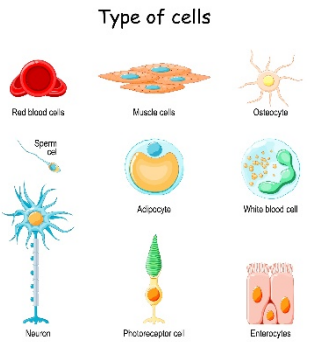
Cytology, the study of cells, is largely concerned with understanding the structure and functions of cells (morphological and physiological).

All cells have certain things in common, but are also quite different to allow them to carry out specialized functions to make their own unique contribution to the needs of the organism to survive in its environment. Microscopes allow us to take a closer look at what makes cells different, specialized, and how they change in disease states (**pathologies**).





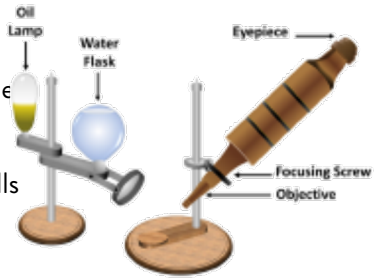




DISCOVERY OF CELLS

It's clear that the discovery of cells would not have happened without the invention of the microscope. Robert Hooke modified the compound microscope to have **three lenses** and a **stage light** to increase the magnification of his specimens.

His first recorded sample was a slice of cork where he described the cork structure similar to monastery "cells" in arrangement. Hooke had discovered plant cells -- more precisely, what he saw were the cell walls in cork tissue. It was Hooke who coined the term "cells." He also reported seeing similar structures in wood and other plants

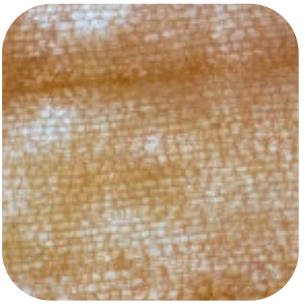


ANALYZING CORK CELLS

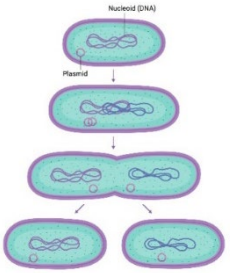
How would you design an experiment to repeat Hooke's experiments by preparing a slide with a sample of cork?

Tips:

- use the tweezers to place a cork slice on your slide
- Change the illumination, condenser, and filters to compare resolution and contrast
- What other skills do you have to try

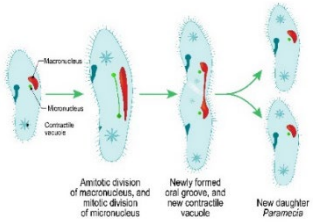


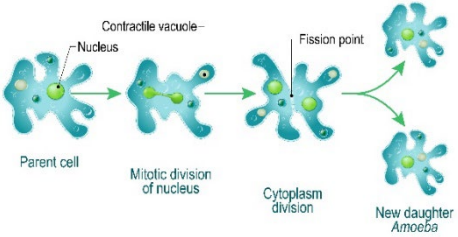
CELL REPLICATION



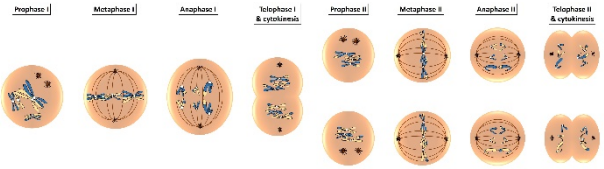
Cells need to replicate to create offspring, growth, or repair.

BINARY FISSION aka "cellular cloning" lets an organism (usually a single celled prokaryote)create an exact copy of itself.





MEIOSIS allows multicellular organisms to pass on assorted genes to crate diversity in offspring.



MITOSIS creates daughter cells identical to the parent cell for repair and growth

Interphase, cell has made copies of its DNA (chromosomes) to pass on.

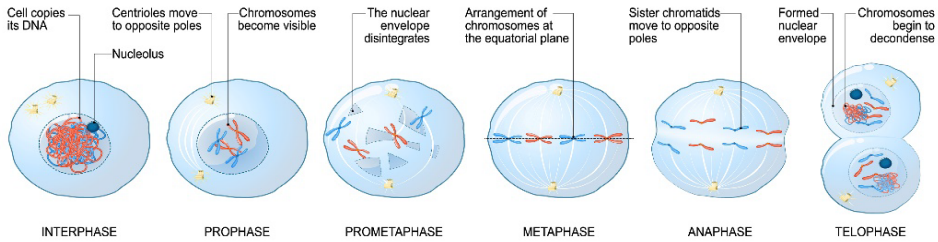
prophase, chromosomes coil up tightly and appear thicker while the membranes get weaker to for a split

Metaphase chromosomes line up at the "equator".

Anaphase chromatids separate into two identical chromosomesbefore being pulled apart to opposite sides of the cell.

Telophase, chromosomes unwind as the nucleus reforms

Cytokinesis the cytoplasm of the mother cell divides forming two daughter cells. Each daughter cell has DNA that is identical to the



Live Fresh Mount

environment or temporary wet mount

Wet mounts are suspend specimens in fluids such as water, brine, glycerin and immersion oil for short term viewing.

Temporary Wet mount slides are best for…

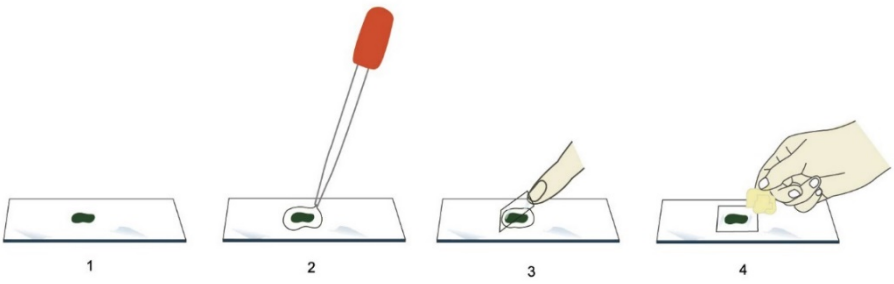
- Preparing specimens for microscopic visualization on the spot
- Removing the refractive index of air
- Aquatic samples, living organisms and natural observations of Specimens collected in their own environment
- Examine tissue that has not been preserved, or that may still be living.
- Watching specimens react in their own environment

KEEP IN MIND…

- Biologic specimens must be keep wet, or they will dry and decay, if only viewing short time. Although, they provide a transitory window as the liquid will dehydrate and living specimens will die. Organisms such as protozoa may only live 30 minutes under a wet mount slide
- Larger protozoan such as paramecium may be too large and/or move too quickly under the wet mount.

In these circumstances:

- Applying petroleum jelly to the outer edges of the cover slip creates a seal that may extend the life of the slide up to a few days.
- Adding a few ring stickers or framing the center of the slide with strips of electrical tape, or in extreme cases, adding ground pieces of cover glass to the water before the slip cover will create added space
- Adding chemicals like glycerol and methylene cellulose, or strands of cotton to slow the movement of paramecium, amoeba at may and ciliates



Be prepared, Wet mount can be messy

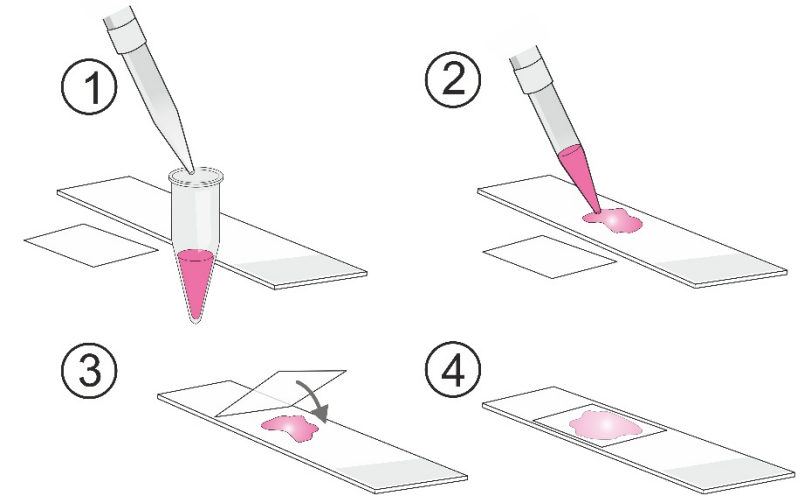
So, its good to have everything ready for a spill like paper towels and gloves if infection or staining is an issue.

1. Start with a glass or transparency sheet slide
2. Collect a Drop of water using pipette or the end of a rod
3. Place it in the center of the slide, Don't use too much or the water will run off the edge and make a mess!
4. Transfer specimen to droplet using tweezers
5. Place the edge of the cover slip on the slide at 45 degree angle , NEAR the side of the water drop.
6. Slowly lower the cover slip on top of the drop being careful to not trap bubbles. Gently tap the coverslip with eraser if bubbles are visible (bubbles will look like dark rings under the scope)
7. Blot excess water with paper towel

You do not need to use the stage clips when viewing wet-mount slides. Place the slide on the stage and view it first with the scanning/low power objective. Once you see the image, you can rotate the nosepiece to view the slide with the different objectives.

Tips:

- Sometimes vegetable oil works better than water
- Making a "donut" of petroleum jelly works to hold water drops before placing slide cover on



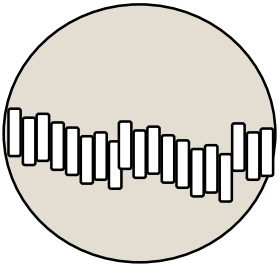
Notebook Practice

Record your observation of hooks cells.

Hypothesize what you will see in other wood specimens

Plan your experiment then conduct it

Label, Sketch, and measure your specimen



Record the total magnification

Determine the size using this formula

- Specimen size = FOV diameter/ ?

If _____ cells are visible and the FOV is _____um

- Width of cell = ______um/19 so the cells are approximately ____um
- What are your conclusions?
- How could use your knowledge to repeat the experiment differently?

Analyzing pencil wood

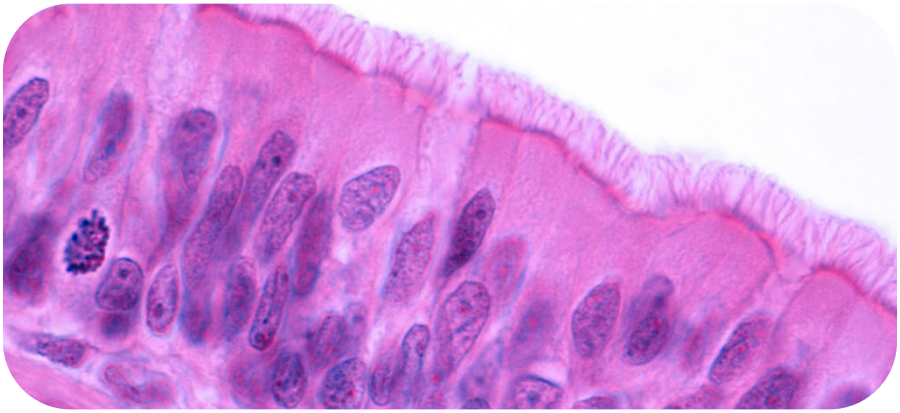
Hmm. I wonder if all tree cells look the same? Repeat the experiment with a pencil shaving.

Analyzing wood cells:

Compare what you see to prepared slices of tree cells

Stains

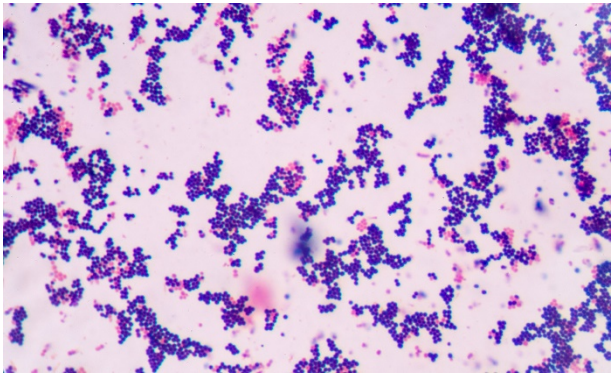
Preparing biological samples for the light microscope often need to be 'stained' or colored in some way to make it easier for users to understand what they're seeing. Stains highlight particular components of the sample and make it easier to interpret. This is important because these samples often lack **contrast**, which makes it hard to distinguish between parts of the sample.



Stains interact with a specific part of the sample turning it a different color from its surroundings. For example H&E stain is used to emphasis the nuclei of the cells (purple) in the trachea image above.

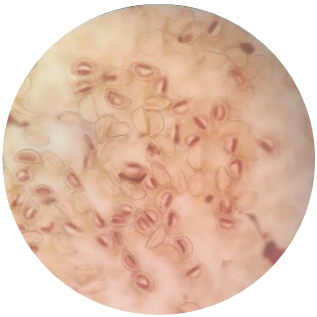
Stains can be used on living or non-living biological material. Some stains require the sample to be treated beforehand, and in this case, the tissue is no longer living. Other stains are used on living tissue, which is important for observing biological processes under the microscope.

Stains are especially useful in the fields of histology, virology and pathology, allowing researchers to study and diagnose diseases



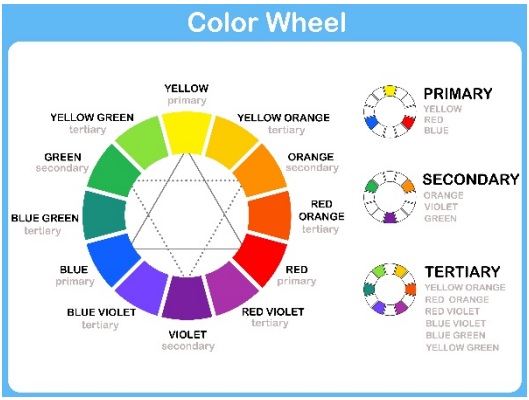
Examples

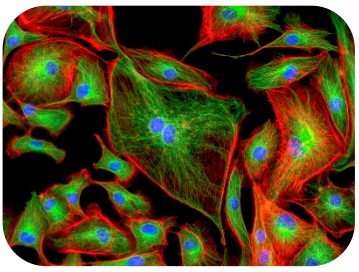
- Iodine likes carbohydrates so its is often used to stain plant cells because it colors the starch stored in the cells blue and other substances a pale brown. It will turn animal sugars red
- Carmine(cochineal in stores) stains proteins and nucleus pink
- Methylene blue likes acidic nuclei and proteins (compared to Eosin that likes alkaline cytoplasm)
- H&E (hematoxylin and eosin), which stains the cell nucleus purple and other tissue components pink
- Lactophenol cotton blue dye turns chitin blue in fungus cell walls
- Gram stain, which divides bacteria into Gram-positive and Gram-negative categories.
- Fluorescent stains are used to highlight specific parts of the cell or tissue In fluorescence microscopy



Using color filters

- Filters with a complementary color to the stain improve contrast
- Complementary colors are any 2 colors that make white light= 1 primary (red, green, blue) used with a secondary (yellow, magenta, cyan)

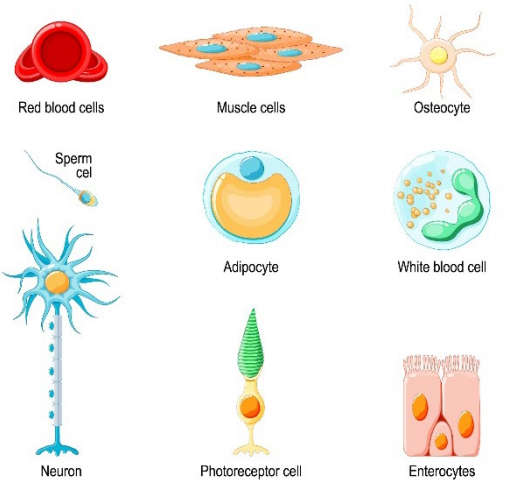




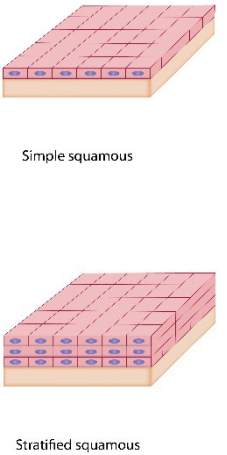
Animal Cells

Observing human cheek cells under a microscope is a simple way to quickly view and learn about eukaryotic animal cells, nuclei, and cell structure. The cells on the inside of your cheek are specialized cells called Squamous Epithelium .

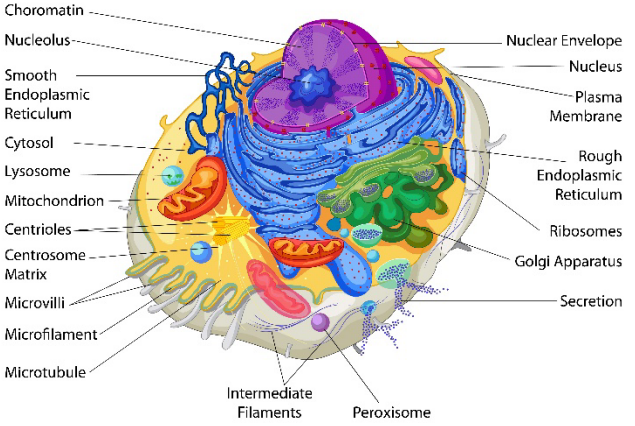
Types of Cells



Squamous epithelium



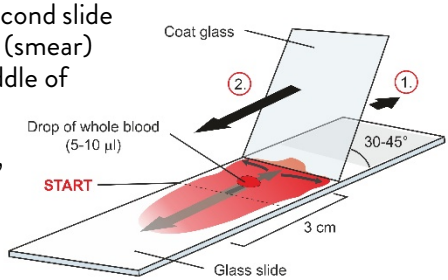
Cells typically have negatively charged cell walls and nuclei, so positive charged chromophores in BASIC dyes tend to stick to the cell walls, making them positive stains. Commonly used BASIC dyes like **fuchsin**, **crystal violet**, **malachite green**, **methylene blue**, and **safranin** typically serve as positive stains. Be careful with the dyes used for the wet mounts as they will stain your skin and clothes.



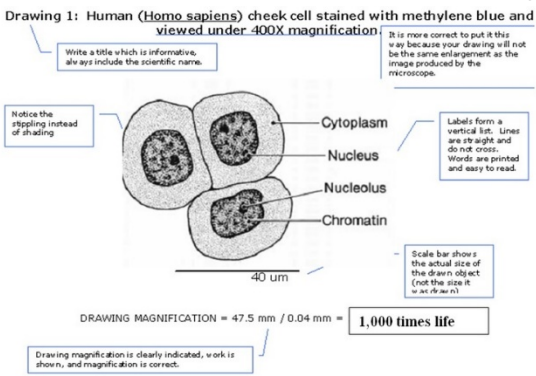
Smear Slides

Smear slides are good for thicker samples that are full of loose cells or a liquidity sample with lots of tiny cells

1. Spread a liquid specimen like cheek "goo", blood or slime onto the center of a GLASS slide
2. To make a cheek smear, take a clean toothpick and gently scrape the inside of your cheek. Then wipe that part of the toothpick in the center of your slide.
3. holding the edge of the cover slip or second slide flush with the sample slide gently wipe (smear) the edge of the coverslip along the middle of the slide's surface.



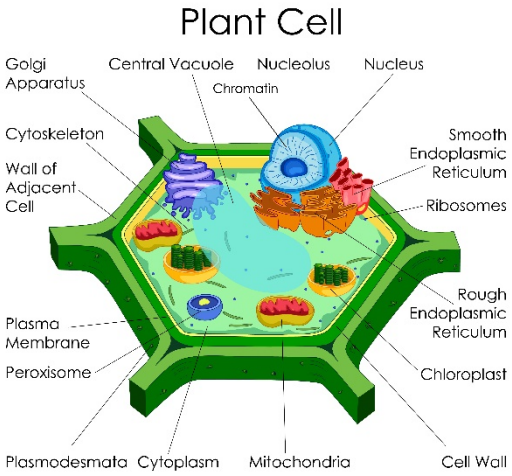
4. This will smear the cells along the slide, making a layer thin enough to view clearly. The angle of the smearing slide determines the length of the smear; a steeper angle creates a shorter smear.
5. Let the smear air dry.
6. Once your smear is dry, add a drop of water or stain (methylene blue for cheek cells 0 to the center of the smear so you will be able to see the cells more clearly.
7. Gently set a coverslip over the smear, careful not to trap air bubbles Blot excess fluid with paper towel (NOT lens paper)
8. Scan your slide under low power to locate the cells, then observe them more closely under high power
    1. Draw 1-3 cells large enough to show the detail
    2. Label its **cell membrane, cytoplasm** and **nucleus.**
    3. Note the magnification used and specimen name.
    4. Estimated cell size in micrometers under your drawing.

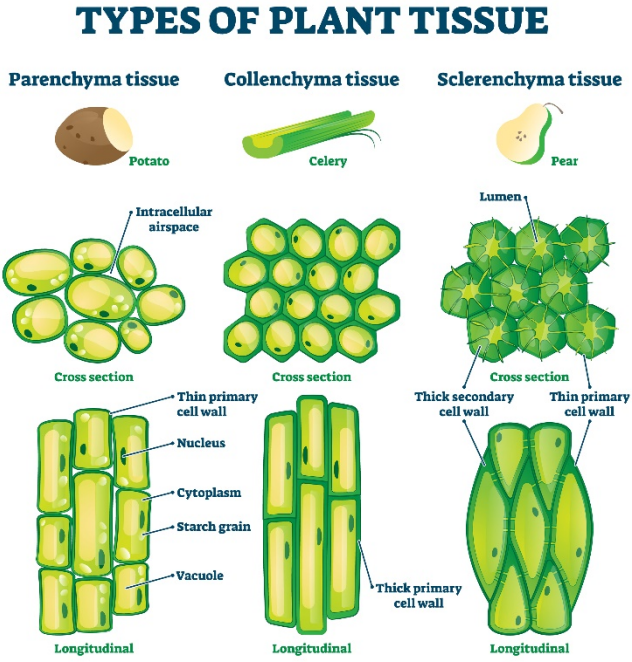


Plant Cell

Plants are unique among the eukaryotes because they can manufacture their own food. Chlorophyll, which gives plants their green color, enables them to use sunlight to convert water and carbon dioxide into sugars and carbohydrates, chemicals the cell uses for fuel.

Unlike animal cells the cell wall of an onion and other plants are made up of cellulose, which protects the cell and maintains its shape





Analyzing Onion Skin

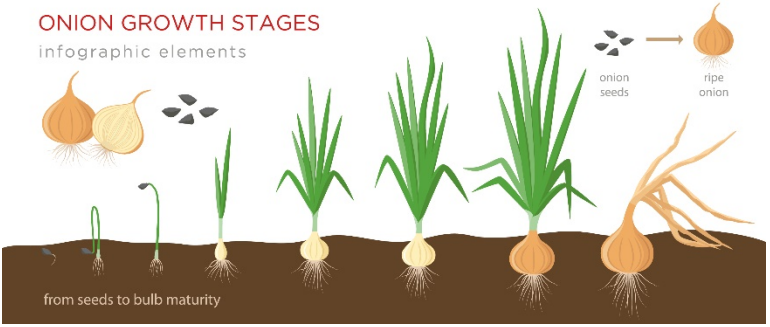
Analyzing onion skin

Onion bulbs are actually swollen leaves that form an underground structure. While not such a good source for viewing chloroplasts, they are an excellent source for viewing eukaryotic plant nuclei, cell walls and starch granules

The layers of an onion contain simple sugars (carbohydrates) some of which are stored as starch (starch granules). Given that iodine tends to bind to starch, it stains the starch granules when the two come in to contact making them visible.

Did you notice the onion cells were not green?!? Why?

Observe the prepared slide of an onion. Can you tell what stain if any was used? Can you identify the cell structures? Record your observation in your notebook'



EXTRA! EXTRA!

Cut a tiny square of one layer of the onion. Use forceps to peel the thin, white, transparent membrane from the inner concave side of an onion section (you only need a small piece, about the size of a pencil eraser) and place on slide. Try to smooth out the transparent onion membrane as flat as possible.

Add **one drop of water** and the coverslip and then observe the onion on each level of magnification.

Slice a very thin piece of skin from an onion and place it on the center of a clean slide. Add a drop of iodine to the membrane and wait 30 seconds. Cover the membrane with a coverslip. Place the slide inside folded paper towel and pat gently for 1 second to remove excess dye.

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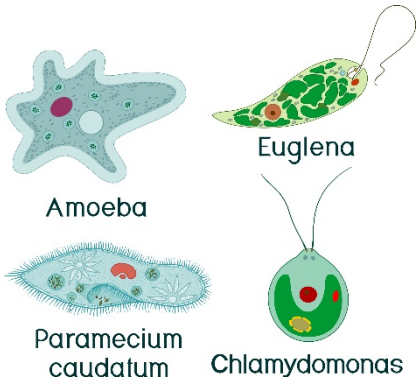
Protists

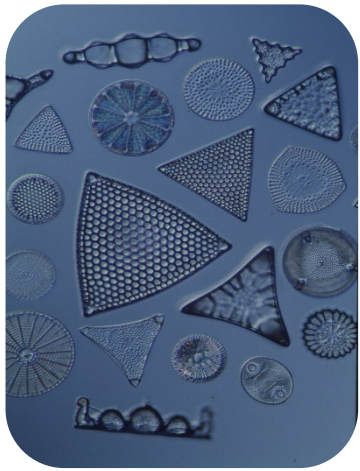
Protists are often classified based on how similar they are to other eukaryotes—animals, plants and fungi.

- **Protozo**a are Animal-like protists. Most consist of a single cell. heterotrophic and capable of moving. Examples of protozoa include amoebas and paramecia.

- **Algae** are Plant-like protists. They include single-celled diatoms and multicellular seaweed. contain chlorophyll and make food by photosynthesis. Types of algae include red and green algae, euglenids, and dinoflagellates.

- **Molds** are Fungus-like protists absorptive feeders, found on decaying organic matter. They resemble fungi and reproduce with spores as fungi do. Examples of fungus-like protists include slime molds and water molds.



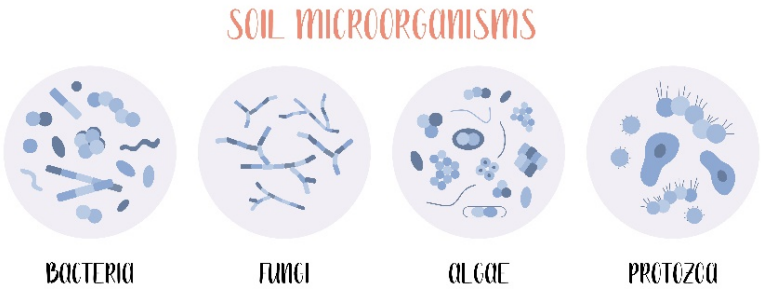


Water Drop Analysis

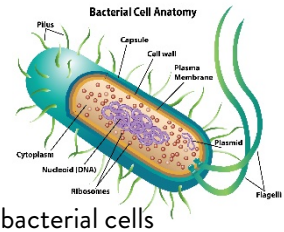
Water can be home to a lot of interesting creatures and microorganisms, especially if it's dirty water found in ponds or nearby plants. Even when you look at crystal clear fresh water with a microscope you will likely see a variety of tiny living things. Great Sources of fresh water samples can include ponds, lakes, rivers, aquarium tanks or even an old rain puddle. If the fresh waer isn't there, you can take samples of dry habitats like Lichen, mosses, soil, rotting wood and more.

Depending on if you are looking at wet or dry origin, you may be able to see some or all of the kindoms represented. Some of the creatures and microorganisms you might be able to see include:

- Animals - like tardigrades or rotifers
- Monera – like bacteria Euglenas - These are between a plant and an animal, they have a long tail called a flagellum which allows them to move.
- Plants – typically sessile eukaryotes that make their own food and have a typical color because of it.
- Algae - Not considered to be plants by most scientists, these organisms might be colored yellowish, greenish or reddish. They may also be found by themselves or in chains.
- Protozoa - They have a flagella (tail) which can be hard to see, the difference between protozoa and algae is often hard to define.
- Amoebas - These microorganisms swim by wobbling. They also surround their food like a blob in order to eat it.
- Rocks or minerals – these give clues to the type of habitat and how it was formed
- There might even my larger creatures such as worms, insects, or brine shrimp in your water samples, depending on where you took them from.



Bacteria



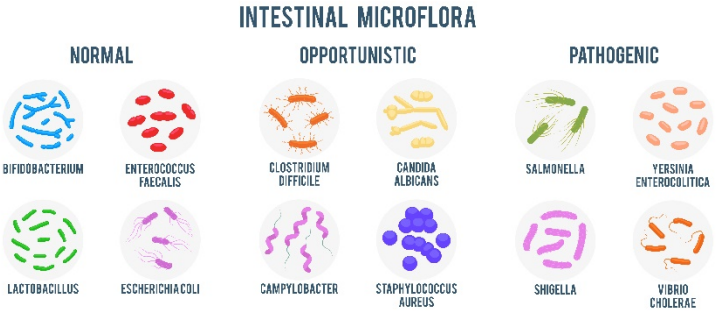
Bacteria are EVERYWHERE! In fact your body has more bacterial cells than human cells! They are prokaryotic cells. This means that they are single-celled organisms without a nucleus membrane (nuclear envelope). While they are very small, they are diverse and vary in shape and size.

There are a wide variety of different shapes, the three main types are cocci, bacilli, and spiral.

- **Cocci** – most common type of bacteria. They have spherical shape, and they commonly appear in groups. For instance, diplococci refer to pairs of cocci, streptococci refer to chains and staphylococci refers to clusters of cocci.
- **Bacilli** –rod-shaped bacteria can be found on their own or grouped. For example, diplobacilli refer to two bacilli next to each other, while streptobacilli refers to chains of bacilli.
- **Spiral** –spiral-shaped bacteria. Examples include spirillium, which are thick, durable spirals and spirochetes, which are slender and flexible.

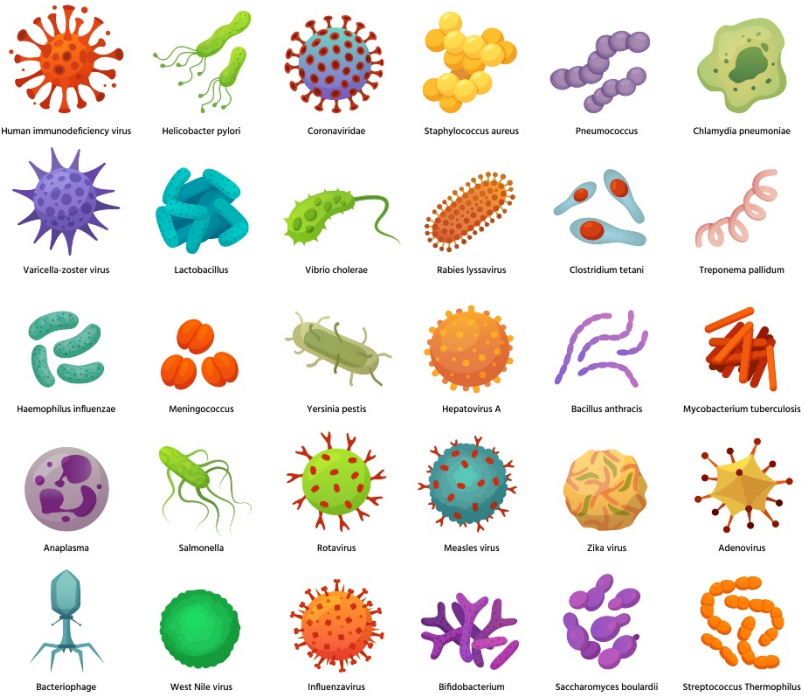
Stains are an important tool for identifying bacterial cell types based on their capsules attraction to dyes. by determining whether it is a gram negative or gram positive is achieved through the staining process and stains such as crystal violet dye, iodine, and the counterstain safranin.

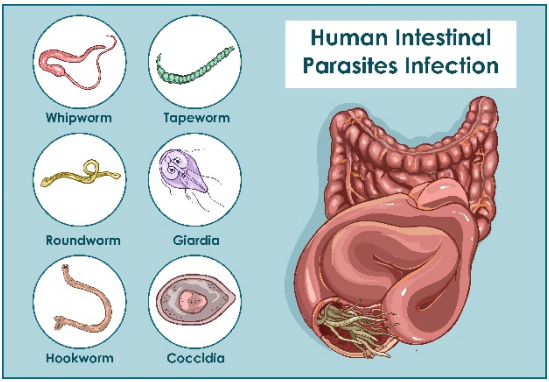
- **Gram Positive Bacteria –** These are bacteria that have a thick peptidoglycan layer which the stain (crystal violet-iodine complex) is attracted to. As a result, the stain is retained resulting in them having a purple/bluish appearance when viewed down the microscope. Examples of gram-positive bacteria include *Listeria, Streptococcus, and Bacillus.*
- **Gram-Negative Bacteria** – These are bacteria that do not have a thick layer of peptidoglycan and so the crystal violet-iodine complex is unable to be trapped within the structure. Instead, the bacteria can hold the safranin, which results in them having a red appearance under the microscope. Examples of gram-negative bacteria include proteobacteria and cyanobacteria.



Pathogens

A pathogen is a biological cause of illness. Pathogens can cause a range of diseases and are divided into four kinds: **bacteria, viruses, fungi and parasites**. When you come down with a cold, sore throat or the flu, it is pathogens which have caused you the trouble. A pathogen is a biological cause of illness.





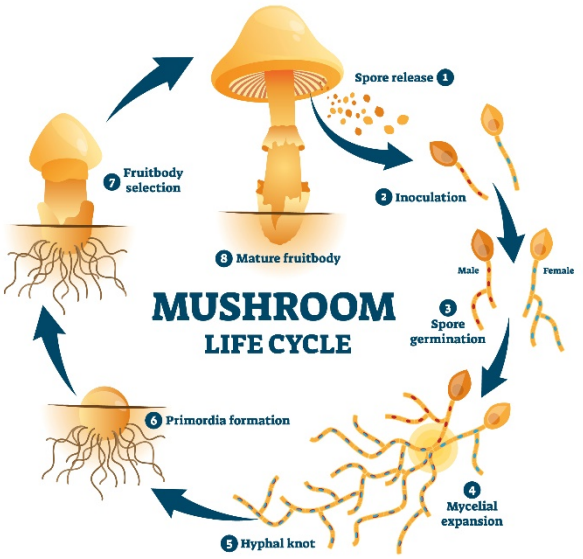
Fungus

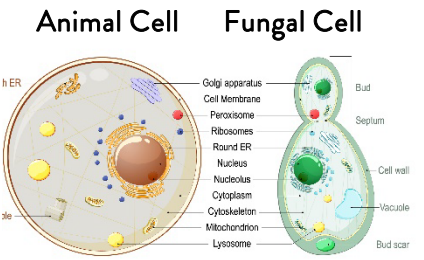
Fungi are a kingdom of mostly microscopic organisms that are closely related to animals. almost always invisible to the naked eye, some fungi will produce large 'fruiting bodies' when its time to release their spores. The reproductive structure is usually what we see, like mushrooms. They are decomposers, and they digest their food outside their body through microscopic root-like Hyphae form mycelium masses, usually under the surface cell walls of fungi are made from a compound called chitin. Fungus have common structures but diverse to live anywhere. They include spore producing organisms such as mushrooms, yeast and molds.

Mushrooms, or toadstools, are the fruiting body common to many species of fungi . Most are almost completely invisible were it not for their large mushrooms. The rest of their tissue is hidden within the soil or the dead plant that they are feeding on

Molds belong to a group of fungi called zygomycetes. fast growing fungi that are responsible for the spoiling of many foods such as bread, fruits, vegetables and dairy products. The hyphae of molds spread across a food source and penetrate into the food

Single-celled fungi are known as **yeasts**. Some yeast have the ability to shift between living as yeasts or in a multicellular form with hyphae. Yeasts do not belong to one particular group of fungi but are found in a range of distantly related fungal groups. easts are able to metabolize carbohydrates into alcohol and carbon dioxide. Humans have utilized the fermentation of carbohydrates by yeasts to create fermented foods and drinks such as bread, beer and wine. Yeasts are found in aquatic environments and on land. They are also found living in and on plants and animals.





Analyzing Lichen habitats

Lichen forms when a fungi and photosynthetic organisms, such as a green algae or cyanobacteria, form a symbiotic relationship.

Mycorrhizae are fungi that live in close association with plant roots and help plants to absorb more nutrients

Like soil, pond water, and moss lichen are a habitat for almost all kingdoms of creature. See if you can find something from each kingdom.

Tardigrades, often called water bears or moss piglets, are near-microscopic animals with long, plump bodies and scrunched-up heads. They have eight legs, and hands with four to eight claws on each. While strangely cute, these tiny animals are almost indestructible and can even survive in outer space.

Analyzing Yeast Cells

Yeast and fungi are two types of organisms, which belong to the kingdom Fungi. Yeast is a type of fungi, which is a unicellular, oval-shaped organism. Fungi are mostly multicellular, consisting of fungal hyphae. ... The main difference between yeast and fungi is their structure

Yeast are great for quick culturing and experimenting with because they are living organisms that go into a hibernation state and quickly come out of it. this is excellent for fast processes like baking bread or even slow processes like making wine or beer.

1. Put a pack of yeast in a Ziploc bag.
2. Add lukewarm water and packet of sugar, mix with a spoon.
3. Seal bag and place where water can pool
4. wait approx. 10min,
5. Record what you see and what happens to the bag
6. Transfer yeast by pipette for observation

Mycology is the study of fungi. Not only do fungi make a large contribution to the clean up of our world, they also make great contributions to medicine, culinary, and chemical industry.

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