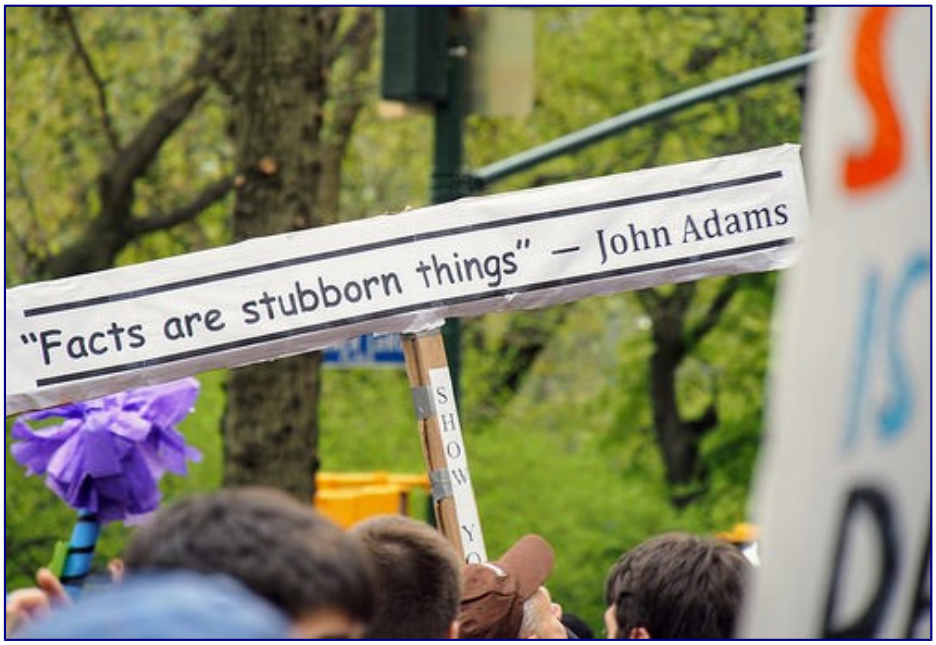
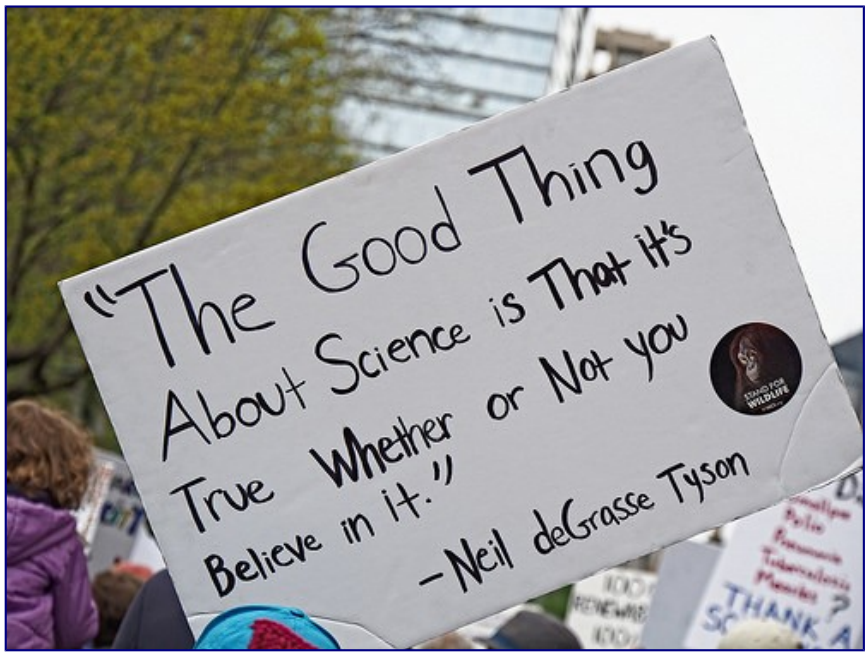
The Scientific Method



Scientists use a methodology for systematically investigating natural phenomena. This method uses existing information or observations to acquire knew information or validate previous knowledge. These knowledge types come from empirical (experiential) or measured information. Empirical and measured data (or knowledge) are referred to as **observations**. While **empirical** data comes from experiences, science has developed into a mode of inquiry using **experimentation**. Experimental science uses the pre-existing base of knowledge to ask a a testable question called a **hypothesis**. As a youngster, we're incorrectly taught that a hypothesis is *an educated guess*. Formulating previous observations and measurements into a cohesive line of inquiry requires no guessing. People often have "theories" on something, when they actually have hypotheses based on their observations and assumptions.



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Experimental Science

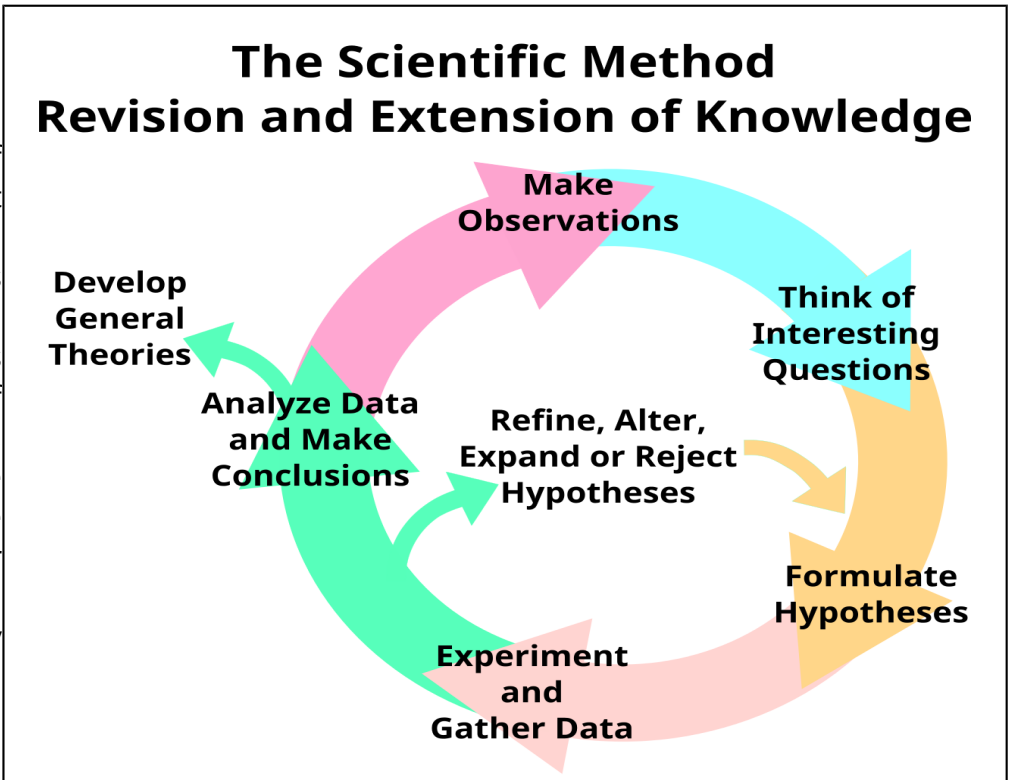
Hypothesis testing is the the means by which experimental science is conducted. Experimental science is designed to enhance the understanding of a problem and removing biases from the interpretation. The goal of hypothesis testing is to try every way possible to disqualify the validity of the hypothesis. By doing so, the experimenter removes any biases in the experimental design. If the experimenter is unable to invalidate the hypothesis, the hypothesis becomes more valid and better able to act as a predictor of phenomena.

Experiments utilize **controls**. In a controlled experiment, there is a positive and negative control. These controls act as references in the experiment. A **positive control** is an experimental condition where the expected outcome that is tested will be produced. This control is necessary to assess the validity of a test or treatment. There can be multiple instances used as a positive control to examine the sensitivity of the experiment. A **negative control** is an experimental condition where the expected outcome is known not to occur. This type of control sometimes comes in the form of a sham or mock treatment such as giving someone a sugar pill (a **placebo**).

Through the use of experimental science and hypothesis testing, an increased refinement of existing knowledge can aid in designing new hypotheses. Hypothesis testing is re-iterative. That is to say, we use new knowledge to continue to enhance our understanding of the universe.

Theories

A **scientific theory** comes from repeated substantiation of multiple tested hypotheses. That is to say, confirmed hypotheses, observations and experiments permit scientists to formulate a cohesive idea that integrates multiple substantiated pieces of evidence. As with hypotheses, theories are designed to be predictive and falsifiable. In the common language, we often hear the word theory to mean a conjecture, and as already discussed, conjectures based on evidence can be formulated into testable hypotheses.



Hypothesis testing is a continuous and repeated process designed to increase knowledge.
Credit: Jeremy Seto [CC-BY-NC-SA]

When a theory is accepted by a predominant population of the specialists, it is referred to as a **scientific principle**. An example of a scientific principle is the ***theory of evolution by natural selection***.

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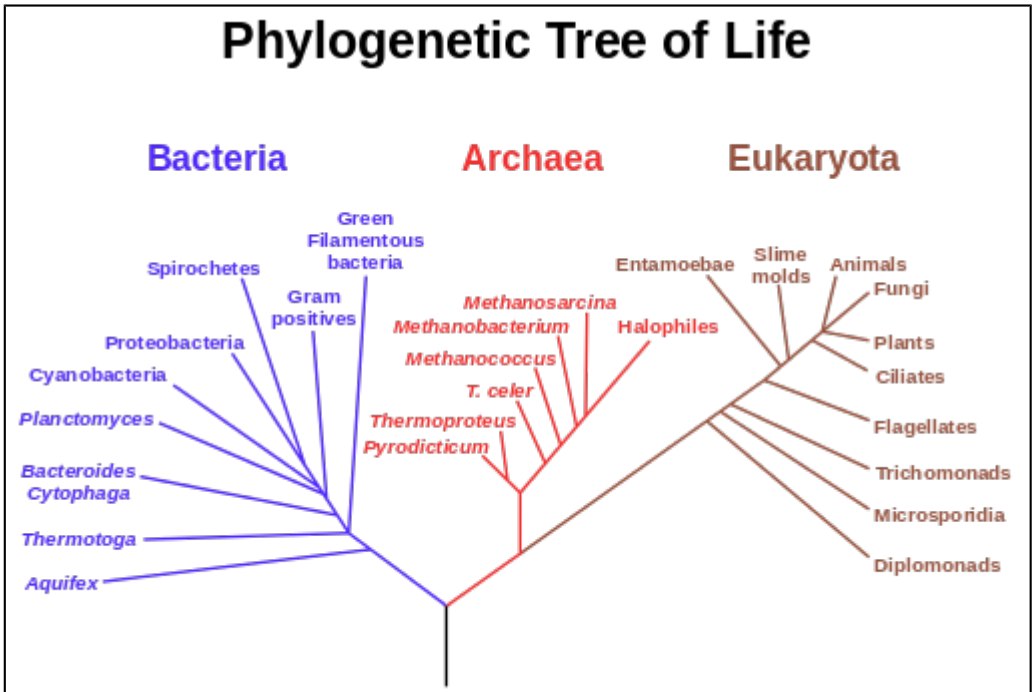
Numerous tested hypotheses have been confirmed that lead to the understanding of natural selection as a method of evolution. This theory allows scientists to understand the underlying relatedness of all living things on the planet. Additionally, it unifies the disparate fields of Biology that can utilize the theory in a predictive manner. It is therefore also referred to as a **unifying principle** of Biology.

Classification of Life

All living things on Earth share a relationship. The rules that govern life processes can be generalized across all **organisms** (living things), as well as non-living biological entities (viruses). The relatedness of organisms is often visualized as a **phylogenetic tree**. This tree is a hierarchical classification system that groups organisms together based on common features that is used to name them and is referred to as **taxonomy**. The most broad category is called a **domain**.



The phylogenetic tree of life describing the inter-relatedness of all living things on Earth. [CC0]

Three domains exist: **Archaea**, **Bacteria** and **Eukarya**. Archaea and Bacteria are also grouped together as **Prokaryotes** (*pro-* before; *karya-* nucleus). Eukarya (*eu-* true; *karya-* nucleus), or eukaryotes, is a group of of organisms that have nuclei. The second most inclusive or broad category is **Kingdom**. Humans are in the Kingdom of animals. The third most inclusive or broad category is **Phylum**. Humans are in the phylum called chordata. Each level of organization can be further subdivided and you may be more familiar with the subphylum called vertebrata. Within the this division, humans fall in the **class** of mammals. Amongst the mammals, humans are in the **order** of primates. Humans are categorized into a narrower group of organisms in the **family** of great apes or hominids. Within this family, humans fall into the **genus** of *Homo*. Biologists use a method of identifying specific organisms called binomial nomenclature. Binomial nomenclature uses the most specific groupings of taxonomy (genus and **species**) as a two part name. While humans are of the species *sapiens*, the species name of humans using binomial nomenclature is *Homo sapiens*.

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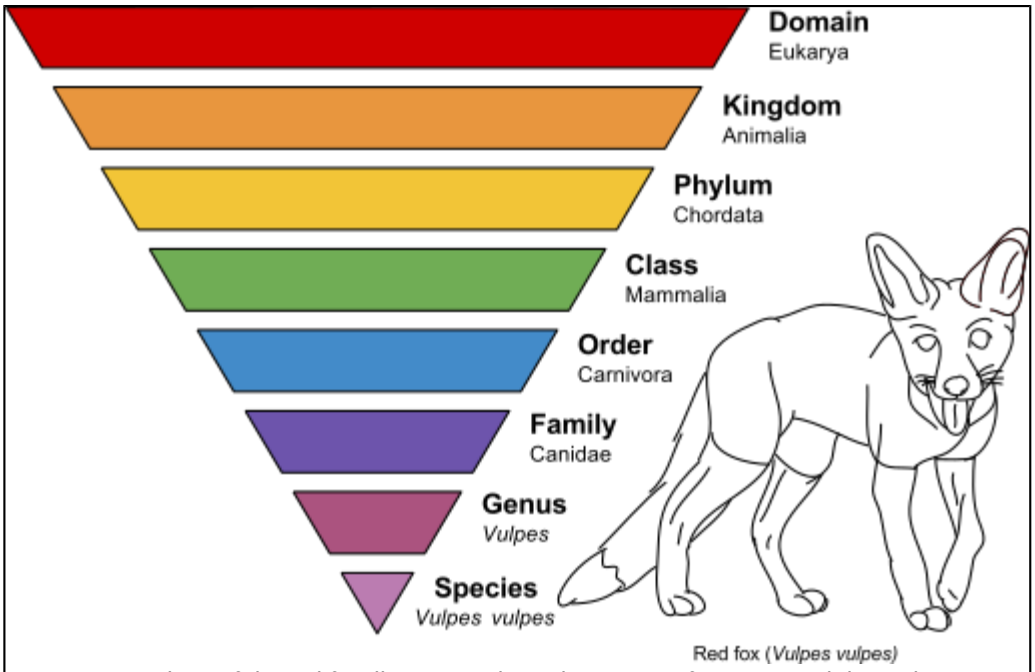


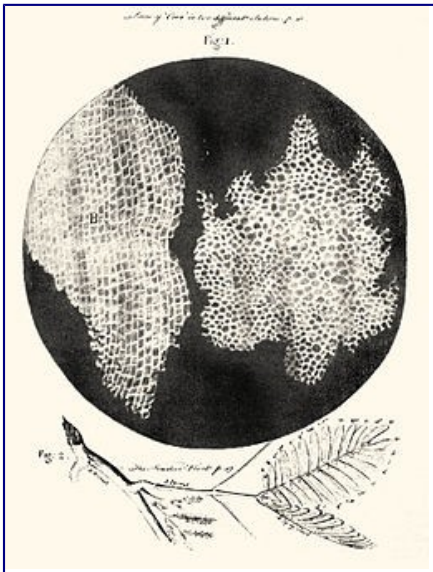
Taxonomic ranking of the red fox illustrating the inclusiveness of Domain and the exclusiveness of the Species level of categorization.

Credit: Annina Breen (CC-BY-SA 4.0)

The Light Microscope

Hooke's Cell

Illustration of Hooke's microscope



The empty chambers of cork inspired Hooke to coin the term "Cell"

In 1665, Robert Hooke published *Micrographia*, a book that illustrated highly magnified items that included insects and plants. This book spurred on interest in the sciences to examine the microscopic world using lenses but is also notable for Hooke's observations of cork where he used the word "cell" in a biological sense for the first time.

The father of Microbiology: van Leeuwenhoek

van Leeuwenhoek's microscope
Credit: Jeroen Rouwkema (CC BY-SA 3.0)

The Dutch tradesman Antonie van Leeuwenhoek used high power magnifying lenses to examine the parts of insects and to examine the quality of fabric in his drapery business. He began to experiment with pulling glass to generate lenses and developed a simple microscope to observe samples. Using a simple single lens with a specimen mounted on a point, he was able to identify the first microscopic "animalcules" (*little animals*) that will be later known as protozoa (*original animals*).

Though van Leeuwenhoek's apparatus was simple, the magnifying power of his lenses and his curiosity enabled him to perform great scientific observations on the the microscopic world. He was ridiculed for fabricating his observations of protists at first. Ever the scientist, van Leeuwenhoek examined samples of his own diarrhea to discover *Giardia intestinalis*. While he did not make the connection of the causative nature of this microorganism, he described the details of the way this organism could propel itself through the medium in great detail.

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Modern micrograph of Giardia intestinalis (Kingdom Protista)
Credit: Doc. RNDr. Josef Reischig, CSc. (CC BY-SA 3.0)

Using the Light Microscope

https://www.youtube.com/watch?v=lo2aC_m2vyo

Unlike van Leeuwenhoek's single lens microscope, we now combine the magnifying power of multiple lenses in what is called a compound microscope.

1. Ocular lens or eyepiece
2. Nose Piece/ Lens Carousel
3. Objective lens
4. Course Focus Knob
5. Fine Focus Knob
6. Stage
7. lamp
8. condenser
9. stage control

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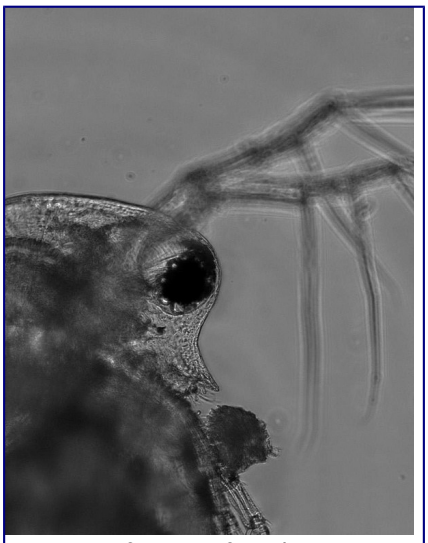
Microscopic World

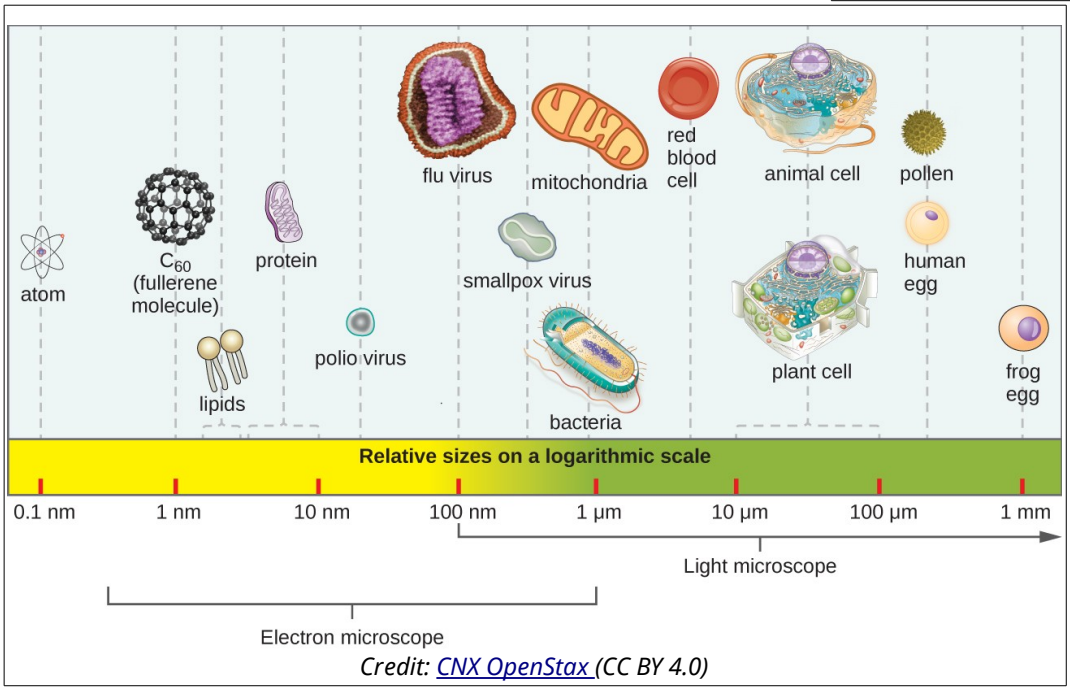
Daphnia magna, the freshwater flea is a small crustacean (scanning magnification)



Low magnification of Daphnia magna

Protozoa. Top: Amoeba Center: Euglena Bottom: Paramecium https://flic.kr/p/WDjKV1

Scale



Credit: CNX OpenStax (CC BY 4.0)

- Discover more about scale and microscopy at this link http://learn.genetics.utah.edu/content/cells/scale/

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Magnification

Magnification is the process of enlarging the appearance of an object. We calculate the magnification of an object by indicating the fold change in size. So if something appears to be double the size of the real item, then it is obviously magnified 2X. Because there is a magnification by the eye-piece (**ocular lens**), as well as the **objective lenses**, our final magnification of an item is the product of those two lenses.

The lowest magnification objective lens (usually 4X or 5X) is referred to as a **scanning lens**. There is also usually a low power lens at 10X and a higher magnification lens at 40X. There may be a higher magnification lens at 100X but these usually require oil to function properly and are often reserved for microbiology labs.

- What is the power of the ocular lens?
- We can calculate that as:
  $\text{Magnification}_{\text{total}} = \text{Magnification}_{\text{objective}} \text{ X } \text{Magnification}_{\text{ocular}}$
- With this in mind, fill in the following table:

	Objective Magnification	Ocular Magnification	Total Magnification
Scanning			
Low Power			
High Power			
Oil Immersion	**100X**		

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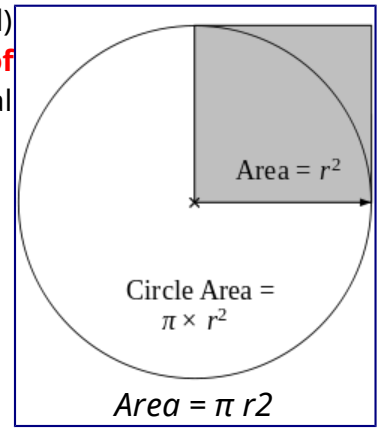
Field of View (FOV)

In a microscope, we ordinarily observe things within a circular space (or field) as defined by the lenses. We refer to this observable area as the **field of view (FOV)**. Understanding the size of the FOV is important because actual sizes of object can be calculated using the Magnification of the lenses.



Area = π r2

FOV can be described as the area of a circle:

What are the effects of magnification on FOV?


1) Lowest Magnification


2) Low Magnification


3) High Magnification


4) Highest Magnification

In image 1, we can see a model of DNA on a table with a water bottle and a large area of the room. Image 2 displays less of the room in the background but the DNA model is larger in appearance because the magnification is greater. In image 3, we no longer see evidence of a door and the DNA model is much larger than before. In image 4, we no longer see the table the model and water bottle rest upon. While the last image is largest, we see less of the surrounding objects. We have higher magnification at the cost of field of view. FOV is inversely related to the magnification level.

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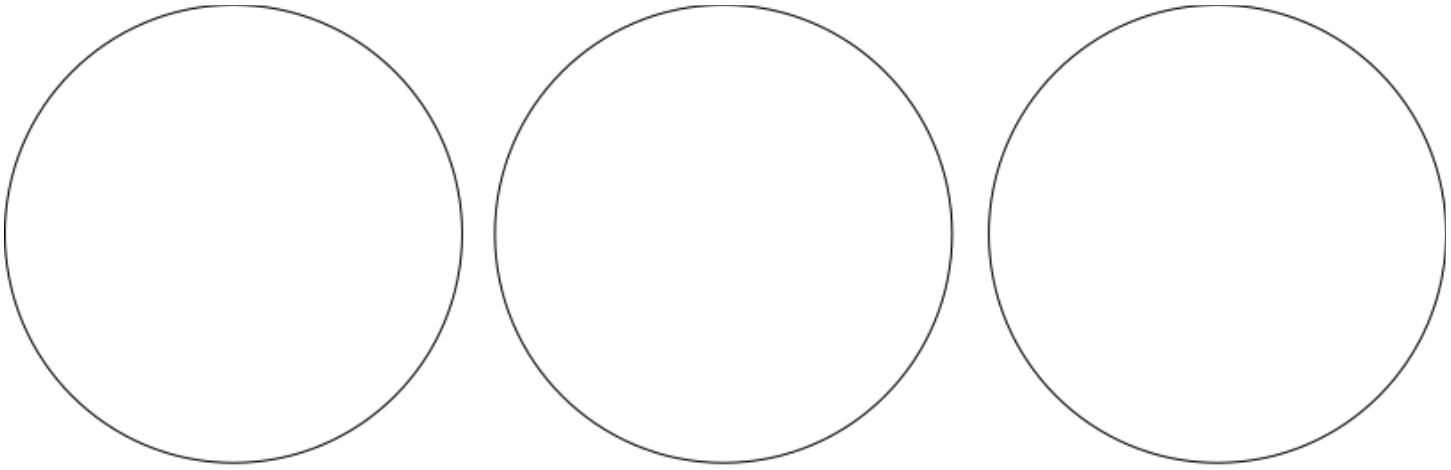
Field of View Calculation

1. Examine a ruler under scanning magnification
    - Measure the diameter in mm
    - diameter= _____
    - radius= _____
    - Calculate the field of view at this magnification= _____
2. Examine a ruler under low magnification (10x)
    - Measure the diameter in mm
    - diameter= _____
    - radius= _____
    - Calculate the field of view at this magnification= _____
3. What is the relationship in the between the magnification and field of view?
4. What is the proportion of change in field of view when doubling the magnification?

The Letter "e"

1. Orient a slide with the Letter "e" so that it is read as an "e" without magnification
2. Draw the "e" at scanning, low and high magnification



5X **10X** **40X**

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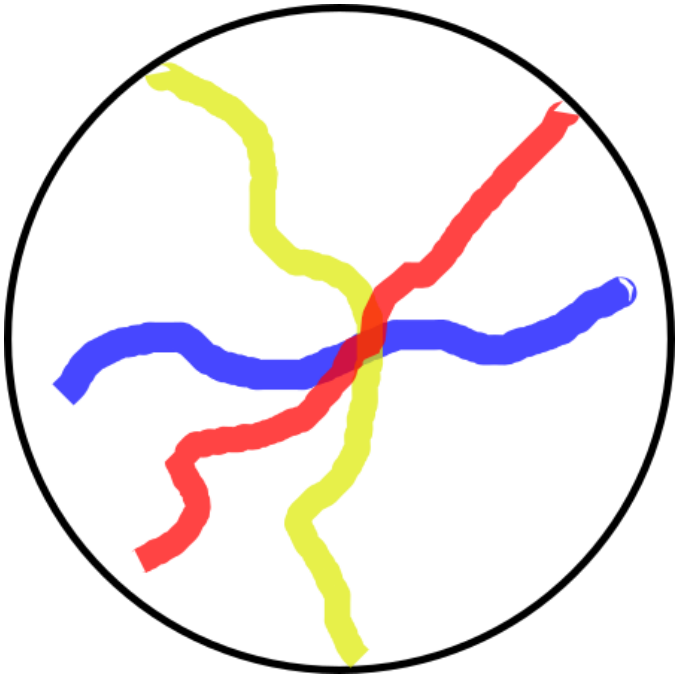
Depth of field

1. Examine the slide of colored threads under scanning power so the cross-point of the threads is at the center of the field



2. Raise the magnification to the low power objective
    - What do we notice about the threads and the focus?
    - How can we explain this observation with respect to the threads?
    - Close the diaphragm so allow a pinpoint of light through the slide. What effect does this have on the image?

We notice that when we observe 3 overlapping threads of different color under a microscope, we can focus on one thread at a time. Similarly, when we zoom in a great deal on the DNA model below, we notice that the print on the water bottle is not sharp.

Highest Magnification with shallow depth of field. Notice how the label on the water bottle is blurry while the lettering on the DNA model is sharp.

We know that the water bottle is behind the DNA molecule. Under the microscope, the threads of differing color are also stacked on top of each other. We recognize that they are in different planes because they are three dimensional. Each thread has depth and do not occupy the same exact space. If we focus on the print of the water bottle on the image above, we would no longer see the lettering on the DNA molecule sharply. We refer to this concept as **Depth of Field (DOF)**. Under the microscope, at a low magnification, we can make out fewer finer details. However, most items appear on the same plane in this case and or comparably sharp. But as we increase the magnification and see finer details, the distances between the various planes in view become more apparent. We can see a similar phenomenon at low magnification of the DNA model. At

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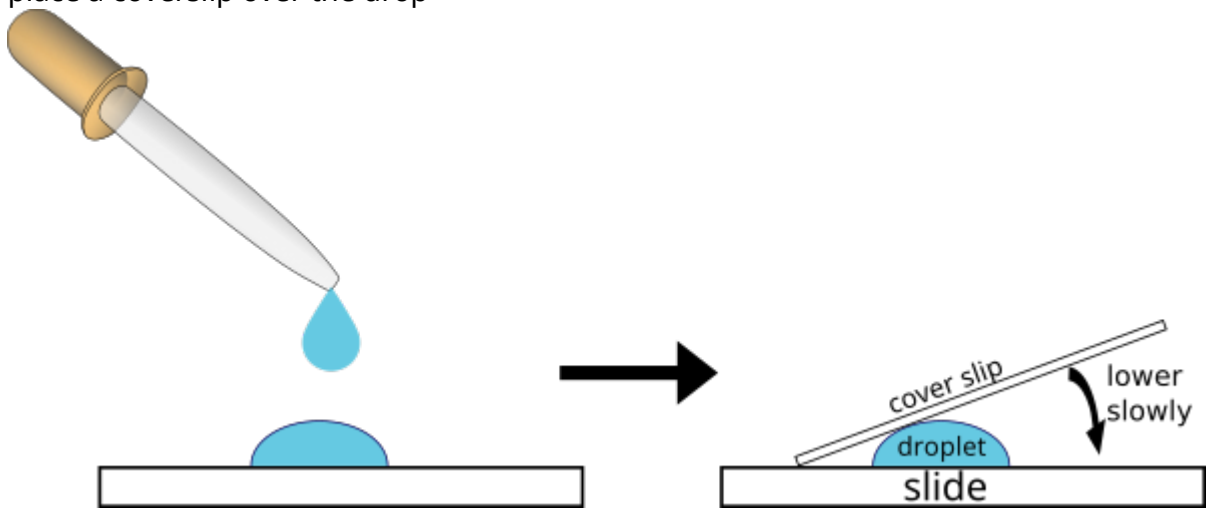
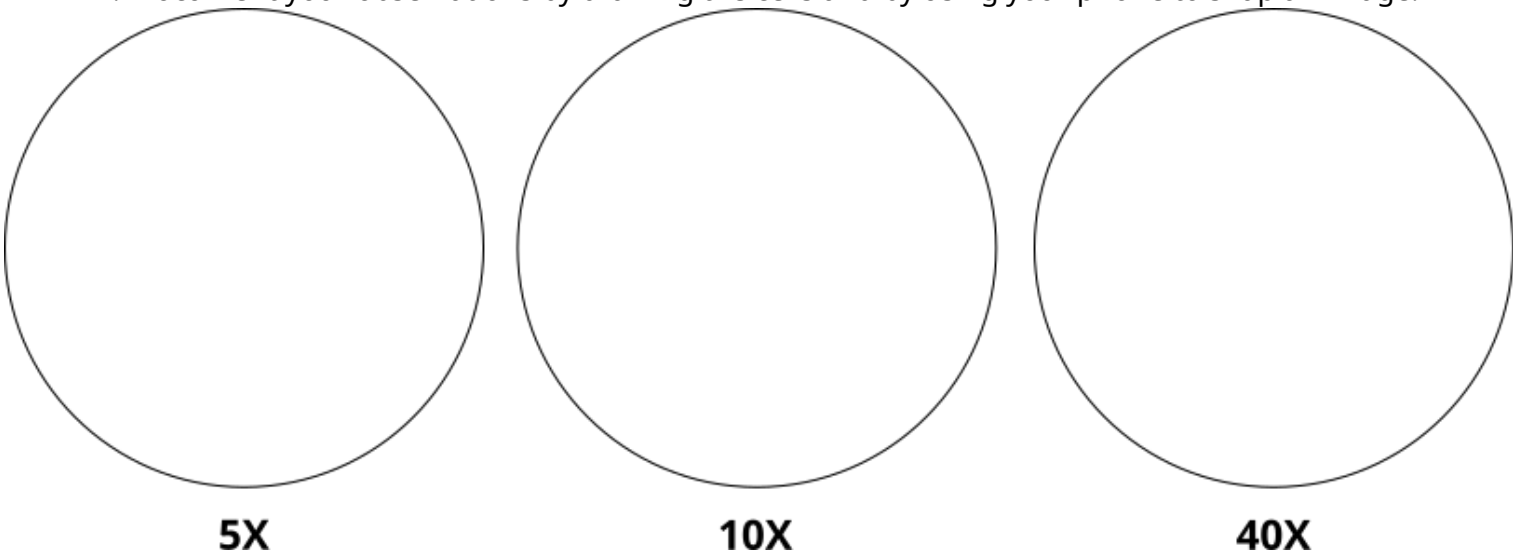
the low magnification, we may not be able to read the print on the water bottle, but the bottle and DNA molecule are of a similar distance from our view that the small difference in apparent depth is not as noticeable. We can still draw on other visual cues to know that the bottle is behind the model, but the sharpness of both items are equivalent.

Examining Cells

1. Choose a prepared slide of a Protist (*Euglena*, *Amoeba*, *Paramecium*)
2. Prepare a wet mount of a drop of pond water and place a cover slip over the drop.
3. Swab the inside of your cheek
    1. Roll the swab across a slide
    2. Drop some methylene blue onto the slide
    3. place a coverslip over the drop



    4. visualize and draw your cheek cells
4. Document your observations by drawing the cells and by using your phone to snap an image.



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Real biological examples

We can see the concepts of FOV and DOF in the following pictures.

In an even more extreme close-up (higher magnification), we would have difficulty focusing on both the eyes and beak since there is depth and distance between those features.

How do we use microscopes

In our lab, we look at some pond water. What do we see? Why is this significant? How does the microscope help us study these items? What is the utility of the the concepts of magnification, FOV and DOF when we use microscopes to study biological samples?

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Lab Reports

Title

A description of the main idea or question of the lab. This can also highlight a key finding or question.

Abstract

A brief summary of the main question, methods and findings. This is usually the last thing written but the first thing presented in order to grab the attention of the reader.
A rough breakdown of an abstract would contain about:

- 3 sentences worth of introduction with the key question
- 2 sentences of major methodology
- 3-6 sentences of the major results and conclusions drawn from them

Lengths will vary, but using this framework, you will not deviate too far from having a reader lose interest.

Introduction

Introduce the background that is relevant for forming the hypotheses being tested. What were the previous observations or prior knowledge used to come to these ideas? State the actual hypotheses to be tested and how it will further the understanding of the issue.

Materials and Methods

This section is a little like a cooking recipe. The main steps taken should be summarized as a standard prose in a manner that anyone could follow and repeat. This is written in the past tense and 3rd person. Do not write in the first person as *you* have nothing to do with the experiments. Explain "What was done with which reagents?"

Results

This section is descriptive of what was observed. Figures and tables serve as a summary of the results to illustrate the data. They also serve as guides to outline the text of the section. Slowly describe each figure or table. Expand these points into sentences and paragraphs. Present the data as fully as possible, including stuff that at the moment does not quite make sense. This is written in the past tense and 3rd person. Conclusions are not provided in this section as they are made from analyzing the information and synthesizing the results.

Discussions

Discussions are the conclusions made through analyzing the results. At this time, you will be able to re-emphasize the original hypotheses made in the introduction. Indicate whether or not the hypotheses were demonstrated sufficiently. If this is not the case, offer alternatives and interpretations. Can you improve or modify your hypotheses? Explain how multiple lines of evidence corroborate each other and help to further the understanding of the problem.

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References

Prior knowledge requires demonstration of strength or validity. The references should be from primary sources and should illustrate the point of the statement. The section is presented in numerous formats as a bibliography, but citations are inserted near the text where knowledge or statements are displayed. Use a reference Manager to insert citations and format the bibliography. An excellent free reference manager with a plug-in to common word processors is Zotero.

Additional resources

- MIT OCW

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