LEARNING GUIDE 3.1

PRINCIPLES OF MICROSCOPY

Subject code: Biology 1

Learning Guide Code: 3.0

Lesson Code: 3.1 Principles of Microscopy

MATERIALS

To complete this module, you need the following:

1. pen and paper
2. phone/tablet/laptop
3. stable internet connection
4. coloring materials
5. scissors and glue

TARGET

At the end of this module, you should be able to: (1) know the concepts of magnification, resolution and contrast, and determine the different types of microscopes, and: (2) make a collage that features the development of the first microscope and its recent advancements.

HOOK

We are in a living world of unimaginable beauty and what makes it more even beautiful is the fact that most of the treasures are invisible to the naked eye. Exploring biology therefore is a challenge when we cannot look at things. But there comes the

power of human creativity that continues to push our boundaries. Watch the video below to know more!

WATCH!

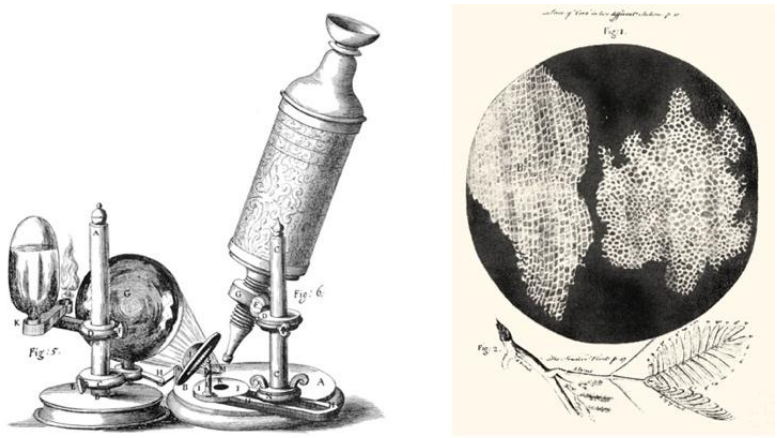
https://www.youtube.com/watch?v=FiZqn6fV-4Y

IGNITE

Microscopes are tools used to enlarge images of small objects so as they can be studied. Historians credit the invention of the microscope to the Dutch spectacle makers, Hans and Zacharias Janssen, around the year 1590. Most of the documented works of that time however are credited to the likes of **Antoine van Leeuwenhoek** and **Robert Hooke**. Leeuwenhoek is considered to be the first person to observe microbes which he called animalcules. Despite using a fairly simple device, it provided better resolution than other microscopes of that time.

Figure 1. Antoine van Leeuwenhoek and his handheld microscope. (Peering into the invisible world (2020). Retrieved from: https://bio.libretexts.org/Courses/City_College_of_San_Francisco/Introduction_to_Microbiology/02%3A_How_We_See_the_Invisible_World/2.01%3A_Peering_into_the_Invisible_World is licensed by CC VY-NC-SA 3.0)

Robert Hooke, van Leeuwenhoek's contemporary, also made significant contributions to microscopy, publishing his book entitled Micrographia in 1665. With a thin sample of cork, he was the first to observe and describe structures that we now know as cells which he first described as "honey-comb" and "small bladders of air".



(a) (b)

Figure 2. Robert Hooke's microscope and the image of the cork cells he observed. (Peering into the invisible world (2020). Retrieved from: https://bio.libretexts.org/Courses/City_College_of_San_Francisco/Introduction_to_Microbiology/02%3A_How_We_See_the_Invisible_World/2.01%3A_Peering_into_the_InvisibleWorld is licensed by CC VY-NC-SA 3.0)

The compound light microscope is an instrument containing two lenses, which magnifies, and a variety of knobs to resolve (focus) the picture. Because it uses more than one lens, it is sometimes called the compound microscope in addition to being referred to as being a light microscope. In this module, we will learn more about the principles of microscopy. In order to understand how microscopes become useful tools in biological observations, it is imperative for us to discuss the principles that govern microscopy which includes magnification, resolution and contrast.

Magnification is the ability to make smaller objects look larger in contrast to their original sizes. For example, a magnification of 10x means that the apparent image is observed 10 times larger as viewed using the naked eye. Van Leeuwenhoek's microscope was composed of two flat and thin metal plates with biconcave lenses sandwiched between the plates. The magnification of this fairly simple microscope

would range from 70x to about 250x depending on the quality of lens. More advanced microscopes at present are now capable of magnifying to about 10,000,000x. The term magnification is often confused with **resolution**, which describes the capability of a system to distinguish details in a sample being viewed. While greater magnification would allow us to observe small microbes, the subcellular parts of these microbes will not be further explored without a high resolution. This is because resolution depends on the distance between two distinguishable points in a field of vision.

Contrast on the other hand would describe how we detect an object against a background. In other words, it is defined as the relative difference in the light intensity between the image and the background it has. Some samples appear to be transparent that's why scientists have developed staining techniques and phase contrast techniques to increase contrast. All these concepts were key factors in the development of different kinds of microscopes we have today.

LIGHT MICROSCOPES

Many types of microscopes can be classified as light microscopes which use light to visualize images. This category includes the widely used brightfield and darkfield microscopes.

Brightfield Microscope

A brightfield microscope creates an image by means of directing light from a light source to the sample or specimen being observed. This is perhaps the most commonly used type of microscope. It uses two or more lenses that produces a dark image in a light background. Like shown in the image below, an ocular lens (usually 10x) works together with objective lenses (4x-100x).

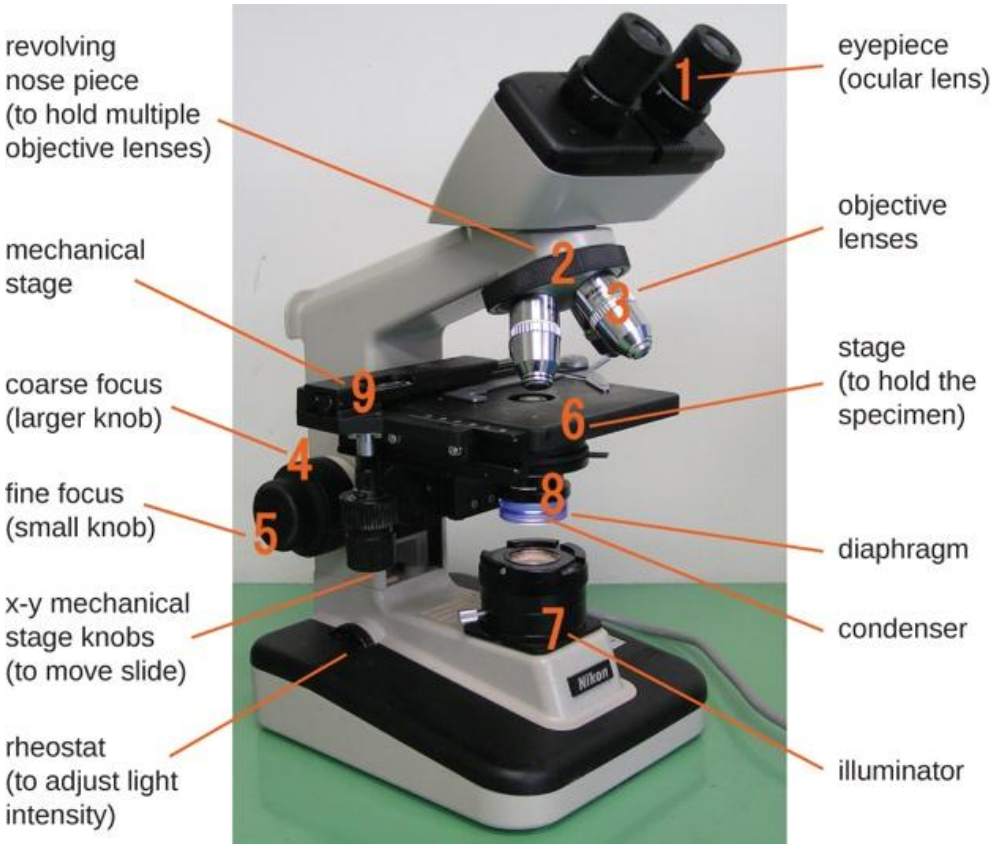


Figure 3. Parts of a compound microscope. (Instruments of Microscopy (2019). Retrieved from:https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible_World/2.3%3A_Instruments_of_Microscopy is licensed by CC VY-NC-SA 3.0)

Dark field Microscope

A darkfield microscope works like a brightfield microscope but has a modification in the part called the condenser. A small opaque light stop or disk (around 1 cm in diameter) is placed between the condenser and the light source which blocks most of the light coming from the light source as it passes through the condenser thus producing a hollow cone of light focused on the specimen. The resulting image shows a bright specimen on a dark background.

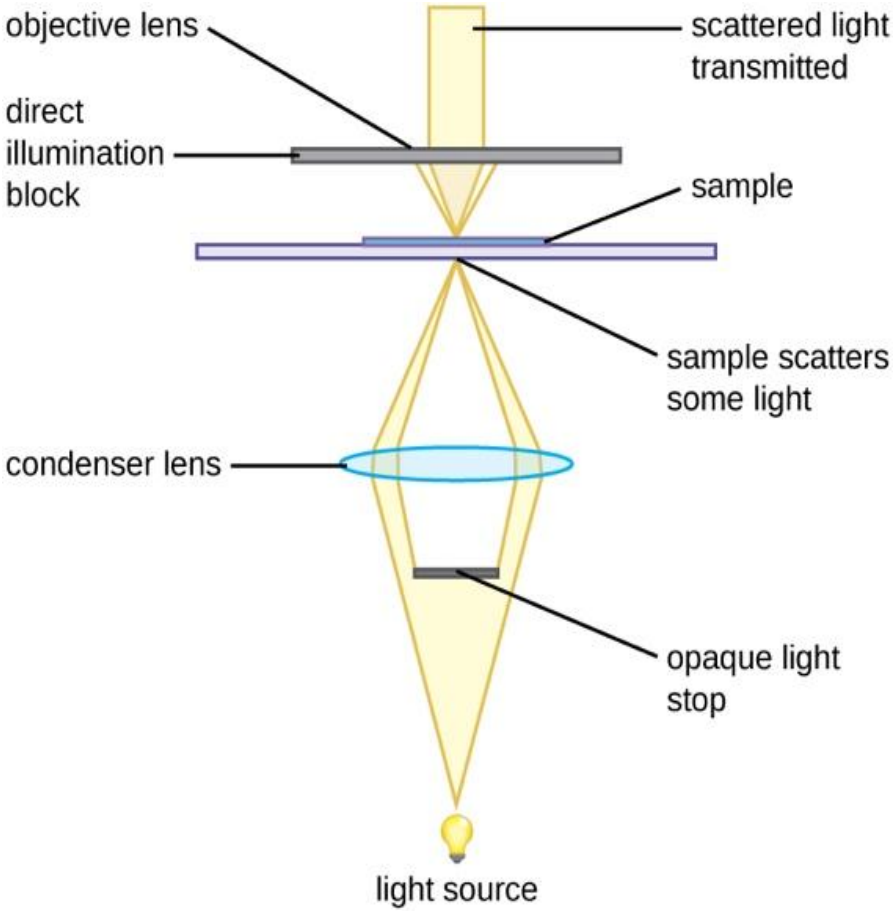
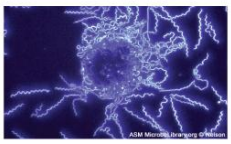
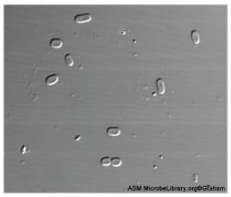
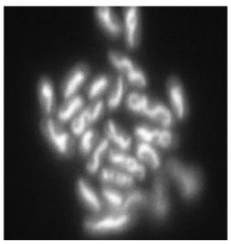
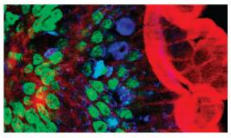


Figure 4. An opaque light stop is inserted to produce a dark field image. (Instruments of Microscopy (2019). Retrieved from: https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible_World/2.3%3A_Instruments_of_Microscopy is licensed by CC VY-NC-SA 3.0)

Table 1. Summary of different kinds of light microscopes.

LIGHT MICROSCOPES **Magnification: up to about 1000×** Use visible or ultraviolet light to produce an image.		
Microscope Type	**Key Uses**	**Sample Images**
Brightfield	Commonly used in a wide variety of laboratory applications as the standard microscope; produces an image on a bright background. **Example:** *Bacillus* sp. showing endospores.	
Darkfield	Increases contrast without staining by producing a bright image on a darker background; especially useful for viewing live specimens. **Example:** *Borrelia burgdorferi*	
Phase contrast	Uses refraction and interference caused by structures in the specimen to create high-contrast, high-resolution images without staining, making it useful for viewing live specimens, and structures such as endospores and organelles. **Example:** *Pseudomonas* sp.	
Differential interference contrast (DIC)	Uses interference patterns to enhance contrast between different features of a specimen to produce high-contrast images of living organisms with a three-dimensional appearance, making it especially useful in distinguishing structures within live, unstained specimens; images viewed reveal detailed structures within cells. **Example:** *Escherichia coli* O157:H7	
Fluorescence	Uses fluorescent stains to produce an image; can be used to identify pathogens, to find particular species, to distinguish living from dead cells, or to find locations of particular molecules within a cell; also used for immunofluorescence. **Example:** *P. putida* stained with fluorescent dyes to visualize the capsule.	
Confocal	Uses a laser to scan mutiple z-planes successively, producing numerous two-dimensional, high-resolution images at various depths that can be constructed into a three-dimensional image by a computer, making this useful for examining thick specimens such as biofilms. **Example:** *Escherichia coli* stained with acridine orange dye to show the nucleoid regions of the cells.	
Two-photon	Uses a scanning technique, fluorochromes, and long-wavelength light (such as infrared) to penetrate deep into thick specimens such as biofilms. **Example:** Mouse intestine cells stained with fluorescent dye.	

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ELECTRON MICROSCOPES

Theoretically, the resolution of images created by light microscopes is limited by the wavelengths of visible light. Some light microscopes could magnify up to 1000-1500x yet some microscopes were able to harness the magnifying power of electrons and use magnets to focus these electron beams. There are two basic types of electron microscopes: the transmission electron microscope and the scanning electron microscope.

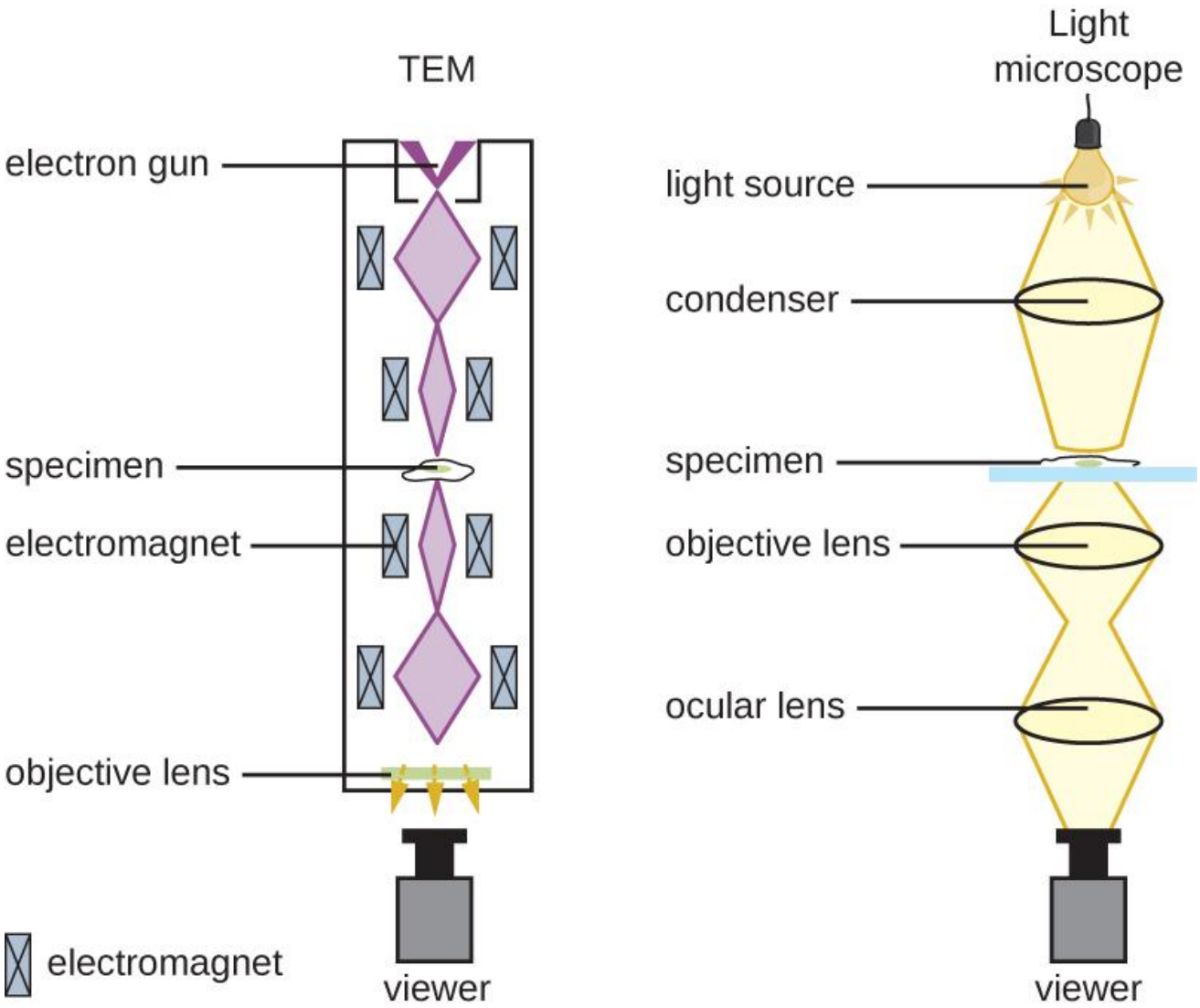

Figure 5.Difference between a light microscope and a Transmission Electron Microscope. . (Instruments of Microscopy (2019). Retrieved from: https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/0

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Transmission Electron Microscopes are used in studying inner morphology of samples. TEM requires ultra-thin slices of samples and then stained with metals like gold or palladium. The densely coated parts will eventually deflect the electron beam while lightly coated portions will most likely pass through this. The transmitted electrons will produce the image.

Scanning Electron Microscopes on the other hand is used to study surfaces of samples thus specimens are not cut into thin sections unlike that of the samples for TEM. The samples for SEM are coated in gold or palladium allowing the electrons to bounce off the surface of a specimen. The scattered electrons would allow us to study the overall surface of our specimen.

Table 2. Summary of Electron Microscope Types

ELECTRON MICROSCOPES **Magnification: 20–100,000× or more** Use electron beams focused with magnets to produce an image.		
Microscope Type	**Key Uses**	**Sample Images**
Transmission (TEM)	Uses electron beams that pass through a specimen to visualize small images; useful to observe small, thin specimens such as tissue sections and subcellular structures. **Example:** *Ebola* virus	
Scanning (SEM)	Uses electron beams to visualize surfaces; useful to observe the three-dimensional surface details of specimens. **Example:** *Campylobactor jejuni*	

Lifted from: Instruments of Microscopy (2019). Retrieved from: https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible_World/2.3%3A_Instruments_of_Microscopy is licensed by CC VY-NC-SA 3.0)

NAVIGATE

Timeline Collage

At the end of this activity, you should be able to make a collage that features the development of the first microscope and its recent advancements. It must feature the scientists and their contribution to the field of microscopy. You may print some pictures and paste them in a bond paper, or you may do a digital collage using the software you're comfortable to use. The collage must feature the scientist, his/her discovery and year he/she made this discovery.

Rubrics:

Criteria	Excellent	Satisfactory	Poor	Score
Creativity	The objects used in the collage gives an effective visual impact. (6-8 points)	Some of the objects in the collage gives an effective visual impact. (3-5 points)	The objects used in the collage gives a limited visual impact. (0-2 points)	
Completeness	The collage is complete with details which includes the name of the scientist, his/her discovery and the date of the discovery. (6-8 points)	1-3 components are missing. (3-5 points)	3 or more components are missing. (0-2 points)	
Timeliness	The collage was submitted on time. (4 points)	-	The collage was not submitted on time. (0 points)	
TOTAL				/20

Homework

Use of Different Microscopes

There are different microscopes that can be used in different ways. Since they work differently, images formed under them look different too. Using online sources, you are tasked to look for images of cheek cells under different kinds of microscopes and fill the table below with the necessary information. Do not forget to properly cite your sources right after the table.

Type of microscope	**How it woks**	**Cheek cell image**	**Description**
Brightfield			
Darkfield			
Phase Contrast			
Differential Interference Contrast (DIC)			
Electron Microscope			

KNOT

In summary, the development of the microscope was a revolutionary step in our observation of the natural world. Several factors like magnification, resolution and contrast guided us in the development of different approaches on how we would properly observe those that are not visible to our naked eye. While brightfield and darkfield microscopes would provide us with valuable data, the use of high-power electron microscopes help us push the boundaries of microscopy and challenge biology and physics experts to develop more powerful microscopes for future use.

References/Sources:

Instruments of Microscopy (2019). Retrieved from: https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible_World/2.3%3A_Instruments_of_Microscopy

Electron Microscopy (2019). Retrieved From: https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Boundless)/3%3A_Microscopy/3.3%3A_Other_Types_of_Microscopy/3.3F%3A_Electron_Microscopy

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