NANOWARE Curriculum

MODULE 4: HOW DO WE SEE NANOPARTICLES?

DELIVERABLE: R1/T1.1

NANOWARE

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Contents

1. Introduction to the Module

After a brief description of the limits of the vision of the human eye, different types and operating principles of microscopes are introduced in this module. Visualization and analysis methods for nanoparticles are explained in detail. The module is complete with an overview of the types of microscopes and their usage areas.

Description

In this module, students will learn visualization and analysis methods for nanoparticles, their description, and operational principles.

Module Goals

The goal of this module is to raise awareness on:

- the limits of vision of the human eye,
- the purpose of using the light microscope and other visualization methods such as SEM, TEM, AFM, STM,
- the differences among all microscopic methods.

Learning Objectives

Students will be able to:

- explain the limits of vision of the human eye,
- describe the purpose of using the light microscope and its operational principles
- describe why we cannot visualize atoms using the light microscope,
- explain visualization methods we can use to visualize atoms and nanoparticles.

Learning Outcomes

On successful completion of this module, the students will learn:

- the limits of vision of the human eye,
- the purpose of using microscopes and their types and operational principles,
- visualization methods we can use to visualize atoms and nanoparticles.

Estimated seat time

The completion of the module will last 1,5 hours. A 30-minute lecture section and a 1-hour lab session are involved.

2. How Do We See Nanoparticles?

Limits of Vision of the Human Eye

The human eye cannot see smaller than 40 µm(micrometer). (1 µm=0,000001 m=10-6 m) We should use different types of microscopes to see objects smaller than that size.

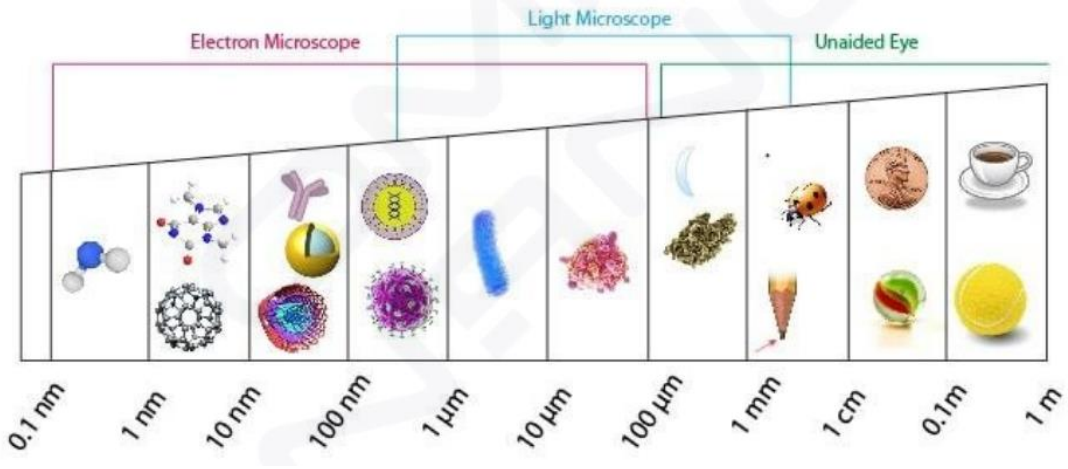

Figure 1. The visualization range of the human eye, the optical/light microscope, and the electron microscope.

Microscopes are optical instruments that allow us to examine living or lifeless objects that are too small to be seen with the naked eye. They bring small objects closer to the visual limits of the eye, making them visible to the naked eye. Eukaryotic organisms and bacteria are measured on the micrometre, viruses on the nanometer, and atoms and molecules on the angstrom scale.

1 mm = 1000 µm (micrometer)

1 µm = 1000 nm (nanometer)

1 nm = 1000 Å (angstrom)

Figure 2. The Relative Size of Particles (https://www.visualcapitalist.com/visualizing-relative-size-of-particles/)

2.1 The Microscope

Microscopes, which have a wide usage area in laboratories, is a combined word consisting of the Greek words "micro" and "scope". Micro, small; Scope means observer, observer. The word means "seeing little things". A microscope is an optical instrument that allows us to examine living or inanimate objects too small to be seen with the naked eye. The microscope is a visual and magnifying device based on the principles of light and optics.

In the 17th century, Dutch Anton van Leeuwenhoek and English Robert Hooke discovered the main principles of the microscope used today. The microscope, which was used first in morphological examinations in 1757, took its place in modern medicine in the middle of the 19th century. The microscope, which showed great developments in this period, is essential of two types a light microscope and an electron microscope according to the illumination source. In the light microscope, the source of illumination is sunlight or a lamp. In the electron microscope, accelerated electron beams are used to illuminate the object.

Live and lifeless objects that can be seen by using light and electron microscopes are shown in the figure below:

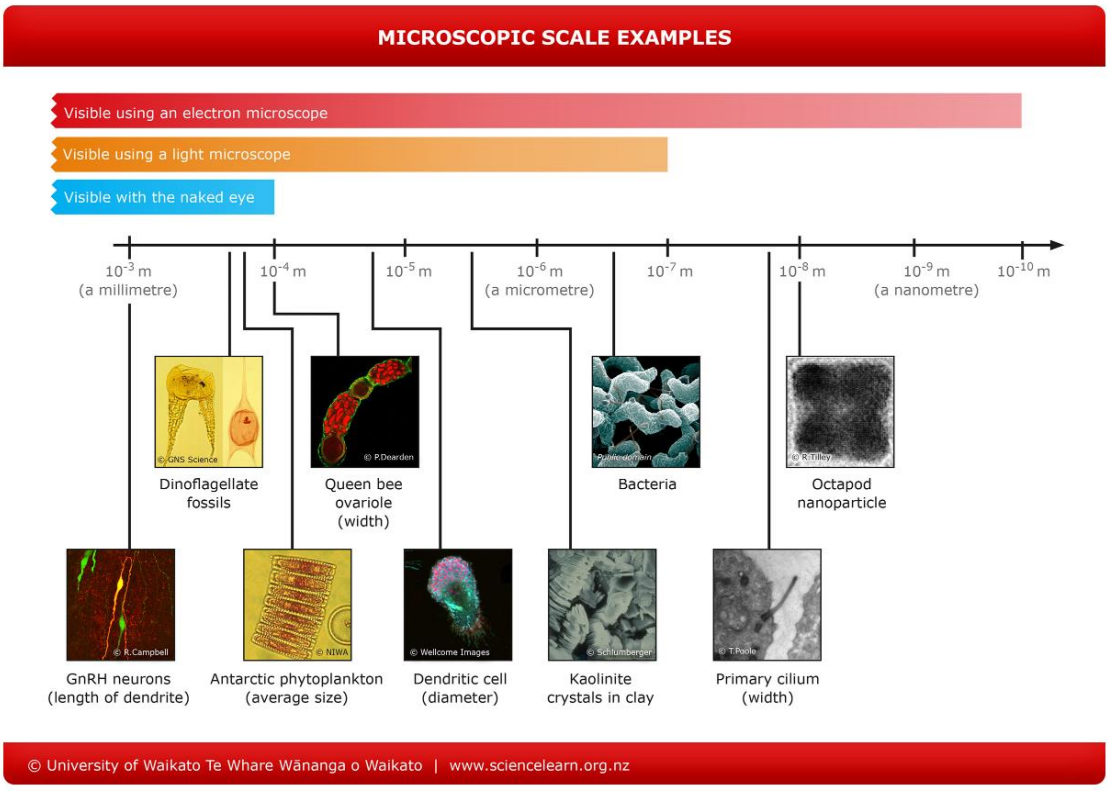

Figure 3. Microscopic Scale Examples (https://www.sciencelearn.org.nz/resources/497-the-microscopic-scale)

3. What Can We See with An Optical/Light Microscope?

The optical microscope, or light microscope, uses visible light with a system of lenses to magnify small objects. The light microscope allows the magnification of objects by using various lenses and arranging these lenses. It is an instrument often used in biological research. Light microscopes are based on light beams in visible wavelengths sent from a light source, passing through the sample to be examined and the perception of the image magnified by the lenses and by the observer according to the contrast, density and thickness differences.

We can see objects of size ranging down to 1 micron with an optical microscope. (For comparison, the diameter of a human hair is approximately 100 microns.) Other objects we can see using a light microscope are flower parts, insects, earthworms, and human skin. The light microscope is ideal for imaging biological specimens because the resolution of the light microscope (the ability of a microscope to distinguish detail) is within the size range of cells.

The quality of the image obtained in the light microscope and other types of microscopes is determined by two features. The first of these features is the magnification ratio (magnification). The magnification ratio indicates how many times larger an image is obtained than the original size of an object. The second property is resolution. Resolution indicates the extent to which conditions such as the distance and density difference between the structures of the observed object are preserved in the magnified image, that is, how clear the image is. In microscopes, resolution and magnification are provided by light and microscope lenses. Lenses allow us to direct the light used for various purposes.

In microscope images, the wavelength (λ) of the microscope light and the numerical aperture (NA), which expresses the light-collecting power of the lens, are two factors that affect the resolution. There is a linear proportionality between Numerical Aperture and resolution. A lens with a high Numerical Aperture value also has a high resolution. The refractive index (n) is a numerical expression that indicates how much light is transmitted/scattered without refraction. The refractive index is the ratio of emitted light to refracted light. The value of n, which indicates the refractive index, expresses the light-gathering capacity of the lens used. In other words, the higher the n value, the greater the light-gathering capacity of the lens used.

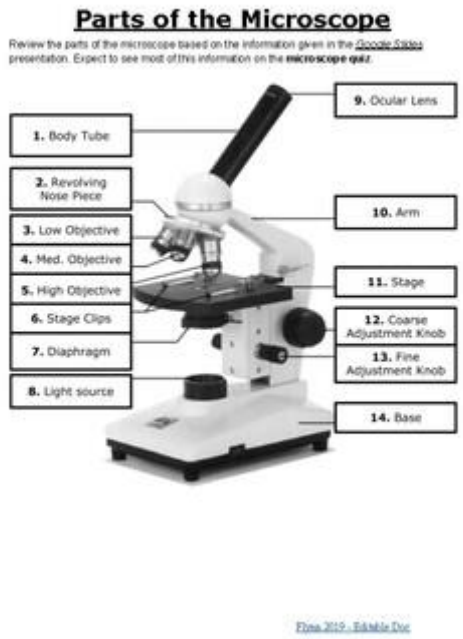

Figure 4. Parts of the Microscope

A high level of contrast is required for the image to be seen with a microscope. It is possible to adjust the contrast by varying the light intensity and the angle at which the light passes through the lenses. The angle of the light coming from the illumination lens (condenser) is adjusted by the phase rings in the microscope called a diaphragm. Another method used to adjust the contrast is filters that can be placed in front of the lighting source. It is possible to strengthen the contrast by staining the sample to be examined with various chemicals.

Two types of light microscopes exist in classrooms. One of them is the stereomicroscope, used to see the surface of the objects, and the other is the compound microscope, used to examine a thin cross-section. One of the advantages of light microscopes (and stereomicroscopes in particular) is that objects can be viewed with little or no prior preparation.

There is a need to increase the visibility of biological samples in the light microscope (especially compound microscopes). For this, some dyeing materials are used to colour the samples since they often lack contrast, making it difficult to distinguish all their parts.

As a dyestuff, iodine is often used to colour plant cells. Iodine dyes the starch stored in the cells blue and the other structures pale brown, allowing them to be separated from each other in colour. Other most commonly used dyestuffs are hematoxylin and eosin. When using fluorescent microscopy, fluorescent dyes are used to highlight certain parts of the cell or tissue. (https://www.sciencelearn.org.nz/resources/501-light-microscopes).

Confocal laser scanning fluorescence microscopy (confocal) allows a thick biological sample to be viewed under a microscope without taking any sections. This feature makes it possible to monitor processes in biological samples such as the movement of mitochondria around cells and the entrance of the drug carrier system across the cell membrane.

The confocal can be used to examine organelles of cells, tissues, or certain components such as proteins. The component to be examined is exposed to a fluorescent substance and becomes brighter with different colours in the image leading to its easiest examination.

When we want to see the minerals in a rock, we use a polarized light microscope. Thus, different minerals appear as different colours under polarized light (light waves vibrating in only one direction). When we look at minerals with a polarized light microscope, it allows us to examine their shape, size, and orientation.

4. How Can We See Atoms? The Electron Microscope

The modern light microscope can achieve magnification up to 2000 times. While this magnification is sufficient to see inside plant and animal cells, it is not enough to see many nanoscale objects because of the wavelength of light. Nanoscale objects are so small that light directed at them cannot hit them, leading to no reflection back for us to see them.

Electron microscopes allow us to look at objects in detail, as they can achieve much greater magnification than a light microscope. Some electron microscopes allow viewing objects as small as molecules or even atoms. Unlike the light microscope, electron microscopes must go through a sample preparation stage before looking directly at living things.

Using an electron microscope, we can see objects as small as an atom (about one ten-millionth of a millimetre or 10-10 m). The size of an atom is a fraction of a nanometer (diameter of an atom=10-10m, 1 nanometer(nm)=10-9m, therefore the size of an atom=0,1 nm). Other examples of objects we can see using an electron microscope are viruses and molecules.

Two German scientists, Ernst Ruska and Max Knoll developed a method in 1931 that would provide better resolution than visible light. In this method, electrons pass through the sample to be examined instead of light and the image is obtained. With this method, the first example of the electron microscope was revealed. Electron beams are directed at a sample and made to strike it. Electrons striking the sample are scattered and used to image the object they hit. In the electron microscope, this image can be magnified 500.00 times. While an electron microscope allows us to see the inside of the cell in detail, a transmission electron microscope allows us to see nanoparticles and atoms. Between the years 1940-1950, thanks to the work done by Albert Claude, Keith Parter and George Palade, the electron microscope also became a field of use in biology.

Electrons are used instead of light as the illumination source in the electron microscope. An electron microscope provides better resolution than a light microscope because the wavelength of electrons is about 0.04 nm. Electron wavelength is about 10000 times smaller than the wavelength of visible light. Since the wavelength of electrons is shorter than the wavelength of visible light, the magnification and resolution provided by the electron microscope are higher than that of standard light microscopes.

In the electron microscope, electrons are released by the electron gun. Condensing lenses then turn the electron beam into a single, thin beam. Thus, electrons are provided to focus on a single point. There are positively charged systems around the sample for electrons to travel downstream and be targeted at the sample. The use of the electron microscope and the preparation of samples for image processing are more complex compared to standard microscopes. The sample to be used in the electron microscope must be in much thinner sections than in the light microscope. By passing electrons directly through this sample, images can be created based on the degree of darkness on the fluorescent surface, or the electrons hit the sample surface and are deflected at different angles and rates according to the depth and density of the sample under study. The stray electrons are detected by the detectors, resulting in a digital image of the sample.

There are many types of electron microscopes:

The transmission electron microscope (TEM) was the first electron microscope to be developed in electron microscopy technology. It works on the principle of detecting electrons that strike a thin slice of a sample and pass it to the other side. TEM is similar to compound light microscopy, allowing us to look at a thin section of a sample at a very high resolution. This functionality provides an even more detailed examination of many small objects.

Electron tomography is a different type of TEM that allows us to see a three-dimensional image of the cell or tissue. Seeing objects and their parts in three dimensions helps us understand the relations between them. Also, electron tomography can give two-dimensional images with higher resolution than conventional TEM.

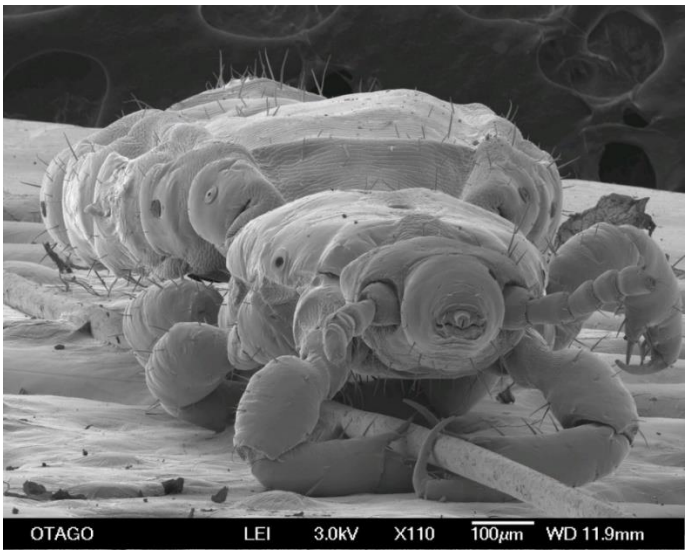

Figure 5. Louse viewed with the SEM (ww.sciencelearn.org.nz/resources/502-types-of-electron-microscope)

The scanning electron microscope (SEM) first scans the surface of the sample with a beam of electrons directed to the object, then uses the electrons reflected from the surface of the sample to obtain a 3-dimensional and high-resolution image. Sometimes, to see objects (insects such as lice, fleas, etc.), low magnification values may be sufficient to obtain 3D images of them.

CryoSEM is a special form of SEM that allows us to see and examine objects that naturally contain water (plants, food, etc.). Unlike sample preparation in conventional SEM, samples are frozen in liquid nitrogen before examination in CryoSEM. Many researchers prefer to work with cryoSEM because it is similar to the shape of the sample before it was prepared.

Electron backscatter diffraction (EBSD) is used to look in detail at the structure of minerals in stones and rocks. EBSD detectors work as part of SEMs. The electron beam is directed towards an object such as a stone/rock, the electrons hitting the object are scattered, and the device detects these electrons. The scattering pattern here gives important information about the structure and crystal shape of the mineral.

4.1 Electron Microscopy

In general, the direct imaging technique of nanoparticles is performed by electron microscopy methods. The basic principles of electron microscopy are described in module 2.4. Two different electron microscopy techniques should be mentioned here. These are Scanning Electron Microscopy (SEM) and Transition Electron Microscopy (TEM). SEM is a method of obtaining the image by reflecting the electron beam sent on the material to be examined from the material surface. In this method information about the surface properties of the material can be obtained. TEM is a technique of taking images by passing a beam of sent electrons through the material.

Both methods have advantages and disadvantages relative to each other. In addition, different physical properties of nanomaterials can be found with both electron microscopy methods by scattering electrons or rays (x-rays) that emerge as a result of the interaction of electrons with the atoms of the material.

In the SEM, the energy of the electrons to be sent to the nanomaterial surface is between 200 eV and 100 keV. This value must be higher for electrons to pass through the material in the TEM. This value varies between about 100 keV and 500 keV. In imaging processes to be performed with SEM, the material must be conductive for the electrons to be oriented. If the material is not conductive, the surface of the material should be covered with a conductive material (Gold, Carbon) a few nanometers thick. This is a disadvantage of the technic in terms of both the difficulty of the measurement preparation stage and the long time it takes, as well as the cost. On the other hand, TEM can be used directly on both conductive and insulating materials. However, for the sent electrons to pass through the material, the material must be very thin or must be prepared with a thickness of a few nm using special techniques. This is one of the disadvantages of this method. In both methods, information can be obtained from nanomaterials about both their imaging and their different physical properties.

4.2 Probe Microscopy

Probe microscopes are indirect imaging methods. In these methods, information about both imaging and physical properties of the material can be obtained with the use of a probe. The most commonly used probe microscopes are Scanning Tunneling Microscopes (STM) and Atomic Force microscopes (AFM).

STM: STMs were first invented in 1981 by G.Binnig and H. Rohrer. In this method, with the help of a piezo-electric material, a current transition occurs between a sharp tip and the surface, and information about the scanned surface atoms is obtained. This method only works on conductive surfaces. Generally, tungsten, platinum-iridium and carbon nanotubes are used as piezoelectric spikes. Thanks to STM, information about the electronic state of the region is obtained in sample materials, surface roughness can be measured and three-dimensional images of metal surfaces are obtained. However, while the microscope setup should be in a vacuum environment, measurements are made at low temperatures. (Figure 6)

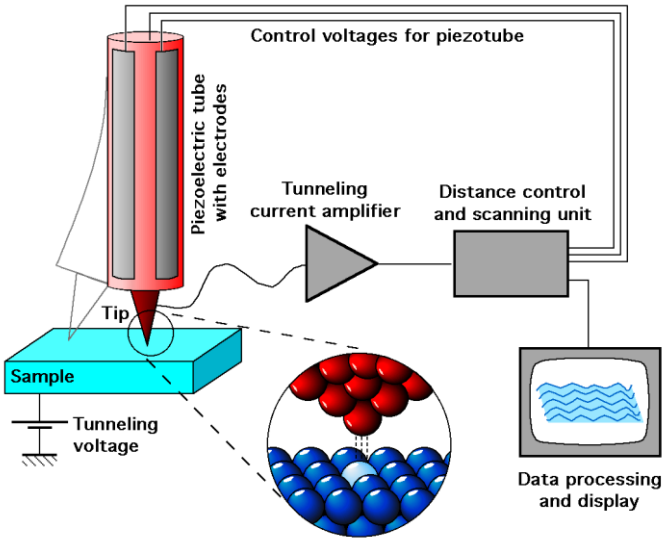

Figure 6. Scanning Tunneling Microscopy structure (https://commons.wikimedia.org/wiki/Category:Scanning_tunneling_microscope)

AFM: AFMs are a relatively easier and more useful method than the STM method. In this method, high-resolution, three-dimensional imaging of the conductive or insulating surface is achieved with the help of a needle tip sharpened to atomic dimensions. Imaging is performed by examining the interaction of

the needle tip with the surface. In this method, the movement of the needle works with the principle of reflecting the laser, which is dropped on the needle surface, to the photodiode (Figure 7).

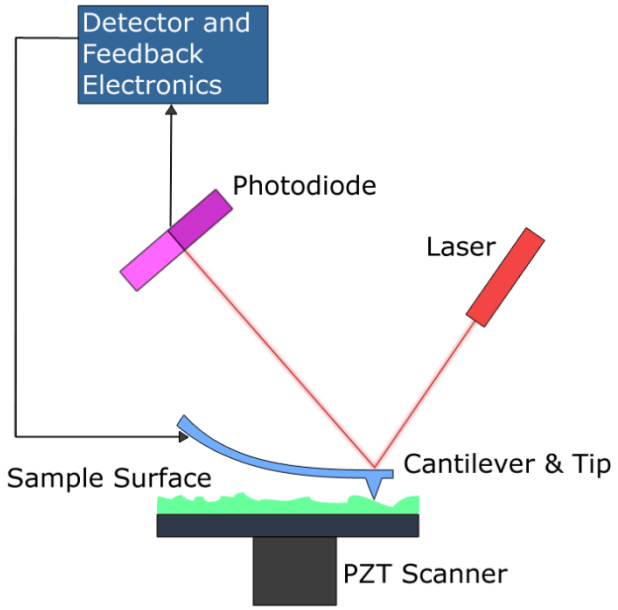

Figure 7. Atomic Force Microscopy struccture (https://upload.wikimedia.org/wikipedia/commons/1/1a/Atomic_force_microscope_block_diagram.png)

4.3 Optical Microscopy Processing

In this section, we will discuss how optical microscopes are used in nanoparticle analysis. As we mentioned in the previous section, direct observation of nanoparticles can be achieved with high resolution only if electron microscopes are used. However, due to the disadvantages of electron microscopes such as requiring a vacuum environment, needing experts, and measuring time and cost, efforts to develop analyzes of nanoparticles by optical microscopes have become increasingly important. First of all, just the use of optical microscopes is not sufficient for the observation of nanoparticles. The maximum magnification possible is only 1000X, and the very best resolution possible is 200 nm by optical microscopes. However, the development of technology and new studies in this field have shown that these devices can be used in certain analyzes of nanoparticles as a result of combining them with certain instruments. Particularly, the digital images obtained as a result of combining Charge-Coupled Device (CCD) cameras with optical microscopes have made it possible to reveal many properties of nanoparticles by processing them with computer programs. The use of optical microscopes in the analysis of nanomaterials due to their advantages such as ease of use, not requiring

a vacuum environment, cost, accessibility and measurement speed has led to an increase in the number of studies in this field. If we talk about some of the methods performed with optical microscopes: are photothermal optical microscopy, Scattering-type scanning near-field optical microscopy (s-SNOM), Raman microscopy, surface plasmon resonance microscopy, dark-field microscopy, and fluorescence microscopy.

4.4 Overview of Microscopes

Types of microscopes can be seen within the figure below:

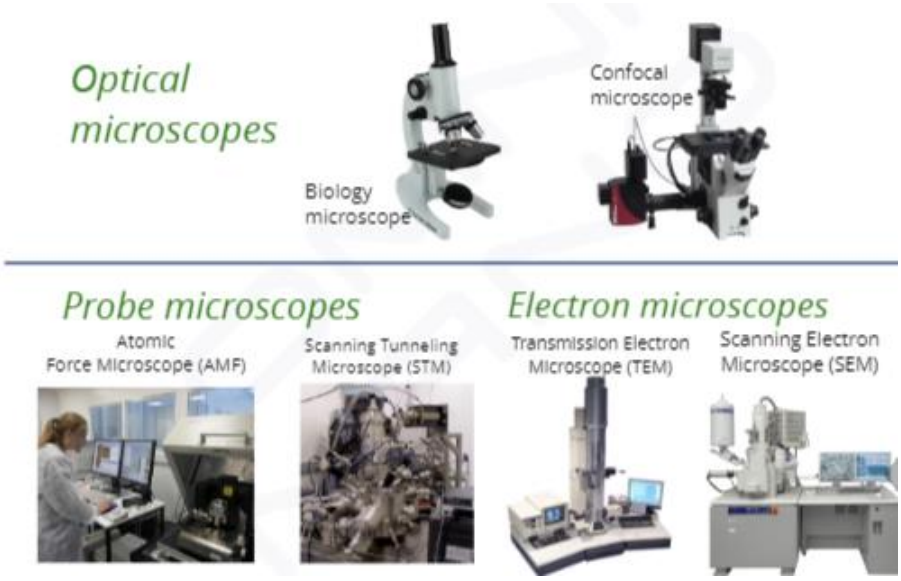

Figure 8. Types of microscopes

5. References

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