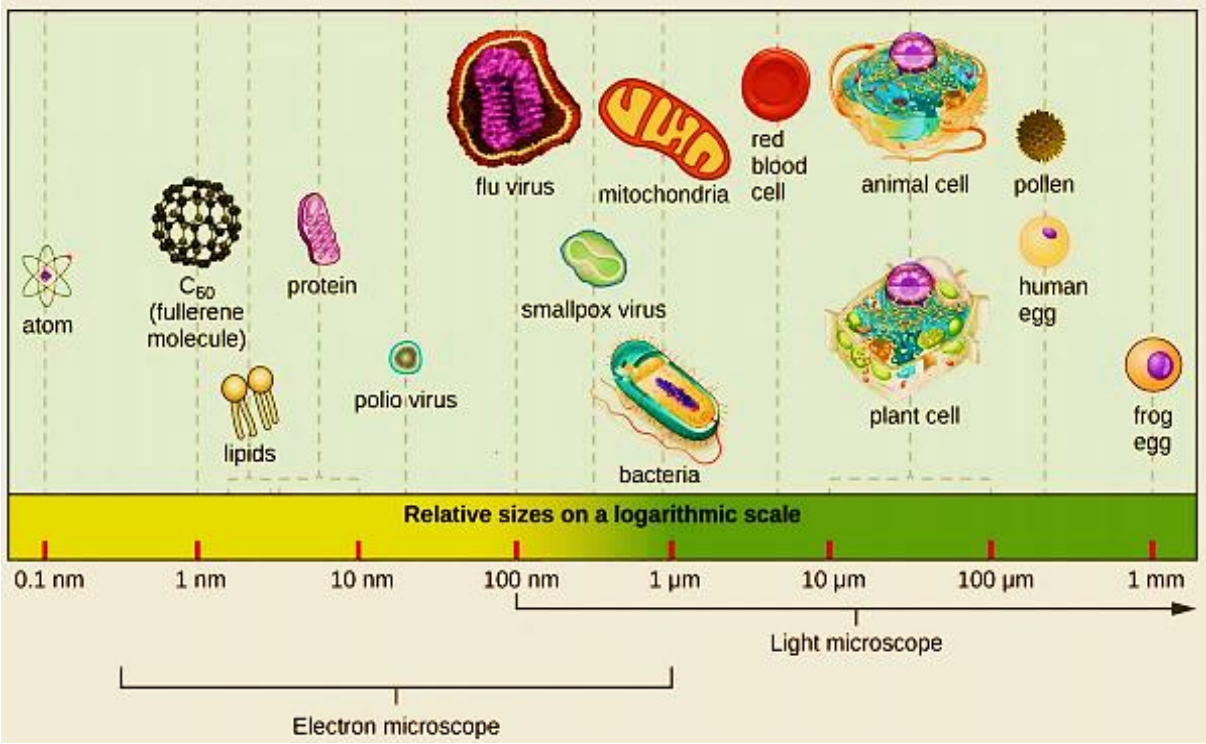
CHAPTER 1. INTRODUCTION AND BRIEF HISTORY OF MICROBIOLOGY

Microbiology is the study of a variety of living organisms which are invisible to the naked eye like bacteria and fungi and many other microscopic organisms. Although tiny in size these organisms form the basis for all life on earth. These microbes are also known to produce the soil in which plants grow and fix the atmospheric gases that both plants and animals use. About 3 billion years ago at the time of the formation of the earth, microbes were the only lives on earth. Microorganisms have played a key role in the evolution of the planet earth. Microorganisms affect animals, the environment, the food supply and also the healthcare industry. Many individuals have made significant contributions to the development of microbiology.



EARLY HISTORY OF MICROBIOLOGY.

Historians are unsure who made the first observations of microorganisms, but the microscope was available during the mid-1600s, **The development of microbiology.** In the late 1800s and for the first decade of the 1900s, scientists seized the opportunity to further develop the germ theory of disease as enunciated by Pasteur and proved by Koch. There emerged a **Golden Age of Microbiology** during which many agents of different infectious diseases were identified. Many of the etiologic agents of microbial disease were discovered during that period, leading to the ability to halt epidemics by interrupting the spread of microorganisms. Despite the advances

in microbiology, it was rarely possible to render life-saving therapy to an infected patient. Then, after World War II, the **antibiotics** were introduced to medicine. The incidence of pneumonia, tuberculosis, meningitis, syphilis, and many other diseases declined with the use of antibiotics. Work with viruses could not be effectively performed until instruments were developed to help scientists see these disease agents. In the 1940s, the **electron microscope** was developed and perfected. In that decade, cultivation methods for viruses were also introduced, and the knowledge of viruses developed rapidly. With the development of vaccines in the 1950s and 1960s, such viral diseases as polio, measles, mumps, and rubella came under control.

ROBERT HOOKE (1635-1703)

He was an English scientist named **Robert Hooke** who made key observations. He is reputed to have observed strands of fungi among the specimens of cells he viewed. He used a **compound microscope**, a microscope with two lenses in tandem, to observe many different objects. In 1665 he published a book by the name of *Micrographia*, with drawing of microbes such as fungi, as well as other organisms and cell structures. His microscopes were restricted in their resolution, or clarity, which appeared to limit what microbes he was able to observe.

ANTONY VAN LEEUWENHOEK (1632-1723)

In the 1670s and the decades thereafter, a Dutch merchant **Anton van Leeuwenhoek** made careful observations of microscopic organisms, which he called **animalcules.** Until his death in 1723, van Leeuwenhoek revealed the microscopic world to scientists of the day and is regarded as one of the first to provide accurate descriptions of protozoa, fungi, and bacteria. He constructed a **simple microscope** (which has a single lens), where the lens was held between two silver plates. He made detailed drawings and notes about his observations and discoveries, sending them off to the **Royal Society of London**, the scientific organization of that time. This invaluable record clearly indicates that he saw both bacteria and a wide variety of protists. Some microbiologists refer to van Leeuwenhoek as the "**Father of Microbiology**," because of his contributions to the field. After van Leeuwenhoek died, the study of microbiology did not develop rapidly because microscopes were rare and the interest in microorganisms was not high.

In those years, scientists debated the theory of **spontaneous generation**, which stated that microorganisms arise from lifeless matter such as beef broth. This theory was disputed

by **Francesco Redi**, who showed that fly maggots do not arise from decaying meat (as others believed) if the meat is covered to prevent the entry of flies.

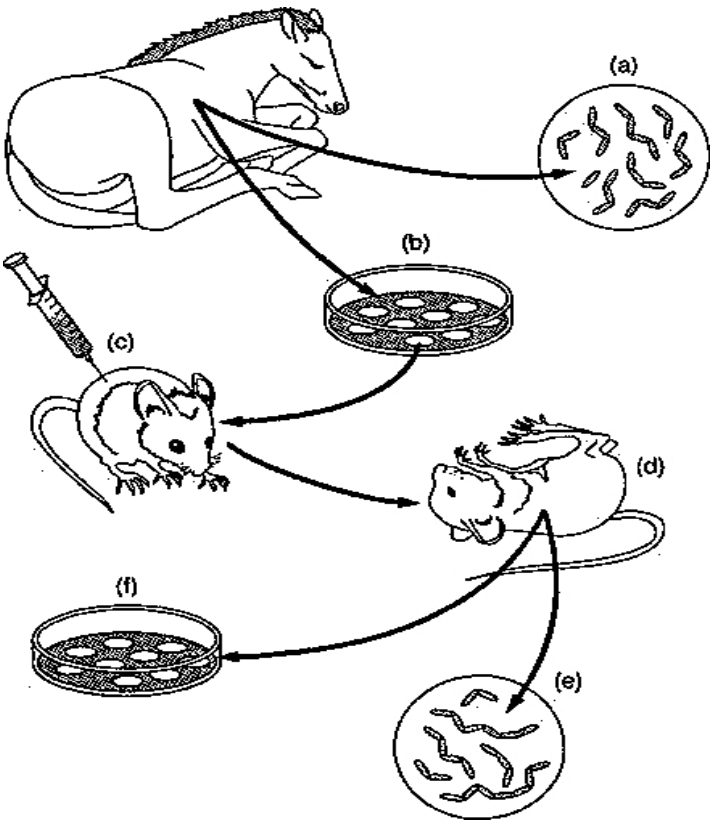
An English cleric named **John Needham** advanced spontaneous generation, but **Lazzaro Spallanzani** disputed the theory by showing that boiled broth would not give rise to microscopic forms of life.

LOUIS PASTEUR AND THE GERM THEORY.

Louis Pasteur worked in the middle and late 1800s. He performed numerous experiments to discover why wine and dairy products became sour, and he found that bacteria were to blame. Pasteur called attention to the importance of microorganisms in everyday life and stirred scientists to think that if bacteria could make the wine "sick," then perhaps they could cause human illness. Pasteur had to disprove spontaneous generation to sustain his theory, and he therefore devised a series of **swan-necked flasks** filled with broth. He left the flasks of broth open to the air, but the flasks had a curve in the neck so that microorganisms would fall into the neck, not the broth. The flasks did not become contaminated (as he predicted they would not), and Pasteur's experiments put to rest the notion of spontaneous generation. His work also encouraged the belief that microorganisms were in the air and could cause disease. Pasteur postulated the **germ theory of disease**, which states that microorganisms are the causes of infectious disease. Pasteur's attempts to prove the germ theory were unsuccessful.

ROBERT KOCH

However, the German scientist **Robert Koch** provided the proof by cultivating anthrax bacteria apart from any other type of organism. He then injected pure cultures of the bacilli into mice and showed that the bacilli invariably caused anthrax. The procedures used by Koch came to be known as **Koch's postulates**. They provided a set of principles whereby other microorganisms could be related to other diseases.



The steps of Koch's postulates used to relate a specific microorganism to a specific disease. (a) Microorganisms are observed in a sick animal and (b) cultivated in the lab. (c) The organisms are injected into a healthy animal, and (d) the animal develops the disease. (e) The organisms are observed in the sick animal and (f) reisolated in the lab.

MODERN MICROBIOLOGY.

Modern microbiology reaches into many fields of human endeavour, including the development of pharmaceutical products, the use of quality-control methods in food and dairy product production, the control of disease-causing microorganisms in consumable waters, and the industrial applications of microorganisms. Microorganisms are used to produce vitamins, amino acids, enzymes, and growth supplements. They manufacture many foods, including fermented dairy products (sour cream, yogurt, and buttermilk), as well as other fermented foods such as pickles, sauerkraut, breads, and alcoholic beverages. One of the major areas of applied microbiology is **biotechnology.** In this discipline, microorganisms are used as living factories to produce pharmaceuticals that otherwise could not be manufactured. These substances include the human hormone insulin, the antiviral substance interferon, numerous blood-clotting factors and clot dissolving enzymes, and a number of vaccines. Bacteria can be reengineered to increase plant resistance to insects and frost, and biotechnology will represent a major application of microorganisms in the next century.

CHAPTER 2. MICROSCOPY AND CHEMICAL BASIS OF MICROBIOLOGY

INTRODUCTION TO MICROSCOPES

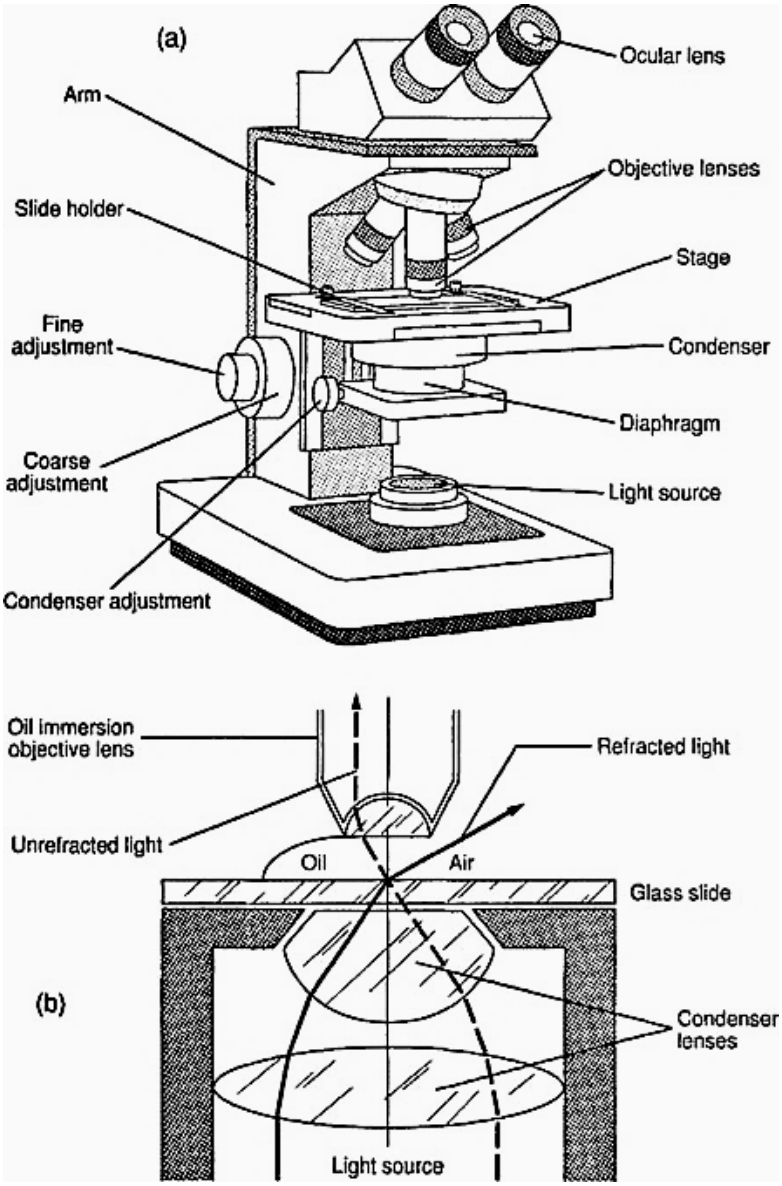
Since microorganisms are invisible to the unaided eye, the essential tool in microbiology is the microscope. During the 1700s, microscopes were used to further elaborate on the microbial world, and by the late 1800s, the sophisticated light microscopes had been developed. The electron microscope was developed in the 1940s, thus making the viruses and the smallest bacteria (for example, rickettsiae and chlamydiae) visible. Microscopes permit extremely small objects to be seen, objects measured in the metric system in micrometers and nanometers. A micrometer (μm) is equivalent to a millionth of a meter, while a nanometer (nm) is a billionth of a meter. Bacteria, fungi, protozoa, and unicellular algae are normally measured in micrometers, while viruses are commonly measured in nanometers. A typical bacterium such as Escherichia coli measures about two micrometers in length and about one micrometer in width,

TYPES OF MICROSCOPES

The microscopes have varied applications and modifications that contribute to their usefulness. **The Light Microscope**. The common light microscope used in the laboratory is called a compound microscope because it contains two types of lenses that function to magnify an object. The lens closest to the eye is called the ocular, while the lens closest to the object is called the objective. Most microscopes have on their base an apparatus called a condenser, which condenses light rays to a strong beam. A diaphragm located on the condenser controls the amount of light coming through it. Both coarse and fine adjustments are found on the light microscope. To magnify an object, light is projected through an opening in the stage, where it hits the object and then enters the objective. An image is created, and this image becomes an object for the ocular lens, which remagnifies the image. Thus, the total magnification possible with the microscope is the magnification achieved by the objective multiplied by the magnification achieved by the ocular lens. A compound light microscope often contains four objective lenses:

- The scanning lens (4X),
- The low-power lens (10X),
- The high-power lens (40 X),
- And the oil-immersion lens (100 X).

With an ocular lens that magnifies 10 times, the total magnifications possible will be 40 X with the scanning lens, 100 X with the low-power lens, 400 X with the high-power lens, and 1000 X with the oil-immersion lens. Most microscopes are parfocal. This term means that the microscope remains in focus when one switches from one objective to the next objective.

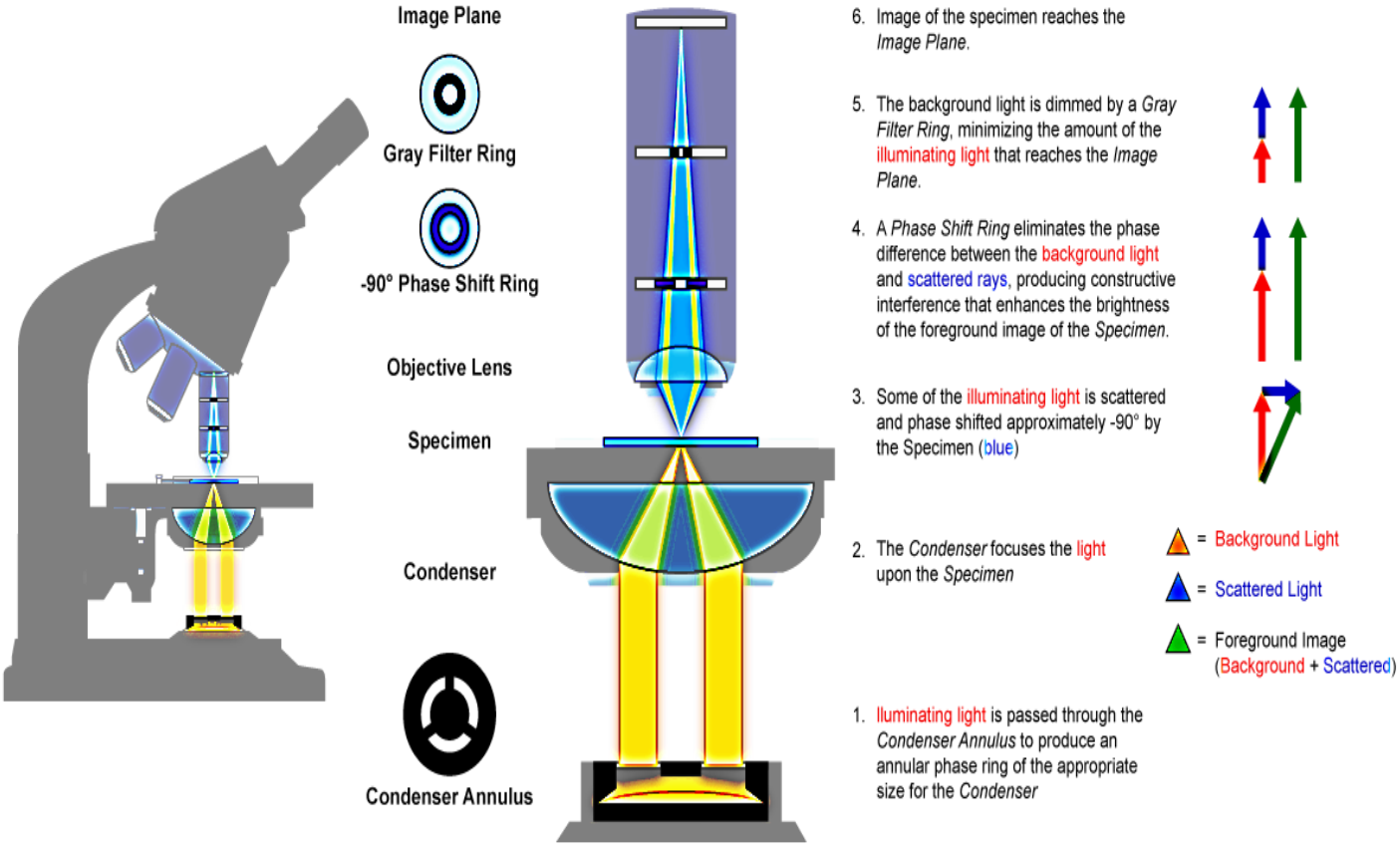


Light microscopy. (a) The important parts of a common light microscope. (b) How immersion oil gathers more light for use in the microscope.

The resolution is determined in part by the wavelength of the light used for observing. Visible light has a wavelength of about 550 nm, while ultraviolet light has a wavelength of about 400 nm or less. The resolution of a microscope increases as the wavelength decreases, so ultraviolet light allows one to detect objects not seen with visible light. However, the oil-immersion lens is exceedingly narrow, and most light misses it. Therefore, the object is seen poorly and without

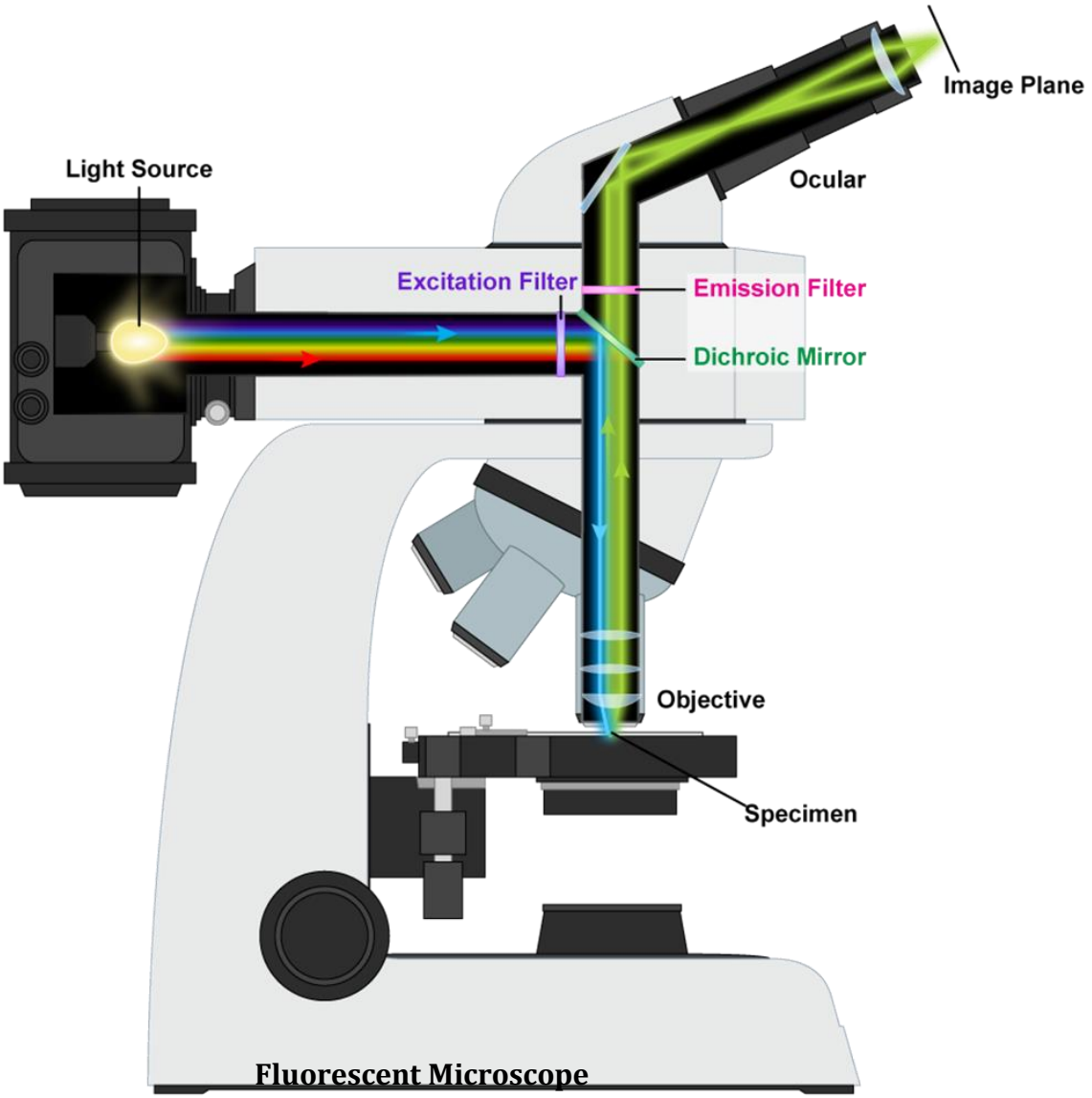
resolution. To increase the resolution with the oil-immersion lens, a drop of immersion oil is placed between the lens and the glass slide. Immersion oil has the same light-bending ability (index of refraction) as the glass slide, so it keeps light in a straight line as it passes through the glass slide to the oil and on to the glass of the objective, the oil- immersion lens. With the increased amount of light entering the objective, the resolution of the object increases, and one can observe objects as small as bacteria

An alternative microscope is the **Dark-Field Microscope**, which is used to observe live spirochetes, such as those that cause syphilis. This microscope contains a special condenser that scatters light and causes it to reflect off the specimen at an angle. A light object is seen on a dark background. A second alternative microscope is the **Phase-Contrast Microscope**. This microscope also contains special condensers that throw light "out of phase" and cause it to pass through the object at different speeds. Live, unstained organisms are seen clearly with this microscope, and internal cell parts such as mitochondria, lysosomes, and the Golgi body can be seen with this instrument.



Phase Contrast Microscope Mechanics.

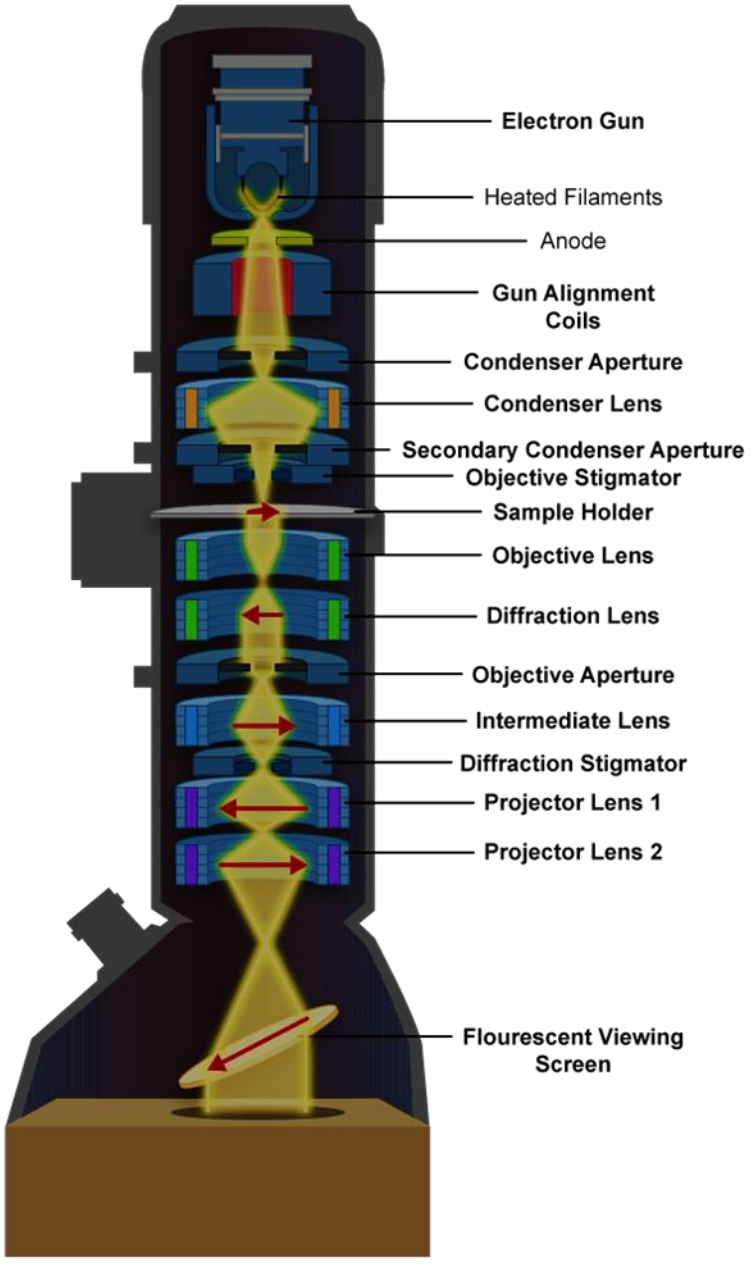
The Fluorescent Microscope uses ultraviolet light as its light source. When ultraviolet light hits an object, it excites the electrons of the object, and they give off light in various shades of colour. Since ultraviolet light is used, the resolution of the object increases. A laboratory technique called the fluorescent-antibody technique employs fluorescent dyes and antibodies to help identify unknown bacteria.



Fluorescent Microscope

Electron microscopy. The energy source used in the electron microscope is a beam of electrons. Since the beam has an exceptionally short wavelength, it strikes most objects in its path and increases the resolution of the microscope significantly. Viruses and some large molecules can be seen with this instrument. The electrons travel in a vacuum to avoid contact with deflecting air molecules, and magnets focus the beam on the object to be viewed. An image is created on a monitor and viewed by the technologist.

The more traditional form of electron microscope is the **Transmission Electron Microscope (TEM)**. To use this instrument, one places ultrathin slices of microorganisms or viruses on a wire grid and then stains them with gold or palladium before viewing. The densely coated parts of the specimen deflect the electron beam, and both dark and light areas show up on the image.

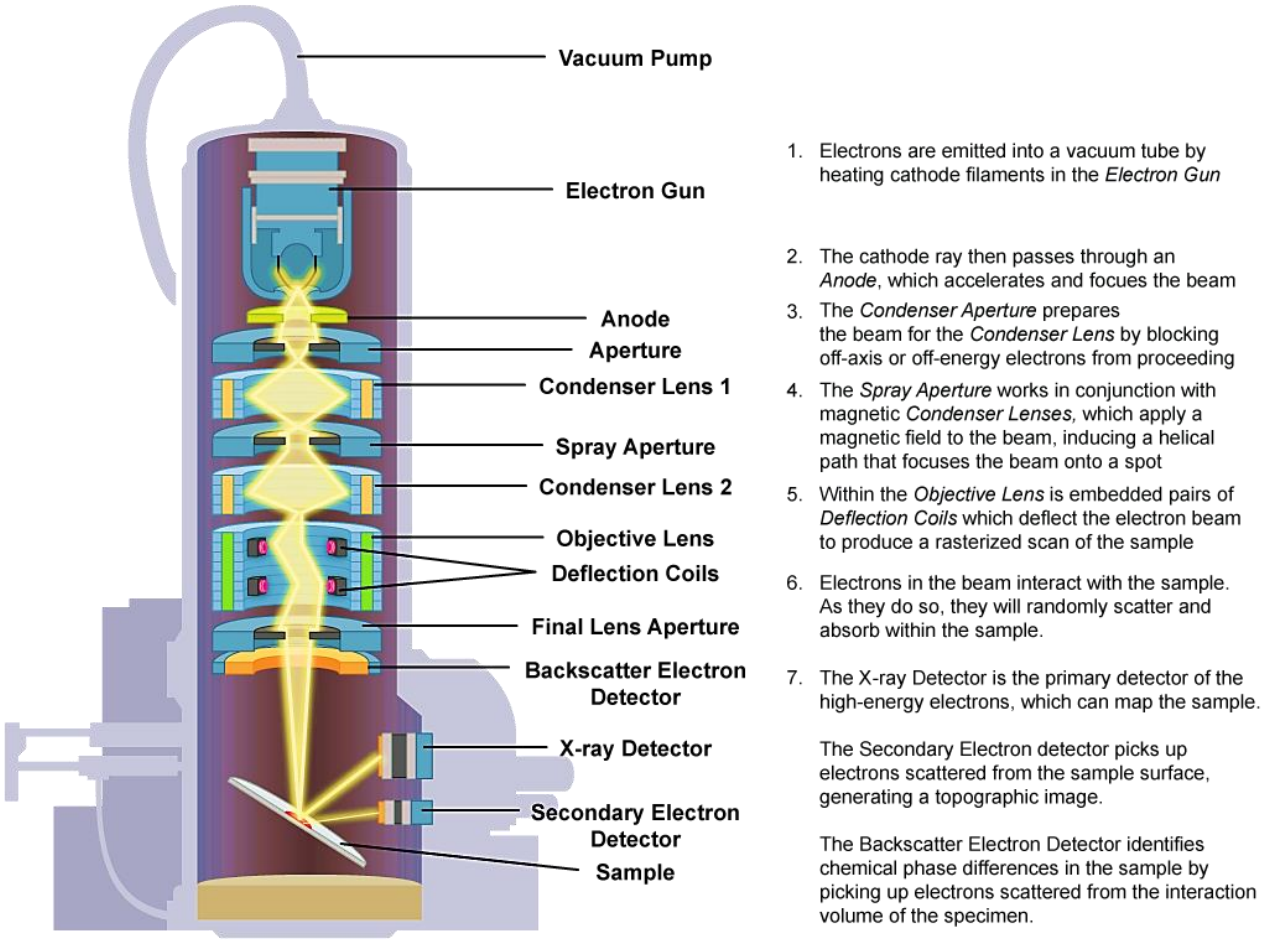


1. Electrons are emitted into a vacuum tube by heating *Cathode Filaments* in the electron gun
2. The cathode ray then passes through an *Anode*, which accelerates and focues the beam; *Alignment Coils* additionally accelerate the beam
3. An adjustable *Condenser Aperture* prepares the beam for the *Condenser Lens* by blocking off-axis or off-energy electrons from proceeding.
4. The magnetic *Condenser Lens* applies a magnetic field, inducing a helical path for the electrons, and leading the cone-shaped electron beam to converge on a spot
5. A *Stigmator* helps to adjust the beam and prevent astigmatism (different foci between rays) in the optical system
6. Electrons pass through the thinly sliced sample, inserted onto a grid-like stage
7. The *Objective Lens* focuses the image of the sample
8. A *Diffraction Lens* is used to apply Bragg Scattering to the electrons
9. The *Objective Aperture*, positioned on the back focal plane of the scattered rays, selects (or excludes) the portion of the sample that produced the scattering
10. *Projector Lenses* calibrate the magnification of the image
11. The image is visualized through oculars or by an image recording system underneath the *Fluorescent Screen*

Transmission Electron Microscope (TEM)

The **Scanning Electron Microscope (SEM)** is the more contemporary form electron microscope. Although this microscope gives lower magnifications than the TEM, the SEM

permits three-dimensional views of microorganisms and other objects. Whole objects are used, and gold or palladium staining is employed.



Scanning Electron Microscope (SEM)

CHEMICAL PRINCIPLES

In the 1700s, scientists discovered the chemical and physical basis of living things, and soon they realized that the chemical organization of all living things is remarkably similar. Microorganisms, as forms of living things, conform to this principle and have a chemical basis that underlies their metabolism.

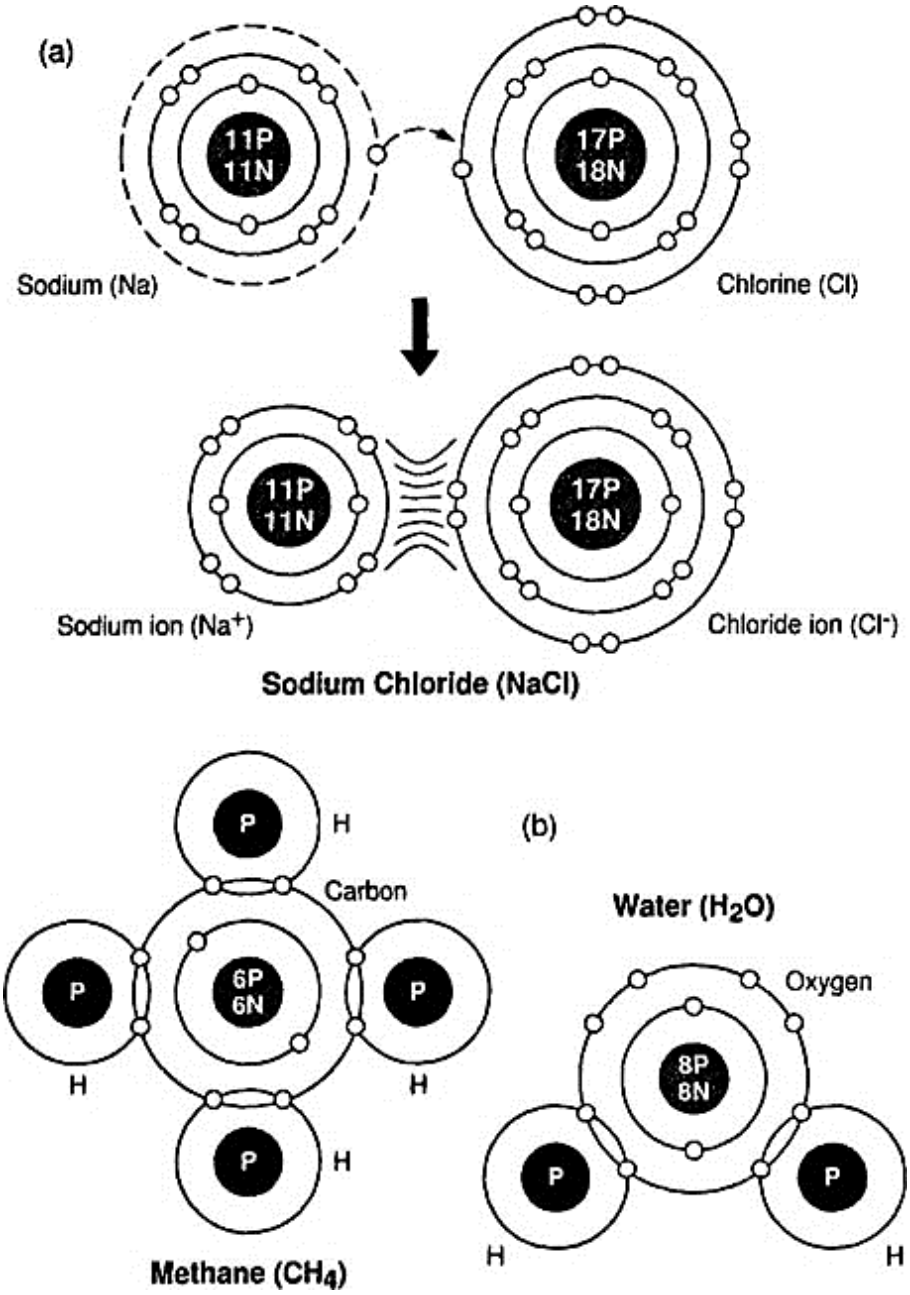
Elements and atoms. All living things on earth, including microorganisms, are composed of fundamental building blocks of matter called elements. Over 100 elements are known to exist, including certain ones synthesized by scientists. An element is a substance that cannot be decomposed by chemical means. Such things as oxygen, iron, calcium, sodium, hydrogen, carbon, and nitrogen are elements. Each **element** is composed of one particular kind of **atom**.

An **atom** is the smallest part of an element that can enter into combinations with atoms of other elements. Atoms consist of positively charged particles called **protons** surrounded by negatively charged particles called **electrons**. A third particle called the **neutron** has no electrical charge; it has the same weight as a proton. Protons and neutrons adhere tightly to form the dense, positively charged nucleus of the atom. Electrons spin around the nucleus in orbits, or shells. The arrangement of electrons in an atom plays an essential role in the chemistry of the atom. Atoms are most stable when their outer shell of electrons has a full quota, which may be two electrons or eight electrons. Atoms tend to gain or lose electrons until their outer shells have this stable arrangement. The gaining or losing of electrons contributes to the chemical reactions in which an atom participates.

Molecules: Most of the microbial compounds of interest to biologists are composed of units called molecules. A molecule is a precise arrangement of atoms from different elements; a compound is a mass of molecules. The arrangements of the atoms in a molecule account for the properties of a **compound**.

The molecular weight is equal to the atomic weights of the atoms in the molecule. For example, the molecular weight of water is 18. The atoms in molecules may be joined to one another by various linkages called bonds. One example of a bond is an ionic bond, which is formed when the electrons of one atom transfer to a second atom, creating electrically charged atoms called ions. The electrical charges attract the ions to one another; the attraction creates the ionic bond. Sodium chloride consists of sodium ions and chloride ions joined by ionic bonds. Bond formation in molecules.

A second type of linkage is called a covalent bond which forms when two atoms share one or more electrons with one another. For example, carbon shares its electrons with four hydrogen atoms, and the resulting molecule is methane (CH_4). If one pair of electrons is shared, the bond is a single bond; if two pairs are shared, then it is a double bond. Covalent bonds are present in organic molecules such as proteins, lipids, and carbohydrates. Acids and bases. Certain chemical compounds release hydrogen ions when the compounds are placed in water.



(a) Formation of an ionic bond in a sodium chloride molecule.

(b) Covalent bonding in methane and water molecules. Syntheses in organic molecules.

These compounds are called acids. For example, when hydrogen chloride is placed in water, it releases its hydrogen ions, and the solution becomes hydrochloric acid. Certain chemical compounds attract hydrogen ions when they are placed in water. These substances are called bases. An example of a base is sodium hydroxide (NaOH). When this substance is placed in water, it attracts hydrogen ions, and a basic (or alkaline) solution result.

CHAPTER 3. INTRODUCTION TO MICROORGANISMS

THE SPECTRUM OF MICROBIOLOGY

WHAT ARE MICROORGANISMS?

Microorganisms are a collection of organisms that share the characteristic of being visible only with a microscope. They constitute the subject matter of **microbiology.** Members of the microbial world are very diverse and include the bacteria, cyanobacteria, rickettsiae, chlamydiae, fungi, unicellular (single-celled) algae, protozoa, and viruses. The majority of microorganisms contribute to the quality of human life by doing such things as maintaining the balance of chemical elements in the natural environment, by breaking down the remains of all that dies, and by recycling carbon, nitrogen, sulfur, phosphorus, and other elements. Some species of microorganisms cause infectious disease. They overwhelm body systems by sheer force of numbers, or they produce powerful toxins that interfere with body physiology. Viruses inflict damage by replicating within tissue cells, thereby causing tissue degeneration. Like all other living things, microorganisms are placed into a system of **classification** which highlights characteristics that are common among certain groups while providing order to the variety of living things. The science of classification is known as **taxonomy**, and **taxon** is an alternative expression for a classification category. Taxonomy displays the unity and diversity among living things, including microorganisms. Among the first taxonomists was **Carolus Linnaeus.** In the 1750s and 1760s, Linnaeus classified all known plants and animals of that period and set down the rules for nomenclature.

THE FIVE KINGDOMS.

The generally accepted classification of living things was devised by **Robert Whittaker** of Cornell University in 1969. Whittaker suggested a five-kingdom classification. The first of the five kingdoms are **Monera** (in some books, **Prokaryotae**). Prokaryotes, such as bacteria and cyanobacteria (formerly, blue-green algae), are in this kingdom; the second kingdom, **Protista**, includes protozoa, unicellular algae, and slime molds, all of which are eukaryotes and single-celled; in the third kingdom, **Fungi**, are the molds, mushrooms, and yeasts. These organisms are eukaryotes that absorb simple nutrients from the soil. The remaining two kingdoms are **Plantae** (plants) and **Animalia** (animals).

BRIEF DESCRIPTIONS OF MICROORGANISMS.

Bacteria are relatively simple, prokaryotic organisms whose cells lack a nucleus or nuclear membrane. The bacteria may appear as rods (bacilli), spheres (cocci), or spirals (spirilla or spirochetes). Bacteria reproduce by binary fission, have unique constituents in their cell walls, and exist in most environments on earth. For instance, they live at temperatures ranging from 0° to 100°C and in conditions that are oxygen rich or oxygen free. A microscope is necessary to see and study them.

Fungi are eukaryotic microorganisms that include multicellular molds and unicellular (single-celled) yeasts. The **yeasts** are slightly larger than bacteria and are used in alcoholic fermentations and bread making. Certain yeasts such as *Candida albicans* are pathogenic (disease causing). **Molds** are filamentous, branched fungi that use spores for reproduction. The fungi prefer acidic environments, and most live at room temperature under oxygen-rich conditions. The common mushroom is a fungus.

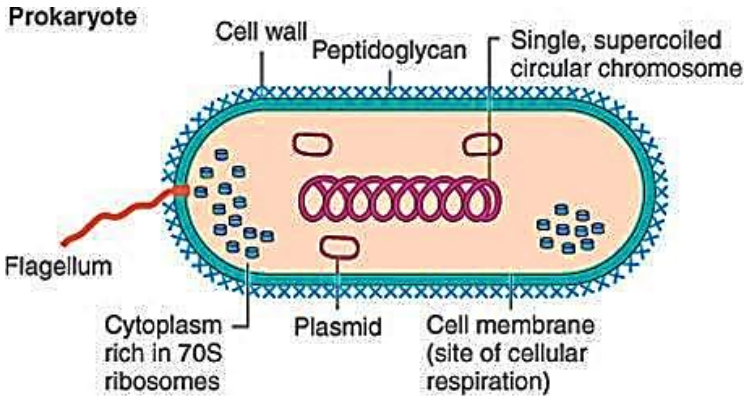
Protozoa are eukaryotic, unicellular organisms. Motion is a characteristic associated with many species, and the protozoa can be classified according to how they move: Some protozoa use flagella, others use cilia, and others use pseudopodia. Certain species are nonmotile. Protozoa exist in an infinite variety of shapes because they have no cell walls. Many species cause such human diseases as malaria, sleeping sickness, dysentery, and toxoplasmosis.

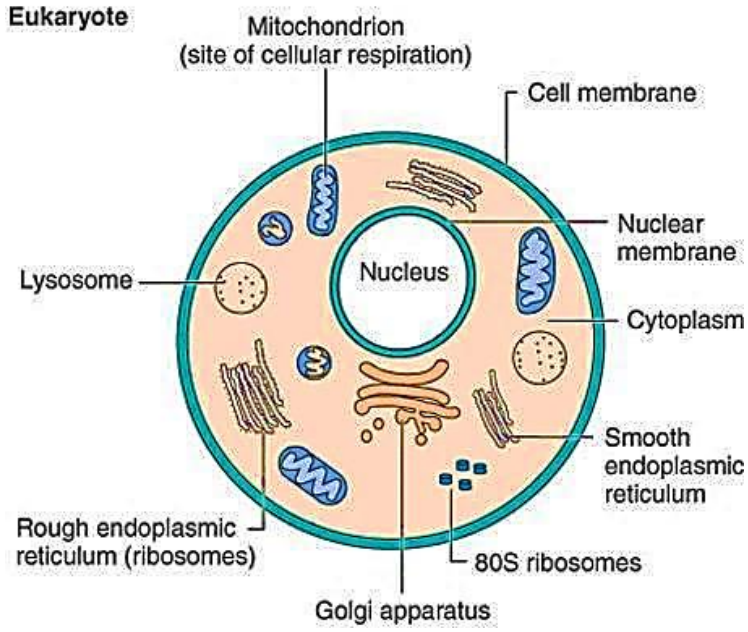
The term **Algae** implies a variety of plant like organisms. In microbiology, several types of single-celled algae are important. Examples are the diatoms and dinoflagellates that inhabit the oceans and are found at the bases of marine food chains. Most algae capture sunlight and transform it to the chemical energy of carbohydrates in the process of photosynthesis.

Viruses are ultramicroscopic bits of genetic material (DNA or RNA) enclosed in a protein shell and, sometimes, a membranous envelope. Viruses have no metabolism; therefore, it is difficult to use drugs to interfere with their structures or activities. Viruses multiply in living cells and use the chemical machinery of the cells for their own purpose. Often, they destroy the cells in the process of replicating.

PROKARYOTES AND EUKARYOTES.

Because of their characteristics, microorganisms join all other living organisms in two major groups of organisms: prokaryotes and eukaryotes. Bacteria are **prokaryotes** (simple organisms having no nucleus or organelles) because of their cellular properties, while other microorganisms such as fungi, protozoa, and unicellular algae are **eukaryotes** (more complex organisms whose cells have a nucleus and organelles). Viruses are neither prokaryotes nor eukaryotes because of their simplicity and unique characteristics.





.S/N.	Character	Prokaryotes	Eukaryotes
1.	Term Origin	Greek for "primitive nucleus"	Greek for "true nucleus"
2.	Definition	Organisms are made up of cell(s) that lack a cell nucleus or any membrane-encased organelles.	Organisms are made up of cells that possess a membrane-bound nucleus as well as membrane-bound organelles.

3.	Major groups	Bacteria, Archaea, and Bluegreen algae	Algae, fungi, protozoa, plants, animals
4.	Origin	Around 3.5 billion years ago.	Around 2 billion years ago.
5.	Size (approximate)	0.5-3.0 µm	>5 µm
6.	Cell Type	Usually unicellular (some cyanobacteria may be multicellular)	Usually multicellular
7.	Complexity	Simple	Complex organization.
8.	Nucleus Location	Free in the cytoplasm, attached to mesosomes	Contained in membrane bound structure
9.	Nuclear membrane	No nuclear membrane.	Classic membrane present.
10.	Nucleolus	Absent	Present
11.	Chromosome number	One	More than one
12.	Chromosome shape	Circular	Linear
13.	Genes	Expressed in groups called operons.	Expressed individually
14.	Genome	**DNA** haploid genome	**DNA** diploid genome
15.	DNA base ratio (G+C %)	28-73	About 40
16.	DNA wrapping on proteins	Multiple proteins act together to fold and condense prokaryotic DNA. Folded DNA is then organized into a variety of conformations that are supercoiled and wound around tetramers of the HU protein.	Eukaryotes wrap their DNA around proteins called histones.
17.	Genome nature	Efficient and compact with little repetitive DNA.	With large amounts of non-coding repetitive DNA.
18.	Membrane-bound organelles	Absent	Present
19.	Ribosomes (sedimentation coefficient)	70S (50S + 30S). Smaller.	80S (60S + 40S). Larger.
20.	Ribosome's location	Free in the cytoplasm or bound to the cell membrane	Attached to the rough endoplasmic reticulum
21.	Mitochondria	Absent	Present
22.	Golgi bodies	Absent	Present
23.	Endoplasmic reticulum	Absent	Present
24.	Mesosomes	Present. Performs the function of Golgi bodies and mitochondria and also helps in the separation of the chromosome during cell division.	Absent
25.	Lysosomes	Absent	Present
26.	Peroxisomes	Absent	Present

27.	Chloroplasts	Absent; chlorophyll scattered in the cytoplasm	Present (in plants)
28.	Fimbriae	Prokaryotes may have pili and fimbriae (appendage that can be found on many Gram-negative and some Gram-positive bacteria).	Absent
29.	Microtubules	Absent or rare	Present
30.	Centrosome	Absent	Present except in flowering plants.
31.	Cytoskeleton	May be absent	Present
32.	Glycocalyx	Present	Only in some
33.	Cytoplasmic streaming	Absent	Present
34.	Cytoplasmic membrane	Does not contain sterols (except *Mycoplasma*)	Contains sterols
35.	Cell wall	Complex structure containing protein, lipids, and peptidoglycans	Present for plant cells and fungi; otherwise absent
36.	Muramic acid	Present	Absent
37.	Movement	Simple **flagellum**, if present	Complex **flagellum**, if present
38.	Respiration	Via cytoplasmic membrane	Via mitochondria
39.	Energy production site	Electron transport chain located in the cell membrane	Within membrane bound mitochondria
40.	Metabolic rate	Higher due to larger surface area to volume ratio	Comparatively slow
41.	Reproduction	Asexual (binary fission)	Sexual and asexual/ Mitotic division
42.	Generation time	Shorter	Comparatively longer
43.	Genetic Recombination	Partial, unidirectional transfer	Meiosis and fusion of gametes
44.	Zygote	Merozygotic (partially diploid)	Diploid
45.	Extrachromosomal DNA	Plasmid	Inside the mitochondria
46.	DNA replication	Occurs in the cytoplasm.	Occurs in the nucleus.
47.	Transcription and translation	Occurs simultaneously.	Transcription occurs in the nucleus and then translation occurs in the cytoplasm.