DEFINITION AND SCOPE OF MICROBIOLOGY

1.1 INTRODUCTION

Microbiology is a branch of science that deals with microbes. The term microbiology derives its name from three Greek words ***mikros*** **[small] bios [life] and logos [study].** Microbiology focus on the occurrence and distribution of microorganisms in nature, their structure, physiology, reproduction, metabolism and classification.

Microbes - Microorganisms are tiny and invisible to naked eye. They can be seen only by magnifying their image with a microscope. Small subcellular or cellular living beings with milli-micron or micron in size and are not visible to our naked eyes are called micro-organisms. Microorganisms include the cellular organisms like **bacteria, fungi, algae and protozoa**. **Viruses** are also included as one of the microorganism but they are acellular.

The following branches that are concerned with the study of morphology, ecology, taxonomy, genetics and physiology of specific groups of microbes.

1. **Bacteriology**-Study of bacteria
2. **Phycology**- Study of algae
3. **Mycology** -Study of fungi [molds and yeasts].
4. **Virology**- Study of viruses
5. **Protozoology-** Study of protozoa

1.2 DIFFERENCE BETWEEN MICROBIAL CELL AND PLANT/ANIMAL CELL

S. No	Microbial cell	Plant/Animal cell
(a)	A microbial cell can **live alone**	Plant or animal cell exist only as part of organisms
(b)	Growth, energy generation and reproduction by a microbial cell are **independent**	Plant or animal cell depend on other cells for all processes

1.3. OCCURRENCE OF MICROORGANISMS

Microbes are widely distributed in the world. They are **ubiquitous i.e. present everywhere** in air, water, soil, in living plants, animals, dead matter etc. They are present on our body, in our body, in the air we breathe, in the food we eat, in the air we drink, in our mouth and in our intestine. Almost all natural surfaces are colonized by microbes. Some microorganisms are even adapted to live comfortably in boiling hot springs and frozen sea ice. Microbes are the **dominant form of life** in the universe. More than 50 per cent of the biomass on earth consists of microorganisms compared to animals which constitute only 15 per cent of the mass of living organisms on earth. Majority of the microorganisms are not dangerous to humans. In fact, microbes help to digest our food and protect our bodies from pathogens. Additionally, they are considered as **beneficial** ones as they keep the biosphere running by carrying out essential functions such as decomposition of dead animals and plants, nutrient cycling which enhances the soil health and crop productivity.

1.4 LEVEL OF ORGANIZATION

The microbes are either unicellular or multicellular or acellular (non-cellular) forms.

- Unicellular – Bacteria and Protozoa
- Multicellular – Algae and Fungi
- Acellular – Virus

1.5 MEMBERS OF THE MICROBIAL WORLD

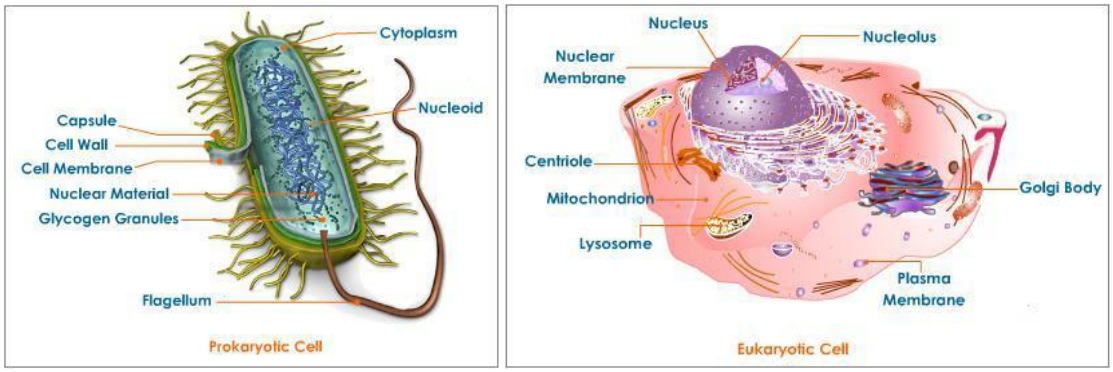
Based on the presence or absence of nuclear membrane microorganisms are basically classified under two groups:

i) PROKARYOTES

Prokayote is a Greek word, *pro* - before and *karyon* - nut or kernel. Prokaryotes are the organism with a primordial nucleus. They have a much simpler morphology than eukaryotic cells and **lack** a true membrane bound nucleus and cell organelles like mitochondria, golgi bodies, endoplasmic reticulum, etc. All **bacteria** and **archaea** are prokaryotic.

ii) EUKARYOTES

Eukaryote is a Greek word, *eu* - true and *karyon* - nut or kernel. Eukaryotes **posses** a membrane enclosed nucleus and cell organelles. They are more complex morphologically and are usually larger than prokaryotes. **Algae, fungi, protozoa, higher plants** and **animals** are eukaryotic.



1.6. MICROBIAL GROUPS

Based on the morphological, phylogenetic and physiological characteristics, microorganisms are divided into six distinct groups, they are as follows

1) Bacteria
2) Archaea
3) Fungi
4) Protozoa
5) Algae
6) Viruses

1) **BACTERIA** are **prokaryotes** that are usually single celled organisms. They multiply by binary fission and reproduces asexually. They are the most **dominant** group of microorganisms in soil, water and air. Some bacteria even live in environment that has extreme temperatures, pH or salinity. Many of them play more **beneficial roles** in nutrient cycling, decomposition of organic matter, production of commercial industrial products like vitamins, antibiotics, etc. Wherein, some of them cause diseases and food spoilage. Ex: *Bacillus, Pseudomonas.*

2) **ARCHAEA** are phylogenetically related **prokaryotes** that are distinguished from bacteria by many features, most notably their unique ribosomal RNA sequences. Many archaea are found in **extreme environments**. Some have unusual metabolic characteristics, such as the **methanogens**, which generate methane gas. Ex: *Methanobacterium.*

3) **ALGAE** are **eukaryotes** that **contain chlorophyll** and are capable of performing photosynthesis. Algae are found most commonly in **aquatic environments**. They reproduce either sexually or asexually. Mostly they are used as food supplements. They are mainly used in the preparation of agar. Ex: *Spirulina, Gelidium.*

4) **FUNGI** are **eukaryotes**. Next to bacteria, they are the most dominant organism in the soil. In general, fungi range in size and shape from single-celled microscopic yeasts to gaint multicellular mushrooms. They possess filamentous **mycelium** composed of individual **hyphae** and reproduce either sexually or asexually by fission, budding or by means of spores borne on fruiting structures. **Unicellular fungi** like **yeast** are involved in the production of alcoholic beverages like wine and beer. **Multicellular fungi** like **molds** are useful for industrial production of antibiotics like penicillin. Ex: *Mucor, Rhizopus*.

5) **PROTOZOA** are unicellular **eukaryotes** that are usually **motile** and **lack cell wall**. Many free living protozoa function as the **primary hunters** and **grazers** of the microbial world. They can be found in many different environments and some are normal inhabitants of the intestinal tracts of animals, where they aid in digestion of complex materials such as cellulose. Some of them are parasitic and can cause diseases. Ex: *Amoeba, Paramecium*.

6) **VIRUSES** are **acellular** (non cellular) organisms that are too small and can be visualized only using electron microscopes. All are **obligate parasites** that require a living cell for reproduction. They are **pathogenic** to plants, animals and humans. At most of the cases they cause human diseases. Ex: Cauliflower mosaic virus, Cucumber mosaic virus.

1.7. IMPORTANCE AND SCOPE OF MICROBIOLOGY

Microbiology is an applied science that has great impact on genetics, biochemistry, food sciences, ecology, immunology, agriculture, medicine and many other disciplines. Despite their small size they form the largest resource for biotechnology. Various microbial genera have been used to study their genetics and molecular biology. **"*Escherichia Coli*"** is a wonderful colon bacterium that has been extensively studied by biotechnologists. They used it for cloning and Microbes play a pivotal role in human welfare majority of the microbes are useful to mankind but some of them are harmful as they cause infectious diseases in human beings, domestic animals and agricultural crops.

1. Biotechnology:

a. Microbes produce very important DNA manipulating enzymes like REN (Restriction Endo Nucleases) and Ligase. These two are used as molecular scissors and stitches in biotechnology/ Genetic Engineering.

b. Some microbes, for example: *E.coli* is used as host organism to clone desired gene for desired product.

2. Agriculture: from the point of agriculture microbes play an important role in the following aspects.

a. Some microbes can be used as **bio-fertilizers** to enrich soil fertility.

b. Some bacteria can fix inert atmospheric nitrogen known as **nitrogen fixing** bacteria. Ex: rhizobium, Azotobacter, Anabaena etc.

c. Some microbes like viruses and bacteria are used as **bio-pesticides** to protect the crop plants from pest and insect eating.

3. Industry: from the point of industry, microbes are extremely useful

a. for the production of industrial chemicals like acetic acid, lactic acid, citric acid etc. by fermentation process.

b. Microbes also find their importance in food industry and dairy industries to produce fermented food products.

c. Microbes also play an important role in the production of ethyl alcohol in brewing industry.

d. Microbes also find their importance in food and dairy industry to produce fermented food products.

4. Medicine:

a. From the point of medicine various kinds of antibiotics used to treat pathogenic diseases of man and animals are derived from microbial group called **actinomycetes.**

b. Some heat killed microbes are used as vaccine against various kinds of pathogenic microbes causing diseases.

5. Environment:

a. Microbes help to clean the environment by degrading all kinds of biodegradable waste products. Hence, the microbes are regarded as scavengers of nature.

b. Microbes play important role bio-geo chemical cycles.

c. Microbes also play an important role in the production of Bio-gas from the biological waste products.

6. Bio-remediation:

Is a method of pollution alleviation using microbes. Several bacteria and fungi are capable of decay the natural waste, toxic chemicals, heavy metals, oil spills etc.

7. Bioleaching:

When the ore contains lower metal content, it is difficult to extract them by direct smelting, in such cases some microbes (*Thiobacillus* species) are used to separate the mineral from crude ores. This process is known as **bioleaching** or microbial leaching.

1.8 BRANCHES OF MICROBIOLOGY

With the accumulation of knowledge about various aspects of microbes since the last century and has spread in to various branches. Thus the various aspects of microbiology study can be divided basically in to following branches.

1. Industrial Microbiology

- It deals with the exploitation of microbes for industrial production. Here the microbes can be considered as mini chemical factories, as they are capable of converting some raw materials into end products which have value for human use.
- Microbes have been used to produce alcohols, antibiotics and organic acids, in industrial

- scale. The study of fermentation by microorganisms has provided booster to beverage industry.
- Recently with great advances in recombinant DNA technologies, provided a better route to manipulate microbes genetically to produce new products.

2. Diary Microbiology

- It deals with the study of harmful and beneficial bacteria present in milk and milk products.
- In diary microbiology the aspects like production of (yogurt) fermented milk products.
- Pasteurization of milk and milk products can be studied. Many such fermented milk products are used in treatment of dysentery and gastro enteritis.

3. Environmental Microbiology

- It is one of the important branches of microbiology where the role of microbes in maintaining quality of environment is studied. Since microbes are found in every environment the air, water, soil and food, they influence the degradation and decay of natural wastes (bioremediation) they also influence the energy flow in ecosystem.
- The study also helps to understand freshwater and marine water and their microbes. Recently it has been shown that some genetically modified microbes can help in cleaning oil spills and this gives an added advantage to the study of environmental microbiology.

4. Food Microbiology

- It is concerned with study of role of microbes in food processing, food preservation and canning. Extensive study of microbes in relation to food products lead to characterization of microbes.
- As a result new methods have developed and old methods have been improved. This branch also provides a platform for the study of food borne microbial diseases and their control.

5. Agricultural Microbiology

- In this branch, the role of microbial activity in plants and their surroundings is studied. Many microbes like fungi, bacteria, and viruses cause many diseases in plants.

- This branch is concerned with study of nitrogen fixation activity. Use of microbes as biofertilizers, use of microbes as bio pesticides and many more aspects.

6. Medical Microbiology

- The study of pathogenic microbes, the etiology, their life cycle, physiology. Genetics, pathogenicity and control are known as medical microbiology. The integral part of medical microbiology is to understand how immune system of vertebrate protects themselves from pathogens and shows response to the pathogen.
- This branch primarily allows the study of morphological and cultural characteristic resistance nature of microbes, their diagnosis, treatment and control of infectious diseases.

7. Air Microbiology

- The branch covers the study of dispersal of pathogenic microbes through air, microbial population in air and control of air borne microbes by chemical agents, radiations, filtration and laminar air flow methods.

8. Aquatic Microbiology

- It encompass the study of microbes present in fresh water, ocean water and estuarine. This branch is of great significance that;
    - Many aquatic microbes are pathogenic to human beings:
    - Most of them are important in food chain in the ecosystem.
    - They take part in recycling processes.
    - They help in exploration of oils and minerals.

9. Immunology

- It is one of the fastest growing areas that covers the practical health problems their nature and treatments.
- It is the study of immunity against invading microbes by a host.

10. Biotechnology

- It is the most significant branch that deals with the application of biological techniques for the benefit of mankind.
- It encompasses the use of microbes for the production of drugs, fermented foods and treatment of waste.
- It also includes developing techniques for the more efficient production of specific compounds.

- It focuses on aspects such as nature of genetic material, regulation, development and function of a cell, the method of production of new microbial cells by recombinant DNA technology which are useful in industrial microbiology..

11. Exo-Microbiology

- It is branch still in its infancy, it includes explore and the study of microbes in outer space and other planets such as moon and mars.

12. Geo-chemical Microbiology

- Study of role of microbes in coal, gas and mineral formation. Exploration of oil, gas and minerals is known as geochemical microbiology.

As each branch of microbiology have got their own specialization that contribute to the development of science and technology, always microbiology are crowned as innovate, ever green branch of biology that have wider scopes for the emerging scientists to be explored.

Reference:

1. General Microbiology – Sullia and Shantharam
2. Microbiology – Prescott
3. Microbiology – Pelczar

HISTORY OF MICROBIOLOGY

1.1 EARLY HISTORY

- Ancient literature abounds with references to diseases of man but both plant and human diseases were attributed to the wrath of gods and people try to get remission from the ailments bye rituals to appease the angry gods that have been suggestions that diseases may result from invasion of the body by external contagion.
- The Romans had God for those were specific for microorganisms. The roman God of mold and mildew was "*Robigus*" and "*Robigo"* which means crop rust. (Rust is one of the plant disease caused by fungus). God Robigus was very much feared because of crop lost.
- Various and Columella - in the first century BC postulated that diseases were caused by invisible beings (Animalia minuta) inhaled and ingested.
- Kircher (1659) - reported finding minute worms in the blood of plague victims but what he observed where only blood cells.
- Babylonians were using yeast to make beer over 8000 years ago and acetic acid bacteria to make vinegar over 6000 years ago. About 5000 years ago, Persia (Now Iran) region recorded the wine making.
- The real microbiology history began when people learn to grind lenses from pieces of glass and combined them to produce magnified images

1.2 HIGHLIGHTS IN THE HISTORY OF MICROBIOLOGY

Effects of Disease on Civilization

- ✓ **Infectious diseases** have played major roles in shaping human history.
- ✓ **Bubonic Plague** epidemic of mid 1300's, the **"Great Plague",** reduced population of western Europe by 25%. Plague bacterium was carried by fleas, spread from China via trade routes and poor hygiene. As fleas became established in rat populations in Western Europe, disease became major crisis.
- ✓ **Smallpox** and **other infectious diseases** introduced by European explorers to the Americas in 1500's were responsible for destroying Native American populations. Example: In the century after Hernan Cortez's arrival in Mexico, the

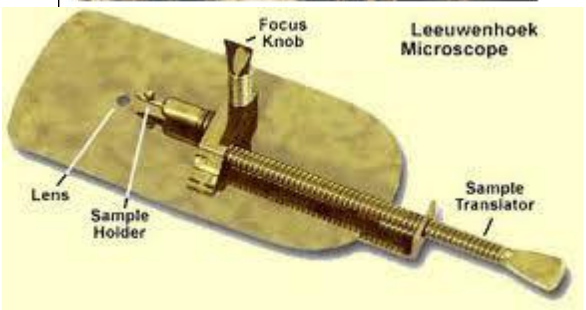
Aztec population declined from about 20 million to about 1.6 million, mainly because of disease.

- ✓ Infectious diseases have killed more soldiers than battles in all wars up to World War II. Example: in U. S. Civil war, 93,000 Union soldiers died in direct combat; 210,000 died as a result of infections.
- ✓ Until late 1800's, no one had proved that infectious diseases were caused by specific microbes, so there is no possibility of prevention or treatment.

Discovery of Microbes

Antony Van Leeuwenhoek (1632 – 1723)

- ➢ To see microbes, you need a microscope. The **first microscope** was invented by **Antony van Leeuwenhoek.**
- ➢ He was always praised as the **Father of Microbiology**. He was a Dutch merchant and his hobby was making lenses and microscopes.
- ➢ Leeuwenhoek took up lens grinding to make magnifiying glasses so he could examine fine weave of fabrics.



- ➢ He examined a diverse variety of materials such as rain water, pepper in fusion, saliva and excreta.
- ➢ His microscopes were simple microscopes composed of double convex glass lenses held between two silver plates that could magnify 50 to 300 times.
- ➢ He was the first to describe the protozoa and bacteria. He observed some bacteria from plagues of his own teeth. He named them as **animalcules (small animals)**.
- ➢ He is regarded as **Father of ''Bacteriology"** and **''Protozoology".**
- ➢ Leeuwenhoek reported discoveries to Royal Society from 1670's on, firmly established existence of microbes.

1.3 THE THEORY OF SPONTANEOUS GENERATION (ABIOGENESIS) Vs BIOGENESIS

Origin of Life Controversy

Where did microbes come from? Many believed they arose from non-living materials by the process of spontaneous generation. This concept had been suggested by Aristotle (382-322 B.C.) and other Greek philosophers in 1700's and 1800's.

From earliest times, people had believed in **spontaneous generation**—that living organisms could develop from **non-living matter**.

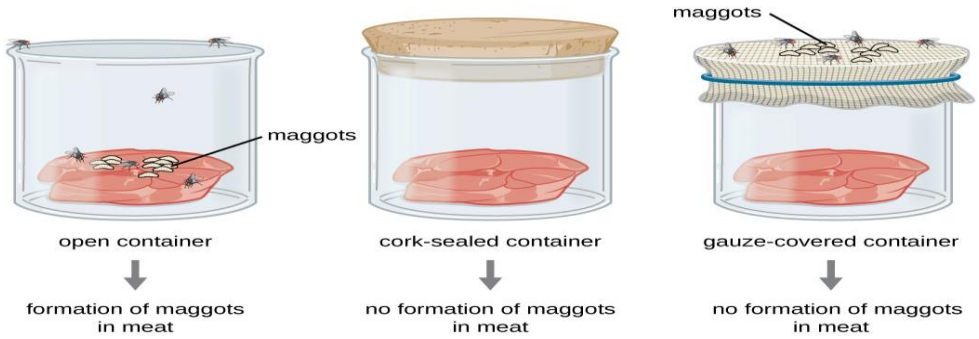
Key people in proving microbes arise from other microbes:

- ❖ Franscesco Redi
- ❖ Lazzaro Spallanzani
- ❖ Louis Pasteur

Francesco Redi (1626–1697)

He **disproved the spontaneous generation**. He carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously. He placed meat in three containers. One was uncovered, a second was covered with paper, and the third was covered with fine gauze that would exclude flies. Flies laid their eggs on the uncovered meat and maggots developed. The other two pieces of meat did not produce maggots spontaneously. However, flies were attracted to the gauze-covered container and laid their eggs on the gauze; these eggs produced maggots.

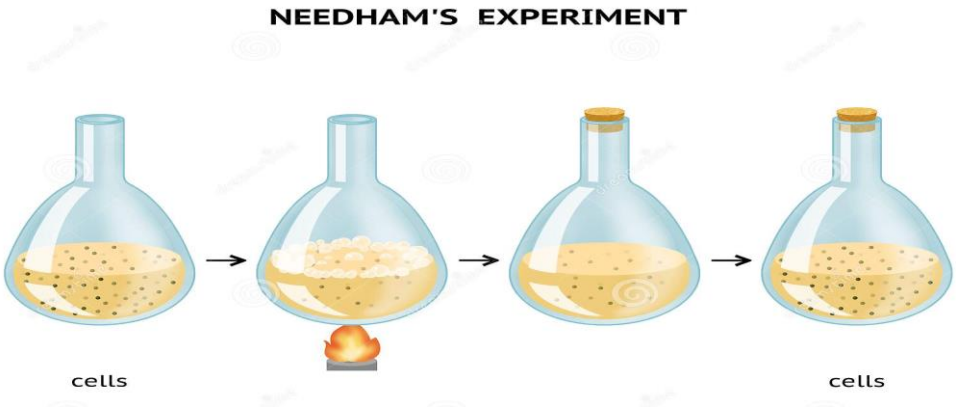
Conclusion: Thus the generation of maggots by decaying meat resulted from the presence of fly eggs, and meat did not spontaneously generate maggots as previously believed. Similar experiments by others helped discredit the theory for larger organisms.



John Needham (1713–1781)

In 1748 the English priest John Needham reported the results of his experiments on spontaneous generation. He **supported the theory of spontaneous generation**. He boiled mutton broth for short period and then tightly stoppered the flasks. Eventually many of the flasks became cloudy and contained microorganisms. He thought organic matter contained a vital force that could confer the properties of life on non-living matter.

Conclusion: He concluded that the microorganisms originated from meat.

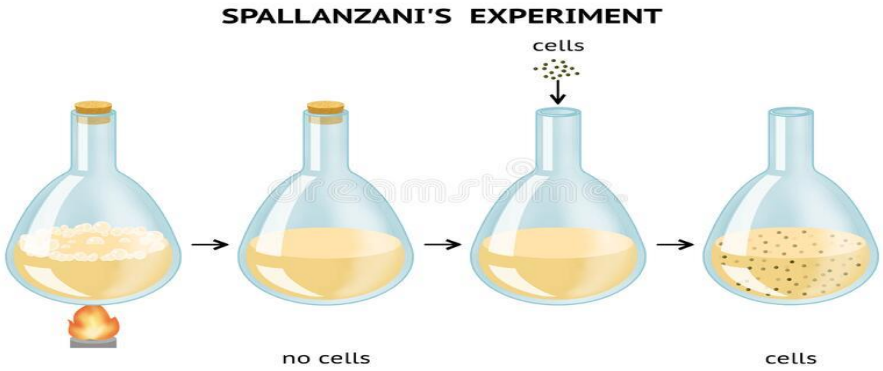


Lazzaro Spallanzani (1729–1799)

A few years later the Italian priest and naturalist Lazzaro Spallanzani improved on Needham's experimental design by boiling the broth for an hour and then sealed the flasks no microbes appeared even after a day or two.

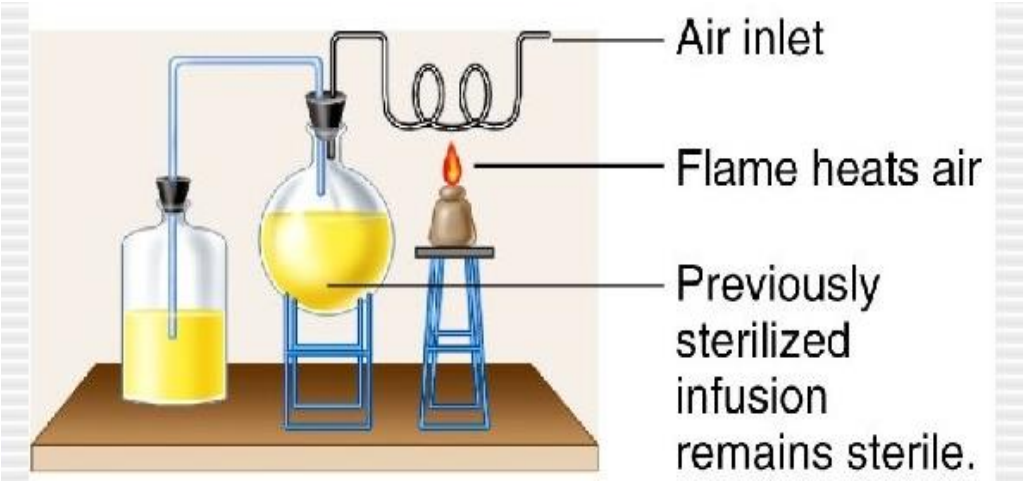
Conclusion: He concluded that air is necessary for the the generation of microorganisms in well heated broths. He **disproved the theory of spontaneous generation or abiotic origin of life** and **proposed**

the theory of biogenesis. He said that every form of life takes its origin from their parents, germ cells or seeds. This theory of biogenesis was later proved and supported by Louis Pasteur.



Theodore Schwann (1810–1882)

He stated that **air was the source of microbes** hence he allowed air to enter a flask containing a sterile nutrient solution after the air had passed through a red hot tube. The flask remained free from microbes (sterile).

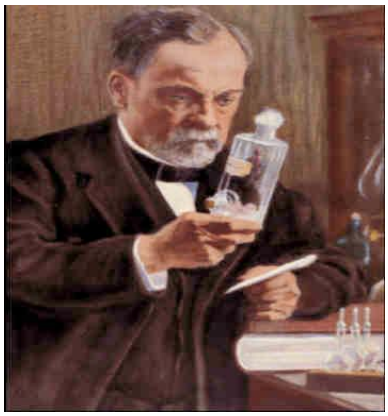


Georg Friedrich Schroder and Theodor von Dusch

They allowed air to enter a flask of heat-sterilized medium after it had passed through sterile cotton wool. No growth occurred in the medium even though the air had not been heated. This was due to filtering of organism by cotton. They introduced the **idea of using cotton plugs** for microbial culture tubes.

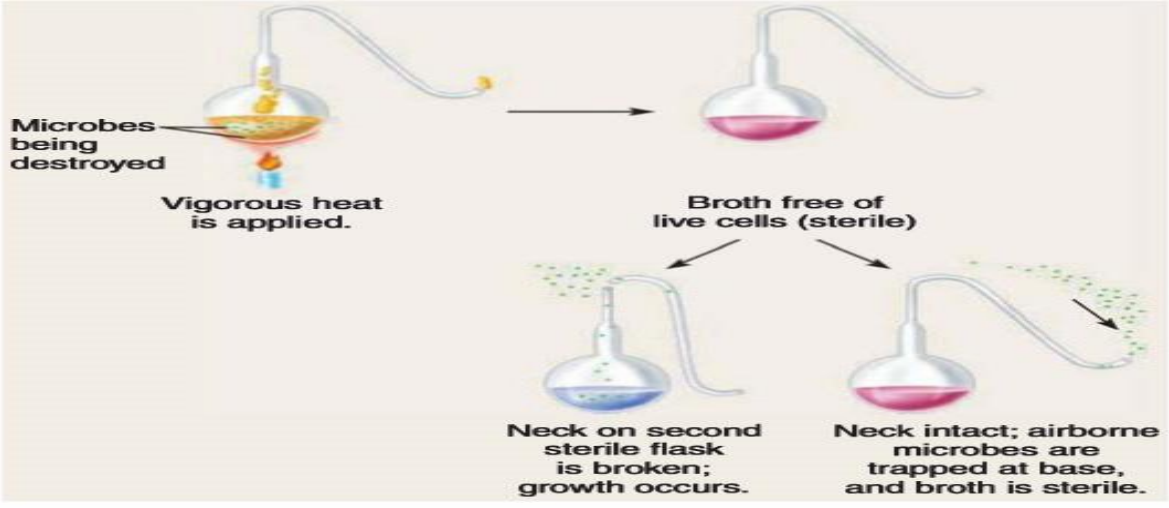
1.4 GOLDEN AGE OF MICROBIOLOGY

Louis Pasteur (1822–1895)



- He was a Professor of Chemistry at the University of Lille, France. He is considered as **"Pioneer of Microbiology",** as his contribution led to the development of Microbiology as a separate scientific discipline.
- He proved the theory of **"Biogenesis"** and disproved the "Theory of spontaneous generation" (Abiogenesis), by using **swan-neck experiment.**.
- Pasteur first filtered air through cotton and found that objects resembling plant spores had been trapped. If a piece of the cotton was placed in sterile medium after air had been filtered through it, microbial growth appeared.
- Next he placed nutrient solutions in flasks, heated their necks in a flame, and drew them out into a variety of curves, while keeping the ends of the necks open to the atmosphere. Pasteur then boiled the solutions for a few minutes and allowed them to cool. No growth took place even though the contents of the flasks were exposed to the air. Pasteur pointed out that no growth occurred because dust and germs had been trapped on the walls of the curved necks.
- If the necks were broken, growth commenced immediately. Pasteur had not only resolved the controversy by 1861 but also had shown how to keep solutions sterile.

Swan neck experiment



Fermentation

He also worked on souring of wine and beer and found that this alcohol spoilage is due to the growth of undesirable organisms (bacteria), while the desirable microorganisms produce alcohol from fruits and grains by a chemical process called **"Fermentation".** He suggested that mild heating at **62.8°C** for **30 minutes** rather that heating could destroy microorganism without ruining the taste of wine. This method is called **"Pasteurization",** now widely used in dairy units, to kill pathogenic microorganisms in milk.

Contributions of Louis Pasteur

- He was a French Biochemist, born on 27 December 1822.
- He is regarded as **''Father of Microbiology and Immunology".**
- He proposed the **''Theory of Germ Disease",** where diseases of plants, viruses, animals and human beings are caused by pathogenic microbes.
- He **disproved the theory of abiogenesis** by conducting **''Swan neck flask experiment"**.
- He discovered the presence of bacteria in the air and classified the bacteria into aerobic and anaerobic forms.
- He **coined** the term **''microbiology", aerobic, anaerobic**.
- He discovered the role of anaerobic microbes in the fermentation of sugar.
- He developed technique to prevent souring of milk and spoilage of wine. His technique is now called **Pasteurization technique**.
- He first isolated bacteria causing **cholera (Vibrio cholerae).**
- He isolated the anthrax causing bacilli from the bloods of cattle, sheep and human being. He developed **anthrax vaccine** to strengthen immunity against anthrax bacteria by injecting weakened anthrax bacteria to healthy animal.
- He discovered that parasites (protozoa) causing **pebrine disease of silk worm**. He suggested that disease free caterpillars can eliminate the disease.
- He also demonstrated the **virulence** (ability of microbe to cause disease) of bacteria
- He developed **rabies vaccine** (a killed or attenuated microbe to induce the immunity) against rabies from the brain and spinal cord of rabbit.

John Tyndall (1820–1893)

- The English physicist John Tyndall dealt a final blow to spontaneous generation in 1877.
- He designed a special chamber to free the dust in the air and kept the sterile broth in the chamber. No microbial growth was observed when a sterilized broth was kept in the chamber. Thus, he proved that dust in the air carried the germs and this is the source for the growth of microorganisms and not the spontaneous generation.
- He also provided the evidence for the existence of **heat-resistant forms of bacteria**.
- He also developed a sterilization method **"Tyndallization",** also called as **intermittent or fractional sterilization**. The subsequent heating and cooling by steam for 3 days will remove the germs and their spores. Heating at 100^{o}C kills the vegetative cells. The spore forms are killed on subsequent heating upon germination of spores.
- Working independently, the German botanist **Ferdinand Cohn** (1828–1898) discovered the existence of **heat-resistant bacterial endospores.**

1.5 GERM THEORY OF DISEASE

- **Pasteur –** demonstrated microbe could be a cause of disease that spoil the wine and make the body sick.
- **Girolamo Fracrstro –** disease caused by minute seed ans spread from person to person.
- **Agastino Bassi –** demonstrated silkworm disease caused by fungal infection.

Joseph Lister (1827-1912)

- He was a professor of surgery at University of Glasgow and Edinburg and later at King's College, London. He was deeply **interested in the prevention of post-operative sepsis.** He was attracted by Pasteur's germ theory of disease and **concluded that sepsis or wound infection may be**

due to microbial growth, derived from the atmosphere.

- He successfully prevented post-operative sepsis by introducing antiseptic techniques. He sprayed carbolic acid (Phenol) in the operation theatre. He also applied dressings soaked in carbolic acid on wounds. As a result, there was a marked reduction of post-operative sepsis, wound inflammation and suppuration. It saved millions of lives from the death due to wound infections.
- He was the first to introduce aseptic technique for the control of microbes by the use of physical and chemical agents. Hence, he is considered as the **"Father of antiseptic surgery".**

Robert Koch (1843-1912)

- He was a German country Doctor who later became the Professor of hygiene and Director of institute of infective diseases at Berlin.
- He postulated the role of bacteria in causing disease.
- He **discovered rod shaped organisms (Bacillus anthracis)** in the blood of animals that died of anthrax. He experimentally obtained the anthrax organisms in pure culture on a depression slide by inoculation of infected blood into the aqueous humour of a bullock's eye. He observed multiplication of bacteria and spore formation. He injected these spores into mice and reproduced the disease. He found that in certain conditions, the anthrax bacillus forms spores, which can survive on earth for years. He passed anthrax bacilli, from the blood of an infected animal, from one mouse to another through twenty generations, and found that they bred true. He worked out its life-history.
- He **discovered Vibrio cholerae,** the **causative agent of cholera disease**.
- He **discovered tubercle bacillus (Mycobacterium tuberculosis)** which is popularly called as **Koch's bacillus**. He injected tubercle bacilli into laboratory animals and reproduced the disease, satisfying all Koch's postulates.
- He **discovered "Old Tuberculin"** (protein extracted from tubercle bacilli) used in the treatment of tuberculosis**.** Koch noted that when tubercle bacilli or its

protein extract was injected into a Guinea-pig already infected with the bacillus, an exaggerated reaction took place and the reaction remains localized. This is popularly called **"Koch Phenomenon"** and it is a demonstration of **cell mediated immunity**. The tuberculin test is based on Koch's phenomenon.

- Koch did a series of experiments to fulfill the criteria laid by his teacher Henle to establish the causative role between a particular microorganism and a particular disease. They are popularly known as **Koch's postulates (Henle-Koch's Postulates)**. They are:

  1. A specific organism should be found constantly in association with the disease.

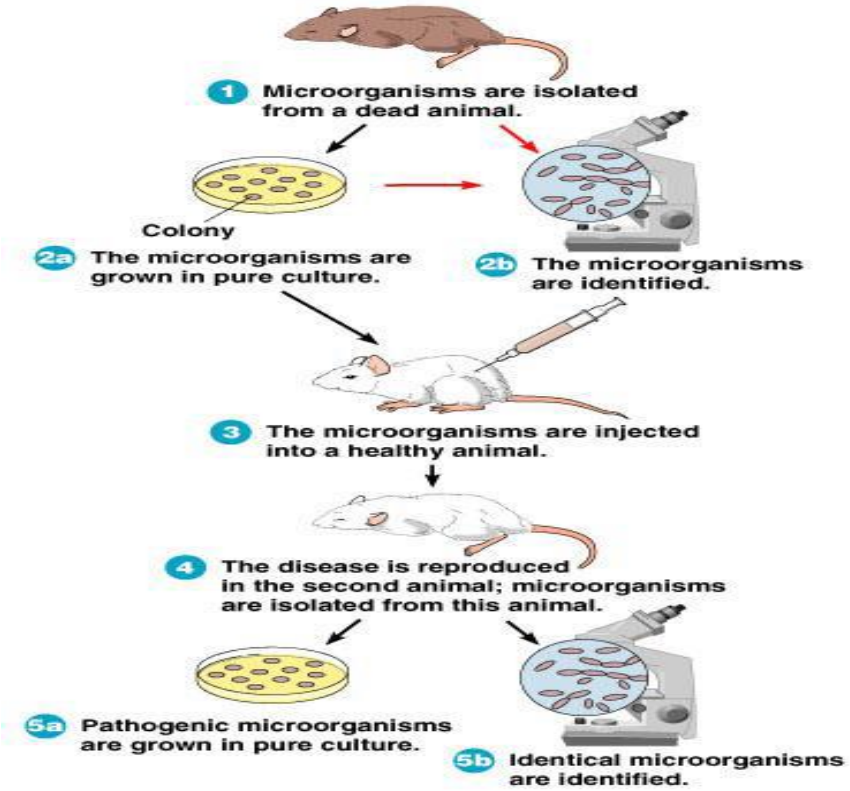
  2. The organism should be isolated and grown in a pure culture in the laboratory.

  3. The pure culture when inoculated into a healthy susceptible animal should produce symptoms/ lesions of the same disease.

  4. From the inoculated animal, the microorganism should be isolated in pure culture.

  5. An additional criterion introduced is that specific antibodies to the causative organism should be demonstrable in patient's serum.

- He was **awarded Nobel Prize of medicine** in 1905, formulating principles regarding diseases called **''Koch Postulates".**



1.6 LABORATORY TECHNIQUES AND PURE CULTURES

- In order to study the characteristics of a particular species it is first necessary to separate it from all other species. The growth of a mass of cells of the same species in a test tube is called **pure culture**.

Joseph Lister

- He **first obtained pure cultures of bacteria by serial dilution technique**.
- He diluted milk containing a mixture of bacteria until a single organism was obtained. After incubation, bacteria that grown were of a single kind, identical to the parent cell. Lister named the organism as **''Bacterium lactis''.**

Robert Koch

- He perfected many bacteriological techniques and known as **"Father of Practical Bacteriology" or ''The Father of Microbial Techniques''.**
- He introduced **staining techniques**. He prepared dried bacterial films (Smears) on glass slides and stained them with aniline dyes. Whereby it increases the contrast of the cells, hence individual cells could be seen more clearly with the microscope.
- He **developed pure culture techniques** by introducing **solid media**.
- He first cultured the bacteria on solid fruits and vegetables such as slices of boiled potato but many bacteria did not grow on such substrates. Then he tried gelatin as a solidifying agent and succeeded in developing solid culture media in order to obtain isolated growth of organisms known as **colonies**.
- **Disadvantage of gelatin** – Gelatin protein was digested by many bacteria that produce gelatinase hydrolyse and it melts when temperature rises above 25°C which is below the optimum growth temperature for pathogens.
- Then he replaced gelatin by agar in 1883-84 on the recommendation of F.E. Hesse.

Fannie Eilshemius Hesse (1883)

- Koch's student wife who had gained experience with the characteristics of agar in the process of making jelly. She suggested the use of agar to Hesse.

- Then agar is used in the preparation of solid bacteriological media as a solidifying agent in microbiological laboratories which is still frequently used.
- The agar-agar can be obtained from dried sea weeds (Gelidium Sp.) which is totally inert with no nutritive value, solidifies at 45°C and melts at 90°C, and was found to be most suitable solidifying agent in the preparation of culture media.
- Koch isolated bacteria in pure cultures on these solid media. It revolutionized bacteriology.

1.7 IMMUNIZATION / IMMUNOLOGY

- **Immunity** - Body develop resistance to further attacks by pathogen is called immunity.
- Immunity can be induced by **vaccination**.

Edward Jenner

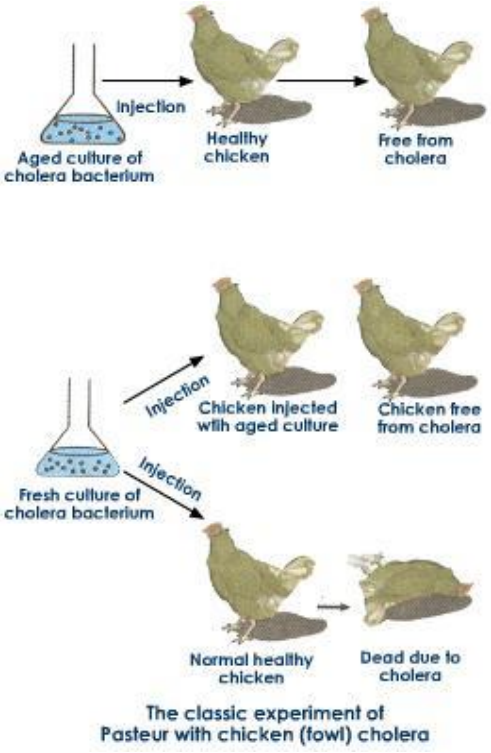
- He was the first to initiate scientific approach to **immunisation** and developed **smallpox vaccine**.
- In 1798, He identified that milkmaids who had contracted the mild disease cowpox were subsequently immune to severe smallpox disease.
- He inoculated the fluid from cowpox pustules to an 8 year old boy to protect him from smallpox. Then intentionally infected the child with smallpox, as he predicted, the child did not contracted the disease smallpox.
- The **process of vaccination** was introduced by Jenner and according to WHO Jennerian vaccination has eliminated small pox totally from the human population.

Louis Pasteur

- He is the **Father of Immunology**
- He demonstrated the principle of **immunisation**. He discovered attenuated vaccine. He called the attenuated cultures as **''Vaccine''** (**Vacca means cow**) to honour Jenner's work with cowpox inoculation.
- Demonstrated the **virulence** (ability to cause disease) of bacteria.
- He worked on common diseases such as **Pebrine** (disease of silkworm), **Chicken cholera** (disease of fowls), **Anthrax** (disease of cattle) and **Rabies.**

a) Pasteur work on chicken cholera

- He succeeded in growing the bacterium **(*Pasteurella aviseptica*)** that causes **fowl cholera** in culture.
- Injected the chickens with this cultured bacterium developed fatal cholera and died.
- After summer vacation, he injected the chickens with old bacterial culture. The chickens became ill but they recovered.
- Then he grew a fresh culture of bacterium and injected into the recovered chickens, now the chickens were completely protected from the disease.
- He hypothesised and proved that **aging had weakened the virulence (ability to produce the disease) of the pathogen such attenuated strain (decreased virulence) retained their capacity to induce antibodies.** These antibodies protect the host against disease or infection.
- He developed the **First attenuated vaccine.**



The classic experiment of Pasteur with chicken (fowl) cholera

b) Pasteur work on Anthrax vaccine

- ***Bacillus anthracis*** was cultivated at 42˚C (high temperature)
- Inoculated into sheep
- Sheep did not develop the disease anthrax
- At normal temperature at 37˚C these bacteria were highly pathogenic
- He concluded that the pathogenic bacteria lost their virulence on cultivation at high temperature.

- **Attenuation -** the process of weakening or reducing the virulence of pathogenic organisms without losing the capacity to induce immunity.

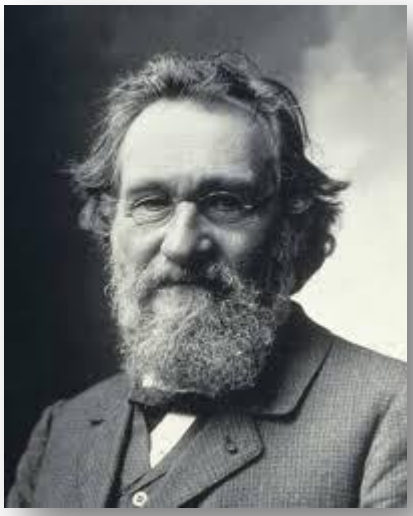
c) Pasteur work on Rabies vaccine

- Rabies causing ***Rabdo virus*** is passed through rabbit many times and vaccine was prepared.
- He collected saliva from rabid dogs and injected into normal healthy rabbits.
- An extract was prepared from the infected rabbit spinal cord.
- Dried for several days at room temperature.
- This extract contains attenuated viruses.
- He proved that passing the microorganism into an unnatural host also reduces its virulence.
- Thus the microorganisms lose their capacity to produce serious disease but retained the capacity to induce immunity.

Kitasato

- He devised a method of producing immunity to infections caused by organisms by injecting their toxins (poisons) into animals so that an antitoxin would develop.

Elie Metchnikoff (1845-1916) (Russian Zoologist)



- He discovered the importance of cells in immunity and **phagocytosis.**
- While observing the transparent starfish larvae, he could see some motile cells. Later he observed some cells (leukocytes) in **Daphnia (water fleas)** that ingest and destroy yeast that are pathogenic to water fleas. These cells are called **phagocytes** and the process is called **Phagocytosis**.
- He also suggested that **inflammation might be a protective process rather than a destructive process.**
- In 1908, he shared his **noble prize with Ehrlich** for his contribution to **immunity**.
- He discovered **cell mediated immunity**. His work on phagocytic cells formed the basis of cellular immunity.

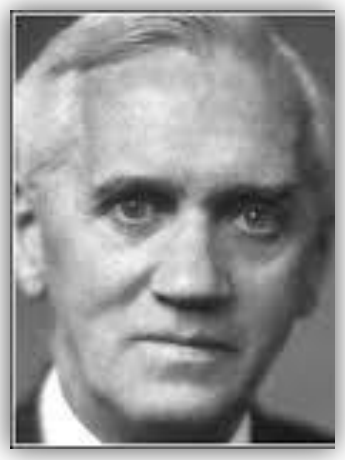
1.8 CHEMOTHERAPY AND WONDER DRUGS

- Scientist initiated the work to search the substances that kill pathogens without harming the patient.

Paul Ehrlich (1854-1915)

- He was a German Bacteriologist, who pioneered the technique of **chemotheraphy in medicine**.
- He introduced **Trypan red** which is active against ***Trypanosoma*** which causes **African sleeping sickness**. This dye with antimicrobial activity was referred to as **magic bullet**.
- He discovered that certain tissues have a specific affinity and the organisms causing diseases could be selectively killed with chemical drugs. This led him to produce **"arsphenamine"** (an arsenic compound), the **first synthetic drug**, which destroyed the syphilis microbe in the body.

Alexander Fleming (1928)



- Flemming was associated with two major discoveries - **lysozyme** and **penicillin**.
- **In 1922, he discovered lysozyme** by demonstrating that the nasal secretion has the power of dissolving or lysing certain kinds of bacteria. Subsequently, he showed that lysozyme was present in many tissues of the body such as saliva, nasal mucous etc.

- **In 1929, Flemming made an accidental discovery that the fungus *Penicillium notatum* produces an antibacterial substance which he called penicillin.** Flemming was culturing Staphylococci in Petridishes and some of his cultures were contaminated with a mold, subsquently identified as *Penicillium notatum*.
- Around the mold colony, there were clear zones, where Staphylococci disappeared. Flemming attributed this to the production of an antibacterial substance by the mold. Flemming cultured the fungus *Penicilium notatum* in broth cultures, filtered the fungal mat and obtained the penicillin in soluble form in the culture filtrate.
- In 1940, **Howard Florey** and **Ernst Boris Chain** demonstrated its antibacterial action in vivo, and produced large quantities of penicillin in pure form.
- In 1945, Flemming, Florey and Chain shared the **nobel prize** in physiology and medicine for the discovery of penicillin.

Selman A Waksman (1945)

- He is an American microbiologist. He isolated ***Thiobacillus thiooxidans*** which is an important discovery before he identified **Streptomycin antibiotic** from soil bacterium.
- In 1939 Waksman and his colleagues undertook a systematic effort to identify soil organisms producing soluble substances that might be useful in the control

of infectious diseases, what are now known as **antibiotics**. Within a decade ten antibiotics were isolated and characterized. **Three of them with important clinical applications** - **Actinomycin** in 1940, **Streptomycin** in 1944, and **Neomycin** in 1949. Eighteen antibiotics were discovered under his general direction.

1.9 SOIL MICROBIOLOGY

Sergey Winogradsky (1856 – 1953)

- He developed **Enrichment Culture Technique for the isolation of Beggiatoa sp**.
- He explained the **chemoautotrophic nature of bacteria** and initially called them as **"anorgoxydants".**
- He also identified the **process of Nitrification**; isolated the nitrifying bacteria ***Nitrobacter*** and isolated ***Azotobacter chroococcum*** and proved its nitrogen fixing capacity.
- Due to his varied contributions in soil microbiology he is considered as the **"Father of Soil Microbiology".**
- Additionally, he identified the **Chemolithotrophic nutrition of soil bacteria** and also discovered **anaerobic nitrogen fixing bacterium *Clostridium pasteurianum.***

Martinus W. Beijerinck (1851-1931)

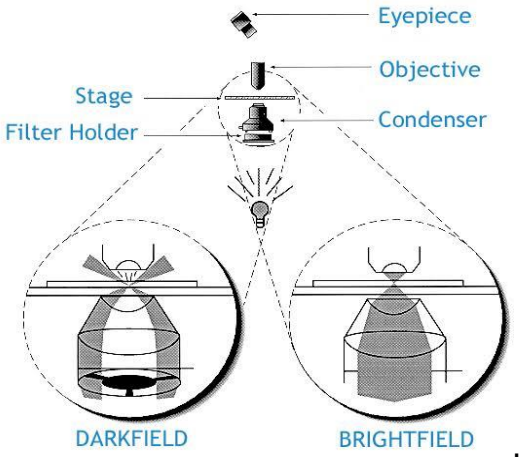
- He developed the **enrichment culture technique**, simultaneously with Sergey Winogradsky, which permits the isolation of highly specialized microorganisms.
- Beijerinck cultivated and isolated ***Bacillus radicicola*** (later named as ***Rhizobium leguminosarum***), a bacteria that fixes free nitrogen and causes the formation of nodules on the roots of Legumes.
- He also characterized ***Azobacter*** as nitrogen-fixing, and isolated the new genus, ***Aerobacter***.
- Isolated sulphur reducing bacteria and sulphur oxidizing bacteria from soil.
- In studying tobacco mosaic disease, he concluded that the filterable pathogen was a contagium vivum fluidum, a term coined to convey his concept of a living infectious agent in a fluid (noncellular) form.

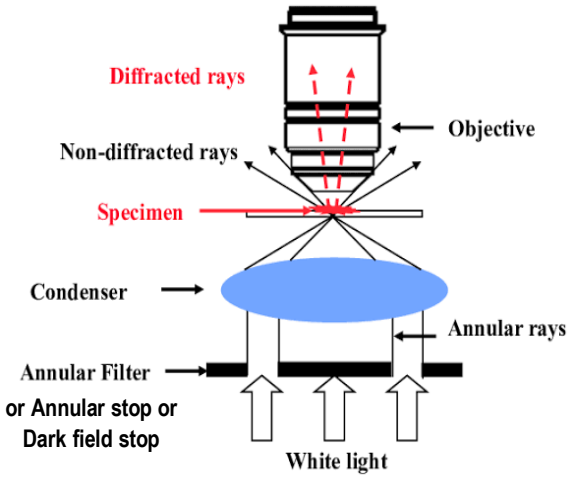
DARK-FIELD MICROSCOPY

This is similar to the ordinary light microscope; however, the condenser system is modified so that the specimen is not illuminated directly. The condenser directs the light obliquely so that the light is deflected or scattered from the specimen, which then appears bright against a dark background. **Live, unstained specimens** can be observed more readily with dark-field than with bright-field microscopy.

Working Principle

In a dark field microscope the **object is brightly illuminated against a dark background**. This is accomplished by equipping a light microscope with a **special kind of condenser with a dark field stop,** which is an opaque disc obstructing the path of light from the light source centrally, but allowing a peripheral ring of light. Thus, the condenser transmits a hollow cone of light from the light source. This cone of light converges on the object and diverges from there again as an inverted hollow cone. Thus, no light enters into the objective and the background remains dark in the absence of any object. If microbial cells are present some of the light rays are scattered (diffracted) by them, these diffracted rays enter into the objective and the object appears bright against the dark microscopic field.





Uses of Dark-Field Microscopy

Dark-field microscopes are used in the microbiology laboratory for the following purposes,

- ✓ Used to observe living and unstained cells.
- ✓ It is more useful in examining external details, such as outlines, edges, grain boundaries and surface defects than internal structure
- ✓ Used to identify bacteria which is thin and distinctively shaped.

- ✓ Used to identify bacteria which are thin and distinctively shaped such as spirochetes (cannot be seen by light microscopy because of their thin dimensions) such as *Treponema pallidum* (syphilis), *Borrelia burgdorferi* (lyme borreliosis) and *Leptospira interrogans* (leptospirosis) in clinical samples.
- ✓ To observe microbial motility; tufts of bacterial flagella can often be seen in unstained cells by dark-field or phase-contrast microscopy
- ✓ To observe internal structure in larger eukaryotic microorganisms such as algae, yeasts, etc.
- ✓ Used to observe the blood cells.
- ✓ A stereo dark-field microscope used to observe the shrimp or other invertebrates.

Advantages of Dark-Field Microscopy

- Resolution by dark-field microscopy is somewhat better than bright-field microscopy
- Improves image contrast without the use of stain, and thus do not kill cells.
- It is useful for those specimens that are transparent and absorb little or no light.
- Direct detection of non-culturable bacteria present in patient samples.
- No sample preparation is required
- Requires no special set up, even a light microscope can be converted to dark field.
- Marine organisms such as algae, plankton, diatoms, insects, fibers, hairs, yeast, and protozoa can observe under a dark-field microscope.
- Dark-field microscope can use to study for minerals and crystals, thin polymers, and some ceramics.

Limitations of Dark-Field Microscopy

- Necessity to examine wet, moist specimens containing living organisms very quickly, because visualization of the moving bacteria is essential to detection.
- An intense amount of light is required for a dark field microscope to work.
- The sample must be very strongly illuminated, which can cause damage to the sample.
- Besides the sample, dust particles also scatter the light and appear bright.

- Sample material needs to be spread thinly, dense preparations can grossly affect the contrast and accuracy of the image.

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PHASE-CONTRAST MICROSCOPE

Introduction

In a compound microscope the object is viewed due to **differences in colour intensity** of a specimen. To create the colour intensity the specimen is stained with suitable dyes which will impart specific colour in a compound microscope. The contrast is obtained when the light rays pass through a stained specimen because different stains absorb different amounts of light. These differential absorption properties of stained specimen modify the intensity or amplitude of the light waves transmitted by different regions of the cells. This ultimately creates contrast in the image does staining is essential to create contrast in a bright field microscope. Moreover the unstained specimen cannot be observed properly through a bright field microscope

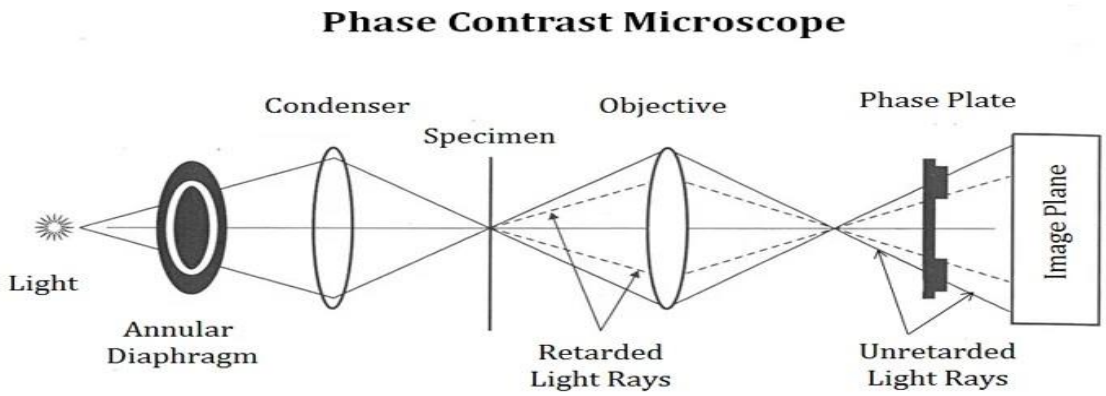
The phase contrast microscope is used to visualise unstained living cells. Most of the stains or staining procedures will kill the cells. Phase contrast microscopy **enables the visualisation of living cells and cellular processes**. The phase contrast microscope was developed by **Zernike** in 1930's. He was awarded **Nobel Prize in physics in 1953**.

Working principle

The phase contrast microscope is based on the principle that small phase changes in the light rays induced by differences in the thickness and refractive index of the different parts of an object can be transformed into differences in brightness for light intensity. i.e. it translate the **invisible phase shift into to visible difference of intensities**. The **phase changes are not detectable to human eye** whereas the **brightness or light intensity can be easily detected** by the human eyes.

The condenser of a phase-contrast microscope has an **annular stop** an opaque disk with a thin transparent ring that **produces a hollow cone of light**. As this cone passes through a cell some light rays are bent due to variation in density and refractive index within the specimen and are retarded by 1/4 wavelength. The **deviated light is focused to form an image of the object.** The **undeviated light rays strike a phase ring in the phase plate,** special optical disks located in the objective, while the deviated rays miss the ring and passed through the rest of the plate. The undeviated light which

strikes the phase ring gets advance by 1/4 wavelength when passing through this ring. The **deviated and undeviated waves become 1/2 wavelength** to each other and will cancel each other to come together to form an image. The **background** formed by **undeviated light is bright** while the **unstained object** appears **dark and well-defined**.



Components of phase contrast microscope

The phase contrast microscope is similar to the bright field microscope in its optical components. It has two additional components **i) Sub stage annular diaphargm and ii) phase plate**

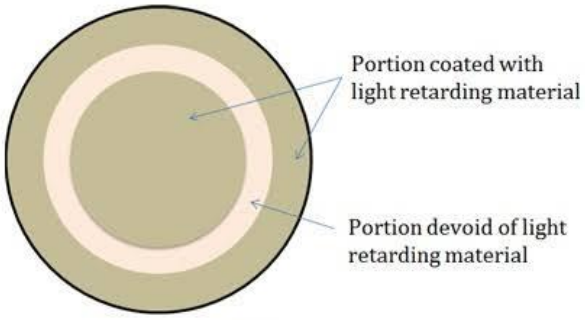
i) Sub stage annular diaphragm

It is located below the sub stage condenser of the microscope. The sub stage diaphragm helps to create a narrow, hollow cone or ring of light to illuminate the object.

ii) Phase plate

It is also called as **diffraction plate or phase retardation plate**. It is located at the back focal plane of the objective lens. The phase retarding components are coated

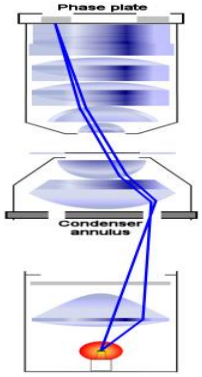
on this plate. The phase plate is a **transparent glass disc with one or few channels**. The channel is coated with the material that can absorb light but cannot retard. The other portion of the phase plate is coated with **light retarding materials** such as **magnesium fluoride**. Phase plate helps to **reduce the phase of the incident light.**



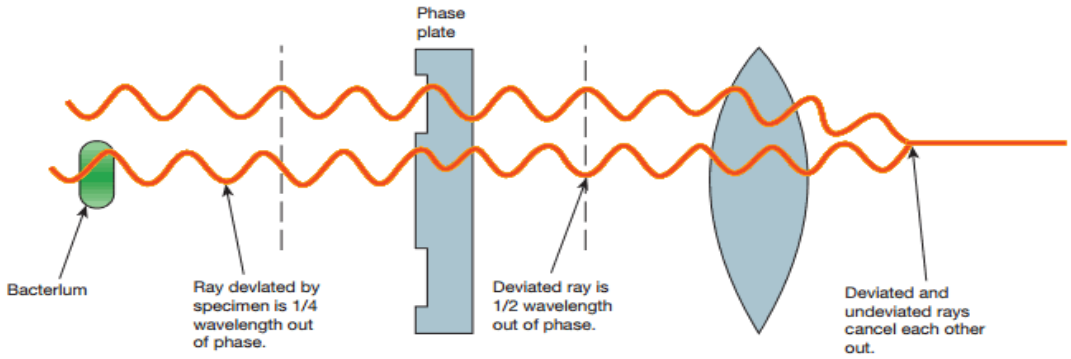
Phase Plate

Unstained cells cannot create contrast under the normal microscope. However when the light pass through an unstained cell it encounters regions in the cells with different refractive indexes and thickness. When light rays pass through an area of high refractive index, it deviates from its normal path and such a light ray experiences phase change or phase retardation. Light rays pass through the area of less refractive index remine undeviated (no phase change).

The difference in the faces between the retarded (deviated) and un-retarded (undeviated) light rays is about 1/4 of original wavelength. Human eyes are not able to detect this minute changes in the phase of light and thus, such small phase changes do not create any contrast in the image. Phase contrast microscope has special devices ie. **annular diaphargm and phase plate** which **convert this minute phase changes into amplitude changes or brightness changes**, so that a contrast difference can be created in the final image, this contrast difference can be easily detected by our eyes.



In order **to get contrast**, the **diffracted waves have to be separated from the direct waves**. This separation is **achieved by the substage annular diaphragm**. The annular diaphragm illuminates the specimen with a hollow cone of light. Some rays (direct rays) pass through the thinner region of the specimen and do not undergo any retardation and they directly enter into the objective lens. The light rays passing through the denser region of the specimen get retarded and they run with delayed phase than the undeviated rays. The retardation of the phase of light is about 1/4 of the lambda of the incident light. Both the retarded and unretarded light has to pass through the phase plate kept on the back focal plane of the objective to form the final image. The phase plate is designed and positioned in such a way that the retarded light rays will pass through the area of phase plate where light retarding materials are coated. When the 1/4 retarded light is passed through this region of phase plate, it is further retarded by 1/4. With this final change or retardation will be ¼ λ + ¼ λ = ½ λ. The un-retarded phase will pass through the channels of the phase plate and their phase is not altered by the phase plate.



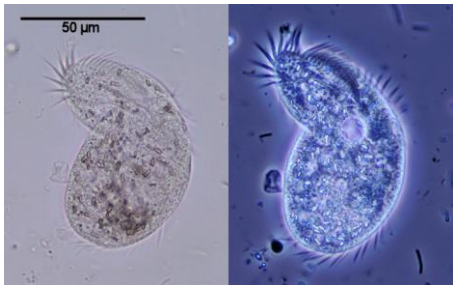
Production of contrast in phase contrast microscopy by phase plate

Applications

- Used to visualise live, unstained cells.
- Used to view various cell organelles such as mitochondria nucleus and vacuoles.
- Helps to study the cellular events such as cell division phagocytosis cyclosis.
- Used to visualise all types of cellular movements such as chromosome and flagellar movements.
- It enables the study of membrane permeability of cells and different organelles.
- It is extensively used to observe living cells in tissue culture to monitor their growth.

Advantages

- ✓ Provide the clear image of unstained cells.
- ✓ Avoid damages off the shelf due to chemical preparation and staining.
- ✓ Provide high contrast images highlighting the fine details of the cells.



- ✓ The optical construction is simple by adding phase condenser and face objective
- ✓ Enables prolonged observation of living cells without losing the viability of the cells
- ✓ Live cell imaging and live process monitoring are possible at affordable cost

Disadvantages

- ❖ It produces a bright halo around the images the formation of the halo is due to the partial or incomplete separation of direct and deviated rays.
- ❖ It is only useful for viewing individual cells or thin layer of cells.

References

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FLUORESCENCE MICROSCOPE

Introduction

Fluorescence microscopy has become one of the most powerful techniques in biomedical research and clinical pathology. **August Köhler** investigated fluorescence microscopy in 1904. **Albert Coons and N.H. Kaplan** developed the **fluorescein isothiocyanate (FITC) immunofluorescence technique** (attaching a fluorochrome to an antibody made against a specific protein) in 1950. Today, the localization of molecules by immunofluorescence is one of the most powerful techniques in light microscopy. In 1991, **B.J. Trask** described a method of fluorescently labeling specific sequences of DNA. This method is known as **fluorescence in situ hybridization (FISH).** In 1992, **D.C. Parsher *et al.*** cloned the gene that codes for the **"green fluorescent protein" (GFP).** The gene is from a chemiluminescent jellyfish. Using biotechnology methods the gene is fused with a host gene of interest and this chimera is transfected into the host genome. The resulting protein that the cell produces is fluorescent. This work has been extended by **Rodger Tsien** into a host of different colors and adapted to a vast array of biological techniques.

Autofluorescence

Some specimens naturally fluoresce when illuminated by a proper wavelength of light. This phenomenon is called **autofluorescence** or **primary fluorescence.** Autofluorescence is often a problem in fluorescence microscopy.

Autofluorescence Compound	Fluorescing Color
Ascorbic Acid	Weak yellow
Carotene (provitamin A)	Intense golden yellow
Pyridoxine (B4) hydrochloride	Intense violet white
Chlorophyll	Intense blood red

Fluorescent Stains or dyes or Fluorochromes

M. Haitinger in 1933 was the first to stain histological specimens with fluorescent dyes called **Fluorochromes**. The fluorochromes absorb light energy from excitation light and fluoresce brightly. Many fluorescent dyes selectively stain various tissue components. The fluorescence that results from this method of staining is **secondary fluorescence**.

Immunofluorescence

Albert H. Coons and N. H. Kaplan were the first to attach a fluorescent dye to an antibody, and this antibody subsequently used to localize its respective antigen in a tissue section.

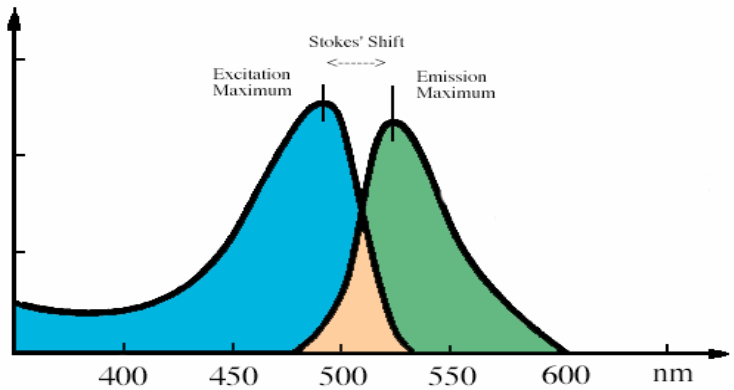
Fluorochrome	Excitation max	Uses
DAPI (Diamidino-2-phenyl indole)	358/461nm	Stains DNA; fluoresces green; binds to A-T rich regions of ds DNA
FITC (Fluorescein isothiocyanate)	495/517nm	Often attached to antibodies that bind specific cellular components; fluoresces green
Rodamine	550/573nm	Often attached to antibodies that bind specific cellular components; fluoresces red

Stokes' law.

The color of the emitted light has a longer wavelength than the color of the exciting light, this relationship is known as **Stokes' law.**

Fluorochromes	Excitation light	Emmisson light
FITC	Blue light	Green light
Rhodamine	Green light	Red light

Fluorescent substances are excited by a range of wavelengths known as their **absorption spectrum**. They also emit a range of wavelengths known as their **emission spectrum**. For any fluorescent substance the two spectra will show an absorption (excitation) maximum and an emission maximum and some portions of the spectra will usually overlap. The difference between the absorption maximum and emission maximum is the **Stokes' shift.**



The absorption and emission spectra for FITC in aqueous solution.

Types of Fluorescence Microscopes

There are two types of fluorescent microscopy

i. **Diascopic Fluorescence**
ii. **Episcopic or epifluorescence microscope**

Epifluorescence microscope

It the most commonly used fluorescence microscopy. In epifluorescence microscopy, the excitation light comes from above the specimen through the objective lens. This is the most common form of fluorescence microscopy today. This type of fluorescence microscopy became feasible with the invention of the dichroic mirror (chromatic beam-splitter) by **E.M. Bromberg in 1953**.

Advantages of epifluorescence microscope over Diascopic Fluorescence

1. High NA objectives are used at their full aperture, therefore the expected resolution is much better, and images are brighter at high magnification.
2. The invention of the **epi-illumination filter cube by J. S. Ploem** in 1970 has made it easy to interchange filter combinations using the episcopic apparatus.
3. It is easy to combine the fluorescent image with a transmitted light image of the specimen.

Components of fluorescent microscope

i) Light source

Light sources for fluorescence microscopy, Field Condenser must produce light within the absorption region of the fluorochrome(s) being used and the intensity of the light should be high. Several light sources are available.

- **Tungsten halogen lamps** can be used for FITC.
- **High-pressure mercury lamps** are a common source since they produce radiation in the UV as well as the visible spectrum (it is not suitable for all fluorochromes).
- **High-pressure xenon lamp** offers an alternative to the Hg lamp, but it has low emission in the UV.
- **CSI lamp,** this is a metal-halide arc lamp.

ii) Filter

Optical filters pass only selected wavelengths of light that is necessary in fluorescence microscopy. Several types of filters accomplish the excitation and emission in fluorescence microscopy. Each type is characterized by the wavelengths and intensity of light that it transmits.

Excitation filter

Transmits only the desired wavelength of excitation light. An excitation filter must select wavelengths of light from a suitable light source that fall in the maximum absorption region of a fluorescent dye.

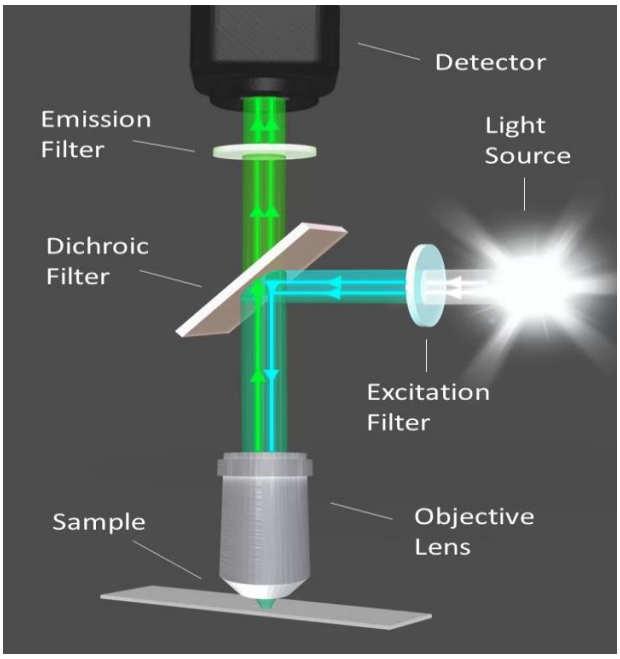
Emission filter or Barrier filter

An emission filter allows the emitted light of longer wavelength to pass through. It blocks out any residual excitation wavelengths which creates dark background.

iii) Dichroic mirror

A special type of filter is the **dichroic mirror or chromatic beam-splitter.** This **interference filter** will reflect light of shorter wavelengths (i.e. the excitation light) but allows the light of longer wavelengths to pass through. Dichroic mirrors have very specific reflection and transmission wavelength characteristics.

The optical set-up of fluorescent microscope



When fluorescent molecules absorb radiant energy, they become excited and later release much of their trapped energy as light and returns to a more stable form. Any light emitted by an excited molecule will have a **longer wavelength (lower energy)** than the radiation originally absorbed. The fluorescence microscope exposes a specimen to UV, violet or blue light and forms an image of the object with the resulting fluorescent light. The objective lens of epifluorescence microscope also acts as a condenser. A **mercury vapour arc or other source** produces an intense beam of light that passes through an exciter filter. The **exciter filter** transmits only desired wavelength of excitation light. The excitation light is directed down the microscope by a special mirror called **dichromatic mirror**. It reflects the light of shorter wavelengths (i.e. the excitation light) but allows the light of longer wavelengths to pass through. The excitation light continues down, passing through the objective lens to specimen, which is stained with fluorochromes. The fluorochromes absorbs light energy from the excitation light and fluoresce brightly. The emitted fluorescent light travels up through the objective lens into the microscope. The emitted florescence light has a longer wavelength, it passes through the dichromatic mirror to a **barrier filter**, which blocks out any residual excitation light. Finally, the emitted light reaches the eyepiece.

Application of Fluorescence Microscope

- The Fluorescence microscope has become an essential tool in **medical microbiology** and **microbial ecology**.
- Bacterial pathogens can be identified after staining them with fluorescent or specifically labeling them with fluorescent antibodies using **immunofluorescence** procedures. **eg. *Mycobacterium tuberculosis***.
- In ecological studies, the Fluorescence microscope is used to observe microorganisms stained with Fluorochrome-label probes or Fluorochromes that bind specific cell constituents.
- **To visualize photosynthetic microbes**, as their pigments naturally fluoresce when excited by light of specific wavelengths.
- **To distinguish live and dead bacteria** by the color they fluoresce after treatment with a mixture of stains.
- Another Important use of the fluorescence microscope is the **localization of specific proteins within the cell.**

Advantages of Fluorescence Microscope

- It is used to study the dynamic behaviour exhibited in live-cell imaging.
- It can trace the location of a specific protein in the cell.
- Due to the presence of higher sensitivity, it can detect the 50 molecules per cubic micrometer.
- It allows multicolor staining of the specimen.

Disadvantages of Fluorescence Microscope

- During the process of photobleaching, the fluorophores lose their ability to fluoresce.
- Fluorescent molecules can generate reactive chemical species during the illumination process which enhances the phototoxic effect.
- The specimen must be stained with the fluorescent dyes.
- Specimen preparation is a costly process.