



The microscopic measurements and identification of bacterial cells

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Abstract

The bacteria are the smallest primitive, simple, unicellular, prokaryotic and microscopic organisms. These organisms cannot be studied with naked eyes because of their minute structure. Therefore, in search for the information about the structure and composition of bacterial cells the cell biologist used light microscopes with a numerical aperture of 1.4 and using wavelength of 0.4 μm separation. There are still certain cellular structures that cannot be seen through naked eyes, and for them electron microscope is used. There are certain improved types of light microscope which can be incorporated to improve their resolving power. Microscopy is playing a crucial role in the field of bacteriology.

Keywords: Measurements, microorganism, bacteria, staining and microscopy

Introduction

To get acquainted with the world of bacteria like small organisms, very effective and advanced techniques are required. The size of bacteria ranges between 0.5-5.0 micrometers in length; the smallest of them are members of mycoplasma which measures 0.3 micrometers. The study of morphology and physiology of bacteria is called bacteriology which includes a large group of typically unicellular prokaryotic and eukaryotic bacteria widely distributed in air, water, soil etc. and therefore bacteriology requires many varied techniques and instruments. Microscopy is a great achievement in this respect and promises to be a valuable tool for diagnosing and measuring bacterial cell surfaces^[1]. The invention of the microscope opened the door to another world for scientists to pass through to look at organisms or things too small to be seen with the naked eye. The Techni microscope of using microscopes for the study of such small samples like bacteria etc. is said to be microscopy. Bacteriology as well as microscopy goes parallel side by side. Microscopy in the field of bacteriology includes bright fields, dark fields, atomic force microscopy, scanning electron microscope and transmission electron microscope^[4]. Microscopy proved as a boon to find and identify causal bacteria and analyze samples to diagnose disease in organism. Microscopic detection of organisms stained with different antibodies labeled with fluorescent dyes or other markers has proved to be very useful for specific identification of bacteria^[9]. We have reviewed different microscopic techniques to study morphological as well as physiological characteristics of various bacterial strains.

Electron microscopy surpasses the use of light microscopy due to high resolution power and hence transmission electron microscopy becomes important to find the number of bacterial cells and their biomass. Recent advancements in microscopy lead to the invention of Atomic Force Microscopy and Scanning Probe microscopy which is evolving from a qualitative imaging tool to quantitative probe of interaction forms to characterize the properties and function of unicellular prokaryotes. Atomic force microscopy overcame the challenge of sample preparation by using contact mode to study cell growth and division.

Bacteriology

The branch of biology that deals with the study of morphology, physiology and cytology of bacterial strains is said to be bacteriology. The beginning of bacteriology paralleled the development of microscopy. The modern methods of bacteriological techniques begin in 1870-85 with the introduction of the use of stains and by the discovery of the method of separating mixtures of organisms on plates of nutrient media solidified with gelatin or agar. Bacteria are prokaryotes and have no nucleus, rather there is a genetic material restricted to an area of cytoplasm called nucleoid. Prokaryotic cells do not have cytoplasmic compartments such as mitochondria and lysosomes. These cells differ in complexity and hence bacteria are divided into two major groups, the gram negative and gram-positive organism, which reflects their cell wall structure. Gram-positive bacteria are relatively thick and featureless contain peptidoglycan which comprises 50% of major component but have no lipid and often no protein content, accessory polymers like teichoic acid are covalently linked to peptidoglycan. On the other hand, gram-negative cells consist of a pair of membranes with thin intermediate layer of peptidoglycan. The outer membrane contains lipopolysaccharide as well as lipids and proteins. LPS is located exclusively in the outer leaflet, lipid embedded in the membrane, polysaccharide protruding. This makes the bacteria appear rather fuzzy under electron microscope. There are more than thousand species of bacteria, but all of them are basically of three different shapes, some are like little balls and called cocci, others are still helical or spiral in shape, some bacterial cells exist as individuals while others cluster together to form pairs, chains, squares or other groupings. Some are rod- or stick-shaped and called bacilli. The external form and structure of bacteria including shape, size and arrangement of bacteria is a defining characteristic called morphology. Bacteria grow and spread widely with variety of sizes and shapes^[8].

Microscopy

Microscopy is the technical field of using microscopes to view samples and objects that cannot be seen with the unaided eye objects that are not within the resolution range of the normal eye. Microscopy is all about seeing and seeing

things with greater knowledge and insight. Microscopy is based on two major principles:

1. Resolution

Resolution and resolving power of a microscope is defined as the minimum distance by which points must be separated for them to be perceived as to separate point rather than a fused image.

2. Magnification

Linear magnification refers to the ratio of image length to object length measured in planes that are perpendicular to the optical axis. A negative value of linear magnification denotes an inverted image. Longitudinal magnification denotes the factor by which an image increases in size, as measured along the optical axis.

There are three well-known branches of microscopy: optical, electron, and scanning probe microscopy. The conventional optical microscopy uses an instrument consisting of one or more lenses that produces an enlarged image of an object placed in the focal plane of lens. Its resolution limit for submicron particles is within visible light i.e. 400 to 700 nm. Optical microscopy is applicable to observe crystal morphology and symmetry as well as identifying phase, purity and homogeneity. Electron microscopy has long been recognized as a key technique in microbiology to elucidate cell surface ultra structure [2]. Optical and electron microscopy involve the diffraction, reflection, or refraction of electromagnetic radiation/electron beams interacting with the specimen, and the subsequent collection of this scattered radiation or another signal to create an image. This process may be carried out by wide-field irradiation of the sample for example standard light microscopy and transmission electron microscopy or by scanning of a fine beam over the sample for example confocal laser scanning microscopy and scanning electron microscopy. Because of the small size of microorganisms, the physical properties of their surfaces have been difficult to study with conventional methods of microscopy hence there is clearly a need for new, nondestructive tools capable of probing single cell surfaces at high resolution. During the last years, atomic force microscopy. The most recent advance technique in atomic force microscopy is reported the use of force distance-based technology to simultaneously image the structure and map the biophysical properties of biological samples at the nanoscale range [6].

Microscopic Study of Bacteria

The typical compound microscope has objective that face downwards and picking up light that is transmitted through the biological specimen on the microscope slide. This is different from the tissue culture microscope that is made especially for viewing cell cultures grown in petri dishes. This type of microscope has inverted objectives that are under the petri dish and face upward to the Petri dish where the culture first begins to grow. Both uses transmitted light microscopy where the light is passed through the specimen. Other methods of viewing bacteria and culture smears may entail the use of the phase contrast microscope. Using phase is often the method of choice when it is desired to not stain the fresh bacterial strain.

1. Preparation of Sample

Living bacteria are difficult to observe under microscope directly because they are of very small dimensions and most bacteria are colorless hence there is utmost need to prepare bacterial samples in such a way to make them visible under microscope.

2. Fixation

It is the first step in sample preparation and has the aim of preserving tissue in its original state. Specimens for light and electron microscopy are commonly fixed with a solution containing chemical that crosslink most proteins and nucleic acid. Fixatives are acids and aldehydes such as acetic acid, picric acid, formaldehyde, and glutaraldehyde.

3. Staining

Most biological materials show little contrast with their surrounding unless they are stained. In the case of light microscopy, contrast can be enhanced by using colored stains which selectively absorbed certain wavelength. Specimens for light microscopy are stained to visualize the structural features. Many chemical stains bind to specific molecules present in the specimen. Hematoxylin binds to basic amino acid lysine and arginine of different proteins, whereas eosin binds to acidic molecules such as DNA and side chains of aspartate and glutamate. Although unstained, amorphous, frozen-hydrated sections provide a faithful, high-resolution representation of living material, the observation of unstained frozen-hydrated Specimens provides a unique method for the measurement of density.

4. Washing

After staining the sample is washed by several buffer solutions specifically, like washing with double distilled water or in a 1% solution of ammonium acetate. Another method for washing the samples for dark field microscopy uses nonfoaming spray-on glass cleaner such as bon-aim, which is wiped off with tissue after a few seconds, to be as effective as acid washing procedures.

5. Dehydration

After fixing and washing the specimens are dehydrated by several alcoholic series. Dehydration is best performed in capped wide-neck vials to accommodate the filters. The specimens are dehydrated through a graded ethanol series 20 40, 60, 80, 95, 100, 100, 100, 100, 100, 100, 100% ethanol on a slowly moving inclined rotary table. Short, 5-10 min intervals are sufficient to dehydrate several filters in a vial. Finally, the filters are critical-point dried. The filter must remain wet all the time. It may be transferred very briefly onto a piece of filter paper to drain residual absolute ethanol before critical point drying.

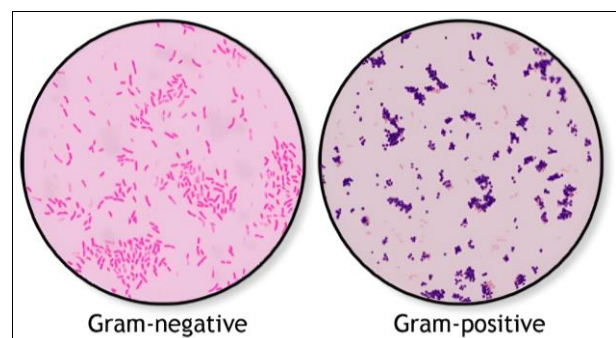


Fig 1

Morphological Study

There are various microscopic techniques which are employed in the field of bacteriology for morphology, anatomy and physiology and various shapes and sizes of bacterial strains which is the basis for the identification of a particular bacteria and diagnosing diseases caused by pathogenic species.

1. Atomic Microscopy

Atomic force microscopy has been used increasingly to investigate microbial surfaces at high resolution. The structure of isolated cell surface layers can now be imaged at sub molecular resolution. By imaging bacteria in aqueous

solution, AFM can provide real-time in situ quantitative morphological information as well as measurement of the interaction forces between AFM tip and modified probe and cell surface. The morphological features of typical bacteria are very well studied by atomic force microscopy. Through AFM it is rather much easier way to distinguish between gram negative and gram-positive bacteria without any pretreatment to the sample. Light microscopy cannot be incorporated to study subcellular details such as murein-like structures hence atomic force microscopy is used to measure elasticity and rigidity properties of bacterial surfaces and thickness of murein sacculi ^[4].

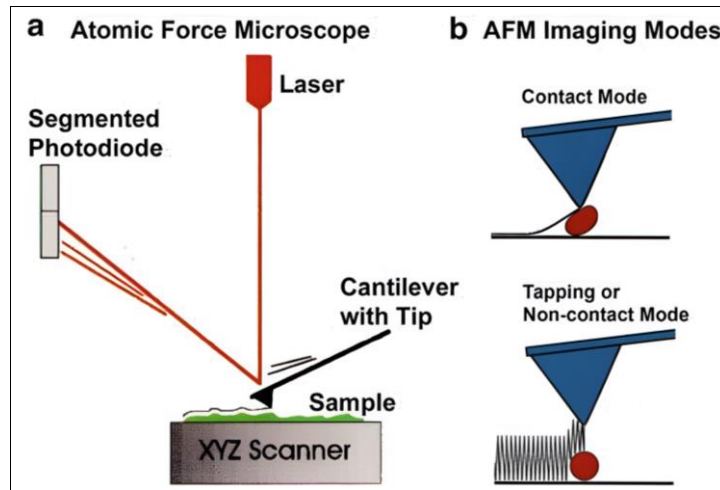


Fig 2

2. Electron Microscopy

Electron microscopy is of two types scanning electron microscopy and transmission electron microscopy:

2.1. Scanning Electron Microscopy

It is provided for producing a scan image at high spatial resolution and in a low acceleration voltage area. The electron beam is generally scanned in a raster scan pattern,

and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than one nanometer. For SEM specimen is normally required to be completely dry, since the specimen chamber is at high vacuum. Electron microscopy revealed ultrastructure of bacteria in which nuclear zone is clearly visible, and excretion of cell wall is also observed ^[5].

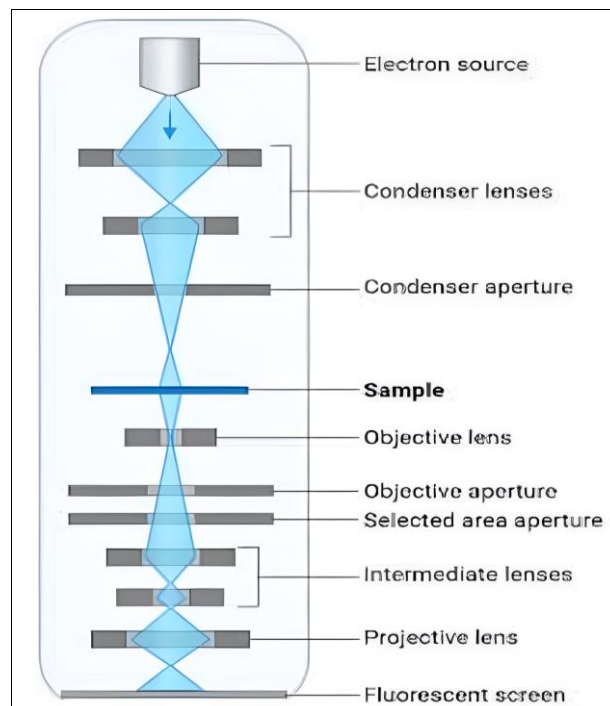


Fig 3

2.2. Transmission Electron Microscopy

In this type of microscopy, a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device. The bilirubin stones with bacterial infection were examined by transmission electron microscopy which shows the presence of mixed microcolonies of gram positive and gram-negative bacteria embedded in an amorphous anionic matrix.

3. Dark field microscopy

Dark field microscopy is a technique used to observe unstained samples causing them to appear brightly against a dark, almost purely black, background. These specimens often have similar refractive indices as their surroundings, making them hard to distinguish with other illumination techniques. It works by illuminating the sample with light that will not be collected by the objective lens, and thus will not form part of the image. Flagellation of bacteria can be studied by dark field microscopy of individual unstained flagella on whole cell. *S. typhimurium* has peritrichous flagella and *Pseudomonas* species are polarly flagellated. The ability to see individual, unstained flagella is a significant advance in the power of dark field light microscopy [3].

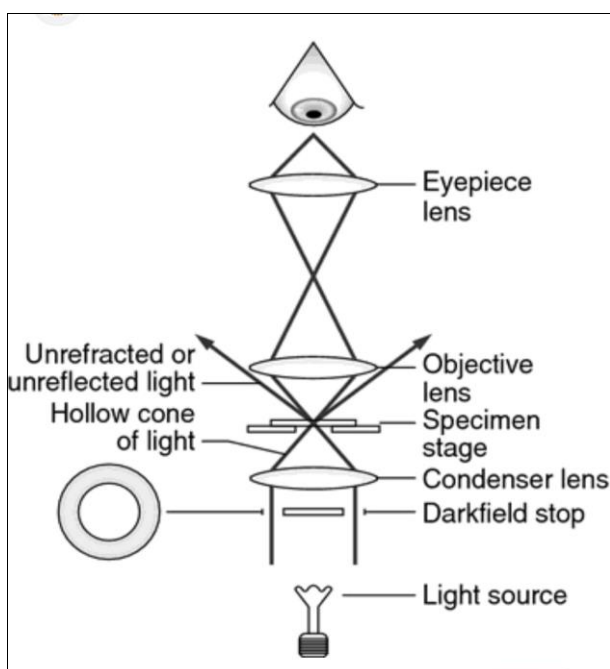


Fig 4

4. Scanning Laser Microscopy

It is employed as an effective tool to microscopy distribution of bacteria in rhizosphere. In this technique nondestructive optical sections of a sample are obtained and disturbing effect from out of focus fluorescence is reduced [7].

5. Nuclear Component Microscopy

Electron microscopy heteroduplex recognizes the presence of insertion sequences on F and R plasmid in bacteria and these sequences on several phage DNA will form duplex

region. The structure of nuclear protein as well as nucleic acid is observed through different microscopic techniques.

Conclusions

The beginning of microbiology by the discovery of bacteria, a new era of microscopic technique has also been developed. It cannot be possible to observe such a small creature like bacteria through our naked eyes. Previously used microscopes were simple light microscope but there came a lot of difficulty in observing submicron particles through light microscope and after that electron microscopy came into existence which had revolutionized the microbial world with the identification of nuclear component of bacterial cells. After that a more advanced technique named atomic force Microscopy overcame with all the difficulty in the study of ultrathin structures of bacterial cells as it could provide real time in situ quantitative morphological information. Many recent microscopic techniques are scanning laser and nuclear component microscopy etc. The difference between different microscopic techniques is based on the source of transmission on the object.

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