Applications of Microscopy in Biological Sciences

Light microscopy

Light microscopy finds a variety of applications in studying biological systems some of which are:

1. **Specimen identification and quality***:* The simplest application of microscopy is to observe the given sample to identify the different components in it. A given sample may have different microorganisms with different morphologies and structures. Viability of cells, and therefore their quality, is ascertained by staining the cells with dyes that distinguish between live and dead cells.

2. **Cell counting***:* Counting of cells using a hemocytometer utilizes light microscopy.

3. **Classification of bacteria***:* Differential staining of the bacterial cell wall by Gram staining method is the basis of classifying the bacteria into Gram positive and Gram negative. The stained cells can easily be observed in a bright-field microscope.

4. **Microscopic analysis of body fluids***:* Microscopic analysis of blood samples is routinely used to determine the blood cell count, to detect the microbial infection , and to identify any changes in the cellular structures.

5. Fecal analysis of domesticated animals: protozoan parasites can easily be identified and quantitated by analyzing the fecal samples using light microscopy.

6- **Histopathology**: Histopathology is the area of pathology that deals with the anatomical changes in the tissues. The tissue samples are sliced into thin sections and stained with a dye. The choice of stain depends on the histological features one needs to study. For example, **hematoxylin** and eosin stain is a routinely used stain to study the morphological features of tissue samples, **congo red** is often used to identify the amyloid plaques, **Giemsa** stain is used for identifying the parasites such as plasmodium.

7- **Cytopathology:**

Cytopathology, is the study of pathological conditions at the cellular level. Any change in the cellular morphology or anatomy following an infection, as a result of a metabolic disorder, or a cellular condition like sickle cell anemia can be studied by staining the cells and analyzing them using any of the light microscopic methods.

8- Cellular membranes and intracellular structures: A cellular feature can be selectively labeled using fluorescently labeled antibodies immunofluorescence, or the fluorescent dyes that selectively bind to the cellular structures. For example, probes that specifically bind to the cellular organelles like nucleus, mitochondria, and lysosomes are commercially available. e.g. DAPI for DNA staining.

9. Live cell imaging*:* Inverted microscopes allow direct microscopy of the cultured cells.

10. Protein dynamics and localization*:* Green fluorescent protein (GFP) and its variants have made it possible to selectively label the proteins within a cell. Live cell imaging using fluorescence microscopy allows studying the dynamics and localization of the proteins in the cells.

Application of Scanning Electron Microscope

1- **Compositional analysis***:* Backscattering of electrons depends on the atomic number of the material. Backscattered electrons reveal the differences in the composition of a material. The regions with high atomic mass elements scatter more electrons thereby giving a brighter image. This kind of analysis allows detection of the contaminants in a specimen, if any.

2- **Energy dispersive X-ray spectroscopy (EDS/EDX/EDXS):** EDXS is one of the several analytical electron microscopic methods. The primary electron beam causes excitation of the atoms in the specimen by ejecting electrons form their inner shells. The excess energy is emitted as the X-rays that are characteristic of the element; determination of their energies allows identification of the elements in the specimen**.**

Applications of Transmission Electron Microscopy

1- Bright-field and dark-field microscopic imaging

TEM images usually are bright-field images. Thicker and electron-rich regions in the specimen produce darker regions in the image. Owing to their sub-micrometer /nanometer dimensions, many of the cellular components are not observed by light microscopy. High resolution TEM can reveal the ultrastructural details of these components. Like light microscopy, TEM can operate in dark-field mode too.

2- **Electron diffraction:** The crystalline regions in the specimen diffract the incident electrons. The diffraction pattern generated provides information about the lattice parameters of the crystalline regions.

3- **Energy dispersive X-ray spectroscopy (EDS/EDX/EDXS)***:* Analytical transmission electron microscopes usually come with several detectors such as detectors for secondary electrons, backscattered electrons, and X-rays. If a TEM is used in scanning mode (Scanning TEM/STEM), a compositional map can be obtained for the specimen.

4- Nanotomography: A TEM micrograph is the two-dimensional projection of a three dimensional object.

Applications Atomic force microscopy (AFM)

1- Imaging of dry samples: The specimen is deposited on an atomically-smooth substrate, typically mica and dried. The ability to provide resolutions comparable to TEM makes AFM a powerful tool in nanotechnology.

2- Cell biology: Owing to its ability to operate on liquid samples, AFM has been used to study the real-time biological processes. Migrating epithelial cells, dynamics of membrane invaginations, conformational changes in membrane proteins, and assembly/disassembly of structural proteins have been studied in real time using AFM.

3- Nucleic acid research*:* AFM has slowly emerged as a powerful tool to analyze the structures of the nucleic acids and the various processes they are involved in. Three-way and four-way DNA junctions have been analyzed using AFM.

4- **Biomolecular interactio*n*:** Biomolecular interactions can be studied by labeling the AFM probe with the ligand for the receptor biomolecule under study.

5- **Protein unfolding***:* AFM has been used to study the mechanical unfolding of proteins.

6- **Nanofabrication***:* An AFM probe has been successfully utilized to oxidize the metal and semiconductor surfaces.

7- **Detection of defects:** AFM can be used to determine the cracks and other deformations in the materials, *e.g.* detection of defects in the semiconductor materials and electronic chips and circuits.