## FREQUENTLY ASKED QUESTIONS

**Q.No.1: Define Microscopy?**

**Ans1:** Microscopy is the technique used to view objects that cannot be seen by the naked eye but can be viewed with the help of microscopes.

**Q.No.2: Name most common and simple microscopic technique used?**

**Ans2:** Optical microscopy is most common and simplest microscopic technique used.

**Q.No.3: What is confocal microscopy and discuss briefly?**

**Ans3:** Confocal microscopy is defined as that microscopic technique in which all out of focus structures are suppressed at image formation. This is obtained by an arrangement of diaphragms, which, at optically conjugated points of the path of rays, act as a point source and as a point detector respectively. .

**Q.No4: Define briefly Dark Field Microscopy?**

**Ans4:** Dark Field Microscopy is a technique in which a specimen is illuminated obliquely, with no direct light entering the objective. Features in the specimen plane which scatter light can clearly be seen against a dark background.

**Q.No5: Define Phase Contrast microscopy?**

**Ans5:** In this technique specimen is illuminated by a hollow cone of light coming through a phase annulus in the condenser. Phase contrast objectives must be used, which have a corresponding phase plate. Light rays passing through the specimen are slightly retarded, and further retardation takes place in the phase plate. When these rays combine with rays which have not taken this path, degrees of constructive and destructive interference occur

which produce the characteristic light and dark features in the image.

**Q.No6: Define Polarised Light Microscopy?**

**Ans6:** Polarised light microscopy uses plane-polarised light to analyse structures that have two different refractive indices at right angles to one another (e.g. cellulose micro fibrils).

**Q.No7: What is the importance of Fluorescence Microscopy?**

**Ans7:** Fluorescence microscopy can be used as a label or tag when preparing specific biological probes. Some biological substances, such as chlorophyll and some oils and waxes, have primary fluorescence (auto-fluorescence). However, most biological molecules do not fluoresce on their own, so they must be linked with fluorescent molecules (fluorochromes) in order to create specific fluorescent probes.

**Q.No8: Give the key feature of fluorescence microscopy?**

**Ans8:** The key feature of fluorescence microscopy is that it employs reflected rather than transmitted light, which means transmitted light techniques such as phase contrast and DIC can be combined with fluorescence microscopy.

**Q.No9: What is scanning probe microscopy?**

**Ans9:** This is another branch of microscopy that involves using a probe to scan the object. Basically it works by being moved around in a rectangular pattern known as raster scanning.

**Q.No10: Define electron microscopy?**

**Ans10:** This is a form of microscopy that uses electron beams to create an image of the object being used. They have a much higher magnification than light microscopes and so a much higher resolution as a result, this allows us to see smaller specimens in

greater detail. The resolution is able to be increased because as the electrons travel faster their wavelength becomes shorter so there is a direct correlation between reducing wavelength and increasing resolution.

**Q.No11: Define Transmission Electron Microscopy?**

**Ans11:** The original form of electron microscopy is Transmission electron microscopy which involves a high voltage electron beam emitted by a cathode and formed by magnetic lenses. The electron beam has been partially transmitted through the very thin specimen who carries information about the structure of the specimen. The spatial variation is then magnified by a series of magnetic lenses until it is recorded by hitting a fluorescent screen, photographic plate, or light sensitive sensor such as a CCD (charge-coupled device) camera.

**Q.No12: What is Scanning Electron Microscopy?**

**Ans12:** Scanning Electron Microscope produces images by detecting secondary electrons which are emitted from the surface due to excitation by the primary electron beam. In the SEM, the electron beam is scanned across the surface of the sample in a raster pattern with detectors building up an image by mapping the detected signals with beam position. SEM image relies on electron interactions at the surface rather than transmission it is able to image bulk samples and has a much greater depth of view, and so can produce images that are a good representation of the 3D structure of the sample.

**Q.No13: Give disadvantages of Electron Microscopy?**

**Ans13:** Electron microscopes are very expensive to buy and maintain. They are dynamic rather than static in their operation, requiring extremely stable high voltage supplies, extremely

stable currents to each electromagnetic coil/lens, continuously-pumped high/ultra-high vacuum systems and a cooling water supply circulation through the lenses and pumps. As they are very sensitive to vibration and external magnetic fields, microscopes aimed at achieving high resolutions must be housed in buildings with special services. A significant amount of training is required in order to operate an electron microscope successfully and electron microscopy is considered a specialised skill.

**Q.No14: What is Reflection Electron Microscopy (REM)?**

**Ans14:** Reflection electron microscope works much similar to the SEMs. Here, the reflected electrons are detected and gathered to study the surface of the specimen object. The Reflection Electron Microscope (REMs) are usually grouped with spin polarized and low energy electron microscopes to image the specimen structure.

**Q.No15: What is Scanning Transmission Electron Microscopy?**

**Ans15:** Scanning transmission electron microscopes pass an electron beam through a very thin slice of an object. The STEM focuses on the beam that passes before hand and constructs an image through raster scanning, instead of focusing on the beam after passing through the object. It is a combination of high magnification of TEM and better surface detail of SEM. STEM is usually used to perform very complex analysis of objects and specimens that is not possible by just using the TEM.