ZOO515 – BIOLOGICAL TECHNIQUES

MID TERM MOST IMPORTANT QUESTIONS

PREPARED BY: SIR MUGHAL (ADMIN, BS ZOOLOGY ANNOUNCEMENT)

GROUP LINK: https://chat.whatsapp.com/BjC6W0jZx8o9yaC2ISh1Gu

1. What are limitations of electron microscope?

Electron microscopes are powerful tools for imaging at very high resolution, but they come with several limitations. Here are the main ones:

1. Cost and Size

- ***Expensive:*** Electron microscopes (especially transmission EM) are very costly to purchase, operate, and maintain.
- ***Large and Bulky:*** They require a dedicated space with vibration isolation and controlled environmental conditions.

2. Sample Preparation

- ***Complex:*** Preparing samples for electron microscopy is often time-consuming and can require special techniques (e.g., dehydration, coating with conductive materials).
- ***Potential Artifacts:*** Preparation steps can introduce artifacts that distort the natural structure of the sample.

3. Imaging Limitations

- ***Vacuum Requirement:*** Samples must be placed in a vacuum, which excludes the study of living organisms or hydrated biological samples in their natural state.
- ***Black and White Images:*** Electron microscopes produce grayscale images. Color must be added artificially during post-processing.
- ***Limited Field of View:*** Very high magnification often means only a tiny portion of the sample is visible at once.

4. Biological Limitations

- ***No Live Imaging:*** Cannot image living cells or organisms due to vacuum and electron beam damage.
- ***Radiation Damage:*** The electron beam can damage or alter delicate biological specimens, especially under prolonged exposure.

5. Material Limitations

- ***Non-Conductive Samples:*** Require coating with a conductive layer (like gold or carbon), which can obscure fine details.

6. Technical Skill

- ***Operator Expertise:*** Requires trained personnel to operate and interpret results accurately.

2. What are types of fixatives and their advantages?

There are several types of fixatives used in microscopy, each with different advantages depending on the tissue type and purpose. Aldehyde fixatives, like formaldehyde and glutaraldehyde, are widely used because they stabilize proteins by forming cross-links. Formaldehyde penetrates tissues well and is commonly used for general histology, while glutaraldehyde preserves ultrastructure more effectively and is preferred for electron microscopy. Alcohol fixatives, such as ethanol and methanol, act quickly and are good at preserving nucleic acids and precipitating proteins. They are often used in cytology, especially for smears. Oxidizing fixatives like osmium tetroxide and potassium permanganate are excellent for fixing lipids and membranes, making them ideal for electron microscopy as they also enhance contrast. Mercurial fixatives, like Zenker's fluid and B-5, provide excellent nuclear detail and are useful for lymphoid and hematopoietic tissues. Picric acid-based fixatives, such as Bouin's solution, are effective in preserving soft tissues and improve staining quality. Acetone is another fixative that works rapidly and is used especially in frozen sections and enzyme preservation studies.

3. Explain Laser and Saw microtome.

Laser microtome is a modern, contact-free cutting technique that uses a focused laser beam, usually a femtosecond laser, to slice thin sections of biological or material samples. Since there is no physical blade involved, there's no mechanical stress or distortion to the sample. It's especially useful for hard tissues like bone, teeth, or implants, and it can cut even through embedded materials without damaging them. It's also safe for samples that are difficult to section by traditional means, and it doesn't require prior embedding or freezing in many cases.

Saw microtome, on the other hand, is designed specifically for hard and mineralized tissues like bone or teeth. It uses a diamond-coated circular saw blade to cut precise sections, usually thicker than what traditional microtomes produce. The saw runs at a slow speed with lubrication (like water or oil) to reduce heat and avoid sample damage. It allows for relatively smooth cutting of undecalcified hard tissues, which are difficult to cut with standard microtomes. This method is particularly used in histological studies involving biomaterials and orthopedic research.

4. Explain principle of Paper Chromatography.

Paper Chromatography – Principle:

Paper chromatography separates components of a mixture based on their solubility and interaction with two phases:

- ***Stationary Phase:*** Water trapped in paper (cellulose).
- ***Mobile Phase:*** A solvent that moves through the paper by capillary action.

Different components travel at different speeds depending on how well they dissolve in the solvent versus how strongly they stick to the paper.

5. What are limitations of phase contrast microscope?

Here are the limitations of a phase contrast microscope:

1. ***Halo Effect:*** Bright or dark halos often appear around the edges of specimens, which can distort details.
2. ***Limited to Thin Specimens:*** Works best with thin, transparent samples; thick specimens can cause image artifacts.
3. ***Cannot Show Color:*** Produces monochromatic (black and white or gray) images, so no natural color information.
4. ***Complex Setup:*** Requires special phase contrast objectives and condensers, making the microscope more expensive and complex.
5. ***Not Suitable for Highly Pigmented Samples:*** Dark or strongly colored specimens may reduce image quality.

6. Compare simple light microscope and light compound microscope.

A **simple light microscope** uses a single lens to magnify objects and typically provides low magnification (up to about 10x). It is straightforward and easy to use but offers lower image clarity and resolution. Simple microscopes are suitable for basic observation of larger objects like insects or leaves.

A **compound light microscope** uses two or more lenses—an objective lens and an eyepiece—to achieve much higher magnification, often ranging from 40x to 1000x or more. This allows for detailed viewing of small specimens like cells and microorganisms with better clarity and resolution. Compound microscopes are more complex, usually equipped with a built-in light source, and require more careful focusing compared to simple microscopes.

7. Differentiate between Magnification and Resolution.

Magnification is the process of making an object appear larger than its actual size using lenses or other optical instruments. It tells you *how much bigger* the image is compared to the real object.

Resolution is the ability of a microscope or optical system to distinguish two close points as separate and clear. It determines the *clarity* and *detail* of the image, showing how sharp or detailed the magnified image is.

8. What are functions of Microtome?

The functions of a microtome are:

1. To cut very thin slices (sections) of biological specimens.
2. To prepare these thin sections for microscopic examination.
3. To produce uniform and consistent sections for better observation of internal structures.
4. To enable the study of tissues in detail by allowing light or electrons to pass through the thin slices.

9. What are function of Acromicrotome?

The acromicrotome is a specialized type of microtome designed to cut ultra-thin sections of biological specimens, often less than 1 micrometer thick. These extremely thin slices are necessary for viewing under an electron microscope, which requires very thin samples so that electrons can pass through them to form detailed images. The acromicrotome allows precise and consistent sectioning of hard or embedded tissues, enabling

scientists to study the fine internal structures of cells, organelles, and tissues at a very high resolution. This makes it essential for advanced research in cell biology and pathology.

10. What is Nuclear Aperture?

The nuclear aperture—more commonly called the nuclear pore—is a tiny channel in the double membrane of the nuclear envelope. The nuclear envelope separates the nucleus (where DNA is stored) from the cytoplasm of the cell. These pores are crucial because they regulate the movement of molecules between the nucleus and the cytoplasm. For example, messenger RNA (mRNA) produced in the nucleus passes out through these pores to the cytoplasm, where proteins are made. At the same time, proteins like enzymes and signaling molecules needed in the nucleus can enter through these pores. The nuclear pores are highly selective, allowing only certain molecules to pass while blocking others, which helps maintain the proper environment for DNA and RNA processes inside the nucleus.

11. Define Vibratome.

A vibratome is a specialized microtome designed to cut thin sections of biological tissue using a vibrating blade. Unlike traditional microtomes that require samples to be frozen or embedded in wax or resin, a vibratome can section fresh, unfixed, or lightly fixed tissue. The vibration of the blade reduces the pressure on the specimen, minimizing damage and distortion, which is especially important when working with soft, delicate tissues like brain, spinal cord, or muscle. These thin slices are typically used for microscopic examination, histological studies, or physiological experiments where preserving the natural structure and viability of cells is crucial. The vibratome is widely used in neuroscience and developmental biology for preparing living tissue sections.

12. What is confocal microscope?

A confocal microscope is an advanced optical microscope that uses laser light to scan specimens and produce high-resolution, sharply focused images. Unlike traditional microscopes, it captures images one tiny point at a time and uses a pinhole to eliminate out-of-focus light, which greatly improves image clarity and contrast. This allows it to create detailed 3D images of thick specimens by stacking multiple focused layers (optical sections). Confocal microscopy is widely used in biological and medical research to study cells, tissues, and small structures with great precision.

13. What factors effect resolution?

1. ***Wavelength of Light:*** Resolution depends on the wavelength of the light used for imaging. Shorter wavelengths (like blue or violet light) can resolve smaller details because they can interact with smaller structures more effectively. Longer wavelengths (like red light) produce lower resolution.
2. ***Numerical Aperture (NA):*** The NA of a microscope objective lens measures its ability to gather light and resolve fine specimen detail at a fixed object distance. Higher NA lenses can collect more light and finer details, improving resolution.
3. ***Quality of Lenses:*** Lenses with fewer optical imperfections (aberrations) such as chromatic or spherical aberration produce clearer images with better resolution. High-quality lenses focus light more precisely.
4. ***Refractive Index of Medium:*** Resolution improves when the space between the specimen and lens is filled with a medium of higher refractive index (like immersion oil), as it reduces light refraction and allows more light to enter the lens.

5. ***Contrast and Illumination:*** Proper lighting techniques and good contrast help differentiate between fine details in the specimen, which can affect how well you perceive resolution.

14. What factors effect Gel Electrophoresis?

Factors affecting gel electrophoresis include:

1. ***Gel Concentration:*** Higher gel concentration creates smaller pores, slowing down larger molecules and separating smaller ones better. Lower concentration gels have larger pores, suitable for bigger molecules.
2. ***Voltage Applied:*** Higher voltage speeds up migration but can cause band distortion or overheating. Lower voltage gives better resolution but takes longer.
3. ***Buffer Composition and pH:*** Buffers maintain consistent pH and conduct electricity; incorrect pH or composition can affect migration and band clarity.
4. ***Size and Charge of Molecules:*** Smaller and more negatively charged molecules move faster through the gel.
5. ***Gel Type:*** Agarose gels are used mainly for DNA, while polyacrylamide gels are used for proteins or small DNA fragments, affecting resolution and separation.
6. ***Running Time:*** Longer runs allow better separation but can cause bands to diffuse or run off the gel.

15. Write fixation methods.

Here are common fixation methods used to preserve biological specimens:

1. **Chemical Fixation:**
   - Uses chemicals like formaldehyde, glutaraldehyde, or alcohols (ethanol, methanol) to preserve tissue by cross-linking proteins and stabilizing cell structures.
   - Common in histology and microscopy.
2. **Physical Fixation:**
   - Involves methods like freezing (cryofixation) or heat (heat fixation) to preserve samples quickly by stopping biological processes.
   - Used for certain staining techniques and electron microscopy.
3. **Coagulative Fixation:**
   - Causes proteins to coagulate or precipitate, often using alcohol or acetone, preserving tissue by hardening it.
4. **Cross-linking Fixation:**
   - Uses chemicals that form covalent bonds between proteins (e.g., formaldehyde), stabilizing the specimen structure.

16. How DNA is detected on gel?

- DNA fragments are invisible after electrophoresis because they're colorless.
- The gel is stained with a dye that binds specifically to DNA, commonly Ethidium Bromide (EtBr).
- EtBr intercalates between DNA base pairs, attaching firmly to the DNA.
- When exposed to UV light, the EtBr-bound DNA fluoresces, making the DNA bands visible as bright bands on the gel.
- Safer alternative dyes like SYBR Green, GelRed, and GelGreen can also be used and work similarly.

- The glowing DNA bands are captured using a UV transilluminator and camera for analysis.
- This method helps determine the size and amount of DNA based on how far fragments travel in the gel.

17. What are limitations of SEM?

Here are the main limitations of a Scanning Electron Microscope (SEM):

- ***Sample Preparation:*** Requires samples to be dry and often coated with a conductive material (like gold), which can alter or damage delicate specimens.
- ***Vacuum Requirement:*** SEM operates under high vacuum, so it can't be used to observe living organisms or wet samples directly.
- ***Limited Depth of Field for Thick Samples:*** While SEM has good depth of field, very thick or uneven samples may still cause focus issues.
- ***No Color Imaging:*** SEM images are grayscale; any color seen in images is artificially added later.
- ***Cost and Complexity:*** SEM instruments are expensive and require skilled operators.
- ***Resolution Limits:*** While SEM offers high resolution, it's generally lower than Transmission Electron Microscopy (TEM) for viewing internal structures.

18. Differentiate between Homogenous and Heterogeneous Mixture.

A **homogeneous mixture** is one in which the components are evenly distributed throughout the mixture, resulting in a uniform composition. The individual substances cannot be seen separately because they are mixed at the molecular or ionic level. Examples of homogeneous mixtures include salt dissolved in water, where the salt particles are completely dispersed, and air, which is a mixture of gases evenly spread out. These mixtures appear the same throughout and have consistent properties in any sample taken.

A **heterogeneous mixture**, on the other hand, has an uneven distribution of its components, meaning the different substances remain physically separate and can often be seen as distinct parts. The composition varies from one part of the mixture to another. Common examples include sand mixed with water, where the sand particles settle or are visible, and a salad, where the ingredients like lettuce, tomatoes, and cucumbers can be identified individually. Heterogeneous mixtures do not have uniform properties throughout.

19. What factor effect rate of migration of DNA?

Factors affecting the rate of migration of DNA during gel electrophoresis include:

1. ***Size of DNA fragments:*** Smaller DNA fragments move faster through the gel pores than larger ones.
2. ***Gel concentration:*** Higher gel concentrations create smaller pores, slowing down DNA movement; lower concentrations allow faster migration.
3. ***Voltage applied:*** Higher voltage increases migration speed but can cause overheating and band distortion; lower voltage slows migration but improves resolution.
4. ***Buffer composition and pH:*** Proper buffer maintains consistent pH and ionic strength, affecting DNA migration speed and band sharpness.
5. ***Conformation of DNA:*** Linear, supercoiled, and circular DNA migrate differently due to their shapes.
6. ***Temperature:*** Higher temperature can increase migration speed but may also cause gel melting or band distortion.

20. Write physical methods of tissue fixation in microtomy.

1. ***Heat Fixation:*** This method involves applying direct heat to the tissue or specimen, usually by passing a slide with a smear over a flame or using a hot plate. The heat coagulates proteins and kills microorganisms, preserving the overall structure. It's commonly used for preparing bacterial smears for staining but is less suitable for delicate tissues as it can cause shrinkage or distortion.

2. ***Freezing (Cryofixation):*** In this technique, tissue samples are rapidly frozen using substances like liquid nitrogen or dry ice. Freezing preserves the cellular structure by halting biological processes almost instantly without chemical cross-linking. This method is especially useful for fresh tissues, enzyme histochemistry, and immunohistochemistry, where chemical fixation might interfere with the sample's properties.

3. ***Microwave Fixation:*** Microwave radiation is used to heat tissue rapidly and uniformly, speeding up the fixation process. It enhances the penetration of fixatives and can reduce fixation time from hours to minutes. This method helps preserve fine cellular details but requires careful control to avoid overheating and damaging the tissue.

21. What factors effect resolving power?

Factors affecting resolving power include:

1. ***Wavelength of Light:*** Shorter wavelengths (like blue or violet light) improve resolving power by allowing finer details to be distinguished.
2. ***Numerical Aperture (NA):*** Higher NA of the objective lens increases resolving power by capturing more light and details.
3. ***Quality of Optical Components:*** Better lenses with fewer aberrations enhance resolving power by producing sharper images.
4. ***Refractive Index of the Medium:*** Using immersion oil (with a higher refractive index than air) between the specimen and lens increases resolving power.
5. ***Contrast and Illumination:*** Proper lighting and contrast techniques help reveal finer details, indirectly influencing resolving power.

22. What is path of light in bright field microscopy?

In bright field microscopy, the path of light follows these steps:

1. ***Light Source:*** The light originates from a lamp or LED below the stage.
2. ***Condenser Lens:*** The light passes through the condenser, which focuses and directs it onto the specimen.
3. ***Specimen:*** Light passes through the transparent specimen; some light is absorbed or scattered, creating contrast.
4. ***Objective Lens:*** The light transmitted through the specimen enters the objective lens, which magnifies the image.
5. ***Body Tube:*** The magnified image travels up through the body tube.
6. ***Ocular Lens (Eyepiece):*** Finally, the image is further magnified by the eyepiece lens before reaching the observer's eye.

23. What is cryomicrotome? Discuss its significance.

A cryomicrotome is a type of microtome that cuts very thin sections of biological tissue that have been rapidly frozen. Unlike traditional microtomes, which require chemical fixation and embedding, a cryomicrotome slices frozen specimens without the need for chemical treatment. The freezing preserves the natural structure and biochemical properties of the tissue.

Significance of Cryomicrotome:

- It allows rapid preparation of tissue sections, saving time compared to conventional methods.
- It preserves enzyme activity, antigenicity, and other sensitive molecules, making it ideal for immunohistochemistry and enzyme histochemistry.
- It avoids chemical artifacts caused by fixation and embedding, providing more accurate representation of tissue morphology.
- Useful for fresh or unfixed tissues, especially when immediate analysis is required.

24. What is multilayer filtration?

Multilayer filtration is a filtration technique that uses several layers of filter materials stacked together, each with different pore sizes or properties. This arrangement allows the removal of particles of varying sizes step-by-step: larger particles are trapped in the upper layers, while smaller particles are captured by the finer layers below. This method improves filtration efficiency, prevents clogging, and extends the lifespan of the filter. Multilayer filtration is commonly used in water purification, air filtration, and industrial processes to achieve thorough cleaning or separation.

25. Discuss hot and cold filtration.

Hot Filtration:

Hot filtration is a technique used primarily during recrystallization to separate insoluble impurities from a hot solution containing a dissolved compound. The solution is kept at a high temperature to ensure that the desired compound remains dissolved while solid impurities, which do not dissolve even at high temperatures, are filtered out. This prevents the premature crystallization of the compound on the filter paper, which could clog the filter and reduce yield. By maintaining the solution's temperature during filtration, hot filtration ensures that only unwanted solids are removed, helping to purify the compound effectively.

Cold Filtration:

Cold filtration is employed after a solution has been cooled and the desired compound has crystallized out. In this process, the solution containing the formed crystals and any insoluble impurities is filtered at a low temperature. The cooling causes the compound to form solid crystals, which can be separated from the liquid and impurities. Filtering the cold mixture helps to isolate pure crystals without redissolving them, while the insoluble impurities remain on the filter paper. Cold filtration is important for collecting pure solid products after recrystallization or precipitation steps.

26. Why we use confocal microscopy?

Confocal microscopy is used because it overcomes the limitations of traditional light microscopy by using a focused laser beam and a pinhole aperture to eliminate out-of-focus light from thick specimens. This results in much clearer, sharper images with higher contrast and resolution. By scanning the specimen point-by-point and layer-by-layer, confocal microscopes can generate detailed optical sections, which can be stacked to create precise three-dimensional images of cells, tissues, or small organisms. This makes confocal microscopy invaluable in fields like cell biology, neuroscience, and developmental biology, where understanding complex structures and interactions inside intact specimens is crucial. Additionally, it allows live-cell imaging with reduced background noise, enabling the study of dynamic biological processes in real time.

27. What are applications of fluorescence microscopy?

Here are some key applications of fluorescence microscopy:

- ***Cell Biology:*** Studying the structure and function of cells by labeling specific proteins, organelles, or nucleic acids with fluorescent dyes or tags.
- ***Medical Diagnostics:*** Detecting specific pathogens, cancer cells, or biomarkers using fluorescent antibodies or probes.
- ***Molecular Biology:*** Tracking gene expression, protein localization, and interactions inside living cells.
- ***Neuroscience:*** Mapping neural circuits and observing neurotransmitter activity with fluorescent markers.
- ***Microbiology:*** Identifying microorganisms and studying their behavior in different environments.
- ***Live-Cell Imaging:*** Monitoring dynamic cellular processes in real time, such as cell division or intracellular transport.
- ***Environmental Science:*** Detecting pollutants or microorganisms in water and soil samples using fluorescence tagging.

28. What is molecular weight of DNA in gel electrolysis?

The molecular weight of DNA itself isn't directly measured during gel electrophoresis. Instead, DNA fragments are separated based on their size (length), typically measured in base pairs (bp) or kilobases (kb)**.** Gel electrophoresis separates DNA fragments by size because smaller fragments move faster through the gel matrix. To estimate the size (and indirectly the molecular weight) of DNA fragments, a DNA ladder (a mixture of DNA fragments of known lengths) is run alongside the samples as a reference. So, while gel electrophoresis doesn't directly give molecular weight, it helps determine the relative size of DNA fragments, which can be correlated to molecular weight since DNA's molecular weight is proportional to its length.

Disclaimer:

For best results, it is advised NOT to rely on this file only. Go through video lectures, handouts and other sources as well.

Regards,
Sir Mughal,
BS Zoology, Virtual University of Pakistan
B.Sc (Honors), University of Karachi.