

MEDICAL BIOLOGY AND GENETICS 1

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1. Essentials of microscopy

Test yourself:

1. How to calculate the final magnification at which we observe the object in the microscope?

The final magnification of the microscope is given by the formula:

magnifying power of the objective lens \times magnifying power of the eyepiece

e.g. If magnification of objective is $\times 40$ and magnification of eyepiece (ocular lens) is $\times 10$, the total magnification is $40 \times 10 = 400$ times

2. What is the function of condenser?

The condenser provides an area of evenly-illuminated light in the field of view at the specimen plane and illuminates the aperture of the objective uniformly with light. Also, it provides a means of regulating contrast of the observed image.

3. The working distance of objective lens is:

The distance between the front edge of the objective lens and the specimen surface when the specimen is focused.

4. The image in a microscope is:

Inverted and upside down. Image “” will be seen as “”.

5. Which parts of microscope are used for adjustment of light intensity?

Light source, Condenser lens, Iris diaphragm

6. What is the principle of fluorescence microscope?

Fluorescence microscope is any microscope that uses fluorescence to generate an image. The specimen is illuminated with light of a specific wavelength which is absorbed by the fluorophores of the specimen, causing them to emit light of longer wavelengths and so of a different color than the absorbed light. Then the illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter. In this manner, the distribution of a single fluorophore (color) is imaged in a specimen (e.g. the distribution of a protein inside a cell).

7. What is the function of revolver holder of objectives?

The function of revolver holder of objectives is to hold a set of objective lenses. It allows the user to switch between objective lenses allowing different magnifications without moving the specimen.

8. What is the principal of observation in “dark field”?

To obtain a "dark field" image, an opaque disc is placed underneath the condenser lens, so that only light that is scattered by objects on the slide can reach the eye. Instead of coming up through the specimen, the light is reflected by particles on the slide. Specimens are visible regardless of color, usually bright white against a dark background. Pigmented objects are often seen in "false colors," that is, the reflected light is of a color different than the color of the object.

9. Which types of electron microscopes are used in biomedicine?

There are two main types of electron microscope (EM) – the transmission EM (TEM) and the scanning EM (SEM). The transmission electron microscope is used to view thin specimens (e.g. tissue sections, molecules position in cell) through which electrons can pass generating a projection image. The scanning electron microscope is used to produce 3D images of specimens (e.g. surface of tissues or whole organisms) by reflection of electrons on the specimen's surface.

10. What is the principal of transmission electron microscope?

The Transmission Electron Microscope (TEM) operates on the same basic principles as the light microscope but uses electrons instead of light. Because the wavelength of electrons is much smaller than that of light, the optimal resolution attainable for TEM images is many orders of magnitude better than that from a light microscope. The source of illumination is a beam of electrons of very short wavelength, emitted from a tungsten filament at the top of a cylindrical column of about 2 m high. The whole optical system of the microscope is enclosed in vacuum. Air must be evacuated from the column to create a vacuum so that the collision of electrons with air molecules and hence the scattering of electrons are avoided. Along the column, at specific intervals magnetic coils are placed. Just as the light is focused by the glass lenses in a light microscope, these magnetic coils in the electron microscope focus the electron beam. The magnetic coils placed at specific intervals in the column acts as an electromagnetic condenser lenses system. The specimen must be stained with an electron dense material and is placed in the vacuum. The electron beams are passes through the specimen and scattered by the internal structures. Then they are recorded by hitting a fluorescent screen, photographic plate, or light sensitive sensor like CCD (charge-coupled device) camera. The image detected by the CCD may be displayed in real time on a monitor or computer.

2. Native and permanent slides of different cell types. Osmotic phenomena and cell.

Test yourself:

1. Which types of preparation are recognized according to way of preparation?

The main types of preparing biological samples for microscope observation are wet mount, dry mount, smear, squash and staining. They can be separated to native slides (fresh of alive material observation) and permanent slides (fixed material).

2. In which occasions do you preferred utilization of native preparation?

When we want to observe natural activity of organisms (e.g. phagocytosis, flagella movement).

3. What does cell fixation mean?

Cell fixation is the process by which internal and external structures of a cell are preserved.

4. Which types of cell fixation are used in biomedicine?

Heat fixation: This helps to preserve the overall morphology of a cell. This is commonly used in smear preparation and preparation of temporary slides.

Chemical fixation: In this method chemicals are used to preserve fine cellular substructures and morphology of more delicate microorganism. Chemical fixatives are commonly used in preparation of permanent slides.

5. Which types of cell staining do you know?

We have used the following types of cell staining:

Lugol solution: fixing cells and staining cell parts (cell wall brown, nucleus yellow-brown, cytoplasm yellow).

Giemsa solution: staining nuclei of blood cells purple

Nile blue dye: a fluorescent dye used for sperm observation.

We also know Gram stain for bacteria identification.

6. Which organelles may be stained with Giemsa solution?

Giemsa solution mainly stains nucleus, nucleolus and chromosomes.

7. What does osmosis mean?

Osmosis is the movement of water molecules from an area of high water concentration (weak/dilute solution) to an area of low water concentration (strong/concentrated solution) through a partially permeable membrane.

8. Explain the differences of osmosis in plant and animal cells.

Animal and plant cells response differently to osmosis because plant cells have a rigid cell wall. In a hypotonic solution an animal cell will lyse and destroy while a plant cell will turgid as the cell wall will not let it burst. On the other hand, in a hypertonic solution animal cells will lose water to their surroundings,

shriveled, and probably die, while plant cells, will lose water and their plasma membrane will pull away from the cell wall at multiple places (plasmolysis) causing the plant to wilt.

9. In which solution cytolysis occurs?

Cytolysis occurs in a hypotonic solution (a solution with lower concentration of dissolved substances than the concentration of the substances inside the cell). If we place a cell in hypotonic solution water enters the cell until it ruptures.

10. The environment with higher osmotic activity than the osmotic activity of solution in the cell is:

A solution with higher osmotic activity is more concentrated; that is it has more molecules dissolved in water than another solution (e.g. cell). So an environment with higher osmotic activity is hypertonic to the cell.