Chapter 7

Haematology Experiments

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EXPERIMENT NO. 1

AIM: 1. TO UNDERSTAND THE WORKING PRINCIPLE OF MICROSCOPES IN GENERAL
2. TO STUDY COMPOUND MICROSCOPE IN DETAIL

Working of Microscope

Magnification

It is defined as the power of enlargement of an object that cannot be viewed by the naked eye and is done by the microscope so as to see clearly and distinctly the details and contours of closely placed structures of an object.

$$M = \frac{\text{Size of an object/image observed under microscope}}{\text{Actual size of an object}}$$

Resolution

It is defined as the ability to distinguish two closely located points as distinct.

Resolution Power

It is the minimum distance at which two close points can be seen as separate so as to improve the details of an image. It is also known as limit of resolution. Resolution power of unaided human eye is 1/60 of a degree/or 100 mm.

$Lm = \frac{0.61 \times \lambda}{NA}$ where, Lm is limit of resolution, λ is wavelength of light.

Resolution power of a lens depends upon wavelength of light and numerical aperture of lens. It can be increased by increasing numerical aperture and decreasing the wavelength of light. NA is numerical aperture of lens.

$NA = n \sin \alpha$

where, n is refractive index of the medium and sine α is sine of semi-angle of light passing through the objective lens from the specimen. Numerical aperture can be increased by increasing the refractive index of the medium. Refractive index of air, oil immersion lens and most of the optical articles is 1.0,1.5 and 1.6 respectively. Oil immersion objective has more numerical aperture and hence resolution power. Numerical aperture of objective is fixed and that of condenser can be varied by increasing or decreasing the amount of light through the material under observation. It can be done by:

a. By moving the condenser to the uppermost or lowermost position.
b. By operating the iris diaphragm (opening or closing).

Working Distance

- It is the distance between the front surface of the objective lens and the surface of cover glass or the object to be seen. Magnification increases with decrease in working distance.
- Working distance for oil immersion objective is 0.15–1.5 mm

- High power objective is 0.5–4 mm
- Low power objective is 5–15 mm.

Types of Microscopes

There are four types of microscopes depending upon the sources of illumination

a. Light microscope
b. Electron microscope
c. Ultraviolet microscope
d. X-ray microscope

There are various types of Light Microscopes e.g. *Compound, Phase Contrast, Dark Field and Differential Interference Microscope.*

Light (Optical) Microscope (Bright Field Microscope)

Principle: It uses light as a source of illumination and lens for magnification of images so as to reveal details of their structures.

In Compound Microscope, glass lenses are used in combination for magnification.

Phase Contrast Microscope

Discovered by Zernike in 1932.

Principle: Various components of cells refract the light to different degrees. This Microscope multiplies the small differences among the phases or refractive indices of different constituents as well as between the cell interior and outside. It converts these differences of refractive indices into differences of brightness of light.

Refractive index is the degree of light velocity retarded by a substance due to its thickness and opacity.

Uses: It is used to study cells and their constituents in the living state and various physical changes during cellular events e.g. spindle formation, *karyokinesis, cytokinesis, pinocytosis phagocytosis, spermatogenesis* etc.

Dark Field Microscope

Discovered by Zsigmondy in 1903.

Principle: The object is illuminated by oblique beam of light which becomes brightly visible against a dark background e.g. cell organelles, bacteria.

Uses: It is used to see the living objects of less than 0.3 mm. It has a special condenser. There is a disc called stop in the centre of the condenser. As the disc/stop does not allow the light to pass, the central field remains dark.

Differential Interference Microscope

Discovered by Nomarski, 1952.

Principle: The image of the living structures appears as stained due to colour contrast produced by the prism.

In this microscope, two beams of light arising from same source are separated by means of two prisms.

The beam that passes through the object undergoes diffraction or phase change. The second beam travels besides the object which does not undergo any change. These two beams come together above the object and give bright contrast.

Uses: Differential interference microscope gives information about

1. Thickness of the objects.
2. Presence of various light absorbing materials like nucleic acids, proteins, lipids etc. and gives better images as compared to phase contrast microscope.

Fluorescent Microscope

Discovered by Coons in 1941.

Principle: A tissue stained with fluorescent dye absorbs radiation of short wavelength i.e. ultraviolet radiation, gets excited and emits back light energy of long wavelength, which is in the visible spectrum and is seen by fluorescent microscope.

Uses: It is used in immunological laboratories .The substances, which can emit fluorescence, when excited by short wavelength light can be used for tagging cellular components and are known as Fluorochromes e.g. acridine orange, FITC.

Electron Microscope

Principle: In Electron Microscope, three electromagnets are used in place of glass lenses i.e. condenser, objective and projector. A beam of electrons is made to pass through high vacuum for illumination of object and image formation. There are two types of electron microscopes

1. Transmission electron microscope: It was invented by Knoll and Ruska in 1931. It magnifies the image 100,000 -300,000 times and resolution power is 1-10A°.
2. Scanning electron microscope: It was discovered by Knoll in 1935. It has magnification range up to 200,000. Resolution 10 nm.

Uses: It is employed in the study of ultra structure of cells and small structures like spores and microorganisms.

Compound Microscope in Detail

Compound Microscope was invented by Antonie van Leeuwenhoek in 1674. It is an optical instrument by which objects that are not visible to the naked eye are magnified. It consists of:

1. *The stand or base:* It comprises of a heavy foot and is connected to the handle, which bears the optical system. It gives mechanical stability to the instrument. It supports the microscope on working table.
2. *Handle:* It is curved and the microscope can be tilted at the hinge when desired.
3. *Tube:* It is a cylindrical tube through which light traverses. Its length determines the mechanical length of the microscope (mechanical length is the distance between upper part of the objective and eye piece). It consists of two parts:
   a. *Outer or external tube:* It bears nosepiece at lower end of the tube to which three objective lenses are fitted
      - Low power 10×
      - High power 40×
      - Oil immersion 100×

b. *Inner tube:* It carries the eyepiece at its upper end having magnification of 5× or 10×. Inner tube can slide inside the outer tube to adjust the mechanical length. Normally it is 160 mm.

4. *Fine and coarse adjustment*: With help of these adjustments, height of the tube can be adjusted so that the objective lens can be positioned at its optimal distance (focal length) so that the object can be examined. A third knob is there to move condenser.
5. *The stage*: It is a platform on which glass slide is accommodated on which the object is mounted. There is an aperture in the centre to permit light to reach the object.
6. *The sub-stage*: It consists of a condenser and a diaphragm.
   a. *Condenser:* It is a system of lenses, which focuses light from light source on the object. The intensity of illumination of the object can be varied by raising or lowering the condenser. Position of condenser is highest when oil immersion is used and lowest when low power objective lens is used.
   b. *Iris diaphragm:* It controls the amount of light reaching the condenser.
7. *Mirror*: At the foot end of the microscope below the condenser, the hinged double reflecting mirror is fitted, which is plane on one side and concave on the other side. Plane mirror is used when condenser is at the highest position and source of light is diffuse e.g. sunlight. Concave mirror is used when source of light is artificial or limited e.g. bulb or tube light as in laboratory.

Calculation of Magnification of Object

Power of eyepiece × power of objective lens × tube factor

Method of Focusing of an Object

- *Under low power (10 × objective):* Place the slide on the mechanical stage and bring the object near the central aperture in the stage and bring the low power objective above the aperture of stage. Using distant light/artificial light and plane/concave mirror focus the light on the object with condenser in its lowest position. Using coarse adjustment, lower the tube and focus the object. Now with the help of fine adjustment the slide can be brought to a sharp focus.
- *Under high power (40 × objective):* Revolve the nosepiece clockwise to bring the high power objective near the central aperture of the stage. Condenser is raised. Iris diaphragm is fully opened and concave mirror is used to focus artificial light on the object under observation. The object is finally focused using fine adjustment.
- *Oil immersion (100 × objective):* Revolve the nosepiece to bring oil immersion objective above the object. Raise the tube and a drop of cedar wood oil is placed on the area of the slide to be focused. Lower the tube again till the objective just touches the drop of oil. Iris diaphragm is fully opened and condenser is in top position. Then using fine adjustment, focus the area to be examined.

Precautions

- Eye piece as well as objectives should be cleaned after every use
- Always clean the objectives with xylene, not with alcohol as alcohol may dissolve the cement used in component of lenses
- Never lower the tube quickly while looking through the eyepiece

- As the tube is lowered using coarse adjustment, visualise from the side so that the coarse adjustment is stopped as the objective just touches the drop of oil. This step is taken to prevent the damage to the objective especially with high power and oil immersion lens
- Care should be taken while handling the microscope. (Always hold upright by handling with a hand below its base).

QUESTIONS

1. Describe the principle of microscopy and explain resolution.
2. Explain the numerical aperture and working distance in microscopy and its relation to magnification.
3. What are different adjustments made in the microscope while using different types of objectives and sources of light?
4. Enumerate the functions of condenser and iris diaphragm.