E-CONTENT PREPARED BY

Dr. Sandip Majumder

Assistant Professor of Department of

Conservation Biology

Durgapur Government College, Durgapur, West Bengal

(*Affiliated to Kazi Nazrul University, Asansol, West Bengal*)

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E-Content prepared for students of

M.Sc.(Semester-IV) in Conservation Biology

Name of Course:
Biostatistics and Bioinstrumentation

Topic of the E-Content:
Microscopic Techniques

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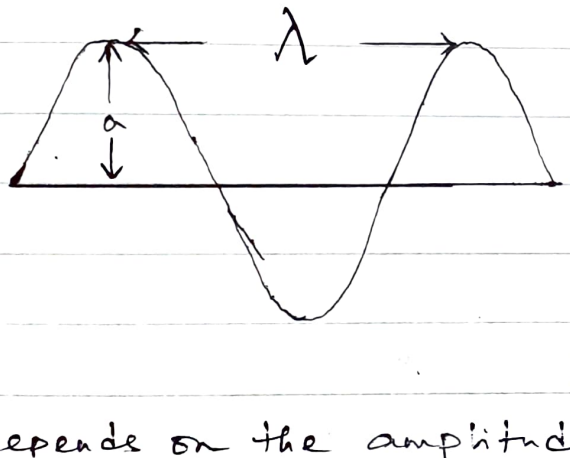
OPTICAL MICROSCOPE

(1) BRIGHT FIELD MICROSCOPE :

(a) Light is an electromagnetic radiation, each region of the electromagnetic spectrum is characterised by its wavelength and the visible region is in the 4000-7000A° (400-700nm). Light is emitted as a series of energy pulses from a source. Each pulse has a maximum and a minimum forming a wave & a phase. The maximum & the minimum energy level forms the peak and trough of a wave.



(b) The distance between the peaks of two successive phase is the wavelength 'λ'. The no. of pulses / time is the frequency 'f'.

(c) The intensity or brightness depends on the amplitude 'a'. In vacuum, light has a constant velocity c which usually decreases as the medium becomes denser.

(d) When two light waves cross each other, they can either reinforce their intensity or cancel each other to darken an object. The phase difference δ between two waves is related directly to the path diff x between the waves & inversely proportional to their

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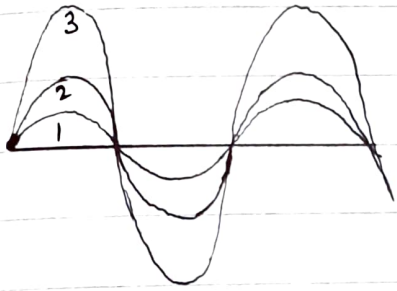
wavelength. $\therefore \delta = \frac{2\pi n}{\lambda}$.

This is called INTERFERENCE.

(i) If the phase of two light waves are in the same phase, coinciding each other in their peaks & troughs, the amplitude a is doubled. This is called CONSTRUCTIVE INTERFERENCE.



(ii) When the waves are superimposed in such a way that the peak of one coincide with trough of the other the amplitude becomes zero, causing a darker background. This is called DESTRUCTIVE INTERFERENCE.

(e) Diffraction is bending of light waves while emerging from the edge of an obstacle or an aperture. A diffraction pattern has an uniformly illuminated central spot & several concentric and alternate bright & dark fringes. This results from interference between light waves that are made out of phase to each other due to diffraction.

(f) Refraction is the change in the direction of propagation of light as they cross the interface between two media of different density. When ever light travels between media, the velocity changes, as a result

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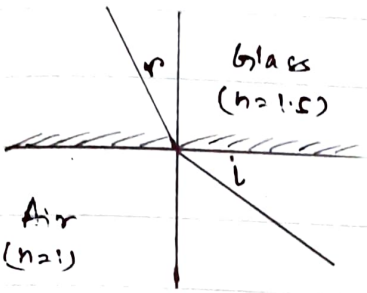
light waves change path, if and when they strike the interface at any angle other than 90°.



$$\sin r = \sin i \times \frac{n_1}{n_2}.$$

n_1 = density of medium 1
n_2 = density of medium 2
i = angle of incidence
r = angle of refraction.

When light passes from lighter to denser medium, refraction occurs towards normal i.e. $i > r$.

PRINCIPLE OF OPTICAL MICROSCOPE:

(i) In bright field optical microscopy, a light source is used to illuminate the object and a lens system focusses the transmitted/scattered light rays from the object, forming its image.

(ii) Rays of light from an illuminating source are condensed on the object by a condenser lens (Kohler illumination) and the objective lens system collects the light coming from the object, to form a real, inverted and magnified image of the object (primary object). The eye piece lens system or Projector lens focusses the diverted rays of the primary image on the retinal plane. as a magnified virtual image.

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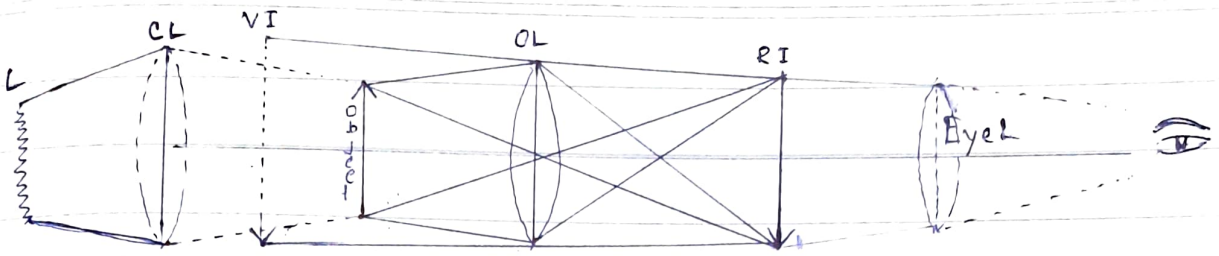

· Principle of Bright field Light Microscope.

L = Light Source, CL = Condenser lens; OL = Objective lens
EL = Eye piece lens; RI = Real Image; VI = Virtual image.

A COMPOUND MICROSCOPE : BRIGHT FIELD :

(1) Bright field microscope uses the visible light rays transmitted through the object to produce an magnified image of the latter.

(2) A plano concave mirror reflects the diffuse light to the condenser lens system located below the stage supporting the specimen.

(3) The substage condenser consists of a combination of achromatic convex & concave lenses for correcting the spherical and chromatic aberration. The condenser converges a cone of bright light to focus it at the plane of the specimen.

(4) An iris diaphragm has an aperture which can be adjusted to regulate the light beam.

(5) Two to six objective lens system are present above the stage and is screwed to a movable circular disc. Each lens is a combination of achromatic convex & concave lenses.

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(6) The objective lens collects light rays from the specimen and forms an inverted and magnified real image just within the focal point of eye piece lens located at the top.

(7) The objective lens not only magnifies the object, but also sets highest resolution.

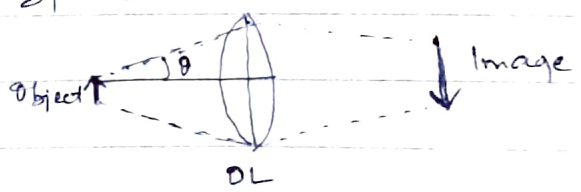
(8) The eye piece lens system uses the magnified real image to produce a more magnified virtual image near the plane of the object. Thus the object is magnified twice but inverted once.

NB: MAGNIFICATION: It is the ratio between the apparent size of object seen in a virtual image and the actual size of the object.

RESOLUTION: It is the capacity of the objective to distinguish between two closely set point in a specimen as separate entities. The shortest distance between such points is the limit of resolution. (y) Smaller the y, higher is the resolution. It depends on the Numerical Aperture (NA).

NA = $n \sin\theta$ Where θ is the semiangle accepted by objective

& $y = \frac{0.61\lambda}{NA}$



Where λ = wavelength of light

Ref: Biophysics & Biophysical Chemistry, Debojyoti Das Academic Publishers.

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