Name: PRECIOUS OROGBU

Matric no:16/MHS/06/060

Course code: MLS 410

Questions

1. Discuss the physics of the light microscope diagrams and illustrations needed

2. Write notes on the biomedical equipment. Add notes on principle, brand, care and maintenance and cost A. Centrifuge B. Automatic Tissues Processor C. Microtome

ANSWER

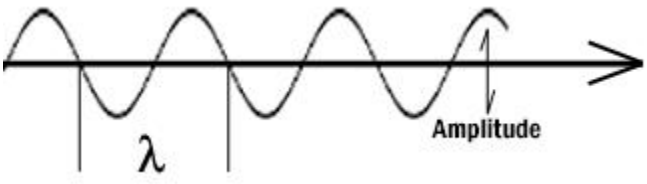
1. Light Microscopy

Introduction

The light microscope is an instrument for visualizing fine detail of an object. It does this by creating a magnified image through the use of a series of glass lenses, which first focus a beam of light onto or through an object, and convex objective lenses to enlarge the image formed

Principles of Light, Electrons, & Microscopy

In microscopy we take advantage of waveform properties of light. These waves when produced at a particular source vibrate at right angles to the line of propagation. Each wave has a peak and trough. The distance traveled forward by the light ray is one wavelength (lambda). Wavelength varies with the color and intensity of the source.



A Schematic diagram of a wave

HOW IMAGE IS FORMED

The structures the light microscope is called upon to resolve exert only a small influence on the light they transmit. What is changed is the phase of momentary vibration. Conventional bright field illumination will lack contrast and the details of the sample remain invisible. When the emerging waves have acquired a larger phase difference due to changes in refractive index, greater contrast is produced. This manifests itself by an edge effect (diffraction, refraction, and reflection). Sample details may be resolved in a number of ways. When a light passes through stained structures intensity is reduced selectively depending on the color and density of the sample as the light is absorbed. Selective absorption of wavelengths of white light produces colored light. Refraction changes the direction of a light ray as it passes from one medium to

another. The shorter the wavelength, the greater the refractive angle. Diffraction is the bending of light rays around objects with sharp edges. A new wave front is created at this edge. Diffraction can be useful, but can also reduce resolution. When light is dispersed it is separated into its constituent wavelengths as a result of refraction on entering a transparent medium. Contrast can be defined as a steep slope between bright and dark image points. Adequate contrast MUST be achieved before the specimen can be resolved.

Two terms that are often confused, but are central to microscopy are magnification and resolution. Magnification is the degree by which dimensions in an image are, or appear to be, enlarged with respect to the corresponding dimensions in the object. Resolution is the act or result of displaying fine detail in an image. Magnification without resolution would be meaningless.

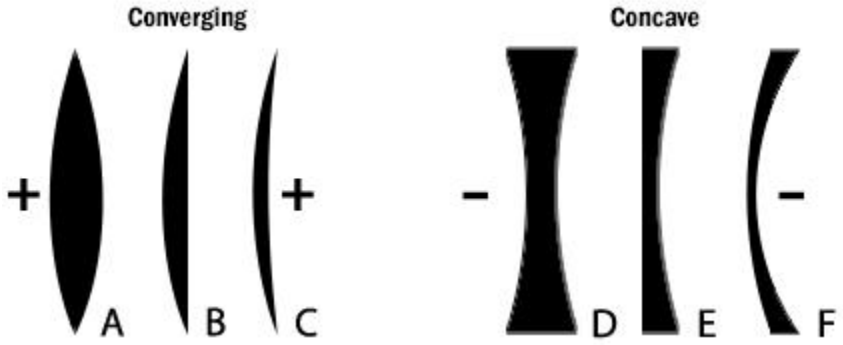
The theoretical resolution of the light microscope was first defined by Abbe in the following equation.

Abbe's equation for theoretical resolution of the light microscope:

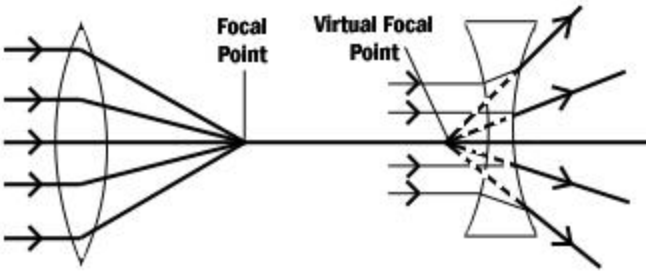
$$d = 1.22 \times \lambda o / n \sin \mu$$

d=distance of resolution
λ=wavelength of incident light
μ= 1/2 angle of incoming light

The Lenses



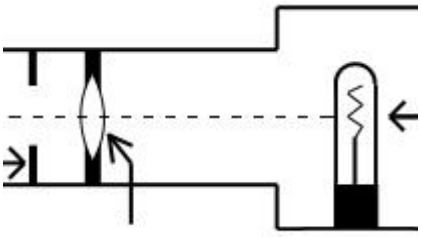
The six simple lenses. A, B, & C are converging or positive lenses. D, E, & F are concave or negative lenses.



Converging and diverging lenses. Parallel rays enter a convex lens converge and meet at the principle focus. The distance between the center of the lens and the principal focus is the focal length. Parallel rays entering a concave lens diverge and never meet.

The Objective Lens is the first part of the imaging system; the objective lens forms a primary, enlarged image of the object. Very fine details are distinguished with the objective lens. The eyepiece sometimes called the ocular lens, is the second lens, which forms a secondary, further enlarged image. By multiplying the magnifying power of the objective lens and the magnifying power of the ocular the final magnification is found. A Substage Condenser lens is the third optical component. It is placed on a platform beneath the object. Light is directed through the substage condenser and converges to a point at the position of the specimen. The light rays diverge as they pass through the specimen and form an inverted cone, whose base is just large enough to fill the aperture of the objective. The size of the light beam is controlled by a diaphragm beneath the condenser called the aperture diaphragm.

The light source should contain both a lens to project an image of the lamp filament called a field condenser and a diaphragm to control the size of the illuminated field called a field diaphragm.



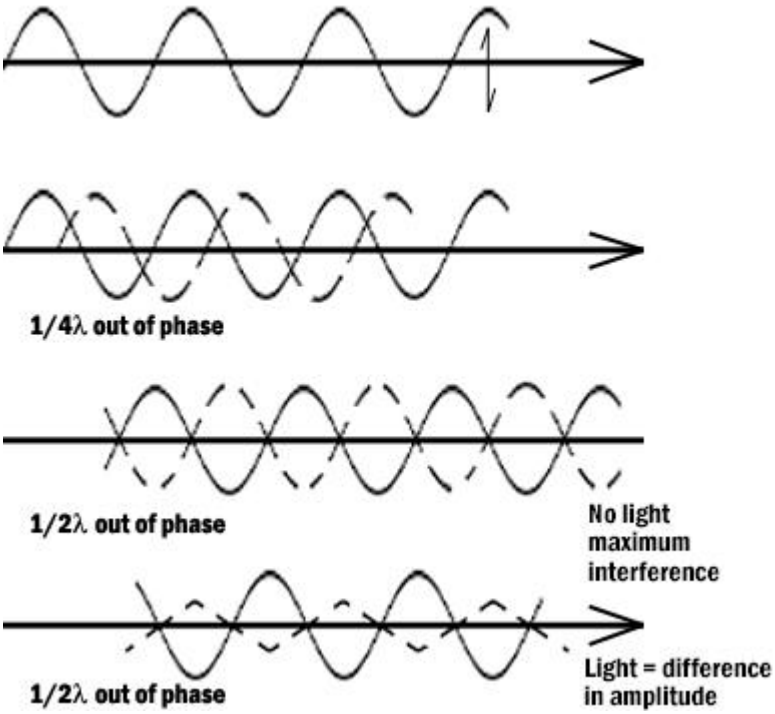
Typical lamp for light microscope.

Kohler Illumination is the most common method of illumination. In Kohler illumination the image of the source is projected by the field condenser onto the substage condenser, to the top of the plane of the object. This method assures even illumination.

Modern light microscopes use several different modes of operation depending on the needs of the investigator. The most common of these being brightfield microscopy in which direct light passes through the objective aperture and illuminates the background against which the image is seen. Since the structural elements being resolved have little variance in refractive index, the

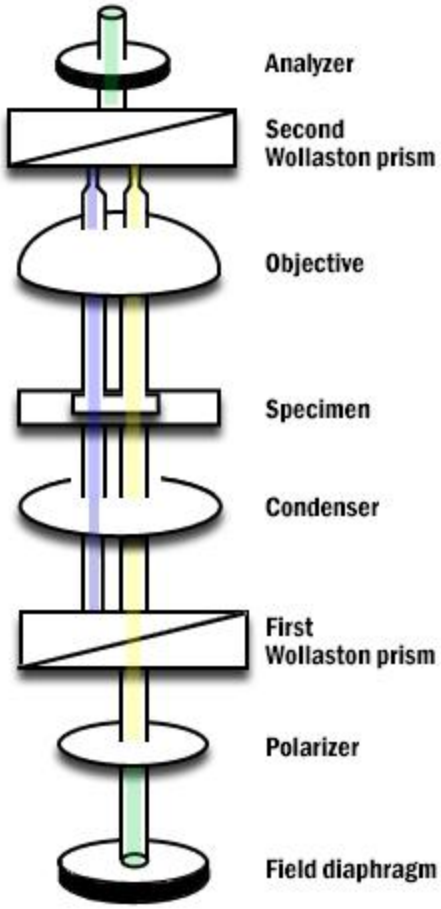
image will lack contrast and the details remain invisible. Small structure detail can be revealed by changing the absorption of the object by means of staining.

Many microscopes have special lenses that allow an investigator to image using phase-contrast. This method reveals details in specimens possessing very slight differences in optical path or refractive index in the surrounding media. Phase contrast requires no staining and can be used with living tissue. Contrast is produced in this method by phase differences in the light leaving the object to amplitude differences in the image. A phase plate alters the optical path length traveled by diffracted light from that traveled by direct light.



Light waves in bright field and phase contrast after passing through an object. Pathway A represents the lightwave before encountering the object. Wave B represents the wave phase after passage in brightfield (unstained mode). C compares the wave phase of an object viewed with phase contrast.

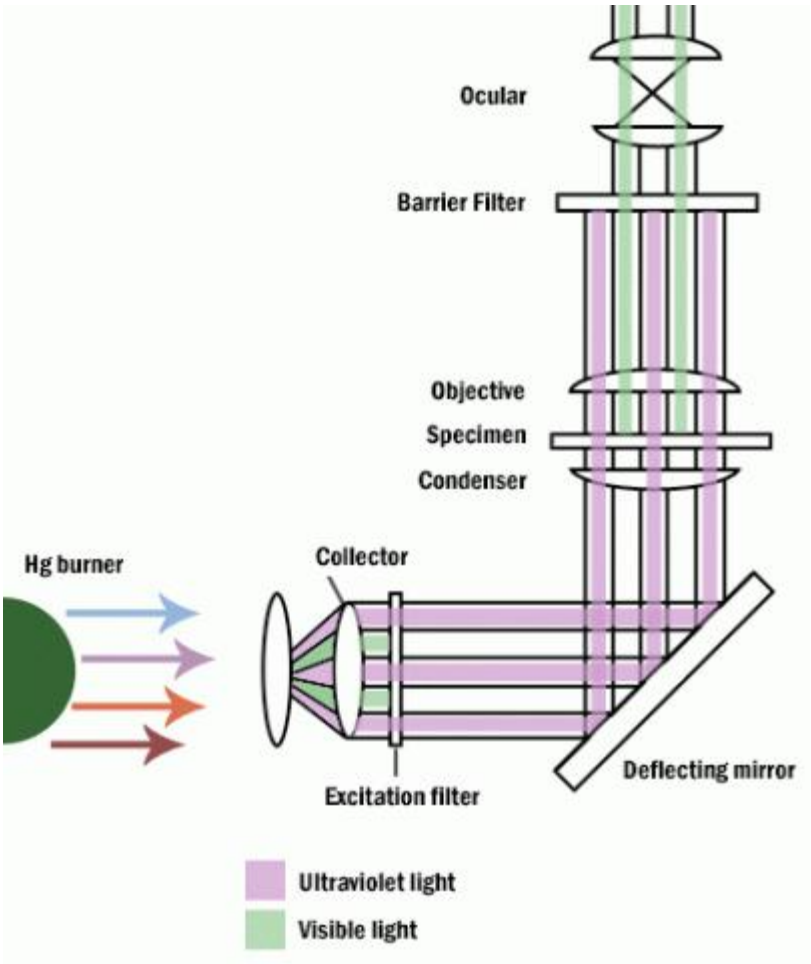
Differential interference contrast (DIC) differs from phase contrast in that the image has a strong relief and three-dimensional appearance. It must be remembered that the impression of surface details are the results of the optics and not the specimen for most biological samples. The optics for DIC consist of a polarizer at the light source and Wollaston prisms in the condenser and above the objectives. The beam passes through the polarizer, enters the first prism where it is split in two. One beam vibration is parallel to the prism and one is perpendicular. Both beams pass through the specimen in parallel in close proximity and are recombined in the second prism.



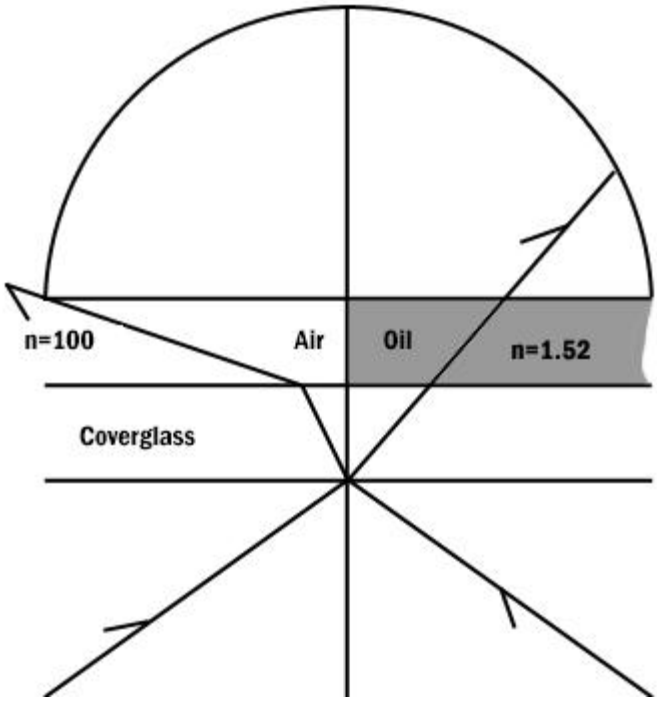
Differential Interference Contrast Schematic.

Darkfield microscopy is a mode in which direct light is prevented from passing through the objective aperture, but a hollow cone of light forms an apex in the plane of the specimen. The image is formed by light scattering from features of the object. Details appear bright white against a dark background. Darkfield can also be used for living bacteria, algae and plankton.

Some materials produce light when excited by short wavelengths of radiation. This effect is called fluorescence or auto-fluorescence. Specimens that do not fluoresce by themselves may be treated with fluorochromes which produce a secondary florescence. By illuminating with a high intensity mercury or xenon source and filtering out all but the desired excitation wave length to contact the specimen, the resulting longer (less energetic) wavelengths of emission from the specimen its self-viewed. Fluorescence microscopy can be used to enhance particular organelles, immunocytochemistry, in-situ hybridization, enzyme cytochemistry and elemental localization.



Fluorescence microscope.



Comparison of a dry and an oil immersion objective.

Abbe in order to ease in identification of lens quality devised an equation for numerical aperture. Numerical aperture numbers can assist in comparing angles of dry, water immersion, and oil immersion objectives. Note the similarity to Abbe's equation for theoretical resolution. This number is found on all objective lenses.

$$N.A. = n \sin u$$

n = refractive index of medium

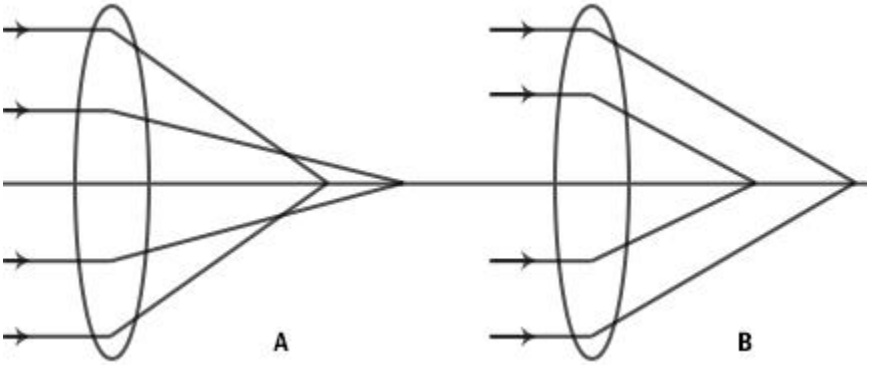
u = 1/2 the angle of light rays taken in when focused on the object.

When choosing an objective another consideration is depth of field. Depth of field is the distance from the nearest part of the subject in acceptable focus to the farthest part of the subject in acceptable focus. The efficiency (resolution) of a lens is inversely proportional to the depth of field

N.A.	.25 .30 .50 .65 .85 .95
Depth (in microns)	8.0 5.5 2.0 1.0 .25 .10

Variation in Depth of Field with Change in N.A.

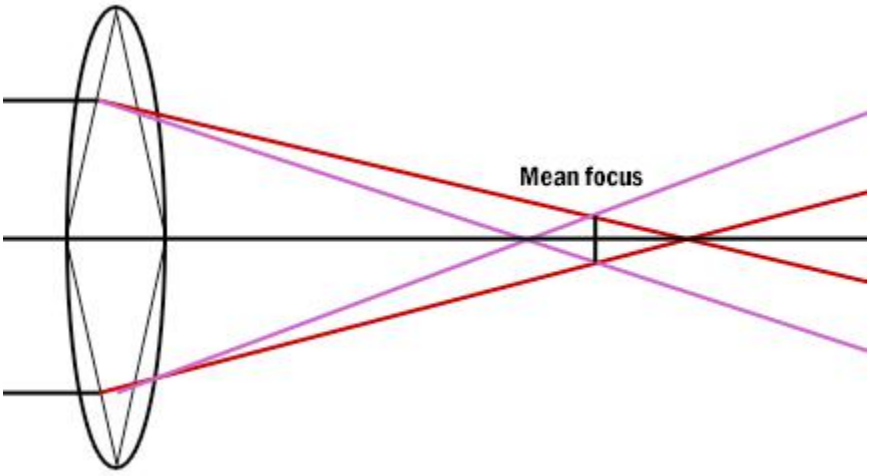
Two aberrations within lenses detract from Abbe's equation of theoretical resolution. These aberrations are called spherical aberration and chromatic aberration. Spherical Aberration occurs when outer rays entering a lens are diffracted differently from those entering near the center. A solution for reducing spherical aberration is introducing a diaphragm or aperture.



Spherical aberration of a simple lens. A. Under correction. B. Over correction.

The thickness of the cover glass should be chosen according to specifications of a particular objective. Deviation from the required thickness results in over correction or under correction of spherical aberration.

Chromatic Aberration occurs as white light entering a lens is broken into a spectrum from red to violet. Violet rays (more energetic) are refracted more than the red rays (less energetic). Consequently, an uncorrected lens will be surrounded by color fringes. The more expensive lenses have a higher degree of correction.



Chromatic aberration of a simple lens. Each spectrum color has a separate focus.

2.

A. CENTRIFUGE

A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a force perpendicular to the axis of spin (outward) that can be very strong. A laboratory centrifuge is a motor-driven device used in laboratories for the purposes of separating the components of a liquids.

PRINCIPLES OF CENTRIFUGATION

Particles suspended in a fluid move, under the influence of gravity, towards the bottom of a vessel at a rate that depends, in general, on their size and density. Centrifugation is a technique designed to utilise centrifugal forces, which are greater than the force of gravity, to speed up the sedimentation rate of particles. This is achieved by spinning the vessel containing the fluid and particles about an axis of rotation so that the particles experience a force acting away from the axis. The force is measured in multiples of the Earth's gravitational force and is known as the relative centrifugal field (RCF) or, more commonly, the '***g***' force.

TYPES OF CENTRIFUGE

- Small Bench Centrifuges
- Large Capacity Refrigerated Centrifuges
- High Speed Refrigerated Centrifuges
- Ultra Centrifuges
- Fixed Angle Rotors
- Vertical Tube Rotors
- Zonal Rotors
- Elutriator Rotors

BRANDS OF CENTRIFUGE

- Corning
- Leica
- Celnee
- McKesson
- Fisher Scientific
- Schott

CENTRIFUGE USE

- ✓ **The work surface must be level and firm**. Do not use the centrifuge on an uneven or slanted work surface.
- ✓ **Balance the tubes in the rotor**! If you want to run a tube with 10 mL of liquid, put another tube with 10 mL of water in the opposing hole on the rotor. If the liquid has a higher or lower density than water, you must balance the tubes by mass, not volume.

- ✓ **Do not open the lid while the rotor is moving**. Even though many centrifuges have a "safety shutoff" if the lid is opened, the only thing this does is stop powering the rotor. The rotor will still spin due to its own inertia for a while until friction slows and eventually stops it.
- ✓ **If you see it wobbling or shaking, turn it off or pull the plug**. A little vibration is normal, but excessive amounts can mean danger. FIRST, double check that you correctly balanced the tubes. If the answer is yes and the wobbling still happens, contact the manufacturer or dealer and get the unit serviced. Do NOT continue to run a centrifuge that wobbles visibly when the rotor is spinning.
- ✓ **Wear a face shield and / or safety goggles** if you have to work anywhere near a centrifuge that's in use.
- ✓ **Do not bump, jar, or move the centrifuge while the rotor is spinning**. Make sure you don't have the cord dangling from a table edge where someone could catch their foot in it and pull down the centrifuge.

COST

The cost of centrifuge depends on the type of centrifuge. It ranges from twenty-one thousand naira to one hundred and thirty-eight thousand naira

B. Automatic Tissues Processor

A tissue processor is a device that prepares tissue samples for sectioning and microscopic examination in the diagnostic laboratory.

Principle

The principle of tissue processing is to remove the extractable water from tissue specimens and replace it with a medium that solidifies to allow sectioning. It consists of 3 stages which are dehydration, clearing and infiltrating.

Types of Automatic Tissues Processor

- ✓ Tissue transfer
- ✓ Fluid transfer

Brands of an Automatic Tissues Processor

- ✓ KEDEE
- ✓ Leica
- ✓ Thermo-scientific
- ✓ SLEE MPT

Care and Maintenance

- ✓ any spillage or overflow should be cleaned immediately.
- ✓ Accumulation of wax on the surface should be removed.

- ✓ Timings should be checked when placing the cassettes in the processor.
- ✓ Clean outside of instrument surf xylene dampened cloth.
- ✓ Ensure the reagent are monitored and changed frequently as per manufacturers instruction

Cost

The price ranges from one million two hundred and ninety-six thousand naira to about f0ur million depending on the brand.

C. MICROTOME

A microtome is a specialized precision cutting instrument, which accurately and repeatedly slices sections from a block of embedded tissue

Principle

microtome is a sectioning instrument that allows the cutting of extremely thin slices of a material known as section. microtome is used in microscopy, allowing for the preparation of sample for observation under transmitted light or electrons radiation.

Types of Microtome

- ✓ Rocking microtome
- ✓ Rotary microtome
- ✓ Rotary rocking microtome
- ✓ Base sledge microtome
- ✓ Sliding microtome
- ✓ Freezing microtome
- ✓ Vibrating microtome
- ✓ Saw microtome
- ✓ Cryostat microtome
- ✓ Ultra microtome
- ✓ Laser microtome

Brands of Microtome

- ✓ Leica
- ✓ Thermo-scientific
- ✓ KDEE
- ✓ Myr
- ✓ SLEE
- ✓ BIOBASE
- ✓ Leedo

CARE AND MAINTENANCE

- ✓ dust accumulation must be prevented by putting a cover when not in use
- ✓ Wipe the moving parts regularly with good neutral oil to lubricate and avoid rust
- ✓ After cutting clean frequently from accumulated paraffin using a soft brush with xylene

- ✓ Never adjust the screws too tightly that they may cause binding
- ✓ Lock wheels and guard and remove the blade from the blade holder after use

COST

The cost of a microtome depends on the type and the automation. It ranges from three hundred thousand to one million naira.