RAMA UNIVERSITY

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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OF ENGINEERING & TECHNOLOGY

LT 1: Light Microscope

Content Outline

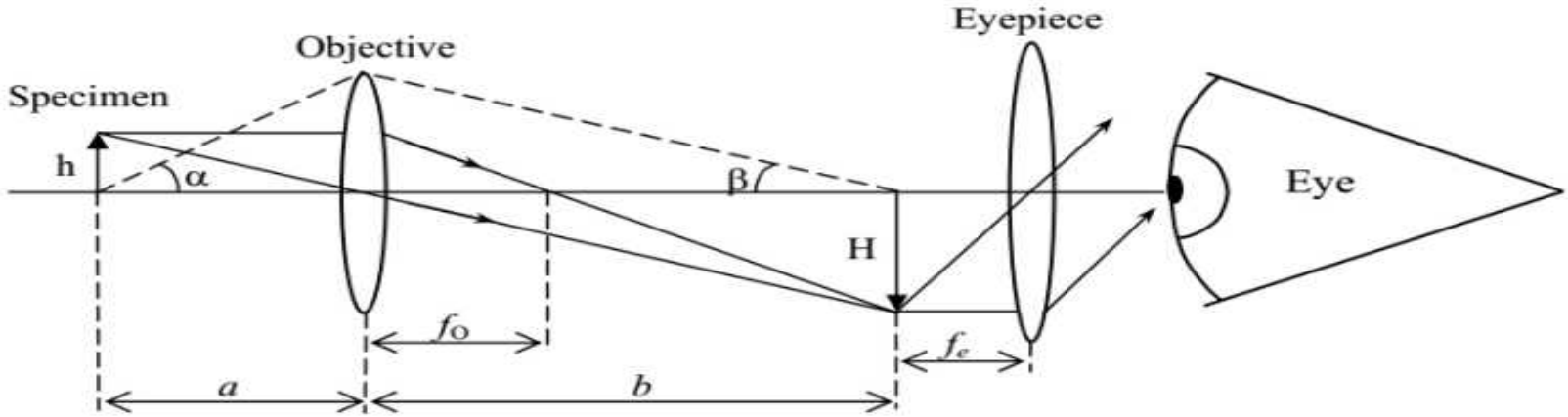
Light microscope principle

Light microscopy

A **light microscope** uses focused light and lenses to magnify a specimen, usually a cell. In this way, a light microscope is much like a telescope, except that instead of the object being very large and very far away; it is very small and very close to the lens.

Types of microscope

Light microscopes can come in several forms. **Simple light microscopes** use a single lens to magnify an object and cannot reach high magnification. **Compound light microscopes** use two sets of lenses - an objective lens and an eyepiece - to produce images. **Monocular** microscopes have one eyepiece, while **binocular** microscopes have two eyepieces and reduce eye strain.



Light microscopes send light through a path that first focuses the light into a tight beam and then passes that light through a sample, which creates an image. That image then passes through one or more lenses to magnify it until it reaches the user's eye or a camera. Because light needs to pass through the sample, it must be either very small or very thin. Most cells (bacterial or otherwise) are both small and transparent, and so light can easily pass through them.

Important Terms & Definition related to Light Microscope

Magnification: Magnification = The angle subtended by a (small) object as seen through the microscope, divided by the angle when the same object is viewed by the naked eye at a distance of 250 mm. Using the denotations in Fig. 1, we can express the magnifications of the objective, M0, and eyepiece, Me, as

$$M_o = \frac{H}{h} = \frac{b}{a}$$

$$M_e = \frac{250\,(\text{mm})}{f_e\,(\text{mm})}$$

➢The total magnification of a microscope is obtained by multiplying the objective and eyepiece magnifications. Typically total magnifications are in the range 100X to 1000X.

•Resolution: **Resolution** is the ability of a lens to separate or distinguish between small objects that are close together. Resolution is best when the distance separating the two tiny objects is small. The minimum distance (*d*) between two objects that reveals them as separate entities is given by the Abbé equation, in which lambda () is the wavelength of light used to illuminate the specimen and *n* sin is the numerical aperture (NA).

$$d = \frac{0.5\lambda}{n \sin \theta}$$

•As *d* becomes smaller, the resolution increases, and finer detail can be discerned in a specimen.

•The wavelength must be shorter than the distance between two objects or they will not be seen clearly.

•Thus the greatest resolution is obtained with light of the shortest wavelength, light at the blue end of the visible spectrum (in the range of 450 to 500 nm).

Resolving power of microscope

d_{min} = 1.22 x wavelength / N.A. $_{objective}$ + N.A. $_{condenser}$

This is the theoretical resolving power of a light microscope. In practice, specimen quality usually limits d_{min} to something greater than its theoretical lower limit.

Numerical aperture: N.A. (Numerical Aperture) (n Sin θ) is a mathematical calculation of the light-gathering capabilities of a lens. The N.A. of each objective lens is inscribed in the metal tube, and ranges from 0.25-1.4. The higher the N.A., the better the light-gathering properties of the lens, and the better the resolution. Higher N.A. values also mean shorter working distances (you have to get the lens closer to the object). N.A. values above 1.0 also indicate that the lens is used with some immersion fluid, such as immersion oil. ‘n’ is refractive index of medium separating object and aperture.

Why Immersion oil is used in microscope for visualization?

The refractive index for air is 1.00. Since sin θ cannot be greater than 1 (the maximum is 90° and sin 90° is 1.00), no lens working in air can have a numerical aperture greater than 1.00. The only practical way to raise the numerical aperture above 1.00, and therefore achieve higher resolution, is to increase the refractive index with immersion oil, a colorless liquid with the same refractive index as glass. If air is replaced with immersion oil, many light rays that did not enter the objective due to reflection and refraction at the surfaces of the objective lens and slide will now do so. An increase in numerical aperture and resolution results.

Light microscope

Modern microscopes are all compound microscopes. That is, the magnified image formed by the objective lens is further enlarged by one or more additional lenses.

Bright field microscope

The ordinary microscope is called a **bright-field microscope** because it forms a dark image against a brighter background.

A typical light microscope

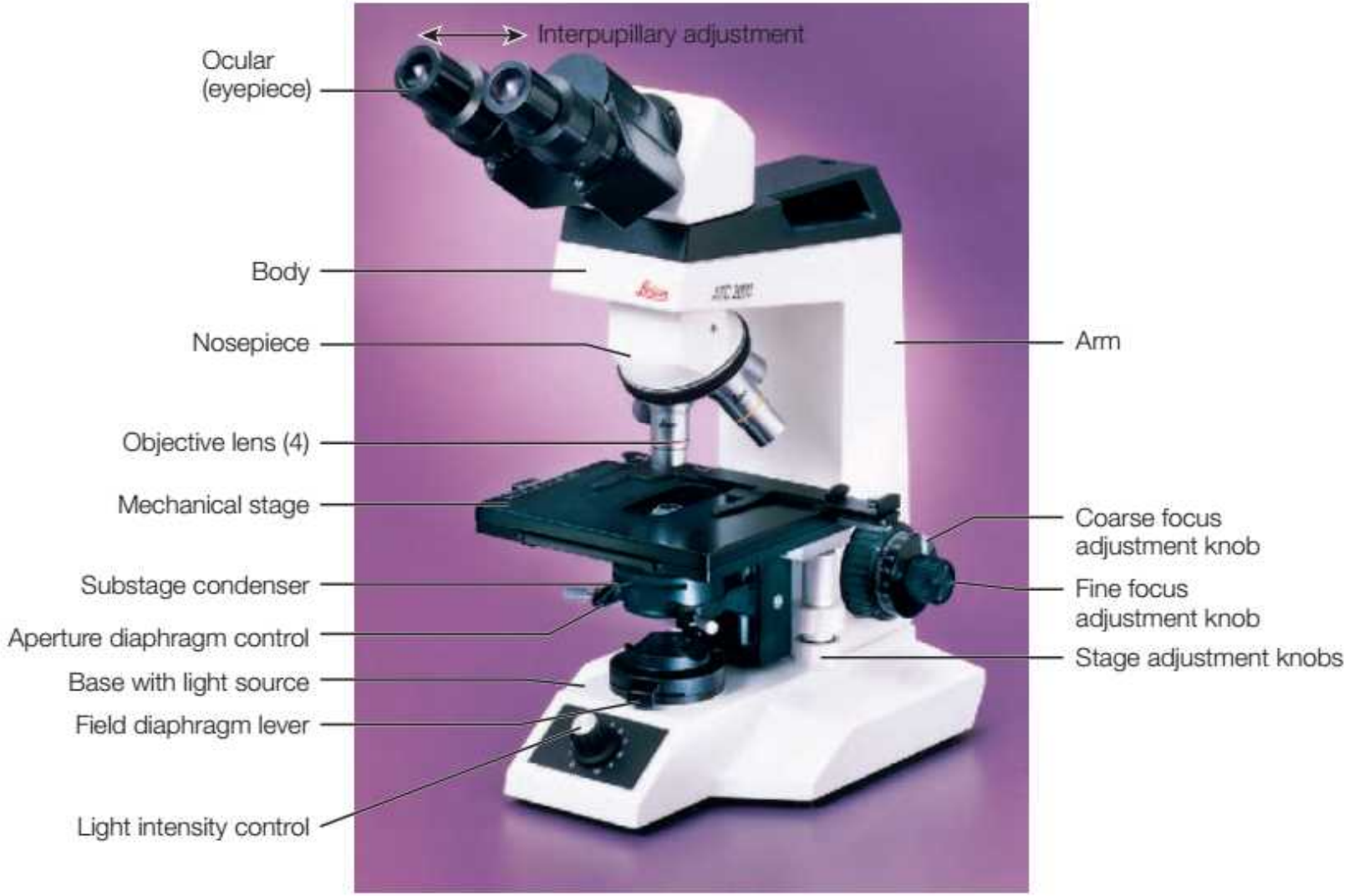
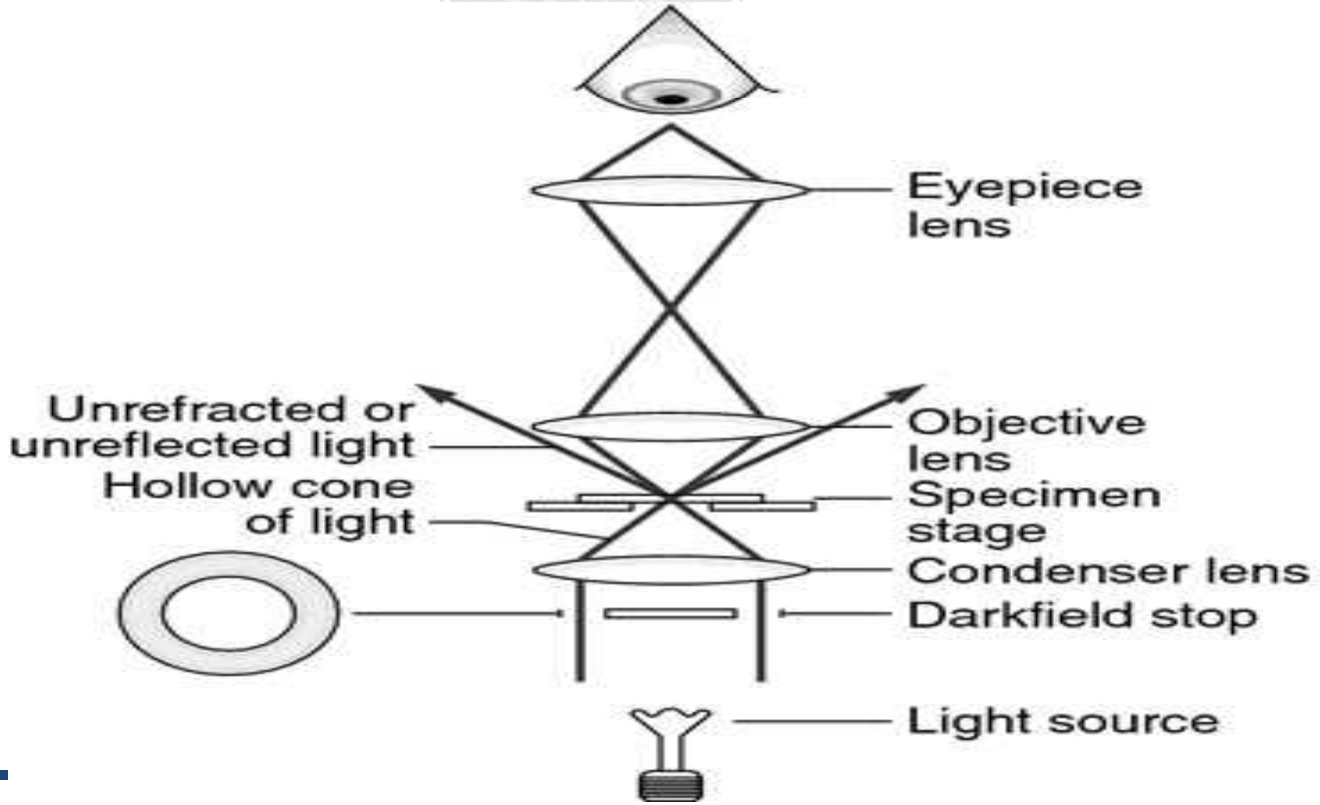


Figure source: Microbiology by Prescott

Image formation in Bright Field Microscope

The objective lens forms an enlarged real image within the microscope, and the eyepiece lens further magnifies this primary image. When one looks into a microscope, the enlarged specimen image, called the virtual image, appears to lie just beyond the stage about 25 cm away. The total magnification is calculated by multiplying the objective and eyepiece magnifications together. For example, if a 45_ objective is used with a 10_ eyepiece, the overall magnification of the specimen will be 45X.



Usage Area/ Applications

The life sciences, particularly microbiology and bacteriology, have always relied on the brightfield technique. This technique can be used to view fixed specimens or live cells. Since many organic specimens are transparent or opaque, staining is required to cause the contrast that allows them to be visible under the microscope. For visualization of specimen under bright field microscope, specimen should be stained with dyes.

Different stains and staining techniques are used depending upon the type of specimen and cell structure being examined.

For example:

•Fuchsin is used to stain smooth muscle cells

•Methylene blue is used to stain cell nuclei

Gram stain is used on bacteria and gives rise to the name gram-negative or gram-positive bacteria based on the reaction of the bacteria to the stain. In fact, many scientific journals will not accept microbiological research for publication that is not supported by gram staining and brightfield illumination methodology. Most routine medical microscopic examination of blood and tissue is performed using this illumination technique.

Advantages & Disadvantages of light microscope

Advantages

•Brightfield microscopy is very simple to use with fewer adjustments needed to be made to view specimens.

•Some specimens can be viewed without staining and the optics used in the brightfield technique don't alter the color of the specimen.

Disadvantages

•Certain disadvantages are inherent in any optical imaging technique.

•By using an aperture diaphragm for contrast, past a certain point, greater contrast adds distortion. However, employing an iris diaphragm will help compensate for this problem.

•Brightfield microscopy can't be used to observe living specimens of bacteria, although when using fixed specimens, bacteria have an optimum viewing magnification of 1000x.

•It has low contrast, therefore staining of specimens are absolutely necessary.

•This method requires a strong light source for high magnification applications and intense lighting can produce heat that will damage specimens or kill living microorganisms.

Test your understanding

References & Further reading

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