

**MICROSCOPE**

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## What Is A Microscope ?

- A **microscope** (from the Ancient Greek: micro- "small" and scope-"to look") is an instrument used to *see objects that are too small for the naked eye*.
- A microscope is an instrument that produces an accurately enlarged image of small objects.
- The science of investigating small objects using such an instrument is called *microscopy*.
- Microscopic** means invisible to the eye unless aided by a microscope.

# HISTORY

- **1st Century** - Romans experimented with glass and found objects appeared larger when viewed
- **12th Century** - Salvino D'Armate from Italy made first eye glass, providing wearer with an element of magnification to one eye.



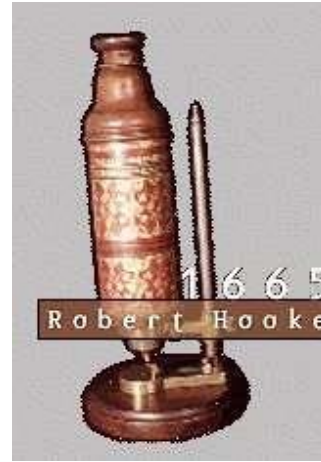
- **1590** - Two Dutch spectacle makers, Zacharias Jansen and his father Hans started experimenting by mounting two lenses in a tube, first compound microscope.



- **1609** - Galileo Galilei developed a compound microscope with convex and a concave lens.



- **1665** - Robert Hooke's book called Micrographia officially documented a wide range of observations through the microscope.



**Hooke Microscope**



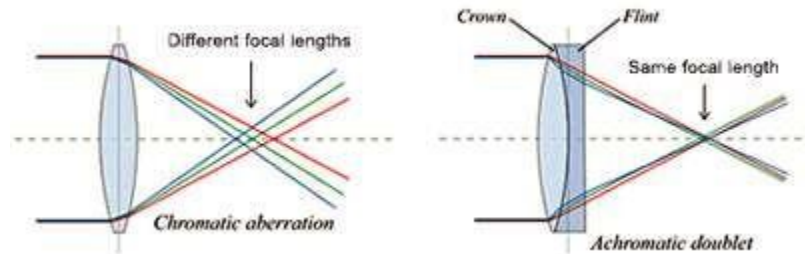
**Robert Hooke  
1635-1703**

- **1674** - Anton van Leeuwenhoek grinded lenses to achieve greater magnification which he utilised to make a microscope, enabling detailed observations to be made of bacteria.



**Anthony van Leeuwenhoek  
1632-1723**

- **1826** - Joseph Jackson Lister created an achromatic lens to eradicating the chromatic effect caused by different wavelengths of light.



- **1931** - Ernst Ruska starts to build the first electron microscope

# CLASSIFICATION OF LIGHT MICROSCOPES

## **Depending on lens system:**

**Simple**

**Compound**

## **Depending on optical technique:**

**Bright Field**

**Dark Field**

**Phase Contrast**

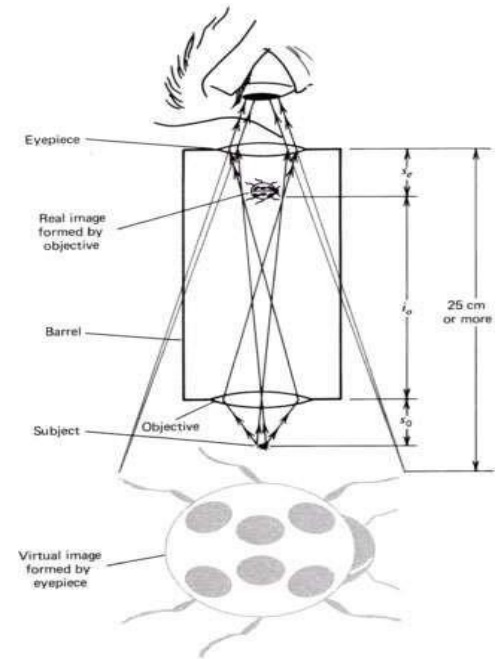
**Interference fluorescence**



# SIMPLE MICROSCOPE

Because of the limited ability of the eye's lens to change its shape, objects brought very close to the eye cannot have their images brought to focus on the retina.

The accepted minimal conventional viewing distance is **10** inches or 250 millimeters (25 centimeters).



***SIMPLE MAGNIFIER. A SIMPLE MAGNIFIER USES A SINGLE LENS SYSTEM TO ENLARGE THE OBJECT IN ONE STEP***

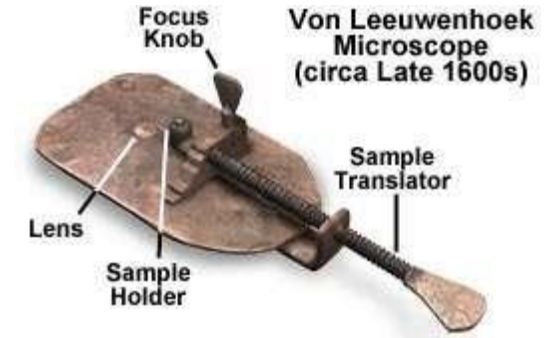
More than five hundred years ago, simple glass magnifiers were developed. These were convex lenses (thicker in the center than the periphery).

The specimen or object could be focused by use of the magnifier placed between the object and the eye.

These "simple microscopes", along with the cornea and eye lens, could spread the image on the retina by magnification through increasing the visual angle on the retina.

The "simple microscope" or magnifying glass reached its highest state of perfection, in the 1600's, in the work of Anton von Leeuwenhoek who was able to see single-celled animals ("animalcules") and even some larger bacteria.

The image produced by such a magnifier, held close to the observer's eye, appears as if it were on the same side of the lens as the object itself.



***VON LEEUWENHOEK  
MICROSCOPE. (CIRCA  
LATE 16002)***

Such an image, seen as if it were ten inches from the eye, is known as a virtual image and cannot be captured on film.

These magnifiers had severe limitations in specimen positioning, illumination, lens aberrations, and construction

# LIGHT MICROSCOPY

## THE COMPOUND MICROSCOPE

Around the beginning of the 1600's, through work attributed to the Janssen brothers in the Netherlands and Galileo in Italy, the compound microscope was developed.

In its basic form, it consisted of two convex lenses aligned in series: an object glass (objective) closer to the object or specimen, and an eyepiece (ocular) closer to the observer's eye—with means of adjusting the position of the specimen and the microscope lenses.

The compound microscope achieves a two-stage magnification. The objective projects a magnified image into the body tube of the microscope and the eyepiece further magnifies the image projected by the objective more of how this is done later).

For example, the total visual magnification using a 10x objective and a **15X** eyepiece is 150X<sup>17</sup>

Conversely, it may be (and often is) all too easy to degrade an image through improper technique or poor equipment.

Essentially, this is how a microscope functions.

Light from a lamp passes through a sub stage condenser and then through a transparent specimen placed over an opening in the stage.

Light is then gathered by the objective.

The objective, together with the built-in tube lens (more of this later), focuses the image of the specimen at the level of the fixed diaphragm of the eyepiece.

The image is then seen by the observer as if it were at a distance of approximately 10 inches (250 millimeters) from the eye.

At the lowest part of the observation tube in infinity-corrected systems for Olympus equipment, there is a tube lens which gathers the parallel beams of light emerging from the objective and focuses the resulting image at the plane of the fixed diaphragm of the eyepiece.

The eye lens of the eyepiece, together with the curved cornea and lens of the eye, focuses the image on the retina of the observer's eye .

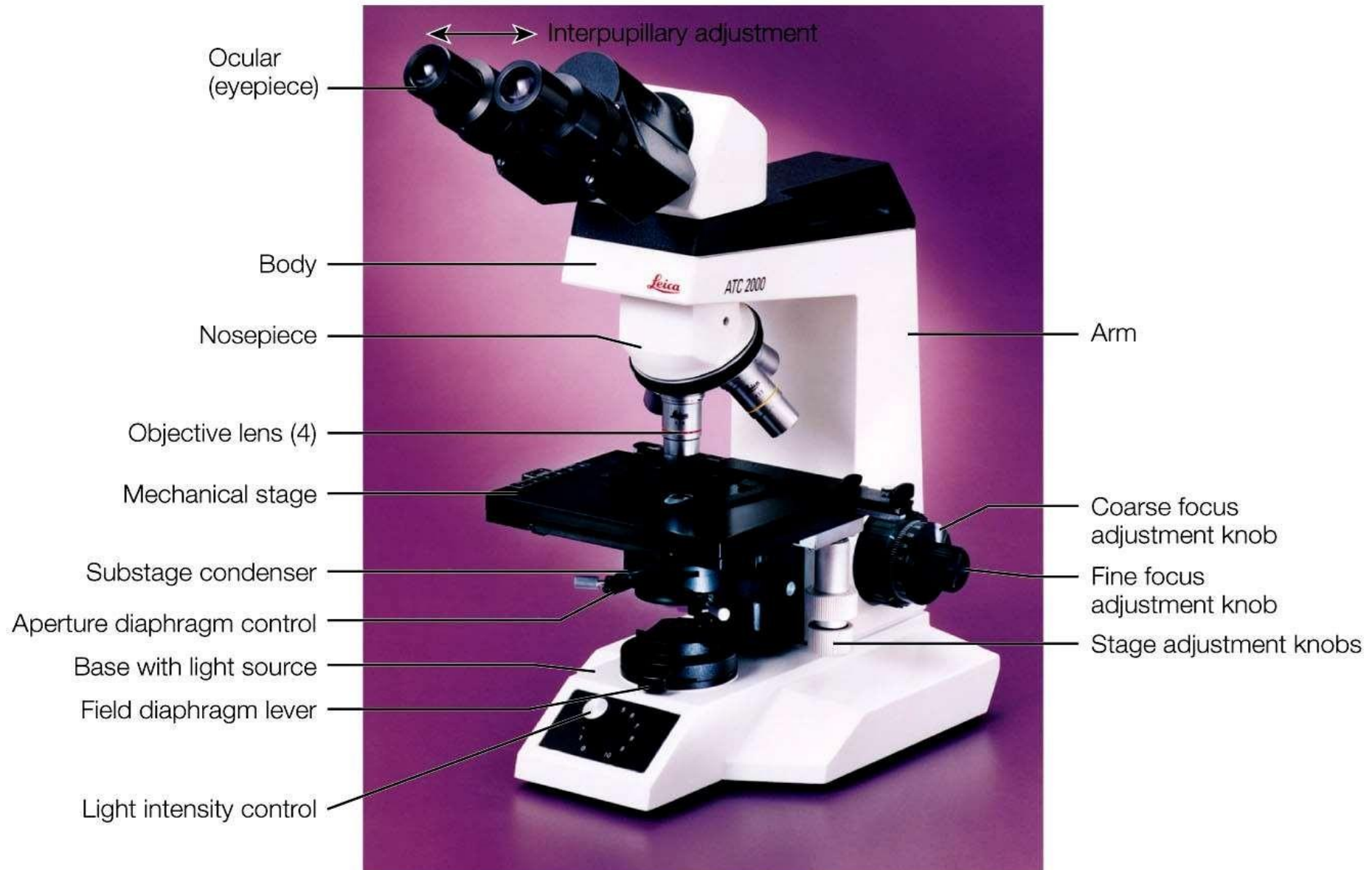
# PARTS OF A COMPOUND MICROSCOPE

**Microscope proper:** Incorporating the body tube with the objective at one end and the eyepiece at the other end.

**The stand** which include the supporting, adjusting and illuminating apparatus



# Binocular microscope



# COMPONENT PARTS OF A MICROSCOPE

## A. SUPPORT SYSTEM

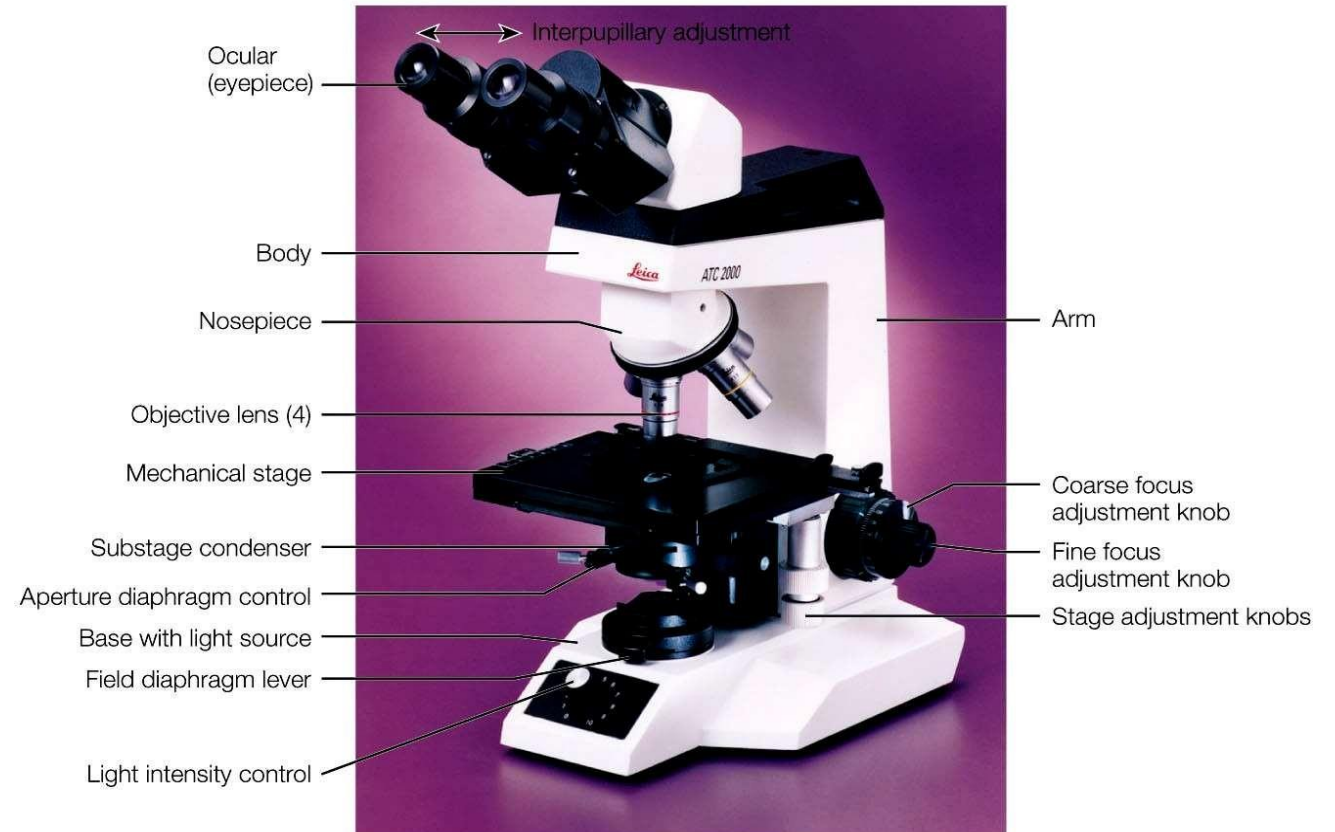
- (i) Base
- (ii) Pillars
- (iii) Handle/Limb

## B. FOCUSING SYSTEM

- (i) Course adjustment screw
- (ii) Fine adjustment screw

## C. THE STAGE

- (i) Fixed Stage
- (ii) Mechanical stage

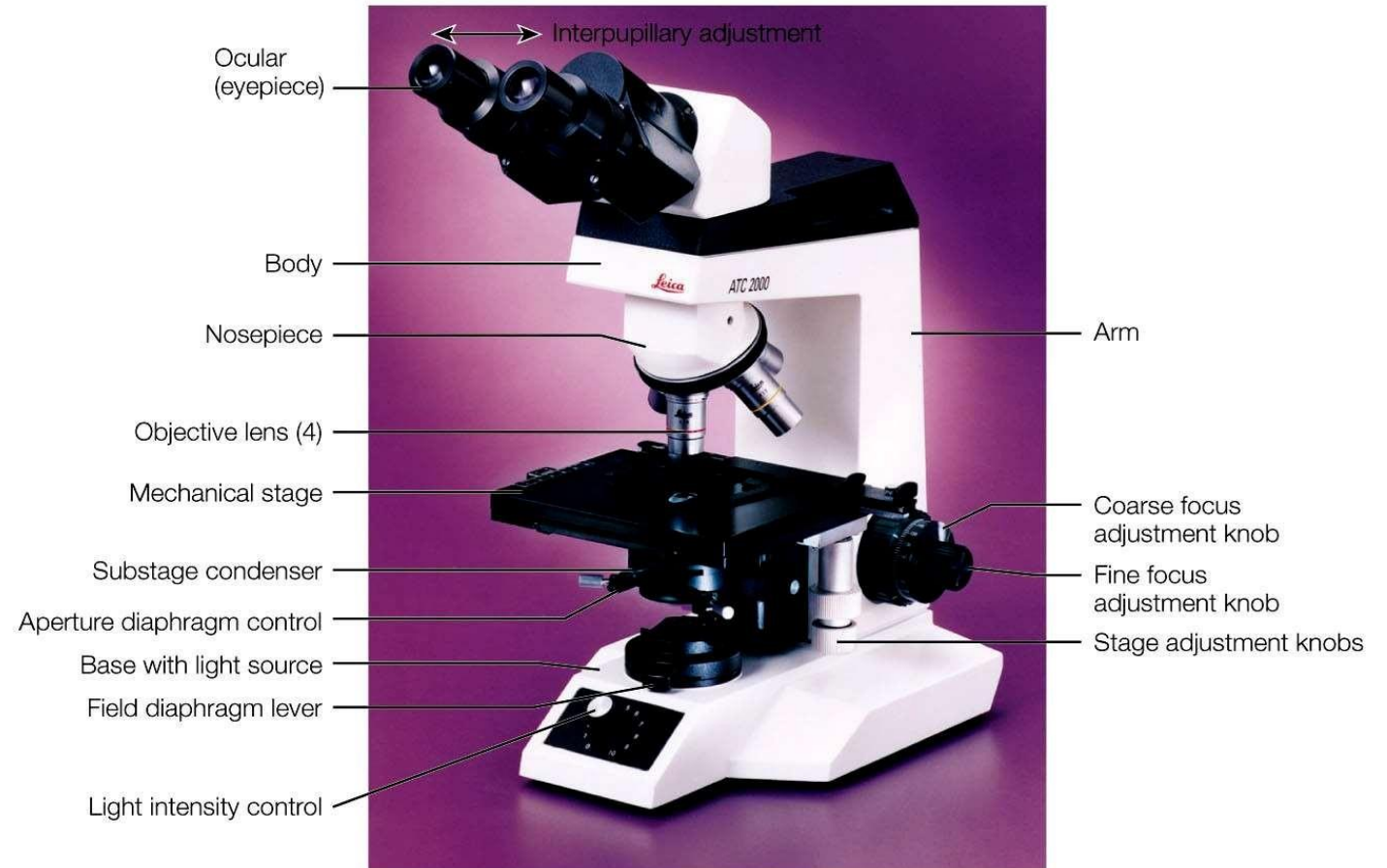


## D. OPTICAL SYSTEM

- (i) Body Tube
- (ii) Nose piece
  - a. Fixed
  - b. Revolving
- (iii) Objective lenses
- (iv) Eye piece

## E. ILLUMINATION SYSTEM

- (i) Source of light
- (ii) The mirror
- (iii) The condenser

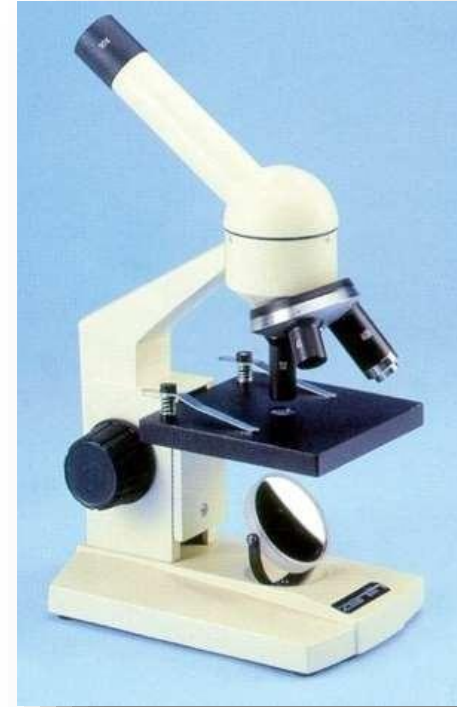


# Light Source

- Light is an essential part of the system.
- Sunlight, low voltage electric lamps, tungsten etc.
- Neutral density filters- excess brightness of light is reduced to comfortable viewing levels through these filters.

The source of illumination should be

- Uniformly intense
- Should completely flood the back lens of the condenser with light when the lamp iris diaphragm is open
- Make the object appear as though it were self-luminous



# OBJECTIVE LENSES





## Color Coding of Objective Lens

Immersion color code a	Immersion type
Black	Oil
Orange	Glycerol
White	Water
Red	Special
Magnification color code b	Magnification
Black	1x, 1.25x
Brown	2x, 2.5x
Red	4x, 5x
Yellow	10x
Green	16x, 20x
Turquoise blue	25x, 32x
Light blue	40x, 50x
Cobalt (dark) blue	60x, 63x
White (cream)	100x

a. Narrow colored ring located near the specimen of objective.

b. Narrow band located closer to the mounting thread than the immersion code.



# **THE MOST IMPORTANT OPTICAL COMPONENT OF THE MICROSCOPE IS THE OBJECTIVE.**

- Its basic function is to gather the light passing through the specimen and then to project an accurate, real, inverted IMAGE of the specimen up into the body of the microscope.
- Other related functions of the objective are to house special devices such as an iris for darkfield microscopy, a correction collar for counteracting spherical aberration (more of this later), or a phase plate for phase contrast microscopy.
- The objective must have the capacity to reconstitute the various points of the specimen into the various corresponding points in the image, sometimes called the "anti-points".

- The objective must be constructed so that it will be focused close enough to the specimen so that it will project a magnified, real image up into the microscope.
- The higher power objectives should have a retractable front lens housing to protect the front lens where the objective requires focusing very close to the specimen.
- To the extent possible, corrections for lens errors (aberrations) should be made within the objective itself.



## *ACTION OF A CORRECTION COLLAR (DIAGRAMMATIC). SECOND IMPORTANT OPTICAL COMPONENT IS THE EYEPIECE*

- Its basic function is to "look at" the focused, magnified real image projected by the objective (and tube lens in infinity-corrected systems) and magnify that image a second time as a virtual image seen as if 10 inches from the eye.
- In recording, a Photoeyepiece "picks up" the real image projected by the objective a second time as a real image able to be captured by a camera,
- The eyepiece houses a fixed diaphragm. It is at the plane of that fixed diaphragm that the image projected by the objective will be "seen".
- On the shelf of the fixed diaphragm, the eyepiece can be fitted with scales or markers or pointers or crosshairs that will be in simultaneous focus with the focused image.

Eyepieces work in combination with microscope objectives to further magnify the intermediate image so that specimen details can be observed. Best results in microscopy require that objectives be used in combination with eyepieces that are appropriate to the correction and type of objective.



**ABERRATION-FREE 10x EYEPIECE WITH DIOPTR  
ADJUSTMENT**

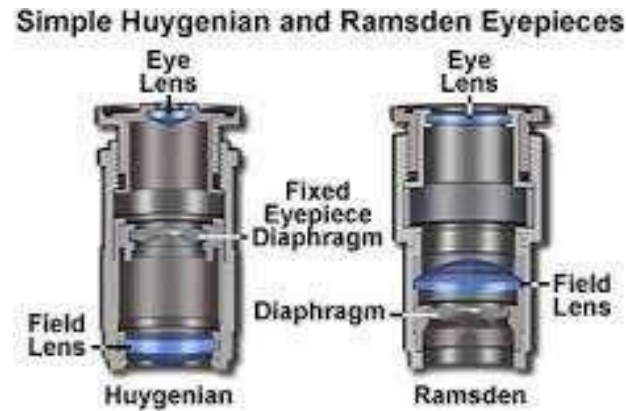
There are two major types of eyepieces that are grouped according to lens and diaphragm arrangement:

the negative eyepieces with an internal diaphragm and positive eyepieces that have a diaphragm below the lenses of the eyepiece.

Negative eyepieces have two lenses: the upper lens, which is closest to the observer's eye, is called the eye-lens and the lower lens (beneath the diaphragm) is often termed the field lens.

In their simplest form, both lenses are plano-convex, with convex sides "facing the specimen.

Approximately midway between these lenses there is a fixed circular opening or internal diaphragm which, by its size, defines the circular field of view that is observed in looking into the microscope.



***RAMSDEN EYEPIECE, HUYGENIAN EYEPIECE.  
BOTH  
ILLUSTRATED IN LONGI- SECTION***

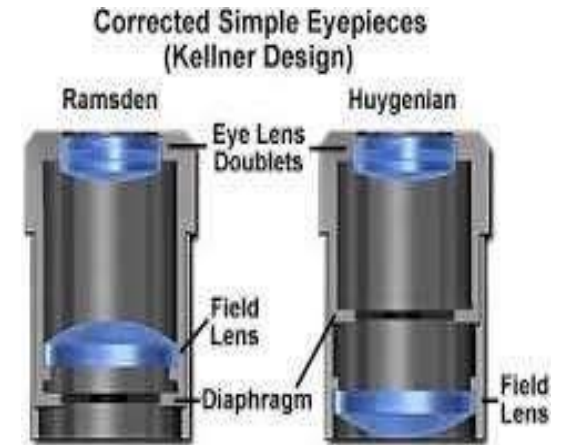
The simplest kind of negative eyepiece, or Huygenian eye-piece, is found on most routine microscopes fitted with achromatic objectives.

Although the Huygenian eye and field lenses are not well corrected, their aberrations tend to cancel each other out.

More highly corrected negative eyepieces have two or three lens elements cemented and combined together to make the eye lens.

If an unknown eyepiece carries only the magnification inscribed on the housing, it is most likely to be a Huygenian eyepiece, best suited for use with achromatic objectives of 5x-40x magnification.

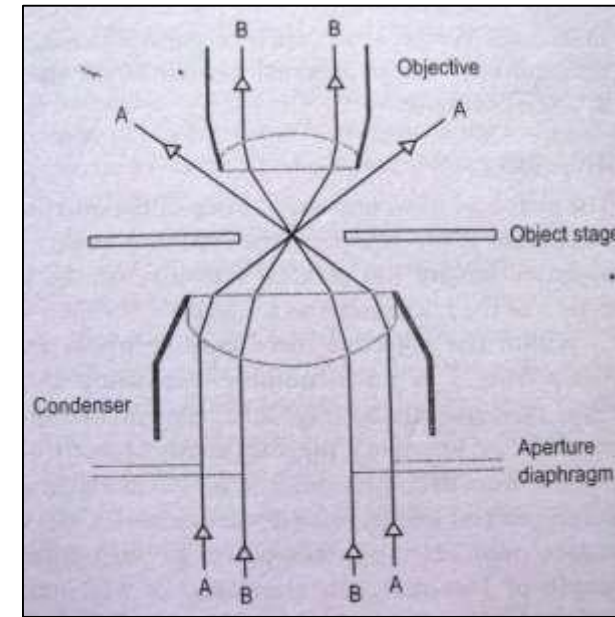
The other main kind of eyepiece is the positive eyepiece with a diaphragm below its lenses, commonly known as the Ramsden eyepiece.



**SIMPLE EYEPIECE**

- This eyepiece has an eye lens and field lens that are also plano-convex, but the field lens is mounted with the curved surface facing towards the eye lens.
- The front focal plane of this eyepiece lies just below the field lens, at the level of the eyepiece diaphragm, making this eyepiece readily adaptable for mounting graticules.
- To provide better correction, the two lenses of the Ramsden eyepiece may be cemented together

# THE THIRD IMPORTANT OPTICAL COMPONENT IS THE SUBSTAGE CONDENSER.



1. Its basic function is to gather the light coming from the light source and to concentrate that light in a collection of parallel beams (from every azimuth) onto the specimen.
2. The light gathered by the condenser comes to a focus at the back focal plane of the objective (later, the explanation of this term).
3. In appropriately set up illumination, it is arranged that the image of the light source, comes to focus at the level of the built-in variable aperture diaphragm of the substage condenser (the front focal plane of the condenser).

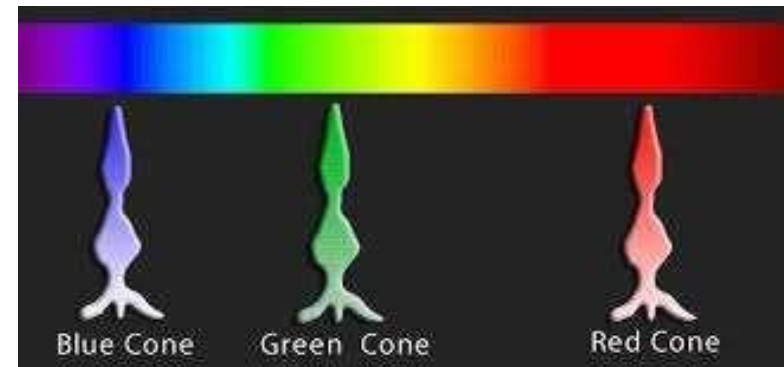
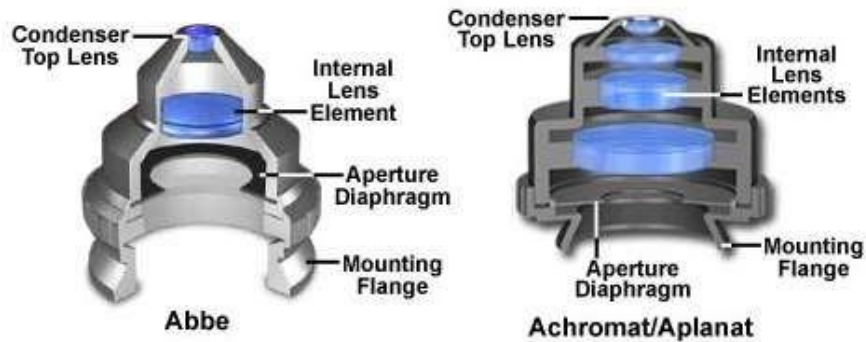
1. Correction for lens errors are incorporated in the finest condensers, an important feature for research and photography.
2. Where desired, the condenser can be designed to house special accessories for phase contrast or differential interference or dark field microscopy.

The substage condenser is fitted below the stage of the microscope, between the illumination lamp and the specimen. Condensers are manufactured according to different levels of correction needed.



The simplest and least well-corrected condenser is the Abbe condenser, numerical aperture up to 1.25. While the Abbe condenser is capable of passing bright light, it is not well-corrected chromatically or spherically.

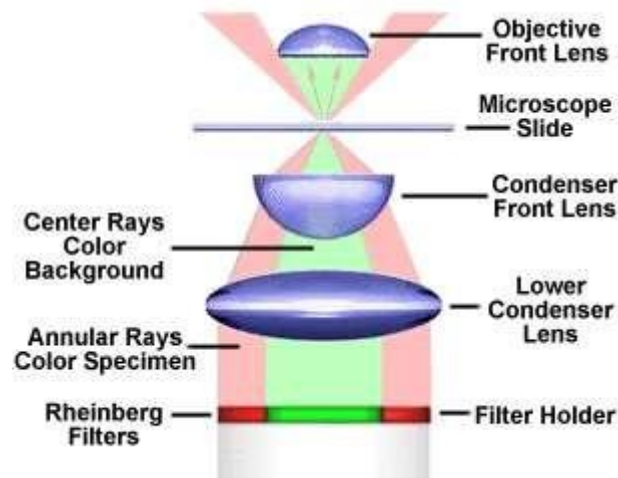
As a result, the Abbe is most suitable for routine observation with objectives of modest numerical aperture and correction.



***A. ABBE AND APLANATIC-ACHROMATIC CONDENSER SYSTEMS.***  
***B. CONES OF LIGHT TRANSMITTED***

The best level of correction in condensers is found in the aplanatic-achromatic condenser. Such a condenser is well-corrected for chromatic aberration and spherical aberration. It is the condenser of choice for use in color observation and recording in white light.

The engraving on the condenser includes its numerical aperture and its correction, if aplanatic-achromatic. Condensers with a numerical aperture above 1.0 perform best when a drop of oil is applied to their upper lens and is brought into contact with the underside of the slide



***CONE OF ILLUMINATION. THE SUBSTAGE CONDENSER MUST BE FOCUSED AND THE DIAPHRAGM ADJUSTED SO THAT THE CONE OF ILLUMINATION COMPLETELY FILLS THE APERTURE OF THE MICROSCOPE OBJECTIVE***

- The condenser aperture and the proper focusing of the condenser are of critical importance in realizing the full potential of the objective in use. Likewise, the appropriate use of the adjustable aperture iris diaphragm (incorporated in the condenser or just below it) is most important in securing excellent illumination and contrast.
- The opening and closing of the aperture iris diaphragm controls the angle of the illuminating rays which pass through the condenser, through the specimen and into the objective.

- For low power objectives (4X or below), it may be necessary to unscrew the top lens of the condenser or to use a condenser with a flip-top upper lens.
- Special low power condensers are also available.
- Specialty condensers are available for dark field microscopy, for phase contrast, polarized light, and for interference microscopy.
- The height of the condenser is regulated by one or a pair of condenser knobs which raise or lower the condenser.
- This adjustment is described in Koehler illumination

## **OTHER OPTICAL COMPONENTS:**

1. The base of the microscope contains a **COLLECTOR LENS**. This lens is placed in front of the light source. Its function is to project an image of the light source onto the plane of the condenser's aperture diaphragm. In some instruments a diffusion or frosted filter is placed just after the collector lens (side closer to the specimen) in order to provide more even illumination.
2. Also in the base of the microscope, under the condenser, is a **FIRST SURFACE MIRROR** (silvered on its front surface only). Its function is to reflect the light coming from the lamp up into the sub stage condenser. Just before that mirror (closer to the lamp side) is another variable diaphragm known as the field diaphragm.

- At the lowest part of the observation tubes (binocular or trinocular) there is incorporated a TUBE LENS.
- Its function is to gather the parallel rays of light projected by the objective (in infinity-corrected systems) and bring those rays to focus at the plane of the fixed diaphragm of the eyepiece. In the instruments of some manufacturers, the tube lens is built into the body of the microscope itself.

## **MECHANICAL/ELECTRICAL COMPONENTS-**

- The **STAND** of the microscope houses the mechanical/electrical parts of the microscope. It provides a sturdy, vibration-resistant base for the various attachments.
- The **BASE** of the Olympus microscopes is Y-shaped for great stability. It houses the electrical components for operating and controlling the intensity of the lamp. The lamp may be placed, depending on the instrument, at the lower rear of the stand or directly under the condenser fitting. The base also houses the variable field diaphragm. The base may also have built in filters and a special circuit for illumination intensity for photomicrography

- Built into the stand is a fitting to receive the microscope STAGE. The stage has an opening for passing the light. The specimen is placed on top of the stage and held in place by a specimen holder. Attached to the stage are concentric X-Y control knobs which move the specimen forward /back or left/right.
- On the lower right and left side of the stand are the concentric COARSE and FINE FOCUSING KNOBS. These raise or lower the stage in larger / smaller increments to bring the specimen into focus.



- Under the stage there is a built-in ring or a U-shaped CONDENSER HOLDER. This holder receives any one of several types of condenser. The holder has a tightening screw to hold the condenser in place and may have 2 small knobs (at 7 o'clock and 5 o'clock positions) for centering the condenser to the optical axis of the microscope in Koehler Illumination (explained later). Adjacent to the condenser holder there are either one or two knobs for raising or lowering the condenser.
- Above the stage, the stand has a NOSEPIECE (may be fixed or removable) for holding the objectives of various magnifications. The rotation of the nosepiece can bring any one of the attached objectives into the light path (optical axis). The nosepiece may also have a slot for special attachments.

- Removable OBSERVATION TUBES, either binocular or trinocular, are attached to the stand above the nosepiece.
- The binocular is used for viewing and the trinocular is used for viewing and /or photography. The observation tubes are usually set at approximately a 30 degree angle for comfortable viewing and may be tiltable or telescoping push-pull for greater flexibility.
- The bottom of the observation tube holds a special lens called the TUBE LENS.
- The tube lens has the function of gathering the parallel beams projected by the objective and bringing the image to focus at the level of the eyepiece diaphragm (intermediate image plane).
- On the instruments of some manufacturers (not Olympus) the tube lens may complete optical corrections not made in their objectives.

## **THE MICROSCOPE STAND HAS THE FOLLOWING FUNCTIONS:**

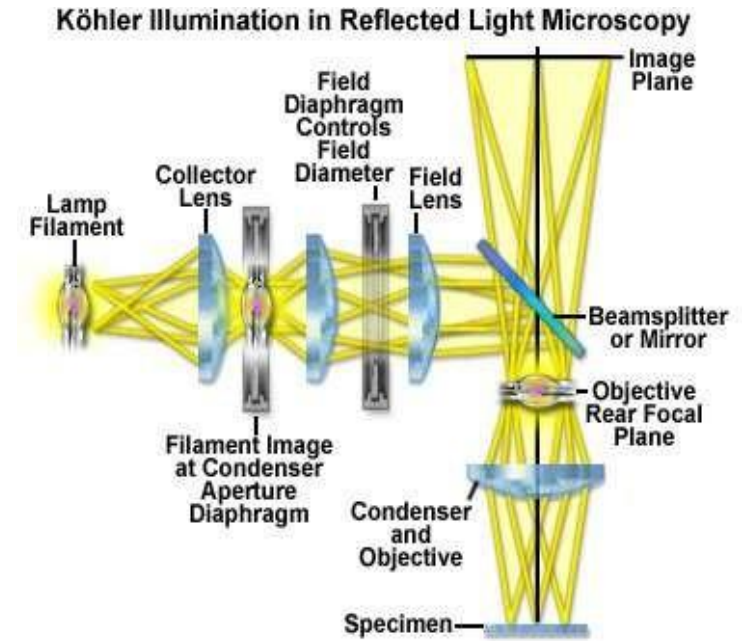
1. To insure stability and rigidity of the microscope.
2. To provide the frame for holding the objectives and eyepieces at opposite ends of the stand.
3. To make it possible, by means of adjustment knobs, to bring the specimen into focus.
4. To hold the specimen on a flat surface stage and to be able to move the specimen on that surface.
5. To carry the moveable substage condenser which receives the light deflected by the built-in mirror and transmits that light up through the specimen.
6. To hold the lamp and the electrical controls to operate the lamp and control its brightness

## ILLUMINATION

Since specimens rarely generate their own light, illumination is usually furnished by means of a built-in lamp. The light beams pass through the substage condenser after deflection by a built-in mirror.

The light transmitted by the condenser then passes through the specimen on the stage, into the objective, thus illuminating the specimen.

If the lamp is of high intensity (tungstenhalogen), its brightness is controlled by a built-in or separate transformer.

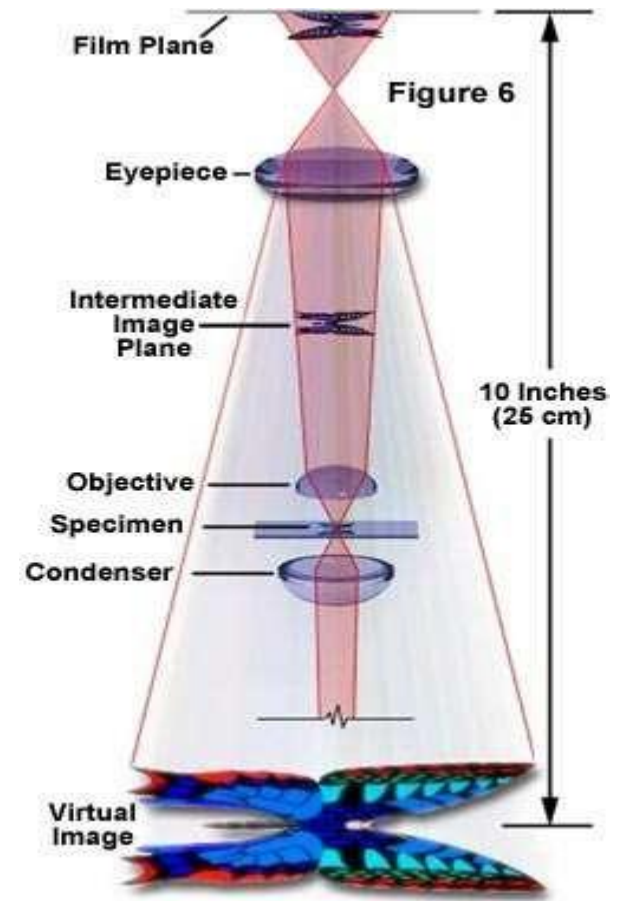


MICROSCOPE  
ILLUMINATOR. THE  
ESSENTIAL ELEMENTS  
OF THE ILLUMINATOR  
ARE THE LAMP, A  
COLLECTOR LENS, AND  
THE FIELD DIAPHRAGM.  
THE DIAPHRAGM IS  
ADJUSTABLE

## TYPES OF ILLUMINATION:-

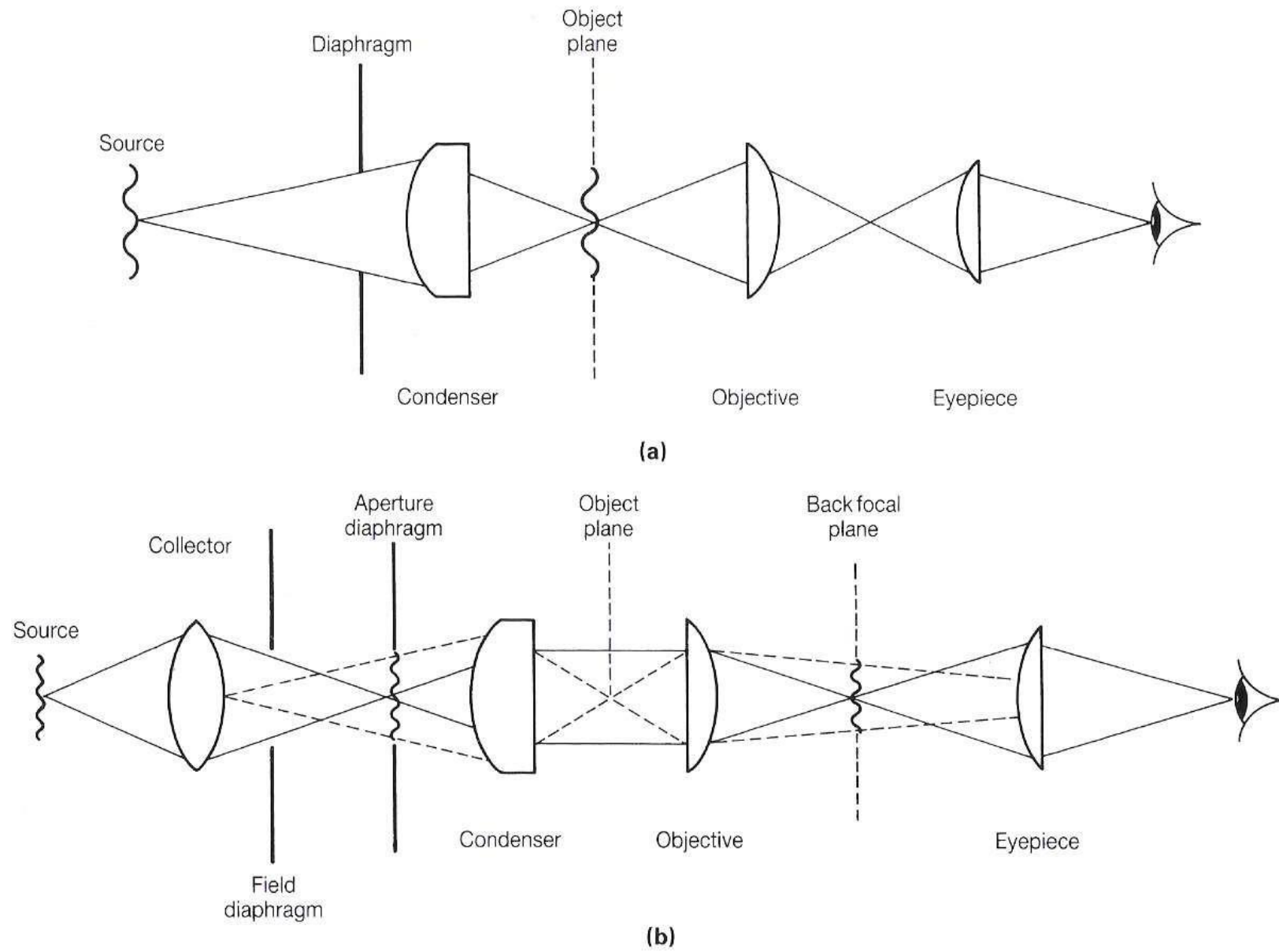
### Critical or Nelsonian or source focused illumination.

- Homogenous light source is used to get this kind of illumination.
- No lamp condenser used
- Normally employed with a base light source.
- Light source be focused on the object plane by racking the substage condenser up and down.
- Light source must be large.
- Used in routine visual microscopy. Easy to set up.



# KOHLER ILLUMINATION

- Non homogenous light source is used.
- Lamp condenser or collector lens is essential.
- A field iris is placed in front of the collector to focus the light from the collector onto the substage iris.
- It is used with compound lamps .
- Used in photomicrography.
- Hence image obtained from the field iris will be found in the primary image plane and retina of the eye.



**Fig. 4.13** (a) Critical illumination. (b) Köhler illumination.