A **microscope**: is a high precision optical instrument that uses a lens or a combination of lenses to produce highly magnified images of small specimens or objects especially when they are too small to be seen by the naked eye.

A light source is used (either by mirrors or lamps) to make it easier to see the subject matter.

Microscopy is the use of a microscope or investigation by a microscope

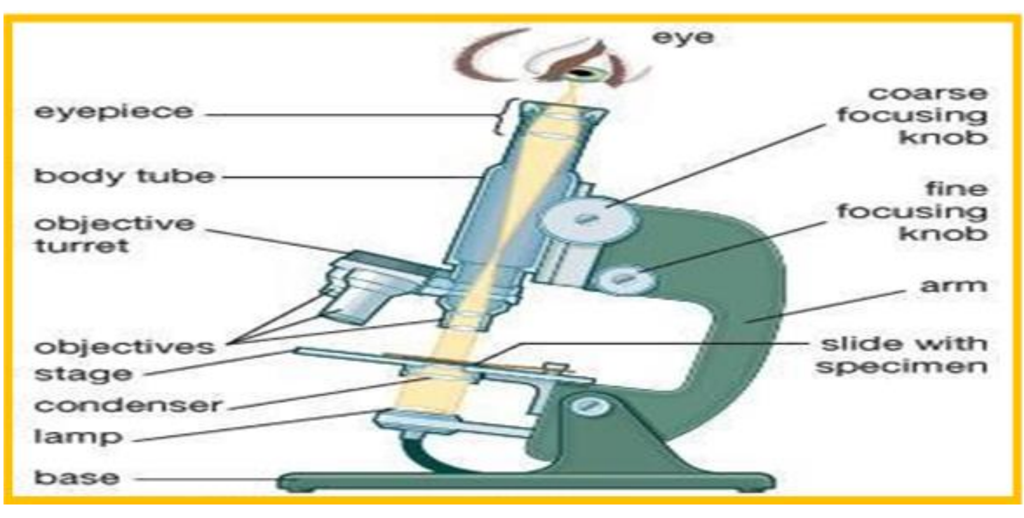
Types of Microscopes

The majority of microscopes are called light (bright field) microscopes since they rely on light to observe the magnified image of a specimen or object. There are two main categories:

(1) Compound (high power microscopes).
(2) Stereo or dissecting (low power microscopes).

1- Compound Microscope:

This is the most common type of microscope. It can also be referred to as a biological or research microscope. The compound microscope is refer to as a high power microscope. The magnification (power) can have a range from about 40x to 1000x and some can go up to 1500x or 2000x. Much serious work of a compound microscope is done at 400x to 500x. Compound refers to the fact that in order to enlarge an image



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Principle:

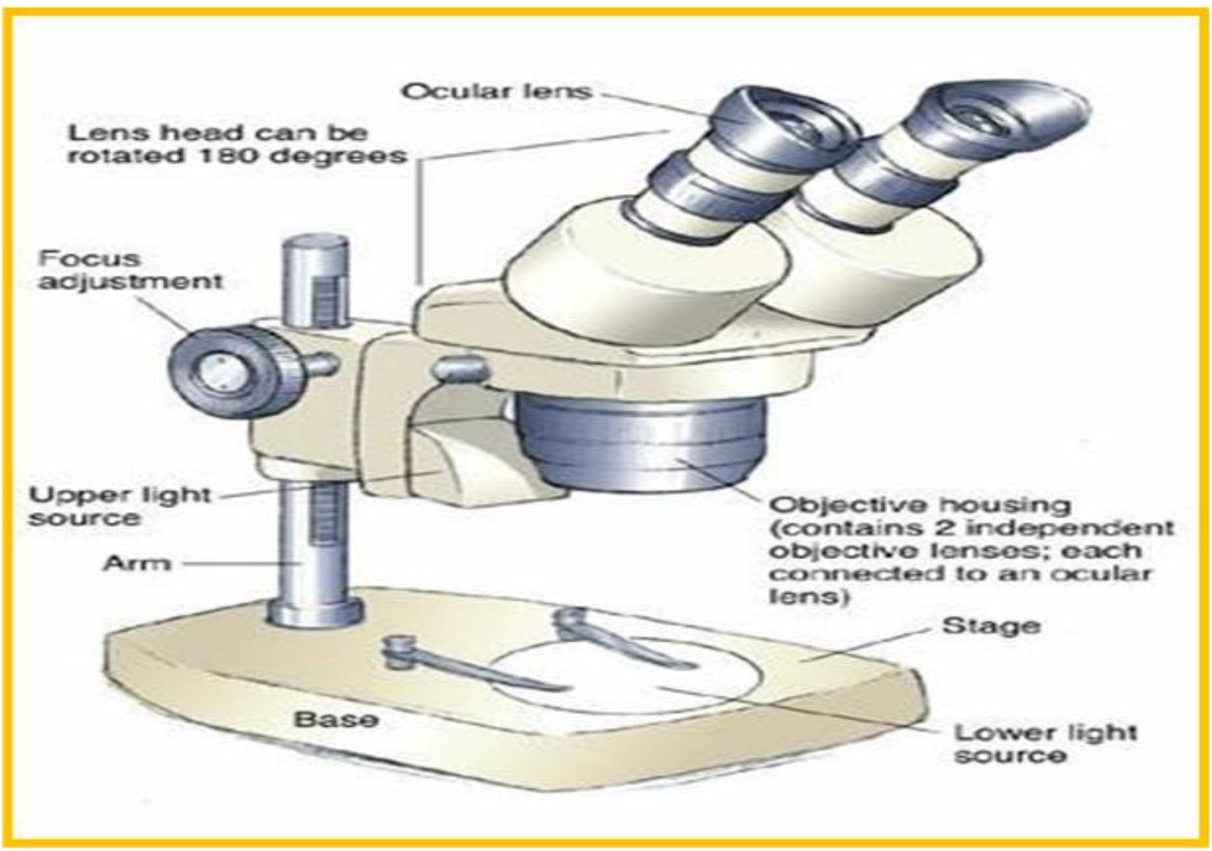
A single light path passes through a series of lenses in a line where each lens magnifies the image over the previous one. In other words, one light path with multiple lenses equals a compound microscope. The image is seen by the observer as if it were only 10"(250mm) from your eye. In the standard form the lenses consist of **an objective lens** (closest to the object or specimen) and an **eyepiece lens** (closest to the observers' eye) and a means of adjusting the focus and position of the specimen or object. In addition, acompound microscope uses **light** (reflected from a mirror, from indirect sunlight, from desk lamps or other interior light sources, or from built-in lamps) to illuminate the specimen or object so that you can see it with your eye. The objective lens usually consists of three or four lenses (sometimes even five) on a **rotating nosepiece** (turret) so that the power can be changed. The image produced at the eye is two dimensional (2-D) and usually reversed and upside down. The most used light method is trans- illumination (light projected from below to pass through the specimen). At 400x much detail can be seen at the cellular level of biological specimens

2- Stereo Microscope:

Stereo microscopes are the second most common type of microscope. They can also be referred to as **dissection**, or **inspection** microscopes. The stereo microscope is referring to as a **low power microscope**. Magnification (power) can have a range from about **10xto 80x** with magnification in the **10x to 40x** the most popular. Also, zoom models from about **10x to 60x** or so are very convenient. Low power **is used** for examining larger sized items like insect parts, flower parts, rocks and fossils, stamps, coins, PC boards, material surfaces, hair, etc.

Principle:

There are **two separate light paths** (as opposed to a single light path in a compound microscope) which produce a true stereo, three dimensional (3-D) image of the specimen or object. Within the **objective lens** you will find two lenses (one for each path of light) side-by-side. The optical design parameters of a stereo microscope limitits 3-D effects to low powers only. Also in the category of low power microscopes isthe single light path (like a compound microscope) type usually referred to as a dissecting microscope. This type is more economical than the stereo type but is very useful for examining large sized specimens and objects.



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In the standard form the lenses consist of **objective lenses** (closest to the object or specimen) and **eyepiece lenses** (closest to the observers' eyes) and a means of adjusting the focus and position of the object or specimen. In addition, a stereo microscope **uses light** (from desk or table lamps, indirect sunlight, other interior light sources, or from built-in or attached lamps) to illuminate the specimen or object so that you can see it with your eyes. The images you see are correct (upright and normal which is the opposite of compound microscopes). Most stereo microscopes have both top and bottom built-in or attached illumination to handle various objects and specimens of all varieties, shapes, and colors.

These are usually **advanced and expensive** type microscopes made for specific usages mainly in advanced medical and research. There are many, many types but some of the more popular types are listed below.

Other Types of microscopes

1- **Phase Contrast**: This is a microscope that uses the differences in the phase of light transmitted or reflected by a specimen to form distinct, contrasting imagesof different parts of the specimen.
2- **Polarizing microscope**: in which the object viewed is illuminated by polarized light for typically analyzing the content and make-up of organic or inorganic material like crystals, chemical microscopy, and optical mineralogy
3- **Fluorescence:** These microscopes use an illumination method that is used to locate fluorescently tagged material (protein, enzyme, genes) by exciting the specimen with one wavelength of light in hopes that the fluorescence will appear by emitting a light at a different wavelength.
4- **Metallurgical** A microscope that is used for identification, inspection, and analysis of different metals and alloys.
5- **Electron Beams**: These microscopes very expensive and use a beam of highly energetic electrons instead of light to examine objects on a very

fine scale. This allows the microscope to surpass the resolution limits of optical microscopes and can magnify specimens up to 250,000x or more. Users can examine the topography of a specimen, its morphology, composition, etc.

6- **Digital**: These are a combination of a microscope and a digital camera. The camera can be integrated (built-in) with the microscope or specialized cameras (imagers) can be purchased separately and adapted to virtually any microscope at economical prices. With basic software provided (with the camera (or using your own photo editing software) the user can display, save, and edit images. Some more expensive software packages allows for a variety of image analysis useful for medical, educational, and sophisticated research usage. Handheld DigitalMicroscopes use new technology for a miniature camera and illuminator in one unit. You use a PC or laptop computer to view and image.

Another division of microscopes:

a) Types according to the number of the eye pieces (early type):

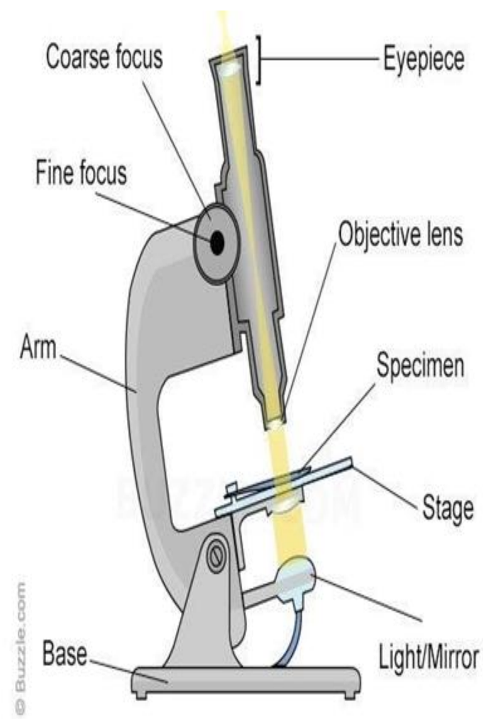
1) Monocular
2) Binocular

b) Types according to the source of light:

1) Day light
2) Electric light

c) Types according to the principle of work(physical):

1) Optical microscope
2) Electron microscope



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d) Types according to their technical uses:

1) Dark field microscope

2) Polarizing microscope

3) Fluorescent

4) Phase contrast.

Parts & Function of each parts of the microscope

1- **Eye pieces**: The eyepiece consists of a series of lenses mounted in a tube (barrel) at the upper end of the microscope**. Its basic function is to** look at the focused, magnified image projected by the objective lens and magnify that image a second time before your eye looks at the image of the specimen. For special applications, eyepieces can have scales, pointers, crosshairs, markers, etc. on them.

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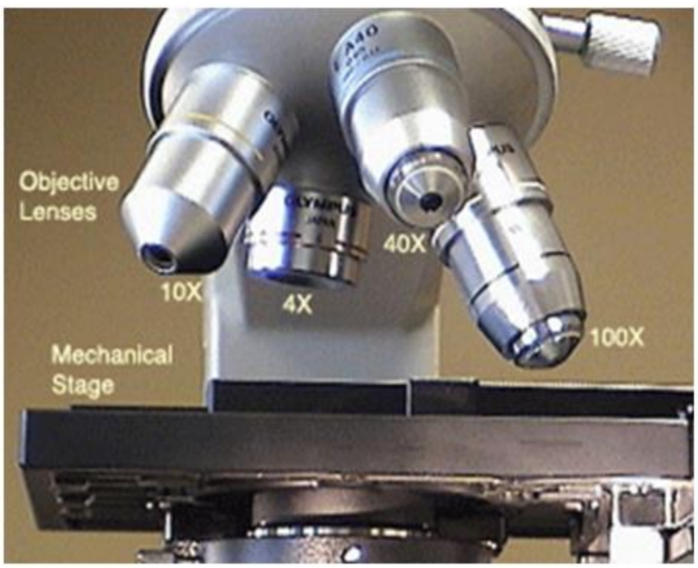
2- Objective lens: there are (3)or (4) with magnification of (x4,x10 low power,x40 high power,x100 oil projector)). The objective lenses are the most important components of microscopes. **Their basic function is to** gather the light passing throughthe specimen and then to project the image up into the body of the microscope.

The objectives are the lens system closest to the specimen. There is one objective for each eyepiece in a compound microscope.

For stereo microscopes, there are objective pairs (one objective lens for each eyepiece lens) which give the 3-D effect.



on compound microscope objectives, there is printed the following information

on each one such as power, tube length. Tube length of the objectives usually have a DIN (interchangeable) of 185mm or 195mm. Objectives vary in power from lx to 160x in compound microscopes but the most common power range is from 4x to 100x. Most compound microscopes have three or four (occasionally five) objectives usually of 4x, 10x, 40x, and 100x (oil immersion) which revolve on a nosepiece (turret) to give different magnifying powers.

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The 4x, 10x, and 40x are called "dry" objectives which means they operate with air between the objective and the specimen. The 100x is called a "wet" objective which means it operates with immersion oil between the lens and the specimen.

For stereo microscopes, they usually have one or two objectives lenses which normally are lx, 2x, 3x, or 4x. In addition, there are zoom models which operate from about 0.5x up to 5x. The extent of corrections for lens errors (aberrations) and flatness of the image field determines the usefulness and cost of the objectives for compound microscopes.

Microscope Objective Anatomy and Specifications

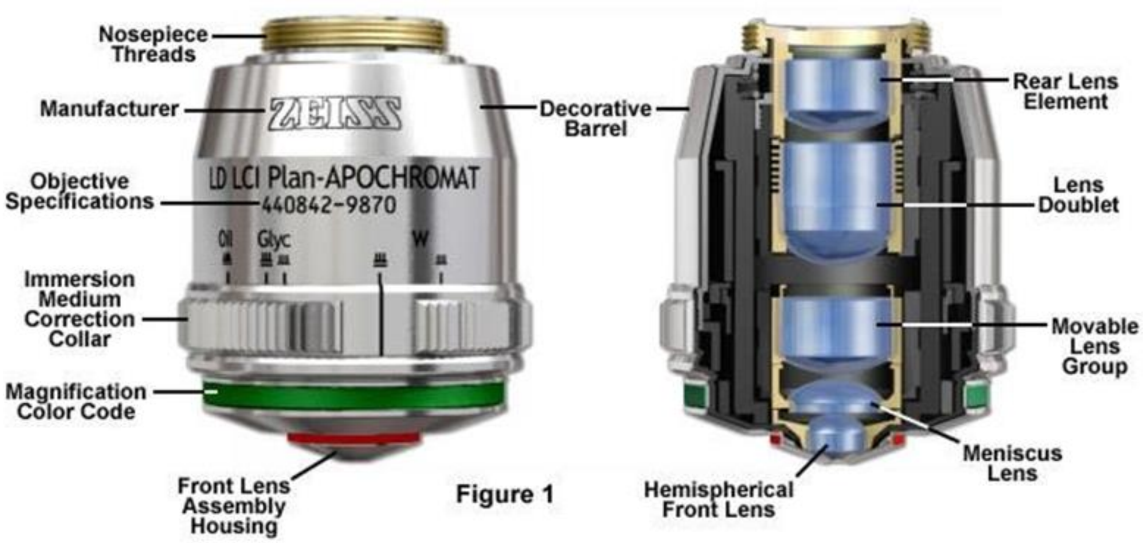


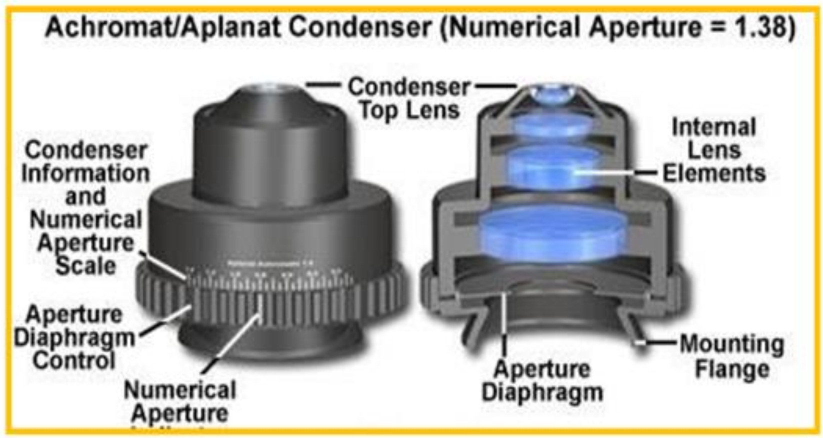
Figure 1

N.A. (numerical aperture) is a number that expresses the ability of a lens to resolve fine details in an object being observed especially those close together. As the N.A. number increases, the resolution becomes better. The N.A. may vary from 0.04 (low power) to1.4 (high power Plan wet objective). The N.A. will be marked on the objective and the typical N.A. for the following are; 4x=0.10, 10x=0.25, 40x=0.65, and 100x=1.25. Resolution (actual not theoretical) is the separation distance of two details (points or lines) lying close together still seen as separate. Resolution comes from the objective and not the eyepieces as the eyepieces only magnify the resolution. Sometimes objectives have a color ring (universally used) to aid in identifying the magnification: black (1x), brown (2x), red (4x), yellow (10x), green (20x), turquoise (25x), light blue (40x), dark blue (60x), white (100x). Another number on the objective (like 0.17).0i1 Immersion concentrates the light path and increases the resolution. Immersion oil is the only suitable oil for this purpose and will allow high magnifications and avoid damage to the objectives. There are two basic types of oil immersion — Type A is for low viscosity and Type B is for high viscosity.

3- **Optical tube**: connects the eye pieces with the objective lens .the standard length(16cm).

4- **Specimen stage** the platform beneath the objectives on which the slide or object tobe observed placed is called a stage. It has a smooth, flat surface and can be rectangular or circular. The simple type of stage is called a plain stage and the more sophisticatedstage is called a mechanical stage.

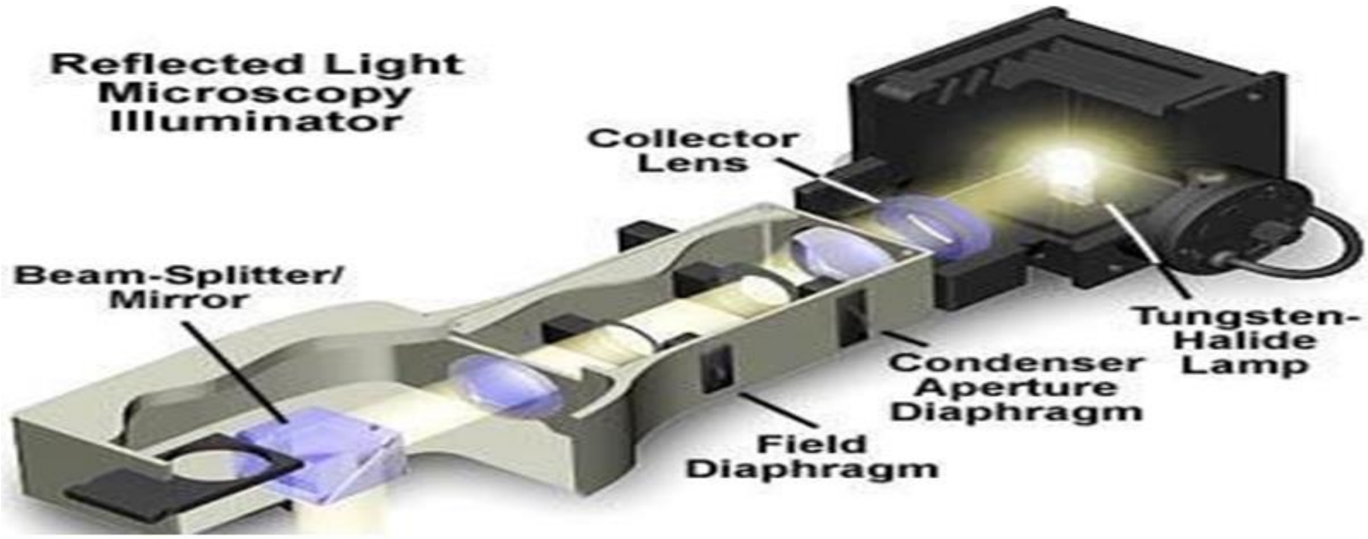
5- Condenser Lens (Sub-stage Condenser) is a glass lens or lens system located within or below the stage on compound microscopes. Its basic function is to gather the light coming in from the light source and to concentrate that light into a light cone onto the specimen.



The diaphragm is also called the sub-stage diaphragm or aperture diaphragm. The diaphragm is normally located under the stage of a microscope and it adjusts the amount of light passing into the slide or specimen. It is most useful at high powers.Most compound microscopes have one of two types of diaphragm:

- **Disc Diaphragm** — is the simplest and least expensive of the two types. It is located between the light source and the slide or specimen. It contains a rotatingdisk (usually fixed) with five to ten openings of differing diameters which limit the amount of light passing through to the specimen.
- **Iris Diaphragm** — is the better and more expensive of the two types. It has a continuously variable diameter (like the iris of an eye or a camera shutter) which has a function to limit the size of the opening through which light passes from thelight source to optimize resolution, contrast, and sharpness. It is usually controlled by a lever.

6- **Illumination Systems** (Light Source): Since specimens rarely generate their own light, illumination is necessary. Illumination is the application of light onto an object or specimen in a microscope. The illuminator is the source of light which illuminates the object or specimen_ to be observed. The simplest means of the illumination can be provided by overhead lights, desk or table lamps, or indirect sunlight. Many compound microscopes are provided with adjustable piano/concave mirrors which reflect an external light source into the microscope. The flat slide (piano) of the mirror usually provides the sharper image but if stronger and brighter illumination is needed then use the concave side.



The more expensive and illumination is by using built-in or attached light sources using' bulbs or lamps that provide direct illumination. It can be from above the specimen or object which is used mainly with low power stereo microscopes and is called incident (reflected) light or from below a specimen (typically a -slide specimen) which is light passing up through the specimen from inside the base and called trans illumination (transmitted light).

Illumination lamps or bulbs come in various types:

- **Tungsten** — is an incandescent bulb filament which is the most common and least expensive. They give off a yellowish hue and give off moderate heat. Theyare typically 15-watt or 20-watt.

- **Halogen** — is a lamp which generally is the hottest light source for a microscope. The light is very bright, very white, and concentrated. The halogen type is more expensive than the tungsten. They are typically 15-watt or 20-watt.
- **Fluorescent -** is a lamp that is cool in temperature. The light is bright and white and very sharp while being comfortable to the eye. The fluorescent is great for observing live specimens. They are typically 5-watt to 10-watt and generate the same brightness as the tungsten or halogens do. They can be built in the base of a microscope or they can be attached (called a ring light) to observe from above.
- **LED –** these are light emitting diodes which provide a bright light source with- virtually no heat. The white beam is brighter and cooler than the other illumination systems. They are typically battery operated and thus are cordless and great for outdoor use also.

7-Focus Systems A focus control allows you to adjust the focus of the microscope. Every microscope includes a focusing control (knob) for quick focusing of the image. More expensive compound microscope models include a quick and fine focusing control. The fine focus is particularly advantageous in high power applications and required for 400x and higher but is not available on stereo microscopes since they are only low power.

8-Head (Body) The head is the upper part of the microscope that connects the eyepieceto the nosepiece or turret. Some heads are fixed in place and allow you to tilt them fromangles of 0° up to 60°.

There are several types of heads:

- Monocular - this is a microscope with a single eyepiece.
- Binocular - this is a microscope with two eyepieces, one for each eye.
- Trinocular - this is a microscope with a binocular head for viewing and anadditional port that can be used for a third eyepiece for a second person

9-Nosepiece (Turret or Revolving Nosepiece): The nosepiece is a rotating turretlocated above the stage on compound microscopes that can hold



multiple objective lenses of various magnifications. By rotating the objectives into the light path and over the specimen you can observe various magnifications of the specimen during your examination. As any of the objectives are rotated they will click when the precise location is reached.

10-Arm: The arm (also called the. stand or limb) is the component of a microscope which contains the focus mechanism and supports the stage, as well as the body or headwhich contains the eyepieces. It provides the rigidity of a microscope as it rises from the base. A few types of arms are:

- Fixed - a type of arm where the arm and the body are integral parts of themicroscope and connected solidly to the base.
- Pillar (Post) - a type of arm which consists of a single post rising c verticallyfrom the base. The microscope body can rotate about the post and also be moved up and down on it.
- Boom (Universal) - a long boom type stand used to support a microscope body. Ithas many adjustments allowing the microscope to be aligned in a wide variety of configurations. This is the least common type of arm.

11- Base: The base is the bottom support part of the microscope. It provides balance andrigidity. It houses electrical components for illumination.

Magnification of the microscope:

Total magnification = magnification of the eye piece X magnification of the objective.

Types of microscope according to their technical use :

Dark —field microscope:

Uses of the dark field:

1) Useful in the examination of treponema palladium, too small to seen by lightmicroscope.
2) Study other living unstained microorganism's motion.
3) Useful in the fluorescent microscopy.

A special dark —field apparatus is installed on an ordinary light microscope, excludes all direct light by the presence of a disc beneath the condenser which diffracts the light from the center of the condenser to the periphery, so the illumination emanates from thesides only.

An opaque ring in the objective cuts all directly transmitted light, the objective picks uponly scattered light, so that the minute objects under study glow brightly against a dark field. The dark fields study only the external morphology.

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B. Polarizing microscope:

Uses of polarizing microscope-

Identify certain crystalline chemicals such as, talk, silica, alum, hematein&crystal in synovial fluid &in chemistry studies& in urine analysis.

Crystals affect the plane of polarization of light passing through it. Polarizing filter transmits light vibrating in only one of these planes, creating polarized light.If two filters (polarizes) were used with their transmission axes oriented at right angles. No light would pass through the pair. Certain crystals rotate a plane of polarized light passing through them. When the crystal is viewed between pair of crossed filters, the crystal is seen magnified against a black background because the rotated plane of light emerging from the crystal is no longer perpendicular to the second filter.

Location of the device:

First polarizing is interposed between the material spacemen &the source of light,second is interpreted between the specimen & the observers eye (on the eye lens).

C. Fluorescent microscope:

Uses:

Because it has Aspasia filters, so the organisms stained with fluorescent dyes are made visible by u.v. Principles: Some substances emit light when stimulated. The source of light is u.v. lamp (high pressure mercury vapor lamp). Exciter filter used to permits only short wave (blue rays)to fall on the object, if the object is auto fluorescent it emits color. Other object can be stained by fluorescent dyes, , making them visible under fluorescent microscope. A barrier filter interposed between object and the observer to absorb or bar the transmission of undesired wave length pass only the fluorescing wave length emitted by the spacemen from the u.v radiation. Usual equipped with interchangeable condenser for both dark- field &bright-field.

Care of the microscope:

1- Kept covered when not in use.
2- Eye pieces and objectives should be cleaned by breathing on the lens ofwiping dry with lens paper.
3- For sticky lens, after the use of immersion of oil, lens paper moistened withXylem or Benzen.
4- Should be professionally cleaned & adjusted once year.

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Electron microscope

The electron microscope can produce images much greater magnification than lightmicroscope.

Types of the E.M.

1- Transmission electron microscope (produce2d.image)

2 - Scanning E.M.(produce 3-d.image)

3- Transmission scanning E.M.

Construction of the E.M.

1- Electron gun
2- Condenser lens
3- Spaceman chamber
4- Objective lens
5- Screen of camera

Physical principles:

The electron microscope consists of evacuated "stack" the electron gun in the top of thisstack emits electrons which directed to the anode (50-100 kV). The focusing of magnification is achieved by a series of (lenses) electromagnetic field. The condenser directed the electrons on the object packed below it. The electrons are scattered according to the thickness &density of various parts of the sp. Then an objective lens gathers the scattered electrons &focuses them to form a real primary magnified image. Two projector lenses (which have the function of the eye piece) magnify the primary. The final image is observed on the fluorescent screen or photographic plate or film held in a camera.

The differences between light microscope & electronic microscopes

Optical microscope	Electronic microscope
1- it has a source of light (Tungsten —halogen lam	1- It has a source of electrons (electron gun)
2- it has a specimen stage	2- It has a specimen chamber
3- It has eyepiece to see imaginary object	3- It has a screen of camera or photographic film to see image of the object.
4-It has optical lens to converge or divergethe light	4- It has a magnate lenses to bend the electron paths
5- It can separate dot of about 0.25 Micronsapart (low resolution power)	5- It can separate dot of about 5A apart (high resolution power)

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