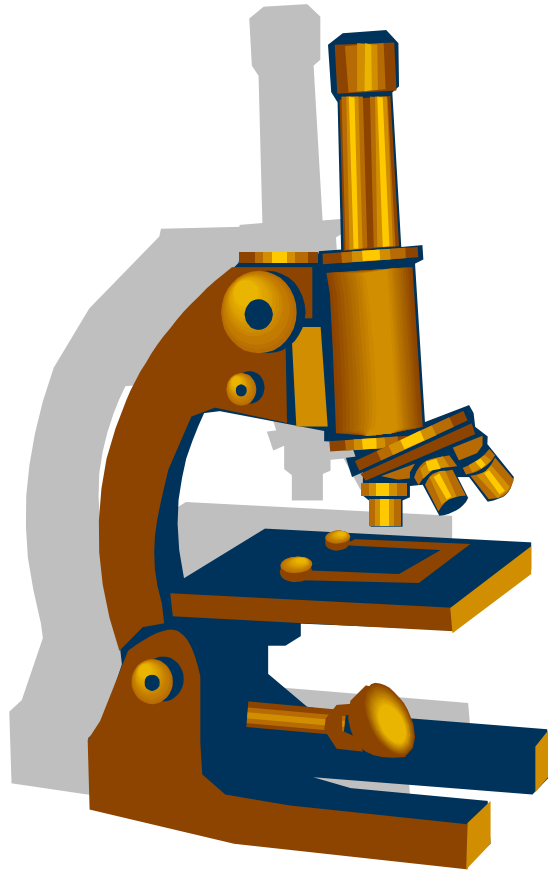


# Microscopes



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# Microscopes



## What is a microscope ?

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

## What is microscopy ?

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

## Who invented the microscope ?

There is no one person who invented the microscope as several different inventors experimented with theories and ideas and developed different parts of the concept as they evolved to what is today's microscopes.

About 1590 two Dutch spectacle makers, Zaccharias Janssen and his son Hans, experimented with a crude concept of a microscope that enlarged objects 10x to 30x or so. In 1609, Galileo (an Italian) improved on the principle of lenses and added a focusing device to improve somewhat upon what the Janssen's had done.

These rudimentary instruments didn't change much until the early 1670s. A Dutchman, Anton van Leeuwenhoek, is considered the father of microscopes because of the advances he made in microscope design and use. He worked as an apprentice in a dry goods store where magnifying lenses were used to count the threads in cloth. Anton was inspired by these glasses and he taught himself new methods for grinding and polishing small lenses which magnified up to 270x. This led to the first practical microscopes. In 1674, Anton was the first to see and describe bacteria, yeast, plants, and life in a drop of water.



**Anton van Leeuwenhoek (1632-1723)**

Not many improvements were made until the 1850's when several inventors in Europe and America made vast improvements. From this time frame, modern compound and stereo microscopes have changed very little.

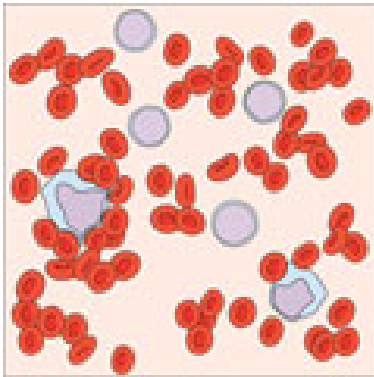
In the early 1930's the first electron beam microscopes were developed which were a breakthrough in technology as they increased the magnification from about 1000x or so up to 250,000x or more. These microscopes use electrons rather than light to examine objects.

## What can you do with a microscope ?

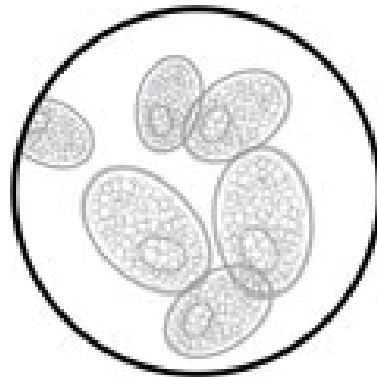
One could write a book answering this question but briefly – a microscope can be used for numerous things:

- hobbyists have an interest in microscopic worlds from learning and having fun to using a microscope for the study of stamps, coins, gems, insects, etc
- children can view a fantastic world of discovery
- students have an interest in pursuing knowledge anywhere from middle school through the university level
- medical uses from analysis to advanced research in many and varied disciplines
- industrial uses as inspection and measurement tools
- government and scientific uses for public safety and discovery
- imaging of whatever you are looking at





**Blood Smear Schematic**



**Mite Eggs**

Normal will become extraordinary when looking through a microscope. You will find that almost everything (alive or that was alive) consists of components called cells and they take on a fabulous view at the cellular structure – like your own blood, molds, yeast, and bacteria. You can observe prepared slides or prepare your own. You can spend hours examining a drop of pond water and observing the enormous amount of life within it. You can study ordinary things like salt, cotton, foods, plants, or flowers. Using stereo (dissecting) microscopes, you can examine details of the specific parts of insects, animals, gems, textiles, paper, fingerprints, sand, milk, bread, etc.

Microscopes are essential tools in creating new medicines and finding cures for diseases.

The same basic microscopes are used for all these various activities. Everyone should own a decent microscope and it will last a lifetime with reasonable care. Many people think that microscopes are very complex and difficult to use and that they are only used in research facilities and universities and that they are expensive --- but they are less expensive than one might think and are really easy to use.

## **What specific categories are microscopes used for or by ?**

- **Hobbyists** – gems, coins, stamps, collectibles, learning and discovery, etc.
- **Education** – chemistry, biology, botany, zoology
- **Medical** – microbiology, hematology, pathology, entomology, dermatology, dental usage, veterinary use, everyday analysis to advanced research. From medical schools to labs to hospitals
- **Industry** – inspection of electronic assembly components and many different materials such as metals, textiles, plastics, etc. Used in agriculture, wineries, breweries, and for fine engravings and mining inspection. Used by jewelers and geologists
- **Teachers and students** – in the educational process starting in elementary school through graduate school
- **Science** – for the study of archeology, oceanography, geology, metallurgy, and numerous other fields
- **Government** – many areas for public health and safety such as water quality, pharmaceuticals, forensics, asbestos, lab work, military applications, etc.



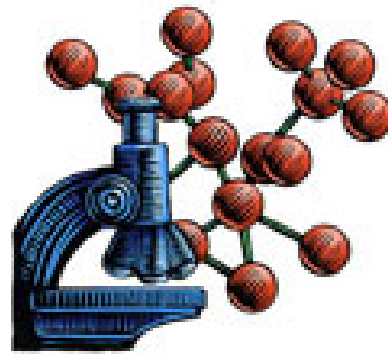
**Education**



**Medical & Research**



**Science**



**Molecular Structure**

## **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. Within this category are two main categories – (1) **compound** (high power microscopes), and (2) **stereo** or dissecting (low power microscopes).

### ***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 what many 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 Microscope**

Compound refers to the fact that in order to enlarge an image, 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, a compound 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. Learning about cells and microorganisms is both educational and important for medical and science applications.

### ***Stereo Microscope***

Stereo microscopes are the second most common type of microscope. They can also be referred to as dissecting, dissection, or inspection microscopes.

The stereo microscope is what many refer to as a low power microscope. Magnification (power) can have a range from about 10x to 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, plant and flower parts, rocks and fossils, stamps, coins, PC boards, material surfaces, hair, etc. However, specimen slides can also be observed albeit at these lower powers.



**Stereo Microscope**

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 limit its 3-D effects to low powers only.

Also in the category of low power microscopes is the 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.

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 (rack and pinion style) 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.

### ***Other Types of Microscopes***

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.

**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 images of different parts of the specimen.

**Polarizing** – A 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.

**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. **Metallurgical** – A microscope that is used for identification, inspection, and analysis of different metals and alloys.

**Electron Beam** – These microscopes typically cost more than \$ 100,000 and sometimes much more 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.

**Digital** – These are a combination of a microscope and a digital camera. The more common types are the digital cameras which can use a CCD or CMOS chip. The camera can be integrated (built-in) with the microscope or **specialized cameras (imagers)** can be purchased separately and adapted to virtually any microscope (like one pictured below) 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 Digital Microscopes** use new technology for a miniature camera and illuminator in one unit. You use a PC or laptop computer to view and image. See the photo below.



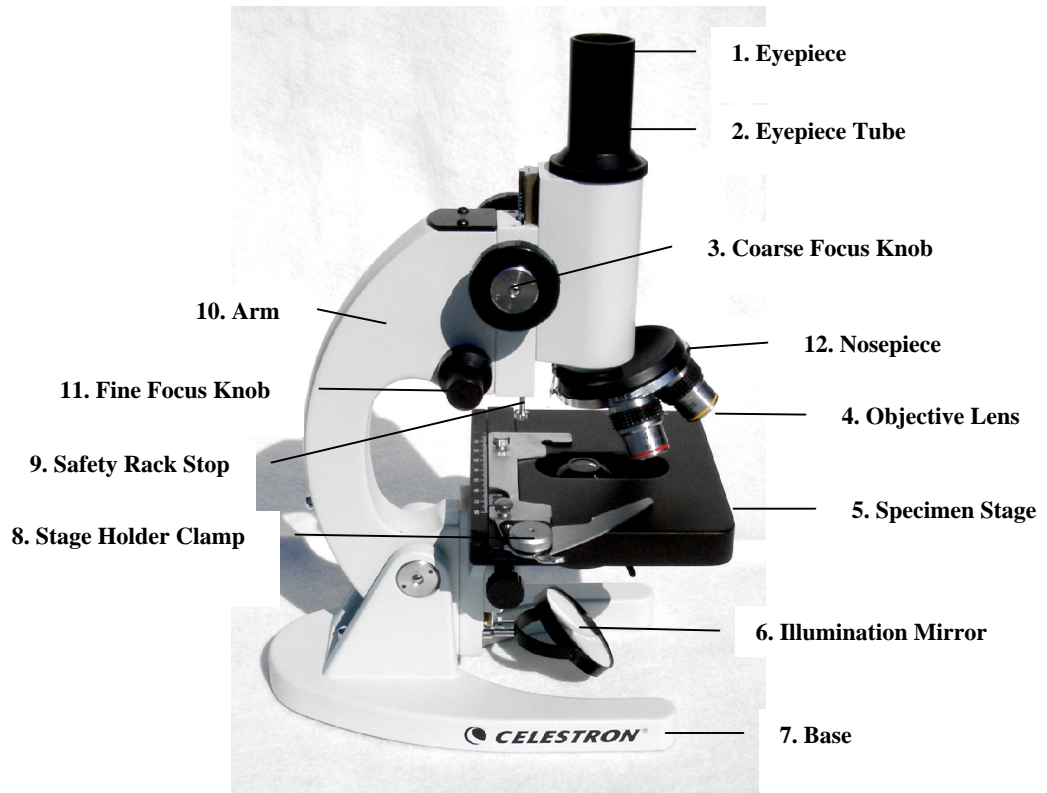
**Handheld Digital Microscope with a Laptop**



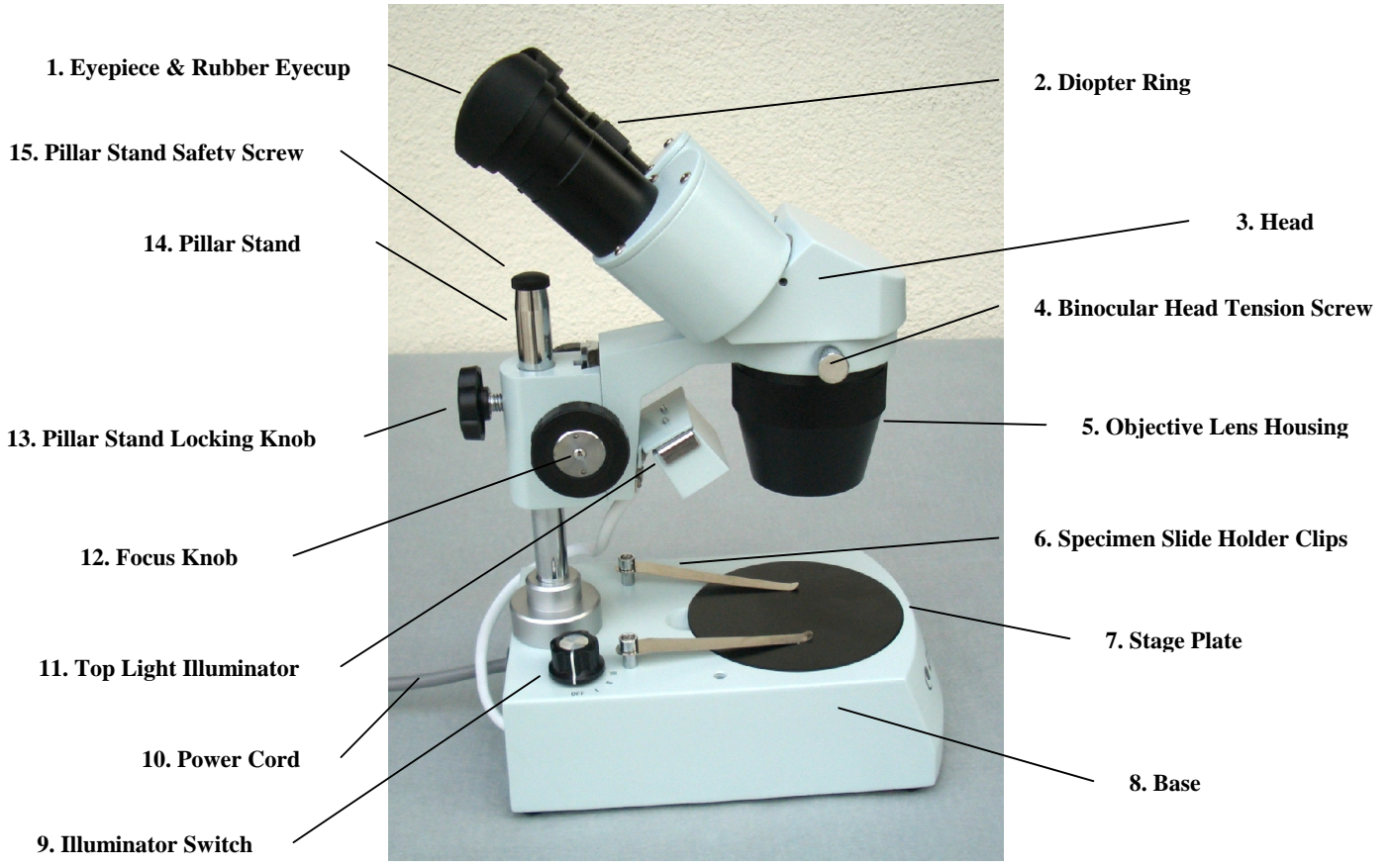
**Microscope VGA Imager attached to a microscope and laptop**

## **Parts of a Microscope**





**Compound Microscope**

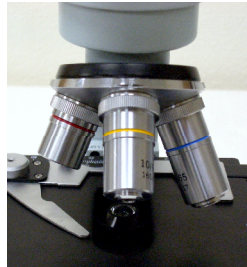


## Stereo Microscope

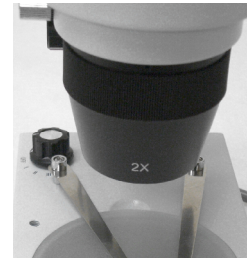
### Objective Lenses



Objectives for compound microscope



Objectives Mounted



Objective on Stereo Microscope

The objective lenses are the most important components of microscopes and thus will be discussed in greater detail here. Their basic function is to gather the light passing through the specimen and then to project the image up into the body of the microscope. Then, the eyepiece lens system further magnifies the image for your eye to see. Most quality microscopes use glass for the objectives and even for beginner microscopes, stay away from plastic objective lenses as the quality level is quite inferior. 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 – power, DIN tube length, N.A., cover slip thickness, universal color ring. Tube length of the objectives usually have a DIN (interchangeable) of 185mm or 195mm.

Objectives vary in power from 1x 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. 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 objective lenses which normally are 1x, 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. The least expensive objectives are achromatic types and these are fine for all microscopes costing under about \$ 750.00. The cost of objectives increases if using fluorites or semi-apochromatic types and the most expensive are the apochromats.

**Flatness of field** (field curvature) refers to how well the specimen is focused across the entire field of view. In achromatic objectives the flatness of field is usually about 50% to 70% of the field where they are in sharp focus in the center and the outside of field becomes out of focus but you can refocus in the outer portions to see that area sharply. Flatter field objectives (micro plan or semi-Plan) are the semi-apochromats which are flat for about 70% to 85% of the field and apochromats (Plan types) are flat for about 90% to 100% of the field. Plan objectives are the ultimate for quality and a microscope typically will cost more than \$ 750.00 to have these type objectives in them.

**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) to 1.4 (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. The higher the resolution, the closer the two points may be to one another and still be distinguished as two points. 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) refers to the thickness in millimeters of the cover glass that was assumed by the lens designer in computing best performance for the objective lens.

**DIN** (Deutsche Institut Fuer Normung) standard objectives are the most popular in international standards which dictates the design capability of the objectives. DIN standard objectives from one manufacturer can be used in another manufacturer's compatible microscope. The standard refers to the 45mm parfocal (explained below) distance and RMS standard 33mm thread for the objective. The 45mm distance is the measurement from the mounting hole in the objective nosepiece to the point of focus on the specimen.

JIS (Japanese Industrial Standard) is a less used international standard for objectives. They use 36mm objective distance.

RMS (Royal Microscopical Society) uses a 33mm parfocal distance for the objective. RMS screw threads are 20.32mm diameter with a pitch of 0.706.

**Parfocal** refers to the ability to change objective lenses with differing powers and have the focus remain sharp or with very little refocusing. **Parcentered** refers to when changing objective lens power, the specimen should stay centered in the field of view.

Oil Immersion concentrates the light path and increases the resolution. A special oil is used with the 100x objective lens and usually at 1000x up to 1500x. This is a technique used to close the air gap between the tip of the objective and the top of the specimen with the oil. Once the objective contacts the drop of oil of matching refractivity, it becomes one unit. 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.

## ***Eyepiece (Ocular)***



**Various Eyepieces**

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. As with objective lenses, stay away from eyepieces where the optics are made of plastic as they will ensure very poor quality. Eyepieces come in several different designs such as Huygens, Ramsden, Kellner, Orthoscopic, Plossl, etc. and all can be good in microscopes and the differences are minor as compared to various optical designs used with astronomical telescopes.

The eyepieces are usually 10x but also come in 5x, 12.5x, 15x, and 20x. The “x” refers to the amount of magnification (power) that this lens adds as a multiplier to the magnification of the objective. They are inscribed with the magnification and its field number (which is the diameter in millimeters of the diaphragm opening (aperture) of the eyepiece). The aperture limits the field of view to the useful coverage of the eyepiece. For special applications, eyepieces can have scales, pointers, crosshairs, markers, etc. on them.

Eyepieces also come in **wide field** styles. They have a large diameter and show a wider field of view than standard eyepieces. Most are 10x although 15x and sometimes 20x are common. **Eye Relief** is the distance (in millimeters) between the observer's eye (cornea) and the nearest surface of the eyepiece lens – longer eye relief being better for eyeglass wearers.

The **eyepoint** is the location (or position) of the eye from the eyepiece which allows for the best possible viewing of the image. Some eyepieces use a pointer to point at a section of the specimen. You can rotate the pointer by turning the eyepiece. An eyepiece reticle (micrometer) is a piece of glass with a pattern printed or etched which is put inside an eyepiece to make measurements of the size of objects seen through the microscope.

The **diopter adjustment** is a focusing characteristic to match the user's eyes on microscopes with binocular heads. Most all people have a different vision in each eye. This allows the user to adjust the focus of each eyepiece separately. For eyeglass wears, it allows them to adjust for their prescription and they can use the microscope without using their glasses. Typically, the diopter is on the left eyepiece and to make the adjustment --- close your left eye and look through the right eyepiece with your right eye and focus the specimen sharply, then close your right eye and look through the left eyepiece with your left eye and focus the specimen sharply. Then you will be able to focus and examine specimens with comfort and ease.

**Rubber eyecups** (eye shields) are typically used with stereo microscopes. They reduce ambient light and provide comfort but should not be used if you are wearing eyeglasses.

## ***Condenser Lens (Sub-stage Condenser)***

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. High power

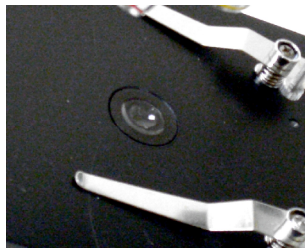


objective lenses have very tiny diameters and require concentrated light to work properly. A **basic condenser** is fixed in place.

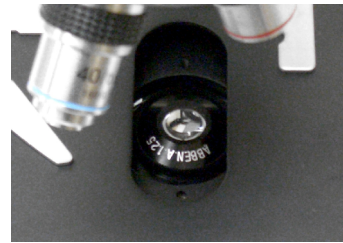
A moveable and more precise and more expensive condenser is the **Abbe condenser**. It usually can be moved up and down vertically, regulating the amount of light from the illuminator. It mounts under the stage and usually has an adjustable iris type aperture to control the diameter of the beam of light entering the lens system. By changing the size of the iris and moving the lens up or down from the stage, the diameter and focal point of the cone of light that goes through the specimen can be controlled. It is most useful at 400x and higher powers.

A condenser should have a N.A. equal to or greater than the N.A. of the objective lens being used. A basic condenser has a N.A. of 0.65 which is fine at 400x and below. But, at higher magnifications, a N.A. of 1.20 or 1.25 is common and you will need an Abbe type condenser. For condensers of a higher quality than the Abbe type, they are usually called aplanatic achromatic types and they have a very flat field and they are very expensive.

Some condensers can be designed to have special accessories for phase contrast, polarized light, differential interference, and dark field microscopy.



**Basic Condenser**



**Abbe Condenser**

### ***Diaphragm***



**Disc Diaphragm**



**Iris Diaphragm**

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:

1. **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 rotating disk (usually fixed) with five to ten openings of differing diameters which limit the amount of light passing through to the specimen.
2. **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 the light source to optimize resolution, contrast, and sharpness. It is usually controlled by a lever.

### ***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. Illumination of the object or specimen should be bright, free of glare, and evenly dispersed in the field of view.

The simplest means of illumination can be provided by overhead lights, desk or table lamps, or indirect sunlight. Many compound microscopes are provided with adjustable plano/concave mirrors which reflect an external light source into the microscope. The flat side (plano) of the mirror usually provides the sharper image but if stronger and brighter illumination

is needed then use the concave side. These methods are the least expensive illumination methods but it can be difficult to direct the light source for proper illumination.

The more expensive and common illumination is by using built-in or attached light sources using bulbs or lamps that provide direct and intense illumination. These light sources 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 transillumination (transmitted light). Lighting from both top and bottom at the same time can provide enough light for the most thick and irregular specimens. These illuminators may be of a fixed intensity, or of a variable intensity, which use a control knob (rheostat) to control the intensity of the light produced.

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. They are 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.

There are various forms of illumination produced by varying the amount of light or the quality of the light allowed to impinge on the specimen:

**Bright Field** – this is the most fundamental and common form of lighting for microscopes. It is a highly directional and intense light source. Light aimed from beneath the stage through a condenser lens, through the specimen, through an objective lens, and through the eyepiece to the eye.

**Diffuse** – this is where you place a ground glass, some translucent plastic, some opalescent material, or other similar material in front of the condenser (between the illuminator source and the condenser lens) and will cause the light of a bright field source to be scattered. Often this broadens the field illumination and brings subtle changes in the image.

**Phase Contrast** – this form is used mainly because a large spectrum of living biological specimens (blood, tissue, and cultured cells), are virtually transparent or have poor contrast when observed with a bright field microscope. By utilizing a phase annulus (ring) mounted in the condensers front focal plane partially modulates the light ray bundles that pass through and around the specimen, where they are slowed  $\frac{1}{4}$  wave, then are retarded another  $\frac{1}{4}$  wave when they pass through the phase plate in the rear focal plane of the objective. This system also diminishes background light about 85% providing a darkened background to contrast with the illuminated structure of the phase object. While the affect diminishes the resolution of the image, it makes detail visible that one could not see without it.

**Dark Field** – this form is a method to examine transparent or semi-transparent specimens which cannot be distinguished from the background. It shuts out background light and allows only scattered light to reach the specimen in order to heighten textural detail.

**Koehler** – this form is a technique to optimize light quality and sharpness by aligning and adjusting each component of the optical system starting with a focusing illuminator. The light quality will be even and bright. The Koehler is the best form of illumination possible with a microscope and is offered on only the most expensive microscopes.



**Koehler Illumination**

## ***Focus Systems***

A focus control allows you to adjust the focus of the microscope. Every microscope includes a focusing control (knob) for **quick (coarse)** focusing of the image. More expensive compound microscope models include a coarse (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.



**Coarse Focus Knob (Top)  
Fine Focus Knob (Bottom)**



**Coaxial Coarse/Fine Focus Knob**

**Coaxial controls** (focus) are where both the coarse and fine focus knobs are on one large control (on top of each other). The larger knob is typically for the coarse focusing and the smaller knob for the fine focusing. The smaller knob is usually centered on the inside of the larger one.

Focusing mechanisms used in microscopes are rack and pinion type. This is a design involving the intermeshing of a geared wheel and matching grooved rack. The rack is a track with teeth and the pinion is a gear that rides on the teeth. By turning a knob, the pinion gear moves along the track.

A **safety rack stop** (rack stop or stop screw) is an adjustable screw located at or near the base of a focus mechanism. When adjusted properly, it prevents the body tube from lowering too far and potentially causing damage to both the high power objective and/or the specimen. Most microscopes have a slip clutch which allows for continuous turning of a focus knob when it is all the way up or down without damaging the gear system.

Focusing can take place in one of two ways. The stage moves vertically to bring it nearer or further from the objective which remains fixed in place. The other method is the drawtube moves vertically with the objective and nosepiece while the stage stays in place.

## ***Head (Body)***

The head is the upper part of the microscope that connects the eyepiece to the nosepiece or turret. Some heads are fixed in place and allow you to tilt them from angles of 0° up to 60°. More expensive microscopes usually have heads that can be rotated 360° allowing two or more users to see the specimen without the microscope itself having to be picked up and rotated.

There are several types of heads:

**Monocular** – this is a microscope with a single eyepiece. These types are the more economical models and are very satisfactory for their usage. A monocular head with a second vertical viewing port is called a teaching head (dual view head) which can be used by a second person (or teacher) to observe the same image as the first person. Or, the second port can be used with various cameras.

**Binocular** – this is a microscope with two eyepieces, one for each eye. They are generally used on high power compound microscopes and all low power stereo microscopes and are generally more comfortable to use than a monocular type. There are different types of heads for adjusting the interpupillary distance (IPD) – see description below -- and the more common types are (1) seidentoff – IPD is adjusted by twisting the eyepieces in an up and down arc motion similar to most binoculars and (2) slider – IPD is adjustable side-to-side by sliding the eyepieces toward and away from each other.



**Binocular Head**

**Trinocular** – this is a microscope with a binocular head for viewing and an additional port that can be used for a third eyepiece for a second person (or teacher) to use or it would be used for various photo applications. On many microscopes, the amount of light can be adjusted (from 30% to 70% or so) for the third port.

Binocular heads contain prisms carrying light rays from the objectives to the eyepieces. The two eyepieces must be lined up with the user's pupils for comfortable viewing and this process is called adjusting the interpupillary distance (IPD). The eyepieces will get further apart or closer together to fit each individual as different people have varying distances between their eyes.

### ***Nosepiece (Turret or Revolving Nosepiece)***

The nosepiece is a rotating turret located 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. There are normally three or four objectives (4x, 10x, 40x, and 100x) and in rare cases there are five. The objective can be of different powers but the ones just mentioned are used in the vast majority of all compound microscopes.

Sometimes the 40x and 100x objectives are spring loaded at the end which allows the objective to be retracted slightly when nearing the slide/cover slip that may be too thick and this then prevents damage to the objective. Some nosepieces may be called “reverse nosepieces” and this is when the objectives are in a tucked position under the head and nosepiece allowing ease of placing slide specimens onto the stage from the front of the microscope.

In a stereo microscope, a nosepiece can be referred to as a single objective housing or a turret that may have two objectives. If a turret type, the turret is rotated all the way in either direction to use the particular objective.

### ***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 head which contains the eyepieces. It provides the rigidity of a microscope as it rises from the base. When moving a microscope, this is the part you should grab with one hand while putting your other hand under the base which will give you good protection. A few types of arms are:

**Fixed** – a type of arm where the arm and the body are integral parts of the microscope and connected solidly to the base.

**Pillar (Post)** – a type of arm which consists of a single post rising vertically from 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. It has many adjustments allowing the microscope to be aligned in a wide variety of configurations. This is the least common type of arm.

### ***Base***

The base is the bottom support part of the microscope. It provides balance and rigidity. It houses electrical components for illumination.

### ***Eyepiece Tubes***

The eyepiece tubes are also called observation tubes or drawtubes. They are attached to the arm above the nosepiece. They are usually set at angles of 45° or 30° for comfortable viewing. The bottom of the eyepiece tubes hold a special lens called

the eyepiece (tube) lens. The tube length is a fixed measurement in millimeters of the distance from the objective shoulder to the seat of the eyepiece which is typically DIN 160mm or 170mm (except on introductory microscopes) where the focused image forms. This distance governs the interchangeability of optical components.

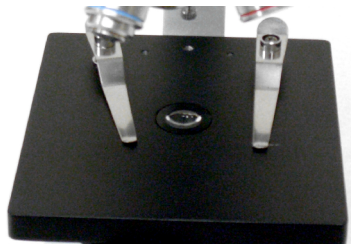
### ***Tube Lens***

At the bottom of the eyepiece tubes is a tube lens. Its function is to gather the parallel rays of light projected by the objective lens and bring those rays to focus at the plane of the fixed diaphragm of the eyepiece. On some microscopes, the tube lens is built into the body of the microscope itself.

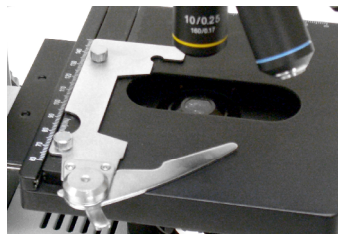
### ***Stage***

The platform beneath the objectives on which the slide or object to be observed is placed is called a stage. It has a smooth, flat surface and can be rectangular or circular. On most compound microscopes, the stage moves up and down and the nosepiece is stationary but on some microscopes just the reverse takes place. The stage has an opening for passing light. The simple type of stage is called a **plain stage** and the more sophisticated stage is called a **mechanical stage**.

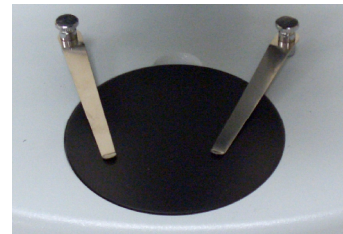
The more sophisticated and expensive mechanical stage makes it much easier to center specimen slides (standard slide size is 1" x 3") with precise movements in two axes with knobs which is critical at high powers (400x or more) where slight movements of the slide by hand moves the slide too much or out of the field of view. A vernier type scale on the stage allows for making measurements of the specimen. The "X" axis moves a slide forward and back (north/south) and the "Y" axis moves the slide side-to-side (east/west or left/right).



**Plain Stage**



**Mechanical Stage**



**Stage Plate**

A **stage plate** is used with stereo microscopes and this plate fits in over the lower illumination in a circular hole. It normally is a frosted glass plate or a dual sided (white and black) plastic plate which can be used for improving contrast on various specimens to be viewed with top illumination.

The working distance of a stage is the vertical area that is available to place specimens for examination.

## **Terms Associated with Microscopes**

**Magnification (power)** – the magnification of a microscope is determined by multiplying the power of the eyepiece by the power of the objective lens being used. As an example, a 40x objective lens times 10x eyepiece = 400x. Another way to look at this is that the magnification is the number of times the image is larger than the object would appear to the unaided (naked) eye.

Lower powers allow for brighter, sharper images combined with a wide field of view. Higher powers, often for examining slide specimens, present larger but dimmer images with a narrower field of view. When observing, always start with the lowest power on your microscope and progress to higher powers. High powers are useless unless the resolution is good. When the magnification is increased but the detail is not increased (due to the limitation of the resolution), it is called "empty magnification". For a given magnification, always use the higher magnification objective when given a choice rather than using a lower power objective with a high powered eyepiece as the results will always be better. For example, using a 40x objective and 10x eyepiece = 400x will give higher quality views than using a 20x objective and 20x eyepiece = 400x.

**Field of View** – the field of view is the diameter of the circle of light that you see when looking into a microscope and it is measured in millimeters. The lowest powers have the widest field of view. As you increase power, the field of view gets smaller. Some wide angle or super wide angle eyepieces increase the field of view over standard eyepieces.



**Depth of Field** – how much depth of field is a function of the objective lenses and means the farthest and nearest points in the field of view are in simultaneous sharp focus. Low magnification objectives have more depth of field than high magnification objectives.

**Depth of Focus** – means the farthest and nearest points in the film plane (photomicrography) or the CCD plane (video microphotography) which are simultaneously in focus. It is just the reverse of the depth of field, where here greater depth of focus occurs with high magnification objectives.

**Flatness of Field** – a quality describing the appearance of the field of view as being flat from edge-to-edge.

**Brightness** – how light or dark is the image? Brightness is related to the illumination system. It is also related to the N.A. of the objective lens where the larger the N.A., the brighter the image at that power.

**Contrast** – is the ratio of light and dark parts of a specimen or object and how well they are defined and the main part of the microscope that affects this is the illumination system.

**Collimation (Alignment)** – this is a condition in which all optical elements are centered on the same axis.

## Microscope Accessories

**Prepared Slides** – for those who do not wish to make their own slides or to supplement their collections, prepared slides are readily available. These slides can open up the world of microorganisms that lead to learning, discovery, and enjoyment. For best results use only glass slides that are 1” x 3” in size.

**Filters** – they can be useful in providing enhanced contrast and color correction for observing but not for photography. In some cases, colored filters can be a simple substitute for staining, which would kill live specimens. They usually lay over the top of illumination systems or on more expensive microscopes they sometimes have a special holder under the condenser. Blue is the most used filter since it absorbs some of the yellow to red light from of the illumination bulbs, used in many microscopes, resulting in a more natural coloration of the specimen. Green, yellow, and frosted filters all give varying effects and all filters should be experimented with to see the actual changes they make in observations of specimens.

**Blank Slides** – these are offered for those who want to make their own slides to observe. The higher quality ones are made of glass. Some may have a small depression or well to hold a few drops of liquid.

**Cover Glass (Cover Slip)** – these are extremely thin, flat glass or plastic covers that go over a specimen on a glass slide that has been made by you to protect the specimen during observation and storage. They come in different thicknesses which are usually matched to a number engraved on an objective lens for best performance.

**Slide Making Kits** – kits can contain blank slides, cover glasses, various types of stains to color objects or specimens, dissecting tools, labels, etc. which are all useful when making specimen slides.

**Imagers and Photo Adapters** – you can do photomicrography (documenting images) through your microscope. The most common form to image (and view) through a microscope is to use digital or CCD cameras. Most imagers are used as an accessory on the microscope and use specific adapters to attach them to the eyepiece tubes of both compound and stereo microscopes – **see page 8**. Various adapters are available to attach digital or film cameras to certain microscopes.

**Specialized Accessories** – some microscopes are set up to use specialized kits or adapters such as a phase contrast kit, a dark field adapter, or a polarizing kit.

## Optical Aberrations

Aberrations are any errors that result in the imperfection of an image. Such errors can result from design, fabrication, or both. It is impossible to design an absolutely perfect microscope. Some aberrations of a minimal amount are present in all microscopes and generally are less apparent as the price of the microscope increases. So, don't be too concerned unless the aberrations are severe or bothersome.

Two extremes are the Plan optical microscopes which provide incredible performance with the least amount of aberrations on a microscope and microscopes with plastic optics which give you the most aberrations and the worst performance.

**Chromatic Aberration** – is a failure to bring light of different wavelengths (colors) to a common focus. You may see color fringes or halos especially at the edges of the field of view or around the specimen you are looking at. Chromatic aberration is inherent in achromatic lenses which are the standard type used in microscope objectives but well designed objectives usually hold it to a minimum and within acceptable limits.

**Distortion** – is generally caused when magnification varies from the center of the field of view to the edges. A straight line will be curved as you look near the edges of the field. If the lines are curved outward, this is called barrel distortion and if the lines are curved inward then this is called pincushion distortion.

**Field Curvature** – is caused by the light rays not all coming to a sharp focus in the same plane. The center of the field may be sharp and in focus but the edges are out of focus and vice versa.

**Spherical Aberration** – is when light rays at different distances from the optical center come to focus at different points on the axis. This will cause a blurred image and you will not be able to focus sharply on any object.

## Recommended Books

To help you in understanding microscopes in greater detail and to learn various experiments and other usages of them, the following are recommended books:

- “*The Microscope Book*” by Shar Levine & Leslie Johnstone (Sterling)
- “*The World Of The Microscope*” by Chris Oxlade & Corrine Stockley (Usborne)
- “*Exploring with the Microscope*” by Werner Nachtigall (Sterling)
- “*The Microscope and how to use it*” by George Stehli (Dover)
- “*Guide to Microlife*” by Kenneth Rainis & Bruce Russell (Watts)
- “*Adventures With A Microscope*” by Richard Headstrom (Dover)

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