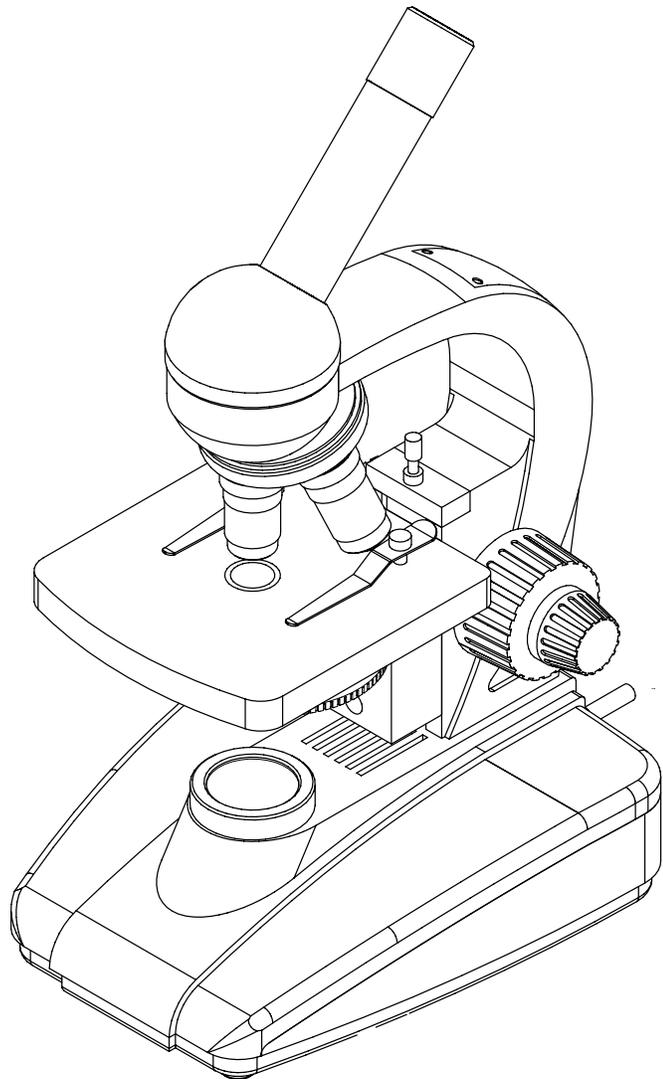


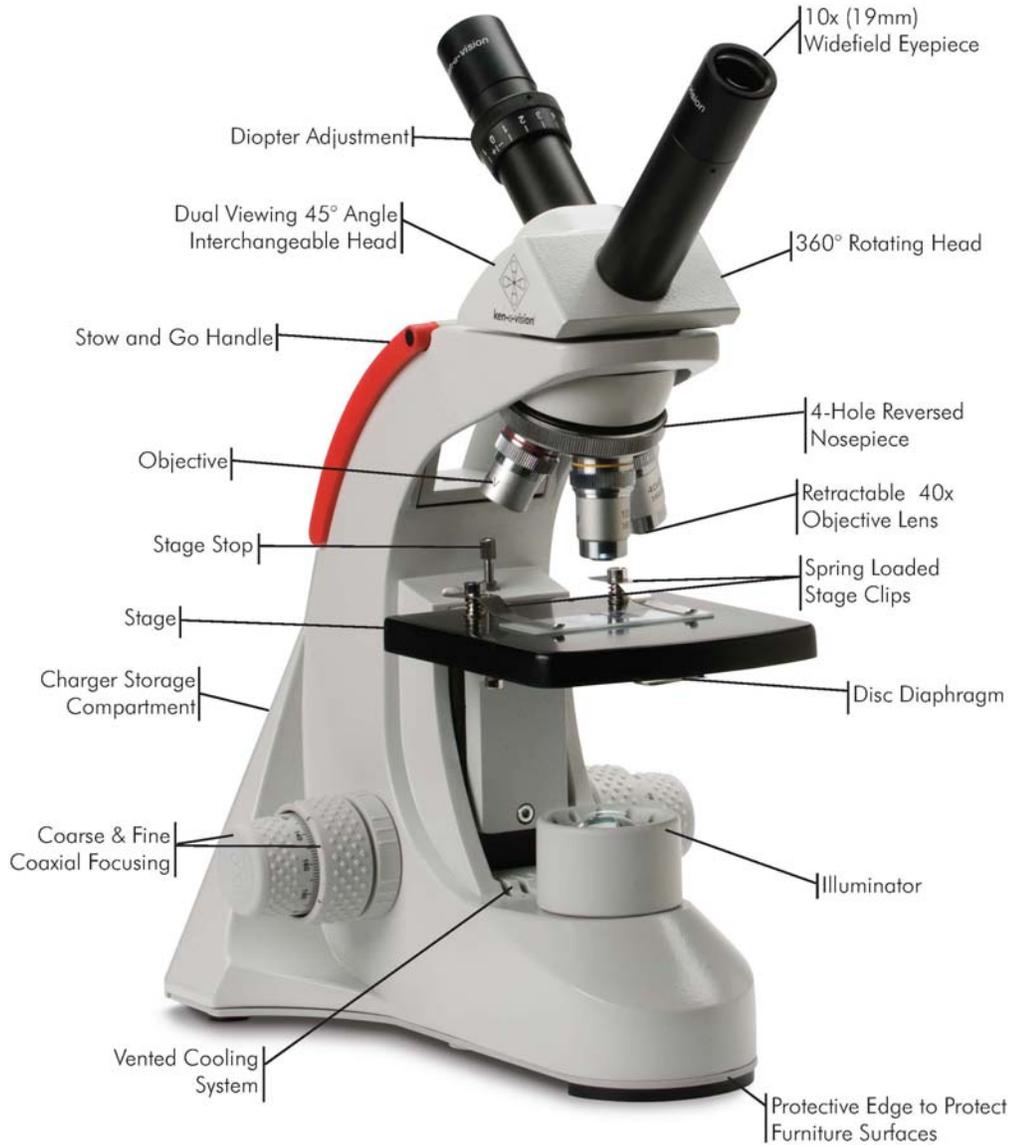
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KNOWLEDGE THROUGH VISION

MICROSCOPE PRIMER

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Anatomy of a Microscope



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Note – Please see generalized instructions on how to use a microscope at the end of this document.

Kinds of Light (Optical) Microscopes

Light – Light refers to use of light to send the image to your eye. This differs from an electron microscope which uses electrons to transmit the image. Light microscopes can magnify images 1,000 to 2,000 times, whereas electron microscopes can magnify images 100,000 times or more. There is an upper limit to the resolving power of both light and electron microscopes. Light can be sent through the specimen in various ways, some of which are described below under illumination.

Microscope – is a composite word from micro meaning *small*, and scope meaning *view*.

Compound Microscope – In the original ‘**simple**’ microscopes, a single light path went through the object viewed and one (1) lens, increasing the visibility of the object by some amount (magnification). (One light path, one lens = **simple** 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. Each lens magnifies the image over the previous one. (One light path, multiple lenses = **compound** microscope) Usually, a modern compound microscope has multiple lenses within the eye tube, and a series of three or four objective lenses on the ‘head’ which can be rotated into place. The image produced is a two dimensional (2-D) image.

Stereo or Dissecting Microscope – In a stereo or dissecting microscope there are two separate light paths, which produce a true stereo, three dimensional (3-D) image of the specimen. There is usually only one objective, but on close examination one will find two lenses (one for each path of light), side-by-side within the objective. This viewing three-dimensionally makes it very easy to visualize large objects, such as whole organisms or whole organs, or any ‘macro’ structure that can fit under the objective. Note, however, a stereo microscope has limited resolution (see definition below) and so is limited in the amount of magnification possible.

Digital Microscope - A microscope and video camera are integrated together with a digital USB output. Ken-A-Vision digital units come with software to allow the display of images to monitors or LCD projectors, and/or simultaneous recording of single images, or a series of time lapse images, or a time lapse movie, or a digital movie. There are two kinds of digital cameras, both of which convert light into electric charge and process it into electronic signals. The performance differences are highly technical, and vary with application. For most microscopes uses, the difference in quality of image is small.

1. **CCD Camera** – a Charge-Coupled Device. The specific technical detail is that in a **CCD** sensor, every pixel's charge is transferred through a very limited



number (often one) of output nodes to be converted to voltage, buffered, and sent off-chip as an analog (video) signal. All of the pixels can be devoted to light capture, and the uniformity of the output (a key factor in image quality) is high.

2. **CMOS Camera** – Complementary Metal Oxide Semiconductor - In a CMOS sensor, each pixel has its own charge-to-voltage conversion, and the sensor often includes digitization circuits, so that the chip outputs digital bits which may reduce the area available for light capture. The capture chip requires less off-chip circuitry for basic operation.

Microscope Frame Types

Upright Microscope – looks down on a specimen with its objectives. This is the most common type of microscope.

Inverted Microscope – looks up at the specimen. The inverted scope is designed to be used when specimens are very large or heavy or when the specimen is affected by gravity. (For example, during development, frog eggs will turn so the blastopore points down. An inverted microscope would allow direct viewing of this important development site.) It is also useful in viewing tissue culture tubes from the bottom up.

Microscope Vocabulary

Magnification – is to make something larger in size than it really is by use of lenses. In microscopes, this is usually indicated by the abbreviation "X". So a 10X eyepiece (see below) will magnify the image on a slide by 10 times. To determine total magnification of a specimen by a microscope, simply multiply the eyepiece magnification times the objective magnification. (For example, if the eyepiece is 10X and the objective is 10X, then the total magnification would be 100X.) As magnification increases, generally more light is needed to maintain the same level of resolution (defined below).

Field – is the area of observation on the slide when viewed through the microscope. As the magnification goes up, the field gets smaller in diameter, and requires more light for visualization of the slide.

Resolution - in microscopy, this refers to the ability of the lens system to separate the image into small parts OR the ability to separate two elements in the



viewing field from each other. As the light increases, resolution should increase or as the light decreases, resolution in general decreases. Since resolution is a function of diffraction the very best optical microscopes are limited to a resolution of 0.2 micrometers. NOTE – in modern usage, the resolution of a projector or a video monitor or any other display unit, has a different meaning, as noted below.

Parfocal – This is the ability of a microscope to stay relatively in focus as the user switches among the objectives. In a good, parfocal microscope, less than 1/8 of a turn of the fine adjust should bring the image back into focus.

Parcentered - When a microscope is parcentered, the specimens will appear centered in the field of view at every magnification. So if a field of a slide is centered at the lowest power, even though the field diameter shrinks at each higher magnification, the desired part of the specimen will remain in the center of the viewing field.

Objective Lenses

There are typically three types of objective lenses found on brightfield microscopes – **Achromat, semi-plan, and plan** objectives. To understand the difference, realize that all microscope lenses are curved, which results in an image that is slightly out of focus at the edges, and may produce chromatic or spherical aberrations due to this curved shape.

Achromat - An **achromatic lens (achromat)** is an objective lens that is designed to limit the effects of **chromatic** and **spherical aberration** (See notes below). Achromatic lenses are corrected by bringing two wavelengths (typically red and blue) into focus in the same plane. An objective that is Achromatic carries the implication (guarantee) that at least 60% of the field of view will have a quality flat focus.

Semi-Plan – **Semi-Plan** objectives are guaranteed to have a quality flat field of focus for 80% of the field of view. A **Semi-Plan Achromat** lens is also corrected for spherical and chromatic aberrations, as described above.

Plan Objectives – **Plan Objectives** guarantee a quality flat field of focus for 100% of the field of view. A **Plan Achromat** lens will also have the same corrections for spherical and chromatic aberrations as noted above. In Plan Objectives, when imaging the field, parts of the specimen at the edge of the visual field are almost as well focused as those in the center. Straight lines on the specimen appear straight throughout the visual field, without any aberrations. Such microscopes are especially good for photomicrography. The better the plan field objective, the more expensive.

Flat Field Objectives. – As one looks at the field of view, there may be slight blurring at the edge of the field. This edge blurring of Flat Field objectives is slight and easily corrected by a small tweak of the fine focus knob as one looks toward the edge of the field, or by moving what is at the edge into the center of the viewing field. Flat Field Objectives tend to be inexpensive when compared to Plan Field.

Plan Field versus Flat Field Objectives - Most educational microscopes are Flat Field Objectives due to this cost difference.

Infinity – Infinity lenses – In microscopy, the term "infinity" means that the objective lenses are designed to project their images to infinity, not to some finite distance. Traditional brightlight microscopes had a "fixed" focal point at 160 mm established by the standard in the 1800's. The infinity optical system provides an "infinite" region of parallel light between objective and eyepiece, pushing the image out approaching infinity.

Aberrations – Chromatic, Achromatic or Spherical – Any change from idealized and/or uniform light conditions coming through a lens is called an aberration.

Chromatic aberration - White light is made up of all the colors or wavelengths of the visible spectrum. These color components travel through the lens at different wave lengths and in doing so the lenses of the microscope may cause the different colors to refract (bend from a straight line) at different angles. This is called chromatic aberration.

Achromatic aberrations – Achromatic aberrations are caused by distortions in the smoothness of the lenses. The image appears blurry in places.

Spherical aberrations - Spherical aberration occurs when the rays of light passing through the center of the lens tend to focus on a different plane than those entering near the edge of the lens. This is because the curvature of the lens is not perfect. The variance in cost of microscopes, in general, is often attributed to the cost of lenses needed to cure this problem. Often, the only way that these problems can be overcome is by using a series of lenses, each lens carefully being designed to counteract the aberrations of the other lenses.

Microscope Parts (please see diagram provided)

Eyepiece or Ocular – lens at top of microscope through which observer looks to see specimen. These most commonly come as a 10X *widfield* lens. (older scopes may have a *Hygenia* eyepiece, which is a very narrow, pin-hole type lens) The 'X'

refers to the amount of magnification that this lens adds as a multiplier to the magnification of the specimen.

Kinds of eyepieces. -

Widefield eyepiece - an eyepiece with an [achromatic doublet lens](#) which allows the observer to view through the whole lens diameter rather than being limited to viewing only in its center. The enlarged opening allows a large non-distorted viewing of the field, making it easier, particularly for novice observers to use the microscope, because the position of the eye does not have to be 'perfect'. Ken-A-Vision microscopes come standard with 10X, widefield eyepieces, though 15X and 20X are available on special order.

Diopter – provided on some eyepiece tubes. A diopter is available on the Ken-A-Vision T-1202C, T-1702C, T-19021C, T-19031C, T-2901 microscopes. A diopter allows the user to focus that eyepiece separately from a second eyepiece on the same microscope without changing the focus of the microscope itself. This will allow (with a T-19021C) for the teacher to focus for their eyesight, rather than the student's, or in the T-19031C, T-29031, microscopes it allows for most users to compensate for their eyeglass prescription, allowing them to use the microscope directly without glasses. Diopters also come on Ken-A-Vision digital microscopes, so that once an image is focused on the computer, the eyepiece allows the user to focus the microscope for direct viewing with their eyes.

Head – the head is the part of the microscope that connects the eyepiece to the nosepiece. In all Ken-A-Vision compound microscopes, the head may be rotated 360°, allowing two or more observers to see into the scope without the microscope itself having to be picked up and rotated.

Monocular head – A single eyepiece, is set at 45° to the head for viewing by one observer. Inexperienced microscopists will tend to close the eye not looking through the eyepiece, which may result in headaches and eye strain to the intense light in one eye and darkness in the other.

Dual Viewing head – Two eye tubes and two eyepieces, each of which are set to rise 45° from the head of the microscope, and are set at 180° from each other. This allows two observers to use the microscope simultaneously, for example a teacher and the student, or two students. Again leaving both eyes open is suggested. One eye tube may have a diopter on it which allows that individual to focus for themselves given differences in user's eyesight acuity.

Binocular head – Two eyepieces, side by side, one for each of the Viewer’s eyes. The two eyepieces are generally set at a comfortable 45° angle. The inter-ocular or inter-pupillary distance may be adjusted to match the distance between an individual’s eyes.

Seidentopf (*sigh-den-top-ff*) **binocular head** - a head design where the increasing or decreasing the distance between the two eyepieces (interpupillary distance) is done by twisting the eyepieces in an up and down arc motion similar to most binoculars.

Slider binocular head – Inter-pupillary distance is adjusted side-to-side, by sliding the eyepieces towards and away from each other.

Trinocular head – This microscope has a binocular head for viewing and an additional eyepiece for a camera mount. Ken-A-Vision trinocular heads have an 80:20 split feature so that when a slide adjustment feature is in position one, 80% of the light comes to the camera and 20% comes to the binocular head, allowing a view of what the camera will see. In the opposite position, the slide allows 100% of the light to get to the binocular eyepieces.

Nosepiece – In a compound microscope there are usually 3 or 4 openings, holding 3-4 objective lenses. Most commonly the objectives come in 4X (usually referred to as ‘scanner’), 10X (usually referred to as ‘low power’) and 40X (usually referred to as ‘high power’) and finally 100X (most commonly an ‘oil emersion’ lens for microbiologicals). The 40X and 100X lens are spring-loaded at the end, which allow the objective lens to be shortened in response to the thickness of the slide/cover slip being observed and preventing lens breakage, lens scratching or slide breaking, or slide scratching. Refer to microscope terms for the meaning of “X” and magnification.

Reversed Nosepiece – a common feature on Ken-A-Vision microscopes, positions the objectives in a ‘tucked’ position under the head and nosepiece, allowing ease of placing slides onto the stage from the front of the microscope.

Objectives – Microscopes commonly have 3 or 4 objectives usually of 4X, 10X, 40X, and 100X Oil Emersion. Other objectives, such as 60X are available as special order on a limited number of Ken-A-Vision microscopes.

DIN Standard Objectives – Stands for Deutsches Institut fuer Normung, which is an international standard which dictates the design compatibility of the objective lens. Therefore DIN standard objectives from one manufacturer can be used in another manufacturer’s DIN standard compatible microscope. All Ken-A-Vision microscopes are DIN except the T-1200C (elementary) series microscopes.



Stage – The platform beneath the objectives on which the slide or object to be observed is placed. In Ken-A-Vision microscopes, the stage moves up and down and the nosepiece is stationary. (In other scopes the opposite may be true).

Stage Clips or Mechanical Stage (Slide Manipulator) – Ken-A-Vision microscopes have patented spring loaded clips for holding a slide in place upon the stage. A more sophisticated way to hold the slide is using a mechanical stage. This is a mechanism mounted on the stage that allows the operator to move the specimen slide in the X or Y direction by turning a knob. This is particularly useful at higher magnifications where slight movement of the slide may produce a large movement in the field of view. A Vernier type scale on the stage allows the exact marking and replication of an object in the field that the viewer may want to come back to. Mechanical stages are optionally available for many models of Ken-A-Vision microscopes.

Diaphragm – located on or below the stage of the microscope and adjusts the amount of light passing into the slide or specimen.

Disc Diaphragm – a rotating disc with 6-10 openings of differing diameter which limit the amount of light passing through the specimen.

Iris Diaphragm – a single opening whose diameter can be varied usually from no light passing through to full diameter of the visual field. It operates similar to the iris of the human eye, or the shutter of a camera.

Condenser – a lens or lens system located either within or below the stage which helps to focus the light coming into the specimen from the microscope's light source. All Ken-A-Vision microscopes have at least a single lens located within the opening of the stage.

Abbé Condenser – a moveable lens system under the stage that can be moved up and down vertically, regulating the amount of light from the illuminator. It contains an adjustable iris to control the beam diameter of the light. An experienced microscopist can have good control over the amount of light by manipulating the iris, or moving the Abbé condenser up or down. It is very useful at higher magnifications.

Coarse and Fine Adjustment knob(s) – Located on the side of the frame, below the stage, allow the observer to adjust the focus of the microscope.

Coaxial Controls – Consists of two knobs where one smaller, knob (fine adjust) is centered on top of another, larger knob (coarse adjust). This is a feature of all Ken-A-Vision compound microscopes except the 1200 series for elementary students.

Singlet Control – one knob does both coarse and fine adjustment. Excellent for novice and young microscope users.

Rack and Pinion – refers to the interlocking of a series of gears and cams that allows the coarse and fine adjustment knobs to interact. All new Ken-A-Vision microscopes have a rack and pinion system.

Microscope Illumination

Light Sources - Ken-A-Vision offers four basic kinds of light sources on microscopes.

Tungsten – optionally available on the research grade (compound) microscopes – T-2900 plus in the top light of the T-2200C and T-2600C stereo microscopes. In general this is a moderately hot light source.

Halogen – optionally available on the T-1200C, T-19001C and T-2400C series microscopes. This is generally the hottest (temperature) light source available on a microscope.

Fluorescent – optionally available on the T-19001C series compound scopes, and as the bottom light on the T-2200 and 2600 stereomicroscopes. This is generally a cooler light, though the transformer needed may get warm.

LED – (“Ken-A-Vision cool light”) – Optionally available on the all Ken-A-Vision microscopes except the research grade compound microscopes (T-2900’s). This is a bright light source, but puts out essentially no heat. Great for live specimen work.

Forms of Microscope Illumination

There are various forms of microscope illumination, produced by varying the amount of light or the quality of the light allowed to impinge on the microscopic slide – **Bright Field, Diffusion Illumination, or Phase Contrast**. Most student grade microscopes are Bright Field or Diffusion Illumination. Research grade microscopes (T-2900’s) are capable of being modified for either bright field or phase contrast microscopy.

Bright Field – in conventional Bright Field microscopy, the light path is as follows: light is aimed towards a lens from beneath the stage through a condenser lens, through the specimen, through an objective lens, and to the eye through a second magnifying lens, the eyepiece. This is the most fundamental lighting of microscopes, and involves a highly directional and intense light source. All



Ken-A-Vision microscopes have condenser lens, though not always moveable. In the higher level microscopes (e.g. KAV T-19031C, T-19541C, T-2900) there is a moveable, focusable condenser (Abbé). An Abbé Condenser may optionally be added to a T-19011C or T-19021C. In the lower, less expensive microscopes (KAV T-1201C, T-1202C, T-1754, T-19011C, T-19021C) a non-moveable, non-focusable stage lens is present. The resultant of Bright Field Illumination is that a very high intensity light can be seen in the microscope field. This light intensity may be modified by various DISC OR IRIS diaphragms (see above)

Diffusion Illumination – Sometimes the presence of ground glass, translucent plastic, opalescent materials or some other suitable materials can be placed 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. This often allows the iris to be left completely open, so that the resolution can be maximized.

Phase Contrast Microscopy – A large spectrum of living biological specimens are virtually transparent when observed in the optical microscope under bright field illumination. To improve visibility and create contrast in such specimens, microscopists often reduce the opening size of the substage condenser **iris** diaphragm. Unfortunately this may be accompanied by a serious loss of resolution and the introduction of artifacts (due to the diffraction of the light). This technique provides an excellent method of improving contrast in unstained biological specimens without significant loss in resolution, and is widely utilized to examine dynamic events in living cells. One of the major advantages of phase contrast microscopy is that living cells can be examined in their natural state without being killed, fixed, and stained. As a result, the dynamics of ongoing biological processes in live cells can be observed and recorded in high contrast with sharp clarity of minute specimen detail. A phase contrast 'kit' can be added to some Ken-A-Vision microscopes particularly the T-19031C, or T-2900.

Specialized Kinds of Microscopy

Fluorescence Microscopy - Fluorescence illumination and observation is the most rapidly expanding microscopy technique employed today, both in the medical and biological sciences, a fact which has spurred the development of more sophisticated microscopes and numerous fluorescence accessories. Fluorescence occurs when susceptible molecules emit light from electronically excited states created by either a physical (for example, absorption of light), mechanical (friction), or chemical mechanism. Generation of luminescence through excitation of a molecule by ultraviolet or visible light photons is a phenomenon termed **photoluminescence**,



which is formally divided into two categories, **fluorescence** and **phosphorescence**, depending upon the electronic configuration of the excited state and the emission pathway. Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength after a brief interval, termed the fluorescence lifetime. The process of phosphorescence occurs in a manner similar to fluorescence, but with a much longer excited state lifetime.

Electron Microscopy – Ken-A-Vision does not manufacture electron microscopes. Electron Microscopes 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. Employing an electron microscope allows the user to examine very fine parts of a specimen including its topography (surface features), its morphology (shape and size of small particles and structures giving structure to specimen), composition (what the specimen is made up of) and even crystallographic information (such as how atoms are arranged in the object, or strength and electrical properties). There are increasingly more variations in electron microscopes, but two basic types are:

Scanning Electron Microscope – patterned after the light microscope, with an electron beam replacing the light and thereby allowing magnifications in excess of 1000X.

Transmitting Electron Microscope – a transmitting electron microscope shines a beam of electrons (like the light) through the specimen. When electrons hit the specimen, they are stopped and others pass through or are transmitted. The ones that are transmitted are projected onto a phosphor screen for the user to see an image.

Learning to use a Light (Optical) Microscope

1. Carrying – To properly carry a microscope, grasp the microscope arm with one hand, and place the other hand under the base of the scope.
2. Place the microscope on your table, with the curvature of the arm facing you.
3. While looking at the stage, turn the coarse adjust knob, so that stage moves down and away from the objective.
4. Revolve the nosepiece until sure that the lowest power (4X generally) is in position, and you can feel (or hear) it click into place.
5. Place your slide or specimen into position, with the object to be viewed centered in the middle of the opening of the stage.
6. Looking through the eyepiece and using the coarse adjust, slowly move the specimen towards the objective (up), until it comes into fairly good focus.
7. Now 'fine-tune' your focus using the fine adjust knob, to get your clearest view. Move the slide using your fingers or a mechanical stage (if available) to center in the viewing field the portion of the specimen you wish to examine.
8. Adjust the diaphragm to get the best image. In general, the lower the magnification you are using, the less light is needed, and the image will be clearest in the least light possible. Continuous use of the highest amount of light often results in the observer getting headaches from the intensity of the light hitting the eye.
9. Now look at the slide from the side (NOT THROUGH THE EYEPIECE) and rotate the next higher powered objective into place – feel/hear it click. Observing from the side prevents the observer from smashing the objective into a too thick slide, breaking the slide and scratching the objective lens.
10. The field you view will now be reduced in diameter, but if you centered in step 7, what you wished to view will be in place. IF the microscope is parfocal, as it should be, a small turn of the fine adjust should bring the image into clear focus. You may have to increase the amount of light coming through the diaphragm, since as magnification increases, the amount of light needed must also increase.
11. If the observer wishes to go to the next higher power, simply repeat steps 9 and 10. (BE CAREFUL TO CHANGE OBJECTIVES WHILE LOOKING FROM THE SIDE!)



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