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MICROSCOPE PRIMER

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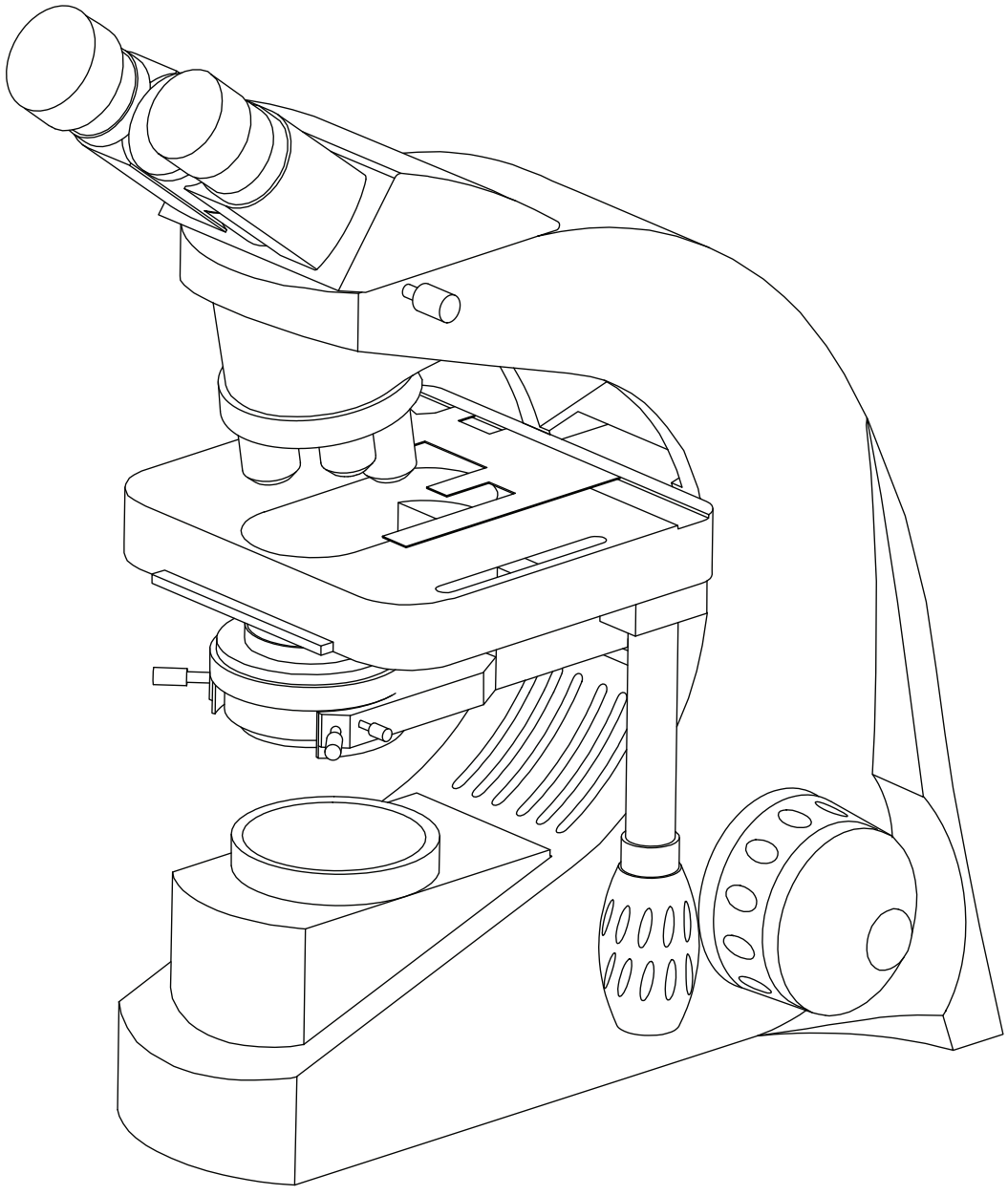

Microscopy Primer

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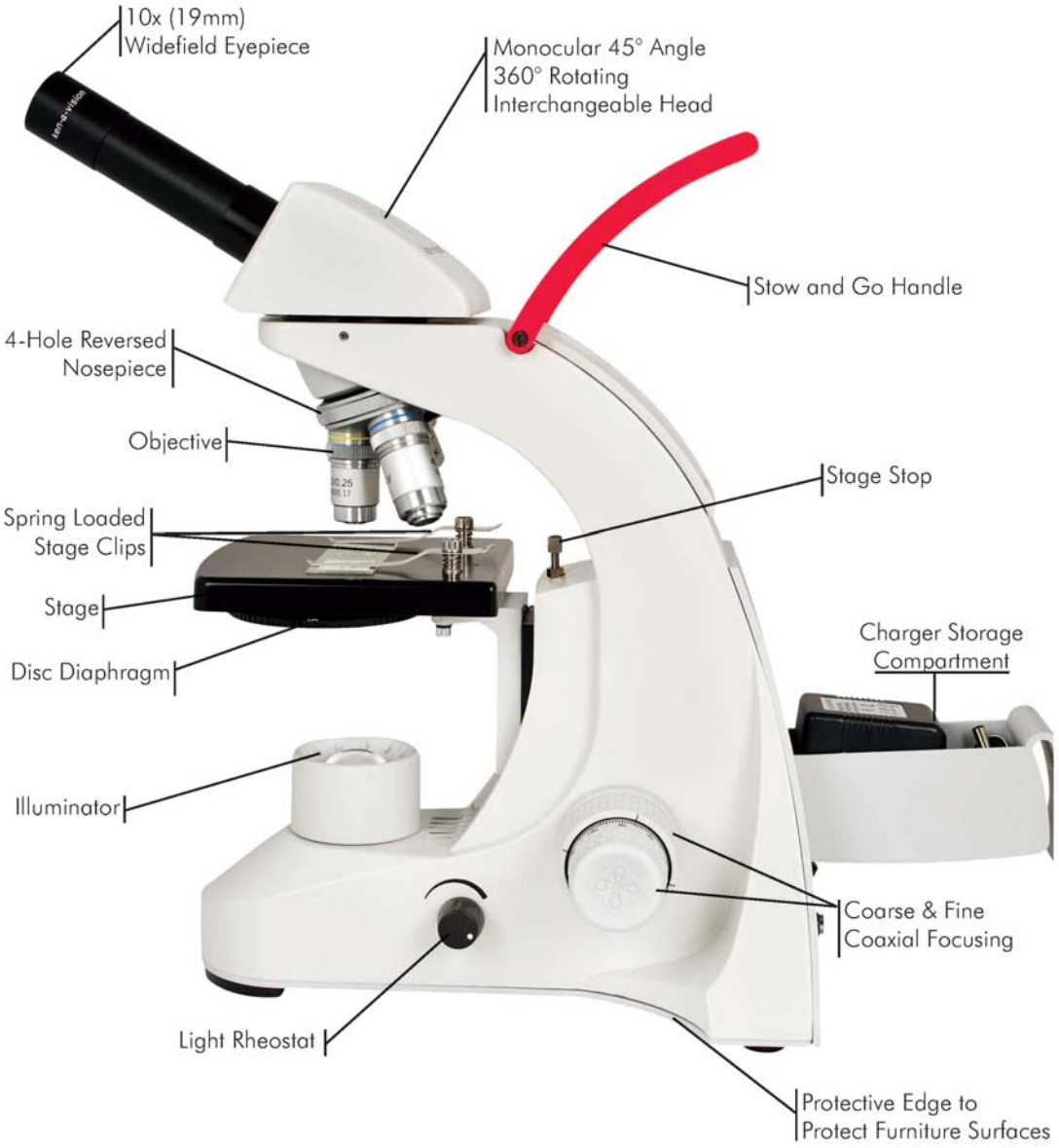
Anatomy of a Microscope



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Types of Microscopes

Stereoscope: binocular viewing of two separate light paths, which produce a three dimensional (3-D) image for viewing larger specimen.

Light Microscope: refers to the use of light to send the image to your eye by refracting the light, which magnifies the object 1,000-2,000 times larger.

Compound Microscope: a single light path passes through multiple lenses within an 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.

Digital Microscope: A microscope and video camera are integrated together with a digital USB output. There are two types of cameras that can be used:

> **CCD Camera:** Charge-Coupled Device. 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 is high resulting in a higher quality image and more pixels than a CMOS camera.
>
> **CMOS Camera:** Complementary Metal Oxide Semiconductor. In a **CMOS** sensor, each pixel has its own charge-to-voltage conversion, and the sensor often also 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. These cameras are more susceptible to noise in the image.

Parts of a Microscope

Coarse and Fine Coaxial Focusing: Located on the side of the frame, below the stage, allows the observer to adjust the focus of the microscope.

> **Coaxial Controls:** two knobs where one smaller, knob (fine adjust) is centered on top of another, larger knob (coarse adjust).
>
> **Rack and Pinion:** refers to the interlocking of a series of gears and cams that allows the coarse and fine adjustment knobs to interact.
>
> **Singlet Control:** one knob does both coarse and fine adjustment.

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.

> **Abbe 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.

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 5-10 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.

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 for the teacher to focus for their eyesight, rather than the student's or it allows for most users to compensate for their eyeglass prescription, allowing them to use the microscope directly without glasses.

Eyepiece or Ocular: 10X *widefield* lens at the top of a microscope through which the observer looks to see the specimen.

Head: the part of the microscope that connects the eyepiece to the nosepiece.

> **Monocular head:** single eyepiece, set at 45° to the head for viewing by one observer.
>
> **Binocular head:** two eyepieces, each of which are at 45° to the head of the microscope, and set at 180° from each other. This allows two observers to use the microscope simultaneously.

Trinocular head: a binocular head for viewing and an additional eyepiece for a camera mount.

Seidentopf binocular head: a head design where the interpupillary distance between the two eyepieces is done by twisting the eyepieces in an up and down arc motion similar to most binoculars.

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

Mechanical Stage: 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.

Vernier Mechanical Stage: a 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.

Nosepiece: in a compound microscope, there are usually 3 or 4 openings, holding 3-4 objective lenses.

Reversed Nosepiece: 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: 3 or 4 objectives on a nosepiece, usually of 4X (scanning), 10X (low power), 40X (high power), and 100X (oil emersion).

Stage: the platform beneath the objectives on which the slide or object to be observed is placed.

Stage Clips: clips for holding a slide in place upon the stage.

Stage Stop: a small bar and screw between the stage and the arm of the microscope to prevent the stage from coming too far up to hit the objective lens.

Magnification

	Magnification	Ocular Lens	Total Magnification
Scanning	4x	10x	40x
Low Power	10x	10x	100x
High Power	40x	10x	400x
Oil Emersion	100x	10x	1000x

Microscope Illumination

The light sources most commonly used, listed from a cool to hot source, are the *light-emitting diode (LED), fluorescent, tungsten,* and *halogen*. There are various forms of microscope illumination, produced by varying the amount of light or the quality of the light allowed to impose on the microscopic slide.

Bright Field: the 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. The resultant is that a very high intensity light can be seen in the microscope field.

Diffusion Illumination: sometimes the presence of ground glass, or translucent plastic, or opalescent 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: to improve visibility and create contrast in specimens, 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.

Learning to Use a Microscope

1. Carry the microscope by grasping 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 adjustment knob, so that stage moves down and away from the objective.

4. Revolve the nosepiece until the scanning 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 adjustment knob, slowly move the specimen towards the objective (up), until it comes into fairly good focus.

7. Use the fine adjustment 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.

9. Look at the slide from the side and rotate to the next higher power objective.

10. The field of view will now be reduced in diameter, but if you centered in step 7, what you wished to view will be in place.

11. A small turn of the fine adjustment knob should bring the image into clear focus. Remember when magnification increases, the amount of light needed must also increase.

How to Care for Your Microscope

General Care

1. Microscopes should always be stored in a clean, dry storage area, as far from any interaction with chemical fumes, or other vapors that might scratch glass.

2. After every use, microscopes should be carefully cleaned to remove any water, dirt or other debris, and any immersion oil that might be on the stage or the ends of the objectives.

3. On objectives only use **lens paper**, not paper toweling. The body of the microscope may be wiped with a dry or damp toweling.

4. Cordless microscopes should always be stored charged, particularly if the unit will not be used again for several weeks. Batteries tend to discharge over time, and if the microscope is stored already partially discharged, by the time the user tries to charge it back up, batteries may be too low to recover. Storing units in a charged condition should give the average cordless unit many years of use without battery repair.

Annual Maintenance

1. Clean all eyepieces and objectives carefully using lens paper, and if necessary lens cleaner. A cotton swab is dipped in lens cleaner and can aid in getting into the edges of the eyepieces and objectives to dislodge any debris or dirt accumulated there.

2. If the eyepieces are still firmly attached with the small set screw, then there should be no need to take them out of the eye tube unless obvious dust has accumulated there. Once unscrewed, the set screw often gets lost, and then the eyepieces become subject to being pulled on and off by students, resulting in significant dust and debris being pulled inside the microscope and potential damage occurring.

3. The nose piece and objectives inserted into the nose piece should be carefully checked for looseness. If the nose piece itself has become loose, often there is a screw located in the middle of the bottom of the nosepiece which can be seen by turning the microscope upside down. If the user has a tool that can reach this without stripping the head or causing other damage, they might be able to tighten the nosepiece.

    - Inability to find the screw, or lacking a tool to easily reach it, is excellent reason for sending the microscope to a microscope repair service for general maintenance and cleaning.

4. The stage should move up and down easily, **but** only when the coarse/fine adjust knob(s) are turned. If the stage moves down on its own weight or the stage is loose, the instrument should be sent in to a microscope repair shop for general maintenance, cleaning, and repair.

5. Mechanical stages will sometimes become loose, or bent. The mechanical part of the stage is screwed on to the stage with 2 screws. Locating these screws and tightening them will often fix a loose mechanical stage. A bent stage generally requires the unit to be sent in to a microscope repair shop for general maintenance, repair, and cleaning.

Maintaining Digital Microscopes

1. As with all computer and electronic equipment, the camera and its USB connection should be kept away from water and other spills.
2. If the USB cord or computer connector gets wet, dry immediately and thoroughly before reconnecting to the computer. Failure to do this could result in a short to either the camera or the computer and possibly result in expensive damage.

Should your microscope and camera have technical problems, first call a service repair shop, and they will try to save the problem or give you instructions on returning the unit for repair.

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