

Microscopy

Danil Hammoudi.MD



Compound by William Salmon (circa 1860)

Care and Handling of the Microscope:

- •A microscope is a **delicate** piece of equipment and should be treated with care.
- •Use **two hands** when carrying the microscope. Place one hand around the arm of the microscope and the other under the base for support.
- •Carry the microscope **upright** and close to the body.
- •Place the microscope **flat on the table**, but not too near the edge where it might be knocked off.
- •DO NOT slide the microscope back and forth on the lab table.
- •If it becomes necessary to clean the lenses on the microscope, ask your facilitator for a piece of **lens paper**. Other materials, such as paper towel, can scratch the surface of the lens.

COLOR BLIND TEST











The test to the left is simpler.

The individual with normal color vision will see a 5 revealed in the dot pattern. An individual with Red/Green (the most common) color blindness will see a 2 revealed in the dots.

- There are two basic types of microscopes commonly used to study anatomy today:
- light microscopes and electron microscopes.
- ullet
- -<u>A light microscope</u> works as light is passed
- through the **specimen** to be observed; the light then passes through one or more glass **lenses**, which magnify and focus the image.
- -<u>An electron microscope</u> shoots a beam of electrons through the specimen. Rather than using glass lenses, magnets are used as lenses to magnify and focus
- the image.









Because the lens systems in a microscope are composed of many lenses it is called compound. The typical illumination of specimens in which light passes through the specimen and travels to your eye is called **bright field microscopy**. Light has the following path.

- •Eye or Camera
- •Ocular
- •Body or Tube
- •Specimen
- •Objective
- •Condenser (Iris)
- •Condenser Lenses
- •Field (Iris) Diaphragm
- •Mirror
- •Light Source

parfocal, which means they are of

equal focus

The relationship between field of view and magnification is said to be

inversely proportional.

Eyepiece Lens: the lens at the top that you look through. They are usually 10X or 15X power.

- Tube: Connects the eyepiece to the objective lenses
- Arm: Supports the tube and connects it to the base
- **Base**: The bottom of the microscope, used for support
- **Illuminator:** A steady light source (110 volts) used in place of a mirror. If your microscope has a mirror, it is used to reflect light from an external light source up through the bottom of the stage. **Stage:** The flat platform where you place your slides. Stage clips hold the slides in place. If your microscope has a mechanical stage, you will be able to move the slide around by turning two knobs. One moves it left and right, the other moves it up and down.
- **Revolving Nosepiece or Turret**: This is the part that holds two or more objective lenses and can be rotated to easily change power.
- **Objective Lenses**: Usually you will find 3 or 4 objective lenses on a microscope. They almost always consist of 4X, 10X, 40X and 100X powers. When coupled with a 10X (most common) eyepiece lens, we get total magnifications of 40X (4X times 10X), 100X, 400X and 1000X. To have good resolution at 1000X, you will need a relatively sophisticated microscope with an Abbe condenser. The shortest lens is the lowest power, the longest one is the lens with the greatest power. Lenses are color coded and if built to DIN standards are interchangeable between microscopes. The high power objective lenses are retractable (i.e. 40XR). This means that if they hit a slide, the end of the lens will push in (spring loaded) thereby protecting the lens and the slide. All quality microscopes have achromatic, parcentered, parfocal lenses.

Observing and Drawing Objects:

Because the light rays from an object cross before reaching your eye, the image you see through our light microscopes will be **inverted** and **upside down**



Rack Stop: This is an adjustment that determines how close the objective lens can get to the slide. It is set at the factory and keeps students from cranking the high power objective lens down into the slide and breaking things. You would only need to adjust this if you were using very thin slides and you weren't able to focus on the specimen at high power. (Tip: If you are using thin slides and can't focus, rather than adjust the rack stop, place a clear glass slide under the original slide to raise it a bit higher)

Condenser Lens: The purpose of the condenser lens is to focus the light onto the specimen. Condenser lenses are most useful at the highest powers (400X and above). Microscopes with in stage condenser lenses render a sharper image than those with no lens (at 400X). If your microscope has a maximum power of 400X, you will get the maximum benefit by using a condenser lenses rated at 0.65 NA or greater. 0.65 NA condenser lenses may be mounted in the stage and work quite well. A big advantage to a stage mounted lens is that there is one less focusing item to deal with. If you go to 1000X then you should have a focusable condenser lens with an N.A. of 1.25 or greater. Most 1000X microscopes use 1.25 Abbe condenser lens systems. The Abbe condenser lens can be moved up and down. It is set very close to the slide at 1000X and moved further away at the lower powers.

Diaphragm or Iris: Many microscopes have a rotating disk under the stage. This diaphragm has different sized holes and is used to vary the intensity and size of the cone of light that is projected upward into the slide. There is no set rule regarding which setting to use for a particular power. Rather, the setting is a function of the transparency of the specimen, the degree of contrast you desire and the particular objective lens in use.

Calculation of the field of view FOV magnification (MAG).

The **field of view** is the part of the observable world that is seen at any given moment.

$FOVA \times MAGA = FOVB \times MAGB$

-"A" refers to a microscope with one objective in place

- the subscript "B"refers to the same microscope with a different objective in place.