How to Achieve the Highest Quality Imaging

Magnification vs. Resolution

Magnification: This is the product obtained when you multiply the (objective magnification) X (eyepiece magnification). Since a magnification of 10X in the eyepiece has come to be standard, the range of magnification depends mainly on your choice of objectives.

Resolution: Magnification determines the apparent size of the image. The quality of the image, or the rendering of detail within the image, is a separate function called resolution. Resolution depends primarily on the quality of the objective and the way in which it is illuminated. To exploit the full resolution of any objective, it must be appropriately illuminated by a converging beam or “cone” of light: the higher the potential resolution of the objective, the wider must be the cone. The maximum width of the cone of light that any lens will admit is indicated by its numerical aperture, abbreviated N.A.

Mathematically, N.A. =(Sin. ½ angle of cone) X (refractive index of the medium between the specimen and objective lens)

The N.A. of an objective is usually marked on the objective lens mount (10 / 0.25, 40 / 0.65). The larger the N.A., the higher the potential resolution of the objective.

You may have noticed that the medium between the specimen and objective is an integral mathematical part of the N.A. formula. The N.A. of oil immersion lenses take into account the use of immersion oil as a medium. If immersion oil is not used the potential resolution of the oil immersion objective can never be achieved.

The N.A. formula also includes the shape of the cone of light entering the microscope objective. Without a condensing system built into the stage or below the stage (substage) to shape the cone of light to the desired angle, the resolution potential of objectives magnifying more than 40X will be greatly hindered. For a 40X/ N.A. 0.65 objective an N.A. 0.65 condenser should be provided. Similarly, a 100X/ N.A. 1.25 oil immersion objective requires an N.A. 1.25 condenser. For the 100X/ N.A. 1.25 objectives, the situation is even more complex than described above. Not only must an adequate condensing system be used but the cone of light must be properly focused.

Empty Magnification:

Keep in mind that there is a limit to meaningful magnification. When you magnify an object beyond 1000x, it will continue to get larger, but the resolution will stay the same. This will actually make the image blurry and unclear, and it is called empty magnification.

This is why it is not professionally recommended to change the WF 10x eyepiece to a higher magnification eyepiece such as the (WF 15x, 20x, or 25x), because 1000x magnification is the maximum limit of clear magnification achieved with an optical system

Using your Student & Advanced Microscope

How to Achieve the Highest Quality Imaging:

A. Student Microscope:

- Select a position to work where little direct light falls on the instrument. The worst position is to face a large window, because light falling directly on the slide will affect contrast and resolution.
- Turn the disc diaphragm so that the number 2 or 3 opening is aligned with the instage condenser.
- Place a low powered objective into position and simply plug it into any grounded receptacle
- Place the microslide specimen to be observed under the spring stage clips. If using a mechanical stage, pull back the lever on the left side of the stage, insert the slide, then bring the crescent shaped holder into contact with the slide. Be certain that the coverslip of the slide is facing toward the objective; otherwise you will not be able to focus your specimen at high magnifications.
- Position your specimen so that it is centered over the instage condenser.
- Focus the objective on your specimen by turning the LARGE COURSE ADJUSTMENT KNOB until the image of your specimen is bright and clear. (Microscopists will always lower the objective to a point which they know is beneath the "focal plane" and focus upwards). Now bring the specimen into sharp focus by turning SMALLER FINE FOCUS KNOB slightly.
- With the specimen now in sharp focus rotate the nose-piece to the other objectives and focus using only the fine focus knob. Since the optics in these microscopes are both parfocaled and parcentered only a slight turn of the fine focus knob will be necessary.

  Note: It is important to note that because of our built-in stop the 4X and 10X objectives can never come into contact with your microslides. The 40XR and 100XR may occasionally touch the microslide but because these lenses are in retractable mounts your slide will not be damaged.
- Adjust the disc diaphragm until proper specimen contrast is reached
- You are ready for viewing!

B. Advanced Microscope

(All models equipped with an Abbe-condesner with or without the 100XR oil immersion objective)

1 – 6. Same as in steps A1 through A6 above

7. FOCUSING THE CONDENSER: There are two different types of Abbe-condensers. One includes a spiral focusing mount that you turn to move it up or down and the other is a rack and pinion system, controlled with a condenser focusing knob. To focus the Abbe-condenser with spiral focusing mount, loosen the large lock screw facing you as you look at the front of the condenser. Twist the base of the condenser to the left to lower and to the right to raise. Adjust the condenser until the illumination of the field is uniform.

8. Adjust the condenser iris diaphragm to match the N.A. of the objective. This is simply done by first closing the iris and then opening it slowly until the entire field is evenly and brightly lit and in good focus. If the objective is changed to a higher power, the iris must be adjusted to the new objective.

9. USE OF THE 100XR OIL IMMERSION OBJECTIVE: Here is how to do it:

Lower the stage and swing out the objective to give yourself room to work. Place one or two drops of quality immersion oil over the slide cover slip, which is needed to gather enough light for viewing with 100xR. Swing the 100xR objective back in place again, and proceed by moving the stage upwards until the lens makes contact with the oil. Continue (slowly) to focus down with coarse adjustment until the color or a blurred outline of the specimen appears. Now complete the focusing with the fine adjustment so that your image details come into sharper focus.

When you are finished using the slides, ensure to clean the oil off of the slides and microscope lens by using lens paper and cleaning solution.

In using the 100xR DIN objective, the most favorable resolution is obtained with the Abbe condenser nearly touching the slide specimen. Ideally, a drop of immersion oil is placed between the condenser and the slide, as well as between the slide and the 100xR DIN objective. Although this practice is not always followed in routine study, it is the only way to take full advantage of the inherent resolution of the 1.25 NA Abbe condenser.

Correcting Basic Mechanical Problems

Drifting:

If the body of your microscope falls toward the stage by the weight of gravity and will not stay in a focused position, it is said to be **"drifting"**. It is the result of loss of tension in the pinion mechanism due to normal and constant use. The tension is easily and quickly adjusted. You need not employ the services of a microscope technician to perform the function.

To correct for drift you will need the small metric allen wrench that is supplied with every microscope. Immediately to the right of the left coarse focus knob there is a pinion tension adjustment ring. Using the allen wrench, loosen the allen screw of the adjustment ring. This will allow you to turn the adjustment ring in a clockwise motion in relation to the coarse focus knob. A ¼ to ½ turn is all that should be necessary. Again tighten the allen screw.

Test the tension. If the microscope body still drifts, repeat the procedure above. If the tension is now too tight, reverse the procedure. A little practice will make you an expert in adjusting the drift control.