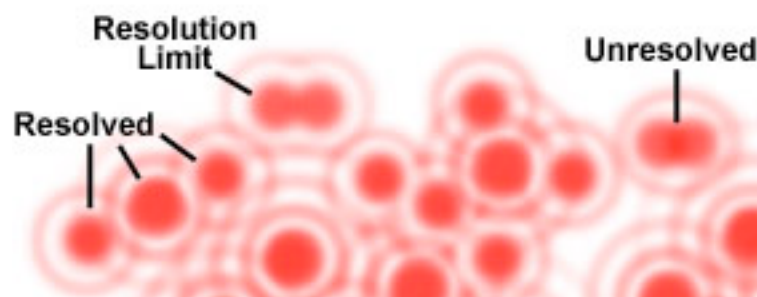


## A BRIEF INTRODUCTION TO MICROSCOPY

The two key properties of a microscope that allow you to see microbes are resolution and magnification.

**Magnification** refers to the enlargement of the specimen when seen through the microscope. For a compound light microscope like we will use in this lab, magnification is achieved through the use of lenses. You will view your microbes using two main lenses. The ocular or eyepiece contains a lens with a magnification power of 10X (ten times larger than when viewed with the naked eye). The microscopes are equipped with three objectives, each of which contains a lens with a different magnification power: 10X, 40X (some of the microscopes have a 45X objective instead of 40X), and 100X OIL. The total magnification is the product of the magnification power of the ocular and the objective (for the 10X ocular with the 45X objective, the total magnification is 450X).

**Resolution** refers to the ability to see objects that are close together as separate, distinct entities and is quantified as the minimum distance between two objects that reveals them as separate objects. You can have a microscope with high magnification, but, if the resolution is not sufficient, you won't be able to see the objects distinctly. Resolving power is a function of the wavelength of light used to illuminate the specimen and the numerical aperture of the lens. The numerical aperture is a measure of the ability of a lens to gather light and resolve fine detail in a specimen. High quality lenses have high numerical apertures and therefore give images with better resolution. Another contributor to resolution is the refractive index of the medium containing the light. The 10X and 40/45X objectives are viewed in air. Oil has a higher refractive index than air and minimizes loss of light rays, making it possible to reach 100X magnification with the oil immersion lens.



**Resolution:** the ability to distinguish two objects as distinct entities is illustrated.

Image from <http://www.microscopyu.com/articles/formulas/formulasresolution.html>

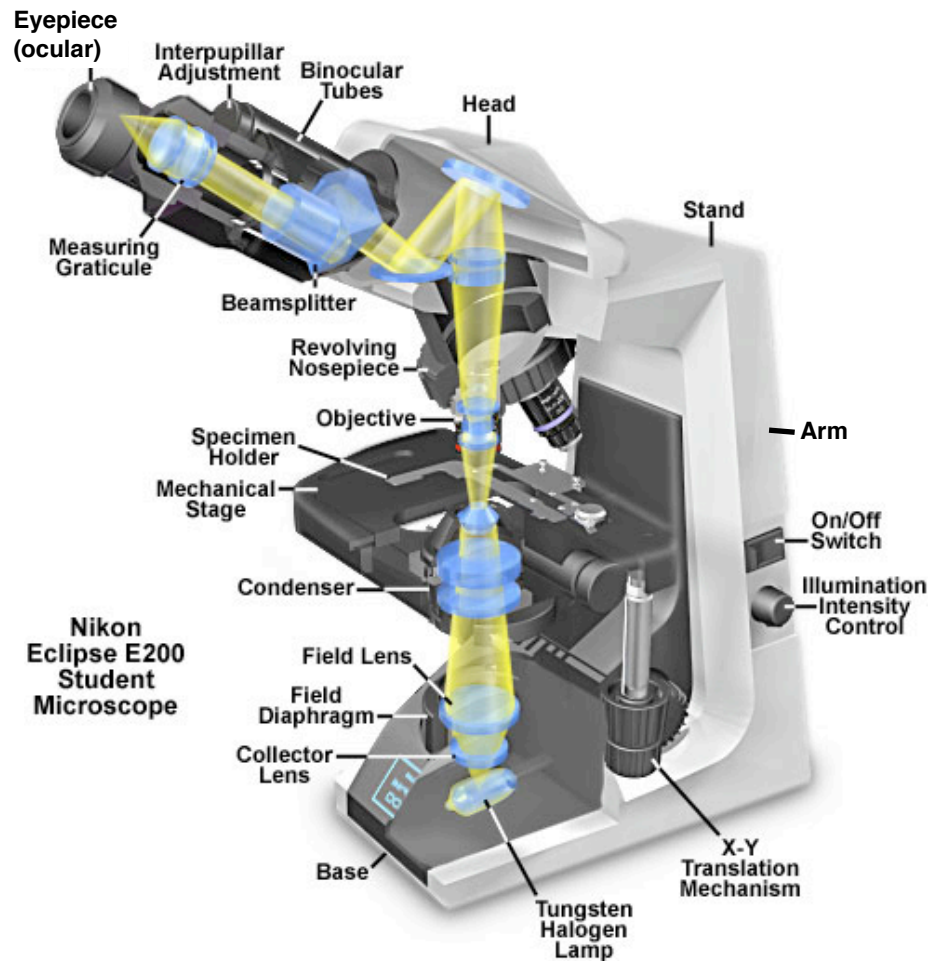
For more information about lenses, magnification, and resolution visit the websites below. They have some good illustrations and interactive tutorials about magnification and resolution.

magnification - <http://micro.magnet.fsu.edu/primer/anatomy/magnification.html>

resolution- <http://micro.magnet.fsu.edu/primer/anatomy/numaperture.html>

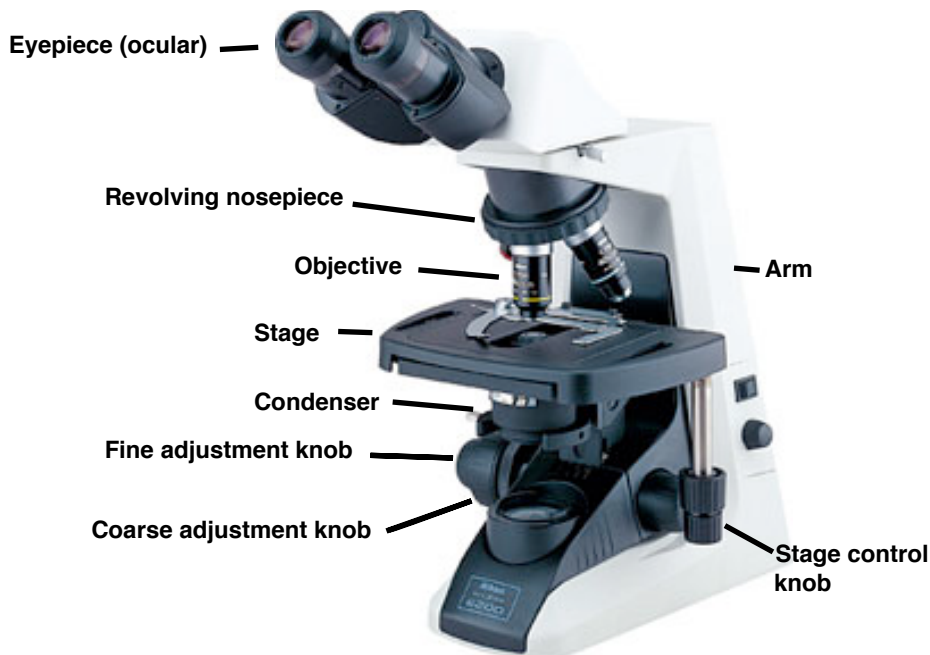
<http://www.microscopyu.com/articles/formulas/formulasresolution.html>

## THE COMPOUND LIGHT MICROSCOPE



**Cut-away View Of The Nikon Eclipse E200 Microscope.**  
The light path is shown.

Image from <http://molecular.magnet.fsu.edu/primer/anatomy/nikone200cutaway.html>



**Nikon Eclipse E200 Microscope.**

Image from <http://www.nikon.com>

## USE OF THE MICROSCOPE

1. Always carry a microscope with two hands- one hand on the arm and the other hand under the base. Microscopes do not survive falls well and are expensive to replace.
2. Once you have the microscope at your station, remove the cover and put it in a safe place, like the cubbies under the lab bench. Orient the microscope with the arm away from you and the stage facing you. Rotate the eyepiece head so that the eyepiece (ocular) is facing you. To put the microscope away, you will reverse this process.
3. Plug in the microscope and turn on the lamp.
4. Rotate the nosepiece so the 10X objective is positioned directly over the slide.
5. Place the slide on the stage with the specimen side facing up, using the slide holder to hold it in place. You won't be able to focus at high magnification if the slide is face down.
6. Turn the coarse adjustment knob (the larger knob) until the slide is as close to the lens as possible. Looking through the eyepiece, rotate the coarse adjustment knob and lower the slide away from the objective. When the cells start to come into focus, turn the fine adjustment knob until the image is in sharp focus.

For many of us, finding the cells is the hardest and often most frustrating part of microscopy. Here are some tips that should help.

- Follow the three steps below
  1. Move the stage so that the edge of the cover slip or edge of the stained area is directly under the objective. Turn the coarse adjustment knob as described above until the edge of the coverslip or staining area comes into focus.
  2. Move the stage slightly with the stage control knob so that the area with the specimen is under the objective.
  3. Adjust the focus using the fine adjustment knob.
- To make sure that you are looking at the slide and not dust on the lens, move the stage. If you are looking at the slide, the things you see will move. If not, you aren't focusing on the slide yet.
- If you find things that look like specks but not cells and move with the stage, you are probably looking the surface of the cover slip. You are close! Keep adjusting the focus with the fine adjustment knob until you see the cells.

7. Once the cells come into focus, you can sharpen the image further by adjusting the focus of the left eyepiece to compensate for differences in vision between your eyes.
8. Close your left eye. Using only your right eye, adjust the fine adjustment to bring the cells into sharp focus. If your cells are already in focus, skip this step.
9. Close your right eye and look through the microscope using only your left eye. If the cells are out of focus, rotate the left ocular until the cells are in focus. Do not touch the fine adjustment knob. Now the image should be in focus for both eyes. If your cells were already in focus for your left eye, you can skip this step too.
10. Now that you have focused on the cells using the 10X objective (100X total magnification = 10X ocular lens x 10X objective lens) rotate the set of lenses so that 40X or 45X objective, depending on your microscope, is over the slide. Now the magnification is 400X or 450X. The cells will still be small, but easier to see than with the 10X objective.
11. The microscopes are parfocal so the images should remain approximately in focus when you change objectives. You should only have to make minor adjustments with the fine adjustment knob. Do not use the coarse adjustment knob. A small turn will move the stage enough to lose focus.
12. If the image was in focus with the 10X objective, but appears fuzzy with the 40/45X objective, it is likely that the objective has oil on it. Use lens paper to wipe any oil off the lens. Only touch the lens with the lens paper, not your fingers.
13. The 100X OIL objective used with immersion oil will give you the best view of your microbes. With this objective in place, you will be viewing your microbe at 1000X magnification.
14. To use the 100X OIL objective, rotate the nosepiece so that no objectives are directly above the slide.
15. Place a drop of immersion oil on the slide in the spot that will be directly under the 100X OIL objective. Now rotate the nosepiece so that the 100X OIL objective slides into the oil drop, and the objective is in place. The oil should form a seal between the slide and the objective.
16. NEVER rotate the nosepiece so that the 40/45X objective contacts the oil. The 40/45X objective is not designed to be used with oil and can be damaged by contact with the oil. If you make a mistake and put the 40/45X objective in the oil, clean it well with lens paper. It is far better to not make this mistake.
17. When the 100X OIL objective is in place, slowly rotate the fine adjustment knob to refocus the microscope if needed. Only a very small adjustment should be

necessary because the focal length or working distance is very short for this objective. Don't use the coarse adjustment knob, even a small rotation of this objective will disrupt the focus. You could also accidentally grind the objective into the slide, which can damage the objective.

18. When you are finished, rotate the nosepiece so that no objectives are over the slide. Remove the slide and wipe the oil off the 100X OIL objective with lens paper.
19. When you are done using the microscope, turn off the lamp, unplug the microscope, put the cover back on and return it to the cabinet.

### **Fine tuning options:**

The image that you see in the microscope can be optimized by adjusting the light that passes through the slide and enters the lens. As the magnification increases, the diameter of the lens decreases, reducing the amount of light captured by the lens. To compensate for this you can increase the amount of light passing through the specimen. Conversely, lower magnification lenses gather more light. To increase the contrast between the cells and the background, you can decrease the amount of light used.

**Light intensity dial:** Most of the microscopes have a dial that can be used to adjust the lamp voltage and thus the intensity of light leaving the lamp. At higher magnifications, you will want to have the lamp adjusted so it produces more light.

**Iris diaphragm:** The iris diaphragm controls the amount of light passing from the lamp through the bottom of the slide. At lower magnifications, the diameter of the iris opening can be decreased to increase the contrast between the cells and the slide. At higher magnifications, the diameter of the opening must be increased to allow more light to pass through the slide.

**Condenser:** The condenser is a lens system that focuses the light coming from the lamp below the slide. It does not contribute to magnification. At lower magnifications, it may help to lower the condenser to increase contrast, especially if the cells are unstained. At higher magnifications, move the condenser up to focus the light to a point on the slide directly below the 100X oil immersion lens.