Evolution and Biodiversity Laboratory
Proper Use of the Compound Microscope

You will be assigned two microscopes to use this semester:

- **stereoscope** (Greek *stereo*, "solid" and *scop*, "observe")
  - for overall, three-dimensional (stereoscopic) view of an opaque or transparent specimen at low magnification.
- **compound microscope** (Greek *micro*, "small" and *scop*, "observe")
  - for viewing very thin, transparent materials for study of fine details at high magnification.

This guide explains proper use of the compound microscope (Figure 1).

Always begin with the lowest magnification on the microscope and progress to the higher powers only as needed.

This will allow you to

- **relate small portions and details to the entire specimen**
- **avoid breaking slides if you accidentally try to focus on high power**

Under no circumstances should you remove any part of the microscope or attempt repairs.

If you have any difficulty operating your microscope, ask your instructor for assistance.

If your microscope has missing, damaged or malfunctioning parts, report these to your instructor immediately.

I. Proper Use of the Microscope

1. Your microscope is located in the table locker beside your chair. The storage areas allow very little clearance: **be careful not to damage the eyepiece when you remove and replace your scopes**.
2. Your microscope is protected by a slippery plastic dust cover. Remove the dust cover *before* you attempt to lift your microscope out of its cabinet.
3. Hold the scope level while lifting to avoid unseating the ocular lens or loose filters.
4. Place one hand beneath the base and hold the arm of the microscope with your other hand. Place solidly on your lab table.
5. Unfold the lamp cord, plug it in and switch the light ON to make sure the bulb works. Turn it on and off as little as possible to increase the life of the bulb.

6. Never touch anything but lens paper to a microscope lens. Even tiny hard particles will permanently scratch the lens.

DO NOT use Kimwipes to clean a lens.
DO NOT use wet lens paper on a lens.
Water can wick into the lens and damage it.
DO NOT allow your objective lens to touch water on a slide for the same reason.

7. **NEVER FORCE A MECHANICAL PART**. If you have problems operating your scope, call your laboratory instructor.
8. No one wearing heavy eye makeup will be allowed to use a microscope.
9. When you have finished using the microscope at the end of lab:
    a. Turn off the lamp and *allow it to cool*. Moving a hot lamp can cause the bulb filament to break.
    b. Remove your slide from the stage.
    c. Rotate the nose piece until the low power objective is over the condenser.
    d. Wipe any liquid or dirt off the stage or body. If a lens is dirty, *carefully* clean it with lens paper.
    e. Unplug the cord and wrap it *loosely* around the base.
    f. Carefully place the scope in its cabinet (Use both hands!)
    g. Replace the dust cover. Pull the dust cover all the way over the base.

II. Parts of the Compound Microscope

The **base** is the support upon which the instrument rests.

The **stage** is the rectangular platform upon which the microscope slide is placed.
A hole in the center of the stage allows light to pass from the light source beneath the stage through the slide specimen, which should be centered over the hole in the stage. Keep the stage dry at all times. Fluids can damage metal parts and also make it difficult to move a slide around on the stage.

The **light source** is housed in the base of the microscope.
A switch located on the right hand side of the base allows you to vary the intensity of the light source. **Always turn the light off when you are not using the microscope.**

The **condenser** is located directly beneath the stage. It concentrates the light beam

The **iris diaphragm** is attached below the condenser.
A small lever allows you to control the diameter of the **iris opening** through which the light passes. When the diameter is decreased to allow only a very narrow cone of light

to pass through the specimen, the **resolution** (sharpness) and **depth of field** of the image are increased.

The **body tube** supports the two lens systems (hence the term "compound" microscope) comprising the optical or magnifying system.

The **oculars,** the lenses closest to your eyes, are at the top of the body tube.
Your ocular magnifies the image l0 times and is called a l0X ocular.
Note that your microscope is **binocular**: it has one ocular for each eye.

The three **objective lenses** are housed in the revolving nosepiece above the stage.
When switching lenses, turn the nosepiece ring while holding its edges.
A "click" indicates when the objective is in position.
DO NOT use the lenses as handles to swivel the ring, as this will gradually unscrew the lens and make focusing impossible!

- The shortest (**low power**) objective magnifies 4X
- The intermediate (**medium power**) objective magnifies 10X
- The longest objective (**high power**) magnifies 40X.

The **focus adjustment knobs** are located on either side of the microscope.
The larger, outer knobs are **coarse adjustment.**
The smaller, inner knobs are **fine adjustment.**
Coarse adjustment should be used only to obtain approximate focus, as it moves the stage a considerable distance with relatively little knob movement. Fine adjustment is used to obtain an exact and clear focus after an approximate image has been obtained with the coarse adjustment.

Never use coarse focus except with the low power objective lens!

It is very easy to drive an objective through a slide. This destroys the slide and can scratch the lens.

Always use <u>both hands</u> when turning the focusing knobs.

The ratchet mechanism for moving the stage is thrown out of alignment when only one knob is turned during focusing. This results in costly repairs.

When properly adjusted, your microscope should be **parfocal**: the image should remain in fairly good focus even when you switch from lower to higher power. To adjust your scope to parfocal setting (zero diopters), turn the ribbed collar of the adjustable ocular until the two white dots on the collar and lens barrel line up. Then use the coarse and fine adjustment knobs to focus.

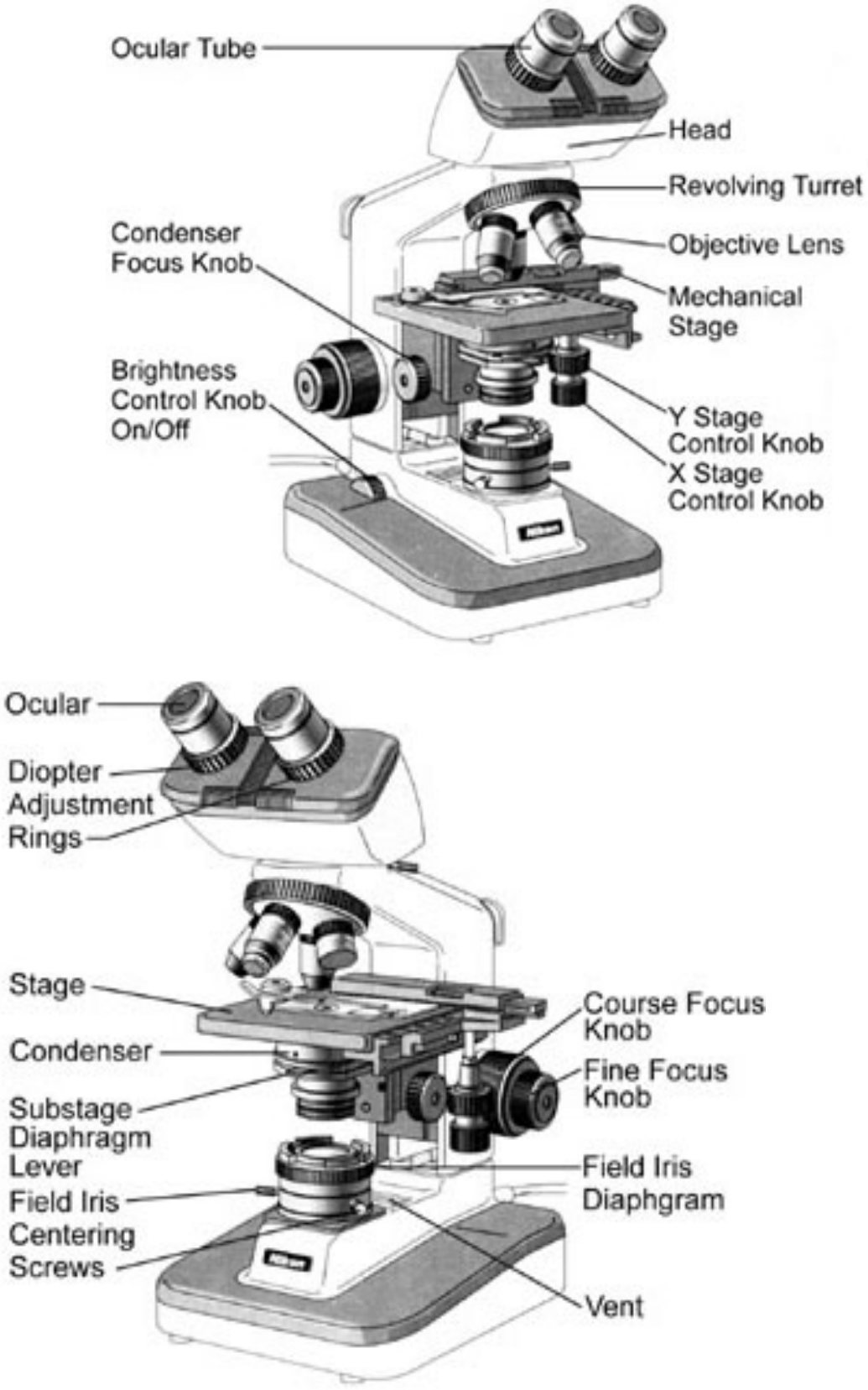

Figure 1. The Alphaphot 2 Nikon Student Microscope.

III. Functions of the Compound Microscope

The compound microscope has two functions: magnification and resolution.

1. Magnification

The microscope **magnifies** (increases apparent size) very small objects. **The total magnification reaching your eye is the *product* of the magnifications of ocular and objective**. What magnifications are possible with your compound microscope?

low power:_____ medium power:_____ high power:_____

2. Resolution

The second function of the microscope is to increase the **resolution** (illumination of detail) of very small objects.

Resolution is a measure of the capacity to distinguish two points as separate objects. You may be familiar with this phenomenon in terms of photography. Low resolution photographs, such as those printed in a newspaper (a what now?), are rather grainy. One can see the individual "dots" (**pixels**) making up the image. Higher-resolution digital cameras create a crisper, more resolved image.

The smallest unit of visible light is the **photon** which travel in waves. The distance between the crest of one wave and the next is known as a photon's **wavelength**.). Units of measurement commonly used to express wavelength are the millimeter (mm), micrometer (mm), nanometer (nm), and Angstrom (Å).

The resolving power of an optical system (your eye or your microscope) is limited by the wavelengths of light it employs. The shorter the wavelength used, the greater the resolving power. The resolution (R)of an optical system is

$$(R) = \frac{0.61\lambda}{N.A.}$$

R = the smallest distance distinguishable between two points.
λ = the wavelength of light used for viewing.
N. A. = the numerical aperture of the objective lens (a dimensionless ratio)
0.61 = a dimensionless constant.

The numerical aperture and magnification of each objective lens is etched on its barrel. Calculate limits of resolution for each of your objective lenses.
(Designate **λ** as 610 nm, approximately the average wavelength of white light.) Note that the units of **λ** will also be the units of **R**.

low power:_____ medium power:_____ high power:_____

Use your microscope with care, treat it with respect, and it will serve you well.