**THE MICROSCOPE:**

Parts of the binocular compound microscope(identify parts, know their functions, know how to clean and care for each part):

Ocular, diopter adjustment, ocular micrometer, pointer, body tube or head, revolving nose piece, objectives (scanning, low-power, high-power, oil immersion), arm, coarse adjustment knob, fine adjustment knob, base, light source, iris diaphragm, iris diaphragm lever, condenser, condenser height control knob, (pull-out phase adapter), stage, graduated mechanical stage controls, power switch, electrical cord and plug.

Computation of total magnification of specimen being viewed:



*Care and handling of microscope:*

1. Locate your microscope by number in the cabinet. Carry with two hands, one under base and other around the arm UNDER the Plastic cover/not on top. Keep the microscope *level* near your body.
2. Place scope gently on the lab table (protect table top with plastic sheet or paper towel). Do not slide it around on the table; lift it up and set it down.
3. Do **not** disassemble your microscope or reorient the oculars. *The eye pieces (oculars) face toward you at the same time that the cord holder also faces you*. Report any problems with your microscope to your instructor IMMEDIATELY.
4. Keep the scope and lens systems clean. Clean **lenses only with the lens paper** from your student drawer. Kim wipes may be used to clean slides, base and stage.

**A. Setting up your microscope at your student station:**

* Remove the plastic dust cover and place cover in your lab drawer.
* Unwrap just enough cord to reach the electrical outlet. Do not let cord dangle off table where it could pull the scope onto the floor.

 Initial focusing of the microscope (for first slide to be studied during a lab period):

1. **Clean slide** to be viewed by wiping gently with a Kimwipe. If oily, place a few drops of 70% ethyl alcohol onto the Kimwipe and gently wipe the cover slip and bottom of slide. **Never** place alcohol directly onto a prepared slide.
2. **Place the slide** down on the front of the stage with the label facing up and in position to be read. Open the spring stage clip by pulling the clip arm out. While keeping the slide flat on the stage, maneuver the slide to rest firmly against the L-shaped guide of the slide holder. Slowly release the stage clip so that it rests against the corner of the slide (***NOT on top*** of the slide).
3. **Align the cover slip** of the slide over the opening in the stage using the graduated mechanical stage control knobs located below and at the left side of stage.
4. With the scanning objective in position, use the **coarse adjustment** **knob** to **move the** **stage up until it stops**. Look from the side of your scope to visually confirm that the objective will not touch the cover slip on top of the slide.
5. **Turn on the light** to sufficient intensity to produce a WHITE background (not yellow). Note that the ***condenser is in the full up position***. Locate the condenser height control knob forward from course / fine adjustment knobs.
6. Adjust for individual eye differences:
7. Viewing through the right eye piece (left eye closed), adjust the coarse adjustment knob away from you until the image is clear. This will involve **lowering the stage** no more than about one-fourth of an inch. An additional slight adjustment (no more than two revolutions) of the fine adjustment knob may be needed to bring the specimen into BEST focus.
8. Then viewing with the left eye (right eye closed) through the left ocular use the **diopter adjustment** or zoom feature at the base of this left ocular to bring the specimen into best focus for your left eye.
9. Viewing with both eyes open, adjust the interpupillary distance by grasping the eye pieces plate and moving the eye pieces closer or farther apart. You should see ONE circular field of light. You may also need to adjust the distance you hold your head from the oculars.

 Now the slide is in best focus for both eyes.

1. Use the **iris diaphragm lever** to adjust the amount of light striking your specimen. More light will be needed for preserved and stained slides and at higher magnifications. Less light is required for thin preparations and unstained slides. Remember the condenser remains in its full uppermost position.
* You are now ready to use the microscope for the day.

**B. Focusing your Specimen** *(after completing A. above)*

1. Make sure your Microscope stage is at its lowest point, and the Scanning objective is clicked into place
2. Center the specimen to be examined in the ***CENTER*** of the field of view. These microscopes are ***parfocal***. This means that if the specimen is focus at the center of the field of view, it will be in partial focus when viewing in the next power objective. *\* NOTE – you need to make sure your specimen is centered EACH time before moving up objectives OR you can “lose sight” of your specimen as it will fall outside the field of view diameter when changing to the next hire objective.*
3. While observing the movement of the objectives ***from the side*** grasp the **revolving nosepiece** (not the objective) and rotate it to the next power (low power – yellow band). Listen/feel the nosepiece settle or click into place.
4. Focus at low power using ONLY **fine adjustment knob** to focus. NOTE: if you cannot find focus rotating more than a few revolutions one direction, reverse and try the other direction. Regardless, excessive rotation means you either did not focus sharp enough on Scanning OR you lost your specimen outside the field of view (meaning you did not center your image before changing objectives). Rotate nosepiece back to scanning and fine tune focus with both the course and fine focus knobs before moving back up to low power.
5. When moving from low to ***high power***, repeat steps #1 and #2, but in #3 and again you may use ONLY **fine adjustment knob** with the high power objective. Watch from the side that it will not touch when sliding it into place. \*Note if you focused well in Scanning, you will eliminate this issue – sloppy focus in scanning results in high power objective scratches. \*Do not move the stage lower if you are worried it will hit – simply back down to Scanning and verify your subject is in the best focus possible.
6. To use the 100X objective, you need to add a drop of **immersion oil** on the slide.This technique will be more fully described below in part C.

After observations have been completed up to High Power (40x):

1. Move the revolving nosepiece backwards through low and then to scanning. Do not rotate from High through Oil as you will drag the oil immersion objective (longest objective) across the cover slip and scratch the objective lens!
2. Lower the Stage.
3. Open the spring stage clip and slide the microscope slide out to the forward edge of the stage.
4. Return the clean slide to where you obtained it (your slide box or the side counter). (if viewing a wet mount, dry off any accidental moisture on the stage with a kim wipe.
5. Repeat Focusing steps in part B. above.
6. After last slide of the day is finished, prepare the microscope for storage: Scanning Objective in place, Stage down, Cord secured, dust cover on, placed in the CORRECT numbered cabinet space.

Storage of your microscope in cabinets:

 1. Scanning objective (4X with red band) in position.

 2. Power switch off.

 3. Stage with NO slide left on it.

 4. All lenses and the stage **must be clean**.

 5. Graduated mechanical stage centered.

 6. Phase contrast slide in the “OUT” position. *\*For microscopes in A&P & Micro labs ONLY\**

 7. Stage in the full down position. (Do not lower the condenser.)

 8. Cord wrapped around the cord holder.

 9. Plastic dust cover is on.

 10. The microscope sits above its number on the cabinet shelf.

**C. Oil immersion techniques (for A&P II, Biology II, and Microbiology Labs ONLY)**:

1. Focus the slide as before under the scanning, low power, and high power objectives. Now the stage and lighting are set for the best resolution of the specimen.
2. Do not lower the stage.
3. Rotate the revolving nosepiece back the way you came to high (back to low, then to scanning). Do not drag the long oil immersion objective over the cover slip.
4. Place a drop of immersion oil (from your drawer) on the cover slip where the light is passing through the slide. Be careful not to allow any oil to flow over the edge of the slide onto the condenser lens or onto the stage.
5. Looking from the side of your scope, visually confirm that the objective will not touch the cover slip of the slide. **Rotate the revolving nosepiece DIRECTLY from Scanning (4X) objective to the 100X objective.** You can see the oil come into contact with the 100X objective.
6. *Slowly* adjust focus using the fine adjustment knob. *(If you focused well at high power, you should not need a full revolution to obtain focus)*
7. You may need more light. Move the iris diaphragm lever to allow more light on the slide.
8. Do not lower the stage if viewing another slide. Your scope should be relatively in focus for the next slide thus you should only need to make minor adjustment in scanning before proceeding back to step 4. Continue 4-8 until finished all observations.

After observations have been completed at Oil Immersion (100x):

1. After study of specimen is complete, turn the revolving nosepiece DIRECTLY from
2. 100X objective BACK the 4X objective. This avoids bringing other long lenses in contact with the oil.
3. Open the spring stage clip and remove slide forward toward the edge of the stage.
4. CLEAN the slide – Remove most of the oil by blotting cover slip with a KimwipeAdd some 70% ethyl alcohol on a clean Kimwipe and remove any remainingoily residue. Return clean slide to where you obtained it (your slide box or sidecounter). Check the stage is also clear of oil.
5. CLEAN the 100X objective if you are through using oil for this lab session.*DO not use Kimwips on OBJECTIVES!* Blot (DO NOT RUB) the objective with clean lens paper to polish the 100X lens until no oily residue is observed. Use lenspaper to check other objectives to be sure no oil is on them.

**\*\*\*Never use any liquid to clean your microscope lenses. \*\*\***

**D. Other Techniques:**

Using phase contrast optics:

This type of microscopy is used when live, unstained specimens are to be viewed.

1. Focus the specimen as you have been instructed above on 4X, 10X, and 40X.
2. When you are in best focus on high power (40X objective), push the phase ring holder into the path of light. Make sure the condenser is raised to its highest position. Also, make sure the lever controlling the amount of light entering the condenser is fully open. You may also have to turn the light source on full.
3. Only the high power (40X) objective may be used with the phase contrast optics.

Using dark field optics:

This type of microscopy is used when studying diatoms and algae.

1. Obtain a dark field adapter. Your lab instructor who will show you where to obtain the adapter numbered for your microscope.
2. Remove the blue filter and snap it onto the bottom of the adapter.
3. Snap the top of the adapter to the bottom of the condenser.
4. Focus normally. Your best resolution will be at low power. Note the different colors.

Measurement of a specimen using the microscope:

Use the ocular micrometer – the small “ruler” in the right eyepiece.

1. For ***small specimens*** that will fit under the ocular micrometer, position ocular micrometer over specimen. Move the slide on stage to position the specimen. You can move the ocular micrometer by rotating the ocular.
* The size of the specimen can be determined by multiplying the number of ocular micrometer spaces covered by the specimen by the conversion factor for that objective as given in the following table:



* Use the graduated mechanical stage markings.
1. For ***larger specimens,*** which are too large to fit in the space of the 100 units on the ocular micrometer, follow these steps:
2. Place the zero line of ocular micrometer at the anterior end of the specimen. You can move the ocular micrometer by rotating the ocular.
3. Record the coordinates to the nearest tenth from the graduated mechanical stage axes.
4. View through the ocular micrometer, move the zero line of the ocular micrometer to the posterior end of the specimen.
5. Record the new coordinates to the nearest tenth.
6. Calculate the difference between the coordinates. The stage is marked ingraduations of one millimeter.
7. Convert you answer in millimeters to microns by multiplying mm by 1,000.