How to Use the Leica CME Microscopes in the A&P Labs

Carrying the Microscope:
1. Always use two hands, one of which should support the base while the other holds the arm of the microscope. Microscopes contain delicate optical structures that could be damaged through impact. Thus, be very careful and gentle when setting down the scope and moving it.

Setting Up:
2. The eyepieces (oculars), condenser lens, and light source should be clean and dust-free. You may want to wipe these surfaces with a lens-grade tissue prior to using the microscope.
3. Ensure that the **lowest power objective** is selected before placing your slide on the stage.

Ocular Distance and Focusing: Preventing Eye Strain, Headaches, and Dizziness
4. Place your slide on the stage with the specimen over the condenser lens. Use the mechanical stage controls to position your slide.
5. Move the Iris Diaphragm Lever to the left and use the condenser lens knob to raise the condenser lens to the highest position possible, which should be immediately beneath the slide.
6. Turn on the microscope and use the rheostat wheel on the side of the stage to adjust the light so that it is not too bright or dim – go for what is comfortable for your eyes.
7. Look through the eyepieces – What do you see? Two circles, one blurry circle, or one clear circle? Do not worry about whether you can see the slide clearly – all you should be focusing on right now is the circle – if you see two circles, you need to push your eyepieces together a bit. If you see a blurry circle, you need to widen them. When you find the right distance for your eyes, look at where the dial is between the eyepieces. You can select the best distance for you using that number whenever you need to use your microscope for the rest of the quarter.
8. Finally, set the ocular focus – look at the slide on 4X power (red objective). Bring it into focus using the coarse focus knob and then the fine focus knob. Rotate both of the eyepieces clockwise or counterclockwise to lower them as close to the headpiece as possible. Look through the right eyepiece with your right eye – close your left eye. Use the fine focus to make the image as sharp as possible. Now look through the left eyepiece with your left eye and close your right eye. If the image is blurry in any way, sharpen it by rotating the left eyepiece clockwise or counterclockwise.

Viewing Slides – Red means “Go.” - Always start with the red objective and work your way up.
9. With each slide, ALWAYS start with the scanning (4X, red) objective. With the 4X objective, you may start with the coarse focus knob and then use the fine focus knob to sharpen the image.
10. Next, look at the slide with the low power (10X, yellow) objective. The objectives should be parfocal, which means that you should **only need to use the fine focus knob**. Note how the objectives increase in length with magnification power. The coarse focus moves the stage up and down, and you may run the slide into the objective if you use coarse focus with the longer objectives.
11. Finally, look at the slide with the high power (40X, blue) objective. Again, only use the fine focus knob – you can turn it in either direction to sharpen the image. If the image does not become clear in a couple rotations in one direction, you should probably rotate the focus knob in the other direction.
12. Once you have focused through the 40X objective, without moving the stage, swivel the lowest power objective back into place and add a drop or two of oil to the slide over the light. The 100X oil immersion objective is right next to the lowest power objective. Swivel the 100X objective into place. The oil should adjoin the lens at the tip of the objective, creating a bridge in the space between the objective and the slide. Use the **fine focus only** to focus on your specimen.
The Condenser Lens and Iris Diaphragm

13. When you are viewing a slide, experiment with the positioning of the condenser lens and the iris diaphragm lever. Find the knob that raises and lowers the condenser lens under the left-hand side of the stage. You should **not** loosen the condenser lens with the pins that are used to hold it under the stage. When you lower the condenser lens at high power, it will sharpen the image. For scanning and low power, it is best to keep the condenser lens in the highest position possible.

14. The iris diaphragm controls how much light passes through the slide to the objectives. View your slide with the lever in various positions – far left, middle, far right, and see how it changes the image that you see. You should not use the lever to control the actual light level. For this, use the rheostat dial on the side of the base.

Putting the Microscope Away

15. Select the scanning (4x, red) objective, and remove your slide from the stage.

16. If you used methylene blue or any other stains, gently wipe the high power (40x, blue) objective with a dry lens tissue. If it is clean and dry, go to step 17. If you see fluid or stain on the tissue, use a small amount of lens cleaner to wipe the objective.

17. If you used immersion oil, use a dry lens tissue to gently wipe the oil from the tip of the 100X objective. If you used proper technique, you should not have oil on any of your other objectives.

18. Wipe the eyepieces and stage; use lens cleaner if necessary.

19. Center the mechanical stage so none of the gearing is hanging out on the side. The electrical cord should be bound with the velcro strap. Do not bend the cord tightly – it damages the cord. It is better to bind the cord into a circle so that it does not have any sharp bends in it.

20. Carefully return your microscope to the cabinet at your workstation with the arm facing outward. When the arm is facing outward, the number is visible, and the scope is more easily retrieved from the cabinet.

Parking Tickets

21. If you put away your microscope improperly, whoever uses it after you may write up a ticket and attach it to your microscope.

22. If you find something wrong with your microscope, notify your instructor. If it was put away improperly, you can write a ticket, and attach it to the arm so that it is visible from outside the cabinet. Your microscope will probably be used by at least 10 other students this quarter. The purpose of the tickets is to foster awareness for proper handling and use of the microscopes. They are very expensive, useful tools for your learning and should be respected as such. Some faculty may deduct points for improper handling of the microscopes.

Troubleshooting

**“The light doesn’t work”**

- If you are working at a table in the center of the lab, ensure that the worktable is plugged in – the worktables should be plugged into overhead electrical outlets. If your table is not plugged in, it won’t have power.
- If you are working at one of the workstations on the side of the lab, try a different outlet – the outlet you are using may be faulty.
- Ensure that your power cord is properly inserted into the base. The power cords are removable and sometimes come loose.
- Ensure that the rheostat is not dialed all the way down.
- If you have tried all of the above, and the light still does not work, notify your instructor. The lightbulb, power cord, or fuse may need to be replaced. Your instructor may have you use a different microscope for the time being. If your instructor is unable to repair the microscope, the lab technician should be notified.

**“I cannot see anything.”**

- Ensure that the scanning (4x, red) objective is fully locked into place.
• Make sure your slide is centered properly and not upside down.
• Check the condenser to make sure the iris diaphragm lever is set to the left. If the iris diaphragm is completely closed, insufficient light may be an issue.
• You will need to use the coarse adjustment knob to raise the stage so that the slide is quite close to the objective before an image can be seen.

“The image is blurry.”
• First, make sure you followed all of the focusing steps described in steps 2 through 11.
• CLEAN EVERYTHING. Clean the ocular lenses, the light source, the condenser lens, the objective, and the slide. DO NOT REMOVE ANYTHING. You should be able to clean the objective without removing it. Make sure the slide is not upside down!
• If you’ve tried all of the above, and the image is still not sharp, please notify your instructor. Your instructor may attempt further cleaning or direct you to put the microscope away and use a different one. The lab technician should be notified in this case.
Parts of the Leica CME

Objectives – these range from 5X (red) to 100X (white). The red one should be set when you put the scope away.

Ocular lens (10X) and rubber eyepiece

Condenser Lens Control Knob (raises and lowers condenser lens; condenser lens should ideally be about 75mm beneath the slide for normal viewing)

Light Intensity Control (Rheostat) (the power switch is in the back of the microscope)

Light Source

Headpiece

Mechanical Stage
Holds the slide, is moved by the mechanical stage control

Mechanical Stage Control moves the slide forward/back, left/right

Condenser lens and blue filter – the iris diaphragm lever is located on the front of the assembly.