forensic TRACE
Training, Research, Analysis, Consulting, Education
Wayne Moorehead

criminalistics@hotmail.com (714)272-4628

Setting Up the Polarized Light Microscope

As with every analytical instrument, the microscope must be set-up, aligned, and calibrated properly in order to maximize the information it can deliver. The polarized light microscope (PLM), an effective forensic analytical tool, must be correctly aligned and adjusted. The process involves two major components

1) centering of the optics (condenser and objectives, where appropriate the stage) and
2) proper Koehler illumination.

Setting up the microscope with proper illumination and centering is most easily achieved when doing both at the same time. The following is for Olympus, Leica, and Nikon microscopes built after 1985.

As you read these step by step instructions below, think about how the changes you are making affect the illumination and alignment of the different microscope components being adjusted. Consider the effect on the object image. Think about the path of the light through the microscope – from the illuminator to your eye.

NOTE: Each time a microscope (PLM or other transmitted light microscope), is used and ideally with each change of objective, Koehler illumination should be established. This process should be followed to make sure the optics are aligned and the proper illumination is established.

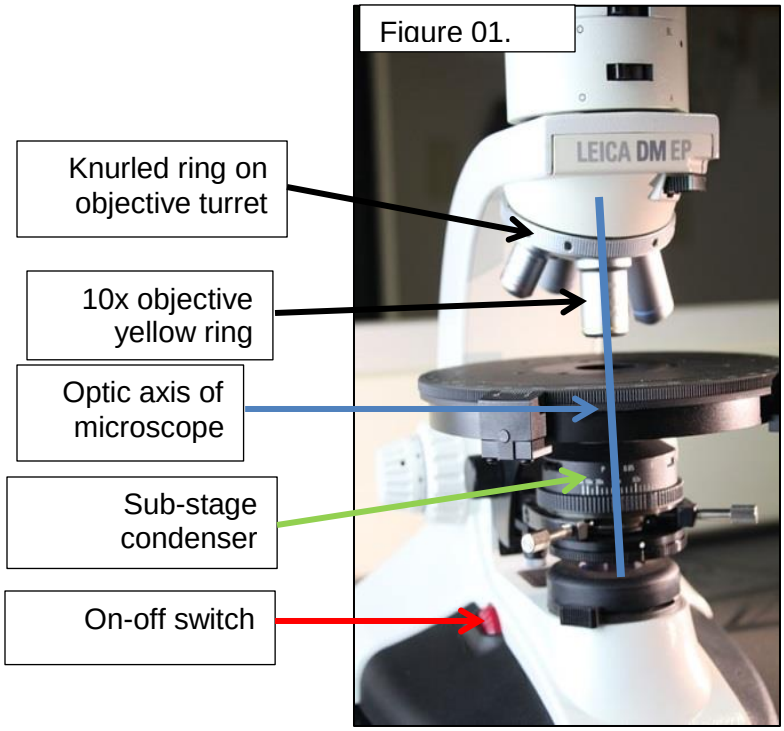
Instructions for Aligning & Establishing Koehler Illumination

1. Turn the microscope illuminator to the “on” position. Adjust the illumination to just get some light, never use full brightness during this process (depending on the specimen adjustment of the light source may be needed). Open the field diaphragm to its maximum.
2. Adjust the eyepieces to suit your interpupillary distance by moving them closer together or farther apart.
3. Place a prepared slide (03 Obsidian or 04 Quartz) onto the rotating stage.
4. Check to see that the 10x objective (modern scopes have a yellow ring and 10x stamped into the barrel of the objective) is in the optic axis of the microscope (Figure 01) If not in the optic axis, use the **knurled ring** on the nosepiece (aka objective turret), to turn the nosepiece so the 10x objective is in place. (**never use the objective to rotate the turret!**)
5. While still looking **from the side – not through the oculars**, (Figure 02) rack the stage to its highest point (thumb at top of focus knob and turn away from you, clockwise) but DO NOT ALLOW THE OBJECTIVE AND COVERSLIP TO TOUCH

forensic TRACE
Training, Research, Analysis, Consulting, Education
Wayne Moorehead

criminalistics@hotmail.com (714)272-4628

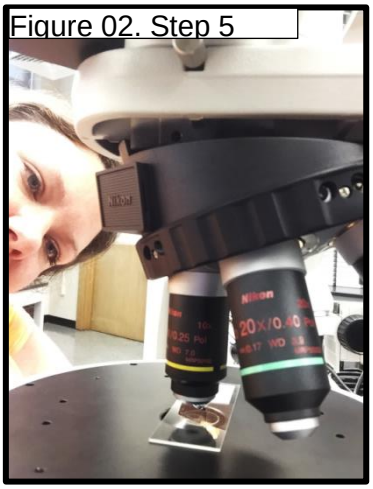
Figure 01.



6. Watch the stage rise to its top position <u>from the side</u> – not through the oculars – to **make sure the coverslip doesn't hit the objective**.

7. To raise the stage to its top position, turn the coarse focusing knob away from you. Put your thumb at the 12 o'clock position on the focus knob and move your thumb away from you (Figure 03). Continue until the stage reaches its top most position. **Do not continue to turn the knob once the stage has reached the top position**

Figure 02. Step 5



8. Once at the top, look through the oculars and use the right side coarse focus knob to slowly begin lowering the stage by turning the right side coarse focusing knob **counter-clockwise** (put your thumb at the 12 o'clock position and turn toward you – Figure 04),to focus on a particle on the slide in the field of view. If you do not see particles, move the slide around using your left hand with the one-hand technique (see Figure 11) until particles of interest come into view. With one polar inserted, this is considered plane polarized light (PPL) viewing (similar to brightfield)). **HINT:** Look for bubbles in the medium to help find your particles.

forensic TRACE
Training, Research, Analysis, Consulting, Education
Wayne Moorehead

criminalistics@hotmail.com (714)272-4628

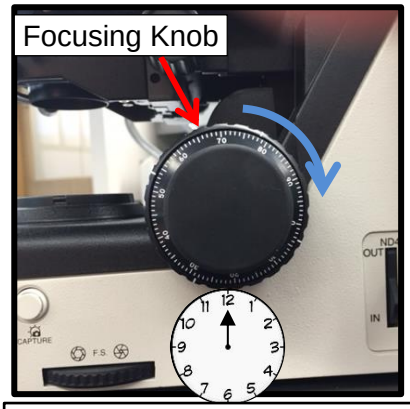


Figure 03. Raise the stage by turning the focus knob on right side clockwise – away.

9. By moving the stage downward (Figure 04), the slide with the specimen moves away from the objective. Lowering the stage is also called "***focusing up***".

10. Once the sample/specimen is in the plane of focus (in focus, move the slide around to find the specimen if necessary), use the fine focus knob to sharpen the focus on the sample.

11. Close the field diaphragm of your microscope **while observing through the oculars**. Watch the direction the diaphragm moves as you make the opening smaller.

Figure 04. Lower the stage by turning focus knob counter-clockwise or your thumb at the 12 o'clock position and moves toward you.

12. If the image in your oculars appears to be similar to Figure 05, open the field diaphragm to permit light across the field of view.

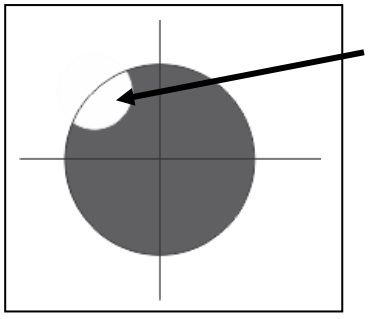
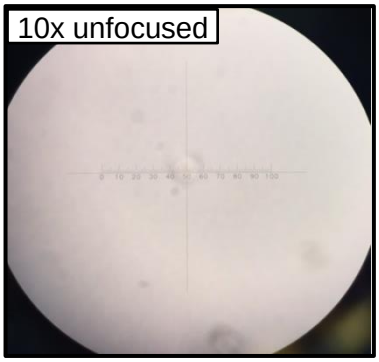
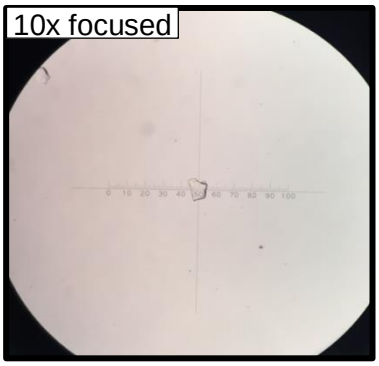


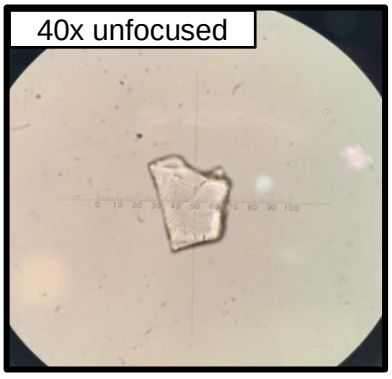
Figure 05. Field diaphragm closed down and uncentered but visible in the field of view.

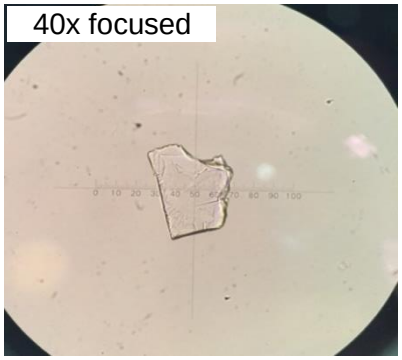
13. Close down the substage condenser diaphragm to increase contrast (yes, reduce resolution for now).

14. Next: **Use the nosepiece ring** (not the objective!) rotate to the 40x objective (blue band) into the microscope viewing axis. **The microscope will be set-up based on the 40x objective. If you plan on using a 60x objective use this one.**









Your particle will not look like this particle!

Figure 06. This figure is to show a particle at the cross hair and an out-of- focus and in-focus particle so you will know the difference and the relative size proportion in magnification.

15. Use the focus knob to focus on a particle.
    a. If there is no particle at the crosshair, move the slide with your **left hand ONLY (see figure 11)** to put a small particle centered at the crosshair. Consider this particle location to be position A .

16. Obtain the objective centering screws for the objective. Put the screws into the holes on the nosepiece near the base of the objective in use (Figure 07).

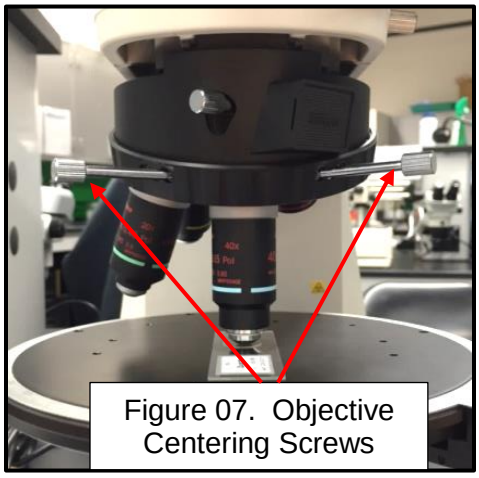


Figure 07. Objective Centering Screws

17. Looking through the oculars, **watch the particle at the crosshair as you rotate the stage 180º**. At the left in Figure 08 is the starting place (Position A) of the particle. Note how only **one part** of the particle is touching the cross hair.

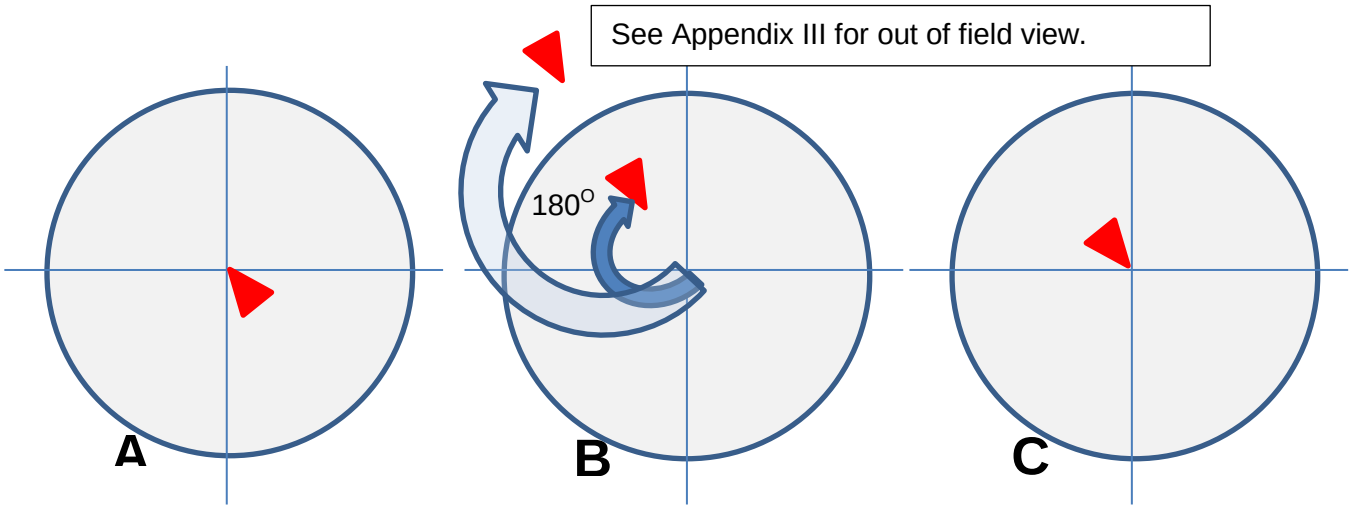


Figure 08. Left (A) is the starting position for your particle. Position B shows two possible outcomes**:** the particle stays in the field of view or leaves the field of view. In C is the ideal possible outcome of rotating the stage 180 degrees. In B, the particle moves away from the cross hair. In C, the particle simply rotates around the cross hair without moving away.

If your objective is **centered**, the particle will remain at the crosshair – not moving away from the center. (Figure 08C above or Appendix I).

If your objective **is not centered**, the particle will move away from the crosshair and the objective needs to be centered (Either scenario of Figure 08B).

Continue reading on how to center the objectives.

There are two possibilities when you rotate the stage 180 degrees:

1) The particle will go out of the field of view

If the particle goes out of the field of view, you must find the center of rotation (approximately ½ way from where the particle started and where it ended up at 180 degrees) and move the center of rotation so the center of rotation is close to the cross hair. Use the centering screws on the objective to adjust the center of rotation of the objective into the field of view. **See Appendix III.**

2) The particle will remain in the field of view.

If your particle moves off the crosshair but remains in your field of view, assume the final location of the 180º stage rotation of the particle to be position 2.

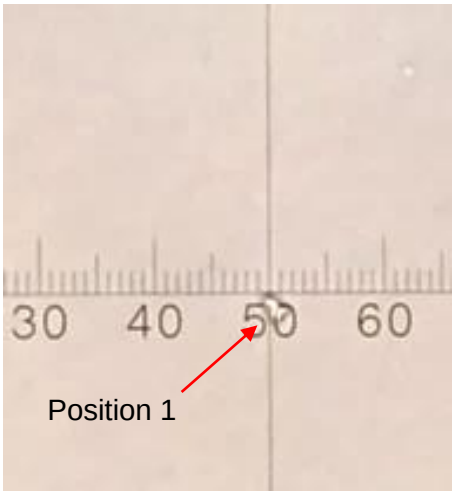
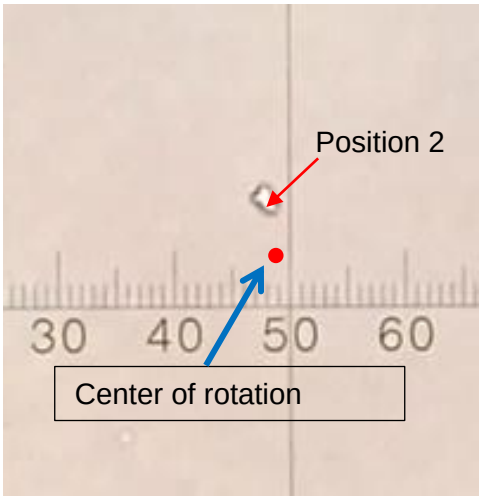


Figure 09.

A B



18. To move the center of rotation to the crosshair, draw an imaginary line between position 1 and position 2 (to help visualize the center you may need to rock or rotate the stage back and forth from the 0º position (position 1) with the particle on the crosshair to the 180º position (position 2) until you can visualize the line between the two and the approximate the center of rotation).

19. Estimate the midpoint of the connecting line (position 1.5 if you will). That is the center of rotation – to center the objective, that midpoint needs to be moved onto the crosshair.

20. Use the objective centering screws to move the **midpoint** to the crosshairs (similar to an Etch A Sketch®). Do not move the particle to the cross hair because the center of rotation will now be on the other side of the cross hair (Figure 10).

21. Next, **manually move the slide** to put the particle at the crosshair center again (the same particle **or any particle**).

22. Repeat steps 16-21 until you can rotate the stage 360º and your particle remains centered at the crosshair.

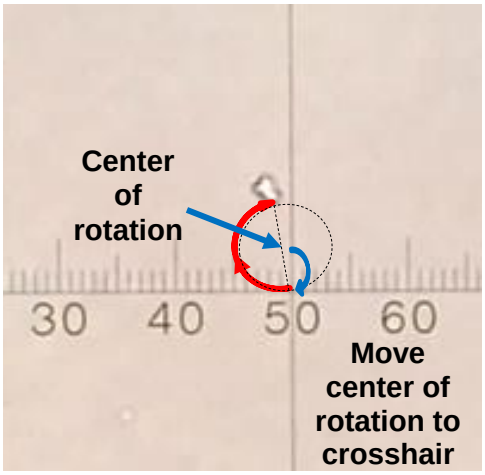


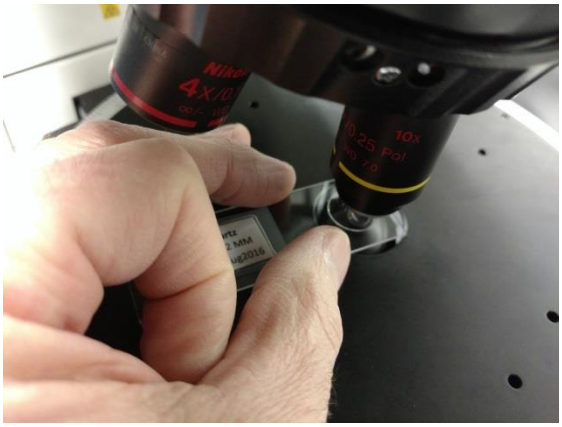
Figure 10. Moving center of rotation back to cross hair

NOTE: This is a series of successive approximations. It can take 3-5 times of repeating steps 16-21 to properly center the objective. When you are first learning how to do center the objective, the process will likely take many more times than 5 until the particle remains centered. **Have patience!**

TWO WAYS OF HANDLING SLIDES

Figure 11a: One hand holds the ends of the slide. With practice, you will be able to move the slide micrometers at a time more quickly than with a mechanical stage! This frees up your other hand to change focus, insert the analyzer, etc. You can quickly scan a slide while adjusting the focus as you search.

Figure 11b: One hand holds the slide **near the sample**. This **affords more control** on the sample being viewed. With practice, you will be able to move the slide micrometers at a time quickly! This frees up your other hand to change focus, insert the analyzer, etc. You can quickly scan a slide while adjusting the focus as you search.



After the 40x objective is centered, set-up the 40x objective for Koehler illumination.

23. While observing through the oculars, close down the leaves of the field diaphragm to a small aperture opening. The opening should be centered on the crosshair. If not, the condenser must be centered. **Watch where the leaves of the diaphragm move (the circle of light).**
    a. You may need to move the slide a little bit so the specimen does not interfere with viewing the aperture opening at the crosshair. Be sure to include the coverslip and mounting medium on the slide.
    b. If as you close the field diaphragm, the diaphragm appears to be leaving the field of view, stop and leave the opening so you can see it.

24. If the condenser is off center (the diaphragm leaves do not surround the crosshair equally), center the condenser to center the field diaphragm. (The field diaphragm is fixed at the base of the microscope).
    a. There are two adjustment screws under the stage attached to the substage condenser. Turn the screws to move the substage condenser until the aperture opening of the field diaphragm is centered on the crosshair (similar to the objective adjustment screws or an Etch A Sketch®).

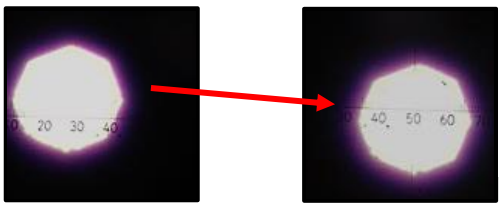


Figure 12. The polygon shape of the aperture is made by the leaves of the diaphragm. Each flat edge indicates one leaf.

25. Using the <u>focusing knob **on the side of the condenser**</u> (NOT the coarse and fine focus for the stage), focus on the field diaphragm leaves (by moving the condenser up and down **slowly**!).
    a. Turn the knob until the edges of the aperture leaves appear sharp and crisp.
    b. Diffraction colors (reddish-orange and/or blue) may be seen along the edge/margin of the leaves. Use the condenser focus knob to minimize the colors (try to find the happy medium in-between the colors).

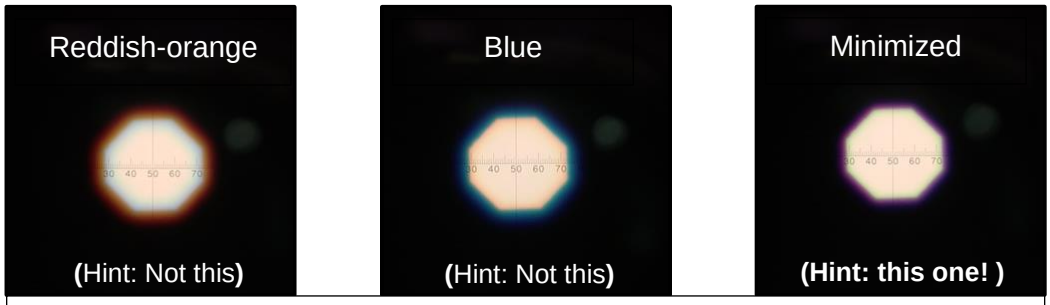


Figure 13. Under, over, and proper focus of condenser. Very important that you are **still focused on the specimen** plane at this point.

26. Viewing through the oculars, open the field diaphragm (open the aperture) until the leaves are **just outside your field of view** on the microscope at 40x. You should not see them anymore when looking through your oculars.

27. Remove the left ocular **(DO NOT put your finger/thumb on the glass lens!)** or you may use your Bertrand lens to look down the back focal plane of the microscope.
    c. Use the diaphragm ring or lever of the substage condenser diaphragm to open the substage condenser diaphragm so that light fills 2/3 of the opening of the back focal plane of the objective.
    d. **DO NOT use the numbers on the condenser** for this – use your eyes and the opening in the back focal plane
    e. Replace the ocular (**do not put your finger/thumb on the lens!**) or next remove the Bertrand lens.

Figure 14

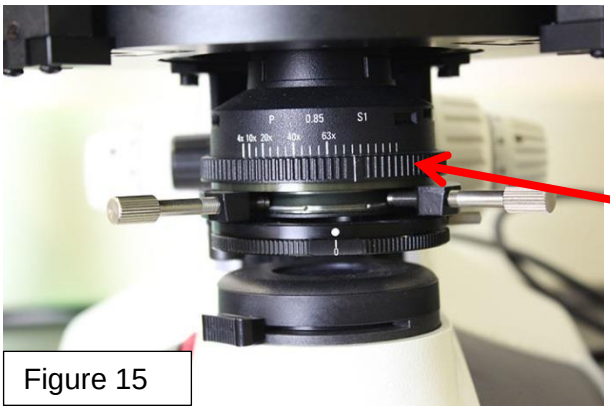


Figure 15

Figure 15. Using the substage condenser diaphragm ring, open the substage diaphragm ~2/3 diameter of the back focal plane of the objective – observe **don't use the numbers on the condenser**

Figure 16. Showing the condenser diaphragm 2/3 open in the back focal plane of the objective look down the ocular tube without the ocular

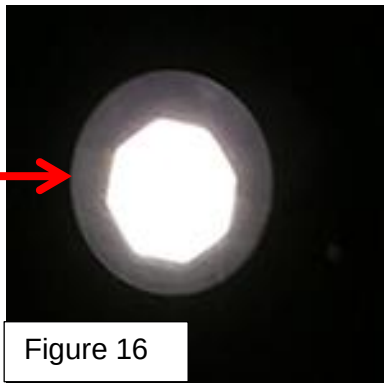


Figure 16

The microscope is now properly aligned and for Koehler illumination established for the 40x objective. The condenser centration should NOT be adjusted again during the remaining objectives. The condenser centration will be consistent across all of the objectives – **DO NOT attempt to re-center the condenser to the other objectives**! **Each of the other lower magnification objectives must be centered to the condenser.**

28. For the 40x objective, **carefully** remove the objective centering screws from the nosepiece without turning them. Be sure to be looking through the oculars and observe your particle at the cross hair to be sure you do not twist or turn the adjustment screws when removing them. Use the nosepiece to rotate to the next objective into the viewing axis.

29. Next center the 20x and 10x objectives on your microscope (or the next lower magnifications) using the objective centering procedures. Just align each objective to the cross hair. Do not adjust the condenser. Insert the objective centering screws into the holes on the nosepiece near the base of the next objective.

30. Repeat the steps above for the each uncentered objective.
    DO NOT redo focusing or centering of the condenser steps again – once you do it the for 40x objective (or whatever the highest objective is that you will be using), you are done.

    For each objective you may have to adjust the field diaphragm in the base of the microscope to fit the field of view.

    Do not adjust condenser focus or try to re-center the condenser – only the field diaphragm needs to be opened or closed more depending on the objective. An adjustment of the substage diaphragm may be needed to increase contrast to see particles better.

    To recap: In order to achieve correct centering and proper Koehler illumination:
    - Center the 40x objective relative to the stage (optic axis of the microscope)
    - Center the condenser to the 40x objective (optic axis of the microscope)
    - Set-up Koehler illumination using the 40x objective (open substage diaphragm to 2/3)
    - Center the 20x and 10x objectives
    - Adjust the substage diaphragm (do not center or re-focus the condenser) and the field diaphragm each time you switch objectives. You should not re-align or re-focus the condenser once this has been performed for the 40x (or highest magnification) objective.

forensic TRACE
Training, Research, Analysis, Consulting, Education
Wayne Moorehead

criminalistics@hotmail.com (714)272-4628

APPENDICES

Appendix I – Objective appears centered

If your center of rotation is at the center of the crosshair, when you rotate the stage 180º the particle will rotate in a circle but not move away from the crosshair. Congratulations – you can align each objective to this standard and then set up Koehler illumination.

There are two ways to check to see if your objective is centered – using a **very** small particle or using the point or edge of a larger particle (small particles are typically better). **Be careful if you choose to use a big particle – it can obscure the true center of rotation** if not used correctly to center the objective.

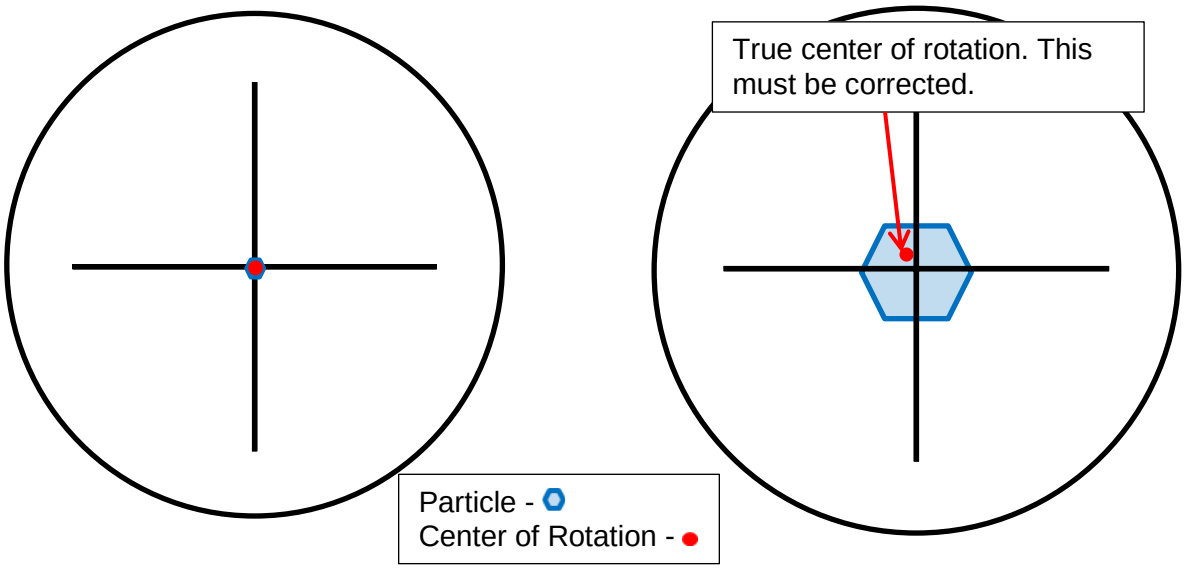


FIGURE A. If you center a small particle on the crosshair, and then rotate the stage 180º and the particle does not move off of the crosshair during the revolution, the objective is centered correctly.

FIGURE B. If the particle is large, the stage rotated 180º, and the particle does not appear to move off of the crosshair during the revolution, it is possible that the objective is off-center, but you cannot tell because the size of the particle makes it appear that the center of rotation is at the apex of the crosshair. **Don't use the center of a particle to determine if objective is centered.**

criminalistics@hotmail.com (714)272-4628

If all you seem to have are larger particles available for centering, then choose an edge or point on the larger particle to put onto the crosshair and center the objective from that point or edge. **Typically among the larger particles, one can find very tiny particles**. Use any of these very tiny particles. They are very acceptable to use. One does not have to use the same particle each time!

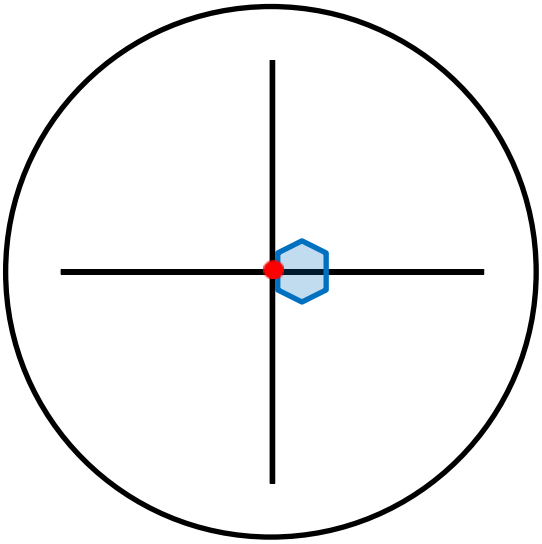


Figure C. Large particle with edge at cross hair.

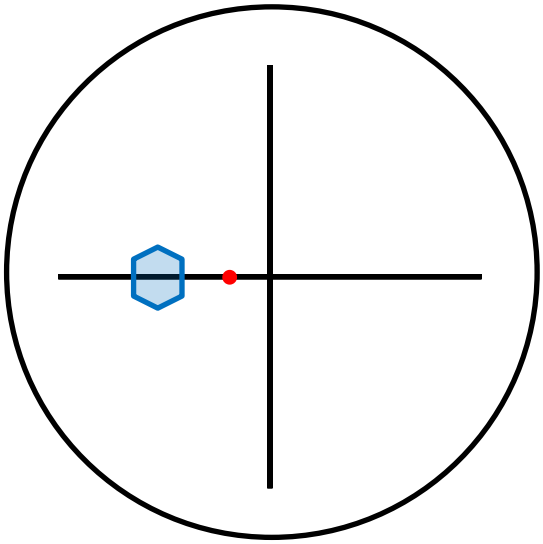


Figure D. Large particle after stage is rotated 180 degrees. Red dot is the center of rotation. Adjust the objective so the red dot is at the cross hairs.

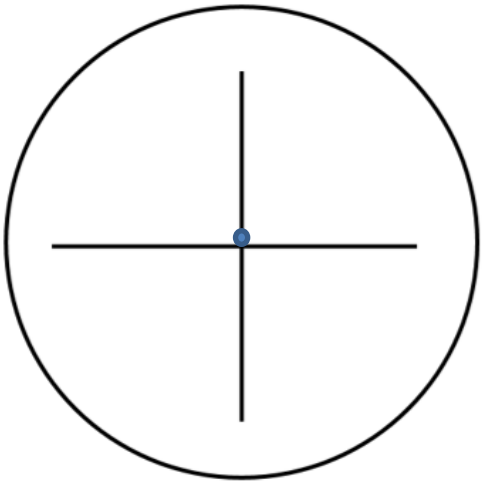
forensic TRACE
Training, Research, Analysis, Consulting, Education
Wayne Moorehead

criminalistics@hotmail.com (714)272-4628

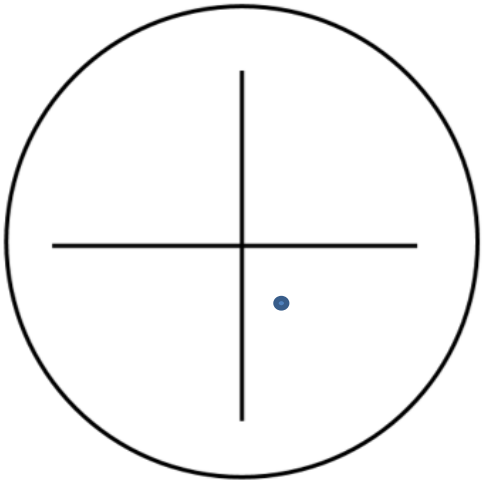
Appendix II – Objective not centered but in field of view

If your objective is not centered, the objective needs to be centered. The particle will move off of the crosshair. If the particle remains in the field of view, try to determine where the center of rotation is by rotating your particle 180 degrees. Once you become proficient, you will likely only need to rotate the particle 180º and then adjust the objective accordingly. When you are learning this skill, you may find it easier to rotate the particle 90º at a time (0º, 90º, 180º, 270º) and sketch the location of the particle around the crosshair to learn how to visualize the center of rotation.

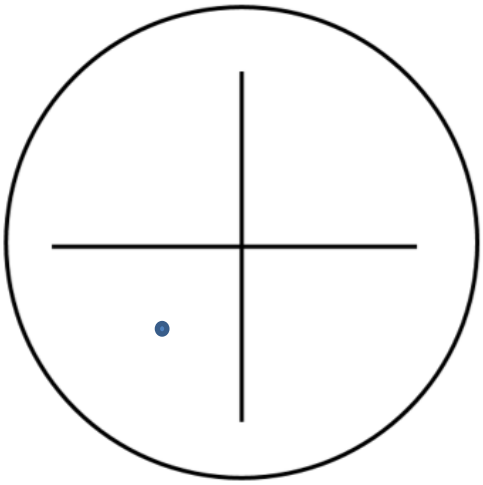
Start on crosshair 0º



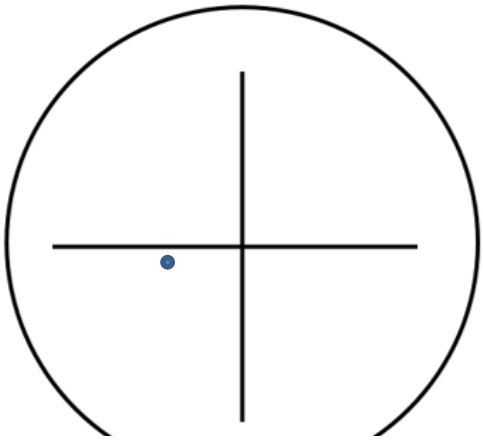
Rotated 90º



Rotated 180º



Rotated 270º

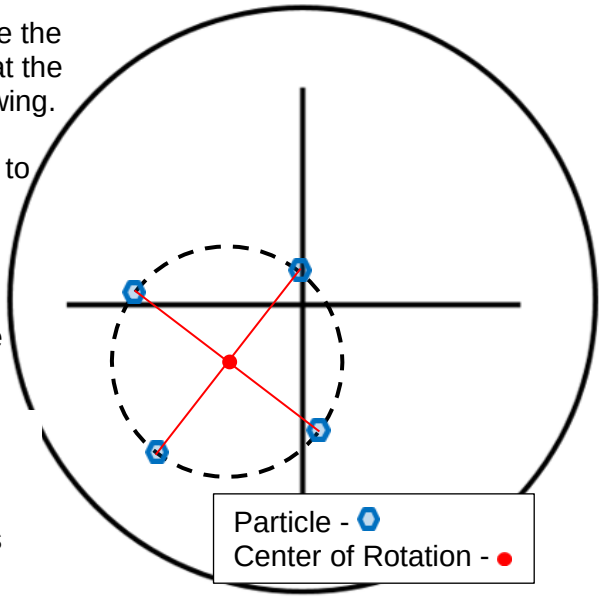


Place a particle on the crosshair - consider this the 0º position. Sketch a crosshair and draw the particle at the 0º position.

Watch the particle as you rotate the stage 90º. Sketch the particle at the 90º position into the same drawing.

Rotate the particle another 90º to the 180º position, and sketch it into the same drawing.

Rotate another 90º to the 270º position and sketch the particle into the drawing.



Draw a circle connecting the drawn-in particles.

Find the center by connecting the 0º and 180º particles and the 90º and 270º particles. The intersection of those lines represents the center of rotation. Use the screws to move that center to the crosshair.

forensic TRACE
Training, Research, Analysis, Consulting, Education
Wayne Moorehead

criminalistics@hotmail.com (714)272-4628

Appendix III – Objective not centered - particle not in field of view

If your objective needs to be centered, the particle will move off of the crosshair. If the particle moves out of the field of view, the objective is far off of center. Determine the center of rotation based on your observations of the particle's movement in the area that you can see.

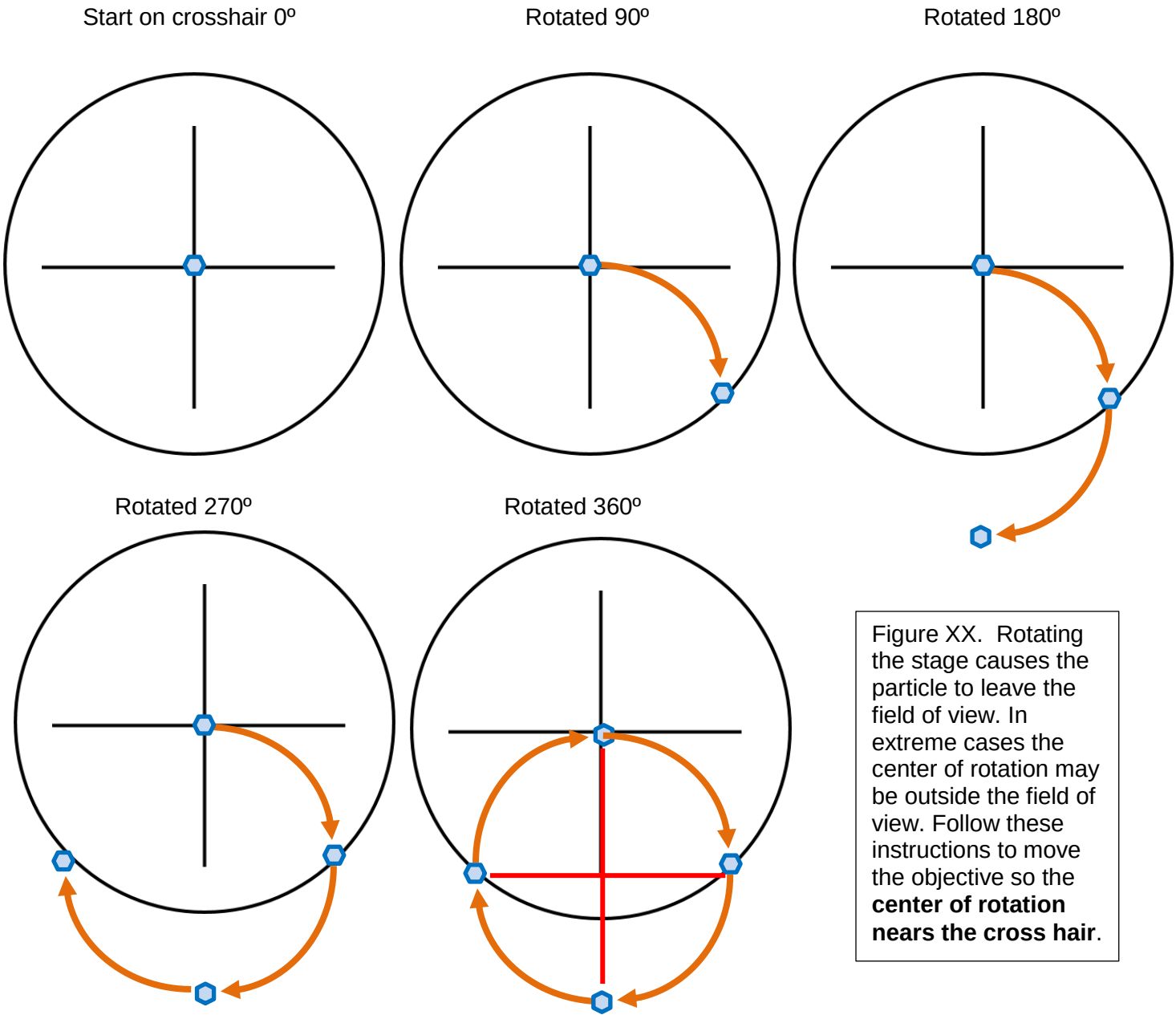


Figure XX. Rotating the stage causes the particle to leave the field of view. In extreme cases the center of rotation may be outside the field of view. Follow these instructions to move the objective so the **center of rotation nears the cross hair**.

forensic TRACE
Training, Research, Analysis, Consulting, Education
Wayne Moorehead

criminalistics@hotmail.com (714)272-4628

Appendix III – Objective not centered - particle not in field of view

When the particle leaves the field of view:

Place a particle on the crosshair - consider this the 0º position. Sketch a crosshair and draw the particle at the 0º position.

Watch the particle as you rotate the stage 90º. Sketch the particle at the 90º position.

Rotate the particle another 90º to the 180º position. The particle disappears from view. You cannot draw the particle into the field of view but you can estimate its location in the drawing.

Rotate another 90º to the 270º position and sketch the particle into the drawing if you can see it.

NOTE: If the centration is too far off, then the 90º and the 270º positions may also be outside the field of view. Look for where the center of rotation might be and move the objective using the adjustment screws so the center is in the field of view. Continue with the following or see Appendix II.

Draw a circle connecting the three drawn-in particles, and an imaginary fourth particle at the 180º position (estimate where it might be based on rotation of stage). Find the center by connecting the 0º and 180º particles and the 90º and 270º particles. The intersection of those two lines is the center of rotation.

Use the screws to move the center of rotation to the crosshair.