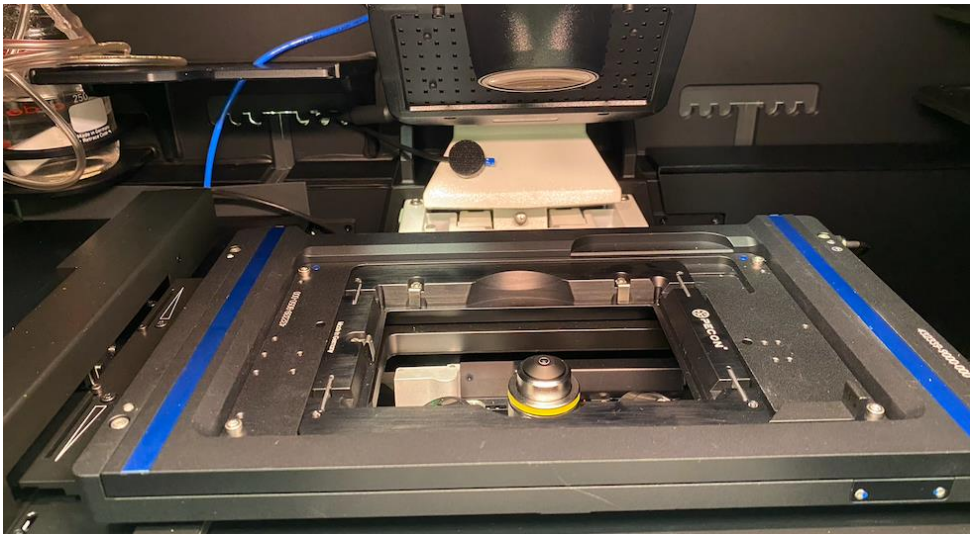
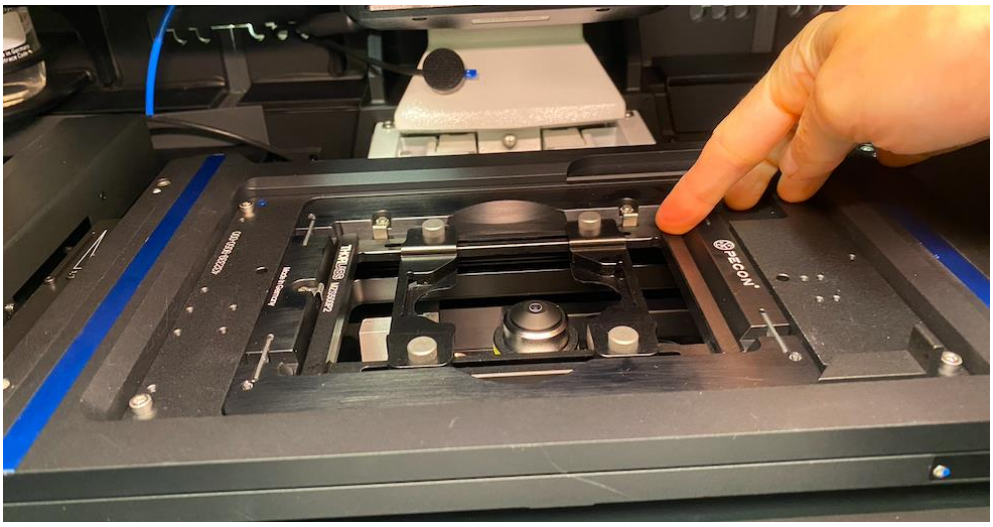
MODULE 6: FOCUSING

Objective: This goal of this module is to ensure students know the steps to finding focus. After this module, students should be able to find focus on a microscope efficiently following the steps below.

1. Begin at lower magnification lens (10X). This increases the chance your sample is in the field of view.

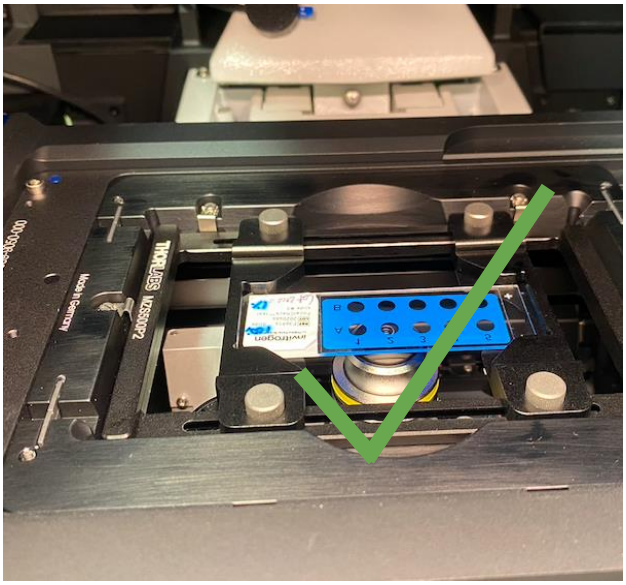


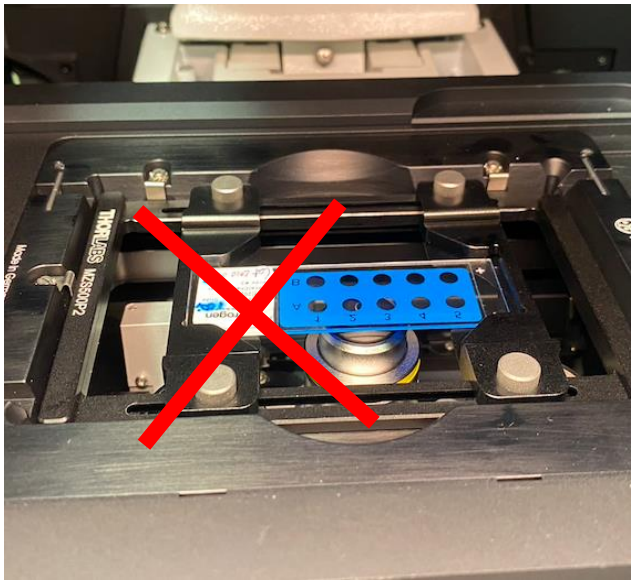
2. Check the stage:
    - Sample holder mounted correctly (check that it is flat on all four corners).



3. Ensure the coverslip is facing the microscope objective lens:
    - Upright microscope - facing up.
    - Inverted microscope - facing down.

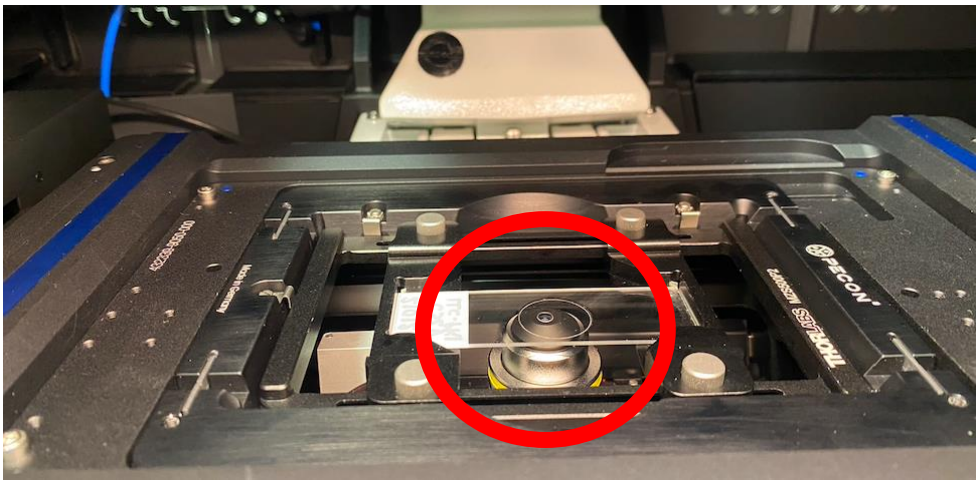
4. Position the sample in the path of the objective.





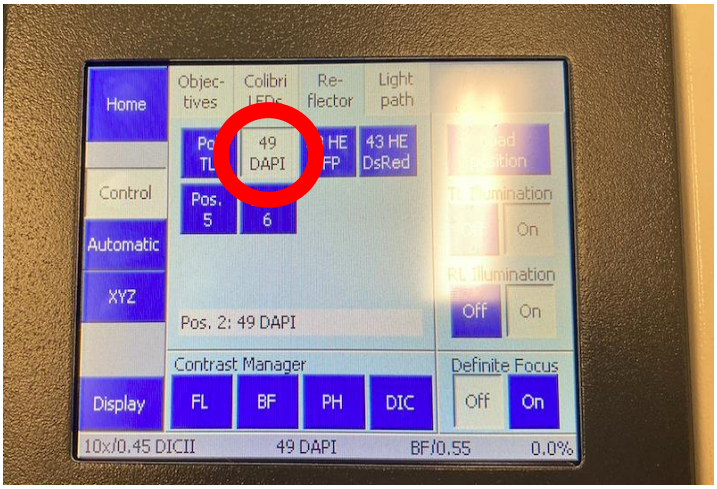
5. Position the objective so that it is very close to the sample but not touching. Then you can move the lens away from the sample to find focus.

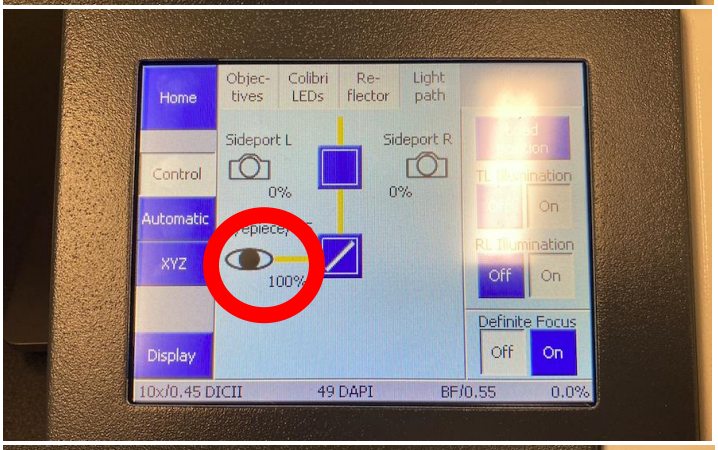
- PRO TIP - Why do we start high and move lower? To minimize the chance of collision.

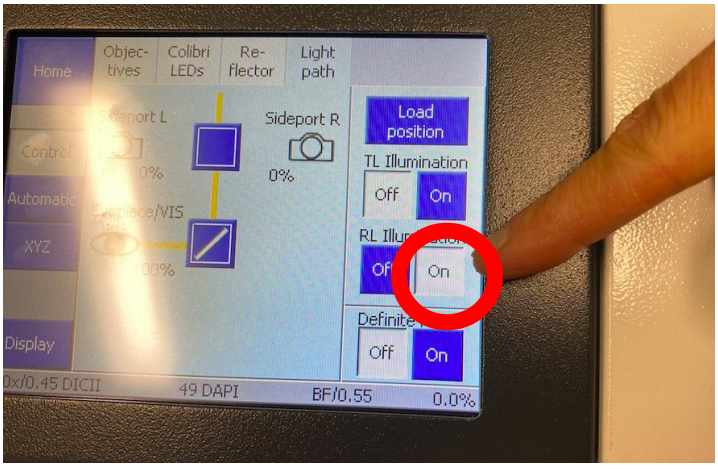


6. Choose the most abundant and brightest fluorophore to find focus, e.g. DAPI nuclear stain. Put DAPI cube in the light path, open the light source shutter, and direct light to the eyeyepiece

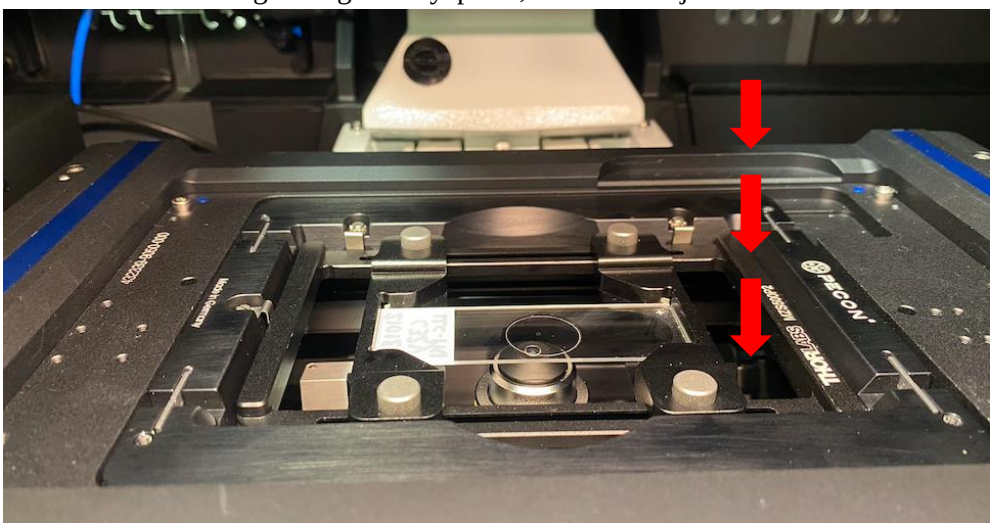
- PRO TIP - Do not choose weak or dim stains to find focus.

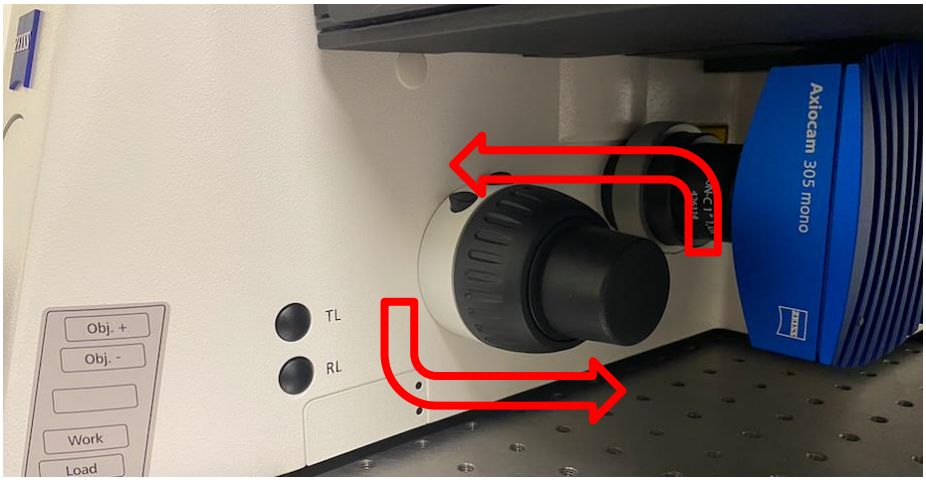






7. While looking through the eyepiece, lower the objectives until focus is reached.





8. Once focused in 10X, move up to a higher objective. Since the objectives are parfocal, you will be close to the correct focal plane and will only need minimal fine adjustments.

- PRO TIP - At each magnification change, center the sample in the middle of the eyepiecee, this will ensure the sample remains in the field of view at higher magnification.

9. If you are still having trouble focusing after these methods/steps, try finding focus on your test slide. Always have a test slide, something very bright and easy, that you have imaged before. This will allow you to determine whether the problem is the microscope/light path, or your sample (not properly stained, not bright enough).