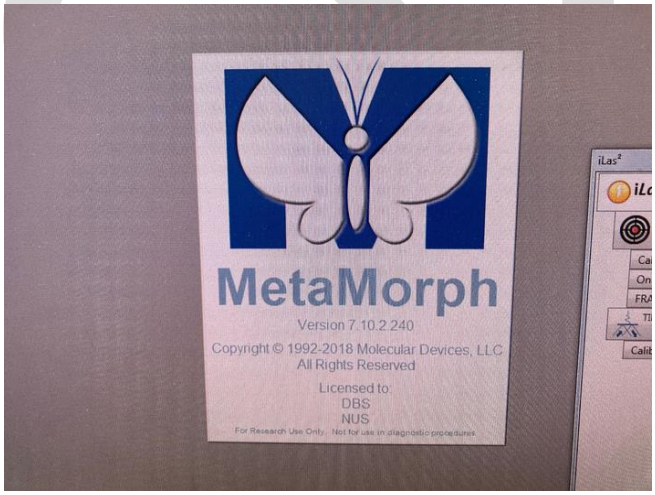
DARK FIELD MICROSCOPY ON TIRF 1

This manual is used as a guideline for users to acquire darkfield images on TIRF microscope using transmitted light for unstained sample only.

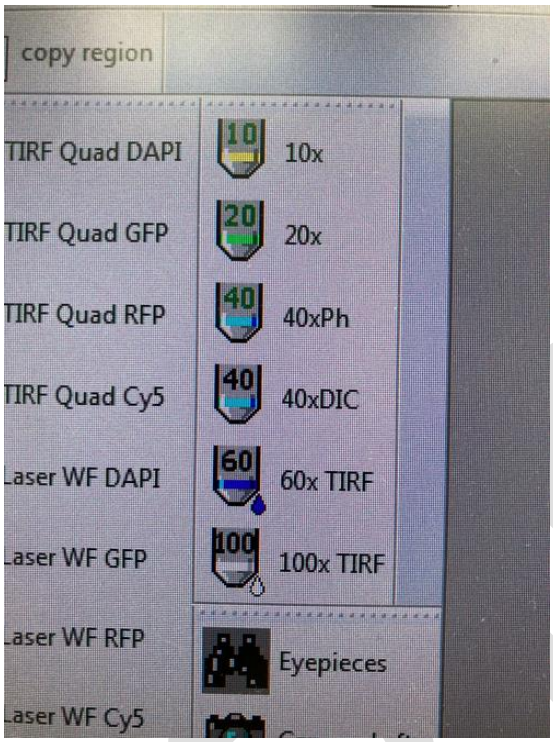
1. Turn on WF switch on the wall. Leave LASER switch off.

2. Turn on computer. Launch MetaMorph (MM) software. Watch for the MetaMorph logo to appear in the software window. If it doesn't appear, it hasn't detected the camera and you have to restart the computer.

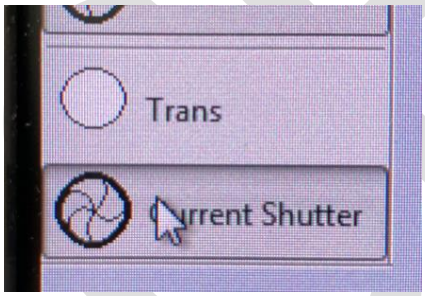


3. Mount sample with coverslip facing down. Apply oil to glass slide if you use oil condenser.

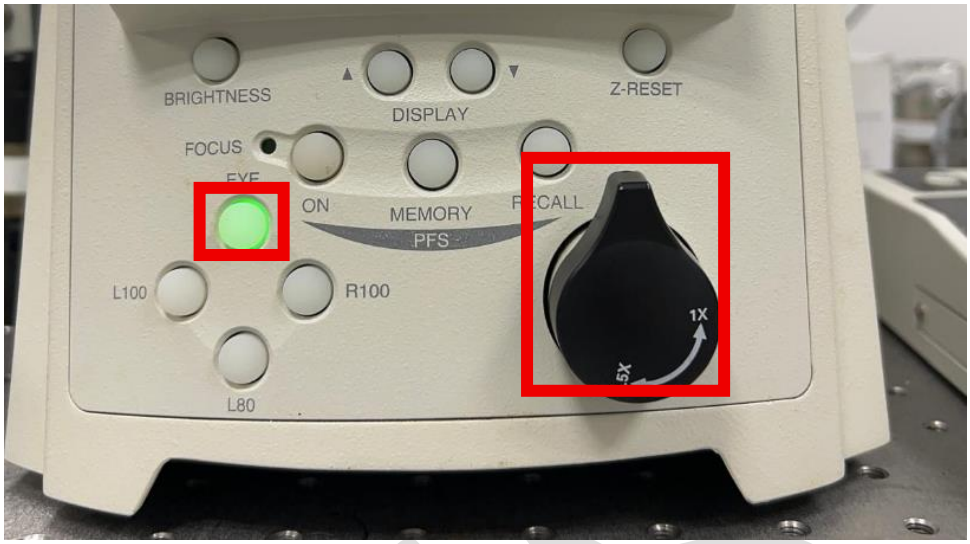
4. Select objective in the software (instead of the microscope touch screen) so the software will embed the correct scale information in the image.



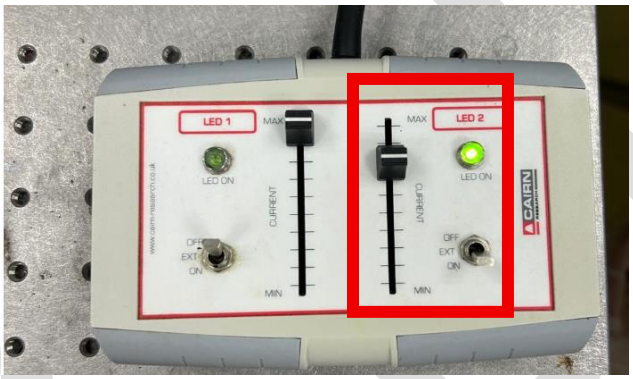
5. Click Trans followed by Current Shutter at lower left. The shutter icons at the top of the window should turn green..



6. Microscope: press “eye”; turn to “1x” magnify position.

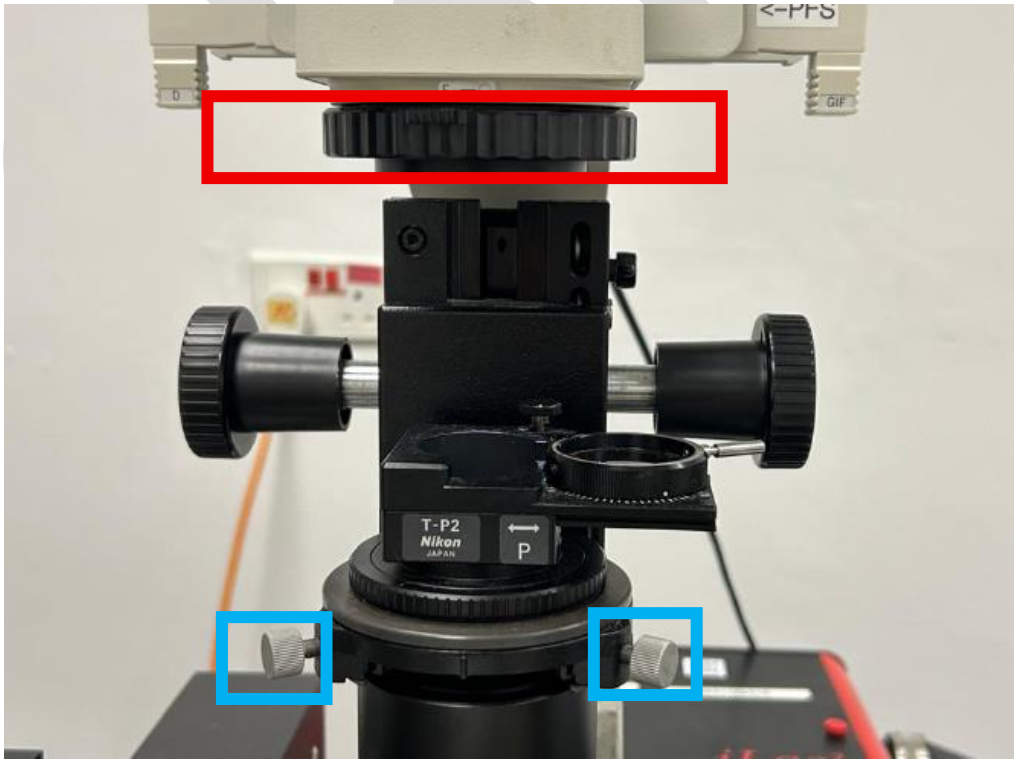


7. Adjust light illumination intensity by changing the slide.



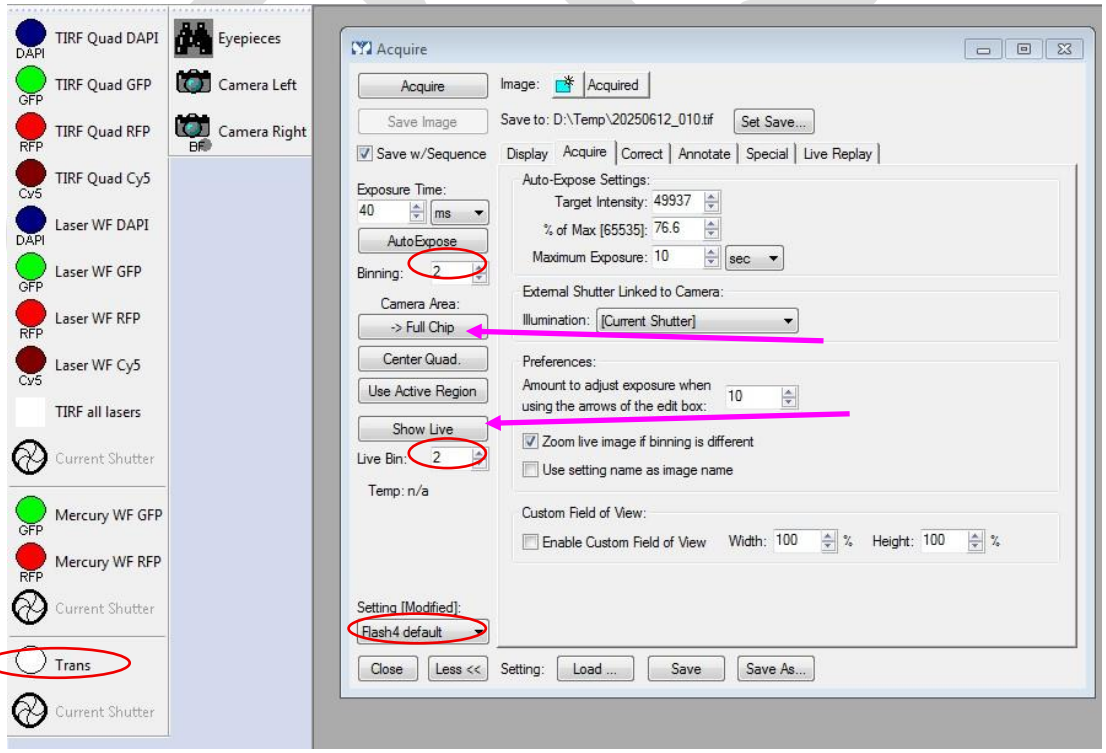
8. Lower the condenser until it touches the oil and you see a flash of light from under the condenser.

9. Moving focus knob to move objective up until sample is in focus.
10. Close **field diaphragm**, using the two **entering pins** to align condenser so that the image of field diaphragm is in the centre of field of view. You need to do this half Köhler illumination only once in each imaging session.

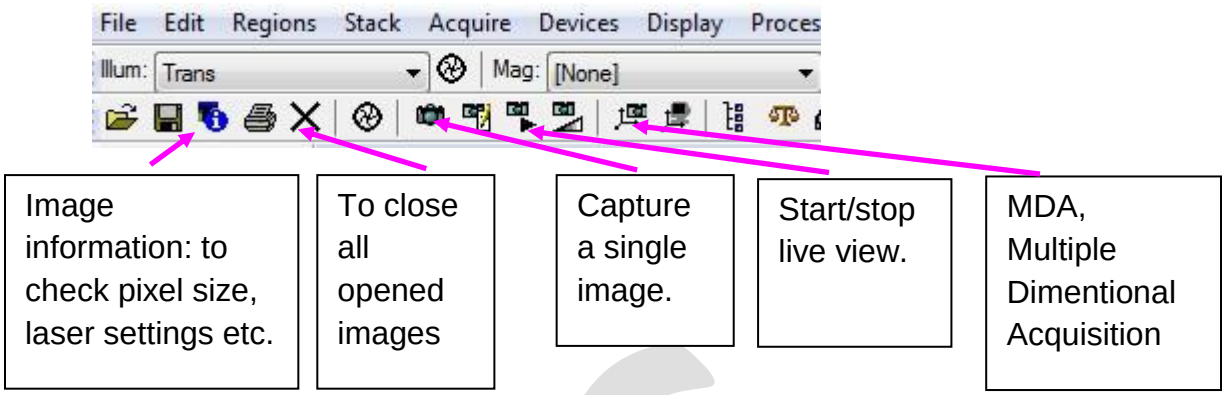


11. Switch to the 40x objective and bring your sample into focus.
12. You may need to adjust the height of the condenser slightly to achieve a dark background.
13. Avoid raising the condenser too high—once it is moved out of the oil immersion, re-immersing it may introduce bubbles into the oil.
14. Even if no bubbles are visible, it's advisable to wait a short while after repositioning the condenser back into the immersion oil. This allows the oil to redistribute evenly around the condenser lens.
15. Press "L100" to switch to camera mode.

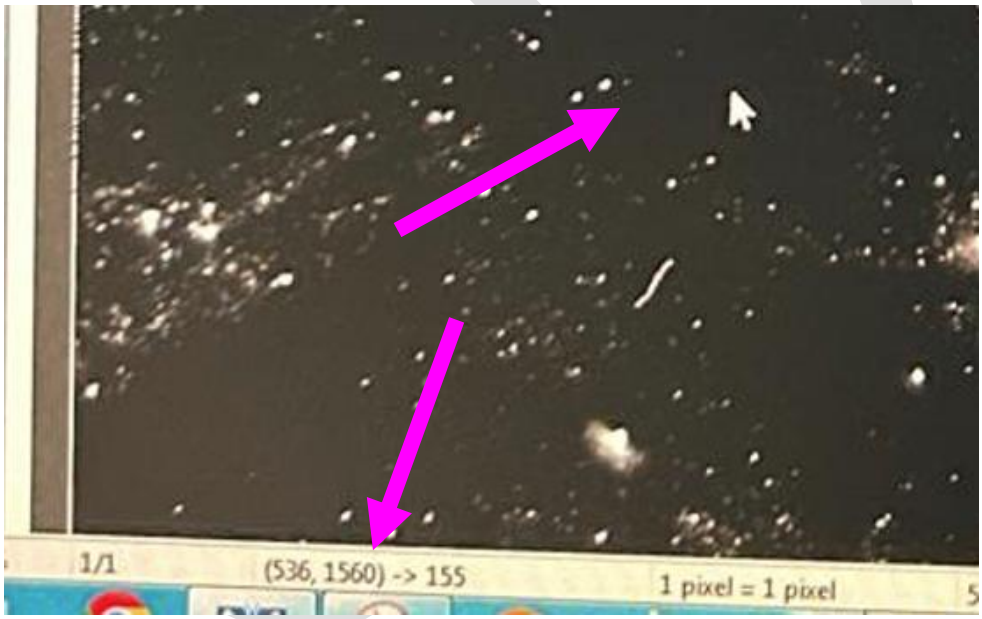
16. MM: Go to Acquire --> Acquire: select Flash4 camera, set both acquisition and live binning to 1. Click on "Trans" light path, full chip and show live. You can see live image now.



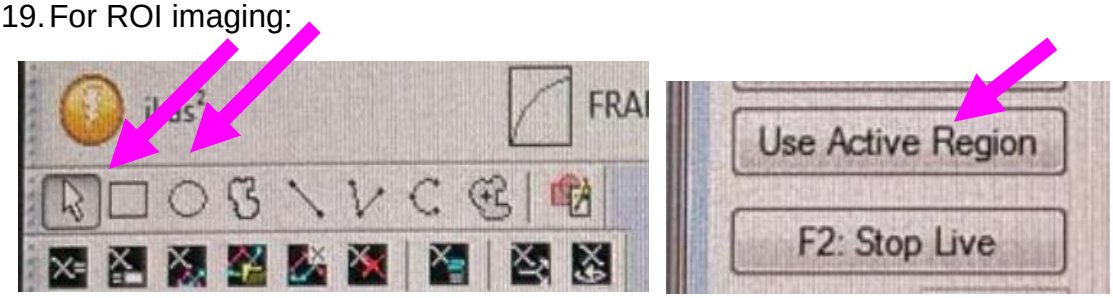
17. Shortcut on MM software:



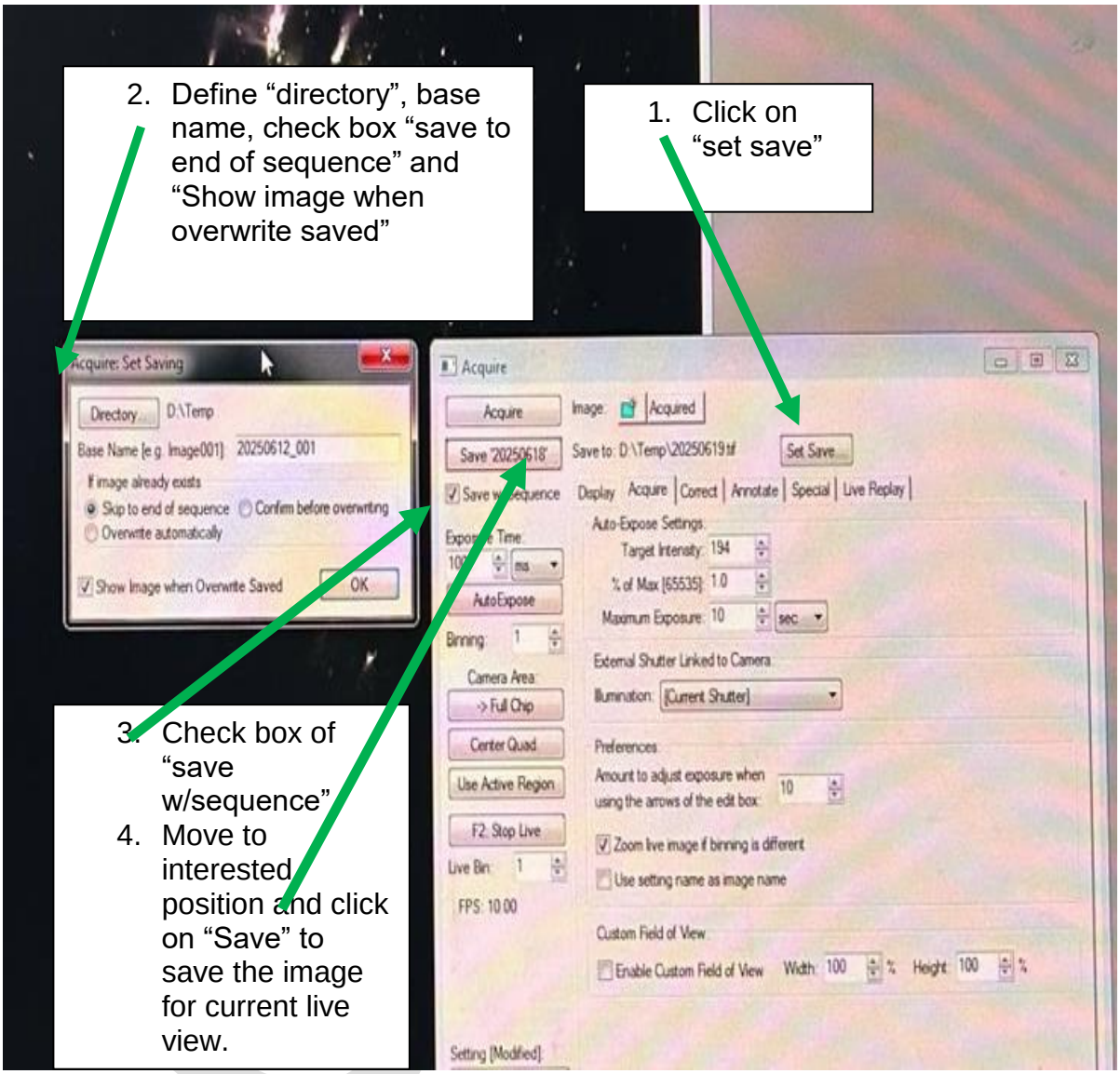
18. After focusing, check background intensity level if needed, for a better understanding of the darkfield setup, including condenser position, field diaphragm size and objective focusing plane. For example, the following image shows a intensity reading for a background is around 155.



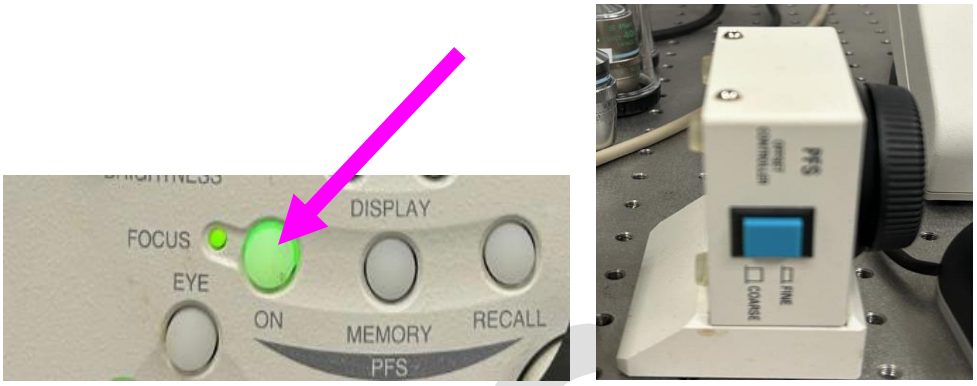
19. For ROI imaging:



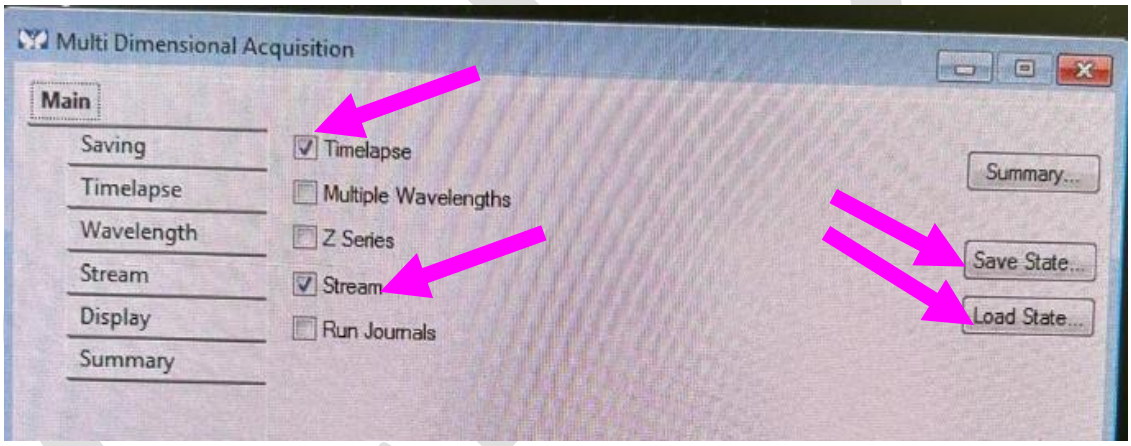
20. To save live view automatically:



21. Time lapse imaging: press “focus” on microscopy body to activate PFS to maintain focus. You need to use “PFS” focus knob when PFS is engaged.



22. To setup time lapse acquisition: Apps-> Multiple Dimensional Acquisition (MDA). In the Main tab, check box for timelapse and stream, if special speed and interval is required. You may click on “Save State” to save imaging protocol and reuse it in next imaging session by using “Load State”. Check each subtab, e.g. saving, wavelength, etc for detailed settings.



23. To end:
    1) Use portable drive to transfer data.
    2) Clean condenser. First use dry lens paper to soak up the oil, then use lens paper with ethanol.
    3) Shut down computer and turn off power switches.