

Olympus IX83 TIRF User Guide

Single Wavelength TIRF Acquisition

1. Turn on Microscope and software following the On-Procedure
2. Locate your sample through the oculars in combination with the appropriate left hand menu option

a. Example: EGFP/FITC/CY2 use FITC.



b. If your sample can't be found using fluorescence due to not having the appropriate cube, try BF or DIC; otherwise you'll have to locate the sample through the software using the proper wavelength selection.

c. Use the  icon to open the shutter.

3. If the wavelength you'd like to use is different from the one you used to find the sample through the eyepiece, make the change in the left hand menu.

a. Make sure the eyepiece slider is pushed IN

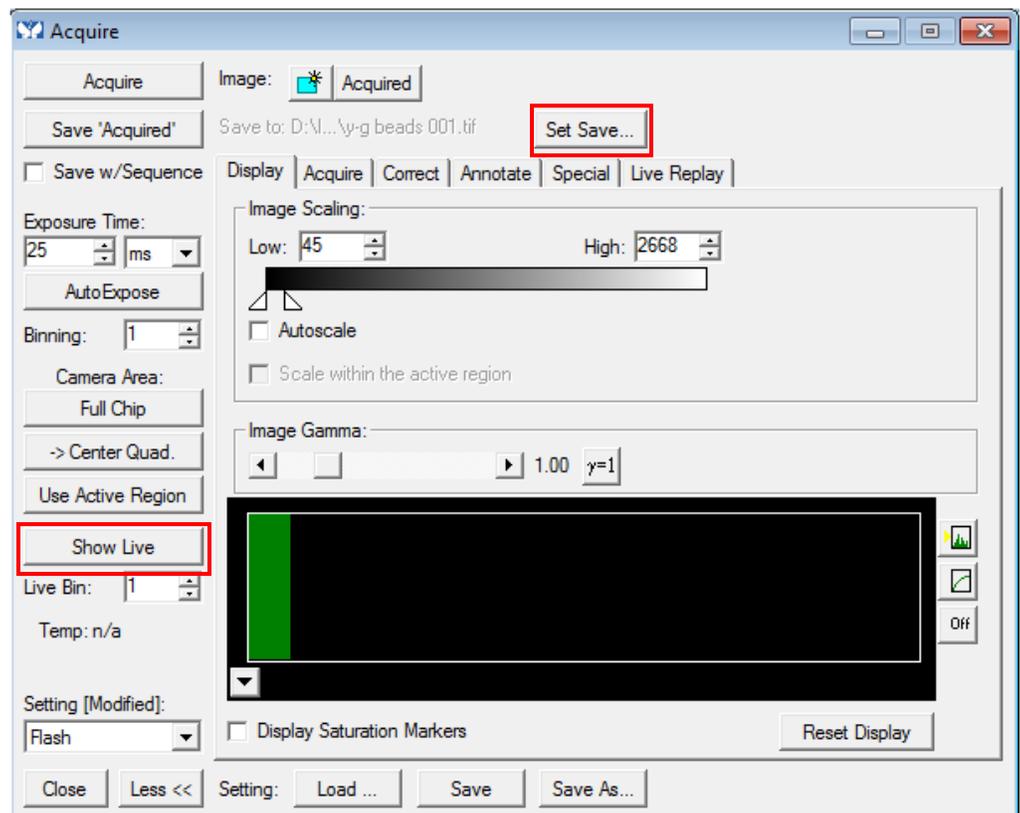
6. Press the Acquire button from the Main Menu floating window

7. Use the  button to select the location of your saved images.

8. Set your Exposure time to the desired time-frame (NOTE: you can use ms, sec, or min options from the drop menu as well as typing in a numerical value)

9. If you'd like to make notes on the file, you can use the Annotate tab.

10. Use the  button to bring the image onto the screen. (Note: That same button will be your Stop Live button.)



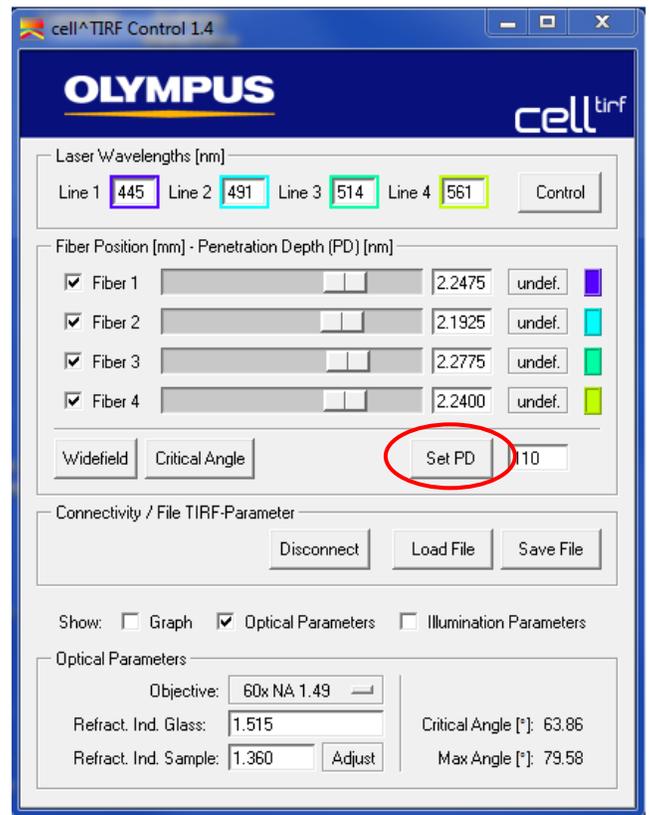
11. Focus on the specimen using the focus knob. Make any necessary XY location changes using the stage controller joystick or fine tuning knobs.

12. In the cell TIRF software main menu select the  button. This bring the machine from widefield mode to TIRF mode.

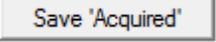
*Note: All of the sliders should shift towards the right. If they're still on the left you are in widefield mode.

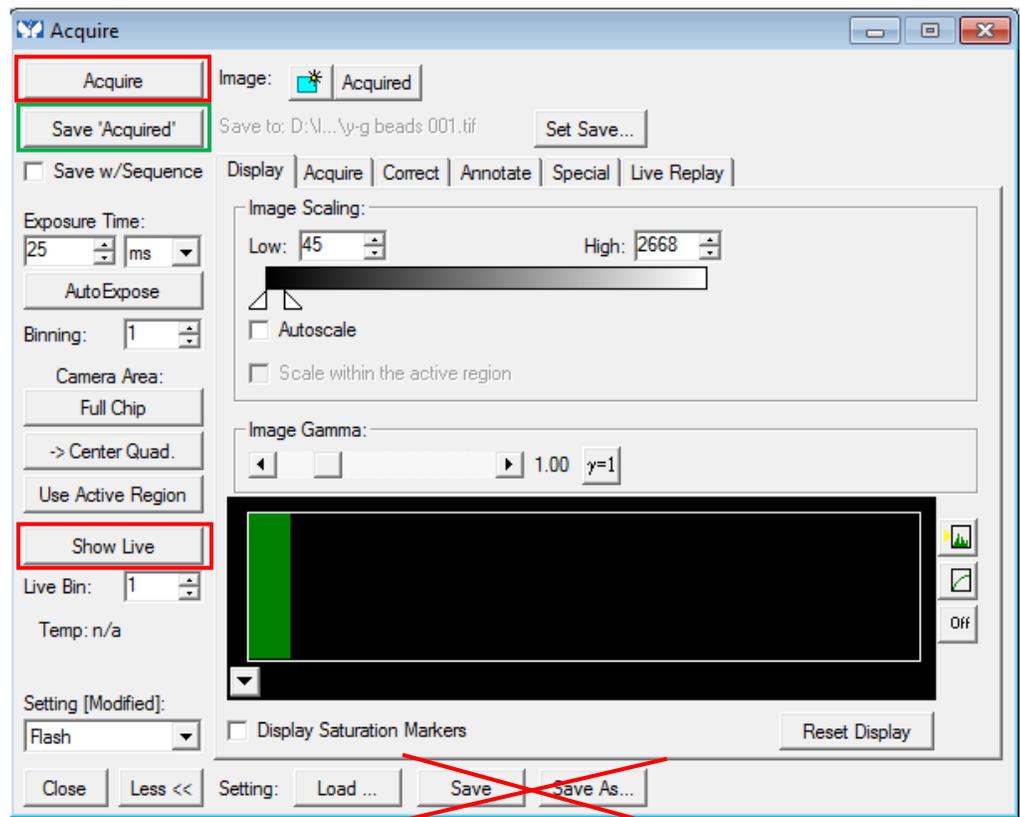
13. Turn on the Continuous Autofocus using either the touchscreen next to the microscope or the  button from the main Metamorph software toolbar.

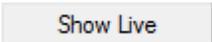
- a. You will hear a single beep once it's activated
- b. Fine tune the focus using the focus knob



14. Select the  button from the Acquire window to take the image

15. Save the image by selecting the  button located below the Acquire button. DO NOT USE EITHER SAVE BUTTONS AT THE BOTTOM OF THE ACQUIRE WINDOW.



16. If desired, find another area on the current coverslip using the  button and stage controller. Repeat steps 14 and 15 to take and save as many images as you'd like. Refocusing is not necessary (unless you want to fine tune the focus) as long as Continuous Autofocus is still engaged.