

Automated TIRF calibration for iMic

Step 1: Focus on a TIRF sample e.g. 100 nm beads

- Focus on a bead sample with a TIRF objective
- Check that the correction collar of the objective is in the right place and that you are using a TIRF compatible coverslip of 0.17 μm thickness

Step 2: Calibrate the TIRF Center

- Choose the TIRF calibration preset, that you want to calibrate e.g. 60X 1.49-Quad-488nm and go to the “settings”, put the laser power to 100 %

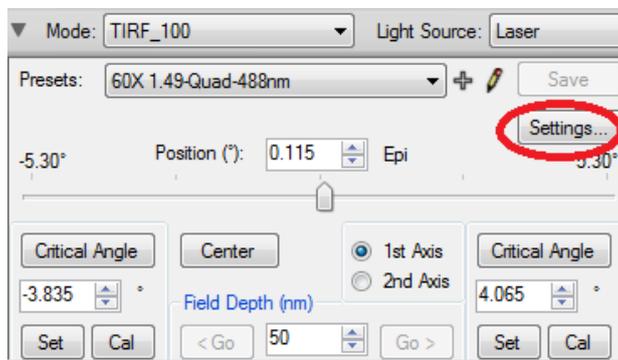


Fig. 1

- in settings press “calibrate”, the Yanus TIRF center will show you the old values

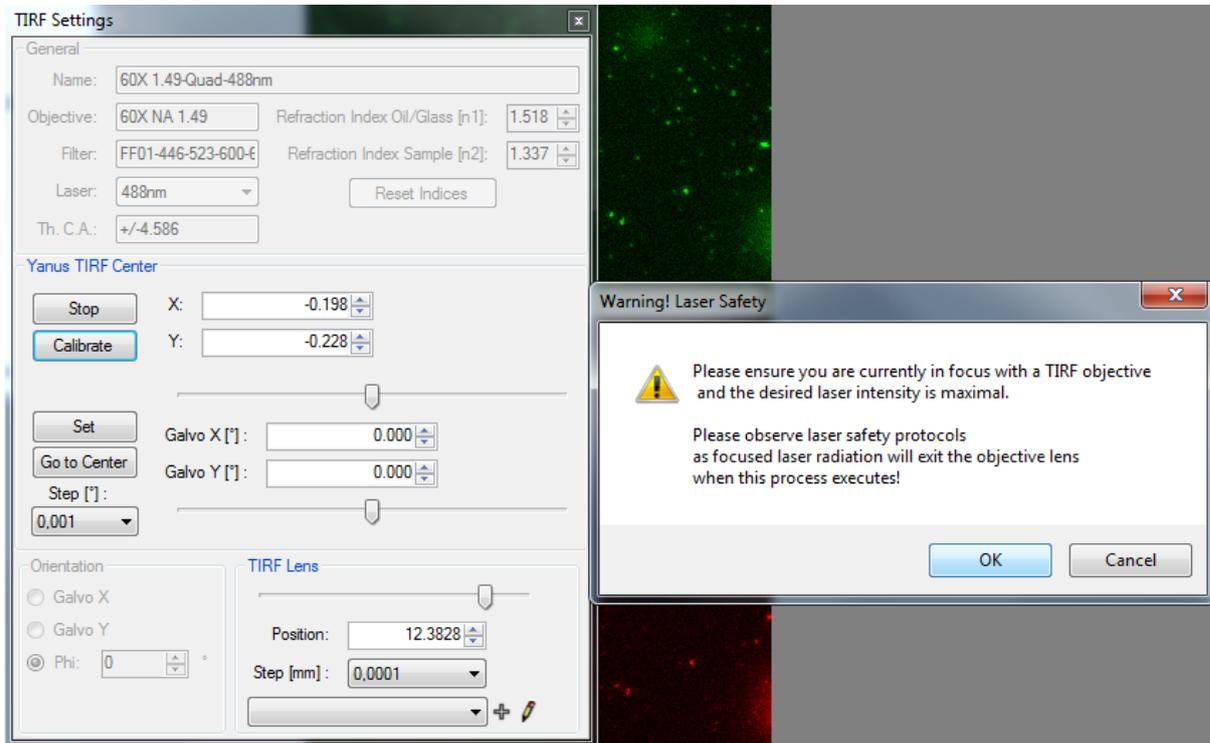


Fig. 2

- after the calibration is done you want to overwrite the old TIRF center values with the new calculated ones
- Press “Go to Center” and “Set” then press “Stop”, values in both windows should match. Close the TIRF settings

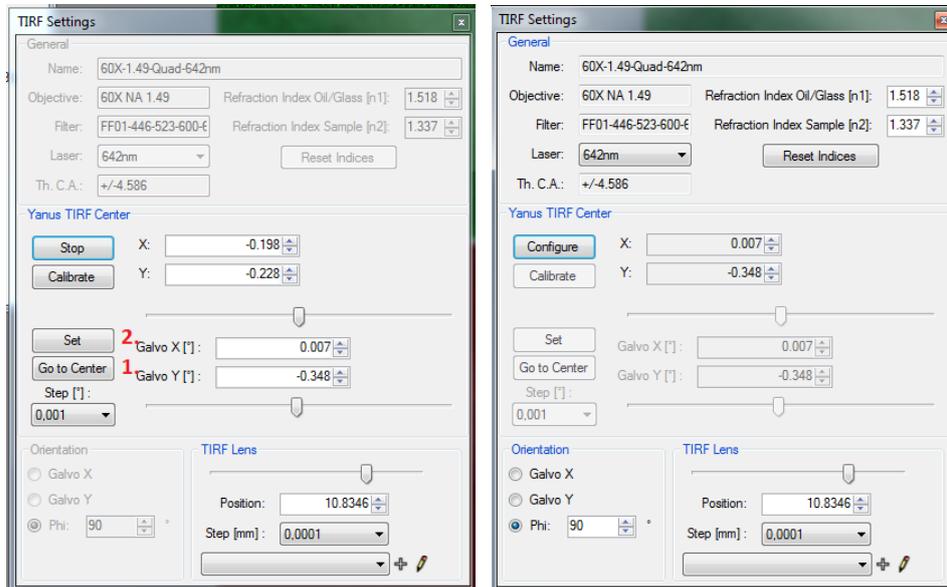


Fig. 3

- Don't touch the TIRF lens position, this can only be adjusted on the open beam!!!

Step 3: Calibrate four TIRF angles on two axis of the ring TIRF

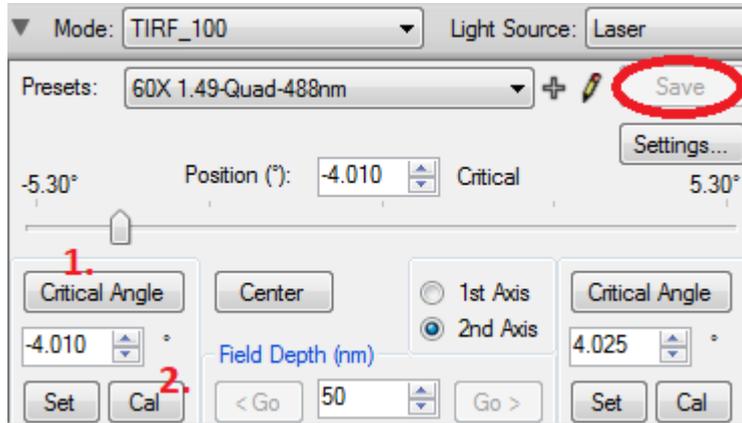


Fig. 4

You are still in Focus with your bead dish, the laser power should be set quite high because the total reflection of the beam is sensed to determine the critical angle.

- Press “Critical Angle” then “Cal”
- Repeat this step for all four angles
- Press center, to check if the TIRF center position is below 1.0, if it's above 1.0 repeat the TIRF Center calibration

Step 4: Overtake TIRF Center values for all laser lines (except 445nm) using the same objective

- The TIRF center value is the same for all laser lines 405nm, 488nm, 561nm, and 642nm, when using the same objective (100x and 60x objective have different TIRF center values) because all lasers are coupled in with the same fiber.

- When you have done the TIRF center calibration of one laser line successfully, the galvo mirror values can be copied to the other TIRF presets
- When you have done the calibration for preset: 60X 1.49-Quad-488nm you can then choose preset: 60X 1.49-Quad-642nm for instance and open the “settings” Fig.3
- Press “Configure” and then just press “Set” to overtake the values from the Yanus TIRF center to the Galvo mirrors. Then press “ Stop” and close the TIRF settings
- Now perform the calibration of the four TIRF angles from Step: 3