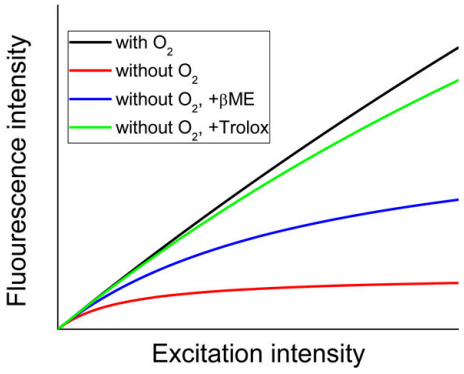
Sonicate TetraSpeck beads and equilibrate them to proper temperature. Take note of the lot number of the TetraSpeck beads (the concentration and actual diameter of the beads vary greatly from lot to lot).

Preprocessing standard fluorescent dye solution:
Tween-20: 1:100
O2-scavenging: PCA: 1:50, PCD: 1:50
Trolox (anti-blinking): 1:100



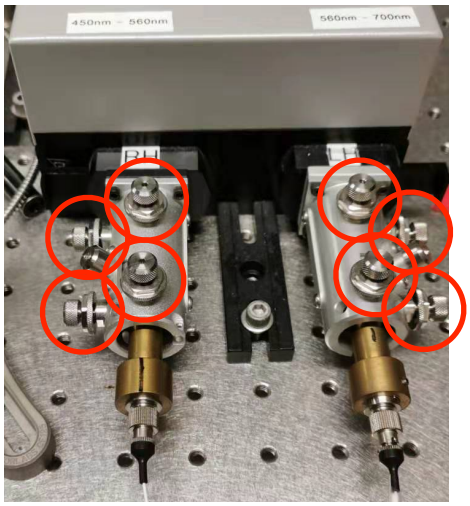
(Ha and Tinnefeld, 2012)

The filter wheel of the Olympus microscope should be set to position #6.

Open the lid of the optical components box and check whether the first filter is fully pushed in [excitation filter wheel should be set to #3 (405/488/561/635 nm)].

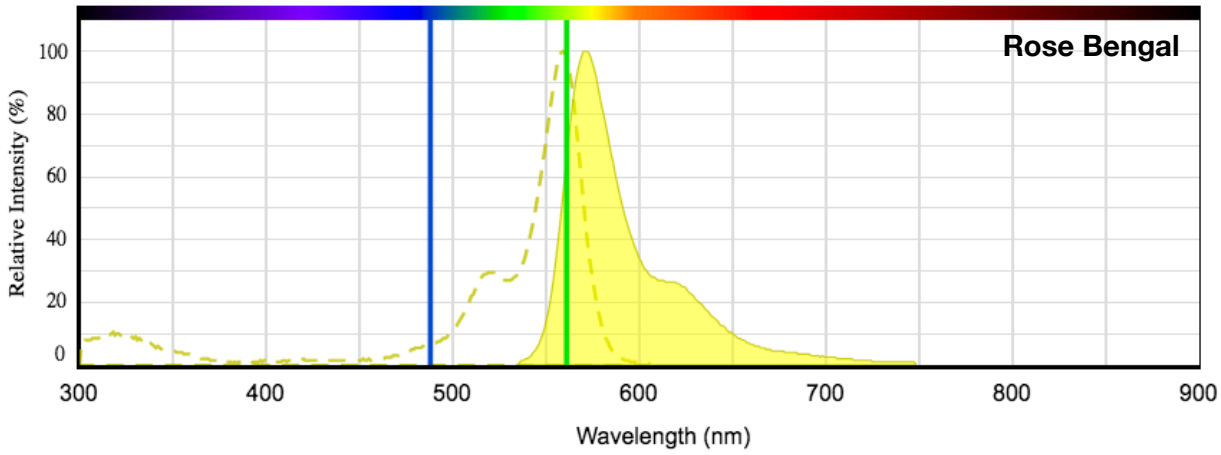
Single GFP Channel (1): #1(531/40 bandpass) <- #5 (653 shortpass) <-
RFP (1) + GFP (2) channels: (Ch 2) #1 (531/40 bandpass) <- #3 (empty) <- #2 (mirror)
|
(Ch 1) #3 (593/40 bandpass) <- #4 (562 nm long pass) <-

Make sure that the excitation light is not going through the TIRF mode fiber. Use the 8 knobs (circled out in the image below) to align and collimate the light.

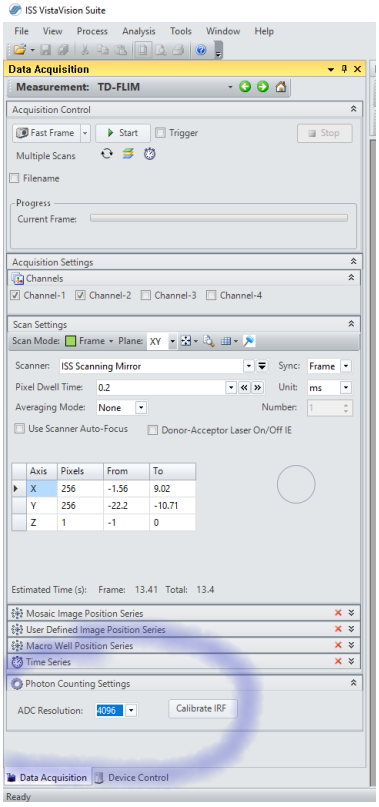


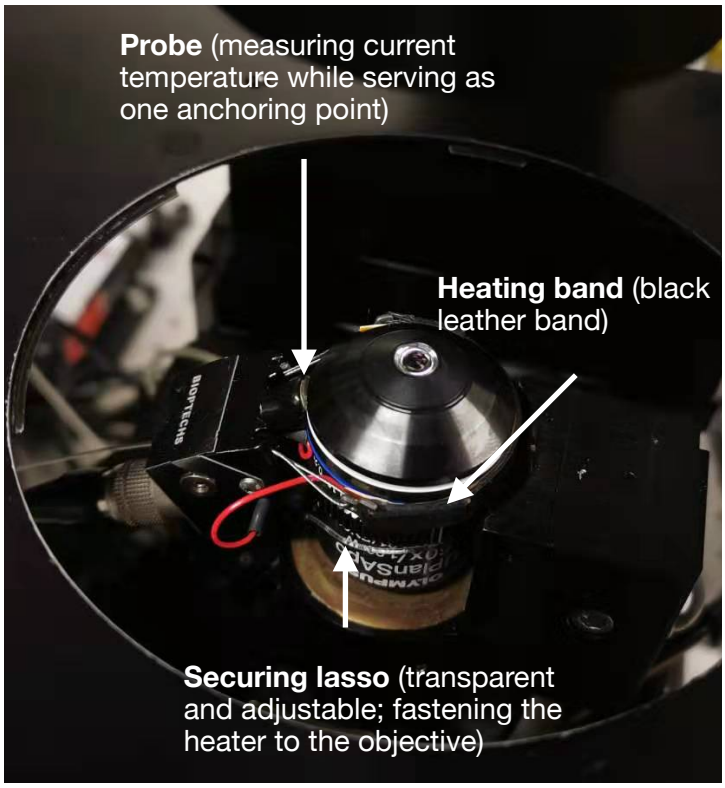
When using different pinhole sizes, re-alignment is necessary.

IRF measurement: 1-10 uM Rose Bengal in saturated KI (aq). Filter setup:
(Ch 2 - for 488 nm) #5 (582/75 bandpass) <- #3 (empty) <- #2 (mirror)
|
(Ch 1 - for 561 nm) #3 (593/40 bandpass) <- #4 (562 nm long pass) <-
Use high power percentage and large pinhole sizes for 488 nm since Rose Bengal has poor excitation at 488 nm.



ADC Resolution in TD-FLIM:





Setting up the objective heater: 1. Use a hex key of the correct size (5/64") to adjust (from the back) the probe indent so that it is firmly touching the objective. 2. Adjust the length of the securing lasso.