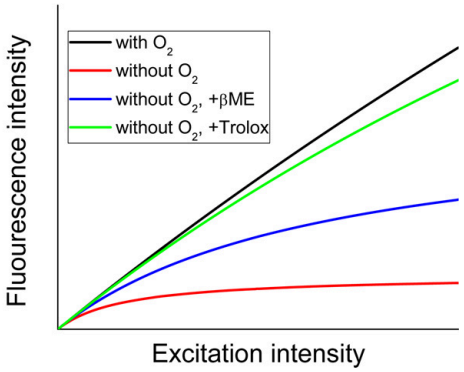
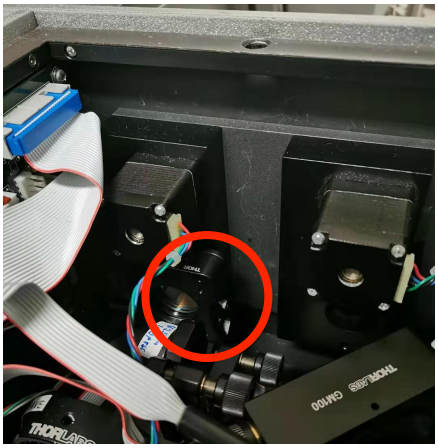
Sonicate TetraSpeck beads and equilibrate them to 37 C. Take note of the lot number of the TetraSpeck beads (the concentration and actual diameter of the beads vary from lot to lot).

Preprocessing standard fluorescent dye solution using Damon's stocks:
Tween-20: 1:100 (final concentration: 0.05% v/v)
O2-scavenging: PCA: 1:50 (final concentration: 2.5 mM), PCD: 1:50 (final concentration: 7 ug/mL or 21 uU/mL)
Anti-blinking: Trolox: 1:100 (final concentration: 1 mM)



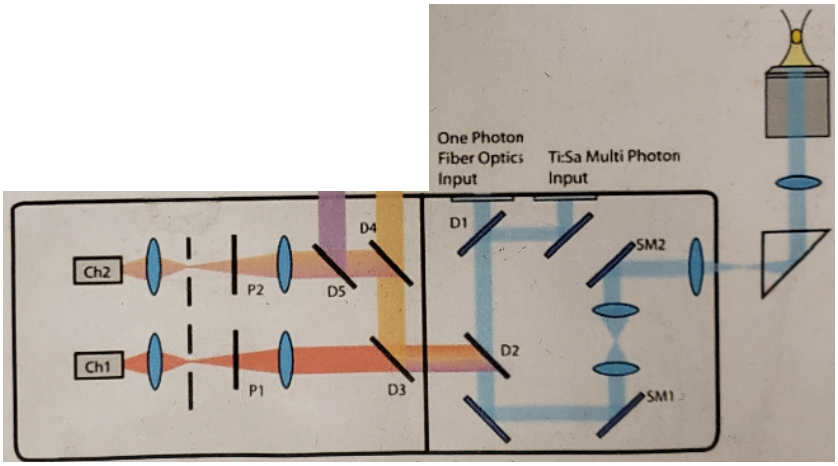
(Ha and Tinnefeld, 2012)

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

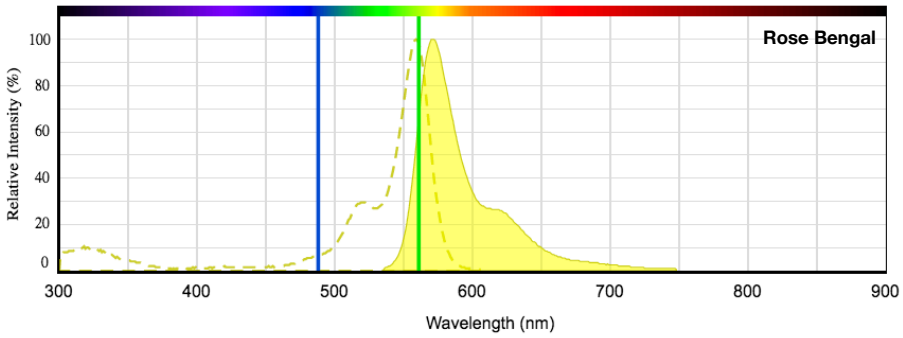


Open the lid of the optical components box and check whether the first filter is fully pushed in [excitation filter wheel should be set to #3 (405/488/561/635 nm)].

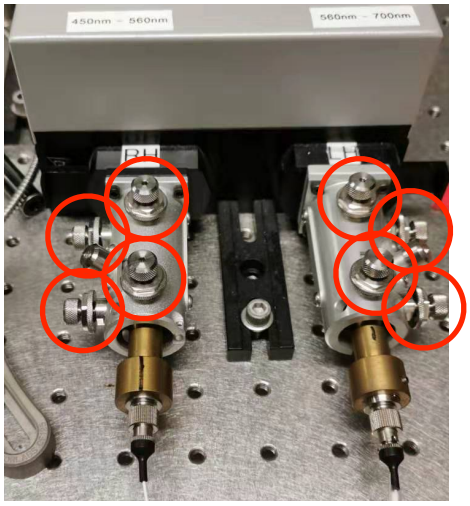
Filter setup:



i. Single GFP Channel (Ch 1):
(Ch 1) P1#1 (531/40 bandpass) <- D3#5 (653 shortpass) <-
ii. RFP (Ch 1) + GFP (Ch 2) channels:
(Ch 2) P2#1 (531/40 bandpass) <- D5#3 (empty) <- D4#2 (mirror)
|
(Ch 1) P1#3 (593/40 bandpass) <- D3#4 (562 longpass) <-
iii. IRF measurement [use 1-10 uM Rose Bengal in saturated KI (aq); Ch 2 for 488 nm IRF and Ch1 for 561 nm IRF]:
(Ch 2) P2#5 (582/75 bandpass) <- D5#3 (empty) <- D4#2 (mirror)
|
(Ch 1) P1#3 (593/40 bandpass) <- D3#4 (562 longpass) <-
Note: use high power percentage and large pinhole sizes for 488 nm to measure Ch 2 IRF since Rose Bengal has poor excitation at 488 nm:

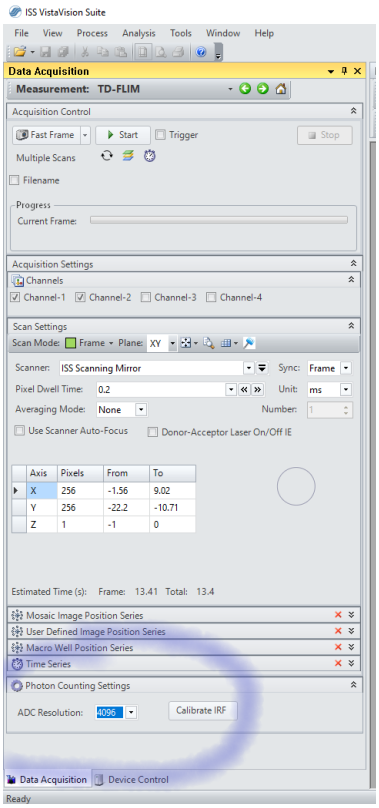


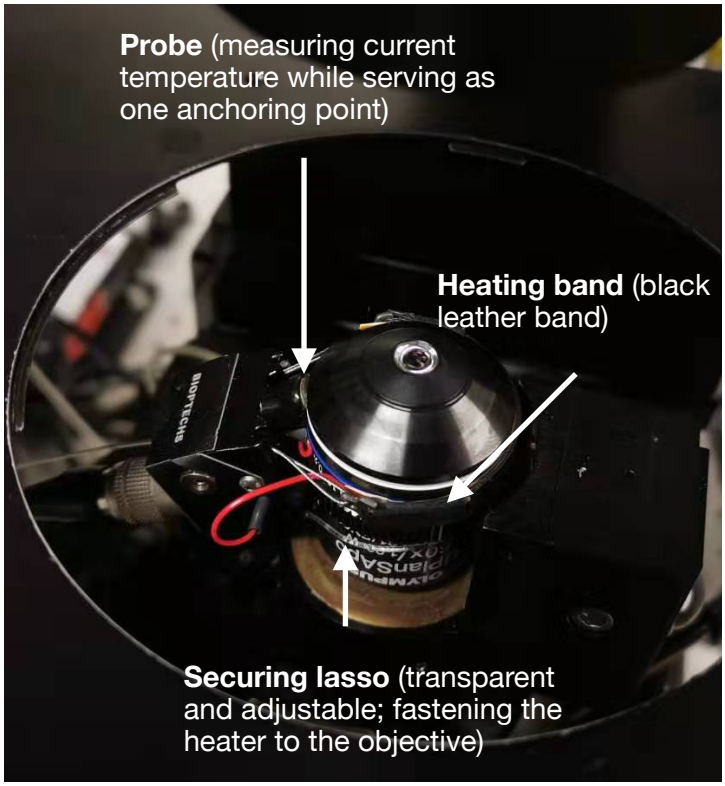
Make sure that correct single-mode fibers are inserted. The 8 knobs (circled out in the image below) can be adjusted to align and collimate the light (ask Damon for help if you find out that the signal is low and suspect that the excitation light is not well collimated).



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

ADC Resolution in TD-FLIM (set to the maximum value 4096):





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.