

ALIGNMENT Procedure of TIRF/STORM Laser

To achieve clean TIRF illumination, the laser should be **focused on the back focal plane** to leave the objective **collimated**. That means, the laser will display a minimal spot on the ceiling mat board target.

ATTENTION: A COLLIMATED laser is dangerous for your eyes! Avoid looking into the laser beam or putting reflective objects into the beam path! NEVER look from above the setup into the laser beam down at the objective! If there are any uncertainties or questions, please contact AMBIO staff!

Alignment procedure:

- Focus on the sample using bright-field, wide-field (lamp) or the perfect focus (it is OK having oil on the lens already for alignment; to not bleach your sample, best to remove it remove before alignment!)
- Choose a laser line and put the laser power on LOW (1%)
- Choose the dichroic you need
- Choose the condenser setting (TIRF – OUT, STORM – IN) in the back

X/Y Alignment

- Put on the laser in NIS (don't forget the laser safety screen on the sample stage)
- X-position (left/right): Adjust in NIS, start with in the EPI Laser Position Number listed below
- Y-position (front/back): Manually adjust with the knob in the back
- Align laser roughly with cross on ceiling mat board
- Loosen set screw in back, focus laser with focus knob (in back), then set the set screw again
- Fine adjust

Laser Position Measurement (23.3.2021)

Laser	Dichroic	Condenser	EPI Laser Position in NIS
647	Quad	IN	3335
647	Quad	OUT	3180
647	647	IN	3260
647	647	OUT	3015