Manual: Optical Forces Set-Up Build and Alignment

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1 Introduction

Starting August 15th, we began a re-build of the optical forces measurement set-up. This is a document to keep track of parts and the procedure for building and aligning. Hopefully it will be some assistance to those continuing this work in the group or those interested in reproducing this work.

2 Components

2.1 Light Sources

The set-up is similar in its core to a classic total internal reflection microscopy (TIRM) set-up. Such a set-up requires optical access of the sample from both the top and bottom of the chamber. The laser beam (660 nm CW) incident from above must be focused by a high-NA water-immersion objective for single-beam optical tweezing. A white LED, focused down through the same objective, provides dark-field lighting over the entire objective field of view.

The detection laser beam (637 nm CW) incident from below must be at an angle such that the light totally internally reflects at the glass-water interface of the chamber. For this purpose, a 60-degree prism is often used to couple light into the glass.

For measurements of optical forces from an evanescent field a second totally-internally reflecting beam (785 nm CW) is incident on the bottom of the sample chamber from the opposite direction. The total number of light sources on this set-up is therefore, currently, three. All except the LED are fiber-coupled for easy maneuvering and realignment.

2.2 Detectors

Three detectors track the three dimensional displacement of the trapped microsphere. The vertical motion (perpendicular to the surface) of the particle is tracked by monitoring the intensity of the light it scatters from the detection (probe) beam by a photodiode.

Motion in the two lateral directions (parallel to the surface) is monitored by tracking the displacement of the reflected trap beam back-scattered by the particle. In a method similar to a quadrant photodiode, for each axis, the reflected beam is divided spatially into two halves, and the difference between the intensities of these two signals is reported by a balanced detector and converted into displacement.

2.3 The Objective Assembly

The current version of the objective assembly uses a piezo objective scanner from Piezosystems Jena designed for microscopy applications. This should afford us significantly more stable positioning and reduced vibrational noise compared to the flexure stage on which our objective was originally mounted.

The goal of this design is to allow macro-movements with the vertical translation stage and micro-positioning with the piezo scanner. The 4 mm travel of the translation stage enables loading, unloading, and manual objective focusing. The piezo scanner is a part of a closed loop involving a capacitive sensor (not pictured) to detect and correct for thermal expansion and drift of the objective focus.

For a full list of parts see Table 2.3.

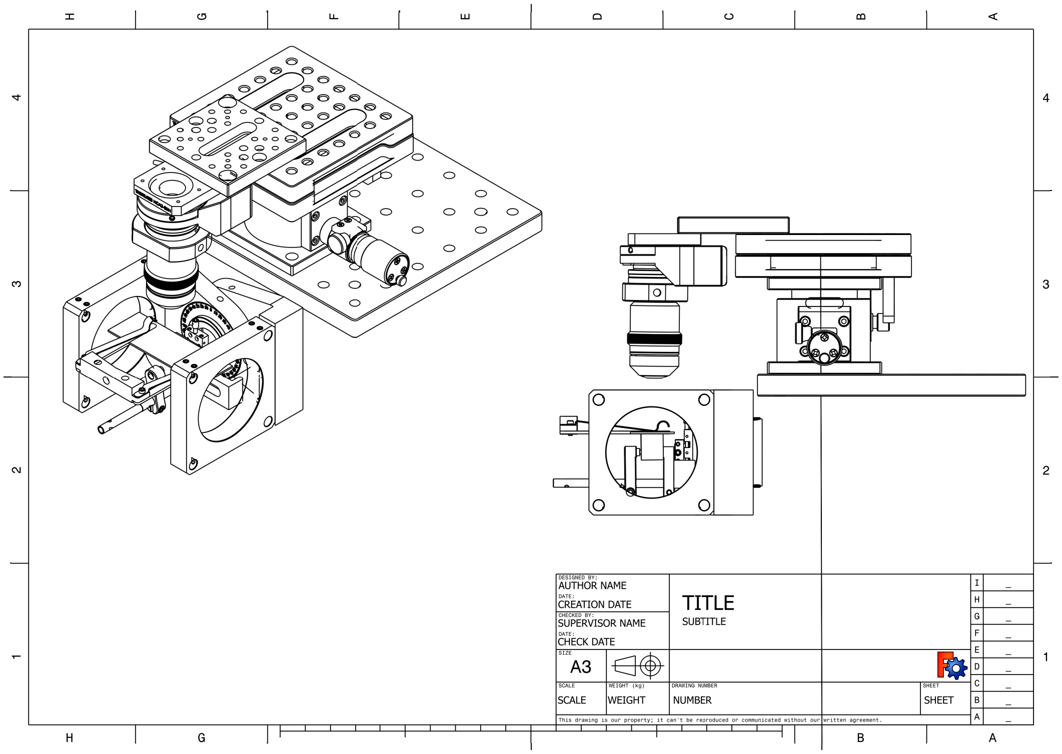

Figure 1: Version 2 of the objective and prism assembly. For a list of parts please see Tables 2.3 and ().

Part	**Description**	**Vendor**
Leica PL APO 63/1.20 WCORR	Water-Immersion NA 1.2 Objective	Leica (belongs to Weitz Group)
MIPOS 100 PL O-323-00	Piezo Objective Scanner	Piezosystems Jena
TSD-653DMUU	Micrometer vertical stage	Optosigma
KBM1	Switchable Magnetic Kinematic Breadboard	Thorlabs
SM1A11	Adapter with External M25 Threads and Internal SM1 Threads	Thorlabs
SM1A12	Adapter with External SM1 Threads and Internal M25 Threads	Thorlabs
SM1L05	SM1 Lens Tube	Thorlabs
SM1RC	Slip Ring for SM1 Lens Tubes 832 Tap	Thorlabs
HCA3-SM1	SM1 Adapter for Mounting Objective	Thorlabs
XT34HP	Dovetail Mount used as right-angle adapter	Thorlabs
9101NF	Right Angle Bracket	Newport

Table 1: Parts for objective assembly mount for low vibrational noise

2.4 The prism assembly

TBD

2.5 Microfluidic Chambers

3 Set-up and Alignment

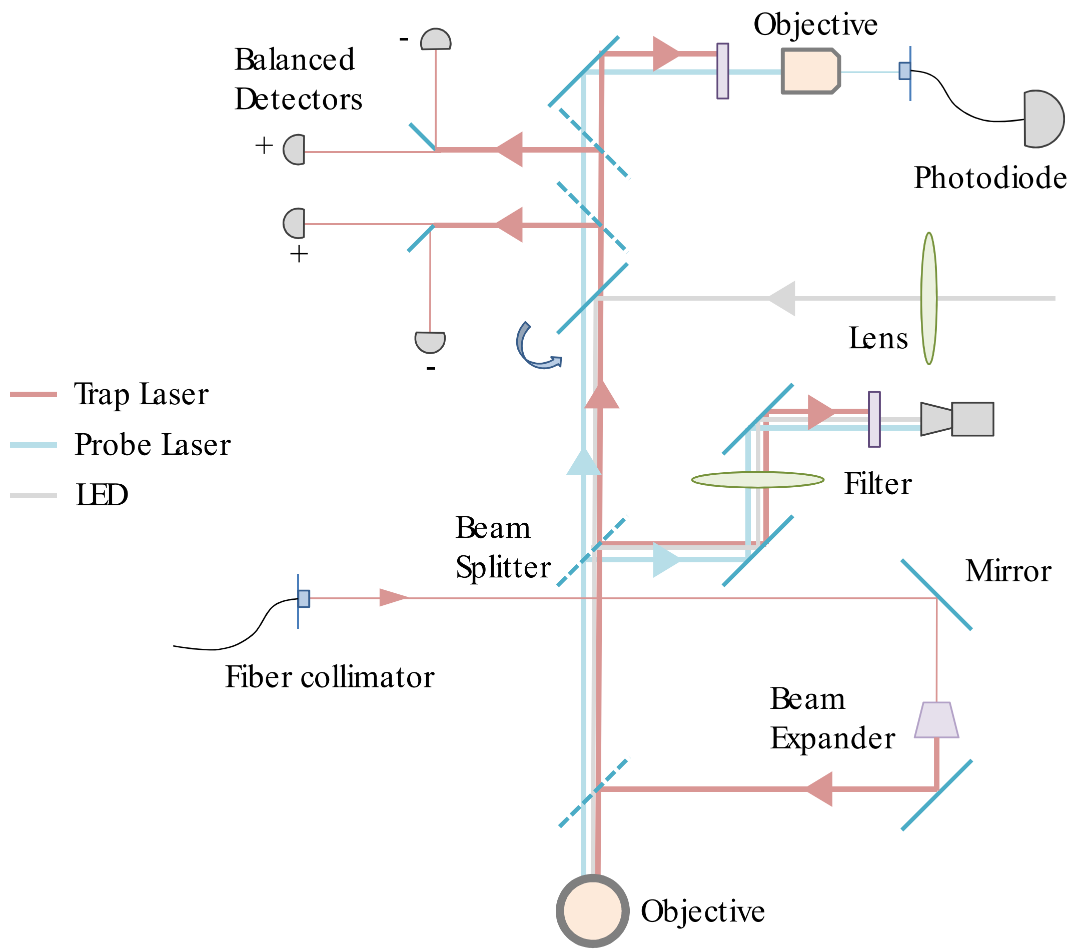

Figure 2: The current optical paths and equipment on the top level of our optical set-up.

3.1 Two-level optics bench

The set-up is built on two levels. Top level is reserved for beam paths to and from the back aperture of the objective (Figure 2) while the bottom level holds the sample stage, optics for accessing the sample back-side, and mounted utility optics such as fiber couplers and the LED. The top surface of the raised breadboard is 11 inches above the optical table top. The optical table is itself floated on four actively-stabilized hydraulic legs for isolation from vibrations of the lab itself, which is on the ground floor of our building.

3.2 45-degree mirror

Besides the objective, the most important piece of optics, which affects the alignment of the whole system, is the 45-degree mirror above the back aperture of the objective, which redirects the laterally traveling beams

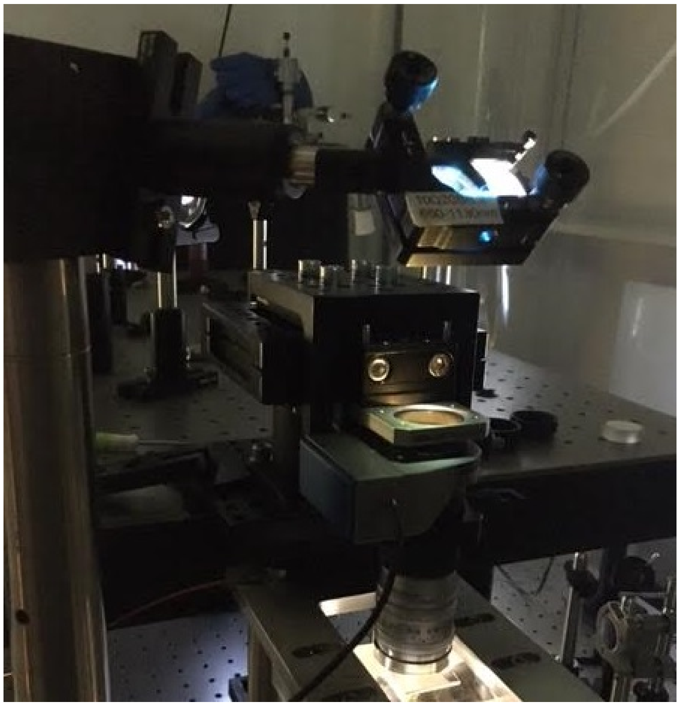

Figure 3: Photo showing the 45-degree mirror in our set-up above the back aperture of the objective.

down into the objective and sample. An improperly aligned 45-degree mirror will introduce stigmation which will negatively affect particle trapping, tracking, and imaging.

To ensure that the mirror is centered properly above the objective and angled at 45 degrees, a temporary alignment beam is set up with the 637 nm fiber-coupled laser and two mirrors. The two mirrors are used to send the laser light straight and flat down the bolt line centered on the objective, using irises as aids. The 45 degree mirror, to be aligned, is inserted into the beam line and placed roughly in order to direct the beam down towards the objective. A mirror laid flat on the back aperture of the objective returns the laser light along the beam line.

The aim is then the following: to overlap the reflected and incident beams by adjusting only the position and tilt of the 45-degree mirror while ensuring that the laser spot is centered on both the 45-degree mirror and the objective aperture. If the objective is mounted squarely, that is, with its back focal plane parallel to the ground, this procedure ensures the proper centering and orientation of the 45-degree mirror.

All other beams will be roughly aligned to this temporary alignment reference beam.

3.3 Imaging LED light

The mounted LED light is about 100 mW at full power. In the current configuration, the LED collimator is set to a distance which produces a beam with a divergence of about 20 degrees. A 2 inch diameter, 500 mm focal length lens (which must be placed out of the central beam path), focuses the LED light into a spot about 0.8 cm in diameter at the back aperture of the objective. The long focal length lens allows the white light to be joined onto the central beam line behind the other beams. Therefore, causes the least power loss, and least disturbance to the more important beam lines for trapping and imaging.

Figure 4: Photo of the LED beam path on the lower level of our optical set-up.

The distance between the LED and the 500 mm FL lens is around 130 cm, and the distance between that same lens and the objective is around 90 cm. The beam starts off on the lower level of the optical set-up and is sent to the upper level by a periscope and two 2 inch mirrors. Only approximately 5 % of the total power of the LED reaches the back aperture of the objective in this scheme. Although this is more than enough power for our needs, a redesign (including a different FL choice for the LED collimator) may dramatically improve power efficiency.

A flip-mounted mirror ultimately joins the white LED light onto the central beam line. However, for initial alignment, it's recommended that a beam-splitter is used instead. A beam-splitter allows the alignment reference beam to be viewed juxtaposed with the white illumination light. Place and orient this beam-splitter such that the reference beam is centered on the white LED spot and they travel collinearly to the back aperture of the objective. This ensures that the white light is also approximately aligned along the optical axis of the objective.

3.4 Imaging and camera

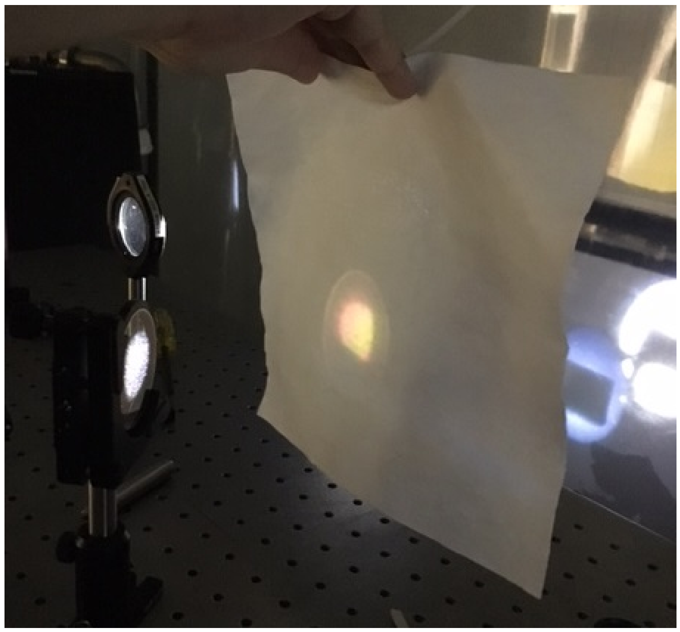

Figure 5: Finding the objective focus without an existing imaging mechanism is possible by monitoring the imaging light reflected from the sample surface. It should reach a maximum when focus is approached.

The objective is infinity-corrected for use with a tube lens of focal length 200 mm, and is coverslip corrected for a #1.5 glass coverslip (170 μm thick). To set-up the correct position of the camera and tube lens, first place the 8% reflective pellicle beam-splitter in the central beam path according to Figure 2. This beam-splitter aims to pick off a portion of the light coming from the objective to send to the ccd camera.

Approach the water-immersion objective to the sample surface while monitoring carefully the LED light coming from the objective lens. The focal height of the objective is determined by the position where the back-reflected beam is brightest, see Figure 6. Direct this beam along the bolt lines to a 200 mm tube lens and ccd camera, placed at the lens focus. Verify that image looks sharp and clean, resembling Figure **??**.

3.5 Optical Tweezer

Fiber couple the 660 nm CW laser (see Section 4). Use the fiber collimator and the 2-5x adjustable beam expander to create a collimated spot with a FWHM of about 3 mm. A screw-on target may be used at the back aperture of the adjustable beam expander, and a set of two mirrors are necessary to direct the beam such that it is passing through the optical axis of the telescope (see Figure 8).

The trap beam is joined onto the central beam path by a 50-50 pellicle beam splitter. The pellicle should be placed and oriented such that the trap beam is collinear with the imaging and reference beams.

At this point, with the imaging system in place, view the trap beam (at low power) under the microscope. The spot should be diffraction-limited, roughly centered in the camera field of view, and spread out symmetrically when de-focused. If the beam is asymmetric, adjust one of the two mirrors after the beam expander.

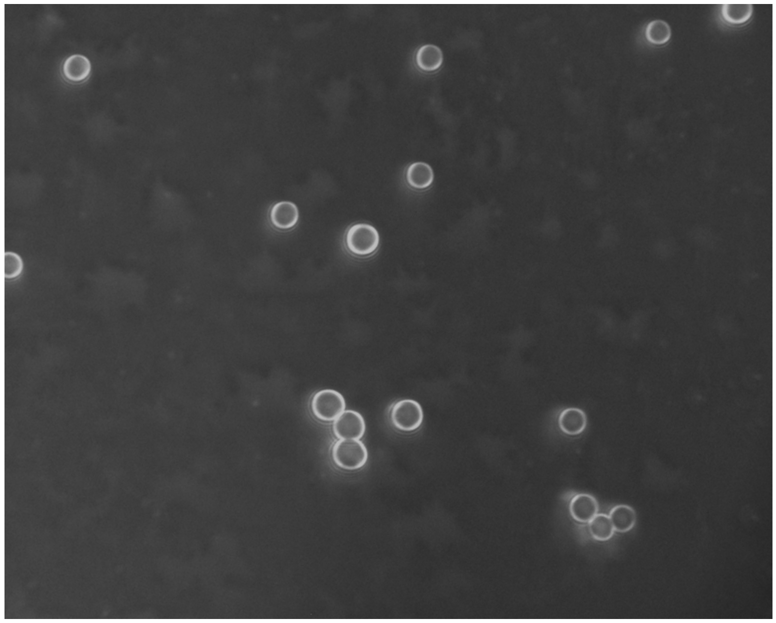

Figure 6: Dark-field image of polystyrene beads in water-filled chamber. Reference for proper focus and resolution.

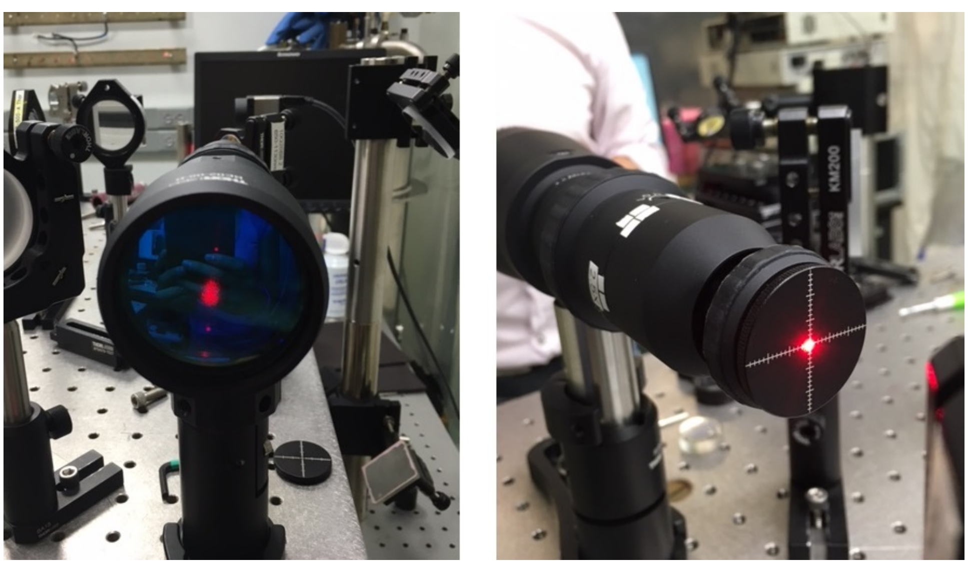

Figure 7: Ensuring that the trap beam goes through the optical axis of the beam expanding telescope minimizes stigmation.

If the beam is off-center in the camera FOV, "walk" the spot towards the desired location adjusting the two closest mirrors. First, adjust the back mirror slightly so that the spot moves in the direction opposite to what is desired. The beam should start to look asymmetric. Then, use the closest mirror (in our case, the 50-50 beam splitter) to restore the symmetry of the beam. Repeat as needed for the two dimensions. When the spot is in the required position, use a semi-transparent lens tissue at the objective back aperture to check that the beam is centered. Also, if necessary, at this point, re-align the imaging beam to the optimized trap beam. This ensures that the LED light is also aligned to the optical axis of the objective.

Place a filter on a flip-mount in front of the ccd camera to block the trap wavelength. Ensure that single beam tweezing is functional by introducing glass beads into the sample chamber.

3.6 Confocal Detection Set-up

4 Fiber Coupling

All free space lasers are fiber coupled on our set-up. Fiber coupled lasers can easily be moved and swapped, facilitating alignment and troubleshooting. In choosing a fiber/lens pair for single-mode coupling, one aims to match the focused beam spot diameter and the mode field diameter of the fiber. The appropriate optics have already been chosen on this set-up. 50% coupling should be achievable.

Fiber coupling can be thought of as a three-step process, most efficiently performed with two lasers of similar wavelength: the free-space laser you wish to couple, and an already fiber coupled laser. Use two low-drift mirrors on kinematic mounts, for control over all four degrees of freedom of the beam.

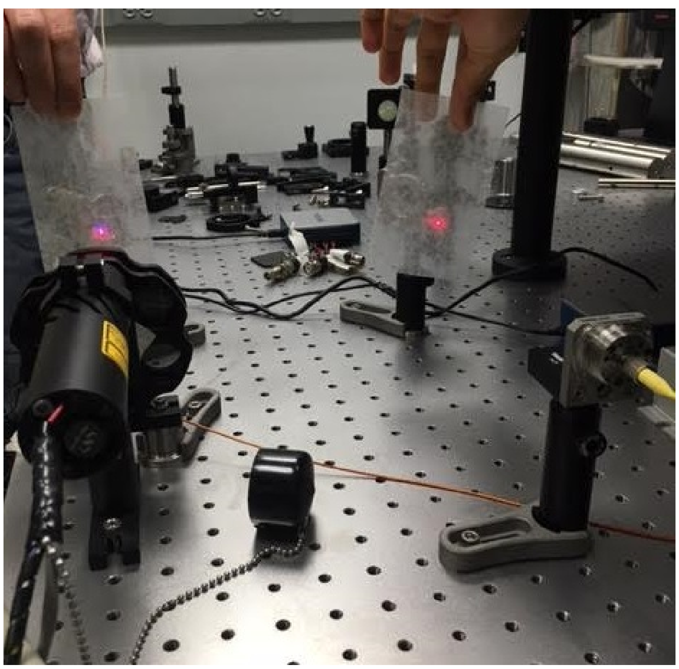

Figure 8: Roughly aligning the output of the guidance beam and the free space laser is essential to start the fiber coupling process. Use two lens tissues to align the spots at two points in the beam path. For each tissue, align the spots using only the farthest mirror.

4.1 Procedure

1. Set up laser and mirrors and fiber coupler so that the beam travels roughly on bolt lines and is reflected at 45 degrees.

2. Attach fiber coupled laser to the fiber port of the fiber coupler, so that a guidance beam comes out of the fiber and travels backwards along the intended beam path. Turn on the free space laser and overlap the two beams at all points along the beam path. The quickest way to do this is to place a

lens tissue between each laser source and the mirror nearest to it (see Figure **??**. Both beams should be visible on each tissue. Start with one tissue, use the mirror farthest from the tissue to overlap the laser spots on it. Iterate, alternating tissues, and the beams should converge.

3. Once rough alignment is established, there should be some light coming out of the fiber coupler. Remove fiber-coupled laser and attach a new optical fiber (FC/PC connector). Connect the free end of fiber to an optical power meter with an FC/PC adapter. Turn on free space laser, and record the power measured. Walk the beam using the two mirrors until the power is maximized:

   - Adjust each of the four degrees of freedom of the mirrors individually, maximizing the power by moving one at a time.
   - Adjust the two lateral degrees of freedom on the two mirrors simultaneously. Similar to fixing stigmation, move the first mirror in some direction until the power is about 1/3 of maximum, and restore the beam's alignment with the second mirror and find the new maximum. If the new maximum is higher than the previous, continue walking beam in the same direction. If not, change directions. Repeat with vertical dimension.

4. Adjust the internal degrees of freedom of the fiber coupler. In the case of the compact fiberport fiber coupler from Thorlabs, this is achieved by alternately adjusting 5 screws in the back of the coupler. In the objective coupler, the longitudinal degree of freedom is separated from the others, and should be only one to be adjusted.