Manual: Optical Forces Set-Up Build and Alignment

Lulu Liu

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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.

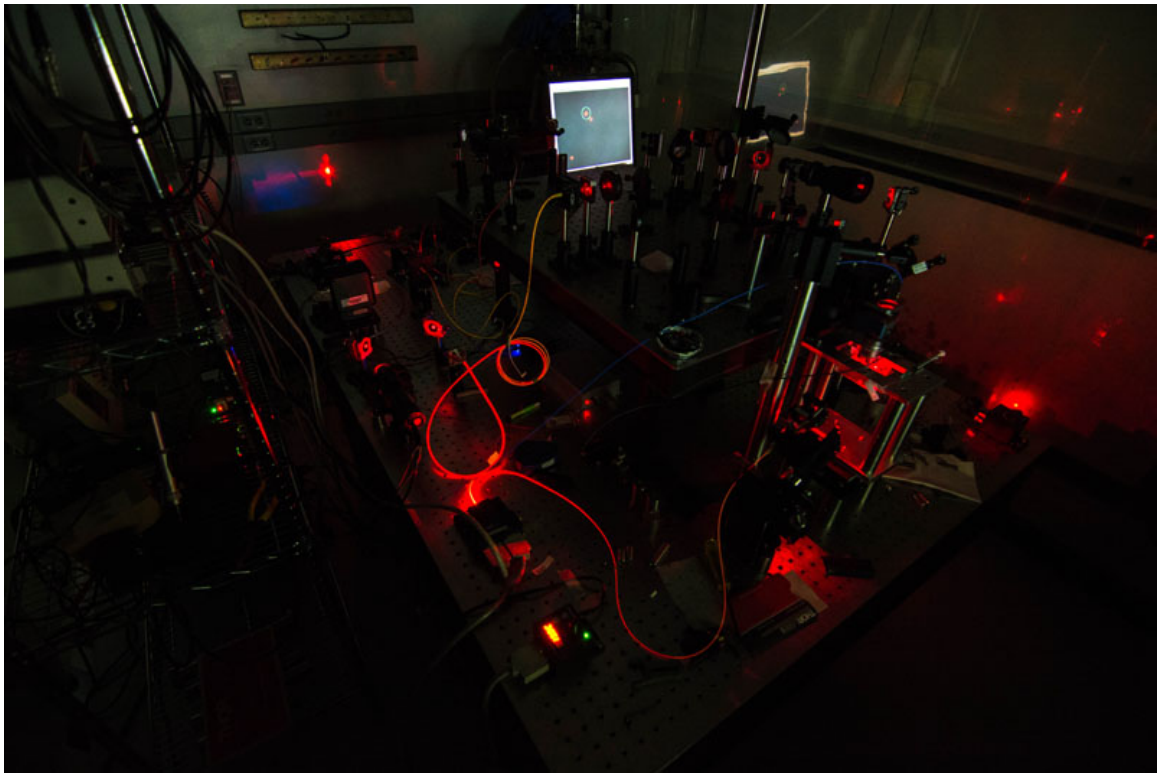

Figure 1: Photo showing the optical forces set-up in operation. Photo credit: Arman Amirzhan

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

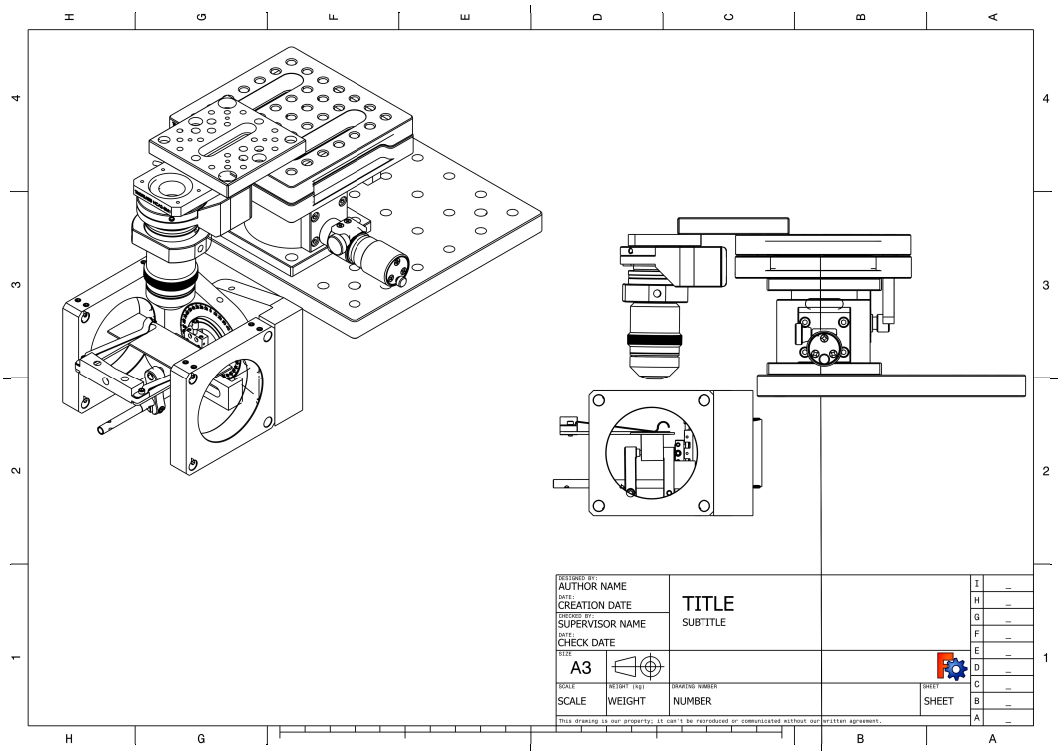

Figure 2: Version 2 of the objective and prism assembly. For a list of parts please see Tables 2.3 and ().

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.

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

3 Set-up and Alignment

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 3) 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 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.

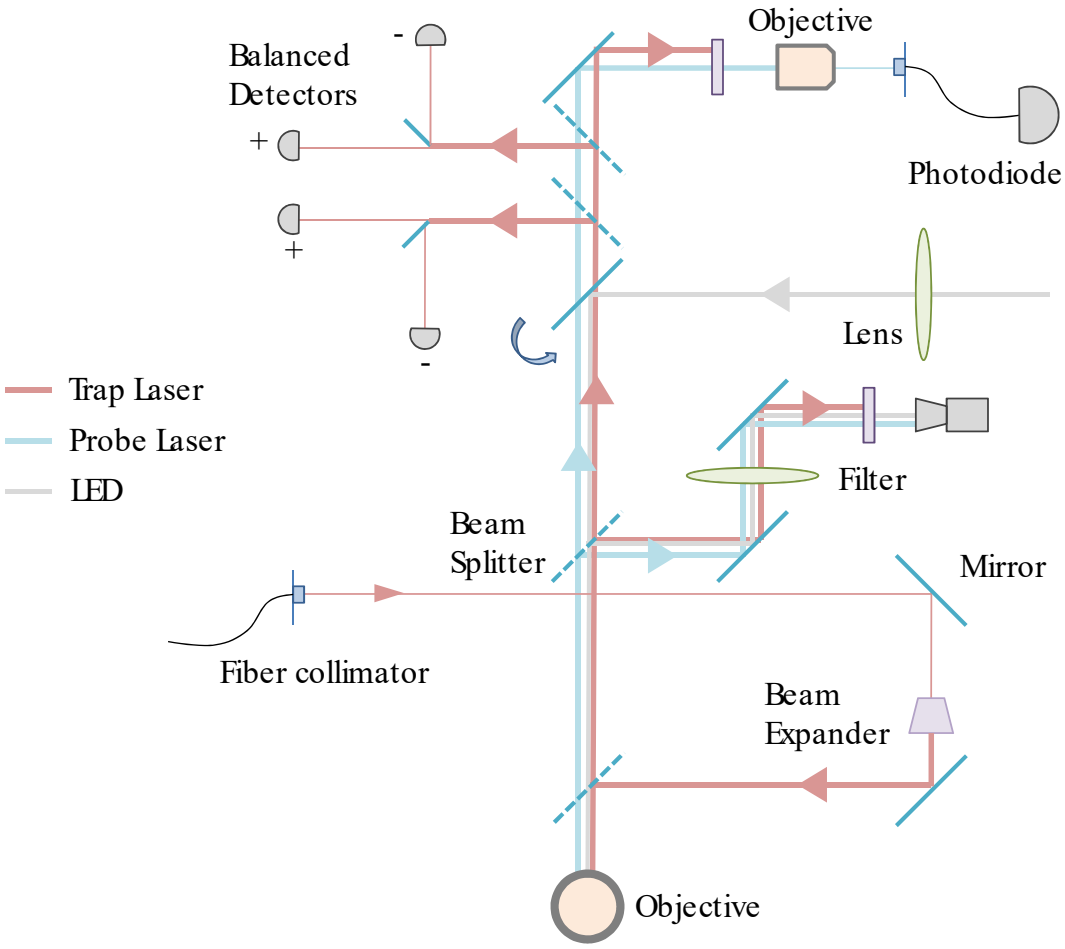

Figure 3: The current optical paths and equipment on the top level of our optical set-up.

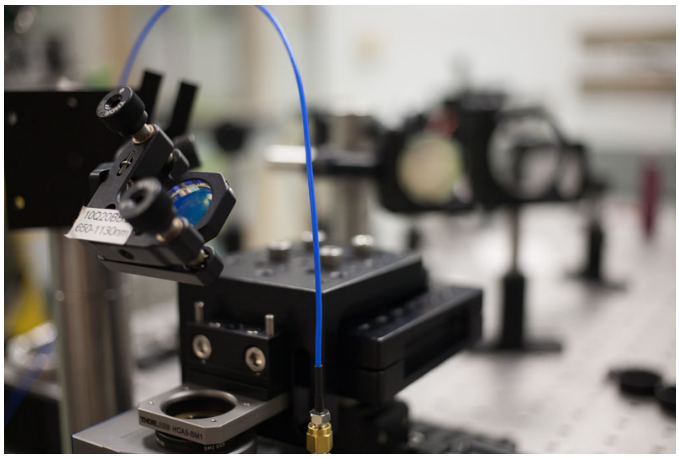

Figure 4: Photo showing the 45-degree mirror in our set-up above the back aperture of the objective.

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.

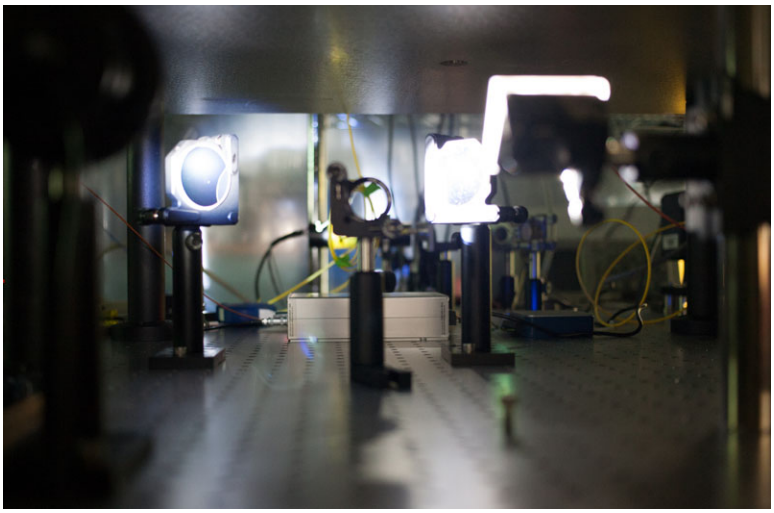

Figure 5: Photo of the LED beam path on the lower level of our optical set-up.

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

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 3. 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 7.

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

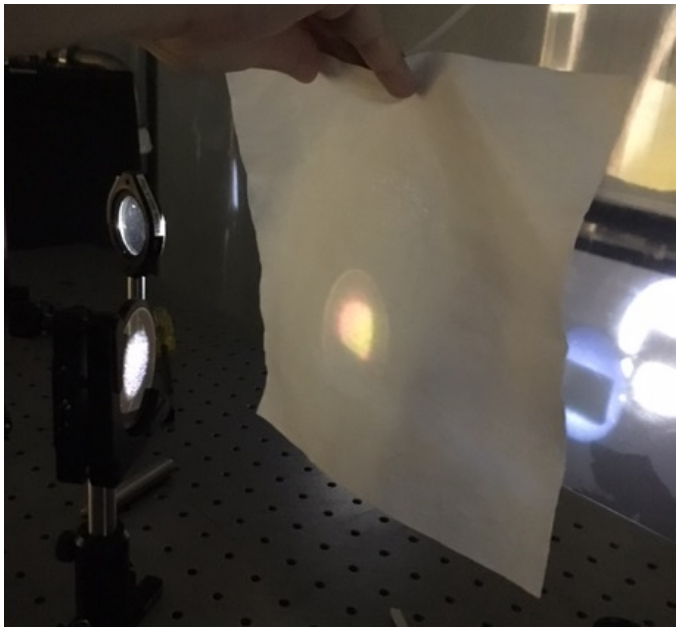

Figure 6: Finding the objective focus without an existing imaging mechanism is possible by monitoring the imaging light reflected from the sample surface. It should reach a maximum when focus is approached.

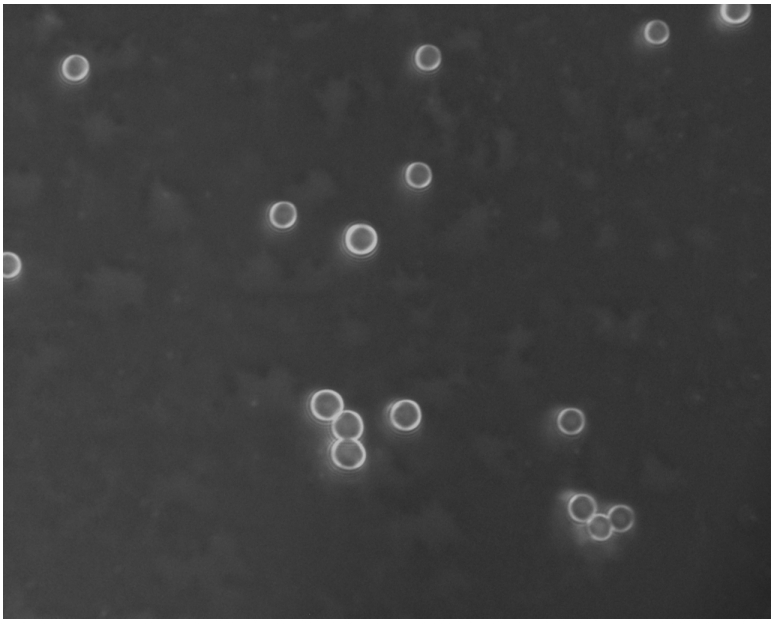

Figure 7: Dark-field image of polystyrene beads in water-filled chamber. Reference for proper focus and resolution.

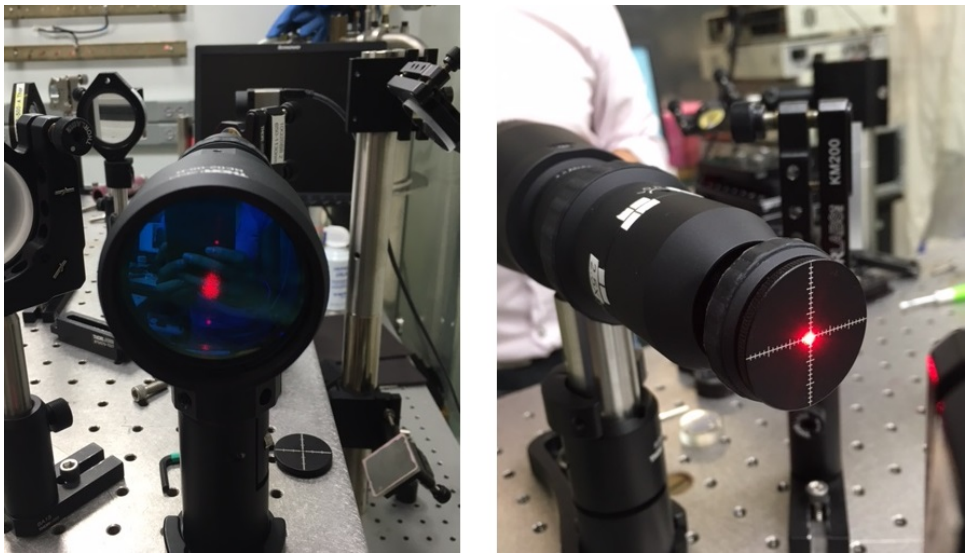

Figure 8: Ensuring that the trap beam goes through the optical axis of the beam expanding telescope minimizes stigmation.

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.

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 The Totally Internally Reflecting Probe Beam

The probe beam, pictured in Figure 3, is a fiber-coupled 637 nm laser. It needs to have access to the bottom-side of the sample chamber to totally internally reflect off the glass-water interface. The evanescent field created, at the position of the trapped bead, offers highly sensitive detection of particle motion perpendicular to the surface.

The probe beam, after exiting the fiber, is focused by an adjustable collimator to a spot of around 300μm in diameter. It must pass through a half-wave plate (to rotate linear polarization direction), followed by an ND filter, before reaching the surface of the prism. To hold all optics in place, a 30mm cage system is used and mounted on a rotation platform for angle adjustments and two translation stages for lateral and vertical adjustments.

To align the probe beam, begin by finding horizontal. Use an iris set at a fixed height or a ruler to ensure the beam is traveling parallel to the table. Mark the spot on the rotation stage as 0°. Rotate to 30° to horizontal, the beam should now be perpendicular to the surface of the prism. Align probe beam assembly such that beam spot overlaps its own reflection from the prism.

Place a sample chamber (full of water) and lower the water-immersion objective until the sample is in focus. Under white light illumination, you should be able to see (looking from below through the prism) a

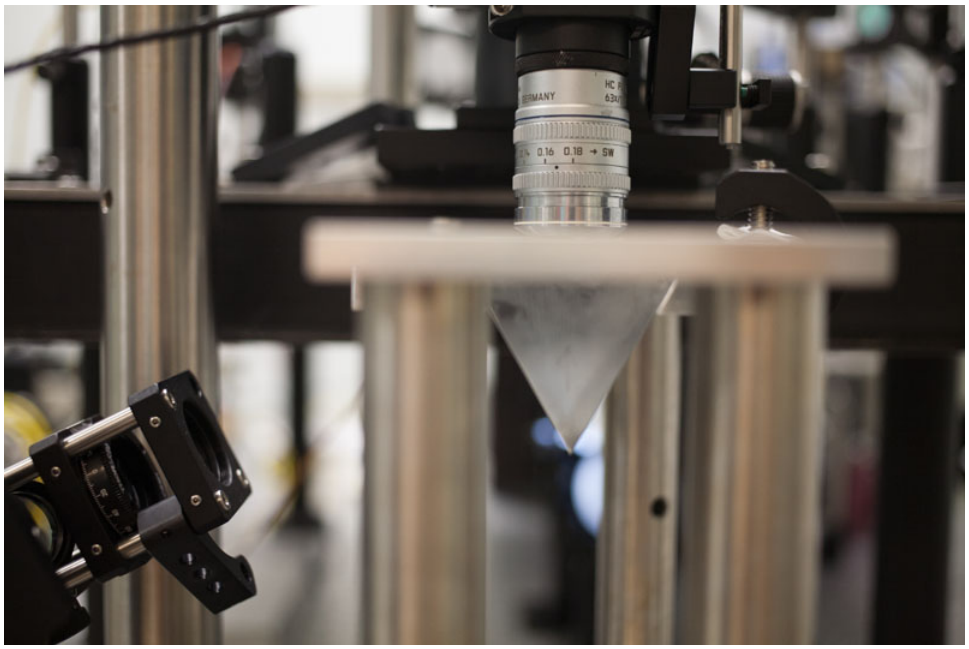

Figure 9: Probe beam entering the prism on the backside of the sample chamber.

white spot where the LED light is focused onto the sample surface. This is the position where you will aim to align the probe beam. Without adjusting angle, using only the translation stage in the two dimensions, move the probe beam until a spot is visible in the chamber from light scattered at the glass-water interface. You will often see two dimmer spots on either side of this brighter spot, from scattering at the imperfectly index-matched fluid at the sample-prism interface.

When alignment is achieved, particles in the field of view of the objective should begin to glow red in the microscope image. However, the probe light is most likely not totally internally reflective. To check for total internal reflection, trap a bead and begin to raise it using the optical tweezer, lifting it away from the surface. If the scattered probe light diminishes quickly to zero (with displacement less than few microns), the bead is scattering light from an evanescent field. Otherwise, the bead is scattering light propagating in the watery chamber.

If an evanescent field is not produced, "walk" the probe beam towards the critical angle. Alternately move the rotation stage and the vertical translator to keep the spot in the field of view at all times. Repeatedly check for total internal reflection using the method above.

3.7 Confocal Detection Set-up

With the scattered probe light visible in the camera, it's time to set up the photo-receiver which will collect this light. We chose a confocal collection scheme (or, an apertured collection scheme) to cut down on background noise by limiting the collection area on the sample surface to a diameter of around 4μm.

The most straight-forward way of aligning the confocal sensitivity region, is, as usual, by sending light backwards through this system. The probe light (637 nm) is end-to-end coupled into output of the multi-mode collection fiber. The trap light, reflecting from a mirror in the back focal plane of the objective, can be used as a reference to roughly align to.

When rough alignment is achieved, the light from the multi-mode fiber should form an image on the sample surface. The image should be a speckled circle about 4μm in diameter. Slightly defocus the objective and observe how the circle expands. If it expands asymmetrically, move the two confocal mirrors to remove the stigmation. Now, using the method discussed in Section 3.5, walk the collection area toward the optical trap until the two overlap, keeping the stigmation minimal.

Remove the in-coupling probe light from the end of the multi-mode fiber, attach the fiber to the photoreceiver, and replace the probe beam in its assembly. There should now be signal in the photoreceiver. Move the two confocal mirrors in two directions to "center" the signal in the collection area (the region of

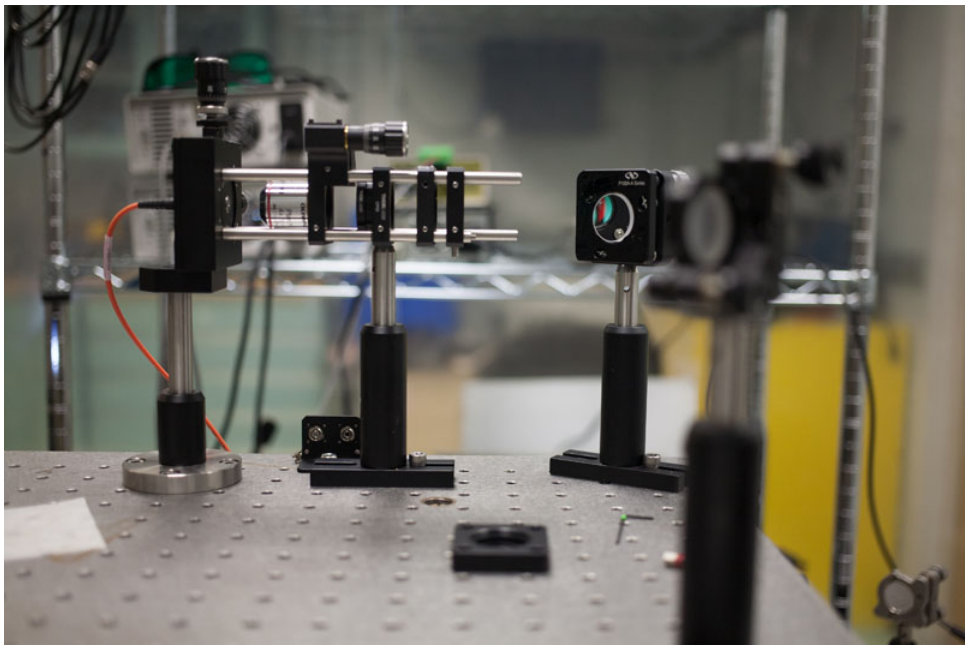

Figure 10: Photo showing the confocal collection scheme. A 4x objective is used to focus bandpass-filtered light from the sample onto a 60 μm diameter optical fiber. The output of this fiber is read by a photo receiver.

max sensitivity should be flat).

Optically tweeze a particle and collect the power spectrum of its motion in the vertical direction. One dimension of particle tracking is in place, two more to go.

3.8 Balanced Detection Set-up

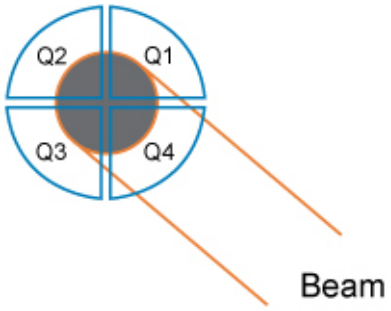

Figure 11: Quadrant photodiode. Image from Thorlabs.com.

Lateral position tracking relies on measuring the deflection of the back-scattered trap beam when a particle is present. Often, a quadrant photodiode is used for 2-dimensional detection (see Figure 11. In our case, we use two balanced detectors sensitive to the two orthogonal directions to make this measurement.

A portion of the back-scattered beam (picked off by a 50-50 beam-splitter) is divided first laterally in half (with a D-shaped mirror), with each half of the beam sent to a photodiode. The difference between these two signals is recorded in volts, related to the particle's lateral displacement in nm by a proportionality constant, C_0. The remainder of the beam is then divided vertically in half for measurement in the orthogonal direction.

A properly aligned balanced detection system should report a mean displacement (or mean voltage) of zero when the D-shaped mirror is symmetrically dividing the back-scattered beam.

First, identify the back-scattered beam from the trapped bead. See Figure 12.

Figure 12: Back-scattered trap beam from trapped polystyrene microsphere. It's recommended to align the balanced detector with PS microspheres due to larger index contrast.

Use a 50-50 beam splitter to pick off a portion of the back-scattered light ahead of the confocal detection. Use bolt holes to ensure the beam is scattering at 45 degrees from the beam splitter.

Place the D-shaped mirror in the center of the beam so that the full beam is entirely reflected. Direct this beam to one of the input photodiodes of the balanced detector using a half-inch mirror. Turn down the gain on the balanced detector and observe on the oscilloscope the intensity readout of the full beam. Move the mirrors until the signal is maximized. There is a range of acceptable angles since the sensor area will be large compared to the beam spot.

Translate the D-shaped mirror entirely out of the beam path until none of the beam is reflected. Use the remaining two half-inch mirrors to direct this beam towards the second input photodiode of the balanced detector. Maximize the intensity reading in the same way, this time, looking for a maximum signal that is equal to the negative of the first photodiode signal during the previous step.

If the alignment is correct, you should be able to move the D-shaped mirror until it reflects only half the beam. Increase the gain to maximum on the balanced detector and look for a signal with a mean of zero. Place the necessary neutral density filters in order to obtain a peak-to-peak signal in the balanced detector of no more than $\pm$ 3 V.

Note: the balanced detector signal is highly sensitive to environmental noise! Be careful when choosing optical components to optimize for low mechanical vibrations!

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.

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.

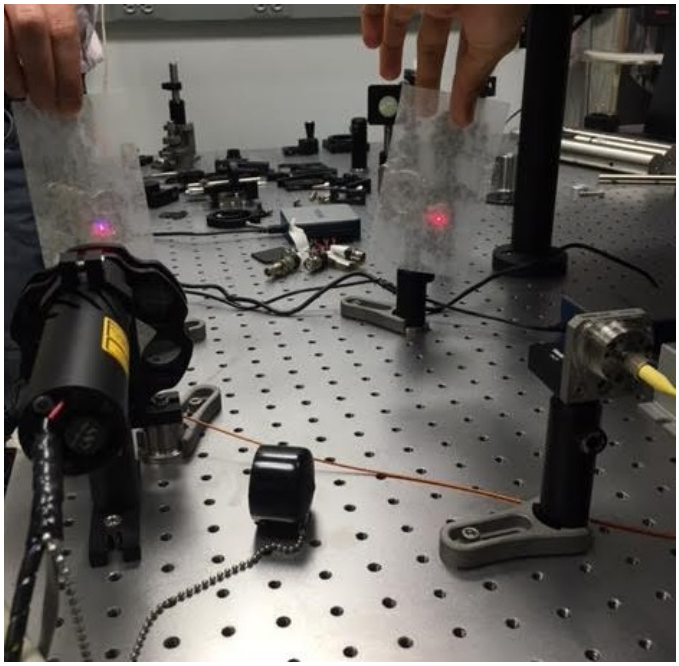

Figure 13: Roughly aligning the output of the guidance beam and the free space laser is essential to start the fiber coupling process. Use two lens tissues to align the spots at two points in the beam path. For each tissue, align the spots using only the farthest mirror.

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 13. 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.

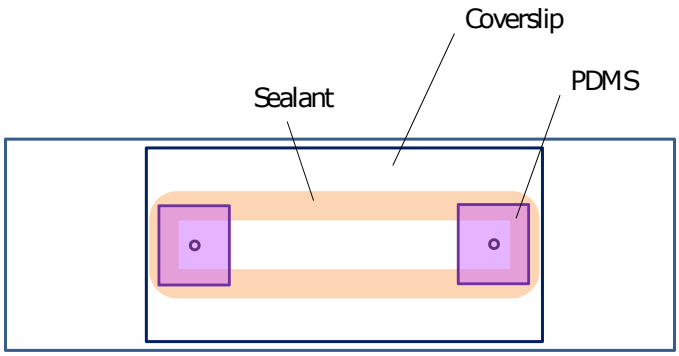

Figure 14: Fully constructed microfluidic sample chamber.

Part	Description	Vendor
BK7 Glass Slide	75mm x 25mm x 1mm	VWR
Glass Coverslips 1.5H	60mm x 24mm x 0.17mm	Schott Nexterion
Meltonix Sealant	25 um thick	Solaronix
BD-309628	1 mL Syringe w/ Luer-Lok Tip	BD / VWR
Z192481-100EA	BD PrecisionGlide Needle 21 gauge	Sigma-Aldrich
EW-06406-60	FEP Tubing 1/16in OD	Cole Parmer
PDMS	-	Soft Materials Clean Room

Table 2: Parts for microfluidic sample chamber.

Figure 15: Coverslip with laser drilled holes.

5 Microfluidic Sample Chambers

5.1 Parts

5.2 Procedure

1. Deposit AR coating on BK7 slides. Use NEXX PECVD tool in CNS clean room. The target index is 1.445, target thickness (for 660 nm AR in water) is 112 nm. According to the current process on the tool, the deposition time should be 10 minutes and 40 seconds, corresponding to a rate of 10.5 nm/min.

2. Drill Holes in 1.5H Coverslips. Use the VersaLaser in G06 and the template saved under LuluLiu_Coverslip. The settings should be, after focusing the laser, 31.5% power, 48% speed, 165 PPI, 1mm Z axis, and the cut should be performed four times for each hole.

3. Cut Solaronix Sealant into shape, using a razor. The sealand should not exceed 60 mm in length or 24mm in width.

Figure 16: Sealant cut into shape.

4. In a hot press, gently press the sealant between coverslip and slide. Careful not to crack the coverslip. Align holes on inside of channel. The press should be applied for 20 minutes minimum at 110C.

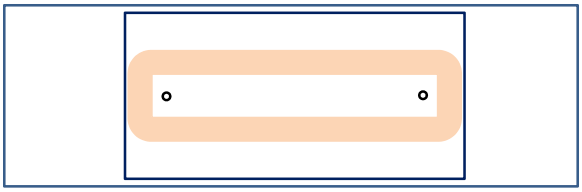

Figure 17: Assembled chamber.

5. Attach PDMS to inlet and outlet. First, punch 1.5 mm holes in PDMS cubes of 1 cm by 1 cm. Sonicate assembled chamber and PDMS blocks in isopropyl alcohol for 10 minutes. Dry afterwards in 60C oven for 3 hours to ensure alcohol is completely evaporated. Bond PDMS to coverslip using plasma oxidizer in soft materials clean room. The power is 25W and the exposure time should be 10s. Read manual for potential bonding procedure updates.