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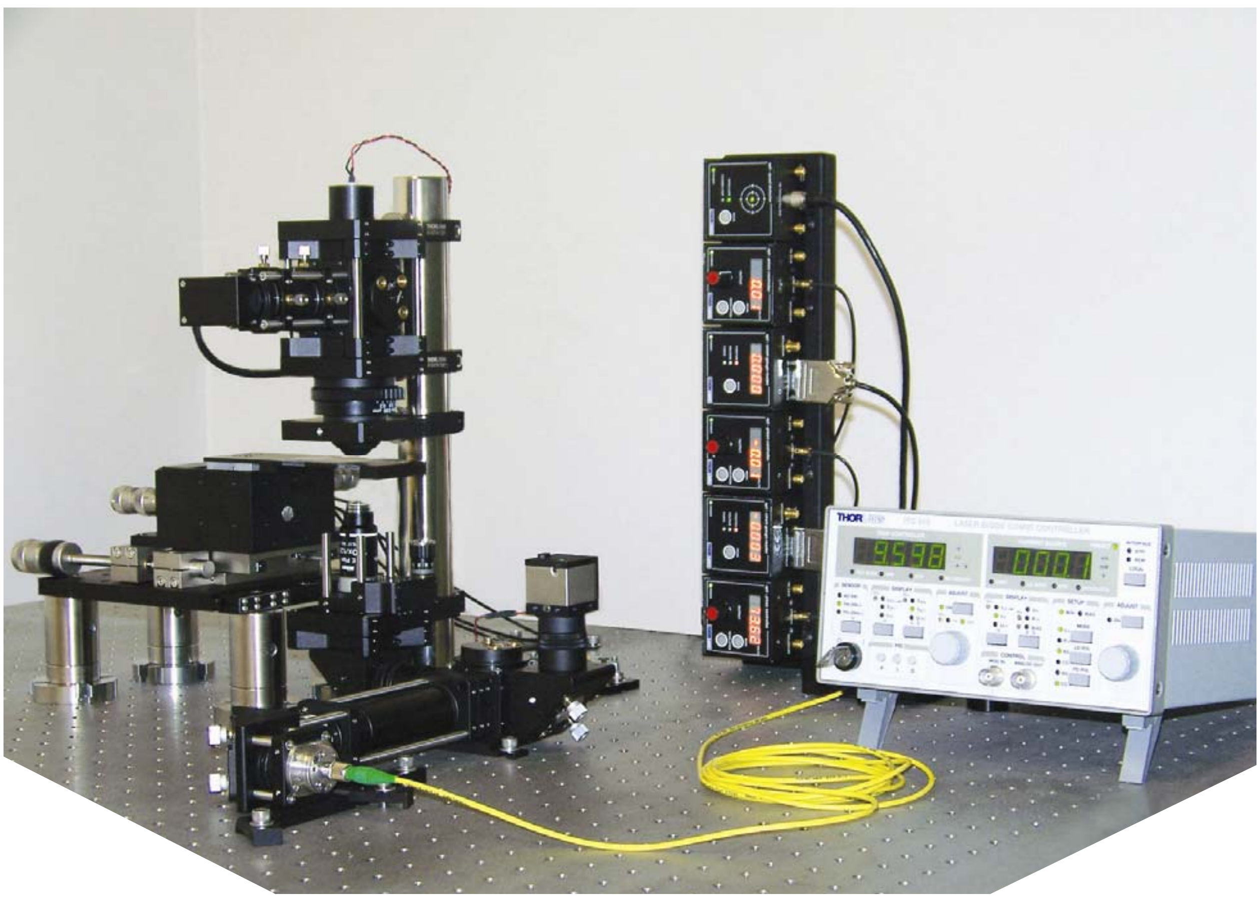
Optical Trap Application Setup



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1. Introduction

In 2007, three researchers in the Department of Biological Engineering at MIT – Steve Wasserman, David Appleyard, and Matthew Lang – built an optical trapping setup for use in teaching labs. Their results were published in the American Journal of Physics [S. Wasserman, D. Appleyard, and M. Lang, *Optical Trapping for Undergraduates*, Am. J. Phys. **75**, (2007)]. Based on their design, Thorlabs has collaborated with the aforementioned authors to design an OTKB optical trapping kit that includes all necessary components and provides the same capabilities. Moreover, since Thorlabs' components are designed to be compatible with each other, the OTKB optical trapping kit is easily modified to provide additional functionalities as your research needs evolve or change with time. For example, users who wish to add the ability to steer / scan the optical trap position can replace the mirror mount before the first relay lens with a 1- or 2-D Galvo scanning mirror. The 2D galvo scanning mirror modification to Thorlab's optical trap kit has been implemented and tested. Alternatively, an excitation light source can be easily added by incorporating a beamsplitting cube into the beam path.

All of the components needed to construct a fully functional optical trapping system are included in this cost effective kit (OTKB contains imperial components while OTKB/M contains their metric counterparts). Previous versions of the OTKB system incorporated a BE02M-B beam expander; this manual, however, describes the latest version of the OTKB kit, which uses two achromatic doublets, AC254-060-B and AC254-150-B, to modify the beam diameter. This choice offers added steering benefit. At the same time, the AC254-150-B lens is used as the imaging lens, making the CCD camera segment more compact.

2. Setup Description

Optical Trapping Kit OTKB (OTKB/M)

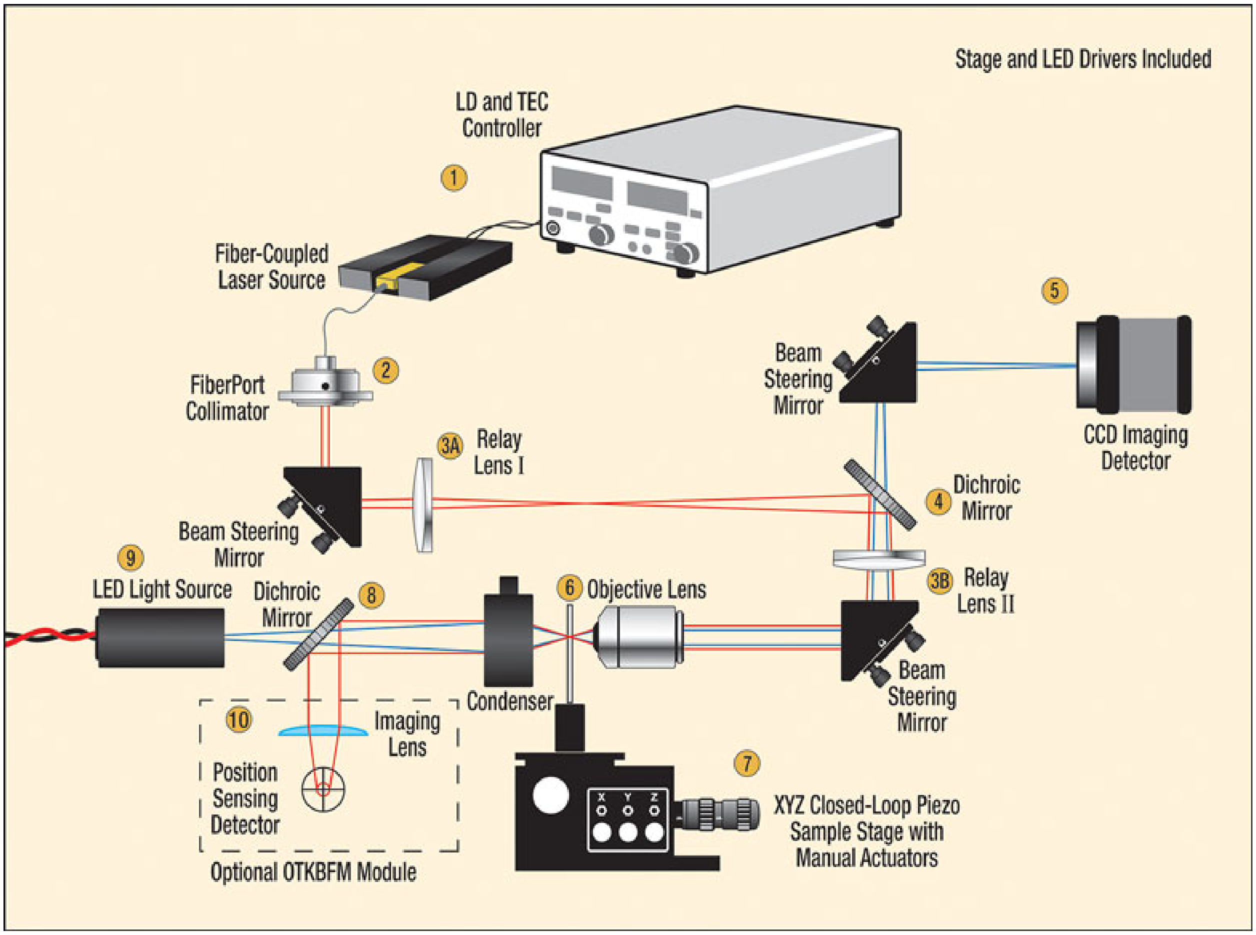
Like many optical trapping systems, this one is based on an inverted microscope design. The structure of the inverted light microscope is constructed using Thorlabs' 60mm cage system, which is supported with a damped ∅1.5″ post (Item # DP14). All of the parts discussed in this section are included in the OTKB kit unless otherwise noted. Some of the parts mentioned below are not universal so the metric version of the part is used in the OTKB/M kit. See the Bill of Material in section 9 of the manual for the complete contents of the OTKB and OTKB/M kits.

1) The 980 nm trapping laser source (Item # PL980P330J) is a pigtailed Fiber Bragg Grating (FBG) stabilized single mode laser diode in a hermetically sealed 14-pin butterfly package. The integrated TEC element and thermistor in the butterfly package allow the temperature of the laser to be precisely controlled when mounted in the LM14S2 laser diode mount and controlled using an ITC510 TEC and Laser Diode Current Driver. This laser, mount, and controller combination was chosen to ensure that the output power (330 mW max) of the laser will be extremely stable, which is important to maintaining a constant trapping force.

2) The FiberPort (Item # PAF-X-7-B) collimates the output of the trapping laser. The FiberPort is a versatile collimator since it allows the aspheric collimation lens to be precisely positioned along 5 axes (X, Y, Z, Pitch, and Yaw). For polarization sensitive applications, the keyway on the FiberPort can be rotated about the optical axis so that the orientation of a linearly polarized collimated beam can be set.

3) The two achromatic doublet relay lenses, AC254-060-B and AC254-150-B, expand the collimated trapping laser beam by a factor of two so that it is approximately 5 mm in diameter. In addition, the relay lenses image the rotation point of the first right angle mirror (Item # KCB1) onto the back aperture of the objective lens so that the KCB1 can be used to position the optical trap. The back

aperture of the focusing objective is 5 mm in diameter, which means that objective is not over filled. However, the stiffness of the trap is still excellent. The second relay lens (3B) also serves to image the sample on the CCD Imaging Detector.

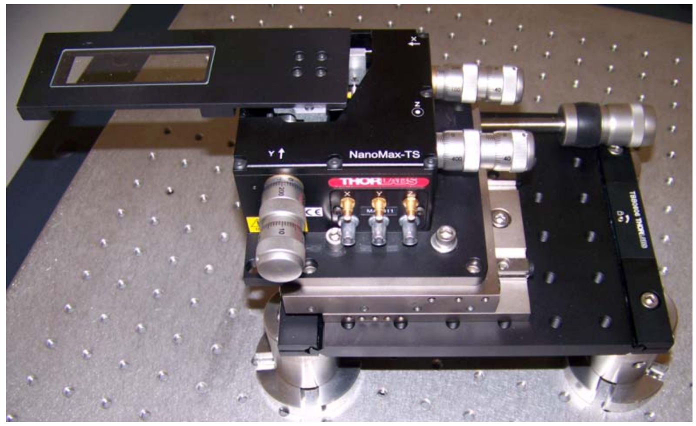


4) The dichroic mirror reflects 980 nm light (trapping source) while passing visible light.

5) Visible light from the LED light source (see segment 9) illuminates the sample and is then imaged on the 1280 x 1024 pixel color CCD camera (Item # DCU224C). The dichroic mirror in the light path in combination with a short pass filter prevents backscattered light from the 980 nm laser from saturating the CCD detector.

6) A 100X oil immersion Nikon objective lens (CDI4390) is used to focus the 980 nm laser beam down to form the optical trap. The calculated diffraction limited trap diameter is 1.1µm. A Nikon Condenser (CDI4391) collimates the beam after the optical trap.

7) The sample stage consist of a microscope slide holder mounted to a 3-axis (X, Y, and Z) translation stage that is mounted on a 1-axis long travel translation stage, which results in the following capabilities:
   A) 2" (50 mm) of travel perpendicular to the beam path. This makes it easy to load the sample and coarsely position it near the trap.
   B) 4 mm of travel in the X, Y, and Z directions using the coarse knobs (0.5 mm/rev) on the 3-axis stage actuators.
   C) 300 µm of travel in the X, Y, and Z directions using the differential knobs (50 µm/rev) on the 3-axis stage actuators.
   D) 20 µm of travel in the X, Y, and Z directions using the piezo actuators (20 nm resolution without using feedback from the internal strain gauge sensors, 5 nm resolution using the internal strain gauges for positional feedback) on the 3-axis stage. Three T-Cube Piezo Drivers (Item # TPZ001) are included in the kit. (Two T-Cube Strain Gauge Readers (Item # TSG001) are included with the OTKBFM force module.)



The stage assembly is mounted on a translating breadboard TBB0606 which facilitates loading/unloading of samples.

8) The visible light emitted from the LED passes through the dichroic mirror and illuminates the sample while the 980 nm laser beam is reflected down the optional OTKBFM Force Calibration Module. (If the OTKBFM is not being used, the laser is blocked using an SM1CP2.

9) A single emitter white light LED (Item # LEDWE10,10 deg half angle forward radiating). The light from the LED illuminates the sample.

10) The OTKBFM force measurement module contains the hardware needed to calibrate the trap. The back focal plane of the condenser is imaged on the PDQ80A Quadrant Position Detector (QPD) using a 40 mm focal length biconvex BK7 lens (Item # LB1027-B). The detector is a silicon based segmented quadrant position-sensing detector with a rise time of 40 nsec and a bandwidth of 150 kHz. The signal generated by the QPD is sensitive to the relative displacement of the trapped particle from the laser beam axis. As a result the output of the detector can be used to calibrate the position, stiffness, and force of the optical trap. A TQD001 T-Cube Quadrant Detector Reader is included with this module.

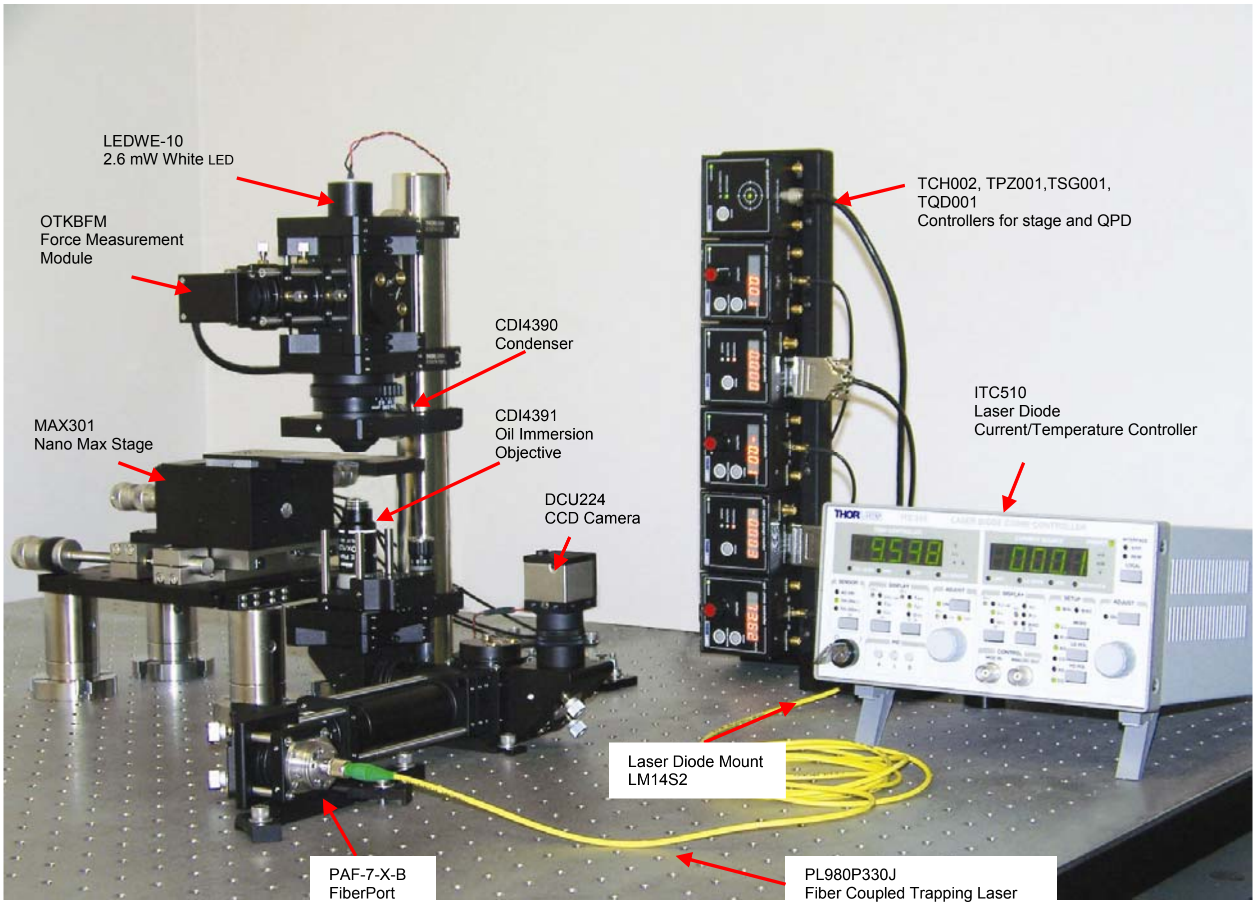


Figure 2.1: Setup with main components labeled

3. Optical Force Measurement Module OTKBFM

The OTKBFM module contains the components that can be used to calibrate the trap using positional detection of the back-focal plane of the condenser. By placing the Quadrant Position Detector (QPD) in a plane conjugate to the back focal plane of the condenser, the signal generated by the QPD is sensitive to the relative displacement of the trapped particle from the laser beam axis. As a result the output of the detector can be used to calibrate the position, stiffness, and force of the optical trap. The detector is connected to the cage cube above the condenser (see picture above). A TQD001 T-Cube Quadrant Detector Reader and two T-Cube Strain Gauge Readers are the main components included in this module. For high bandwidth measurements the QPD signal can be read out from the controller cube directly via a DAQ card (not included).

Figure 3.1: Force Measurement Module OTKBFM consisting of two TGS001, TQD001, PDQ80A and the cage assembly for connecting the detector and optics to the base kit. The shaded controllers and the USB hub TCH002 are included with the base kit OTKB

4. Initial Setup and Alignment

Unless otherwise noted, all of the parts mentioned in this section are included. **Please observe proper laser safety procedures. IR laser beams are particularly dangerous because they cannot be seen. Always wear the appropriate type of laser glasses (not included) when working with laser beams.**

1) Select the correct laser type on the laser diode mount (LM14S2). When used with the fiber laser that is part of the kit (PL980P330J) you will have to put the 'Type 1' PCB card into the LM14S2 mount, please refer to the LM14S2 manual for details.

2) Mount the laser diode in the butterfly controller mount and connect to the laser diode controller (ITC510). Make sure the correct pin style is set on the controller and mount. Set the temperature sensor on the ITC510 to "TH < 20kOhm" and the Laser Diode polarity to 'AG Ground'. Set the current limit to 700mA. Please check individual manuals for controller, mount and diode for additional information.

4.1 Collimating Trap beam from FiberPort

1) Connect the FiberPort (PAF-7-X-B) to the FiberPort Cage Adapter (CP02FP) and mount the Adapter on the optical table using a CPB1 Cage Plate Mounting Base. Secure this assembly to an optical breadboard or table (not included) using two CL6 mounting cleats (if the mounting slots on the CPB1 align with the hole pattern on the optical table they may be used instead of the CL6 cleats).

2) Thread the SM1L03 lens tube into the CP02FP FiberPort cage Adapter.

3) Attach the SM1T2 coupler to the SM1L03 lens tube.

4) Temporarily attach a ring iris (Item # SM1D12D) to the SM1T2 coupler.

5) Temporarily attach two ER6 or ER8 cage rods to the CP02FP (use the top two cage rod holes).

6) Create an eye safe environment prior to turning on the laser and collimating it. In addition, use a low power beam for this alignment step. Turn on the laser and aim the beam at an IR card (not included) on a wall about 5 m away.

7) Adjusts the FiberPort to collimate the beam. (Two people may be required.)

   A) Attach a connectorized fiber source to the bulkhead of the FiberPort and examine the output.

   B) Adjust the X-Y screws to center the output beam in the tilt plate aperture.

   C) Trace the beam away from the FiberPort to check for collimation.

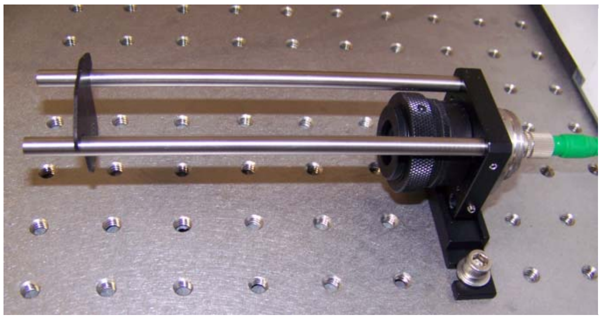
      a) For a converging beam (beam comes to a focus): The lens is too far away from the fiber. Alternately turn the Socket Head Cap Screws (SHCS) clockwise in small, equal increments. **Be sure to adjust all screws in equal increments.**

      b) For a diverging beam (beam diameter continually increases): The lens is too close to the fiber. Alternately turn the SHCS counter clockwise in small, equal increments. **Be sure to adjust all screws in equal increments.**

D) Check the beam path and adjust the X-Y screws as needed to re-center the beam in the output aperture.

E) Use progressively smaller adjustments until collimation is achieved and the desired beam centration is obtained. Do not force the screws past their normal operating range, if collimation is not easily achieved please contact Tech Support for assistance.

8) Ensure that beam is parallel to the cage structure by sliding the alignment plate (Item # VRC4CPT) along the ER6 cage (temporarily attached) rods as shown on Figure 4.1.1. with the iris adjusted to its smallest aperture. The intensity of the beam transmitted through the hole in the alignment plate should be constant as the distance the alignment plate is from the iris is changed.



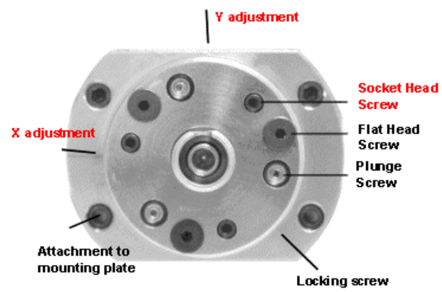


Figure 4.1.1 (Right) FiberPort Alignment Configuration, (Left) FiberPort with adjustment screws.

9) Turn off the laser and remove the ER6 cage rods and ring iris.
10) Attach four ER1 cage rods to a KCB1 right angle mirror mount.
11) Join the KCB1 assembly to the FiberPort assembly. The ER1 cage rods should be secured in the CP02FP adapter and the SM1T2 lens tube coupler should be threaded into the KCB1 right angle mirror mount. The beam path from the FiberPort to the KCB1 should be closed.
12) Insert the PF10-03-P01 mirror into the KCB1 and attach the SM1D12D ring iris used previously to the output port of the KCB1.
13) Temporarily attach two ER6 cage rods to the top two tapped holes on the output face of the KCB1.
14) Turn the laser on to ensure that beam is aligned through the center of the iris on the output port of the KCB1 and along the cage structure using the VRC4CPT. Remove this KCB1 with mirror and put it aside as it will later be used for the coupling to the vertical path of the microscope.
15) Attach the second KCB1 (with a PF10-03-P01 new mirror and SM1D12D ring iris) provided in the kit in the same way and align it.

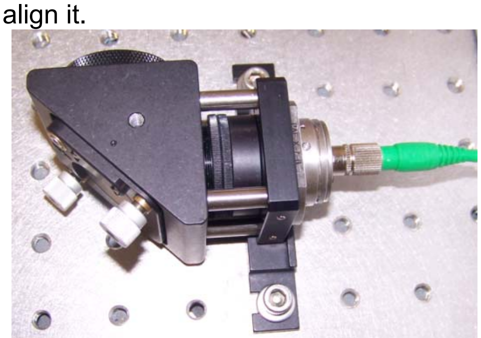


Figure 4.1.2 Adjust Right Angle Mirror Mounts KCB1

4.2 Beam Expander Section

1. Mount a CP02 onto a CPB1
2. Mount the achromatic doublet AC254-060-B into a SM1V10 lens tube and thread it into the CP02 (from the previous step), such that external retaining rings (with the extra SM1NT-1-CT) are on both sides of CP02 as in figure 4.2.1. (Make sure that the lens is mounted in the lens tube so that the collimated beam from the FiberPort is incident on the curved surface of the doublet.)
3. Connect a SM1L03 to a SM1T2, threading it fully in.
4. Attach the SM1L03 from the previous step to the SM1V10 holding the achromatic doublet.
5. Remove one threaded stud from the end of an ER2 cage rod. Repeat with a second ER2 rod.
6. Use two ER2 rods to connect the CP02 to KCB1 as shown in Figure 4.2.1 (rods are diagonally across from each other).
7. Clamp CPB1 attached to the CP02 to table.
8. Adjust the SM1V10 threaded into the CP02 cage plate such that the distance between SM1V05 surface and front surface of KCB1 is 25 mm (important!) and lock in this position by tightening the external retaining rings on both sides of the CP02.
9. Use the SM1T2 between the iris and the achromatic lens to close the beam path. This is a little difficult given the tight space.
10. Fully thread a SM1V05 into an SM1L30
11. Connect the SM1V05 from Step 10 to the SM1V10 on the opposite side of the CP02 mount that the achromatic lens is.
12. Thread a SM1D12D ring iris into a CP02 cage plate.
13. Thread the SM1L30 lens tube from Step 10 onto the SM1D12D. To get the CP02 cage plate oriented correctly, you may unthread the SM1L30 from the SM1V10 a little (Step 10).
14. Connect an ER4 cage rod to an ER1 cage rod to produce a 5″ long cage rod. Repeat so that you have two 5″ cage rods. Remove the threaded studs from the ends of the 5″ cage rods.
15. Use the 5″ cage rods to hold the CP02s from Steps 1 and 10 together as shown in figure 4.2.1. The cage rods are located diagonally across the cage from each other. Note that part of the ER4 + ER1 combination will extend beyond CP02 from Step 10.

Figure 4.2.1: Building beam expander segment

16. Mount a CP02T Cage Plate on a CPB1 Cage Plate Mounting Base
17. Close off the bottom of a C6W cube using a B1C.
18. Using an SM1T2 connect a new KCB1 with a new PF10-03-P01 mirror to the right side of the C6W. This is not the one prepared in Step 14 of Section 4.1. Adjust the KCB1 such thate the free port of the KCB1 faces upward.
19. Connect the C6W to the CP02T from Step 16 using 2 ER1 cage rods with the threaded studs removed. The cage rods should be located diagonally across the cage from each other. Do not use the same orrientation that was used in Step 15. Only a small part of the cage rods should extend into the C6W, otherwise the rods may not allow you to freely rotate the FM01 mirror after it

has been installed inside the C6W cube. The cube face attached to the CP02T should be perpendicular to the face that the KCB1 was attached to.

20. Connect assembly create in Steps 16 through 19 to the existing assembly by attaching the CP02 to the CP02T.
21. Temporarily attach two ER6 cage rods to the C6W on the opposite side of the cube as the completed lens tube assembly. Use the upper mounting holes.
22. Use the iris nearest the cube and a VRC4CPT alignment guide resting on the ER6 cage rods to ensure that the beam is still aligned to the center of the cage system.
23. Mount the FM01 hot mirror into a B5C.
24. Mount the B5C onto a B4C
25. Insert the B4C into the top of the C6W and coarsely align it so that the beam is reflected 90˚ to the left.
26. Remove the temporary ER6 cage rods and close the port on that face of the C6W cube using a SM1CP2
27. Mount the second AC254-150-B achromatic doublet into a SM1L10 lens tube.
28. Attach the SM1L10 to the C6W cube on the face of the cube that serves as the exit port for the laser reflected off of the hot mirror. (The beam reflected from the hot mirror should be incident on the flat side of the achromatic lens.) Note that the distance between the two achromatic doublets must be the sum of their focal lengths (or 210mm in this case).
29. Thread a SM1V05 completely into the SM1L10 from Step 27.
30. Temporarily attach two ER6 or ER8 cage rods to this (Step 27) side of the C6W and place the cage system VRC4CPT alignment guide on the rods.
31. Adjust the B4C so that the reflected beam is aligned to the center of the cage system. Then use a SM1D12D connected to the SM1V05 and ensure that beam is centered through iris and alignment plate. Remove the ER6 cage rods and SM1D12D.

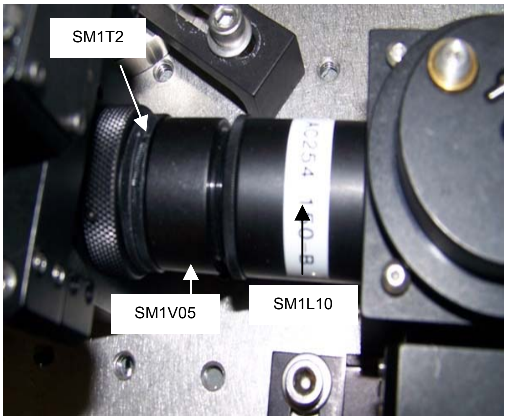


Figure 4.2.2: Assembly between the cage cube and the vertical segment that contains the second relay lens.

4.3 Vertical segment

Figure 4.3.2: The vertical segment during alignment.

1. Attach a C1500 clamp to an LCP01 (ensure that the C1500 face is parallel to the LCP01 before locking) and slide-hold to the DP14.
2. Attach an SM1D12D to the previously adjusted KCB1 from step 14 of Section 4.1.
3. Fully thread an SM1T2 to the SM1D12d and use one of the locking rings to lock to SM1D12D.
4. Slide this KCB1under the LCP01-C1500 combination (that has been lowered on DP14) such that the SM1D12D and SM1T2 face the SM1V05 that has been connected to the SM1l10 holding the AC254-150-B as in step 28 of Section 4.2
5. Connect the SM1T2 from step above to the SM1V05 from Step 29 in section 4.2. The SM1V05 will unthread from the SM1L10 as it threads onto the SM1T2. Lock it in place using the locking ring on the SM1V05.
6. Lock the C1500 to the DP14.
7. The DP14 Post can be locked down to the table along with the CPB1 bases. Use CL6 table clamps when the holes in the bases do not align.
8. Attach four ER03 cage rods on the side of the KCB1 that is facing vertically.
9. Stack an LCP02 on top of an LCP01 and connect them using four ER01 cage rods (remove the threaded studs from both ends of the cage rods). Note that the 4 ER03 rods will slide though LCP02 when the set screws have been slightly released.
10. Attach an SM1D12D to the LCP02.
11. Temporarily attach four ER8 cage rods to the ends of the four ER1 cage rods used to hold the LCP01 and LCP02 plates together.
12. On the other end of the ER8 cage rods attach an LCP02.
13. Attach an additional SM1D12D iris to the upper LCP02.
14. Use this arrangement to align the beam to the center of the vertical cage segment. Make sure you use protective glasses when doing this. The B4C is used to align the beam through the lower diaphragm while the knobs on the KCB1 are used to align the beam through the upper diaphragm. Neither should require much adjustment but the B4C should be used to optimize the alignment through the lower iris while the KCB1 is used to optimize the alignment through the upper aperture.

Centering of the beam along this path is very critical. A Thorlabs power meter (not included) with an SM1 threaded measurement head (e.g., PM100D with S121C) will allow you to verify the alignment by maximizing the transmitted power when both apertures are nearly closed. As a guideline, using the 330mW laser PL980P330J, at a drive current of 100 mA and the diaphragms 8˝ apart, both SM1D12D closed, the measured power should be around 0.7 mW.

15. Thread the SM1A10 C-Mount to SM1 adapter onto the CDI4390 Nikon objective.
16. Now mount the objective on to the SM1Z cage translator so that both the objective lens and the adjuster on the translator point upwards.
17. Remove one ER8 rod, where the SM1Z adjuster is situated and slide the SM1Z onto the ER3 cage rods from Step 7 as shown in Figure 4.3.3.

As a guideline, with the S121C sensor placed about 0.5˝ above objective (see Figure 4.3.3), with diaphragm below objective fully open, the measured power should be around 6mW or more when driving the PL980P330J with 100 mA of current (14 mW at 130 mA drive current).

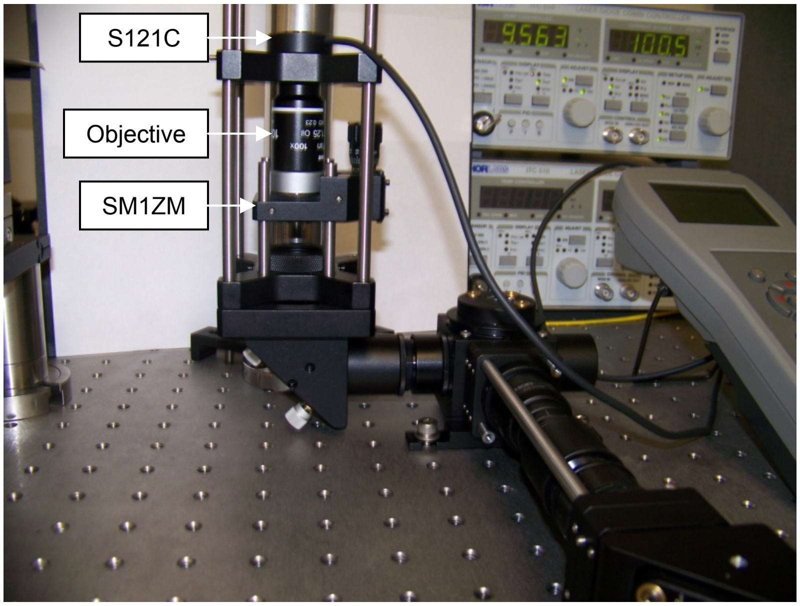


Figure 4.3.3: Power measurement above objective

4.4 Mounting Objective and Condenser

1. Mount the CDI4391 Nikon condenser to the the LCP03-1.45CH-SP condenser adapter plate and clamp it in place with the SS8N025 nylon tipped 8-32 set screw.
2. With the condenser secured, use the same hole to attach a C1500 clamp to the condenser adapter plate.
3. Slide the condenser adapter plate and clamp onto the cage rods and DP14, respectively. Lower the assembly until the clamp is 20.1 cm (disregarding the additional 2 or so mm on standard rulers) above the table surface.
4. Lock the C1500 clamp to the DP14 post.
5. Remove the remaining three ER8 cage rods.

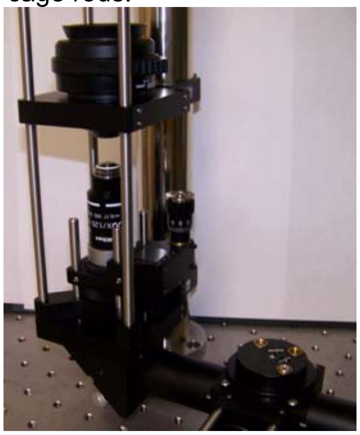


Figure 4.4.1: Objective and condenser mounted.

6. Remove the threaded studs from eight ER05 cage rods.
7. Place an LCP02 on a flat surface and insert four of the ER05 cage rods in the 30 mm cage rod holes and secure them.

8. Connect a C6W cube to the LCP02 from Step 7. Lock the cube onto the ER05 cage rods.
9. Place a second LCP02 on a flat surface and insert the four remaining ER05 cage rods in the 30 mm cage rode holes and secure them.
10. Flip the LCP02 over and secure it to the top of the C6W cube from Step 8.
11. Attach a B1C and a B4C to the C6W
    a. If you are using the optional force measurement module (OTKBFM), then mount a hot mirror on the B4C as in Step 23 of Section 4.2.
12. Close off the horizontal ports using two SM1CP2 caps. (Only close one port when using the OTKBFM)
13. Remove one half of the SM1Q quick release adapter from the OTKB-LS-SP light source and attach it to the upper LCP02 cage plate.
14. Now stack an LCP01, the C6W assembly and a second LCP01.
15. Lock them all together using four ER4 cage rods. Make sure to Align the LCP01 plates so that the tapped mounting holes are on a side of the cube that has a SM1CP2 cap to close the port.
16. Attach C1500 to both of the LCP01 cage plates.
17. Slip the C1500 clamps onto the DP14 post and lower the assembly so that it sits right over the condenser. (Make sure the LCP02 with the quick release adapter is facing upward.)
18. Mount the OTKB-LS-SP LED source onto the LCP02 as shown in Figure 4.4.2.

Now we will complete the camera segment

19. Mount the FES0750 into a SM1L10.
20. Attach it to the KCB1 from Step 18 of Section 4.2.
21. Thread an SM1T2 to the SM1L10.
22. Attach an SM1A9 to the SM1T2
23. Attach the DCU223M CCD camera to the SM1A9 as shown in Figure 4.4.2 (Right).

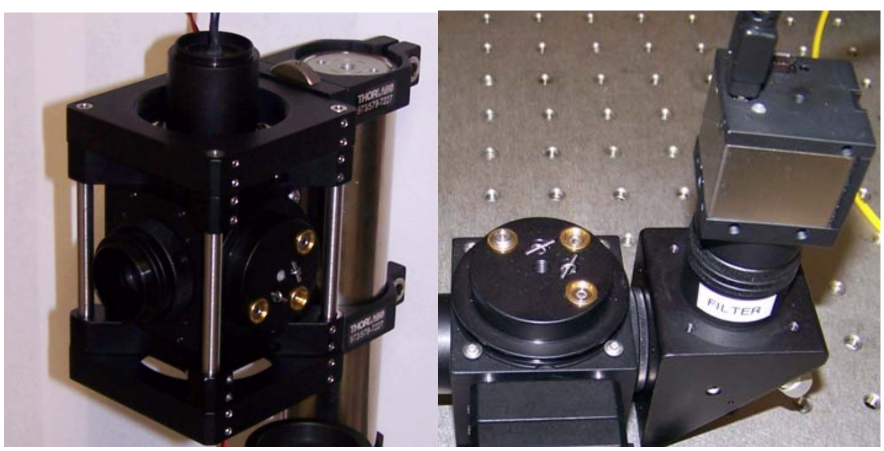
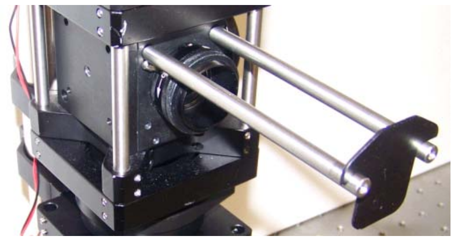


Figure 4.4.2: (Left) C6W cube and visible Light Source. (Right) The camera segment mounted.

Your trapping system is now complete. If you use the force measurement module, continue below for the quadrant detector setup guidelines or skip to section 4.6 for instructions on how to assemble the sample stage.

4.5 Quadrant Detector Segment (optional Force Measurement Module)



1. Make sure to wear laser glasses and take all other nece steps in this section.
2. Make sure that Step 11a was completed in Section 4.4.
3. Thread an SM1D12D onto the port on the C6W that is opp
4. Temporarily thread two ER4, ER6 or ER8 cage rods into guide on the rods as shown in figure 4.5.1.
5. Adjust the B4C so that the trap laser beam is rougly aligne
6. Adjust the objective lens position so that the trap laser bea
7. Readjust the alignment of the trap laser beam using the B4C.
8. Remove the ER rods you used for alignment.
9. Mount an LB1027-B in an HPT1 XY translating cage lens mount.
10. Using an SM1T2 connect the HPT1 with the lens to the SM1D12D iris. Using four ER3 rods (with studs removed at one end), connect these to C6W through the holes on the HPT1 whose orientation is adjusted to fit C6W hole pattern.
11. Adjust HPT1 until beam is centered on the alignment guide.
12. Connect an SM1T2 lens tube coupler to the PDQ80A quadrent photodetector (QPD) with an SM1A1 adapter.
13. Mount the NE06B ND filter in a second HPT1 cage mount. Depending on the trap laser power, this filter may need to be substituted. Please use a filter that limits the beam power incident on the PDQ80A to less than 10 mW.
14. Conncet the HPT1 cage mount with ND filter to the SM1T2 from Step 11.
15. Connect a SM1D12D iris to the HPT1 from Step 12 as shown in Figure 4.5.2 (left). The SM1D12D will spatially filter the beam in front of the detector.

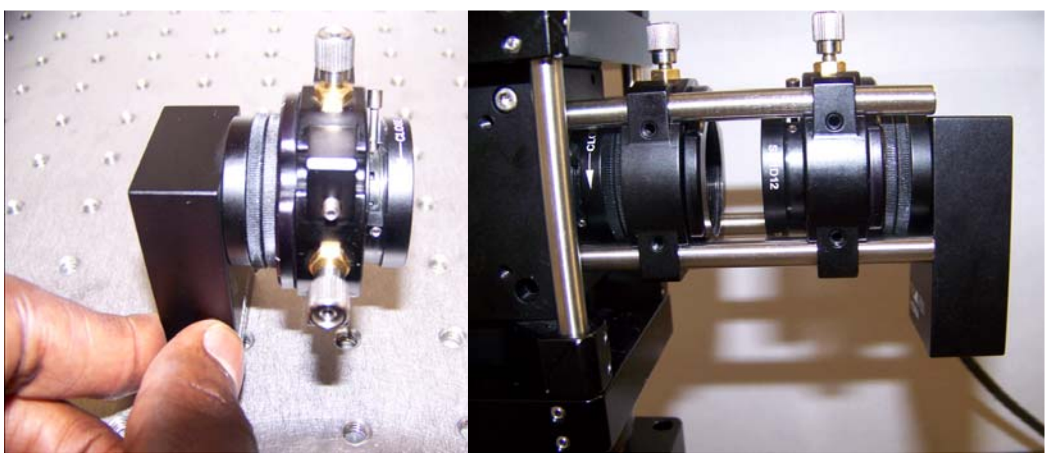


Figure 4.5.2 (Left) the quadrant detector connected to an HPT1 (note that the lever actuated iris has been replaced with a ring activated iris since the picture was taken). (Right) Mounting the Force calibration segment to the C6W

16. Slide the detector segment (Steps 11 to 14) onto the ER3 rods until the beam is imaged onto the detector.
17. The first HPT1 is used to make small adjustments to the focusing lens while the second HPT1 give small adjustments to the quadrant detector.

For optimum results, it is advisable to keep the power on the quadrant detector between 250 μW and 1 mW. In our application, since we have an NE06B filter, this corresponds to a power range at the QD position of 1 mW to 4 mW. For reference, when we use the PL980P330J we have the following power when the objective is at sample position: 100 mA gives 553 uW, 150 mA gives 1.6 mW, 200 mA gives 2.7 mW, 250 mA gives 3.8 mW, and 300 mA gives 4.9 mW. Note that the power was measured with the detector and filter removed but the lens used to image the beam on the detector was left in the beam path. If you are using the components included in the kit, please keep drive current for the PL980P330J at or below 300 mA.

4.6 Sample Stage and Holder

1. Connect the four PS3 spacers to each P2 and attach the PB2 bases.
2. Mount the TBB0606 translating breadboard onto the post assemblies.
3. Mount the LNR50D to the translating breadboard as shown in Figure 4.6.1.
4. Mount the MAX301 stage to the LNR50D. Three holes will line up.
5. Remove the top plate (the plate has an array of mounting holes and two groves that split the plate into four quadrants) from the MAX301 stage (four screws, use a 2 mm hex key)
6. Attach the AMA-SLH slide holder to the MAX301 stage. Attach the three DRV002 drives.
7. Since the oil immersion objective might push the slide from the bottom during the imaging process we used tape at both ends of the glass slide to keep it in place.

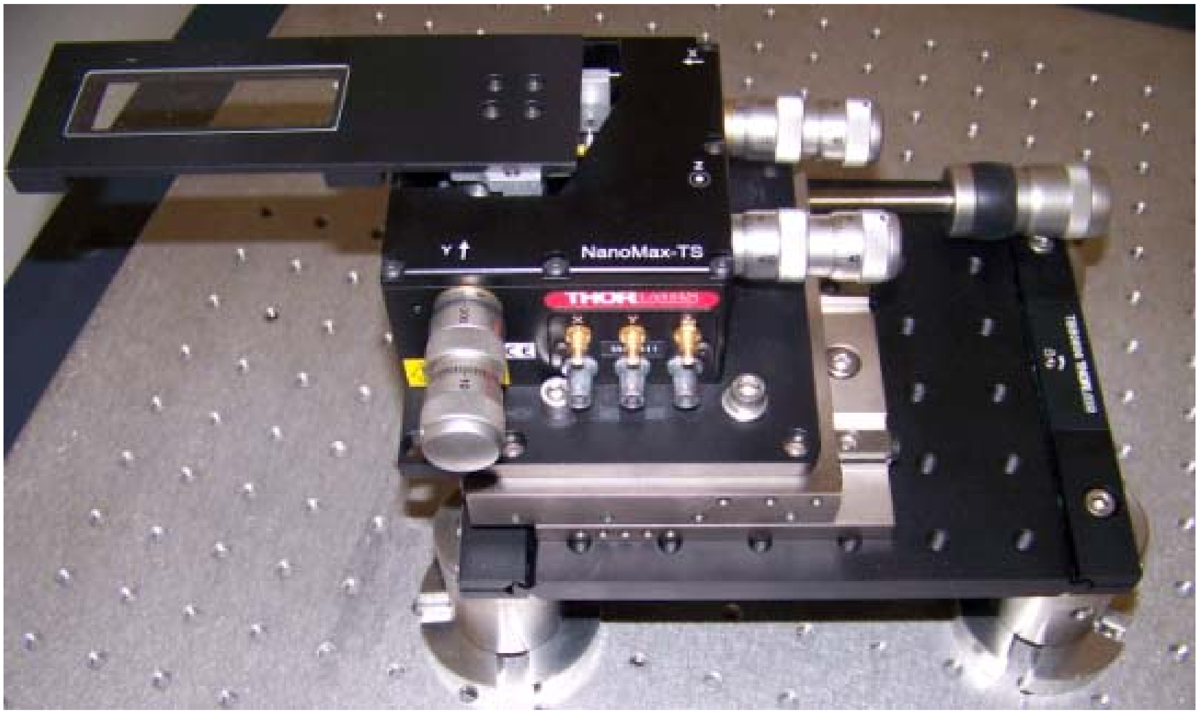


Figure 4.6.1: The sample stage and holder

5. Software Package

The optical trapping kit comes with the standard software which is included with the electronic and nano positioning parts, i.e. it includes the powerful APT software package to control the sample positioning stage and to read out the quadrant detector signal as well as the application software for the DCU camera. The kit does not include any routines that will analyze the data and calculate force/stiffness values. The ActiveX based software modules can be used to develop custom applications (e.g. using LabWindows CVI, Visual C++, Matlab, Visual Basic, HPVEE, any programming software that supports ActiveX). A procedure of how to approach this data analysis can be found in 'Calibration of optical tweezers with positional detection in the back-focal-plane, Review of Scientific Instruments 77, 103101, 2006'. The screen shots on the right hand side show the QPD and Strain Gauge Controller software on top (included with OTKBFM) and the piezo controller software and CCD camera software at the bottom (included with OTKB (OTKB/M)

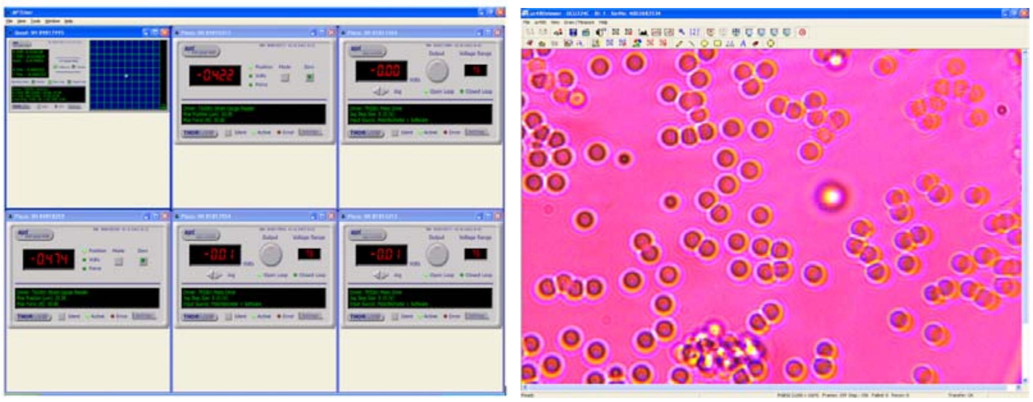


Figure 5.1 (Left) Application Screen (Right) CCD camera application, 1 μm Silica beads. The bright spot near the center of the picture is the trapped bead.

6. Sample preparation and measurement

Sample and sample preparation materials are not included in the kit. We used 1 μm and 2 μm silica beads (Bangs Laboratories, Inc., product code SS04N/8620, SS04N/7995), as well as polystyrene beads in a phosphate buffer with Tween (0.1% Tween-20). Polystyrene beads were provided by D. Appleyard, Biological Engineering, MIT. Silica beads were diluted with de-ionized water.

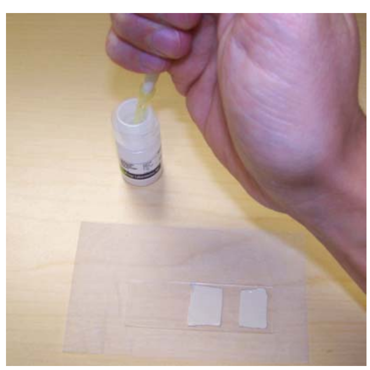


Figure 6.1 Beads are diluted using a pipette and placed between strips of double sided tape

For test measurements, a solution was mixed by first loading a bead solution and then some DI water into a pipette using an Accumax FA-1000 fixed volume pipettor (Lab Depot Inc.) The sample liquid was placed in a channel created between two strips of double sided tape on a microscope slide, after which a cover glass was added on top. The channel width was around 3 to 4 mm. Nail polish or vacuum grease can be used to seal the channel. The slide is placed upside down, cover glass facing towards objective, in the sample holder. For test measurements it will not be necessary to use DI water nor seal the sample.
Fix the slide with tape to the sample holder since the objective otherwise may lift the glass slide when it touches it. In order to load the sample use the translating breadboard TBB0606 (TBB0606/M) to position the sample holder between the objective and condenser. The glass slide will be about 2mm away from the condenser. Now slowly move the SM1Z cage translator with the Nikon objective upwards. As soon as you see that the immersion oil gets in contact with the cover glass you will need to use the smallest possible adjustments up and down until you see a picture of the beads. If you use the same samples as described above make sure you see particles with about the same size, particle with different shapes and sizes usually indicate that you are looking at the immersion oil. Fix the SM1Z cage translator using the 4-40 screws and use the fine adjustment on the SM1Z and the MAX301 stage to optimize the focus of the picture.

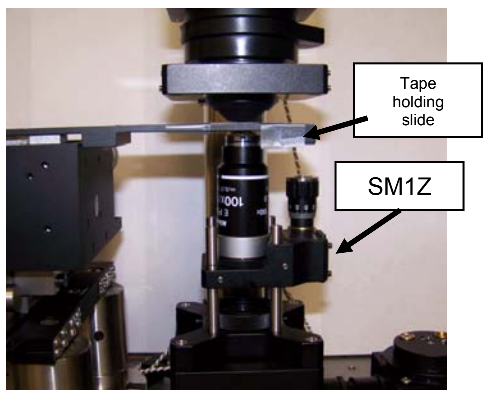


Figure 6.2 Setup after loading sample

7. Additional Accessories

Following parts will be helpful to setup and operate the trap. They are not part of the 'Optical Trap Kit':

a) Immersion Oil, e.g. Cargille non-drying immersion oil for microscopy, Type B, Cat. No. 16484. **Immersion oil is required to able to use the Nikon Oil Immersion objective.**

b) Power meter PM100D with S121C measurement head
http://www.thorlabs.com/NewGroupPage9.cfm?ObjectGroup_ID=3341&pn=PM100D

c) Appropriate laser goggle
http://www.thorlabs.com/newgrouppage9.cfm?objectGroup_ID=762

d) Fiber coupled laser source emitting in visible range
http://www.thorlabs.com/NewGroupPage9.cfm?ObjectGroup_ID=1500&pn=S1FC635

e) Shearing Interferometer with Viewing Accessory
http://www.thorlabs.com/NewGroupPage9.cfm?ObjectGroup_ID=2970&pn=SIVS

8. Acknowledgement

A special thanks to Matthew Lang, Dave Appleyard and S. Wasserman from Department of Biological Engineering, MIT who provided many suggestions and gave many useful hints.

9. Bill of Material (Imperial, 110V Electronics)

Some Components will appear several times in the list since they are shown in the sequence as used in the setup. If Imperial/110V electronic items differ from Metric/230V electronic item the latter are shown in brackets.

Qty	Item/Imperial	Item/Metric	Description
1	PL980P330J		**Trapping laser source**
1	ITC510		
1	LM14S2		
4	ER1		**Laser Collimation**
1	CP02FP	CP02FP/M	
1	SM1T2		
1	SM1L03		
1	PF10-03-P01		
1	CPB1	CPB1/M	
1	KCB1		
1	PAF-X-7-B		
2	CL6		
2	SM1D12D		**Beam expander and steering**
1	SM1T2		
1	SM1L03		
1	SM1V05		
1	AC254-060-B		
2	ER2		
2	CP02	CP02/M	
1	CPB1	CPB1/M	
1	SM1V10		
1	SM1L30		
2	ER4		
2	ER1		
1	C6W		**Connect to vertical path**
1	CPB1	CPB1/M	
1	CP02T	CP02T/M	
2	ER1		
1	B1C	B1C/M	
1	B4C	B4C/M	
1	B5C		

1	FM01		
1	SM1L10		
1	AC254-150-B		
1	SM1V05		
1	SM1T2		
2	CL6		
1	SM1CP2		
1	DP14	DP14/M	**Vertical Segment**
2	SM1D12D		
1	KCB1		
1	PF10-03-P01		
4	C1500	C1500/M	additional clamp for custom LCP03
3	LCP01		
3	LCP02		
4	ER1		
4	ER3		
1	SM1Z		
1	SM1A10		
1	CDI4390		Nikon Objective
1	LCP03-1.45CH-SP		
1	CDI4391		Nikon Condensor
1	SS8N025		
8	ER05		
4	ER4		
1	C6W		
1	B1C	B1C/M	
1	B4C		
1	B5C		
1	FM01		
2	SM1CP2		
4	ER8		initial alignment
3	CL5		
1	SM1T2		**Camera Segment**
1	KCB1		
1	PF10-03-P01		
1	FES0750		
1	SM1L10		
1	SM1T2		

1	SM1A9		
1	DCU224C		
4	P2	P50/M	**Sample Positioning**
4	PB2	PB2/M	
4	PS3	PS15/M	
1	LNR50D	LNR50D/M	
1	MAX301	MAX301/M	
1	AMA-SLH		
3	DRV002		
3	TPZ001		
1	TCH002		
1	TBB0606	TBB0606/M	
1	OTKB-LS-SP		**Visible Light Source**
1	LD1255-CAB		
1	PS-12DC-US	PS-12DC-EU	
1	LD1255P		
1	LEDWE-10		
1	LD1255R		

OTKBFM Parts List:

Qty	Item/Imperial	Item/Metric	Description
1	SM1D12D		
1	SM1T2		
1	HPT1		
1	SM1D12D		
4	ER3		
1	LB1027-B		
1	SM1A1		
1	SM1T2		
1	HPT1		
1	NE06B		
1	PDQ80A		
1	TQD001		
2	TSG001		

10. Optional Galvo Scanning Mirror Integration (OTKGALVO)

This section gives instructions on how to integrate Thorlabs part number OTKGALVO, which is the galvo scanning mirror system for the optical trap. The instructions assume that you have collimated the trap beam on the fiber port. For instructions on how to do this, please refer to section 4.1 above. The component to be replaced with the galvo scanning mirrors is the first KCB1 mirror, and that is what we describe below.

Follow the instructions that come with the GCM002 to mount the galvo mirrors (section 1.2 Installation), up to step 6. Since alignment is crucial, step 7 has to be done in the setup. Using 4 ER05 rods and an SM1T2, connect the fiberport to the GCM002 as shown in Fig. 10.1. Then attach an SM1D12D to the lower hole on the GCM002. Attach 2 ER6 or ER8 rods through the CP02 to the top holes on the GCM002 as in Fig. 1. Using an alignment plate mounted as shown, adjust the X-axis and Y-axis mirrors such that the beam is going through the SM1D12D and hitting the center of alignment plate. Ensure that you have good alignment even when the SM1D12D is at its smallest aperture size. This will take a few back and forth tweaking of the galvo mirrors. Once the alignment is good, you can then proceed by tightening the pinch bolts on both clamps for the mirrors. It is advisable to have the cables from the driver board to mirrors connected before finalizing this alignment. Note that first time this is done, you may lose the alignment and the mirror adjustments will have to be done again.

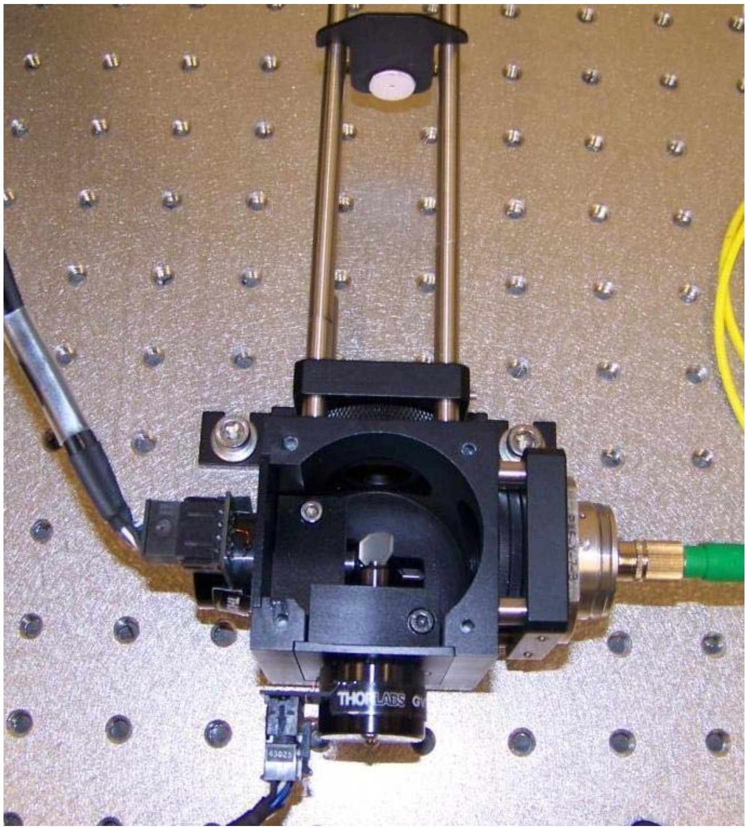


Figure 10.1: Galvo scanning mirrors integration

Once this alignment is done, remove the ER6 or ER8 rods. Using two ER2 rods with studs at each end only, thread these to the diagonal andconnect an SM1L03 lens tube on the other side of the CP02 as shown in Fig 10.2.

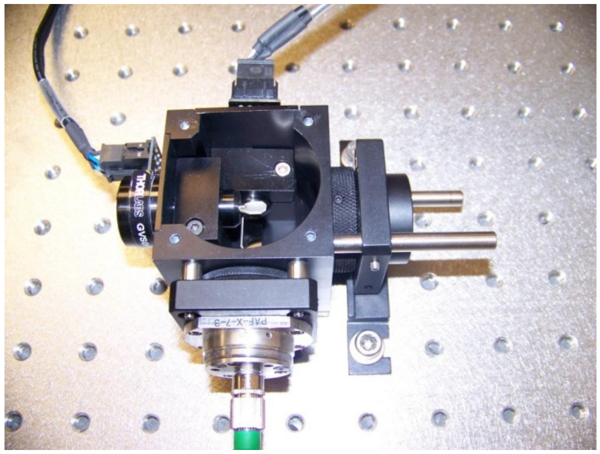


Figure 10.2: Connecting ER2 rods for beam expander segment.

Then slide this to the assembly that has the first lens for the beam expander, as shown in Fig. 10.3. This will give you the right distance between the first lens and the center of the galco mirror (60mm).

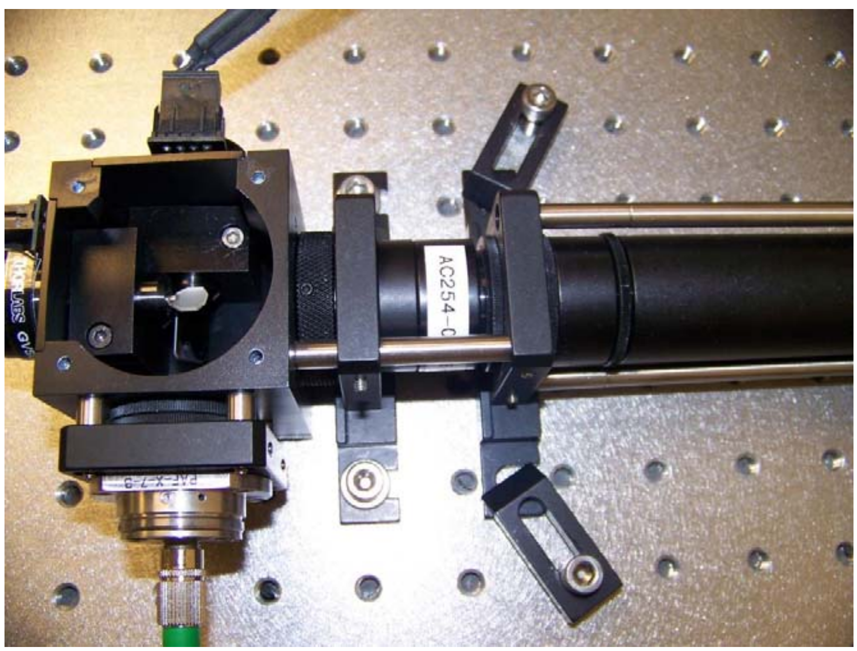


Figure 10.3: Coupling to beam expander segment

This completes the integration of the galvo mirrors. You can now cover the top of the GCM002 to prevent dust particles from getting to the mirror surfaces. Then continue with the alignment as in section 4.2 in this manual.