THORLABS

Discovery

EDU-OT1
EDU-OT1/M
Portable Optical Tweezers
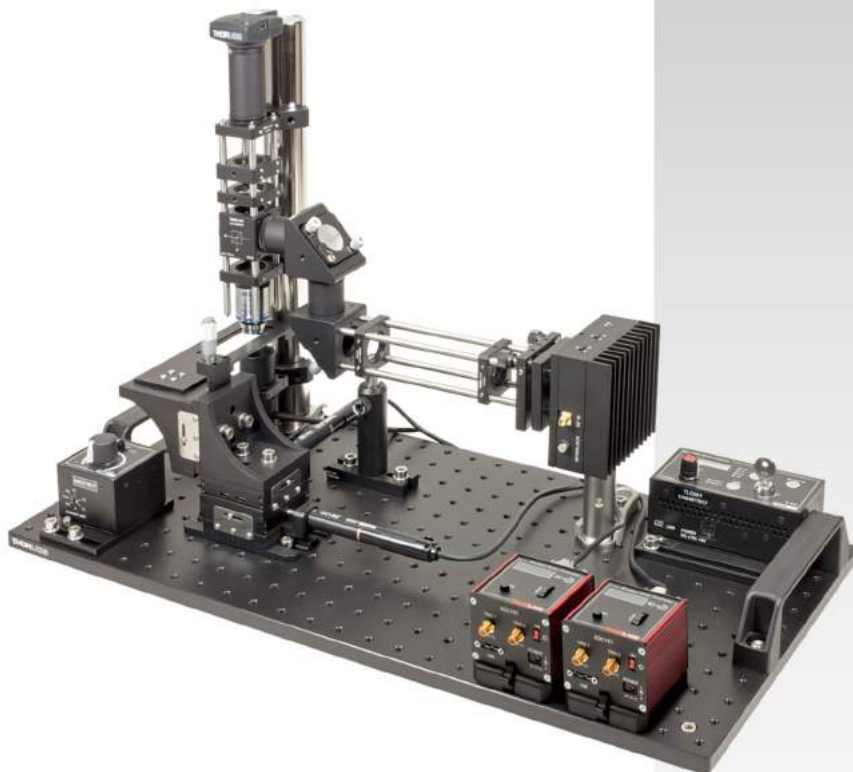
User Guide

Table of Contents

Chapter 1 Warning Symbol Definitions

Below is a list of warning symbols you may encounter in this manual or on your device.

Symbol	Description
	Direct Current
	Alternating Current
	Both Direct and Alternating Current
	Earth Ground Terminal
	Protective Conductor Terminal
	Frame or Chassis Terminal
	Equipotentiality
	On (Supply)
	Off (Supply)
	In Position of a Bi-Stable Push Control
	Out Position of a Bi-Stable Push Control
	Caution: Risk of Electric Shock
	Caution: Hot Surface
	Caution: Risk of Danger
	Warning: Laser Radiation

Chapter 2 Safety

<table>
<tr><td>⚠</td><td>Warning</td><td>⚠</td></tr>
<tr><td colspan="3">The class 3B laser diode used in this kit emits up to 42 mW of optical power, which can cause damage to the eyes if viewed directly. The laser driver is equipped with a key switch and safety interlock, which should be used appropriately to avoid injury. Additionally, we recommend wearing appropriate laser safety glasses when using this kit.</td></tr>
</table>

LASER RADIATION

AVOID EXPOSURE TO BEAM
CLASS 3B LASER PRODUCT

Chapter 3 Product Description

For many people, moving and controlling objects with a light beam sounds more like the "tractor beams" of science fiction than reality. However, optical tweezers are devices that allow precisely that kind of manipulation. Many areas of research use them, e.g., to measure very small forces in the order of piconewtons[1] or to control tiny microgears[2]. Biologists use optical tweezers to manipulate many different types of molecules or complete cells[3]. *In-vitro* fertilization of ovules is a typical application example – sperm can be inserted into ovules without mechanical contact and, therefore, a sterile environment can be sustained.

In a lab course, various demonstrations and experiments can be performed with an optical tweezers setup. Besides the experience of moving small spheres or cells selectively through an aqueous medium with a single laser beam, more advanced experiments can be carried out. The Brownian motion of objects can be investigated and the optical forces of the tweezers can be measured.

The working principle can be explained via geometric optics, basic theory of Brownian motion, and Stokes' friction, concepts usually known to undergraduate students. It is an intriguing experience to be able to control objects with a laser beam – and not only for students!

This Optical Tweezers Kit can be assembled into complete and fully operating experimental setup with which particles in the order of Ø1-10 µm can be trapped and moved. The beam path is schematically depicted in Figure 1. It is possible to perform a variety of experiments using a number of different particles such as polystyrene or glass beads or starch grains gained from customary corn flour. A special feature of this setup is that it is portable. It can be moved from room to room without needing disassembly or readjustment, making it ideally suited to demonstrate the principle of optical tweezers to students in seminars or lecture halls.

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[1] K. SVOBODA, S.M. BLOCK: Optical trapping of metallic Rayleigh particles, Optics Letters 19 (1994) 13, 930-932

[2] S.L. NEALE, M.P. MACDONALD, K. DHOLAKIA, T.F. KRAUSS: All-optical control of microfluidic components using form birefringence, Nature materials 4 (2005), 530-533

[3] J.E. MOLLOY, M.J. PADGETT: Lights, action: optical tweezers, Cont. Phys. 43 (2002) 43, 241-258
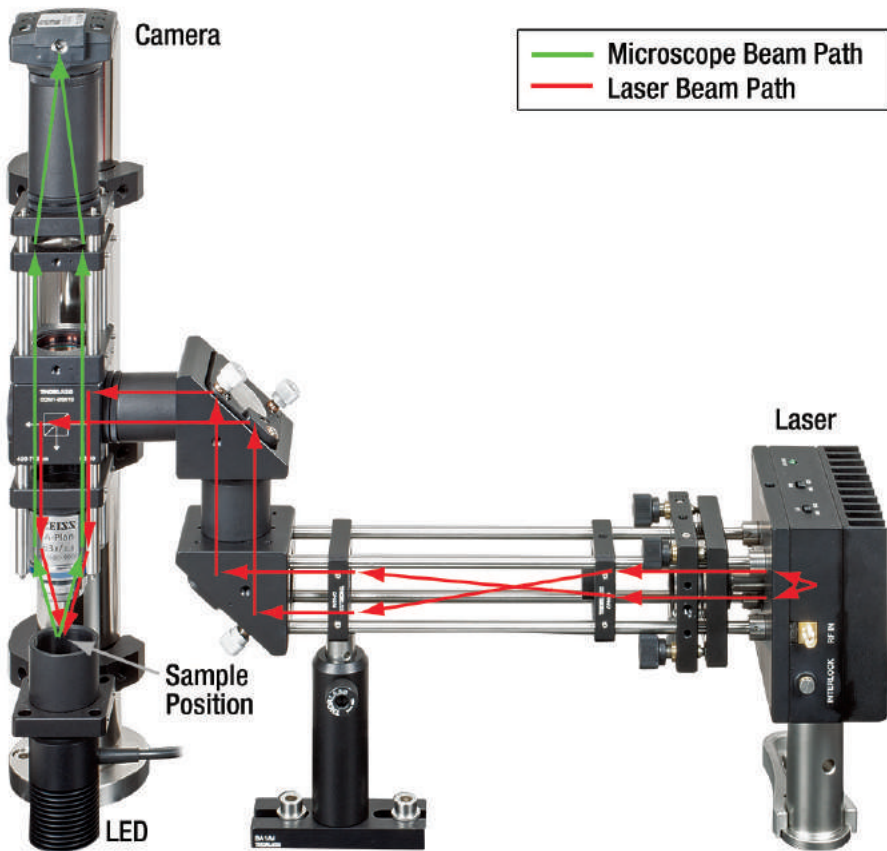
Figure 1 The light path through the optical tweezers experiment.

Chapter 4   Principles of Optical Tweezers

To describe the function of optical tweezers, we will examine the force that a focused laser beam with a Gaussian intensity profile (the TEM$_{00}$ mode) exerts on an object, which is near or in the focus. Usually one also assumes that the object is a bead, which consists of a dielectric, linear, isotropic, and spatially and chronologically non-dispersive material. In the experiments described below, micron-sized beads made of polystyrene are primarily used. It is customary to describe the force of the laser on the object by separating it into two components. One component, the scattering force, acts along the direction of beam propagation. The second component acts along the intensity gradient and is therefore called the gradient force. The gradient force can act in different directions with respect to the beam. As the laser has a Gaussian intensity profile, the gradient force can act orthogonally to the beam, but it can also act parallel to the beam, as the laser is focused and therefore also has an intensity gradient along the beam axis. These two components and their relationship to one another are the defining factor for whether or not a particle can be trapped by the optical trap. Stable optical tweezers are only obtained if the gradient force, which pulls the object in the direction of the focus, is greater than the scattering force, which pushes the particle in the direction of the beam away from the focus.

The various theoretical approaches to describe optical trapping can roughly be divided according to the areas in which they are valid. The relationship of the radius R (or diameter d) of the bead to the wavelength of the incident laser beam is the dividing factor. The case $R \approx \lambda$ is theoretically very complex and shall therefore not be dealt with here. The two extreme cases for very large and very small particles are summarized below:

4.1.   Dipole Approach in the Rayleigh Scattering Regime R << λ

The first case we will consider is when the radius R of the bead is significantly smaller than the wavelength λ of the incident laser beam. Then, the electrical field $\vec{E}(\vec{r})$ is approximately spatially constant with respect to the particle and the situation can be portrayed as follows:

As the bead is assumed to be dielectric, one can imagine it as a collection of N point dipoles. Due to their polarizability, a dipole momentum $\vec{p}_i$ is induced in each of the point dipoles by the incident laser beam. Due to the linearity of the material, the following applies:

$$\vec{p}_i = \alpha \cdot \vec{E}\left(\vec{r}_i\right) \qquad (1)$$

Here, $\vec{r}_i$ is the location of the *i-th* point dipoles and $\vec{E}(\vec{r}_i)$ is the electrical field strength at this location. In addition, the electrical field of the laser appears to be approximately spatially constant for the bead due to the condition $R \ll \lambda$, meaning that at a certain point in time t_0 the strength of the electrical field is equally great for all point dipoles of the

bead. As a result, the induced dipole momentum is equally great for all N point dipoles. The polarization $\vec{P}$ resulting from the induced dipole momentums is then

$$\vec{P} = \frac{1}{V}\sum_i \vec{p} = \frac{N}{V} \cdot \alpha \cdot \vec{E} = \chi \cdot \epsilon_0 \cdot \vec{E} \tag{2}$$

whereas χ the electrical susceptibility, ϵ_0 is the electrical constant, and V is the volume of the bead. The potential energy U_i of one of the point dipoles with dipole momentum $\vec{p}$ in the electrical field $\vec{E}$ is $U_i = -\vec{p}\vec{E}$. Because there are N point dipoles in a bead with the volume V, the energy density in the bead is defined by:

$$U = \frac{N \cdot U_i}{V} = -\underbrace{\frac{N}{V} \cdot \vec{p}}_{\vec{P}} \cdot \vec{E} = -\vec{P} \cdot \vec{E} \tag{3}$$

The occurrence of the gradient force, therefore a force component that is directed in the direction of the intensity gradient of the incident electrical field, can be explained when one observes this potential energy U of the bead in the electrical field. Equation (2) states that $\vec{P}$ is proportional to $\vec{E}$. Therefore, according to equation (3), U is proportional to $\vec{E}^2$ and thus to intensity $I \propto \vec{E}^2$ of the incident field. The force exerted on the particle by the incident field is proportional to the gradient of the potential energy ∇U and therefore proportional to the intensity gradient ∇I. The following equations describe the gradient force:

$$F_{Grad} = \frac{2\,\pi\,\alpha}{c\,n_m^2}\,\nabla I \tag{4}$$

$$\alpha = n_m^2\,R^3\left(\frac{m^2 - 1}{m^2 + 2}\right) \tag{5}$$

$$m = \frac{n_p}{n_m} \tag{6}$$

Here, α is the polarizability of the dipoles and m is the relationship of the refraction index of the particles, n_p (polystyrene in our case) to the refraction index of the surrounding medium, n_m (water in our case). The destabilizing scattering force component is explained by the scattering of the incident light at the particle. The force action is created by the absorption and isotropic re-emission of the light by the bead. As $R \ll \lambda$, the conditions are fulfilled for Rayleigh scattering. The resulting force can be stated as follows:

$$F_{Scattering} = \frac{\sigma\,n_m}{c} \cdot I \tag{7}$$

$$\sigma = \frac{128\,\pi^5\,R^6}{3\,\lambda^4}\left(\frac{m^2-1}{m^2+2}\right)^2 \tag{8}$$

Here, I is the incident intensity and σ is the scattering cross-section of the incident light. It is important to note that the scattering force is proportional to the intensity and points in the beam direction.

4.2. Ray Optical Approach in the Mie Regime R >> λ

This ray optical approach deals with the second possible extreme case. Here, we will assume that the radius of the bead is much larger than the wavelength of the incident laser. In this range, the conditions of geometric (ray) optics are fulfilled and one can think of the laser beam as a bundle of rays. Typically, this assumption is valid for beads with a radius R that is greater than $\sim 10\lambda$. The basics of the theoretical derivation of the gradient and scattering force in accordance with this model can be found in a work by Ashkin[4].

The particle properties of light must now be taken into account, namely that light can transfer momentum to an object in the form of photons. The force action of a beam on a particle can be explained using Newton's second law: the force on a particle is exactly equal to the change in the momentum of the particle over time:

$$F = \frac{dp}{dt} \tag{9}$$

The following equation describes the change in momentum $\frac{dp}{dt}$ of a beam over time in a medium with a refractive index n_m:

$$\frac{dp}{dt} = \frac{n_m}{c}P_{Beam} = \frac{n_m}{c}I(r)dA \tag{10}$$

Here, c is light speed in a vacuum and $I(r)$ is the intensity distribution in the beam cross-section. Often, a Gaussian profile is used, in which the intensity decreases in a Gaussian distribution from the center of the beam outward. This is also the case in our setup.

If a beam with the power P_{Beam} hits a sphere at an angle of ϑ, part of the beam will be reflected and part of it will reach the interior of the sphere through transmission (see Figure 2). For the power of these two partial beams, the following is in effect:

$$P_{refl} = P_{Beam} \cdot R_r \tag{11}$$

$$P_{trans} = P_{Beam} \cdot T \tag{12}$$

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[4] Ashkin A., Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime. In: Biophys. J. 61 (1992) 2, 569-582

Here, R_r is the reflectivity and T is the transmissivity. The transmitted beam transports momentum into the sphere in accordance with the equation (10).
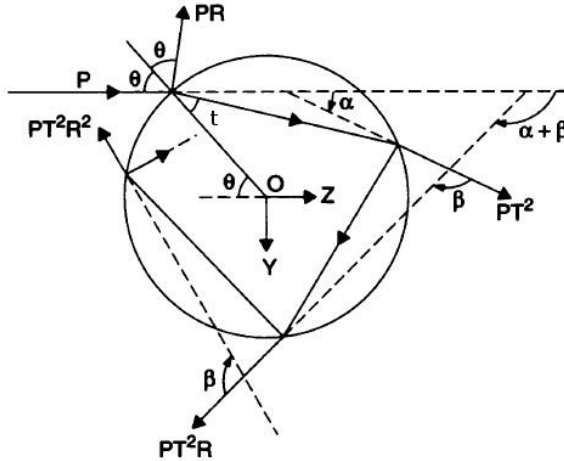
Figure 2 Reflection and Transmission of an Incident Partial Beam on the Inside and Outside Surfaces of a Sample Bead.

Inside the sphere, the beam is reflected and transmitted numerous times. Part of the beam is repeatedly reflected on the sphere's internal wall and remains in the sphere, while the rest exits the sphere again through transmission. The beams which exit the sphere again were thus subject to a change in momentum $\frac{dp}{dt}$. The force on the sphere is equal to the momentum per time unit that remains in the sphere, based on the equation (9). Now, the force on the sphere is once again divided into two components: a component in the direction of the incident beam (corresponds to the $z-$direction) and a perpendicular component (corresponds to the $y-$ axis). This results in the following for both forces:

$$F_s := F_z = n_m \frac{P_{Beam}}{c} Q_s \tag{13}$$

with the Q factor

$$Q_s = 1 + R_r \cos(2\theta) - \frac{T^2\big(\cos(2\theta - 2t) + R_r \cos(2\theta)\big)}{1 + R_r^2 + 2R_r \cos(2t)} \tag{14}$$

and

$$F_g \equiv F_y = n_m \frac{P_{Beam}}{c} Q_g \tag{15}$$

with the Q factor

$$Q_g = R_r \, \sin(2\theta) - \frac{T^2 \left(\sin(2\theta - 2t) + R_r \cos(2\theta) \right)}{1 + R_r^2 + 2R_r \cos(2t)} \tag{16}$$

Here, t is the angle at which the first transmitted beam is refracted toward the normal (see Figure 2). According to Snell's law of refraction, the following relationship is in effect for the angles θ and t:

$$\frac{\sin(\theta)}{\sin(t)} = \frac{n_p}{n_m} \tag{17}$$

Q_s and Q_g are dimensionless Q factors, which state what percentage of the incident momentum that contributes to the force parallel or perpendicular to the beam, respectively. These factors depend heavily on the angle of incidence of the beam, as one can see from the equations. This angle becomes larger the more heavily the beam is focused, which occurs when a higher numerical aperture objective is used.

The component of the beam that points in the incident direction ($z-$direction) ultimately causes the scattering force F_s. The component perpendicular to this ($y-$direction) is mainly responsible for the gradient force F_g. In order to obtain the overall power, one must naturally consider all partial beams and integrate all of them.
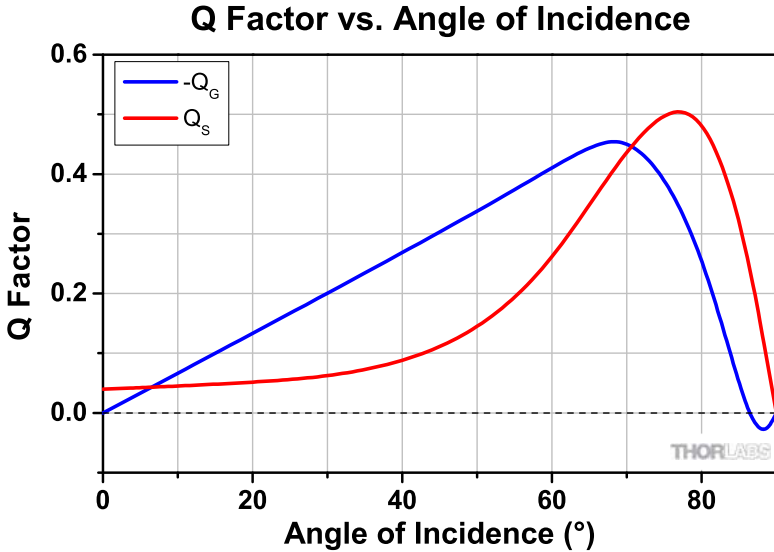
Q Factor vs. Angle of Incidence

Figure 3 Q Factor Angular Dependence[5]

Figure 3 shows the values of the two $Q-$factors, depending upon the angle of incidence θ when the focus is located slightly above the surface of the sphere. One can see here Q_g is negative through almost the entire range, meaning the force acts in the negative $y-$direction, which is upwards in Figure 2. The factor Q_s is always positive. The $z-$component of the force therefore always points in the beam direction. If the beam were not to hit in the upper half of the sphere, but rather in the lower half, one can easily conclude for reasons of symmetry that the direction of the $y-$component would reverse, the direction of the $z-$component would remain the same. This allows us to formulate a simple rule, which will somewhat simplify our further considerations: if one observes the path of a beam from left to right (e.g. in Figure 2), it can be stated:

- F_s always points in the direction of the beam
- F_g points upward when the normal from the center of the sphere O to the impact point of the beam is located above the beam
- F_g points downward when the normal is located below the beam

In short: The sphere always moves into the focus or the point of highest intensity. In order to ultimately achieve a stable optical trap, the following must be true:

$$F_g > F_s \tag{18}$$

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[5] Ashkin A., Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime. In: Biophys. J. 61 (1992) 2, 569-582

Chapter 5 Kit Components

In cases where the metric and imperial kits contain parts with different item numbers, metric part numbers and measurements are indicated by parentheses unless otherwise noted.

5.1.   Trapping Laser Source

1 x TCLDM9 Laser Diode Mount	**1 x L658P040** 658 nm, 40 mW A Pin Code Laser Diode	**1 x TLD001** T-Cube Laser Diode Driver
1 x TPS002 ±15 V / 5 V T-Cube Power Supply	**1 x RS3P(/M)** Ø1" (Ø25 mm) Pedestal Post, 3" (75 mm) Tall	**1 x CF125** Small Clamping Fork
1 x CAB400 Cable for Laser Diode Driver		

5.2.   Laser Collimation

1 x **KC1-T(/M)** Ø1" Cage-Compatible Mirror Mount	1 x **A230TM-B** Mounted Aspheric Lens, f = 4.51 mm	1 x **E09RMS** RMS to M9 x 0.5 Adapter
1 x **SM1A3** Adapter with External SM1 Threads and Internal RMS Threads		

5.3.   Beam Expander

4 x **ER8** Ø6 mm Cage Assembly Rod, 8" Long	2 x **CP02(/M)**[6] SM1-Threaded Cage Plate	1 x **LA1074-A** Ø1/2" N-BK7 Plano- Convex Lens, f = 20 mm

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[6] A total of 6 CP02(/M) Cage Plates are included with this kit.

1 x **LA1509-A** Ø1" N-BK7 Plano-Convex Lens, f = 100 mm	1 x **SM1A6** Adapter with External SM1 Threads and Internal SM05 Threads	1 x **SM05L03** Ø1/2" Lens Tube, 0.3" Long
1 x **TR3 (TR75/M)** Ø1/2" (Ø12.7 mm) Post, 3" (75 mm) Long	1 x **PH3 (PH75/M)** Ø1/2" (Ø12.7 mm) Post Holder, 3" (75 mm) Long	1 x **BA1(/M)** Post Holder Mounting Base, 1" x 3" x 3/8" (25 mm x 58 mm x 10 mm)

5.4. Right-Angle Mirrors

2 x **KCB1** Right Angle Kinematic Mirror Mount	2 x **PF10-03-P01** Ø1" Protected Silver Mirror	2 x **SM1L10**[7] Ø1" Lens Tube, 1" Long
2 x **SM1T2**[8] Ø1" Lens Tube Coupler		

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[7] A total of 3 SM1L10 lens tubes are included with this kit.

5.5.　　Sample Positioning System

2 x MT1(/M)-Z8 Motorized Translation Stage, 1/2" (12 mm) Travel	**1 x MT1(/M)** Manual Translation Stage, 1/2" (13 mm) Travel	**2 x KDC101** DC Servo Motor Module
Location-Specific Adapter Provided **2 x KPS101** K-Cube Power Supply	**1 x MT402** Right Angle Bracket	**1 x MT402-SLH-SP** Modified MT402 for Mounting **AMA-SLH**
1 x AMA-SLH Slide Holder	**1 x MT401** Mounting Base for Translation Stages	

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[8] A total of 4 SM1T2 couplers are included with this kit.

5.6.   Microscope

1 x **DCC1645C** High Resolution CMOS Camera	2 x **SM1T2**[9] Ø1" Lens Tube Coupler	3 x **C1498(/M)** Slip-On Ø1.5" Post Clamps
1 x **SM1L20** Ø1" Lens Tube, 2" Long	4 x **CP02(/M)**[10] SM1-Threaded Cage Plate	1 x **CP02T(/M)** SM1-Threaded Cage Plate, 0.5" Thick
2 x **FGB37** Ø25 mm BG40 Colored Glass Bandpass Filter, 335 – 610 nm	1 x **FES0650** Ø1" Shortpass Filter, 650 nm Cut Off	1 x **LB1676** Ø1" N-BK7 Bi-Convex Lens, f = 100 mm
1 x **SM1A9** Adapter with External C-Mount Threads and Internal SM1 Threads	1 x **CCM1-BS013(/M)** Cube-Mounted 50:50 Beamsplitter Cube	1 x **SM1CP2** SM1 End Cap

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[9] A total of 4 SM1T2 couplers are included with this kit.

[10] A total of 6 CP02(/M) Cage Plates are included with this kit.

1 x DP14A(/M) Damped Ø1.5" Post	**4 x ER3** Ø6 mm Cage Assembly Rod, 3" Long	**4 x ER2** Ø6 mm Cage Assembly Rod, 2" Long
1 x MCWHL5 Cold White Mounted LED	**1 x LEDD1B** T-Cube LED Driver	Location-Specific Adapter Provided **1 x KPS101** Power Supply
1 x SM1L10[11] Ø1" Lens Tube, 1" Long	Zeiss Microscope Objective 63X, 0.8 NA	**1 x SM1A17** Adapter with External SM1 Threads and Internal M27 x 0.75 Threads

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[11] A total of 3 SM1L10 lens tubes are included with this kit.

5.7.   Additional Components

1 x **MB1224 (MB3060/M)** Aluminum Breadboard, 12" x 24" x 1/2" (300 mm x 600 mm)	1 x **RDF1** Rubber Dampening Breadboard Feet	4 x **ERSCA** Ø6 mm Cage Rod Adapters
1 x **BBH1** Breadboard Handles (Pack of 2)	1 x **CPA1** 30 mm Cage System Alignment Plate with Ø1 mm Aperture	1 x **BD-3/16L (BD-5ML)** 1/4-20 (M6) Balldriver 0.050", 5/64", 3/32", 1/8", and 2.5 mm Hex Keys (1 x 1.3 mm, 2 mm, 2.5 mm, and 3 mm Hex Keys in Metric Version)

5.8.   Screws Included

Imperial Kit

Type	Quantity	Type	Quantity
1/4-20 Cap Screw, 5/8" Long	30	8-32 Cap Screw, 1/2" Long	3
1/4-20 Cap Screw, 3/4" Long	4	8-32 Set Screw, 5/8" Long	3
#1/4 Washer	30	8-32 Set Screw, 1" Long	1
#1/4 Nut	4	M3 x 0.5 Cap Screw, 6 mm Long	4
1/8" x 1/4" Steel Dowel Pin	4	SD1 8-32 to 1/4 Counterbore Adapter	3

Metric Kit

Type	Quantity	Type	Quantity
M6 x 16 mm Cap Screw	30	M4 x 10 mm Cap Screw	3
M6 x 20 mm Cap Screw	4	M4 x 16 mm Setscrew	3
M6 Washer	30	M4 x 25 mm Setscrew	1
M6 Nut	4	M3 x 0.5 Cap Screw, 6 mm Long	4
1/8" x 1/4" Steel Dowel Pin	4	SD1 M6 to M4 Counterbore Adapter	3

Chapter 6 Setup and Adjustment

6.1.   Assembly

The tweezers are set up on a MB1224 (MB3060/M) breadboard. Use a stable desk without vibrating noise nearby. Further damping, such as using an optical table, is not necessary. Figure 4 shows the breadboard, and the positions of components to be mounted are indicated with cap screws. Attach the BBH1 breadboard handles to the ends of the breadboard so the assembled tweezers can be moved. Attach the RDF1 rubber breadboard feet to the underside of the breadboard using 1/4″-20, 3/4″ long (M6 x 20 mm) cap screws in the counterbores at the corners of the breadboard.
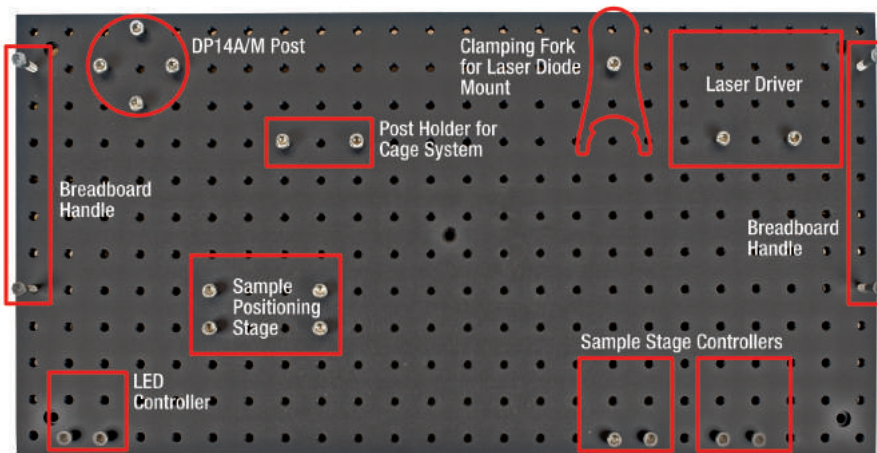
Figure 4 Positions of Components on the Breadboard

6.1.1.   Trapping Laser Source

Figure 5 shows the components that are necessary for setting up the laser source. First, place the L658P040 laser diode into the TCLDM9 laser mount. To prevent electrostatic damage to the laser diode, wear a grounding bracelet during this procedure. In order to place the diode in the mount, first remove the front plate of the mount. Then, insert the diode into the pin connectors. Insert the laser diode so that the bottom pin hole on the mount is unused. Replace the front plate and **move the LD switch on the top of the mount to the AG position and the PD switch to CG**. Thread the four ERSCA adapters into the front of the laser diode mount. Next, the laser mount must be attached to an RS3P(/M) mounting base. Use the 8-32, 1" long (M4 x 25 mm) setscrew and the included 8-32 to 1/4″-20 (M4 to M6) adapter that is included with the RS3P(/M) to attach the mounting post to the bottom of the laser diode mount. Bolt the mounting base to the breadboard using a CF125 clamping fork.

Figure 5 Laser Source Components

6.1.2.   Laser Collimator

Figure 6 shows the laser collimator components. First, the threaded portion of the lens (A230TM-B) should be screwed into the silver side of the adapter (E09RMS). The lens should be oriented so that the engraved portion of its mount is inside the E09RMS adapter (see Figure 7).

Figure 6 Laser Collimator Components
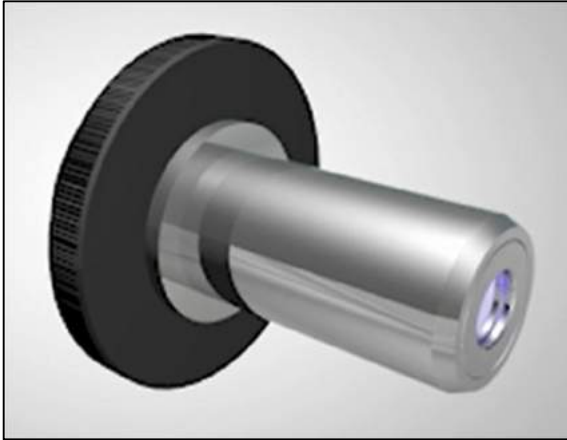
Figure 7   E09RMS Correct Lens Orientation

Then, attach the collimator to the KC1-T(/M) adjustable mount using the SM1A3 adapter. During adjustment, you can precisely orient the collimator with the adjustment screws on the mount. The locking screws, which are located on the top of the mount, are used to attach the cage system rods.

6.1.3.   Beam Expander

In the following, the cage system is set up to house the beam expander. The individual components are assembled first, and then mounted in the cage system.

Lens 1

The elements that are necessary for the setup of lens (1), are shown in Figure 8. First, place the LA1047-A lens into the SM05L03 Ø1/2" lens tube using the included SM05 retaining ring. Ensure that the convex side of the lens is inserted into the lens tube first Then, screw the lens tube into the SM1A6 adapter, and attach it to the CP02(/M) cage plate.

Figure 8 Beam Expander Lens 1 Components

Lens 2

The components for lens (2) are shown in Figure 9. The lens (LA1509-A) must be attached to the CP02(/M) cage plate using two SM1 retaining rings.

Figure 9 Beam Expander Lens 2 Components

Adjustment mirror

In the next step, mount the mirrors (PF10-03-P01) in the right angle mirror mounts (KCB1) (Figure 10). Assemble two adjustments mirrors.

Figure 10 Angle Mirrors

Base

Attach the PH3 (PH75/M) post holder to the BA1(/M) mounting base using a 1/4″-20, 5/8″ long (M6 x 16 mm) cap screw, as shown in Figure 11.

Figure 11 Post Holder Base

Cage System

Attach the four ER8 Ø6 mm cage rods to the housing of the adjustment mirror, as shown in Figure 12. Be sure that the cage rods are completely screwed into the threaded holes of the mirror mount.

Figure 12 Cage Rods Attached to the Angle Mirror Mount

Push the cage plate holding lens 2 onto the rods. Here, the curved side of the lens must face the mirror. Then, push the cage plate holding lens 1 onto the four rods. The lens tube attached to this cage plate should also face in the direction of the mirror. The mount with the collimator is the last component that must be placed on the rods. The collimator should face away from the mirror. Finally, attach the TR3 (TR75/M) post to the cage plate holding lens 2. The complete cage system assembly is shown in Figure 13 Each of the individual cage plates or mounts have small locking screws on the top for locking the components to the rods. However, these should not be tightened yet, as the precise position of the brackets will only be determined through subsequent adjustment.
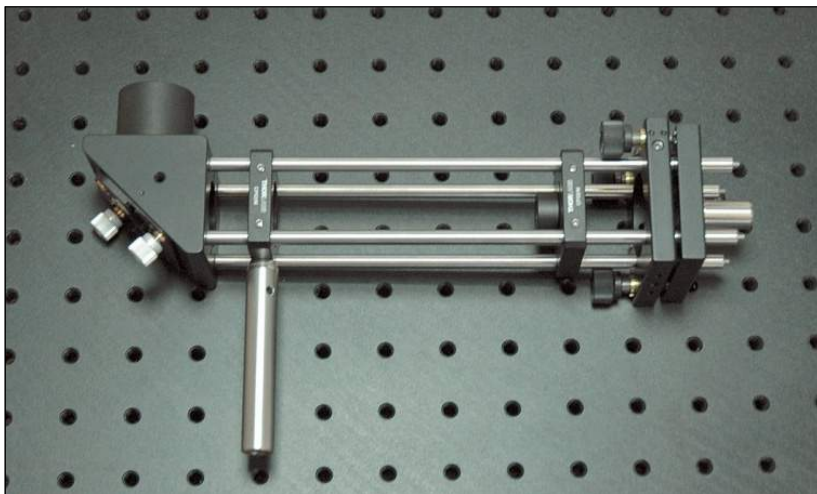
Figure 13 Cage System on Breadboard

The cage system must be attached to the breadboard using the post holder and base. Next, screw the rods into the ERSCA adapters on the laser mount, as shown in Figure 14. Ensure that the collimator is aimed directly at the laser diode and that the laser diode mount is oriented perpendicular to the rods.
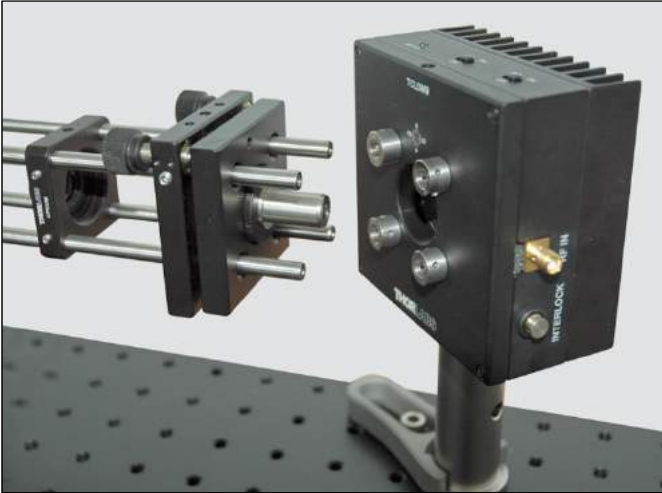
Figure 14 Attaching the Laser to the Cage System

Next, attach a lens tube (SM1L10) to the free threads of the first adjustment mirror. Attach the second adjustment mirror to the lens tube using a lens tube coupler (SM1T2). Attach a lens tube (SM1L10) with an adapter (SM1T2) to the free thread of the second mirror mount, as shown in Figure 15.

Note: Use a level to ensure that the cage rods are aligned parallel to the breadboard. Adjust the height of the post in the post holder to level the rods. Adjust the **KCB1** right-angle mirror mounts so that they are parallel to each other.
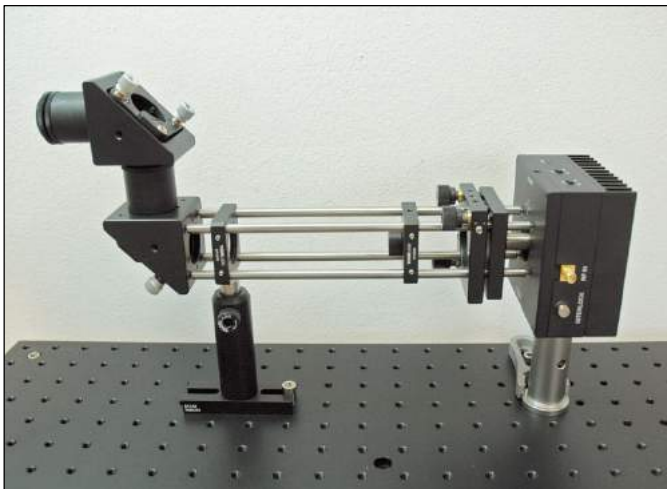
Figure 15 Mirror and Cage System on the Breadboard

6.1.4.   **Microscope**

Attach the damped DP14A(/M) Ø1.5" mounting post to the breadboard using the mounting holes in its base. In the following procedure, the individual parts of the microscope must once again be built before the microscope system can be created.

Beamsplitter

Attach an SM1CP2 end cap to the left side of the CCM1-BS013(/M) beamsplitter cube as shown in Figure 16.

Figure 16 Beamsplitter Cube Components

Objective

Figure 17 shows the components of the objective assembly. First, attach the objective to the CP02(/M) cage plate using the SM1A17 adapter. Then, mount the cage plate to the C1498(/M) post clamp using an SD1 counterbore adapter and an 8-32, 1/2″ long (M4 x 10 mm) cap screw. Ensure that you do not touch the bottom of the objective with your fingers, in order to prevent possible contamination.

Figure 17 Microscope Objective Components

LED Assembly

The components for the LED assembly are shown in Figure 18. First, attach the MCWHL5 LED to the CP02(/M) cage plate using the lens tube coupler (SM1T2). Connect the lens tube to the CP02(/M) cage plate. Mount the cage plate to the C1498(/M) post clamp using an SD1 counterbore adapter and an 8-32, 1/2″ long (M4 x 10 mm) cap screw.

Figure 18 LED Components

Lens 3

Figure 19 shows the components for the lens 3 assembly. Mount the LB1676 lens to the CP02(/M) cage plate using two retaining rings.

Figure 19 Lens 3 Components

Bandpass filter

Attach the FES0650 bandpass filter to the CP02T(/M) thick cage plate. Figure 20 shows the necessary components. Note that the filter has a defined direction of light propagation. This is marked on the frame of the filter with a small white arrow. This arrow should be pointing up during assembly.

Figure 20 Bandpass Filter Components

Camera System

Figure 21 shows the components for assembling the camera system. First, install the two FGB37 filters in the SM1L20 lens tube using retaining rings. Screw one retaining ring into

the lens tube, then insert the first filter, and then secure it with a second retaining ring. Insert the second filter, and then insert a third retaining ring to secure it (using the unused retaining ring from the LED's cage plate. Then, connect the DCC1645C camera to the lens tube using the SM1A9 adapter. After this, screw the other side of the lens tube onto the CP02(/M) cage plate with the SM1T2 lens tube coupler. Finally, attach the cage plate to the C1498(/M) post clamp using an SD1 counterbore adapter and an 8-32, 1/2″ long (M4 x 10 mm) cap screw.

Figure 21 Camera System Components

Microscope Assembly

Now the individual components can be assembled into the microscope. First, screw the CCM1-BS013(/M) beam splitter to the SM1T2 lens tube coupler on the end of the SM1L10 lens tube attached to the second adjustment mirror. Then, attach the four shorter ER2 rods to the bottom of the beam splitter and the four longer ER3 rods to the top.

Note: Use a level to ensure that the cage rods are aligned parallel to the breadboard. Adjust the height of the post in the post holder to level the rods.

Figure 22 Microscope Assembly

Next, push the cage plate with the objective onto the shorter ER2 rods from the bottom and attach the cage plate to the DP14A(/M) post by tightening the setscrew of the attached C1498(/M) post clamp. Once again, take care not to touch the optical components of the objective with your fingers. Attach the LED assembly beneath of the microscope by attaching the C1498(/M) post clamp to the DP14A(/M) post and tightening the setscrew. Figure 22 shows the current setup of the microscope.

Install the bandpass filter onto the upper cage rods. Tighten the setscrew on the post clamp to lock it into place. Next, place lens 3 approximately 5 cm above the beamsplitter by pushing it onto the upper cage rods. Tighten the setscrews on the cage plate to lock it into place. After this, attach the camera system above the lens by sliding the cage plate onto the upper cage rods and attaching the post clamp to the mounting post.

The optical tweezers are now complete except for the sample positioning table and the control elements. Figure 23 shows the current assembly.

Figure 23 Complete Trap Laser and Microscope System

6.1.5.   Sample Positioning System

Like the previous units, the control and positioning unit consists of several individual subassemblies, which are constructed first.

Stage

Figure 24 shows the components to construct the sample positioning stage. First, install the MT1(/M)-Z8 motorized stage on the MT401 base plate so that the actuator points to the right. Then, install the second MT1(/M)-Z8 motorized stage so that its actuator points to the rear. After this, attach the MT402 angle bracket to the top of the second motorized stage. Attach the MT1(/M) stage with the micrometer screw to the back of the angle bracket, with the micrometer screw pointing upward. The small dowel pins can be used to ensure the stage is attached correctly. Attach the second MT402-SLH-SP angle

bracket to the micrometer-driven stage, and attach the AMA-SLH slide holder to it. Figure 25 shows the finished stage.
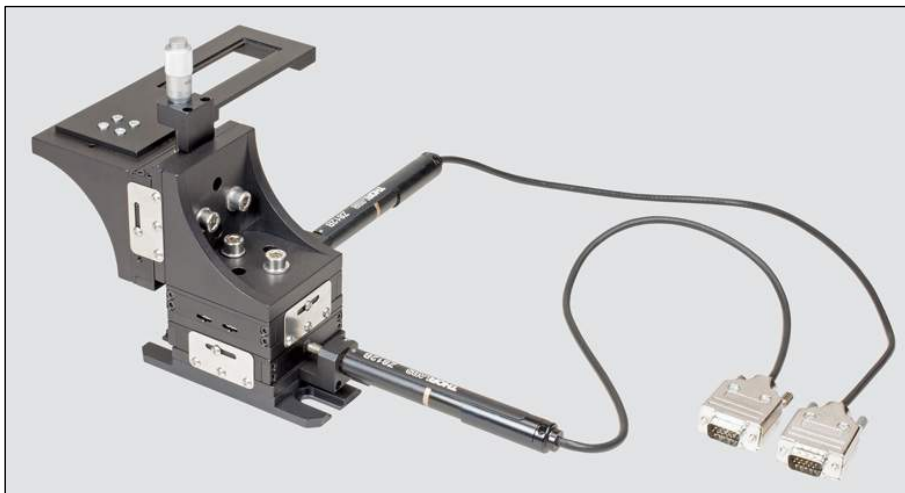

Figure 24 Stage 1 Components

Figure 25 Assembled Stage

Now, attach the assembled stage on the breadboard. Ensure that the sample holder is centered under the objective. Next, install the electronic controls onto the breadboard. These are shown in Figure 26.

Figure 26 Control Components: 2x KDC101, LEDD1B and TLD001 Controller

First, connect the LEDD1B LED Driver to the MCWHL5 LED and position it on the breadboard. Then, place the two KDC101 K-Cube Stage controller cubes in their positions and connect them to the stage and to the computer using USB cables. Next, connect the TLD001 laser diode controller to the LDM9T mount, and mount it on the breadboard. In addition, you must install the supplied software programs for the operation of the DCC1645C camera and the cubes (KDC101, TLD001). In order to use the optical tweezers, they must still be adjusted. You can find the instructions for this in section 6.3, Adjustment.

6.2.   Controller and Software Setup

6.2.1.   LED Controller

The front of the LED Controller has a current limit adjuster. Set the maximum current of 1A when using the MCWHL5 LED. The mode switch on top of the controller should be set to CW.

6.2.2.   Laser Controller

The back of the laser diode driver has six small switches, which set the correct current range to the laser diode. Set up these switches to match Figure 27 for correct laser diode operation.
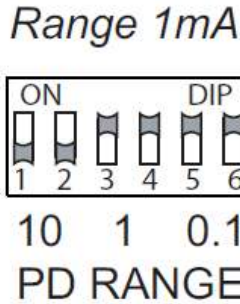
Figure 27 Laser Controller Switch Settings

Next, install the Kinesis or APT control software[12], which will allow for control of the laser diode controller and sample positioning stages. The software can be installed using the provided CD or downloaded from the Thorlabs website. Connect the laser controller to the PC only after you were asked to during the installation. Afterwards, open the newly installed program.
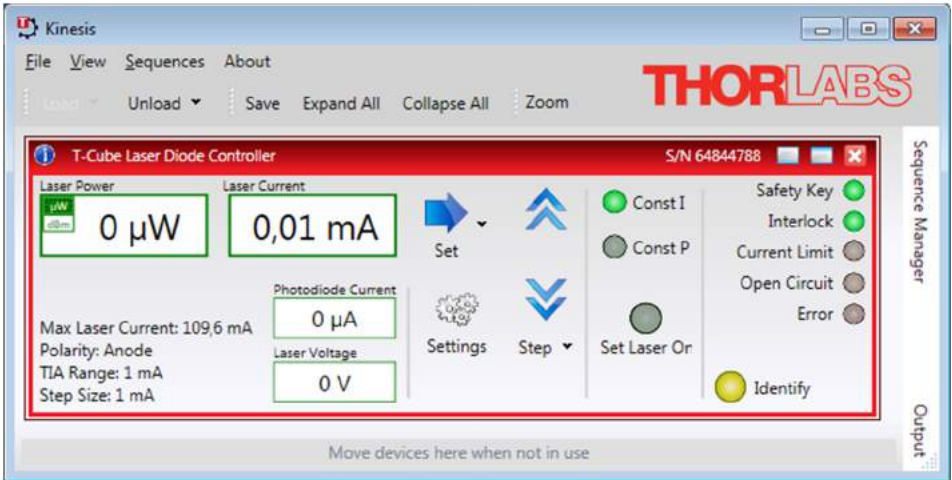

Figure 28 Kinesis Laser Controller Panel.

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[12] Thorlabs offers two platforms to drive our wide range of motion controllers: our legacy APT™ (Advanced Positioning Technology) software package or the new Kinesis software package. The legacy APT System Software platform features ActiveX-based controls. The Kinesis Software features new .NET controls. The software packages allow two methods of usage: graphical user interface (GUI) utilities for direct interaction with the controllers 'out of the box', and a set of programming interfaces that allow custom-integrated positioning and alignment solutions.
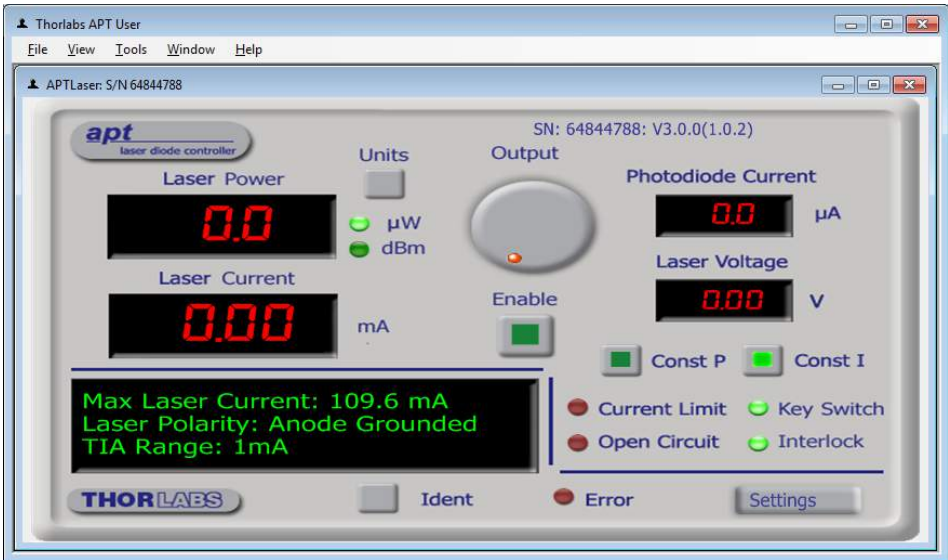
Figure 29 APT-Laser Controller Panel

In the Kinesis (Figure 28) or APT (Figure 29) control panel press "settings". To operate the laser properly, the polarity setting of the diode will need to be switched to "Anode (Grounded)". Additionally you should also set the maximum current for safe operation of the diode in the tab "Max (Laser) Current" to 110 mA (Figure 30 and Figure 31). By activating the "Persist Settings to Hardware" checkbox, you can store the settings on the device.

The current range of L658P040 laser diode is 75 mA (typical) to 110 mA (maximum). It should be operated within this range. We recommend an operating current just below 100 mA.
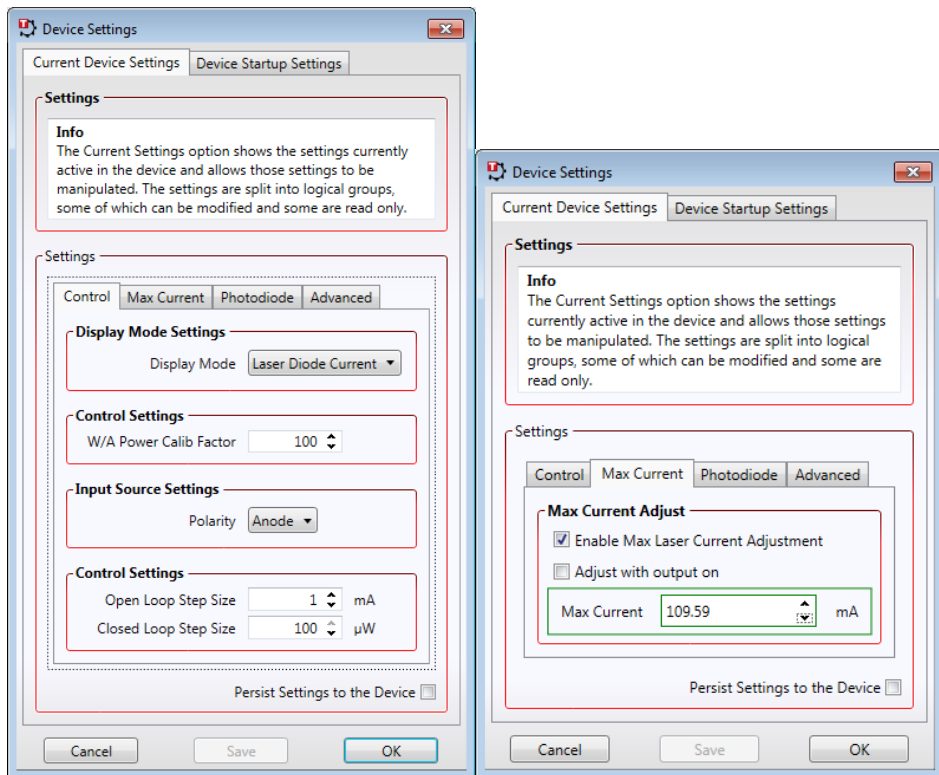
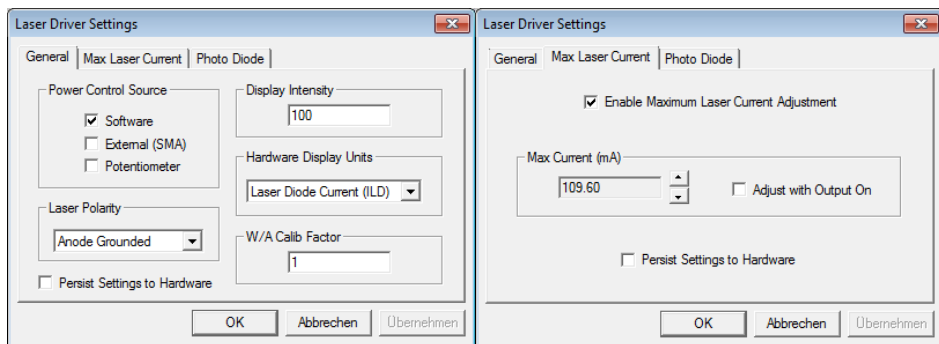**Figure 30 Settings of the Laser Diode Controller with the Kinesis Software**

Figure 31 Settings of Laser Diode Controller with the APT Software

6.2.3.   Camera

To operate the camera download the latest ThorCam Software from the Thorlabs website or use the installation CD included with the camera.[13] Follow the installation guide and select *DCU/DCC Series Compact CMOS and CCD Cameras*. After finishing the installation reboot and connect the camera to your computer. After the driver initialization you can start the ThorCam Software.
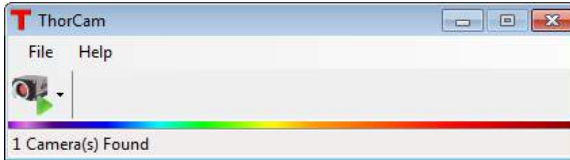

Figure 32 ThorCam start panel

Choose the camera you want to activate and press the start button (see Figure 32). This will start the software front panel with a black screen shown in Figure 33.
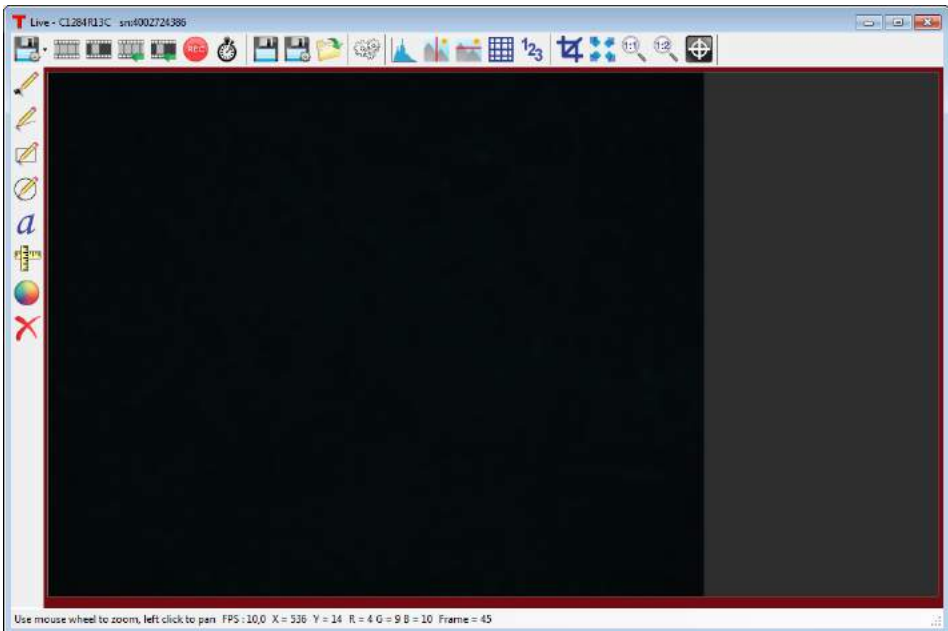

Figure 33 Home Screen of the ThorCam DCC front panel

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[13] https://www.thorlabs.de/software_pages/ViewSoftwarePage.cfm?Code=ThorCam

To see live transmission from the camera, select 🎞️ *Start Capture* from the command panel. You can change the brightness of the LED to check the signal coming from the camera.

The image properties need to be changed such that we can observe the laser spot later on. To do so, open the ⚙️ *Settings* to open the window shown in Figure 34.
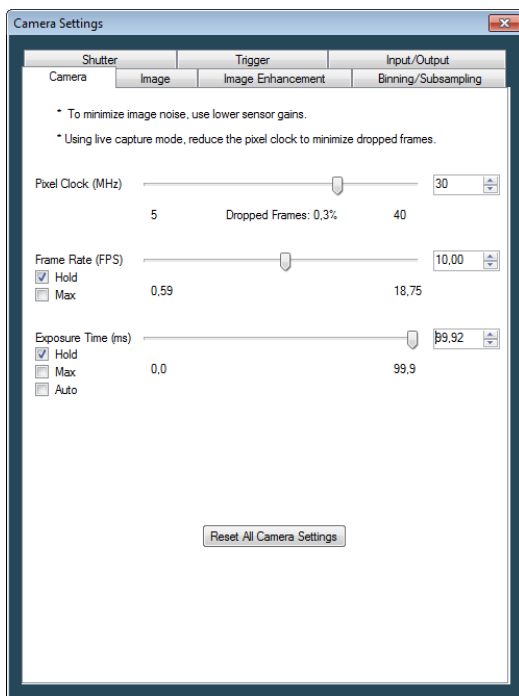
Figure 34 Settings of ThorCam DCC Camera Software

In the Camera tab you can choose the *Pixel Clock*, *Frame Rate* and *Exposure Time*. The basic parameter for camera timing is the pixel clock. It determines the speed at which the sensor pixels are read out.

We recommend setting the **pixel clock** no higher than necessary to achieve the desired frame rate. Adjust the pixel clock while observing the percentage of dropped frames shown beneath the Pixel Clock slider.

Frame Rate: The possible range of settings for the frame rate depends on the currently selected pixel clock. You can select a lower frame rate without changing the pixel clock. To set a higher frame rate, however, the pixel clock might need to be increased. For the optical tweezers application, we recommend a frame rate of 10 FPS or higher.

Exposure Time: The exposure time depends on the currently selected frame rate and is preset to its reciprocal value. A shorter exposure time can be set without changing the frame rate. A longer exposure time will reduce the frame rate.

For **image improvements** adjust the settings in the *Image* tab. The recommended settings are shown in Figure 35. The high red gain accommodates for the red bandpass filter in front of the camera (to avoid laser damage).
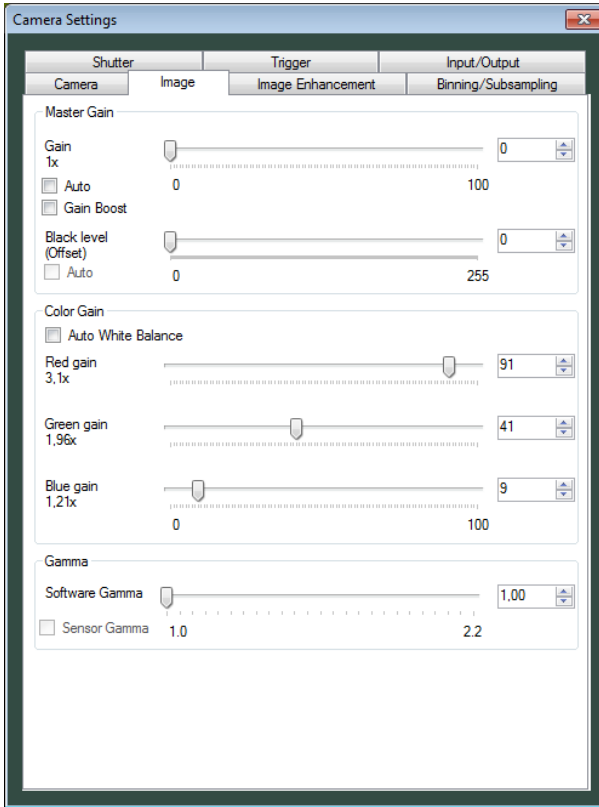
Figure 35 Image Settings

With the ThorCam software you can also save 🖫🖫 snapshots during live imaging or 🔴🔴 record videos. The recording dialog box shown in Figure 36 gives you the option of limiting the recording size by setting the maximum number of frames or the maximum file size in MB.
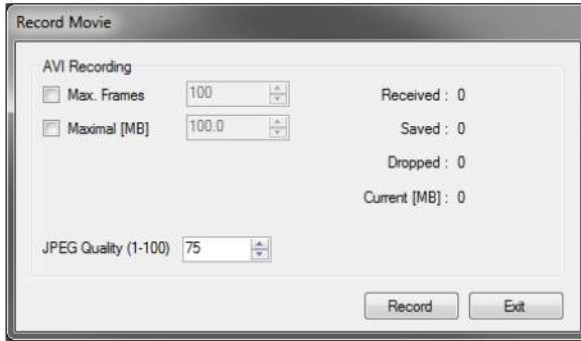
Figure 36 Record Video Settings

For further information on the settings check the ThorCam user guide installed in the program's folder.

Former uc480 software:

In the current revision we recommend using the ThorCam software described in the previous section. Former kit revisions included the uc480 software package . The package contained the "uc480 Manager" and the "uc480 Viewer".[14] If you want to fall back on this software install the software and connect the camera to the PC. After your PC has detected the camera you can open the "uc480 Viewer" program. The first dialog is depicted in Figure 37.

Figure 37 Camera Software Initialization

Choose "Live Video" to proceed to the viewing window depicted in Figure 38.

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[14] http://www.thorlabs.de/software_pages/ViewSoftwarePage.cfm?Code=ThorCam# in Tab: Archive
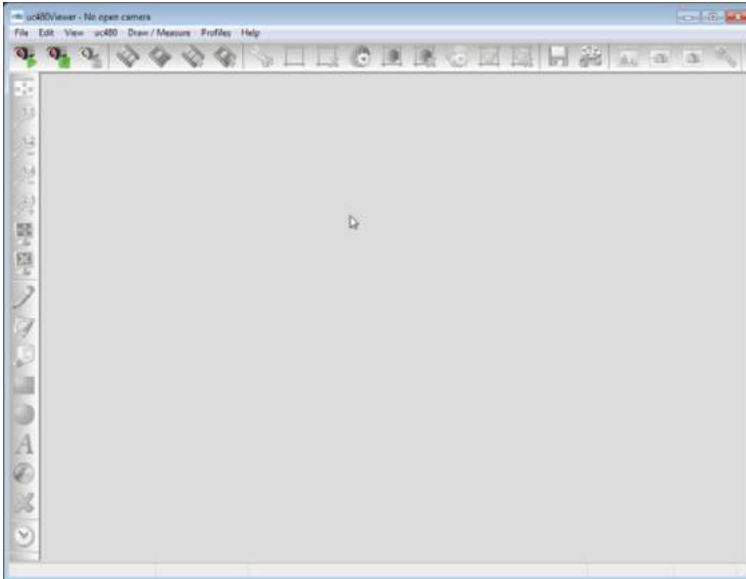
Figure 38 Camera Software Home Screen

Next, the camera has to be initialized. For that, choose the uc480->Initialize, or press the play button in the upper left corner. The image will appear in the window.

Then, the image properties need to be changed such that we can observe the laser spot later on. To do so, open the menu uc480->Properties to open the window shown in Figure 39.
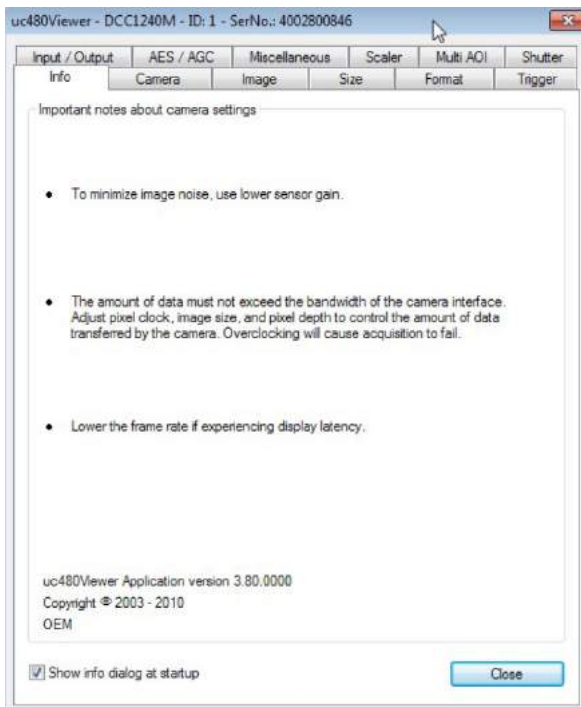
Figure 39 Camera Software Properties Dialog

Choose the Image tab. The red, green and blue gain can be set. Values such as the ones in Figure 40 have shown good results in our testing.
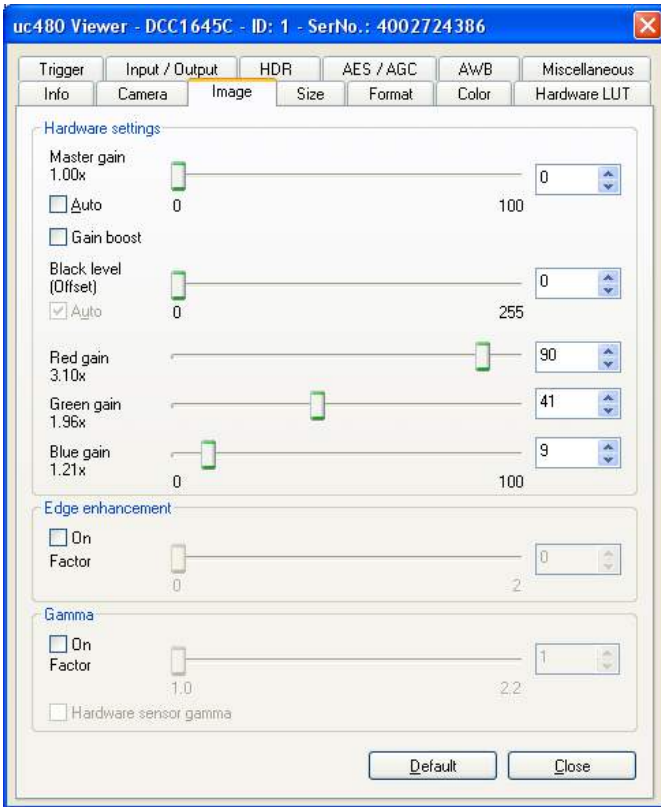
Figure 40   Camera Software Image Properties

The control buttons in the "uc480Viewer" can also:

     take a snapshot.

     record a video.

To record a video, either choose the button shown above or you choose File->Record Video Sequence. The window shown in Figure 41 will pop up.

Figure 41 Camera Software Record Dialog

Using "Create" a new file with the desired title of the video can be generated. After pressing the "Save" button, the video can be started ("record") or stopped ("stop") any time.
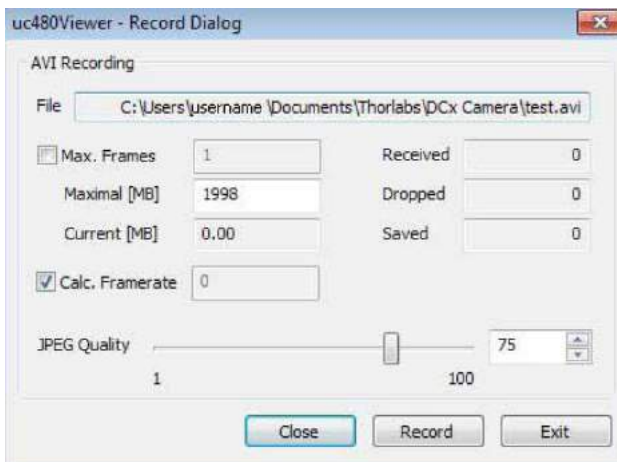
Figure 42 Camera Software Record Dialog

6.2.4.   Sample Positioning Stages

The stage is driven by Z812B brushed motors, which are controlled by two KDC101 devices. For optical trapping, low driving speeds are necessary. To adjust the velocity of

the servo motors you can either use the display of the KDC101 or the configuration software. You can find the software via Thorlabs product page of KDC101.[15]

Method 1: Configuration directly via KDC101 display and wheel: Power up the controller and open the menu on the KDC101 controller via the menu button, select "set velocity" with the wheel and confirm with the menu button. You can now set the velocity to the recommended 0.01 to 0.05 mm/s. Furthermore select "Joystick Mode" in the menu and chose "Velocity Control".

Method 2: Configuration via Software: The controllers can also be configured via Kinesis or APT software as shown for the laser diode controller. If not installed yet, download the desired software and follow the installation instructions. Connect the controllers via USB when asked or after the complete installation.

Start the *Kinesis* or *APT User* software. The controllers are shown automatically. Check the device serial numbers in the software and on the K-cubes.

To change the settings of the controllers, open the program "Kinesis" or "APT User".

The two windows, shown in Figure 44 and Figure 43, correspond to the two servo motors. To establish which window controls which controller cube, press the "*Ident*" or "*Identify*" button in the software, which will flash the display a few times on the corresponding controller.
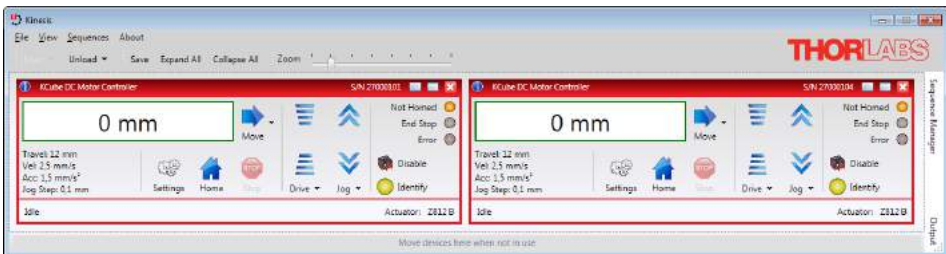
Figure 43 Kinesis Software User Interface

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[15] www.thorlabs.com/newgrouppage9.cfm?objectgroup_ID=2419

Figure 44 APT User Interface

Kinesis Software: Press the *Settings* button in the Kinesis software and select the "Advanced" tab (Figure 45). In the *MMI Controls* field, choose Velocity control. The recommended *Max. Velocity* is set to 0.05 mm/s and *Acceleration* to 1 - 2 mm/s^2. The recommended *Drive Array Velocities* are set to *Velocity 1*: 0.01 *Velocity 2*: 0.02 *Velocity 3*: 0.03 *Velocity 4*: 0.05. This defines the different velocities which can be accessed via drive buttons in the software as shown in Figure 46. The controller wheel deflection is proportional to the max. velocity. The settings need to be entered for each servo motor separately. All other settings remain default values.
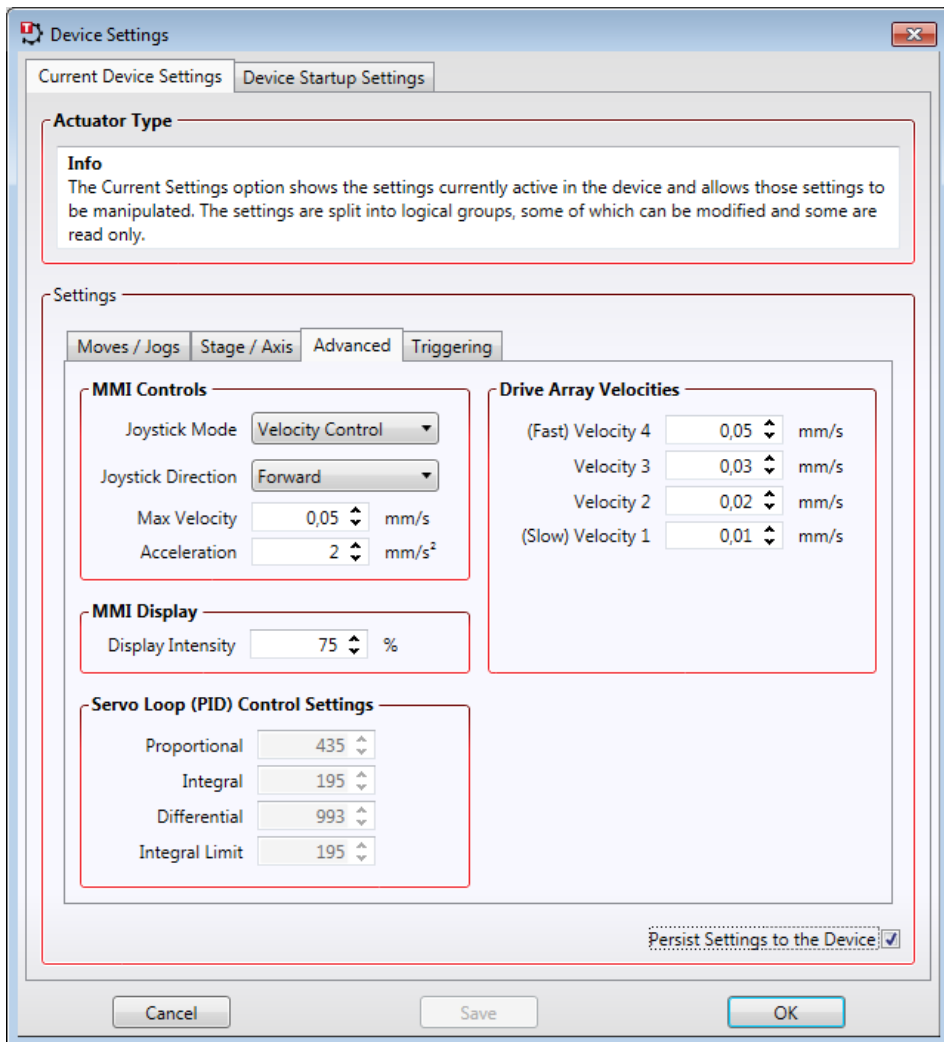
Figure 45 Kinesis KDC101 Settings
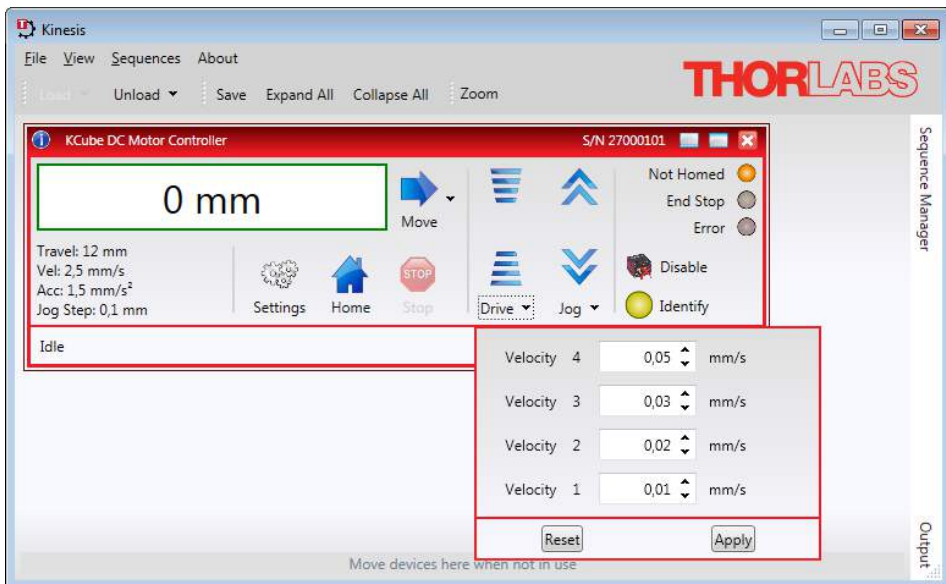
Figure 46 Kinesis Drive Array Velocity

APT Software: Press the *Settings* button in the APT software and select "Panel/Triggering" tab (Figure 47). The recommended Wheel Max. Vel. is set to 0.05 (mm/s) and Wheel Accn to 2. The settings need to be entered for each servo motor separately. All other settings remain default values.
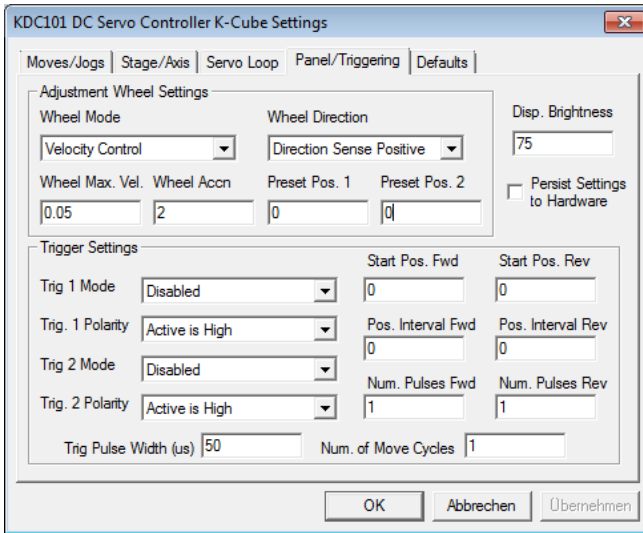
Figure 47 APT User Settings

Important Note: By clicking OK, the settings are sent to the device. By checking the *Persist Settings to the Device* checkbox the settings are stored in the device and loaded after each restart of the controllers. In the Kinesis Software you can also enter all parameters under the *Device Startup Settings* tab. By clicking *Save*, this will store the settings on your computer as well. When the controllers are reconnected to this computer they will download the stored settings immediately. You can find further information in the KDC101 manual.

Previous version T-cube TDC001 APT settings: To specify the speed of the actuator motors, press the "Settings" button and go to the "Advanced" tab In total, 4 velocities ("Velocity 1-4") can be entered, in units of $\frac{mm}{s}$. Make sure that "Velocity 1" is the smallest value and "Velocity 4" is the largest value. The chosen values correspond to the motor velocities for different displacements of the lever. When the lever is at its maximum displacement, the stage moves with the velocity 4. The settings only apply to the physical levers when the box "Persist Settings to Hardware" is checked and "OK" is pressed. The settings need to be entered for each servo motor separately. Figure 48 shows the default values of the software which are too high for the optical tweezers setup. A recommended set of parameters is: Velocity 1: 0.005, Velocity 2: 0.010, Velocity 3: 0.030, and Velocity 4: 0.050.
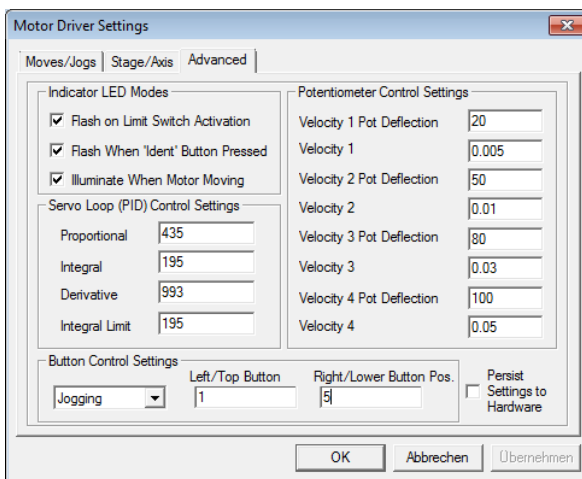
Figure 48 APT Settings with Default Values

6.3.   Beam Adjustment

In order to adjust the laser beam, both lens 1 and lens 2 must be removed from the beam path. In the case of lens 1, the lens can be removed from the cage plate by removing the entire lens tube. In order to remove lens 2 from the beam path, a retaining ring must first be removed from the cage plate.

The second (upper) adjustment mirror must also be removed from its mount. To do this unscrew the set screw, place a piece of adhesive tape on the back of the mirror and pull it out of the mount.

Warning

The class 3B laser diode used in this kit emits up to 42 mW of optical power, which can cause damage to the eyes if viewed directly. The laser driver is equipped with a key switch and safety interlock, which should be used appropriately to avoid injury. Additionally, we recommend wearing appropriate laser safety glasses when using this kit.

Now, switch the laser on. First, the collimator must be positioned to properly collimate the beam. The collimator is adjusted by sliding it along the cage rods. Place the cage system alignment target (CPA1) on the cage rods to precisely adjust the laser beam. Place the target directly in front of the mirror and align the collimator so there is a sharp spot in the center of the target, as in Figure 49. It is important to note that the collimator should be positioned as close as possible to the laser diode mount and moved from there till the beam is collimated properly. Positions further away from the laser diode mount can also

lead to a collimated beam but may not lead to a good alignment and lead to problems with laser trapping. To check the collimation move the CPA1 along the cage rods to make sure the beam stays in the center of the target. Use the tip/tilt mount holding the collimator lens to make adjustments, if necessary.

Figure 49 Laser Spot on the CPA1 Cage Alignment Tool

After this, remove the target from the beam path and observe the laser spot on the ceiling. It should also be sharply visible and have a diameter of a few millimeters. In addition, it should run vertically upward. Adjust the first (lower) mirror if necessary.

Then, switch off the laser and reinstall the second mirror. Remove the cover on the left side of the beam splitter. Switch the laser on and check whether the laser beam exits in the center of the opening on the beam splitter. If this is not the case, correct the position of the beam using the adjuster screws on the mirror. Switch the laser off again.

Now, reinstall the two lenses, which serve to expand the laser beam Lens 1 must be installed with the lens tube facing to the left. Lens 2 must also be installed with the convex side facing to the left. Slide the cage plate holding lens (1) along the cage rods so the distance between them is approximately 11 cm (measured from the center of the cage plates). After this, remove the upper adjustment mirror from its bracket again and switch the laser on.

The laser spot on the ceiling should now have a diameter of approximately 2 cm and should still exit vertically. After this, replace the mirror. Ensure that the input of the objective is completely filled by the beam. The laser spot should overlap with the aperture of the objective such that a several mm thick red margin is observable around the aperture. This overlap is extremely important because if the entire laser beam is focused into the objective, a large enough gradient force for trapping will not be present.

6.3.1.   Microscope Focus and Final Alignment

Now, make a sample with, for example, polystyrene-beads (see Chapter 7: Experiments). Switch on all devices (computer, APTs, LED lighting, laser), start the operating programs (camera, ATP's) and proceed as follows:

- Place the sample positioning table as low as necessary with the aid of the micrometer screw for the z-direction and place the selected sample under the objective

- Now, raise the sample positioning table with the micrometer screw and pay close attention to the camera image on the monitor of your computer

After certain distance, you will see a laser spot in the form of a red, distorted spot on the monitor. This is not the sample, but rather the top of the cover glass. The second visible spot, which is found by approaching the sample, shows the bottom of the cover glass. The third spot shows the sample. Then, the focus is at the right level. Figure 50 shows a schematic representation of the situations.
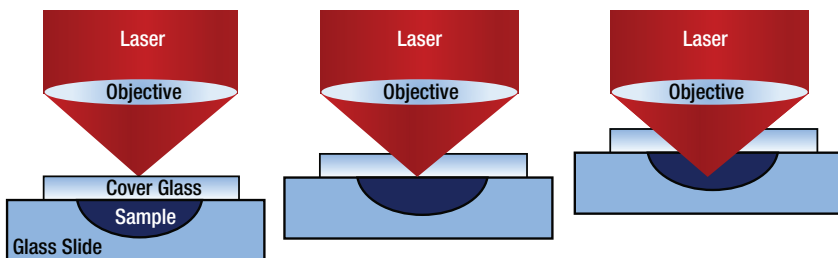
Figure 50 Focus Level Settings

If the laser has not yet been correctly adjusted, you may not see any laser spot on your screen. Position the sample under the objective by means of the micrometer screw so that you can clearly see the beads on the screen. Try to adjust the laser beam with the aid of the locking screws on the second adjustment mirror so that you can see them on the screen as a small, red spot. Here, ensure that you have set the red amplification to maximum when adjusting the live image. If you have trouble seeing the laser spot, try dimming the LED.

Laser Focal Plane vs. Microscope Focal Plane

The position of the laser focus in the z-direction is defined by the focal length of the objective (red curve). For ideal observation of both the laser focus and the trapped

sample beads it is necessary to adjust the microscope (tube) lens (shown in Figure 51) in such a way that the focal plane of the observing camera system (green curve) - consisting of the objective, the tube lens and the CCD camera chip - coincides with the fixed focal plane of the laser. When adjusted correctly, the focused laser spot, as well as the sample beads in the plane of the focus, are both visible through the microscope.

Position the tube lens roughly as shown in Figure 23. Place a sample onto the sample stage, observe the camera image and move the stage up and down tentatively. You might find the laser focus in a different plane as the beads – that means that further adjustment is required. Move the tube lens in the z-direction until you have a sharp image of both the beads and the laser spot. If beads are pulled out of the focal plane when trapped, the focal planes do not coincide.
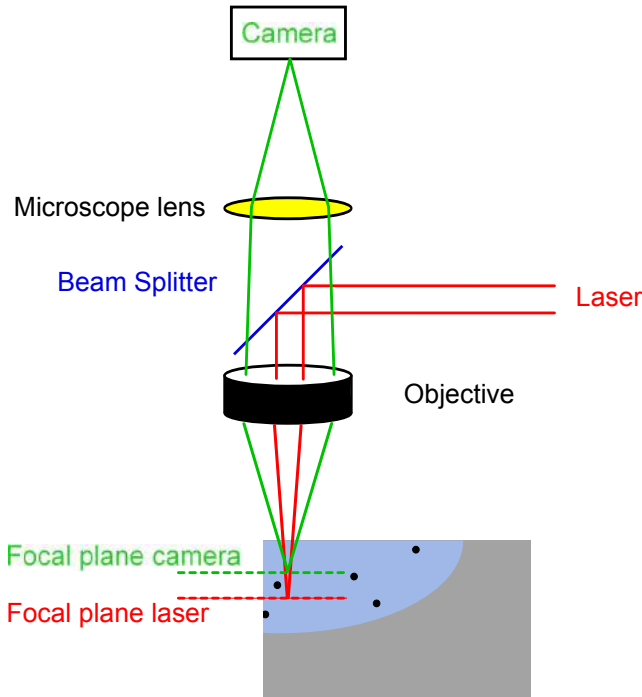

Figure 51 Laser and Camera Focal Planes

The laser is optimally adjusted when the beads can be trapped and moved using the two motorized stages.

Chapter 7 Experiments

In this chapter, we discuss exercises using glass beads as samples. The material can be found in the **OTKBTK** preparation kit, which is sold separately on the Thorlabs webpage. However, these experiments can also be conducted with several other materials, including polystyrene beads or lipids in aqueous solution.
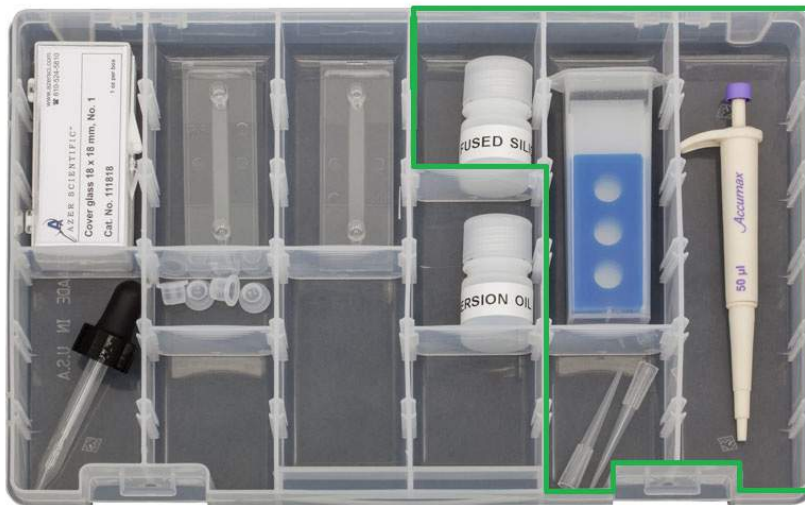
Figure 52 Content of the OTKBTK preparation Kit with tools for glass bead sample preparation (green highlighting).

7.1.   Creating a Sample

A sample containing 1 µm or 3 µm silica beads is useful, as these are well-suited for getting to know the operation and handling of the optical tweezers. The following materials are necessary to create the sample:

- Microscope slide with around 20 µm deep cutouts

- Cover glass

- Pipette

- Solution with fused silica (glass) beads

First, shake glass bead solution and place a small droplet (approx. 25 µl) in a cutout on the microscope slide using a pipette. Place a cover glass over the sample, starting with the glass tilted at a steep angle and lowering it so that there are no air bubbles between the glass and the sample (see Figure 53). Remove excess solution with a tissue.

Figure 53 Droplet of Bead Solution on Glass Slide

The samples can either be prepared before each experiment or they can be sealed between the slide and the coverglass with a UV adhesive. We recommend allowing students to prepare new samples as an educational exercise.

7.2.   Setting the Correct Focus Level

Place the sample positioning table as low as necessary using the micrometer screw on the z-axis stage and place the selected sample under the objective. Now, raise the sample positioning table once again using the micrometer screw and pay close attention to the camera image on the monitor of your computer.

After a certain distance, you will see a laser spot in the form of a red, distorted spot on the monitor. This is not the sample, but rather the top of the cover glass. The second visible spot, seen after moving the objective closer to the sample, shows the bottom of the cover glass. Moving the objective closer to the sample, the third visible spot is inside the sample. Once this is in view, the focus is at the right level. Figure 54 shows a schematic representation of the laser focus levels.

Note: It is possible that large red laser reflections are observed which do not change even when the z-axis (focal plane) is changed. They originate from the beamsplitter and have nothing to do with the laser spot of the optical trap. Also, do not move the sample positioning table to far upwards. Otherwise, there is a risk of destroying the cover glass by touching it with the objective. This will damage the objective and would entail unnecessary cleaning work.
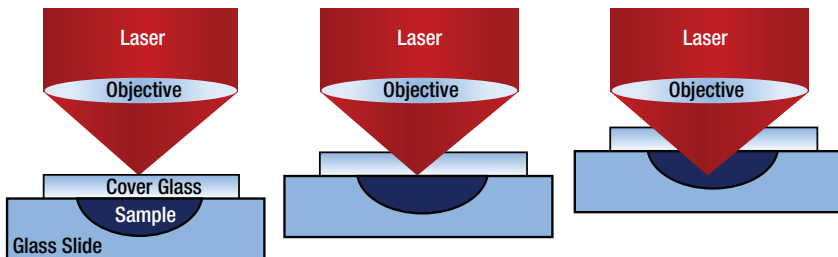
Figure 54 Focus Level Settings

Set the laser focus so that you see the sample beads clearly and sharply. By moving the laser spot onto a bead using the servomotors and levers on the K-cube controllers, you can trap a bead and move it to another location. If you are able to capture one of the beads, you have found the correct focus level.

At this point, if the trapped beads move out of the focal plane, it indicates that the focal planes of the laser and camera system are not aligned. See Section 6.3.1, above, for details

7.3.   Arranging the Silica Beads

In order to get to know the optical tweezers better, try next to move as many beads as possible to one location using the K-cube controllers as shown in Figure 55. These controllers allow you to set different speeds. You can use this to your advantage when releasing the beads.

If you experience lagging of the camera image, try to reduce the frame rate in the camera settings.

Hint

With older samples, one can observe that some beads no longer move, but instead "stick" in place.
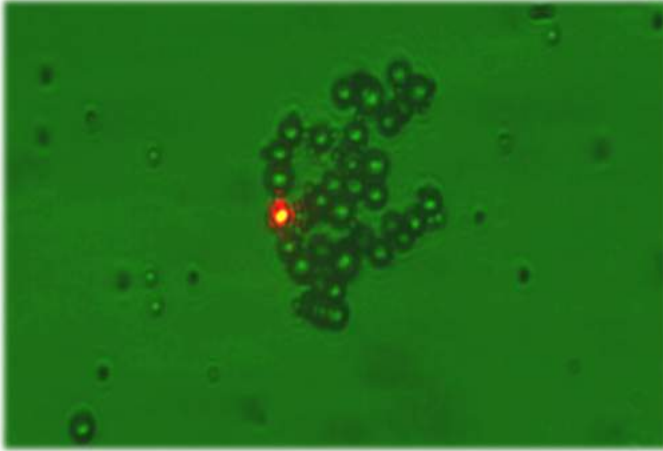
Figure 55 Beads as Viewed Through the Microscope

7.4.   Manipulation of a Dairy Cream Particle within a Cream / Water Emulsion

Mix a drop of dairy cream with sufficient water so that the solution is still slightly milky. Now, create a finished sample for the optical tweezers as described in Section 7.1, above. Observe the solution under the optical tweezers.

Exercise

What do you observe? Try to bring a particle into the beam path. What happens? Then, try to track the particle by changing the $z-$ position. Where is it? Switch the laser off and observe the particle. What is happening here?

Solution

In this part of the experiment, a sample must first be created out of a cream/water emulsion. If one attempts to trap the cream particles with the laser, it is observed that they disappear from the focus and can no longer be clearly seen on the monitor (see Figure 56). The explanation for this observation can be found by examining more closely the cream/water emulsion. Cream consists primarily of fat, which collects on the surface when mixed with water. The cream particles, which are trapped by the laser, are therefore located on the surface of the water. However, the laser focus is located at a deeper level, so when the cream particles are trapped, they are pulled down into the emulsion. This effect can be observed when the particle at which the laser is directed is tracked with the aid of the z-axis screw.
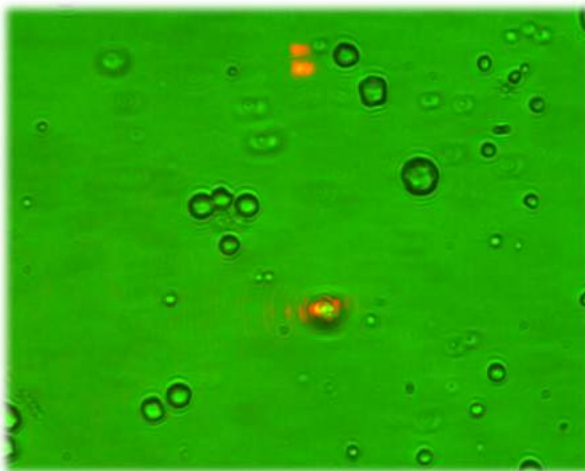
Figure 56 Cream Particles in a Cream / Water Emulsion

If a cream particle, which is located in the optical trap, has been brought into focus with the z-axis screw and thus can clearly be seen on the monitor, the laser can be switched off and the particle observed. Switching off the laser causes the cream particle to no longer be located in the optical trap and thus to move upward once again to the surface of the water. This procedure can be observed again with the aid of the z-axis screw.
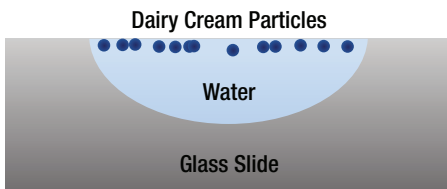
Cream particles collect on the surface of the water.
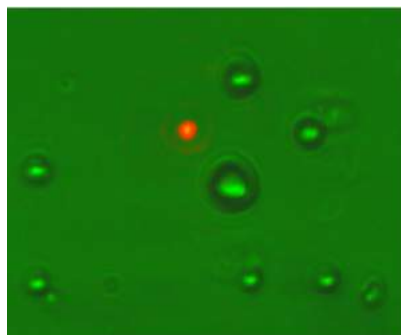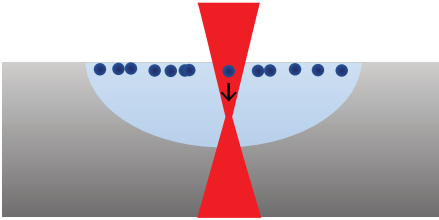
A cream particle is pushed down by the laser focus.

Cream particle is trapped at the laser's focus.

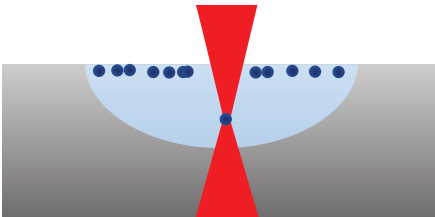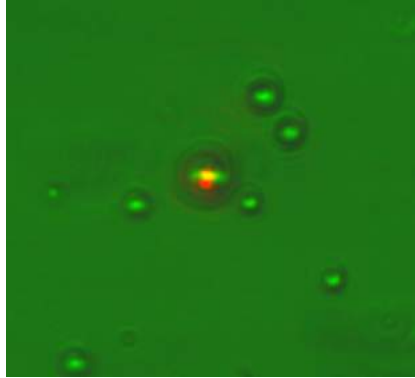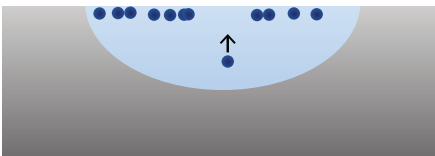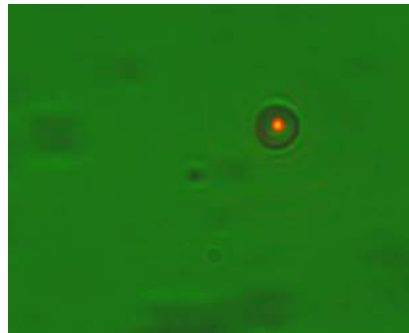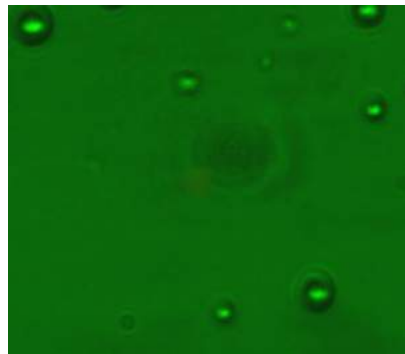Once the laser is switched off, the fat particle moves back to the surface of the water.

7.5.   The Holding Force of the Optical Trap

7.5.1.   Brownian Motion

The proper motion or vibrating motion of particles, also referred to as Brownian motion, can be observed under the optical tweezers. Samples can be found at www.polysciences.com "Microspheres and Particles".

Exercise

Explain briefly the causes of Brownian motion and what role it plays in the experiments with the optical tweezers.

Solution

Brownian motion is the statistic vibrating motion (translation and rotation) of microscopic free particles. Under the microscope, the paths of particles are seen as short, straight lines (see Figure 57). The Brownian motion can be observed in experiments under the optical tweezers. The polystyrene beads are located in a medium that consist of molecules. These molecules are constantly moving in all directions. Because of this, the molecules repeatedly bump into the beads, which causes a vibrating motion of the beads that can be observed under the optical tweezers. The molecules move more the higher the temperature.
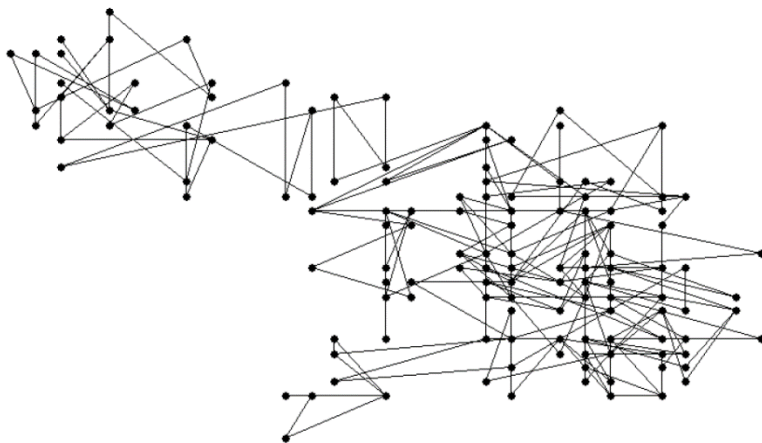
Figure 57 Brownian Motion

Use the sample with the 3 µm polystyrene spheres. You must first switch the laser off so that you can observe only Brownian motion. For evaluation, a video sequence with a duration of 2 minutes or more must be recorded. During this period, about 5 particles, which do not touch each other, should be in the image. A similar video should be recorded with the 1 µm spheres. The videos must be evaluated with the aid of image

analysis software, which provides the x and y position of a particle over time. A program that makes evaluation possible and is offered for free is "Viana", available at http://www.viananet.de/en.

Note: Before taking the video sequence, make sure that the beads do not show a drift in any direction, which can be caused by an air bubble. If necessary, prepare another sample.

We recommend evaluating the data obtained with the aid of a table calculation program and to show the results in a graph. First, the mean squared displacement $r^2(t_i)$ of the beads must be determined. This can be calculated with the positions $(x_i(t_i), y_i(t_i))$ of the particles at different times t_i, which must be taken from the video:

$$r^2(t_i) = \big(x(t_i) - x(0)\big)^2 + \big(y(t_i) - y(0)\big)^2 \tag{19}$$

The time mean value $\langle r^2 \rangle_{time}$ at time t_n results from the averaging of all measured values $r^2(t_1), \ldots, r^2(t_n)$ at the measurement times:

$$\langle r^2 \rangle_{time}(t_n) = \frac{1}{n} \sum_{i=1}^{n} r^2(t_i) \tag{20}$$

In order to eliminate statistically possible deviations of individual particles, it is recommended to once again determine the mean value over M various particles. We recommend doing this for at least 5 particles.

$$\langle r^2 \rangle(t_n) = \frac{1}{M} \sum \frac{1}{n} \sum_{i=1}^{n} r^2(t_i) \tag{21}$$

The obtained values for average displacement, $\langle r^2 \rangle(t_n)$, are now plotted with respect to time. Figure 58 shows an example of mean squared displacement over time for three different sizes of polystyrene beads. Here, each straight line is the mean value of several particles of the same sample. It is recommended to perform a linear fit through the resulting curves in order to obtain the slope, m, of the straight lines.

Exercise

What differences do you expect between the Brownian motion of the 3 μm and 1 μm spheres and why?

Solution

The slope of the lines in Figure 58 decreases with increasing diameter of the beads, meaning that larger beads move less. This result can be easily explained through Brownian motion. The 1 μm spheres can be more easily sent into motion by impact with the water molecules than larger spheres. Therefore, a 1 μm bead travels more in a certain time interval than a larger bead.
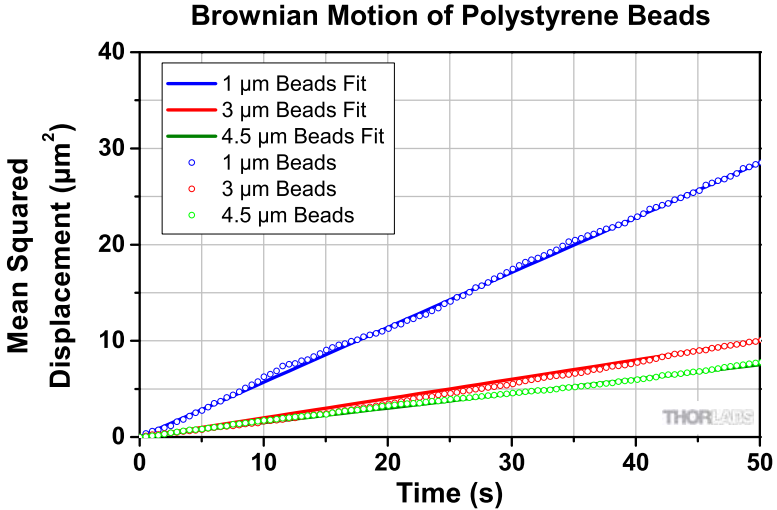
Brownian Motion of Polystyrene Beads

Figure 58 Mean Squared Displacement for Polystyrene Beads of Different Sizes

7.5.2.   The Maximum Holding Force

In the following, we shall determine how well the optical tweezers can hold a polystyrene bead. The goal is to determine the maximum holding force of the optical trap.

If one observes an individual polystyrene bead under the microscope which is moving freely, frictional force acts on the particle. This inhibits the particle in its movement through any liquid. This frictional force F_R is directly proportional to the speed v, with which the spheres move and can be described by the following equation:

$$F_R = 6\pi\eta_{eff}Rv \tag{22}$$

Here, R describes the radius of the bead and η_{eff} describes the effect of viscosity of the suspension. The latter indicates how "thick" the combination of water and beads is and is different for each sample. As the viscosity depends on various factors, it must be determined through experimentation. It depends upon the mean squared displacement of the particles, which was determined in the previous exercise. This resulted in a straight line, whose gradient can be determined by means of the following equation:

$$m = \frac{2k_BT}{3\pi\eta_{eff}R} \tag{23}$$

Here, η_{eff} denotes the effective viscosity, R is the radius of the PS-bead, T is the temperature of the sample in Kelvin (corresponds to room temperature), and k_B is the Boltzmann constant, which is a natural constant and has a value of $1.38 \cdot 10^{23} \frac{J}{K}$.

Exercise

Determine the effective viscosity η_{eff} of the sample with the 3 µm polystyrene spheres, by solving the equation (23) according to viscosity and using the gradient m of the measured curve from the previous exercise.

Solution

The equation for the calculation of the effective viscosity η_{eff} is:

$$\eta_{eff} = \frac{2k_B T}{3\pi R m} \tag{24}$$

Here, T is the room temperature, k_B the Boltzmann constant, a the radius, and m the previously determined gradient of the PS-beads used.

The determined effective viscosity should be in the range of a few $10^{-3}\frac{Ns}{m^2}$.

Exercise

After which speed can the PS-bead no longer be held? Determine the maximum holding force of the optical tweezers.

Solution

If the PS-bead is in the optical trap, two forces act on it. First of all, the frictional force F_R, which is caused by the suspension in which the PS-bead is located, and thus works against the other force, the holding force F_H of the optical trap. The following equation describes the frictional force F_R:

$$F_R = 6\pi\eta_{eff} R v \tag{25}$$

Here, η_{eff} is the effective viscosity of the suspension, R is the radius of the bead, and v is the speed. The maximum holding force is said to have been reached precisely, when the PS-bead at a certain speed v_{max} can just be held. This is the case when both forces are in balance:

$$F_{Stokes} = F_{H,max} = 6\pi\eta_{eff} R v_{max} \tag{26}$$

The holding force is in the range of a few pN.

Chapter 8 Teaching Tips

In this chapter we present a simplified approach to help students in understanding the basic physical principles of optical tweezers. In the following, we discuss how a particle is trapped in a focused laser beam and how a three-dimensional optical trap works.

For keeping an object in place, a restoring force has to act as soon as the object leaves its equilibrium position. Since the object is trapped only by the laser, the restoring force has to be applied by the laser itself.

An essential precondition for trapping an object is that it is transparent to the laser light. This means that at least part of the light has to be transmitted. Part of the laser light is reflected off the object's surface, while the other part is refracted and transmitted. Thereby, the laser faces a change in direction which corresponds to a change in the momentum. This in turn means that there has to be a force between the laser light and the object. Intuitively, one would expect the particle to be deflected. However, in an optical trap the object is drawn into the laser focus.

The laser beam exhibits a certain intensity profile. In order to create an optical trap, this profile needs to possess a point with maximal intensity, such as in a Gaussian profile.
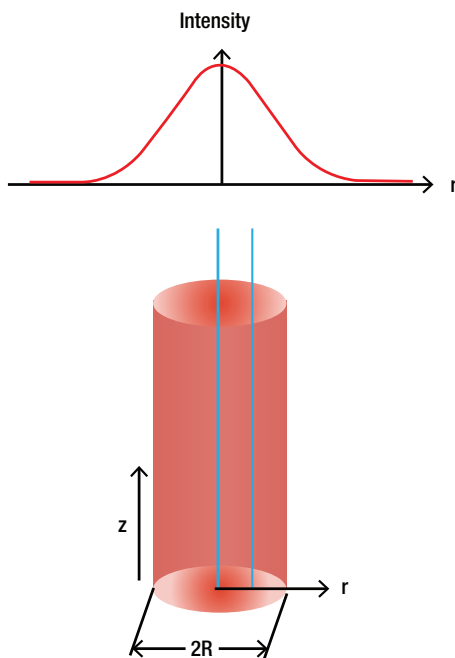

Figure 59 Gaussian Intensity Profile

As shown in Figure 59, the cross section of a laser beam has a radius R. The intensity varies radially: the intensity is maximal in the center of the beam and declines towards the edge. Comparing a ray in the middle of the profile to a ray outside, the inner one shows the maximal intensity while the outer one exhibits a lower intensity. This is demonstrated by the thickness of the blue lines in Figure 59.

The entire laser beam is the sum of all rays. For simplicity, we will only consider two rays in the following which are symmetrical around the beam axis. The insights we gain with these two rays can be transferred to the entire beam.

First, we have a look at an unfocused (collimated) laser beam that is incident on a polystyrene bead. We have to distinguish between two cases:

- The center of the bead is on the beam's main axis
- The center of the bead is not on the beam's main axis

Note: The size of the vector arrows in the following diagrams do not match from diagram to diagram, and instead are enlarged where possible for clarity.

Unfocused Laser Beam, Particle on the Beam's Main Axis:

We consider two partial beams which are symmetrically arranged around the beam axis, as shown in Figure 60. The beams are labeled s and t.
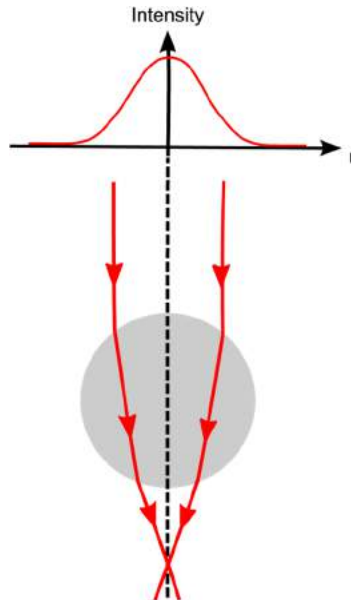

Figure 60 Unfocused Laser Beam Strikes a Polystyrene Bead Located on the Beam Axis

Both partial beams have the same direction and intensity (since they both have the same radial distance to the beam center with highest intensity) and carry the same momentum. In Figure 61, the momentum vectors of the beams are depicted at some positions using arrows in blue and green. The direction of the vector arrow corresponds to the direction of the momentum and its length corresponds to the momentum's magnitude.
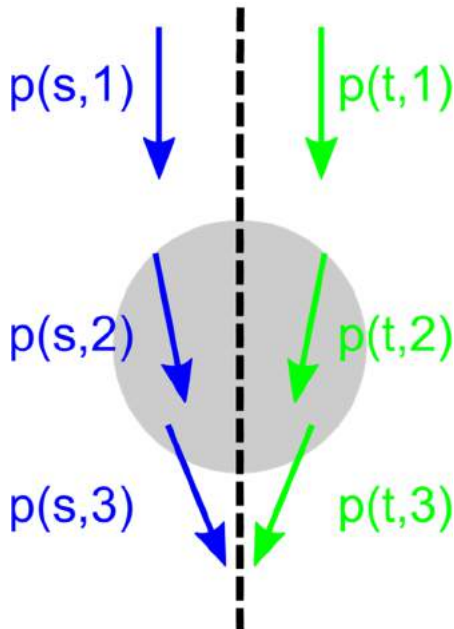

Figure 61   Unfocused Laser Beam Momentum

For example, the vector labeled with $p(s,1)$ depicts the momentum of the beam s at position 1. It important to note that the magnitude of the momentum, i.e., the length of the arrows, does not change. However, the direction changes. In Figure 62, the vectors before the entry of the beam into the sphere (position 1) and after the exit (position 3) are subtracted to see how the momentum changes. The momentum change is depicted as orange arrow.
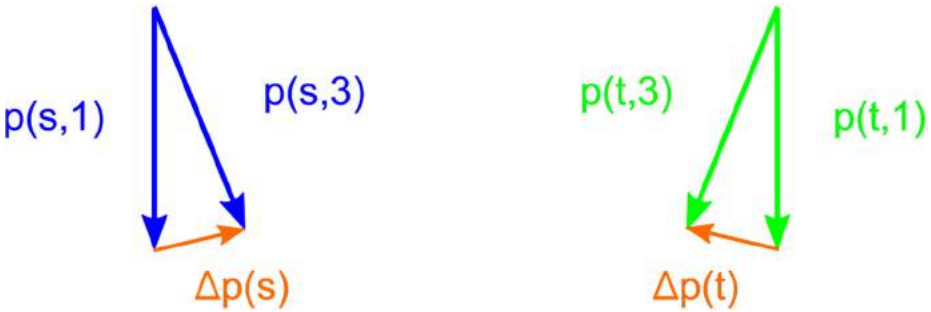
Figure 62 Unfocused Laser Beam Momentum Changes

To obtain the net momentum change of both partial beams, we add both orange change of momentum vectors in Figure 63. This line of argument can be transferred qualitatively to the entire bundle of partial beams.
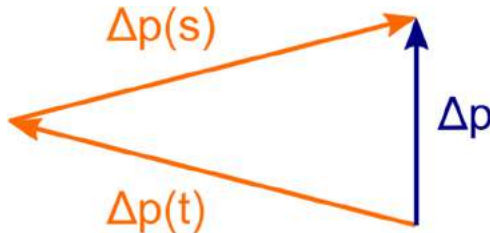

Figure 63 Unfocused Laser Beam Total Momentum Change

In Figure 63 we observe that the total laser beam experiences a momentum change in the opposite direction of its direction of incidence. Momentum conservation then demands a momentum change of the bead in downward direction. This means that the bead is pushed away by the beam, along the optical axis. The beam experiences no momentum change orthogonal to the optical axis, so it stays in the center of the beam.

Unfocused Laser Beam, Particle is not on the Beam's Main Axis:

In the following, we follow the same train of thought as above but with the particle outside the center of the beam. This is depicted in Figure 64.
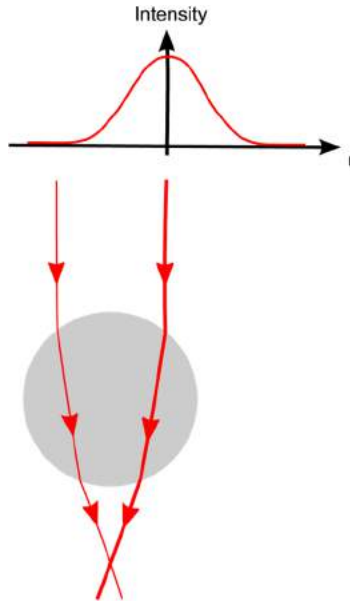
Figure 64 Unfocused Laser Beam Incident on a Polystyrene Beam Located Off the Beam Axis

In this case, the two partial beams do not feature the same intensity. Therefore, they do not have the same momentum, as shown in Figure 65.
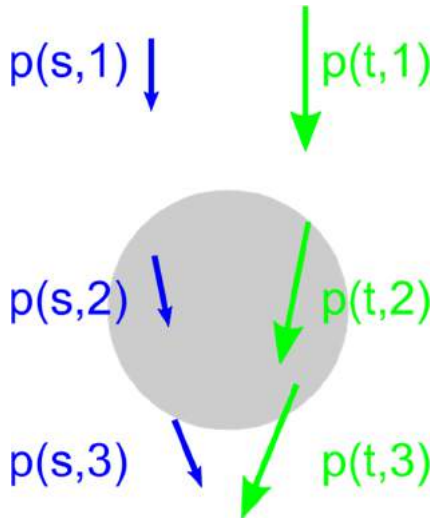

Figure 65 Unfocused Off-Axis Laser Beam Momentum

Both partial beams then exhibit the momentum change displayed in Figure 66.

Figure 66 Unfocused Off-Axis Laser Beam Momentum Changes

As shown, the partial beams exhibit momentum changes that differ in magnitude. Figure 67 shows the resulting net momentum change of the entire laser beam.
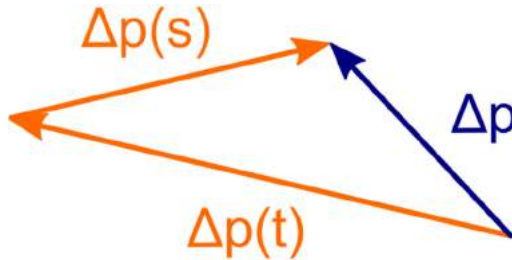

Figure 67 Unfocused Off-Axis Laser Beam Total Change in Momentum

Figure 67 demonstrates that the laser beam experiences a momentum change to the left and upwards. This results in a momentum change of the bead to the right and downwards. Therefore, the bead is drawn to the position with maximum intensity, forming a two-dimensional trap. The bead, however, is still pushed downwards which means that it is not trapped in once place.

Focused Laser Beam, Particle on the Beam's Main Axis Below the Laser Focus:

We will next consider a focused laser beam. The particle is assumed to be in the center of the beam but below the focus of the laser. The laser is assumed to be highly focused which implies that the incident partial beams are no longer parallel.

Figure 68 depicts two partial beams of the focused laser. The center of the bead is assumed to be below the focus.
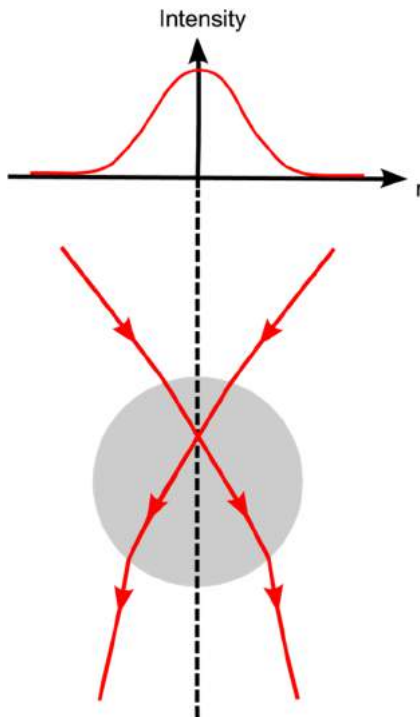
Figure 68 Focused Laser Beam Incident on a Polystyrene Bead

Since the bead is in the geometrical center both partial beams have the same intensity and, therefore, the same momentum amplitude. The direction off the momentum vectors is different since the incident laser beam is focused.
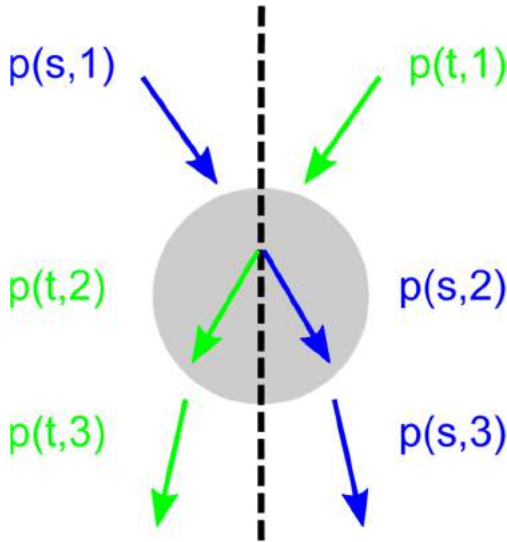
Figure 69 Focused Laser Beam Momentum

Figure 70 displays the momentum change of both partial beams.

Figure 70 Focused Laser Beam Momentum Changes

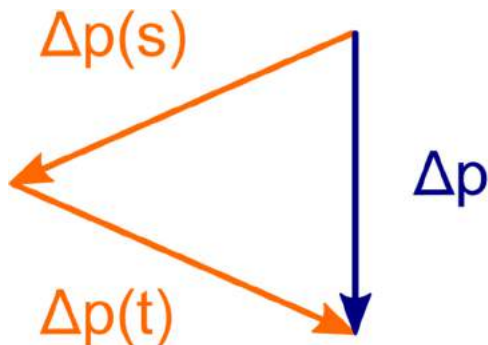The net momentum change of the entire beam is depicted in Figure 71.

Figure 71 Focused Laser Beam Change in Momentum

The laser beam exhibits a momentum change downwards. Hence, the bead's momentum is changed upwards. The bead moves against the beam's main propagation direction and into the laser focus. Analogously, one can show that the bead is pushed downwards when the laser focus is beneath it.

In essence, a single laser beam can create an ideal three-dimensional optical trap. One thing is crucial for functioning optical tweezers: in order to trap an object in a three-dimensional trap, a highly focused laser beam with a suitable intensity profile is required. The object is always pushed in direction towards the point with highest intensity:

- Radially into the middle of the laser beam due to the (Gaussian) intensity profile
- Vertically into the direction of the focus

As soon as a particle moves away from the focus, a restoring force acts on it to push it back into the focus.

Chapter 9 Regulatory

As required by the WEEE (Waste Electrical and Electronic Equipment Directive) of the European Community and the corresponding national laws, Thorlabs offers all end users in the EC the possibility to return "end of life" units without incurring disposal charges.

- This offer is valid for Thorlabs electrical and electronic equipment:
- Sold after August 13, 2005
- Marked correspondingly with the crossed out "wheelie bin" logo (see right)
- Sold to a company or institute within the EC
- Currently owned by a company or institute within the EC
- Still complete, not disassembled and not contaminated

Wheelie Bin Logo

As the WEEE directive applies to self contained operational electrical and electronic products, this end of life take back service does not refer to other Thorlabs products, such as:

- Pure OEM products, that means assemblies to be built into a unit by the user (e.g. OEM laser driver cards)
- Components
- Mechanics and optics
- Left over parts of units disassembled by the user (PCB's, housings etc.).

If you wish to return a Thorlabs unit for waste recovery, please contact Thorlabs or your nearest dealer for further information.

9.1.   Waste Treatment is Your Own Responsibility

If you do not return an "end of life" unit to Thorlabs, you must hand it to a company specialized in waste recovery. Do not dispose of the unit in a litter bin or at a public waste disposal site.

9.2.   Ecological Background

It is well known that WEEE pollutes the environment by releasing toxic products during decomposition. The aim of the European RoHS directive is to reduce the content of toxic substances in electronic products in the future.

The intent of the WEEE directive is to enforce the recycling of WEEE. A controlled recycling of end of life products will thereby avoid negative impacts on the environment.

Chapter 10   Thorlabs Worldwide Contacts

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