

Table of Contents

Chapter 1	Safety	1
1.1.	<i>Warning Symbol Definitions.....</i>	1
1.2.	<i>Laser Radiation Warning</i>	1
Chapter 2	Product Description	2
Chapter 3	Principles of Optical Tweezers.....	4
3.1.	<i>Dipole Approach in the Rayleigh Scattering Regime $R \ll \lambda$.....</i>	4
3.2.	<i>Geometrical Optics Approach in the Mie Regime $R \gg \lambda$.....</i>	6
Chapter 4	Kit Components	16
4.1.	<i>Trapping Laser Source.....</i>	16
4.2.	<i>Beam Expander.....</i>	17
4.3.	<i>Right-Angle Mirrors</i>	18
4.4.	<i>Sample Positioning System</i>	19
4.5.	<i>Microscope</i>	20
4.6.	<i>Additional Components.....</i>	22
4.7.	<i>Included Hardware</i>	23
4.7.1.	Imperial Kit.....	23
4.7.2.	Metric Kit	24
Chapter 5	Setup and Adjustment.....	25
5.1.	<i>Assembly</i>	25
5.1.1.	Trapping Laser Source.....	26
5.1.2.	Beam Expander	28
5.1.3.	Microscope.....	33
5.1.4.	Sample Positioning System	40
5.2.	<i>Controller and Software Setup</i>	44
5.2.1.	LED Controller.....	44
5.2.2.	Laser Controller.....	44
5.2.3.	Camera	46
5.2.4.	Sample Positioning Stages	50
5.2.5.	Kinesis Settings.....	53
5.3.	<i>Camera Adjustment</i>	54

5.4. Beam Adjustment	55
5.5. Trapping, Microscope Focus, and Final Alignment	58
Chapter 6 Experiments	63
6.1. Creating a Sample	63
6.2. Setting the Correct Focus Level.....	64
6.3. Arranging the Silica Beads	65
6.4. Manipulating a Dairy Cream Particle in a Cream/Water Emulsion	66
6.5. The Holding Force of the Optical Trap	68
6.5.1. Brownian Motion.....	68
6.5.2. The Maximum Holding Force	71
6.6. Other Samples	72
Chapter 7 Teaching Tips	74
Chapter 8 Control via Game Controller	83
Chapter 9 Troubleshooting	86
Chapter 10 Regulatory	88
Chapter 11 Thorlabs Worldwide Contacts.....	89

Chapter 1 Safety

1.1. Warning Symbol Definitions

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



Warning: Laser Radiation

1.2. Laser Radiation Warning

 **WARNING** 

The class 3B laser diode used in this kit can emit more than 50 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.

LASER RADIATION
AVOID EXPOSURE TO THE BEAM
CLASS 3B LASER PRODUCT

Chapter 2 Product Description

For many people, moving and controlling objects with a beam of light 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 to measure small forces on the order of piconewtons¹. More exotic applications include the control of tiny microgears². Biologists use optical tweezers to manipulate different types of molecules and cells³. *In-vitro* fertilization of ova is a typical application example – sperm can be inserted into ova without mechanical contact, thus maintaining a sterile environment.

In a lab course, various demonstrations and experiments can be performed with an optical tweezers setup. This kit can be used to carry out basic experiments such as moving small spheres or cells through a solution. The kit can also be used for more advanced experiments such as investigating the Brownian motion of objects, and measuring the optical forces of the tweezers.

The working principle can be explained using concepts usually known to undergraduate students, such as geometric optics, basic theory of Brownian motion, and Stokes' friction. 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 a complete and fully operating experimental setup with which particles on the order of microns 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 beads, glass beads, or starch grains from ordinary 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 major readjustment, making it ideally suited to demonstrate the principle of optical tweezers to students in seminars or lecture halls.

Optical tweezers are not only intriguing scientific devices. Their inventor, Arthur Ashkin, also received the 2018 Nobel Prize "for the optical tweezers and their application to biological systems." As he wrote: "It is surprising that this simple [...] experiment, intended only to show simple forward motion due to laser radiation pressure, ended up demonstrating not only this force but the existence of the transverse force component [...] and stable three-dimensional particle trapping."⁴

We recommend using the OTKBTK sample kit with the setup. The performance was optimized for the sample slides and the cover glasses provided with the OTKBTK. For simplicity, we designed the tweezers system to work without immersion oil.

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

⁴ *Proc. Natl. Acad. Sci. USA*, Vol. 94, pp. 4853-4860 (1997)

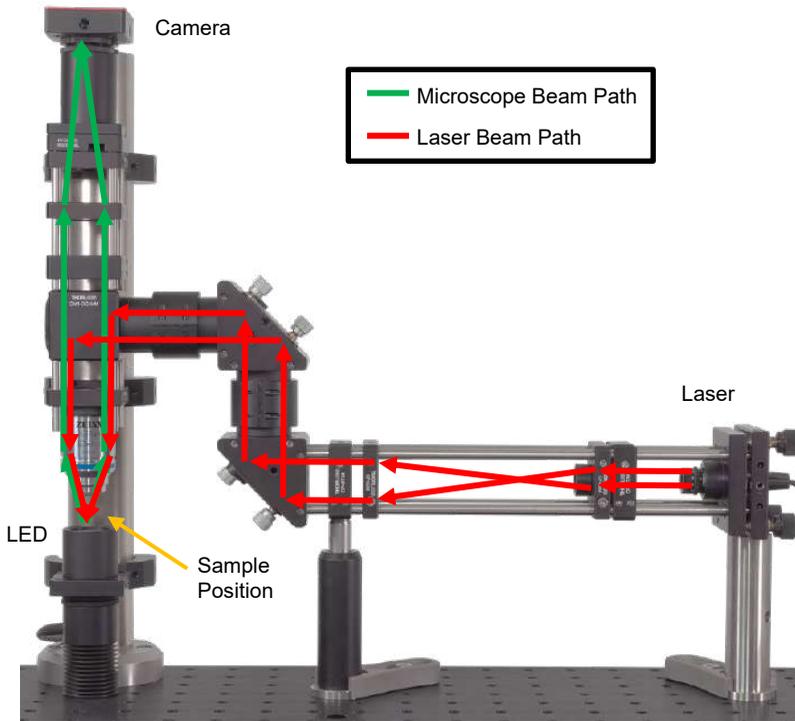


Figure 1 The light path through the optical tweezers experiment.

Chapter 3 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 TEM₀₀ 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 factors 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 λ 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:

3.1. Dipole Approach in the Rayleigh Scattering Regime $R \ll \lambda$

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 moment \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:

$$\vec{p}_i = \alpha \cdot \vec{E}(\vec{r}_i) \quad (1)$$

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 moment is equally great for all N point dipoles. The polarization \vec{P} resulting from the induced dipole moments is then

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

where χ is 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 moment \vec{p} in the electrical field \vec{E} is $U_i = -\vec{p} \cdot \vec{E}$. Because there are N point dipoles in a bead with the volume V , the energy density in the bead is defined by:

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

The occurrence of the gradient force, which is 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:

$$F_{Grad} = \frac{2 \pi \alpha}{c n_m^2} \nabla I \quad (4)$$

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

$$m = \frac{n_p}{n_m} \quad (6)$$

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:

$$F_{Scattering} = \frac{\sigma n_m}{c} \cdot I \quad (7)$$

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

Here, c is the speed of light in vacuum and 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.

3.2. Geometrical Optics Approach in the Mie Regime $R \gg \lambda$

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 Ashkin⁵.

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:

$$F = \frac{dp}{dt} \quad (9)$$

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 :

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

Here, $I(r)$ 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:

$$P_{refl} = P_{Beam} \cdot R_r \quad (11)$$

$$P_{trans} = P_{Beam} \cdot T \quad (12)$$

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).

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

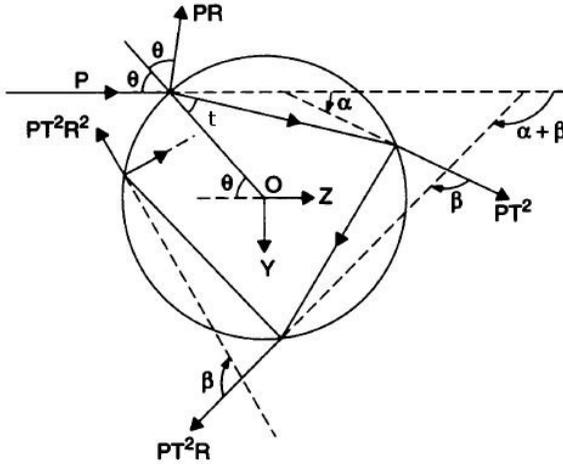


Figure 2 Reflection and Transmission of an Incident Partial Beam on the Inside and Outside Surfaces of a Sample Bead with a Refractive Index Higher than the Immersion Medium

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 unit time that remains in the sphere, based on 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

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

and

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

with the Q factor

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

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 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. That will be discussed in detail below.

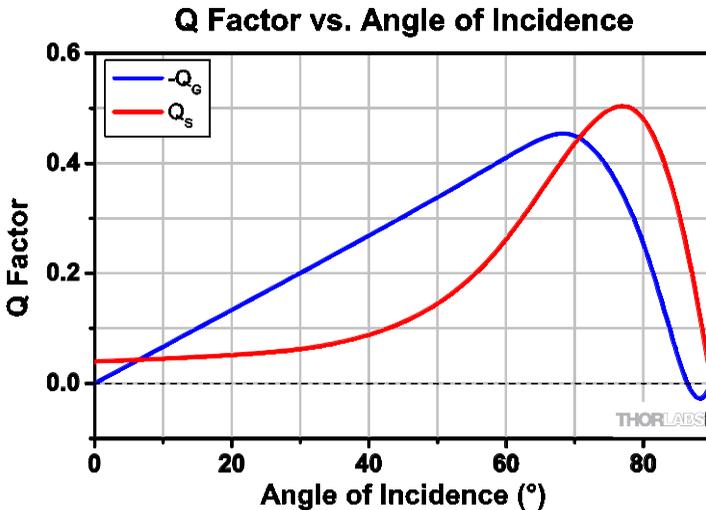


Figure 3 Q Factor Angular Dependence⁵

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.

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:

$$F_g > F_s \quad (18)$$

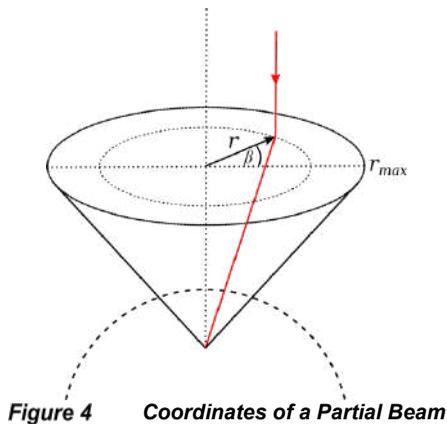
In the following, we'll discuss these forces in more detail.

Total force on the sphere

To obtain the total force acting on the sphere, we have to sum over all partial beams that hit it.

For that, we integrate (i) over the distance r between the partial beam and the symmetry axis of the whole beam, ranging from 0 to r_{max} , and (ii) over its angular coordinate β , ranging from 0 to 2π .

Figure 4 shows how the coordinates r and β of a partial beam (in red) are defined. The dashed line stands symbolically for the sphere the laser is focused on.



So far, the force was given as a function of θ which now has to be expressed in terms of r and β . Figure 5 shows a partial beam incident on the sphere with angle θ from the side. The following statements hold true:

$$X = \sin \phi \cdot S = \sin \theta \cdot R \tag{19}$$

and

$$\sin \phi = r / \sqrt{r^2 + l^2} \tag{20}$$

l roughly corresponds to the focal length of the objective. Effectively, you can use the objective's working distance for this parameter.

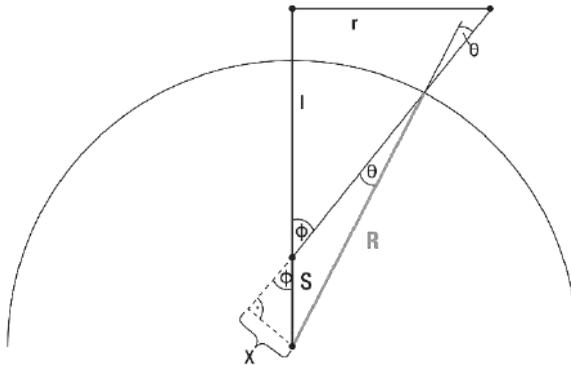


Figure 5 Relation Between r and θ .⁶

Then, θ can be expressed as

$$\theta(r) = \arcsin\left(\frac{S}{R} \frac{r}{\sqrt{r^2 + l^2}}\right) \tag{21}$$

Next, the forces need to be summed. For that, we start by observing Figure 6. A partial beam with distance r to the symmetry axis of the whole beam falls on the sphere under an angle θ . As discussed above, we can split the resulting force in two perpendicular components, F_s and F_g . For clarity, we can now add another partial beam, namely the one mirrored on the symmetry axis, denoted “mirror beam”. As sketched in Figure 6, this partial beam falls on the sphere on the other side and results in “mirrored” force vectors $F_{s,mirror}$ and $F_{g,mirror}$ in the right part of the sphere (which were not drawn to avoid an overcrowded figure).

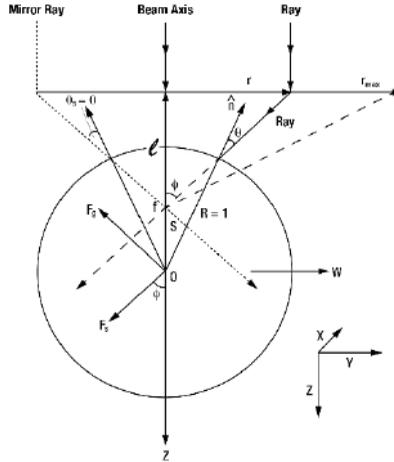


Figure 6 The Contribution of One Ray to the Total Force^{5,6}

When you consider all of the partial beams with the same condition around the symmetry axis, it immediately becomes clear that all force components in X and Y direction vanish and only resulting force components along the Z axis remain.

These can be written as

$$F_{g,z} = -F_g \cdot \sin \phi = -F_g \cdot \frac{r}{\sqrt{r^2 + l^2}} \tag{22}$$

$$F_{s,z} = F_s \cdot \cos \phi = F_s \cdot \frac{l}{\sqrt{r^2 + l^2}} \tag{23}$$

where $F_{g,z}$ is pointing in the negative Z direction and is, therefore, negative. Hence, each infinitesimal force contributing to the total force is given by

$$dF = F_s \cdot \cos \phi - F_g \cdot \sin \phi \tag{24}$$

⁶ Adapted from A. Langendörfer: "Aufbau einer Optischen Pinzette für das Landesmuseum für Technik und Arbeit in Mannheim", wissenschaftliche Arbeit, KIT, Karlsruhe, 2009

The total force F_{tot} is the sum of the total scattering force $F_{s,tot}$ and the total gradient force $F_{g,tot}$, where

$$F_{s,tot} = \int_0^{r_{max}} \int_0^{2\pi} F_s \cdot \cos \phi r dr d\beta \tag{25}$$

and

$$F_{g,tot} = \int_0^{r_{max}} \int_0^{2\pi} -F_g \cdot \sin \phi r dr d\beta \tag{26}$$

In Figure 7, we plotted the gradient force, the scattering force and the resulting total force as a function of the focus position S relative to the radius R of the sphere. The direction is the Z axis, meaning that the plot shows the behavior when the focus is moved through the center of the sphere from bottom to top. The curves were calculated with the above equations with a set of typical parameters since we only want to discuss the curve's general form. Therefore, the forces are given in arbitrary units (a.u.). On the horizontal axis, “+1” corresponds to the focus position on the sphere’s outer surface right above the sphere’s center while “-1” corresponds to the lower surface below the center.

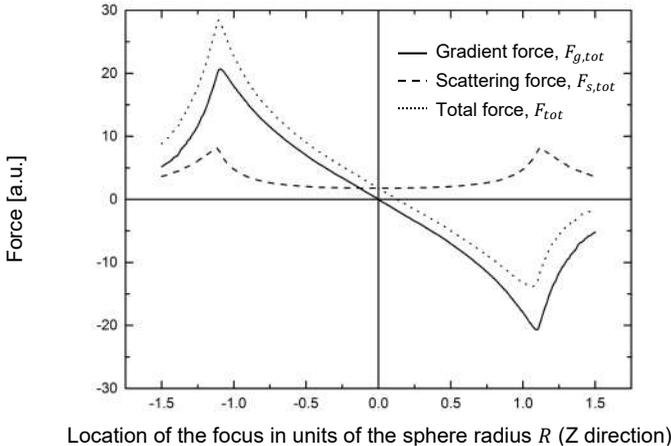


Figure 7 Overview of All Forces When Focus is Changed in Z-Direction⁶

From Figure 7, we learn that

1. The gradient force always points towards the focus. For example, when $S/R \geq 0$, the focus is above the sphere’s center. Then $F_g \leq 0$ and the gradient force pushes the sphere upwards into the focus.
2. The scattering force always points in the direction of propagation of the incident beam, in this case downwards. Note that $F_s \geq 0$ in the entire range of the plot.

- When the focus is below the sphere's center, i.e. $S/R \leq 0$, scattering and gradient force act in the same direction. When the focus is above the sphere's center, i.e. $S/R \geq 0$, both forces point in opposite directions.

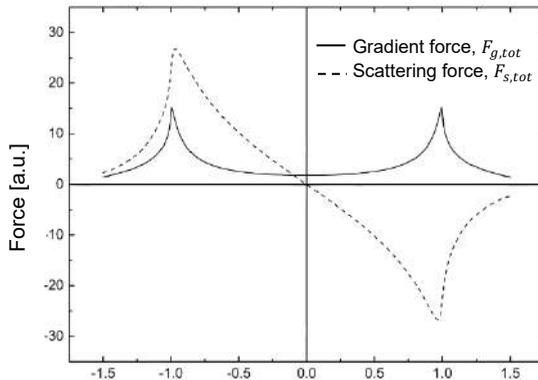
We also note that the absolute value of the gradient force is always higher than the absolute value of the scattering force. This is the requirement for any stable optical tweezers trap. The fundamental quantity to fulfill this requirement is the microscope objective's numerical aperture, which defines the angle of the focus which we will discuss next.

Sometimes the focus is not above or below the sphere's center but along the y-axis instead. Again, the reference axis goes through the sphere's center, and we plot the occurring forces as a function of the focus' distance S to the sphere's center in units of the sphere's radius.

Figure 8 shows the gradient force and the scattering force. Plotting the sum of both forces would not make sense as they point in different directions ($F_{s,tot}$ in Z direction and $F_{g,tot}$ in Y direction).

From Figure 8, we learn that:

- The general form of the curves is similar to the focus' movement on the Z axis. Only outside of the sphere the force decreases a little faster than compared to the Z axis.
- The maximal gradient force is larger than the maximal scattering force (absolute values). This is the case when the parameters such as the numerical aperture allow optical trapping.
- The maximal gradient force are located just within the sphere, close to the surface. The maximal value of the scattering force is found right on the surface.



Location of the focus in units of the sphere radius R (Y direction)

Figure 8 Scattering and Gradient Force with the Focus Changed in Y Direction⁶

Influence of the Numerical Aperture

As we have seen above, the angle of incidence of the partial rays plays a crucial role in optical tweezing. The angle is defined by the numerical aperture of the objective: the numerical aperture (NA) describes the acceptance cone of an objective and is given by

$$NA = n \cdot \sin(\phi) = n \cdot \sin \phi \tag{27}$$

where n is the refractive index of the material between the objective and the focus and ϕ is half of the angle of the maximum light cone.

As discussed, the gradient force $F_{g,tot}$ needs to exceed the scattering force $F_{s,tot}$ to get a stable trap. Next, we want to investigate a measure for the trap's strength. For that, we can have a look at the ratio of $F_{g,tot}$ and $F_{s,tot}$ at the point $S/R = 1$ since we have shown that scattering and gradient force are strongest when the laser focus is at/near the sphere's surface (i.e., $|S/R| = 1$). So for a stable trap, we can assume the condition⁷:

$$\left| \frac{F_{g,tot}(S/R=1)}{F_{s,tot}(S/R=1)} \right| \geq 1 \tag{28}$$

Absolute values are used since $F_{g,tot}$ is negative. Figure 9 shows how this strength depends on the numerical aperture. Again, we plotted a curve with typical parameters to show the general behavior of the curve; therefore, we do not focus on the concrete numbers.

Figure 9 shows the fundamental behavior that the strength of the trap increases with increasing numerical aperture. Also, it becomes apparent that there is a lower limit for the numerical aperture of the objective. Below that, no trapping occurs since the gradient force never exceeds the scattering force.

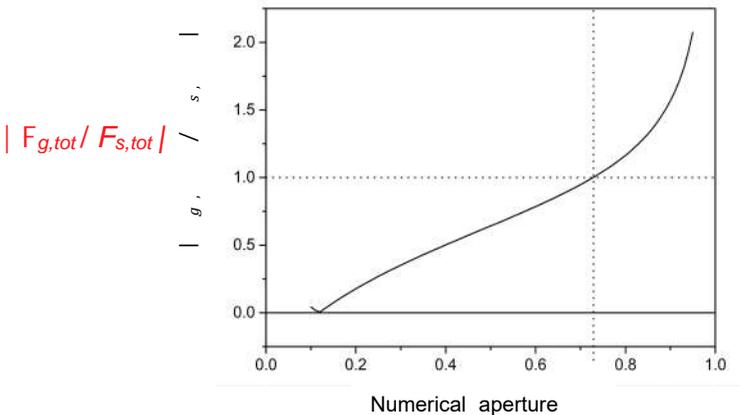


Figure 9 Behavior of the Trap's Strength with Respect to the Numerical Aperture⁶

⁷ The absolute value of the forces is used since $F_{g,tot}$ is negative.

Influence of the Laser Power

While the numerical aperture affects the ratio of gradient and scattering force, the laser power directly influences the individual forces. In Equations (13)

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

and (15),

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

we see that both forces increase proportionally with the laser power P . When the laser power is increased, each individual force gets stronger. However, their ratio (which essentially determines if optical tweezing occurs or not) remains the same, see Equation (28).

Combining what we know about the numerical aperture and laser power, we can note that the increase in trapping strength can be achieved through two factors:

- Increase of the *numerical aperture* of the objective so that the gradient force exceeds the scattering force.
- Increase of the *laser power*, which increases the tweezer's strength linearly if the condition $F_{g,tot} > F_{s,tot}$ is met.

This also tells us that increasing the numerical aperture is the more effective way to improve trapping than increasing the laser power. While the laser power increases the trapping force linearly, Figure 9 shows a stronger than linear behavior above the trapping limit.

Chapter 4 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.

4.1. Trapping Laser Source

 <p>1 x SR9A-DB9 ESD Protection and Strain Relief Cable</p>	 <p>1 x L658P040-S 658 nm, 40 mW, Ø5.6 mm, A Pin Code Laser Diode⁸</p>	 <p>1 x LTN330-A Adjustable Collimator for Ø5.6 mm Laser Diodes, AR Coated: 350 – 700 nm</p>
 <p>1 x KLD101 K-Cube Laser Diode Driver</p>	 <p>1 x TPS002 ±15 V / 5 V K-Cube Power Supply</p>	 <p>1 x RS3.5P8E (RS3.5P4M) Ø1" (Ø25 mm) Pedestal Post, 3.5" (90 mm) Tall</p>
 <p>1 x CF125 Small Clamping Fork</p>	 <p>1 x KC1-T(M) Ø1" Cage-Compatible SM1-Threaded Mirror Mount</p>	 <p>1 x AD15F SM1-Threaded Adapter for Ø15 mm Components</p>

⁸ The L658P040-S is a wavelength-screend L658P040. This ensures that the center wavelength is in the range between 656 nm and 660 nm.

4.2. Beam Expander

 <p>2 x ER10 Ø6 mm Cage Assembly Rod, 10" Long</p>	 <p>2 x ER1 Ø6 mm Cage Assembly Rod, 1" Long</p>	 <p>2 x ER3 Ø6 mm Cage Assembly Rod, 3" Long</p>
 <p>2 x ER6 Ø6 mm Cage Assembly Rod, 6" Long</p>	 <p>2 x CP45(M) 30 mm Removable Segment Cage Plate</p>	 <p>2 x CP45T(M) 30 mm Removable Segment Cage Plate, Thick</p>
 <p>1 x LA1509-A Ø1" N-BK7 Plano-Convex Lens, f = 100 mm AR Coating: 350-700 nm</p>	 <p>1 x SM1A6 Adapter with External SM1 Threads and Internal SM05 Threads</p>	 <p>1 x SM05L03 Ø1/2" Lens Tube, 0.3" Long</p>
 <p>1 x LA1074-A Ø1/2" N-BK7 Plano- Convex Lens, f = 20 mm, AR Coating: 350-700 nm</p>	 <p>1 x TR3 (TR75/M) Ø1/2" (Ø12.7 mm) Post, 3" (75 mm) Long</p>	 <p>1 x PH3 (PH75/M) Ø1/2" (Ø12.7 mm) Post Holder, 3" (75 mm) Long</p>



4.3. Right-Angle Mirrors



4.4. Sample Positioning System

 <p>2 x MT1(M)-Z8 Motorized Translation Stage, 1/2" (12 mm) Travel</p>	 <p>1 x MT1B(M) Manual Translation Stage, 1/2" (13 mm) Travel⁹</p>	 <p>2 x KDC101 K-Cube DC Servo Motor Module</p>
 <p>2 x KPS201 K-Cube Power Supply</p>	 <p>1 x MT402 Right Angle Bracket</p>	 <p>1 x MT401(M) Mounting Base for Translation Stages</p>
 <p>2 x MT405 Side-Mounted Actuator Adapter for MT1(M)-Z8</p>	 <p>1 x Slide Holder Part 1</p>	 <p>1 x Slide Holder Part 2</p>

⁹ The screw of the MT1B(M) allows for a travel of 150 μm per revolution, which is much finer than the 500 μm per revolution provided by a similarly-sized micrometer.

4.5. Microscope

 <p>1 x CS165CU(M) 1.6 MP Color CMOS Camera</p>	 <p>2 x SM1T2 Ø1" Lens Tube Coupler</p>	 <p>1 x SM1NT1 Slotted Locking Ring</p>
 <p>1 x SM1L15 Ø1" Lens Tube, 1.5" Long</p>	 <p>1 x SPT1C(M) Coarse ±1 mm XY Slip Plate Positioner</p>	 <p>4 x CP33(M) SM1-Threaded Cage Plate</p>
 <p>3 x C1498(M) Slip-On Ø1.5" Post Clamps</p>	 <p>1 x LB1676 Ø1" N-BK7 Bi-Convex Lens, f = 100 mm</p>	 <p>1 x CP33T(M) SM1-Threaded Cage Plate, 0.5" Thick</p>
 <p>1 x FES0650-IC Ø1" Shortpass Filter, 650 nm Cut-Off¹⁰</p>	 <p>1 x CM1-DCH(M) 30 mm Cage Cube with Dichroic Filter Mount</p>	 <p>1 x DMSP605R 25 mm x 36 mm Shortpass Dichroic Mirror, 605 nm Cutoff</p>

¹⁰ The filter FES0650 is superseded by the filter FESH0650 in our product offering. Since the FESH0650 does not have the necessary optical properties, we keep using the FES0650 filter in this kit under the item number FES0650-IC.

 <p>1 x SM1CP2 SM1 End Cap</p>	 <p>1 x SM1A17 Adapter with External SM1 Threads and Internal M27 x 0.75 Threads</p>	 <p>1 x Zeiss Microscope Objective 63X, 0.8 NA</p>
 <p>4 x ER05 Ø6 mm Cage Assembly Rod, 0.5" Long</p>	 <p>4 x ER3 Ø6 mm Cage Assembly Rod, 3" Long</p>	 <p>4 x ER1.5 Ø6 mm Cage Assembly Rod, 1.5" Long</p>
 <p>1 x MCWHL7 Cold White Mounted LED 1300 mA¹¹ Max</p>	 <p>1 x SM1L10 Ø1" Lens Tube, 1" Long</p>	 <p>1 x DG10-600 Ø1" N-BK7 Ground Glass Diffuser, 600 Grit</p>
 <p>1 x LEDD1B T-Cube LED Driver</p>	 <p>1 x KPS201 Power Supply</p>	 <p>1 x DP14A(/M) Damped Ø1.5" Post, 14" Long</p>

¹¹ Please note that the LED is rated RG2 in the risk group classification, according to the standard IEC 62461:2006, Photobiological safety of Lamps and Lamp Systems. RG2 = Moderate Risk Group.

4.6. Additional Components

 <p>1 x MB1224 (MB3060/M) Aluminum Breadboard, 12" x 24" x 1/2" (300 mm x 600 mm x 12.7 mm)</p>	 <p>1 x RDF1 Rubber Dampening Breadboard Feet</p>	 <p>1 x CPA1 30 mm Cage System Alignment Plate with Ø1 mm Aperture</p>
 <p>1 x BBH1 Breadboard Handles (Pack of 2)</p>	 <p>1 x SPW606 SM1 Spanner Wrench, 1" Long</p>	 <p>1 x ADF1 Fluorescent Alignment Disk, Blue</p>

4.7. Included Hardware

4.7.1. Imperial Kit

Type	Qty.	Type	Qty.
1/4"-20 Cap Screw, 0.315" Long	2	8-32 Cap Screw, 3/8" Long	2
1/4"-20 Cap Screw, 3/8" Long	10	8-32 Cap Screw, 1/2" Long	3
1/4"-20 Cap Screw, 1/2" Long	12	#8 Washer	2
1/4"-20 Cap Screw, 5/8" Long	8	8-32 Setscrew, 1/2" Long	1
1/4"-20 Cap Screw, 3/4" Long	4	1/8" x 1/4" Steel Dowel Pin	8
1/4" Washer	22	SD1 1/4" to #8 Counterbore Adapter, 10-Pack	1
 <p>1 x BD-5/64 5/64" Balldriver</p>		 <p>1 x SPW502 Spanner Wrench for Slotted SM1</p>	
 <p>1 x BD-3/16L 3/16" Balldriver</p>		 <p>1 x CCHK Imperial Hex Key Set</p>	

4.7.2. Metric Kit

Type	Qty.	Type	Qty.
M6 x 8 mm Cap Screw	2	M4 x 10 mm Cap Screw	2
M6 x 10 mm Cap Screw	10	M4 x 12 mm Cap Screw	3
M6 x 12 mm Cap Screw	12	M4 Washer	2
M6 x 16 mm Cap Screw	8	M4 x 12 mm Set Screw	1
M6 x 20 mm Cap Screw	4	1/8" x 1/4" Steel Dowel Pin	8
M6 Washer	22	SD1 M6 to M4 Counterbore Adapter, 10-Pack	1
 <p>1 x BD-2M M2 Balldriver</p>		 <p>1 x SPW502 Spanner Wrench for Slotted SM1</p>	
 <p>1 x BD-5ML M6 Balldriver</p>		 <p>1 x CCHK/M Metric Hex Key Set</p>	

Chapter 5 Setup and Adjustment

5.1. Assembly

The tweezers will be set up on an MB1224 (MB3060/M) breadboard. Set everything up on a stable desk or table without any sources of vibration nearby. Further damping, such as using an isolated optical table, is not necessary. First, unpack the balldriver BD-3/16L (BD-5ML), the SM1 spanner wrench SPW606, and the hex keys CCHK(/M).

Figure 10 shows the breadboard, and the positions of components to be mounted are indicated with cap screws and red outlines. Attach the BBH1 breadboard handles to the edges of the breadboard using four 1/4"-20, 5/8" long (M6 x 16 mm) cap screws with washers so the assembled tweezers can be moved. Attach the RDF1 rubber breadboard feet to the underside of the breadboard at the positions indicated by the red stars in the diagram below using four 1/4"-20, 1/2" long (M6 x 12 mm) cap screws without washers. Use the threaded hole in the breadboard and not the counterbored holes.

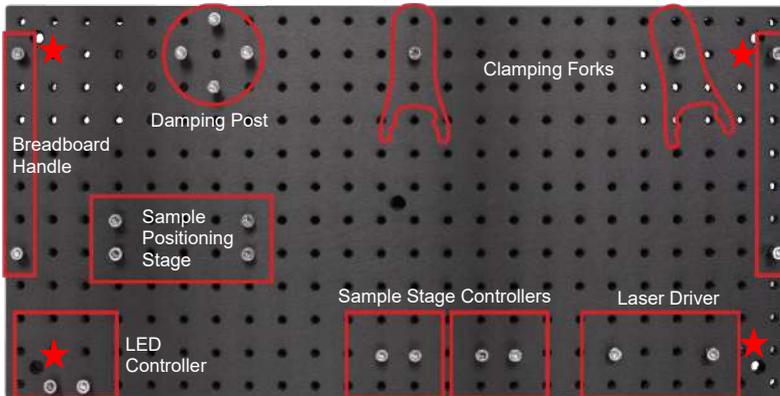


Figure 10 Positions of Components on the Breadboard

Teaching Note:

In the previous version of this kit, EDU-OT1(/M), the laser segment had to be assembled first and the microscope section built step by step by attaching components to the laser segment. We received feedback that some customers prefer to build the microscope section first to demonstrate the fact that optical tweezers can be built by coupling a laser into an existing microscope.

For this kit, we changed some parts so the microscope and the laser assembly can be built separate from each other. The laser assembly now features variable tube elements that allow for a retroactive height match between the two assemblies. Therefore, you may choose your preferred way of teaching & assembling this kit. Either build the microscope first and attach the cage segment/laser source to it or the other way around. In this manual, we present the instructions on how to assemble the laser source and cage segment first.

5.1.1. Trapping Laser Source

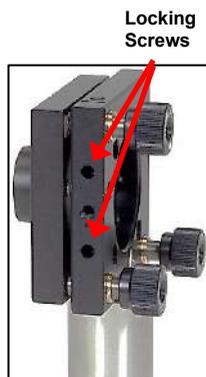
Figure 11 shows the components that are necessary for setting up the laser source.



Figure 11 Laser Source Components

First, screw the 8-32, 1/2" long (M4 x 12 mm) setscrew into the RS3.5P8E (RS3.5P4M). Screw on the KC1-T(M) in the orientation depicted to the right (note the position of the tip/tilt adjusters). This orientation is critical because you will not be able to access the cage rod locking screws if the KC1-T(M) is in a different orientation.

Next, remove one retaining ring from the mount. Screw in the AD15F until the threaded region is within the mount and its locking screws face upwards. Tighten the KC1-T's remaining retaining ring against the AD15F using the SPW606. Now feed the SR9A-DB9 through the mount as shown below.





Warning



We recommend using a grounding bracelet when handling the laser diode to prevent damage.

Take the L658P040-S laser diode from its package and place it in the silver retention ring of the LTN330-A, see the image to the right.



Remove the retaining ring from the LTN330-A housing. Then, insert the diode with the silver retention ring into the LTN330-A.



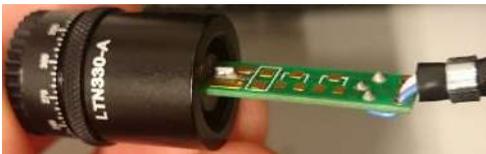
Fix the assembly in the LTN330-A using the black retaining ring (the retaining ring can be tightened using a thin screwdriver). Make sure to use the thick retaining ring and not the one you unscrewed in the previous step. Also, ensure that the laser diode's legs fit through the center hole so they are not damaged, see image to the right.



In the next step, insert the laser diode's legs into the socket of the SR9A-DB9. Please be careful in this step to avoid damage to the diode.



When the diode's legs are in the socket, the cover can be screwed onto the LTN330-A. **Make sure to screw on the part labeled "SR9A-DB9" instead of rotating the part with the printed circuit board!**



Finally, secure the assembly in the AD15F by engaging the two nylon-tipped setscrews, see image to the right.



5.1.2. 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.

Adjustment Mirror

Mount the mirrors (PF10-03-P01) in the right angle mirror mounts (KCB1C(/M)) with the reflective surface facing away from the knobs (Figure 12).



Figure 12 Mirror and Right Angle Mount

Base

Screw the BE1(/M) base into the PH3 (PH75/M) post holder as shown in Figure 13.



Figure 13 Post Holder Base

Lens 1

The elements that are necessary for the setup of lens (1), are shown in Figure 14. First, place the LA1074-A lens into the SM05L03 $\text{\O}1/2$ " 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 CP45(/M) cage plate.



Figure 14 Beam Expander Lens 1 Components

Lens 2

The components for lens 2 are shown in Figure 15. The lens (LA1509-A) must be attached to the CP45(/M) cage plate using two SM1 retaining rings.



Figure 15 Beam Expander Lens 2 Components

Cage Assembly

The cage assembly features a removable segment that comprises the two lenses for beam expansion. Step by step assembly instructions follow below. Figure 16 demonstrates the working principle of the removable cage plate assembly.

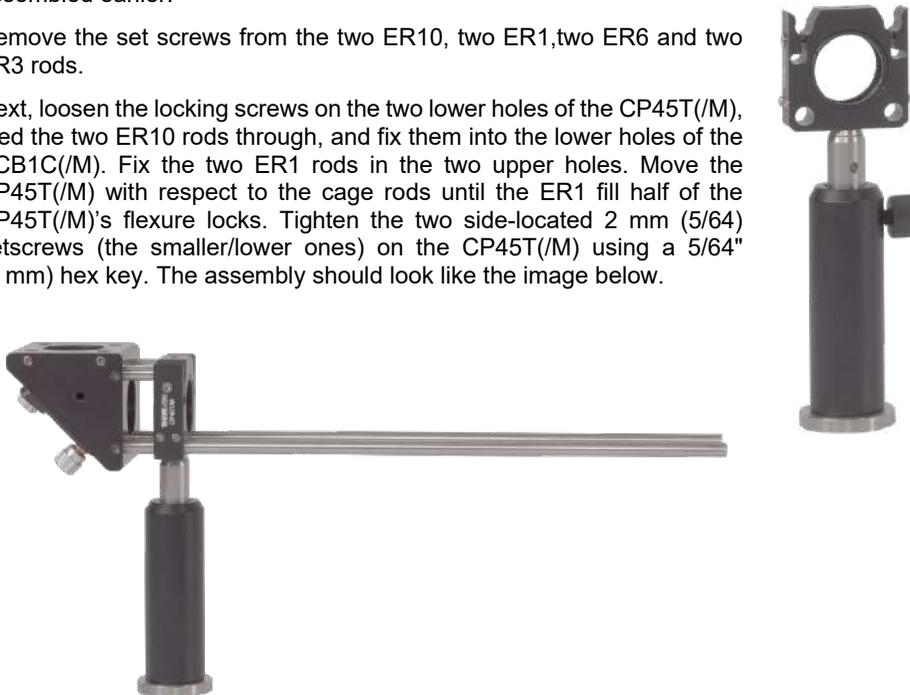


Figure 16 Function of the Removable Cage Segment

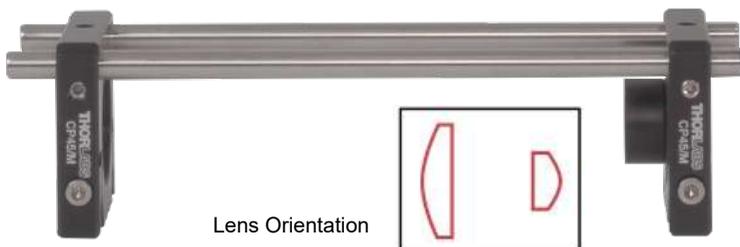
To assemble the cage segment, first screw an empty CP45T(/M) onto the TR3 (TR75/M) in the orientation shown in the image to the right and insert it into the post holder assembled earlier.

Remove the set screws from the two ER10, two ER1, two ER6 and two ER3 rods.

Next, loosen the locking screws on the two lower holes of the CP45T(/M), feed the two ER10 rods through, and fix them into the lower holes of the KCB1C(/M). Fix the two ER1 rods in the two upper holes. Move the CP45T(/M) with respect to the cage rods until the ER1 fill half of the CP45T(/M)'s flexure locks. Tighten the two side-located 2 mm (5/64) setscrews (the smaller/lower ones) on the CP45T(/M) using a 5/64" (2 mm) hex key. The assembly should look like the image below.



To assemble the beam expander segment, take the two lenses (1) and (2), see above, and feed the ER6 rods through their cage holes as shown in the photo below. The distance between the lens centers should be about 12 cm.



Next, click the beam expander segment into the assembly. Note that the cap screws of the CP45(/M)'s and CP45T(/M)'s flexure mechanism should not be tightened. Take the remaining CP45T(/M) cage plate and slip it onto the assembly. *Again, the cage rods should only fill half of the flexure locks.*



Finally, attach the kinematic mount with the laser source from Section 5.1.1. To do this, feed the ER10 cage rods through the lower holes of the kinematic mount (you may need to loosen the KC1-T's rod locking screws first). Insert the ER3 cage rods into the upper holes so that they fill the rest of the flexure locks. You may want to leave a small gap between the adjacent cage rods. Fix all of the KC1-T's cage rod locking screws. You may fix the CP45(/M)'s and CP45T(/M)'s flexure locks. However, in our tests, the flexure locks have demonstrated a sufficient clamping force without tightening the flexure mechanism (which makes taking the expander section out easier).

Use a level to adjust the height of the post in its post holder. The cage segment needs to be horizontal to avoid serious problems during alignment/adjustment!



Figure 17 Fully Assembled Cage Segment

Mirror Position

The mirrors are connected to each other and to the microscope with the help of a lens tube flexure sleeve coupler, SM1CPL10. This allows for freedom in height and distance adjustment when connecting the laser/cage assembly to the microscope assembly.

For the vertical connection, please use SM1L05 lens tubes. For the horizontal connection, please use the longer SM1L10 tubes. This ensures that there is sufficient space for manual adjustment between the stage assembly and the mirrors. Remove the retaining rings from all four lens tubes.

Screw an SM1L05 lens tube into the KCB1C(/M) of the assembly above. Take the second KCB1C(/M) and screw in another SM1L05. Connect them with an SM1CPL10 by slipping it over the tubes and tightening its locking screws. For them to stay parallel, you can press a flat object like a book or level on their side.

Screw an SM1L10 lens tube to the free side of the upper KCB1C(/M). Slip the second SM1CP10 on it. Later, that will be connected to a lens tube attached to the beamsplitter.

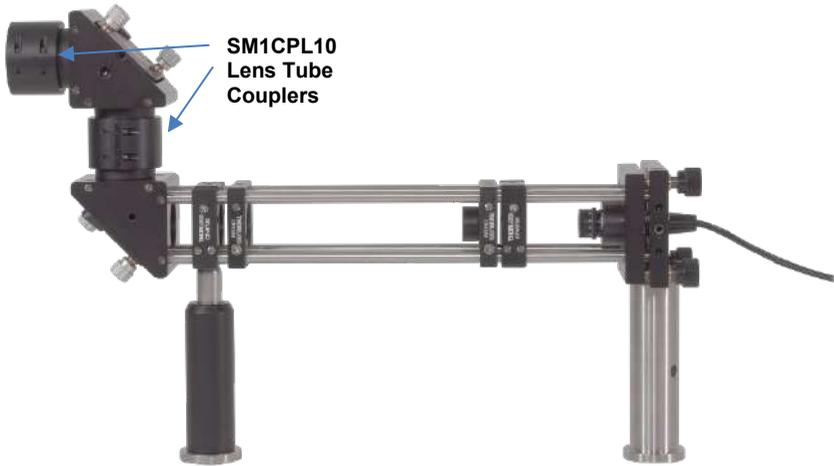


Figure 18 Laser & Cage Segment Fully Assembled

5.1.3. Microscope

Attach the damped DP14A(/M) $\varnothing 1.5$ " mounting post to the breadboard using the mounting holes in its base and four 1/4"-20, 3/4" long (M6 x 20 mm) cap screws. Choose the position shown in Figure 10. In the following procedure, the individual parts of the microscope must be built before the microscope system can be created.

Dichroic Mirror

The DMSP605R shortpass dichroic mirror has to be installed in the CM1-DCH(/M) housing. To do so, remove the small black screws on the side of the housing with the included hex key and pull off the top of the housing.

Next, install the DMSP605R dichroic mirror such that the part number engraving is in the upper left corner, see Figure 19. For doing so, push the slides marked with the blue arrows in Figure 19. Place the housing back on and fix it with the previously removed screws. Make sure the orientation of dichroic mirror and housing match Figure 19.

Finally, screw in four ER05 at the top and four ER1.5 at the bottom; see Figure 21. Remove the cage rods' setscrews that are pointing away from the beamsplitter cube. Attach an SM1CP2 end cap to the left side of the CM1-DCH(/M) cube.



Figure 19 Beamsplitter Cube

Objective Assembly

Figure 20 shows the components of the objective assembly. First, remove the retaining rings from the CP33(/M) cage plate and attach the objective to it 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 12 mm) cap screw. Make sure not to mistake the thick SD1 adapter with a thin washer. Also, the C1498(/M)'s label should face upwards. Ensure that you do not touch the bottom of the objective with your fingers, in order to prevent possible contamination.



Figure 20 Microscope Objective Components



Figure 21 Assembled Microscope Objective

Slip the objective's cage plate onto the cage rods below the beamsplitter, at about half length of the cage rods. Tighten the plate's locking screws to secure the objective. *Make sure to handle the objective with care and do not lay it on the table with the optic facing downward.*

LED Assembly

The components for the LED assembly are shown in Figure 22. First, remove the two retaining rings from the CP33(/M) cage plate and attach the MCWHL7 LED to it using the lens tube coupler (SM1T2). Connect the SM1L10 lens tube to the CP33(/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 12 mm) cap screw. Finally, install the DG10-600 diffuser. The diffuser is supposed to sit at the very top of the SM1L10 lens tube. For that, the retaining ring needs to be positioned close to the edge. Place the DG10-600 on top. Take one of the two retaining rings from the CP33(/M) and fix the DG10-600 with it.



Figure 22 LED Components

Camera Assembly

Figure 23 shows the components for the lens 3 assembly. Mount the LB1676 lens to the CP33(/M) cage plate using two retaining rings.



Figure 23 Lens 3 Components

Next, attach the FES0650-IC bandpass filter to the CP33T(/M) thick cage plate. Figure 24 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 24 Bandpass Filter Components

Figure 25 shows the components for assembling the top part of the camera system.

- First, unscrew one of the locking rings from the SM1T2 lens tube coupler. Next, screw the SM1L15 lens tube to the coupler and tighten the locking ring. Screw the SM1NT1 slotted locking ring onto the coupler (do not tighten it).
- Second, screw the SM1T2 lens tube coupler to the CS165CU(/M) camera with the slotted locking ring facing the camera but do not tighten it yet. After this, screw the other side of the lens tube onto the SPT1C(/M) slip plate positioner (with the locking screws facing towards the camera).
- Third, connect the CP33(/M) cage plate to the C1498(/M) post clamp using an SD1 counterbore adapter and an 8-32, 1/2" long (M4 x 12 mm) cap screw.
- Finally, place the SPT1C(/M) and CP33(/M) onto each other and slip four ER3 cage rods through them (with the rods' setscrews removed on both sides). Ideally, the cage rods ends should be flush with the upper side of the SPT1C(/M). Attach them both to the rods by tightening the cage locking screws at the side.



Figure 25 Camera System Components

Slip lens 3 from Figure 23 onto the cage rods and secure it with the locking screws on the side. Make sure the locking screws do not point towards the side where the damped post will be (i.e., the side of the C1498(M) clamp).

Next, slip on the filter in the cage plate from Figure 24. Also make sure no locking screws face towards the C1498(M) clamp. The cage rods should be inserted into the CP33T(M) **only half way**. Make sure that the upper locking screws fix the cage rods to the thick cage plate. The lower locking screws need to be left free (so the camera segment can be attached to the beamsplitter assembly). The fully assembled camera segment can be seen in Figure 26.

The SM1NT1 slotted locking ring coupler allows the user to choose the optimal camera orientation. Hold the camera in position with one hand and use the SPW502 spanner wrench to fix the camera such that the camera cable is on the left side. If the camera is not parallel to the cage plates, the motion of the stage will result in a skewed motion of the camera image.

Double check that the orientation of all cage plates and the camera is identical to Figure 26.



Figure 26 Camera System

Microscope Assembly

- Attach the LED assembly to the damped post. The LED can be in contact with the breadboard. Try to align the cage plate of the LED assembly parallel to the breadboard hole pattern.
- Attach the objective assembly to the damped post. The distance from breadboard top to the bottom of the objective should be about 11.5 cm.

Use a level at the beamsplitter cube to make sure the assembly is vertical. If not, you have to adjust the angle in which the C1498(M) clamp is screwed to the cage plate.

- Put the camera assembly on top of the objective assembly. The short cage rods on top of the beamsplitter should fit easily into the thick cage plate at the bottom of the camera assembly. If not, adjust the angle at which the C1498(M) clamp is screwed to the respective cage plate.

The microscope assembly is now complete and should look like the image on the right.



Next, connect the laser assembly and the microscope. For that, screw the remaining SM1L10 into the side of the beamsplitter. Then, adjust the position and height of the KCB1C(/M) right-angle kinematic mirror mount by loosening the SM1CLP10 flexure sleeve couplers. When they are in place, make sure the KCB1C(/M) mounts are still parallel to each other (to check that, you can use a flat object like a book or level). Clamp the posts supporting the cage segment and the laser to the breadboard using the two CF125 clamps and two 1/4"-20, 3/8" long (M6 x 10 mm) cap screws with washers.

Note: If the objective/microscope was assembled with a height that differs strongly from the aforementioned 11.5 cm (distance from the breadboard to bottom of the objective), the SM1CPL10 might not be long enough to cover both tubes. In that case, you need to change the height of the entire microscope by moving the objective assembly and the camera assembly either up or down.

The optical tweezers are now complete except for the sample positioning table and the control elements. Figure 27 shows the current assembly.

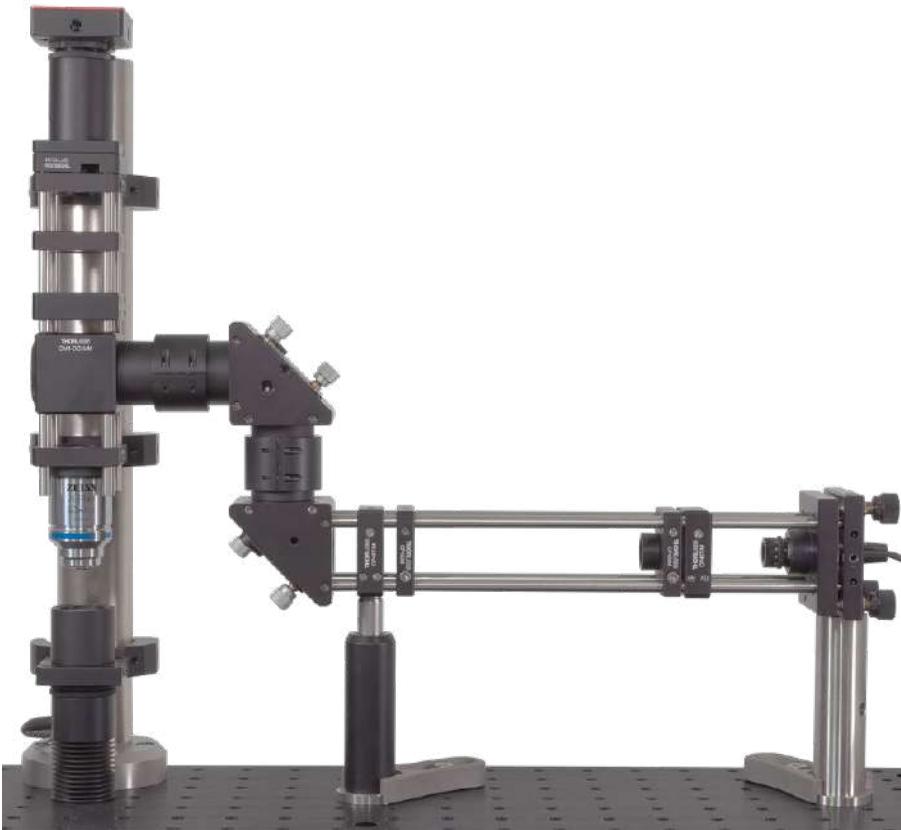


Figure 27 Complete Trap Laser and Microscope System

5.1.4. Sample Positioning System

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

First, take the MT401(/M) base plate and add 4 dowel pins.



In this setup, we use the MT405 to side-mount the motors to the MT1(/M) stage. Do the following for the two MT1(/M)-Z8 stages:

First, take the 3/32" hex key from the MT405. Remove the end-mount adapter by removing the two inner screws.



Do not remove the locking plate and attempt to mount an actuator on that side of the stage.



First, screw the two parts of the MT405 to the side of the stage. The hollow part that holds the barrel/motor is mounted to the thin, lower part of the stage. The part with the silver inset is screwed to the thick, upper part of the stage. Make sure to tighten the respective screws symmetrically. Insert the motor and tighten the clamping screw.



Customers with the metric kit should make sure to label and store the MT405's 3/32" (imperial) hex key for future use.

Screw the first MT1(/M)-Z8 with side-mounted motor to the base plate in the orientation shown to the right. Use two 1/4"-20, 3/8" long (M6 x 10 mm) screws.

Insert 2 dowel pins into the top of the stage.



Screw the second MT1(/M)-Z8 with side-mounted motor onto the first one in the orientation shown in the image to the right using two 1/4"-20, 3/8" long (M6 x 10 mm) cap screws.

Add 2 dowel pins to the top.



Screw the MT402 right angle bracket to the stage assembly using two 1/4"-20, 5/8" long (M6 x 16 mm) cap screws with washers.

Add 2 dowel pins to the thick side of the MT1B(/M) stage and screw it to the MT402 using two 1/4"-20, 5/8" long (M6 x 16 mm) cap screws with washers.



Connect the two pieces of the sample holder using two 8-32", 3/8" long (M4 x 10) mm screws with washers.



Screw the adapter assembly to the stage using two 1/4"-20, 0.315" long (M6 x 8 mm) cap screws with washers and 4 dowel pins (you may need to use the screws to pull the stages together once you have aligned the dowel pins).



Now, attach the assembled stage on the breadboard at the position depicted in Figure 10 using four 1/4"-20, 3/8" long (M6 x 10 mm) cap screws with washers.

The next step is to install the electronic controls, see Figure 28, onto the breadboard.



Figure 28 2 x KDC101, LEDD1B, and KLD101 Controllers

First, connect the LEDD1B LED Driver to the MCWHL7 LED and position it on the breadboard using two 1/4"-20, 1/2" long (M6 x 12 mm) cap screws with washers (the same type of screws and washers should be used with the following controllers as well). Then, place the two KDC101 K-Cube Stage controller cubes in their positions, mount them to the breadboard, and connect them to the computer using USB cables. Next, connect the KLD101 laser diode controller to the SR9A-DB9 mount, and mount it on the breadboard. In addition, you must install the supplied software programs for the operation of the CS165CU camera and the K-Cube controllers (KDC101, KLD101). Further adjustment of the camera and beam path are required for operation. You can find the instructions for this in Sections 5.3 to 5.5.

5.2. Controller and Software Setup

5.2.1. LED Controller

Connect the LED to the LEDD1B driver with the cable. Note that the LED is designed for a current up to **1300 mA**. Set the current limit to 1200 mA on the LED driver by turning the arrow on the current limit adjuster with a narrow screwdriver (see the photo on the right).

The mode switch on top of the controller should be set to CW.



5.2.2. Laser Controller

Install the Kinesis control software, 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 are asked to during the installation. Afterwards, open the newly installed program.



Figure 29 Kinesis Laser Controller Panel

In the Kinesis control panel, see Figure 29, press “Settings”.

- To operate the laser properly, the polarity setting of the diode will need to be switched to anode grounded in the “Control” tab. For that, select “Anode” in the drop-down menu labeled “Polarity”, see Figure 30.
- Set the maximum current for safe operation of the diode in the tab “Max Current” to 100 mA, see Figure 30. By activating the “Persist Settings to Hardware” checkbox, you can store the settings on the device.
- It is possible to monitor the laser diode with the included photodiode, see Figure 31. This is not necessary for this kit and should not be enabled.
- Once the polarity and the maximum current are set and persisted to the hardware, you can operate the laser driver with the controls on the controller itself (rather than controlling it by software). The current can be set by turning the potentiometer knob at the top of the controller. For that, the “Advanced” settings should look like in Figure 31.

The current range of the L658P040-S laser diode is 75 mA (typical) to 110 mA (maximum). The actual output for a certain current depends on the diode at hand. You have two options:

- Set the current to approximately 80 mA and measure the output power with a power meter. Adjust the voltage until you reach 40 mW of output power.
- Set the operating current just below 100 mA. According to our test measurements, this will result in an output power above 50 mW. The advantage is a stronger optical trap, the downside is that operating a laser diode above the specified output power may decrease its lifetime.¹²

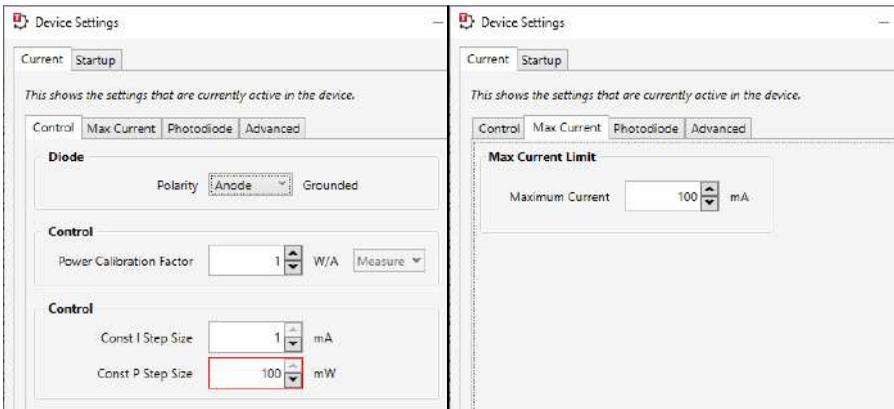


Figure 30 Settings of the Laser Diode Controller

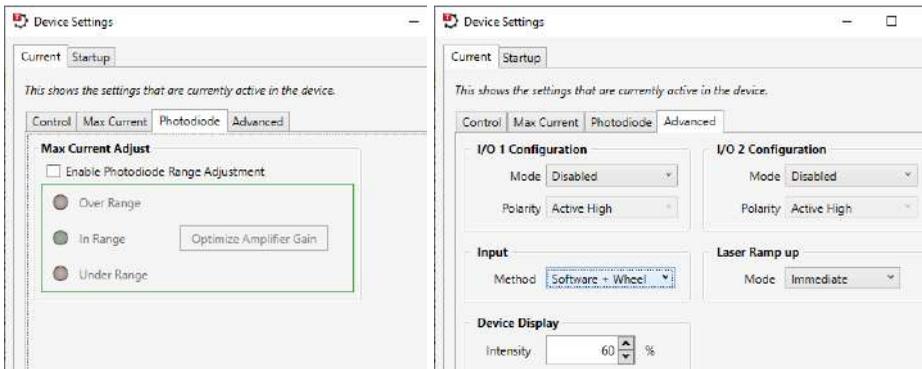


Figure 31 More Settings for the Laser Diode Controller

¹² So far, we have not seen a significantly increased failure rate with an operating current near 100 mA. However, we cannot guarantee that the diode reaches the typical lifetime when driven near its current limit.

Figure 32 shows the Kinesis software with the laser switched on and a current of 95.0 mA.



Figure 32 Kinesis Software for Laser Diode Controller with Laser Switched On

5.2.3. Camera

To operate the camera download the latest ThorCam Software from the Thorlabs website¹³ or use the installation CD included with the camera, then follow the installation guide. After finishing the installation reboot and connect the camera to your computer. After the driver initialization you can start the ThorCam Software.

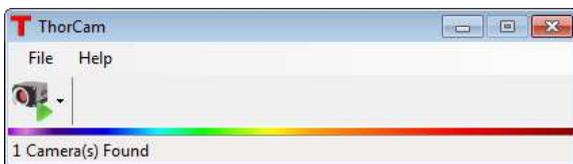


Figure 33 ThorCam Start Panel

Choose the camera you want to activate and press the start button (see Figure 33). This will start the software front panel with a black screen shown in Figure 34.

¹³ https://www.thorlabs.com/software_pages/ViewSoftwarePage.cfm?Code=ThorCam

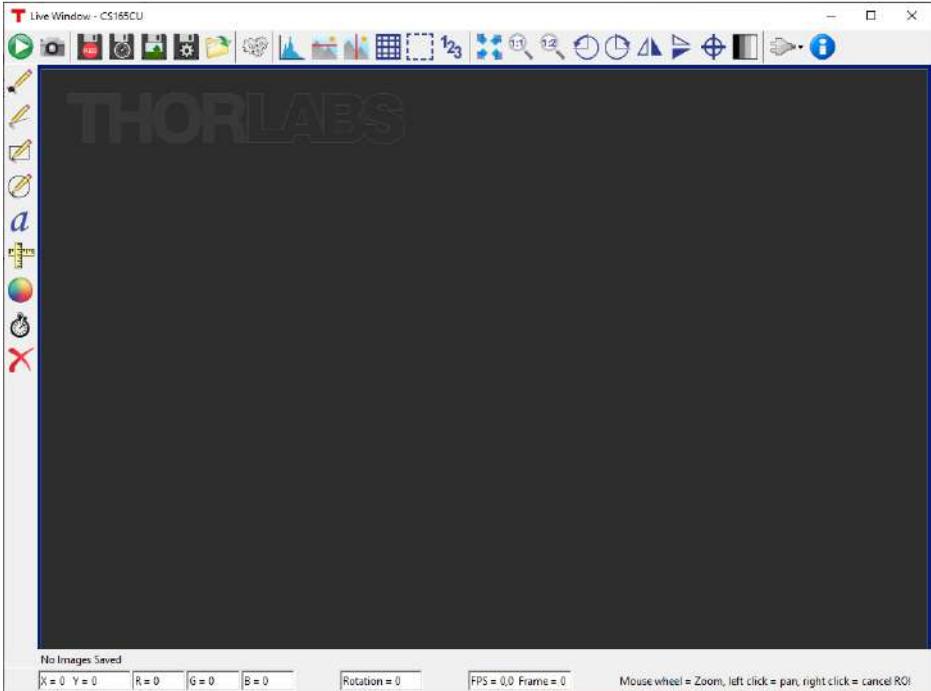


Figure 34 Home Screen of the ThorCam Software

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.

A helpful feature is the “Draw Circle” option . You can use it to mark the location of your laser spot. This can be helpful when you move out of the trapping plane but still want to know the location of the laser focus. An example can be seen in Figure 35. There is also the possibility to label the laser focus using the text insert function via the button .



Figure 35 “Draw Circle” Option to Mark the Location of the Laser Spot

The image properties need to be changed so that we can observe the laser spot later on.

To do so, open the  **Settings** to open the window shown in Figure 36.

In the general settings tab, you can set the frame rate and the exposure time. Also, you can activate the Autoexposure feature. However, please note that this function results in frame rates of about 10 FPS. We recommend the following procedure: use the Autoexposure feature to find a realistic exposure time range. Then, deactivate Autoexposure and make fine adjustments later to account for your laser and LED intensity.

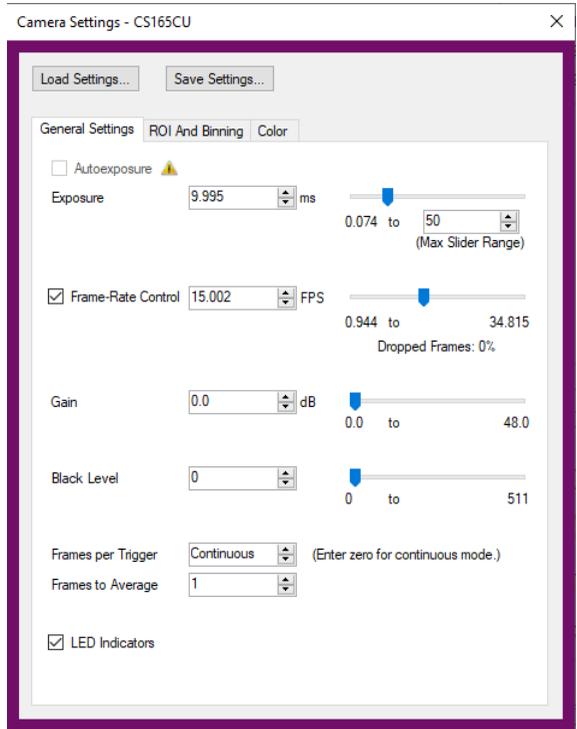


Figure 36 Settings of ThorCam Software

For **image improvements** adjust the settings in the *Color* tab. The recommended settings are shown in Figure 37. The high red gain compensates for the red bandpass filter in front of the camera (to avoid saturation by the laser).

Note: in previous versions of this kit, a cube beamsplitter was used instead of a dichroic mirror. This resulted in less laser light in the focus and more laser light on the camera.

Both the laser diode’s center wavelength and the dichroic mirror’s cut-off wavelength can vary slightly depending on the manufacturing lot. This will affect the Red Gain settings you have to choose for your setup. To do so, vary the Red Gain during the adjustment process and choose the optimal setting that allows to observe both the sample and the laser spot. In very rare cases, the image and laser intensity might have an unfavorable combination that cannot be compensated for using the gain settings. Should that be the case, please contact Tech Support. For an example of the desired results, please see Figure 44.

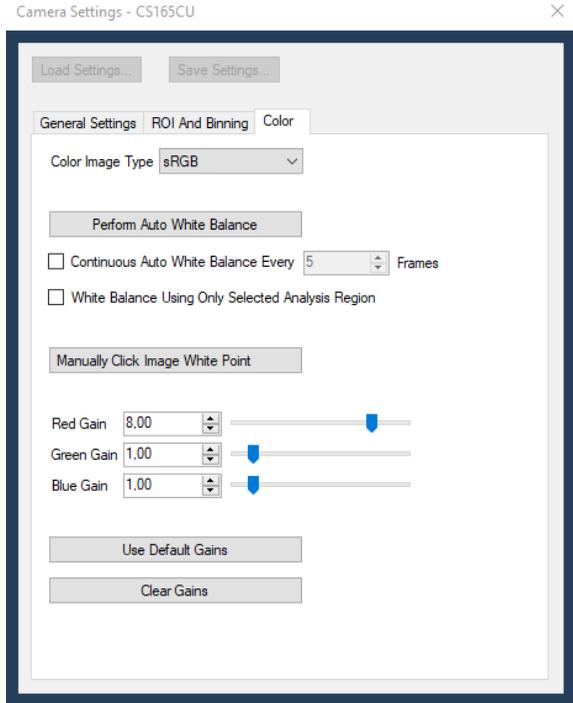


Figure 37 Camera Settings

With the ThorCam software you can also save snapshots during live imaging or record videos and timed series,   . The recording dialog box shown in Figure 38 lets you set file type, path and name.

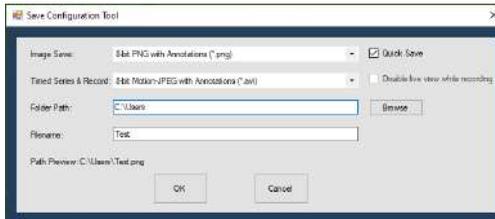


Figure 38 Record Video Settings

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

5.2.4. 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 the Thorlabs product page¹⁴ of either the KDC101 or the EDU-OT2(/M).

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 0.005 to 0.03 mm/s. Furthermore select “Joystick Mode” in the menu and chose “Velocity Control”. This corresponds to a continuous motion of the stage when the wheel is actuated. Confirm with the menu button.

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

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

The two windows, shown in Figure 39, 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.

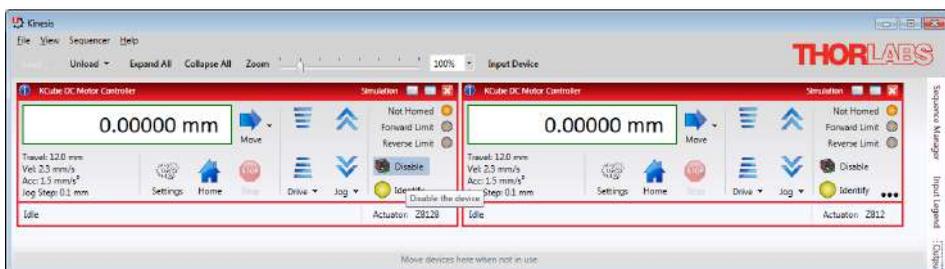


Figure 39 Kinesis Software User Interface

¹⁴ www.thorlabs.com/newgrouppage9.cfm?objectgroup_ID=2419

Settings:

- Press the *Settings* button in the Kinesis software and select the “Advanced” tab (Figure 40).
- In the *Device Controls* field, choose “Velocity control” for the *Wheel Mode* (sometimes also referred to as Joystick Mode). This corresponds to a continuous motion of the stage when the wheel is actuated.
- The recommended *Max. Velocity* is set to 0.03 mm/s and *Acceleration* to 1 – 2 mm/s².
- The recommended¹⁵ *Drive Array Velocities* are set to
 - *Velocity 4*: 0.03
 - *Velocity 3*: 0.02
 - *Velocity 2*: 0.01
 - *Velocity 1*: 0.005

This defines the different velocities which can be accessed via drive buttons in the software as shown in Figure 41. 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.

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.

You can operate the motors either by hand via the controllers or by software. When you choose the latter, you can move the stage by pressing the “Drive” buttons in the main window, see Figure 41. Here, you can also open the Drive Array Velocities in a drop-down menu. Each of the horizontal blue bars in the “Drive” button field corresponds to one of the velocities.

¹⁵ These velocities are good start values to ensure that the particles are not lost when moving at maximum speed. If the Optical Tweezer is adjusted very well, trapping is possible at even higher velocities.

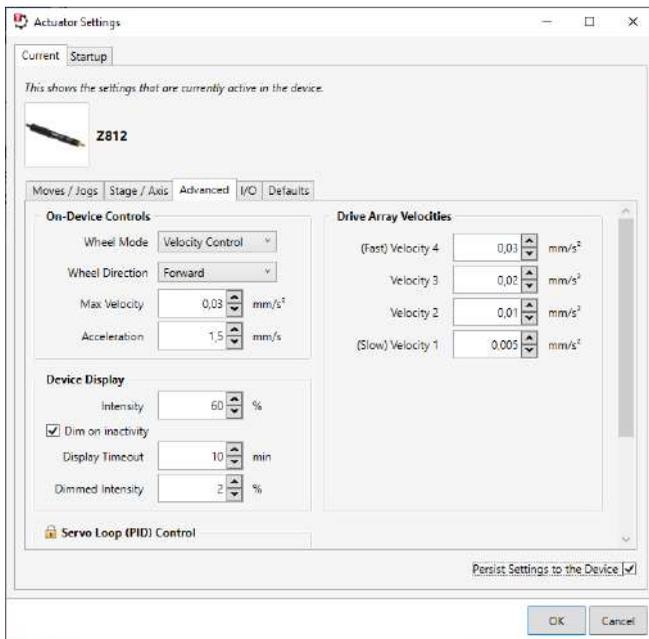


Figure 40 Kinesis KDC101 Settings

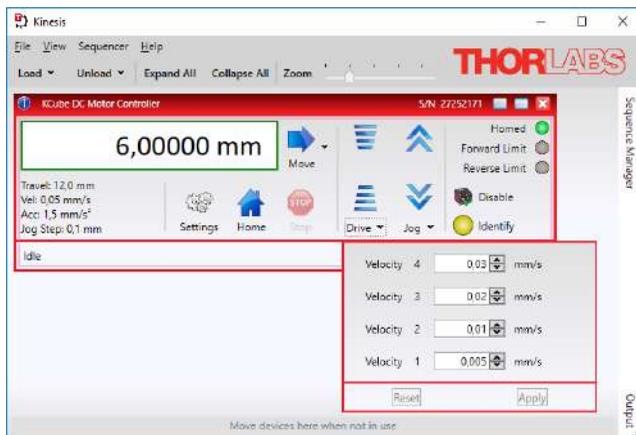


Figure 41 Kinesis Drive Array Velocity

5.2.5. Kinesis Settings

Thorlabs Kinesis software can be set to grab the current settings from the connected devices on startup. This option can be activated via the dropdown menu *File -> Options* by checking the box *Use device persisted settings* (see Figure 42).

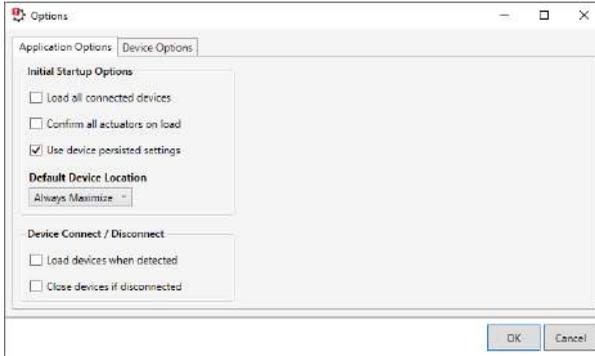


Figure 42 Use Device Persisted Settings

In most cases for the optical tweezers setup you would want to read the settings of the device, so before connecting the controllers, open the Kinesis software and select *Use device persisted settings*. This will be applied for all connected devices.

5.3. Camera Adjustment

The objective used in this kit is infinity-corrected. That is a great advantage and eases the adjustment significantly. It means that the light from the objective's focal plane leaves the objective effectively parallel. This plane can be mapped onto a camera with a lens at an almost arbitrary distance to the objective. To find the correct distance between lens and camera chip, one only needs to map a distant object onto the chip. Proceed the following way:

Step 1: Preparation

- Remove the camera segment from the microscope (that includes all parts starting from the camera until the cage plate above the beamsplitter). For that, unscrew the cage locking screws from the CP33T(/M) and loosen the screw on the C1498(/M) post clamp.
- Start the camera software.
- Open the settings and set the gain to maximum.

Step 2: Lens Adjustment

- Aim the camera at an object more than 4 m away. Make sure the room is well lit or activate the gain boost in the camera settings (beneath the regular gain). You could also increase the exposure time.
- Move the lens in the cage segment until you see a sharp image of the object. Below is a sample image of a clock at 4 meters distance.



Step 3: Installation of the camera

- Place the camera segment back into the microscope. Tighten the cage locking screws of the CP33T(/M) and fix the screw on the C1498(/M) post clamp.

5.4. Beam Adjustment



Warning



The class 3B laser diode used in this kit can emit more than 50 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.

Step 1: Preparation

- Remove the removable cage segment with the two lenses forming the beam expander.
- Take the laser out of its mount by opening the locking screws on the holder. Guide it through the CP45T(M) cage plate.
- Screw in the collimation lens' retaining ring as far as possible.
- Screw in the collimation lens as far as possible (as close to the diode as possible), see image to the right.



Step 2: Collimation

- Turn the safety key on the KLD101 laser driver to ON.
- Press the “Laser On” button.
- Turn the knob to set the diode current to 50 mA.¹⁶
- After ensuring that the area is clear of other people, point the laser at a wall, preferably more than 3 m away.
- Slowly screw the collimation lens away from the diode until you see the laser light distribution on the wall has turned into a small spot. Note that the pattern will be slightly asymmetrical. That is not a problem.
- Fix the collimation lens with the collimator's locking ring.
- Turn off the laser by pressing the button near the “Laser On” light once again.

¹⁶ Make sure you have set the maximum current to protect the laser diode, see Chapter 5.2.2.

Step 3: Laser Installation

- Place the alignment plate into the cage system right before the 45° mirror, see image to the right.
- Insert the laser into the mount without securing it with the locking screws yet.
- Turn the laser on and observe the pattern on the alignment target. You will see an elliptical shape.
- Rotate the collimator in the housing until the long side of the ellipse is oriented in vertical direction.
- Fix the locking screws. Make sure the locking screws fix the collimator close to the thread, see image below.



Step 4: Laser Mount Alignment

- Turn the tip and tilt screws on the KC1-T mount until the brightest spot of the laser light distribution hits the hole in the alignment plate.
- Turn off the laser.
- Remove the alignment plate.



Step 5: Adjustment Mirror Alignment, Part 1

- 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.
- Insert the ADF1 disk into the KCB1C(/M), ideally with the reticle aligned to the axis of the mount.
- Turn on the laser.
- Adjust the lower adjustment mirror so the laser beam exits the upper KCB1C **vertically**. For that, make sure the laser spot is centered on the reticle.
- Turn off the laser.
- Remove the reticle and place the mirror back into the mount and fix the locking screw.



Step 6: Insert Beam Expander

- Adjust the distance between the lenses: the distance between the CP45(M) cage plates should be 12 cm (distance between adjacent edges).
- Place the beam expander section back into the cage segment.
- Turn on the laser. It should look like the photo below. Note that the elliptical beam is oriented with the long arm vertical.

**Step 7: Final Adjustment**

- Increase the diode current to approximately 95 mA.
- Observe the expanded laser spot on the objective aperture. Adjust the upper alignment mirror to move the laser spot into the center of the objective aperture.



Note: Ideally, an optical tweezers system consists of a single mode laser that enters the objective as centered and vertical as possible. Also, the laser beam should be as parallel as possible, with a small divergence angle. In our setup, this is not the case: After following the instructions above, remove the end cap at the beamsplitter and examine the beam shape using a viewing card or piece of paper. Notice that it converges at a distance of about 1 to 2 meters. In other words, the light is not very parallel.

However, keep in mind that we do not have a single mode laser. Since we optimized the setup for low cost, the laser diode used is relatively cheap. The intensity distribution of the laser diode is elliptical and differs quite a bit from the ideal, Gaussian case. In our trials, the trapping was clearly better when the laser showed the described convergence. Therefore, the adjustment instructions above were written to yield optimal trapping results rather than a minimum divergence angle.

5.5. Trapping, Microscope Focus, and Final Alignment

The table is now set to trap microscopic particles.

Step 1: Preparation

- Switch on your computer and start the camera software (see Section Figure 32).
- Set the velocity of the motors to 0.02 mm/s (see Section 5.2.4, you may increase the speed when you have trapped a particle).
- Set the LED lighting to maximum (see Section 5.2.1).
- Switch on the laser with a diode current of 95 mA (or the diode current you measured to obtain 40 mW of output power, see Section 5.2.2)

Step 2: Sample

- Prepare a sample with polystyrene-beads (see Section 6.1).
- Place the sample positioning table as low as necessary with the aid of the micrometer screw for the Z direction. Make sure to leave enough space to avoid a collision with the objective when the sample slide is inserted into its holder.
- Place the sample in the holder with the selected well under the objective.

Step 3: Approach

- Raise the sample positioning table with the micrometer screw and pay close attention to the camera image on the monitor of your computer. Note that the objective's working distance is below 0.5 mm due to the high numerical aperture.
- As you raise the sample, you will see a distorted red laser spot forming on the monitor. This is not the sample, but rather the top of the cover glass. Continue slowly raising the sample. The laser spot will disappear and reappear. The second time you see the spot indicates the bottom of the cover glass. Raise the sample higher, and the spot will disappear and reappear again. The third time you see the spot indicates that the laser focus is in the sample. At this point, the focus is at the right level. Figure 43 shows a schematic representation of the situations.

Please note that reflections of the laser may occur. In the camera image, these will appear with varying diameter (small spots or larger shapes). These are not a sign of bad adjustment and can be ignored.

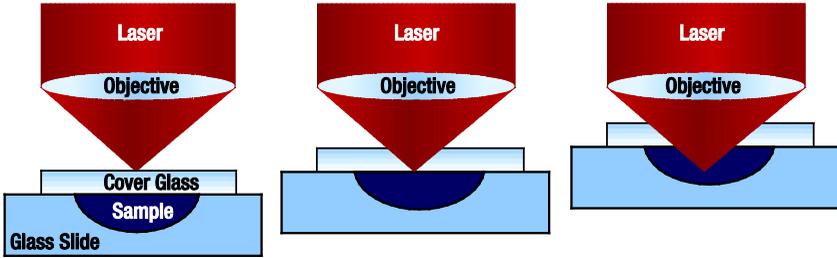
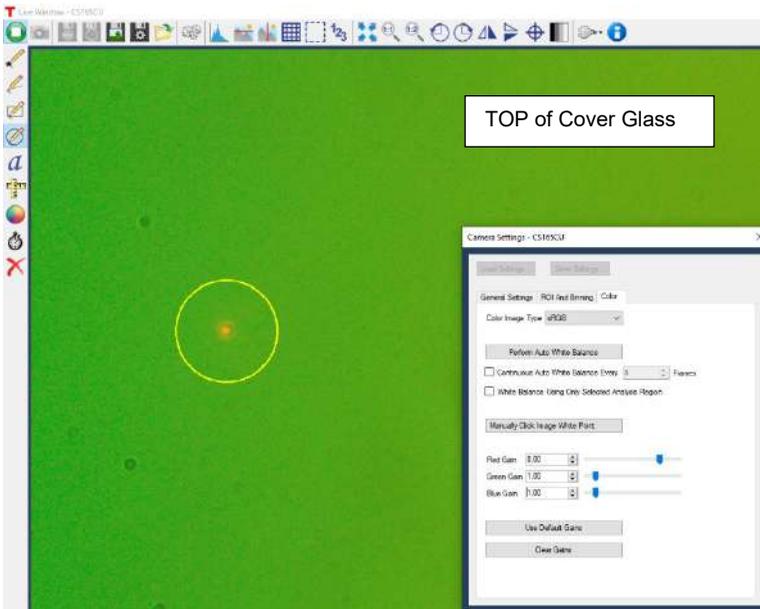


Figure 43 Focus Level Settings

- The camera image should look similar to Figure 44.



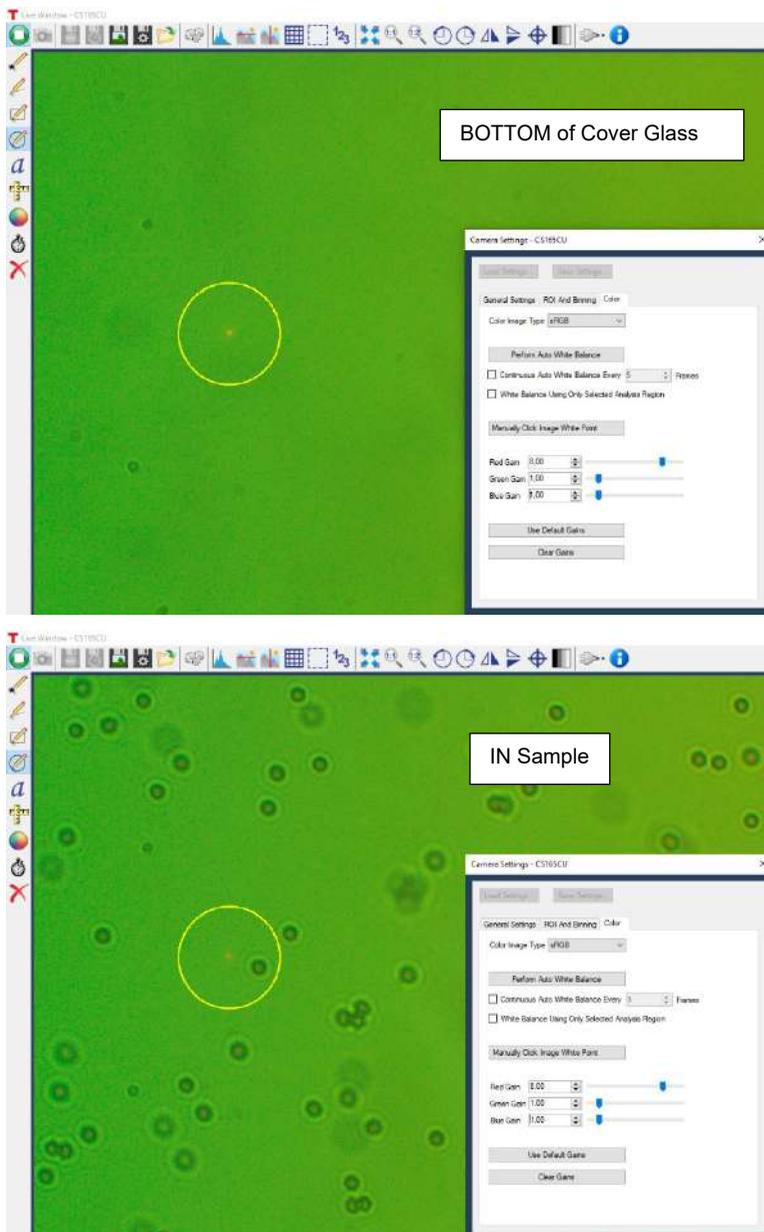


Figure 44 Camera Images During the Approach (2 μ m Beads)

Step 4: Camera Position

- Depending on the angle of the optical elements, the laser spot may not be in the center of the camera image. In fact, this is hardly ever the case and does NOT constitute bad alignment.
- You can move the laser spot closer to the center of the camera image by physically moving the camera. The camera's lens tube is mounted on an SPT1C slip plate that allows travel of ± 1 mm in each direction. Loosen the two screws at the top, move the slip plate with the attached lens tube/camera and fix the screws again.
- In rare cases, you may initially not see a laser spot in the camera image at all. In these cases, you may find it within the travel range of the SPT1C. Make sure you have the correct plane by getting the beads in focus and move the SPT1C's slip plate to find the laser spot.

Step 5: Trap a Bead

- Move the stage using the wheels on the controller cubes. Move the sample so that a bead comes to rest under the laser spot, see Figure 45.
- When the bead is close to the spot, you should see it getting pulled into the focus.
- Move the sample around by moving the stage. The bead should stay trapped in the laser focus while the sample is moved.

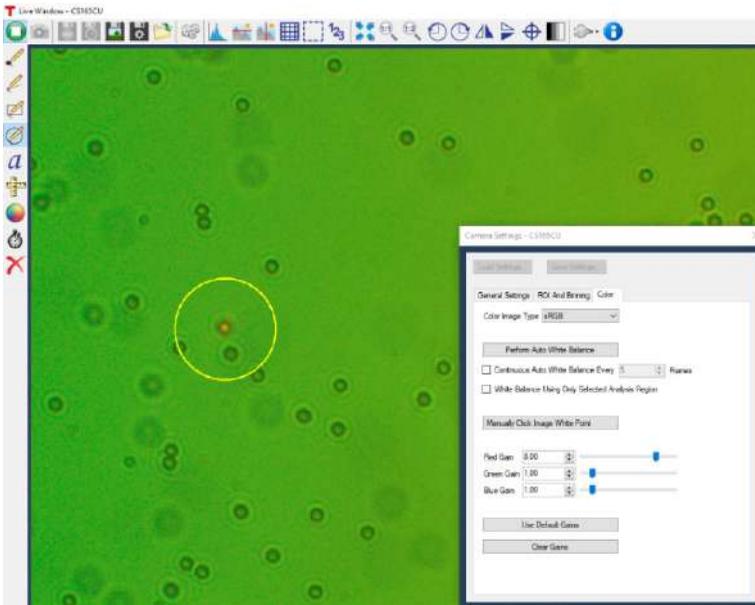


Figure 45 2 μ m Bead is Trapped in the Laser Focus

Step 6: Final Corrections and Potential Errors

- If you still have trouble seeing the laser spot, set the red amplification to maximum when adjusting the live image. You can also try dimming the LED.
- If trapping is weak, slightly move the sample up or down to find the optimal plane of trapping.
- If the laser spot is in a significantly different plane than the plane where the beads can be seen sharply, you may have to adjust the focal planes, see Figure 46 and the explanation below. Move the lens in front of the camera in the Z direction along the cage rods 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.
- If you want the movement in Z direction to be smoother, a hex key can be inserted in the adjuster of the MT1B. This allows you to move the stage up and down with less vibration in the system.

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 in Figure 46). 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 46) 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.

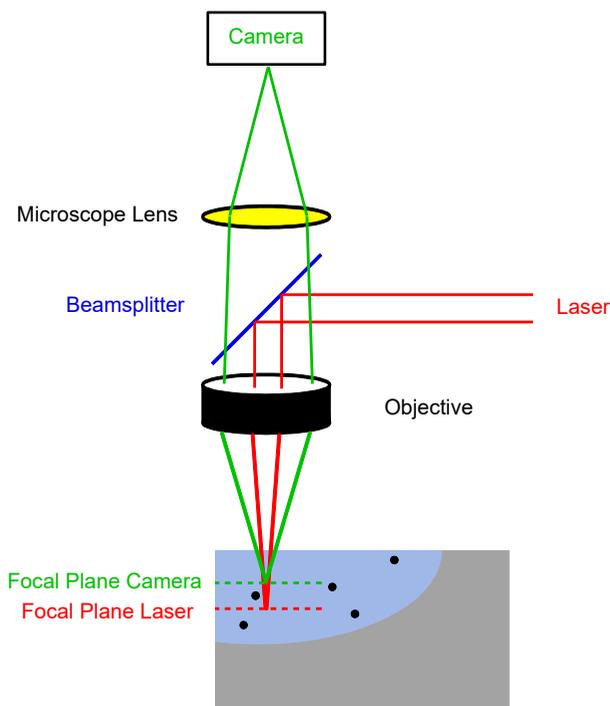


Figure 46 Laser and Camera Focal Planes

Chapter 6 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.

We do, however, recommend the use of the OTKBTK since the setup was optimized for the sample slides and the cover glasses provided with the OTKBTK.



Figure 47 Content of the OTKBTK Preparation Kit with tools for glass bead sample preparation (green highlighting). Do not use immersion oil.

6.1. Creating a Sample

A sample containing $1\ \mu\text{m}$ or $3\ \mu\text{m}$ 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 $20\ \mu\text{m}$ deep cutouts
- Cover glass
- Pipette
- Solution with fused silica (glass) beads
- **Do NOT use immersion oil. The supplied objective is an air objective and immersion oil is neither needed nor recommended in this setup.**

First, shake the glass bead solution and place one or two droplets (approx. 30-40 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 48). Remove excess solution with a tissue.



Figure 48 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.¹⁷

6.2. 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. Move the sample stage to center the sample under the objective. For this step, it may be convenient to temporarily set the maximum velocity of the KDC101 controllers to a higher value, see Section 5.2.4. 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 49 shows a schematic representation of the laser focus levels.

The beads might show a drift motion in one direction. This should stop once the sample is settled for a minute.

Note: Do not move the sample positioning table too far upwards. Otherwise, there is a risk of destroying the cover glass by touching it with the objective. This may damage the objective and would entail unnecessary cleaning work.

¹⁷ Replacement pipette tips can be found here <http://www.accumaximum.com/tips-ordering.html>

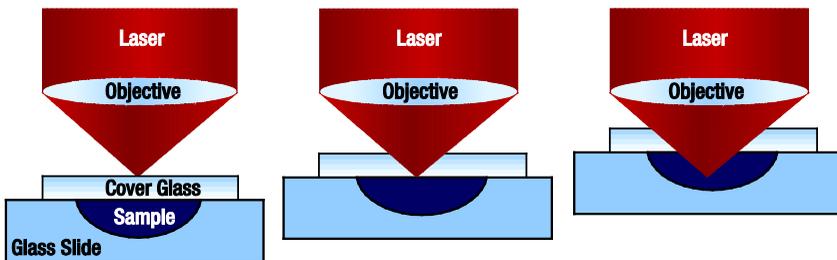


Figure 49 Focus Level Settings

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.

Please note that reflections of the laser may occur. In the camera image, these will appear with varying diameter (small spots or larger shapes). These are not a sign of bad adjustment and can be ignored.

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 5.5, above, for details.

6.3. 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 50. 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 or slightly dried samples, one can observe that some beads no longer move, but instead "stick" in place.

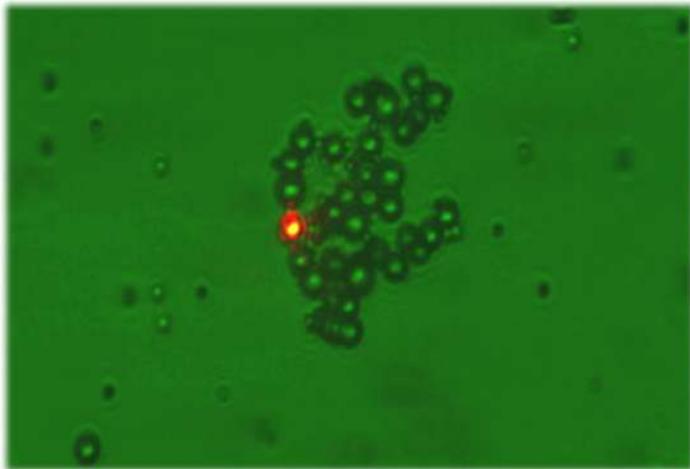


Figure 50 Beads as Viewed Through the Microscope

6.4. Manipulating a Dairy Cream Particle in 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 6.1, 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 51). 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.

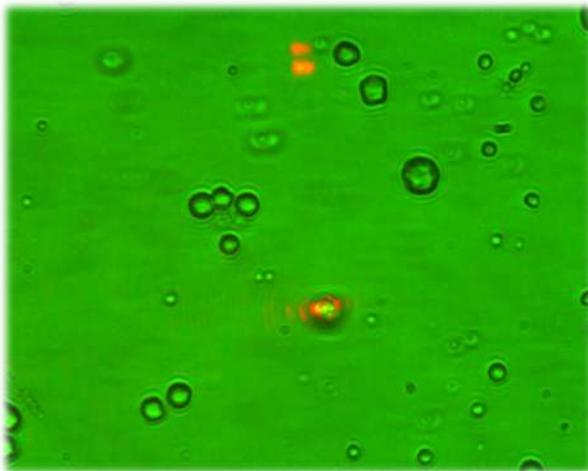
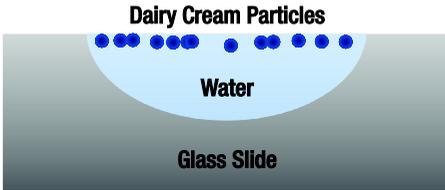


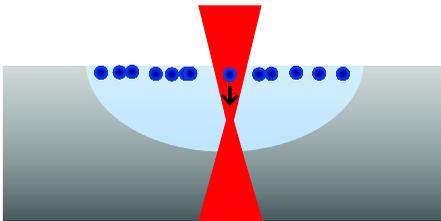
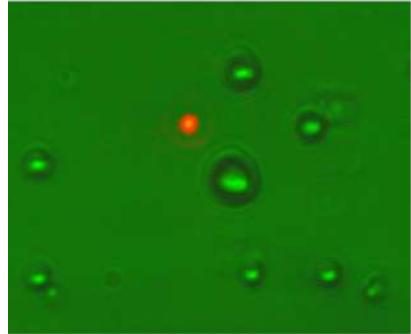
Figure 51 Cream Particles in a Cream / Water Emulsion

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

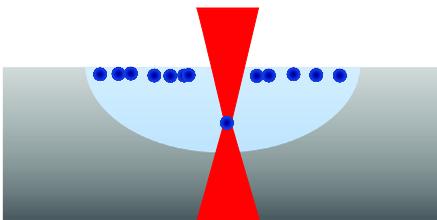
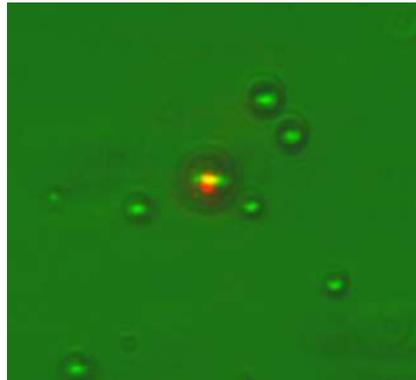
be located in the optical trap and thus to move upward once again to the surface of the water. This procedure can be observed again with the aid of the z-axis screw.



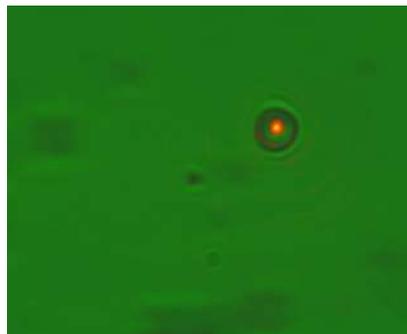
Cream particles collect on the surface of the water.

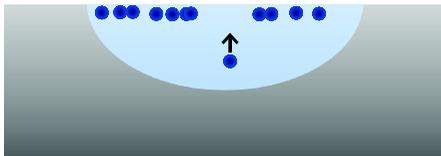


A cream particle is pushed down by the laser focus.

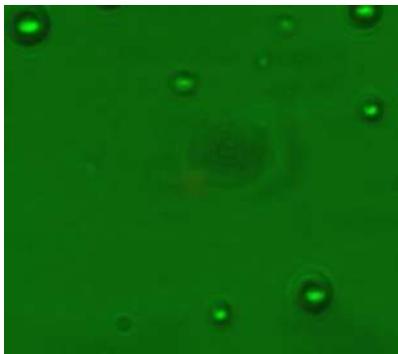


Cream particle is trapped at the laser's focus.





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



6.5. The Holding Force of the Optical Trap

6.5.1. 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 52). 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.

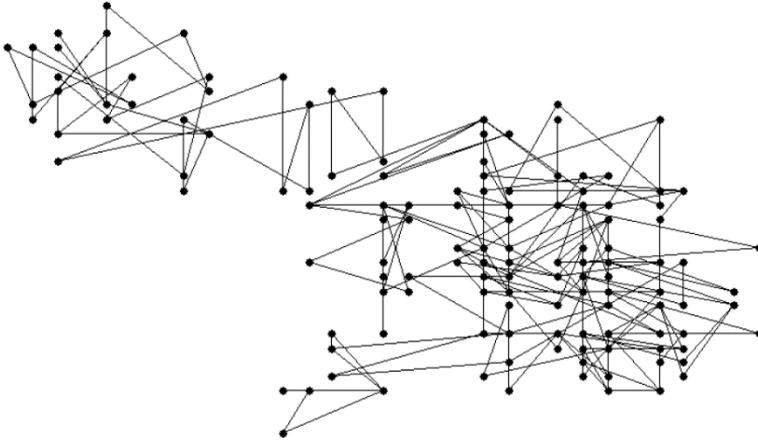


Figure 52 *Brownian Motion*

Use the sample with the $3\ \mu\text{m}$ 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 $1\ \mu\text{m}$ 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. Allow the sample to settle for a minute. If necessary, prepare another sample. Sometimes, collecting shorter video sequences with more particles is helpful.

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:

$$r^2(t_i) = (x(t_i) - x(0))^2 + (y(t_i) - y(0))^2 \quad (29)$$

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

$$\langle r^2 \rangle_{time}(t_n) = \frac{1}{n} \sum_{i=1}^n r^2(t_i) \quad (30)$$

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.

$$\langle r^2 \rangle(t_n) = \frac{1}{M} \sum \frac{1}{n} \sum_{i=1}^n r^2(t_i) \quad (31)$$

The obtained values for average displacement, $\langle r^2 \rangle(t_n)$, are now plotted with respect to time. Figure 53 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.

Scaling of the camera image: To accurately determine the movement of the particles, the real, physical dimensions need to be known. There are two options:

(i) use a microscopic ruler to determine how many pixels correspond to a micrometer. Standard test/resolution targets such as the USAF pattern or the NBS 1952 pattern can be found on our website.

(ii) you can calculate the theoretical dimensions. For that, it is important to note that the objective magnification always depends on the tube lens. The objective used in this setup is made by Zeiss and is labeled as a “63x” objective. However, Zeiss’ standard for tube lenses is a focal length of 165 mm while our system’s tube lens has a 100 mm focal length. To obtain the “effective objective magnification”, you multiply the design magnification with the actual tube lens focal length and divide by the design tube lens focal length. That means that the effective magnification in our system is $63x \cdot 100 \text{ mm} / 165 \text{ mm} = 38.2x$. The sample area is then retrieved by dividing the camera sensor dimensions by the system magnification. The CS165CU camera’s CMOS sensor has an imaging area of 4.968 mm x 3.726 mm. Thus, the imaged sample area is

$$\frac{4.968 \text{ mm}}{38.2x} \times \frac{3.726 \text{ mm}}{38.2x} = 130 \mu\text{m} \times 98 \mu\text{m}$$

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 53 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.

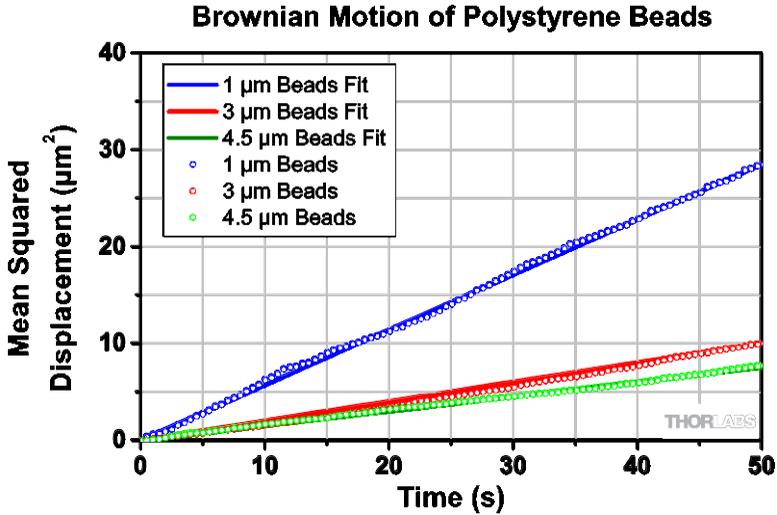


Figure 53 Mean Squared Displacement for Polystyrene Beads of Different Sizes

6.5.2. 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:

$$F_R = 6\pi\eta_{eff}Rv \tag{32}$$

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:

$$m = \frac{2k_B T}{3\pi\eta_{eff}R} \tag{33}$$

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 (33) 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:

$$\eta_{eff} = \frac{2k_B T}{3\pi R m} \quad (34)$$

Here, T is the room temperature, k_B the Boltzmann constant, R 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 $10^{-3} \frac{\text{Ns}}{\text{m}^2}$

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 :

$$F_R = 6\pi\eta_{eff} R v \quad (35)$$

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:

$$F_{Stokes} = F_{H,max} = 6\pi\eta_{eff} R v_{max} \quad (36)$$

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

6.6. Other Samples

There is a wide range of samples that can be investigated with the optical tweezers.

- Water from a pond or a hay infusion features microscopic organisms, some of which can be trapped with the tweezers.

- We were able to trap and move toothpaste matter (we are not sure whether or not this is microplastic). To prepare a sample, mix a small bit of toothpaste with distilled water.



- Yeast cultures can be trapped.
- Live lactobacillus are sold as dietary supplements. We were able to trap these as well.

Chapter 7 Teaching Tips

To engage students, it is particularly helpful to point out that they have a Nobel winning experiment in front of them! 48 years after his paper entitled “Acceleration and Trapping of Particles by Radiation Pressure” (Physical Review Letters, 24, No. 4, Jan. 26, 1970 (pp. 156–159)), Arthur Ashkin was awarded the Nobel prize “for the optical tweezers and their application to biological systems”. This emphasizes the profound meaning this technology has in fundamental research. Students may also enjoy the Nobel lecture on optical tweezers, found via:

<https://www.nobelprize.org/prizes/physics/2018/ashkin/lecture/>

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.

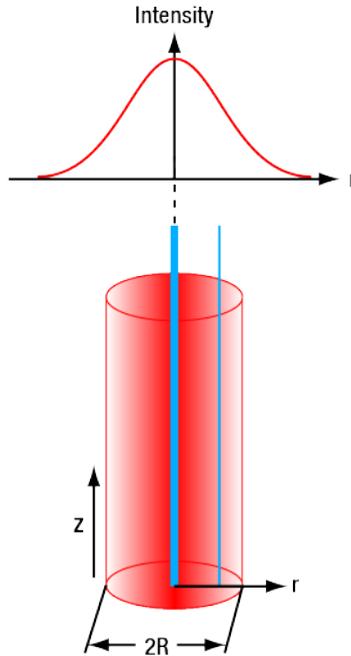


Figure 54 Gaussian Intensity Profile

As shown in Figure 54, 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 54.

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 55. The beams are labeled s and t .

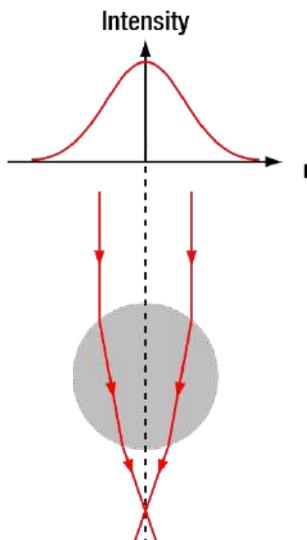


Figure 55 *Unfocused Laser Beam Strikes a Polystyrene Bead Located on the Beam Axis*

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 56, 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.

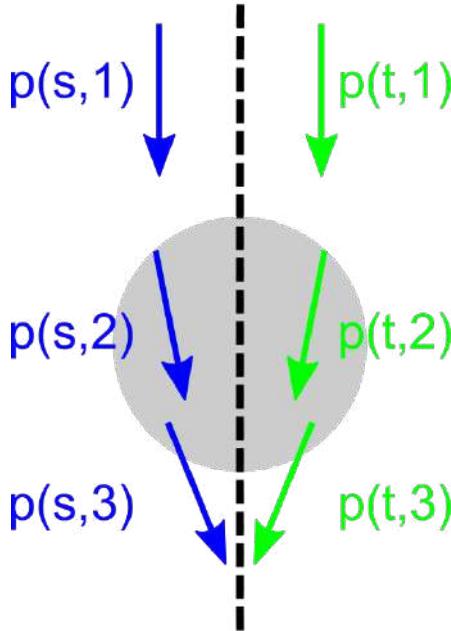


Figure 56 Unfocused Laser Beam Momentum

For example, the vector labeled with $p(s,1)$ depicts the momentum of the beam s at position 1. It is 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 57, 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 an orange arrow.

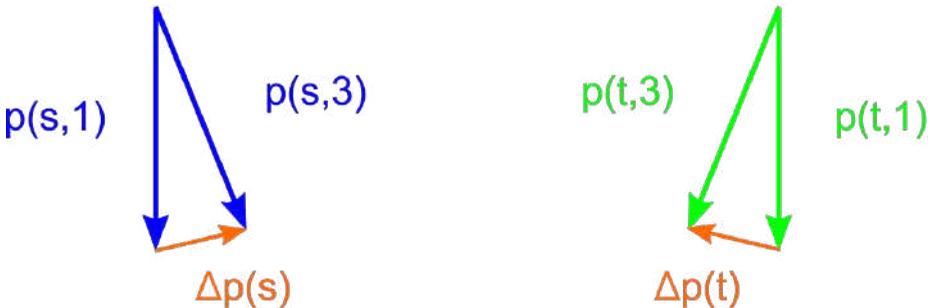


Figure 57 Unfocused Laser Beam Momentum Changes

To obtain the net momentum change of both partial beams, we add both orange change of momentum vectors in Figure 58. This line of argument can be transferred qualitatively to the entire bundle of partial beams.

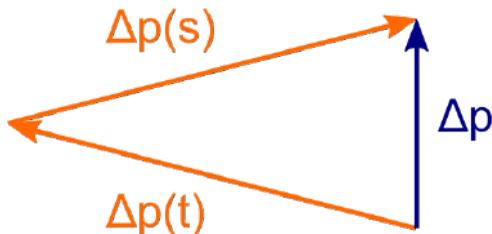


Figure 58 Unfocused Laser Beam Total Momentum Change

In Figure 58 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 59.

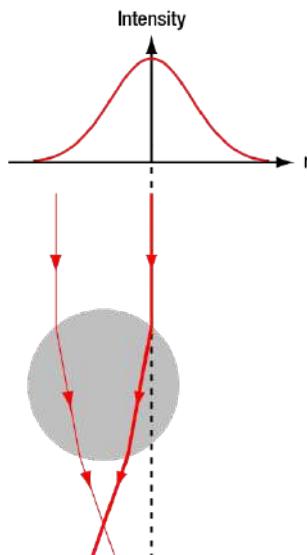


Figure 59 Unfocused Laser Beam Incident on a Polystyrene Beam Located Off the Beam Axis

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 60.

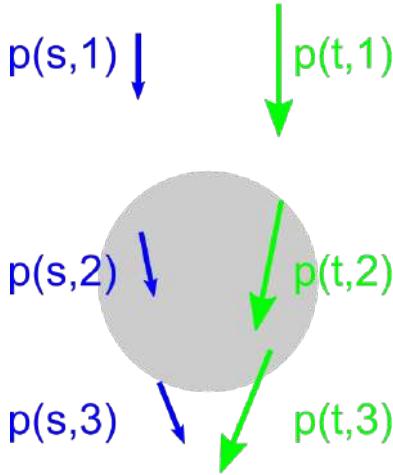


Figure 60 Unfocused Off-Axis Laser Beam Momentum

Both partial beams then exhibit the momentum change displayed in Figure 61.



Figure 61 Unfocused Off-Axis Laser Beam Momentum Changes

As shown, the partial beams exhibit momentum changes that differ in magnitude. Figure 62 shows the resulting net momentum change of the entire laser beam.

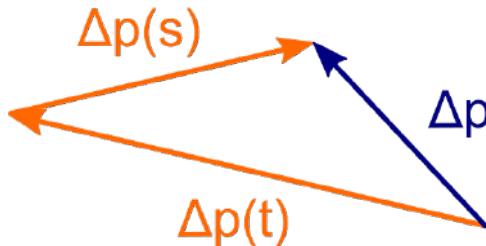


Figure 62 Unfocused Off-Axis Laser Beam Total Change in Momentum

Figure 62 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 63 depicts two partial beams of the focused laser. The center of the bead is assumed to be below the focus.

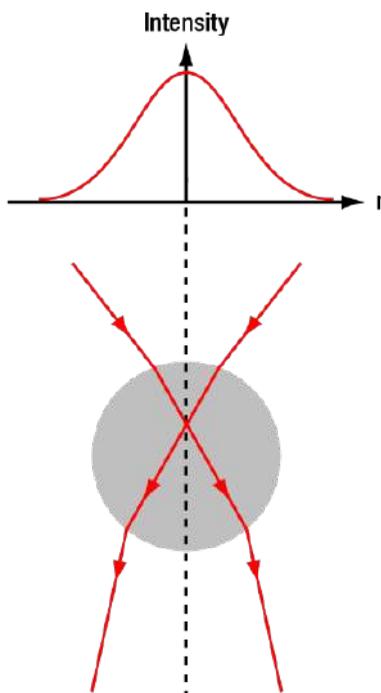


Figure 63 Focused Laser Beam Incident on a Polystyrene Bead

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.

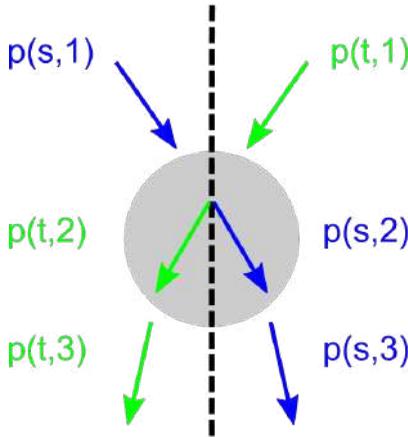


Figure 64 Focused Laser Beam Momentum

Figure 65 displays the momentum change of both partial beams.

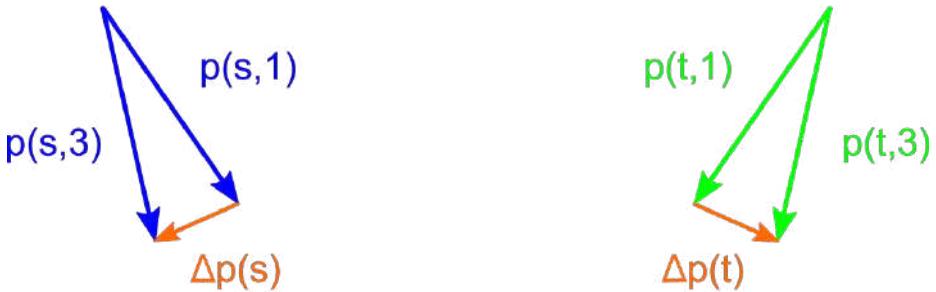


Figure 65 Focused Laser Beam Momentum Changes

The net momentum change of the entire beam is depicted in Figure 66.

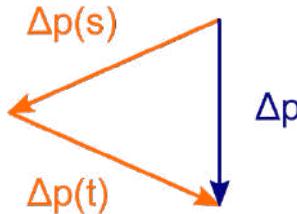


Figure 66 Focused Laser Beam Change in Momentum

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 8 Control via Game Controller

The Thorlabs Kinesis Software includes the possibility of controlling various components via external input devices. For the Optical Tweezer kit, this allows to drive the KDC101 Servo Motor Controllers and in turn the sample stage movement via a game controller. Please note that no game controller is provided with the kit. In the following, the required settings are explained. They have been tested with a Logitech F310 controller under Windows 10. While the feature could work with similar devices, we do not guarantee this.

1. Connect both KDC101 controllers as well as the game controller to your PC via USB, then start the Kinesis software.
2. From the main menu of the Kinesis software, choose *File -> Input Devices*.
3. In the upper right corner of the window that has opened, select your controller from the dropdown menu.
4. Optionally, you can turn the laser on/off via the game controller. To do this, connect your KLD101 to the PC via USB, select its serial number in the *Target* dropdown menu behind *Button A* and choose *Laser On* in the *Action* dropdown menu. Do the same for *Button B* and select *Laser Off* as Action.
5. On the right side of the same window, select the *Target* dropdown menu of *Left Analog Y*, then choose the serial number of the KDC101 that is responsible for a movement of the camera image on the screen in vertical direction. Choose the other KDC101 in the *Target* dropdown menu of *Left Analog X*.
6. In the *Action* dropdown menus of both *Left Analog Y* and *Left Analog X*, select *Move Continuous*. The settings should now look similar to Figure 67.
7. Click on the *Calibrate*-Button next to *Left Analog Y*. In the window that opens, check the box in front of *Use Custom Banding Values* (see Figure 68).
8. In the table appearing below, you can now customize the movement speed of the stage in relation to the displacement of the analog stick. This is done by pairing a lower boundary of the joystick displacement with a corresponding movement speed. Such value pairs can be created by clicking the *Add* button on the right side of the table. If the joystick displacement is larger than the boundary, the stage will move at that speed until the displacement of the next higher boundary is reached.

The velocity is expressed as a percentage of the maximum speed of the sample stage motors, which is about 2.5 mm/s. We recommend the setup displayed in Figure 68 for both axes (Note the checked *Invert Sense* checkbox for the Y-axis). Depending on the controller and the intended experiment, other values may be more suitable.

Before using the game controller, the KDC101 controllers should be homed. To do this, press the *Home* Button for both controllers in Kinesis (see Figure 69). After homing, the stage will be in one corner of its travel range and should be moved in positive direction for both axes to center the sample under the objective.

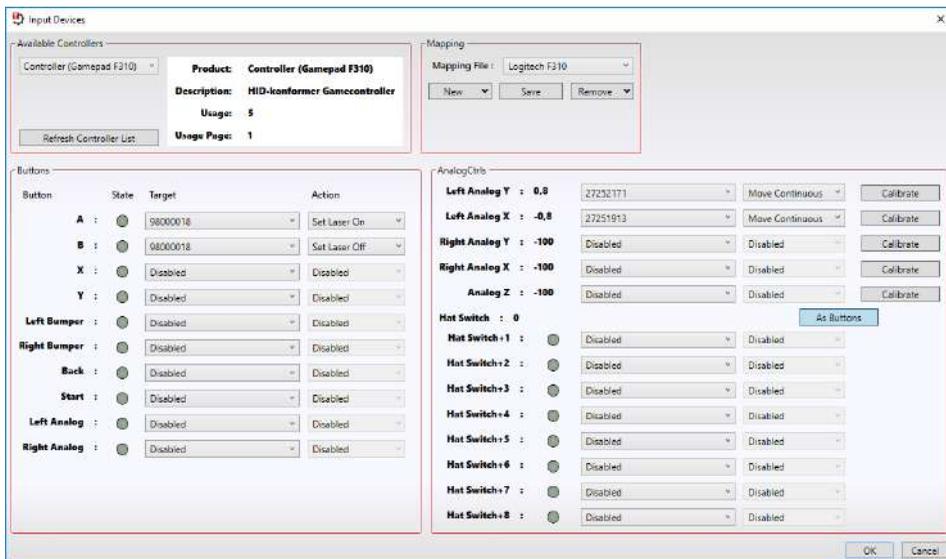


Figure 67 Input Devices Settings in Kinesis Software

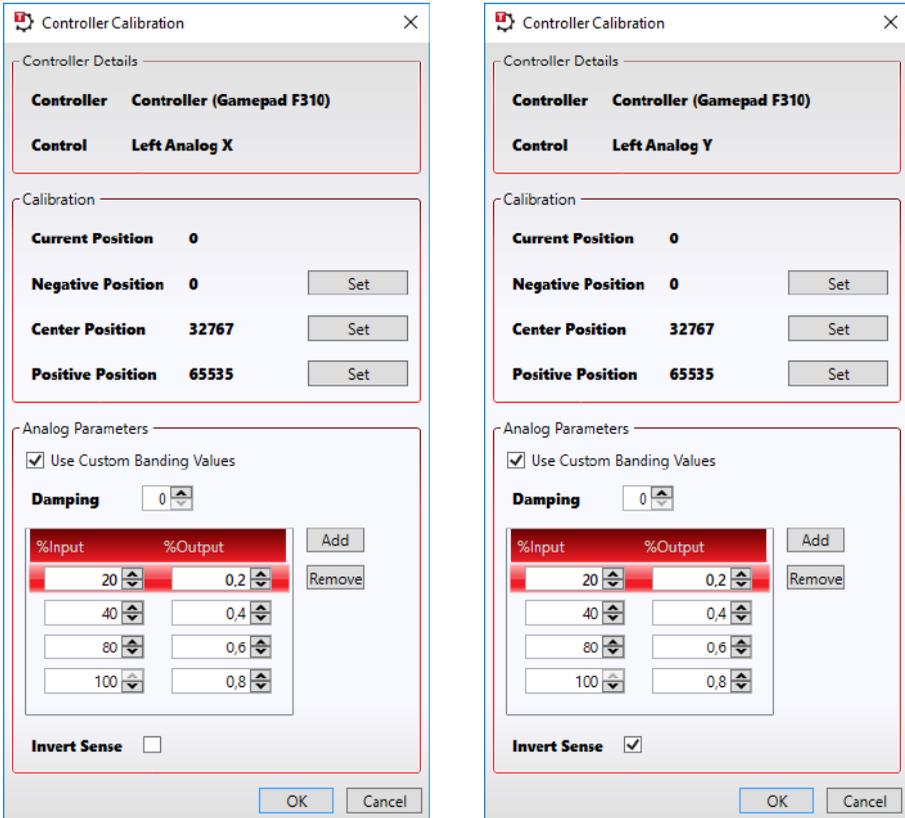


Figure 68 Recommended Controller Calibration Settings

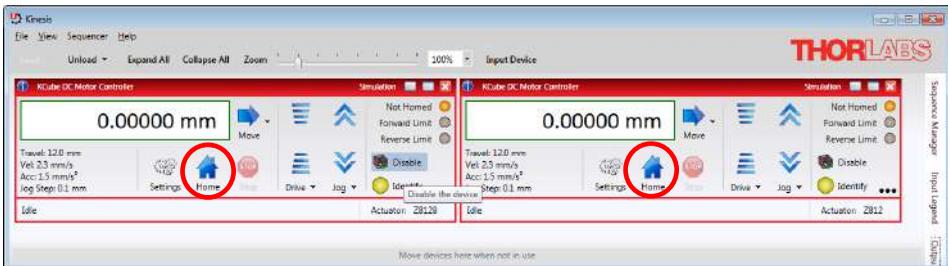


Figure 69 Homing the KDC101 controllers

Chapter 9 Troubleshooting

- *When the stage is moved, the sample quickly moves out of focus and loses the bead.*

We have seen this behavior when the screw on the locking plate is tightened too much.



- *The camera image is stuck/frozen.*

If the image is stuck (framerate in status bar not counting), close the program. Take the USB out and plug it back in. Restart the ThorCam software. Enter the settings and reduce the pixel clock to 8 - 10 MHz, exposure to 100 ms, and restart the stream from the camera.
- *Actuating the knob on the KLD101 does not change the laser diode current.*

Make sure you are in the “Const I” mode. Make sure all settings are correct, especially the anode grounding. If the knob still does not show an effect, unplug the USB from the PC, turn the power of the KLD101 off and back on again.
- *All settings are correct, but the laser diode still doesn’t emit light.*

Switch of the KLD101, then remove the laser diode from its mount and reinsert it (see Section 5.1.1).
- *KDC101 controller shows the message “Overload”.*

This message means that the motor was driven to the maximum extent. First, try to switch the KDC101 off and back on again. Then, move the stage back. If this does not work, you need to remove the motor from the MT1(/M) stage. Loosen the screw that holds the barrel and remove the motor **while holding both parts of the stage to avoid rapid retraction and potential damage**. When the motor is removed, turn the KDC101 off and back on. Then, actuate the motor towards the middle position. Afterwards, insert the motor back into the MT1(/M).
- *When the stage is actuated, the camera image moves at an angle.*

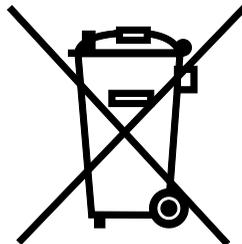
Make sure the camera is mounted parallel to the cage system.
- *The optical tweezers don’t trap beads.*
 - Check your laser diode’s current. It should be about 95 mA.

- You can try to vary the distance between the lenses in the beam expander section. 12 cm is the maximum distance (measured from the lens centers); you should not exceed it. You should also not go below 11 cm.
- Make sure your camera's plane is set correctly, see Section 5.3.
- Verify that the beam expander cage assembly is parallel to the breadboard using a level.
- You may try to slightly vary the collimation lens' distance to the laser diode.
- Make sure the laser spot hits the objective's aperture at its center.
- Is the objective clean? Are you able to find the plane with the beads in their solution? If not, you may need to carefully clean the objective.
- Have you used immersion oil? Please note that this setup does NOT require immersion oil. The objective is an air objective so please refrain from using immersion oil.
- Make sure there is enough liquid in the sample, see Figure 48. If there is not enough liquid, the beads will not be able to move.
- After a while, the beads will stick. You may need to prepare a new sample, or you can try to move the coverslip slightly to dislodge the beads (make sure to move the stage away from the objective first to avoid damage).
- If all of the above points do not show an improvement, please carefully repeat all steps in the adjustment procedure, Sections 5.4 and 5.5.

Chapter 10 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



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.

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.

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 11 Thorlabs Worldwide Contacts

For technical support or sales inquiries, please visit us at www.thorlabs.com/contact for our most up-to-date contact information.



USA, Canada, and South America

Thorlabs, Inc.
sales@thorlabs.com
techsupport@thorlabs.com

Europe

Thorlabs GmbH
europe@thorlabs.com

France

Thorlabs SAS
sales.fr@thorlabs.com

Japan

Thorlabs Japan, Inc.
sales@thorlabs.jp

UK and Ireland

Thorlabs Ltd.
sales.uk@thorlabs.com
techsupport.uk@thorlabs.com

Scandinavia

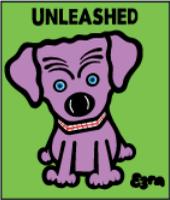
Thorlabs Sweden AB
scandinavia@thorlabs.com

Brazil

Thorlabs Vendas de Fotônicos Ltda.
brasil@thorlabs.com

China

Thorlabs China
chinasales@thorlabs.com



THORLABS
www.thorlabs.com
