OTKBFM-CAL Calibration and Measurement Module for OTKB with OTKBFM

User Guide

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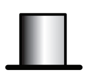
Chapter 1 Warning Symbol Definitions

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

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

Chapter 2 Safety

2.1. Safety Information

For the continuing safety of the operators of this equipment and the protection of the equipment itself, the operator should take note of the warnings, cautions, and notes throughout this user guide, and where visible, on the product itself.

The following safety symbols may be used throughout the user guide and on the equipment.

SHOCK WARNING
Given when there is a risk of injury from electrical shock.

CAUTION
Caution is given when there is a possibility of damage to the product.

WARNING
Given when there is danger of injury to users.

2.2. General Warnings

WARNING
If the product is used in a manner not specified in this user guide, the equipment may be damaged. Specific care must be taken to ensure that the applied input voltage levels do not exceed the product specifications and that any equipment connected to the output ports of the device is compatible with the supplied output voltage levels.

WARNING
Before connecting the included power supply to the AC mains, make sure the correct line input voltage for your region is selected. The selector is located on the back of the power supply housing.

Chapter 3 Introduction

The capability of optical tweezers to exert measurable forces on micron-scale, dielectric particles offers a unique and valuable tool for studying cell components, such as biological polymers and molecular motors. In many investigations, optical tweezers need to apply precise force to functionalized microspheres that have been attached to molecules of interest. For small displacements from the center of the trap, optical tweezers apply a force toward the focus of the trapping laser beam with a magnitude proportional to the distance of the particle from the focus. This allows the optical tweezers to be modeled by Hooke's law, $F_i = -k_i x_i$, where k_i is the spring constant and x_i is the displacement from the center of the trap.

In order to allow quantitative measurements the optical tweezers system needs to be calibrated. While there are a couple of different approaches, the most common technique is based on back focal plane detection.

As the laser beam passes through the sample plane interference occurs between the light which is transmitted through the trapped particle and the remainder of the light. As a consequence the interference pattern at the back focal plane of the condenser depends on the distance of the trapped particle to the trap center. The deflection is converted to an electrical signal by a quadrant photodiode, which produces a voltage proportional to particle position for small displacements from the center of the trap. Accurate force measurements depend on precise calibration of the spring constant (also called stiffness), k_i, and the sensitivity of the particle position detector, which vary with laser power and particle properties.

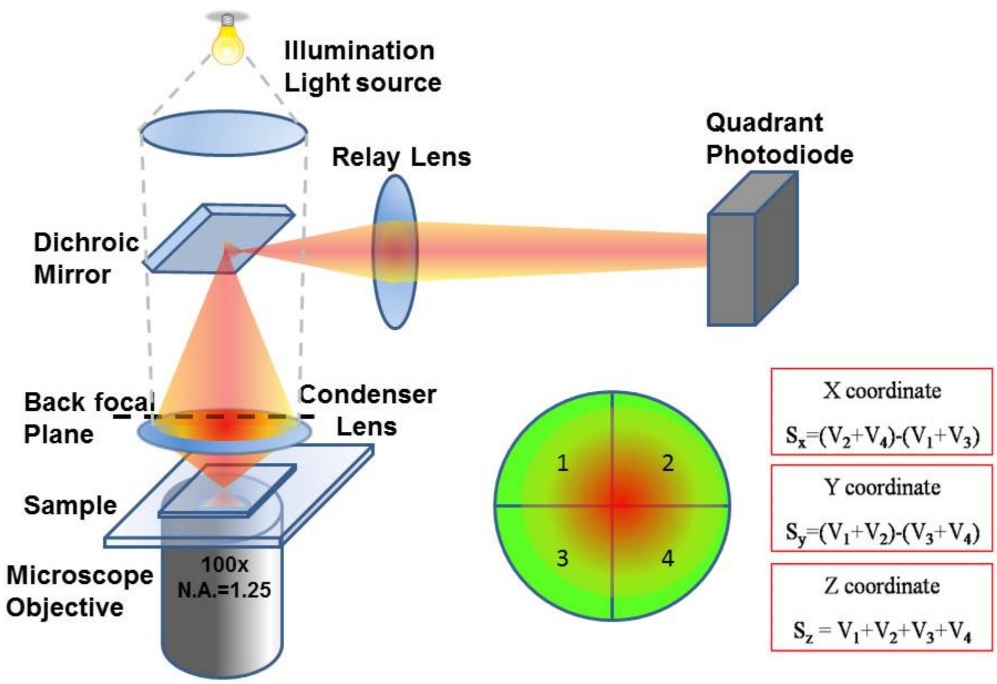


Figure 1 Schematic of the optical train of a tweezers setup with back focal plane detection. The inset illustrates how the X, Y, and SUM (S_x,S_y,S_z) signals are calculated.

In the following sections, two methods to determine the trap stiffness are described. Since each method relies on a different physical principle, the combined results provide a convenient way to verify the calibration.

3.1. Position Calibration

The data acquired from the QPD detector is given in volts. For quantitative force and position measurements it is necessary to determine the detector responsivity factor. The method used with the OTKBFM-Cal requires a stuck bead to be moved across the location of the trap while recording the detector voltage. Using the sample stage, steps of known size are used which then allow plotting of the position signal in volts versus the position signal in microns. When the bead moves across the trap position an S-shaped curved is formed as shown in Figure 2. Note that the conversion factor only applies if the distance of the trapped particle to the trap center lies within the linear range.

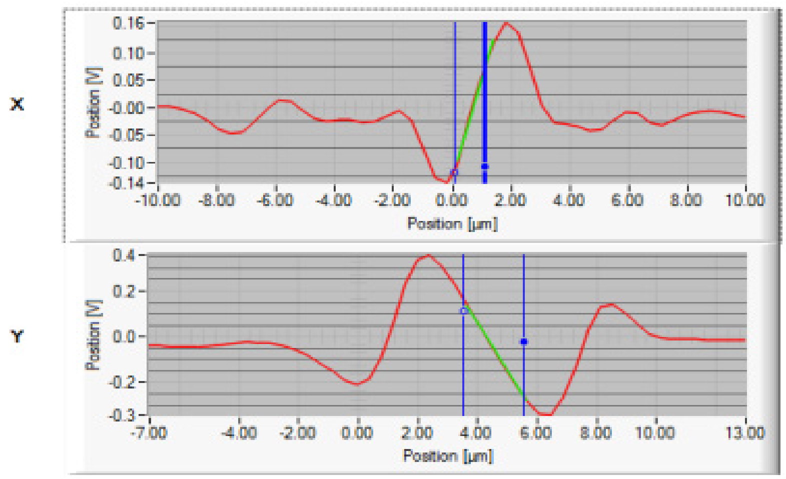


Figure 2 Typical position calibration curve. QPD voltage data is acquired while a stuck bead is moved across the focal spot / trap position.

3.2. Stiffness Calibration

The OTKBFM-CAL software determines the trap stiffness using two approaches: the so called PSD Roll-Off method and the equipartition theorem. The first approach is based on the frequency analysis of the thermal fluctuations of a trapped bead with a known damping. The equipartition theorem calibration on the other hand equates the known thermal energy per degree of freedom with the energy associated with the fluctuations of the particle.

This method requires the hydrodynamic drag coefficient to be known. On the other hand it does not require a position calibrated detector.

3.2.1. Equipartition Method

The equipartition theorem states, that each degree of freedom in a physica system at thermal equilibrium will have an energy of

$$\frac{1}{2}k_B T = \frac{1}{2}\mathrm{k}_i\langle x_i^2\rangle$$

Where k_i is the trap stiffness, k_B is the Boltzmann constant and $<x_i^2>$ is the statistical variance of the particle position. By recording the particle position and with k_B and the temperature known, it allows the stiffness factor to be determined. It should be noted that for this approach it is necessary to first determine the position calibration for the detector.

3.2.2. PSD Roll-Off Method

While the Roll-Off method also makes use of the Brownian Motion, it uses a frequency analysis of the fluctuations to determine the stiffness calibration factor. In the low Reynolds number regime, where most optical tweezers are operated, a microscopic bead in an optical trap can be described by the equation of motion of a damped oscillator with Brownian motion in the $\mathrm{x_i}$ direction with a corresponding velocity $\dot{\mathrm{x}}_\mathrm{i}$:

$$\beta\dot{\mathrm{x}}_\mathrm{i}(\mathrm{t}) + \mathrm{k_i x_i(t)} = \mathrm{F(t)},$$

where

$$\beta = 6\pi\eta\mathrm{a}$$

is the drag coefficient, η is the fluid viscosity, a is the radius of the bead, k_i is trap stiffness, and F(t) is thermal fluctuation induced force. If the fluid is water then we can take: $\eta = 8.90 \times 10^{-4}$ *Pa s* at room temperature. The power spectrum of the position fluctuations in this case is a Lorentzian

$$S(f) = \frac{k_b T}{\pi^2\beta(f^2 + f_c^2)}$$

with a Roll-Off frequency f_c of

$$f_c = \frac{\mathrm{k}_i}{2\pi\beta}$$

By fitting the power spectrum the Roll-Off frequency (also called corner frequency) can be calculated and hence the stiffness k_i is found. Note that it is not necessary to convert the detector data from voltage to distance, hence no position calibration is required and the PSD plot typically is expressed as $\mathrm{Volt}^2 \cdot \mathrm{s}$ versus frequency. The approach does however require the drag coefficient to be known.

Chapter 4 Setup

The OTKBFM-CAL is used in combination with Thorlabs force measurement module OTKBFM. The following installation procedure assumes that you have already completed the setup of the force module and that it is working in combination with your optical tweezers setup.

1. Use the included CD/DVD to install the OTKBFM-CAL software on your PC or download the latest version from the Thorlabs web page **www.thorlabs.com/manuals**. Make sure you are logged in with administrative user rights for the installation.

2. The Thorlabs APT software should already have been installed on your PC during the installation of the OTKBFM force module. If not, please download the APT software from our web page and install it.

3. Use the SMA-to-BNC cables (included) to connect the OTKBFM-CAL to the K-Cube piezo and strain gauge controllers. See Figure 3 below for details.

4. Connect the OTKBFM-CAL module via the USB cable to the PC and connect the power supply to the OTKBFM-CAL module. Ensure that the power supply is set to the correct voltage level for your region and connect the power supply to the OTKBFM-CAL control box. Switch on the power supply.

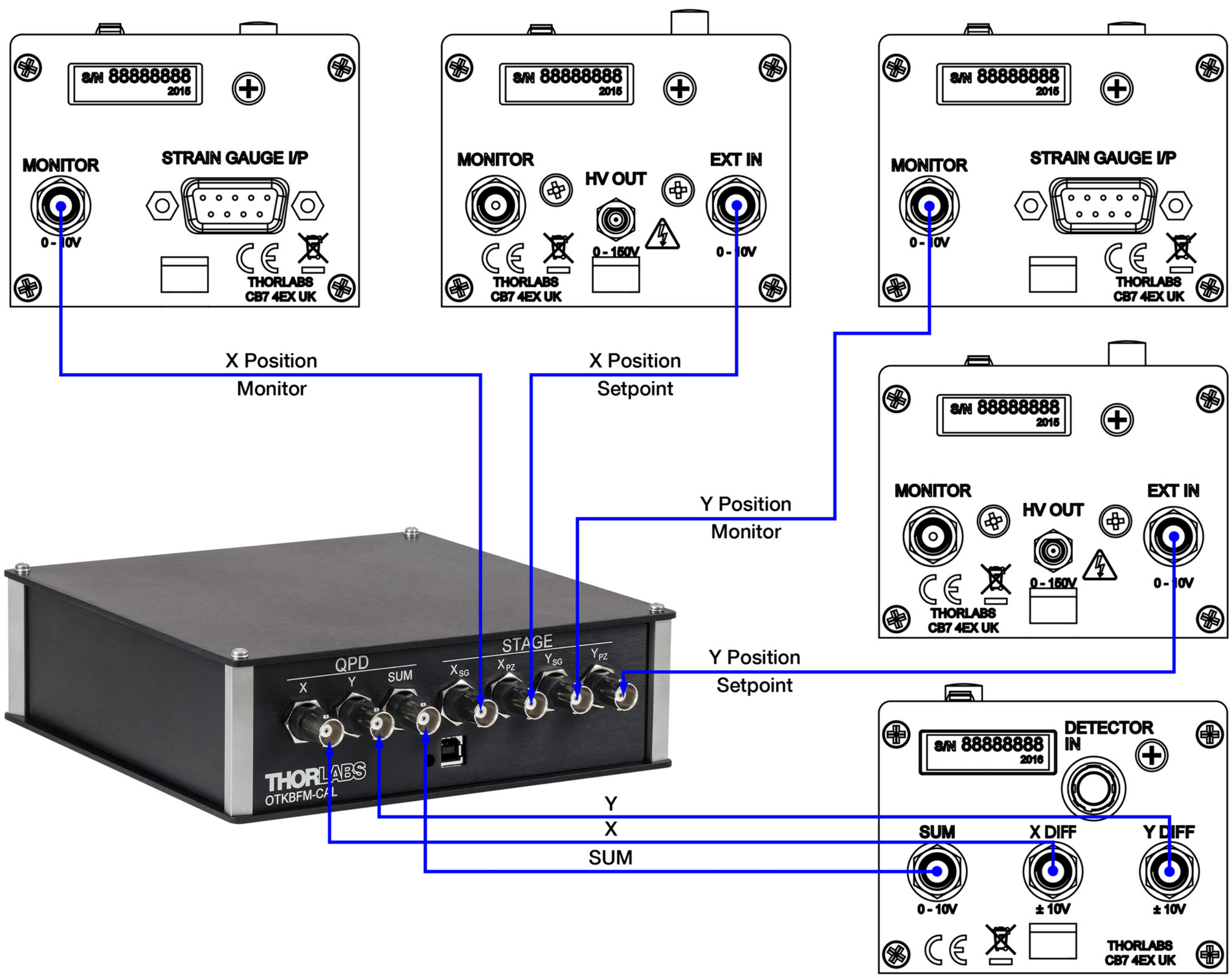


Figure 3 Connections Between OTKBFM-CAL Control Box and Piezo/Strain Gauge Controllers

5. Start the "APT User" software to configure the controller set. The program should show you a window for each of the K-Cube controllers. The OTKBFM-CAL module requires two piezo controllers, two strain gauge controllers and the PSD controller cube.

6. Select "Settings" in one of the piezo controller windows and adjust the settings as shown in Figure 4. Close the window and do the same for the second piezo controller.

7. Click "Zero" on the strain gauge readers control windows. Alternatively, you can press the control button on the strain gauge K-Cube for at least two seconds to start the zeroing process. The cube will count down to zero on the K-Cube display to indicate the duration of the procedure.

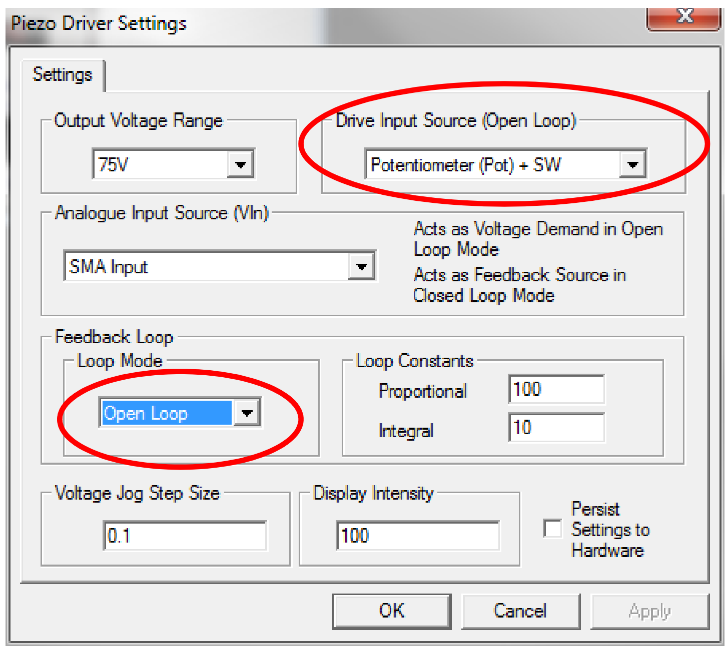


Figure 4 Piezo Controller Panel, Settings Required During Zeroing of Strain Gauge Controllers

8. After the strain gauge controllers have been set to zero, you can set the piezo controllers to closed loop mode. Select each of the piezo controller Setting windows and adjust the settings as shown in Figure 5. Make sure that the “Persist Settings to Hardware” flag is set; this will save the configuration to the controller’s EEPROM.

WARNING

If the Piezo Driver is switched to closed loop mode without a feedback signal applied, the piezo drive voltage will ramp up to the maximum limit. If the limit is set to 75 V, a protective circuit limits the voltage to 75 V. If 100 V or 150 V is selected, the output will ramp to 150 V. Using the standard piezo stage supplied with the OTKB, the output voltage may not exceed 75 V. Higher voltages will damage the stage.

9. For the PSD controller cube remember to **set the operation mode to ‘Monitor’**.

10. Close the APT User software.

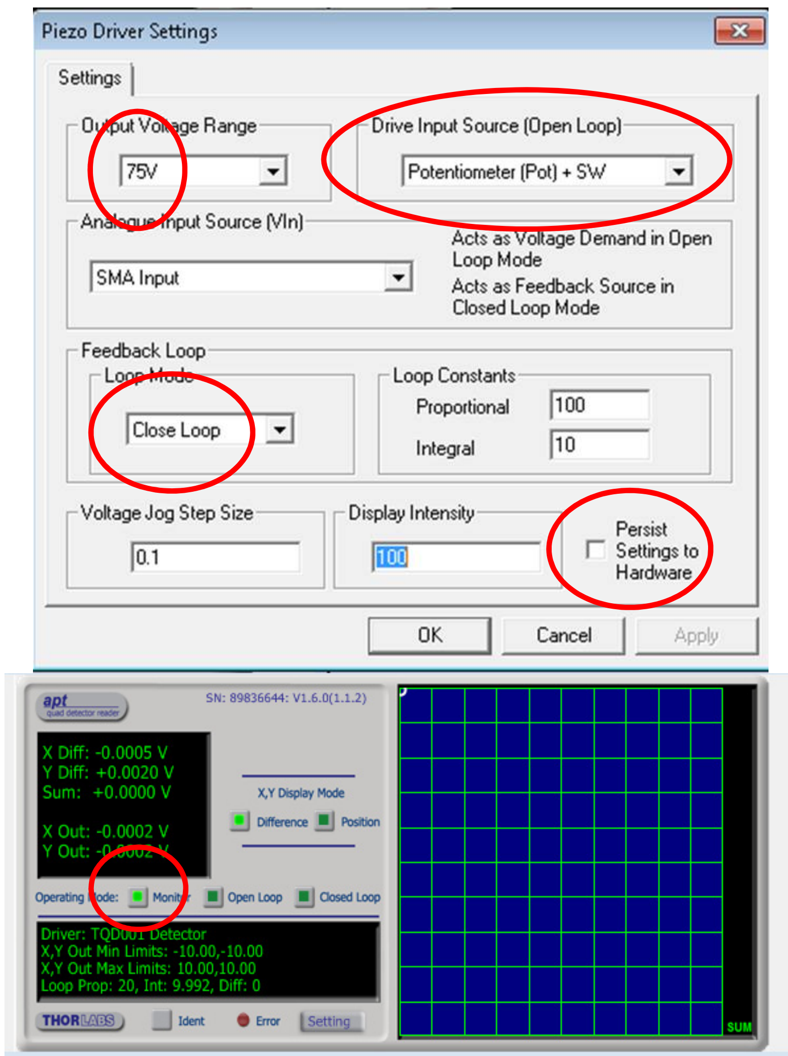


Figure 5 Piezo Controller (KPZ101) Settings Window and PSD Aligner (KPA101) Settings Window

11. During the OTKBFM-CAL software installation the necessary National Instruments software had been automatically added to your PC (if it was not present already). Start the “NI MAX” tool, typically a link is found in your windows “All Program” list. Right click on the “NI USB-6212” device and rename it to “OTKB”. See figure 6. Close “NI MAX.”

12. Start the OTKBFM-CAL software. You are now ready to calibrate your system and run force measurements.

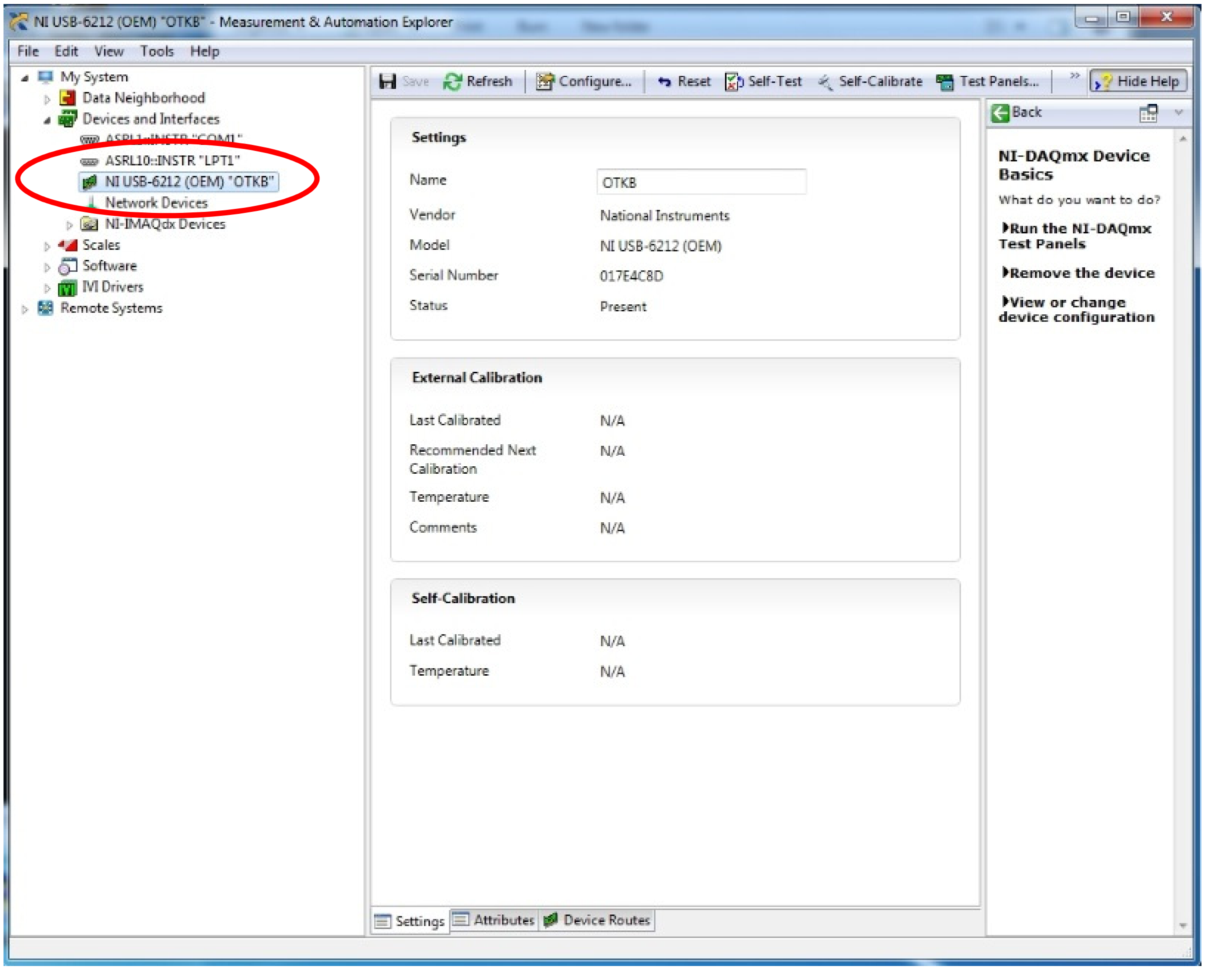


Figure 6 Configuration Window for DAQ Hardware using NI MAX

Chapter 5 Operation

Before quantitative measurements can be performed with the optical tweezers system, it is necessary to determine the factor to convert detector voltages to distances and to find the trap stiffness. Changing the trapping laser power during this procedure will affect the calibration parameters. Therefore it often makes sense to run the calibration sequence described below for various laser power settings. Further on, the distance of the laser trap to the cover slip will affect the trap stiffness significantly. Again you can determine the calibration for different z-positions using the positioning system of the stage. During an initial calibration make sure that your trap is located several microns above the cover glass. This can be achieved by moving a trapped bead towards the cover glass until it contacts the glass and goes out of focus. The z-axis adjustment knob on the stage has micrometer markings, which you can then use to move the trap away from the cover glass surface. If the bead is too close to the wall, hydrodynamics effects or physical constraints with the coverslip will restrict the Brownian motion, while at heights above 5 µm the optical trap loses its tight focus.

We assume that you have setup your tweezers system and aligned the detector to monitor the back focal plane of the condenser. Further on you need to load a sample with a combination of stuck and freely diffusing microspheres. See Section 5.3 for details.

5.1. Position Calibration

When a bead is located close to the trap center a linear relationship exists between the quadrant detector voltage and the distance of the bead to the trap center. To find the corresponding conversion factor we use a stuck bead and move it across the trap at a constant speed. Plotting the detector voltage versus stage position a curve can be fitted to the linear range, providing the conversion factor. A sample plot is shown in Figure 7 below.

1. Move the sample stage until you see a free bead on your camera image. Enable the trapping laser and trap the bead. Mark the position on your monitor, e.g. using a small piece of tape. Disable the laser to release the bead.

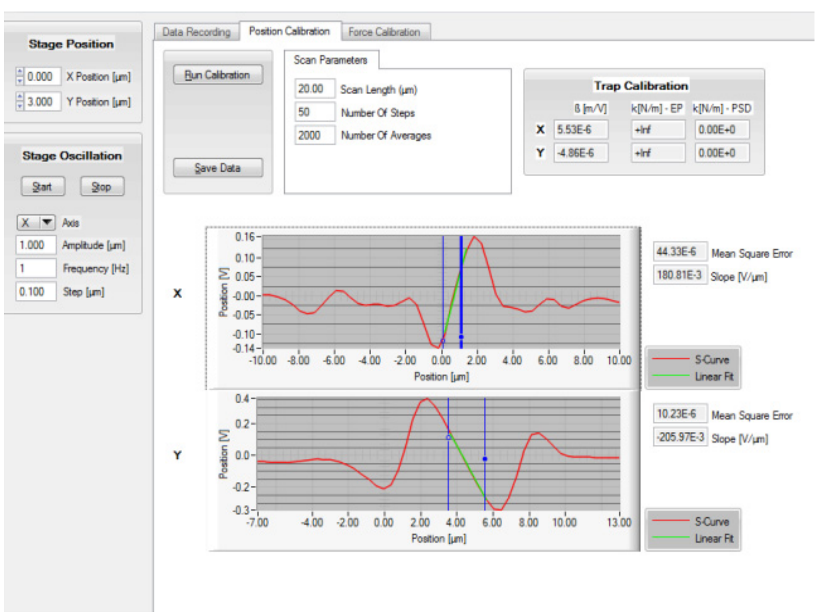


Figure 7 Example Position Calibration Plot Showing Fit to the Linear Range

2. Find a stuck bead and move it to the position marked as the trap position on the screen.

3. Select the “Data Recording” Tab and click on “Start Tracking”. You will see a curve similar to the example shown in Figure 8 on Page 13.

4. Start the Stage Oscillation in the OTKBFM-CAL software, use an amplitude appropriate for the bead size, e.g. 3 µm for a 1 µm bead, and set the frequency to 1 Hz.

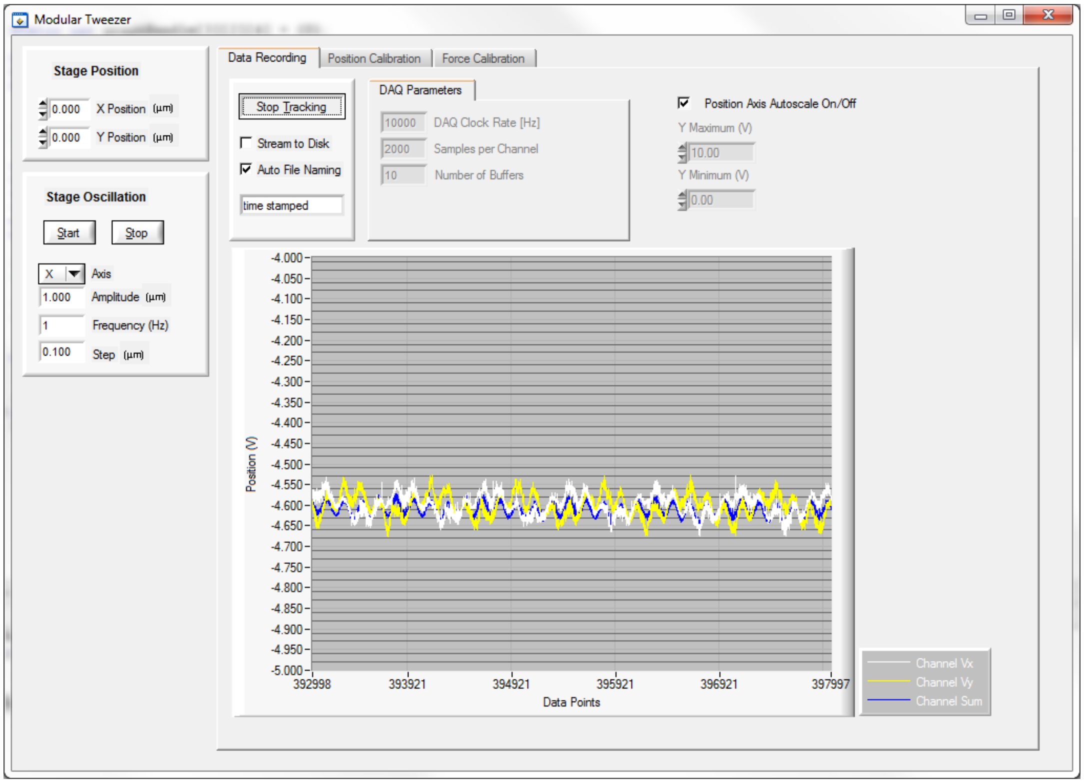


Figure 8 Example Trace of Detector Voltage Signals

5. Use the “Stage Position” adjusters to change the bead position relative to the trap position until you see a significant change in the voltage signals during the state oscillation. (Hint: The stuck bead is aligned with the optical trap center when the voltage signal changes most significantly for Vx and Vy.)

6. Stop the stage oscillation and select the “Position Calibration” tab in the software. Click “Calibration”.

7. If the X and Y plot versus position show the crossing of the bead through the trap, click on the left and right side of the graph to move the limits for the automatic linear fit. The beta value is shown on the right hand side in the box labeled “Slope”.

5.2. Stiffness Calibration

The OTKBFM-CAL software provides two stiffness calibration methods, the so-called PSD Roll-Off and Equipartition calibration. The first approach uses the fact that the thermal motion of a spherical bead of known size suspended in water is well characterized. As the laser power is increased, the Brownian motion of the bead is constrained more and more by the increasing trap force restoring the bead to the center of the trap. A frequency analysis of the particle position can therefore be used to extract the stiffness parameter. The second approach is based on the equipartition theorem, which relates the energy of the particle to the temperature at which the experiment is conducted. Both methods are applied to the data collected from the detector during a force calibration allowing the user to verify the result.

1. Find a free bead and trap it.
2. Use the data recording tool to verify that the X and Y voltages of the quadrant detector are close to zero. If this is not the case then adjust the detector to minimize the voltages.
3. Select the “Force Calibration” tab and run the calibration. Depending on the calibration length and the number of averages which are set, this measurement can take several seconds. The Fourier Transform of the data is displayed and you can click on the graph to set the lower/upper limit for the fit routine. Each time you change a limit, the fit is automatically recalculated and the stiffness determined.
4. It is possible to adjust the scale of the plots by holding down the CTRL key and clicking the left mouse button on the graph to draw a rectangle which defines the new range. In order to zoom out again, hold the CTRL key and press the right mouse button on the graph surface.

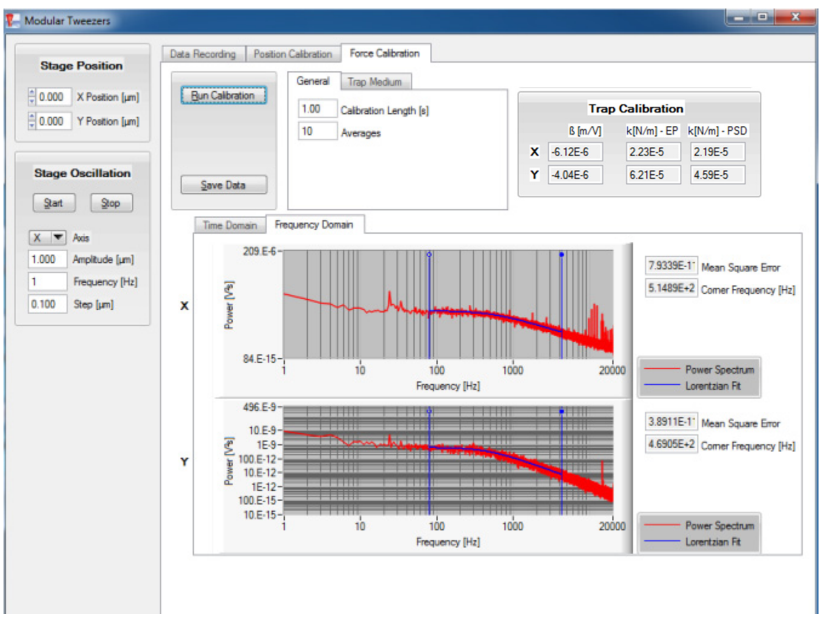


Figure 9 Example PSD Curve Acquired During Force Calibration

5.3. Sample Preparation

Follow the steps below to prepare a sample for measurement.

1. Using the OTKBTK, prepare a sample with 1 um or 2 um beads. In most experiments, the trapped objects are typically microspheres because of their symmetry and standardized characteristics, such as refractive index, size, shape, etc. In this case, the QPD's calibration is done with silica beads of 1 or 2 um in diameter.

2. The sample solution can be loaded into the channel using a microscopy slide with built-in channel (offered via our optical trapping accessories kit, Thorlabs item number OTKBTK, sold separately), or you can build a simple channel by placing double-sided tape on a standard slide, and adding a cover glass on top. Liquid can be pipetted in-between. The two open sides can be sealed off with nail polish, to prevent the sample from drying out. (Hint: To obtain a sample with many stuck beads, add a high concentration of NaCl (table salt) to the solution. Salt will reduce the Debye screening length between the beads and the glass surface, thereby increasing the probability of beads sticking onto the glass surface.)

3. Place the slide onto the sample holder and carefully place the slide between objective and condenser. Make sure to either use immersion oil on the bottom of the slide or to apply it to the objective before trying to image the sample.

4. Assess the quality of the sample. There should be a small number of stuck beads in most fields of view. It should take at least a minute to find a free bead. Too many free microspheres will make it difficult to trap only one sphere for the duration of a measurement.

5.4. Saving Data

If the "Stream to Disk" flag is set on the Data Tracking screen, a set of files will be saved. Files can be named automatically using a time stamp or the user can provide a file name. The file will include the data acquisition rate used during the measurement, followed by the raw detector voltage data X,Y and SUM.

On the calibration tab it is further on possible to save the data acquired during the PSD Roll-Off Calibration. Three sets of files are saved and can be identified by their file extension:

".TDdat": Time domain data file. It includes in the header the sample rate, number of samples, number of averages. After the header four columns include the acquired data. Column 1 is time in seconds, Column 2 to Column4 are X, Y and SUM detector voltage data. The X and Y detector voltage signals are normalized by the SUM before saving to the file.

".FDdat": Fourier domain data file. It includes in the header the number of frequencies and the measurement time used during the calibration. After the header four columns include the data. Column1 is the frequency in Hertz, Column 2 to Column 4 are power spectral data based on the detector's X, Y and SUM signal. The data is derived from the time domain data using a discrete FFT transformation. The raw FFT data is converted to absolute squared values and devided by the measurement time. For background details please refer to publications, such as "K. Berg-Sorensen et al., Rev.Sci.Instrum., Vol75, N0. 3, March 2004"

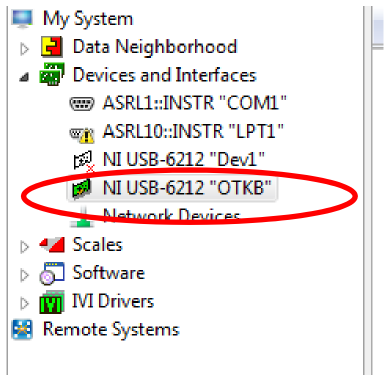
".LSdat": Line Scan data file. It includes in the header the number of samples and the number of averages acquired. After the header four columns include the data. Column 1 is the stage position in µm, Column 2 to Column 4 includes the detector X, Y and SUM voltage data. X and Y detector voltage signals are normalized by the SUM before saving to the file.

Chapter 6 Frequently Asked Questions

1. The OTKBFM-CAL software will not start. What should I do?

    a. Check that the system is connected to the power source and the computer through the provided power supply and the USB cables respectively.

    b. Check that you have changed the default name of the DAQ card through NI MAX software. The default name the USB-6212 card used is “OTKB”.



2. How come I cannot control the NanoMax stage with OTKBFM-CAL software?

    a. Make sure that all cable connections are correct. Check the connection between calibration module and the K-Cube controllers. Make sure the piezo and strain gauge cables connect to the corresponding axis on the stage. Check the USB connection between the calibration module and the PC.

    b. Using the APT software, make sure that the K-cubes are functional, i.e. they show up automatically after starting APT User. If the APT User software cannot identify any of the K-cubes in the hub, power cycle the K-cube hub. Then identify the K-cube piezo and strain gauge controller pairs connected to the X and Y axis on the stage. Set the piezo cubes to ‘Open Loop’ temporarily and adjust the piezo voltage to zero.

    Next use the NI MAX softare and select ‘Test Panels’. Set a DC value of 0V for analog output ao0 and ao1.

    At this point you can verify that the cabeling between controller and stage is correct: temporarily adjust the piezo voltage to some positive value and observe if the strain gauge controller shows the position change. If the strain gauge does not show a position change check the cables to the stage. Finally set the piezo voltage back to 0V.

    Now select the ‘Zero’ button on the strain gauge controllers and wait until the controller has found its zero setting. Afterwards switch the piezo controllers for X and Y back to closed loop.

    Close the NI MAX software and start using the OTKBFM-CAL software.

3. There is an offset when I start oscilating the NanoMax stage. What is the reason?

    a. This can be caused by zeroing the strain gauge while a voltage signale other than 0V is applied to the corresponding external input of the piezo controller. Please follow the setup described under point 2 to remove any such offset.

4. When I start data tracking in the OTKBFM-CAL software the X,Y,SUM data is zero (0 V)?

    a. Check that you have switched on the laser.

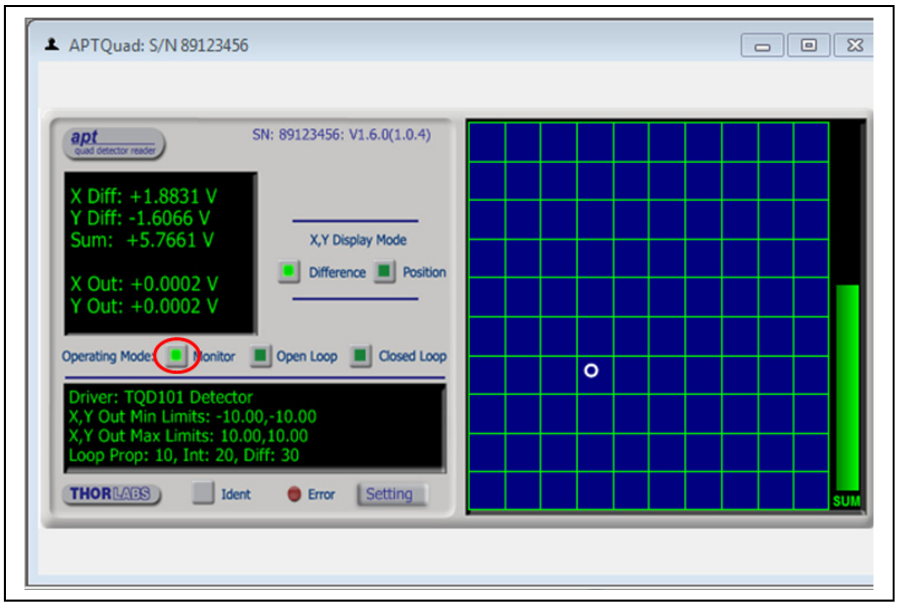
    b. Make sure the laser beam is aligned to the center of the QPD.

    c. Check the connections of the cables from the PSD K-Cube to the OTKBFM-CAL module.

    d. Check the PSD K-Cube is set to “Monitor Mode” as shown in Fig.4 . Use the Thorlabs APT User software to access the panel shown and adjust this setting.

5. When I start data tracking in the OTKBFM-CAL software only the SUM signal changes.

    Make sure that the K-Cube controller for the PSD detector is set to “Monitor Mode”. Use the APT User software to check this setting. See Figure below with the Monitor setting marked by a red circle.



6. Why are there multiple roll off frequencies in the power spectrum plot?

    a. You have more than one bead in the optical trap.

Chapter 7 Specifications

7.1. Shipping List

The OTKBFM-Cal product includes the following items:

- OTKBFM-CAL Control Box
- ±12 VDC Linear Power Supply
- 7x SMA Male Straight to BNC Male Straight Cables, 24"
- Software DVD
- Power Cable
- User Guide

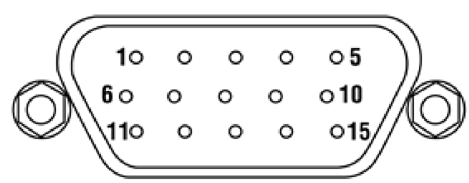
7.2. Specifications

OTKBFM-CAL Specifications	
Supply Voltage	±12 V (±0.5 V)
Inputs	
Input Connector Receptacle	BNC Female
XY Detector Signal Inputs	-10 to 10 V
SUM Detector Input	0 to 10 V
X_{SG} , Y_{SG} Strain Gauge Inputs	0 to 10 V
DAC Resolution	16 bit
Sampling Range	400 kS / s / channel
Outputs	
DAQ Manufacturer and Item Number	National Instruments / USB-6212 OEM

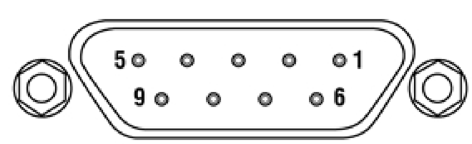
7.3. Pin Diagrams

The following images describe the connections available on the back side of the OTKBFM-CAL unit. Name of the pins is based on the convention used by the manufacturer of the DAQ card used in the unit, which is part number USB-6212 from National Instruments.

PIN	EXT	SYNC
1	AI 4	PF 10
2	AI 12	PF 11
3	AI 5	D GND
4	AI 13	N/C
5	AI 6	
6	AI 14	
7	AI 7	
8	AI 15	
9	P 0.0	
10	P 0.1	
11	P 0.2	
12	AO GND	
13	D GND2	
14	AO 0	
15	AO 1	



Pin Out of EXT output



Pin Out of SYNC output

Figure 10 Pin Out Description for Back Side Connector Panel of the OTKBFM-CAL Module

Chapter 8 CE compliance

THORLABS
www.thorlabs.com

EU Declaration of Conformity

in accordance with EN ISO 17050-1:2010

We: Thorlabs Inc.

Of: 56 Sparta Avenue, Newton, New Jersey, 07860, USA

in accordance with the following Directive(s):

2006/95/EC	Low Voltage Directive (LVD)
2004/108/EC	Electromagnetic Compatibility (EMC) Directive
2011/65/EU	Restriction of Use of Certain Hazardous Substances (RoHS)

hereby declare that:

Model: ***OTKBFM-CAL***

Equipment: ***Calibration & Measurement Module for OTKB/OTKBFM***

is in conformity with the applicable requirements of the following documents:

EN 61010-1	Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use.	2010
EN 61326-1	Electrical Equipment for Measurement, Control and Laboratory Use - EMC Requirements	2013

and which, issued under the sole responsibility of Thorlabs, is in conformity with Directive 2011/65/EU of the European Parliament and of the Council of 8th June 2011 on the restriction of the use of certain hazardous substances in electrical and electronic equipment, for the reason stated below:

does not contain substances in excess of the maximum concentration values tolerated by weight in homogenous materials as listed in Annex II of the Directive

I hereby declare that the equipment named has been designed to comply with the relevant sections of the above referenced specifications, and complies with all applicable Essential Requirements of the Directives.

Signed: *On:* 29 July 2015

Name: Ann Strachan

Position: Compliance Manager

EDC - OTKBFM-CAL -2015-07-29

CE 15

Chapter 9 Regulatory

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

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

Wheelie Bin Logo

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

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

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

9.1. Waste Treatment is Your Own Responsibility

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

9.2. Ecological Background

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

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

Chapter 10 Thorlabs Worldwide Contacts

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

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