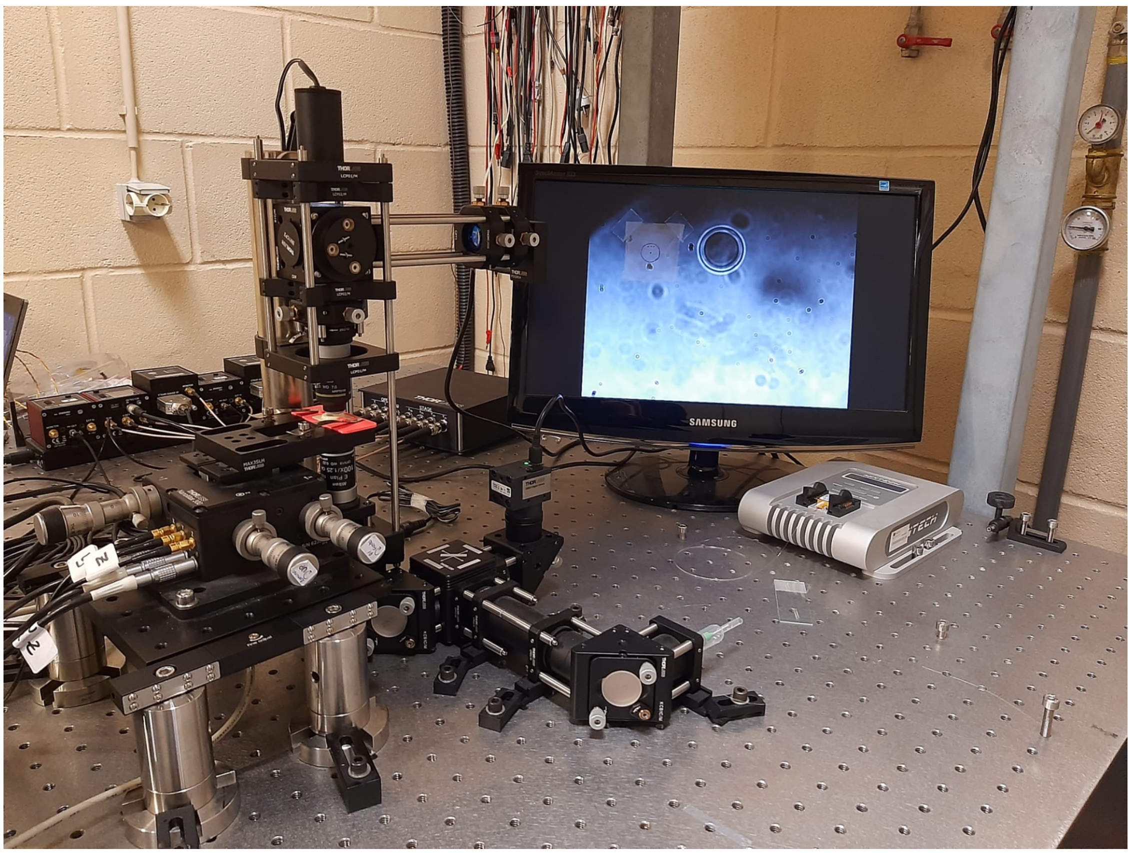
Conventional Optical Tweezers System OTKB Thorlabs



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January 2022

Contents

1 Introduction-physical model

Optical tweezers explore optically-induced forces generated by a tightly focused laser beam. The trapping results from the balance of the gradient force with the scattering force, creating an effective three-dimensional (3D) potential. Considering the target specimen fulfils certain criteria regarding its refractive index and size, the manipulation in three dimensions is possible (Neuman and Block, 2004; Jones et al., 2015).

Recent results at the Center of Applied Photonics (CAP) at INESC TEC demonstrate the suitability of optical tweezers as tools that can select and analyze single cells. Optical fiber tweezers combined with backscattered signal analysis and machine learning strategies, allowed for high accuracy discrimination and quantification of different cells and nanostructures (Paiva et al., 2018).

Some of the developments regarding conventional and fiber optical tweezers systems can be found in the literature (Ribeiro, 2017; Paiva, 2019; Rodrigues, 2019; Carvalho, 2021), along with some examples of the papers published over the years (Ribeiro et al., 2015; Rodrigues et al., 2018; Ribeiro et al., 2017; Paiva et al., 2018; Jorge et al., 2021; Carvalho et al., 2021)

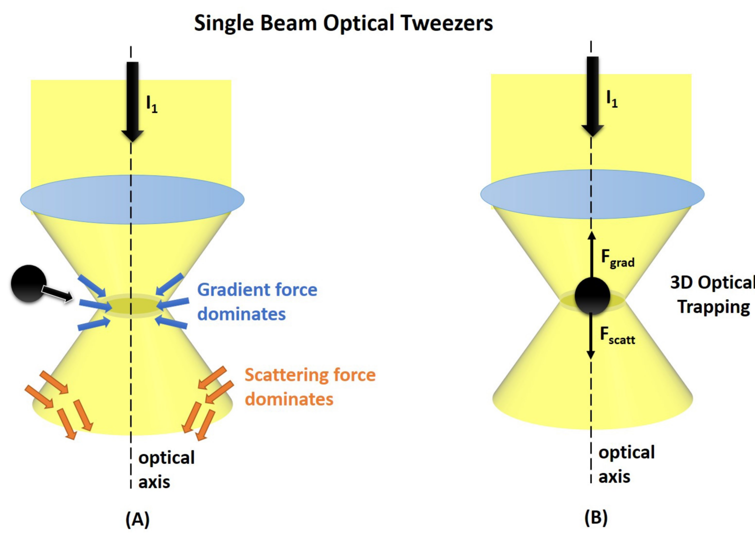

Figure 1: Representation of the optical forces in a conventional optical tweezers configuration (Paiva, 2019).

An optically trapped particle experiences a change in momentum that results in the balancing between the corresponding forces that tend to restore the bead towards the centre of the trap (Ashkin, 1970). In the most typical conditions, the force in each dimension is reasonably approximated as proportional to the displacement, as given by the Hooke's law (Jones et al., 2015), as:

$$F_x = -k_p^x(x - x_{eq}) \tag{1}$$

where x_{eq} is the position of the particle in equilibrium, x is the particle position, and k_p^x is the trap stiffness (Jones et al., 2015). From this model, the trapping potential is then approximated to an harmonic potential (Gieseler et al., 2021) given by

$$U(x) = \frac{1}{2}k_p^x(x - x_{eq})^2 \tag{2}$$

Regarding the physical origin of the optically-induced forces, we can highlight two fundamental regimes depending on the size of the particle relatively to the wavelength of the trapping laser beam (Ashkin et al., 1986).

The ray optics regime, also called as Mie scattering regime, is valid for trapped particles with radius (a) much larger than the wavelength of the trapping laser (λ) and simple ray optics can be employed. On the other hand, for particles with radius (a) much smaller than the wavelength (λ) of the trapping laser, the Rayleigh scattering regime is considered since $a \ll \lambda$, and the particle is treated as a point dipole.

1.1 Theory of Brownian motion

From a microscopic perspective, a particle with a size comparable to that of the molecules of the immersion fluid is permanently moving in random directions. This characteristic movement, Brownian motion, occurs in a solution due to the collisions between the solute particle with medium molecules (Jones et al., 2015; Bérut, 2015).

The physical model for the motion of a free Brownian particle immersed in a fluid (Bérut, 2015), is given by the Langevin equation

$$m\ddot{\boldsymbol{r}}(t) = -\gamma\dot{\boldsymbol{r}}(t) + \sqrt{2k_B T\gamma}\boldsymbol{W}(t) \tag{3}$$

where m is the mass of the immersed particle, $\boldsymbol{r}$ represents the vector position of the particle, γ is the particle friction coefficient, k_B is the Boltzmann constant, T is the absolute temperature and $\boldsymbol{W}(t)$ is a stochastic vector term accounting for random collisions (Jones et al., 2015; Bérut, 2015). The first term on the right-hand side of the last equation is related to the drag force felt by a spherical particle of radius a, given by the Stoke's law, $\gamma = 6\pi\eta a$, where η is the viscosity of the medium.

In the presence of a trapping potential, the model for the Brownian particle includes an additional deterministic contribution of the optical-induced force (Jones et al., 2015; Volpe and Volpe, 2013), resulting into

$$m\ddot{\boldsymbol{r}}(t) = -\gamma\dot{\boldsymbol{r}}(t) - \boldsymbol{k}_p \odot \boldsymbol{r}(t) + \sqrt{2k_B T\gamma}\boldsymbol{W}(t) \tag{4}$$

where k_p is the trap stiffness constant represented as a vector (k_p^x, k_p^y, k_p^z) and $\odot$ stands for the Hadamard product.

Optical tweezers exploit systems of low Reynolds number, allowing to drop the inertial term $m\ddot{r}$ (Jones et al., 2015; Volpe and Volpe, 2013) in Equation 4. The result is an over-damped Langevin equation given by

$$\gamma\dot{\boldsymbol{r}}(t) + \boldsymbol{k}_p \odot \boldsymbol{r}(t) = \sqrt{2k_B T\gamma}\boldsymbol{W}(t) \tag{5}$$

which simplifies the modelling of the dynamics of the trapped particles.

After a brief description of the theory of Brownian motion, three methods used for the determination of the stiffness and optical force will be described.

1.2 Equipartition method

The equipartition method (EP) assumes that the position of a trapped particle in a harmonic potential follows a Gaussian distribution (Figure 2). In this method, the stiffness constant is obtained through the statistical variance of the particle position (Gieseler et al., 2021; Sarshar et al., 2014; Neuman and Block, 2004). The equipartition method is given by

$$\frac{1}{2}k_p^x \left\langle (x - x_{eq})^2 \right\rangle = \frac{1}{2}k_B T \tag{6}$$

where $<(x - x_{eq})^2>$ corresponds to the statistical variance of the particle position derived from the fluctuations over time (Sarshar et al., 2014; MIT Department of Physics , 2017).

At thermal equilibrium, $K_B T$ constant, the higher the stiffness constant, the smaller the statistical variance of the particle position.

To compute the trap stiffness, the equiparition method is a fast and simple approach, only requiring the position of the trapped particle over time. This method requires previous position calibration of the quadrant photodetector for the determination of the trap constant (Sarshar et al., 2014; Neuman and Block, 2004).

As a major disadvantage, this method is unable to compute the friction properties of the particle, essential for a complete and precise characterization of the system.

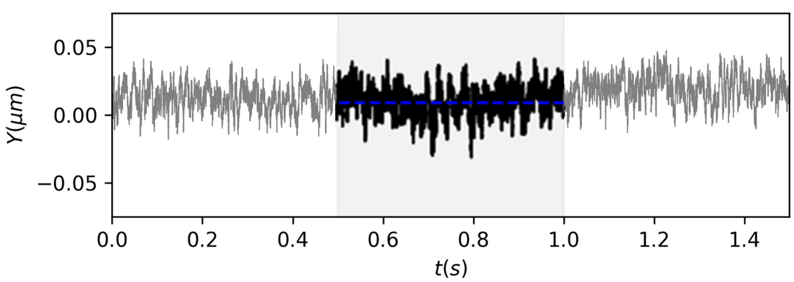

Figure 2: Stiffness constant determination for 3 μm PMMA particle through the Equipartition method, for an optical power of 22.575 mW (Carvalho, 2021).

1.3 Power spectral density method

The power spectral density (PSD) method lays on the analysis of the power spectrum of the fluctuations of a trapped particle (Figure 3) (Sarshar et al., 2014). By fitting the one-sided power spectral density of a trapped bead to a Lorentzian function (Equation 61), the corner frequency (f_0) and the particle friction coefficient (γ) can be determined (Appleyard et al., 2007; Sarshar et al., 2014). Finally, the stiffness constant is computed (Equation 8).

$$S_{xx}(f) = \frac{k_B T}{\pi^2 \gamma (f_0^2 + f^2)} \tag{7}$$

$$k_p^x = 2\pi\gamma f_0. \tag{8}$$

where f stands for the frequency. The parameters extracted are related with the properties of the particle and of the optical trap.

This method provides additional information on the particle friction parameter, in comparison to the EP method and it is independent on calibration factors.

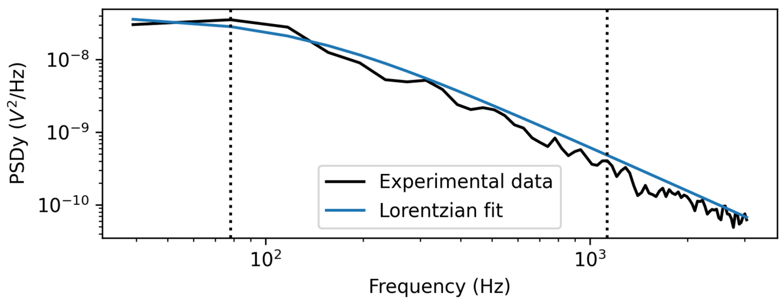

Figure 3: Stiffness constant determination for 3 μm PMMA particle through the Power Spectral Density method, for an optical power of 22.575 mW (Carvalho, 2021).

1.4 Boltzmann statistics method

The probability density $\rho(x)$ of the particle position (x) can be described as a function of the trapping potential $U(x)$ (Jones et al., 2015; Gieseler et al., 2021) (Equation 9), where ρ_0 is a constant normalization (Sarshar et al., 2014). This distribution can be computed by analysing the position of the trapped particle and constructing the corresponding histogram, which approximates the probability density function, $\rho(x)$.

$$\rho(x) = \rho_0 e^{\left(-\frac{U(x)}{k_B T}\right)} \tag{9}$$

The optical potential can be obtained by inverting Equation 9 for the equilibrium potential $U(x)$ (Jones et al., 2015; Gieseler et al., 2021)

$$U(x) = -k_B T ln \frac{\rho(x)}{\rho_0} \tag{10}$$

The main advantage of the Boltzmann statistics method is that it does not require the trap potential to be harmonic, as in the previously described methods. This method does not demand information on medium viscosity or particle radius for stiffness determination. By fitting an harmonic function to the resulting potential curve, the constant can be computed.

2 Modular Optical Tweezers System (OTKB)

A conventional optical tweezers system is based on an inverted microscope configuration where the trapping laser is focused with a microscope objective onto the sample. Additionally, an imaging system is used to image the sample and the forward scattered radiation is collected by a photodetector. The functionalization of the system requires calibration procedures, such as force and stiffness determination

2.1 System description

The OTKB system explores an inverted microscope configuration mounted on an optical table (**Figure 4**). The laser diode (@980nm) (Driver- **Figure 5**) is fiber coupled and connected to the system, being properly collimated. The laser beam is reflected along the system and expanded which provides an expansion factor of ≈ 3 of the beam diameter, essential to fill the back aperture of the objective lens (**Figure 6** and **Figure 7**). The laser beam is directed to the 100X oil immersion objective that focuses the beam to a diffraction-limited spot onto the sample creating the optical trap (**Figure 8**). A positioning stage (**Figure 9**) is connected to the cube modules, being useful for the manipulation of the sample holder which contains the sample. The objective lens should be used after the oil immersion is placed on its top. After using the system, the immersion oil is cleaned with a [7:3 EtO_2:EtO_1] solution.

Then, the transmitted beam is recollimated by a 10x air condenser lens and a dichroic mirror redirects the beam towards the quadrant photodetector, which is place in a plane conjugate to the back focal plane of the condenser (**Figure 10** and **Figure 11**). The objective lens is used both for trapping and imaging the sample, which in turn, is illuminated by the LED at the top and the respective image is captured by a camera. To prevent camera damage, a shortpass filter is placed, with a cut-off wavelength of 750 nm, and a 200 mm focal length lens to image the sample plane.

To ensure the best performance and stability of the OTKB system, the precise alignment of the system and of the laser beam through the modules is necessary. To accomplish that, several tools were necessary, namely a power meter with a thermal power sensor head, laser safety glasses, a laser sensible target, a laser viewing card, and an infrared viewer.

2.2 External modules and controllers

For system control and data acquisition, different modules are available. The K-cube piezo controller KPZ (**Figure 12** and **Figure 13**), the T-cube strain gauge reader TSG (**Figure 14** and **Figure 15**) and the K-cube beam position aligner KPA (**Figure 16** and **Figure 17**) are the cube modules.

To power up individually the three cube modules, the Hub Controller module KCH601 (**Figure 18**) is used. The cubes are all connected to the KCH601 module. In particular, the KPZ and TSG modules are connected to the positioning stage and the KPA cube is connected to the quadrant photodetector. Additionally, a data acquisition card USB6212 (**Figure 19**) is connected to the cube modules. The USB6212 device acquires the signals from the detector and enables fast stage positioning (closed loop operation mode).

2.2.1 K-cube piezo controller (KPZ)

The piezo actuator provides local and computerized control along a single direction through high voltage operation. The voltage set controls the expansion of the piezo actuator resulting into displacement of the positioning stage. This cube module supports different output ranges, which can be user selected to 75 V, 100 V or 150 V of drive voltage. The control of the displacement can be conducted through open or closed loop operation mode. When the KPZ is operated with the TSG cube, high-precision closed-loop operation is possible.

2.2.2 T-cube strain gauge reader (TSG)

The strain gauge reader is used to monitor/read the displacement of the positioning stage for a given axis. Depending on the mode operation set, the error between the demanded and the real displacement is different. In open loop mode, the selected input drives the high voltage amplifier directly. On the other hand, in closed loop mode, the selected input receives the feedback position signal from the external position reader TSG.

For calibration purposes, the piezo driver must be set to closed loop operation, allowing precise control and positioning of the system. For that, the TSG cubes must be zeroed before operating.

2.2.3 K-cube beam position aligner (KPA)

The beam position aligner is used for beam alignment, position monitoring and even beam stabilization. The quadrant photodetector outputs signals related with the position and intensity of the beam. When Operation Mode is set to Monitor mode and the X,Y Display Mode to Difference, these signals can be monitored.

2.2.4 Quadrant photodetector (QPD)

The quadrant photodetector retrieves three signals, the $XDiff$ and $YDiff$ normalized signals and the SUM signal. When a symmetric beam is perfectly centered onto the detector, a value close to zero output voltage is displayed. To center the beam use the fine adjustment of X,Y cage adjusters which are connected to the QPD. The total intensity signal is used for normalization purposes, to normalize X_{Diff} and Y_{Diff} signals, which are related to the position of the beam detected in the quadrants (MIT Department of Physics , 2017).

Consequently, the normalized coordinates (X, Y) for the position of the laser beam can be expressed, where X_{Diff} and Y_{Diff} correspond to the numerator and SUM signal to the denominator,

$$X = \frac{(Q2 + Q3) - (Q1 + Q4)}{(Q1 + Q2 + Q3 + Q4)} \tag{11}$$

$$Y = \frac{(Q1 + Q2) - (Q3 + Q4)}{(Q1 + Q2 + Q3 + Q4)} \tag{12}$$

For lateral position calibration purposes, X and Y can be converted into distances, with the detector conversion factor, considering the linear range.

Auxiliary Figures

OTKB system

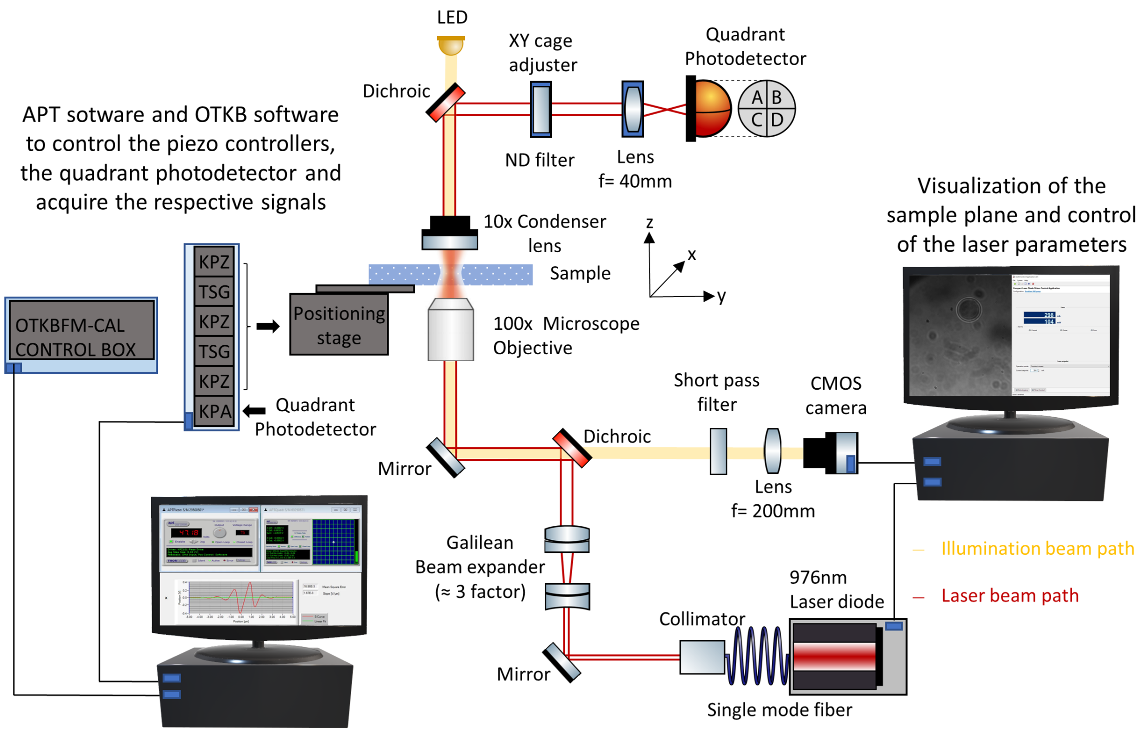

Figure 4: Schematic of the conventional optical tweezers system.

Figure 5: Compact Laser Driver Diode (CLDD)

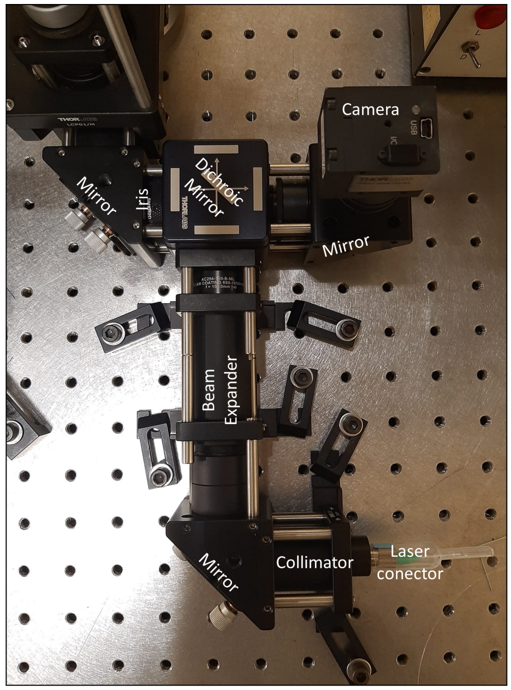

Figure 6: OTKB system- Beam Expander.

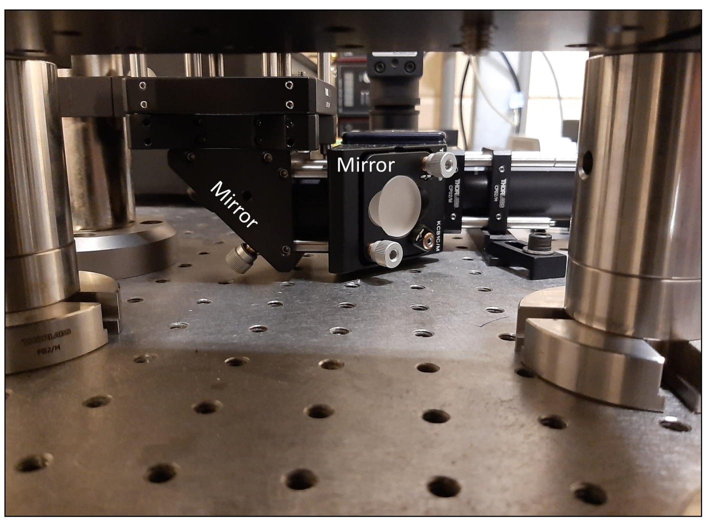

Figure 7: OTKB system- Horizontal, vertical mirrors.

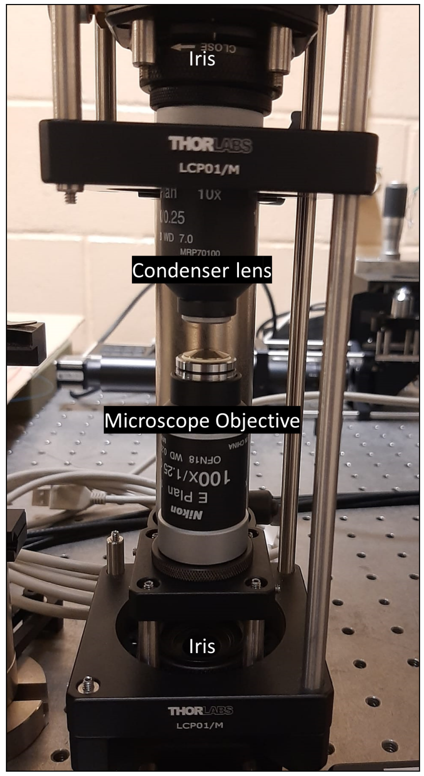

Figure 8: OTKB system- Optical trapping.

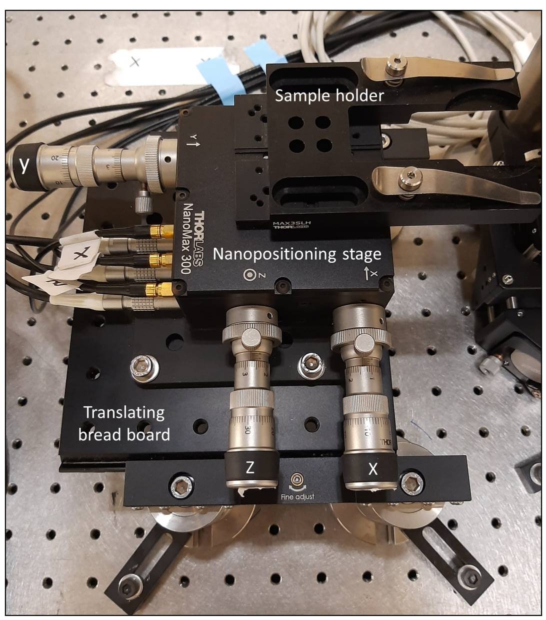

Figure 9: OTKB system- Nanopositioning stage.

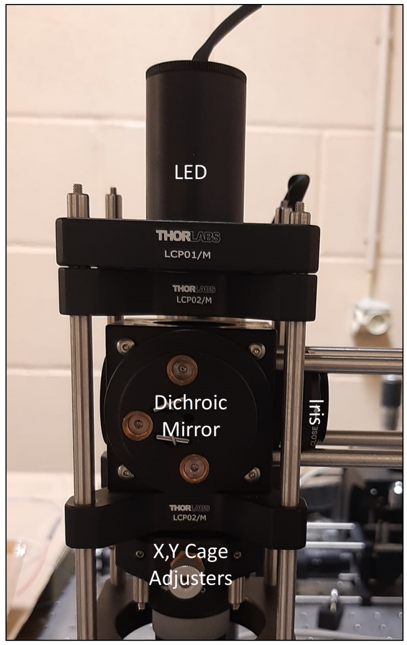

Figure 10: OTKB system- Dichroic mirror.

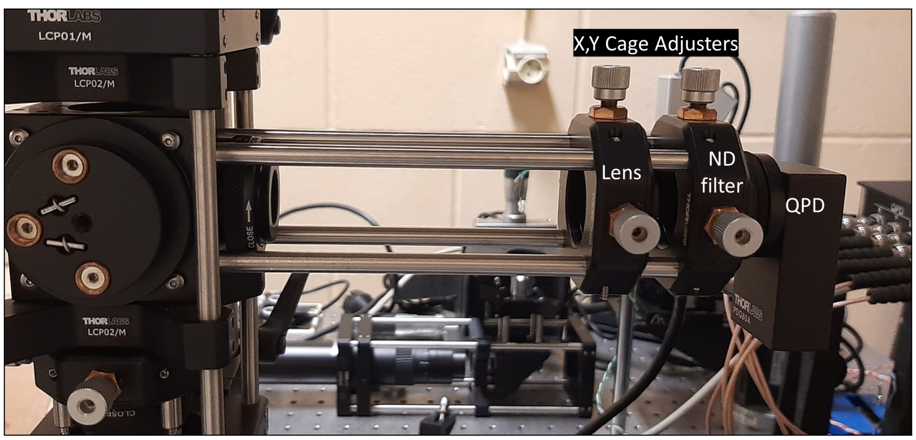

Figure 11: OTKB system- QPD detection.

External modules/devices

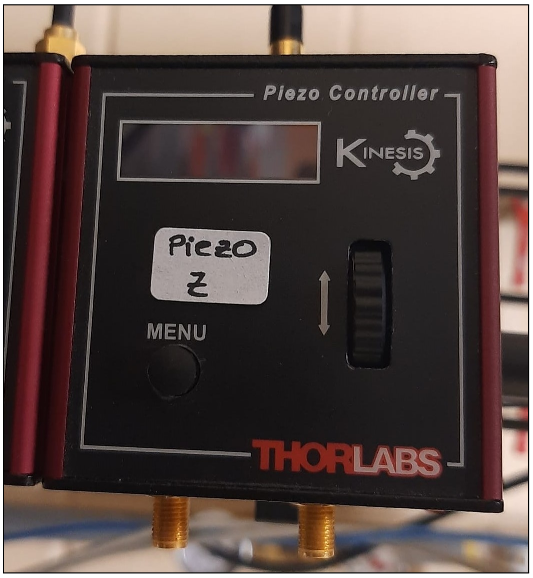

Figure 12: K-cube piezo controller KPZ.

Figure 13: K-cube piezo controller KPZ- red switch.

Figure 14: T-cube strain gauge reader TSG.

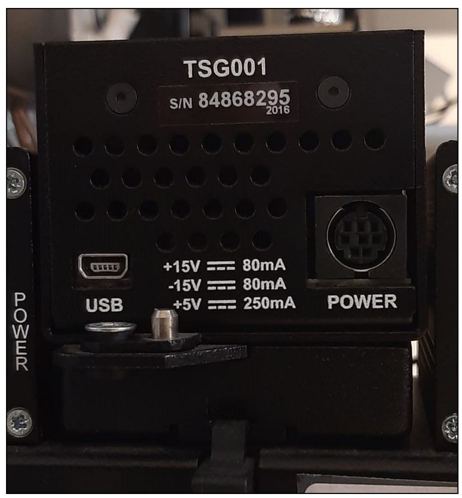

Figure 15: T-cube strain gauge reader TSG.

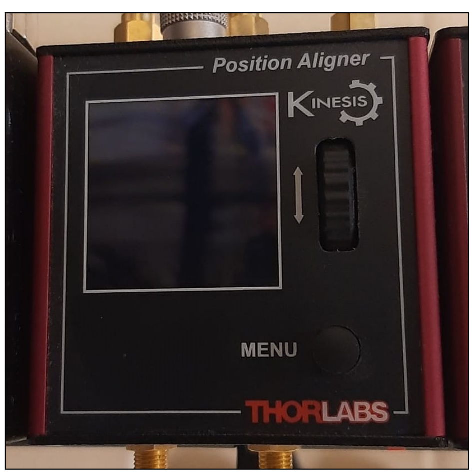

Figure 16: K-cube beam position aligner KPA.

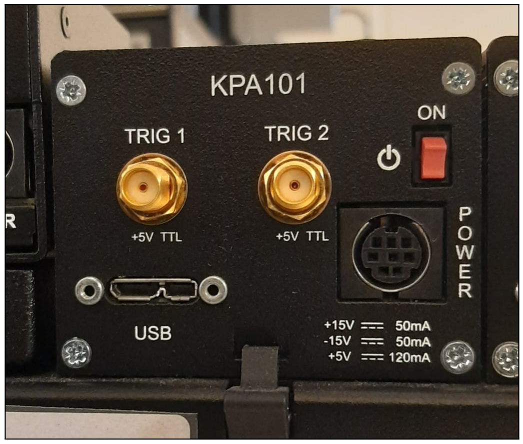

Figure 17: K-cube beam position aligner KPA- red switch.

Figure 18: Hub Controller module KCH601.

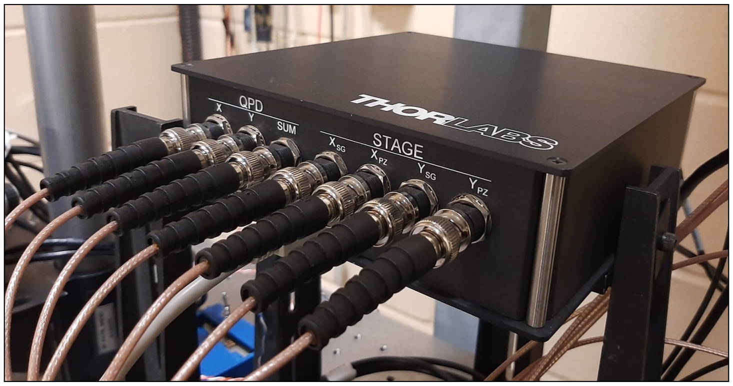

Figure 19: Data Acquisition card- DAQ (USB6212).

Figure 20: DAQ power supply.

3 Software

The characterization and calibration of the OTKB system makes use of the CLDD Software, ThorCam Software, APT User Software and OTKB/OTKBFM-CAL Software. In a nutshell:

- CLDD Software controls and monitors the laser diode parameters.
- ThorCam Software allows sample plane visualization.
- APT User Software allows control of all settings and operating modes. The Software generates GUI panels essential to monitor the KPZ, TSG and KPA cubes.
- OTKB/OTKBFM-CAL Software determines the stiffness by two approaches, the equipartition and the power spectral density method. The Software monitors and acquires the detector signals over time.

3.1 CLDD Software

CLDD Software is used to set and control the parameters of the laser diode. The Software allows monitoring the laser diode current, the output power (for a given calibration constant), the laser mode operation, the TEC temperature and other parameters.

Following steps:

1. Run the Software installer.
2. Connect the laser to the corresponding power supply. Connect the USB cable from the laser to the PC.
3. Select the button Next. In "Select the USB Port" select the correct USB port. If no device is found select the button Re-scan ports and find the correct device (**Figure 21** and **Figure 22**).

   (NOTE: When connecting the other modules and devices to the PC, some confusion can occur in CLDD Sofwtare or APT Software. In this case, re-start the CLDD Software and Re-scan ports).
4. Select the button Next after the USB port selected.
5. Configure the laser diode parameters with CLDD Software before operating the laser unit. In "Selected laser configuration" select "Bookham 980 pump" (**Figure 23)**.

   (NOTE: Do not selected the Bookham 980 pump hours related).
6. Then, to configure the device, select the button Edit. These parameters are available in the datasheet (**Figure 24**).
7. The warnings and alarms must be set. Finally, select the button OK (**Figure 25**).

8. Select the button Next after proper configuration and alarms selected.

9. Laser setpoint: select "Constant current" for "Operation mode". TEC setpoint: select "Constant temperature" for "Operation mode". Select the button Next. Select the button Finish (**Figure 26**).

   (NOTE: Next time using the laser unit, if properly selected at "Selected laser configuration", no re-configuration is necessary. The first configuration must be done before starting operating).

10. When the laser is ready for operation, the CLDD driver display shows the message "system ready for operation...".

    (NOTE: Do not press the button Laser enable before configuring the unit. This may result in permanent damage to the laser diode).

11. Check: Laser setpoint: "Constant current" for "Operation mode". When the laser is disabled, the laser current drivers and TEC are disabled (**Figure 27**).

12. Start with 50 mA at "Current setpoint" and then increase the current value to the desirable one. This can be performed all at once or by increasing the current in smaller steps. The behaviour of the laser must be observed and the best option chosen.

    (NOTE: If the laser is disabled, select the current value. Nothing will happen as the laser is disabled).

13. Select the button Laser enable. The laser current drivers and TEC are active and in the process of locking the control loops (**Figure 28** and **Figure 29**).

    (NOTE: The laser current values controlled by the user can not exceed the maximum limits (laser datasheet). The TEC temperature can not exceed the range of 24.9-25.1ºC.

14. The laser diode is enabled. The CLDD Software monitors the output power, the TEC current and TEC temperature (**Figure 30**).

15. Select the button Laser disable to turn off the laser (**Figure 31**).

16. If the threshold values are exceeded, the alarms are activated (**Figure 32**).

    (NOTE: The laser operation must be performed bellow the threshold values, ensuring the best performance of the unit. If the laser parameters are not correct or if the laser is not stable, the TEC temperature can exceed the range of 24.9-25.1ºC. If the problem persists communicate with your supervisor).

17. To power off the CLDD unit, first select the button Hibernate. The CLDD Software shows the message "It's now safe to turn down your CLDD unit." and, at the same time, the display of the CLDD driver shows the message "system ready for shutdown..." (**Figure 33** and **Figure 36**).

18. Then, press the button Quit. Finally, the power supply can be disconnected from the CLDD driver (**Figure 37**). The CLDD unit is turned off.

Connect/Disconnect CLDD unit

Power ON

1. Connect the laser to the corresponding power supply.
2. Connect the USB cable from the laser to the PC.
3. The CLDD display shows the message "system ready for operation...".
4. Enable the laser. The CLDD unit is ON.

Power OFF

1. Disable the laser. Then, hibernate the laser.
2. The CLDD display shows the message "system ready for shutdown..."
3. Disconnect the laser to the corresponding power supply.
4. Disconnect the USB cable from the laser to the PC.

Auxiliary Figures

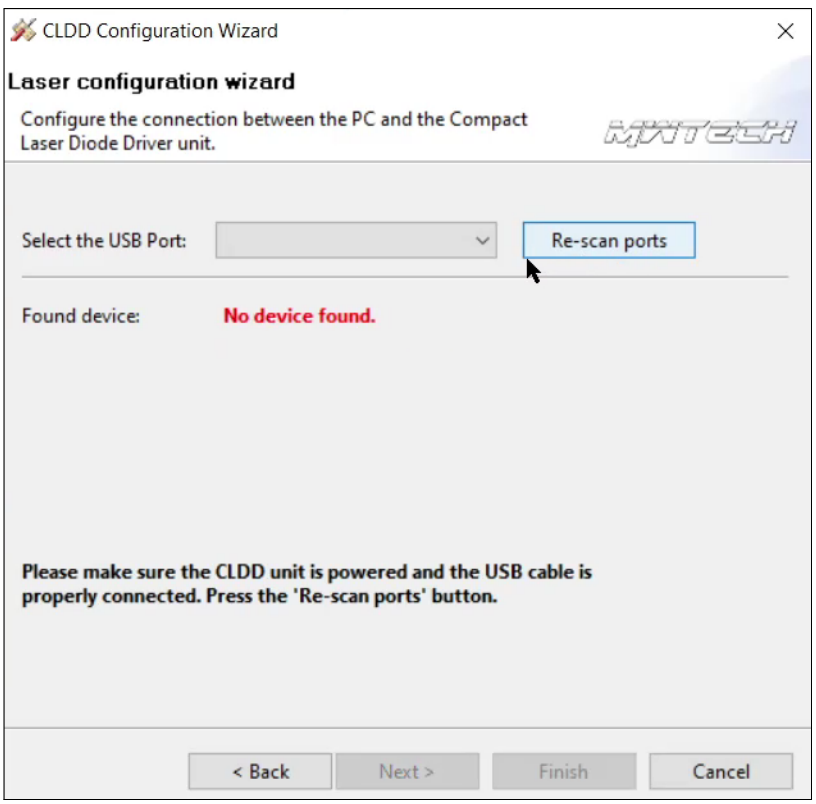

Figure 21: "Select the USB Port" select the correct USB port. If no device is found select the button Re-scan ports.

Figure 22: Select the correct USB port. In this case, only the CLDD device is connected to the PC.

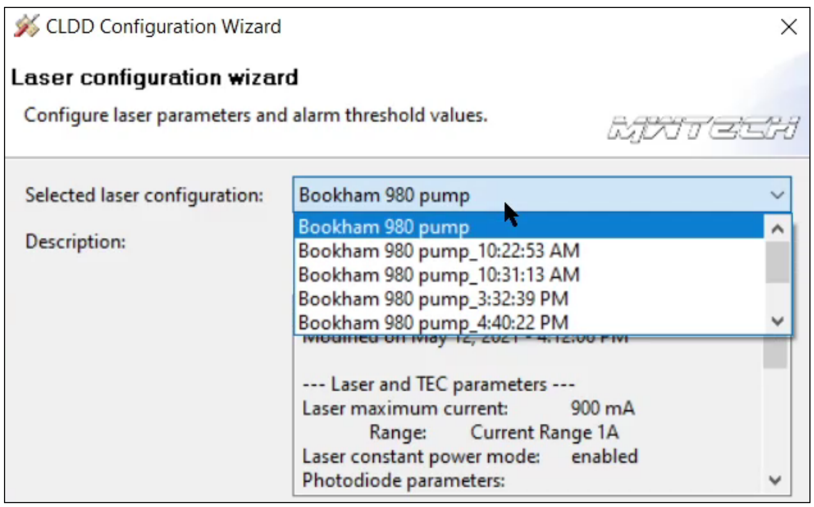

Figure 23: "Selected laser configuration" select "Bookham 980 pump". Do not selected the Bookham 980 pump hours related.

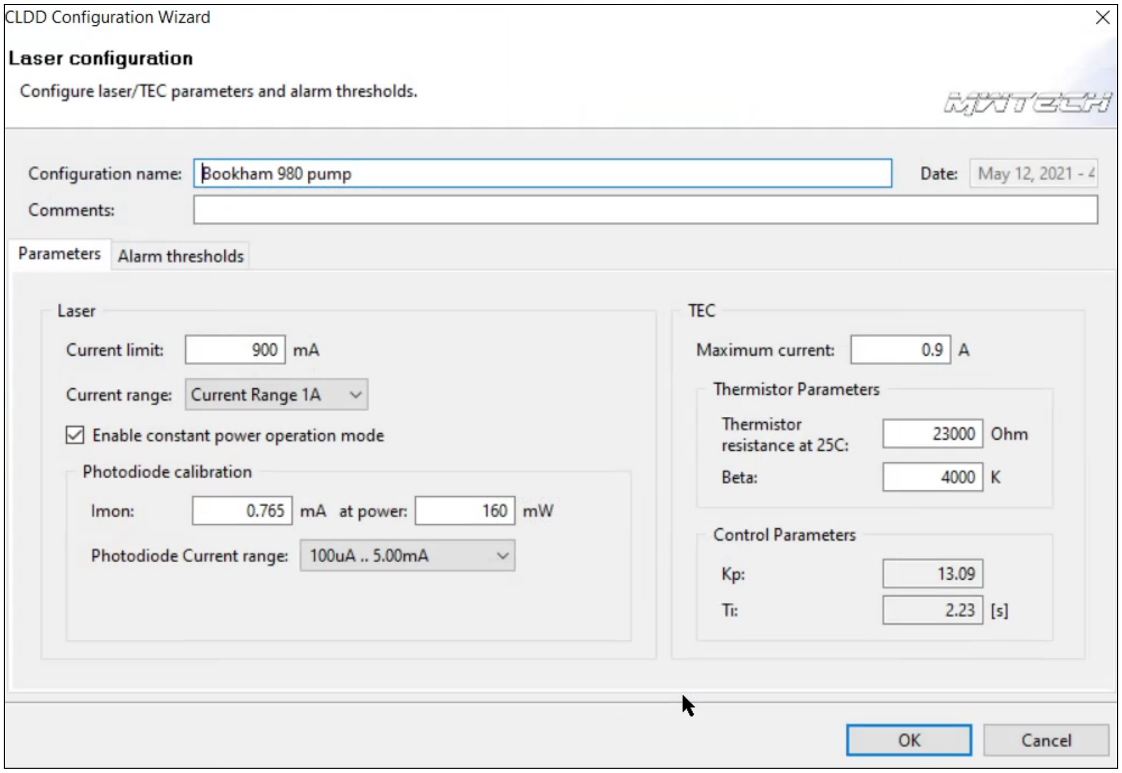

Figure 24: Laser diode parameters configuration.

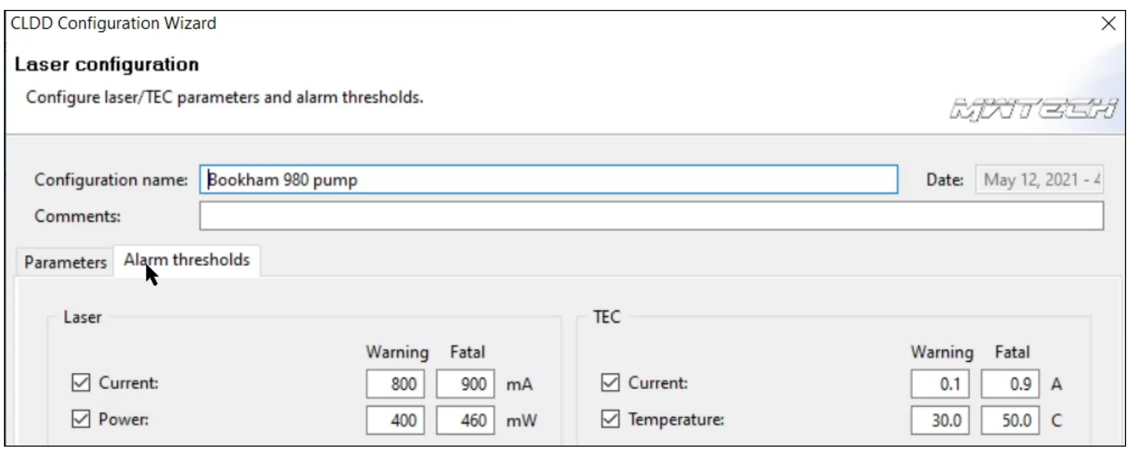

Figure 25: CLDD device warnings and alarms set.

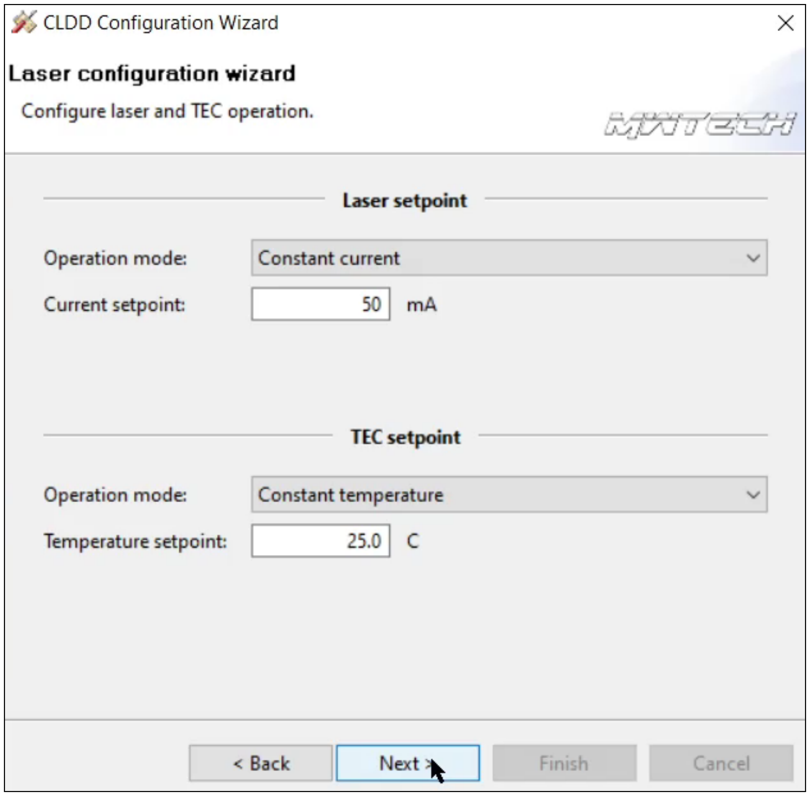

Figure 26: Laser and TEC setpoint and operation mode.

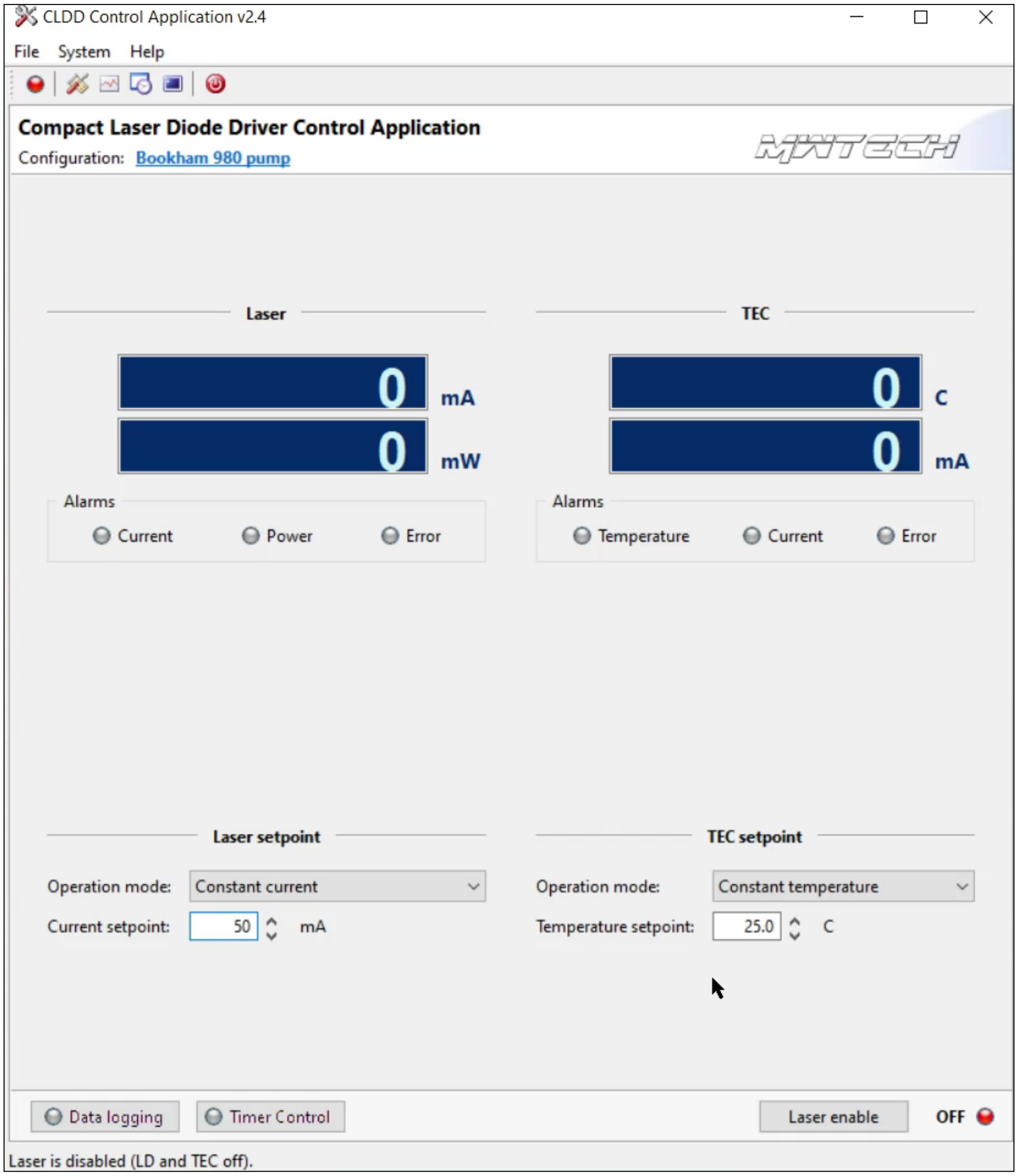

Figure 27: Laser is disable. The laser current drivers and TEC are disabled.

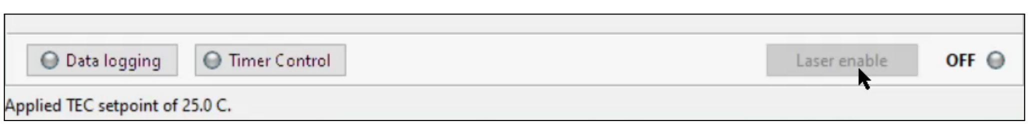

Figure 28: Select the button ENABLE.

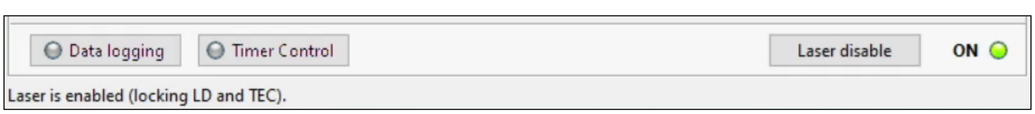

Figure 29: Laser current and TEC drivers are active and in the process of locking.

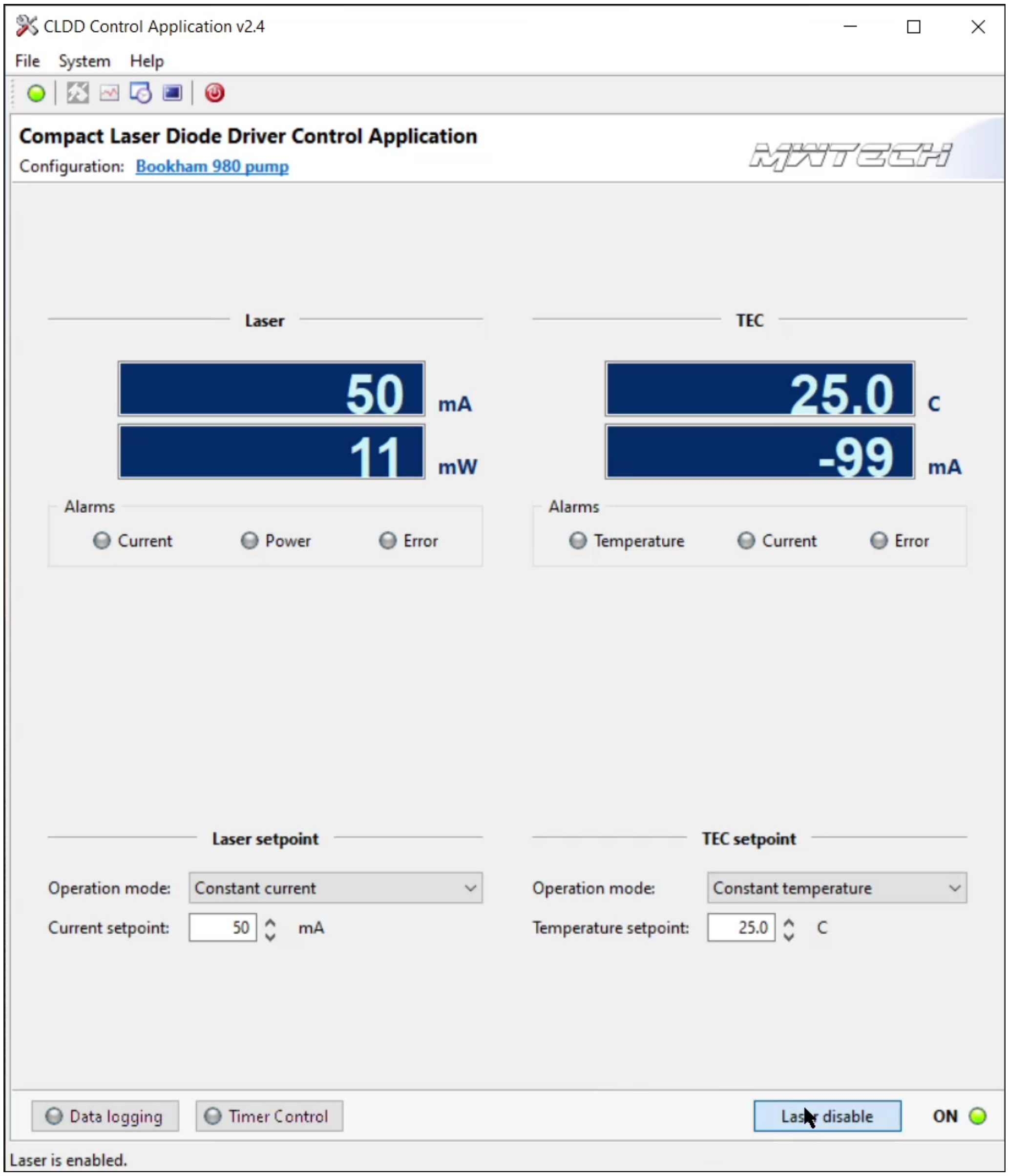

Figure 30: CLDD unit is ENABLE.

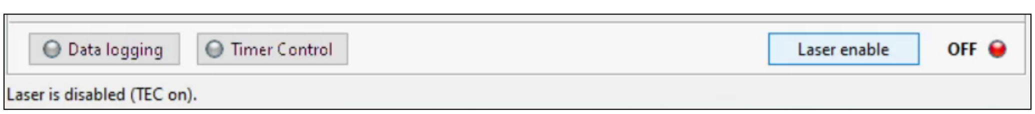

Figure 31: Select the DISABLE button. Laser is now DISABLE.

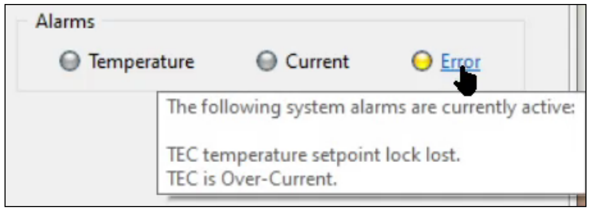

Figure 32: Threshold values are exceeded and alarms detected.

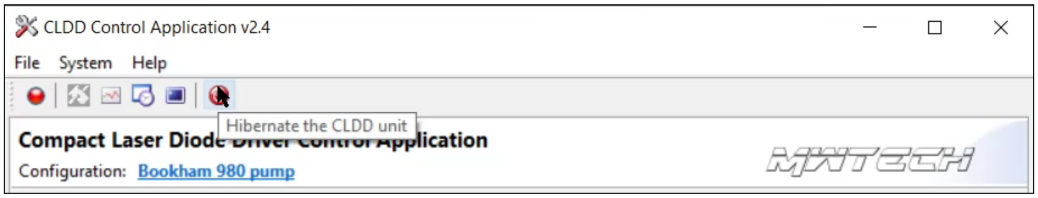

Figure 33: select the button Hibernate.

Figure 34: CLDD Software. "It's now safe to turn down your CLDD unit."

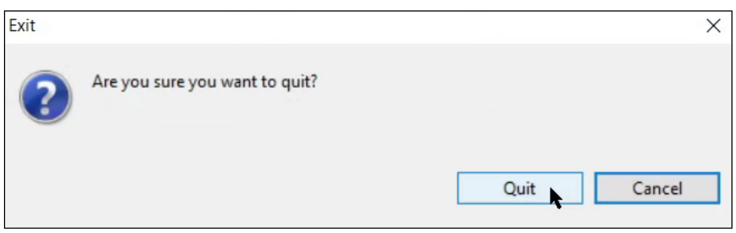

Figure 35: Press the button Quit.

For more information please consult CLDD User's Guide and the laser datasheet

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3.2 ThorCam Software

ThorCam Software allows sample visualization. Additionally, the position of the IR trapping laser can be identified at the specimen plane, when a particle/cell is trapped.

Following steps:

1. Run the Software installer.
2. Connect the camera to the USB cable. Connect the USB cable from the camera to the PC.
3. Start ThorCam Software (**Figure 36**).
4. If no camera is detected, refresh to find the camera available (**Figure 37**).
5. The software recognizes the camera. Select the camera available (**Figure 38** and **Figure 39**).
6. Start capture by pressing the button Start Capture (red highlighted) (**Figure 40**)

   (NOTE: Do not forget to place immersion oil in the objective lens, otherwise you will not see the sample).
7. Save images by pressing the respective button (blue highlighted) and select the desirable folder. Record video by pressing the respective button (green highlighted), then press Record and select the path (**Figure 41** and **Figure 42**).
8. To support the position calibration procedure, histogram lines are available. With these, the positioning of the stuck particle in the desirable location can be achieved (**Figure 43** and **Figure 44**).

   (NOTE: The position of the histogram lines can be modified by clicking with the mouse in the desirable site).
9. If this window appears is because the ThorCam Software is opened twice. Please close one window (**Figure 45**).

Connect/Disconnect ThorCam unit

Power ON

1. Connect the camera to the USB cable.
2. Connect the USB cable from the camera to the PC.
3. Start ThorCam Software.

Power OFF

1. Close ThorCam Software.
2. Disconnect the camera.

Auxiliary Figures

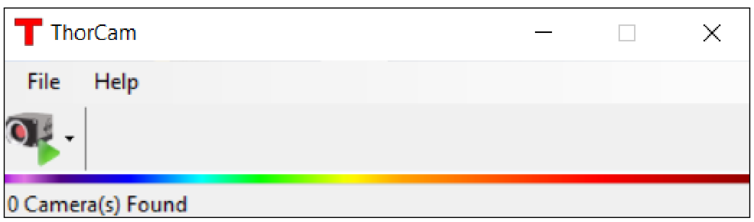


Figure 36: ThorCam software.

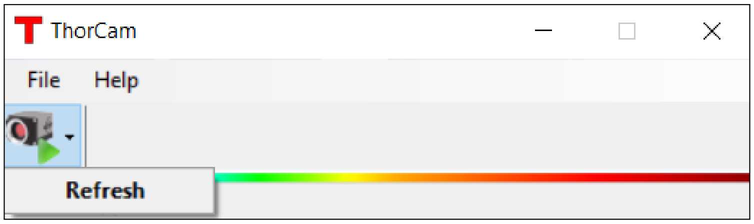


Figure 37: Refresh to find the available camera.

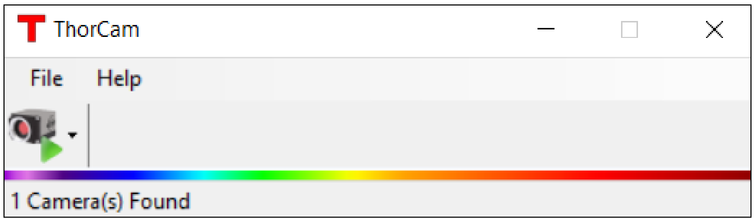


Figure 38: The software recognizes the available camera.

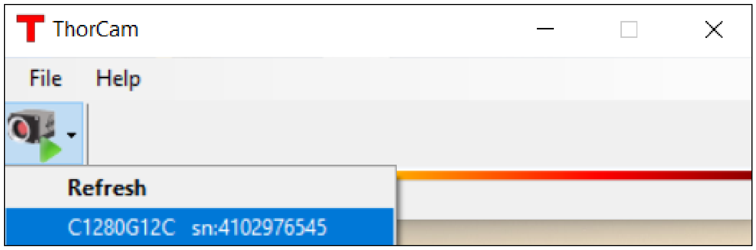


Figure 39: Select the available camera.

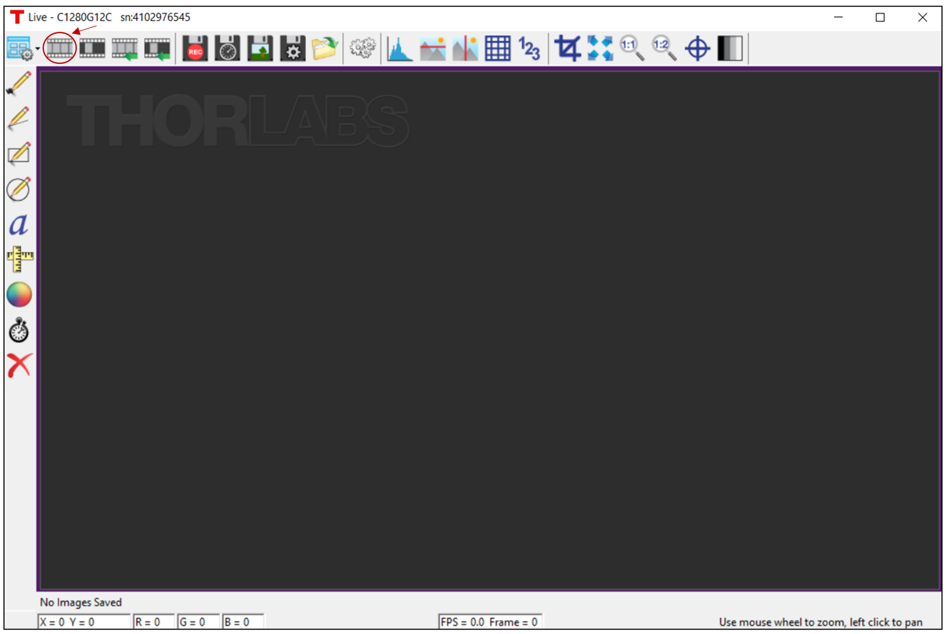


Figure 40: Press the Start Capture button.

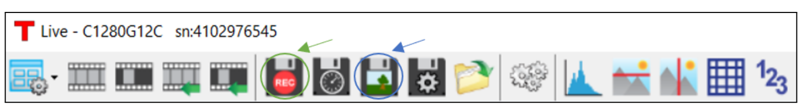


Figure 41: Acquire image or record video.

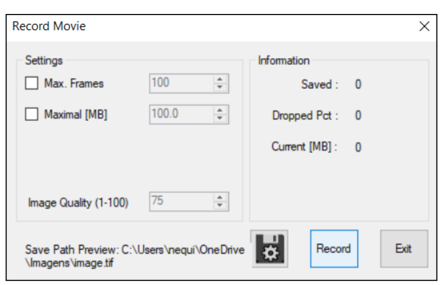


Figure 42: Acquire image or record video.

Figure 43: Select histogram lines.

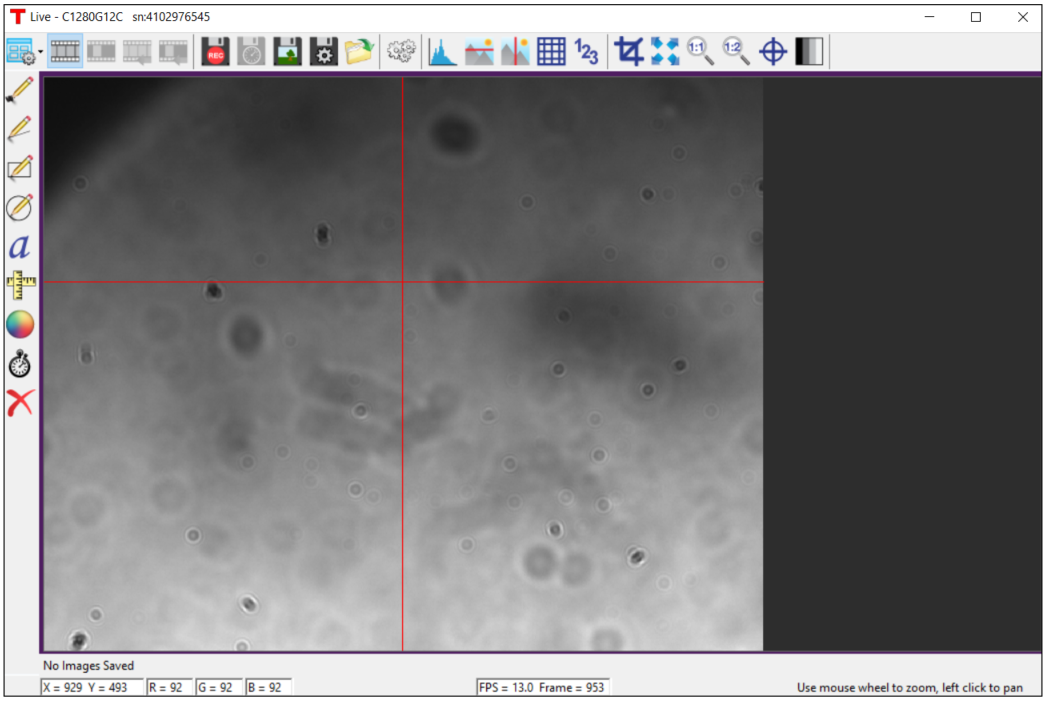


Figure 44: Select histogram lines.

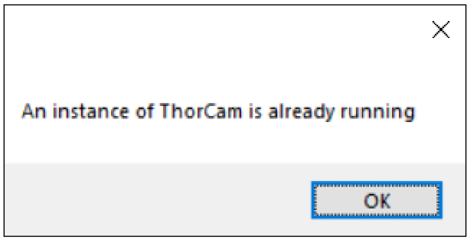


Figure 45: Error Window.

For more information please consult Quick Start Guide, Software Manual or Manual by Thorlabs.

—

3.3 APT User Software

APT User Software is used to control the cubes, the K-cube piezo controllers (KPZ101), the T-cube strain gauge readers (TSG001), the K-cube beam position aligner (KPA101), and the positioning stage. In particular, the APT User Software sets the operation mode of the piezo controllers (open or closed loop operation mode).

Following steps:

1. Run the Software installer.

   (NOTE: National Instruments installation might be necessary).

2. Start APT Software.

   (NOTE: When connecting the other modules and devices to the PC, some confusion can occur. If APT User Software does not recognize all devices, re-start the system. Power OFF all devices and then power ON).

3. The GUI panel of each type of device is different. Serial number of each cube: KPZ_X-29500489, KPZ_Y-29500501, KPZ_Z-29500495, TSG_X-84868288,TSG_Y-848682295, KPA-69250571 (**Figure 46**).

4. KPA cube. In Operation mode select Monitor mode. In X,Y Display Mode select Difference. Before starting the measurements, the laser beam must be placed at the center of the photodetector array ($XDiff$, $YDiff$ signals $\approx 0V$) (**Figure 47**).

5. TSG cube. Click the button Mode to change the display mode. The displayed position reading changes to a position, voltage or force value depending on the selection. The position mode displays the position of the sensor in μm. The voltage mode displays a voltage proportional to the signal measured by the strain gauge, ranging from 0 to $10V$ (**Figure 48**).

6. When the KPZ device is at zero position with zero Volts applied (KPZ $= 0\ V$) and an offset is detected (TSG $\neq 0\ \mu m$), this offset value can be removed by selecting the button Zero. During the NULL process, the unit counts down to zero (**Figure 49**).

7. KPZ cube. The KPZ operates in open or closed loop operation. In open loop operation, to enable piezo expansion, the voltage is set using the Jog button, the Output button or the voltage display. Finally, click Enable (**Figure 50**).

   (NOTE: Close loop- only available the Output button. More information available in section 4).

8. If the brightness limits are exceeded (error window), select KPZ settings and adjust the Idle Level of Display Brightness. For default, the option Persist Settings to Hardware does not work (**Figure 51**).

   (NOTE: The display of the KPZ cubes is damaged.)

Connect/Disconnect APT/OTKB modules

Power ON

1. Connect the USB cable from the DAQ to the PC.
2. Connect the USB cable from the Hub Controller to the PC.
3. Connect the Hub Controller to the corresponding power supply.
4. Ensure the power of all devices is switched OFF (Hub Controller and DAQ/OTKBFM-CAL control box).
5. Ensure the power switch on the front panel of the K-cube unit is switched OFF.
6. Re-check if all K-cubes are switched OFF.
7. Turn ON the main supply (Transformador- **Figure 20**).
8. Turn ON the Hub Controller unit. After this, you should see the TSG cubes starting, as they are now connected.
9. Turn ON the unit using the red switch on the front panel of the K-cubes (KPZ101 and KPA101 cubes).
10. The system is ready to start. Start APT User Software and then OTKB Software.

    (NOTE: If APT User Software does not recognize three KPZ101 cubes (KPZ_x,KPZ_y,KPZ_z), two TSG001 cubes (TSG_x,TSG_y) and one KPA101 cube, re-start the system. For that power OFF all devices and then power ON).
11. To power OFF:

(NOTE: The devices must be connected before starting the APT User Software and OTKB Sofwtare.)

Power OFF

1. Close APT User software. Close OTKB Software.
2. Switch OFF the unit using the switch on the front panel of the K-cubes (KPZ101 and KPA101 cubes).
3. Turn OFF the Hub Controller unit.
4. Turn OFF the main supply (Transformador- **Figure 20**).
5. Disconnect the USB cables.
6. The system is disconnected. The system is now ready to re-start (Power ON).

Auxiliary Figures

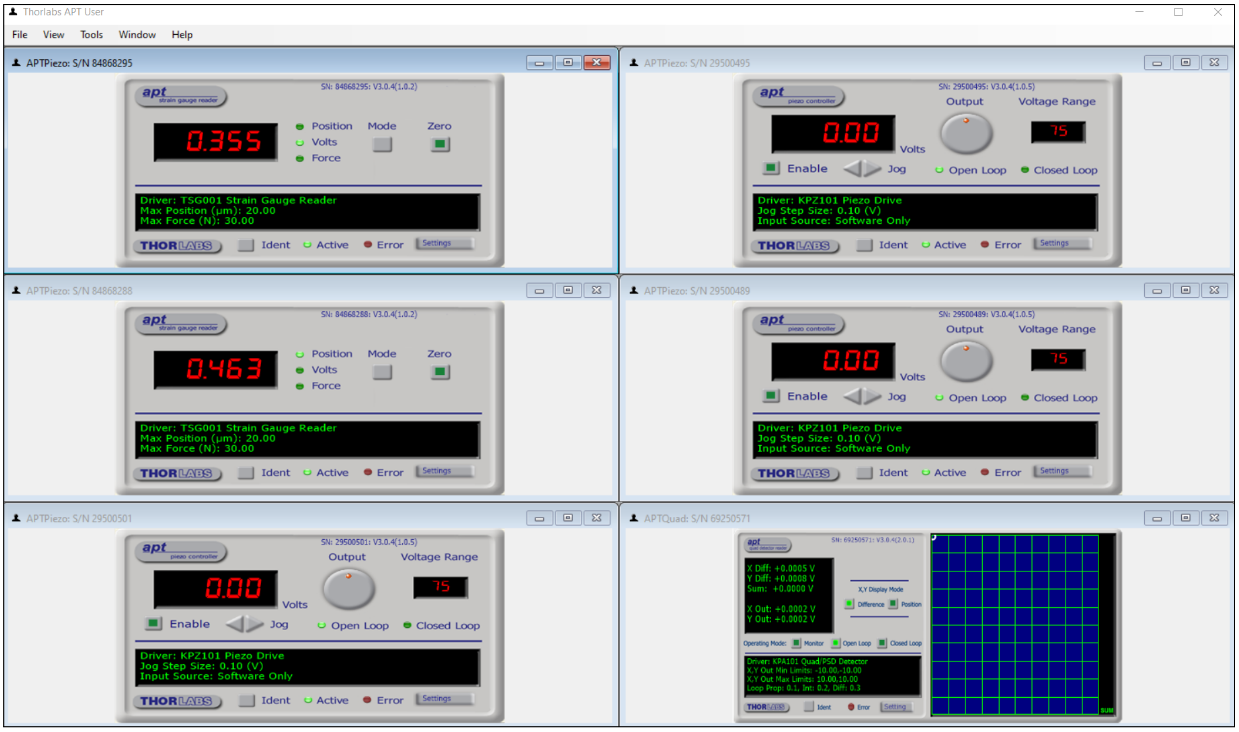


Figure 46: APT User Software. GUI panel of each device.

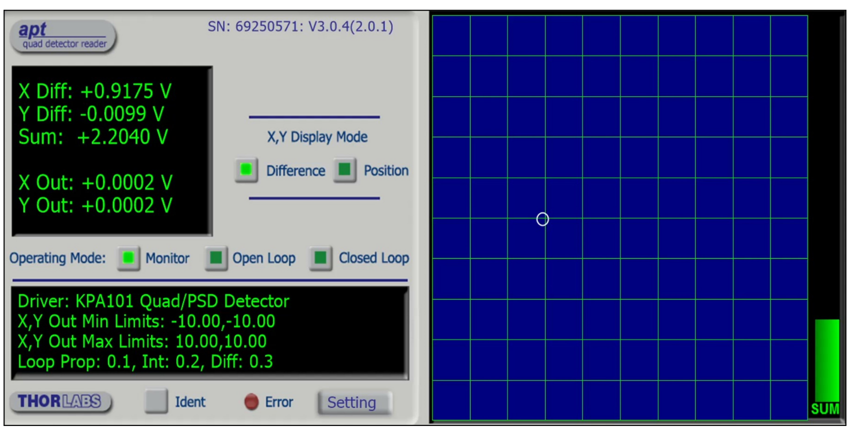


Figure 47: KPA- APT User Software. In Operation mode select Monitor mode. In X,Y Display Mode select Difference.

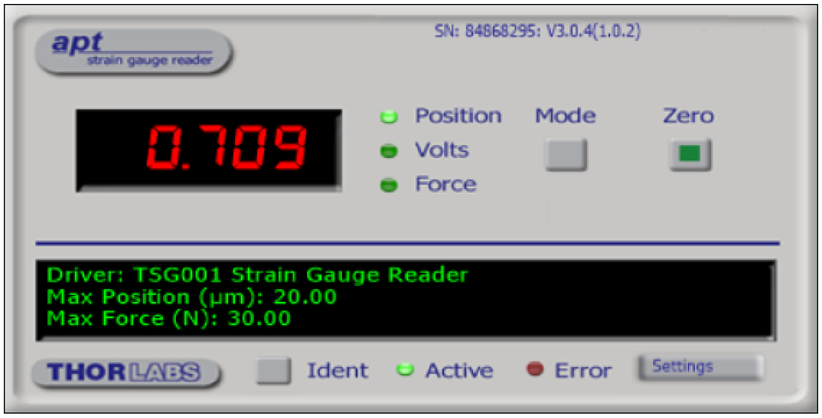


Figure 48: TSG- APT User Software. Select the display mode.

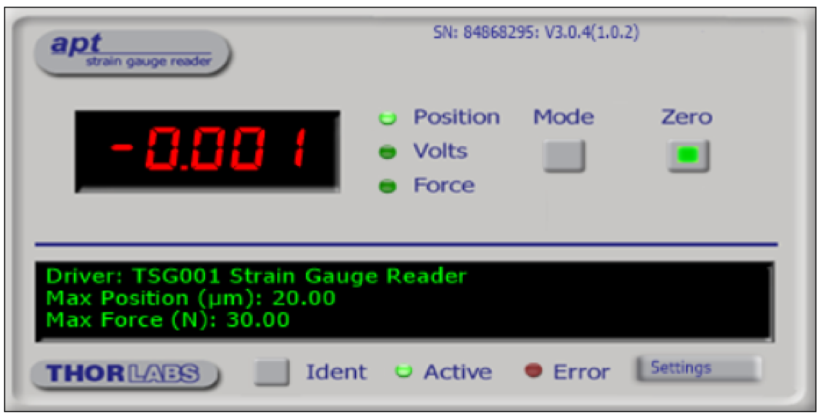


Figure 49: Zeroing the TSG device.

Figure 50: KPZ- APT User Software. Select the voltage.

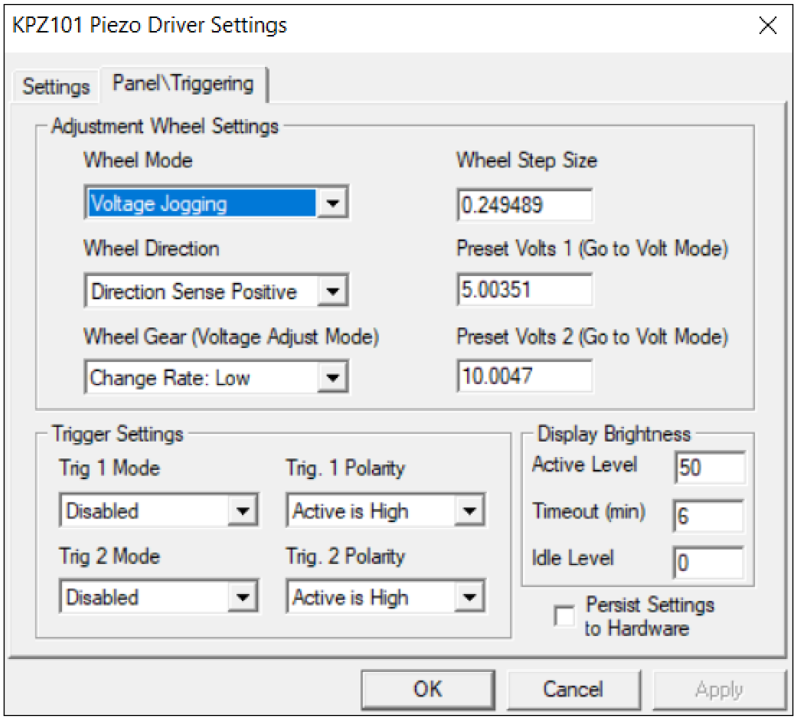


Figure 51: KPZ settings. Adjust Idle Level of Display Brightness.

For more information please consult OTKBFM Manual, OTKB(/M) Manual or OTKBFM-CAL Manual by Thorlabs.

3.4 OTKB Software

OTKB Software acquires signals over time used to determine the trap stiffness. Additionally, the OTKB Software is useful for calibration purposes.

Following steps:

1. Run the Software installer.

   (NOTE: National Instruments installation might be necessary unless it was automatically installed).

2. Open NI MAX, select "NI USB-6212" device and rename it to "OTKB" (**Figure 52**).

3. If you do not follow the step above, the OTKB Software <u>will not</u> run. For default, the DAQ's name only has to be changed once (**Figure 53**).

4. Start OTKB Software (**Figure 54**).

5. OTKB Software is able to acquire four file types, .dat file (detector raw voltage), .LSdat file (position calibration), .TDdat file (EP-stiffness), .FDdat file (PSD-stiffness).

6. Ensure Monitor mode is selected in APT User Software (**Figure 47**).

7. In Data Recording select Stream to Disk. Select Auto File Naming, chose the folder and the path. This must be followed for all measurements (**Figure 55**).

   (NOTE: If you <u>do not</u> select Stream to Disk, the files <u>will not</u> be saved.)

8. Define DAQ Clock Rate and Samples per Channel. This must be followed for all measurements (**Figure 56**).

9. <u>.dat file</u>; In Data Recording, select Start Tracking. After the desirable time, select Stop Tracking (**Figure55**).

10. .dat file includes the data acquisition rate used during the measurement and, three columns which represent the raw detector voltage data X,Y and SUM respectively (**Figure 57**).

11. <u>.LSdat file</u>; For lateral position calibration, follow the steps indicated in section 4.2.

    (NOTE: If .LSdat file is empty verify if you followed all steps required).

12. .LSdat file includes the number of samples, the number of averages acquired and, four columns: Column 1 is the the stage position in μm for X, Column 2 is the X voltage data, Column 3 is the the stage position in μm for Y and Column 4 is the the Y voltage data (**Figure 72**).

13. To determine the trap stiffness, two methods can be used, the EP method and the PSD method. The .TDdat file is representative of the EP method, while the .FDdat file is representative of the PSD method. In Force calibration, in General select the values desirable (**Figure 58**).

14. .TDdat file; In Force calibration, select Run Calibration. Select Run Calibration. Finally, to save the files select Save Data (**Figure 71**).

    (NOTE: In Force Calibration, for stiffness determination with EP method, the beta value is required (lateral position calibration)).

15. .TDdat file includes the sample rate, number of samples, number of averages and, four columns: Column 1 is time in seconds, Column 2 is X voltage, Column 3 is Y voltage and Column 4 is SUM voltage. The X and Y signals are normalized by the SUM (**Figure 60**).

16. .FDdat file; In Force calibration, define the Trap Medium parameters (**Figure 59**). Select Run Calibration. Finally, to save the files select Save Data (**Figure 71**).

17. .FDdat file includes the number of frequencies, the measurement time used during the calibration and, four columns: Column1 is the frequency (Hz), Column 2 to Column 4 are power spectral data based on X, Y and SUM signal (**Figure 61**).

18. In Position Calibration or Force Calibration-Frequency Domain click on the left and right side of the graph to move the limits.

19. Whenever you select Save Data in Force Calibratrion, after Run Calibration, all three files, .LSdat, .TDdat and FDdat will be saved by default.

20. For accurate data analysis, you can select the parameters in OTKB Software, or you can use a programming language for data analysis.

*__erro mesa dll__

Auxiliary Figures

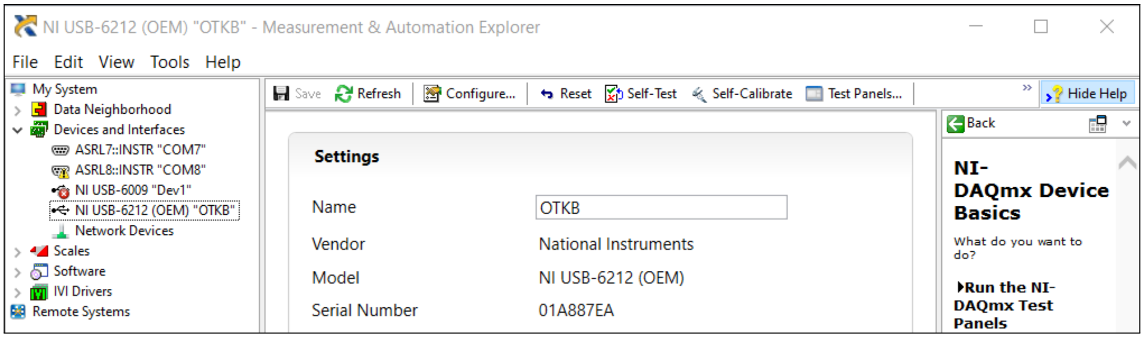


Figure 52: NI MAX tool. Select “NI USB-6212” device and rename it to “OTKB”.

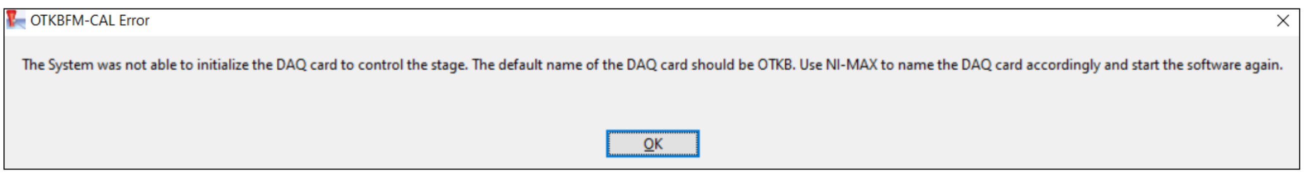


Figure 53: OTKBFM-CAL Error window. Change the default name of the DAQ to ”OTKB”.

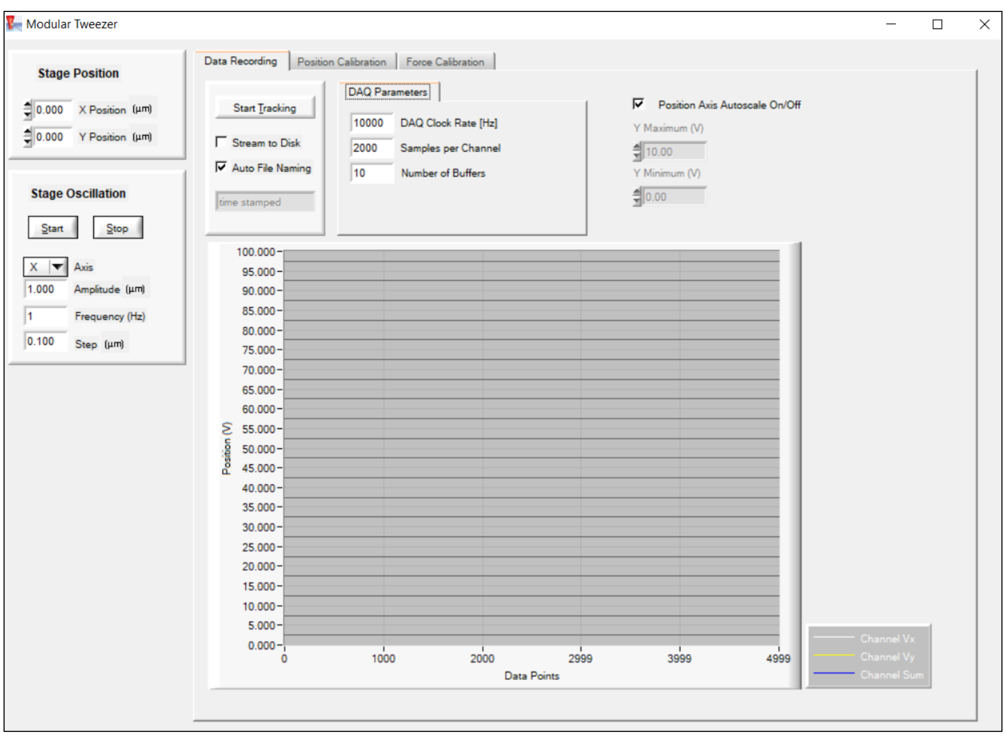


Figure 54: OTKB Software.

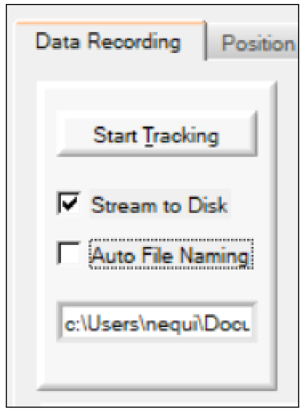


Figure 55: In Data Recording select Stream to Disk and select Auto File Naming.

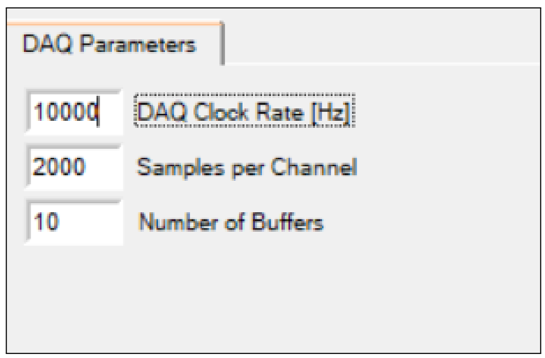


Figure 56: Define DAQ Clock Rate and Samples per Channel.

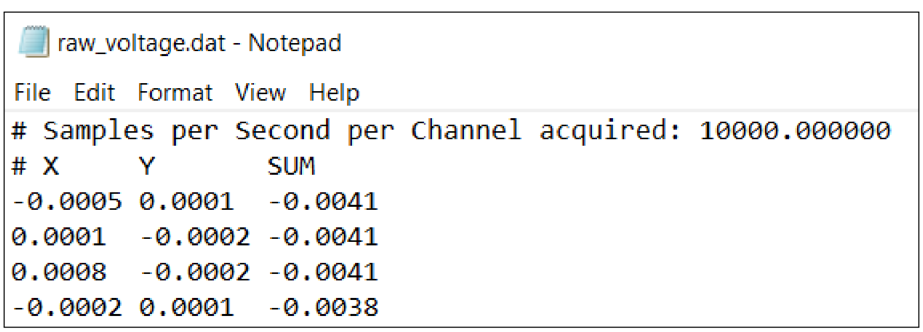


Figure 57: .dat file. The three columns represent the raw detector voltage data X,Y and SUM.

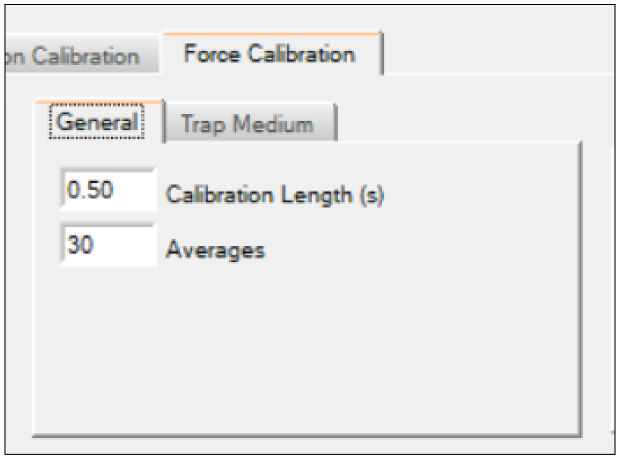


Figure 58: Define the Calibration Length and Averages.

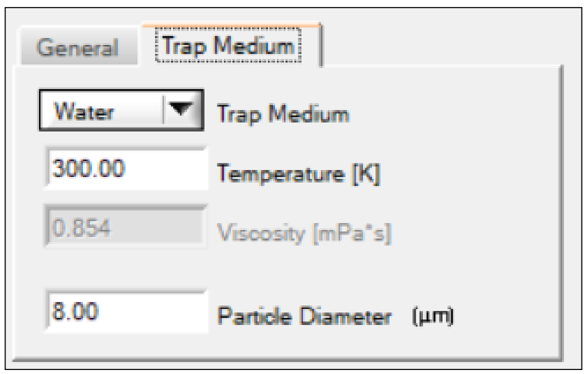
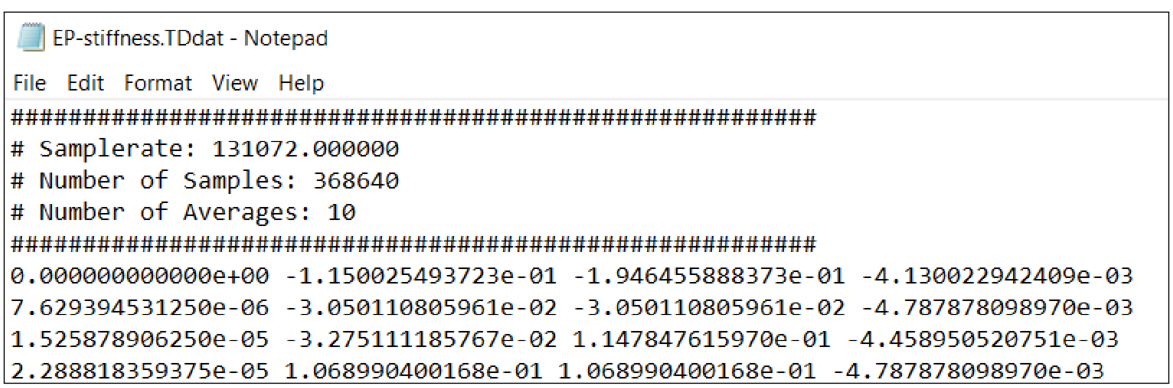


Figure 59: Define the Trap Medium parameters.


EP-stiffness.TDdat - Notepad

File Edit Format View Help

```
########################################################
# Samplerate: 131072.000000
# Number of Samples: 368640
# Number of Averages: 10
########################################################
0.000000000000e+00 -1.150025493723e-01 -1.946455888373e-01 -4.130022942409e-03
7.629394531250e-06 -3.050110805961e-02 -3.050110805961e-02 -4.787878098970e-03
1.525878906250e-05 -3.275111185767e-02 1.147847615970e-01 -4.458950520751e-03
2.288818359375e-05 1.068990400168e-01 1.068990400168e-01 -4.787878098970e-03
```

Figure 60: .TDdat file. The four columns represent: Column 1 is time in seconds, Column 2 is X voltage, Column 3 is Y voltage and Column 4 is SUM voltage.

PSD-stiffness.FDdat - Notepad

File Edit Format View Help

```
########################################################
# Number of Frequencies: 18432
# Measurement Time [s]: 0.281250
########################################################
0.000000000000e+00 2.704293942966e-05, 4.887061823887e-04 4.950308438033e-06
3.555555555556e+00 1.026713696717e-06, 1.526390301374e-06 2.052520203786e-11
7.111111111111e+00 5.072603347095e-07, 5.248537338262e-07 5.672804275866e-12
1.066666666667e+01 3.240718975580e-07, 2.860198095213e-07 3.283225438682e-12
```

Figure 61: .FDdat file. The four columns represent:Column1 is the frequency (Hz), Column 2 to Column 4 are power spectral data based on X, Y and SUM signal.

For more information please consult OTKBFM Manual, OTKB(/M) Manual or OTKBFM-CAL Manual by Thorlabs.

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4 KPZ operation mode

4.1 Open loop operation mode

For the measurement of the position fluctuations over time, open loop operation is implemented. The position fluctuations are used to determine the trap stiffness of the trap and particle properties. During the signal acquisition the positioning stage is static.

To implement the open loop operation mode, only precautions regarding the QPD display in APT User Software must be taken into consideration. The position aligner must be set to Difference in X,Y Display Mode, so that the display plots the respective $XDiff$ and $YDiff$ signals. Additionally, the operating mode must be set to Monitor mode so that the difference signals are fed through the correct way.

4.2 Closed loop operation mode

APT User Software and OTKB Software work together to implement the closed loop operation mode. In particular, this operation mode is useful to characterize the beam profile and for lateral position calibration of the QPD.

The piezo controllers (KPZ) must be set to closed loop operation (APT User Software) to enable the measurement of the displacement and of the voltage over time. The values are acquired and saved by OTKB Software. Following steps:

APT User Software

1. Start OTKB Software. Start APT User Software.

   (NOTE: If not this method will not be successful).

2. The piezo actuators (KPZ$_{x,y}$) must be in open loop operation mode (**Figure 62**).

3. In piezo settings → Drive Input Source (Open Source) → SW (Software Only).

4. Open loop → Set 35Volts to the piezo controllers (**Figure 63**).

5. Click the button ENABLE.

6. Zero the strain gauge readers (TSG$_{x,y}$). NULL count from 10 to 0 (**Figure 49**).

7. Change the operation mode (KPZ$_{x,y}$). First set Analog Input Source (VIn) → SMA Input (**Figure 64**).

8. Then, set the piezo controllers to closed loop operation (**Figure 65**).

9. After that, KPZ settings → Drive Input Source (Open Source) → VIn + Wheel + SW (**Figure 66**).

10. If you select the KPZ display, the position setpoint must be 46,66% (**Figure 67**).

    (NOTE:This steps must be followed, to allow the usage of the close loop operation.)

OTKB Software

1. In Data recording set 10kHz for DAQ Clock Rate (**Figure 56**).
2. In Data recording set 200 Samples per Channel (**Figure 56**).
3. In Position Calibration set 10 μm to the Scan Length and set 2000 Number of Averages. Define Number of steps (**Figure 68** and **Figure 69**).
4. In Position Calibration for the knife-edge method use 300 to Number of Steps.
5. In Position Calibration for lateral position calibration use 200 to Number of Steps.
6. Click Run Calibration (**Figure 69**). The S-curve obtained in OTKB Software must be similiar to **Figure 70**.

   (NOTE: To save the files do not forget to select the option Stream to Disk. While performing the Run Calibration you will the piezo display in GUI panel changing the values due to the movement of the piezo controllers.)
7. In Position Calibration, the Save Data button does not work correctly. To save the .LSdat files, in Force Calibration select Run Calibration and then select Save Data. Finally, the .LSdat must be saved (**Figure 71**).
8. The .LSdat file, Line Scan data file, includes the number of samples and the number of averages acquired. For position calibration, Column 1 is the the stage position in μm for X, Column 2 is the X voltage data. Column 3 is the the stage position in μm for Y, Column 4 is the the Y voltage data. X and Y detector voltage signals are normalized by the SUM before saving to the file (**Figure 72**).

Auxiliary Figures

APT User Software

Figure 62: KPZ- Open loop operation mode.

Figure 63: KPZ- Select the voltage, 35 Volts (Software only).

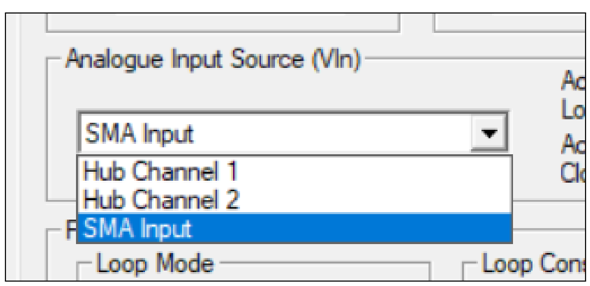

Figure 64: KPZ- Set SMA Input.

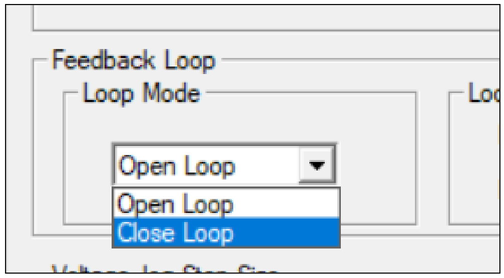

Figure 65: KPZ- Set closed loop operation.

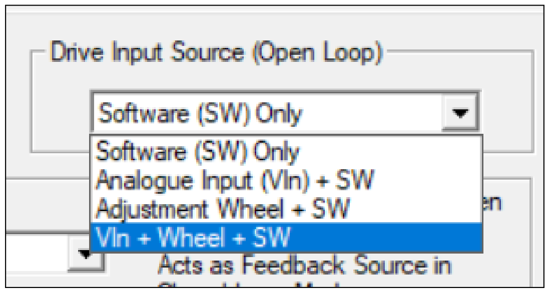

Figure 66: KPZ- VIn + Wheel + SW.

Figure 67: KPZ- 46.66% position feedback.

OTKB Software

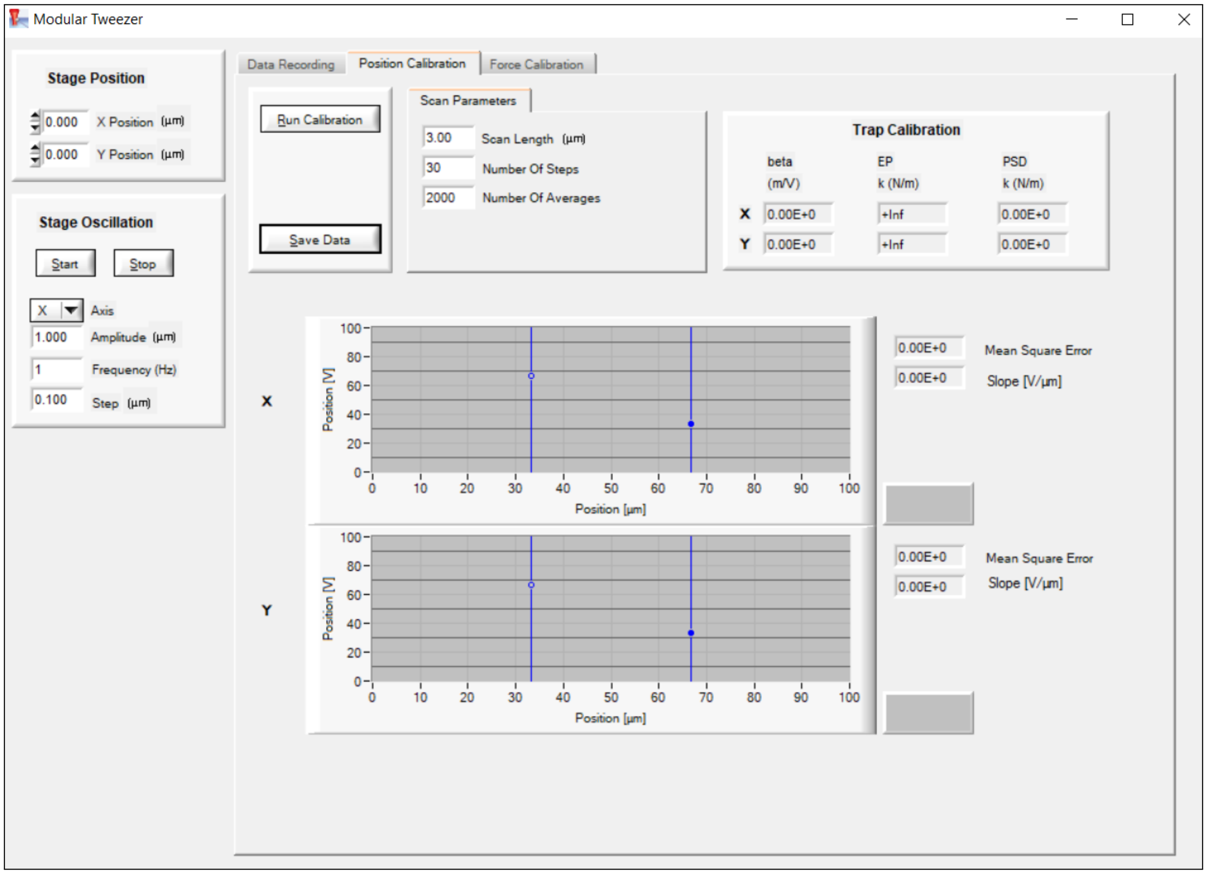

Figure 68: OTKB- Position calibration window.

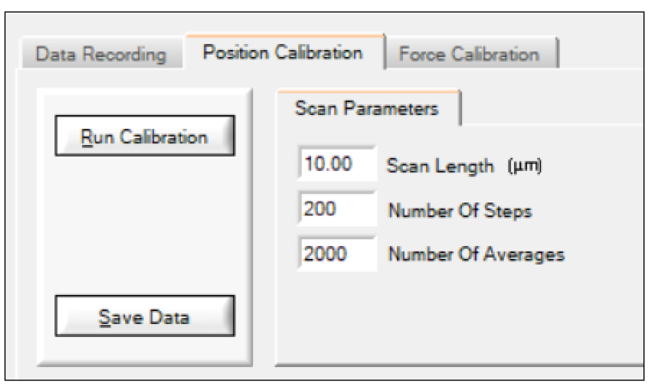

Figure 69: In Position Calibration define Scan Length and Number of Averages. Define Number of Steps. Select Run Calibration.

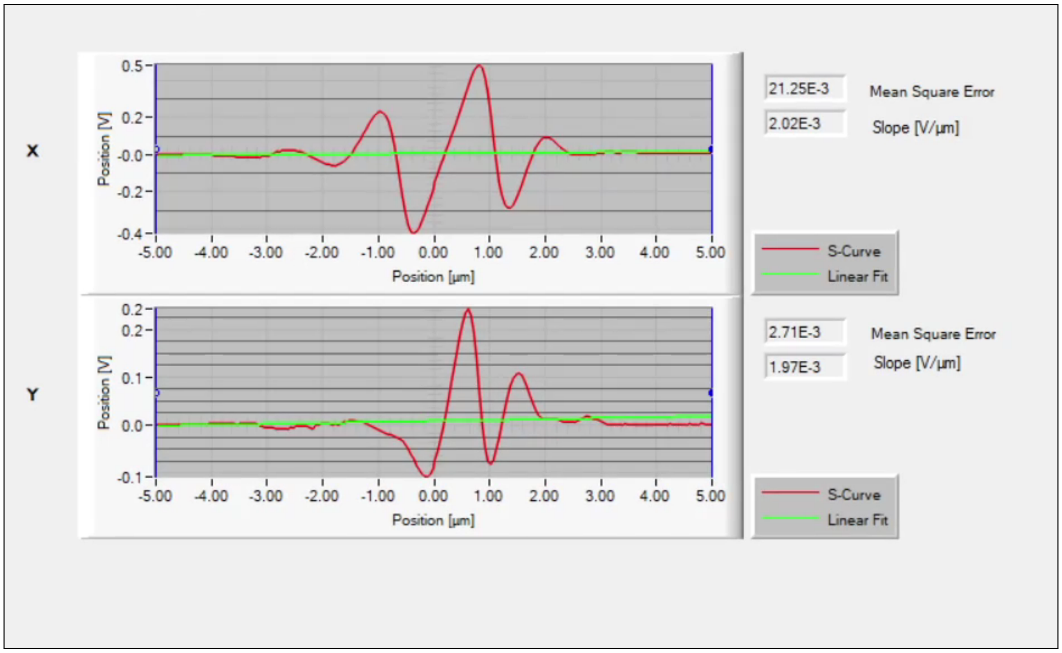

Figure 70: Position calibration- X and Y signal.

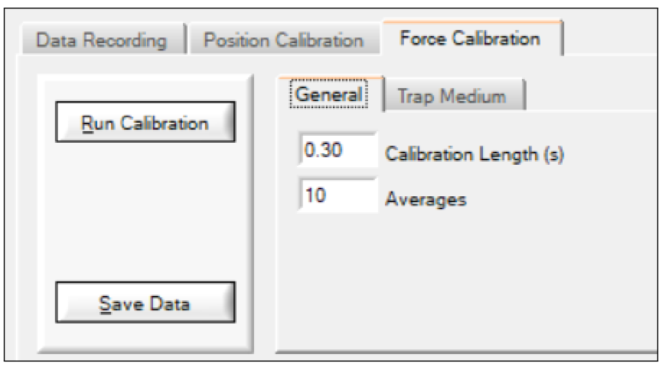

Figure 71: In Position Calibration select Run Calibration. Then, select Save Data.

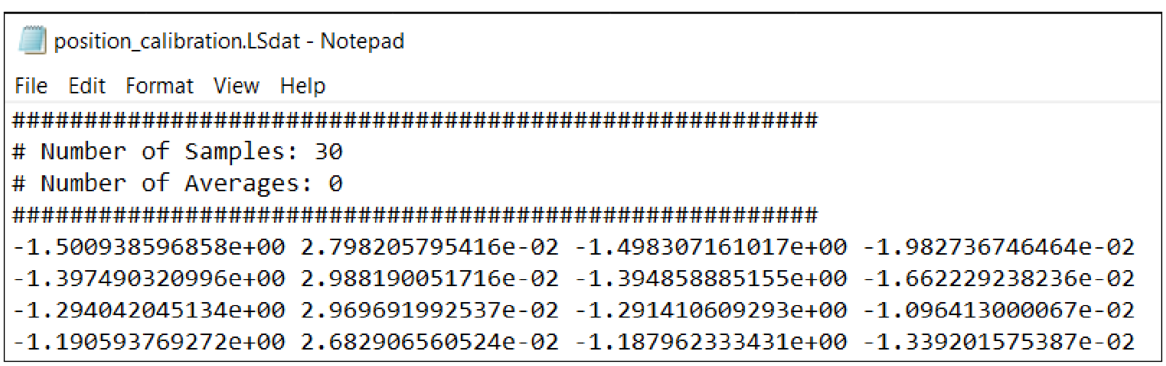
```
position_calibration.LSdat - Notepad
File  Edit  Format  View  Help
########################################################
# Number of Samples: 30
# Number of Averages: 0
########################################################
-1.500938596858e+00 2.798205795416e-02 -1.498307161017e+00 -1.982736746464e-02
-1.397490320996e+00 2.988190051716e-02 -1.394858885155e+00 -1.662229238236e-02
-1.294042045134e+00 2.969691992537e-02 -1.291410609293e+00 -1.096413000067e-02
-1.190593769272e+00 2.682906560524e-02 -1.187962333431e+00 -1.339201575387e-02
```

Figure 72: .LSdat file. The four columns represent: Column 1 X position in μm, Column 2 X voltage data, Column 3 Y position in μm, Column 4 Y voltage data,.

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