Matlab OTKB GUI Manual:

Preface: This is the manual for the OTKB GUI. This GUI can be used to control stage position as well as perform sensitivity and stiffness calibrations on the trap. This manual will document how to use all of the possible features of this GUI.

Table of Contents:

- 1. Getting Started
 - 1. Download appropriate software and install appropriate hardware
 - 2. Opening the GUI figure and Matlab script and entering cube serial numbers
 - 3. Running the GUI
 - 4. GUI layout
 - 5. Turning on the active-x controls
 - 6. Camera control
- 2. Controlling Piezo Position
 - 1. Zeroing the strain gauge cubes
 - 2. Centering the piezo cubes
 - 3. Nanometer accuracy 3-axis positioning
- 3. Calibration Data Table
- 1. Initializing XY position calibration values
- 4. Quad-Photodiode Detector
 - 1. Starting QPD alignment
 - 2. Stopping QPD alignment
- 5. XY Position Calibration
 - 1. Initializing parameters
 - 2. Centering bead
 - 3. Starting XY position calibration
 - 4. Stopping XY position calibration
 - 5. Saving data
 - 6. DNA Tether Stretch
- 6. Voltage Waveform and Equipartition and PSD-Rolloff Stiffness Calibrations
 - 1. Initializing parameters
 - 2. Starting data collection
 - 3. Saving data
- 7. Stokes Drag Calibration
 - 1. Initializing parameters
 - 2. Starting stokes calibration
 - 3. Stopping stokes calibration
 - 4. Saving data
- 8. Exiting the GUI
- 9. Hardware Block Diagram

1: Getting Started

1.1: Download appropriate software and install appropriate hardware:

This GUI utilizes ThorLabs's T-Cube APTUser as well as ThorLabs's DCx camera software. Before you attempt to run this GUI, make sure both of these are installed on your computer (the disks with these software should be included and the software can also be found on the ThorLabs website). While the GUI requires these components to run, you will receive errors if you try to run either APTUser or the camera software, uc480 Viewer, with the GUI.

This GUI also makes use of a National Instruments DAQ card for data collection. Make sure you setup the DAQ card as instructed in the hardware block diagram included at the end of this manual.

Once you have completed these two steps, the GUI can be run.

1.2: Opening the GUI figure and Matlab script and entering cube serial numbers: In order for this GUI to run properly, several Matlab files must be in the same directory (do not change the name of these files!). The list is as follows:

- 1. OtkbCalibration.m (main GUI script)
- 2. OtkbCalibration.fig (figure for the GUI)
- 3. EquipartitionAnalysis.m (used for the equipartition calibration)
- 4. FitPsdFunction.m (used for the PSD-rolloff calibration)
- 5. TransferFunc.m (used for the PSD-rolloff calibration)

Start Matlab and set the current directory to be the directory in which you placed the above files. Open the OtkbCalibration.m file. In order for the GUI to work with the 6 T-cubes that you are using in your setup, you will need to change the serial numbers currently entered in the Matlab script. In order to do this, go to the "turnOn_Callback" function part of the code. The first part of the function initializes the 6 T-cubes. Enter the serial numbers from your cubes into the corresponding T-cube initialization block in the command line: set(handles.object, 'HWSerialNumber', serial number). Hit save.

Then, open the OtkbCalibration.fig file by right clicking on the file name and selecting "open in GUIDE". You can choose to launch the GUI from either of these files.

1.3: Running the GUI:

From either the Matlab figure or script file, press the green arrow to launch the GUI. Maximize the figure that appears for position consistency (you will see why this is important in section 1.6).

1.4: GUI layout:

When you run the GUI, you will notice several different panels, which will be used to control the stage and perform the aforementioned calibrations, and several axes whose purposes will be explained later.

1.5: Turning on the active-x controls:

The GUI is initially launched with none of the piezo, strain gauge, or camera active-x controls displayed. In order to turn them on, press the "Turn On" push button in the upper left corner of the GUI in the "Piezo Controls" panel. You will notice that 6 small piezo, strain gauge, and QPD active-x controls will be initialized in the lower right of the GUI and the camera active-x control will be initialized in the middle right of the GUI. For the sake of this GUI, you can ignore the piezo and strain gauge controls as they will not be manually adjusted.

1.6: Camera control:

The camera active-x control is initialized with the 'AutoGain' and 'AutoExposure' properties turned on. If you need to adjust these properties, or any other camera properties, simply right click on the camera active-x object and select properties.

It is highly recommended that at this time you mark the location of the center of your trap on the computer screen. This can be done by making a bead slide, as documented on the OTKB wiki, and trapping a free bead. Make an arrow-shaped piece of tape and use it to mark the center of the bead on the screen. This will make trapping much easier because you will have a rough physical reference to the location of the laser.

2: Controlling Piezo Position:

This section discusses how to use the GUI to zero the strain gauges, center the piezos, and use the nanometer precision positioning panel.

2.1: Zeroing the strain gauge cubes:

After you complete section 1.5, you need to null the position reading on the strain gauge cubes. In order to do this, press the "Zero" push button in the upper left corner of the GUI in the "Piezo Controls" panel. This button will set the x and y piezo control outputs to 0V, set the strain gauge position to 0V, and then set both the x and y piezos in closed loop (feedback) mode. In addition, it will move the piezos to the center of their output (roughly 37.5 volts, but values can vary between 37 and 39 volts).

2.2: Centering the piezo cubes:

This property of the GUI allows you to set all 3 piezo cubes (x, y, and z axes) to the middle of their output range, the approximate value of which is mentioned at the end of the previous section. In addition, it will zero the output from the two DAQ analog output channels. In order to execute this property, press the "Reset" push button in the upper left corner of the GUI in the "Piezo Controls" panel.

2.3: Nanometer accuracy 3-axis positioning:

The three edit text boxes in the upper left corner of the GUI in the "Piezo Controls" panel allow you to move the stage by a specific displacement in nanometers. In order to move the stage, type the desired displacement in nanometers in the edit text box corresponding to the cardinal axis upon which you would like to move. Hit enter in order to move the stage. You can press enter multiple times to move the stage in increments of the entered value. This works for both positive and negative displacements. If you exceed the range of the piezos, which is set to be between 0

and 75V (this value should not be changed), you will no longer be able to move the stage via this method. In order to counteract this, try to do the majority of the movement using the rough and fine adjust knobs on the actual stage, and use this property for final small displacements.

3: Calibration Data Table:

This data table is where all of your calibration data will be recorded. The table will be filled as you execute the various calibrations. The only cells that can be manually entered are those corresponding to the row labeled "QPD Sensitivity".

3.1: Initializing XY position calibration values:

If you have already run a position calibration and do not wish to run another, you may enter the QPD stiffness values you obtained into the GUI. The default value is set to 0.001 V/nm. This value is stored once you complete section 1.5 so it is imperative that you either replace these values or run a position calibration before you perform any of the other calibrations. Otherwise, your data will be inaccurate. In order to enter values for the x and y axis QPD sensitivity, type the value into the corresponding edit boxes titled "Enter X-axis Sensitivity (V/nm)" and "Enter Y-axis Sensitivity (V/nm)" (make sure the values you are entering are in the specified units!). After you type the value in and press enter, you will notice the data table will update with the recently entered value.

4: Quad-Photodiode Detector:

This section explains how to use the GUI's version of ThorLabs's part TQD001, the T-Cube Quad Detector Reader.

4.1: Starting QPD alignment:

In order to start data collection from the QPD, make sure no other calibrations are running and press the "QPD Alignment Start" push button on the left side of the GUI. Once started, the axes labeled "QPD alignment" below the button will start to display a voltage signal corresponding to the position of the laser on the QPD.

When first running this GUI, it is recommended that you center the QPD with a bead trapped. This is done by turning on the QPD alignment and adjusting the screws on the HPT1 such that the voltage signal is centered at zero on both axes.

4.2: Stopping QPD alignment:

In order to stop data collection from the QPD, press the "QPD Alignment Stop" button to the right of the start button. Make sure to stop the QPD Alignment before you run any of the calibrations (XY Position Calibration, Waveform and PSD-Rolloff, or Stokes Drag) otherwise you will receive an error.

5: XY Position Calibration:

This section explains how to execute the first of the OTKB trap calibrations. The procedure for making slides for this calibration is documented on the OTKB wiki page. The control panel that is responsible for executing this calibration is titled "Position Calibration" and is located in the upper right hand corner of the GUI.

5.1: Initializing parameters:

There are several user input parameters that control different aspects of the calibration. They are as follows:

-Axis (note, the object that controls this parameter is not located inside the control panel mentioned in the previous section, but rather to the left of the edit text box used to enter x-axis sensitivity): Selects along which axis to scan. This is controlled by a popup menu, and either the X or Y axis can be selected. The X-axis has been set as the default axis.

-Number of Lines: This parameter controls the number of scans that will be run along the scanning axis. The default value for this parameter is set to 100.

-Scan Freq. (Hz): Controls the scanning frequency. The default value for this parameter is 1 Hz. -Scan Amplitude: Controls the amplitude of the scans in volts. The amplitude of the scan in micrometers is the amplitude in volts multiplied by 2.26. The default value for this parameter is 0.5V.

-Samples per Line: Controls the number of samples taken by the DAQ card per scan. The default value of this parameter is 250.

-Save: Chooses the save name under which the collected data will be saved. See section 5.5 for more information on saving data. Make sure the save name ends in '.txt' so that the data are saved in a text document.

5.2: Centering bead:

Before you start the XY position calibration, it is important that the laser is centered on the stuck bead being used. Turn on the QPD Alignment, and using the piezo controls, center the voltage signal at 0. Turn off the QPD Alignment. You are now ready to start the position calibration.

5.3: Starting XY position calibration:

In order to initiate the XY position calibration, press the "Position Cal. Start" push button. The stage will start to move. After each scan, you will see the X and Y voltage signals from the scan displayed on the axes titled "X-axis Voltage Signal" and "Y-axis Voltage Signal" respectively. Once all of the scans are finished, the stage will return to its initial position, a surface plot of a compilation of the X and Y axis voltage signals from all of the scans will be displayed on the axes titled "X Position Calibration" and "Y Position Calibration" respectively, and the calculated sensitivity values for both axes will be updated in the table described in section 3.

5.4: Stopping XY position calibration:

If for any reason you need to stop the XY position calibration while it is running, press the "Position Cal. Stop" push button. This will stop the DAQ input and output channels and reset the position of the stage to its starting position.

5.5: Saving data:

Once all of the scans have finished, Matlab will save a copy of the collected data under the name specified by the parameter "Save" from the 4 input channels in the specified order: X strain gauge position (in V), Y strain gauge position (in V), X QPD position (in V), and Y QPD position (in V). These data can be loaded back into Matlab if further analysis is needed.

5.6: DNA Tether Stretch:

An additional feature of the Position Calibration control panel allows for the measuring of the tether length of a piece of DNA. Initialize the parameters listed in section 5.1 in addition to the parameter "Bead Rad. (um)", which sets the bead radius (in micrometers). The execution, stop, and saving procedures are the same as for the XY Position Calibration. Once the tether stretch has finished, the calculated tether length in nanometers will be displayed in the static text box labeled "Tether Length (nm)". It is recommended that you set the scan amplitude to a higher number than the default value; for 3500bp DNA and 1 μ m diameter beads, a scan amplitude of 1.25V is recommended.

6: Voltage Waveform and Equipartition and PSD-Rolloff Stiffness Calibrations:

This section explains how to run the Equipartition and PSD-Rolloff stiffness calibrations as well as collect a waveform of QPD voltage data.

6.1: Initializing parameters:

There are 3 parameters that control data acquisition for the voltage waveform data, and they are: **-Axis**: Set the axis you wish to collect data from, see section 5.1.

-Sampling Time (s): Enter how long for which you would like to take data. The default value is 3 seconds.

-Sampling Frequency (Hz): Enter what sampling frequency you would like to take data at. The default value is 100,000 Hz.

In addition, this process saves data under the save name specified in the XY position calibration control panel, so make sure you change that name if you don't want to overwrite previous data!

6.2: Starting data collection:

To initialize data acquisition, press the "PSD start" push button in the Waveform and PSD control panel. The DAQ will acquire data for the specified time and display the resulting waveform on the axes labeled "Voltage Waveform". In addition, a PSD of the waveform data will be taken and displayed on the axes labeled "Power Density Spectrum of Waveform Data" along with a first order transfer function fit. Finally, the table mentioned in section 3 will be updated with the calculated Equipartition and PSD-rolloff stiffness values for the axis from which data was collected.

6.3: Saving data:

Data is saved in the same manner specified in section 5.5 in the following order: A time vector in increments of the sampling period for the duration of the data acquisition, waveform data corresponding to the QPD voltage signal from the selected axis, a frequency axis used for the PSD, the PSD data, the transfer function data, and the cutoff frequency of the transfer function (this is the value used for calculating the trap stiffness via the PSD-rolloff method). These data can be loaded back into Matlab for further analysis.

7: Stokes Drag Calibration:

This section explains how to run the stokes drag calibration to determine trap stiffness.

7.1: Initializing parameters:

There are 5 parameters which control the data acquisition and stage movement for the stokes drag calibration, and they are:

-Axis: Select the axis you wish to scan along, see section 5.1.

-Amplitude (set in the "Stokes" control panel): Sets the amplitude of the line scans; higher amplitudes mean larger velocities. The default value is 1.25V (see section 5.1 for converting a voltage amplitude into a distance amplitude). Make sure you enter the amplitude in the Stokes control panel and not the Position Calibration control panel.

-Number of Scans: This controls the number of scans. The default value is 10.

The final two parameters used to control stage movement and data acquisition are "Scan Frequency" and "Samples per Line" (see section 5.1) located in the Position Calibration control panel. Also, this process saves data under the save name specified in the XY position calibration control panel, so make sure you change that name if you don't want to overwrite previous data! **-Bead Radius (um)**: This parameter is used to set the radius of the bead in micrometers. Note, the edit text box used to enter this parameter is in the Position Calibration control panel.

7.2: Starting stokes calibration:

In order to start the stokes drag calibration, press the "Start" push button in the Stokes control panel. The stage will start scanning the axis you selected in section 7.1. Once it has finished scanning, it create a scatter plot on the axes that are also used for the position calibration voltage signals per scan (though the axes on which these data are plotting is relabeled either "X-axis displacement (m) vs. velocity (m/s)" or "Y-axis displacement (m) vs. velocity (m/s) depending on selected scan axis). In addition, the data table mentioned in section 3 will be updated with the stokes drag trap stiffness value corresponding to the scanning axis.

7.3: Stopping stokes calibration:

In order to stop the stokes drag calibration, press the "Stop" push button in the Stokes control panel. This will stop the DAQ input and output channels and set the stage position to its starting position.

7.4: Saving data:

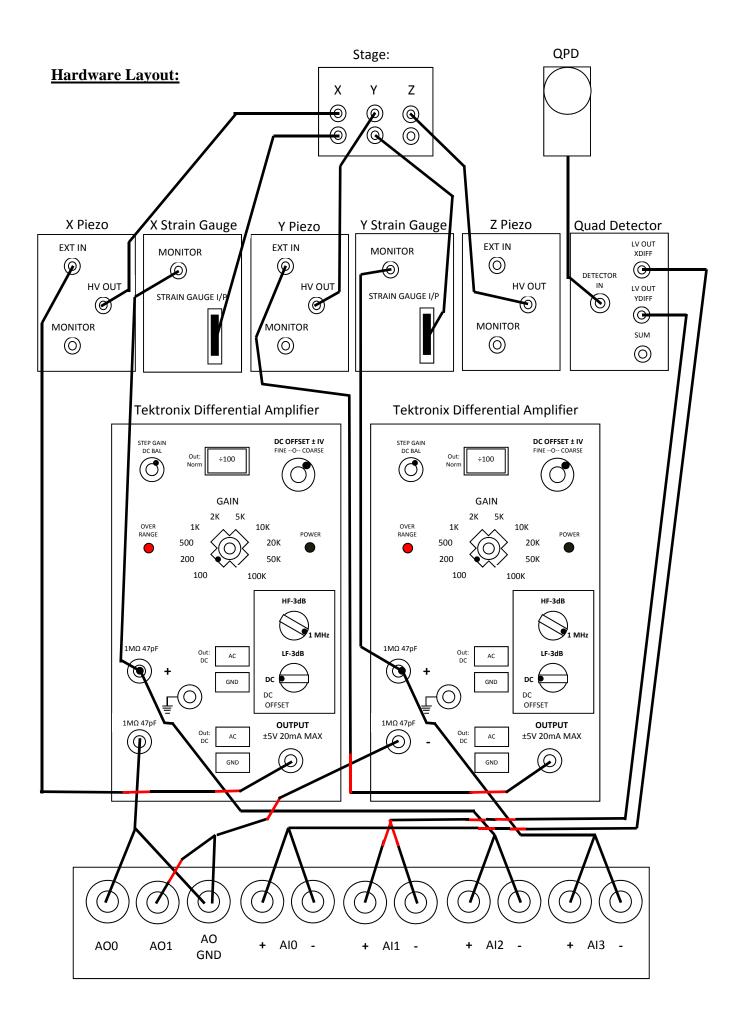
Data is saved in the same manner as described in section 5.5 in the following order: QPD voltage data from scanning axis, piezo voltage signal from scanning axis, velocity data, and displacement data. These data can be loaded back into Matlab if further analysis is required.

8: Exiting the GUI:

In order to exit the GUI, simply close the figure.

9: Hardware Block Diagram:

This section will demonstrate how to setup the hardware required for this GUI to run. The setup is as pictured in the diagram on the following page. Note, you can use any differential amplifier, as the amplifier in this setup may not be easily obtainable. Make sure the settings follow what is pictured below. Also, note the how the DAQ card is set up: the two output channels are driving the X and Y piezos and the 4 input channels are collecting data from the X and Y piezos as well as the X and Y axes from the QPD. In addition, a picture of the GUI is displayed on the page following the hardware block diagram.



Picture of GUI:

