Standard Operation Procedures (SOP) for Optical Tweezers

This is a simplified version that focuses mainly on routine operation. For more detailed instructions, please refer to the technician (Dr. Jinyuan CHEN, jychen06@connect.hku.hk)

1. Scope

1.1 This document provides the basic SOP and requirements to Optical Tweezers. Please refer to “Olympus X81 Inverted Microscope” manual for the imaging procedure.

1.2 **Booking System:** https://tanglab.hku.hk/main/resources/

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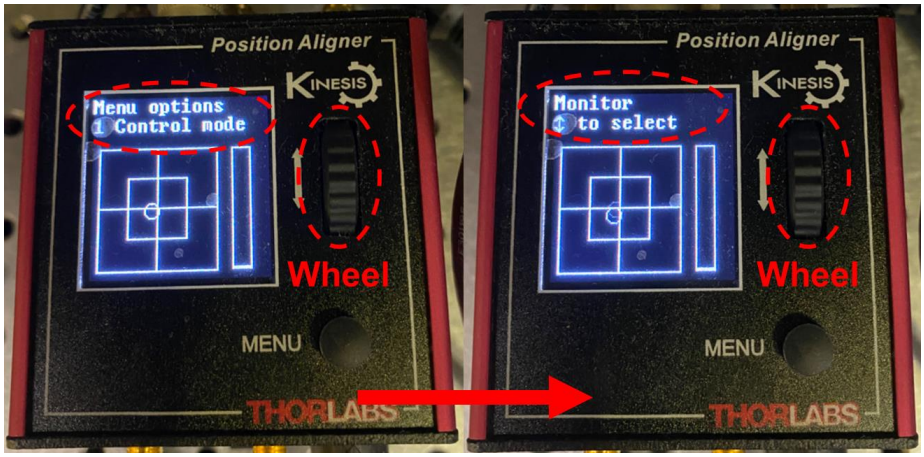
2. Start system

2.1 Turn on the Olympus IX81 Inverted Microscope system (Computer, IX2_UCB controller), Laser Power (key: off → on), Galvo Scanning System Power, Shuttle Switches and QPD successively.

2.2 Choose “1 Control mode” by pressing the “Menu” bottom and using the “Wheel” on the panel, then, choose “Monitor” to open the QPD measurement mode by the same process.

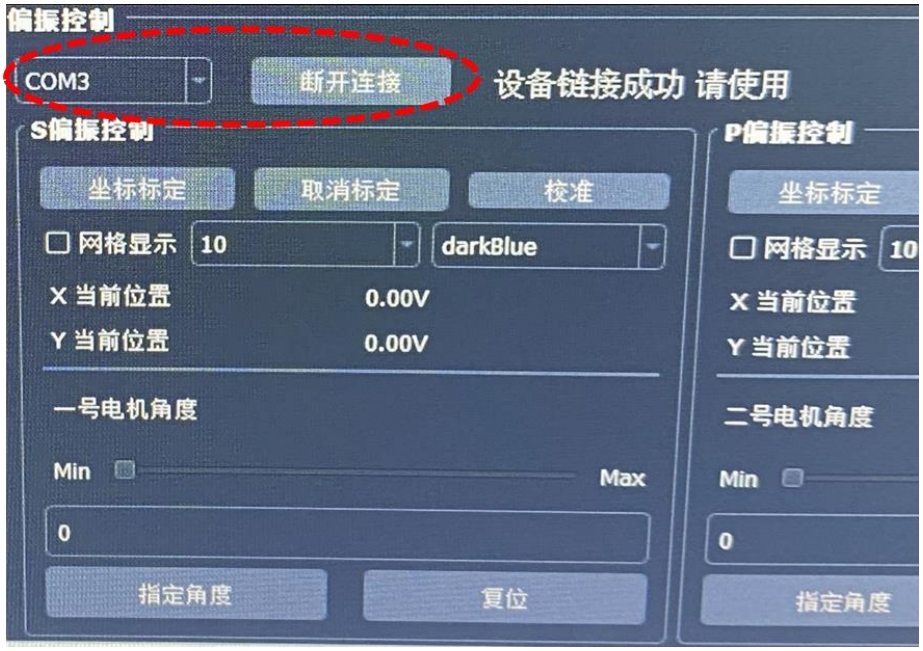


2.3 Start the control software by double-clicking “HAYEAR”  (assistant camera for observation of laser beam) and “K-DWell”  (Control position and power of laser beams) on the desktop.

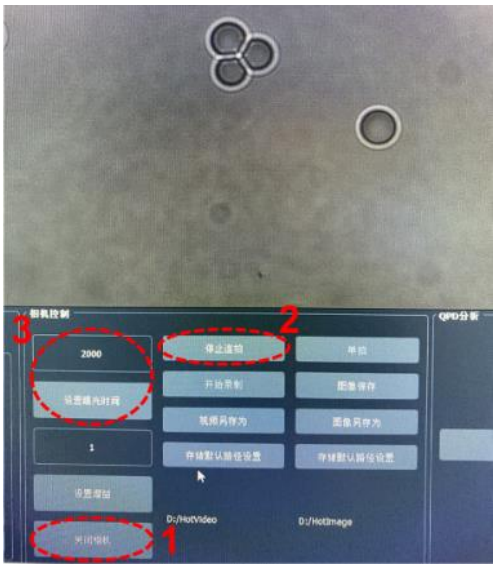
2.4 Choose the “COM4” serial port and click the “Link” in the “Light source control” panel of the interface in “K-DWell” software to control laser power. Then, input numbers (0 - 1500) of laser power into the (Laser Power) panel and click the “Setting” to control the output power of laser of optical tweezer.

(Warning: change the 50% ND filter in the condenser to when the light power over 1000 mW to protect the QPD sensor.)

2.5 Choose the “COM3” serial port and click the “Link” in the “Polarization Control” panel of the interface in “K-DWell” software to control the position of laser beam and intensity (the angle of electric rotor).



2.6 Click the “Open Camera”, “Burst Mode” and “Setting Exposure Time” to visualize the sample and laser beam.

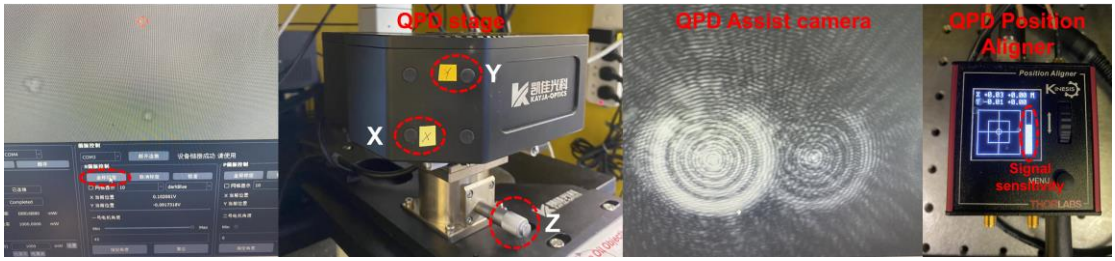


3. Calibration

3.1 Stick your test samples (colloids) on a glass substrate and inject your experimental solution to immerse your samples, then seal it and placed on the microscope.

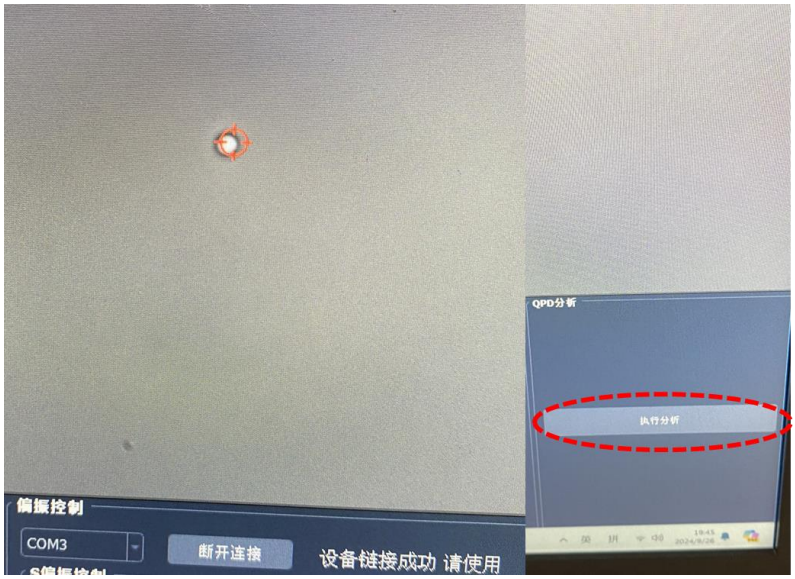
3.2 Click “Position Demarcation” in the “Polarization Control” panel, the bright laser beam shows in the red circle on center of the screen. Move the z position of QPD stage to get the pattern of laser beam shown in the assist camera as

the picture and make the signal sensitivity over 50% in the QPD position aligner. Then, Move X and Y axis of the mirror of QPD to align the white ring (indicating the laser beam position) in the center of QPD position aligner.



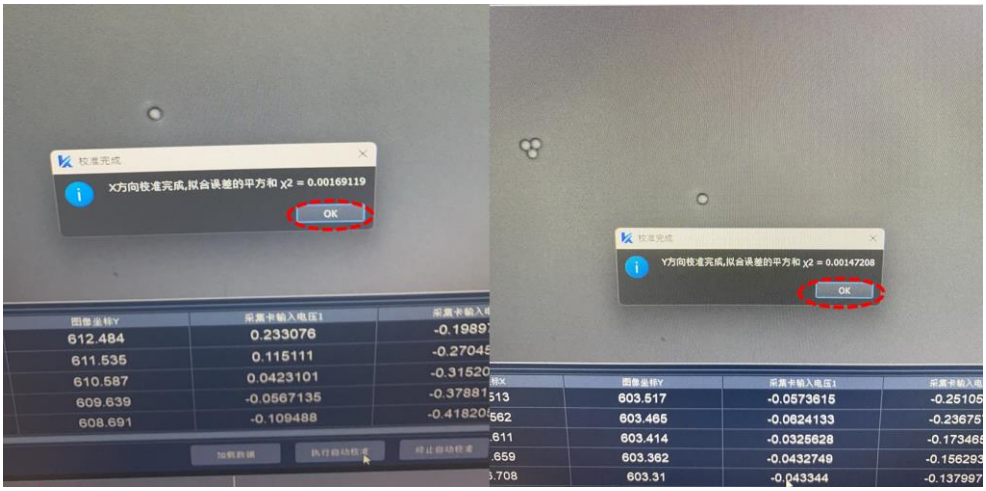
(Warning: Don't move the QPD stage after finished laser beam alignment)

3.3 Move the microscope stage to align the particle's center with laser beam center, then, click the "Perform Analysis" in the QPD analysis panel.



3.4 Click "Equipment Calibration", "Perform Automatic Calibration" and "Calibration Area Selection" successfully. Then, chose a preoperative area which is cover 2/3 central region of the particle (indicating as green square) by mouse in the new window. Finally, click "OK" to start the automatic calibration by equipment.

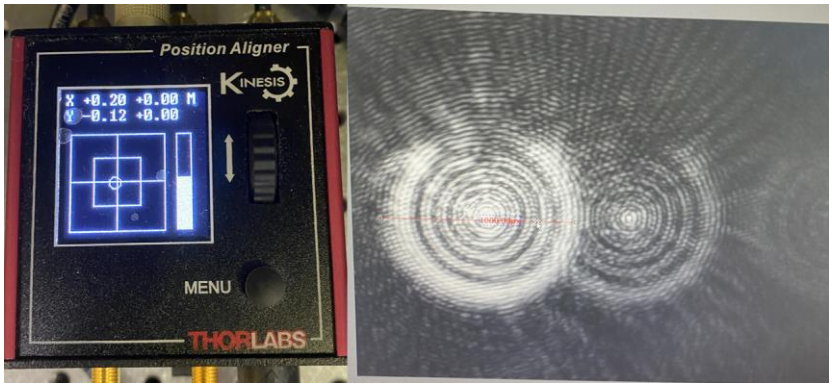
3.5 Click “OK” to finish x axis calibration and start y axis calibration automatically, if the sum of squares due to error χ^2 was below 0.01. Otherwise, redo the calibration step 3.4. Similarly, the y axis calibration should be finished as same as x axis.



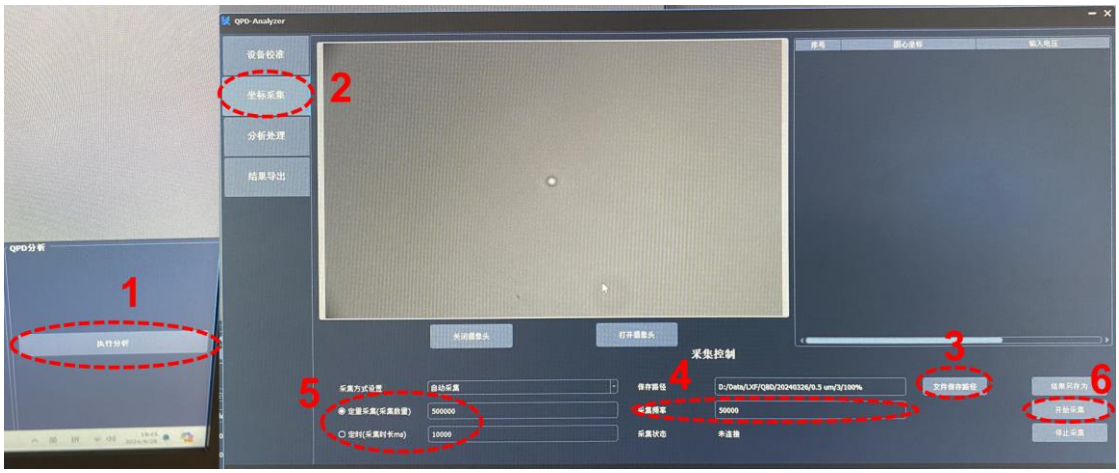
(Warning: the input voltages from capture card should linearly increase or decrease with position scanning during automatic calibration, if not, the calibration area was large than the particle.)

4. Measurement

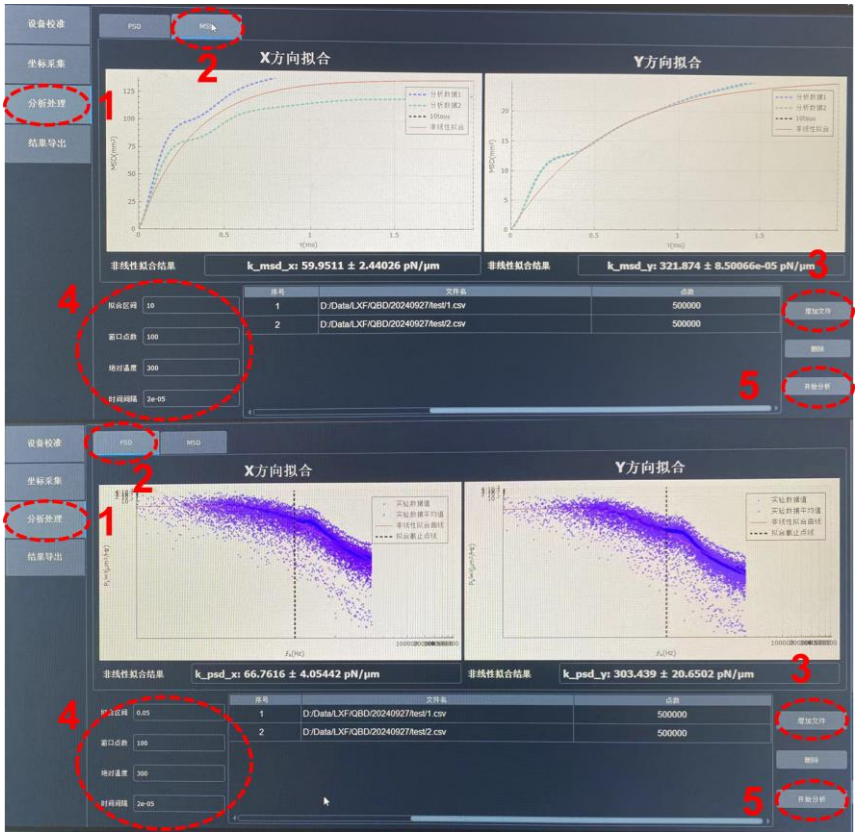
4.1 Place your sample on the microscope and to trap your sample by optical tweezer. Then move the laser beam to let the light spot in QPD position alignment at the center. Maximum the signal intensity and make the laser beam size around 1000 pixel in the assist camera by moving z axis of microscope lens and QPD stage.



4.2 Click “Perform Analysis” and “Coordinate Collection” to start take coordinate data of trapped particle. Click “File Save Path” to select the save path of coordinate data file as .CSV format. Input numbers of “Collection Frequency” and “Collection Quantity” or “Collection Time” to select total data quantity and frequency. Finally, click “Start Collection” to automatic data collection.



4.3 Click “Analysis and Processing” to do the analysis of stiffness. Choose “MSD” or “PSD” method to do computation. Click “Add File” to add the files and analysis. Then, choose appropriated parameters to do fitting (“Fitting Interval”, “Window Points”, “Absolute Temperature” and “Time Interval”). Finally, click “Start Analysis”.



5. Shutdown

5.1 The lens turns to 4X and lower the lens to lowest position at z axis. (If used oil lens, clean oil firstly.) Then, turn off QPD, Shuttle Switches, Galvo Scanning System Power, Laser Power (key: off → on) and Olympus IX81 Inverted Microscope system (Computer, IX2_UCB controller) successively.

5.2 Take away your sample and clean the desk.