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Research paper

Axial Calibration of QPD Signal based on Stuck Bead Method for Optical Trapping Applications

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Abstract

Calibration of axial quadrant photodetector (QPD) signal to the trapped bead position in an optical tweezer is important to measure the quantitative mechanical parameter in axial (laser propagation) direction. An alternative calibration based on the Stuck Bead Method (SBM) was proposed in this study. 3 µm polystyrene beads were stuck at the surface of glass coverslip and moved axially around the laser focus. QPD was used to obtain the position dependent intensity profile at three different laser powers (19.8 mW, 34.1 mW, 48.5 mW). The QPD signal-to-distance calibration value was consistent at 26 mV/µm for the used bead at the three laser powers. It was found that the calibration values are independent of laser powers and limited by the resolution of distance adjustment.

Keywords: *Axial QPD Signal; Optical Tweezer; Stuck Bead Method.*

1. Introduction

Optical tweezers (OT) had been invented three decades ago by Arthur Ashkin and friends. Since then, OT has undergone a lot of development and continues to advance our understanding about the microscopic world. Application of OT has been proven significant in the field of physics, biology and chemistry, manipulation of micro- to nanosized particles were made possible by using OT as tool to apply piconewton force to the particles. In a typical OT, a tightly focused laser beam creates an intense gradient force that can be used to trap the particle of interest, while at the same time measure its displacement down to nanometer scale. In recent years, OT has been used to manipulate, rotate and assemble nanostructures[1] such as carbon nanotubes[5,7], graphene flakes[8], nanowires[11] and polystyrene microbeads[10].

For force related measurements, OT requires calibration to translate light-based detection into its mechanical interpretation. As application of OT commonly involve micro- to nanosized particles, the calibration needs to be extremely precise. There are two main calibrations in the OT which is lateral (proportional to laser propagation direction) and axial (laser propagation direction) calibration. In comparison, the lateral calibration for OT is easier to achieve than axial calibration. One of the reasons for this situation is the refractive index mismatch between the sample and surrounding medium, which leads to focal shift and spherical aberrations that tend to elongate the focus of the laser in the axial direction[9]. Most of calibration in the lateral direction involves the estimation of the optical trap stiffness, k_{OT}. There are several methods to estimate k_{OT} which are passive power spectrum method, equipartition theorem, active power spectrum method and force measurement through momentum change[4]. However, these methods are not suitable for axial calibration as the spherical aberrations will cause the k_{OT} to vary[9].

Several methods had been established to calibrate the axial signal from QPD as the signal can be used in estimation of k_{OT}. Unzipping force-extension data of double stranded DNA (dsDNA) were used in determining the location of trap center along the axial direction, axial displacement of the bead from the trap center and axial force exerted on the bead. This method utilized the known force-extension relation for unzipping a DNA molecule to measure the trap height relative to the position of the cover glass[2]. Problem exists if the data for force-extension relation is not readily available for the targeted particle. Another method is back focal plane interferometry, where a charge coupled device (CCD) camera was used to visualize the interference pattern of the forward-scattered laser light, within the back focal plane of the condenser[12]. The axial positions of the bead were successfully determined to within a few nanometers. However, CCD camera's temporal resolution of the measurements is limited to ~1 kHz, therefore restricting a better estimation of the k_{OT}. In this study, we propose an alternative method to calibrate an optical tweezer along the axial direction of laser beam by using Stuck Bead Method (SBM)[6]. The quadrant photodiode (QPD) signal variation to the trapped bead position in an optical tweezer is important to measure the quantitative mechanical parameter in axial (laser propagation) direction.

2. Experimental setup

2.1. Optical system setup

The optical tweezer setup was illustrated in Fig. 1. A near infrared laser beam (wavelength λ = 915 nm) was emitted from the laser diode, coupled through the fiber coupler and passed through the beam expander. After that, the laser was reflected by dichroic mirror into the objective lens (100× N.A 1.25 WD 0.25 mm, oil immersion type, Olympus) which focuses the laser at the sample stage. The scattered laser beam was then collected by condenser lens (10× N.A 0.25 WD 7 mm, air type, Olympus) and reflected towards a quadrant photodetector (QPD) (PDQ80A, Thorlabs). The laser intensity variation due to bead movement were detected by the QPD. The bead was observed by using CCD camera installed behind the objective.

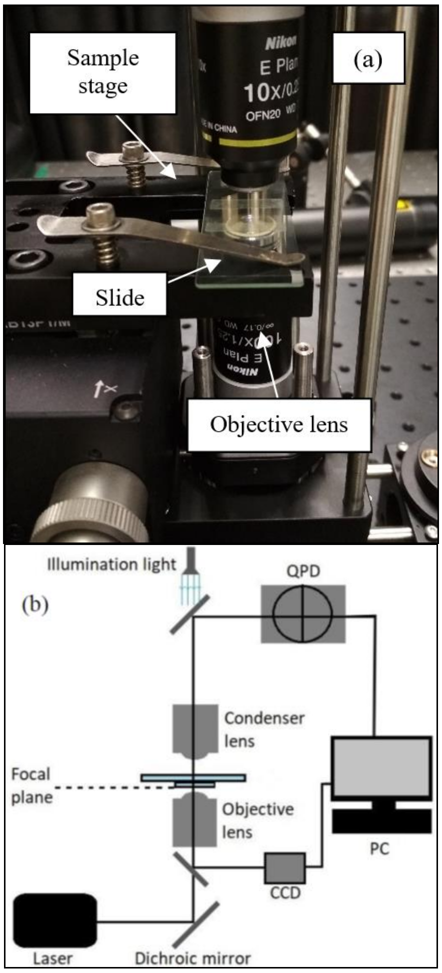

Fig. 1: (a) The experimental setup for axial calibration of QPD signal. (b) Schematic diagram of optical tweezer setup.

2.2. Sample preparation

The bead was prepared by diluting stock solution of polystyrene microbeads (Polysciences, Inc.) with deionized water by ratio 1:1000. The diluted solution was then dropped on the glass slide. The glass slide was prepared beforehand by placing two small strips of double-sided tape 2 cm apart to create a space for the solution. After dropping the solution, a glass cover was placed onto the double-sided tape to create a sample microchamber. The sample was left to set for two hours, allowing the bead to stuck at the bottom wall of the chamber. Next, the slide was placed on the sample stage which can be controlled by either manually or using the PC. Confirmation of the stuck condition of the bead can be done by turning on the optical tweezer as the stuck bead will not be affected by the optical force.

2.3. Stuck bead method

After the stuck bead was found, the sample stage was moved in the z-direction to analyze the effect of axial positioning of the bead on the QPD signal. The position where the bead is in focus (as seen as sharp image by the CCD) was set as the origin ($z = 0$), whereas the position below and above the focus were set as -z and +z respectively. Fig. 2 illustrates the position of the bead with respect to the origin.

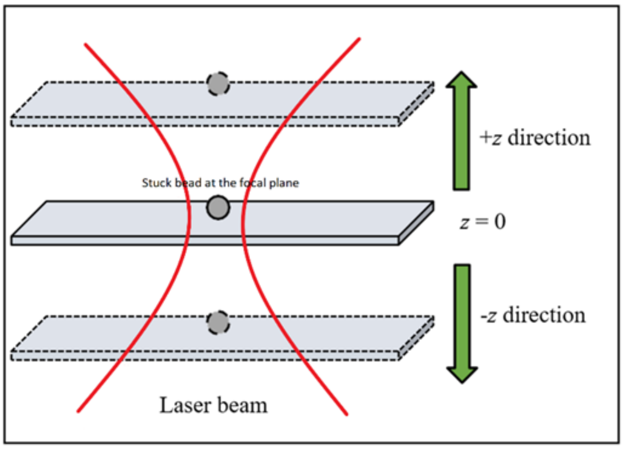

Fig. 2: Position of the bead with respect to the origin (z = 0). At z = 0, the bead was at the focal plane. Position of the bead were controlled by using sample stage.

3. Results and Discussion

Fig. 3 shows the sum signal from QPD for bead position along beam propagation direction for three laser powers which are 19.8 mW, 34.1 mW and 48.5 mW. It is important to note that all other experimental components are kept fixed except for the sample stage. The objective and QPD distance were not changed and fixed at their location during measurement.

During the measurement, the differential signals detected by the QPD was adjusted to minimum (~0V) to make sure the bead is at located at the central axis of the beam. For each power, a pair of shown arrows indicates the size of the bead (3 µm). This is the area which the light scattered due to the refractive index mismatch between the bead (n_b = 1.5) and water (n_w = 1.3) as shown in Fig. 4(b).

In this region, the signal started to increase (along +z) due to the focusing effect of the bead which act as a lens (see Fig. 4(a)). Beyond that, the defocusing effect of the object was dominant thus reducing the intensity received by the QPD. Below the focal plane (below z=0), the laser light was diverged by the bead, causing decrement in the QPD signal (see Fig. 4(c)). signal reached its maximum value above the focus as it was intensely focused by the bead onto the QPD.

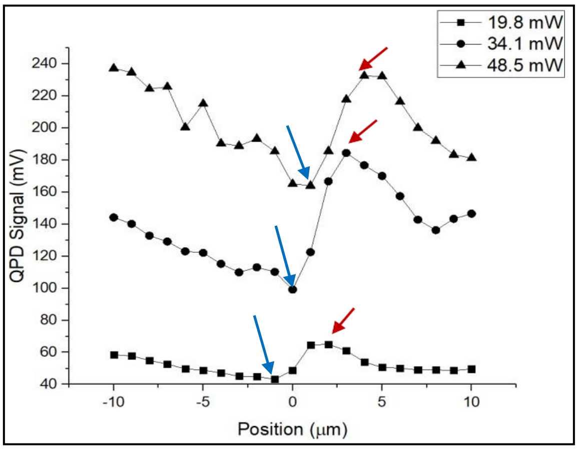

Fig 3: Axial signal obtained from the QPD for three different laser powers.

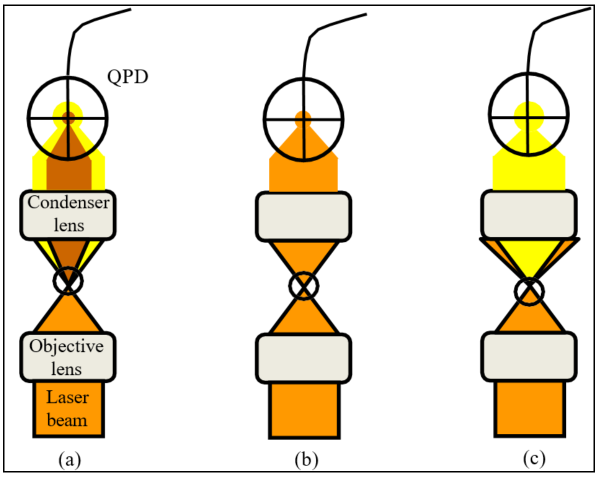

Fig. 4: (a) The bead was displaced above the focal plane (+z), converging the laser onto the QPD. (b) The bead was at the focal plane (z = 0), minimum divergence of the laser. (c) The bead was displaced below the focal plane (-z), diverging the laser and causing the signal to decrease. The changes in intensity were indicated by change in color. Yellow indicates decrease in intensity while brown indicates increase in intensity.

Even though the intensity detected by the QPD depends on the laser power, the intensity gradient from Fig. 3 was consistent at ≈ 26 mV/µm. The intensity gradient was calculated at the region near the focus of the laser. This implies that the calibration is independent of laser intensity. In optical trapping applications, the trapped bead is confined in an optical focus are which is in the submicron regions. The optical force is linearly proportional the bead displacement within the small volume at the trap center. This displacement is usually in nanometer scale[3]. Therefore, this proposed calibration scheme is still valid for the 3 µm bead or larger as long as the bead is within the focus region. The calibration procedure was limited by the resolution of the stage, currently manually adjusted using micrometer at resolution of 1 µm. For the smaller bead, higher resolution is needed which can be achieved using piezostage control. This is our motivation for the next level of higher precision of calibration.

4. Conclusion

The QPD signal-to-distance calibration value was consistent at 26 mV/µm for 3 μm bead at laser power 19.8, 34.1 and 48.5 mW. For calibration using smaller bead, higher resolution of displacement adjustment is required. This calibration is not limited to the optical trapping applications but those experimental methods which rely on the use of spherical bead as a probe of measurement.

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