A simple method for constructing and calibrating an optical tweezer

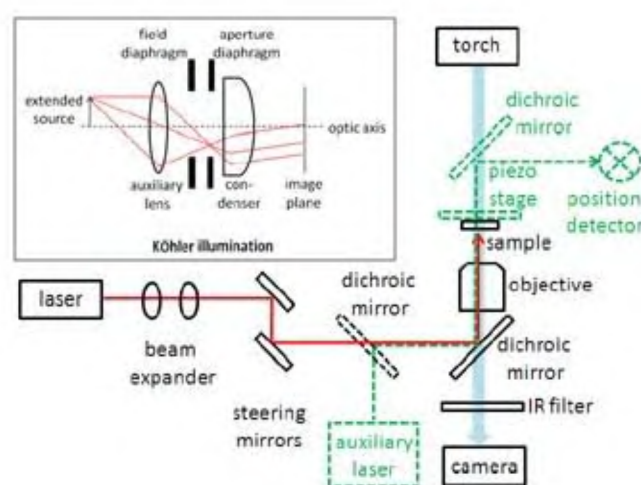Since the realization of implementing photon momentum to exert pressure and hold objects three-dimensionally in space[1] and its subsequent demonstration[2], optical tweezers or single beam gradient optical traps have been used in diverse applications ranging from atom trapping and cooling to study the properties of Bose–Einstein condensate[3] to manipulating cells[4]. There is a wealth of literature concerning the construction of a tweezer[5]. A comprehensive treatise on optical trapping may be found elsewhere[6]. In an optical tweezer a trapped particle is held in space by a harmonic force, the force constant (known as 'trap stiffness') of which is a measure of the strength of the trap. In conventional method for calibrating trap stiffness, one measures the threshold velocity needed to detach the bead from the trap and calculates the force from Stokes' formula of viscous force. This requires a controllable piezo-stage on which the sample is mounted. The fluctuations of the particle within the trap are measured using a position detector (e.g. a quadrant photo-diode). This may require an additional laser beam to co-propagate with the original trapping beam. All these equipments add to the cost of the system which can easily be circumvented by using a pulsed excitation for calibration as described here. The method shows good agreement with the values measured by other methods.

In our experiment (Figures 1 and 2), we used a Ti : saph laser (Mira900-F pumped by Verdi5, Coherent). We operated the laser both in continuous (CW) and pulsed mode. We used ~150 fs pulsed excitation centred at 800 nm and having 76 MHz repetition rate for the latter case. For blanking the excitation, a rotating-disk optical chopper (MC1000A, Thorlabs) with 50% duty cycle (i.e. having 1 : 1 mark/space ratio) was introduced in the excitation path. The expanded laser light was sent to a home-built inverted microscope after passing through a pair of steering mirrors. The microscope consists of a tight-focusing oil-immersion objective (100× 1.4 NA Olympus). To get a clear bright field image we uniformly illuminated the sample by Köhler illumination instead of critical illumination by using a solitary condenser[7]. One can alternatively use a point source such as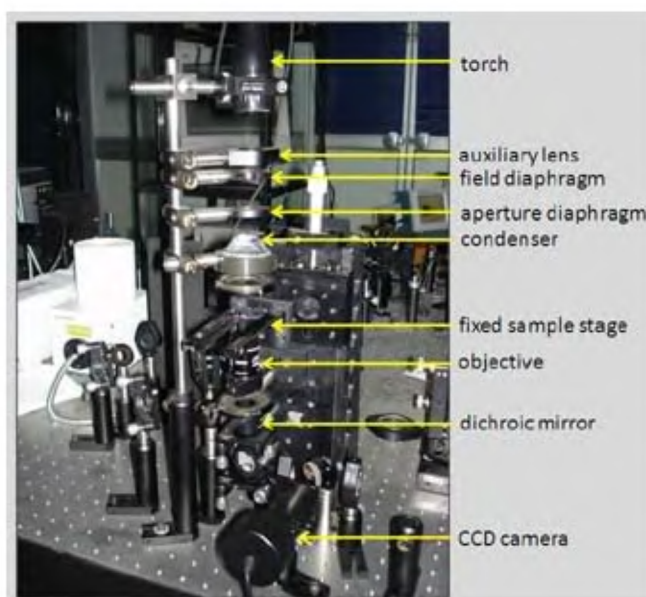 an optical fiber illuminator and use critical illumination[5]. We used dilute aqueous solution of (1 : 50 v/v) 4.1 micron sized fluorophore coated polystyrene microbeads (Molecular Probes Inc.) for trapping. The CCD camera (350 K pixels, e-Mark Inc.) was connected to a computer and the trapping events were directly monitored and recorded (Figure 3).

As mentioned earlier, a trapped bead executes oscillations due to the harmonic potential created by a tightly focused Gaussian laser beam. Since the ampli-

Figure 1. Schematic of the experimental set up. The laser excitation path is shown in dark red line and the bright field illumination and image forming path is shown as light blue line. Additional optical and electronic components required for conventional calibration (not present in the present set up) are shown as dashed green legends. The Köhler illumination scheme is shown in the inset.

tude of this motion (few nanometers) is very small, the trapped particle appears as a static entity. An optical chopper turns a CW input into pulsed excitation having alternative 'on' and 'off' light inputs. Since the chopper has a 50% duty cycle, decreasing the chopper frequency results in increase in both the pulse duration and the time lag between the pulses to the same extent. Now at high frequency, the trapped bead recognizes the excitation almost as a CW input. Starting from a

Figure 2. The experimental set-up.
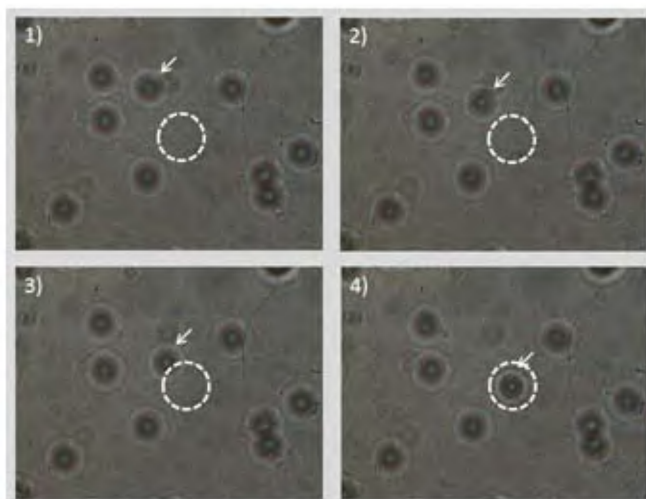
Figure 3. Gradual movement of a bead (indicated by a white arrow) towards the trap centre (shown white dashed circle).
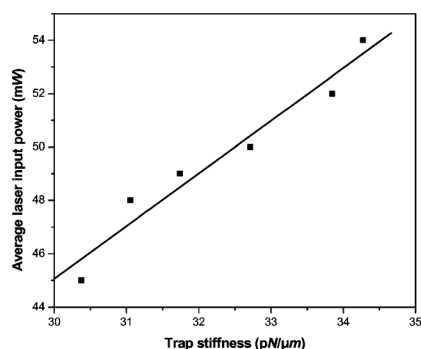
Figure 4. Plot of trap stiffness with average laser input power.

maximum blanking frequency at 5 kHz, we slowly decreased the frequency and monitored the process on the computer screen. We noticed that at a certain frequency the bead undergoes a rapid oscillation viewed as a sudden 'wriggling' motion on the monitor (as soon as the blanking frequency starts matching that of the oscillating frequency of the trapped bead); this occurs due to a well-known phenomenon described in classical mechanics: 'resonance'. Blanking introduces new frequencies which may match with the natural frequency of the system. This is resonance, found in forced (or driven) harmonic oscillator, that manifests itself as a 'blow-up' in the magnitude of amplitude[8]. The calculated trap stiffness was found to be ~30 pN/µm for

~50 mW input laser power; this is of the same order of magnitude as obtained by other methods.

At even further low frequencies, the dead time of the excitation becomes large compared with the time period for oscillation of the trapped bead. Therefore, the bead can easily come out of the trap and the trap is said to be 'unstable'.

When the laser was operated in pulse mode, the same trap stiffness values were observed as reported earlier[9]. Since in the pulse mode the laser repetition rate is several orders of magnitude higher than the blanking frequency, the effect is same as the CW mode; in other words, a pulsed excitation with ~100 MHz repetition rate can well be regarded as a quasi-continuous excitation[10].

The linear variation of the trap stiffness with input optical power is shown in Figure 4; this is a consequence of the linearity of Maxwell's equation and the equations for the transverse scattering (i.e. gradient) force[11]. This also shows the validity of the present calibration method.

Thus we show how intelligent illumination results in faster and cheaper calibration methods for an optical tweezer. The measured trap stiffness also agrees well with the typical values measured by standard methods. The work reflects how simple ideas borrowed from a well-known phenomenon in classical physics can be implemented to extract dynamical information within an optical trap.

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