Measuring the complete force field of an optical trap

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The use of optical traps to measure or apply forces on the molecular level requires a precise knowledge of the trapping force field. Close to the trap center, this field is typically approximated as linear in the displacement of the trapped microsphere. However, applications demanding high forces at low laser intensities can probe the light-microsphere interaction beyond the linear regime. Here, we measured the full nonlinear force and displacement response of an optical trap in two dimensions using a dual-beam optical trap setup with back-focal-plane photodetection. We observed a substantial stiffening of the trap beyond the linear regime that depends on microsphere size, in agreement with Mie theory calculations. Surprisingly, we found that the linear detection range for forces exceeds the one for displacement by far. Our approach allows for a complete calibration of an optical trap. © 2011 Optical Society of America

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Optical traps are widely used to probe forces on the piconewton level or to measure displacements down to a few angstrom, for example in biological systems [1,2]. The application of an optical trap as an accurate force and position sensor crucially depends on the detection and calibration method used. A sensitive detection method, back-focal-plane interferometry [3], monitors alterations of the light field due to a trapped microsphere on a position-sensitive detector. Two physical quantities are inferred from this detector signal: the displacement of the microsphere with respect to the center of the trap and the optical forces the microsphere experiences. To precisely set the scale of these quantities in units of force and length, one typically performs a thermal calibration. Here, thermal fluctuations of the microsphere around the center of the optical trap are measured and compared with theories of Brownian fluctuations in a confining harmonic potential [4,5]. Although the assumption of a linear force field with constant trap stiffness, κ, holds true in the vicinity of the trap center, this linear force–displacement relation breaks down at larger displacements. Exact mapping of the complete optical force field is not only necessary to determine the validity of the linear approximation, but enables the use of the full force range of an optical trap.

Here we report on a procedure to characterize the interaction between the trapped microsphere and the trapping laser in terms of accurate force and position measurements in two dimensions. We use a strong thermally calibrated trap in its linear operating regime as a precise sensor of force (and position) for characterizing a weaker, uncalibrated trap of interest. Note that this differs qualitatively from a pure detection laser, which senses position but not force. Our approach extends preceding studies [6–8] and can be readily implemented in dual-beam optical traps. Neither DNA tethers [6] nor extra instrumentation such as additional lasers [7] or a laminar flow system [8] are required. We observe force–displacement relationships that depend on microsphere size and that are in excellent agreement with numerical Mie theory calculations [9]. Thus, our method allows for an absolute calibration of high-resolution optical traps.

To measure the landscape of the forces exerted by one optical trap, we used a dual-beam optical trap apparatus (Fig. 1) based on an instrument previously described [10]. Here the positions and intensities of two optical traps, separated by polarization, can be independently adjusted [11]. While keeping the strong, thermally calibrated [4] trap, T_C, stationary, we scanned the weaker, to-be-analyzed trap, T_A, in 10 nm steps over the whole microsphere-interaction regime. At each step—with stationary traps and measurement times long enough to average over thermal fluctuations—the balance of

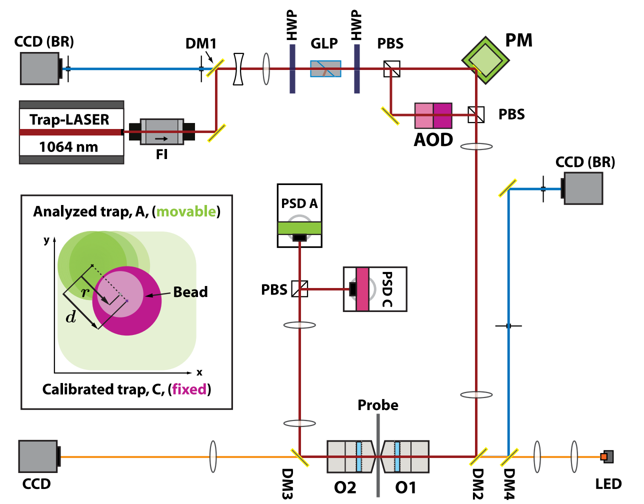

Fig. 1. (Color online) Setup: beam path, starting at Trap-LASER (red), back reflection path, ending at CCDs (blue), imaging path, from LED to CCD (orange). FI, Faraday isolator; DM1–DM5, dichroic mirrors; HWP, half-wave plate, GLP, Glan–Laser polarizer; PBS, polarizing beam splitter; AOD, acousto-optical deflector; PM, piezo mirror; O1 and O2, water immersion objectives (NA = 1.20); PSD C and PSD A, position-sensitive devices; CCD (BR), camera-to-monitor back-reflections [11] Inset: schematic of the experiment. **d**, intertrap distance; **r**, displacement of microsphere from center of T_A.

the optical forces yields $\langle \mathbf{F}_C(\mathbf{r})\rangle_{t_{av}} = -\langle \mathbf{F}_A(\mathbf{r})\rangle_{t_{av}}$. This allows for measuring the force–displacement relation of the trap of interest. To never leave the linear operating regime of the calibration trap, we adjusted the relative intensities in both traps such that T_C had at least a five times higher trap stiffness than T_A. Under these conditions, we have

$$\hat{\kappa}_{\mathrm{C}}\langle \mathbf{r} - \mathbf{d}\rangle_{t_{av}} = \langle \mathbf{F}_{\mathrm{A}}(\mathbf{r})\rangle_{t_{av}}, \quad (1)$$

where the diagonal matrix $\hat{\kappa}_C$ contains the trap stiffness of T_C in the x and y directions. The distance vector between the two traps is denoted by $\mathbf{d}$ and is changed by scanning T_A. Allowing the microsphere to relax to its new equilibrium position, we sampled the complete optical force profile for arbitrary displacement $\mathbf{r}$ relative to the center of T_A. In all experiments, the microsphere displacement in T_C was less than 150 nm and 100 nm in the lateral and axial directions, respectively. This ensured the validity of the linear force–displacement approximation for T_C to within 5% (see below). The averaging time at each grid point (10 ms) was longer than the characteristic equilibration time (<5 ms). Microsphere sizes were measured to within 5% accuracy using a combined drag-force-power-spectral-analysis method [5].

We determined a two-dimensional (2D) map of the optical forces exerted by T_A on a polystyrene microsphere of diameter 1.26 μm [Fig. 2(a)]. The net force, obtained by combining the parallel (F_x) and perpendicular (F_y) force components relative to the trap polarization, demonstrates that for this microsphere size the optical forces are nearly radially symmetric [Fig. 2(b)] as expected [12]. We therefore restrict our remaining discussion to cross sections of the force map in x [dashed line in Fig. 2(c)]. Results for the other directions are qualitatively the same.

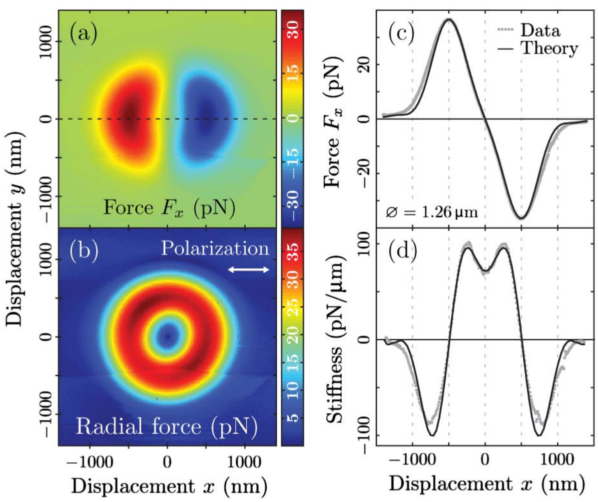

Fig. 2. (Color online) 2D maps of (a) optical forces on a 1.26 μm microsphere in the direction of polarization, (b) magnitude of radial force $|\mathbf{F}| = \sqrt{F_x^2 + F_y^2}$. Force magnitudes are color-coded by corresponding heat maps. (c) Complete force response along the polarization axis [dashed line in (a)]. (d) Numerical differentiation, $\kappa(x) = -\partial_x F(\mathbf{r})$, yielded trap stiffness with respect to microsphere displacement.

We asked whether the measured force maps are consistent with predictions based on Mie scattering. Therefore, we fit the experimental data using numerical calculations based on the T matrix method [9]. We found that data and theory are in excellent agreement [Fig. 2(c)]. Compared to the theoretical force profile, the experimental one is slightly broader beyond the extrema. We attribute this difference to diffraction effects at the back aperture of the trapping objective not directly taken into account by this theoretical model [9].

Close to the origin, a constant trap stiffness—assuming Hooke's law—is expected. However, numerical differentiation of the measured force curve (Savitzky–Golay filtered, fourth order, 400 nm filter width) proved that the trap stiffness continuously deviated from its value at the origin, $\kappa_0 = 72 \pm 3\,\mathrm{pN}/\mu\mathrm{m}$ [Fig. 2(d)]. Displacing the microsphere from the center, the trap stiffness increased moderately within the first 300 nm toward a maximum, before it fell off and eventually became negative. In this region, the analogy between optical traps and mechanical springs fails; the trap stiffness is negative for a decreasing yet still restoring force. Note that the stiffening effect was substantial even for small displacements. A displacement from the trap center of 250 nm already leads to a deviation of the trap stiffness of more than 30% as compared with its value at the origin, which would be probed by thermal calibration. Thus, without any preliminary assumptions about the trap of interest (T_A), we measured its full force field and analyzed the validity of the linear force-displacement approximation.

A distinct second linear regime of higher constant trap stiffness was recently reported for 2.01 μm microspheres [8]. To study the microsphere size dependence of the observed stiffening effect in more detail, we use our setup to compare the 1.26 μm microspheres with larger ones of diameters 2.01 μm and 2.40 μm in Figs. 3(a) and 3(b). Indeed, the stiffening was more pronounced for larger microspheres and depended sensitively on their exact size. Figure 3(b) shows that the measured stiffness landscapes are complex, displaying ripples yet no extended linear regime. Importantly, these nonlinear effects are well described by Mie theory calculations [9]. Therefore, our approach allows for a detailed investigation of these optical phenomena and could, for example, be used to verify that this nonlinear behavior near the trap center is less apparent when using Rayleigh scatterers [13].

Next, we used our assay to evaluate the accuracy of the back-focal-plane detection method [3]. This method infers both the displacement and the force from a single differential voltage signal on a position-sensitive photodetector. For small displacements, both measures are well approximated as linear functions of the differential voltage signal. For larger displacements, we found that the linear force-displacement relation broke down [Figs. 2(c) and 3(a)]. However, it remains elusive to what extent each single quantity (force or displacement) can still be accurately described by a linear dependence of the detector signal. To address this, we correlated the calibrated force and displacement signals of the strong trap with the detector signal of the weak trap. As depicted in Figs. 3(c) and 3(d), for microspheres of diameter 2.01 μm, assuming a linear relationship between force and voltage signal is correct to within $\pm 5\%$, even for very large

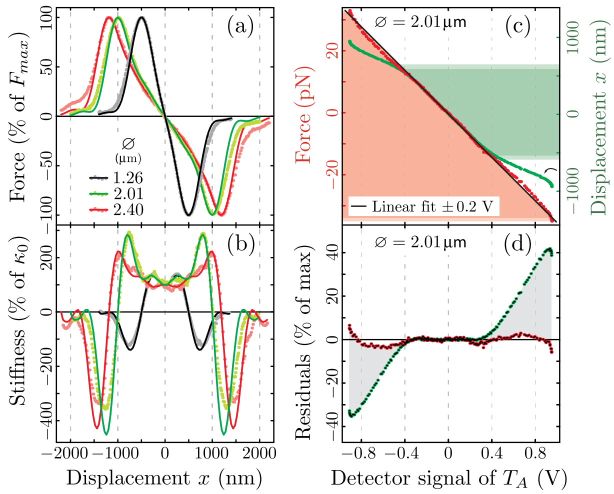

Fig. 3. (Color online) Data (points) and theory (lines) of (a) normalized force-extension curves and (b) thereof-derived trap stiffnesses for indicated microsphere sizes. For experimental purposes, different microspheres were investigated with different laser intensities; $F_{\rm max}:(37, 35, 48)\,{\rm pN}$; κ_0:(72,22,30)pN/μm for microspheres of diameter $(1.26, 2.01, 2.40)\,\mu$m, respectively. (c) Comparing forces (dotted line, red) and displacements (dashed line, green) to the detector signal of T_A for a 2.01 μm microsphere. Shaded areas indicate where residuals [see (d)] of linear fit are less than 5% (dark) or less than 10% (bright). (d) Force (dotted line, red) and displacement (dashed line, green) residuals of the fit in (c) normalized to $F(V_{\rm max}) = 33\,{\rm pN}$ and $x(V_{\rm max}) = 950\,{\rm nm}$, respectively.

displacements close to the force maximum. On the other hand, inferring microsphere position from the same voltage signal—again assuming linearity—leads to significantly larger errors of up to 40% [Fig. 3(d)]. To conclude, an optical trap with back-focal-plane detection is foremost a sensor of force and not of position [3]. Positional information is inferred from a linear approximation of the optical force field, which does not hold for large microsphere displacements [Figs. 2(c) and 3(a)]. These findings have implications for detection methods that infer force from microsphere position, such as high-speed videomicroscopy [14]. Without a full characterization of the force field, accurate force measurements are in this case limited to the range where the linear force–displacement relation holds.

In summary, we have shown how to measure the complete 2D force field of an optical trap with a dual-beam optical trap setup. A strong calibration trap in its linear regime acts as an accurate force sensor. An extension of this procedure into three dimensions by varying the relative collimation of T_A is possible [15]. Our calibration strategy directly addresses the difficulties associated with inferring physical quantities from optical tweezers measurements that are based on the assumption of a linear trapping force field. The treatment of optical tweezers as springs is an approximation, valid only close to the trap center, and its validity depends sensitively on the size of the trapped object. By reporting a simple calibration scheme for absolute forces and displacements in an optical trap, we provide a robust means for the study of the complete light–microsphere-interactions for accurate tweezers measurements beyond the linear regime.

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