We report an improved method for calibrating the nonlinear region of a single-beam gradient optical trap. Through analysis of the position fluctuations of a trapped object that is displaced from the trap center by controlled flow we measure the local trap stiffness in both the linear and nonlinear regimes without knowledge of the magnitude of the applied external forces. This approach requires only knowledge of the system temperature, and is especially useful for experiments involving trapped objects of unknown size, or objects in a fluid of unknown viscosity. Several prior nonlinear trap calibration techniques have been reported [8–10]. One approach used a dual beam optical trapping system to hold two particles connected via a polymer tether between a stiff trap, which had a well-known linear stiffness, and a soft trap, whose full nonlinear force profile could be determined [9]. A second method also employed a dual beam trap, again with one calibrated stiff trap and a second, weaker trap that was raster scanned over the interaction area of the trapped microsphere and the strong laser. This allowed a single particle to interact with both traps, permitting the nonlinear regime of the weaker trap to be explored [10]. In both approaches, only the soft trap was fully calibrated, while the stiff trap was operated exclusively in its linear regime in order to apply controlled forces. This prevents the experimenter from directly measuring the highest possible forces using the available lasers.

An alternate method for probing the nonlinear range of the optical trap is to impose an external drag force on the trapped particle by moving the microscope stage (and therefore the fluid surrounding the particle) at constant speed. If the particle drag coefficient is known, it is possible to estimate the drag force and establish the force profile of the optical trap; this is commonly referred to as the “Stokes’ drag method” of calibration [11]. Although in principle the particle can be displaced into the nonlinear region of the optical trap by flow, calibration of this regime is not typically performed due to challenges in quantifying the external drag force, which depends on a number of factors through the particle’s drag coefficient, including the exact particle size, the height of the particle above the sample chamber surface, and the viscosity of the surrounding media, which are difficult to measure accurately. Rather, the Stokes’ drag method is used to approximate the spatial extent of the linear region of the optical trap, and to provide an independent confirmation of the linear stiffness, which is then compared to that measured by other established methods that rely on thermal fluctuations of the particle only [1]. All existing nonlinear calibration methods have the drawback that they require the application of controlled, pre-determined forces.

In this Letter, we report an improved method that allows calibration of the full nonlinear range of the optical trap without knowledge of the magnitude of the applied external forces. By analyzing the position fluctuations of a trapped particle while its average position is displaced from the center by an (unknown) external force, we obtain the local value of the trap stiffness.

Optical traps allow the precise application of forces to soft and biological materials [1]. Many biophysics applications, including most single-molecule measurements of motor proteins and biopolymers, require forces in the range of one to a few tens of picoNewtons (pN) of force, which is easily achieved using a standard single-beam gradient optical trap. However, higher forces are often desired, e.g., to study collective behavior of multiple motor proteins [2–4], activate mechanotransduction pathways in cells [5], or probe the viscoelastic properties of cytoskeletal networks and other stiff polymeric materials [6]. In principle, it is possible to increase force by increasing the laser power, objective lens numerical aperture, or index of refraction of the trapped object. In practice, even after these inputs are optimized, the application of forces >100 pN remains challenging.

An alternate way to increase the applied force is to increase the offset distance between the center of the trapped object and the center of the focused laser beam. Experimenters often limit this distance to <100 nm to ensure that the particle remains within the linear range of the optical trap, where the trapping potential can be reasonably approximated as a harmonic well. In this regime, a single value of the trap stiffness (linear stiffness) is used to relate offset distance and applied force, and numerous well-established calibration methods exist to determine its value [1]. However, expanding the calibration into the nonlinear range of the trap allows a substantial force increase without the need for new instrumentation or exotic particle types [7].
stiffness at that displaced position. Repeating this procedure for different positions covering the full range of the trap, we obtain the full, nonlinear trap force profile. To verify the accuracy of this approach, we compare our results to those obtained using the Stokes’ drag method and show that we can properly calibrate the stable nonlinear range without the need for a second optical trap, and without knowledge of the applied external force.

The key concept of our approach is to shift the average position of the particle in the trap using a constant external force (e.g., a fluid drag force) and then record the fluctuations of the particle around this position [Fig. 1(a)]. It is well-known that the thermal fluctuations of a particle in the center of the trap provide a direct readout of the linear trap stiffness [1]. By a similar principle, if the amplitude of a particle’s fluctuations about its shifted position is small compared to the spatial extent of the stable trapping range, then these local fluctuations provide a measurement of the local trap stiffness at that position [Figs. 1(b) and 1(c)]. The spatial resolution of the measurement of local stiffness is determined by the standard deviation of the distribution of particle displacements at that position. For applications requiring large trap forces and therefore stiff traps, the typical amplitude of the fluctuations around an equilibrium position is 5–10 nm. In this case, we measure the trap stiffness with a spatial resolution of 10–20 nm, much smaller than the typical trap range of 300 nm. Because the stiffnesses are measured directly from the distribution of the particle’s positions, it is not necessary to know the magnitude of the constant external force that is applied. This is particularly useful when constant fluid flow is used to displace the particle because the exact drag coefficient need not be determined.

To describe the distribution of the particle’s fluctuations when its position is shifted by an external force, we write the Fokker–Planck equation for the probability distribution $\rho(x, t)$ of finding the particle at position $x$ at time $t$, namely $\partial_t \rho + \vec{v} \cdot \nabla \rho - \frac{\kappa}{2} \nabla^2 \rho = 0$. The probability flux $\vec{j}$ is given by $\vec{j} = \dot{x} \rho(x, t) - \nu \dot{x} \rho(x, t)$, where $\nu$ is the diffusion constant of the particle and $\dot{x}$ is the net particle velocity caused by the external forces. Although it is possible to use a variety of methods to apply forces on the particle and shift its position, in our particular experimental setup we apply a drag force on the particle by moving a feedback-controlled nanopositioning stage at constant velocity $\vec{v} = \nu \hat{\xi}$. The net motion of the particle is determined by the balance of forces acting on it. Given that the particle’s motion is overdamped, force balance on the particle reads

$$-\xi (\dot{x} - \nu) + F_{\text{trap}}(x) = 0,$$

where $\xi$ is the drag coefficient of the particle and $F_{\text{trap}}(x)$ is the position-dependent trap force.

At equilibrium, the net velocity of the particle vanishes and the trap force balances the drag force on the particle. The equilibrium average particle position, $x^*$, is given implicitly by Eq. (1), namely $\xi \nu + F_{\text{trap}}(x^*) = 0$. Once the equilibrium particle position is known, we obtain the probability distribution of the particle position fluctuations around the equilibrium position by integrating the Fokker–Planck equation. If the amplitude of the fluctuations in the particle position around the average position $x^*$ is small compared to the spatial range of the trap (Fig. 1), the trap force around $x^*$ can be approximated by $F_{\text{trap}}(x) \approx F_{\text{trap}}(x^*) - \kappa(x^*) (x - x^*)$, where the local trap stiffness $\kappa(x^*)$ is defined as positive and corresponds to the derivative of the trap force at $x = x^*$: $\kappa(x^*) \equiv \lim_{x \to x^*} F_{\text{trap}}(x) / (x - x^*)$. Using the linear expansion of the trap force around the average particle position $x^*$, as well as the Einstein relation $\nu = k_B T / \xi$, the equilibrium probability distribution of the particle position, $\rho_{\text{eq}}(x)$, reads

$$\rho_{\text{eq}}(x) = \sqrt{\frac{2\pi k_B T}{\kappa(x^*)}} \exp \left[ -\frac{\kappa(x^*)}{2k_B T} (x - x^*)^2 \right].$$

The amplitude of the fluctuations around the equilibrium position $x^*$ is given by the standard deviation $\sigma(x^*)$ of the equilibrium distribution Eq. (2) and reads

$$\sigma(x^*)^2 = \frac{k_B T}{\kappa(x^*)}.$$

Therefore, the local trap stiffness $\kappa(x^*)$ at $x^*$ can be directly obtained from the amplitude of the particle fluctuations around $x^*$ Eq. (3). It should then be possible to obtain the local stiffness $\kappa(x^*)$ at every point of the trap by simply shifting the equi-

![Fig. 1.](image-url)
librium position of the particle and measuring the distribution of the particle’s position fluctuations around the shifted position. Once the local trap stiffnesses have been measured at several positions covering the full nonlinear range of the trap, and knowing that the trap stiffness is defined by $dF_{\text{trap}}/dx \equiv -\kappa$, the full nonlinear trap force profile, $F_{\text{trap}}(x)$, is given by

$$F_{\text{trap}}(x) = -\int_x^0 \kappa(x')dx' + C,$$

where $C$ is an integration constant set by the condition $F_{\text{trap}}(x = 0) = 0$.

To experimentally test this approach, a single-beam gradient optical trap was formed by focusing a 1064 nm, 5 W Nd:YAG laser (Spectra-Physics) through a high numerical aperture objective lens (Nikon, NA = 1.49). A separate, low-power, non-trapping 830 nm laser (Melles Griot) and a position sensitive detector (PSD) (Pacific Silicon Sensors Inc.) were used to detect the displacement of the trapped particle from the center of the detection beam [12]. The voltage signals from the PSD were normalized by total light intensity using custom-built electronics and were calibrated by raster scanning the trapped particle over a matrix of known positions using a pair of acousto-optic deflectors (IntraAction) [12]. The radius of the calibrated detection region was typically >300 nm. The output signals were sampled at 100 kHz and low-pass filtered using a programmable filter (Krohn-Hite) at the Nyquist frequency. A sample chamber was created by placing two strips of double-sided tape on a slide, and then securing a #1.5 coverslip on top. A high-viscosity mixture of glycerol, water, and polystyrene beads with a mean diameter of 1.1 ± 0.035 μm (Invitrogen), was flowed into the chamber, and the ends of the chamber were sealed with vacuum grease.

A constant fluid drag force was applied to a trapped bead by commanding a precision piezoelectric nanopositioning stage (Physik Instrumente) to execute a step change in the stage velocity from zero to a constant value, typically in the range of 10 to 300 μm/s, which was then maintained for the full range of motion of 100 μm. Upon application of the drag force, the average bead displacement increased before reaching an asymptote at position $x^*$, where the drag force and restoring trap force balanced [Fig. 2(a)]. Typically 40,000 to 200,000 samples of the equilibrium position of the particle were recorded over time, and their equilibrium distribution $\rho_{eq}(x)$ was fitted with a Gaussian function. The variance of the distribution, $\sigma(x^*)$, was then used to calculate the local trap stiffness, $\kappa(x^*)$, as given by Eq. (3). The experiment was repeated for a range of velocities, giving rise to the spatial profile $\kappa(x)$ [Fig. 2(b)]. The full nonlinear trap profile, $F_{\text{trap}}(x)$, was then obtained by numerical integration of the measured spatial profile, $\kappa(x)$ Eq. (4), and is shown in Fig. 3.

To validate our approach and confirm the accuracy of this new method of calibration, we independently measured the force profile of the trap using the Stokes’ drag method. To do so, the same displacement data were used, but the fluid drag forces were directly calculated using Stokes’ law, $F_{\text{drag}} = 6\pi \eta v_s$, and plotted against the mean positions of the bead under the constant external force, allowing us to obtain the spatial force profile directly. Force profiles obtained by the traditional Stokes’ drag method, and by our new method are compared in Fig. 3, and show good agreement over the full force range. By calibrating the nonlinear range of the trap, the experimenter can take advantage of a higher maximum trap force, as well as an extended displacement range. As shown in Fig. 3, we measure an

![Fig. 2.](image-url)
particular, the solution viscosity may be sensitive to temperature through regions of varying temperature or surface roughness. In the experiment, which may not be the case as the bead is moved together. We do require an estimate of the system temperature to no knowledge of the drag coefficient, avoiding these issues altogether. We require an estimate of the system temperature to calculate $k_B T$, however this is fairly insensitive to temperature fluctuations. With our new method, a change from 20°C to 25°C (293 to 298 K) results in only a 2% uncertainty in the stiffness measurement; by contrast, the same 5°C change causes a 12% change in the viscosity of water corresponding to a 12% uncertainty in the forces calculated in the Stokes’ drag method. These viscosity changes can be even larger in other fluids, e.g., glycerol.

In summary, the method we describe allows robust measurement of the nonlinear force profile of the trap and allows the experimenter to easily and accurately extend the force and displacement range of an existing single-beam optical trap. Direct measurement of the entire force profile avoids extrapolation of the linear range past its valid boundary and can account for minor asymmetries in the trapping beam. In contrast to prior methods, our approach does not require independently calibrated forces or knowledge of the fluid viscosity, the particle radius, and height of the bead above the surface, considerably simplifying the calibration. Our method works best when the local trap stiffness is reasonably high, and therefore the extent of the particle fluctuations $\sigma(x')$ is small, providing good spatial resolution of the trap force profile. Although a stiff trap provides good spatial resolution of the force profile, in this regime, the amplitude of the fluctuations from background sources (i.e., mechanical vibrations and electronic noise) can compete with that of the desired particle fluctuations, so care must be taken to identify and eliminate possible sources of undesired fluctuations for best results. This approach can be extended to three dimensions (3D) by measuring the 3D fluctuations in the particle’s position and relating these to the local 3D force profile, allowing the 3D nonlinear calibration of the full range of an optical trap.

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**REFERENCES**