Calibration of optical traps by dual trapping of one bead

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We introduce a method for optical trap calibration that is suitable for viscoelastic material. The method is designed for use on experimental setups with two optical tweezers and is based on pulling a trapped particle with one trap while holding it with the other. No piezo stage is needed, and only one optical trap must be movable with galvo mirrors, piezo mirrors, or acousto-optical defectors. The method combines advantages of commonly known PSD-fitting and fast-sweeping methods, allowing calibration of a completely fixed trap in a fluid of unknown viscosity/viscoelasticity. A detailed method description, a theoretical derivation, and an experimental comparison to other methods are reported. © 2013 Optical Society of America

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While micro rheology [1] is becoming increasingly popular in biophysics [2,3] and polymer science [4], new methods of particle tracking with a wide frequency range are required for capturing effects of medium viscoelasticity [5], compressibility [6], fluid and particle inertia [7], and so on. The most common methods of micro rheology are video particle tracking (VPT) [8] and laser scattering (LS) [9]. Studying multicomponent viscoelastic fluids, such as bio-networks, polymer solutions, and micelles, where structure size might be comparable to probe size, requires active and/or two-point micro rheological experiments. Optical tweezers [10] (also known as optical trap/laser tweezers/laser particle tracking) have the frequency range of LS methods, the analysis simplicity of VPT methods but also allow implementation of active [11] and two-point micro rheological techniques [12]. The optical trapping phenomenon relies on the momentum conservation principle: a focused laser beam is deflected while passing through the bead and therefore gives the bead momentum toward the center of the trap [10]. The beam can be collected and projected on a quadrant photodiode (QPD), and the output voltages would contain the information about the bead’s displacement from the center of the trap, and forces acting on the bead.

Converting detector signals to particle displacement and force acting on the particle requires the calibration of two trap parameters: sensitivity and stiffness. Sensitivity [V/m] is the relation between the signal and the displacement of the particle from the center of the trap. Stiffness is the relation between the force pulling the particle toward the center of the trap and the displacement of the center of the trap. Both the sensitivity and stiffness depend on several experimental parameters and might need to be recalibrated every time one changes the optical density of the media or the particle, size of the particle, distance from the coverslip, sample chamber size, laser beam power, etc. [13].

Several methods for trap calibration exist [14–22]. However, most methods are based on fitting an experimental power spectral density (PSD) to a theoretically calculated one, which requires having a viscous fluid with known viscosity. There are methods suitable for viscoelastic fluids, but with certain limitations. A recent method by Vermeulen et al. [19] is based on moving the trap across the probe sufficiently fast that inertia and drag make it effectively fixed in space. However, fast-acting acousto-optic defectors (AODs) are required for this method, adding extra cost to an experimental setup and sometimes introduces high-frequency noise due to amplifiers and frequency generators. A method described by Visscher and Block [20] assumes the presence of an additional fixed laser of very small intensity used for position detection only. That beam must be realigned every time the trap is moved. Allersma et al. measured sensitivity by fixing a particle to a coverslip and moving the whole stage across the trap waist [14]. Even though this method is very common, its precision is questionable—aberration, caused by the proximity of the glass coverslip, might introduce significant error [13]. Also, a piezo-electric stage is required. The most recent method by Shindel et al. [22] simultaneously combines two calibration methods [17,19] with viscoelasticity theory in order to determine trap stiffness and material properties simultaneously; however, determination of trap sensitivity was not addressed.

In this letter, we introduce a new method of trap calibration (i.e., determination of sensitivity and stiffness), which is suitable for simultaneous calibration of two independent optical traps (including one possibly fixed trap) and does not require AODs, a piezo-electric stage or a Newtonian fluid with known viscosity.

The idea behind most sensitivity calibration methods is to create a known displacement of the bead from the center of the trap and measure the change in output signal. Our proposed method relies on detecting one bead near two traps of known rate-of-change of separation. We then use averages and fluctuations of signals from both traps, exploiting the fact that the underlying signals come from the same bead.

We first place the traps such that they are nominally overlapping and then move one trap for a known distance (or with known velocity) [Fig. 1(a)]. If the distance is sufficiently small (usually, 20% to 30% of the bead diameter) such that the bead is still in the linear mode of both
traps [Fig. 1(d)], one can calculate displacement of the bead from the center of each trap knowing the trap stiffness ratio. In fact, in the case when both traps are equally stiff, the average displacement of the bead would be exactly one half of the distance the trap has been moved. Therefore, desired sensitivity would be equal to the slope of the signal/displacement plot [Fig. 1(d)] multiplied by 2.

In the more general case of traps having different stiffness, we must additionally measure the variance of thermal fluctuations of the bead in each trap [Figs. 1(b), 1(c) and Figs. 1(c), 1(d)] to account for an additional degree of freedom in the system.

Consider first the 1D case with one fixed trap, $x^f(t)$, and one movable trap, $x^m(t)$, which starts moving in the positive $x$ direction at time $t = 0$ with velocity $v_x^m$. Throughout all derivations, we assume that (1) the traps are sufficiently close that $x^f(t) - x^m(t) < 0.3D$ ($D$ is the diameter of the trapped bead), and we can assume a Hookean force law for each trap; (2) trap motion is sufficiently slow, so that the average drag force exerted by the fluid on the bead is negligible. Note that as far as assumption (1) holds, more precise knowledge of trap positions or trap separation is not required. The only important parameter is the velocity of the trap movement $v_x^m$.

We express the QPD voltages as

$$V^i_x(t) = c^i_x[x_b(t) - x^i(t)] + d^i_k + \delta V^i_x(t),$$

where $i = f, m$ is the trap index of the fixed or movable trap, respectively, $x^i_b(t)$ is bead position, $c^i_x$ are the (desired) sensitivities, $d^i_k$ are constant offsets due to optical system alignment, and $\delta V^i_x$ are signal fluctuations from electronic noise.

We estimate the slopes of the QPD signals [Fig. 1(d)]

$$\alpha^i_x = \frac{1}{\nu^m_x} \frac{d\delta V^i_x(t)}{dt},$$

where overbar means short-time average (see supplementary materials for all detailed derivations and definitions). Then, exploiting the Hookean law, we express the slopes as

$$\alpha^f_x = \frac{c^f_x k^m_x}{k^f_x + k^m_x}; \quad \alpha^m_x = -\frac{c^m_x k^f_x}{k^f_x + k^m_x}.$$

where $k^f_x$ and $k^m_x$ are the (desired) stiffnesses of the fixed and movable traps, respectively.

Using the second moment of Brownian bead displacement in a harmonic potential $\langle \delta x(t)^2 \rangle = k_BT/k$ (where $k_BT$ is the thermal energy and $k$ is the spring constant), we express variances $\sigma^i_x$ of QPD voltages $V^i_x$ as

$$\langle \sigma^i_x \rangle^2 = \left(\frac{c^i_x k^m_x}{k^f_x + k^m_x}\right)^2 \frac{k_BT}{k^m_x}.$$ 

Now, solving Eqs. (3) and (4) together, we get

$$\begin{align*}
\sigma^f_x &= \left(\frac{c^f_x}{\nu^m_x}\right)^2 \frac{\langle \delta x^m(t)^2 \rangle}{\langle \delta x^m(t)^2 \rangle} + \left(\frac{\alpha^f_x}{\nu^m_x}\right)^2 \left(\frac{c^m_x}{\nu^m_x}\right)^2 \\
\sigma^m_x &= -\left(\frac{c^f_x}{\nu^m_x}\right)^2 \left(\frac{\alpha^f_x}{\nu^m_x}\right)^2 + \left(\frac{\alpha^m_x}{\nu^m_x}\right)^2 \left(\frac{c^m_x}{\nu^m_x}\right)^2 \\
k^f_x &= k_BT \left(\frac{1}{\nu^m_x}\right)^2 \frac{\langle \delta x^m(t)^2 \rangle}{\langle \delta x^m(t)^2 \rangle} + \left(\frac{\alpha^f_x}{\nu^m_x}\right)^2 \left(\frac{c^m_x}{\nu^m_x}\right)^2 \\
k^m_x &= k_BT \left(\frac{1}{\nu^m_x}\right)^2 \frac{\langle \delta x^m(t)^2 \rangle}{\langle \delta x^m(t)^2 \rangle} + \left(\frac{\alpha^m_x}{\nu^m_x}\right)^2 \left(\frac{c^m_x}{\nu^m_x}\right)^2.
\end{align*}$$

To apply the described method, one needs to perform three independent experiments [Figs. 1(a)–1(c)] while recording QPD signals [Figs. 1(d)–1(f)]. Then, by using linear fitting and calculation of standard deviation, one can gain enough data to calculate sensitivities and stiffnesses using Eq. (5).

In our experiments, we used the commercially available optical tweezers setup called NanoTracker (JPK Instruments, Berlin, Germany) with a 3 W 1064 nm laser, split into two traps by polarization [23]. For the following experiments, Trap 1 was fixed (no steering), and Trap 2 had either galvo mirrors or AODs installed for steering.

For comparison with the PSD-fitting calibration method and the fast-sweeping method, experiments were performed in water (Fig. 2). 1.53 µm polystyrene spheres (Polybead Polystyrene Microspheres, mean diameter 1.53 µm, standard deviation ±5%, Polysciences, Inc., Warrington, Pennsylvania) were used as probes. Experiments were performed at room temperature (24°C).
Please refer to supplementary materials for a detailed description of the experimental procedures.

To demonstrate performance of the dual trapping method, we first validate it by comparing with the PSD-fitting method \[^{15}\] and the fast-sweeping method \[^{19}\] in water (Fig. 2) and then in a viscoelastic solution of polyacrylamide in water (2% w/w, 5–6 MDa, Sigma-Aldrich, Co., St. Louis, Missouri) (Fig. 3), where the PSD-fitting method could not be used. Note that the fixed trap can be calibrated in a viscoelastic fluid (Fig. 3) with the dual trapping method only.

One can notice that even though all methods agree within uncertainty, the fast-sweeping method systematically shows lower values of sensitivity by about 5%–10%. The fast-sweeping method relies on inertia to prevent the bead from following the trap. However, this assumption is expected to be better for the more viscous PAM solution than for DI water.

Uncertainty of the PSD-fitting method is 20%, according to JPK Instruments AG. However, we suspect that rigorous error propagation might yield smaller values. Uncertainty of the fast-sweeping method is 5%, according to the authors \[^{19}\]; however, it does not seem to include systematic error of the bead following the trap. We estimated the actual uncertainty for the dual trapping method by calculating uncertainties in fit parameters using linear regression and \(\chi^2\) methods \[^{24}\] and then propagating uncertainties through Eq. (5). See supplementary materials for details on propagation of uncertainty.

We introduce a new method of optical trap calibration, designed for use in viscoelastic fluids and capable of precise calibration of fixed and movable traps simultaneously without using AOD or a piezo-actuated stage. Our method shows good agreement with the PSD-fitting \[^{15}\] and the fast-sweeping \[^{19}\] methods in both viscous and viscoelastic fluids.

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