Note: Three-dimensional linearization of optical trap position detection for precise high speed diffusion measurements

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Studies of the details of Brownian motion, hydrodynamic of colloids, or protein diffusion measurements all require high temporal and spatial resolution of the position detector and a means to trap the colloid. Optical trap based thermal noise imaging employing a quadrant photodiode as detector provides such a method. However, optical trapping requires an objective with high numerical aperture resulting in highly nonlinear position signal and significant cross-dependence of the three spatial directions. Local diffusion measurements are especially susceptible to distance errors. Here, we present a position calibration method, which corrects nonlinearities sufficiently to allow precise local diffusion measurement throughout the entire trapping volume. This correction permits us to obtain high-resolution two- and three-dimensional diffusion maps. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4890384>]

Optical traps widely use back focal plane mounted quadrant photo diodes (QPD) to monitor the position of a trapped probe with nanometer spatial and microsecond temporal resolution. The method utilizes the interference between light scattered of the probe and unscattered laser light (Fig. 1). The probe's relative lateral position is measured as difference signal between the detector halves,¹ while the relative axial position is determined from the total laser intensity.² However, for probes smaller than the laser focus and position deflections larger than a few nanometers the QPD signal is highly nonlinear and position sensitive. In particular, when off-axis position measurements are analyzed to track protein motion or map spaces within the trapping volume, the cross-dependence of the directional position sensing interferes with the result.

Thermal noise imaging (TNI) exploits the Brownian thermal position fluctuations of the probe within a shallow trapping profile to thermally explore a volume of $300\text{ nm} \times 300\text{ nm} \times 600\text{ nm}$ with minimal perturbation.³⁻⁶ The position fluctuations are analyzed to map the local viscosity, the accessible volume, and local elasticity within the trapping volume. As these can be sampled in voxels on the order of $(10\text{ nm})^3$, TNI provides extremely high-resolution data about local interaction potentials, diffusion, and stiffness. However, now the deflections of the probe from the trap minimum are large compared to the trapping volume and the probe size. Therefore, the nonlinearity of the QPD detection system needs to be corrected.

Using a second laser and a low NA condenser can stretch the linear range, but requires aligning two lasers in register and provides limited improvement for sub-micron probes.⁷ It is possible to measure and computationally correct the nonlinearity as long as the viscosity of the medium is homogeneous.⁸ This approach suffices for larger probes and purely two-dimensional motion orthogonal to the axis

of laser light propagation. However, signal noise for small, weakly scattering probes, and for displacements along the laser propagation is often too large. Here, we present a refinement of the approach, which provides local diffusion measurements in volumes as small as $(10\text{ nm})^3$ and performs well for small probes. The approach significantly increases the corrected volume to almost the entire trapping volume, most significantly improving the measurement of the vertical diffusion along the optical axis.

As long as the probe is far from any surface its hydrodynamic drag depends only on the medium's homogeneous and isotropic viscosity, and the viscous drag should be also uniform across the trap volume. To measure the local viscous drag the local mean square displacement (MSD_l) is calculated for all trace segments of lag time Δt , and originating in a specific voxel (Fig. 2),

$$MSD_l = \frac{1}{n} \sum_i (r_{i+i_{lag}} - r_i)^2, \quad (1)$$

$x_0 - \frac{1}{2}\Delta x < r_i < x_0 + \frac{1}{2}\Delta x$

where x_0 is the center of the local voxel with width of Δx ; r_i are measured particle positions falling within the chosen voxel; n is the number of segments (square displacements) included in the sum; and $i_{lag} \cdot \delta t = \Delta t$ with δt being the acquisition interval. The high temporal and spatial resolution of the QPD detector x_0 permits the calculation of local MSD_l in voxels as small as $(5\text{ nm})^3$.

The local detector sensitivity $s(x_0)$ at position x_0 is calculated from the ratio of the local diffusion coefficient to the expected D . By using a line fit of the MSD_l curve to determine rather than just one individual square displacement measurement as has been done before,⁸ the influences of the positional noise in determining the local sensitivity can be minimized (Fig. 2(b)). This is crucial because the corrected particle's position is reconstructed by integrating over all the local detector sensitivities $s(x)$ from the center x_c to the apparent current

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FIG. 1. Laser trap based position sensing. (a) The bottom objective lens (100 $\times$, NA 1.3) focuses an infrared laser ($\lambda = 1064$ nm) to form the trap. The top objective lens (63 $\times$, NA 1.0) collects scattered and unscattered light. QPD is placed at conjugate back focal plane of the top objective. Lateral signals are obtained from intensity difference between half planes, while the axial position is derived from the total intensity. (b) Signal response for a 216 nm particle moved through the laser trap. (Top) X axis (dashed) and Y axis (continuous); (Bottom) Z axis.

position x_a ,

$$x'_a = \int_{x_c}^{x_a} s(x) dx. \quad (2)$$

The potential trapping minimum, x_c , is the “resting” position of the bead in the trap. Repeated iterations correct the crosstalk between x, y, and z and extend the useful range to the edge of the laser trap. This method greatly improves local diffusion or velocity maps which are particularly sensitive to position errors.

In calculating local sensitivities, the local MSD_l is fit from 1 μ s to 16 μ s (Fig. 2(b)). The voxels are cubes with side lengths equal to the square root of 150% MSD at lag time 16 μ s (~ 13 nm). Reliable calibration requires at least 200 (700) data points per voxel in X and Y (Z) direction. In areas with fewer position measurements, the edge length of the voxels is doubled. Voxels expand until sensitivities are calculated for the entire trapping volume. Before the reconstruction, the calculated sensitivities were Gauss smoothed over neighboring voxels. These steps are repeated until the corrected position measurements converge.

The nonlinearity of the QPD sensitivity leads to particularly large position measurement errors far from the center and at 45° between two detection axes. In Figure 3, we plot the distribution of the three-dimensional diffusion coefficient

FIG. 2. Local MSD calculation. (a) Solid line: trace of a brownian particle. Dashed line: Trace segments that are within the pixel (gray-dashed-square). Circles: The end of each displacement whose start is within the pixel (or on the dashed line). The circles are allowed to be outside of the pixel. (b) Local MSD is calculated for several lag time to plot MSD curve for diffusion coefficient or sensitivity calculation ($r_{\text{bead}} = 108$ nm, 1 MHz sampling rate, 5.5 μ m above the cover glass).

FIG. 3. Distribution of three-dimensional D_{xyz} (a) and axial D_z (b) coefficients in $(30 \text{ nm})^3$ voxels covering the trapping volume. The black values are within $\sim 10\%$ of the expected value. (Left: without linearization; Middle: with simplified linearization; Right: with the refined method. The edge of the trap volume was set to 200 position measurements per voxel. The local MSD was fitted from 1 μ s to 16 μ s.

values D_{xyz} and that of the axial D_z extracted by the three methods: using only the central detector sensitivity (1998),⁹ an earlier diffusion based approach (2004),⁸ and the new approach (2014) (Fig. 3).

The new method results in distributions of D_{xyz} values and of axial D_z values that are symmetric around the correct value and are limited by the statistical variations of random diffusion. Earlier methods resulted in the distributions that were skewed towards an incorrect average. To quantify the volume corrected by the various methods, we calculate the number of voxels, which show a D_{xyz} (Fig. 3(a)) or an axial D_z (Fig. 3(b)) within a 10% of the expected D. If the non-linearity of the detector is not corrected (1998 approach),⁹ the local diffusion coefficient deviates from the correct value by at least 10% in more than half the voxels. The earlier correction⁸ narrowed the distribution of D_{xyz} significantly, but the values are still not normal distributed around the expected D. That approach provides the correct three-dimensional D_{xyz} in 73% of the $(30 \text{ nm})^3$ voxels of the trap. However, the correction of the axial position sensitivity is incomplete, providing a correct D_z only in 29% of the voxels (Fig. 3(b)). In the original publication using larger probes, which scatter more, the correction worked a little better. Using the refined method, the three-dimensional local diffusion coefficient is determined anywhere in the trapping volume to within 25% of the expected diffusion coefficient. In 87% of the voxels, the local D_{xyz} value is corrected to within 10% of the expected D. We have confirmed by simulations that 87% is close to the statistical limit for the number of data points. D_z is corrected to close to the statistical limit: in 68% of the voxels the local D_z agrees to within 10% with the expected value.

The improvement over previous methods varies spatially. Therefore, we compare two-dimensional plots of the local D obtained with our approach to the best previously available method. In Figures 4(a)–4(d), two slices of the local lateral D_z mapped through the trap center (a), (b) and through a point 100 nm laterally off center (c), (d). Our results are shown

FIG. 4. Slices of the local axial D_z through the trap center (a), (b) and through a point 100 nm laterally off center (c), (d). Slices of the local lateral D_{xy} mapped through the center (e), (f) and through a point 150 nm axially off center (g), (h). (Pixel size = (15 nm);² slice thickness = 15 nm; pixels with fewer than 20 measurements = omitted; data length = 11s).

in Figures 4(a) and 4(c), and compared to the prior method (Figs. 4(b) and 4(d)). The new approach reduces the voxel-to-voxel variation, results in a normal distributed D , and pushes the usable range almost to the trap edge. Figures 4(e)–4(h) show the comparison for the D_{xyz} . Here, the improvements are less striking as the lateral signals have a larger signal-to-noise ratio than the axial one.

The presented refined method corrects the nonlinearity of the QPD detection system to within statistically variation of diffusive motion. Hence, this allows the QPD detection system to be used for precise local diffusion measurement especially with weak laser trap strength, resulting in a larger probing volume and weaker influence of the trap on the particle. The method also has higher tolerance on the positional noise level, nonlinearity, and crosstalk among the three axes, which commonly appear in the recorded trace. Our method is robust against the randomness of diffusive motions because the use of various voxel sizes eliminates optimizing the voxel size to the number of available position measurements and covers sparsely visited volumes. Internal thresholds on the number of

position measurements control the quality of recovered sensitivities, which is important for repetitive reconstructions to converge. The MSD fitting range is chosen for the axis with highest positional noise for the same reason. Assuming slow variation of sensitivities spatial gauss filter may be applied. Varying the distance between voxels' centers and the center of the trap may reduce any voxel digitization. Technically, the motion of the particle is not completely diffusive.¹⁰ The measurement speed is sufficiently high that hydrodynamic corrections should be considered. According to the Hinch theory, the expected deviation of the calculated diffusion coefficient through a line fit is 5% (216 nm probe; $\rho_p = 1.06$ g/ml).¹⁰ However, those corrections would be the same everywhere in the trapping volume and hence do not affect the linearization but result in a single scaling factor.

Overall, we present a method to completely correct the nonlinearities and cross-talk intrinsically associated with backfocal QPD position detection in optical traps. The increased precision over a larger volume will benefit measurements of surface hydrodynamic coupling, relevant for drug-delivery vesicles or nanoparticle interaction with cell surfaces, studies of non-Brownian diffusion and others.^{11–13}

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