Recently, Huang, Wu and Florin posted a Comment \[1\] on our preprint \[2\] describing nonequilibrium circulation of a colloidal sphere trapped in an optical tweezer. The Comment suggests that evidence for toroidal probability currents obtained from experiments and simulations in \[2\] should be considered inconclusive. The authors’ concerns are based on two claims: (1) that Brownian dynamics simulations of the trapped particle’s motions reveal no statistically significant circulation, and (2) that a realistic description of the radiation pressure acting on the trapped sphere is inconsistent with the motion described in Ref. \[2\]. In this Reply, we demonstrate both of these claims to be incorrect, and thus the original results and conclusions in Ref. \[2\] to be still valid.

The system, shown schematically in Fig. 1, consists of a single colloidal sphere trapped in a conventional optical tweezer formed by bringing a beam of light to a diffraction-limited focus \[3\]. In Ref. \[2\], we modeled the trap as a radially symmetric harmonic well within which radiation pressure exerts an additional force directed along $\hat{z}$:

$$F_0(r) = -k r + f_1 \exp \left( -\frac{r^2}{2\sigma^2} \right) \hat{z}. \tag{1}$$

The particle’s position $r$ is measured from the trap’s focus, $k$ is the trap’s stiffness, $f_1$ sets the scale for the radiation pressure, and $\sigma$ is the effective range over which the focused light exerts forces on the particle. We assume that the particle is stably trapped, so that $\epsilon = f_1/(k\sigma)$ may be treated as a small parameter.

Were these the only forces acting on the sphere, the particle would come to a stable mechanical equilibrium at a distance $z_0 \approx \epsilon \sigma$ downstream of the focus. The particle also is acted on by random thermal forces, however, which displace it away from its equilibrium point. Reference \[2\] demonstrates analytically that the second term in Eq. \(1\) biases the trapped particle’s thermal fluctuations in favor of toroidal circulation in the sense depicted in Fig. 1.

Such a bias toward nonequilibrium circulation would occur in any model for the radiation pressure whose curl does not vanish. The particular choice in Eq. \(1\) facilitates an analytic treatment of the effect. On this basis, we have claimed \[2\] that a particle trapped in an optical tweezer does come to equilibrium, but rather acts as a Brownian motor \[4\ \[5\ \[6\], with the nonconservative component of $F_0(r)$ biasing thermal fluctuations in the manner of a thermal ratchet.

The authors of Ref. \[1\] do not call this result into question, but rather claim that it is not conclusively demonstrated by the simulations and experiments presented in Ref. \[2\]. To observe the predicted circulatory bias in a trajectory $r(t)$ discretely sampled over time intervals $\tau = 1/30$ s, we introduced a measure of the mean circulation rate \[2\]

$$\Omega(t) = \frac{1}{2\pi} \frac{(r(t + \tau) \times r(t)) \cdot \hat{z}}{\sqrt{(\rho - \rho(t))^2 + (\dot{z} - \dot{z}(t))^2}}. \tag{2}$$

where $r = (\rho, \phi, z)$ is measured in cylindrical coordinates centered on the trap’s focal point, with $\hat{z}$ pointing along the optical axis. The predicted nonequilibrium circulation corresponds to clockwise rotation in the $(\rho, z)$ plane, and to positive values of $\Omega(t)$.

Huang et al. claim \[1\] that Brownian dynamics simulations corresponding to the experimental conditions in Ref. \[2\] show no statistically significant trend in $\Omega(t)$, and thus no evidence for circulation. Our numerical simulations, whose results are presented in Fig. 1 demonstrate this claim to be incorrect. Here, we have performed fourth-order Runge-Kutta integration of a particle’s trajectory evolving according to the Langevin equation

$$\gamma \dot{r}(t) = F_0(r) + F_1(t), \tag{3}$$

where $\gamma = 6\pi \eta a$ is the Stokes drag coefficient for a sphere of radius $a$ moving through a fluid of viscosity $\eta$, and $F_1(t)$ is a zero-mean stochastic force whose variance is the thermal energy scale. Although the simulations in

![FIG. 1: The probability $P(\Omega)$ compiled from 1000 Brownian dynamics simulations for a particle to circulate in an optical tweezer at an average rate $\Omega$ over the course of 1000 s. Inset: the experimental geometry, with a colloidal sphere localized near the focus of a beam of light propagating in the $\hat{z}$ direction. The broad arrow indicates circulation in the positive direction.](image-url)
were performed with the isotropic model in Eq. (1), the authors of Ref. [1] generalize the harmonic restoring force to account for different trap stiffnesses in the three Cartesian directions, using $k_x = 0.467$ pN/$\mu$m, $k_y = 0.4$ pN/$\mu$m and $k_z = 0.08$ pN/$\mu$m. In responding to their criticism, we adopt the same anisotropic force law in simulating the motions of a sphere of radius $a = 1.1$ $\mu$m in a trap of width $\sigma = a$. Following Refs. [1] and [2], we also set $\epsilon = 0.1$ and adopted time steps of $10^{-4}$ s. Taking the suspending medium to be water at room temperature, $\eta = 10^{-3}$ Pa.s.

Figure 1 shows the distribution of mean circulation rates, $\Omega = \langle \Omega(t) \rangle$, obtained from 1000 independent runs, each of 1000 s duration. As Huang et al. point out [1], individual realizations can display either positive or negative circulation. Contrary to their assertion, however, these variations do not occur with equal probability. Out of 1000 realizations, only 131 showed negative circulation. A similarly small proportion of retrograde circulation is observed experimentally. The ensemble-averaged circulation rate $\Omega = 0.08$ Hz agrees quantitatively both with the experimental results and also with the analytic predictions presented in Ref. [2].

Although the principal claim by Huang et al. is thus shown to be incorrect, their Comment raises the valid point that the scattering force experienced by a colloidal sphere in a real optical trap is likely to be more complicated than the idealized model in Eq. (1), particularly for spheres larger than the wavelength of light. The detailed form of $F_\theta$ is less important, however, than the presence of a rotational component, $\nabla \times F_\theta \neq 0$, for biasing the system out of equilibrium. It is this rotational component that breaks the spatiotemporal symmetry of the particle’s fluctuations to create a net flux in its probability density [3]. The particular form in Eq. (1) was selected more for its analytic tractability than for its accuracy as a model for radiation pressure in optical traps. The form for the scattering force presented in Ref. [1] also has a rotational component, and so will give rise to circulation in the particle’s trajectory. Unlike the scattering force in Eq. (1), which is peaked on the optical axis, the ray-optics calculation in Ref. [1] increases with distance from the optical axis, and so would induce retrograde circulation. This observation raises the interesting point that optically trapped particles’ behavior may be more complicated than is predicted by the idealized model in Eq. (1).

If this model for the radiation pressure were relevant to the experiments in Ref. [2], then the measured trajectories also should have displayed retrograde circulation. Huang et al. suggest that the discrepancy can be ascribed to insufficient statistics in the experimental analysis. We argue instead that the result for the scattering force presented in Fig. 2 of Ref. [1] reflects only the $\hat{z}$ component of the optical force that a particle would experience at the optical tweezer’s focal point. In fact, the silica sphere is more than twice as dense as the water in which it is suspended, and so settles roughly 2 $\mu$m below the focal point. In this region of the beam, the total optical force computed by a fully vectorial theory [7] has a uniformly positive curl. Consequently, the particle should undergo positive circulation, as reported. This is not to say that Eq. (1) is an accurate representation for the optical forces experienced by the sphere, but rather that the form proposed in Fig. 2 of Ref. [1] is not.

In conclusion, we have demonstrated that the concerns raised by Huang, Wu and Florin in Ref. [1] can be ascribed to inadequate statistical analysis of their simulations and to an incomplete analysis of the scattering force acting on optically trapped spheres. The results and conclusions presented in Ref. [2] therefore remain unchanged.

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