



## High bandwidth optical force clamp for investigation of molecular motor motion

Subhrajit Roychowdhury, Tanuj Aggarwal, Srinivasa Salapaka, and Murti V. Salapaka

Citation: [Applied Physics Letters](#) **103**, 153703 (2013); doi: 10.1063/1.4824816

View online: <http://dx.doi.org/10.1063/1.4824816>

View Table of Contents: <http://scitation.aip.org/content/aip/journal/apl/103/15?ver=pdfcov>

Published by the [AIP Publishing](#)

---

### Articles you may be interested in

[Distinguishing cells by their first-order transient motion response under an optically induced dielectrophoretic force field](#)

Appl. Phys. Lett. **103**, 183702 (2013); 10.1063/1.4827300

[Dual-trap optical tweezers with real-time force clamp control](#)

Rev. Sci. Instrum. **82**, 083102 (2011); 10.1063/1.3615309

[High bandwidth force estimation for optical tweezers](#)

Appl. Phys. Lett. **94**, 153114 (2009); 10.1063/1.3116621

[Separation of a single cell by red-laser manipulation](#)

Appl. Phys. Lett. **75**, 2671 (1999); 10.1063/1.125114

[A scanning force microscope for simultaneous force and patch-clamp measurements on living cell tissues](#)

Rev. Sci. Instrum. **68**, 2583 (1997); 10.1063/1.1148063

---

The image shows the cover of the journal Applied Physics Reviews. It features a blue and orange color scheme with a molecular structure in the background. The text 'AIP Applied Physics Reviews' is at the top left, and 'NEW Special Topic Sections' is in large white letters in the center. Below this, it says 'NOW ONLINE' and 'Lithium Niobate Properties and Applications: Reviews of Emerging Trends'. The AIP logo and 'Applied Physics Reviews' are at the bottom right.

**NEW Special Topic Sections**

**NOW ONLINE**  
Lithium Niobate Properties and Applications:  
Reviews of Emerging Trends

**AIP** Applied Physics Reviews

# High bandwidth optical force clamp for investigation of molecular motor motion

Subhrajit Roychowdhury,<sup>1</sup> Tanuj Aggarwal,<sup>2</sup> Srinivasa Salapaka,<sup>3</sup> and Murti V. Salapaka<sup>4,a)</sup>

<sup>1</sup>Department of Electrical and Computer Engineering, University of Minnesota-Twin Cities, 2-270 Keller Hall, Minneapolis, Minnesota 55455, USA

<sup>2</sup>Cymer Inc., 17075 Thornmint Ct, San Diego, California 92127, USA

<sup>3</sup>Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, 362C Mechanical Engineering Bldg., 1206 W. Green St., Urbana, Illinois 61801, USA

<sup>4</sup>Department of Electrical and Computer Engineering, University of Minnesota-Twin Cities, 5-161 Keller Hall, Minneapolis, Minnesota 55455, USA

(Received 24 April 2013; accepted 20 September 2013; published online 10 October 2013)

Use of optical tweezers for load force regulation on processive motors has yielded significant insights into intracellular transport mechanisms. The methodology developed in this letter circumvents the limitations of existing active force clamps with the use of experimentally determined models for various components of the optical tweezing system, thus making it possible to probe motor proteins at higher speeds. This paradigm also allows for real-time step estimation for step sizes as small as 8 nm with dwell time of 5 ms or higher without sacrificing force regulation. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4824816>]

Motor proteins such as kinesin and dynein are critical components of intracellular transport. Their study is important to understand cellular functionality, where malfunction in their transport may cause neuro-degenerative diseases such as Alzheimer's.<sup>1,2</sup> Optical traps provide an efficient way of probing the motor proteins via bead handles as they enable nm spatial resolution and fN force resolution capabilities.<sup>3</sup> Challenge in furthering the interrogative capabilities of optical tweezer based studies of motor-proteins stem from the significant impact of thermal noise and the nonlinear dynamics of the system.

In an optical trap based interrogation system, polystyrene beads are attached to motor proteins which are then probed by the laser trap (see Figure 1). When the motor-protein takes a step on the microtubule (where the position of the motor-protein on the microtubule is denoted by  $x_m$ ), the resulting extension of the stalk exerts a force on the polystyrene bead which in turn displaces the bead from the trap center  $x_T$ . The trap exerts a restoring force towards the trap center. Here, equilibrium position of the bead  $x_b$  is determined by the balance of the trap force (proportional to  $x_T - x_b$ ) and the force exerted by the motor protein (proportional to  $x_b - x_m$ ) (see Figure 1). For small displacements, force-displacement relationships above admit a linear Hookean spring approximation.<sup>4</sup>

One of the main objectives in the study of motor protein is to detect the stepping motion  $x_m$ . The major impediments in estimating the stepping motion accurately are the nonlinear force-extension relationship of the motor proteins, and the large influence of thermal noise on the bead. Constant force clamps<sup>4,5</sup> are designed to make the bead follow the protein motion by regulating  $x_b - x_T$  to a desired constant value. The efficacy of this method depends on the *disturbance rejection bandwidth* (the disturbance in this case being the motor stepping motion  $x_m$ ), that is, how fast the effects of

the protein motion are countered to maintain a constant trapping force. However, the state-of-the-art force clamps have low bandwidths. Therefore, they are effective only for slow stepping motions<sup>4,5</sup> and fail to regulate the trapping force when the protein is moving at a much higher speed (elaborated later). Passive clamps have been used to increase bandwidth, however, they are not suitable for probing fast moving motor proteins due to reduced trapping force<sup>6</sup> and limited extent of constant force region.<sup>7</sup>

The speed limitations in active clamps primarily arise due to the dynamics of the actuators that manipulate the trap position. In existing force clamp designs, the latencies caused by the physics of the actuators are not modeled appropriately, which forms one of the primary causes for limited speeds of operation of these designs. In an acousto-optic deflector (AOD) (which is typically used to manipulate trap position in state-of-the-art optical trapping systems), a diffraction grating is created by propagating sound waves through a crystal. The frequency of the sound wave is controlled by an input radio frequency (RF) wave. The first

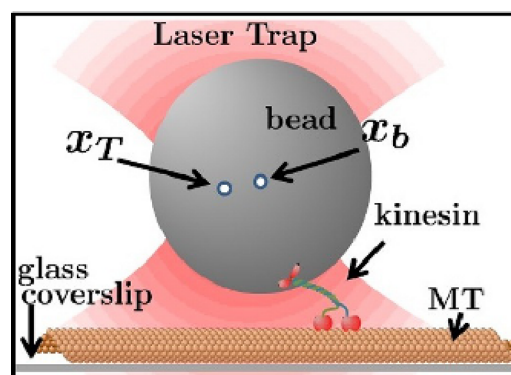


FIG. 1. A high resolution method to study kinesin motion is to optically trap the cargo (the bead) carried by the kinesin while it walks on microtubule. The bead position  $x_b$  is changed in response to the change in trap position  $x_T$  and the force exerted by the kinesin as it moves.

a)murtis@umn.edu and nanodynamics.ece.umn.edu

order diffracted spot created by passing a laser through this grating contains the majority of the light and is used to create a trap. The trap position can be manipulated by altering the input RF frequency, which affects the spacing of the grating caused by a change in the frequency of the sound wave traveling through the crystal. Here, the position of the laser spot at the output of the AOD does not settle until the propagating sound wave has crossed the entire width of the laser beam. Thus, the limitation in response time of the desired change in the trap location is determined by the velocity of the sound wave traveling in the crystal and the diameter of the laser beam. Another significant issue is caused by the partial reflection of the sound waves at the crystal boundaries. When the input frequency is changed, a mixture of waves of different frequencies exists in the crystal till the reflected waves die out completely. Thus, the time taken by the trap position to settle to its commanded position also depends on how well the sound waves get absorbed at crystal boundaries and the time taken by the reflected waves to die out.<sup>8</sup>

In this work, we incorporate models of components of the optical trapping system (including the AOD) into the design. Here the trap location  $x_T$  manipulable via the AOD exerts a trapping force depending on  $x_T - x_b$ ,  $x_b$  being the bead position which is sensed by a photosensitive detector (PSD). Figures 2(a)–2(c) illustrate the responses of a bead in a trap when the AOD is actuated with known signals. Figure 2(a) shows the amplification in the amplitude of the sinusoidal bead motion (as measured by the PSD) obtained when

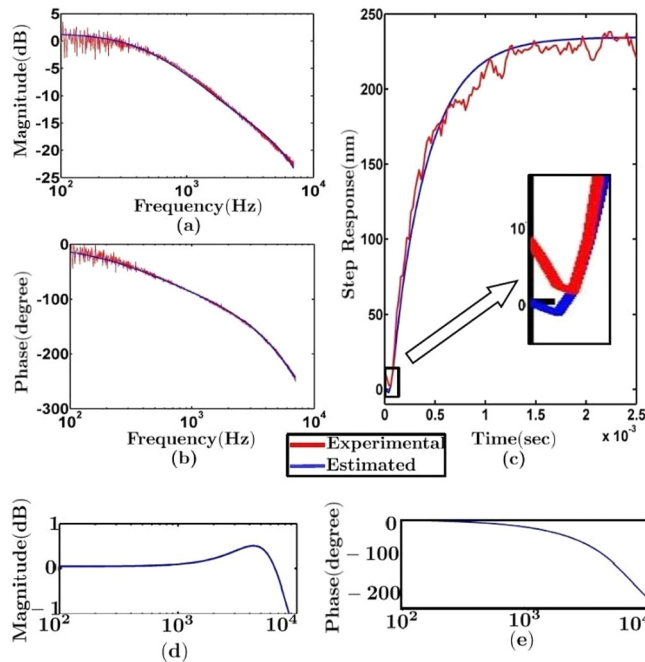


FIG. 2. The characterization of the system by frequency sweep method is demonstrated here. The red plots in (a) and (b) shows the experimentally obtained magnitude and phase response of the system while the blue curve corresponds to the transfer function fit of the same. (c) validates the estimated transfer function by comparing the simulated step response with the estimated transfer function (blue) with that obtained experimentally. The initial negative kick present in both the experimental and simulated step response (shown inset) is a signature of the delay present in the actuator. (d) and (e) show the magnitude and phase response of the instrument dynamics, respectively, which is extracted from the estimated transfer function and using estimated values for viscous drag coefficient and trap stiffness.

the AOD is commanded to change the trap location sinusoidally over a range of frequencies. Here, the phase shown in Figure 2(b) is the phase lag of the response sinusoid with respect to the command sinusoid provided to the AOD. This frequency response data can be used to obtain the *transfer function* (Laplace domain input-output relationship that describes the dynamic relationship between the actuation input to the AOD and the PSD measurement).<sup>9</sup> As alluded to earlier, the AOD physics describing the latencies can be quite complicated. However, the model of the AOD obtained using the experimentally obtained frequency response is accurate; indeed, as is evident from Figure 2(c), for the AOD and the experimentally obtained responses when the trap location is commanded to change by a step, quantitatively matches the response predicted by the model. It is also evident from the inset in Figure 2(c) that the bead initially moves in a direction opposite to the commanded direction which is a signature of delays present in the system.<sup>9</sup> Such behavior cannot be explained by the spring-mass-damper<sup>3,4</sup> models typically used to describe trap dynamics alone (see supplementary material text for more details). The transfer function  $D(s)$  from the trap movement command  $u$ , provided as an input to the AOD, and the actual trap movement  $x_T$ , is determined to be  $D(s) = \frac{0.66(s^2 - 7.98 \times 10^4 s + 2.78 \times 10^9)}{s^2 + 5.51 \times 10^4 s + 1.81 \times 10^9}$  (also see supplementary material text for details). The corresponding frequency response  $D(j\omega)$  is shown in Figures 2(d) and 2(e), which is obtained by setting  $s = j\omega$  at various angular frequencies  $\omega$ .  $|D(j\omega)|$  signifies the amplification and  $\tan^{-1}D(j\omega)$  signifies the phase lag from input to output for an input sine-wave of frequency  $\omega$ . It can be seen that as the phase lag becomes significant at higher frequencies, the assumption that trap position moves instantaneously in response to the input command (or, in other words  $D(s)$  can be treated as unity) remains valid only for low frequencies. Violation of this assumption would not only prevent from achieving desired performance at higher frequencies, but may introduce instability in the system as well.<sup>7</sup>

We emphasize that unlike current works, it is not assumed that the trap position  $x_T$  is manipulable directly. However, as we have modeled the AOD dynamics, the trap position  $x_T$  can be estimated from the trap command  $u$  using the model  $D(s)$ . For slow stepping motion, the disturbance effects on the trap are predominantly in the low frequency and therefore approximating  $D(s)$  by unity (and thus  $u = x_T$ ) is valid whereas for fast stepping motion it is not. For fast stepping motion,  $x_T \neq u$  and thus the error in force regulation  $e_f = f_d - k_T(x_b - x_T)$  (where  $f_d$  is the desired force to be maintained and  $k_T$  is the stiffness of the trap) cannot be directly estimated, rendering active force clamps which depend on error  $e_f$  ineffective for high bandwidth studies. To circumvent this issue, in this letter we provide a method that does not rely on the regulated variables (such as  $e_f$ ) to be measurable to achieve the goals of regulating a constant trapping force.

As mentioned before, one of the main objectives of force regulation is to allow for the estimation of the motor protein stepping motion accurately. In our method, it is possible to pose the estimation of stepping motion as the primary objective, with force regulation and reduction of noise on the estimate as secondary objectives. The high magnitude





proteins moving slowly (400 nm/s, similar to what is achieved in Ref. 5) but not on motor proteins moving at significantly higher velocities (10  $\mu$ /s).

Histograms in Figures 5(a) and 5(b) show the force regulations achieved for the same simulated motor velocities as in the previous case with an optimal controller designed following our method which incorporates the estimated  $D(s)$ , where the only objective is force regulation. Here, the controller is suitable for force regulation on motor proteins moving at velocities that are an order of magnitude or more than achieved in Refs. 4 and 5. The limitation on achievable bandwidth is now caused by the bead-handle dynamics (how fast the bead can respond to the motor motion), which is common to all active as well as passive clamps. Clearly, the limitations of low disturbance rejection bandwidth and instability about feedback based force clamps, as mentioned in Ref. 7 can be overcome. Another limitation arises due to the presence of right half plane zeros in the AOD dynamics (see  $D(s)$ ), which poses a fundamental limit on the achievable performance.<sup>13</sup>

Figures 6(a) and 6(b) demonstrate the *real-time* step estimation capability achieved by our method while Figures 6(e) and 6(f) show that corresponding force regulations are within satisfactory limit so that bead displacement can be used to infer motor motion without the need for linkage corrections.<sup>4</sup> The corresponding noisy bead position traces are shown in Figures 6(c) and 6(d) from which the steps are typically estimated offline.

Small error in estimating the stepping motion (that is small  $\|T_{x_m\hat{x}_m}\|_\infty$ ) requires the transfer function  $T_{x_m\hat{x}_m}$  from the actual to the estimated motion be such that  $|T_{x_m\hat{x}_m}(j\omega)| \approx 1$  for the range of frequency of interest  $\Omega_r$ . This would, however, increase the effect of thermal noise on the estimate  $\|T_{\eta\hat{x}_m}\|_2$  where noise is integrated over  $\Omega_r$ , since  $|T_{\eta\hat{x}_m}| = \frac{1}{k_m}|T_{x_m\hat{x}_m}|$ . Thus, from practical considerations,  $\Omega_r$  needs to be chosen carefully so that we achieve satisfactory noise reduction at the step estimate  $\hat{x}_m$  along with step estimation in the desired frequency range. This also explains why the noise level increases as we increase the bandwidth of step estimation.

To summarize, in this letter, we have precisely characterized the instrument dynamics and adopted a model based

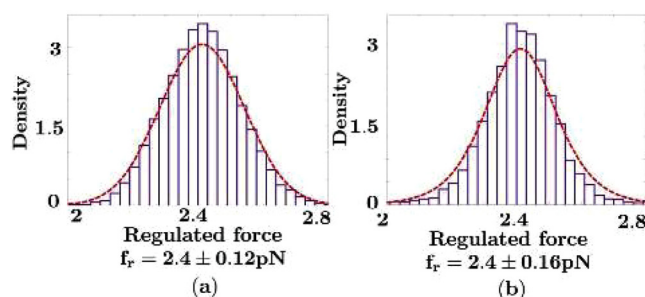


FIG. 5. Histograms showing the force regulation achieved with an *optimal* controller with the only objective of force regulation for different square pulse disturbances: (a) amplitude 8 nm and dwell time 20 ms, which corresponds to a motor velocity of 400 nm/s and (b) amplitude 25 nm and dwell time 2.5 ms, which corresponds to a motor velocity of 10  $\mu$ m/s. It is evident from the histograms that the controller maintains the force within 6.7% of desired value even for high frequency high magnitude disturbances (higher velocity).

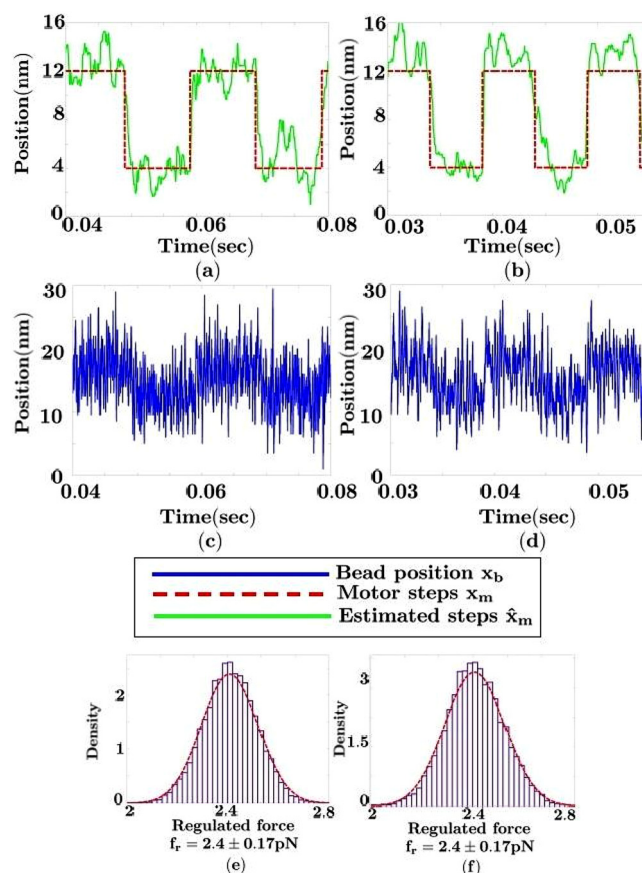


FIG. 6. The real time step estimation for step size 8 nm is demonstrated for (a) dwell time 10 ms, which corresponds to a motor velocity 800 nm/s and (b) dwell time 5 ms, which corresponds to a motor velocity 1600 nm/s. (c) and (d) show the corresponding noisy bead position measurements, from which the steps are typically estimated via post-processing, while (e) and (f) show the corresponding force regulation achieved.

modern control paradigm<sup>11</sup> to design an active force clamp that is capable of probing motor proteins with satisfactory force regulation close to their native cellular environment (where vesicles are transported at close to 4  $\mu$ m/s) (Ref. 14) and also motor proteins having larger step sizes and hence higher velocities (such as ciliary dynein with velocities close to 7  $\mu$ m/s).<sup>15</sup> This is much higher than the state-of-the-art capability<sup>5</sup> shown to operate satisfactorily on kinesin moving at velocities up to 450 nm/s. Simultaneously, this paradigm allows for estimation of motor stepping motion in real-time for high motor velocities (more than what is achieved for kinesin *in vitro*<sup>14</sup>). We demonstrated estimation of square pulses of magnitude as small as 8 nm with dwell time of 5 ms, without sacrificing the force regulation. Although long time averaging and median filtering is employed<sup>10</sup> to estimate steps in real time, it is limited to the cases with dwell time between steps of 1 s or higher, thus rendering them unsuitable for motor protein related applications. Thus the approach discussed here enables exploration of spatial and temporal realms of motor protein investigation which are not currently possible.

This research was supported by NSF grant award No. ECCS 1202411.

<sup>1</sup>N. Hirokawa, Y. Noda, Y. Tanaka, and S. Niwa, *Nat. Rev. Mol. Cell Biol.* **10**, 682 (2009).

- <sup>2</sup>G. B. Stokin, C. Lillo, T. L. Falzone, R. G. Bruschi, E. Rockenstein, S. L. Mount, R. Raman, P. Davies, E. Masliah, D. S. Williams *et al.*, *Science* **307**, 1282 (2005).
- <sup>3</sup>W. J. Greenleaf, M. T. Woodside, and S. M. Block, *Annu. Rev. Biophys. Biomol. Struct.* **36**, 171 (2007).
- <sup>4</sup>K. Visscher and S. M. Block, *Methods Enzymol.* **298**, 460 (1998).
- <sup>5</sup>M. J. Lang, C. L. Asbury, J. W. Shaevitz, and S. M. Block, *Biophys. J.* **83**, 491 (2002).
- <sup>6</sup>R. Nambiar, A. Gajraj, and J.-C. Meiners, *Biophys. J.* **87**, 1972 (2004).
- <sup>7</sup>W. J. Greenleaf, M. T. Woodside, E. A. Abbondanzieri, and S. M. Block, *Phys. Rev. Lett.* **95**, 208102 (2005).
- <sup>8</sup>A. Knight, G. Mashanov, and J. Molloy, *Eur. Biophys. J.* **35**, 89 (2005).
- <sup>9</sup>K. Ogata, *Modern Control Engineering*, 5th ed. (Prentice-Hall, Upper Saddle River, NJ, USA, 2010).
- <sup>10</sup>E. A. Abbondanzieri, W. J. Greenleaf, J. W. Shaevitz, R. Landick, and S. M. Block, *Nature* **438**, 460 (2005).
- <sup>11</sup>C. Scherer, P. Gahinet, and M. Chilali, *IEEE Trans. Autom. Control* **42**, 896 (1997).
- <sup>12</sup>C. Lee and S. Salapaka, *IEEE/ASME Trans. Mechatron.* **14**, 414 (2009).
- <sup>13</sup>T. Aggarwal, H. Sehgal, and M. Salapaka, *Rev. Sci. Instrum.* **82**, 115108 (2011).
- <sup>14</sup>J. Gagliano, M. Walb, B. Blaker, J. C. Macosko, and G. Holzwarth, *Eur. Biophys. J.* **39**, 801 (2010).
- <sup>15</sup>R. D. Vale, F. Malik, and D. Brown, *J. Cell Biol.* **119**, 1589 (1992).
- <sup>16</sup>See supplementary material at <http://dx.doi.org/10.1063/1.4824816> for detailed description of experimental setup, system model, experimental realization of stepping motion, and control system design.