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Measuring Molecular Forces Using Calibrated Optical Tweezers in Living Cells

Adam G. Hendricks and Yale E. Goldman

Abstract

Optical tweezers have been instrumental in uncovering the mechanisms motor proteins use to generate and react to force. While optical traps have primarily been applied to purified, in vitro systems, emerging methods enable measurements in living cells where the actively fluctuating, viscoelastic environment and varying refractive index complicate calibration of the instrument. Here, we describe techniques to calibrate optical traps in living cells using the forced response to sinusoidal oscillations and spontaneous fluctuations, and to measure the forces exerted by endogenous ensembles of kinesin and dynein motor proteins as they transport cargoes in the cell.

Keywords

Optical trap; Optical tweezers; Kinesin; Dynein; Intracellular transport; Microtubules; Cell mechanics; Live-cell assays; Biological materials

1 Introduction

Through the ability to exert piconewton-level forces on microscopic refractile particles, such as micron-sized polymer beads, and to make precise measurements of forces and displacements, optical traps, also termed optical tweezers, have provided fundamental insights into motor protein mechanochemistry [1], microtubule dynamics [2, 3], protein folding [4], and biological materials [5, 6]. Yet how motor protein function is modulated by the cellular environment, where motor proteins operate in teams, interact with binding partners and effectors, and navigate complex cytoskeletal networks (Fig. 1), is not well understood. The use of optical traps in living cells has been limited due to the lack of methods to reliably calibrate the instrument in the cytoplasm (Fig. 1). Active processes in the cell (motor proteins, cytoskeletal dynamics, etc.) continually exert fluctuating forces on the trapped object, precluding the use of standard calibration methods based on predictable thermal fluctuations. The index of refraction of the cytoplasm varies among cell types and may depend on the local environment in different regions of a cell. In addition, due to the presence of cross-linked cytoskeletal filaments, the cellular environment is highly viscoelastic, such that the response to forces applied by the optical trap includes contributions from both the cytoplasm and the optical trap that must be resolved (Fig. 2). The elastic and viscous components of the cytoplasm are frequency-dependent, further complicating quantification.

Measurement of biophysical force using an optical trap often involves attaching the biological target specimen, such as a molecular motor, to a small plastic bead and illuminating it with tightly focused infrared (IR) light which constrains its position near the beam waist. The position or scattering of the IR light by the bead is detected with a differential photodetector, usually a quadrant (QPD) or lateral-effect photodiode. The classical method to calibrate the force sensitivity, in units of pN per volt deflection of the detector signal, uses the frequency spectrum of thermal fluctuations of the bead within the trap [7, 8]. The power density spectrum ($P(f)$) of the photodiode signal in this situation is a Lorentzian curve given by:

$$P_{\text{th}}(f) = \frac{k_{\text{B}} T}{\beta^2 \gamma \pi^2 (f^2 + f_c^2)}, \text{ with } f_c = \frac{k_{\text{trap}}}{2\pi \gamma},$$

where β is the position sensitivity of the photodiode signal in nm/V, k_{trap} is the Hookean stiffness of the optical trap [pN/nm], f is the frequency [Hz], f_c is the cutoff (half-power) frequency of the spectrum [Hz], and γ is the viscous drag coefficient [N s/m]. The force sensitivity of the photodiode signal, C [pN/V], is then given by βk_{trap} . If the radius, r , of the bead and viscosity, η , of the fluid are known, then γ is calculated using Stokes' equation ($\gamma = 6\pi\eta r$). Alternatively, a sinusoidal oscillation can be applied to measure the drag coefficient [7]. Note that reliable values of r and η are required to define γ , and their uncertainties are propagated into the value of the force sensitivity.

The power spectrum method assumes that fluctuations are entirely due to thermal motion in a purely viscous fluid. To account for the actively fluctuating, viscoelastic environment in living cells, several methods have been proposed. The effect of the cellular refractive index on the stiffness of the optical trap can be approximated by performing calibrations in vitro using a medium that has a similar index of refraction [9, 10]. To determine the refractive index of the cytoplasm, oils of varying refractive indices are microinjected into cells [10] or the refractive index of the cytosol is measured using an Abbe-type refractometer [9]. This method is straightforward to implement and allows the calibration to be performed outside of the cell. However, refractive index matching does not account for variations in properties between cells or at different locations in a cell, and variability in the size or refractivity of the cargo must be estimated through image analysis. Cellular viscosity has also been noted to be temperature dependent [11].

An alternative method is based on the conservation of light momentum. Instead of imaging the bead on the detector, the distribution of the trap's IR light transmitted through the sample and arriving at the back-focal plane of the collecting condenser is imaged onto the photodetector. Using this back-focal plane interferometry [7], the lateral displacement of the light that is scattered and refracted by the trapped object is proportional to the displacement of the bead from the center of the trap, i.e., proportional to the force, rather than the position. In the case that all or a large fraction of the light is collected, the force calibration is insensitive to the size of the trapped object, the refractive index and viscosity of the medium, and the absolute position of the trap [11]. The light momentum method has significant advantages, as the calibration performed in water remains valid regardless of the media in

which the measurement is performed. To maximize the amount of light captured, a high numerical aperture (NA) condenser is paired with an objective of lower numerical aperture. Scattering by the media or other objects in the cell will result in errors, so measurements must be performed near the coverslip or slide surface of the flow cell closest to the condenser. One disadvantage of light momentum methods is that these constraints make them more difficult to use with total internal reflection fluorescence microscopy, which requires the use of high NA, oil-immersion objectives. Also, light momentum methods provide the calibration factor for force (C) but not for position (β), so camera-based tracking is often used in parallel to obtain or calibrate position data [11].

To explicitly account for the viscoelastic environment in living cells and enable calibration to be performed in situ, active calibration methods impose a series of sinusoidal oscillations through either the stage or the trap position and analyze the deflection of the trapped object [12, 13]. The correlated response to sinusoidal oscillations is insensitive to random disturbances from biological processes in the cell (motor proteins, cytoskeletal dynamics, etc.) as these occur stochastically and do not strongly or systematically affect the sinusoidal response at the frequency of the imposed oscillations. The calibration is performed in the cell on the same object that is used for measurements, thereby accounting for variations in the properties of the trapped object or the local viscoelastic environment. The relationship between the QPD signal and the position and force on the trapped object are determined by comparing the measured responses to a simple model of cellular viscoelasticity. This calibration also provides a measurement of the viscoelastic properties of the cellular environment.

Most endogenous intracellular components have refractive indices too close to the cytoplasm to be reliably trapped by laser tweezers. Lipid storage droplets in early *Drosophila* embryos are an exception that have been used to study motions and forces generated by intracellular molecular motors [9]. Some mammalian cells, such as macrophages, will readily take up plastic beads by phagocytosis. The resulting phagosomes are encased in a native lipid membrane containing endogenous motors, which transport them within the cell [14]. These latex-bead containing phagosomes are particularly amenable for studying intracellular transport by native kinesin and dynein molecular motors [10, 13, 15].

Live cell optical trapping techniques have been used to measure the forces exerted by microtubule-based motor proteins on intracellular cargoes such as lipid droplets and phagosomes. Forces indicate that motor proteins function collectively, working in teams of several motors to transport these cargoes [9, 10, 13, 15]. Teams of up to ten dynein motors drive transport from the cell cortex toward the cell center where multiple motors, each producing ~ 1.6 pN, are often simultaneously engaged [10, 13]. In contrast, transport towards the cell periphery is driven by kinesin motors, each of which produce ~ 6 pN and rarely function collectively [10, 13]. The cargoes demonstrate fast switching between plus- and minus-end-directed movements typical of bidirectional motility, suggesting that most commonly only one type of motor is engaged [13]. However, there is evidence that kinesin and dynein may sometimes oppose each other's motions during transport [15]. Future studies will focus on how motor proteins are regulated to achieve targeted trafficking of intracellular cargoes, and how transport is misregulated in disease [16–18].

The ability to perform calibrated optical trapping measurements in living cells has also enabled researchers to quantify the mechanical properties of the cellular environment [6, 13]. Unlike microrheological measurements based on tracking the spontaneous fluctuations of particles in the cytoplasm, optical tweezers-based measurements use the coherent response to known mechanical perturbations and are thus less sensitive to the stochastic active processes in the cell [12]. These measurements indicate that the cellular environment is viscoelastic, with viscosity dominating at low frequencies and elasticity becoming more prominent at higher strain rates [12, 13]. The cytoplasm of benign cells tends to be stiffer than malignant cells, suggesting that cell mechanics are perturbed in disease [6].

2 Materials

2.1 Cell Culture

1. J774A.1 mouse macrophage cells (ATTC).
2. Complete medium: Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % heat-denatured newborn calf serum, 1 % glutamine.
3. Imaging medium: Complete medium supplemented with 10 mM HEPES.
4. Polystyrene beads: 0.5–1 μm , carboxylated, 2.68 % solids (Fluorospheres, Molecular Probes). Pellet and resuspend in blocking buffer (10 mM K-PIPES, 50 mM potassium acetate, 4 mM MgSO_4 , 1 mM EGTA, 1 mg/ml bovine serum albumin, pH 7.0). Store for up to 2 weeks at 4 °C.
5. 50 mm $\times$ 0.17 mm glass coverslips (Bioprotech).

2.2 Force Measurements

1. Optical trapping microscope (*see* Subheading 2.4).
2. Data acquisition and control instrumentation (*see* Subheading 2.5).
3. Live cell chamber (*see* Subheading 2.6).

2.3 Optical Trap Calibration

1. Optical trapping microscope (*see* Subheading 2.4).
2. Data acquisition and control instrumentation (*see* Subheading 2.5).
3. Live cell chamber (*see* Subheading 2.6).

2.4 Optical Trapping Microscope

A combined optical trap and Total Internal Reflection Fluorescence (TIRF) microscope is used to perform simultaneous imaging, manipulation, and force measurements (Fig. 3). Briefly, the output of a 10 W, 1064 nm laser is expanded to overfill the back aperture of an oil immersion objective. Acousto-optic deflectors, located conjugate telecentric to the back-focal plane of the objective, are used to control the position of the trap and impart sinusoidal oscillations. The light scattered and refracted by the trapped object is collected using an oil-immersion condenser. A quadrant photodiode detector is positioned conjugate to the back-

focal plane of the condenser such that the output signal is proportional to displacement of the object from the trap center. For more details on the design and alignment of optical tweezers, see [8] and [19]. A live cell chamber is used to maintain cells at 37 °C in buffered medium (*see* Subheading 2.6). The thermal gradients caused by heating the sample require active stabilization of the focus to counteract drift. We monitor the reflection of a low-intensity TIRF beam using a quadrant photodiode and use a laboratory-built controller to maintain a stable focus.

Components include:

1. Inverted microscope (Nikon).
2. 1064 nm diode-pumped, solid-state laser, 10 W maximum output (Spectra Physics).
3. Acousto-optic deflectors controlled via a 30-bit direct digital synthesizer (Gooch and Housego).
4. Custom dichroic mirror mounted below the objective to couple the 1064 nm beam into the optical path (Chroma Technology Corp.).
5. 100×, oil-immersion objective (Nikon 1.49 NA, CFI APO TIRF).
6. Oil-immersion condenser lens, NA 1.4 (Nikon).
7. Quadrant photodiodes (Electro Optical Components, model JQ 50P). The QPD bias voltage is set to −150 V to narrow the electron/hole depletion layer near the P-N junctions of the photodiodes which markedly improves their frequency response [20]. Although the maximum specified bias voltage is 20 V, the photodiode operates stably under the high bias voltage.
8. Piezoelectric nano-positioning stage (Mad City Labs).
9. EMCCD camera (Cascade II, Photometrics).

2.5 Data Acquisition and control Instrumentation

A field-programmable gate array (FPGA) is used to control the piezoelectric nanostage via an analog voltage and an acousto-optic deflector through a 30-bit direct digital synthesizer. The signals from the nanostage and quadrant photodiode are amplified to utilize the full range of the analog-to-digital converters, low-pass filtered to avoid aliasing, and acquired using a simultaneous-sampling data acquisition card which preserves phase information between the signals. Custom LabVIEW (National Instruments) software is used to monitor signals, apply feedback control to conduct force-clamp experiments, and to apply sinusoidal oscillations over a range of frequencies to the stage and laser trap position for calibration in living cells.

Components include:

1. Field programmable gate array card (PXI-7851R, National Instruments).
2. Simultaneous-sampling data acquisition card (PXI-6143, National Instruments).

3. 8-pole Bessel low-pass filters.
4. Custom LabVIEW software.

2.6 Live Cell Chamber

Cells are plated on a 50-mm diameter coverslip, then mounted in a customized live cell chamber which maintains the sample at 37 °C during imaging. The chamber consists of the 50-mm coverslip and a 1-mm thick glass disk spaced apart by a 2 mm-thick silicone gasket, allowing access for the oil-immersion imaging objective underneath (the coverslip side) and the oil-immersion condenser above. The chamber is used in combination with an objective heater to provide increased thermal stability.

Components include:

1. Live cell chamber (FCS2 with custom top plate to allow access with an oil-immersion condenser, Bioprotech Inc.).
2. 50-mm × 1-mm glass disk (Bioprotech Inc.).
3. 50-mm × 0.17 coverslips (Bioprotech Inc.).
4. 2-mm thick silicone gasket (Bioprotech Inc.). 5. Objective heater (Bioprotech Inc.).

3 Methods

3.1 Cell Culture

1. Maintain mouse macrophage cells (J774A.1) in 10 cm dishes in complete medium at 37 °C, 5 % CO₂. Passage by scraping with a plastic policeman.
2. For optical trapping assays, plate cells on 50 mm glass coverslips and grow to ~50 % confluence.
3. On the day of the experiment, sonicate polystyrene beads for 1 min to disrupt aggregates. Dilute beads 1:50 in complete medium.
4. Replace cell medium with bead-containing medium. The concentration of beads is titrated to result in only one or two phagosomes present in each cell to avoid interference during experiments. Incubate cells at 37 °C and 5 % CO₂ for 10 min to allow the cells to phagocytose beads.
5. Wash cells three times with complete media to remove any beads that have not been internalized.
6. Incubate cells at 37 °C for an additional 60 min while phagosomes mature (*see* Note 1).
7. Transfer coverslip to live cell chamber and add imaging media.

¹Phagosomes undergo a series of biochemical changes as they are internalized and ultimately fuse with lysosomes. These biochemical changes correlate with changes in motility and motor protein activity [24].

8. Transport cells to the microscope in a styrofoam box filled with warm (37 °C) aluminum pellets. Once positioned on the microscope in the live cell chamber, allow 10–15 min for thermal equilibration.

3.2 Force Measurements

1. Once the cells have equilibrated in the live cell chamber, select a phagosome undergoing robust bidirectional transport in a cell with extended morphology so that the direction of movement and polarity of the microtubule cytoskeleton is well defined.
2. With the optical trapping laser shuttered, adjust the stage and acousto-optic deflector to center the selected phagosome at the position the IR beam will appear and activate the focus feedback to minimize focus drift (*see* Subheading 2.4). Open the laser shutter and use the nanostage to make fine adjustments to center the trap over the phagosome, using the *x*- and *y*-photodiode signals as a reference.
3. Record quadrant photodiode signals at 2 kHz for 250 s intervals.
4. Record an image of the cell.

3.3 Optical Trap Calibration

3.3.1 Response to Spontaneous Fluctuations and Sinusoidal Oscillations

1. After recording force and displacements produced by the motor proteins driving motility of the phagosome, initiate the calibration procedure. First, record the spontaneous fluctuations of the bead at 20 kHz for 25 s.
2. Apply a series of sinusoidal oscillations of position either through a piezoelectric stage (frequencies <50 Hz) or the acousto-optic deflector (frequencies ~50 Hz) (Fig. 4a). To obtain accurate calibrations, frequency data must be recorded over a large enough range (~5–500 Hz) to capture the sigmoidal shape of the mechanical transfer function (Fig. 4d).
3. For each frequency, adjust the amplitude of the oscillation such that (1) the coherence, a measure of the fraction of energy in the response that is correlated with the excitation signal [21], is greater than 0.98; and (2) the magnitude of harmonics in the response at multiples of the excitation frequency are less than one tenth of the response (20 dB below) that at the excitation (fundamental) frequency to obtain linearity (*see* Note 2; Fig. 4b,c). Typically, excitation amplitudes that meet these constraints range from 100 to 250 nm, gradually decreasing with frequency.
4. Apply the excitation for 2 s before recording data to allow transients in the response to decay.

²The calibration procedure requires that the response to sinusoidal oscillations is in the linear range, i.e., the magnitude of the response (Eq. 1) does not depend on the amplitude of the excitation. Nonlinear effects, as evidenced by the presence of harmonics in the response (Fig. 4c) become more pronounced at high amplitudes of excitation. For this reason, and to minimize perturbing the cellular components near the phagosome, the minimum amplitude of excitation needed to obtain a coherent response is used.

5. Record signals (quadrant photodiode, piezo stage position, and acousto-optic deflector position) at 20 kHz for 25 s at each frequency of excitation.
6. To test for possible degradation of motor protein function or damage due to exposure of the IR laser during the calibration procedure, remove the sinusoidal perturbations and record force and displacements of the phagosome for 250 s as in Subheading 3.2. Our measurements indicate that the calibration procedure does not substantially alter the motility and dynamics of phagosomes in the J774A.1 macrophage cell line.

3.3.2 Analysis of the Bead Response to Spontaneous Fluctuations and Forced Oscillations

1. Calculate the power spectrum of spontaneous bead fluctuations recorded while the optical trap and stage are stationary using MATLAB (Natick, MA). The power spectra measured in living cells deviate significantly from power spectra obtained in buffer, particularly for frequencies <300 Hz (Fig. 4e). This excess noise has two sources: (1) Active processes in the cell including motor proteins and cytoskeletal dynamics exert forces on the phagosomes at these timescales. (2) The viscoelastic environment of the cytoplasm produces strong mechanical coupling between the bead and the coverslip, resulting in increased sensitivity to stage vibrations.
2. Calculate the frequency response of the bead to sinusoidal oscillations. The response of the bead position (measured by the quadrant photodiode), to sinusoidal perturbations of the stage or trap position, i.e., the mechanical transfer function, is characterized by the ratio of the magnitudes and the phase between the response and the sinusoidal excitation (Fig. 4, *see Note 3*). The magnitude and phase of the response is calculated at each frequency of excitation using MATLAB (scripts available upon request). See Tolic-Norrelykke et al. [7] for an excellent discussion of data handling and frequency domain analysis in optical trap calibration experiments.
3. To estimate trap parameters (trap stiffness, position sensitivity) and the viscoelastic properties of the cellular environment, fit the power spectrum of spontaneous fluctuations and response to sinusoidal oscillations to a simple viscoelastic model (*see subheading 3.3.3 and Note 4*).

³In minimum phase systems (no distributed transport components), a zero in a transfer function is defined as a root of its numerator (*see Eq. 1*), and a pole is a root of the denominator, i.e., the power density is zero at that frequency. In the frequency response, or Bode diagram, a zero corresponds to a change in the asymptotic slope of the magnitude of +20 dB/decade and a change in phase of +90°. A pole corresponds to a change of -20 dB/decade in slope of the magnitude and -90° in phase (Fig. 5).

⁴To determine the model parameters that result in the best fits of Eqs. 1 and 4 to the forced and spontaneous responses, use a Levenberg-Marquardt algorithm (implemented in MATLAB using the `lsqcurvefit()` function) to perform a global fit of the model responses to the magnitude of the full sinusoidal frequency response (Fig. 4d) and the power spectrum of spontaneous oscillations (Fig. 4e) at frequencies of 300 Hz and above. The random fluctuations are driven primarily by thermal motions in this range. A weighting function is used to account for differences in the number of data points between the forced and spontaneous responses. Initial estimates of α , $k_{\text{Cyt},0}$, k_{trap} , and β are calculated based on estimating the corner frequencies in the frequency response and the slope of the power spectrum.

3.3.3 Simple Model of Cellular Viscoelasticity—To interpret the viscoelastic response of the bead to spontaneous and forced oscillations, we developed a minimal dynamic model that describes the responses observed in cells [13]. The forces on the trapped object are assumed to originate from (1) the optical trap acting as a Hookean spring with stiffness k_{trap} , (2) the viscous and elastic components of the cytoplasm, (3) thermal fluctuations, and (4) random active forces from biological processes in the cell (Fig. 2). The mechanical components of the cellular environment are described by a linear elasticity ($k_{\text{cyt},0}$) and viscosity (γ), as well as frequency-dependent viscoelasticity ($k_{\text{cyt},1}t^{-(\alpha-1)}$) characteristic of cross-linked semi-flexible polymers. At steady-state, the force response to sinusoidal oscillations ($s \rightarrow j\omega$) is given by

$$\frac{\Delta X}{X_{\text{stage}}}(s) = -\frac{\Delta X}{X_{\text{trap}}}(s) = \frac{s + \frac{1}{\gamma}(k_{\text{cyt},0} + k_{\text{cyt},1}s^\alpha/\Gamma(\alpha))}{\frac{m}{\gamma}s^2 + s + \frac{1}{\gamma}(k_{\text{cyt},0} + k_{\text{cyt},1}s^\alpha/\Gamma(\alpha) + k_{\text{trap}})} \quad (1)$$

where $X = X_{\text{bead}} - X_{\text{trap}} = \beta V_{\text{PD}}$, β is the position sensitivity of the detector in nm/V, and Γ is the gamma function. The force sensitivity, in pN/V, is given by $C = \beta k_{\text{trap}}$. The steady-state response to thermal fluctuations is

$$\frac{\Delta X}{F_{\text{th}}}(s) = \frac{1}{ms^2 + \gamma s + k_{\text{cyt},0} + k_{\text{cyt},1}s^\alpha/\Gamma(\alpha)/k_{\text{trap}}} \quad (2)$$

for spherical particles in a homogeneous medium. Stokes law relates Eq. 2 to the viscous ($G''(\omega)$) and elastic ($G'(\omega)$) moduli of the cellular environment [22].

$$G(\omega) = G'(\omega) + jG''(\omega) = \frac{1}{6\pi R} \left[\frac{\Delta X}{F_{\text{th}}}(\omega) \right]^{-1} \quad (3)$$

and the power spectrum is given by [23].

$$P_{\text{th}}(\omega) = \beta^2 \langle |V_{\text{PD}}(\omega)|^2 \rangle = \frac{2k_{\text{B}}T}{6\pi R} \frac{G''(\omega)}{\omega |G(\omega)|^2} \quad (4)$$

Equations 1 and 4 are fit to the responses to sinusoidal perturbations (Fig. 4d, Note 4) and thermal fluctuations (Fig. 4e). The frequency response enables the contributions to the elastic response from the optical trap and the cytoplasm to be determined separately, as the critical frequency of the zero in the transfer function (*see* Note 3, the zero corresponds to the upward change in slope at ~5 Hz in Fig. 4d) is defined by the cellular elasticity (Fig. 5b,c), while the critical frequency of the pole in the transfer function (*see* Note 3, the pole

corresponds to the leveling off of the slope at ~60 Hz in Fig. 4d) is defined by the total elasticity (from the optical trap and cellular components, Fig. 5a–c). The exponent α determines the slope of the response to sinusoidal oscillations for frequencies between the zero and the pole, as well as the downward slope of the thermal response at high frequencies (Fig. 5d). The slope of the thermal response is 2 (when plotted on a log-log) in the familiar situation that $\alpha = 1$.

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Fig. 1.

Teams of motor proteins transport cargoes along dense, cross-linked networks of cytoskeletal filaments. Many factors regulate the activity of motor proteins to direct transport in the cell: (1) the microtubule cytoskeleton is organized by microtubule-associated proteins which also directly modulate motor function, (2) the dense, dynamically cross-linked actin network constrains diffusion of cargoes, and (3) opposing teams of motor proteins interact to enable bidirectional transport. The activity of motor proteins is further regulated by interactions with binding partners and scaffolding proteins that mediate cargo binding. Note that an actual cell is much more densely packed than this cartoon

Fig. 2.

The cellular environment is viscoelastic. Forces are exerted on intracellular cargoes by the optical trap (k_{trap}), active forces including motor proteins and cytoskeletal dynamics (F_{active}), thermal motion (F_{th}), and the viscoelastic cytoplasmic environment ($k_{\text{cyt}}(f)$ and $b_{\text{cyt}}(f)$). The positions of the trap and bead are indicated by x_{trap} and x_{bead}

Fig. 3.

Combined optical tweezers and total internal reflection fluorescence (TIRF) microscope. The optical trap is based on a 1064 nm diode-pumped, solid-state laser. A half-wave plate and a polarizing beam splitter (PBS) are used for fine adjustments of the laser power or to divide the power between two orthogonally polarized beams. The two beams can be used to form two optical traps by recombining the light using a second polarizing beam splitter. Acousto-optic deflectors (AODs) enable fast, high-resolution control of the trap position. Achromatic lenses (L1–L3) serve to expand the beam $\sim 3\times$ and to position a conjugate back focal plane near the AODs such that angular deflections produced by the AODs result in translation of the optical trap at the image plane. A custom dichroic mirror (DM2) mounted beneath the objective couples the optical trapping laser into the optical path. A 100 $\times$, oil immersion objective is used to focus the laser beam to a narrow beam waist and for fluorescence and brightfield imaging. An oil immersion condenser, mounted on a custom 3D translation stage, collects the light that is transmitted, scattered and refracted by the trapped particle. The dichroic mirror (DM3) directs the IR light to quadrant photodiodes (QPD1,2). Lenses L4–L7 are used to relay the back focal plane to the quadrant photodiodes causing the photodiode signal to report force exerted on the trapped object and become insensitive to the trap position [7]. For live cell assays, a heated sample chamber is used, along with an objective heater. A custom auto-focus system monitors the position of a totally reflected beam from a low-intensity 532 nm laser using a quadrant photodiode (QPD3) and adjusting the sample position using the piezo stage

Fig. 4.

The forced response of the bead to oscillations of the stage and optical trap position enables calibration in the cellular environment. (a) To obtain the forced response, sinusoidal oscillations are applied using a piezoelectric stage (for frequencies <50 Hz) or by moving the optical trap position using an acousto-optic deflector ($f \geq 50$ Hz). The trace shows the trap position (blue) and the quadrant photodiode voltage (red), which corresponds to the force exerted on the trapped object, at excitation frequency 280 Hz. (b) The power spectral density of the trap position shows a peak at 280 Hz. (c) The differential photodiode voltage also shows a dominant peak at 280 Hz (see Note 2). (d) Forced responses were obtained for a range of excitation frequencies (blue circles). The red line indicates the fitted theoretical response (Eq. 1, see Notes 3 and 4). (e) The power spectrum of spontaneous fluctuations (no sinusoidal excitation) indicates that thermal motions dominate at frequencies >300 Hz. At lower frequencies, the fluctuations are influenced by active processes in the cell, such as molecular motors. Vibrations of the stage also affect the passive response at low frequencies. The red line indicates a fit of Eq. 4 at $f > 300$ Hz

Fig. 5.

The properties of the optical trap and cellular environment influence the forced response and thermal oscillations. Each plot shows the effect of changing the parameter by $\pm 50\%$ (blue to red) from the nominal value $k_{\text{trap}} = 0.17$ pN/nm, $k_{\text{cyt},0} = 0.04$ pN/nm, $k_{\text{cyt},1} = 0.005$ pN/nm, $\alpha = 0.73$. (a) Increasing optical trap stiffness (blue to red) causes the pole (see Note 3) of the forced response to move to higher frequencies, while the zero is unaffected. The corner frequency of the thermal fluctuations increases with increasing trap stiffness. (b) The elasticity of the cellular environment affects the zero in the forced response, and only weakly

influences the thermal response. (c) Increasing the frequency-dependent viscoelasticity of the cytoplasm shifts the zero and pole of the forced response and the corner frequency of the thermal response rightward. (d) The exponent α describes the scaling of the viscoelasticity with frequency. As α increases, the viscous component dominates such that the total elasticity in the system is decreased and the zero and pole in the forced response occur at lower frequencies. α also determines the slope of the thermal response at high frequencies, such that subdiffusive behavior is observed at low values of α , while $\alpha = 1$ corresponds to pure diffusion