Fiber based optical tweezers for simultaneous \textit{in situ} force exertion and measurements in a 3D polyacrylamide gel compartment

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Abstract: Optical tweezers play an important role in biological applications. However, it is difficult for traditional optical tweezers based on objective lenses to work in a three-dimensional (3D) solid far away from the substrate. In this work, we develop a fiber based optical trapping system, namely inclined dual fiber optical tweezers, that can simultaneously apply and measure forces both in water and in a 3D polyacrylamide gel matrix. In addition, we demonstrate \textit{in situ}, non-invasive characterization of local mechanical properties of polyacrylamide gel by measurements on an embedded bead. The fiber optical tweezers measurements agree well with those of atomic force microscopy (AFM). The inclined dual fiber optical tweezers provide a promising and versatile tool for cell mechanics study in 3D environments.

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1. Introduction

Optical tweezers have become a versatile and flexible tool in manipulating micro or nanoscale particles and measuring nanometer scale displacements [1]. Optical tweezers have enjoyed a wide range of applications in biological and physical researches, such as the study of the motion of individual motor proteins [2, 3] and mechanical properties of polymers [4]. Conventional optical tweezers are based on high numerical aperture objective lenses, which are bulky and expensive. Particularly, the limited working distances and the requirement of substrate transparency significantly hinder the application of conventional optical tweezers in emerging biophysical topics such as cellular mechanics in a three-dimensional (3D) environment.

Both cell generated forces [5] and external forces applied on cells [6] have been shown to regulate biological development such as proliferation [7] and differentiation [8]. Currently, cellular force characterization has been carried out by deformation measurements of homogeneous two-dimensional (2D) cell substrates [9, 10]. These techniques, also called traction force microscopy, involve either micropost arrays [11] or embedded fluorescent nanoparticles [12] in the substrate. Most of these techniques can only measure cellular responses on 2D substrates. Recently, 3D traction forces of cells on 2D substrates have been measured by traction force microscopy [13]. Atomic force microscopy (AFM) has also been used to measure cell stiffness [14]. However, physical contact with cells is required, and the resolution of lateral force measurements is limited.

The abovementioned techniques cannot measure cellular forces in a 3D compartment. It has been shown that cells behave very differently in a native environment, which is always inhomogeneous and 3D, compared on a 2D substrate [15]. Legant et al quantitatively measured traction forces of cells fully encapsulated in elastic hydrogel matrices by tracking fluorescent beads around the cells [16]. However, post-processing of experimental data is time consuming and can be difficult to provide real-time measurements. In addition, a homogeneous elastic medium is required in order to back out the cellular forces from the medium displacement field.
In this paper, we developed a versatile fiber optical trapping system for measuring forces on and applying forces to particles embedded in a 3D compartment, without any physical contact with the particles required. The fiber optical trapping system, namely the inclined dual fiber optical tweezers (DFOTs) [17, 18], can reach anywhere in a liquid solution and are not constrained by the substrate, so it can reach particles located high above the substrate and encapsulated in a solid 3D compartment. The inclined DFOTs can provide the maximum force of around 20 pN on a 4.63 μm silica bead at an optical power of 100 mW at each fiber tip. By comparison, conventional optical tweezers have maximum trapping forces on the scale of 10 pN per 100 mW with micrometer-sized beads [1]. The maximum forces are comparable in the inclined DFOTs and conventional optical tweezers. Moreover, the inclined DFOTs could allow a higher optical power than conventional optical tweezers before possible photodamage occurs to a trapped cell. This is because of the following two reasons: 1) the inclined DFOTs distribute the optical power over a large area of cell surfaces from both sides and hence have low power density on the cell, compared with conventional optical tweezers focusing all the power in a sub-micron spot; 2) the optical beam size in the inclined DFOTs is on the order of 100 μm at the trap position (beam intersection), so the effective trapping power delivered on a 5 μm bead is less than 10 mW out of 100 mW at the fiber tip.

As a proof of concept, we calibrated the optical trapping spring constant on microscale silica beads in water as well as ones encapsulated in polyacrylamide gel matrices. We experimentally characterized polyacrylamide gel stiffness by in situ optical trapping measurements, and the results agree well with AFM measurements. Since the optical trapping measurements do not require the polyacrylamide gel to be mechanically homogeneous, our results imply that the inclined DFOTs can characterize local mechanical properties of a 3D inhomogeneous, nonlinear medium. In addition, by varying optical forces, we successfully changed the effective spring constant on the particles embedded in a polyacrylamide gel matrix. These results suggest that the inclined DFOTs provide a powerful tool to apply forces to cells and measure cellular responses simultaneously in a 3D inhomogeneous environment.

2. Methodology

2.1 Working principles and system setup

Optical forces arise from the momentum change of light resulting from scattering or refraction [19]. A light beam shining on a sphere with a refractive index higher than the medium exerts both a scattering force directed along the light beam and a gradient force pointing to the region of maximum light intensity. For the inclined DFOTs, the two inclined optical beams apply two sets of gradient forces and scattering forces, as shown in Fig. 1(a). A 3D optical trap is achieved when the four optical forces balance with all the other forces that the particle is subject to, such as gravity and drag force.

Details of the inclined DFOTs setup can be found in our previous work [17]. Briefly, the inclined DFOTs were set up on a microscope platform. Each tapered fibers were attached to a common aluminum board via a micro 3D translational stage and a micro one-dimensional (1D) rotational stage, as shown in Fig. 1(a). These stages allow the alignment of the fibers with respect to each other and control of parameters such as the fiber inclination angle and fiber separation. Once the two fibers were properly aligned, only the aluminum board was moved by another 3D translational stage to move the position of the optical trap. In this case, the inclined DFOTs served as a building block and the position of the optical trap was controlled by a single 3D translational stage.
Fig. 1. (a) Working principles and the fiber setup of the inclined DFOTs. $F_s$ = scattering force; $F_g$ = gradient force. The inclined DFOTs can be moved by a single translational stage that controls the position of the aluminum board (yellow plate in the figure). (b) Schematic of the measurement setup of the inclined dual fiber optical trapping system.

The calibration setup is shown in Fig. 1(b). Light from a single-mode 980 nm laser diode (AC 1405-0400-0974-SM-500, Eques) was split into two tapered fibers (Nanonics Imaging, Ltd) through a 3dB coupler. Attenuators were employed to ensure equal optical power outputs from the two tapered fiber ends. When a bead in water or polyacrylamide gel was trapped, the light scattered by the bead was collected by an objective lens and detected by a position-sensitive detector (PSD) (DL100-7-PCBA3, First Sensor). The signal from the PSD enabled precision measurements of bead displacements in the $x$, $y$, and $z$ directions with a nanometer resolution. All of the fiber components worked at the single mode at the wavelength 980 nm.

2.2 Power spectrum calibration of optical spring constants

In this paper, calibration of the optical tweezers was accomplished by the power spectrum analysis method [20]. The forces applied by the optical trap as well as the surrounding medium (such as the polyacrylamide gel) can be considered as restoring “spring” forces on the trapped particle. The particle thus undergoes a confined Brownian motion, and the one-sided power spectrum of the particle displacement can be expressed as [19]

$$S_{xx}(f) = \frac{k_B T}{\pi^2 \gamma(f_0^2 + f^2)},$$

(1)

where $k_B$ is Boltzmann’s constant, $T$ is the absolute temperature, $\gamma$ is the hydrodynamic drag coefficient of the object ($\gamma = 6\pi \eta a$ for Stokes drag on a sphere with a radius $a$ in a medium with a viscosity $\eta$), and $f_0$ is the corner frequency or roll-off frequency, which is the characteristic of the fitted experimental curve, and provides the trapping spring constant $k$ by

$$k = 2\pi \gamma f_0.$$  (2)

In our experiments, the power spectra were obtained by complex Fourier transform of the time domain PSD signals [20] that were recorded in a period of 5 seconds at a sampling rate of 50 kHz. The frequency domain power spectrum data were then blocked in order to transform exponential distribution of the data to Gaussian distribution, the latter of which is necessary for the following least square curve fitting. The blocked power spectra were fitted to a Lorentzian in Eq. (1) with a frequency range of 10 Hz to 800 Hz, which provided the calibration results of the spring constants following Eq. (2). The calibration of the spring constant of the inclined DFOTs doesn’t require actuation of measured particle at any specific frequencies, and can be performed in situ in real time.
2.3 Experiments in water

We first investigated the dependence of the spring constant of the inclined DFOTs on the optical power, which has not been studied so far. This investigation enables better characterization of the inclined DFOTs, which also sets the base for polyacrylamide gel measurements described below. Silica beads (Bangs Laboratories, Inc.) with a diameter of 4.63 μm and density of 2.0 g/cm³ were used for the experiments. The bead solution was diluted by adding 1 μl of original bead solution (10.2%, 0.5 g in weight) into 6 ml of deionized (DI) water. An ultrasonic cleaner was used to reverse bead aggregation. One drop (0.2 ml) of the prepared bead solution was then added on a coverslip, where the trapping experiment was carried out. To prevent the water on the coverslip from drying up under the illumination light, water was added to the coverslip frequently [17]. The calibration of the optical trap was performed with beads trapped in three dimensions around 35 μm above the coverslip to reduce the sidewall effects [1].

2.4 Experiments in polyacrylamide gel

The inclined DFOTs were used to apply tunable forces on a bead embedded in the polyacrylamide gel in a noncontact fashion. In the meantime, the response of the bead was measured by the PSD to enable spring constant calibration of both the optical trap and the polyacrylamide gel. The optical force was readily tuned by varying the optical power, and the resultant effective spring constant on the embedded bead was calibrated.

2.4.1 Preparation of polyacrylamide gel with embedded beads

Polyacrylamide gels were prepared on 25 × 25 mm² coverslips. The polyacrylamide gel solution of the desired concentration of 5% acrylamide and 0.04% bisacrylamide was prepared in a pH 8.2 HEPES buffer. The polymerization process was initiated by adding 3 μl tetramethylethylenediamine (TEMED) and 10 μl of 10% ammonium persulfate (APS) solution for each 1 ml of polyacrylamide gel solution. The polyacrylamide gel solution was then quickly transferred to a glutaraldehyde-activated coverslip and covered by a second plasma-cleaned coverslip, on which silica beads with a diameter of 4.63 μm were attached previously. A desired thickness of around 50 μm of polymerized polyacrylamide gel was achieved by controlling the volume of polyacrylamide gel solution on each coverslip to be 30 μL. The solution was left undisturbed for 15 min at the room temperature until gelation was complete. The second coverslip was then peeled off, resulting in a layer of polyacrylamide gel on the first coverslip with silica beads embedded on the top. Due to the deformation of the polyacrylamide gel during the polymerization process, not all the beads are on the polyacrylamide gel surface. Beads close to the surface of the polyacrylamide gel were chosen for the optical trapping experiments. The polyacrylamide gel elastic modulus (E') and the viscous modulus (E''), also referred to as the storage and loss moduli, were measured by AFM to be 1469.9 ± 555.9 Pa and 533.2 ± 243.4 Pa, respectively (see Section 3.2.2).

2.4.2 Optical trapping experiments in polyacrylamide gel

Once a separate bead close to the polyacrylamide gel top surface was identified, the inclined DFOTs were moved so that the bead was in the optical trap. The power spectrum calibration then provided an effective spring constant combining the effects of both the optical trap and the polyacrylamide gel. By varying the optical power, we tuned the optical trapping spring constant, which enabled the calibration of polyacrylamide gel stiffness.

2.4.3 AFM measurements in polyacrylamide gel

After the inclined DFOTs measurements, AFM based microrheology measurements [21, 22] were performed to characterize local viscoelasticity of polyacrylamide gel around the bead that was studied in the inclined DFOTs. An Asylum Research MFP3D-BIO AFM (Asylum...
Research, CA) was used for the measurements. Briefly, the AFM cantilever was first positioned above the gel around the bead that was studied by the inclined DFO Ts. The cantilever was lowered down to create a constant (DC) indentation of approximately 2 μm in the polyacrylamide gel. Without retrieving it, the cantilever was then driven to oscillate sinusoidally with an amplitude of 25 nm and a frequency \( f = 10 \text{ Hz} \) to create a small sinusoidal (AC) indentation at the tip. The force and indentation was calculated to provide measurements of the polyacrylamide gel elastic and viscous moduli. The viscosity of the polyacrylamide gel was calculated using

\[
\eta = \frac{E''}{2\pi f}.
\]

3. Experimental results and discussion

3.1 Experimental results in DI water and discussion

We will show in this section the capability of both applying and measuring 3D forces in aqueous environments with the inclined DFO Ts.

3.1.1 3D trapping of yeast cells in water

Optical tweezers are well known for their capability of manipulating microscale particles. In our previous work [17], 3D trapping of silica beads were demonstrated with the inclined DFO Ts. Here, we demonstrate that the inclined DFO Ts are also suitable to apply 3D forces to living cells. In the experiment, a living yeast cell was trapped and manipulated in all three dimensions in DI water, as shown in Fig. 2. The movements of the trapped yeast cell were controlled by a single 3D stage that moved the aluminum board (see Fig. 1(a)). The maximum speed of the trapped cells was dependent on the optical power. At the power of 6.8 mW from each fiber, the maximum moving speed was around 20 μm/s for the yeast cells to remain in the trap. By manipulating the 3D positions of living cells, the inclined DFO Ts can enable relocation and assembly of living biological particles.

![Fig. 2. Experimental demonstration of 3D trapping of living yeast cells with the inclined DFO Ts. (a-d) Consecutive microscope images of the trapping experiment. The red arrows indicate the trapped yeast cell, and the black arrows point to a free reference yeast cell. The yeast cell was (a) trapped and (b) moved in the -x direction, followed by (c) +z and (d) +y directions. The positions of the fibers and trapped beads in (b-d) are shown in the schematics (e-g), respectively. The next movements of the optical trap are shown in the lower left corners of (a-c) and at the bottom of (e-f). The black shadow on the left-hand side of (a-d) is the tapered fiber tip. The optical power from each fiber taper was 6.8 mW.](image)
3.1.2 Optical trapping spring constant calibration in water

The calibration of the trapping spring constant was carried out with 4.63 μm silica beads. Silica beads, which are uniform in shape and material properties, serve as appropriate samples to evaluate the capability of the inclined DFOTs.

When a silica bead is trapped in three dimensions in water, the optical trapping spring constant can be measured by the bead displacement power spectrum. Typical power spectrum data of a bead trapped in water in the x and y axes are shown in Fig. 3(a) and (b), respectively. The Lorentzian fitting of the power spectra provides corner frequencies \( f_0 \) of 86.0 ± 3.3 Hz in the x axis (Fig. 3(a)) and 74.4 ± 3.1 Hz in the y axis (Fig. 3(b)). The spring constants in the x and y directions are different, which results from different optical field distributions along the two directions, as shown in Fig. 1(a). By varying the optical power, we obtained the dependence of optical spring constant on optical power along the x and y directions, as shown in Fig. 3(c) and 3(d). The maximum spring constants provided by the inclined DFOTs are 22.1 ± 1.0 pN/μm and 21.5 ± 0.9 pN/μm in the x and y directions, respectively. The tunable optical forces can be obtained based on the displacement measurements of the trapped particle. According our previous results [17], the maximum bead displacement in the inclined DFOTs is around 2 μm before it escapes from the trap, and the linear range of the force-displacement relationship is −1 μm to 1 μm. Bead displacements between 1 μm and 2 μm might cause the trapping to be unstable and the bead easy to escape.

Since the optical force can be calculated as the product of optical trapping spring constant and the bead displacement, the results in Fig. 3(c) and 3(d) imply that the inclined DFOTs can simultaneously apply and measure forces ranging from sub-pN to tens of pN. For example, at the optical power of 100 mW, the spring constant is 22 pN/μm and the maximum force is around 20 pN. Various cellular forces such as those generated by neural growth cone [23] and by a single actin filament [24] lie in this range. The inclined DFOTs can find applications to...
measure these cellular forces as well as apply forces to cells in aqueous environments, not limited by the working distance of the objectives. Compared with the AFM force sensing range of 10 pN to 100 nN [25], the inclined DFOTs can measure and apply forces that are too small for AFM, which may enable the inclined DFOTs to extend the range of measurable quantities and the biological systems that can be interrogated with AFM.

The error bars shown in Fig. 3(c) and 3(d) are the 95% confidence interval range of the Lorentzian curve fitting. It can be seen that there is a linear dependence of the optical spring constant on the optical power in both $x$ and $y$ directions. We notice some data points in Fig. 3(c) and 3(d) are scattered around the fitted linear progression. One possible reason is that the $z$-dimensional equilibrium position (see Fig. 1(a)) of the trapped bead changes with different optical power, which could influence the linearity of the dependence of the spring constant on optical power.

The results in water confirm that the optical force can be well characterized, which enables the exertion of controlled optical forces as well as measurements of external forces on the trapped particles.

3.2 Experimental results in polyacrylamide gel and discussion

3.2.1 Experimental results measured by inclined DFOTs in polyacrylamide gel

The effective spring constant on a silica bead embedded in the polyacrylamide gel matrix at different powers in $y$ direction (see Fig. 1) is shown in Fig. 4(b), with a typical set of power spectrum data shown in Fig. 4(a). Each single data point in Fig. 4(b) is acquired from the Lorentzian fitting of a set of power spectrum data at a fixed power as described in Section 2.2. The results indicate a linear relationship between the effective spring constant and the optical power, and the intrinsic stiffness of the polyacrylamide gel with no laser illumination can be obtained to be $0.012 \pm 0.005 \text{ N/m}$ based on the intersection with the vertical axis. The uncertainty is determined by the standard deviation from four independent polyacrylamide gel stiffness measurements, with one of the spring constant-power curves shown in Fig. 4(b). This bestows upon the inclined DFOTs the capability of in situ characterization of mechanical properties of solid media. Once the equivalent spring constant of polyacrylamide gel is characterized by the inclined DFOTs, a force on embedded beads can be measured by monitoring the bead displacement.

Since the calibration of the material equivalent spring constant is done by monitoring the bead Brownian motion, the inclined DFOTs can measure forces in nonlinear materials. The effective mean-square displacement of the Brownian motion is around 0.6 nm for a bead confined by a material equivalent spring constant of 0.012 N/m at the room temperature. As long as the material properties are approximately linear in the displacement range of around 0.6 nm, the spring constant can be calibrated in situ. Therefore, the optical trapping measurement of the material properties (and hence the force) is not dependent on the linearity or homogeneity of the materials, unlike other measurements where material linearity is important, such as traction force microscopy.

In addition, no physical contact is needed for the inclined DFOTs. Traditional AFM requires physical contact, and hence difficult to work with particles embedded in 3D compartments. Although the traction force microscopy have been demonstrated in a homogenous 3D medium, the requirements of tracking large numbers of fluorescent beads constrains its application in real-time measurements [16]. By comparison, the inclined DFOTs can provide a real-time, versatile, and non-invasive way to characterize the material properties inside a 3D heterogeneous and nonlinear medium.
It is noted that the mechanical properties of polyacrylamide gel can change under laser illumination. For example, it has been demonstrated that the viscosity of medium can be influenced by local temperature changes originating from optical absorption of the trapped particle [26]. Polyacrylamide gel stiffness has also been observed to change under localized laser illumination [27, 28]. Due to the nature of polyacrylamide gel swelling, polyacrylamide gel mechanical integrity can also be changed [27]. In addition, the imperfect contact condition at the interface between bead and surrounding polyacrylamide gel may cause effective polyacrylamide gel property to change. In our experiments, we noticed differences in data obtained in water and polyacrylamide gel, mainly in the slope of spring constant-power curve. Although we don’t fully understand how the polyacrylamide gel has been changed by laser illumination, we observed that the corner frequencies of the power spectrum data were linear with the optical power, because the spring constant-power data in polyacrylamide gel can be well fitted by linear functions. We also noticed the polyacrylamide gel changes are reversible according to the repeatability of our optical tweezers measurements of the polyacrylamide gel stiffness. As a result, the laser induced polyacrylamide gel changes will not influence the capability of the dual DFOTs to measure the intrinsic polyacrylamide gel stiffness, which is determined by the intersect of the linear fitting curve on the vertical axis.

3.2.2 Experimental results measured by AFM in polyacrylamide gel

AFM serves as an alternative polyacrylamide gel characterization method to verify the fiber optical tweezers measurements. AFM measurements were carried out at 19 different locations on the polyacrylamide gel in the vicinity of the bead of interest. Figure 5(a) shows AFM oscillatory force and indentation curves obtained in a typical measurement. The force and indentation raw data can be fitted by sinusoidal curves to remove noise. The force was plotted as a function of indentation in Fig. 5(b), where the hysteresis indicates that polyacrylamide gel is viscoelastic. The blue curve in Fig. 5(b) is the raw data corresponding to Fig. 5(a), and the red curve in Fig. 5(b) is obtained from the sinusoidal fitting of the force and indentation curves. Using the analysis method in ref [21], we obtained from the force-indentation curve the elastic and viscous moduli of the polyacrylamide gel to be 1469.9 ± 555.9 Pa and 533.2 ± 243.4 Pa, respectively. The mean values and standard deviations of the moduli were obtained from 19 independent measurements. The viscosity of the polyacrylamide gel was calculated from the viscous modulus to be 8.5 ± 3.9 Pa·s.
Fig. 5. AFM microrheology measurements of the polyacrylamide gel. (a) The sinusoidal force (red) and indentation (blue) as functions of time in a typical measurement. (b) Force as a function of indentation. The blue curve is obtained from the raw data in (a), and the red curve is from the sinusoidal fitting of the data in (a).

3.2.3 Comparison of AFM and inclined DFOTs results

The AFM microrheology measures the elastic modulus (with a unit of Pa). However, the optical trapping experiments measures the effective spring constants (with a unit of N/m) on a silica bead, which is dependent on the elastic modulus of polyacrylamide gel, the bead size, and the bead location. To compare them, we used commercial finite element analysis software (COMSOL) to calculate the spring constant on a bead embedded in polyacrylamide gel using the elastic modulus measured from the AFM experiments. We then compare the calculated spring constant with the inclined DFOTs measurements.

In the simulation, the Kelvin-Voigt model is used to describe the polyacrylamide gel. The displacement of a silica bead embedded in the polyacrylamide gel is calculated with a 20 nN static body force applied parallel to the surface. The polyacrylamide gel stiffness is then obtained by the ratio of the applied force to the resultant bead displacement.

According to the polyacrylamide gel preparation in section 2.4.1, the beads we chose for optical trapping measurements were always close to the top surface of polyacrylamide gel. However, the precise position of the bead with respect to the polyacrylamide gel top surface was not known due to the polyacrylamide gel deformation during the polymerization process. Therefore, we investigated the dependence of the polyacrylamide gel stiffness on the depth of the bead, with the simulation results shown in Fig. 6. The polyacrylamide gel stiffness in Fig. 6(a-c) are 0.0154 N/m, 0.0177 N/m, and 0.0206 N/m, respectively. The deeper the bead, the larger the stiffness. The rate of change of the stiffness is smaller with deeper bead positions because of weaker polyacrylamide gels surface effects. The results shown in Fig. 6(d) indicate the variation of the stiffness is small when the bead is deeper than 10 μm. This is because the polyacrylamide gel displacement field is not influenced much by the surface when the bead is far away from the polyacrylamide gel surface, as shown in Fig. 6(c).

As shown in Fig. 6(d), the optical trapping measurement of polyacrylamide gel stiffness agrees well with the simulation results based on AFM measurements when the bead top surface is in the range from −1 μm to 2 μm above the polyacrylamide gel surface. This confirms that the inclined DFOTs provide faithful, in situ, and non-invasive characterization of local material properties and forces in a 3D medium. The spring constant of optical trapping is dependent on the optical restoring force applied on the embedded bead. The fact that we can vary the spring constant by tuning optical power demonstrates the capability of applying tunable forces in 3D compartments.
Fig. 6. (a-c) COMSOL calculated the displacement field of a bead embedded in polyacrylamide gel when the bead depth is (a) 0, (b) −2 μm, and (c) −10 μm. The bead top surface is flush with the polyacrylamide gel top surface when the bead depth is 0. The top and bottom figures are the side and top views, respectively. The polyacrylamide gel thickness used in the simulation is 50 μm. (d) Calculated dependence of the polyacrylamide gel stiffness on the bead depth. The three red data points correspond to the results shown in (a-c), respectively. The inclined DFOTs measurements are also shown for comparison.

The ability of simultaneous application and measurements of forces bestows upon the inclined DFOTs great potential for cell mechanics study in 3D compartments. The spring constant measured by the inclined DFOTs can directly allow one to measure forces on the bead if the displacement is monitored. Force measurements of live cells in 3D environments with inclined DFOTs are under way.

4. Conclusion

We have demonstrated that the inclined dual fiber optical tweezers (DFOTs) can simultaneously apply and measure optical forces on both particles in water and those embedded in a 3D polyacrylamide gel matrix. Moreover, we showed in situ characterization of the local polyacrylamide gel stiffness by the optical trapping measurements, and the result agrees well with the simulation results based on the AFM measurements. Since the measurements are not dependent on the homogeneity of the medium, the inclined DFOTs can...
measure the local properties of heterogeneous and nonlinear medium. The ability of simultaneous application and real-time measurements of forces in a 3D compartment makes the inclined DFOTs an attractive tool for biomechanics and mechanobiology study.

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