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Erythrocyte deformation in high-throughput optical stretchers

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Abstract

Optical stretchers can be used to quantify elastic and homeostatic properties of cells. Because they can apply forces to cells without requiring direct contact, they may noninvasively measure mechanical properties related to cell and membrane health. Present-day optical stretchers are, however, limited to measurements on individual stationary cells, limiting throughput. To overcome this limitation and allow study of variations in cell populations, we recently developed and tested a microfluidic chamber that measures optical stretching parameters for erythrocytes under dynamic flowing conditions. The method uses a single linear diode laser bar and permitted measurements at low flow rates and higher throughput. Here, we numerically investigate the feasibility of further increasing the measurement rates of the optical stretcher in parameter domains where hydrodynamic and optical forces are of comparable magnitude. To do this we couple a recently implemented dynamic optical ray-tracing technique with a fluid-structure interaction solver to simulate the deformation of osmotically swollen erythrocytes in fluid flow of variable rate. Our results demonstrate that a detectable steady-state stretch is induced at nominal optical powers and flow rates. In addition, we find that flow rates can be increased significantly with no major effect on net cell stretch showing the feasibility of application of this technique at greatly increased throughputs.

I. INTRODUCTION

The elastic properties of cells are determined by membrane composition, scaffolding proteins, cell volume, viscoelastic properties of soluble constituents of cells, organelles within the cell, and other factors. Because of this, cell-mechanical property measurement can provide significant insight into the biology of the cell with diagnostic potential for diseases ranging from cancer [1,2] to various infections [3–5]. A number of methods have been proposed and used to measure cellular elastic properties [2,6,7]. One of these, optical

tweezers or stretchers, offers the advantage that they can measure cellular properties without directly contacting the cell and can be used to apply deforming forces to a single cell in a controlled manner [7,8]. Ideally, applied forces for stretching range from 10 to 1000 pN depending on cell type, but should not be so large that they alter the membrane properties of the cell.

Human erythrocytes are an important and readily accessible cell type for measurements of mechanical properties. Their surface consists of a heterogeneous lipid bilayer that is supported by a scaffolding of cytoskeletal proteins that include both soluble and integral membrane proteins [9]. Under static conditions in isotonic serum-containing media, healthy erythrocytes assume a biconcave discoid shape that is maintained by an organized lattice of cytoskeletal proteins [10] and an excess of surface lipid for the enclosed volume. This structure is critical for maintaining erythrocyte deformability and ensuring efficient movement through capillary beds which may have inside diameters as small as $2.5\ \mu\text{m}$. The excess surface area also improves loading and unloading of solutes as the erythrocyte transits through various organs. These physical features can be described with two elastic moduli, a dilational modulus that accounts for resistance to area changes contributed by surface lipids, and an extensional modulus that accounts for resistance to local shape changes attributed to the cytoskeletal network. These moduli have been measured using various experimental methods over the years [11,12]. Reported values of the dilational modulus range from 0.0025 to 0.013 dyn/cm, while values of the extensional modulus range from 300 to 500 dyn/cm. The structure and deformability of erythrocytes can be adversely affected by changes in membrane composition [13], temperature [14], extracellular osmolarity and solution composition [15], cell age [16], and infection. Of note, infection by the malaria parasite, *Plasmodium falciparum*, is known to alter erythrocyte membrane properties in drastic ways [17,18] from the effects of exported parasite proteins [19] and marked increases in erythrocyte permeability [20].

To measure their elasticity, red blood cells (RBCs) have been optically trapped and stretched by other researchers using divergent counterpropagating light sources in which cells are elongated along the beam axis [8,21]. In this approach, however, each cell is sequentially trapped and then stretched leading to throughput rates of 10–100 cells/h in microfluidic systems [22,23]. Efforts to increase the rate and performance by extending this method to flowing systems [21] are limited as both fluid drag and measurement rate are proportional to fluid velocity; increasing the throughput requires increasing laser power to optical intensities that result in cell damage [24,25]. Stretching in flow has other complications; in a planar shear flow, for example, observations and simulations have shown that RBCs first tumble [26,27], then breathe or vacillate when the viscosity contrast is above unity [26], and finally tank tread as shear rate increases [9,28]. These different types of motion require a focusing method to align cells single file at the channel centerline prior to optical stretching.

Recently, Sraj *et al.* implemented a different approach to achieve single-cell resolved measurements on larger numbers (10^3 or more) [29]. This significantly simplified optical stretcher employs an anisotropic optical trap consisting of a linear diode laser bar [30,31] that stretches individual cells in continuous confined microfluidic flow. Their approach in using only a single bar-shaped rather than dual laser beam for cell manipulation and sorting

in flowing systems eliminated the need for alignment and expensive associated optics that provides an easily integrated tool within microscale platforms. Also, a hydrodynamic flow focusing method was used to align cells at the channel centerline where the shear rate is zero, prior to entering the optical test section [31]. Sraj *et al.* used this technique to stretch cells at a constant fluid flow speed of $50 \mu\text{m/s}$ [29]. In their experiments, 1275 bovine RBCs were stretched and their deformation measured in less than 20 min providing stretching measurement rates of ~ 1.2 cells/s, significantly faster than previous single-cell stretching demonstrations [22,23]. The development of a high-throughput optical stretcher must consider the following. The intensity of the laser beam must be high enough to cause detectable deformation while it is necessary to avoid thermal damage in order to obtain mechanical property measurements of cells in their native state. Increasing the throughput rate in a microfluidic based optical stretcher requires increasing the flow velocity. Current cell cytometry measurements based on detecting fluorescence signals typically operate at flow velocities of 1–10 m/s, significantly beyond the range seen physiologically. As flow velocity is increased the effect of increased hydrodynamic forces on cellular deformation as well as the inherent response time of the cellular structure must be accounted for in both the experimental protocol and the mathematical model in order to obtain accurate measurements of cellular mechanical properties.

We numerically model a high-throughput optical stretcher to account for the effects of hydrodynamics discussed above as we investigate the feasibility of significantly increasing the measurement rates and improve the design of the optical stretcher. Here, we increase the applied flow rate while imparting experimentally obtainable optical forces characteristic of the linear optical stretcher and investigate the resulting influence on net cell stretching. In this, the interplay of optical and hydrodynamic forces becomes apparent, as both forces can be significant for cells within these dynamic, flowing environments. In this paper, we present a model to simulate cell deformation under both optical forces and external fluid flow. We use the immersed boundary method (IBM) to describe the hydrodynamics of cell-fluid interaction under external flowing conditions [32] and couple this with a dynamic ray-tracing method (DRT) for calculating the optical forces generated by the diode laser bar on different cell morphologies [33]. DRT is also used to calculate the transient change in optical forces as the RBC shape changes in response to both optical and hydrodynamic forces. We use these techniques to now investigate the influence of flow rate and laser power on the net deformation of RBCs of different initial shape, modeled as capsules immersed in a fluid of equivalent viscosity but different index of refraction. To more accurately describe RBC mechanical response, we implement the membrane model of Evans and Skalak (ES) [34] where both the dilation and extensional elastic modulus are included.

II. METHODOLOGY

We model the deformation of erythrocytes in high-throughput optical stretchers [29] using a single diode laser bar [30,31]. In this device, cells flow within microfluidic channels into an optical trap where light from a diode laser is refracted leading to optical forces at the cell membrane. The resulting forces from a single diode laser bar deform the cell and induce both opposing elastic forces at the membrane and viscous forces due to the fluid inside and outside of the cell. At the same time, cells continue to translate along the length of the diode

bar due to the external flow until they exit the trap where they relax due to the optical forces being removed.

Under high-throughput conditions, external flow creates viscous forces that alter the shape of trapped cells [35] which may lead to additional cell deformation depending on both the external flow strength and the laser optical power. To resolve the deformation of erythrocytes due to hydrodynamics, fluid-cell interaction, and light-cell interaction, a numerical methodology has been described and validated in a recent publication for static conditions [33]. The method combines the IBM, a hydrodynamic solver that accounts for fluid-cell interaction [9,32], with a newly developed optical DRT technique to determine the optical force distribution on deformable cells of arbitrary shape [33]. Here, we modify the DRT method to enable the calculation of optical forces in flowing conditions and the IBM to impose an external flow using a superposition method. We also implement a more sophisticated membrane model to simulate RBCs and briefly review it here to highlight the improvement in the method.

We modeled an RBC as an elastic membrane of constant properties surrounding a fluid of refractive index n_p immersed in an incompressible Newtonian fluid of the same density ρ and viscosity η as the cytoplasmic fluid but of a different index of refraction n_m . These assumptions are valid as cytoskeleton remodeling is only expected in cells stretched with high laser powers for periods of more than 2.5 s [36]. The RBC quiescent shape is a biconcave discoid; however, upon swelling, an RBC can assume a spherical or oblate spheroid shape of radius a . The constitutive law governing the cell membrane is described by a strain energy function W . Here we employ the commonly used Evans and Skalak membrane model [9,34] characterized by a dilation modulus that determines the resistance to local changes in area and an extensional modulus that determines the resistance to shearing stresses. In this, the strain energy function is given by

$$W = \frac{K}{2} (\lambda_1 \lambda_2 - 1)^2 + \mu \left(\frac{\lambda_1^2 + \lambda_2^2}{2\lambda_1 \lambda_2} - 1 \right), \quad (1)$$

where λ_1 and λ_2 are the principal strains calculated from a finite element method approximation for a given shape, K is the dilation modulus, and μ the extensional modulus. When the ratio $C = K/\mu \gg 1$ the membrane is nearly incompressible.

A model cell is situated within a microfluidic channel that is represented with a cube of dimension H at least four times larger than the radius of the cell a with periodic boundary conditions in all directions. Wall boundary conditions with no-slip velocity are assumed at the top and the bottom of the domain at the planes $z = 0$ and $z = H$. The high-throughput optical stretcher is initiated by an external flow along the length of the diode laser bar in the y direction as illustrated in Fig. 1. To accurately simulate erythrocyte deformation in the high-throughput optical stretcher, we simulate an individual cell translating through three different stages. In the first stage [Fig. 1(A)], the cell is exposed to Poiseuille flow of maximum velocity $u_{\max}$ at the centerline representing the hydrodynamic pretrap stage where the cell reaches a steady-state shape [Fig. 1(1)]. Once a steady-state shape due to hydrodynamics is reached, the cell translates into the trap composed of a laser sheet from a

diode laser bar of wavelength λ and total optical power P [Fig. 1(B)] where the cell is deformed by optical forces. This laser is of rectangular shape of dimension w in the x axis and length L in the y axis. The length of the diode laser bar allows the cell to reach a new steady-state shape while subjected to both optical and hydrodynamic forces. The final stage is then the relaxation of the cell as it exits the optical trap and optical forces are removed but the forces due to the Poiseuille flow remain [Fig. 1(C)]. Figure 1 illustrates this process where the transient deformation is characterized by the competition between the shear stresses and optical stresses acting to deform the cell and the elastic forces opposing the deformation.

IBM has been previously used to simulate the fluid-structure interaction in biological systems [9,28,37] by splitting the numerical solution onto two grids: a stationary Eulerian fluid grid which does not change after each time step and a moving Lagrangian boundary grid (representing a two-dimensional membrane mesh made of triangular elements). Solution involves solving the unsteady Stokes equation (in the limit of low Reynolds number) and the continuity equation at the fluid grid:

$$\begin{aligned}\rho \frac{\partial u(x)}{\partial t} &= -\nabla p(x) + \eta \nabla^2 u(x) + F(x), \\ \nabla \cdot u(x) &= 0,\end{aligned}\quad (2)$$

where u is the velocity, p is the pressure, and F represents body forces from the membrane or fluid. The restoring forces generated due to deformation of the elastic membrane are then calculated using the membrane constitutive equations by employing a finite element model developed by Charrier *et al.* [38] and Shrivastava and Tang [39]. The membrane forces are then distributed to the nearby fluid grid using an appropriately chosen three-dimensional discrete delta function [40]. The Stokes equation is then modified to include the interpolated restoring forces exerted by the membrane nodes and solved with the coupled continuity equation to give the fluid velocity for the next time step. No-slip conditions at the membrane surface are satisfied by moving the membrane nodes with the local fluid velocity. The velocity of a membrane node is found as the sum of the velocities at the fluid grid nodes weighted by the same discrete delta function used before. Only fluid grid nodes in the sphere of influence of the membrane node will make a contribution to its velocity. A membrane node moves at this velocity for one time step to a new position. Given the new membrane shape, the corresponding membrane forces are calculated and the procedure is repeated to advance the flow for another time step.

Under static conditions, optical forces are the only forces inducing deformation; however, for high-throughput measurements, an external flow is imposed and the cell both moves and deforms due to the imposed flow and the induced optical forces. To add flow to our numerical model [32] we employ a superposition method. Chorin's projection method [41] was used to solve the Stokes equations and the continuity equation was solved using Fourier-Toeplitz matrix inversion [42] which requires the flow field to be periodic. To include the effects of the channel, a wall boundary condition at the top and bottom of the domain was also modeled as in [32] where each node has a resting position x_0 . This node is perturbed from this position to x by the flow field in the same manner as the nodes in the membrane of the cell. However, this node then exerts a reaction force.

Optical forces induced due to momentum transfer from light to the cell surface upon refraction are determined using a recently developed DRT method. This approach calculates optical forces in the ray optics (RO) regime suitable for large particles [8,29,43,44]. In the RO regime, light can be decomposed into individual rays, each with specific intensity and direction [45] where geometrical optics can then be applied. DRT considers the influence of cell deformation on the optical forces calculations as it is capable of calculating the optical force distribution on deformable particles of arbitrary cell shape [33]. This is opposed to the traditional implementation of RO [8,44,45] where cells are assumed rigid and spherical; forces are calculated using analytical solutions derived for the initial undeformed shape [33,45]. In addition, DRT considers the effect of fluid drag on the cell as deformation may not remain symmetrical [8,45].

Briefly, in this method a finite number of rays are issued from a light source with a given intensity and known direction until they intersect a surface. Our membrane surface can assume an indefinite shape due to the external applied flow and cell deformability. The membrane already divided into triangular elements is used to determine the optical forces. A ray-triangle intersection algorithm is employed to determine the point of intersection and the corresponding parameters required in the general trapping efficiency Q (a dimensionless factor representing the amount of momentum transferred). The trapping efficiency Q is independent of the laser power used and depends only on the object geometry and reflectance of the medium. Determination of Q requires geometric calculations based on cell shape and the direction of incoming rays including the incident, refracted, and reflected ray angles at any point of ray-surface intersection. Elemental optical forces at any part of the cell are therefore found regardless of the initial cell shape. Rays are traced at any surface where Q is multiplied by a factor to account for energy loss from previous refractions and reflections [33]. Internal and external reflections within the cell are neglected as their effects rapidly diminish. Using this method the optical force is expressed as

$$F_{\text{optical}} = \frac{n_m Q P}{c}, \quad (3)$$

where c is the speed of light in vacuum. From this equation, we note that a small difference in the index of refraction between the cell and the suspending medium leads to forces that range from 1 to 100 pN depending on the laser power.

In this work, DRT is adapted to accurately calculate the optical force distribution on flowing cells. As flow in our model is acting upon a different axis from the optical forces (Fig. 1), cells can be deformed in different directions, an issue not considered in the case of static optical stretchers [33]. This variation in shape due to both external applied flow and cell deformability induced by optical forces requires updating the force distribution at smaller time steps, requiring a different procedure in allocating the initial rays. To address this in this study, we dynamically allocate the initial optical rays to accommodate for the diode bar length along the flow direction, an approach that saves significant computational time.

The deformation of capsules under external flow is characterized by the capillary number $Ca = \eta\gamma a/\mu$, representing the ratio of external viscous forces to elastic forces where γ is the

local shear rate of the applied flow. In the case of Poiseuille flow the y component of the velocity can be rewritten as $u = 4u_{\max}[(z/H) - (z/H)^2]$ where $u_{\max}$ is the maximum, centerline velocity. Local shear rate can be then found as $\gamma = u/z = 4u_{\max}[1/H - 2z/H^2]$ having a maximum value of $4u_{\max}/H$ at the channel walls. For the case of $H \approx 4a$, the capillary number can then be expressed as $Ca = \eta u_{\max}/\mu$, providing a direct measure of the strength of the flow. For our considered system, an RBC with $a \approx 3\text{--}4\text{ }\mu\text{m}$ deforming under a Poiseuille flow of $u_{\max} = 50\text{--}500\text{ }\mu\text{m/s}$ has a capillary number $Ca = 0.0012$ to 0.012 . Since the capsule has both shear modulus and viscosity it is possible to define an intrinsic time scale for the capsule defined as $t_c = \eta a/\mu$. For the purpose of quantifying deformation from optical forces or external flow, we use the Taylor deformation parameter defined as $D = (L - B)/(L + B)$, where L and B are the major and minor semiaxes in a specific plane. When viscous stresses, elastic forces, and optical forces are balanced, cells adopt a steady-state shape denoted by D_{∞} .

III. RESULTS

A. RBC stretching under static conditions

The surface of a red blood cell (RBC) consists of a heterogeneous lipid bilayer supported by a scaffolding of cytoskeletal proteins typically modeled by employing the Evans and Skalak (ES) model [34]. To employ the ES membrane model, we need to use a high value of the ratio $C = K/\mu$ as shear modulus μ is fixed. To test and validate the cell membrane model, we first consider the stretching of a swollen RBC by a single diode laser bar in the absence of external flow ($Ca = 0$) [29].

A spherical RBC of initial radius $a = 3\text{ }\mu\text{m}$ is assumed initially trapped at the center of the diode laser bar of length $L = 200\text{ }\mu\text{m}$, capable of producing a total power P . The laser power P is varied from 2.6 to 6 W ($13.3\text{--}30\text{ mW}/\mu\text{m}$). The extensional elastic modulus μ is fixed to 0.033 dyn/cm while the ratio C is changed, varying the area dilatational modulus K . We vary C from $1, 3, 10, 30$ to 100 until we predict an insignificant change in deformation.

We first assume a cell membrane of ratio $C = 1$ and calculate the optical force distribution due to a single diode laser bar of power P . This calculation results in a nonzero net force in the z axis and, due to symmetry, a zero net force in the other directions. The net force pushes the cell away from the diode laser bar, both stretching and translating the cell. The cell, however, reaches a steady-state shape with a terminal velocity when optical forces are opposed by elastic forces from the membrane and viscous forces from the suspending fluid. The two-dimensional (2D) steady-state shape is shown in Fig. 2 at the highest laser power used of $30\text{ mW}/\mu\text{m}$ colored by the normalized elastic energy. The cell, however, continues to translate in the z axis until the simulations are stopped. Using this $30\text{ mW}/\mu\text{m}$ laser power the net deformation measured in the y - z plane (the plane of both optical deformation and flow when present) is $D_{yz} = 0.047$ while the computed translation terminal speed in the z direction is $\sim 10\text{ }\mu\text{m/s}$. However, in the 5 ms needed to reach a steady state, the cell moves a distance of only 5 nm . With this approach we are able to monitor the dynamics of the process as shown in Fig. 3(a) where the evolution of net deformation D_{yz} is shown at different laser powers until the cell reaches a steady-state shape and a constant D_{yz} value.

At higher C [Fig. 3(b)] the initial optical force distribution remains the same; however, the higher cell stiffness results in less net deformation. Using $30 \text{ mW}/\mu\text{m}$ laser power, at $C = 100$ the net deformation reached at steady-state was 0.027, 42% less than that at $C = 1$. The computed terminal velocity increased but only to $\sim 11 \mu\text{m/s}$ with an increase in frontal area negligible compared to the change in the net deformation. In Fig. 4, we compare steady-state deformation as a function of laser power for different cell stiffness where we note the linear deformation response at the given laser powers for all values of C . The increase in cell stiffness is clearly seen here due to the increase in the area dilational modulus and the corresponding decrease in the net deformation.

While a sphere is the shape of minimum surface area for a fixed volume, enforcing local area conservation for initially spherical cells results in a cell that cannot deform. Thus, the high C value of 100 does not necessarily represent a limiting condition. However, to show that our value $C = 100$ can be used for a nearly incompressible membrane we simulate the stretching of ellipsoidal cells under the same optical forces from a single laser diode bar. Experimentally, RBCs are typically swollen osmotically to an oblate spheroid morphology with an average initial net deformation $D_0 = 0.126$ [29] in the x - z plane while in the y - z plane the cross section is circular, of radius $a = 2.5 \mu\text{m}$. In our studies here, the laser power is once again varied from 13.3 to $30 \text{ mW}/\mu\text{m}$ and the ratio C from 1 up to 100. In Figs. 5 and 6 we note that, for the same laser power and same C , the net deformation is less than the spherical cell case. This is attributed to the ellipsoidal shape that induces less drag and less resistance to flow and/or forces. However, the same pattern is noticed with C where local area conservation is satisfied as C approaches 100 while the net deformation approaches a constant value based on the specified laser power. Deformation of ellipsoidal cells of incompressible membrane leads to nonzero net deformation as opposed to spherical shapes.

To further investigate local area conservation, we create a strain histogram to quantify the percent area change for each finite element in the membrane model. Here, the percent change in the area of each element, $E_h = (E/E_0 - 1) \times 100\%$, is calculated where E_0 is the initial elemental area and E is the value at steady state. The range of area changes is divided into 20 bins, centered at zero to create a histogram normalized to unity. With this, the sum of the weighted elemental area for a single bin provides the proportion of the undeformed surface area that falls within that deformation range; thus, a perfectly local-area-conserving membrane would have a value of 1 for $-\epsilon < E_h < \epsilon$ and 0 elsewhere. We calculate the strain histograms at the highest laser power used, $30 \text{ mW}/\mu\text{m}$, for $C = 3, 10, 30$ (Fig. 7) where, as C increases, the membrane becomes stiffer. Local area conservation is best for $C = 100$ with 91% of the original area having local deformations of less than 0.1%. From this we can conclude that a C value of 100 can be used to model an RBC membrane.

B. RBC in Poiseuille flow

Experimental high-throughput measurements require the use of external flow to guide cells in and out of the optical trap [29]. The reported maximum velocity $u_{\text{max}} = 50 \mu\text{m/s}$ in a recent experiment may suggest significant deformation due to flow and may influence the stretching measurements and interpretation. In addition, a desired push to higher velocities and faster stretching measurements motivates a study of higher shear deformation; therefore

we study the influence of external applied flow on cell deformation before reaching the optical trap (A in Fig. 1) where there are no applied optical forces. At a speed $u_{\max} = 50 \mu\text{m/s}$, Sraj *et al.* were able to stretch approximately 100 cells/min and the deformation was attributed to only optical forces [29]. Here, we vary this speed and Ca from 0.0012 to 0.012, corresponding to velocities from $u_{\max} = 50 \mu\text{m/s}$ up to $500 \mu\text{m/s}$.

We first simulate cell deformation due to external Poiseuille flow with a membrane of $C = 1$ varying maximum flow velocities. With a nominal flow velocity $u_{\max} = 50 \mu\text{m/s}$ the resulting deformation is very small, $D_{yz} = 0.00077$, but does increase as the flow maximum velocity is increased to $500 \mu\text{m/s}$ where the maximum net deformation is 0.00335. This net deformation is, however, still one order of magnitude less than the deformation due to optical forces at a laser power of $13 \text{ mW}/\mu\text{m}$ ($D_{yz} = 0.023$). As we vary C in Fig. 8, we observe that the deformation remains in the linear regime as well as a decrease in deformation with increasing C due to the increased resistance to area change.

It is apparent here that the deformation D_{yz} caused by external flow can be neglected at low Ca ($\text{Ca} = 0.0012$) but may become important at higher values ($\text{Ca} = 0.012$). The slight change in shape due to deformation by external flow can influence the calculation of optical forces, as they are shape dependent [33]. The net deformation when combining both at high velocities is discussed in the following section.

C. Cell stretching under high-throughput conditions

In typical high-throughput measurements, cells are hydrodynamically focused by a Poiseuille flow into a linear diode laser bar optical trap [29]. A cell is then exposed to both optical forces and viscous forces from the applied flow when reaching the trap. After flowing along the length of the diode, the cell exits the trap guided by the external applied flow but now relaxes due to the removal of optical forces. The cell is not expected to relax to the initial shape, however, as the Poiseuille flow is not turned off. The net deformation is determined by observing the steady-state shape just before and right after exiting the trap.

To study cell behavior under combined optical and hydrodynamic forces, optical forces from the diode laser bar are applied at the cell surface after reaching steady state by the Poiseuille flow. Here, we vary the diode laser bar power P at a fixed length ($200 \mu\text{m}$), width ($1 \mu\text{m}$), and wavelength (833 nm). The rays from the diode laser bar are assumed parallel to the laser axis (the direction of the light beam) along the z axis. Following Fig. 1, a spherical cell representing an RBC is deformed in two stages after reaching a steady-state shape; it then relaxes back to its initial morphology. Figure 9 illustrates the shape at the end of the second stage due to both applied optical and viscous forces using a laser power of $P = 13.3 \text{ mW}/\mu\text{m}$ and $\text{Ca} = 0.012$. In Fig. 10(a), we show the evolution of deformation where these stages are observed: $0 < t^* < 1$ where the cell is deformed by stretching in flow, $1 < t^* < 2$ where the cell enters the trap, and $2 < t^* < 3$ where the cell exits the trap and relaxes from the applied optical forces. The net cell deformation is then calculated by subtracting the cell deformation at the end of stage 1 from the cell deformation at the end of stage 2, $D_{yz} = D_{2yz} - D_{1yz}$ (Fig. 11). We see from this figure that the net deformation decreases with increasing flow rate. Compared with the quiescent external flow case at the lowest laser power used ($13.3 \text{ mW}/\mu\text{m}$, $D_{yz} = 0.0154$), the net deformation calculated due to the presence

of flow of $50 \mu\text{m/s}$ ($\text{Ca} = 0.0012$) is 0.0154 at the same laser power, a decrease of only 3.51%. However, if we increase the flow to $500 \mu\text{m/s}$, ($\text{Ca} = 0.012$) the net deformation decreases to 0.0138, a decrease of 10.4% compared to the quiescent case. This is a significant difference for elastic property calculation of this deformation. Cells deform by Poiseuille flow such that they are compressed along the flow direction and stretched across it. When optical forces act on the cell, the resulting deformation is in the same direction (compression along y and stretched along z) resulting in less net deformation during stage 2. At higher laser powers, the decrease in deformation due to flow compared to the quiescent case is lower, 7.46% at the highest flow rate due to the dominance of optical force deformation over that due to flow.

Second, we determine again net cell deformation but now by subtracting cell deformation at the end of stage 3 from cell deformation at the end of stage 2, $D_{yz} = D_{2yz} - D_{3yz}$, as done in experimental measurements [29]. We see from Fig. 12 that net deformation decreases with an increase in flow rate. The amount of decrease is slightly different, however, as the cell does not relax back to the initial shape. This method is more practical in the experimental setup as it requires only a single camera to view and capture the cell shape at two different positions [Fig. 1(C)] opposed to the previous method that would require two cameras due to the long distance separating the two cell positions [Fig. 1(B)]. However, there is a need to wait ~ 0.1 s for relaxation limiting the speed of measurement; therefore, it is recommended that we use the first method to calculate the net cell deformation.

Finally, we compute the net optical forces at different flow rates to develop a master curve needed to calculate cell elastic properties from experimental measurements (Fig. 13) [33].

The master curve shows the variation of net dimensionless optical force $F^* = F_{\text{optical}}/a\mu$ with Ca of initially spherical RBC for different laser powers. From the definition of the dimensionless optical force, Eq. (6), and net optical force, Eq. (5), the cell shear modulus can be calculated as $\mu = n_m PQ/acF^*$. Based on the operating flow rate of experimental measurements one can now use the appropriate master curve and find the individual cell shear modulus in addition to the mean shear modulus. Using these data we can predict error in determining the shear modulus of a cell due to neglecting hydrodynamic forces. At the nominal flow rate of $50 \mu\text{m/s}$, $\text{Ca} = 0.0012$, if one neglects flow (assuming $\text{Ca} = 0$) the error in the shear modulus is 7.3%, while at a flow rate of $500 \mu\text{m/s}$, $\text{Ca} = 0.012$, neglecting flow leads to an error of 15.1% at the highest optical power considered, $30 \text{ mW}/\mu\text{m}$. Lowering the optical power would decrease the deformation due to optical forces and increase the error.

IV. SUMMARY AND CONCLUSION

In this work, we employ an improved numerical tool to investigate the feasibility of increasing cell stretching measurement rates in a high-throughput optical stretcher. At high flow rates, hydrodynamic forces are of comparable magnitude to optical forces and must be taken into account as they can influence cell morphology within the stretcher. For this, we simulated the deformation of osmotically swollen RBCs by combined optical and hydrodynamic forces. We used a recently implemented dynamic ray-tracing method to find the optical force distribution on a deformable cell of arbitrary morphology, coupled with the

immersed boundary method to solve for cell mechanics and cell-fluid interaction. Using single linear diode laser bar optical stretchers, we showed that at nominal flow rates (center velocities of $50 \mu\text{m/s}$) the deformation caused by external flow is insignificant compared to deformation caused by the optical forces and can be neglected. However, at very high velocities (of $500 \mu\text{m/s}$) desired for faster cell throughput, the decrease in net deformation due to hydrodynamic forces was 10.4%, corresponding to an error of 15% in the shear modulus when one neglects flow effects, and thus the effect of external flow must be considered in cell elastic property measurements. Our results show the effectiveness of the simulated optical stretcher and practical feasibility of increasing the external flow rate to achieve higher throughput. Our results also demonstrate the flexibility of the approach for modeling cells of different morphology and structure.

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FIG. 1.

(Color online) Cross section of the stretching process where cells are exposed to light along the z axis and to fluid flow down the y axis.

FIG. 2.

(Color online) 2D view of steady-state shape from three-dimensional (3D) simulations of a spherical cell stretched with $30 \text{ mW}/\mu\text{m}$ of laser power and ratio $C = 1$ colored by the elastic energy W , normalized by shear modulus μ . Laser source is shown in red (not to scale).

FIG. 3.

(Color online) Spherical cell stretching under optical forces with no external flow. Evolution of deformation as a function of dimensionless time $t^* = t/t_c$ for different laser powers at (a) $C = 1$ and (b) $C = 100$. The inset represents the initial spherical cell shape.

FIG. 4.
(Color online) Deformation with increasing laser power. The inset represents the initial spherical cell shape.

FIG. 5.

(Color online) Ellipsoidal cell stretching under optical forces with no external flow (a) $C = 1$ and (b) $C = 100$. The inset represents the initial ellipsoidal cell shape in the x - y plane.

FIG. 6.
(Color online) Deformation as power is increased. The inset represents the initial ellipsoidal cell shape in the x - y plane.

FIG. 7.
(Color online) Strain histograms at the equilibrium shape for $P = 23.3 \text{ mW}/\mu\text{m}$ for the Evans-Skalak membrane with increasing dilational modulus.

FIG. 8.
(Color online) Deformation at steady state in the absence of optical forces. The inset represents the initial spherical cell shape.

FIG. 9.
 (Color online) Steady-state shape from 3D simulations of spherical cell stretching colored by the elastic energy W , normalized by shear modulus μ for $C = 100$, $P = 23.3 \text{ mW}/\mu\text{m}$, and $\text{Ca} = 0.012$.

FIG. 10.
 (Color online) Evolution of deformation for a spherical cell in a high-throughput optical trap of laser power (a) $P = 13.3 \text{ mW}/\mu\text{m}$ and (b) $P = 30 \text{ mW}/\mu\text{m}$.

FIG. 11.
 (Color online) Net cell deformation $\Delta D_{yz} = D_{2yz} - D_{1yz}$.

FIG. 12.
 (Color online) Net cell deformation $\Delta D_{yz} = D_{2yz} - D_{3yz}$.

FIG. 13.
(Color online) Variation of optical force F^* with Ca of initially spherical RBC for different laser power.