Laser-guidance based detection of cells with single-gene modification

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An optical method with single-gene sensitivity for cell detection based on laser guidance was explored. Guided by the optical force from a weakly focused laser beam, a cell will move along the laser axis. Cells with different properties experience different optical forces and thus guidance speeds. The guidance speeds of the TC-1 cell and its genetically modified counterpart with only one gene change, L-10 cell, were studied under the same conditions. The results demonstrated that this laser guidance-based speed-measurement method can precisely distinguish cells that differ by only one gene. © 2008 American Institute of Physics. [DOI: 10.1063/1.2938020]

Cell sorting has played a vital role in cell biology studies, biomedical research, disease diagnoses, and cell therapies for at least four decades.¹ There are numerous cell-sorting methods, each of which employs a series of two procedures: cell detection, which distinguishes different cell types within the cell mixture, and cell separation, which separates the detected cell types according to their differences. In flow cytometry, by far the most commonly used cell sorting method,² cell detection is accomplished by use of fluorescent markers. However, flow cytometry cannot be used when corresponding fluorescent markers are not available or their application is contraindicated by clinical restriction. For example, flow cytometry is used to sort cells that have been collected from blood samples and genetically modified for autologous cancer therapy.³ However, the attachment and subsequent removal of the fluorescent markers result in loss of cell viability.^{4,5} Thus, techniques and instruments to detect cells accurately and consistently without using fluorescence-based cell markers are critical to clinical applications.

Changes in size, shape, internal structure, or composition of a cell cause changes in its optical properties (such as scattering cross sections) and thus the optical force experienced by the cell when it interacts with a laser beam. Therefore, several cell-detection techniques are based on optical force, including optical chromatography,⁶ optical lattice,⁷ optophoresis,⁸ optical stretcher,⁹ and micro-optical elements.^{10,11} Here, we report another optical force-based approach to distinguish cells by measuring speed of cells driven by optical force during laser guidance.

Cells located in the focal region of a weakly focused laser beam experience an optical force with two components: a radial component directed toward the beam axis and an axial component in the direction of beam propagation. Con-

sequently, cells can be drawn into the beam center and propelled along the axis: this is laser guidance. The optical force, which determines the guidance speed of the cell, is dependent on the parameters of the cell being guided, such as size, shape, composition, and refractive index. Therefore, by measuring the guidance speed of the cell along the laser beam, cells with different properties can be effectively distinguished.

Two theories, ray-optics theory^{6,12} and generalized Lorentz-Mie theory (GLMT),^{13,14} can be applied to the study of the optical force exerted on a cell by a Gaussian beam that is the laser beam used here. GLMT is the more complicated one, but it can be used to explore several phenomena that ray-optics cannot. These include the resonance effect, which indicates that the optical force is not a monotonic function of the cell size.¹⁵ Unlike use of GLMT theory involving TEM₀₀ mode structure in laser trapping¹⁶ (where the optical field in the tightly focused region is very complex), TEM₀₀ mode structure produces an effective approximation of the optical field of a weakly focused Gaussian beam. Thus, we used GLMT theory to estimate guidance forces in this study.

According to classical theory for transverse optical waves, the momentum of the wave is equal to the ratio of the Poynting vector over the speed of the wave $\mathbf{S}_s/c$. This momentum may be considered as the electromagnetic form of the momentum defined in quantum mechanics for a single photon $p = h\nu/c$. When an optical wave is scattered by a particle (e.g., a cell), an optical force is produced due to the momentum exchange between the wave and the particle. The net optical force exerted by the wave on the particle is given by the net momentum removed from the wave. It can be calculated by integrating $\mathbf{S}_s/c$ for the scattered beams over the surface of the particle, $\int_s (\mathbf{S}_s/c) \cdot d\mathbf{r} \cdot \mathbf{S}_s$ can be calculated by using scattering theories. Based on the earlier work of Lorenz, a scattering theory (Lorenz-Mie theory or LMT) was developed by Mie and Debye that described the scattering of a plane wave by an isotropic and homogenous spherical particle. GLMT was developed to solve the light scatter-

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ing of a nonplane wave (e.g., a Gaussian beam) by an isotropic and homogenous spherical particle. The GLMT for a Gaussian beam was developed by expanding the beam partially as an infinite set of beam-shape coefficients. After substituting the expanded form of the Gaussian beam travelling in the medium along z with a power of P into $\int_s (\mathbf{S}_r/c) \cdot d\mathbf{r}$, the optical force in the propagation direction (z) can be expressed as

$$F_l(z) = \frac{2n_{\text{medium}}P}{\pi w_0^2 c} C_{pr,z}, \quad (1)$$

where $C_{pr,z}$ is the radiation pressure cross section along the propagation direction, with a detailed expression determined by the GLMT theory as

$$C_{pr,z} = \frac{\lambda^2}{\pi} \sum_{n=1}^{\infty} \sum_{m=-n}^{+n} \left\{ \frac{(n+1+|m|)!}{(n+1)^2(n-|m|)!} \text{Re}[(a_n + a_{n+1}^* - 2a_n a_{n+1}^*)g_{n,\text{TM}}^m g_{n+1,\text{TM}}^{m*} + (b_n + b_{n+1}^* - 2b_n b_{n+1}^*)g_{n,\text{TE}}^m g_{n+1,\text{TE}}^{m*}] \right. \\ \left. + m \frac{(2n+1)(n+|m|)!}{n^2(n+1)^2(n-|m|)!} \text{Re}[i(2a_n b_n^* - a_n^* b_n)g_{n,\text{TM}}^m g_{n,\text{TE}}^{m*} - b_n^* g_{n,\text{TM}}^m g_{n,\text{TE}}^{m*}] \right\},$$

which is related to the beam-shape coefficients $g_{n,\text{TM}}^m$, $g_{n,\text{TE}}^m$, and the classical scattering coefficients of LMT a_n , b_n . By calculating those coefficients, the force can be estimated for each point.

While the cell with a mass m is moving along the laser beam axis in the cell suspension medium driven by the optical force, it also experiences the gravitational force $F_g = mg = \rho_{\text{cell}} Vg$, buoyancy force $F_b = \rho_{\text{medium}} Vg$ and the drag force $F_d = 6\pi\mu a v$, where ρ_{cell} and ρ_{medium} are the density of the cell and the density of the medium respectively, V is the volume of the cell, μ is the viscosity of the medium, a is the radius of the cell, and v is the guidance speed of the cell. Since ρ_{cell} and ρ_{medium} are nearly the same, the resultant force (subpiconewton) from the buoyancy force and the gravity force (their action is along the same vertical line but in opposite directions) is negligible comparing to the guidance force (tens of piconewtons); however, if the guidance is in horizontal, this resultant force may pull the cell guidance away from the optical axis and thus change the guidance force indirectly. To diminish this effect on the guidance trajectory and assure the cell to move along the beam axis, the cell is guided vertically. Consequently, according to the Newton's second law, we have

$$\frac{dv}{dt} = \frac{1}{m} [F_l(z) - 6\pi\mu a v], \quad (2)$$

where, $F_l(z)$ is the guidance force along the optical axis z , which can be estimated using GLMT theory as described in Eq. (1). From the homogeneous equation corresponding to Eq. (2), we can calculate the characteristic time $\tau = m/6\pi\mu a$, which suggests how long a cell can attain its terminal speed under a constant guidance force. For a typically cell, $m \approx 1$ ng, $a = 5 \mu\text{m}$, $\mu = 1.002$ cPoise, and thus $\tau < 10 \mu\text{s}$. It means that the cell attains its terminal speed of motion in the order of microseconds and hence the drag

FIG. 1. (Color online) Schematics of the testing system.

force will increase from, for instance 0, and finally balance the guidance force with no time comparing to the measurement time scale at millisecond. Therefore, the guidance force is considered to be balanced by the drag force at each measurement position and, consequently, the speed can be calculated based on the formula $v = F_l(z)/6\pi\mu a$.

The laser guidance system schematically shown in Fig. 1 was composed of a tunable Ti-sapphire laser (3900S, Spectra Physics) operated in the TEM₀₀ mode with a wavelength of 800 nm. A spatial filter assembly was used to remove the undesirable noise while transmitting most of the beam's energy. The beam was then collimated onto a right-angled prism to form a vertical guidance. Using a 60 mm planoconvex lens, the collimated beam was then focused into the chamber with a cell feeding mechanism. The beam waist, experimentally evaluated by the knife-edge method,¹⁵ was found to be 7.6 μm . The intensity of the beam inside the chamber was experimentally determined to be 150 mW. The main body of the chamber had two bolt-nut mounted parts, made of composite carbon. Coverglasses were glued to the main body to form the guidance channel. The cross section of the guidance channel was built to be $3 \times 3 \text{ mm}^2$ to minimize the convection force. The cell suspension was pumped by a microsyringe into the guidance chamber within the focal region through a hollow glass fiber (ID=50 μm). The guidance was recorded using a $10\times$ long working-distance objective and a high-resolution charged-coupled device camera (Sony) with an IR filter that blocked the scattered guiding beam. Cell images were captured using the Matrox Imaging frame grabber at the rate of 30 frames/s. The guidance speed was calculated from the positions of the cell in two separate frames and the time intervals between those two frames.

In this study, TC-1 cells, mouse lung-carcinoma tumor cells, and their genetically modified counterpart L-10 cells were used to compare differences in guidance speeds. L-10 cells are genetically modified TC-1 cells that stably express a fusion protein of the extracellular domain of mouse UL16-binding proteinlike transcript 1 (MULT1) and the transmembrane and intracellular domains of Fas. L-10 cells are resistant to an antibiotic, Zeocin. Under a high-magnification microscope (Zeiss Microscope, $63\times$, NA=1.4), L-10 cells

FIG. 2. (Color online) Cell guidance profile (L10 cell). The speed reached the peak value around the beam waist and then fell.

and TC-1 cells showed no difference in phenotype. For example, cell-diameter measurements demonstrated no statistical difference between the two cell diameters (D): $D_{TC-1} = 11.5 \pm 0.7 \mu\text{m}$ and $D_{L-10} = 11.5 \pm 1.1 \mu\text{m}$.

To ensure the same conditions during tests, the two cell types were fed one immediately after another. Phosphate buffered saline (PBS), a clear cell-handling saline, was used as the guidance medium; the rate of cell feed was controlled to maintain single cell guidance. A typical guidance trajectory is shown in Fig. 2. For each cell-guidance event, average speed was calculated by averaging the speeds at each position along the recorded guidance path; the maximum speed was selected at the beam waist position. As shown in Table I, the results demonstrated that modification of cells by only one gene resulted in approximately 40% decrease in average speed and maximum speed. Based on the formula of $F_l(z_0) = 6\pi\eta v_m a$, the optical forces at beam waist position, where the maximal guidance ($v = v_m$) occurred, also decreased about 40%, which resulted from the change of the refractive index between the two cell types. Because cells were inhomogeneous, here we consider the effective refractive index, which is the value we used in the GLMT theory. Assuming the central effective refractive index of the cell is 1.36, the optical force was calculated using GLMT with various index values around the central value with an increment of ± 0.005 . The 40% change in peak optical force corresponded to a 0.4% change in effective refractive index. Reference 17 shows a typical guidance movie for 1) a TC-1 regular cell (left column) and 2) an L-10 modified cell (right column).

Flow cytometry assessment demonstrated that the genetic-modification efficiency of the test cells was 90%. In fact, results showed that one datum from the L-10 group ($n=15$) fell into the data range of the TC-1 cells, approximately matching the genetic modification efficiency. Our data demonstrated this laser guidance method's sensitivity to changes in cell phenotype due to modification of a single

gene, a level of sensitivity that is not achievable in conventional high resolution microscopy. We believe that this is due to the sensitivity of the laser-guidance process, which integrates the microscopic interactions among photons and molecules throughout the entire cell to affect a single macroscopic outcome, guidance speed. In addition, the speed-measurement procedure accumulates the force differences at each time point, the sum of which is reflected in the final results. To conclude, cell detection based on speed measurement during laser guidance is a sensitive, accurate method for certain cell-sorting applications. Cell guidance's intrinsically slow speed may not be practical for high-throughput cell separation. However, when combined with conventional (e.g., microfluidic based) cell-separation methods, laser guidance can be used to develop a unique cell-sorting technique: Distinguishing cells with high sensitivity and subsequently using microfluidic-based, high-throughput cell separation without the use of additional cell markers.

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¹⁷See EPAPS Document No. E-APPLAB-92-045822 for a movie file of laser guidance for speed comparison between a TC-1 regular cell (left column) and an L-10 modified cell (right column). For more information on EPAPS, see <http://www.aip.org/pubservs/epaps.html>.

TABLE I. Comparison of the guidance speeds for the two cell types ($N=15$, $p<0.001$)

Cell type	Average speed ($\mu\text{m/s}$)	Maximum speed ($\mu\text{m/s}$)
TC-1 Regular Cell	12 ± 1.7	14 ± 2.5
L-10 Modified Cell	7.1 ± 0.56	8.6 ± 0.96