Optical manipulation and detection of biological cells in bio-microfluidic devices


CFD Research Corporation
215 Wynn Drive
Huntsville, AL 35805

*Genoptix, Inc.
3398 Carmel Valley Road
San Diego, CA 92121

**Worcester Polytechnic Institute
ME/CHSLT-NEST
Worcester, MA 01609

ABSTRACT

Focused laser beams can be used to trap and manipulate small particles and biological cells without mechanical contact. Particles trapped by such a laser beam can be used as local force probes in biomicrofluidic environment. Optical manipulation and separation of biological cell is a flexible technology with important potential biomedical applications, like cancer cell detection. Optical forces are in the picoNewton range, which makes them suitable for study of physics of biological objects like cells, viruses, bacteria, and research in genetics. The optical manipulation system presented and simulated in this paper uses a time-programmed scanning beam to segregate biological cells. The optical system generates a computer-controlled electromagnetic field, i.e., a "moving optical gradient field" which is precisely positioned and moved over a target cell population. As the optical gradient field translates across cells, it moves each cell a characteristic distance. Relative motion measurements capture this characteristic response for each cell under those specific conditions. This facilitates cell-by-cell analysis and separation of cells into distinct populations and is accomplished without the need for prior staining or labeling of the cells. The paper will present the experimental results and numerical modeling of the optical manipulation of cells based on the electromagnetic T-Matrix theory and a plane-wave expansion of the laser beam optical field.

Keywords: optical forces, optical trap, cancer cell detection, multiphysics modeling, bio-microfluidics.

1. INTRODUCTION

Optical trapping and manipulation of small particles and biological cells by laser beams is based on the forces of radiation pressure [1]. These forces, acting on matter from angstrom-sized atoms to micron-sized biological objects, plastic, silica, or metallic [2] beads, originate from momentum exchanges between radiation and matter. Originally proposed as an atom trap [3], optical gradient traps have been used extensively in biology, chemistry, and colloid physics [4-6].

Using lasers, the forces can be made large enough to accelerate, decelerate, deflect, guide, and even trap small particles. This is a direct consequence of high intensities and high intensity gradients achievable with continuous-wave coherent laser light beams. Laser manipulation techniques apply to particles as diverse as atoms, large molecules, small dielectric spheres, and to biological objects such as viruses, single living cells, and organelles within cells. Use of laser trapping and manipulation techniques permits control over the dynamics of both the individual particles/cells and the groups of objects [5].

In biological applications of optical trapping and manipulation, it is possible to remotely apply controlled forces on living cells, internal parts of cells, and large biological molecules without inflicting detectable damage. With the optical traps, forces generated by single motor molecules can be measured, and their stepping motion, of about 10 nm per step as they move along the submicron microtube and actin strands of the cytoskeleton, can be resolved [5].
Theoretical models of optical forces acting on particles much smaller than the wavelength of light (i.e., in the Rayleigh regime) are based on a dipole model of interaction of light with a dielectric particle. The total optical force on the particle can be resolved into two components: the dissipative scattering force pointing in the direction of the incident light [3,7,8] and the conservative gradient force along the incident gradient [3,8-10]. The scattering force pushes the particle in the direction of Poynting vector of laser light, i.e., moves it with the beam, while the gradient force drags the particle toward maximum of laser beam intensity in the focal region. Ratio of the scattering force to gradient force increases with a particle radius. A stable trap is obtained, with a single beam, when the gradient force is greater than the scattering force. In this case, a potential well produced by the optical force field is equal to or greater than other forces acting on the particle (e.g., viscous, Brownian, or biological interactions) and the particle remains in the trap. Optical manipulation of microbeads attached to a complex biomolecule allows to rotate, stretch, and cut it, measure kinetic constants of binding, and study behavior of molecular motors. Modeling of optical manipulation of nanoparticles (Rayleigh regime) was a subject of our previous paper [11]. In this paper, we consider a direct optical manipulation of biological cells. In this case (scattering on a large dielectric object) dipole approximation is no longer valid, and more sophisticated electromagnetic methods have to be applied.

Scattering on objects of 1-10 micrometer radius is the most difficult to model due to strong manifestation of vector electromagnetic effects. Scattering on biological cells belongs to this group of problems. Rigorous results can be obtained using Mie theory of scattering on spheres [12], based on analytical expansions of plane wave incident electric field and scattered field into vector spherical functions. Generalized Lorentz-Mie Theory (GLMT) extends this to higher order Gaussian approximation is no longer valid, and more sophisticated electromagnetic methods have to be applied.

Other electromagnetic scattering theories were developed for nonspherical objects [15]. Most of them, although very powerful and general (e.g. Finite-Difference Time-Domain (FDTD), [16]) are computationally too costly to be applied for computation of optical forces exerted on biological cells, especially for a purpose ofcell manipulation in microfluidic devices. Methods based on analytical models can be much faster. Two of them were especially successful: Discrete Dipole Approximation (DDA) [17] and T-Matrix [18,19,20]. Application of DDA for scattering on biological cells is limited by dimensions of matrix to be inverted. T-matrix method developed in astrophysics can be efficiently applied to rotationally symmetric objects (including spheres, spheroids, cylinders), and closely related theories allow to model spherical inclusions. T-matrix method is based on expansion of the incident plane wave, transmitted, and the scattered field into spherical wave functions. The T-matrix depends only on the incident wavelength, particle shape, refractive index, and placement in the coordinate system. Thus once calculated, T-matrix can efficiently be used to calculate the scattered field and the scattering characteristics at a set of orientations.

A few attempts have been made to model the electromagnetic Mie scattering of incident waves different than plane waves. These involve a direct decomposition of the incident beam into an infinite series of regular vector spherical functions (VSWFs) [13,14,] or analytical expansion into plane waves followed by an expansion of plane waves into VSWFs [20]. The former approach has been applied in the aforementioned GLMT method. Both approaches based on the analytical expansions, although fast and accurate, are limited to symmetric Gaussian or Gaussian-Davis beams [22]. Approach based on the numerical summation of the scattered-field contributions from individual plane wave components was applied to spherical objects smaller than wavelength [23,24]. This method is applicable to “arbitrary” incident beams including practical cases of asymmetric, aberrated, off-axis beams in high numerical aperture focusing systems. In this paper we further extend the method on large non-axisymmetric objects and demonstrate its application to biological cell manipulation in microfluidic devices. In the presented simulations, cell motion powered by the optical forces is, in addition, bi-directionally coupled with the surrounding fluid motion (induced flow, drag forces exerted on cells).

A method based on a plane-wave decomposition of the incident optical field, T-matrix computation of the individual-plane-wave contribution to the scattered field, and numerical integration of the total scattered field and optical forces is presented in Section 2. Applications of the method to computation of forces exerted on spherical and cylindrical objects in optical beams are described in Section 3. Section 4 addresses simulation of biological cell manipulation in scanning optical fields and compares simulations with experiments on optical trapping and manipulation, and separation of biological cell in a microfluidic device.

2. SCATTERING ON BIOLOGICAL CELLS MODELED USING T-MATRIX THEORY AND PLANE-WAVE-SPECTRUM

We have adapted the electromagnetic, rigorous, and powerful T-matrix method [18,19] to compute scattering and optical forces exerted on particles and biological cells in arbitrary vectorial optical beams. T-matrix method can be efficiently applied to rotationally symmetric objects (including spheres, spheroids, cylinders), and closely related theories allow to model spherical inclusions. T-matrix is calculated only once for a given particle, since it is independent of the propagation directions and polarization states of the incident and scattered fields. Original T-matrix method assumes plane wave illumination, expands incident plane wave into vector spherical wave functions and computes scattered field expansion coefficients from linear relationship involving T-matrix.
In our approach the incident field is represented as a superposition of plane waves. Calculated T-Matrix is used to compute scattered field for each incident plane wave. Total scattered field is a coherent vectorial superposition of the scattered fields coming from each of the incident plane waves. Plane-wave spectrum representation of the incident field can be applied for aberrated nonsymmetrical beams and will allow for modeling of practical devices (e.g. including effects of media interfaces). It can also be applied to higher-order (Davis) Gaussian beams being a high-numerical-aperture (high-angle focusing, small laser beam waist, strong manifestation of polarization effects) counterpart of paraxial Gaussian beam. Knowing the scattered field, we can compute radiation forces from the Maxwell stress tensor \[25\]. Other characteristics, e.g. extinction and scattering cross-sections, and trapping efficiency can also be evaluated. The remainder of this Section will describe how the plane wave spectrum in the focal region is determined from the electric field distribution in the lens pupil and how the optical forces exerted on a biological cell are calculated.

The electric field distribution in the focal region of a lens can be obtained by taking the 2D inverse Fourier transform of the product of plane-wave spectrum in the pupil and the coherent transfer function for propagation from the pupil to the focal region:

\[
\mathbf{E}^{\text{inc}}(x, y, z) = \int \int \mathcal{E}^{\text{pupil}}(k_{1x}, k_{1y}) \times \exp \left[i \sqrt{k_1^2 - k_{1x}^2 - k_{1y}^2} \right] \\
\times \exp \left[ i \left( k_{1x} x - k_{1y} y \right) \right] dk_{1x} dk_{1y}
\]  

The angular spectrum in the pupil can be separated into functions that describe the field strength \( A(k_1) \), the transmission and phase \( \mathcal{P}(k_1) \), the polarization \( \mathcal{P}(k_1) \), and the apodization \( \mathcal{B}(k_1) \) of the incident electric field

\[
\mathcal{E}^{\text{pupil}}(k_{1x}, k_{1y}) = E_0(k_{1x}, k_{1y}) A(k_{1x}, k_{1y}) \mathcal{P}(k_{1x}, k_{1y}) \mathcal{B}(k_{1x}, k_{1y}).
\]

The above functions can be arbitrary. For example, apodization function can obey the sine-condition and is then written as

\[
\mathcal{B}(k_1) = \left( k_1 / k_{1z} \right)^{1/2} = 1 / \sqrt{\cos \theta_1}.
\]

The polarization function \( \mathcal{P}(k_1) = [P_x(k_1), P_y(k_1), P_z(k_1)] \) describes the components of the electric field vector of a polarized beam that changes its direction from \( k_1 = (0,0,1) \) to \( k_1 = (k_{1x}, k_{1y}, k_{1z}) \). The three components of \( \mathcal{P}(k_{1x}, k_{1y}) \) for an incident linearly x-polarized electric field \( E_0 = (1,0,0) \) are provided by the following equations \[26, 27\]

\[
P_x(k_{1x}, k_{1y}) = 1 - \frac{k_{1y}^2}{k_{1x}^2 + k_{1y}^2}, \quad P_y(k_{1x}, k_{1y}) = -\frac{k_{1x} k_{1y}}{k_{1x}^2 + k_{1y}^2}, \quad P_z(k_{1x}, k_{1y}) = -k_{1x} / k_1.
\]

This is a representation of arbitrary vector optical field as an integral over plane waves for a known incident field in the pupil. Alternative approach can start from the known optical field in the focal plane (e.g. higher-order Gaussian beam) and follow with its expansion into plane waves. These are then propagated and the optical field is reconstructed in any position in the focal region.

The T-matrix method \[19\] is used then to compute scattered field for each incident plane wave \( \mathcal{E}^{\text{sc}}(r)_{\mathcal{E}_{1\text{in}}} \). Total scattered field \( \mathcal{E}^{\text{sc}}(r)_{\mathcal{E}_{1\text{in}}} \) is an integral over all incident wave angular spectrum components (a coherent vectorial superposition of the scattered fields coming from each of the incident plane waves)

\[
\mathcal{E}^{\text{sc}}(r)_{\mathcal{E}_{1\text{in}}} = \mathcal{E}^{\text{sc}}(r)_{\mathcal{E}_{1\text{in}}} = \frac{e^{i \theta r}}{r} \int \mathcal{E}^{\text{sc}}(r)_{\mathcal{E}_{1\text{in}}} \mathcal{E}^{\text{inc}}(r) \, d\mathbf{n}^{\text{inc}} = \frac{e^{i \theta r}}{r} \mathcal{E}^{\text{inc}}(r).
\]
Scattered and incident field are used to compute scattering $C_{sca}$ and extinction $C_{ext}$ cross sections, and averaged directional cosines for the incident $\langle \cos \Theta \rangle$ and scattered field $\langle \cos \Theta \rangle$. Radiation pressure cross section is related to momentum vectorial difference between the change of the incident wave momentum and momentum transfer to the scattered wave. The component in the z direction can be expressed as:

$$C_{prz} = \langle \cos \Theta \rangle C_{ext} - \langle \cos \Theta \rangle C_{sca}$$  \hspace{1cm} (5)

The z-component of force $F(r)$ exerted on cells can be expressed by

$$F_z = \frac{n_1}{c} I^{inc}(0,0,0) C_{prz}.$$  \hspace{1cm} (6)

where intensity of the incident field at the biological cell center

$$I^{inc}(0,0,0) = \frac{1}{2} \sqrt{\frac{\varepsilon_1}{\mu_0}} |E^{inc}(0,0,0)|^2 = \frac{C\varepsilon_1 n_1}{2} |E^{inc}(0,0,0)|^2.$$  \hspace{1cm} (7)

Formulas for the transverse force components are

$$F_x = \frac{n_1}{c} I^{inc}(0,0,0) C_{prx}, \quad F_y = \frac{n_1}{c} I^{inc}(0,0,0) C_{pry}$$  \hspace{1cm} (8)

These forces include both the scattering and the gradient forces commonly occurring separately in formulations for small particles.

Trapping efficiency, $Q_z$ is defined as:

$$Q_z(x,y,z) = F_z(x,y,z) \frac{c}{n_1 P},$$  \hspace{1cm} (9)

where $P$ is a total beam power and $c$ is velocity of light, and $n_1$ is the refractive index of surrounding medium. Similar expressions define trapping efficiencies for $x$ and $y$ directions.

### 3. OPTICAL FORCE CALCULATIONS FOR SPHERICAL AND CYLINDRICAL CELLS

The method described in Section 2 was tested on a plane wave and Gaussian beam scattering on spherical and cylindrical objects. The results of these validation tests are presented in this Section. The computations helped to understand the differences between the scattering properties of cylinders and spheres of the same volume, and were motivated by the fact, that cylindrical beads of micron size can be easier and more precisely manufactured than the spherical ones.

#### 3.1. Plane-wave scattering on cylinders

The code has been successfully tested on parameters like scattering and extinction cross-sections, anisotropy and characteristics like phase function and amplitude function using as a benchmark web available software tool from Oregon Medical Laser Center (OMLC, [http://omlc.ogi.edu](http://omlc.ogi.edu)). Tests were performed for single plane waves incident on oblate cylinders at different angles. Figs. 2 and 3 present phase function (scattered intensity vs. scattering zenith angle) for oblate cylinder of diameter to length ratio $D/L$ equal to 4 and surface equivalent radius equal to $2.79 \lambda_0$.

![phase_function](image1.png)

![phase_function](image2.png)

Fig. 2. Phase function for plane wave scattering on oblate cylinder of relative refractive index =1.04. Incidence azimuth angle = 0°. Left-incidence zenith angle = 0°, right –incidence zenith angle = 20°.
3.2. Gaussian beam scattering on spheres

We have validated our code against results obtained using Generalized Lorenz-Mie Theory (GLMT) [13,14] and published in paper [28]. The test parameters were as follows: sphere radius $r = 0.5 \, \mu m$, Gaussian-Davis beam waist radius $\omega_0 = 0.4 \, \mu m$, wavelength $\lambda = 1.06 \, \mu m$, refractive index 1.45/1.33 (silica/water). Incident light had linear polarization in transverse direction. Fig. 4 presents axial trapping efficiency obtained using each of the solvers. Note that, for this case, a stable trap exists at small positive z position (Force = 0). For other particle positions, shown in the z-axis range, the forces will drag the particle to that trap position.

Small differences may be related to different methods of computing the incident optical field. Davies beam has been used in paper [28], which is not an exact Maxwell equations’ solution in the whole focal region. Plane-wave spectrum is the exact solution, but integration over discrete plane waves may be a source of error.

3.3. Gaussian beam scattering on cylinders

We have performed parametric simulations of Gaussian-Davis beam scattering on cylinders and spheres to study how a scatterer shape influences trapping efficiency, and scattering force spatial distribution. We have assumed the following simulation input:
• Scatterer: sphere of diameter \( r = 1.0 \, \mu m \) or oblate cylinder of the same volume and height \( h = \frac{4}{3} \, \frac{r}{3} = \frac{4}{3} \, \mu m \), refractive index 1.45/1.33,
• Laser beam of wavelength \( \lambda = 1.064 \, \mu m \), power \( P = 50 \, mW \), and beam waist radius \( \omega_0 = 1.0 \, \mu m \).

Figure 5a presents the laser beam intensity in the focal region, where z-axis is directed along the beam axis, and x-axis is transverse to the laser beam propagation direction. Figure 5b illustrates variables used to describe orientation of cylinder in the focused laser beam.

Transverse trapping efficiency \( Q_x = \frac{f_x}{P \, n} \) is a normalized measure of transverse component of trapping force \( f_x \), where \( c \) is the speed of light, \( P \) is a laser beam power, and \( n \) is the host medium refractive index. For cylinder orientation \( \beta = 0^\circ \) we observe about 25% smaller values of trapping efficiency for cylinders as compared to spheres (see Fig. 6). This is due to a better fit of the high refractive index of scatterer to the high electric field values for spheres than oblate cylinders of this orientation. Such a fit minimizes the electromagnetic field energy in the system.

Axial trapping efficiency \( Q_z = \frac{f_z}{P \, n} \) is a measure of axial trapping force \( f_z \). The minimum of the axial force is located at positive \( z \) (behind the focal point), for this case at \((x = 0, z = 3.5 \, \mu m)\), as can be seen in Fig. 7. Magnitude of the transverse forces is several times higher than magnitude of axial forces.
Another convenient parameter describing the optical manipulator forces is the trap stiffness $\kappa$. Transverse trap stiffness is defined by the following formula: 

$$f_x = \kappa \times x.$$ 

Trap stiffness at scatterer position $(x = 0, z = 3.5 \mu m)$ is equal to $\kappa = 21.8 \, pN/\mu m$ for sphere, and increases with zenith angle for cylinder: $\kappa = 16.5 \, pN/\mu m$, $\kappa = 18.0 \, pN/\mu m$, $\kappa = 26.8 \, pN/\mu m$ for $\beta = 0^\circ$, $\beta = 45^\circ$, and $\beta = 90^\circ$, respectively.

Figure 8 shows asymmetry of trapping forces for cylinders if their orientation is different than $\beta = 0^\circ$ and $\beta = 90^\circ$.

4. CELL SEPARATION IN SCANNING OPTICAL FIELDS

The optical gradient field can be time-programmed to implement more complicated optical-manipulation functions. Genoptix has applied a sawtooth-scan-velocity profile to a line-shaped gradient optical field moving over a target cell population in a microfluidic device. Relative cell-motion measurements capture the characteristic response for each cell. This facilitates cell-by-cell analysis and separation of cells into distinct populations and is accomplished without the need for prior staining or labeling of the cells. The left-hand-side (LHS) snapshots in Fig. 9 show experimental results on cell collection during down stroke, while LHS snapshots in Fig. 10 present experiments on cell sorting during up stroke. The laser beam (visible as a bright line in these Figures) propagates along a normal to the image. Right-hand-side images in Figs 9 and 10 present simulations of the Genoptix experiment. Computations were performed at CFDRC using the T-Matrix and plane-wave-spectrum based approach presented in preceding Sections. We have observed that cell response is dependent on optical driving forces, cell inertia, and drag forces. Closely positioned cells can influence motion of each other through the induced
fluid motion and drag forces. We have demonstrated that simulations are in good agreement with experiments and that computations can be a useful analysis and optimization tool in such a complex multiphysics environment.

Fig. 9 Comparison of the experiment (LHS) with simulation (RHS). Smooth downward motion of scanning beam traps and moves all cells to bottom
**Fig. 10** Comparison of the experiment (LHS) with simulation (RHS). Cell sorting during up stroke. Results shown at the end of each upwards step jump of the scanning beam.
5. CONCLUSIONS AND FUTURE WORK

Computational simulation and experimental results on optical trapping and manipulation of biological cells in gradient laser fields were presented. Method of computing the optical forces exerted on cells in non-Gaussian, complicated incident optical field patterns has been described. The CFDRC approach based on the electromagnetic T-matrix method, plane-wave spectrum decomposition of the optical field, direct numerical integration of force contributions from each plane wave component, and full coupling of the fluids with optics in a bio-microfluidic device is very general. We have demonstrated several validation cases including scattering on cylinders and spheres, and computation of trap stiffness. The method has been demonstrated on simulation of experimental results obtained from Genoptix showing powerful manipulation and segregation of cells using time-programmed scanning optical fields in the individual cell-based diagnostic assays. These computational advances will be of significant importance during designs and optimization of biomicrofluidic devices utilizing optical manipulation technique.

6. REFERENCES