Optical tweezers: light for manipulating microscopic world

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Abstract: Optical tweezers make use of a tightly focused laser beam to trap, move, guide, rotate and even sort microscopic objects solely with light. Although the basic laser tweezers, making use of a TEM00 laser beam to create a single trap point, have proved to be useful for any applications in areas ranging from physics to biology, a major breakthrough in this field came as the use of computer generated holograms enabled researchers to create multiple trap sites from single laser source (holographic optical tweezers). Coupled with microfluidic techniques, holographic optical tweezers have promised development of optical techniques for high throughput sorting of different cell types under a single micro-chip platform. The holographic methods have also helped the use of specialized laser beams like Laguerre-Gaussian beams instead of the conventional laser beam for interesting applications like orienting/rotating the trapped objects or trapping cells with minimum photodamage. Further, combining optical tweezers with Raman spectroscopy is becoming increasingly popular for studying single cell biochemistry as use of optical forces to immobilize the cells under investigations not only avoids the negative effects of fixing the cells onto substrate but also improve the quality of the recorded spectra. These advanced optical trapping techniques as outlined above along with some illustrative biophotonics applications have been explored.

1. Introduction
During the past two decades after its invention, single-beam optical gradient force trap, commonly known as optical tweezers [1], has become an indispensable tool for biophotonics research [2]. When a microscopic particle is illuminated by a laser beam, it experiences two types of forces: the scattering force acts along the direction of the laser beam; and the gradient force, proportional to the gradient of light intensity (as exists in a Gaussian profiled beam), pulls the particle to the beam axis. Near the focus of a laser beam, there also exists an axial gradient force pulling the particle towards the focus, and for a sufficiently tightly focused laser beam, this force overcomes the scattering force resulting trapping of the particle at beam focus. Unlike mechanical micro tools, the optical trap is gentle and absolutely sterile and can be used to capture, move and position single cells or sub cellular particles without direct contact or significant damage.

2. Optical sorting with holographic optical tweezers
In the last decade the field of optical trapping has seen several new developments. One very significant advancement in optical trapping technique is the holographic optical tweezers [3], that uses computer generated holograms to create three-dimensional array of optical traps out of a single laser beam. The computer-generated holograms splits the incident laser beam into any desired fan-out of beams, each of which is relayed to a strongly converging objective lens and focused into a distinct optical trap. This
approach can project hundreds of simultaneous optical traps in arbitrary three-dimensional configurations. By offering trap array comprising of number of traps placed with micrometer-scale spatial resolution and real-time reconfigurability, holographic optical tweezers provide unsurpassed access to the microscopic world. For example the holographic trapping technique has proven to be useful for a wide range of filtering and sorting methods that are important for lab-on-a-chip applications. One interesting approach is to exploit the difference in degree of interactions of flowing colloidal particles (having varying size, shape or composition) with the array of traps to selectively deflect a particular set of particles away from the fluid flow direction. This approach has been successfully used for sorting of objects from a co-flowing mixture of two types of colloids using either a linear array pattern [4] or a three dimensional optical lattice [5]. We recently studied the influence of trap separation, within an array of holographic traps, on the force experienced by flowing colloids [6]. The force required by a microsphere to move from a trapping position to the next was observed to strongly depend on the ratio of the trap separation and the microsphere size as the inter traps separation was reduced. For some particular ratios the force reaches minima and a linear array of traps behaves like a potential trench consisting of small potential barriers which can be surmounted when a small external force is applied and thus particle can be guided along the array. Such size selective response of particles in an array could form a strong basis for sorting of particles of different size. In a two dimensional trap array, the trap separations in two orthogonal directions could be tailored to simultaneously lower the potential energy barriers for two different sizes of particles in two orthogonal directions. When a mixed suspension of 3 and 5 µm particles was flown diagonally to a suitably designed two dimensional array, the 3 and 5 µm particles moved along vertical and horizontal directions respectively in the array.

3. Optical manipulation with Laguerre-Gaussian beams

Another important improvement in optical trapping technique is the use of special laser beams like Laguerre-Gaussian (LG) beams, Bessel beams etc that can be generated from conventional TEM00 laser beam by inserting a suitable hologram [7]. Like for example, we have recently shown that use of LG beams provides a more efficient and gentler means for trapping biological cells [8]. Due to its annular light profile, LG01 beam is known to have better trapping efficiency as confirmed with spherical particles. Further, LG modes have lower peak intensities compared to the usual Gaussian mode for identical beam power. We realized that these properties of LG beams can offer important advantage for manipulating biological cells by reducing the level of photodamage. Since nowadays spermatozoa are being routinely manipulated by laser tweezers for assessing quality of sperm samples and assisting the in vitro fertilization method, we chose spermatozoa as the model biological cells. The results of our studies.

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\begin{align*}
    l &= 0 & l &= 5 & l &= 10 \\
\end{align*}
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![Figure 1. Trapping LG beam profiles for topological charges 0, 5 and 10 (A-C) and the corresponding orientation of a trapped RBC (a-c) respectively. Scale bar, 2.5 μm. (A-C) and 6 μm (a-c).](image)
revealed two important advantages: firstly, LG01 mode offers better trapping efficiency than TEM00 mode for highly motile spermatozoa and second, the light damage on the cells is significantly reduced with LG beams. The reason for reduced light damage on the cells is believed to be due to lowered degree of nonlinear absorption of trapping light and experiments performed with acridine orange stained cells supported this conjecture. Considering high level of concern over the genetic purity of spermatozoa the results should motivate evaluation of the special laser beams for manipulating the cells.

Another very interesting application of LG trapping beams was their use for orientation and rotation of trapped red blood cells (RBCs) [9]. It was observed that since the size of the intensity annulas of LG modes increases with the topological charge the cells get trapped at larger tilt angle with respect to the beam axis and thus provided additional control on the orientation of the cells under trap. Further, the RBCs could also be driven as micro-rotors by a transfer of orbital angular momentum from the LG trapping beam having large topological charge or by rotating the profile of LG mode having fractional topological charge. The technique for controlling the orientation of the trapped RBC may find useful applications while studying polarized Raman spectra of the cells.

4. Studying red blood cells with Raman optical tweezers

Optical tweezers are also being used for spectroscopic studies on single cells, which helps account for the problem of heterogeneity present in bulk cell samples. In particular Raman spectroscopy is receiving considerable current interest for studies of the chemical composition and conformation of macromolecules in individual cells since this technique avoids the necessity of any exogenous stain. However, due to the inherent weak nature of the Raman signal, a long acquisition time, often tens of seconds to few minutes, is required to acquire spectra with a good signal to noise ratio. The cell should therefore be immobilized. But the physical or chemical methods used for immobilization of cells in micro-Raman technique often lead to undesirable surface-induced effects on the cells or lead to strong background spectra originating from the substrate medium. The use of optical tweezers to immobilize cell without direct contact helps to avoid these problems and therefore Raman optical tweezers or a setup facilitating acquisition of Raman spectra from an optically trapped cell, are receiving much attention [10]. In particular the use of near infrared (NIR) radiation for Raman studies is gaining rapid interest due to much reduced fluorescence background that often obscures the small but important Raman bands. Raman optical tweezers have already been utilized for several interesting studies such as monitoring the real-time heat denaturation of yeast cells [11], the transition from the oxygenated to deoxygenated condition of a RBC on application of mechanical stress [12], sorting and identification of microorganisms [13] etc. Raman optical tweezers are being extensively used for studying RBCs since Raman spectroscopy is a powerful technique to monitor the oxygen carrying capacity of RBCs because the binding or the dissociation of oxygen with heme leads to significant conformational changes of hemoglobin that can be sensitively monitored by this technique [14]. For recording Raman spectra from a single optically trapped RBC, we used a Raman tweezers incorporating a 785 nm cw beam from a Ti:Sapphire laser for both trapping the cells and exciting the Raman spectra. In Fig 2 we show the mean Raman spectrum of RBCs collected from blood samples of five healthy volunteers as well as from five patients suffering from malaria (Plasmodium vivax infection). For the malaria samples investigated, the number of parasitized...
cells was ~ 1-2%, as confirmed by acridine orange staining. For this study no distinction was made between parasitized and non-parasitized cells. As compared to RBCs from healthy donors, in RBCs taken from malaria patients (IRBCs), a significant decrease in the intensity of the low spin (oxygenated-haemoglobin) marker Raman band at 1223 cm$^{-1}$ (ν13 or ν42) along with a concomitant increase in the high spin (deoxygenated-hemoglobin) marker bands at 1210 cm$^{-1}$ (ν5+ν18) and 1546 cm$^{-1}$ (ν11) was observed. These changes suggest a reduced hemoglobin-oxygen affinity for the IRBCs [15]. For this study all the spectra were acquired with trap laser beam power of ~ 2 mW and an integration time of 30 s since it was observed that at higher trap beam power the intensity of Raman bands at 975 cm$^{-1}$, 1244 cm$^{-1}$ and 1366 cm$^{-1}$, increases with exposure time, first slowly until a critical exposure time, beyond which it increase rapidly and then again levels off. The time interval at which the steep increase in intensity occurred was 90-100 s, for excitation power of ~ 5 mW and reduced to 30-40 s, at ~ 9 mW. These changes in the Raman spectra suggest photoinduced aggregation of intracellular heme [16]. Further the simultaneous decrease in intensity of the Raman band at 1544 cm$^{-1}$ (ν11) suggests photo induced hemichrome formation in the cells [17].

5. Conclusion
The important recent developments in the field of optical tweezers have been briefly discussed alongwith some useful applications of these. It is pertinent to note here that being fairly new techniques the full potentials of holographic optical trapping and Raman optical tweezers are yet to be explored.

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