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# Optical Deformation of Biological Cells using Dual-Beam Laser Tweezer

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***Abstract*— Optical tweezer is a non-contact tool to trap and manipulate microparticles such as biological cells using coherent light beams. In this study, we utilized a dual-beam optical tweezer, created using two counterpropagating and slightly divergent laser beams to trap and deform biological cells. Human embryonic kidney 293 (HEK-293) and breast cancer (SKBR3) cells were used to characterize their membrane elasticity by optically stretching in the dual-beam optical tweezer. It was observed that the extent of deformation in both cell types increases with increasing optical trapping power. The SKBR3 cells exhibited greater percentage deformation than that of HEK-293 cells for a given trapping power. Our results demonstrate that the dual-beam optical tweezer provides measures of cell elasticity that can distinguish between various cell types. The non-contact optical cell stretching can be effectively utilized in disease diagnosis such as cancer based on the cell elasticity measures.**

## I. INTRODUCTION

The cellular cytoskeleton, which is a complex network of microfilaments, microtubules, intermediate filaments and linkers, contributes to the mechanical characteristics of the cell [1]. Malignant transformations of cells are closely associated with remodeling of the cytoskeleton, which also contribute to the alterations in mechanical stiffness of the cell membrane [2–4]. An optical tweezer (OT) [2] uses laser beams for non-contact trapping of microparticles. When a particle, such as a biological cell, comes in the path of light, part of the momentum from the light beam is transferred to the particle. This momentum transfer exerts a proportional force on the particle's surface in the direction of light [3],[4], which is responsible for trapping, and potentially deforming, the particle. The interactions of light with dielectric matter such as biological cells result in two types of forces [2]: The gradient force, which works to move particles towards regions of greater intensity of the laser beam, and the scattering force, which is the result of momentum transfer from photons to the cell. The OT uses the scattering forces to trap and deform biological cells. These forces are caused by radiation pressure from light leaving one medium and entering a dielectric material with a different refractive index, particularly from low to high refractive indices.

The cell deformation in an OT can be quantified to provide a comprehensive characterization of cell's elasticity. By integrating microfluidics into the design of the OT, it is possible to achieve great efficiency in cell trapping and analysis. The optical deformation of cells in an OT has been shown to be a useful mechanical marker for distinguishing between healthy and diseased cells [5].

In this study, we utilized a dual-beam OT setup by employing two counter-propagating laser beams to trap and stretch cells for characterizing their membrane elasticity. We hypothesized that the alteration of actin filaments, which are one of the constituents responsible for cell shape and structure would contribute to changes in cytoskeletal elasticity that are detectable by trapping and stretching of cells in an OT.

## II. METHODS

### A. Dual beam optical tweezer theory

Light is made up of electromagnetic radiation that carries momentum in the direction of propagation which is proportional to its energy. If a cell in a buffer solution bends light, the momentum of light beam changes, and since the momentum is conserved, the object experiences an equal and opposite change in momentum [6]. The momentum change, $\Delta p$, is given by,

$$\Delta p = \Delta p_i - (\Delta p_r + \Delta p_t) \quad \text{(1)}$$

where $\Delta p_i$ , $\Delta p_r$ and $\Delta p_t$ are the momentums of the incident, reflected, and transmitted rays, respectively.

By Newton's second law, a force, *F*, called scattering force, is exerted on the cell surface in the direction of light, which is proportional to $\Delta p$, as given below.

$$F = \frac{\Delta p}{\Delta t} = \frac{n_1 * \Delta E}{c * \Delta t} = \frac{n_1 * Q * P}{c} \quad \text{(2)}$$

where $n_1$ is the lower refractive index of the two media, $E$ is the energy in the light beam, $c$ is the speed of light in vacuum, $P$ is the power of incident light and $Q$ is the trapping efficiency. Since momentum transfer occurs due to light scattering at both the front and back surfaces of the cell, the cell experiences force on both its front and back surfaces. This results in two components, $Q_{front}$ and $Q_{back}$ , corresponding to the trapping efficiency at the front and back surfaces, respectively. Figure 1A illustrates the reflected and transmitted components when a ray of light is incident on a spherical cell.

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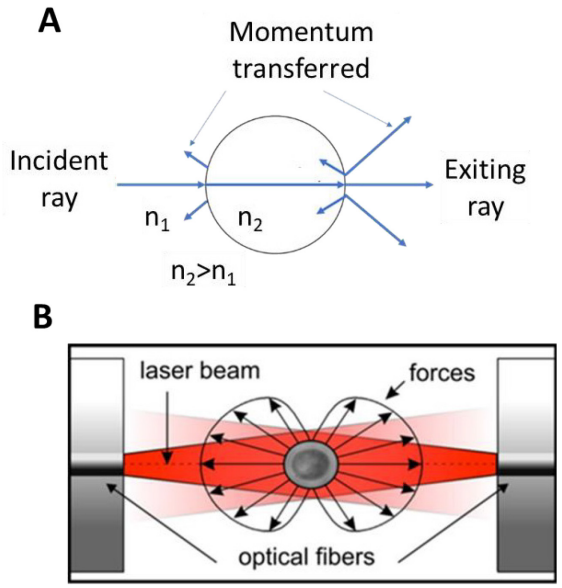


Figure 1. A) Reflected and transmitted components of a light ray incident on a spherical cell. B) Trapping and stretching forces on the surface of a cell trapped in a dual-beam OT [10]. Cell rotation occurs when the forces are out of balance.

Therefore, it is possible to leverage the symmetric surface forces acting on the trapped dielectric object surface to stretch the object along the beam axis when two counter-propagating, slightly divergent laser beams are employed. The stress on surface of the trapped biological cell is given by:

$$\sigma = \frac{n_1}{c} * \frac{P}{A} Q = \frac{n_1}{c} * I * Q \,, \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where A is the biological cell's surface area, and $I$ is the Gaussian beam's intensity. This surface stress is sufficient to deform the trapped cell at higher optical powers. Figure 1B illustrates the forces experienced by the surface of a cell when trapped in a dual-beam OT. This surface stress may be used to stretch biological cells and determine the stiffness/elasticity of their membranes. The phenomenon of cell stretching in a dual-beam laser tweezer is useful in the study and detection of disorders affecting the cytoskeleton composition such as breast cancer.

*B. Experimental Setup*

We implemented a dual-beam optical tweezer setup (shown in Figure 2) to optically trap and deform cells. The system uses two counterpropagating laser beams (975 nm wavelength which exhibits low water absorption and biological heating [7]) derived from two separate sources, with a maximum power of 600mW and 700mW, respectively. The beams were carried to the trapping stage via multi-mode optical fibers. Two multi-axis positioning stages were used to precisely align the counter-propagating optical fibers under a microscope (Zeiss Axioscope40), equipped with a 20x objective for stage viewing throughout the experiment. Before the trapping experiments, the fibers were cleaved, and the output optical power was calibrated using an optical power meter and a photodetector. For the live recording of video and images captured during the experiment, a digital CMOS AmScope camera was mounted on the microscope. The cells were suspended using a drop of Hank's Balanced Salt Solution (HBSS) with no calcium, magnesium or Phenol Red on polydimethylsiloxane (PDMS) substrate (7x7x3 mm dimensions). Because PDMS is hydrophobic, it enables the drop to create a dome shape, allowing the cells to move freely throughout the trapping experiment. The fibers were then brought closer to each other inside the drop, creating a gap of approximately 110 µm between them where the cells were trapped and stretched. The average optical power in the fibers was varied between 80 and 500 mW. The trap was maintained for 20 seconds at each power and images were captured at every 1 second. Thus, for each power, the camera recorded 20 frames at an interval of 1 second each.

The images recorded during the trapping experiments were processed in MATLAB to delineate cell borders and calculate the percent deformation. The deformation of cell causes its shape to change from spherical to ellipsoid. Aspect ratio of the deformed cell in each recorded frame was calculated as the ratio of lengths of the longest-axis to shortest-axis of the ellipse.

$$Aspect\ ratio = \frac{xy}{wz} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where $xy$ represents the length of the longest axis and $wz$ represents the length of the shortest axis of the cell image. The longest axis $xy$ may not necessarily be parallel to the beam axis, especially when cells rotate in the trap. Optical deformation was then determined as:

$$D(\%) = (\frac{xy}{wz} - 1) * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

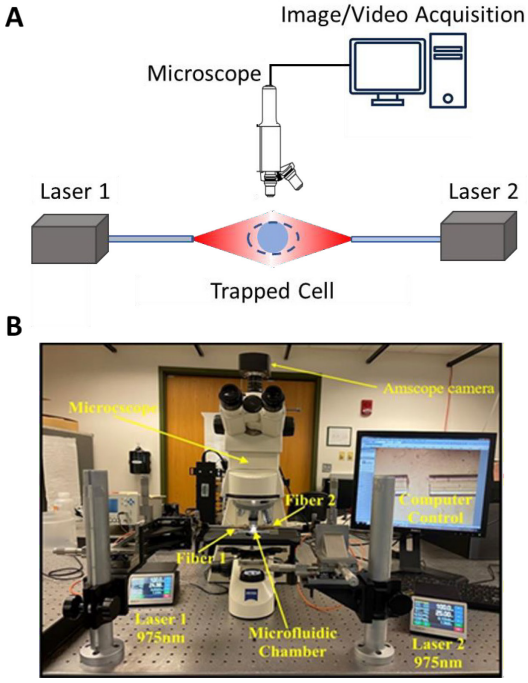


Figure 2. A) Schematic and B) photograph of the dual-beam OT setup used to trap and stretch biological cells.

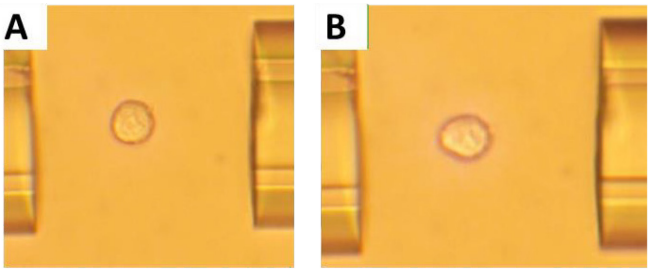


Figure 3. Snapshots of a trapped SKBR3 cell (A) at an initial trapping power of 117.88mW and (B) stretched at higher trapping power of 412.01mW.

### *C. Cell Preparation*

Two adherent cell lines were grown and used for experiments, namely, HEK-293 cells derived from Homo sapiens embryonic kidney cells (ATCC, CRL-1573), and human epidermal growth factor receptor, HER2, positive human breast cancer cell line SKBR3 (ATCC, HTB-30). The HEK-293 cells were grown in Eagle's Minimum Essential Medium EMEM (ATCC, 30-2003) and SKBR3 cells were grown in McCoy's 5A medium (ATCC, 30-2007) plus 10% Fetal Bovine Serum (Fisher Scientific, 16-143-063) in a humidified 37ºC incubator in the presence of 5% $CO_2$. Cells were fed every 2-3 days and were passaged in a 1:6 ratio for HEK-293 and 1:2 ratio for SKBR3 when they become 70 – 80% confluent. To passage the cells, 0.25% Trypsin-EDTA was used. Trypsinized single cells were washed in 10 mL HBSS without Phenol Red, calcium or magnesium (Fisher Scientific, MP091810554) by centrifuging at 2000 rpm for 5 minutes. The pellet was resuspended in 10 mL HBSS and kept on ice during the trapping experiments. The lack of phenol red prevents optical interference with the laser; lack of calcium and magnesium reduces the chance of cells adhering to each other. To alter the expression of actin, the cells were treated with an actin inhibitor, cytochalasin D (Tocris Bioscience, 1233/1) using 10mM in Dimethyl sulfoxide (DMSO) for 2 hours prior to trypsinization. Cells treated with the same volume of DMSO for 2 hours were used as control.

## III. RESULTS AND DISCUSSION

Figure 3 shows an example of a trapped SKBR3 cell at an initial trapping power (117.88 mW, Panel A) and at higher trapping power (412.01 mW, Panel B). The stretching of the cell in the direction of light (horizontal) at higher optical powers is evident from the figure. The aspect ratio of cell in Panel A is closer to 1 because the cell is still maintaining its spherical shape. However, as the cell starts deforming (e.g., in Panel B), the aspect ratio become greater than 1.

Figure 4A shows deformation of both cell types treated with DMSO (Control) at varying trapping powers. The error bars at each data point represent standard deviation in the percentage deformation based on 20 frames acquired for each trapping power. It is evident that as power increases, the extent of deformation in both cell types increased monotonically. However, the SKBR3 cells exhibited greater deformation than the HEK-293 cells for a given power. This implies that the cytoskeletal elasticity of SKBR3 cells is higher than that of HEK-293 cells.

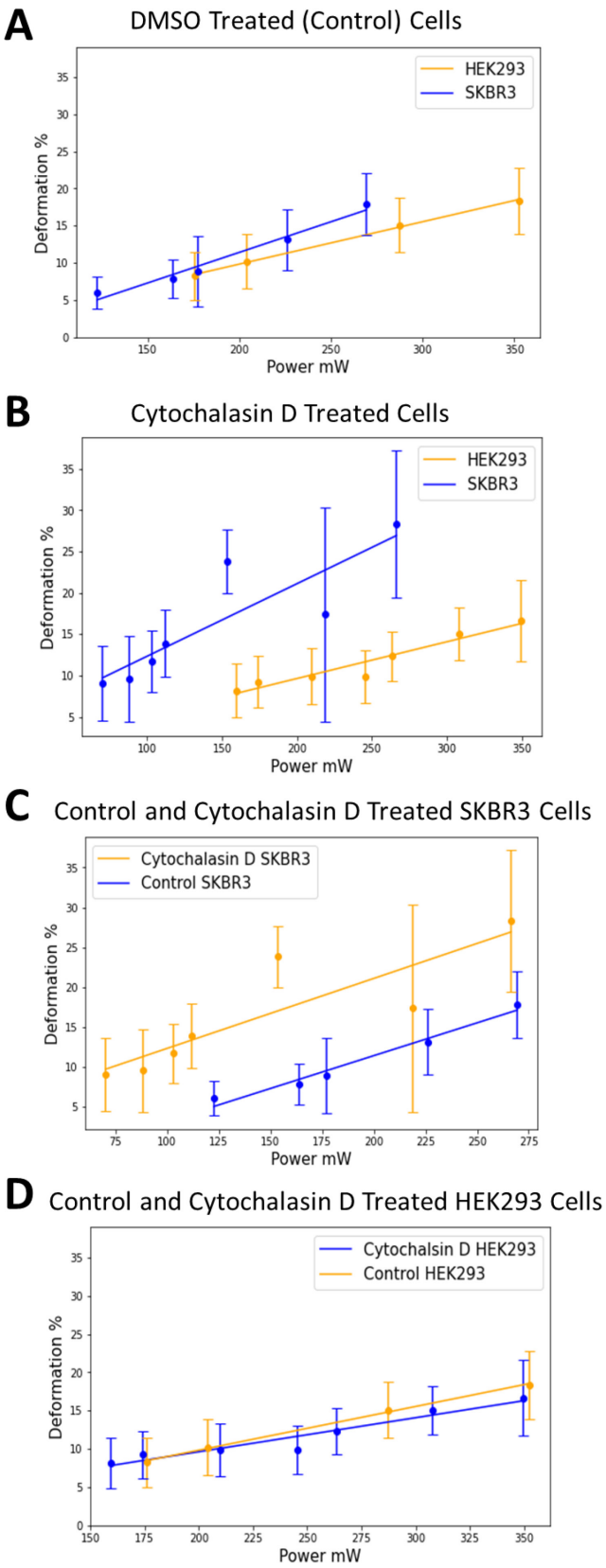


Figure 4. Percentage deformation of cells in dual-beam OT at varying trapping powers. A) DMSO-treated (Control) HEK-293 (n=2) and SKBR3 (n=4) cells, B) Cytochalasin D-treated HEK-293 (n=4) and SKBR3 (n=2) cells, and comparison of Control and Cytochalasin D-treated C) SKBR3 and D) HEK-293 cells.

We further tested our hypothesis that the cytoskeletal elasticity of a cell is inversely proportional to the actin expression and thus, the actin expression can be quantified based on the cell deformation in the OT. Figure 4B shows the percentage deformation in the two cell types when they were treated with actin inhibitor cytochalasin D. The HEK-293 cells exhibited slightly increased deformation at each power when treated with cytochalasin D compared to control. However, it can be observed from Figure 4D that the extent of deformation in cytochalasin D-treated HEK-293 cells is not significantly different than that in Control. Cytochalasin D-treated SKBR3 cells, on the other hand, exhibited significantly higher deformation at each trapping power when compared to control. For example, the Control SKBR3 cells had a mean deformation of 17.5 % at trapping power of 270 mW whereas cytochalasin D-treated SKBR3 cells had mean deformation of 26% for the same power, an increase of ~9 percentage points. Figure 4C shows a direct comparison of percentage deformation in control vs. cytochalasin D -treated SKBR3 cells. It is clear from the plot that the cytoskeletal elasticity of SKBR3 cells increases substantially when actin expression was reduced. Further analysis needs to be done to ascertain if there exists a threshold of deformation beyond which the cell membrane cannot return to its original shape. Our OT setup used simplistic method of trapping cells inside a drop instead of passing the cells through a microfluidic channel. This puts constraints on the stability of traps in that the trapped cells were free to rotate around the axis of laser beams as well as perpendicular to the light path due to slight misalignment of the optical fibers. The cell rotation at 90 degrees caused reversal of deformation which led to decrease in the aspect ratio measurements. The effects of cell rotation are reflected in our results in the form of large standard deviations when a mean of 20 frames was calculated. We used two separate laser sources for better control on the power in individual beams, however, it may also cause minor optical mis-alignment. We quantified the cell deformation in terms of aspect ratio, however, further analysis based on volumetric changes would be more insightful. Notwithstanding these limitations, our results demonstrate a clear ability of our optical trap to distinguish between the two cell types based on their membrane elasticity.

Cell's cytoskeletal changes are significant in diseases such as cancer [8]. The malignant transformation, which can progress to infiltration of tumor cells into tissue, and metastatic spread of cancer cells involves dramatic spatial and temporal reorganization of cell's cytoskeleton. It has been reported that more aggressive breast cancer types are associated with alterations in actin components of the cytoskeleton which affects the biomechanical properties, and thus aggressiveness and metastatic potential, of tumor cells [9]. Our results suggest that it may be possible to quantify the effects of actin filamentation in cells based on their behavior in the dual-beam OT. The actin filament formation and expression in malignant cells may be sufficiently different and therefore be distinguishable from non-malignant cells using OT, as demonstrated here. Further experimentation is warranted to mechanistically ascertain these preliminary observations.

## IV. Conclusions

The optical stretcher is a non-invasive and label-free tool that can be used to quantify cell's elasticity based on the cell deformation in OT. We utilized a dual-beam OT to trap and deform two cell types, namely, HEK-293 and SKBR3 cell lines. In both cell types, the percentage cell-deformation increased with increasing trapping powers. The extent of cell-deformation increased significantly in SKBR3 cells when actin-inhibitor cytochalasin D was used, suggesting increased elasticity. The increase in deformation in cytochalasin D-treated HEK-293 cells was insignificant, indicating a cell-type difference in response to optical stretching. Using OT-dependent cytoskeletal elasticity measures as a malignancy indication might contribute to detection strategies in cancer research.

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