Optoelectronic Tweezers (OET) trap stiffness with HeLa cells

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ABSTRACT

Optoelectronic Tweezers (OET) creates patterned electrical fields by selectively illuminating a photoconductive layer sandwiched between two electrodes. The resulting electrical gradients are used to manipulate microscopic particles, including biological cells, using the dielectrophoresis (DEP) force. Previously it has been shown that up to 15,000 traps can be created with just 1 mW of optical power[1], and that OET traps are 470 times stiffer than traps created with optical tweezers of the same power[2]. In this paper we explore the use of OET for trapping HeLa cells. First, experiments are performed using glass beads as a model particle, and the results are compared with numerical simulations to confirm our ability to model the electrical field gradients in the OET device. We then track trapped HeLa cells in different sizes of traps, showing maximum cell velocities of 60 μm s^{-1} using an illumination intensity of just 2.5 W cm^{-2}. We measure the electrical properties of the cell's membrane by analyzing the cell's DEP frequency response and use this information to model the forces on the cell. We find that it is possible to create a trap with a stiffness of $3x10^{-6}$ N m^{-1} that does not vary with position within the trap.

Keywords: Micromanipulation, Optoelectronic Tweezers (OET), Dielectrophoresis, HeLa cells

1. INTRODUCTION

The manipulation of microscopic particles is an expanding field consisting of many complementary techniques. As the trend of miniaturization continues in many fields, established techniques including Optical Tweezers (OT)[3] and Dielectrophoresis (DEP)[4,5] are finding many new applications from the positioning of nanowires[6] to the study of biology[7,8]. A technique that combines some of the benefits of both of these techniques is Optoelectronic Tweezers (OET). Here the ability of OT to continually reconfigure the shape of the applied forces is combined with the low power requirements and ability of DEP to control the sign of the force by varying the AC frequency. This is achieved by selectively illuminating a photoconductor in the desired pattern whilst placing an electrical field across it, resulting in light induced dielectrophoresis.

OET has been shown to be capable of manipulating cells[9] and in this report we will further explore this capability. An ideal manipulation method would allow not just single cell control but precise control over each cell being trapped as well as the ability to trap many cells at one time. To fully describe the control afforded by the OET trap it is necessary not just to find the trap stiffness of a trapped cell but to move the cell around within the trap and study how the force on the cell changes with position. Although such experiments have been conducted using trapped beads as a model particle, the forces experienced by a cell will be different due to the difference in conductivity, size, and complexity of the cellular structure.

1.1 Optoelectronic Tweezers (OET)

The OET chamber consists of two indium-tin-oxide (ITO) coated glass substrates, one of which is capped by a layer of photoconductive material (Figure 1). The two substrates are used to sandwich a layer of liquid containing the particles under manipulation. When a voltage is placed across the ITO layers, the electric field pattern in the liquid is controlled by selectively illuminating the photoconductor.

Any non-uniformity in the electric field results in a gradient that induces a dielectrophoretic force on any polarizable particle in the liquid (see section 1.2). By altering the light pattern, we can change where these forces act, and thus continuously control the position of the particle.

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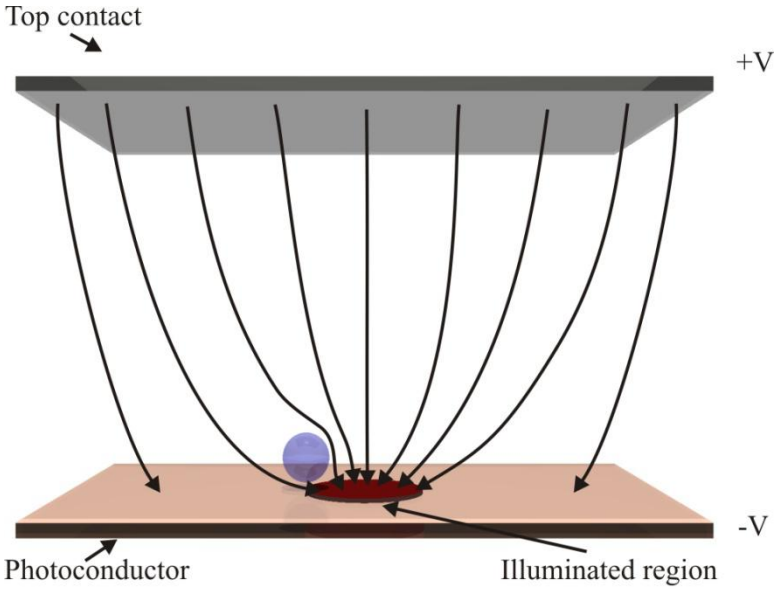

Fig. 1. The OET chamber consists of particles suspended in a fluid sandwiched between a top contact of ITO-coated glass and a bottom contact of ITO-coated glass topped by a photoconductive layer. When a voltage is placed across the contacts and an area of the photoconductor is illuminated, an electrical field pattern is created with large field gradients near the edges of the illuminated area.

1.2 Dielectrophoresis (DEP)

Dielectrophoresis (DEP) is the electrical force experienced by a neutral but polarizable particle exposed to electric field gradients. An electric field creates a charge dipole within the particle. If the electric field is uniform, the force on one pole is equal and opposite to the force on the other pole. However, if there is a non-uniformity in the field, the forces do not cancel and a net DEP force is experienced. The magnitude of the force can be calculated from[5]:

$$F = 2\pi r^3 \varepsilon_m \,\mathrm{Re}[k(\omega)]\nabla E^2 \tag{1}$$

where r is the radius of the particle, ε_m is the permittivity of the particle suspension media, Re[$k(\omega)$] is the real part of the Clausius-Mossotti factor and ∇E^2 is the gradient of the electric field squared. Re[$k(\omega)$] is a term that depends on the relative permittivity of the particle and the suspension medium, given by:

$$k(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \tag{2}$$

where ε_p^* and ε_m^* are the complex permittivities of the particle and medium respectively. If the particle is more polarizable than the medium, the Clausius-Mossotti factor is positive, resulting in a positive force, or a force directed towards a high electric field region. If the particle is less polarizable, a negative force results, and particles are repelled from a high electric field region. However, due to the frequency dependant nature of the permittivity of some combinations of particle and medium materials, the Clausisu-Mossotti factor can change from positive to negative at different frequencies. This is the case for HeLa cells in the isotonic medium used for OET trapping (10 mSm^{-1} isotonic solution, see section 4.2). The frequency at which the force switches from positive to negative can be used to determine some electrical properties of the cells, as will be discussed later.

2. OPTICAL SETUP

The setup used to create the optical patterns is shown in figure 2. A DLP data projector (Dell 2400MP) was adapted by removing the projection optics in front of the digital micromirror device (DMD) chip. The low illumination intensity requirement of OET allows us to use the standard projector lamp as an excitation source. This greatly simplifies the optical setup as the DMD is already well aligned to the light source in the projector, making it easier to align the projector with the rest of the optical setup.

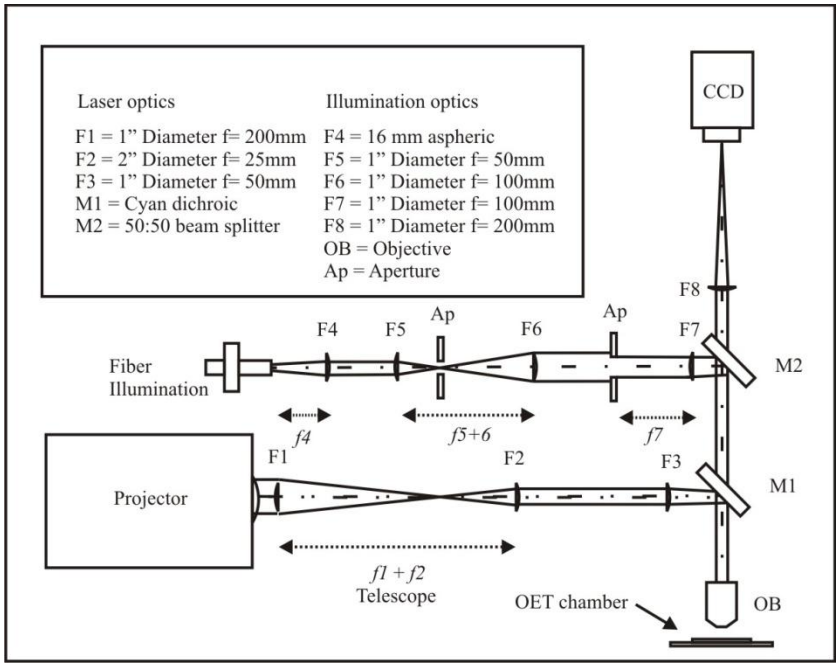

Fig. 2. The optical setup used to create the OET trapping patterns. The pattern created by the projector reduced in size, then focused by the objective onto the OET chamber. A fiber illuminator is used to provide illumination for observation. The image of the particles under manipulation is focused onto a CCD camera.

The rest of the setup is a simple microscope created using the 30-mm cage system from Thorlabs. Illumination light from a fiber illuminator is controlled using Köhler illumination before being reflected down through an infinity-corrected objective by a 50:50 beam splitter. The Köhler illumination focuses the light through the first aperture, which can be adjusted to control the brightness of the illumination. The illumination light is then collimated before passing through the second aperture, which can be used to control the size of the field being illuminated. The images from the projector are projected in red, so the cyan dichroic reflects them through the objective onto the sample chamber. When images of the particle being trapped are viewed with the CCD camera, this dichroic filters out most of the light from the projector, resulting in the projected pattern being visible, yet faint enough to not obscure viewing of the cells under manipulation.

3. SIMULATION

The OET chamber was modeled with COMSOL Multiphysics using the "Quasi Statics, Electric" section of the Electromagnetics module. This section uses the quasistatic approximation to simplify Maxwell's equations using two separate approximations. First, it is assumed that the fields vary slowly in space. This is true if the wavelength associated with the applied AC voltage is much larger than the geometry we are considering. We use AC frequencies in the hundreds of kHz, which results in associated wavelengths of hundreds of meters. These wavelengths are much larger than the geometry under consideration, which is a cube 100 μm along each edge. The second approximation is that the coupling between the electric and magnetic fields can be ignored. This is true if the skin depth for each layer of the

chamber is much larger than the layer's thickness. For the materials used, all of the skin depths are larger than the respective layer's thickness. ITO has the thinnest skin depth, at 1.1 mm, but this is still much larger than the 200nm thickness of the ITO layer.

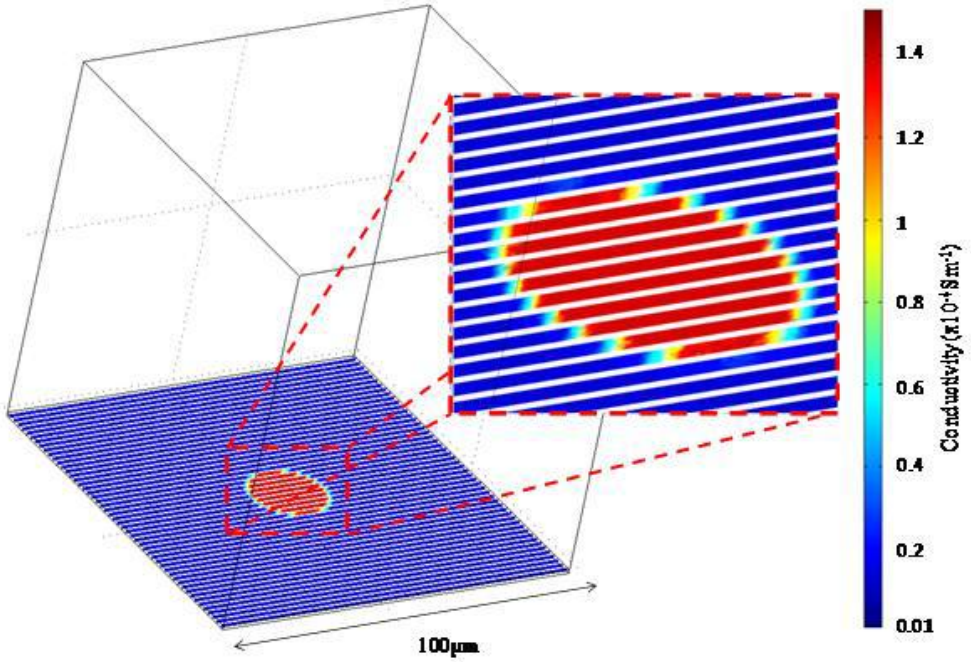

Fig. 3. Numerical simulations were performed using COMSOL Multiphysics. The effect of the light on the photoconductive layer is modeled as a change in conductivity with a saturated Gaussian profile that matches the measured optical profile.

Each layer of the OET chamber is defined as a geometry object with an associated electrical permittivity and conductivity. The effect of the light is modeled as a saturated Gaussian of the conductivity profile in the photoconductive layer (Figure 3). Boundary conditions are set to represent the voltage applied across the chamber. Using finite element modeling (FEM) COMSOL splits each geometry object into small elements and solves Maxwell's equations across them (using the aforementioned approximations). This calculates the electric fields within the chamber, from which the DEP force can be determined.

4. RESULTS

To study the trapping force as a function of the particle's position within the trap, experiments were performed where a trapped particle is dragged at a constant velocity through the fluid medium. This is accomplished by moving the OET chamber on a motorized stage. The videos of the trapped particle can then be analyzed using particle tracking software to calculate the force versus position profile of the OET trap[2].

4.1 Negative OET trapping

The illuminated pattern on the photoconductor produces the greatest electrical gradients when the resistance of the medium within the chamber is such that the majority of the electrical field is across photoconductor when it is dark and across the liquid when the photoconductor is illuminated. The photoconductor used in these experiments is amorphous silicon (a-Si), which has been measured to give a dark conductivity of $1x10^{-6}$ S m^{-1} and an illuminated conductivity of $1.5x10^{-4}$ S m^{-1}, at an illumination intensity of 2.5 W cm^{-2}. This gives an optimal liquid conductivity of 10 mS m^{-1}. Glass beads suspended in liquid of this conductivity give a negative DEP response and can be trapped by illuminating a ring around the particles (Figure 4).

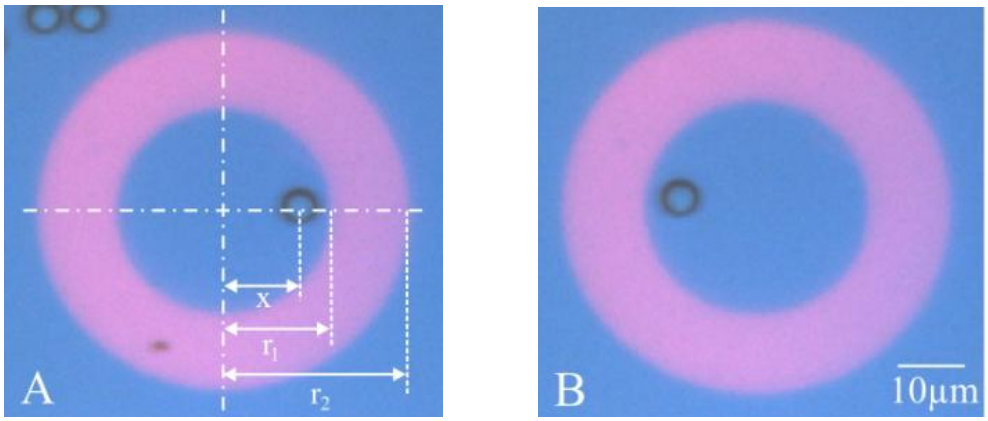

Fig. 4. Under the negative DEP conditions, the particle is repelled by the illuminated regions. A trap can be created by illuminating a ring around the particle. When the stage is translated to the right, the particle sits within the trap as shown in A. When the stage is translated to the left, the particle sits within the trap as shown in B.

In these experiments, 5μm diameter glass beads were used suspended in a KCl solution of the desired conductivity. This results in a negative DEP force on the particle, such that it may be trapped by illuminating a ring around the particle. The stage was moved at an increasing velocity until the viscous drag on the particle was greater than the trapping force, causing the particle to fall out of the trap. An AC voltage of 10Vp-p at 100 kHz was used, which resulted in a maximum velocity of 25 μm s^{-1}.

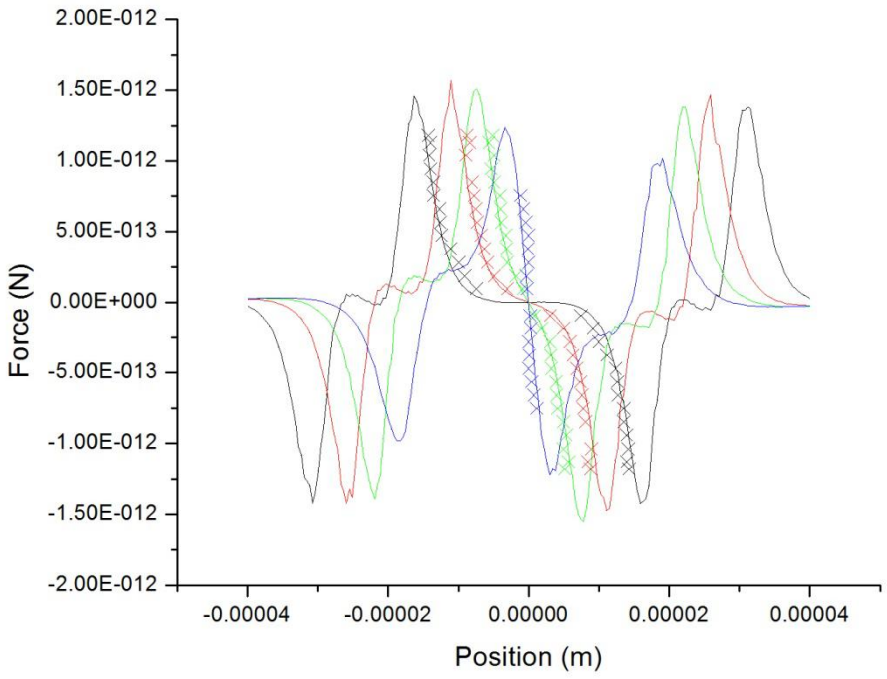

Fig. 5. The experimental results (crosses) are compared with simulated results (solid lines) for the force profile of the ring traps of different radius. The width of the illuminated line was kept constant (13 μm) and the radius r_1 was varied: r_1 = 3.9 (blue), 8.2 (green) 11.9 (red), 17.2μm (black). The error in the measured positions is roughly equal to one pixel of the analyzed image, or 0.2 μm.

As the stage velocity increases the particle is forced further from the center of the trap. For the larger traps it can be seen that the force versus position profile is highly non-linear, with a small force moving the particle far from the center of the trap (Figure 5). Even as the particle approaches the illuminated region, the force does not increase linearly with distance, but follows a curved profile. However, as the trap size is reduced, the trap approaches the ideal case where the force is linearly proportional to the distance from the trap center, resulting in a constant trap stiffness of 8×10^{-7} N m^{-1}. This linear operation is at the cost of a reduced maximum force that can be exerted on the particle. The reduction in force is due to the increase in the vertical DEP force that pushes the particle away from the substrate and thus away from the areas of high lateral DEP force. This vertical DEP force is increased when the trap size is reduced due to the additive effect of the vertical force from each side of the trap.

4.2 Positive OET trapping

For live cells to remain viable it is necessary to balance the osmotic pressure of the liquid and the cells. The OET device has an additional requirement that the osmotically-balanced solution has a relatively low electrical conductivity. This is achieved by re-suspending the cells in an isotonic sugar solution of 8.5% sucrose and 0.3% dextrose. Cells can be manipulated using OET in higher conductivity solutions using a photo-transistor based device[10], however this does necessitate a more complicated microfabrication process to produce the OET chamber.

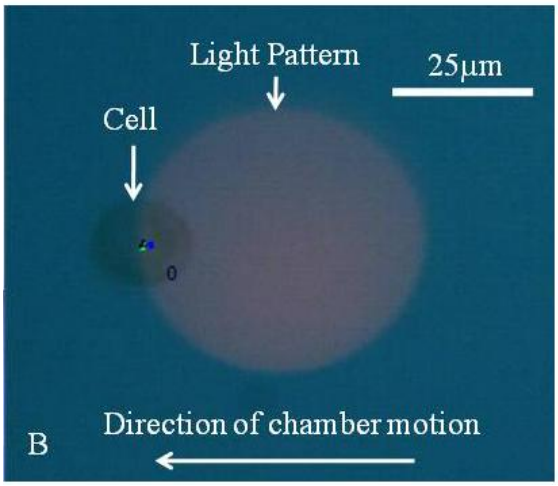

Fig. 6. HeLa cells in an isotonic sugar solution can exhibit a positive DEP force. This attracts the cell to the illuminated region where the electric field is strongest. The maximum force on the cell is exhibited at the edge of the illuminated region where the electrical gradient is the highest.

It is possible to calculate the DEP response of the cells by considering them as an insulating shell (the cell membrane) surrounding a conductive core (the cytoplasm). We do this by defining an effective permittivity for the cell using[5];

$$\varepsilon_{1eff}^{*} = \varepsilon_{2}^{*} \frac{\left(\frac{r_2}{r_1}\right)^3 + 2\frac{\varepsilon_1^{*} - \varepsilon_2^{*}}{\varepsilon_1^{*} + 2\varepsilon_2^{*}}}{\left(\frac{r_2}{r_1}\right)^3 - \frac{\varepsilon_1^{*} - \varepsilon_2^{*}}{\varepsilon_1^{*} + 2\varepsilon_2^{*}}} \tag{3}$$

Where ε_1^{*} and ε_2^{*} are the complex permittivities of the core and shell respectively and r_1 and r_2 are their radii. We can then put this effective permittivity into equation 2 to calculate the DEP force. The frequency dependence of the complex permittivities give a frequency dependence of the DEP force on the cell, which goes from a negative force at low frequencies to a positive force at high frequencies. The point at which the force crosses from negative to positive is dependent on the relative permittivity of the cell and the medium, and is known as the crossover frequency. Thus, by suspending cells in solutions of different conductivities, (the isotonic sugar solution was mixed with different amounts of growth medium to increase its conductivity) the size and conductivity of the cells membrane can be deduced. Figure 7 shows the results of experiments to measure the crossover frequency as a function of medium conductivity. These are

plotted against models written in Matlab, with the thickness and conductivity of the insulating shell varied to match to the experimental results. From these simulations it was found that the best match was for a shell 5 nm thick shell that has a conductivity of 0.87 μS m^{-1}. This agrees well with the literature where the membrane thickness has been measured as 4.97 ± 0.2 nm by scanning electron microscopy[11].

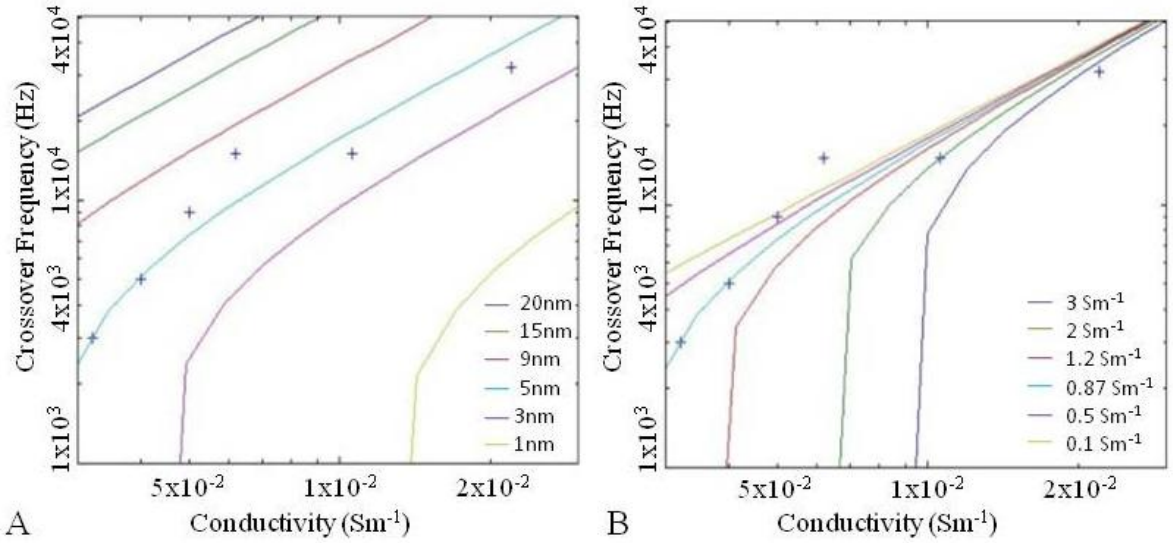

Fig. 7. The frequency at which the DEP force crosses from positive to negative was measured for cells suspended in a variety of medium conductivities. These are plotted as points and compared to models of a single insulating shell of A) various membrane thicknesses and B) various membrane conductivities, surrounding a conductive core.

Using these fitted values for the thickness and conductivity of the cell's membrane and values for the cell's cytoplasm from the literature (cytoplasm $\varepsilon_r = 50$, $\sigma = 0.53\,Sm^{-1}$, membrane $\varepsilon_r = 7$[11,12]) a value of 0.8 was calculated for the Clausius-Mossotti factor for 1x10^5 Hz. Using this value it is then possible to calculate the DEP force if the gradient of the electric field is known. Simulations were performed as described in section 3 for the four cases that were experimentally measured, e.g. optical spot sizes of 12 μm, 31 μm, 49 μm, and 73 μm in diameter.

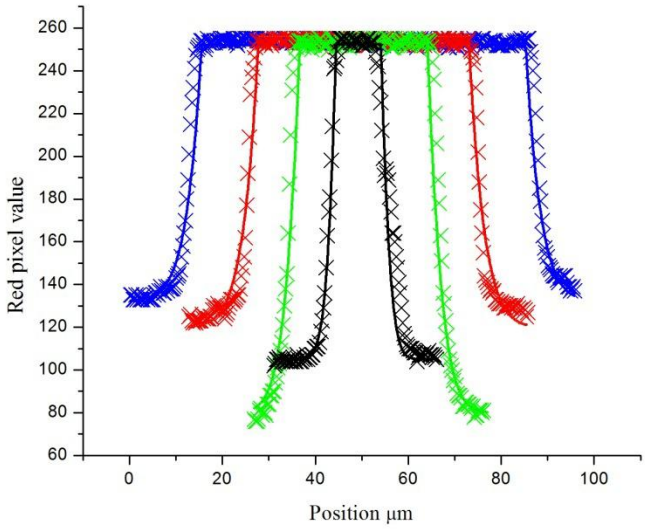

Fig. 8. The optical spot sizes were measured by taking images with the CCD camera and plotting the values of the red pixels versus their positions (points), FWHM were blue 73μm, red 49μm, green 31μm, black 12μm. These were then fitted to saturated Gaussians (lines) the parameters of which were then used in the simulations.

To get an accurate model of the force it was also necessary to consider how the intensity varies at the edges of the optical spots. This is crucial as it is at the edges of the light pattern where the horizontal electrical gradients are produced. Thus the trap created by a Gaussian laser beam (as has been previously studied[2]) will be very different from the traps created here by an image from a data projector. By analyzing images of the optical spots it was found that their profiles matched well to a saturated Gaussian (Figure 8). The parameters of these Gaussian fits were then entered into the conductivity of the photoconductive layer in the COMSOL simulations.

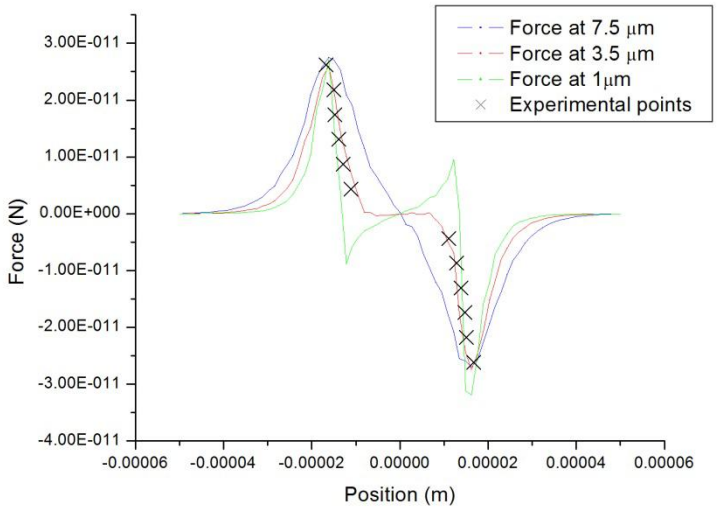

Fig. 9. The experimentally measured points are plotted and compared to simulated forces (reduced to match the magnitude of the experimentally measured forces) for different heights from the photoconductive surface.

The simulated electrical field gradients were used to calculate the trapping force and then compared to the forces experimentally measured on the trapped cells. The forces were calculated from the measured cell velocities using the Stokes drag equation with Faxen's correction for the proximity of the substrate[7]. The HeLa cells have a diameter of 15 μm, so at first we compared the electrical gradients at 7.5 μm from the surface as an average over the volume of the cell. Although this approach gave a good agreement for the smaller 5 μm beads, it was found that the simulated and experimentally-measured force profiles for the cells were very different (Figure 9).

It was found that the shape of the force profile at 3.5 μm above the photoconductive surface fitted the experimentally measured profile well, although the magnitude of the simulated force was 4 times that of the measured force. Thus, Figure 9 shows the simulated forces for a height 3.5 μm above the photoconductive surface, reduced by a factor of 4.

This gives good agreement for all four spot sizes (see figure 10). The fact that the cells act as if they were closer to the surface is reasonable, as the forces closer to the substrate are stronger, and have a greater effect on the force profile the cell experiences. This also explains why we have to reduce the force, as one of the assumptions we make in calculating the DEP force is that the gradient of the field does not change significantly over the volume of the particle. This assumption is not correct in this case. As the forces closer to the surface are greater than the average force on the cell's volume, assuming that the force is equal over the volume of the cell to the force at 3.5 μm from the surface will give an overestimate of the force. The reduction factor of 4 is chosen as it gives good agreement between the experimental and theoretical results.

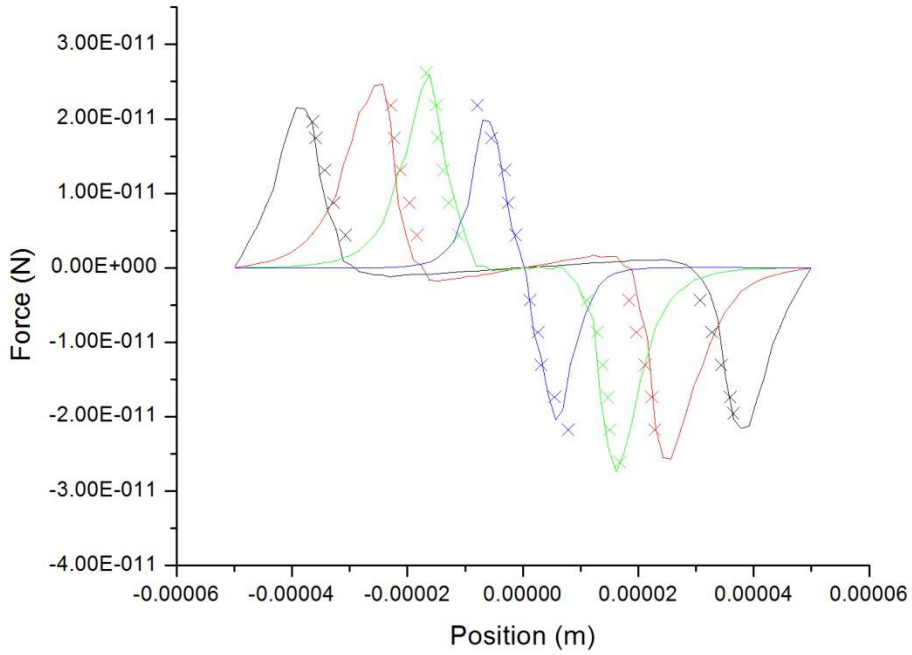

Fig. 10. For four optical spot sizes the experimental results are plotted as points and compared to simulated forces, plotted as lines, at 3.5 µm from the photoconductive surface. The 73µm spot is in black, the 49µm spot in red, the 31µm spot in green and the 12µm spot in blue.

Figure 10 shows the results for the trapping of HeLa cells using four optical spot sizes. The maximum velocity a cell could be transported at was 50 µm s^{-1} using the smallest (12 µm diameter) spot size. This was found to be the optimum size of trap, as the force is not much less than the larger traps, and it gives the constant trap stiffness (force proportional to distance from trap center) that gives precise control over the position of the cell. This ideal trap was found to have a trap stiffness of $3x10^{-6}$ N m^{-1} using an illumination intensity of 2.5 W cm^{-2} and an applied AC voltage of 10Vp-p at 100kHz.

5. CONCLUSIONS

In this paper we have shown that it is possible to create OET traps that have a constant trap stiffness giving a force that is proportional to the distance from the center of the trap using images from a data projector. These ideal traps allow particles to be positioned accurately, as there is only a single point in the center of the trap where the particle feels no force. We show traps with a stiffness of $3x10^{-6}$ N m^{-1} capable of moving cells up to 50 µm s^{-1}. To demonstrate this and highlight the ability of OET to manipulate many particles at once, we chose to reproduce the Cal logo in HeLa cells (Figure 11).

We also show that the force profile of the traps can be predicted with simulations by considering the electrical gradients produced at a distance of 3.5 µm from the surface (not 7.5 µm as might be expected for a 15-µm-diameter object) and reducing the magnitude by a factor of four. The importance of these results comes from the ease of which this system allows us to manipulate many single calls at once. The data projector is an inexpensive, easy-to-align alternative to a spatial light modulator that is used for optical tweezing. The reduced illumination intensity necessary for OET reduces the focusing requirement allowing for the manipulation of more particles over a larger field of view, while still retaining a high level of control over each cell.

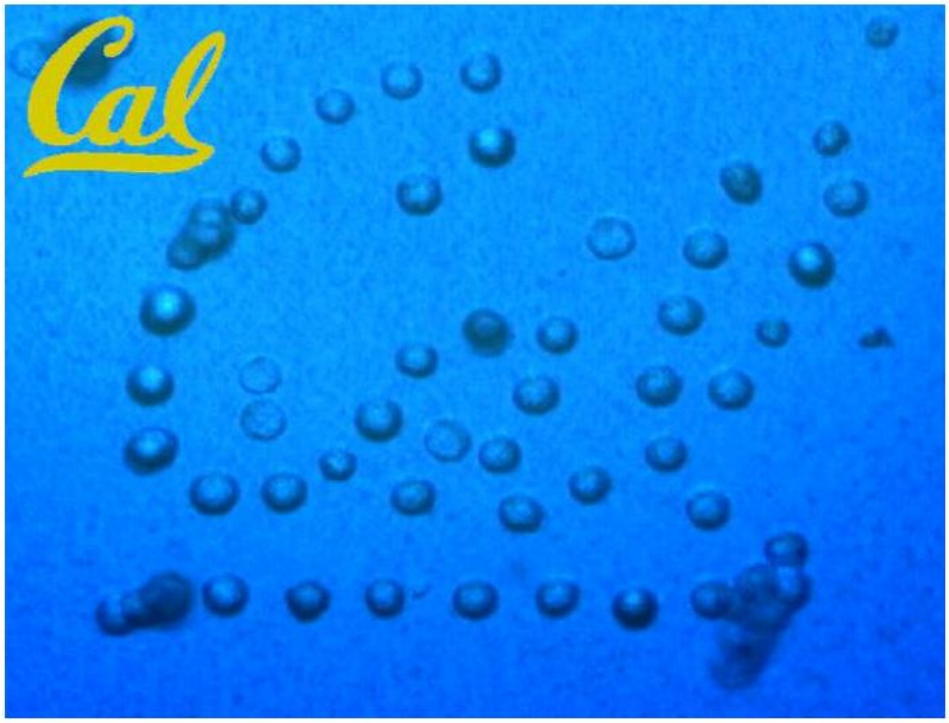

Fig. 11. HeLa cells arranged into a Cal logo. Live HeLa cells are attracted to a light pattern that is filtered out. A few dead HeLa cells can also be seen which are not attracted to the light and can be identified by their lighter appearance.

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