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Utilization of Nano-Deep Evanescent Field for Particles Optophoresis

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ABSTRACT

Separation and sorting of microparticles have set foot in critical diagnostics, advanced chemical and biomedical assessment. By utilizing specific characteristics for sorting, various techniques have been developed and optimized. Evanescent field optical trapping of microparticles is one of the emerging technologies to sort and separate in a non-mechanical and non-destructively several particles simultaneously. This paper describes the studies carried out, both theoretically and experimentally, to optimize the propulsion of polymer particles on copper ion-exchanged channel waveguides, to ultimately allow for the trapping and separation of mammalian adipose tissue derived stem cells (AT-SCs) according to their size and refractive index. The propulsion of polymer particles was observed to increase with the supplied input power and with laser polarization at transverse electric (TE) mode. The propulsion of particles was demonstrated to peak on a 4μm channel width of a 1μm thick copper ion-exchanged waveguide. The work carried out provides the optimal optical and waveguide parameters to be exploited for trapping and sorting AT-SCs on copper ion-exchanged waveguides.

Introduction

There is a significant potential for stem cells to be exploited for regenerative medicine. Stem cells can be defined as cells with self-renewal and pluripotency properties (Schwartz, Hubschman et al., 2012). Self-renewal in this case refers to the ability to maintain undifferentiated state after numerous cell division cycles. Pluripotency, on the other hand, is the capacity to differentiate into specialized cell types. Stem cells act as a repair system for the body by differentiating into specialized cells and replenishing cells in regenerative organs such as skin or intestinal tissues (Tomasetti and Vogelstein, 2015). Hence stem cells can be utilized to develop into many different cell types in the body for medical therapies. Recent derivation of mammalian adipose tissue derived stem cells (AT-SCs) opens new opportunities for regenerative medicine as well as having the advantage of simple procedures, less invasive and can be collected repeatedly in abundance (Buang & Shahimin, 2010; Reshak, Shahimin et al., 2013; Buang et al., 2014). Currently, the number of people needing a transplant for diseased or destroyed organs far exceeds the number of donated organs or tissues available for transplantation. Theoretically, AT-SCs therapy has the potential to dramatically change the treatment of a myriad of diseases, conditions, and disabilities including Parkinson's and Alzheimer's diseases, diabetes, leukemia, spinal cord injuries, muscle damage and rheumatoid arthritis (Zuk et al., 2002; Strem et al., 2005).

There has been extensive research on AT-SCs therapy, yet remarkably little is known about the molecular mechanisms that underlie the pluripotency of AT-SCs (Miranville et al., 2004). Hence, there is a need for an approach to provide a pure population of AT-SCs that is free from mechanical (fluid sheer stress, cyclic stretch and pressure), electrical (field induced) or chemical (need for labelling) induced cellular response. Such an approach will be able to provide the effective characterization and study of different AT-SCs populations and ultimately clearer strategies for regenerative medicine. Optical trapping and propulsion is seen as a potential candidate as a sorting technique that avoids detrimental effects on the AT-SCs.

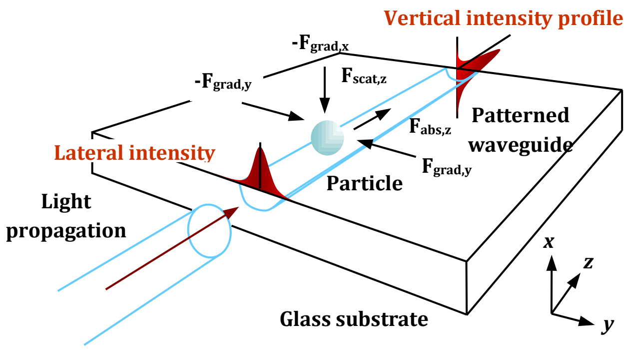

Fig. 1: Schematic of optical forces acting on a particle on a channel waveguide.

The optical trapping technique has proven to be a very useful tool that offers a non-contact method for precise particle handling. The pioneering work by Ashkin in 1970 showed that the forces of radiation pressure from focused laser beams could be used to significantly affect the dynamics of small transparent micrometer-sized neutral particles (Ashkin, 1970). It was shown experimentally that, using just these forces, small micrometer-sized neutral particles could be accelerated, decelerated and even stably trapped using focused laser beams. The utilization of evanescent waves for biological cells has so far been limited, as reviewed in (Shahimin et al., 2011) due to fabrication complexity and feasibility. The most comprehensive work to date was done by Shahimin involving adipose tissue derived stem cells (Shahimin et al., 2015).

This motivates the use of the evanescent wave trapping technique to develop a novel method for sorting particles and biological cells according to their optical properties under optimized conditions. Using optical trapping and propulsion on a channel waveguide device allows the manipulation of several particles and cells simultaneously. This has a significant clinical advantage for biological applications where high throughput is required. Furthermore, the channel waveguide configuration permits integration with microsystems for a lab-on-a-chip device. This is expected to pave the way for low cost, robust and simple integrated optical devices to be fabricated and optimized, allowing this technology to become applicable to a practical system.

In the evanescent field above an optical channel waveguide, a particle or a biological cell in the optical field experiences three main forces as illustrated in Fig. 1; an axial scattering force, F_{scat}, a gradient force, F_{grad}, which can act in both the axial (due to losses along the propagation of light) and the radial direction (due to variation in the intensity profile), and an absorption force, F_{abs}, which is dependent upon the complex refractive index of the particle. A particle in the evanescent field will be propelled and trapped with a dependence on the intensity gradient (a property dependent upon the physical characteristic of the waveguide and the laser configurations). Thus the light reflected and absorbed by the particle will be a function of the relative intensity gradient, the particle size and refractive index of the particle. The particle is drawn into the region of highest light intensity and propelled in the direction of the propagating light. In this paper, we report our studies to establish the optimum optical and waveguide parameters for propulsion of polymer particles on copper ion-exchanged waveguides.

Experimental Procedures

In order to investigate the propulsion of polymer particles under evanescent field illumination, a suspension is placed in a reservoir (fabricated in house in polydimethylsiloxane (PDMS)) on top of the channel waveguide, as illustrated in Fig. 2. The waveguide used for the experiments was fabricated from BK7 glass (Lanjut Pertiwi; 50mm x 50mm x 1mm), by ion-exchange process. The substrate was masked with aluminum having photolithographically-defined channels of 2-10μm width and immersed in $ZnCl_2+Cu_2Cl_2$ as ion source at 500ºC for 5-30 minutes and annealed for the next 3-4 hours; the resultant channel waveguides have a ~3-12μm modal width, as described earlier in (Huang et al., 2007; Reshak et al., 2013). The waveguide was ensured to produce a satisfactory evanescent field and moderate losses via optical characterization prior to the experimentation with polymer particles. Evanescent field illumination was powered by a polarized HeNe laser (HNL210L-EC, Thorlabs) operating at 632.8nm. The laser output was coupled into the waveguide in free space via a 20x microscope objective lens. The propulsion of particles was imaged using a stereomicroscope (Olympus) equipped with a cooled CCD camera. The particles were tracked between frames and the propulsion characteristics is established using in-house written software.

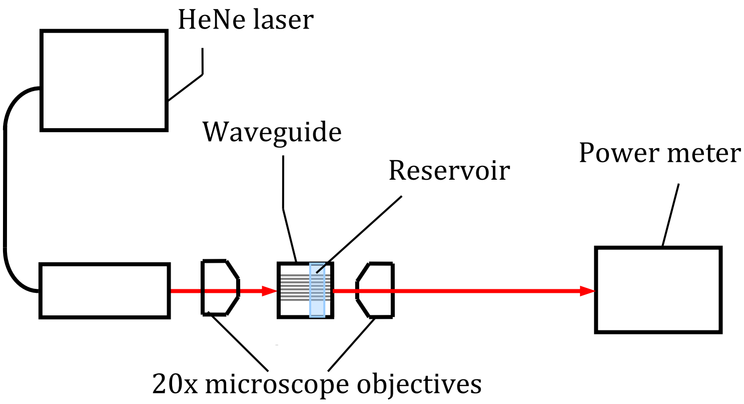

Fig. 2: Schematic of the experimental set-up used for particle trapping and monitoring

Polymer particles (polystyrene, refractive index 1.59, Sigma Aldrich) of diameter 3μm (std dev <0.1 μm), 5μm (std dev <0.1 μm) and 10μm (std dev <0.2 μm), suspended in deionized water were pipetted into the PDMS reservoir. All polymer particle solutions used in this paper were prepared by diluting the particles in deionized water until a concentration of 1 x 10^6 particles per ml was reached. The concentration was such as to achieve a low density of particles in order to avoid the formation of long particle chains (Ng, 2000; Hole, 2005). Each of the experimental data was compared with a theoretical model. OptiBPM software is used to simulate the electric field distribution in the channel waveguide. OptiBPM is the software that implemented FD-BPM algorithm or finite difference beam propagation method in the simulation of field distribution. OptiBPM has provided parameter scan feature with VB script programming which allow the user to write program to scan parameter automatically and thus reduce effort on manually change the parameter value. This feature has significant advantage for the research in order to obtain the optimized parameter. Step index profile of the channel waveguide is used instead of gradient index profile for the simplicity of simulation (unless stated otherwise). Note that the model assumed that there is no power loss due to the Fresnel scattering, modal mismatch or propagation loss along the channel waveguide. Furthermore, it is also assumed that the propulsion of particles is not affected by any non-optical forces.

Results and Discussion

i. Optical Configurations

One of the crucial optical parameter in the particle trapping and propulsion is the laser polarization. There are two polarization modes, transverse electric (TE) and transverse magnetic (TM). TE mode signifies that the electric field vector is directed along the y-direction (parallel to the waveguide channel) as illustrated in Fig. 1. Likewise, TM mode signifies that the magnetic field vector is directed along to the y-direction. In determining the effect of polarization on particle velocity, a simulation has been carried out in determining the evanescent field across different width and different waveguide thickness (which is directly related to the time of immersion in ion source during fabrication). The evanescent field is directly related to the optical forces acted on the particle, which in turn translates into the particle propulsion velocity. Fig. 3 shows the overall output of the simulation exercise. Evanescent field resulted from TM polarization has the same parabola trend with increasing thickness with simulation result obtained by using TE polarization as demonstrated in Fig. 3 a). Evanescent field resulted from TM polarization has the optimum thickness of 1µm. While in Fig. 3 b), evanescent field obtained from TM polarization is lower than TE polarization with varied width. Polarization of laser will only influence the magnitude of evanescent field without affecting optimum width.

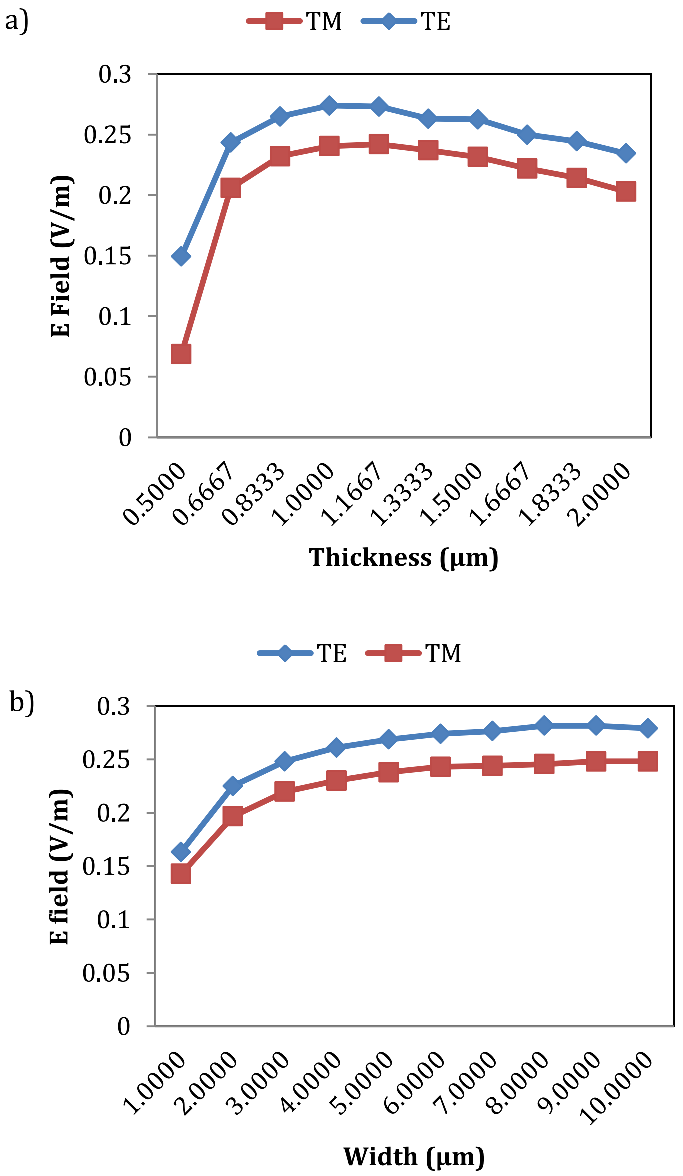

Fig. 3: Plot of evanescent field a) versus thickness and b) versus width for TE and TM polarization

The simulation results are then compared with experimental output. A 10μm polystyrene particle solution was pipetted to the reservoir and the propulsion on all fabricated waveguides (immersion time 5, 10, 20 and 30 minutes) across all channel widths (2 μm, 4 μm, 6 μm, 8 μm, 10 μm) was monitored. A polarizer was used to set the light to the correct mode before the beginning of each experiment. Fig. 4 shows the plotted distribution of propulsion velocity of 10μm polystyrene particle across different width and different waveguides.

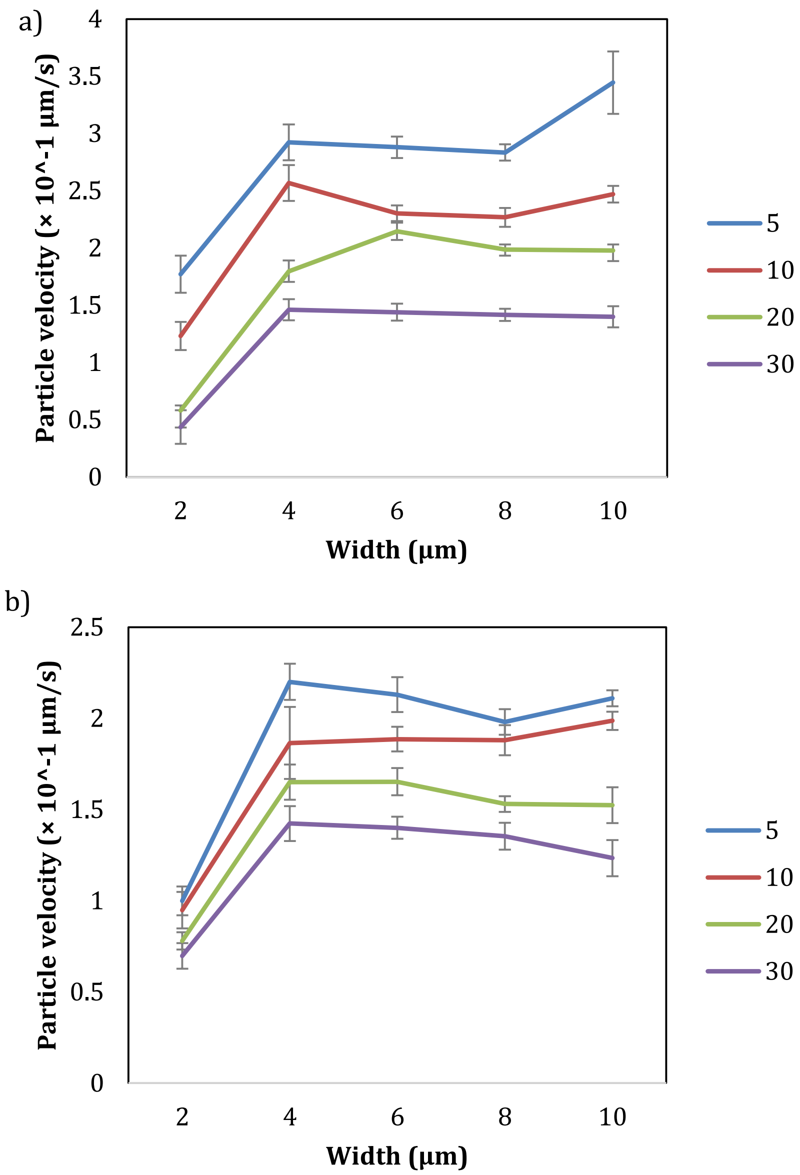

Fig. 4: 10 μm particle propulsion across different widths and different waveguides at a) TE and b) TM polarization

The velocity trend for TM and TE modes was in accordance with the simulation results as well as observed in (Reshak et al., 2013). The greater overall velocity for the TE mode across all waveguides indicates that the mode exhibits a higher surface intensity, as expected from several published papers (Nan & Shahimin, 2011; Shahimin et al., 2011; Khor et al., 2013; Lau et al., 2014a; Lau et al., 2014b; Martham et al., 2014; Reshak et al., 2016). Hence, the TE mode was used for all subsequent experiments unless stated otherwise. It was observed that the highest velocity reached by 10 μm polystyrene particles was on waveguide immersed in ion source for 5 minutes vis-à-vis other waveguides at 3.4×10^{-1} μm/s. As observed in the simulation, the evanescent field peaked at approximately 1 μm thick before gradually decreasing with increasing thickness. This is observed in the experiment with the increasing immersion duration. Fig. 5 shows the simulated evanescent field intensity against different particle diameter based on Mie theory for both polarizations. The TE mode polarization is observed to remain higher than TM with increasing size; in agreement with the previous simulation and experiments. It is also interesting to note that the intensity is sharply reduced with decreasing diameter. Experiment is carried out for propulsion of 3 µm, 5 µm and 10 µm polystyrene particles on 5-minute waveguide against all waveguide widths. Particle velocity consistently shows a decrement with smaller diameter particle with maximum velocity for 3 µm is 1.7 × 10^{-1} µms^{-1} and for 5 µm is 2.1 × 10^{-1} µms^{-1}.

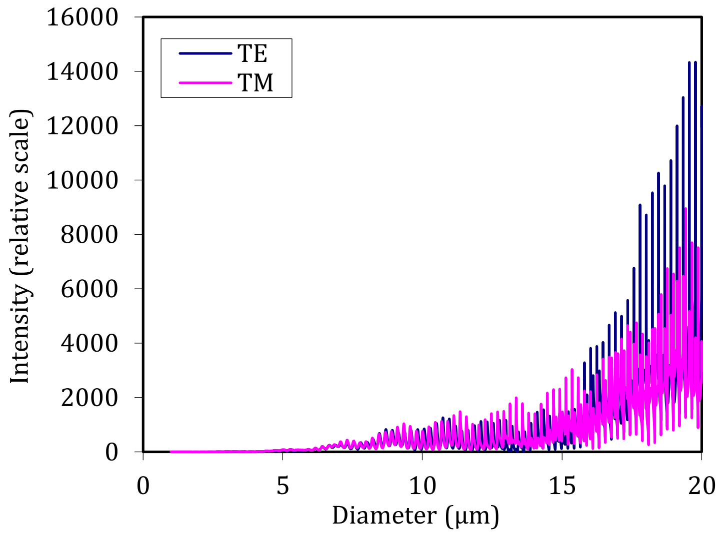

Fig. 5: Evanescent field intensity versus particle diameter based on Mie theory for both polarizations

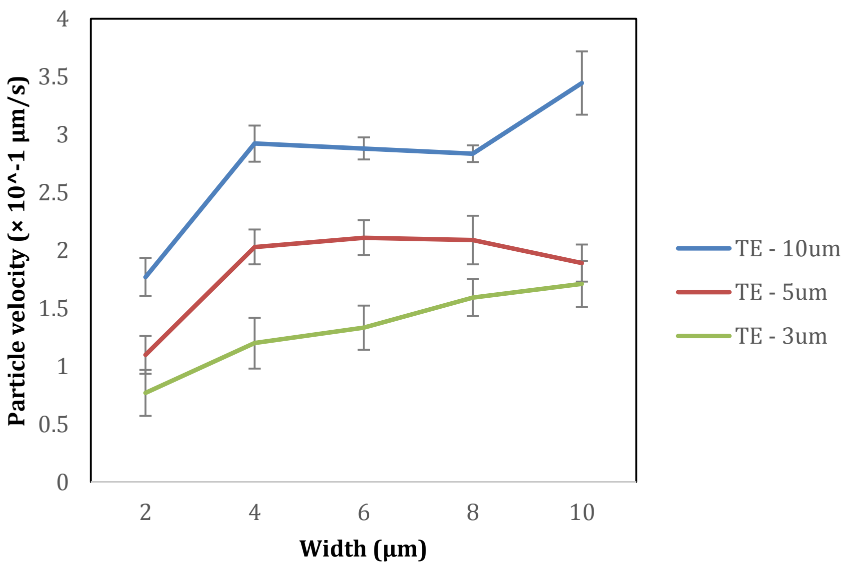

Fig. 6: Propulsion of 3 µm, 5 µm and 10 µm polystyrene particles on 5-minute waveguide against all waveguide widths

ii. Waveguide Parameters

Several waveguides have been fabricated to investigate the effect of ion-exchange time on the particle propulsion. By varying ion-exchange time, the waveguide depth is also varied. 10 µm polystyrene particles were used for this investigation. The particle was diluted in deionized water before being pipetted into the reservoir. The same optical and imaging setup was used as described in previous sections. Out of all 4 waveguides tested in this investigation, the 2 μm width waveguide consistently shows the slowest velocity based on Fig. 4. This indicates that the width was not enough to properly formed a waveguide depth that permits light to propagate in a confined mode. In contrast, with increasing width of the waveguide, the propulsion velocity was observed to reach a plateau and remained the same across all waveguides.

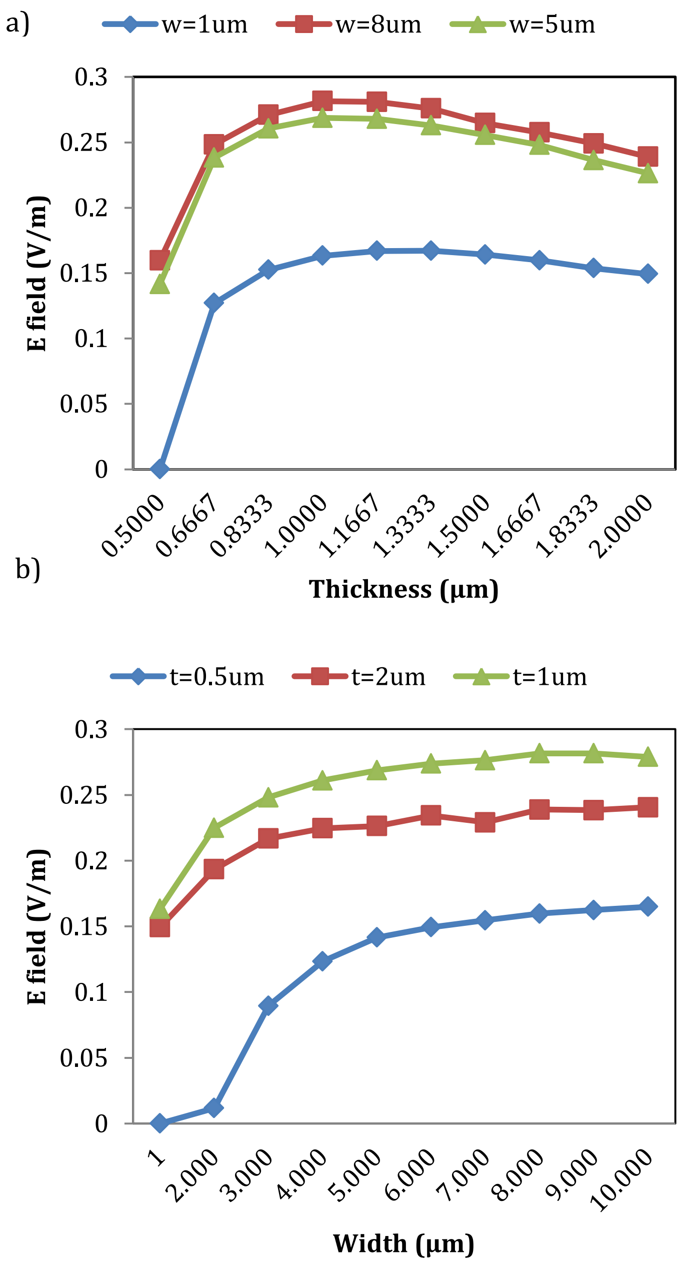

Fig. 7: Plot of evanescent field a) versus thickness at different width and b) versus width at different thickness.

The investigation on waveguide width and depth (thickness) was further carried out via simulation. As shown in Fig. 7, evanescent field follow a parabola trend with increasing thickness which is in agreement with the result obtained in Fig. 3. As shown in Fig. 7, evanescent field generally increased with increasing width. Fig. 8 shows the 3D plot of evanescent field versus thickness and width which allows accurate study of relationship between evanescent field and the physical geometry of channel waveguide. At any fixed width, the parabola trend is observed between evanescent field and increasing thickness. At any fixed thickness, evanescent field increases with width generally. Based on the range investigated in the simulated results, the optimum width is in 10μm while the optimum thickness is 1μm.

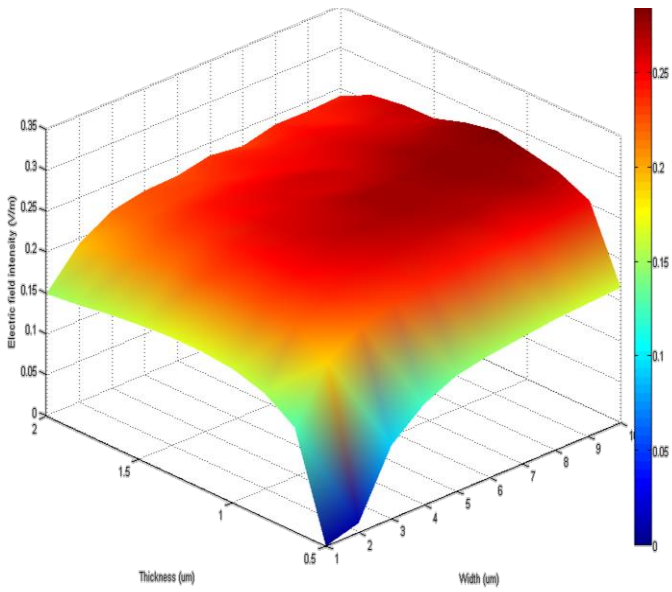

Fig. 8: The theoretical estimation of the evanescent field versus width and thickness

Conclusion

The optical trapping and propulsion of polymer particles, namely polystyrene were presented in this paper. Characterization of particle propulsion against optical and waveguide parameters is used to investigate the optimum parameters for propulsion on cesium ion-exchanged waveguides. The effects of particle size and laser polarization upon propulsion were also investigated. 10μm polystyrene particles were observed to propel the fastest at a rate of 3.4×10^{-1} μms^{-1} on 5-minute waveguide and in the TE polarization. In contrast, the rate of propulsion velocity of 10μm particles in the TM polarization is 2.2×10^{-1} μms^{-1}. Evanescent field resulted from TM polarization has the same trend with result obtained from TE polarization albeit a lower magnitude. Both polarization modes also show same optimum thickness for propulsion. Thus, polarization of the laser will only influence the magnitude of the evanescent field without affecting optimum thickness. This observation confirms the theoretical evaluation and experimental findings of several papers as quoted in previous sections. The velocity of particle propulsion was also observed to decrease with decreasing diameter. Experimental characterization of the waveguide parameters of copper ion-exchanged waveguides was also carried out. Propulsion of polystyrene particles was conducted on several waveguides, investigating the effect of waveguide width and the waveguide ion-exchanged time (which is related to the waveguide depth). Results from this research found that the optimum propulsion is observed on a 5-minute copper ion-exchanged waveguide. Variation in propulsion velocity of particles with different channel widths concludes that the optimum propulsion is observed on a 10 μm nominal copper ion-exchanged channel waveguide width. The work carried out here determined the optimum optical and waveguide parameters to be further exploited for trapping and sorting of ATSCs on copper ion-exchanged channel waveguides. These studies will continue with a view to developing a new stem cell sorting device.

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