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EXPERIMENTAL INVESTIGATION OF DIELECTROPHORETIC BEHAVIOR OF MYOGLOBIN AND SILICA PARTICLES ON A MICROELECTRODE CHIP

Naga Siva Kumar Gunda
Micro and Nano-scale Transport Laboratory
Department of Mechanical Engineering
University of Alberta
Edmonton, Canada, T6G 2G8
Email: nagasiva@ualberta.ca

Sushanta Kumar Mitra*
Micro and Nano-scale Transport Laboratory
Department of Mechanical Engineering
University of Alberta
Edmonton, Canada, T6G 2G8
Email: sushanta.mitra@ualberta.ca

ABSTRACT

Dielectrophoresis (DEP) is one of the nondestructive electrokinetic techniques that has immense capability for manipulating nano-sized biomolecules like myoglobin. The present study investigates the behavior of myoglobin molecules on a microelectrode surface under the influence of dielectrophoresis. Microelectrodes are fabricated in transparent borofloat glass wafers with a sequence of microfabrication steps like piranha cleaning, metal deposition, optical lithography and etching. A detailed description of experimental setup to conduct DEP experiments on myoglobin is presented with a brief overview of myoglobin preparation. Silica particles are used to mimic the myoglobin molecules. Both positive DEP and negative DEP effects on silica particles is observed and positive DEP effect on myoglobin is also observed. Positive DEP on silica particles is observed at applied voltage range of $5-10V$ *and frequency range of DC to* $1KHz$*. Negative DEP effect on silica particles is observed at* $10V$ *applied voltage and* $10KHz$ *to* $40KHz$ *frequency range. The positive DEP effect of myoglobin is observed at applied voltage of* $5V$ *and frequency of* $5kHz$*.*

1 INTRODUCTION

The application of dielectrophoresis (DEP) in micro or nano-scale have increased in the last few decades due to improved nanofabrication facilities [1]. DEP is a phenomena in which the dielectric particles can be manipulated under nonuniform electric fields because of polarization effects. With the availability of dielectric properties of most biological molecules, the importance of DEP technique has increased in biomedical applications. DEP technique has been used to manipulate bioparticles such as cells, bacteria, viruses, DNA, and proteins [1–14]. The ability of DEP to manipulate such bioparticles is utilized for separating, trapping, concentrating, mixing, and sorting selective bioparticles [1–4, 15]. The effect of DEP on nano-sized particles is small because the DEP force is directly proportional to the cube root of a particle radius and also due to significant Brownian motion of nanoparticles. Due to these limitations, the use of DEP technique at nano-scale is limited. Very few researchers have worked on nanoparticle manipulation and the work on protein manipulation using this DEP technique is also not so much. In the present study, the dielectrophoretic behavior of nano-sized biomolecules such as myoglobin molecules, has been investigated experimentally. Myoglobin is a single chain globular protein of 153 amino acids containing heme (iron containing porphyrin) prosthetic group in the center, around which remaining apoprotein folds are present [18–23]. It has a molecular weight of 16.7 kDa and an approximated size of 1.53 nm radius [18–23]. Myoglobin is one of the important cardiac markers to identify the early detection of acute myocardial infarction [16, 17]. The findings of this work will be useful for separating the myoglobin from blood and detecting it, which will be helpful in predicting the heart attacks.

*Address all correspondence to this author.

The initial experimentation on protein manipulation under DEP effects was conducted by Washizu et al. [7]. An array of 1 μm width corrugated microelectrodes with a minimum gap of 4, 15, and 55 μm between the electrodes was used for creating nonuniform electric fields. The trapping of avidin (68 kDa), concanavalin (52 kDa), chymotrypsinogen (25 kDa), and ribonuclease A (13.7 kDa) proteins at the edges of the electrodes under positive DEP was showed. They measured protein concentration using a fluorescence detection unit and plotted a graph showing fluorescence intensity vs time, indicating a relation between the molecular weight and fluorescent intensity of proteins. Their work showed only the positive DEP of proteins. Bakewell et al. [8] and Hughes et al. [9] demonstrated both positive and negative DEP effects on proteins. Manipulation of avidin protein molecules on 2 μm center gap polynomial electrode surface was observed. Later, Zheng et al. [10, 11] showed the manipulation of bovine serum albumin (BSA) protein under positive DEP effects. They trapped BSA proteins at the edges of the quadrupole gold microelectrodes which were fabricated with a center gap of 5, 10, 20, and 50 μm. Mathematical calculation of the Clausius-Mossotti factor for BSA protein was also presented. They modeled BSA protein as sphere of 5 nm diameters and the crossover frequency of 100 MHz was calculated for the protein solution of conductivity 1 mS/m. Clarke et al. [12] reported the dielectrophoretic concentration of proteins with nanopipette technique. Fluorescent labeled protein G and IgG concentration was observed with fluorescence detection. They used borosilicate pipettes of a 3.6^o half-cone angle and an inner diameter of 100-150 nm with an electrode both inside and outside of the pipette. They demonstrated both types of DEP effects with protein G at $1V$ and $0.1Hz$.

Recently, Gunda and Mitra [13, 14] presented the theoretical investigation of dielectrophoretic behavior of myoglobin in a microchannel housing rectangular microelectrodes at the bottom surface. They approximated the shape of the myoglobin as sphere, oblate and prolate spheroids. Their results shows that the effect of the shape of myoglobin on the DEP is not significant. They also calculated the efficient DEP force for manipulating myoglobin under given conditions. In addition, Gunda and Mitra [24] showed the different fabrication procedures for making a dielectrophoretic microfluidic device. They presented the different equipment required for conducting DEP experiments. They also provided results of positive DEP on polystyrene microparticles. Dalton et al. [25] provided the complete experimental setup with details of microfluidic chip platform required for DEP experiments. They eliminated the requirement of multiple layer fabrication for their platform. Their system also provided easy chip changes.

The remaining sections of the paper are organized as follows: In section 2, materials and methods required to conduct the DEP experiments are described with the emphasis on microelectrode fabrication, equipment, electrical connections and myoglobin preparation; in section 3, future study of this work is presented.

2 MATERIALS AND METHODS

2.1 Microelectrode chip Fabrication

Fabrication of microelectrodes on glass wafer is described in this section. The NanoFab facility available at University of Alberta was used to fabricate the chip. Initially, the layout of microelectrode configuration (rectangular array of electrodes) was designed with the L-Edit MEMS Design software (MemsPRO v6, Tanner Research, Inc., CA) and then exported to Laser Pattern Generator (Heidelberg DWL-200, Heidelberg Instruments, Germany) for fabricating the chrome mask. Next, piranha (H_2SO_4:H_2O_2 of 4:1 by volume)cleaned borofloat glass wafer (Paragon Optical Company, Inc., PA) of $4in \times 4in$ and $1.1mm$ thick was taken and a layer of $40nm$ chromium and $200nm$ gold sequentially deposited using planar magnetron sputtering system. The gold coated glass wafer was then spin-coated with a layer of $1-2\mu m$ positive photoresist(PPR)(HPR 504, Fujifilm Electronic Materials, Inc., Arizona) using Solitec resist spinner (Model 5110-CD, Solitec Wafer Processing, Inc., CA). Then, the PPR coated wafer was soft-baked at 110^oC for $90sec$ on a Solitec vacuum hot plate (Solitec Wafer Processing, Inc., CA). Using mask aligner, the spin-coated wafer was exposed to UV illumination (350-400 nm) through chrome mask for $2-3$ sec to transfer the electrode structures on PPR. Then, the wafer was developed using PPR developer (HPRD 429, Fujifilm Electronic Materials, Inc., Arizona) for $20-30$ sec. Later, gold and chromium were etched using gold etchant ($KI + I_2 + H_2O$) and chromium etchant ($K_3[Fe(CN)_6] + NaOH + H_2O$), respectively. Next, acetone was used to strip off the PPR. Finally, the wafer was cleaned in isopropyl alcohol and dried with nitrogen spray. The different number of layouts of electrodes on glass wafer were diced using diamond cutter or diamond touch dicing saw.

2.2 Experimental conf guration

This section explains the equipment required for conducting DEP experiments. Figure. 1 shows the complete experimental configuration. Arbitrary function generator (Fluke 294, Fluke Electronics, ON) was used to generate sinusoidal varying alternating current (AC) signal of 0^o and 180^o with voltage amplitude V. The function generator has capacity of generating sine waves of amplitude $10V_{p-p}$, frequency $40MHz$ and four output channels. The applied voltage V was amplified using the high voltage amplifier (Tabor 9400, Tabor Electronics Inc., CA). The high voltage amplifier can amplify the voltages up to $400V_{p-p}$ and can connect up to 4 channels. The generated sinusoidal AC signal was applied on microelectrode chip via copper electrical wires and verified the applied AC signal on the chips using oscilloscope (Tektronix MSO2024, Tektronix, Inc., OR). Electrical

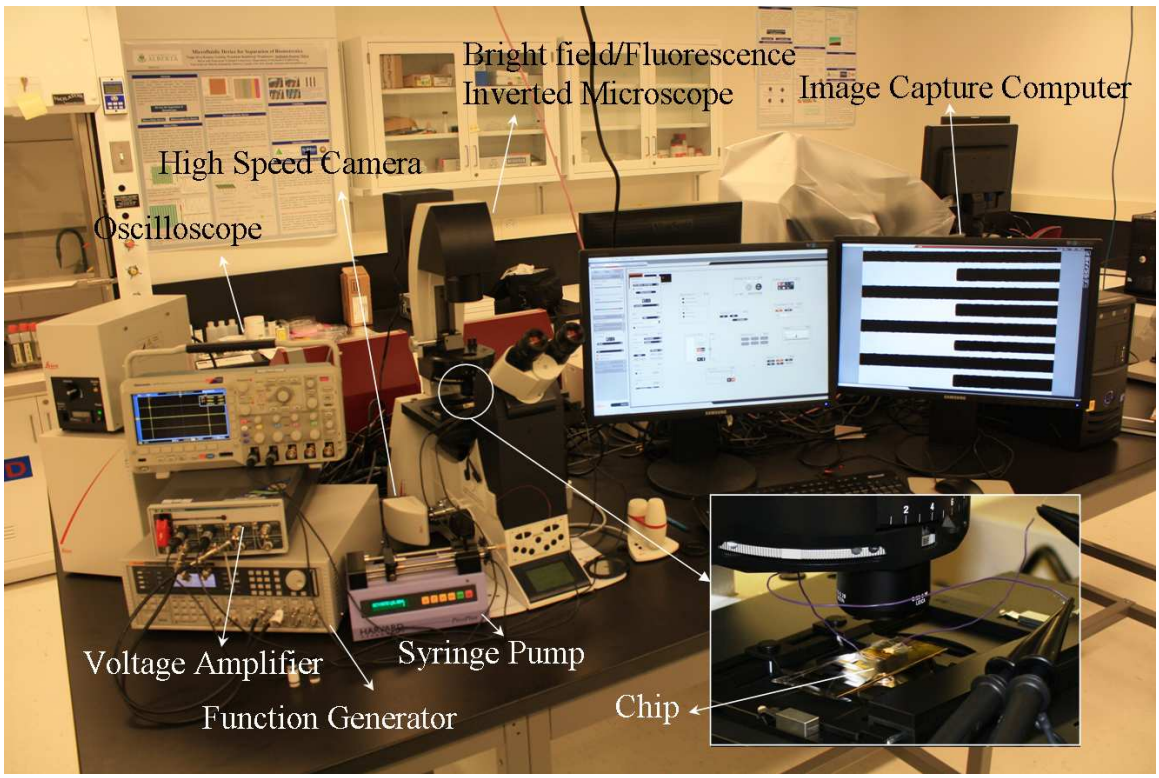


FIGURE 1. EXPERIMENTAL CONFIGURATION FOR DEP

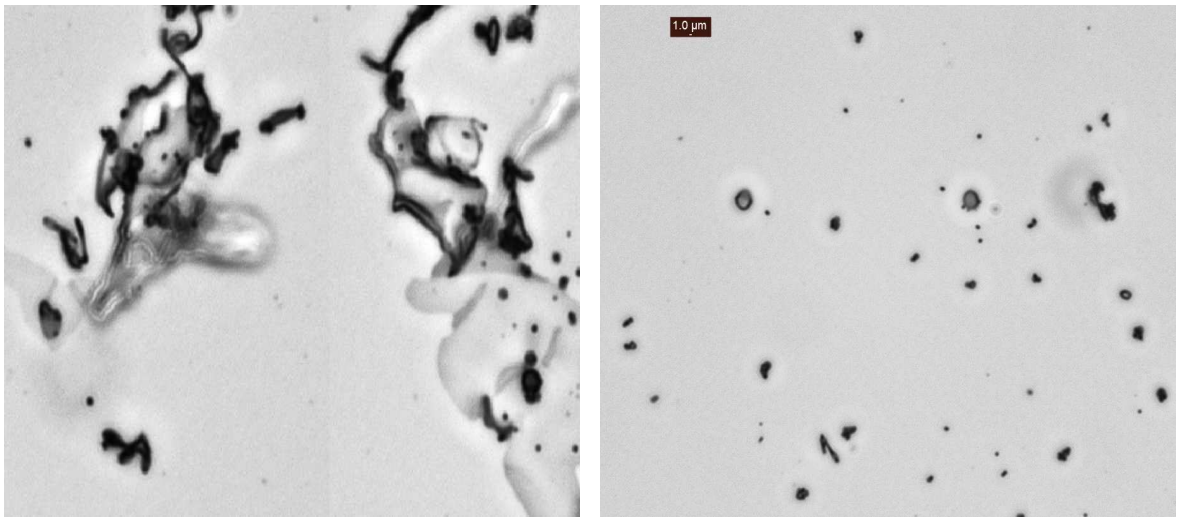
connections between the function generator and voltage amplifier were provided using BNC cable with BNC male connectors at the ends. The connections of voltage amplifier and oscilloscope to microelectrode chip were carried using BNC cable with BNC male adapter at one end and BNC plug at the other end. The copper wires were soldered to the gold pads of microelectrode chip using silver indium wire. A syringe pump (Pump 11 Pico Plus, Harvard Apparatus, QC) or pipette (Fisherbrand Finnpipette, Fisher Scientific, Canada) was used to inject the aqueous solution of myoglobin on the microelectrode surface. A fully automated bright field and /or fluorescence inverted microscope (Leica DMI6000 B, Leica Microsystems Inc., ON) was used to observe the myoglobin molecule behavior under DEP and ultra fast, high-resolution monochrome image acquisition digital camera (Leica DFC360X, Leica Microsystems Inc., ON) was used to capture the movements.

2.3 Sample preparation

The lyophilized form of myoglobin (Myoglobin 8M50, Hytest Ltd, Finland) sample was taken and then dialyzed against deionized water (resistivity $> 1M\Omega cm$) to make the globular structure of myoglobin molecules to monomers. Then, the sample was diluted by adding deionized water to get required concentration. Figure. 2 shows the comparison of myoglobin molecules before and after dialysis. Proper dialysis of myoglobin molecules is required to break the myoglobin into individual molecules. Then the aqueous solution of myoglobin was dropped on the microelectrode surface. Here, the chip was cleaned using ethanol solution before placing the drop of myoglobin solution. Some of the experiments are also conducted on silica particles (Polysciences, Inc., Warrington, PA) to mimic the myoglobin molecules.

3 RESULTS AND DISCUSSIONS

In this section, the results obtained for silica particles as well as myoglobin is provided. Silica particles of 5μ diameter are used to mimic the myoglobin particles for the preliminary experiments. Both types of DEP effects are observed for the silica particles and positive DEP effect only is observed for the myoglobin particles. Figure 3 shows the positive DEP effect of silica particles under $5-10V$ applied voltage and for the frequency range of DC to $1KHz$. It is clearly observed that the particles are trapped at the electrode edges. The image is acquired from the inverted microscope where the light source is placed at the top and capturing camera is placed at the bottom of the chip. Figure 4 shows the negative DEP effect of silica particles under $10V$ ap-



(a) (b)

FIGURE 2. LYOPHILIZED FORM OF MYOGLOBIN (a)BEFORE DIALYSIS; (b) AFTER DIALYSIS

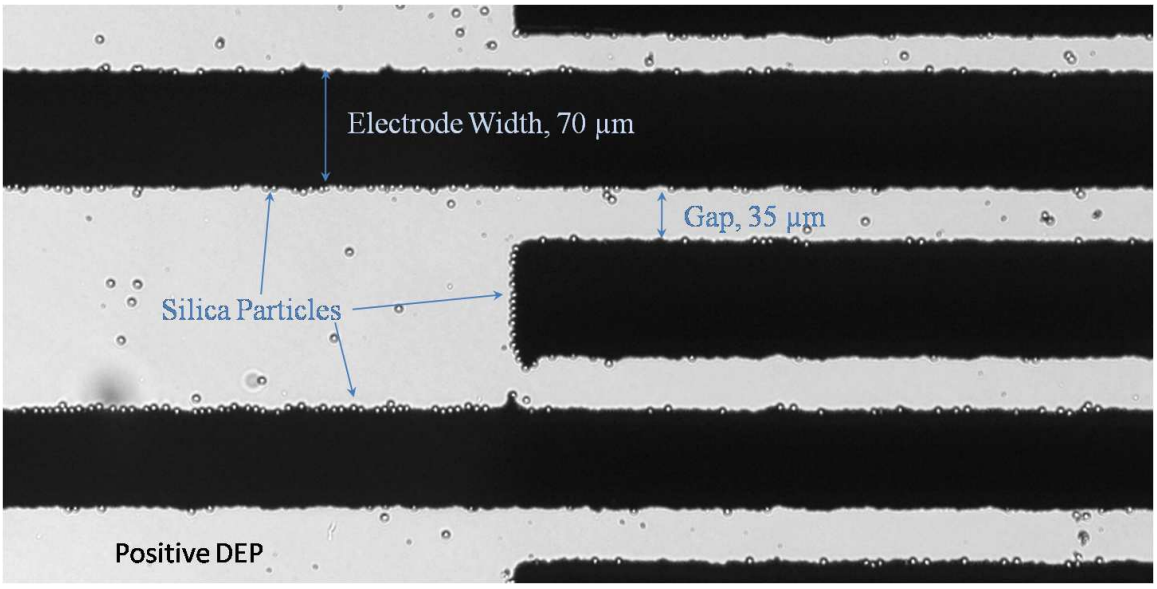


FIGURE 3. POSITIVE DEP OF SILICA PARTICLES

plied voltage for the frequency range of 10KHz to 40KHz. Under negative effects, the particles are moved from edges of the electrode to the middle of the electrodes and the mid-point of the gap between the electrodes. The spreading of the particles on the electrode surface is also observed while the frequency is shifted from the 40KHz to 60KHz. Figure 5 shows the positive DEP effect of myoglobin. The polymeric structure of myoglobin molecules collecting at the electrode edges is observed under the conditions of 5kHz frequency and 5V. In Fig. 5, small number of silica particles are also observed and which are not moved to edge of the electrode. The movement of small monomeric structure of myoglobin is not observed under these conditions. And also the repulsion of myoglobin is not observed for the range of DC to 10MHz. The results indicate that the requirement of higher frequencies to capture the negative DEP effect of myoglobin. It is required to label the myoglobin molecules with FITC to observe them clearly and possible to quantify the amount of myoglobin in the solution.

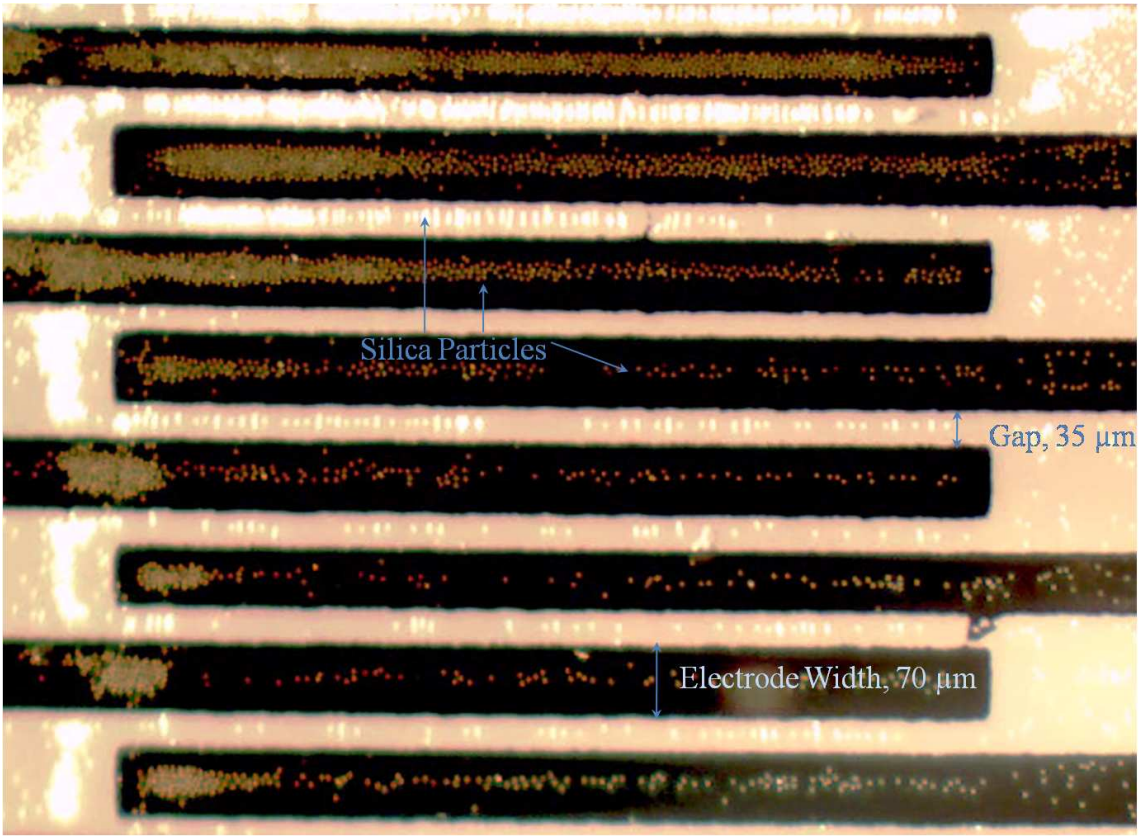


FIGURE 4. NEGATIVE DEP OF SILICA PARTICLES

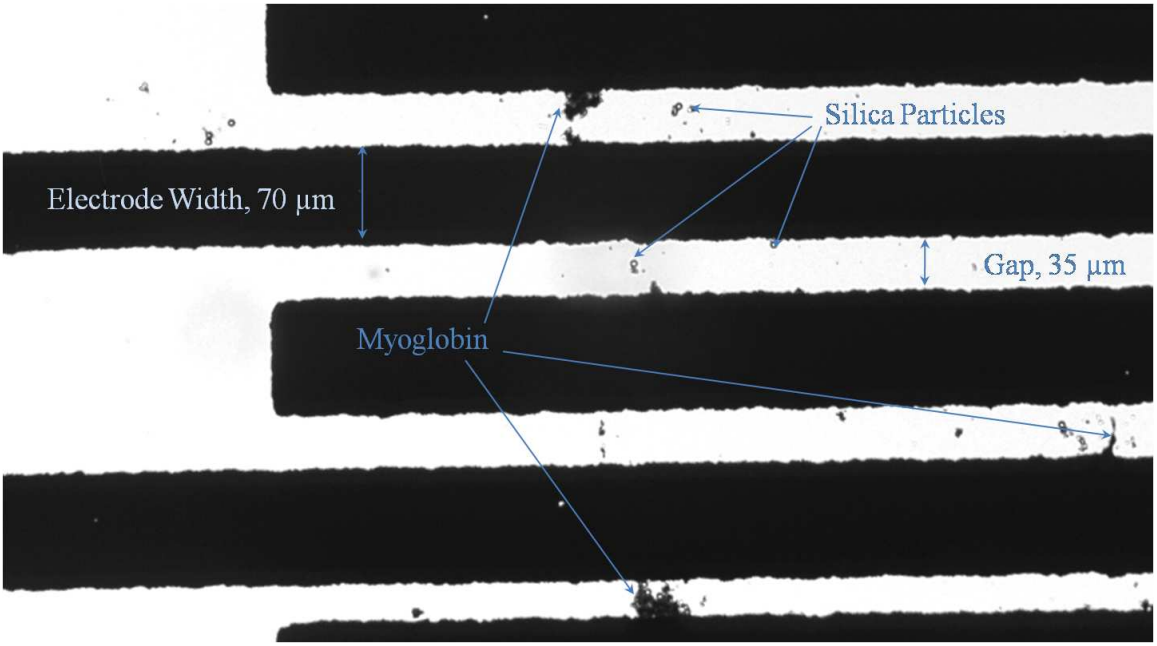


FIGURE 5. POSITIVE DEP OF MYOGLOBIN MOLECULES

4 CONCLUSION

The present study investigated the behavior of myoglobin molecules on a microelectrode surface under the influence of dielectrophoresis. Using nanofabrication facilities, the microelectrode structure is fabricated on the transparent borofloat glass wafer. Equipments required to conduct DEP experiments is described. Myoglobin sample preparation is also discussed. Both positive DEP and negative DEP effects oo silica particles is observed. Positive DEP is observed at DC to $1KHz$ frequency range and $5-10V$ applied voltage and negative DEP is observed at $10KHz$ to $40KHz$ frequency range and $10V$ applied voltage. The positive DEP effect of myoglobin is observed at $5kHz$ frequency and $5V$. This work shows the positive DEP effect on polymeric structure of myoglobin. Further work of this study will be observing both type of DEP effects on monomer structure of myoglobin.

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