International Journal of Nanoelectronics and Materials
Volume 13 (Special Issue) Dec 2020 [33-44]

Determination of Dielectrophoretic Unique Crossover Frequency by Velocity of Enterobacter Aerogenes Trajectory

Muhammad Khairulanwar Abdul Rahim[1], Nur Mas Ayu Jamaludin[1], Jacinta Santhanam[2], Azrul Azlan Hamzah[1] and Muhamad Ramdzan Buyong[1*]

[1]*Institute of Microengineering and Nanoelectronics (IMEN), Level 4, Research complex, Universiti Kebangsaan Malaysia (UKM), 43600 Bangi, Selangor Darul Ehsan, Malaysia.*
[2]*Biomedical Science Programme, Faculty of Health Sciences, Universiti Kebangsaan Malaysia (UKM), Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.*

ABSTRACT

This study presents the Dielectrophoresis (DEP) technique for the detection of Enterobacter Aerogenes (EA) pathogen by their velocity bacteria trajectory. The DEP particle manipulation was used as non uniform AC electric field. It was capable to manipulate and separate the EA pathogen from their medium deionised (DI) water. It was important to determining the DEP force (F_{DEP}) and the unique crossover frequency range (COF_u) of EA pathogen. The F_{DEP} and Claussius-Mossotti Factor (CMF) or polarization factor of EA were analysed and simulated by finite element method (FEM) first. The parameters of DEP experimental were fixed at 10 Volt peak to peak (V_{pp}) with frequency range starting from 300 kHz to 15000 kHz. The DEP microelectrode with one dropped of 0.2µL EA suspension was monitored and recorded by eyepiece camera for observed the EA movement. The DEP experimental was successfully conducted. The EA species displayed one peak for P_{DEP} and one peak for N_{DEP}. The highest P_{DEP} velocity was 80 µm/s at 1100 kHz and the highest N_{DEP} velocity was 40 µm/s at range frequency of 8000 kHz until 10000 kHz. Thus, the COF_u was ranged from 1200 kHz until 1300 kHz.

Keywords: Dielectrophoresis (DEP), Enterobacter Aerogenes (EA), Unique Crossover Frequency (COF_u), Velocity Bacteria.

1. INTRODUCTION

Approximately, the infection of microbial globally around 1.5 million and over 4.6 million deaths was recorded. A recent study was stated that multidrug-resistant bacteria infections more to 700,000 deaths in the world in the year 2016 [1]. In undeveloped countries or regions, technology related to the diagnosis of the disease is a bit behind. This has led to the long-term presence of pathogens as well as reduced efficiency of antibiotic chemotherapy in infected patients. [2]. The trial and error techniques are commonly inaccurate detection. Also, the patients have high risks due to contributing the pathogenic bacteria to multidrug resistance and less the antibiotics' lifetime. The time constraint and high cost are a major problem to make the research to find the proper treatment or to develop the right antibiotic [3, 10]. Normally, the current biomedical technique is required to culture the microbial sample for the identification of pathogenic bacteria, it may take a longer time from hours until weeks [10]. This project aims to overcome this limitation of current methods. The electrokinetic method is also known as dielectrophoresis (DEP) is very effective for manipulation, isolation, and detection of microbial [9]. It is selective manipulation of bacteria based on their physiological and phenotype state, without the biomarkers, chemical/materials added or additives [4-9]. Also, the DEP technique flexibility to do early detection of *EA* that is rapid, portable, and real-time.

*Corresponding Author: *muhdramdzan@ukm.edu.my*

1.1 DEP Theoretical

In the general, the dielectrophoresis force (F_{DEP}) is a force that caused the particle polarized motion due to the difference gradient of electric field intensity (non-uniform electric field) [11, 12, 14 - 20]. If the particles are more polarized than medium, it called positive DEP (P_{DEP}) or otherwise if the particles less polarized than the medium that is Negative DEP (N_{DEP}) [4]. The effectiveness dipole moment (m) of dielectric particles is proportional to their electric field, were given by [4]:

$$m = 4\pi\varepsilon_0\varepsilon_m R^3 p \quad (1)$$

Based on equation (1), the value of medium permittivity is $\varepsilon_o\varepsilon_m$. The ε_o is permittivity of free space, that value equivalent to 8.854 x 10^{-12} F/m, but the ε_m is the relative permittivity value of the medium or it called as the dielectric constant. The p is representing the Clausius-Mossotti Factor (CMF), it is the ability of electrical effectiveness to particle for polarizable [4, 14, 15]. However, the difference of electric field distribution, that caused it to produce the potential energy, W based on their dipole moment. The W is represented as

$$W = -m.E \quad (2)$$

The forces are acting on the polarized particles in non-uniform electric field known as F_{DEP}. The use of F_{DEP} with work energy as equation (2), gives [4]:

$$F_{DEP} = -\nabla W = (m.\nabla)E \quad (3)$$

From equation (1) and (3), F_{DEP} gave as:

$$F_{DEP} = 4\pi\varepsilon_0\varepsilon_m R^3 p(E.\nabla) \quad (4)$$

The general equation of F_{DEP} can be determined by:

$(E.\nabla)E = \frac{1}{2}\nabla E^2$; to form the equation:

$$F_{DEP} = 2\pi\varepsilon_0\varepsilon_m R^3 p\nabla E^2 \quad (5)$$

2. MATERIAL AND METHODS

2.1 MATLAB (formula)

The analytical modelling polarisation factors for *EA* bacteria were done by used MATLAB software. The calculation of the physiological state properties of *EA* was divided into permittivity and conductivity values. The permittivity and conductivity values can be defined as f_{xo}. Based on equation (5), we have modelled the *EA* bacteria according to their physical shape and size to estimated their dielectric properties as showed in figure 1. The *EA* bacteria have rod-shaped and their dimension of about 1.45 µm x 0.70 µm [13]. Based on this *EA* physical size, it can be translated into their uniqueness of dielectric properties. It was useful for the identification of *EA* COF_u.

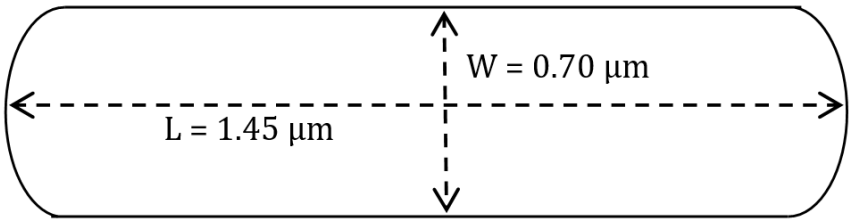

Figure 1. Rod-shaped modelled of *EA* bacteria for dielectric properties estimation.

2.2 COMSOL Multiphysics Software

To design the DEP microelectrode with a dimension of 4240 µm by 2080 µm, we were used the COMSOL Multiphysics Software version 5.3 a. This FEM analysis was important to make a mathematical prediction for electric field distribution and DEP forces direction of DEP microelectrode. The effectiveness of DEP microelectrode for manipulation of *EA* bacteria can be predicted by this analysis before started the device fabrication.

2.3 Fabrication of DEP Microelectrode

DEP microelectrode architecture with a dimension of 4240 µm by 2080 µm is moderately complex. The silicon substrate is used in the manufacturing process of the Aluminum Microelectrode Arrays (TAMA) platform. The PECVD technique is used to manufacture the TAMA platform. Silicone oxide (SiO2) is deposited approximately 1.15 µm as an insulator on the top layer of the silicone substrate. The physical-vapor deposition (PVD) technique is used to deposit approximately 60nm/30 nm of a thin titanium / titanium nitrite (Ti / TiN) adhesion layer. The PVD is used to deposit a film of aluminum / silicon / copper Al / Si / Cu (98/1/1 wt percent) with a thickness of 4.0 µm following the Ti / TiN deposition. To move the structure of the square array to the layer Al/Si/Cu, photolithography is performed with a resistant thickness of 4.0 um including UV cured for hardened photoresist process. Finally, Al / Si / Cu is etched by the use of inductively coupled plasma (ICP) etcher with an advanced plasma resistant strip.

2.4 Experimental Setup

The experimental setup consisted of measuring microscope model STM-6 Olympus Japan, Function Generator (IWATSU SG-4105), glass slide, a couple of probes, coupled with wires prober, Microscope eyepiece camera Model AM7025X Dyno-Eye Edge and tapered DEP microelectrode were setup as Figure 2. The DI water with low permittivity of 78 F/m and conductivity of 0.0002 S/m was used for medium preparation to reduce the joule heating effect. Initially, the eyepiece camera has replaced the lens of a measuring microscope (either left or right lens). Then the eyepiece camera is connected properly to the computer by using its USB cable. After that, the tapered DEP microelectrode is placed under a measuring microscope. Attached properly the needle of probes at both terminals of DEP microelectrode, and then connected the probe's cables to both proper terminals at Function Generator (source terminal and ground terminal). Run the Dino Edge apps on the computer to startup the eyepiece camera for visualize. Lastly, the focusing is adjusted using 20x magnification lens until get a clearer image of the region of interest (ROI).

To ensure no contamination occurred in the sample, all equipment is properly sterilized like wire loop, agar petri dish content individual colonies of *EA* bacteria with the heat of bunsen burner. The wire loop is heated up until it looks like "red flammable" and then reduced the temperature in a few seconds. After that directly scratch the white spot on the agar dish. Scratch gently the white spot on the surface only, no needed scratch deeply in an agar petri dish. The flame of Bunsen burner is retained open to ensure the environment still in sterilizes condition during make the

bacteria suspension medium. Next transfer the tiny dot of a white spot from the agar incubated dish to a test tube containing 1mL DI water. The DI water stirred with a wire loop inside the test tube until all tiny white spots on the wire loop completely dissolved in DI water. Remove the wire loop and immediately close the cap of the test tube. Shake test tube gently to ensure the bacteria suspension completely dissolved. Finally used the pipette to be dropped one drop of bacteria suspension on a glass slide to put it in DEP tapered microelectrode device.

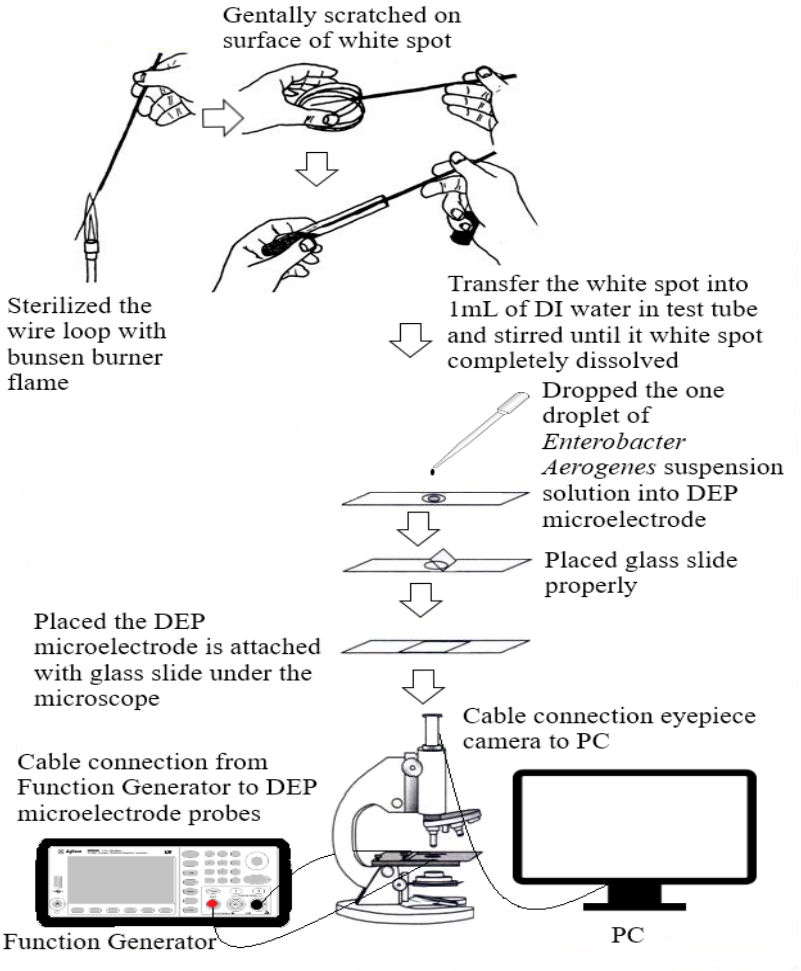

Figure 2. Sample preparation and experimental setup.

The 0.2 µL droplet of *EA* bacteria suspension was dropped into DEP microelectrode and then placed properly glass slide on top of the bacteria droplet. Secondly, focus the measuring microscope using the lens of 20x objection to determine the clearer visualize of ROI and the position of *EA* colony. Thirdly, setting up the Function Generator for AC voltage at 10 V_{pp} about 300 kHz frequency, then turn on the Function Generator for 5 seconds and after that switch off the Function Generator. This procedure was repeated for 400 kHz until 15000 kHz of input frequencies. Finally, all of F_{DEP} responses were observed and analyzed.

2.5 Measurement of *EA* Velocity

The measurement of *EA* velocity was based on video recording captured by an eyepiece camera. From the video frame, the displacement of *EA* pathogen was identified with recorded time for velocity measurement analysis. The displacement 5 µm between two points was done under condition P_{DEP} and N_{DEP}. In detail, for the case of P_{DEP}, the *EA* bacteria were positioned at 5 µm from the edge of the microelectrode. When the voltage is applied the *EA* bacteria started moving

from the middle of between microelectrode to the top surface of the microelectrode. In contrast, N_{DEP} was from a top surface of microelectrode to edge in between microelectrode with the same displacement of 5 µm

3. RESULTS AND DISCUSSION

3.1 MATLAB Simulation Result

The MATLAB software was used to simulate the CMF for *EA* pathogen. The simulation and experimental showed in figure 3, f_{xo} on 10 V_{pp} at 300 kHz to 15000 kHz was 760 kHz and 1200 kHz – 1300 kHz respectively.

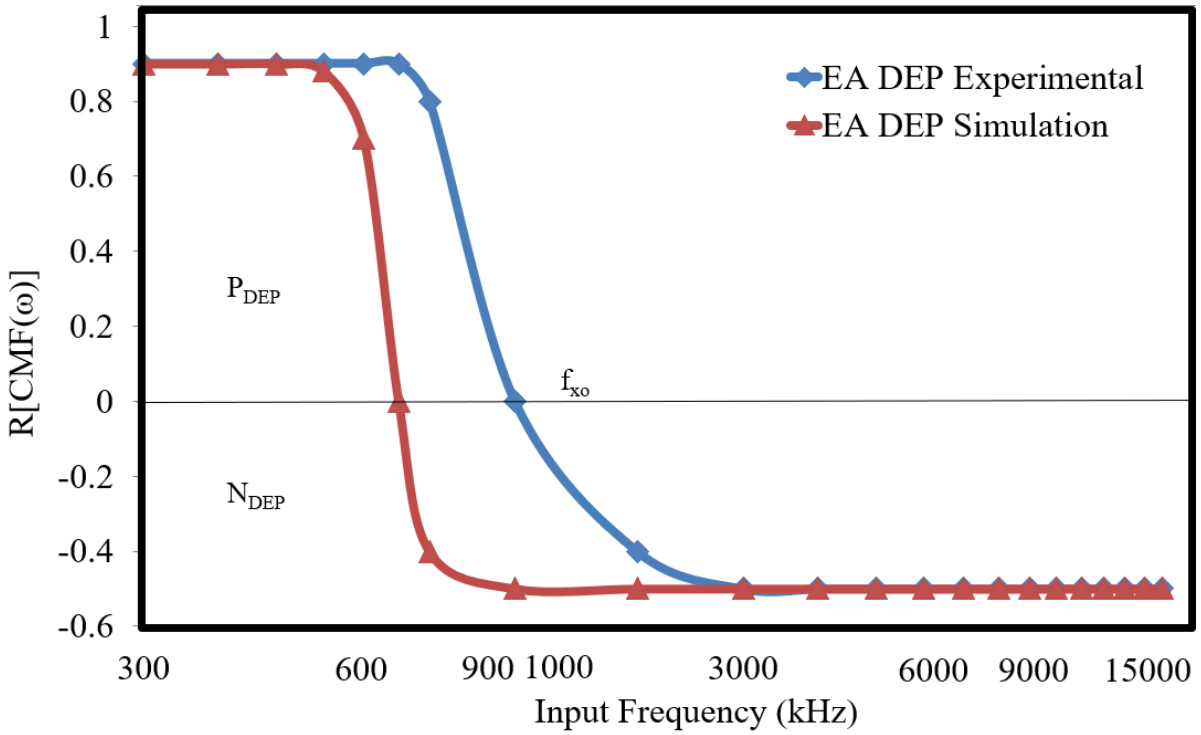

Figure 3. CMF for Simulation and Experimental of *EA.*

3.2 COMSOL Multiphysics Simulation Result

The FEM analysis was indicated that the tapered DEP microelectrode has two spots of high intensity of the electric field. It was located at the edge of the top surface and edge of the bottom surface on DEP microelectrode. These two spots of high intensity of electric field caused the DEP microelectrode to produce the two types of F_{DEP}, there are lateral and vertical of F_{DEP}. The closeup left-hand side of DEP microelectrode was illustrated in figure 4, it was represented the two spots of higher intensity of electric field (yellow circle colored), electric field distribution (red lines), DEP forces direction (green arrow) due to the *EA* bacterium (rod silver colored).

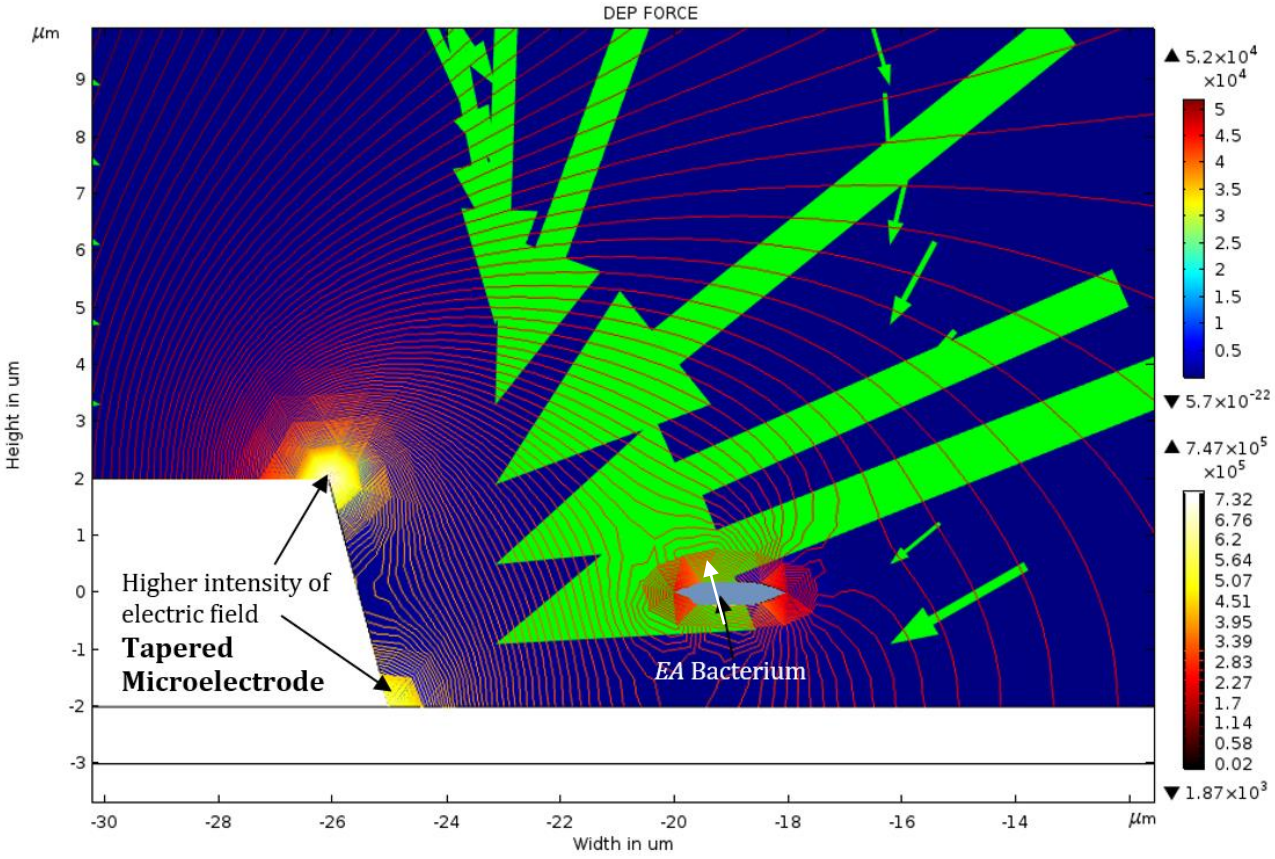

Figure 4. The simulation of electric field distribution and F_{DEP} induced by DEP microelectrode to *EA* bacterium.

3.3 Experimental Result

The experiment showed that F_{DEP} response of *EA* bacteria in figure 5 (a) and 5 (b) were P_{DEP} responses. The experimental result showed that on 10 V_{pp} at 300 kHz until 1100 kHz of input frequencies the *EA* bacteria were attracted to top of DEP microelectrode from the middle region. Meanwhile, in figure 5 (c) and 5 (d) the 10 V_{pp} at 1400 kHz until 15000 kHz of input frequencies were given N_{DEP} responses. The distribution of *EA* bacteria was accumulated in the middle between the DEP microelectrode region. But at frequency input, 1200 kHz and 1300 kHz are f_{xo} (P_{DEP} = N_{DEP}) because of no movement of *EA* bacteria. Table. 1 was summarized all the results of DEP response for *EA* bacteria.

Table 1 Result DEP responses for *EA* bacteria

Input frequency	Mobility	Mobility Direction	F_{DEP} Types
300 kHz	Yes	Attraction	P_{DEP}
400 kHz	Yes	Attraction	P_{DEP}
800 kHz	Yes	Attraction	P_{DEP}
1000 kHz	Yes	Attraction	P_{DEP}
1100 kHz	Yes	Attraction	P_{DEP}
1200 kHz	No	No	f_{xo}
1300 kHz	No	No	f_{xo}
1400 kHz	Yes	Yes	N_{DEP}
2000 kHz	Yes	Yes	N_{DEP}
3000 kHz	Yes	Yes	N_{DEP}
4000 kHz	Yes	Yes	N_{DEP}
5000 kHz	Yes	Yes	N_{DEP}
6000 kHz	Yes	Yes	N_{DEP}
7000 kHz	Yes	Yes	N_{DEP}
8000 kHz	Yes	Yes	N_{DEP}
10000 kHz	Yes	Yes	N_{DEP}
14000 kHz	Yes	Yes	N_{DEP}
15000 kHz	Yes	Yes	N_{DEP}

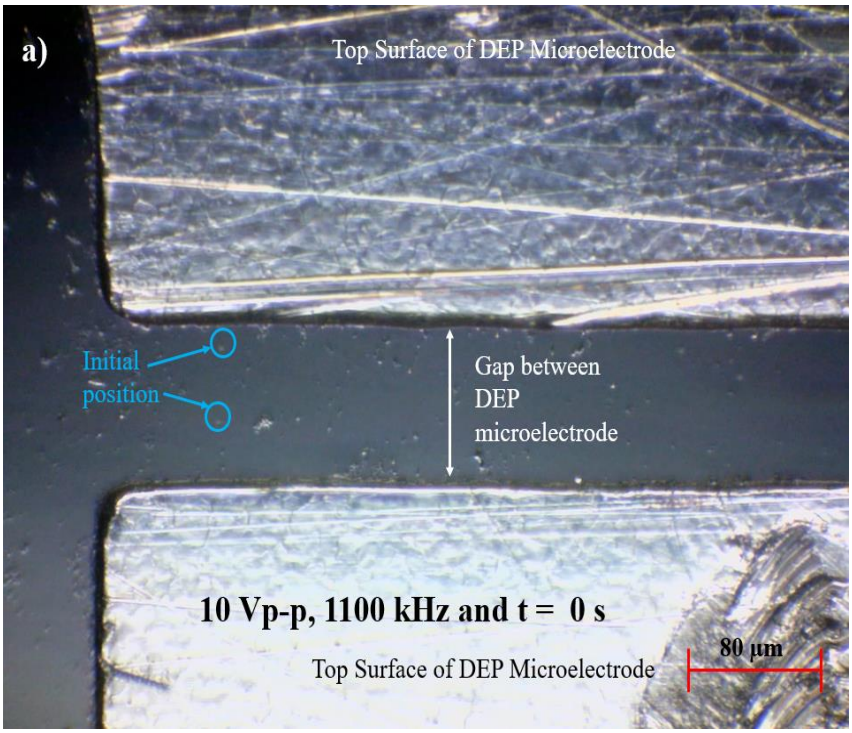

Figure 5 (a). Response P_{DEP} on 10 V_{pp} at 1100 kHz for *EA* Initial position at 0 second.

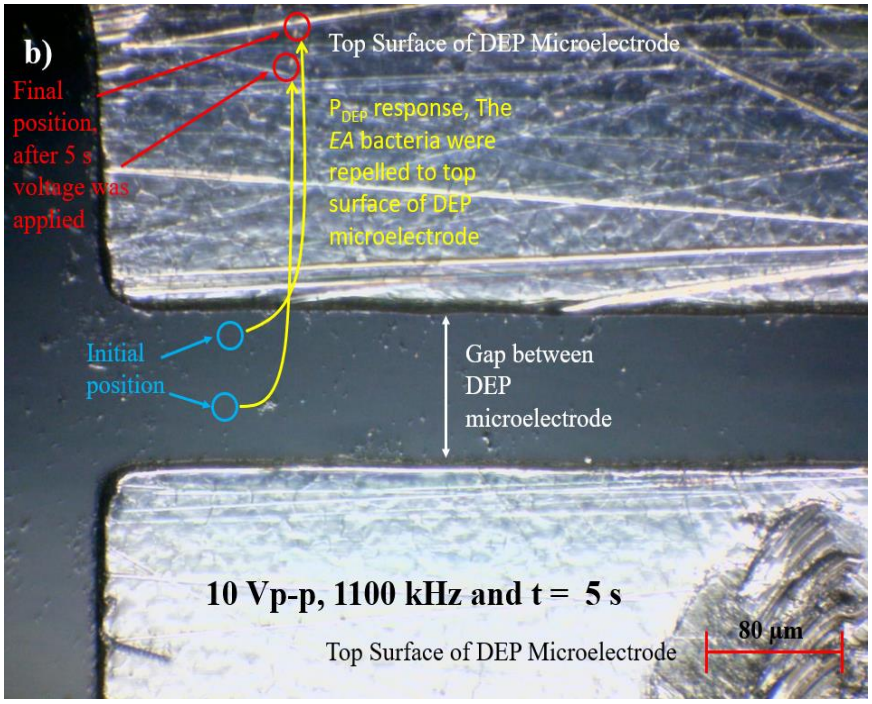

Figure 5 (b). Response P_{DEP} on 10 V_{pp} at 1100 kHz for *EA* final movement P_{DEP} at 5 second.

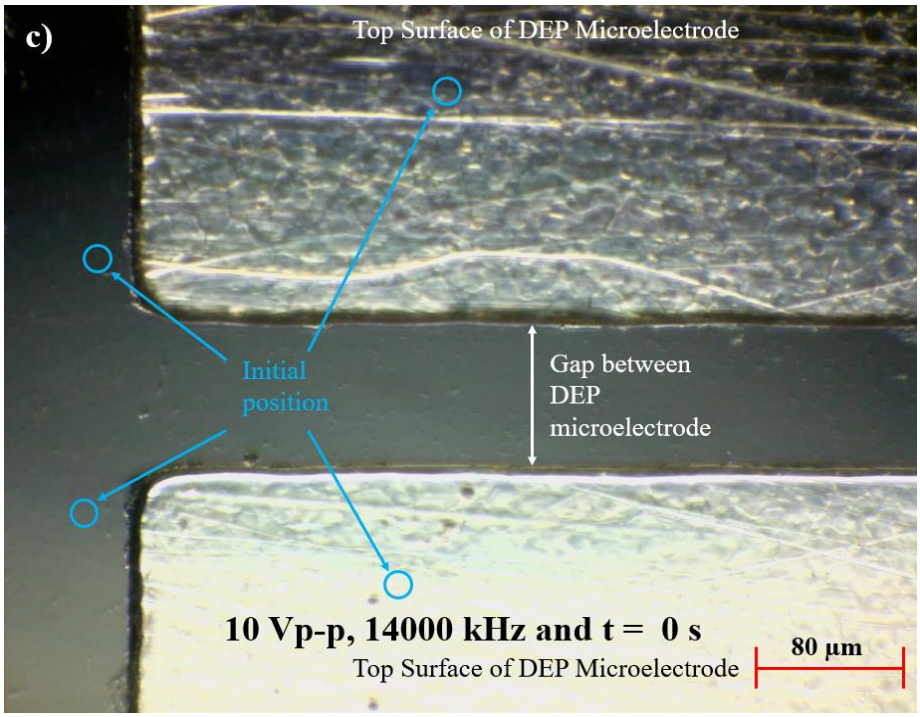

Figure 5 (c). Response N_{DEP} on 10 V_{pp} at 14000 kHz for *EA* initial state at 0 second, the *EA* bacteria were randomly at ROI.

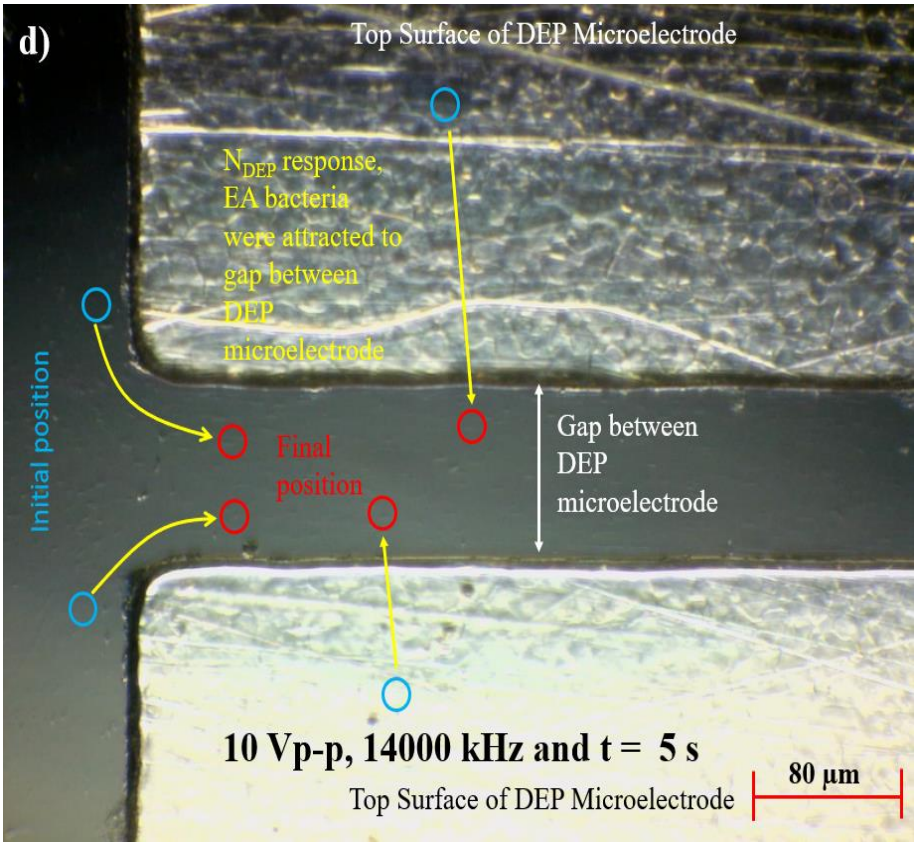

Figure 5 (d). Response N_{DEP} on 10 V_{pp} at 14000 kHz for *EA* final state, after 5second the *EA* was repelled and accumulated at between DEP microelectrode.

Based on the DEP experimental, the *EA* bacteria were given the P_{DEP} and N_{DEP} responses as an illustrated blue left-right arrow in figures 6 (a) and 6 (b). The *EA* bacteria were attracted to the top of the DEP microelectrode surface when applied the 10 V_{pp} at 300 kHz until 1100 kHz of input frequencies. Due to that having the high intensity, two spots of the electric field caused the *EA* bacteria was experienced as P_{DEP}. In contrast for 10 V_{pp} at 1400 kHz until 15000 kHz were experienced as N_{DEP} responses. All *EA* bacteria were repelling to the middle in between the DEP microelectrode. This region has a low intensity of the electric field, it caused these *EA* bacteria to accumulate in between the electrode. Since the input frequencies were at 1200 kHz and 1300 kHz on 10 V_{pp}, the *EA* was experienced as f_{xo} (no movement) because the DEP forces P_{DEP} equal N_{DEP} no net charges distorted. The f_{xo} range was illustrated in figure 6 (a) and 6 (b) as a red vertical rectangle. To illustrate crossover frequency clearly, we were close up figure 6 (a) by reducing the graph scaling from the range 300 kHz – 15000 kHz to 300 kHz – 3200 kHz as shown in figure 6 (b). Figures 6 (a) and 6 (b) also were showed the *EA* bacteria have one peak for P_{DEP} and one peak for N_{DEP}. The highest P_{DEP} velocity was 80 µm/s at 1100 kHz and the highest N_{DEP} velocity was 40 µm/s at range frequency of 8000 kHz until 10000 kHz. Thus, the COF_u frequency was ranged from 1200 kHz until 1300 kHz.

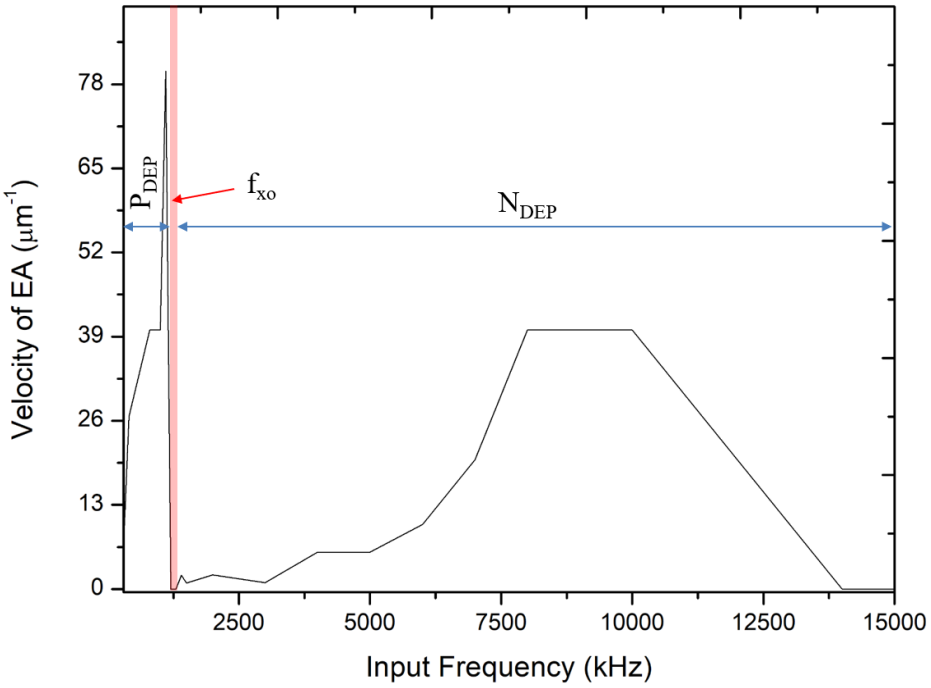

Figure 6 (a). Overall experimental result for the curve of *EA* velocity versus input frequency at 10 V_{pp} 300 kHz to 15000 kHz.

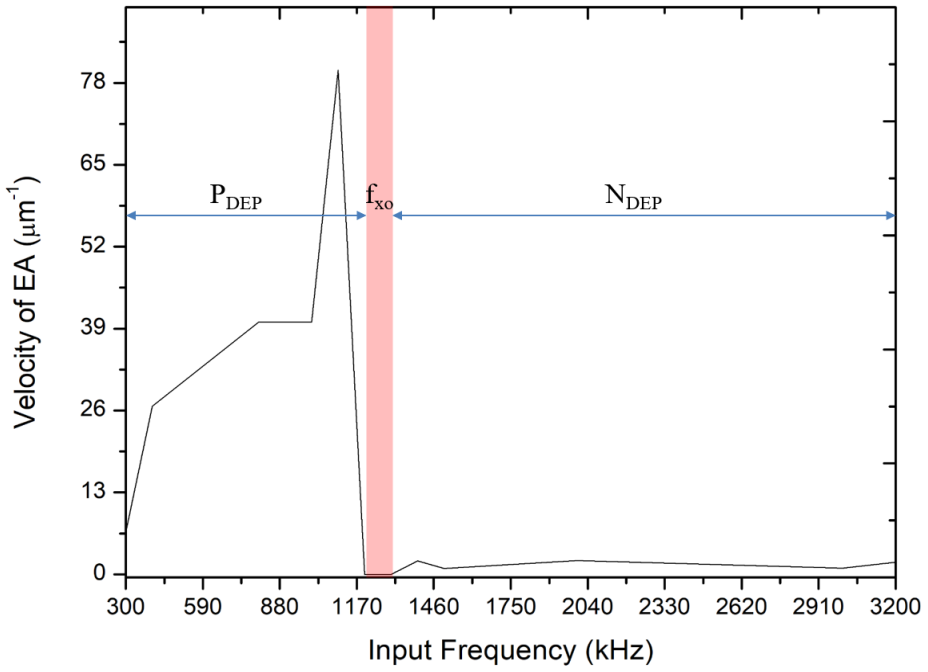

Figure 6 (b). The close up of experimental result for *EA* COF_u ranges from 1200 kHz to 1300 kHz.

The originality of this work is using contactless DEP technique in detection of EA. Simple and clean method without additional marker caused contamination. The alternative solution to culture technique that takes a long time for detection purposes.

4. CONCLUSION

This study was successfully conducted the DEP experimental to manipulate and isolate the *EA* on 10 V_{pp} at 300 kHz until 15 MHz, the result indicated the F_{DEP} responses on 10 V_{pp} at 300 kHz until 1100 kHz were P_{DEP} lateral attraction. The frequencies input 1400 kHz until 15000 kHz at 10 V_{pp} was N_{DEP} vertical repulsion. Thus, the COF_u responses were at 1200 kHz and 1300 kHz on 10 V_{pp}. These COF_u played an important role for the detection of *EA* bacteria.

ACKNOWLEDGEMENTS

The author would like to acknowledge with gratitude the sponsor of Geran Galakan Penyelidik Muda (GGPM-2017-028), grants funded by Universiti Kebangsaan Malaysia (UKM) as a Research University and AKU254: HICOE (FASA2) for Artificial Kidney grant funded by the Ministry of Higher Education (MOHE) Malaysia.

REFERENCES

[1] Brooks, L.E., Ul-Hasan, S., Chan, B. K. & Sistrom, M.J., Quantifying the evolutionary conservation of genes encoding multidrug efflux pumps in the ESKAPE pathogens to identify antimicrobial drug targets. *Ecological and Evolutionary Science.* **3**, 3 (2018) e00024-18.

[2] Renner, L. D., Zan, J., Hu, L. I., Martinez, M., Resto, P. J., Siegel, A. C., Torres, C., Hall, S. B., Slezak, T. R., Nguyen, T. H. & Weibel, D. B., Detection of ESKAPE bacterial Pathogens at the point of care using isothermal DNA-based assays in a portable degas-actuated microfluidic diagnostic assay platform. *Applied and Environmental Microbiology.* **83**, 4 (2017) e02449-16.

[3] Santajit, S., & Indrawattana, N., Mechanisms of antimicrobial resistances in ESKAPE pathogens. *Biomed Research International*. **2016**, 1155 (2016) 1 – 8.

[4] Pethiq, R., Dielectrophoresis: An assessment of its potential to aid the research and particle of drug discover and delivery. Advance Drug Delivery Reviews. **65**, 11 - 12 (2013) 1589-1592.

[5] Honegger, T., & Peyrade, D., Moving Pulsed Dielectrophoresis. Lab on a chip. **13**, 8 (2013) 1538 - 1545.

[6] Buyong, M. R., Larki, F., Caille, C. E., Takamura, Y., Hamzah, A. A., & Majlis, B. Y., Determination of lateral and vertical dielectrophoresis force using tapered microelectrode array. Micro & nano letters. **13**, 2 (2018) 143 – 148.

[7] Buyong, M. R., Yunas, J., Hamzah, A. A., Majlis, B. Y., Larki, F., & Aziz, N. A., Design, fabrication and characterization of dielectrophoresis microelectrode array for particle capture. Microelectronics International. **32**, 2 (2015) 96-102.

[8] Buyong, M. R., Larki, F., Takamura, Y., & Majlis, B. M., Tapered microelectrode array system for dielectrophoretically filtration: fabrication, characterization, and simulation study. Journal of Micro/Nanolithography, MEMS, and MOEMS. **16**, 4 (2017) 044501.

[9] Mohammad, K., Buchanan, D. A., Braasch, K., Butler, M. & Thomson, D. J., CMOS single cell dielectrophoresis cytometer. *Sensors and Actuators B: Chemical*. **249** (2017) 246-255.

[10] D'Amico, L., Ajami, N. J., Adachi, J. A., Gascoyne, P. R. C. & Petrosino, J. F., Isolation and concentration of bacteria from blood using microfluidic membraneless dialysis and dielectrophoresis. *Lab on a Chip*. **17**, 7 (2017) 1340-1348.

[11] Yunus, F. W., Buyong, M. R., Yunas, J., Majlis, B. M., Hamzah, A. A., 3-Dimensional Electric Field Distributions of Castellated and Straight Dielectrophoresis Electrodes for Cell Separation. *Sains Malaysiana*. **48**, 6 (2019) 1239-1249.

[12] M. I. Samad, M. R. Buyong, S. S. Kim, & B. Y. Majlis., Dielectrophoresis velocities response on tapered electrode profile: simulation and experimental. Microelectron. Inter. **36** [2019] 45-53.
[13] Diene, S. M., Merhej, V., Henry, M., El Filali, A., Roux, V., Robert, C., Azza, S., Gavory, F., Barbe, V., La Scola, B. & Raoult, D., The rhizome of the multidrug-resistant Enterobacter aerogenes genome reveals how new "killer bugs" are created because of a sympatric lifestyle. Molecular biology and evolution. **30**, 2 (2013) 369 – 383.
[14] Pohl HA, Dielectrophoresis: The behavior of neutral matter in nonuniform electric fields (Cambridge Monographs on physics). Cambridge/New York: Cambridge University Press, (1978).
[15] Pethig R. R, Dielectrophoresis: Theory, methodology and biological applications. John Wiley & Sons, (2017).
[16] Rahim, M. K. A., Buyong, M. R., Jamaludin, N. M. A., Hamzah, A. A., Siow, K. S., Majlis, B. Y., "Characterization of permittivity and conductivity for ESKAPE pathogens detection", in IEEE International Conference on Semiconductor Electronics (ICSE), Kuala Lumpur, Malaysia, (2018) 132 – 135.
[17] Yunus, F. W., Hamzah, A. A., Norzin, M. S., Buyong, M. R., Yunas, J., Majlis, B. Y., "Dielectrophoresis: Iron Dificient Anemic Red Blood Cells for Artificial Kidney Purposes", in IEEE International Conference on Semiconductor Electronics (ICSE), Kuala Lumpur, Malaysia, (2018) 5 – 8.
[18] Jamaludin, N. M. A., Buyong, M. R., Rahim, M. K. A., Hamzah, A. A., Mailis, B. Y., & Bais, B. "Dielectrophoresis: Characterization of Triple-Negative Breast Cancer using Clausius-Mossotti Factor", in IEEE International Conference on Semiconductor Electronics (ICSE), Kuala Lumpur, Malaysia, (2018) 85 – 88
[19] Samad, M. I. A., Buyong, M. R., Yunus, F. W., Siow, K. S., Hamzah, A. A., & Majlis, B. Y., "Voltage characterization on dielectrophoretic force response to hematologic cell manipulation", in IEEE International Conference on Semiconductor Electronics (ICSE), Kuala Lumpur, Malaysia, (2018) 13 – 16.
[20) Buyong, M. R., Larki, F., Aziz, N. A., Yunas, J., Hamzah, A. A., & Majlis, B. Y., "Dielectrophoretic force response for lactobacillus casei", in IEEE International Conference on Semiconductor Electronics (ICSE), Kuala Lumpur, Malaysia, (2016) 38 – 42.