

Bacterial Concentration Detection with Dielectrophoresis and Capacitive Measurement

Jaehyuck Yoo, Misun Cha and Junhoon Lee

Department of Mechanical and Aerospace Engineering and Institute of Advanced Machinery and Design
Seoul National University, Seoul, Korea, jleenano@snu.ac.kr

ABSTRACT

The concentration of *Escherichia coli* (*E. coli*) cells in 1 μ l droplet of water was detected by depositing them on an array of electrodes using dielectrophoresis (DEP). Electrowetting was used to effectively concentrate the *E. coli* in a droplet of water which dried out during the DEP deposition, enabling a simple but powerful system without complicated microfluidic components. By measuring the capacitance after the deposition and evaporation, it was possible to quantitatively evaluate the cell population correlated with the concentration.

Keywords: dielectrophoresis (DEP), droplet, bacteria cell concentration, capacitive measurement

1 INTRODUCTION

The quantity estimation of microorganisms such as pathogenic bacteria cells is important in variety of fields including bioscience research, environmental monitoring, and hazard analysis in food industry. Impedance measurement and quantitative analysis after focusing cells by DEP have been proposed [1-4]. DEP has also been used for selective separation of micro particles and biological cells inside an evaporating droplet [5]. However the impedance method needed complicated microfluidic devices, and was affected by various factors such as adhesion, medium conductivity, and flow velocity. In this study, we use a DEP deposition of bacteria on an array of electrodes directly from a droplet. The concentration of bacterial cells was estimated by capacitance measurement after the deposition. Our approach demonstrates an effective system free from the issues mentioned above.

2 PRINCIPLE

When an AC voltage is applied between electrode pairs in a medium, an electric field is created. The electric field depends on the shape of the electrode pair and the properties of particle and medium, causing electrokinetic effects such as DEP, AC-electroosmotic flow (AC-EOF), and AC electrothermal (AC-ET) effect [7-10]. According to basic principle, the effective range of the DEP force is shorter than that of the AC-EOF force. Thus the AC-EOF is

used to focus particles near the intended area, and the DEP force is used to firmly attach the particles on the electrodes. AC-ET effect is not considered in this work because it becomes significant only when the medium conductivity and the voltage are both high.

2.1 Dielectrophoresis on Particles

In DEP process, an attractive force is exerted on a polarized particle in a non-uniform electric field. For example, a force produced on a sphere of radius R suspended in a medium of permittivity ϵ_m can be expressed given by

$$F_{DEP} = 2\pi R^3 \text{Re}[K(\omega)] \nabla E_{rms}^2, \quad (1)$$

where E_{rms} is the magnitude (RMS) of the applied field and $\text{Re}[K(\omega)]$ the real component of the Clausius-Mossotti factor

$$K(\omega) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*}, \quad (2)$$

where ϵ_p^* and ϵ_m^* are the complex permittivity of the particle and surrounding medium, respectively [2, 3]. For a real dielectric material, the complex permittivity is defined as

$$\epsilon^* = \epsilon - j \frac{\sigma}{\omega}, \quad (3)$$

where $j = \sqrt{-1}$, ϵ is the permittivity, σ the conductivity of the dielectric, and ω the angular frequency of the applied field. The Clausius-Mossotti factor is dependent on the frequency of applied electric field, resulting in either a pDEP (attractive) or an nDEP (repulsive). Moreover, the DEP force can be altered due to the variation of its field gradient related to electrode shape. The DEP force is usually strongest near electrode edge where electric field gradient is steepest. Therefore, many particles can be deposited along the electrode edge by the strong pDEP force.

2.2 AC Electroosmotic Flow by Capacitance

A nonuniform AC electric field generates steady fluid flow on coplanar electrode structure. The induced charge in the electrical double layer on the electrode surfaces generates the flow driven by the tangential component of the electric field [7-9]. Preliminary experimental results showed that the AC-EOF caused the polystyrene particles along the axis of electrode edge to rotate. The rotation speed was faster near the electrode where the electric field is stronger than far from the edge. In addition, the AC-EOF occurred only below the charge relaxation frequency. This AC-EOF made the entire circulation of the fluid that increases the possibility of group of particles existing within the effective range of DEP force even when the particles were initially far from the electrode edge. Figure 1 shows that the polystyrene particles attracted along the electrode edge by pDEP. The particles were also aligned along the middle section of the electrode because of the circulation caused by the AC-EOF.

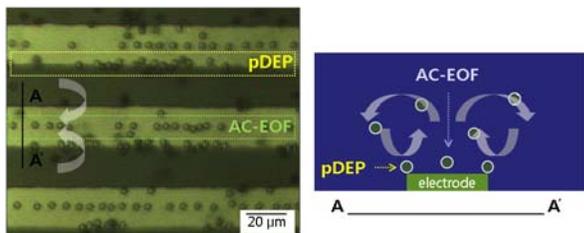


Figure 1: Polystyrene particles (3 μm) deposited by AC-EOF and pDEP (0.5 V_{pp} @ 10 kHz)

3 MATERIALS AND METHODS

3.1 Preparation of Bacterial Cell Media

E. coli DH5- α was initially incubated on agar plates. At the beginning of experiment, cells were harvested from the agar plates and were cultured in LB broth medium (Biosesang, Korea) at 37 $^{\circ}\text{C}$ for 18 hours. Cultured *E. coli* cells were washed several times in NaCl 0.85 % to remove the LB broth medium by centrifugation. After several washing steps by centrifugation, the cells were finally resuspended in a normal saline solution. The *E. coli* cells with five different concentrations (10^1 ~ 10^5 colony forming unit (cfu)/ μl) were prepared by serial dilution.

3.2 Fabrication of Electrodes

Two kinds of electrode devices were fabricated by lift-off as shown in Figure 2. The gap between the adjacent electrodes was 2 μm which is the average size of the bacterial cells to be detected. The electrodes in (a) consist of 100 pairs of opposing fingers between which the *E. coli* was to be deposited. The parallel electrode was designed to contain a prescribed amount of droplet (1 μl) at the center

of the electrode without overflowing. In this case, we expected that the dynamic range could be enhanced because of the large capacitance due to the wide area when almost all the *E. coli* in the medium were deposited.

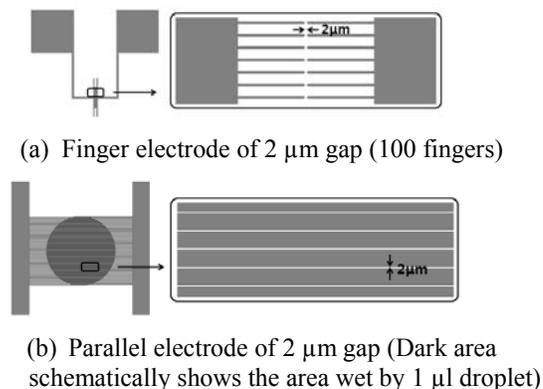


Figure 2: Schematics of electrode devices

3.3 Procedure

1 μl droplets with various concentrations of *E. coli* were dropped on the electrode devices while the voltage was applied with a function generator (AFG3101, Tektronix). The DEP voltage was experimentally tuned to be 1 MHz and 5 V_{pp} for the best pDEP deposition of the bacteria. In fact, this condition has been used to separate viable cells from unviable cells in a previous work [3]. After washing and drying up the bacterial solution, the capacitance was measured using digital capacitance meter (Model 3000, GLK instruments). Humidity was controlled during the capacitance measurement to increase the sensitivity.

4 RESULTS

E. coli was remained between the electrode fingers after drying as shown in Figure 3. When the deposition was performed with 1 μl droplet of 10^2 cfu/ μl concentration medium, theoretically one *E. coli* could be assigned on each finger pairs. Indeed single deposited *E. coli* was found in some cases, and five successive deposition of single *E. coli* was discovered as show in Figure 4.

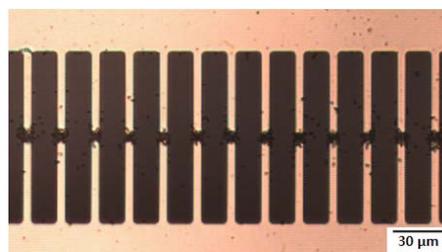


Figure 3: *E. coli*'s densely deposited between fingers after evaporation @ 1MHz and 5 V_{pp}

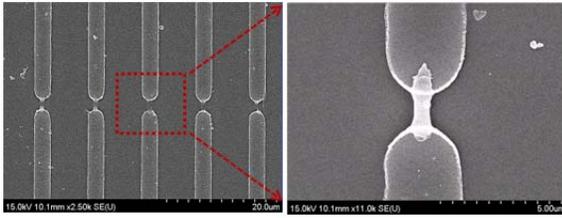


Figure 4: SEM images of five successive deposition of single *E.coli* with 10^2 cfu/ μ l concentration medium

The deposited *E. coli* at different concentrations appear in Figure 5. The quantity of the deposited *E. coli* was correlated with the concentration of *E. coli* solutions.

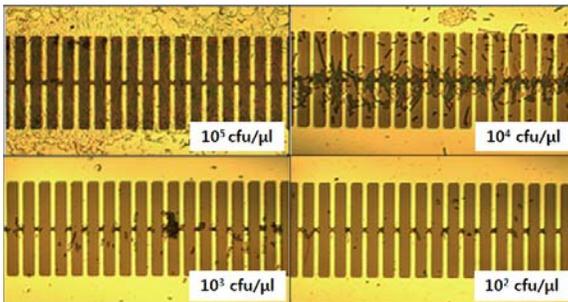


Figure 5: Deposited *E. coli* with various concentrations.

It was discovered that large capacitive variation occurred between different concentrations at a high humidity condition. Capacitance value after stabilization was correlated with the concentration of *E. coli* solution as shown in Figure 6.

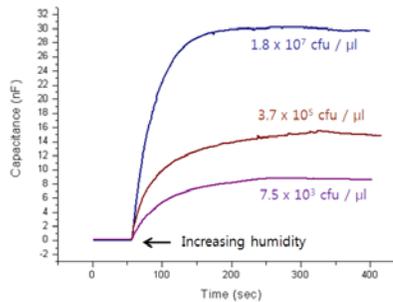


Figure 6: The transient capacitance response with concentration using the circular parallel electrode device

5 DISCUSSION

The humidity is the key to enhancing the signal strength. The capacitance of two parallel plates with area A separated by a distance d is approximately equal to the following,

$$C = \epsilon_r \frac{A}{d}, \quad (4)$$

where ϵ_r is the relative permittivity.

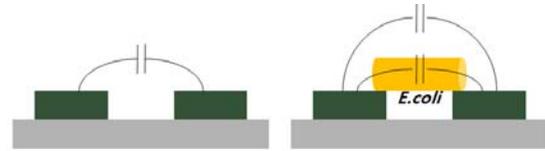


Figure 7: Capacitive modeling after *E. coli* deposition

As modeled in Figure 7, the humidity change affects the permittivity of the bridge with deposited *E. coli*, substantially increasing the measurable signal.

The deposition of bacterial cell in an evaporating droplet is affected by the competition between the pDEP force and the drag force of evaporating droplet. Therefore, the design of optimal electrode shape is important to increase the DEP force for the best performance. Our preliminary experiment confirmed that the finger electrodes can exert stronger DEP forces than the parallel electrodes.

The pattern of drying sequence is also critical for good deposition results. The electrowetting phenomenon was automatically involved, and helped effectively concentrating the deposition on active area [11]. Figure 7 shows the sequence of droplet evaporation guided by electrowetting, which did not occur without the application of the electric potential. The concentration of the liquid meniscus helps the deposition of *E. coli* cells by focusing evaporating droplet on the conducting electrodes.

Impedance measured by LCR meter (E4980A, Agilent) during deposition was decreased, in accordance with previous research [1-4]. However, the amount of impedance change was so small because 1μ l of droplet was not enough to produce effective signals. Previous research based on microfluidics and impedance suffered from similar issues. In most cases, bulky external apparatus was needed for the operation of fluids with fine control of speed. Surface treatment of the microfluidic channels to avoid bio fouling problem was also a concern, especially at low concentrations. In addition, many bacterial cells might have bypassed the trapping, and reduced the sensitivity of the impedance change. We believe our approach can resolve many of these issues with the droplet deposition and direct capacitive measurement.

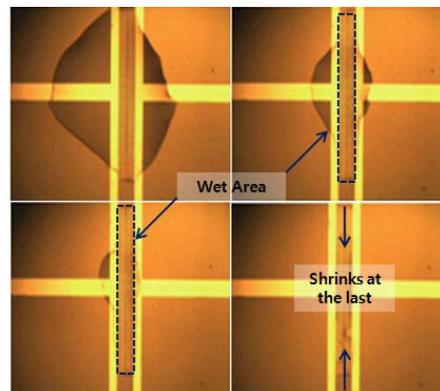


Figure 7: The sequence of droplet evaporation affected by electrowetting @ 1MHz, 10V

6 CONCLUSIONS

This paper presents bacterial cell deposition from a droplet driven by DEP and electrowetting. The concentration of the bacterial cells was estimated using capacitive measurement at a high humidity. Improved deposition and concentration sensitivity is expected with optimal design and material choice, enabling a new type bacteria cell sensor is under development based on the result of this work.

REFERENCES

- [1] Junya Suehiro, Ryuichi Yatsunami, Ryo Hamada and Masanori Hara, "Quantitative estimation of biological cell concentration suspended in aqueous medium by using dielectrophoretic impedance measurement method", *J. Phys. D: Appl. Phys.*, 32, 2814–2820, 1999.
- [2] T.B. Jones, "Electromechanics of Particles", Cambridge University Press, Cambridge, 1995.
- [3] Junya Suehiro, Ryo Hamada, Daisuke Noutomi, Masanori Shutou, and Masanori Hara, "Selective detection of viable bacteria using dielectrophoretic impedance measurement method", *Journal of Electrostatics*, 57, 157–168, 2003
- [4] Mats JoInsson, Ken Welch, Sven Hamp, and Maria Strømme, "Bacteria Counting with Impedance Spectroscopy in a Micro Probe Station", *J. Phys. Chem. B*, 110, 10165-10169, 2006
- [5] Jung-Yeul Jung and Ho-Young Kwak, "Separation of Microparticles and Biological Cells Inside an Evaporating Droplet Using Dielectrophoresis", *Anal. Chem.*, 79, 5087-5092, 2007.
- [6] Darren S. Gray, John L. Tan, Joel Voldman, and Christopher S. Chena, "Dielectrophoretic registration of living cells to a microelectrode array", *Biosensors and Bioelectronics*, 19, 1765–1774, 2004.
- [7] N. G. Green, A. Ramos, A. Gonza'lez, H. Morgan, and A. Castellanos, "Fluid flow induced by nonuniform ac electric fields in electrolytes on microelectrodes. III. Observation of streamlines and numerical simulation", *PHYSICAL REVIEW E*, 66, 026305, 2002
- [8] Jie Wu, "Biased AC Electro-Osmosis for On-Chip Bioparticle Processing", *IEEE TRANSACTIONS ON NANOTECHNOLOGY*, VOL. 5, NO. 2, MARCH 2006
- [9] A. Ramos, H. Morgan, N.G. Green and A. Castellanos, "The role of electrohydrodynamic forces in the dielectrophoretic manipulation and separation of particles", *Journal of Electrostatics*, 47, 71-81, 1999
- [10] M. Lian, N. Islam and J. Wu, "AC electrothermal manipulation of conductive fluids and particles for lab-chip applications", *IET Nanobiotechnol.*, 1, (3), 36–43, 2007
- [11] Lee J, Moon H, Fowler J, Kim CJ, Schoellhammer T, "Electrowetting and electrowetting-on-dielectric for microscale liquid handling", *Sensors and Actuators A*, 95, 259-268, 2002