Improvement of dielectrophoretic impedance measurement method by bacterial concentration utilizing negative dielectrophoresis

R Hamada^1,2, H Takayama^2, Y Shonishi^2, T Hisajima^2, L Mao^2, M Nakano^2, J Suehiro^2

1 R & D Center, Panasonic Healthcare Co., Ltd., 2131-1, Minanikata, Toon, Ehime, Japan
2 Department of Electrical and Electronic Engineering, Graduate School of Information Science and Electrical Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka, Japan

E-mail: hamada.ryo@jp.panasonic.com

Abstract. In this study, the concept design for the improvement of the bacterial detection sensitivity of the DEPIM (Dielectrophoretic Impedance Measurement) method has been proposed. The cells in the micro-chamber are repelled and concentrated by n-DEP (negative dielectrophoresis). The concentrated cells are captured by p-DEP (positive DEP) and detected by measuring the change in the electrical impedance. The numerical simulations and the preliminary test were performed to investigate the effectiveness of the n-DEP concentration. When n-DEP concentration was employed, the increase in the rate of the conductance became approximately two times higher than that obtained without n-DEP.

1. Introduction

To inspect the level of bacterial contamination in the production process and products is a crucial matter for the food and beverage industry because an outbreak of food poisoning results in health hazard to consumers, and the company may lose its image. Conventionally, bacterial count using the cultivation and colony counting method is adopted as a standard method for quantification of bacterial contamination. The culturing method is well established but the cultivation process requires typically one to several days to complete the inspection, so the development of a rapid bacterial quantification method is required in the industrial fields. In recent years, several rapid bacterial counting methods alternative to conventional cultivation methods, including Adenosine Tri-Phosphate (ATP) bioluminescence [1] and the direct-count technique using epifluorescence microscopy (EFM) [2] have been proposed. In most cases, alternative methods require operation with a reagent, so the process of bacterial detection is rather complicated. The authors have previously proposed DEPIM (Dielectrophoretic Impedance Measurement) method utilizing DEP (dielectrophoresis) as a simple and rapid technique for detection of bacteria without any reagents [3]. DEP is a motion of dielectrically polarized particles in non-uniform electric fields [4]. As most bacteria behave as polarizable particles in external AC electric fields, DEP allows the manipulation of the bacteria in a liquid suspension. The direction of the DEP force depends on dielectric properties of particles and the surrounding medium,
as well as frequency of applied electric field. In case a particle moves toward the high electric field region it is referred to as positive DEP (p-DEP), while it is referred to as negative DEP (n-DEP) for a particle repelled from the high field region. The DEPIM utilizes p-DEP in order to capture bacteria onto an electrode chip. Since bacteria have intrinsic electrical impedance, so the trapping of bacteria using p-DEP at the electrode results in a change in the electrical impedance of the electrode. Higher cell population results in faster bacteria trap and the electrode impedance change. By monitoring the temporal variation of the electrode impedance, the cell population can be quantitatively evaluated. DEPIM can realize simple and rapid bacterial inspection because it utilizes only the electrical phenomenon, also it can realize high sensitive detection combined with electropermeabilization [5] and selective detection of bacteria based on viability [6] or species [7].

In the DEPIM inspection, the lower the bacterial concentration, the longer it takes for the bacteria trapping process to sense the measurable change in the electrical impedance. Therefore, improving bacteria trapping efficiency by p-DEP is essential to achieve more rapid bacterial count by DEPIM. In the conventional DEPIM method, bacteria suspended in an aqueous medium are trapped onto a thin microelectrode, which is fabricated on a glass substrate to generate high electric field. The p-DEP force acting on a particle decreases rapidly in accordance with the increasing distance between the electrode and the particle. In these situations, one strategy to increase the trapping efficiency is to decrease the distance between the electrode surface and the lid aligned at the opposite side of the electrode. However, the narrower the flow path, the smaller the amount of bacteria which can pass through the chamber. This results in an increase in the time to trap sufficient amount of bacteria, which causes a measurable electrical impedance change. Another strategy is using n-DEP to concentrate bacteria to enhance trapping efficiency at p-DEP electrode [8].

In this study, an improved technique of the DEPIM for higher bacterial detection sensitivity was proposed and its effectiveness was experimentally confirmed.

2. Principle and theoretical prediction

Figure 1 shows the concept design of the bacterial concentration by n-DEP combined with the conventional DEPIM method. The concentrator consists of the microelectrode, which induces the n-DEP force on the cells passing through the chamber. The cells experience the n-DEP force and move towards the lower substrate. The detector is aligned at the downstream of the flow, and the structure is the same as the conventional DEPIM electrode system. As the cells reach the detector area to be concentrated in the vicinity of the lower substrate by the preceding n-DEP electrode, the cells are efficiently captured onto the microelectrode fabricated on the lower substrate and an impedance change arising from the captured cells will become larger in comparison with the conventional DEPIM method.

The numerical simulations were carried out to investigate the effectiveness of the bacterial concentration using n-DEP combined with the conventional DEPIM method. The DEP force acts on a spherical particle of radius \( r \) suspended in a medium of permittivity \( \varepsilon \), is given by [9]

\[
F_{\text{DEP}} = 2\pi r^3 \varepsilon_s \text{Re}[K(\omega)]\nabla E^2
\]

where \( E \) is the magnitude (RMS) of the applied field and \( \text{Re}[K(\omega)] \) is the real component of the Clausius–Mossotti factor given by

\[
K(\omega) = \frac{\varepsilon_p^* - \varepsilon_s^*}{\varepsilon_p^* + 2\varepsilon_s^*}
\]

where \( \varepsilon_p^* \) and \( \varepsilon_s^* \) are the complex permittivity of the particle and the surrounding medium, respectively. For a real dielectric, the complex permittivity is defined as

\[
\varepsilon^* = \varepsilon - j\frac{\sigma}{\omega}
\]
where $\varepsilon$ is the permittivity, $\sigma$ is the conductivity of the dielectric, and $\omega$ is the angular frequency of the applied field.

A particle moves in an aqueous medium by the DEP force, it experiences the drag force, $F_{\text{drag}}$, which can be written as

$$F_{\text{drag}} = -6\pi\eta av$$

where $\eta$ is the dynamic viscosity of the surrounding medium, $v$ is the particle velocity relative to the surrounding medium. Assuming Brownian motion and buoyancy force can be negligible, the equation of motion for a particle with mass $m$ in a medium can be written as

$$m\frac{dv}{dt} = F_{\text{DEP}} + F_{\text{drag}}$$

The electric field distribution, DEP force and bacteria motion trajectory were numerically calculated by a finite element method using COMSOL Multiphysics. The following values of the geometry of the interdigit electrode inducing n-DEP were employed on the numerical simulations: the electrode width was 50 $\mu$m, gaps between adjacent electrodes were 5 $\mu$m, and thickness of the electrode was 100 nm. We assumed the geometry of bacteria as a double-shelled structure, i.e. the conductive cytoplasm is covered by the thin insulation membrane. The values of the dielectric properties and geometry of bacteria were obtained from literature [10].

In order to study influences of the height of the chamber $h$, and the applied voltage $V$, the simulations were carried out at two different heights of the chamber; $h = 10, 20$ $\mu$m, and two different applied voltage; $V = 20, 50$ $V_{\text{peak-peak}}$. Figure 2 shows calculation results of the electric field distribution and bacteria trajectories. In the case of the thinner chamber, $h = 10$ $\mu$m and lower voltage, $V = 20$ $V_{\text{peak-peak}}$, bacteria trajectories were focused within about 5 $\mu$m above the lower substrate of the chamber after bacteria pass through the four electrode gaps (Figure 2(a)). Increasing voltage to 50 $V_{\text{peak-peak}}$ results in the enhancement of bacterial concentration so that bacteria were focused within 1 $\mu$m above the lower substrate after they passed the three electrode gaps (Figure 2(b)). On the other hand, increasing height of the chamber decreased concentration efficiency. In the case of the higher chamber, $h = 20$ $\mu$m, the applied voltage was lower, $V = 20$ $V_{\text{peak-peak}}$, bacteria could not arrive the region within 10 $\mu$m above the lower substrate even after passing the four electrode gaps (Figure 2(c)). At higher voltage, $V = 50$ $V_{\text{peak-peak}}$, the bacteria was barely concentrated within 10 $\mu$m above the lower substrate after passing the four electrode gaps (Figure 2(d)).
3. Materials and Methods

Figure 3 depicts an outline of the DEPIM system combined with the n-DEP concentrator. We employed the 66-gap n-DEP electrode followed by the 4-gap p-DEP electrode arranged at opposite sides of each other. Due of the limitation of the manufacturing of the chamber, the height of the chamber was 200 $\mu$m, which was larger than that assumed in the theoretical model. The frequencies of the applied electric field to n-DEP and p-DEP electrodes are 1 kHz and 100 kHz, respectively, which were determined by the investigation of $\text{Re}[K(\omega)]$ calculated in our previous work [11]. These values were suitable for n-DEP and p-DEP in a low conductivity medium. The cell suspension liquid was stored in a reservoir tank and circularly fed to the test chamber using a peristaltic pump. Sinusoidal AC voltage was generated by a function generator (WF 1945, NF Corporation, Japan) and applied to the n-DEP electrode for repelling the bacteria towards the p-DEP electrode. AC voltage was applied to the p-DEP electrode by a lock-in amplifier, which also monitors the current passing through the p-DEP electrode via shunt resistance (1 k$\Omega$), and the data was transferred to the PC to calculate the impedance change. *E. coli* strain K-12 (NBRC3301), which has a high growth rate and has been successfully employed in previous work [3, 5-7], was employed in order to improve the efficiency of the experiments. *E. coli* was incubated at 30°C on agar plates for 24 hours. Before each measurement, cells were harvested from the agar and suspended in a 0.1 M mannitol solution. After several washings...
by centrifugation, they were finally resuspended in a 0.1 M mannitol solution (conductivity 1 µS/cm) at various diluted concentrations as determined by a colony counting method.

![Figure 3. Outline of the DEPIM system combined with the n-DEP concentrator.](image)

4. Results and discussion

Figure 4 depicts the DEPIM results with and without the n-DEP concentration. When the n-DEP concentration was employed, the increase rate of conductance was approximately two times faster compared to that obtained without the n-DEP concentration. This result proves that the number of bacteria trapped onto the p-DEP electrode increases as a result of n-DEP concentration as theoretically predicted in Figure 2.

In the present study, we aimed to improve DEPIM sensitivity by utilizing n-DEP concentrator, in which bacteria are repelled downward from the n-DEP electrode surface towards the p-DEP electrode surface. It is expected that energizing the n-DEP electrode with higher electrical potential can enhance the n-DEP force and the resultant concentration of cells. According to numerical calculation results shown in Figure 2, it was revealed that an increase in the applied voltage enlarged high field regions near the n-DEP electrode and could drive more bacteria toward the p-DEP electrode. In a real situation, however, there is an upper limit of applied voltage due to electrochemical stability of the thin metal electrode. More n-DEP concentration may be alternatively achieved by increasing the number of electrode gap, where high electric field and strong n-DEP force arises. In order to confirm if this idea works, computer simulation of cell trajectory was conducted again for various number of the electrode gap. As shown in Figure 5, a cell can be driven closer to the bottom surface of the chamber as the number of the electrode gap. This result shows that an increase in the number of the gaps of the n-DEP electrode can enhance the efficiency of the bacteria concentration by n-DEP.

According to Figures 2 and 5, it would seem that making the chamber height smaller has an advantage because bacteria can be driven closer to the chamber bottom with less number of electrode and lower electrode energizing voltage. However, lower chamber height or smaller channel cross section decreases the number of bacteria flowing into the chamber per unit time for a constant flow rate of bacteria suspension and bacteria concentration. If the suspension flow rate is increased to compensate the negative effect of the smaller height, the drag force will be comparable with n-DEP force so that n-DEP effect might be suppressed. In the future work, these parameters should be optimized to make DEPIM sensitivity as high as possible.

5. Concluding remarks

In this study, we proposed a new concept of the n-DEP bacterial concentration combined with the conventional DEPIM method to improve DEPIM sensitivity for on-site inspection of bacterial contamination. Results of numerical simulations and preliminary DEPIM tests show that the concept of the n-DEP concentration works.
This work was partly supported by a Grand-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (No. 20360184, No. 21651063 and No. 22110510).

**Figure 4.** DEPIM results with and without n-DEP concentration.

**Figure 5.** Effects of the number of n-DEP electrode gap on cell position.

**References**

[1] Jinping Luo, Xiaohong Liu, Qing Tian, Weiwei Yue, Jing Zeng, Guangquan Chen and Xinxia Cai 2009 Disposable bioluminescence-based biosensor for detection of bacterial count in food *Analytical Biochemistry* **394** 1–6

[2] L. KEPNER JR. and JAMES R. PRATT 1994 Use of Fluorochromes for Direct Enumeration of Total Bacteria in Environmental Samples: Past and Present *MICROBIOLOGICAL REVIEWS* Dec. 603-615

[3] J. Suehiro, R. Yatsunami, R. Hamada and M. Hara 1999 Quantitative estimation of biological cell concentration suspended in aqueous medium by using dielectrophoretic impedance measurement method *J. Phys. D: Appl. Phys.* **32** 2814-2820.

[4] R. Pethig 1996 Dielectrophoresis: using inhomogeneous ac electrical fields to separate and manipulate cells *Crit. Rev. Biotechnol.* **16** 331-348

[5] J. Suehiro, T. Hatano, M. Shutou and M. Hara 2005 Improvement of electric pulse shape for electropermeabilizationassisted dielectrophoretic impedance measurement for high sensitive bacteria detection *Sensors and Actuators B* **109** 209-215

[6] J. Suehiro, R. Hamada, D. Noutomi, M. Shutouand and M. Hara 2003 Selective detection of viable bacteria using dielectrophoretic impedance measurement method *J. Electrostat.* **57** 157-168

[7] J. Suehiro, D. Noutomi, M. Shutou and M. Hara 2003 Selective detection of specific bacteria using dielectrophoretic impedance measurement method combined with an antigen–antibody reaction *J. Electrostat.* **58** 229-246

[8] F. Aldaeus, Y. Lin, J. Roeraade and G. Amberg 2005 Superpositioned dielectrophoresis for enhanced trapping efficiency *Electrophoresis* **26** 4252-4259

[9] T. B. Jones 1995 Electromechanics of Particles *Cambridge University Press*

[10] M. Llamas, V. Giner and M. Sancho 1998 The dynamic evolution of cell chaining in a biological suspension induced by an electrical field *J. Phys. D: Appl. Phys.* **31(21)** 3160-3167

[11] R. Hamada, J. Suehiro, M. Nakano, T. Kikutani and K. Konishi (inpress) Development of rapid oral bacteria detection apparatus based on dielectrophoretic impedance measurement method *IET Nanobiotechnol.*