DNA Detection Method based on the Microbead Velocity under Traveling Wave Dielectrophoresis

Zhenhao Ding[1], Michihiko Nakano[2] and Junya Suehiro[2]
[1]*Graduate School of Information Science and Electrical Engineering, Kyushu University, 744 Motooka, Fukuoka, Japan*
[2]*Faculty of Information Science and Electrical Engineering, Kyushu University, 744 Motooka, Fukuoka, Japan*

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Abstract: Polymerase chain reaction (PCR) is a highly specific and sensitive detection method for bacterial and viral infections by amplifying the specific regions of DNA or RNA via enzymatic reaction. The authors have developed a rapid DNA detection method based on the dielectrophoresis (DEP) characteristic of DNA labeled microbeads for the rapid detection of the DNA that amplified by PCR. This method is based on the change of the Clausius-Mossotti (CM) factor *K* of DEP when DNA is attached onto microbeads. In former studies, we developed a new DNA detection method based on the change of the real part of K (Re[*K*]). However, this method requires a large amount of DNA attaching to a microbead to alter the microbead DEP behaviour for DNA detection. In this study, we focus on the imaginary part of K (Im[*K*]), which theoretically will change more dramatically than Re[*K*] when DNA is attached onto the microbeads. Since the traveling wave dielectrophoresis behaviour is based on the Im[*K*], we propose a new method for DNA detection based on the traveling wave dielectrophoresis (twDEP) of microbeads.

1 INTRODUCTION

There are various diseases caused by bacterial or viral infections. In order to select an appropriate treatment for an infectious disease, the early detection and identification are extremely important. A nucleic acids amplification assay is a highly sensitive and specific method among various diagnosis methods for infectious diseases. The polymerase chain reaction (PCR) is a type of nucleic acids amplification assay, which is well developed for clinical applications. The PCR is used to amplify specific regions of DNA or RNA of a target pathogen via enzymatic reaction. The amplicons amplified by PCR are generally detected by the agarose gel electrophoresis, which is well established and reliable. However, the agarose gel electrophoresis requires time-consuming manual operation by experts.

The authors develop and demonstrate a novel electrical method for rapid detection of amplicons by dielectrophoresis (DEP) of microbeads (Nakano et al. 2014). In the method, the amplicons are chemically attached to dielectric microbeads so that the DNA attaching alters the surface conductance of microbeads and result in the change of Clausius–Mossotti (CM) factor *K* of DEP. When enough amount of DNA is attached to the microbeads, the real part of *K* (Re[*K*]) will change from negative to positive, which means the DNA-labeled microbeads will be trapped on a microelectrode under the action of positive DEP, whereas pristine ones are not trapped. Combining this dramatic alteration in DEP phenomena with impedance measurement allows rapid and quantitative detection of the amplicons, and can be used for bacterial detection (Ding et al. 2016). However, this method requires a large amount of DNA attaching to a microbead to alter the microbead DEP behaviour for DNA detection.

In this study, we propose a new DNA detection method based on the traveling wave dielectrophoresis (twDEP), which is a phenomenon affected by the imaginary part of *K* (Im[*K*]). Since the Im[*K*] will change more dramatically than Re[*K*] against DNA attachment on the microbeads when microbeads surface conductance is small, the twDEP can lead to a more sensitive detection of DNA. Since the velocity of microbeads will change due to different twDEP forces, we measured the velocity of DNA labeled microbeads under twDEP force using computer-based image analysis.

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2 THEORY

DEP is the electrokinetic motion of dielectrically polarized materials in non-uniform electric fields, and it is currently an active area of research for manipulation of biological particles and nanomaterials, including bacterial cells and DNA molecules (Pethig, 2010, Hughes, 2000, Washizu and Kurosawa, 1990). The DEP force acting on a spherical dielectric particle of radius r suspended in a medium of absolute permittivity ε_m is given by as follows

$$F_{DEP} = 2\pi r^3 \varepsilon_m \mathrm{Re}[K(\omega)]\nabla E^2 \quad (1)$$

where E is the magnitude of the applied field. Re[$K(\omega)$] is the real component of the Clausius–Mossotti (CM) factor, given by

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \quad (2)$$

where ε^*_p and ε^*_m are the complex permittivities of the particle and the surrounding medium, respectively. For a real dielectric, the complex permittivity is defined as $\varepsilon^* = \varepsilon - j(\sigma/\omega)$, where ε is the permittivity, σ is the conductivity of the dielectric, and ω is the angular frequency of the applied electric field. When Re[$K(\omega)$] has a positive value, the particle is propelled toward the high field region (positive DEP, p-DEP). With a negative value of Re[$K(\omega)$], the particle is repelled from the high field region (negative DEP, n-DEP).

The conductivity of a solid dielectric particle, σ_p, can be expressed by the following equation (Zhou et al. 1995, Ermolina and Morgan, 2005).

$$\sigma_p = \sigma_b + \frac{2K_s}{r} \quad (3)$$

where σ_b and K_s are the bulk conductivity and the surface conductance of the particle. Equations 1–3 imply that the dielectric properties and the resultant DEP force acting on a smaller particle should be more dependent on the surface conductance K_s. Hughes *et al.* reported that antibody (protein) coating of submicrometer latex spheres altered the surface conductance and DEP spectrum of the particles, enabling the separation of unlabeled and protein-labeled particles (Hughes and Morgan, 1999).

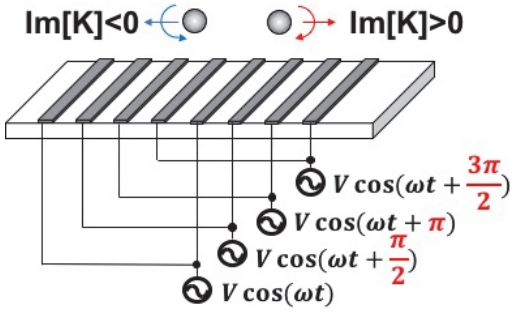

Figure 1: The twDEP of microbeads under four voltage waveforms with shifted phase.

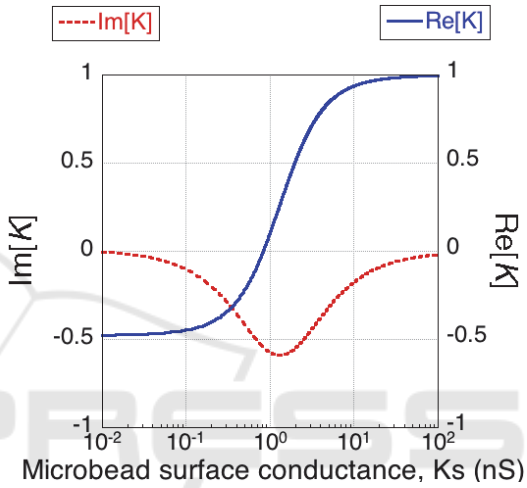

Figure 2: Calculation results of relationship between microbeads surface conductance and Re[K], Im[K].

The twDEP will be generated when the electric field has a spatially varying phase. When the electrodes are equally spaced, the phase angle change across each electrode will be the same. The twDEP force is given by (Cheng et al. 2009, Fathy et al. 2017):

$$F_{twDEP} = -\frac{4\pi^2 r^3 \varepsilon_m}{\lambda} \mathrm{Im}[K(\omega)]E^2 \quad (4)$$

where λ is the distance between every four electrodes. Microbeads will experience twDEP force against or along the direction of field travel when the Im[K] is positive or negative as shown in Fig. 1. The theoretical calculation results of relationship between microbeads surface conductance and Re[K] as well as Im[K] are shown as Fig. 2. The voltage frequency used in the calculation is 100 kHz. The Im[K] changed more dramatically than Re[K] when microbeads surface conductance is small (less than 0.2 nS), which means Im[K] will change more dramatically when less DNA was attached on the microbeads. Since the twDEP force is associated with the Im[K] and change the velocity of the

microbeads, the target DNA can be detected more sensitively by measuring the velocity of microbeads under the twDEP.

3 EXPERIMENTS

We used pUC 19 DNA as the template for PCR. The 5' end of forward primers were labeled with biotin. As the results of PCR, 391 bp DNA were amplified and the amplicons were confirmed by standard agarose gel electrophoresis.

Magnetic microbeads (Dynabeads® M-280, Life Technologies 2.8 µm in diameter) were used in this experiment. The surface of the microbead was coated with streptavidin, which binds specifically to biotin. Microbeads ($3x10^4$ beads/µl) were mixed with the reaction solution (5 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 1 M NaCl), including the amplicons. The amplicon concentrations in the solution were $3.5x10^7$ copies/µl and $3.5x10^8$ copies/µl, which make the ratio of DNA to microbeads as 10^3 : 1 and 10^4 : 1, respectively. The mixtures of the amplicons and microbeads were incubated at room temperature for 30 minutes. Hence, the microbeads were labeled with amplicons via biotin-streptavidin interaction. Then, the DNA labeled microbeads were suspended in deionized water (conductivity 4×10^{-4} S/m).

In order to manufacture the Indium Tin Oxide (ITO) microelectrode with 30 fingers, the glass substrate with ITO thin film (Narika, Inc.) was used. The microelectrode was fabricated by photolithography and wet etching. The schematic diagram and microscope image of the micro electrode were show as figure 3.a. The ITO microelectrode, which is transparent, was chosen in this study in order to simplify the process of velocity analysis by computer-based image analysis.

20 µl of the solution containing the DNA-labeled microbeads was placed on the microelectrode and covered with a cover slip. The microscope image of the microbeads solution at the detection region was shown as figure 3. b. Then the voltage (6 Vp-p, 100 kHz) was applied on the microelectrode with 90-degree phase shift between each electrode fingers to generate twDEP and the twDEP was confirmed by microscope observation. As a result, the microbeads, which were shown in figure 3.b. moved horizontally to the left. The movements of microbeads were recorded by a CCD camera at 100 fps and used for velocity analysis.

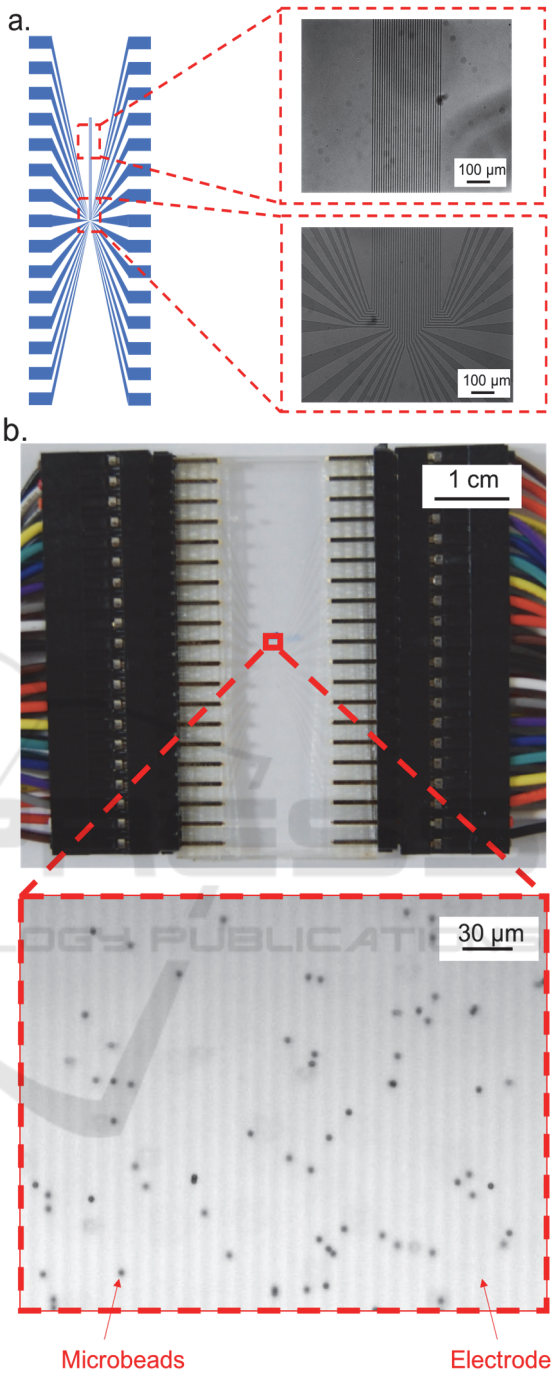

Figure 3: a. The schematic diagram and microscope image of the microelectrode; b. The photo of the electrode and the microscope image of detection region after the microbeads solution was placed on the microelectrode.

The velocities of microbeads were analysed by image analysis software Image-Pro (Media Cybermetics Inc). The trajectories of microbeads

inside the detection region were automatically tracked and analysed.

4 RESULTS AND DISCUSSION

The velocity of bare microbeads and DNA labeled microbeads at ratio of 10^3 and 10^4 copies DNA to 1 microbead was shown in figure 4.a. b. and c. respectively. The velocity of microbeads distributed in a range of velocities. The main reason was that the amount of DNA attached to each microbead was not perfect uniform.

The average velocity of bare microbeads and DNA labeled microbeads at ratio of 10^3 and 10^4 copies DNA to 1 microbead was calculated and shown in figure 5. The average velocity of microbeads increased as the labeled DNA amount increased. This is because the increase of labeled DNA will result in the increase of the surface conductance of microbeads. As shown in figure 2, the Im[K] will increase against the increase of microbead surface conductance when the surface conductance is small. Hence, the twDEP force that microbeads experienced would increase along with the increase of labeled DNA and result in the increase of the average velocity as shown in the figure 5.

As shown in figure 5, the velocity of microbeads under twDEP force can only be measured when less than 10^4 copies of DNA attached on 1 microbead. This was because the twDEP would only occur when the Re[K] was negative. When the Re[K] was positive, the microbeads would experience positive DEP and be trapped to the gap of microelectrode. Hence, the microbeads would not be able to move upon the electrode. Since the microbeads would experience positive DEP when the ratio of DNA to microbeads was above 10^5 :1, the twDEP would only occur when the labeled DNA was less than 10^5 copies on 1 microbead. Hence, the velocity of microbeads under twDEP force can be measured when less than 10^4 copies of DNA were labeled on 1 microbead as shown in figure 5. However, when the Re[K] was positive, which means the microbeads would be trapped between the microelectrode and induce the impedance change of the microelectrode as former proposed method (Nakano et al. 2014). Hence, the detection sensitivity can be increase by applying the twDEP and the detection range can be increase by combine the former method and twDEP based method.

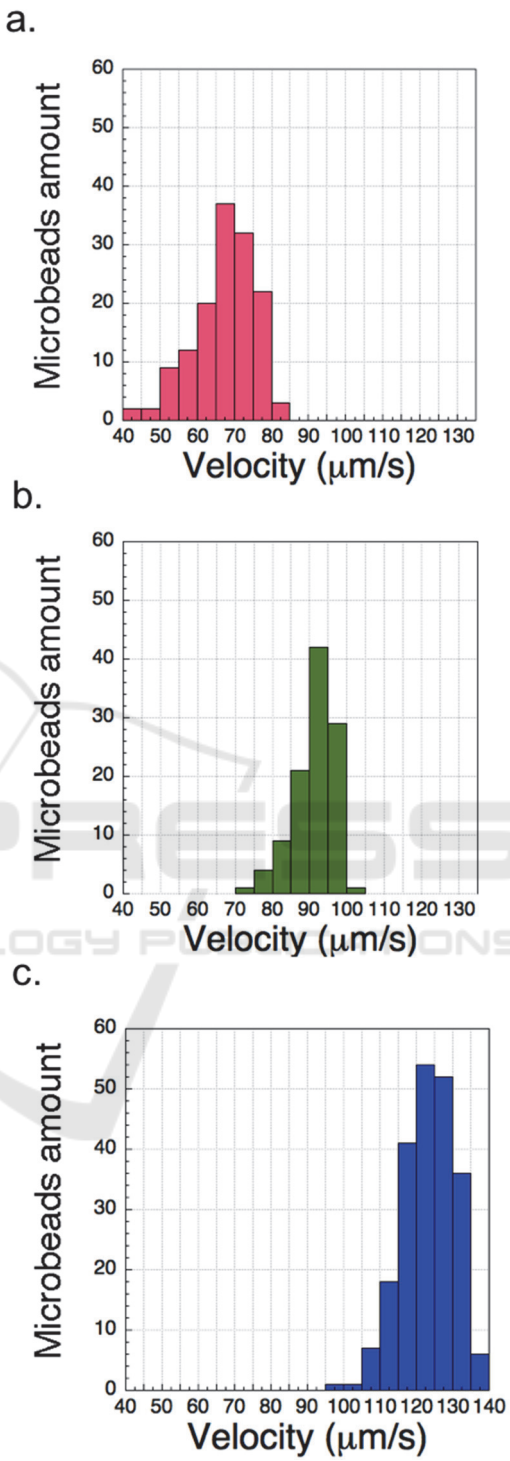

Figure 4: The velocity of a. bare microbeads; b. DNA labeled microbeads at ratio of 10^3 copies DNA to 1 microbead; c. DNA labeled microbeads at ratio of 10^4 copies DNA to 1 microbead.

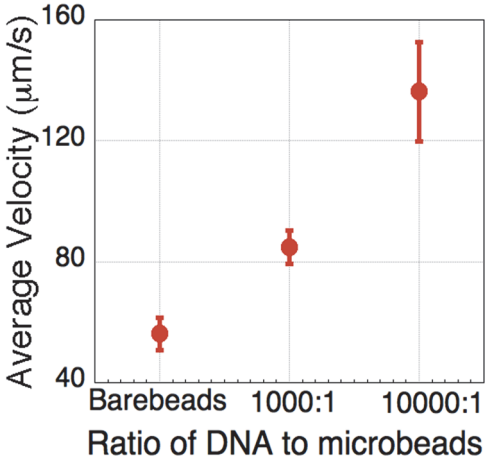

Figure 5: The velocity of bare microbeads and DNA labeled microbeads at ratio of 10^3 and 10^4 copies DNA to 1 microbead.

5 CONCLUSION

The velocity of DNA labeled microbeads under twDEP was measured and analysed by image analysis. The average velocity of DNA labeled microbeads would increase along with the increase of the amount of the labeled DNA when the ratio of DNA to microbead is above 10^3: 1. Since the former proposed method required the amount of DNA to alter the DEP force from negative to positive, which requires the amount of DNA to achieve the ratio of DNA to microbeads above 10^5 : 1, this method can increase the sensitivity of rapid DNA detection based on the twDEP. Furthermore, by combing this method with the former proposed method, the detection range of DNA can be increased as well. For example, by measuring the impedance change of the electrode as well as the velocity of the microbeads after unknown amount of DNA labeled microbeads placed on the microelectrode, the DNA can be detected as long as there were more than 10^3 copies of DNA labeled on one microbeads.

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