

# Measurement of dielectrophoretic mobility of single micro-particles in a flow channel

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A new device to measure the dielectrophoretic mobility  $\alpha$  of a single micro-particle in a micro-flow system has been proposed. From the velocity analysis of the micro-particle flowing between a pair of parabolic electrodes, the value of  $\alpha$  could be obtained.

There have been many analytical separation methods developed for molecules and ions in solution.<sup>1</sup> However, analytical separation methods for higher molecular weight proteins than 10<sup>5</sup> Da, larger DNA than 40 kbp and biological cells in solution are still very limited. For the separation analysis of biological cell, centrifugation, ultra filtration and electrophoresis have been widely used. However, these methods are not enough to separate various bio-particles. For example, it is reported that the separation between live and dead cells is difficult by ordinary electrophoresis.<sup>2</sup>

Recently, various new analytical methods for micro-particles, including dielectrophoresis (DEP),<sup>3–12</sup> laser photophoresis and magnetophoresis, have been proposed from our laboratory. These new methods are characteristic in utilizing various external fields<sup>13</sup> to manipulate and separate micro-particles in liquids. DEP is an interesting dielectric phenomenon originally reported by Pohl.<sup>3</sup> It is the migration of a micro-particle in non-uniform electric field, which is caused by an interaction between an induced dipole in the particle and the gradient of electric field intensity. The DEP migration velocity depends on the size of micro-particle, the conductivity and permittivity of both micro-particle and medium, and the frequency and voltage of the alternating current which generates the non-uniform electric field. The migration from weaker electric field to stronger electric field is called “positive dielectrophoresis”. In reverse, the migration from stronger electric field to weaker electric field is “negative dielectrophoresis”.

Recently, we have studied the dielectrophoretic behavior of commercial baker's yeast cell (*Saccharomyces cerevisiae*) with a planar-quadrupole electrode and reported that the binding of protein, *concanavalin A*, by yeast cells and the death of the cells by heating could be detected from a change in DEP behavior.<sup>12</sup> We also investigated experimentally the dependence of dielectrophoretic mobility,  $\alpha$ , on the diameter, conductivity and permittivity of sample particles.<sup>9</sup> The value of  $\alpha$  is useful to characterize the property of micro-particles. However, the planar-quadrupole electrode used previously was not compatible with high throughput analysis. To solve this problem, we combined a planar electrode and a micro-flow system for the dielectrophoretic

characterization of particles in liquids. In the present study, we utilized a computer-controlled laser ablation method for the fabrication of di-parabolic dielectrophoretic electrodes<sup>11</sup> and an imprinting method<sup>14</sup> for the fabrication of a micro-flow channel for sample solutions. The dielectrophoretic mobility,  $\alpha$ , for the positive dielectrophoresis of yeast cells and the negative dielectrophoresis of polystyrene particles was investigated to demonstrate the utility of the newly constructed micro-flow-dielectrophoresis device.

In this work, we fabricated the di-parabolic electrode shown in Fig. 1 to generate a non-uniform electric field. A part of this electrode looks like the “isomotive electrode” reported by Pohl and coworkers<sup>3,4</sup> and Feeley and McGovern,<sup>5</sup> but the whole shape is completely different. The gradient of the electric field intensity,  $\nabla|E|^2$ , generated along the center axis,  $x$ , of the parabolic electrode is approximated by;

$$\nabla|E|^2 = \frac{V^2}{d^4}x \quad (1)$$

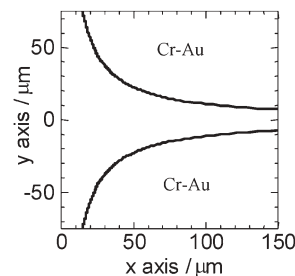
where  $d$  is the radius of an inscribed circle of the di-parabolic electrodes,  $V$  is the applied voltage, and  $x$  is a position on the center axis of the parabolic electrode. This equation indicates that the gradient of the electric field strength  $\nabla|E|^2$  linearly increases with the distance  $x$ . This relationship was utilized to analyze the dielectrophoretic behavior.

To analyze the dielectrophoretic behavior of the particles, we considered two forces acting on them; dielectrophoretic force,<sup>6</sup>  $F_{\text{DEP}}$ , and the drag force,  $F_{\text{D}}$ , which are expressed as follows, respectively;

$$\langle F_{\text{DEP}} \rangle = 2\pi r^3 \epsilon_m \text{Re}[K\epsilon] \nabla|E_{\text{rms}}|^2 \quad (2)$$

$$F_{\text{D}} = 6\pi\eta r \frac{dx}{dt} \quad (3)$$

where  $r$  is the radius of the particle,  $\epsilon_m$  is the permittivity of the medium,  $\text{Re}[K\epsilon]$  is the real part of the Clausius–Mossotti factor



**Fig. 1** The di-parabolic shaped electrode ( $y = \pm d^2/x$ ) for the flow dielectrophoresis. The Cr–Au coating metal is removed from a slide glass by irradiation using a pulsed laser. In this experiment  $d$  is about 33  $\mu\text{m}$ .

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( $Ke$ ),  $\eta$  is the viscosity of the medium. In this system, the Reynolds number was much less than 1, therefore eqn (3) is applicable.<sup>15</sup> From eqn (1), (2) and (3), we obtained a relationship between the velocity  $dx/dt$  and the distance  $x$  as follows:

$$\frac{dx}{dt} = \frac{2r^3 \epsilon_m V_{rms}^2}{3\eta d^4} Re[Ke]x \quad (4)$$

This equation shows a simple linear relationship between the velocity,  $dx/dt$ , and the distance,  $x$ . The slope of the plot of  $dx/dt$  against  $x$  is defined as the dielectrophoretic mobility,  $\alpha$ .

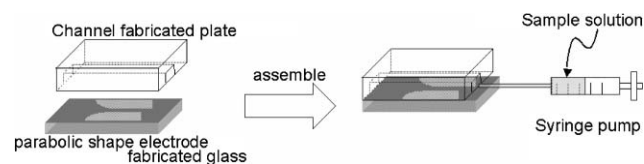
$$\alpha = \frac{2r^3 \epsilon_m V_{rms}^2}{3\eta d^4} Re[Ke] \quad (5)$$

The physical meaning of  $\alpha$  is the same as that obtained from dielectrophoresis in the planar-quadrupole electrode, although the form of equation is different from that reported previously.<sup>9</sup> We used eqn (5) to analyze the dielectrophoretic behavior of a single particle in the flow channel.

A common baker's yeast (Ogura kasei kogyo) was purchased from a local supermarket. A suspension of polystyrene particles (2.57 wt%,  $15.86 \pm 2.34 \mu\text{m}$  in diameter) was purchased from Funakoshi (Tokyo, Japan). 2-[4-(2-Hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES) known as a Good's buffer was purchased from Dojindo (Kumamoto, Japan). Potassium chloride (GR) was purchased from Nacalai Tesque (Kyoto, Japan). The water used to prepare all of the sample solutions was distilled and purified with a Milli-Q system (Milli-Q Sp, Toc., Millipore).

Powder yeast cell and a suspension of polystyrene particles were dispersed and diluted by  $1 \text{ mmol dm}^{-3}$  HEPES buffer solution containing  $1 \text{ mmol dm}^{-3}$  KCl. The conductivity and pH of the sample solutions were  $18.2\text{--}18.5 \text{ mS m}^{-1}$  and  $7.2\text{--}7.3$ , respectively.

We used an imprinting method<sup>14</sup> for the fabrication of the flow channel for sample solutions in a plastic plate. The depth and width of the flow channel was about  $375 \mu\text{m}$ . A dielectrophoretic electrode was fabricated on a Cr–Au coated glass slide (EMF corporation, New York) by the computer-controlled laser ablation method reported previously.<sup>11</sup> A pulsed laser ( $355 \text{ nm}$ ,  $14 \mu\text{J pulse}^{-1}$ ) was focused at the surface of the Cr–Au coated glass slide placed on the  $xy$ -stage, to remove the coated metals with a thickness of  $100 \text{ nm}$ . During the irradiation of the pulsed laser, the  $xy$ -stage was moved precisely, controlled by a computer program with  $1 \mu\text{m}$  step, following a parabolic shape to fabricate dielectrophoretic electrode. Fig. 2 shows a schematic drawing of the experimental setup. The parabolic electrode fabricated on the Cr–Au coated glass slide and the plastic plate with a flow channel were assembled and a sample solution containing particles was introduced to the flow channel through a glass capillary (GL science, Tokyo, Japan), which has  $75 \mu\text{m}$  inner diameter and



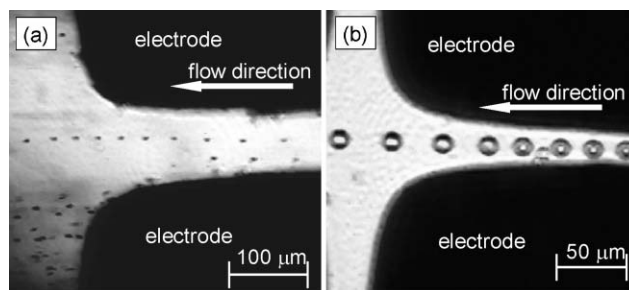
**Fig. 2** Schematic drawing of the experimental setup for the flow dielectrophoresis. The channel plate and the di-parabolic shaped electrode glass plate were assembled and the solution that contained sample particles was introduced into the channel with the syringe pump.

$150 \mu\text{m}$  outer diameter, connected to a syringe pump (Pump II, Harvard Apparatus, Holliston, MA).

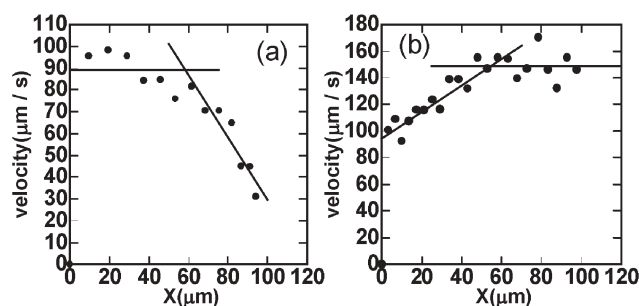
A phase-contrast microscope (Arch company, BX-3500T) equipped with an objective lens ( $10\times$ ,  $20\times$ ) and a digital camera (Nikon, COOLPIX 4500) were used to observe the track of sample particles in the flow channel. Microscope images were recorded on a video recorder and these images were transferred to AVI image file with a personal computer. These AVI images were analyzed on a personal computer with image analysis software, image J. A function generator (SG-4105, Iwatsu Co.) was utilized to apply alternating current to the dielectrophoretic electrode. The conductivity and pH of sample solutions were measured with a conductivity meter (CM-40 V, TOA ELECTRONICS CO.) and a pH meter (F-14, HORIBA Co.), respectively.

The assembly of micro channel and parabolic shaped electrode was placed on a stage of the phase-contrast microscope and the track of the sample particle in the micro channel was observed. Fig. 3 is a micrograph that shows dielectrophoresis of a yeast cell and a polystyrene particle in a flow channel. If no dielectrophoretic force influences the sample particles, the velocity of sample particles should be equal to the linear velocity of the flow. However, as shown in Fig. 3, the flow rate of sample particles increases or decreases in the “estuary” of the electrode depending on the nature of the particle. Under the condition of  $f = 1 \text{ MHz}$ , it was predicted from the quadrupole electrode experiment that yeast cells should show a positive dielectrophoresis in the di-parabolic electrode and that the direction of the dielectrophoretic force should be opposite to the flow direction. Therefore, it was reasonable that the migration velocity of a yeast cell was decreased at the “estuary” of the electrode. On the other hand, the polystyrene particle, which should show a negative dielectrophoresis at  $1 \text{ MHz}$ , was accelerated by a negative dielectrophoretic force and increased its flow velocity.

The velocity and the distance of a sample particle that flows along the center axis at  $V_{p-p} = 10 \text{ V}$  and  $f = 1 \text{ MHz}$  is plotted in Fig. 4. The plots in Fig. 4 are divided into two regions; a constant velocity region and a velocity changing region. The constant velocity was equal to the linear flow rate of the solution. From eqn (4), it was found that the slope of these plots referred to the dielectrophoretic mobility  $\alpha$ . From the slopes of Fig. 4, the values of  $\alpha$  were determined as  $1.1 \pm 0.3$  for yeast cells and  $-1.3 \pm 0.3$  for polystyrene particles. The signs of the slopes of Fig. 4 are



**Fig. 3** Superposition of consecutive images of dielectrophoresis of (a) yeast cell and (b) polystyrene particle. Alternating current ((a)  $f = 1 \text{ MHz}$ ,  $V_{p-p} = 20 \text{ V}$ , (b)  $f = 1 \text{ MHz}$ ,  $V_{p-p} = 10 \text{ V}$ ) is applied to the electrodes. Sampling rate of the images is (a)  $7.5 \text{ Hz}$  and (b)  $5 \text{ Hz}$ . Sample particles are dispersed in  $1 \text{ mM}$  HEPES buffer containing  $1 \text{ mM}$  KCl.  $\sigma_m = 18.2\text{--}18.5 \text{ mS m}^{-1}$ , pH  $7.2\text{--}7.3$ .



**Fig. 4** Decreasing or increasing of velocity for the micro-particles with position,  $x$ , in the flow channel, (a) positive dielectrophoresis of yeast cells ( $f = 1$  MHz,  $V_{p-p} = 10$  V) and (b) negative dielectrophoresis of polystyrene particles ( $f = 1$  MHz,  $V_{p-p} = 10$  V). The slope corresponds to the dielectrophoretic mobility,  $\alpha$ .

reversed to those of the values of  $\alpha$ , because the velocity is decreased or increased corresponding to the positive or negative dielectrophoresis, respectively. These  $\alpha$  values of the same particles were also obtained by using the planar-quadrupole electrode under  $V_{p-p} = 10$  V and  $f = 1$  MHz, which gave  $\alpha$  values of  $0.81 \pm 0.45$  for yeast cells and  $-2.4 \pm 0.4$  for polystyrene particles, respectively. For yeast cells, both positive  $\alpha$  values are relatively close. But, for polystyrene particles, the absolute value obtained by a planar-quadrupole electrode was about 2 times larger than that obtained by the flow dielectrophoresis. The reason for this discrepancy seems to depend on the distance between the particle and the glass wall of the electrode. It was observed that some particles levitated a little from the parabolic electrode in the negative dielectrophoretic condition. Therefore, these particles were not in the effective non-uniform electric field and were affected by a field smaller than the field exerted in a planar-quadrupole electrode. In order to evaluate the effect of the vertical distance from the planar electrode, the dependence of  $\nabla|E|^2$  on the distance from the glass wall of the electrode was estimated by the Laplace equation and calculus of finite differences method. This simulation predicted that the  $\nabla|E|^2$  value decreases to half of the original value at a distance of about  $13 \mu\text{m}$  from the glass wall of the di-parabolic electrode. This was consistent with the observation in a preliminary experiment that negative  $\alpha$  values obtained by flow dielectrophoresis were about one half of those obtained by the planar-quadrupole electrode. Therefore, the polystyrene particle should flow while floating in the range of about  $5\text{--}13 \mu\text{m}$  from the wall.

In conclusion, a new flow device to measure the dielectrophoretic mobility  $\alpha$  of micro-particles in a flow system could be fabricated by the imprinting method and computer controlled laser ablation. Under the conditions of  $V_{p-p} = 10$  V and  $f = 1$  MHz, the measurement of dielectrophoretic mobility  $\alpha$  of yeast cells and polystyrene particles was demonstrated. Analysis of the velocity changes afforded  $\alpha$  values for yeast cells and polystyrene particles rather easily. The  $\alpha$  values measured by flow dielectrophoresis and by the planar-quadrupole electrode were almost comparable. However, for negative dielectrophoresis of polystyrene particles, the  $\alpha$  value was lower than that obtained by a static quadrupole electrode method. Floating of sample particles was suggested during the flow. To improve this situation, the fabrication of a parabolic electrode of larger thickness (about  $10\text{--}50 \mu\text{m}$ ) or a flow channel sandwiched by double di-parabolic electrode plates will be recommended. Also, it will be necessary to investigate the dependence of dielectrophoretic behavior on voltage and frequency of applied ac electric field in this device. Combination of this method with any sorting technique will provide a powerful separation device for micro-particles based on the difference between their dielectric properties.

## Notes and references

- 1 J. C. Giddings, *Unified Separation Science*, John Wiley & Sons, New York, 1991.
- 2 D. W. Armstrong and H. Lingfeng, *Anal. Chem.*, 2001, **73**, 4551.
- 3 H. A. Pohl, *Dielectrophoresis*, Cambridge University Press, Cambridge, 1978.
- 4 H. A. Pohl and R. Pethig, *J. Phys. E: Sci. Instrum.*, 1977, **10**, 2, 190–3.
- 5 C. M. Feeley and F. McGovern, *J. Phys. E: Sci. Instrum.*, 1986, **19**, 11, 923.
- 6 L. Benguigui and I. J. Lin, *J. Appl. Phys.*, 1982, **53**, 1141.
- 7 Y. Huang and R. Pethig, *Meas. Sci. Technol.*, 1991, **2**, 1142.
- 8 P. Gascoyne, J. Satayavivad and M. Ruchirawat, *Acta Trop.*, 2004, **89**, 357.
- 9 H. Watarai, T. Sakamoto and S. Tsukahara, *Langmuir*, 1997, **13**, 2417.
- 10 X. B. Wang, J. Ying, J. Vykoukal, F. F. Becker and P. R. C. Gascoyne, *Anal. Chem.*, 2000, **72**, 832.
- 11 I. Ikeda, S. Tsukahara, H. Monjushiro and H. Watarai, *Bunseki Kagaku*, 2002, **51**, 767.
- 12 I. Ikeda, S. Tsukahara and H. Watarai, *Anal. Sci.*, 2003, **19**, 1, 27.
- 13 H. Watarai, H. Monjushiro, S. Tsukahara, M. Suwa and Y. Iiguni, *Anal. Sci.*, 2004, **20**, 423.
- 14 L. Martynova, L. E. Locascio, M. Gaitan, G. W. Kramer, R. G. Christensen and W. A. MacCrehan, *Anal. Chem.*, 1997, **69**, 4783.
- 15 V. L. Streeter and E. B. Wylie, *Fluid Mechanics*, McGraw-Hill Book Company, New York, 7th edn, 1979, p. 226.