Phase separation techniques in two-phase microchannel flow

To cite this article: K Hishida et al 2009 J. Phys.: Conf. Ser. 147 012056

View the article online for updates and enhancements.
Phase Separation Techniques in Two-Phase Microchannel Flow

K Hishida 1, M Ichiyanagi 1, Y Sato 1
1 Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, 223-8522 JAPAN
hishida@sd.keio.ac.jp

Abstract. Two kinds of phase separation techniques in a microchannel have been developed to advance microfluidic devices. One is the technique to combine acoustic radiation and electrostatic forces, and these forces act on particles in aqueous solution perpendicular to the flow direction simultaneously. The acoustic radiation force trapped both 9.6 \(\mu\)m and 4.0 \(\mu\)m diameter particles at nodal positions in the standing wave field, and the electrostatic force moved only 4.0 \(\mu\)m diameter particles towards the anode. The magnitudes of each force were evaluated by using particle images and particle tracking velocimetry for particle separation in the flow field. On the other hand, another technique is to utilize bilayered acoustic radiation forces. The ultrasonic transducers with 5 and 2.5 MHz were equipped parallel to the flow direction. Both 15.0 \(\mu\)m and 4.0 \(\mu\)m diameter particles in the aqueous solution were trapped at the nodes of the upstream in 5 MHz sound field, and 2.5 MHz transducer was radiated to move only 15.0 \(\mu\)m diameter particles toward a nodal position of its sound field. The exposure time and time cycle of 2.5 MHz transducer were determined to be 0.1 s and 1 s from the migration times of large and small particles transfer to the nodal positions, respectively. It is confirmed that the continuous and selective separation based on particle diameter was accomplished by the present techniques.

1. INTRODUCTION

Material separation and dispersion control are significant techniques for product quality enhancement in various fields including engineering, medical science and sitology (Petersson et al. 2004; Scampicchio et al. 2004). These techniques are used for pre-processing, sample dilution or extracting impurities. The recent development of microfabrication technology has yielded microfluidic devices, which allows to possess numerous functions on a single miniature device (Auroux et al. 2002; Reyes et al. 2002; Sinton 2004). The conventional techniques using a centrifugal separation or membrane separation have a number of difficulties in implementing within these miniaturized devices due to their larger apparatus in comparison with the devices. Furthermore, many are contact-type techniques with the subject matter, which may cause the disruption of subject and is difficult to handle samples of very small quantity. Thus a non-intrusive and continuous separation technique is required to implement in microfluidic devices.

A number of non-intrusive techniques such as the magnetic force (Relle and Grant 1998; Ueno et al. 1990) and laser radiation pressure (Ashkin et al. 1986; Ito et al. 2002) were applied to microfluidic devices, and the separation technique utilizing acoustic radiation force gives a significant attention in the recent years due to their possession of numerous unique properties (Hawkes et al. 2002; Petersson et al. 2004). The acoustic radiation force affects particles in proportional to physical parameters such as particle diameter, density and compressibility, and the force moves particles toward the nodal
positions of the standing wave field perpendicular to the flow direction. Furthermore, the electrostatic force is one of the most non-intrusive separation techniques in microfluidic devices (Harrison et al. 1993; Woolley et al. 1998), and the electrophoresis is commonly used for material transport and separation. However, since this technique depends on the electrical properties of the subjects, its applicability is limited. For further enhancement of the separation efficiency in microfluidic devices, it is expected to develop an innovative separation technique.

The objective of the present work is to develop two kinds of novel separation techniques based on particle diameter. One is the technique to combine acoustic radiation and electrostatic forces. The acoustic radiation force traps both large and small size particles at nodal positions in the standing wave field, and the electrostatic force moves only small size particles towards the anode. The magnitudes of each force were evaluated by using particle images and particle tracking velocimetry for particle separation in the flow field. On the other hand, another technique is to utilize bilayered acoustic radiation forces. This technique utilizes two ultrasonic transducers with 5 and 2.5 MHz and depends on only physical parameters such as the particle diameter, density and compressibility. Two kinds of particles with the different diameters are selected, and both the migration times of particle transfer to the nodal positions and the acoustic radiation force are estimated from the particle velocity data. 5 MHz ultrasonic sound traps both the large and small particles at the nodal positions and subsequently only large particles are moved toward the nodal position of 2.5 MHz sound field, because the 2.5 MHz transducer is radiated at the pulse time to trap only large particles which is determined by the migration times of particles transfer to the nodal positions.

2. PRINCIPLES OF PHASE SEPARATION TECHNIQUE

2.1 Effect of Acoustic Radiation Force on Particles

When an acoustic standing wave is set up in fluid, particles within the standing wave are affected by acoustic radiation force, $F_{ac}$ (Laurell et al. 2007). The particles are translated to either nodes or anti-nodes of the standing wave depending on their physical properties relative to the ones of the suspended fluid. Acoustic radiation force is given by:

$$F_{ac} = -\frac{4}{3}\pi r^3 kE_{ac} A \sin(2kl) ,$$  \hspace{1cm} (1)

where $r$ [m] is the particle radius, $k$ [-] is the wave number, $E_{ac}$ [J/m$^3$] is the time-averaged acoustic energy density, $l$ [m] is the distance from the nearest node, and $A$ [-] is the acoustic constant factor which is derived from both the particle density and its compressibility. Figure 1 illustrates the schematic of the particle motion affected by the acoustic radiation force within the standing wave. Particles with different diameter are suspended in a channel as shown in Figure 1 (a). Within the standing wave field, nodes with small acoustic pressure amplitude and anti-nodes with large amplitudes exist one after another as represented in Figures 1 (b) and (c). The direction of the force is determined by the sign of $A$ factor of equation 1; a positive $A$ factor results in movement toward a pressure node and a negative in movement toward a pressure anti-node. Generally, solid particles in aqueous solutions are transported to nodes. Furthermore, the acoustic radiation force is proportional to third power of particle radius as described in equation 1. When the acoustic radiation force is constant, firstly large size particles are moved toward the nodes of the sound in shorter time than small size particles as exhibited in Figure 1 (b), and subsequently small size particles are collected at nodes as shown in Figure 1 (c).

2.2 Phase Separation Technique Utilizing Acoustic and Electrostatic Force

The acoustic standing wave field was yielded by equipping the ultrasonic transducer and reflector plate perpendicular to the flow direction. Electrodes were located at four corners of the microchannel.
When applying the acoustic radiation force, particles are collected at the nodes as shown in Figure 2 (a). When applying the electrical field, particles with a negative charge are moved towards the positive electrode by electrostatic force, \( F_e \). Thus, both the acoustic radiation and electrostatic forces act on the particles simultaneously. When the acoustic radiation force is larger than the electrostatic force as shown in Figure 2 (b), large and small size particles are confined at nodal positions in the standing wave field. When the electrostatic force is dominant as shown in Figure 2 (c), large and small size particles are transferred towards the positive electrode. Finally, it is observed in Figure 2 (d) that large size particles were collected at the node of the sound field and small size particle were transferred towards the positive electrode. This is caused by controlling the balance of the acoustic radiation and electrostatic force, that is, \( F_{ac} > F_e \) for large size particles and \( F_{ac} < F_e \) for small size particles, which yields to move only small size particles towards the positive electrode, because the acoustic radiation force is proportional to third power of particle radius. Thus it is possible to separate the large size particles from small size particles by setting appropriate acoustic radiation and electrostatic forces which are important parameters of the present separation technique.

Figure 1. Schematic of particle motion affected by acoustic radiation force in standing wave field. (a) Particles are suspended in a channel prior to an application of acoustic radiation force \( t = 0 \). (b) Only large size particles are collected at nodes of a sound field at \( t = t_0 \) after an application of acoustic radiation force. (c) Both large and small size particles are collected at \( t = t_0 + \Delta t \).

Figure 2. Concept of separation technique utilizing acoustic radiation and electrostatic forces. (a) Particles are trapped in the standing wave field. (b) Particle motion when \( F_{ac} > F_e \) for both large and small size particles. (c) Particle motion when \( F_{ac} < F_e \) for both large and small size particles. (d) Particle motion when \( F_{ac} > F_e \) for large size particles and \( F_{ac} < F_e \) for small size particles.
2.3 Phase Separation Technique Utilizing Bilayered Acoustic Force

Figure 3 represents the concept of the present separation technique. Acoustic standing wave fields were generated by using two ultrasonic transducers and a reflector plate, which were placed parallel to each other in the span-wise direction of the channel as illustrated in Figure 3 (a). Both large and small size particles were randomly suspended within in a flow field. The oscillating frequency of ultrasonic transducer at upstream was two times as large as that at downstream, because two and one nodes were generated at the upstream and downstream, respectively. Figure 3 (b) indicated that when entering the upstream sound field, both particles were collected at the two nodes of standing wave because the upstream transducer was constantly radiated. On the other hand, it is observed at the downstream of Figure 3 (c) that large size particles were collected at the node of the downstream sound field and small size particle were kept at the nodal positions of upstream sound field. This is caused by controlling the exposure time of downstream ultrasonic transducer which yields to move only large size particles towards the node, because the acoustic radiation force is proportional to third power of particle radius and the migration time to move large size particles is shorter than that to move small size particles as shown in Figure 1. Thus it is possible to separate the particles into three threads by setting an appropriate exposure time of downstream transducer. Since the exposure time is determined from the migration time to move particles towards the node, the migration time, $t_{\text{steady}}$, is an important parameter of the present separation technique. The present work defined the following equation as the separable condition of particles:

$$t_{\text{trap}} \geq t_{\text{steady}} \quad \text{for large size particles,}$$
$$t_{\text{trap}} \leq t_{\text{steady}} \quad \text{for small size particles,}$$
$$t_{\text{trap}} = \frac{Y_l}{v} \times \frac{t_{\text{ex}}}{t_{\text{per}}},$$

where $Y_l$ [m] is the length of downstream transducer, $v$ [m/s] is the bulk velocity and $t_{\text{ex}}$ [s] and $t_{\text{per}}$ [s] are the exposure time and time cycle of exposure of the downstream transducer, respectively. $Y_l / v$ is

![Figure 3. Concept of separation technique utilizing bilayered acoustic radiation forces.](Image)
the time for particles to pass through the downstream transducer, and \( t_{\text{exposure}}/t_{\text{per}} \) is the duty ratio for the exposure of the downstream transducer.

### 2.4 Evaluation Technique of Acoustic Radiation Force and Migration Time

The conventional measurement techniques for the acoustic radiation force such as the hydrophone method are not suitable for measuring within micro-scale field, because they require the insertion of probes and involve sensitive axis alignments for accurate results. In the present work, since it is difficult to directly measure the acoustic radiation force, they were obtained by measuring the particle velocities affected by the acoustic force. Within the Stokes region, the relaxation time of particles, \( \tau_p \), is given by Clift et al. (1978):

\[
\tau_p = \frac{\rho (2 \rho_p + \rho_m)}{9 \mu},
\]

where \( \rho_m \) and \( \rho_p \) [kg/m\(^3\)] are the density of fluid and particles, respectively, and \( \mu \) [Pa·s] is the fluid viscosity. The relaxation time of the largest particle used in this work was \( O \left(10^{-6}\right) \) s, while the relaxation time of fluid flow, \( \tau_f \), was \( O \left(10^{-1}\right) \) s which was derived from the channel width divided by the bulk velocity. Since \( \tau_p \ll \tau_f \), the particle motion is assumed to follow the flow field. In addition, when the volume concentration of particles is less than 2 %, the fluid motion generated by the particle motion is negligible (Probstein 1994). Thus, the acoustic radiation force described in equation 1 can be equal to the drag force, \( F_d \), that particles receive from the fluid. The drag force is provided by:

\[
F_d = 6\pi \mu u,
\]

where \( u \) is the particle velocity, as the experiments were performed in a stationary fluid. The effects of gravity and buoyancy can be ignored because the direction of sound is perpendicular to the direction of gravity. The particle velocity was obtained by particle tracking velocimetry (PTV), and the acoustic radiation force was evaluated by substituting the particle velocity to equation 4.

Furthermore, particles are separated by trapping at the nodal positions within the acoustic standing wave fields, so that the migration time to move particles towards the nodes, \( t_{\text{steady}} \), is an important parameter. The present work evaluated the migration time by using PTV, and defined as the steady-state condition when the averaged span-wise velocity is less than 0.6 \( \mu m/s \), which is the measurement uncertainty in 95 % confidence level of PTV.

### 3. EXPERIMENT APPARATUS

#### 3.1 Microchannel

Figure 4 shows the schematic of the I-shaped flow channel for the particle separation with combining the acoustic radiation and electrostatic forces. The channel is composed of acrylic and glass, and is 2 mm width and 25 mm length with a depth of 1 mm. The ultrasonic transducer with oscillating frequency of 8.125 MHz was equipped at the center of the channel perpendicular to the flow direction. The oscillating frequency was set by considering transmission rate of sound, \( D \), through surface boundaries (Takeda and Kikura 2002), which is given as:

\[
D = \sqrt{1 + \frac{1}{4} \left( m - \frac{1}{m} \right)^2 \sin^2 \frac{2\pi d}{c} f}, \quad \text{when } m = Z_1/Z_2
\]

where \( d (= 2 \text{ mm}) \) is the thickness of acrylic wall, \( c \) is the sound velocity within acrylic, \( f \) is the sound
frequency, and $Z_1$ and $Z_2$ are the acoustic impedance of the materials at the interface. Table 1 compiles the acoustic impedance of each material. The transmission rate between water and glass is 0.23, and the transmission rate between water and acrylic is 0.76, so that the ultrasonic wave oscillated by the transducer penetrates the acrylic wall and fluid in the channel, and is reflected by the glass wall across the channel. In addition, four platinum electrodes with 100 μm diameter were placed along the microchannel wall, and those at the glass wall were set to be positive, and those at acrylic were set to be negative.

Figure 5 shows the schematic diagram of the I-shaped microchannel for the particle separation with bilayered acoustic radiation forces. The channel is composed of acrylic and glass, and is 300 μm width and 55 mm length with a depth of 1.5 mm. Ultrasonic transducers with oscillating frequency of 5 and 2.5 MHz were equipped perpendicular to the flow direction. The oscillating frequency was derived from equation 5. The point of origin was set at the center of transducer, and $X$, $Y$, and $Z$-axis were positioned at span-wise, stream-wise and depth-wise direction, respectively. 5 MHz sound field of upstream transducer provides two nodes at $x = 75$ and $x = 225$ μm, and the oscillating frequency of 2.5 MHz yields one node at the center of the channel ($x = 150$ μm).

Table 1. Acoustic impedance of various materials

| Material               | Acoustic impedance [$\times 10^6$ kg/m²s] |
|------------------------|------------------------------------------|
| Acrylic                | 3.26                                     |
| Glass                  | 12.8                                     |
| Water at 20 degrees    | 1.48                                     |

Figure 4. (a) Top view and (b) cross-sectional view of a microchannel for the particle separation with acoustic radiation and electrostatic forces.

Figure 5. (a) Top view, (b) cross-sectional view and (c) side view of a microchannel for the particle separation with bilayered acoustic radiation forces.
3.2 Flow Visualization and Control System

Figure 6 (a) illustrates a schematic of the optical measurement system. A transmitted light by LED lamp (Ohm Electric Inc, LL-10W) was set below the stage and emitted towards the channel to project the shadow of particles. The particle images were captured by the CCD camera (SONY Co., Ltd, XCDL-X710CR, 1024 × 768 pixels, 24 bits, 30 fps) through the 10× magnification objective lens. This set-up corresponds to a measurement area of 433 × 325 μm². Figure 6 (b) shows the control system for the particle separation with the acoustic radiation and electrostatic forces. The transducer was oscillated at a sinusoidal wave by a function generator (Micronix Co., Ltd, MFG206). Platinum electrodes were connected to a DC voltage which was generated by high-voltage power supply (KEPCO Inc. BOP1000M). Figure 6 (c) exhibits the control system for the particle separation with the bilayered acoustic radiation forces. Each of the transducers was oscillated at a sinusoidal wave by a function generator. Relay switch driven by pulse generator (Quantum Composers Inc, 9300 series) was equipped to the 2.5MHz transducer because of cutting the signal from the function generator at intervals set on the pulse generator.

3.3 Particle and Fluid Properties

Table 2 lists the properties of the polystyrene particles. Three kinds of particles with 2.0, 4.0 and 15 μm diameters (Invitrogen Corp., FluoSpheres Sulfate Microspheres) were fluorescent, and the particle with 9.6 μm diameter (Interfacial Dynamics Co., White Sulfate Latex) was non-fluorescent. The particles were suspended in ion-exchanged water and were negatively charged because the particle surface was modified by sulfo groups (−SO₃H).

![Diagram](image)

Figure 6. (a) Schematic of optical measurement system. (b) Control system to generate acoustic radiation and electrostatic forces. (c) Control system to generate bilayered acoustic radiation forces.

| Diameter [μm] | 2.0  | 4.0  | 9.6  | 15   |
|---------------|------|------|------|------|
| Density [g/cm³] | 1.055| 1.055| 1.055| 1.055|
| Volume concentration [%] | 0.020| 0.020| 0.168| 0.336|
| Absorption wavelength [nm] | 505  | 505  | –    | 540  |
| Emission wavelength [nm] | 515  | 515  | –    | 560  |
4. RESULTS AND DISCUSSION

4.1 Particle Separation Utilizing Acoustic Radiation and Electrostatic Forces

4.1.1 Evaluation of Acoustic Radiation and Electrostatic Forces

The aqueous solutions with 2.0 \( \mu \text{m} \), 4.0 \( \mu \text{m} \) and 9.6 \( \mu \text{m} \) diameter particles were injected through the channel inlet, and the acoustic radiation force acting on particles was evaluated by measuring particle velocities in a liquid phase flow and substituting particle velocities to equation 4. The particle velocities were averaged by 5 pixel intervals along \( X \)-axis. Figure 7 shows the profiles of drag force acting on the particles. It is found that the sinusoidal pattern with twice the oscillating frequency was observed and the acoustic radiation force was increasing with an increase in the particle diameter.

The electrostatic force acting on particles was evaluated by measuring particle velocities in a liquid phase flow and substituting particle velocities to equation 4. It is observed that the velocity distribution in the measurement area is uniform and the standard deviation of the velocity measurement was approximately 2.5 \%. The relationship between the applied voltage and drag force is plotted in Figure 8. The particle velocities are summation of electrophoretic and electroosmotic flow velocities. The electroosmotic flow velocity was estimated to be approximately 10 \( \mu \text{m/s} \) at 15 V/cm by using the numerical simulation. Thus, it was evaluated that around 20 to 30\% of the force shown in Figure 8 was due to electroosmotic flow.

The applied voltage has been determined from the relationship between the acoustic radiation and electrostatic forces. For 2.0 \( \mu \text{m} \) diameter particles, the amplitude of drag force and applied electric field, which the electrostatic force is equal to the acoustic radiation force, are 0.5 pN and 12 V/cm, respectively. This is the point where the dominant force of the particle motion changes from acoustic radiation force to electrostatic force. For 4.0 \( \mu \text{m} \) diameter particles, 15 V/cm is the point of equilibrium. For 9.6 \( \mu \text{m} \) diameter particles, the acoustic radiation force is dominant factor, because the acoustic radiation force is five times larger than the electrostatic force. Thus, their balances of the acoustic radiation and electrostatic forces enable to separate large size particles of 9.6 \( \mu \text{m} \) from small size particles of 2.0 and 4.0 \( \mu \text{m} \).

![Figure 7. Profile of force acting on particles induced by acoustic radiation force.](image1.png)

![Figure 8. Relationship between electric field and force acting on particles induced by electrostatic force.](image2.png)
4.1.2 Demonstration of Particle Separation Utilizing Acoustic Radiation and Electrostatic Forces

For the demonstration of the particle separation, the flow field was formed by injecting the aqueous solutions with 2.0 μm and 9.6 μm diameter particles in the I-shaped channel as shown in Figure 4. The acoustic radiation force with the frequency of 8.125 MHz and the electric field of 14 V/cm were simultaneously applied. Bulk velocity was set at 45 μm/s by a pressure-driven flow which was generated by the syringe pump. Figure 9 shows the instantaneous particle images with velocity-vectors which were obtained by the PTV measurement. It is observed that 2.0 μm diameter particles were moved towards the span-wise direction, while 9.6 μm diameter particles were trapped at the nodal position of the standing wave field. The averaged span-wise velocity of 2.0 μm diameter particles was 23.1 μm/s, so that these particles were transferred to the location of half channel width in approximately 45 s. Thus, it is found that the particle separation utilizing the acoustic radiation and electrostatic forces is realized by evaluating the acoustic radiation and electrostatic forces. The separation efficiency of this technique will be improved by optimizing several factors, that is, the channel design, the transducer positioning, the flow velocity of continuous phase and the amplitude of applied voltage.

4.2 Particle Separation Utilizing Bilayered Acoustic Radiation Forces

4.2.1 Evaluation of Migration Time of Particles

In the separation technique utilizing the bilayered acoustic radiation forces, particles are selectively separated by using the difference of migration time to move towards the node of 2.5 MHz standing wave. Migration time of particles from nodal positions of 5 MHz standing wave to nodal position of 2.5 MHz was evaluated by the PTV measurement. Instantaneous images of 15 μm diameter particles in 2.5 MHz standing wave were presented in Figure 10, and black circles indicate particles. The present work defined as \( t = 0 \) s, when the acoustic radiation force of 2.5 MHz standing wave was applied to the channel as shown in Figure 10 (a). When the span-wise velocity becomes less than 0.6 μm/s as shown in Figure 10 (b), the particles were assumed to be steady-state. Figures 11 (a), (b) and (c) give profiles of brightness intensity of 4.0 μm, 9.6 μm and 15 μm diameter particles, respectively, which were obtained after each 10 iterative measurements. Black and white circles indicate brightness intensity when applying the acoustic radiation force by the sound field of 2.5 MHz and when reaching

![Image](image-url)

Figure 9. Instantaneous images of (a) 2.0 and (b) 9.6 μm diameter particles with velocity-vectors which were obtained by the PTV measurement.
the migration time to move particles towards the nodes, respectively. Figure 12 summarizes the migration time in terms of particle diameter, and exhibits that the migration time of 15 μm and 9.6 μm is approximately fifth part of that of 4.0 μm. Their difference of migration time enables to separate large size particles of 15 μm and 9.6 μm from small size particles of 4.0 μm.

Figure 10. Instantaneous images of 15 μm diameter particles in sound field of 2.5 MHz.

Figure 11. Profiles of brightness intensity of (a) 4.0 μm, (b) 9.6 μm and (c) 15 μm diameter particles. Black and white circles are brightness intensity when applying the acoustic radiation force by the sound field of 2.5 MHz and when reaching the migration time to move particles at the nodes, respectively.
4.2.2 Demonstration of Particle Separation Utilizing Bilayered Acoustic Radiation Forces

For the application of the separation technique, the flow field was formed by injecting the aqueous solutions in the I-shaped channel as shown in Figure 5. The experiments were performed under two conditions, that is, a flow with 15 μm and 4.0 μm diameter particles and a flow with 9.6 μm and 4.0 μm diameter particles. Bulk velocities, \( v \), were set at 750 μm/s, 450 μm/s, 250 μm/s and 100 μm/s by a pressure-driven flow which was generated by the difference of the water surface in both reservoirs, and the Reynolds number, \( Re \), were 0.37, 0.22, 0.12 and 0.05, based on the hydraulic diameter of 0.5 mm, respectively. The parameters in equation 2 were set below. The length of downstream transducer, \( Y_L \), is 11 mm, and the exposure time, \( t_{ex} \), and time cycle of the exposure of the downstream transducer, \( t_{per} \), are 0.1 s and 1 s, respectively. The bulk velocity of 750 μm/s, 450 μm/s and 250 μm/s qualifies as the separable condition as described in equation 2, and that of 100 μm/s does not qualify.

Figure 13 shows the spatial distributions of particles, which were obtained after each 10 iterative measurements and located at the center of 2.5 MHz transducer (\( x = 0 \) mm). The black and white circles indicate large and small size particles, respectively. As typical results, Figures 13 (a) exhibit the separation of 15 μm and 4.0 μm diameter particles with bulk velocities of 750 μm/s, and Figures 13 (b) plot the separation of 9.6 μm and 4.0 μm diameter particles with bulk velocities of 100 μm/s. From Figures 13 (a) which qualifies as the separable condition, large particles were collected at the nodal...
position of 2.5 MHz sound field and small particles were existed at the nodes of 5 MHz, and the particle separation by size was successful. On the other hand, the experiments with 100 μm/s show that both large and small particles were accumulated at the span-wise position from 100 μm to 200 μm. Thus, it is obvious that the particle separation utilizing the acoustic radiation force with two ultrasonic transducers is advanced by evaluating the migration time of particles as shown in Figure 12 and qualifying the separable condition of equation 2.

5. CONCLUSIONS
Two kinds of novel non-intrusive and continuous separation techniques for suspended particles in a microchannel were proposed for the application to the microfluidic device. One is the technique to combine acoustic radiation and electrostatic forces, and another technique is to utilize bilayered acoustic radiation forces. The important conclusions obtained from this work are summarized below.

1. The separation technique utilizing the acoustic radiation and electrostatic forces was applied to a flow field with 9.6 μm and 2.0 μm diameter particles. The transducer and electrodes were equipped at the location perpendicular to the flow direction. 2.0 μm diameter particles were transferred towards the side wall of the microchannel by the electrostatic force, and 9.6 μm diameter particles were trapped at the nodal position of the acoustic field. This is caused by evaluating the acoustic radiation and electrostatic forces acting on particles and investigating the relationship where the dominant force of each particle changes from the acoustic radiation force to the electrostatic force.

2. The separation technique utilizing the bilayered acoustic radiation forces was applied to a flow field with 15 μm and 4.0 μm diameter particles. 5 MHz and 2.5 MHz ultrasonic transducers were equipped at upstream and downstream, respectively. The acoustic radiation force acts on particles in proportion to third power of particle radius and moves particles towards the node of standing wave field. So that the large size particles have short migration time of transfer to the nodal positions in comparison with the small size particles. By controlling the exposure time of downstream ultrasonic transducer, large size particles were collected at the node of the downstream sound field and small size particles were kept at the nodal positions of upstream sound field. The migration times of each particle were evaluated by using the PTV measurement, and the exposure time and time cycle of 2.5 MHz transducer was determined to be 0.1 and 1 s, respectively, which were derived from the relationship between their difference of migration time and the bulk flow velocity. Finally, it is observed that the small size particles were trapped at the nodal position of upstream transducer, and large size particles were moved at the nodal position of downstream transducer.

ACKNOWLEDGEMENTS
The authors would like to thank Messrs. H. Ishida and H. Nakanosono at Keio University for performing their experiments. This work was subsidized by Grant-in-Aid for Scientific Research (No. 18206024) from Ministry of Education, Culture, Sports, Science and Technology in Japan. The second author is supported by Research Fellowships for Young Scientists (No. 19-8799) from Japan Society for the Promotion of Science.

NOMENCLATURE
\[ c \] sound velocity
\[ d \] wall thickness
\[ D \] transmission rate of sound
REFERENCES

Ashkin A; Dziedzic JM; Bjorkholm JE; Chu S (1986) Observation of a single-beam gradient force optical trap for dielectric particles. Opt Lett 11: 288–290

Auroux PA; Iossifidis D; Reyes DR; Manz A (2002) Micro total analysis systems. 2. Analytical standard operations and applications. Anal Chem 74: 2637–2652

Clift T; Grace JR; Weber ME (1978) Bubbles, Drops, and Particles., New York: Academic Press

Harrison DJ; Fluri K; Seiler K; Fan Z; Effenhauser CS; Manz A (1993) Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip. Science 261: 895–897

Hawkes JJ; Coakley WT; Gröschl M; Benes E; Armstrong S; Tasker PJ; Nowotny H (2002) Single half-wavelength ultrasonic particle filter: Predictions of the transfer matrix multilayer resonator model and experimental filtration results. J Acoust Soc Am 111: 1259–1266

Ishida H; Sato Y; Hishida K (2006) Continuous separation technique of suspended particles by utilizing acoustic radiation and electrostatic force. Trans JSME Ser B 72(722): 2473–2480

Ito S; Yoshikawa H; Masuhara H (2002) Laser manipulation and fixation of single gold nanoparticles in solution at room temperature. Appl Phys Lett 80: 482–484

Laurell T; Petersson F; Nilsson A (2007) Chip integrated strategies for acoustic separation and manipulation of cells and particles. Chem Soc Rev 36: 492–506
Petersson F; Nilsson A; Holm C; Jonsson H; Laurell T (2004) Separation of lipids from blood utilizing ultrasonic standing waves in microfluidic channels. Analyst 129: 938–943

Probstein RF (1994) *Physicochemical Hydrodynamics, 2nd edn.*, John Wiley & Sons

Reyes DR; Iossifidis D; Auroux PA; Manz A (2002) Micro total analysis systems. 1. Introduction, theory, and technology. Anal Chem 74: 2623–2636

Relle S; Grant SB (1998) One-step process for particle separation by magnetic seeding. Langmuir 14: 2316–2328

Scampicchio M; Wang J; Mannino S; Chatrathi MP (2004) Microchip capillary electrophoresis with amperometric detection for rapid separation and detection of phenolic acids. J Chromatography A 1049: 189–194

Sinton D (2004) Microscale flow visualization. Microfluid Nanofluid 1: 2–21

Takeda Y; Kikura H (2002) Flow mapping of the mercury flow. Exp Fluids 32: 161–169

Ueno S; Shimohata K; Yamada T; Iwamoto M (1990) Magnetic separation of fine particle dusts using a superconducting magnet and wire meshes. J Appl Phys 67: 5904–5906

Woolley AT; Lao K; Glazer AN; Mathies RA (1998) Capillary electrophoresis chips with integrated electrochemical detection. Anal Chem 70: 684–688.