Proceedings of
The 5th National Congress on Fluids Engineering
March 26-28. 2008, Jeju, Korea

Generation of sheath-free particle beam: application to micro-flow cytometry

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외피유체 없이 입자 빔의 발생: 유세포 분류기 응용

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Key Words: *Particle beam(입자빔), 3-D particle focusing(3 차원 입자집속), Microchannel(마이크로채널) Flow cytometry (유세포 분류기)*

Abstract

A generation of a particle beam is the key technique in a flow cytometry that measures the fluorescence and light scattering of individual cell and other particulate or molecular analytes in biomedical research. Recent methods performing this function require a laborious and time-consuming assembly. In the present work, we propose a novel device for the generation of an axisymmetrical focusing beam of microparticles (3-D focusing) in a single capillary without sheath flows. This work uses the concept that the particles migrate toward the centerline of the channel when they lag behind the parabolic velocity profile. Particle focusing of spherical particles was successfully made with a beam diameter of about 10 μm. Proposed device provides crucial solutions for simple and innovative 3-D particle focusing method for the applications to the MEMS-based micro-flow cytometry. We believe that this device can be utilized in a wide variety of applications, such as biomedical/ biochemical engineering.

1. Introduction

For the past four decades, the flow cytometry has been a versatile instrument that measures the fluorescent signal of individual cell and other particulate or molecular analytes in biomedical research and clinical diagnostic applications [1] such as clinical hematology diagnosis, bacteria analysis, gene diagnosis and so forth. One of the key components for these functions is to hydrodynamically focus samples within the sensing area of the optical systems in order to optimize detection signals. In general, a flow cytometry utilizes two concentric cylinders to tightly focus samples [2]. In practice, a conventional flow cytometry contains delicate optical components and complicated control circuits, making the system bulky, expensive and mechanically complex [2]. Furthermore, the operation of conventional flow cytometry often suffers from the need for skilled hand, delicate pre-treatment procedures and high volume requirement of samples.

Accordingly, there has been much effort to miniaturize the conventional flow cytometry on lab-on-a-chip devices [3, 4] to overcome these drawbacks, since the miniaturization of diagnostic components in these devices offers many potential benefits including reduction in sample volume and small system size, disposability, development of low cost, improved portability and many others [5]. In an early work, two-dimensional hydrodynamic focusing using pressure-driven flows [3, 6] or electrokinetic flow [7] has been achieved for lateral focusing by two neighboring sheath flows in planar microchannels. In this case, the particles are not focused vertically, and spread in the out-of-plane direction. This effect is the major complication for the better performance of micro-flow cytometry [8].

In an ideal approach, 3-D hydrodynamic focusing can be achieved by two coaxial-jet devices with two concentric capillary tubes, where the samples introduced from the inner cylinder is axisymmetrically focused by the completely surrounding sheath flows from the outer cylinder. In practice, a fabrication of such a cylindrical cross-section of micro-devices is difficult using conventional fabrication processes. However, in planar micro-devices, Yu et al (2005) demonstrated a 3-D particle/cell focusing using 3-D dielectrophoretic (DEP) force with the array of elliptic-like electrodes fabricated on the circumference of elliptic-like microchannels [9]. Other than this approach involving complicated 3-D geometries, piecewise approximation has been more frequently employed for 3-D particle focusing. For example, once particles are horizontally focused by sheath flows, they are vertically focused by the additional external force, such as negative DEP [10] or other sheath flows [11]. These methods require either laborious assembly [9, 10], individual fabrication [9~11], or additional patterning of planar electrode [10] or electrode with complex O-type geometry [9]. Further, these methods involve multi-layer channel devices, which make their integration into lab-on-a chip systems problematic [8].

More recently, Mao et al (2007) have demonstrated 3-D particle focusing using a single layer planar microchannel [12]. However, many sheath flows are still inevitable. In addition, acoustic wave is used to focus particles in a microchannel, where a pair of the interdigital transducers is deposited on piezoelectric substrate [13]. In summary of recent state-of-the-art in 3-D particle focusing methods in planar microchannels, previous methods inevitably involve many sheath flows at the channel inlet and/or additional fabrication process to generate external force, where the massive production of lab-on-a-chip devices would be difficult.

In the present work, we develop a novel particle focusing device that uses a single circular capillary. Our device neither uses any sheath

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flows, such as coaxial-jet flows nor involves additional fabrication processes to generate force components.

2. Methods

The concept utilized for generating a 3-D particle focusing in the present study has been traced back to the observation of radial migration of non-neutrally buoyant particles ($\rho_p > \rho_w$) subject to a vertical ascending Poiseuille flow, where ρ_p is the particle density and ρ_f is the fluid density as shown in Figure 1. In this fluidic situation, a particle migrates toward the center of the channel axis, since the particle lags behind the Poiseuille velocity profile as shown in Figure 1. This phenomenon has been demonstrated in a tube [14] and in a rectangular channel [15] experimentally [14] and numerically [16]. As shown in Figure 1(b), we constructed the same fluidic situation as that of Figure 1(a) by replacing the gravitational force F_g due to the non-neutrality of the particle in a vertical ascending flow, with the electrophoretic force F_{ep} of a neutrally-buoyant and negatively charged particle in electrolyte solution, by applying an electric field between two reservoirs at both ends of the test channel. This fluidic circumstance represents that the particle lags behind the Poiseuille flow due to the electrophoretic mobility, which indicates that the particle is radially focused.

Experimental apparatus is illustrated in Figure 2. Fluorescence-labeled microspheres were imaged using an epi-fluorescent microscope (IX50, Olympus, Japan) and 10x air immersion objective lens with a numerical aperture of 0.35. Illumination was provided by 100-W mercury lamp, which is specifically filtered by a filter cube of the microscope. The test particle with a green-fluorescent microsphere in the flow field absorbs the blue light of wavelength 488 nm and emits the green light of wavelength 530 nm, recorded on the CCD sensor. A cooled-CCD camera (SensiCam, PCO, Germany) with a 1300 × 1024 array of square pixels, 12-bit resolution with a pixel dimension of 6.4 × 6.4 μm, connected to the image-grabbing board (PCO PCI interface board 520/525) on a personal computer was used to capture particle image frames. On the other hand, in order to produce particle electrophoresis in the capillary, DC voltage generated from a function generator (Tektronix, Japan) was amplified by high-voltage power supply (Matsusda Precision, Japan) and was applied to each platinum electrode.

3. Results and discussion

Once particle image frames are obtained, identification of the particles in the flow field is performed using the method described earlier, and the locations of the particle centers are determined by using the 2-D Gaussian curve fitting of the particle image intensity with sub-pixel resolution using. In order to evaluate a focusing of the test particles, probability density function (PDF) has been adopted to present their spatial distribution along the lateral direction, which can be defined as follows:

$$f(r) = \frac{\sum_{k=1}^{k=700} N_k(r, r+dr)}{\sum_{r=-R}^{r=R} \sum_{k=1}^{k=700} N_k(r, r+dr)}, \qquad (2)$$

where $N_k(r,r+dr)$ is the number of particles between the radial positions r and $r+dr$ in the k-th image frame at a given experimental condition. Further, the denominator of the above equation represents the total number of particles, summed over the radial positions and the number of particles between r and $r+dr$. Meanwhile, there are possible experimental errors in determining spatial distribution of the test particles: pixel reading error ε_r, and error ε_p due to the particle mislocation in the 2-D Gaussian fitting. It is proved that ε_p is less than

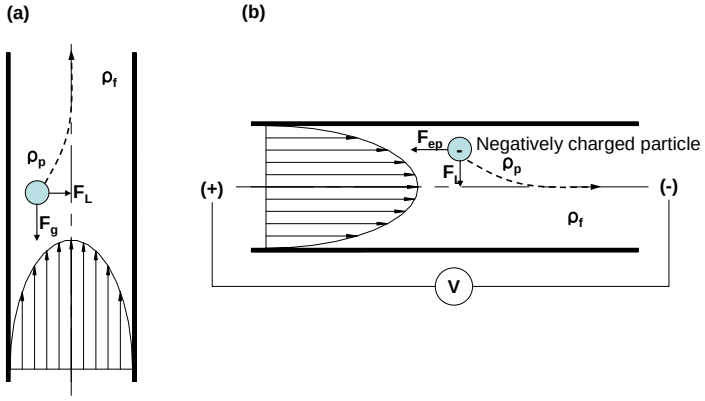

Figure 1 A concept of the particle focusing method utilized in the present study. If a particle moves against the Poiseuille flow direction due to (a) gravity or (b) electrophoresis, the particle travels along the center of the channel (dotted line is the particle trajectory).

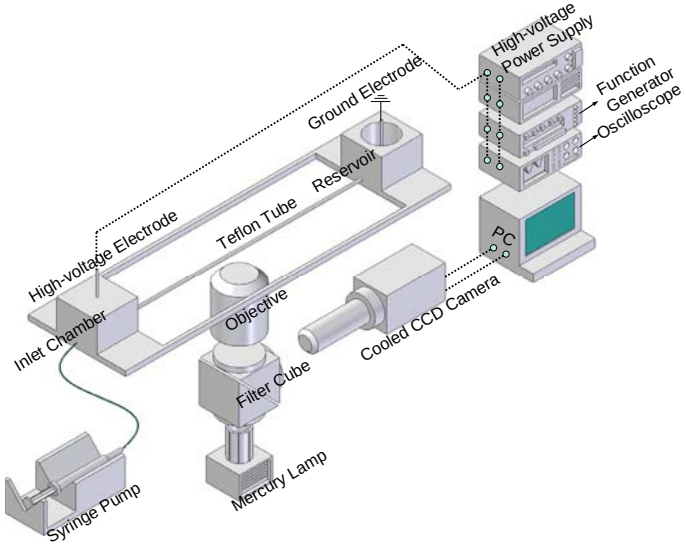

Figure 2 Experimental setup consists of an epi-fluorescent microscope, a cooled-CCD camera, and high-voltage power supply assembly.

5% of the particle diameter.

Accordingly, overall error $\varepsilon_t = \varepsilon_r + \varepsilon_p$ and percent errors according to the channel size ($\varepsilon_t/D_t \times 100\%$) is less than 2% of the capillary diameter D_t. In other words, a spatial resolution of each bar in the particle distribution, i.e., Figure 2, is less than ± 1.96 μm.

3.1 Generation of a particle beam

Figure 3 shows spatial distributions of the test particles at various field strengths. When the pressure-driven flow is only introduced as shown in Figure 3(a), particles are uniformly distributed across the cross section. However, when the voltage of 42.8 V/cm was applied between the electrodes, particles drifted away from the center of the test channel, and as a result they are clustered near the walls. There was a time gap of 30 sec between the start of the power supply and the instant when the recording of the images started. Images were recorded for 60 sec. As the voltage slightly increases up to E = 64.3 V/cm (Figure 3(b)), the peak values near the walls slightly decayed and a number of particles are seen near the center of the test channel. Similarly, in the flow of a non-neutrally buoyant particle (the density of a particle is heavier than that of the fluid) subject to a vertical ascending Poiseuille

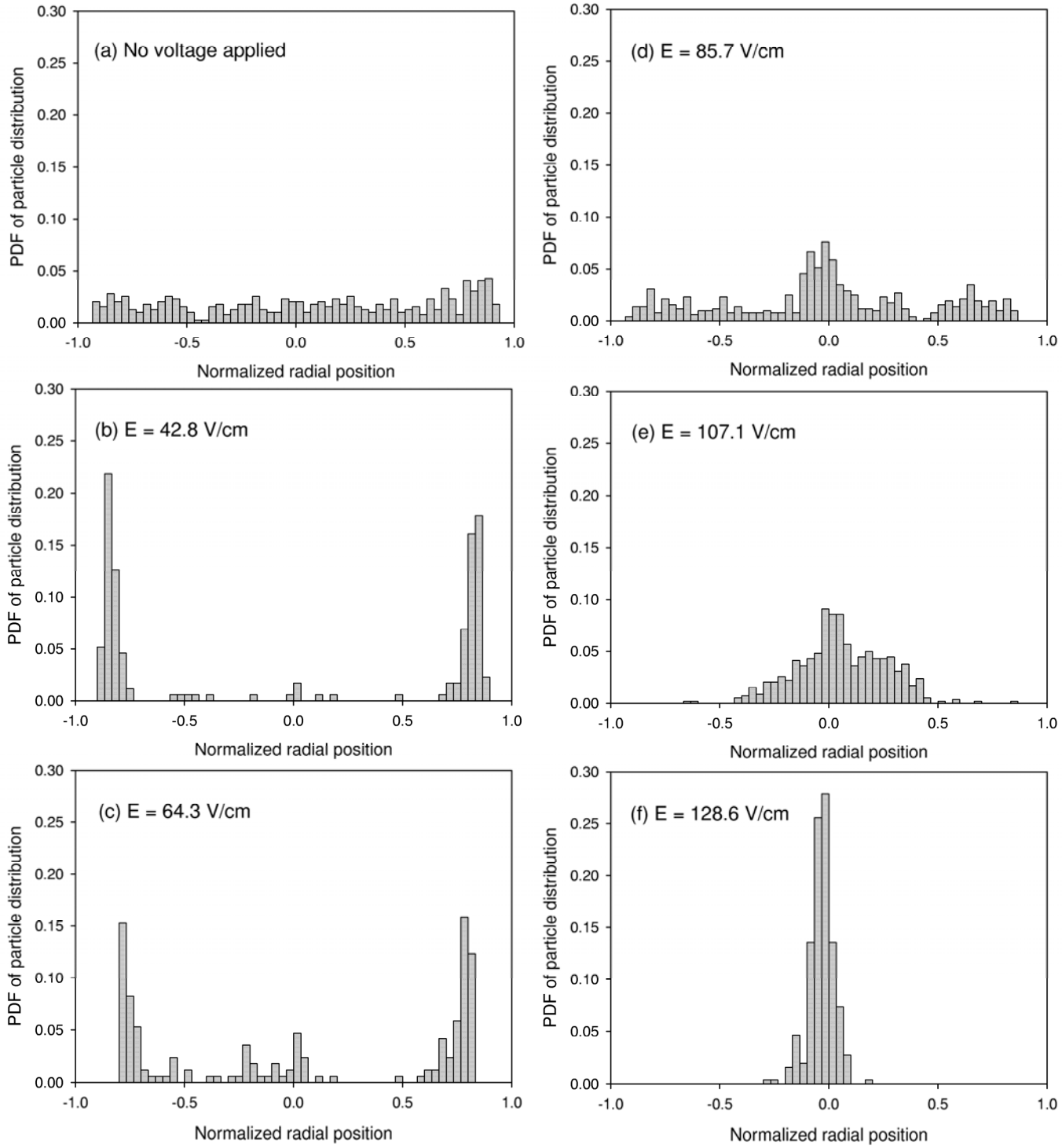

Figure 3 Evolution of the particle focusing at various electric field strengths is presented in terms of the probability density function. As the field strength increases, particles are tightly focused into a very narrow region.

flow in a tube, particles migrate toward off-centerline positions when the lagging effect or the particle buoyancy is small [15, 16].

Actually, a non-neutrally buoyant particle shows a complicated motion of the radial migration depending on the density difference between the fluid and the particle [16]. For example, considering the particle heavier than the fluid in a vertical ascending Poiseuille flow, if the density difference between the fluid and the particle is small, the particle travels along the off-centerline position, while much denser particle (producing larger lagging motion against Poiseuille flow) travels along the centerline of the channel. It is noticed that higher electric field strength in the present work is equivalent to the larger particle lagging motion against the Poiseuille flow. In other words, a particle slip velocity or a particle velocity due to electrophoresis where the particle is subjected to the electric field, becomes larger when the electric field strength increases due to the relation of $v_{slip} = E\mu_{ep}$, where v_{slip} is the particle slip velocity and μ_{ep} is the electrophoretic mobility. On the other hand, as the electric field increases up to E= 85.7 V/cm, we have a spreading of particle distribution rather than a sharp peak (Figure 3(d)). As a result, the peak values dramatically decayed. As the voltage further increases, i.e., electric field of 128.6 V/cm in Figure 3(f), three-dimensional particle focusing was successfully achieved axisymmetrically. In Figure 3(f), more than 90% of the total number of

the particles is tightly constrained within the focusing diameter less than two times the diameter of a single particle or less then 11% of the channel diameter. On the other hand, outward particle migration toward the wall as shown in Figures 3(b) and 3(c) has been reported by Zheng and Yeung (2002) using this concept. However, this is only a transient case toward the 3-D particle focusing [17].

In practice, the beam diameter of the conventional flow cytometer is approximately 10 ~ 15 μm surrounded by the outer cylinder with a diameter of 100 μm. The beam diameter of our device was measured to be approximately 9.9 μm, which holds for the practical applications. Actually, microfluidic channels formed by photolithography process have a rectangular cross-sectional geometry. However, the feature of the lateral migration in a circular cross-sectional tube is very similar to those in a plane channel or square channel. Thus, our device can be directly applied to the planar microfluidic channels (probably in square microchannels), for 3-D focusing.

4. Conclusion

We have presented a novel 3-D particle focusing technique that enables microparticles to be axisymmetrically focused in a single micro-capillary without sheath flows, such as coaxial-jet flows. Our device provides crucial solutions for simple and innovative 3-D particle focusing method by overcoming current limitations of complicated fabrication techniques required for 3-D focusing. We believe that this device can be utilized in a wide variety of applications, such as biomedical/biochemical engineering.

Acknowledgements

This work was supported by the Small and Medium Business Administration funded by the Korean Government (0420-2007-017-00).

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