19th Australasian Fluid Mechanics Conference
Melbourne, Australia
8-11 December 2014

Particle Sizing Using Dielectrophoresis-active Hydrophoresis

S. Yan[1], J. Zhang[1], Y. Yuan[2], G. Alici[1], H.P. Du[3], Y. Zhu[4,5] and W.H. Li[1]

[1] School of Mechanical, Materials and Mechatronic Engineering University of Wollongong, Wollongong, NSW 2522, Australia

[2] School of Materials Science and Engineering, University of New South Wales, Sydney, NSW 2052, Australia

[3] School of Electric, Computer and Telecommunication Engineering, University of Wollongong, Wollongong, NSW 2522, Australia

[4] CSIRO Manufacturing Flagship, Private Bag 33, Clayton, VIC, 3169, Australia

[5] Melbourne Centre for Nanofabrication/Australian National Fabrication Facility, 151 Wellington Road, Clayton, VIC, 3168, Australia

Abstract

A particle sizing technique using dielectrophoresis-active hydrophoresis is presented in this study. This method can measure particle diameters without complex optical tools and electric impedance, and can be adjusted by modulating the external voltage. The separation region is designed to amplify the slight differences in the lateral positions of the particles. The calibration line is obtained from the lateral positions of standard beads and then utilised to measure the distribution of 8 μm beads with a high coefficient of variation (CV). This calibration line is tunable when distinguished voltages are applied. The diameter estimated using DEP-active hydrophoretic sizing is compared with the data obtained using the transmission electron microscope (TEM) method, and it is shown that our DEP-active hydrophpretic technique can potentially be a versatile method for sizing particles inside a microfluidic device.

Introduction

The need to size individual particles or cells exists in fields such as industrial testing [1] and clinical diagnostics [2]. Traditionally particle size is measured either manually by analysing the transmission electron microscope (TEM) images or automatically by a Coulter counter [3]. However, both methods typically require complicated and expensive facilities. The recent advance in microfluidics technology has provided impetus for developing microfluidics for particle analysis. The unique properties of microfluidics such as low sample volume, fast response, low cost and portability are attractive in developing portable and hand-held devices for detecting the properties of particles and cells.

Microfluidic Coulter counters have been developed to count and size cells and particles, and researchers are making on-going efforts to improve their performance such as high throughput technique using multiple-orifice designs [4]. Unlike a conventional Coulter counter, the sizing range of an on-chip Coulter counter has been extended to the nanoscale so that nanoparticles such as DNA molecules can be detected in an on-chip Coulter counter with nanoscale apertures [5, 6]. Sun *et al*. [7] proposed a MOSFET-based microfluidic Coulter counter with a sensing channel for polystyrene particles and yeast sizing. However, most on-chip Coulter counters monitor the alteration of DC resistance in the sensing channel, but the interface between the electrodes and the liquid generates an electrical double layer which makes it difficult to apply DC signals [8]. Meanwhile, due to the different electrode layouts and channel geometry, the model used in a conventional Coulter to measure particle size is no longer applicable to an on-chip Coulter counter [9]. As well as on-chip Coulter counters, microfluidic flow cytometry has been developed to analyse particles and cells. Gawad *et al*. [10] proposed an impedance flow cytometry using coplanar, but the impedance altered differentially when the micro-beads passed the detection area, which means the variation of the levitation height in the non-uniform electric field may affect the detection results. To solve this problem, facing electrodes patterned on the top and bottom of the channel were developed [11], so now, when a cell appears between one pair of electrodes, the other pair measured the electric signals as a reference. Holmes *et al*. [12] demonstrated a microfluidic impedance cytometry that conducted a white blood cell differential counting.

Microfluidic impedance cytometry involves the inherent complexity of optical components, and accurate laser alignment needs skilled technicians to ensure that the laser light illuminates the sample perpendicularly. Choi *et al*. [13] proposed a hydrophoretic technique to size micro-particles without electric impedance and optical detection techniques. Since the hydrophoretic movements of particles depended on their size, a standard calibration curve for hydrophoretic sizing was obtained using micro-particles with known diameters. Then, 10.4 μm beads with a high coefficient of variation (CV) of 8.7% were used to demonstrate the validity of hydrophoretic sizing. Although the diameters using hydrophoretic sizing agreed with the result measured by a commercial Coulter counter, the operational range of the hydrophoretic device was limited and fixed because a hydrophoretic channel has a fixed operational range which cannot be adjusted unless a new channel with a different height has been fabricated.

In this paper we present a technique for dielectrophoresis (DEP)-active hydrophoretic particle sizing. The technique uses grooves patterned on top of the channel and electrodes embedded at the bottom of the channel. The concept of DEP-active hydrophoresis was reported previously in a previous study [14]. In this study, a widened channel was added after the pinched channel to 'amplify' the distance between the different particles (Fig. 1). The operational range of the DEP-active hydrophoretic device was flexible and could be adjusted by varying the external voltage. Particles were sized by analysing the images captured without complex optical detection and electric impedance analysis. This study deals with the effect of a sudden expansion on the movement of particles such that the particle trajectories and flow characteristics in the DEP-active hydrophoretic device were calculated using the finite element software (COMSOL Multi-physics 4.3 COMSOL, Burlington, MA). The 8 μm beads were measured using the calibration line obtained from the lateral positions of standard beads. Our DEP-active hydrophpretic technique has the potential to be a versatile method for sizing particles.

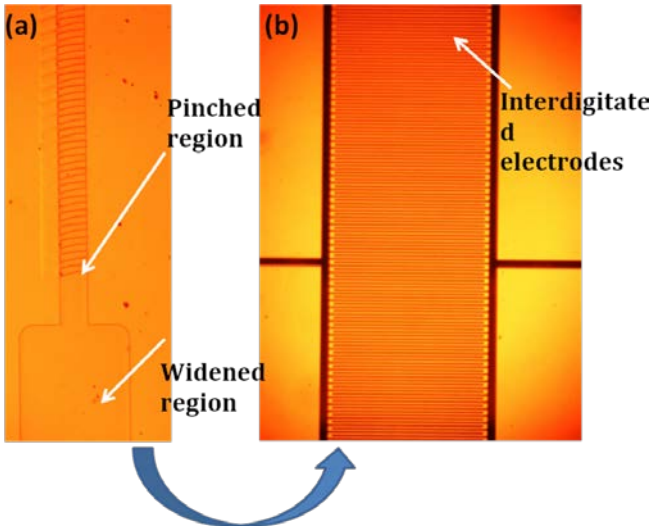

Figure 1. Overview of DEP-active hydrophoretic device. (a)PDMS micro-channel consisting of pinched and widened channel. The slight difference of particle trajectories can be expanded after entering the widened channel. (b) Interdigitated electrodes were patterned onto the bottom of the channel which generated a negative DEP force to push particles upwards.

Experimental Methods

Methodology

The micro-channel was consisted of pinched and widened regions. In the pinched region, interdigitated electrodes were patterned onto the bottom of the channel so that particles exerting a negative DEP force were pushed into a narrow space. As a result, the particles have more opportunity to interact with the grooves and induce hydrophoretic ordering by steric hindrance. Hence, large and small particles can be focused simultaneously in pinched region under an appropriate flow rate and applied voltage. The widened region was used to expand the relative distance between the different particles. Because the flow profile between the pinched and widened micro-channel spread, the slight difference in the positions of the particles in the pinched region can be amplified substantially in the widened region. This means that particles have obviously different lateral positions according to their sizes using the DEP-active hydrophoretic channel. The calibration line was obtained from the lateral positions of standard beads and then uncertain particles can be measured according to their lateral positions in the DEP-active hydrophoretic channel.

Fabrication

The interdigitated electrodes were fabricated using standard lift-off technique. Two-step photolithography of SU-8 photoresist (SU8-2010; Microchem Corp., MA) was employed to form the double-layer mold. The first layer served as the main channel, whereas, the second layer were seated on the top of the first layer to generate the grooves.

Material Preparation and Equipments

Beads of different sizes (3, 5, 8, 10 μm) were purchased from Thermo Fisher Scientific and suspended in DI water. The final concentration of bead solution was ~1 × 10^5 particles per millilitre.

The waveform generator (33250A, Agilent, USA) was used to generate AC fields with a frequency of 1 MHz. A syringe pump (Legato 100, Kd Scientific) was utilised to inject the bead mixtures into the microfluidic channel. An inverted microscope (CKX41, Olympus, Japan) was used to monitor the particle trajectories, which were recorded by a CCD camera (Rolera Bolt, Q-imaging, Australia). The lateral positions of particles were analysed using an image processing program, Q-Capture Pro 7 (Q-imaging, Australia).

Results and Discussion

Calibration Line for Sizing

The lateral position was inversely proportional to the diameter of particles. A standard line for sizing particles was fitted from the lateral positions of the 3, 5, and 10 μm particles whose CVs were around 5%. Fig. 2a shows the typical lateral position of the 3, 5, and 10 μm particles in the widened channel. These figures were acquired at a flow rate of 5 μl min^{-1} and a voltage of 24 V_{p-p}. The lateral position of the particles depends on their size; as their diameters increased, they tended to move closer to side 1 of the channel. The linear calibration line could be adjustable by modulating the voltages (Fig. 2b). Actually, the 3 μm beads were not focused very well at 18 V_{p-p}. Its position was measured within 213.5 ± 29.8 μm, which had overlapped with the 5 μm particles whose position was 201.9 ± 5.0 μm. The sizing range was fixed at the conventional hydrophoretic channel and it can be changed by changing the height of the channel [13]. In our tunable system, the limit of the sizing range can be overcome by changing the voltage. To modulate the sizing range, another calibration was carried out at 24 V_{p-p}, and the particle trajectories no longer overlapped with each other. The equation for the linear calibration line was defined [13]: $D = (P-P_0)/R_{P-D}$, where D is the diameter, R_{P-D} is the conversion ratio from the diameter to the lateral position, P is the lateral position in the widened channel, and P_0 is the intercept when the diameter was zero. R_{P-D} and P_0 were -11.4 and 226.9, respectively, at a voltage of 24 V_{p-p}.

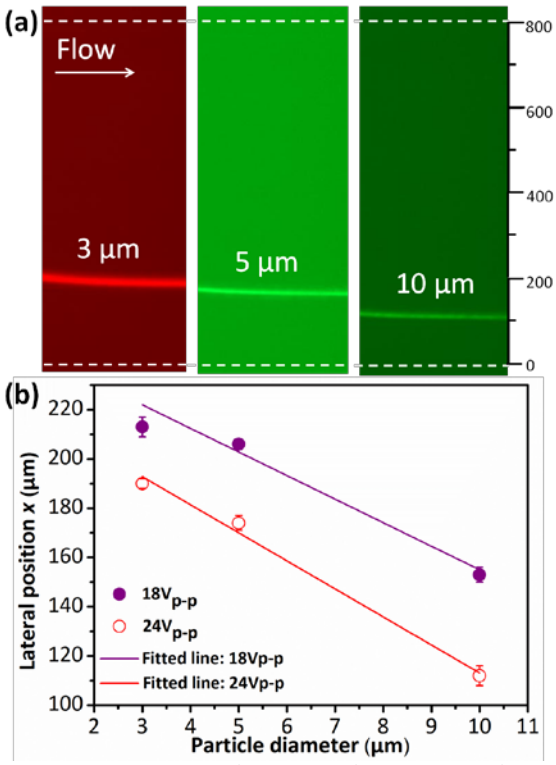

Figure 2. Calibration of the microfluidic device for DEP-active hydrophoretic sizing. (a) Optical microscopy images showing position distributions of 3, 5, 10 μm beads in the widened channel. (b) Lateral positions of particles as a function of their diameters. The solid lines were linear regression lines to obtain calibration lines for particle sizing at 18 and 24 Vp-p, respectively. The average experimental value of lateral positions was measured 3 times.

DEP-active Hydrophoretic Sizing

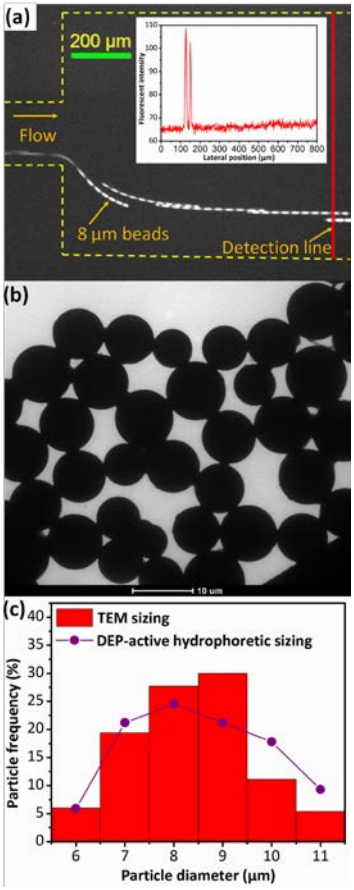

Figure 3. DEP-active hydrophoretic sizing of 8 µm bead with CV of 18% for its diameter. (a) Optical images showing the lateral position of 8 µm beads into the widened channel. The inset shows the fluorescent intensity acquired from the detection line. Each peak value corresponds to a lateral position. (b) The image of particles captured by TEM. (c) The distributions of 8 µm particles measured using the DEP-active hydrophoretic method and TEM sizing method. The plot for particle sizing was obtained from the measurement over 200 particles.

The calibration line for DEP-active hydrophoretic sizing was obtained (Fig. 2b), and then utilised to compare with the TEM sizing method. To verify the concept of DEP-active hydrophoretic sizing, 8 µm beads with high CV of 18% were injected into the channel at a flow rate of 5 µl min^{-1} and a voltage of 24 V_{p-p}. As Fig. 3a shows, the beads were distributed differently in the widened channel, depending on their specific diameters. The inset of Fig. 3a shows the measured fluorescent intensity of particle trajectories such that the peak value of each fluorescent intensity corresponded to a lateral position of the particles. This lateral position was measured to within 130 ± 20 µm, which was then converted into a diameter of 8.5 ± 1.8 µm. In this experiment, the CV of 8 µm beads was 21.1%. Fig. 3b shows the image of particles captured by TEM. The diameter of the beads estimated by DEP-active hydrophoresis agreed reasonably well with the result measured by the TEM method (Fig. 3c).

The concentration of injected particles was ~10^5 beads per millilitre. At such a moderate concentration the particle trajectories were separated rather than overlapped with each other, which can be easily recognized and detected by the detection line. Even though the head of one particle trajectory had overlapped with another particle's rear trajectory, their lateral position could still be measured at their middle part. At a higher concentration the particle-particle interaction may affect the sizing performance by colliding with each other. Meanwhile, particles with same or close lateral positions passed through the detection line at one time, so the measured fluorescent intensity was the superposition of several particles. By this method the lateral position of a particle would not be detected accurately so the optimised concentration will be investigated in future work. In the current work, measurements were performed manually, so proper programming to measure the lateral position and then automatically convert it into particle diameter will be developed further.

Conclusions

The DEP-active hydrophoretic technique was applied to size particles without any complicated detection equipment. When the lateral position of particles was amplified in the separation region, its focusing width expanded simultaneously, but once the particles were focused well in the pre-focusing region, they could still focus well in the separation region. In our experiments, the limited sizing range meant that the smallest particles could be focused. The sizing range can be adjusted by varying the voltage without changing the height of the channel. Unlike other methods used to size particles which need expensive equipment and precise physical parameters, our DEP-active hydrophoretic sizing approach is low cost and robust because the particle diameters can be obtained by analysing the captured pictures. All the analysing procedures only involve software without any specific hardware. Our DEP-active hydrophoretic sizing technique has the potential to be a sensor that will measure the diameters of particle.

Acknowledgments

This work is supported by the University of Wollongong -China Scholarship Council joint scholarships and the CSIRO Office of Chief Executive Top-up Scholarship. This work was performed in part at the Melbourne Centre for Nanofabrication (MCN) in the Victorian Node of the Australian National Fabrication Facility (ANFF).

References

[1] Garcia-Cordero, J.L., Barrett, L.M., O'Kennedy, R. & Ricco, A.J., Microfluidic sedimentation cytometer for milk quality and bovine mastitis monitoring, *Biomed. Microdevices*, **12**, 2010, 1051-1059.

[2] Alt, C., Veilleux, I., Lee, H., Pitsillides, C.M., Côté, D. & Lin, C.P., Retinal flow cytometer, *Opt. Lett.*, **32**, 2007, 3450-3452.

[3] Wyatt, P.J., Submicrometer particle sizing by multiangle light scattering following fractionation, *J. Colloid Interface Sci.*, **197**, 1998, 9-20.

[4] Zhe, J., Jagtiani, A., Dutta, P., Hu, J. & Carletta, J., A micromachined high throughput Coulter counter for bioparticle detection and counting, *J. Micromech.Microeng.*, **17**, 2007, 304-313.

[5] Carbonaro, A. & Sohn, L.L., A resistive-pulse sensor chip for multianalyte immunoassays, *Lab Chip*, **5**, 2005, 1155-1160.

[6] Ateya, D.A., Sachs, F., Gottlieb, P.A., Besch, S. & Hua, S.Z., Volume cytometry: Microfluidic sensor for high-throughput screening in real time, *Anal. Chem.*, **77**, 2005, 1290-1294.

[7] Sun, J., Gao, Y., Isaacs, R.J., Boelte, K.C., Charles Lin, P., Boczko, E.M. & Li, D., Simultaneous on-chip DC dielectrophoretic cell separation and quantitative separation performance characterization, *Anal. Chem.*, **84**, 2012, 2017-2024.

[8] Zheng, Y., Nguyen, J., Wei, Y. & Sun, Y., Recent advances in microfluidic techniques for single-cell biophysical characterization, *Lab Chip*, **13**, 2013, 2464-2483.

[9] Saleh, O.A., Quantitative sensing of nanoscale colloids using a microchip Coulter counter, *Rev. Sci. Instrum.*, **72**, 2001, 4449-4451.

[10] Gawad, S., Schild, L. & Renaud, P., Micromachined impedance spectroscopy flow cytometer for cell analysis and particle sizing, *Lab Chip*, **1**, 2001, 76-82.

[11] Cheung, K., Gawad, S. & Renaud, P., Impedance spectroscopy flow cytometry: On-chip label-free cell differentiation, *Cytometry A*, **65**, 2005, 124-132.

[12] Holmes, D., Pettigrew, D., Reccius, C.H., Gwyer, J.D., van Berkel, C., Holloway, J., Davies, D.E. & Morgan, H., Leukocyte analysis and differentiation using high speed microfluidic single cell impedance cytometry, *Lab Chip*, **9**, 2009, 2881-2889.

[13] Choi, S. & Park, J.-K., Continuous hydrophoretic separation and sizing of microparticles using slanted obstacles in a microchannel, *Lab Chip*, **7**, 2007, 890-897.

[14] Yan, S., Zhang, J., Li, M., Alici, G., Du, H., Sluyter, R. & Li, W., On-chip high-throughput manipulation of particles in a dielectrophoresis-active hydrophoretic focuser, *Sci. Rep.*, **4**, 2014.