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2017

# On-Chip Microparticle and Cell Washing Using Coflow of Viscoelastic Fluid and Newtonian Fluid

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## Publication Details

Yuan, D., Tan, S. Hwa., Sluyter, R., Zhao, Q., Yan, S., Nguyen, N., Guo, J., Zhang, J. & Li, W. (2017). On-Chip Microparticle and Cell Washing Using Coflow of Viscoelastic Fluid and Newtonian Fluid. *Analytical Chemistry*, 89 (17), 9574-9582.

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## Abstract

This work investigates the on-chip washing process of microparticles and cells using coflow configuration of viscoelastic fluid and Newtonian fluid in a straight microchannel. By adding a small amount of biocompatible polymers into the particle medium or cell culture medium, the induced viscoelasticity can push particles and cells laterally from their original medium to the coflow Newtonian medium. This behavior can be used for particle or cell washing. First, we demonstrated on-chip particle washing by the size-dependent migration speed using coflow of viscoelastic fluid and Newtonian fluid. The critical particle size for efficient particle washing was determined. Second, we demonstrated continuous on-chip washing of Jurkat cells using coflow of viscoelastic fluid and Newtonian fluid. The lateral migration process of Jurkat cells along the channel length was investigated. In addition, the cell washing quality was verified by hemocytometry and flow cytometry with a recovery rate as high as 92.8%. Scanning spectrophotometric measurements of the media from the two inlets and the two outlets demonstrated that diffusion of the coflow was negligible, indicating efficient cell washing from culture medium to phosphate-buffered saline medium. This technique may be a safer, simpler, cheaper, and more efficient alternative to the tedious conventional centrifugation methods and may open up a wide range of biomedical applications.

## Disciplines

Medicine and Health Sciences

## Publication Details

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# **On-chip microparticle and cell washing using co-flow of viscoelastic fluid and Newtonian fluid**

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## **Abstract**

This work investigates the on-chip washing process of microparticles and cells using co-flow configuration of viscoelastic fluid and Newtonian fluid in a straight microchannel. By adding a small amount of biocompatible polymers into the particle medium or cell culture medium, the induced viscoelasticity can push particles and cells laterally from their original medium to the co-flow Newtonian medium. This behavior can be used for particle or cell washing. First, we demonstrated on-chip particle washing by the size-dependent migration speed using co-flow of viscoelastic fluid and Newtonian fluid. The critical particle size for efficient particle washing was determined. Second, we demonstrated continuous on-chip washing of Jurkat cells using co-flow of viscoelastic fluid and Newtonian fluid. The lateral migration process of Jurkat cells along the channel length was investigated. In addition, the cell washing quality was verified by hemocytometry and flow cytometry with a recovery rate as high as 92.8%. Scanning spectrophotometric measurements of the media from the two inlets and the two outlets demonstrated that diffusion of the co-flow was negligible, indicating efficient cell washing from culture medium to phosphate-buffered saline medium. This technique may be a safer, simpler, cheaper, and more efficient alternative to the tedious conventional centrifugation methods, and may open up a wide range of biomedical applications.

**Keywords:** Viscoelastic fluid, viscoelastic force, cell washing, cell lateral migration.

## Introduction

Washing of microparticles and cells have broad applications in biology, medicine and clinical analysis <sup>1-3</sup>. Washing is often necessary for the preparation of samples prior to experimentation or analysis <sup>4-6</sup>. For example, the extraction of stained leukocytes from whole blood samples requires conventional steps such as mixing reagents, centrifugation, and resuspension or microfluidic washing steps to remove cell lysates or other cell components <sup>7-9</sup>. Transferring particles or cells from a high background to a low background can improve the measurement accuracy in flow cytometry <sup>7</sup>.

Centrifugation is the conventional cell washing method. Although simple and ubiquitous, centrifugation has many limitations. For example, the centrifugation processes are often carried out in batches and are not continuous in nature. This method is also time-consuming, labour-intensive, and the high speed rotation may alter the analytes of interest. The recovery rate and purity of the collected cells is limited because of the manual pipetting steps, which varies significantly based on experience and skill of operators.

Microfluidic techniques, which can manipulate particles and control fluids at micron and submicron dimensions <sup>10</sup>, have been used in various biomedical and biochemical fields <sup>11-14</sup>, and present alternative methods for particle/cell washing. According to their operating principle, microfluidic techniques are classified as active and passive methods. Active methods rely on external force fields such as dielectrophoresis (DEP) <sup>15, 16</sup>, optical <sup>17</sup>, magnetic <sup>18, 19</sup> and acoustophoresis <sup>20-23</sup>. Whereas passive methods rely on intrinsic hydrodynamic forces induced in microchannels with specialized geometry or structures <sup>6, 9, 24</sup> such as deterministic lateral displacement <sup>6, 8</sup>, pinched-flow fractionation <sup>25</sup>, differential inertial focusing in the channels with shifting aspect ratios <sup>7, 26-28</sup>, trapping particles or cells by vortex technology using expansion and contraction cavity arrays <sup>29, 30</sup>, inertial flow

deformation induced by sequences of simple micro-pillar arrays to switch fluid streams<sup>31,32</sup>, and fluid transfer around particles induced by particles rotation<sup>33</sup>.

The above microfluidic methods for particle/cell washing are all performed in Newtonian fluids, which employ either external force fields or specially designed complex channels. Recently, the interest in particle manipulation in viscoelastic fluids has been growing because of its superior focusing performance in the relatively simple channel geometry. In viscoelastic fluids, the dilute polymer within the fluid can induce the first normal stress difference ( $N_1$ ). The suspended particles or cells flowing in the micro-channels can migrate laterally under the effect of  $N_1$ <sup>34-36</sup>. Many researchers have studied particle/cell focusing or separation in viscoelastic fluids with or without the aid of sheath flow<sup>37-42</sup>. In those experiments that with the aid of sheath flow to do particles/cells separation<sup>38, 39, 43, 44</sup>, the sample and sheath streams are both viscoelastic fluids. The separation mechanism is that all the particles/cells pre-focused by sheath flow are prone to migrate to the centre of the channels in the viscoelastic fluid, while the migration speed is different according to their sizes. However, the particles/cells lateral migration behaviours in sample-sheath flow with different properties are rarely studied.

Recently, our group<sup>45</sup> and others<sup>46</sup> have proposed and explored the lateral migration of particles using the co-flow configuration of viscoelastic and Newtonian fluids. Ha et al. implemented particle separation using  $\lambda$ -DNA viscoelastic and Newtonian fluids<sup>46</sup>.  $\lambda$ -DNA is linear, doubled-stranded DNA from an *Escherichia coli* bacteriophage. Instead of using expensive  $\lambda$ -DNA as the diluted polymer to form viscoelastic fluid, our group has investigated particle lateral transfer from poly(ethylene oxide) (PEO)-containing viscoelastic fluid to a Newtonian fluid<sup>45</sup>. In the present work, we further characterise particle manipulation properties using this configuration, and apply them for on-chip microparticle and cell washing. The phenomenon of lateral migration of particles with different sizes using

co-flow of viscoelastic fluid and Newtonian fluid is studied, and the critical blockage ratio for efficient particle washing is determined. Secondly, the continuous on-chip washing of leukaemic Jurkat cells is demonstrated using co-flow of viscoelastic fluid and Newtonian fluid. Finally, the cell washing quality was tested by using hemocytometry and flow cytometry.

Compared with other particle/cell washing methods, our method can be performed in simple straight channels, without any external force. The simplicity of the method is due to the viscoelastic force that is induced by the medium intrinsic property. Although inertia-based cell washing can work in straight channel as well <sup>7, 26</sup>, with higher flow rate, the particles/cells size for efficient migration and washing is limited. However, our method can work with smaller and wider range of particles/cells sizes. This paper investigates a new method for particles/cell washing, and can deepen the understanding of particle behaviour in coflows with different properties. Moreover, the PEO polymer, which is added to the medium to tune its elasticity, is biocompatible and of low cost. In summary, the technique presented here can be safer, simpler, cheaper and more efficient than the tedious conventional medium exchange and washing methods. This method has the potential to allow for direct processing of various native biofluids and may be suitable for a wide range of biomedical applications.

## Theory

### Elastic force

In viscoelastic fluid, the polymer within the medium can induce an additional elastic force on particles.  $W_i$  <sup>37</sup> was used to characterize the elastic effects of the viscoelastic fluid, which is the ratio of two time constants:

$$W_i = \frac{\lambda}{t_f} = \lambda \dot{\gamma} = \lambda \frac{2V_m}{w} = \frac{2\lambda Q}{hw^2} \quad (1)$$

where  $\lambda$  defines the relaxation time of the fluid, the average velocity and characteristic time of the channel flow are defined by  $V_m$  and  $t_f$ , respectively. The characteristic time is  $2V_m / w$

or  $2\lambda Q/hw^2$  in a rectangular channel. In viscoelastic fluid, both the first normal stress  $N_1$  ( $=\tau_{xx} - \tau_{yy}$ ) and second normal stress  $N_2$  ( $=\tau_{yy} - \tau_{zz}$ ) contribute to particle migration.  $\tau_{xx}$ ,  $\tau_{yy}$ , and  $\tau_{zz}$  are normal stresses that are exerted in the flow, for the velocity gradient and vorticity direction, respectively. However, the effects of  $N_2$  can be neglected in diluted PEO solutions<sup>47, 48</sup>, because  $N_1$  is much larger than  $N_2$ . The elastic force  $F_E$ , which originates from an imbalance in the distribution of  $N_1$  ( $=\tau_{xx} - \tau_{yy}$ ) over the size of the particle, can be expressed as<sup>49</sup>:

$$F_E = C_{eL} a^3 \nabla N_1 = C_{eL} a^3 (\nabla \tau_{xx} - \nabla \tau_{yy}) = -2C_{eL} a^3 \eta_p \lambda \nabla \dot{\gamma}^2 \quad (2)$$

Where  $C_{eL}$  is the non-dimensional elastic lift coefficient,  $a$  is the particle size,  $\eta_p$  is the polymeric contribution to the solution viscosity.

It should be noted that particle focusing positions in viscoelastic fluids are controversial. Many researchers believe that under purely viscoelastic effect, particles migrate towards four corners and centreline in a rectangular channel, corresponding to the lower shear rate regions. However, under the synergetic effect of inertia and viscoelasticity<sup>37</sup>, the multiple equilibrium positions can be reduced to one at the centreline. Moreover, Del Giudice et al.<sup>50</sup> stated that the PEO medium can always exhibit purely elastic effects, because they believe the elastic effects are much stronger than the inertial effects, no matter whether the inertia is negligible or not. In their experiments, in both polyvinylpyrrolidone (PVP) and PEO fluids, the particles can always migrate to the channel centerline in all experimental conditions. In the current work, the maximum particle Reynolds number  $R_p$

$$(R_p = R_c \frac{a^2}{D_h^2} = \frac{\rho_f U_m a^2}{\mu_f D_h} \approx 0.09, \text{ where } \rho_f, U_m \text{ and } \mu_f \text{ are the fluid density, mean velocity,}$$

and dynamic viscosity, respectively;  $a$  is the particle size;  $D_h = 2wh/(w + h)$  is the hydraulic diameter for a rectangular channel.  $w$  and  $h$  is the width and height of the channel cross



section.) is much smaller than  $1^{-51}$  and the effect of inertial lift force is negligible. According to the experiments, the elastic force only directs to the Newtonian fluid, as no particle migration towards the channel wall was observed.

### Drag force

Assuming a spherical particle travelling in a uniform Stokes fluid, and there is a velocity difference between particle and fluid, a drag force can arise, which can be expressed as<sup>52, 53</sup>:

$$F_D = 3\pi\mu_f a(v_f - v_p) \quad (3)$$

where  $v_f$  and  $v_p$  are the velocities of the fluid element and particles, respectively.  $a$  is the particle size, and  $\mu_f$  represents the fluid viscosity.

### Schematic diagram of particles' on-chip washing using co-flow of viscoelastic fluid and Newtonian fluid

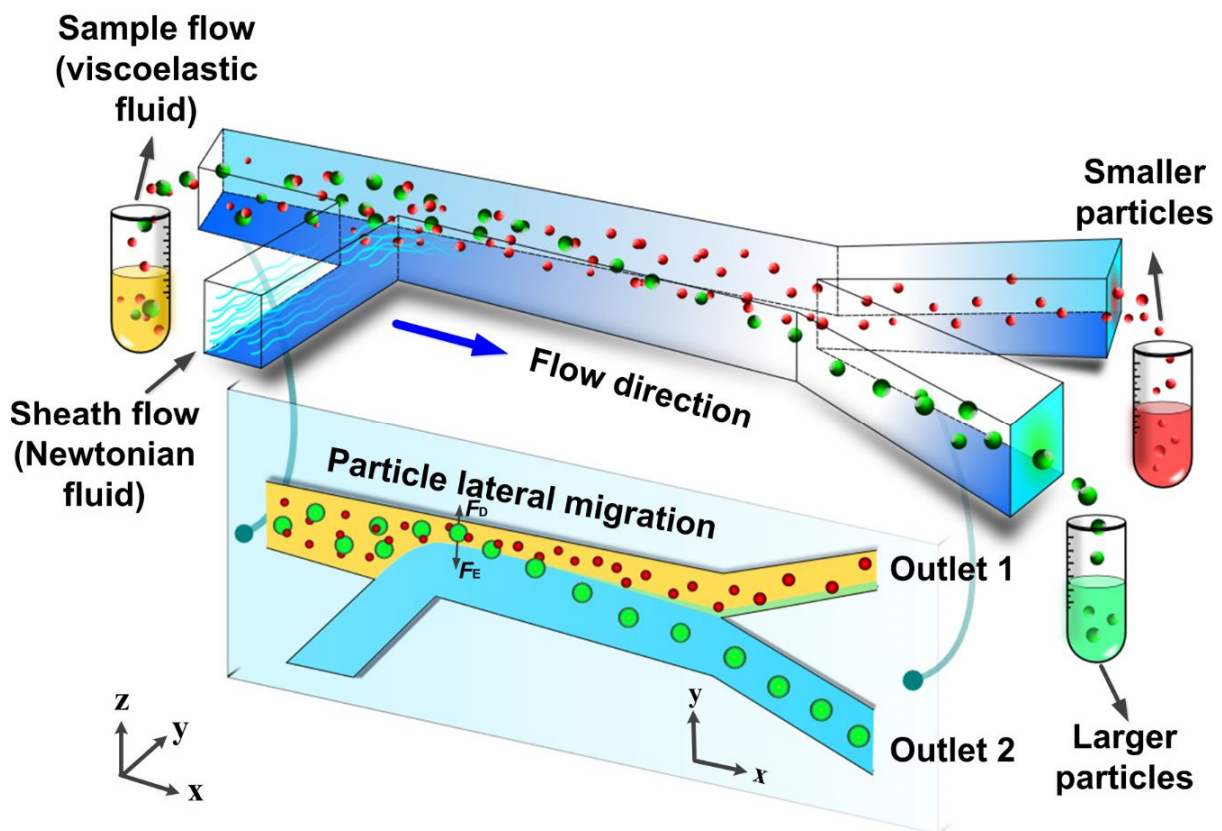


Figure 1: Schematic diagram of on-chip particle washing using co-flow of a viscoelastic fluid and a Newtonian fluid.

Figure 1 shows the concept of on-chip particle washing using co-flow of a viscoelastic fluid and a Newtonian fluid. The mixture of particles with two different sizes in the viscoelastic fluid is injected from one inlet, while the Newtonian fluid is introduced from the other inlet as a sheath flow. The flow rate ratio is 1:3 with the flow rate of sample flow  $Q_s$  and sheath flow  $Q_{sh}$  at 2  $\mu\text{l}/\text{min}$  and 6  $\mu\text{l}/\text{min}$ , respectively. At the inlet section of the channel, the particles are dispersed in the original viscoelastic fluid, and are squeezed to the upper side of the channel due to the Newtonian sheath flow. As the fluid moves, the particles with their blockage ratio above a certain value are focused into a tight streak, and transported laterally. Progressively, all the particles move across the boundary of the PEO medium (viscoelastic fluid) and migrate into deionized water (Newtonian fluid). However, the particles with their blockage ratio below the threshold cannot migrate to the Newtonian medium, and stay in their original viscoelastic medium. The migration phenomenon is attributed to the elastic force  $F_E$  in viscoelastic fluid. The migration direction is from viscoelastic fluid to Newtonian fluid, which has been validated in our previous work<sup>45</sup>. The particles are affected by elastic force  $F_E$  (points to the other Newtonian fluid) and drag force  $F_D$  with the opposite direction of elastic force. For particles with a blockage ratio above a threshold, the elastic force is strong enough to push the particles laterally across streamlines and enter the other fluid, as the elastic force is proportional to particle size. After the particles transfer across the viscoelastic fluid and Newtonian fluid interface, and completely migrate out of PEO solution, the elastic force disappears. However, particles can still migrate a certain lateral distance within the deionized water. This phenomenon might be caused by the inertia of the particles. For particles with their blockage ratio below a threshold (e.g., nanoscale molecules in the culture medium), the elastic force is not strong enough to drive particles to the other medium, so they remain within the viscoelastic fluid stream. Therefore, the lateral migration and on-chip washing of particles or cells can be achieved. The critical particle size for efficient

particle or cell washing can be determined. For the particles that can migrate out of viscoelastic fluid, the lateral migration distance in Newtonian fluid highly depends on the particle size. Larger particles migrate further in the Newtonian fluid than smaller particles do. The particle migration process in sample viscoelastic fluid and sheath Newtonian fluid are analysed in detail according to Newton's second law in supplementary information. Using the same method, particle separation according to different blockage ratios can be realized using this co-flow configuration of viscoelastic fluid and Newtonian fluid.

## **Materials and methods**

### **Design and fabrication of a microfluidic device**

The device is a simple straight rectangular channel with two inlets and two outlets. Its cross section is  $30 \times 50 \mu\text{m}$  (width  $\times$  height), and length 5 mm. Standard photolithography and soft lithographic techniques were used to fabricate the device<sup>54,55</sup>.

### **Preparation of the PEO medium**

For particle washing, PEO (2,000,000 Da; Sigma-Aldrich) was diluted to 1000 ppm in deionized water (DI water) containing 0.01% (v/v) Tween 20 (Sigma-Aldrich) to form the viscoelastic fluid, which acts as the sample flow. The sheath stream in particle washing experiment was DI water, which served as the Newtonian fluid. Tween 20 was added to both fluids to prevent particle aggregation. For on-chip Jurkat cell washing, PEO was added to a phosphate-buffered saline (PBS) (Sigma-Aldrich) at 2000 ppm. Before the cell experiments, the same volume of PBS containing 2000 ppm PEO was added to Jurkat cell culture medium (complete RPMI 1640 medium), thus the PEO concentration of the Jurkat cell culture medium is 1000 ppm, and PBS in sheath fluid worked as the Newtonian fluid. The viscosity of the Newtonian fluid and 1000 ppm PEO solution as a function of the shear rate were shown in Figure S2.

### **Particles and cells preparation**

For particle washing experiments, particle suspensions were prepared by diluting the mixture of 0.8- $\mu\text{m}$  internally red dyed fluorescent polystyrene microspheres (ThermoFisher Scientific, CV 5%) with 2- $\mu\text{m}$ , 3- $\mu\text{m}$ , 5- $\mu\text{m}$  and 10- $\mu\text{m}$  internally green dyed fluorescent polystyrene microspheres (Thermo Fisher Scientific, CV 5%) in the 1000 ppm PEO medium, respectively. The 0.8- $\mu\text{m}$  particles have a different fluorescence spectrum than that of particles of other sizes, and are too small to migrate laterally. Thus, the 0.8- $\mu\text{m}$  particles are used here as an indicator of the distribution of the original medium. The blockage ratio  $\alpha$  for 0.8- $\mu\text{m}$ , 2- $\mu\text{m}$ , 3- $\mu\text{m}$ , 5- $\mu\text{m}$  and 10- $\mu\text{m}$  particles is 0.02, 0.05, 0.08, 0.13, and 0.27, respectively.

For cell washing experiments, leukaemic Jurkat cells (ATCC), an immortalized human T cell line (average diameter of approximately 15  $\mu\text{m}$ ), were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (ThermoFisher Scientific) containing 10% fetal bovine serum (Bovogen Biologicals) and 2 mM L-glutamine (ThermoFisher Scientific) in a humidified incubator (Thermo Scientific) at 37°C and 95% air/5% CO<sub>2</sub>. Before commencing each experiment, the particle mixture was re-suspended by vortex and cell samples were manually stirred to provide uniform suspensions in a complete medium containing PEO. Absorbance spectra of fluids were assessed using a microplate reader (SPECTROstar<sup>Nano</sup>, BMG Labtech).

### **Experimental setup**

The sample medium (the mixture of PBS containing 2000ppm PEO with the same volume of Jurkat cell and RPMI 1640 culture medium), and sheath medium (PBS) were transferred to the chip from two 1-ml syringes, with silicon tube connected between them. The flow rate was controlled by syringe pumps (Legato 100, Kd Scientific, USA). An inverted microscope (CKX41, Olympus, Japan) mounted with a CCD camera (Optimos, Q-imaging, Australia) was used to observe and capture the images of the fluorescent particles,

cells and fluids. The fluorescent images were post-processed and analysed with Q-Capture Pro 7 (Q-imaging, Australia) software.

The volumetric flow rates of sample flow  $Q_s$  and sheath flow  $Q_{sh}$  were 2  $\mu\text{l}/\text{min}$  and 6  $\mu\text{l}/\text{min}$ , respectively, so that the ratio of the flow rate of sheath flow to sample flow ( $\alpha = Q_{sh}/Q_s$ ) was fixed at 1:3. The absorbance spectra of the media from the two inlets and the two outlets were tested by plate reader.

## Results and discussion

### On-chip particle washing

As described above, the particles are affected by an elastic force  $F_E$  (pointing to the Newtonian fluid) and drag force  $F_D$  which is in the opposite direction of the elastic force. The particle lateral migration for mixtures of either 0.8- $\mu\text{m}$  ( $\alpha = 0.02$ ) + 2- $\mu\text{m}$  ( $\alpha = 0.05$ ), 0.8- $\mu\text{m}$  + 3- $\mu\text{m}$  ( $\alpha = 0.08$ ), 0.8- $\mu\text{m}$  + 5- $\mu\text{m}$  ( $\alpha = 0.13$ ) or 0.8- $\mu\text{m}$  + 10- $\mu\text{m}$  ( $\alpha = 0.27$ ) particles at the inlet, 1.5 mm from the inlet, 3 mm from the inlet, and the outlet are shown in Figure 2. The red fluorescent streams in Figure 2(a) and red curves in Figure 2(b) indicate the distribution of 0.8- $\mu\text{m}$  particle medium, while the green fluorescent streams in Figure 2(a) and green curves in Figure 2(b) indicate the distribution of particles with other sizes.

Particle mixtures of either 0.8- $\mu\text{m}$  + 2- $\mu\text{m}$ , 0.8- $\mu\text{m}$  + 3- $\mu\text{m}$ , 0.8- $\mu\text{m}$  + 5- $\mu\text{m}$  or 0.8- $\mu\text{m}$  + 10- $\mu\text{m}$  in 1000 ppm PEO medium were injected into one inlet, while Newtonian fluid was injected into the other inlet. From the fluorescent images and corresponding normalized fluorescent intensity profiles, the mixture of 0.8- $\mu\text{m}$  + 2- $\mu\text{m}$  particles remain mixed together in the viscoelastic fluid along the channel from the inlet to the outlet, and both exited together into outlet 1 [Figure 2 (ai) and Figure 2 (bi)]. For particle mixtures of 0.8- $\mu\text{m}$  + 3- $\mu\text{m}$ , the 3- $\mu\text{m}$  particles started to focus and migrated laterally. Some of the 3- $\mu\text{m}$  particles migrated out of the original medium, thus 3- $\mu\text{m}$  particles moved in both the viscoelastic and Newtonian fluids, and exited from both outlets (Figure 2 (aii) and Figure 2 (bii)). As the particle size

continued to increase, the elastic force become strong enough to push the particles to migrate laterally and distinctively across streamlines to enter the Newtonian fluid. Figure 2 (a-iii)/(b-iii) and (a-iv)/(b-iv) show that 5- $\mu\text{m}$  and 10- $\mu\text{m}$  particles experienced stronger elastic forces, so that they began to be focused into a single streak and migrate laterally at 1.5 mm from the inlet. Further downstream, the particles continued to migrate laterally further, detaching from the original medium and enter the Newtonian fluid, which eventually exited into outlet 2. The 0.8- $\mu\text{m}$  particles remained dispersed and followed in the original viscoelastic fluid and come out from outlet 1. Particle washing can be realized using co-flow of viscoelastic and Newtonian fluids. However, the separation or washing performance depends on the particle size or particle blockage ratio. In our device, the critical particle blockage ratio for efficient particle washing is 0.08; when the particle blockage ratio exceeds 0.08, lateral migration starts.

In Figure 2(b), the two red curves in each sub-figure indicate the distribution of 0.8- $\mu\text{m}$  particles at the inlet and the outlet, as well as the original medium. It can be seen that the red curve at the outlet shift slightly along the lateral direction compared with that at the inlet, indicating the 0.8- $\mu\text{m}$  particles and original medium slightly diffuses as they flow through the whole channel.

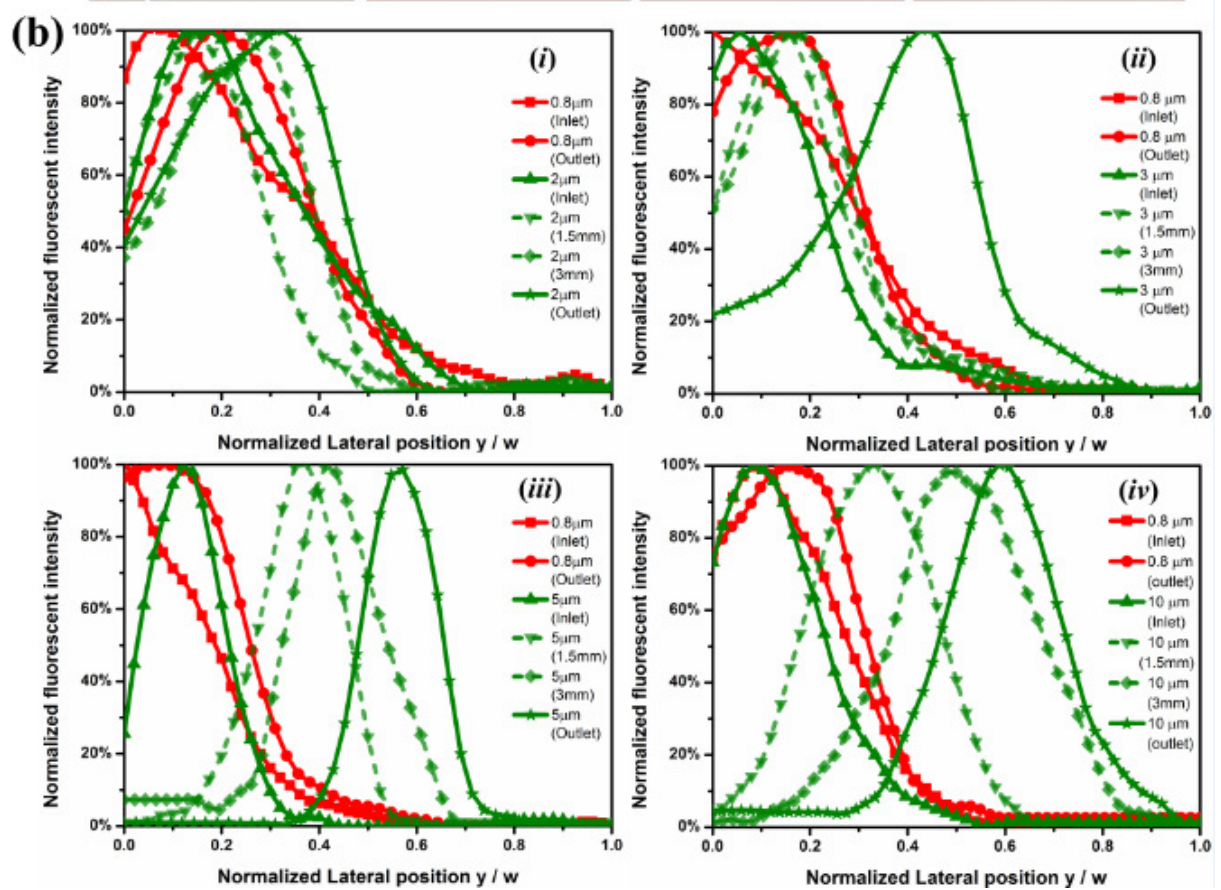
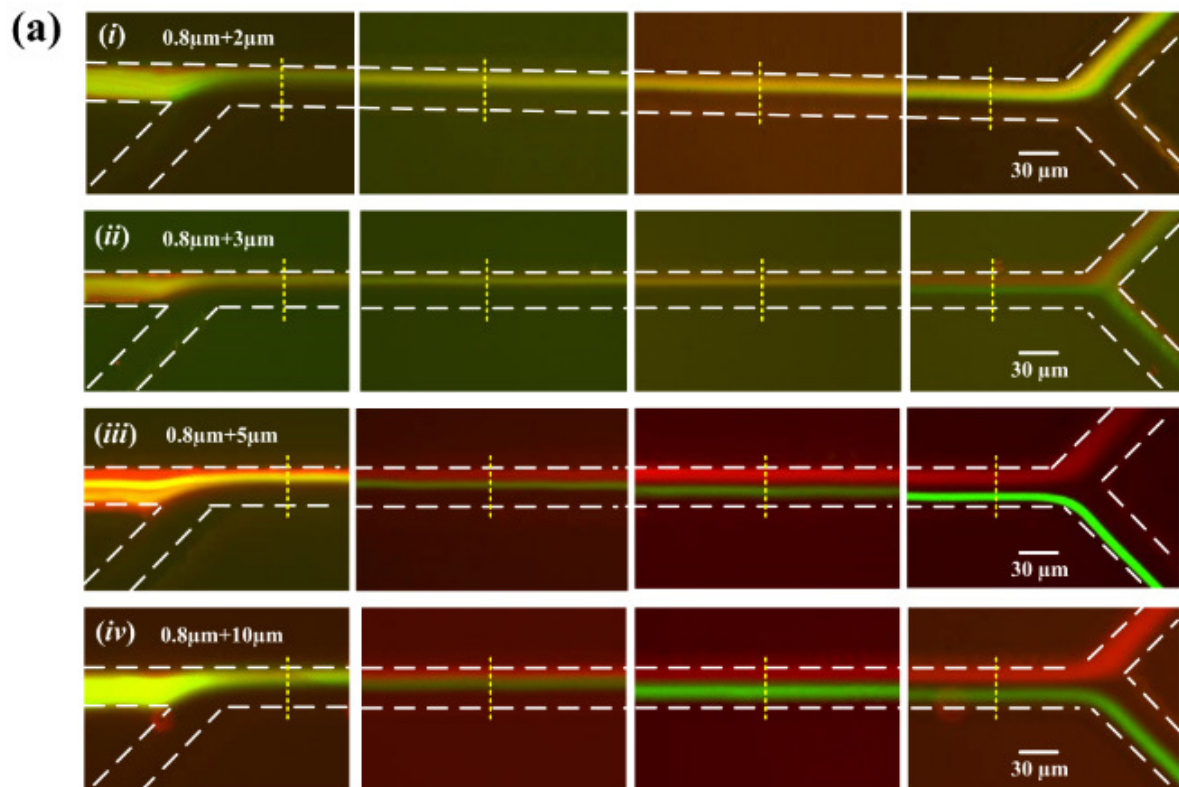


Figure 2: On-chip particle washing. (a) Fluorescent images of particle migration processes for mixture of 0.8- $\mu\text{m}$  + 2- $\mu\text{m}$ , 0.8- $\mu\text{m}$  + 3- $\mu\text{m}$ , 0.8- $\mu\text{m}$  + 5- $\mu\text{m}$ , 0.8- $\mu\text{m}$  + 10- $\mu\text{m}$  particles at inlet, 1.5mm from inlet, 3mm from inlet, and outlet. (b) Corresponding normalised fluorescent intensity profiles.

In a co-flow of viscoelastic and Newtonian fluids, particles with different sizes in the viscoelastic fluid either migrate gradually to the other fluid or remain in the original fluid. The migration speed of particles with different sizes can differ. Using this rule of thumb, size-dependent separation can also be achieved besides particle washing. Figure 3 shows the normalized particle lateral positions along the channel length at the inlet, 1.5 mm from inlet, 3 mm from the inlet, and the outlet. As the particle size and channel length increase, the lateral migration distance increases correspondingly. According to the lateral positions of different particle sizes at different channel lengths, apart from 0.8- $\mu\text{m}$  + 5- $\mu\text{m}$  and 0.8- $\mu\text{m}$  + 10- $\mu\text{m}$  particle mixture separation at the outlet, separation of 0.8- $\mu\text{m}$ , 2- $\mu\text{m}$  or 3- $\mu\text{m}$  particles with 5- $\mu\text{m}$  or 10- $\mu\text{m}$  particles can also be achieved after a proper channel length (1.5 mm or 3 mm from the inlet). By adjusting the channel length, multiple particle separation and washing steps can be achieved using co-flow of viscoelastic fluid and Newtonian fluid in simple straight channels.



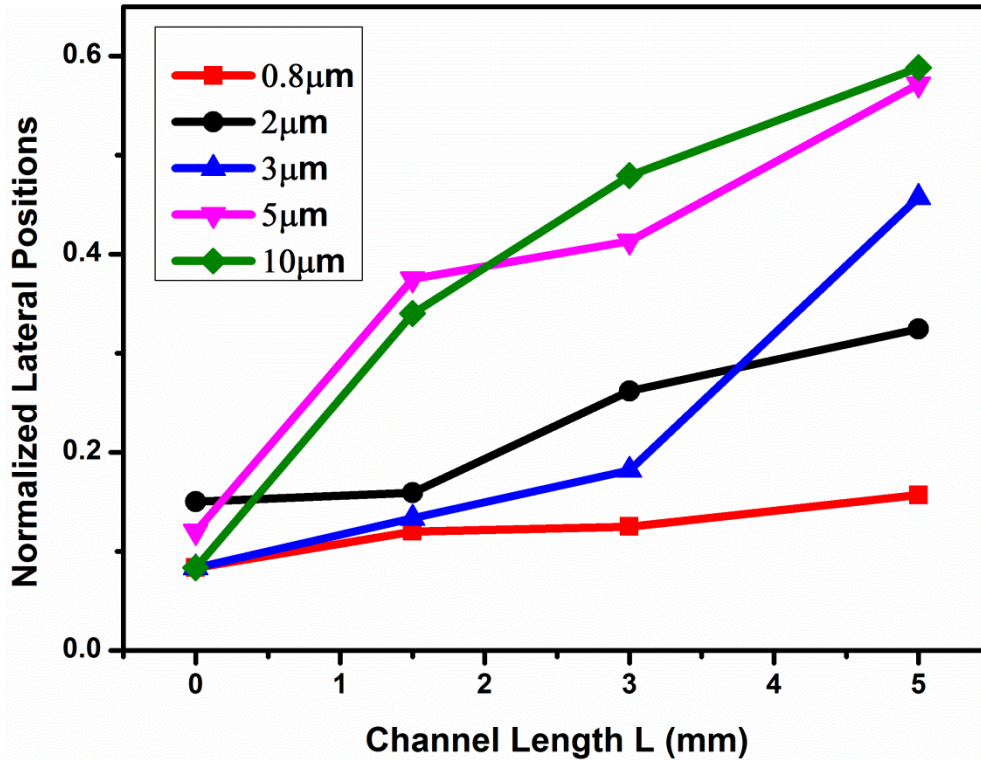


Figure 3: Particle lateral positions along the channel length at the inlet (0 mm), 1.5 mm from the inlet, 3 mm from the inlet, and the outlet (5 mm).

In washing experiments, particles with a blockage ratio exceeding 0.08, will start lateral migration and exit at outlet 2. Figure 4 shows the fluorescent images of media from the inlet, and outlets 1 and 2 for particle mixture of 0.8- $\mu\text{m}$  + 2- $\mu\text{m}$ , 0.8- $\mu\text{m}$  + 3- $\mu\text{m}$ , 0.8- $\mu\text{m}$  + 5- $\mu\text{m}$ , and 0.8- $\mu\text{m}$  + 10- $\mu\text{m}$  visualized on a hemocytometer, respectively. For 0.8- $\mu\text{m}$  + 2- $\mu\text{m}$  particle mixture, the particles are too small. Thus, no particles migrate to the Newtonian fluid, and no particles come out from outlet 2, Figure 4(a). However, for 3- $\mu\text{m}$  particles, a small number of them exit at outlet 2, Figure 4(b). For particle mixtures of 0.8- $\mu\text{m}$  + 5- $\mu\text{m}$ , and 0.8- $\mu\text{m}$  + 10- $\mu\text{m}$ , almost all of the 5- $\mu\text{m}$  and 10- $\mu\text{m}$  particles enter the Newtonian fluid and exit at outlet 2 (Figure 4(c) and Figure 4(d)). The recovery rates for 2- $\mu\text{m}$ , 3- $\mu\text{m}$ , 5- $\mu\text{m}$ , and 10- $\mu\text{m}$  particles at outlet 2 were 0%, 9%, 94.4% and 100%, respectively.

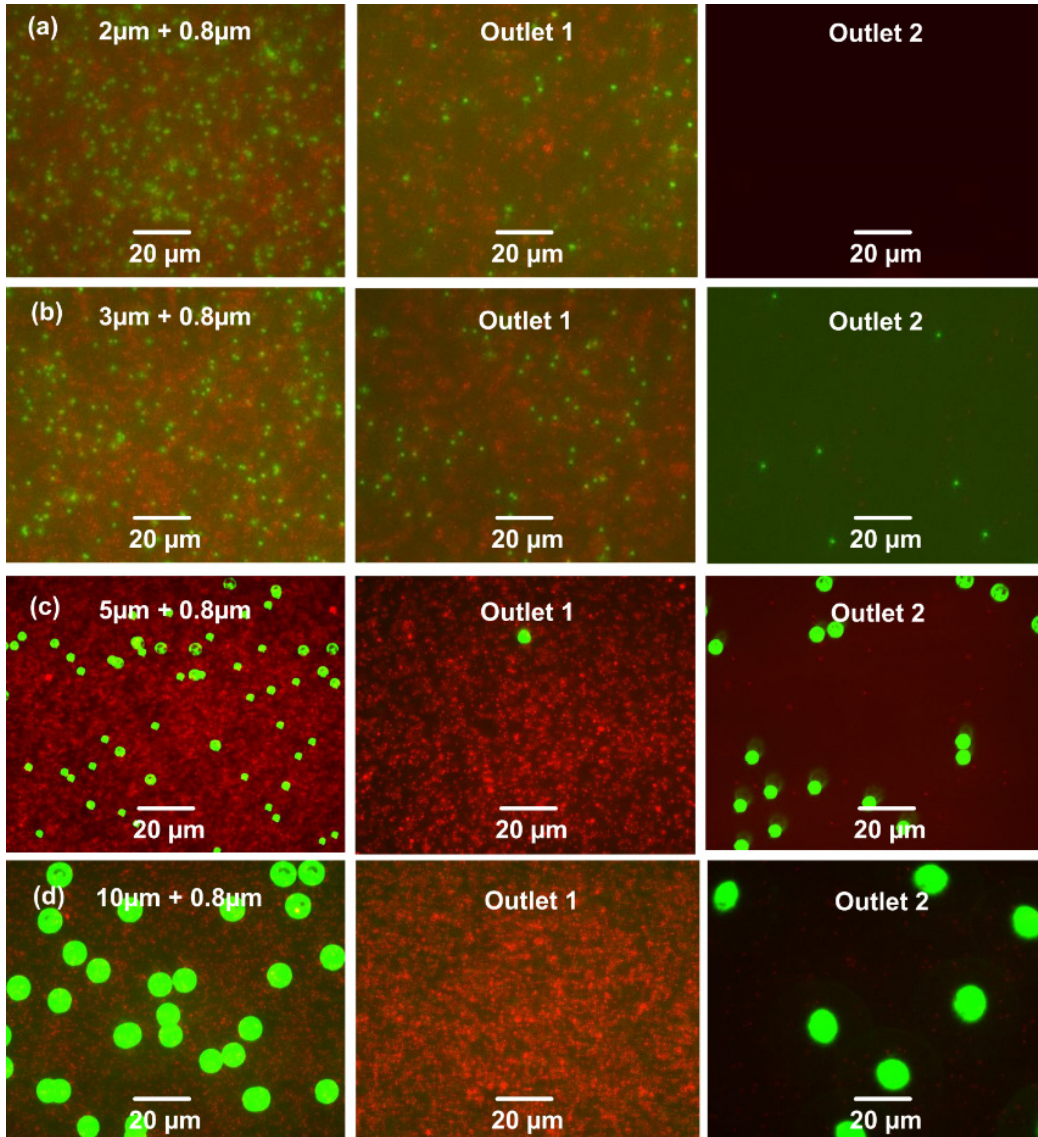


Figure 4: Fluorescent images of mediums from the inlet (left panels), and outlets 1 and 2 (as indicated) for particle mixtures of (a) 0.8- $\mu\text{m}$  + 2- $\mu\text{m}$ , (b) 0.8- $\mu\text{m}$  + 3- $\mu\text{m}$ , (c) 0.8- $\mu\text{m}$  + 5- $\mu\text{m}$ , and (d) 0.8- $\mu\text{m}$  + 10- $\mu\text{m}$  on a hemocytometer.

### On-chip Jurkat cell washing

After studying the critical particle size for efficient particle washing, on-chip Jurkat cells washing was conducted as shown in Figure 5. The blockage ratio of the Jurkat cells was  $\alpha = 0.4$ , which exceeds the critical blockage ratio, thus Jurkat cells should have sufficient migration speed to transfer to the washing medium. Like the particles in viscoelastic fluid, Jurkat cells experience an elastic force which points to the Newtonian fluid and a drag force  $F_D$  acting in the opposite direction. As the average diameter of Jurkat cells is 15  $\mu\text{m}$ , the elastic force is dominant, and the cells start to migrate laterally at the position of 1.5 mm,

Figure 5 (a) (Jurkat cells are labelled by blue circles). At the outlet, almost all Jurkat cells are transferred from the viscoelastic fluid to the Newtonian fluid, and exit at outlet 2. The medium from inlet 1 and the two outlets were both collected, Figure 5 (b). The viscoelastic fluid containing cells (cell culture medium + PEO) and the Newtonian fluid (PBS) were injected from inlet 1 and inlet 2, respectively. In this experiment, the flow rate ratio of sample and sheath flow is 1:3, and the width ratio of the two outlets is 1:2. As the fluid flow in this channel is laminar, the cell culture medium containing PEO at inlet 1 will exit at outlet 1, while the PBS medium at inlet 2 will flow into both outlets, and the cell culture medium will be diluted further. This phenomenon can be seen from the change in the colour of the medium, which becomes lighter at outlet 1 compared with the colour at inlet 1. However, Jurkat cells do not remain in the original cell culture medium, but migrate into the PBS medium and flow out from outlet 2. The hemocytometer test indicated that Jurkat cells in inlet 1 have been successfully transferred to outlet 2, Figure 5 (c). Flow cytometric data [Figure 5 (d)] displays a forward scatter (FSC-A: relative event size) and a side scatter (SSC-A: relative cell surface and intracellular complexity), validating the washing performance of this technique. Figure 5(d) shows the events number in the two outlets, the dots inside the red gating area indicate the Jurkat cells (231 in outlet 1, 2958 in outlet 2). The recovery rate for Jurkat cell on-chip washing was 92.8%. The cells' viability before and after solution exchange experiments were tested using 7AAD method. The cells' viability before experiment is about 96.9%. The viability of cells from inlet after 2h is about 96.5%, while the viability of cells from outlet after PEO exposure is about 91%. It means that PEO has little effect on the cells' viability.

In the co-flow of viscoelastic fluid and Newtonian fluid, diffusion is inevitable. Particles can migrate across the boundary of the two fluids when the velocity of the migrating particles is higher than the speed of diffusion for the PEO medium. Each substance in the fluid has a

characteristic absorption spectrum. Therefore, according to the absorption spectrum, the substances in the fluid and if there are any contaminants can be determined. The heights of the peaks vary according to the concentration of the fluid. Scanning spectrophotometry was used to assess the diffusion extent of the co-flow. The absorbance spectra of the media from the two inlets and the two outlets were tested, Figure 6. The medium in inlet 2 is PBS and has minimal absorbance within the tested wavelength range, thus its absorbance spectrum is relatively flat. In contrast, the absorbance spectrum of the original cell culture medium in inlet 1 has two major peaks. These peaks show a reduction in the medium from outlet 1, indicating that the cell culture medium in outlet 1 was partially diluted by PBS. Moreover, the medium from outlet 2 displays a slight increase in absorbance compared to PBS indicating diffusion of the cell culture medium may have occurred. The relationship between absorbance and concentration of an absorbing species is linear. One of the molecules in the culture medium has the highest absorbance. The absorbance of this molecule in inlet 1 is about 0.175, while the absorbance of this molecule in outlet 2 (target washing outlet) is about 0.04. It means that the concentration of this molecule in culture medium has dropped by about 77% in the new Newtonian fluid after washing. It's indicated that little culture medium diffused to the new medium.

Moreover, the 10nm and 100nm size molecule's Peclet numbers were calculated as  $3.84 \times 10^5$  and  $3.84 \times 10^6$ , respectively; and migration distance as 3.4 $\mu\text{m}$  and 1.1 $\mu\text{m}$ , respectively. The calculated details are also included in the supplementary information. The calculated results indicate neglectable diffusion. However, in the actual experiments, this migration distance may be larger according to the Scanning spectrophotometry test.

Nevertheless, this spectra data reveal that diffusion of the co-flow is minimal, confirming efficient cell washing from culture medium to PBS medium.

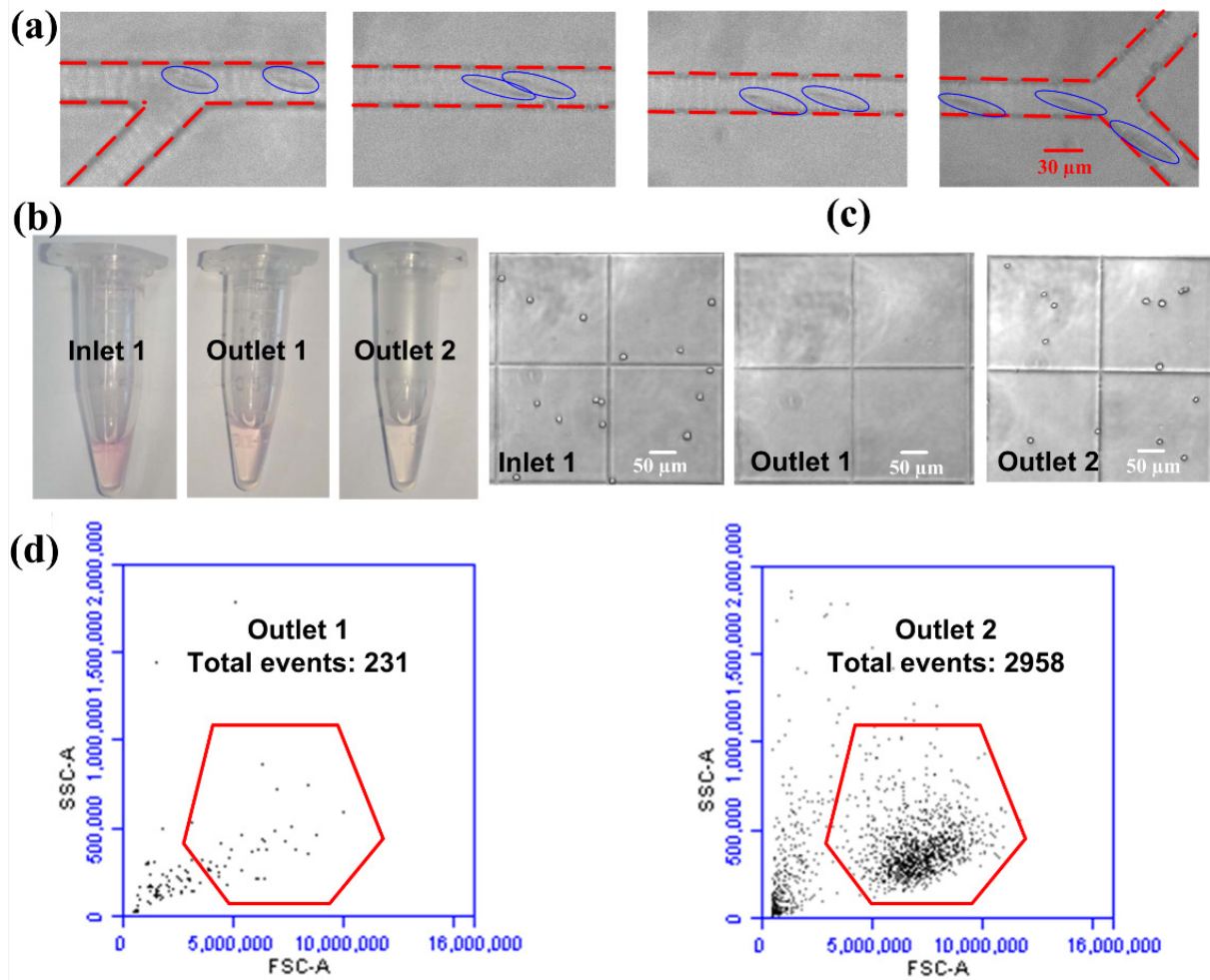


Figure 5: On-chip Jurkat cells washing. (a) Jurkat cell migration processes at inlet, 1.5 mm from the inlet, 3mm from the inlet, and the outlet. (b) The original Jurkat cell culture medium and the mediums exit from outlet 1 and outlet 2 after cell washing process. (c) Hemocytometer images of the inlet and the two outlets after cell washing. (d) Flow cytometer results of the two outlets.

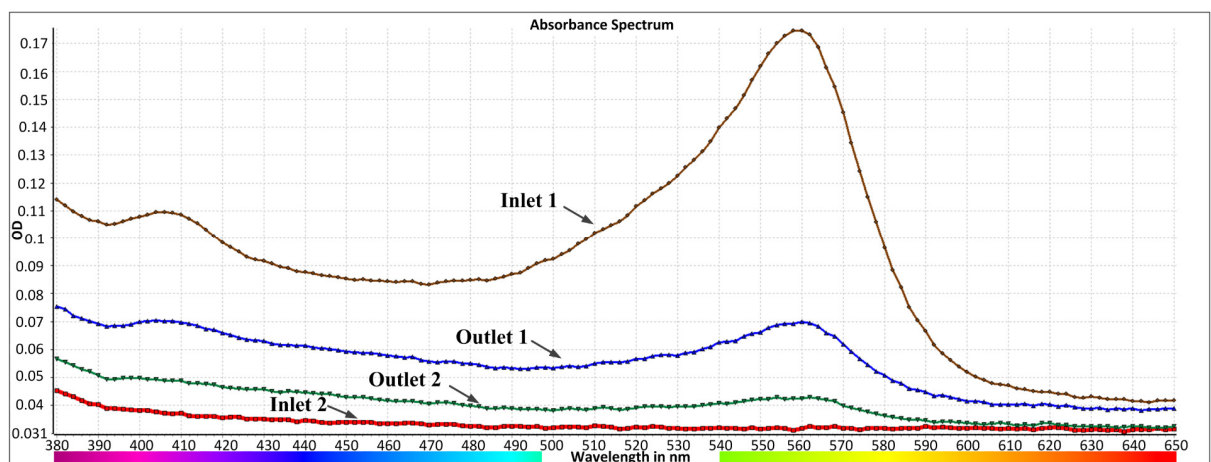


Figure 6: The absorbance spectra of the mediums from the two inlets and the two outlets tested by plate reader.

### **Concluding remarks**

In summary, we demonstrated on-chip washing of microparticles and cells using co-flow of viscoelastic fluid and Newtonian fluid. By simply adding a biocompatible polymer into the native biofluids, continuous on-chip particle and cell washing can be realized with a high recovery rate. After the investigations on the size-dependent particle lateral migration, the critical particle blockage ratio for efficient washing was determined as 0.08. Cell washing performance was verified by hemocytometry and flow cytometry. According to the absorbance spectra of the mediums from the two inlets and the two outlets, diffusion of the co-flow is negligible, indicating efficient cell washing from culture medium to PBS medium. The reported technique can be performed in a simple straight channel, without any external force fields. The technique is a more efficient alternative for tedious conventional medium exchange and washing methods. It has also huge potential to allow direct processing of various biofluids, and holds numerous biomedical applications.

## **Acknowledgements**

This work is supported by a University of Wollongong - China Scholarship Council joint scholarship and the Natural Science Foundation of Jiangsu Province (BK20170839). This work was performed in part at the Queensland Node of the Australian National Fabrication Facility, a company established under the National Collaborative Research Infrastructure Strategy to provide nano and micro-fabrication facilities for Australia's researchers. S. H. Tan gratefully acknowledges the support of the Australian Research Council (ARC) Discovery Early Career Researcher Award (DECRA) DE170100600.

The authors have declared no conflict of interest.

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