Mixing Enhancement in a Lamination Micro Mixer for Cell Sorting

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Abstract

Lagrangian particle tracking simulation is performed to investigate the motion and mixing of cells and magnetic beads suspended in a buffer fluid flow introduced into a lamination micro mixer. The mixing is enhanced, under the condition of low Reynolds number (*Re*) by spitting and recombining fluids. Performance of the mixing is evaluated through visualization of particle distribution and a proposed quantitative analysis. The distribution of magnetic beads and cells on cross sectional views in the streamwise direction shows that at *Re* = 1, periodic unmixed regions of accumulating particles are observed, and two remedies tried are found effective for improving the mixing. With increasing *Re*, the influence of secondary flow considerably improves the mixing. With an alternating rotation mixer proposed, the unmixed regions is avoided even at *Re* = 1, and the mixing is improved by about 48%.

Keywords: Micro mixer, Cell sorting, Magnetic beads.

1. Introduction

With growing up of the demand for treatment strategies, scientists from many fields have much paid attention in the researches of biochemical assays, such as regenerative medicine, which offers the alternative strategy for disease treatments and repairing organs. To do such research, target cells need to be effectively separated from bio-fluidic mixture. Among a number of cell sorting methods, Magnetic Activated Cell Sorting system (MACS system) is an efficient method, and gives low operation cost.

Concept of MAC system is explained by following procedure. Firstly, fluid of micro magnetic beads, which are coated with ligand or antibody reacting with only with target cells, are mixed with bio-fluidic mixture, as shown in Fig. 1. Afterward, both are imposed into magnetic fields. Only target cells attached to magnetic beads could be trapped by the magnetic force. Consequently, the target cells are selectively separated from the other cells.

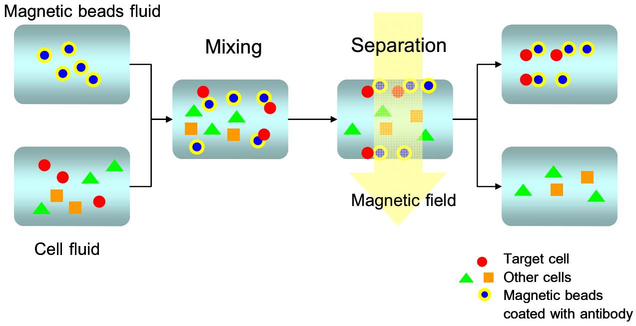


Fig. 1 Schematics of magnetic activated cell sorting system.

With the use of micro fluidic processes, which give high ratio of surface to volume in fluids, we can obtain higher accuracy of product, and reduce a large amount of cell samples and operation cost. However, due to inherent flow of very low Reynolds numbers ($Re \sim O(1)$) in micro scale devices, efficient mixing becomes extremely difficult to be promoted in compact length.

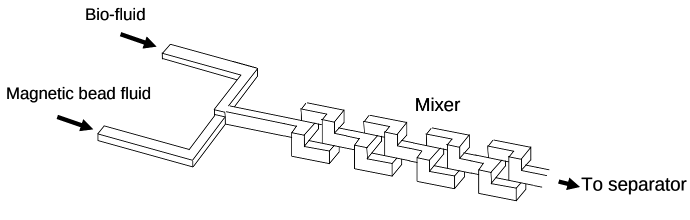
To date, many kinds of micromixers have been proposed and fabricated for overcoming any difficulties of mixing at low Reynolds number. Usually, those can be classified into two categories; active and passive micromixers. For complete mixing, active micromixers reduce the length of the channel dramatically through the use of various kinds of the external forces, such as magnetic force [1], dielectrophoresis force [2], and pressure disturbance [3]. Although external forces can be suitably adjusted when the operating conditions are changed, external force devices are quite difficult to fabricate and to integrate with the other micro components. On the other hand, passive micromixers use the structure of the microchannel to promote the efficient mixing at a suitable flow condition without the use of external forces. By increasing the number of interfaces between different fluids, passive micromixers have been able to use the stretching and folding of interfaces induced by chaotic advection [4, 5] as well as lamination of interfaces [6, 7, 8] as mixing mechanism. Lamination mixers, so-called split-and-recombine (SAR) mixers, increase interfaces exponentially by laminating interface continually along the channel. After passing a unit of mixer, the number of fluid layers increases to 2^{n+1}-1. Due to micro scale device, the evaluation of the mixing in experiment is difficult to assess. Most of the researches use the change of color dye to qualify the mixing of two fluids, however, mixing in transverse direction is not known. Other conventional method is to use the concentration of particles on the cross sections, e.g., intensity of segregation which relies on the sizes of the sample, for quantifying the degree of the mixing [e.g., 6, 9, 10].

The goals of our work are to evaluate and to improve the mixing performance the passive micromixers used for bio-fluidic processes with magnetic particles. In this study, we numerically demonstrated and enhanced the mixing in the three-layer lamination micro mixer proposed by Tan et al. [6].

2. Split-and-recombine micromixer

We consider a passive micromixer proposed by Tan et al. [6]. This micromixer is composed of three-layer structure, as shown in Fig.2. One unit of the micromixer is 800 μm long (L_x), and has a constant cross-sectional area of 200×200 μm^2 ($L_y \times L_z$). Concept of the mixing is to split the flow of two fluid layers perpendicularly to the interface, which is introduced in two separate channels, and then to merge them downstream. Theoretically, if flow is laminar, after passing a unit of a mixer, the thickness of interface between different fluid layers will be reduced into a half, i.e., thickness of fluid layer $\propto 2^n$, where n is number of mixer units. Therefore, mixing becomes 4^{n-1} times faster. Whereas, an exponential decrease of lamellae thickness is obtained, the increase in total pressure drop, Δp, is linear. In addition, without any abrupt change in cross-sectional area, bio-molecules in the fluid is not subjected to large shear stress, thus no damage of cells is expected. Since specific density of the magnetic beads is much larger than unity, the sedimentation loss should be concerned. In this micro mixer using three separate layers of flow passages, fluids are rotated 180 degrees, resulting in low sedimentation [6].

(a)



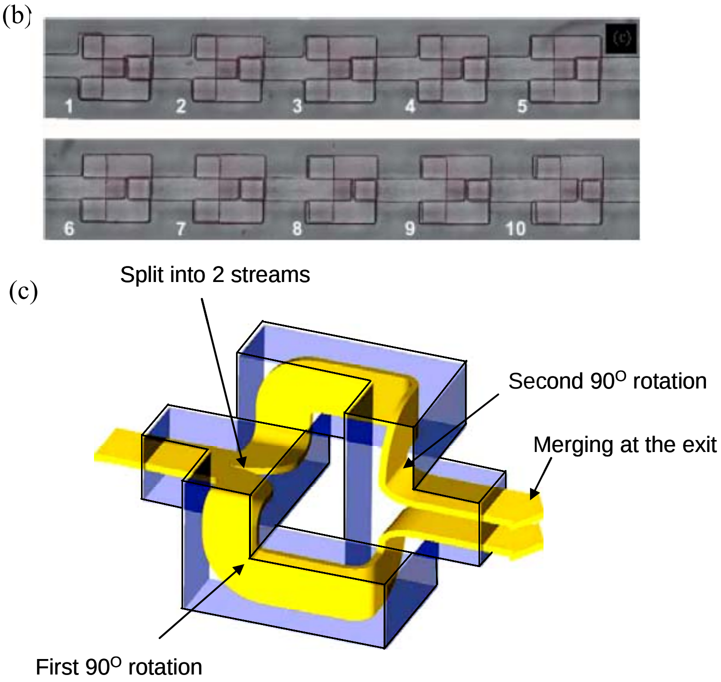


Fig. 2 Schematics of the lamination micro mixer proposed by Tan et al. [6]: (a) a serial micro mixer; (b) mixer with 10 units; (c) a unit of the micro mixer.

3. Numerical procedure

The numerical simulations are used for analyzing the mixing of magnetic beads and cells in the steady laminar flow of micro mixer. . The velocity of fluid in the flow passage is obtained by computing the continuity and Navier-Stokes equations. The incompressible flow of Newtonian fluids is computed in spatially periodic domain by using the finite difference method on a staggered grid system. The computational domain is $8\delta \times 6\delta \times 6\delta$ (x, y, z), where δ is a half width of flow passage, and the numbers of computational grids are $96 \times 72 \times 72$ in the streamwise (x), spanwise (y), and normalwise (z) directions, respectively. The bulk Reynolds number is defined as $Re_b = U_b L_z/\nu$, where U_b is a bulk mean velocity of fluid, L_z is the width of channel, and ν is kinematic viscosity of water. We have used the immersed boundary method (see e.g., [11]) to compute the force acting from wall or obstacles onto fluid. The time integration method is used for the continuity and Navier-Stokes equations based on a fractional-step method. The 3rd order Runge-Kutta and Crank-Nicolson methods are applied to convective and diffusion terms, respectively.

For indicating the mechanisms of mixing, the one-way coupling Lagrangian particle tracking simulations are performed on the motion of the magnetic beads and cells in the mixer. We assume, for simplicity, that both beads and cells are rigid spheres of 1 μm in diameter. The density of beads and cells are ρ_p = 1500 and 1000 kg/m^3, respectively. The relaxation time of beads and cells, $\tau_p = d^2_p S/(18\nu)$ (where $S = \rho_p / \rho_f$ is the density of particle to fluid) is on order of $\tau_p \sim 1 \times 10^{-7}$. Due to very small particle size (diameter to length-scale << 1), the other force terms in equation of particle motion [12] are neglected. Thus, the simplified particle equation of motion reads,

$$\frac{d\vec{u}_{\mathrm{p}}}{dt} = \frac{1}{\tau_{\mathrm{p}}}\left(\vec{u}_{\mathrm{f}} - \vec{u}_{\mathrm{p}}\right) \qquad (1)$$

where $\vec{u}_{\mathrm{f}}$ and $\vec{u}_{\mathrm{p}}$ are fluid and particle velocities, respectively. The fluid velocity at the particle position is interpolated from the grids by using the tri-linear interpolation scheme. The time integration for the velocity and position of particles is done by the Crank-Nicolson scheme.

In the simulations, the effects of the Brownian motion and the interaction collisions are neglected. As shown in Fig. 3a, ten thousand particles of magnetic beds and cells with uniform spacing are initially introduced from the lower and upper halves of the inlet in unit 1, respectively. The computational time for tracking particles approximately is ~ 200 mixer units.

4. Results and discussions

4.1 Qualitative analysis

To demonstrate chaotic particle motion in the transverse direction, we use the standard dynamical systems diagnostic known as the Poincaré section. To construct Poincaré sections, the positions of particles are tracked as they advect down the conduit, and their location in the cross sectional views in the flow direction is plotted after each mixer unit. By using this Poincaré section, we can determine the number of mixer units required for achieving the efficient mixing. Since all cross-sectional areas of the mixer are a square and initial positions of magnetic bead fluid and bio-fluid are equally located on lower and upper halves, the distributions of the positions of cells and beads on the cross sections are the mirror of each other. In addition, the effect of sedimentation of our beads does not much influence in this mixer [6]. Therefore, to clearly present the development of the mixing, we plot only the distribution of beads. Initial positions of particles are shown in Fig. 3(a).

Simulation results showing cross sectional views of the exit of unit 1, 2 and 9 are given in Fig 3(b) – 3(d). The number of stacked layers increases double when fluids pass through a mixer unit. However, the splitting-and-recombining principle is imperfectly realized. Due to internal friction from many sharp bends, the stacked layers do not preserve ideal lamination pattern, i.e., non-uniform and distorted thickness. This leads the number of required mixer units for completed mixing not to follow the prediction. Moreover, at the downstream units, four regions of accumulating particles, which have same species, are observed near the center of the flow passage. Since we deal with the spatial chaotic mixing, the fluids should often mix together near the center more than the other regions. Therefore, these unmixed regions will much influence the overall performance of the mixing in this micro mixer.

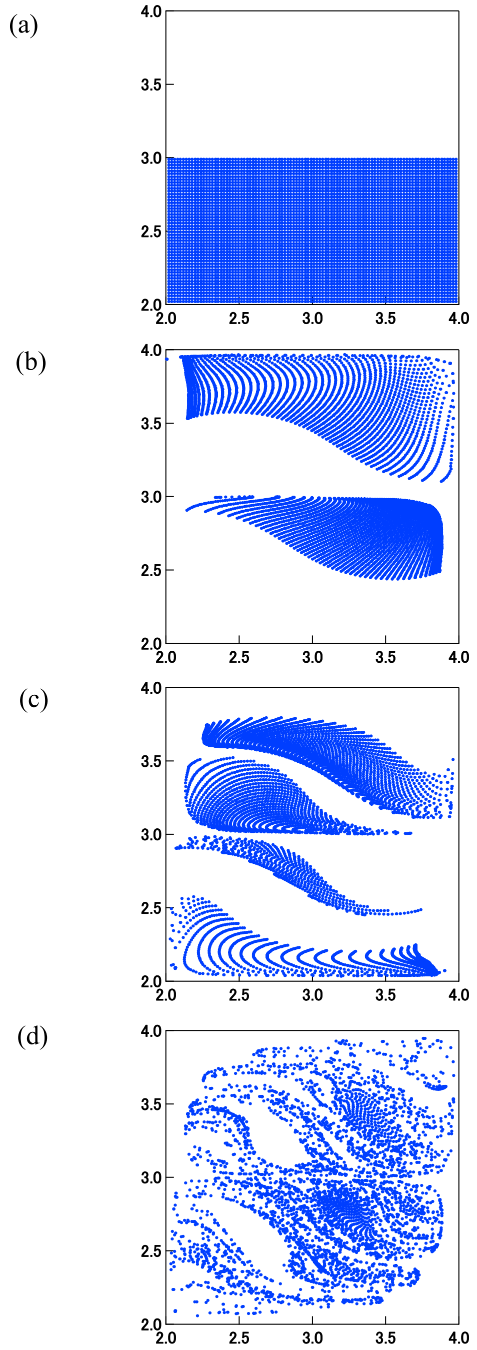


Fig. 3 Distribution of beads at *Re* = 1: (a) initial positions at the inlet; (b) exit plane of unit 1; (c) unit 2; (d) unit 9.

Of more significance is the behavior of these unmixed regions in this micro mixer. To understand this, we plot the positions of particles, which locate only in a lower half (quadrant 3), as shown in Fig. 4 (a) - (d). It is found that in every 4 units of mixer, some of the particles still accumulate together and they come back to the quadrant 3 again. Furthermore, we investigated the motion of particles in this accumulating particles area, and found that a particle still periodically locates in the same coordinate (y-z coordinates), and that the rest particles always orbit around this coordinate.

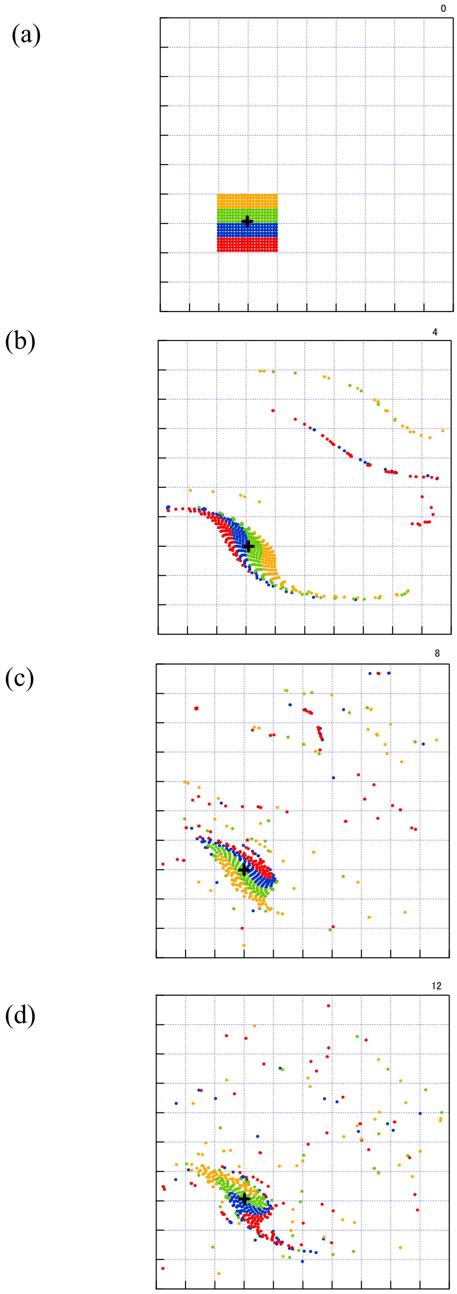


Fig. 4 Positions of an unmixed region on the cross sectional plane: (a) initial positions; (b) – (d) the exit of unit 4, 8, and 12.

The other unmixed regions are also investigated by this procedure. Figure 5 plots the mapping positions of periodic positions in unmixed regions. This shows that the motion of periodic positions on the exit planes is $(y, z) = \Phi_{n+4}(\mathbf{Y}, \mathbf{Z})$, where n is the mixer unit. This means that these unmixed regions form stream tubes and the mixing is prevents by these periodic unmixed regions or periodic islands. Without effects of diffusion and gravitation, the particles will not move away from these periodic unmixed regions, called Kolmogorov–Arnold–Moser (KAM) boundary. A similar case also was found in the partitioned-pipe mixer [9].

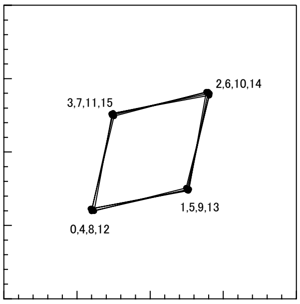


Fig. 5 The mapping of positions (cross mark in Fig.4) locating in an unmixed region for every 4 unit of mixer

Increase of *Re*

Figure 6(a) - (b) show the mixing with the Reynolds numbers at 24 and 66, respectively. When *Re* = 24, the unmixed regions of accumulating particles move close to the wall, and the mixing around the center of conduit is better. However, effect of secondary flow is not strong enough to destroy these unmixed regions. When *Re* = 66, the positions of particles significantly disperse. In addition, particles much mix together and any unmixed regions are not observed.

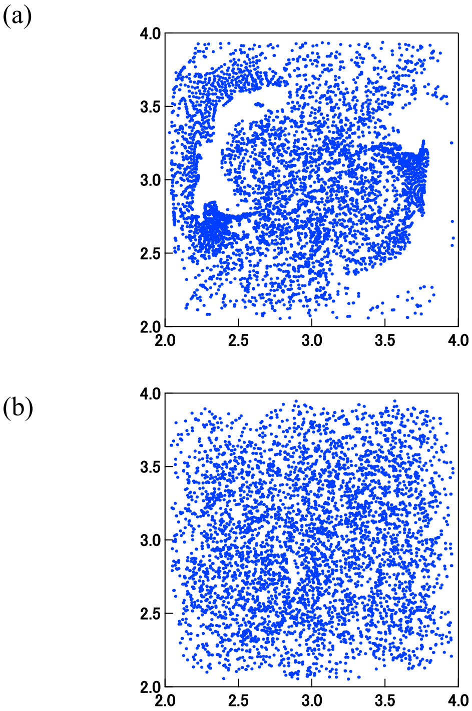


Fig. 6 Distribution of beads on the exit plane of unit 9 in the designed mixer: (a) designed *Re* = 24; (b) at *Re* = 66.

4.2 Quantitative analysis

In this study, we also propose a quantitative analysis to approximate the size of unmixed regions and to evaluate the degree of the lamination mixing. Basically, this method is to measure the spacing between the different species of particles, i.e., the nearest distance between magnetic beads and cells on the cross-sectional area. An advantage of this method over the conventional measures, e.g., the degree of intensity, is that our proposed method does not rely on the number of sub areas or sample sizes. The equation writes

$$r_{\min,\mathrm{i}} = \min\left|\vec{x}_{\mathrm{bead,i}} - \vec{x}_{\mathrm{cell,j}}\right|, \qquad (2)$$

where $\vec{x}_{\mathrm{bead,i}}$ and $\vec{x}_{\mathrm{cell,j}}$ are the positions of bead and cell, respectively. Moreover, the radius of the unmixed region (island) is quantified by selecting the maximum value of $r_{\min,\mathrm{i}}$, i.e.,

$$\max r_{\min} = \max\left[r_{\min,\mathrm{i}}\right]. \qquad (3)$$

Moreover, the mixing performance is evaluated by computing the average thickness of fluid layers. To consider the effective mixing, the average value of $r_{\min,i}$ is weighted by velocity factor, i.e.,

$$\left\langle r_{\min}\right\rangle = \frac{1}{N_{\mathrm{p}}}\sum_{\mathrm{i}=1}^{N_{\mathrm{p}}}\frac{u_{\mathrm{p,i}}}{U_{\mathrm{b}}} r_{\min,\mathrm{i}}, \qquad (4)$$

where N_{p} is the number of beads on each Poincaré section, and $u_{\mathrm{p,i}}$ is the velocity of beads in the streamwise direction. Thus, the average thickness of fluid layers is equal to $2\left\langle r_{\min}\right\rangle$.

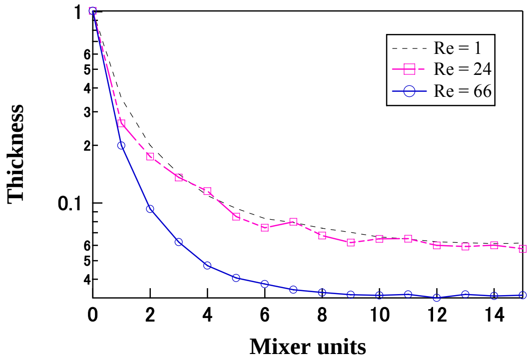


Fig. 7 Average thickness of fluid layer in various Reynolds numbers.

With Eq. (3), the size of an unmixed region proximately is 28 μm in diameter. Figure 7 plots the average thickness of fluid layers, which are computed by Eq. (4). Through the nearest distance method, the average thickness tends to give a constant value of 4.4 μm. Accordingly, increasing number of mixer units more than 15 units will not much improve the mixing.

With increasing the *Re* = 66, the thickness of fluid layers is much decrease. Therefore, beads and cells are much mixed together. Based on thickness of *Re* = 1, the influence of secondary flow of *Re* = 24 and 66, improve the mixing by about 7% and 47%, respectively

5 Improved design

With concerning high shear stress due to high flow rate, we proposed a lamination micro mixer to enhance the mixing at *Re* = 1. To promote chaotic flow, we manipulate flow with modifying the geometry of the mixer. As shown in Fig. 8, we reverse the direction of flow at the branches of channel in every 2 units. This causes the fluids not to fold at the same positions (y – z coordinates). Consequently, the streamlines of fluids are more across, and do not confine to the stream surface.

Figure 9 (a) shows the distribution of particles in our modified mixer at *Re* = 1. The modified mixer achieves the efficient mixing in 9 units, and the unmixed region is not observed. To compare the periodic point between the original design and modified mixer, we plot the mapping positions of same particle number. As shown in Fig. 9 (b), the periodic points found in the designed mixer [6] randomly locate in the mapping position of the modified mixer. This shows that no periodic point takes place in our modified mixer, that mixing is much improved.

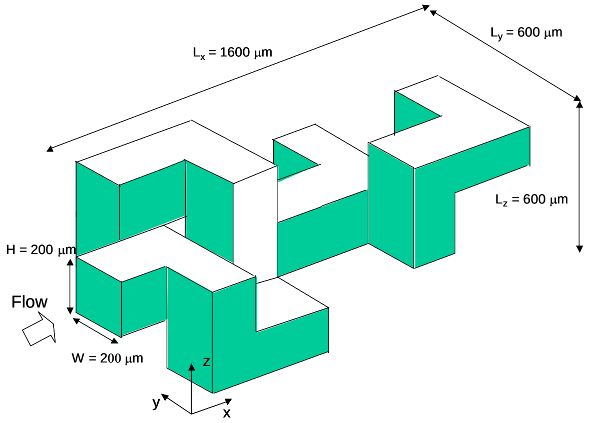


Fig. 8 Schematics of modified micro mixer alternating rotation.

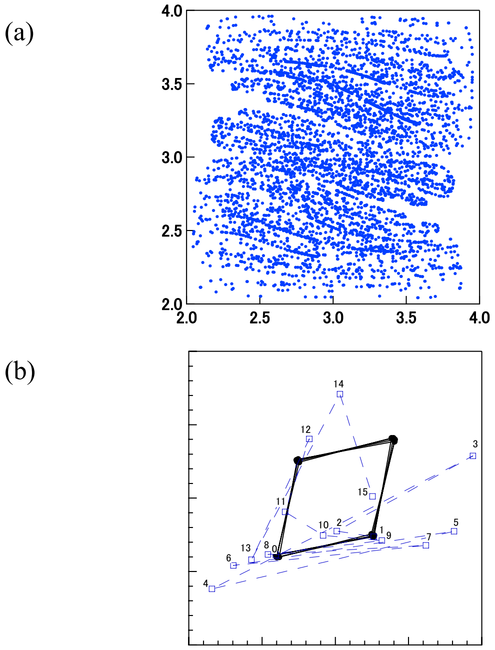


Fig. 9 Mixing in the modified mixer at *Re* = 1: (a) distribution of particle on the exit plane of Unit 9; (b) Comparison of mapping position obtaining from the designed mixer (solid line) and the modified mixer (dotted line).

With the modified mixer alternating rotation, the unmixed island is not observed. In this modified mixer, fluids are folded in the different positions; hence their streamlines more cross together. The average thickness is plot in Fig. 10. With our proposed measure, the performance is improved around 48%.

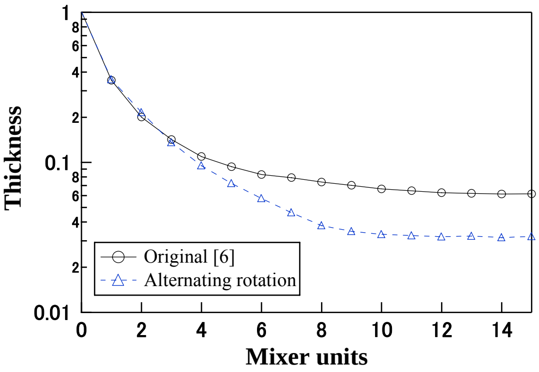


Fig. 10 Average thickness of fluid layers in a proposed mixer at $Re = 1$.

6 Conclusions

Performance of the mixing of magnetic beads and cells in the serial lamination micro mixers for micro magnetic cell sorting system was evaluated through numerical simulations. By plotting the distribution of particles on the exit plane of each mixer unit, it was found that in the designed micro mixer, which proposed by Tan et al. [6], there were some unmixed islands, where a large number of same species of particles accumulated together in a long length of the mixer. Due to these unmixed islands, increasing the number of mixer units more than 15 units did not much improve the mixing. These unmixed islands appeared periodically and formed by the effect of streamlines confining to stream surface. This causes the particles in these islands not to move out from these regions even in long length mixer, resulting in prevention of mixing.

We proposed a method for quantitative analysis of mixing, named as the nearest distance method. This method provides a reliable measure, which is consistence with the scale of segregation, but it does not rely on the sample size. When $Re = 1$, unmixed islands occupied about 6% of the cross-section area on the exit plane of unit 15.

Two strategies were examined for destroying the unmixed islands. With influence of secondary flow at $Re = 24$, and 66 mixing is improved about 7%, 25%, respectively. And with a modified mixer alternating rotation, fluids were not folded at the same position. The unmixed island was suppressed, and the mixing is improved about 48%.

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