論 文 内 容 の 要 旨

論文題目 Performance Assessment of Micromixers for Bio-fluidic Processing with Magnetic Beads
（磁性粒子を用いたバイオフルイディック・プロセッシングのためのマイクロ混合器の性能評価に関する研究）

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Introduction

With continuously growing demand for treatment strategies, the researchers in bioengineering and medical fields have paid much attention on regenerative medicine. By using stem cells of patient, we can heal severe illness and repair damaged organs. Doing research of stem cells needs efficient separation processes to specifically separate stem cells from bio-fluidic mixture. Micro magnetic-activated-cell-sorting (MACS) process can give effective selection, high purification of product, and reduction in a large amount of cell sample and operating cost. Performance of MACS process relies on the number of cells attached to magnetic beads, thus mixing of cells and magnetic beads is a crucial process. In the last decade, researchers have proposed and fabricated many kinds of passive and active micromixers, which can hopefully overcome any difficulties of mixing in micro-scale devices. Those can be categorized into active and passive micromixers.

Evaluating the mixing efficiencies is vital to reveal the potentials of the mixers for designing the other components of bio-fluidic processing and also to improve the mixing. Due to micro-scale devices, monitoring the performances becomes more difficult in experiments. In addition, most experimental results are two-dimensional information. This may cause us to lack or to misinterpret some important mechanisms of mixing. With numerical simulations, we can obtain more details of mixing as well as three-dimensional information.

The objectives of this study are to numerically assess and to analyze the performances of a passive and an active micromixers that are used for enhancing the attachment of magnetic particles onto biological molecules suspended in the medium. Two selected mixers are the serpentine micromixer using time-dependent magnetic force [1] and the lamination micromixer [2]. With these mixers, efficient mixing is possible to achieve even at *Re* ~ 1, and cells will not be damaged by operation conditions of the mixers. Consequently, these mixers have high potentials to be used in micro bio-fluidic processes with magnetic beads.

2. Methodology

The stationary flow of Newtonian fluids is computed in spatially periodic domain. Since variables such as concentration of particle on each cross section are not spatially periodic and must be calculated throughout the whole units of a mixer, we conduct the mixing calculations in the mixer units, which are serially connected.

For indicating the mechanisms of mixing, the one-way coupling Lagrangian particle tracking simulations are performed on the motion of the magnetic bead and cell particles in both mixers. The beads and cells are assumed to be rigid spheres of 1 μm in diameter. In addition, the Brownian motion is neglected

Performances of the mixer are evaluated by conventional measures of chaotic system such as the largest Lyapunov exponent and Poincaré section, and also by alternative measures such as the contact ratio, which is the ratio of number of cells attached by beads to total number of cell at the outlet of mixer, and the nearest distance, which is a proposed quantitative measure.

3. Active mixer: serpentine micromixer using time-dependent magnetic actuation

The Idea of mixing is to utilize magnetic forces to move magnetic beads across the streamlines of fluid. With alternating the magnetic-control signal, the beads can be stirred and dispersed in the mixer. The magnetic forces are generated from electrodes, which are embedded in the bottom wall of mixer. A unit of mixer is composed four electrodes and a cycle of magnetic actuation has four phases.

In present simulations, a mixer is composed of 9 units and magnetic forces are applied from unit 4 to 8. Controlling parameters are amplitude and frequency of magnetic actuation. Normalized amplitude is the ratio of maximum velocity due to magnetic force to bulk mean velocity of fluid, α. Normalized frequency denotes as Strouhal number, $Str = f L/(4U)$, where L and U are length of mixer in one unit, and bulk mean velocity of fluid, respectively. The operation conditions of magnetic actuation are in the range of $0.25 \leq Str \leq 2.0$ and $0.39 \leq \alpha \leq 6.19$, and the introducing rate of bead to cell particle is unity.

In two-dimensional simulations, *Re* is equal to 0.0032. From the simulation, we have reached the following conclusions:

3.1 Operating the sequence of magnetic actuation in the direction same as flow gives mixing better than in the opposite direction. With performing the sequence in opposite direction, magnetic force induces beads to move against flow therefore beads move slower than cell particles, which is not influenced by magnetic flow.

3.2 In order to evaluate the efficiency of mixing of beads and cells, the number of cell attached by magnetic to the total number of cells is more suitable measure than the largest Lyapunov exponent (λ). For instance, large amplitude force of $\alpha = 6.19$ when $Str = 1.43$, gives a negative value of λ even if a large number of cell particles is attached by beads.

3.3 Movement of magnetic beads toward the region of cells depends on the relative velocity of beads increased by the magnetic force. With larger amplitude force, beads suddenly get access into the region of cells. When $\alpha \sim 1.0$ and $Str = 1.0$, the, magnetic beads can have high drift to increasingly disperse from upstream to downstream, and also to deeply move into the region of cells, resulting in maximized value of contact ratio. With this condition, the number ratio of bead attaching on a cell is ~ 2:1. In addition, extending more mixer units, which utilize magnetic force gives higher value of contact ratio.

3.4 The number of cells attached to beads increases as the increase of α. The Strouhal number, *Str*, directly affects the dispersion of beads. A large number of dispersion (thus, a higher contact ratio) is attained when $Str = 1.0$ except for the case with very large amplitude of magnetic actuation, where magnetic beads are trapped around the inner edges of electrodes. Even though, the contact ratio is maximized when $\alpha = 6.19$ and $Str = 1.43$, the number ratio of beads attaching on a cell is large (~ 6:1).

In three-dimensional simulation, the Reynolds numbers are in the range of $0.0032 \leq Re \leq 0.048$. From simulations, we found that due to magnetic force in gravity direction magnetic beads were driven toward the bottom wall. In addition, influences of lift forces and the Brownian motion on beads are not strong to push beads up to the region of high velocity of fluid. Consequently, beads sink and move on the bottom wall: the mixing is not good in three-dimensional simulation.

3. Passive mixer: lamination micromixer

Idea of the mixing is to split and to recombine flows. Splitting and recombining flows can reduce the thickness (distance) between layers of two fluids. After passing a unit of mixer, the thickness between different fluid layers will be reduced in half. Ideally, the decrease of the thickness is proportional to 2 power of $-n$, where n is number of mixer units. Mechanisms of the mixing at Re = 1 are clarified through both ideal topological analysis and Poincaré section.

From Poincaré section, thickness of fluid layers distorts and is not uniform. This is due to effect of shear force from many sharp bends in the mixer. In addition, the existence of four periodic unmixed islands, where a large number of same particles accumulate together, is found near the centre of conduit. Particles inside these islands have high velocities. In every four units of mixer, these islands come back to the same position. To quantify performances of this mixer, we measure the scale of segregation and the nearest distance. Results from both mixing indices are consistent each other, and tend to reach the constant values. Accordingly, increasing number of mixer units more than 15 units will not much improve the mixing.

These periodic unmixed islands are occurred because streamlines confine to stream surface, so-called Kolmogorov-Arnold-Moser (KAM tube). This causes particles inside the islands to not move away from the islands. From the nearest distance method, sizes of unmixed islands on the exit plane of unit 15 occupy ~ 6 % of cross section area, and the average thickness of fluid layer is ~ 4.4 μm.

In order to destroy the unmixed islands, we have performed three strategies, i.e., increasing Reynolds number, using uneven cross-section mixer, and alternating rotation of flow. First results show that when Re = 24, effect of secondary flow dominates on the motion of particles. Regions of accumulating particles move close to the wall and the mixing around the centre of conduit is better. From the nearest distance method, performance of mixer is a bit improved, i.e., ~ 6.7 %. Next, in uneven cross-section mixer, the unmixed islands still exist and most particles move in the bigger cross section. Mixing becomes worse and the average thickness is larger when the aspect ratio is smaller. This is because mechanism of the mixing exhibits similar in the original design. Finally, by using the alternating rotation mixer at Re = 1, the unmixed islands found the original design are not observed. With alternating rotation, streamlines are gradually crossed together, hence the periodic unmixed islands do not occur in this mixer. The average thickness is smaller ~ 48%.

5. Conclusion

In two-dimensional simulations of active mixer, mixing of bead and cell particles can be promoted by controlling the amplitude and frequency of magnetic actuation at the same order of fluid flow, i.e. $\alpha \sim 1$ and Str = 1. Due to high drift on the motion of beads, beads can much disperse and deeply get access into the region of cells. When α = 6.19 and Str = 1.43, the value of contact ratio is maximized but the number ratio of bead attaching to a cell is large. In three-dimensional simulations, due to influence of magnetic force in gravity direction, magnetic beads sink on the bottom wall, resulting in bad mixing.

In the passive mixer at *Re* = 1, existence of periodic unmixed islands is observed and in every four units of mixer, these islands will come back to the same positions. A proposed quantitative measure, named the nearest distance method, can use to evaluate the performance of lamination mixer. Three strategies are performed for enhancement of the mixing. When *Re* = 24, effect of secondary flow dominates on the motion of particles, and mixing is improved by ~ 6.7%. With mixer alternating rotation, the unmixed island is not observed even at *Re* = 1, and mixing is improved by ~ 48 %. But with uneven cross section, mixing becomes worse.

Reference

[1] H. Suzuki, C. M. Ho, and N. Kasagi, *J. Microelectromech. Syst.*,13(5), 2004, pp.779-790.

[2] Tan, W.-H., Suzuki, Y., Kasagi, N., Shikazono, N., Furukawa, K., and Ushida, T., *Int. J. JSME*, Ser. C, 48(4), 2005, pp. 425-435.