ABSTRACT
This paper reports an improved method for achieving on-chip parallel sorting of fluorescently labeled bioparticles. We have integrated 8-parallel sheath flow sorters, in which a sample stream is sheathed by two buffer streams to focus the sample stream, into a single chip. Each sorter integrated in the chip successfully sorted fluorescent microspheres and *Escherichia coli* (*E. coli*) cells expressing fluorescent proteins with 10-20 ms of flow switching time and an 85-95% recovery ratio.

KEYWORDS: Microfluidics, Parallel sorting, Multi-layer PDMS, Sol-gel transition

INTRODUCTION
An on-chip sorter is expected to be one of the attractive methods to enrich bioparticles in the field of organelle proteomics because the sorter can separate fluorescently labeled bioparticles with sensitive detection. However, it requires a long time to collect sufficient bioparticles for following analysis. Although a sensitive and high speed sorter to enrich bioparticles was reported [1], it could not collect enough organelles for analysis of the components. We previously reported a parallel sorting system for the rapid collection of bioparticles [2]. Here we report that the performance of parallel sorting has been greatly improved by installing a sheath flow in the sorting system.

THEORY
The principle of the sorting method of our sorter is based on the sol-gel transition of a thermoreversible gelation polymer (TGP) (Figure 1) [1-2]. The TGP is liquid at low temperature and turns to a gel immediately upon heating. Sample and buffer solutions containing TGP are introduced into microchannels. Upon detection of the fluorescence signal from a target, the sol-gel transition of the TGP is induced at a waste channel by heating with infrared laser irradiation. This operation allows a fluorescent target to flow into a collection channel. In an 8-parallel chip, the fluorescence signals...
of targets are detected by 16-channel photomultiplier tubes which are capable of performing independent detection of the target in each channel, and the infrared laser beam heats each waste channel to separate individual targets.

**EXPERIMENTAL**

In our previous study, an 8-parallel sorter separated microbeads with a 40-50% recovery ratio [2]. To improve the recovery ratio, we designed and fabricated an 8-parallel sheath flow chip with lower fluidic resistance. Figure 2-(a, b) shows the design and microphotograph of the 8-parallel sheath flow chip. The chip consists of a glass cover plate and a PDMS block. The PDMS block has a multilayer structure with two inlets and two outlets. The structure makes it possible to form a sheath flow in each 8-parallel channel by introducing sample and buffer solutions from the two inlets. Metal dots, which are evaporated on the glass plate, absorb the laser beam and function as a heater upon sorting. To examine whether sheath flow was formed as expected, we introduced Rhodamine 6G (R6G) solution into inlet 1 for specimen and water into inlet 2 for carrier solution. Figure 2-(c) shows a fluorescence micrograph of sheath flow. R6G solutions ran into the waste channel in a waste mode because the fluidic resistance of the waste channel was lower than that of the collection channel.

![Figure 2. Illustration and micrograph of 8-parallel sheath flow chip](image)

Next, microbeads or *E. coli* cells were sorted. We used 0.5 μm beads with excitation/emission maxima of 505/515 nm or *E. coli* cells expressing GFP. Microbeads or *E. coli* cells were mixed with TGP solutions and introduced into inlet 1 while carrier solutions containing only TGP were introduced into inlet 2.

**RESULTS AND DISCUSSION**

Figure 3 shows the micrographs of microbead sorting. In the waste mode, all microbeads sandwiched by carrier flows ran into waste channels. In the collection mode, microbeads were detected by photomultiplier tubes and sorted into collection channels. *E. coli* cells were also successfully sorted. Figure 4 shows the recovery ratio of microbeads or *E. coli* cells as a function of irradiation time of the laser. The sorter could separate microbeads and *E. coli* cells with an 85-95% recovery ratio, and an irradiation time of 10 ms was enough for accurate separation.
Figure 3. (a) Fluorescence micrograph of channels in waste mode. Exposure time was 16.7 s. (b) Sequential micrographs of bead sorting. Exposure time was 33 ms.

Figure 4. Relation between recovery ratio and laser-on time. Recovery ratio is calculated as the number of target particles recovered in the collection channels divided by the total number of fluorescent particles detected.

CONCLUSIONS

We greatly improved the performance of the parallel sorting system by reducing fluidic resistance and introducing sheath flow. The 8-parallel sorter is a promising device for rapid collection of bioparticles. We will separate organelles such as mitochondria using this parallel sorter in the near future.

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