PARTICLE MEASUREMENT BY USING TWISTED MICRO SHEATH FLOW CELL

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ABSTRACT

We have been developing a compact and low-cost flow cytometer available for continuous monitoring of bacteria in drinking water. Key elements of the flow cytometer are a sheath flow cell to make 3-dimensional (3D) hydrodynamic focusing flow and a pretreatment part to dye bacteria. To integrate those elements and fabricate simply, we used an imprint fabrication process of Polydimetylsiloxane (PDMS), and in order to realize 3D focusing flow, we proposed a twisted micro-channel structure by applying the unique elastic property of PDMS. An observation system with a photomultiplier tube (PMT) was constructed and an experiment for checking the measurement ability was carried out by using standard fluorescent particles. Consequently, most of the fluorescent signal from each particle holds the value within the narrow range, which means that the twisted micro sheath flow cell can realize 3D focusing flow and is available for the flow cytometric analysis.

KEYWORDS:flow cytometer, 3D hydrodynamic focusing flow, sheath flow, water monitor

INTRODUCTION

Flow cytometers have been widely used to characterize biological cells statistically. In biological application, they are required to classify and select just target cells among many kinds of cell's population with high accuracy. Key elements of those systems are a sheath flow cell to realize 3D focusing flow and a pretreatment part to dye those cells. In general, the integrated systems with the elements become complicated and expensive. As a result, the application field might be limited. Thus, we have been developing a compact and low-cost flow cytometer and, as an application, we chose the monitoring system of bacteria in drinking water as shown in Figure 1. To integrate those elements and fabricate simply, we used an imprint fabrication process of PDMS, and in order to realize 3D focusing flow, we proposed a twisted micro-channel structure by applying the unique property of PDMS, that is, "easily bent and twisted"[1] [2]. An observation system with a photomultiplier tube (PMT) was constructed and an experiment was carried out to check the measurement ability. In this paper, we introduce the twisted micro sheath flow cell and demonstrate the ability of particle measurement.

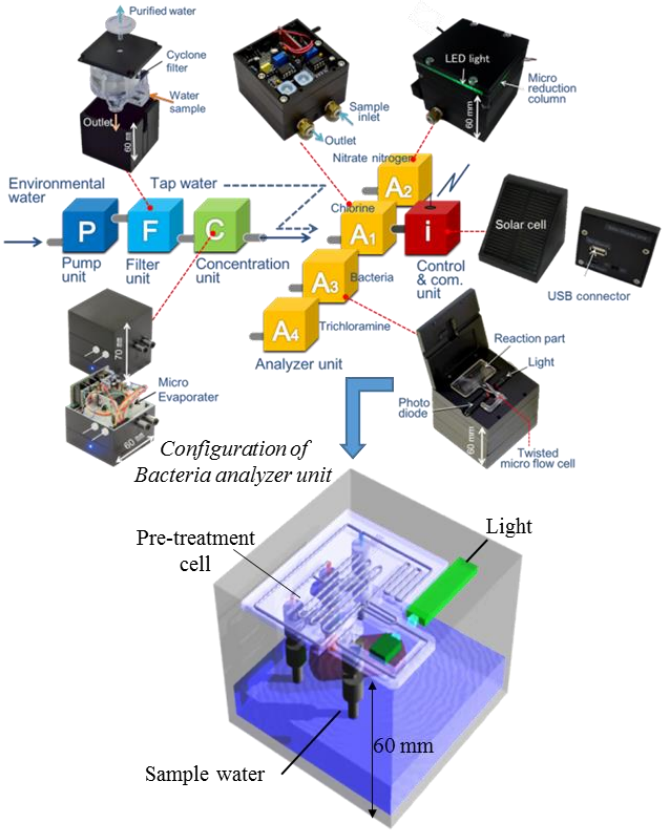

Figure 1. Unit Modular system for water monitoring

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Figure 1 shows the concept of unit modular system for water monitoring and the configuration of the bacteria analyzer unit. The cubic unit is 60 mm on a side. On top of the unit, the micro sheath flow cell with pretreatment cell is installed. More detail structure is shown in Figure 2. A sample water is introduced from the upper inlet in the figure. A reagent is introduced from the lower inlet. A sheath fluid is introduced from the port located in the left side and flows into both of branched channels. The sample water and the reagent are mixed and reacted in the

following meandering channel (pretreatment cell). The mixed fluid enters the first junction and is sandwiched by the sheath fluids from both channels. In the downstream, the fluid flow again enters the second junction and is enveloped by the sheath fluids from both channels. In order to realize 3D hydrodynamic focusing flow, the micro-channel between the two junction is twisted by 90 degrees as indicated in the figure. Here, as shown in Figure 3, we fabricated a prototype of micro flow cell to check the performance. The mold for the channel was made of SU-8 photoresist. Then, the base material of PDMS was poured on the top of the mold. Then, the channel structure was transferred. After that, the cover plate made of PDMS was bonded over the channel. Finally, the channel was twisted as shown in Figure 3(b). Figure 4 shows a result of numerical simulation that is the trajectory of particles in the twisted micro-channel. It was observed that the particles were focused into the center of the channel after second junction. In addition, the histogram of transit time of each particle at the position after the first junction and that after the second junction is shown in the figure. The histogram after the second junction becomes narrower. It is expected that 3D focusing flow can be realized.

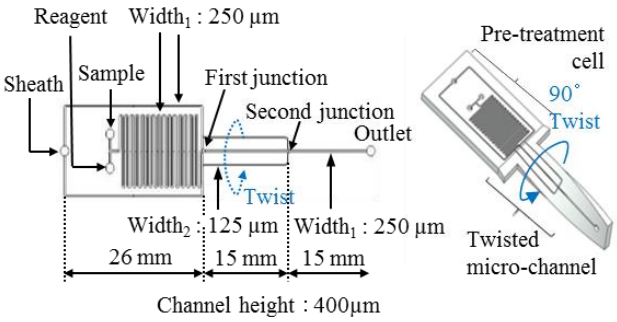
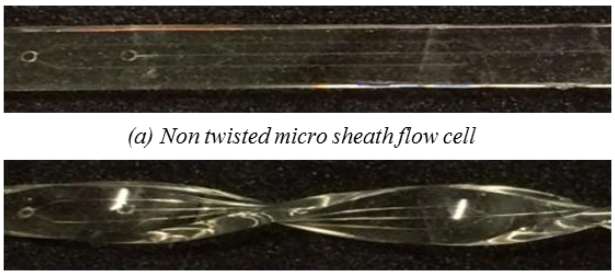


Figure 2. Configuration of twisted micro flow cell with pre-treatment cell



(a) Non twisted micro sheath flow cell

(b) Twisted micro sheath flow cell

Figure 3. Fabricated twisted micro

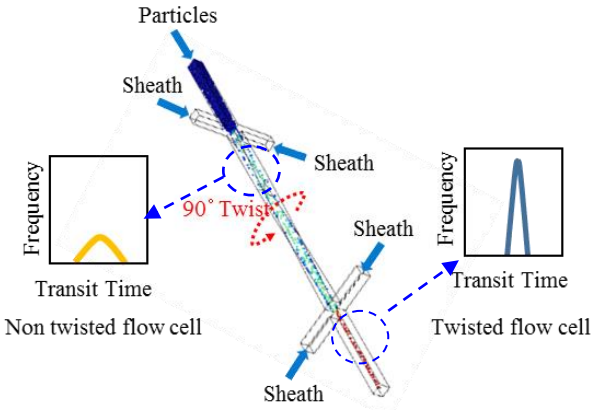


Figure 4. Twisted micro-channel Model and results

EXPERIMENT

We checked the forming of 3D focusing flow by using the standard fluorescent particles. Here, an ethanol was used as the sheath liquid, and a solution including fluorescent particles (φ6μm, polystyrene) was used as the sample liquid. Each flow rate of the sheath liquid and the sample liquid was set to be 2.1μl/sec and 0.017μl/sec respectively. Therefore, the mean flow velocity of observation point was calculated to be 21mm/sec. Figure 5 shows a schematic of a counting system of particles with a photomultiplier tube (PMT). As a illumination, laser light(465 nm) was used, and the fluorescent light from each particle was collected by a microscopic lens, dichroic mirror and a long pass filer. The experiment was done by comparing the signal before twisting the channel with that after twisting it.

RESULTS AND DISCUSSION

Figure 6 shows signal of particles (6(a)), histogram of signal (6(b)) and the histogram of full width at half maximum (FWHM) at each peak (6(c)). In case of the non twisted micro sheath flow cell, the counts of signal are distributed over the wide rage. In case of the twisted micro sheath flow cell, most of the fluorescent signal from each particle holds the value within the narrow range around 0.15V. Moreover, FWHM of peak at the twisted micro sheath flow cell is smaller than that at the non twisted one. This means the velocity of each particle in the twisted sheath flow cell is almost constant. Compared with coefficient of variation (CV) of FWHM, it decreases from 82% to15% when the micro-channel is twisted as was predicted by the simulation (Figure 4). According to the above results, the twisted micro sheath flow cell can realize 3D focusing flow, and is available for flow-cytometric analysis.

CONCLUSION

In order to realize 3D focusing flow in the chip fabricated by using an imprint process, we proposed a twisted micro-channel structure by applying the unique elastic property of PDMS. The experiment to check the performance with using standard fluorescent particles were carried out. Most of the fluorescent signal from each particle holds the value within the narrow range, which means that the proposed flow cell can realize 3D focusing flow and is available for flow-cytometric analysis.

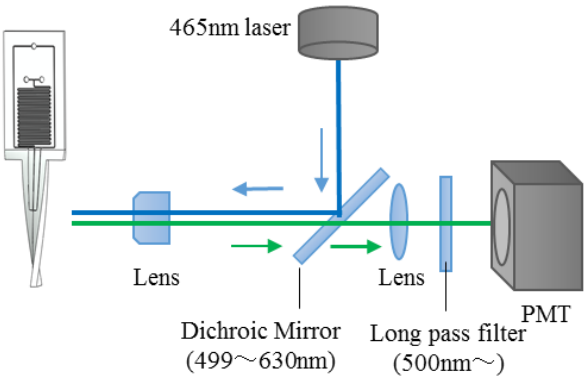
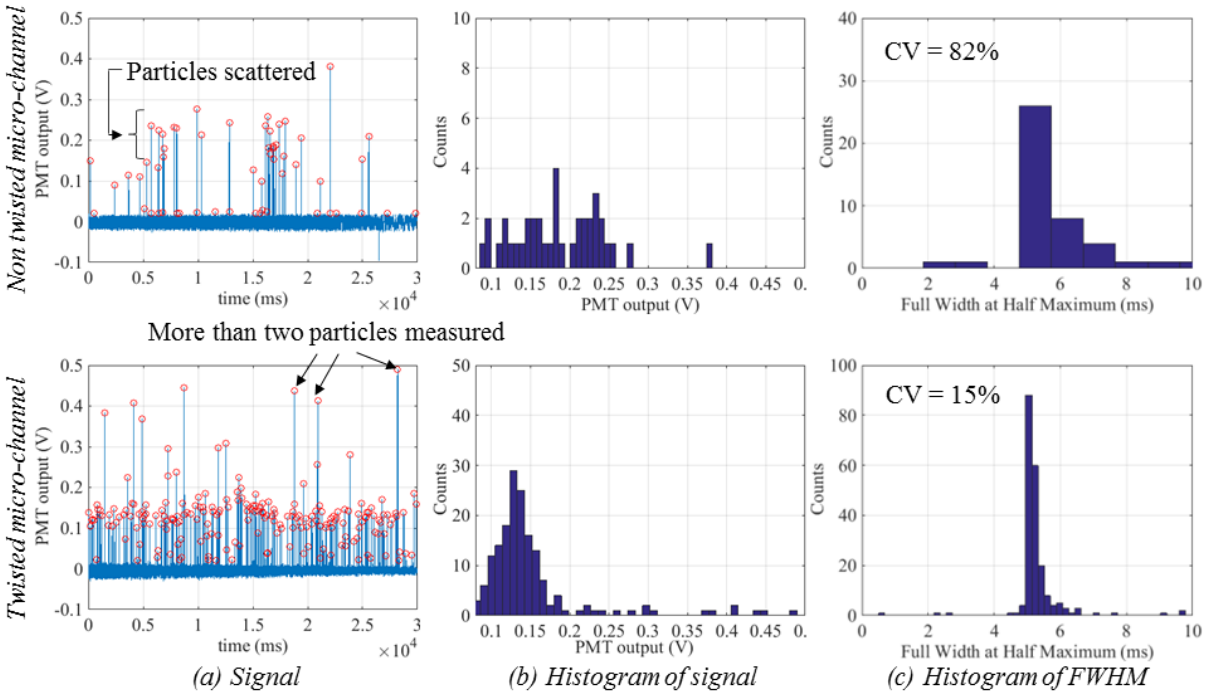


Figure 5. schematic of measurement system



(a) Signal *(b) Histogram of signal* *(c) Histogram of FWHM*

Figure 6. Results of measurement

ACKNOWLEDGEMENTS

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