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Development of Local Environmental Control System by Combination of Microfluidic Chip and Pipette

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***Abstract*—Bio-actuated, especially flagellated bacteria-driven, micro/nanorobots have been actively studied to develop novel technologies such as drug delivery systems (DDS), though the characteristics of the actuator (flagellar motor) have not been sufficiently investigated. For analysis or control of the bacterial flagellar motor, we previously proposed the local environmental control system with nano/micro dual pipettes to dynamically generate arbitrary local ion/reagent concentration change. In the result, transient-state and steady-state of local Na^+ concentration were manipulated with high facultativity. However, in this system, over time, the local ion/reagent could not be fully flushed out due to the increase of the diffusion rate caused by high external (in the bath) ion/reagent concentration. Therefore, in this paper, to achieve more arbitrary local environmental control, we propose the local environmental control system by combination of microfluidic chip with world-to-chip interface (WtCI) and pipette. We fabricate the WtCI microfluidic chip by two-step photolithography, and develop the system by using precisely controllable syringe pump. And, we demonstrate the basic feasibility of the local environmental control system by combination of WtCI microfluidic chip and pipette.**

I. Introduction

Recently, flagellated bacteria have attracted much scientific attention as actuators [1-6] because of their useful features. First, the bacterial self-propagation makes mass production of actuators quite easy and low time-cost. Second, bacteria have the metabolic function, which enables to produce the proteins and to replace the impaired proteins with the new proteins by themselves, i.e. self-repairing. Third, the bacterial flagellar motors, which convert ion-motive force into mechanical force at high energy efficiency of up to near 100 %, excel in the energy efficiency. Fourth, bacterial well-characterized sensor systems (called "taxis systems"), which most of bacteria have in order to regulate the rotation of the flagellar motors in response to the environmental stimuli [7], are expected to be utilized to control the bacterial driving force artificially. Fifth, some genetically well-studied flagellated bacteria can be modified genetically to add or delete some functions depending on the requirements.

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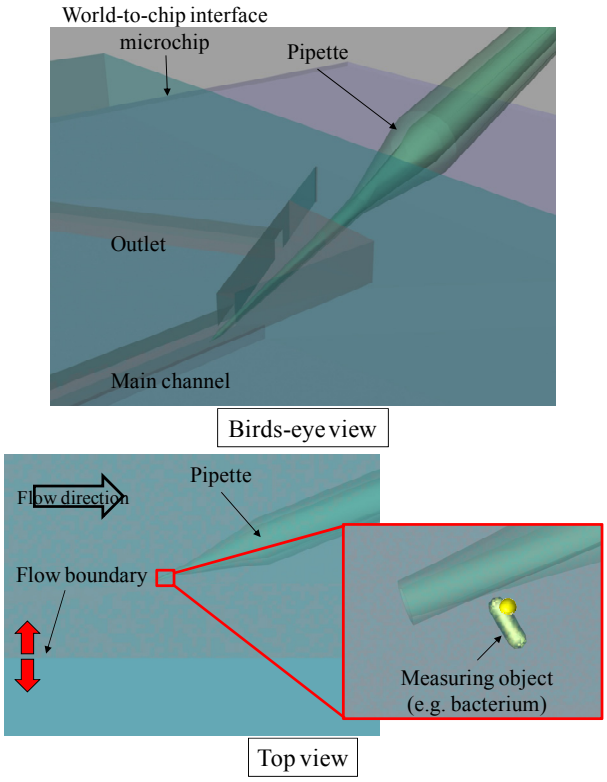

Figure 1. Schematic of the concept of the local environmental control system by combination of WtCI microfluidic chip and pipette.

In fact, flagellated bacteria have been used as actuators for various purposes in many fields, though they are still in the research phase. Kaehr *et al.* used flagellated bacteria in microfabricated devices for transportations of microobjects [2]. Kim *et al.* used flagellated bacteria as microfluidic pumps by flow deposition of bacteria onto the bottom surfaces in microfabricated fluid systems [3]. Flagellated bacteria were also used as driving forces for micro motors/gears utilizing the collision between bacteria and micro motors/gears [4-6].

And, especially, bacteria-driven microobjects have been actively studied toward micro/nanorobots to develop novel technologies such as drug delivery systems (DDS), micro/nano surgery inside the human body [8-12]. In these conventional researches, the bacteria-driven microobjects were fabricated by random or limited attachment of bacteria. To enhance the controllability of the bacteria-driven microobjects, we previously proposed the method to assemble single bacterium onto a microobject using optical tweezers [13, 14]. From the quantitative analysis of behaviors of the

assembled bacterium-driven microobjects, it was shown that the mobility of the bacterium-driven microobjects was higher and their movements reflected the action of the intact single bacterium.

While at the same time, the characteristics of the flagellar motor have not been sufficiently investigated. In fact, even the mechanism of the flagellar rotation has not been fully revealed. If detailed characteristics/mechanism of the flagellar rotation are revealed, intact bacterium-like action of the bacterium-driven microobjects can lead to more efficient and fine control utilizing bacterial natural features, such as chemotaxis. Therefore, for analysis or control of the bacterial flagellar motor, we previously proposed local environmental control system with nano/micro dual pipettes to dynamically generate arbitrary local ion/reagent concentration change [15, 16]. However, in this system, over time, the local ion/reagent could not be fully flushed out due to the increase of the diffusion rate caused by high external (in the bath) ion/reagent concentration, because the solution in the chamber was not refreshed.

Therefore, in this paper, we propose the local environmental control system by combination of microfluidic chip with world-to-chip interface (WtCI) and pipette (Fig. 1). We fabricate the WtCI microfluidic chip, develop a local environmental control system, and verify the feasibility of the proposed system experimentally.

II. Local Environmental Control System by Combination of Microfluidic Chip and Pipette

A. Concept

By using microfluidic chip, discrete concentration control (switching between certain prepared solutions whose concentrations are different) can be done by manipulating the flow boundary. Concentration gradient also can be generated by well-designed mixing channels [17]. However, it is difficult to dynamically generate arbitrary local ion/reagent concentration change by microfluidic chip alone.

Using local environmental control system with nano/micro dual pipettes we previously proposed [15, 16], transient-state and steady-state of local Na^+ concentration were manipulated with high facultativity by switching between / ejecting simultaneously ion/reagent- containing and -free solutions with changing the spouting velocities independently. However, in this system, the solution in the chamber was not refreshed. Therefore, over time, the local ion/reagent could not be fully flushed out due to the increase of the diffusion rate caused by high external (in the bath) ion/reagent concentration.

WtCI microfluidic chip was previously proposed by Arai *et al.* as a microfluidic device which concurrently can refresh

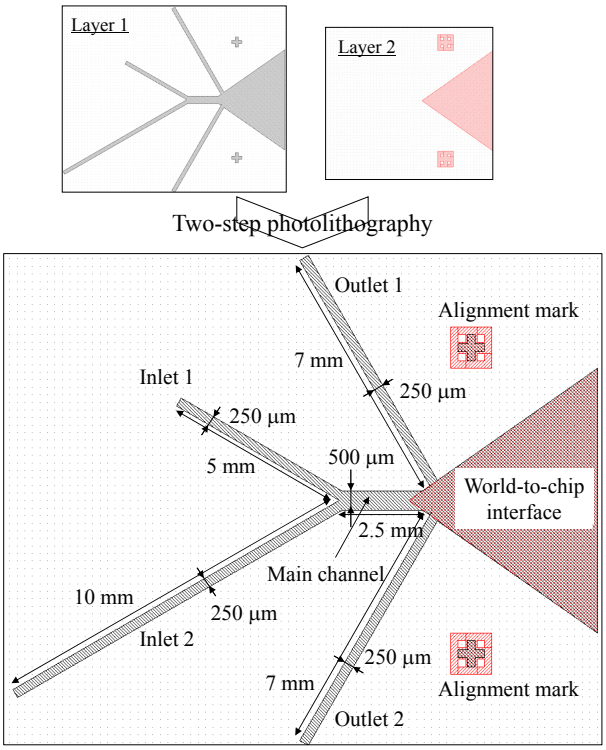

Figure 2. Design and dimension of WtCI microfluidic chip.

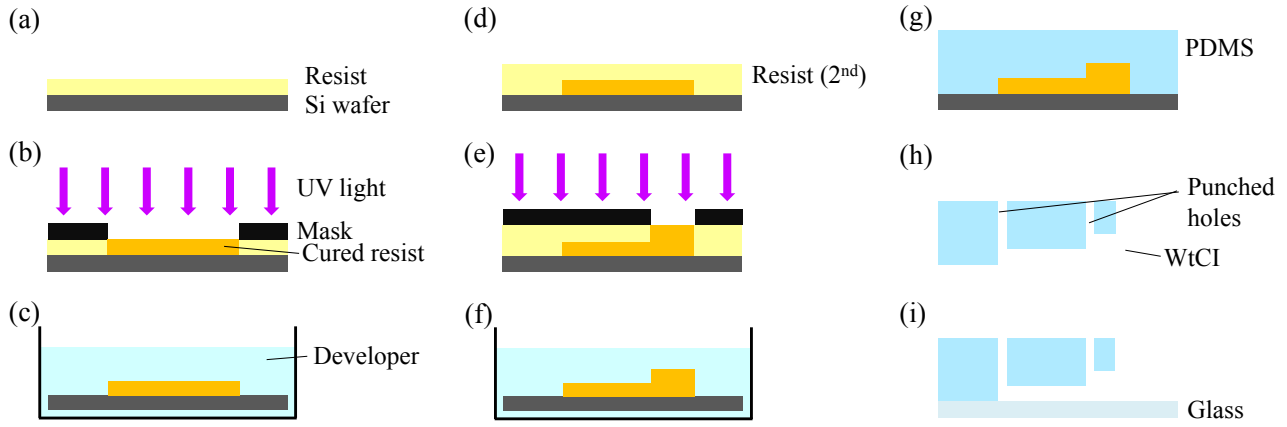

Figure 3. Schematic of fabrication process of the WtCI microfluidic chip. (a) Spin coat silicon wafer with photo resist, (b) expose photo resist to UV light, (c) develop photo resist, (d) spin coat 2nd photo resist layer, (e) expose 2nd photo resist layer to UV light, (f) develop 2nd photo resist layer, (g) mold PDMS, (h) detach, punch holes for tubing and cut PDMS to open WtCI, (i) bond PDMS to glass substrate.

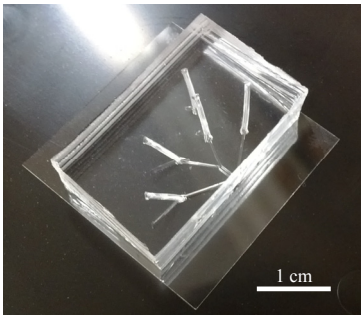

Figure 4. Photo of a fabricated WtCI microfluidic chip.

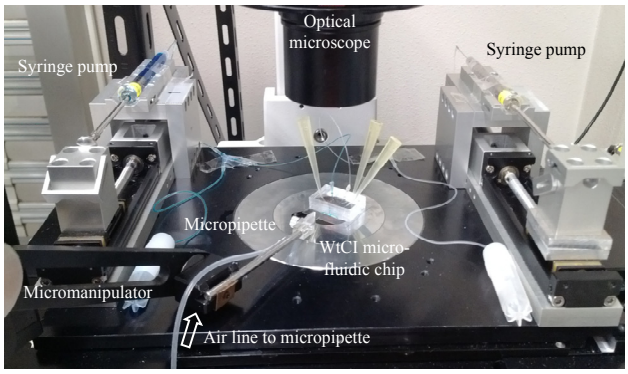

Figure 5. Photo of the system configuration.

the solution, can control the flow boundary and can insert probe type devices from its "world-to-chip interface" [18]. Threfore, as shown in Fig. 1, the local ion/reagent concentration might be able to be controlled arbitrarily and dynamically by combining the manipulation of flow boundary (discrete (digital) control) and the mixing of ion/reagent-containing and -free solutions (to be exact, it might be caused by not mixing but interplay between flushing and diffusion) utilizing the ejection from the pipette (indiscrete (analog) control).

B. Design and Fabrication of WtCI Microfluidic Chip

Figure 2 shows the design and the dimension of the WtCI microfluidic chip we made in this paper. This WtCI microfluidic chip had two inlets to generate a two-layer flow, main channel, two outlets just behind the main channel to drain the solutions, and WtCI to insert pipette into it. The heights of inlets, outlets and main channel were ~400 μm. To make it less difficult to insert a micropipette into the chip, the height of WtCI was made taller than other parts by two-step photolithography. Other dimensions were determined based on desired flow rate, the height and width of the main channel and the performance of syringe pumps used in this paper.

Figure 3 shows the schematic of the fabrication process of the WtCI microfluidic chip. At first, negative photo resist SU-8 3050 (Kayaku Microchem) was spin coated on a silicon wafer at 400 rpm for 60 sec (Fig. 3(a)), and baked using a hot plate at 95 ºC for 14 hours (thickness: ~400 μm). Prebaked SU-8 was exposed to UV light using a photo mask with negative channel pattern shown in Fig. 2 (Fig. 3(b)), and

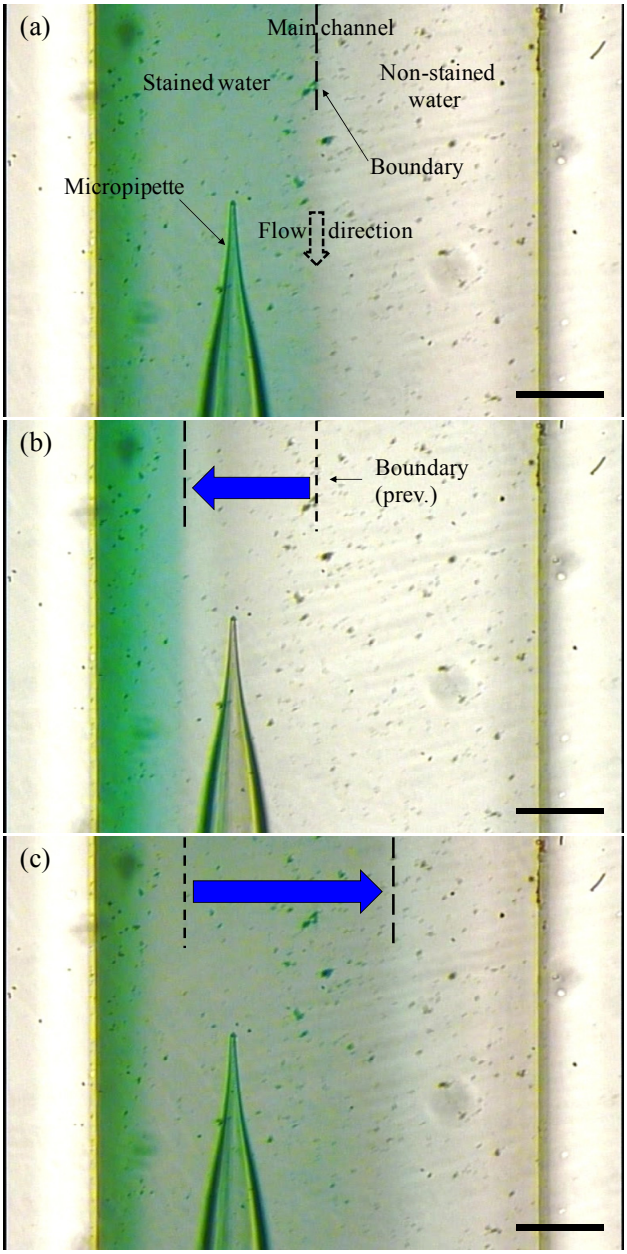

Figure 6. Sequence images of the experiment of the flow boundary manipulation. (a) Initial state, (b) moved to the left, (c) moved to the right. Scale bars are 100 μm.

baked using a hot plate at 65 ºC for 1 min & 95 ºC for 5 min. SU-8 was developed to make the channel pattern by dissolving the unexposed part (Fig. 3(c)). Developed SU-8 was baked using a hot plate at 150 ºC for 2 hours for the purpose of hard bake. Then, SU-8 3050 was spin coated again on the silicon wafer with patterned first SU-8 layer at 300 rpm for 30 sec (Fig. 3(d)), and baked using a hot plate at 95 ºC for 20 hours. Prebaked second SU-8 layer was exposed to UV light using a photo mask with negative pattern of WtCI shown in Fig. 2 upper right (Fig. 3(e)), and baked using a hot plate at 65 ºC for 1 min & 95 ºC for 5 min. Second SU-8 layer was developed (Fig. 3(f)), and baked using a hot plate at 150 ºC for 2 hours for the purpose of hard bake. Next, polydimethyl-siloxane (PDMS; SILPOT 184, Dow Corning Toray) was

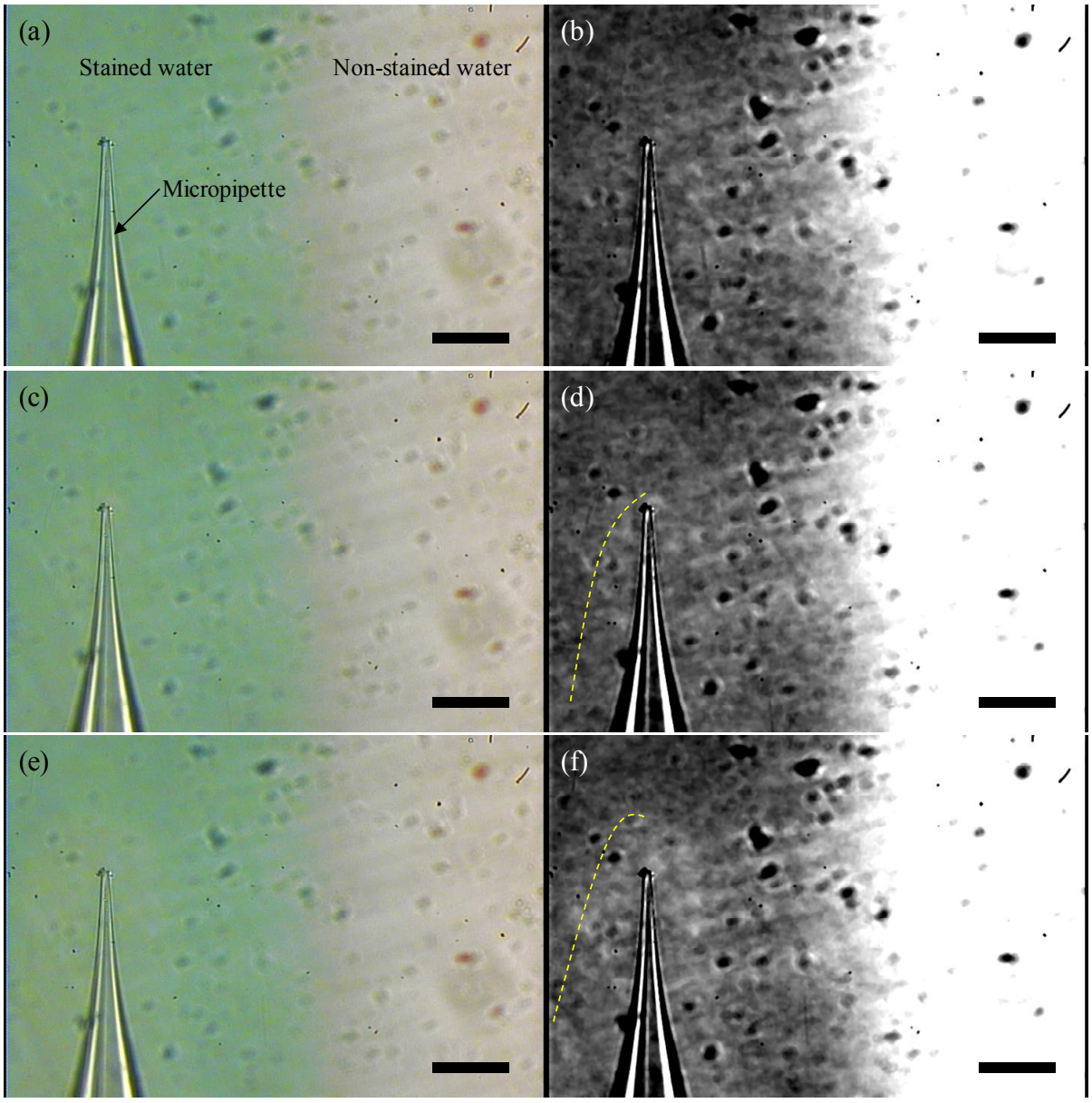

Figure 7. Experimental results of indiscrete control of local stain concentration by ejection of non-stained water from the micropipette. (a), (c) and (e) are the original images w/o ejection, with weak ejection and with strong ejection, respectively. (a), (c) and (e) were binarized and enhanced in contrast, and the results are (b), (d) and (f), respectively. The dotted lines in (d) and (f) indicate the half of the low concentration area. Scale bars are 50 μm.

poured onto the silicon wafer with patterned two SU-8 layers, and cured for ~1 day at room temperature (Fig. 3(g)). The cured PDMS was detached, punched holes at the end of inlets and outlets to connect tubes, and cut to open the WtCI (Fig. 3(h)). Then, to seal the channels, the PDMS chip was bonded to a glass substrate by O_2 plasma bonding technique (Fig. 3(i)). A fabricated WtCI microfluidic chip is shown in Fig. 4.

C. System Configuration

Figure 5 shows the system configuration of the local environmental control system by combination of WtCI microfluidic chip and glass micropipette. Inlet and syringe pump were connected by a connection tube which had a bifurcation to a waste line to decrease the flow volume into the chip. The flow rates of each inlet can be controlled independently, finely and dynamically by using precisely controllable syringe pumps. The WtCI microfluidic chip was set on the stage of an inverted optical microscope. The glass micropipette was fixed on a micromanipulator, and an air pressure controller was connected to the micropipette to eject a solution by air pressure.

III. Experimental Results

The capabilities of local environmental control system by combination of WtCI microfluidic chip and pipette, discrete control by the manipulation of flow boundary and indiscrete control by the mixing of the ion/reagent-containing and -free

solutions utilizing the ejection of the ion/reagent-free solution from the micropipette, were verified experimentally.

In the following experiments, stained water and non-stained (pure) water were used to visualize the boundary and to measure the concentration qualitatively. And, the non-stained water was filled into the micropipette.

A. Discrete Control by Manipulation of Flow Boundary

At first, discrete control of the local concentration was demonstrated by manipulation of the boundary between the stained water layer and non-stained water layer. Figure 6 shows the experimental sequence images of the flow boundary manipulation. As shown in this figure, the boundary of the stained water layer and non-stained water layer was successfully manipulated by changing the flow rates of two syringe pumps. And, the boundary was almost straight. It means that the flow boundary was not largely affected by the insertion of the micropipette and two-layer flow was sustainably generated.

From these results, it can be concluded that the ion/reagent concentration at a certain local target point in the main channel can be controlled discretely by flow boundary manipulation. Therefore, the capability of discrete control of local ion/reagent concentration by flow boundary manipulation was verified.

B. Indiscrete Control by Mixing of Ion/Reagent-containing/ -free Solutions

Next, indiscrete control of the local concentration was demonstrated. The non-stained water was ejected into the stained water layer to generate arbitrary concentration by the mixing of the stained and non-stained water. The ejection rate from the micropipette was changed in three levels (without ejection, with weak ejection and with strong ejection) by changing the applied air pressure to the micropipette. Qualitative evaluations of the stain concentrations were visually conducted. Original experimental images w/o ejection, with weak ejection and with strong ejection are shown in Fig. 7(a), (c) and (e), respectively. To make the difference clearer, these images were binarized. Subsequently, contrast was enhanced and brightness was adjusted. The results of this image processing for Fig. 7(a), (c) and (e) are shown in Fig. 7(b), (d) and (f), respectively. As shown in this figure, the areas where the stain concentrations were changed depended on the ejection rate of the non-stained water from the pipette.

For more detailed evaluation, the grey values on the line along the flow direction, which is ~5 μm away from the tip of the micropipette, were plotted in Fig. 8. The data for W/O ejection/weak ejection/strong ejection in Fig. 6 correspond to the images in Fig. 7(b)/Fig. 7(d)/Fig. 7(f), respectively. With ejection, grey values around the tip of the micropipette are bigger (brighter). As compared to each other condition, the grey values behind the micropipette were gradually changed depending on the ejection rate. In addition, with strong ejection, the grey values above the micropipette changed depending on the distance.

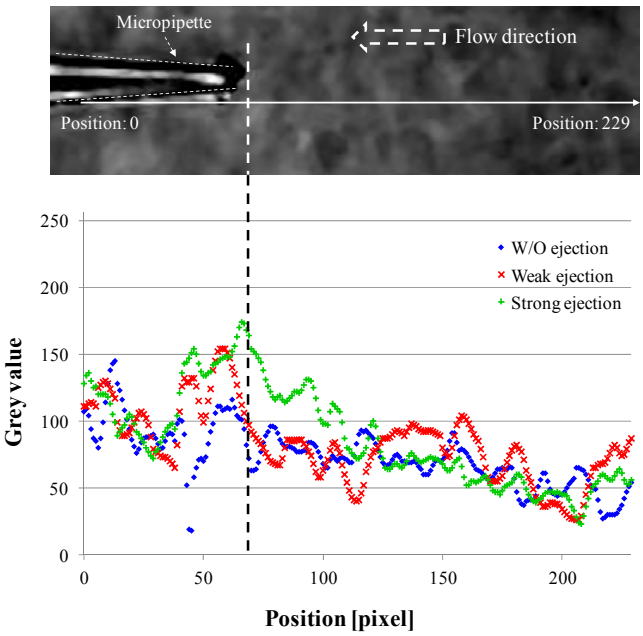

Figure 8. Grey value (qualitative concentration of stain) plots along the flow direction. W/O ejection/weak ejection/strong ejection correspond to Fig. 7(b)/Fig. 7(d)/Fig. 7(f), respectively. The above image is the extraction from Fig. 7(b) as an illustration.

From these results, it can be concluded that the ion/reagent concentration at a certain local target point in the main channel can be arbitrarily controlled depending on the ejection rate and the position relative to the micropipette. Therefore, the capability of indiscrete control of local ion/reagent concentration by mixing of ion/reagent-containing and -free solutions was verified.

IV. Conclusion

In this paper, to achieve more arbitrary local environmental control, we proposed the local environmental control system by combination of world-to-chip interface (WtCI) microfluidic chip and pipette. We fabricated the WtCI microfluidic chip using two-step photolithography, and develop the system by utilizing precisely controllable syringe pumps. And, we demonstrated the basic feasibility of the proposed local environmental control system, discrete control by the manipulation of flow boundary and indiscrete control by the mixing of the stained and non-stained water utilizing the ejection of the non-stained water from the micropipette.

As the future work, we will evaluate the performance of the system, e.g. response times of flow boundary manipulation and the mixing, stability of the local ion/reagent concentration generated by mixing, etc. We will also identify the local ion/reagent concentration depending on the ejection rate and the position relative to the micropipette. And, finally, we will utilize our local environmental control system by combination of WtCI microfluidic chip and pipette for some practical biological application.

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