**I. INTRODUCTION**

Chemical waves can be observed in diverse kinds of chemical reaction systems [1–7]. All of them are driven by a non-linear chemical reaction, in which an autocatalytic reaction is essentially involved. Since the first discovery of chemical waves [8], many experimental and theoretical investigations have been done in order to make clear their mechanism. Among them, one intriguing scientific interest is an effect of gravity on the chemical wave propagation dynamics [3]. Since the chemical wave front is consists of interface between two solutions (reacted and unreacted solutions) with different density. The gravity causes an asymmetric wave front interface, which promote the reaction at the interface and thus accelerate the propagation dynamics [7].

In addition to such scientific and basic researches, there are growing interests on the applicational use of the chemical waves, e.g., image processing [9–12], signal transport [13–16], and mass transport [17, 18]. Furthermore, stationary chemical wave, i.e., chemical oscillation, can induce periodic change of stimuli-responsive molecules [19, 20]. For example, periodic switching of DNA conformation has been induced by using pH oscillation, in which the reaction solution underwent periodic pH change between 5 and 7 [19]. The use of traveling and stationary chemical wave makes it possible to manipulate stimuli-responsive molecules by spatiotemporal patterns or oscillations caused by the chemical waves.

Taking the best advantage that the chemical wave propagation is driven by an internal stimuli brought from the non-linear chemical reaction involved in the system, there are no needs to input any external stimuli such as water flow and electric field to transport the chemical signals. Thus, an additional external control system such as pump sets, electrodes, power suppliers are no more needed, which can reduce the devise size and energy consumption. For this reason, the chemical wave would be effectively used in future micro- and nano-scale devices.

**II. EXPERIMENTAL**

Glass substrate was washed with H$_2$SO$_4$ and rinsed with boiled water. After being dried, PDMS microchannel was put on the substrate. The channel widths were 100, 600, and 2000 μm, and the channel depth was 100 μm (Fig. 1). Mixed solution containing 70 mM Na$_2$S$_2$O$_3$, 100 mM KBrO$_3$, and 2 mM NaOH was filled in the microchannel. As pH indicator, a small amount of methyl orange was also added in the solution. Then, 100 mM H$_2$SO$_4$ was dropped in the inlet of the microchannel. The propagating behavior of the pH front was observed under...
optical microscope (Olympus BX53).

Fluorescein isothiocyanate (FITC) and 3-aminopropyltrimethoxysilane (APTMS) were used without further purification. To obtain FITC-APTMS, 333 μl APTMS and 50 mg FITC were dissolved in 33.3 ml absolute ethanol and stirred for 24 h in darkness. After washing the obtained crystal with absolute ethanol three times, aqueous solution with almost saturated concentration was prepared. For the surface modification, FITC-APTMS aqueous solution was poured on the washed glass surface and the reaction was to continue for 13 h in darkness at room temperature. After washing the substrate with water, the substrate was heated at 120°C for 30 min. This substrate was also used for pH wave propagation experiment. The generation of pH wave was done with the same protocol above but without the addition of pH indicator.

III. RESULTS AND DISCUSSIONS

First we have checked the surface modification of pH-responsive FITC-ATPMS on the glass substrate. It is known that FITC-APTMS immobilized on SiO₂ surface exhibit strong emission at high pH [21]. In our system, we have also observed strong emission only when the surface was in contact with high pH solution containing NaOH (Fig. 2). The emission was strongly quenched by changing the solution to acidic condition. The emission intensity histogram revealed that the emission intensity was reduced about one-half by decreasing pH from 11 to 4. These results imply that FITC molecules immobilized glass surface sustain their pH-dependent fluorescence property.

We used and compared non-modified and FITC-immobilized glass substrates in the pH propagation experiments. When we use the non-modified glass, we can characterize the pH of the solution from the color of added pH indicator (Fig. 3(a)). In the present system, the initial color of solution is yellow because the pH of the original solution is ca. 11. However, after adding a small amount of H₂SO₄, autocalytic proton production reactions occurred. On the analogy of iodate-sulfite-thiosulfate system [22], transient formation of bisulfite takes place at relatively high pH condition (initial state).

\[
2\text{BrO}_3^- + 3\text{S}_2\text{O}_3^{2-} + 3\text{H}_2\text{O} \rightarrow 2\text{Br}^- + 6\text{HSO}_3^- \quad (1)
\]

The addition of proton initiates the oxidation of bisulfate ion.

\[
\text{BrO}_3^- + 3\text{HSO}_3^- + \text{H}^+ \rightarrow \text{Br}^- + 3\text{SO}_4^{2-} + 4\text{H}^+ \quad (2)
\]

The process (2) is autocatalytic and the presence of a small amount of proton increase proton rapidly, which induces rapid decrease in pH. In the present system, the addition of H₂SO₄ initiates the autocatalytic reaction (2) and cause rapid pH decrease at the inlet region. pH decrease changes the color of solution near inlet region (left edge of each figure in Fig. 3) to red, indicating pH of this region becomes below ca. 4. Autocatalytically produced proton diffuses to nearby region and low proton concentration by proton concentration gradient, which leads to the propagation of autocatalytic reaction (2) to nearby region. As such, the region with high proton concentration, i.e., low pH region, propagate like waves in the microchannel as shown in Fig. 1 left, which is the origin of the name of this phenomenon, “pH wave”. Although reports on pH wave propagation in milli-meter scale capillary have been vigorously reported so far, it is the first time to demonstrate the pH wave propagation in the micro-meter scale space.

Similar experiments were done by using FITC-immobilized glass substrate instead of non-modified glass. In this case, pH indicator was not added in the reaction solution. Before adding H₂SO₄, the microchannel exhibited bright green fluorescence from FITC molecules in contact with original solution (pH 11). As mentioned above, the addition of H₂SO₄ induces autocatalytic production of proton and reduces the pH below 4. Since the fluorescence of FITC is strongly reduced in contact with low pH solution, the propagation of low pH region, which had been observed as the expansion of red region in in Fig. 3(a), would cause a propagation of quenched region from left to right. As is clearly shown in Fig. 3(b), the region near the H₂SO₄ inlet was first quenched. Then, the quenched region propagated in the microchannel with time. This finding strongly indicates that the pH wave can modulate pH-responsive molecule that is immobilized on the channel wall. The observed propagation of quenched region can be regarded as a wave of chemical reaction that is triggered by the pH wave propagation.

In order to examine the propagation dynamics of non-modified and FITC-immobilized systems, we tracked the position of boundary between high- and low-pH region,
which corresponds to yellow/red boundary, and emissive/quenched boundary for non-modified and FITC-immobilized systems, respectively. In the case of 100 μm-width microchannel, pH wave propagate for longer than 2 μm at 250 s in both non-modified and FITC-immobilized systems (Figs. 4(a) and (d)). It should be noted here that the propagation dynamics is slightly different even under the same experimental condition. For example, the propagation length differs from 2.0 to 2.6 μm at 250 s for non-modified glass (Fig. 4(a)). This seems to be due to the difficulty in controlling the mixing behavior at the inlet between reaction solution (Na₂S₂O₃, KBrO₃, and NaOH) and H₂SO₄, although the mixing behavior determines the local proton concentration that triggers autocatalytic reaction dynamics through reaction (2). Since no stirring or mixing operations was done upon adding the H₂SO₄, the proton concentration in the inlet is not homogeneous. In the case that initial proton concentration near the junction between the inlet and channel regions becomes high enough just after the H₂SO₄ addition, the proton concentration reaches much faster at the level need for the proton propagation step. This results in fast pH wave propagation. If we could make the solution after the addition of H₂SO₄ homogeneous, we are able to define results on the propagation dynamics without such significant deviations. Since the situation is the same for FITC-immobilized glass system, we also observed relatively large deviation in the experimental results under the same experimental condition (Fig. 4(d)). However, the propagation lengths were similar between non-modified and FITC-immobilized systems. This result strongly supports our assumption that the propagation of quenched region is caused by the pH wave propagation in the microchannel, i.e., it is possible to induce a propagation of pH-responsive chemical reaction in the microchannel by the pH wave propagation.

We further investigated channel-width dependence on the propagation dynamics. As the general tendency, we have found that the propagation velocity was slower at wider microchannel. For example, the propagation length at 250 s was reduced from 2 to 1 μm by increasing the channel width from 100 to 2000 μm (Fig. 4, from (a) to (c)). As the origin of faster propagation at narrower channel, we propose the effect of diffusion confinement of proton. That is, autocatalytically produced proton is not able to diffuse beyond the microchannel wall and confined in the channel, which results in the accumulation of proton in the reacted solution. Such diffusion confinement increase an apparent rate constant of proton production that accelerates the propagation of proton production reaction, causing accelerated pH wave propagation at the narrower channels. This is the first report on the acceleration of pH wave propagation at narrower channel.

The channel width dependence has also already been reported by using glass capillaries and proved that wider capillary exhibited faster propagation [1], which is com-
Fig. 4: Propagation length of pH wave with using (a)-(c) non-modified glass substrates and (d)-(f) FITC-immobilized glass substrates. Channel widths are (a, d): 100 μm, (b, e): 600 μm, and (c, f): 2000 μm.

completely opposite to our results. The difference between the present and previous results is come from the fact that we used microchannels and the previous system used glass capillaries. Former enables to control the channel width and channel depth independently, whereas the channel depth and channel width is equivalent, i.e., both are equivalent to the channel diameter, in the capillary system. Thus, the previous system cannot distinguish the effects of channel width and channel depth. Based on several experimental results, they finally concluded that the observed diameter dependence should be attributed to the depth dependence, not the width dependence, which was discussed in terms of gravity effect [1, 7]. Thus, we can exclude the gravity effect since we use microchannels with the same depth.

The channel width dependence is also observed on the FITC-immobilized glass system, in which the propagation length decreased from 2 to below 1 μm by increasing the channel width from 100 to 2000 μm (Fig. 4, from (d) to (f)). Quantitative agreement in the propagation lengths between non-modified and FITC-immobilized systems again support our assumption that the pH wave propagating in the microchannel induced the structural change of pH-responsive FITC molecule immobilized on the glass surface, indicating that the pH wave propagating in the microchannel can induce pH-driven chemical reaction and pH-driven structural change both in the microchannel solution and on the microchannel wall. The present finding opens up novel system that enables to establish freestanding propagation of pH-driven reaction in the microscopic space, i.e., any external power supply such as an electric field or water flow are no more needed to induce chemical propagations.

IV. CONCLUSIONS

In conclusion, we have succeeded in the appearance of pH wave propagation in the microchannel systems. The microchannel width was found to be an important parameter that determines the pH wave propagation dynamics, i.e., narrower channel can exhibit faster propagation. The pH wave in the microchannel can induce a pH-driven structural change of molecules immobilized on the microchannel substrate.

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