

# FUNCTIONALIZED HYDROGEL SURFACE PATTERNED IN A CHIP FOR LOCAL PH SENSING

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## ABSTRACT

We developed a novel technique to investigate local pH distribution on a chip using patterned hydrogel film made of UV photosensitive resin. The hydrogel is first patterned by photolithography in any shape on a glass, and later functionalized with the pH indicator, for example, Bromocresol Green (BCG). The hydrogel is polymerized by UV-ray illumination (300-400 nm) on the glass substrate, which is spin coated with the water-soluble prepolymer solution, and immobilized on the glass surface. Unexposed part can be removed easily by water. The hydrogel is hydrophilic, transparent, biocompatible, and is impregnated with the BCG. Then, the Polydimethylsiloxane (PDMS) microchannel is assembled and bonded on the functionalized surface. In this way, we can make patterned pH sensitive surface in the microchannel. Local pH is measured from the color of the hydrogel impregnated with BCG based on the calibrated color information in YCrCb color space. We succeeded in measuring local pH distribution in the microchip.

## 1. INTRODUCTION

There are great demands to investigate unknown properties of microorganisms and cells. Analysis based on the batch culture has been commonly used. However, we can get averaged information of specimens. Therefore, single cell analysis on a chip has been studied actively, since the change of individual specimen is highlighted microscopically [1], [2]. We can get dynamic information by controlling the ambient conditions as well as spatial distribution of specimens.

To control the conditions around specimens, measurement of the local ambient conditions in microchips is necessary and has been in great demand in recent years. Conventionally, the measurement was performed with a probe with the mechanical micromanipulator. However, disturbances in the environment caused by the motion of the probe are a problem as well as evaporation of liquid in an open space such as a petri dish. Moreover, control of the probe position by the micromanipulator is also influenced by vibration. To solve these problems, microfluidic chip is employed since the closed space inside the chip is robust against such disturbances.

Fluorescence reagent is commonly used for sensing in a chip [3], [4]. However, fluorescent method is not suitable for long-time measurement because it has major problems with absorption, quenching, and photo-degradation. Microparticles modified with a pH indicator were developed [5]. Such microparticles can be used for long-time measurements because of the absence of photo-degradation. However, surface modification of

microparticles by chemical treatment takes a long time. On the other hand, pH measurements using electrochemical reactions have been developed [6], however, the measurement area of the electrochemical pH sensor is limited by the sensor position. These sensing methods are unsatisfactory to know ambient conditions around a cell.

We have proposed to use hydrogel microbeads made by salting-out for local sensing [7], [8]. The microbead impregnated with an indicator is patterned at the desired point in a chip. However, patterning process is quite time-consuming. In this paper, we proposed functionalized hydrogel surface for sensing with high special resolution. Compared with the conventional methods, proposal technique is quite easy to make patterned sensitive surface.

## 2. PATTERNING OF pH SENSITIVE SURFACE

### Hydrogel Formation by UV Exposure

Figure 1 shows mechanism of hydrogel formation by UV exposure. Bonding force is strong enough for microfluidic experiments (flow speed < 2000  $\mu\text{m}/\text{s}$ ). This hydrogel is water-soluble and we can make any pattern on glass surface by lithography. In the lithography process, we can adjust its thickness by spin coating and develop using deionized water after UV exposure. The pattern made by the lithography keeps good shape in water and organic solvent, for example methanol and THF, more than 1 week. This patterned hydrogel formation has a good water-absorbing property and it is easy to dye by water-soluble chemicals.

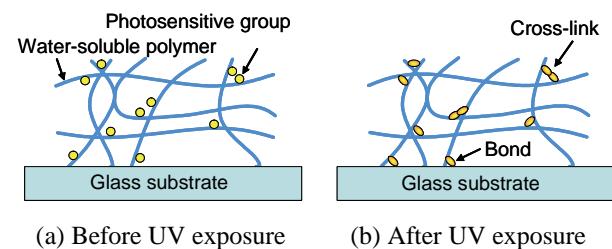


Figure 1: Mechanism of hydrogel formation by UV exposure.

### Property of pH Sensitive Surface

Figure 2 shows pH sensitive surface coated on the glass surface. The surface was modified by BCG, which is a pH indicator. The photometric properties of the pH sensitive surface were evaluated using a spectrophotometer as shown in Fig. 3. The maximum transmittance of the surface was 565 at pH4 and 477 nm at pH7, which corresponds to that of protonated and deprotonated form of BCG.

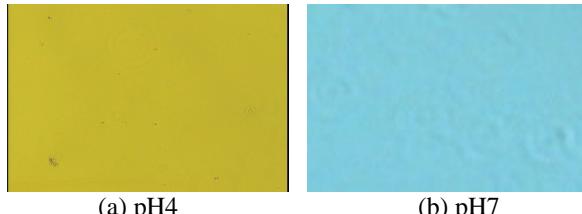


Figure 2: Photographs of pH sensitive surface on glass

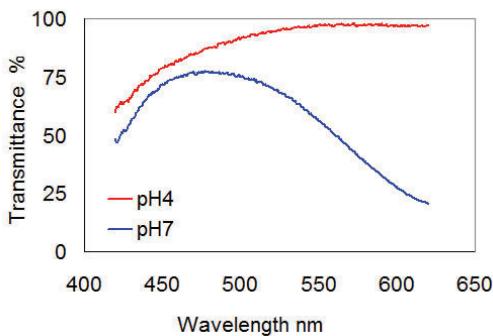


Figure 3: Spectra of pH sensitive surface

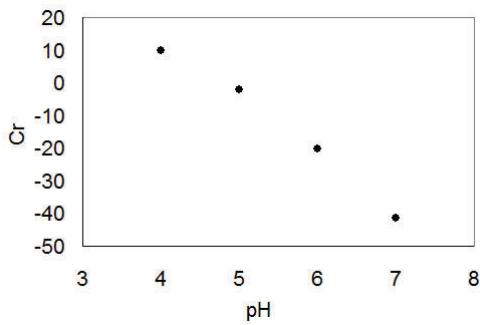


Figure 4: Calibration result of pH sensitive surface with Cr

pH is measured by the image processing of the color information obtained by CCD. Calibration of pH with the color of the surface was performed. The color information from CCD is generally RGB information. RGB are influenced by brightness which is included in each RGB value. To reduce the influence of brightness, the RGB information was converted to YCrCb information using equation (1) [9].

$$\begin{aligned} Y &= 0.299R + 0.587G + 0.114B \\ Cr &= 0.5000R - 0.419G - 0.081B \\ Cb &= -0.169R - 0.331G + 0.500B \end{aligned} \quad (1)$$

Here, the Y shows brightness, the Cr shows the color difference for red, and the Cb shows the color difference for blue. The color of BCG changes from yellow to blue with increasing pH. Therefore we calibrated pH with Cr and Cb. In this paper, Cr was used for pH calibration. The calibration results are shown in Fig. 4. There were proportional relationships between the pH and Cr. Equation 2 shows the linear approximation formulas for the plots. The correlation coefficient was about 0.98. From

this result, pH value is measured by the image processing (accuracy: about 0.2). Response of the color change is less than 1 second. The speed depends on the permeation speed of the liquid.

$$pH = -5.8 \times 10^{-2} \times Cr + 4.7 \quad (2)$$

Figure 5 shows pH sensitive surface tested for demonstration. Any 2D pattern is made by photolithography.

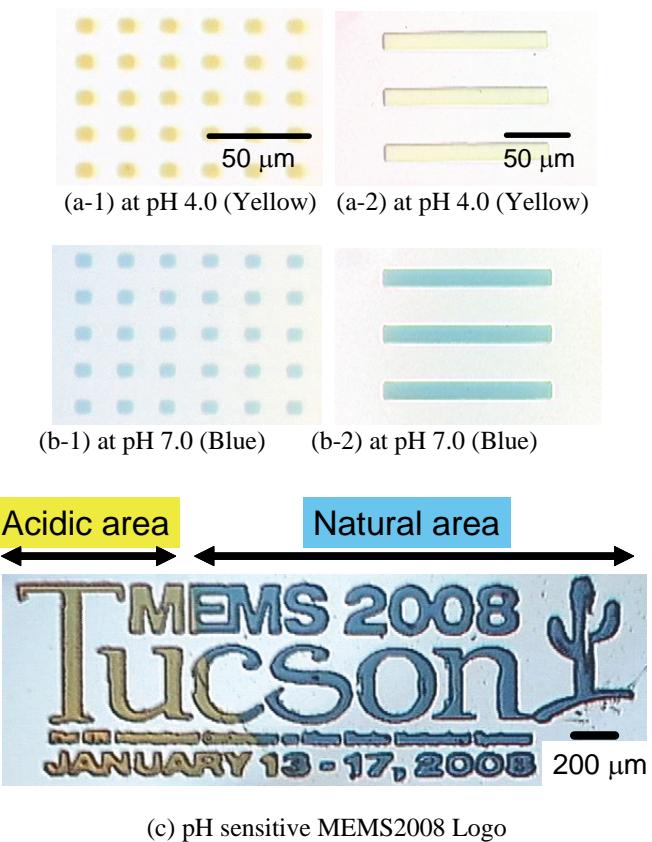


Figure 5: pH sensitive surface patterned on the glass surface (a),(b) and on the Si wafer(c).

### 3. pH MEASUREMENT ON A CHIP

#### Experimental Setup

Using this method, we measured pH distribution at the two-layer flow in a microchannel. Figure 6 shows a schematic of the chip. It has two input ports. There is a micro-stirrer with a micro-rotor made of composite of magnetite nano particles and PDMS, and the rotor is rotated by the external rotating magnetic field [10]-[12]. Hydrogel sensor array is patterned along the microchannel in a PDMS chip and pH distribution is measured.

Figure 7 shows a fabrication method to produce magnetic micro-rotors. The detailed description was described in the past study [10]-[12] and which are summarized as follows; the resist mold for magnetic micro-rotor was fabricated by patterning the KMPR 1050 resist layer. Then PDMS-magnetite composite has put in the patterned hole and baked at 80 °C. Finally the patterned substrate is put in the stripper liquid bath in order to

collect the magnetic micro-rotors. By using the techniques, high-accuracy magnetic micro-rotors were fabricated as shown in Fig. 8. The thickness of them was approximately 80  $\mu\text{m}$ .

Figure 9 shows photos of mixing operation with rotating speed of 1000-5500 rpm. The solutions used for this experiment were composed of dyes and DI water. The blue solution contained of methylene blue, and the yellow solution contained yellow food coloring. It is clear to see that two input solutions are mixed properly from color change. Quantitative evaluation of mixing was shown in ref. [12].

### pH Measurement at the Two-layer Flow

Figure 10 shows the pH measurement at the two-layer flow (a) and mixed flow (b), where liquid 1 (pH 4.0) and liquid 2 (pH 7.0) are flowing respectively. These liquids are transparent but the color of pH sensitive surface is changed depends on pH of the liquid. It is clear to see the pH distribution is measured by the pH sensitive surface.

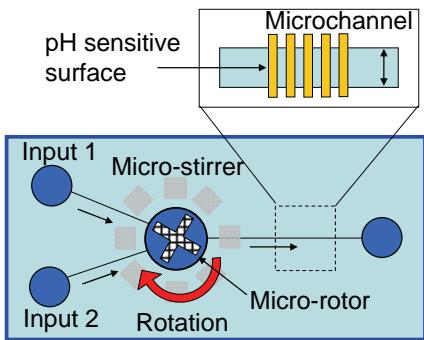


Figure 6: A schematic of the microfluidic chip with patterned pH sensitive surface and micro-stirrer.

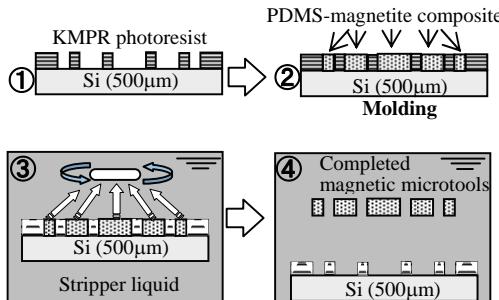


Figure 7: Process flows to fabricate polymeric magnetic micro-rotors.



Figure 8: Photos of magnetic micro-rotors used in the micro-stirrer on a chip.

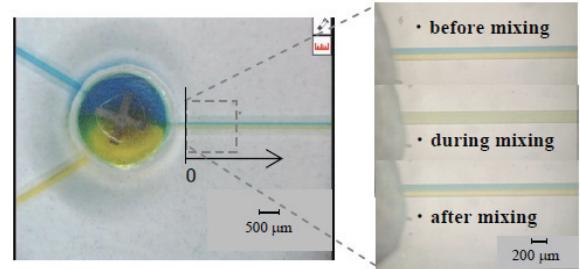
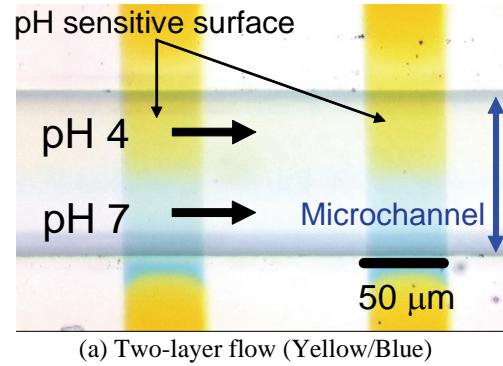
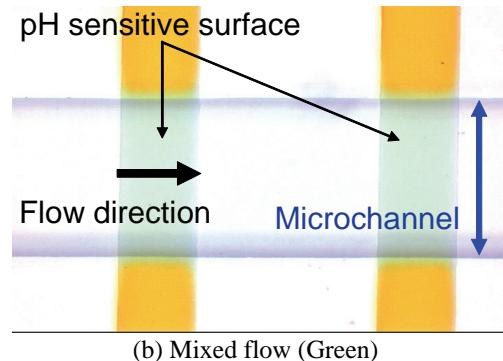


Figure 9: Photos of mixing operation of a micro-stirrer in a chip.



(a) Two-layer flow (Yellow/Blue)



(b) Mixed flow (Green)

Figure 10: pH measurement at the two-layer flow.

## 4. ON-CHIP MONITORING OF CELLS

### Fabrication of a Chip for On-chip Monitoring of Cells

Next, we measured pH distribution around cells in micro-chambers.

Figure 11 shows fabrication process of this chip. We did spin coating with hydrogel on glass substrate, baking and UV exposure (a). The thickness of hydrogel formation layer was 4  $\mu\text{m}$ . We dyed with saturated BCG aqueous solution and later baked again (b). Microchannel which has micro-chamber and micro-pillars was fabricated by PDMS molding of SU-8 pattern on Si wafer (c), (d). The channel height was 10  $\mu\text{m}$ . We made two inputs and an output by using a punch (e). Finally, we did O<sub>2</sub> plasma treatment for bonding between PDMS and pH sensitive surface and connected teflon tubes and silicone tubes into the PDMS chip (f).

Figure 12 (a) shows micro-chambers in a PDMS chip. The chip has two inputs and the main channel is separated by a thin PDMS wall (thickness: 7  $\mu\text{m}$ ) to form two micro-chambers.

There are several micro-pillars to capture cells. Cells adhere to the pillars and they are monitored by CCD camera at the same time.

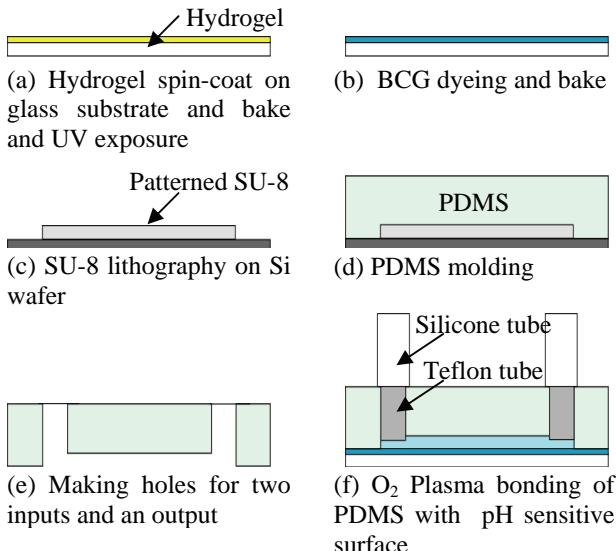


Figure 11: Fabrication of pH sensitive chip.

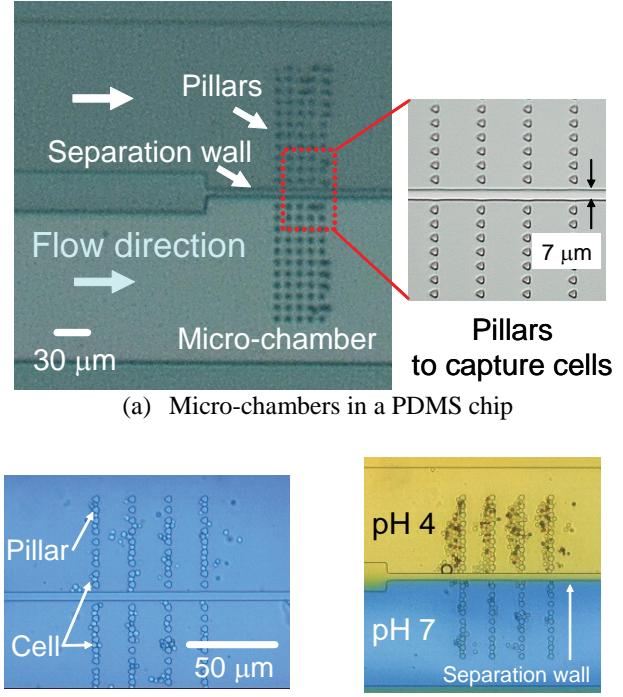


Figure 12: pH measurement around cells immobilized in micro-chambers.

### Experiment of Monitoring of Cells on a Chip

Two different kinds of cells (one of them is genetically controlled) are immobilized at the pillar, and different pH liquids are applied respectively. Then, properties of cells

are monitored in real time. We will have another opportunity to report on these results.

The proposed method is applicable for investigation of the individual properties of cells by changing the indicator. The proposed method will be applicable to measure the change of each individual cell at different conditions.

## 5. CONCLUSIONS

We developed a novel technique to investigate local pH distribution on a chip using patterned hydrogel film made of UV photosensitive resin. The hydrogel is first patterned by photolithography in any shape on a glass surface, and later functionalized with BCG. We can make patterned pH sensitive surface in the microchannel. Local pH is measured from the color of the hydrogel impregnated with BCG based on the calibrated color information in YCrCb color space. We succeeded in measuring local pH distribution in the microchip. The proposed method is quite easy to make patterned sensitive surface.

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