2007 IEEE International Conference on
Robotics and Automation
Roma, Italy, 10-14 April 2007

Gel-tool Sensor Positioned by Optical Tweezers for Local pH Measurement in a Microchip

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***Abstract*— A local pH measurement method in a microchip using a functional gel-tool was developed. We used salting-out gel-tools impregnated with Bromothymol Blue (BTB), which is a pH indicator. The gel-tool is made of hydrophilic photo-crosslinkable resin. The primary constituent of this photo-crosslinkable resin is polyethyleneglycol. A solution mixed with photo-crosslinkable resin, BTB, high concentrations of an electrolytic solution are stirred, and gel beads impregnated with BTB are obtained. Gel-tools are cured by UV-ray and adhere to the glass plate, but we can manipulate them by the optical tweezers. We can measure pH value locally from the color of gel-tool using calibrated color information in YCrCb color space. We succeeded in measuring local pH value with the pH sensing gel-tool by manipulating and locating it at the desired point in the microchip.**

I. Introduction

THE measurement of environment inside a microchip is important for on-chip experiments such as biological analysis on investigating unknown properties of cells and microorganisms and chemical production. In recent years, many types of microchips for cell analysis have been proposed [1]-[3]. Various cell experiments such as screening, culture, and observation of their activities are operated in the microchip [4], [5]. Miniaturization of experiment space is expected to provide many potential advantages such as increased reaction speed, efficiency, portability and reduced consumption through the merits of scale. Moreover, microchip can avoid the disturbance of environment and achieve single cell level experiment easily.

Cell experiment in a microchip needs some techniques, such as manipulation, separation, immobilization, observation, and measurement. Many researches have been done on these techniques [6]-[10].

We have studied the cell experiment microchip by employing non-contact manipulation techniques [11]-[13]. In our cell experiment microchip, optical tweezers and dielectrophoresis are used for cell manipulation and separation, thermal gelation and local photofabrication are used for cell immobilization on the microchip. The characteristic point of our study is to use a microtool for manipulation of cells.

Microtool was proposed for manipulation of microobject such as cells. Optical tweezers can manipulate individual cell, however, direct irradiation of focused laser may damage cells. In previous studies of photodamage to cells, the action spectrum for photodamage depends on the wavelength and intensity of laser [14]. To prevent this problem, we developed microtool and manipulated the target indirectly by optically controlled microtool. Direct irradiation of focused laser on the cell is avoided by using the microtool. This manipulation technique is called indirect laser micromanipulation (ILM) [15]. We developed manipulation of multiple targets by high-speed scanning of single laser. Laser scanning is suitable for attitude and trajectory control, since it is easy to create and change multiple laser trap potentials. We call this technique synchronized laser micromanipulation (SLM) for independent trajectory control of multiple objects [16]. By using ILM and SLM, attitude control and simultaneous manipulation of cells are achieved. Moreover, we developed in-situ photofabrication of functional microtool to add the special functions to microtool [17].

Environment measurement in a microchip is important to the on-chip experiments such as cell analysis and chemical production. Conventionally, the environmental dependence of fluorescent reagents was used for measurement of the environmental condition [18]. Laser-induced fluorescence (LIF) has been widely used to investigate the temperature and pH in macro-scale flow and microchannel flow. However, this method requires filling the reagent in the microchip at measurement. Although measurement is possible by a microsphere whose surface is modified by fluorescent reagents and indicators, it takes time to modify the surface [19]. A number of microsensors have been developed specifically for measuring a pH in a microchannel. One of them is the ion-sensitive field effect transistor (ISFET), which is fabricated on a microchip and which determines the pH, based on the interface voltage. Although offering high accuracy, it is difficult to obtain a three dimensional pH distribution using this device. In case that fabricating sensors in a microchip by microprocessing, it is difficult to measure the desired location and make the microchip disposable.

Manuscript received September 13, 2006. This research is supported by The Ministry of Education, Culture, Sports, Science and Technology Grant-in-Aid for Scientific Research (17040017).

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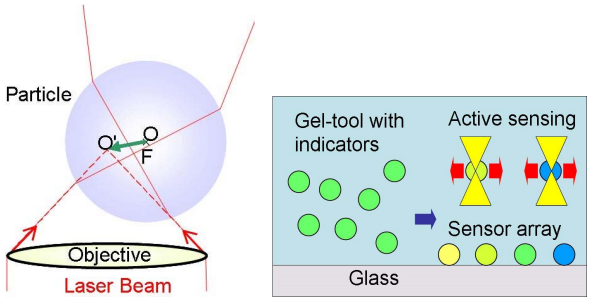
This paper reports a novel functional gel-tool which contains an indicator to measure the local environmental condition in a microchip. We used gel microbeads, which are obtained by salting-out of hydrophilic photo-crosslinkablre resin. BTB was contained in the gel-tools as a pH indicator. Gel-tool is manipulated by optical tweezers and pH value is calculated from color information acquired by CCD (Fig. 1).



(a) Principle of optical tweezers (b) active pH sensing

Fig. 1 A schematic of pH sensing gel-tool

II. Materials and Methods

A. *Microtool based microchip for on-chip cell experiment*

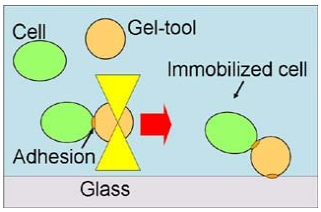
We have studied on microtool based microchip for on-chip cell experiment. Microtool is used for improving the cell experiment in the microchannel. At first, we classified the role of microtool in 4 categories as shown in Fig. 2.

(a) Micromanipulation:
Microtool is used for manipulating cells and microobjects.

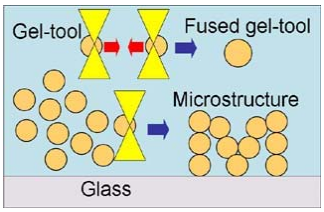
(b) Microfabrication:
Microstructure is fabricated by microtool.

(c) Measurement
Local environment such as pH and temperature in a microchip is measured by microtool.

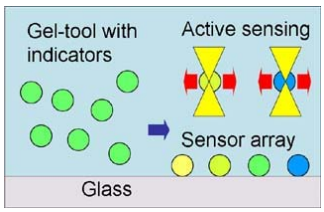
(d) Local injection
Microtool is injected at desired area in a microchip.



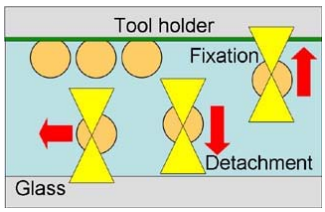
(a) Micromanipulation



(b) Microfabrication



(c) Local measurement



(d) Local injection

Fig. 2 Elements for microtool based cell experiment microchip

In most on-chip cell experiments, cell is immobilized in the microchannel and treatment of cell is operated by not manipulation of cell but reagent flow. However, controls of position, attitude, and combination of cells are necessary for the detailed analysis of cell properties. Moreover, local environment measurement is also important technique for more detailed analysis. By fabricating and employing microtools, we can achieve elements for the cell experiment such as manipulation of cell, fabrication of microstructure, and measurement of environment.

B. *pH sensing gel microtool impregnated a pH indicator*

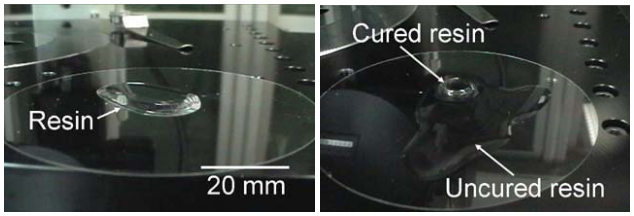
The material of pH sensing gel-tool is photo-crosslinkable resin (ENT–3400, Kansai Paint, Japan), which is used for cell immobilization, and BTB. This photo-crosslinkable resin consists chiefly of polyethyleneglycol (PEG) prepolymer and it is hydrophilic. This resin is polymerized by irradiating near-ultraviolet rays around 366 nm as shown in Fig. 3. Hydrophilic resin salts out in the high concentration electrolyte solution. Salting-out gel microbead generates in the over 20 wt% KCl solution. Fig. 4 shows a photograph of salting-out gel microbead. This microbead can adhere to the cell physically. We can manipulate salting-out gel microbead by laser in water because the relative refractive index of PEG (1.42) is higher than that of water (1.33). BTB is a pH indicator. BTB indicates yellow in a acidic solution (pH < 6), green in a neutral solution, and blue in an alkaline solution. Color shift of BTB is repeatable depends on pH value of the solution.

Fig. 5 shows the generating process of pH sensing gel-tool.

(1)Generating salting-out gel-tool by agitation. Agitating the mixture of hydrophilic photo-crosslinkable resin and BTB about 1 minute. Then injection of the gel-tool into microchip.

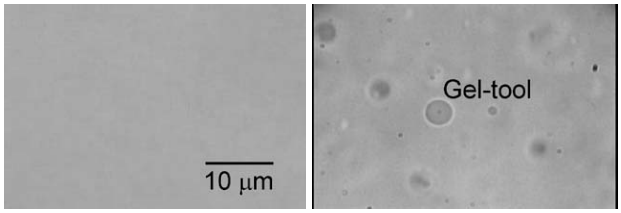
(2)Manipulation of the gel-tool by optical tweezers or positioned the gel-tool on the glass plate. pH measurement is operated by observing the color of gel-tool with color CCD.

We can easily introduce reagents in to the gel-tool when generating salting-out gel microbead as shown. The pH sensing gel-tool is fabricated by agitating mixture of 0.9 g ENT-3400, 0.3g BTB, and 2.4g 20 wt% KCl solution. We used a mercury lamp for curing photo-crosslinkable resin. Uncured gel microbeads are easily fused by contact other gel microbeads as shown in Fig. 6. On the other hand, cured microbead is adhered to glass plate by the contact as shown in Fig. 7. A solution can pass through inside the gel-tool as shown in Fig. 8.



(a) Before cure of the resin. (b) After cure of the resin

Fig.3 Photographs of ENT-3400 (Resin content: 40%) in macro-scale.



(a) KCl density: 0% (b) KCl density: 20%

Fig. 4 Generation of salting-out gel microbead

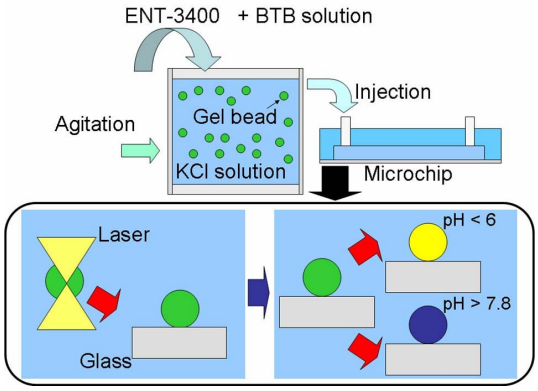


Fig. 5 A schematic of use of salting-out gel microtool

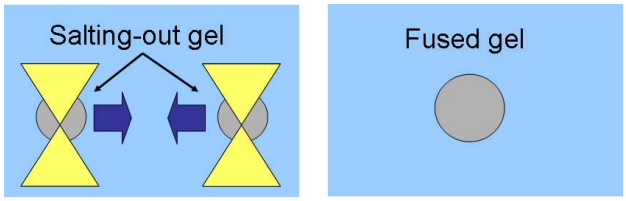


Fig. 6 A schematic of fusing gel-tool

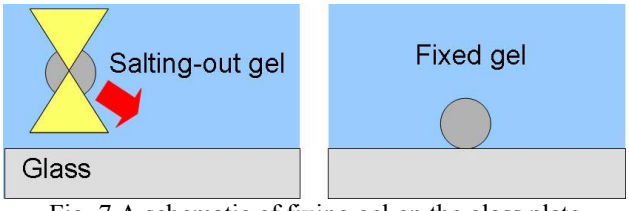


Fig. 7 A schematic of fixing gel on the glass plate

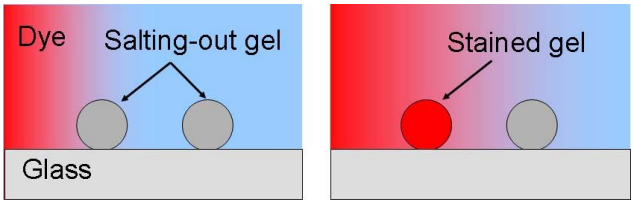


Fig. 8 A schematic of staining gel-tool

C. *Experiment system*

We built a laser scanning tele-manipulation system. Fig. 9 shows a schematic of our laser scanning micromanipulator. We can integrate 3 independent lasers at maximum. The focus of each laser is adjusted independently, and is scanned by the Galvano mirrors in the observation plane. In this paper, we integrated the CW Nd: YVO_4 laser (wavelength: 1064 nm, power > 4W, mode: TEM_{00}, M^2 < 1.1) for laser micromanipulation. The main body was made by the die casting to have the laser scanning micromanipulator and the inverted microscope. The microchip was set on the X-Y-Z stage of the inverted microscope. The stage was controlled by the stepping motors. The operator can control focal points of laser using a force feedback joystick, and can manipulate the trapped object by observing it in the monitor. We integrated the mercury lamp with the mechanical shutter and mirror unit for fluorescent observation and cure the photo-crosslinkable resin. Color information on gel-tools is acquired by color CCD (XC-555, Sony).

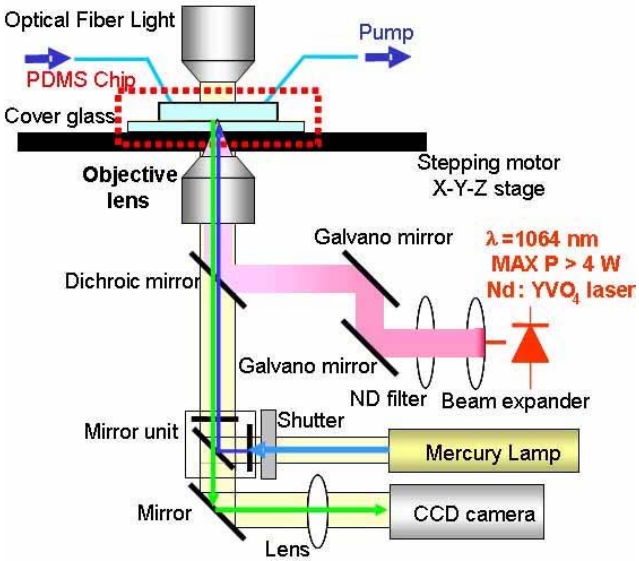


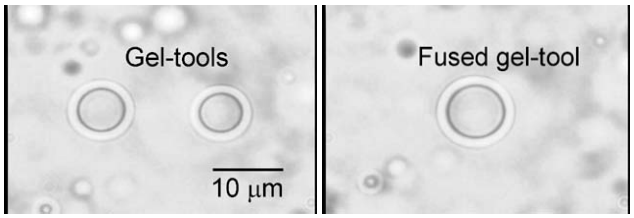
Fig. 9 Schematic of laser scanning micromanipulator

III. EXPERIMENTAL RESULTS

A. *Manipulation of salting-out gel tool*

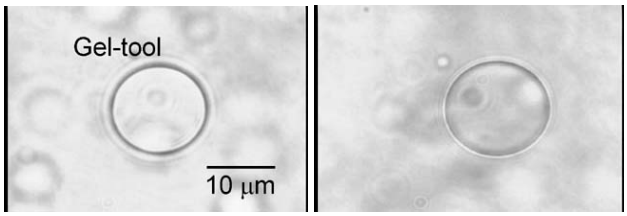
In experiments of this section, salting-out gel-tool is made of 1g ENT-3400 and 2g 20wt% KCl solution and image is acquired by monochrome CCD (XC-ST50, Sony). Fig. 10 shows fusion of salting-out gel-tool. Uncured gel-tools are fused and we can adjust the gel-tool size. Fig. 11 shows burst of salting-out gel. Burst gel can be used as a cell immobilization point because the salting-out gel adheres to cell. Fig. 12 shows binding cured gels. Cured gel-tools are bound by contacting other gel and the bonds of the gels are kept in the water solution. Fig. 13 shows cell manipulation using salting-out gel-tool using optical tweezers. We could bind the gel-tool to a yeast cell and transport the cell at 150 μm/s. After transport, we can release the cell by bursting the gel-tool. If immobilization of the cell is needed, we can

immobilize the cell by contacting the burst gel. We can also immobilize the cell on the arbitrary point of the gel-tool. As shown in Fig. 14, we fixed the cured gel-tool at a desired point on the glass plate and we manipulate a yeast cell and immobilized the cell on the gel-tool. The gel-tool is dyed by congo red for improving visibility of gel-tool.



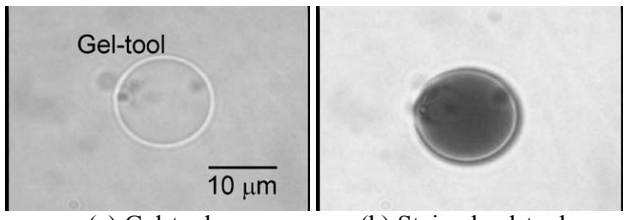
(a) Trapped gel-tools (b) Fused gel-tool

Fig. 10 Fusion of the uncured gel microtool



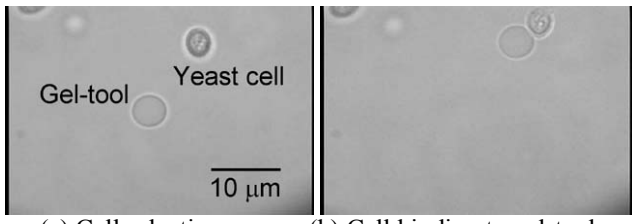
(a) Trapped gel-tool (b) Fixed gel-tool

Fig. 11 Fixation of gel-tool on the glass plate

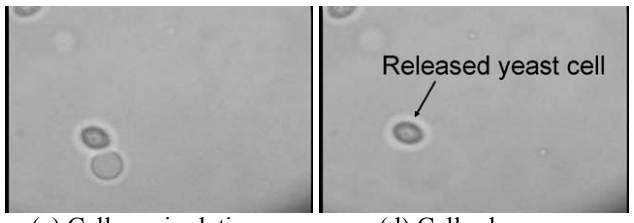


(a) Gel-tool (b) Stained gel-tool

Fig. 12 Staining cured gel-tool

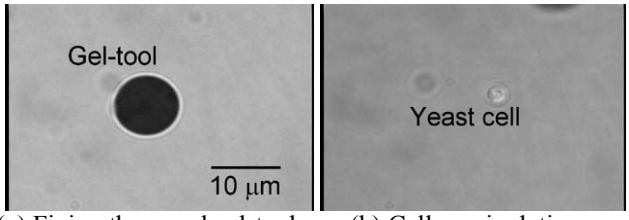


(a) Cell selection (b) Cell binding to gel-tool

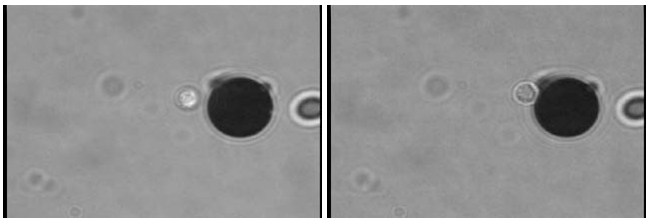


(c) Cell manipulation (d) Cell release

Fig. 13 Cell manipulation and release of microtool



(a) Fixing the cured gel-tool (b) Cell manipulation



(c) Cell immobilization (d) After immobilization

Fig. 14 Cell immobilization using gel-tool

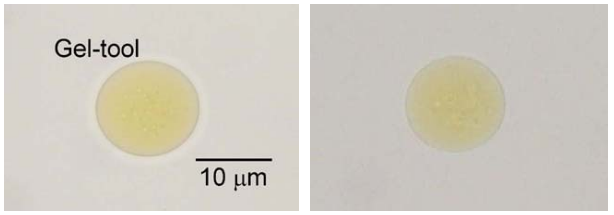
B. Calibration of pH sensing gel tool

A color of pH sensing gel-tool is obtained as RGB information by color CCD. Fig. 15 shows photographs of pH sensing gel-tools. RBG values are influenced by brightness which is included in each RGB value. To prevent the influence of brightness, RGB information was converted to YCrCb information by equation 1. Y value shows brightness, Cr value shows a color difference of red, and Cb value shows a color difference of blue. Cr value decreases with increase of pH value. On the other hand, Cb value increases with increase of pH value. The pH value can be obtained by calibrating Cr and Cb values against a pH of the solution.

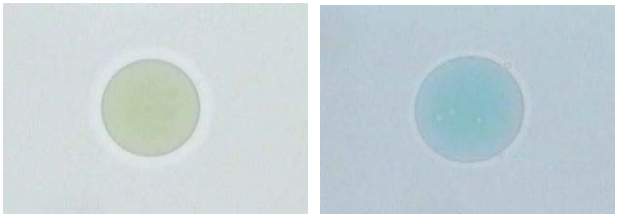
Before pH measurement, we calibrated the pH sensing gel-tool. The color of gel-tool was obtained in the state that focus was adjusted in the equatorial plane. Calibration results are shown in Figs. 16, 17. CX-555 (Sony) was used all experiments. Color temperature of CCD was 3200K, Gain was zero, Gamma was on, and illuminance was adjusted at 2000 lux. Sample pH values are 5.8, 6.7, 7.8 and 9.0. The sizes of sample gel tool were 5-15 μmϕ.

pH value is decreasing monotonously with increase of Cr value. The pH value is increasing monotonously with increase of Cb value. There are proportional relations between pH and Cr and between pH and Cb. Equations 2, 3 showed the linear approximation formula in Figs, 16 and 17. Both correlation coefficients of Figs. 16 and 17 are about 0.97. The dispersion of Cb is larger than that of Cr. The maximum dispersion of each pH in Fig. 16 is about 0.1 and maximum difference of pH value from equation 2 is about 0.3. On the other hand the maximum dispersion of each pH in Fig. 17 is about 0.25 and maximum difference of pH value from equation 3 is about 0.6. Therefore, we employed Cr value and equation 2 to calculate pH value.

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



(a) pH 5.8 (b) pH 6.7



(c) pH 7.8 (d) pH 9.0

Fig.15 Color shift of pH sensing gel-tool

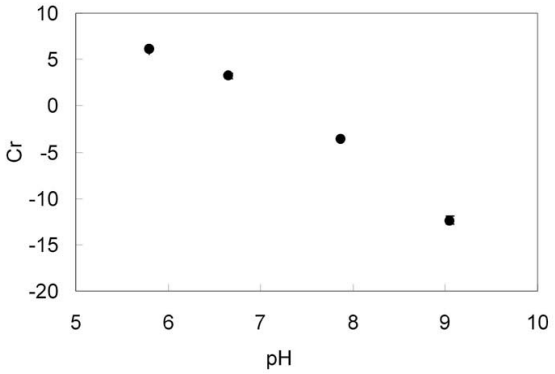


Fig. 16 Relation between pH and Cr value

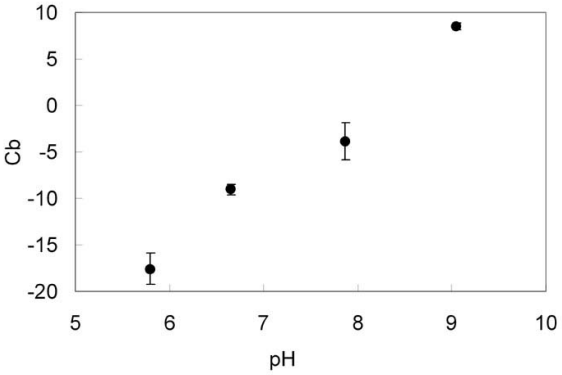


Fig. 17 Relation between pH and Cb value

$$pH = -1.7\times10^{-1}\times Cr + 7.1 \quad (2)$$
$$pH = 1.3\times10^{-1}\times Cb + 8.0 \quad (3)$$

C. Active pH sensing by pH sensing gel tool

pH value in the microchip was measured actively by pH sensing gel-tool controlled by optical tweezers. I scanned 8 μmϕ gel-tool between pH 6 and pH 9 in a range of 200 μm as shown in Figs. 18, 19. And the color of gel tool was measured every 50 μm. Gel-tool could be manipulated at over 150 μm/s. The result of measurement was shown in Figs. 20. pH value was calculated from equation 2. Active pH sensing was confirmed by manipulating pH sensing gel-tool by optical tweezers.

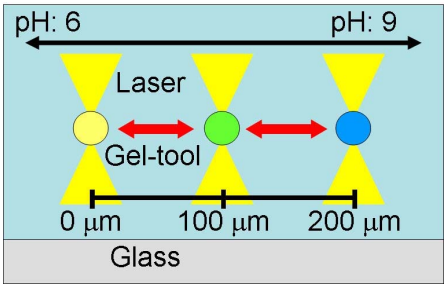
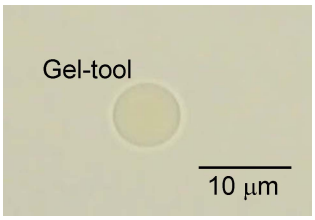
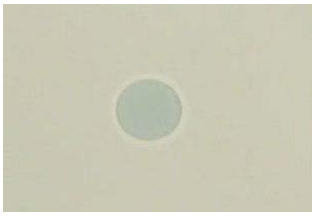


Fig. 18 A schematic of active pH sensing



(a) Start point (b) 100 μm moved



(c) 200 μm moved (d) Return to start point

Fig. 19 Active pH sensing

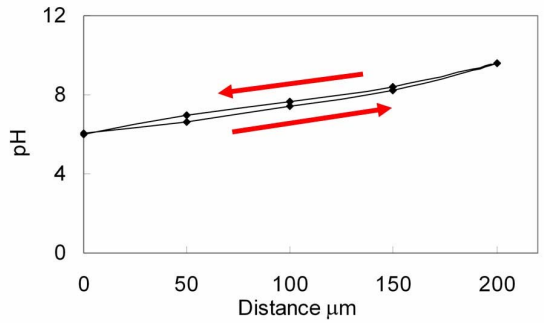
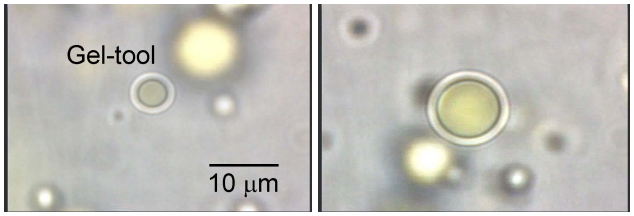


Fig. 20 Change of pH value

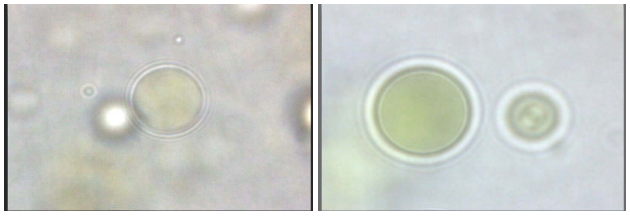
D. pH sensing by sensor pattern of pH sensing gel tool

Fig. 21 shows the experiment result of pH measurement by the gel sensor array in the microchip. After the size

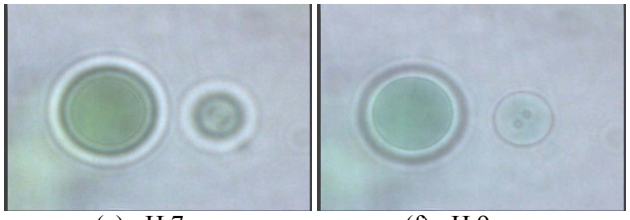
adjustment of gel-tool and polymerization by UV-ray illumination, gel-tools were positioned on the glass. Adhered gel-tools did not be removed from glass even by 894 mm/s flow speed. The pH sensor pattern of gel-tools in the microchip was constructed by positioning of gel tool repeatedly. Then the shift of their color depending on pH of a solution was observed. Local environment measurement was confirmed by manipulating and positioning pH sensing gel-tool in a microchip.



(a) Manipulation of gel -tool (b) Size adjustment



(c) Fixation of gel-tool (d) Gel-tool pattern (pH 6)



(e) pH 7 (f) pH 9

Fig. 21 On-chip pH sensing using pH sensing gel-tool

IV. Conclusion

Gel-tool sensor has been developed to measure the local pH in the microchip, and active pH sensing and pH sensing by the gel sensor array were demonstrated. Salting-out gel-tool which is generated by salting out the hydrophilic photo-crosslinkable resin was used as a carrier of a pH indicator. In this research, BTB was used as a pH indicator. pH sensing gel-tool is obtained with the simple and short process, which is only agitation of the mixture of hydrophilic photo-crosslinkable resin and BTB in the 20 wt% KCl solution. The obtained RGB color information was converted to YCrCb information. Converted color information was calibrated against the pH of the solution. Our proposed gel microbead sensor has near accuracy against a commercial pH meter. This measurement method enables us to make the on-chip measurement easy and it will make great contributions for cell biology.

Acknowledgment

This research is supported by The Ministry of Education, Culture, Sports, Science and Technology Grant-in-Aid for Scientific Research (17040017).

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