An Integrated Micro-Electrophoretic Chip Fabricated Using a New Stereolithographic Process

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ABSTRACT

We have fabricated an integrated micro-electrophoretic chip by implementing an acrylic microfluidic channel directly on top of a photosensor array using a new micro-fabrication process. The cross section of the microchannel is 100 μm high × 100 μm wide, and the effective length is 4.5 cm. The photosensor consists of 147 × 147 pixels, each measuring 39 μm × 39 μm. The integrated microchip is able to detect electrophoretic signals in real time along the whole microchannel as a two-dimensional image. The microfabrication process named "stereolithography with double controlled surface (SD method)" has been newly proposed in order to realize a highly transparent micro-channel with a smooth surface without assembly processes such as bonding of two plates. The accuracy of fabrication is within 5% of the design values.

We have also evaluated the performance of the fabricated microchip and confirmed its functionality. After a sample (Blue Dextran) was injected into the microchannel, significant values of absorbance were obtained from the photosensor along the whole microchannel. The absorbance was proportional to the concentration of Blue Dextran.

1. INTRODUCTION

Recently, miniaturized total chemical analysis systems (μ-TAS) implementing various kinds of chemical components such as fluidic channels and chemical reactors on a micro scale have been developed. This concept has attracted considerable attention worldwide because it is possible to reduce the analysis time and simplify analysis processes. Further extension of the concept has been proposed as a micro integrated fluid system (MIFS) [1,2], which integrates these chemical components and a semiconductor sensor for signal detection. It has the potential to develop new analysis methods.

In this study, we present a novel micro-electrophoretic chip. The microchip is based on the concept of the MIFS and integrates a capillary (microfluidic channel) and a signal detection device (semiconductor sensor) into one chip for capillary electrophoresis [3], which is one of the separation and analysis techniques with high speed and high resolution for DNA and proteins. A large number of papers on electrophoretic microchips have been reported [4-6] so far. However, with conventional microchips, all the samples

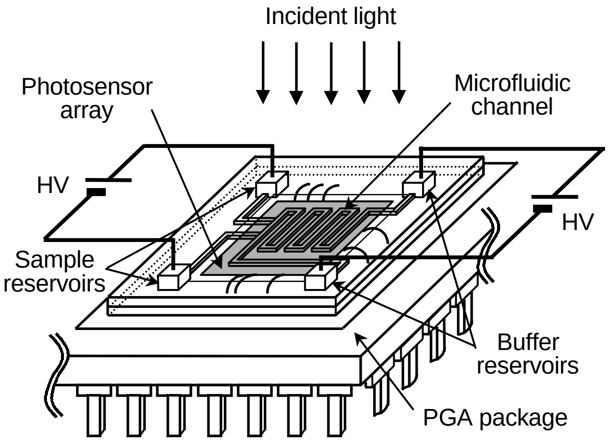

Figure 1: Concept of an integrated micro-electrophoretic system combining a microfluidic channel and a photosensor array.

have to reach the end of the microchannel to be detected even after separation is completed, resulting in a long analysis time. The proposed micro-electrophoretic chip shown in Figure 1 is able to detect signals in real time along the whole microchannel as a two-dimensional image because the microchannel is implemented directly on top of a photosensor array. Therefore, the analysis process is accelerated and simplified for capillary zone electrophoresis and isoelectric focusing.

As one of the fabrication methods of the microchannel, etching of a glass substrate [4,5] or casting of silicone elastomer (PDMS) from a microfabricated master mold [6] have generally been used. Although these methods enable us to easily fabricate two-dimensional structures, they require assembly processes such as bonding of two plates for complicated three-dimensional structures such as the microchannel. Other fabrication methods using a sacrificial etch technique [7] or stereolithography [1,2,8-10] have also been reported. These methods do not require the assembly processes, unlike the casting of PDMS and the etching of glass substrates. However, the sacrificial etch process is rather complicated. Stereolithography has also the serious problem that an opaque ceiling with a rough surface is produced due to a lack of resin surface control whenever three-dimensional structures with voids like the microchannel are fabricated. A rough surface is not desirable in optical signal detection since it causes the dispersion of an incident light. Therefore, a new microfabrication method is needed in order to realize a microchannel with high quality.

A microfabrication process named "stereolithography with double controlled surface (SD method)" has been newly proposed here. The SD method is based on the conventional stereolithography, which has advantages compared with other fabrication processes. With the SD method, the surface of the liquid resin is controlled in order to achieve a highly transparent microchannel with a smooth surface. Furthermore, an integrated micro-electrophoretic chip has been fabricated by implementing an acrylic microfluidic channel directly on top of a photosensor array using the SD method. In this paper, the stereolithography with double controlled surface and the integrated microchip are presented.

2. STEREOLITHOGRAPHY

2.1. Principle

Stereolithography is one of the technologies to fabricate three-dimensional microstructures by solidifying a photopolymerizable resin layer-by-layer via the scanning of a laser beam. The principle of the method is shown in Figure 2a. First, a three-dimensional structure is designed and an arbitrary number (*n*) of two-dimensional sliced shapes of the final structure, the so-called "slice data", are created using a CAD/CAM system. Then, a first layer is fabricated by solidifying the liquid resin via the scanning of the laser beam according to the coordinate information in the slice data. After lifting the *Z*-stage up one layer with the solidified first layer, a second layer is fabricated on top of the first layer in the same way. The final structure is completed by repeating the same operation until the final *n-th* layer is created.

The advantages of the stereolithography are as follows: (1) any complicated three-dimensional microstructure is easily fabricated without assembly processes; (2) microstructures with high-aspect ratio are produced [10]; (3) the fabrication process is simple, and the apparatus is cheap and small. However, when three-dimensional structures with voids such as a microchannel are fabricated, a rough surface is produced due to a lack of resin surface control (Figure 2b).

2.2. Apparatus

Figure 3 shows the schematic diagram of the stereolithographic system developed. The system consists of a resin chamber, *Z*-stage, *XY*-stage, mechanical shutter, ultraviolet light source (325 nm He-Cd laser system; TEM_{00}; max: 12mW), and light power controller with two ND filters. After the power of the laser beam is adjusted by the two ND filters and the beam is focused by the collimator unit, an optimal laser beam is projected from under the resin chamber where a quartz glass window is installed at the bottom. The exposure of resin to the laser beam is controlled by the mechanical shutter. The power can be dynamically adjusted to an arbitrary value in the range of 0.1 μW to 1 mW by the two ND filters. Both the *XY*-stage for the scanning of the laser beam and the *Z*-stage are controlled by stepping motors with 1 μm resolution. The stepping motors, mechanical shutter, and light power controller are commanded by programmed signals from a host computer.

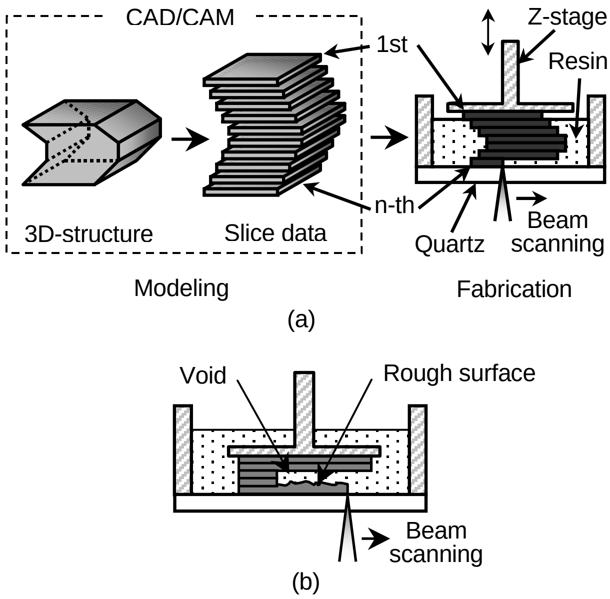

Figure 2: (a) Principle of a stereolithographic process. (b) Rough surface produced when three-dimensional structures with voids are fabricated using stereolithography.

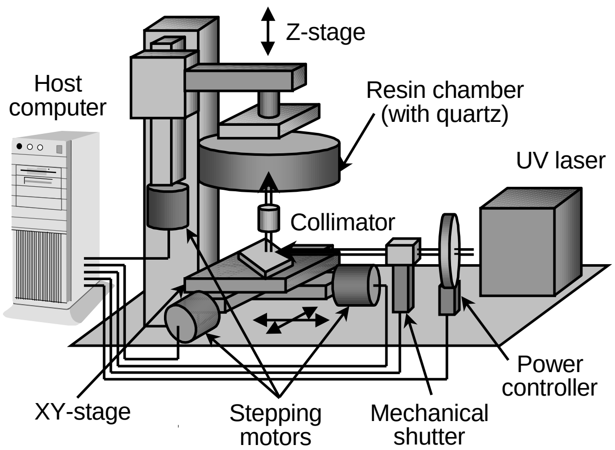

Figure 3: Schematic diagram of a stereolithographic system.

3. NEW STEREOLITHOGRAPHY

3.1. Concept

A stereolithography with double controlled surface (SD method) is described below. The concept of the new method is to control the resin surface using a metal plate having a smooth surface when the ceiling (cover plate) of the microchannel is fabricated. The surface conditions of the metal plate are molded onto the surface of the fabricated cover plate. Therefore, the SD method enables us to easily fabricate a highly transparent microchannel with a smooth surface, which is difficult to realize with conventional stereolithography because of a lack of resin surface control.

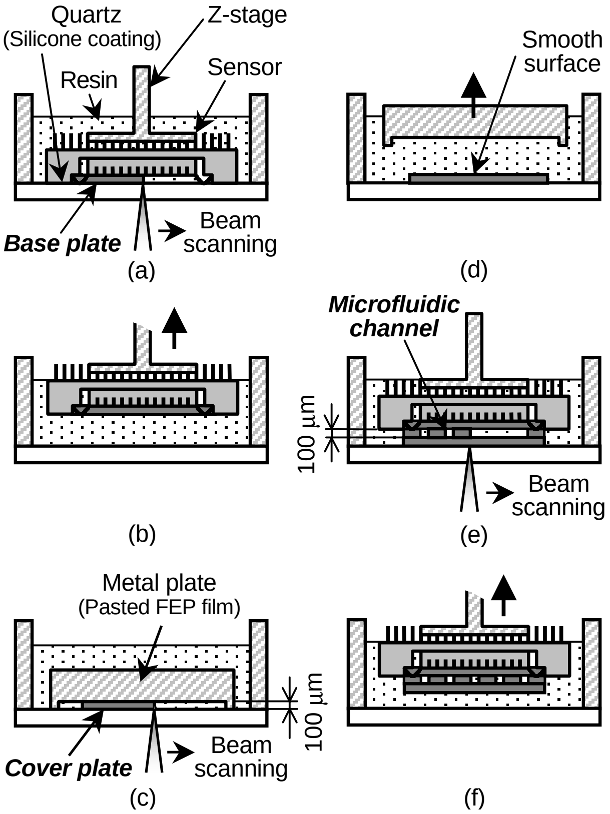

Figure 4: Fabrication procedure of the integrated micro-electrophoretic chip using a new stereo lithographic process with double controlled resin surface.

3.2. Fabrication Procedure

The fabrication procedure of the integrated micro-electrophoretic chip using the SD method is as follows (Figure 4).

Step (a) **:** The photosensor which is fixed in an inverted fashion at the bottom of the *Z*-stage is sunk into the resin chamber, and then a base plate is fabricated on the photosensor surface by the scanning of the laser beam projected from under the resin chamber.

Step (b) **:** The photosensor is lifted with the base plate.

Step (c) **:** After putting a metal plate into the resin chamber, a cover plate is fabricated between the metal plate and the quartz glass.

Step (d) **:** The metal plate is removed. At this time, the cover plate is kept on the quartz glass.

Step (e) **:** After bringing the photosensor back down close to the cover plate, a channel wall is fabricated between the base plate and the cover plate.

Step (f) **:** The completed structure is elevated off the quartz glass. Finally, the liquid resin is sucked out of the microchannel, and the channel is rinsed with ethanol and water by applying vacuum to one of the holes.

In this procedure, it is necessary to keep the structure on the photosensor surface covered with a glass in step (*b*) and (*f*), or on the surface of the quartz glass installed at the bottom of the resin chamber in step (*d*). For that purpose, the surface adhesion strength between each material used in the SD method and the acrylic resin has to meet the following condition: (the adhesion strength of the sensor

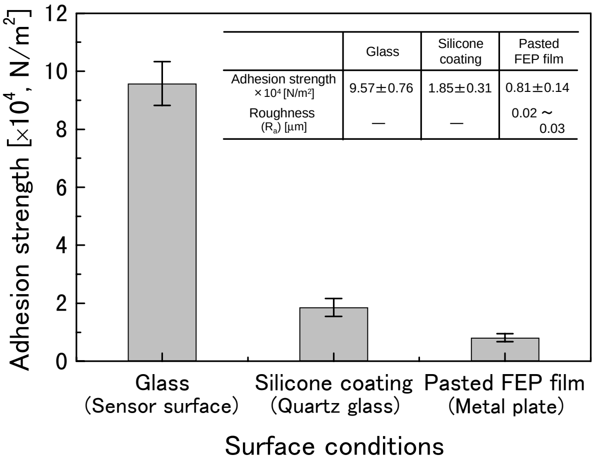

Figure 5: Adhesion strength measured for three materials.

surface) > (the adhesion strength of the quartz glass) > (the adhesion strength of the metal plate). It was achieved by coating silicone on the quartz glass and pasting an FEP film on the metal plate (Figure 5). In addition, it is important to keep the surface of the FEP film and the metal plate smooth since these surface conditions are molded onto the fabricated cover plate.

3.3. Evaluation of SD method

In order to evaluate the SD method, firstly, the cover plate fabricated using the SD method was compared with that fabricated using the conventional method in terms of surface roughness and transparency. The cover plates were fabricated under the following conditions: power of the laser beam (P_L)=0.1 mW; beam scanning speed (V_s)=2.2 mm/sec; beam radius (W_f)=123 μm; and the beam hatch spacing (h_s) was in the range of 1 μm to 200 μm. To evaluate the surface roughness of the cover plate, average roughness (R_a) was measured. For evaluation of transparency, a printed letter "*M*" was put under the cover plate, and the quality of the letter "*M*" was observed through the cover plate. Secondly, a test structure fabricated using the SD method was used in order to evaluate the accuracy of the SD method. The structure consists of several microchannels fabricated on the cover plate. The microchannel was designed to have a cross section of 100 μm high×100 μm wide. The cover plate was fabricated under the following conditions: P_L=0.1 mW; V_s=2.2 mm/sec; W_f =123 μm; and h_s=10 μm. The channel walls were fabricated under the following conditions: P_L=0.06 mW; V_s=2.2 mm/sec; W_f =123 μm; and h_s=10 μm. In addition, an acrylic photopolymerizable resin was used in this study.

3.4. Results and Discussion

Figure 6 shows the results of the surface roughness measurements. While R_a did not significantly decrease in the conventional method even if h_s was reduced, R_a became less than 0.1 μm when h_s/W_f<0.08 in the SD method. This result indicated that the surface roughness decreased more than 140-fold compared with the cover plate fabricated using the conventional method. The photographs in Figure 6 show the surface of the cover plates fabricated under the condition of h_s/W_f =0.08. It is clear that the surface

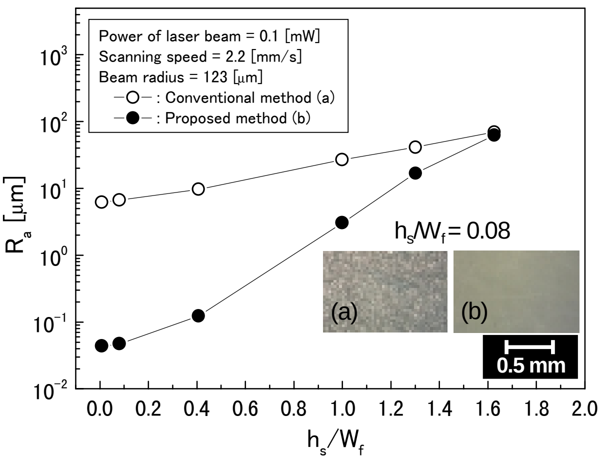

Figure 6: Results of surface roughness measurements (**R_a**: average roughness; h_s: beam hatch spacing; W_f: beam radius). Photographs show the surface fabricated using the conventional method (a) and the proposed method (b).

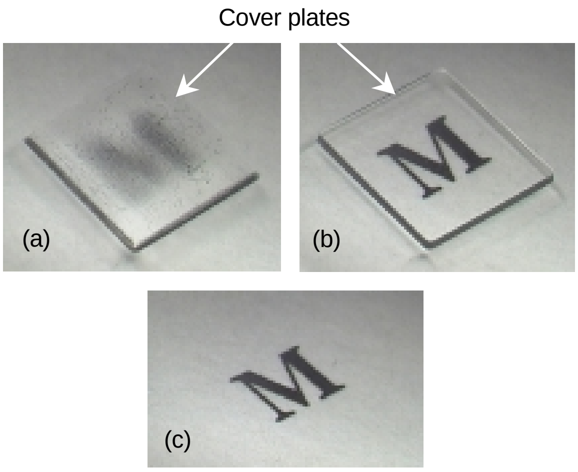

Figure 7: Photographs of the cover plates. (a) Fabricated using the conventional method. (b) Fabricated using the proposed method. (c) Print to show and to compare the quality of the plates.

condition was improved in the SD method. Figure 7 shows photographs of the cover plate fabricated using the conventional method (Figure 7a) and the SD method (Figure 7b). While the printed letter was illegible through the cover plate fabricated using the conventional method, the letter "*M*" was clearly observed through the cover plate fabricated using the SD method.

Figure 8 shows measurement results of a cross-sectional shape of the microchannel in the test structure shown in the inner photograph. The measured dimensions of the channel width and height were 99.8 ± 3.5 μm (n=8) and 95.2 ± 0.4 μm (n=8), respectively. The size accuracy with respect to the designed value was less than 5%. Moreover, the inclined angle of the channel walls was 85.9 ± 2.3 degrees (n=16), which was very close to perpendicularity. The inclined angle depends on the energy distribution of the laser beam in the liquid resin, which is determined by the fabrication conditions. As the inclined planes cause dispersion of the incident light, it is desirable that the

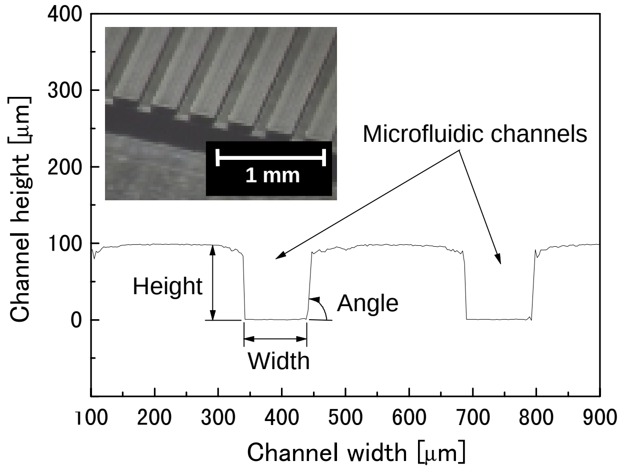

Figure 8: Evaluation of a cross-sectional shape of the microfluidic channels in a test structure shown in the inner photograph.

inclined angle is as close to 90 degrees as possible.

These results indicate that the SD method enables fabrication of a highly transparent microchannel with a smooth surface and high accuracy of dimension.

4. MICRO-ELECTROPHORETIC CHIP

4.1. Chip Design

Figure 9 shows the structure of the integrated micro-electrophoretic chip. The microchip is composed of an acrylic microfluidic channel and a photosensor array. The microchannel consists of a base plate, a channel wall 100 μm high, and a cover plate 100 μm thick. The distance between the bottom of the microchannel and the surface of the photosensor is 500 μm. The microchannel consists of the channel for electrophoresis and the channel for sample injection. The cross section of the microchannel is 100 μm high × 100 μm wide. The effective length of the microchannel for electrophoresis is 4.5 cm. The injection volume of a sample for electrophoresis is only 10 nl (100 μm high × 100 μm wide × 1 mm long). Holes of 1 mm × 1 mm in size were fabricated at the end of each micro-channel to interface with the reservoirs. The photosensor consists of 147 × 147 pixels, each measuring 39 μm × 39 μm. It was fabricated using a 2 μm CMOS technology. In order to keep the same relative position of the microchannel with respect to the photosensor over the whole microchip, the spacing between adjacent paths of the microchannel was chosen as 351 μm, which was a multiple of the pixel size of the photosensor. The microchip was fabricated using the SD method explained in section 3.2.

4.2. Experiments

In order to evaluate the basic functionality of the fabricated micro-electrophoretic chip, two types of experiments were carried out. Firstly, after the whole microchannel was filled with 25 mg/ml of Blue Dextran sample by applying vacuum to one of the holes, absorbance values of Blue Dextran with respect to distilled water were measured by the photosensor. The spread of an incident light was also evaluated to investigate the effect of diffraction and refraction at the microchannel. Secondly,

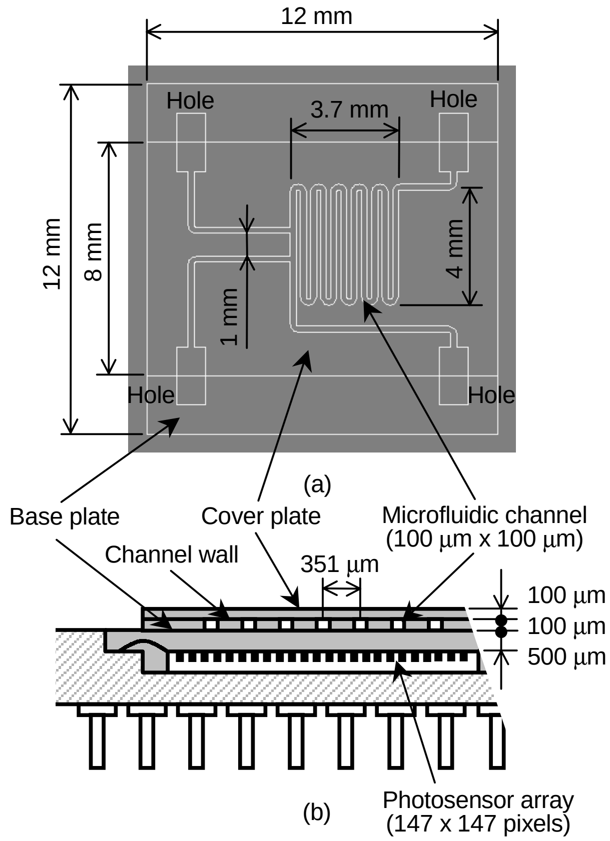

Figure 9: Structure of the integrated micro-electrophoretic chip. (a) Top view of the microfluidic channel. (b) Cross-sectional view of the integrated microchip.

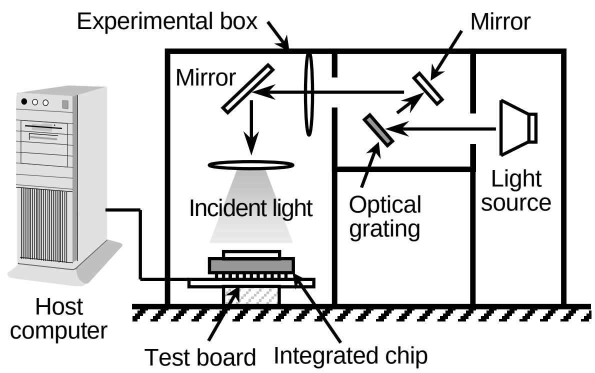

Figure 10: Experimental setup for optical measurements.

absorbance values measured by the photosensor were compared with the values measured by a spectrometer at various concentrations of Blue Dextran ranging from 5 to 25 mg/ml. Regarding the values obtained from the photosensor, an average of 11 measurements at different positions along the microchannel was used.

Figure 10 shows the experimental setup which consists of a light source (halogen lamp), a monochrometer (optical grating), and an experimental box. The fabricated microchip mounted on a test board with control circuits was fixed at the bottom of the experimental box. The monochromatic light was projected on the microchip. The

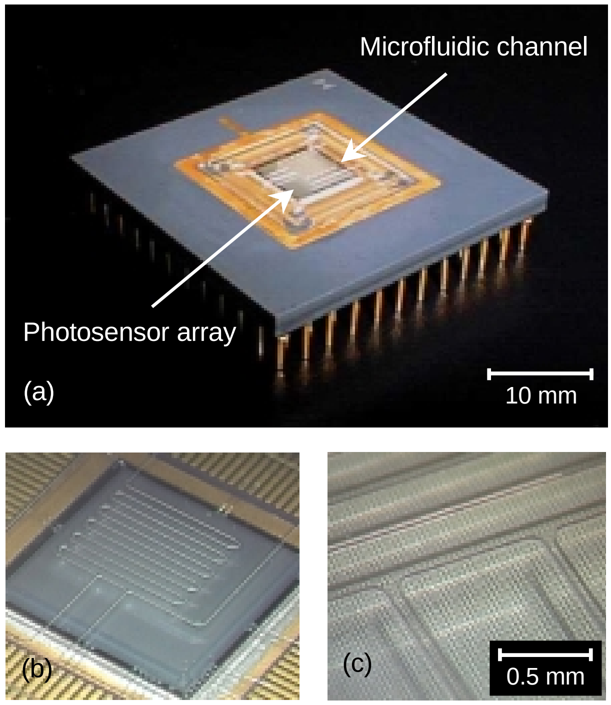

Figure 11: Photographs of the integrated micro-electrophoretic chip fabricated using the proposed method. (a) Complete view. (b) Close-up view of the microfluidic channel. (c) Close-up view of the microchannel for sample injection.

wavelength was set to 620 nm. The output voltages obtained from the photosensor were transferred to a host computer for further analysis.

4.3. Results and Discussion

Figure 11 shows the photographs of the integrated micro-electrophoretic chip fabricated using the SD method. Black dots observed below the microchannel in Figure 11c represent the photosensor pixels. The size of the microchip including the PGA sensor package is 3 cm wide × 3 cm deep × 0.6 mm high, and the size of the microchannel is 12 mm wide × 12 mm deep. The time required for fabricating the microchannel was only 2.5 hours.

Figure 12 shows photographs (top row) and images obtained from the photosensor (bottom row) depicting distilled water (left side) and Blue Dextran (right side) injected into the microchannel. Significant values of absorbance were measured for Blue Dextran only along the whole microchannel. These results indicated that there was no leak in the microchannel and that the photosensor was not damaged during the fabrication of the microchannel.

Figure 13 shows the absorbance values obtained from the pixels located on the line shown in Figure 12. Closed circles on the graph represent the signals obtained from the pixels of the photosensor, and the 11 peaks correspond to the microchannel. Each peak consists of signals from three or four pixels. There should be two or three pixels under the microchannel theoretically, considering that the micro-channel and the sensor pixel are 100 µm and 39 µm in width, respectively. This result suggests that diffraction and refraction of the incident light at the microchannel is negligible.

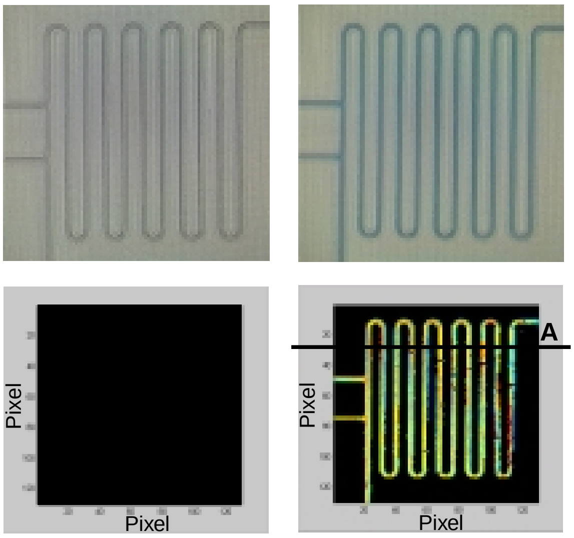

Figure 12: Photographs (top row) and sensor images (bottom row) of distilled water (left side) and Blue Dextran (right side) injected into the microfluidic channel

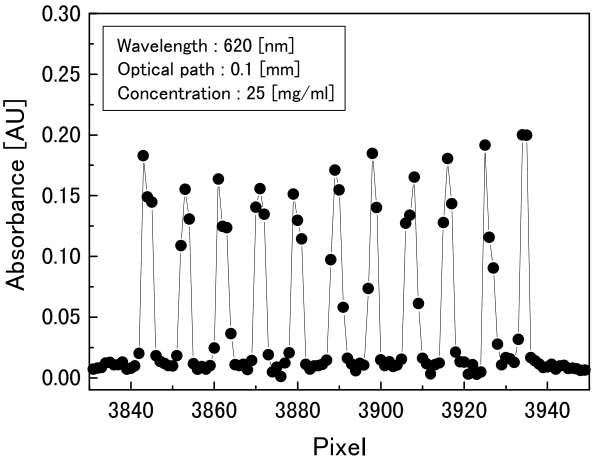

Figure 13: Absorbance values obtained from the pixels located on the line shown in the figure 11.

Absorbance values at various concentrations of Blue Dextran obtained using the fabricated microchip and a spectrometer are shown in Figure 14. Absorbance values obtained from the photosensor increased proportionally to the concentration of Blue Dextran. The efficiency of the signal detection was about 70% that of the spectrometer.

5. CONCLUSIONS

A stereolithography with double controlled surface (SD method) has been newly proposed in order to realize a highly transparent microfluidic channel with a smooth surface. Using the new method, an integrated micro-electrophoretic chip has been fabricated by implementing an acrylic microfluidic channel directly on top of a photosensor array, and its functionality confirmed. The microchip is able to detect signals in real time along the whole microchannel as a two-dimensional image.

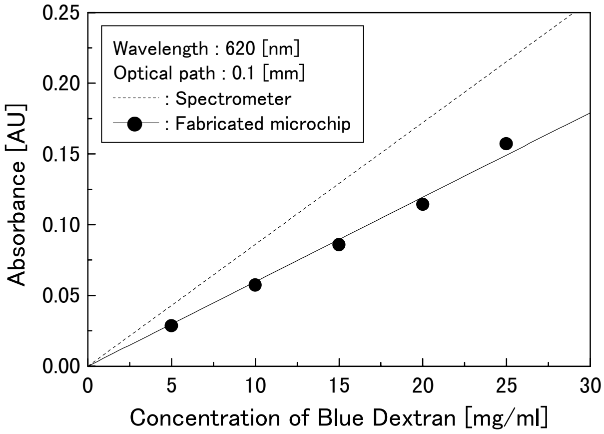

Figure 14: Absorbance at various concentrations of Blue Dextran obtained using the fabricated microchip (solid line) and a spectrometer (broken line).

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