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Micro-Polymerase Chain Reaction Chip Construction for Fluorescence Detection Test

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Abstract. This paper proposes the optimal structure of a Printed Circuit Board-based micro-PCR chip constructed on a PCB substrate using commercial adhesive tapes and plastic films. The proposed micro-PCR chip was composed of four layers. In a previous experiment, the chip had been made with a green PCB, yielding an unwanted reflection that prevented accurate analysis. In this study, the solder mask of the PCB substrate was coated black for a higher signal-to-noise ratio, and the area where the reaction chamber was attached was legend-printed with white silk for better reflection of the fluorescence light. The top cover was also colored black to minimize the noise during fluorescence detection.
As a result of carrying out PCR, it was found that the fluorescence brightness increased with increasing numbers of cycles.

Keywords: Micro-PCR chip, Double-sided tape, Polymerase Chain Reaction, Acrylic adhesive, Microfluidic channel, Fluorescence Detection Test, Black PCB

1 Introduction

A variety of inexpensive and disposable lab-on-a-chip (LOC) devices are being developed for miniaturization, integration, and automation and for point-of-care diagnosis of routine biochemical process [1, 2]. LOC devices are used for a variety of applications in biotechnology, medical diagnosis and treatment, and basic research [3, 4]. The most important characteristics of LOC devices are their ability to stably process body fluids of cells and aqueous solutions of biomolecules, their cost-effectiveness, and their ability to process smaller amounts of samples [1, 2, 5, 6, 7]. Because of these requirements, development of microfluidic channels has been actively carried out to process such small amounts.

In a previously reported study, a micro-PCR chip fabricated with double-sided tape showed results similar to those of regular tube-type PCR chips. However, in the regular PCR chip, PCR amplification products can be confirmed at the end point only through the electrophoresis process; however, there is high risk of contamination in

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the electrophoresis process. However, the real-time PCR method is a technology that can monitor and analyze the increase of PCR amplification products in real-time. Moreover, its advantage is that accurate quantification of DNA and RNA is possible, allowing quick and easy analysis because electrophoresis is not necessary, and the risk of contamination is low as compared to the conventional PCR method. An experiment was carried out by changing the color of the PCB, the chip substrate, to matte black in order to decrease the signal-to-noise ratio (SNR) that occurs when light is reflected in a PCR chamber during fluorescence detection in the process of implementing a real-time PCR with a biochip. In this study, by conducting the fluorescence detection according to the PCB color, the frequencies of fluorescence brightness are shown. By carrying out a PCR with a new chip structure using a black PCB, the results for PCR performance are shown as a function of the frequency of fluorescence brightness for each cycle. In Section 2, the micro-PCR driving system and black PCB-based structure are discussed, and in Section 3, the results of carrying out brightness experiments using a conventional chip and a PCR cycle with the proposed chip are presented. The conclusion is summarized in Section 4.

2 Black PCB-based micro-PCR chip

The local-host structure using PC is chosen for the chip control system architecture. The PCR chip's temperature is controlled in the local system through temperature measurement of the chip thermistor and the periodic control of the heater and fan.

The local-host system using the PC has the advantage of reducing the total cost because major functions can be performed in the host [8, 9, 10, 11].

The micro-PCR chip proposed in this paper consists of a four-layer structure. At the very bottom, a heating circuit for heating, with the PCB base and a thermal sensor for sensing temperature, are attached [12, 13, 14].

To prevent a fluorescence substance from sticking to the PCB, a box tape of PP material was attached. To set the height of the microfluidic channel to 400 μm, 200-μm-thick double-sided tape was attached in two layers. The chamber was fabricated by covering it with a 180-μm-thick PP material cover.

In the case of implementing a real-time PCR system with the conventional chip structure, when light was received in the fluorescence detector at a green PCB, the light was reflected, which acted as a noise, which reduced the overall signal-to-noise ratio (SNR), which adversely affected the measurement. To prevent the reflection on the parts other than the chamber, after cutting black matte aluminum tape into the diamond shape, it was attached on the cover. When DDW and PCR were finished, the brightness was compared.

When PCR was performed by fabricating a chip, because the black matte aluminum tape attached on the cover conserved heat, it became an obstacle to controlling the temperature, acting sensitively in the process of carrying out PCR. Therefore, the micro-PCR chip proposed in this paper used a non-light-reflective black matte-type PCB. White-silk legend printing was used only on the chamber where the fluorescence detection was performed to easily distinguish the change of fluorescence amount while performing the PCR cycle.

To prevent heat conservation inside the chip while performing PCR, the cover was colored with a black marker pen, so that parts other than the chamber could be easily distinguished.

A double-sided tape as the chamber in the micro-PCR chip, was the YT-7720(510) model manufactured by YOUNGWOO (South Korea). As reported in the past, it can withstand high temperatures of 95°C and pressure when expanding. The adhesive was acrylic adhesive, and the carrier of the double-sided tape was composed of PET. The chamber in which the PCR was performed in the middle was fabricated by drilling a hole in a diamond shape.

3 Experiments and Results

Images were captured with DSLR (Canon 1100D), and to detect FAM fluorescence, a blue LED (9600 mcd) was illuminated diagonally.The chip was fabricated into a structure in which an increase or decrease of frequency could easily be observed by printing a white-silk legend on the chamber to make the distinction of fluorescence measurement easy. In the experiment, the PCR process was carried out according to protocol. To carry out temperature control in the proposed system, the PCR process, i.e., the denaturation, renaturation, and extension processes, was carried out. A total of 40 cycles were carried out for 3 min at 95°C, 15 s at 95°C, and 1 min at 60°C.

As for the reagent used in the PCR, the experiment was carried out by adding 1 ng/11 μL of DNA (Chlamydia trachomatis), 12.5 μL of master mix, 10 pM/0.5 μL of primer F, 10 pM/0.5 μL of primer R, and 10 pM/0.5 μL of probe.

The images captured after performing DDW and PCR in the 400 μm chamber by changing the TV value. With the green PCB, there is some reflection, and the differences of fluorescence brightness cannot be easily distinguished. However, the fluorescence brightness of the PCR products of DDW and CT (Chlamydia trachomatis) DNA can be distinguished.The photographs of the changes of fluorescence brightness of the PCR cycle performed with the PCR protocol provided above were captured with DSLR whenever a cycle was proceeded at the stage of 72°C (DNA extension state of PCR) which was the middle amplification part in the PCR process excluding the initial stage. Because the images were captured manually, photographs could not be taken for about three cycles. The brightness of fluorescence increased as the PCR progressed.

The mean and Bhattacharyya distance, excluding the median value, all showed that as the PCR progressed, the brightness values decreased slightly as compared to those before PCR. After the 15th cycle, an exponential increase, but at the 27th cycle, the values sharply decrease. The values also decrease sharply at the 33rd cycle, and at the 35th cycle, a decreasing trend is shown again. Furthermore, although the mean and Bhattacharyya distance show similar patterns, the Bhattacharyya distance, expressing the distance to two distributions, shows a more exponential increase. At the latter half of the PCR cycle, the graph shows a saturated form. The brightness of fluorescence can be compared with the mean values of selected areas in the images cap-

tured for each cycle; however, because the quantitative brightness is difficult to show with only the mean values, it is also shown with Bhattacharyya distance.

4 Conclusion

It was reported in past studies that PCR is possible with a micro-PCR chip fabricated by using double-sided tape. It is sufficient to fabricate it with a green PCB, which is frequently used, when carrying out a conventional PCR. However, when carrying out a fluorescence detection test, the blue LED light is reflected by the PCB, which acts as noise and reduces the signal-to-noise ratio (SNR). Therefore, the noise was minimized by changing the color of PCB to matte black. While carrying out real-time PCR, the fluorescence brightness changes could be seen as a function of PCR cycle.

Because it was difficult to make a quantitative comparison by mentioning only the sum of the means, which is used frequently in the process of analyzing fluorescence brightness, the increase of fluorescence was analyzed by using the Bhattacharyya distance and comparing the similarities shown by a histogram of images. The Bhattacharyya distance showed more exponential increases than the mean values, and it was very similar to the curve drawn by the amplified amount of PCR products at the actual real-time PCR. If an exponential increase graph can consistently show real-time PCR results, it seems it would also be possible to carry out a real-time PCR with the proposed chip structure.

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