Chamber Temperature Measurement of Micro PCR Chip Using Thermocouple

Jong-Dae Kim[1,2], Chan-Young Park[1,2,*] , Sang-Yoon Kim[2,3], Ok-Dong Gwak[3], Deuk-Joo Lee[2,3], Yu-Seop Kim[1,2] and Hye-Jung Song[1,2]

[1] *Dept. of Ubiquitous Computing, Hallym University, Chuncheon, South Korea*
[2] *Bio-IT Research Center, Hallym University, Chuncheon, South Korea*
[3] *Dept. of Computer Engineering, Hallym University, Chuncheon, South Korea*

**Corresponding Author: cypark@hallym.ac.kr*

Abstract

The polymerase chain reaction (PCR) is a method utilized in most of the experiments handling genetic materials. PCR amplifies the target genetic material that is to be analyzed. This paper demonstrates the accuracy of the temperature measured by the thermistor. Since the thermistor is attached to the bottom of the micro PCR chip, there becomes a gap between the chamber and the thermistor. This experiment measures the temperature difference due to the gap. Micro PCR chip has a chamber made of double-sided tape and OHP film integrated upon a PCB substrate printed with a heater pattern. The thermistor to measure the chamber temperature is attached to the bottom of the substrate. In the experiment, a thermo-couple was inserted into the chamber directly to measure the actual temperature. The results showed no significant difference between the actual chamber temperature measured by the thermo-couple and the temperature measured by the thermistor attached at the bottom of the substrate.

Keywords: *polymerase chain reaction, micro PCR, temperature measurement*

1. Introduction

Polymerase chain reaction (PCR) is a technique to amplify a certain sequence of a complex DNA such as the human genome from a very small amount of DNA solution [1, 2]. Many PCR machines are available on the market. Recently, micro PCR which can amplify the sequence faster using less amounts of reagents conveniently than the conventional PCR machines is taking the center stage [3-9]. We have suggested a micro PCR chip which was designed to use the already existing PCB efficiently. The micro PCR chip has a chamber on top of a cover glass that was put on a PCB substrate [5-7]. The thermistor that measures the temperature is soldered at the bottom of the PCB substrate, where the heater pattern is printed [5, 7]. Therefore, the temperature measured by the thermistor might differ from the actual chamber temperature due to the gap between the thermistor and the chamber. However, it is difficult to measure the internal temperature of the chamber through common means. Since the chamber is made with a thermal tape, cover glass, double-sided tape, and OHP film on top of the printed substrate, the total height of the chamber is only about 400 ㎛. Thus, it is very hard to insert a common temperature measuring equipment [7].

This paper utilizes a thermo-couple to measure the temperature of chamber by inserting it between the cover glass and chamber of the PCR chip. Because the thermo-couple is made with a metallic wire, it is easy to change the shape and generally has a small diameter. Therefore it is predicted that if a wire is thin enough, the thermo-couple will be able to be inserted between the double-sided tape and the cover glass that makes up the chamber, or between the chamber and the substrate [10, 11]. The thinnest thermo-couple was selected to prevent the leakage of the solution during the PCR process that occurs when the thermo-couple was inserted inside the PCR chip. This enables the thermo-couple to measure the temperature periodically all throughout the PCR process. The measurement was executed in an embedded environment, identical to the actual micro PCR environment.

2. System Structure

The micro PCR system was constructed as a local-host system consisting of a PCR chip drive with an embedded environment, and a PC providing GUI to control the PCR. The diagram is illustrated in Figure 1.

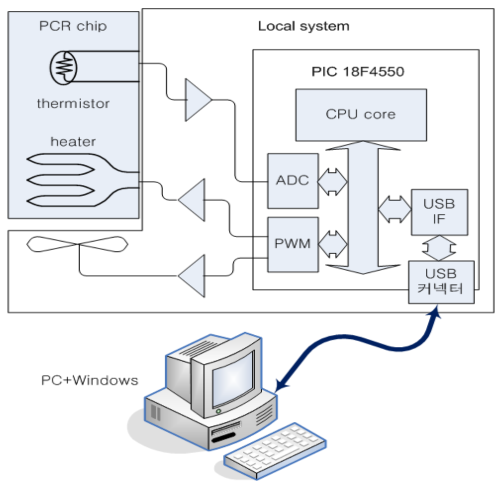

Figure 1. PCR Chip Drive System

The PCR chip drive system which corresponds to the local system can be divided into three parts: the thermistor that measures the temperature, the PCR chip with the heater that heats the chamber, and the embedded board that receives the command from the host PC and controls the PCR. We select the PIC18F4550 system for the local system. It is an 8-bit microcontroller with all of ADC, PWM, and USB interface. ADC is used to read the temperature from the thermistor, and PWM controls the heater and the fan to heat and cool down the PCR chip, and USB interface is used for the communication with the host PC.

The PCR chip is constructed with a thermistor attached to a PCB having a printed heater pattern [3, 7] and a slide glass is attached on top of the PCB using a thermal tape [6]. The chamber is made from a double-sided tape on top of the slide glass, and OHP film made of PET was employed to cover the chamber [7]. Figure 2(a) illustrates the diagram of the PCR chip.

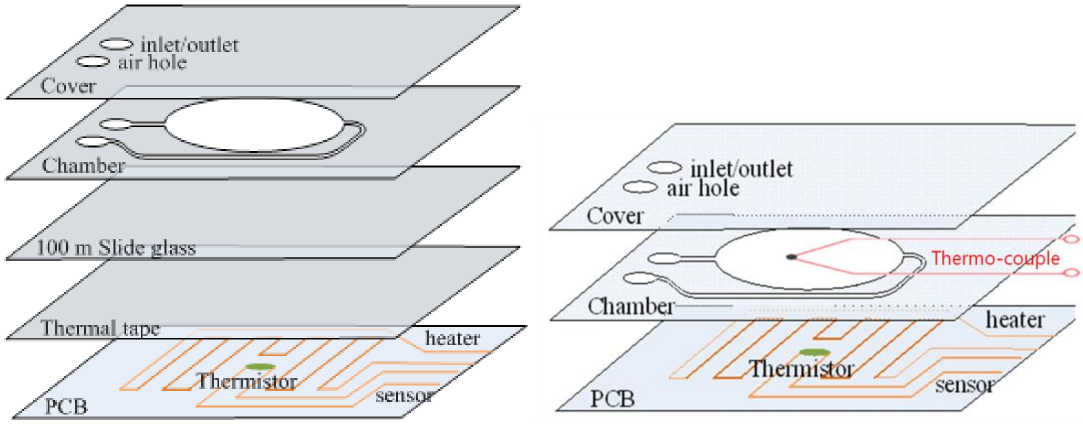

Figure 2. (a) PCR chip diagram
(b) Disposition of the inserted thermo-couple

In the aforementioned local system, the temperature of the chamber is measured by the thermistor attached to the PCB. The temperature is read using the thermistor and ADC and transmitted to the host PC through the USB interface.

GUI was implemented in the host system using MFC to visualize the chamber temperature. The temperature was measured in a 200ms period, in order to match the conversion time of MAX6674 [12]. Since micro PCR runs in the unit of 50ms and has a rapid temperature change of 10℃ per second due to its low heat capacity[7, 9], it is most likely to predict that the optimum time period should be less than 100ms for the most efficient temperature change. Nevertheless, 200ms is considered to be sufficiently short enough to measure a 2~3℃ change per second. Based on the temperature measured, the host PC controls the PCR process.

However, as can be seen in Figure 2(a), the thermistor and the chamber are not in contact directly having a slide glass and the PCB in between. This might cause an error in the measurement of the temperature. To check and overcome such problems, a method of measuring the actual temperature of the chamber by inserting a thermo-couple is presented.

Thermo-couple consists of two different types of metallic wires that are welded at a single junction. It measures the temperature using the thermoelectromotive force produced when a temperature difference is applied to the junction. The specific thermo-couple utilized in this experiment, which was flexible and thin enough to be inserted in the chamber, was chosen from a common K-type thermo-couple.

The thermo-couple was inserted both on top of the chamber between the cover OHP film and on the bottom of the chamber between the slide glass. The disposition of the inserted thermo-couple is shown in Figure 2(b).

The inserted thermo-couple measured the temperature using the MAX6674 control chip. The contact terminals of the thermo-couple and the input pins of MAX6674 should be patterned with copper or anything of the same sort in order to prevent noise [12]. MAX6674 was attached to the substrate of the drive system part of the PCR chip. The local system reads the temperature measured by the thermo-couple from MAX6674 and transmits the data to the host PC. Figure 3 shows the local system after the thermo-couple was applied.

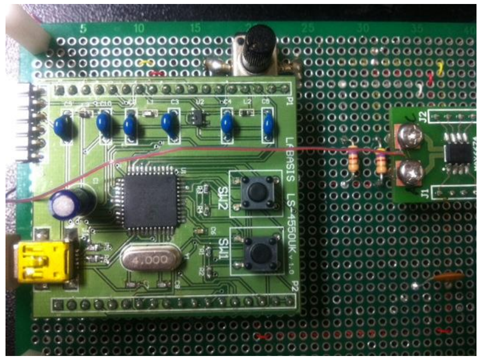

Figure 3. Local System

3. Experiments and Results

This paper demonstrates a method using a thermo-couple to measure the internal temperature of the chamber during the PCR process of the PCB based micro PCR. The PCR process was conducted with water inside the chamber where the thermo-couple was inserted. The micro PCR system and the thermo-couple system were connected to the host PC with an USB connector. During the micro PCR process, the micro PCR system records the temperature measured by the thermistor attached at the bottom of the PCB substrate, and the thermo-couple system records the internal temperature of the chamber. To eliminate the possibility of errors that might occur due to the thermo-couple itself, the reliability of the thermo-couple was verified beforehand by measuring the temperature of the water inside a constant-temperature water bath for a certain amount of time. When measuring the temperature of the water bath, the thermo-couple was protected with a thin waterproof plastic bag, and coins were used to give weight to the bag to prevent it from floating up. Each section of the temperature graph showed that the temperature measured by the thermo-couple inside the constant-temperature water bath was generally 2℃ higher than the actual temperature shown in the display of the water bath. In the high temperature range (90℃), the temperature measured by the thermo-couple was 4℃ higher. However the standard deviation of the temperature in overall was within 1℃, and in the low temperature range the measured temperature differed within 0.5℃.

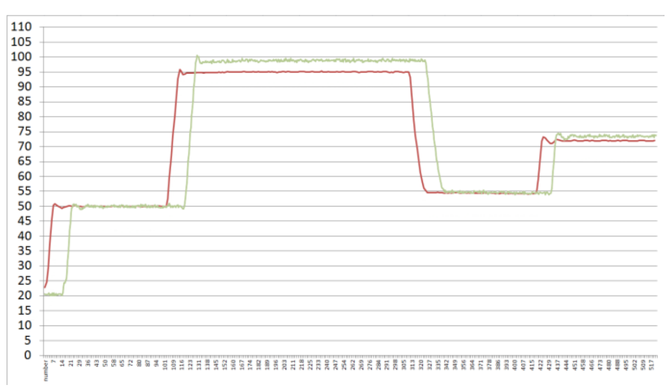

Figure 4. Measured temperature of micro PCR chamber

Figure 4 illustrates the results of the temperature measurement of the micro PCR. The red line indicates the temperature measured with the thermistor, and the green line is that with the thermo-couple. The insignificant temperature difference between the thermistor attached to the bottom of the PCB and the thermo-couple is thought to have resulted from the contact of the double-sided printed heater pattern to both the water inside the chamber and the thermistor. Since the heat is rapidly conducted to both sides, there is almost no difference in the temperatures measured by the thermistor and the thermo-couple. By applying a thermo-couple with the diameter of 25μm, the leakage of the chamber was prevented.

In Figure 4, a temperature difference of about 4℃ can be observed in the 90℃ section. However, regarding the results from the constant-temperature water bath which showed 4℃~5℃ temperature difference at 95℃, it can be said that there is no significant difference between the internal chamber temperature and that of the bottom side of the PCR chip.

Nevertheless, the fact that the experiment was done with water instead of the actual solution and excluded a slide glass attached on top of the PCB using a thermal tape in order to simplify the PCR chip should be considered. Thermal tape was adapted to provide fast and even heat conduction to the chamber from the heater pattern printed on the PCB substrate. The overall thickness including the thermal tape and slide glass was 150μm, which is thin enough for rapid conduction of heat. However, the chances of temperature difference due to the low heat capacity of the micro PCR chamber should be kept open.

4. Conclusion

The experiment verified that there was no significant difference between the temperature of the chamber measured by a thermo-couple and the temperature measured from the thermistor which was attached to the bottom of the PCR chip. The temperature measured with the thermo-couple showed higher temperatures than the actual temperature, as demonstrated in the thermo-couple verification experiment. The errors in the temperature could have occurred because the thermo-couple was manually made, or because of a contact failure between the PCB and the thermo-couple.

Such problems can be solved through calibration in the host system. Since the deviation of the measured and actual temperature increase linearly, calibration can be executed by software in the host. On the other hand, if the manually made thermo-couples show different error properties or values, it becomes very difficult to calibrate the temperature. Therefore, the calibration might be reliable only with the thermo-couple products that are out in the market.

In the future research, the presented method will be employed under the conditions where there is a possibility of a decrease in heat conduction to the chamber according to the different constructions of the PCR chip, such as the method of attaching the slide glass and so on. Additionally, the proposed scheme is applicable to measure the temperature difference due to different heat conducting properties of different materials rather than the PCB by constructing the chip with various materials.

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Authors

Jong-Dae Kim

He received the PhD degrees in Electrical Engineering from Korea Advanced Institute of Science and Technology, Seoul, Korea, in 1984 and 1990, respectively. He worked for Samsung Electronics from 1988 to 2000 as an electrical engineer. He is a Professor in Department of Ubiquitous Computing, Hallym University. His recent interests focus on biomedical system and bioinformatics.

Chan-Young Park

He received a B.S and M.S. from Seoul National Univ. and a Ph.D degree from Korea Advanced Institute of Science and Technology in 1995. From 1991 to 1999, he worked at Samsung Electronics. He is currently a Professor in the Department of Ubiquitous Computing of Hallym University, Korea. His research interests are in Bio-IT convergence, Intelligent Transportation System and sensor networks.

Ok-Dong Gwak

He received a B.S and M.S. in Computer Engineering from Hallym University. He is currently a Software engineer in Tobesoft Company, South Korea. His research interests are in the areas of bioinformatics, Bio-IT convergence, and server framework development.

Sang-Yoon Kim

He received a master's degree in Computer Engineering from Hallym University. He is getting a PhD in Computer Engineering at Hallym University

Duek-Joo Lee

He received a B.S degree in Computer Engineering from Hallym University. He is getting a master's in Computer Engineering at Hallym University.

Yu-Seop Kim

He received the PhD degree in Computer Engineering from Seoul National University. He is currently a Professor in the Department of Ubiquitous Computing at Hallym University, South Korea. His research interests are in the areas of bioinformatics, computational intelligence and natural language processing.

Hye-Jeong Song

She received the PhD degree in degree in Computer Engineering from Hallym University. She is a Professor in the Department of Ubiquitous Computing, Hallym University. Her recent interests focus on biomedical system and bioinformatics