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Temperature Change Analysis of Internal Channel and Outside Sensor for PCR Chips

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Abstract

This paper presents the analysis of the steady state error and the time delay of the temperature of the chip sensor in a micro PCR chip and that of the reagent. The chambers with the various heights and sizes are compared in order to examine the differences in temperature and speed. A NTC-thermistor was employed as the chip sensor and the thermocouples were used to examine the temperature of the reagent in the chamber. From the result, we confirmed that the steady state error was negligible for all of constructions and the delay times showed no difference in the range of 60–72℃, while in the other ranges, the speed was higher for the lower height construction.

Keywords: *microfluidic channel internal temperature, polymerase chain reaction, NTC-thermistor, thermocouple*

1. Introduction

In polymerase chain reaction (PCR), two strands of DNA are separated by the application of heat in denaturation; at a low temperature, the primer is annealed to the sequential terminal for amplification, and at slightly higher temperature, the DNA is synthesized—this process is called polymerization or extension. A test involving PCR is referred to as a PCR test. A PCR test is advantageous because only a small amount of DNA is needed to run a test, and it can be applied for diagnosing many diseases [1, 3-7]. However, current commercial PCR devices have disadvantages such as long testing time, poor transportability because of their large sizes, and high cost [1, 2]. With the active development of lab-on-a-chip systems, which are analytical systems that overcome the disadvantages of the existing genetic diagnostic techniques and allow on-site genetic diagnoses, for overcoming such disadvantages, micro PCR chips that contain microfluidic channels are also being developed [1, 2]. A lab-on-a-chip system employs technology such as the MEMS (Micro Electro Mechanical System) and performs all preprocessing and analytical steps, including the dilution, mixing, reaction, separation, and determination for a sample on a single chip [1, 2].

A micro PCR chip, one of the lab-on-a-chip systems, can quickly draw various pieces of genetic information with a very small amount of DNA, enables genetic analyses, and is used for diagnosing various diseases through reactions to the internal components of a test subject. A more accurate PCR test needs to secure reliability on reproducibility, sensitivity, and such other factors, for which an accurate temperature control and temperature sensor correction may be necessary, and the heater and temperature sensors need to be installed as close as possible to the chamber in order to control temperature [2].

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One of the most important factors involved in PCR amplification is temperature. Temperature control in micro PCR, which will be examined in this study, is achieved by means of the heating pattern of a micro PCR chip and by the temperature sensor of an NTC-thermistor (Negative Temperature Coefficient). If the temperature is effectively controlled, a quicker PCR test is possible even with the same protocol. Further, the miniaturization of PCR devices makes them better than the existing PCR devices by increasing transportability and portability, thus, increasing efficiency.

Because a thermistor is installed on the bottom of a micro PCR chip, however, it is necessary to confirm whether the temperature of a testing sample inside the chamber is actually the same as that of the temperature sensor. In this study, for experiments, we installed two thermocouples inside the chamber of the micro PCR chip and performed experiments to examine if the sensors show exactly the same temperature and to compare the times of delay of the two temperatures.

The temperature sensor used in the micro PCR chip was an NTC-thermistor with a resistance error of 1%. The NTC-thermistor was corrected for a more precise temperature measurement prior to use, and the characteristics of temperature conductivity depending on the thickness of a double-sided tape composing the chip chamber, *i.e.*, the thickness of the chamber, were examined.

This paper describes the materials and methods in Section 2, presents the results in Section 3, and summarizes the conclusions in Section 4.

2. Materials and Methods

2.1. Micro PCR Chip

The structure of the micro PCR chip that was examined in this study is presented in Figure 1(a). A heating pattern to heat up a sample in the chamber is layered above the PCB that forms the bottom layer of the chip. An NTC thermistor is attached below the bottom layer to measure temperature. Because the epoxy coating of the PCB is a PCR inhibitor, a polypropylene (PP) box tape is layered above the PCB that is overlaid with a double-sided tape. The double-sided tape is cut out in the middle in the shape of the chamber. A 180-μm PP film with inlet and outlet holes form the top layer of the chip.

The chip is installed in the local system of the host-local system, as shown in Figure 2. The local system measures and sends the temperature of the thermistor to the host system. Then, based on the target temperature, the host system calculates the PWM to heat up the heater, and the calculated PWM is sent back to the local system so that the PWM of the heater or fan can be set. A Windows PC was selected as the host, and the local system has PIC18F4550.

(a) Micro Fluidic Chip for Internal Temperature Measurement (b) Bottom of the Chip

Figure 1. Chip Structure

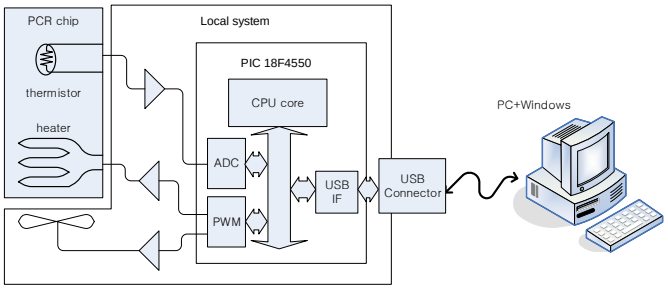


Figure 2. PCR Chip and Control System

The thermistor in the micro PCR chip has a resistance error of 1% and operates in the range -40–125℃ (NCP15XH103F03RC, Digikey). By referring to the resistance values for four temperatures (50, 60, 70, and 95℃), which were provided with the basic data sheet for the pertinent part number, we determined the Steinhart-Hart calibration coefficients and temperature values by using the Steinhart-Hart equation ($1/T=A+B \ln(R)+C(\ln(R))^3$).

2.2. Installation of the Thermocouples

In this study, in addition to measuring the internal temperatures of the chamber, two USB-type thermocouple readers (UTC-USB, Omega Engineering Inc.) were used to examine the time of delay in transferring the temperature between the surface temperature of the box tape (above the NTC thermistor) and the bottom temperature of the cover film: that is, the time delay in transferring the temperature from the bottom of a DNA sample to the top of the chip. The thermocouple was selected for its very thin wire (AWG 40; 5SC-TT-K-40-36, Omega Engineering Inc.).

If a thermocouple is installed inside a channel as it is, the thickness of its wire insulation will prevent the wire from being installed tightly, causing leaks. For preventing leaks, the wire insulation should be peeled off before installation, as shown in Figure 3. The insulation comprises two layers: one layer comprising a covering each for chromel—the plus wire—and aluminum—the minus wire, and the other layer covering the plus and minus wires together. Peeling off both layers will prevent leaks. If a bare thermocouple is used, the task of peeling off the insulation can be done away with; however, a connector will have to be installed with the thermocouple, and extra care will have to be taken to ensure that the extremely thin wires of the thermocouple are not broken. Moreover, the channel has to be trashed with the installed bare thermocouple because separating it from the channel is difficult after an experiment. Therefore, in this experiment, we selected 55C-TT-L-40-36, the thinnest product with a pre-installed connector. Figure 3 presents the micro PCR chip that we developed, considering the abovementioned information, with thermocouples installed inside the channel.

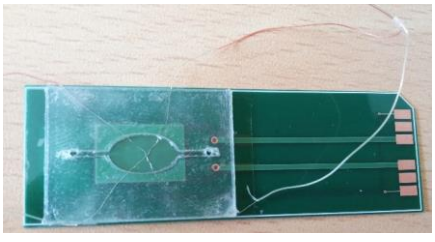


Figure 3. The Developed Micro PCR Chip with Two Thermocouples Installed Inside the Channel

2.3. Temperature Measurement Experiment

To examine the time of transferring the internal temperature of the channel and the bottom temperature of the channel to the top, as shown in Figure 4 and Figure 5, we created channels by installing two thermocouples, one placed above the box tape and the other below the cover film; the heights of the channels were 200 µm and 400 µm. In addition, for experiments with different amounts of samples inside each channel, as shown in Figure 6, chambers of three sizes—A, B, and C—were used. Table 1 presents the amount of sample in each chamber. During the actual experiments, the amounts of samples were slightly different because of the errors in cutting the shapes of the chambers on the box tapes.

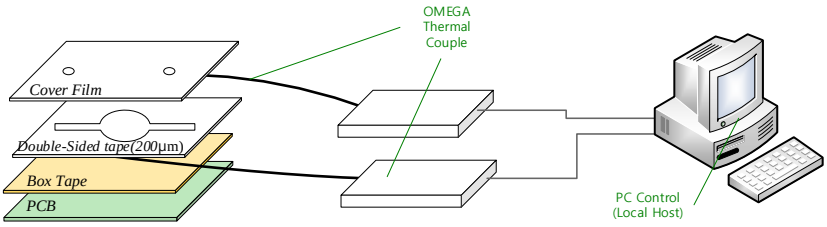


Figure 4. 200-µm Temperature Measurement Experiment

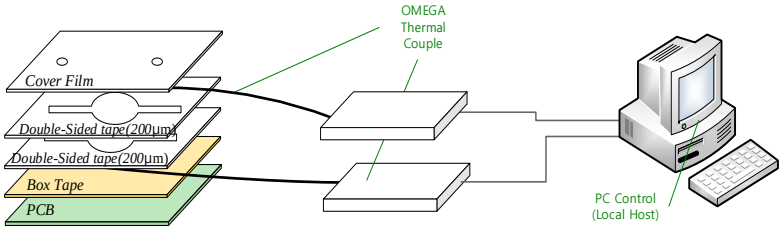
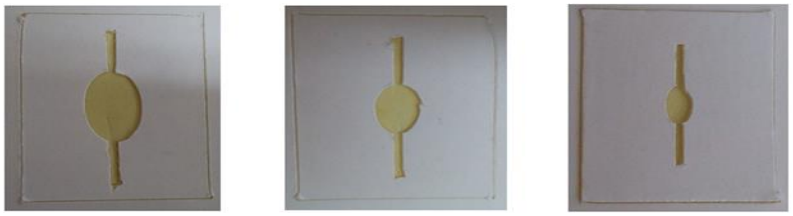


Figure 5. 400-µm Temperature Measurement Experiment



(a) Large Chamber (b) Medium Chamber (c) Small Chamber

Figure 6. Shapes of the Chambers by Size

Table 1. Amounts of Samples in Different Chambers

	A. Large	B. Medium	C. Small
200µm	17µl	9 µl	5 µl
400µm	34 µl	18 µl	10 µl

To examine temperature errors of the thermocouples, they were put in a constant water bath, and their temperatures were measured at 50, 60, 72, and 95℃. A TRH Central program, basically provided by Omega Engineering, to show the temperatures of a

thermocouple was used to examine temperatures, and we confirmed that the temperatures of the thermocouples were the same as those of the constant water bath. However, because the temperature resolution of the thermocouples 1℃, no precision error was found at an increment of 0.5℃. The temperature measurement of the TRH Central program was at intervals of 1 s, and hence, we used our own program to measure temperatures faster and to record the temperatures of the NTC thermistor and of the thermocouples simultaneously.

Figure 7 presents the GUI of our program for measuring the temperatures of the thermocouples and the NTC thermistor simultaneously. “Device State” at the upper left corner of the GUI indicates whether a device is connected, and “A1” area, which is shown in a blue box, indicates the connection status, temperatures, etc., of the two thermocouples connected to the serial port. That is, the temperature of a sample inside the chamber is indicated in this area. “Serial Port1” indicates the temperature in the upper chamber, while “Serial Port2” indicates the temperature in the bottom chamber. When “RS232 Recording” is clicked, the temperature values are filed. “A2” area indicates the temperature of the NTC thermistor. The experiment to measure the internal temperature was performed using the PCR protocol processing algorithm; therefore, “A3” area lists the protocol, which is necessary for measuring the internal temperature, as the PCR protocol, and “A4” area shows the buttons that control the experiment, including the start and stop of an experiment.

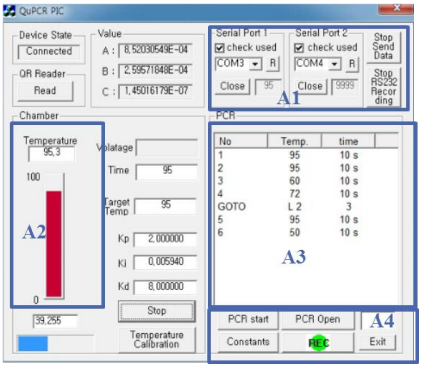


Figure 7. The GUI for Measuring Temperatures inside the Channel

The protocol that was used in the experiment for measuring the internal temperatures was composed of four cycles of the PCR protocol, as shown in Table2. The temperatures and times shown in the table were shortened values of the times and cycles of the protocol that is used in a PCR experiment.

Table 2. Protocol Used For Internal Temperature Measurement

PCR Process	Temperature(℃)	Time Duration
1 Cycle	95℃	10 s
4 Cycle	95℃	10 s
	60℃	10 s
	72℃	10 s
1 Cycle	95℃	10 s
PCR Process	50℃	10 s

3. Results

The graphs shown in Figure 8 indicate the temperature change for each chamber height, 200 μm and 400 μm. Regardless of the sizes of the chambers, all four PCR cycles showed

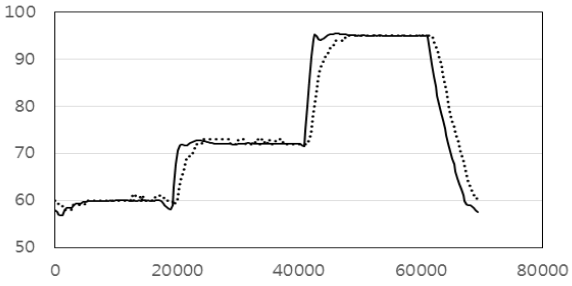
the same temperature change and had almost no steady state error; therefore, Figure 8 presents only the temperature change of the first cycle of one of the chambers. The horizontal axis indicates time in units of milliseconds, while the vertical axis temperatures in Celsius. As the graphs suggest, no steady state error in temperature was observed for both 200 μm and 400 μm. However, as expected, the temperatures of the chamber changed faster than those of the chip thermistor.

Figure 8 indicates that the time taken for temperature transfer for the greater chamber height appeared to be longer; Table 3 and Table 4 present the speeds of temperature changes in the 200-μm-high chamber and the 400-μm-high chamber by the chamber size to examine this phenomenon. As suggested by the data in these tables, excluding the event in which a fan was used to lower the temperature, when the temperature increased, the speed of temperature change of the thermistor was higher by more than a factor of two, and the speed for the 200-μm-high chamber was much higher than that for the 400-μm-high chamber.

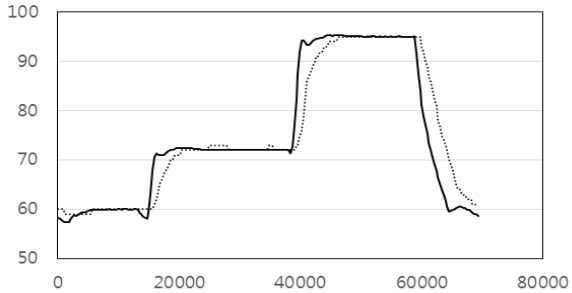
When the fan was used for cooling, the thermistor and thermocouples had a similar speeds in terms of temperature change in the case of the chip with a 200-μm-high chamber; this is attributed to the fact that the top and the bottom of the chip were cooled off at the same speed by the fan that blew air in the sides of the chip.

On the other hand, for 200-μm-high chamber, the speed of temperature change was not necessarily proportional to the size of the chamber; the speed in the medium chamber was the lowest. We attribute this phenomenon to the larger heating area of the large chamber because the dimensions of the heating pattern of the PCB substrate remained constant.

Because the overall temperature change in the chamber was insensitive to overshooting, even when the thermistor's temperature change was overshot, the thermocouples were not subject to overshooting. Because we received a quick reaction when the thermocouples were put in the pre-heated constant water bath, the reaction speed of the thermocouples was irrelevant. Therefore, during temperature control, it seems fine even if we control the temperature quickly to the degree where a bigger overshooting occurs as a reaction from the thermistor.



(a) 200-μm-High Chamber



(b) 400-μm-High Chamber

Figure 8. The Thermistor (Solid Line) and the Thermocouple (Dashed Line) Temperature Variation during 1 PCR Cycle

Table 3. Temperature Change Speeds for the Chamber Size with Height 200 μm

	thermistor			thermocouple		
	large	medium	small	large	medium	small
60-72	14.4	14.4	15.4	4.4	3.4	5.1
72-95	17.2	16.9	18.1	6.9	4.9	7.1
95-60	-6.0	-5.4	-6.5	-5.5	-5.0	-6.0

Table 4. Temperature Change Speeds for the Chamber Size with Height 400 μm

	thermistor			thermocouple		
	large	medium	small	large	medium	small
60-72	14.0	14.8	15.6	3.3	2.6	2.6
72-95	16.4	17.1	17.6	5.5	4.5	3.7
95-60	-6.6	-5.5	-5.1	-4.9	-4.5	-4.6

4. Conclusion

This study observed temperature changes of the thermistor in a PCR chip in which a reaction chamber was installed with commercial tapes over a PCB substrate and of the samples inside the chamber. Measurements of temperature changes were made for two different chamber heights and three chamber sizes. The results showed that regardless of the height or size of a chamber, there was almost no steady state error. As expected, greater the chamber height, the lower is the speed of temperature transfer of a sample; however, because the size of the heating area remained constant, the speed was not proportional to the chamber size. Because the direction of air of the cooling fan was in parallel with the chip, the lower and upper parts of the chip cooled down at the same time; therefore, the temperature changes of the chip's thermistor and the sample within the chip were similar.

Because the temperature change in the chamber was insensitive to overshooting, even if the thermistor's temperature change overshot, the thermocouples were not subject to overshooting.

As we received a quick reaction when the thermocouples were put in a pre-heated constant water bath, the reaction speed of the thermocouples was found to be irrelevant. Therefore, during temperature control, it is fine even if we control the temperature quickly to the degree where a bigger overshooting occurs as a reaction from the thermistor.

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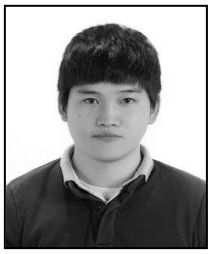

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