

Effect of Thermal Sensor Calibration on PCR Chip Performance

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Abstract

The fabrication of microfluidic channels in previously proposed polymerase chain reaction (PCR) chips by using polydimethylsiloxane, polycarbonate, and poly(methyl methacrylate) is expensive. To reduce the cost of the disposable chips, a polymer film is employed on the polychlorinated biphenyl (PCB) substrate for the bottom, a carved double-sided tape is used for the wall, and a film with inlet and outlet holes is used for the ceiling of the reaction chamber. The chip temperature is controlled by the heater pattern of the substrate and the thermistor on the opposite face. Commercial chip thermistors with a 1% resistance tolerance are adapted. In this paper, the effect of the thermistor calibration on PCR performance is investigated. A comparison of results on the band brightness of gel images indicated that the calibration seemed to deliver a better performance.

Keywords: PCR, Micro-PCR chip, calibration, thermistor

1. Introduction

A polymerase chain reaction (PCR) test has advantages in that the test can be conducted with a small amount of DNA and is used in the diagnosis of various diseases. However, the current commercialized PCR devices have several weaknesses: the test takes a long time, moving the devices is difficult owing to their size, and the devices are expensive [1]. Now that lab-on-a-chip systems are being used for DNA analysis, the development of a PCR chip that has a microfluidic channel is being carried out to overcome the previous weaknesses [1–3]. In a PCR chip, by using a very small amount of DNA, various data about the gene can be obtained quickly, and the gene can be analyzed. The PCR chip is used to diagnose various diseases through a test subject's reaction with its internal component [3]. To commercialize this capability, the PCR chip must be reliable, reproducible, and sensitive. Therefore, accurate temperature control and calibration of the temperature sensor may be necessary [4, 5].

Manufacturing costs are high for previously proposed PCR microfluidic channel chips that used polydimethylsiloxane, polycarbonate, and poly(methyl methacrylate). In this paper, to reduce the manufacturing costs of the PCR chip, a study was carried out for the temperature measurement and calibration of a PCR chip fabricated by using a thin polychlorinated biphenyl (PCB) substrate, polymer film, and double-sided tape, respectively, for the chamber floor, ceiling, and walls.

Temperature is one of the most important factors in the PCR amplification process. Controlling the temperature of PCR amplification is accomplished by using the PCB's heating pattern and a negative-temperature-coefficient (NTC) thermistor sensor. When the temperature is effectively controlled, since a PCR can operate fast even in the same cycle and can be smaller in size, the mobility of the PCR will be more effective than that of

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conventional devices.

The PCR chip studied in this paper uses an NTC thermistor, which has a 1% resistance error, as a temperature sensor. In this paper, PCR performance was compared for cases when the NTC thermistor was calibrated and not calibrated. The effect of thermistor calibration on PCR performance was investigated by comparing the band brightness of gel images obtained through electrophoresis for amplified DNA in both cases.

This paper introduces the materials and methods in Section 2 and presents the results in Section 3. The conclusion is made in Section 4.

2. Materials and Methods

2.1. Micro PCR Chip

The structure of the microPCR chip proposed in this study consists of four layers, as shown in Figure 1(a). When the experiment is carried out with only the PCB base, results are poor because DNA or fluorescent substances prone to be adsorbed. By attaching box tape (#309 mini clear, 3M) made of PP (polypropylene) material on top, and 400- μ m double-sided tape (5620A, Nitto) in the middle part of the chamber as chamber walls, a space inside the chamber was secured. The cover of the chamber was fabricated with a material using PP, which is a material of the tube actually used in a biology laboratory.

Figure 1(b) shows the bottom part of the PCB, which is on the opposite side of where the chamber will be located. To heat up the reagent, a heating pattern was constructed with copper wire. At the center of the heating pattern, an NTC thermistor sensor was attached for temperature measurement of the chamber. When the NTC thermistor sensor used in the chip measured temperature, the resistance value was measured and converted to the temperature using Steinhart-Hart (SH) equation.

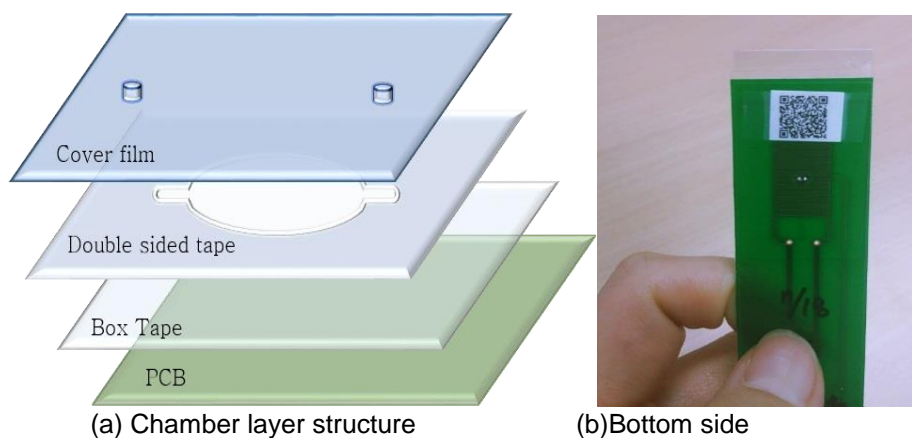


Figure 1. Chip Structure

2.2. NTC Thermistor

The NTC thermistors employed in this study were chip thermistors with a resistance error of 1% (NCP15XH103F03RC, DigiKey). Considering the possibility of lot variations among the thermistors, they were supplied by two companies (Mouser and DigiKey) and the thermistors from each company formed a group of thermistors. The previously supplied sensors from DigiKey with the same part number compose another group resulting in three groups of thermistors. For the experiment, the newly purchased thermistors from Mouser were marked as Group M, those purchased from DigiKey as Group D, and the previously purchased DigiKey thermistors as Group R. The number n after each group's alphabetic designator indicates the n -th thermistor in its group.

Using the resistance values for four important temperatures (50°C, 60°C, 70°C, and

95°C) in the PCR from the temperature-resistance tables provided by the thermistor suppliers, the uncalibrated SH coefficients of A, B, and C were obtained. Using these values and the Steinhart-Hart formula ($\frac{1}{T} = A + B \ln(R) + C (\ln(R))^3$), the uncalibrated temperature values were calculated. The calibrated SH coefficients were determined by measuring the resistance values for each of four temperatures in a constant-temperature water tank. The calibrated temperature values were obtained by these coefficients.

In the PCR, the temperatures of three processes—denaturation, annealing, and extension—are important. In the current experiment protocol, the temperatures corresponding to the three stages are 95°C, 60°C (or sometimes 50°C), and 72°C. At 95°C, eight chips that had thermistors showing the temperature values corresponding to the overall average value, and four chips showing maximum values and minimum values, were selected, for a total of 16 chips (Table 1).

The selected thermistors were divided into four groups, as shown in Table 2, considering that there were four units of micro PCR systems used in the experiment. Each group consisted of one chip showing a maximum value, one chip showing a minimum value, and two chips showing the values corresponding to the average value. For each group, the PCR tests were carried out in four systems simultaneously. As shown in Table 3, four rounds of experiments were conducted. Throughout each round, the maximum-value and minimum-value chips were mounted once each in the four systems, randomizing the system variation. In the table, MAX, MIN, and AVG are the chips showing maximum value, minimum value, and average value, respectively. In each round of experiments, two experiments were carried out using the uncalibrated and the calibrated coefficient alternatively.

Table 1. Maximum, Minimum, and Average Values at a Temperature of 95°C, and the Corresponding Chip Numbers of the Three Groups

	Temperature (°C)	Corresponding sensor chip numbers of the three groups
MAX	98.26	D1
	96.77	M29, M73
	96.42	M27
MIN	94.50	D17
	94.57	R69
	94.73	M6, M22
AVG	95.01	D5, D21, D61, D85, R21, R29
	95.02	R3, M9

Table 2. Sixteen Chips Divided into Four Groups

	Group 1	Group 2	Group 3	Group 4
MAX	D1	M29	M73	M27
MIN	M6	M22	R69	D17
AVG	D5	D21	D61	D85
AVG	R21	R29	R3	M9

Table 3. Arrangement of Thermistors for each Round of Experiments

	Group 1	Group 2	Group 3	Group 4
PCR_4	MAX	AVG	AVG	MIN
PCR_3	MIN	MAX	AVG	AVG
PCR_2	AVG	MIN	MAX	AVG
PCR_1	AVG	AVG	MIN	MAX

2.3. Micro PCR Device

As shown in Figure 2, a PC, which is a host system, read the temperature values from the local system and calculated pulse width modulation (PWM) values on the basis of the proportional plus integral plus derivative (PID) control mechanism. In other words, the graphical user interface (GUI) was processed for the hardware task command and device status; and the micro PCR hardware, which is a local system, performed hardware control including ADC and PWM with the PIC18F4550 microcontroller. A universal serial bus (USB), which is a standard link, was used to connect the host and local systems.

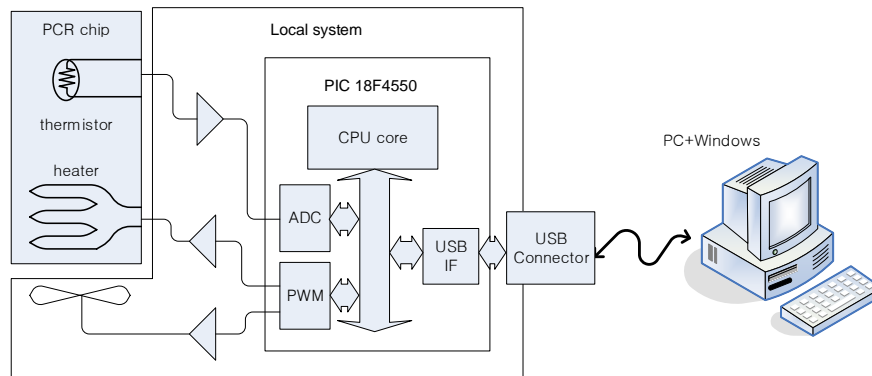


Figure 2. PCR Chip and Control System

With the four micro PCR systems configured as shown in Figure 3, two PCR experiments were carried out: one using the uncalibrated SH coefficients for 12 chips, and the other using the calibrated ones which were in the quick-response (QR) codes. In a PCR chip, as shown in Figure 1(b), the calibrated SH coefficients were converted to a QRcode; and when the PCR chip was mounted on the micro PCR system, the QRcode was read and the calibrated SH coefficients were applied to the system. In the mean while, the uncalibrated coefficients, which could be pre-calculated from the resistance-temperature table provided by the manufacturer.

Since the PCR results according to the four micro PCR devices may be different from each other, a total of two sets were carried out. A set was defined as carrying out the experiment from Group 1 to Group 4, in which the chips corresponding to the maximum, minimum, and average values were selected on the basis of not being calibrated at 95°C. This is shown in Table 3. Therefore, eight sets of results were produced in total: four sets for calibrated groups and four sets for uncalibrated groups.



Figure 3. Four Units of the Micro PCR System Device

2.4. Protocol and Reagent

In this paper, the protocol was as follows: 3 min at 95°C for pre-heating; 39 cycles of 10 sec at 95°C, 30 sec at 60°C, and 30 sec at 72°C; and 3 min at 95°C and 30 sec at 50°C for the final extension.

The following materials were used in the experiment: 11 μl of 1 ng/ $1\mu\text{l}$ DNA (CT), 12.5 μl of the master mix, 10 pM/ $0.5\mu\text{l}$ of primer F, 10 pM/ $0.5\mu\text{l}$ of primer R, and 10 pM/ $0.5\mu\text{l}$ of the probe.

2.5. Electrophoresis and UV Image Capturing

The electrophoresis was carried out for 25 min at 135 V in the TBE 0.5X agarose gel. And to capture UV images, a Canon EOS 540D camera was used, and the images were captured by setting the f-stop at 5.6, ISO intensity at 1600, and focal distance at 42mm.

3. Results

Figure 4 shows the gel images for all four rounds of the experiments. The topmost gel image of the figure shows the first round of the experiment. The 1st and the last columns of each round image are the ladders, and the 2nd and 3rd columns show the bands of the amplicons from the commercial thermal cycler. The even ones show the bands of the amplicons from the calibrated chips, and the odd ones show the bands of the uncalibrated chips. In the 3rd round of experiments, it was found that the band for one of uncalibrated chips (7th column of the 3rd image) was not shown. With the naked eye, it was difficult to distinguish between the band brightness of the amplified amplicon in a calibrated chip and that of an uncalibrated chip.

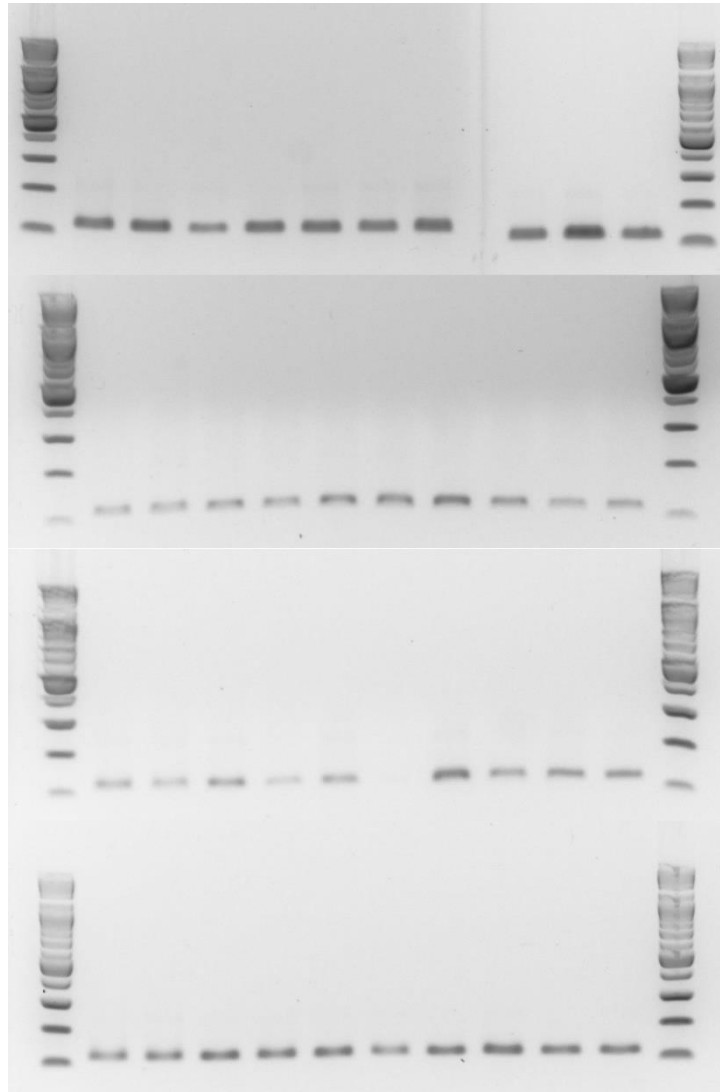


Figure 4. Gel Images for all Four Experimental Rounds. (The Topmost Gel Image Shows the Results of the First Round)

To quantitatively analyze the brightness of the bands, freeware called Gel Analyzer was used. As shown in the figure, because the background brightness changed vertically, the change in background was compensated for by using the rolling-ball algorithm before detecting a peak (the ball radius was 120 pixels). This example is shown in Figure 5. In the upper graph of the figure, the brightness profile of the lane located on the 8th column in the 2nd gel image was drawn by overlaying it on the trajectory of the rolling ball. In the lower lane image, the peak zone and ridge zone for calculating the band intensity volume are marked in red lines.

In Table 4, the intensity volumes of bands extracted as above were summarized. In the first row of the table, the column numbers are shown. From the second row on, the brightness volumes of the bands are summarized sequentially, starting from the top gel image. The first and last columns show the volumes of the last bands of the ladder. The 7th column of row R3 was left blank because a band was not detected.

As shown in Figure 4, the ladder brightness of each gel image was different. In other words, even if the DNA amounts are the same, the band brightness will be different. To compensate for this, it was normalized with the volume of the last ladder band, which is the ladder band that is the most similar to the molecular weight of amplicons. This is

shown in Table 5, where the bands of calibrated chips and the bands of uncalibrated chips from Table 4 were grouped and re-arranged. As shown in Table 5, the average brightness volumes of amplicons produced from the calibrated chips were slightly larger. This fact becomes clear when looking at the box plot shown in Figure 6.

However, as a result of the t-test or ranksum test, the band volumes from the calibrated chips were not significantly larger (t-test p-value=0.45, ranksum test p-value=0.41). There is no basis for assuming that significant results will be obtained if more experiments are conducted. However, based on the box-plot result for the case of measuring temperatures with the thermistor, it is expected that better results will be obtained through calibration.

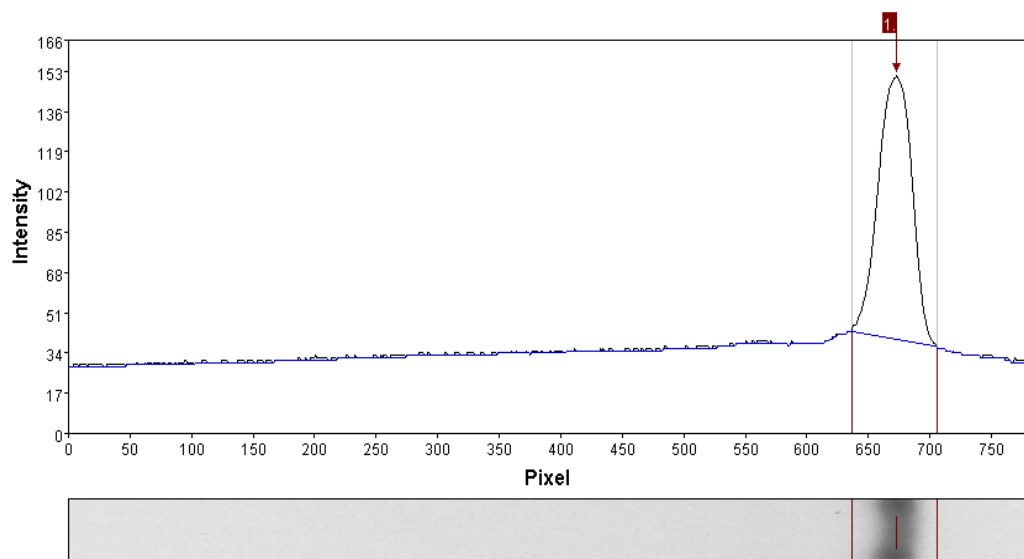


Figure 5. Band Detection Example using Gel Analyzer. Upper Graph: the Profile of the 8th Column of the 2nd Gel Image of Figure4.Lower Image: the Extracted Lane Image and Band Volume Calculation Area (Red Lines)

Table 4. Calculated Band Volume

	1	2	3	4	5	6	7	8	9	10	11	12
R1	2313	3569	3798	2286	3497	3641	3157	3879	3731	5238	3737	2881
R2	437	995	1152	1586	1390	2132	2412	2911	1865	1142	1132	690
R3	644	1208	830	1640	565	1294		3084	1599	1979	1947	1290
R4	2234	2168	2471	3075	2896	3086	2084	3041	3532	2833	2849	2642

Table 5. Band Volumes Normalized by the Last band Volume of the Ladder

	<i>Band volumes for the calibrated chips</i>				<i>Band volumes for the uncalibrated chips</i>			
R1	0.88	1.40	1.49	2.02	1.35	1.22	1.44	1.44
R2	2.81	3.78	5.17	2.03	2.47	4.28	3.31	2.01
R3	1.70	1.34	3.19	2.05	0.58	0.00	1.65	2.01
R4	1.26	1.27	1.25	1.16	1.19	0.85	1.45	1.17
mean	2.05				1.65			
std	1.15				1.03			

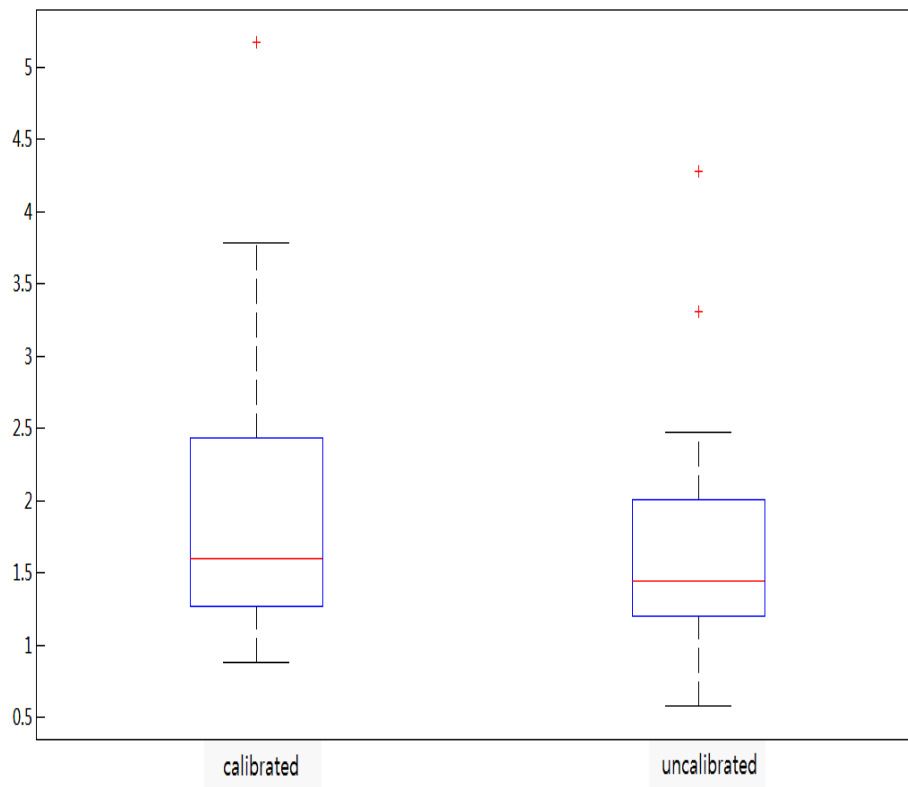


Figure 6. Box Plot of Band Volumes Shown in Table

4. Conclusion

In this study, the effect of thermistor calibration on the performance of a PCR was investigated by conducting experiments of the PCR process with chips that were calibrated and not calibrated with a NTC thermistor that has a 1% resistance error. This is required for measuring temperature in a micro PCR chip, which is a reaction chamber constituted on a thin PCB substrate.

Using the Steinhart-Hart formula, eight chips in which a thermistor showed a temperature value corresponding to the overall average value at 95°C, and four chips showing maximum and minimum values, were selected for a total of 16 chips. Using four units of the micro PCR as local systems, experiments were carried out.

The average brightness volume (1.15) of amplicons produced from the calibrated chips using GelAnalyzer was slightly larger than the average brightness volume (1.03) of amplicons produced from the uncalibrated chips. This was more apparent in a box plot. However, as a result of a t-test or ranksum test, the band volume from the calibrated chips was not significantly large (t-test p-value=0.45, rank test p-value=0.41).

Although significant results were not obtained, as a result of the box plot, it is expected that better results will be obtained through calibration when the temperature is measured with a thermistor.

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