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PCR Chip Temperature Sensor Calibration System

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Abstract. In this paper, to get accurate temperature measurements of a negative-temperature coefficient thermistor which is mounted on a micro-polymerase chain reaction (PCR) chip in a micro-PCR system that requires accurate temperature measurement and control, we realize a Steinhart-Hart calibration-factor value measurement system. When getting the calibration-factor value, if the measured resistance error is not set to 1%, measuring and controlling the temperature accurately is impossible. Taking into account this characteristic, we use the sensor of beforehand-calibrated chip to get correct temperature control of pre-calibrated chip, and we measure the resistance value that coincide to the temperature of the heating pattern of the pre-calibrated chip at one time. After attaching a thermal pad on the heating pattern of the pre-calibrated micro-PCR chip for heat transfer, we press it as close as possible against the thermal pad on the heating pattern of the to-be-calibrated chip. When the temperature goal is reached in each of the four sections, we measure the resistance value of the to-be-calibrated chip sensor several times in order to obtain their average values. The results obtained show that the temperature error rate was within the tolerance range when the sensor of the to-be-calibrated chip measured the temperature of the heating pattern in the pre-calibrated chip. We obtained three calibration factor values from the four average resistance values. The calibration system proposed in this paper requires a short time to reach each target temperature, and we therefore confirm that the calibration task time is less than that of conventional calibration systems.

Keywords: Polymerase Chain Reaction (PCR), Lab-on-a-chip, Micro-PCR, NTC-thermistor, Calibration.

1 Introduction

PCR is one of the most commonly used methods for generating an identical copy of certain DNA segments by amplifying a DNA sample through a heat-cycling stage at three specific temperatures. Therefore, DNA extraction from a raw sample is a required stage [1]. A micro-PCR chip obtains different types of genetic information in a short time, and facilitates genetic analysis. In addition, it is used in the diagnosis of various diseases by reacting with sample components of body fluid. Micro-PCR chips are required to have good reproducibility, sensitivity, and reliability, and to realize this, there is a need for accurate temperature measurement and control in addition to calibration for the micro-PCR chip sensor [2, 3, 4, 5].

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The micro-PCR chip used in this paper was fabricated using a thin printed circuit board (PCB). We heated the chamber using a heating pattern, and we measured the resistance value for the chamber temperature using anegative-temperature coefficient (NTC) thermistor at the center of the heating pattern. The measured resistance value was converted to a temperature value using the Steinhart-Hart formula. However, even if the same NTC thermistor sensor is used, there is a 1% resistance-measurement error rate. Therefore, to accurately measure the temperature of an NTC thermistor sensor, the Steinhart-Hart calibration-factor value is required, and we need a system that measures this calibration factor value. The conventionally used Steinhart-Hart calibration-factor value-measurement system obtained the NTC thermistor-sensor calibration-factor value of the micro-PCR chip using a constant-temperature water bank. By using the characteristic of the micro-PCR chip, which can increase the temperature quickly, the proposed system is an improvement on the weakness of conventional Steinhart-Hart calibration-factor value measurement system that uses a constant-temperature water bank.

The proposed method is as follows. With this method, the error-tolerance range should be considered when measuring the temperature of the pre-calibrated chip and that of the to-be-calibrated chip. However, when constructing the system proposed in this paper, and then experimentally measuring the error range between two chips, the error range was found to lie within the error tolerance range. Consequently, using the four averaged resistance values measured at each target temperature from the NTC thermistor sensor, three calibration-factor values could be obtained using the Armadillo library. Because the measurement error was within the tolerance range when the pre-calibrated chip's temperature was measured by a non-calibrated chip, we conclude that there is no need to calibrate the chip using a constant-temperature water bank.

2 Materials and Methods

We fabricated the micro-PCR using a thin PCB. On the opposite side of the part at which the chamber is located, the heating pattern is printed with a copper wire for the heating reagent, and at the center of the heating pattern, an NTC thermistor sensor was mounted to measure and control the temperature of the chamber. The NTC thermistor sensor is a device whose resistance changes with temperature.er However, the NTC thermistor product mentioned in this paper has a resistance measurement error of about 1%, and when this resistance value is calculated as a temperature in degrees Celsius, there is a small error when compared to the actual temperature.

The conventional temperature-sensor calibration method employed by micro-PCR chips uses a constant-temperature water tank. In this method, when it reaches the four temperature values required for each PCR amplification, the NTC thermistor sensor records the current resistance value. With a total of four resistance values, we obtain three calibration-factor values (A, B, C) of the Steinhart-Hart formula.

The NTC thermistor mounted on the micro-PCR is a sensor that measures the temperature as a resistance value. In the Arduino chip, because the resistance value is obtained from the sensor as an ADC value that is within the range 0–1,023, the ADC

value is again converted to a resistance. In addition, using the Steinhart-Hart formula shown in Equation (1), we obtain the temperature in Celsius as follows.

$$\frac{1}{T} = A + B\ln(R) + C(\ln(R))^3 \quad (1)$$

In the Steinhart-Hart formula, A, B, and C play the role of calibrating the error rate of the temperature when a resistance value with an error is converted to temperature using the Steinhart-Hart calibration-factor value. To calibrate the chip-temperature sensor, the A, B, and C values of the above formula are obtained using the resistance value for each temperature of the four sections.

To prevent the leakage of heat from the lower and upper parts of the entire apparatus, we used a thermal insulator with a low thermal conductivity, and we attached a thermal pad between the micro-PCR chip and the chip to transfer heat effectively. The NTC thermistor chips located at the center of the heating pattern must not interfere with each other, and they are positioned as close as possible to each other. Finally, by applying pressure to the top thermal insulator, all of the components are positioned to be as close as possible to each other.

The firmware of the MCU performs the following functions. When the ADC value is obtained from the NTC thermistor of the chip calibrated in advance, we obtain the temperature value using the Steinhart-Hart formula. When the target temperature is attained using the temperature value obtained from the sensor and proportional-integral derivative (PID) control, it is implemented to continuously maintain it like the function of a constant-temperature water tank. PID control is the most commonly used control method of the automatic control methods, and it enables control that maintains a constant value with combinations of three types, proportional, integral, and differential. In the proportional PID control term, we used the temperature value obtained by performing calculations using an ADC value obtained from the sensor.

The system proposed in this paper does not require the direct application of heat to a to-be-calibrated chip. Moreover, instead of using an environment where a constant temperature is maintained, as in a constant-temperature water tank, heat is applied, and the temperature of the calibrated chip is measured using a pre-calibrated chip. This chip is placed at a specific distance outside, and there is therefore an error in the temperature value to be actually measured.

To do this, we configured the system proposed in this paper, and we measured the error range using the pre-calibrated chip instead of the to-be-calibrated chip. In other words, we used two pre-calibrated chips, and the chip that was used instead of the to-be-calibrated chip functions as the to-be-calibrated chip. However, the calibrated chip that performs the role of the to-be-calibrated chip calculates the temperature value using the calibration factor of the pertinent chip. From each chip, two temperature values are sent to a PC via a serial communication link, and data are saved every 100 ms for 3 min. The data obtained for every temperature value are compiled as an Excel spreadsheet.

3 Results

The time that is required to reach each of the four target temperatures was measured in seconds, which is relatively short. In the experiment to measure the error range of the to-be-calibrated chip, because the error was shown to be 0.1°C, we were able to perform the calibration task. Therefore, we confirmed its potential to replace the chip-calibration system of a constant-temperature water tank.

4 Conclusion

In our study, heat was not directly applied to the to-be-calibrated micro-PCR chip. In the experiment, where the temperature of the other chip that was heated up by itself was measured and where the error was confirmed for temperature values between two chips, the average error of the two chips was found to be 0.1°C. Because this error lies within the temperature error range of the PCR process, it appears to be suitable as a replacement for the conventional chip calibration system that uses a constant-temperature water tank. However, after calibrating the micro-PCR chip using the system proposed in this paper, the verification was not performed to determine whether it had been normally calibrated. This verification has to be performed using the conventional chip-calibration system with a constant-temperature water tank. Finally, by considering the result after completing the verification experiment for the PCR task, we can confirm whether the conventional calibration system can be replaced.

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