Temperature Uniformity and DNA Amplification Efficiency in Micromachined Glass PCR chip

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ABSTRACT

This paper presented the relationship between the temperature uniformity and the DNA amplification efficiency in glass PCR chip. The top glass slide was laser drilled to form liquid access holes. The bottom glass slide was wet etched to get 100μm depth trench and deposited platinum thin-film as heater and sensor. Two Pyrex 7740 glass slides were thermally bonded together to form a chip of 4μl PCR reaction chamber. The DNA replications are successfully demonstrated in these chips by precision temperature control and oil sealing. Chips were fabricated with different patterns of heater and sensor. Thermochromic liquid crystal (Hallcrest, Chicago, IL) and Agilent Bioanalyzer 2100 were used to check temperature uniformity and amplification efficiency of each chip. According to the experimental result, we did not only design a micro-PCR chip with higher efficiency, but also provide a convenient and cheap temperature measurement solution in micro technology.

Keywords: micro PCR, glass chip, thermal mapping, amplification efficiency, thermochromic liquid crystal

1 INTRODUCTION

The polymerase chain reaction (PCR) has been a revolutionized molecular biology, and is at the forefront of many current efforts to document and understand human genetic diversity. While a wide variety of commercial PCR thermocycling systems have been described, most machines with relatively massive metal block limit the maximum achievable temperature transition rates, significantly increasing the time required to complete PCR cycling.

Use of microfabrication techniques for the creation of various integrated functional modules are being developed as miniaturized total analysis systems (μTAS). The system had been successfully demonstrated for amplification of nucleic acids using micro PCR [1-3], whose accurate temperature control and fast thermal speed are achieved.

Some researchers applied computer CAD analysis method to analyze temperature distribution on micro chip [4], and imaged the temperature uniformity on microchip by using small thermistors in different positions [5] or IR camera thermal imaging system [6]. However, the relation of the temperature distribution of a single reaction chamber in micro-PCR chips and the DNA amplification efficiency had not been reported.

In this study, micro-PCR chips are fabricated by two slides of Pyrex 7740 glass thermal bonded together. Each chip had a backside heater, sensor, and a 4μl reaction chamber. We used various patterns of the heater to test the different temperature distribution. Thermochromic liquid crystal was used to show the temperature distribution of water in those chips. The results of 750-bp DNA amplification were analyzed in Agilent Bioanalyzer 2100. The amplification efficiency was improved about 2.3 due to different heater route design. According this approach method, we could not only design a micro-PCR chip with higher efficiency, but also provide a convenient and cheap temperature measurement solution in micro technology. Using the high efficiency thermal reaction, we could decrease the cycle number and the reaction volume with higher DNA concentration. This result indicated that the micro-PCR could integrate with micro capillary electrophoresis (μCE) without losing the detection signals.

2 CHIP DESIGN AND FABRICATION

To present influence of the heater pattern on micro-PCR amplification efficiency, different heater and sensor patterns are designed and fabricated in micro-PCR chips.

2.1 Chip Design

A schematic structure of the micro-PCR is shown in Figure 1. The heater and sensor is designed at the backside of the chamber to avoid to over-heat PCR reagent.

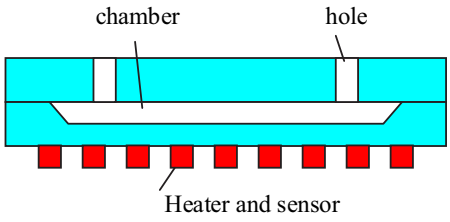


Figure 1: Schematic structure of glass micro-PCR chip

In this design, the chamber was heated only by the glass heat conduction. The trench in the bottom glass slide that was near the heater and sensor could decrease heat transfer path length.

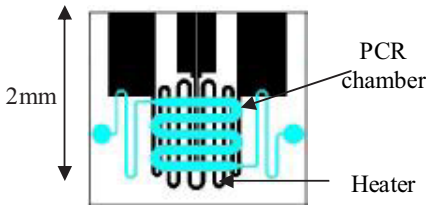


Figure 2: Layout of micro-PCR chip A

Traditionally, a heater was placed uniformly on the chip surface. In our design, different distances between heater route turns were placed on the chip. According to the power generation needed for heater and the chip dimension, only a few heater route methods could be used. The reaction chamber was designed as channel shape to avoid bubble generation when injecting liquid. There was the same reaction chamber of 4μl in all the chips.

2.2 Fabrication

The substrate was Pyrex 7740 glass. First, the bottom glass slide of the micro-PCR chip was wet etched by HF and another side of it was protected by polysilicon. After the 100 μm deep trench was achieved, all the polysilicon for protection was removed in KOH. Second, the heater and sensor were patterned on the protected side using lift-off method. Using photoresist as sacrificial layer, 200Å titanium and 2KÅ platinum are sequentially sputtered on it.

Third, the holes on the top glass slide of the micro-PCR chip were formed by laser drilling. Each hole is about 2mm in diameter. Forth, the diced chips were then thermally bonded together in high temperature about 650℃.

3 EXPERIMENTAL RESULTS AND DISCUSIONS

3.1 Temperature control

The rapid thermal cycle system with precision temperature control was constructed of three main parts, which include a computer-based controller, a power unit and a data acquisition system.

The schematic structure of entire PCR operation system is showed in Figure 3. The controller algorithm was a PID method written in Labview 6.0. The ON/OFF and PWN signals were linked to the fan and relay for cooling and heat generation. The resistance of the RTD on the chip was measured to present the chip temperature.

The RTD on the chip fabricated by microfabrication techniques was calibrated in hot bath (Hart Scientific, American Fork, UT) to get the relation between resistance and temperature. Three temperature steps are required in this PCR reaction and the details are showed as Table 1. The fan was turned on only in the cooling transition time.

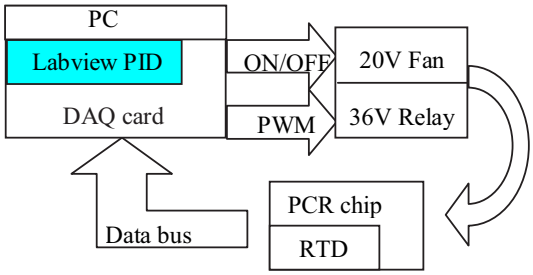


Figure 3: Schematic structure of entire PCR operation system.

	1 cycle	30 cycle			1 cycle
Temperature (℃)	94	94	56	72	72
Time (sec)	180	15	30	30	600

Table 1: The temperature and time for thermal cycle.

3.2 PCR mixtures

The PCR mixtures were prepared with the following reagents in 10ul volume. 0.2 ng pUC18 plasmid, 1X PCR buffer, 0.25mM dNTP, 1.5mM MgCl2 , 0.5 uM forward and reverse primer pairs (pUC18-750-F : 5'-GCTTAATCAGTGAGGCACCT -3' ; pUC18-750-R : 5'-AAGATCAGTTGGGTGCAC-3', product size: 750 bp), and 0.1u Taq DNA polymerase.

3.3 Thermochromic liquid crystals

Thermochromic liquid crystals (TLC) are a class of organic cholesteric that has a phase between the solid and liquid states. In this phase, the reflectivity of TLC is wavelength dependent. And the wavelength of maximum reflectivity increases with lowering temperature within a range. TLC encapsulated by a nonreactive polymer can be used longer in solid surface coatings. Because of the wavelength and temperature relation, TLC can be used to image the thermal gradient.

Figure 4: Lookup table of TLC of red starting at 92℃ and blue starting at 98℃

In our experiments, the TLC slurries for coating were sprayed through spray gun. Three different color change formulations of TLC are employed in the temperature measure for micro-PCR chip. Their red start temperature were 53℃, 70℃ and 92℃, respectively, and the blue start temperature are 57℃, 74℃ and 98℃ respectively. The illumination method for imaging TLC is not critical because of the narrow color change bandwidth of the TLC that we specified.

Although the TLC data sheets had indicated the color change data of each TLC formulation, we calibrate each formulation to get the lookup table, as shown in Figure 4. The procedure and apparatus of calibration were the same as that used for the RTD sensor on micro-PCR chip.

3.4 Thermal mapping and micro-PCR

The micro-PCR chip needs precision temperature control for successful DNA replication. Although the RTD sensor was calibrated in the hot bath, the temperature that changed the RTD resistance was an average value of the area covered by the RTD sensor. Three lookup tables were established and used to calibrate the RTD temperature readout when micro-PCR is running.

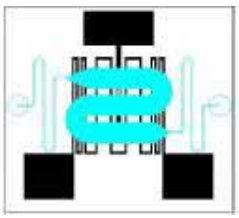
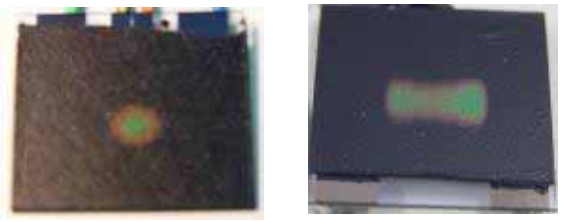


Figure 5: Layout of micro-PCR chip B

The temperature uniformity of chip A, whose layout is showed in Figure 2, was compared with that of chip B, whose layout is showed in Figure 5.



(a) chip A (b) chip B

Figure 6: Temperature distribution of chip A and chip B, red starting at 70℃ and blue starting at 74℃.

The thermal mapping results of these two chips are showed in Figure 6. These images were captured by a CCD camera when the temperature was in steady state. The reaction chamber was filled with water as dummy DNA reagent. The thermal mapping of these two chips were tested by TLC with red starting at 70℃ and blue starting at 74℃. The other two temperature steps for PCR reaction were not tested.

3.5 Result and discussions

These two chips were connected to PCR operation system as described before. And the thermal cycle condition used is presented in table 1. The cooling method was utilizing a fan that blew air up and beneath the chip. Some mineral oil was filled as sealing material in the laser drilled holes, and the PCR reagents were filled carefully to avoid generate bubbles between reagent and oil.

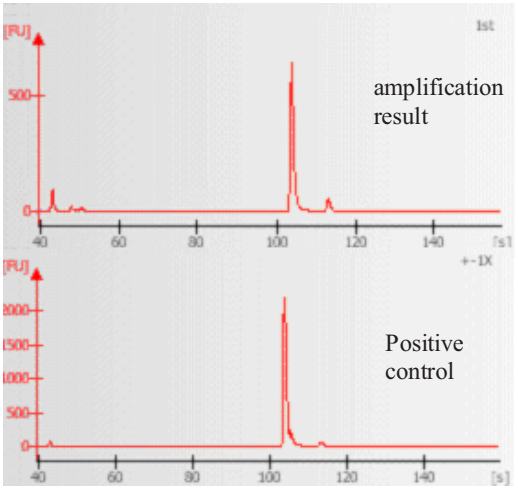


Figure 7: CE test for chip A amplification result

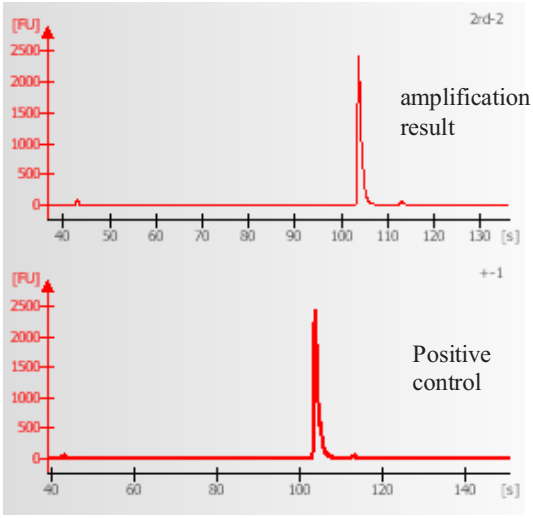


Figure 8: CE test for chip B amplification result

The DNA amplification results with 750 bp were checked by Agilent Bioanalyzer 2100, showed as figure 7 and figure 8. The data of each Spectrum are presented in table 2. The amplification efficiency was improved to multiple of 2.3. Although the route of the heater in chip A is more uniform than chip B, chip B is much more efficiency.

	Positive control	DNA amplification result concentration
CE test for chip A	52.14(ng/μl)	19.44(ng/μl)
CE test for chip B	57.62(ng/μl)	49.42(ng/μl)

Table 2: Bioanalyzer 2100 CE test of chip A and chip B PCR result

4 CONCLUSION

The glass micro-PCR chips with platinum resistors were micromachined to quickly amplify DNA. Different chips of different heater route were designed and compared in this study. The relation between temperature uniformity of single reaction chamber and micro-PCR efficiency was verified. TLC and Agilent Bioanalyzer were used to image the temperature uniformity and check the PCR reaction efficiency, respectively. The DNA amplification efficiency could be improved 2.3 fold in this experiment.

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