Simulation and Optimization of a Flow-Through Micro PCR Chip

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ABSTRACT

A flow-through micro polymerase chain reaction (PCR) chip was optimized by using coupled multiphysics computational tools. Comprehensive simulations of temperature and flow profiles were presented for the denaturation, extension, and annealing zones on the flow-through thermocycling chip with ANSYS. The flow simulation shows that the room temperature fluid in the 100 μm deep microchannels reached the designated temperature after traveling a few hundred μm at the flow rate of 140nl/s. The air gaps between the heating blocks were found to be at least 1mm for proper thermal isolation. The joule heating simulation of the initial design shows a drop of 1.6ºC on both ends of the long heating blocks due to the larger convection on the ends, as well as the less heating efficient aluminum conducting pads and the non-uniform current at the U-turn. After heating compensation by locally alternating the heater patterns, we achieved a perfect flat temperature profile over the 50 mm span of heating blocks.

Keywords: flow-through reactor, polymerase chain reaction (PCR), thermocycling, microchannel, lab-on-a-chip, multiphysics simulation

1 INTRODUCTION

The polymerase chain reaction (PCR) [1] has become one of the most important techniques for amplifying nucleic acid sequences. Since the first PCR chip was introduced [2], microsystem and microfluidic technologies have facilitated DNA amplification with higher efficiency, higher throughput, and lower reagent consumption [3]. In flow-through PCR microfluidics [4], the amplification mixture repeatedly flows through the three temperature zones. The residence time in which a sample is exposed to each of the three temperatures, as well as the number of thermal cycles, is determined by the pattern of the serpentine microchannels rather than by the heating and cooling processes as required by most commercial thermocyclers. It has the advantage of very rapid thermal cycling to achieve a total run time in the order of minutes [5] for a typical reaction of 30 cycles.

The temperature uniformity in each zone affects the DNA amplification efficiency of a PCR microfluidics [6]. The temperature homogeneity depends on the substrate material, the arrangement of heating elements, and on the structure and dimension of the chip [3]. Reliable computational modeling tools can quickly predict the thermal characteristics of different designs without tedious fabrication processes and experimental measurements. Most numerical simulation studies contributed to chamber stationary PCR devices [7-12], where the reagent is kept stationary in the reaction chamber and the chamber is cycled between three different temperatures. A system level design environment is used to evaluate the performance for flow-through PCR systems [13]. However, the simulation of temperature and flow fields is still a lack of flow-through PCR microfluidics.

In this paper, we present the three-dimensional simulation and optimization of a flow-through micro PCR chip. The effect of material, architecture, and heater pattern were investigated to achieve the ideal temperature distribution.

2 MULTIPHYSICS ANALYSIS

ANSYS® Multiphysics (ANSYS, Inc. Canonsburg, PA) was used for the computational analysis of the micro PCR chip. Since the 3-D fluidic-thermal element FLUID142 can not be used together with any other ANSYS elements, we first neglected the fluidic effects, assuming the fluidic regions having the same material properties as the walls. Without considering the flow, multi-fields iterative coupling of electrical-thermal were employed to simulate the joule heating from the platinum heaters, heat transfer in the silicon and glass devices, and heat convection to the environment. SHELL157, a 3-D element having in-plane thermal and electrical conduction capability, was chosen to simulate the platinum heaters; SOLID70, a 3-D element having thermal conduction capability, was used to present the solid materials—silicon and glass. Thus we were able to determine the voltages that should be applied on platinum heaters in order to achieve designated denaturation, extension, and annealing temperature profiles of the PCR chip. The simulation results discussed in sections 3 and 5 adopted this non-fluidic simulation model.

Based on the non-fluidic simulation results, we re-simulated the PCR chip, only using fluidic-thermal element FLUID142, instead of SHELL157 and SOLID70. Fluidic regions (microchannel) and non-fluidic regions (silicon and glass) were specified by their material properties. The

temperatures obtained from the previous non-fluidic simulation were applied on the heating surfaces as the boundary conditions in the new simulation. With a constant flow rate through the microchannel, we were able to predict what temperature profile the flow would be and what pressure drop that needed. The fluidic-thermal simulation is discussed in detail in section 4.

The material properties of the silicon, glass, solution sample, platinum (heater), and aluminum (conducting pad) are shown in Table 1. In all studies, we assume that the natural convection coefficient is $15W(m^2 \bullet K)^{-1}$ and that the surrounding temperature is 20ºC.

Table 1: Material properties

Materials	Density (kg/m3)	Conductivity (W/m·K)	Specific Heat (J/kg·K)	Thickness (μm)	Resistivity (μΩ·cm)	Viscosity (mPa•s)
Silicon	2330	150	700	600		
Glass	3000	1	1681	500		
Sample	1000	0.6	4200	100*		1
Pt	21440	70	133	0.2	10.6	
Al	2700	237	900	0.3	2.65	

* microchannel height

3 PCR CHIP DESIGN

As illustrated in Figure 1, the flow-through micro PCR chip comprises a serpentine microfluidic channel and three thermally-isolated heating zones: denaturation (~95ºC), extension (~72ºC), and annealing (~60ºC). Each heating zone is composed of heating elements and temperature sensors. The external thermal controller can set the three zones to different characteristic temperatures necessary for DNA amplification. The microchannel passes repetitively through three heating zones with the pre-defined number of 40 cycles per run on the 54 mm by 20 mm chip area.

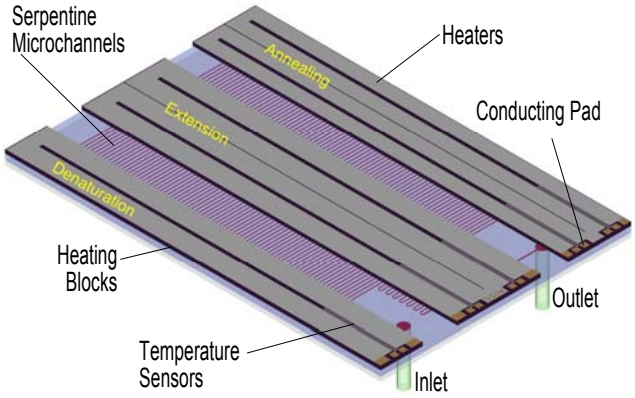

Figure 1: Design of the flow-through micro PCR chip.

3.1 Material and Structure

Glass substrate was selected for microchannels fabrication to provide good optical access. A thin metal layer was deposited and patterned on a cover substrate to create heating and sensing elements. We first studied the temperature distributions of simple two-layer structures. Since the lateral heat transfer between adjacent cycles is so small (will be verified in section 5), we simplified the model by only simulating one cycle. Figure 2(a) shows the results from a one cycle glass/glass structure. Due to low thermal conductivity of glass, it provides enough temperature gradient between the three zones. However it can not generate a homogeneous temperature zone defined by the heaters, as shown in the temperature profiles in Figure 2(d). When we replaced the cover substrate with silicon as shown in Figure 2(b), we obtained a flatter temperature profile caused by high thermal conduction of the silicon substrate (Figure 2(d)). The lowest temperature is still higher than 88ºC even without using the heaters for the extension and the annealing zones. It appears that the silicon/glass structure is not suitable for this particular application.

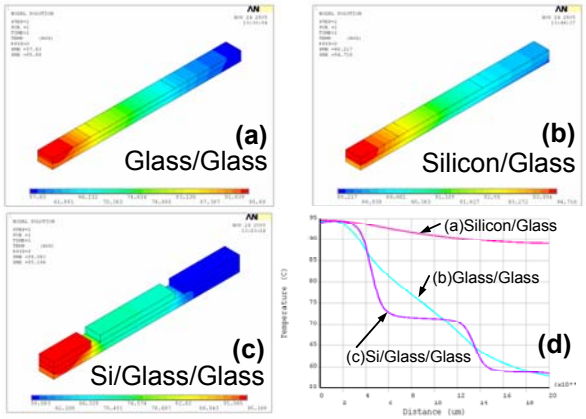

Figure 2: Simulated temperature distributions of different materials and structures.

To maintain the three individual thermal zones in a small chip, a three layer structure was proposed, as depicted in Figure 2(c). A third layer of silicon was placed on top of the glass/glass layers. Part of the silicon layer without heating elements was removed to form silicon strips and isolation air gaps. This arrangement assures good thermal resistance between zones and high thermal conduction within each zone. It is found that the air gap has to be at least 1mm to obtain good thermal isolation. The simulated profiles in Figure 2(d) shows three beautiful plateaus of the isothermal zones.

4 CONTINUOUS FLOW STUDY

Flow-through microchannels were etched 100-μm-deep into the glass substrate with repeated expansion and contraction regions to define the denaturation, annealing, and extension zones. The structure is the same as the optimized design shown in Figure 2(c) except for the new defined flow region. The wider microchannel width in each zone reduced the flow velocity and thus increased the residence time of PCR sample. To determine the flow

information, such as its temperature profile, and the relationship between pressure drop and flow rate, a fluidic-thermal simulation was performed based on the previous non-fluidic optimized design shown in Figure 2(c).

The temperature distribution of the flowing-through sample is shown in Figure 3 when a 140nl/s flow passes through the microchannels with inlet temperature at 20ºC. With the relatively small thermal mass flow, the sample layer has the same three-plateau profile of the heating blocks (Figure 2(d)), except for a small deviation near the inlet region of the first, as shown the in Path 1 of Figure 3(c). It is due to the low inlet flow temperature of 20 ºC introduced to the first cycle. The fluid sample reaches the denaturation temperature within a few hundred μm.

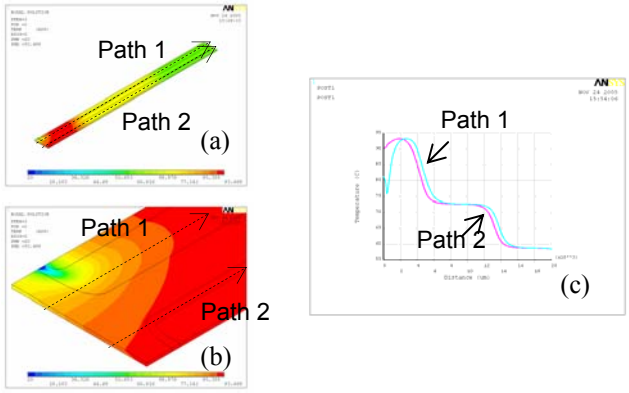

Figure 3: (a) temperature distribution at half channel depth, (b) a close view at inlet region, (c) simulated temperature distributions of flowing-through sample in the first cycle along the two paths defined in (a) and (b).

Figure 4 (a) shows that the flow velocity information near the inlet and Figure 4 (b) gives the information about how much pressure drop that is needed to drive the flow at a mass flow rate of 140nl/s. The simulated pressure drop is 874Pa per cycle, corresponding to a total pressure of 35kPa for 40 cycles. The ratio of flow velocities in expansion and contraction microchannels is about 1:5.

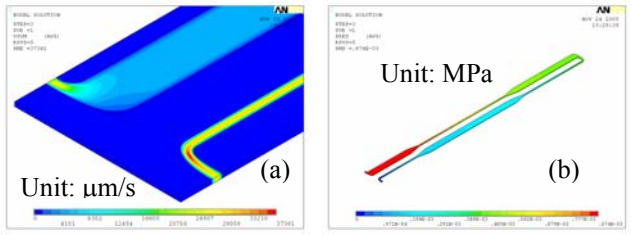

Figure 4: (a) a flow velocity map located the inlet of the first cycle, (b) pressure drop through the first cycle.

5 HEATING ELEMENT OPTIMIZATION

The efficiency of the flow-through PCR reaction depends on the temperature uniformity. Optimization of the heating elements can greatly improve the temperature homogeneity of each isothermal zone. In our original design of the denaturation zone, we used the same platinum line width (1750 μm) throughout the heating block, as depicted in Figure 5. The temperature simulation shows a drop in the temperature on both ends of the long heating block, and especially on the rear end. It is caused by larger convection on the ends, as well as the less heating efficient conducting pads and non-uniform current at the U-turn.

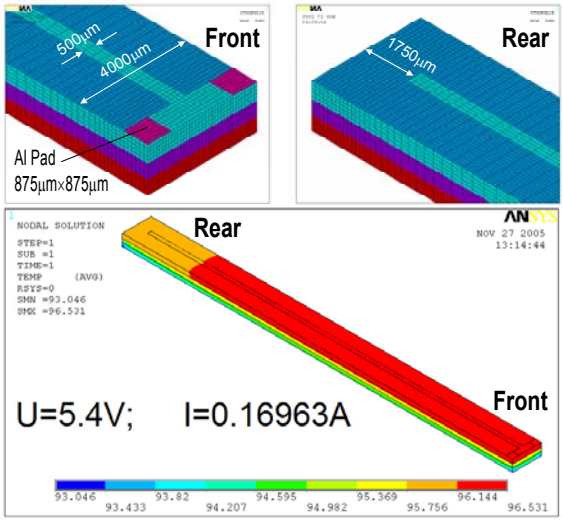

Figure 5: Heater layouts and simulated temperature distribution of original design (denaturation zone).

We tried different heater patterns on both ends to compensate the additional heat loss by locally increasing resistance, i.e. reducing the width of platinum line. Figure 6 shows the optimized heater design of the denaturation zone. Width of 600-μm long platinum line is reduced from 1750 μm to that of the aluminum pad (875 μm) to generate more heat at the front end. Meanwhile, the width of the U-turn at the rear end is reduced to 1000 μm.

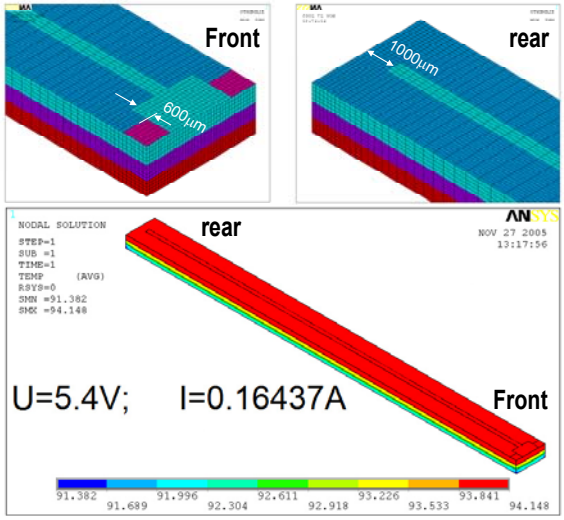

Figure 6: Heater layouts and simulated temperature distribution of optimized design (denaturation zone).

The optimized heater provided temperature uniformity that was superior to that of the original one, as shown in Figure 7. It has nearly zero variation while the original design had as much as a 1.6 ºC over a 50-mm heater span. The temperature difference between original and optimized results is due to different electric currents. The same voltage (5.4 V) was applied to both heaters, but the optimized one has a slightly higher electric resistance and thus generates a lower current and power output. The optimized temperature distribution from the inlet (first cycle) to the outlet (last cycle) is so uniform which assures all the cycles has the same thermal conditions for DNA amplification to achieve higher efficiency.

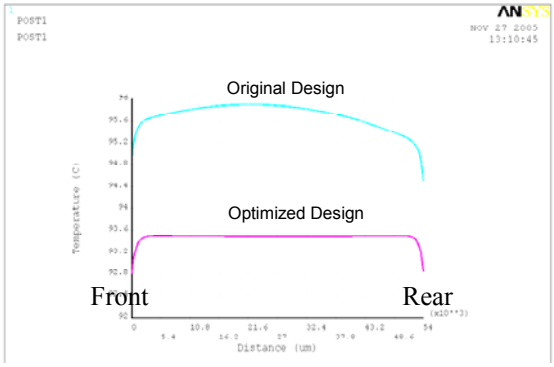

Figure 7: Comparison of temperature profiles of the original and optimized designs.

6 CONCLUSIONS

We have demonstrated a multiphysics simulation and optimization of a flow-through micro PCR chip with a three-layer structure comprised of one silicon and two glass substrates. A 100 μm deep serpentine microchannel between two glass substrate provided rapid thermal cycling while it repeatedly passes through three isothermal zones defined by the silicon heating blocks. We found good thermal isolation by air gaps of at least 1mm between heating blocks. We obtained perfect temperature uniformity over the 50 mm span of heating blocks by optimizing the heater patterns to compensate additional heat loss on both ends.

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