

Simulation and Experimental Verification of Micro Polymerase Chain Reaction Chips

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ABSTRACT

This study used finite element analysis to simulate the temperature characteristics of a micro polymerase chain reaction (PCR) chip. The micro-PCR chip was fabricated on a silicon wafer and Pyrex glass using photolithography, wet etching, and anodic bonding methods.

The main goal of this study was to analyze the temperature uniformity and distribution of the micro-PCR chip, the temperature distribution of the DNA sample, and the transient temperature difference between the heater and DNA sample. The finite element analysis results were also confirmed by one-dimensional theoretic analysis.

The simulation results were used to improve the thermal cycling time of a rapid micro-PCR system, consisting of a rapid thermal cycling system and a micro-PCR chip. The improved thermal cycles of the rapid μ PCR system were verified using serum samples from patients with chronic hepatitis C. The hepatitis C virus (HCV) amplicon of the rapid μ PCR system was analyzed by slab gel electrophoresis with DNA marker separation in parallel.

Keywords: PCR, DNA, FEA

1 INTRODUCTION

There has been an increasing interest in developing miniature devices for applications in biology [1]. After Northrup and his coworkers successfully amplified DNA in a microfabricated reaction chamber [2,3], great progress has been made in miniaturizing micro-polymerase chain reaction (PCR) chips and shortening thermal cycle time [4,5]. Using transient liquid crystals to study the temperature uniformity in microfabricated PCR wells provides a useful experimental method for characterizing micro-PCR systems [6]. Applying CAD analysis to PCR chips was reported in 1999. That research focused on the affect on PCR containment during temperature cycling [7]. However, analyzing the temperature distribution of micro-PCR chips and the transient temperature response has not been reported in detail.

This study used an ANSYS finite element analysis package to analyze the temperature distribution and uniformity of the micro-PCR chip, the temperature pattern of the DNA sample, and the transient temperature difference between the heater and DNA sample. One-

dimensional theoretic analysis was also used in this study to further confirm the transient temperature response results from the finite element analysis. Applying the simulation results to improve the thermal cycling time of a rapid micro-PCR system was also a goal of this study. The rapid micro-PCR system, which includes a micro-PCR chip, has been used to amplify the HCV cDNA to test its performance.

In this study the simulation, theoretic analysis, and experimental verification of the micro-PCR chips are reported in detail. This work has demonstrated the techniques in simulating the thermal characteristics and shortening the thermal cycling time of micro-PCR chips.

2 MICRO-PCR CHIP DESIGN

Micro-PCR chips are designed to fabricate the reaction well on a silicon wafer. The hole-drilled Pyrex glass dies are anodically bonded to the etched and diced pieces to form the reaction chamber for PCR. A schematic drawing of the DNA sample filled micro-PCR chip with a heat source is shown in Figure 1. The reaction chamber size is $3.2 \text{ cm} \times 3.2 \text{ cm} \times 50 \mu\text{m}$ to form a $50 \mu\text{l}$ volume. The micro-PCR chips are heated from the bottom of the silicon.

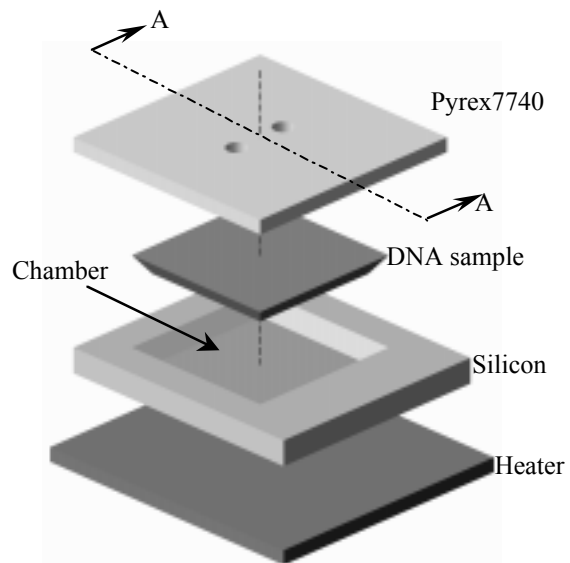


Fig. 1: Schematic drawing of the DNA sample filled micro PCR chip with a heat source.

3 FINITE ELEMENT ANALYSIS

ANSYS 5.5 (Swanson Analysis Systems Inc., Houston, PA) running on a PC was used for the analysis. The material properties of the silicon, Pyrex7740 glass, and DNA sample are shown in Table 1. The finite element simulation analyzes one complete thermal cycle. Each thermal cycle of the PCR has four steps, including denaturation at 94°C, primer annealing at 37°C and 55°C, and primer extension at 72°C. The simulation analyzes the temperature distribution in steady state and the temperature deviation in transient response.

The steady state analysis studies the temperature distribution of the micro-PCR chip in the holding temperatures at 94°C, 37°C, 55°C, and 72°C in detail. In the transient temperature response analysis, the temperature deviations between the DNA sample and the heating surface were examined.

Table 1: Material properties.

	Thermal conductivity (W/mm°C)	Specific heat (J/gm°C)	Convection heat transfer coefficient (W/mm ² °C)
Silicon	0.157	0.7	
DNA sample	0.643	4.182	
Pyrex 7740 glass	1.13×10^{-3}	0.753	
Air		1.008	5.39×10^{-5}

4 RESULTS

4.1 Steady State Analysis

The steady state temperature characteristics provide information to understand the temperature difference between the DNA sample and the heating surface, and the temperature distribution of the DNA sample. This information helps to improve the micro-PCR chip design. Figure 2 shows the temperature distribution of a micro-PCR chip when the heating surface is at 94°C. The temperature deviation of the whole micro-PCR chip is within 1.7°C.

In the steady state, the temperature of the whole silicon reaction well was found uniformly at 94°C, which is the same as the temperature of the heating surface. To further understand the temperature difference inside the chip in the steady state, the temperature distribution of the A-A section without silicon is shown in Figure 3. The data shows that the temperature distribution of the sample is uniform and the same as that in the silicon well. The temperature deviation appears on the glass surface. The location of the

temperature sensor will cause a temperature disagreement between the sensor and the heating surface if the sensor is placed on the top surface of the glass. In other steady state temperatures, i.e., 37°C, 55°C, and 72°C, the temperature of the DNA sample still matched the silicon reaction well, and the temperature difference between the glass surface and the heating surface became smaller than that at 94°C.

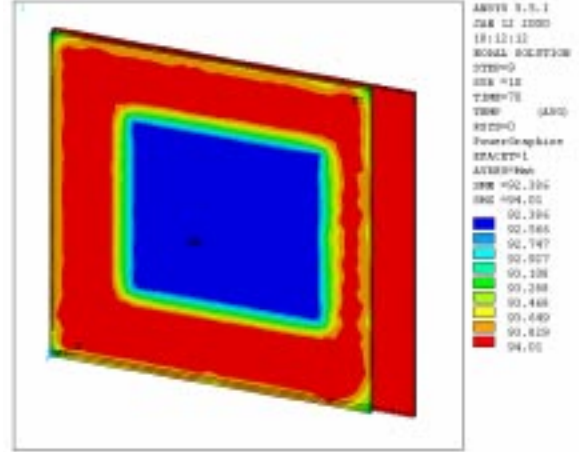


Fig. 2: Temperature distribution of the micro-PCR chip.

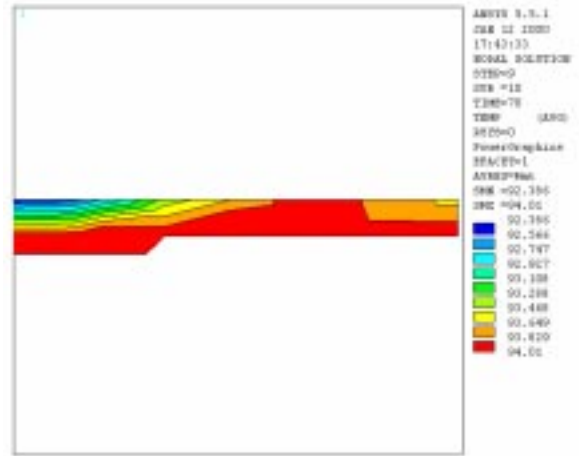


Fig. 3: Temperature distribution of the glass and DNA sample of the A-A section in Figure 1.

4.2 Transient Response Analysis

In the study of the transient temperature response, the temperature of the heating surface was set to the desired temperature profile for PCR performance. The time step was set to 0.3 seconds in the simulation. Figure 4 shows the temperature comparison of the center point of the DNA sample and the heating surface in one thermal cycle. The maximum temperature difference of these points was 0.007°C in the simulation, so the temperature of the DNA sample always followed that of the heating surface. Figure 5 shows a close-up of the transient temperature response around 94°C.

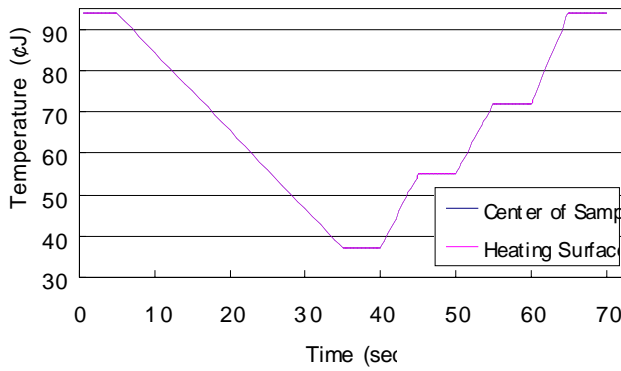


Fig. 4: The comparison of temperatures of the center of the DNA sample and the heater surface in one thermal cycle.

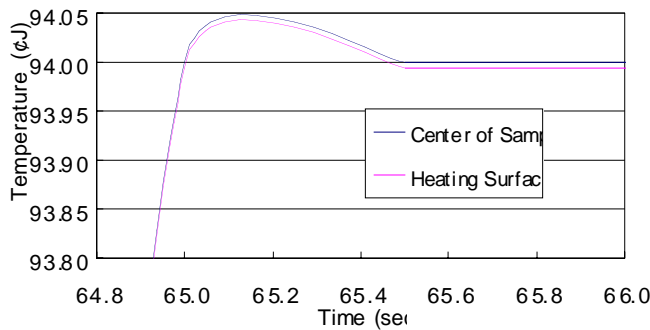


Fig. 5: The close-up of the transient temperature response around 94°C.

4.3 One-dimensional Theoretical Analysis

Using the diffusion equation to solve heat transport in a one-dimensional direction:

$$\frac{fT}{ft} = \alpha \frac{f^2T}{f^2x^2}$$

Where T is the temperature, t the diffusion time, x the thickness, and α the thermal diffusivity. The diffusion equation can be further simplified to

$$t = \frac{x^2}{\alpha}$$

Where x is the thickness for heat to diffuse. In this model heat diffuses through 0.45 mm silicon and then reaches the DNA sample. The thermal diffusivity (α) is 0.9 cm²/sec for silicon. So

$$t = \frac{x^2}{\alpha} = \frac{(0.045)^2}{0.9} = 2.25 \times 10^{-3} (\text{sec})$$

The diffusion time for heat to diffuse through the silicon well to the DNA sample only takes 2.25×10^{-3} second. In the transient thermal response simulation, the time step was set to 0.3 second and the data showed that the DNA sample temperature followed that of the heating surface. So, the simulation results are confirmed by the theoretical analysis.

4.4 Experimental Verification

The DNA sample temperature follows the temperature of the heating surface within 0.007°C based upon the results of the transient temperature response simulation. The time step was set to 0.3 seconds in the simulation. The one-dimensional heat transfer theoretical analysis shows that the sample reaches the temperature of the heating surface within 0.003 seconds.

These results were applied to decrease the holding time of the thermal cycle in performing a reaction from 15 seconds to 5 seconds. The improved thermal cycles was verified using serum samples from patients with chronic hepatitis C. The HCV cDNA sample was successfully amplified for 5 seconds holding time case after 30 thermal cycles. Figure 6 shows the image of separation result in slab gel of the HCV cDNA amplified in a micro-PCR chip.

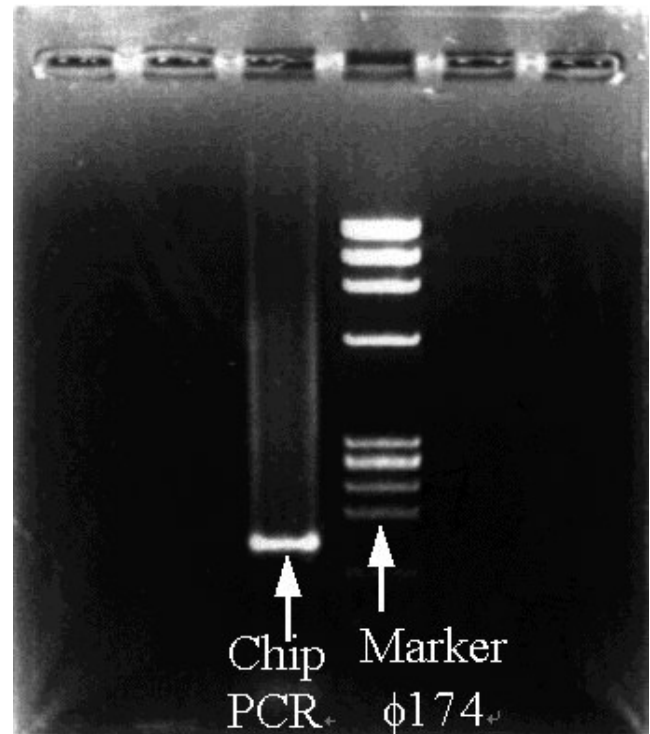


Fig. 6: Image of separation result in slab gel of HCV cDNA amplified by a micro-PCR chip.

5 CONCLUSIONS

A detailed study of the temperature distribution in steady state and transient response was conducted to improve the thermal cycling time of a rapid micro-PCR system. Under finite element analysis and one-dimensional heat transfer theoretical analysis, the DNA sample temperature distribution was uniform and can be seen the same as that of the heating surface in a silicon well under both steady and transient conditions in the current micro-PCR chip configuration. The temperature of the glass surface shows some deviation. This deviation may cause a temperature disagreement between the temperature sensor and the heating surface if the sensor is placed on the top surface of the glass.

The simulation results were used to shorten the thermal cycling time of the micro-PCR chip. This improved micro-PCR system thermal cycling time successfully amplified HCV cDNA. This work has demonstrated the techniques for simulating the thermal characteristics and shortening the thermal cycling time of micro-PCR chips.

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