The Design and Manufacture of Functional Micro-stationary PCR Chip

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Abstract. This study presents a novel microfabricated polymerase chain reaction (PCR) chip based on silicon. A microheater utilizing doped semiconductors as heating resistors and a temperature sensor made of Pt are integrated on the chip to make up a thermal module. The micro-stationary PCR chip is fabricated on a silicon wafer using photolithography, wet etching and ion implantation technology. The package is created without complex processes. Three types of configurations for the microheater are designed and simulated to analyze the temperature distribution by the finite element analysis so as to enhance the temperature uniformity in the reaction chamber. With this approach, the microheater is optimized. Finally, the simulation results are validated by infrared images from experiments.

Keywords: PCR, doped semiconductor, temperature distribution, temperature uniformity, MEMS.

1 Introduction

Polymerase chain reaction (PCR) has been an effective method for amplifying nucleic acid molecules, which typically modulates repeated thermal cycling to complete the procedure, involving denaturing (90–95°C) for separation of double-stranded DNA, annealing (50–65°C) for hybridization of primers, and elongation (70–75°C) for replication of the DNA targets [1]. The PCR technology is widely used in many fields, including clinical diagnostic, life science, forensic medicine, military affairs and aerospace. However, the conventional equipments based on PCR are usually bulky, time-consuming and require a large number of samples and reagents, which limits their practical applications. Therefore, the development of a micro-PCR chip has great practical significance.

Bio-micro-electro-mechanical-system (Bio-MEMS) technology, integrating biological sciences and MEMS technology, has enabled the miniaturization of biomedical devices and systems. Micromachined biomedical devices or systems have several advantages over their large-scale counterparts such as a shorter assay time, disposability, low reagent and sample consumption, portability, and lower power consumption [2]. Since the first demonstration of a functional PCR device, various types of micro-devices have been presented in the literature [3,4]. Typically, micro-PCR chips can be

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classified into two major categories in accordance with the method of changing temperature, namely micro-stationary PCR chips [5,6] and micro-fluidic PCR chips [7,8]. Compared with the micro-fluidic PCR chips, the micro-stationary PCR chips have simple structure, smaller volume and lower power consumption, which is important for practical application.

However, the disposable usage of the micro-PCR chip would cause high operational cost. Besides, the temperature uniformity and power consumption of the micro-stationary PCR chips also need improvement.

To tackle these problems, the present study proposes a novel micro-stationary PCR chip which integrates microheaters utilizing doped semiconductors made by ion implantation technology as heating resistors. The relationship between the configuration of microheaters and the temperature uniformity of micro-chips is simulated and validated by experiments. A simple and feasible packaging method is designed. The development of the new micro-stationary PCR chip may provide a practical tool for the rapid and accurate amplification of the nucleic acid.

2 Design and Simulation

2.1 Design of the Chip

In order to increase the heating efficiency and enhance the temperature uniformity of the reaction area, a new design for microheaters made by ion implantation technology is adopted and integrated into the PCR chip. As shown in Fig.1, the PCR chip is comprised of four micro reaction chambers and four thermal modules corresponding to the chambers.

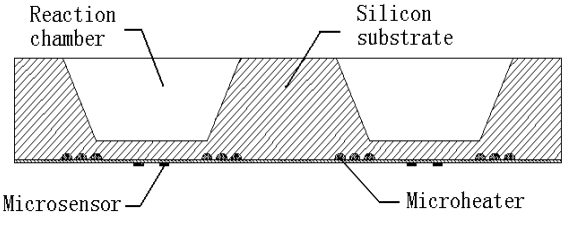

Fig. 1. One exploded view of the micro-PCR chip

The chambers with 2.5 mm in side length of upper surface, about 2.0 mm in side length of lower surface and 350 μm in depth are distributed symmetrically on the silicon substrate. Each of them has a volume of about 1.8 μL. To facilitate temperature calibration, open-type reaction chambers are adopted. It is reported that some materials such as silicon would cause the inhibition of the DNA amplification and silicon oxide could weaken this inhibition, so a silicon dioxide (SiO_2) layer is deposited on the inner surface of the chambers.

Corresponding to each chamber, a thermal module composed of a microheater and a temperature sensor is integrated underneath the reaction chamber to maintain precise and uniform temperature conditions for the PCR processes, including denaturing,

annealing and extension. The microheaters utilize doped semiconductors made by ion implantation technology as heating resistors, so they can be embedded into the silicon substrate, which increases the heating efficiency for the heat dissipation is reduced and the heat conduction is enhanced. Besides, the thickness of the silicon where the microheaters are located is only 50 μm and the thermal conductivity of silicon is as high as 128.6 w/(m·k), so the heating rate is increased and the temperature hysteresis of PCR mixture is decreased. Pt is used to make the sensitive element of the temperature sensor for its resistance value is linearly dependent on temperature. The heating resistors of microheaters are located underneath the reaction chamber apart. As shown in Fig 2, three designs for the microheaters are used in this study.

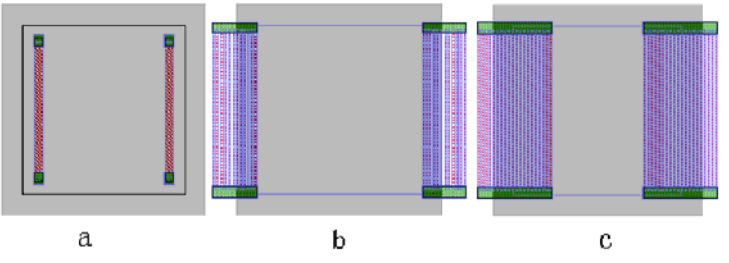

Fig. 2. Three designs of the microheaters

2.2 Simulation on Temperature Distribution

The simulation on temperature distribution of three designs is carried out by finite element analysis. In the simulation, the heat transfer between the chip and air is natural convection at the heating process, and is forced convection at the cooling process. The boundary conditions are as follows: natural convection heat transfer coefficient h_1=10 W/(m^2·k), forced convection heat transfer coefficient h_2=100 W/(m^2·k), ambient temperature T_f=26℃. In order to simplify the simulation, water is used to substitute the PCR mixture. The physical attribute of relevant materials is shown in Table 1.

Fig 3 shows the analysis results of each design. Fist, two heating resistors are used to heat the reaction area, and the result shows that non-uniformity of the temperature is obvious because the temperature decreases rapidly with the increase of distance away from the heating resistors. In order to reduce the drop in temperature around the resistors, twenty-four heating resistors are arrayed apart underneath the reaction area with an interval and width of 20 μm. According to the simulation, this design has effectively improved the temperature uniformity. Then, forty heating resistors are used and the two areas with highest temperature in the second design have connected together. Therefore, the third design could provide a quite uniform temperature for PCR processes.

Table 1. The physical attribute of relevant materials

Material	Specific Heat Capacity [J/(kg·k)]	Thermal Conductivity [W/(m·K)]	Density (kg/m^3)
silicon	737.8	128.6	2210.0
water	4201.0	0.6410	974.3

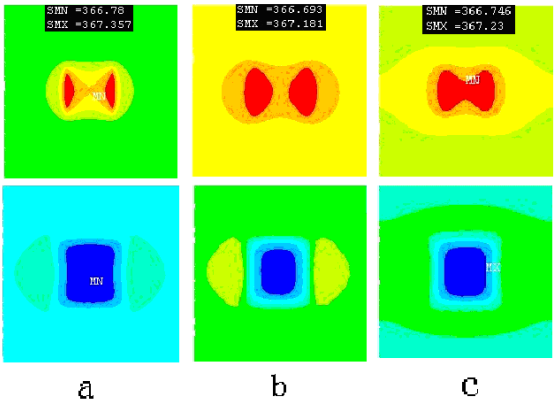

Fig. 3. The temperature distribution of three designs

3 Fabrication and Packaging

3.1 Fabrication

The PCR chip is fabricated using MEMS technology, as shown in Fig 4. A 4 inch silicon wafer with a thickness of 400 μm is used as the substrate. First, the thermal module is fabricated on the silicon. A positive photoresist is spin-coated and patterned on the cleaned silicon substrate using a standard photolithography process. Then heating resistors with a width of 20 μm are formed using ion implantation technology. The same ion implantation technology is used once more to form an area with low resistivity as a junction at the end of the heating resistors by increasing the concentration of boron ion. A silica layer is then deposited to protect the resistors mentioned above by LPCVD. A Pt layer with a thickness of about 100 nm is deposited by an electro-beam evaporation process. Prior to this process, a layer of Al Cu Bimetal is deposited and used as a sacrificial layer. The Pt layer is then patterned using a standard lift-off process to form the temperature-sensing resistors. A layer of 200 nm Au is deposited and patterned as electrical leads. Next, four open chambers are formed by wet etching. Finally, a silicon dioxide (SiO_2) layer is deposited on the inner surface of the chambers by PECVD. The size of fabricated PCR chip is 12000 μm ×12000 μm ×400 μm.

3.2 Packaging

The packaging of the micro-PCR chip is important for its application, which provides the mechanical and electrical connection with the equipment for the chip. However, the electrical leads are not on the operating side but the back of the chip, and it is unsuitable to achieve mechanical connection on the operating side, so a simplified flip-chip bonding method is adopted, as shown in Fig 5. Fist, electrical leads are welded on the bonding pads of the chip. A bit of adhesive is then dripped around the solder joint. After the adhesive solidifies, the chip is bonded with the Printed Circuit Board (PCB) by adhesive with a glass block between them. When the adhesive is solidified, the electrical leads are welded on the bonding pads of the PCB.

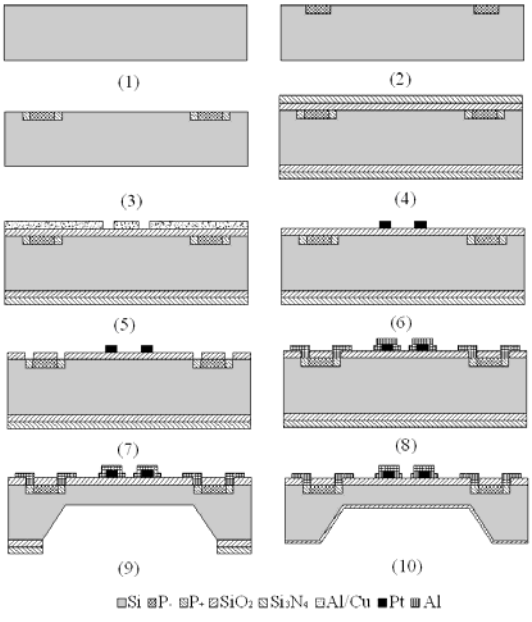

Fig. 4. Simplified fabrication process of the chip

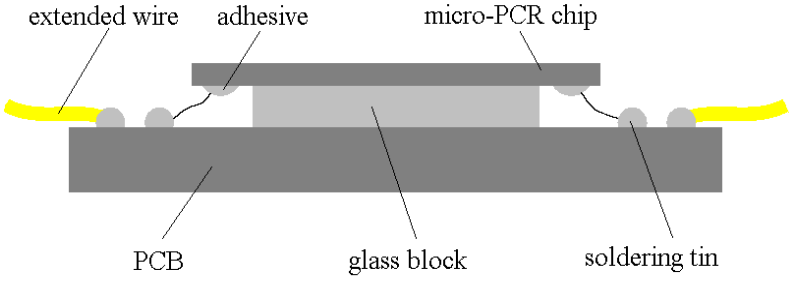

Fig. 5. Structure of packaged chip

4 Results and Discussion

The temperature uniformity and distribution in the reaction chamber can affect the efficiency of the PCR process. Hence, it is important to improve the temperature uniformity, which could increase the PCR efficiency.

Experiments are carried out in order to validate the simulation results which are mentioned above. It must be declared that four microheaters are supplied with the same power but only one of the temperature sensors is connected with the temperature control system. In other words, only one thermal module is in the normal working condition. Fig 6, Fig 7 and Fig 8 show the infrared images of the temperature distributions for three types of microheaters respectively while operating at the elongation

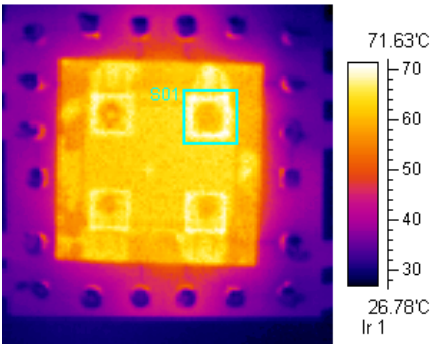

Fig. 6. The infrared image of the temperature distribution for two heating resistors

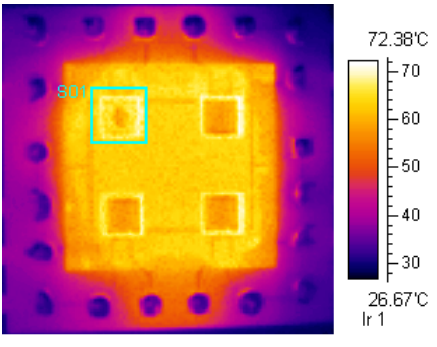

Fig. 8. The infrared image of the temperature distribution for twenty-four heating resistors

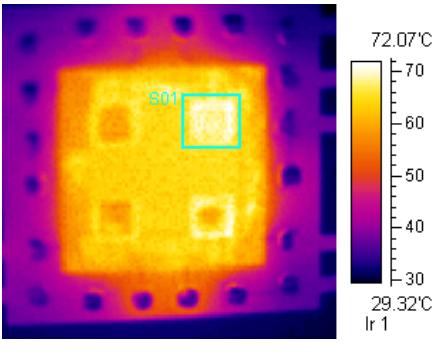

Fig. 9. The infrared image of the temperature distribution for forty heating resistors

temperature of 72°C. In the images, the thermal modules marked with green square frame are working normally. It is obvious that the results of the infrared images accord with the simulation results mentioned above. The well-distributed microheaters would enhance the temperature uniformity in the reaction chamber. For a same chip,

there exist evident temperature differences between the four thermal modules under the same power supply. The reason may be the differences in resistances of the microheaters caused by non-uniformity of the chip fabrication process.

5 Conclusions

This study developed a new micro-PCR chip, including the design, fabrication and packaging. Three types of configurations for microheaters are designed to enhance the temperature uniformity which is important for the PCR procedure. Finite element analysis is used to simulate the temperature distribution of the three designs. The simulation results are then verified by infrared images from experiments, which reveals that the temperature distribution is related to the configurations of microheaters and the well-distributed microheaters would enhance the temperature uniformity. This work is crucial for further modification of the micro-PCR chip.

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