A THERMALLY IMPROVED QUANTITATIVE PCR CHIP

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ABSTRACT

Here we present a thermally improved parylene-C based qPCR chip. The reaction chamber resides on a thermally isolated silicon island with an integrated heater connected to the main body via parylene-C bridges. This design allowed fast and efficient thermal cycling. By measuring the fluorescence of Sybr Green I during thermal cycling, detection of the M13 virus is demonstrated.

INTRODUCTION

The polymerase chain reaction (PCR) is a well-established biochemical tool that can greatly benefit from miniaturization, leading to improved speed, portability, and ease of use. Although PCR has long been implemented on chips, quantitative PCR (qPCR) chips are less prominent. Polycarbonate [1], SU-8 [2], silicon-glass [3] and PDMS-based chips [4,5] have been realized; however, most utilize external, active heating and cooling elements. As these chips continue to mature, it becomes continually important to optimize thermal performance in terms of maximum heating and cooling rates with minimum power consumption.

DESIGN

We choose parylene-C (poly(monochloro-p-xylylene)) as the channel material. It is biocompatible (USP Class VI) for minimal interference with the PCR reaction, optically transparent which allows DNA detection by optical fluorescence, and conformally deposited using a room temperature CVD process making it compatible with many standard MEMS procedures.

To optimize the thermal design, we use a unique technology developed by our group [6] to create a reaction chamber that sits on a 2mm x 2mm Si island surrounded by air and suspended by low-thermal-conductivity parylene bridges (Figs. 1 and 2). This design eliminates the need to heat the entire chip. Mechanical stability of the island was excellent as no relative movement with respect to the chip's main body was observed throughout the fabrication and testing phases of the device.

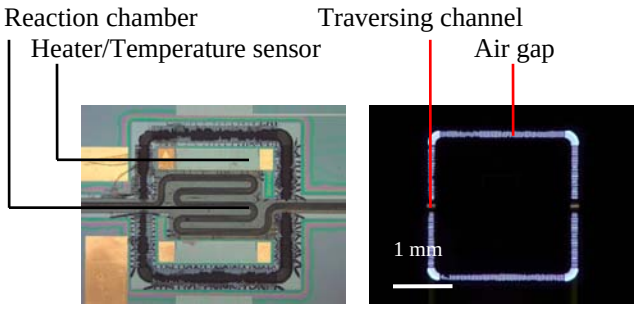

Figure 1: Thermally isolated reaction chamber with (left) front side lighting and (right) backlighting. The air gap is connected to the main body via parylene bridges. In this picture, wire bonds were removed for clarity

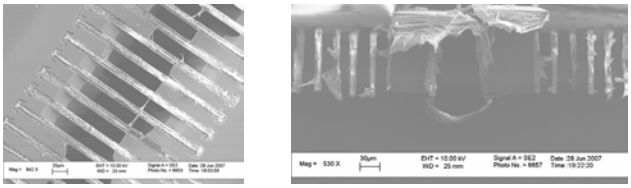

Figure 2: Air gap. (Left) top view of gap with parylene stitches. (Right) Cross sectional view showing bridges and air-gap-traversing channel

The 70nL qPCR reaction chamber is a serpentine channel embedded in the silicon. It is fabricated using a process that results in a clean parylene surface by avoiding the use of a photoresist sacrificial layer [7]. The channel is still fluidically connected to the main body via a parylene channel section that traverses the air gap (Fig. 1 and 2, right) to continue to the main body.

The integrated heater/temperature sensor is a 2.3 kOhm Ti/Pt metal trace. It is electrically connected to the main body by gold bridges formed by wire bonding. The main body (Fig. 3) contains the electrical contact pads and fluidic inlet and outlet holes. A customized Ultem and acrylic jig (Fig 4) is designed and fabricated in-house using a computer numerical control (CNC) milling machine.

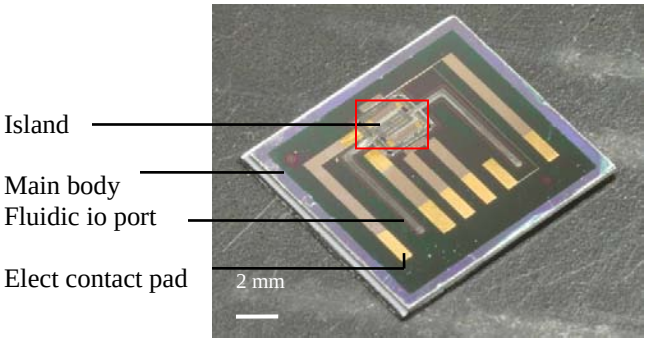

Figure 3: Entire chip view. Red square is the thermally isolated island enlarged for figure 1

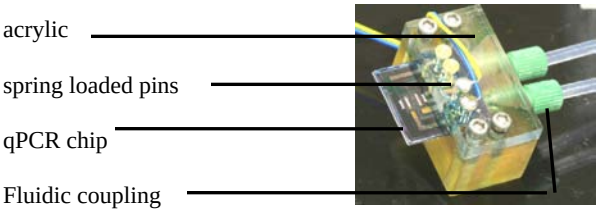

Figure 4: Customized jig. Amber section is Ultem polymer while clear top plate is acrylic. Spring loaded conductive pins provide electrical contact with the chip

FABRICATION

The fabrication is illustrated in figure 5. After growing 1um of thermal oxide as an electrical insulation layer, the metal layer (300Å Ti , 2000Å Pt, 2000Å, Au) is deposited and patterned using a lift off technique. The gold is etched everywhere except at contact pads. Trenches for channel sidewalls and stitches are then etched using deep reactive ion etching (DRIE). The first parylene layer is then deposited to fill the trenches. The air gap and inlet/outlet holes are then simultaneously formed by etching the backside silicon using DRIE. Next, the parylene layer is etched to expose silicon and XeF_2 is used to etch the exposed silicon about

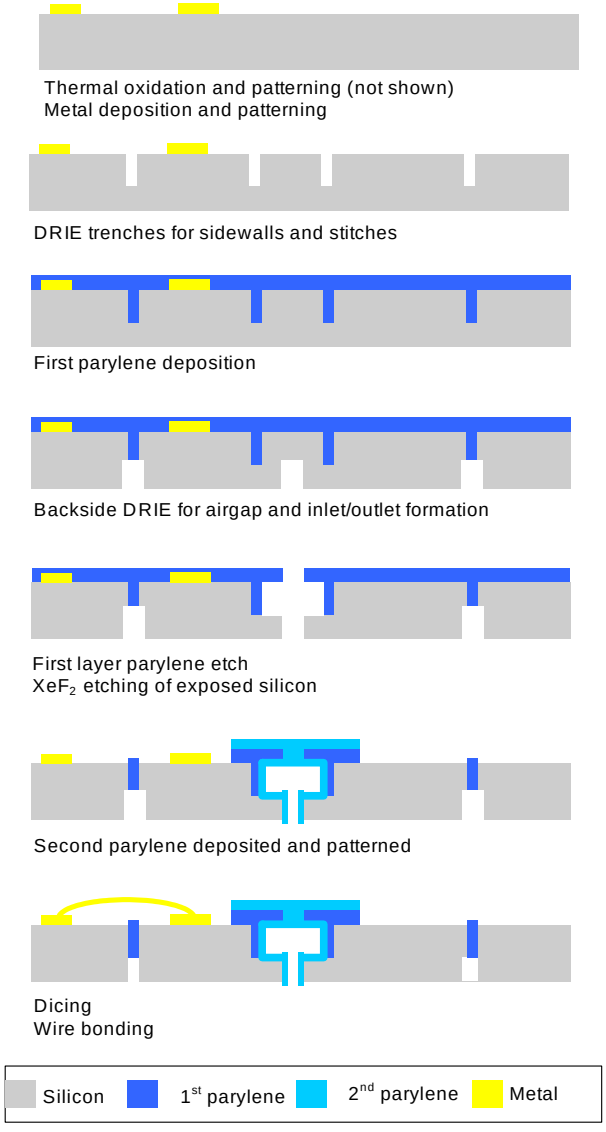

Figure 5: Fabrication process flow

100 µm deep forming the fluidic channels. The sidewalls protect XeF_2 from excess lateral etching due to its isotropic silicon etching characteristic. Next, a second parylene layer is deposited and patterned to seal the channels. This embedded channel technology enables channels of arbitrary length by eliminating the diffusion-limited sacrificial material release step required for surface micromachining of parylene channels. After dicing, wire bonding is performed to electrically connect the heaters on the island to the main body.

TESTING RESULTS AND DISCUSSION

Thermal Characterization

The temperature coefficient of resistance was 2.3×10^{-3} $/^0C$ from the calibration curve (Fig 6). From this relationship between resistance and temperature, LabView was used to implement a PID scheme to feedback control a power source and a multimeter to supply programmed power and temperature profiles.

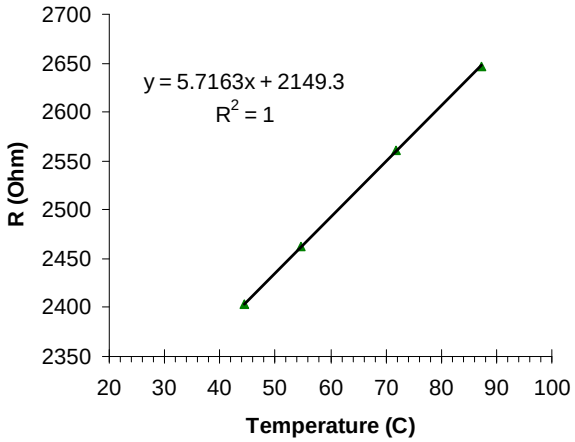

Figure 6: Temperature calibration. Resistance showed a highly linear relationship with temperature

The basic thermal characteristics of the dry chip (with empty channels) can be described using a simple analogy to a parallel RC circuit driven by a constant current source (figure 7).

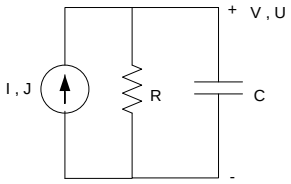

Figure 7: Variables for electrical, thermal circuit

In the electrical case, the voltage difference, V, observed in response to the applied current, I, is characterized by equation (1).

$$V(t) = V_0 e^{-t/RC} + IR(1 - e^{-t/RC}) \qquad (1)$$

Transferring to the thermal case, the voltage difference is replaced by the temperature difference, U, between the chip and room temperature (taken as 23.5^0C here). The applied current is replaced by the applied power, J.

$$U(t) = U_0 e^{-t/RC} + JR(1 - e^{-t/RC})$$
$$U = T - T_{room} \qquad (2)$$
$$\tau = RC$$

It should be noted that this simple model serves only to provide a framework to quantitate the thermal properties of the chip. A more thorough model would address assumptions relating to higher order effects such as differences in temperature, thermal conductivity, and heat capacities between the silicon island,

parylene channels, and PCR fluid.

The thermal resistance, R, is the resistance to the natural convection heat flow from the chip into the air. Hence, R = 1/hA where h is the heat transfer coefficient and A is the heat transfer area which is the top and bottom surface of the 2mm x 2mm island. R can be determined by measuring the steady state temperature for a given applied power. From figure 8, the thermal resistance is 0.34 ^{0}C/mW corresponding to a heat transfer coefficient of 735 W/m^2 ^{0}C.

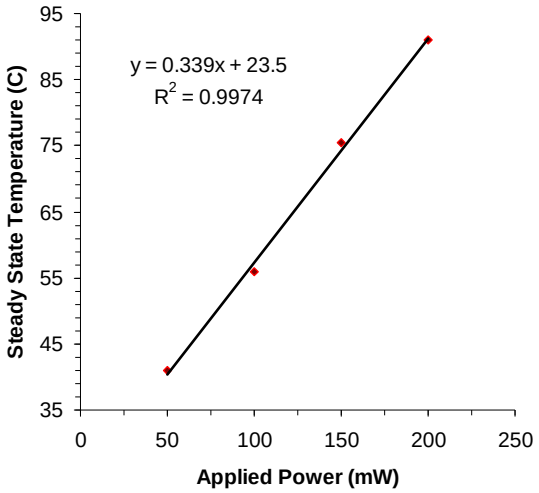

Figure 8: Steady State Power Response

The thermal time constant, τ, was determined by fitting the model to the temperature response of the chip when the applied power is suddenly and instantaneously removed (figure 9). This yielded a time constant of approximately 2 seconds. From the values of τ and R, the calculated thermal capacitance, C is 5.9 mJ/^{0}C. Given the rough nature of the model, the value for C was in fair agreement with the expected value of 3.6mJ/^{0}C calculated from the heat capacity, density, and volume of the thermal island assuming it is comprised of only silicon.

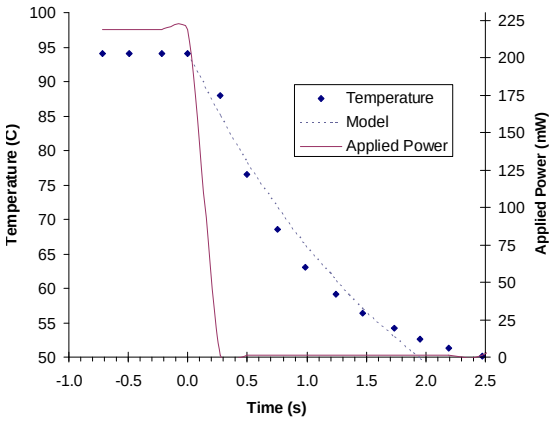

Figure 9: Step response to zero applied power

PCR

Although the temperature could increase at about 10^{0}C/sec and decrease at about 14^{0}C/sec using only resistive heating and passive cooling, for simplicity all ramping times were set to 5 seconds for the PCR tests runs (Fig. 11). The rates were programmed as linear increases and decreases instead of aggressive step functions to minimize instability from the PID control algorithm, although better control and faster ramping times should be easily achieved with further experimentation with the control parameters.

We tested our qPCR chip using the M13 virus which has been shown to be an excellent model system for qPCR analysis [8]. The test solution was a freeze-dried seed sample of M13 from the American Type Culture Collection (ATCC #15669-B1) resuspended in 1mL of Tris-EDTA buffer pH 8.0. A standard Sybr Green I qPCR protocol was used with viral lysis occurring during the initial 2 min at 95^{0}C "hot-start" procedure. Thermal cycling then proceeded with the following temperature schedule: 95^{0}C for 15 sec, 51^{0}C for 15sec, and 72^{0}C for 15 sec for a total of 40 cycles. A fluorescence microscope and CCD camera were used to capture images of the chip and an image analysis program, ImageJ, [9] was used to extract the fluorescence data. Figure 10 shows that this device successfully discriminated between the no primers control and the test solution of 10^7 viral particles/ul. Based on the chamber volume, the total viral load was 7x10^5 viral particles.

Improvements to the device can be made from both the engineering and biological standpoints. Although this work focuses on thermal optimization, optical performance is also an important factor for this device. Studies of the optical behavior of parylene and silicon will lead to future designs.

From the biological standpoint, total cycling time can be greatly reduced by optimizing the hold times for each temperature. Hold times down to 5 seconds at each temperature have been reported for on-chip PCR [10]. Although basic viral detection is demonstrated here further work needs to be done to determine the sensitivity of the device both in terms of minimum viral load and minimum DNA load that can be detected. It would also be natural to test the device with RNA samples.

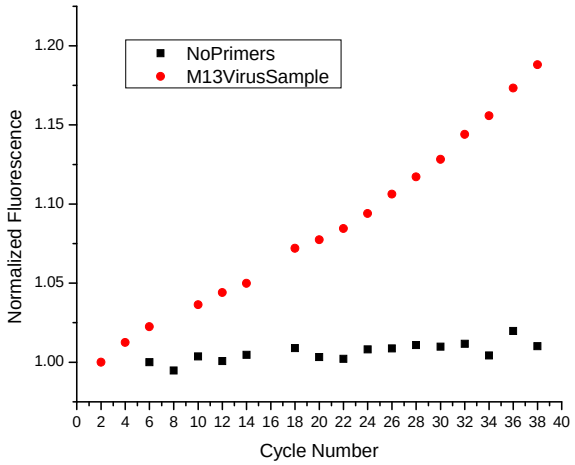

Figure 10: Example qPCR result. A fluorescence photograph of the chip was taken at the end of the 72^{0}C extension step every 2 cycles. The image was then analyzed using the image analysis program ImageJ

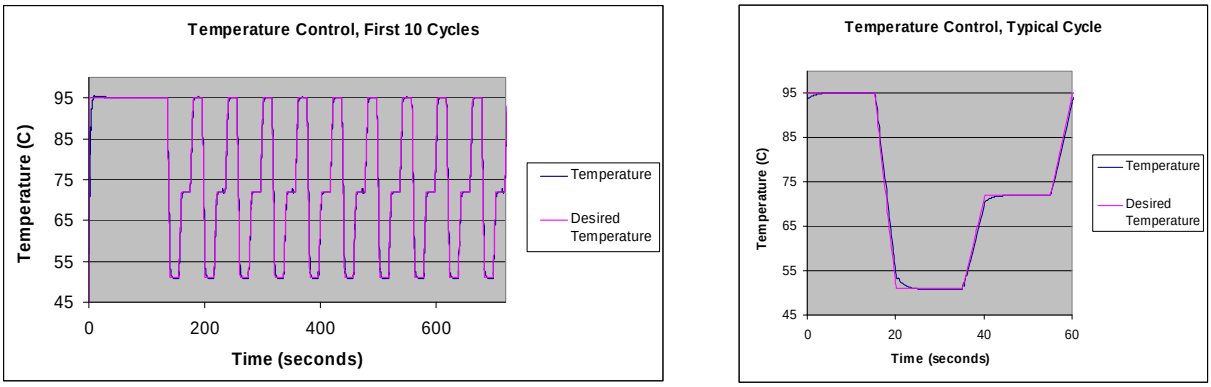

Figure 11: Temperature profiles for qPCR chip. Actual temperatures matched desired temperatures within 0.1°C. Left: First 10 cycles including initial 'hot-start' and viral lysing at 95 °C for 2 min. Right: Typical cycle

CONLUSIONS

These results demonstrate a thermally improved quantitative PCR chip based on parylene technology capable of detecting a model virus. The fast thermal response with low power consumption is crucial to the development of battery-powered portable instruments for quantitative PCR while the use of parylene allows future integration with the various other parylene based MEMS devices.

ACKNOWLEDGEMENTS

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