# Highly integrated transparent microchannel chips for continuous-flow PCR

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## ABSTRACT

A transparent microchannel chip for continuous-flow polymerase chain reaction (PCR) was developed. The microchannel was fabricated on a cover glass substrate using standard photolithography and wet-etching techniques, and sealed by another cover glass substrate. A heat source consisting of two indium-tin-oxide (ITO) films was deposited on the etched substrate. An insulation layer of $SiO_2$ was then deposited on the ITO heaters. Thin, patterned ITO films deposited on the insulation layer served as temperature sensors. Linear relationship between the thin ITO film sheet resistivity and the temperature was confirmed and calibrated. An amplication of a 97-bp segment of rat tongue cDNA was successfully performed with the chip.

***Keywords***: microchannel; PCR; ITO; heater; sensor

## 1 INTRODUCTION

Polymerase chain reaction (PCR) has developed into a popular technique since its discovery by Kary B. Mullis et al. in 1985. To date, many micro-PCR chip instruments have been developed to perform rapid thermal cycling and analysis with reduced reagent [1-5]. Summarily, There are two general types of micro-PCR chips, which use different reactors. One of them is based on chambers [1], while the other is a fluidic system [3]. The chambers of the former could be made very small on silicon or glass substrates and have rapid temperature ramp rates of 3~4 ℃/s for cooling and of 6℃/s or even of 30℃/s for heating [2]. In some studies integration of PCR and electrophoresis on small chips was demonstrated [4,5]. Microfluidic systems have a novel and simple structure, which makes it possible to achieve similar rapid temperature ramp rates without need for bulky cooling units. In contrast to the chamber-based systems, their fabrication and operation, namely handling of the sample transport, are simple.

Integration of heaters and thermal sensors is important, considering the need for high heat-transfer coefficient and accurate temperature control. A wide variety of investigations have been carried out to study integrated heaters and sensors for microreactors [1,6-10]. These heaters utilize metal [6] or polysilicon [1] thin film as a resistor to generate heat over a large surface area. ITO has been used as a material for heating small devices [7], because of its advanced features, including low resistivity ($\rho$=~$\times 10^{-4}\Omega\cdot$cm), strong adhesion to glass substrates, and good transmittance in visible regions (> 80%). Conventional thermal sensors consist of a thin metallic wire or a thermistor bead. Miniature thermal sensors usually involve a thin film and patterned using photolithography and deposition technique [8-10], thus it is smaller than conventional ones and is easily integrated. However, to avoid blocking the detection light, these sensors are always mounted at a small distance from the reactors. Thus the temperature of the reactors cannot be measured and controlled well.

Our work is aimed at developing a continuous flow PCR chip from transparent materials with low fabrication costs and high device performance. Barium borosilicate glass was used as the substrate of the chip, and two layers of indium tin oxide (ITO) films were employed as thermal sources and temperature sensors for the PCR. To test its operation, a 97-bp segment of rat tongue cDNA was amplified using the developed microchannel chip.

## 2 EXPERIMENTAL

### 2.1 Microfabrication

Figure 1 shows the structure of the chip reactor and the integrated heaters and temperature sensors. The starting material is a barium borosilicate substrate (30 mm × 30 mm × 500 μm). The substrate is first sonicated in acetone and methanol, rinsed with deionized water, and blow-dried with nitrogen gas. A metal layer of 1000 Å chromium is then coated on the substrate by magnetron RF sputtering. Subsequently, microchannel is photolithographically patterned on the front side, followed by glass etching in HF solution (HF: 46%) for 1 min to the depth of 20 μm (50 μm wide). The microchannel incorporates 40 cycles with inlet and outlet portions on the two sides of the substrate. The

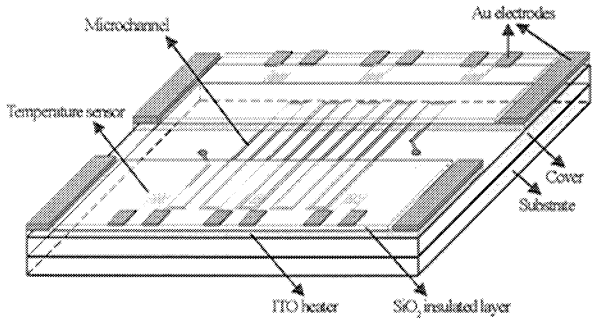


Figure 1: The structure of the microchannel chip for PCR.

bonding process consists of several steps. First, the substrates are cleaned in acetone to remove particles, dust and organic contamination. Then, a sulphuric acid peroxide mixture ($H_2SO_4$ : $H_2O_2$ = 5:1) is used to increase the hydrophilicity of the glass substrates. Immediately after cleaning, the cover plate is brought into contact with the glass substrate. It is very important to squeeze out the air between the two plates until no seeing of Newton rings. The bonding is carried out in the furnace at a constant pressure of 3 MPa and a temperature of 430℃ for 15 hours. The furnace is then turned off, and the structures are allowed to cool inside overnight.

The ITO thin films, served as heaters, are sputtered on the back side of the etched substrate at substrate temperature 300℃ followed by sputtering deposition of 2000 Å of gold on top of 100 Å of chromium. The thickness of the films are 400 nm, and their resistivity is $1.6\times10^{-4}$ Ω·cm. The ITO films are fabricated by means of standard photolithographic and wet-chemical etching techniques. The etchant is composed of 6 M HCl and 0.2 M $FeCl_3$, and the etching rate is about 150 Å/min. A layer of $SiO_2$ is then deposited on the ITO heaters by electron beam (EB) evaporation to form an insulation layer. At last, a layer of ITO film is deposited on the top of the insulation layer, and is patterned by using the same method as was described above. The substrate temperature of sputtering and annealing temperature are optimized in order to obtain more sensitive and stable temperature sensors.

## 2.2 Quantitative PCR conditions

The thermal control system is composed of three main units including microchannel chip, the temperature sensors data acquisition unit and the heaters control unit. The schematic diagram of the whole setup is shown in Figure 2. The temperature sensors are connected to a $6^1/_2$-digit high-performance digital multimeter (DMM: Model 2000, KEITHLEY), and the conversion from resistance to temperature is performed by a software based on the calibration curve of the temperature sensors after the data is transmitted to a computer through RS232 interface from the DMM. The heaters are driven by a DC power supply and are controlled by solid-state relays (SSR), a D/A board, and computer-controlled proportional/integral/derivative (PID) arithmetic.

The fluorescence detection system contains a microscope (Olympus IX70) and ICCD camera (Andor DH534). The microscope is equipped with a mercury burner lamp and an excitation filter (Omega BP460-490) to illuminate the sample. The filter (Omega BA515-550) in front of ICCD camera is for cutting reflected excitation light and passing only the desired fluorescence from the sample. A precision syringe pump (Harvard, Model 975) is used to deliver the solution into the microchannel, and fluorescence is measured in-situ under a continuous solution flow.

SYBR® Green PCR Master Mix (ABI), including SYBR® Green I dye, AmpliTaq Gold DNA Polymerase, dNTP with dUTP, Passive Reference and optimized buffer, was used. Sensitive experiments were performed using rat tongue cDNA as a template, and the primers (each 100 nM) designed to produce a 97-bp segment of Rat Chymase. All reactions were performed in 20 μl volumes. The template

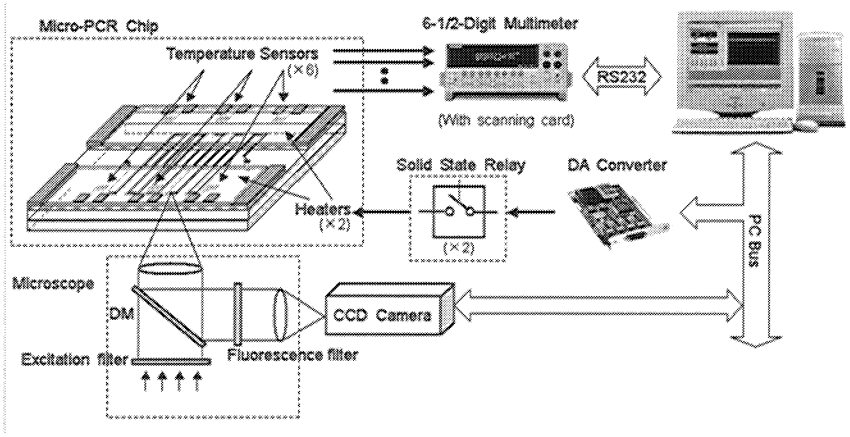


Figure 2: Schematic diagram of the instrumentation.

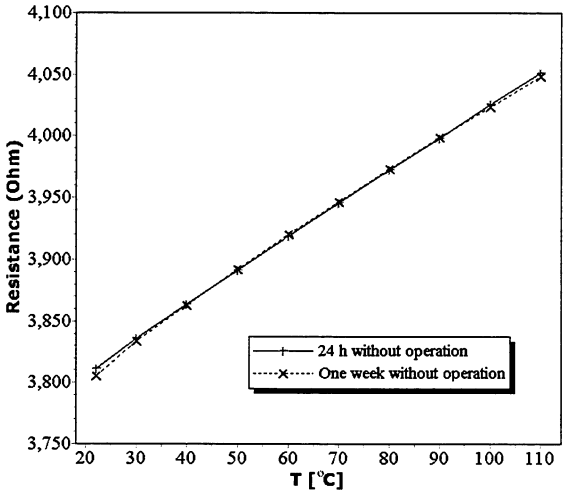


Figure 3: Calibration of the ITO temperature sensor.

was diluted to the concentration of 50ng, 25ng, 10ng, 5ng,and 2.5ng per micro liter. The mixture flowed through the channel with 40 cycles at 95℃ 10 s for denaturation and 15 s annealing at 60℃. The quantitative PCR analysis using SYBR® Green I dye was performed by taking and saving an image of the PCR during each annealing/extension phase.

## 3 RESULTS AND DISCUSSION

### 3.1 Calibration of temperature sensor

Two of the most important requirements for sensors are sensitivity and stability. All the experiments were finished in still air with the assumption that the surface temperature of samples is equal to the ambient gas temperature. And all the data were obtained by temperature cycling (TC) approach [10], which means that the sensor ambient temperature varies in an appropriate range, and a time period of about 30 min is required for each temperature. Two kinds of ITO thin films were used, one is sputtered at the substrate temperature of 300℃ (hereafter called Sensor 1), the other sputtered at room temperature (hereafter called Sensor 2). A linear rise with increasing temperature is found in the curves of Sensor 1. Although the Sensor 2 after annealing at 200℃ for 1 h also showed a similar linear relationship, it needed longer time to stabilize after being heated, and could not be repriduced well. Annealing has an interesting influence on Sensor 2. After the annealing at 300℃ its resistance decreases when temperature is above 70℃-80℃. Also, Sensor 2 shows about one percent of negative drift error in its repeatability. The surface topography of ITO thin films deposited on cover glass substrate at substrate temperature of 300℃ and RT are observed by AFM. The mean roughness at 300℃ and RT are 6.1 and 11.4 nm. Here, we think low substrate temperature during sputter deposition will result in amorphous or partially amorphous material. Moreover, it is clear that the crystallinity improves with increasing temperature. The improvement may be due to an increase in carrier density with increasing substrate temperature and this in turn can be attributed to the improvement of the crystallinity which results in decreasing donor sites trapped at the dislocations and grain boundaries [11].

It is well known that an important aspect of a sensor operation is its stability (drift) during operation and its repeatability over an extended period of time. The difference between calibration curves 24 h delay without operation of sensor 1(Figure 3) is less than 0.07%. Hence, we adopted the ITO thin film sputtered at substrate temperature of 300℃ as temperature sensor. In the case of resistor with positive temperature coefficient, its resistivity is expressed as follows:

$$R_T = R_0 e^{A(T+273)} \quad (1)$$

Where $R_T$ is the resistance of the sensor at temperature (T, ℃), $R_0$ is a constant, which depends on the size and physical characteristics of the material, and $A$ is a constant, equal in value to the temperature coefficient of resistance (TCR, /℃) of the sensor. Here, the TCR of ITO sensor is 0.7 $\times 10^{-3}$/℃. To simplify the calibration, a linear temperature -resistance relationship is used according to Figure 3. The linearity of correlation is represented by:

$$R_T = 3.26 + 2.72T \qquad (20 \le T \le 110).$$

This relationship was used for the calibration of temperature sensors in PCR experiment.

### 3.2 PCR amplification

The analysis of this set of reaction is shown in Figure 4. SYBR® Green I dye has been shown to produce results that

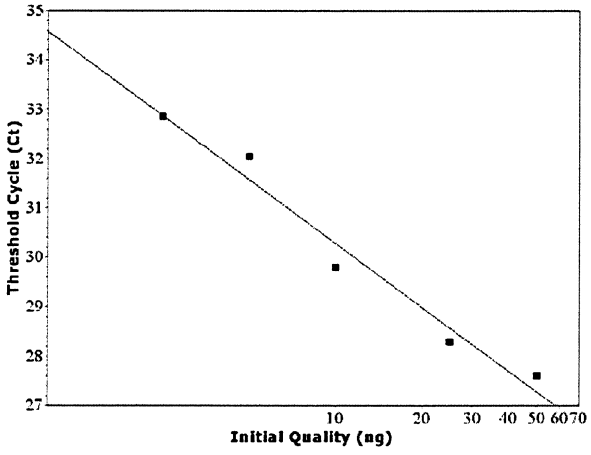


Figure 4: Linearity of template dilutions with SYBR® Green.

are linear over 5 logs of initial template concentrations. The PCR products were also detected using electrophoresis. The gel was stained for 60 min with SYBR® Green I nucleic acid gel stain (using a 1:10,000 dilution of the stock reagent) and not destained. The SYBR® Green I dye-stained gel was then excited using 254 nm transillumination, and was photographed with CCD camera (using a SYBR® Green gel stain photographic filter). The 97-bp PCR products with different dilutions of templates were observed and non-specific products were not found. Thus, the microchannel chip performed the PCR amplification successfully.

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