MICRO-MANUFACTURING OF A NANO-LITER-SCALE, CONTINUOUS-FLOW POLYMERASE CHAIN REACTION SYSTEM

Wei Zheng and Shaochen Chen*
Department of Industrial and Manufacturing Systems Engineering
Iowa State University
Ames, Iowa 50011

ABSTRACT

In recent years, many micromachined polymerase chain reaction (PCR) chips have been reported. These miniaturized PCR chips have great advantages such as the significant reduction in reagent costs and vastly reduced reaction times over the conventional PCR reactions. In this paper a micro analysis system that will allow nano-liter scale, continuous-flow PCR to be conducted in a glass chip has been presented. This glass chip is fabricated through thermally bonding two Pyrex 7740 glass wafers. One Pyrex wafer is etched to form the 20-cycle microchannel with 80 microns wide and 30 microns deep. Another Pyrex wafer is thermally bonded to the etched wafer to produce a closed continuous microchannel for PCR. The total length of the microchannel is 0.5 m and the size of the chip is 56 mm $\times$ 24 mm $\times$ 1 mm. This PCR chip has a significant reagent reduction with a volume of less than 1 micro-liter. With 1 micro-liter reagent we obtain a total reaction time of 0.5 min to 3 min depending on various flow rates. This analytical chip is fabricated using standard micromachining techniques.

INTRODUCTION

Polymerase chain reaction (PCR) is a technique to amplify the number of copies of a specific region of DNA in order to produce enough DNA to be tested. Since the introduction of the PCR technique, PCRs have become one of the most useful and versatile processes in biological science (Mullis et al., 1986). Recently, microfabrication technology has led to the realization of miniature PCR devices. The first micromachined PCR device was presented in 1993 (Northrup et al., 1993). It used a relatively large reagent volume of 25~50 μl. Silicone adhesive was used for the sealing of the chambers. Since then, great progress has been made in miniaturizing, shortening thermal cycle time, and reducing reagent volume. More recently, several groups have reported PCR devices with various designs of micromachined heating chambers (Woolley et al., 1996; Daniel et al., 1998; Northrup et al., 1998; Wilding et al., 1998; Chaudhari et al., 1998; Taylor et al., 1998; Waters et al., 1998). However, these devices lack the flexibility of changing the reaction rate, resulting in more cycling and heating time. Instead of using a microchamber, Kopp et al. utilized a microfabricated device to perform continuous flow PCR at a higher speed (Kopp et al., 1998). The micromachined “chemical amplifier” relied on the movement of PCR reaction samples through a microchannel (40 μm deep and 90μm wide) across defined temperature zones on a glass microchip. Using 20-cycle of PCR amplification, a 176-base pair fragment from the gyrase gene of Neisseria gonorrhoeae was successfully amplified at various flow rates, resulting in total reaction times of only 90 seconds to 18.7 minutes. One can control the sample injection volume from a few nanoliter to several microliter, which brings the flexibility to the application of this device and results in a significant reduction in reagent costs and reaction times. But, this device has some difficulty in the integration of fabrication. It requires extra devices for heating and cooling and controlling reaction temperatures, thus increasing the fabrication cost.

In this paper, we have developed a fully integrated system in glass wafer for continuous flow, nano-liter scale PCR for the analysis of plant genomes. This glass chip is achieved through thermally bonding two Pyrex 7740 glass wafers. One Pyrex wafer is etched to form the 20-cycle microchannel with 80 microns wide and

30 microns deep. Another cover Pyrex wafer is thermally bonded to the etched wafer to produce closed continuous microchannel for PCR. The total microchannel length is about 0.5 m. Nickel micro-heaters and temperature sensors have been integrated onto the chip.

DESIGN

For DNA amplification, it is necessary to cycle reaction mixtures through three temperatures (e.g., 95°C, 72°C and 60°C) multiple times. This is realized by using three independent thin film heating elements fabricated on the surface of the glass wafer (Fig.1). Hence, each time a PCR reaction volume flows through its microchannel across the microchip, it will experience three distinct temperature zones. Since the rate of fluid flow is constant across the microchannel, the amount of time each reaction volume is exposed to the three temperatures can be controlled by varying the pattern of the microchanel. The number of temperature cycles that the PCR device experiences can be controlled by varying the number of cycles of the microchannel crossing the three temperature regions.

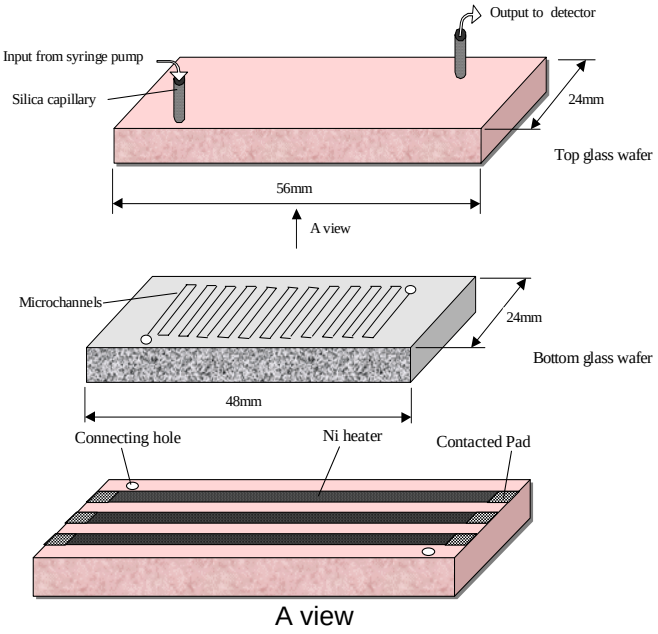


Fig.1 Layout of the PCR chip

Three PID (proportional, integral, and derivative) digital temperature controllers were used to control the Ni heaters to obtain three required temperatures. The temperature of each heater was monitored by a RTD resistor. The heater resistance can be calculated from:

$$R = \frac{L}{A\sigma} \quad (1)$$

where σ is the electrical conductivity of the Ni, A is the area of cross section (8×10^{-10} m^2) and L is the length of the heater (0.056 m).The heat capacity of the heater is found from:

$$C_T = \rho A L C_m \quad (2)$$

where ρ is the mass density of Ni and C_m is the specific heat per unit mass (444 J/kg-K).
To estimate the thermal resistance, we use the highly simplified mode (Senturia, 2001):

$$R_T = \frac{T_R}{I_Q} \quad (3)$$

where T_R is the heater temperature and I_Q is the total heat current through the boundary layer.
The steady-state temperature rise T_{SS} of the micro-heater for a steady current I is:

$$T_{ss} = \frac{R_0 R_T I^2}{1 - \alpha_R R_0 R_T I^2} \quad (4)$$

where R_0 is the resistance at the reference temperature of 296K, and α_R is the temperature coefficient of the resistance, or TCR. The results of T_{ss} for a range of currents are shown in Fig. 2.

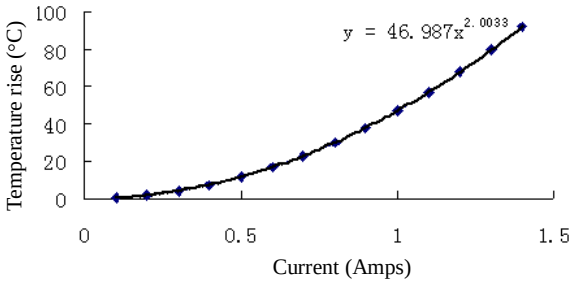


Fig. 2 Temperature rise vs. current of the heater

The temperature of 92°C requires a drive current of about 1.2 A, corresponding to a power supply of 7 W.

When the fluid moves through the microchannel, the temperature zones are fixed. So heat transfer is mainly by conduction and convection. Considering a section of the fluid channel located in a region of uniform wall temperature T, we can calculate the wall-to-fluid time constant for this section of the channel as:

$$\tau = \frac{\rho C_m A}{2k\pi^2} \quad (5)$$

where ρ is the mass density of water (1000 kg/m^3) and C_m is the specific heat per unit mass (4182 J/kg-K), k is the thermal conductivity of water (0.6 W/K-m), and A is the cross section area of the channel (30 μm × 80 μm). Calculation shows that the thermal time constant τ is about 0.85 ms. Based on the time constant τ, if a segment of fluid at temperature T_f moving with an average fluid velocity v enters a region with uniform wall temperature T_w, it will take a time of order τ for the center of the fluid to reach temperature Tw. We can get the corresponding distance $v\tau$ that the fluid must flow before it reaches the temperature Tw. Fig. 3 shows the thermal equilibrium distances with respect to different flow velocities.

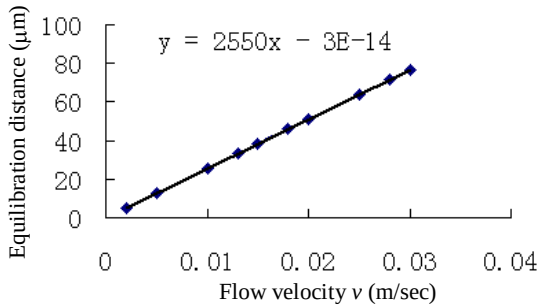


Fig. 3 Distance vs. flow velocity

From Fig. 3, we can see that a distance of about 80 μm is needed to reach the equilibrium temperature. Since the minimum length of every channel zone is 2 mm that is much greater than 80 μm, we can assume that the fluid sample fully satisfies the thermal requirement.

In this device, 20 identical cycles were used with a theoretical amplification factor of 2^{20}. The microchannel has a length ratio of 4:4:9 for melting:annealing:extension. The glass chip consists of two Pyrex 7740 glass wafers thermally bonded together. The microchannel etched into the bottom pyrex is designed to give the continuous reaction channels for reagents. The microchannel has a dimension of 80 μm in width and 30 μm in depth. The total length of the microchannel is about 0.5 m, which gives the reagent volume from 10 nanoliter to 1.5 microliter. The heaters that are integrated onto the top Pyrex wafer are made of nickel of 200 nm thick. The top Pyrex has two connection holes, through which the reagent can be injected into the channel on the bottom Pyrex wafer, and also the silica capillary can be glued in. The two wafers have different sizes such that the heaters can be controlled by the outside circuit. The bottom Pyrex wafer has a size of 48 mm × 24 mm. The top Pyrex wafer has a size of 56 mm × 24 mm.

FABRICATION

The device was fabricated using 0.5 mm thick, 4- inch Pyrex 7740 glass wafers. Figures 4 and 5 show the fabrication procedures.

Fabrication of the microchannel

Pyrex wafers were cleaned before deposition of metal mask of 15 nm Cr and 200 nm Au using an e-beam evaporator. The AZ5206 photoresist was spun onto the wafer at 4000 rpm. Then the metal mask was patterned using mask #1 and developed in MIF-312 1:1.2 developer for about one minute. The metal mask was etched using KI:I_2:H_2O(4:1:40) for Au and HCl:Glycerine: H_2O (1:1:3) for Cr. The exposed glass area was etched in HF (49%):HNO_3 (69%):H_2O [20:14:66] for 30 min. Finally the photoresist and metal mask were removed. The microchannel was measured by SEM (Fig.6).

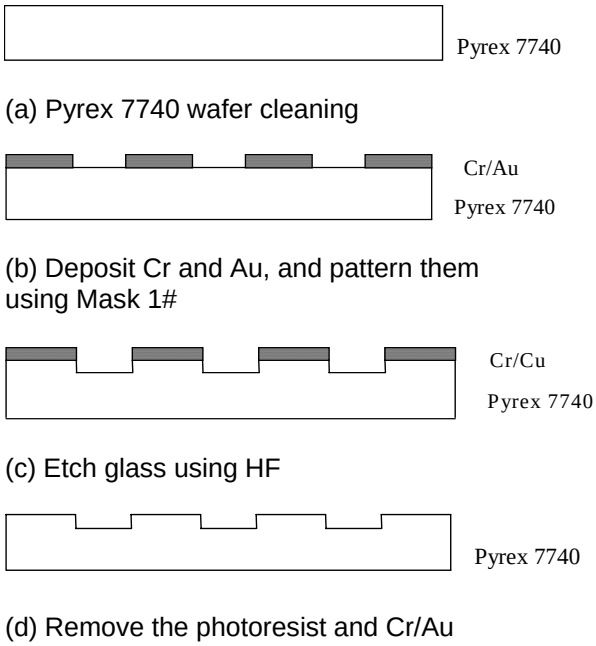


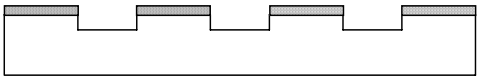
Fig.4 Fabrication procedures of the microchannel

Fabrication of microheaters and through holes

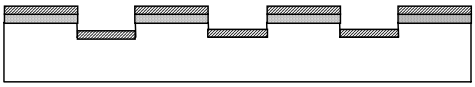
First, Pyrex wafers were cleaned. Then the AZ5206 photoresist was spun onto the wafer at 4000 rpm. The photoresist was patterned using mask #2 and developed in MIF-312 1:1.2

developer for about one minute. The exposed glass area was etched in BOE for 70 minutes to get a 1.0 μm deep channel. A 20 nm thick Cr film and a 300 nm thick nickel film were deposited using an e-beam evaporator. After that, the photoresist was removed and the unwanted metal was lifted off. The 1.0 μm thick SiO_2 layer was deposited using PECVD. Then the SiO_2 film was patterned using mask 3 # and etched using BOE. Finally two holes of 400 μm diameter were ultrasonically drilled.

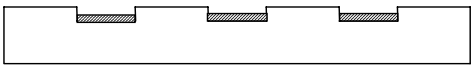
(a) Pyrex 7740 wafer



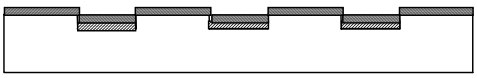
(b) Deposit photoresist and pattern them using Mask 2# and etch glass



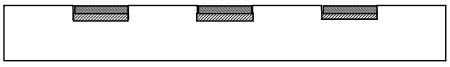
(c) Deposit Cr and Ni layers



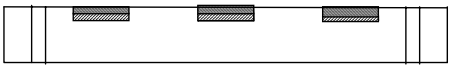
(d) Remove the photoresist



(e) Deposit 1.0 μm thick SiO_2 layer



(f) Pattern the SiO_2 layer using Mask3 #



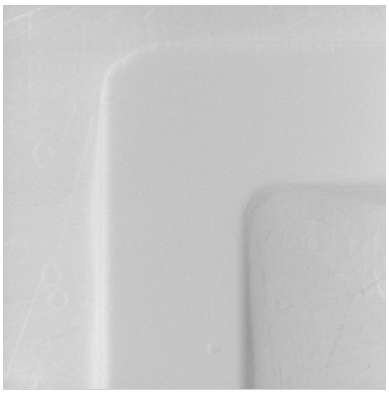
(g) Drill two holes

Fig.5 Fabrication procedures of the heater

Bonding of two wafers

The two wafers were cleaned before bonding. The Ni heaters face the microchannel that was pre-fabricated on the bottom wafer. The two wafers were clamped with a uniform external pressure and then put into a programmable furnace. The heating program was as follows: 20°C/min ramp to 400 °C, 10°C /min ramp to 650 °C held for 4 hours, 10°C /min ramp to 450 °C, and then cooled to the room temperature (Lapos and Ewing, 2000). Frequently, some regions were not properly bonded. Our approach is to repeat the heating/cooling cycle again.

(a)



(b)

Fig.6 SEM images of the microchannel: (a) a large view of the microchannel, (b) a closed-up view

TESTING

A syringe pump (KDS 53200, 10μl) was used to inject sample to the device through the silica capillary (OD 400 μm, ID 320 μm). Three PID temperature controllers were used to control three Ni heaters to obtain the required temperatures. The temperature of each heater was monitored by a RTD resistor. For each amplification, 1μl mixture was injected. The device was run with different flow rates controlled by the syringe pump. As a result, we obtained different total reaction times from 14 s to 3 min.

CONCLUSIONS

We have designed and fabricated a micro device that can perform continuous flow PCR on glass wafers. This glass chip is fabricated through thermally bonding two Pyrex 7740 glass wafers. One Pyrex wafer is etched to form the 20-cycle microchannel with 80 microns wide and 30 microns deep. Another Pyrex wafer is thermally bonded to the etched wafer to produce a closed continuous microchannel for PCR. The total length of the microchannel is 0.5 m and the size of the chip is 56 mm $\times$ 24 mm $\times$ 1 mm. Miniaturization of bioanalytical reaction chambers brings obvious advantages over conventional analytical method. The fast and small single device will offer an enormous market potential to different application areas such as medical and biological sciences and environment monitoring.

ACKNOWLEDGMENTS

This work was supported by the Plant Science Institute of Iowa State University (ISU). The authors would like to thank Dr. Tony Witt and Prof. Gary Tuttle in the Microelectronics Research Center at ISU for their help with the microfabrication facilities.

*****Present address: Department of Mechanical Engineering, The University of Texas at Austin, ETC 5.160, Austin, TX 78712.

REFERENCES

Chaudhari, A.M., T.M. Woudenberg, M. Albin and K.E. Goodson, (1998),"Transient liquid crystal thermometry of microfabricated PCR vessel. Arrays", *J. Microelectromech. Systems*. Vol.7, p345–355.

Daniel, J.H., S. Iqbal, R.B. Millington, D.F. Moore, C.R. Lowe, D.L.Leslie, M.A. Lee and M.J. Pearce, (1998), Silicon microchambers for DNA amplification, *Sensors and Actuators*, A 71, p81–88.

Kopp, M.U., de Mello, A.J., and Manz, A., (1998),"chemical amplification: Continuous-Flow PCR on a chip", *Science,* Vol.280, p1046-1048.

Lapos, J. A. and A. G.Ewing, (2000) "Injection of fluorescently labeled analyses into microfabricated chips using optically gated electrophoresis", *Anal. Chem*. Vol.72, p4598-4602.

Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn and H. Erlich, (1986), " Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction", *Cold Spring Harbor Symp. Quant. Biol*. Vol.51, 263–273.

Northrup, M.A., M.T. Ching, R.M. White and R.T. Watson, (1993) "DNA Amplification in a microfabricated reaction chamber", *Transducers '93, in: Proceedings of the 7th International Conference on Solid State Sensors and Actuators*, Yokohama, Japan, p.924-926.

Northrup, M.A., B. Benett, D. Hadley, P. Landre, S. Lehew, J. Richards and P. Stratton, (1998),"A miniature analytical instrument for nucleic acids based on micromachined silicon reaction chambers", *Anal. Chem*. Vol.70, p 918–922.

Taylor, T.B., S.E. Harvey, M. Albin, L. Lebak, Y. Ning, I. Mowat, T. Schuerlein and E. Principe, (1998),"Process control for optimal PCR performance in glass microstructures", *Biomedical Microdevices* Vol.1, p65–70.

Senturia, S.D. (2001), *Microsystem Design,* Kluwer Academic Publishers

Waters, L.C., S.C. Jacobson, N. Kroutchinina, J. Khandurina, R.S. Foote and J.M. Ramsey, (1998), "Multiple sample PCR amplification and electrophoretic analysis on a microchip", *Anal. Chem.* Vol.70, p5172–5176.

Wilding, P, L.J. Kricka, J. Cheng, G. Hvichia, M.A. Shoffner and P. Fortina, (1998), "Integrated cell isolation and polymerase chain reaction analysis using silicon microfilter chambers", *Anal. Biochem*. Vol.257, p95–100.

Woolley, A.T., D. Hadley, P. Landre, A.J. de Mello, R.A. Mathies and M.A. Northrup, (1996) "Functional integration of PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device", *Anal. Chem.* Vol.68, p4081–408