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Silicon microchambers for DNA amplification

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Abstract

Microchambers have been fabricated in silicon by bulk micromachining using anisotropic wet etching. Rapid temperature cycling and control at low power is achieved using a special thermal design of the chambers, combined with thin film platinum resistors as temperature sensors and heaters. The chambers have been used for the polymerase chain reaction (PCR) in order to amplify DNA. Rapid temperature cycling and small size are promising attributes for portable high efficiency analytical systems. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Silicon micromachining; Microchambers; Thermal isolation; Bioanalysis; Polymerase chain reaction

1. Introduction

The miniaturization of analytical devices by micromachining technology is destined to have a major impact on the medical and bioanalytical field [1]. There have been many reports of miniaturized systems, including microvalves, micropumps, microelectrophoresis devices and even fluid handling systems [2–7]. All this work is aimed at a so-called Micro-TAS (total chemical analysis system) [8]. DNA analysis has become an important analytical method for the detection of diseases, in forensics and for large scale gene mapping such as is required in the Human Genome project. However, in these cases the amount of sample is often small and direct detection would be impossible. The polymerase chain reaction (PCR) is a widely used method to amplify DNA [9]. A single DNA molecule can be typically copied a billion times giving quantities which are easily detected.

The process of the PCR involves a number of heat cycles (25–30) which can be performed in a thermocycler. During each cycle a double stranded target DNA is first separated (denatured) into two single strands at a high temperature (~90°C). Oligonucleotide primers that flank the DNA region to be amplified are then annealed at a low temperature (~50°C). Finally these DNA strands are extended in the presence of a polymerization enzyme and deoxynucleotide

triphosphates (dNTPs) at an intermediate temperature (~70°C). Each cycle doubles the amount of DNA.

In this paper we focus on miniaturized reaction chambers in silicon which are designed to allow the temperature of PCR reagents inside the chambers to be changed rapidly. The advantages of miniaturizing PCR devices are many [10–14]. Small size means a cost reduction because smaller quantities of the rather expensive reagents are needed. Having shorter analysis times due to faster operation, and a better performance are also significant advantages. An important factor is, of course, the possibility of manufacturing portable or hand-held devices which would be of great use for environmental screening and also in the medical sector. The mass production of silicon reactors could possibly make single-shot throw-away devices economically viable. Silicon as a material for the microchambers has the attraction that the fabrication technology is well developed due to the existence of the semiconductor industry. The shape can be precisely controlled with photolithography and etching technology. Silicon can easily be combined with thin film technology for implementing sensors. The thermal characteristics of silicon are good because of the high thermal conductivity. The availability of low-stress silicon nitride thin films allows chambers with transparent membranes for optical analysis of the reaction products to be made. Silicon is also ideal for arrays of microchambers on a wafer with the possibility to address the chambers individually. This feature is of significance for

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optimization of PCR amplifications or in large scale screening applications. Miniaturized PCR devices have been fabricated, as pioneered by the work of Northrup et al. [11,12]. Most of them are chips which employ wafer bonding to seal the chambers, designed for multiple use. Liquid volumes are typically above 10 μl and the concept of thermal management has only been recently addressed [14]. We focus on throw-away silicon chambers for reagent volumes around 1 μl [15]. The chosen thermal design allows low power operation for handheld analytical systems and arrays of PCR chambers. The microchambers can be easily filled with reagents and they are compatible with optical readout methods. The fabrication process is simple.

2. Thermal design

One disadvantage of conventional thermocyclers is the large thermal mass of the system. This results in high power requirements and slow heating and cooling rates. For efficient and rapid heat transfer it is desirable to have a system with low heat capacity which transfers heat quickly to the liquid upon heating, and quickly away if the heater is switched off. Miniaturization means that the thermal mass of the heating block is reduced. Silicon is an ideal starting material as its thermal conductivity λ is high with $\lambda \sim 150 \text{ W}/^\circ\text{C m}$ (at 20°C) and it can be precisely shaped. Fig. 1 shows a microchamber in silicon for the thermal cycling of liquids and Fig. 2 is a schematic of the layout along with the equivalent thermal network. The reaction chamber is suspended on four beams and thus partially thermally isolated from the substrate as seen in Figs. 1 and 3. The small dimensions of the chamber keep the heat capacity of the chamber low. The chamber and the liquid inside can then be rapidly heated by applying the heat through resistors directly to the chamber walls. Rather low power is necessary if the thermal isolation is good. During cooling the heat has to be conducted away from the chamber to the substrate (which is connected to a heat sink and thus kept at a constant low temperature). The degree of thermal isolation determines how fast the cooling occurs. The system can be described by a lumped-heat-capacity model [16,17] and the thermal time constant τ characterizes the system (Fig. 2):

$$\tau = R_{th} C_{th} = (l/4\lambda A) c\rho V \quad (1)$$

C_{th} is the thermal capacity of the chamber and R_{th} is the thermal resistance between the thermally insulated chamber and the surrounding substrate. C_{th} is expressed as $C_{th} = c\rho V$, with the specific heat c , density ρ and volume V (of the silicon chamber and the liquid inside the chamber). The thermal resistance $R_{th} = l/4\lambda A$ of the four suspension beams depends on the thermal conductivity λ of silicon, the beam cross-section A and the beam length l . The thermal time constant is analogous to the RC time constant for charging a capacitor in an electrical circuit. In Eq. (1), τ is the time when the temperature difference $T - T_0$ has a value of 37% of the initial

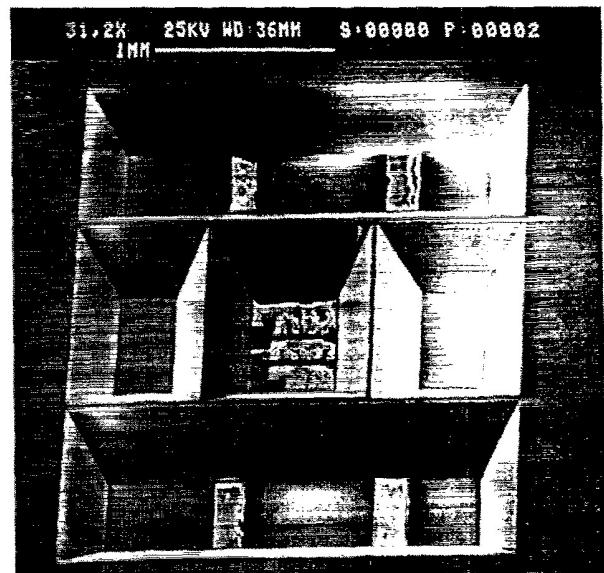


Fig. 1. Scanning electron micrograph (SEM) of a silicon microchamber which is thermally insulated from the surrounding wafer.

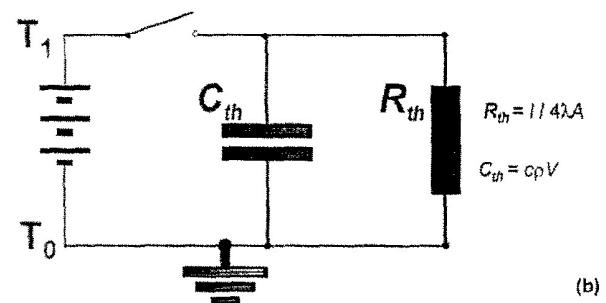
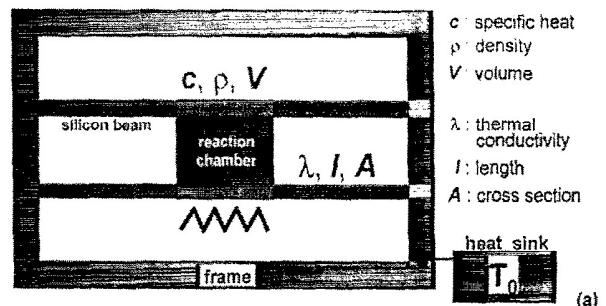


Fig. 2. (a) Schematic of the layout of a microchamber and (b) thermal network for the lumped-heat-capacity analysis.

difference $T_{in} - T_0$ upon heating or cooling. A thermal time constant which is not too big is required to ensure rapid cooling and not too small to keep the input power low. We aimed at a thermal time constant of the order of half a second. Taking the chamber design of Fig. 3 with 3 mm long suspension beams of cross-sectional area $A = 1.26 \times 10^{-7} \text{ m}^2$, we calculate a thermal resistance of $R_{th} = 40 \text{ K/W}$. The thermal capacity of the silicon chamber walls and the liquid inside the chamber (1.5 μl water and 1 μl oil) gave a calculated value of $C_{th} = C_{th}(\text{silicon}) + C_{th}(\text{liquid}) = 3.2 \text{ mJ/K} + 8.4 \text{ mJ/K} = 11.6 \text{ mJ/K}$. With these values the calculated thermal

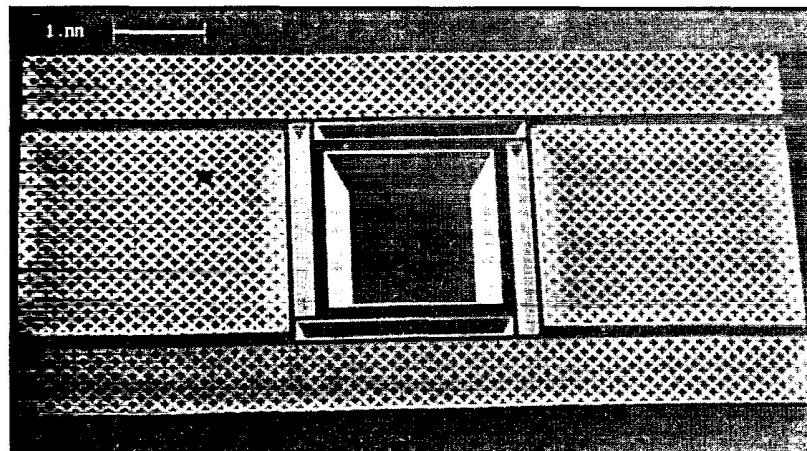


Fig. 3. SEM of a microchamber with webbing over the surrounding chambers for thermal isolation. The four silicon beams that thermally isolate the reaction chamber from the surrounding silicon substrate are each 3 mm long.

time constant is about $\tau \approx 0.46$ s. In our calculations contributions from thermal convection and radiation are neglected. Conduction through the silicon nitride membrane is negligible.

3. Temperature regulation

For regulating the temperature of the microchamber thin film platinum resistors are patterned on the underside of the chamber (Fig. 4). Two resistor lines (heater 1 and heater 2) which may be operated in series or in parallel act as resistive heaters. They apply the heat directly to the silicon walls of the reaction chamber. The temperature sensor is a platinum resistor on the underside of the chamber (T-sensor 1). The resistor is patterned on the bottom silicon nitride window in order to give an accurate temperature readout for the liquid inside the chamber. As the resistor lines can be kept narrow they do not block a significant proportion of the light that goes through the chamber in the case that an optical readout method is used. The temperature is regulated by a proportional feedback electronic circuit which is driven by a 15 V power supply. The temperature setting and the data acquisition is controlled by a computer with LabVIEW[®] software.

We found that to obtain a fast feedback it is effective to place a temperature sensor on the heated silicon beams, rather close to the heaters. Because of the high thermal conductivity of the silicon this gives a fast feedback and reduces temperature overshooting. Overshooting is a potential problem as it can affect the reagents or cause boiling when temperatures over 90°C have to be regulated. The temperature of liquid inside the chamber has been calibrated with a small thermocouple and cross-checked with organic salts with well known melting points, as suggested by Poser et al. [14].

4. Fabrication process

The fabrication process is designed to be simple and suitable for manufacturing. It is shown schematically in Fig. 5. The starting material is a 400 μm thick (100) silicon wafer which is coated on both sides with a low-stress silicon nitride layer. The top and bottom layers had thicknesses of 1 μm and 3 μm respectively. The process sequence was: (a) patterning of the top silicon nitride layer using photolithography and reactive ion etching (RIE); (b) anisotropic wet etch of the silicon through the wafer in aqueous KOH with the silicon nitride acting as a mask; (c) patterning of sputter deposited

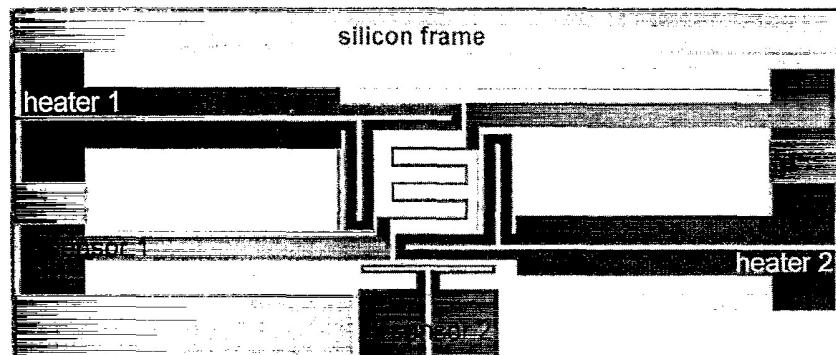


Fig. 4. Schematic of the bottom view of a microchamber. Platinum resistor lines are used as heaters and as temperature sensors for temperature control. They are patterned on the bottom silicon nitride layer.

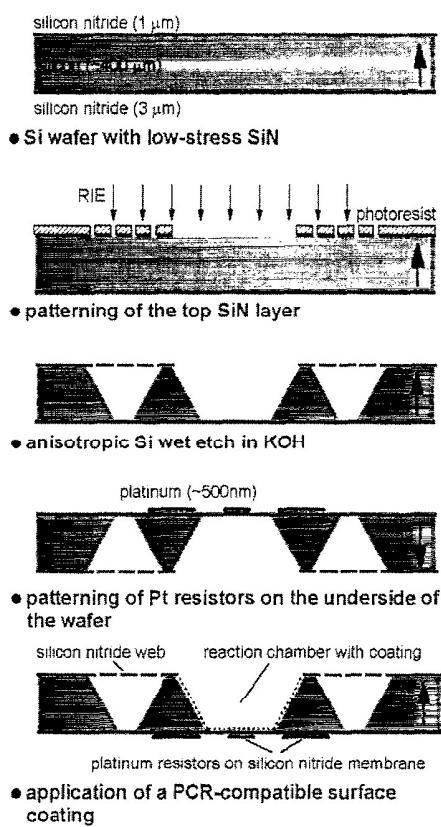


Fig. 5. Fabrication process for a microchamber.

Pt thin film resistors with a lift-off technique on the underside of the wafer, a thin layer of Cr between the Pt and the silicon nitride serving as an adhesion layer; (d) annealing of the Pt to decrease and stabilize the resistance value; (e) deposition of a PCR-compatible surface coating, such as a 200 nm silicon dioxide by plasma enhanced chemical vapour deposition (PECVD), followed by a bovine serum albumin (BSA) treatment. The process can be modified in order to avoid the patterning on the thin nitride membrane by using a front-to-backside wafer alignment method and first patterning the resistors. One drawback of simple wet etching is that the area required on the wafer for a well-isolated microchamber is inconveniently large (due to the fact that the cross-section of the beams for thermal isolation is always a trapezoid). This can be improved by using either deep reactive ion etching to etch the silicon or by taking (110) silicon wafers. As the

cross-section of the beams becomes smaller, the length can be reduced.

5. Filling and sealing of microchambers

The microchambers were filled by first pipetting the aqueous solution of reagents into the chamber. Then a drop of silicone oil on top seals the chamber and prevents the evaporation of the reagents (Fig. 6). There is a risk of inadvertently filling the chambers for thermal isolation which are surrounding the reaction chamber, especially if the pipetting is done manually. In order to prevent this, a novel silicon nitride web has been patterned above these cavities (Figs. 3 and 7). If the surface is made hydrophobic and the holes of the web are small enough, aqueous solutions are repelled due to their high surface tension and excess liquid is rejected off the device. The hole size of the web can be precisely adjusted in the patterning step. The webbing works less well for oil because the surface tension of oil is lower than for aqueous solutions. To prevent the oil from spreading out, small grooves were patterned around the reaction chamber in the layout of Fig. 3. If the silicon nitride web is made hydrophilic, it actually can be used to fill microchambers to a precise level. If a drop of aqueous solution touches the web, the liquid is sucked into the chamber due to capillary force until the chamber is full. The top level of the liquid is determined by the web and a meniscus does not form.

6. Biology and surface treatment

In order to perform PCR in the microchambers, special attention must be paid to the condition of the internal surfaces. For microchambers, surface effects are generally pronounced because the surface to volume ratio increases upon miniaturization. In particular the adsorption of reaction components on the chamber surfaces can lead to an inhibition of the PCR. The compatibility of silicon based materials with PCR has been addressed previously [18]. In order to get reproducible results for the PCR, first a layer of PECVD oxide (200 nm thick) has been deposited. Then the chambers were incubated in BSA and rinsed with water.

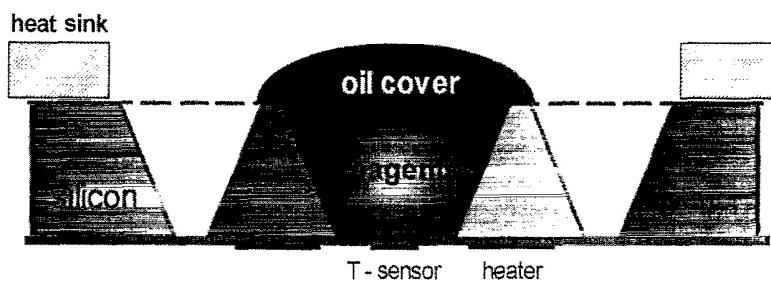


Fig. 6. Schematic of a cross-section through a microchamber. An oil cover seals the chamber and prevents evaporation of the reagents. A heat sink is connected to the frame for fast cooling.



Fig. 7. Detailed SEM of the silicon nitride web over the side chambers. The crosses have to be arranged so that the anisotropic etch cannot stop at a (111) plane. Depending on the hydrophobicity of the web, it either stops aqueous solutions from filling the chambers or it sucks liquids into the chambers until they are filled.

7. Results and discussion

Fig. 8 demonstrates rapid temperature cycling of the reaction chamber for typical PCR cycles. The chamber was filled with 2 μl water and covered with about 1 μl oil. The recorded temperature was measured on the silicon nitride window at the bottom of the chamber. The microchamber of Fig. 3 was used. For this chamber the transition from the denaturation temperature (92°C) to the annealing temperature (55°C) takes about 0.5 s which corresponds to a cooling rate of $74^\circ\text{C}/\text{s}$. The heating rate lies around $60\text{--}90^\circ\text{C}/\text{s}$ but depends on the available input power. Fig. 9 shows the efficiency of the thermal isolation. The same chamber layout of Fig. 3 with 3 mm long beams for thermal insulation has been taken. The frame was connected to a heat sink ($\sim 5^\circ\text{C}/\text{W}$) with heat conductive paste for these tests and it would also be heat sunk in a production device. The chamber temperature and the temperature of the silicon frame were then measured upon heating and cooling. The chamber temperature was measured

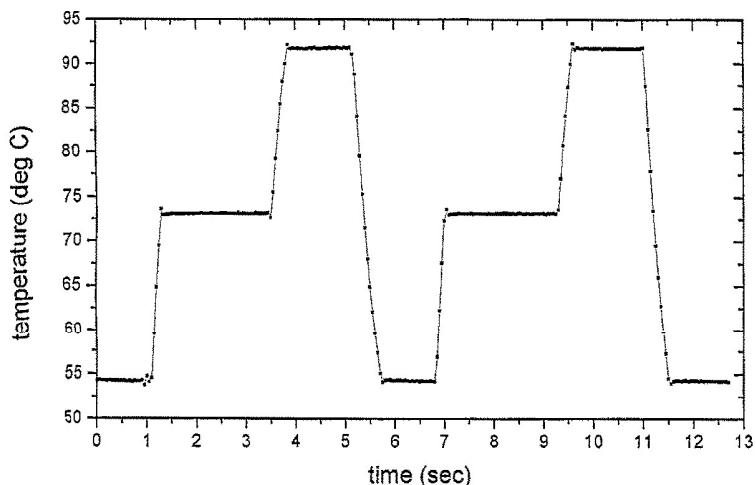


Fig. 8. Measured temperature using the Pt sensor for two rapid temperature cycles which are typical for the PCR. The chamber in Fig. 3 with 2 μl water and oil cover was used.

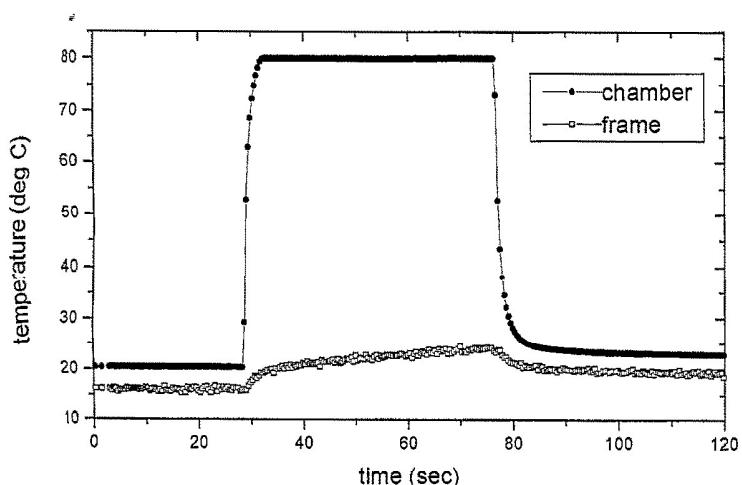


Fig. 9. Graph showing the temperature of the chamber and the frame as a function of time, demonstrating the efficiency of the thermal isolation. The silicon frame is connected to a heat sink and changes only slightly in temperature (there is an offset of a few degrees between the temperature sensors for the frame and the chamber). Rapid heating and cooling is achieved.

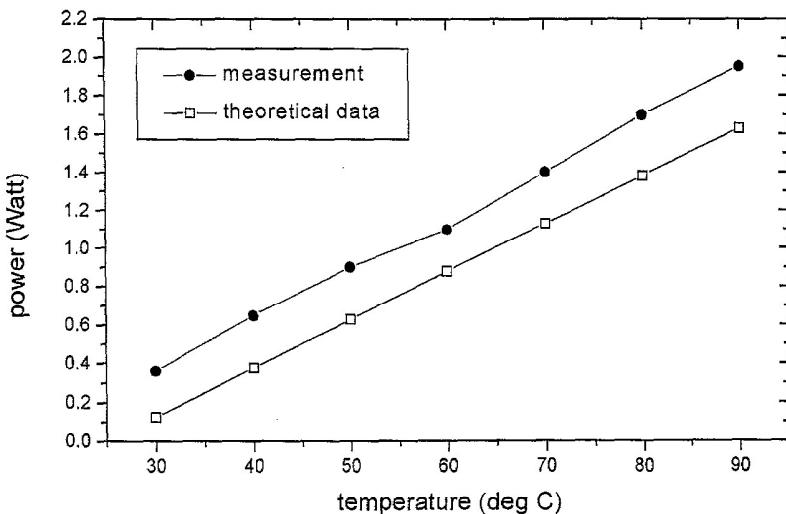


Fig. 10. Power consumption for the chamber in Fig. 3 with 1.5 μ l water and about 1 μ l silicone oil. The values are low enough to allow battery-operation for a portable PCR device.

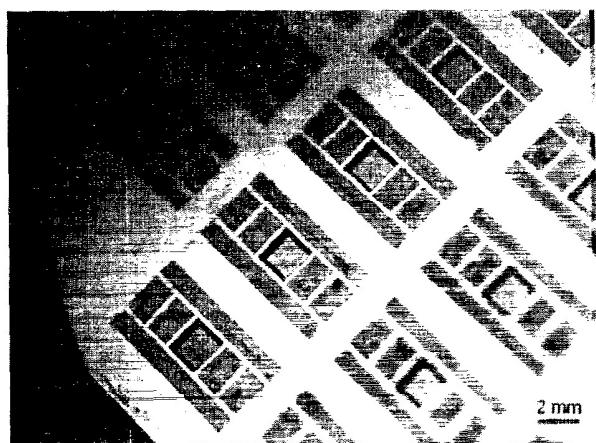


Fig. 11. Photograph of an array of microchambers. As the chambers are thermally isolated from each other each chamber can be individually heated/cycled.

in this case with a platinum resistor located on the chamber walls in order to measure as closely as possible the temperature of the silicon. The frame temperature was measured with a small thermocouple on the frame close to the end of the thermal isolation beams. After heating the chamber to 80°C for 45 s there is still a temperature difference of 55°C present between chamber and frame. This guarantees fast cooling when the heater is switched off. From this curve the thermal time constant for the chamber was extracted. For the chamber filled with 1.5 μ l water and about 1 μ l silicone oil we get a thermal time constant of $\tau \sim 0.4$ s. This is in good correspondence with a theoretical value of $\tau \sim 0.46$ s from the lumped-heat-capacity model. The specific heat of oil was taken as half of the specific heat of water. Fig. 10 shows the power that is necessary to hold the chamber at a certain temperature. The values were taken about 30 s after setting a temperature. The values are slightly higher than the theoretical value. However, the model neglects heat loss due to

convection and we also do not take the thermal mass of the four silicon beams into account. The measured power requirement is sufficiently modest to allow battery driven operation of the microchamber.

Successful PCR reactions were performed in the microchambers on a linearised target DNA from the plasmid pBluescript. The reaction mixture was made with 10 \times PCR buffer (Bioline, UK), a 5 mM dNTP mixture, 0.25 μ M pUC/M13 forward and reverse primers (Pharmacia Biotech, UK). For a 20 μ l sample, 0.2 units of *Taq* DNA polymerase and 10 ng DNA template were used. Together, the PCR primers define a ~ 260 bp PCR product. The PCR was successfully performed on a 1.5 μ l sample with 30 cycles at 94°C for 5 s, 55°C for 7 s and 72°C for 5 s. The initial denaturation was at 94°C and the final extension at 72°C, each for one minute. For an even faster PCR the system would need optimization of the reagents and the temperature profile. The reaction products were extracted from the chamber by means of a pipette and analysed by gel-electrophoresis and ethidium bromide stain. In future pipetting should be avoided by combining the chambers with a fluorescence detection system as in Refs. [19,20]. This would also allow in situ monitoring of the PCR. The transparent silicon nitride window at the bottom of the reaction chamber would be ideal for this readout method. As shown in Fig. 11, the chamber design is also suitable for arrays of microchambers. The chambers are thermally isolated from each other on the wafer and can be addressed individually with different thermal programmes. Such arrays have scope for fast optimization of a PCR or for large scale screening applications.

8. Conclusions

Micro-reaction chambers for the thermal cycling of DNA by the polymerase chain reaction have been fabricated.

Although materials such as glass or plastics are becoming popular for bioanalysis, silicon has some distinctive advantages: for example it can be precisely shaped by existing etching methods and the low stress silicon nitride allows for thin transparent membranes for optical readout based on fluorescence or light absorption to be constructed. The thermal conductivity of silicon is high and, in combination with thin film heaters and temperature sensors, precise temperature control of silicon devices is possible. It has been shown in this report that the thermal characteristic of a microchamber can be adjusted by thermally isolating it from the wafer. The thermal time constant is an important parameter that describes the system: if it is designed to have an appropriate value, the microchambers perform fast thermal cycling at low power consumption. Such microchambers are a candidate for portable DNA analysis systems or for arrays of chambers on a wafer.

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