Integrated MEMS-based Heat Sources for Micro-PCR Systems

Dan Echizenya
Laboratory for Manufacturing Automation (LMA)
Department of Mechanical Engineering
University of California at Berkeley

Abstract: The polymerase chain reaction (PCR) method has become a widely used technique for the rapid amplification of DNA. The use of MEMS-based PCR systems has recently gained popularity for its high throughput, low cost, space saving potential, and ease of use. However, a great deal of work needs to be done in the area of component integration, especially for the heaters used to control the PCR reaction. A novel PCR system is proposed with integrated silicon microheaters for improved temperature control of the PCR reaction.

Introduction:

The polymerase chain reaction (PCR) method has gained widespread usage as a DNA amplification technique. PCR has enabled the rapid synthesis of DNA for genotyping and other characterization techniques. Recent advances in MEMS fabrication technology have allowed for the miniaturization of PCR reactor chambers. Northrup et. al. fabricated what many people called the first micro-PCR system, which consisted of a series of mm-scale chambers micromachined in silicon (see figure 1) [1].

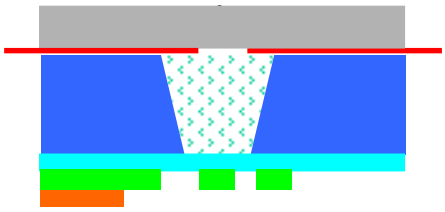

Figure 1: Northrup micro-PCR design.

One main problem with the Northrup design is the chamber walls; they are formed by the silicon wafer itself, adding to the thermal mass of the system and increasing the heating and cooling rate of the system. The heat-flow path from the heater to the substrate passed through the fluid volume, but the sidewalls of the chamber could be significantly cooler than the nitride membrane, also increasing the heating rate. Daniel et. al. demonstrated the usage of micromachined silicon for PCR reactors with deep-etched trenches for thermal isolation, and integrated microfluidic channels directly into the wafer. This design isolates the PCR chamber from the rest of the silicon wafer using a silicon nitride membrane on the backside of the wafer and a thin mesh of silicon nitride on the front side (see figure 2) [2].

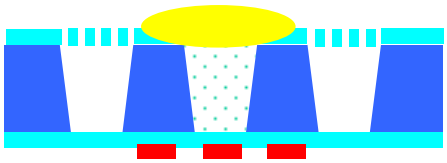

Figure 2: Daniel micro-PCR design.

With the isolation channels around the main PCR chamber, Daniel's design improved upon the thermal isolation problem, hence decreasing the heating and cooling rate of the chamber. But, there are sealing problems with this particular design. In addition, the heat source was physically isolated from the actual PCR chamber in both designs, greatly increasing the thermal time constant. Also, insufficient thermal isolation led to an increased thermal mass, which tended to increase the thermal time constant. Since the PCR process requires a very specific thermal cycle, any imprecision in the heating and cooling cycle would result in inefficiencies in DNA amplification [3]. A new MEMS-based PCR design with integrated heat sources is proposed.

Layout and Fabrication:

The first design uses a single-mask deep reactive ion etch (DRIE) process, and has two sets of silicon heaters per PCR chamber (see figure 3).

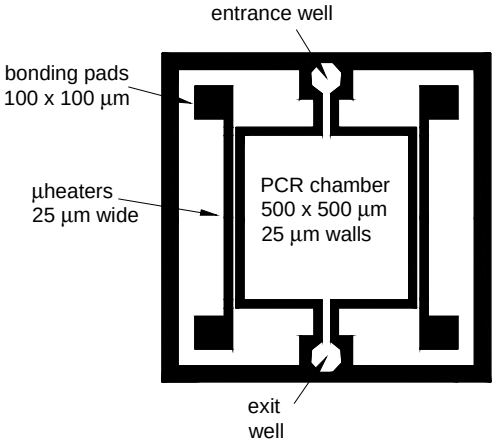

Figure 3: Initial layout for PCR chamber.

This design relies on the DRIE process on a SOI wafer to create deep trenches that act as thermal isolators between the PCR chamber and the remainder of the device hence lowering the thermal mass of the system (see figure 4).

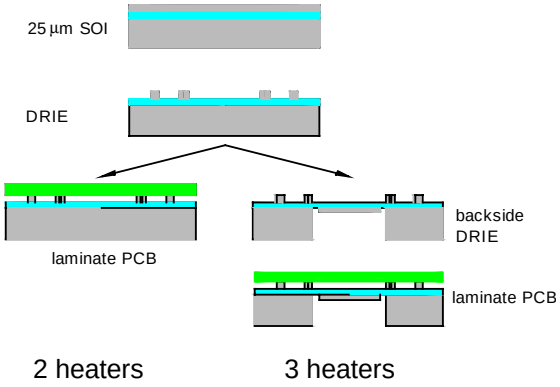

Figure 4: Process flow for PCR device.

Note that there is a 10 micron air gap between the heaters and PCR chamber walls; in order to minimize electro-phoresis and electro-osmosis processes, the heaters need to be electrically isolated from the PCR chamber. The entire die size is 1 mm by 1 mm, with a main PCR chamber of 500 by 500 microns. The inlet and outlet channels are 50 microns wide. Circular reagent and waste reservoirs with diameters of 100 microns (corresponding to the smallest diameters of commercially available injectors in bioassay systems) are included at the ends of the inlet and outlet channels. As there are no moving components, a release etch does not have to be done. After the DRIE etch, a prefabricated PCB with the necessary sealant, control electronics, and electrical interconnects with the heater bonding pads is attached via thermal bonding with a gold eutectic compound, plastic lamination, or epoxy. Electrical operating parameters for each heater are 15 V at 10 milliamps, which yields a power of 0.15 W. A second design involves a backside DRIE etch using Seth Hollar's proprietary BSAC process. To minimize thermal gradients and reduce heating time within the PCR chamber, a third heater can be etched into the base of the SOI wafer with a backside etch (see figure 4). The channel depth will be the same as the thickness of the top layer of silicon on the SOI wafer (typically ranging from 20-50 microns). A 25 micron SOI wafer is used for the purpose of analysis in this paper.

Test Structure Layout:

The single die shown in figure 3 can be laid out in a 12 by 8 pattern, as shown in figure 5. This layout can also be used for creating a variety of test structures. One primary design parameters is the resistivity of the heaters (which control Joule heating rate), and can be adjusted by changing the geometry of the heaters. The other design parameter is the geometry of the PCR chamber, which will be varied from 500 to 1000 microns in length. The 96 well pattern is standard in many bioassay systems, such as the ACLARA Arteas 96 and Agilent Bioanalyzer 2100. The automated fluid injection and extraction capabilities of commercial bioassay systems such as these can be used to inject and extract reagent into each device.

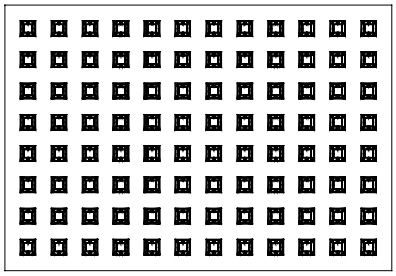

Figure 5: 96 assay well pattern.

Analysis:

For simplicity, only the two-heater design will be analyzed in depth. Since there is no

externally induced flow in the system during a PCR cycle, the heat transfer rate due to forced convection will effectively be zero. Since the aspect ratio (height/width) of the PCR chamber is only 0.05, free convection at the vertical sidewalls, as well as any radiation effects, can be neglected. Hence, heat transfer occurs primarily by a conduction mechanism. The Biot number of the system, defined as

$$Bi = \frac{hL}{k} \qquad (1)$$

(where h is the convective heat transfer rate, L is a characteristic length of the chamber, and k is the thermal conductivity of water, or 0.6 W/m*K) indicates whether significant temperature gradients will be present in the system. A Bi<0.1 usually indicates that temperature gradients are negligible, and the system can be treated as a lumped capacitance system. Since h is effectively zero, the Biot number will effectively be zero, and temperature gradients aren't expected to be present.

The thermal time constant must be minimized to reduce any imprecision in the PCR thermal cycle. The time constant τ_c can be calculated as

$$\tau_c = \frac{\rho_{fluid} C_{fluid}}{2k_{water}\pi^2} L^2 \qquad (2)$$

where ρ_{fluid} is the density of water (1000 kg/m^3), C_{fluid} is the specific heat capacity of water (4138 J/kg*K), L is a characteristic length of the chamber (conservatively taken as 500 microns), and k is the thermal conductivity of water. The time constant for this design was found to be approximately 0.088 seconds, which is significantly less than the time constants of many commercial macroscale PCR systems (which have τ_c on the order of several seconds). Note that as a first approximation, all thermal properties were taken at the homologous temperature since the total temperature change in the system is relatively small; a more accurate analysis would account for variation in material properties as a function of temperature.

As an approximation, 2D heat transfer analysis is done in the plane of the die, since the depth of the PCR chamber is small compared to its lateral dimensions. The primary governing equation for transient conductive systems can be written as

$$\nabla^2 T = \frac{1}{\alpha}\frac{\partial T}{\partial t} \qquad (3)$$

where α is the thermal diffusivity of water. However, a full form solution for the temperature distribution (which would account for $\partial T/\partial t$) would require FEM. A finite-difference method was used to find the temperature distribution as a function of the changing boundary conditions at the sidewalls by setting $\partial T/\partial t=0$. Hence, the governing equation reduces to $\nabla^2 T=0$. The solution to this 2D steady-state conduction model can be found with a partial differential equation solver package in MATLAB, if the proper transient boundary conditions are known. An index of time of 0.001 seconds was chosen, and the solution to the temperature distribution was found at discrete instances of time, which then yielded the transient temperature distribution. To find the temperature of the heated sidewalls, a 1D nodal analysis using a conduction thermal circuit was used to approximate the transient thermal behavior of the PCR chamber sidewalls due to heating (see figure 6). A 1D analysis was used since attempts at using a 2D circuit did not converge to a meaningful solution.

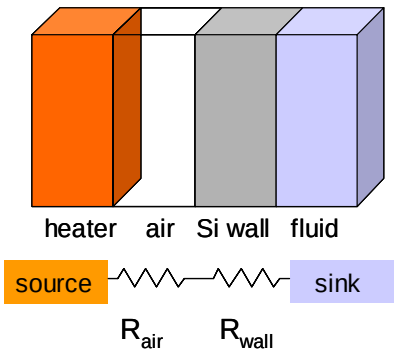

Figure 6: 1D nodal circuit analysis for PCR chamber sidewalls.

The temperature variation at the sidewall was found with the nodal equation

$$T_m^{p+1} = Fo(T_{m+1}^p + T_{m-1}^p) + (1-2Fo)T_m^p \qquad (4)$$

where T is the temperature, Fo is the Fourier number (non-dimensionalized time), and the p/m indexes indicate time and nodal position, respectively. The transient behavior of the sidewalls was calculated in MATLAB, and these boundary conditions were then used

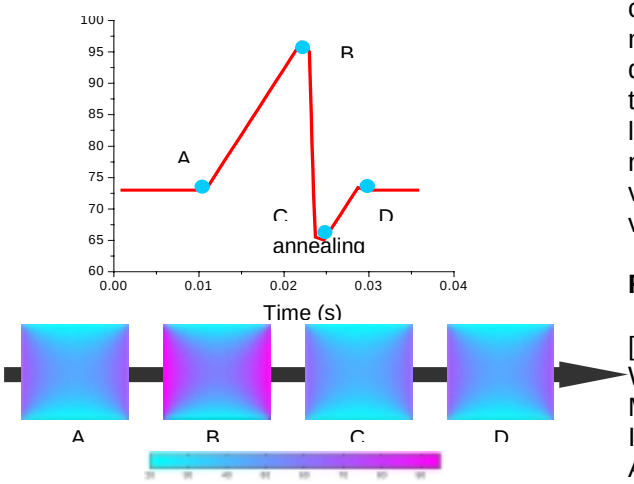

Figure 7: Transient 2D temp. distribution.

to get the transient 2D temperature distribution (see figure 7).

Of particular concern are the sharp temperature gradients evident in the chamber at each step of the cycle. This can be accounted for by the lack of knowledge of the boundary conditions at the entrance and exit sidewalls. A more detailed 2D thermal circuit needs to be made in order to obtain a more accurate thermal analysis of the PCR chamber.

Conclusions/Future Work:

A micro-PCR system with integrated heaters for improved thermal cycling has been demonstrated. Lower thermal time constants were observed in the simulation, and further testing should prove that this system yields more efficient PCR reactions over previous designs. This design can be integrated in pre-existing commercial bioassay systems as well.

However, it is important to note that all theoretical considerations given here are based on first-order approximations. Transience in the thermal properties of the system needs to be accounted for in future analyses. The boundary conditions also need to be more rigorously modeled, since accurate knowledge of the boundary conditions is needed for a more realistic model of the temperature distribution. In order to take all these into account, a commercial FEM packages like ANSYS is needed. There is also room to improve this design in terms of minimal size, high throughput, low time constant, and heater locations. Finally, a series of test structures needs to be fabricated to experimentally verify the functionality and temperature variation of the actual device.

References:

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