Design and microfabrication of a molded polycarbonate continuous flow polymerase chain reaction device

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DESIGN AND MICROFABRICATION OF A MOLDED POLYCARBONATE CONTINUOUS FLOW POLYMERASE CHAIN REACTION DEVICE

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science in Mechanical Engineering in The Department of Mechanical Engineering

by
Michael Mitchell
B.S., Louisiana State University, 2000
December, 2002
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First let me give GOD the praise and accept the glory of having accomplished an educational goal set for myself four and half years ago. I would like to take this opportunity to express my gratitude to my family, friends and colleagues who have always been a source of strength and encouragement. The moment I had been waiting for has finally arrived and I am excited to start a new phase in my life.

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LIST OF SYMBOLS

\( \rho_w \)  
Thermal power density, \( \text{W/in}^2 \)

\( \rho \)  
Fluid density

\( \rho_R \)  
Specific resistivity

\( \varepsilon \)  
Thermal emissivity

\( \delta_{ij} \)  
Tensor notation

\( \phi \)  
Viscous heat dissipation function

\( \alpha \)  
Thermal diffusivity

\( \alpha_0 \)  
Temperature coefficient of resistivity

\( \alpha_i \)  
Aspect Ratio

\( \Delta t \)  
Time difference

\( \xi \)  
Radiation absorptivity

\( \nu \)  
Kinematic viscosity

\( \nu_1 \)  
Electrical conductivity

\( \beta \)  
1/Temperature

\( \Delta T \)  
\( T_{\text{Hot}} - T_{\text{Cold}} \)

\( \sigma \)  
Boltzman’s constant

\( \Delta P \)  
Pressure difference, psi

\( \alpha_r \)  
Aspect ratio, \( \alpha_r < 1 \)

\( \alpha_{\text{scr}} \)  
Temperature coefficient of resistance

\( \rho_R \)  
Resistivity

\( \Delta T \)  
Temperature difference, \( ^\circ\text{C} \)
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<thead>
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<tr>
<td>$\mu$</td>
<td>Dynamic viscosity</td>
</tr>
<tr>
<td>$a$</td>
<td>Channel semi-width, $\mu$m</td>
</tr>
<tr>
<td>$A$</td>
<td>Cross-sectional area, $m^2$</td>
</tr>
<tr>
<td>$b$</td>
<td>Channel semi-depth, $\mu$m</td>
</tr>
<tr>
<td>$C$</td>
<td>Thermal capacitance</td>
</tr>
<tr>
<td>$c_p, c_v$</td>
<td>Specific heat capacity constant pressure and constant volume</td>
</tr>
<tr>
<td>$D_h$</td>
<td>Hydraulic diameter, $m$</td>
</tr>
<tr>
<td>$e(t)$</td>
<td>Effort variable for a bond graph port</td>
</tr>
<tr>
<td>$f(t)$</td>
<td>Flow variable for a bond graph port</td>
</tr>
<tr>
<td>$g$</td>
<td>Acceleration due to gravity, $m/s^2$</td>
</tr>
<tr>
<td>$h_{con}$</td>
<td>Convective heat transfer coefficient</td>
</tr>
<tr>
<td>$h_r$</td>
<td>Radiation heat transfer coefficient</td>
</tr>
<tr>
<td>$I$</td>
<td>Current, $A$</td>
</tr>
<tr>
<td>$i$</td>
<td>Current density, $A/m^2$</td>
</tr>
<tr>
<td>$inv \Delta p$</td>
<td>Reciprocal of pressure difference, $m^2/N$</td>
</tr>
<tr>
<td>$k$</td>
<td>Thermal conductivity</td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>Equivalent thermal conductivity</td>
</tr>
<tr>
<td>$K_P$</td>
<td>Proportional gain</td>
</tr>
<tr>
<td>$K_I$</td>
<td>Integral gain</td>
</tr>
<tr>
<td>$K_D$</td>
<td>Derivative gain</td>
</tr>
<tr>
<td>$k_{Th}$</td>
<td>Thermal conductivity</td>
</tr>
<tr>
<td>$l$</td>
<td>Length of fluid path, $m$</td>
</tr>
<tr>
<td>$L$</td>
<td>Length of channel, $m$</td>
</tr>
<tr>
<td>$L_c$</td>
<td>Characteristic length, $m$</td>
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LHW  Length of heater wire, m
m  Mass, kg
Mwt  Molecular weight
n  Number of turns
P  Fluid pressure, N/m²
PE  Electrical power, W
P(t)  Power flowing into or out of a bond graph port
PT  Thermal power, W
PT  Thermal power, W
q  Heat flux
Qcon  Conductive heat transfer, W
Qcv  Convective heat transfer, W
Qrad  Radiation heat transfer, W
Ra  Rayleigh number (Ra),
Rcond  Resistance for conductive heat transfer, Ohm
Rconv  Resistance for convective heat transfer, Ohm
RE  Electrical resistance, Ohm
RE,T  Electrical resistance at temperature T, Ohm
Re  Reynolds number
RT  Thermal resistance, Ohm
Se  Effort source for input into or output from a bond graph
Sf  Flow source for input into a bond graph, m³/s
t  Time, s
T∞  Ambient temperature, °C
\begin{itemize}
\item \( T_{\text{ref}} \) \quad Reference temperature, °C
\item \( T_s \) \quad Surface temperature, °C
\item \( u \) \quad Fluid velocity, m/s
\item \( u_{(x, y)} \) \quad Fluid velocity in the \((x, y)\) direction, m/s
\item \( U_{\text{max}} \) \quad Maximum fluid velocity
\item \( v \) \quad Mean fluid velocity, m/s
\item \( T_{\text{ref}} \) \quad Reference temperature, °C
\item \( V_E \) \quad Electrical voltage, V
\end{itemize}
ABSTRACT

The polymerase chain reaction (PCR) is an amplification technique that is used with deoxyribonucleic acid (DNA) and can produce millions of copies of the starting material even from single molecules. Since its discovery by Kary Mullis in the mid-1980s, PCR has been one of the most important techniques in the arsenal of tools used for genetic analyses.

For this research a continuous flow polymerase chain reaction (CFPCR) system was designed, microfabricated from molded polycarbonate, and tested. Finite element modeling was used to model the system thermal and microfluidic response. X-ray LIGA was used to fabricate mold inserts for the microfluidic channels. Polycarbonate was used instead of PMMA because polycarbonate has a higher glass transition temperature and is better able to withstand the sustained high operating temperature of the continuous flow PCR system. Commercial thin film heaters under PID control were used to supply the necessary heat flux to maintain the steady-state temperatures in the PCR device.

There are a number of advantages to performing molecular biology on a small scale. The savings in time and money are the most prominent. A reduction of the cost of these experiments is accomplished on a variety of levels. First, this continuous flow microreactor can be mass-produced using existing microfabrication techniques (LIGA). The small sample sizes require much smaller volumes of reagents. Many of the enzymes used in molecular biology are quite costly and reducing the volume required per reaction will significantly decrease the cost of the experiment. By achieving a quicker and less expensive DNA amplification, DNA analysis could become a
technique used routinely in clinical medicine, leading to a vast improvement in medical care.
CHAPTER 1: INTRODUCTION

The polymerase chain reaction (PCR) is widely used as a molecular biology tool to amplify DNA (deoxyribonucleic acid). DNA is a double-stranded helix of nucleotides, which carries the genetic information of a cell. PCR is a common method used for creating copies of specific fragments of DNA by the following three steps, denaturation, hybridization (annealing), and enzymatic polymerization (extension) [Stephen, et al. 1994].PCR can amplify a single DNA molecule into many billions of molecules. The three steps of the polymerase chain reaction are typically carried out in the same vial, but at different temperatures.

The first part of the process is to separate the two DNA chains. The target genetic material must be denatured, i.e., the strands of its helix must be unwound from a double-strand, into a single-strand by heating the vial to 95 °C. A primer that normally binds to the DNA cannot operate at such a high temperature, so the vial is cooled to 55 °C. At this temperature, the primers are "annealed" to the ends of the DNA strands. The final step of the reaction is to make a copy of the templates. This is known as the extension step where the DNA synthesizes by extending the primer down the template DNA, i.e., making copies of the templates, this is performed at temperature of 72°C.

Since its discovery by Kary Mullis in the mid 1980s [Saiki, et al.1998], PCR has become one of the most important techniques in the arsenal of tools available for genetic analyses [Mullis, et al, 1994]. In its short life, PCR has utterly transformed the life sciences. From the daily practical uses of medical diagnosis to the theoretical framework of science and from courts of law to field studies of animal behavior. PCR takes analysis of minute amounts of genetic material to a new level of precision and reliability. It is far
simpler and less expensive than previous techniques for duplicating DNA [Saiki, et al.1998], so PCR has democratized genetic research, putting it within reach of all biologists, even those with little or no training in molecular biology.

1.1 REQUIREMENTS FOR PCR DEVICES

The polymerase chain reaction involves processing a mixture of buffers, template DNA, primers (short oligonucleotides that hybridize to specific locations on the target DNA and define the amplifying region), and a thermostable DNA polymerase. The temperatures that are typically used in the PCR reaction include 90-94°C for denaturation of the double-stranded DNA molecule, 50-68°C for hybridization of the primers to the single-stranded DNA template and 70-75°C for enzymatic polymerization (extension) of the primers as shown in Figure 1.1. This temperature sequence is then cycled many times (~10-50) to provide an exponential amplification of the starting material.

According to Innis, (1988) it is possible to have an amplification time per cycle of approximately 12 s for a 1,000 base amplicon (PCR product). For 25-35 PCR cycles, a processing time of only 5-7 minutes would be required since the denaturing and annealing of nucleic acids requires less than 1 s and the extension speed for Taq polymerase, a thermally stable enzyme used in most PCR reactions, is on the order of 35-100 bases per second. A typical PCR device must be capable of obtaining the required temperature sequence in the smallest possible time. The device must be able to produce the temperature in the cycle in sequence and maintain them for the required duration in order to get optimal performance.
1.2 CONVENTIONAL PCR DEVICE

Conventional PCR is typically performed by thermocycling the PCR mixture in a thin-walled plastic vial embedded in a temperature controlled aluminum heat sink (also known as a block thermal cycler). Due to the high thermal capacitances of the material, the ramp rate of the temperature is typically on the order of 1°C per second for heating and cooling. For the temperature transition between the 95 °C and 55 °C zones the time would be approximately 40 sec. The cycle speed is then limited by the thermal capacitance of the heat sink and the heat transfer rate between the heat sink and the PCR sample. To complete this analysis in a conventional PCR block thermal cycler, approximately 2 hrs is necessary.

1.3 CONTRIBUTION TO PCR DEVICE PRODUCTION

Presently conventional PCR devices are limited by their sizes, rate of heating and cooling, and cost. Because of these factors, a study was performed for the manufacturing of micro-fluidic components capable of performing the same function as the conventional PCR device. For this research a molded polycarbonate continuous flow polymerase chain reaction device was design and micro-fabricated. There were several benefits of the miniaturization of PCR devices.
Due to the small sample sizes, much smaller volume of reagents was required. Enzymes used in molecular biology are quite costly; therefore by reducing the volume per reaction the cost of experiment was dramatically decrease. The power consumption is reduced, which also reduces cost. The rate of heating and cooling is much faster because of its small thermal capacitance and less power to dissipate. Through molding the device can be produced in large quantities and used as a disposables instrument.

In general the performance of PCR equipment was enhanced, where heating cooling and material transport were much more efficient, leading to better yield and faster analysis. By making DNA amplification and analysis quicker and cheaper, the analysis itself could become more widespread in medicine, leading to an improvement of medical care. Even though such experimental chip-based devices are not yet ready for public use, engineers are greatly anticipating the day when doctors can take the devices on the road and patients will be able to get on the spot diagnosis based on readouts of their DNA.

1.4 THESIS OUTLINE

The main objective was to design and micro-fabricate a molded polycarbonate continuous flow polymerase chain reaction device capable of the required temperature sequence in the lowest possible time. The device must be able to produce the desired temperature cycles and maintain these temperatures for the time duration required.

This thesis is arranged as follows: Chapter 2-The background of PCR and a review of the literature on the most resent micro-fabricated PCR devices; Chapter 3-Design and simulation, where detailed three and two-dimensional numerical modeling of microscale fluid flows (pressure driven) and heat transfer simulation using ANSYS (v5.7, ANSYS, Inc., Houston, PA) were performed. The most feasible device configuration was selected
based on the simulation results; Chapter 4-Micro-mold inserts were fabricated using the LIGA microfabrication technique for the hot embossing of polycarbonate with micro-fluidic channels; Chapter 5-Mathematical models of the thermal characteristics of the PCR chip were developed and used to determine the thermal power input requirement and construct the necessary control circuit to maintain the temperatures-required; Chapter 6-The devices were assembled and experiments were performed using a CCD camera to capture the various temperature contours displayed in a thin layer of liquid crystal applied over the PCR channel. These images were compared with those obtained from the simulation. This chapter also includes the discussion, conclusion and recommendations for future development of this project.
CHAPTER 2: BACKGROUND

The polymerase chain reaction (PCR) is a powerful technique, which results in the rapid production of multiple copies of a target DNA sequence (DNA is a double-stranded helix of nucleotides). PCR requires a template molecule (the desired DNA) and two primer molecules to begin the copying process. The primers are short chains of four different chemical components that make up any strand of genetic material. These four components commonly known as nucleotides, or bases [Mullis, et al, 1994], are the building blocks used to construct genetic molecules.

The first step in this process is to separate the two DNA chains of the double helix. As the two strands separate, DNA polymerase makes a copy using each strand as a template. The four nucleotide bases, the building blocks of every piece of DNA, are represented by the letters A, C, G, and T, which stand for their chemical names: adenine, cytosine, guanine, and thymine. The A on one strand always pairs with the T on the other, whereas C always pairs with G. The two strands are said to be complementary to each other [http://www.accessexcellence.org ]

To copy DNA, polymerase requires two other components: a supply of the four nucleotide bases and something called a primer. DNA polymerases, whether from humans, bacteria, or viruses, cannot copy a chain of DNA without a short sequence of nucleotides to "prime" the process, or get it started. So the cell has another enzyme called a primase that actually makes the first few nucleotides of the copy. This stretch of DNA is called a primer. Once the primer is made, the polymerase can take over making the rest of the new chain. Because the products of one PCR cycle can act as templates for the next
PCR cycle, the number of new identical molecules produce doubles with each repetition of the cycle.

The three parts of the polymerase chain reaction are carried out at different temperatures. The first part of the process separates the two DNA chains in the double helix. This is done at 95 °C degrees centigrade for about 30 seconds. The primers cannot bind to the DNA strands at such a high temperature, so the vial is cooled to 55 °C at this temperature; the primers bind or "anneal" to the ends of the DNA strands for about 20 seconds. The final step of the reaction is to make a complete copy of the templates. This works best at around 75 °C. The Taq polymerase begins adding nucleotides to the primer and eventually makes a complementary copy of the template. If the template contains an A nucleotide, the enzyme adds on a T nucleotide to the primer. If the template contains a G, it adds a C to the new chain, and so on to the end of the DNA strand. This completes one PCR cycle. The three steps in the polymerase chain reaction - the separation of the strands, annealing the primer to the template, and the synthesis of new strands - take can be completed in less 12 sec Innis, (1988).

2.1 APPLICATIONS

It is difficult to exaggerate the impact that the polymerase chain reaction (PCR) has on our world today. The polymerase chain reaction (PCR) is an amplification technique that is used for nucleic acids and produces billions of copies of the starting material even from single molecules. PCR is a quick and easy method for generating unlimited copies of any fragment of deoxyribonucleic acid (DNA). The technique's unmatched ability to identify and copy the smallest, oldest and even most damaged DNA has proven
exceptionally valuable in criminal courts. For instance, PCR is an indispensable tool used by forensic scientists to conduct a form of DNA-typing commonly known as DNA fingerprinting [U.S., Congress, Genetic Witness, 1990]. To type DNA, blood is extracted from a suspect or victim’s clothing. Scientists then study various areas on the DNA where inconsistencies are found. This helps the scientists to determine if the sample matches the DNA of a specific person, that of the victim or the assailant. In its earlier stages, DNA typing was considered controversial. However, laboratory standards have been established and DNA typing is now accepted as strong evidence in court cases around the world [U.S., Congress, Genetic Witness, 1990]. Although DNA typing is only one of many pieces of evidence that can lead to a conviction, it is invaluable in demonstrating innocence.

2.2 GENETICS

The genetic material of all living organisms including plants, animals, bacterium and viruses have sequences of nucleotide building blocks that are uniquely present in its respective species. Correspondingly, complex organisms such as human beings possess DNA sequences that are uniquely and specifically present only in particular individuals [Saiki, at el.1998].

These unique variations make it possible to trace genetic material back to its origin, identifying with precision the species of organism and often a particular member of that species. A study of such magnitude requires that an ample amount of the DNA under investigation is available for analysis, which is where PCR comes in.

PCR utilizes natural enzymes known as polymerases. These enzymes are present in all living things and their job is to duplicate genetic material. Sometimes referred to as
"molecular photocopying," it can be used to characterize, analyze, and synthesize any specific piece of DNA [Stephen, et al. 1994]. It works even on extremely complicated mixtures by seeking out, identifying, and duplicating a particular bit of genetic material from blood, hair, or tissue specimens. These tissue samples can be from any species, even samples that are thousands or possibly even millions-of years old.

2.3 LITERATURE REVIEW OF PCR DEVICE’S

Conventional laboratory techniques for performing PCR are expensive and time consuming. The performance of micro fluidic devices capable of doing these functions are significantly better in terms of speed, costs, flexibility and performing multiple functions on the same device. The proliferation of manufacturing techniques for building micro-fluidic components has led to a virtual explosion in the development of micro-scale chemical and biological analysis systems (BioMEMS). Numerous studies have unveiled chip-sized devices capable of conducting complex analytical procedures including capillary electrophoresis, and the polymerase chain reaction. Some of the most publications are tabulated in Table 2.1. This table identifies the different groups, sample volume used, reaction time, the primary materials used for device construction, and the number of cycles incorporated on the chip.

To enhance the speed of the PCR reaction, researchers have investigated alternative methods for heating and cooling by reducing the thermal capacitance of the system. For example, In 1989 Wittwer and his group used thin-walled capillaries (total volume = 100 \( \mu \text{L} \)) containing the PCR mixture, which were then placed in an air-thermal cycler [Wittwer, et al. 1989 and 1990]. Air was used as the heat source because of its low density. Air was pumped into the cycling chamber at the appropriate temperature. The
air mixed rapidly to achieve a fast temperature transition. Amplifying a 536-bp fragment from the β-globin gene was accomplished in ~25 mins.

Table 2.1: Cited Works of Prior Micro PCR Devices (see references list for more details)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sample Volume</th>
<th>Reaction time</th>
<th>Material</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheng, et al. 1996</td>
<td>12 µl</td>
<td>30min</td>
<td>Silicon-glass</td>
<td>30</td>
</tr>
<tr>
<td>Shoffner, et al. 1996</td>
<td>28 µl</td>
<td>30min</td>
<td>Silicon-glass</td>
<td>35</td>
</tr>
<tr>
<td>Woolley, et al.1996</td>
<td>50 µl</td>
<td>26 min 15sec</td>
<td>Si-glass</td>
<td>35</td>
</tr>
<tr>
<td>Kalinia, et al. 1997</td>
<td>10 nl</td>
<td>30min</td>
<td>Quartz glass</td>
<td>40</td>
</tr>
<tr>
<td>Landers, et al. 1998</td>
<td>5-15 µl</td>
<td>14min</td>
<td>Glass</td>
<td>30</td>
</tr>
<tr>
<td>Kopp, et al. 1998</td>
<td>5-20 µl</td>
<td>50min</td>
<td>Glass-copper</td>
<td>30</td>
</tr>
<tr>
<td>Friedman, et al 1998</td>
<td>5 µl</td>
<td>20 min</td>
<td>Glass</td>
<td>35</td>
</tr>
<tr>
<td>Waters, et al. 1998</td>
<td>12 µl</td>
<td>216 min</td>
<td>Glass</td>
<td>24</td>
</tr>
<tr>
<td>Ferrance, et al. 2000</td>
<td>10 µl</td>
<td>7 min 44sec</td>
<td>Silicon</td>
<td>30</td>
</tr>
<tr>
<td>Lagally, et al. 2000</td>
<td>10 µl</td>
<td>15 min</td>
<td>Glass</td>
<td>20</td>
</tr>
<tr>
<td>Khandurina, et al. 2000</td>
<td>7 µl</td>
<td>20 min</td>
<td>Cr-Glass</td>
<td>10</td>
</tr>
<tr>
<td>Giordano, et al. 2000</td>
<td>5 µl</td>
<td>4 min 50 sec</td>
<td>Polyimide</td>
<td>15</td>
</tr>
<tr>
<td>Nagai, et al. 2001</td>
<td>1.3 pl -32 µl</td>
<td>120min</td>
<td>Silicon</td>
<td>40</td>
</tr>
<tr>
<td>Schneega, et al. 2001</td>
<td>50 µl</td>
<td>35 min</td>
<td>Glass</td>
<td>7</td>
</tr>
</tbody>
</table>
Friedman and Meldrum used thin-walled micro-capillaries to contain the PCR mix and then coated the micro capillaries with a thin-film of indium-tin oxide (ITO) that not only acted as the heating element, but also a temperature sensor [Friedman and Meldrum, 1998]. They were able to demonstrate amplification of a 777-bp PCR product in 12 minutes (35 cycles, 20.6 s/cycle). Landers and his group also used micro-capillaries as the reactor, but performed infrared-mediated temperature control, in which a tungsten lamp generated IR-radiation to excite IR-active vibration modes in the aqueous solvent [Oda, et al. 1998][Giordano, 2001]. Amplifying genomic DNA in a sample volume of 5 µl (β-chain of T-cell receptor, 216 base pair amplicon) required approximately 30 min.

Several researchers have focused on improving PCR performance by reducing the reaction size with a smaller reaction chamber [Cheng, et al. 1996]. For example, some scientists are using capillaries with hot-air thermal cyclers [Woolley, 1996] or infrared-mediated heating [Huhmer and Landers, 2000] as their heat source. Scientists are also directly integrating PCR reactors into microchips. Several groups have recently been successful at including on a single chip both the PCR reactor and the capillary electrophoresis device for subsequent analysis of the reaction products [Burns, et al. 1996 &1998],

A flow through type of PCR system based on microfabrication technology was a contribution of Kopp and Manz (1998). A glass chip with a 40 µm by 90 µm by 2.2 m channel was built using a lithographic technique. The PCR mixture was pumped by a syringe along the channel through three different temperature zones at flow rates ranging from 5.8 to 72.9 nL/s. The PCR products were collected and analyzed on a polyacrylamide gel stained with ethidium bromide. The continuous flow PCR chip
showed less cross-contamination than a stationary-tube format because of continuous washing. The flow through chip could be reused without significant cleaning. Their results indicate that the PCR product could be visualized at a total processing time of 50 minutes producing a cycling time of 7.5 s/cycle.

Most recently, Schneegass created a silicon and glass chip, flow through PCR device [Schneegass, et al. 2001]. The silicon and glass chip with a 250 µm by 100 µm by 1.512 m channel etched in glass. A syringe pump was used to drive the PCR mixture through the chip. For each experiment a sample volume of 10 µl was used. The optimal flow rate was 0.0167 µl/s, which give a total time of 35min for 20 cycles. Their amplification was 606 bp, which was larger than Kopp’s 176 bp.
CHAPTER 3: DESIGN OF A TEST DEVICE AND FINITE ELEMENT (THERMAL) ANALYSIS

3.1 INTRODUCTION TO THE CONTINUOUS FLOW STRAIGHT CHANNEL PCR DEVICE

The continuous flow straight channel PCR device was based on the movement of small packets of fluid between constant temperature zones in the manner of Kopp (1998). This significantly simplified the temperature control problem, since the transient heat transfer in the device was confined to start-up and the effect of the thermal capacitance of the components on the response time as a packet moved through the PCR cycle was eliminated.

A steady state thermal analysis was performed to evaluate the temperature profile along the PCR channel. The principal underlying assumptions were that the heating elements could be configured to create distinct nearly-constant temperature zones of the correct length in the fluid channel and that the fluid packets would attain equilibrium within a short distance, relative to the length of the section, of entering each constant temperature zone. A series of finite element simulations using ANSYS/CFD-FLOTRAN (vers. 5.7, ANSYS, Inc., Canonsburg, PA) was carried out to evaluate these assumptions and to define a prototype device configuration for experimental evaluation.

Different materials were investigated for their workable temperature range and formability. Also, different methods of thermal power input were analyzed in order to determine method of heating, which would lead to the simplest fabrication process and reduce production cost. The effects of increasing fluid velocity were investigated since high speed processing is a goal for clinically useful devices.
3.2 MATERIAL SELECTION

Polycarbonate was selected as the material for the PCR module. The desired maximum steady-state temperature was 95 °C in the denaturation zone. This temperature was too high for the PMMA (glass transition temperature approximately 100 °C [http://www.matweb.com, 2002] which was previously used to mold capillary electrophoresis devices by our group [Soper et al., 2000]. For polycarbonate the glass transition temperature is typically in the range of 145 °C – 148 °C, which was suitable for the higher operating temperature, while the thermal conductivity of the two materials was essentially the same (PMMA 0.19 W/m–°K, PC 0.22 W/m–°K) [1]. The low thermal conductivity compared to glass (1.1 W/m–°K and silicon (124 W/m–°K) [http://www.matweb.com, 2002] meant that less power would be required to maintain the steady-state temperatures needed in the module. Initial studies also showed that microfluidic channels could be hot embossed in polycarbonate and sealed by thermal bonding of a polycarbonate cover plate.

3.3 DEVICE CONFIGURATION

The test device consisted of a single straight 50 µm by 150 µm fluidic channel. This continuous flow polymerase chain reaction (CFPCR) device was designed, and fabricated from molded polycarbonate. The device had two cover plates, which incorporated air pockets, electrical resistive heaters, and a hot embossed microfluidic channel. Figure 3.1 shows the cross-section of the test device configuration. All of the dimensions of the test device configuration can found in Chapter 6 and Appendix C.

The continuous flow PCR was based on the movement of small packets of fluid analyte between constant temperature zones. These isothermal zones, through which the
fluid flows, are very important for achieving and maintaining the desired profile. There were two aspects of maintaining constant temperatures in the isothermal zones. The first task was to minimize heat transfer to the environment. In the future, the PCR device will be stacked with other modules in forming multi-functional instruments; therefore it is imperative that the heat transfer to the environment is minimized. The second task was to determine the optimal location for the resistive heaters used as the thermal energy source in each zone. Spacing between heaters affects the width of the transition band between temperature zones.

Figure. 3.1: A cross-sectional schematic view of the straight channel test device (not to scale). Configuration is similar to the spiral device.
3.4 FINITE ELEMENT ANALYSIS (ANSYS /FLOTRAN)

Finite Element Analysis (FEA) is a way to simulate loading conditions on a design and determine the response to those conditions. The model is composed of discrete building blocks called elements. Each element has exact differential equations that describe how it responds to a certain load. The sum of the responses of all of the elements in a model gives the total response of a system.

In order to obtain a correct solution using the finite element analysis package, (ANSYS vs. 5.7, Canonsburg, PA), the following were completed: (1). The equation (or system of equations); boundary and initial conditions were clearly defined to ensure that a well-defined problem was formulated; (2). An element type was chosen which defined the approximation function used in the solution, (3). A mesh was created that adequately defines the regions where large changes in the solution were expected, which allows the boundary conditions to be properly imposed.

The FEA package (ANSYS) was capable of formulating the finite element algorithm that was used to solve the resulting system of algebraic equations. It also calculates the error in the approximation to determine if the solution was converging or if a more refined solution was needed (meaning a reduction in mesh size).

Finite Element Analysis (FEA) was carried out separately on three different sections of the device. First it was used to analyze the air pocket for both heat convection and thermal radiation effects. Second the method was used to do two dimensional heat conduction analyses for preliminary studies of heater placement. Third coupled thermal
and fluid analysis of the test device was carried out. This analysis was the combination of steady-state heat conduction through the solid region of the model and forced convection through the micro channel.

3.5 STEADY-STATE THERMAL

A steady-state thermal analysis calculates the effects of steady thermal loads on a system or component. Steady-state thermal analysis can be used to determine temperature distribution. Such loads include, convection, radiation, heat fluxes, heat generation rates, and constant temperature boundaries. Steady-state thermal analysis may be either linear, with constant material properties, or nonlinear due to radiation. If the material properties of the system are temperature dependent then the system may be nonlinear. Radiation effects are nonlinear because it varies with the fourth power of the absolute temperature.

3.6 ANALYSIS OF THE AIR POCKETS

Air pockets were incorporated in the design to maximize the thermal insulation above and below the heated microfluidic channels. Air is a poor conductor of heat and the convective effects could be neglected.

It is possible to obtain heat conduction and thermal radiation across the air pocket by neglecting the effects of heat convection. For a sub-layer of fluid of thickness L confined between two parallel, isothermal plates and heated from below, the heat transfer through the layer is governed by the difference in temperature between the two plates, \((T_H - T_C)\). For smaller values of \((T_H - T_C)\), the fluid is stationary and the heat transfer through the layer is assumed to be by conduction only. However, if \((T_H - T_C)\) is increased to a critical value, the fluids will become unstable, and a cellular flow pattern is established. The circulation of fluid within a cell convects warm fluid upward and cold
fluid downward. To accommodate the higher rate of heat transfer across the layer, the isotherms adjacent to each plate move closer together. If \((T_H - T_C)\) is further increased, there are transitions to increasingly more complex flow patterns until a chaotic turbulent motion replaces cellular flow. The core of the fluidic layer is then almost isothermal, with the major temperature variations confined to a very thin viscous sub-layer adjacent to each plate, where viscosity dampens the turbulent motions [Mills 1999].

![Figure 3.2: Natural-convection flow regimes for a layer of fluid between two horizontal isothermal plates. The temperature difference \((T_H - T_C)\) increases from regime (a) through to regime (d) [Mills 1999].](image)

The criterion for instability and the onset of cellular convection is a critical value of the Rayleigh number \(Ra\), which is a dimensionless parameter.
$Ra = g \beta \Delta T L^3 \rho^2 C_p / k\mu = 1708 \quad 3.1$

where:

g = gravitational acceleration (m/s$^2$),

$\beta = 1/T \ (1/K)$, $\Delta T = T_{Hot} - T_{Cold} \ (^0C)$

$L = $ thickness of the cavity (m),

$\rho = $ density (kg/m$^3$),

$\mu = $ viscosity (kg/m s),

$k = $ thermal conductivity (W/m K)

and $C_p = $ specific heat (J/kg/K)

Finite element simulations using ANSYS (CFD-FLOTRAN) (vers. 5.7, ANSYS, Inc., Canonsburg, PA) were carried out to evaluate the assumption that heat transfer across the insulating air layer was primarily by conduction and radiation.

3.7 PROBLEM DESCRIPTION

The simulation evaluated a buoyancy-driven flow in a square cavity (air pocket) heated from below. The thermal conductivity of the air and the movement of the fluid governed the heat transfer through the layer. A laminar, steady-state analysis that used the FLUID141 element was performed. FLUID141 is two-dimensional element used in FLOTRAN to model transient or steady-state fluid/thermal systems that involve fluid and/or non-fluid regions [ANSYS/FLOTRAN 5.4, Analysis Guide 1997].
The dimensions of the simulated cavity were 20mm wide x 1mm deep and the operating conditions were reference temperature of 300K, a reference pressure 1.0135 x 10^3 Pa, and gravitational acceleration 9.81 m/s^2. The units for all air properties are in the SI system. No slip boundary conditions were assumed for the walls (Vx = Vy = 0). At the two ends, adiabatic (meaning \( \frac{\partial T}{\partial x} = 0 \)) boundary conditions were assumed because the ratio of the length to the depth of the cavity was 20:1 and the end effects could be neglected. A heat source Q = 400W/m^2K was used to ensure that the air was in the cavity was heated to our maximum working temperature of 95°C, which was also enough to induce a cellular flow pattern if possible. The top surface of the polycarbonate was exposed to natural convection where \( h_{\infty} \), the convective heat transfer coefficient, was taken as 50W/m^2, the value normally used for conservative design.
Figure 3.4 shows a stationary fluid with evenly spaced isotherms because convection is not a factor. This is in agreement with the case shown in the schematic in Figure 3.2 (a) and validates the assumption of conduction dominated heat transfer.

The pressure increase on the inside of the pocket was another concern. This may cause expansion, which would induce stress on the bonded joints and the heating elements. Based on the results obtained from the simulation, shown in Figure 3.5, the maximum pressure change would be negligible when compared to the reference pressure of $1.0135 \times 10^3$ Pa.
3.8 THERMAL RADIATION AND CONDUCTION

The dominant heat transfer mechanisms through the air pocket were radiation and conduction. The heat flow due to radiation varies with the fourth power of the absolute temperature of a body. In heat conduction, which is governed by Fourier’s Law, the local heat flux is proportional to the negative of the local temperature gradient, which is linear.

In order to combine these two parallel but separate heat flows, the radiation heat transfer was linearized from:

\[ Q_{12} = A\sigma \frac{(T_1^4 - T_2^4)}{\varepsilon_1 + \varepsilon_2 - 1} \]

where \( \varepsilon \) is emissivity of each wall, \( \sigma \) is Stefan-Boltzmann constant \((5.67 \times 10^{-8} \text{ W/m}^2 \text{K}^4)\), \( A \) is the surface area and \( T_1, T_2 \) are the temperature of the top and bottom surface respectively. The emissivity of polycarbonate is \( \varepsilon = 0.9 \approx 1 \) therefore Equation 3.6 is simplified to:

\[ Q_{12} = A\sigma (T_1^4 - T_2^4) \]

According to Mills [1999] if the difference between \( T_1 \) and \( T_2 \) is small when compared to the absolute temperature, Equation 3.3 can be linearized to yield an approximate radiation heat transfer coefficient of

\[ h_r = \sigma 4T_m^3 \]

where \( T_m \) is the mean temperature between \( T_1 \) and \( T_2 \) and heat flow between each radiating surface is now:

\[ Q_{12} = Ah_r (T_1^4 - T_2^4) \]
By using the electrical analogy of heat transfer for two parallel resistors, the total heat resistance across the air pocket was formulated as shown in Figure 3.6 where:

\[
R_{\text{cond}} = \frac{L_T}{k_{\text{Th}} A} \quad 3.6
\]

\[
R_{\text{rad}} = \frac{1}{h_t A} \quad 3.7
\]

and the total heat resistance across the air pocket is

\[
R_{\text{total}} = \frac{L_T}{A(k_{\text{Th}} + h_t L_T)} \quad 3.8
\]

where \( L_T \) is the characteristic length between the two radiating surfaces. This resistance was used to calculate the equivalent thermal conductivity for the air pocket, which is \( K_{\text{eq}} = (k_{\text{TH}} + h_t L_T) \). For air at a temperature of 95°C this equivalent thermal conductivity is 0.07 W/m²K, which is one third of the thermal conductivity of polycarbonate of (0.23 W/m²K), which makes air the better insulator.

Figure 3.6: These are two parallel resistors, radiation and conduction across the air pocket
In order to validate the assumptions used in the above radiation analysis, laminar, thermal, steady state FLOTRAN analysis was performed using the model shown in Figure 3.3. The radiosity solution method was used with surface-to-surface radiation for the buoyancy-driven flow in the heated cavity. All the walls of the cavity were taken to be radiating surfaces because the cavity was fully closed. The walls of the cavity were of polycarbonate which an emissivity of 0.9. The final temperature distribution in the cavity was affected by the presence of the radiating walls in the fluid domain and the heat conducted through the fluid.

A steady-state ANSYS thermal conduction simulation was done for the same air filled cavity. Treating the air as solid with an equivalent thermal conductivity of $K_{\text{eq}} = (k_{TH} + h_{L}r_{T})$. The heat load and the boundary conditions remained the same as in the convective analysis. The temperature distributions across the cavity for both simulations were compared to each other in order to observe the differences (see Figure 3.7). Figure 3.7 shows that the difference between the two model predictions was less than 1% at any point across the cavity, therefore the assumptions and linearized model were considered valid.

3.9 FINITE ELEMENT ANALYSIS FOR PRELIMINARY SIMULATIONS

All of the preliminary simulations were carried out for conduction heat transfer only, meaning that forced convection through the channel was neglected because of slow moving fluid (2mm/s) and small fluid volume which convects negligible thermal energy. In doing so, the problem was simplified significantly. The problem was further simplified
Figure 3.7: The comparison of the temperature distribution across the air pocket, when simulated with radiation and conduction and conduction only with the equivalent thermal conductivity. The enlarged section shows clearly the temperature differences.

by setting the temperature on the outer surface of the device to a constant 22 °C.

ANSYS was used to simulate two-dimensional steady-state conduction heat transfer across the section (see Figure 3.8).
\[
\frac{\partial}{\partial x} \left( k \frac{\partial T}{\partial x} \right) + \frac{\partial}{\partial y} \left( k \frac{\partial T}{\partial y} \right) + \frac{\partial}{\partial z} \left( k \frac{\partial T}{\partial z} \right) + \dot{q} = \rho c \frac{\partial T}{\partial t}
\]

Equation 3.9 is the general heat conduction equation with variable thermal conduction in the rectangular coordinate system [Kakac, 1993]. Since there was no heat generation and steady state condition: \( \dot{q} = 0 \) and \( \frac{\partial T}{\partial t} = 0 \) respectively. The equation simplified to:

\[
\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2} = 0
\]

3.10

\[
\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} = 0 \quad \text{for} \quad 2D
\]

The selected ANSYS element for the steady-state heat conduction model was PLANE55. It can be used as an axisymmetric element with a two-dimensional thermal conduction capability. The element has four nodes with a single degree of freedom, temperature, at each node. The element is applicable to a two-dimensional, steady-state, or transient thermal analysis.

3.10 HEATER POSITIONING AND LOCATION

Simulations were used to determine the optimal number and locations of the heaters needed to obtain the constant temperature zones for the PCR cycle as shown in Figure 1.1. The three functional regions represent nominally constant temperature zones in the continuous flow device. There were three temperature transitions: (1) from 95\(^\circ\)C to 55\(^\circ\)C; (2) from 55\(^\circ\)C to 72\(^\circ\)C; and (3) from 72\(^\circ\)C to 95\(^\circ\)C. Two principal questions were addressed. First was to determine the spacing between the heaters that would maintain the
temperature in each zone with the narrowest transition band. Second was an evaluation of whether heaters would be needed on the top only or on both the top and bottom of the device, in order to ensure a uniform temperature profile across the microchannels.

The critical section of the device was the transition from 95°C to 55°C therefore preliminary simulations was carried for this section only. A straight section of the device containing two constant temperature zones and one transition zone was used to the model the system. Two-dimensional, steady-state heat transfer was assumed. The models were executed in ANSYS (vers 5.7). The heaters were represented by constant heat flux as the sources. Each cycle for PCR device was repeated at 95 °C, 55 °C and 72 °C, therefore the inlet and outlet temperature of the critical section (95 °C to 55 °C) was considered to be 72 °C.

In order to obtain a short PCR cycle time, the durations of the temperature transitions between the zones must be minimized. The spacing between the heaters, influences the temperature gradients and the transition time between each temperature zone. Due to the goal of a small sized device, the spacing between heaters must also be
kept to a minimum provided that it does not cause any excessive temperature non-uniformity in the transition region.

Heat conduction away from the channel in the unheated section between temperature zones was the only source of cooling between the denaturation (95°C) and hybridization (55°C) sections. There was a concern that, in relying on only ambient cooling, the distance between heaters required for the temperature to decrease to the desired level would be unacceptably long. Therefore, the use of metal cooling fins in the transition zone to accelerate the temperature drop was evaluated. ANSYS was used to simulate two modes, one with fins and the other without fins. The simulated results are shown in; Figure 3.9 (a) for the channel with cooling fins, and in Figure 3.9 (b) for a system without fins.

With the fins, the temperature in the transition zone dropped below the hybridization temperature before recovering. When the fins were removed, a monotonic decrease in temperature from the denaturization (95°C) level to the hybridization (55°C) was observed. Therefore the fins were eliminated from the design, which simplified the fabrication and assembly of the final device. By iteratively varying the positions of the heaters, an acceptable temperature profile across the transition zone was obtained.

The objective of this device was to have as close to a constant temperature in each zone as possible and a uniform temperature profile across the microchannel so that all of the analyte would be exposed to the same temperature-time profile. Models of two different heater configurations were simulated, with heaters on one side or on both sides of the channel. The simulation results for the two different models are shown in Figure 3.10 (a) and (b). The device with heaters on both sides showed a more uniform
temperature distribution across the channel, but the final assessment of each configuration will be made after the simulation of the final design.

Figure 3.9: ANSYS simulated temperature contour obtained for the device, each color representing approximately a 10°C temperature bond where different colors show the temperature variation. (a) The device with fins and (b) The device without fins.

Figure 3.10: ANSYS simulated temperature contour obtained for the device, where different colors showing the temperature variation with a 10°C temperature bond. (a) The device with heaters one top and bottom and (b) The device with heaters on top only.
For the simulation of the final device a combination of steady-state heat conduction and forced convection through the microchannel was used. Cooling fins were eliminated from the design. However both heater configurations, with heaters on one side and heaters on both sides of the channel were simulated. The model with heaters on one side would be easier to fabricate, particularly for modular components, so the choice would be made after the final simulations were completed.

3.11 FLUID ANALYSIS OF THE TEST DEVICE

A fluid analysis was performed to investigate the entrance effect of the velocity. This was distance the fluid traveled along the channel before it would be hydrodynamically fully developed. If the entrance effect could be neglected, the flow would be simulated as being fully developed flow.

To validate the simulation, a comparison was made between the analytical and simulated velocity profiles for three different mesh sizes. The mesh that gave the closest approximation to the analytical solution was used for the final device simulation.

For a mean velocity of 2 mm/s, the cycle duration was 30 sec of which the respective time for denaturation (95°C), hybridization of the primers (55°C) and enzymatic polymerization (extension 72°C) are Δt, Δt and 4Δt respectively, hence Δt was 5 sec.

Water has characteristics similar to the analyte so the properties of water were assumed to be that of the actual mixture. The average steady-state temperature of the working fluid is 74°C therefore all of the properties were taken at this temperature.

With the above parameters and fluid properties, the Reynolds (Re) Number and the
Table 3.1: The design parameters and specifications

<table>
<thead>
<tr>
<th>Zones</th>
<th>Time</th>
<th>Dimensions</th>
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<tbody>
<tr>
<td>Denaturation (95°C)</td>
<td>5 sec</td>
<td>Length (6 m)</td>
</tr>
<tr>
<td>Hybridization (55°C)</td>
<td>5 sec</td>
<td>Width (50µm)</td>
</tr>
<tr>
<td>Extension (72°C)</td>
<td>20 sec</td>
<td>Depth (150µm)</td>
</tr>
<tr>
<td>Total time</td>
<td>30 sec</td>
<td>Volume = 0.45µl/cycle</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Material properties</th>
<th>Symbols</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinematic viscosity</td>
<td>ν</td>
<td>0.44*10^-6 m²/s</td>
</tr>
<tr>
<td>Density</td>
<td>ρ</td>
<td>975 kg/m³</td>
</tr>
<tr>
<td>Thermal diffusivity=</td>
<td>α</td>
<td>1.6*10^-7 m²/s</td>
</tr>
</tbody>
</table>

Hydraulic diameter were calculated. For a rectangular duct, the equivalent hydraulic diameter is \( D_h = \frac{4wd}{2(w+d)} \), and Reynolds (Re) Number \( Re = \frac{u*D_h}{v} \). The calculated values of hydraulic diameter, and Reynolds’s (Re) Number were 75µm and 0.351, respectively. This showed that the flow was laminar because \( Re < 2300 \). For laminar flow, the hydrodynamic development length in entrance region is:

\[
0 \leq \frac{L_e}{D_h Re} \leq 0.05 
\]

In this application the velocity development length was calculated to be 1.32 µm, which was negligible when compared to the total length of the channel. Fully developed flow was assumed for the simulation.
Three-dimensional analyses for fluid flow and heat transfer through the rectangular duct were carried out for fully developed laminar flow. Consider the cross section of a rectangular duct shown in Figure 3.11 with the flow direction along the x axis perpendicular to the plane of the paper.

According to Dryden (1959), such a section is characterized by its aspect ratio, b/a. For the rectangular section where \(-a \leq y \leq a\) and \(-b \leq z \leq b\), the velocity profile is given by:

\[
u(y,z) = \frac{16a^2}{\mu \pi^5} \left(-\frac{dp}{dx}\right) \sum_{i=1,3,5,}^{\infty} (-1)^{i-1/2} \left[1 - \frac{\cosh(i \pi y / 2a)}{\cosh(i \pi b / 2a)}\right] \times \frac{\cos((i \pi y / 2a) / i^3)}{\pi^5} \tag{3.12}
\]

where \(dp/dx\) is the pressure gradient given in terms of the mean velocity, which is:

\[
\left[\frac{dp}{dx}\right]^{-1} = \frac{a^2}{3} \left(-\frac{1}{u_{\text{men}}}\right) \left[1 - \frac{192a}{\pi^4 b} \sum_{i=1,3,5,}^{\infty} \tanh\left(\frac{i \pi b / 2a}{i^5}\right)\right] \tag{3.13}
\]

Matlab 2000 (The Mathworks, Natick MA) was used to solve the above equations and the result is shown in Figure 3.12.
Figure 3.12: Three-dimensional velocity profile of the pressure driven flow in the PCR channel

The flow rate is given by:

\[
Q = \frac{4ba^3}{3} \left[ -\frac{dp}{dx} \left( 1 - \frac{192a}{\pi^5 b} \sum_{i=1,3,5,}^{\infty} \frac{\tanh(i\pi b/2a)}{i^5} \right) \right]
\]

3.13

The maximum velocity (\(U_{\text{max}}\)) at the center of the section was 3.727 mm/s and the corresponding pressure gradient (\(dp/dx\)) at the average velocity of \(U_{\text{mean}}\) 2 mm/s was -1.2152*10^4 (Pa/m).
3.12 COUPLED ANALYSIS OF THE TEST DEVICE

Thermal Analysis Element type: The coupled analysis of the test device was the combination of steady-state heat conduction through the solid region of the model and forced convection through the microchannel. Equation 3.9 was solved in three-dimensions using ANSYS and the resulting temperature distribution was used as the boundary condition for the fluid simulation. The element selected was SOLID187. This element is a higher order 3-D thermal element. It has a quadratic displacement behavior and is well suited to modeling irregular meshed objects. Ten nodes defined the element, which had three degrees of freedom at each node: translations in the nodal x, y, and z directions. Temperatures may be input as element body loads at the nodes. If all of the corner node temperatures are specified, each midside node temperature will default to the average temperature of its adjacent corner nodes.

3.13 FLOTRAN (CFD) ELEMENT TYPE

Flotran CFD uses the finite element method for fluid flow analysis. Computational fluid dynamics uses computers to solve the equations that described the motion of fluids, i.e., both liquids and gases. The equations that govern the motion of a fluid are based on the principles of conservation of mass, momentum and energy.

The most general form of the mass conservation or continuity equation, using indicial form, is:

\[ \frac{\partial \rho}{\partial t} + \frac{\partial \rho u_j}{\partial x_j} = 0 \]  

3.14
where $\rho$ is the fluid density, $u_j$ is the velocity (here $j=1,2,3$ denoted $u, v$ and $w$ in the $x, y$ and $z$ direction, respectively), and $t$ denoted time. [Kundu, 1990] If the fluid is incompressible

Equation 3.9 reduces to \( \frac{\partial u_j}{\partial x_j} = 0 \)

The conservation of momentum equation for an incompressible, Newtonian, viscous fluid can be written as:

\[
\rho \left( \frac{\partial u_i}{\partial t} + u_j \frac{\partial u_i}{\partial x_j} \right) = - \frac{\partial p}{\partial x_i} + \frac{\partial}{\partial x_j} \left( \mu \frac{\partial u_j}{\partial x_j} \right) + \rho B_i
\]

where $p$ is the static pressure, $\mu$ is the molecular viscosity of the fluid and $\rho B_i$ are the gravitation and body forces in the $i$ direction. Kundu [1990]

The energy conservation equation is based on the first law of thermodynamics. Conservation of energy for an incompressible fluid is written in terms of internal energy per unit mass, as:

\[
\rho c_p \left( \frac{\partial T}{\partial t} + u_j \frac{\partial T}{\partial x_j} \right) = \frac{\partial}{\partial x_j} \left( k \frac{\partial T}{\partial x_j} \right) + \phi + f
\]

where $\phi$ is the viscous heat dissipation function and is defined as

\[
\phi = \left[ \mu \left( \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right) + \lambda \frac{\partial u_k}{\partial x_k} \delta_{ij} \right] \frac{\partial u_i}{\partial x_j}
\]

where $k$ is the fluid thermal conductivity and $f$ if a volumetric heat source or sink. In many applications involving incompressible fluids, the dissipation term is very small and is often neglected. [Dryden, 1956]

The element selected was FLUID142. This is a 3-D element that can be used to model transient or steady-state fluid/thermal systems that involve fluid and/or non-fluid
regions. The conservation equations for viscous fluid flow and energy are solved in the fluid region, while only the energy equation is solved in the non-fluid region. The FLOTRAN CFD element can be used to solve for flow and temperature distributions within a region.

For the FLOTRAN CFD elements, the velocities are obtained from the conservation of momentum principle, and the pressure is obtained from conservation of mass. The temperature is obtained from the conservation of energy. FLOTRAN used a segregated sequential solver algorithm, the matrix system, derived from the finite element discretization of the governing equation each degree of freedom is solved separately. The flow problem was nonlinear and the governing equations were coupled. The sequential solution of all the governing equations, combined with the update of any temperature or pressure dependent properties, constitute a global iteration ANSYS [vs. 5.4 Thermal Analysis Guide, 1197].

3.14 PROBLEM CONFIGURATION

The model system was comprised of two cover plates, which were exposed to convective heat transfer. The convective heat transfer coefficient was assumed to be 50\,\text{w/m}^2\text{k} and the ambient air temperature was 22^\circ\text{C}. There were six air pockets, two for each temperature zone, one at top and one at the bottom. The core of the device consisted of a microfluidic channel sandwiched between polycarbonate and the air pockets. Constant heat fluxes were used to simulate the electrical heaters as the heat source. Water was used as the working fluid.

Figure 3.13 is a cross-sectional schematic view of the model with the applied boundary conditions. The ratio of the width of the channel to the width of the device was
on the order of 1000:1, so the heating surface was assumed to be infinitely long. This led to the assumption that the boundaries in the z direction were adiabatic. By modeling the device as a closed loop, which was cut through the 72°C zone and straightened out, the boundary condition at each end was also understood to be adiabatic (in the x-direction).

Figure 3.13: Two cross-sectional views of the model: (a) In the xy plane, and (a) in the yz plane.

\[
\frac{\partial T}{\partial z} = 0
\]

\[
\frac{\partial T}{\partial x} = 0
\]
Figure 3.14: This is a three-dimensional view of the simulated section of which two views are shown in Figure 3.13

3.15 MESH SIZES

Three different mesh sizes were evaluated to ensure that the simulated results obtained are independent of element size. These different meshes were drawn using ANSYS, with different distribution of cells along the channel wall and the temperature transition zones. The model was meshed with finer cells close to the channel wall and between each transition zone, where the respective velocity gradient and thermal transition length are important.

Figure 3.15: The cross sections of the right end for each of the three different mesh sizes of the fluid channel and its surrounding solid region.
Figure 3.16: The cross section of the three different mesh sizes for each temperature transition zones

Table 3.2: The different element dimensions of the three mesh configurations

<table>
<thead>
<tr>
<th>Mesh</th>
<th>Micro-channel (µm)</th>
<th>Air Pocket (µm)</th>
<th>Transition (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(10 x 5 x 20)</td>
<td>(10 x 10 x 20)</td>
<td>(10 x 10 x 20)</td>
</tr>
<tr>
<td>2</td>
<td>(40 x 40 x 50)</td>
<td>(40 x 40 x 50)</td>
<td>(40 x 40 x 50)</td>
</tr>
<tr>
<td>3</td>
<td>(10 x 10 x 10)</td>
<td>(20 x 20 x 20)</td>
<td>(20 x 20 x 40)</td>
</tr>
</tbody>
</table>
3.16 FINITE ELEMENT SIMULATION RESULTS

In order to verify the validity of the numerical simulation, a comparison was done between the analytical and the numerically simulated velocity profiles for the three different mesh sizes. The closest velocity profile to the analytical solution was Mesh (1), with a maximum velocity difference of 0.005 mm/s, which was negligible.

After the validation of the numerical simulation with the analytical results for the velocity profile shown in Figure 3.17, ANSYS was used to evaluate the thermal transition length between temperature zones using Mesh (1).

![Figure 3.17 (a): The comparison of the analytical velocity profile to the simulated result obtained from ANSYS](image)
The first two simulations were done with zero fluid velocity for two different heater configurations. Single-sided heating as shown in Figure 3.18 gave an adequate temperature profile with longer temperature transition (see Table 3.3). The extent to which the amount of temperature non-uniformity that can be allowed is still unknown and experiments will be needed to determine this.

Figure 3.17 (b): An enlargement of the comparison of the analytical and simulated velocity profiles.

The first two simulations were done with zero fluid velocity for two different heater configurations. Single-sided heating as shown in Figure 3.18 gave an adequate temperature profile with longer temperature transition (see Table 3.3). The extent to which the amount of temperature non-uniformity that can be allowed is still unknown and experiments will be needed to determine this.
Table 3.3: The temperature transition length between each zone.

<table>
<thead>
<tr>
<th>Temperature transition zones</th>
<th>72-95°C</th>
<th>95-55°C</th>
<th>55-72°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>One side heating temperature transition length</td>
<td>1.8 cm</td>
<td>1.5 cm</td>
<td>1.8 cm</td>
</tr>
<tr>
<td>Two side heating temperature transition length</td>
<td>1 cm</td>
<td>1.1 cm</td>
<td>0.8 cm</td>
</tr>
</tbody>
</table>

Figure 3.18: The comparison of the temperature distribution along the channel for the model with heaters on one side and with heaters on both sides.
The objective was to achieve a simulation as close as possible to the real device, by applying temperature constraints at specific locations, where the thermocouples for the control system were located. These temperatures were kept at 95°C, 55°C and 72°C, respectively, by varying the different heat fluxes (q_{95}, q_{55} and q_{72}) to reflect the closed loop control of the temperature at those locations. Table 3.4 and Figure 3.20 shows the variation of the heat fluxes and the temperature distribution for the different velocities.
Table 3.4: The variation in the input heat fluxes for the different velocities to obtain the applied temperature constraint.

<table>
<thead>
<tr>
<th>Velocities (mm/s)</th>
<th>q_{95} (W/m^2)</th>
<th>q_{55} (W/m^2)</th>
<th>q_{72} (W/m^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1900</td>
<td>100</td>
<td>900</td>
</tr>
<tr>
<td>1</td>
<td>1925</td>
<td>85</td>
<td>920</td>
</tr>
<tr>
<td>2</td>
<td>1947</td>
<td>70</td>
<td>927</td>
</tr>
<tr>
<td>3</td>
<td>1970</td>
<td>48</td>
<td>935</td>
</tr>
<tr>
<td>4</td>
<td>2000</td>
<td>15</td>
<td>942</td>
</tr>
<tr>
<td>5</td>
<td>2100</td>
<td>0</td>
<td>950</td>
</tr>
</tbody>
</table>

Figure 3.20: The variation of temperature distribution along the microfluidic channels, showing how increasing velocity affect the temperature profile, with the design temperature constraint.
One of our design objectives was to increase the speed of the amplification. Higher average velocity means higher throughput faster results. The study of increasing velocity through the channel was performed to determine what the costs were for this particular device configuration.

The residence time in each zone was defined as the time spent by the fluid in each isothermal zone. With a temperature bandwidth of $\pm 5^0\text{C}$, the same used by Kopp [1998] for the $72^0\text{C}$ and the $55^0\text{C}$ zones and $0$ to $-4^0\text{C}$ for the $95^0\text{C}$, the residence times in each zone were calculated and the ratios of the actual to the design time spent in each zone were evaluated: $\frac{\Delta t_{72}}{3\Delta t}$, $\frac{\Delta t_{95}}{\Delta t}$ and $\frac{\Delta t_{55}}{\Delta t}$. The residence time in each of the temperature transition zones was quantified by the ratios of the transition time to the design time ($\Delta t = 5\text{sec}$) for a mean velocity of $2\text{ mm/s}$, which were: $\frac{\Delta t_{72-95}}{\Delta t}$, $\frac{\Delta t_{95-55}}{\Delta t}$ and $\frac{\Delta t_{55-72}}{\Delta t}$. These times are shown on Figure 3.21 where: $\Delta t_{72}$, $\Delta t_{95}$ and $\Delta t_{55}$, are the time the fluid spent in the $72^0\text{C}$, $95^0\text{C}$ and $55^0\text{C}$ zones, respectively. Also $\Delta t_{72-95}$, $\Delta t_{95-55}$ and $\Delta t_{55-72}$, are the time the fluid sent in transition from $72^0\text{C}$ to $95^0\text{C}$, $95^0\text{C}$ to $55^0\text{C}$ and $55^0\text{C}$ to $72^0\text{C}$. The estimated results from the simulation were plotted versus the respective velocities and are shown in Figure 3.22 and Figure 3.23. Figure 3.22 shows time spent in each isothermal zone as a function of velocity and Figure 3.23 also shows the time spent in temperature as a function of velocity. After analyzing the velocity effect on of temperature profile along the channel, the assumed maximum velocity was determined. Plots of the temperature at the channel wall and center are shown in Figure 3.24.
Figure 3:21 Residence times $\Delta t_{72}$, $\Delta t_{95}$ and $\Delta t_{55}$, are the time the fluid spent in the 72°C, 95°C and 55°C zones, respectively, while $\Delta t_{72-95}$, $\Delta t_{95-55}$ and $\Delta t_{55-72}$, are the times the fluid spent in transition from 72°C to 95°C, 95°C to 55°C and 55°C to 72°C.
Figure 3.22: The ratio of the residence times of the fluid in the 72°C, 95°C and 55°C zones as function of mean velocity.

Figure 3.23: The ratio of the transition times for the fluid from 72°C to 95°C, 95°C to 55°C and 55°C to 72°C in the 72°C, 95°C and 55°C as function of mean velocity.
3.17 CONCLUSION OF FINITE ELEMENT SIMULATION

Simulation results show that single-sided heating produced an adequate temperature profile but has longer thermal transition lengths. Two sided heating would increase complexity of the fabrication process with the payoff of more uniform temperature zones while single side heating reduced complexity. Operating the chip at velocities of higher magnitude would increase thermal power requirement. This leads to an increase in the temperature just beneath the heaters, which may get too close to the polycarbonate glass.
transition temperature, therefore two side heating would be more favorable at velocities greater than 2 mm/s.

The mean velocity in the channel was increased from 0 mm/s to 5 mm/s. For every increase in the fluid velocity, more convective heat was removed from the 95°C and the 72°C zones so the constraint temperature tended to fall. However, heat was transferred from the 95°C to the 55°C zone, which resulted in a temperature increase. For higher velocity the fluid spends more time in transition, than in the constant temperature zones. The maximum recommended mean velocity for this device is 4 mm/s (maximum velocity of 7.5 mm/s). The results of comparison between the temperature at the channel wall and center showed a difference of 1.3°C between the maximum temperatures.
CHAPTER 4: FABRICATION OF THE MOLD INSERT BOTH PCR DEVICES

4.1 INTRODUCTION to (LIGA) MOLD INSERT

Mold inserts were fabricated using X-ray LIGA microfabrication techniques for the test (straight channel) [Bejat, 2001] and the (final spiral) devices. Initially, UV-LIGA was used to fabricate a mold insert for the spiral design configuration but the height was limited to 100 µm [Liu, 2003]. Deep X-ray lithography permits fabrication of microstructures with aspect ratios greater than 100:1 and height over 1 mm.

X-ray LIGA was used because of the ability to fabricate high, smooth, vertical sidewalls at high aspect ratio. The X-ray mask was used to reproduce the desired features in poly methyl methacrylate (PMMA X-ray resist), which was attached to a stainless steel plating base. Following X-ray exposure, the exposed PMMA was dissolved in a chemical developer (GG developer) and Ni was electroplated into the pattern to produce the desired mold insert structure. The structure was lapped and polished to obtain the desired final height. These mold inserts were used to hot embosse high aspect ratio microstructures of polycarbonate. The embossed polycarbonate devices were then used as the microfluidic channels.

4.2 BACKGROUND OF LIGA FABRICATIONS

LIGA (an acronym from the German words for lithography, electroplating, and molding) is a micromachining technology used to produce micro-electromechanical systems (MEMS) mainly in metals, ceramics or plastic [Kovaces, 1998]. This processes uses synchrotron X-ray radiation as a lithographic light source. Highly parallel X-rays from the synchrotron impinge on a mask patterned with high radiation absorbers. The absorbers on the mask are thick enough to prevent the penetration of X-rays. In the open
areas of the mask, the radiation passes through and exposes the photoresist typically poly methyl methacrylate (PMMA). In most cases the PMMA is attached to the substrate that is used later as an electroplating base. Bond scissions occur in the region of the PMMA that is exposed to the X-ray, which are selectively dissolved in a chemical developer. Once the PMMA is developed, the resulting pattern is filled with metal by electrodeposition from its conductive base.

LIGA processing contains two distinct electroplating applications. One electroplating process is the deposition of gold onto a mask substrate to serve as the X-ray absorber during synchrotron exposure. The gold thickness is typically about ten to twenty microns and must be dense, homogeneous, and uniformly thick to provide an adequate contrast ratio. The absorber stress must also be minimized otherwise it can cause pattern displacement errors. The second application of electroplating in LIGA is the fabrication of the metal device, which requires plating in a pattern of thick X-ray resist, with lateral dimensions on the order of tens of microns or more, and heights on the order of millimeters.

4.3 OPTICAL LITHOGRAPHY

The first step was to fabricate an optical lithography mask and transfer the pattern to an X-ray mask [Desta, 2000]. Optical lithography is the first step in LIGA, where the design is transferred from paper to a physical entity in the form of an optical mask. The optical mask consists of a radiation-absorbing component laid out in a pattern based upon the device design. For ultraviolet (UV) light based optical lithography, 300 Å of chromium forms the radiation-absorbing region. Dark field regions on the mask are covered with chrome and block the UV light. Clear field regions allow the UV light to
pass through and modify the photoresist. The light either polymerizes a negative resist into chains or causes molecular scissions in a positive resist. The masks where dark field regions define the pattern are called dark field masks, while those where the clear filled areas define the pattern are called clear field masks. Dark field and clear field masks for the same pattern have chrome on complimentary areas and are in “opposite tone” to each other.

4.4 OPTICAL MASK DESIGN

An optical lithography mask pattern of a straight channel PCR device was laid out using AutoCAD 2000 (AutoDesk, San Rafael, CA). The minimum size feature on the mask was 50 µm, which was the channel width and the channel length was 6.5 cm. The AutoCAD drawing was converted to the .dxf file format. This .dxf file was then converted to the .gds file format using the DXF2GDS® (DFX, Artwork Conversion, Santa Cruz, CA) conversion software then viewed using the GDSVUE® software. By doing these conversions of the mask both the dark and clear field images of the mask were assessed for errors before sending them out for bid. After the assessment, the industry standard chrome on soda lime glass, 0.09” thick and each side, 5” by 5”, optical masks were purchased from LSI Photomask (Scottsdale, AZ).

The mask, shown in Figure 4.1 (b) consists of straight micro fluidic channel in the center length 6.5 cm and width 50 µm. There were 20 other channels on either side of the fluidic channel. They 300 µm by 4 cm and were used to support in section of the temperature-sensing devices (thermocouples) for the control circuit assembly. At each end there was a reservoir; these were used as input and output ports for fluidic interconnects.
Figure 4.1(a): Optical mask pattern for the PCR straight channel designs

Figure 4.1(b): Optical mask purchased from LSI Photomask (Scottsdale, AZ).

For the spiral design, Yannick Bejat laid out the mask, which was also purchased from LSI Photomask (Scottsdale, AZ). The mask for the spiral device can be seen in [Liu, 2003] or in Figure 4.2.
4.5 X–RAY LITHOGRAPHY

X-ray lithography uses synchrotron X-ray radiation as a lithographic light source. The highly parallel X-rays from the synchrotron impinge on a mask patterned with X-ray high radiation absorbers. The absorbers on the mask are thick enough to prevent the penetration of X-rays. Hard X-rays are used as the exposure radiation. The synchrotron ring at the Center for Advanced Microstructures and Devices (CAMD) at Louisiana State University was used for this application. This is an electron storage ring with electron storage energies of 1.3 GeV and 1.5 GeV. Due to the use of shorter wavelengths and the insignificance of diffraction, X-ray lithography is far superior to optical lithography for
producing aspect ratios in the order of 20:1 or higher. The use of PMMA as an X-ray resist has been well documented [Pan, L.W et. al, 2001] [Madou 1997]. The exposure and development of PMMA resists can be done as long as the X-ray source provides 4 to 25 KJ/cm³ of energy for a maximum top to bottom dose ratio of 5 [Madou 1997]. The exposure dose was carefully calculated using Transmit® [http://www.camd.lsu.edu, 2000]. Overexposure can cause swelling and cracking which may result in deformation or delamination of the desired structures. Underexposure may cause PMMA to be undeveloped, resulting in the inability to electroplate metal on the surface of the substrate.

4.6 X-RAY MASK FABRICATION TECHNIQUES

The mask for X-ray lithography mask consists of a substrate patterned with a high radiation absorber. The substrate must be adequately transparent and the absorber opaque to the incident radiation. Some of the commonly used mask substrate materials are Silicon (Si), Silicon Carbide (SiC), Silicon Nitride (Si₃N₄), Beryllium (Be), Titanium (Ti) and Graphite (C) (Madou, 1997). For this application graphite was used as the substrate for the mask. Graphite also acts as a filter for X-rays of shorter wavelength, thus transmitting a specific bandwidth of X-ray radiation to the PMMA. High radiation absorption is proportional to molecular weight cubed. Gold, with a high molecular weight and density (M_wt=196, ρ=19300 kg/m³), with an absorption coefficient, α, ranging from 0.8 – 11 µm/m (Madou, 1997), and can be electrodeposited was used for this application.

The X-ray mask was constructed directly on a graphite membrane using SU-8 25 negative resist (MicroChem, Newton MA). For a negative resist that the exposed areas of the resist cross-link into long chains. Prior to resist application graphite wafers [Poco
Decatur, TX, DFP-3] were polished and cleaned thoroughly using acetone, IPA, and DI water to remove all unwanted particles from the wafer surface. To avoid sagging of the graphite wafer under its unsupported weight plus the weight of the resist, the wafer was bonded to a standard NIST (National Institute of Standards and Technology) aluminum adapter ring after processing the resist and gold electrodeposition. Sagging generally causes underexposure because of the gap change in the between the mask and the wafer. This generally leads to resist being under developed during resist development.

4.7 SPIN COATING AND PRE-BAKE OF RESIST

The backside of the graphite wafer was placed on a spin coating machine, and fixed into place via a vacuum chuck. Approximately 15 ml of SU-8 25 (MicroChem Corp., Newton, MA) negative photoresist was placed on the wafer and spun at 1000 rpm for 20 seconds to give a uniform coat of the resist. A thickness of 50-60 µm of resist was obtained on the graphite wafer. The SU-8 coat was pre-baked in a convection oven, starting at a temperature of 60°C, then ramped up to 95°C at a rate of 4°C/min and maintained at this temperature for about 1 hour, then slowly cooled down to 30°C at 2°C/min in the oven. Final cooling from 30°C to room temperature (~23°C), took place outside the oven. The slow cooling was necessary to reduce the internal stresses generated in the SU-8 layer during solidification.

4.8 UV EXPOSURE, POST BAKE AND DEVELOPMENT OF RESIST

The SU-8 coated wafers were exposed to ultraviolet light through the optical mask for 18.3 seconds. The wafers were exposed to 330 mJ/cm² of UV light, which was the required energy for exposing a 50 µm thick layer of SU-8. After exposure the wafers were post-baked in a convection oven, starting at a temperature of 60°C, ramped up to
95°C at a rate of 4°C/min and maintained there for 15 minutes, after which they were cooled down to 70°C at 1°C/min. Cooling to room temperature was done in ambient air.

The post-baked, patterned wafer was developed in the SU-8 developer (MicroChem Corp., Newton, MA) for a total of 6-8 min, moving back and forth from a cleaning bath to a rinse in IPA in 3 steps. The appearance of a white residue indicated incomplete development of SU-8. Whenever there was any white residue the wafers were re-immersed in the developing solution for a few more seconds. After complete development they were rinsed and dried with compressed air.

4.9 GRAPHITE SUBSTRATE PREPARATIONS AND GOLD PLATING

A layer of gold was electroplated in the developed pattern on the graphite wafer to produce the absorber layer for the X-ray masks. A commercially available neutral gold sulfite solution (Technic Inc, Irving TX), containing 1 troy oz gold per gallon was used for gold plating. Before plating 7.5g/l of sodium sulphate (Na2So3) was added to the gold solution. The gold sulfite (Na3Au(SO3)2) solution, was mildly acidic, with a pH of 6 – 7. Plating was carried out at a current density of 5 mA/cm², using a pulsed current (1 Hz) for four hours to get a 12-15 µm coat of gold. The plating rate was approximately 4 µm per hour. A platinized titanium mesh was used as the anode. The bath temperature was maintained 25°C and the amount of gold in the solution was maintained at no less than 80% of its initial value. This was done by more concentrated commercially available neutral gold sulfite solution (Technic Inc, Irving TX), containing 1 troy oz gold per 500ml. The amount of gold used in plating was from the gold sulfite (Na3Au(SO3)2) bath was 1 troy oz gold for every 4 mA/hr.
The structure of the mask was composed of SU-8 and with gold embedded in it. The exposed SU-8 resist was left on the wafer to provide structural stability to the mask. The mask was now ready for use. It was attached using super glue (Loctite 4210, Loctite Corporation, Newington, CT) to an NIST (National Institute of Standards and Technology) aluminum adapter ring mask is shown in Figure 4.3. The striations in the gold are due to secondary roughness arising from the graphite surface.

4.10 PREPARATION OF STAINLESS STEEL MOLD INSERT SUBSTRATE

In order to keep costs low for individual nickel electroforms, it was necessary to find an inexpensive substrate, which the raised microstructures could be plated upon. Other properties such as machinability and thermal the coefficient of expansion were
considered as well. Good machinability was necessary in order to easily cut the substrate to the desired dimensions and obtain an initial surface finish to rms value of 1μ. Finally, thermal expansion characteristics had to be considered because nickel microstructures electroformed directly onto a substrate with a large difference in coefficient of thermal expansion could debond under the elevated temperatures used during embossing. The coefficient of thermal expansion of 316 stainless steel (16 μm/m-°C) and nickel (13 μm/m-°C) are very similar. Therefore, stainless steel was judged as an acceptable substrate for the present application.

The 316 stainless steel (Ryerson-Tull, Dallas, TX) was cut and machined into 5-inch diameter base plates. Using a Harig 618 surface grinder (Elgin, IL), the base plate was ground flat and sandblasted to create a roughened surface with rms value of approximately 2 μ. The stainless steel base plate was activated in C-12 activator (Puma Chemical, Warne, NC) with a current density of (20 mA/cm²), for 2 minutes forward and 2 minutes backward. Approximately 10 ml of a 9% PMMA in cyclobenzene solution (MicroChem, MA, USA) was placed on the wafer and spun onto the base at 1000 rpm for about 30 seconds. This was allowed to melt on the stainless steel surface at 180 °C in a conventional oven and cooled slowly to room temperature.

4.11 PMMA BONDING

The resist, 500 μm thick PMMA sheet stock (CQ grade Goodfellow, UK) was cut to the size of the substrate and bonded to its coated surface. A solvent bonding process, using a MMA bonding solution was used to achieve the bond. The constituents of the bonding solution are shown in Table 4.1
In order to mix the solution, the powdered PMMA was dissolved in the MMA. This process took a fairly long time during which some of the MMA may evaporate. It was kept in a closed container and the weight of the container was recorded before and after the PMMA dissolved in the MMA so that the correct amount of MMA could be added to maintain the correct proportions if any had escaped from the closed container. The DMA acted as a starter for polymerization and BPO was used as the hardener. The BPO was not added until a few minutes before use. After adding BPO the mixture hardened within 15 minutes. The above solution was thoroughly mixed and degassed in a fume hood for 12 hours.

A few drops of this MMA glue were applied to the wafer surface and the 500 µm thick PMMA resist was pressed onto the substrate. Care was taken to ensure that no bubbles were trapped in the glue. This sandwiched wafer was placed in a pneumatic press for 12 hrs under a pressure of 10-12 psi at room temperature. Finally, the PMMA was fly cut to the final desired height of the microstructures, which in this case was 200 µm, which leaves us with 50 µm for surface grinding and polishing. The thickness of the PMMA resist needed to be known to enable accurate calculation of the dose for X-ray exposure.

<table>
<thead>
<tr>
<th>Table 4.1 Composition by mass of MMA bonding glue</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMA (Methyl methacrylate)</td>
</tr>
<tr>
<td>Powdered PMMA</td>
</tr>
<tr>
<td>BPO (Benzoyl peroxide)</td>
</tr>
<tr>
<td>DMA (Dimethyl aniline)</td>
</tr>
<tr>
<td>MEMO (Methacryloxpropyltrimethoxysilane)</td>
</tr>
</tbody>
</table>
4.12 X-RAY EXPOSURE OF PMMA

To pattern structures in PMMA accurately by the shadow printing process, a high energy, highly collimated light source was required. A synchrotron provides such a light source. A synchrotron is an electron storage ring where relativistic particles travel at nearly the speed of light, emitting a continuous spectrum from infrared to the hard X-ray range.

X-ray exposures were performed on the XRLM-3 beamline at CAMD. A 125-µm Beryllium filter was installed in the beamline to act as an initial filter for low energy X-rays. The intensity of X-rays decreases as they pass through the different filters, and mask. The exposure dose must be calculated as shown in Appendix 4.A. Overexposure can cause swelling and cracking resulting in deformation of desired structures, while underexposure results in the inability to electroplate a metal at the substrate surface. For full exposure of PMMA, the bottom surface of the resist must receive at least 3000 mJ/cm³ of X-ray energy and the top surface should be less the 20000 mJ/cm³. Thicker samples will require longer exposure times in order to deposit the necessary 3000 mJ/cm³ at the bottom. In this instance, the thickness of PMMA to be exposed was 200 µm.

Combinations of 6 µm and 15 µm thick aluminum filters were used to improve the contrast between the top and bottom surfaces of the resist. The filters also served as an additional barrier for low energy X-rays, thus improving resolution. The more filtration used, the better the contrast between the exposed and the unexposed resist, although the time for complete exposure can increase significantly.
4.13 PMMA RESIST DEVELOPMENT

X-ray radiation causes scissioning of the polymer chains. The broken chains have lower molecular weight and can be dissolved in a molecular weight-sensitive organic solvent. To fully utilize the accuracy potential of synchrotron radiation lithography the resist-developer system should have a ratio of dissolution rate in the exposed and unexposed areas of approximately 1000. This would result in minimal dissolution of the unexposed, high molecular weight cross-linked PMMA and a sufficient dissolution rate in the exposed area. See Table 4.2 for the composition by volume of the GG developer and GG rinse used to dissolved exposed PMMA

Table 4.2: Composition by volume of the GG Developer formulated at the Institute for Microstructure Technology at the Forschungszentrum, Karlsruhe. This solvent is used to dissolve PMMA that were exposed to X-rays.

<table>
<thead>
<tr>
<th>Developer:</th>
<th>(ml)</th>
<th>(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-(2-Butoxyethoxy) ethanol / (diethylene glycol butyl ether)</td>
<td>1500</td>
<td>900</td>
</tr>
<tr>
<td>Morpholine</td>
<td>500</td>
<td>300</td>
</tr>
<tr>
<td>2-aminoethanol</td>
<td>125</td>
<td>75</td>
</tr>
<tr>
<td>Water</td>
<td>375</td>
<td>225</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>2500</strong></td>
<td><strong>1500</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rinse</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-(2-Butoxyethoxy) ethanol / (diethylene glycol butyl ether)</td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
For every 200 μm of PMMA thickness, the wafer was run through a development cycle of 20 min in the GG developer and a rinse cycle of 40 min in the GG rinse at room temperature for three repetitions. The GG developer was filtered continuously. After development the microstructures were rinsed with deionized water and dried with compressed air before electrodeposition.

4.14 NICKEL ELECTRODEPOSITION

In order to achieve a good bond between the plated nickel and the stainless steel substrate, the substrate was activated with C-12 and Wood’s strike before nickel was electrodeposited. A nickel sulfamate electroplating solution (see Table 4.3) was used to electrodeposit nickel into the exposed and developed PMMA mold.

Before plating the stainless steel base plate with the PMMA patterned was activated again using a C-12 activator to remove any dirt or residual oxide prior to electroplating. The C-12 bath was composed of 35 of gallons water and 5 of gallons of C-12 activator with 2% HCL to reach a pH of 1.5. By using nickel pellets as the anode and the work piece as the cathode, the substrate was activated with a current density of (20 mA/cm²) at room temperature. This was done with cycle of two minutes forward and two minutes backward.

The substrate was treated with the Wood’s strike because the chromium atoms in stainless steel have a tendency to form an oxide layer across the surface of the substrate [Stefanov, 2000]. This oxide layer creates a weak bond between the substrate and the electroformed nickel. Debonding is a significant problem when structures are plated directly onto this oxide layer. In order to minimize this problem, an acid Wood’s strike composed of 240g of nickel chloride (NiCl₂*6H₂O), 80g of hydrochloric acid (HCl) and
Table 4.3: Composition by mass of the nickel sulfamate solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Chemical formula</th>
<th>Amount (g)/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel sulfamate</td>
<td>Ni ((SO_3NH_2)_{2.4}H_2O)</td>
<td>450</td>
</tr>
<tr>
<td>Boric acid</td>
<td>(H_3BO_3)</td>
<td>37.5</td>
</tr>
<tr>
<td>Sodium lauryl sulfate</td>
<td>(C_{12}H_{12}O_4Sna)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

One liter of distilled water at a pH of 1.5 was used. This was done at room temperature \((23 \, ^{0}\text{C})\) with reverse plating for two minutes at a current density of 50 mA/cm², then switched to forward plating for six minutes at 50 mA/cm². The nickel strike had two effects on the stainless steel substrate. First, the acidic nature of the bath etched away the oxide layer. Secondly, a thin layer (~ 1 µm) of nickel was plated onto the oxide free surface giving excellent adhesion between the stainless steel substrate and the plated microstructures. The substrate was then placed directly into the nickel-plating bath without rinsing.

Nickel sulfamate electroplating solution was used to electrodeposit nickel into the exposed and developed PMMA molds directly after the Wood’s strike. Nickel electrodeposition using a nickel sulfamate solution has been widely used for electroplating into microstructures (Madou, 1997). Nickel sulfamate has the advantages of low stress in electroplated microstructures, as compared to a nickel sulfate or nickel chloride electroplating solutions.

Six liters of 1.8 M nickel sulfamate electroplating solution was prepared by using the compounds listed in Table 4.3. The concentration of the hydroxyl ions (OH⁻) increases as the plating progresses and increases the pH of the solution. A starting pH of
3.8 was used to account for the increase in pH during electroplating. Boric acid was used as a buffering agent to minimize the pH rise of the solution while electroplating. Lauryl sulfate served as a wetting agent reducing the surface tension of the solution, which helped the solution to reach deep recesses in microstructures and also contributed to low stress plating. Also plating at lowering current densities can reduce stress, by reducing grain sizes of the plated nickel. Plating nickel to a height of 200 µm, to allow for post plating finishing required only 10-12 hours using a current density of 25 mA/cm².

The components were mixed and DI water was added to increase the volume to six liters. The solution was heated on a hot plate, with constant stirring, to a temperature of about 50-60 °C. It required about two hours to mix and dissolve the salt completely. After mixing, the nickel sulfamate plating bath was filtered with a 0.45-um-cellulose filter prior to electrodeposition.

4.15 LAPPING AND POLISHING

The final step in producing the mold insert was lapping or surface grinding of the substrate to the final thickness and then polishing. This was done using diamond slurries with an ENGIS lapping machine (Model 15LM11V Engis Wheeling, IL). Thickness tolerances were plus or minus five microns, and the surface finish was one-micron rms. The surface-grinding step was used to ensure that the surfaces were parallel to each other was able to produce a flatness of under 5 µm across the 5 inches diameter of the stainless steel substrate. Once ground parallel to the bottom of the substrate at the desired height, the tops of the electroformed structures were polished using diamond coated sand paper. The smoother the surface the easier is demolding.
Finally, all of the PMMA on the substrate was flood exposed and developed in the GG developer. The mold insert was then complete and ready for hot embossing.

Both mold inserts were inspected and scanning electron microscope images of them were taken using the SEM and Electron Microprobe Laboratory at the Department of Geology and Geophysics at LSU. Figure 4.4 shows the SEM image of the straight channel mold insert and Figure 4.6 shows the SEM image for spiral design mold insert. Figure 4.5 and 4.7 are actual photos of the straight channel and the spiral design mold inserts respectively.

Figure 4.4: SEM of straight channel mold insert

![SEM Image of Straight Channel Mold Insert](image)
Figure 4.5: Actual photo of the straight channel mold insert

Figure 4.6: SEM of spiral channel mold insert
Figure 4.7: Actual photo of the spiral design mold insert
CHAPTER 5: THERMAL SYSTEM DYNAMICS AND CONTROL

5.1 INTRODUCTION TO SYSTEM DYNAMICS OF THE PCR DEVICE

The system dynamics of the PCR device were studied to determine: (1) the required thermal input for the selection or design of the heater; (2) to determine the controller needed to maintain the steady state temperatures for the PCR cycle and (3) to check the stability and time constant.

One of the design criteria for the PCR was to achieve one-dimensional heat transfer, which required making the device as thin as possible. The heater that would be selected or designed needed to meet several specifications to satisfy the assumption of one-dimensional heat flow, including: (1) thin < 50 µm so that heat flow in the lateral directions could be neglected, (2) capable of providing the required power for each constant temperature zone. The area of the heater was also restricted to cover the spiral path of the CFP PCR prototype.

5.2 PSEUDO BOND GRAPH MODELING FOR THERMAL SYSTEMS

In order to study the dynamics of the system a lumped parameter thermal model of the PCR device was developed using the pseudo-bond graph method [Karnopp, 2000]. Thermal systems have no inertial element so that the only one-port elements that were used in this system model were sources, resistors and capacitors. In the case of thermal systems, the product of temperature and heat flow is not equal to power. Heat flow itself has the dimensions of power. For this reason, a thermal bond graph with temperature as the effort variable and heat flow as the flow variable is called a pseudo bond graph.

The PCR was assumed to be symmetric with the same power input on either side, which was modeled as a constant heat flux (flow source). The ambient condition was
taken as constant temperature, $T_{\infty}$, which was represented by an effort source. Since the ratio of the thickness compared to the length and width of the device was less than 0.001, one-dimensional heat transfer was assumed.

The PCR was reticulated into three sub-systems capable of storing energy; these represented the air in the air pockets, the polycarbonate cover plate, and the core of the system, which was comprised of water (to simulate the analyte) and polycarbonate. The capacitances were evaluated using Equation 5.1.

$$C = mc_p$$  \hspace{1cm} 5.1

where $c_p$ was the heat capacity at constant pressure, and $m$ was the mass. Upon evaluation, the capacitance of air was four orders of magnitude less than all the other capacitances as shown in Table 5.1 and energy storage in the air was neglected.

Heat transfer to the environment was assumed to be by convection, and the resistance was represented by (Equation 5.2):

$$R_{\text{conv}} = \frac{L}{h_cA_{\text{surf}}}$$  \hspace{1cm} 5.2

where $h_c$ is the local convective heat transfer coefficient, and $A_{\text{surf}}$ is the surface area.

Table 5.1: Numerical values for the resistors and capacitors for the lumped parameter model

<table>
<thead>
<tr>
<th>$R_1$=R$_5$</th>
<th>$R_2$=R$_4$</th>
<th>$R_3$</th>
<th>$C_1$=C$_3$</th>
<th>$C_2$</th>
<th>$C_{\text{air}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>32 m$^2$/K/W</td>
<td>114.8 m$^2$/K/W</td>
<td>14.55m$^2$/K/W</td>
<td>3.8 J/k</td>
<td>1.5155 J/k</td>
<td>1.59x10$^{-5}$ J/k</td>
</tr>
</tbody>
</table>
Heat flow between the sub-systems was modeled by resistive elements for conduction (Equation 5.3) and radiation (Equation 5.4).

\[ R_{\text{cond}} = \frac{L}{kA} \quad 5.3 \]

where \( L \) is a characteristic length, \( k \) is the thermal conductivity of the material, and \( A \) is the cross-sectional area. Radiation is a nonlinear function of temperature, so the relationship was linearized by assuming that the temperature difference between any two surfaces was small when compared to the absolute temperatures of the two surfaces. A linearized radiation heat transfer coefficient was calculated (\( h_r = 4\sigma T_{\text{surf}}^3 \))

\[ R_{\text{rad}} = \frac{1}{h_r A_{\text{surf}}} \quad 5.4 \]

The total resistance across the air pocket was:

\[ R_{\text{total}} = \frac{L_T}{A(k_{\text{Th}} + h_r L_T)} \quad 5.5 \]

as shown in Section 3.4, where both the radiation resistance and the conduction resistor through the air were parallel to each other. After simplification, the model was reduced to three capacitances and five resistances. The electrical analogy was used to layout the equivalent circuit diagram and a bond graph was drawn for the system as shown in Figure 5.1 and 5.2 respectively.
Figure 5.1: Equivalent electrical circuit for one dimensional heat flow for each zone

Figure 5.2: Pesude bond graph of the system

The linearized state equations were rearranged in matrix format [Equation 5.6]

\[
\begin{bmatrix}
\frac{dT_1}{dt} \\
\frac{dT_2}{dt} \\
\frac{dT_3}{dt}
\end{bmatrix} =
\begin{bmatrix}
-(R_1 + R_2) & \frac{1}{C_1 R_1 R_2} & 0 \\
\frac{1}{R_2 C_2} & -\frac{1}{C_2 \left( \frac{1}{R_3} + \frac{1}{R_2} + \frac{1}{R_4} \right)} & \frac{1}{R_4 C_2} \\
0 & \frac{1}{R_4 C_3} & -(R_4 + R_3) \frac{1}{C_3 R_4 R_5}
\end{bmatrix}
\begin{bmatrix}
T_1 \\
T_2 \\
T_3
\end{bmatrix} +
\begin{bmatrix}
\frac{1}{R_1 C_1} \\
0 \\
0
\end{bmatrix}
\begin{bmatrix}
T_1 \\
T_2 \\
T_3 \\
e_i
\end{bmatrix}
\]

Equation 5.6
5.3 CONTROL SYSTEMS BACKGROUND

In a regulated system, the setpoint specifies the desired response level at the system output. In this case the output is temperature. In a perfect world, you would provide the setpoint signal as the input to a system and it would immediately provide the desired output level. This configuration is called open loop control and is illustrated in Figure 5.3.

A real process does not normally operate in this way. The output may drift away from the setpoint for a variety of reasons, or may respond too slowly to changes, or may oscillate too much in response to transient changes. In these situations, it is necessary that the output of the system be measured and processed by a separate controller (sometimes called a regulator). The controller compares the system output to the setpoint level to determine a new control input for the system. Often the difference between the output of the process under control and the reference input is amplified and used to control the process so the difference is continually reduced. This configuration, called a closed loop feedback control system, is illustrated in Figure 5.4. Closed loop control handles disturbances better and the time constant is adjustable.

Figure 5.3. Ideal system with open loop control
Figure 5.4: Typical system under closed loop control.

Figure 5.5: The response of the system for a step input power of 1.415 W and 2.5 W which are flow source.
The open loop step response of the system was evaluated using MATLAB™ (ver. 5.4, The Mathworks, Natick, MA) for the power input needed to reach the maximum required steady state temperature of the device (95 °C) and the glass transition temperature of polycarbonate (148 °C), which are plotted in Figure 5.5. These temperature achieved by constantly adjusting the power input until the desired temperatures were reached.

5.4 STABILITY

A system is stable if all the poles of the function have negative real parts [Franklin, et al., 1994]. The poles of the system were -0.0510, -0.088 and -0.0167, which were all negative and real so the time response will decay.

5.5 HEATER POWER REQUIREMENT

For the open loop analysis, of the double side-heating configuration, 1.415W of thermal power was needed obtained the maximum working temperature (95 °C) and 2.5W for the glass transition temperature (148 °C) of PCR device. The assumed power inputs for the single side-heating configuration were approximately 3W for (95 °C) and 5W for (148 °C) respectively. The average of these values (4W) was chosen as the required power rating for the heaters. The time constant for the system is 48 sec.

5.6 PID CONTROLLER PARAMETERS

The power (W) from the PID controller is:

\[ W(t) = K_p \times (T_s - T_o) + K_D \frac{dT}{dt} (T_s - T_o) + K_I \int (T_s - T_o) dt \]  

where \( T_s \) and \( T_o \) are the final and initial temperature of the system, respectively. Multipliers \( K_p \), \( K_I \) and \( K_D \) are referred to as the proportional, integral and the derivative.
gains. The effects of each of the controller gains on a closed-loop system are summarized in Table 5.2. MATLAB (SIMULINK) was used to determine the required gains and the block diagram used is shown in Figure 5.6.

Selecting optimal values of $K_P$, $K_I$ and $K_D$ parameters of a closed loop control system is an iterative process. This process is called PID tuning. The MATLAB Controls Toolbox was used to simulate the time response of the PCR device. The time response for each zone is shown in Figure 5.7.

Table 5.2: The effects of each of the controller gain on a closed-loop system

<table>
<thead>
<tr>
<th>CLOSED LOOP RESPONSE</th>
<th>RISE TIME</th>
<th>OVERSHOOT</th>
<th>SETTLING TIME</th>
<th>STEADY-STATE ERROR</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_P$</td>
<td>Decrease</td>
<td>Increase</td>
<td>Small change</td>
<td>Decrease</td>
</tr>
<tr>
<td>$K_I$</td>
<td>Decrease</td>
<td>Increase</td>
<td>Increase</td>
<td>Eliminate</td>
</tr>
<tr>
<td>$K_D$</td>
<td>Small change</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Small change</td>
</tr>
</tbody>
</table>

Figure 5.6: The block diagram used in SIMULINK to determine the correct gains for the simulation
The results from the simulation show that 200 sec after the system was powered up the PCR process could be started. It also shows that the temperature as measured by the thermocouple, in each zone can be held constant using a PID controller with the correct gains. Three Series 96 PID controllers were bought from Watlow (Winona, Minnesota). They were capable of self-tuning. Self-tuning allowed the controller to explore the responsiveness of the system to determine an effective set of gains for PID control. The controllers could attain PID gains over the full range of gains selected based on the simulation results so the PCR temperatures could be maintained within the desired bands.
5.7 HEATER SELECTION

The next step was to find an off-the-shelf heater that could deliver the 4W defined by the open loop analysis. Alternatively, the heaters could be designed and fabricated. An extensive search was carried out for commercially available heaters with the desired characteristics shown in Table 5.2. The heaters that were available were deposited on, Kapton or silicone rubber with maximum temperatures of 200°C and 235°C respectively. The Kapton polyimide heater (Model K005020C5, Watlow, Dallas, TX) was chosen because it was the only one that was small (2.54 cm x 2.54 cm) and relatively thin (~200µm) to meet specifications. These heaters were still 150 µm thicker than desired, therefore it would be best to deposit the heating element directly onto the device.

Table 5.3: The comparison of the specifications for the electrical heaters to commercially available devices from Watlow (Dallas, TX).

<table>
<thead>
<tr>
<th>Desire Specifications</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>Thickness (µm)</th>
<th>Power (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiral Configuration</td>
<td>2.54</td>
<td>1.5</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Straight Channel Configuration</td>
<td>1.00</td>
<td>1.00</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Commercially Available</td>
<td>Length (cm)</td>
<td>Width (cm)</td>
<td>Thickness (µm)</td>
<td>Power (W)</td>
</tr>
<tr>
<td>Spiral Configuration</td>
<td>2.54</td>
<td>2.45</td>
<td>200</td>
<td>5</td>
</tr>
<tr>
<td>Straight Channel Configuration</td>
<td>3.5</td>
<td>1.2</td>
<td>200</td>
<td>5</td>
</tr>
</tbody>
</table>
5.8 INTRODUCTION TO HEATER DESIGN

Ideally the heating element will be deposited directly onto the polycarbonate by activating the surface. Exposing the polycarbonate substrate to UV radiation via a patterned quartz mask will carry out an activation process as shown by [Henry and McCarley, 2001] on PMMA. Electroless plating was used to deposit gold on the activated surface. After the gold was deposited on the surface, electroless plating was used to deposit copper onto the patterned gold. The final heating element, a Nichrome alloy of composition 80% Ni and 20% Cr will be electroplated onto the electroless copper.

The dimensions of the heating element were determined based on the analytical and simulation analysis of the heating element. A quartz mask was laid out (see Figure 5.8) so that the heater could be patterned by UV lithography. The current required to power the heater was determined.

5.9 RESISTIVE HEATING

Every current-carrying conductor loses electrical energy by $I^2R$ losses; this rate of energy loss is the electrical power that is converted into heat. When a heater is connected to an electric power supply, current flows through the high resistance heating element. The heating element resists the flow of electric current through it, converting electrical energy into heat energy.

The resistance of a conductor of length (L) and cross-sectional area (A) normal to the direction of current flow is

$$R_E = \frac{\rho L}{A}$$

5.8
where $\rho_R$ is the specific resistivity ($\Omega \cdot \text{m}$). For metals, specific resistivity increases approximately linearly with temperature over moderate temperature ranges ($20^\circ\text{C}$ to $500^\circ\text{C}$). This variation is most often written as

$$R_{T_f} = R_{T_0} (1 + \alpha_0 (T_f - T_0))$$ \hspace{1cm} (5.9)

where $\alpha_0$ is the temperature coefficient of resistivity and has units of inverse temperature ($\text{T}^{-1}$). Because the maximum temperature on the PCR chip is only $95^\circ\text{C}$ the minor change in resistance can be disregarded, hence the specific resistivity ($\Omega \cdot \text{m}$) of the heating element was assumed constant. The length and cross-sectional area of the heating element depends upon the specific resistivity of material and the required power density. Equation (5.10) gives the power density, $P_E$ for a wire of resistance $R_E$ with a voltage $V_E$ applied across its ends or a current $I_E$ flowing through it.

The power density for a heater is the ratio of the heat generated by the heating elements to the surface area of the heater as in Equation 5.11.

$$\text{(Power Density (P_E))} \frac{\text{Watt}}{m^2} = \frac{I_E^2 R_E}{A_{\text{surf}}}$$ \hspace{1cm} (5.10)

The temperature rise in a material is a function of: (1) The bulk resistance, which depends on the resistivity and thermal conductivity; (2) the magnitude of the electrical current; and (3) the convection and radiation energy loss to the surroundings. The maximum, steady state temperature rise of a heating element in still air was calculated using Equation 5.11.
\[ \Delta T = (T_H - T_\infty) = \frac{I^2 t^2}{2kvA^2} + \frac{I^2 t}{\nu h A^2} \]

where \( \Delta T \) temperature rise in °C, \( I \) current in amps, \( t \) element thickness in meters, \( A \) the cross-sectional area of the heating element, \( \nu \) electrical conductivity \((1/\rho)\), \( k \) the thermal conductivity \((\text{Wm}^{-1}\text{K}^{-1})\) and \( h \) is the natural convective heat transfer coefficient \((\text{W/m}^2\text{K}^{-1})\). This equation gave a conservative estimate of temperature rise because the heat loss via radiation was neglected [Kakac, et al., 1993].

For the resistive heating applications, the most commonly used heating material is a nickel-chromium alloy (about 80% nickel and 20% chromium), which carries the trade name "Nichrome". Nichrome is the most commonly used metallic alloys in resistors used at elevated temperatures.

All heaters eventually fail or burn out, even when using Nichrome as the heating element. Resistive heaters will often fail if the heating element is damaged or contaminated with chemicals, but the primary cause of failure is oxidation. At high temperatures, oxygen from the air chemically reacts with the element, which causes a hard oxide coating to form on it. This oxidation process continues until the heating element eventually disintegrates or burns out. If the heater is maintained at a constant temperature, the hard oxide coating actually slows the oxidation process and extends the life of the heater.

Periodic heating and cooling cycles of the heater may cause the resistance element to expand and contract. This will cause cracking of the hard oxide coating and expose more metal to oxygen attack. Therefore temperature cycling through abrupt temperature changes will greatly accelerate the oxidation process and cause the heating element to
burn through much more quickly. Even though there is no clear cut way to really stop this oxidation process, it can be easily slowed down.

One way this was done was to use PID controllers to minimize the temperature cycling of the heating element. Another way of extending the service life of the heater was to use it at temperatures lower than the maximum. The maximum temperature on the PCR chip is 95°C and the maximum temperature of the Nichrome heating elements is about 870°C. By using the heater so far below its maximum temperature the heater element life will be prolonged.

5.10 DESIGN SPECIFICATIONS AND MASK LAYOUT

The maximum power required from each heater was 4 W. The surface area for each heater was (length 2.54cm and width 1.52 cm as shown in Figure: 5.8) 3.82 cm², which was the area of the spiral the needed to be covered by each heater, and a maximum temperature increase of \((\Delta T_{\text{max}})\) 135 °C. The variable parameters were the length \((L_h)\), width \((W_h)\), and thickness \((t_h)\) of the heating element. Equation 5.13 gives the length of the heating element, as a function of the system specifications.

\[
L_h = \frac{V_e^2 A}{\rho_h P_e} \tag{5.12}
\]

The dimensions chosen for the heater were width \(W_h\): 200 µm, thickness \(t_h\): 10 µm and Equation 5.12 was used to calculate the length \(L_h\): 0.29 m. The 10 µm thickness was chosen because it was the maximum thickness of Ni-Cr that can be plated with direct current, without cracking (K-Lin, C-Hsu, I-Hsu, T-Chang, 1992). For a heating of cross section 200 µm by 50 µm, using Equation 5.13 the calculated element length \((L_h)\) was 0.29 m for the required power of 4W and an applied voltage of 25V. Table 5.4 consist of the electrical properties of commercial Ni-Chrome alloy [Goodfellow, UK]
Table 5.4: Electrical Properties of Ni-Chrome alloy [Goodfellow, UK]

<table>
<thead>
<tr>
<th>Metal</th>
<th>Specific resistivity $\rho_0$ at 25$^\circ$C ($\mu\Omega$-cm)</th>
<th>Coefficient of resistivity $\alpha (^\circ$C$^{-1})$</th>
<th>Thermal Conductivity (W/m-K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni-Chrome</td>
<td>108</td>
<td>0.0004</td>
<td>13.4</td>
</tr>
</tbody>
</table>

The predicted resistance of this heater was 156$\Omega$ as shown in Figure 5.9 at a current flow 0.160 amps. The number of turns of the heater coil ($n$) was:

$$n = \frac{L_0}{\Delta l}$$  \hspace{1cm} 5.13

where $\Delta l$ (0.015m) was the length of one turn of the coil. For the above dimensions the number of turns ($n$) was 20. For the actual design (see Figure 5.8) 24 turns were used to make allowance for changes in design parameters during the fabrication process, such as the cross sectional area due to overplating, or the combinations of Nichrome, copper and gold, which might result in a lower specific resistivity. By keeping the thermal power constant the required resistance of the heating element was calculated as function of the applied voltage. The relationship between voltage and resistance of the heating element for a constant power of 4W is shown in Figure 5.9.
Figure 5.8: The mask pattern for the Ni-Cr heater with 24 turns all units are in µm

Figure 5.9: The variation of heating element (Ni-Cr) resistance with voltage for a power of 4W
ANSYS (5.7, ANSYS, Inc., Houston, PA) was used to simulate the temperature in still air (air pockets) as a function of current. The convective heat transfer coefficient was assumed to be 50W/m²K and the ambient air temperature to be 300K. Figure 5.10 shows the comparison between the theoretical and simulated predictions of the temperature increase with an increase in current flow through the heating element. Also the heat flux from the heating element to the surrounding air was simulated and shown in Figure 5.11.

Figure 5.10: The temperature increase with an increase in current flow through the heating element Comparison between the Lumped parameter and finite element results simulated results.
The heat flux from the heating element to the surrounding air, which is equivalent to the heaters’ Power density.

The calculated average value for the power density was $96517 \text{ W/m}^2$ and the maximum value obtained from the simulation was $97768 \text{ W/m}^2$. The result of the lumped parameter and finite element models for the temperature variation and the power density of the heater were within 4% of each other. The error obtained was a result of the assumption of one dimensional heat transfer for the lumped parameter analysis, which neglected heat losses along the other two axes. The finite element simulations accounted for all heat losses, therefore the values obtained from the ANSYS simulation are slightly less and more accurate. Since all of the design parameters were met, the design is thus complete and the next step is fabrication.

5.11 DIRECT ELECTRODEPOSITION ON POLYCARBONATE

Schlesinger and his group Schlesinger, [2000] state that it is possible to obtain patterned or selective deposition of electroless copper by UV radiation of a nonconductive substrate through a quartz mask. It is possible to achieve electrodeposition on the irradiated areas by using a negative mask image. This was also demonstrated by Henry and McCarley [2001] where copper was deposited on PMMA. This process
involves cleaning, surface modification, sensitization, and subsequent catalyzing, and activating.

This procedure was carried out on polycarbonate, where wet chemical deposition techniques (electroless plating) will be used to deposit copper. Dr. McCarley’s group will carry out the process in the Department of Chemistry at Louisiana State University. This process was not completely optimized. Experiments are still being carried out on the surface modification of polycarbonate. Therefore the complete fabrication process for the heaters will be completed in the near future.

5.12 ELECTRODEPOSITION OF NI-CR ALLOY

A series of experiments were conducted to determine the correct plating conditions for Ni-Cr alloy of composition 80%Ni 20%Cr on copper. These conditions were the pH of the solution, current density, the bath composition, and the plating temperature.

The objective of the Ni-Cr plating was to obtain an alloy composition of 80%(Ni) and 20%(Cr) that had equivalent properties to the commercially available Ni-Cr alloy shown in Table 5.4 of section 5.10. It was reported by Lin and Ho, [1991] that, through the application of DC plating, smooth dense Ni-Cr alloy of up to 20% Cr and 80% Ni could be obtained. They also stated that percentage composition of Ni to Cr was a function of temperature, pH, and current densities for the bath composition shown in Table 5.5. They used a pH of 4 with a current density of 0.005/cm² and a plating temperature of 10°C to obtain Cr content of up to 30%.
Another group from Taiwan (Lin, et al. 1992) electroplated Ni Cr on steel using the same bath composition as Lin and Ho. The plating of chromium produced cracks if the thickness was over 0.5 µm. The main reason for this was the formation of chromium hydride, which tended to decompose during electroplating. By using pulse-plating Lin, et al. (1992) were able to obtain the release of hydrogen during the off period before it reached the critical thickness of 0.5 µm. They were able to achieve smooth crack-free plating up to 15 µm of up to 65% chromium with a forward pulse of 5 ms and 3 ms at zero current.

### Table 5.5: The bath composition for Ni-Cr alloy plating

<table>
<thead>
<tr>
<th>Names</th>
<th>Chemicals Formulas</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium Chloride</td>
<td>CrCl(_3).6H_2O</td>
<td>100</td>
</tr>
<tr>
<td>Nickel Chloride</td>
<td>NiCl(_2).6H_2O</td>
<td>30</td>
</tr>
<tr>
<td>Ammonium Chloride</td>
<td>NH(_4)Cl</td>
<td>50</td>
</tr>
<tr>
<td>Boric Acid</td>
<td>H(_3)BO(_3)</td>
<td>30</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>C(_3)H(_4)(OH)(COONa)(_3).2H_2O</td>
<td>80</td>
</tr>
<tr>
<td>Fermic Acid</td>
<td>HCOOH, 98%</td>
<td>35ml/l</td>
</tr>
</tbody>
</table>

5.13 PLATING EXPERIMENTS

By using the same bath composition as in Table 5.5 a series of depositions were carried out in a Hull cell shown in Figure 5.12. The pH was held constant at 2.8 so the two variables were current density and temperature. The Hull Cell achieved the variation of the current density so the only parameter that was physically adjusted was the temperature. Equation 5.14 [West, et al. 1991] was used to calculate the current density.
variation along the working electrode (Cathode) for an average current density of 12.5 mA/cm² and the results shown in Figure 5.13.

\[
\frac{i(x)}{I_{avg}} = \frac{x^{1.273}}{L^{0.359}} \left( 1.733 - 0.763 \frac{x}{L} \right) \left( 1 + 0.0659 \left( \frac{x}{L} - \left( \frac{x}{L} \right)^2 \right) \right)
\]

Figure 5.12: The Hull Cell bath in Cartesian coordinates
For a current density of 12.5 mA/cm\(^2\) and a plating temperature of 6-8 °C an alloy composition of 21.2% Cr and 78.8% Ni was achieved. The variation of the Nichrome percentage composition is shown in Figure 5.14.

Figure 5.13: Hull Cell current distribution along the working electrode with average current of 12.5mA/cm\(^2\)
Figure 5.14: Nichrome composition for the current distribution along the working electrode with average current density of 12.5-mA/cm².

Based on the calculated results for plating temperature range of 6-8 °C, an alloy composition of 21.2 % Cr and 78.8% Ni was achieved. This composition was obtained at highest current density in the Hull Cell bath, which was the 43.5mA/cm² with a pH of 2.8.
6.1 INTRODUCTION TO FABRICATION AND ASSEMBLY OF TEST DEVICE

The mold inserts were used to hot embossed several microfluidic channels in polycarbonate substrates. These substrates were then bonded to 250 µm thick polycarbonate sheets to form closed channels. The air pockets were machined from 3 mm thick polycarbonate sheet at depth 1mm as shown in Figure 6.4. These polycarbonate sheets (with air pockets) were cut to specific dimensions as the cover plates. All the cover plates were machined at the Chemical Engineering workshop at Louisiana State University (Baton Rouge La).

The cover plates and the closed micro channels were assembled with heaters and micro fluidic interconnects. The fluidic interconnects used to connect the PCR device to a syringe pump (the source of flow).

For the experiment Liquid crystal would be used to display the steady state temperature profile along the channel. A camera will then be used to capture the image displayed by the Liquid crystal. These images will then compared to the industrial chart to assign values to the different colors displayed by Liquid crystal. The temperature contours obtained from the Liquid crystal were then compared to those achieved from ANSYS simulation to ensure that the predictions from the simulations were valid.

6.2 POLYCARBONATE MOLDING

Embossing of the microfluidic channels was performed on a PHI (City of Industry, CA) press equipped with a machined vacuum chamber. Polycarbonate sheets from (Goodfellow, UK)) of thickness 5 mm and 2 mm were cut into 13.456 cm diameter round
disks. So that it would be fixed in the vacuum chamber. After washing with isopropanol and distilled water the polycarbonate plates were dried with compressed air and pre-baked in an oven at 80°C overnight for 12 hours to drive off excess moisture and residual monomer. The rounds were placed in a vacuum fixture and held in place with an aluminum ring. The mold insert was attached to the opposite side of the vacuum fixture with screws. The complete fixture was then placed between the two platens of the PHI press shown in Figure 6.1 with the side of the vacuum fixture containing the molding tool in contact with the top platen and the polycarbonate disk in contact with the bottom platen. The press was closed with enough pressure for both sides of the vacuum fixture to be in contact with the platens.

The press was heated with different temperatures applied to the platens. The upper platen with the mold insert was heated to 190°C and the bottom platen with the polycarbonate disk to 90°C. When heated 850 lbs force was applied for 5 minutes and then the part was demolded. After molding, the polycarbonate plates were placed between two glass plates and kept in an oven at 130°C for one half hour, then allowed to slowly cool at 2°C/min to room temperature. This annealing process was used to reduce the residual internal stress in the polycarbonate chips.

SEM photos of the molded polycarbonate chips for both the test device and the spiral device are shown in Figures 6.2 and 6.3, respectively. The sidewalls of the channel were straight and smooth but the bottom of the channels is relatively rough because of the sandblasted surface of the mold insert.
Figure 6.1: PHI press equipped with a machined vacuum chamber used for hot embossing of the microfluidic channels

Figure 6.2: SEM of molded the polycarbonate using straight channel mold insert
6.3 POLYCARBONATE BONDING

A polycarbonate sheet (Goodfellow, UK) of thickness 0.25 mm was cut into a 12.7cm diameter disk and used to seal the microchannels. Before bonding, two 0.25 mm diameter holes were drilled through the molded disk at the inlet and outlet positions of the device for the assembly of the fluidic interconnects. The molded disk and its 0.25 mm disk cover plate were thermally bonded together to form the closed channels.

Before bonding these disks were ultrasonically cleaned in distilled water for 1 hour, then rinsed thoroughly with distilled water and dried with compressed air. Immediately before bonding, the two bonding surfaces were coated with isopropanol and dried with compressed air. The embossed substrate and its cover were clipped between two highly polished glass plates and placed in a convection oven. The optimized bonding conditions for the polycarbonate chip were heating for 30 minutes at 150°C, followed by a slow cool down to room temperature a rate of 5 °C/min. The enclosed micro fluidic channel was now complete.

Figure 6.3: SEM of molded the polycarbonate using spiral channel mold insert
6.4 FLUID INTERCONNECTS

The two 0.5 mm diameter holes that were drilled in the molded disk were the inlet and outlet ports for the chip. Polycarbonate female adapters with 5 cm inside diameter were designed and built for the connection of the fluidic assembly. These adapters were bonded to the device with epoxy. Fluidic assemblies from Upchurch Scientific (Oak Harbor, WA) were used to connect capillaries (Polymicro Technologies, Phoenix, AZ) of 20 cm length, 75 µm i.d. 375 µm o.d to the device. A syringe pump with a syringe-to-capillary adapter was used to make the connection between the pump and capillary from the device. This syringe pump (Harvard 22, Harvard Apparatus, Holliston, MA) was used to pump the analyte through the microchannel during experiments.

6.5 ASSEMBLY OF THE TEST DEVICE

Commercial thin film, resistance heaters (5 W, 24 VDC, K010030C5, Watlow, Columbia, MO) were used to provide the heat fluxes to maintain the temperatures in the isothermal zones of the PCR device. Theses heaters were fixed in their respective positions with high thermal conductivity element (Omegatherm 201, Omega, Stamford, CT). The cover plates with air pockets were place directly over their respective heaters and fixed in position with mechanical clips. Figure 6.4 is a drawing of the cover plate with air pockets.
6.6 CONTROL SYSTEM CIRCUIT ASSEMBLE

The heaters were connected to a control circuit which consist of; (1) computer to measure on collect data of the transient response of the system, (2) electrical power supply, which power the heaters, (3) (PID) controllers to control the temperature in each isothermal zone, (4) temperature sensors (thermocouple) for the closed loop feedback as shown in Figure 6.5.

The control circuit consists of a power supply, thermocouple, electrical relays, PID controllers and thin film electrical resistive heaters as shown if the schematic of Figure 6.6. K type thermocouples (HYPO-33-1-T-G-60-SMP-M, Omega, Stamford, CT) were used for temperature feedback to the PID controllers (Series 96, Watlow, Winona, MN).
The controllers were equipped with relays that were used to regulate the current to the heaters during the temperature control process (see Figure 6.6).

![Control Circuit Diagram](image)

Figure 6.5: A schematic diagram of the control circuit used to control the temperature of the PCR device.

After the test device was assembled it was coated with liquid crystals (LC) (Davis Liquid Crystals, San Leandro, CA). These were used to measure the temperature distribution along the channel and completely characterize the thermal performance of the device. LC thermography is a temperature measurement method that uses the color-temperature response of thermochromic liquid crystals (TLCs) to determine the actual temperature distribution along the PCR channel.

The activation temperature and bandwidth of a TLC formulation are determined by its chemical composition at the time of manufacture. The TLC activation temperature is the temperature at which a TLC will begin to reflect visible light. The temperature
bandwidth defines the relative color response range for the TLC formulation. TLC formulations with activation temperatures ranging from -30°C to 120°C and bandwidths ranging from 0.5°C to 30°C are commercially available, (See Davis Liquid Crystals, San Leandro, CA) and (Hallcrest, Inc., Glenview, IL) for more detailed information).

Two separate batches of liquid crystals were purchased from Davis Liquid Crystals, San Leandro, CA of temperature ranges of 53.4 °C to 75 °C and 74 °C to 95 °C respectively. For the temperature transition from 55 °C to 95 °C the surface will be coated with both set of liquid crystals. Because both set of liquid crystals have to different response time the CCD camera will be used to capture of the heated surface for the two separate temperature displayed. The other two temperature transition 55 °C to 72 °C and 72 °C to 95 °C liquid crystal of temperature range 53.4 °C to 75 °C and 74 °C to 95 °C will be used respectively. The image would be compared to the standard commercial chart for the actual temperature measurement.

In the future liquid crystal would be used to display the steady state temperature profile alone the channel. A camera will then be used to capture the image displayed by the Liquid crystal. These images will then compared to the industrial chart to assign values to the different colors displayed by Liquid crystal. The temperature contours obtained from the Liquid crystal were then compared to those achieved from ANSYS simulation to ensure that the predictions from the simulations were valid.

The only experiment that was performed for the thesis was for the transient temperature response of the system. The temperatures were plotted against the simulated results for comparison (see Figure 6.7). The experimental results showed a time lag which resulting from the thermal capacitance of the heaters that was neglected in the
numerical simulation. Experiment for the steady state temperature contour using liquid crystals will be carried out at a later time.

6.7 EXPERIMENTAL SET UP AND RESULTS

Figure 6.6: A schematic diagram of the experimental setup to test for the transient response and capture the steady state temperature contour of the PCR device.
Figure 6.7: The comparison between simulated and experimental transient temperature response
CHAPTER 7: DISCUSSIONS AND CONCLUSION

It was showed that it was possible to fabricate a PCR device from polycarbonate, which produced amplification comparable to the conventional laboratory-scale device. An analytical tool was developed to predict the temperature distribution for this specific continuous flow PCR device configuration. By using air pockets it confirm by the simulation that it was possible to achieve a relatively uniform temperature zone of tolerances $\pm 5 ^{\circ} C$ the design value for an average fluid velocity of 2mm/s. This simulation tool showed that the thinner the device the easier it is to obtained the desired temperature profile. The simulation tool was used for parametric studies and prediction of the device performance for increasing fluid velocity.

Different materials were investigated for their workable temperature range and formability and polycarbonate was selected based on its ability to be used for mass production and working temperatures of up to 148 $^{\circ} C$. The polymer chips have better mechanical properties than the silicon and glass chips and can be mass-produced.

The heating and cooling rates of most microfabricated PCR devices depends on their thermal capacitance, which dictate the PCR reaction time. Kopp in (19980 showed that continuous flow PCR devices were independent of their thermal capacitance because they were used at steady state temperatures. The thermal cycle for a continuous flows PCR device depend on the device configuration, the thermal capacitance of the fluid and velocity.

Since high speed processing is a necessity for clinically useful devices a series of finite element simulations using ANSYS/CFD-FLOTTRAN (vers. 5.7, ANSYS, Inc., Canonsburg, PA) was performed to evaluate the effects of increasing fluid velocity. It
was observed that as the speed increases the fluid spends more time in transition than in the temperature zones. As the fluid velocity increases another trend was observed, the entire fluid stream was converging on an average temperature of 74 °C. For a fluid velocity of 5 mm/s, no heat input was needed to achieve temperature of 60 °C in the annealing (55 °C) zone. This meant that at fluid velocity of 5 mm/s and higher it was not possible to control the temperature for annealing, because there would be no control over the thermal power input. The maximum recommended mean velocity for this particular device configuration is 4 mm/s with maximum velocity at the center of channel to be 7.5 mm/s, which result in a reaction time of 15 sec per cycle. At this speed it was possible to maintain temperature within ± 5 °C of design value in each zone. The comparison of the temperature along the wall of the channel and its center shows a maximum difference of 1.3 °C. This is the maximum lateral temperature variation in each temperature zone for an average fluid velocity of 4mm/s.

Simulation results showed that single-sided heating produced an adequate temperature profile, but that it has a thermal transition length approximately twice that of double side heating. Operating the PCR device at average velocities higher than 2 mm/s would increase the magnitude of the thermal power requirement. Leading to an increase in the temperature beneath the heaters, which may get too close to the polycarbonate glass transition temperature. The main disadvantage of single-sided heating is that as the velocity increase the fluid will always be in transition rather than reaching a steady state temperature. Two-sided heating is recommended for velocities greater than 2 mm/s for this device configuration.
By using X-Ray LIGA to fabricate the mold inserts straight and smooth sidewalls were achieved. These mold inserts were used to emboss multiple devices. The mold inserts were fabricated of nickel and steel they are relatively durable, which may permit mass production of the devices. SEM photos of the microchannels produced by X-Ray LIGA mold insert shows that the quality is much better than that produced by UV LIGA mold insert.

Based on the similarity of the experimental and analytical transient temperatures response, the assumptions made during the system dynamics simulation were validated. The controllers and heaters that were chosen to maintain the temperatures in the zones were appropriate for this application. This device can complete a PCR reaction as fast as 15 sec/cycle at volume of 0.45 µl / cycle.

7.1 RECOMMENDATIONS AND FUTURE WORKS

The heating element should be deposited directly on the polycarbonate this will help to ensure that uniform heating is achieved. It will also reduce the effect of the thermal capacitance of the heaters. Depositing the heating element directly on the polycarbonate cover plate will increase the accuracy of the approximation of one-dimensional heat flow. Improvement in the present molding process so that thinner polycarbonate can be hot embossed would also help in achieving one-dimensional, heat flow. The device should be tested for speed of 4 mm/s to determine what amplification would be achieved at this velocity.

To improve the speed of the device, the first stem would be to use a smaller footprint to construct the device. This will shorten the channel length, resulting in a
shorten cycle time. Development of fluidic interconnects for the attachment of the PCR to the LDR device and simulates their temperature response.
REFERENCES


http://www.accessexcellence.org, November, 14, 2002


Ivonne Schneegass, Reiner Brautigam and Johann Michael Kohler, “Miniaturized flow-through PCR with different template types in a silicon chip thermocycler”


APPENDIX A: A SCHEMATIC OF THE FABRICATION PROCEDURES USED TO MAKE THE GRAPHITE X-RAY MASK

Spin coating of 40 µm SU 8-25

UV Light

Optical Cr Mask

Development of SU 8-25

15 µm Gold Electroplating At 4 µm per hour & 65°C
APPENDIX B: SCHEMATIC OF THE FABRICATION PROCEDURES USED TO MAKE THE TWO MOLD INSERTS USING THE X-RAY MASK

- Resist PMMA
- Steel Plating Base

X-Ray Radiation

- X-Ray mask

Exposed Resist

- Developed Resist

Electrodeposition of Nickel which was Lapped and polished

Flood exposure and Resist Development to produced Mold Insert
APPENDIX C: A COMPLETE DIMENSION DRAWING OF THE TEST DEVICE

[Diagram showing dimensions and labels for cover plate with air pockets and optical mask pattern]
Michael Mitchell was born on May 12, 1974, in Clarendon Jamaica. He received his Bachelor of Science degree in mechanical engineering in December of 2000 from Louisiana State University, Baton Rouge La, USA.

He joined the graduate program in mechanical engineering at Louisiana State University in 2000 as a member of the Microsystems Engineering Team with the Mechanical Engineering Department and started working on this project in June 1999. He expects to receive the degree of Master of Science in Mechanical Engineering in December 2002.