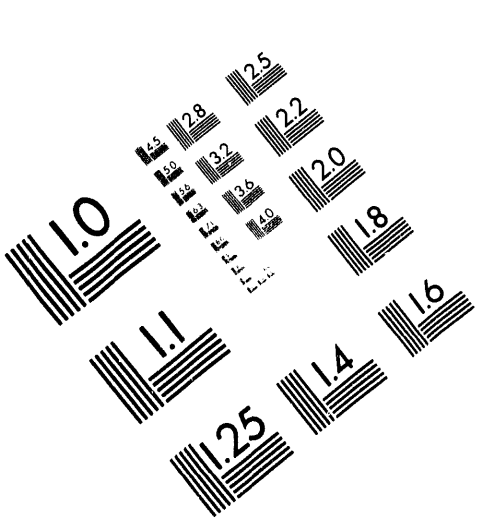

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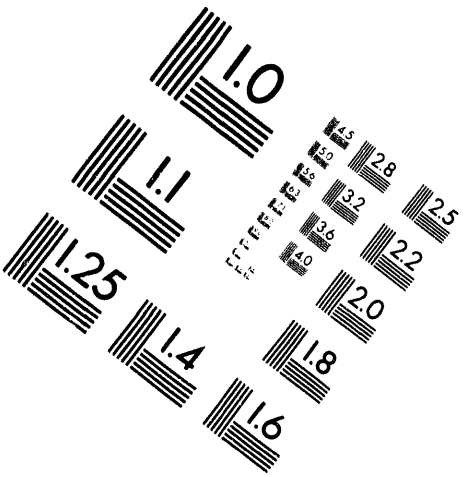

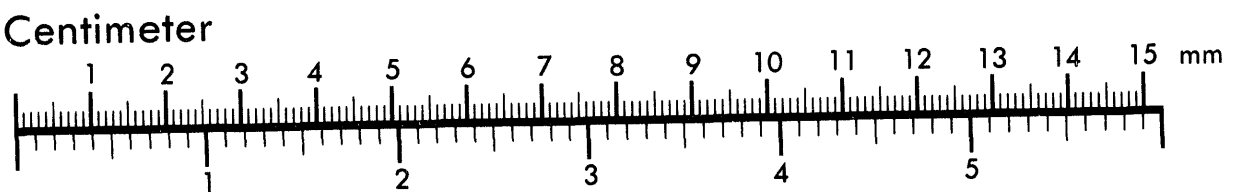

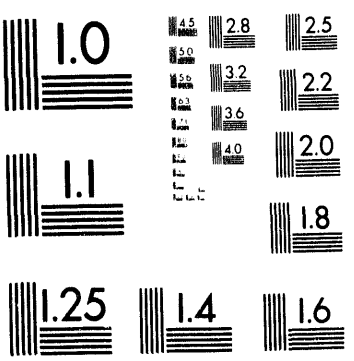

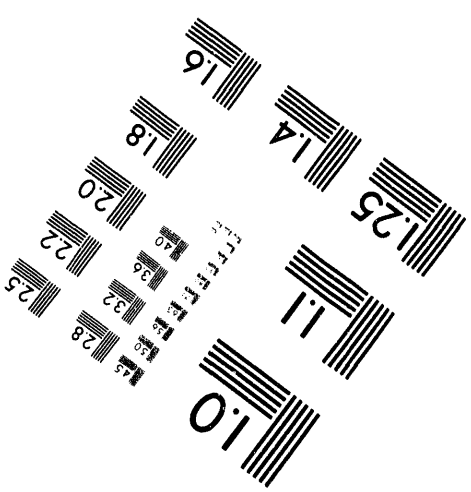

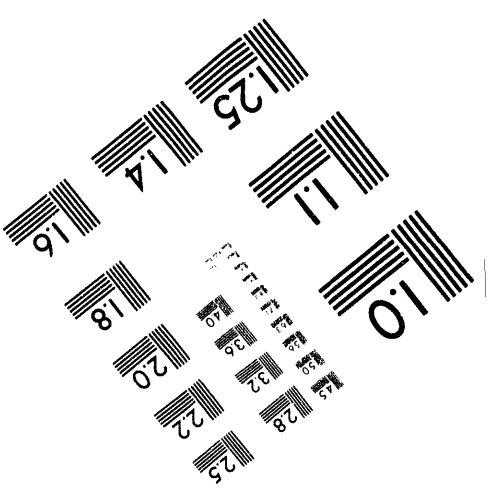

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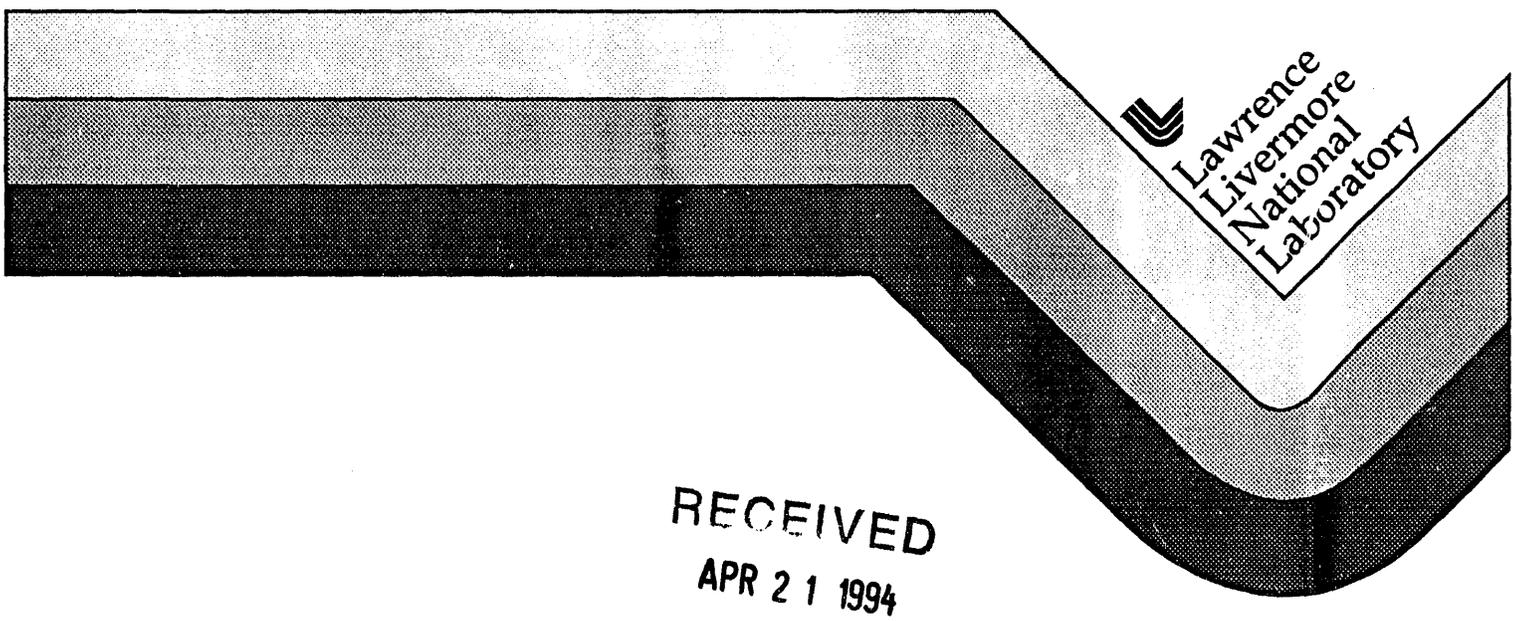
Microfabricated DNA Analysis System

Semi-Annual Report

M. Allen Northrup

January 1994



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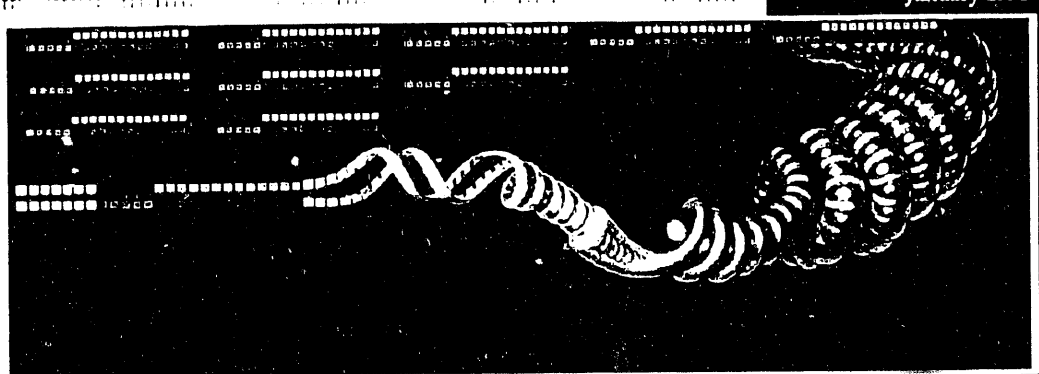
Microfabricated DNA Analysis System

Semi-Annual Report
January 1994



Covering the period:
August 1993 - January 1994

By
M. Allen Northrup Ph. D.
Principal Investigator
Engineering Research Division
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P.O. Box 808, Livermore, CA 94551

Submitted to: Microelectromechanical Systems Program
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3701 North Fairfax Drive
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ARPA

Table of Contents

Executive Summary

We are miniaturizing instrumentation for the polymerase chain reaction (PCR) - a bioanalytical technique that amplifies target sections of DNA through thermal cycling. This first report, representing 5 months of a 3-year project, focuses on delineating reaction chamber design parameters through computer modeling and infrared imaging. We have also continued micro-chamber-based PCR experiments and have successfully amplified three different targets from two different biological sources. Specifically, we have amplified DNA from ß-globin (a subunit of hemoglobin), and two different-sized targets from the Human Immunodeficiency Virus (HIV). These experiments were performed in newly-designed chambers. Reaction chambers are also being designed with detection in mind, specifically for the detection of fluorescent DNA labels. Finally, some new developments in PCR technology are described as they represent potential biological diagnostics to be evaluated in microfabricated DNA analysis systems.

Microfabricated DNA Analysis System

Progress Report No. 94. 1

Period Covered: August 1993 - December 1993

M. Allen Northrup, Ph.D.
Lawrence Livermore National Laboratory
Engineering Research Division
L-222, POB 808
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Introduction:

The design and implementation of a microfabrication-based, miniaturized analytical instrument is a challenging task. In the present project we have chosen to pursue the miniaturization of a bioanalytical system that is relatively simple; that is, the instrumentation is inherently basic, yet the power of the analysis is significant. Specifically, we are miniaturizing the instrumentation associated with the polymerase chain reaction (PCR). The instrumentation that drives this analytical technique is centered around a reaction chamber that provides thermal cycling conditions for the synthesis of specific pieces of DNA. It is the power of the PCR technique, as implemented in a miniature instrument, that will provide the significance in analyses.

This first report, describing the initial 5 months of effort under the 3-yr ARPA contract, will outline the research effort for the first year, provide a brief description of the PCR technique, describe previous results in a silicon-based reaction chamber, provide first modeling results of systems being considered for micro-reaction chamber design, show several examples of infrared images of different thermal cycling reaction chambers, discuss detection concepts, show new DNA amplification results from micro-chambers, and explain several potential new biological systems for analysis.

Background:

The Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a biochemical technique invented in 1985 at Cetus Corporation as a means to amplify, to over a billion-fold, specific sections of target genetic material (i.e., deoxyribonucleic acid, DNA; or ribonucleic acid, RNA) from biological sources.

PCR provides a highly specific means to identify a biological entity from minute amounts of original sample. The primary alternative method for the identification of pathogens of biological origin include the culturing of infected materials and microbiological identification through a series of tests. The cell culturing technique can involve several days to weeks to obtain sufficient identifying data. Other methods of pathogen identification involve the identification of symptoms in an infected individual or the use of antibody-based tests in the blood sera of such individuals. The PCR technique has been identified as nothing less than revolutionary in the promise that it has realized and continues to realize in the identification of biological agents. Its application spans the range from environmental to clinical analyses. PCR allows the rapid and unequivocal identification of pathogens in ambient samples far in advance of the onset of symptoms or the growth of sample colonies.

For a detailed description of the PCR technique, the reader is referred to a recent book entitled Diagnostic Molecular Microbiology: Principles and Applications, by D.H. Persing, T.F. White, F. C. Tenover, and T.J. White (eds),1993. A physical description of the process is provided here (Figure 1). All living organisms contain genetic material such as DNA (or RNA). DNA is a double-stranded, long-chain polymer made up of alternating sequences of four different DNA building blocks called nucleotides. The particular sequence of these bases is the genetic basis of individual and species identification. Each chain is complementary to the other providing two copies of that individual's genetic code. The two strands are held together by thermally-labile hydrogen bonds. The polymerase chain reaction involves the repetitive denaturing (separation of the two strands at high temperature) and cooling (annealing of the strands). The polymerase enzyme will rebuild

targeted pieces of DNA during the heating and cooling cycles of the reaction. The following reagents are required for the rebuilding process: an excess of the four nucleotides; the thermally-active polymerase enzyme; two small pieces of DNA that bracket a specific, species-identifying section of the target DNA (oligonucleotide primers); the target DNA from the organism to be identified; and various cofactors. This repeated cycle will cause the enzyme to identify, copy, manufacture, and verify that part of the target DNA that lies between the primers. This extension part of the process usually occurs in the intermediate part of the thermal cycle (i.e., around 60-70 °C). Each thermal cycle produces a doubling of the target (first cycle) and then copies of the copies, leading to a 2^n (n equals number of cycles) multiplication of the identifying target sequence.

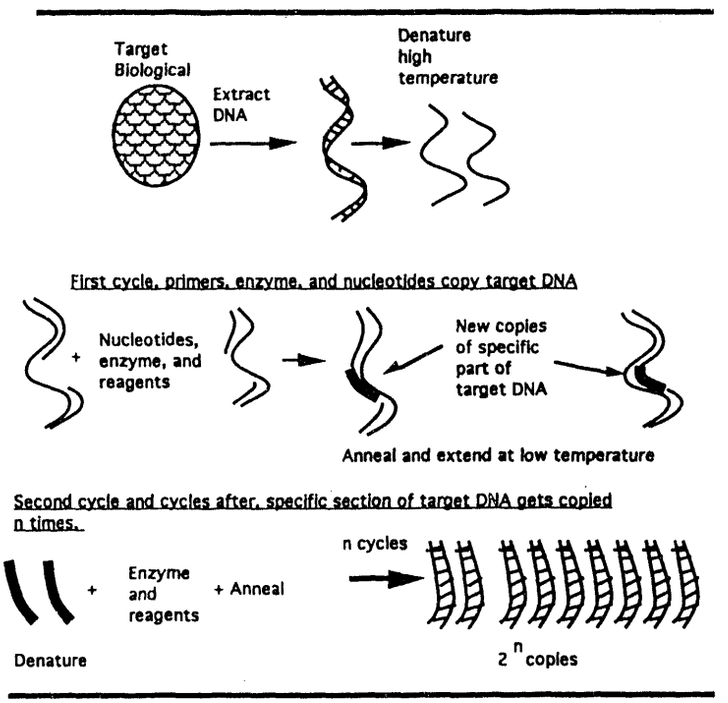


Figure 1. Schematic diagram of PCR methodology for amplification of target DNA, for details see text.

This amplification process brings minute undetectable amounts of target DNA to millions or billions of copies after 20 to 40 cycles which can then be detected. This entire process typically involves 30 to 40, 2-minute thermal cycles, and a 1-hour electrophoretic separation (i.e., total analysis time is about 3 hours). Present instrumentation for this process (Perkin Elmer Model 9600) is a 120

VAC, 12 A, thermal cycling instrument that is 12 in. by 20 in. by 24 in. and weighs 90 lbs. The heating rate specification of that instrument at 120 VAC is 1.0 °C/s. It can handle up to 96 reaction test tubes at once.

Standard PCR Product Detection Techniques:

Standard identification of the amplified DNA involves slab-gel (agarose) electrophoretic separation and staining with ethidium bromide (ETBr). ETBr intercalates (adsorbs between the coils if the DNA strands) into the amplified DNA which enhances it's fluorescence (UV or Visible excitation, infrared emission). The electrophoretic separation provides verification that the size of the target piece of DNA is the size expected. Transillumination of the gel with ultraviolet light is usually used to fluoresce the electrophoretically separated PCR products. Black and white photographic images of the stained gel is the typical record of verification. Electrophoretic equipment is typically tens of inches on a side and requires 110 V, 300 mA power supplies.

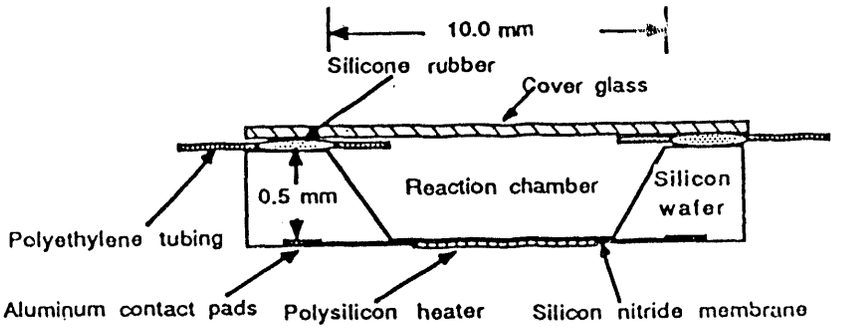
Other efforts are on-going to develop real-time detection of PCR product synthesis. One example utilizes the ETBr fluorescence technique to monitor DNA production in a real-time scenario by imaging the fluorescence intensity of the ETBr as it intercalates into the PCR products after each cycle (Higuchi et al, 1993). This real-time detection scheme may be adaptable to the micro-reaction chamber.

Previous Supporting Results:

Various experiments were performed in conjunction with Roche Molecular Systems in support of the present project over the past year and one-half. These experiments and results are described in Northrup et al. (1993), but will also be outlined here: First, a series of biocompatibility studies were performed to verify that the silicon-based materials being used in the reaction chamber did not inhibit the reaction. Then, miniature silicon-based reaction chambers were fabricated with integrated polysilicon heaters and temperature control. These chambers were used to perform PCR amplification of a 142 base pair sized target that is specific for the detection of the Human Immunodeficiency Virus (HIV). The reaction

products from the prototype micro-reaction chamber (Figure 2a) were compared to a commercial PCR thermal cycling instrument and verified with standard gel electrophoresis (Figure 2b). The PCR cycling time was reduced by a factor of 4 in these experiments with the micro-device over commercial instrumentation and power consumption was 0.5 W for the entire circuit including the controller. The heating rates for the microdevice-based reaction chamber far exceed those of commercial instruments and the potential for speeding up the PCR analyses from hours to minutes is a reality. We have found heating rates of over 35°C/sec for 25 μL volumes of reaction mixture, the Model 9600 commercial instrument specifies a rate of 1°C/sec. These rates will further increase with the incorporation of dual heaters, for example.

a)



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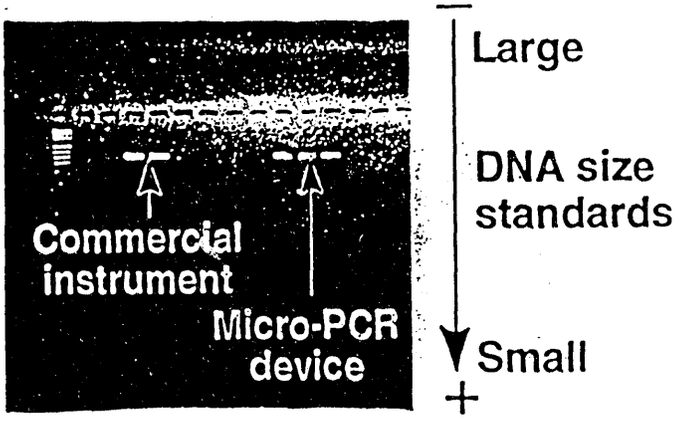


Figure 2. a) Prototype micro-PCR reaction chamber consisting of a silicon chamber with silicon nitride membrane and polysilicon heater. A glass coverslip and silicone rubber was to seal the chamber. Polyethylene tubing was used for reactant and product input and output (note drawing is not to scale). b) Gel electrophoretic results indicating that micro-reaction chamber produced same results as commercial instrument in one-quarter the time. Analyses was from an HIV-specific target.

First Year Plans and Recent Accomplishments

First year goals:

In this first year, we expect to continue the solidification of the infrastructure, personnel, and collaborators of this project. We are bringing on board postdoctoral fellows, biologists, and engineers for the project. As well, we are establishing our collaborative work with the University of California campuses and the Armed Forces Institute of Pathology. As well we are defining our cooperative efforts with Roche Molecular Systems of Alameda, CA., and Perkin Elmer Corporation of Foster City, CA and Norwalk, CT. These companies will play a critical role in the productization and commercialization of the technology in this project.

Technically, the first year of the project is focused on the implementation and refinement of the reaction chamber, and will begin investigation and development of the detection and fluidic systems. We expect to provide background information on the associated techniques of the research, demonstration of the miniaturized reaction chamber, modeling of the thermal characteristics of the chamber, fabrication designs for the reaction chamber, confirmation of the biocompatibility of the device, and verification of the reaction with standard detection techniques. Several biological systems will be the test species for the PCR microinstrument. Examples will include HIV, ß-globin, and others to be determined. A prototype of the PCR reactor and a potentially integratable detection scheme will be demonstrated at the end of year 1.

Specifics of first year technical goals:

Modeling:
Modeling is particularly useful for highly miniaturized devices to provide an additional means of analyzing experimental results in the fabricated devices. Direct measurements of performance variables such as local temperature, pressure, and concentration are usually not available to allow diagnosis and interpretation of results. The design variables include chamber geometry, heater geometry and

locations, heater power and waveform, chamber materials, and device packaging. Critical to the reaction chamber heating design is the ability have integrated temperature sensing and feedback to avoid overshooting, heat excursions, and to ensure precise control of cycling temperatures.

Modeling of the heat and mass transfer in the reaction chamber and the experimental verification of these models are being performed in order to augment the design of optimal devices. This is important to the successful application of these devices to PCR, since temperature profiles are critical to reaction efficiency. It is known, for example, that the upper temperature of the PCR methodology should be reached quickly and accurately. Overshooting or spending extended times at this upper temperature can be damaging to the enzyme, and therefore will degrade the reaction.

Optimal design specifications of the reaction chamber must be developed, including temperature cycling range, cycling time, accuracy, uniformity, and repeatability. Considerations of the heater materials must also be considered. For example, silicon, with its relatively high thermal conductivity, may or may not be able to provide the specifications for the reaction chamber within the power limitations of portable power sources such as batteries. In order to obtain sufficient bulk heating, for example, areas on the heater element may become too hot. Unique placement of the heater elements in the reaction chamber is one possible solution to some of the thermal limitations of resistive heating.

Once mass and heat transfer, and temperature control have been characterized in the reaction chamber, detailed studies of heating rates versus reaction efficiency can be performed. From these studies, the usefulness of or need for active pumping versus convective mixing can be determined.

Recent Accomplishments

Modeling Results:
The thermal conductivities of the chamber materials can be used to augment the temperature changes and/or stability of micron-sized chemical reaction chambers. For example, representative thermal conductivities of crystalline silicon is 150 W/m °K and silicon nitride is 19 W/m °K. Therefore, a thin film of the silicon nitride can act as a thermal insulator, just as the silicon can be used as

heat sink. Computer-based, finite element analysis of several reactor designs have been performed using LLNL's TOPAZ 2D modeling software.

Specifically, for the initial analyses, three designs have been modeled in order to understand and predict the thermal characteristics of actively heated, and both passively and actively cooled silicon-based microreaction chambers. The first one assumes that the silicon will be used to help transfer the heat into the reaction liquid. The specific design is proprietary, but the results of modeling its thermal cycling capabilities will be described.

Model 1: Silicon as a heat sink:

For this analysis, speed of heating was the parameter that was maximized and therefore there was no attempt to limit the power. We did this in order to understand and predict how fast it would be possible to cycle an optimized silicon-based reaction chamber. The model was performed on the smallest symmetrical unit of this design, i.e, one-half of one chamber. The parameters for this analysis were: 1) The power was ramped from 0 to 60 W per cm^2 in 0.6 sec. 2) boundary condition of 5000 W/m^2 at the heater, 3) airflow rates in actively cooled model of 0.6 L/min, and 4) reaction volume of 50 μL. This system modeled was without cooling but with heating (a) and with active cooling (b) (air flow). It was determined that in (a) the maximum temp (106 °C) was reached in 0.6 sec. In (b), 91.5 °C was reached in 2.2 sec. Cooling was also modeled by exploring the effect of flowing air in an adjacent chamber. With active cooling, ambient temperature (19.6 °C) was reached from over 100 °C in 3.2 sec.

Model 2: Heater isolated from Silicon.

Two variations of analyses (Figures 3 and 4) were performed for this model, one assuming a single heater with a glass top and sealed with silicone as in previous results (Figure 2), and the other with dual heaters (Figure 5).

The single heater device was further modeled in two ways: one assuming a constant heater temperature of 96 °C (Figure 3), the

other with a heat flux similar to the previous experimental device (0.64 W per 1 cm^2) (Northrup et al, 1993). The latter device model included convective losses (1.9 W/m^2 °K) to the air surrounding the exposed silicon. The sizes and dimensions of the model devices are also on the figures. The models show one-half of a 1 cm x 1 cm x 0.5 mm silicon reaction chamber with a 1.5 μm thick, low stress, silicon nitride membrane, doped polysilicon heater on the membrane, 1 mm thick silicone gasket, and 0.5 mm thick glass cover slip. The double heater device is two of the above silicon devices bonded together by a 50 μm thick layer of polyimide.

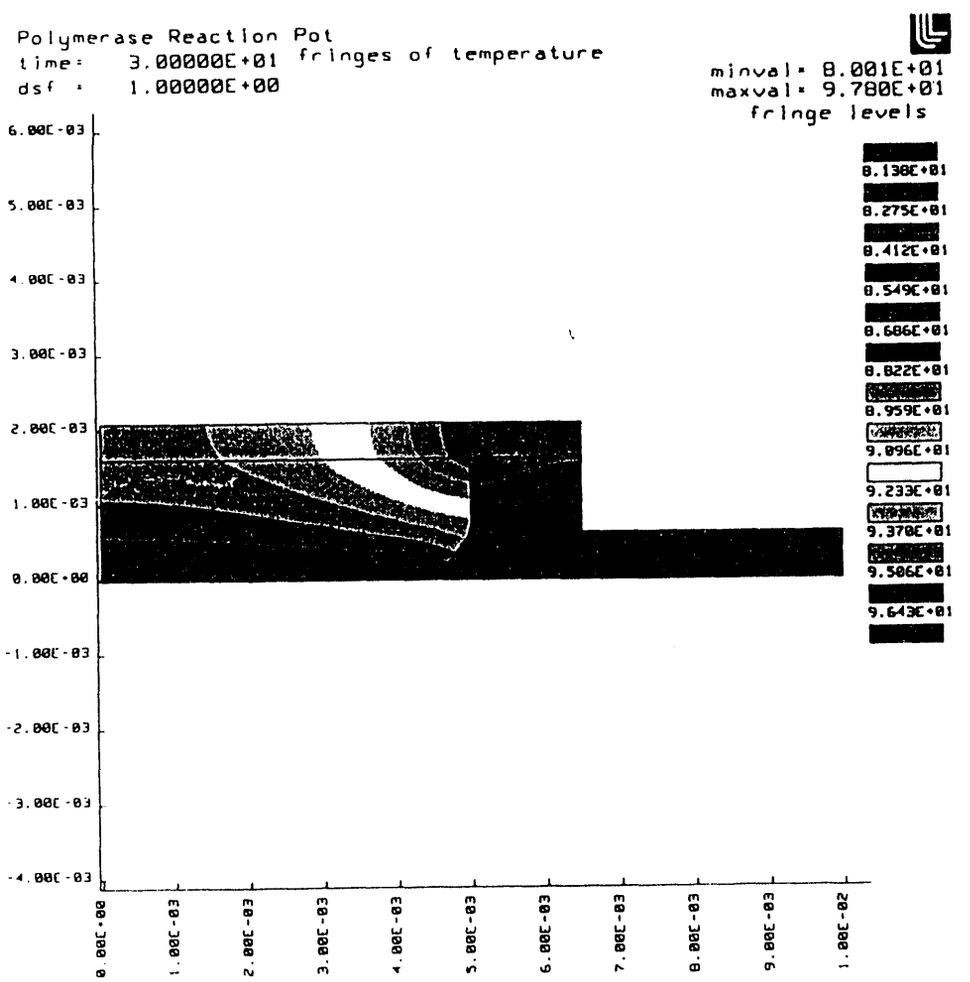
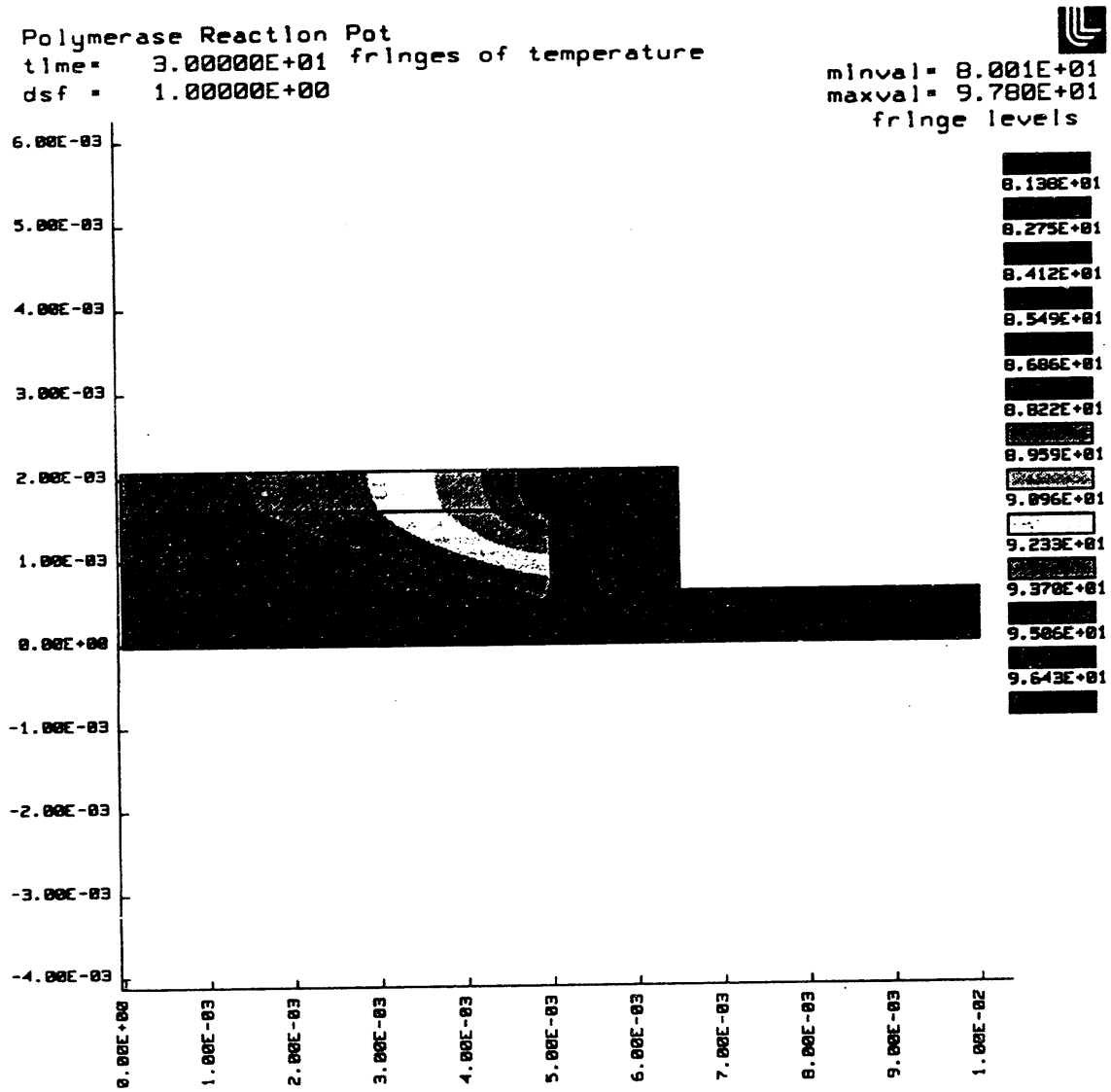


Figure 3. Thermal modeling results from single heater and glass top. This plot assumes a constant temperature at the heater (96 °C). The temperature fringes shown are after 30 secs. Maximum temperature reached was 96.3 °C.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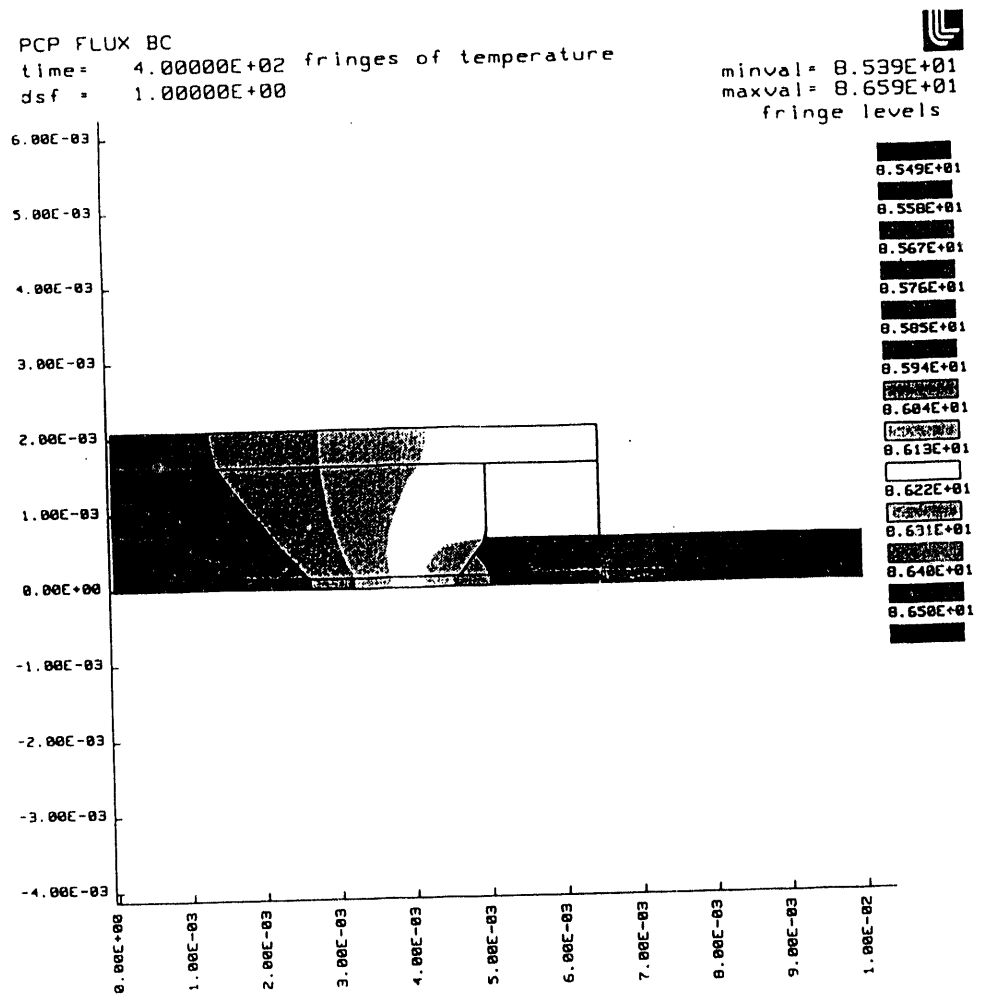
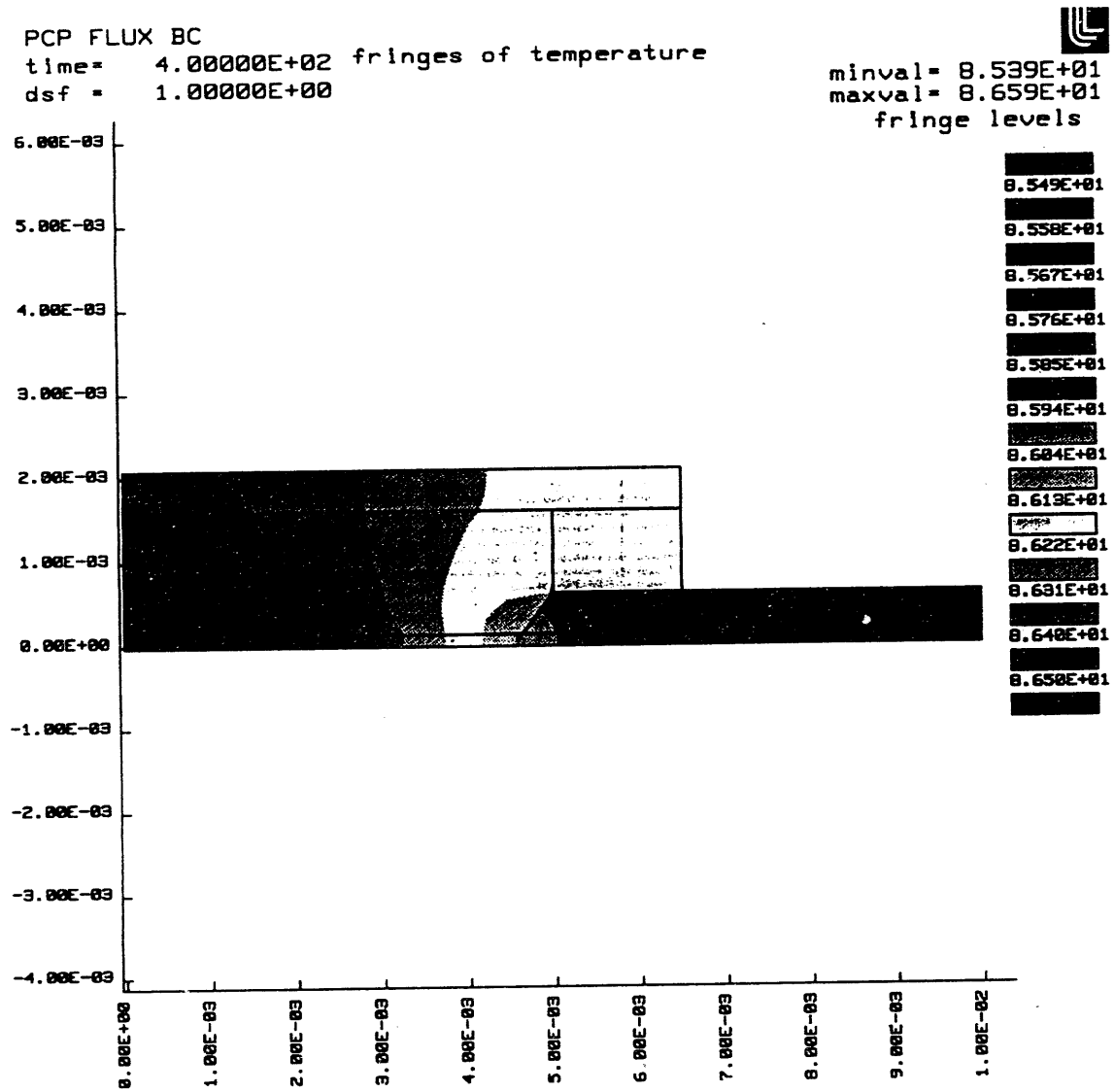


Figure 4. Temperature fringes of the single heater device with a heat flux (0.64 W/cm^2). The resistance of the polysilicon heater was 20 ohms and the current was 0.180 amps. It was also assumed that 100 % of the heat went into the silicon nitride membrane and convective loss to the air of 1.9 W/cm^2 °K. These contours were reached in 400.0 secs. The maximum temperature reached was 86.5 °C.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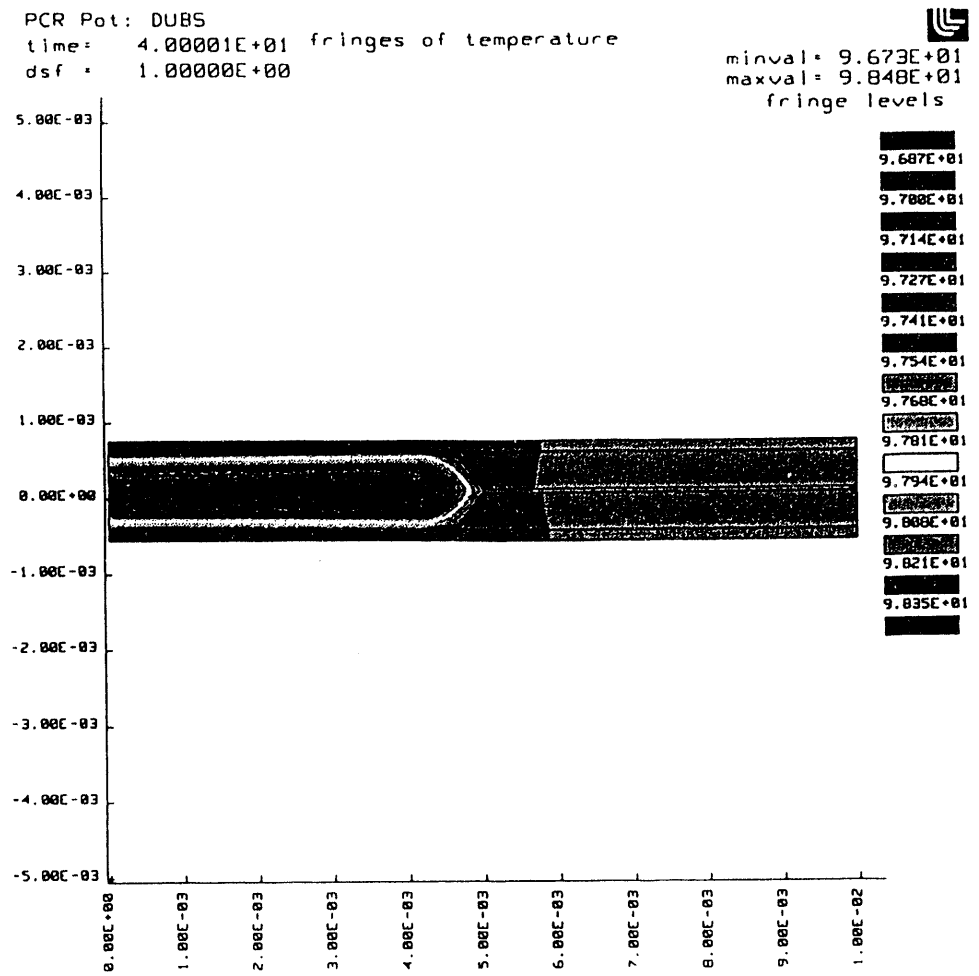
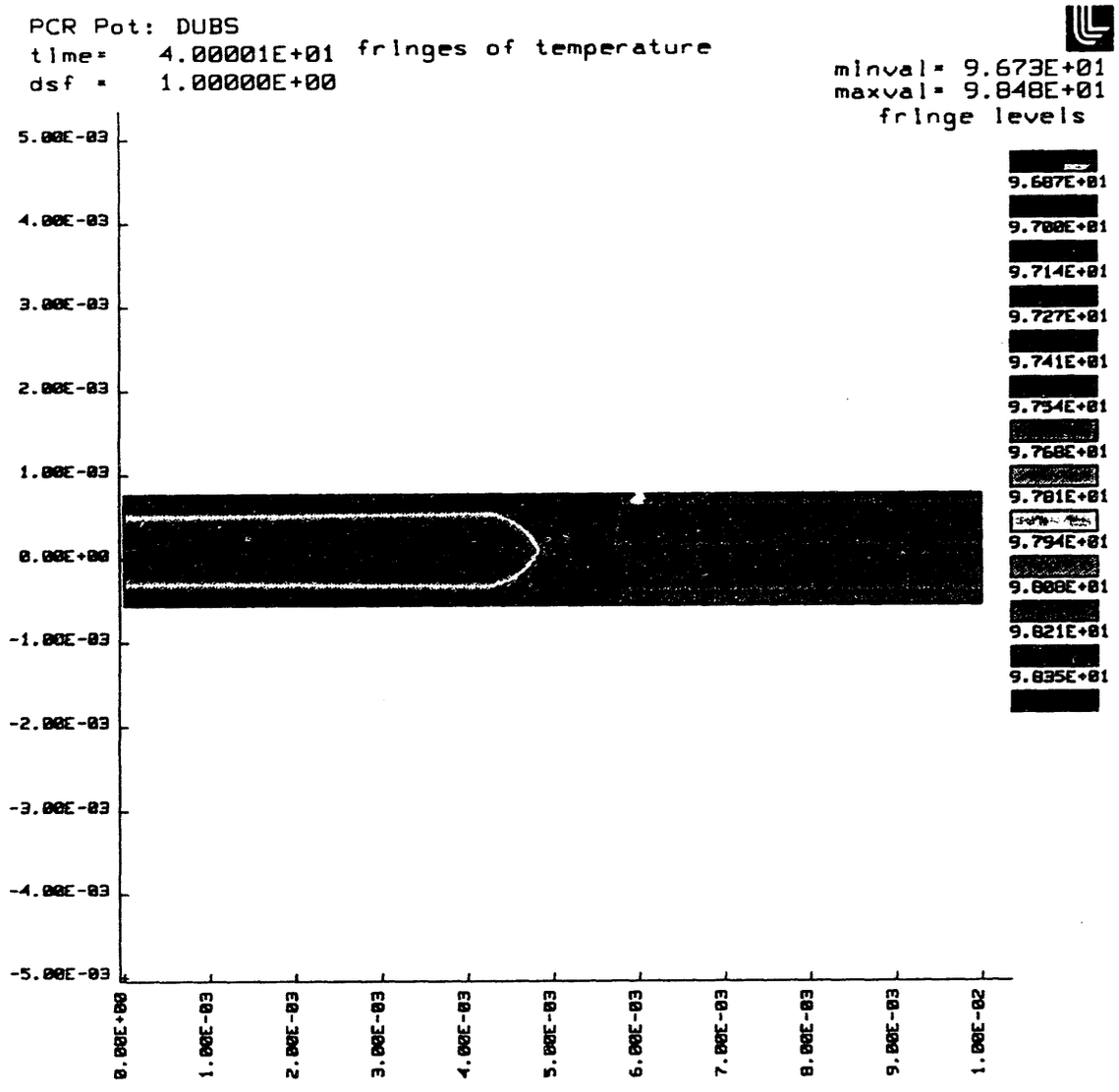


Figure 5. Double-sided heater design temperature fringes (max temp 98 °C) after 40 sec from 20 °C. Power to the heaters was 1.5 W. This model used a heater flux, rather than a constant temperature, and no convective losses.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Summary of first modeling results:

Modeling was performed on several reaction chamber designs ranging from power intensive with potentially extremely rapid thermal cycling capabilities to low power single and double heater designs. These do not cover all the possibilities, but provides a first analysis of what thermal cycling rates may be achievable. For example, the power intensive, silicon as a heat sink design, without active cooling such as flowing air can reach 96+ °C in 0.6 secs from ambient air, or over 126 °C per sec. With active cooling (air flow at 0.6 L/min) this design can achieve the same temperature in about 2.2 secs or over 34 °C per sec, and cooling back to 20 °C in 3.2 secs. Therefore a typical complete cycle (55 to 95 °C and back to 55 °C) could be performed in approximately 3 secs. These types of cycling speeds could be too fast for the polymerase enzyme, but would allow detailed reaction kinetics studies. It is expected that aspects of this geometry will be incorporated in low power micro-reactor designs.

As expected, thermal models of the low power, isolated heater designs indicate slower cycling speeds. These speeds are still faster than commercial, high power devices, however. For example, the double-sided heater design reaches over 96 °C from 20 °C in 40 secs, or nearly 2 °C per second at 0.64 Watts utilizing 0.180 amps of current at approximately 12 volts. Commercial instruments such as the Perkin Elmer Model 9600 draws over 12 amps (120 VAC), and heats at about 1°C per second. Of course, the 9600 has 96 wells for reaction vessels and the silicon device as described has only one. For medical diagnostic PCRs, however, only a few wells are actually ever used at once.

Thermal uniformity is also of significance for the PCR technique. It appears that both the silicon heat sink and double-sided heater designs provide good uniformity, however further analysis of this important parameter will be performed.

Through some preliminary infrared imaging of some early designs (single heaters) during heating has revealed very important information about uniformity and the usefulness of single-point thermocouple measurements. These are described in the next section.

Thermal Images of Reaction Chambers:

An infrared (IR) camera and video recording system (AGEMA Thermovision Model 870) was used to obtain images of sealed, 1 cm^2 by 0.5 mm and 0.25 cm^2 by 0.5 mm silicon/silicon nitride, single-heater, reaction chambers during thermal cycling. These images provide critical information about temperature uniformity in these devices, and the role of insulation during PCR experiments. The IR images in Figures 6 - 9 are a few examples of results that show the effects on reaction chamber temperature uniformity by: a) no thermal isolation, b) air bubbles, c) heater material, and d) thermal isolation of a device. Note that the colors represent different temperature ranges in each image and are therefore not directly comparable. The temperature ranges described in the captions. A small thermocouple was placed the chamber, but it's position was variable.

Summary of Imaging results:

Thermal imaging results have provided key information on the silicon-based reaction chamber thermal environments and their effects on PCR viability in reaction chamber designs. Factors such as air bubbles not only effect the thermal uniformity and ranges of the chambers, but also chemical reactions themselves. Heater materials and designs, and the importance of multi-point temperature analysis have also been delineated.

Captions for Figures 6-9

Figure 6. Infrared image of a reaction chamber showing the temperature fringes of an non-insulated, water-filled, 1 cm x 1 cm by 0.5 mm deep silicon well with silicone sealant and glass cover slip. The heater material is 0.3 um thick polysilicon on 1.5 μm silicon nitride. The total volume of this chamber was ~150 μL. The thermocouple reading was 68.7 °C. The temp range across the chamber was 56.4 to 76. 8 °C. Chamber image is at a ~45 ° angle, and ends along the inner edge of the green contour line.

Figure 7. IR image of a 0.5 cm x 0.5 cm x 0.5 mm reaction chamber mounted on a 2.0 mm thick piece of teflon. The heater material was nickel-chrome patterned in a tight serpentine pattern on a1.5 μm-thick silicon nitride membrane. The water volume was ~150 μL. The bright white spot is an air bubble in the water that reached a temperature of over 100 °C, while the thermocouple read 62.5 °C. The variation outside the of the bubble was approximately 44.9 - 71.6 °C. Chamber image ends along the inner edge of the green contour line.

Figure 8. Similar reaction chamber as Figure 6, without an air bubble. Over 20 °C variation can be observed. The chamber ends along the inner edge of the brown contour line.

Figure 9. Same device as in Figure 7, except device was moderately insulated. Both temperature variation and uniformity are significantly improved. The chamber ends along the inner edge of the brown contour line in the figure.

Figure 6.

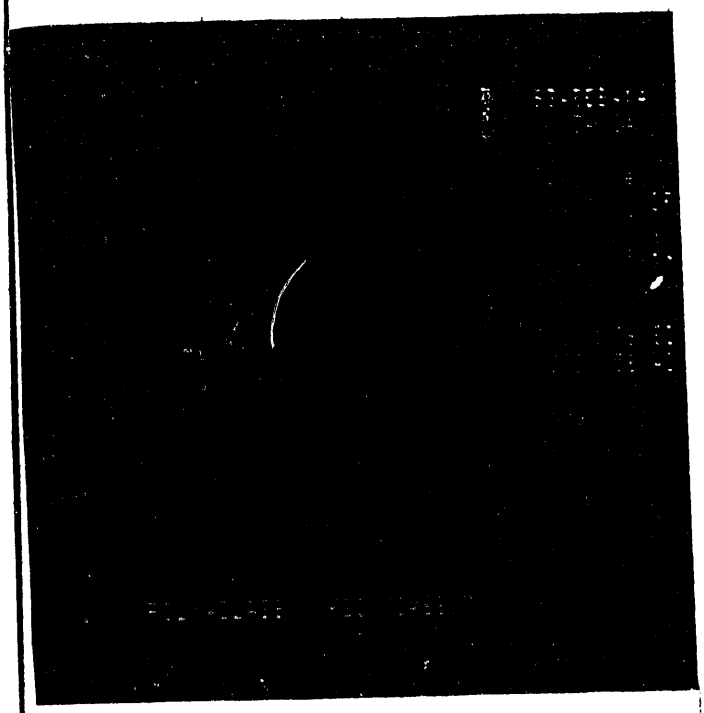


Figure 7.

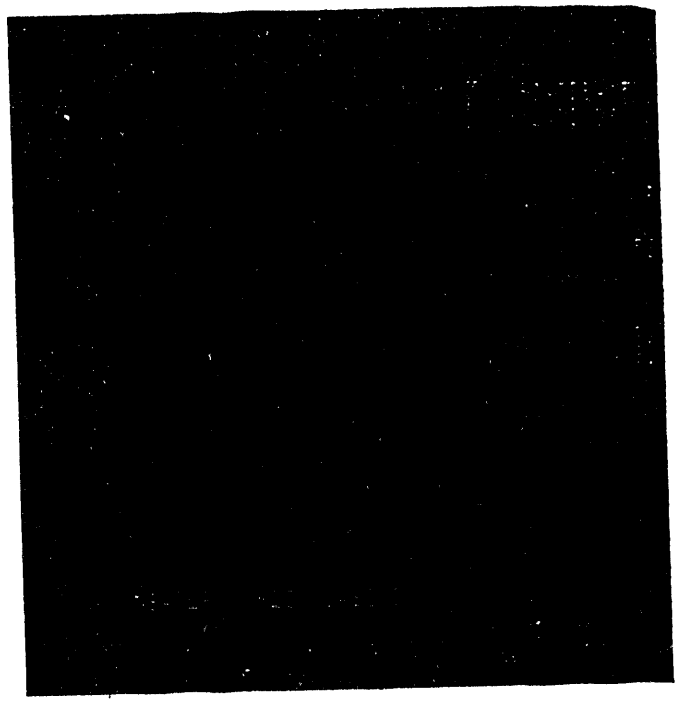


Figure 8.

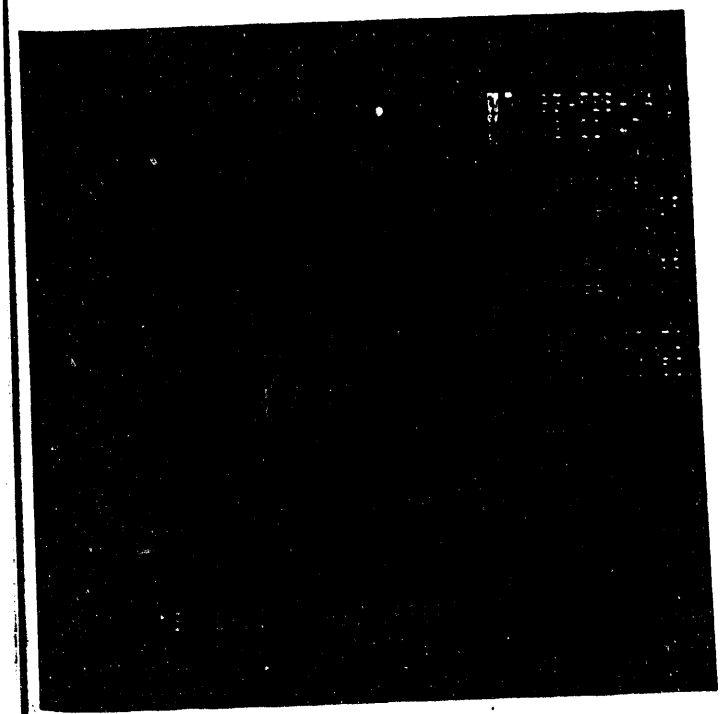
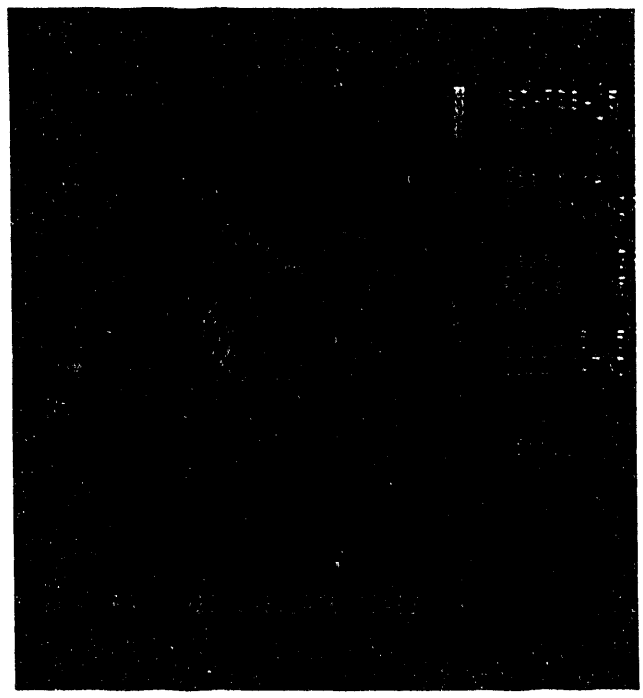


Figure 9.



Reaction Chamber Designs and Recent DNA Amplifications:

Several reaction chamber designs have been fabricated. Sealing of the chambers has thus far have included teflon, polyimide, silicone, lexan, and others. Wafer bonding techniques are also being implemented. Different designs of both single and double sided chambers have been evaluated. The variations tried are too numerous to be described here.

The following PCR results were produced in double-sided chambers separated by a thin layer of teflon material. The cycling rate for over 100 μL of reaction fluid was three cycles per minute at a maximum power consumption of 1.3 Watts. Figure 10 shows a photograph of an ETBr stained electrophoresis gel indicating the micro-device-based amplification of 3 different primer/target sets: a 268 base pair (bp) target representing ß-globin (a subunit of hemoglobin); and 2 from the Human Immunodeficiency Virus (HIV), (112-bp target, and 142-bp target). In these examples, only the ß-globin was amplified in the commercial instrument (Perkin Elmer Model 9600).

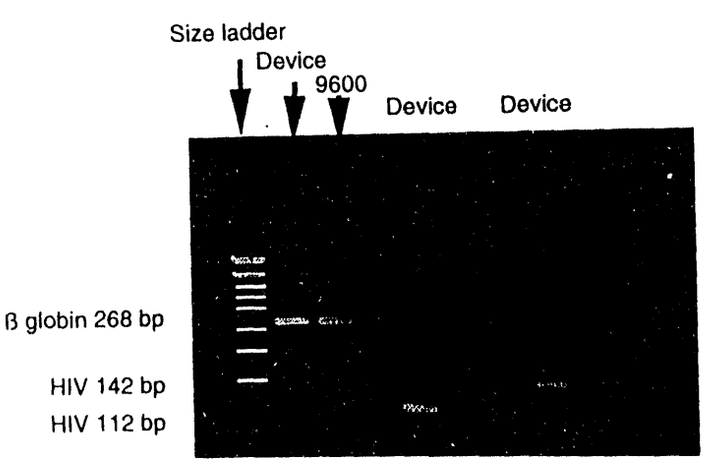


Figure 10. ETbr stained electrophoresis gel showing the amplification of a) 268 base pair (bp) ß-globin, and 2 (HIV) targets, (112-bp and 142-bp). Size ladder (enhanced) is on the left, the standard is the 3rd band in from the left.

Summary of PCR amplification results:

Microfabricated, double-sided, silicon/silicon nitride, with polysilicon heaters have been shown to successfully amplify several different PCR-based biological diagnostic systems. The increased efficiency of the micro-reaction chamber amplification over a commercial bench-top PCR thermal cyclers continues to be verified.

Detection:

Several detection methods are being investigated, including fluorescence and electrochemical. Proper optical transmissivity is being incorporated into reaction chamber material design. Silicon nitride for example, acts as long-pass, cut-off filter for fluorescent molecular tags that are excited in the blue and emit in the red. The transmission spectrum of a low-stress silicon nitride membranes along with the fluorescence emission spectra of a commonly used DNA tag, ETBr, is shown in Figure 11.

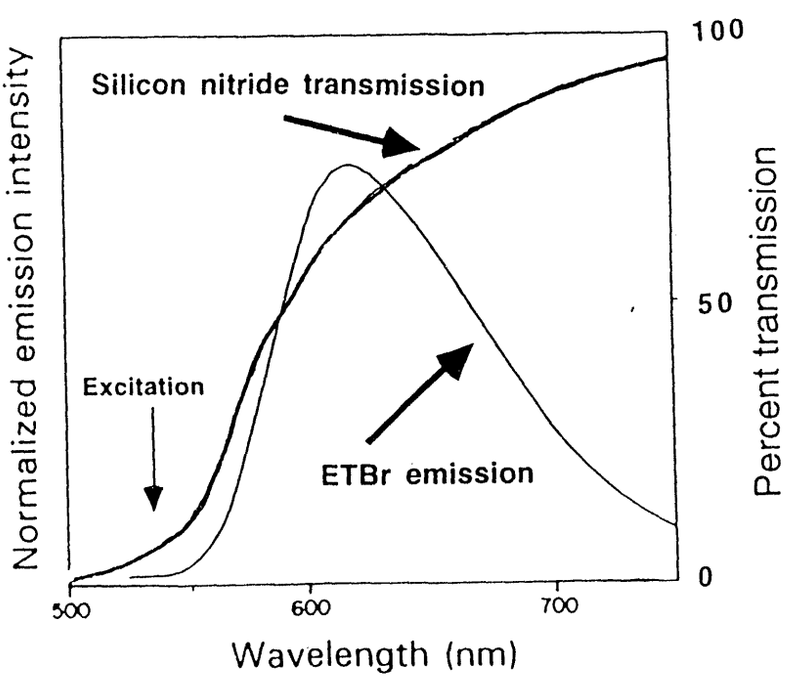


Figure 11. Transmission spectrum of a 3.0 μm-thick low stress silicon nitride membrane (Y axis on right) along with the emission spectrum (axis on left) of the DNA intercalating dye ethidium bromide (ETBr).

Examples of New PCR Developments

The following results performed in commercial thermal cycling instruments, are provided by Roche Molecular Systems, and can be viewed as recent PCR-based diagnostic techniques that lend themselves to micro-reaction chamber analyses. Figures 12 and 13 show the results of Higuchi et al. (1993) illustrating the quantitative, real-time PCR detection obtained by CCD-based imaging of ETBr during thermal cycling.

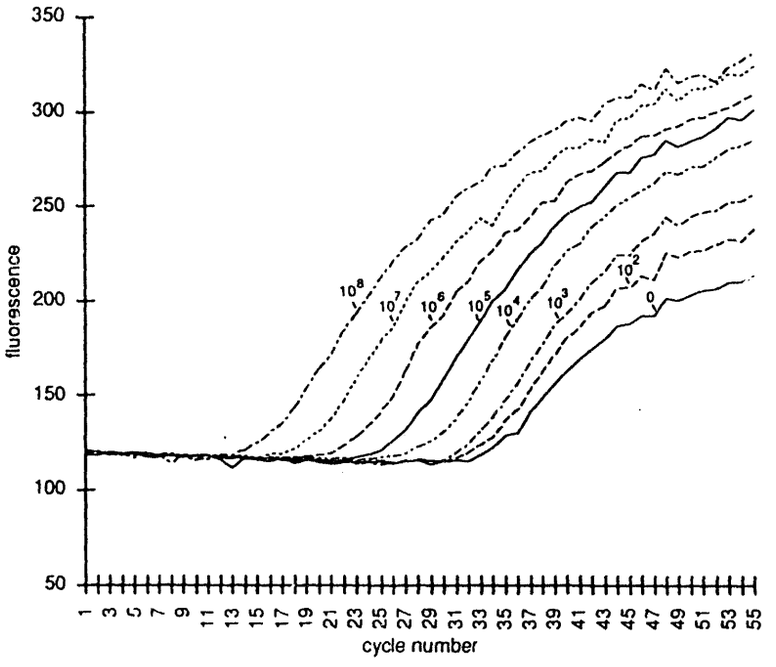


Figure 12. Normalized fluorescence data obtained for different starting target copy numbers (10^0 to 10^8) and cycle number.

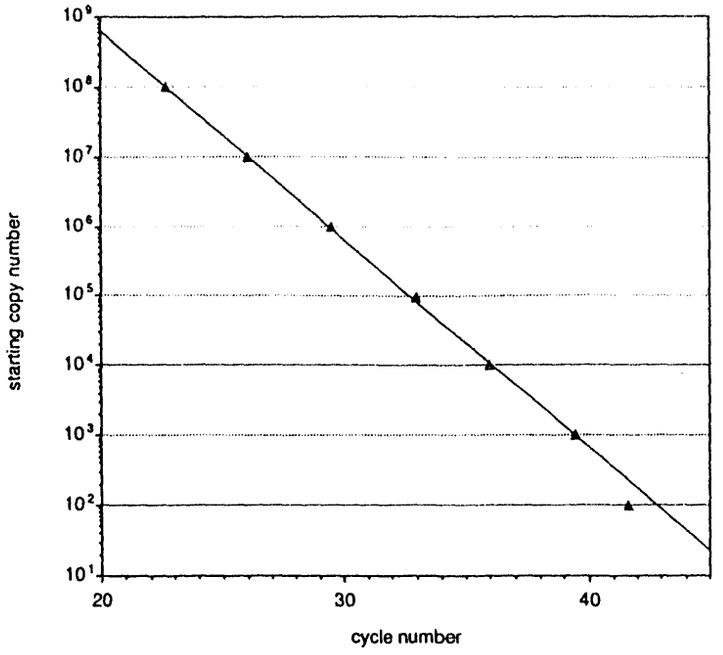
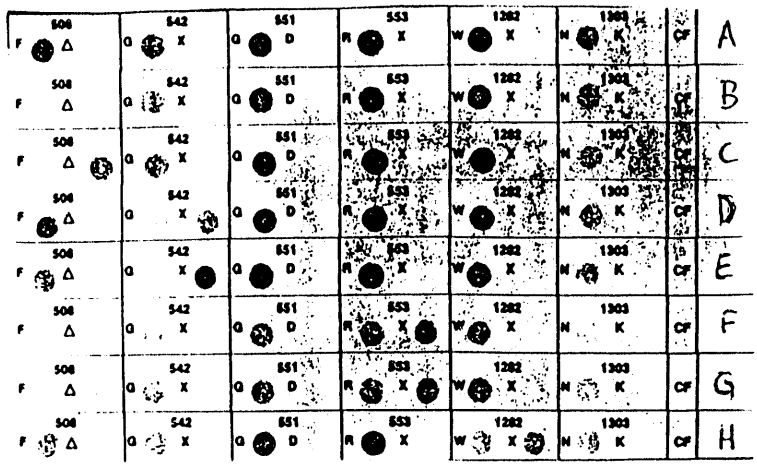


Figure 13. The data in Figure 12 plotted as a quantitative calibration of copy number versus cycle number.

One other recent development in PCR-based diagnostics include the use of multiple amplicons (amplification centers) simultaneously in conjunction with immobilized reagents. Cystic fibrosis (CF) is a relatively common genetic-based disease that can be typed by amplification of a 6-mutation detection system (Saiki, 1993, unpublished results). Figure 14 shows a reverse dot plot screening, PCR-based analysis of amniotic fluid, indicating the presence or absence of the CF mutation.



A	Normal
B	ΔF508 carrier
C	ΔF508 / ΔF508
D	G542X carrier
E	G542X / G542X
F	R553X carrier
G	ΔF508 / R553X
H	W1282X carrier

Figure 14. Reverse dot-plot of PCR amplification of a 6-mutation screening results for cystic fibrosis under development at Roche Molecular Systems.

Some Biology and a Summary

For those unfamiliar with these biotechnological techniques, a short description of what it is that is actually amplified in these experiments is in order.

In the previous examples of this report, a PCR-based analysis was performed in the microfabricated reaction chamber. In particular, we amplified the 142-bp target of the HIV DNA as illustrated in Figure 15. This Figure shows the genes that have been identified on the HIV DNA (gag, pol, etc...). The enlarged region of the "gag" gene delineated by the the shaded areas such as SK462 are specifc primer locations.

The specific sequences such as the "gag" region are cloned onto another (innocuous) pieces of DNA for analysis, such as in this research. These cloned versions of the target are what is amplified in these experiments. They are essentially the analytical standards for the specific PCR-based diagnostic.

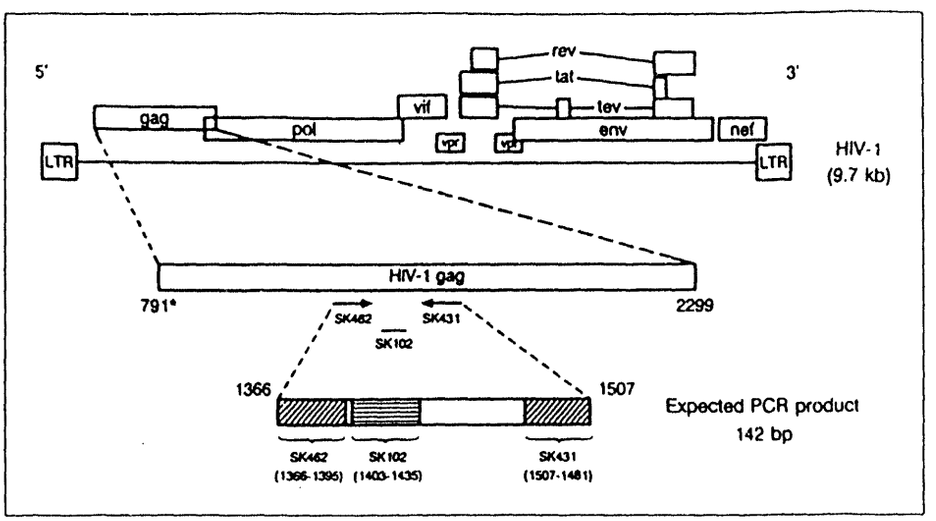


Figure 15. PCR primers and probes developed for detection of HIV-1. Shaded areas represent specific primer sets. (Adapted from Kwok and Sninsky, 1993)

In summary, during these first few months we have been able to extend the previous work into a more concerted effort to build efficient microfabricated reaction chambers. In this report we have provided a description of the PCR technique, previous results in a silicon-based reaction chamber, first modeling results of systems being considered for micro-reaction chamber design, and several examples of infrared images of different cycling reaction chambers. We have discussed detection concepts, verified DNA amplification from recent chamber designs, and have explained several potential new biological systems for analysis. We expect to continue to produce new PCR data in new and improved micro-reaction chambers, while incorporating detection and fluidics.

References:

R. Higuchi, C. Fockler, G. Dollinger, and R. Watson, *Kinetic PCR Analysis: Real-time Monitoring of DNA Amplification Reactions*, Biotechnolgy,11, Sept., 1993. p 026-1030.

S. Kwok and J.J. Sninsky, *in* Diagnostic Molecular Microbiology: Principles and Applications, D.H. Persing, T.F. White, F.C. Tenover, and T.J. White (eds), American Society of Microbiology, Washington, DC., 1993, p. 309-316.

M.A. Northrup, M.T. Ching, R.M. White and R.T. Watson, *DNA Amplification with a Microfabricated Reaction Chamber*, 7th Intern. Conf. on Solid State Sensors and Actuators, Yokohama, Japan, June, 1993, p 924-926.

R. Saiki, 1993
(Personel Communication)

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